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DisQuE Dispersive Sample Preparation Kit

For Pesticide Residue Analysis

DisQuE™ Dispersive Sample Preparation

Page 43

This new solid-phase extraction technology is Integral to LC/MS screening of pesticide residues in foods and can be used in accordance with the Association of Analytical Communities (AOAC) Official Method 2007.01 for residual pesticide analysis. The pre-assembled kit simplifies the process of performing trace-level analysis by giving scientists an all-in-one sample preparation method for the reproducible screening of pesticides in a variety of food commodities, including fruits and vegetables.

PoraPak RXN Cartridges

Superior Cleanup of Synthetic Reactions

PoraPak™ Rxn Post Synthesis Cleanup

Pages 44-49

PoraPak™ Rxn Cartridges are single-use, syringe-barrel cartridges that offer medicinal chemists an effective and simpler method of isolating target compounds in synthetic reaction mixtures. They are a cost-effective alternative to flash chromatography or liquid/liquid extraction for fractionating reaction mixtures, taking much of the complexity out of the process and consuming significantly less solvent per procedure.

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3. When will I receive my order?

The majority of items in this catalog are in stock and will normally be shipped within 24 hours of receipt of your order. We can also arrange blanket or standing orders to meet your needs.

4. How will my order be shipped?

You select the transportation best suited to your needs. Waters offers FedEx and UPS as our standard carriers offering 3-day ground saver, Priority 1, 10:30 a.m. Next Day; Standard, 3:00 p.m. Next Day; and Economy Two-Day Service.

5. What are the payment terms?

Net 30 days. Shipment on U.S. orders is FOB, Franklin, MA.

6. How do I return an item?

A return authorization number (RA#) must be obtained to return an item. If an item is being returned for a warranty reason, please contact Technical Support at 1-800-252-4752, extension 8360. If the return is being requested due to an incorrect order, shipping error, or damage claim, please contact Customer Support at 1-800-252-4752, extension 8365.

Outside the U.S.A., please refer to page 312 for the address and telephone number of your local Waters office, or visit **www.waters.com**

Ordering On-line

Whether researching product information or making on-line purchases, shopping on the Waters Website has never been easier. The Waters Chromatography Columns and Supplies catalog is integrated into the product pages on the site, where you can now **Add to Cart**, **Request Quote** or **Contact Waters**. You can also create a favorites or shopping list and email the product information to colleagues, purchasing or a local sales representative. Let Waters continue to be your on-line resource for the best chromatography columns, sample preparation products, accessories, and Waters Quality Parts. For your convenience, please find our Shopping FAQs on the following page.

Waters Corporation—eCommerce

- Waters maintains a dedicated staff with over 5 years experience in the ecommerce area with vendors such as Ariba, Sciquest, Direct Commerce, Perfect Commerce, Hubwoo and Quadrem.
- We are capable of integrating with your backoffice application or spend management system for orders and invoices.
- Waters is capable of providing static and dynamic catalogs - xls, cif, custom, PunchOut. Catalog contains over 8,500 items for your ordering convenience with images and url links.
- Waters PunchOut offers the following:
  - Secure site
  - Capable of Edit, Inspect and Cancellations
  - Acceptance of Pcards, ghosted Pcards and purchase orders
  - Real-time availability, product pictures and specifications
  - Contained navigation
  - Auto registration

If you are interested in discussing or establishing an electronic relationship for orders or invoices with Waters, please contact Nancy Resteghini at 508/482-8615.

How do I get started?

Click **Login** in the upper right corner of the website.

- If you have previously registered: enter your **Email Address**, **Password** and click **Login**.
- If you have never registered: Click **Sign Up** and complete the waters.com registration form.

Registering allows you ongoing access to Waters events, information, shopping, and support.

How do I find chemistry-related consumables when I don't know what I am looking for?

1. Click the **Products** tab > **Chromatography Consumables & Columns** > and select a Product Category (for example: **Analytical & Preparative Columns**).
2. Select a value from the new filter bar design (**Chemistry, Particle Size etc.**) displaying relevant facets and value selections that can be used to drill down to your desired result.
3. Continue to select values from the various menus to refine the results until you locate the desired product, click on the product header (for more details) or simply fill in a **Quantity** and click **Add to Cart**.

* If you select a Product within the Product Category or through the left-hand navigation (for example: XBridge Columns), click on **Find Products** first and the proceed to step 2 (above).

I already know my part number.

1. Click **Cart** in the upper right corner of the website.
2. Using the Quick Order box: enter the **Product Number**, **Quantity** and click **Add to Cart**. Your items are added directly to the shopping cart.

How do I use Favorites?

You can identify products as a Favorite to expedite ordering in the future.

To add an item to your Favorites:

1. Click **Cart** to display your shopping cart.
2. Place a check mark next to the **Product Name** and click **Add to Favorites**.

To remove an item in your Favorites:

1. Click **My Account** and select **Favorites**.
2. Place a check mark next to the **Product Name** and click **Remove**.

To order using Favorites:

1. Click **My Account** and select **Favorites**.
2. Place a check mark in the box next to the **Product Name** and click **Add to Cart**. A message displays: *The selected item(s) have been successfully added to your cart*.
3. Click **Return to Favorites**. Click the **Close** button then **Cart** to return to your shopping cart.

What if I am unable to purchase on-line or have a purchase approval process?

Waters has provided the ability to email a copy of the Cart contents to someone else with your comments. Sending this information is especially helpful for those who do not have the ability to directly purchase on-line. You can inform your purchasing department, local distributor or *Waters Representative* of your interest or needs. It should not be considered an ordering process but may help you to expedite your order.

To use this feature:

1. Click **Cart**.
2. Click **Email**, enter the email address and a brief message if desired.
3. Click **Send**.

How do I see related items?

1. Click the **Products** tab > **Chromatography Consumables & Columns** > then select a **Product Area**.
2. Select **value(s)** to refine your results.
3. When you find your Product, click the **Product Name** and if there is a Related product, a **Related Product** tab is displayed. Click **Related Products** Tab.

   NOTE: Not all products have Related Products available.

How do I view my order history?

To access your order history, from the Waters Home Page (after login):

1. Click **My Account**, then **Order History**.
2. Find your orders by indicating one of the following: Date, Order Number or Purchase Order Number, then click **Search**.

To access your order history from your **Cart**: click the **Close** button returning you to your previous page, select **My Account** and follow the same instructions above.

How do I find instrument spare parts when I don't know what I am looking for?

There are two ways to find instrument spare parts:

Using the Graphical Parts Locator:

1. Click the **Services & Support** tab > **Find Spare Parts** > and **Graphical Parts Locator**.
2. Select a product from one of the dropdown lists, then select **Waters Quality Parts Locator**.
3. Move your cursor over the picture of the instrument to discover orderable parts, or select a part from the displayed list. Continue to explore and drill down through the photos and graphics until you find the part you need. Click the **Part Description**, fill in a **Quantity** and click **Add to Cart**.

Using Spare Parts Catalog:

1. Click the **Services & Support** tab > **Find Spare Parts** > and **Spare Parts Catalog**.
2. Select a **Value** from one of the Menus (**Product Category** etc.) to begin.
3. Continue to select values from the various menus to refine the results until you locate the desired part. Fill in a **Quantity** and click **Add to Cart**.

ACQUITY UPLC Columns Brochure
Lit. Code 720001140EN

XBridge HPLC Columns Brochure
Lit. Code 720001255EN

SunFire HPLC Columns Brochure
Lit. Code 720000875EN

Prep OBD Columns Brochure
Lit. Code 720002336EN

Atlantis Columns Brochure
Lit. Code 720000793EN

Oasis Sample Extraction
Products Brochure
Lit. Code 720001692EN

Sample Vials and
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Lit. Code 720001818EN

PoraPak RXN for
Post Synthesis Cleanup Brochure
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Sample Preparation Brochure
Lit. Code 720000848EN

Environmental Anaylsis Catalog
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Application Notebook
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Methods Guide
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Waters Vials Selector
www.waters.com/vials

eSelectivity Chart Version 3.0
www.waters.com/selectivitychart

Preparative OBD Column Calculator
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To view and download our latest literature, simply visit **www.waters.com** and enter the literature code in the search field *(top right)*

Sorbent Selection Guide
for SPE Wall Chart
Lit. Code 720002007EN

Analytical Columns
Wall Chart
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Prep OBD Wall Chart
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Click anywhere on page. Type desired page number. Click OK.

Sample Preparation

Oasis Solid-Phase Extraction (SPE) Products

A Breakthrough in SPE

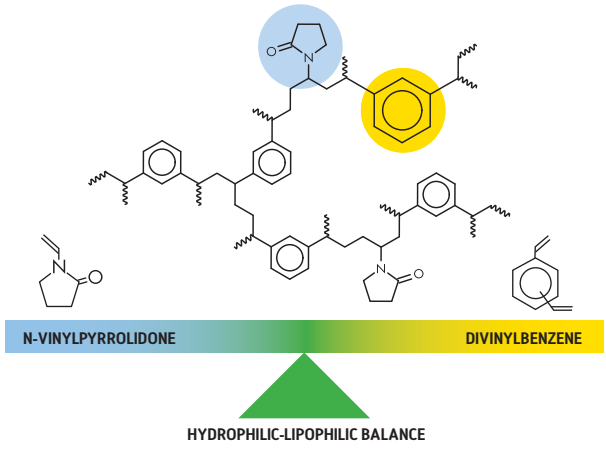
Introduction

In October 1977, Waters designed the first miniature cartridge columns (Sep-Pak® cartridges) containing silica-based adsorbents for SPE.

Demands for sample preparation led to the development of a specially-designed polymeric sorbent that performs optimally for reversed-phase SPE. The Oasis® HLB copolymer with unique Hydrophilic-Lipophilic Balance is unlike traditional SPE sorbents.

Today's goals for modern solid-phase extraction (SPE) are faster throughput, higher recovery and reproducibility, and stronger retention and selectivity. Now SPE can outpace high-throughput techniques such as LC/MS/MS.

Unique Water-Wettable Oasis HLB Copolymer



Specific Surface Area: 810 m^2/g, Average Pore Diameter: 80Å
Total Pore Volume: 1.3 cm^3/g, Average Particle Diameter: 30 mm or 60 mm

The Oasis HLB sorbent is a macroporous copolymer made from a balanced ratio of two monomers, the lipophilic divinylbenzene and the hydrophilic N-vinylpyrrolidone. It provides reversed-phase capability with a special "polar hook" for enhanced capture of polar analytes and excellent wettability.

High and Consistent Recoveries

Oasis sorbents are water-wettable maintaining high retention and capacity for a wide spectrum of analytes, especially when the SPE column runs dry. When the sorbent pores dry out, the chromatographic retention (capture) of the analytes is reduced, resulting in poor recovery. Traditional, silica-based C_{18} sorbents can easily dry out, especially on a vacuum manifold if a particular cartridge flows quickly and allows air to be drawn in. Oasis sorbents maintain proper wetting for more consistent performance (especially important for 96-well plates). Even if air passes through, the Oasis pores do not dry out.

Pore Dewetting Mechanism of Sorbent Pores (Silica Based C_{18})

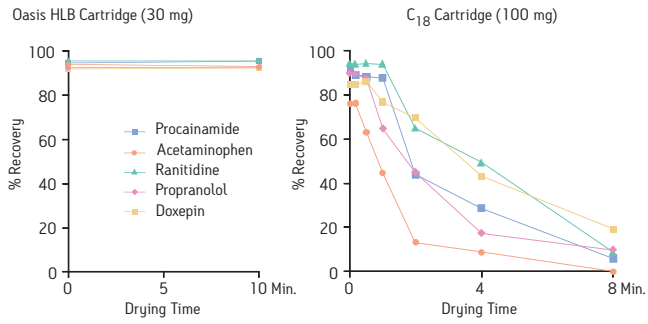
Properly Wetted Pore

Dewetted Pore
If air passes through traditional C_{18} cartridge the pores dry out. When sample is loaded, the analytes are not retained resulting in poor recoveries.

Current Oasis Patents:

Patent No. 5,882,521 (1996), Patent No. 5,976,376 (1998), Patent No. 6,106,721 (1999), Patent No. 6,254,780 (2001) Patent No. 6,322,695 (2001), Patent No. 6,468,422 (2002), Patent No. 6,726,842 (2004), Patent No. 6,773,583 (2004) Patent No. 6,723,236 (2004), Additional Patents Pending

Effect of Drying on Recovery—Oasis HLB Versus C_{18} Sorbents



Oasis HLB 1 cc/30 mg and 1 cc/100 mg C_{18} cartridges were conditioned on a Waters vacuum manifold. When the methanol reached the top of the upper frit in each cartridge, vacuum was maintained for different times to vary the cartridge drying time. The SPE protocol was then continued. The data shown are the average of three replicate extractions.

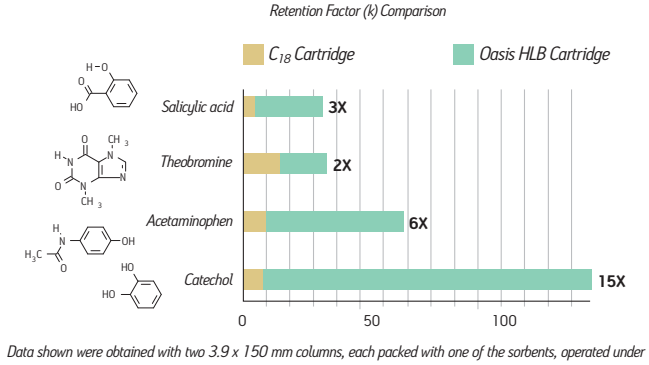
The variable recoveries seen with the C_{18} sorbents, due to the drying out effect, are often the cause for "retests", reducing laboratory productivity. In some laboratories 10% of samples are retests—this can be reduced using Oasis sorbents.

Also, Oasis sorbents retain polar compounds far better than bonded silica SPE sorbents. Note the poor recovery of the polar analyte Acetaminophen for C_{18}. Oasis sorbents work especially well when you need to capture metabolites (see figure above).

High Capacity—Use Less Sorbent

When transferring methods from a C_{18} bonded phase to Oasis products, keep in mind the greater capacity of the Oasis sorbent. The Oasis sorbent has 2-3X more surface area and shows a dramatic increase in k values compared to silica-based C_{18}, This reduces breakthrough potential. In addition, you may be able to use 2/3 less sorbent than you would with C_{18} (30 mg Oasis HLB gives equivalent capacity to 100 mg C_{18}).

Higher Retention Means Greater Capacity, No Breakthrough



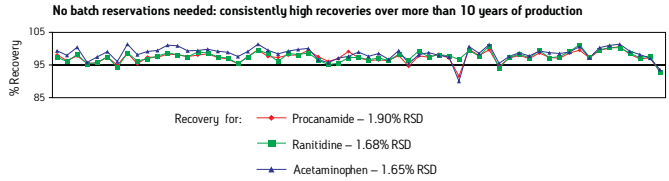
Data shown were obtained with two 3.9 x 150 mm columns, each packed with one of the sorbents, operated under the same conditions: mobile phase: 20 mM potassium phosphate, pH 7.0/methanol (95/5 v/v); temperature: 30 °C; flow rate: 1.0 mL/min; detection: UV @ 254 n

Exceptional Batch-to-Batch Reproducibility

Because of poor stability at pH extremes and relatively low ionic capacity traditional silica-based, mixed-mode sorbents don't have long-term batch-to-batch reproducibility and therefore require reservations of specific lots of sorbent for large projects. Oasis sorbents have demonstrated excellent long-term, batch-to-batch reproducibility for over ten years. As a result of careful process design and stringent quality controls, a new standard has been set in batch-to-batch and lot-to-lot reproducibility for SPE sorbents. The Oasis family of sorbents and devices are manufactured in a Waters ISO 9002 registered facility in compliance with cGMP guidelines of the U.S. Food and Drug Administration for class 1 medical devices.

Multiple batches of each Oasis HLB, MCX, and MAX have been successfully used on validated bioanalytical assays in a regulated laboratory environment.

Batch-to-Batch Reproducibility of Oasis HLB Sorbent



Oasis SPE Applications

Oasis products come in a full range of device formats to meet your SPE requirements—µElution plates, on-line columns, 96-well plates, and single-use cartridges.

Try Oasis and successfully meet your SPE challenges.

Download your free Oasis Applications Notebook
at **www.waters.com/oasis**

Literature References

Oasis Sample Extraction Products Brochure, Literature Reference 720001692EN

Oasis µElution Plate Brochure, Literature Reference 720000476EN

Topics in Solid-Phase Extraction. Part 1. Ion Suppression in LC/MS Analysis White Paper, Literature Reference 720001237EN

Sample Prep Solutions Brochure, Literature Reference 720000848EN

Oasis WAX Sorbent for UPLC/MS Determination of PFOS and Related Compounds in Waters and Tissue, Literature Reference 720001871EN

SPE Sample Preparation for UPLC®–MS Determination of Enrofloxacin (Baytril) in Chicken, Literature Reference WA43206

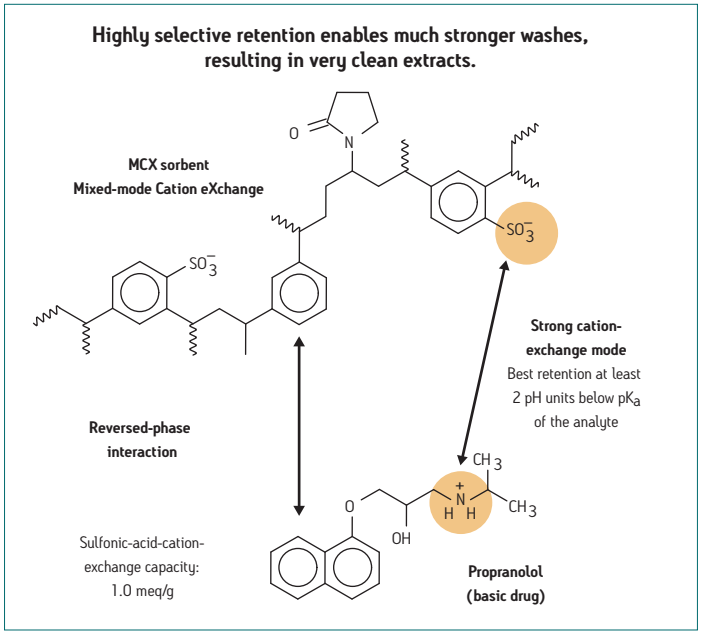
A Sensitive Method for the Determination of Endocrine-Disrupting Compounds in River Water by LC/MS/MS, Literature Reference 720001296EN

Oasis MCX for Basic Compounds

Oasis MCX Mixed-Mode Cation eXchange and Reversed-Phase Sorbent for High Selectivity and Sensitivity for Basic Compounds

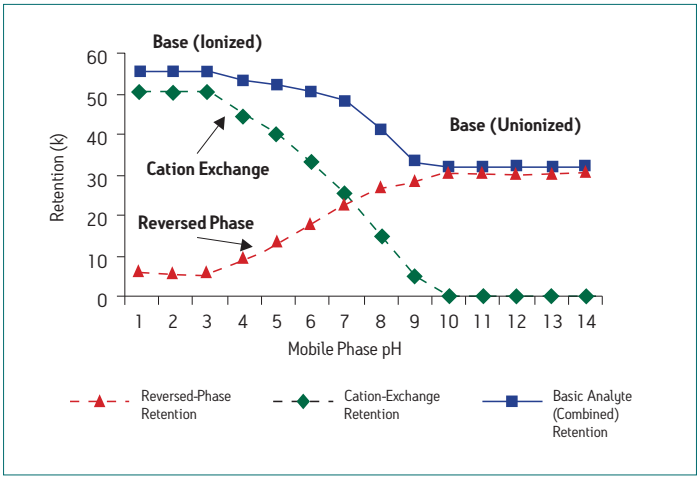
Obtain selective retention of basic drugs with cation-exchange groups on the sorbent surface. The Oasis MCX sorbent has a tightly controlled ion-exchange capacity (1 meq/gram). There are no silanol groups to complicate the retention mode or method development. This novel, water-wettable, polymeric sorbent is stable from pH 0 to 14, making method development simple and fast.

Drug/Sorbent Interactions on Oasis MCX Sorbent



Since this ion-exchange sorbent is synthesized from the reversed-phase Oasis HLB copolymer, it features two retention mechanisms (cation exchange and reversed phase) which can be manipulated very predictably (please refer to the Oasis MCX Retention Map).

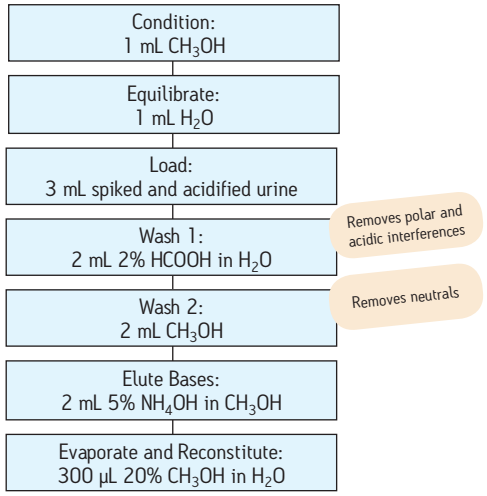
Oasis MCX Retention Map for Basic Analytes Impact on Retention Factor (k) for a Base by Changing Mobile Phase pH in Cation-Exchange and Reversed-Phase Mode



This Retention Map plots the total k or capacity (retention) of a basic analyte relative to pH. Note that the total k, is the sum of the two retention mechanisms. At low pH, the analyte is charged and experiences maximum retention primarily from the ion-exchange mechanism, however, there is also a slight amount of reversed-phase contribution for the combined retention. If your goal is to capture basic analytes and then wash out interferences aggressively, the Load and Wash steps should be at low pH to obtain maximum capture.

At high pH, the ion-exchange retention mechanism shuts-off because the analyte becomes un-ionized. Only reversed-phase retention is present, but since the analyte is now un-ionized, we get the maximum of the reversed-phase retention. We can elute with a combination of high pH and high organic concentration.

Generic Oasis MCX Method for Extraction of Basic Compounds

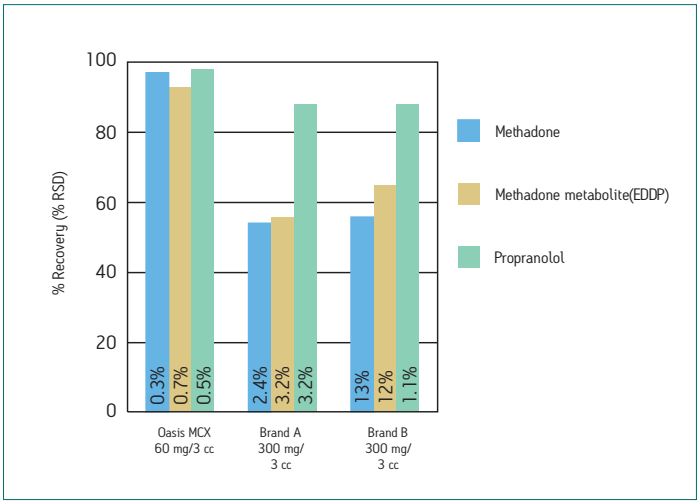


Oasis MCX 3 cc/60 mg cartridge

As shown, one protocol with minimum wash steps gives extractions fast enough to keep pace with your analytical system.

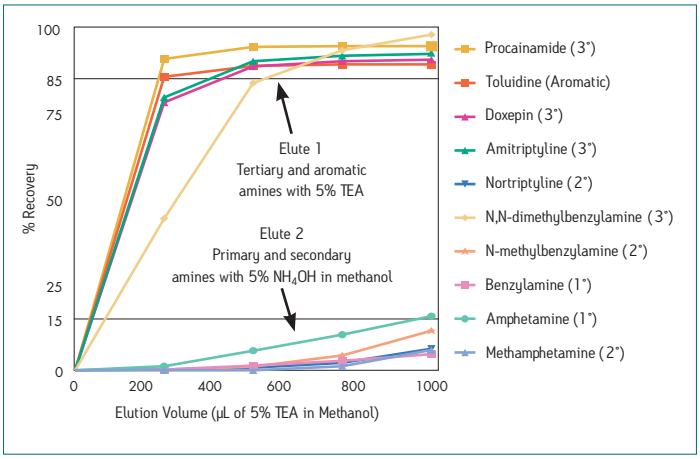
In some cases, with urine samples, no conditioning or equilibration of cartridges is required to achieve excellent results for basic drugs.

Comparison of Oasis MCX Cartridges to other SPE Silica-Based Products Using the Generic Oasis MCX Method



The use of the 2-D approach can be very successful for the Oasis MCX sorbent as well. A pH modified wash system allows optimization of the method and it produces a very selective protocol for basic compounds.

Selectively Separate Primary and Secondary Amines from Tertiary and Aromatic Amines Using an Oasis MCX Cartridge



We can also fractionate the tertiary and aromatic amines in an Elute 1 step using 5% TEA (triethyl amine) in methanol, with very little release of the primary and secondary amines. To elute the primary and secondary amines ammonium hydroxide is used.

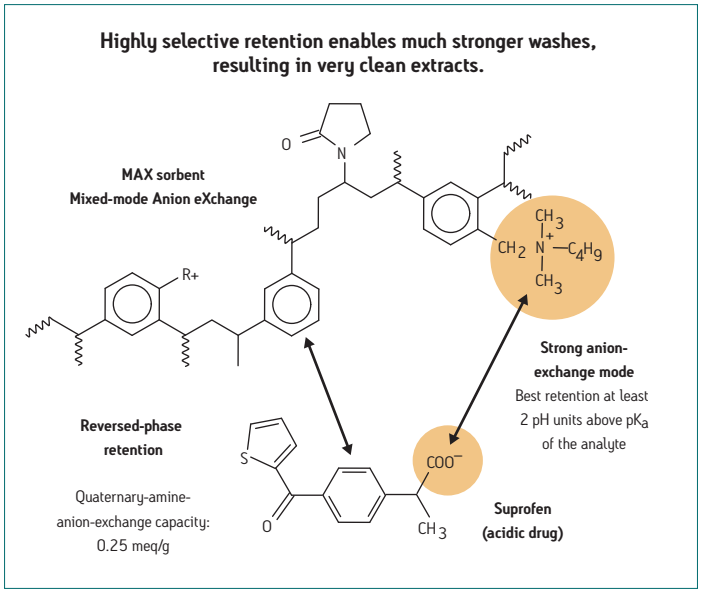
Oasis MAX for Acidic Compounds

Oasis MAX Mixed-Mode Anion eXchange and Reversed-Phase Sorbent for High Selectivity and Sensitivity for Acidic Compounds

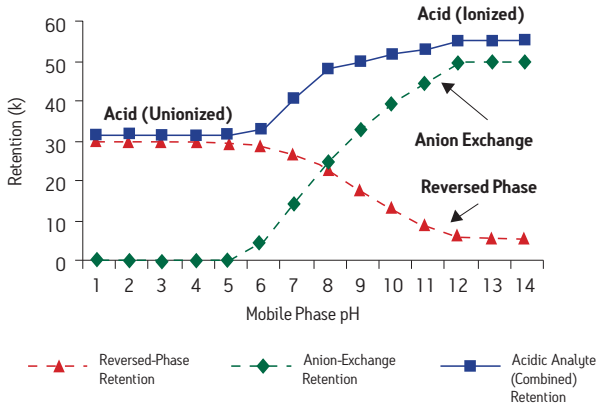
The Oasis MAX sorbent has a tightly controlled ion-exchange capacity of 0.3 meq/gram ensuring reproducible SPE protocols for extraction of acidic compounds and metabolites from biological fluids. There are no silanol groups to complicate the retention mode or method development. This novel, water-wettable, polymeric sorbent is stable from pH 0 to 14, making method development simple and fast.

Since this ion-exchange sorbent is synthesized from the reversed-phase Oasis HLB copolymer, it features two retention mechanisms (anion exchange and reversed phase) which can be manipulated very predictably (please refer to the Oasis MAX Retention Map, page 12).

Drug/Sorbent Interactions on Oasis MAX Sorbent



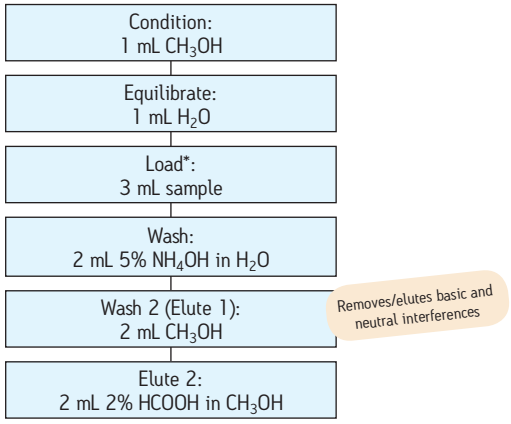
Oasis MAX Retention Map for Acidic Analytes Impact on Retention Factor (k) for an Acid by Changing Mobile Phase pH in Anion-Exchange and Reversed-Phase



The Retention Map plots the total *k* or capacity (retention) of an acidic analyte relative to pH. Note that the total *k*, is the sum of the two retention mechanisms. At high pH, the analyte is charged and experiences maximum retention primarily from the ion-exchange mechanism, however, there is also a slight amount of reversed-phase contribution for the combined retention. If your goal is to capture acidic analytes and then wash out interferences aggressively, the Load and Wash steps should be at high pH to obtain maximum capture.

At low pH, the ion-exchange retention mechanism shuts-off because the analyte becomes un-ionized. Only reversed-phase retention is present, but since the analyte is now un-ionized, we get the maximum of the reversed-phase retention. We can elute with a combination of low pH and high organic concentration.

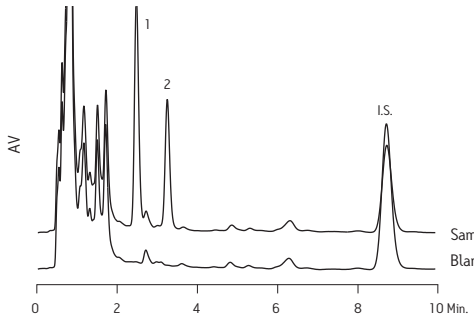
Generic Oasis MAX Method for Extraction of Acidic Drugs



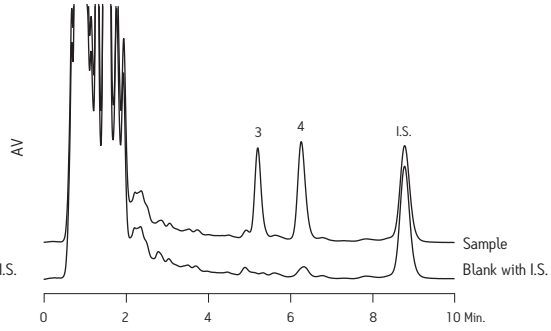
As shown below, acidic compounds can be separated from basic and neutral compounds on the same Oasis MAX cartridge following the recommended protocol.

High Recovery of Acidic, Basic, and Neutral Compounds Using the Generic Oasis MAX Method

Elute 1 (basic and neutral drugs)



Elute 2 (acidic drugs)



Column:	SymmetryShield™ 3.5 µm, 4.6 x 100 mm SymmetryShield Guard column, 5 µm, 3.9 x 20 mm
Mobile Phase:	20 mM potassium phosphate, pH 2.7/methanol, 52:48 v/v
Detector:	UV @ 214 nm
Temperature:	30 °C
Flow Rate:	2 mL/min
Injection Volume:	10 µL of extract with internal standard

Peak Identification
1. Nortriptyline
2. Secobarbital
3. Ketoprofen
4. Naproxen
I.S. Butylparaben

Results (n=6)

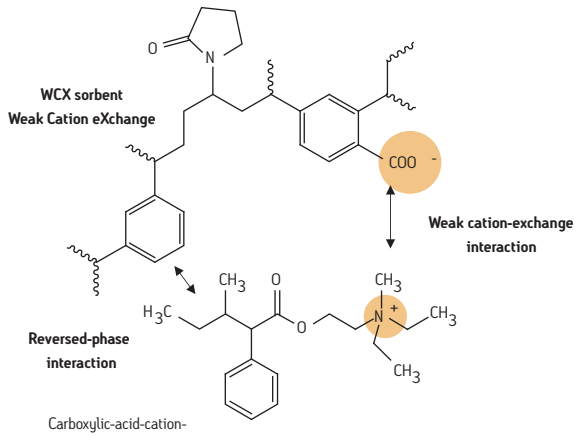
Drugs	µg/mL	Type of Drug	% Recovery	% RSD
Nortriptyline	5	Basic (Elute 1)	94.34	0.78
Secobarbital	10	Neutral (Elute 1)	88.53	1.03
Ketoprofen	5	Acidic (Elute 2)	91.64	2.35
Naproxen	2.5	Acidic (Elute 2)	103.43	3.04

* *1 mL urine hydrolyzed (1M KOH 60 °C, 15 minutes) and adjusted to pH 2 with phosphoric acid; diluted 1:1 with 10 mM sodium acetate, pH 2.*

Oasis WCX for Strong Basic Compounds

High Selectivity and Sensitivity for Strong Basic Compounds

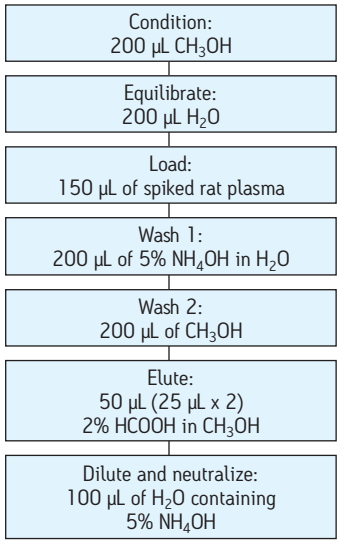
Highly selective retention enables much stronger washes, resulting in very clean extracts.



Carboxylic-acid-cation-exchange capacity: 0.75 meq/g

The Oasis WCX (Weak Cation eXchange) SPE material was developed to provide better sample preparation for strong bases and quaternary amines. The retention mechanism is mixed mode, both ion exchange and reversed phase, which improves retention for all types of basic analytes, especially strong bases.

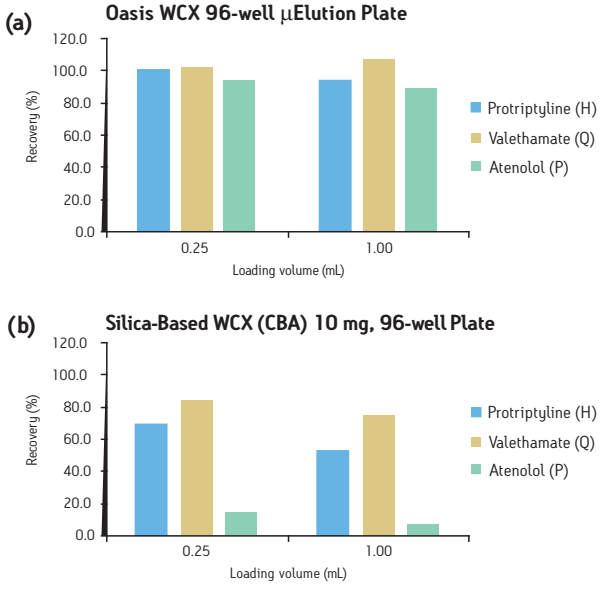
Generic Oasis WCX Method for Plasma Samples Format: Oasis 96-well Elution Plate Used



Sample Pre-Treatment

Spike rat plasma separately with valethamate, protriptyline, and atenolol (each 10 ng/mL). Acidify with H_3PO_4 (2% of total sample volume) for protriptyline only.

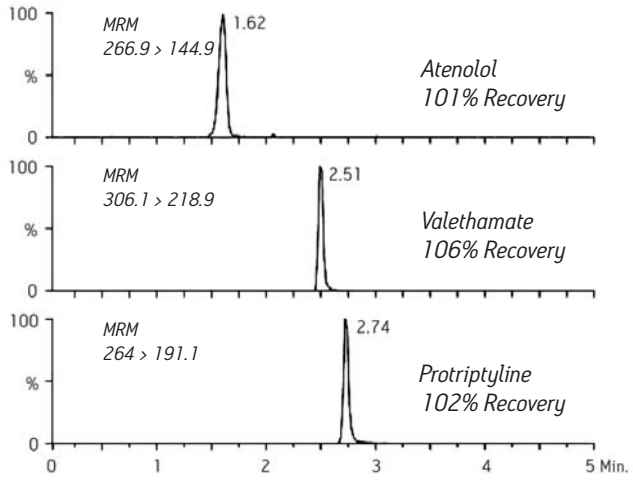
Recovery Data for Three Basic Compounds from Oasis WCX vs. Silica-Based WCX Products



SPE recoveries for three types of bases versus volume of spiked saline solution loaded onto (a) the Oasis WCX sorbent and (b) a commercially available silica-based WCX sorbent. H is hydrophobic base, Q is quaternary amine, and P is polar base.

Excellent Recovery for Quaternary Amines as well as Polar and Hydrophobic Bases

Column:	XTerra® MS C_{18} *5*™ 2.1 x 20 mm, 3.5 µm	Instruments:
Mobile Phase A:	10 mM NH_4HCO_3, pH 10	Waters 2777 Sample Manager,
Mobile Phase B:	MeOH with 10 mM NH_4HCO_3, pH 10	and Waters 1525µ binary HPLC pump,
Gradient:	5 to 95% B in 3 min	Waters Quattro Ultima™
Flow Rate:	0.4 mL/min	(ESI in MRM)
Injection Volume:	10 µL	

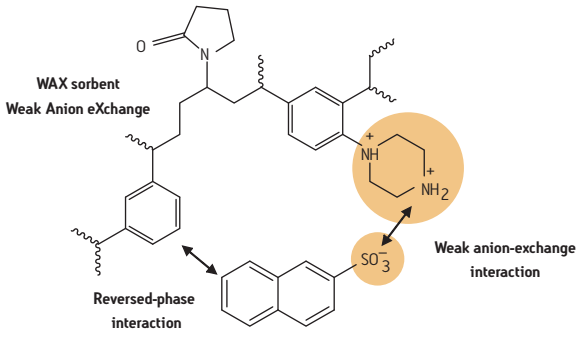


LC/MS/MS chromatograms of the analytes (polar base, quaternary amine, and hydrophobic base) extracted from rat plasma with Oasis WCX 96-well µElution plate. The SPE recoveries were calculated by comparison with postextracted spiked plasma samples.

Oasis WAX for Strong Acidic Compounds

High Selectivity and Sensitivity for Strong Acidic Compounds

Highly selective retention enables much stronger washes, resulting in very clean extracts.



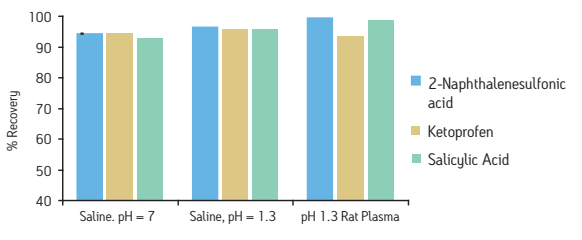
Piperazine-anion-exchange capacity: 0.6 meq/g

The Oasis WAX (Weak Anion-eXchange) SPE material was developed to provide sample preparation for strong acidic compounds. The retention mechanism is mixed mode, both ion exchange and reversed phase, which improves retention for strong acidic compounds.

Generic Oasis WAX Method for Plasma Samples Format: Oasis 96-well µElution Plate

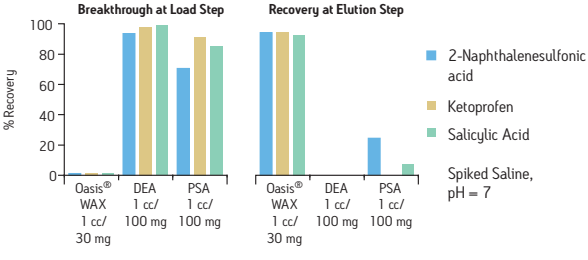
Condition: 200 µL of CH_3OH
Equilibrate: 200 µL of H_2O
Load: 100 µL 1:1 diluted plasma with 2% H_3PO_4 (pH = 1.3)
Wash 1: 200 µL 2% HCOOH in H_2O
Wash 2: 200 µL of CH_3OH
Elute: 50 µL (25 µL x 2) in CH_3OH 5% NH_4OH
Dilute: 50 µL H_2O with 2% HCOOH

Recovery Data for Three Acidic Compounds from Oasis WAX 1 cc, 30 mg Cartridge



SPE recoveries for three types of acidic compounds spiked in saline pH 7 and 1.3, and in rat plasma pH 1.3.

Recovery Data of Three Acidic Compounds for Oasis WAX vs. Silica-Based WAX Products



SPE recoveries for three types of acidic compounds versus volume of spiked saline solution and rat plasma loaded onto (a) the Oasis WAX sorbent and (b) a commercially available silica-based WAX sorbent.

Excellent Recovery for Strong Acid: Camphorsulfonic Acid

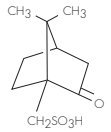
HPLC Conditions:
Column: SunFire™ C_{18} 2.1 x 20 mm *IS*, 3.5 µm
Mobile Phase A: H_2O with 10 mM $CH_3COO\text{-}NH_4^+$, pH 7
Mobile Phase B: MeOH with 10 mM $CH_3COO\text{-}NH_4^+$, pH 7
Flow Rate: 0.4 mL/min
Gradient:

Time (min)	Profile %A	%B
0.0	95	5
3.0	5	95
4.0	5	95
4.1	95	5
5.0	95	5

Injection Volume: 10 µL
Temperature: Ambient
Instrument: Waters 2777 Sample Manager, Waters 1525µ Binary HPLC Pump and Waters Quattro Premier™

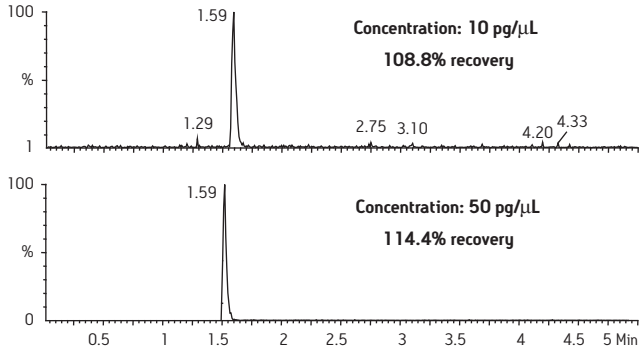
MS/MS Conditions:
Waters Quattro Premier
ESI-

Source Temp:	150 °C
Desolvation Temp:	350 °C
Cone Gas Flow:	50 L/Hr
Desolvation Gas Flow:	600 L/Hr
Collision Cell:	2.2e-3 bar (Ar gas)

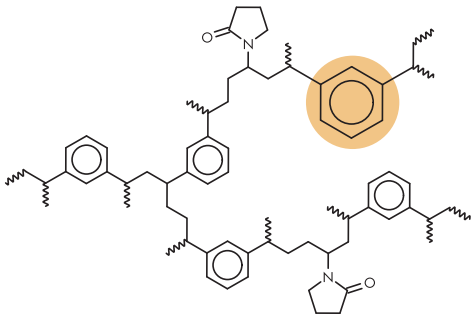


(1S)-Camphor-10-sulfonic acid
MW 232.3, $C_{10}H_{16}O_4S$

MRM Transition	Cone (V)	CID (eV)
m/z 231.1 _ 79.8	60	30



Oasis HLB for Reversed-Phase SPE



Oasis HLB Sorbent

Oasis HLB sorbent makes it fast and easy to develop sample preparation methods that deliver high, reproducible recoveries especially suited to LC/MS/MS analysis by providing the required selectivity and sensitivity.

A Simple and Fast Generic SPE Reversed-Phase Protocol for Rapid Method Development for a Wide Range of Compounds (1-D)

Limitations of traditional silica-based sorbents make you evaluate several different bonded phases and brands to obtain acceptable results. With Oasis HLB, acidic, basic, and neutral compounds, whether polar or nonpolar, can be isolated reproducibly (RSDs <5%) with high recovery (>85%), using the same simple SPE protocol (see below).

This generic, 1-D method (1-Dimensional – only the organic strength is changed) has proven useful for a wide variety of compound types and may be the only protocol required, reducing method development time.

Recommended Generic Oasis HLB SPE Method (1-D)*

Condition: 1 mL CH_3OH
Equilibrate: 1 mL H_2O
Load: 1 mL spiked sample**
Wash (Mild Wash): 1 mL 5% CH_3OH in H_2O
Elute (Strong Elution): 1 mL CH_3OH
Evaporate and Reconstitute: 40 °C/under nitrogen stream 200 µL mobile phase

** Volumes are given for the Oasis HLB cartridge 1 cc/30 mg*

Sample Pretreatment Suggestion

Applying one or more of the following steps before loading your sample may improve your results:

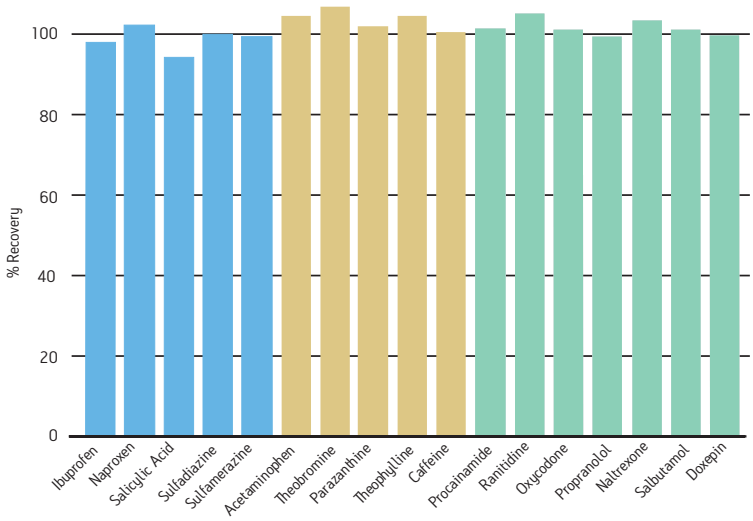
- *Dilute sample 1:1 with buffer to improve flow during loading*

** *Add 20 µL (H_3PO_4) to disrupt drug-protein interaction/ binding*

- *Dilute 1:1 or greater with 0.1 N HCl or other acids*
- *Filter through 0.45 µm membrane*
- *Centrifuge @ ≥3000 rpm*

One Simple Procedure: Many Applications on a Universal Sorbent—Reduced Methods Development Time

With silica-based sorbents (C_{18}) you would need several different chemistries to achieve the results shown here



Acids
Neutrals
Bases

n=6
RSDs < 3.5%

1-D Method

Seventeen drugs with a wide range of polarities were spiked in porcine serum, then extracted with 1 cc/30 mg Oasis HLB cartridges, using the same SPE method.

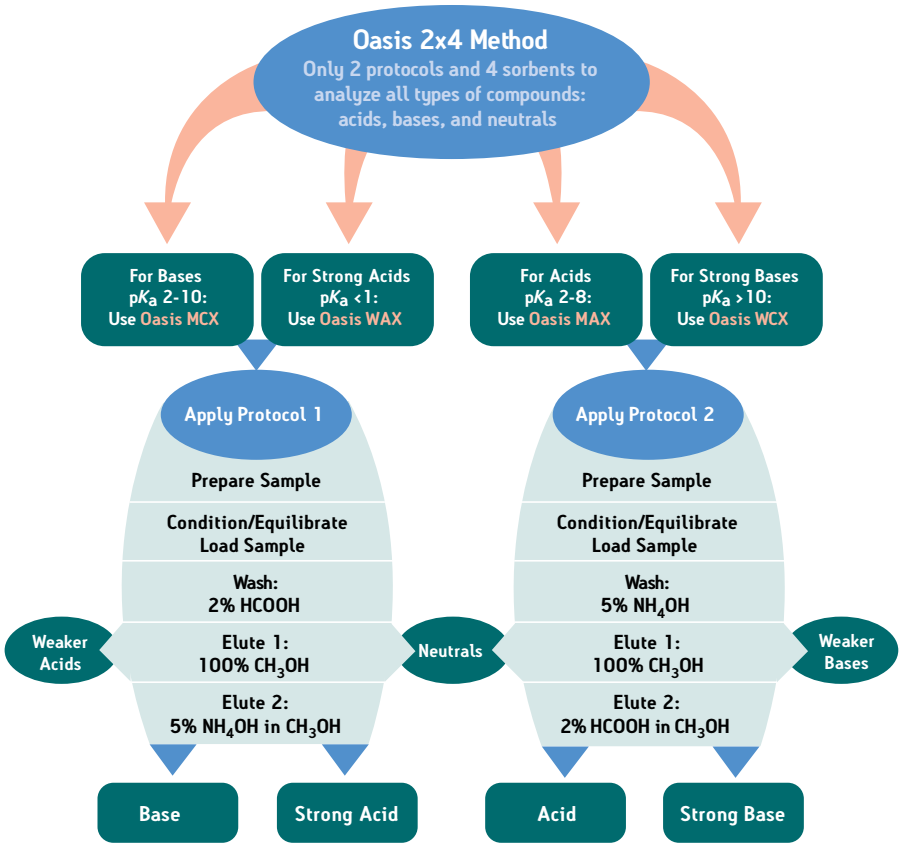
(20 µL/mL H_2PO_4 was used to disrupt drug-protein binding)

Oasis 2x4 Methodology

The Oasis 2x4 Method is a simple, logical approach to the selection of an SPE sorbent and protocol. Two protocols and four sorbents provide the flexibility to extract acids, bases, and neutrals with high SPE recoveries while removing matrix components that may interfere with analysis.

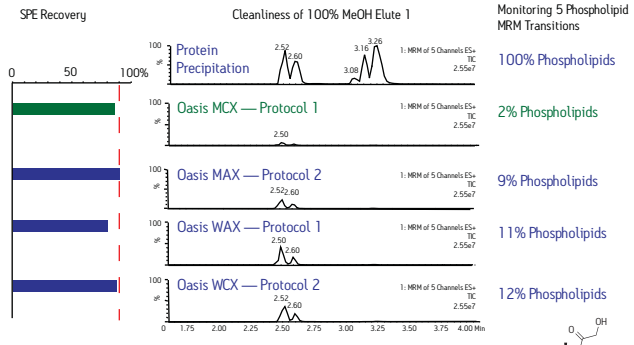
Follow the simple steps outlined in this flow chart to achieve high recoveries and the cleanest extracts:

- Characterize your analyte [Neutral, Acid, or Base, pK_a]
- Select one of the four Oasis sorbents
- Apply the indicated Protocol [1 or 2]
- Determine SPE recoveries by LC analysis



Note that neutral analytes can be isolated from any of the four sorbents in the Elute 1 step of either protocol. Choose the particular ion-exchange sorbent that is best at removing specific matrix interferences. A good example of this is shown below.

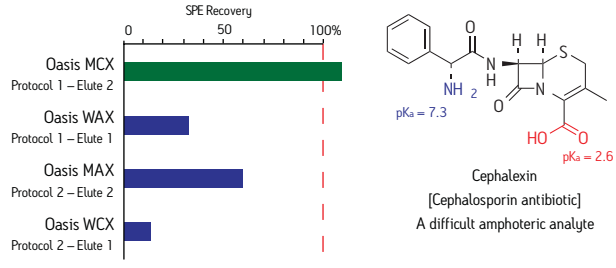
Choosing Optimum Sorbent and Protocol for Neutral Compounds
Example: Prednisone in Plasma



Recovery is high on all four Oasis sorbents, but removal of phospholipids, a primary cause of matrix ion suppression, is best on Oasis MCX. Protein precipitation is a poor choice for sample preparation prior to LC/MS/MS analysis.

Oasis sorbent selection plate and cartridge kits enable rapid development of SPE methods for LC/MS analysis. Having all four Oasis ion-exchange sorbents [MCX, MAX, WAX, and WCX] in a single plate is convenient for scouting the best ways to accomplish efficient isolation of unknowns, zwitterionic compounds, or mixtures of analytes with different retention/elution properties.

Oasis Sorbent Selection Plate:
Evaluating Oasis 2x4 Method for Cephalexin



Aliquots of prepared sample processed using Oasis 2x4 Method protocol designated for each of 4 sorbents. Eluates from Elute 1 and Elute 2 steps analyzed by LC/MS/MS.

Clearly, Oasis MCX is the sorbent of choice.

Oasis 2x4 Method—Proof-of-Concept

To demonstrate the logic, simplicity, and effectiveness of the Oasis 2x4 Method, five rat plasma samples were prepared, each containing one of these characterized test analytes:

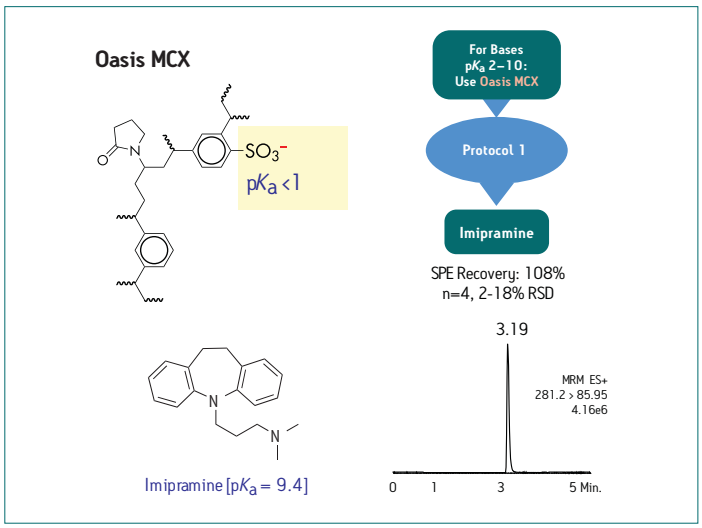
- Imipramine, a base – pK_a of conjugate acid = 9.4
- Ibuprofen, an acid – pK_a = 5.2
- Decanesulfonic acid, a strong acid – pK_a < 0.5
- Valethamate, a quaternary amine [strong base] – pK_a of conjugate acid > 12
- Prednisone, a neutral compound

Each plasma sample was diluted [1:1, v/v] and acidified with phosphoric acid [4% in water]. Respective aliquots were then processed using the protocol and the Oasis ion-mixed-mode sorbent designated by the Oasis 2x4 Method for the corresponding sample type. LC/MS/MS analysis was used to determine SPE recoveries.

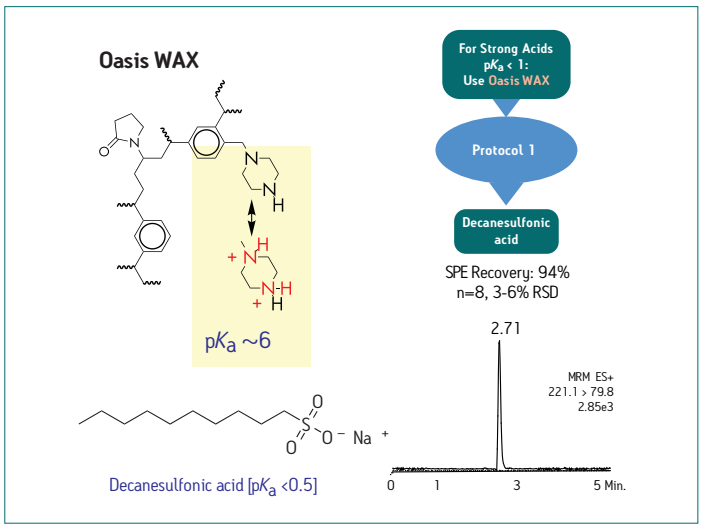
The neutral analyte was processed on all four sorbents, as shown on the previous page. Of the four method options, Oasis MCX with Protocol 1 proved superior at removing nearly all the phospholipids, eliminating this major source of matrix effects, a known cause of ion suppression, loss of sensitivity, and inaccurate quantitation in LC/MS analysis.

Essentially, quantitative recovery and excellent cleanup efficiency were achieved for each of the ionic or ionizable test analytes when the recommended Oasis 2x4 Method sorbent/protocol combination was used. These results are shown in the four figures below.

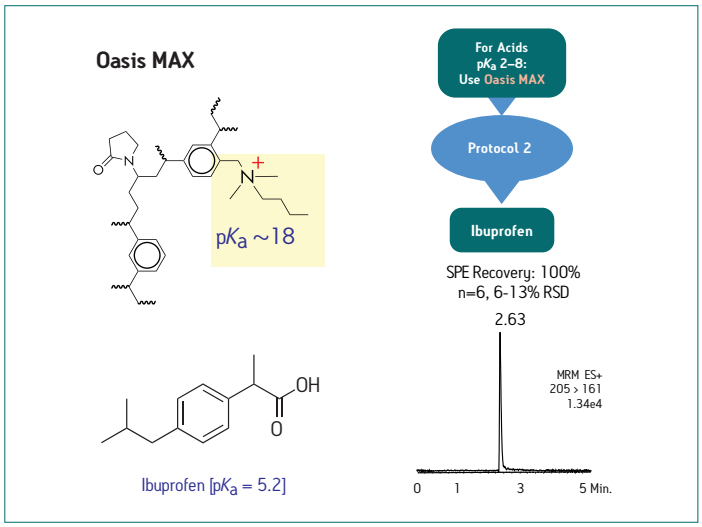
Oasis 2x4 Method Test on MCX: Base Isolation



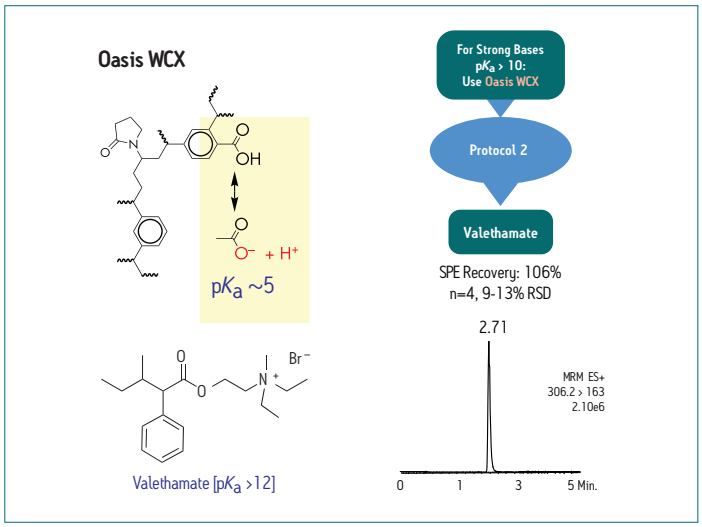
Oasis 2x4 Method Test on WAX: Strong Acid Isolation



Oasis 2x4 Method Test on MAX: Acid Isolation



Oasis 2x4 Method Test on WCX: Strong Base Isolation



Oasis µElution Plates for Ultra Low Elution Volumes

- Elution volume as low as 25 µL
- No evaporation and reconstitution
- Ideal for small sample volumes
- Up to a 15X increase in concentration

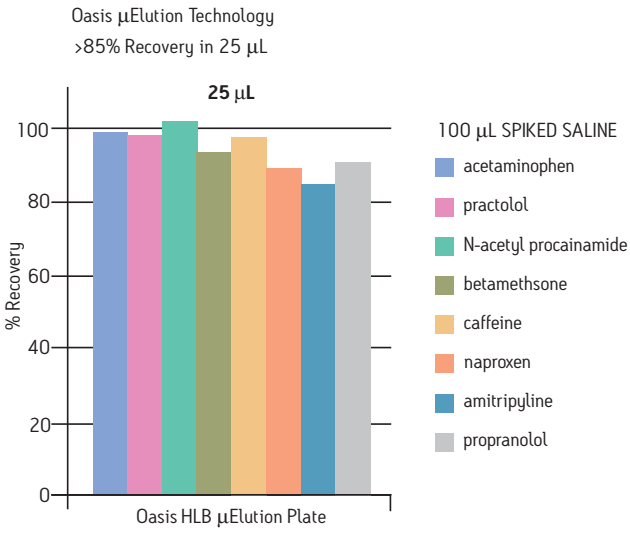
Eluting in 25 µL without evaporation provides up to a 15-fold increase in analyte concentration, enabling sensitive, robust, and reproducible SPE results. Scientists in both pharmaceutical drug discovery and drug development can prepare the cleanest biological sample extracts for more sensitive LC/MS/MS analysis.

*U.S. Patent 6,723,236

Waters Newest Innovation in SPE Technology

Now you can confidently perform SPE cleanup and analyte enrichment of very small sample volumes (10-25 µL) up to a maximum of 375 µL. The Waters Oasis µElution plate combines patented* plate design, proven Oasis chemistries, and straightforward protocols that deliver high analyte recovery and clean extracts in elution volumes as low as 25 µL. Using the Oasis µElution plate achieves superior results compared to other conventional SPE formats in less time. This plate format produces concentrated extracts that can be directly injected into your LC/MS/MS, eliminating the need for the time-consuming evaporation step.

Excellent Recovery in 25 µL Elution



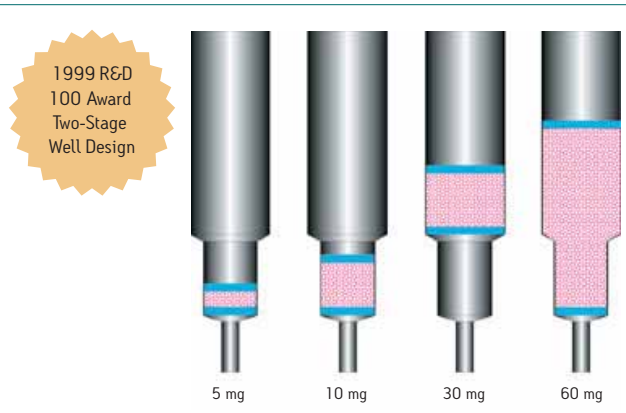
The innovative features of Oasis µElution plates enable sensitive, robust, reproducible results without evaporation and reconstitution.

Oasis 96-Well High-Throughput Extraction Plates

Versatile, High-Throughput Oasis 96-Well Extraction Plates

Waters award-winning plate design, with five chemistry and four sorbent-mass options, provides flexible high-throughput SPE in a single device. The Oasis 96-well plates are designed to be used on many manifold configurations and most robotic liquid handling systems. Oasis sorbents' unique balance of hydrophobicity and water-wettability means you will never have to worry about poor results if individual wells of the 96-well plate dry out. As always, you can expect Oasis SPE products to perform reliably, delivering high and reproducible recoveries for a wide range of analytes, including polar and basic compounds, with RSDs less than 5% (n=96).

Waters 96-Well Plate Design



By varying frit size and/or placement, the same plate may be filled with various quantities of sorbent per well. Our design permits optimal recoveries, even with low sorbent weights for smaller elution volumes.

Speed and Throughput

Comparison of Oasis µElution Plate and Protein Precipitation—Superior Results, Less Time and Effort

The Oasis µElution plate optimizes the configuration of the highly efficient Oasis sorbents (HLB, MCX, MAX, WCX, and WAX), enabling elution volumes as low as 25 µL, providing fast cleanup with improved performance over protein precipitation. Time consuming evaporation and reconstitution steps are eliminated, compressing preparation cycle time and increasing throughput capabilities.

Extraction Protocols

The protein precipitation generic method includes both a centrifugation and an evaporation step, which produces the cleanest sample extract possible for protein precipitation. The final sample volume (75 µL) is the same for all three generic methods. The Oasis generic methods produce cleaner extracts than protein precipitation, demonstrated by the improved sensitivity with the Oasis HLB (4X) and the Oasis MCX method (9X to 25X). The Oasis method enables improved sensitivity by eliminating matrix effect and reducing ion suppression. Achieve superior results compared to protein precipitation in less time using the Oasis µElution plate.

Protein Precipitation Generic Method	**Oasis HLB Method** *Oasis HLB µElution Plate** Part Number 186001828*	**Oasis MCX Method** *Oasis MCX µElution Plate** Part Number 186001830*
Add 50 µL spiked plasma (1 to 1000 pg/µL), 50 µL IS (10 pg/µL) in H_2O	Condition with 200 µL CH_3OH	Condition with 200 µL CH_3OH
Add 1 mL CH_3CN to precipitate plasma	Equilibrate with 200 µL H_2O	Equilibrate with 200 µL H_2O
Centrifuge the plasma at 3600 rpm for 30 minutes*	Load 50 µL spiked rat plasma, 50 µL IS (10 pg/µL) in H_2O	Load 50 µL spiked rat plasma, 50 µL IS (10 pg/µL) in H_2O
Transfer the supernatant to another 2 mL 96-well container	Wash with 200 µL 5% CH_3OH in H_2O	Wash 1 with 200 µL H_2O + 2% COOH
Evaporate the solution to dryness	Elute with 25 µL CH_3CN:$(CH_3)_2CHOH$ 40:60 + 2% HCOOH	Wash 2 with 200 µL CH_3OH
Reconstitute with 25 µL CH_3CN:$(CH_3)_2CHOH$ (40:60) + 2% NH_4OH and 50 µL H_2O	Dilute with 50 µL H_2O	Elute with 25 µL CH_3CN:$(CH_3)_2CHOH$ 40:60 + 5% NH_4OH
Inject 20 µL	Inject 20 µL	Dilute with 50 µL H_2O
		Inject 20 µL

Increase in Selectivity (More Selective Methods)

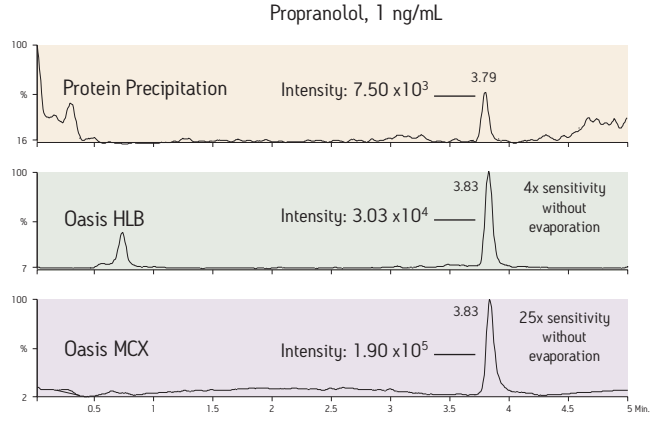
*** Tomtec Quadra 96 used.*

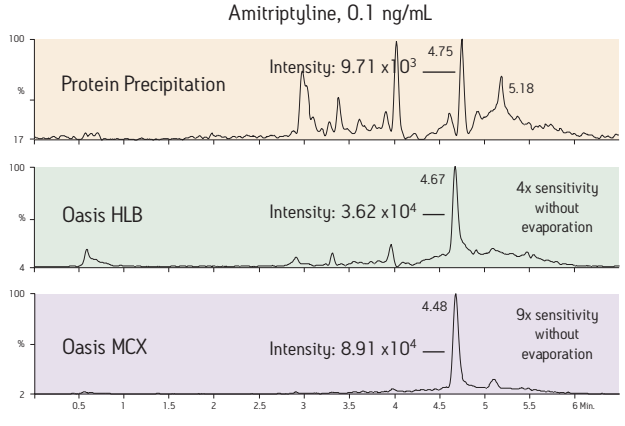
Oasis HLB and MCX µElution Plate versus Protein Precipitation

LC Conditions	
Column:	XTerra MS C_{18}, 2.1 x 30 mm, 3.5 µm
Mobile Phase A:	Water + 0.5% NH_4OH
Mobile Phase B:	ACN + 0.5% NH_4OH
Flow Rate:	0.2 mL/min
LC conditions:	5% - 95% B 1 min.
Temperature:	Room temperature
LC Instrument:	Waters Alliance® 2795

MS Conditions	
MS Instrument:	Quattro Triple Quadrupole
Ion Source:	ESI (+)
Source Temperature:	150 °C
Gas Cell:	2.0 e^{-3} bar Argon
Desolvation Temperature:	350 °C
Drying Gas Flow:	500 L/hr
Cone Gas Flow:	50 L/hr
Cone Voltage:	25 volts
Collision Energy:	20
Capillary Voltage:	3.5 Kv

MRM Transition:		
Metoclopramide (IS)	m/z 299.8	226.7
Propranolol	m/z 259.9	154.9
Amitriptyline	m/z 278.1	232.9
Nortriptyline	m/z 263.9	190.8



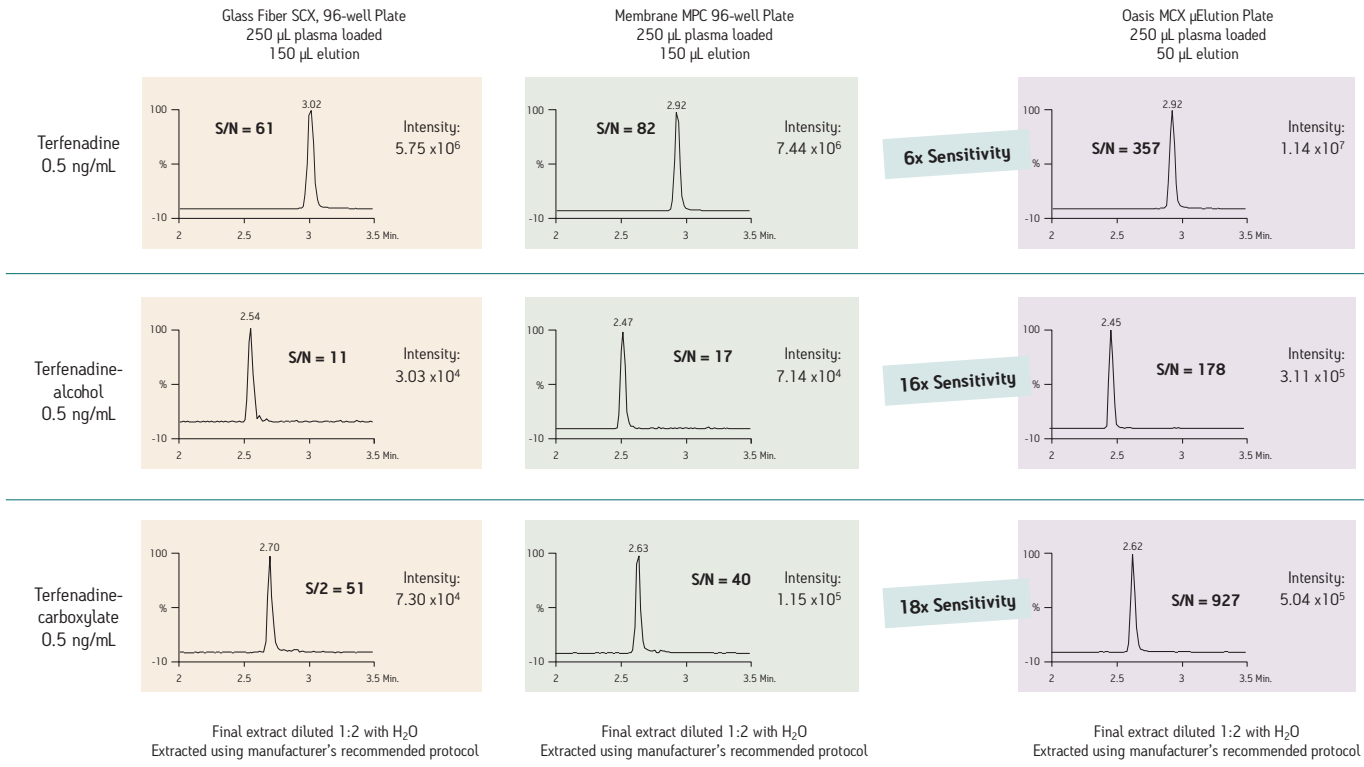


Sensitivity and Precision

Comparison of Oasis MCX µElution Plate and Glass Fiber and Membrane 96-well Disk Plates—Up to 18X Increase in Sensitivity

The Oasis µElution plate shows superior performance when compared to other low elution disk plate products used according to recommended protocols. A generic Oasis MCX method for terfenadine with 50 µL elution, dilution and direct injection shows better sensitivity than the membrane and glass fiber plates, which require a 150 µL elution volume for complete analyte recovery. The Oasis µElution plate enables sensitivity gains and delivers high performance without the time consuming evaporation step.

Oasis MCX µElution Plate vs. Glass Fiber and Membrane 96-well Disk Plates



Validation Assay of Terfenadine and Its Metabolites Using The Oasis MCX µElution Plate

- Calibration range: 0.5-200 ng/mL, 7 levels
- 6 Calibration curves per day over four days
- Analyte recoveries over calibration range: ≥ 95%
- Inter-day %RSD of back-calculated standard concentrations over four days (24 values) ≤ 5.6%
- Intra-day %RSD of back-calculated standard concentrations within one day (6 values) ≤ 8.0%

Sorbent Amount and Solvent Selection for the Generic Method

The suggested amount of sorbent in a cartridge or a plate required for your application, is given in the table below. Remember, because of the increased capacity of the Oasis sorbents, you can use less sorbent than you would normally need if you used a silica-based packing. The solvent used for the elution step should be selected based on the polarity of the analyte. The second table below gives a selection of elution solvents and each solvent gives you different selectivity and elution strength.

Capacity and Elution Volume of Oasis 96-Well Plates and Cartridges

Sorbent per device	Maximum Mass Capacity	Typical Sample Volumes	Elution Volume
µElution Plate*	60 to 400 µg	10 to 375 µL	25 µL**
5 mg*	0.15 to 1 mg	10 to 100 µL	≤ 150 µL
10 mg	0.35 to 2 mg	50 to 200 µL	≤ 250 µL
30 mg	1 to 5 mg	100 µL to 1 mL	≥ 400 µL
60 mg	2 to 10 mg	200 µL to 2 mL	≥ 800 µL

* Available only in 96-well plates
** µElution Plate requires no evaporation step

When converting from C_{18} silica-based to Oasis SPE sorbents, use approximately 2/3 less Oasis sorbent (100 mg C_{18} sorbent = 30 mg Oasis sorbent).

Note: Larger capacity cartridges are also available.

Tips for Selecting Elution Solvents for the Generic SPE Method (1-D)*

The elution solvent is selected based on polarity of analyte.

Solvent	Solvent Type	Relative Elution Strength**	Comments
Methanol	proton donor	1.0	disrupts H-bonding
Acetonitrile	dipole-dipole	3.1	medium polarity drugs
Tetrahydrofuran	dipole-dipole	3.7	medium polarity drugs
Acetone	dipole-dipole	8.8	medium polarity drugs
Ethyl Acetate	dipole-dipole	high	nonpolar drugs and GC compatible
Methylene Chloride	dipole-dipole	high	nonpolar drugs and GC compatible

*When using solvents other than methanol, add 10-30% (of proton donor solvent like methanol) to disrupt H-bonding on the Oasis HLB sorbent.
** High-Purity Solvent Guide. Burdick & Jackson Laboratories, Inc. Solvent Properties of Common Liquids, L.R. Snyder, J. Chromatogr., 92, 223 (1974); J. Chromatogr. Sci. 16, 223 (1978)

The generic method (1-D) is an excellent starting protocol for methods development. If you were not able to meet all of your SPE goals, then the advanced 2-D protocol will provide a rapid way to chromatographically determine an even cleaner, more selective and sensitive result.

Automation

Automation of Oasis Sample Extraction Cartridges and 96-Well Plates

Oasis SPE products are compatible with the following liquid handling and/or SPE automation systems:

- Perkin Elmer Robotic liquid handling system, MultiProbe II
- Tomtec Quadra3 and Quadra3SPE
- Hamilton Microlab SPE Workstation
- Beckman Biomek 2000 Laboratory Automation Workstation
- Caliper Life Science RapidTrace Automated SPE Workstation, AutoTrace
- Gilson ASPEC XL4
- Gilson ASPEC XL
- Gilson 215 SPE Liquid Handler
- Tecan Genesis FE500
- Spark Holland Symbiosis

Waters ACQUITY UPLC MS/MS System for Bioanalysis Featuring the Waters Xevo TQ MS

For high sensitivity analyses, such as those employing UPLC/MS/MS, proper sample preparation can be critical for minimizing matrix effects and concentrating analytes of interest. Oasis sample preparation can be used with UPLC/MS/MS systems to provide the cleanest extracts.

The system shown here integrates the Xevo™ TQ MS, a highly advanced mass spectrometer, combining MassLynx™ informatics with innovative ScanWave™ and IntelliStart technologies, and the Waters ACQUITY UPLC system.

Oasis On-Line Columns

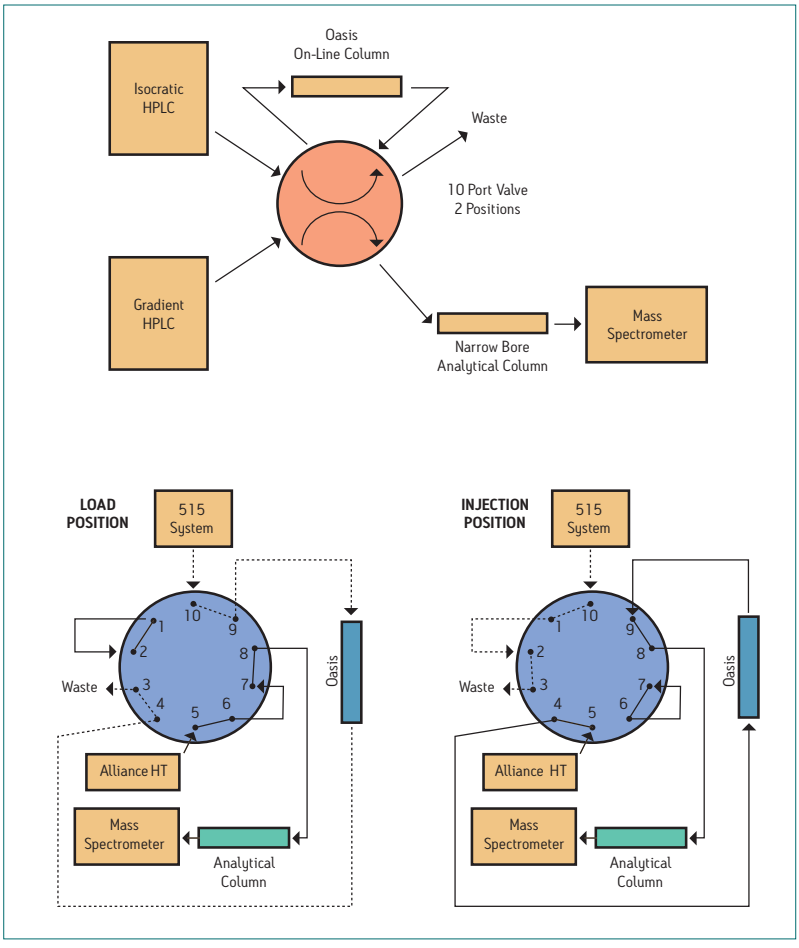
On-Line SPE Columns for LC/MS/MS

There are three HPLC Oasis on-line column configurations designed to fit all your on-line-analysis needs.

- The Oasis cartridge column fits into a Sentry™ holder that features a finger-tight fitting for fast, convenient replacement[1]
- The Oasis Direct-Connect Column can be screwed directly into a switching valve or connected to fittings like those for a conventional HPLC column[2]
- The Oasis Column features traditional HPLC column fittings and hardware[3]

All of these formats are available with the five Oasis patented sorbents (HLB, MCX, MAX, WCX, and WAX) in a wide choice of particle sizes and dimensions. The Oasis on-line columns make it possible to analyze a specific analyte in a sample matrix when combined with appropriate Waters narrow-bore analytical columns (such as XBridge™, SunFire™, Atlantis®, XTerra®, or Symmetry® columns).

On-Line System Configuration



Oasis Glass Cartridges for PPT Detection Levels

Waters Oasis glass cartridges are available in 5 cc (200 mg) configuration with Teflon® frits for trace analysis at parts per trillion level. Each lot is tested for the presence of bisphenol A and other phenols and phthalates, assuring that endocrine disruptors in water samples can be analyzed to part per trillion levels.

See the full application in the Oasis Applications Notebook available for download at w**ww.waters.com/oasis**

Endocrine Disruptors

PPT Recovery of Estrogens from River Water, LC/MS, 5 ng/L Spike Level, n=4

Results	Ions Monitored (m/z)	Recovery	% RSD
1. bisphenol A	227	113	11
2. 17b-estradiol	271	93	15
3. 17a-ethynylestradiol	295	96	12
4. estrone	269	87	5
5. diethylstilbestrol	267	75	5

Recovery of Phthalates and Nonylphenol from River Water, GC/MS, 200 ng/L Spike Level, n=4

Results	Recovery	% RSD
1. dimethylphthalate	130	15
2. dirthylphthalate	86	12
3. n-nonylphenol	90	11
4. dibutylphthalate	110	11
5. benzylbutylphthalate	110	8
6. bis(ethylhexyl)phthalate	60	8
ISTD. o-terphenyl (internal standard)		

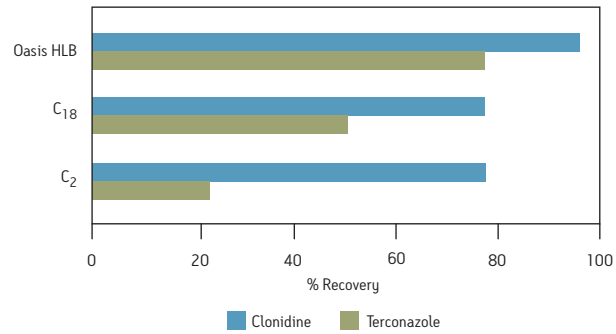
Oasis Symbiosis On-Line Cartridges

On-Line SPE with Oasis Sorbent in Symbiosis Cartridges

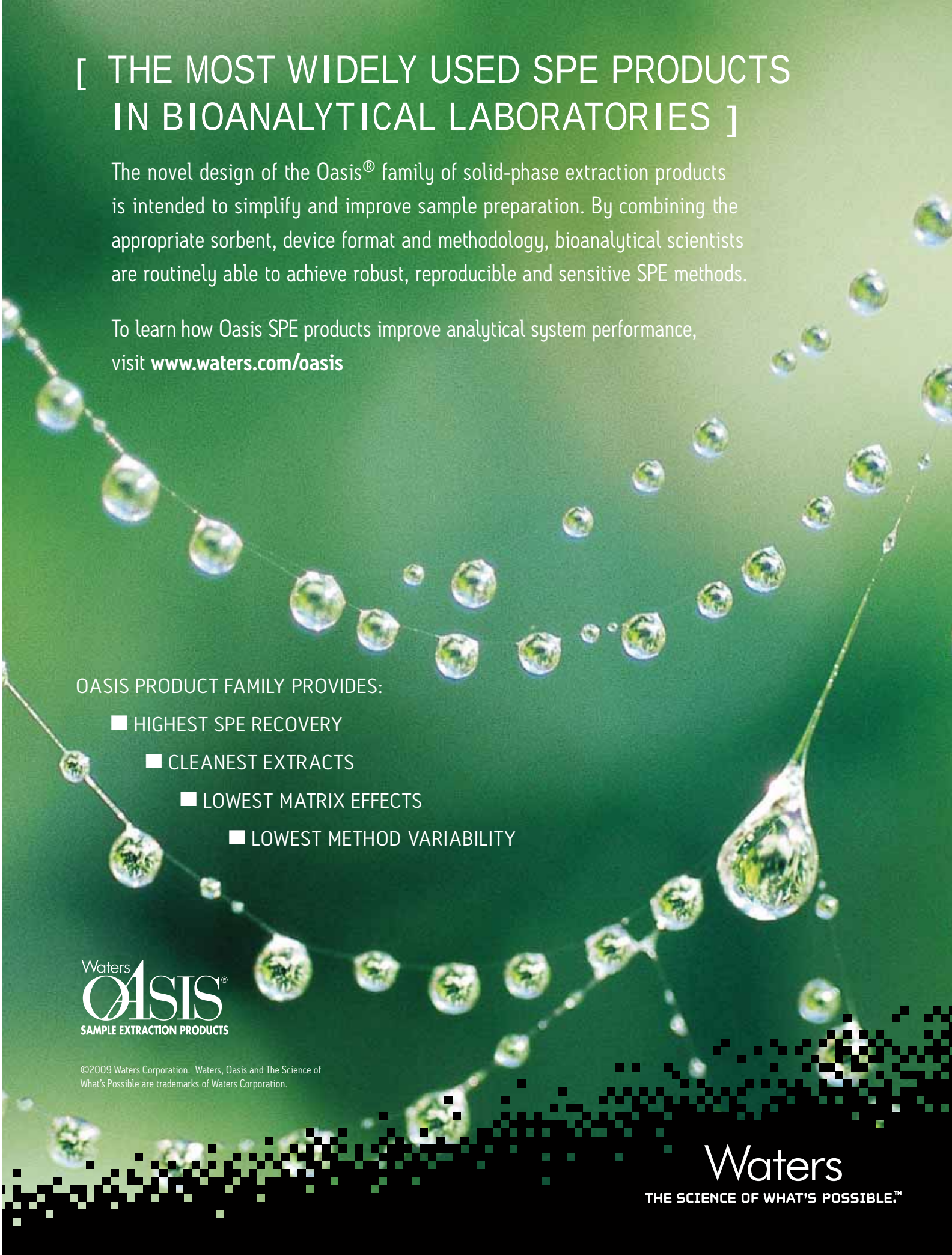
Waters Oasis sorbents are available in Symbiosis cartridges for use with Spark Holland Symbiosis systems. Each narrow-bore PVDF cartridge is available in either 1.0 x 10 mm or 2.0 x 10 mm format, and all five patented Oasis sorbents—HLB, MCX, MAX, WCX, and WAX.

The Oasis sorbents in Symbiosis cartridges show the same performance advantages as in other Oasis formats for pharmaceutical compounds when compared to silica-based sorbents.

Recovery Comparison: Oasis HLB vs. Bonded-Silica Symbiosis Cartridge



*Data provided by Dr. Francis Beaudry, Principal Research Scientist, Phoenix International Life Sciences, Inc., Montreal, Quebec, Canada.


[THE MOST WIDELY USED SPE PRODUCTS IN BIOANALYTICAL LABORATORIES]
The novel design of the Oasis® family of solid-phase extraction products is intended to simplify and improve sample preparation. By combining the appropriate sorbent, device format and methodology, bioanalytical scientists are routinely able to achieve robust, reproducible and sensitive SPE methods.
To learn how Oasis SPE products improve analytical system performance, visit www.waters.com/oasis
OASIS PRODUCT FAMILY PROVIDES:
HIGHEST SPE RECOVERY
CLEANEST EXTRACTS
LOWEST MATRIX EFFECTS
LOWEST METHOD VARIABILITY
Waters OASIS® SAMPLE EXTRACTION PRODUCTS
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Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Oasis Product Selection Guide

	1 cc/ 10 mg	1 cc/ 30 mg	1 cc/30 mg Flangeless	1 cc/30 mg Gilson Adapter	3 cc/ 60 mg	3 cc/60 mg Flangeless	3 cc/60 mg Gilson Adapter	3 cc/ 540 mg	3 cc/540 mg Flangeless
Sorbent	100/box	100/box	100/box	500/box	100/box	100/box	500/box	100/box	100/box
Oasis HLB 30 µm	186000383	WAT094225	186001879	WAT058882	WAT094226	186001880	WAT058883	—	—
Oasis HLB 60 µm	—	—	—	—	—	—	—	186004134	186003852
Oasis MCX 30 µm	186004648	186000252	186001881	186001888	186000254	186001882	—	—	—
Oasis MCX 60 µm	—	186000782	—	—	186000253	—	—	—	—
Oasis MAX 30 µm	186004649	186000366	186001883	—	186000367	186001884	—	—	—
Oasis MAX 60 µm	—	—	—	—	186000368	—	—	—	—
Oasis WCX 30 µm	186004650	186002494	—	—	186002495	—	—	—	—
Oasis WCX 60 µm	—	186002496	—	—	186002497	—	—	—	—
Oasis WAX 30 µm	186004651	186002489	—	—	186002490	—	—	—	—
Oasis WAX 60 µm	—	186002491	—	—	186002492	—	—	—	—

Oasis 96-well Plates

Description	5 mg/ 96-well 1/pkg	10 mg/ 96-well 1/pkg	30 mg/ 96-well 1/pkg	60 mg/ 96-well 1/pkg
Oasis HLB 30 µm	186000309	186000128	WAT058951	—
Oasis HLB 60 µm	—	—	—	186000679
Oasis MCX 30 µm	—	186000259	186000248	—
Oasis MCX 60 µm	—	—	186000250	186000678
Oasis MAX 30 µm	—	186000375	186000373	186001256
Oasis MAX 60 µm	—	—	—	186001205
Oasis WCX 30 µm	—	186002501	186002503	—
Oasis WAX 30 µm	—	186002502	186002504	186003915

Oasis 96-well µElution Plates

Description	µElution 96-well 1/pkg
Oasis HLB 30 µm	186001828BA
Oasis MCX 30 µm	186001830BA
Oasis MAX 30 µm	186001829
Oasis WCX 30 µm	186002499
Oasis WAX 30 µm	186002500

6 cc/ 150 mg	6 cc/ 200 mg	6 cc/400 mg Flangeless	6 cc/ 500 mg	12 cc/ 500 mg	20 cc/ 1 g	35 cc/ 6 g	Plus 225 mg	Vac RC 30 mg	Vac RC 60 mg	Glass Cartridge 5 cc/200 mg
30/box	30/box	100*/500**/box	30/box	20/box	20/box	10/box	50/box	50/box	50/box	30/box
186003365	WAT106202	—	—	—	—	—	—	186000382	186000381	—
186003379	—	—	186000115	186000116	186000117	186000118	186000132	—	—	186000683
186000256	—	186001216**	—	—	—	—	—	—	186000261	—
186000255	—	—	186000776	—	186000777	186000778	186003516	—	186000380	—
186000369	—	186001855*	—	—	—	—	—	186000372	186000371	—
186000370	—	—	186000865	—	—	—	186003517	—	186000378	—
186002498	—	—	—	—	—	—	—	—	—	—
—	—	—	186004646	—	—	—	186003518	—	—	—
186002493	—	—	—	—	—	—	—	—	—	—
—	—	—	186004647	—	—	—	186003519	—	—	

Oasis Sorbent Selection Tools

The Oasis Sorbent Selection Tools enable the rapid development of SPE-LC-MS-MS methods. These Sorbent Selection Tools have all the Oasis sorbents, allowing you the ability to extract your analyte of interest.

Description	Particle Size	Part No.
Oasis µElution Sorbent Selection Plate, 96-well	30 µm	186004475
Oasis Sorbent Selection Plate,10 mg/96-well	30 µm	186003249
Oasis Sorbent Selection Kit, 30 mg/1 cc cartridge	30 µm	186003463

Oasis HLB Sample Extraction Products

Description		Particle Size	Qty.	Part No.
Oasis HLB cartridge	1 cc/10 mg	30 µm	100/box	186000383
Oasis HLB cartridge	1 cc/30 mg	30 µm	100/box	WAT094225
Oasis HLB cartridge	1 cc/30 mg	30 µm	1000/box	186003908
Oasis HLB flangeless cartridge	1 cc/30 mg	30 µm	100/box	186001879
Oasis HLB cartridge with Gilson ASPC adapter	1 cc/10 mg	30 µm	500/box	186000988
Oasis HLB cartridge with Gilson ASPC adapter	1 cc/30 mg	30 µm	500/box	WAT058882
Oasis HLB cartridge	3 cc/60 mg	30 µm	100/box	WAT094226
Oasis HLB flangeless cartridge	3 cc/60 mg	30 µm	100/box	186001880
Oasis HLB cartridge with Gilson ASPC adapter	3 cc/60 mg	30 µm	500/box	WAT058883
Oasis HLB cartridge	6 cc/200 mg	30 µm	30/box	WAT106202
Oasis HLB	3 cc/400 mg	60 µm	100/box	186003849
Oasis HLB cartridge	3 cc/540 mg	60 µm	100/box	186004134
Oasis HLB flangeless cartridge	3 cc/540 mg	60 µm	100/box	186003852
Oasis HLB cartridge	6 cc/150 mg	30 µm	30/box	186003365
Oasis HLB cartridge	6 cc/150 mg	60 µm	30/box	186003379
Oasis HLB cartridge	6 cc/500 mg	60 µm	30/box	186000115
Oasis HLB cartridge	12 cc/500 mg	60 µm	20/box	186000116
Oasis HLB cartridge	20 cc/1 g	60 µm	20/box	186000117
Oasis HLB cartridge	35 cc/6 g	60 µm	10/box	186000118
Oasis HLB Plus cartridge	225 mg	60 µm	50/box	186000132
Oasis HLB Vac RC cartridge	20 cc/30 mg	30 µm	50/box	186000382
Oasis HLB Vac RC cartridge	20 cc/60 mg	30 µm	50/box	186000381
Oasis HLB glass cartridge	5 cc/200 mg	60 µm	30/box	186000683
Oasis HLB Symbiosis cartridge*	1.0 x 10 mm	30 µm	96/box	186001196
Oasis HLB Symbiosis cartridge*	2.0 x 10 mm	30 µm	96/box	186003925
Reservoir 30 cc for Oasis cartridges			48/box	WAT011390
Reservoir 60 cc for Oasis cartridges			12/box	WAT024659
Reservoir adapter for 1 cc, 3 cc, 6 cc cartridges			10/box	WAT054260
Reservoir adapter for 12 cc, 20 cc, 35 cc cartridges			10/box	WAT048160
Reservoir adapter for 5 cc cartridges, Teflon			10/pkg	405000934
Oasis HLB column	2.1 x 20 mm	5 µm	1/pkg	186002034
Oasis HLB column	3.0 x 20 mm	5 µm	1/pkg	186002037
Oasis HLB column	3.9 x 20 mm	5 µm	1/pkg	186002040
Oasis HLB cartridge column	3.9 x 20 mm	5 µm	1/pkg	186001413
Oasis HLB column	4.6 x 20 mm	5 µm	1/pkg	186002043
Oasis HLB column	2.1 x 20 mm	15 µm	1/pkg	186002035
Oasis HLB column	3.0 x 20 mm	15 µm	1/pkg	186002038
Oasis HLB column	3.9 x 20 mm	15 µm	1/pkg	186002041
Oasis HLB cartridge column	3.9 x 20 mm	15 µm	1/pkg	186001414
Oasis HLB column	4.6 x 20 mm	15 µm	1/pkg	186002044
Oasis HLB column	2.0 x 15 mm	25 µm	1/pkg	186001792
Oasis HLB column	2.1 x 20 mm	25 µm	1/pkg	186002036
Oasis HLB cartridge column	2.1 x 20 mm	25 µm	1/pkg	186000706
Oasis HLB column	3.0 x 20 mm	25 µm	1/pkg	186002039
Oasis HLB column	3.9 x 20 mm	25 µm	1/pkg	186002042
Oasis HLB column	4.6 x 20 mm	25 µm	1/pkg	186002045
Holder kit for 2.1 x 20 mm cartridge column			1/pkg	186000262
Holder kit for 3.9 x 20 mm cartridge column			1/pkg	WAT046910
Extraction column connector			1/pkg	WAT082745
Inline precolumn filter kit			1/pkg	WAT084560
Replacement filters			5/pkg	WAT005139
Replacement steel gaskets			1/pkg	WAT084567
Oasis HLB µElution plate	2 mg/96-well	30 µm	1/pkg	186001828BA
Oasis HLB plate	5 mg/96-well	30 µm	1/pkg	186000309
Oasis HLB plate	10 mg/96-well	30 µm	1/pkg	186000128
Oasis HLB plate	30 mg/96-well	30 µm	1/pkg	WAT058951
Oasis HLB plate	60 mg/96-well	60 µm	1/pkg	186000679

Oasis MCX Sample Extraction Products (Cation Exchange)

Description		Particle Size	Qty.	Part No.
NEW Oasis MCX cartridge	1cc/10mg	30 µm	100/box	186004648
Oasis MCX cartridge	1 cc/30 mg	30 µm	100/box	186000252
Oasis MCX flangeless cartridge	1 cc/30 mg	30 µm	100/box	186001881
Oasis MCX cartridge	1 cc/60 mg	60 µm	100/box	186000782
Oasis MCX cartridge	3 cc/60 mg	30 µm	100/box	186000254
Oasis MCX flangeless cartridge	3 cc/60 mg	30 µm	100/box	186001882
Oasis MCX cartridge	3 cc/60 mg	60 µm	100/box	186000253
Oasis MCX cartridge	6 cc/150 mg	30 µm	30/box	186000256
Oasis MCX cartridge	6 cc/150 mg	60 µm	30/box	186000255
Oasis MCX cartridge	6 cc/500 mg	60 µm	30/box	186000776
Oasis MCX cartridge	20 cc/1 g	60 µm	20/box	186000777
Oasis MCX cartridge	35 cc/6 g	60 µm	10/box	186000778
Oasis MCX Plus cartridge	225 mg	60 µm	50/box	186003516
Oasis MCX Vac RC cartridge	20 cc/60 mg	30 µm	50/box	186000261
Oasis MCX Vac RC cartridge	20 cc/60 mg	60 µm	50/box	186000380
Oasis MCX Symbiosis cartridge	10 x 1 mm	30 µm	96/box	186002098
NEW Oasis Symbiosis MCX cartridge	2.0 x 10 mm	30 µm	96/box	186004653
Oasis MCX direct connect column	2.1 x 15 mm	30 µm	1/pkg	186002050
Oasis MCX column	2.1 x 20 mm	30 µm	1/pkg	186002046
Oasis MCX cartridge column	2.1 x 20 mm	30 µm	1/pkg	186002051
Oasis MCX column	3.0 x 20 mm	30 µm	1/pkg	186002047
Oasis MCX column	3.9 x 20 mm	30 µm	1/pkg	186002048
Oasis MCX column	4.6 x 20 mm	30 µm	1/pkg	186002049
Oasis MCX µElution plate	96-well	30 µm	1/pkg	186001830BA
Oasis MCX plate	10 mg/96-well	30 µm	1/pkg	186000259
Oasis MCX plate	30 mg/96-well	30 µm	1/pkg	186000248
Oasis MCX plate	30 mg/96-well	60 µm	1/pkg	186000250
Oasis MCX plate	60 mg/96-well	60 µm	1/pkg	186000678

* For use with Spark Holland Symbiosis systems

Literature References

Oasis Sample Extraction Products Brochure, Literature Reference 720001692EN

Oasis µElution Plate Brochure, Literature Reference 720000476EN

Topics in Solid-Phase Extraction. Part 1. Ion Suppression in LC/MS Analysis White Paper, Literature Reference 720001237EN

Sample Prep Solutions Brochure, Literature Reference 720000848EN

Oasis WAX Sorbent for UPLC/MS Determination of PFOS and Related Compounds in Waters and Tissue, Literature Reference 720001871EN

SPE Sample Preparation for UPLC–MS Determination of Enrofloxacin (Baytril) in Chicken, Literature Reference WA43206

A Sensitive Method for the Determination of Endocrine-Disrupting Compounds in River Water by LC/MS/MS, Literature Reference 720001296EN

Oasis MAX Sample Extraction Products (Anion Exchange)

Description		Particle Size	Qty.	Part No.
NEW Oasis MAX cartridge	1 cc/10 mg	30 µm	100/box	186004649
Oasis MAX cartridge	1 cc/30 mg	30 µm	100/box	186000366
Oasis MAX flangeless cartridge	1 cc/30 mg	30 µm	100/box	186001883
Oasis MAX cartridge	3 cc/60 mg	30 µm	100/box	186000367
Oasis MAX cartridge	3 cc/60 mg	60 µm	100/box	186000368
Oasis MAX flangeless cartridge	3 cc/60 mg	30 µm	100/box	186001884
Oasis MAX cartridge	6 cc/150 mg	30 µm	30/box	186000369
Oasis MAX cartridge	6 cc/150 mg	60 µm	30/box	186000370
Oasis MAX cartridge	6 cc/500 mg	60 µm	30/box	186000865
Oasis MAX Plus cartridge	225 mg	60 µm	50/box	186003517
Oasis MAX Vac RC cartridge	20 cc/30 mg	30 µm	50/box	186000372
Oasis MAX Vac RC cartridge	20 cc/60 mg	30 µm	50/box	186000371
Oasis MAX Vac RC cartridge	20 cc/60 mg	60 µm	50/box	186000378
Oasis MAX Symbiosis cartridge*	10 x 1 mm	30 µm	96/box	186002099
NEW Oasis Symbiosis MAX cartridge	2.0 x 10 mm	30 µm	96/box	186004654
Oasis MAX direct connect column	2.1 x 15 mm	30 µm	1/pkg	186002056
Oasis MAX column	2.1 x 20 mm	30 µm	1/pkg	186002052
Oasis MAX cartridge column	2.1 x 20 mm	30 µm	1/pkg	186002057
Oasis MAX column	3.0 x 20 mm	30 µm	1/pkg	186002053
Oasis MAX column	3.9 x 20 mm	30 µm	1/pkg	186002054
Oasis MAX column	4.6 x 20 mm	30 µm	1/pkg	186002055
Oasis MAX µElution plate	2 mg/96-well		1/pkg	186001829
Oasis MAX plate	10 mg/96-well	30 µm	1/pkg	186000375
Oasis MAX plate	30 mg/96-well	30 µm	1/pkg	186000373
Oasis MAX plate	60 mg/96-well	30 µm	1/pkg	186001256
Oasis MAX plate	60 mg/96-well	60 µm	1/pkg	186001205

* For use with Spark Holland Symbiosis systems

Oasis WCX Sample Extraction Products (Weak Cation Exchange)

Description		Particle Size	Qty.	Part No.
NEW Oasis WCX cartridge	1 cc/10 mg	30 µm	100/box	186004650
Oasis WCX cartridge	1 cc/30 mg	30 µm	100/box	186002494
Oasis WCX cartridge	3 cc/60 mg	30 µm	100/box	186002495
Oasis WCX cartridge	6 cc/150 mg	30 µm	30/box	186002498
Oasis WCX cartridge	1 cc/30 mg	60 µm	100/box	186002496
Oasis WCX cartridge	3 cc/60 mg	60 µm	100/box	186002497
NEW Oasis WCX cartridge	6 cc/500 mg	60 µm	30/box	186004646
Oasis WCX Plus cartridge	225 mg	60 µm	50/box	186003518
Oasis WCX µElution plate	2 mg/96-well	30 µm	1/pkg	186002499
Oasis WCX 96-well plate	10 mg/96-well	30 µm	1/pkg	186002501
Oasis WCX 96-well plate	30 mg/96-well	30 µm	1/pkg	186002503
Oasis WCX Symbiosis cartridge*	1.0 x 10 mm	30 µm	96/box	186002892
NEW Oasis Symbiosis WCX cartridge	2.0 x 10 mm	30 µm	96/box	186004655
Oasis WCX 2.1 x 20 mm column		30 µm		186002505
Oasis WCX 3.9 x 20 mm column		30 µm		186002507
Oasis WCX 2.1 x 20 mm column		5 µm		186002510
Oasis WCX 3.9 x 20 mm column		5 µm		186002512

Oasis WAX Sample Extraction Products (Weak Anion Exchange)

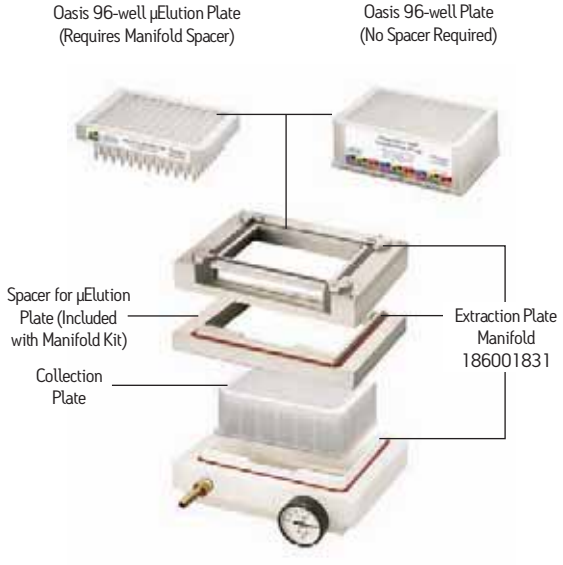
Description		Particle Size	Qty.	Part No.
NEW Oasis WAX cartridge	1 cc/10 mg	30 µm	100/box	186004651
Oasis WAX cartridge	1 cc /30 mg	30 µm	100/box	186002489
Oasis WAX cartridge	3 cc /60 mg	30 µm	100/box	186002490
Oasis WAX cartridge	6 cc /150 mg	30 µm	30/box	186002493
Oasis WAX cartridge	1 cc /30 mg	60 µm	100/box	186002491
Oasis WAX cartridge	3 cc /60 mg	60 µm	100/box	186002492
NEW Oasis WAX cartridge	6 cc/500 mg	60 µm	30/box	186004647
Oasis WAX Plus cartridge	225 mg	60 µm	50/box	186003519
Oasis WAX µElution plate	96-well	30 µm	1/pkg	186002500
Oasis WAX 96-well plate	10 mg/96-well	30 µm	1/pkg	186002502
Oasis WAX 96-well plate	30 mg/96-well	30 µm	1/pkg	186002504
Oasis WAX 96-well plate	60 mg	30 µm	1/pkg	186003915
Oasis WAX Symbiosis cartridge*	1.0 x 10 mm	30 µm	96/box	186002893
NEW Oasis Symbiosis WAX cartridge	2.0 x 10 mm	30 µm	96/box	186004656
Oasis WAX 2.1 x 20 mm column		30 µm		186002508
Oasis WAX 3.9 x 20 mm column		30 µm		186002509
Oasis WAX 2.1 x 20 mm column		5 µm		186002511
Oasis WAX 3.9 x 20 mm column		5 µm		186002513

Oasis Method Development Kits

Description		Particle Size	Part No.
Oasis sorbent selection plate 3 rows each: MCX, MAX, WCX and WAX	96-well	30 µm	186003249
Oasis µElution Sorbent Selection Plate, 3 rows each: MCX, MAX, WCX and WAX	96-well	30 µm	186004475
Oasis sorbent selection cartridge kit, 10 each: MCX, MAX, WCX and WAX	1 cc/30 mg	30 µm	186003463
Oasis Symbiosis Sorbent Selection Kit 24 1.0 x 10 mm Cartridges of MCX, MAX, WCX ,and WAX, 96/box		30µm	186004657

Manifold for Extraction Plate

Description	Qty.	Part No.
Extraction plate manifold for Oasis 96-well plates	1/box	186001831
Extraction plate manifold kit A (includes extraction plate manifold, reservoir tray, sealing cap and 350 µL sample collection plate)		WAT097944
Extraction plate manifold kit B (as kit A, with 1 mL sample collection plate)		WAT097945
Extraction plate manifold kit C (as kit A, with 2 mL sample collection plate)		WAT097946



Manifold for Extraction Cartridges

Description	Part No.
Waters extraction manifold, 20-position without rack (includes 20 needle tips, 25 plugs, and ejector tool)	WAT200677
Waters extraction manifold, 20-position (complete with rack for 13 x 75 mm tubes)	WAT200606
Waters extraction manifold, 20-position (complete with rack for 13 x 100 mm tubes)	WAT200607
Waters extraction manifold, 20-position (complete with rack for 16 x 75 mm tubes)	WAT200608
Waters extraction manifold, 20-position (complete with rack for 16 x 100 mm tubes)	WAT200609

Waters Extraction Manifold

Accessories for Extraction Cartridge Manifold

Description	Qty.	Part No.
30 cc Reservoir	48/pkg	WAT011390
60 cc Reservoir	12/pkg	WAT024659
Reservoir Adapters for 1, 3 and 6 cc VAC	12/pkg	WAT054260
Reservoir Adapters for 12, 20 and 35 cc VAC	10/pkg	WAT048160
Male-Male Adapter	100/pkg	WAT024310
Male Luer Plugs		WAT044395
Female Luer Plugs		WAT044385

Accessories for Extraction Plate Manifold

Description	Qty.	Part No.
Disposable reservoir tray	25/box	WAT058942
Sample collection plate, 350 µL	50/box	WAT058943
Sample collection plate, 2 mL	50/box	WAT058958
Sealing cap for 96-well collection plate	50/pkg	WAT058959
SPE vacuum pump 115 V 60 Hz		725000417
SPE vacuum pump 240 V 50 Hz		725000418
Vacuum box gasket kit Kit includes: 2 foam top gaskets 2 orange O-rings		186003522

Vacuum Box Gasket Kit

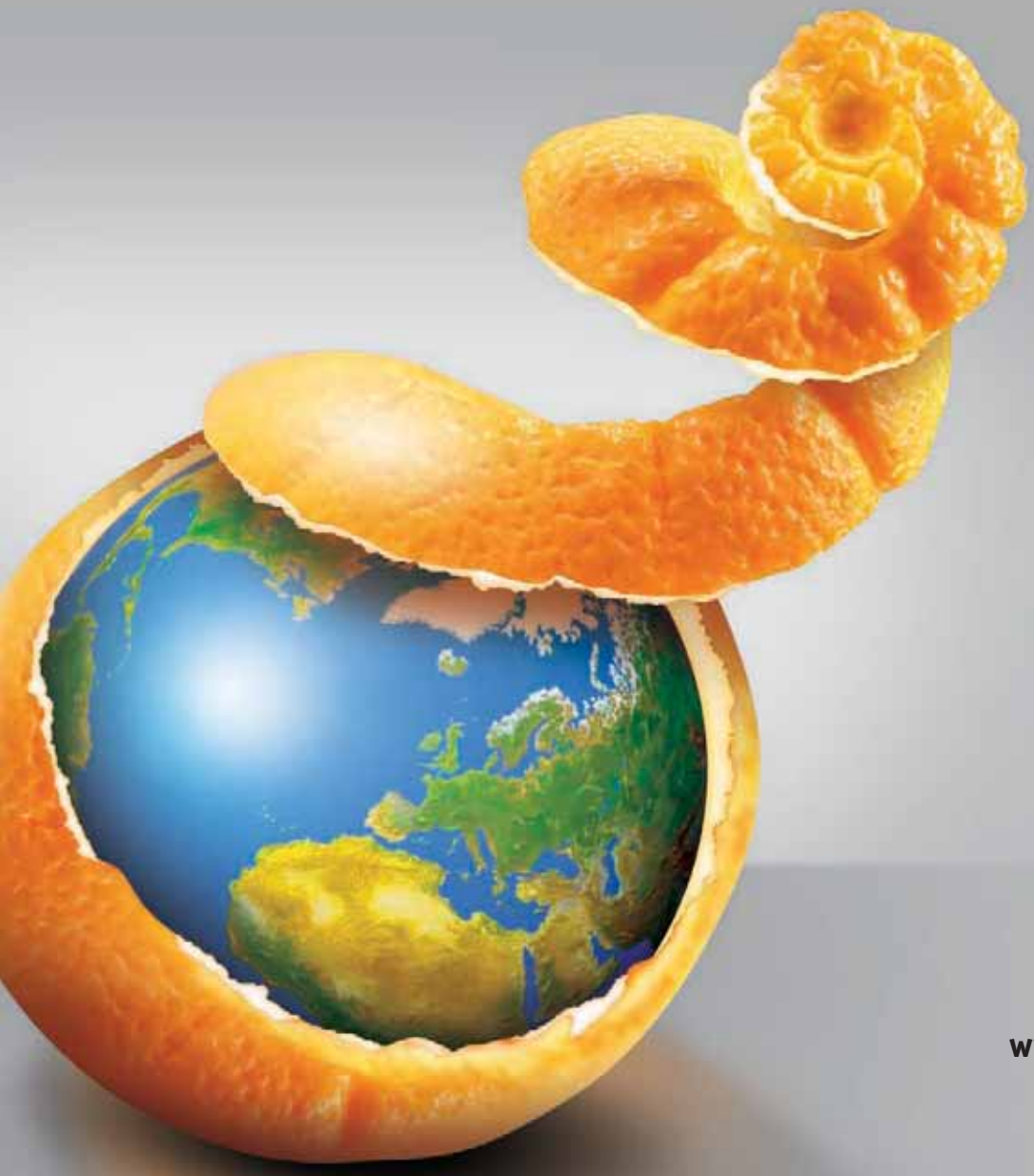
Accessories for Extraction Columns and Cartridges

Description	Qty.	Part No.
Holder kit for 2.1 x 20 mm cartridge column	1/pkg	186000262
Holder kit for 3.9 x 20 mm cartridge column	1/pkg	WAT046910
Extraction column connector	1/pkg	WAT082745
Inline precolumn filter kit	1/pkg	WAT084560
Replacement filters	5/pkg	WAT005139
Replacement steel gaskets	1/pkg	WAT084567
SPE vacuum pump 115 V 60 Hz		725000417
SPE vacuum pump 240 V 50 Hz		725000418
Reservoir, 30 cc (for Oasis Plus, Light, Vac and Classic cartridges)	48/pkg	WAT011390
Reservoir, 60 cc (for Oasis Plus, Light and Vac cartridges)	12/pkg	WAT024659
Adapter, male-male Luer (for Oasis Classic cartridges)	100/pkg	WAT024310
Adapter (to attach reservoir to 1, 3 and 6 cc Oasis Vac cartridges)	12/pkg	WAT054260
Adapter (to attach reservoir to 12, 20 and 35 cc Oasis Vac cartridges)	10/pkg	WAT048160

SPE Vacuum Pump
(Includes two gauges and pressure regulator)

[SIMPLIFY QuEChERS]

Pesticide analysis is now simple and easy. Waters DisQuE™ Dispersive Sample Preparation Kit simplifies the extraction of pesticide residues from any food or agricultural product. The easy-to-use kit, with pre-weighed sorbents and buffers, and easy-to-follow instructions provides reliable, reproducible results that conform to official AOAC methods.



DisQuE™
Dispersive Sample Preparation

www.waters.com/disque

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Sep-Pak 96-Well Plates

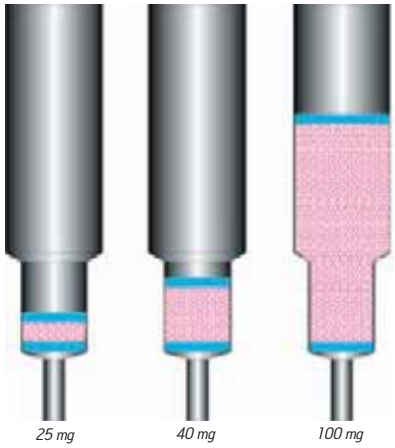
Sep-Pak 96-Well Plates are available with chemistries that have been proven for years in Sep-Pak cartridges. These plates have been introduced for high volume applications where the need to process 100's of samples is a requirement. Plates can be used on most vacuum manifolds, robotic and automated sample handling systems. Sep-Pak Plates are available with different sorbent masses for different sample loads.

Sep-Pak 96-Well Plates are extensively tested for quality control. Each plate is shipped with a certificate of analysis that covers tests performed on raw silica and the bonded silica phase. Each lot is tested for bonding, cleanliness and chromatographic selectively. The plates are packed and tested to ensure consistent flow properties and sorbent mass across the 96 wells.

Sep-Pak tC_{18} is also available in the patented* µElution Plate. This plate is designed for small volume samples. The recommended protocol for elution from this plate is as low as 25 µL. The low volume extracts from the plate can be injected directly, eliminating time consuming evaporation steps.

The 96-well plate has two stage diameter well design. This design allows for varying quantities of sorbent within the well. The lower portion of the well has a smaller diameter which accommodates smaller sorbent masses, packed to desirable aspect ratios. This means the sorbent is packed to desirable height to width ratios, resulting in good sample distribution and bed depth. The upper portion of the well has a larger diameter. Both well diameters are used for larger sorbent masses, resulting in good sample distribution, bed height and sample load for larger sample sizes.

Waters 96-Well Plate Design



By varying frit size and/or placement, the same plate may be filled with various quantities of sorbent per well. This design permits optimal recoveries, even with low sorbent weights for smaller elution volumes.

Sep-Pak 96-Well Plates

Description	Part No.
Sep-Pak tC_{18} µElution Plate	186002318
Sep-Pak tC_{18} 25 mg Plate	186002319
Sep-Pak tC_{18} 40 mg Plate	186002320
Sep-Pak tC_{18} 100 mg Plate	186002321
Sep-Pak Accell™ Plus QMA 100 mg Plate	186001917
Sep-Pak C_{18} 40 mg Plate	186003966

* Patent Pending

Waters Extraction Manifold for Use With Solid-Phase Extraction Cartridges

The vacuum manifold has the capacity to process up to twenty samples simultaneously. The extraction manifold has enhanced features designed for use with conventional silica-based, solid-phase extraction cartridges as well as modifications that allow you take full advantage of the unique performance characteristics of our Oasis HLB extraction cartridges.

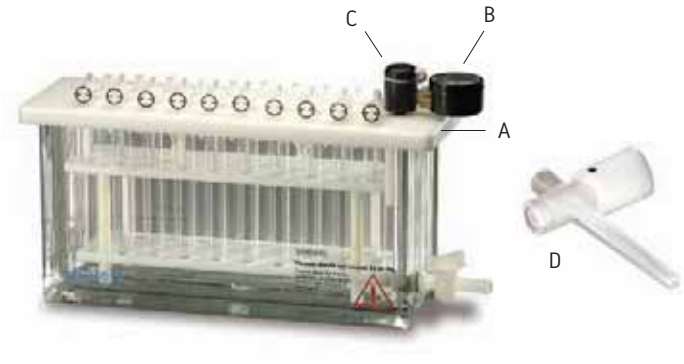
Just look at what this manifold has to offer:

A Precision-machined Delrin® cover with alignment posts for quick and easy alignment with test tube rack.

B Vacuum gauge placement on cover, not in fluid path allows for quick and easy waste removal at bottom by vacuum.

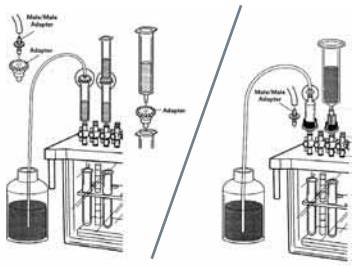
C Enhanced vacuum control valve designed for use with Waters Oasis HLB extraction cartridges, allows for a quick and momentary rise in vacuum above the frit bubble point at the touch of a finger.

D High purity polypropylene needle valves and needle tips with minimum dead volume *(Opening and closing the valves is required to prevent silica-based SPE cartridges from drying out).*



Sep-Pak Cartridge Accessories

Description	Qty.	Part No.
30 cc Reservoir	48/pkg	WAT011390
60 cc Reservoir	12/pkg	WAT024659
Reservoir Adapters for 1, 3, and 6 cc VAC	12/pkg	WAT054260
Reservoir Adapters for 12, 20, and 35 cc VAC	10/pkg	WAT048160
Male-Male Adapter	100/pkg	WAT024310
Male Luer Plugs		WAT044395
Female Luer Plugs		WAT044385



Extraction Manifolds (Includes 20 needle tips, 25 plugs, and ejector tool)

Description	Part No.
20 position extraction manifold without rack	WAT200677
20 position extraction manifold with 13 x 75 mm test tube rack	WAT200606
20 position extraction manifold with 13 x 100 mm test tube rack	WAT200607
20 position extraction manifold with 16 x 75 mm test tube rack	WAT200608
20 position extraction manifold with 16 x 100 mm test tube rack	WAT200609

Accessories and Spare Parts for the Waters Extraction Manifolds

Description	Qty.	Part No.
Needle valves, 20/pkg (required when using silica-based SPE cartridges) (not required for use with Oasis Extraction Cartridges)		WAT200685
Needle tips, 20/pkg		WAT200691
Cover, 20 position without gauge assembly		WAT200686
Gauge assembly, vacuum		WAT200687
Reservoir, glass with outlet valve		WAT200688
Outlet valve kit		WAT200689
Gasket for cover		WAT200690
Ejector tool		WAT058839
Luer Plugs, 25/pkg		WAT058851
Rubber ball ring (for vacuum gauge assembly)		WAT058840
Reversible Vial Rack for 1 mL or 4 mL autosampler vials		WAT058871
13 x 75 mm test tube rack		WAT200678
13 x 100 mm test tube rack		WAT200679
16 x 75 mm test tube rack		WAT200680
16 x 100 mm test tube rack		WAT200681
Reservoir, 30 cc (for Sep-Pak Plus, Light, Vac, and Classic cartridges)	48/pkg	WAT011390
Reservoir, 60 cc (for Sep-Pak Plus, Light, and Vac cartridges)	12/pkg	WAT024659
Adapter, male-male Luer (for Sep-Pak Classic cartridges)	100/pkg	WAT024310
Adapter (to attach reservoir to 1, 3, and 6 cc Sep-Pak Vac cartridges)	12/pkg	WAT054260
Adapter (to attach reservoir to 12, 20, and 35 cc Sep-Pak Vac cartridges)	10/pkg	WAT048160
Vacuum pump (110 V, 60 Hz)		WAT085114
Vacuum pump (220 V, 50 Hz)		WAT085115

SPE Vacuum Pump
(Includes a single gauge. Pressure can be controlled using relief valve on manifold.)

Sep-Pak Cartridge Connections Kit

Contains a selection of the most commonly needed fittings, adapters, valves, and tubing for use with Sep-Pak cartridges.

Description	Part No.
Sep-Pak Connections Kit	WAT011400

Sep-Pak Cartridge Selection Guide







	Plus Short	Plus Long	Plus Light	Classic Short/Long	Vac 1 cc/ 50 mg	Vac 1 cc/ 100 mg	Vac RC/ 100 mg
	50/box	50/box	50/box	50/box	100/box	100 /box	50/box
Sorbent	Part No./ Mass/Volume*	Part No./ Mass/Volume*	Part No./ Mass/Volume*	Part No./ Mass/Volume*	Part No./ Volume*	Part No./ Volume*	Part No./ Volume*
C_{18}	WAT020515 360 mg/0.7 mL	WAT023635 820 mg/1.6 mL	WAT023501 130 mg/0.3 mL	WAT051910 360 mg/0.85 mL	WAT054955 0.13 mL	WAT023590 0.2 mL	WAT036935 0.2 mL
tC_{18}	WAT036810 400 mg/0.8 mL	WAT036800 900 mg/1.4 mL	WAT036805 145 mg/0.4 mL	—	WAT054960 0.11 mL	WAT036820 0.25 mL	WAT043410 0.25 mL
C_8	WAT036775 400 mg/0.8 mL	—	WAT036770 145 mg/0.4 mL	—	WAT054965 0.11 mL	WAT036785 0.25 mL	WAT043415 0.25 mL
tC_2	WAT052720 400 mg/0.8 mL	—	WAT052725 145 mg/0.4 mL	—	—	WAT052710 0.25 mL	—
Silica	—	WAT020520 690 mg/1.6 mL	WAT023537 120 mg/0.4 mL	WAT051900 690 mg/2.0 mL	WAT054980 0.15 mL	WAT023595 0.25 mL	WAT036940 0.25 mL
Florisil	—	WAT020525 910 mg/1.4 mL	WAT023543 145 mg/0.3 mL	WAT051960 900 mg/1.7 mL	WAT054985 0.12 mL	WAT023600 0.2 mL	—
Accell Plus CM	WAT020550 360 mg/0.8 mL	—	WAT023531 130 mg/0.4 mL	WAT010910 360 mg/1.1 mL	—	WAT023625 0.25 mL	—
Accell Plus QMA	WAT020545 360 mg/0.8 mL	—	WAT023525 130 mg/0.4 mL	WAT010835 360 mg/1.1 mL	—	WAT023620 0.25 mL	WAT043460 0.25 mL
Alumina A	—	WAT020500 1710 mg/1.2 mL	WAT023549 280 mg/0.35 mL	WAT051800 1850 mg/1.8 mL	—	WAT023575 0.1 mL	—
Alumina B	—	WAT020505 1710 mg/1.2 mL	WAT023555 280 mg/0.35 mL	WAT051820 1850 mg/1.8 mL	—	WAT023580 0.1 mL	—
Alumina N	—	WAT020510 1710 mg/1.2 mL	WAT023561 280 mg/0.35 mL	WAT051810 1850 mg/1.8 mL	—	WAT023585 0.1 mL	—
Amino Propyl (NH_2)	WAT020535 360 mg/0.7 mL	—	WAT023513 130 mg/0.3 mL	WAT010830 360 mg/0.85 mL	—	WAT023610 0.2 mL	WAT043475 0.2 mL
PSA	186004538 360 mg/0.7 mL	—	186004578 130 mg/0.3 mL	186004560 360 mg/0.85 mL	186004562 0.1 mL	186004561 0.2 mL	186004567 0.2 mL
Cyano Propyl (CN)	WAT020540 360 mg/0.7 mL	—	WAT023507 130 mg/0.3 mL	WAT010823 360 mg/0.85 mL	WAT054975 0.13 mL	WAT023615 0.2 mL	—
Diol	WAT020530 360 mg/0.8 mL		WAT023519 130 mg/0.4 mL	—	—	WAT023605 0.25 mL	WAT043480 0.25 mL

Vac 3 cc/ 200 mg	Vac 3 cc/ 500 mg	Vac RC/ 500 mg	Vac 6 cc/ 500 mg	Vac 6 cc/ 1 g	Vac 12 cc/ 2 g	Vac 20 cc/ 5 g	Vac 35 cc/ 10 g	
50/box	50/box	50/box	30/box	30/box	20/box	20/box	10/box	
Part No./ Volume*	Part No./ Volume*	Part No./ Volume*	Part No./ Volume*	Part No./ Volume*	Part No./ Volume*	Part No./ Volume*	Part No./ Volume*	Sorbent
WAT054945 0.42 mL	WAT020805 0.8 mL	WAT036945 0.8 mL	WAT043395 1.2 mL	WAT036905 2.0 mL	WAT036915 3.6 mL	WAT036925 8.0 mL	WAT043345 16.8 mL	C_{18}
WAT054925 0.34 mL	WAT036815 1.0 mL	WAT043425 1.0 mL	WAT036790 1.1 mL	WAT036795 1.9 mL	WAT043380 3.5 mL	WAT043365 7.8 mL	WAT043350 16.3 mL	tC_{18}
WAT054940 0.34 mL	WAT036780 1.0 mL	WAT043430 1.0 mL	WAT054525 1.1 mL	WAT054570 1.9 mL	WAT054615 3.5 mL	WAT054660 7.8 mL	WAT054700 16.3 mL	C_8
—	WAT052715 1.0 mL	—	—	WAT052705 1.9 mL	—	—	—	tC_2
WAT054930 0.53 mL	WAT020810 1.2 mL	WAT036950 1.2 mL	WAT043400 1.2 mL	WAT036910 1.9 mL	WAT036920 3.9 mL	WAT036930 11.0 mL	WAT043355 23.4 mL	Silica
—	WAT020815 0.8 mL	WAT043435 0.8 mL	WAT043405 1.2 mL	WAT043390 2.0 mL	WAT043385 3.6 mL	WAT043370 8.0 mL	WAT043360 16.8 mL	Florisil
—	WAT020855 1.1 mL	WAT054505 1.1 mL	WAT054545 1.2 mL	WAT054590 1.9 mL	WAT054635 3.5 mL	WAT054675 7.8 mL	WAT054720 16.3 mL	Accell Plus CM
—	WAT020850 1.1 mL	WAT054500 1.1 mL	WAT054550 1.2 mL	WAT054595 1.9 mL	WAT054640 3.5 mL	WAT054680 7.8 mL	WAT054725 16.3 mL	Accell Plus QMA
—	WAT020820 0.4 mL	—	WAT054535 0.5 mL	WAT054580 0.8 mL	WAT054620 1.8 mL	WAT054670 3.9 mL	WAT054710 8.2 mL	Alumina A
—	WAT020825 0.4 mL	—	WAT054540 0.5 mL	WAT054585 0.8 mL	WAT054625 1.8 mL	WAT054665 3.9 mL	WAT054715 8.2 mL	Alumina B
—	WAT020830 0.4 mL	WAT043485 0.4 mL	WAT054530 0.5 mL	WAT054575 0.8 mL	WAT054630 1.8 mL	WAT043375 3.9 mL	WAT054705 8.2 mL	Alumina N
—	WAT020840 0.8 mL	WAT054515 0.8 mL	WAT054560 1.2 mL	WAT054605 2.0 mL	WAT054650 3.6 mL	WAT054695 8.0 mL	WAT054740 16.8 mL	Amino Propyl (NH_2)
—	186004536 0.8 mL	186004568 0.8 mL	186004563 1.2 mL	186004537 2.0 mL	186004564 3.6 mL	186004565 8.0 mL	186004566 16.8 mL	PSA
WAT054935 0.42 mL	WAT020835 0.8 mL	—	WAT054555 1.2 mL	WAT054600 2.0 mL	WAT054645 3.6 mL	WAT054685 8.0 mL	WAT054730 16.8 mL	Cyano Propyl (CN)
—	WAT020845 1.0 mL	WAT054520 1.0 mL	WAT054565 1.1 mL	WAT054610 1.9 mL	WAT054655 3.5 mL	WAT054690 7.8 mL	WAT054735 16.3 mL	Diol

* Hold-up Volume

Sep-Pak Sorbent Selection Guide

Reversed Phase

	Description	Applications	Chemistry
C_{18} $Si(CH_3)_2C_{18}H_{37}$	Silica-based bonded phase with strong hydrophobicity; used to adsorb analytes of even weak hydrophobicity from aqueous solutions.	• Drugs and their metabolites in serum, plasma or urine • Desalting of peptides • Trace organics in environmental water samples • Organic acids in beverages • Similar in behavior to reversed-phase HPLC columns	1. Particle Size: 55-105 µm 2. Pore Size: 125Å 3. Surface Area: 325 m^2/g 4. Carbon Load: 12%
tC_{18} $SiC_{18}H_{37}$	Silica-based bonded phase with strong hydrophobicity; trifunctional bonding chemistry gives it an increased hydrolytic stability over C_{18}.	• Similar to C_{18}	1. Particle Size: 37-55 µm 2. Pore Size: 125Å 3. Surface Area: 325 m^2/g 4. Carbon Load: 17%
C_8 $Si(CH_3)_2C_8H_{17}$	Silica-based bonded phase with moderate hydrophobicity; use for methods requiring less retention than C_{18}.	• Drugs and their metabolites in serum plasma or urine • Peptides in serum • Plasma	1. Particle Size: 37-55 µm 2. Pore Size: 125Å 3. Surface Area: 325 m^2/g 4. Carbon Load: 9%
tC_2 SiC_2H_5	Silica-based bonded phase with low hydrophobic character; use for methods requiring less retention than C_8.	• Similar to C_{18} and C_8	1. Particle Size: 37-55 µm 2. Pore Size: 125Å 3. Surface Area: 325 m^2/g 4. Carbon Load: 2.7%

Reversed or Normal-Phase

	Description	Applications	Chemistry
Amino Propyl $Si(CH_2)_3NH_2$	Silica-based polar bonded phase with basic character; can be used as a polar sorbent, like silica, with different selectivity for acidic/basic analytes or as weak anion exchanger in aqueous medium.	• Phenols and phenolic pigments • Petroleum fractionation • Saccharides • Drugs and metabolites	1. Particle Size: 55-105 µm 2. Pore Size: 125Å 3. Surface Area: 325 m^2/g 4. Carbon Load: 3.5%
Cyano Propyl $Si(CH_3)(CH_2)_3(CN)$	Silica-based polar bonded phase; can be used as less polar alternative to silica in normal-phase applications or as less hydrophobic alternative to C_{18} or C_8 in reversed-phase.	• Drugs • Drug metabolites • Pesticides	1. Particle Size: 55-105 µm 2. Pore Size: 125Å 3. Surface Area: 325 m^2/g 4. Carbon Load: 6.5%
Diol $Si(CH_2)_3OCH_2CH(OH)CH_2OH$	Silica-based polar bonded phase with neutral character; can be used as an alternative to silica in normal phase applications, where the acidic character of silica is undesirable or as very weakly interacting phase in aqueous applications.	• Antibiotics from cosmetics • Isolation of proteins or peptides by hydrophobic interaction chromatography	1. Particle Size: 37-55 µm 2. Pore Size: 125Å 3. Surface Area: 325 m^2/g 4. Carbon Load: 9%

Normal Phase

	Description	Applications	Chemistry
Silica SiO_2	Polar sorbent, used primarily to adsorb analytes from non polar solvents like hydrocarbons, chloro- or fluoro-substituted hydrocarbons or less polar esters and ethers; elution with more polar solvents like polar esters, ethers, alcohols, acetonitrile or water; the binding mechanism can be hydrogen bonding or dipole-dipole interaction; silica can also be used in aqueous medium as a cation exchanger of intermediate strength.	• General normal phase	1. Particle Size: 55-105 µm 2. Pore Size: 125Å 3. Surface Area: 325 m^2/g 4. Activity Grade: high
Alumina (A, B & N) Al_2O_3	Similar in use to silica; available in acidic, basic and neutral high activity grades; alumina also exhibits specific interactions with the π-electrons of aromatic hydrocarbons. More stable under high pH conditions than silica.	• Crude oil fractionation • Acidic and basic grades can also be used as low capacity ion-exchangers	1. Particle Size: 50-300 µm 2. Pore Size: 120Å 3. Activity Grade: high • Al N pH: 7.5 • Al A pH: 4.5 • Al B pH: 10.0
Florisil $MgO•SiO_2$	Polar, highly active, weakly basic sorbent for adsorption of low to moderate polarity species from non-aqueous solutions.	• Specifically designed for the adsorption of pesticides using official AOAC and EPA methods • Other polychlorinated biphenyls (PCB's) in transformer oil	1. Particle Size: 50-200 µm 2. Pore Size: 60Å 3. Activity Grade: high

Ion-Exchange

	Description	Applications	Chemistry	
Accell Plus QMA Strong Anion-Exchanger $C(O)NH(CH_2)_3N(CH_3)_3^+ Cl^-$	Silica-based, hydrophilic, strong anion-exchanger with large pore size. Used for extraction of anionic analytes in aqueous and non-aqueous solutions.	• Isolation of anionic proteins, e.g., immunoglobulins, enzymes • Acidic pigments from wines, fruit juices and food extracts, isolation of phenolic compounds • Peptide pool fractionation	1. Particle Size: 37-55 µm 2. Pore Size: 300Å 3. Carbon: 6% 4. Counter Ion: Cl 5. pH Range 2-9	6. Loading Capacity: 200 mg BSA/gram sorbent 7. Small Molecule loading: 1.8-2.8 meq/gram 8. Ligand Density: 220 µmoles/g
Accell Plus CM Weak Cation-Exchanger $CO_2^-Na^+$	Silica-based hydrophilic weak cation-exchanger with large pore size; extraction of cationic analytes in aqueous and non-aqueous solutions	• Isolation of cationic proteins • Pesticides • Herbicides • Steroids	1. Particle Size: 37-55 µm 2. Pore Size: 300Å 3. Carbon: 5.5% 4. Counter Ion: Na 5. pH Range 2-9	6. Loading Capacity: 175 mg Cytochrome C/ gram sorbent 7. Small Molecule loading: 3.1-4.2 meq/gram 8. Ligand Density: 350 µmoles/g
PSA $–SiC_2H_4NHC_2H_4NH_2$	Silica-based bonded phase containing primary and secondary amines. Similar selectivity to aminopropyl but with higher pKa's and increased ion-exchange capacity.	• Strong affinity for fatty acids, polar pigments, and sugars • Potential for adsorption by chelation	1. Particle sizes: 37-55 µm 2. Pore size: 60Å 3. Surface area: 450 m^2/g	4. pH range: 2-9 5. IEX capacity: 1.75 meg/g

Literature References

A Sample Preparation Primer and Guide to Solid-Phase Extraction Methods Development, Literature Reference WA20300

Sample Prep Solutions Brochure, Literature Reference 720000848EN

96-well Collection Plate Options for the Waters Extraction Plate Manifold, Literature Reference 720001263EN

Waters Sep-Pak Sample Extraction Products Brochure, Literature Reference 720000860EN

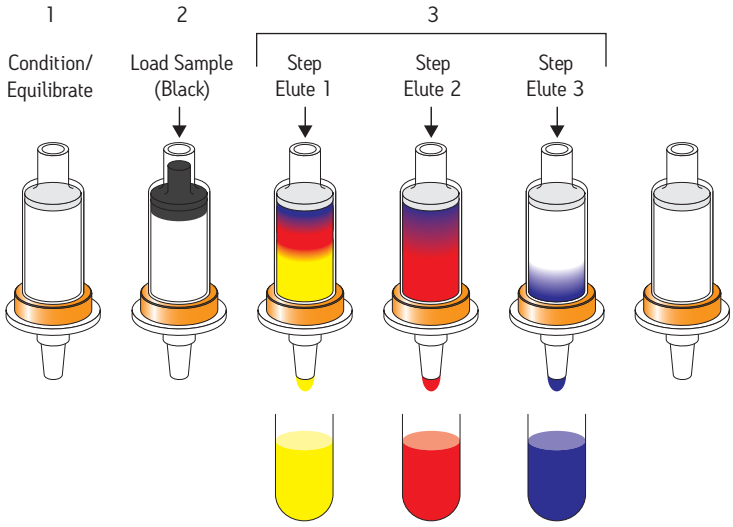
Waters 96 and 384-Well Collection Plate specifications, Literature Reference WA41941

Sorbent Selection Guide for SPE Wall Chart, Literature Reference 720002007EN

Sep-Pak Elution Guidelines

Basic Steps for SPE

1. Condition and equilibrate the cartridge (not required for normal phase)
2. Load sample
3. Elute components—increase strength of mobile phase in steps



	Reverse Phase	Normal Phase	Ion Exchange
Analyte	Low to moderate polarity Hydrophobic	Moderate to highly polar/uncharged	Charged or Ionized
Matrix	Aqueous	Non-polar organic solvent	Aqueous/ Low ionic strength
Condition/ Equilibrate	1. Solvate polar organic 2. Water	Non-polar organic	Low ionic strength buffer
Wash	Aqueous/buffer	Non-polar	Low ionic strength buffer
Elution Steps	Increase polar organic content in steps	Increase moderate to high polarity organic content in steps	Stronger buffers—ionic strength or pH to neutralize the charge

	Reverse Phase	Normal Phase	Ion Exchange	
			AX (Anion)	CX (Cation)
Sorbent	C_{18}, tC_{18}, C_8, tC_2, CN, NH_2, PSA	Silica, Alumina, Florisil, Diol, CN, NH_2, PSA	Accell Plus QMA, NH_2 , PSA	Accell Plus CM
Surface Polarity	Low to Medium	High to Medium	High	High
Solvent Polarity	High	Low	High	High
Typical Loading Solvent	Water, low strength buffer	Hexane, chloroform, $MeCl_2$	Water, low strength buffer	Water, low strength buffer
Elution Solvent	MeOH/water, ACN/water	% ethyl acetate, acetone	Buffers, salts with high ionic strength, increase pH	Buffers, salts with high ionic strength, decrease pH
Sample Elution Order	Most polar first	Least polar first	Most weakly ionized sample component first	Most weakly ionized sample component first
Change Required to Elute Compounds	Decrease solvent polarity	Increase solvent polarity	Increase ionic strength, increase pH	Increase ionic strength, or lower pH

Note: The steps shown are used to illustrate how to elute components of the sample using different chromatographic modes.

Sep-Pak Specialty Chemistries

	Description	Part Number	Mass/Volume/Type	Chemistry
Sep-Pak Dry Sodium Sulfate	For removal of residual water from the SPE extract.	WAT054265 50/box	2.85 g/1.6 mL/Plus Long	1. 2.85 g anhydrous sodium sulfate
Ozone Scrubber Potassium/Iodine	For removing ozone interference with the analysis of carbonyl compounds. Use in series with Sep-Pak DNPH or XPoSure cartridge.	WAT054420 20/box	1.4 g/1.6 mL/Plus Short	1. 1.4 g of potassium iodide 2. Capacity: 4.2 mmoles of ozone/cartridge
Porapak™ RDX DVB - Vinylpryrrolidone	For the analysis of explosives in surface and ground water. Meets or exceeds requirement of EPA Method 8330. Reduces use of organic solvent by 10-fold. PoraPak RDX is a divinylbenzene/vinylpyrrolidone copolymer.	WAT047220 30/box	500 mg/1 mL/6cc Vac	1. Particle Size: 125-150 µm 2. Pore Size: 200Å
DNPH Short	DNPH and XPoSure contains acidified dinitrophenylhydrazine reagent coated on a silica sorbent. Used for the collection of air samples and subsequent quantitation of aldehydes and ketones by reaction to form the hydrazone derivative, and analysis by HPLC. DNPH-Silica is specified in several EPA procedures for the analysis of carbonyl compounds in air.	WAT037500 20/box	350 mg/0.7 mL/Plus Short	1. Particle Size: 55-105µm 2. Pore Size: 125Å 3. Coating: 14 µmoles/g or 5 µmoles/cartridge
DNPH Long	DNPH and XPoSure contains acidified dinitrophenylhydrazine reagent coated on a silica sorbent. Used for the collection of air samples and subsequent quantitation of aldehydes and ketones by reaction to form the hydrazone derivative, and analysis by HPLC. DNPH-Silica is specified in several EPA procedures for the analysis of carbonyl compounds in air.	WAT039550 20/box	800 mg/1.6 mL/Plus Long	1. Particle Size: 55-105 µm 2. Pore Size: 125Å 3. Coating: 14 µmoles/g or 10 µmoles/cartridge
XPoSure™	Larger particle dinitrophenylhydrazine coated silica for use with personal air monitors.	WAT047205 20/box	350 mg/0.7 mL/Plus Short	1. Particle Size: 500-1000 µm
AC2 Fine Activated Carbon	Low ash content activated carbon, packed in a Sep-Pak Plus short body cartridge. Common applications include the concentrate of pesticides and herbicides in water or food samples. Sep-Pak AC2 is used in Japan Pesticide Method #17.	JJAN20229 50/box	400 mg/0.7 mL/Plus Short	1. Particle Size: 85 µm 2. 400 mg/cartridge activated carbon
PS2 Styrene - DVB	Styrene – divinylbenzene copolymer packed in a Sep-Pak Plus short body cartridge. PS2 applications include concentration of pesticides and herbicides in water. This cartridge is used in Japan pesticide methods #5, 6, and 9. AC2 connected in series with PS2 was adopted as the official method for the extraction of pesticides from water by JPMHW.	JJAN20131 50/box	300 mg/0.8 mL/Plus Short	1. Particle Size: 80 µm 2. 300 mg cartridge
Carbon Black/ Amino Propyl	500 mg of carbon black/amino propyl bonded silica separated by a frit. Typical application includes pesticide clean-up from food matrixes. Particularly useful for removing coloring from samples before GC analysis.	186003369 30/box	500 mg carbon black , 500 mg Amino Propyl /1.4 mL/6 cc Vac	1. 500 mg Carbon black top layer, 37-105 µm 2. 500 mg Amino propyl, 55-105 µm
Carbon Black/ PSA Primary-Secondary Amine Silica	Two-layer sorbent bed used for pesticide clean-up in food matrices prior to GC analysis. PSA provides alternative selectivity compared to aminopropyl.	186004590 30/box	500 mg carbon black , 500 mg PSA /1.4 mL/6 cc Vac	1. Particle Size: 37-105 µm [carbon-black, top layer] 2. 37-55 µm [PSA, bottom layer]
Accell Plus QMA Carbonate	Silica-based, hydrophilic, strong anion-exchanger with a carbonate counter-ion. Used for extraction of anionic analytes in aqueous and non-aqueous solutions when the standard chloride counter-ion will interfere with the analysis.	186004051 50/box	150 mg/0.4 mL/Plus Light	1. Particle Size: 37-55 µm 2. Pore Size: 300Å 3. Counter-ion: Carbonate 4. pH Range: 2-9 5. Ligand Density: 220 µmoles/g

Sep-Pak DNPH-Silica Cartridges

for Analyzing Formaldehyde, Other Aldehydes and Ketones in Air

Formaldehyde and other aldehydes are receiving increasing attention both as toxic substances and as promoters in the photochemical formation of ozone in air. Sources of aldehydes in residential buildings include plywood and particle board, insulation, combustion appliances, tobacco smoke, and various consumer products. Aldehydes are released into the atmosphere in the exhaust of motor vehicles and other equipment in which hydrocarbon fuels are incompletely burned.

The most sensitive and specific method for analyzing aldehydes and ketones is based on their reaction with 2,4-dinitrophenylhydrazine (DNPH) and subsequent analysis of the hydrazone derivatives by HPLC. The hydrazones may be detected by absorbance in the ultraviolet region, with maximum sensitivity obtained between 350 and 380 nm.

Airborne aldehydes have traditionally been collected by drawing a sample through an impinger containing a solution of DNPH. However, the impinger collector is generally cumbersome to use and is not well suited for high flow rates or extended collection times due to solvent evaporation.

The new Sep-Pak DNPH-silica cartridges meet the requirements of EPA Method TO-11A and provide a convenient device for sample collection. Using a vacuum pump, an air sample is drawn through the new Sep-Pak DNPH-silica cartridge. The aldehydes and ketones react with the DNPH and form the hydrazone derivative, which is retained on the cartridge. Later, the hydrazones are eluted from the cartridge with acetonitrile and analyzed by HPLC. Detection limits can be as low as 3 ppbv for a 100 liter sample.

Advantages of Waters Sep-Pak DNPH-Silica Cartridges

These cartridges provide you with significant advantages when compared to other techniques, such as liquid impingers, for the analysis of aldehydes and ketones. In addition, a new high speed, high resolution HPLC application has been developed to provide excellent quantitation capability in the low parts-per-billion range.

- Sep-Pak DNPH-silica cartridges meet the requirements of EPA Method TO-11A and ASTM-D-5791-1
- Results from impingers and these cartridges are in excellent agreement
- Solvent consumption, solvent exposure and hazardous waste disposal costs are reduced
- Sep-Pak DNPH-silica cartridges provide superior convenience and reproducibility, making them ideal for field sampling and process monitoring applications
- Sep-Pak DNPH-silica cartridges can save time and increase productivity
- Increased safety.

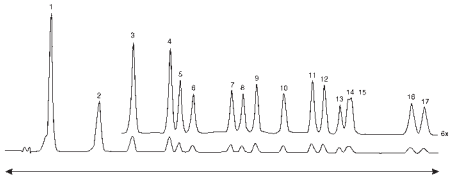
Sep-Pak DNPH-Silica Cartridge

Description	Qty	Part No.
Sep-Pak DNPH-silica cartridge	20/box	WAT037500
Sep-Pak DNPH-silica long body cartridge	20/box	WAT039550

HPLC Separation of DNPH Derivatives of Common Aldehydes and Ketones

HPLC Conditions

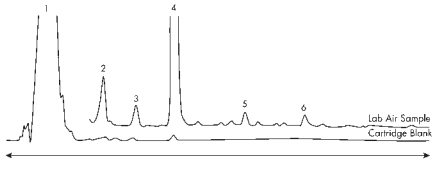
Column:	Nova-Pak® C_{18} 3.9 x 150 mm
Sample:	Mixture of DNPH and DNPH derivatives in acetonitrile.
Mobile Phase:	A: Water/Acetonitrile/Tetrahydrofuran 60/30/10 v/v/v B: Water/Acetonitrile 40/60 v/v 3 mL
Gradient:	100% A for 1 minute, then linear gradient from 100% A to 100% B in 10 minutes
Flow Rate:	1.5 mL/min
Injection:	20 µL
Detection:	360 nm



1. DNPH
2. Formaldehyde-DNPH
3. Acetaldehyde-DNPH
4. Acetone-DNPH
5. Acrolein-DNPH
6. Propionaldehyde-DNPH
7. Crotonaldehyde-DNPH
8. Butanone-DNPH
9. Butyraldehyde-DNPH
10. Benzaldehyde-DNPH
11. Isovaleraldehyde-DNPH
12. Valeraldehyde-DNPH
13. o-Tolualdehyde-DNPH
14. m-Tolualdehyde-DNPH
15. p-Tolualdehyde-DNPH
16. Hexaldehyde-DNPH
17. 2,5-Dimethylbenzaldehyde-DNPH

Low-Level: Aldehyde Profile from Laboratory Air

Lab Air Sample		Cartridge Blank (calculated for 100 liter sample)	
1. DNPH		1. DNPH	
2. Formaldehyde-DNPH	4.8 ppbv	2. Formaldehyde-DNPH	0.35 ppbv
3. Acetaldehyde-DNPH	1.2 ppbv	3. Acetaldehyde-DNPH	0.27 ppbv
4. Acetone-DNPH	118.0 ppbv	4. Acetone-DNPH	0.34 ppbv
5. Butanone-DNPH	0.8 ppbv		
6. Isovaleraldehyde-DNPH	0.7 ppbv		

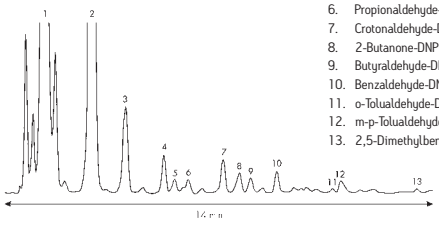


The sample was obtained in a chemical research laboratory using a portable sampling pump. One hundred liters of air was drawn through the cartridge at 0.65 L/min. The sample was found to contain low concentration of formaldehyde (4.8 ppbv), acetaldehyde (1.2 ppbv) and methyl ethyl ketone (0.8 ppbv), but a significant amount of acetone (118 ppbv). Significant concentrations of acetone are frequently found in laboratories because of its widespread use as a solvent.

High-Level: Aldehyde Profile from Diluted Auto Exhaust Emissions

Sample (8.4 L at 1 L/min) collected at US EPA Atmospheric Research Assessment Lab. Courtesy of Dr. S. B. Tejada.

1. DNPH	
2. Formaldehyde-DNPH	2290 ppbv
3. Acetaldehyde-DNPH	367 ppbv
4. Acetone-DNPH	26 ppbv
5. Acrolein-DNPH	8 ppbv
6. Propionaldehyde-DNPH	12 ppbv
7. Crotonaldehyde-DNPH	36 ppbv
8. 2-Butanone-DNPH	24 ppbv
9. Butyraldehyde-DNPH	15 ppbv
10. Benzaldehyde-DNPH	32 ppbv
11. o-Tolualdehyde-DNPH	5 ppbv
12. m-p-Tolualdehyde-DNPH	11 ppbv
13. 2,5-Dimethylbenzaldehyde-DNPH	3 ppbv



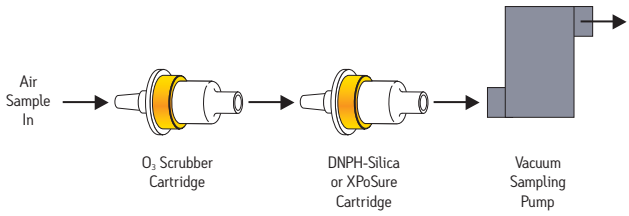
Ozone Scrubber Cartridges

Ozone has been shown to interfere with the analysis of carbonyl compounds in air samples that have been drawn through cartridges containing silica-coated with 2,4-dinitrophenylhydrazine (DNPH). Waters Ozone Scrubber cartridges are designed to remove this ozone interference.

These disposable devices are intended for use in series combination with the Waters Sep-Pak DNPH-Silica cartridges or XPoSure Aldehyde Sampler cartridges. One Ozone Scrubber cartridge replaces the 1/4" diameter by 36" long copper ozone denuder located in the heated zone of sampling systems used for outdoor air monitoring (PAMS program).

Each Ozone Scrubber cartridge contains 1.4 g of granular potassium iodide. When air containing ozone is drawn through this packed bed, iodide is oxidized to iodine, consuming the ozone. The theoretical capacity of a single cartridge is 4.2 mmoles of ozone (200 mg). The particle size of the potassium iodide granules is optimized for good mass transfer and flow characteristics.

Flow Schematic for Air Sampling System



Ozone Scrubber

Description	Qty.	Part No.
Ozone Scrubber	20/box	WAT054420

Waters XPoSure Aldehyde Sampler Cartridges

for Monitoring Aldehydes in Indoor Air

Based on an extension of our DNPH coating technology, XPoSure aldehyde sampler cartridges are the most sensitive active samplers available today.

Highest Sensitivity

Compared to existing sampling tube technology which have high and variable backgrounds, XPoSure cartridges are guaranteed to give consistent low aldehyde backgrounds, cartridge-to-cartridge, lot-to-lot.

High-Collection Efficiencies

You can achieve > 95% collection efficiencies for all aldehydes at flows of up to a liter per minute. And, you only need to use one cartridge—no breakthrough bed is necessary.

Low Pressure Drop—Use with Portable Personal Sampling Pumps

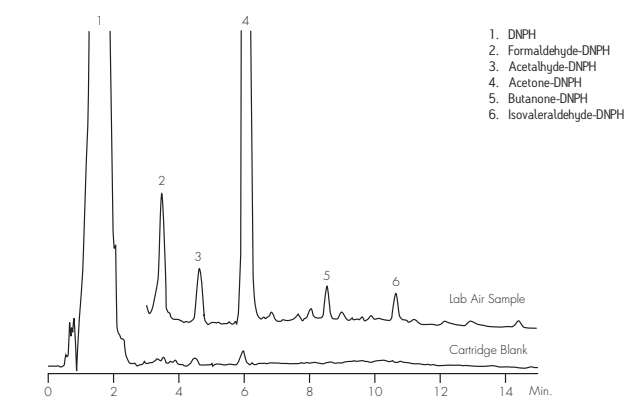
Large particle size and higher porosity frits make the XPoSure cartridge compatible with personal sampling pumps.

Easy-to-Use

Sample, elute and shoot, it's that easy. You'll never have to break open and manipulate a glass tube again. And because the cartridges are made from high density polyethylene (HDPE), breakage is not a concern.

The figure on the right shows two traces. An actual cartridge blank demonstrating extremely low background levels and as an actual laboratory air sample.

Low-Level Example: Aldehyde Profile from Laboratory Air



The above sample was collected in a chemical research laboratory using a portable sampling pump. One hundred liters of air was drawn through the cartridge at 0.65 L/min. The chromatogram shows ormaldehyde (4.8 ppbv), acetaldehyde (1.2 ppbv), acetone (118 ppbv), butanone (0.8 ppbv), and isovaleraldehyde (0.7 ppbv).

XPoSure Aldehyde Sampler Cartridge

Description	Qty.	Part No.
XPoSure Aldehyde Sampler Cartridge	20/box	WAT047205

PoraPak Rdx Sep-Pak Extraction Cartridge

for the Analysis of Explosives in Surface and Ground Waters

Designed to meet or exceed the QA/QC requirements of EPA method 8330, it is ideal for environmental testing laboratories supporting Department of Defense remediation programs.

High Sensitivity

PoraPak Sep-Pak cartridges contain PoraPak RDX resin, a specially prepared, specially cleaned divinylbenzene/vinylpyrrolidone copolymer, packed in a high purity polypropylene syringe barrel. With the lowest guaranteed backgrounds and the highest cartridge-to-cartridge, lot-to-lot consistency, the Waters PoraPak RDX column is the most sensitive technology available today and allows you to perform analysis at sub ppb levels.

Unmatched Recoveries

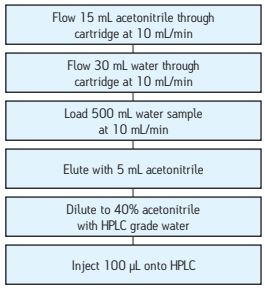
The specially prepared resin is highly selective for nitroaromatic and nitramine compounds, resulting in recoveries of 90% or greater. Recovery data from preconcentrating 500 mL of explosives standards in sterile water at two concentrations on PoraPak RDX Sep-Pak Vac columns. Number of replicates = 7.

Compound	1 ppb % Recovery	% RSD	10 ppb % Recovery	% RSD
HMX	100.5	6.7	100.5	3.9
TNB	95.9	3.5	99.3	3.3
RDX	90.9	6.4	98.7	3.2
DNB	99.5	3.2	99.2	3.2
TNT	97.0	3.0	102.	3.7
TETRYL	89.0	6.4	102.8	4.7
NB	96.5	2.5	97.9	2.8
3,5-DNA	91.2	3.3	98.2	3.6
2,4-DNT	97.3	3.4	99.9	3.4
2,6-DNT	94.5	3.4	98.7	3.4
2-Am-DNT	92.4	5.2	98.0	3.7
4-Am-DNT	90.0	4.9	97.2	4.1
4-NT	89.5	4.3	100.4	3.7
2-NT	96.8	6.6	93.4	3.0

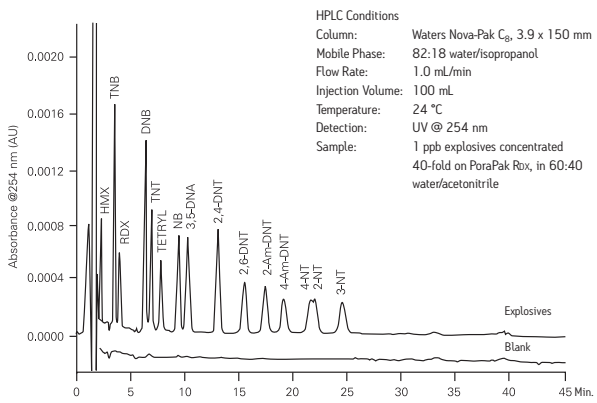
Increase Productivity and Reduce waste

By using PoraPak RDX cartridges, you can reduce the amount of organic solvent used per sample by 10-fold and decrease your sample prep time by 3X.

Activate, Load, Elute, and Shoot



Isocratic Separation of Method 8330 Analytes



Achieve the lowest method detection limits using Waters PoraPak RDX cartridges. The chromatogram above shows the analysis of reagent H_2O spiked at the 1 ppb level. The bottom chromatogram is a reagent (H_2O) blank.

PoraPak RDX Cartridges and Accessories

Description	Qty.	Part No.
PoraPak RDX cartridges	30/box	WAT047220
Tubing, Tefzel, 1/8 inch o.d. x 0.040-inch i.d.		WAT023344
Sep-Pak Vac adapter	12/box	WAT054260
60 cc Sep-Pak reservoir	12/box	WAT024659
Male-male adapter		WAT024310

Sep-Pak Dry SPE Cartridge

Waters Sep-Pak Dry cartridges are packed with 2.85 g of anhydrous sodium sulfate. These cartridges are designed to remove residual water from the SPE extract.

Sep-Pak Dry Cartridge

Description	Qty.	Part No.
Sep-Pak Dry Cartridge	50/box	WAT054265

NEW DisQuE Dispersive Sample Preparation Kit

DisQuE™
Dispersive Sample Preparation

Dispersive sample preparation, commonly referred to as "QuEChERS", is a simple and straightforward sample preparation technique suitable for multi-residue pesticide analysis in a wide variety of food and agricultural products. Waters DisQuE™ Dispersive Sample Preparation Kit contains conveniently packaged centrifuge tubes with pre-weighed sorbents and buffers designed for use with AOAC official methods.

DisQuE dispersive sample preparation is a well proven, high throughput sample preparation method for a wide array of pesticide in produce samples.*

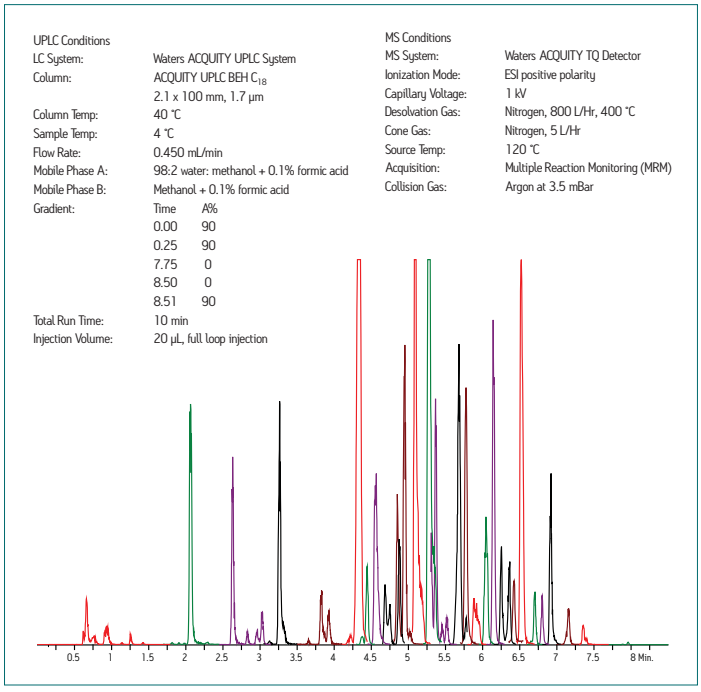
- Easy and straightforward method to implement, requiring little training
- Conforms to the AOAC official method for determining pesticide residues in fruits in vegetables
- Cost effective
- Reliable, high quality product in a simple kit format

Extraction Procedure

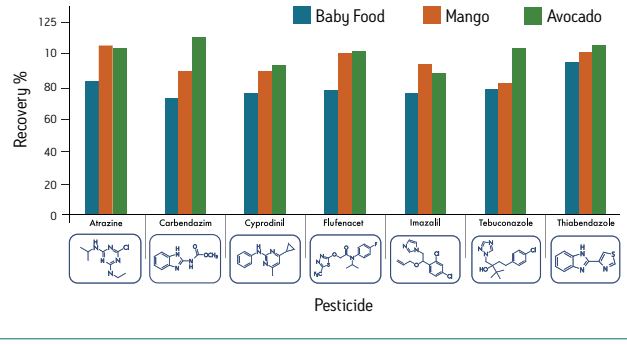
1. Add 15 g of homogenized sample to the 50 mL DisQuE extraction tube containing 1.5 g of sodium acetate and 6 g of magnesium sulphate.
2. Add 15 mL of 1% acetic acid in acetonitrile.
3. Add any pre-extraction internal standards.
4. Shake vigorously for 1 minute and centrifuge >1500 rcf for 1 minute.
5. Transfer 1 mL of the acetonitrile extract into the 2 mL DisQuE extraction tube containing 50 mg PSA and 150 mg of magnesium sulphate.
6. Shake for 30 seconds and centrifuge >1500 rcf for 1 minute.
7. Transfer 250 µL of final extract into an autosampler vial.
8. Add any post-extraction internal standards.
9. Dilute as needed with an appropriate buffer or solvent.

*Reference: Lehotay, J.AOAC Int. 90(2) 2007, 485-520.

Chromatogram Showing 402 Pesticide Residues at 10 ppb ng/g In One 10 Minute Run



Recovery Data for Three Types of Sample Matrices Fortified at 10 ng/g



DisQuE Dispersive Sample Preparation Kit

Description	Qty.	Part No.
DisQuE Dispersive Sample Preparation Kit	100/pk	176001676

Literature References

A Rapid Method for the Screening and Confirmation of over 400 Pesticide Residues in Food, Literature Reference 720002628EN

Determination and Confirmation of Priority Pesticide Residues in Baby Food, Literature Reference 720002812EN

NEW PoraPak Rxn Cartridges

for Post Synthesis Cleanup

PoraPak™ Rxn
Post Synthesis Cleanup

Waters now offers PoraPak Rxn, a family of polymer-based chromatography products for superior cleanup of synthetic reactions. PoraPak Rxn products are available in two chemistries:

- PoraPak Rxn CX, a strong cation-exchange sorbent
- PoraPak Rxn RP, a reversed-phase sorbent

PoraPak Rxn sorbents are available in fritted syringe-barrel devices in 6, 20, and 60 cc volumes. The resins are also sold in bulk units, and custom configurations are available on request.

New Solutions for Faster Results

PoraPak Rxn sorbents are based on copolymers that exhibit the following properties:

- Hard material that does not develop increasing back pressure with flow
- Little swelling or shrinking across a range of solvents and pH extremes
- Low hydraulic resistance enables flow by gravity
- pH extreme tolerance without dissolution or hydrolysis, both limitations of silica-based sorbents

This combination of physical and chemical properties makes PoraPak Rxn cartridges ideal for synthesis cleanup. The polymers characteristics and particle size maintain gravity, pressure -or vacuum-assisted flow; even when reaction mixtures contain precipitate that may contribute additional resistance to flow. The sample will still pass through the cartridge.

The polymer used in PoraPak Rxn products is resistant to shrinking or swelling in the organic solvents typically used in synthetic reactions. Tests with the following solvents demonstrate that the packed bed maintains good flow properties:

- DCE
- DCM
- DMSO
- DMF
- THF
- Acetone

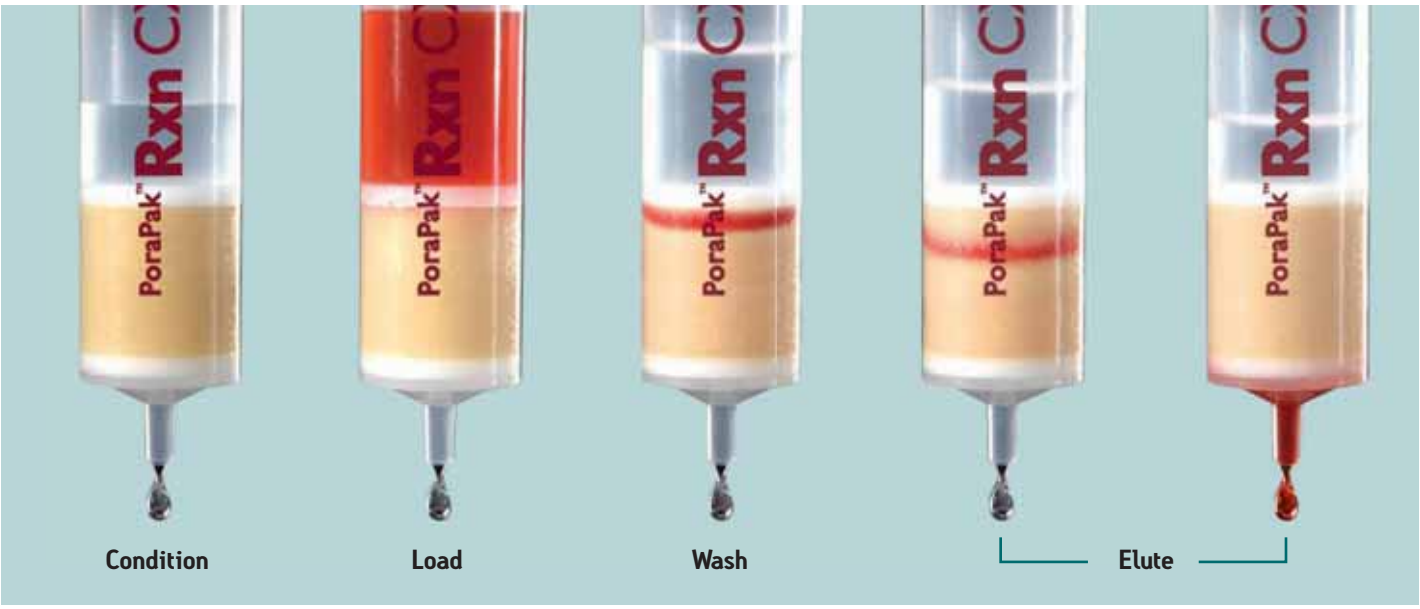
Some medicinal chemists are familiar with silica-based chromatographic products for reaction cleanup. One of the limitations of these silica-based ion-exchange materials is pH. Silica will dissolve at high pH, while bonded phases are hydrolyzed at low pH; both conditions result in loss of sample and/or impurities (silica and bonded phase) collected in product fractions. PoraPak Rxn polymer-based chromatographic phases are stable at extreme pH. This feature permits using pH as a very powerful tool to create a separation, particularly in ion-exchange mode.

Providing Separations Solutions

Waters is highly respected worldwide for its expertise in chromatography. Coupled with our ability to seamlessly link critical instrumentation, chemistries, separation technologies, and software, this expertise puts us in a unique position to deliver value-added solutions to our customers.

Manufacturing

Our world-class manufacturing facilities are continuously expanded and upgraded to keep pace with market demand for our new and existing products. We manufacture under the highest quality standards in the industry, including ISO9001:2000, ISO 13485:2003 and Current Good Manufacturing Practices (cGMP).

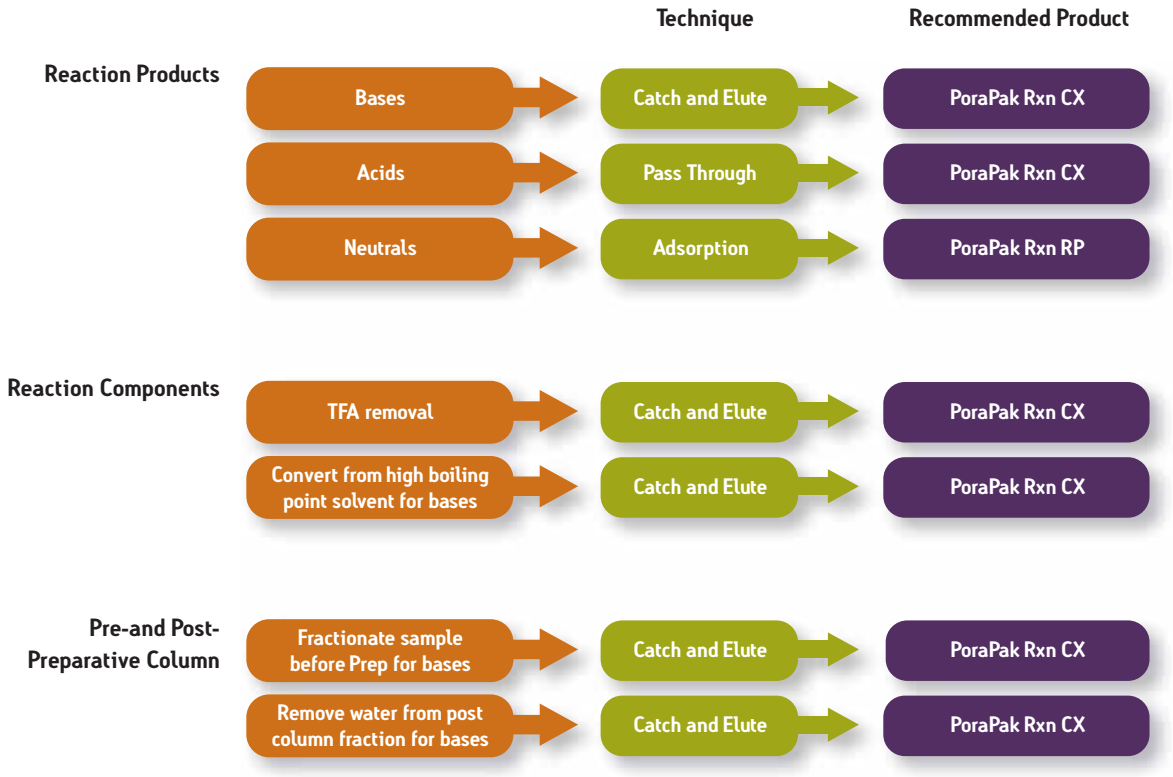


Cleanup Challenges

Challenge	Solution
Convert or remove high boiling solvent before next reaction step - product is a base.	Load reaction mix on PoraPak Rxn CX cartridge and pass through high boiling point solvent such as DMSO. Wash with methanol. Elute using 5% ammoniated methanol. Achieve sample enrichment and save time by evaporative removal of a low boiling point solvent before the next step.
Separate product (bases) from reactants and reaction solvent.	Load on PoraPak Rxn CX cartridge using a catch-and-elute strategy. Wash with methanol and elute with ammoniated methanol.
Remove TFA used in the reaction from bases.	Load reaction mixture on PoraPak Rxn CX cartridge using a catch-and-elute strategy. TFA passes through unretained. Wash with methanol and elute with ammoniated methanol.
Convert from a solvent used in the last synthesis before Prep LC to get sample in the starting mobile phase conditions.	Load on PoraPak Rxn CX cartridge using a catch-and-elute strategy. Wash with methanol and elute with ammoniated methanol. Evaporate the fraction and reconstitute the product in the Prep LC starting solvent.
Remove water from fractions collected from Prep LC for faster dry down.	Load on PoraPak Rxn CX cartridges using a catch-and-elute strategy. Wash with methanol and elute with ammoniated methanol.

Choose the Best Product and Technique for Your Cleanup

PoraPak Rxn products are available in two sorbents: CX, a strong Cation-eXchange resin and RP, a Reversed-Phase resin. The illustrations below recommend the sorbent to use and the separation technique to apply during cleanup. The choice of the appropriate sorbent and technique depends upon the nature of the reaction products, reactants, solvents, and/or preparative columns.



Catch and Elute

In the "catch and elute" technique, the reaction mixture is loaded onto the cartridge; the analytes of interest are retained by the sorbent. A wash step follows to remove additional reaction components from the cartridge. A strong solvent is used to elute the analytes from the cartridge. Sample concentration results when the final elution volume is smaller than the load volume.

Pass Through

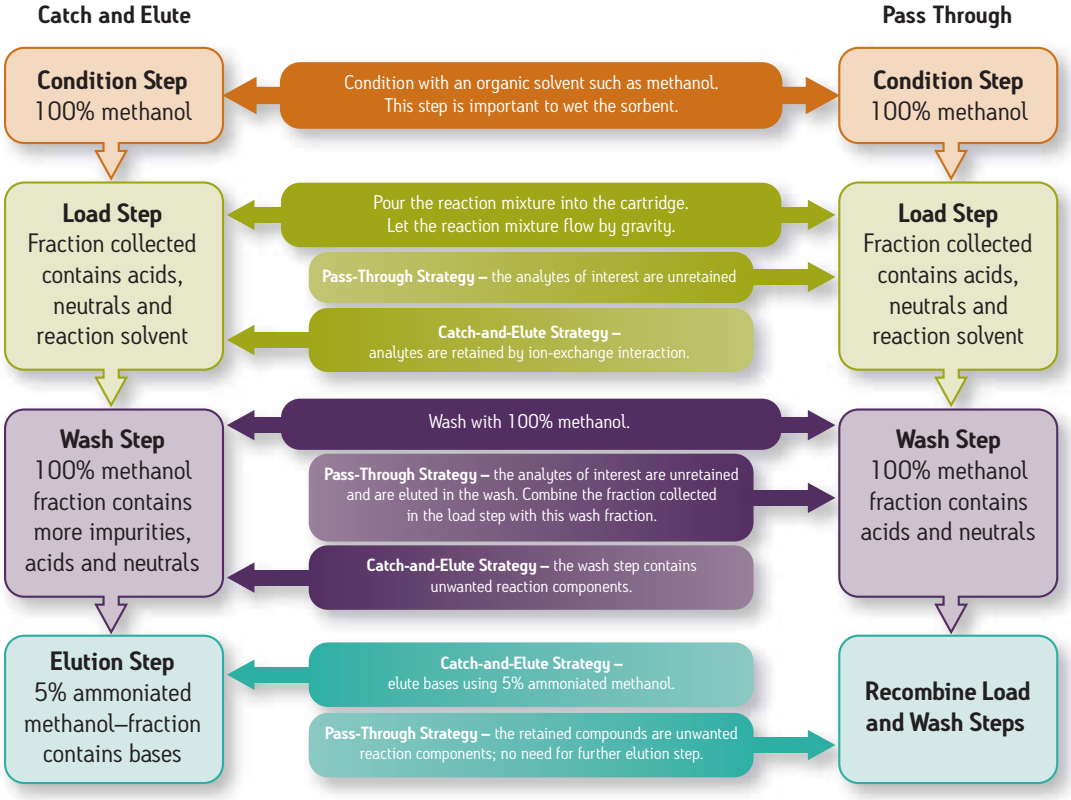
In the pass-through technique, the sorbent does not retain the compounds of interest, but does retain some class(es) of compounds and impurities, that can be discarded with the column. Fractionation is achieved by the interaction between the impurities in the reaction mixture and the sorbent. No sample enrichment occurs during the solid-phase extraction (SPE) step.

Adsorption

In the adsorption-chromatography technique on a reversed-phase sorbent, the neutral compounds are moving down the chromatographic column in the presence of organic solvent, albeit slowly, for small-percent organic washes. Load the reaction mixture in high water content (75% water for example), wash to remove polar compounds, and then increase the organic content in large increments to elute neutrals. The increase is determined by the hydrophobicity and MW of the compounds.

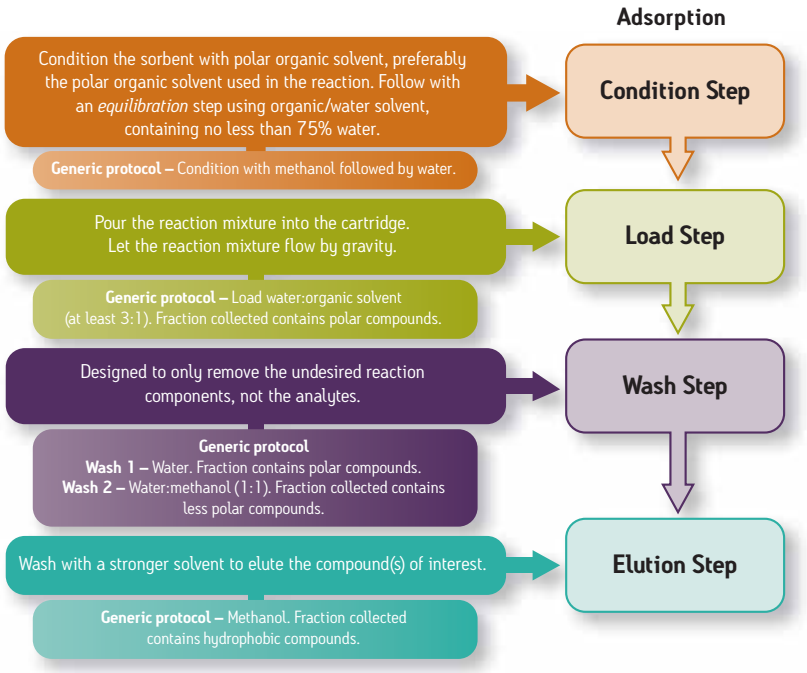
Cleanup Procedures

Using PoraPak Rxn CX: Strong Cation-Ion Exchange Catch-and-Elute and Pass-Through Protocols



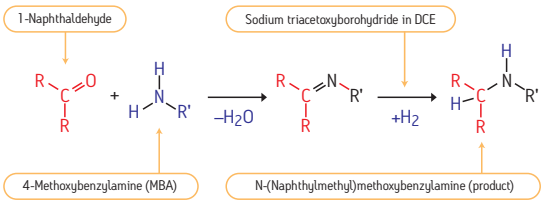
For PoraPak Rxn RP: Reversed-Phase Protocol

For a compound to be retained, a reaction, carried out in a polar organic solvent will have to be concentrated by evaporation and/or diluted with water (3-to-1 or greater). This dilution brings the organic content down to 25% or less so as to enable reversed-phase retention. The generic protocol, shown to the right, can be used as a starting point to clean up reaction mixture.

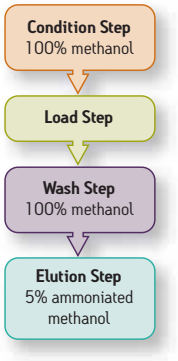


Cleanup Of A Reductive Amination Mixture

Reductive amination is a common reaction carried out in medicinal chemistry laboratories. In this example, a PoraPak Rxn CX cartridge is used to fractionate the reaction mixture.



Catch-and-Elute Procedure



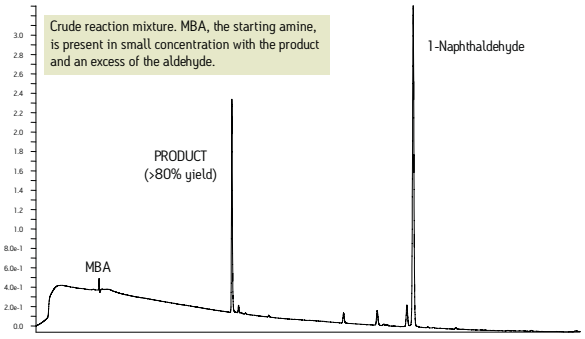
UPLC Conditions

Instrument:	ACQUITY UPLC System
Column:	ACQUITY UPLC BEH Shield RP18, 2.1 X 100 mm, 1.7 µm
Column Part Number:	186004049
Mobile Phase A:	0.01% formic acid
Mobile Phase B:	100% acetonitrile
Gradient and Flow Rate:	

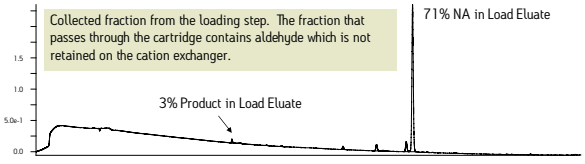
Time (min)	Flow Rate (mL/min)	A(%)	B(%)	Curve
Initial	0.42	95	5	Initial
7	0.42	1	99	6

Injection Volume:	2 µL
Column Temperature:	30 °C
Detector:	ACQUITY UPLC PDA
UV Wavelength:	250-340 nm (total absorbance mode)

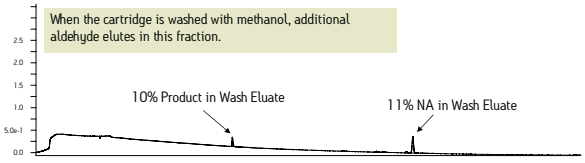
Reaction Mixture Before Cleanup



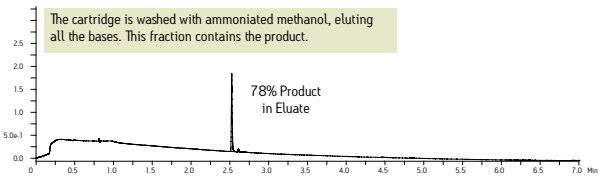
Load Step



Wash Step



Elution Step



Using the 'catch-and-elute' procedure to clean up this synthesis mixture enables a quick collection of a limited number of fractions, increasing the certainty of finding the product rapidly. Assay the product fraction for confirmation, then begin the evaporation of the more volatile elution solvent.

Product Guidelines

The following tables should be used as guidelines for loading capacity, flow rates, and volumes of wash and elution solvents. These tables take into consideration the dimension of the device and the bed volumes suggest appropriate flow rates and volumes for the condition, wash and elute steps.

Maximum Capacity Guidelines	PoraPak Rxn RP	PoraPak Rxn CX
Configuration	Retained Compounds	Retained Base
6 cc/150 mg	15 mg/50 µmole	15 mg/50 µmole
6 cc/400 mg	40 mg/150 µmole	40 mg/150 µmole
20 cc/2 g	200 mg/350 µmole	200 mg/350 µmole
60 cc/5 g	500 mg/2000 µmole	500 mg/2000 µmole

The capacity is for the total of all compounds in the reaction that will be retained. For cation exchange, the capacity is for all bases in the reaction mixture including product and reactants.

Consider smaller loads than maximum capacity guidelines or choose a larger device to avoid compound break through.

Flow Rate Guidelines (mL/min)	
6 cc cartridge	2 mL/min
20 cc cartridge	5 mL/min
60 cc cartridge	9 mL/min

Flow rate guideline is meant to provide a flow for reaction loading step.

The reaction mix can be poured into the cartridge and allowed to flow by gravity. If the flow is too slow, simply add a small amount of pressure or vacuum to increase it. If there is precipitate in the reaction vessel, rinse the vessel with more loading solvent and add to the top of the cartridge. During the washing steps, product trapped by the precipitate may be dissolved and can be retained on the packed bed for later elution under the right solvent conditions.

Wash and Elution Volumes (mL)			
Device	Condition	Wash	Elute
6 cc/150 mg	3 mL	4 mL	4 mL
6 cc/400 mg	5 mL	10 mL	10 mL
20 cc/2 g	20 mL	20 mL	20 mL
60 cc/5 g	45 mL	45 mL	45 mL

This table provides guidelines for volumes used in the condition, wash and elution steps for the various sizes of PoraPak Rxn cartridges.

PoraPak Rxn Cartridges and Bulk Material

Description	Part No. PoraPak Rxn CX	Part No. PoraPak Rxn RP
6 cc Flanged cartridges, 400 mg, 30/pkg	186004541	186004545
6 cc Flangeless cartridges, 400 mg, 30/pkg	186004542	186004546
20 cc cartridges, 2 g, 20/pkg	186004543	186004547
60 cc cartridges, 5 g, 10/pkg	186004544	186004548
Bulk, 200 mL /container	186004569	186004570

[CLEAN]

- Fast Cleanup of Synthesis Reactions
- Efficient Fractionation
- Cost Effective

PoraPak™ Rxn products are designed to ensure fast and thorough cleanup of most synthesis reactions. The polymer used in PoraPak Rxn material is tolerant of pH extremes, shows low hydraulic resistance and displays very little shrinking or swelling in organic solvents. This combination of physical and chemical properties makes PoraPak Rxn an ideal solution for your most challenging synthesis cleanup.

PoraPak™ Rxn
Post Synthesis Cleanup

www.waters.com/medicinalchemists

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Click anywhere on page. Type desired page number. Click OK.

Sample Vials and Accessories

Waters Autosampler Vials

LCGC and LCMS certified vials are now available

Waters is a leading manufacturer of analytical instrumentation and consumable products. We understand the importance of autosampler vials for the performance of analytical instrumentation. There are many factors to consider in selecting the proper vial:

- Needle design
- Autosampler tray design
- Chemical compatibility
- Cleanliness
- Optic and robotic specifications
- Volatility
- Sample volume

At Waters, we take all of these factors into consideration in the design, manufacture and delivery of our vials and accessories. Unlike our competition, who offer Type I, 33-expansion glass in North America and Type I, 51-expansion glass in Europe or Japan, Waters single source manufacturing produces Type I, 33-expansion glass, the lowest free ion glass available, for worldwide distribution.

Waters LCGC Certified Vials

Vials are usually manufactured by glass artisans and engineers who don't understand the requirements for their use in HPLC and GC. As a manufacturer of autosamplers and chemistry consumables, we understand the dimensional and chemical requirements of vials. We reviewed the manufacturing process, anticipated possible problem areas, and developed tests to ensure the delivery of a problem-free product. The HPLC test to ensure the delivery of residue clean vials is a radically different form of test for the vials industry.

Waters LCMS Certified Vials

In 2006, we added Waters LCMS certified vials to the product line. This is a continuation of our approach to offer a product suitable for the demands of LCMS. We took an unbiased approach in developing this product, looking for any ionized masses regardless of the source. The vials are tested by MS with specifications for total ion count and presence of clusters in the high mass range. The product introduced is cleaner than any product we tested from vendors around the globe.

Septumless Polyethylene Screw Cap

Waters septumless polyethylene screw caps are available with 12 x 32 mm polypropylene or glass vials. This innovative cap design incorporates a needle entry path, removing the need for a septum. The cap is offered alone or comes packaged in combination pack of 100 vials and caps. Look for part numbers on pages 56, 60, and 64

Vials and Plates for ACQUITY UPLC Systems

Waters offers a selection of premier supplies including vials, plates, and accessories, suited for the ACQUITY UPLC System and its sample manager. Recommended vials and plates for ACQUITY UPLC systems are listed on pages 56 through 59 including details of needle depth settings.

IMPORTANT: ACQUITY UPLC systems can be equipped with injector needles made from different materials: PEEK™ and metal. The recommended vial and plate options are different for each needle. Please refer to the appropriate vial and plate selections, depending on the needle installed in your ACQUITY UPLC system.

Vials

Waters recommends vials with pre-slit or self venting septa for ACQUITY UPLC systems. Pre-slit septa are designed to provide proper venting, resulting in reproducible and accurate sample draw volumes. This is crucial as the sample volumes become smaller.

Plates and Accessories

Waters offers a selection of 96 and 384-well plates with different sealing options for ACQUITY UPLC systems. The plates with cap mats and heat seals are located on pages 58 and 59 with the appropriate needle selection.

ACQUITY UPLC system with sample organizer

Vials and Accessories for Waters HPLC Systems

Vials and accessories for Waters HPLC systems are listed on pages 60 through 63 and include ordering information as well as maximum injectable volumes and estimated residual volumes for each vial and insert.

Pre-slit septa are designed to provide proper venting, resulting in reproducible sample draw volumes. Waters recommends their use whenever possible.

Vials and Accessories for Other Manufacturers' Autosamplers

Many of Waters vials and accessories also fit other manufacturers' autosamplers. These vials are held to the same high quality standards we demand for our systems.

Alliance® HPLC System

Literature References

Sample Vials & Accessories Brochure, Literature Reference 720001818EN

Waters LC/MS Certified Sample Vials Whitepaper, Literature Reference 720001517EN

Determination of the Level of Ion Suppression from LC/MS Vials, Literature Reference WA60004

Waters Certified Sample Vials Technical Whitepaper, Literature Reference 720001303EN

Choosing the Right Vial and Septum for Your Application

There are three decisions you need to make when choosing the correct vial for your application: the septum, the closure and the vial itself. Read through the selection options below to determine the proper combination for your application. For your convenience, Waters offers many of these choices as combination packs. The vial, cap, and septum come pre-packaged as packs of 100 for ease and convenience in ordering.

Septa Selection Guide

PTFE

- Recommended for single injection applications
- Ideal for use in MS applications
- Excellent solvent resistance and chemical compatibility
- Does not reseal upon puncturing
- Not recommended for long-term sample storage

PTFE/Silicone

- Recommended for multiple injections and sample storage
- Demonstrates excellent resealing characteristics
- PTFE chemical resistance until punctured, then the septum will have the chemical compatibility of silicone
- Working temperature range from -40 °C to 200 °C
  Pre-slit PTFE/Silicone
- Provides adequate venting to prevent vacuum formation in sample vial, delivering excellent sample-draw reproducibility
- Eliminates coring from bottom draw-port needles
- Good resealing capabilities
- Recommended for multiple injections
- PTFE chemical resistance until punctured, then the septum will have the chemical compatibility of silicone
- Working temperature range from -40 °C to 200 °C

PE Septumless

- Same advantages as PTFE

Vial Closures Guide

Vials are available in three closure types: crimp, snap, and screw cap. Each closure has its advantages and disadvantages.

Crimp caps squeeze the septum between the rim of the glass vial and the crimped aluminum cap. This forms an excellent seal preventing evaporation. The septum stays seated during piercing by the autosampler needle. The crimp cap vial requires crimping tools to carry out the sealing process. For few samples, manual crimper tools are the best choice. For large numbers of sample, automated crimpers are available.

Snap caps are an extension of the crimp cap system of sealing. A plastic cap is stretched over the rim of the vial to form a seal by squeezing the septum between the glass and the stretched plastic cap. The plastic cap creates tension when trying to return to its original size. This tension forms the seal between glass, cap and septum. Plastic snap caps do not require any tools to assemble.

Snap caps are not as effective a seal as other closures.

- If the fit of the cap is very tight, the cap is hard to apply and may be subject to crack.
- If the fit is too loose, the seal is poor and the septum may dislodge.

Screw cap vials are universal. Screwing the cap applies a mechanical force that squeezes the septum between the glass rim and the cap. Screw caps form an excellent seal and mechanically hold the septum in place during piercing. No tools are required for assembly.

LectraBond™ screw caps are available through Waters. This screw cap has a PTFE/silicone septum bonded to the polypropylene cap, using a non-solvent bonding process. This bonding technology is designed to keep the septum/cap together during shipment and assembly onto vials. The bond will aid in preventing dislodging of the septum during use, but the primary sealing mechanism is the mechanical force applied by tightening the cap to the vial.

Cap tightening is the mechanism that forms the seal and holds the septum in place during needle insertion. There is no need to over-tighten the cap, as it can compromise the seal and lead to dislodging. The septum starts to cup or indent when you begin to over-tighten.

Cap Design	Strength Design	Comment
Crimp	Excellent seal	Requires tools
Snap	Moderate seal	Fast, no tools, some cap cracking
Screw	Excellent seal	Universal

Vial Selection Guide

Type 1, 33-Expansion Borosilicate Glass

The most chemically-inert glass available, generally used in high precision laboratories to prevent alteration of test results. It has an expansion coefficient of approximately 33x10–7 °C and is composed primarily of silicon and oxygen, with trace amounts of boron and sodium.

Type 1, 51-Expansion Glass

More alkaline than 33-expansion glass and is adequate for many laboratory uses. It has an expansion coefficient of 51x10–7 °C and is composed primarily of silicon and oxygen, with trace amounts of boron. All amber glassware is 51-expansion glass.

Deactivated Glass (DV)

For glass-sensitive compounds, glass vials are treated with gas phase reactive organosilane to produce a hydrophobic glass surface. Vials treated by this procedure can be stored indefinitely.

Polypropylene Plastic

Polypropylene is a non-reactive plastic and can be used where glass is not an appropriate option. Polypropylene vials can be incinerated while still sealed, minimizing exposure to potentially hazardous substances. The maximum temperature use is: 135 °C.

Deactivated Glass Vials (DV) and Inserts

Eliminates adsorbtion of compounds onto the glass surface when working with biological or pharmaceutical compounds, natural products, pesticides and herbicides. The surface modification is permanent, resulting in an indefinite shelf life.

Waters Alliance Total Recovery Vial

Specifically designed for the side draw-port needle and the factory needle draw depth settings of the Waters Alliance 2690/2695 HPLC. This vial delivers maximum sample capacity (~1 mL) with minimum residual volume (~9 µL).

Waters Maximum Recovery Vial

Specifically designed for the bottom draw-port needle of the Waters ACQUITY UPLC and Alliance HT HPLC Systems. This vial delivers maximum sample capacity (~1.5 mL) with minimum residual volume. The 9 mm cap makes it ideal for use with Agilent HPLC and GC Systems.

NEW Waters Vials Selector

The new Waters Vials Selector is designed to simplify the process of selecting the best vial solutions for your system and application requirements.

The selector offers vial options matching the criteria entered, such as the system you are using, sample volume, detection method and light sensitivity of analytes. As a registered user, you will be notified of all future updates.

For more information about Waters Certified Vials, or to download your FREE copy, please visit

www.waters.com/vials

This interactive tool requires that the user has Adobe Flash Player 8.

Screw Cap 12 x 32 mm Vials for ACQUITY UPLC Systems

For PEEK and Metal-Tipped Needles

LCMS Certified Combination Packs					
Vial, Cap and Pre-slit Silicone/PTFE Septum	600000668CV	600000669CV	600000670CV	600000755CV	
LCGC Certified Combination Packs					
Bonded Pre-slit Silicone/PTFE Septum	186000307C	186000847C	186000327C	186003886C	
Bonded Pre-slit Silicone/PTFE Septum Deactivated	186000307DV	186000847DV	186000327DV		
Combination with PE Septumless Cap	186004132C	186004133C	186004168C		186004112
Combination Packs					
Bonded Pre-slit Silicone/PTFE Septum					186002639
Injectable Volumes ACQUITY UPLC					
Max	1600 µL	1600 µL	1100 µL	1100 µL	210 µL
Residual	165 µL	165 µL	22 µL	22 µL	20 µL

All items come in quantities of 100 unless otherwise noted.

For Amino Acid ACQUITY UPLC systems, the use of total recovery vials is recommended but is only suitable for PEEK needles.

ACQUITY UPLC Vial Holder

Description	Part No.
48-Well Vial Holder	405003743

ACQUITY UPLC Vial Descriptions

Screw Cap 12 x 32 mm Vials for ACQUITY UPLC Systems	
1	Clear 12 x 32, Type 1, 33-Expansion Glass, Screw Neck with Quick Thread Design, (6 mm Opening, 9 mm Cap).
2	Amber 12 x 32, Type 1, 51-Expansion Glass Screw Neck with Quick Thread Design (6 mm Opening, 9 mm Cap).
3	Waters Maximum Recovery Vial, 12 x 32, Type 1, 33-Expansion Glass, Screw Neck with Quick Thread Design (6 mm Opening, 9 mm Cap).
4	Waters Amber Maximum Recovery Vial, 12 x 32, Type 1, 51-Expansion Glass, Screw Neck with Quick Thread Design (6 mm Opening, 9 mm Cap).
5	Polypropylene 12 x 32, 300 µL Screw Neck with Quick Thread Design (6 mm Opening, 9 mm Cap). Reformulate Clean PP Vial.

Settings for ACQUITY UPLC Vials

Vial Column Number*	Description	Total Height (mm)	Vial Depth (mm)	Residual Volume	Needle Placement	Comments
1,2	Screw Cap Vial	38.3	32.0	165 µL	2 mm	Preset
1,2	150 µL or 300 µL Insert Inside Vial 1 or 2	38.3	31.0	4 µL	3 mm	Advanced Settings
3,4	Max Recovery Vial	38.3	32.0	22 µL	2 mm	Preset
5	300 PP Vial	38.3	31.0	20 µL	3 mm	Advanced Settings

*Please reference the listing of Vials Descriptions on page 55 for more details.

The table above shows vial depths, residual volumes and needle placements for several products. In the ACQUITY UPLC System, the default needle placement for 48-well vial holders with a 2 mL vial (ANSI 48 vial 2 ml holder) is automatically preset and brings the needle down to a depth which is 2 mm from the bottom of the vial. The residual sample volume remaining in the vial for a 2 mm offset is recorded in the table above.

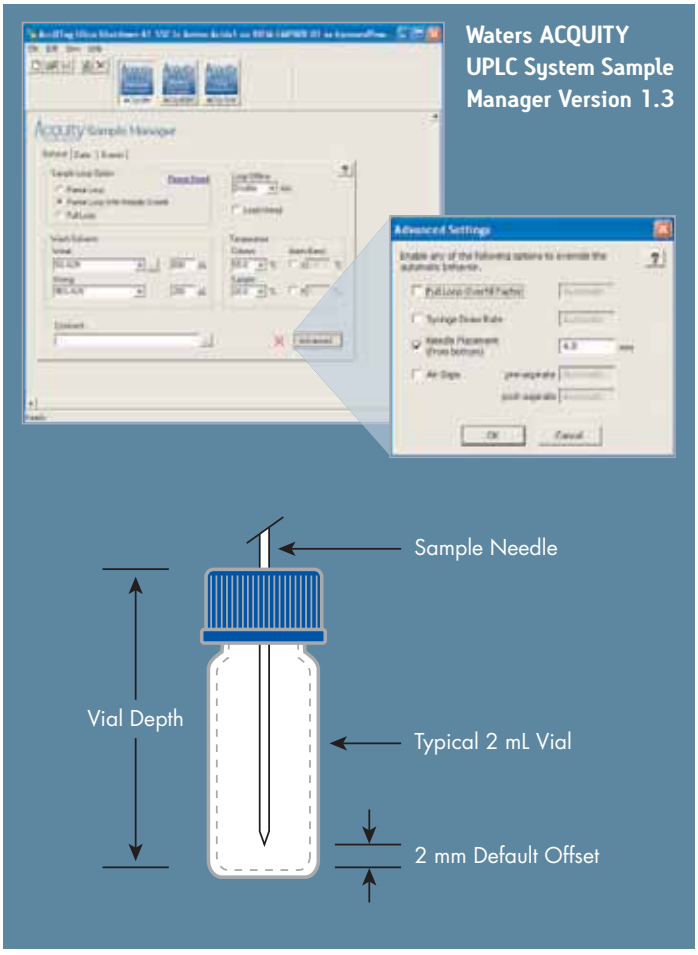
For situations where you need to access more of the sample, or if you need to keep the needle further from the bottom of the vial, go to the advanced setting screen in the Sample Manager Instrument Method Editor to change the needle placement.

Please note that you must go to the advanced settings and change the needle placement to 3 mm for vial numbers 5 and 6 in the table above. You will also have to review needle settings when using glass inserts.

Waters ACQUITY UPLC System Sample Manager

The Waters ACQUITY UPLC System Sample Manager incorporates several technology advancements. Low dispersion is maintained through the injection process, using a series of pressure transducers to facilitate self-monitoring and diagnostics. It uses needle-in-needle sampling for improved ruggedness and a needle calibration sensor for increased accuracy. A variety of sample holder formats (vials or tubes) and micro-liter plate formats (deep-well, mid-height) can also be accommodated in a thermostatically-controlled environment. Within the ACQUITY UPLC Sample Manager Instrument Method Editor, a number of parameters can be customized for your specific task, including depth, as shown here, to confer maximum sample format flexibility.

For further information on setting vial depth offsets, see the ACQUITY UPLC Operator's Guide (information documentation set for ACQUITY UPLC– part number 716001664) or visit the ACQUITY UPLC Sample Manager Instrument Method Editor On-Line Help.



Plates for ACQUITY UPLC Systems

For PEEK and Metal-Tipped Needles

	96-Well Plate			384-Well Plate	
Plate	186002643	186002481	186002482	186002632	186002631
• Well Volume	350 µL	1 mL	2 mL	250 µL	100 µL
• Number of Plates in Sample Organizer	21	10	7	10	21
• Shape	Round	Round	Square	Square	Square
• Bottom	Round	Conical	Conical	Conical	Conical
• Material	PP	PP	PP	PP	PP
• Height of Plate	14 mm	31 mm	42.5 mm	22 mm	15.5 mm
• Well Depth	11.25 mm	27 mm	39 mm	19.5 mm	12.3 mm
• Pack Size	100	50	50	50	50
• Estimated Residual Volume ACQUITY UPLC	35 µL	15 µL	20 µL	15 µL	15 µL
Seal Options					
PP, 50/pk (For Metal-Tipped Needles ONLY)	186002483	186002483	186002484		
Heat Seal (For All Needles)					
Clear Polyester, 100/pk	186002788	186002788	186002788	186002788	186002788
Aluminum Foil Laminate, 100/pk	186002789	186002789	186002789	186002789	186002789

Glass Inserts for 96-Well Plates

Description (For Metal-Tipped Needles ONLY)	Part No.	Max Volume	Residual Volume
Plates for Quick-Load Glass – Widest Opening for Inserts, 20/pk	186001438		
• 700 µL Glass – Quick-Load, 1/pk	186001437 (DV)[1]	650 µL	15 µL
• 1 mL Glass – Quick-Load, 1/pk	186001436 (DV)[1]	850 µL	15 µL
96-Well Plate with 700 µL Glass Insert, 1/pk	186000349 (DV)[1]	650 µL	15 µL
96-Well Plate with 1 mL Glass Insert, 18/pk	186000855 (DV)[1]	850 µL	15 µL
Sealing Cap for 700 µL Glass Insert – Square Well Seals Against the Well Wall PTFE/Silicone, 5/pk	186000857		
Sealing Cap for 1 mL Glass Inserts – Seals in the Glass PTFE/Silicone, 10/pk	186000856		

[1] When (DV) appears beside the part number, a deactivated version of this product can be ordered by adding DV to the right of the part number.

Quick-Load Inserts for 96-Well Plates

Quick-load packs are the easy and fast way to load your plate with glass inserts

Heat Seal for ACQUITY UPLC Systems

Heat Sealer	Part No.
115 Volt	186002786
240 Volt	186002787

Heat sealer dimensions: 5.75 x 13 x 6”
(140 x 330 x 150 mm)

Heat Sealer Operating Guidelines

For more information regarding heat sealers, download the data sheet number 720001330EN at http://www.waters.com/library

Heat Seal	Temperature Range	Solvent Range	Recommendation
Clear Polyester	From -80 °C to 80 °C	Good for Most Lab Solvents	
Aluminum Foil Laminate	From -200 °C to 90 °C	Good for Most Lab Solvents	Best for Long Term Storage

The aluminum foil laminate heat seal is good for most solvents used in laboratories. For applications requiring DMSO, the plates should be stored at 4 °C.

Position the seal with the white side facing up. Apply heat using the Waters Heat Sealer for 2 to 3 seconds in both directions following the instructions found on page 9 in the heater manual, part number: 720001330EN.

The clear polyester heat seal is a non-conductive seal. For applications requiring DMSO, the plates should be stored at 4 °C.

Position the seal with the shiny side facing up. Apply heat using the Waters heat sealer for 2 to 3 seconds in both directions following the instructions found on page 9 in the heater manual, part number: 720001330EN.

Both of these seals can be peeled off by hand. For plate storage, apply a new unpierced seal or polypropylene cap mat.

ACQUITY® TQD System featuring the TQ detector and sample organizer

Literature References

Sample Vials and Accessories Brochure, Literature Reference 720001818EN

Waters Heat Sealer User Manual, Literature Reference 720001330EN

96-well Collection Plate Options for the Waters Extraction Plate Manifold, Literature Reference 720001263EN

Waters 96 and 384-Well Collection Plate Specifications, Literature Reference WA41941

Waters LC/MS Certified Sample Vials Whitepaper, Literature Reference 720001517EN

Determination of the Level of Ion Suppression from LC/MS Vials, Literature Reference WA60004

Waters Certified Sample Vials Technical Whitepaper, Literature Reference 720001303EN

Screw Cap 12 x 32 mm Vials for Alliance Systems


Clear
6


Amber
7


Max Recovery
8


300 µL PP
9


10 mm Cap Clear
10


Total Recovery
11


Amber Max
12

LCMS Certified Combination Packs							
Vial, Cap and Silicone/PTFE Septum	600000751CV	600000752CV	600000749CV			600000750CV	600000754CV
Vial, Cap and Pre-slit Silicone/PTFE Septum	600000668CV	600000669CV	600000670CV			600000671CV	600000755CV
LCGC Certified Combination Packs							
Bonded Silicone/PTFE Septum	186000272C	186000846C	186000326C	186002640[3]	WAT270946C[2]	186000384C	186003885C
Combination Deactivated	186000272DV	186000846DV	186000326DV		WAT270946DV[2]	186000384DV	
Bonded Pre-slit Silicone/PTFE Septum	186000307C	186000847C	186000327C	186002639[3]		186000385C	186003886C
Combination Deactivated	186000307DV	186000847DV	186000327DV			186000385DV	
Combination with PE Septum-less Cap	186004132C	186004133C	186004168C	186004112		186004167C	
Vials Only							
Vials Only	186000273	186000848	186002802	186002626	WAT063300	186002805	
Deactivated Vials Only	186000273DV	186000848DV			WAT063300DV		
Injectable Volumes Alliance 2690/2695							
Max	1100 µL	1100 µL		280 µL	1100 µL	950 µL	
Residual	750 µL	750 µL		20 µL	750 µL	9 µL	
Injectable Volumes Alliance 2790/2795							
Max	1700 µL	1700 µL	1500 µL	290 µL	1700 µL		1500 µL
Residual	170 µL	170 µL	22 µL	10 µL	170 µL		22 µL
Inserts							
300 µL with Poly Spring	WAT094170(DV)[1]	WAT094170 (DV)[1]			WAT094170 (DV)[1]		
Max Volume Injection/Max Residual Volume	230 µL/20 µL	230 µL/20 µL			230 µL/20 µL		
150 µL with Poly Spring	WAT094171 (DV)[1]	WAT094171 (DV)[1]			WAT094171 (DV)[1]		
Max Volume Injection/Max Residual Volume	144 µL/6 µL	144 µL/6 µL			144 µL/6 µL		
Cap and Septum							
PE Septumless Caps	186004169	186004169	186004169	186004169		186004169	186004169
Cap Black					WAT058875		
Septa Silicone/PTFE					WAT058874		
Screw Cap and Septum – Silicone/PTFE							
PE Septum-less Cap	186004169	186004169	186004169	186004169		186004169	
Blue LectraBond	186000274	186000274	186000274	186000274		186000274	
Red LectraBond	186002129	186002129	186002129	186002129		186002129	
Green LectraBond	186002130	186002130	186002130	186002130		186002130	
White LectraBond	186002456	186002456	186002456	186002456		186002456	
Screw Cap and Pre-slit Septum – Silicone/PTFE							
Blue LectraBond	186000305	186000305	186000305	186000305		186000305	
Red LectraBond	186002128	186002128	186002128	186002128		186002128	
Green LectraBond	186002127	186002127	186002127	186002127		186002127	
White LectraBond	186002457	186002457	186002457	186002457		186002457	
For Dissolution System							
Pre-assembled Vial, Cap and Pre-slit Septum	186000989(DV)[1]	186003455					

Compatible Systems							
Alliance 2690/2695	•	•		•	•	•	
Alliance 2790/2795	•	•	•	•	•		•

All items come in quantities of 100 unless otherwise noted. [1] When (DV) appears beside the part number, a deactivated version of this product can be ordered by adding DV to the right of the part number. [2] Septum not bonded. [3] Vials not certified.

Snap Cap 12 x 32 mm Vials for Alliance Systems

Clear Glass	Amber	Max Recovery	PP 300 µL	Clear Glass Crimp	Amber Crimp	Total Recovery
13	14	15	16	17	18	19

Combination Packs							
Vial, Cap and Silicone/PTFE Septum				186002642			186000234(DV)[1]
Vial, Cap and Pre-slit Silicone/PTFE Septum				186002641			
Vials							
Vials Only	WAT094219	WAT094220	186000984	186002628	WAT094222	WAT094223	186000302
Deactivated Vials Only	WAT094219DV	WAT094220DV	186000984DV		WAT094222DV	WAT094223DV	186000302DV
Injectable Volumes Alliance 2690/2695							
Max	1100 µL	1100 µL		280 µL	1100 µL	1100 µL	950 µL
Residual	750 µL	750 µL		20 µL	750 µL	750 µL	9 µL
Injectable Volumes Alliance 2790/2795							
Max	1700 µL	1700 µL	1500 µL	290 µL	1700 µL	1700 µL	
Residual	170 µL	170 µL	22 µL	10 µL	170 µL	170 µL	
Inserts							
300 µL with Poly Spring	WAT094170(DV)[1]	WAT094170 (DV)[1]			WAT094170 (DV)[1]	WAT094170 (DV)[1]	
Max Volume Injection/Max Residual Volume	230 µL/20 µL	230 µL/20 µL			230 µL/20 µL	230 µL/20 µL	
150 µL with Poly Spring	WAT094171 (DV)[1]	WAT094171 (DV)[1]			WAT094171 (DV)[1]	WAT094171 (DV)[1]	
Max Volume Injection/Max Residual Volume	144 µL/6 µL	144 µL/6 µL			144 µL/6 µL	144 µL/6 µL	
Snap Cap and Septum – Silicone/PTFE							
Blue	186000303	186000303	186000303	186000303			186000303
Black	186002649	186002649	186002649	186002649			186002649
Red	186002650	186002650	186002650	186002650			186002650
Snap Cap and Pre-slit Septum – Silicone/PTFE							
Blue	186000304	186000304	186000304	186000304			186000304
Black	186002648	186002648	186002648	186002648			186002648
Red	186002647	186002647	186002647	186002647			186002647
Snap Cap and PTFE Septum							
Blue	186000328	186000328	186000328	186000328			186000328
Black	186002645	186002645	186002645	186002645			186002645
Red	186002646	186002646	186002646	186002646			186002646
Crimp Cap							
Crimp Cap Silicone/PTFE Septum					PSL404219	PSL404219	
Crimp Cap PTFE/Silicone/PTFE Septum					PSL404231	PSL404231	
Crimper					PSL904301	PSL904301	

Compatible Systems							
Alliance 2690/2695	•	•		•	•	•	•
Alliance 2790/2795	•	•	•	•	•	•	

All items come in quantities of 100 unless otherwise noted.

[1] When (DV) appears beside the part number, a deactivated version of this product can be ordered by adding DV to the right of the part number.

Holder for 12 x 32 mm Vials

Description	Part No.
Holder for12 x 32 mm Vials (5/pk)	186004487

(Vials not included)

Plates for Alliance HT Systems

	96-Well Plate						384-Well Plate	
Plate	186002643	186002481	186002482	WAT058958	WAT058957	WAT058943	186002632	186002631
Well Volume	350 µL	1 mL	2 mL	2 mL	1 mL	350 µL	250 µL	100 µL
Shape	Round	Round	Square	Square	Square	Square	Square	Square
Bottom	Round	Conical	Conical	Pyramid	Pyramid	Pyramid	Conical	Conical
Material	PP	PP	PP	PP	PP	PP	PP	PP
Height of Plate	14 mm	31 mm	42.5 mm	44.4 mm	44.4 mm	44.4 mm	22 mm	15.5 mm
Well Depth	11.25 mm	27 mm	39 mm	39.9 mm	24.5 mm	15.3 mm	19.5 mm	12.3 mm
Pack Size	100	50	50	50	50	50	50	50
Estimated Residual Volume 2795 or ACQUITY	35 µL	15 µL	20 µL				15 µL	15 µL
Cap Mats								
PP, 50/pk	186002483	186002483	186002484					
PE, 50/pk				WAT058959	WAT058959	WAT058959		
Silicone/PTFE 5/pk				186000857	186000857	186000857		
Heat Seal								
Clear Polyester, 100/pk	186002788	186002788	186002788	186002788	186002788	186002788	186002788	186002788
Aluminum Foil Laminate, 100/pk	186002789	186002789	186002789	186002789	186002789	186002789	186002789	186002789

Heat Sealer

Description	Part No.
115 Volt	186002786
240 Volt	186002787

Roller for Cap Mats

Description	Part No.
Roller for Mats	186002633

Glass Inserts for 96-Well Plates

Description	Part No.
Plates for Quick-Load Glass – Widest Opening for Inserts, 20/pk	186001438
• 700 µL Glass – Quick-Load, 1/pk	186001437(DV)[1]
• 1 mL Glass – Quick-Load, 1/pk	186001436(DV)[1]
96-Well Plate with 700 µL Glass Insert, 1/pk	186000349(DV)[1]
96-Well Plate with 1 mL Glass Insert, 18/pk	186000855(DV)[1]
Sealing Cap for 700 µL Glass Square Well PTFE/Silicone, 5/pk	186000857
Sealing Cap for 1 mL Round Well – Seals in the Glass PTFE/Silicone, 10/pk	186000856

[1] When (DV) appears beside the part number, a deactivated version of this product can be ordered by adding DV to the right of the part number.

Selection of plates and cap mats

Roller for cap mats

Heat sealer

Vials for Waters 717 Autosampler

15 x 45 mm Vials

48 Position Carousel


4 mL Screw Neck
20


Amber Screw Neck
21


Total Recovery
22


PP Screw Neck Vial
23


PP Conical
24


Glass Shell Vial
25


Amber Glass Shell Vial
26

Combination Packs							
Vial, Cap and LectraBond PTFE/Silicone Septum	186000838C	186001133C	186002629C				
Combination Deactivated	186000838DV	186001133DV					
Vial, Cap and LectraBond Pre-slit PTFE/Silicone Septum	186000839C	186001134C	186002630C				
Combination Deactivated	186000839DV	186001134DV					
Vial and PE Snap Cap					186004031	WAT025051	WAT025050
Components							
Vials Only	186000840(DV)[1]	186001135(DV)[1]	186002520	186000999[4]			
Max Volume Injection/Max Residual Volume	2400 µL/1600 µL	2400 µL/1600 µL	3000 µL/40 µL	2000 µL/1000 µL	2950 µL/50 µL	2400 µL/1600 µL	2400 µL/1600 µL
Cap LectraBond PTFE/Silicone 100/pk	186000841	186000841	186000841	186000841			
Screw Cap with Bonded PTFE/Silicone Septum 1000/pk	186000965	186000965	186000965	186000965			
Cap LectraBond Pre-slit PTFE/Silicone 100/pk	186000842	186000842	186000842	186000842			
Black Phenol Cap 144/pk	WAT072711	WAT072711	WAT072711	WAT072711			
PTFE Septum 1440/pk	WAT073005	WAT073005	WAT073005	WAT073005			
PTFE Septum 144/pk	WAT072714	WAT072714	WAT072714	WAT072714			
Self Sealing Septum 144/pk	WAT022861	WAT022861	WAT022861	WAT022861			
250 µL Glass Insert[5]	WAT072704(DV)[1]	WAT072704(DV)[1]		WAT072704			
Max Volume Injection/Max Residual Volume	244 µL/6 µL	244 µL/6 µL					
250 µL Glass Insert 144/pk[5]	WAT015199(DV)[1]	WAT015199(DV)[1]					
Max Volume Injection/Max Residual Volume	230 µL/20 µL	230 µL/20 µL					
250 µL Plastic Conical Insert (PMP) 144/pk[5]	WAT072030	WAT072030					
Max Volume Injection/Max Residual Volume	230 µL/20 µL	230 µL/20 µL					
Springs for LVI 100/pk	WAT072708	WAT072708					
250 µL PP Insert 1000/pk[5]	186001729	186001729					

8 x 40 mm Vials

96 Position Carousel


1 mL Shell
27


Amber
28


Total Recovery
29


PP Conical
30

Vials for 96 Position Carousel				
Shell Vial and Snap Cap	WAT025054C	WAT025053C	186000837C	WAT022476[3]
Shell Vial and Snap Cap Deactivated	WAT025054DV	WAT025053DV	186000837DV	
Pack Size	250	250	100	100
Max Volume Injection/Max Residual Volume	600 µL/400 µL	600 µL/400 µL	700 µL/6 µL	650 µL/50 µL
150 µL Glass Insert	WAT072294(DV)[1]	WAT072294(DV)[1]		
Spring for LVI	WAT072289	WAT072289		
Max Volume Injection/Max Residual Volume	144 µL/6 µL	144 µL/6 µL		
PE Snap Cap 1000/pk	WAT078515	WAT078515	WAT078515	WAT078515
200 µL PE Grad Insert with Poly Spring 1000/pk	186001728	186001728		
1 mL Shell Vial Assembled 500/pk for Dissolution System	WAT022479			

All items come in quantities of 100 unless otherwise noted.

[1] When (DV) appears beside the part number, a deactivated version of this product can be ordered by adding DV to the right of the part number.

[3] Vials not certified.

[4] 1000/pk

[5] Inserts requires springs (Part No. WAT072708)

Screw Cap 12 x 32 mm Vials for Compatible Systems

31 32 33 34 35 36 37 38 39

LCMS Certified Combination Packs									
Vial, Cap and Silicone/PTFE Septum	600000751CV	600000752CV	600000754CV	600000749CV					
Vial, Cap and Pre-slit Silicone/PTFE Septum	600000668CV	600000669CV	600000755CV	600000670CV					
LCGC Certified Combination Packs									
Bonded Silicone/PTFE Septum	186000272C	186000846C	186003885C	186000326C	186001126C	186001130C		WAT270946C[2]	
Combination Deactivated	186000272DV	186000846DV		186000326DV	186001126DV	186001130DV		WAT270946DV[2]	
Bonded Pre-slit Silicone/PTFE Septum	186000307C	186000847C	186003886C	186000327C	186001128C	186001131C			
Combination Deactivated	186000307DV	186000847DV		186000327DV	186001128DV	186001131DV			
LCGC Combination Packs									
Bonded Silicone/PTFE Septum							186002640		
Bonded Pre-slit Silicone/PTFE Septum							186002639		
Vials Only									
Vials Only	186000273	186000848		186002802	186002804	186002803	186002626	WAT063300	WAT094172
Deactivated Vials Only	186000273DV	186000848DV						WAT063300DV	
Inserts									
300 µL with Poly Spring	WAT094170	WAT094170						WAT094170	
300 µL with Poly Spring Deactivated	WAT094170DV	WAT094170DV						WAT094170DV	
150 µL with Poly Spring	WAT094171	WAT094171						WAT094171	
150 µL with Poly Spring Deactivated	WAT094171DV	WAT094171DV						WAT094171DV	
Cap and Septum									
PE Septumless Caps	186004169	186004169	186004169	186004169	186004169	186004169	186004169		
Black Cap								WAT058875	WAT210684
Cap and Septum, Silicone/PTFE, Assembled									WAT094174
Septum Only, PTFE/Silicone, Pre-slit									WAT058876
Septum Only, Silicone/PTFE								WAT058874	WAT210685
Septum Only, PTFE									WAT058886
Screw Cap and Septum – Silicone/PTFE									
Blue LectraBond	186000274	186000274		186000274	186000274	186000274	186000274		
Red LectraBond	186002129	186002129		186002129	186002129	186002129	186002129		
Green LectraBond	186002130	186002130		186002130	186002130	186002130	186002130		
Screw Cap and Pre-slit Septum – Silicone/PTFE									
Blue LectraBond	186000305	186000305		186000305	186000305	186000305	186000305		
Red LectraBond	186002128	186002128		186002128	186002128	186002128	186002128		
Green LectraBond	186002127	186002127		186002127	186002127	186002127	186002127		

Compatible Systems									
Agilent Technologies	•	•		•	•	•	•		
Alcott, Antek, CTC, Spark, Thermal Separations								•	•
Beckman, Dynatech, Finnigan, Fisons, Gilson	•	•		•	•	•	•		
Hitachi, LDC, Perkin-Elmer, Shimadzu, Spectra-Physics, Thermo, Varian	•	•		•	•	•	•	•	•

All items come in quantities of 100 unless otherwise noted.

[2] Septum not bonded.

Snap and Crimp Cap 12 x 32 mm (9 mm Cap) Vials for Compatible Systems

Clear	Amber	Max Recovery	Qsert Vial	PP 300 µL	Clear Crimp	Amber Crimp
						
40	41	42	43	44	45	46

Combination Packs							
Vial, Cap and Silicone/PTFE Septum				186001124(DV)[1]	186002642		
Vial, Cap and Pre-Slit Silicone/PTFE Septum				186001125(DV)[1]	186002641		
Vial, Cap and PTFE Septum				186001127(DV)[1]			
Vials Only							
Vials Only	WAT094219	WAT094220	186000984		186002628	WAT094222	WAT094223
Deactivated Vials Only	WAT094219DV	WAT094220DV	186000984DV			WAT094222DV	WAT094223DV
Inserts							
300 µL with Poly Spring	WAT094170(DV)[1]	WAT094170(DV)[1]				WAT094170(DV)[1]	WAT094170(DV)[1]
150 µL with Poly Spring	WAT094171(DV)[1]	WAT094171(DV)[1]				WAT094171(DV)[1]	WAT094171(DV)[1]
Snap Cap and Septum – Silicone/PTFE							
Blue	186000303	186000303	186000303	186000303	186000303		
Black	186002649	186002649	186002649	186002649	186002649		
Red	186002650	186002650	186002650	186002650	186002650		
Snap Cap and Pre-slit Septum – Silicone/PTFE							
Blue	186000304	186000304	186000304	186000304	186000304		
Black	186002648	186002648	186002648	186002648	186002648		
Red	186002647	186002647	186002647	186002647	186002647		
Snap Cap and PTFE Septum							
Blue	186000328	186000328	186000328	186000328	186000328		
Black	186002645	186002645	186002645	186002645	186002645		
Red	186002646	186002646	186002646	186002646	186002646		
Crimp Cap							
Crimp Cap Silicone/PTFE Septum						PSL404219	PSL404219
Crimp Cap PTFE/Silicone/PTFE Septum						PSL404231	PSL404231
Crimper						PSL904301	PSL904301

Compatible Systems							
Agilent Technologies, Beckman, Dynatech, Finnigan, Fisons, Gilson, Hitachi, LDC, Perkin-Elmer, Shimadzu, Spectra-Physics, Varian	•	•	•	•	•	•	•
CTC, Spark, Thermal Separations						•	•

All items come in quantities of 100 unless otherwise noted.

[1] When (DV) appears beside the part number, a deactivated version of this product can be ordered by adding DV to the right of the part number.

Holder for 12 x 32 mm Vials

Description	Part No.
Holder for12 x 32 mm Vials (5/pk)	186004487

(Vials not included)

15 x 45 mm Vials for Compatible Systems

15 x 45 mm Vials and Accessories

4 mL Screw Neck	Amber Screw Neck	Total Recovery	PP Screw Neck Vial	PP Snap Cap	Glass Shell Vial	Amber Glass Shell Vial
						
47	48	49	50	51	52	53

Combination Pack							
Vial, Cap and LectraBond PTFE/Silicone Septum	186000838C	186001133C	186002629C				
Combination Deactivated	186000838DV	186001133DV					
Vial, Cap and LectraBond Pre-slit PTFE/Silicone Septum	186000839C	186001134C	186002630C				
Combination Deactivated	186000839DV	186001134DV					
Vial and PE Snap Cap					186004031	WAT025051	WAT025050
Components							
Vials Only	186000840	186001135	186002520	186000999[4]			
Deactivated Vials Only	186000840DV	186001135DV					
LectraBond Cap and Septum							
Black Cap PTFE/Silicone 100/pk	186000841	186000841	186000841				
Screw Cap with Bonded PTFE/Silicone Septum 1000/pk	186000965	186000965	186000965	186000965			
Black Cap Pre-slit PTFE/Silicone 100/pk	186000842	186000842	186000842				
Caps, Septa, and Inserts							
Black Phenol Cap 144/pk	WAT072711	WAT072711	WAT072711				
PTFE Septum 144/pk	WAT073005	WAT073005	WAT073005				
PTFE Septum 144/pk	WAT072714	WAT072714	WAT072714				
Self Sealing Septum 144/pk	WAT022861	WAT022861	WAT022861				
250 µL Glass Insert	WAT072704	WAT072704	WAT072704				
250 µL Glass Insert Deactivated	WAT072704DV	WAT072704DV	WAT072704DV				
250 µL Glass Insert 144/pk	WAT015199	WAT015199	WAT015199				
250 µL Glass Insert 144/pk Deactivated	WAT015199DV	WAT015199DV	WAT015199DV				
250 µL Plastic Conical Insert (PMP) 144/pk	WAT072030	WAT072030	WAT072030				
Springs for LVI 100/pk	WAT072708	WAT072708	WAT072708				
250 µL PP Insert 100/pk	186001729	186001729	186001729				

Compatible Systems							
Bruker, Kontron, Perkin-Elmer, Shimadzu, Tosoh, Unicam	•	•	•	•	•	•	•

[4] Item contains 1,000 vials

GPC 2000 Vials

4 mL Screw Cap	10 mL Screw Neck
	
54	55

Components		
Vial	186000840	186001420
Black Screw Cap	600000162	186001421
PTFE Septum	WAT072714[6]	186001422
Aluminum Crimp Cap, Aluminum/PFTE/Silicone		

Aqua Analysis System Vials

Components	
22 mL Vial with pre-slit silicone/PTFE septum, 100/pk	186004108
Solid Cap, PTFE/silicone liner, 100/pk. Solid cap for shipping water samples.	186004109
Mailing Box dor 22 mL vials, 100/pk. Box for shipping samples to lab.	186004111

Settings for the Alliance 2690 and 2695 Vials and Low Volume Inserts (LVI)

The Waters 2690 Separations Module is set initially to accept vials with a bottom thickness <1.6 mm. Any vial that does not meet this criteria should not be used without first adding a positive needle offset to the "sample draw depth." Failure to do so can cause vial breakage or needle damage.

Vial Column Number*	Description	Average Thickness	Needle Offset	Notes*	Comments
6	Screw Cap Glass Vial	0.037" (0.93 mm)	0	1,3	Add at least 1 mm offset when used with LVI
7	Screw Cap Glass Vial	0.037" (0.93 mm)	0	1,3	Add at least 1 mm offset when used with LVI
9	Polypropylene Screw Neck Vial (300 µL)	0.037" (0.93 mm)	1 mm		
16	Polypropylene Snap Cap Vial (300 µL)	0.037" (0.93 mm)	1 mm		
11	Total Recovery Vial	0.037" (0.93 mm)	0		
19	Total Recovery Vial	0.037" (0.93 mm)	0		
10	Screw Cap Glass Vial	0.037" (0.93 mm)	0	1,3	Add at least 1 mm offset when used with LVI
13	Snap Cap Glass Vial	0.063" (1.59 mm)	0	1,3	Add at least 1 mm offset when used with LVI
17	Crimp Cap Vial	0.068" (1.72 mm)	1 mm	2,3	Variable thickness; Add at least 1 mm offset when used with LVI
39	Screw Cap 'V' Vial	0.058" (1.46 mm)	0	1	Low volume (250 µL) vial
13	Low Volume Insert (300 µL)	0.024" (0.61 mm)	–	4	Use with vials with neck opening, 6 mm
17	Low Volume Insert (150 µL)	0.028" (0.71 mm)	–	4	Use with vials with neck opening, 6 mm
6 10	LVI (300 µL) in Screw Cap Vial (7 mm neck)	0.062" (1.57 mm)	0 –	1	Recommended configuration for this LVI
6 10	LVI (150 µL) in Screw Cap Vial (7 mm neck)	0.065" (1.65 mm)	1 mm –	1	Recommended configuration—has the lowest sample volume requirement
17 18	LVI (300 µL) in Snap/Crimp Vial (6 mm neck)	0.090" (2.27 mm)	1 mm –	2	Variable thickness. Add at least 1 mm offset
18 18	LVI (150 µL) in Snap/Crimp Vial (6 mm neck)	0.091" (2.30 mm)	1 mm –	2	Variable thickness. Add at least 1 mm offset
18	Snap/Crimp Vial	0.068" (1.72 mm)	1 mm	2,3	Variable thickness; Add at least 1 mm offset when used with LVI
13	Snap Cap Vial	0.063" (1.59 mm)	0	1,3	Add at least 1 mm offset when used with LVI

Please reference the Listing of Vials Descriptions on page 55 for more details.

***Notes:**

1. Clears needle tip—no offset required. Meets the criteria of a bottom thickness < 1.6 mm.
2. Does not clear needle tip—positive needle offset should be used.
3. Designed to accept Waters low volume inserts (LVI's)—at least 1 mm offset should be added.
4. This dimension should be added to vial bottom thickness and result checked against the criteria for bottom thickness.

Literature References

Sample Vials & Accessories Brochure, Literature Reference 720001818EN

Waters LC/MS Certified Sample Vials Whitepaper, Literature Reference 720001517EN

Determination of the Level of Ion Suppression from LC/MS Vials, Literature Reference WA60004

Waters Certified Sample Vials Technical Whitepaper, Literature Reference 720001303EN

Vial Descriptions

Vials for Alliance Systems

Screw Cap 12 x 32 mm Vials for Alliance Systems	
6	Clear 12 x 32, Type 1, 33-Expansion Glass, Screw Neck with Quick Thread Design (6 mm Opening, 9 mm Cap).
7	Amber 12 x 32, Type 1, 51-Expansion Glass Screw Neck with Quick Thread Design (6 mm Opening, 9 mm Cap).
8	Clear Maximum Recovery Vial 12 x 32, Type 1 33-Expansion Glass, Screw Neck with Quick Thread Design (6 mm Opening, 9 mm Cap).
9	Polypropylene 12 x 32, 300 µL Screw Neck with Quick Thread Design (6 mm Opening, 9 mm Cap). Reformulate Clean PP Vial.
10	Clear 12 x 32, Type 1, 33-Expansion Glass, Screw Neck (6 mm Opening, 10 mm Cap).
11	Total Recovery Vial Clear 12 x 32, Type 1, 33-Expansion Glass, Screw Neck with Quick Thread Design (6 mm Opening, 9 mm Cap).
12	Amber Maximum Recovery Vial, 12 x 32, Type 1 51-Expansion Glass, Screw Neck with Quick Thread Design (6 mm Opening, 9 mm Cap).

Snap Cap 12 x 32 mm Vials for Alliance Systems	
13	Clear 12 x 32, Type 1, 33-Expansion Glass, Snap Cap Vial (6 mm Opening, 9 mm Cap).
14	Amber 12 x 32, Type 1, 51-Expansion Glass Snap Cap Vial (6 mm Ppening, 9 mm Cap).
15	Clear Maximum Recovery Vial 12 x 32, Type 1, 33-Expansion Glass, Snap Cap Vial (6 mm Opening, 9 mm Cap).
16	Polypropylene 12 x 32, 300 µL Snap Cap Vial (6 mm Opening, 9 mm Cap). Reformulate Clean PP Vial.
17	Clear 12 x 32, Type 1, 33-Expansion Glass, Crimp Top Vial (6 mm Opening, 12 mm Cap).
18	Amber 12 x 32, Type 1, 51-Expansion Glass, Crimp Top Vial (6 mm Opening, 12 mm Cap).
19	Total Recovery Vial Clear 12 x 32, Type 1, 33-Expansion Glass, Snap Cap Vial (6 mm Opening, 9 mm Cap).

Vials for Waters 717 Autosampler: 15 x 45 mm Vials	
20	Clear 15 x 45, Type 1, 33-Expansion Glass, Screw Neck Vial.
21	Amber 15 x 45, Type 1, 51-Expansion Glass, Screw Neck Vial.
22	Total Recovery Screw Neck Vial Clear Glass 15 x 45, Type 1, 33-Expansion Glass.
23	Polypropylene 15 x 45, 3 mL Round Bottom Screw Neck Vial 1,000/pk.
24	Polypropylene Snap Cap Vial with Conical Bottom, PE Snap Caps.
25	4 mL Glass Shell Vial with Polyethylene Snap Cap, Type 1, 51-Expansion Glass.
26	4 mL Amber Shell Vial with Polyethylene Snap Cap, Type 1, 51-Expansion Glass.

Vials for Waters 717 Autosampler: 8 x 40 mm Vials	
27	1 mL Clear Glass Shell Vial (8 x 40 mm) Type 1, 51-Expansion Glass with Polyethylene Snap Cap 250/pk.
28	1 mL Amber Glass Shell Vial (8 x 40 mm) Type 1, 51-Expansion Glass with Polyethylene Snap Cap, Type 1, 250/pk.
29	Total Recovery Clear Glass Vial (8 x 40 mm) with Polyethylene Snap Cap, Type 1, 51-Expansion Glass.
30	700 µL Polypropylene Vial (8 x 40 mm) with Polyethylene Snap Cap.

Vials for Compatible Systems

Screw Cap 12 x 32 mm Vials for Compatible Systems	
31	Clear 12 x 32, Type 1, 33-Expansion Glass, Screw Neck with Quick Thread Design (6 mm Opening, 9 mm Cap).
32	Amber 12 x 32, Type 1, 51-Expansion Glass Screw Neck with Quick Thread Design (6 mm Opening, 9 mm Cap).
33	Amber Maximum Recovery Vial 12 x 32, Type 1, 51-Expansion Glass, Screw Neck with Quick Thread Design (6 mm Opening, 9 mm Cap).
34	Clear Maximum Recovery Vial 12 x 32, Type 1, 33-Expansion Glass, Screw Neck with Quick Thread Design (6 mm Opening, 9 mm Cap).
35	Qsert Vial Clear Screw Cap Glass Vial, Quick Thread Design with Fused in Glass Insert (6 mm Opening, 9 mm Cap).
36	Qsert Vial Amber Screw Cap Glass Vial, Quick Thread Design with Fused in Glass Insert (6 mm Opening, 9 mm Cap).
37	Polypropylene 12 x 32, 300 µL Screw Neck with Quick Thread Design (6 mm Opening, 9 mm Cap). Reformulate Clean PP Vial.
38	Clear 12 x 32, Type 1, 33-Expansion Glass, Screw Neck (6 mm Opening, 10 mm Cap).
39	Polypropylene 12 x 32, 250 µL Screw Neck Vial (6 mm Opening, 8 mm Cap).

Snap and Crimp Cap 12 x 32 mm (9 mm Cap) Vials for Compatible Systems	
40	Clear 12 x 32, Type 1, 33-Expansion Glass, Snap Cap Vial (6 mm Opening, 9 mm Cap).
41	Amber 12 x 32, Type 1, 51-Expansion Glass Snap Cap Vial (6 mm Opening, 9 mm Cap).
42	Maximum Recovery Vial 12 x 32, Type 1, 33-Expansion Glass, Snap Cap Vial 6 mm Opening, 9 mm Cap).
43	Qsert Vial Clear Snap Cap Glass Vial with Fused in Glass Insert (6 mm Opening, 9 mm Cap).
44	Polypropylene 12 x 32, 300 µL Snap Cap Vial (6 mm Opening, 9 mm Cap). Reformulate Clean PP Vial.
45	Clear 12 x 32, Type 1, 33-Expansion Glass, Crimp Top Vial (6 mm Opening, 12 mm Cap).
46	Amber 12 x 32, Type 1, 51-Expansion Glass, Crimp Top Vial (6 mm Opening, 12 mm Cap).

15 x 45 mm Vials for Compatible Systems: Vials and Accessories	
47	Clear 15 x 45, Type 1, 33-Expansion Glass, Screw Neck Vial.
48	Amber 15 x 45, Type 1, 51-Expansion Glass, Screw Neck Vial.
49	Waters Total Recovery Screw Neck Vial Clear Glass 15 x 45, Type 1, 33-Expansion Glass.
50	Polypropylene 15 x 45, 3 mL Screw Neck Vial 1,000/pk.
51	Polypropylene Snap Cap Vial with Conical Bottom, PE Snap Caps.
52	4 mL Glass Shell Vial with Polyethylene Snap Cap, Type 1, 51-Expansion Glass.
53	4 mL Amber Shell Vial with Polyethylene Snap Cap, Type 1, 51-Expansion Glass.

15 x 45 mm Vials for Compatible Systems: GPC 2000 Vials	
54	4 mL Glass Screw Neck Vial, Type 1, 33-Expansion Glass for GPC 2000.
55	10 mL Screw Neck Glass Vial for GPC 2000.

Vials Troubleshooting Guide

Waters offers solutions that help eliminate common problems that conventional sample vials have been known to cause in the laboratories.

Problem	Impact	Solution
Septum dislodged during shipment or use	• Need to insert septum or need to rerun analysis • Loss of time	• Check to see if needle is piercing in center of septa. • Check to see if needle is sharp.
Vacuum forms in vial during sample draw	• Sample spill over • Sample draw reproducibility problems	• Use pre-slit septa which provides proper venting, eliminating sample spill over and insuring reproducible sample draw volumes.
Sample-limited applications require the use of cumbersome low-volume inserts	• Increased labor required for inserting the LVI into the vial leads to delays in sample processing • Increased labor time and difficulty when pipetting into small neck opening of LVI • Additional handling increases chance of contamination • Increased costs from purchasing multiple components: vial, cap and LVI	• Use Waters Total Recovery vial and Maximum Recovery vial: - No need to use LVIs. - Wide neck opening for easy sample pipetting. - One less handling step reduces chance of contamination. - Only need one component, saving storage space and costs.
Need to perform multiple injections with minimum residual volume in each vial requires LVI to obtain minimum residual volume, but maximum capacity is only 300 µL	• Increased labor to fill additional sample vials • Increased cost to purchase additional sample vials and LVIs	• Use Waters Total Recovery vial and Maximum Recovery vial: The increased capacity and low residual volume allows you to perform multiple injections with minimum residual volume in a single vial.
Need to use glass inserts in a 96-well plate format but it requires capping each insert one-at-a-time	• Delay in sample processing	• The glass inserts in the Waters 96-well format allows for the use of a sealing cap mat, saving time and labor.
Frequent needle damage	• Downtime causing missed deadlines • Cost of repairs	• All Waters vials have dimensional specifications that eliminate the potential of needle damage.
Laboratory owns HPLC instruments from several different manufacturers	• Purchasing several different vials • Increased number of purchase orders • Unable to take advantage of quantity discounts, leading to higher costs	• The tight dimensional tolerances on all Waters vials and accessories make them ideal for use with virtually all HPLC systems. • Reduce the number of purchase orders and take advantage of quantity discounts by buying all your sample vials from Waters.
Analyte compounds are sticking to the glass surface of the vial	• Loss of sample • Loss of time • Need to run the analysis again	• Deactivated glass vials and inserts: Waters uses a gas phase deactivation process that renders the glass surface inert. Unlike other deactivated vials, the surface modification is permanent, resulting in an indefinite shelf life.
Inconsistent quality between laboratory sites		• Waters vials are distributed world wide from the same source.

Beware of Poor Quality Look-alike Vials

- Only Waters Alliance Total Recovery vials and Maximum Recovery vials utilize a proprietary manufacturing process, ensuring that the slope of the internal taper will deliver all of the sample to the bottom of the vial.
- The bottom thickness is held to a close tolerance eliminating needle damage caused by bottoming out.

Waters is involved in the entire sample analysis process from sample preparation to reliable analytical results.

We understand the importance of quality, reliable vials.

Avoid ghost peaks, dislodged septa or damaged needles which would compromise your test results. Work with confidence by choosing Waters Certified Vials.

Learn more at **www.waters.com/vials**

[CERTIFIED]

CERTIFIED VIALS FOR HPLC AND LC/MS

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Filtration

Waters Sirocco Protein Precipitation Plate

Sirocco™
Protein Precipitation Plate

- State-of-the-art design for maximum analyte recovery
- Unique valve design prevents flow until you are ready to process
- Eliminates time-consuming centrifugation and pipetting steps
- Design ensures no sample cross talk
- Maximize mass spec uptime by eliminating cloudy filtrates
- Low extractables, no interferences in UV or ion suppression

Waters, in collaboration with Pall Life Sciences, is pleased to introduce the most technologically-advanced protein precipitation plate on the market. The Sirocco™ Protein Precipitation (PPT) Plate enables high-throughput "in-well" protein precipitation of biological samples without the fear of cloudy filtrates, clogged devices, cross-talk, or leaking during use. This is due to a unique filter system, sealing cap mat and patented valve technology. It provides optimum performance in faster processing time by reducing steps in 'in-well' sample processing and complete recovery of clean filtrate from smaller plasma sample volumes.

Sirocco Patented Plate Technology

This patented plate technology includes:

- Valve mat on the tips of the wells to prevent flow or drips from the plate until vacuum is applied
- Gradient of purpose-selected filtration media for clean, precipitate free filtration
- Vented cap mat for processing during mixing and vacuuming

The plate was validated for performance using a variety of polar and non-polar acids, bases, and neutral compounds spiked into porcine and rat plasmas looking for high recoveries. The plates are highly reproducible and linear, with the analytes examined having >95% recovery.

All materials used in the Sirocco plate were chosen after careful and intensive evaluation for:

- Ion suppression
- Ion enhancement
- UV extractables
- MS extractables
- Recovery of analytes

Typical Plasma Precipitation

1. Place the Sirocco PPT Plate on a collection plate, this suspends the valve mat tips in the center of the collection plate wells during processing.
2. Add crash solvent to the wells.
3. Add plasma.
4. Apply the vented cap mat.
5. Vortex the filter plate/collection plate stack.
6. Filter on a vacuum manifold at 8-10" Hg or centrafuge.

Typical processing of 96 samples takes less than 15 minutes. Note, there is no need to remove the cap or valve mat. The slits in the valve tips are designed to open under several inches of Hg vacuum. The cap mat is pre-slit to allow processing steps of vortexing and vacuum filtration. The entire Sirocco plate assembly is disposed of after the vacuum filtration step. It is designed for 'in-well' sample preparation—no mat removal is required; therefore, the risk of cross-talk is reduced or eliminated, as compared to other commercially available plates.

Specification

The Sirocco plate has 1 ml sample wells. With the cap mat applied, the combined plasma/crash solvent volume should not exceed 600 µL. This allows enough volume above the sample to ensure complete mixing during the vortex step. The package contains plates assembled with a valve mat, and pre-slit cap mats. There are no other parts required for the 'in-well' processing of plasma.

The Sirocco Protein Precipitation Plate is suitable for automated processing.

Description	Qty.	Part No.
Sirocco Protein Precipitation Plate	1/pk	186003873
Sirocco Protein Precipitation Plate	5/pk	186002448

For more information visit us at www.waters.com/sirocco and download the Sirocco Interactive Product Guide.

Pall Life Sciences Sample and Solvent Filtration Products

Filtration of samples and solvents is a preventative maintenance procedure that saves lab time and money. Filtration provides immediate protection for the components of column and instrumentation by minimizing down time.

Pall Life Sciences filters have been Certified for Compliance; which means they have been designed and developed to assist customers in complying with their regulatory and quality objectives.

Waters carries a broad range of Pall Life Sciences filter products, a range of different membranes for solvent and sample compatibility, and a variety of devices for various filtration applications.

Choosing the right filter for your application.

To choose the right filter you need to consider sample characteristics, volume, pore size and decide if the sample may require prefiltration because it is laden with particulate matter.

Membrane Choices

GHP Acrodiscs
Hydrophilic propylene membrane suitable for aqueous, organic and has low protein binding.

Nylon Acrodiscs
Hydrophilic nylon membrane

GHP Acrodics GF and Nylon Acrodics GF
Designed with a glass fiber prefilter over the membrane for hard to filter samples laden with particulate matter.

Glass Fiber Acrodiscs
Can be used alone or as a prefilter with another Acrodics in series.

Acrodisc LC (PVDF)
Hydrophilic polyvinylidene fluoride good for aqueous and organic solvents.

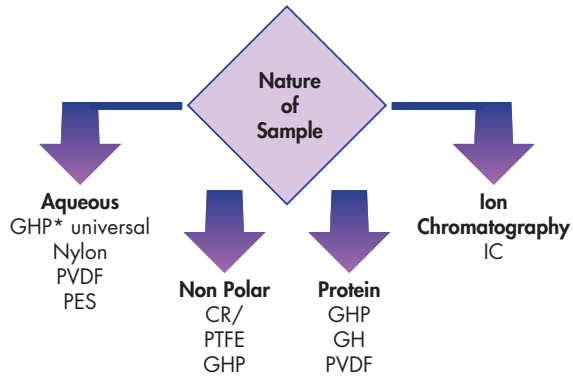
Acrodisc CR(PTFE)
Used for aggressive organic solvents

Ion Chromatography (IC) Acrodisc
Certified to contain low ionic backgrounds

Supor (PES)
Hydrophilic polyethersulfone for biological, pharmaceutical or sterilizing. Can be gamma sterilized or autoclaved.

Concerned about particulate matter in your sample?

Step 1: What is the nature of your sample?



* *For samples with laden particulate that are difficult to filter, it is best to use a syringe filter with a glass fiber pre-filter over the membrane. These are available in GHP and Nylon.*

Step 2: What micron size are the particles in the column are you using?

Column	Pore Size of Filter
> 3 µm	0.45 µm
< 3 µm	0.20 µm

Step 3: What is the volume of your sample?

Volume	Acrodisc Size	Hold Up Volume
< 2 mL	4 mm	< 10 µL
< 10 mL	13 mm minispike	< 14 µL
< 10 mL	13 mm male luer	< 30 µL
< 100 mL	25 mm	< 100 µL

Example 1: 1.5 mL of aqueous sample to be filtered for injection on a 5 µm column:

Step	Question	Answer	Choice
1	Sample	Aqueous	GHP and others
2	Particle size in column	5 µm	0.45 µm
3	Volume	1.5 mL	4 mm or larger

Choice: Membrane 0.45 µm GHP Acrodisc in 4 mm or larger. You can also use the Nylon, PVDF or PES (other choices of hydrophilic membranes under the aqueous sample path). In terms of device size, if you are injecting only a few uL of sample on the column, you can use any device size. The 13 and 25 mm Acrodiscs have hold up volumes of at most 100 µL, leaving plenty of filtered sample for the application.

Filter Design and Membrane Choices

R = RESISTANT LR = LIMITED RESISTANCE NR = NOT RESISTANT • = INSUFFICIENT DATA	Acetone	Acetonitrile	Acetic acid, glacial	n-Butanol	Chloroform	Dioxane	Dimethyl formamide	Dimethyl sulfoxide	Ethanol	Ethyl acetate	Ethyl ether	Freon TF	Hydrochloric acid (1N)	Hexane, dry	Methanol	Methylene chloride	Methyl ethyl ketone	N-Methylpyrrolidone	Isopropanol	Sodium hydroxide (5N)	Tetrahydrofuran	Tetrahydrofuran/ water (50/50)	Toluene	Water
GH Polypro Syringe Filters																								
GHP Acrodisc® 13 (13 mm)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
GHP Acrodisc (25 mm)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
GHP Acrodisc GF (25 mm)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
PTFE Syringe Filters																								
Acrodisc 4CR PTFE (4 mm)	R*	R	R	R	LR	R	R*	R*	R	R*	R	R	R	R	R	LR	R*	R*	R	LR	LR	•	LR*	R
Acrodisc 13CR PTFE (13 mm)	R*	R	R	R	R	R	R*	R*	R	R*	R	R	R	R	R	R	R*	R*	R	R	R	R	R*	R
Acrodisc CR PTFE (25 mm)	R*	R	R	R	R	R	R*	R*	R	R*	R	R	R	R	R	R	R*	R*	R	R	R	R	R*	R
PVDF Syringe Filters																								
Acrodisc LC13 PVDF (13 mm)	NR*	R	R	R	R	R	NR*	NR*	R	R*	R	R	R	R	R	R	NR*	NR*	R	NR	R	R	R*	R
Acrodisc LC PVDF (25 mm)	NR*	R	R	R	R	R	NR*	NR*	R	R*	R	R	R	R	R	R	NR*	NR*	R	NR	R	R	R*	R
Nylon Syringe Filters																								
Nylon Acrodisc 4 (4 mm)	R*	R	R	R	NR	•	R*	R*	R	R*	NR	R	NR	R	R	NR	R*	R*	R	R	NR	LR	R*	R
Nylon Acrodisc 13 (13 mm)	R*	R	R	R	NR	•	R*	R*	R	R*	NR	R	NR	R	R	NR	R*	R*	R	R	NR	LR	R*	R
Nylon Acrodisc (25 mm)	R*	R	R	R	NR	•	R*	R*	R	R*	NR	R	NR	R	R	NR	R*	R*	R	R	NR	LR	R*	R
Nylon Acrodisc GF (25 mm)	R*	R	R	R	NR	•	R*	R*	R	R*	NR	R	NR	R	R	NR	R*	R*	R	R	NR	LR	R*	R
Ion Chromatography Syringe Filters																								
IC Acrodisc (13 mm & 25 mm)	NR	LR	NR	R	NR	•	NR	NR	•	LR	R	LR	•	LR	R	NR	•	NR	•	•	NR	•	R	R
Glass Fibre Syringe Filters																								
GF Acrodisc	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	LR	R	R	R	R
Acrylic Copolymer Syringe Filters																								
Non-sterile Acrodisc (25 mm)	NR	NR	NR	R	NR	NR	NR	NR	R	NR	NR	R	LR	NR	R	NR	NR	NR	R	R	NR	NR	NR	R
Disc Filters																								
GH Polypro	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
FP Verciel (PVDF)	NR	R	R	R	R	LR	NR	NR	R	R	R	R	R	R	R	R	LR	NR	R	NR	LR	•	R	R
Nylaflo (Nylon)	R	R	NR	R	NR	R	R	R	R	R	R	LR	NR	•	LR	NR	NR	R	R	R	R	R	NR	R
TF (PTFE)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

Note:

R = Resistant

No significant change was observed in flow rate or bubble point of the membrane.

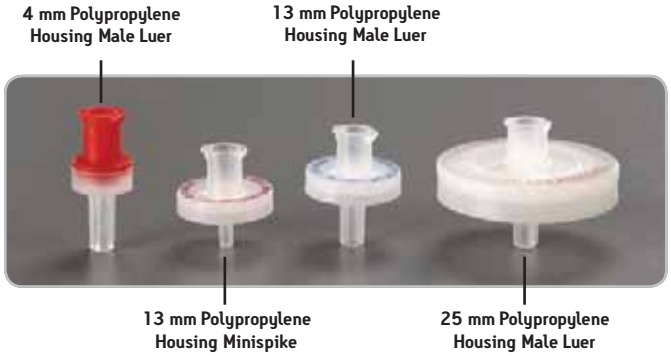
* UV absorbance was set at 254 nm

LR = Limited Resistance

Moderate changes in physical properties or dimension of the membrane were observed. The filter may be suitable for short term, non-critical use at room temperature.

NR = Not Resistant

The membrane is basically unstable. In most cases, extensive shrinkage or swelling occurs. The filter may gradually weaken or partially dissolve after extended exposure.



Part Number Cross Reference

Pall Life Sciences Description	Whatman Part No.	Millipore Part No.	Waters Part No.
PTFE, 4 mm, 0.45 µm	6783-0404	SJFHL04NS	WAT200508
PTFE, 13 mm, 0.2 µm	6783-1302	SJFG013NS	WAT200506
PTFE, 13 mm, 0.45 µm	6783-1304	SJFH013NS	WAT200502
PTFE, 25 mm, 0.2 µm	6784-2502	SLFG025NS	WAT200504
PTFE, 25 mm, 0.45 µm	6784-2504	SLSR025NS	WAT200500
PVDF, 13 mm, 0.45 µm	6792-1304	SJHV013NS	WAT200512
PVDF, 25 mm, 0.45 µm	—	SLHV025NS	WAT200510
GHP, 13 mm, 0.45 µm	6784-1304	SJFH013NS	WAT200516
GHP, 25 mm, 0.45 µm	6785-2504	SLCR025NS	WAT200514
Nylon, 13 mm, 0.2 µm	6790-1302	—	WAT200524
Nylon, 13 mm, 0.45µm	6790-1304	—	WAT200520
Nylon, 25 mm, 0.2 µm	—	—	WAT200522
Nylon, 25 mm, 0.45 µm	—	—	WAT200518
Supor®, 25 mm, 0.45 µm	6780-2504	SLHA025OS	WAT200528
Supor, 25 mm, 0.2 µm	6780-2502	SLGS025OS	WAT200529
PVDF, 47 mm, 0.45 µm	—	HVLP04700	WAT200530
PVDF, 47 mm, 0.2 µm	—	—	WAT200531
Nylon, 47 mm, 0.45 µm	7404-004	—	WAT200532
Nylon, 47 mm, 0.2 µm	7402-004	—	WAT200533
PTFE, 47 mm, 0.45 µm	7585-004	FHLP04700	WAT200534
PTFE, 47 mm, 0.2 µm	7582-004	FGLP04700	WAT200535
GHP, 47 mm, 0.45 µm	7002-0447	FHLC04700	WAT200537
Supor, 47 mm, 0.2 µm	—	GSTF04700	WAT200539
Supor, 47 mm, 0.45 µm	—	HATF04700	WAT200538
Supor, 13 mm, 0.45 µm	—	HATF01300	WAT200540
Glass Filter Holder	—	XX1504700	WAT200543
Forceps	—	XX6200006	WAT200544
300 mL Funnel	—	XX1004704	WAT200545
Glass Base, Tabulated Cap	—	XX1504702	WAT200546
Ground Joint Flask	—	XX1504705	WAT200547
Thick Glass Prefilter	—	AP2501000	WAT200541
A/E Glass Prefilter	—	AP4001000	WAT200542

Solvent Filtration Membranes

Description	Qty.	Diameter	Pore Size	Part No.
PVDF Filter	100/pkg	47 mm	0.45 µm	WAT200530
Nylon Filter	100/pkg	47 mm	0.45 µm	WAT200532
PTFE Filter	100/pkg	47 mm	0.45 µm	WAT200534
	100/pkg	13 mm	0.45 µm	WAT200536
GH Polypro Filter	100/pkg	47 mm	0.45 µm	WAT200537
Supor (PES) Filter	100/pkg	47 mm	0.45 µm	WAT200538
	100/pkg	13 mm	0.45 µm	WAT200540
PVDF Filter	100/pkg	47 mm	0.2 µm	WAT200531
Nylon Filter	100/pkg	47 mm	0.2 µm	WAT200533
PTFE Filter	100/pkg	47 mm	0.2 µm	WAT200535
GHP	100/pkg	47 mm	0.2 mm	186003524
Supor (PES) Filter	100/pkg	47 mm	0.2 µm	WAT200539
Thick Glass Filter	100/pkg	10 mm	1.0 µm	WAT200541
A/E Glass Filter	100/pkg	10 mm	1.0 µm	WAT200542

Solvent Filtration Apparatus

The 300 mL capacity 47 mm Glass Filter Funnel and 1 Litre capacity 47 mm Glass Funnel/Support Assembly are ideal for vacuum filtration of liquids and degassing of HPLC solvent and mobile phases. The 100% borosilicate glass construction assures resistance to even the most aggressive solvents.

Description	Part No.
Solvent Filtration Apparatus 110 V, 60 Hz	WAT085113
Solvent Filtration Apparatus 220 V, 50 Hz	WAT085102
All Glass Filter Holder 47 mm, complete	WAT200543
Forceps, SS	WAT200544
Funnel, 300 mL	WAT200545
Glass Base, tabulated cap	WAT200546
Ground Joint Flask	WAT200547
Swinney Holder	WAT200566
Vacuum Pump 110 V, 60 Hz	WAT085114
Vacuum Pump 110 V, 50 Hz	WAT085123
Vacuum Pump 220 V, 50 Hz	WAT085115

Syringe Filters Product List

Acrodisc 13 mm

		0.2 µm			0.45 µm		
	Pack Size	100	300	1000	100	300	1000
Aqueous	NYLON	WAT200524	WAT200525	WAT200834	WAT200520	WAT200521	WAT200832
	PVDF	WAT200806	WAT200807	—	WAT200512	WAT200513	WAT200827
Non Polar	CR	WAT200506	WAT200507	WAT200823	WAT200502	WAT200503	WAT200821
Protein	PVDF	WAT200806	WAT200807	—	WAT200512	WAT200513	WAT200827
Ion Chromatography	PES	WAT200810	WAT200811	WAT200844	WAT200812	WAT200813	WAT200842

Acrodisc 13 mm Mini spike

		0.2 µm			0.45 µm		
	Pack Size	100	300	1000	100	300	1000
Aqueous	GHP	WAT097962	WAT097963	—	WAT200516	WAT200517	WAT200830
	NYLON	WAT200562	WAT200563	WAT200835	WAT200564	WAT200565	WAT200836
	PVDF	WAT200804	WAT200805	WAT200838	WAT200560	WAT200561	WAT200828
Non Polar	CR	WAT200556	WAT200557	WAT200824	WAT200558	WAT200559	WAT200825
	GHP	WAT097962	WAT097963	—	WAT200516	WAT200517	WAT200830
Protein	IC	WAT200804	WAT200805	WAT200838	WAT200560	WAT200561	WAT200828

Acrodisc 25 mm

		0.2 µm			0.45 µm		
	Pack Size	50	200	1000	50	200	1000
Aqueous	GHP	WAT097964	WAT097965	—	WAT200514	WAT200515	WAT200829
	NYLON	WAT200522	WAT200523	WAT200833	WAT200518	WAT200519	WAT200831
	PVDF	WAT200808	WAT200809	WAT200839	WAT200510	WAT200511	WAT200826
	GHP GF*	—	—	—	WAT200802	WAT200803	WAT058853
	NYLON GF*	—	—	—	WAT200800	WAT200801	WAT200846
	GF**	—	—	—	WAT200818	WAT200819	WAT200840
	Versapor	—	—	—	—	—	WAT200841
	Sterile Syringe PES	WAT200529	—	—	—	—	—
Non Polar	CR	WAT200504	WAT200505	WAT200822	WAT200500	WAT200501	WAT200820
	GHP	WAT097964	WAT097965	—	WAT200514	WAT200515	WAT200829
Protein	PVDF	WAT200808	WAT200809	WAT200839	WAT200510	WAT200511	WAT200826
Ion Chromatography	IC	WAT200814	WAT200815	WAT200845	WAT200816	WAT200817	WAT200843

* GHP GF and Nylon GF are Glass Fiber prefilters in combination with GHP and nylon filters.

** GF Glass Fiber only 1 µm

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ACQUITY UPLC Columns and Consumables

ACQUITY UltraPerformance LC Columns

Since the 1970's chromatographers have been limited to LC systems that were capable of operating at maximum system pressures of only 6000 psi (400 bar). This pressure limitation, coupled with large system volumes and slow data acquisition rates, hindered the ability of separations scientists to fully realize the speed and efficiencies promised by using small (< 2 µm) particles. The ACQUITY UPLC® system shatters these operating limitations and is designed to routinely operate at a maximum pressure of up to 15,000 psi (1000 bar).

The heart of any LC system is the column and the heart of the ACQUITY UPLC system is the ACQUITY UPLC column. The ACQUITY UPLC columns are the most advanced LC columns ever created: capable at operating at pressures up to 15,000 psi (1,000 bar) while producing efficiencies of greater than 200,000 N/m. ACQUITY UPLC columns are available in two particle substrates (Ethylene Bridged Hybrid [BEH] and High Strength Silica [HSS]) and eight column chemistries. ACQUITY UPLC columns also feature eCord™ technology which stores all of the column's manufacturing information as well as its entire history: the day the column was first installed; number of injections and sample sets; maximum pressure and temperature that the column was subjected to; and the last time the column was used. These data cannot be erased and are unique to the individual column.

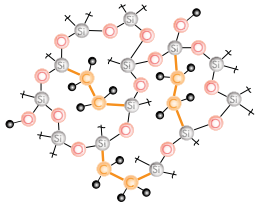
ACQUITY UPLC Particle Technology

There is more to creating a UPLC® particle than synthesizing a small particle. Many HPLC particles do not possess the mechanical stability and structural integrity to withstand UPLC operating pressures (e.g., 15000 psi/1000 bar). In order to realize the efficiency gains of sub-2 µm particles, the ability to routinely operate at higher linear velocities (e.g., higher flow rates) is required. These higher linear velocities combined with small, sub-2 µm particles results in higher operating backpressures. Waters has created two highly efficient, pressure-tolerant UPLC particles: the 1.7 µm BEH particle and the 1.8 µm HSS particle.

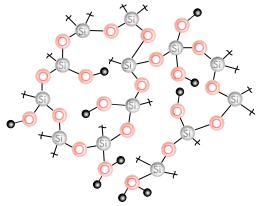
The first ACQUITY UPLC particle created was the 1.7 µm Ethylene Bridged Hybrid (BEH) particle. This second generation hybrid particle is one of the key enablers behind UPLC technology and is available in five column chemistries for small molecule separations (C_{18}, C_8, Shield RP18, Phenyl and HILIC), and two column chemistries for large molecule separations (C_{18} and C_4). Because this is a hybrid particle, a wider usable pH range (up to pH 1-12) makes method development faster and easier. BEH particles are also available in HPLC particle sizes (2.5, 3.5, 5, and 10 µm) in the XBridge™ family of HPLC columns, thus allowing seamless transfer between HPLC and UPLC separations.

The second and newest UPLC particle from Waters is the 1.8 µm HSS particle. This new ACQUITY UPLC HSS particle is not an HPLC particle since most HPLC particles do not posses the mechanical stability necessary to withstand the high column packing and operating pressures encountered in UPLC separations. This is the first and only 100% silica particle designed specifically for 15,000 psi (1000 bar) applications. ACQUITY UPLC HSS column chemistries include C_{18}, C_{18} SB, and T3.

ACQUITY UPLC BEH Columns



ACQUITY UPLC HSS Columns



Particle Type	Ethylene Bridged Hybrid (BEH)		High Strength Silica (HSS)
Particle Size	1.7 µm		1.8 µm
Maximum Rated Pressure	15000 psi (~1000 bar)		15000 psi (~1000 bar)
Pore Diameter/Volume	130Å / 0.7 mL/g	300Å / 0.7 mL/g	100Å / 0.7 mL/g
Surface Area	185 m^2/g	90 m^2/g	230 m^2/g
Available Chemistries	C_{18}, C_8, Shield RP18, Phenyl, HILIC	C_{18}, C_4	C_{18}, C_{18} SB, T3
pH Range	1-12; (RP18: 2-11); (HILIC: 1-8)	C_{18}: 1-12; C_4: 1-10	C_{18}: 1-8; C_{18} SB, T3: 2-8

Ultra Resolution

Chromatographers are always looking for solutions that will deliver more resolution and more robust separations. To meet this need for higher efficiency, Waters developed 150 mm length ACQUITY UPLC columns. With these longer columns, challenging separations such as impurity profiling, metabolite ID analyses and drug stability monitoring can be run routinely with high resolution and moderate analysis times.

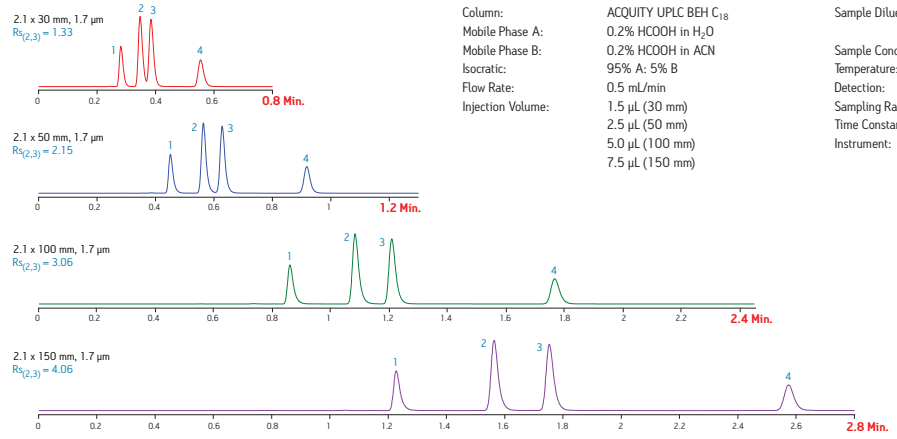
The resolving power of an LC column can be expressed by its length to particle size ratio (L/dp). Columns with the highest L/dp ratios provide greater efficiencies (N) and are normally used in the most difficult separations. The L/dp ratio of the 150 mm length columns is more than 88,000, thus producing efficiencies of >35,000 plates per separation. As a point of reference, a 4.6 x 150 mm, 5 µm HPLC column has an L/dp ratio of 30,000 and can produce efficiencies of only 12,000 plates.

Choosing a UPLC column based upon the relationship between UPLC column length, length to particle size ratio (l/dp), efficiency (N) and separation type.

UPLC Column Length	L/dp*	Efficiency (N)	Separation Type
30 mm	17,600	5,875	Easy
50 mm	29,400	11,750	Moderately Challenging
100 mm	58,800	23,500	Difficult
150 mm	88,200	35,000	Extremely Difficult

* dp = 1.7 µm

Ultra-Resolution Separations with 150 mm Length ACQUITY UPLC Columns

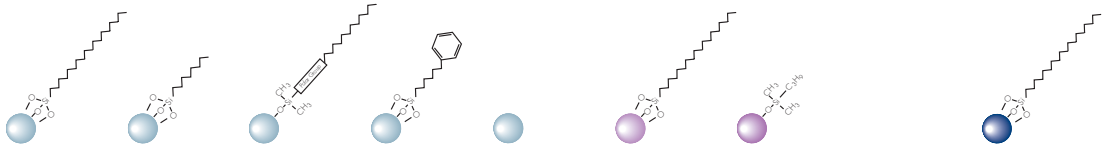


Column:	ACQUITY UPLC BEH C_{18}
Mobile Phase A:	0.2% HCOOH in H_2O
Mobile Phase B:	0.2% HCOOH in ACN
Isocratic:	95% A: 5% B
Flow Rate:	0.5 mL/min
Injection Volume:	1.5 µL (30 mm) 2.5 µL (50 mm) 5.0 µL (100 mm) 7.5 µL (150 mm)
Sample Diluent:	95:5 H_2O: ACN with 0.2% HCOOH
Sample Conc.:	25 µg/mL
Temperature:	50 °C
Detection:	UV @ 280 nm
Sampling Rate:	40 pts/sec
Time Constant:	0.1
Instrument:	ACQUITY UPLC with ACQUITY UPLC TUV detector

Caffeine Metabolites Compounds
1. 1-methylxanthene
2. 1,3-dimethyluric acid
3. theobromine
4. 1,7-dimethylxanthene

150 mm length ACQUITY UPLC columns provide high resolution in less time than HPLC.

UPLC Column Chemistries



	130Å BEH Particle					300Å BEH Particle		HSS Particle		
Chemistry	C_{18}	C_8	Shield RP18	Phenyl	HILIC	C_{18}	C_4	C_{18}	C_{18} SB	T3
Ligand Type	Trifunctional C_{18}	Trifunctional C_8	Monofunctional Embedded Polar Group	Trifunctional C_6 Phenyl	Unbonded	Trifunctional C_{18}	Monofunctional C_4	Trifunctional C_{18}	Trifunctional C_{18}	Trifunctional C_{18}
Available Particle Sizes	1.7, 2.5, 3.5, 5, 10 µm	1.7, 2.5, 3.5, 5, 10 µm	1.7, 2.5, 3.5, 5 µm	1.7, 2.5, 3.5, 5, 10 µm	1.7, 2.5, 3.5, 5 µm	1.7, 3.5, 5, 10 µm	1.7, 3.5 µm	1.8 µm	1.8 µm	1.8 µm
Ligand Density*	3.1 µmol/m²	3.2 µmol/m²	3.3 µmol/m²	3.0 µmol/m²	—	3.2 µmol/m²	2.5 µmol/m²	3.2 µmol/m²	1.6 µmol/m²	1.6 µmol/m²
Carbon Load*	18%	13%	17%	15%	—	13%	8%	15%	8%	11%
Endcap Style	Proprietary	Proprietary	TMS	Proprietary	—	Proprietary	None	Proprietary	None	Proprietary
pH Range	1-12	1-12	2-11	1-12	1-8	1-12	1-10	1-8	2-8	2-8

** Expected or Approximate Values*

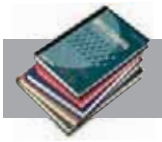
Ultra Speed

How do you produce a short separation that does not sacrifice efficiency and/or peak shape? The simple answer lies in chromatographic theory. If you can reduce the column length, while simultaneously reducing the particle size by the same ratio (i.e., maintain column length to particle size (L/dp) ratio), the resolving power of the column, as well as the resolution or peak capacity of the resulting chromatogram, remains intact. However, this separation can be achieved in less time. The practical and commercial application of this simple chromatographic theory, however, has proven to be challenging.

For the first time, UPLC technology allows separation scientists to fully realize the speed and efficiencies promised by short columns packed with very small particles:

- Ultra-low system volume (low bandspreading)
- Fast, efficient detectors and/or mass spectrometers
- Short cycle time sample manager with low carryover
- Small, rugged, highly efficient particles
- Advanced column hardware and packing techniques.

Application areas that benefit from the fast separations that are possible with 30 mm length ACQUITY UPLC columns include confirmatory Active Pharmaceutical Ingredient (API) QC, stability monitoring, cleaning protocol validation, early drug development screening, bioanalytical analyses, content uniformity, drug release assays, and process monitoring.



Literature References

ACQUITY UPLC Columns Brochure,
Literature Reference 720001140EN

ACQUITY UPLC HSS Columns
Care and Use Manual,
Literature Reference 715001429

ACQUITY UPLC BEH Columns
Care and Use Manual,
Literature Reference 715001371

Interactive Waters Reversed-Phase
Column Selectivity Chart
www.waters.com/selectivitychart

Atlantis® T3 and ACQUITY UPLC
HSS T3 Columns Brochure,
Literature Reference 720001887EN

T3 Technology Whitepaper,
Literature Reference 720001889EN

Ultra-Fast SPE-UPLC/MS/MS Determination of Risperidone and Metabolite

Chromatographic Conditions

Column:	ACQUITY UPLC BEH C_{18} 2.1 x 30 mm, 1.7 µm
Part Number:	186002349
Mobile Phase A:	10 mM CH_3COONH_4 pH 9.0
Mobile Phase B:	MeOH

Gradient:

Time (min)	Flow mL/min	Profile %A	%B
0.0	1.0	60	40
0.5	1.0	5	95
0.55	1.5	5	95
0.85	1.5	5	95
0.9	1.0	60	40
1.0	1.0	60	40

Injection Volume:	8 µL
Column Temp.:	50 °C
Sample Temp.:	15 °C
Sample Concentration:	0.1 ng/mL
Sample Diluent:	50/50 H_2O/MeOH
Strong Needle Wash:	60:40 ACN:IPA + 0.5% HCOOH
Weak Needle Wash:	95:5 H_2O/MeOH
Instrument:	ACQUITY UPLC with Quattro Premier MS

MS Conditions
Waters Quattro Premier
ES+

Capillary:	3.0 kV
Source Temp:	120 °C
Desolvation Temp:	350 °C
Cone Gas Flow:	50 L/Hr
Desolvation Gas Flow:	700 L/Hr
Collision Cell Pressure:	2.59 e^{-3} mbar
Dwell time:	30 msec
Transfer Tubing:	0.005"

SPE Protocol for Risperidone Using Oasis MCX µElution Plate

Oasis MCX 30 µm µElution Plate
Part Number: 186001830BA

Condition
0.2 mL MeOH

Equilibrate
0.2 mL H_2O

Load: 0.4 mL diluted Human Plasma
(0.2 mL plasma diluted 1:1 with 4% H_3PO_4 in H_2O)

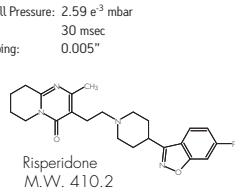
Wash 1
0.2 mL 2% HCOOH in H_2O

Wash 2
0.1 mL MeOH

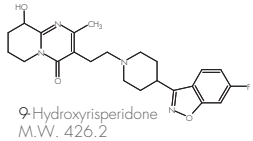
Elute: 50 µL (25 µL x 2)
5% NH_4OH in 90:10 MeOH:H_2O

Dilute
50 µL H_2O

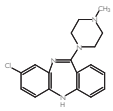
Recoveries
Risperidone 96%, 9-OH Risperidone 96%
Clozapine 94%



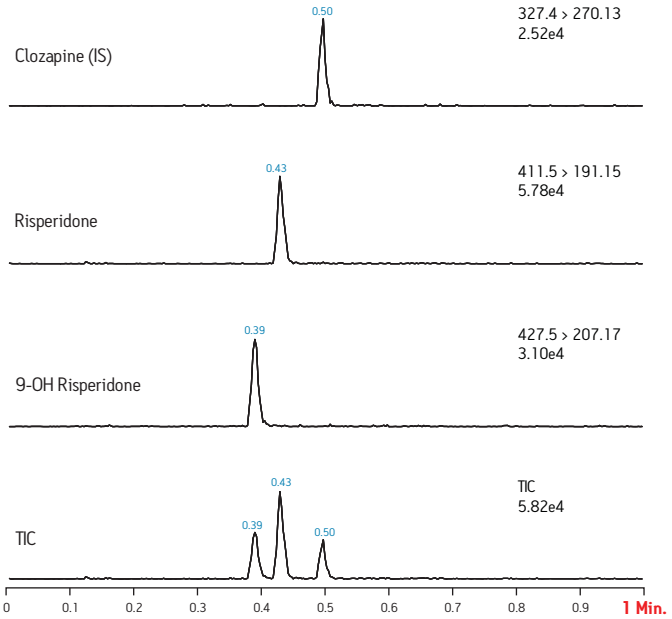
Risperidone
M.W. 410.2



9-Hydroxyrisperidone
M.W. 426.2



Clozapine , IS
M.W. 326.1



The antipsychotic drug risperidone is rapidly metabolized into 9-hydroxyrisperidone and is the predominant circulating species with same activity as parent drug. Automated sample processing time is 15 min. for 96 samples (~9 sec/sample). Peak widths are approximately 1.7 sec. wide, Pmax= 8900 psi.

ACQUITY UPLC BEH Reversed-Phase Columns

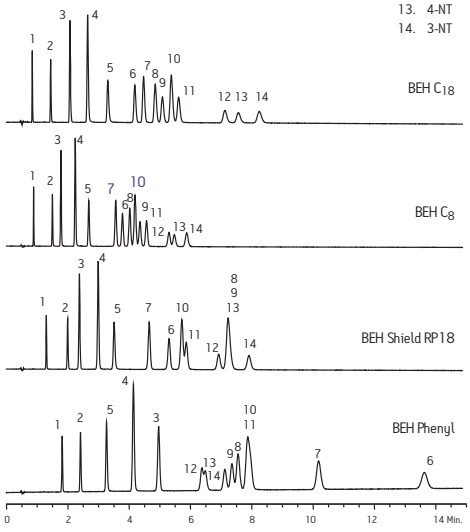
ACQUITY UPLC BEH columns are available in four reversed-phase column chemistries (C_{18}, C_8, Shield RP18, and Phenyl) aimed at small molecule separations. ACQUITY UPLC BEH C_{18}, C_8, and Phenyl columns incorporate trifunctional ligand bonding chemistries which produce superior low pH stability and ultra-low column bleed. ACQUITY UPLC BEH Shield RP18 columns provide alternate selectivities by utilizing Waters patented Shield Technology* which incorporates an embedded carbamate group into the bonded phase. All of these innovative UPLC column chemistries produce the sharpest peaks, highest efficiencies and maximum MS sensitivities. These chemistries are available in larger particle sizes (2.5, 3.5, 5, and 10 µm) in the XBridge family of HPLC columns for seamless method transfer between HPLC and UPLC separations.

* Patent Number # 5,374,755

EPA Method 8330 Separations on ACQUITY UPLC BEH Columns

Column:	ACQUITY UPLC BEH 2.1 x 100 mm, 1.7 µm
Mobile Phase A:	H_2O
Mobile Phase B:	MeOH
Flow Rate:	0.5 mL/min
Isocratic Mobile Phase Composition:	72% A; 28% B
Injection Volume:	5 µL
Sample Conc.:	10 µg/mL
Temperature:	50 °C
Detection:	UV @ 254 nm
Instrument:	ACQUITY UPLC with PDA detector

Compounds
1. HMX
2. RDX
3. 1,3,5-TNB
4. 1,3-DNB
5. NB
6. Tetryl
7. TNT
8. 2-Am-4,6 DNT
9. 4-Am-2,6 DNT
10. 2,4 DNT
11. 2,6-DNT
12. 2-NT
13. 4-NT
14. 3-NT



ACQUITY UPLC BEH HILIC Columns

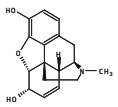
Hydrophilic-Interaction Chromatography (HILIC) is a technique used to retain and separate very polar compounds that cannot be retained using reversed-phase chromatography. HILIC is also referred to as 'aqueous normal phase' or 'reverse, reversed phase' since the elution order is that of normal phase (non-polar analytes elute first) and the solvents are similar to those of reversed-phase chromatography.

The ACQUITY UPLC BEH HILIC columns contain the rugged 1.7 µm unbonded BEH particles and are designed to retain and separate very polar basic compounds. These unique columns are optimized and tested to produce efficient and reproducible separations under UPLC HILIC conditions. ACQUITY UPLC BEH HILIC columns overcome a major weakness of HILIC stationary phases: chemical stability. Silica-based HILIC phases are often chemically unstable. The rugged BEH particle's wide usable pH range overcomes this chemical instability and results in long column lifetimes. This chemistry is also available in larger particle sizes (2.5, 3.5, and 5 µm) in the XBridge family of columns.

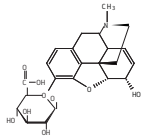
Separation of Very Polar Bases Using ACQUITY UPLC BEH HILIC Columns

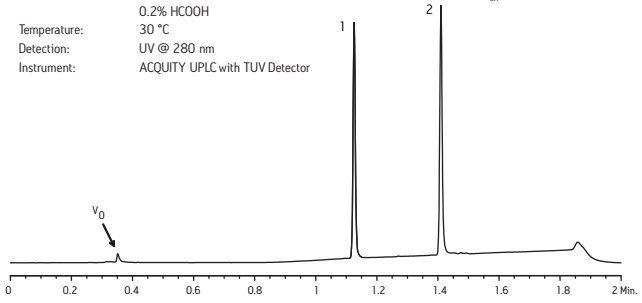
Column:	ACQUITY UPLC BEH HILIC 2.1 x 100 mm, 1.7 µm
Part Number:	186003461
Mobile Phase A:	10 mM NH_4COOH, 0.2% HCOOH in 50:50 ACN:H_2O
Mobile Phase B:	10 mM NH_4COOH, 0.2% HCOOH in 90:10 ACN:H_2O
Flow Rate:	0.788 mL/min
Gradient:	Time (min) / %A / %B Profile: 0.0 / 0.1 / 99.9; 0.37 / 0.1 / 99.9; 1.46 / 99.9 / 0.1; 1.50 / 0.1 / 99.9; 2.00 / 0.1 / 99.9
Injection Volume:	2.1 µL
Sample Conc.:	125 µg/mL
Sample Diluent:	75:25 ACN:MeOH with 0.2% HCOOH
Temperature:	30 °C
Detection:	UV @ 280 nm
Instrument:	ACQUITY UPLC with TUV Detector

1. Morphine



2. Morphine 3-fl-D-Glucuronide





ACQUITY UPLC HSS Reversed-Phase Chemistries

ACQUITY UPLC HSS columns are available in three bonded phases: C_{18}, C_{18} SB and T3.

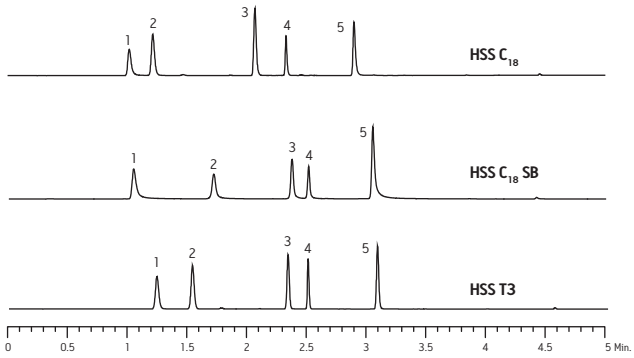
ACQUITY UPLC HSS C_{18} chemistry is an ultra-performance, general-purpose C_{18} bonded phase that provides superior peak shape for bases, increased retention (vs. ACQUITY UPLC BEH C_{18} columns), and extremely long column lifetimes at low pH. The selectivities and retention observed with HSS C_{18} columns will resemble that of most modern, fully endcapped silica-based C_{18} HPLC columns.

ACQUITY UPLC HSS C_{18} SB (Selectivity for Bases) chemistry is an unendcapped C_{18} bonded phase that was designed specifically to be "different" in terms of selectivity. The HSS C_{18} SB chemistry is optimized for low pH method development applications and provides alternate selectivities, especially for basic compounds, as compared to most modern, fully endcapped C_{18} chemistries.

ACQUITY UPLC HSS T3 columns are designed to solve a common problem facing separations scientists: retaining small, water soluble, polar organic molecules. The HSS T3 chemistry is an aqueous mobile phase compatible C_{18} bonded phase designed to retain and separate polar organic compounds, much like Atlantis T3 HPLC columns.

Caffeic Acid Derivatives Separations on ACQUITY UPLC HSS Columns

Column:	ACQUITY UPLC HSS 2.1 x 50 mm, 1.8 µm				Compounds
Mobile Phase A:	0.1% CF_3COOH in H_2O				1. Caftaric acid
Mobile Phase B:	0.08% CF_3COOH in ACN				2. Chlorogenic acid
Flow Rate:	0.5 mL/min				3. Cynarin
Gradient:	Time	Profile			4. Echinacoside
	(min)	%A	%B	Curve	5. Cichoric acid
	0.0	92	8	6	
	0.1	92	8	6	
	4.45	50	50	7	
	4.86	10	90	6	
	5.0	92	8	6	
	6.0	92	8	6	
Injection Volume:	1.0 µL				
Sample Diluent:	50:50 H_2O: MeOH with 0.05% CF_3COOH				
Sample Conc.:	100 µg/mL				
Temperature:	40 °C				
Detection:	UV @ 330 nm				
Instrument:	ACQUITY UPLC with ACQUITY UPLC TUV				

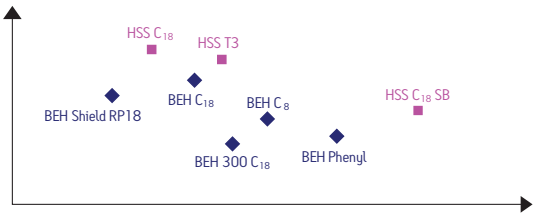


ACQUITY UPLC Columns Reversed-Phase Selectivity Choices

One of the major driving forces in UPLC technology is increasing resolution by maximizing efficiency. The ultra efficiencies of UPLC separations are a result of the ultra-low dispersion UPLC system, small (< 2 µm) particles, higher pressure capability and well-designed columns. Efficiency alone, however, is not always enough to provide the desired separation. Resolution can also be greatly improved by changing selectivity or capacity factor. Selectivity and capacity factor are affected by bonded phase, particle substrate, pH range, and retentivity. Waters has maximized resolution by effectively combining the physically-influenced efficiency of the UPLC system and columns with the chemically-influenced selectivity and capacity factor of the bonded phases and particle substrates.

In the past, lower efficiency separations required chromatographers to randomly try many of the hundreds of different types of HPLC column chemistries in order to obtain the desired separation. Because the efficiencies of UPLC separations are 2-3 times higher, a smaller number of high-quality ligands and particles are necessary. The higher efficiencies and speed of UPLC Technology, when combined with multiple < 2 µm particle substrates and bonded phases, allow chromatographers to more efficiently develop faster and more robust separations.

Low pH UPLC Selectivities



Waters offers three < 2 µm UPLC particle substrates and nine unique UPLC column chemistries (including BEH HILIC) that provide a wide range of selectivity choices.

VanGuard Pre-Columns

Separation scientists working in demanding application areas (such as bioanalysis, food/beverage, natural products, environmental, and industrial chemicals) analyze complex, unpredictable and challenging samples on a routine basis. These types of samples can have a negative impact on column lifetimes when appropriate sample preparation/cleanup procedures are not implemented. VanGuard™ pre-columns are designed for these types of application areas where chemical and/or particulate contamination can shorten the lifetime of a UPLC column.

VanGuard pre-columns are the result of over two years of product development and are the first guard column devices designed for routine use at pressures up to 15000 psi (1000 bar) in applications run on the ACQUITY UltraPerformance LC® system. VanGuard Pre-columns feature a 2.1 mm i.d. x 5 mm length, ultra-low volume design which efficiently protects UPLC column performance. This patent-pending design does not compromise the UPLC holistic design approach to higher efficiency, greater resolution and increased throughput since the same ACQUITY UPLC column stationary phases and column frits are used in VanGuard pre-columns. Since the VanGuard Pre-column connects directly to the inlet of the ACQUITY UPLC column, extra-column volumes are minimized and mobile phase leaks due to additional connections are all but eliminated.

VANGUARD™ PRE-COLUMNS

Key Features and Benefits of VanGuard Pre-Columns

FEATURE	BENEFIT
First pre-column for UPLC applications	Guaranteed compatibility with pressures up to 15000 psi
Patent pending, ultra-low volume design	Minimal chromatography effects
Manufactured using UPLC column hardware, particles, and chemistries	Superior UPLC column protection and performance
Connects directly to UPLC column	Leaks and connection voids are eliminated

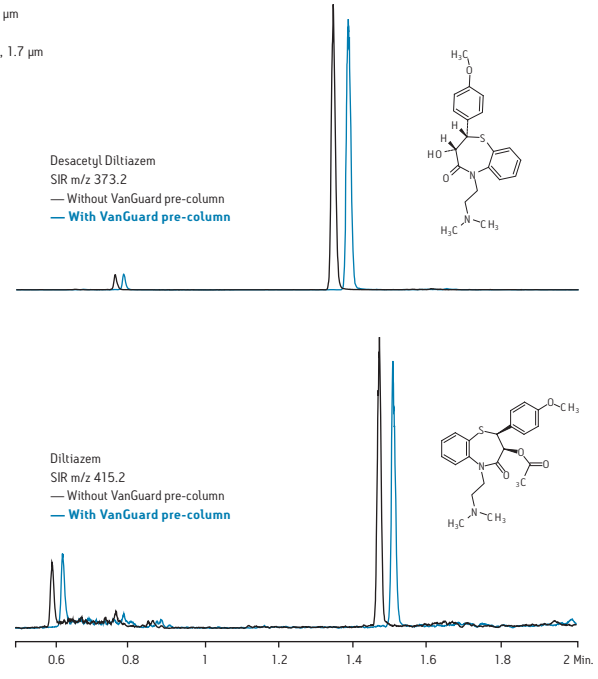
Minimal Chromatographic Effects With VanGuard Pre-Columns

VanGuard pre-columns are uniquely designed to protect and prolong ACQUITY UPLC column performance while contributing minimal chromatographic effects.

Column:	ACQUITY UPLC BEH C_{18}, 2.1 x 50 mm, 1.7 µm
Part Number:	186002350
Pre-column:	VanGuard pre-column, BEH C_{18}, 2.1 x 5 mm, 1.7 µm
Part Number:	186003975
Mobile Phase A:	0.2% NH_4OH in H_2O
Mobile Phase B:	0.2% NH_4OH in ACN
Flow Rate:	0.8 mL/min
Gradient:	Time Profile

(min)	%A	%B
0.0	95	5
2.0	5	95
2.5	5	95
2.6	95	5
3.0	95	5

Injection Volume:	2 µL
Temperature:	40 °C
Detection:	UV @ 254 nm
Sampling Rate:	40 pts/sec
Time Constant:	0.05
Instrument:	ACQUITY UPLC with ACQUITY UPLC TUV detector and ACQUITY SQ detector
ACQUITY SQ Detector	
ES+	
Capillary:	3.5 kV
Cone:	35 V
Source Temp:	150 °C
Desolvation Temp:	500 °C
Cone gas Flow:	50 L/Hr
Desolvation Gas Flow:	850 L/Hr
SIR:	373.2 m/z, 415.2 m/z
MS Interscan Delay:	0.005 sec
Dwell:	0.005 sec



Protein Precipitation Procedure

Spike plasma with 500 ng/mL of diltiazem and desacetyl diltiazem

↓

Take 200 µL of spiked plasma and add to 1.5 mL centrifuge tube

↓

Add 600 µL of acetonitrile to the centrifuge tube containing the spiked plasma

↓

Centrifuge for 10 minutes at 13000 RPM

↓

Take 650 µL of supernatent and evaporate to dryness with nitrogen

↓

Reconstitute with 400 µL of a 50:50 MeOH:H_2O solution (results in a 203.125 ng/mL conc.)

ACQUITY UPLC Columns

Chemistry	Dimensions	Particle Size	Part No. Individual Column	Part No. 3 pk
BEH C_{18}	1.0 x 50 mm	1.7 µm	186002344	176000861
	1.0 x 100 mm	1.7 µm	186002346	176000862
	1.0 x 150 mm	1.7 µm	186002347	176001044
	2.1 x 30 mm	1.7 µm	186002349	176001304
	2.1 x 50 mm	1.7 µm	186002350	176000863
	2.1 x 100 mm	1.7 µm	186002352	176000864
	2.1 x 150 mm	1.7 µm	186002353	176001048
BEH Shield RP18	1.0 x 50 mm	1.7 µm	186002851	176000874
	1.0 x 100 mm	1.7 µm	186002852	176000875
	1.0 x 150 mm	1.7 µm	186003373	176001045
	2.1 x 30 mm	1.7 µm	186003909	176001309
	2.1 x 50 mm	1.7 µm	186002853	176000876
	2.1 x 100 mm	1.7 µm	186002854	176000877
	2.1 x 150 mm	1.7 µm	186003376	176001049
BEH C_8	1.0 x 50 mm	1.7 µm	186002875	176000882
	1.0 x 100 mm	1.7 µm	186002876	176000883
	1.0 x 150 mm	1.7 µm	186003374	176001046
	2.1 x 30 mm	1.7 µm	186003910	176001310
	2.1 x 50 mm	1.7 µm	186002877	176000884
	2.1 x 100 mm	1.7 µm	186002878	176000885
	2.1 x 150 mm	1.7 µm	186003377	176001050
BEH Phenyl	1.0 x 50 mm	1.7 µm	186002882	176000905
	1.0 x 100 mm	1.7 µm	186002883	176000906
	1.0 x 150 mm	1.7 µm	186003375	176001047
	2.1 x 30 mm	1.7 µm	186003911	176001311
	2.1 x 50 mm	1.7 µm	186002884	176000907
	2.1 x 100 mm	1.7 µm	186002885	176000908
	2.1 x 150 mm	1.7 µm	186003378	176001051
BEH HILIC	1.0 x 50 mm	1.7 µm	186003457	176001089
	1.0 x 100 mm	1.7 µm	186003458	176001090
	1.0 x 150 mm	1.7 µm	186003459	176001091
	2.1 x 50 mm	1.7 µm	186003460	176001092
	2.1 x 100 mm	1.7 µm	186003461	176001093
	2.1 x 150 mm	1.7 µm	186003462	176001094
HSS C_{18}	1.0 x 50 mm	1.8 µm	186003529	176001121
	1.0 x 100 mm	1.8 µm	186003530	176001122
	1.0 x 150 mm	1.8 µm	186003531	176001123
	2.1 x 30 mm	1.8 µm	186003987	176001398
	2.1 x 50 mm	1.8 µm	186003532	176001124
	2.1 x 100 mm	1.8 µm	186003533	176001125
	2.1 x 150 mm	1.8 µm	186003534	176001126
HSS C_{18} SB	1.0 x 50 mm	1.8 µm	186004114	176001556
	1.0 x 100 mm	1.8 µm	186004115	176001557
	1.0 x 150 mm	1.8 µm	186004116	176001558
	2.1 x 30 mm	1.8 µm	186004117	176001559
	2.1 x 50 mm	1.8 µm	186004118	176001560
	2.1 x 100 mm	1.8 µm	186004119	176001561
	2.1 x 150 mm	1.8 µm	186004120	176001562
HSS T3	1.0 x 50 mm	1.8 µm	186003535	176001127
	1.0 x 100 mm	1.8 µm	186003536	176001129
	1.0 x 150 mm	1.8 µm	186003537	176001130
	2.1 x 30 mm	1.8 µm	186003944	176001375
	2.1 x 50 mm	1.8 µm	186003538	176001131
	2.1 x 100 mm	1.8 µm	186003539	176001132
	2.1 x 150 mm	1.8 µm	186003540	176001133

Mixed ACQUITY UPLC Chemistries Column 4-Packs

Description	Dimensions	Part No.
High & Low pH, Widest Selectivities UPLC Columns Kit	2.1 x 50 mm	176001042
BEH C_{18}, BEH C_8, BEH Shield RP18, BEH Phenyl	2.1 x 100 mm	176001043
UPLC Method Development Scouting Kit	2.1 x 50 mm	176001603
BEH C_{18}, BEH Shield RP18, BEH Phenyl, HSS T3	2.1 x 100 mm	176001604
L1 UPLC Columns Kit	2.1 x 50 mm	176001605
BEH C_{18}, BEH Shield RP18, HSS C_{18}, HSS T3	2.1 x 100 mm	176001606
Mass Spec UPLC Columns Kit	2.1 x 50 mm	176001607
BEH C_{18}, HSS C_{18}, HSS C_{18} SB, HSS T3	2.1 x 100 mm	176001608
Low pH, Widest Selectivities UPLC Columns Kit	2.1 x 50 mm	176001609
BEH Shield RP18, BEH Phenyl, HSS C_{18}, HSS C_{18} SB	2.1 x 100 mm	176001610

ACQUITY UPLC Columns Method Validation Kits*

Chemistry	Dimensions	Particle Size	Part No.
BEH C_{18}	2.1 x 50 mm	1.7 µm	186004044
	2.1 x 100 mm	1.7 µm	186004045
BEH C_8	2.1 x 50 mm	1.7 µm	186004046
	2.1 x 100 mm	1.7 µm	186004047
BEH Shield RP18	2.1 x 50 mm	1.7 µm	186004048
	2.1 x 100 mm	1.7 µm	186004049
BEH Phenyl	2.1 x 50 mm	1.7 µm	186004050
	2.1 x 100 mm	1.7 µm	186004052
BEH HILIC	2.1 x 50 mm	1.7 µm	186004053
	2.1 x 100 mm	1.7 µm	186004054
HSS C_{18}	2.1 x 50 mm	1.8 µm	186004057
	2.1 x 100 mm	1.8 µm	186004058
HSS C_{18} SB	2.1 x 50 mm	1.8 µm	186004137
	2.1 x 100 mm	1.8 µm	186004138
HSS T3	2.1 x 50 mm	1.8 µm	186004055
	2.1 x 100 mm	1.8 µm	186004056

* Contains 3 columns, each packed with a different batch of packing material

VanGuard Pre-Columns for UPLC Column Protection

Chemistry	Dimensions	Particle Size	Part No.
BEH C_{18}	2.1 x 5 mm	1.7 µm	186003975
BEH Shield RP18	2.1 x 5 mm	1.7 µm	186003977
BEH C_8	2.1 x 5 mm	1.7 µm	186003978
BEH Phenyl	2.1 x 5 mm	1.7 µm	186003979
BEH HILIC	2.1 x 5 mm	1.7 µm	186003980
HSS C_{18}	2.1 x 5 mm	1.8 µm	186003981
HSS C_{18} SB	2.1 x 5 mm	1.8 µm	186004136
HSS T3	2.1 x 5 mm	1.8 µm	186003976

ACQUITY UPLC Column In-Line Filter Unit

Description	Part No.
In-line filter holder and six 0.2 µm stainless steel replacement filters	205000343
Five 0.2 µm stainless steel replacement filters and End Nuts for 205000343	700002775

ACQUITY UPLC Column Replacement Parts

Description	Part No.
Three 0.2 µm Inlet/Outlet Frits for 2.1 mm i.d. UPLC Columns	700003776
Three 0.2 µm Inlet/Outlet Frits for 1.0 mm i.d. UPLC Columns	700003775
One Inlet End Nut for 2.1 mm i.d. UPLC Column	700003779
One Outlet End Nut for 2.1 mm i.d. UPLC Column	700003780
One Inlet End Nut for 1.0 mm i.d. UPLC Column	700003777
One Outlet End Nut for 1.0 mm i.d. UPLC Column	700003778

Vials for ACQUITY UPLC Systems, Screw Cap 12 x 32 mm

Maximum Recovery Vials

- Most commonly used vial in ACQUITY UPLC systems
- Best choice when sample volume is limited
- This center draining vial allows access to most of the sample
- Preset needle placement of 2 mm from the bottom of the vial leaves 22 µL residual volume in the vial
- Using ACQUITY UPLC Sample Manager Advanced Setting, adjust the needle placement to access more sample, leaving less residual volume

Description	Part No.
LCMS-Certified Maximum Recovery Vial	600000670CV
LCMS-Certified Amber Maximum Recovery Vial	600000755CV
LCGC-Certified Maximum Recovery Vial	186000327C
LCGC-Certified Amber Maximum Recovery Vial	186003886C

All items come in quantities of 100 unless otherwise stated. For Amino Acid ACQUITY UPLC Systems, the use of total recovery vials is recommended but is only suitable for PEEK needles.

Waters LCMS-Certified Vials

Waters LCMS-certified vials are a continuation of our approach to offer products suitable for the demands of LCMS. We took an unbiased approach in developing this product, looking for any ionized masses regardless of the source. The vials are tested by MS with specifications for total ion count and presence of clusters in the high mass range. The product introduced is cleaner than any product we tested from vendors around the globe.

Waters LCGC-Certified Vials

Vials are usually manufactured by glass artisans and engineers who don't understand the requirements for their use in LC and GC. As a manufacturer of autosamplers and chemistry consumables, Waters understands the dimensional and chemical requirements of vials. We reviewed the manufacturing process, anticipated possible problem areas, and developed tests to ensure the delivery of problem-free products. The LC test, to ensure the delivery of residue-clean vials, is a radically different form of test for the vials industry.

Deactivated Glass Vials

Deactivated glass vials eliminate adsorption of compounds onto the glass surface when working with biological or pharmaceutical compounds, natural products, pesticides, and herbicides. The surface modification is permanent, resulting in an indefinite vial shelf life.

2 mL Vials

- Best Choice where sample volume is not limited
- Large residual volume at preset 2 mm needle placement–165 µL

Description	Part No.
LCMS-Certified 2 mL Vial	600000668CV
LCMS-Certified 2 mL Amber Vial	600000669CV
LCGC-Certified 2 mL Vial	186000307C
LCGC-Certified 2 mL Amber Vial	186000847C
Deactivated Clear Glass 2 mL Vial	186000307DV
Deactivated Amber Glass 2 mL Vial	186000847DV

All products listed are 12 x 32 mm combination packs of 100, screw top vials, caps and pre-slit silicone / PTFE septa

Polypropylene Vials

- Good choice for applications where there is a concern that sample can bond to glass; alternative choice to deactivated glass
- Can be incinerated while still sealed to minimize exposure to hazardous substances
- 300 µL vial requires needle placement of 3 mm (see ACQUITY UPLC System Sample Manager—advanced settings below), leaving a residual volume of 20 µL

Description	Part No.
300 µL Polypropylene (PP) Vial	186002639

All products listed are 12 x 32 mm combination packs of 100, screw top vials, caps and pre-slit silicone / PTFE septa

ACQUITY UPLC Vial Holder

Description	Part No.
48-Well Vial Holder	405003743

Waters ACQUITY UPLC System Sample Manager

The Waters ACQUITY UPLC System Sample Manager incorporates several technology advancements. Low dispersion is maintained through the injection process, using a series of pressure transducers to facilitate self-monitoring and diagnostics. It uses needle-in-needle sampling for improved ruggedness and a needle calibration sensor for increased accuracy. A variety of sample holder formats (vials or tubes) and micro-liter plate formats (deep-well, mid-height) can also be accommodated in a thermostatically-controlled environment. Within the ACQUITY UPLC Sample Manager Instrument Method Editor, a number of parameters can be customized for your specific task, including depth, as shown here, to confer maximum sample format flexibility.

For further information on setting vial depth offsets, see the ACQUITY UPLC Operator's Guide (information documentation set for ACQUITY UPLC–part number 716001664) or visit the ACQUITY UPLC Sample Manager Instrument Method Editor On-Line Help.

Literature References

Waters Heat Sealer User Manual, Literature Reference 720001330EN

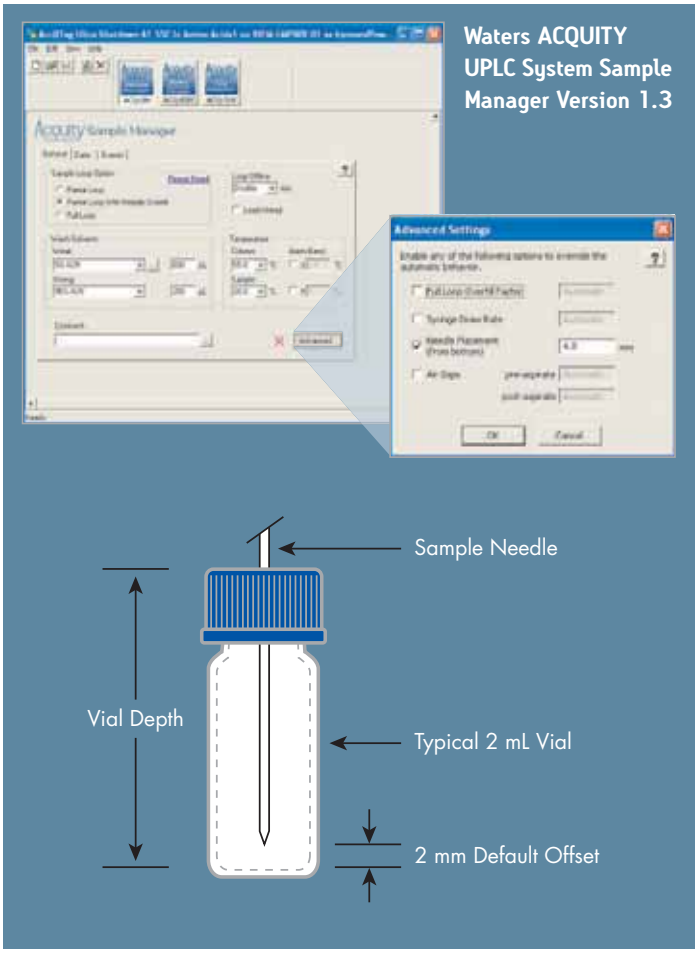
96-well Collection Plate Options for the Waters Extraction Plate Manifold, Literature Reference 720001263EN

Waters 96- and 384-well Collection Plate Specifications, Literature Reference WA41941

Waters LCMS Certified Sample Vials White Paper, Literature Reference 72001517EN

Determination of the Level of Ion Suppression from LCMS Vials, Literature Reference WA60004

Waters Certified Sample Vials Technical White Paper, Literature Reference 720001303EN



96- and 384-Well Plates for ACQUITY UPLC Systems

For PEEK and Metal-Tipped Needles

	96-Well Plate			384-Well Plate	
Plate	186002643	186002481	186002482	186002632	186002631
■ Well Volume	350 µL	1 mL	2 mL	250 µL	100 µL
■ Number of Plates in Sample Organizer	21	10	7	10	21
■ Shape	Round	Round	Square	Square	Square
■ Bottom	Round	Conical	Conical	Conical	Conical
■ Material	PP	PP	PP	PP	PP
■ Height of Plate	14 mm	31 mm	42.5 mm	22 mm	15.5 mm
■ Well Depth	11.25 mm	27 mm	39 mm	19.5 mm	12.3 mm
■ Pack Size	100	50	50	50	50
■ Estimated Residual Volume ACQUITY UPLC	35 µL	15 µL	20 µL	15 µL	15 µL
Seal Options					
PP, 50/pk (for Metal-Tipped Needles ONLY)[1]	186002483	186002483	186002484		
Heat Seal (for All Needles)					
Clear Polyester, 100/pk	186002788	186002788	186002788	186002788	186002788
Aluminum Foil Laminate, 100/pk	186002789	186002789	186002789	186002789	186002789

[1] With ACQUITY UPLC system driver V1.30 and Y-Carriage assembly.

Heat Sealer	Part No.
115 Volt	186002786
240 Volt	186002787

Heat sealer dimensions:
5.75 x 13 x 6 in.
(140 x 330 x 150 mm)

Glass Inserts for Multi-Well Plates

Description (for Metal-Tipped Needles ONLY)	Part No.	Max Volume	Residual Volume
Plates for Quick-Load Glass – Widest Opening for Inserts, 20/pk	186001438		
■ 700 µL Glass – Quick-Load, 1/pk	186001437(DV)[2]	650 µL	15 µL
■ 1 mL Glass – Quick-Load, 1/pk	186001436 (DV)[2]	850 µL	15 µL
96-Well Plate with 700 µL Glass Insert, 1/pk	186000349 (DV)[2]	650 µL	15 µL
96-Well Plate with 1 mL Glass Insert, 18/pk	186000855 (DV)[2]	850 µL	15 µL

[2] When (DV) appears beside the part number, a deactivated version of this product can be ordered by adding DV to the right of the part number.

Recommended Supplies for Use in Conjunction With the ACQUITY UPLC System

Filters

Waters recommends you filter buffers and samples using a 0.2 µm filter prior to use. Filtration protects the column and instrument components from a build-up of particulate matter, improving column lifetime and minimizing system down time. 0.2 µm GHP Filters are recommended for filtering aqueous, non-polar solvents and proteins.

Description	Qty.	Part No.
Solvent filtration membranes 47 mm disc	100/pk*	186003524
13 mm Mini spike (< 14 µL hold up volume)	100/pk	WAT097962
25 mm (< 100 µL hold up volume)	50/pk	WAT097964

* requires solvent filtration apparatus, WAT200543 or equivalent

Sirocco Protein Precipitation Plate

Sirocco™ Protein Precipitation Plate enables high throughput "in-well" protein precipitation. The unique filter system with vented cap mat and patented valve technology, allows the user to process samples "in-well" and collect clean filtrate from smaller plasma volumes without transfer steps.

Description	Qty.	Part No.
Sirocco Protein Precipitation Plate	1/pk	186003873
Sirocco Protein Precipitation Plate	5/pk	186002448

BEH C_{18}

BEH C_8

BEH SHIELD RP18

BEH PHENYL

BEH HILIC

HSS C_{18}

HSS C_{18} SB

HSS T3

PEPTIDES

OLIGONUCLEOTIDES

PROTEINS

ACCQ•TAG ULTRA

VANGUARD

[INNOVATION]

First and Only UPLC-Certified Columns and Pre-Columns

1.7 µm BEH UPLC Particles

1.8 µm HSS UPLC Particles

Reversed-Phase and HILIC

Ten Bonded Phases

VanGuard Pre-Columns

Acquity
UltraPerformance LC®

ACQUITY UPLC® columns and VanGuard™ Pre-Columns are designed, tested and guaranteed for use in applications up to 15000 psi (1000 bar). Unsurpassed efficiency, ruggedness and throughput. Available in over sixty combinations of column configurations and chemistries. Combine faster separations with higher resolution by harnessing the full potential of small particles.

To learn more about ACQUITY UPLC columns and VanGuard Pre-Columns, visit www.waters.com/acquitycolumns

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

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Analytical Chromatography

Introduction

Choosing an HPLC column can be a very difficult process. The Chromatographic Packing Material (particle "brand name" and functional group) plays a major role in the success of a separation, both initially, when the method is first developed, as well as long term, if the method will need to be reproduced for many years.

The "Analytical Chromatography" section of this catalog is structured in two parts:

- General/Technical Information
- Product and Ordering Information

If you know the brand of column you need, go directly to the page shown in the "Product and Ordering Information Index" for that brand.

If you would like to learn more about HPLC column particle technology (packing materials), including better ways to choose packing materials for your application needs, visit www.waters.com/selectivitychart

US Pharmacopeia LC "L1" (C_{18}) Column Designation Subclassification Testing by Brand Name*

Column Brand Name	Hydrophobicity (k, Ethylbenzene)	Chelating (Tailing Factor (TF), Quinizarin)	Silanol Activity (k, Amitriptyline)	Silanol Activity (TF, Amitriptyline)	Shape Selectivity (Bonding Density, µmol/m^2)
ACQUITY UPLC® BEH C_{18}	1.63	1.10	3.79	1.10	3.1
ACQUITY UPLC BEH Shield RP18	1.30	1.36	2.37	1.13	3.3
ACQUITY UPLC HSS C_{18}	2.54	1.36	5.96	1.41	3.2
ACQUITY UPLC HSS C_{18} SB	1.09	1.07	22.2	7.2	1.6
ACQUITY UPLC HSS T3	2.23	1.19	6.50	3.15	1.6
Atlantis® dC_{18}	1.7	1.1	5.1	2.4	1.6
Atlantis T3	1.87	0.98	5.32	1.35	1.6
µBondapak® C_{18}	1.0	6	7.5	4	1.1
Delta-Pak™ C_{18} 100Å	2.0	2	6.3	1.9	3.6
Nova-Pak® C_{18}	1.5	7.5	4.6	3	2.7
SunFire™ C_{18}	2.5	1.2	6.3	1.1	3.5
Symmetry® C_{18}	2.25	1.7	5.1	1.7	3.2
SymmetryShield™ RP18	1.65	1.5	3.1	1.2	3.3
Waters Spherisorb® ODS-1	0.8	no peak	23	3	1.7
Waters Spherisorb ODS-2	2.0	no peak	11.5	7	2.6
XBridge™ C_{18}	1.63	1.10	3.79	1.10	3.1
XBridge Shield RP18	1.30	1.36	2.37	1.13	3.3
XTerra® RP18	1.0	1.2	1.7	1.1	2.3
XTerra MS C_{18}	1.5	1.1	3.3	1.3	2.2
Resolve™ C_{18}	2.0	no peak	35	8	2.8

* For use in C_{18} column brand name selection when referencing USP monographs which specify the characteristics/performance of the C_{18} column used in the method. Choose a brand as close as possible to the referenced column values for the best chance of duplicating the analytical results of the method.

USP "L" Column Listing

L1

Octadecyl silane (ODS or C_{18}) chemically bonded to porous silica or ceramic particles - 1.5 to 10 µm in diameter. See new subclassification table on previous page.

Brand	Particle Size	Type	Page
AccQ•Tag™ Ultra	1.7	Spherical	219
ACQUITY UPLC BEH C_{18}	1.7	Spherical	84
ACQUITY UPLC Shield RP18	1.7	Spherical	84
nanoACQUITY UPLC BEH130	1.7	Spherical	190
nanoACQUITY UPLC BEH300	1.7	Spherical	190
ACQUITY UPLC OST C_{18}	1.7	Spherical	84
ACQUITY UPLC HSS C_{18}	1.8	Spherical	84
ACQUITY UPLC HSS C_{18} SB	1.8	Spherical	84
ACQUITY UPLC HSS T3	1.8	Spherical	84
Atlantis T3	3, 5, 10	Spherical	110
Atlantis dC_{18}	3, 5, 10	Spherical	110
BioSuite™ PA-B	3	Spherical	194
BioSuite PA-A	3	Spherical	194
µBondapak C_{18}	10	Irregular	129
µBondapak C_{18} Radial-Pak	10	Irregular	167
Delta-Pak C_{18}	5	Spherical	130
Nova-Pak C_{18}	4, 6	Spherical	127
Prep Nova-Pak HR C_{18}	6	Spherical	164
Resolve C_{18}	5, 10	Spherical	132
SunFire C_{18}	3.5, 5, 10	Spherical	104
Symmetry C_{18}	3.5, 5, 7	Spherical	117
SymmetryPrep™ C_{18}	3.5, 5, 7	Spherical	163
Symmetry300™ C_{18}	3.5, 5	Spherical	121
SymmetryShield RP18	3.5, 5	Spherical	119
Waters Spherisorb ODS1	3, 5, 10	Spherical	123
Waters Spherisorb ODS2	3, 5, 10	Spherical	123
Waters Spherisorb ODSB	3, 5, 10	Spherical	123
XBridge C_{18}	2.5, 3.5, 5, 10	Spherical	100
XBridge Shield RP18	2.5, 3.5, 5, 10	Spherical	100
XBridge BEH130	3.5, 5, 10	Spherical	190
XBridge BEH300	3.5, 5, 10	Spherical	190
XBridge OST C_{18}	2.5	Spherical	224
XTerra MS C_{18}	2.5, 3.5, 5, 10	Spherical	113
XTerra RP18	3.5, 5, 10	Spherical	113

L2

Octadecyl silane (ODS or C_{18}) chemically bonded to silica gel of a controlled surface porosity bonded to a solid spherical core - 30 to 50 µm in diameter.

Brand	Particle Size	Type	Page
Bondapak® Prep C_{18}	50	Irregular	164

L3

Porous silica particles - 1.5 to 10 µm in diameter.

Brand	Particle Size	Type	Page
ACQUITY UPLC BEH HILIC	1.7	Spherical	84
Atlantis HILIC Silica	3, 5	Spherical	110
BioSuite HR SEC	5, 8	Spherical	212
BioSuite SEC	7.5	Spherical	212
Nova-Pak	6, 4	Spherical	128
µPorasil™	10	Irregular	131
Resolve	5, 10	Spherical	132
SunFire Silica	5, 10	Spherical	159
Waters Spherisorb	5, 10	Spherical	123
XBridge HILIC	2.5, 3.5, 5	Spherical	100

L4

Silica gel of a controlled surface porosity bonded to a solid spherical core - 30 to 50 µm in diameter.

Brand	Particle Size	Type	Page
Porasil™ Prep Silica	50	Irregular	165

L5

Alumina of controlled surface porosity bonded to a solid spherical core - 30 to 50 µm in diameter.

L6

Strong cation exchanger packing - sulfonated fluorocarbon polymer coated on a solid spherical core - 30 to 50 µm in diameter.

L7

Octyl silane (C_8) chemically bonded to porous silica particles - 1.5 to 10 µm in diameter.

Brand	Particle Size	Type	Page
ACQUITY UPLC BEH C_8	1.7	Spherical	84
Nova-Pak C_8	4, 6	Spherical	127
Resolve C_8	5, 10	Spherical	132
Waters Spherisorb C_8	3, 5, 10	Spherical	123
SunFire C_8	3.5, 5, 10	Spherical	104
Symmetry C_8	3.5, 5, 7	Spherical	117
SymmetryShield RP8	3.5, 5	Spherical	119
SymmetryPrep C_8	7	Spherical	163
XBridge C_8	2.5, 3.5, 5, 10	Spherical	110
XTerra MS C_8	2.5, 3.5, 5, 10	Spherical	113
XTerra RP8	3.5, 5, 10	Spherical	113

L8

An essentially monomolecular layer of aminopropylsilane (NH_2) chemically bonded to totally porous silica gel support - 3 to 10 µm in diameter.

Brand	Particle Size	Type	Page
µBondapak NH_2	10	Irregular	129
High Performance Carbohydrate Analysis	3, 5		143
Waters Spherisorb NH_2	3, 5, 10	Spherical	123

L9

3 to 10 µm irregular, totally porous silica gel having a chemically bonded strongly acidic cation exchanger coating (SCX).

Brand	Particle Size	Type	Page
Spherisorb SCX	5, 10	Spherical	123

L10

Nitrile groups (CN) chemically bonded to porous silica particles - 3 to 10 µm in diameter.

Brand	Particle Size	Type	Page
µBondapak CN	10	Irregular	129
Nova-Pak CN HP	4	Spherical	127
Resolve CN	5, 10	Spherical	132
Waters Spherisorb CN	3, 5, 10	Spherical	123

L11
Phenyl groups chemically bonded to porous silica particles - 1.5 to 10 µm in diameter.

Brand	Particle Size	Type	Page
ACQUITY UPLC BEH Phenyl	1.7	Spherical	84
µBondapak Phenyl	10	Irregular	129
Nova-Pak Phenyl	4	Spherical	127
XBridge Phenyl	2.5, 3.5, 5	Spherical	100
Waters Spherisorb Phenyl	3, 5, 10	Spherical	123
XTerra Phenyl	3.5, 5	Spherical	113

L12
A strong anion exchanger packing made by chemically bonding a quaternary amine to a solid silica spherical core - 30 to 50 µm in diameter.

Brand	Particle Size	Type	Page
AccellPlus™ QMA	50	Irregular	209

L13
Trimethylsilane (C1) chemically bonded to porous silica particles - 3 to 10 µm in diameter

Brand	Particle Size	Type	Page
Waters Spherisorb C_1	3, 5, 10	Spherical	123

L14
Silica gel, 5 to 10 µm in diameter having a chemically bonded, strongly basic quaternary ammonium anion exchanger (SAX) coating.

Brand	Particle Size	Type	Page
Waters Spherisorb SAX	5, 10	Spherical	123

L15
Hexylsilane (C_6) chemically bonded to a totally porous silica particle - 3 to 10 µm in diameter.

Brand	Particle Size	Type	Page
Waters Spherisorb C_6	3, 5, 10	Spherical	123

L16
Dimethylsilane (C_2) chemically bonded to a totally porous silica particles - 5 to 10 µm in diameter.

L17
Strong cation exchange resin consisting of sulfonated, cross-linked styrene divinylbenzene copolymer in the hydrogen form, 7 to 11 µm in diameter.

Brand	Particle Size	Type	Page
Fast Fruit Juice	N/A	N/A	145
IC-Pak™ Ion Exclusion	7	Spherical	147
IC-Pak Cation	10	Spherical	147
Shodex® RSpak DC-613	(6)	Spherical	133

L18
Amino (NH_2) and Cyano (CN) groups chemically bonded to porous silica particles - 3 to 10 µm in diameter.

L19
Strong cation exchange resin consisting of sulfonated, cross-linked styrene divinylbenzene copolymer in the calcium form - about 9 µm in diameter.

Brand	Particle Size	Type	Page
Sugar-Pak™ 1	9	Spherical	141
Shodex SC-1011	(7)	Spherical	141

L20
Dihydroxypropane groups chemically bonded to porous silica particles - 3 to 10 µm in diameter.

Brand	Particle Size	Type	Page
BioSuite 125, 250, 450	4, 5, 8, 10, 13, 17	Spherical	212
Insulin HMWP		N/A	197
Protein-Pak™ 60	10	Irregular	206
Protein Pak 125	10	Irregular	206
Protein-Pak 300SW	10	Irregular	206
Protein-Pak KW -802.5	7	Irregular	206
Protein-Pak KW -803	7	Irregular	206
Protein-Pak KW -804	7	Irregular	206

L21
A rigid, spherical styrene-divinylbenzene copolymer - 5 to 10 µm in diameter.

Brand	Particle Size	Type	Page
Shodex RSpak 613	6	Spherical	133
Styragel® HR 0.5, 1, 2, 3, and 4		Spherical	174
Styragel HR 4E		Spherical	174
Styragel 5E		Spherical	174

L22
A cation-exchange resin made of porous polystyrene with sulfonic acid groups - about 10 µm in size.

Brand	Particle Size	Type	Page
IC-Pak Ion Exclusion	7	Spherical	147
Shodex RSpak DC 613	6	Spherical	133
Shodex SP-0810	8	Spherical	141

L23
An anion exchange resin made of porous polymethacrylate or polyacrylate gel with quaternary ammonium groups - about 10 µm in size.

Brand	Particle Size	Type	Page
BioSuite Q AXC	10, 13	Spherical	205
BioSuite DEAE	2.5, 10, 13	Spherical	205
BioSuite Q-PEEK	10	Spherical	205
IC-Pak Anion	10	Spherical	146
Protein-Pak Q 8HR	8	Spherical	188

L24
A semi-rigid hydrophilic gel consisting of vinyl polymers with numerous hydroxyl groups on the matrix surface - 32 to 63 µms in diameter.

L25
Packing having the capacity to separate compounds with a molecular weight range from 100 to 5,000 (as determined by polyethylene oxide), applied to neutral, anionic and cationic water-soluble polymers. A polymethacrylate resin base, cross-linked with polyhydroxylated ether, (surface contained some residual carboxyl groups) was found suitable.

Brand	Particle Size	Type	Page
Ultrahydrogel™ DP, + 120	10	Spherical	181

L26
Butyl silane (C4) chemically bonded to porous silica particles - 3 to 10 µm in diameter.

Brand	Particle Size	Type	Page
ACQUITY UPLC BEH300 C_4	1.7	Spherical	203
Delta-Pak C_4	5	Spherical [100+300Å]	131
Symmetry300 C_4	3.5	Spherical	121
XBridge BEH300 C_4	3.5	Spherical	203

( ) - Denotes particle sizes available outside of L class.

L27
Porous silica particles, 30 to 50 µm in diameter.

Brand	Particle Size	Type	Page
Porasil	37-55	Irregular	131

L28
A multifunctional support which consists of a high purity, 100Å, spherical silica substrate that has been bonded with anionic (amine) functionality in addition to a conventional reversed-phase C_8 functionality.

L29
Gamma alumina, reversed-phase, low carbon percentage by weight alumina-based polybutadiene spherical particles - 5 µm in diameter with a pore diameter of 80Å.

L30
Ethyl silane chemically bonded to a totally porous silica particle - 3 to 10 µm in diameter.

L31
A strong anion-exchange resin-quaternary amine bonded on latex particles attached to a core of 8.5 µm macroporous particles having a pore size of 2,000Å and consisting of ethylvinylbenzene cross-linked with 55% divinyl benzene.

L32
A chiral-ligand exchange packing - L proline copper complex covalently bonded to an irregularly shaped silica particles - 5 to 10 µm in diameter.

L33
Packing having the capacity to separate proteins of 4,000 to 400,000 daltons. It is spherical, silica-based and processed to provide pH stability.

L34
Strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the lead form, about 9 µm in diameter.

Brand	Particle Size	Type	Page
Shodex SP0810	N/A	Spherical	141

L35
Zirconium-stabilized spherical silica packing with a hydrophilic (diol-type) molecular mono layer bonded phase having a pore size of 150Å.

L36
3,5-dinitrobenzoyl derivative of L-phenylglycine covalently bonded to a 5 µm aminopropyl silica.

L37
Packing having the capacity to separate proteins by molecular size over a range of 2,000 to 40,000 daltons. It is a polymethacrylate gel.

Brand	Particle Size	Type	Page
Ultrahydrogel 250	N/A	Spherical	141

L38
A methacrylate-based size-exclusion packing for water soluble samples.

Brand	Particle Size	Type	Page
Ultrahydrogel	N/A	Spherical	141

L39
A hydrophilic-polyhydroxymethacrylate gel of totally porous spherical resin.

Brand	Particle Size	Type	Page
Ultrahydrogel	N/A	Spherical	141

L40
Cellulose tris-3,5-dimethylphenylcarbamate coated porous silica particles, 5 to 20 µm in diameter.

L41
Immobilized α_1-acid glycoprotein on spherical silica particles, 5 µm in diameter.

L42
Octylsilane and octadecylsilane groups chemically bonded to porous silica particles, 5 µm in diameter.

L43
Pentafluorophenyl groups chemically bonded to silica particles, 5 to 10 µm in diameter.

L44
A multifunctional support, which consists of a high purity, 60Å, spherical silica substrate that has been bonded with a cationic exchanger, sulfonic acid functionality in addition to a convention reversed-phase C_8 functionality.

L45
Beta cyclodextrin bonded to porous silica particles, 5 to 10 µm in diameter.

L46
Polystyrene/divinylbenzene substrate agglomerated with quaternary amine functionalized latex beads, 10 µm in diameter.

L55
A strong cation-exchange resin made of porous silica coated with polybutadiene-maleic acid copolymer, about 5 µm in diameter.

Brand	Particle Size	Type	Page
IC-Pak C M/D			147
Waters Spherisorb SCX	5	Spherical	124

L59
Packing having the capacity to separate proteins by molecular weight over the range of 10 to 500 kDa. It is spherical (10 µm), silica-based, and processed to provide hydrophilic characteristics and pH stability.

Brand	Particle Size	Type	Page
Biosuite 125, 250, 450 Series	4-17	Spherical	212

Source: United States Pharmacopeia

( ) - Denotes particle sizes available outside of L class.

Physical Characteristics of HPLC and UPLC Packing Materials

Brand	Chemistry	Particle Shape	Particle Size(s)	Pore Size*	Surface Area [m^2/g]	Pore Volume [cc/g]	% Carbon Load	Endcapped
AccQ•Tag Ultra	C_{18}	Spherical	1.7 µm	135Å	185	0.70	17	yes
ACQUITY UPLC BEH	C_{18}	Spherical	1.7 µm	135Å	185	0.70	17	yes
	C_8	Spherical	1.7 µm	135Å	185	0.70	13	yes
	Shield RP18	Spherical	1.7 µm	135Å	185	0.70	17	yes
	Phenyl	Spherical	1.7 µm	135Å	185	0.70	15	yes
	HILIC	Spherical	1.7 µm	135Å	185	0.70	0	n/a
nanoACQUITY UPLC BEH130	C_{18}	Spherical	1.7 µm	135Å	185	0.70	17	yes
nanoACQUITY UPLC BEH300	C_{18}	Spherical	1.7 µm	300Å	86	0.66	12	yes
ACQUITY UPLC HSS	T3 C_{18}	Spherical	1.8 µm	100Å	230	0.70	11	yes
	C_{18}	Spherical	1.8 µm	100Å	230	0.70	15	yes
	C_{18} SB	Spherical	1.8 µm	100Å	230	0.70	8	no
Atlantis	T3 C_{18}	Spherical	3, 5, 10 µm	100Å	330	1.00	14	yes
	dC_{18}	Spherical	3, 5, 10 µm	100Å	330	1.00	12	yes
	HILIC	Spherical	3, 5 µm	100Å	330	1.00	n/a	n/a
µBondapak	C_{18}	Irregular	10 µm	125Å	330	1.00	9.8	yes
	Phenyl	Irregular	10 µm	125Å	330	1.00	9.3	yes
	CN	Irregular	10 µm	125Å	330	1.00	6.0	yes
	NH_2	Irregular	10 µm	125Å	330	1.00	4.0	no
Bondapak	C_{18}	Irregular	15-20 µm	125Å	330	1.00	10.0	yes
	C_{18}	Irregular	15-20 µm	300Å	100	1.00	3.5	yes
Delta-Pak	C_4	Spherical	5, 15 µm	100Å	300	1.00	7.3	yes
	C_{18}	Spherical	5, 15 µm	100Å	300	1.00	17.0	yes
	C_4	Spherical	5, 15 µm	300Å	125	1.00	2.6	yes
	C_{18}	Spherical	5, 15 µm	300Å	125	1.00	6.8	yes
Nova-Pak	C_{18}	Spherical	4, 6 µm	60Å	120	0.30	7.3	yes
	C_8	Spherical	4 µm	60Å	120	0.30	4.0	yes
	Phenyl	Spherical	4 µm	60Å	120	0.30	4.6	yes
	CN HP	Spherical	4 µm	60Å	120	0.30	3.0	yes
	Silica	Spherical	4, 6 µm	60Å	120	0.30	n/a	n/a
µPorasil	Silica	Irregular	10 µm	125Å	330	1.00	n/a	n/a
Porasil	Silica	Irregular	15-20 µm	125Å	330	1.00	n/a	n/a
Resolve	C_{18}	Spherical	5, 10 µm	90Å	200	0.50	10.2	no
	C_8	Spherical	5 µm	90Å	200	0.50	5.1	no
	CN	Spherical	10 µm	90Å	200	0.50	3.0	no
	Silica	Spherical	5, 10 µm	90Å	200	0.50	n/a	n/a
SunFire	C_{18}	Spherical	2.5, 3.5, 5, 10 µm	100Å	340	0.90	16	yes
	C_8	Spherical	2.5, 3.5, 5, 10 µm	100Å	340	0.90	11.5	yes
	Silica	Spherical	5, 10 µm	100Å	340	0.90	n/a	n/a
Symmetry	C_{18}	Spherical	3.5, 5 µm	100Å	335	0.90	19.1	yes
	C_8	Spherical	3.5, 5 µm	100Å	335	0.90	11.7	yes
SymmetryShield	RP8	Spherical	3.5, 5 µm	100Å	335	0.90	15.0	yes
	RP18	Spherical	5 µm	100Å	335	0.90	17.0	yes
SymmetryPrep	C_{18}	Spherical	7 µm	100Å	335	0.90	19.1	yes
	C_8	Spherical	7 µm	100Å	335	0.90	11.7	yes
Symmetry300	C_{18}	Spherical	3.5, 5 µm	300Å	110	0.80	8.5	yes
	C_4	Spherical	3.5, 5 µm	300Å	110	0.80	2.8	yes

Brand	Chemistry	Particle Shape	Particle Size(s)	Pore Size*	Surface Area [m^2/g]	Pore Volume [cc/g]	% Carbon Load	Endcapped
Waters Spherisorb	Silica	Spherical	3, 5, 10 µm	80Å	220	0.50	n/a	n/a
	ODS_2	Spherical	3, 5, 10 µm	80Å	220	0.50	11.5	yes
	ODS	Spherical	3, 5, 10 µm	80Å	220	0.50	6.2	no
	ODSB	Spherical	5 µm	80Å	220	0.50	11.5	yes
	C_8	Spherical	3, 5, 10 µm	80Å	220	0.50	5.8	yes
	C_6	Spherical	3, 5, 10 µm	80Å	220	0.50	4.7	yes
	C_1	Spherical	3, 5, 10 µm	80Å	220	0.50	2.2	no
	Nitrile	Spherical	3, 5, 10 µm	80Å	220	0.50	3.1	no
	Amino	Spherical	3, 5, 10 µm	80Å	220	0.50	1.9	no
	Phenyl	Spherical	3, 5, 10 µm	80Å	220	0.50	2.5	no
	OD/CN	Spherical	5 µm	80Å	220	0.50	5.0	yes
	SAX, SCX	Spherical	5, 10 µm	80Å	220	0.50	4.0	no
XBridge	C_{18}	Spherical	2.5, 3.5, 5, 10 µm	135Å	185	0.7	17.5	yes
	C_8	Spherical	2.5, 3.5, 5, 10 µm	135Å	185	0.7	17.5	yes
	Shield RP18	Spherical	2.5, 3.5, 5, 10 µm	135Å	185	0.7	17.5	yes
	Phenyl	Spherical	2.5, 3.5, 5 µm	135Å	185	0.7	17.5	yes
XBridge BEH130	C_{18}	Spherical	3.5, 5, 10 µm	135Å	185	0.7	17.5	yes
XBridge BEH300	C_{18}	Spherical	3.5, 5, 10 µm	300Å	86	0.66	12.0	yes
XTerra	RP18	Spherical	3.5, 5, 10 µm	125Å	175	0.70	15.0	yes
	RP8	Spherical	3.5, 5, 10 µm	125Å	175	0.70	13.5	yes
	MS C_{18}	Spherical	2.5, 3.5, 5, 10 µm	125Å	175	0.70	15.5	yes
	MS C_8	Spherical	2.5, 3.5, 5, 10 µm	125Å	175	0.70	12.0	yes
	Phenyl	Spherical	3.5, 5 µm	125Å	175	0.70	12.0	yes

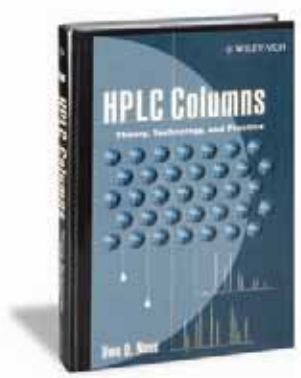
* Nominal value

HPLC Columns Theory, Technology, and Practice

Uwe D. Neue, Waters Corporation, Milford, MA, published by John Wiley & Sons

High-performance liquid chromatography and its derivatives techniques have become the dominant analytical separation tools in the pharmaceutical, chemical, and food industries, environmental laboratories, and therapeutic drug monitoring.

Although the column is the heart of the HPLC instrument and essential to its success, until now no single book has focused on the theory and practice of column technology.



"HPLC Columns" provides thorough, state-of-the-art coverage of HPLC column technology for the practicing technician and academician alike. Along with a comprehensive discussion of the chemical and physical processes of the HPLC column, it includes fundamental principles, separation mechanisms, available technologies, column selection criteria, and special techniques.

Special features include:

- Explanation of the underlying principles of HPLC columns
- Methods for selecting columns
- Practical advice on using and applying columns, including examples
- Section by M. Zoubair El Fallah on methods development
- Special techniques, including preparative chromatography, continuous chromatography, and the simulated moving bed
- Troubleshooting

Description	Part No.
HPLC Columns – Theory, Technology, and Practice	WAT038216

XBridge Columns

A New Milestone in Chromatography

First introduced in 1999 in XTerra columns, Waters patented organic/inorganic Hybrid Particle Technology [HPT] surmounted significant limitations of silica-based, reversed-phase packing materials, particularly their hydrolytic instability at high pH. In 2005, second-generation HPT, branded as BEH Technology™, embodied in new ACQUITY UPLC BEH columns and in Waters XBridge HPLC columns, marks a new milestone in chromatography.

With an order-of-magnitude improvement in high pH stability and a higher level of chromatographic performance, BEH Technology columns define the new benchmark for LC method development.

BEH Technology

BEH Technology, the second generation patented hybrid particle*, is a product of Waters commitment to continued investment in particle Research and Development, providing reliable solutions to complex method development problems. Based upon crucial customer feed-back, three goals for this second-generation hybrid particle were considered paramount.

- Maximize efficiency
- Further improve high-pH stability
- Improve column reliability

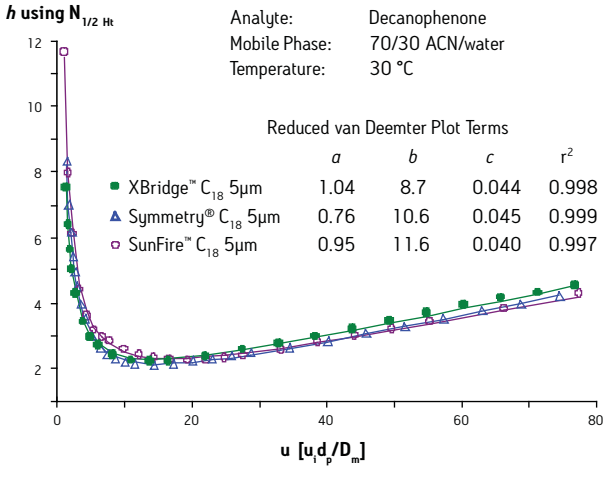
Maximizing Column Efficiency

One of the most important parameters in the design of the new hybrid particle was to significantly improve the chromatographic equivalence to state-of-the-art silica based materials. The origins of band spreading, which decreases separation efficiency, are described by the van Deemter equation:

$$h = a + b/\upsilon + c\upsilon$$

Data for both silica and BEH Technology packings, fitted to the van Deemter equation demonstrate effective equivalence in efficiency. The c term for XBridge C_{18} is virtually identical to that for the two state-of-the-art C_{18} silica columns, indicating that all these columns are comparable in mass transfer characteristics.

van Deemter Curve Comparison



The reduced plate height, h, is a function of the reduced linear velocity, υ, [both normalized for particle size] and a, b, and c summarize the contributions of eddy diffusion, longitudinal diffusion, and the sum of stationary- and mobile-phase mass transfer terms, respectively.

* Patent # 6,686,035 B2

pH Stability

Improved Low pH Performance

The predominant cause of shortened column lifetime in low pH mobile phases relates to the acid hydrolysis of the bonded phase. This hydrolysis can lead to significant changes in analyte retention time, making method suitability requirements difficult to achieve.

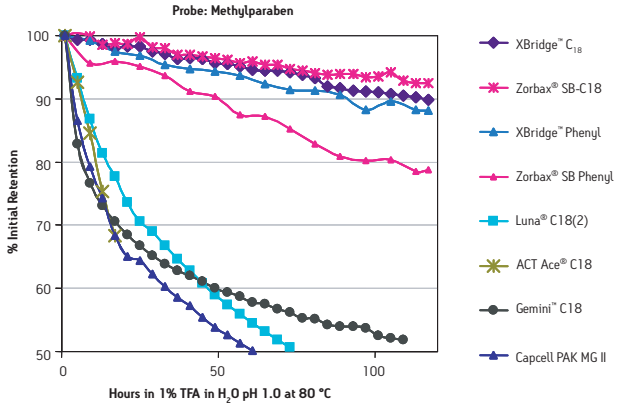
XBridge packings incorporate the use of well-characterized, state-of-the-art, proprietary procedures for bonding and endcapping. Bonded phases prepared using these procedures are much more stable and reproducible at low pH than conventionally prepared materials and resist the typical ligand hydrolysis failure. In an accelerated low pH stability test, XBridge C_{18} columns show very little retention loss and exhibit a column lifetime equivalent to that of a sterically hindered C_{18} silica-bonded phase.

Over 1000% Longer High pH Endurance

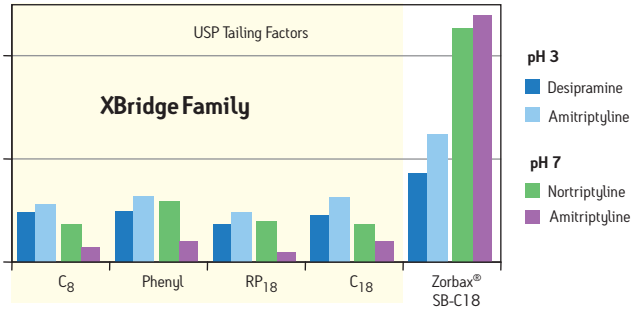
XBridge columns have been specifically designed to be the most pH stable high-performance chromatographic phases commercially available. Unlike approaches which claim high pH resistance due to special surface modifications, XBridge columns have stability built in as part of the particle synthetic process.

Under accelerated pH 10 stability test conditions, a direct comparison to some of the most popular chromatographic phases, claimed to have extended high pH stability, clearly shows the XBridge C_{18} column lifetime exceeding that of the best silica column by over 1000% with very little degradation in chromatographic performance.

Accelerated Low pH Stability Tests of Competitive Columns

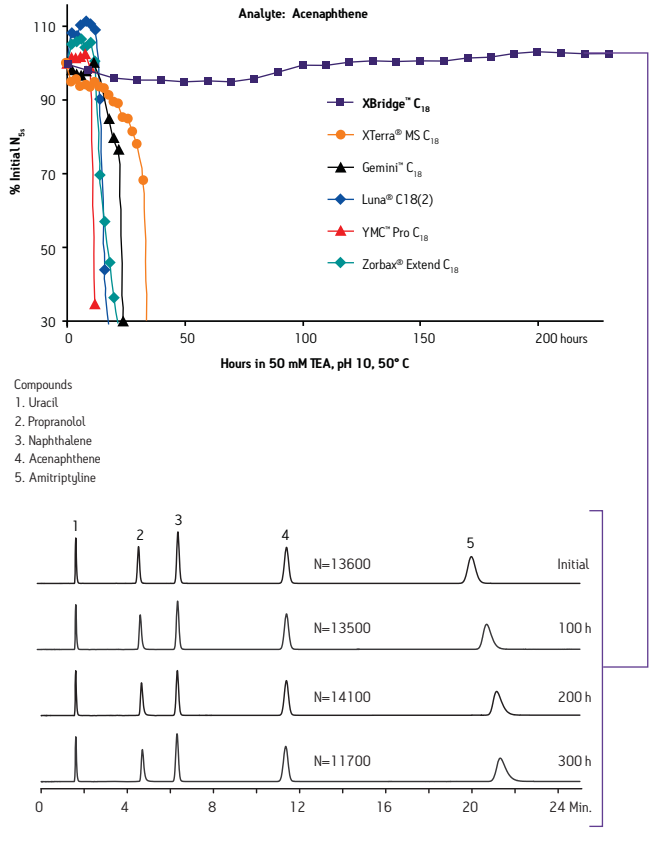


USP Tailing Factors at pH 3 and pH 7



The combination of excellent particle and ligand stability as well as high chromatographic efficiencies makes XBridge columns an ideal choice for low and intermediate pH methods.

Accelerated High pH Stability Test of Competitive Columns



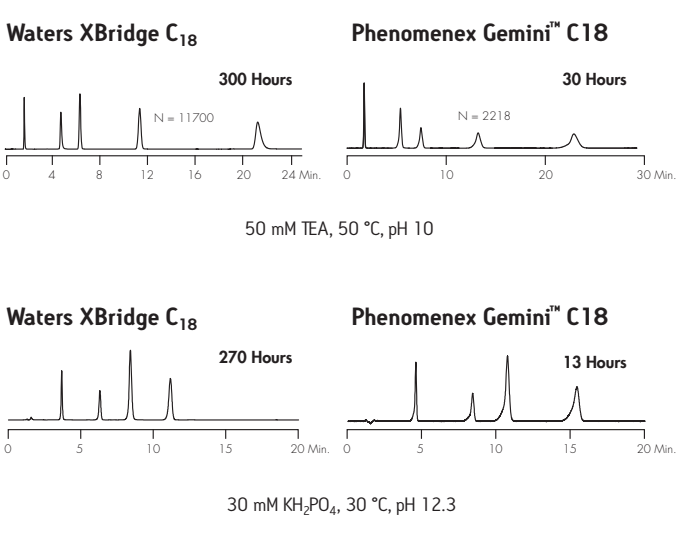
Chromatograms, run at regular intervals during the high-pH lifetime study, verify that 86% of the original XBridge column efficiency remains after 300 hours at pH 10 and elevated temperature, with little change in peak shape or retention time.

Column Lifetime and Buffer Choice

The choice of mobile phase buffer and concentration, particularly at elevated pH, has a profound impact not only on chromatographic peak shape but also on column lifetime. In order to be successful, the method development chemist requires the flexibility to choose the appropriate buffer for selectivity and detection technique.

The XBridge family of columns exhibit maximum stability in the widest range of volatile and non-volatile buffer types.

Chromatographic Lifetime Comparisons



Method Development Flexibility

The Universal Family of Columns

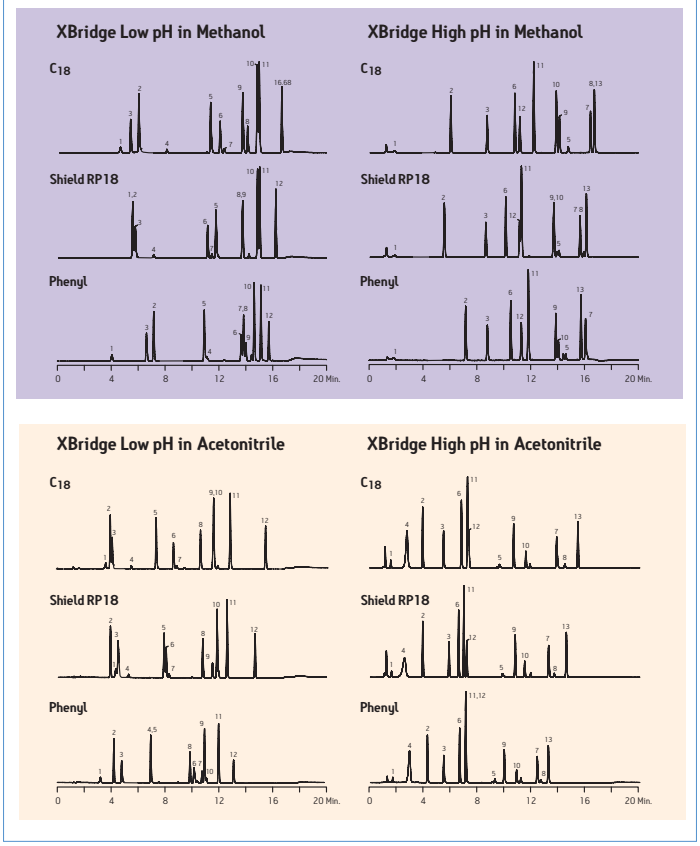
The development route to a final, robust LC method may require investigation of many selectivity tools, such as bonded ligands, solvent type, modifiers, temperature and most importantly pH. This route is complicated further by the fact that modern column chemistries usually are designed to excel in only a limited combination of these conditions, making the selection of the most suitable column extremely difficult.

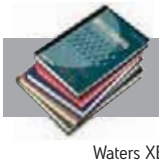
The XBridge family of columns is designed to eliminate this compromise and deliver the flexibility to work under any mobile phase, temperature and pH conditions, thus speeding up the process to an optimum and rugged final method.

Efficient Method Development

The XBridge family of columns introduces a new level of chromatographic performance and confidence to the method development chemist. Robust, rugged methods can now be achieved across the entire pH range of 1-12, simplifying the validation and method transfer process.

Utilizing a simple method development protocol, consisting of XBridge columns, two mobile phase pHs, and two organic solvents, even the most challenging of sample mixtures can be quickly resolved.





Literature References

Waters XBridge HPLC Columns Brochure, Literature Reference 720001255EN

Waters XBridge HPLC Columns White Paper, Literature Reference 720001159EN

Utlizing XBridge HPLC Columns for Method Development at pH Extremes Application Note, Literature Reference WA43181

Interactive Waters Reversed-Phase Column Selectivity Chart, www.waters.com/selectivitychart

XBridge and Phosphate Buffer

With the introduction, and subsequent widespread adoption, of high purity reversed-phase silica columns in the early 1990's, the method development chemist has been unable to freely utilize phosphate buffers (pH 7 and above) due to their extremely aggressive nature. The combination of phosphate, intermediate pH and relatively low temperatures (40 °C) is well recognized to lead to short column lifetimes for most modern phases.

Phosphate buffers however do have quite desirable qualities for a non-MS based assay:

- Excellent UV transparency
- Unique peak shape and selectivity characteristics
- Good buffering capacity at multiple pKa values

Particle Sizes and Dimensions for Method Optimization

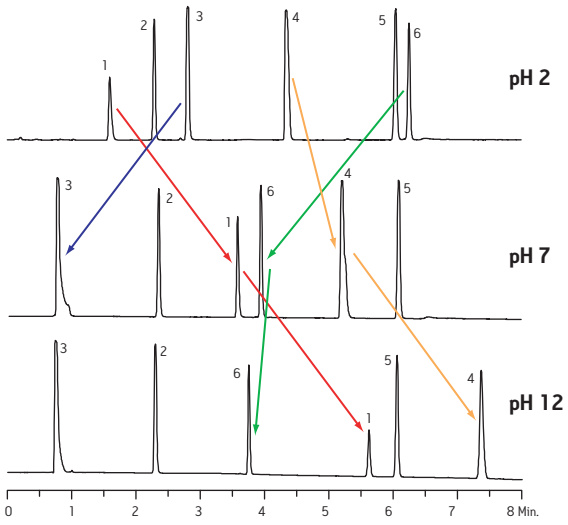
The increasing productivity demands on the chromatography laboratory present an additional tough challenge to the method developer. Once an initial method has been established, optimization of the total cycle time becomes the key parameter.

The availability of multiple particle sizes and dimensions allows the optimization of the total cycle time without sacrificing resolution. Methods can easily be transferred from HPLC to UPLC and from UPLC to HPLC.

Creating Selectivity Changes with Phosphate Buffer

Column:	XBridge C_{18}, 4.6 x 100 mm, 3.5 µm
Mobile Phase:	A1: 30 mM potassium phosphate buffer (pH 2) A2: 30 mM potassium phosphate buffer (pH 7) A3: 30 mM potassium phosphate buffer (pH 12)
Mobile Phase B:	Acetonitrile
Flow Rate:	1.4 mL/min
Gradient:	Time (min) / %A / %B 0.0 / 90 / 10 7.0 / 20 / 80 8.0 / 20 / 80
Injection Volume:	20 µL
Sample Concentration:	50 µg/mL each in 80:20 Water:Methanol
Temperature:	30 °C
Detection:	UV @ 210 nm (pH 2,7); 220 nm (pH 12)
Instrument:	Waters Alliance® 2695 with 2996 PDA

Compounds
1. Doxylamine
2. Benzamide
3. Hydroxyisophthalic Acid
4. Doxepine
5. Flavone
6. Fenoprofen

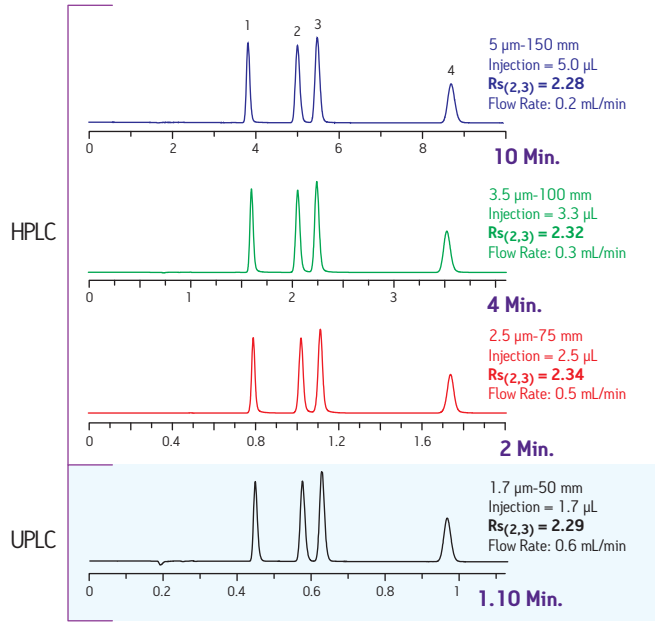


XBridge columns are unique in their ability to withstand these aggressive conditions (even at pH 12) allowing complete flexibility for the method development chemist, while maintaining the highest efficiency values associated with silica columns.

Maintaining Resolution with Constant Length/Particle Size Ratio

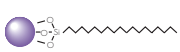
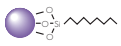
Mobile Phase A:	0.1% FA in H_2O
Mobile Phase B:	0.1% FA in ACN
Isocratic:	95% A: 5% B

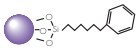
Compounds
1. 1-methylxanthene
2. 1,3-dimethyluric acid
3. theobromine
4. 1,7-dimethylxanthene



ACQUITY UPLC systems and BEH chemistries are specifically designed to enable the quickest method development procedures, fastest cycle times while delivering maximum resolution.

XBridge Column Characteristics




Bonded Phase	Ligand Type	Trifunctional C_{18}	Trifunctional C_8	Monofunctional Embedded Polar Group	Trifunctional C_6 Phenyl	Unbonded BEH
	Available Particle Sizes (µm)	2.5, 3.5, 5, 10 µm	2.5, 3.5, 5, 10 µm	2.5, 3.5, 5, 10 µm	2.5, 3.5, 5 µm	2.5, 3.5, 5 µm
	Ligand Density*	3.1 µmol/m^2	3.2 µmol/m^2	3.3 µmol/m^2	3.0 µmol/m^2	N/A
	Carbon Load*	18%	13%	17%	15%	N/A
	Endcap Style	Proprietary	Proprietary	TMS	Proprietary	N/A
	pH Range	1-12	1-12	2-11	1-12	1-8
	Low pH Temp. Limits	80 °C	60 °C	30 °C	80 °C	45 °C
	High pH Temp. Limits	45 °C	45 °C	45 °C	45 °C	45 °C
BEH Particle	Pore Diameter*	135Å	135Å	135Å	135Å	130Å
	Pore Volume*	0.7 mL/g	0.7 mL/g	0.7 mL/g	0.7 mL/g	0.7 mL/g
	Surface Area*	185 m^2/g	185 m^2/g	185 m^2/g	185 m^2/g	185 m^2/g

** Nominal Values*

XBridge Analytical Columns

Dimensions	Type	Particle Size	C_{18}	C_8	Shield RP18	Phenyl	HILIC
1.0 x 50 mm	Column	2.5 µm	186003118	186003164	186003136	186003306	—
2.1 x 10 mm	Guard	2.5 µm	186003056[1]	186003074[1]	186003065[1]	186003359[1]	186004455
2.1 x 20 mm *IS*™	Column	2.5 µm	186003201	186003167	186003139	186003307	—
2.1 x 30 mm	Column	2.5 µm	186003084	186003099	186003091	186003308	186004456
2.1 x 50 mm	Column	2.5 µm	186003085	186003101	186003092	186003309	186004457
3.0 x 20 mm *IS*	Column	2.5 µm	186003087	186003168	186003140	186003310	—
3.0 x 20 mm	Guard	2.5 µm	186003057[2]	186003075[2]	186003066[2]	186003360[2]	—
3.0 x 30 mm	Column	2.5 µm	186003121	186003169	186003141	186003311	—
3.0 x 50 mm	Column	2.5 µm	186003122	186003170	186003142	186003312	186004458
4.6 x 20 mm *IS*	Column	2.5 µm	186003088	186003172	186003144	186003313	—
4.6 x 20 mm	Guard	2.5 µm	186003058[2]	186003076[2]	186003067[2]	186003361[2]	186004459[2]
4.6 x 30 mm	Column	2.5 µm	186003089	186003173	186003145	186003314	—
4.6 x 50 mm	Column	2.5 µm	186003090	186003174	186003096	186003315	186004460
4.6 x 75 mm	Column	2.5 µm	186003124	186003175	186003146	186003316	186004461
1.0 x 50 mm	Column	3.5 µm	186003126	186003177	186003148	186003317	186004429
1.0 x 100 mm	Column	3.5 µm	186003127	186003178	186003149	186003318	—
1.0 x 150 mm	Column	3.5 µm	186003128	186003179	186003150	186003319	—
2.1 x 10 mm	Guard	3.5 µm	186003059[1]	186003077[1]	186003068[1]	186003362[1]	186004430[1]
2.1 x 20 mm *IS*	Column	3.5 µm	186003019	186003180	186003151	186003320	—
2.1 x 30 mm	Column	3.5 µm	186003020	186003046	186003035	186003321	186004431
2.1 x 50 mm	Column	3.5 µm	186003021	186003047	186003036	186003322	186004432
2.1 x 100 mm	Column	3.5 µm	186003022	186003048	186003037	186003323	186004433
2.1 x 150 mm	Column	3.5 µm	186003023	186003049	186003038	186003324	186004434
3.0 x 20 mm *IS*	Column	3.5 µm	186003024	186003181	186003152	186003325	—
3.0 x 20 mm	Guard	3.5 µm	186003060[2]	186003078[2]	186003069[2]	186003363[2]	—
3.0 x 30 mm	Column	3.5 µm	186003025	186003182	186003153	186003326	—
3.0 x 50 mm	Column	3.5 µm	186003026	186003050	186003039	186003327	186004435
3.0 x 100 mm	Column	3.5 µm	186003027	186003051	186003040	186003328	186004436
3.0 x 150 mm	Column	3.5 µm	186003028	186003052	186003041	186003329	—

[1] Requires Universal Sentry™ Guard Holder - 2.1 x 10 mm WAT097958
[2] Requires Universal Sentry Guard Holder - 3.0 x 20 mm/4.6 x 20 mm WAT046910

XBridge Analytical Columns

Dimensions	Type	Particle Size	C_{18}	C_8	Shield RP18	Phenyl	HILIC
4.6 x 20 mm *IS*	Column	3.5 µm	186003029	186003183	186003154	186003330	—
4.6 x 20 mm	Guard	3.5 µm	186003061[2]	186003079[2]	186003070[2]	186003364[2]	186004437[2]
4.6 x 30 mm	Column	3.5 µm	186003030	186003184	186003155	186003331	186004438
4.6 x 50 mm	Column	3.5 µm	186003031	186003053	186003042	186003332	186004439
4.6 x 75 mm	Column	3.5 µm	186003032	186003185	186003043	186003333	—
4.6 x 100 mm	Column	3.5 µm	186003033	186003054	186003044	186003334	186004440
4.6 x 150 mm	Column	3.5 µm	186003034	186003055	186003045	186003335	186004441
4.6 x 250 mm	Column	3.5 µm	186003943	186003963	186003964	186003965	—
2.1 x 10 mm	Guard	5 µm	186003062[1]	186003080[1]	186003071[1]	186003366[1]	186004442[1]
2.1 x 20 mm *IS*	Column	5 µm	186003107	186003186	186003156	186003336	—
2.1 x 30 mm	Column	5 µm	186003129	186003187	186003157	186003337	186004443
2.1 x 50 mm	Column	5 µm	186003108	186003011	186002999	186003338	186004444
2.1 x 100 mm	Column	5 µm	186003109	186003012	186003002	186003339	186004445
2.1 x 150 mm	Column	5 µm	186003110	186003013	186003003	186003340	186004446
3.0 x 20 mm *IS*	Column	5 µm	186003130	186003188	186003158	186003341	—
3.0 x 20 mm	Guard	5 µm	186003063[2]	186003081[2]	186003072[2]	186003367[2]	—
3.0 x 30 mm	Column	5 µm	186003111	186003189	186003159	186003342	—
3.0 x 50 mm	Column	5 µm	186003131	186003190	186003160	186003343	186004447
3.0 x 100 mm	Column	5 µm	186003132	186003191	186003004	186003344	186004448
3.0 x 150 mm	Column	5 µm	186003112	186003014	186003005	186003345	—
3.0 x 250 mm	Column	5 µm	186003133	186003192	186003161	186003346	—
4.6 x 20 mm *IS*	Column	5 µm	186003134	186003193	186003162	186003347	—
4.6 x 20 mm	Guard	5 µm	186003064[2]	186003082[2]	186003073[2]	186003368[2]	186004449[2]
4.6 x 30 mm	Column	5 µm	186003135	186003194	186003163	186003348	186004450
4.6 x 50 mm	Column	5 µm	186003113	186003015	186003006	186003349	186004451
4.6 x 75 mm	Column	5 µm	186003114	186003195	186003007	186003350	—
4.6 x 100 mm	Column	5 µm	186003115	186003016	186003008	186003351	186004452
4.6 x 150 mm	Column	5 µm	186003116	186003017	186003009	186003352	186004453
4.6 x 250 mm	Column	5 µm	186003117	186003018	186003010	186003353	186004454

[1] Requires Universal Sentry Guard Holder - 2.1 x 10 mm WAT097958
[2] Requires Universal Sentry Guard Holder - 3.0 x 20 mm/4.6 x 20 mm WAT046910

Also Available in Optimum Bed Density (OBD) Preparative Columns, See Page 157

XBridge Column Method Validation Kits

Each Method Validation Kit contains 3 columns, each from a different batch.

Dimensions	Type	Particle Size	C_{18}	C_8	Shield RP18	Phenyl
2.1 x 100 mm	MV Kit	3.5 µm	186003766	186003777	186003788	186003799
3.0 x 100 mm	MV Kit	3.5 µm	186003767	186003778	186003789	186003800
3.0 x 150 mm	MV Kit	3.5 µm	186003768	186003779	186003790	186003801
4.6 x 100 mm	MV Kit	3.5 µm	186003769	186003780	186003791	186003802
4.6 x 150 mm	MV Kit	3.5 µm	186003770	186003781	186003792	186003803
2.1 x 150 mm	MV Kit	5 µm	186003771	186003782	186003793	186003804
3.0 x 100 mm	MV Kit	5 µm	186003772	186003783	186003794	186003805
3.0 x 150 mm	MV Kit	5 µm	186003773	186003784	186003795	186003806
4.6 x 100 mm	MV Kit	5 µm	186003774	186003785	186003796	186003807
4.6 x 150 mm	MV Kit	5 µm	186003775	186003786	186003797	186003808
4.6 x 250 mm	MV Kit	5 µm	186003776	186003787	186003798	186003809

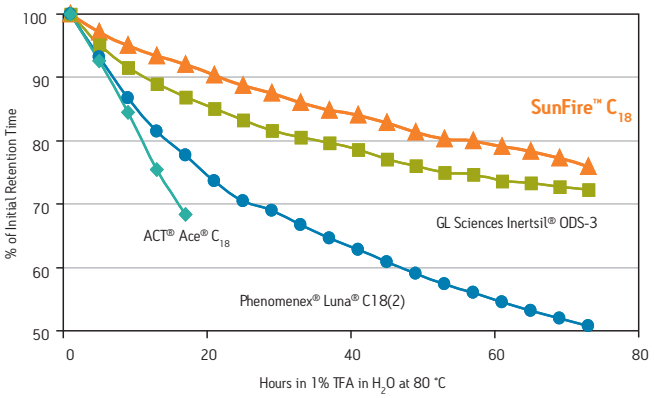
SunFire™ COLUMNS

SunFire C_{18} and C_8 Analytical Columns

SunFire™ columns set the standard for the state-of-the-art bonded C_{18} and C_8 silica HPLC columns. Benefiting from years of research and product development, SunFire columns represent the best in particle and bonding expertise and deliver the industry-leading level of chromatographic performance.

Excellent Low pH Stability

Column lifetime is improved by low pH stability superior to that of many silica-based HPLC column brands



High Efficiency

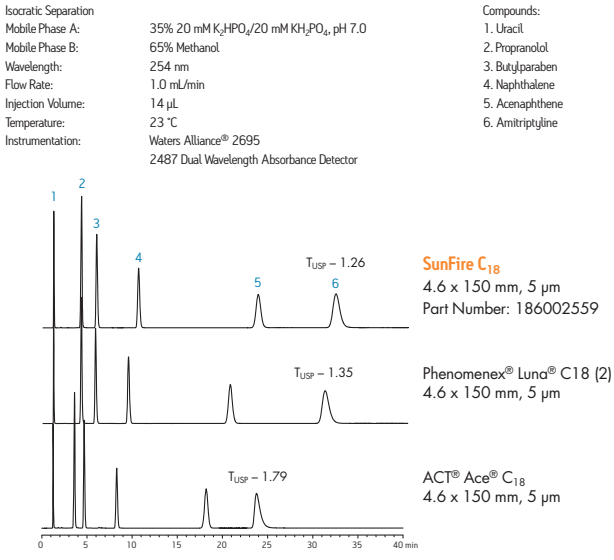
A combination of proprietary, state-of-the-art silica synthesis, bonding, end-capping, and packing technologies produce SunFire columns with high efficiency. One important benefit of these efforts is greater sensitivity.

MS-Compatibility

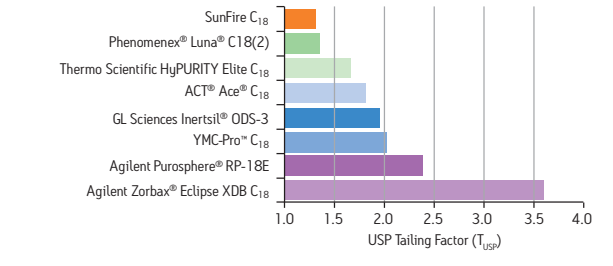
SunFire columns are compatible with mass spectrometry applications, providing sharp peaks, excellent sensitivity, high peak capacity, and ultra-low bleed. In addition, the speed, excellent resolution, and low backpressure offered by SunFire Intelligent Speed (*IS*) columns reduce costs and analysis times.

Superior Peak Shape

With new bonding and new end-capping technologies for the SunFire columns, Waters has developed a sorbent with superior peak shape performance. SunFire columns provide symmetrical peaks for improved resolution and quantitation of acidic, neutral, and basic compounds at low and intermediate pH ranges.



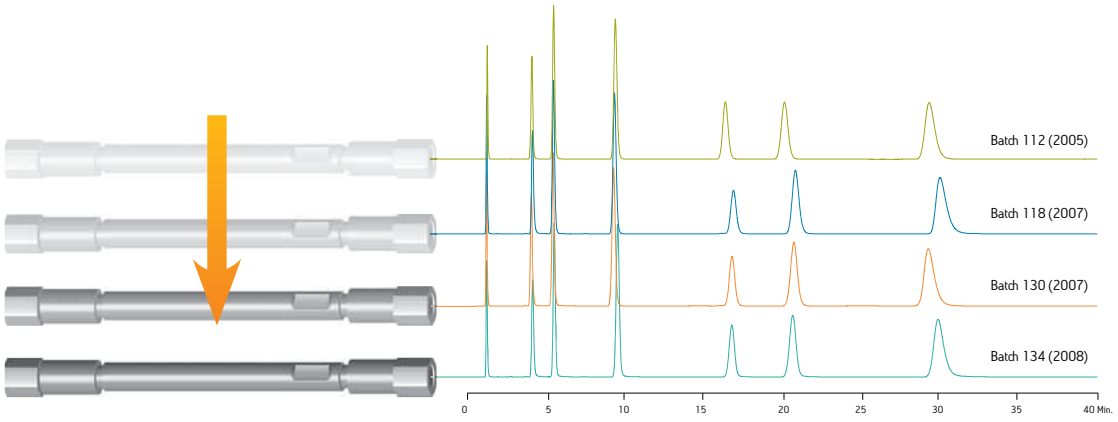
Comparison of C_{18} 5 µm HPLC Columns



In this comparison test of columns packed with 5 µm particles, SunFire C_{18} column has the lowest USP tailing factor for the basic probe amitriptyline under the most demanding pH 7 mobile-phase conditions.

Batch-to-Batch Reproducibility

In establishing new analytical methods for the latest pharmaceutical and biopharmaceutical products, the selection of a reproducible HPLC column is essential. The selected column needs to provide the same chromatographic results over the life of the method and the new drug product. SunFire columns have demonstrated superior reproducibility over many years. Batches randomly selected over the past 4 years show excellent reproducibility in the example below.

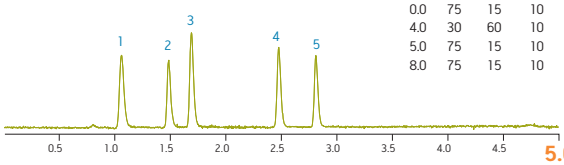


This excellent reproducibility is a result of our commitment to maintaining the tightest specifications in the HPLC column industry. SunFire columns start with high purity raw materials, and are produced using tightly controlled manufacturing processes and column packing procedures that provide today's scientists with the best, most reproducible HPLC columns available.

SunFire 2.5 µm Columns

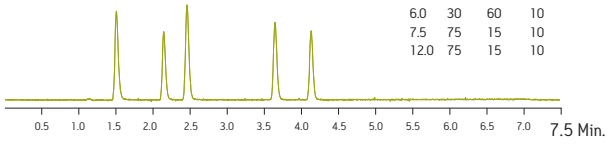
This smaller particle size allows chromatographers to gain improved sensitivity and greater efficiency. SunFire columns with 2.5 µm particle size enable faster run times while maintaining the same resolution.

SunFire C_{18}, 4.6 x 50 mm, 2.5 µm
ΔP = 1800 psi
Pc = 48
$Rs_{2/3}$ = 3.5



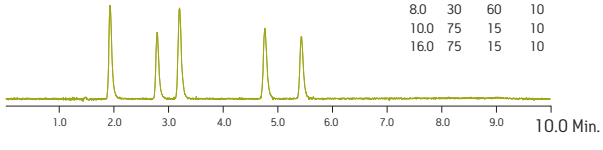
Min	% A	% B	% C
0.0	75	15	10
4.0	30	60	10
5.0	75	15	10
8.0	75	15	10

SunFire C_{18}, 4.6 x 75 mm, 3.5 µm
ΔP = 1360 psi
Pc = 55
$Rs_{2/3}$ = 3.8



Min	% A	% B	% C
0.0	75	15	10
6.0	30	60	10
7.5	75	15	10
12.0	75	15	10

SunFire C_{18}, 4.6 x 100 mm, 5 µm
Δ
P = 880 psi
Pc = 49
$Rs_{2/3}$ = 3.6



Min	% A	% B	% C
0.0	75	15	10
8.0	30	60	10
10.0	75	15	10
16.0	75	15	10

Columns:	SunFire C_{18}, 4.6 x 50 mm, 2.5 µm (186003417); SunFire C_{18}, 4.6 x 75 mm, 3.5 µm (186002552); SunFire C_{18}, 4.6 x 100 mm, 5 µm (186002558)
Mobile Phase A:	Water
Mobile Phase B:	Acetonitrile
Mobile Phase C:	100 mM CH_3COONH_4, pH 5.0
Flow Rate:	1 mL/min
Sample:	Sulfanilamide, sulfathiazole, sulfamerazine, sulfamethoxazole and sulfaquinoxaline dissolved in water at concentration of 10 µg/mL each
Injection Volumes:	5, 7, 10 µL
Column Temperature:	Ambient
Detection:	UV @ 270 nm
Sampling Rate:	5 pt/sec
Instrument:	Waters Alliance 2695 with 2996 PDA (no instrument modifications)

Physical Characteristics

Chemistry	Particle Size	Particle Shape	Pore Size	Carbon Load	End-capped
C_{18}	2.5, 3.5, 5, 10 µm	Spherical	100Å	16%	Yes
C_8	2.5, 3.5, 5, 10 µm	Spherical	100Å	11.5%	Yes
Silica	5, 10 µm	Spherical	100Å	N/A	N/A

SunFire 2.5 µm Analytical Columns

Particle Size	Dimensions	C_{18}	C_8
2.5 µm	1.0 x 50 mm	186003392	186003394
2.5 µm	2.1 x 20 mm *IS*	186003397	186003398
2.5 µm	2.1 x 30 mm	186003399	186003400
2.5 µm	2.1 x 50 mm	186003401	186003402
2.5 µm	3.0 x 20 mm *IS*	186003403	186003404
2.5 µm	3.0 x 30 mm	186003407	186003408
2.5 µm	3.0 x 50 mm	186003409	186003410
2.5 µm	4.6 x 20 mm *IS*	186003411	186003412
2.5 µm	4.6 x 30 mm	186003415	186003416
2.5 µm	4.6 x 50 mm	186003417	186003418
2.5 µm	4.6 x 75 mm	186003419	186003420

SunFire 3.5 µm Analytical Columns

Particle Size	Dimensions	C_{18}	C_8
3.5 µm	1.0 x 50 mm	186002526	186002705
3.5 µm	1.0 x 150 mm	186002528	186002706
3.5 µm	2.1 x 20 mm *IS*	186002531	186002697
3.5 µm	2.1 x 30 mm	186002532	186002709
3.5 µm	2.1 x 50 mm	186002533	186002710
3.5 µm	2.1 x 100 mm	186002534	186002711
3.5 µm	2.1 x 150 mm	186002535	186002712
3.5 µm	3.0 x 20 mm *IS*	186002686	186002701
3.5 µm	3.0 x 30 mm	186003254	Custom
3.5 µm	3.0 x 50 mm	186002542	186002719
3.5 µm	3.0 x 100 mm	186002543	186002720
3.5 µm	3.0 x 150 mm	186002544	186002721
3.5 µm	4.6 x 20 mm *IS*	186002549	186002699
3.5 µm	4.6 x 30 mm	186002550	186002728
3.5 µm	4.6 x 50 mm	186002551	186002729
3.5 µm	4.6 x 75 mm	186002552	186002730
3.5 µm	4.6 x 100 mm	186002553	186002731
3.5 µm	4.6 x 150 mm	186002554	186002732

Literature References

SunFire Columns-The Standard in Silica-Based HPLC Column Performance, Literature Reference 720000875EN

Bridging the Performance Gap From Analytical to Prep Optimum Bed Density (OBD) Preparative Columns Brochure, Literature Reference 720002336EN

A Sensitive Method for the Determination of Endocrine-Disrupting Compounds in River Water by LC/MS/MS, Applications Note, Literature Reference 720001296EN

A New State-of-the-Art Silica C_{18} Column for Improved Peak Shape of Basic Analytes, Applications Note, Literature Reference WA40526EN

Interactive Waters Reversed-Phase Column Selectivity Chart, www.waters.com/selectivitychart

SunFire 5 µm Analytical Columns

Particle Size	Dimensions	C_{18}	C_8
5 µm	1.0 x 150 mm	186002529	186002707
5 µm	2.1 x 20 mm *IS*	186002537	186002698
5 µm	2.1 x 30 mm	186002538	186002714
5 µm	2.1 x 50 mm	186002539	186002715
5 µm	2.1 x 100 mm	186002540	186002716
5 µm	2.1 x 150 mm	186002541	186002717
5 µm	3.0 x 20 mm *IS*	186002685	186002702
5 µm	3.0 x 50 mm	186002545	186002723
5 µm	3.0 x 100 mm	186002546	186002724
5 µm	3.0 x 150 mm	186002547	186002725
5 µm	3.0 x 250 mm	186002548	186002726
5 µm	4.6 x 20 mm *IS*	186002555	186002700
5 µm	4.6 x 30 mm	186002556	186002734
5 µm	4.6 x 50 mm	186002557	186002735
5 µm	4.6 x 100 mm	186002558	186002736
5 µm	4.6 x 150 mm	186002559	186002737
5 µm	4.6 x 250 mm	186002560	186002738

SunFire Method Validation Kits

Particle Size	Dimensions	C_{18}	C_8
3.5 µm	2.1 x 100 mm MV Kits	186002674	186002739
3.5 µm	4.6 x 100 mm MV Kits	186002675	186002740
3.5 µm	4.6 x 150 mm MV Kits	186002676	186002741
5 µm	4.6 x 100 mm MV Kits	186002677	186002742
5 µm	2.1 x 150 mm MV Kits	186002678	186002743
5 µm	4.6 x 150 mm MV Kits	186002679	186002744
5 µm	4.6 x 250 mm MV Kits	186002680	186002745

SunFire Analytical Guard Columns

Particle Size	Sentry Guard Columns (2/pk)	C_{18}	C_8
2.5 µm	2.1 x 10 mm	186003395[3]	186003396[3]
2.5 µm	3.0 x 20 mm	186003405[4]	186003406[4]
2.5 µm	4.6 x 20 mm	186003413[4]	186003414[4]
3.5 µm	2.1 x 10 mm	186002530[3]	186002708[3]
3.5 µm	3.0 x 20 mm	186002681[4]	186002718[4]
3.5 µm	4.6 x 20 mm	186002682[4]	186002727[4]
5 µm	2.1 x 10 mm	186002536[3]	186002713[3]
5 µm	4.6 x 20 mm	186002684[4]	186002733[4]
5 µm	3.0 x 20 mm	186002683[4]	186002722[4]

[3] Requires Universal Sentry Guard Column Holder - 2.1 x 10 mm WAT097958

[4] Requires Universal Sentry Guard Column Holder - 3.0 x 20 mm WAT046910

Also available in Optimum Bed Density (OBD) Preparative Columns, See Page 159

Atlantis® Columns

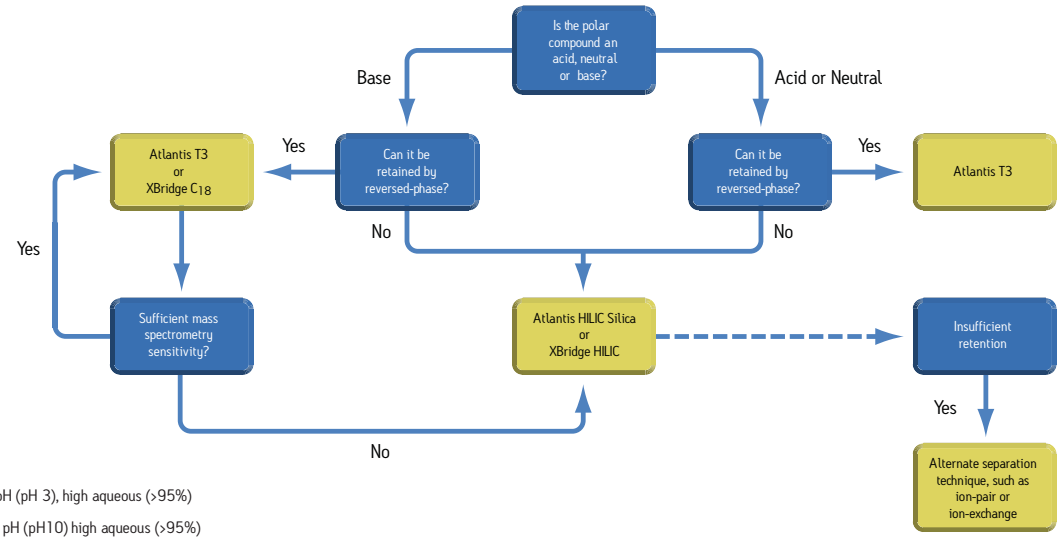
Atlantis Columns

The Atlantis® family of HPLC columns was created to solve one of the most challenging chromatographic problems, retaining polar compounds. Through historical experience and synthetic innovation, Atlantis columns lead the industry in providing exceptional performance, versatility, retention and chromatographic stability for polar compounds, while also affording balanced retention for broad analyte mixtures.

For retention and separation of polar compounds via reversed-phase HPLC, Atlantis T3 columns set the standard for polar compound retention. Atlantis T3 columns are a universal, silica-based, reversed-phase C_{18} line of HPLC columns that not only retain and separate small, water-soluble polar organic compounds, but also provide superior performance across a wider pH range. Atlantis columns are the culmination of over 30 years of bonded phase research and are a vast improvement upon the highly successful Atlantis dC_{18} line of columns. For reversed-phase applications, Atlantis T3 columns should be considered the first choice when developing a separation of polar (and non-polar) compounds. The advantages of T3 bonding technology is extended to UPLC in the ACQUITY UPLC HSS T3 columns.

Atlantis HILIC silica columns retain and separate very polar compounds, such as actives and metabolites using Hydrophilic-Interaction Chromatography (HILIC). Why HILIC? HILIC provides retention when traditional chromatographic techniques will not work. HILIC also offers advantages such as improved LC/MS response, direct compatibility with solid-phase extraction (SPE) solvents and complementary selectivity as compared to reversed-phase HPLC.

Guide to Retaining Polar Compounds



Key:

Atlantis T3 = low pH (pH 3), high aqueous (>95%)

XBridge C_{18} = high pH (pH10) high aqueous (>95%)

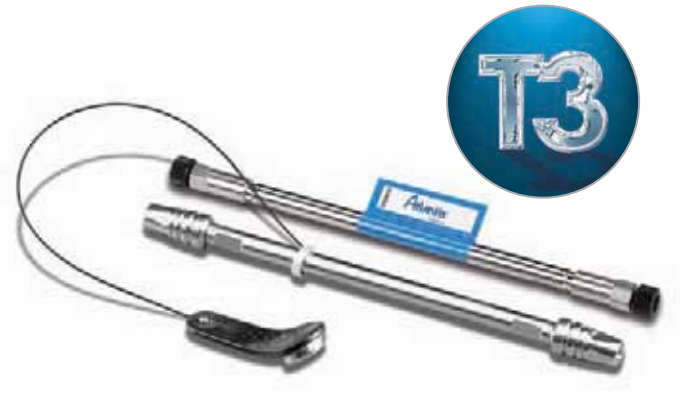
Atlantis HILIC Silica = low pH (pH 3), 10 mM ammonium formate with 0.2% formic acid in 95% ACN: $5H_2O$

XBridge HILIC = mid pH (pH 5), 10 mM ammonium acetate with 0.02% acetic acid in 95% ACN: $5H_2O$

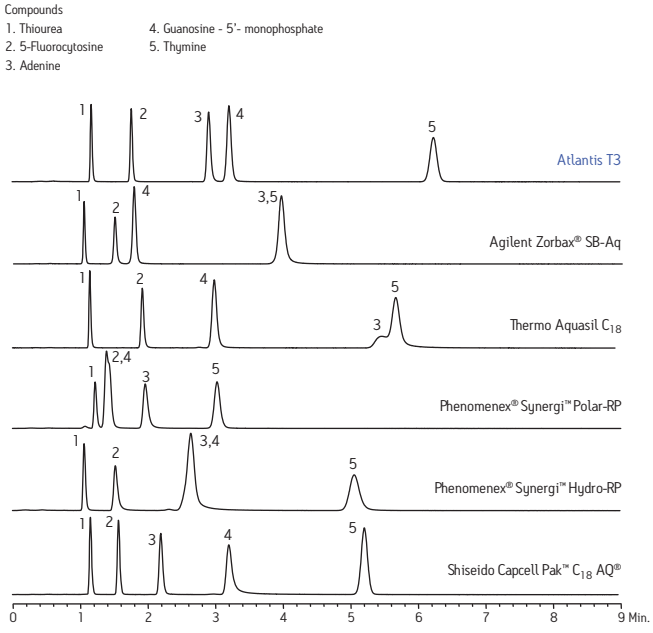
T3 Bonding

The superior performance of Atlantis T3 HPLC columns and the ACQUITY UPLC HSS T3 columns is a result of Waters advanced T3 bonding process. T3 bonding utilizes a trifunctional C_{18} alkyl phase bonded at a ligand density that promotes polar compound retention and aqueous mobile-phase compatibility. The proprietary T3 endcapping process is much more effective than traditional trimethyl-silane (TMS) endcapping.

This unique combination of bonding and endcapping provides superior polar compound retention and aqueous compatibility while also enhancing column performance, lifetime, peak shape, and stability.



Retention and Peak Shape Under 100% Aqueous Conditions
10 mM Ammonium Formate, pH 3.0, 30 °C



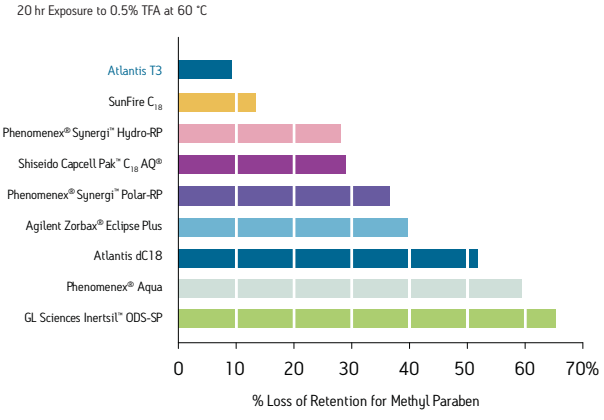
Atlantis T3 columns provide 100% aqueous compatibility, superior polar compound retention and excellent peak shape for acids, neutrals and bases.

Long Column Lifetimes at Low pH

The creation of highly retentive, reversed-phase C_{18} columns for polar compound retention involves bonding at a ligand density that is less hydrophobic and, therefore, more compatible with the weak, highly aqueous mobile phases required for retaining polar compounds. When exposed to strongly acidic mobile phases (i.e. < pH 2.0), traditional C_{18} columns can exhibit bonded phase hydrolysis resulting in gradual loss of retention, loss of efficiency as well as change in selectivity.

Atlantis T3 columns resist ligand cleavage by utilizing a trifunctional attachment of the C_{18} phase to the particle surface, thus providing exceptional column life at low pH.

Superior Low pH Stability

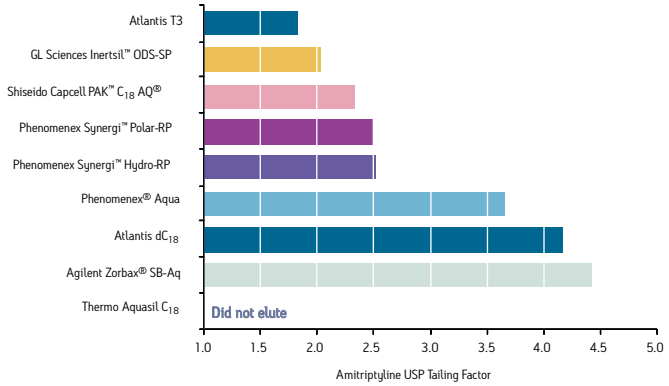


T3 bonding allows Atlantis T3 columns to provide long column lifetimes under harsh, low pH conditions.

Improved pH 7 Performance

At pH 7, poor peak shape for amine-containing bases and shortened column lifetimes are encountered when using intermediate ligand density C_{18} columns designed for polar compound retention. Poor peak shape is due to secondary interactions with unreacted silanols that remain present after bonding and endcapping. The proprietary T3 endcapping procedure reacts with more of these active silanols thereby dramatically improving peak shape for bases. Shortened column lifetimes are due to the dissolution of the silica particle substrate by the high pH mobile phase. The more effective and efficient T3 bonding helps "protect" the silica substrate from dissolution, thus providing longer column lifetimes.

Improved Peak Shape for Bases at pH 7



When compared to other reversed-phase "polar compound retention columns", Atlantis T3 columns provide superior pH 7 peak shape.

Balanced Retention of Diverse Analyte Mixtures

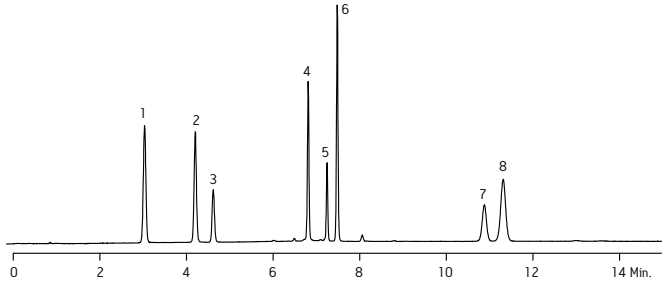
When faced with the task of analyzing a number of compounds encompassing a broad range of polarity, few options exist that will result in adequate retention of polar species without excessive retention of the hydrophobic components. In drug discovery, a large number of candidates need to be screened, often with little or no previous characterization of their chemical properties. Many polar candidates are often overlooked due to their elution in the void space of the column, while hydrophobic species may be permanently bound to the stationary phase.

Atlantis T3 columns are designed to deliver exceptional performance for an expansive range of compound polarities, thus being ideally suited as a universal reversed-phase column for open access environments.

Exceptional Performance for Hydrophobic Compounds

Column:	Atlantis T3, 4.6 x 150 mm, 5 µm
Mobile Phase A:	H_2O
Mobile Phase B:	ACN
Mobile Phase C:	1% HCOOH, pH 2.3
Mobile Phase D:	MeOH
Flow Rate:	2 mL/min
Gradient:	Time (min) / Profile %A %B %C %D
	0.00 55 17.5 10 17.5
	4.00 35 27.5 10 27.5
	6.00 9 40 10 41
	15.00 9 40 10 41
	16.00 55 17.5 10 17.5
	20.00 55 17.5 10 17.5
Injection Volume:	10 µL
Column Temp.:	30 °C
Detection:	UV @ 280 nm

Compounds:
1. Propyl gallate
2. 2,4,5-Trihydroxybutyrophenone
3. Tert-butylhydroquinone
4. Butylated hydroxyanisole
5. 2,6-Di-tert-butyl-4-hydroxymethylphenol
6. Octyl gallate
7. Butylated hydroxytoluene
8. Lauryl gallate



ACQUITY UPLC HSS T3 Columns for Polar Compound Retention

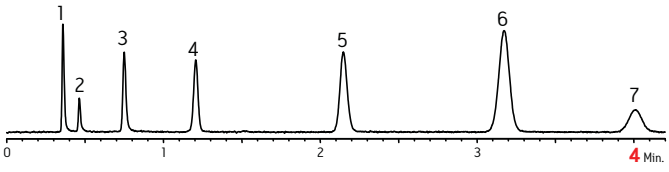
ACQUITY UPLC HSS (High Strength Silica) columns contain the first and only 100% silica particle designed, tested and intended for use in applications up to 15000 psi/1000 bar. The ACQUITY UPLC HSS particle is not an HPLC particle. High pore volume HPLC particles do not posses the mechanical stability necessary to withstand the high column packing and operating pressures of UPLC separations.

The first ligand chemistry in this new UPLC-certified column family utilizes T3 bonding in order to retain and separate polar organic compounds. ACQUITY UPLC HSS T3 columns possess the superior polar-compound retention, aqueous mobile-phase compatibility and ultra-low MS bleed of Atlantis T3 columns.

Superior Retention of Polar Compounds

Column:	ACQUITY UPLC HSS T3 2.1 x 100 mm
Mobile Phase A:	10 mM CH_3COONH_4, pH 5.0
Mobile Phase B:	ACN
Flow Rate:	0.438 mL/min
Isocratic Mobile Phase Composition:	2% B
Injection Volume:	0.7 µL
Temperature:	30 °C
Detection:	UV @ 280 nm
Instrument:	ACQUITY UPLC System with ACQUITY UPLC 2996 PDA

Compounds
1. Norepinephrine
2. Epinephrine
3. Dopamine
4. 3,4- Dihydroxyphenylacetic acid (DOPAC)
5. Serotonin (5-HT)
6. 5-Hydroxy-3-indoleacetic acid (5-HIAA)
7. 4-Hydroxy-3-methoxyphenylacetic acid (HVA)



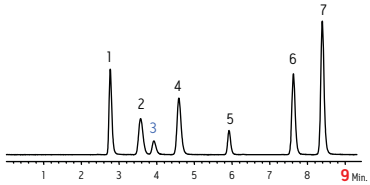
Easier Method Transfer

Since Atlantis T3 HPLC and ACQUITY UPLC HSS T3 columns utilize T3 bonding, LC methods can be transferred from HPLC to UPLC separations. Because Atlantis and HSS particles are different substrates, some method modification and/or optimization may be required. A compelling benefit of UPLC technology is the ease and speed at which this method optimization can occur since UPLC separations offer greater resolution in less time.

Moving from XBridge HPLC to ACQUITY UPLC BEH columns is nearly seamless, as the hybrid particle substrates differ only in particle size. However, because of T3 bonding it should be straightforward to make simple adjustments to successfully transfer a separation from an Atlantis T3 HPLC column to an ACQUITY UPLC HSS T3 column.

T3 Bonding Enables HPLC to UPLC Transfer

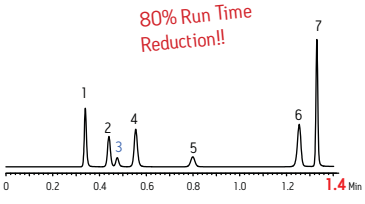
HPLC Separation on Atlantis T3



Compound	Retention Time (min)	USP Resolution
1. Cytosine	2.77	
2. 5-Fluorocytosine	3.58	4.56
3. Uracil	3.93	1.71
4. 5-Fluorouracil	4.59	3.41
5. Guanine	5.92	7.76
6. Thymine	7.63	11.01
7. Adenine	8.39	4.70

Similar Selectivities

UPLC Separation on ACQUITY UPLC HSS T3



Compound	Retention Time (min)	USP Resolution
1. Cytosine	0.34	
2. 5-Fluorocytosine	0.44	5.02
3. Uracil	0.48	1.50
4. 5-Fluorouracil	0.56	3.17
5. Guanine	0.80	8.16
6. Thymine	1.25	14.65
7. Adenine	1.33	3.50

T3 bonding allows for the transfer of HPLC methods to UPLC.

Atlantis HILIC Silica

Hydrophilic-Interaction Chromatography (HILIC)

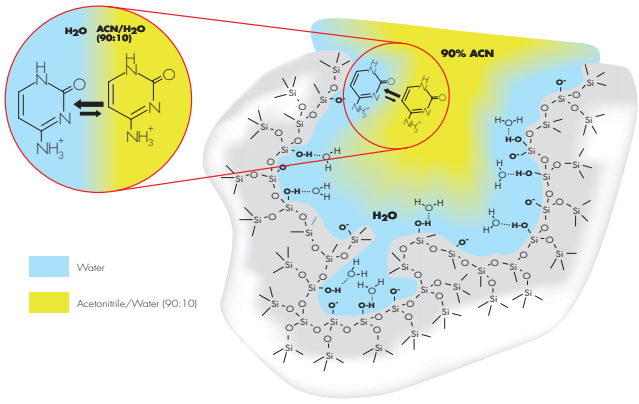
HILIC is an alternative chromatographic technique that offers complementary selectivity to RP and often retains very polar species that cannot be retained by traditional means. Atlantis HILIC Silica columns were designed to retain very polar, organic molecules that are too polar to retain by RP[3]. Unlike the highly aqueous mobile phases required for polar retention in RP separations, Atlantis HILIC Silica columns employ highly volatile (>80% organic) mobile phases which are ideal for mass spectrometry (MS) response and sensitivity. Additionally, direct compatibility with high organic SPE eluates dramatically increases the number of samples that can be handled, thus substantially increasing sample throughput.

HILIC retention mechanisms are a complex combination of partitioning, ion-exchange and hydrogen bonding, resulting in enhanced retention for polar analytes[3,4]. Atlantis HILIC silica columns, used in combination with high organic (>80% acetonitrile) mobile phases, result in retention of analytes that are simply too polar to retain by traditional RP chromatography.

[3] E.S. Grumbach et al LCGC N. Am. 2004, 22, 1010-1023

[4] E.S. Grumbach et al J. Sep. Sci. 2008, 31, 1511-1518

Retention Mechanisms in Hydrophilic-Interaction Chromatography (HILIC)



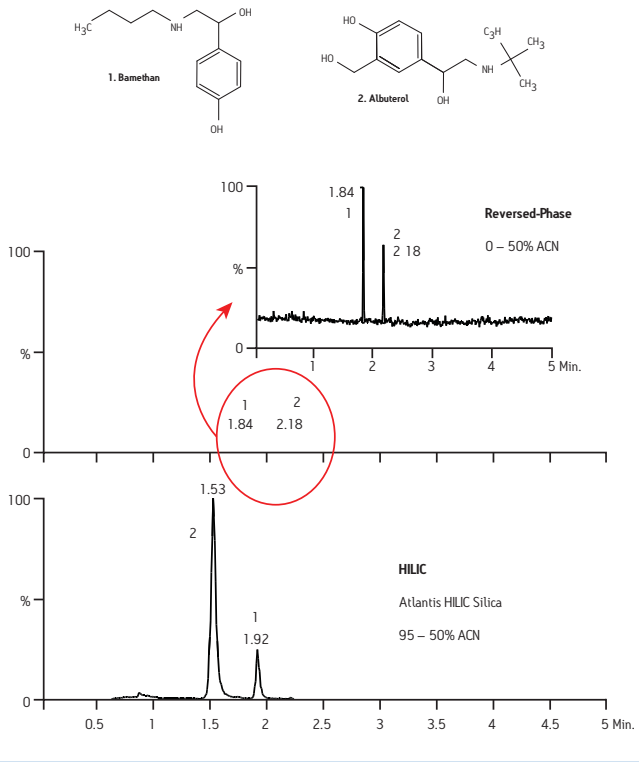
The combination of partitioning and weak cation-exchange results in retention of polar bases with Atlantis HILIC Silica columns.

Enhanced Mass Spectrometry Response

Utilization of HILIC has grown in popularity, primarily due to the extensive adoption of mass spectrometry as a detector and the necessity of improving sensitivity for the quantitation of polar analytes. Unlike reversed-phase which utilizes high aqueous mobile phases to induce retention, HILIC employs an acetonitrile-rich mobile phase. This high organic mobile phase is easily desolvated, resulting in improved ionization efficiency and mass spectrometry response.

For these reasons, HILIC has become a popular technique for bioanalytical laboratories.

Reach Lower Limits of Detection



Atlantis Analytical Columns

Dimensions	Type	Particle Size	T3	dC_{18}	HILIC Silica
1.0 x 50 mm	Column	3 µm	186003713	186001279	186002003
1.0 x 150 mm	Column	3 µm	186003714	186001283	—
2.1 x 10 mm	Guard	3 µm	186003756[1]	186001377[1]	186002005[1]
2.1 x 15 mm	Direct Connect	3 µm	—	186002064	186002007
2.1 x 20 mm	Guard	3 µm	—	186001381[2]	—
2.1 x 20 mm *IS*	Column	3 µm	186003715	186002058	—
2.1 x 30 mm	Column	3 µm	186003716	186001287	186002009
2.1 x 50 mm	Column	3 µm	186003717	186001291	186002011
2.1 x 100 mm	Column	3 µm	186003718	186001295	186002013
2.1 x 150 mm	Column	3 µm	186003719	186001299	186002015
3.0 x 20 mm *IS*	Column	3 µm	186003720	186002060	—
3.0 x 50 mm	Column	3 µm	186003721	186001389	186002017
3.0 x 100 mm	Column	3 µm	186003722	186001303	186002019
3.0 x 150 mm	Column	3 µm	186003723	186001307	—
3.9 x 20 mm	Guard	3 µm	186003757[3]	186001313[3]	186002021[3]
3.9 x 50 mm	Cartridge	3 µm	—	186001385[4]	—
3.9 x 100 mm	Column	3 µm	—	186001393	—
3.9 x 150 mm	Column	3 µm	—	186001317	—
4.6 x 20 mm	Guard	3 µm	186003758[3]	186001321[3]	186002023[3]
4.6 x 20 mm *IS*	Column	3 µm	186003724	186002062	—
4.6 x 30 mm	Column	3 µm	186003725	186001325	186002025
4.6 x 50 mm	Column	3 µm	186003726	186001329	186002027
4.6 x 75 mm	Column	3 µm	186003727	186001333	—
4.6 x 100 mm	Column	3 µm	186003728	186001337	186002029
4.6 x 150 mm	Column	3 µm	186003729	186001342	186002031
1.0 x 50 mm	Column	5 µm	186003730	186001281	186002004
1.0 x 150 mm	Column	5 µm	186003731	186001285	—
2.1 x 10 mm	Guard	5 µm	186003759[1]	186001379[1]	186002006[1]
2.1 x 15 mm	Direct Connect	5 µm	—	186002065	186002008
2.1 x 20 mm	Guard	5 µm	—	186001383[2]	—
2.1 x 20 mm *IS*	Column	5 µm	186003732	186002059	—
2.1 x 30 mm	Column	5 µm	186003733	186001289	186002010
2.1 x 50 mm	Column	5 µm	186003734	186001293	186002012
2.1 x 100 mm	Column	5 µm	186003735	186001297	186002014
2.1 x 150 mm	Column	5 µm	186003736	186001301	186002016
3.0 x 20 mm *IS*	Column	5 µm	186003737	186002061	—
3.0 x 50 mm	Column	5 µm	186003738	186001391	186002018
3.0 x 100 mm	Column	5 µm	186003739	186001305	186002020
3.0 x 150 mm	Column	5 µm	186003740	186001309	—
3.0 x 250 mm	Column	5 µm	186003741	186001311	—
3.9 x 20 mm	Guard	5 µm	186003760[3]	186001315[3]	186002022[3]
3.9 x 50 mm	Cartridge	5 µm	—	186001387[4]	—
3.9 x 100 mm	Column	5 µm	—	186001395	—
3.9 x 150 mm	Column	5 µm	—	186001319	—
4.6 x 20 mm	Guard	5 µm	186003761[3]	186001323[3]	186002024[3]
4.6 x 20 mm *IS*	Column	5 µm	186003742	186002063	—
4.6 x 30 mm	Column	5 µm	186003743	186001327	186002026
4.6 x 50 mm	Column	5 µm	186003744	186001331	186002028
4.6 x 75 mm	Column	5 µm	186003745	186001335	—
4.6 x 100 mm	Column	5 µm	186003746	186001340	186002030
4.6 x 150 mm	Column	5 µm	186003747	186001344	186002032
4.6 x 250 mm	Column	5 µm	186003748	186001346	186002033

Atlantis Columns Method Validation Kits

Dimensions	Particle Size	T3	dC_{18}	HILIC Silica
4.6 x 150 mm	3 µm	186003751	186002312	186002315
4.6 x 150 mm	5 µm	186003754	186002311	186002314
4.6 x 250 mm	5 µm	186003755	186002313	186002316

Literature References

Atlantis Columns Brochure,
Literature Reference 720000793EN

Atlantis Columns Applications Notebook,
Literature Reference 720000472EN

Atlantis T3 and ACQUITY UPLC
HSS T3 Columns Toss Sheet,
Literature Reference 720001887EN

Topics in Liquid Chromatography: Part 1.
Designing a Reversed-Phase Column for Polar
Compound Retention White Paper,
Literature Reference 720001889EN

Interactive Waters Reversed-Phase
Column Selectivity Chart
www.waters.com/selectivitychart

Also available in Optimum Bed Density (OBD) Preparative Columns, See Page 161

[1] Requires Sentry Guard Holder WAT097958
[2] Requires Sentry Guard Holder 186000262
[3] Requires Sentry Guard Holder WAT046910
[4] Requires Cartridge Fittings WAT037525

XTerra® COLUMNS

XTerra Columns

The Most Successful Column in Over 30 Years of Stationary-Phase Technology

The universal acceptance by pharmaceutical scientists in drug discovery, method development, and isolation and purification has enabled XTerra columns to become the fastest selling and most successful column product in the world of chromatography. The reason: the enabling science of Hybrid Particle Technology. Drug discovery scientists benefit from the DMSO injection-resistance of the rugged XTerra particle. Method development scientists are able to operate at whatever pH is necessary for optimal selectivity due to XTerra columns' wide pH range. Isolation and purification scientists can load up to 60x more material per injection because of the high mass loading capacity of XTerra columns.

Physical Characteristics

Packing	Particle Shape	Particle Size(s)	Pore Size	Carbon Load	End-capped
XTerra MS C_{18}	Spherical	2.5, 3.5, 5, 10 µm	125Å	15.5%	yes
XTerra MS C_8	Spherical	2.5, 3.5, 5, 10 µm	125Å	12.0%	yes
XTerra RP18	Spherical	3.5, 5, 10 µm	125Å	15.0%	yes
XTerra RP8	Spherical	3.5, 5, 10 µm	125Å	13.5%	yes
XTerra Phenyl	Spherical	3.5, 5 µm	125Å	12.0%	yes

Hybrid Particle Technology

For over 30 years, scientists have been forced to work within certain boundaries when performing HPLC separations. Restrictions on speed, resolution, pH, temperature, and loading capacity were imposed upon the chromatographer by limitations of the stationary phase material. The patented* Hybrid Particle Technology of XTerra columns allows chromatographers to break these boundaries and realize the full potential of their analytical and preparative separations.

Patent # 6,686,035 B2

In Hybrid Particle Technology, one out of every three silanols is replaced with a methyl group during synthesis. This hydrophobicity is distributed throughout the entire structure of the particle backbone. The result is a rugged hybrid (inorganic/organic) particle that can be operated at high speeds, high temperatures, and high pH. The presence of 33% fewer residual silanols (after endcapping and bonding) also means that XTerra columns give exceptionally sharp, high-efficiency peaks for basic compounds.

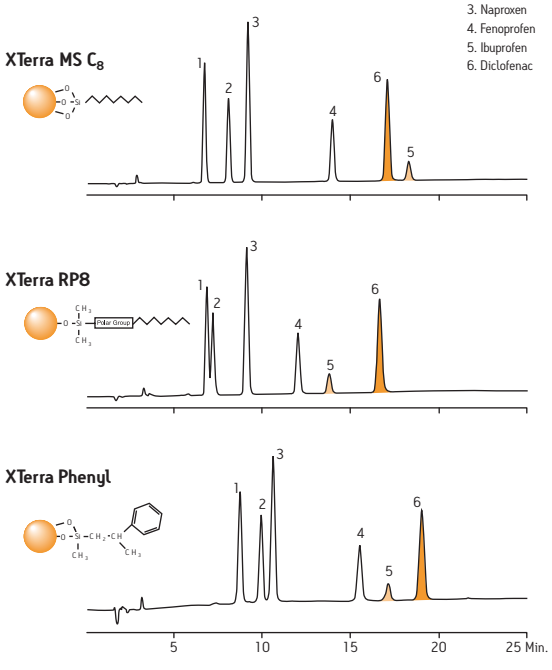
Effect of XTerra Column Chemistry on Selectivity and Retention

Column: 4.6 x 150 mm, 5 µm
Mobile Phase A: H_2O
Mobile Phase B: MeOH
Mobile Phase C: 50 mM HCOOH, pH 2.45
Gradient:

Time (min)	%A	%B	%C
0.0	40	50	10
20.0	25	65	10
35.0	25	65	10

Flow Rate: 1.0 mL/min
Injection Volume: 15 mL
Temperature: 30 °C
Detection: UV @ 254nm
Instrument: Waters Alliance 2695, 996 PDA

Peaks:
1. Suprofen
2. Tolmetin
3. Naproxen
4. Fenoprofen
5. Ibuprofen
6. Diclofenac



The column stationary phase chemistries of XTerra columns are key components in an overall method development plan.

Ordering Information

XTerra Capillary and Narrowbore Columns

i.d. (mm)	Length (mm)	Particle Size	Type	MS C_{18}	MS C_8	RP18	RP8	Phenyl
75 µm	50 mm	3.5 µm	Capillary	186002198	—	—	—	—
75 µm	100 mm	3.5 µm	Capillary	186002199	—	—	—	—
75 µm	150 mm	3.5 µm	Capillary	186002200	—	—	—	—
100 µm	50 mm	3.5 µm	Capillary	186002210	—	—	—	—
100 µm	100 mm	3.5 µm	Capillary	186002211	—	—	—	—
100 µm	150 mm	3.5 µm	Capillary	186002212	—	—	—	—
150 µm	50 mm	3.5 µm	Capillary	186002469	—	—	—	—
150 µm	100 mm	3.5 µm	Capillary	186002470	—	—	—	—
150 µm	150 mm	3.5 µm	Capillary	186002471	—	—	—	—
300 µm	50 mm	3.5 µm	Capillary	186002599	—	—	—	—
300 µm	100 mm	3.5 µm	Capillary	186002600	—	—	—	—
300 µm	150 mm	3.5 µm	Capillary	186002601	—	—	—	—
300 µm	50 mm	5 µm	Capillary	186002602	—	—	—	—
300 µm	100 mm	5 µm	Capillary	186002603	—	—	—	—
300 µm	150 mm	5 µm	Capillary	186002604	—	—	—	—
1.0 mm	50 mm	2.5 µm	Column	186000979	—	—	—	—
1.0 mm	50 mm	3.5 µm	Column	186000386	186000387	186000388	186000389	—
1.0 mm	100 mm	3.5 µm	Column	186000390	186000391	186000392	186000393	—
1.0 mm	150 mm	3.5 µm	Column	186000394	186000395	186000396	186000397	—
2.1 mm	10 mm	3.5 µm	Guard	186000632[1]	186000633[1]	186000634[1]	186000635[1]	186001188
2.1 mm	10 mm	5 µm	Guard	186000648[1]	186000649[1]	186000650[1]	186000651[1]	186001192
2.1 mm	15 mm	2.5 µm	Direct Connect Column	186000900	—	—	—	—
2.1 mm	15 mm	3.5 µm	Direct Connect Column	186001908	—	—	—	—
2.1 mm	15 mm	5 µm	Direct Connect Column	186001907	—	—	—	—
2.1 mm	15 mm	10 µm	Direct Connect Column	186001906	—	—	—	—
2.1 mm	20 mm	2.5 µm	*IS* Column	186001921	186001922	—	—	—
2.1 mm	20 mm	3.5 µm	*IS* Column	186001923	186001924	186001925	186001926	—
2.1 mm	20 mm	3.5 µm	Guard	186000636[2]	186000637[2]	186000638[2]	186000639[2]	186001189
2.1 mm	20 mm	5 µm	*IS* Column	186001979	186001980	186001982	186001983	—
2.1 mm	20 mm	5 µm	Guard	186000652[2]	186000653[2]	186000654[2]	186000655[2]	186001193
2.1 mm	30 mm	2.5 µm	Column	186000592	186000593	—	—	—
2.1 mm	30 mm	3.5 µm	Column	186000398	186000399	—	—	
2.1 mm	50 mm	2.5 µm	Column	186000594	186000595	—	—	—
2.1 mm	50 mm	3.5 µm	Column	186000400	186000401	186000402	186000403	186001179
2.1 mm	50 mm	3.5 µm	Cartridge	186000498[3]	186000499[3]	186000500[3]	186000501[3]	—
2.1 mm	50 mm	5 µm	Column	186000446	186000447	186000448	186000449	186001185
2.1 mm	50 mm	5 µm	Cartridge	186000538[3]	186000539[3]	186000540[3]	186000541[3]	—
2.1 mm	100 mm	3.5 µm	Column	186000404	186000405	186000406	186000407	186001180
2.1 mm	100 mm	3.5 µm	Cartridge	186000502[3]	186000503[3]	186000504[3]	186000505[3]	—
2.1 mm	100 mm	5 µm	Column	186000450	186000451	186000452	186000453	186001186
2.1 mm	100 mm	5 µm	Cartridge	186000542[3]	186000543[3]	186000544[3]	186000545[3]	—
2.1 mm	150 mm	3.5 µm	Column	186000408	186000409	186000410	186000411	186001181
2.1 mm	150 mm	3.5 µm	Cartridge	186000506[3]	186000507[3]	186000508[3]	186000509[3]	—
2.1 mm	150 mm	5 µm	Column	186000454	186000455	186000456	186000457	186001187
2.1 mm	150 mm	5 µm	Cartridge	186000546[3]	186000547[3]	186000548[3]	186000549[3]	—
2.1 mm	250 mm	5 µm	Column	186000458	186000459	186000460	186000461	—
2.1 mm	250 mm	5 µm	Cartridge	186000550[3]	186000551[3]	186000552[3]	186000553[3]	—

[1] Needs Guard Holder WAT097958 [2] Needs Cartridge Column Holder 186000262 [3] Needs Cartridge Column End Connector Kit 700000117

Also available in Optimum Bed Density (OBD)

Preparative Columns, See Page 162

XTerra Analytical Columns

i.d. (mm)	Length (mm)	Particle Size	Type	MS C_{18}	MS C_8	RP18	RP8	Phenyl
3.0 mm	20 mm	2.5 µm	*IS* Column	186001972	186001973	—	—	—
3.0 mm	20 mm	2.5 µm	Cartridge	186000588[4]	186000589[4]	—	—	—
3.0 mm	20 mm	3.5 µm	*IS* Column	186001974	186001975	186001976	186001977	—
3.0 mm	20 mm	3.5 µm	Guard	186000640[4]	186000641[4]	186000642[4]	186000643[4]	186001190
3.0 mm	20 mm	5 µm	*IS* Column	186001984	186001985	186001986	186001987	—
3.0 mm	20 mm	5 µm	Guard	186000656[4]	186000657[4]	186000658[4]	186000659[4]	186001194
3.0 mm	30 mm	2.5 µm	Column	186000596	186000597	—	—	—
3.0 mm	30 mm	3.5 µm	Column	186000412	186000413	—	—	
3.0 mm	50 mm	2.5 µm	Column	186000598	186000599	—	—	—
3.0 mm	50 mm	3.5 µm	Column	186000414	186000415	186000416	186000417	186001141
3.0 mm	50 mm	3.5 µm	Cartridge	186000510[5]	186000511[5]	186000512[5]	186000513[5]	—
3.0 mm	50 mm	5 µm	Column	186000462	186000463	186000464	186000465	186001148
3.0 mm	50 mm	5 µm	Cartridge	186000554[5]	186000555[5]	186000556[5]	186000557[5]	—
3.0 mm	100 mm	3.5 µm	Column	186000418	186000419	186000420	186000421	186001142
3.0 mm	100 mm	3.5 µm	Cartridge	186000514[5]	186000515[5]	186000516[5]	186000517[5]	—
3.0 mm	100 mm	5 µm	Column	186000466	186000467	186000468	186000469	186001149
3.0 mm	100 mm	5 µm	Cartridge	186000558[5]	186000559[5]	186000560[5]	186000561[5]	—
3.0 mm	150 mm	3.5 µm	Column	186000422	186000423	186000424	186000425	186001143
3.0 mm	150 mm	3.5 µm	Cartridge	186000518[5]	186000519[5]	186000520[5]	186000521[5]	—
3.0 mm	150 mm	5 µm	Column	186000470	186000471	186000472	186000473	186001150
3.0 mm	150 mm	5 µm	Cartridge	186000562[5]	186000563[5]	186000564[5]	186000565[5]	—
3.0 mm	250 mm	5 µm	Column	186000474	186000475	186000476	186000477	186001151
3.0 mm	250 mm	5 µm	Cartridge	186000566[5]	186000567[5]	186000568[5]	186000569[5]	—
3.9 mm	20 mm	2.5 µm	*IS* Column	186001899	186001897	—	—	—
3.9 mm	20 mm	3.5 µm	*IS* Column	186001900	186001898	186001902	186001901	—
3.9 mm	20 mm	3.5 µm	Guard	186000644[4]	186000645[4]	186000646[4]	186000647[4]	186001191
3.9 mm	20 mm	5 µm	*IS* Column	186001988	186001989	186001990	186001991	—
3.9 mm	20 mm	5 µm	Guard	186000660[4]	186000661[4]	186000662[4]	186000663[4]	186001195
3.9 mm	50 mm	3.5 µm	Cartridge	186000817[5]	186000818[5]	—	—	186001204
3.9 mm	50 mm	5 µm	Cartridge	186000815[5]	186000816[5]	—	—	186001203
3.9 mm	100 mm	3.5 µm	Column	186000426	186000427	186000428	186000429	186001177
3.9 mm	100 mm	3.5 µm	Cartridge	186000522[5]	186000523[5]	186000524[5]	186000525[5]	—
3.9 mm	100 mm	5 µm	Column	—	—	—	—	186001183
3.9 mm	150 mm	3.5 µm	Column	—	—	—	—	186001178
3.9 mm	150 mm	5 µm	Column	186000478	186000479	186000480	186000481	186001184
3.9 mm	150 mm	5 µm	Cartridge	186000570[5]	186000571[5]	186000572[5]	186000573[5]	—
4.6 mm	10 mm	3.5 µm	Guard	186001927[6]	—	—	—	—
4.6 mm	10 mm	5 µm	Guard	186001920[6]	186001434[6]	—	—	—
4.6 mm	20 mm	2.5 µm	*IS* Column	186001889	186001890	—	—	—
4.6 mm	20 mm	2.5 µm	Cartridge	186000590[4]	186000591[4]	—	—	—
4.6 mm	20 mm	3.5 µm	*IS* Column	186001891	186001892	186001893	186001894	—
4.6 mm	20 mm	5 µm	*IS* Column	186001992	186001993	186001994	186001995	—
4.6 mm	30 mm	2.5 µm	Column	186000600	186000601	—	—	—
4.6 mm	30 mm	3.5 µm	Column	186000430	186000431	186001910	186001912	—
4.6 mm	30 mm	5 µm	Column	186000878	186000879	186001909	186001911	—
4.6 mm	50 mm	2.5 µm	Column	186000602	186000603	—	—	—
4.6 mm	50 mm	3.5 µm	Column	186000432	186000433	186000434	186000435	—
4.6 mm	50 mm	3.5 µm	Cartridge	186000526[5]	186000527[5]	186000528[5]	186000529[5]	—
4.6 mm	50 mm	5 µm	Column	186000482	186000483	186000484	186000485	186001144
4.6 mm	50 mm	5 µm	Cartridge	186000574[5]	186000575[5]	186000576[5]	186000577[5]	—
4.6 mm	100 mm	3.5 µm	Column	186000436	186000437	186000438	186000439	186001139
4.6 mm	100 mm	3.5 µm	Cartridge	186000530[5]	186000531[5]	186000532[5]	186000533[5]	—
4.6 mm	100 mm	5 µm	Column	186000486	186000487	186000488	186000489	186001145
4.6 mm	150 mm	3.5 µm	Column	186000440	186000441	186000442	186000443	186001140
4.6 mm	150 mm	3.5 µm	Cartridge	186000534[5]	186000535[5]	186000536[5]	186000537[5]	—
4.6 mm	150 mm	5 µm	Column	186000490	186000491	186000492	186000493	186001146
4.6 mm	150 mm	5 µm	Cartridge	186000578[5]	186000579[5]	186000580[5]	186000581[5]	—
4.6 mm	250 mm	3.5 µm	Column	186001470	186001471	186001472	186001473	186001474
4.6 mm	250 mm	5 µm	Column	186000494	186000495	186000496	186000497	186001147
4.6 mm	250 mm	5 µm	Cartridge	186000582[5]	186000583[5]	186000584[5]	186000585[5]	—

[4] Needs WAT046910 (Universal, use with standard columns) or WAT046905 (Integrated into cartridge column)
[5] Needs End Connector Kit WAT037525 [6] Requires Inline Guard Cartridge Holder PSS830008

XTerra Oligonucleotide Purification Columns

i.d. (mm)	Length (mm)	Particle Size	Type	MS C_{18}
4.6 mm	50 mm	2.5 µm	Column	186000602
10 mm	50 mm	2.5 µm	Column	186000982

XTerra Method Validation Kits

i.d. (mm)	Length (mm)	Particle Size	Type	MS C_{18}	MS C_8	RP18	RP8	Phenyl
2.1 mm	100 mm	3.5 µm	MV Kit	Custom	186000831	Custom	186000834	Custom
2.1 mm	150 mm	5 µm	MV Kit	186000827	Custom	Custom	Custom	Custom
3.9 mm	150 mm	5 µm	MV Kit	186000828	Custom	Custom	186000836	Custom
4.6 mm	100 mm	3.5 µm	MV Kit	Custom	186000832	Custom	186000835	Custom
4.6 mm	150 mm	3.5 µm	MV Kit	186000826	Custom	186000861	Custom	186002234
4.6 mm	150 mm	5 µm	MV Kit	186000829	Custom	186000862	Custom	186002235
4.6 mm	250 mm	5 µm	MV Kit	186000830	186000833	186000863	Custom	186002236

All other Method Validation kits are custom made on request

Also available in Optimum Bed Density (OBD)

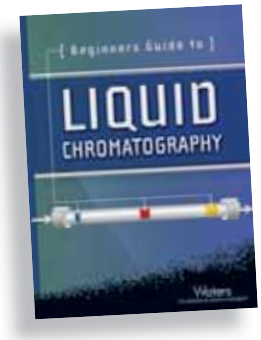
Preparative Columns, See Page 162

Literature References

XTerra Columns Brochure,
Literature Reference 720000424EN

XTerra Applications Notebook,
Literature Reference 720000502EN

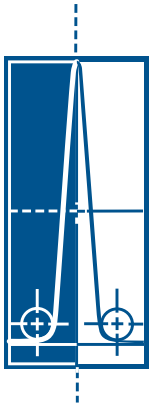
eSelectivity Chart Version 3.0
www.waters.com/selectivitychart



Waters HPLC Primer

- A primer on High-Performance Liquid Chromatography (HPLC) and related topics
- Brief history, definition, and the basics of HPLC and UPLC
- HPLC Column Hardware (design and performance)
- HPLC Separation Mechanism
- HPLC Detector Overview and Types
- Glossary of HPLC Terms

www.waters.com/hplcprimer



Symmetry Columns

Setting the Standard for Reproducibility

As today's chemists establish new analytical methods for the latest pharmaceutical and biopharmaceutical products, the selection of a reproducible HPLC column is essential. The selected column needs to provide the same chromatographic results over the life of the new drug product. Symmetry columns demonstrate superior reproducibility over many years. Batches randomly selected over a five year period demonstrate excellent reproducibility in the example shown.

Reproducibility is our number one priority in supplying Symmetry columns. This excellent reproducibility is a result of our commitment to maintaining the tightest specifications in the HPLC column industry. Symmetry columns are engineered with high purity raw materials, tightly controlled manufacturing processes and column packing procedures that provide today's scientists with the best, most reproducible HPLC column available.

Our goal is to supply a family of HPLC columns that you can rely on for rugged and robust methods. Symmetry columns let you increase your laboratory's productivity, and allow easier method transfer between labs and around the globe.

Physical Characteristics

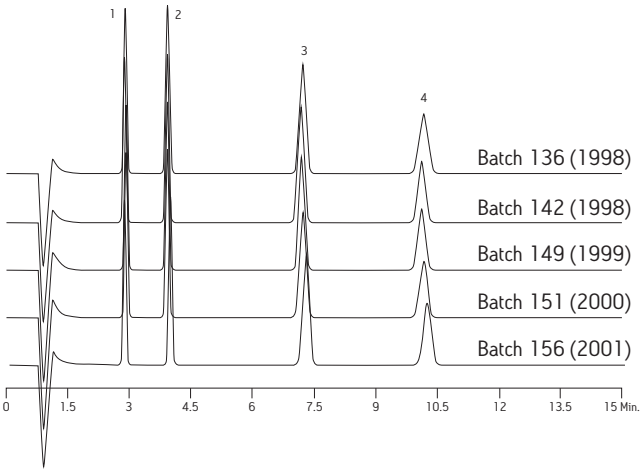
Packing	Chemistry	Particle Size	Particle Shape	Pore Size	Carbon Load	End-capped
Symmetry	C_{18}	3.5, 5 µm	Spherical	100Å	19%	Yes
	C_8	3.5, 5 µm	Spherical	100Å	12%	Yes
SymmetryPrep	C_{18}	7 µm	Spherical	100Å	19%	Yes
	C_8	7 µm	Spherical	100Å	12%	Yes
SymmetryShield	RP18	3.5, 5, 7 µm	Spherical	100Å	17%	Yes
	RP8	3.5, 5, 7 µm	Spherical	100Å	15%	Yes
Symmetry300	C_{18}	3.5, 5 µm	Spherical	300Å	8.5%	Yes
	C_4	3.5, 5 µm	Spherical	300Å	2.8%	Yes

Unmatched Year-to-Year Reproducibility

Column: Symmetry C_{18}, 4.6 x 150 mm, 5 µm
Part No.: WAT045905
Mobile Phase A: H_2O
Mobile Phase B: MeCN
Mobile Phase C: pH 3.75; 100 mM NH_4COOH in H_2O
Flow Rate: 1.4 mL/min
Isocratic: 30% A; 60% B; 10% C
Injection Volume: 5.0 µL
Temperature: 30 °C
Detection: UV @ 233 nm
Instrumentation: Waters Alliance HT 2795
Waters 996 PDA detector
Sampling Rate: 5 pts./sec

RSD's for Retention Times

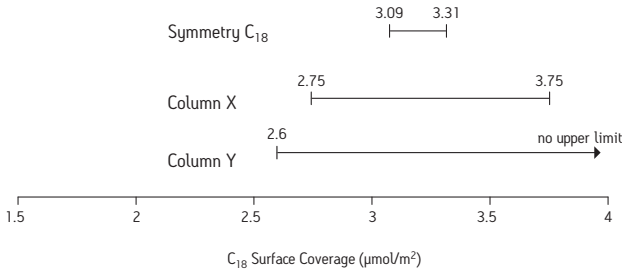
1. Terbinafine HCl	*0.7%*
2. Ibuprofen	*0.8%*
3. Lovastatin	*0.6%*
4. Simvastatin	*0.7%*



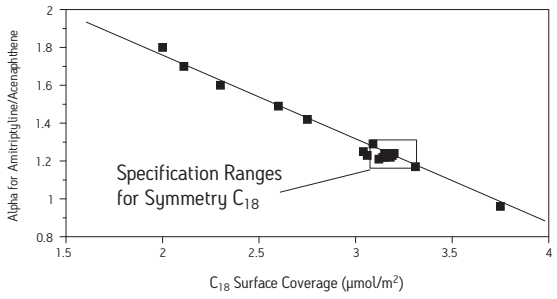
The Benefit of Narrow Specification Ranges

Symmetry columns have the narrowest ligand surface coverage specifications in the industry. Surface coverage (ligand density) is one of the most important parameters affecting reproducibility. The impact of a small variation in surface coverage on the amitriptyline/acenaphthene alpha specification for Symmetry C_{18} columns is shown here. Narrow column specifications benefit the chromatographer in achieving reproducible results. The surface coverage requirements of Symmetry are the tightest specifications in the industry, resulting in minimal shifts in resolution and more reproducible batch-to-batch and column-to-column results.

C_{18} Ligand Surface Coverage Specification Ranges



Narrow Specification Ranges Provide Smaller Shifts in Alpha (α)

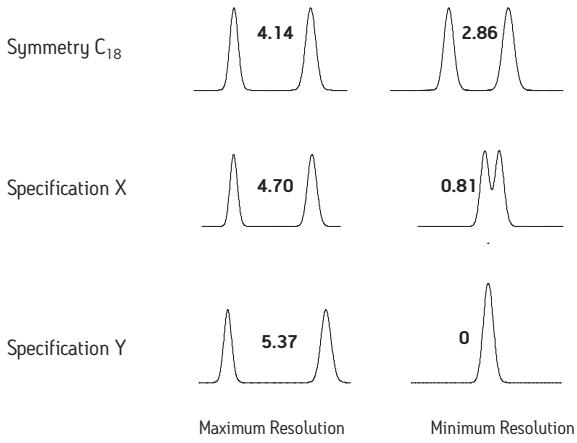


Smaller Shifts in Alpha (α) Reduce Changes in Peak Resolution

$$R=\left(\frac{\sqrt{N}}{4}\right)\left(\frac{\alpha-1}{\alpha}\right)\left(\frac{K_2}{K_2+1}\right)$$

R = resolution
N = number of peaks
α = alpha value
k = peak retention

Minimal Changes in Resolution Means More Reproducible Results*



* Based on vendors column product specifications for ligand density

Long Lifetime

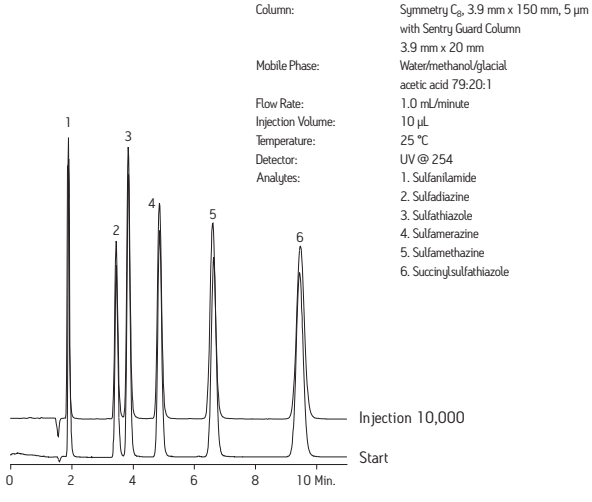
Columns that last longer save you money. Symmetry columns can last over 10,000 injections with minimal loss in efficiency, minimal increase in backpressure and minimal change in retention time.

These columns deliver guaranteed consistent performance optimizing the two key factors that control column lifetime which are hydrolytic stability of the packing material and mechanical stability of the packed bed.

Comprehensive Certificate of Analysis

A three-page Certificate of Analysis is included with every Symmetry column. We report both our specifications, which are the tightest in the industry, and the results of the 22 critical tests each column must pass before it carries the Symmetry brand.

Outstanding Packed Bed Stability—10,000 Injections



The long-term mechanical stability of Symmetry columns is demonstrated in this analysis of sulfa drugs. Over 10,000 injections were run with minimal loss in column efficiency. The use of the Sentry guard column is critical to achieving these types of column lifetimes.

Symmetry Columns and Cartridges

Symmetry Analytical Columns

Dimension	Particle Size	Part No. C_{18}	Part No. C_8
1.0 x 50 mm	3.5 µm	WAT106056	WAT106052
1.0 x 150 mm	3.5 µm	WAT248059	WAT248072
2.1 x 30 mm	3.5 µm	WAT058973	WAT058977
2.1 x 50 mm	3.5 µm	WAT200650	WAT200624
2.1 x 100 mm	3.5 µm	WAT058965	WAT058961
2.1 x 150 mm	3.5 µm	WAT106005	WAT106011
3.0 x 50 mm	3.5 µm	186002612	186002613
3.0 x 100 mm	3.5 µm	186000696	186000698
3.0 x 150 mm	3.5 µm	186000695	186000697
4.6 x 30 mm	3.5 µm	186000271	186000270
4.6 x 50 mm	3.5 µm	WAT200625	WAT200620
4.6 x 75 mm	3.5 µm	WAT066224	WAT066200
4.6 x 100 mm	3.5 µm	WAT066220	WAT066204
4.6 x 150 mm	3.5 µm	WAT200632	WAT200630
2.1 x 50 mm	5 µm	186000206	186000212
2.1 x 100 mm	5 µm	186002608	186002609
2.1 x 150 mm	5 µm	WAT056975	WAT056955
3.0 x 150 mm	5 µm	WAT054200	WAT054230
3.0 x 250 mm	5 µm	186000690	186000691
3.9 x 150 mm	5 µm	WAT046980	WAT046970
4.6 x 50 mm	5 µm	186000207	186000213
4.6 x 100 mm	5 µm	186002616	186002617
4.6 x 150 mm	5 µm	WAT045905	WAT045995
4.6 x 250 mm	5 µm	WAT054275	WAT054270

Symmetry Cartridge Columns

(Requires endfittings, see page 136)

Dimension	Particle Size	Part No. C_{18}	Part No. C_8
2.1 x 20 mm	3.5 µm	186000269	186000268
2.1 x 50 mm	3.5 µm	186000152	186000149
2.1 x 100 mm	3.5 µm	186000151	186000153
2.1 x 150 mm	3.5 µm	186000150	186000148
4.6 x 75 mm	3.5 µm	WAT066260	WAT066210
4.6 x 100 mm	3.5 µm	WAT066265	WAT066215
3.9 x 50 mm	5 µm	WAT054220	WAT054240
3.9 x 150 mm	5 µm	WAT054205	WAT054235
4.6 x 150 mm	5 µm	WAT054210	WAT054255
4.6 x 250 mm	5 µm	WAT054215	WAT054245

Symmetry *IS* Columns

Dimension	Particle Size	Part No. C_{18}	Part No. C_8
2.1 x 20 mm	3.5 µm	186002066	186002067
2.1 x 20 mm	5 µm	186002070	186002071
3.0 x 20 mm	3.5 µm	186002074	186002075
3.0 x 20 mm	5 µm	186002078	186002079
3.9 x 20 mm	3.5 µm	186002082	186002083
3.9 x 20 mm	5 µm	186002086	186002087
4.6 x 20 mm	3.5 µm	186002090	186002091
4.6 x 20 mm	5 µm	186002094	186002095

Symmetry Sentry Guard Columns (2/pk)

(Requires Sentry Guard Holders, see page 136)

Dimension	Particle Size	Part No. C_{18}	Part No. C_8
2.1 x 10 mm	3.5 µm	WAT106127	WAT106128
3.9 x 20 mm	5 µm	WAT054225	WAT054250

Symmetry Column and Cartridge Column Method Validation Kits

Three columns from three different batches to test reproducibility.

Dimension	Type	Particle Size	Part No. C_{18}	Part No. C_8
4.6 x 150 mm	Column	3.5 µm	WAT094240	WAT094237
2.1 x 150 mm	Column	5 µm	WAT094234	WAT094231
3.0 x 150 mm	Column	5 µm	WAT054446	WAT054434
3.9 x 150 mm	Column	5 µm	WAT047210	WAT046955
4.6 x 150 mm	Column	5 µm	WAT054448	WAT054435
4.6 x 250 mm	Column	5 µm	WAT054450	WAT054438
3.9 x 150 mm	Cartridge*	5 µm	WAT054452	WAT054440
4.6 x 150 mm	Cartridge*	5 µm	WAT054454	WAT054442
4.6 x 250 mm	Cartridge*	5 µm	WAT054456	WAT054444

* Requires endfittings, see page 136

Literature References

Symmetry Family of Columns Brochure, Literature Reference 720000454EN

Symmetry Family of Columns Applications Notebook, Literature Reference 720000593EN

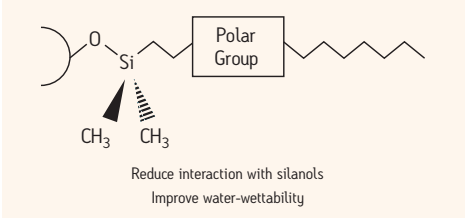
Interactive Waters Reversed-Phase Column Selectivity Chart, www.waters.com/selectivitychart

SymmetryShield Columns

Excellent Peak Shape

SymmetryShield HPLC columns are an excellent choice to achieve superior peak shapes for basic compounds. These reversed-phase columns are based on Waters patented embedded polar group technology that literally "shields" the silica's residual surface silanols from highly basic analytes.

Shield Technology



The embedded polar group, which is in close proximity to the silica surface, further reduces the activity of the surface silanols in SymmetryShield packing materials.

As a result, SymmetryShield columns:

- Deliver significantly improved peak shape and resolution over a broad pH range
- Exhibit less retention of basic compounds than conventional C_{18} and C_8 columns.

In fact, SymmetryShield columns deliver USP Tailing Factors of less than 1.2 over a pH range between pH 2 and pH 8. By delivering lower limits of detection and quantitation, SymmetryShield columns will enable you to develop new methods faster and improve the productivity of your laboratory.

Unique Selectivity

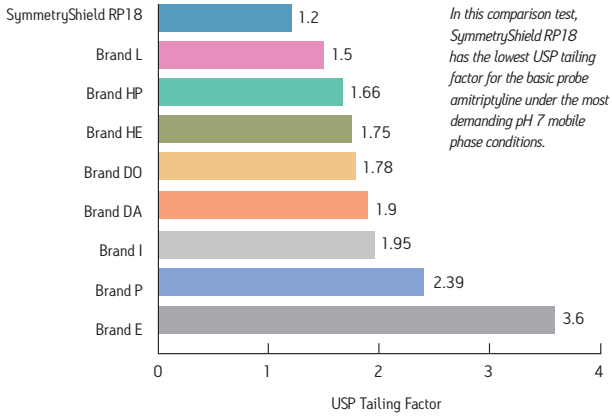
Obtaining a good separation is paramount. SymmetryShield columns exhibit less retention for basic compounds due to lower silanol activity than conventional C_{18} and C_8 columns. SymmetryShield columns are an excellent methods development tool.

In the example shown, a leading pharmaceutical company was faced with a challenging separation. It was only when a SymmetryShield column was substituted for a conventional C_8 column that a much better separation was achieved without having to change mobile phase conditions.

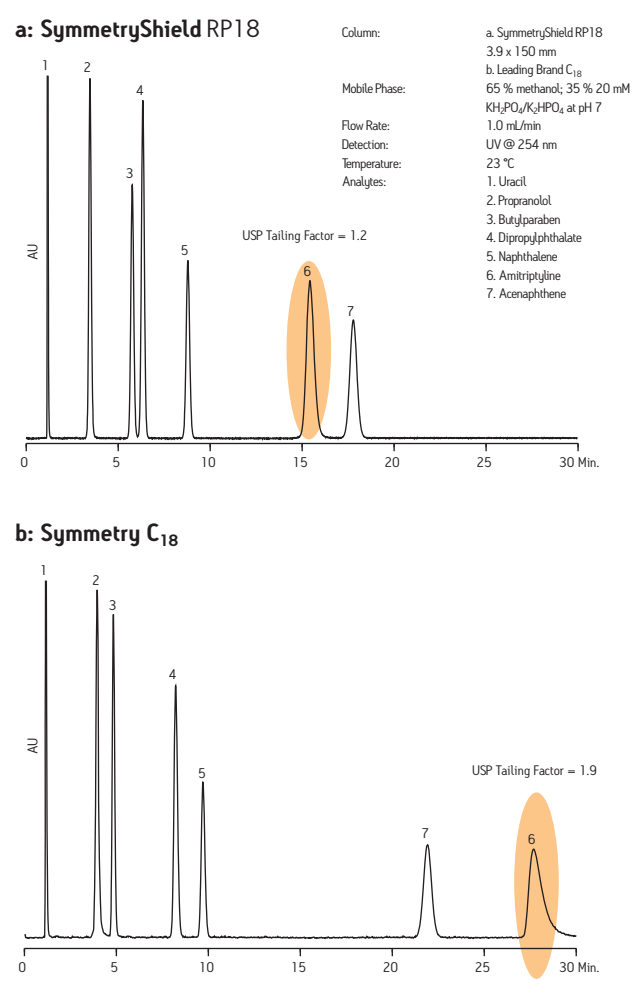
Perfect for Use with Highly Aqueous Mobile Phases

SymmetryShield columns with Shield Technology are the perfect choice for applications that require low organic, highly aqueous mobile-phase conditions. These reversed-phase particles are water-wettable allowing better interaction between the particles and your sample. The result is stable retention in highly aqueous mobile phases with superior peak shape.

Industry Leader for Peak Shape of Basic Compounds

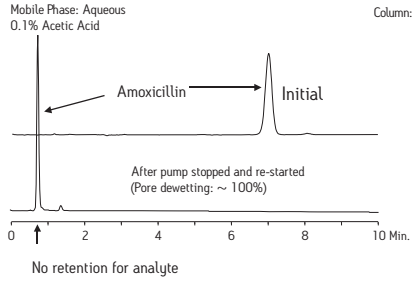


SymmetryShield Columns Deliver Unique Selectivity



SymmetryShield Columns–No Pore Dewetting in Highly Aqueous Mobile Phases

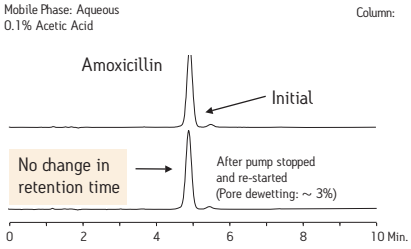
Standard Silica C_8 Columns—Pore Dewetting



Column: Standard C_8 Silica Column
4.6 x 150 mm, 5 µm

Most HPLC columns lose their retention when running low or 0% organic mobile phases if the flow is stopped. When this happens the bonded particle pores become de-wetted. The result is termed "pore dewetting" and you lose the rententivity of your analyte.

SymmetryShield RP8 Columns—No Pore Dewetting!



Column: SymmetryShield RP8
4.6 x 150 mm, 5 µm

SymmetryShield columns prevent this from happening because the embedded polar group maintains the wetted pore surface, ensuring retention of your analyte and eliminating the need for a lengthy re-wetting and column re-equilibration process.

SymmetryShield Columns

Dimension	Particle Size	Part No. RP_{18}	Part No. RP_8
1.0 x 50 mm	3.5 µm	186000175	WAT106060
1.0 x 150 mm	3.5 µm	186000176	WAT106048
2.1 x 30 mm	3.5 µm	186000171	WAT106001
2.1 x 50 mm	3.5 µm	186000172	WAT094257
2.1 x 100 mm	3.5 µm	186000173	WAT058969
2.1 x 150 mm	3.5 µm	186000174	WAT106008
3.0 x 50 mm	3.5 µm	186002614	186002615
3.0 x 100 mm	3.5 µm	186000700	186000703
3.0 x 150 mm	3.5 µm	186000699	186000702
4.6 x 50 mm	3.5 µm	186000177	WAT094260
4.6 x 75 mm	3.5 µm	186000178	WAT094263
4.6 x 100 mm	3.5 µm	186000179	WAT094266
4.6 x 150 mm	3.5 µm	186000180	WAT094269
2.1 x 50 mm	5 µm	186000217	186000223
2.1 x 100 mm	5 µm	186000998	186002611
2.1 x 150 mm	5 µm	186000111	WAT094245
3.0 x 150 mm	5 µm	186000692	WAT094243
3.0 x 250 mm	5 µm	186000693	186000694
3.9 x 150 mm	5 µm	186000108	WAT200655
4.6 x 50 mm	5 µm	186000218	186000224
4.6 x 100 mm	5 µm	186002618	186002619
4.6 x 150 mm	5 µm	186000109	WAT200662
4.6 x 250 mm	5 µm	186000112	WAT200670

SymmetryShield Cartridge Columns

(Requires endfittings, see page 136)

Dimension	Particle Size	Part No. RP_{18}	Part No. RP_8
2.1 x 50 mm	3.5 µm	186000168	186000147
2.1 x 100 mm	3.5 µm	186000167	186000146
2.1 x 150 mm	3.5 µm	186000166	186000145
4.6 x 75 mm	3.5 µm	186000183	WAT094272
4.6 x 100 mm	3.5 µm	186000170	WAT094275
3.9 x 50 mm	5 µm	—	WAT094248
3.9 x 150 mm	5 µm	186000106	WAT200658
4.6 x 150 mm	5 µm	186000110	WAT200665
4.6 x 250 mm	5 µm	186000113	WAT200661

SymmetryShield *IS* Columns

Dimension	Particle Size	Part No. RP_{18}	Part No. RP_8
2.1 x 20 mm	3.5 µm	186002068	186002069
2.1 x 20 mm	5 µm	186002072	186002073
3.0 x 20 mm	3.5 µm	186002076	186002077
3.0 x 20 mm	5 µm	186002080	186002081
3.9 x 20 mm	3.5 µm	186002084	186002085
3.9 x 20 mm	5 µm	186002088	186002089
4.6 x 20 mm	3.5 µm	186002092	186002093
4.6 x 20 mm	5 µm	186002096	186002097

SymmetryShield Sentry Guard Columns (2/pk)

(Requires Sentry Guard Holders, see page 136)

Dimension	Particle Size	Part No. RP_{18}	Part No. RP_8
2.1 x 10 mm	3.5 µm	186000169	WAT106129
3.9 x 20 mm	3.5 µm	186000701	186000704
3.9 x 20 mm	5 µm	186000107	WAT200675

SymmetryShield Column and Cartridge Column Method Validation Kits

Three columns from three different batches to test reproducibility.

Dimension	Type	Particle Size	Part No. RP_{18}	Part No. RP_8
2.1 x 150 mm	Column	3.5 µm	186000182	—
4.6 x 150 mm	Column	3.5 µm	186000181	WAT094278
2.1 x 150 mm	Column	5 µm	186000100	WAT094254
3.0 x 150 mm	Column	5 µm	—	WAT094251
3.9 x 150 mm	Column	5 µm	186000104	WAT210594
4.6 x 150 mm	Column	5 µm	186000103	WAT210588
4.6 x 250 mm	Column	5 µm	186000102	WAT210591
3.9 x 150 mm	Cartridge*	5 µm	186000105	WAT210582
4.6 x 150 mm	Cartridge*	5 µm	186000101	WAT210585
4.6 x 250 mm	Cartridge*	5 µm	186000114	WAT210579

* Requires endfittings, see page 136

Symmetry300 Columns

High Recoveries of Proteins and Peptides

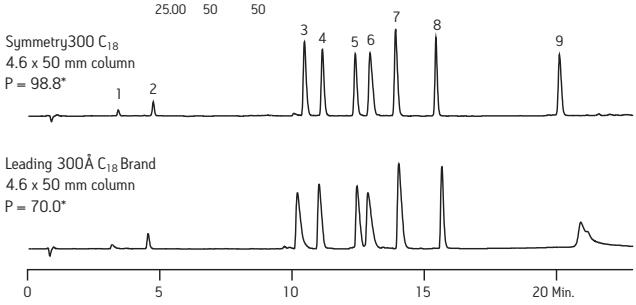
Symmetry300 columns are wide-pore, reversed-phase columns which are built on the Symmetry column platform, the standard that the pharmaceutical industry has come to rely on for sensitive, rugged and robust HPLC analyses.

Peptide Separation at High Mass Load (0.1% TFA)

Columns:	4.6 x 50 mm, 5 µm	Temperature:	40 °C
Mobile Phase A:	0.1% TFA in H_2O	Detection:	UV 220 nm
Mobile Phase B:	0.08% TFA in ACN	Instrument:	Waters Alliance 2690
Flow Rate:	0.75 mL/min		

Gradient:

Time (min)	Profile %A	%B
0.00	100	0
25.00	50	50



Biologically Active Peptide Mixture[1]

Name	MW	pI	Hydrophobicity (Meek)	Number of Amino Acids
1. L2275	986.6	12.04	2.8	9
2. A6677	1001.5	9.34	3.0	10
3. Bradykinin	1060.2	12.00	42.6	9
4. Angiotensin II	1046.2	7.35	43.7	8
5. Angiotensin I	1296.5	7.51	54.0	10
6. Substance P	1347.6	11.20	52.7	11
7. Renin substrate	1759.0	7.61	63.5	14
8. Insulin B chain	3495.9	7.51	129.4	30
9. Melittin	2845.8	12.06	92.3	26

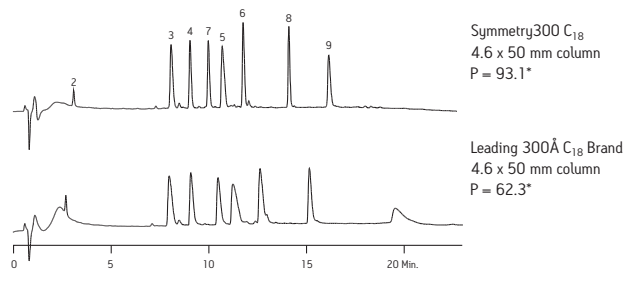
[1] J. L. Meek and Z. L. Rossetti, J. Chromatogr., 211 (1981) 15-28. pI = Isoelectric pH

Peptide Separation at Low Mass Load (0.1% Formic Acid)

Columns:	4.6 x 50 mm, 5 µm	Gradient:	
Mobile Phase A:	0.1% formic acid in H_2O		
Mobile Phase B:	0.08% formic acid in ACN		
Flow Rate:	0.75 mL/min		

Time (min)	Profile %A	%B
0	100	0
25	50	50

Injection Volume:	20 µL
Temperature:	40 °C
Detection:	UV 220 nm
Instrument:	Waters Alliance 2690



* Note: Peak Capacity (P) is defined as the number of theoretical peaks that can fit into a chromatographic run within a certain gradient time.

$P = 1 + (t_g/w_{5\sigma})$ Where tg = gradient time $w_{5\sigma}$ = peak measured at 4.4% of peak height

Symmetry300 C_{18} columns provide better peak shape and peak capacity compared to other leading brands when separating biologically active peptides. In the examples shown, Symmetry300 columns demonstrate superior performance when simultaneously separating hydrophilic and hydrophobic peptides, as well as neutral and highly basic peptides. Also, hydrophobicity (Meek retention prediction 1) is included. The lower hydrophobicity values indicate the peptide is more hydrophilic.

Symmetry300 columns perform better at high and low mass load conditions. For mass spectrometry applications, we recommend low TFA mobile phases or 0.1% formic acid.

Column-after-column, no matter who uses your methods or where they are used around the world, the Symmetry300 columns are the best solution for validation compliance.

Symmetry300 columns are available in two particle sizes: 3.5 µm and 5 µm, and in two chemistries: C_4 for large peptides and proteins, and C_{18} for smaller peptides.

Sharpest Peaks for Highest Resolution

The heart of the column is the high purity base deactivated silica. The silica used in Symmetry300 columns is synthesized using ultrapure organic reagents, resulting in high purity with very low silanol activity.

In addition, when combined with the high surface coverage of the bonded phase the result is higher recoveries of proteins and peptides even when injected at low concentrations.

Symmetry300 columns deliver the sharpest peaks for biomolecules and deliver more peak capacity for complex tryptic maps. They are packed to the highest efficiency and reproducibility of any product on the market. This results in improved precision for impurity and stability assays, more accurate quantitation, and adaptability to more rugged methods.

Unsurpassed Reproducibility

Cytochrome *c* Tryptic Map Q.C. Test

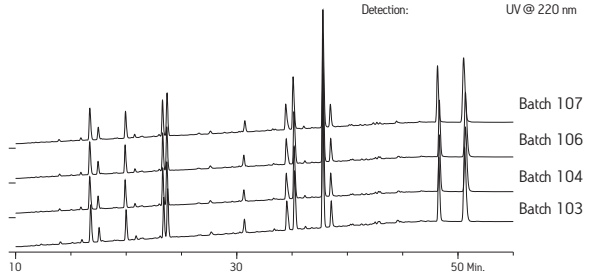
Waters batch release protocol includes a tryptic map of cytochrome c which is used to test for reproducibility of retention time and resolution. In this test, the tryptic map was run on four Symmetry300 columns, each containing a different batch of packing.

Column:	Symmetry300 C_{18} 3.9 x 150 mm, 5 µm
Part No.:	WAT106154
Mobile Phase A:	0.05% TFA in water
Mobile Phase B:	0.1% TFA in acetonitrile
Flow rate:	1.0 mL/min

Gradient:

Time (min)	Profile %A	%B
0	100	0
45	72	28

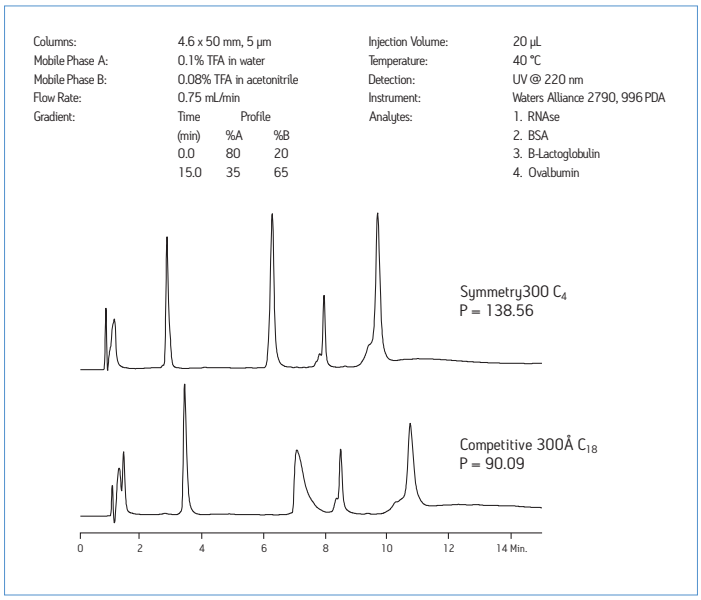
Injection volume:	20 µL
Temperature:	35 °C
Detection:	UV @ 220 nm



Excellent Protein Separations

Symmetry300 C_4 columns have the highest average peak capacity when compared to over 20 leading brands of HPLC columns. In the example shown, which is considered to be a difficult protein separation, the Symmetry300 C_4 column provided a better separation with better peak shape compared to a leading competitive 300Å column.

Protein: Symmetry300 C_4 Versus Competitors



Symmetry300 Analytical Columns

Dimension	Particle Size	Part No. C_{18}	Part No. C_4
1.0 x 150 mm	3.5 µm	186000185	186000276
2.1 x 50 mm	3.5 µm	186000187	186000277
2.1 x 100 mm	3.5 µm	186000188	186000278
2.1 x 150 mm	3.5 µm	186000200	186000279
4.6 x 50 mm	3.5 µm	186000201	186000280
4.6 x 75 mm	3.5 µm	186000189	186000281
4.6 x 100 mm	3.5 µm	186000190	186000282
4.6 x 150 mm	3.5 µm	186000197	186000283
2.1 x 150 mm	5 µm	WAT106172	186000285
3.9 x 150 mm	5 µm	WAT106154	186000286
4.6 x 50 mm	5 µm	WAT106209	186000287
4.6 x 150 mm	5 µm	WAT106157	186000288
4.6 x 250 mm	5 µm	WAT106151	186000289

Symmetry300 Cartridge Columns

(Requires endfittings, see page 136)

Dimension	Particle Size	Part No. C_{18}
2.1 x 50 mm	3.5 µm	186000199
2.1 x 100 mm	3.5 µm	186000191
2.1 x 150 mm	3.5 µm	186000196
4.6 x 75 mm	3.5 µm	186000192
4.6 x 100 mm	3.5 µm	186000193
3.9 x 150 mm	5 µm	WAT106169
4.6 x 150 mm	5 µm	WAT106163
4.6 x 250 mm	5 µm	WAT106160

Symmetry300 Sentry Guard Columns (2 per pack)

(Requires Sentry Guard Holders, see page 136)

Dimension	Particle Size	Part No. C_{18}	Part No. C_4
2.1 x 10 mm	3.5 µm	186000198	186000275
3.9 x 20 mm	5 µm	WAT106166	186000284

Symmetry300 Column and Cartridge Column Method Validation Kits

Three columns from three different batches to test reproducibility.

Dimension	Type	Particle Size	Part No. C_{18}	Part No. C_4
2.1 x 150 mm	Column	3.5 µm	186000194	186000290
4.6 x 150 mm	Column	3.5 µm	186000195	186000291
2.1 x 150 mm	Column	5 µm	WAT106193	186000292
3.9 x 150 mm	Column	5 µm	WAT106187	186000293
4.6 x 150 mm	Column	5 µm	WAT106190	186000294
4.6 x 250 mm	Column	5 µm	WAT106184	186000295
3.9 x 150 mm	Cartridge*	5 µm	WAT106181	—
4.6 x 150 mm	Cartridge*	5 µm	WAT106175	—
4.6 x 250 mm	Cartridge*	5 µm	WAT106178	—

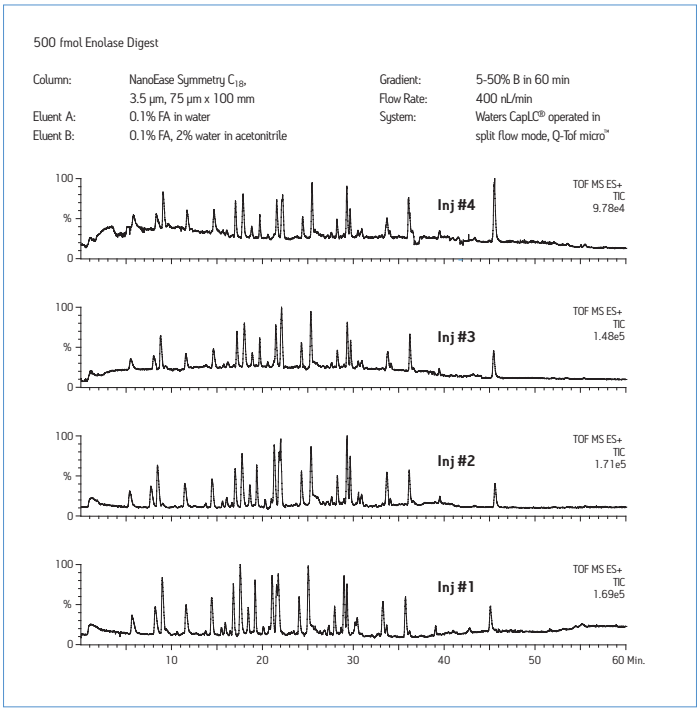
* Requires endfittings, see page 136

Symmetry NanoEase Nano and Capillary Columns

NanoEase columns contain high performance, small particle sorbents. Exact manufacturing and QC production procedures ensure consistent column performance. Superior column longevity is ensured by a novel frit technology that minimizes bed disturbance throughout the life of the column.

- Superior chromatographic performance
- Available in variety of Waters stationary phases
- Robust and easy to handle
- Can be used with any capillary LC system

NanoEase LC/MS Nano Column Reproducibility



Description	Column i.d.	Length	Particle Size	Part No.
Symmetry C_{18}	75 µm	50 mm	3.5 µm	186002188
Symmetry C_{18}	75 µm	100 mm	3.5 µm	186002189
Symmetry C_{18}	75 µm	150 mm	3.5 µm	186002190
Symmetry300 C_{18}	75 µm	50 mm	3.5 µm	186002191
Symmetry300 C_{18}	75 µm	100 mm	3.5 µm	186002192
Symmetry300 C_{18}	75 µm	150 mm	3.5 µm	186002193
Symmetry C_{18}	100 µm	50 mm	3.5 µm	186002201
Symmetry C_{18}	100 µm	100 mm	3.5 µm	186002202
Symmetry C_{18}	100 µm	150 mm	3.5 µm	186002203
Symmetry300 C_{18}	100 µm	50 mm	3.5 µm	186002204
Symmetry300 C_{18}	100 µm	100 mm	3.5 µm	186002205
Symmetry300 C_{18}	100 µm	150 mm	3.5 µm	186002206
Symmetry C_{18}	150 µm	50 mm	3.5 µm	186002459
Symmetry C_{18}	150 µm	100 mm	3.5 µm	186002460
Symmetry C_{18}	150 µm	150 mm	3.5 µm	186002461
Symmetry300 C_{18}	150 µm	50 mm	3.5 µm	186002462
Symmetry300 C_{18}	150 µm	100 mm	3.5 µm	186002463
Symmetry300 C_{18}	150 µm	150 mm	3.5 µm	186002464
Symmetry C_{18}	300 µm	50 mm	3.5 µm	186002581
Symmetry C_{18}	300 µm	100 mm	3.5 µm	186002582
Symmetry C_{18}	300 µm	150 mm	3.5 µm	186002583
Symmetry C_{18}	300 µm	50 mm	5 µm	186002584
Symmetry C_{18}	300 µm	100 mm	5 µm	186002585
Symmetry C_{18}	300 µm	150 mm	5 µm	186002586
Symmetry300 C_{18}	300 µm	50 mm	3.5 µm	186002587
Symmetry300 C_{18}	300 µm	100 mm	3.5 µm	186002588
Symmetry300 C_{18}	300 µm	150 mm	3.5 µm	186002589
Symmetry300 C_{18}	300 µm	50 mm	5 µm	186002590
Symmetry300 C_{18}	300 µm	100 mm	5 µm	186002591
Symmetry300 C_{18}	300 µm	150 mm	5 µm	186002592

Waters Spherisorb Columns

One of the great advantages of choosing Waters Spherisorb columns is the range of choices available to you. Waters Spherisorb columns are produced in a wide range of sizes and bonded phases to meet your chromatographic needs. Silica is produced in 3, 5 and 10 micron particle sizes. The high quality bonded phases give many different and unique separation selectivities.

Physical Characteristics

Product Description	Particle Shape	Surface Area	Pore Size	Pore Volume	Particle Size	Carbon Load	Ligand Coverage	End-capped
Waters Spherisorb Phases								
Waters Spherisorb ODS2 (C_{18})	Spherical	220 m^2/g	80Å	0.50 ml/g	3, 5, 10 µm	11.5%	2.98 µMol/m^2	yes
Waters Spherisorb ODS1 (C_{18})	Spherical	220 m^2/g	80Å	0.50 ml/g	3, 5, 10 µm	6.2%	1.49 µMol/m^2	no
Waters Spherisorb ODSB (C_{18})	Spherical	220 m^2/g	80Å	0.50 ml/g	5 µm	11.5%	2.98 µMol/m^2	yes
Waters Spherisorb C_8	Spherical	220 m^2/g	80Å	0.50 ml/g	3, 5, 10 µm	5.75%	3.12 µMol/m^2	yes
Waters Spherisorb C_6	Spherical	220 m^2/g	80Å	0.50 ml/g	3, 5, 10 µm	4.7%	3.36 µMol/m^2	yes
Waters Spherisorb C_1	Spherical	220 m^2/g	80Å	0.50 ml/g	3, 5, 10 µm	2.15%	2.97 µMol/m^2	no
Waters Spherisorb NH2 (Amino)	Spherical	220 m^2/g	80Å	0.50 ml/g	3, 5, 10 µm	1.9%	2.64 µMol/m^2	no
Waters Spherisorb Phenyl	Spherical	220 m^2/g	80Å	0.50 ml/g	3, 5, 10 µm	2.5%	1.72 µMol/m^2	no
Waters Spherisorb CN (Nitrile)	Spherical	220 m^2/g	80Å	0.50 ml/g	3, 5, 10 µm	3.1%	3.29 µMol/m^2	no
Waters Spherisorb OD/CN	Spherical	220 m^2/g	80Å	0.50 ml/g	5 µm	5%	1.15 µMol/m^2	yes
Waters Spherisorb W (Silica)	Spherical	220 m^2/g	80Å	0.50 ml/g	3, 5, 10 µm	—	—	—
Waters Spherisorb Ion Exchangers								
Waters Spherisorb SCX	Spherical	220 m^2/g	80Å	0.50 ml/g	5, 10 µm	4%	—	no
Waters Spherisorb SAX	Spherical	220 m^2/g	80Å	0.50 ml/g	5, 10 µm	4%	—	no

Waters Spherisorb Analytical Columns

Waters Spherisorb analytical columns come with industry standard endfittings. The threaded female fitting requires a Parker-style compression screw and ferrule to connect the HPLC tubing to the analytical column. If your instrument is set up for Waters columns, replace your ferrule on the connector tubing and set it for the Parker standard.

Waters Spherisorb Cartridge Columns

Waters Spherisorb cartridge columns require reusable cartridge endfittings (Part No. PSS614100). These endfittings should be ordered the first time a cartridge is purchased. The reusable endfittings are a Parker-style standard endfitting. Coupler fittings are also available to allow you to link a guard cartridge to your analytical cartridge.

Waters Spherisorb Columns (10 µm packing)

Chemistry	4.6 mm x 100 mm	4.6 mm x 150 mm	4.6 mm x 200 mm	4.6 mm x 250 mm
S10 ODS2	—	PSS832513	PSS832514	PSS832515
S10 ODS1	PSS830712	PSS830713	PSS830714	PSS830715
S10 C8	—	PSS832813	—	PSS832815
S10 C6	—	PSS833213	—	PSS833215
S10 C1	—	PSS833013	—	PSS833015
S10 NH2	PSS833612	PSS833613	—	PSS833615
S10 P (Phenyl)	—	PSS833813	—	PSS833815
S10 CN	PSS833512	PSS833513	PSS832514	PSS833515
S10 W (Silica)	PSS830212	PSS830213	PSS830214	PSS830215
Ion Exchange				
S10 SAX	PSS833912	PSS833913	—	PSS833915
S10 SCX	—	PSS837613	—	PSS837615

Waters Spherisorb 5 µm Columns and Cartridges

	Columns				Cartridge Columns					
Chemistry	4.0 mm x 125 mm	4.0 mm x 250 mm	4.6 mm x 50 mm	4.6 mm x 100 mm	4.6 mm x 150 mm	4.6 mm x 250 mm	4.6 mm x 50 mm	4.6 mm x 100 mm	4.6 mm x 150 mm	4.6 mm x 250 mm
S5 ODS2	PSS845543	PSS845277	PSS831911	PSS831912	PSS831913	PSS831915	PSS839536	PSS839537	PSS839538	PSS839540
S5 ODS1	PSS845541	PSS845542	PSS830611	PSS830612	PSS830613	PSS830615	PSS839506	PSS839507	PSS839508	PSS839510
S5 ODS B	—	—	—	—	—	—	—	—	PSS839613	PSS839615
S5 C8	PSS845280	PSS845281	PSS831811	PSS831812	PSS831813	PSS831815	PSS839531	PSS839532	PSS839533	PSS839535
S5 C6	PSS845284	PSS845285	PSS831011	PSS831012	PSS831013	PSS831015	PSS839521	PSS839522	PSS839523	PSS839525
S5 C1	PSS845288	PSS845289	PSS832611	PSS832612	PSS832613	PSS832615	PSS839566	PSS839567	PSS839568	PSS839570
S5 NH2	PSS845300	PSS845301	PSS831111	PSS831112	PSS831113	PSS831115	PSS839526	PSS839527	PSS839528	PSS839530
S5 P (Phenyl)	PSS845292	PSS845293	—	PSS830812	PSS830813	PSS830815	PSS839511	PSS839512	PSS839513	PSS839515
S5 CN Normal Phase	PSS845296	PSS845297	PSS830911	PSS830912	PSS830913	PSS830915	PSS839516	PSS839517	PSS839518	PSS839520
S5 CN Reversed Phase	—	—	—	—	PSS830908	PSS830909	—	—	—	—
S5 W (Silica)	PSS845539	PSS845540	PSS830111	PSS830112	PSS830113	PSS830115	—	PSS839502	PSS839503	PSS839505
Ion Exchange										
S5 SAX	PSS845304	PSS845305	PSS832711	PSS832712	PSS832713	PSS832715	PSS839571	PSS839572	PSS839573	PSS839575
S5 SCX	PSS845308	PSS845309	PSS837511	PSS837512	PSS837513	PSS837515	PSS839651	PSS839652	PSS839653	PSS839655
Mixed Mode										
S5 OD/CN	—	—	—	PSS837812	PSS837813	PSS837815	—	—	PSS839643	PSS839645

Waters Spherisorb 3 µm Columns and Cartridges

	Columns				Cartridge Columns			
Chemistry	4.6 mm x 50 mm	4.6 mm x 60 mm	4.6 mm x 100 mm	4.6 mm x 150 mm	4.6 mm x 30 mm	4.6 mm x 50 mm	4.6 mm x 100 mm	4.6 mm x 150 mm
S3 ODS2	PSS832111	PSS839853	PSS832112	PSS832113	PSS830065	PSS839546	PSS839547	PSS839548
S3 ODS1	PSS833411	—	PSS833412	PSS833413	—	—	PSS839587	PSS839588
S3 C8	PSS832211	PSS839852	PSS832212	PSS832213	—	PSS839551	PSS839552	PSS839553
S3 C6	PSS833111	—	PSS833112	PSS833113	—	PSS839581	PSS839582	PSS839583
S3 C1	PSS832911	—	PSS832912	PSS832913	—	PSS839576	PSS839577	PSS839578
S3 NH2	PSS832311	—	PSS832312	PSS832313	—	PSS839556	PSS839557	PSS839558
S3 P (Phenyl)	PSS833711	—	PSS833712	PSS833713	—	PSS839591	PSS839592	PSS839593
S3 CN Normal Phase	PSS832411	—	PSS832412	PSS832413	—	PSS839561	PSS839562	PSS839563
S3 W (Silica)	PSS832011	—	PSS832012	PSS832013	PSS830068	PSS839541	PSS839542	PSS839543

Waters Spherisorb 5 µm Cartridge Columns

Chemistry	3.0 mm x 125 mm	3.0 mm x 250 mm	4.0 mm x 125 mm	4.0 mm x 250 mm
S5 ODS2	PSS838529	PSS838522	PSS845553	PSS845554
S5 ODS1	PSS838530	PSS838523	PSS845551	PSS845552
S5 C8	PSS838533	PSS838525	PSS845555	PSS845556
S5 C6	—	—	PSS845557	PSS845558
S5 C1	—	—	PSS845559	PSS845560
S5 NH2	PSS838535	PSS838526	PSS845565	PSS845566
S5 P (Phenyl)	—	—	PSS845561	PSS845562
S5 CN Normal Phase	PSS838531	PSS838524	PSS845563	PSS845564
S5 CN Reversed Phase	—	—	—	—
S5 W (Silica)	PSS838534	PSS838521	PSS845549	PSS845550
Ion Exchange				
S5 SAX	—	—	PSS845567	PSS845568
S5 SCX	—	—	PSS845569	PSS845570

Waters Spherisorb 5 µm Semiprep Columns

Chemistry	10 mm x 250 mm	20 mm x 250 mm	10 µm Chemistry	10 mm x 250 mm	20 mm x 250 mm
S5 ODS2	PSS831985	PSS831995	S10 ODS2	PSS832585	PSS832595
S5 ODS1	PSS830685	PSS830695	S10 ODS1	PSS830785	PSS830795
S5 C8	PSS831885	PSS831895	S10 C8	PSS832885	PSS832895
S5 C6	PSS831085	PSS831095	S10 C6	PSS833285	PSS833295
S5 C1	PSS832685	PSS832695	S10 C1	PSS833085	PSS833095
S5 NH2	PSS831185	PSS831195	S10 NH2	PSS833685	PSS833695
S5P (Phenyl)	PSS830885	PSS830895	S10 P (Phenyl)	PSS833885	PSS833895
S5 CN	PSS830985	PSS830995	S10 CN	PSS833585	PSS833595
S5 W (Silica)	PSS830185	PSS830195	S10 W (Silica)	PSS830285	PSS830295
Ion Exchange					
S5 SAX	PSS832785	PSS832795	S10 SAX	PSS833985	PSS833995
S5 SCX	PSS837585	PSS837595	S10 SCX	PSS837685	PSS837695

Waters Spherisorb 3 µm Microbore Columns

Chemistry	1 mm x 100 mm	1 mm x 150 mm	5 µm Chemistry	1 mm x 100 mm	1 mm x 150 mm	1 mm x 250 mm
S3 ODS2	PSS832132	PSS832133	S5 ODS2	PSS831932	PSS831933	PSS831935
S3 ODS1	—	PSS833433	S5 ODS1	PSS830632	—	PSS830635
S3 C8	PSS832232	PSS832233	S5 C8	PSS831832	—	—
			S5 C6	PSS831032	—	—
S3 NH2	—	PSS832333	S5 NH2	PSS831132	—	PSS831135
S3 CN	PSS832432	PSS832433	S5 CN	PSS830932	—	—
S3 W (Silica)	PSS832032	PSS832033	S5 W (Silica)	—	—	PSS830135

Waters Spherisorb 3 µm Narrow-Bore Columns

Chemistry	2 mm x 100 mm	2 mm x 150 mm	5 µm Chemistry	2 mm x 100 mm	2 mm x 150 mm	2 mm x 250 mm
S3 ODS2	PSS832122	PSS832123	S5 ODS2	PSS831922	PSS831923	PSS831925
S3 ODS1	PSS833422	PSS833423	S5 ODS1	PSS830622	PSS830623	PSS830625
S3 C8	PSS832222	PSS832223	S5 C8	PSS831822	PSS831823	PSS831825
S3 NH2	PSS832322	PSS832323	S5 NH2	PSS831122	PSS831123	PSS831125
S3 CN	PSS832422	PSS832423	S5 CN	PSS830922	PSS830923	PSS830925
S3 W (Silica)	PSS832022	PSS832023	S5 W (Silica)	PSS830122	PSS830123	PSS830125

Cartridge Fittings and Accessories

These endfittings and accessories can be used with 4.6 mm, 4.0 mm, or 3.0 mm i.d. cartridges.

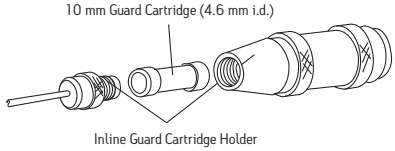
Description	Qty.	Part No.
Removable Column Endfitting	2/pk	PSS614100
Frit Assembly (2 µm)	5/pk	PSS614103
Frit Assembly (0.5 µm)	5/pk	PSS614104
Column Coupler	2/pk	PSS614102
Long Tail Endfitting	2/pk	PSS614101
Extended Endfitting for use with 10 mm Integral Guard	1/pk	PSS614108
Nylon Column Plugs for Storage of Complete Column	1/pk	WAT015674
Nylon Column Caps for Storage of Replacement Cartridge Column	10/pk	PSS614113
Inline 10 mm Guard Cartridge Holder Kit for use with above items		PSS830008

1. 30 mm Stand Alone Guard/Column (endfittings not included)
2. Extended endfitting for use with 10 mm Integral Guard: PSS614108
3. 10 mm Integral Guard Column
4. Column Coupler: PSS614102

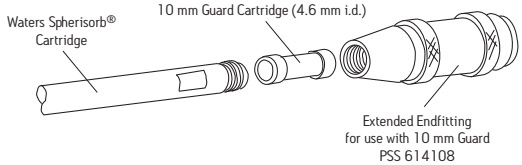
Waters Spherisorb Guard Cartridges

Chemistry	10 x 4.6 mm 3 Pack	30 x 4.6 mm Guard 3 Pack
S5 ODS2	PSS830053	PSS839458
S5 ODS1	PSS830073	PSS839472
S5 C8	PSS830074	PSS839473
S5 C6	PSS830075	PSS839474
S5 C1	PSS830076	PSS839475
S5 CN	PSS830077	PSS839476
S5 Phenyl	PSS830078	PSS839477
S5 NH2	PSS830079	PSS839478
S5 W Silica	PSS830051	PSS839451
S5 SAX	PSS830055	PSS839465
S5 SCX	PSS830057	PSS839471
S5 ODSB	PSS830059	PSS839470

Inline Guard Cartridge Holder



Extended Endfitting for use with 10 mm Guard Cartridges



Nova-Pak Packings

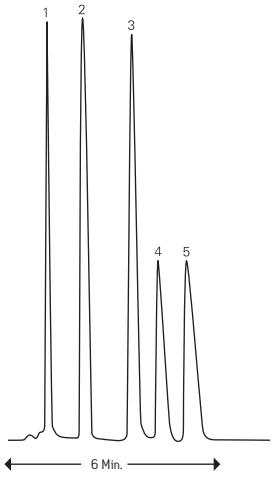
The 4 µm small particle size of Nova-Pak® bonded phases offers high resolution as well as more efficient and faster chromatography. This is accomplished by using shorter columns, thereby reducing solvent consumption or by using longer columns to resolve even the most complex mixture. Analytical columns with 4 µm particle size packings are available in 75, 150, and 300 mm length steel columns. Steel cartridge columns with reusable endfittings are available in 50, 100, 150, and 250 mm lengths. Semi-preparative columns with 6 micron particle size packings give you an unparalleled range of separation possibilities. Stringent QC procedures in our cGMP manufacturing facility ensure batch-to-batch reproducibility.

Physical Characteristics

Packing	Chemistry	Particle Size	Particle Shape	Pore Size	Carbon Load	End-capped
Nova-Pak	C_{18}	4 µm	Spherical	60Å	7%	Yes
	C_8	4 µm	Spherical	60Å	4%	Yes
	Phenyl	4 µm	Spherical	60Å	5%	Yes
	CN HP	4 µm	Spherical	60Å	2%	Yes
	Silica	4 µm	Spherical	60Å	N/A	N/A
Prep Nova-Pak HR	C_{18}	6 µm	Spherical	60Å	7%	Yes
	Silica	6 µm	Spherical	60Å	N/A	N/A

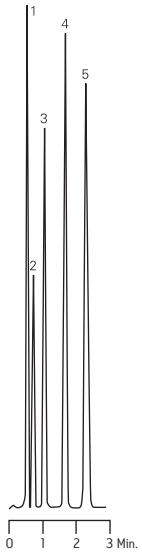
Rapid Analysis of Soft Drink Additives and Artificial Sweeteners on Nova-Pak C_{18} Column

Column: Waters Nova-Pak C_{18}, 3.9 x 150 mm
Part Number: WAT086344
Mobile Phase: Acetonitrile: Ammonium Acetate with 2.5% acetic acid 10:90
Flow Rate: 1.0 mL/min
Detection: 254 nm
Sample:
1. Saccharin
2. Caffeine
3. Benzoic acid
4. Aspartame
5. Sorbic Acid



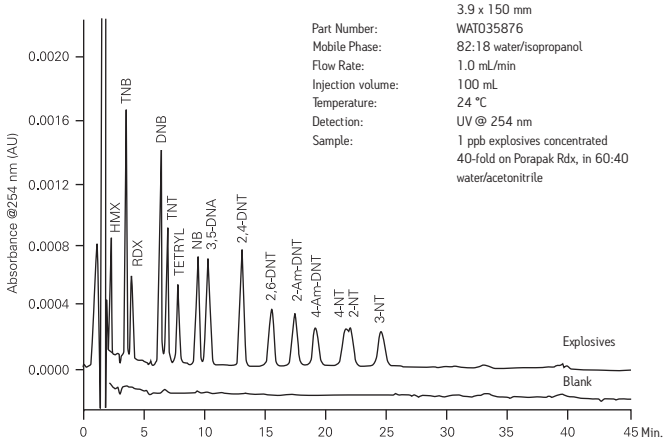
Anticonvulsants

Column: Waters Nova-Pak C_8, 3.9 x 50 mm
Part No.: WAT052775
Sample: Anticonvulsants
In elution order:
1-Succinic acid imide
2-Primidone
3-Phenobarbital
4-Mephenytoin
5-Mephobarbital



Isocratic Separation of Method 8330 Analytes (Explosives)

Column: Waters Nova-Pak C_8, 3.9 x 150 mm
Part Number: WAT035876
Mobile Phase: 82:18 water/isopropanol
Flow Rate: 1.0 mL/min
Injection volume: 100 mL
Temperature: 24 °C
Detection: UV @ 254 nm
Sample: 1 ppb explosives concentrated 40-fold on Porapak Rdx, in 60:40 water/acetonitrile



Nova-Pak Analytical Columns

Analytical columns with 4 µm Nova-Pak packing material gives you an unparalleled range of separation possibilities.

Nova-Pak columns are available in a variety of sizes to meet your applications needs. Manufactured exclusively by Waters, columns packed with Nova-Pak material give you the efficiency you expect from today's analytical columns and the reproducibility and durability you need.

Nova-Pak Analytical Columns

Description	Particle Size	Dimensions	Part No.
Nova-Pak C_{18}	4 µm	2.1 x 150 mm	WAT023655
		3.9 x 75 mm	WAT011670
		3.9 x 150 mm	WAT086344
		3.9 x 300 mm	WAT011695
		4.6 x 150 mm	WAT044375
Nova-Pak C_8	4 µm	2.1 x 150 mm	WAT052735
		3.9 x 75 mm	WAT035877
		3.9 x 150 mm	WAT035876
Nova-Pak Phenyl	4 µm	2.1 x 150 mm	WAT052740
		3.9 x 75 mm	WAT011675
		3.9 x 150 mm	WAT010656
Nova-Pak CN HP	4 µm	3.9 x 75 mm	WAT010270
		3.9 x 150 mm	WAT044245
		3.9 x 300 mm	WAT056920
Nova-Pak Silica	4 µm	2.1 x 150 mm	WAT052745
		3.9 x 75 mm	WAT011680
		3.9 x 150 mm	WAT010025

Nova-Pak Analytical Method Validation Kit

The Method Validation Kit includes three Nova-Pak C_{18}, 4 µm, 4.6 x 150 mm steel columns. Two of these columns are packed with material from one particular batch of material and the other column is packed with material from a different batch of the bulk material. All three columns are identified with the batch number. The columns are ideal for testing the ruggedness of new applications for regulated methods. Nova-Pak batch-to-batch reproducibility is guaranteed.

Nova-Pak Analytical Method Validation Kit

Description	Particle Size	Dimensions	Part No.
Method Validation Kit (includes 3 Nova-Pak C_{18} columns)	4 µm	3.9 x 150 mm	WAT052770

Nova-Pak Cartridge Columns

The cartridge column requires reusable finger-tightened endfittings which are purchased separately. When you change columns, just replace the cartridge and use the endfittings again. The cartridge column assembly includes one disposable cartridge column and reusable endfittings.

The prepacked, disposable cartridge column requires reusable finger-tightened endfittings which can be purchased separately. When the performance of the cartridge column declines, you just replace the cartridge and use the endfittings again.

Nova-Pak Cartridge Column Assembly

(Requires a set of re-usable endfittings)

Nova-Pak Cartridge Columns

(Requires endfittings, Part No. WAT037525)

Description	Particle Size	Qty.	Dimensions	Part No.
Nova-Pak C_{18}	4 µm	1/box	3.9 x 50 mm	WAT052780
		3/box	3.9 x 50 mm	WAT052834
		1/box	3.9 x 100 mm	WAT052810
		1/box	3.9 x 150 mm	WAT036975
		3/box	3.9 x 150 mm	WAT037520
		1/box	4.6 x 150 mm	WAT052845
		1/box	4.6 x 250 mm	WAT052840
Nova-Pak C_8	4 µm	1/box	3.9 x 50 mm	WAT052775
		1/box	3.9 x 100 mm	WAT052805
		1/box	3.9 x 150 mm	WAT036985
		3/box	3.9 x 150 mm	WAT054870
		1/box	4.6 x 150 mm	WAT052855
		1/box	4.6 x 250 mm	WAT052850
Nova-Pak Phenyl	4 µm	1/box	3.9 x 50 mm	WAT052790
		1/box	3.9 x 100 mm	WAT052800
		1/box	3.9 x 150 mm	WAT036970
		3/box	3.9 x 150 mm	WAT054890
Nova-Pak CN HP	4 µm	1/box	3.9 x 50 mm	WAT052785
		1/box	3.9 x 100 mm	WAT052795
		1/box	3.9 x 150 mm	WAT044243
		3/box	3.9 x 150 mm	WAT044445
		1/box	4.6 x 150 mm	WAT044455
		1/box	4.6 x 250 mm	WAT044460
Nova-Pak Silica	4 µm	1/box	3.9 x 150 mm	WAT036980
		3/box	3.9 x 150 mm	WAT054880

Endfittings (includes 1 pair of reusable endfittings, 2 c-clips and coupling)	WAT037525

Analytical Kits

Description	Dimensions	Part No.
Waters Reversed-Phase Scouting Kit (includes 1 each Nova-Pak C_{18}, C_8, and Phenyl cartridge column)	3.9 x 50 mm	WAT044360
Waters Method Development Scouting Kit (includes 1 each Nova-Pak C_8, Phenyl, and CN HP cartridge column)	3.9 x 50 mm	WAT044255
Waters Method Validation Kit (includes 3 Nova-Pak C_{18} columns representing 2 different bonded-silica bulk packing batches)	3.9 x 150 mm	WAT052770

Nova-Pak Sentry Guard Columns 2/pkg

(Requires Sentry Guard Holder, Part No. WAT046910)

Description	Particle Size	Dimensions	Part No.
Nova-Pak C_{18}	4 µm	3.9 x 20 mm	WAT044380
Nova-Pak C_8	4 µm	3.9 x 20 mm	WAT046830
Nova-Pak Phenyl	4 µm	3.9 x 20 mm	WAT046835
Nova-Pak Silica	4 µm	3.9 x 20 mm	WAT046845
Nova-Pak CN HP	4 µm	3.9 x 20 mm	WAT046840

Sentry Guard Holders

(50 mm and 75 mm long cartridge columns must use the Universal Guard Holder)

Description	Part No.
Integrated Guard Holder (for Waters 3.9 mm and 4.6 mm cartridge columns)	WAT046905
Universal Guard Holder (for any 3.9 mm and 4.6 mm HPLC column)	WAT046910

Nova-Pak Guard-Pak Inserts 10/pkg

(Requires Guard-Pak Holders)

Description	Particle Size	Pore Size	Part No.
Nova-Pak C_{18}	4 µm	60Å	WAT015220
Nova-Pak C_8	4 µm	60Å	WAT035880
Nova-Pak Phenyl	4 µm	60Å	WAT020795
Nova-Pak CN HP	4 µm	60Å	WAT020800
Nova-Pak Silica	4 µm	60Å	WAT020790
Guard-Pak Holder			
Guard-Pak Holder			WAT088141

Prep Nova-Pak HR Packings

Prep Nova-Pak HR 6 µm packing is available in both economical column segments and preparative columns. The dimensions of the columns have been chosen specifically to make scale-up from analytical to prep scales simple and fast. The high efficiency packing gives you faster separations, using less solvent, with the added advantage of more concentrated fractions, all of which reduce the cost of your preparative chromatography.

Prep Nova-Pak HR 6 µm packing for milligram scale purifications and predictable transfer of methods at an affordable price.

Prep Nova-Pak HR Analytical and Preparative Columns

Semipreparative and preparative columns for predictable scale-up from lab scale to process chromatography.

Description	Particle Size	Pore Size	Dimensions	Part No.
Prep Nova-Pak HR C_{18}	6 µm	60Å	3.9 x 300 mm	WAT038500
			7.8 x 300 mm	WAT025820
			19 x 300 mm	WAT025822
Prep Nova-Pak HR Silica	6 µm	60Å	3.9 x 300 mm	WAT038501
			7.8 x 300 mm	WAT025821
			19 x 300 mm	WAT025823

µBondapak/Bondapak Packings

If your method calls for µBondapak C_{18} packings, there is only one µBondapak C_{18} packing material. Almost anything can be made once, but making a packing material reproducible year-to-year for over 30 years demonstrates true manufacturing and QC capability. Many companies claim to have columns with "µBondapak-like selectivity" but not one has ever passed Waters stringent QC batch test. Waters has set the standard for reproducible, high-performance chromatographic columns. Waters µBondapak columns are still the most widely referenced and requested columns in the world.

µBondapak and Bondapak packings are available in different column configurations designed for speed, resolution or sensitivity.

Physical Characteristics

Packing	Chemistry	Particle Size	Particle Shape	Pore Size	Carbon Load	End-capped
µBondapak	C_{18}	10 µm	Irregular	125Å	10%	Yes
µBondapak	Phenyl	10 µm	Irregular	125Å	8%	Yes
µBondapak	CN	10 µm	Irregular	125Å	6%	Yes
µBondapak	NH_2	10 µm	Irregular	125Å	3.5%	No
Bondapak	C_{18}	15-20 µm	Irregular	125Å	10%	Yes

µBondapak/Bondapak Analytical Columns

Description	Particle Size	Pore Size	Dimensions	Part No.
µBondapak C_{18}	10 µm	125Å	2.1 x 300 mm	WAT086609
			3.9 x 150 mm	WAT086684
			3.9 x 300 mm	WAT027324
			4.6 x 150 mm	WAT044370
			4.6 x 300 mm	186000925
Bondapak C_{18}	15-20 µm	125Å	3.9 x 300 mm	WAT025875
µBondapak CN	10 µm	125Å	3.9 x 150 mm	WAT086688
µBondapak NH_2	10 µm	125Å	3.9 x 300 mm	WAT084040
µBondapak Phenyl	10 µm	125Å	3.9 x 150 mm	WAT086680
			3.9 x 300 mm	WAT027198

µBondapak Cartridge Column

(Requires endfittings)

Description	Particle Size	Pore Size	Dimensions	Part No.
µBondapak C_{18}	10 µm	125Å	4.6 x 250 mm	WAT052860
Endfittings (includes 1 pair of reusable endfittings, 2 c-clips and coupling)				WAT037525

µBondapak/Bondapak Preparative Columns

Description	Particle Size	Pore Size	Dimensions	Part No.
µBondapak C_{18}	10 µm	125Å	7.8 x 300 mm	WAT084176
			19 x 150 mm	WAT088500
			19 x 300 mm	WAT025828
Bondapak C_{18}	15-20 µm	125Å	7.8 x 300 mm	WAT025832
			19 x 300 mm	WAT025834
µBondapak CN	10 µm	125Å	7.8 x 300 mm	WAT084177
µBondapak NH_2	10 µm	125Å	7.8 x 300 mm	WAT084178
µBondapak Phenyl	10 µm	125Å	7.8 x 300 mm	WAT084179

µBondapak Sentry Guard Columns (3.9 x 20 mm) 2/pkg

(Requires Sentry Holders)

Description	Pore Size	Part No.
µBondapak C_{18}	10 µm	WAT044480
µBondapak CN	10 µm	WAT046855
µBondapak NH_2	10 µm	WAT046865
µBondapak Phenyl	10 µm	WAT046850

Sentry Guard Holders

(50 mm and 75 mm long cartridge columns must use the Universal Guard Holder)

Description	Part No.
Integrated Guard Holder (for Waters 3.9 mm and 4.6 mm cartridge columns)	WAT046905
Universal Guard Holder (for any 3.9 mm and 4.6 mm HPLC column)	WAT046910

µBondapak Guard-Pak™ Inserts 10/pkg

(Requires Guard-Pak Holders)

Description	Particle Size	Pore Size	Part No.
µBondapak C_{18}	10 µm	125Å	WAT088070
µBondapak NH_2	10 µm	125Å	WAT026760
µBondapak Phenyl	10 µm	125Å	WAT026745
µBondapak CN	10 µm	125Å	WAT026750
Guard-Pak Holder			
Guard-Pak Holder			WAT088141

Delta-Pak Columns

Delta-Pak columns, ideal for the separation of peptides, proteins and natural products, are based on a highly stable, bonded, endcapped 5 µm or 15 µm spherical silica. Delta-Pak columns are available in two different pore size materials (100Å and 300Å) with a C_{18} or C_4 bonded phase.

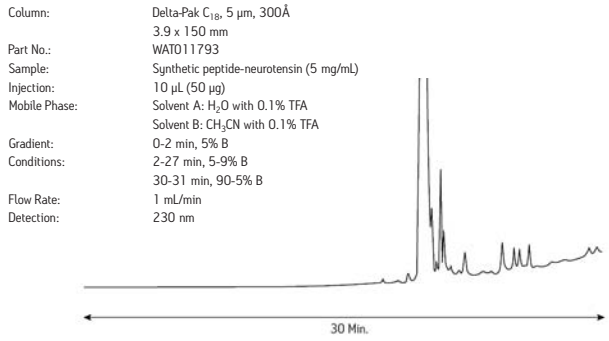
Delta-Pak columns are available in different cartridge and column configurations, providing consistent and predictable scale-up capabilities for milligram-to-gram scale purifications. Also, by having the same chemistry available on a high-efficiency 5 µm particle, you can easily check the purity of your fractions.

Delta-Pak columns provide consistent and predictable scale-up from analytical to preparative scale chromatography. Ideal for complex separations requiring high resolution chemistries.

Physical Characteristics

Packing	Chemistry	Particle Size	Particle Shape	Pore Size	Carbon Load	End-capped
Delta-Pak	C_{18}	5 µm	Spherical	100Å	17%	Yes
	C_{18}	5 µm	Spherical	300Å	7%	Yes
	C_4	5 µm	Spherical	100Å	7%	Yes
	C_4	5 µm	Spherical	300Å	3%	Yes
	C_{18}	15 µm	Spherical	100Å	17%	Yes
	C_{18}	15 µm	Spherical	300Å	7%	Yes
	C_4	15 µm	Spherical	100Å	7%	Yes
	C_4	15 µm	Spherical	300Å	3%	Yes

Purification of a Synthetic Peptide Using Delta-Pak C_{18} Packing



Delta-Pak Analytical Columns

Description	Particle Size	Pore Size	Dimensions	Part No.
Delta-Pak C_{18}	5 µm	300Å	2.1 x 150 mm	WAT023650
		100Å	3.9 x 150 mm	WAT011795
		300Å	3.9 x 150 mm	WAT011793
Delta-Pak C_4	5 µm	100Å	3.9 x 150 mm	WAT011796
		300Å	3.9 x 150 mm	WAT011794

Delta-Pak Preparative Columns

Description	Particle Size	Pore Size	Dimensions	Part No.
Delta-Pak C_{18}	15 µm	100Å	3.9 x 300 mm	WAT011797
		300Å	3.9 x 300 mm	WAT011802
		100Å	7.8 x 300 mm	WAT011798
		300Å	7.8 x 300 mm	WAT011803
		100Å	19 x 300 mm	WAT011799
		300Å	19 x 300 mm	WAT011804
		100Å	30 x 300 mm	WAT011800
		300Å	30 x 300 mm	WAT011805
		100Å	50 x 300 mm	WAT011801
Delta-Pak C_4	15 µm	100Å	3.9 x 300 mm	WAT011807
		300Å	3.9 x 300 mm	WAT011812
		100Å	7.8 x 300 mm	WAT011808
		300Å	7.8 x 300 mm	WAT011813
		100Å	19 x 300 mm	WAT011809
		300Å	19 x 300 mm	WAT011814
		100Å	30 x 300 mm	WAT011810
		300Å	30 x 300 mm	WAT011815

Delta-Pak High Pressure Inert (HPI) Analytical Columns (PEEK)

Column (non metallic)	Particle Size	Pore Size	Dimensions	Part No.
Delta-Pak HPI C_{18}	5 µm	100Å	2.1 x 150 mm	WAT052750
		300Å	2.1 x 150 mm	WAT052765
Delta-Pak HPI C_4	5 µm	100Å	2.1 x 150 mm	WAT052760
		300Å	2.1 x 150 mm	WAT052755
Delta-Pak HPI C_{18}	5 µm	300Å	3.9 x 150 mm	WAT035571
PEEK™ tubing and fitting kit (to minimize damage by stainless steel fittings) 1/16" o.d. x 0.005" i.d. x 5 feet (1.6 mm o.d. x 0.127 mm i.d. x 1.5 mm). Kit contains 5 each of short and long compression screws, 12 poly knobs and 12 PEEK ferrules.				WAT022999

Delta-Pak Sentry Guard Columns (3.9 x 20 mm) 2/pkg

(Requires Sentry Guard Holders)

Description	Particle Size	Pore Size	Part No.
Delta-Pak C_{18}	5 µm	100Å	WAT046880
Delta-Pak C_{18}	5 µm	300Å	WAT046890
Delta-Pak C_4	5 µm	100Å	WAT046875
Delta-Pak C_4	5 µm	300Å	WAT046885

Sentry Guard Holders

(50 mm and 75 mm long cartridge columns must use the Universal Guard Holder)

Description	Part No.
Integrated Guard Holder (for Waters 3.9 mm and 4.6 mm cartridge columns)	WAT046905
Universal Guard Holder (for any 3.9 mm and 4.6 mm HPLC column)	WAT046910

Delta-Pak Guard-Pak Inserts

(Requires Guard-Pak Holders)

Description	Particle Size	Pore Size	Quantity	Part No.
Delta-Pak C_{18}	5 µm	100Å	10/pkg	WAT036870
Delta-Pak C_{18}	5 µm	300Å	10/pkg	WAT036875
Delta-Pak C_4	5 µm	100Å	10/pkg	WAT036860
Delta-Pak C_4	5 µm	300Å	10/pkg	WAT036865

Guard-Pak Holder	
Guard-Pak Holder	WAT088141

µPorasil/Porasil Silica Packings

Waters µPorasil 10 micron packing material provides high-efficiency and low backpressure. Porasil silica packing material is available in 15-20 and 37-55 micron particle size.

Physical Characteristics

Packing	Chemistry	Particle Size	Particle Shape	Pore Size	Carbon Load	End-capped
µPorasil	Silica	10 µm	Irregular	125Å	N/A	N/A
Porasil	Silica	15-20 µm	Irregular	125Å	N/A	N/A

µPorasil/Porasil Analytical and Preparative Columns

Description	Particle Size	Pore Size	Dimensions	Part No.
µPorasil	10 µm	125Å	3.9 x 150 mm	WAT086692
			3.9 x 300 mm	WAT027477
			7.8 x 300 mm	WAT084175
			19 x 150 mm	WAT091648
			19 x 300 mm	WAT025829
Porasil	15-20 µm	125Å	3.9 x 300 mm	WAT025874
			19 x 300 mm	WAT025835

µPorasil Sentry Guard Columns (3.9 x 20 mm) 2/pkg

(Requires Sentry Guard Holders)

Description	Particle Size	Part No.
µPorasil	10 µm	WAT046860

Sentry Guard Holders

(50 mm and 75 mm long cartridge columns must use the Universal Guard Holder)

Description	Part No.
Integrated Guard Holder (for Waters 3.9 mm and 4.6 mm cartridge columns)	WAT046905
Universal Guard Holder (for any 3.9 mm and 4.6 mm HPLC column)	WAT046910

Resolve Columns

The non-endcapped Resolve packings are significantly different from Nova-Pak and Bondapak packing materials. The change in chromatographic behavior is most commonly noticed with polar compounds which are typically more retained. Basic compounds can be chromatographed using mobile phase modifiers such as ion-pairing reagents which reduce excessive tailing. Resolve C_{18} and Silica columns are available for applications requiring high resolution.

Physical Characteristics

Packing	Chemistry	Particle Size	Particle Shape	Pore Size	Carbon Load	Endcapped
Resolve	Silica	5 µm	Spherical	90Å	N/A	N/A
	C_{18}	5 µm	Spherical	90Å	10%	No
	C_{18}	10 µm	Spherical	90Å	10%	No
	C_8	10 µm	Spherical	90Å	5%	No
	CN	10 µm	Spherical	90Å	3%	No
	Silica	10 µm	Spherical	90Å	N/A	N/A

Resolve Columns

Description	Particle Size	Pore Size	Dimensions	Part No.
Resolve C_{18}	5 µm	90Å	3.9 x 300 mm	WAT011740
			3.9 x 150 mm	WAT085711
Resolve Silica	5 µm	90Å	3.9 x 150 mm	WAT086016

Resolve Sentry Guard Columns 2/pkg

(Requires Sentry Guard Holders)

Description	Particle Size	Pore Size	Part No.
Resolve C_{18}	5 µm	90Å	WAT046915
Resolve C_8	5 µm	90Å	WAT046920

Sentry Guard Holders

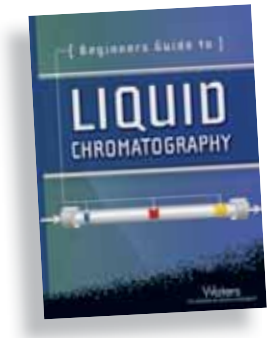
(50 mm and 75 mm long cartridge columns must use the Universal Guard Holder)

Description	Part No.
Integrated Guard Holder (for Waters 3.9 mm and 4.6 mm cartridge columns)	WAT046905
Universal Guard Holder (for any 3.9 mm and 4.6 mm HPLC column)	WAT046910

Resolve Guard-Pak Inserts 10/pkg

(Requires Guard-Pak Holders)

Description	Particle Size	Pore Size	Part No.
Resolve C_{18}	10 µm	90Å	WAT085824
Resolve Silica	10 µm	90Å	WAT085825
Resolve C_8	10 µm	90Å	WAT026755
Resolve CN	10 µm	90Å	WAT085826
Guard-Pak Holder			
Guard-Pak Holder			WAT088141



Waters HPLC Primer

- A primer on High-Performance Liquid Chromatography (HPLC) and related topics.
- Brief history, definition, and the basics of HPLC and UPLC
- HPLC Column Hardware (design and performance)
- HPLC Separation Mechanism
- HPLC Detector Overview and Types
- Glossary of HPLC Terms

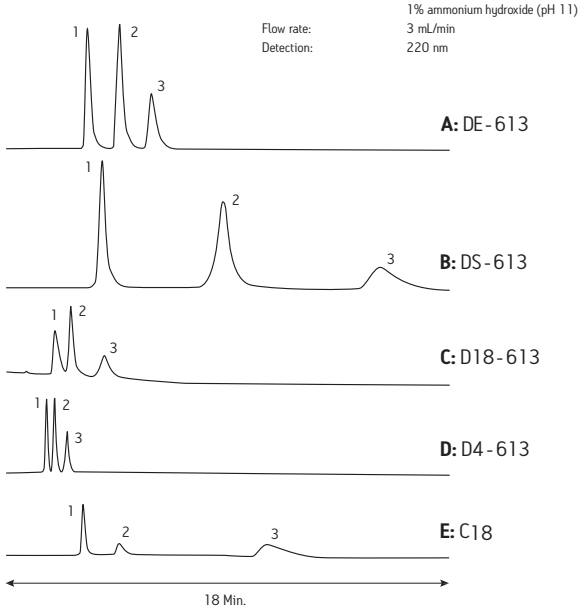
www.waters.com/hplcprimer

Shodex RSpak Polymer Reversed-Phase Columns

Shodex RSpak columns are packed with porous polymeric particles that are stable at a pH range of 2-12 and have a different selectivity from silica-based columns. The wide pH range makes the chromatography of basic compounds much easier because it is possible to chromatograph at high pH without ion-pairing salts. Waters offer six different types of polymeric packings. One of these, the DS-613, is based on a styrene divinylbenzene copolymer which is similar to "conventional" polymer-based columns and works well with more hydrophobic samples requiring high concentrations of organic modifiers.

The DE-613 polymethacrylate and the family of functionalized packings (D18-613 and D4-613) are naturally more hydrophilic than the DS-613 packing and function well in mobile phases containing high concentrations of water. The least hydrophobic of these packings is the DE-613 followed by the D4-613 and the longer carbon chain length derivative columns. The DC-613 is a cation exchanger and has a unique selectivity (mixed chromatographic mode ion exchange and reversed-phase partition) for weakly cationic species.

Comparison of Basic Compounds on Five Different RSpak Columns and C_{18} Based Column



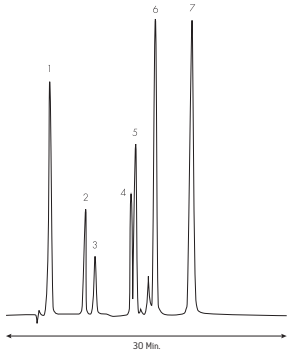
Above is a comparison of the separation of three local anesthetics using RSpak columns DE-613, DS-613, D18-613 and D4-613. Mobile phase contains ammonium hydroxide at pH 11. The compounds separated are lidocaine, procaine and tetracaine. The pK values of the anesthetics are greater than 9.0, a mobile phase with a high pH is therefore needed to deprotonate these compounds. Once deprotonated, they are easily separated by a reversed-phase mechanism. Eluents are simple and easier to reproduce. Compare these to Figure E, a separation performed on a silica-based C_{18} column using an ion pairing reagent.

RSpak D Series

Column Type	Base Polymer	Functional Group
DS-613	Polystyrene	None
DE-613	Polymethacrylate	None
DC-613	Polystyrene	Sulfonated
D18-613	Polymethacrylate	C_{18}
D4-613	Polymethacrylate	C_4

Proteins and Peptides

Column: RSpak D18-613, 6 x 150 mm
Part No.: WAT036576
Mobile Phase: A: 0.05% TFA: CH_3CN (80:20)
B: 0.05% TFA: CH_3CN (30:70)
Linear gradient A to B in 30 mins
Flow Rate: 1 mL/min
Detection: 220 nm
Sample:
1. Bradykinin
2. Neurotensin
3. Substance P
4. Insulin
5. Insulin B chain
6. Lysozyme
7. Myoglobin



Choosing the Appropriate RSpak Column for your Separation

If you had no prior experience with Polymeric Reversed-Phase (PRP) packing materials, the efficient 6 µm polymethaceylate based RSpak DE-613 column would be a good starting point for most applications. This column exhibits a mid-range hydrophobicity index equivalent to a C_8 bonded silica phase.

Should increased retention be required for the analyte of interest, the more hydrophobic SDVB based DS-613 column can be used. Alternatively, decreased retention is observed with the functionalized methacrylate based packings such as D18-613 and D4-613 and should be used if analytes are very hydrophobic.

RSpak D Series Columns

Column	Dimensions	Part No.
DS-613	6 x 150 mm	WAT034220
DE-613	6 x 150 mm	WAT034221
DC-613	6 x 150 mm	WAT034223
DS-G pre-column	4.6 x 10 mm	WAT034224
DE-G pre-column	4.6 x 10 mm	WAT034225
DC-G pre-column	4.6 x 10 mm	WAT034227

Guard Products

Guide to Guard Column Selection and Use

Guard columns are used primarily to protect expensive analytical and preparative columns by removing particulate and strongly retained sample components which ultimately decrease the lifetime of the analytical column. Although economy alone is a persuasive argument for the use of guard columns, the need for a long, stable life in the analytical column in order to obtain reliable and reproducible results is perhaps even more important.

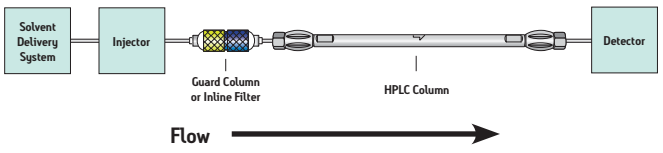
Guard columns are installed between the injector and the analytical column of a liquid chromatographic system. Guard columns may also be used to perform automated, on-line sample cleanup. In situations where a guard column is not needed, or where no suitable guard column is available, an in-line filter should be used to protect the analytical column from microparticulate contamination. The in-line filter is placed between the injector and the column.

Guard Column Design

Guard columns are designed with relatively small total volumes and minimal dead volumes so that they minimize band broadening. The ratio of guard column to analytical column volume should be such that adding a guard column decreases system efficiency by no more than 5-10%. Generally, a ratio of 1:10 to 1:25 is satisfactory.

Although larger guard columns would have the capacity to trap impurities from more sample injections, excessive band broadening would result. Waters offers two configurations for guard columns—the Sentry guard holders and Guard-Pak holder. The Sentry guard holder is available in two designs: one to be used as an integrated part of the cartridge column with reusable endfittings; the other for use with any HPLC column. Both are installed without tools. The Guard-Pak holder is a convenient and inexpensive device since you can use prepacked Guard-Pak inserts matched to the chemistry of your analytical column.

Positioning of a Guard Column in an LC System



Waters Sentry Guard Holders and Sentry Guard Columns

Guard columns are widely used as a cost effective means of prolonging HPLC column life. Guard columns protect the column from particulate matter and chemical contaminants.

The high-performance Sentry guard column was developed for customers needing the increased capacity required for biological samples and/or temperature control. When placed in series with an analytical column, Waters guard column increases overall efficiency. This efficiency increasing phenomenon was observed when the Sentry guard column was used with both short (100 mm) and standard length analytical columns.

The Sentry guard column gives you double the capacity of traditional guard columns so that you need to change the column less frequently. In addition to extending the productive lifetime of your column, the Sentry guard column enhances the reproducibility giving you consistent, reliable, reproducible results time after time.

Sentry guard columns contain the same high-performance packing materials that are used in all Waters analytical columns. These convenient, easy-to-use, disposable guard columns are an economical means of protecting your more expensive analytical column. You can change the guard column in seconds.

Two holder designs are offered, one to be used as an integrated part of the Waters cartridge column with reusable endfittings, the other for use with any HPLC column. Both allow Sentry guard columns to be replaced without tools.

Sentry Guard Holders

(50 mm and 75 mm long cartridge columns must use the Universal Guard Holder)

Description	Part No.
Integrated guard holder (for Waters cartridge columns)	WAT046905
Universal guard holder (for any HPLC column)	WAT046910
Sentry 2.1 mm guard holder kit (includes guard holder and rigid connector)	WAT097958
O-Ring kit (2/pkg) for Sentry 2.1 mm guard holder	WAT097954
Rigid connector for Sentry 2.1 mm guard holder	WAT022681
2.1 x 20 mm guard holder	186000262

Sentry Guard Columns

XBridge Sentry Guard Columns, 2/pkg

Description	Dimensions	2.5 µm	3.5 µm	5 µm
XBridge C_{18}	2.1 x 10 mm	186003056	186003059	186003062
	3.0 x 20 mm	186003057	186003060	186003063
	4.6 x 20 mm	186003058	186003061	186003064
XBridge C_8	2.1 x 10 mm	186003074	186003077	186003080
	3.0 x 20 mm	186003075	186003078	186003081
	4.6 x 20 mm	186003076	186003079	186003082
XBridge Shield RP_{18}	2.1 x 10 mm	186003065	186003068	186003071
	3.0 x 20 mm	186003066	186003069	186003072
	4.6 x 20 mm	186003067	186003070	186003073
XBridge Phenyl	2.1 x 10 mm	186003359	186003362	186003366
	3.0 x 20 mm	186003360	186003363	186003367
	4.6 x 20 mm	186003361	186003364	186003368

SunFire Sentry Guard Columns, 2/pkg

Description	Dimensions	2.5 µm	3.5 µm	5 µm
SunFire C_{18}	2.1 x 10 mm	186003395	186002530	186002536
	3.0 x 20 mm	186003405	186002681	186002683
	4.6 x 20 mm	186003413	186002682	186002684
SunFire C_8	2.1 x 10 mm	186003396	186002708	186002713
	3.0 x 20 mm	186003406	186002718	186002722
	4.6 x 20 mm	186003414	186002727	186002733

Atlantis Sentry Guard Columns, 2/pkg

Description	Dimensions	3 µm	5 µm
Atlantis T3	2.1 x 10 mm	186003756	186003759
	3.9 x 20 mm	186003757	186003760
	4.6 x 20 mm	186003758	186003761
Atlantis dC_{18}	2.1 x 10 mm	186001377	186001379
	2.1 x 20 mm	186001381	186001383
	3.9 x 20 mm	186001313	186001315
	4.6 x 20 mm	186001321	186001323
Atlantis HILIC	2.1 x 10 mm	186002005	186002006
	3.9 x 20 mm	186002021	186002022
	4.6 x 20 mm	186002023	186002024

XTerra Sentry Guard Columns, 2/pkg

Description	Dimensions	3 µm	5 µm
XTerra MS C_{18}	2.1 x 10 mm	186000632	186000648
XTerra MS C_{18}	2.1 x 20 mm	186000636	186000652
XTerra MS C_{18}	3.0 x 20 mm	186000640	186000656
XTerra MS C_{18}	3.9 x 20 mm	186000644	186000660
XTerra MS C_8	2.1 x 10 mm	186000633	186000649
XTerra MS C_8	2.1 x 20 mm	186000637	186000653
XTerra MS C_8	3.0 x 20 mm	186000641	186000657
XTerra MS C_8	3.9 x 20 mm	186000645	186000661
XTerra RP18	2.1 x 10 mm	186000634	186000650
XTerra RP18	2.1 x 20 mm	186000638	186000654
XTerra RP18	3.0 x 20 mm	186000642	186000658
XTerra RP18	3.9 x 20 mm	186000646	186000662
XTerra RP8	2.1 x 10 mm	186000635	186000651
XTerra RP8	2.1 x 20 mm	186000639	186000655
XTerra RP8	3.0 x 20 mm	186000643	186000659
XTerra RP8	3.9 x 20 mm	186000647	186000663

Symmetry Sentry Guard Columns, 2/pkg

Description	Particle Size	Dimensions	Part No.
Symmetry C_{18}	3.5 µm	2.1 x 10 mm	WAT106127
Symmetry C_{18}	5 µm	3.9 x 20 mm	WAT054225
Symmetry C_8	3.5 µm	2.1 x 10 mm	WAT106128
Symmetry C_8	5 µm	3.9 x 20 mm	WAT054250
SymmetryShield RP18	3.5 µm	2.1 x 10 mm	186000169
SymmetryShield RP18	5 µm	3.9 x 20 mm	186000107
SymmetryShield RP8	3.5 µm	2.1 x 10 mm	WAT106129
SymmetryShield RP8	5 µm	3.9 x 20 mm	WAT200675
Symmetry300 C_4, 300Å	3.5 µm	2.1 x 10 mm	186000275
Symmetry300 C_4, 300Å	5 µm	3.9 x 20 mm	186000284
Symmetry300 C_{18}, 300Å	3.5 µm	2.1 x 10 mm	186000198
Symmetry300 C_{18}, 300Å	5 µm	3.9 x 20 mm	WAT106166

Nova-Pak Sentry Guard Columns, 2/pkg

Description	Particle Size	Dimensions	Part No.
Nova-Pak C_{18}	4 µm	3.9 x 20 mm	WAT044380
Nova-Pak C_8	4 µm	3.9 x 20 mm	WAT046830
Nova-Pak Phenyl	4 µm	3.9 x 20 mm	WAT046835
Nova-Pak CN HP	4 µm	3.9 x 20 mm	WAT046840
Nova-Pak Silica	4 µm	3.9 x 20 mm	WAT046845

µBondapak Sentry Guard Columns, 2/pkg

Description	Particle Size	Dimensions	Part No.
µBondapak C_{18}	10 µm	3.9 x 20 mm	WAT044480
µBondapak CN	10 µm	3.9 x 20 mm	WAT046855
µBondapak NH_2	10 µm	3.9 x 20 mm	WAT046865
µBondapak Phenyl	10 µm	3.9 x 20 mm	WAT046850

Porasil Sentry Guard Columns, 2/pkg

Description	Particle Size	Dimensions	Part No.
Porasil	10 µm	3.9 x 20 mm	WAT046860

Delta-Pak Sentry Guard Columns, 2/pkg

Description	Particle Size	Dimensions	Part No.
Delta-Pak C_{18} 100Å	5 µm	3.9 x 20 mm	WAT046880
Delta-Pak C_{18} 300Å	5 µm	3.9 x 20 mm	WAT046890
Delta-Pak C_4 100Å	5 µm	3.9 x 20 mm	WAT046875
Delta-Pak C_4 300Å	5 µm	3.9 x 20 mm	WAT046885

High Performance Carbohydrate Sentry Guard Columns, 2/pkg

Description	Particle Size	Dimensions	Part No.
High-Performance Carbohydrate Sentry Guard Column	4 µm	3.9 x 20 mm	WAT046895

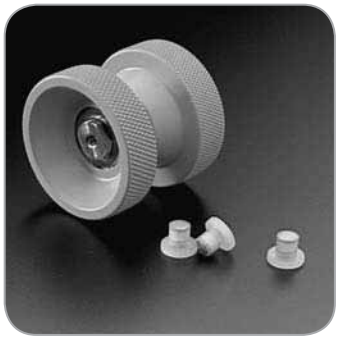
Resolve Sentry Guard Columns, 2/pkg

Description	Particle Size	Dimensions	Part No.
Resolve C_{18}	5 µm	3.9 x 20 mm	WAT046915
Resolve C_8	5 µm	3.9 x 20 mm	WAT046920

Guard-Pak: Inexpensive Column Protection

Guard-Pak Holder

Waters Guard-Pak Holder is a compact, stand-alone housing for our unique disposable Guard-Pak inserts. Installed on-line with your HPLC system immediately before the analytical column, the Guard-Pak holder and inserts protect analytical LC columns against the gradual accumulation of particulates and chemical contaminants originating from the sample.



Guard-Pak inserts prolong column life by removing contaminants from the sample and mobile phase, giving you enhanced reproducibility and performance. They are packed with the same high performance packings used in Waters analytical columns.

Description	Part No.
Guard-Pak Holder	WAT088141
Guard-Pak Holder Connector	WAT080046

Guard-Pak Inserts

Guard-Pak inserts protect your analytical columns from damage due to irreversibly adsorbed chemical contaminants or particulates. The result is enhanced chromatographic reproducibility and improved column lifetime. The inexpensive, disposable plastic inserts are prepacked with analytical grade 4, 5, and 10 µm packing materials and sealed with 2 µm filters. Unlike hand-packed guard columns, Guard-Pak inserts have a consistent, densely packed bed and are designed to eliminate voids thus ensuring column-to-column reproducibility.

Description	Qty.	Particle Size	Pore Size	Part No.
Nova-Pak C_{18}	10/pkg	4 µm	60Å	WAT015220
Nova-Pak C_8	10/pkg	4 µm	60Å	WAT035880
Nova-Pak Phenyl	10/pkg	4 µm	60Å	WAT020795
Nova-Pak Silica	10/pkg	4 µm	60Å	WAT020790
Nova-Pak CN HP	10/pkg	4 µm	60Å	WAT020800
Bondapak C_{18}	10/pkg	10 µm	125Å	WAT088070
Bondapak NH_2	10/pkg	10 µm	125Å	WAT026760
Bondapak Phenyl	10/pkg	10 µm	125Å	WAT026745
Bondapak CN	10/pkg	10 µm	125Å	WAT026750
Resolve C_{18}	10/pkg	10 µm	90Å	WAT085824
Resolve Silica	10/pkg	10 µm	90Å	WAT085825
Resolve C_8	10/pkg	10 µm	90Å	WAT026755
Resolve CN	10/pkg	10 µm	90Å	WAT085826
Delta-Pak C_{18}	10/pkg	5 µm	100Å	WAT036870
Delta-Pak C_{18}	10/pkg	5 µm	300Å	WAT036875
Delta-Pak C_4	10/pkg	5 µm	100Å	WAT036860
Delta-Pak C_4	10/pkg	5 µm	300Å	WAT036865
Guard-Pak In-Line Filters	5/pkg			WAT032472

Waters Cartridge and Guard Column Guide

Guard Columns

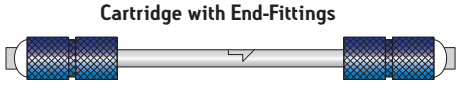
Universal Sentry Guard Holder (Use on any column)

2.1 x 10 mm – use Part Number WAT097958
2.1 x 20 mm – use Part Number 186000262
3.0 x 20 mm – use Part Number WAT046910
3.9 x 20 mm – use Part Number WAT046910
4.6 x 20 mm – use Part Number WAT046910

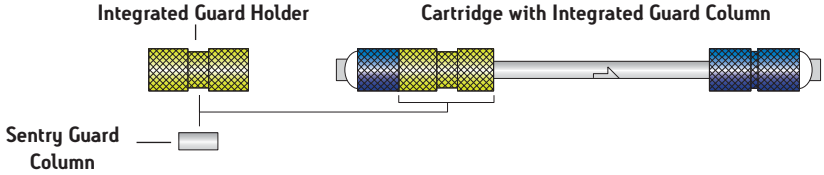
Cartridge Columns

Cartridge End-Fittings

2.1 x 50 mm, 2.1 x 100 mm, 2.1 x 150 mm, 2.1 x 250 mm – use Part Number 700000117
3.0 x 50 mm, 3.0 x 100 mm, 3.0 x 150 mm, 3.0 x 250 mm – use Part Number WAT037525
3.9 x 50 mm, 3.9 x 100 mm, 3.9 x 150 mm, 3.9 x 250 mm – use Part Number WAT037525
4.6 x 50 mm, 4.6 x 100 mm, 4.6 x 150 mm, 4.6 x 250 mm – use Part Number WAT037525



Integrated Sentry Guard Holder to go with cartridge end-fittings (see above)



Paired-Ion Chromatography (PIC Reagents)

An easy solution to the problem of analyzing samples containing ionic species, Waters PIC® reagents allow ionic compounds to be separated by reversed-phase chromatography while eliminating problems of precise pH and temperature control, reproducibility and short column life associated with ion exchange. PIC reagents versatility can save time by allowing simultaneous assay of acids, bases, amphoteric and neutral compounds. Waters PIC reagents are:

- Purified and quality controlled by liquid chromatography to guarantee consistent results
- Preformulated for ease of use
- Buffered to an appropriate pH for most acidic and basic compounds

The accompanying table lists some typical compounds which can be chromatographed using Paired-Ion Chromatography. A µBondapak C_{18} column with a water/methanol solvent system was used in all cases.

Use Low UV PIC to analyze ionic compounds at low UV (200 nm). Low UV PIC is specially formulated to eliminate background interference in the low UV range and provides:

- Methods development opportunities for ion pairs which do not have a chromophore in the working range of regular PIC reagents
- Increased sensitivity for compounds ordinarily used with existing PIC reagents
- Potential for selectivity changes due to alternative buffers as compared to regular PIC reagents

Compound or Class	PIC Reagent	Compound or Class	PIC Reagent
Alkaloids		Antitussives	B
Cephaeline	B	B Vitamins	B5/B7
Emetine	B	Calcium Leucovorin	A
Ergot alkaloids	B	FD&C Dyes	A
Analgesics/Antipyretics		Folic Acid	A
APC	A	Germicides	
Aspirin	A	Benzalkonium Chloride	B
Codeine	B	Cetylpyridinium	B
Indomethacin	A	Imides	A
Meperidine	B	Methotrexate	A
Methadone	B	Petroleum Sulfonates	A
Morphine	B	Surfactants	
Propoxyphene	B	Quaternary amines	B
Antibiotics		Sulfonates	A
Cephalosporins	A or B	Sympathomimetics	
Penicillins	A or B	(Decongestants)	
Antihistamines		Amphetamine	B
Chlorpheniramine	B	Ephedrine	B
Diphenhydramine	B	Epinephrine	B
Methapyrilene	B	Isoproterenol	B
Phenimdamine	B	Naphazoline	B
Pheniramine	B	Nylidrin	B
Pyrilamine	B	Phenylephrine	B
Tripelennamine	B	Phenylpropanolamine	B

Choice of B Reagent will depend upon the mixture to be separated.

B7 is the recommended initial choice.

Use PIC A for rapid separation of acids. FD&C sulfonic acid dyes are classic examples of compounds that are conventionally separated by ion-exchange chromatography. Using PIC Reagent A and reversed-phase chromatography, the sulfonic acid dyes and their impurities can be separated easier, faster, and more reliably.

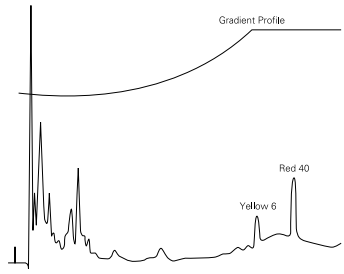
Use PIC B for rapid separation of bases. Quaternary amines, another class of compounds that is usually separated by ion-exchange chromatography, can be more easily separated by Paired-Ion Chromatography employing one of the PIC Reagent B series.

Use Reagent PIC D-4 as a mobile phase modifier when chromatographing basic pharmaceutical compounds to improve peak shape and reduce retention times.

FD&C Dyes Cough Syrup

Column: µBondapak C_{18}, 3.9 x 300 mm
Part No.: WAT027324
Solvent: A) H_2O with PIC Reagent A
B) CH_3OH with PIC Reagent A
40% B - 55% B
Sample: 80 µL Cough Syrup (diluted 1/1 with water)
Flow Rate: 2 mL/min
Detection: 254 nm

PIC techniques can be used to determine trace impurities in bulk dyes or to quantitate dyes in a complex mixture, such as a cough syrup



	Workable UV Range in Mobile Phase (nm)	Quantity (Vials/ Pkg)	Part No.
PIC A (tetrabutylammonium hydrogen sulfate)			
Low UV PIC A	200+	5	WAT084189
PIC A (tetrabutylammonium phosphate	240+	5	WAT085101
PIC B5 (pentane sulfonic acid)			
Low UV PIC B5	200+	5	WAT084198
PIC B5	240+	5	WAT085110
PIC B6 (hexane sulfonic acid)			
Low UV PIC B6	200+	5	WAT084199
PIC B6	240+	5	WAT085140
PIC B7 (heptane sulfonic acid)			
Low UV PIC B7	200+	5	WAT084282
PIC B7	240+	5	WAT085103
PIC B8 (octane sulfonic acid)			
Low UV PIC B8	200+	5	WAT084283
PIC B8	240+	5	WAT085142
PIC Sample Kit (One vial each: PIC A, B5, B6, B7 and B8)		5	WAT085144
PIC Reagent D4 (dibutylammonium phosphate)	215+	5	WAT085466

Sugar and Carbohydrate Analysis

Carefully controlling the content of commercial foods and beverages is the fundamental task of any manufacturer. Quality control is of premier importance, not only for compliance with regulatory agencies, but for customer satisfaction as well.

The primary ingredients of foods and beverages, which must be analyzed and quantitated, are sugars and carbohydrates, fatty acids, triglycerides and cholesterol, pesticides, organic acids, alcohols, caffeine, and other additives.

Waters offers a range of columns for analysis of each of these classes of compounds.

Sugars constitute a very important class of compounds which are ubiquitous in nature. They are found in food, microorganism, and paper pulp, and are involved in many biochemical interactions. Sugars present a challenge for separations because of the small differences in their chemical and physical properties. In order to exploit these minor differences and effect a separation, several modes of chromatography exist for the analysis of sugars.

Selecting the Separation Mode

There are many different modes of chromatography used to characterize carbohydrates and many different columns from which to choose. Before choosing a column, an understanding of the types of columns that are available and the mode of chromatography that can be used to exploit the differences between sugars would be beneficial.

Ion Exchange

This mode provides selectivity to separate mono- and some disaccharides, as well as sugar alcohols and low molecular weight alcohols, by hydroxyl coordination to the metal cation. Ion exchange uses sulfonated styrene divinylbenzene resins in lead form (Shodex SP-0810) or calcium form (Shodex SC-1011, or our Sugar-Pak I). The mobile phase is water at 80-90 °C.

Partition

Partition chromatography is best suited for low molecular weight sugars such as mono-, di-, and trisaccharides. This mode uses a covalently bonded amino packing in a column such as our Carbohydrate Analysis column, or a dynamically-coated silica cartridge employing Silica Amine Modification (SAM) reagents such as SAM I. Typical mobile phases range from 65-85% acetonitrile in water, and the separations are carried out at temperatures ranging from ambient to 70 °C.

Reversed Phase

Reversed-phase is best suited to the separation of mixtures containing monosaccharides and oligosaccharides up to a degree of polymerization (DP) of 8 as applies to monitoring acid or enzyme hydrolysates of large oligosaccharides. This mode is also amenable for the separation of derivatized sugars.

Size Exclusion

Gel-Permeation Chromatography (GPC) is used to determine the molecular weight distribution of food polymers such as starches and gums. The Waters Ultrahydrogel columns are well suited for this application. The Shodex KS Series (7 µm) and robust S Series (10 µm) packings are based on a sulfonated styrene divinylbenzene polymer. Designed to analyze and characterize water-soluble polymers, they are particularly well suited for mono, di and polysaccharides. The KS-800 Series is also useful for separation of intermediate and small DP sugars.

Selecting the Column

The table on page 139 lists the various columns along with the associated separation mode, typical mobile phases, and expected order of carbohydrate elution.

For Shodex columns, the name of a particular column contains all the pertinent information necessary to define the column. These factors are listed in the second table.

Typical Applications for Sugar and Carbohydrate Columns

Cartridge/Column	Mode	Eluent	Application	Elution Order
Carbohydrate analysis column	Partition	65 to 85% CH_3CN:H_2O ambient to 70 °C	Mono, di and trisaccharides up to DP 8 Sugars and sugar alcohols	Smallest elute first
SAM I reagent with silica cartridge	Partition	70 to 80% CH_3CN:H_2O 0.1% SAM I ambient	Mono, di and trisaccharides	Smallest elute first
Sugar-Pak I, SC-1011, SP-0810	Ion exchange/ size exclusion	H_2O 75-95 °C	Mono, di, oligosaccharides and sugar alcohols	Largest elute first
SH-1011, IC-Pak ion exclusion Fast fruit juice	Ion exchange/ size exclusion	0.01N H_3PO_4 50-60 °C	Sugar acids, sugar alcohols organic acids	Largest and most acidic elute first
Dextro-Pak™	Reversed-phase	H_2O ambient	Hydrolysed syrups derivatized sugars	Smallest elute first
KS-800 series	Size exclusion		Mono through oligosaccharides such as syrups	Largest elute first

A Guide to Shodex Sugar Columns

S	C	18	2	1
Type of column S = sugar	Cation H = H+ C = Ca2+ P = Pb2+ Z = Zn2+	% cross linkage	Pore Size 1 = 20Å 2 = 50Å 3 = 100Å 4 = 500Å 5 = 1000Å	0 - Gel type 1 - Semimacropore gel 2 - Permanent pore gel

Example:

S	C	10	1	1
Sugar column	Ca2+	10% cross linkage	20Å pore size	semimacropore gel

Retention Times for Sugar and Carbohydrates

Column Type	Carbohydrate Analysis	Sugar-Pak	Dextro-Pak	Silica-Pak	KS-801	SC-1011	SP-0810	SH-1011
Column Temp:	Ambient	90 °C	Ambient	Ambient	75 °C	80 °C	80 °C	60 °C
Mobile Phase:	80:20 Acetonitrile: Water	Water	Water	SAM I Reagent 75:25 Acetonitrile: Water	Water	Water	Water	Water
Flow Rate:	2 mL/min	0.5 mL/min	1 mL/min	4 mL/min	1 mL/min	1 mL/min	1 mL/min	1 mL/min
Compound	Approximate Elution Time in Minutes							
2-Deoxygalactose						8.4	10.7	7.9
2-Deoxyribose						9.2	10.8	8.6
2-Deoxy-glucose	3.9	8.8	3.4	1.5		7.7	9.3	7.4
Adonitol (Ribitol)	4.8	11.0	2.9	1.8		9.8	13.5	7.7
Arabinose	4.8	10.4	3.0	1.8	8.3	9.0	11.1	7.5
Arabitol						11.1	16.5	8.0
Butanol-1	N/R	18.8	N/R					
Cellobiose	15.3	6.7	3.7	4.1	5.7	5.4	7.9	5.5
Deuterium Oxide					11.1			
Digitose						9.3	10.6	
Digitoxose								8.4
Dulcitol						12.7	20.8	7.6
Ethylene Glycol	N/R	12.6	3.5	1.0	9.3			
Ethanol	N/R	12.8	6.8		10.0			
Fructose	5.7	10.1	2.9	2.0	7.7	9.0	11.5	7.1
Fucose	4.2	10.4	4.1	1.6	8.1	8.9	11.2	7.8
Galactose	7.7	9.4	2.8	2.5	7.6	8.2	10.4	6.9
Glucoheptose						9.6		6.6
Glucose	7.3	8.5	2.8	2.4	7.2	7.6	9.1	6.6
Glucose-6-phosphate	N/R	4.8	2.1					
Glycerol	N/R	12.1	3.2	1.2	8.5			
Inositol						9.0	13.6	7.0
Isomaltitol						8.7	15.3	
Isomaltose					5.9	6.4	8.1	5.6
Lactose	18.0	7.1	3.0/3.2*	4.8	5.9	6.6	8.6	5.8
Lactulose	16.3	7.6	3.8	4.0		7.1	9.9	
Lyxose	4.1	10.7	3.0			9.3	11.4	7.4
Maltitol					6.0		13.2	6.1
Maltoheptaose							6.6	4.6
Maltohexaose						5.4	6.8	4.6
Maltopentaose					4.7	5.3	7.2	4.7
Maltose	15.7	6.8	3.5	4.1	5.9	5.5	8.3	5.6
Maltotetraose						5.8	7.5	4.9
Maltotriose	N/R	6.0	4.4/4.7*	7.8	5.3	6.1	7.9	5.1
Maltotriol						5.9	11.3	5.4
Mannitol	7.0	12.2	2.9	2.2	7.2	11.1	16.5	7.4
Mannose	6.4	9.6	2.9	2.2	7.6	8.3	11.4	6.9
Melibiose	21.6	7.0	3.1	5.3	5.9	6.4	8.7	5.6
Melizitose					5.1	6.0	7.3	5.1
meso-Erythritol						10.1	13.3	8.4
Methanol	N/R	12.5	3.9					
myo-Inositol							13.5	7.0
Pannose		5.9	5.0					
Pentaerythritol						9.8	11.5	9.1
Propanol-2	N/R	12.6	N/R					
Raffinose	N/R	6.0	7.0	7.5	5.2	6.1	7.5	5.1
Rhamnose		9.5	3.3		7.3	8.3	10.3	7.3
Ribose	3.6	14.9	3.1	1.4	9.1	13.5	19.9	7.9
Sorbitol	6.9	14.2	2.9	2.2	7.5	13.2	22.3	7.6
Sorbose	5.6	9.4	2.8		7.4	8.2	10.2	6.7
Stachyose	N/R	5.4	6.3	17.5	4.8	5.7	7.1	4.9
Sucrose	11.9	6.8	4.4	3.4	5.8	6.4	8.0	
Trehalose					5.7	6.4	8.1	5.6
Turanose						6.6	8.5	5.8
Xylitol	5.1	14.3	2.9	1.8	8.0	13.0	20.5	8.2
Xylose	4.3	9.3	2.9	1.6	7.7	8.1	9.7	7.0
Xylulose	3.3	10.6	3.1					

N/R — *Not recommended under these conditions due to insufficient or excessive retention.*

* — *Elution times of anomers*

Blank — *No data exists*

High-Performance Carbohydrate Analysis Cartridge Column

Waters High-Performance Carbohydrate cartridge column with reusable endfittings is based on a 4 µm, spherical silica. This column was developed to separate 5 monosaccharide and disaccharides with base line resolutions in less than 12 minutes. The High-Performance Carbohydrate cartridge column can be run at 1.4 mL/min to achieve 12-minute separations and is available in a 4.6 mm i.d. x 250 mm cartridge column. This column dimension was found to be the optimum for speed, resolution and longevity. The prepacked, disposable cartridge column requires reusable endfittings which can be purchased separately. When you change columns, just replace the cartridge and use the endfittings again.

You can increase the lifetime of the column by using a Sentry guard column. Contaminants are removed from the sample and mobile phase before they reach the column.

Description	Dimensions	Part No.
High-Performance Carbohydrate cartridge column (requires endfittings)	4.6 x 250 mm	WAT044355
Endfittings (End Connector Kit) (contains 1 pair endfittings, and c-clips)		WAT037525
Sentry High Performance Carbohydrate guard column (2/pkg)	3.9 x 20 mm	WAT046895
Sentry Integrated Guard Holder (for Waters cartridge columns)		WAT046905

Columns and Accessories

Description	Dimensions	Part No.
Sugar-Pak 1 column	6.5 x 300 mm	WAT085188
Sugar-Pak 1 Guard-Pak inserts, 10/pkg		WAT015209
Carbohydrate Analysis column	3.9 x 300 mm	WAT084038
SC-1011 column	8 x 300 mm	WAT034238
SP-0810 column	8 x 300 mm	WAT036954
SC-1011P precolumn	6 x 50 mm	WAT034244
SP-0810P precolumn	6 x 50 mm	WAT034245
Silica-Pak™ HP cartridge, 4 µm	8 x 100 mm	WAT010932
Silica-Pak cartridge, 10 µm	8 x 100 mm	WAT010935
SAM I Reagent, 5/box		WAT010873
Dextro-Pak cartridge column	8 x 100 mm	WAT085650

Shodex KS-800 Columns

Column	Exclusion Limit	Dimensions	Part No.
KS-801	1×10^3	8 x 300 mm	WAT034276
KS-802	1×10^4	8 x 300 mm	WAT034277
KS-804	4×10^5	8 x 300 mm	WAT034279
KS-800P pre-column		6 x 50 mm	WAT034282

Waters Ultrahydrogel Columns

Column	Pore Size	Exclusion Limit (PEO)	Dimensions	Part No.
Ultrahydrogel 120	120Å	5×10^3	7.8 x 300 mm	WAT011520
Ultrahydrogel 250	250Å	8×10^4	7.8 x 300 mm	WAT011525
Ultrahydrogel 500	500Å	4×10^5	7.8 x 300 mm	WAT011530
Ultrahydrogel 1000	1000Å	1×10^6	7.8 x 300 mm	WAT011535
Ultrahydrogel 2000	2000Å	7×10^6	7.8 x 300 mm	WAT011540
Ultrahydrogel Linear	Blend	7×10^6	7.8 x 300 mm	WAT011545
Ultrahydrogel DP	120Å	5×10^3	7.8 x 300 mm	WAT011550
Ultrahydrogel Guard Column				WAT011565
Ultrahydrogel Guard Column DP				WAT011570

Fatty Acid Analysis

Fatty acid analysis can be carried out by the reversed-phase mode. The Free Fatty Acid HP column uses a phenyl-bonded packing and a simple isocratic elution method to separate free fatty acids based on carbon chain length and degree of saturation. The short column dimension (3.9 x 150 mm) significantly reduces analysis time and increases sensitivity. Typical applications include free fatty acids, fatty acid derivatives, and some fatty amides, such as saponified coconut oil, tall oils, fatty alcohols, and fatty acid isomers.

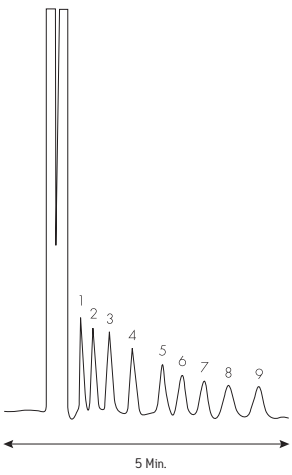
- Straight chain saturated acids elute in order of increasing carbon number
- Unsaturated acids elute before the analgous saturated compound
- For a given carbon number and chain configuration, the greater the unsaturation, the earlier the elution

Fatty Acid Standards

Column: Free Fatty Acid HP
Part No.: WAT011690
Mobile Phase: CH_3CN: THF: H_2O 45:20:35
Flow Rate: 1.5 mL/min
Detection: RI

Sample

1. Capric Acid
2. Lauric Acid
3. Myristic Acid
4. Palmitic Acid
5. Stearic Acid
6. Nonadecanoic Acid
7. Arachidic Acid
8. Heneicosanoic Acid
9. Behenic Acid



Pesticide Analysis

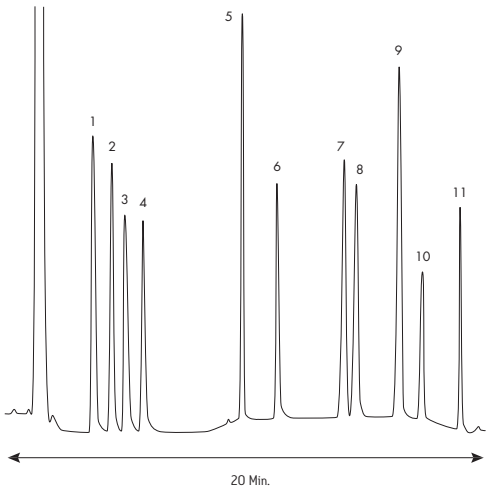
When used with the Waters Carbamate Analysis System, the Carbamate Analysis column provides a guaranteed analysis of the carbamate pesticides that exceeds the sensitivity required by AOAC Method 985.23.

The baseline resolution and high sensitivity of this separation, coupled with the optimized system configuration, provide state-of-the-art analysis of carbamates. The separation of eleven carbamate pesticides and carbamate metabolites is accomplished in 20 minutes.

Carbamate Analysis

Column: Carbamate Analysis column 3.9 x 150 mm
Part No.: WAT035577
Mobile Phase: Water/methanol/acetonitrile complex gradient
Flow Rate: 1.5 mL/min
Sample: 10 ng of each analyte on column
Injection Volume: 400 µL
Post Column Addition: OPA/NaOH @ 0.5 mL/min
Detector: Model 470 Fluorescence Detector
Excitation: 339 nm lambda
Emission: 445 nm lambda

1 Aldicarb sulfoxide
2 Aldicarb sulfone
3 Oxamyl
4 Methomyl
5 3-Hydroxycarbofuran
6 Aldicarb
7 Propoxur
8 Carbofuran
9 Carbaryl
10 1-Naphthol
11 Methiocarb



Free Fatty Acid HP Column

Column	Particle Size	Dimensions	Part No.
Free Fatty Acid HP	4 µm	3.9 x 150 mm	WAT011690

Carbamate Analysis Column for Pesticides

Column	Dimensions	Part No.
Carbamate Analysis	3.9 x 150 mm	WAT035577

Waters PAH Columns

Waters PAH Columns Improve Analysis of PAH Compounds

Polynuclear Aromatic Hydrocarbons (PAHs) are among the most frequently monitored environmental contaminants. Standard and official methods for the analysis of PAHs are found in compendia for air, drinking water, waste-water, solid waste, and food analysis[1].

Many of these methods specify HPLC, usually with UV and fluorescence detection, as the recommended analytical procedure.

Waters PAH columns are optimized for the HPLC analysis of PAHs. The chromatogram (top right) shows 16 PAH compounds, listed as target pollutants by the U.S. EPA. The Waters PAH columns achieve baseline resolution and excellent peak symmetry for all 16 target analytes in less than 25 minutes, while employing a simple water; acetonitrile binary gradient. The resolving power of the PAH Columns provides superior peak identification and quantitation for PAHs.

Florida Administrative Code 17.700 includes 2 additional compounds (1-methyl naphthalene and 2-methyl naphthalene) in addition to the 16 compound EPA 610 mix that we currently use to show the proficiency of Waters instrumentation to analyze PAH compounds (bottom right). The new Waters PAH columns resolve these two compounds along with the other 16.

Waters PAH columns come in seven different dimensions (including a capillary format), and two particle sizes. Each column comes with a complete Certificate of Analysis backed by a world-class ISO 9002 registered documentation trail.

Reference:

1. AOAC 973.30; Deutsche DIN TVO; UK ISBN 0 11 & 752032 2;
U.S. EPA Methods TO-13, 550 & 550.1, 610, 8310

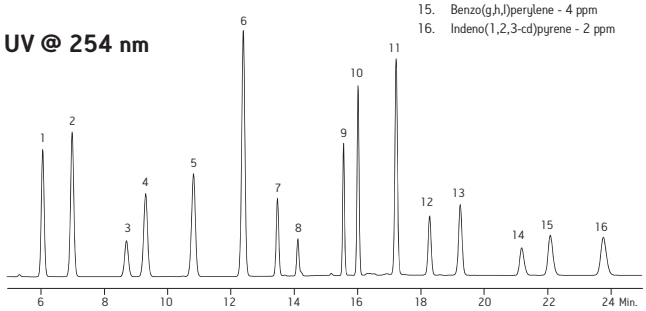
Waters PAH Columns

Description	Particle Size	Dimensions	Part No.
Waters PAH Column	5 µm	4.6 x 250 mm	186001265
Waters PAH Column	5 µm	4.6 x 150 mm	186001264
Waters PAH Column	3 µm	4.6 x 50 mm	186001260
Waters PAH Column	5 µm	3.0 x 250 mm	186001263
Waters PAH Column	5 µm	2.1 x 250 mm	186001262
Waters PAH Column	5 µm	2.1 x 150 mm	186001261
Waters PAH Column	5 µm	0.32 x 150 mm	186001259

PAH Analysis using Waters PAH Columns

Column: Waters PAH Column 5 µm, 4.6 x 250 mm @ 27 °C
Part No.: 186001265
System: Waters Alliance System with 2996 Photodiode Array Detector
Eluent A: Water
Eluent B: Acetonitrile
Gradient: 60% B to 100% B using curve 9 in 12 minutes, hold 11 minutes, back to initial conditions
Flow Rate: 1.2 mL/min
Injection: 20 µL
Sample: EPA-610 mixture

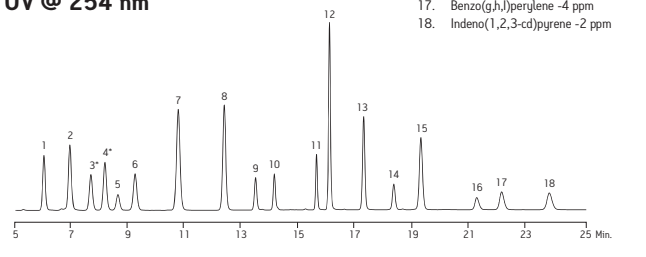
Peaks :
1. Naphthalene - 20 ppm
2. Acenaphthylene - 40 ppm
3. Acenaphthene - 20 ppm
4. Fluorene - 4 ppm
5. Phenanthrene - 2 ppm
6. Anthracene - 2 ppm
7. Fluoranthene - 4 ppm
8. Pyrene - 2 ppm
9. Benzo(a)anthracene - 2 ppm
10. Chrysene- 2 ppm
11. Benzo(b)fluoranthene - 4 ppm
12. Benzo(k)fluoranthene - 2 ppm
13. Benzo(a)pyrene - 2 ppm
14. Dibenzo(a,h)anthracene - 4 ppm
15. Benzo(g,h,I)perylene - 4 ppm
16. Indeno(1,2,3-cd)pyrene - 2 ppm



PAH Analysis According to Florida Administrative Code 17.700

Column: Waters PAH Column 5 µm, 4.6 x 250 mm @ 27 °C
Part No.: 186001265
Eluent A: Water
Eluent B: Acetonitrile
Gradient: 60% B to 100% B using curve 9 in 12 minutes, hold 11 minutes, back to initial conditions
Flow Rate: 1.2 mL/min
Injection: 20 µL
Sample: EPA-610 mixture plus two compounds*

Peaks :
1. Naphthalene - 20 ppm
2. Acenaphthylene - 40 ppm
3*. 1-methyl naphthalene - 25 ppm
4*. 2-methyl naphthalene - 25 ppm
5. Acenaphthene - 20 ppm
6. Fluorene - 4 ppm
7. Phenanthrene - 2 ppm
8. Anthracene - 2 ppm
9. Fluoranthene - 4 ppm
10. Pyrene - 2 ppm
11. Benzo(a)anthracene - 2 ppm
12. Chrysene - 4 ppm
13. Benzo(b)fluoranthene - 4 ppm
14. Benzo(k)fluoranthene - 2 ppm
15. Benzo(a)pyrene - 2 ppm
16. Dibenzo(a,h)anthracene - 4 ppm
17. Benzo(g,h,I)perylene -4 ppm
18. Indeno(1,2,3-cd)pyrene -2 ppm

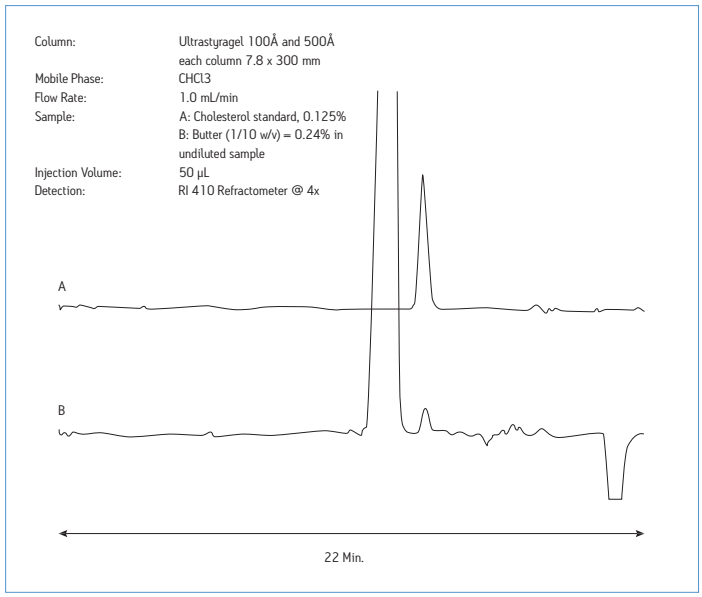


Triglycerides and Cholesterol Analysis

Non-aqueous, reversed-phase chromatography is the best method of separating triglycerides into classes based on carbon number and degree of unsaturation. This method aids in measuring oil degradation and adulteration. Waters Nova-Pak C_{18} column is an excellent choice for this separation due to its high efficiency and carbon surface area. It can also be used for cholesterol determination from samples, such as meat following saponification.

The analysis of high levels of cholesterol in samples like butter and antioxidants in oil, without saponification, can be effectively performed by size exclusion using Waters Ultrastyragel™ columns.

Cholesterol From Butter



Nova-Pak Column for Triglycerides and Cholesterol Analysis

Column	Particle Size	Dimensions	Part No.
Nova-Pak C_{18}	4 µm	3.9 x 150 mm	WAT086344

Ultrastyragel Column for Cholesterol Analysis

Pore Size	Effective Molecular Weight Range	Dimensions	Packed in Toluene	Packed in THF
100Å	50-1,500	7.8 x 300 mm	WAT085500	WAT010570
500Å	100-10,000	7.8 x 300 mm	WAT085501	WAT010571

Fermentation Analysis, Organic Acids, Alcohols, and Carbohydrates

The ion-exclusion mode is ideally suited for the separation of monosaccharides, organic acids, or sugar acids. The column packings are sulfonated styrene divinylbenzene resins in the hydrogen form (IC-Pak Ion Exclusion or SH-1011), and the mobile phase is a dilute acid such as 0.01N H_3PO_4 at 50-60 °C.

Using this mode, the Fast Fruit Juice column can effectively separate glycerol, acetic acid, and ethanol in grape or other fruit juices. The degree of microbial defect, the extent of natural fermentation in grapes and the amount of sulfite present in various foods and beverages can also be analyzed with the Fast Fruit Juice column. The IC-Pak Ion-Exclusion column can separate a wide range of organic acids while the Shodex SH column separates acids as well as larger carbohydrates.

The analysis of alcohols and organic acids is important as these compounds typically play a role in determining the flavor characteristics of beverages such as wine, beer and some spirits. The presence of alcohols in fruit juices can indicate product deterioration. The Shodex KC-811 column is packed with a sulfonated rigid styrene divinylbenzene copolymer providing high efficiency separations of low molecular weight organic acids and water soluble organics such as alcohols, aldehydes and nitriles. The column provides ion exclusion and reversed-phase mode of chromatography. Typical mobile phases are aqueous solutions containing 1% phosphoric, acetic or perchloric acid run 1 mL/min. at 45-80 °C.

Ordering Information

Column	Dimensions	Part No.
Fast Fruit Juice Analysis	7.8 x 150 mm	WAT010639
Fast Fruit Juice Guard-Pak Inserts 10/pkg		WAT015207
IC-Pak Ion Exclusion	7.8 x 300 mm	WAT010290
SH-1011	8 x 300 mm	WAT034236
SH-1011P pre-column	6 x 50 mm	WAT034243
KC-811	8 x 300 mm	WAT034298
KC-811 pre-column	6 x 50 mm	WAT035501

KC-811 Column Retention Chart for Organic Acids

Eluent: Water with 0.1% Phosphoric Acid
Temperature: 60 °C
Flow rate: 1 mL/min

Sample	Retention Time	Sample	Retention Time
Oxalic Acid	5.20	ß- Hydroxypropionic Acid	8.60
Maleic Acid	5.80	D-Glucuronic Acid	8.65
a- Ketoglutaric Acid	5.90	Fumaric Acid	8.95
Citric Acid	6.20	Formic Acid	9.20
Tartaric Acid	6.55	Acetic Acid	9.80
Pyruvic Acid	6.65	Adipic Acid	9.80
trans-Aconitic Acid	6.95	Levulinic Acid	10.00
Glyoxylic Acid	7.00	Mesaconic Acid	10.40
Malic Acid	7.05	Pyroglutamic Acid	10.70
Malonic Acid	7.07	Propionic Acid	11.25
Citraconic Acid	7.20	Acrylic Acid	11.60
Succinic Acid	8.00	Pivalic Acid	14.05
Glycolic Acid	8.40	Methacrylic Acid	14.10
Itaconic	8.50	trans-Crotonic Acid	15.65
Lactic Acid	8.60		

Ion Analysis

Waters offers an array of products for ion chromatography as well as innovative capillary electrophoresis products for ion analysis (see the Capillary Electrophoresis section). In ion chromatography, anions and cations are typically measured in two separate analyses. The columns offered for each type of analysis are briefly described below.

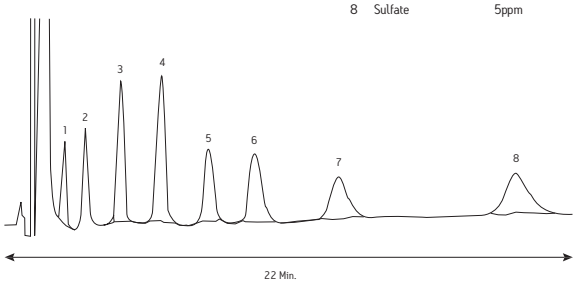
Waters IC-Pak resin-based columns allow you to analyze a full range of ions from numerous sample matrices, both simple and complex. They offer an exceptional linear loading range of less than 1.0 ppb to greater than 400 ppm without dilution and without pH limitations on eluent or sample. The flexibility exists for accurate and reproducible anion and cation analyses at all concentration levels. The IC-Pak series of resin-based columns shares the same chemistry and gives you identical elution order profiles.

The IC-Pak Anion series of columns is used for the analysis of inorganic anions, while the IC-Pak Ion-Exclusion columns are used for organic acid analysis.

The selection of a cation analysis column depends on the type of cation being measured. The IC-Pak C M/D column separates alkali and alkaline earths more efficiently than the traditional IC-Pak Cation column. The IC-Pak C M/D column also separates ethanolamine-related organic cations. Transition metals can be separated using a Delta-Pak C_{18} column. The lanthanide series can be separated using Resolve C_{18} (a nonend-capped C_{18} material). Finally, metallocyanides can be analyzed on a Nova-Pak C_{18} column.

IC-Pak Anion HC Column

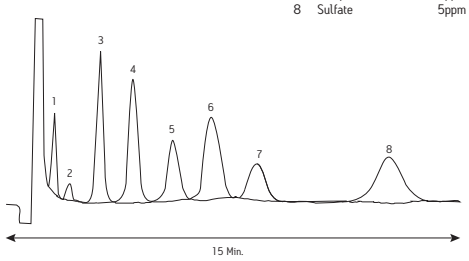
Column:	IC-Pak Anion HC column, 4.6 x 150 mm	1	Fluoride	1ppm
Part No.:	WAT026770	2	Bicarbonate	—
Sample:	Seven anion standard	3	Chloride	2ppm
Eluent:	Borate/gluconate	4	Nitrite	4ppm
Detection:	Waters 431 Conductivity Detector	5	Bromide	4ppm
		6	Nitrate	4ppm
		7	Phosphate	6ppm
		8	Sulfate	5ppm



IC-Pak Anion HC (high capacity) columns use the same packing material as the IC-Pak Anion columns and are best suited for applications where there are orders of magnitude differences in the concentrations of the analytes. This is often the case with water and soil samples where you may have to measure ppb levels of phosphate in the presence of ppm levels of chloride and nitrate. The IC-Pak Anion HC column gives excellent resolution, even with injection volumes up to 500 µL.

IC-Pak Anion Column

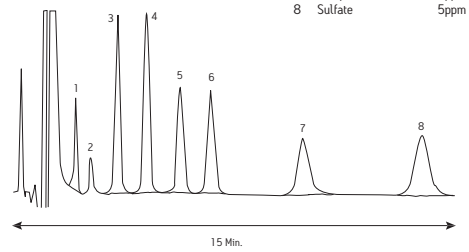
Column:	IC-Pak Anion, 4.6 x 50 mm	1	Fluoride	1ppm
Part No.:	WAT007355	2	Bicarbonate	—
Sample:	Seven anion standard	3	Chloride	2ppm
Eluent:	Borate/gluconate	4	Nitrite	4ppm
Detection:	Waters 431 Conductivity Detector	5	Bromide	4ppm
		6	Nitrate	4ppm
		7	Phosphate	6ppm
		8	Sulfate	5ppm



The IC-Pak Anion column is a configuration of 10 µm anion exchange packing material and a short column length which makes this the column of choice for rapid routine analyses.

IC-Pak Anion HR Column

Column:	IC-Pak Anion HR, 4.6 x 75 mm	1	Fluoride	1ppm
Part No.:	WAT026765	2	Bicarbonate	—
Sample:	Seven anion standard	3	Chloride	2ppm
Eluent:	Borate/gluconate	4	Nitrite	4ppm
Detection:	Waters 431 Conductivity Detector	5	Bromide	4ppm
		6	Nitrate	4ppm
		7	Phosphate	6ppm
		8	Sulfate	5ppm



IC-Pak Anion HR (high resolution) columns are packed with smaller 6 µm particles. This column is designed for the most demanding separations where the column's high efficiency gives enhanced resolution and outstanding sensitivity.

IC-Pak Anion Analysis Columns

A family of anion exchange columns with different characteristics has been developed to meet the needs of even the most demanding separations.

Description	Dimensions	Part No.
IC-Pak Anion	4.6 x 50 mm	WAT007355
IC-Pak Anion HR	4.6 x 75 mm	WAT026765
IC-Pak Anion HC	4.6 x 150 mm	WAT026770
IC-Pak Anion Guard-Pak Kit (Guard-Pak Holder and 5 inserts)		WAT007357
IC-Pak Anion Concentrator Inserts*, 5/pkg		WAT007358
IC-Pak Anion Guard-Pak Inserts*, 5/pkg		WAT010551
Guard-Pak Holder		WAT088141

* Requires Guard-Pak Holder

Ion Analysis

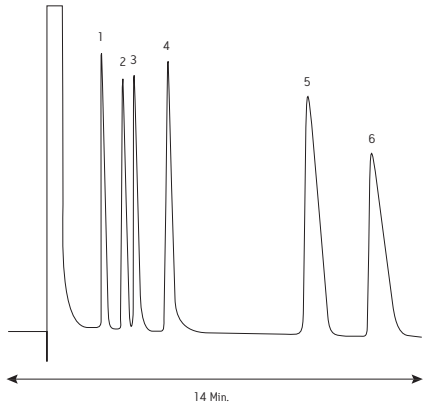
The silica-based IC-Pak C M/D column allows a simultaneous analysis of monovalent and divalent cations under isocratic conditions. This technology provides the analytical chemist with the most sensitive means of analyzing monovalent cations such as Na+, K+, Li+ and NH_4+.

The IC-Pak Cation column is packed with 10 µm sulfonated styrene divinylbenzene particles. Monovalent and divalent cations may be analyzed in two separate runs. Different mobile phase compositions are used for the two analyses.

IC-Pak C M/D

Method: Waters IC-Method C-207
Column: Waters IC-Pak C M/D, 3.9 x 150 mm
Part No.: WAT036570
Eluent: 0.1 mM EDTA/3.0 mM Nitric Acid
Flow Rate: 1 mL/min
Injection: 100 µL
Detection: Conductivity

Cation	Standards
1 Lithium	0.54 ppm
2 Sodium	1.0 ppm
3 Ammonium	2.0 ppm
4 Potassium	4.0 ppm
5 Magnesium	2.0 ppm
6 Calcium	3.0 ppm



IC-Pak Cation Analysis Columns

Description	Dimensions	Part No.
IC-Pak C M/D column	3.9 x 150 mm	WAT036570
IC-Pak C M/D Guard-Pak inserts*, 10/pkg		WAT044250
IC-Pak Cation column	4.6 x 50 mm	WAT007354
IC-Pak Cation Guard column	4.6 x 50 mm	WAT007356
IC-Pak Cation Concentrator inserts*, 5/pkg		WAT010565

Ion Exclusion Columns

Waters IC-Pak Ion Exclusion columns are used for the analysis of weak acid anions such as fluoride and short chain organic acids from formate to butyrate.

Description	Dimensions	Part No.
IC-Pak Ion Exclusion column	7.8 x 150 mm	WAT010295
IC-Pak Ion Exclusion column	7.8 x 300 mm	WAT010290
IC-Pak Ion Exclusion Guard-Pak inserts*, 10/pkg		WAT020770
Guard-Pak Holder		WAT088141

* Requires Guard-Pak Holder

Transition Metal Analysis

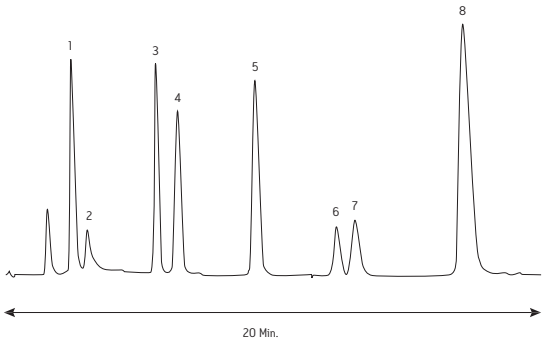
Transition metals can be separated on a dynamically coated C_{18} column and detected at low ppb levels using post-column addition of PAR reagent with UV detection. This method provides excellent resolution with good selectivity and analysis time.

Transition Metal Analysis using Post-Column Derivatization

Column:	Delta-Pak C_{18}, 5 µm, 100Å, 3.9 x 150 mm
Part No.:	WAT011795
Injection:	100 µL
Flow Rate:	0.8 mL/min
Eluent:	2 mM Sodium Octanesulfonate, 35 mM Sodium Tartrate, 5% Acetonitrile, adjust to pH 3.65 with NaOH
Post-column Reagent:	0.2 mM PAR [4-(2-pyridylazo)resorcinol], 1 M Acetic Acid, 3 M Ammonium Hydroxide, 0.5 mL/min

Working Standard

1	Cu	=	0.5 ppm
2	Pb	=	1.0 ppm
3	Zn	=	0.5 ppm
4	Ni	=	1.0 ppm
5	Co	=	0.5 ppm
6	Cd	=	1.0 ppm
7	Fe(II)	=	2.0 ppm
8	Mn	=	1.0 ppm



Ordering Information

Description	Particle Size	Pore Size	Dimensions	Part No.
Delta-Pak C_{18}	5 µm	100Å	3.9 x 150 mm	WAT011795

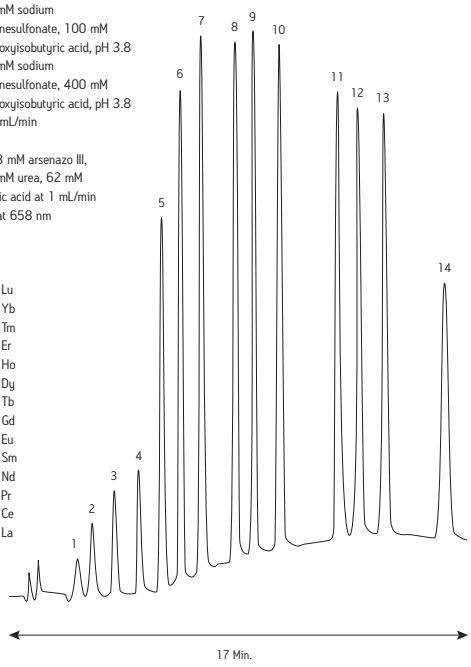
Lanthanide Analysis

Lanthanide Analysis Using a Resolve C_{18} Radial-Pak Cartridge

Column:	Resolve C_{18}, 8 x 100 mm Radial-Pak™ cartridge
Part No.:	WAT084624
Sample:	Lanthanides
Injection:	10 µL
Eluent A:	10 mM sodium octanesulfonate, 100 mM hydroxyisobutyric acid, pH 3.8
Eluent B:	10 mM sodium octanesulfonate, 400 mM hydroxyisobutyric acid, pH 3.8
Flow Rate:	2.0 mL/min
Post-column Reagent:	0.13 mM arsenazo III, 10 mM urea, 62 mM acetic acid at 1 mL/min
Detection:	UV at 658 nm

Working Standard
2 ppm of each

1	Lu
2	Yb
3	Tm
4	Er
5	Ho
6	Dy
7	Tb
8	Gd
9	Eu
10	Sm
11	Nd
12	Pr
13	Ce
14	La



Ordering Information

Particle Description	Pore Size	Size	Dimensions	Part No.
Resolve C_{18} *	5 µm	90Å	8 x 100 mm	WAT084624
8x10 Cartridge Holder				WAT082887

* Requires 8x10 Cartridge Holder

Gas-Chromatography Packings

Waters versatile Porapak™ gas-chromatography column packing materials simplify the analysis of a wide variety of complex compounds from atmospheric gases to organics. Consisting of polymer beads, these unique packings are chemically and physically stable and have consistent particle size, porosity and surface area to guarantee analytical reproducibility. They also provide unequaled separation capability with high resolution and low, constant retention volumes.

Versatility for Specialty Applications

To optimize separation of even the most complex matrices, several physical and chemical variations are available within the Porapak packing materials. Special characteristics of Waters unique GC packings include:

- Fast analysis with compounds eluting in distinctive bands with no tailing
- The ability to sustain elevated temperatures permitting temperature programming without adverse effects to retention, reproducibility, and column life
- The ability to accommodate large sample loads required for preparative and trace analysis while maintaining characteristically high column efficiency

GC Porapak Porous Polymer Packing

Type	Polarity	Surface Area (m^2/g)	Density (g/cm^3)	Single Temp Prog.	Particle Size Mesh	Qty.	Part No.
P	Nonpolar	100-200	0.26	250 °C	50-80	20 g	WAT027053
					80-100	20 g	WAT027054
					100-120	20 g	WAT027055
PS	Nonpolar	100-200	0.26	250 °C	50-80	20 g	WAT027083
					80-100	20 g	WAT027084
					100-120	20 g	WAT027085
Q	Slightly nonpolar to moderate	500-600	0.34	250 °C	50-80	26 g	WAT027059
					80-100	26 g	WAT027060
					100-120	26 g	WAT027061
QS	Slightly nonpolar to moderate	500-600	0.34	250 °C	50-80	26 g	WAT027089
					80-100	26 g	WAT027090
					100-120	26 g	WAT027091
R	Moderate Polar monomer: vinyl pyrrolidone	450-600	0.32	250 °C	50-80	24 g	WAT027065
					80-100	24 g	WAT027066
					100-120	24 g	WAT027067
S	Moderate Polar monomer: vinyl pyridine	300-450	0.35	250 °C	50-80	26 g	WAT027071
					80-100	26 g	WAT027072
					100-120	26 g	WAT027073
N	Polar Polar monomer: vinyl pyrrolidone	250-350	0.41	190 °C	50-80	29 g	WAT027047
					80-100	29 g	WAT027048
					100-120	29 g	WAT027049
T	Highly polar Polar monomer: ethyleneglycol dimethacrylate	225-350	0.39	190 °C	50-80	31 g	WAT027077
					80-100	31 g	WAT027078
					100-120	31 g	WAT027079

[PERFORMANCE]

UNRIVALED VERSATILITY AND PERFORMANCE
FOR METHOD DEVELOPMENT

XBridge™ COLUMNS

The XBridge™ family of columns is designed for maximum method development flexibility. Robust methods can be developed across the entire pH spectrum using an unparalleled range of mobile phases and temperatures. XBridge columns have been shown to provide consistent performance and extended lifetime under even the most demanding conditions. Utilizing revolutionary technology and the highest levels of manufacturing and quality assurance, XBridge columns are a trusted solution for your most challenging chromatographic separations.

To learn more about XBridge HPLC columns, visit www.waters.com/xbridge

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

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Preparative Chromatography

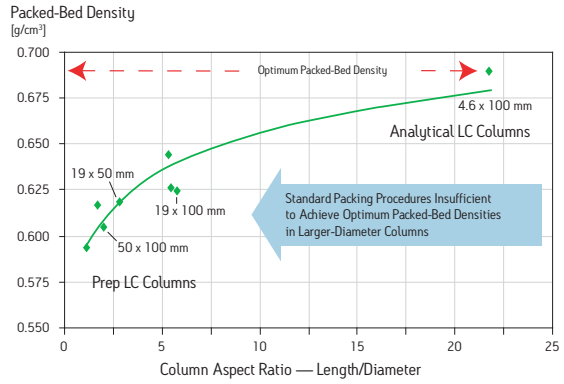
Column Performance—Identifying the Problem

Laboratory-scale HPLC purification presents many challenges to the chromatographer. One of the most frustrating challenges relates to the preparative column itself. Inconsistencies in column-to-column performance and lifetimes often result in lost samples, repeat purification runs, and poor scalability from small to large volume columns. Scientists at Waters recognized this problem and, over a 3-year period, studied all aspects of the packing processes as well as the column design. Launched in 2003, the Waters proprietary* Optimum Bed Density (OBD™) design is a direct result of these investigations.

For a column to remain stable during operation, the bed must be packed sufficiently dense to withstand the compressive fluid forces encountered during use. In the case of analytical column dimensions, the necessary packed bed density can generally be achieved using traditional slurry packing methods. As the diameter and length of the column increases, it becomes increasingly difficult to reach the bed density required for stable, long-term performance when using small particles. Optimizing the bed density depends on the specific properties of the chromatographic particles and column design being used.

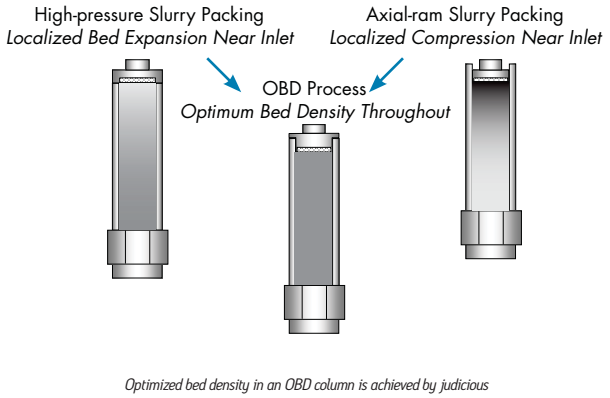
*UK Patent # GB2408469

Average Bed Density for Conventional Analytical-LC-Column-Slurry-Packing Procedures vs. Column Aspect Ratio

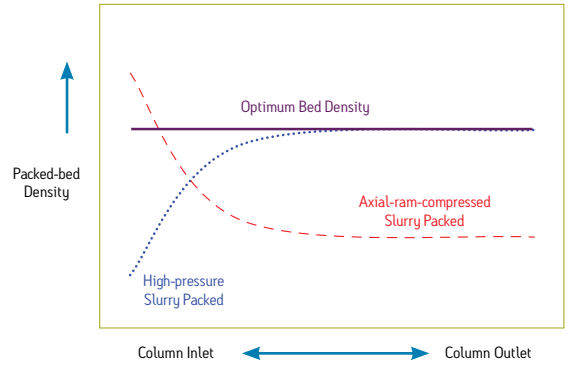


Waters OBD Column Design

Waters has combined high-pressure slurry packing with a carefully calculated axial compression element localized at the less-dense inlet end of the bed.



Optimized bed density in an OBD column is achieved by judicious application of a tuned amount of axial force only at the column inlet.



Effect of packing method on average bed density across column length.

With careful tuning of the packing process for each particle type and column geometry, the Prep OBD design and process results in predictable, uniform density profiles throughout the column. During the final capping process, Waters Column Packing Operation follows carefully established procedures designed not to over-compress or disrupt, in any non-uniform way, this portion of the bed. Waters scientists have established that too much axial compression applied at the inlet can break particles, build bridges, and lower local bed permeability.

The Prep OBD column is designed to incorporate a pair of specially-designed distributors and chemically-inert seals made to prevent leaks at high operating pressures.

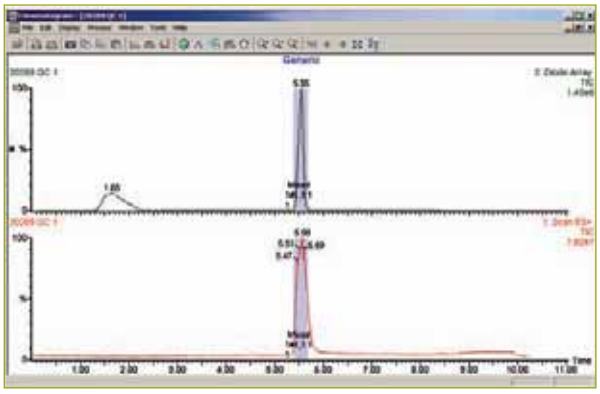
An exploded view of the elements of an empty OBD column.

Column Stability And Reliability—Long, Predictable Lifetimes

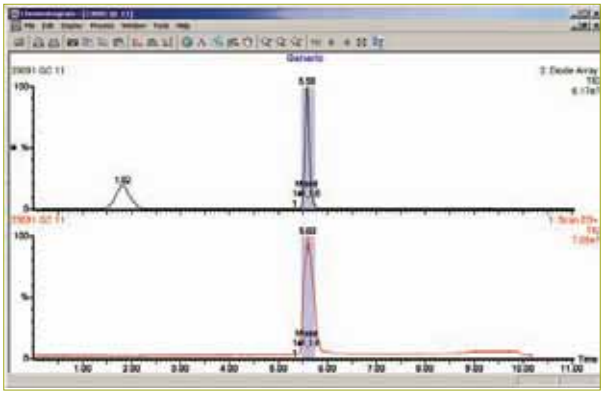
The demand for rapid high purity compound isolation places strong emphasis on the integrity and stability of the preparative column. Complex, sparingly-soluble starting materials are often dissolved with strong solvents, such as DMSO. This combination of poor solubility and pressure shocks associated with large injection volumes of pure organic solvent are the primary contributors to early column failure and chromatographic bed collapse.

The OBD design exhibits exceptional resistance to mechanical chromatographic bed failure and delivers consistent column-to-column performance, reducing cost through extended lifetimes.

Data from a High-throughput Drug Discovery Laboratory: 7,000 Injections on an XBridge Prep OBD C_{18} 5 µm 19 x 50 mm Column



Injection 1



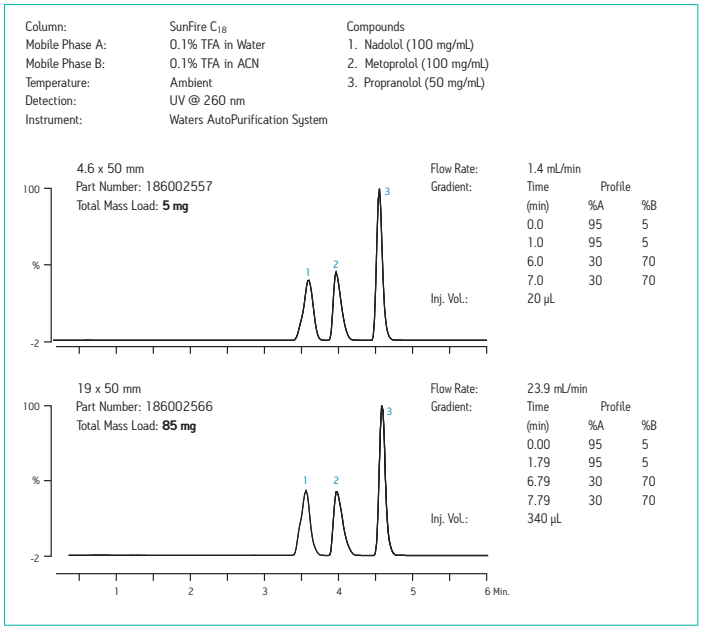
Injection 7,000

Scalability—Analytical Performance In A Prep OBD Column

Successful scaling of methods from analytical to preparative dimensions requires the use of chromatographically equivalent columns. All too often, even when the same chemistry phase and particle size are used, methods do not scale either due to loss of resolution and/or lower than expected loading. By matching the analytical and preparative column bed densities, scalability is assured, eliminating the need for any time consuming method re-development.

OBD preparative columns are packed to bed densities which closely match the equivalent analytical column. This innovative procedure produces preparative columns with excellent stability, reproducibility, and efficiency.

Accurate Scale-up on SunFire Columns in 0.1% TFA



Selecting the Column Packing

Waters column packing materials bring you the best in preparative chromatography options. For optimum flexibility, our column packing materials are available in a range of steel columns. A complete list of preparative packing materials and their characteristics is shown in the following table.

Preparative Packing Characteristics

Packing	Chemistry	Particle Size	Pore Size	Particle Shape	Carbon Load	End-capped
XBridge™	C_{18}	5, 10 µm	135Å	Spherical	17.5%	Yes
	C_8	5, 10 µm	135Å	Spherical	12.8%	Yes
	Shield RP18	5, 10 µm	135Å	Spherical	16.7%	Yes
	Phenyl	5 µm	135Å	Spherical	14.6%	Yes
SunFire™	C_{18}	5, 10 µm	100Å	Spherical	16%	Yes
	C_8	5, 10 µm	100Å	Spherical	11.5%	Yes
	Silica	5, 10 µm	100Å	Spherical	N/A	Yes
Atlantis®	T3	5, 10 µm	100Å	Spherical	14%	Yes
	dC_{18}	5, 10 µm	100Å	Spherical	11%	Yes
	HILIC	5 µm	100Å	Spherical	0%	No
XTerra® MS	C_{18}	5, 10 µm	125Å	Spherical	15.5%	Yes
	C_8	5, 10 µm	125Å	Spherical	12.0%	Yes
XTerra RP	C_{18}	5, 10 µm	125Å	Spherical	15%	Yes
	C_8	5, 10 µm	125Å	Spherical	13.5%	Yes
Symmetry®	C_{18}	5, 7 µm	100Å	Spherical	19%	Yes
	C_8	5, 7 µm	100Å	Spherical	12%	Yes
SymmetryShield™	C_{18}	5, 7 µm	100Å	Spherical	15%	Yes
	C_8	5, 7 µm	100Å	Spherical	17%	Yes
Symmetry300™	C_{18}	5, 7 µm	300Å	Spherical	8.5%	Yes
	C_4	5, 7 µm	300Å	Spherical	2.8%	Yes
Prep Nova-Pak® HR	C_{18}	6 µm	60Å	Spherical	7%	Yes
	Silica	6 µm	60Å	Spherical	N/A	N/A
µBondapak®	C_{18}	10 µm	125Å	Irregular	10%	Yes
	Phenyl	10 µm	125Å	Irregular	8%	Yes
	CN	10 µm	125Å	Irregular	6%	Yes
	NH_2	10 µm	125Å	Irregular	3.5%	No
Bondapak®	C_{18}	15-20 µm	125Å	Irregular	10%	Yes
		37-55 µm	125Å	Irregular	10%	Yes
Bondapak HC_{18} HA	C_{18}	37-55 µm	125Å	Irregular	14%	Yes
µPorasil™	Silica	10 µm	125Å	Irregular	N/A	N/A
Porasil™	Silica	15-20 µm	125Å	Irregular	N/A	N/A
		37-55 µm	125Å	Irregular	N/A	N/A
Delta-Pak™	C_{18}	15 µm	100Å	Spherical	17%	Yes
			300Å	Spherical	7%	Yes
Delta-Pak	C_4	15 µm	100Å	Spherical	7%	Yes
			300Å	Spherical	3%	Yes
Prep	C_{18}	55-105 µm	125Å	Irregular	11%	Yes
	Silica	55-105 µm	125Å	Irregular	N/A	N/A

No matter how you collect fractions, whether by mass spec, UV, ELS, or other detector signals, the Waters AutoPurification systems will meet your needs. These systems provide simplicity, scalability, and speed with a variety of available configurations. The market leading FractionLynx™ Application Manager provides an intuitive interface to manage the purification process from sample acquisition to reporting.

Pictured here is the Waters AutoPurification™ system with a 2767 Sample Manager for inject/collect/re-inject, 2545 Binary Gradient Module for high pressure mixing, System Fluidics Organizer for software-controlled valving, fluidics, and column management, 2998 PDA, 2424ELS and 3100 Mass Detectors.

Choosing Preparative Columns

Step 1

Once the analytical separation has been optimized, a loading study on the analytical column is performed to determine the capacity of the particular packing material. Because the large scale separation should be identical to the small scale separation, the maximum sample load will be dependent upon the complexity of the analytical separation.

Step 2

Determine how much mass you need to purify or isolate.

Step 3

Once the desired purified mass is established, some simple equations may be used to determine the required column size for purification. In addition, preparative HPLC system maximum flow rate and back pressure need to be considered and can limit column size.

Scale-up factor

$$\text{Scale-up factor} = \frac{(\text{Diameter prep})^2 \times \text{Length prep}}{(\text{Diameter analytical})^2 \times \text{Length analytical}}$$

Consider scaling up from a **4.6 x 150 mm** column to a **19 x 150 mm** column:

$$\text{Scale-up factor} = \frac{(19)^2 \times 150}{(4.6)^2 \times 150} = 17.1$$

Applying the scale-up factor, we can predict that approximately 17-135 mg of sample could be applied to the larger column (packed with the same material as the analytical column). This range is based on an analytical (4.6 mm i.d.) mass load of 1-8 mg.

Flow Rate

$$\text{Flow rate (prep)} = \text{Flow rate (anal)} \times \frac{(\text{Diameter prep})^2}{(\text{Diameter anal})^2} \times \frac{\text{Particle Size (anal)}}{\text{Particle Size (prep)}}$$

The calculated flow rate may be used for the larger column to ensure the same linear velocity of mobile phases as used in the analytical run. However, reasonable flow rates are based on column diameters. Systems will be limited by increasing backpressure with increasing column length and decreasing particle size.

Gradient Duration (GD)

$$\text{GD (prep)} = \frac{\text{GD (anal)} \times \text{Length (prep)}}{\text{Length (anal)}} \times \frac{\text{Diameter (prep)}^2}{\text{Diameter (anal)}^2} \times \frac{\text{Flow rate (anal)}}{\text{Flow rate (prep)}}$$

Mass Loading

Approximate Mass Loading Capacity (mg) for Prep OBD Columns (Gradient Mode)

	Diameter (mm)				
Length (mm)	4.6	10	19	30	50
30	–	–	27	–	–
50	3	15	45	110	310
75	–	–	–	165	–
100	5	25	90	225	620
150	8	40	135	335	930
250	13	60	225	560	1550
Reasonable Flow Rate (mL/min)	1.4	6.6	24	60	164
Reasonable Injection Volume (µL)	20	100	350	880	2450

The calculated prep gradient duration is entered into the pump's gradient separation over the same number of column volumes as was used in the analytical run.

Many factors affect the mass capacity of preparative columns. The listed capacities represent an "average" estimate.

Capacity is:

- Higher for strongly retained material
- Higher for simple mixtures
- Lower where higher resolution is required
- Very strongly dependent on loading conditions
  - Limited by loading volume
  - Limited by diluent solvent strength

Reasonable flow rates are based on column diameter. Systems will be limited by increasing backpressure with increasing column length and decreasing particle size.

Reasonable injection volumes are based on column diameter at a length of 50 mm with relatively strong solvents. Increased length is compatible with larger injection, but not proportionately so. Weaker solvents significantly increase injection volume.

Mass loading capacities for peptide purifications depend strongly on the sequence and may be estimated at 5-20% of listed values.

XBridge Prep OBD Columns

Bridging the Performance Gap from Analytical to Prep

A Milestone in Chromatography

First introduced in 1999, Waters patented Hybrid Particle Technology (HPT) surmounted significant limitations of silica-based, reversed-phase packing materials, particularly their hydrolytic instability at high pH. In 2005, the introduction of a second generation HPT, branded as XBridge Prep columns marks a new milestone in chromatography.

With an order of magnitude improvement in high pH stability and a higher level of chromatographic performance, XBridge Prep columns define the new benchmark for LC purification.

XBridge Prep C_{18} and C_8

C_{18} and C_8 phases remain to be the workhorse of the purification chemist. The second generation HPT phases deliver not only unmatched pH stability, but also an equivalent chromatographic performance and peak efficiency to that of the best silica based product. A testament to the enhanced mass transfer characteristics of this new particle. These attributes, combined with the OBD packing design, make the XBridge Prep C_{18} and C_8 phases ideal for robust purification procedures.

XBridge Prep RP18

The XBridge Prep RP18 phase combine the HPT process with the Waters patented Shield Technology. This embedded polar group reverse-phase material delivers maximum peak efficiency and a unique alternate selectivity, particularly useful in the isolation of phenolic analytes. An additional feature of this phase is the ability to routinely operate in 100% aqueous mobile phases without exhibiting the problems associated with phase dewetting.

XBridge Prep Phenyl

Phenyl chromatographic phases have always been of interest to the chromatographer due to the alternative selectivity, particularly with respect to polyaromatic compounds. Unfortunately, routine use of these phases is not common due to their inherent poor stability and reproducibility.

A combination of the second generation HPT and a tri-functional bonding approach in the XBridge Prep Phenyl delivers, for the first time, a truly pH stable and reproducible phase, ideal for drug purification.

A Particle Designed for Purification

Preparative packing materials are routinely offered with a much wider particle size distribution than the equivalent analytical particle. While this approach does reduce the cost of material manufacturing, the chromatographic performance is almost certainly compromised.

XBridge Prep packing materials are fully optimized to deliver maximum efficiency and the highest loadability at the lowest possible back-pressures. These attributes allow the purification chemist the flexibility to choose the best combination of particle size and column dimension to easily purify even the most complex of samples.

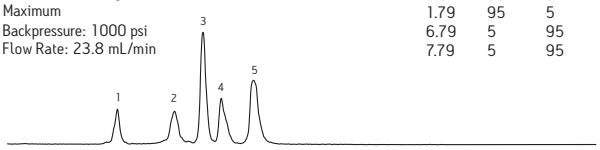
Maximum Efficiency/30% Lower Backpressure

Mobile Phase A:	0.1% DEA in water	Compounds
Mobile Phase B:	0.1% DEA in acetonitrile	1. Labetolol (50 mg/mL)
Sample		2. Quinine (50 mg/mL)
Concentration:	300 mg/mL in DMSO	3. Diltiazem (50 mg/mL)
Instrument::	Waters AutoPurification System	4. Verapamil (100 mg/mL)
Detection:	UV @ 260 nm	5. Amitriptyline (50 mg/mL)

XTerra Prep MS C_{18}, 19 x 50 mm, 5 µm
Injection Volume: 660 µL
Total Load: 198 mg
Maximum
Backpressure: 1000 psi
Flow Rate: 23.8 mL/min

Gradient:

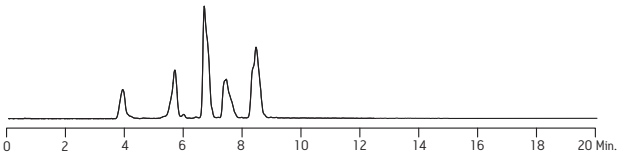
Time (min)	Profile %A	%B
0.0	95	5
1.79	95	5
6.79	5	95
7.79	5	95



XBridge Prep C_{18}, 19 x 50 mm, 5 µm
Injection Volume: 660 µL
Total Load: 198 mg
Maximum
Backpressure: 700 psi
Flow Rate: 23.8 mL/min

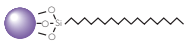
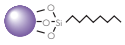
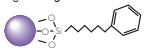
Gradient:

Time (min)	Profile %A	%B
0.0	95	5
1.79	95	5
6.79	5	95
7.79	5	95



XBridge Prep Columns deliver the same high loading capacity and reliability expected of our XTerra Prep products, with a significantly reduced column backpressure.

XBridge Column Characteristics

Chemistry	Ligand Density	Carbon Load	pH Range	Pore Diameter	Surface Area
Trifunctional C_{18} 	3.1 µmol/m^2	18%	1-12	135Å	185 m^2/g
Trifunctional C_8 	3.2 µmol/m^2	13%	1-12	135Å	185 m^2/g
Monofunctional Embedded Polar Group (Shield RP18) 	3.3 µmol/m^2	17%	2-11	135Å	185 m^2/g
Trifunctional C_6 Phenyl 	3.0 µmol/m^2	15%	1-12	135Å	185 m^2/g

Literature References

Waters XBridge HPLC Columns Brochure, Literature Reference 720001255EN

BEH Technology White Paper, Literature Reference 720001159EN

Optimum Bed Density (OBD)] Columns: Enabling Technology for Laboratory-Scale Isolation and Purification White Paper, Literature Reference 720001939EN

Bridging the Performance Gap From Analytical to Prep Optimum Bed Density (OBD) Preparative Columns Brochure, Literature Reference 720002336EN

Interactive Waters Reversed-Phase Column Selectivity Chart www.waters.com/selectivitychart

XBridge Prep OBD Columns

Dimensions	Type	Particle Size	C_{18}	C_8	Shield RP18	Phenyl
10 x 10 mm	Guard	5 µm	186002972[1]	186002991[1]	186002983[1]	186003354[1]
10 x 50 mm	Column	5 µm	186002973	186003264	186003257	186003271
10 x 100 mm	Column	5 µm	186003255	186003265	186003258	186003272
10 x 150 mm	Column	5 µm	186002974	186003266	186003259	186003273
10 x 250 mm	Column	5 µm	186003256	186003267	186003260	186003274
19 x 10 mm	Guard	5 µm	186002975[2]	186002992[2]	186002984[2]	186003355[2]
OBD 19 x 30 mm	Column	5 µm	186002976	186003268	186003261	186003275
OBD 19 x 50 mm	Column	5 µm	186002977	186002993	186002985	186003356
OBD 19 x 100 mm	Column	5 µm	186002978	186002994	186002986	186003357
OBD 19 x 150 mm	Column	5 µm	186002979	186002995	186002987	186003358
OBD 19 x 250 mm	Column	5 µm	186004021	186004023	186004022	186004024
OBD 30 x 50 mm	Column	5 µm	186002980	186002996	186002988	186003277
OBD 30 x 75 mm	Column	5 µm	186002981	186003269	186003262	186003278
OBD 30 x 100 mm	Column	5 µm	186002982	186002997	186002989	186003279
OBD 30 x 150 mm	Column	5 µm	186003284	186003083	186002990	186003276
OBD 30 x 250 mm	Column	5 µm	186004025	—	—	—
OBD 50 x 50 mm	Column	5 µm	186003933	186003934	186003935	186003936
OBD 50 x 100 mm	Column	5 µm	186003937	186003938	186003939	186003940
OBD 50 x 150 mm	Column	5 µm	186003929	—	—	—
OBD 50 x 250 mm	Column	5 µm	186004107	—	—	—
10 x 10 mm	Guard	10 µm	186003889[3]	186004003[3]	186003988[3]	—
10 x 150 mm	Column	10 µm	186003890	186004004	186003989	—
10 x 250 mm	Column	10 µm	186003891	186004005	186003990	—
19 x 10 mm	Guard	10 µm	186003892[4]	186004006[4]	186003991[4]	—
OBD 19 x 50 mm	Column	10 µm	186003893	186004007	186003992	—
OBD 19 x 100 mm	Column	10 µm	186003901	186004008	186003993	—
OBD 19 x 150 mm	Column	10 µm	186003894	186004009	186003994	—
OBD 19 x 250 mm	Column	10 µm	186003895	186004010	186003995	—
OBD 30 x 100 mm	Column	10 µm	186003930	—	—	—
OBD 30 x 150 mm	Column	10 µm	186003896	186004011	186003996	—
OBD 30 x 250 mm	Column	10 µm	186003897	186004012	186003997	—
OBD 50 x 50 mm	Column	10 µm	186003898	186004013	186003998	—
OBD 50 x 100 mm	Column	10 µm	186003902	186004014	186003999	—
OBD 50 x 150 mm	Column	10 µm	186003899	186004015	186004001	—
OBD 50 x 250 mm	Column	10 µm	186003900	186004016	186004002	—

Purification and Isolation Cartridge Holders

Description	Part No.
10 x 10 mm Cartridge Holder	289000779
19 x10 mm Cartridge Holder	186000709
Replacement o-ring 7.8 mm, 2/pkg	700001019
Replacement o-ring 10 mm, 2/pkg	700001436
Replacement o-ring 19 mm, 2/pkg	700001020

For Purification of Peptides, See Pages 186-193

[1] Requires 10 x 10 mm Prep Guard Holder 289000779
[2] Requires 19 x 10 mm Prep Guard Holder 186000709

SunFire Prep OBD Columns

Bridging the Performance Gap from Analytical to Prep

SunFire columns set the standard for the state-of-the-art bonded C_{18} and C_8 silica HPLC columns. Benefiting from years of research and product development, SunFire columns represent the best in particle and bonding expertise and deliver the industry-leading level of chromatographic performance.

Loadability by Design

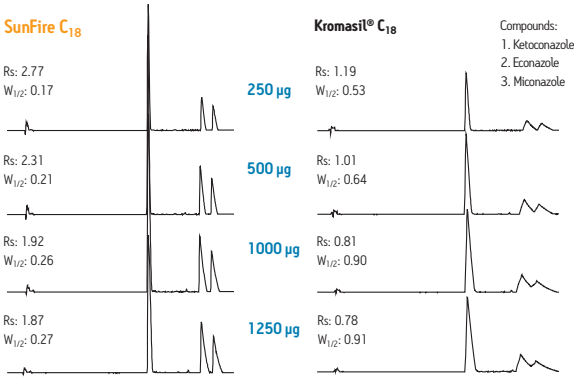
The physicochemical characteristics of sorbents used for preparative separations must be designed and/or chosen not only to achieve the selectivity necessary for optimal separations but also the load capacity that enables maximum throughput. This becomes especially tricky for ionizable compounds in mobile phases with low to moderate pH. SunFire sorbents are made from an engineered synthetic silica with proprietary surface-chemistry modifications, all done under cGMP protocols, that extend loadability far beyond that of competitive packings.

High Mass Loading of Sunfire Sorbents Enables the Use of Smaller Preparative Column Dimensions

Mobile Phase A:	0.1% TFA in water
Mobile Phase B:	0.1% TFA in acetonitrile
Gradient:	15 min linear gradient from 20% B to 85% B, 2 min initial holding time
Flow Rate:	1 mL/min
Injection Volume:	10 µL
Column:	4.6 x 150 mm, 5 µm
Detection:	UV @ 254 nm
Instrument:	Alliance HT 2795

Sample Concentration:
As indicated in the table, dissolved in DMSO

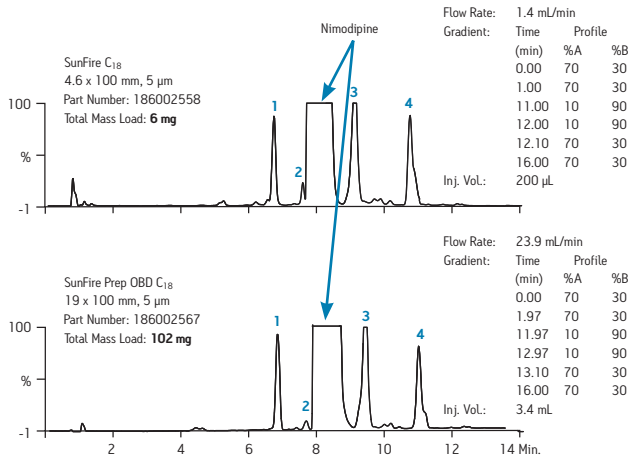
Analyte	mg/mL	mg/mL	mg/mL	mg/mL
Ketoconazole	25	20	10	5
Econazole	50	40	20	10
Miconazole	50	40	20	10
Total Concentration	125	100	50	25



Isolation of Nimodipine and its Impurities

Mobile Phase A:	Water with 0.1% FA
Mobile Phase B:	ACN with 0.1% FA
Analyte:	Nimodipine at 30 mg/mL in DMSO
Detection:	UV @ 290 nm
Instruments:	Waters AutoPurification Systems

Impurity levels:	Peak	% by Area
	1.	3.1
	2.	0.6
	3.	6.5
	4.	4.9



Normal-Phase SunFire Prep Silica Columns

SunFire Prep silica columns allow you to use normal-phase protocols. This gives you a significant selectivity alternative to reversed-phase preparative chromatography on bonded silicas. Analytes that are highly hydrophilic or that are insoluble/unstable in water are ideal candidates for normal-phase purification. Unlike the more viscous, higher-boiling aqueous-organic mobile phases used in reversed phase, typical normal-phase eluents are lower-viscosity, lower-boiling organic solvents. As a consequence, SunFire Silica separations yield several key benefits:

- High efficiency (plate counts similar to those of analytical columns)
- Excellent scale-up capability
- Lower back pressure
- Faster analyte recovery (rapid solvent-evaporation rates)

SunFire Prep columns, available in 5 µm and 10 µm particle sizes, exhibit enhanced column life and stability due to the OBD design as well as excellent peak shapes and high mass-load capacity.

Literature References

SunFire Columns Brochure, Literature Reference 720000875EN

Optimum Bed Density (OBD) Columns: Enabling Technology for Laboratory-Scale Isolation and Purification White Paper, Literature Reference 720001939EN

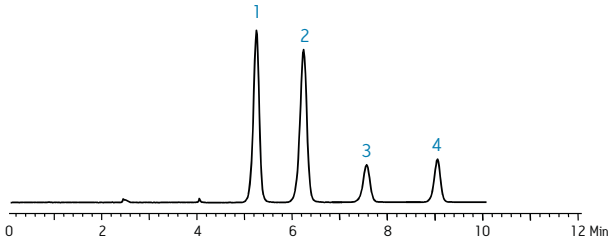
Bridging the Performance Gap From Analytical to Prep Optimum Bed Density (OBD) Preparative Columns Brochure, Literature Reference 720002336EN

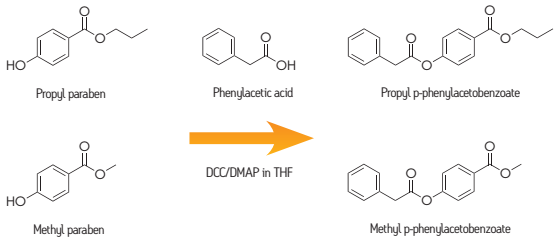
Interactive Waters Reversed-Phase Column Selectivity Chart www.waters.com/selectivitychart

Purification of Diesters from Standard DCC/DMAP Protocol*

Conditons
Column: SunFire Silica, 19 x 150 mm, 5 µm
Part Number: 186002568
Mobile Phase: 80/20 Hexane/ Ethyl acetate
Flow Rate: 18 mL/min
Total Load: 100 mg

Analytes:
1. Propyl p-phenylacetobenzoate
2. Methyl p-phenylacetobenzoate
3. Propyl paraben
4. Methyl paraben





*A base-catalyzed (DMAP) esterification reaction, driven to completion using a dehydrating agent (DCC). **RCOOH + R^{1}OH ↔ RCOOR^1 + H_2O**

DMAP = 4-Dimethylaminopyridine; DCC = 1,3-Dicyclohexylcarbodiimide; THF = Tetrahydrofuran

SunFire 5 µm Prep Columns

Particle Size	Dimensions	C_{18}	C_8	Silica
5 µm	10 x 50 mm	186002561	186002746	186003425
5 µm	10 x 100 mm	186002562	186002747	186003426
5 µm	10 x 150 mm	186002563	186002748	186003427
5 µm	10 x 250 mm	186002564	186002749	186003428
5 µm	OBD 19 x 30 mm	186002879	186002881	186003430
5 µm	OBD 19 x 50 mm	186002566	186002751	186003431
5 µm	OBD 19 x 100 mm	186002567	186002752	186003432
5 µm	OBD 19 x 150 mm	186002568	186002753	186003433
5 µm	OBD 30 x 50 mm	186002570	186002755	186003435
5 µm	OBD 30 x 75 mm	186002571	186002756	186003436
5 µm	OBD 30 x 100 mm	186002572	186002757	186003437
5 µm	OBD 30 x 150 mm	186002797	186002795	186003438
5 µm	OBD 30 x 250 mm	186003969	—	—
5 µm	OBD 50 x 50 mm	186002867	186002868	186003439
5 µm	OBD 50 x 100 mm	186002869	186002870	186003440
5 µm	OBD 50 x 150 mm	186003941	—	—
5 µm	OBD 50 x 250 mm	186003970	—	—

SunFire 10 µm Prep Columns

Particle Size	Dimensions	C_{18}	C_8	Silica
10 µm	10 x 50 mm	186003840	186003841	Custom
10 µm	10 x 150 mm	186002664	186002759	186003442
10 µm	10 x 250 mm	186002665	186002760	186003443
10 µm	OBD 19 x 50 mm	186002667	186002762	186003445
10 µm	OBD 19 x 150 mm	186002668	186002763	186003446
10 µm	OBD 19 x 250 mm	186002669	186002764	186003447
10 µm	OBD 30 x 50 mm	186003854	186003853	186003855
10 µm	OBD 30 x 100 mm	186003971	—	—
10 µm	OBD 30 x 150 mm	186002670	186002765	186003448
10 µm	OBD 30 x 250 mm	186002671	186002766	186003449
10 µm	OBD 50 x 50 mm	186002871	186002872	186003450
10 µm	OBD 50 x 100 mm	186003972	—	—
10 µm	OBD 50 x 150 mm	186002672	186002767	186003451
10 µm	OBD 50 x 250 mm	186002673	186002768	186003452
10 µm	OBD 100 x 250 mm	186003928	—	—

SunFire Prep Guard Columns

Particle Size	Dimensions	C_{18}	C_8	Silica
5 µm	10 x 10 mm	186002565[1]	186002750[1]	186003429[1]
5 µm	19 x 10 mm	186002569[2]	186002754[2]	186003434[2]
10 µm	10 x 10 mm	186002663[1]	186002758[1]	186003441[1]
10 µm	19 x 10 mm	186002666[2]	186002761[2]	186003444[2]

[1] Needs 10 x 10 mm Cartridge Holder Part No. 289000779
[2] Needs 19 x 10 mm Cartridge Holder Part No. 186000709

SunFire Prep Scouting Columns

Particle Size	Dimensions	C_{18}	C_8	Silica
5 µm	4.6 x 150 mm	—	—	186003453
5 µm	4.6 x 250 mm	—	—	186003454
10 µm	4.6 x 150 mm	186003390	Custom	186003467
10 µm	4.6 x 250 mm	186003391	Custom	186003468

Purification and Isolation Cartridge Holders

Description	Part No.
7.8 x 10 mm Cartridge Holder	186000708
10 x 10 mm Cartridge Holder	289000779
19 x 10 mm Cartridge Holder	186000709
Replacement o-ring 7.8 mm, 2/pkg	700001019
Replacement o-ring 10 mm, 2/pkg	700001436
Replacement o-ring 19 mm, 2/pkg	700001020

Atlantis Prep OBD Columns

Bridging the Performance Gap for Polar Compound Retention

First introduced in 2002, the Atlantis product range has rapidly gained acceptance as the industry standard for polar compound retention and purification. Available for both reversed-phase and HILIC modes of chromatography, these phases exhibit significantly increase retention characteristics as compared to conventional C_{18} or embedded polar group column chemistries.

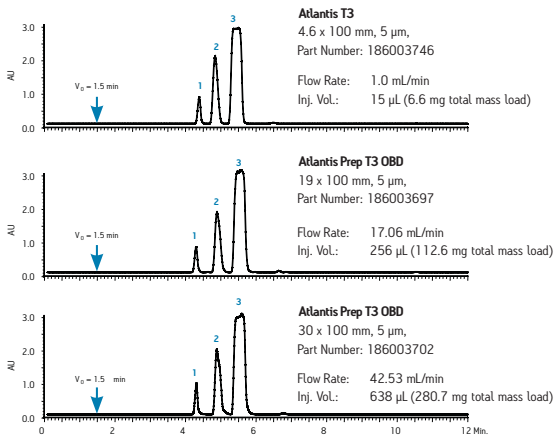
Atlantis dC_{18}

The first in the family to be introduced, Atlantis dC_{18} is a reversed-phase packing bonded with a difunctional C_{18} ligand to an intermediate ligand density (1.6 µmoles/m^2) and fully end-capped. For maximum column lifetime, the dC_{18} phase is recommended for mobile phase pH values of 2.5-6.

Atlantis T3

An evolution of the dC_{18} product, Atlantis T3 columns retain all of the benefits of its predecessor but exhibit significantly improved stability in low pH mobile phases as well as improved peak shape for amine containing bases at pH 7. T3 bonding utilizes a trifunctional C_{18} ligand, (1.6 µmoles/m^2), and a proprietary end-capping process which has been proven to be much more effective than the traditional trimethyl silane (TMS) end-capping.

Beta Blockers



Analytical Gradient Table

Gradient:	Time (min)	Profile %A	%B
	0.0	100	0
	1.0	100	0
	2.0	82	18
	6.0	28	72
	8.0	28	72
	8.1	0	100
	10.0	0	100
	10.1	100	0
	15.0	100	0

Preparative Gradient Table

Gradient:	Time (min)	Profile %A	%B
	0.0	100	0
	2.0	100	0
	3.0	82	18
	7.0	28	72
	9.0	28	72
	9.1	0	100
	11.0	0	100
	11.1	100	0
	16.0	100	0

Compounds	
1. Atenolol	40 mg/mL in DMSO
2. Metoprolol	200 mg/mL in DMSO
3. Propranolol	200 mg/mL in DMSO
Mobile Phase A:	0.1% formic acid in water
Mobile Phase B:	0.1% formic acid in acetonitrile
Detection:	UV @ 280 nm
Instrument:	2525 Binary Gradient Module, 2767 Sample Manager, 2996 PDA (AutoPurification flow cell)
Temperature:	Ambient

Literature References

Atlantis T3 and ACQUITY UPLC HSS T3 Columns Brochure, Literature Reference 720000793EN

Optimum Bed Density (OBD) Columns: Enabling Technology for Laboratory-Scale Isolation and Purification White Paper, Literature Reference 720001939EN

Bridging the Performance Gap From Analytical to Prep Optimum Bed Density (OBD) Preparative Columns Brochure, Literature Reference 720002336EN

Interactive Waters Reversed-Phase Column Selectivity Chart www.waters.com/selectivitychart

Atlantis HILIC Silica

HILIC is a variation of normal-phase chromatography where a polar stationary phase is used with a mobile phase which contains a high concentration of organic (non-polar) solvent and a low concentration of aqueous (polar) solvent. In HILIC, the organic portion of the mobile phase is the weak solvent, the aqueous portion the strong solvent and the compounds elute in the order of increasing hydrophilicity. Due to the high organic, low viscosity mobile phases that are employed, HILIC provides low column backpressure. Additionally, the collected fractions of interest are inherently high in organic, enabling faster solvent evaporation compared to reversed phase.

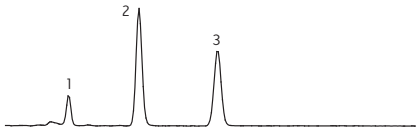
Nucleic Acid Bases Scale-up on Atlantis HILIC Columns

Columns:	Atlantis HILIC, 4.6 x 50 mm, 5 µm (SN: 010536275106 02) Atlantis HILIC OBD Prep, 19 50 mm, 5 µm (SN: 01061631910F 01)
Mobile Phase A:	10 mM NH_4COOH, pH 3 buffer
Mobile Phase B:	Acetonitrile/100 mM NH_4COOH, pH 3 (90/10) v/v
Gradients:	As indicated on chromatograms
Flow Rates:	1.4 mL/min (analytical); 23.9 mL/min (preparative)
Sample Concentration:	25 µg/mL each in ACN/MeOH (75/25) v/v
Injection Volumes:	60 µL (analytical); 1020 µL (preparative)
Weak Wash Solvent:	95/5 ACN/H_2O
Temperature:	Ambient
Detection:	UV @ 280 nm
Instrument:	Waters AutoPurification System with 996 PDA

Compounds:
1. Uracil
2. 5-Fluorocytosine
3. Cytosine

Void is at 0.56 min prep, 0.55 min analytical

Atlantis HILIC, 4.6 x 50 mm, 5 µm

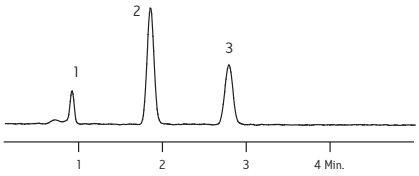


Gradient:

Time (min)	Profile %A	%B
0.0	0	100
5.0	10	90
6.0	10	90
6.1	0	100
10.0	0	100

Injection Volume: 60 µL
Total Loading: 4500 ng

Atlantis HILIC OBD Prep, 19 x 50 mm, 5 µm



Gradient:

Time (min)	Profile %A	%B
0.0	0	100
0.74	0	100
5.74	10	90
6.74	10	90
6.84	0	100
10.74	0	100

Injection Volume: 1020 µL
Total Loading: 76.5 µg

Atlantis Prep Columns

Dimensions	Type	Particle Size	T3	HILIC	dC_{18}
10 x 10 mm	Guard	5 µm	186003695[1]	—	186002300[1]
10 x 50 mm	Column	5 µm	186003691	—	186002298
10 x 100 mm	Column	5 µm	186003692	—	186002299
10 x 150 mm	Column	5 µm	186003693	—	—
10 x 250 mm	Column	5 µm	186003694	—	—
19 x 10 mm	Guard	5 µm	186003699[2]	186003956[2]	186001361[2]
19 x 50 mm	OBD Column	5 µm	186003696	186003957	186001365
19 x 100 mm	OBD Column	5 µm	186003697	186003958	186001367
19 x 150 mm	OBD Column	5 µm	186003698	186003959	186002800
19 x 250 mm	OBD Column	5 µm	186004026	—	186004030
30 x 50 mm	OBD Column	5 µm	186003700	186003960	186001373
30 x 75 mm	OBD Column	5 µm	186003701	—	186002455
30 x 100 mm	OBD Column	5 µm	186003702	186003961	186001375
30 x 150 mm	OBD Column	5 µm	186003703	186003962	186002801
50 x 50 mm	OBD Column	5 µm	186004080	—	—
50 x 100 mm	OBD Column	5 µm	186004081	—	—
50 x 150 mm	OBD Column	5 µm	186004082	—	—
10 x 10 mm	Guard	10 µm	186003706[1]	—	186002452[1]
10 x 150 mm	Column	10 µm	186003704	—	186002453
10 x 250 mm	Column	10 µm	186003705	—	186002454
19 x 10 mm	Guard	10 µm	186003710[2]	—	186001363[2]
19 x 50 mm	OBD Column	10 µm	186003707	—	
19 x 150 mm	OBD Column	10 µm	186003708	—	186001369
19 x 250 mm	OBD Column	10 µm	186003709	—	186001371
30 x 150 mm	OBD Column	10 µm	186003711	—	186002417
30 x 250 mm	OBD Column	10 µm	186003712	—	186002418
50 x 50 mm	OBD Column	10 µm	186004083	—	—
50 x 100 mm	OBD Column	10 µm	186004084	—	—
50 x 150 mm	OBD Column	10 µm	186004085	—	—
50 x 250 mm	OBD Column	10 µm	186004086	—	—

Purification and Isolation Cartridge Holders

Description	Part No.
10 x 10 mm Cartridge Holder	289000779
19 x10 mm Cartridge Holder	186000709
Replacement o-ring 7.8 mm, 2/pkg	700001019
Replacement o-ring 10 mm, 2/pkg	700001436
Replacement o-ring 19 mm, 2/pkg	700001020

[1] Requires 10 x 10 mm Prep Guard Holder 289000779
[2] Requires 19 x 10 mm Prep Guard Holder 186000709

XTerra Prep OBD Columns

Bridging the Performance Gap from Analytical to Prep

XTerra Chemistries

XTerra MS

XTerra MS C_{18} and MS C_8 columns were designed to be compatible with Mass Spectrometry applications and provide sharp peaks, good sensitivity, and large peak capacities. These chemistries exhibit peak shape usually only achievable with columns employing embedded polar group technology (see XTerra RP columns). The tri-functional bonding chemistries of XTerra MS columns deliver the longest lifetimes over the widest pH range (pH 1-12) and provide maximum throughput with excellent resolution and ultra-low bleed.

XTerra RP

XTerra RP18 and RP8 columns combine Hybrid Particle Technology with Shield Technology by incorporating an embedded polar group to deliver the best possible peak shape. These general purpose columns allow fast method development by offering unique selectivity. Like all XTerra columns, the shielded XTerra RP columns exhibit excellent water wettability, even in 100% aqueous mobile phases. The combination of a greatly widened pH range for selectivity choices and the additional selectivity options of two ligands are often the only tools necessary for successful method development.

XTerra Prep Columns

Dimensions	Type	Particle Size	MS C_{18}	MS C_8	RP18	RP8
7.8 x10 mm	Cartridge	5 µm	186001168[6]	186001169[6]	186001170[6]	186001171[6]
7.8 x 10 mm	Cartridge	10 µm	186001172[6]	186001173[6]	186001174[6]	186001175[6]
7.8 x 50 mm	Column	5 µm	186001152	186001153	186001154	186001155
7.8 x 100 mm	Column	5 µm	186001156	186001157	186001158	186001159
7.8 x 150 mm	Column	5 µm	186001475	186001476	186001477	186001478
7.8 x 150 mm	Column	10 µm	186001160	186001161	186001162	186001163
7.8 x 300 mm	Column	10 µm	186001164	186001165	186001166	186001167
10 x 10 mm	Cartridge	5 µm	186001001[7]	186001004[7]	186001006[7]	186001008[7]
10 x 10 mm	Cartridge	10 µm	186001002[7]	186001005[7]	186001007[7]	186001009[7]
10 x 30 mm	Column	5 µm	186001010	186001011	186001012	186001013
10 x 50 mm	Column	5 µm	186001014	186001015	186001016	186001017
10 x 100 mm	Column	5 µm	186001018	186001019	186001020	186001021
10 x 150 mm	Column	5 µm	186001479	186001480	186001481	186001482
10 x 150 mm	Column	10 µm	186001022	186001023	186001024	186001025
10 x 250 mm	Column	10 µm	186001026	186001027	186001028	186001029
10 x 300 mm	Column	10 µm	186001030	186001031	186001032	186001033
19 x 10 mm	Cartridge	5 µm	186001104[8]	186001105[8]	186001106[8]	186001107[8]
19 x 10 mm	Cartridge	10 µm	186001034[8]	186001035[8]	186001036[8]	186001037[8]
19 x 30 mm	OBD Column	5 µm	186002383	186002384	186002385	186002386
19 x 50 mm	OBD Column	5 µm	186001930	186001931	186001932	186001933
19 x 50 mm	OBD Column	10 µm	186002254	—	—	—
19 x 100 mm	OBD Column	5 µm	186001934	186001935	186001936	186001937
19 x 150 mm	OBD Column	5 µm	186002379	186002380	186002381	186002382
19 x 150 mm	OBD Column	10 µm	186002255	186002256	186002257	186002258
19 x 250 mm	OBD Column	10 µm	186002259	186002260	186002261	186002262
19 x 300 mm	OBD Column	10 µm	186002263	186002264	186002265	186002266
30 x 50 mm	OBD Column	5 µm	186001938	186001939	186001940	186001941
30 x 75 mm	OBD Column	5 µm	186002387	186002388	186002389	186002390
30 x 100 mm	OBD Column	5 µm	186001942	186001943	186001944	186001945
30 x 150 mm	OBD Column	10 µm	186002267	186002268	186002269	186002270
30 x 250 mm	OBD Column	10 µm	186002271	186002272	186002273	186002274
30 x 300 mm	OBD Column	10 µm	186002275	186002276	186002277	186002278
50 x 50 mm	OBD Column	5 µm	186002218	186002219	186002220	186002221
50 x 50 mm	OBD Column	10 µm	186002279	186002280	186002281	186002282
50 x 100 mm	OBD Column	5 µm	186002222	186002223	186002224	186002225
50 x 150 mm	OBD Column	10 µm	186002843	186002844	186002845	186002846
50 x 250 mm	OBD Column	10 µm	186002847	186002848	186002849	186002850

[6] Needs 7.8 x 10 mm Cartridge Holder Part No. 186000708
[7] Needs 10 x 10 mm Cartridge Holder Part No. 289000779
[8] Needs 19 x 10 mm Cartridge Holder Part No. 186000709

Symmetry Columns

Symmetry columns provide the highest standard of reproducibility for total confidence in the long term compliance of your HPLC methods. Symmetry columns demonstrate excellent peak symmetry for maximum sensitivity and accurate quantitation. Use SymmetryPrep columns for lead generation and optimization for development of validated assays for stability testing and impurity profiles.

SymmetryShield Columns

SymmetryShield columns with Shield Technology contain a patented chemistry for reversed-phase HPLC that delivers significant changes in selectivity for polar and basic compounds. SymmetryShield columns deliver unique reversed-phase selectivity with significantly improved peak shape and resolution even for the most challenging analytes.

Symmetry300 Columns

Symmetry300, reversed-phase columns are designed specifically for high recoveries of peptides and proteins. Symmetry300 columns are the best solution for validation compliance regarding identity, purity, and stability.

SymmetryPrep Columns

SymmetryPrep 7 µm columns are manufactured for the chemist who needs to purify new chemical entities or isolate impurities or degradants. The new SymmetryPrep 7 µm products are a family of high-capacity, high-efficiency high-performance liquid chromatography (HPLC) columns that have identical selectivity to the Symmetry 3.5 and 5 µm particle size analytical columns. This guarantees direct method scale-up with no further development. SymmetryPrep columns deliver the advantages of unmatched peak shape and resolution using simple volatile mobile phase buffers such as TFA and ammonium acetate. All of these features result in a tremendous time reduction in your research and development programs.

SymmetryPrep Columns for Purification and Isolation

Particle Dimension	Size	C_{18}	C_8
7.8 x 10 mm	5 µm	186000711	186000712
7.8 x 50 mm	5 µm	186000208	186000214
7.8 x 100 mm	5 µm	186000209	186000215
19 x 10 mm	5 µm	186000715[1]	186000716[1]
19 x 50 mm	5 µm	186000210	186000216
19 x 100 mm	5 µm	186000211	186000229
30 x 50 mm	5 µm	186000235	186000237
30 x 100 mm	5 µm	186000236	186000238
7.8 x 10 mm	7 µm	186000713	186000714
7.8 x 150 mm	7 µm	WAT066288	WAT066285
7.8 x 300 mm	7 µm	WAT066235	WAT066225
19 x 10 mm	7 µm	186000717[1]	186000718[1]
19 x 150 mm	7 µm	WAT066240	WAT066228
19 x 300 mm	7 µm	WAT066245	WAT066230

SymmetryShield Columns for Purification and Isolation

Particle Dimension	Size	RP18	RP8
19 x 10 mm	5 µm	186001835[1]	186001841[1]
19 x 50 mm	5 µm	186001836	186001842
19 x 100 mm	5 µm	186001837	186001843
19 x 150 mm	5 µm	186001838	186001844
19 x 150 mm	7 µm	186001839	186001845
19 x 300 mm	7 µm	186001840	186001846

Symmetry300 Columns for Purification and Isolation

Particle Dimension	Size	C_{18}
19 x 10 mm	5 µm	186001847[1]
19 x 50 mm	5 µm	186001848
19 x 100 mm	5 µm	186001849
19 x 150 mm	5 µm	186001850

Symmetry300 is also available as C_4 chemistry and in a 7 µm particle size. Please inquire.

[1] Requires 19 x 10 mm Prep Guard Cartridge Holder 186000709

Purification and Isolation Cartridge Holders

Description	Part No.
7.8 x 10 mm Cartridge Holder	186000708
10 x 10 mm Cartridge Holder	289000779
19 x 10 mm Cartridge Holder	186000709
Replacement o-ring 7.8 mm, 2/pkg	700001019
Replacement o-ring 10 mm, 2/pkg	700001436
Replacement o-ring 19 mm, 2/pkg	700001020

Waters Spherisorb Columns

Waters Spherisorb® columns are the most widely referenced HPLC columns in scientific literature. There are over 2,000 abstracts published on Spherisorb columns, providing a tremendous range of validated methods and applications to assist you in your method development process.

Waters Spherisorb 10 µm Semiprep Columns

Chemistry	10 x 250 mm	20 x 250 mm
S10 ODS2	PSS832585	PSS832595
S10 ODS1	PSS830785	PSS830795
S10 C8	PSS832885	PSS832895
S10 C6	PSS833285	PSS833295
S10 C1	PSS833085	PSS833095
S10 NH2	PSS833685	PSS833695
S10 P	PSS833885	PSS833895
S10 CN	PSS833585	PSS833595
S10 W	PSS830285	PSS830295
Ion Exchange		
S10 SAX	PSS833985	PSS833995
S10 SCX	PSS837685	PSS837695

Prep Nova-Pak HR Columns

Prep Nova-Pak HR 6 µm, ultra high-efficiency packing materials can be used in shorter columns to obtain the same resolution as longer columns with larger particle size packing materials. Shorter columns mean faster separation, lower solvent consumption and more concentrated fractions. Prep Nova-Pak HR has the same selectivity and retention characteristics as the analytical 4 µm Nova-Pak material. The Prep Nova-Pak HR packing materials are ideal for the separation of a wide range of compounds such as organic synthesis intermediates or natural products.

Prep Nova-Pak HR Columns

Description	Particle Size	Pore Size	Dimensions	Part No.
Prep Nova-Pak HR C_{18}	6 µm	60Å	3.9 x 300 mm	WAT038500
			7.8 x 300 mm	WAT025820
			19 x 300 mm	WAT025822
Prep Nova-Pak HR Silica	6 µm	60Å	3.9 x 300 mm	WAT038501
			7.8 x 300 mm	WAT025821
			19 x 300 mm	WAT025823

Bondapak/Porasil Columns

The popular Bondapak C_{18} chemistry and Porasil silica packing materials are available in three different particle sizes, 10, 15-20, and 37-55 µm, providing easy transfer of chromatography methodology from one particle size to another. You can utilize your existing 10 µm Bondapak or Porasil chromatography as a starting point for scale-up or, if you are developing a new purification protocol, investigate which preparative particle size provides the best balance between resolution, throughput and cost.

The preparative Bondapak HC_{18} HA (high-carbon load, high-activity silica) is a highly carbon-loaded packing that provides different selectivity over the standard Bondapak packing materials. The higher carbon load on the silica surface typically results in a higher loading capability. Bondapak HC_{18} HA is available in a 37-55 µm particle size.

The Prep family of packing materials is based on a 55-105 µm Porasil silica and provides a cost effective means for scale-up of preparative processes. The selectivity of Prep silica is identical to 10, 15-20, and 37-55 µm Porasil while Prep C_{18} has a slightly different selectivity than Bondapak packings.

Bondapak/Porasil Columns

Description	Particle Size	Pore Size	Dimensions	Part No.
µBondapak C_{18}	10 µm	125Å	3.9 x 300 mm	WAT027324
			7.8 x 300 mm	WAT084176
			19 x 150 mm	WAT088500
			19 x 300 mm	WAT025828
Bondapak C_{18}	15-20 µm	125Å	3.9 x 300 mm	WAT025875
			7.8 x 300 mm	WAT025832
			19 x 300 mm	WAT025834
µBondapak Phenyl	10 µm	125Å	3.9 x 300 mm	WAT027198
			7.8 x 300 mm	WAT084179
µBondapak CN	10 µm	125Å	7.8 x 300 mm	WAT084177
µBondapak NH_2	10 µm	125Å	3.9 x 300 mm	WAT084040
			7.8 x 300 mm	WAT084178
µPorasil	10 µm	125Å	3.9 x 300 mm	WAT027477
			7.8 x 300 mm	WAT084175
			19 x 150 mm	WAT091648
			19 x 300 mm	WAT025829
Porasil	15-20 µm	125Å	3.9 x 300 mm	WAT025874
			19 x 300 mm	WAT025835

Delta-Pak Packing

Delta-Pak packing materials are ideal for the separation of peptides, proteins, and natural products. The isolation and purification of a peptide is usually a multi-step procedure with the fractions from the first run being re-chromatographed on the same preparative column to obtain pure product. Delta-Pak packing materials are based on a highly stable, bonded, endcapped 5 and 15 µm packing. The 5 µm packing is available in analytical scale dimensions for preliminary preparative chromatographic studies, peptide mapping, and fraction purity assays. The chemistry characteristics of the packing materials are independent of the particle size.

Delta-Pak Columns

Description	Particle Size	Pore Size	Dimensions	Part No.
Delta-Pak C_{18}	15 µm	100Å	3.9 x 300 mm	WAT011797
		300Å	3.9 x 300 mm	WAT011802
		100Å	7.8 x 300 mm	WAT011798
		300Å	7.8 x 300 mm	WAT011803
		100Å	19 x 300 mm	WAT011799
		300Å	19 x 300 mm	WAT011804
		100Å	30 x 300 mm	WAT011800
		300Å	30 x 300 mm	WAT011805
		100Å	50 x 300 mm	WAT011801
Delta-Pak C_4	15 µm	100Å	3.9 x 300 mm	WAT011807
		300Å	3.9 x 300 mm	WAT011812
		100Å	7.8 x 300 mm	WAT011808
		300Å	7.8 x 300 mm	WAT011813
		100Å	19 x 300 mm	WAT011809
		300Å	19 x 300 mm	WAT011814
		100Å	30 x 300 mm	WAT011810
		300Å	30 x 300 mm	WAT011815

Preparative Bulk Material

Waters offers a number of different bulk packing materials for lab-to-process-scale purifications. All our packing materials are manufactured under cGMP (current Good Manufacturing Practices) ensuring a reproducible material for long term reproducibility. Our manufacturing facilities are ISO 9002-certified and are registered with the FDA as a medical device manufacturing facility. Bulk materials are available packaged in quantities of 100 g up to 5 kg. For larger quantity purchases, contact us for pricing and availability.

All bulk packings are carefully quality controlled using a wide array of physical and chemical tests to ensure consistent batch-to-batch reproducibilit

Normal Phase Bulk Packings

Description	Particle Size	Pore Size	Dimensions	Part No.
Porasil Silica	15-20 µm	125Å	100 g	WAT020731
			1 kg	WAT020732
			5 kg	WAT020733
Porasil Silica	37-55 µm	125Å	100 g	WAT020721
			1 kg	WAT020722
			5 kg	WAT020723
Prep Silica	55-105 µm	125Å	100 g	WAT020587
			1 kg	WAT010004
			5 kg	WAT020588

Reversed-Phase Bulk Packings

Description	Particle Size	Pore Size	Dimensions	Part No.
Bondapak C_{18}	15-20 µm	125Å	100 g	WAT020739
			1 kg	WAT020740
			5 kg	WAT020741
Bondapak C_{18}	37-55 µm	125Å	100 g	WAT030632
			1 kg	WAT030633
			5 kg	WAT030634
Bondapak HC_{18} HA	37-55 µm	125Å	100 g	WAT035672
			1 kg	WAT035674
			5 kg	WAT035676
Prep C_{18}	55-105 µm	125Å	100 g	WAT020594
			1 kg	WAT010001
			5 kg	WAT020595

Radial Compression Modules

We carry a complete inventory of accessories and spare parts for the Waters patented Radial Compression Modules for use with the 5 mm and 8 mm i.d. Radial-Pak™ column segments, the 25 mm and 40 mm i.d. PrepLC™ column segments, and the 47 mm and 57 mm i.d. PrepPak® cartridges.

8x10 Holder

8x10 Holder for 8 x 100 mm and
5 x 100 mm Radial-Pak column segments

Description	Part No.
8x10 Holder	WAT082887
8x10 Extension Kit (includes 1 extension tube, union, o-rings)	WAT038846
Cartridge Union	WAT038849

PrepLC 25 mm Module

PrepLC 25 mm Module for
25 x 100 mm column segments

Description	Part No.
PrepLC 25 mm Module	WAT015814
PrepLC 25 mm Extension Kit (includes 1 extension tube, union, o-rings)	WAT022180
Extension tube	WAT019311

PrepLC Assemblies

PrepLC Assembly for
40 x 100 mm and
25 x 100 mm
column segments

Description	Part No.
PrepLC 40 mm Assembly (includes PrepLC Universal Base and PrepLC 40 mm Chamber)	WAT022441
PrepLC Universal Base	WAT027577
PrepLC 40 mm Chamber (includes o-rings, spacer and union)	WAT027578
PrepLC 40 mm Extension Kit (includes extension tube, union, o-rings)	WAT022365
PrepLC 25 mm Chamber (includes o-rings, spacer and union)	WAT033994
PrepLC 25 mm Extension Kit (includes 1 extension tube, union, o-rings)	WAT022180
PrepLC Scale-up Kit with capability for 40 mm or 25 x 300 length includes: 1 - PrepLC Universal Base 2 - PrepLC Chambers (one each of 40 mm and 25 mm) 2 - PrepLC 25 mm Extension Kits 2 - PrepLC 40 mm Extension Kits	WAT022440

*** Note: Up to two extension tubes can be attached for a combined cartridge length of 300 mm**

Nova-Pak and Prep Nova-Pak Radial Compression Column Segments and PrepPak Cartridges

Column	Particle Size	Pore Size	Dimensions	Part No.
Nova-Pak Radial-Pak Column Segments				
Nova-Pak C_{18}	4 µm	60Å	5 x 100 mm	WAT080100
			8 x 100 mm	WAT086342
Nova-Pak C_8	4 µm	60Å	5 x 100 mm	WAT035890
			8 x 100 mm	WAT035884
Nova-Pak Phenyl	4 µm	60Å	5 x 100 mm	WAT010657
			8 x 100 mm	WAT010658
Nova-Pak CN HP	4 µm	60Å	5 x 100 mm	WAT010224
			8 x 100 mm	WAT010223
Nova-Pak Silica	4 µm	60Å	5 x 100 mm	WAT010986
			8 x 100 mm	WAT010987
Prep Nova-Pak HR Radial-Pak Column Segments				
Prep Nova-Pak HR C_{18}	6 µm	60Å	8 x 100 mm	WAT025843
Prep Nova-Pak HR Silica	6 µm	60Å	8 x 100 mm	WAT025844
Prep Nova-Pak HR PrepLC 25 mm Column Segments				
Prep Nova-Pak HR C_{18}	6 µm	60Å	25 x 100 mm	WAT038510
Prep Nova-Pak HR Silica	6 µm	60Å	25 x 100 mm	WAT038511
Prep Nova-Pak HR 25x10 Guard-Pak Inserts (2/pkg)				
Prep Nova-Pak HR C_{18}	6 µm	60Å	25 x 10 mm	WAT038528
Prep Nova-Pak HR Silica	6 µm	60Å	25 x 10 mm	WAT038530
Prep Nova-Pak HR PrepLC 40 mm Column Segments				
Prep Nova-Pak HR C_{18}	6 µm	60Å	40 x 100 mm	WAT037704
Prep Nova-Pak HR Silica	6 µm	60Å	40 x 100 mm	WAT037708
Prep Nova-Pak HR 40x10 Guard-Pak Inserts (2/pkg)				
Prep Nova-Pak HR C_{18}	6 µm	60Å	40 x 10 mm	WAT037854
Prep Nova-Pak HR Silica	6 µm	60Å	40 x 10 mm	WAT037857

Resolve™ Radial Compression Column Segments and PrepPak Cartridges*

Column	Particle Size	Pore Size	Dimensions	Part No.
Resolve C_{18}	5 µm	90Å	8 x 100 mm	WAT084624
Resolve C_{18}	10 µm	90Å	5 x 100 mm	WAT084620
			8 x 100 mm	WAT084720
Resolve C_8	10 µm	90Å	5 x 100 mm	WAT085672
			8 x 100 mm	WAT085670
Resolve CN	10 µm	90Å	5 x 100 mm	WAT084626
			8 x 100 mm	WAT084636
Resolve Silica	5 µm	90Å	8 x 100 mm	WAT084634
Resolve Silica	10 µm	90Å	5 x 100 mm	WAT084630
			8 x 100 mm	WAT084730

*** All column segments and cartridges require the appropriate holder/module, see page 168**

Bondapak/Porasil Radial Compression Column Segments and PrepPak Cartridges*

Column	Particle Size	Pore Size	Dimensions	Part No.
Bondapak/Porasil Radial-Pak Column Segments				
µBondapak C_{18}	10 µm	125Å	8 x 100 mm	WAT085721
µBondapak NH_2	10 µm	125Å	8 x 100 mm	WAT085724
µBondapak Phenyl	10 µm	125Å	8 x 100 mm	WAT085722
µPorasil	10 µm	125Å	8 x 100 mm	WAT085720
Bondapak C_{18}	15-20 µm	125Å	8 x 100 mm	WAT025841
Bondapak HC_{18} HA	37-55 µm	125Å	8 x 100 mm	WAT036561
Bondapak/Porasil PrepLC 25 mm Column Segments				
µBondapak C_{18}	10 µm	125Å	25 x 100 mm	WAT038505
Bondapak C_{18}	15-20 µm	125Å	25 x 100 mm	WAT038503
		300Å	25 x 100 mm	WAT038575
Bondapak HC_{18} HA	37-55 µm	125Å	25 x 100 mm	WAT056970
µPorasil	10 µm	125Å	25 x 100 mm	WAT038504
Porasil	15-20 µm	125Å	25 x 100 mm	WAT038502
Bondapak/Porasil Prep 25x10 Guard-Pak Inserts (2/pkg)				
µBondapak C_{18}	10 µm	125Å	25 x 10 mm	WAT038518
Bondapak C_{18}	15-20 µm	125Å	25 x 10 mm	WAT038514
µPorasil	10 µm	125Å	25 x 10 mm	WAT038516
Porasil	15-20 µm	125Å	25 x 10 mm	WAT038512
Bondapak/Porasil PrepLC 40 mm Column Segments				
µBondapak C_{18}	10 µm	125Å	40 x 100 mm	WAT037684
Bondapak C_{18}	15-20 µm	125Å	40 x 100 mm	WAT037676
		300Å	40 x 100 mm	WAT037712
µPorasil	10 µm	125Å	40 x 100 mm	WAT037680
Porasil	15-20 µm	125Å	40 x 100 mm	WAT037672
Bondapak/Porasil Prep 40x10 Guard-Pak Inserts (2/pkg)				
µBondapak C_{18}	10 µm	125Å	40 x 10 mm	WAT037839
Bondapak C_{18}	15-20 µm	125Å	40 x 10 mm	WAT037833
µPorasil	10 µm	125Å	40 x 10 mm	WAT037836
Porasil	15-20 µm	125Å	40 x 10 mm	WAT037830

*** All column segments and cartridges require the appropriate holder/module, see page 168**

Delta-Pak Radial Compression Column Segments and PrepPak Cartridges*

Column	Particle Size	Pore Size	Dimensions	Part No.
Delta-Pak Radial-Pak Column Segments				
Delta-Pak C_{18}	15 µm	100Å	8 x 100 mm	WAT025846
		300Å	8 x 100 mm	WAT025845
Delta-Pak C_4	15 µm	100Å	8 x 100 mm	WAT025848
		300Å	8 x 100 mm	WAT025847
Delta-Pak PrepLC 25 mm Column Segments				
Delta-Pak C_{18}	15 µm	100Å	25 x 100 mm	WAT038506
		300Å	25 x 100 mm	WAT038507
Delta-Pak C_4	15 µm	100Å	25 x 100 mm	WAT038508
		300Å	25 x 100 mm	WAT038509
Delta-Pak Prep 25x10 Guard-Pak Inserts (2/pkg)				
Delta-Pak C_{18}	15 µm	100Å	25 x 10 mm	WAT038520
		300Å	25 x 10 mm	WAT038522
Delta-Pak C_4	15 µm	100Å	25 x 10 mm	WAT038524
		300Å	25 x 10 mm	WAT038526
Delta-Pak PrepLC 40 mm Column Segments				
Delta-Pak C_{18}	15 µm	100Å	40 x 100 mm	WAT037688
		300Å	40 x 100 mm	WAT037692
Delta-Pak C_4	15 µm	100Å	40 x 100 mm	WAT037696
		300Å	40 x 100 mm	WAT037700
Delta-Pak Prep 40x10 Guard-Pak Inserts (2/pkg)				
Delta-Pak C_{18}	15 µm	100Å	40 x 10 mm	WAT037842
		300Å	40 x 10 mm	WAT037845
Delta-Pak C_4	15 µm	300Å	40 x 10 mm	WAT037851

PrepPak Cartridges

Requires PrepPak 1000 Module (Part No. WAT089592)

Column	Particle Size	Pore Size	Dimensions	Part No.
Bondapak C_{18}	15-20 µm	125Å	47 x 300 mm	WAT091784
Bondapak C_{18}	15-20 µm	300Å	47 x 300 mm	WAT038571
Bondapak C_{18}	37-55 µm	125Å	47 x 300 mm	WAT025873
Bondapak HC_{18} HA	37-55 µm	125Å	47 x 300 mm	WAT038570
Bondapak NH_2	55-105 µm	125Å	47 x 300 mm	WAT091631
Delta-Pak C_{18}	15 µm	100Å	47 x 300 mm	WAT015401
		300Å	47 x 300 mm	WAT010988
Delta-Pak C_4	15 µm	100Å	47 x 300 mm	WAT011633
		300Å	47 x 300 mm	WAT011669
Prep C_{18}	55-105 µm	125Å	47 x 300 mm	WAT025876
Porasil Silica (single) (10/case)	15-20 µm	125Å	47 x 300 mm	WAT025852 WAT025878
Porasil Silica (single) (10/case)	37-55 µm	125Å	47 x 300 mm	WAT025853 WAT025877
Prep Silica (single) (10/case)	55-105 µm	125Å	57 x 300 mm	WAT050041 WAT050040

PrepPak 1000 Module for 47 x 300 mm and 57 x 300 mm PrepPak Cartridges	WAT089592

Accessories and Spare Parts for Waters Radial Compression Cartridge Holders and Modules

We carry a complete inventory of accessories and spare parts for Waters patented Radial Compression Modules for use with the 5 mm and 8 mm i.d. x 100 mm long Radial-Pak column segments, 25 mm and 40 mm i.d. x 100 mm long PrepLC column segments, and 47 mm or 57 mm i.d. x 300 mm long PrepPak cartridges.

8x10 Cartridge Holder, Parts and Accessories

Description	Part No.
8x10 Holder	WAT082887
8x10 Extension Kit (Includes 1 extension tube, union, o-rings)	WAT038846
Column segment union	WAT038849
O-ring for extension tube	WAT038851
Connector assembly (non-metallic)	WAT015898
Connector tubing assembly (non-metallic)	WAT088919
Connector assembly (stainless steel)	WAT082892
Washer for connectors, 10/pk	WAT005147
Pressure relief plug	WAT088027
Check valve	WAT082888
O-ring (large) for connector, 10/pk	WAT005130
O-ring (small) for connector (normal phase), 4/pk	WAT015797
O-ring (small) for connector (reversed-phase), 10/pk	WAT005129
O-ring for filling port, 10/pk	WAT005129
O-ring for pressure piston	WAT088494
Gripper ring replacement kit (Includes 10 gripper rings, 20 washers, 10 ferrules and tool)	WAT021908

PrepLC 25 mm Module, Parts and Accessories

Description	Part No.
PrepLC 25 mm Module	WAT015814
PrepLC 25 mm Extension Kit (Includes 1 extension tube, union, o-rings)	WAT022180
Extension tube	WAT019311
O-ring for extension tube	WAT015831
O-ring (large) for connector	WAT015833
O-ring (small) for connector (normal phase)	WAT015848
O-ring (small) for connector (reversed-phase)	WAT015834
O-ring for filling port, 10/pk	WAT005129
O-ring for pressure piston	WAT015854
Union coupling assembly	WAT015860
Union, 1/8 to 1/16 inch tubing, 5/pk	WAT005137

PrepLC Spare Parts

Description	Part No.
PrepLC Universal Base Spare Parts	
O-ring removal tool	WAT082853
O-ring for pressure piston	WAT022281
O-ring for filling port	WAT005129
Filling port plug	WAT027509
Ferrules and compression fittings (stainless steel) 5/pkg	WAT025604
PrepLC 40 mm Chamber Spare Parts	
Column segment union	WAT033996
Cartridge spacer	WAT033997
O-ring, base plate (small)	WAT022453
O-ring, base plate (large)	WAT022454
O-ring, chamber top	WAT022280
O-ring (normal phase) cartridge top and bottom, spacers and unions	WAT027519
O-ring (normal phase) chamber bottom	WAT022299
O-ring (normal phase) inner connector, top and bottom	WAT022297
O-ring (reversed-phase) cartridge top and bottom, spacers and unions	WAT027518
O-ring (reversed-phase) chamber bottom	WAT022283
O-ring (reversed-phase) inner connector, top and bottom	WAT015835
O-ring, extension tube	WAT022454
Tubing Fluid Path Kit* (PEEK) (Includes inner connectors, tubing, ferrules and compression screws)	WAT022398
PrepLC 25 mm Chamber Spare Parts	
Column segment union	WAT015860
Segment spacer	WAT015859
O-ring, base plate (small)	WAT022276
O-ring, base plate (large)	WAT015831
O-ring, chamber top	WAT015833
O-ring, (normal phase) cartridge top and bottom, spacers and unions	WAT015848
O-ring (normal phase) chamber bottom	WAT022298
O-ring (normal phase) inner connector, top and bottom	WAT022297
O-ring (reversed-phase) cartridge, top and bottom, spacers and union	WAT015834
O-ring (reversed-phase) chamber bottom	WAT022282
O-ring (reversed-phase) inner connector, top and bottom	WAT015835
Tubing Fluid Path Kit* (PEEK) (Includes inner connectors, tubing, ferrules and compression screws)	WAT022400

* For applications where a metal-free flow path is needed.

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Size Exclusion Chromatography—GPC & GFC

Introduction

The Gel Permeation Chromatography (GPC) technique, pioneered by J.C. Moore of the Dow Chemical Company[1] in the early 1960s, is a mode of HPLC for separating molecules based upon their effective size in solution.

Originally patented and licensed to Waters as a method for characterizing polymers, GPC is also useful for separating small molecules from interfering matrices such as those found in foods, pharmaceutical preparations, and natural products. In addition, GPC is often used as the first step in the sequential analysis of complex, unknown, organic mixtures. In these analyses, the unknown mixture is separated by size in the GPC mode, purified fractions are collected, and the fractions are subsequently separated based on chemical differences by normal, or reversed-phase chromatography.

Small Molecules in Organic or Aqueous Solution

Small molecules (less than 2,000 MW) can generally be separated if their size in solution differs by approximately 10%. GPC can also easily detect the presence of high molecular weight components in a sample matrix—indicating the need for sample cleanup prior to analysis of small molecules by normal, or reversed-phase chromatography.

GPC of Organic Soluble Polymers

GPC is a practical, easy, and convenient tool for determining the complete molecular weight distribution of a polymer. Any oligomers, monomers and additives in a complex polymeric solution can also be separated as long as there exist significant size differences among these components.

GPC is an officially recognized method in the plastics industry and has been adopted by the American Society for Testing and Materials (ASTM, Committee D-20) for determining the molecular weight distribution of polymers.[2]

Gel Filtration of Water Soluble Polymers and Proteins

Water soluble polymers and proteins can be routinely separated by size in solution. Size separations in aqueous solutions, often called gel filtration, are commonly used as the first step in the analysis of a complex protein mixture and as an important tool to characterize the molecular weights of water soluble polymers such as polyacrylamide.

Selecting a Column

For over 40 years, Waters has been the market leader in GPC analysis, providing the highest quality GPC products and expert applications support. To solve unique separation problems across a broad spectrum of applications, we manufacture a wide range of GPC columns and accessories. Our series of Ultrastyragel™ and µStyragel™ columns have been the standard in GPC columns for the separation of organic-soluble samples. To solve your specific problems in aqueous separations, you can select from our Ultrahydrogel columns.

Calibration Standards

Waters offers calibration standards for organic and aqueous GPC/SEC. Standards are available as individual molecular weights, or come in kits containing a range of MW standards.

Waters Styragel Columns for Polymer Characterization

Designed specifically for polymer characterization, the Styragel® columns are grouped into the HR series for low-to-mid molecular weight samples, the HT series for high-temperature applications, and the HMW series for ultra-high molecular weight samples. Specially controlled styrene divinylbenzene formulations provide reproducible performance in your GPC applications.

[1] J.C. Moore, J. Polymer Science, A2, 835 (1964)

[2] ASTM D3536-76, D3593-80 and D3016-78

Waters Styragel HR Columns

The Styragel HR (high resolution) series of columns were specifically developed for the analysis of low-to-mid molecular weight samples. The columns are packed with rigid 5 µm styrene divinylbenzene particles and deliver the maximum resolution and efficiency required for low molecular weight analysis.

Waters Styragel HT Columns

The Styragel HT (high temperature) series of columns were specifically developed for use in the mid-to-high molecular weight range. The columns are packed with rigid 10 µm styrene divinylbenzene particles and can be used at ambient or high temperature while still maintaining excellent resolution. The narrow particle size distribution results in a more stable packed bed structure making Styragel HT columns extremely durable.

Waters Styragel HMW Columns

The Styragel HMW (high molecular weight) series of columns were specifically developed for the analysis of shear sensitive, ultra-high molecular weight polymers. Styragel HMW columns are packed with rigid 20 micron styrene divinylbenzene particles and fitted with specially designed high porosity frits which minimize polymer shear effects. The columns may be used at either ambient or elevated temperature and exhibit excellent column lifetime.

Your choice of conventional 7.8 mm i.d. or solvent-efficient 4.6 mm i.d. columns In addition to the conventional 7.8 mm columns, the three Styragel series include 4.6 mm i.d. columns in both the single-pore and mixed-bed columns. These Styragel narrow-bore columns can cut your solvent consumption and disposal costs by as much as two thirds. When used with low-dispersion GPC systems, our 4.6 mm columns match the high-performance of our 7.8 mm columns.

We also carry a full range of high resolution aqueous GPC columns, preparative GPC columns and calibration standards.

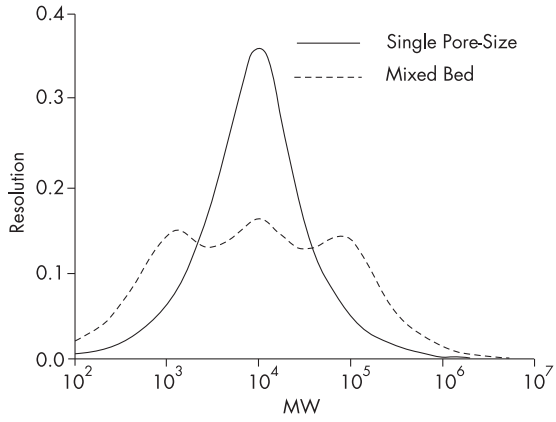
Styragel Guard Column

The Styragel 4.6 mm i.d. x 30 mm guard column is designed to increase the lifetime of your Styragel analytical column. The guard column can be used in series with any Waters conventional organic GPC column.

Column Bank Optimization

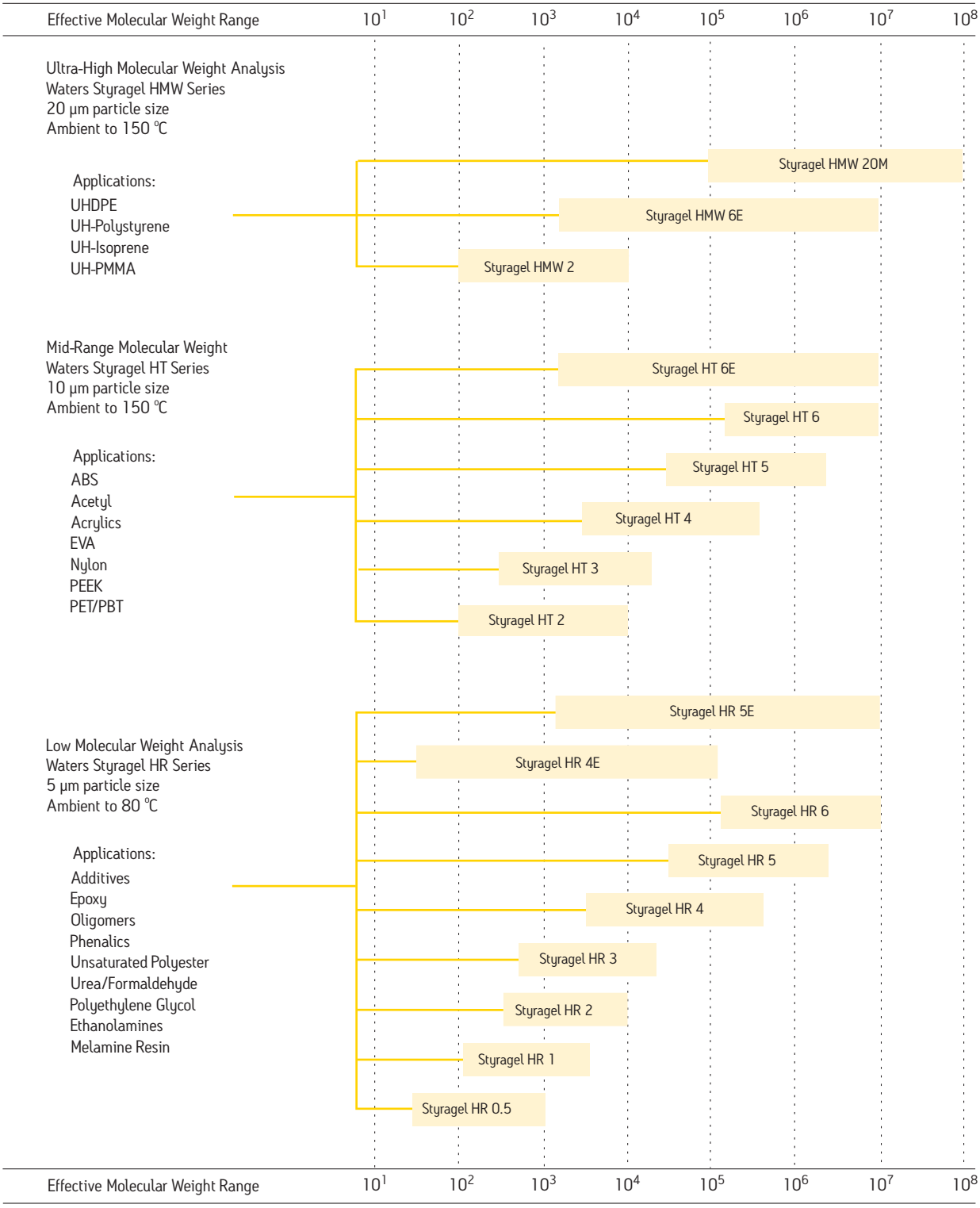
Using the proper column is essential to optimum performance. The rule for selecting the best column(s) for an analysis is straightforward—provide separation only for the molecules which you wish to separate. Never specify a column with a higher exclusion limit than the exclusion limit required by the largest molecules you wish to separate. When the measurement of broad distributions is desired, mixed bed or extended range columns are appropriate, thereby resulting in separation power that is constant at all molecular weight sizes.

Waters Styragel column offering is comprised of mixed-bed and narrow molecular weight range columns. The mixed -bed columns, designated "E" for extended range, are ideal for use as scouting columns when the molecular weight range of your sample is unknown or for the measurement of samples with broad distributions. The narrow molecular weight range columns deliver greater pore volume and resolution in a more concentrated molecular weight range and are a much more powerful tool for obtaining more precise molecular weight information.

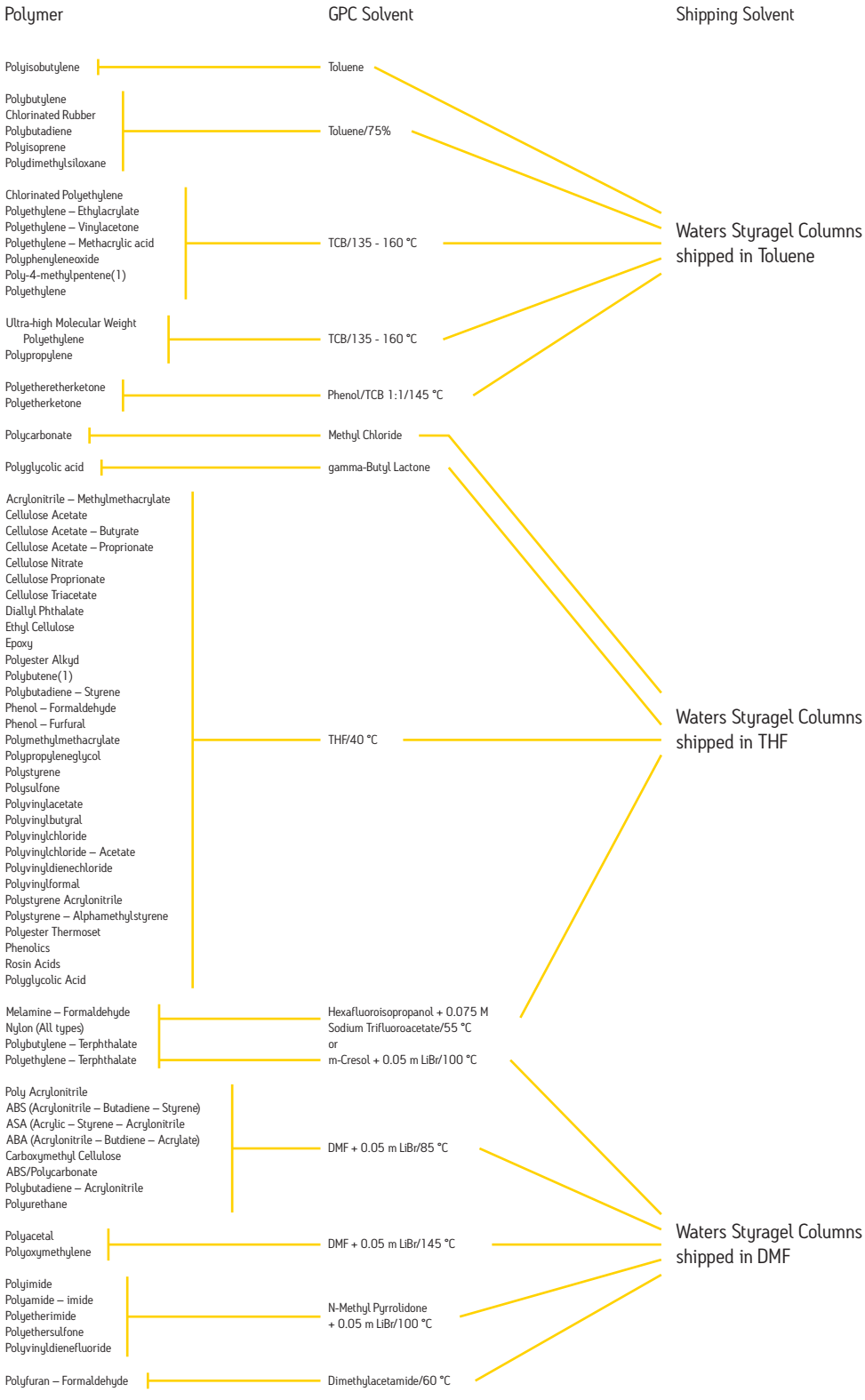


Individual pore-size columns deliver up to 2.5X the resolution of mixed-bed columns.

GPC Column Selection Guide



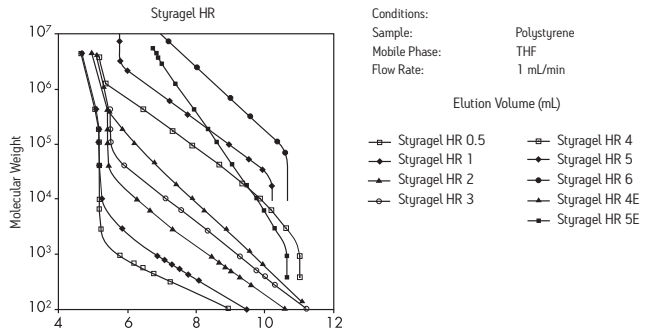
GPC Solvent Selection Guide



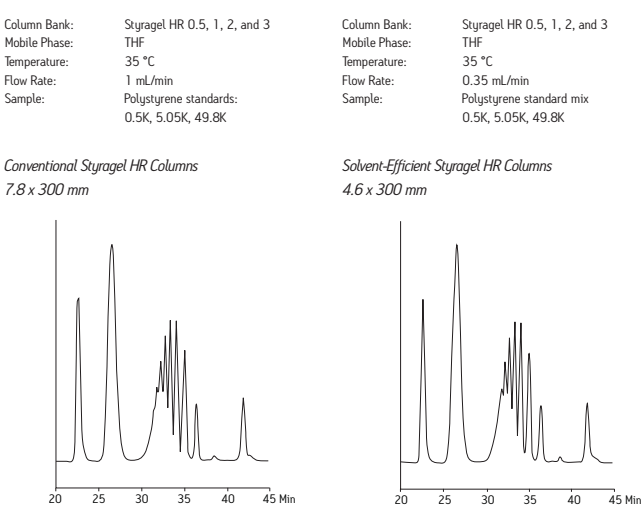
Styragel HR High-Resolution Columns

Designed particularly for low molecular weight samples, the Waters Styragel HR (high resolution) columns are ideal for the analysis of oligomers, epoxies, and polymer additives where high resolution is critical. Packed with rigid 5 µm particles, these columns deliver unrivaled resolution and efficiency in the low-to-mid molecular weight region.

Calibration Curves for the Waters Styragel HR Series of High-Resolution Columns



Styragel HR High Resolution Columns for Unrivaled Resolution of Low Molecular Weight Samples



The 4.6 x 300 mm solvent-efficient Styragel columns deliver the same high resolution as our conventional 7.8 x 300 mm Styragel columns, with the added advantage of reducing solvent consumption by two-thirds.

Styragel HR Columns (7.8 mm i.d. x 300 mm)

Column	Effective Molecular Weight Range	Part No. THF	Part No. DMF	Part No. Toluene
Styragel HR 0.5	0-1,000	WAT044231	WAT044232	WAT044230
Styragel HR 1	100-5,000	WAT044234	WAT044235	WAT044233
Styragel HR 2	500-20,000	WAT044237	WAT044238	WAT044236
Styragel HR 3	500-30,000	WAT044222	WAT044223	WAT044221
Styragel HR 4	5,000-600,000	WAT044225	WAT044226	WAT044224
Styragel HR 4E	50-100,000	WAT044240	WAT044241	WAT044239
Styragel HR 5	50,000-4 x 10^6	WAT054460	WAT054466	WAT054464
Styragel HR 5E	2,000-4 x 10^6	WAT044228	WAT044229	WAT044227
Styragel HR 6	200,000-1 x 10^7	WAT054468	WAT054474	WAT054470
Styragel Guard Column 4.6 x 30 mm		WAT054405	WAT054415	WAT054410

Styragel HR Columns (4.6 mm i.d. x 300 mm)

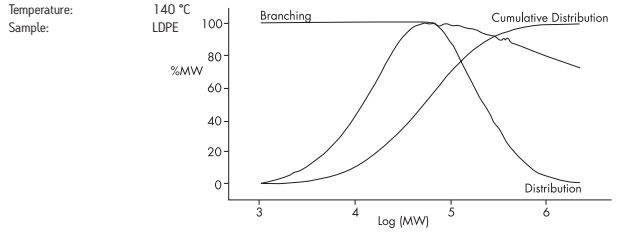
The same high performance as our conventional Styragel HR columns* with the added advantage of reducing your solvent consumption by two-thirds.

Column	Effective Molecular Weight Range	Part No. THF	Part No. DMF	Part No. Toluene
Styragel HR 0.5	0-1,000	WAT045835	WAT045840	WAT045830
Styragel HR 1	100-5,000	WAT045850	WAT045855	WAT045845
Styragel HR 2	500-20,000	WAT045865	WAT045870	WAT045860
Styragel HR 3	500-30,000	WAT045880	WAT045885	WAT045875
Styragel HR 4	5,000-600,000	WAT045895	WAT045900	WAT045890
Styragel HR 4E	50-100,000	WAT045805	WAT045810	WAT045800
Styragel HR 5E	2,000-4 x 10^6	WAT045820	WAT045825	WAT045815

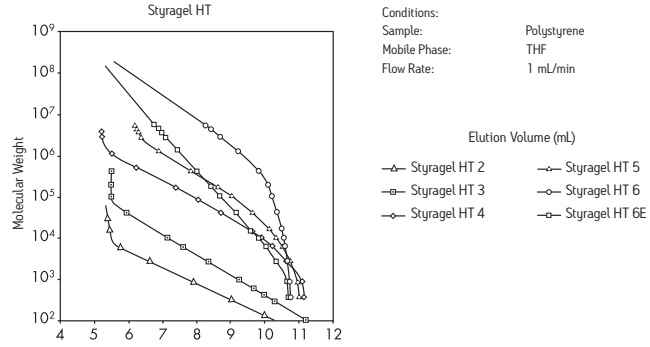
Styragel HT High-Temperature, High-Stability Columns

The Waters Styragel HT (high temperature) columns can be used with aggressive solvents at high temperatures without sacrificing resolution or column lifetime. Packed with rigid 10 µm particles, they have a typical plate count greater than 10,000 plates per column, these columns are extremely durable due to a narrow particle size distribution that results in a very stable column bed. Suitable for both ambient and high-temperature analysis, the Styragel HT columns offer excellent resolution of polymers in the mid-to-high molecular weight range.

Styragel HT Columns Deliver Superior Performance — Even at High Temperatures



Calibration Curves for the Waters Styragel HT Series of High-Temperature Columns



Styragel HT Columns (7.8 mm i.d. x 300 mm)

Column	Effective Molecular Weight Range	Part No. THF	Part No. DMF	Part No. Toluene
Styragel HT 2	100-10,000	WAT054475	WAT054480	WAT054476
Styragel HT 3	500-30,000	WAT044207	WAT044208	WAT044206
Styragel HT 4	5,000-600,000	WAT044210	WAT044211	WAT044209
Styragel HT 5	50,000-4 x 10^6	WAT044213	WAT044214	WAT044212
Styragel HT 6	200,000-1 x 10^7	WAT044216	WAT044217	WAT044215
Styragel HT 6E	5,000 - 1 x 10^7	WAT044219	WAT044220	WAT044218
Styragel Guard Column 4.6 x 30 mm		WAT054405	WAT054415	WAT054410

Styragel HT Columns (4.6 mm i.d. x 300 mm)

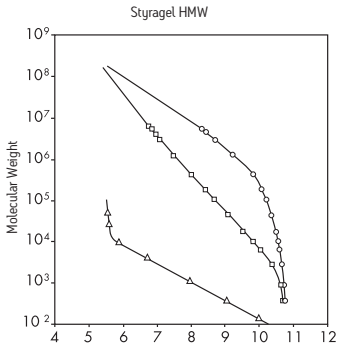
The same high performance as our conventional Styragel HT columns with the added advantage of reducing your solvent consumption by two-thirds.

Column	Effective Molecular Weight Range	Part No. THF	Part No. DMF	Part No. Toluene
Styragel HT 3	500-30,000	WAT045920	WAT045925	WAT045915
Styragel HT 4	5,000-600,000	WAT045935	WAT045940	WAT045930
Styragel HT 5	50,000-4 x 10^6	WAT045950	WAT045955	WAT045945
Styragel HT 6	200,000-1 x 10^7	WAT045965	WAT045970	WAT045960
Styragel HT 6E	5,000-1 x 10^7	WAT045980	WAT045985	WAT045975

Styragel HMW High Molecular Weight Columns

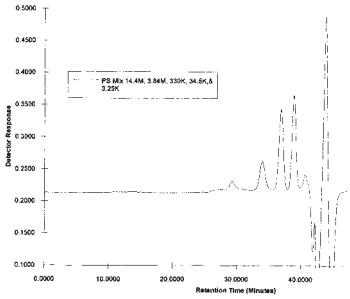
The Styragel HMW (high molecular weight) columns were specifically designed for analysis of ultra-high molecular weight polymers susceptible to shearing. Combining high-porosity 10 µm frits and 20 µm particles, the Styragel HMW columns minimize polymer shear effects. These state-of-the-art columns can be used at either ambient or elevated temperatures and exhibit excellent column lifetime

Calibration Curves for the Waters Styragel HMW Series of High Molecular Weight Columns

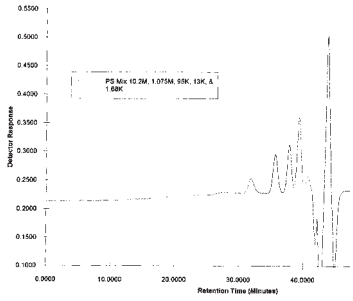


Styragel HMW Columns are Optimized for Analysis of Shear-sensitive, Ultra-high Molecular Weight Polymers

Column Bank: 2 Styragel HMW 7 and 2 Styragel HMW 6E
Column Dimensions: 7.8 x 300 mm
Polystyrene Stds: 14.4M, 3.84M, 330K, 34.5K, 3.25K
Flow Rate: 1 mL/min
Temperature: 145 °C
Solvent: TCB



Column Bank: 2 Styragel HMW 7 and 2 Styragel HMW 6E
Column Dimensions: 7.8 x 300 mm
Polystyrene Stds: 10.2M, 1.075M, 95K, 13K, 1.68K
Flow Rate: 1 mL/min
Temperature: 145 °C
Solvent: TCB



Styragel HMW Columns (7.8 mm i.d. x 300 mm)

Column	Effective Molecular Weight Range	Part No. THF	Part No. DMF	Part No. Toluene
Styragel HMW 2	100-10,000	WAT054488	WAT054494	WAT054490
Styragel HMW 7	500,000-1 x 10^8	WAT044201	WAT044202	WAT044200
Styragel HMW 6E	5,000-1 x 10^7	WAT044204	WAT044205	WAT044203
Styragel Guard Column 4.6 x 30 mm		WAT054405	WAT054415	WAT054410

Styragel HMW Columns (4.6 mm i.d. x 300 mm)

The same high performance as our conventional Styragel HMW columns with the added advantage of reducing your solvent consumption by two-thirds.

Column	Effective Molecular Weight Range	Part No. THF	Part No. DMF	Part No. Toluene
Styragel HMW 7	500,000-1 x 10^8	WAT046805	WAT046810	WAT046800
Styragel HMW 6E	5,000-1 x 10^7	WAT046820	WAT046825	WAT046815

**System dead volume must be minimized for maximum column performance.*

HSPgel Columns for High Speed GPC Analysis

Waters HSPgel™ Column offering for high-speed GPC analysis, provides for accurate and precise molecular weight determination, increased sample throughput, and greatly reduced solvent consumption and disposal. Waters offers four series of high speed GPC columns.

- HSPgel HR series is used for high resolution, room temperature GPC
- HSPgel RT series for routine room temperature GPC
- HSPgel HT series for high temperature GPC
- HSPgel AQ series for aqueous SEC

The column dimension for all columns is 6.0 x 150 mm.

The HSPgel HR series is designed for high resolution, room temperature, organic polymer GPC. These columns come packed in THF and can be converted once to toluene, methylene chloride, or chloroform.

The HSPgel RT series are designed for room temperature, routine work of organic polymer GPC. These come packed in THF and can be converted multiple times from THF to toluene, chloroform, methylene chloride, DMF, DMSO etc.

The HSPgel HT series are designed for room temperature to high temperature (180 °C) organic GPC. The columns come shipped in either THF or ODCB. The ODCB packed column should be used for direct conversion to TCB. These columns can withstand multiple solvent switches.

The HSPgel AQ series are designed for room temperature analysis of water soluble polymers.

Name	Solvent	Particle Size	MW Range	Part No.
Ultra-High Resolution GPC				
HSPgel HR 1.0	THF	3 µm	100-1,000	186001741
HSPgel HR 2.0	THF	3 µm	500-10,000	186001742
HSPgel HR 2.5	THF	3 µm	1,000-20,000	186001743
HSPgel HR 3.0	THF	3 µm	2,000-60,000	186001744
HSPgel HR 4.0	THF	3 µm	10,000-400,000	186001745
HSPgel HR MB-L	THF	3 µm	500-700,000	186001746
HSPgel HR MB-M	THF	3,5 µm	1,000-4,000,000	186001747
HSPgel HR MB-H	THF	10 µm	5,000->10,000,000	186001748
Room-Temperature GPC				
HSPgel RT 1.0	THF	3 µm	100-1,000	186001749
HSPgel RT 2.0	THF	3 µm	500-10,000	186001750
HSPgel RT 2.5	THF	3 µm	1,000-20,000	186001751
HSPgel RT 3.0	THF	3 µm	2,000-60,000	186001752
HSPgel RT 4.0	THF	3 µm	10,000-400,000	186001753
HSPgel RT 5.0	THF	3 µm	25,000-4,000,000	186001754
HSPgel RT 6.0	THF	5 µm	50,000-10,000,000	186001755
HSPgel RT 7.0	THF	5 µm	100,000->15,000,000	186001756
HSPgel RT MB-L	THF	3 µm	100-10,000	186001757
HSPgel RT MB-L/M	THF	3 µm	500-400,000	186001758
HSPgel RT MB-M	THF	3 µm	1,000-4,000,000	186001759
HSPgel RT MB-H	THF	3,5 µm	5,000->10,000,000	186001760
Aqueous GPC				
HSPgel AQ 2.5	Water	4 µm	500-2,000	186001785
HSPgel AQ 3.0	Water	4 µm	1,000-60,000	186001786
HSPgel AQ 4.0	Water	6 µm	10,000-400,000	186001787
HSPgel AQ 5.0	Water	7 µm	50,000-4,000,000	186001788
HSPgel AQ 6.0	Water	9 µm	100,000->10,000,000	186001789
HSPgel AQ MB-H	Water	9 µm	500-10,000,000	186001790

Name	Solvent	Particle Size	MW Range	Part No.
High-Temperature GPC				
HSPgel HT 1.0	THF	5 µm	100-1,000	186001761
HSPgel HT 2.0	THF	5 µm	500-10,000	186001762
HSPgel HT 2.5	THF	5 µm	1,000-20,000	186001763
HSPgel HT 3.0	THF	5 µm	2,000-60,000	186001764
HSPgel HT 4.0	THF	5 µm	10,000-400,000	186001765
HSPgel HT 5.0	THF	5 µm	25,000-4,000,000	186001766
HSPgel HT 6.0	THF	5 µm	50,000-10,000,000	186001767
HSPgel HT 7.0	THF	5 µm	100,000->15,000,000	186001768
HSPgel HT MB-L	THF	5 µm	100-1,000	186001769
HSPgel HT MB-L/M	THF	5 µm	500-400,000	186001770
HSPgel HT MB-M	THF	5 µm	1,000-4,000,000	186001771
HSPgel HT MB-H	THF	5 µm	5,000->10,000,000	186001772
HSPgel HT 1.0	ODCB	5 µm	100-1,000	186001773
HSPgel HT 2.0	ODCB	5 µm	500-10,000	186001774
HSPgel HT 2.5	ODCB	5 µm	1,000-20,000	186001775
HSPgel HT 3.0	ODCB	5 µm	2,000-60,000	186001776
HSPgel HT 4.0	ODCB	5 µm	10,000-400,000	186001777
HSPgel HT 5.0	ODCB	5 µm	25,000-4,000,000	186001778
HSPgel HT 6.0	ODCB	5 µm	50,000-10,000,000	186001779
HSPgel HT 7.0	ODCB	5 µm	100,000->15,000,000	186001780
HSPgel HT MB-L	ODCB	5 µm	100-1,000	186001781
HSPgel HT MB-L/M	ODCB	5 µm	500-400,000	186001782
HSPgel HT MB-M	ODCB	5 µm	1,000-4,000,000	186001783
HSPgel HT MB-H	ODCB	5 µm	5,000->10,000,000	186001784

All columns are 6.0 x 150 mm

* MW ranges for HR and RT are based on polystyrene chain lengths, and based on polyethylene oxide chain lengths for the AQ series.

** Exclusion limits for AQ series extrapolated from highest MW PEO standard, (~900,000).

HR — High Resolution
RT — Room Temperature
AQ — Aqueous
MB — Mixed Bed
L — Low MW Range
M — Medium MW Range
L/M — Low/Medium MW Range
H — High MW Range

Calibration curves for HSPgel are available online at www.waters.com

Shodex GPC Columns

High efficiency columns for GPC analysis. Featuring a very reproducible styrene divinylbenzyne technology, Shodex® GPC columns have more than twenty years of history and have been used by customers all over the world.

K-800 Series (8 x 300 mm)

These are ultra-high efficiency columns designed for high resolution performance. They are available in THF, DMF or chloroform.

HFIP-800 Series (8 x 300 mm)

These columns have the same high efficiency as the K series columns but are available in HFIP.

HFIP-800 Series (8 mm i.d. x 300 mm)

Type	Polystyrene Exclusion Limit	Part No.
HFIP-803	7×10^4	WAT035605
HFIP-806M (linear)	(4×10^7)	WAT035611
HFP-LG precolumn (8 x 50 mm)		WAT035612

Shodex GPC Columns

Type	Polystyrene Exclusion Limit	Part No.
KF-800 (THF)		
KF-801	1.5×10^3	WAT030697
KF-802	5×10^3	WAT030698
KF-802.5	2×10^4	WAT030699
KF-803	7×10^4	WAT034100
KF-804	4×10^5	WAT034101
KF-805	4×10^6	WAT034102
KF-807	(2×10^8)	WAT034104
KF-806M (linear)	(4×10^7)	WAT034105
KF-G pre-column (4.6 x 10 mm)		WAT034106
K-800 (Chloroform)		
K-802.5	2×10^4	WAT034109
K-803	7×10^4	WAT034110
K-804	4×10^5	WAT034111
K-805	4×10^6	WAT034112
K-G precolumn (4.6 x 10 mm)		WAT035524
KD-800 (DMF)		
KD-801	2.5×10^3	WAT034116
KD-802	5×10^3	WAT034117
KD-802.5	2×10^4	WAT034118
KD-803	7×10^4	WAT034119
KD-804	4×10^5	WAT034120
KD-806	(4×10^7)	WAT034122
KD-807	(2×10^8)	WAT034123
KD-806M (linear)	(4×10^7)	WAT034124
KD-G prec-olumn (4.6 x 10 mm)		WAT034125

Envirogel High-Resolution GPC Cleanup Columns

The Envirogel™ high-efficiency GPC cleanup columns are specifically designed to remove low volatility, high molecular weight interferences, such as lipids and natural resins, from environmental samples as specified in EPA Method 3640A*. In the past, the cleanup procedure for environmental samples was performed on low-efficiency GPC columns based on packing particle diameters of 37-75 µm (200 to 400 mesh) Bio-Beads S-X resins. The high-efficiency Envirogel GPC Cleanup columns increase the speed of this process while simultaneously reducing solvent consumption.

Envirogel GPC Cleanup Columns Packed in Methylene Chloride

Column	Dimensions	Part No.
Envirogel GPC Cleanup	19 x 150 mm	WAT036555
Envirogel GPC Cleanup	19 x 300 mm	WAT036554
Envirogel GPC Guard	4.6 x 30 mm	186001913

* EPA Method 3640A requires both columns.

Envirogel GPC Cleanup Columns Packed in Cyclohexane/Ethyl Acetate

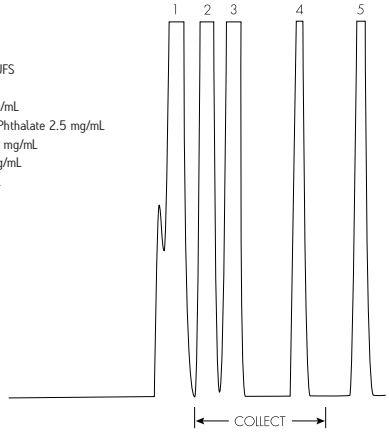
Column	Dimensions	Part No.
Envirogel GPC Cleanup	19 x 150 mm	186001915
Envirogel GPC Cleanup	19 x 300 mm	186001916
Envirogel GPC Guard	4.6 x 30 mm	186001914

* EPA Method 3640A requires both columns.

Column Optimization

Column: Two Envirogel GPC columns 19 x 300 mm and 19 x 150 mm
Sample: 2000 µL
Solvent: Methylene Chloride
Flow Rate: 5 mL/min
Detection: UV at 254 nm, 1.5 AUFS

1. Corn oil, 62 5 mg/mL
2. Bis(2-Ethylhexyl) Phthalate 2.5 mg/mL
3. Methoxychlor, 0.5 mg/mL
4. Perylene, 0.05 mg/mL
5. Sulfur, 0.2 mg/mL



For optimum capacity and resolution, a 150 mm column is used in series with the 300 mm column. The use of both the 150 mm column and the 300 mm column provides maximum loading capacity while the 300 mm column provides maximum throughput and reduction in solvent consumption when used alone.

Preparative GPC Columns

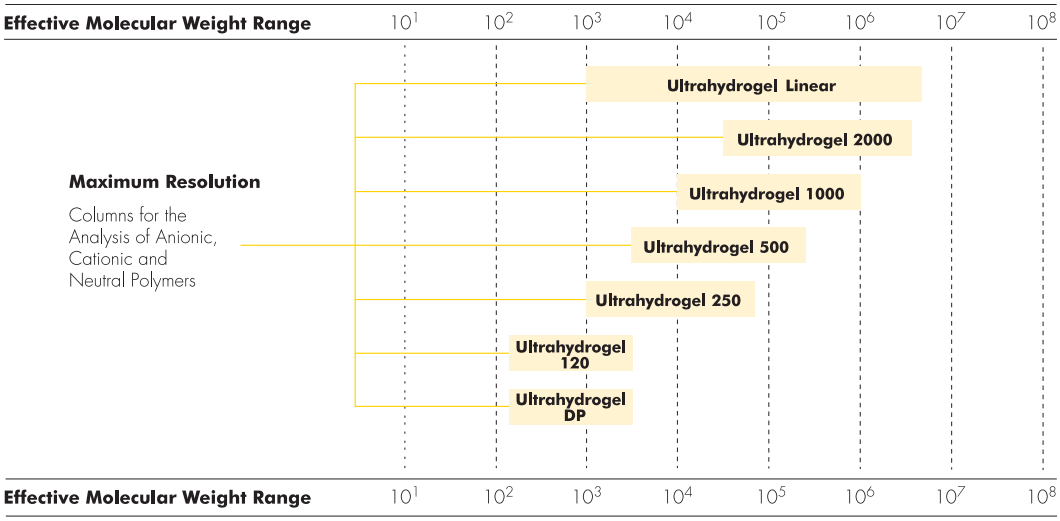
Ultrastyragel Columns (19 mm i.d. x 300 mm)

For high resolution preparative applications, these columns are available in toluene or THF.

Pore Size	Effective Molecular Weight Range	Flow Rate mL/Min	Part No. Toluene	Part No. THF
100Å	50-1,500	4-10	WAT025866	WAT025859
500Å	100-10,000	4-10	WAT025867	WAT025860
103Å	200-30,000	4-10	WAT025868	WAT025861
104Å	5,000-600,000	4-10	WAT025869	WAT025862
105Å	50,000-4M	4-10	WAT025870	WAT025863
106Å	200,000-10M	4-10	WAT025871	WAT025864
Linear	2,000-4M	4-10	WAT025872	WAT025865

[Effective linear functional range: 2K-4M]

Aqueous SEC Column Selection Guide



Eluent Selection—Aqueous SEC with Ultrahydrogel Columns

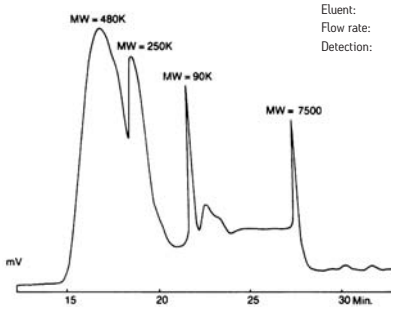
Class	Polymer	Eluent
Nonionic	Polyethylene Oxide Polyethylene Glycol Polysaccharides Pullulans Dextrans Cellulosics (H_2O Soluble) Polyvinyl Alcohol Polyacrylamide	0.10M $NaNO_3$
Nonionic Hydrophobic	Polyvinyl Pyrrolidone	80/20, 0.10M $NaNO_3/CH_3CN$
Anionic	Polyacrylic Acid (Na) Polyalginic Acid (Na) Hyaluronic Acid Carrageenan	0.10M $NaNO_3$
Anionic Hydrophobic	Polystyrene Sulfonate (Na) Lignin, Sulfonated	80/20, 0.10M $NaNO_3/CH_3CN$
Cationic	DEAE Dextran Polyvinylamine Polyepiamine N-Acetylglucosamine	0.80M $NaNO_3$ 0.10M TEA 0.10M TEA/1% HOAc
Cationic Hydrophobic	Polyethyleneimine Poly (n-Methyl-2-Vinyl Pyridinium) Lysozyme Chitosan Polylysine Peptides	0.50M NaOAc/0.50M HOAc 0.5M CH_3COOH/0.3M Na_2SO_4 0.50M HOAc/0.30M Na_2SO_4 5% NaH_2PO_4 with 3% CH_3CN(pH 4.0) 0.10% TFA/40% CH_3CN
Amphoteric	Collagen/Gelatin	80/20, 0.10M $NaNO_3/CH_3CN$

Ultrahydrogel Columns

Packed with hydroxylated polymethacrylate-based gel, Waters Ultrahydrogel™ SEC columns are ideal for the analysis of aqueous-soluble samples, such as oligomers; oligosaccharides; polysaccharides; and cationic, anionic, and amphoteric polymers. Measuring 7.8 x 300 mm, these high-resolution columns offer many advantages over conventional aqueous SEC columns, such as:

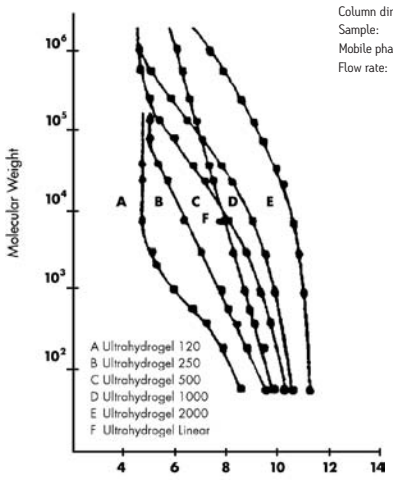
- A wide pH range (2-12)
- Compatibility with high concentrations of organic solvents (up to 20% organic, 50% organic if the mobile phase is introduced by gradient)
- Greater flexibility for the mobile phase
- Minimal non-size-exclusion effects

Gelatin Sample



Column:	Waters Ultrahydrogel 250, 500 and 1000
Temperature:	80 °C
Eluent:	H_2O, pH 7, phosphate buffer
Flow rate:	1 mL/min
Detection:	Waters 410 Differential Refractometer

Ultrahydrogel Columns Calibration Curves



Column dimensions:	7.8 mm i.d. x 300 mm
Sample:	PEG and PEO standards
Mobile phase:	Distilled water
Flow rate:	1 mL/min

Ultrahydrogel Columns (7.8 x 300 mm)

Column	Pore Size	Exclusion Limit	Part No.
Ultrahydrogel 120	120 Å	5 x 10^3	WAT011520
Ultrahydrogel 250	250 Å	8 x 10^4	WAT011525
Ultrahydrogel 500	500 Å	4 x 10^5	WAT011530
Ultrahydrogel 1000	1000 Å	1 x 10^6	WAT011535
Ultrahydrogel 2000	2000 Å	7 x 10^6	WAT011540
Ultrahydrogel Linear	Blend	7 x 10^6	WAT011545
Ultrahydrogel DP*	120 Å	5 x 10^3	WAT011550
Ultrahydrogel Guard Column	N/A	N/A	WAT011565
Ultrahydrogel Guard Column DP*	N/A	N/A	WAT011570

* DP = Degree of Polymerization, choice of column when working with glucose oligomers.

Calibration Standards

Organic Calibration Standards

Organic Standards Kits

Description	Qty./ MW	Part No.
Polystyrene Kit Low-Mid MW*	4 x 10^2/10 g, 5.3 x 10^2/10 g, 9.5 x 10^2/10 g, 2.8 x 10^3/5 g, 6.4 x 10^3/5 g, 1 x 10^4/5 g, 1.7 x 10^4/5 g, 4.3 x 10^4/5 g, 1.1 x 10^5/5 g, 1.8 x 10^5/5 g	WAT011588
Polystyrene Kit Mid-High MW	4.3 x 10^5/5 g, 7.8 x 10^5/5 g, 1.3 x 10^6/1 g, 2.8 x 10^6/1 g, 3.6 x 10^6/1 g, 4.3 x 10^6/1 g, 5.2 x 10^6/1 g, 6.2 x 10^6/1 g, 8.4 x 10^6/1 g, 2 x 10^7/1 g	WAT011610
Polystyrene Kit SL-105	5.8 x 10^2, 9.5 x 10^2, 1.2 x 10^3, 1.8 x 10^3, 2.47 x 10^3, 3.77 x 10^3, 5.1 x 10^3, 7.6 x 10^3, 1.25 x 10^4, 1.7 x 10^4	500 mg/each WAT034208
Polystyrene Kit SM-105	1.2 x 10^3, 3.25 x 10^3, 1.02 x 10^4, 2.8 x 10^4, 6.8 x 10^4, 1.95 x 10^5, 4.9 x 10^5, 1.08 x 10^6, 1.75 x 10^6, 2.75 x 10^6	500 mg/each WAT034209
Polystyrene Kit SH-75	4.5 x 10^5, 1.27 x 10^6, 2.3 x 10^6, 3.26 x 10^6, 4.34 x 10^6, 8 x 10^6, 1.5 x 10^7	500 mg/each WAT034210
Polymethylmethacrylate Mid MW Kit	2.4 x 10^3, 9.5 x 10^3, 3.1 x 10^4, 5.2 x 10^4, 1 x 10^5, 1.7 x 10^5, 2.7 x 10^5, 4.91 x 10^5, 7.3 x 10^5, 1 x 10^6	500 mg/each WAT035706
Polymethylmethacrylate Low MW Kit	1 x 10^3, 1.7 x 10^3, 2.5 x 10^3, 3.5 x 10^3, 5 x 10^3, 7 x 10^3, 1 x 10^4, 1.3 x 10^4, 2 x 10^4, 3 x 10^4	500 mg/each WAT035707
Polybutadiene Kit	1 x 10^3, 3 x 10^3, 7 x 10^3, 1 x 10^4, 3 x 10^4, 7 x 10^4, 1 x 10^5, 3 x 10^5, 7 x 10^5, 1.1 x 10^6	500 mg/each WAT035709
Polyisoprene Kit	1 x 10^3, 3 x 10^3, 1 x 10^4, 3 x 10^4, 7 x 10^4, 1 x 10^5, 3 x 10^5, 5 x 10^5, 1 x 10^6, 3 x 10^6	500 mg/each WAT035708

* Approximate molecular weights

Polystyrene (PS) Organic Standards (Individual Standard)

Approximate Molecular Weight Range LS*	GPC	Qty.	Part No.
	4 x 10^2	10 g g	WAT011590
	5.3 x 10^2	10 g	WAT011592
	9.5 x 10^2	10 g	WAT011594
2.8 x 10^3	2.8 x 10^3	5 g	WAT011596
6.2 x 10^3	6.4 x 10^3	5 g	WAT011598
1.03 x 10^4	1.01 x 10^4	5 g	WAT011600
1.67 x 10^4	1.73 x 10^4	5 g	WAT011602
4.39 x 10^4	4.30 x 10^4	5 g	WAT011604
1.07 x 10^5	1.06 x 10^5	5 g	WAT011606
1.86 x 10^5	1.84 x 10^5	5 g	WAT011608
4.22 x 10^5	4.27 x 10^5	5 g	WAT011612
7.75 x 10^5	7.91 x 10^5	5 g	WAT011614
1.26 x 10^6	1.30 x 10^6	1 g	WAT011616
2.86 x 10^6	2.80 x 10^6	1 g	WAT011618
3.84 x 10^6	3.61 x 10^6	1 g	WAT011620
4.48 x 10^6	4.27 x 10^6	1 g	WAT011622
5.48 x 10^6	5.20 x 10^6	1 g	WAT011624
6.77 x 10^6	6.20 x 10^6	1 g	WAT011626
8.42 x 10^6		1 g	WAT011628
2.0 x 10^7		1 g	WAT011630

* Light scattering

ReadyCal Polystyrene Standards

Thirty autosampler vials which contain 4 polystyrene standards per vial. There are three separate molecular weight ranges in each kit, 10 units of each of the three molecular weight standards. Just add solvent to the vial, let stand for two hours, shake gently and load into your autosampler for analysis. Each kit comes with detailed instructions for proper usage.

Type	Standards	Approximate Molecular Weight	Part No.
ReadyCal, 4 mL Autosampler Vial	12	4 x 10^2 x 2 x 10^6	WAT058930
ReadyCal, 2 mL Autosampler Vial	12	4 x 10^2 x 2 x 10^6	WAT058931

Aqueous Calibration Standards

Aqueous Standards Kits

Description	Qty./ MW	Part No.
Pullulan Kit	* 5.8 x 10^3, 1.22 x 10^4, 2.37 x 10^4, 1 x 10^5, 1.86 x 10^5, 3.8 x 10^5, 8.53 x 10^5	200 mg /each WAT034207
Dextran Kit	5 x 10^3, 1.2 x 10^4, 2.4 x 10^4, 4.8 x 10^4, 1.48 x 10^5, 2.73 x 10^5, 4.1 x 10^5, 7.5 x 10^5	500 mg/each WAT054392
Polyethyleneoxide (PEO) Kit	2.4 x 4 x 10^4, 8 x 10^4, 1.6 x 10^5, 3.4 x 10^5, 5.7 x 10^5, 8.5 x 10^5	500 mg/each WAT011572
Polyethylene Glycol (PEG) Kit	1 x 10^2, 2 x 10^2, 4 x 10^2, 6 x 10^2, 1 x 10^3, 1.5 x 10^3, 4.3 x 10^3, 7 x 10^3, 1.3 x 10^4, 2.2 x 10^4	1 gram/each WAT035711
Polyacrylic Acid Kit	1 x 10^3, 3 x 10^3, 7 x 10^3, 1.5 x 10^4, 3 x 10^4, 7 x 10^4, 1 x 10^5, 3 x 10^5, 7 x 10^5, 1 x 10^6	250 mg/each WAT035714

* Approximate molecular weights

Polyethyleneoxide (PEO) Aqueous Standards

Approximate Molecular Weight Range LS*	GPC	Qty.	Part No.
2.5 x 10^4	2.4 x 10^4	0.5 g	WAT011574
4.0 x 10^4	4.0 x 10^4	0.5 g	WAT011576
7.3 x 10^4	7.9 x 10^4	0.5 g	WAT011578
1.5 x 10^5	1.6 x 10^5	0.5 g	WAT011580
2.8 x 10^5	3.4 x 10^5	0.5 g	WAT011582
6.6 x 10^5	5.7 x 10^5	0.5 g	WAT011584
8.5 x 10^5	8.5 x 10^5	0.5 g	WAT011586

* Light scattering

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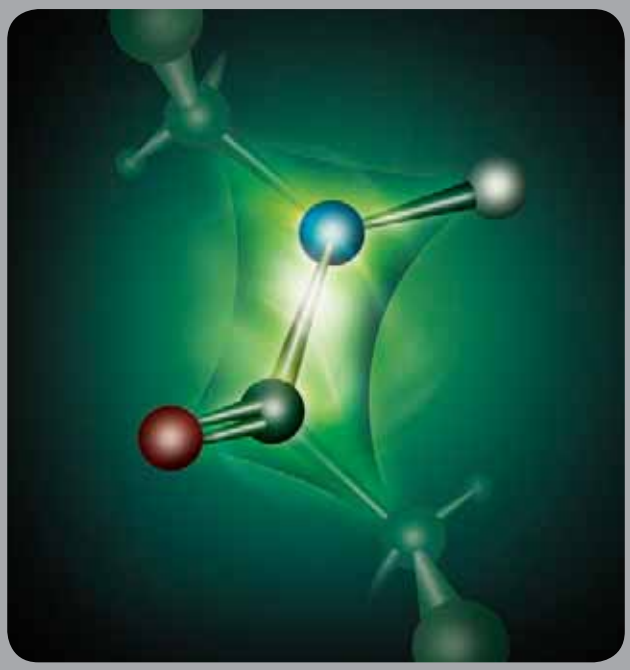
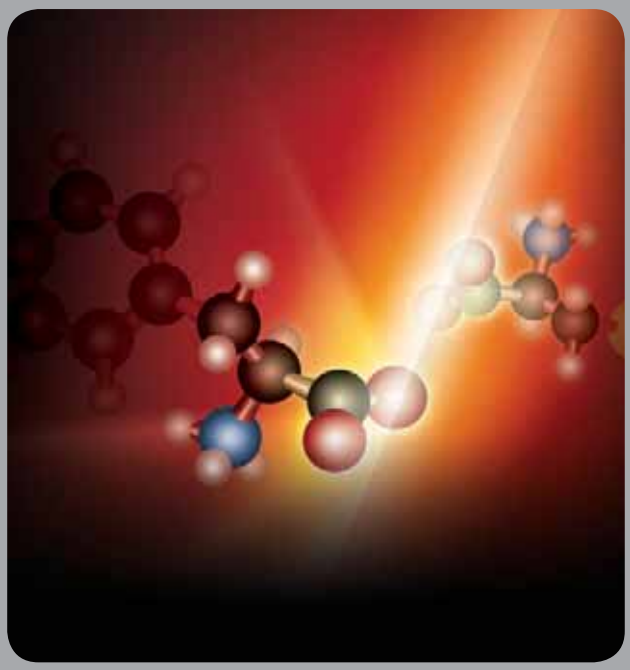
Bioseparations and Analysis

Innovative Technologies From the Leaders in Separation Science and Analytical Biochemistry



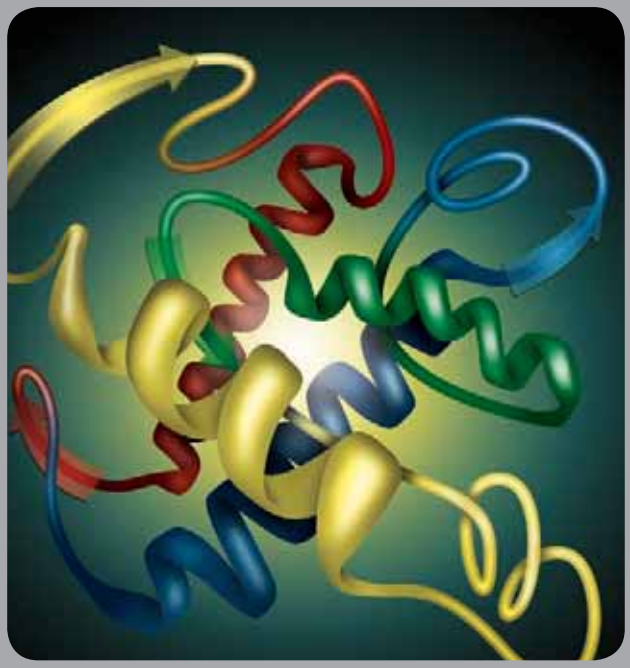


Advances in the areas of genomics, proteomics, metabanomics, and molecular and system biology continue to revolutionize the diagnosis and treatment of disease and increase our fundamental understanding of biological processes.

As a leading analytical supplier of instrumentation, software, service and support, and chemistry products, Waters is uniquely positioned to provide researchers the tools, technologies, and integrated solutions desired to tackle the formidable challenges involving various biomolecules. Beginning with a keen understanding of today's biomolecule-related challenges, Waters scientists and engineers continuously seek purposeful innovations that help deliver impactful solutions in applications ranging from proteomics and biomarker discovery through the commercialization of advanced biopharmaceuticals. We continue to develop new, innovative columns and sample preparation consumables that support the HPLC, UPLC® technology, and LC/MS analyses of peptides, oligonucleotides, proteins, and amino acids.

Waters comprehensive chemistry and consumables family includes:

- Peptide Separation Technology columns for nano, capillary, analytical, and preparative peptide applications
- Protein Separation Technology reversed-phase columns, BioSuite™ SEC, IEX, RP, and HIC columns for analytical and purification applications
- AccQ•Tag™ Ultra chemistry specific for Waters UPLC Amino Acid Analysis Solution, as well as Pico•Tag™ and AccQ•Tag for HPLC-based amino acid analyses
- Oligonucleotide Separation Technology columns for synthetic oligonucleotide and DNA/RNA fragment isolations and analyses
- MassPREP™ chemistry consumables and kits for MS and LC/MS applications of peptides, proteins, and other biomolecules



Peptide and Oligonucleotide Isolations and Analyses

Synthetic peptides and oligonucleotides can be effectively separated by various chromatographic techniques including reversed-phase, ion-exchange, and size-exclusion chromatography. Waters Peptide Separation Technology and BioSuite columns are designed to address various peptide analysis and lab-scale purification needs. Our Oligonucleotide Separation Technology reversed-phase and GenPak™ Fax IEX columns address various high-resolution analysis and lab-scale isolation challenges involving various DNA and RNA species.

Amino Acid Analysis

Waters solutions in this area have evolved from more than 25 years of experience in amino acid analysis and include both HPLC and UPLC -based offerings. AccQ•Tag and Pico•Tag pre-column derivatization chemistries and columns are designed for traditional HPLC applications. In 2006, we introduced our UPLC Amino Acid Analysis Solution that uses our next generation AccQ•Tag Ultra chemistry to provide superior component resolution and separation speed compared to results possible using established HPLC-based methods.

Protein Analysis and Characterization

As the major building blocks of life, many biopharmaceutical advances have resulted from the detailed knowledge and characterization of these molecules. Consequently, detailed protein characterization studies are necessary that incorporate powerful analytical techniques, such as peptide mapping and mass spectrometry using a variety of HPLC and UPLC column chemistries that include size exclusion, ion exchange, hydrophobic interaction, and reversed phase. Waters Protein Separation Technology reversed-phase, BioSuite SEC, IEX, HIC, and RP HPLC columns, offer outstanding performance when applied to the separation of these macromolecules.

Proteomics, Protein Expression, and Biomarker Discovery

Experiments in proteomics begin with samples that are both very complex and limited in volume and concentration. Good sample preparation and separation of the various analytes prior to MS analysis can greatly enhance detection sensitivity and provide more meaningful results. Waters MS and LC/MS consumables that include NanoEase™ and nanoACQUITY® trap, capillary, and nano columns, *Rapi*Gest™ SF reagent, as well as MassPREP standards and kits help ensure the best results from MS and LC/MS instrumentation.

Peptides Isolation and Analysis

The separation of peptides plays a fundamental role in many areas of research and development. Some peptides have significant biological activity and require analysis in a complex matrix. It may be necessary to isolate these molecules as natural products or as synthetic peptides. In addition, the separation of peptides is a fundamental tool for the analysis of protein structure because the small, reproducible fragments are a tool for dissecting the structural details of the much larger protein. The same principle underlies the use of peptide separations for analyzing proteomics, protein expression, and biomarker discovery. All of these applications focus on a family of molecules with similar properties such that the separation can be approached in the same way. The principles behind each separation mechanism can be considered in selecting a column for a particular application. The choice of chromatographic mode will depend on the type of peptide sample you are separating. The guidelines below will help direct you to the appropriate column selection.

Reversed-Phase Peptide Separations

Mechanisms

Reversed-phase separations generally give the highest resolution of peptide mixtures, often allowing for effective separation of peptides differing by a single amino acid. In reversed-phase separations, the hydrophobicity of the peptide determines the elution order, with the least hydrophobic peptides eluting first. This hydrophobicity is related to the size of the peptide, with larger molecules being more hydrophobic in general, and also to the hydrophobicity of the side chains of the constituent amino acids. Both the retention and the separation selectivity are also influenced by the three-dimensional structure of the peptide. The packing material interacts with these peptide properties based foremost on the hydrophobicity of the column surface, reflecting bonded phase chain length and density. Other column properties that can strongly influence the separation selectivity include the pore size of the packing material and the surface chemistry of the particle of the packing material.

Column Selectivity

The retention mechanism of peptide separations is similar on reversed-phase HPLC columns in general. The selectivity of the separation is significantly affected by the base sorbent, pore size, alkyl ligand density, and residual silanol endcapping. These small differences in separation selectivity can be a part of developing a peptide separation. If it is difficult to achieve a desirable separation on a particular RP-HPLC column, then the use of an alternative RP-HPLC column chemistry may be tested. For example, different separation selectivities will be observed using XBridge™ BEH300 C_{18} compared to Atlantis T3.

Mobile-Phase Selectivity

Peptides are charged molecules that do not freely interact with the hydrophobic surface of reversed-phase columns. The mobile phase is typically, therefore, acidified to protonate the peptide carboxyl groups, increasing retention. Trifluoroacetic acid is often preferred for this purpose because it also forms ion pairs with the basic groups of peptides. The combination of protonation and ion pairing leads to the best retention and solubility for peptides. It also gives the best peak shape since the peptide basic groups are shielded from secondary interactions with the surface of the packing material.

TFA, however, reduces the sensitivity of MS detection of peptides. While usable signal is usually obtained by using lower concentrations of TFA, many investigators prefer to use formic acid as the mobile phase modifier. In this case, the column surface has a larger effect on the separation. Retention is often much less, and many of the peptide peaks will be asymmetrical and tailing. It is common practice to choose columns designated as "no TFA" or "low TFA" for these separations. The BioSuite PA-A column described below was developed for this purpose. More recently the development of Ethylene-Bridged Hybrid (BEH Technology™) packing materials provided an improved option. The ethane bridges reduce the surface concentration of silanol groups so that there is little secondary interaction between peptides and the base particle. Packing materials based on these particles can be used for peptide separations in either TFA or formic acid containing mobile phases without significant loss of retention or band-broadening. The BEH Technology particles for the basis of the Peptide Separation Technology family of columns described below.

With the Peptide Separation Technology columns, the minimal secondary interactions create the greatest number of options for adjusting selectivity with mobile phase manipulation. The selectivity of a peptide separation is different for mobile phases containing TFA and formic acid. The use of columns that give good performance with either modifier create an option for developing methods in addition to the desirable impact on sensitivity in MS detection. Furthermore, because peak shape and resolution are not dependent on the presence of TFA, the concentration of TFA can be adjusted to fine tune the selectivity of a peptide separation.

More dramatic changes in selectivity can be induced by changing the pH of the separation from low pH to pH 7 or even pH 10 (e.g., using XBridge BEH130 C_{18} columns).

Ion-Exchange Chromatography

In ion-exchange chromatography, the charge on the amino acid side chains is the determining factor in the separation. The benefits of ion-exchange chromatography are the high-capacity packings and the ability to easily load ion-exchange fractions onto a reversed-phase column for desalting or final purification. While ion-exchange separations of peptides are not generally as highly resolving as reversed phase, the technique can provide an orthogonal selectivity for difficult samples. Peptides most often separate best on strong cation exchangers. The ion-exchange column packings described in the section on protein chromatography are suitable for peptides.

Size-Exclusion Chromatography

Size-exclusion chromatography is sometimes used for peptide separations. The peptides are separated based on their size in solution. The resolution seldom compares favorably to either reversed phase or ion exchange, but it can provide an alternative for some samples. The composition of the separation buffer will require careful adjustment for each particular peptide sample. Elevated ionic strength and the addition of some organic solvent, typically methanol or propanol, may be necessary for good peak shape. The

size-exclusion packings, particularly those with smaller pore sizes, described in the section on protein chromatography, are suitable for peptides.

Applications of Peptide Separations

Peptide Mapping

Peptide mapping continues to be the preferred technique for the comprehensive characterization of biopharmaceutical products and proteins in general. Waters provides an array of products that facilitate the development of information-rich peptide maps. Before applying the separation techniques discussed above, the purified protein sample must be digested. This step often proves difficult because of incomplete and irreproducible digestion, non-specific cleavages, precipitation of large core peptides, and slow digestion. *Rapi*Gest SF is a surfactant that unfolds proteins so that the sites of enzymatic cleavage are freely accessible for digestion. This improves reproducibility and speed. It also helps to keep all the sample components in solution throughout the digestion process and up to the analysis. The properties and use of *Rapi*Gest are fully described below in the section on MS and LC/MS consumables. This section also describes several standard mixtures of peptides and protein digests that are used to test and confirm the performance of columns and instruments before committing any samples to the peptide mapping system.

The primary choice for the separation component of peptide mapping is the Peptide Separation Technology family of columns. This packing material is based on BEH Technology particles to minimize secondary interactions and to maximize column lifetime. These materials are available with either 130Å or 300Å pores to adapt to the sizes of peptides being analyzed. Both pore sizes are available in a range of column dimensions suited to the sample size. The same separation chemistry is provided with either 3.5 µm particles for HPLC analysis or with 1.7 µm for use with ACQUITY UPLC systems. Peptide Separation Technology columns are specifically QC-tested with protein digests to confirm batch to batch reproducibility. This column chemistry is, therefore, suitable for the development of methods that are to be used routinely for extended periods.

Other Waters columns also have proven suitable for peptide mapping. The BioSuite Peptide Analysis columns are two premier reversed-phase column chemistries specifically optimized for peptide mapping. The BioSuite PA-A column is especially suitable for polar peptides and for use with MS-compatible eluents. The BioSuite PA-B column has 300Å pores for the analysis of large and hydrophobic peptides. Both BioSuite Peptide Analysis columns are specifically QC-tested with protein digests to ensure batch-to-batch product consistency and column-to-column performance reproducibility.

Many other Waters columns have been used for particular peptide mixtures. It is often observed that most peptides will give reasonable chromatography on many different columns. There is, however, often a particular column that, for some subtle and unpredictable reason, works better than any other for a particular pair of peptides.

Isolation of Natural and Synthetic Peptides

Purification of large amounts of individual peptides is most often achieved with reversed-phase chromatography using the same principles and ideas described above. The columns are often chosen to provide economical separations on a larger scale so they are packed with larger size particles. The Peptide Separation Technology columns described above are available in larger particle sizes and larger dimension columns for this purpose. The minimal secondary interactions mean that these columns can be used for peptides representing a wide range of sizes and chemical properties. The surface chemistry of these packings is consistent and scalable across the range of particle sizes. As necessary, separations may be optimized on small scale columns and then transferred to columns large enough for the required sample.

For some complex mixtures, two step separations may give the highest yield and purity. In general, the best first step is cation-exchange chromatography followed by a reversed-phase step for final isolation and desalting.

Proteomics, Analysis of Protein Expression, and Biomarker Discovery

For analyses used in proteomics and biomarker discovery, the available samples are extremely limited so the requirements of chromatographic separation must be coupled with extreme sensitivity. The sample chromatographic tools and principles described above are implemented on the smallest scale in the nanoACQUITY UPLC® system with nanoACQUITY columns. The application solutions are described below in the section on MS and LC/MS consumables.

Preparative Peptide Separations

The isolation and purification of peptides from both natural and synthetic sources have been the focus of many scientists in research and development. This process has been difficult because of unpredictable separations, short column lifetime, and lack of scalability.

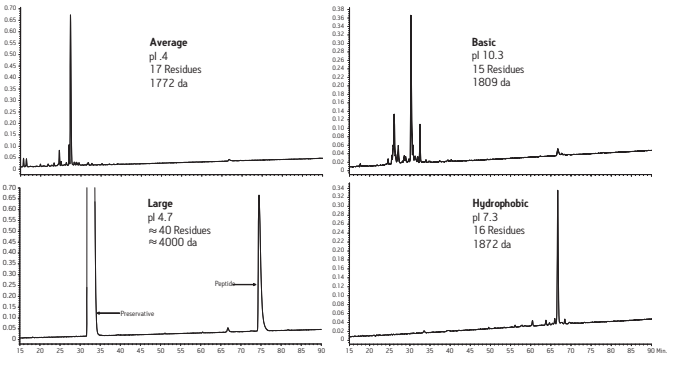
Samples can be complex so separations must be developed and optimized. These experiments are usually performed on pilot or analytical-scale columns, but inconsistent performance of preparative columns complicates the development of purification protocols. Peptide Separation Technology columns provide a stable surface chemistry that can be used for most peptides. Scaling the optimized separation requires identical column chemistry and performance across a range of column dimensions. Peptide Separation Technology column chemistry is consistent across all particle sizes and column dimensions.

The loss of samples due to early column failure must be minimized. Waters developed the patent-pending Optimum Bed Density (OBD™) preparative column design. This format provides the most rugged, efficient, and reproducible preparative columns. When this column design is coupled with the stable surface chemistry of Peptide Separation Technology columns in a range of particle sizes, the process of peptide isolation and purification becomes more efficient, and overall costs are reduced.

Utility and Suitability for a Wide Range of Peptides

Peptide Separation Technology columns are suitable for a wide variety of peptides, including acidic and basic, long and short, hydrophilic and hydrophobic, and modified sequences. There is little need for screening columns to match a particular sample, or for maintaining an inventory of different column chemistries. Peptide Separation Technology columns are compatible with alternative mobile phases for flexibility in developing purification methods. Good peak shapes and separations can be obtained with both TFA and short chain organic acids as mobile phase modifiers, and the columns can be used with both acetonitrile and alcohols. Good purification and yield can be obtained with bio-compatible solvents so that the isolated peptide can be used in bio-assays.

Versatility for Peptide Isolation and Purification



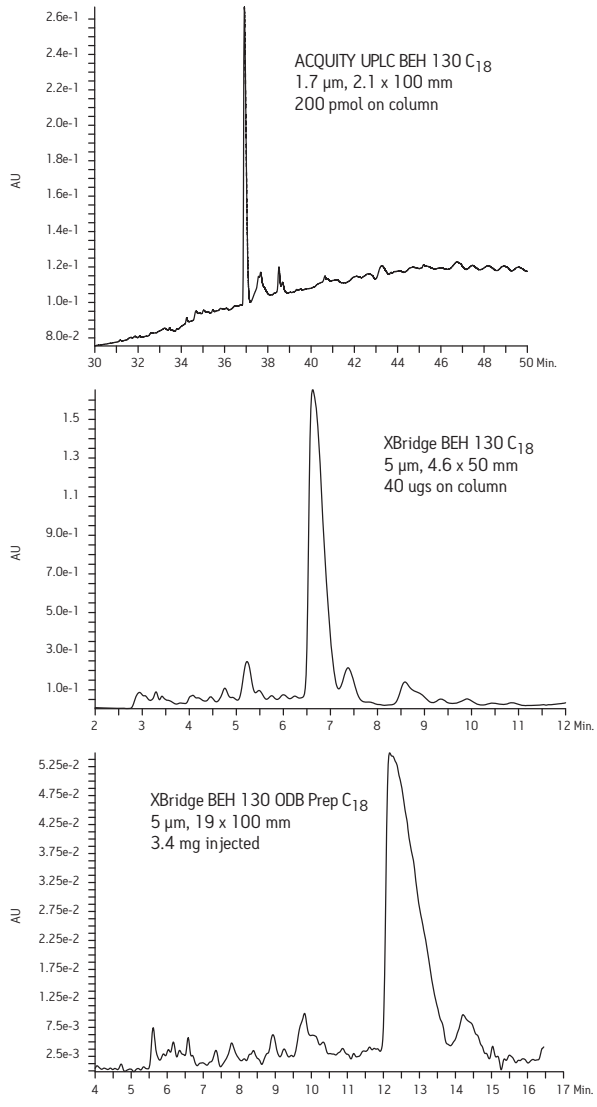
Peptide Separation Technology columns are compatible with a broad range of peptides. A single column can be used to purify the wide range of sample types generated in a peptide synthesis laboratory. There is no need to switch columns for separating peptides of extreme sizes, isoelectric point, or hydrophobicity.

Scale-up

Some peptide samples may require an optimum isolation method. Test runs on an analytical-scale column define conditions to transfer to a larger column. OBD columns are an integral part of the process of transferring a separation because the packed bed is stable and uniform. If preferred, large particles are available with the same selectivity as all other particule sizes. Peptide Separation Technology packings are available in a wide range of particle sizes and column dimensions, all with the same, consistent chemistry.

Separation of 13 Residue Peptide at Various Sample Loads

Scalable Peptide Purification

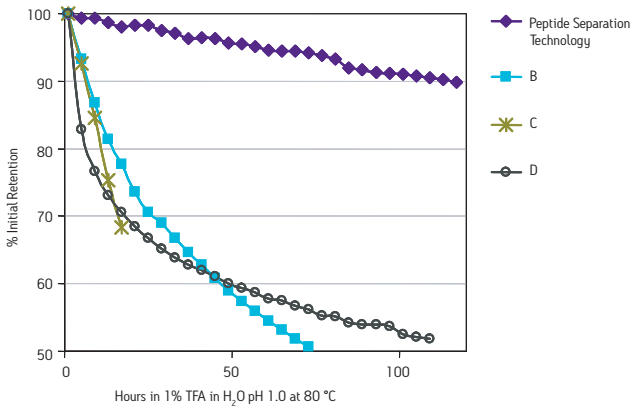


Waters Peptide Separation Technology columns, available in different particle size and column configurations, are well-suited for various lab-scale purification needs.

Column Lifetime

Columns fail both physically and chemically. One major cause of short column lifetime in low pH mobile phases is hydrolysis of the bonded phase that leads to significant changes in peptide retention. BEH Technology columns incorporates proprietary procedures for bonding and endcapping that yield stable bonded phases. In low pH stability test, BEH C_{18} columns showed very little retention loss. The physical stability of these columns is ensured by the OBD design that was developed to produce the most stable packed beds.

Long-Term Stability



Several columns were tested with repeated injections. Retention was monitored to determine column lifetime.

Reduce Cost of Isolation

Peptide purification is expensive but Peptide Separation Technology columns can reduce the cost per sample. The same column can be used for many sample types and mobile phases. Column life is extended with the stable bonding chemistry and OBD packing. Consistent stability ensures that the best methods and column dimensions are used for each sample.

Cation-Exchange Peptide and Polypeptide Separations

For most analytical and preparative peptide separations, cation-exchange chromatography is used mainly when alternative selectivity is required. In some large-scale purifications, cation exchange can take on a more central role. In these cases, cation exchange is frequently used as the first step in the separation, followed by a secondary purification step using reversed-phase methods.

Waters offers Protein-Pak™ SP HR packings for cation-exchange separations. These packings are useful both for analytical and preparative work. They are based on rigid, hydrophilic polymethacrylate particles with large 1000Å pores. The naturally hydrophilic polymer reduces non-specific adsorption, resulting in better recovery of peptide/polypeptide mass and bioactivity. These packings are stable in the pH range of 2-12.

Protein-Pak SP HR 8 and 15 µm packing material is available in prepacked glass columns.

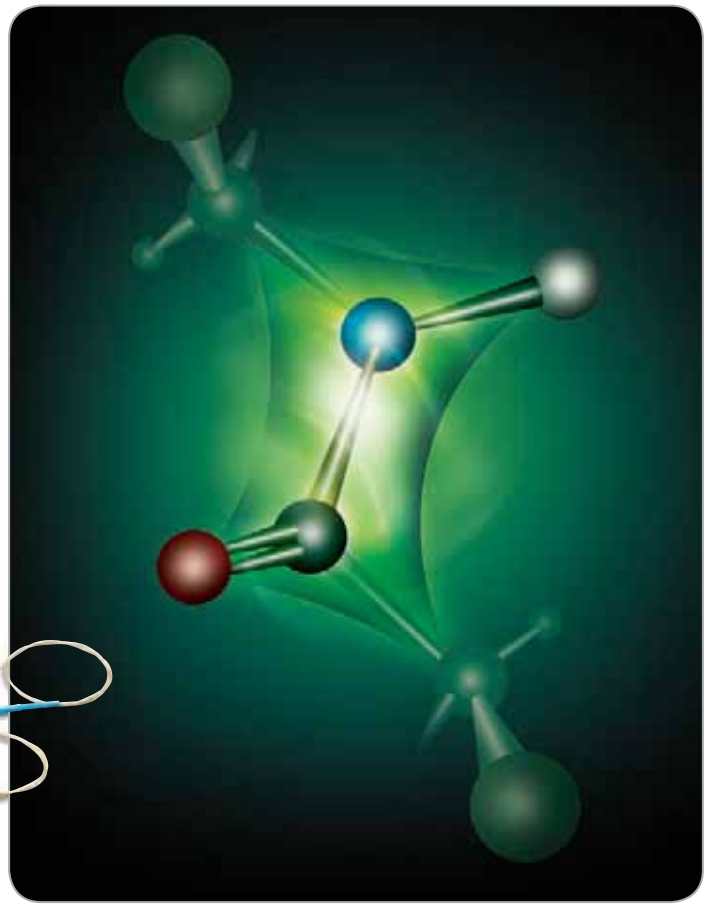
UPLC for Complementing Isolation and Purification

UPLC technology provides useful information on the composition of a peptide sample. UPLC analytical methods suggest appropriate conditions for isolation, and analysis with UPLC identifies the fractions to pool. Purity of the final product can then be confirmed.

Peptide Separation Technology

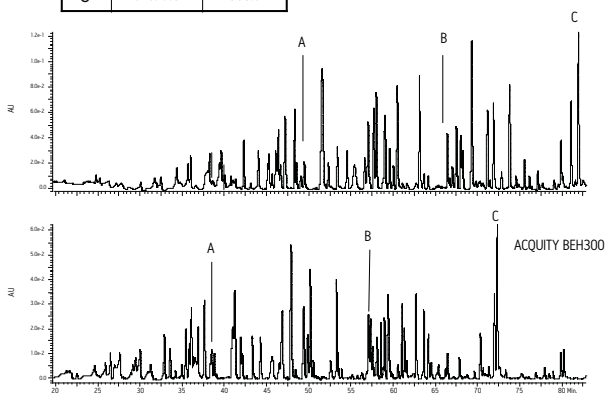
Waters Peptide Separation Technology consists of a unique family of columns that meet the demanding requirements of peptide separations, from proteomics, to peptide mapping and isolation. Key product characteristics include:

- pH and temperature tolerant BEH Technology particles
- 130Å and 300Å pore sizes for small and large peptides
- 1.7, 3.5, 5, and 10 µm offerings for analytical through preparative
- Over 125 HPLC, UPLC, and Nano LC column choices



Two Pores Sizes for Flexible Method Development

A	4 residue	507.3
B	25 residue	2447.2
C	20 residue	1565.8



Comparison of the separation of tryptic digest of phosphorylase b on ACQUITY UPLC BEH130 and BEH300 1.7 µm Peptide Separation Technology columns.

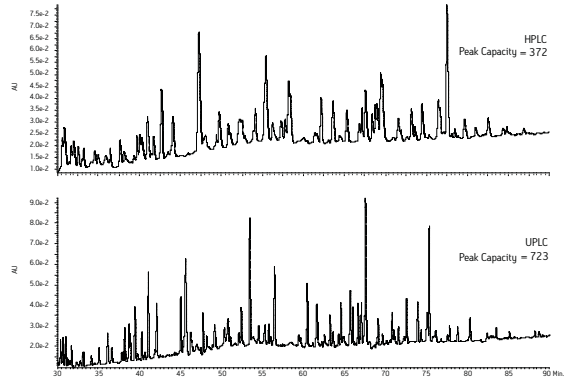
Peptide Separation Technology provides:

- Improved chromatography of peptides
  - Narrow, symmetrical peaks for best resolution
  - Successful separation of a wide range of peptides: large and small, acidic and basic, hydrophobic and hydrophilic
  - Good peak shape and retention in formic acid and trifluoroacetic acid for optimal chromatography and detection
  - Available in 130Å and 300Å pore sizes for varying sample requirements
- Wide range of particle sizes and column dimensions for consistent separations in applications from from very sensitive proteomic analyses to. high mass load purification
  - Chemically stable bonding for extended column life at low pH and high temperatures
  - BEH Technology particles stable to pH 12 for peptide chromatography at high pH
- Unique column chemistry combined with the benefits of sub-2 µm particles of UPLC Technology for the ultimate resolution

BEH Technology

Peptide Separation Technology columns are based on synthetic particles to ensure the highest quality and the most consistent performance. This second generation of Waters patented BEH Technology is a result of Waters commitment to continued investment in chromatography research and development. The columns not only meet the demanding manufacturing and performance specifications for the BEH Technology particles, but are also specifically QC-tested with a peptide map.

Higher Resolution Peptide Mapping with UPLC

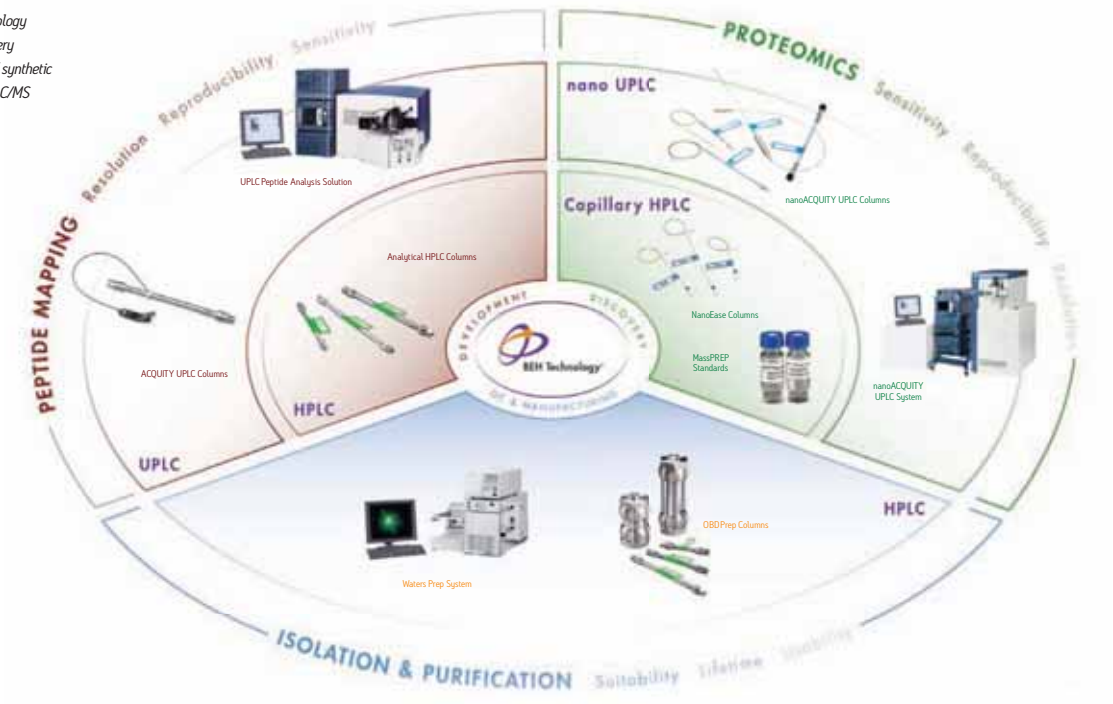


Resolution of complex peptide digests phosphorylase b as shown here, improves when BEH Technology columns are used. Peptide Separation Technology columns with 1.7 µm particles produce highest resolution with the expected increase in sensitivity and decrease in length.

Peptide Separation Technology Columns

Peptide Separation Technology provides a consistent set of chromatographic tools usable across all research and development applications that require analysis and isolation of peptides. Peptide Separation Technology columns are based on the C_{18} BEH Technology particles and are available from sizes 1.7 µm to 10 µm. The range of column dimension spans from i.d. of 75 µm to 30 mm and lengths from 50 to 250 mm. There are over 125 standard columns and others can be custom prepared. There is no need to compromise in matching the column to the application. These columns are specifically QC-tested with a peptide map ensuring the stability of well-developed peptide separation methods used in biotechnology development as well as predictable behavior with many different samples encountered in proteomics, protein characterization, and peptide synthesis. The surface chemistry of all particle sizes, in either the 130Å or 300Å pore sizes, gives sharp symmetrical peaks over a wide range of peptide properties and application requirements. Peptide Separation Technology columns are integral elements of Waters Assured Performance Solutions, combination of instrumentation, chemistry and software optimized for particular applications.

Peptide Separation Technology applications: From discovery proteomics to isolation of synthetic peptides; From capillary LC/MS to HPLC and UPLC



Peptide Nano and Capillary Columns

Peptide Separation Technology nanoACQUITY UPLC® Columns (10,000 psi)

Description	Inner Diameter	Length	Particle Size	Part No.
nanoACQUITY UPLC BEH130 C_{18}	75 µm	100 mm	1.7 µm	186003542
nanoACQUITY UPLC BEH130 C_{18}	75 µm	150 mm	1.7 µm	186003543
nanoACQUITY UPLC BEH130 C_{18}	75 µm	200 mm	1.7 µm	186003544
nanoACQUITY UPLC BEH130 C_{18}	75 µm	250 mm	1.7 µm	186003545
nanoACQUITY UPLC BEH130 C_{18}	100 µm	100 mm	1.7 µm	186003546
nanoACQUITY UPLC BEH130 C_{18}	150 µm	100 mm	1.7 µm	186003550
nanoACQUITY UPLC BEH300 C_{18}	75 µm	100 mm	1.7 µm	186003810
nanoACQUITY UPLC BEH300 C_{18}	100 µm	100 mm	1.7 µm	186003811
nanoACQUITY UPLC BEH300 C_{18}	150 µm	100 mm	1.7 µm	186003812
nanoACQUITY UPLC BEH300 C_{18}	75 µm	150 mm	1.7 µm	186003813
nanoACQUITY UPLC BEH300 C_{18}	75 µm	200 mm	1.7 µm	186003814
nanoACQUITY UPLC BEH300 C_{18}	75 µm	250 mm	1.7 µm	186003815

Peptide Analytical Columns

Peptide Separation Technology NanoEase™ Columns

Description	Inner Diameter	Length	Particle Size	Part No.
XBridge BEH130 C_{18}	75 µm	50 mm	3.5 µm	186003588
XBridge BEH130 C_{18}	75 µm	100 mm	3.5 µm	186003589
XBridge BEH130 C_{18}	75 µm	150 mm	3.5 µm	186003590
XBridge BEH130 C_{18}	100 µm	50 mm	3.5 µm	186003591
XBridge BEH130 C_{18}	100 µm	100 mm	3.5 µm	186003592
XBridge BEH130 C_{18}	100 µm	150 mm	3.5 µm	186003593
XBridge BEH130 C_{18}	150 µm	50 mm	3.5 µm	186003594
XBridge BEH130 C_{18}	150 µm	100 mm	3.5 µm	186003595
XBridge BEH130 C_{18}	150 µm	150 mm	3.5 µm	186003596
XBridge BEH130 C_{18}	300 µm	50 mm	3.5 µm	186003597
XBridge BEH130 C_{18}	300 µm	100 mm	3.5 µm	186003598
XBridge BEH130 C_{18}	300 µm	150 mm	3.5 µm	186003599
XBridge BEH300 C_{18}	75 µm	50 mm	3.5 µm	186003632
XBridge BEH300 C_{18}	75 µm	100 mm	3.5 µm	186003633
XBridge BEH300 C_{18}	75 µm	150 mm	3.5 µm	186003634
XBridge BEH300 C_{18}	100 µm	50 mm	3.5 µm	186003635
XBridge BEH300 C_{18}	100 µm	100 mm	3.5 µm	186003636
XBridge BEH300 C_{18}	100 µm	150 mm	3.5 µm	186003637
XBridge BEH300 C_{18}	150 µm	50 mm	3.5 µm	186003638
XBridge BEH300 C_{18}	150 µm	100 mm	3.5 µm	186003639
XBridge BEH300 C_{18}	150 µm	150 mm	3.5 µm	186003640
XBridge BEH300 C_{18}	300 µm	50 mm	3.5 µm	186003641
XBridge BEH300 C_{18}	300 µm	100 mm	3.5 µm	186003642
XBridge BEH300 C_{18}	300 µm	150 mm	3.5 µm	186003643
XBridge BEH130 C_{18}	300 um	50 mm	5 µm	186003682

Peptide Separation Technology ACQUITY UPLC Columns

Description	Inner Diameter	Length	Particle Size	Part No.
ACQUITY UPLC BEH130 C_{18}	2.1 mm	50 mm	1.7 µm	186003554
ACQUITY UPLC BEH130 C_{18}	2.1 mm	100 mm	1.7 µm	186003555
ACQUITY UPLC BEH130 C_{18}	2.1 mm	150 mm	1.7 µm	186003556
ACQUITY UPLC BEH300 C_{18}	2.1 mm	50 mm	1.7 µm	186003685
ACQUITY UPLC BEH300 C_{18}	2.1 mm	100 mm	1.7 µm	186003686
ACQUITY UPLC BEH300 C_{18}	2.1 mm	150 mm	1.7 µm	186003687

ACQUITY UPLC VanGuard Pre-Column and Column In-Line Filter Unit

Description	Part No.
BEH300, C_{18}, 1.7 µm, 2.1 x 5 mm, 3/pk	186004629
BEH130, C_{18}, 1.7 µm VanGuard Pre-Column, 2.1 x 5 mm, 3/pk	186003975
In-line filter holder and six 0.2 µm stainless steel replacement filters	205000343
Five 0.2 µm stainless steel replacement filters and End Nuts for 205000343	700002775

Peptide Separation Technology HPLC Columns

Description	Inner Diameter	Length	Particle Size	Part No.
XBridge BEH130 C_{18}	1 mm	50 mm	3.5 µm	186003560
XBridge BEH130 C_{18}	1 mm	100 mm	3.5 µm	186003561
XBridge BEH130 C_{18}	1 mm	150 mm	3.5 µm	186003562
XBridge BEH130 C_{18}	2.1 mm	50 mm	3.5 µm	186003563
XBridge BEH130 C_{18}	2.1 mm	100 mm	3.5 µm	186003564
XBridge BEH130 C_{18}	2.1 mm	150 mm	3.5 µm	186003565
XBridge BEH130 C_{18}	2.1 mm	250 mm	3.5 µm	186003566
XBridge BEH130 C_{18}	4.6 mm	50 mm	3.5 µm	186003567
XBridge BEH130 C_{18}	4.6 mm	100 mm	3.5 µm	186003568
XBridge BEH130 C_{18}	4.6 mm	150 mm	3.5 µm	186003569
XBridge BEH130 C_{18}	4.6 mm	250 mm	3.5 µm	186003570
XBridge BEH130 C_{18}	1 mm	50 mm	5 µm	186003571
XBridge BEH130 C_{18}	1 mm	100 mm	5 µm	186003572
XBridge BEH130 C_{18}	1 mm	150 mm	5 µm	186003573
XBridge BEH130 C_{18}	2.1 mm	50 mm	5 µm	186003574
XBridge BEH130 C_{18}	2.1 mm	100 mm	5 µm	186003575
XBridge BEH130 C_{18}	2.1 mm	150 mm	5 µm	186003576
XBridge BEH130 C_{18}	2.1 mm	250 mm	5 µm	186003577
XBridge BEH130 C_{18}	4.6 mm	50 mm	5 µm	186003578
XBridge BEH130 C_{18}	4.6 mm	100 mm	5 µm	186003579
XBridge BEH130 C_{18}	4.6 mm	150 mm	5 µm	186003580
XBridge BEH130 C_{18}	4.6 mm	250 mm	5 µm	186003581
XBridge BEH300 C_{18}	1 mm	50 mm	3.5 µm	186003604
XBridge BEH300 C_{18}	1 mm	100 mm	3.5 µm	186003605
XBridge BEH300 C_{18}	1 mm	150 mm	3.5 µm	186003606
XBridge BEH300 C_{18}	2.1 mm	50 mm	3.5 µm	186003607
XBridge BEH300 C_{18}	2.1 mm	100 mm	3.5 µm	186003608
XBridge BEH300 C_{18}	2.1 mm	150 mm	3.5 µm	186003609
XBridge BEH300 C_{18}	2.1 mm	250 mm	3.5 µm	186003610
XBridge BEH300 C_{18}	4.6 mm	50 mm	3.5 µm	186003611
XBridge BEH300 C_{18}	4.6 mm	100 mm	3.5 µm	186003612
XBridge BEH300 C_{18}	4.6 mm	150 mm	3.5 µm	186003613
XBridge BEH300 C_{18}	4.6 mm	250 mm	3.5 µm	186003614
XBridge BEH300 C_{18}	1 mm	50 mm	5 µm	186003615
XBridge BEH300 C_{18}	1 mm	100 mm	5 µm	186003616
XBridge BEH300 C_{18}	1 mm	150 mm	5 µm	186003617
XBridge BEH300 C_{18}	2.1 mm	50 mm	5 µm	186003618
XBridge BEH300 C_{18}	2.1 mm	100 mm	5 µm	186003619
XBridge BEH300 C_{18}	2.1 mm	150 mm	5 µm	186003620
XBridge BEH300 C_{18}	2.1 mm	250 mm	5 µm	186003621
XBridge BEH300 C_{18}	4.6 mm	50 mm	5 µm	186003622
XBridge BEH300 C_{18}	4.6 mm	100 mm	5 µm	186003623
XBridge BEH300 C_{18}	4.6 mm	150 mm	5 µm	186003624
XBridge BEH300 C_{18}	4.6 mm	250 mm	5 µm	186003625

Peptide Preparative Columns

Peptide Separation Technology HPLC Columns

Description	Inner Diameter	Length	Particle Size	Configuration Style	Part No.
XBridge BEH130 C_{18}	10 mm	50 mm	5 µm	Column	186003582
XBridge BEH130 C_{18}	10 mm	100 mm	5 µm	Column	186003583
XBridge BEH130 C_{18}	10 mm	150 mm	5 µm	Column	186003584
XBridge BEH130 C_{18}	10 mm	250 mm	5 µm	Column	186003585
XBridge BEH130 C_{18}	19 mm	50 mm	5 µm	OBD Column	186003586
XBridge BEH130 C_{18}	19 mm	100 mm	5 µm	OBD Column	186003587
XBridge BEH130 C_{18}	19 mm	150 mm	5 µm	OBD Column	186003945
XBridge BEH130 C_{18}	4.6 mm	50 mm	10 µm	Column	186003648
XBridge BEH130 C_{18}	4.6 mm	100 mm	10 µm	Column	186003649
XBridge BEH130 C_{18}	4.6 mm	150 mm	10 µm	Column	186003650
XBridge BEH130 C_{18}	4.6 mm	250 mm	10 µm	Column	186003651
XBridge BEH130 C_{18}	10 mm	50 mm	10 µm	Column	186003652
XBridge BEH130 C_{18}	10 mm	100 mm	10 µm	Column	186003653
XBridge BEH130 C_{18}	10 mm	150 mm	10 µm	Column	186003654
XBridge BEH130 C_{18}	10 mm	250 mm	10 µm	Column	186003655
XBridge BEH130 C_{18}	19 mm	50 mm	10 µm	OBD Column	186003656
XBridge BEH130 C_{18}	19 mm	150 mm	10 µm	OBD Column	186003657
XBridge BEH130 C_{18}	19 mm	250 mm	10 µm	OBD Column	186003658
XBridge BEH130 C_{18}	30 mm	50 mm	10 µm	OBD Column	186003659
XBridge BEH130 C_{18}	30 mm	100 mm	10 µm	OBD Column	186003660
XBridge BEH130 C_{18}	30 mm	150 mm	10 µm	OBD Column	186003661
XBridge BEH130 C_{18}	30 mm	250 mm	10 µm	OBD Column	186003662
XBridge BEH300 C_{18}	10 mm	50 mm	5 µm	Column	186003626
XBridge BEH300 C_{18}	10 mm	100 mm	5 µm	Column	186003627
XBridge BEH300 C_{18}	10 mm	150 mm	5 µm	Column	186003628
XBridge BEH300 C_{18}	10 mm	250 mm	5 µm	Column	186003629
XBridge BEH300 C_{18}	19 mm	50 mm	5 µm	OBD Column	186003630
XBridge BEH300 C_{18}	19 mm	100 mm	5 µm	OBD Column	186003631
XBridge BEH300 C_{18}	19 mm	150 mm	5 µm	OBD Column	186003946
XBridge BEH300 C_{18}	4.6 mm	50 mm	10 µm	Column	186003663
XBridge BEH300 C_{18}	4.6 mm	100 mm	10 µm	Column	186003664
XBridge BEH300 C_{18}	4.6 mm	150 mm	10 µm	Column	186003665
XBridge BEH300 C_{18}	4.6 mm	250 mm	10 µm	Column	186003666
XBridge BEH300 C_{18}	10 mm	50 mm	10 µm	Column	186003667
XBridge BEH300 C_{18}	10 mm	100 mm	10 µm	Column	186003668
XBridge BEH300 C_{18}	10 mm	150 mm	10 µm	Column	186003669
XBridge BEH300 C_{18}	10 mm	250 mm	10 µm	Column	186003670
XBridge BEH300 C_{18}	19 mm	50 mm	10 µm	OBD Column	186003671
XBridge BEH300 C_{18}	19 mm	150 mm	10 µm	OBD Column	186003672
XBridge BEH300 C_{18}	19 mm	250 mm	10 µm	OBD Column	186003673
XBridge BEH300 C_{18}	30 mm	50 mm	10 µm	OBD Column	186003674
XBridge BEH300 C_{18}	30 mm	100 mm	10 µm	OBD Column	186003675
XBridge BEH300 C_{18}	30 mm	150 mm	10 µm	OBD Column	186003676
XBridge BEH300 C_{18}	30 mm	250 mm	10 µm	OBD Column	186003677

Peptide Separation Technology Guard Columns

Description	Inner Diameter	Length	Particle Size	Configuration Style	Part No.
XBridge BEH130 C_{18}	10 mm	10 mm	5 µm	Guard Column	186004469
XBridge BEH130 C_{18}	19 mm	10 mm	5 µm	Guard Column	186004468
XBridge BEH130 C_{18}	10 mm	10 mm	10 µm	Guard Column	186004465
XBridge BEH130 C_{18}	19 mm	10 mm	10 µm	Guard Column	186004464
XBridge BEH300 C_{18}	10 mm	10 mm	5 µm	Guard Column	186004471
XBridge BEH300 C_{18}	19 mm	10 mm	5 µm	Guard Column	186004470
XBridge BEH300 C_{18}	10 mm	10 mm	10 µm	Guard Column	186004467
XBridge BEH300 C_{18}	19 mm	10 mm	10 µm	Guard Column	186004466

Peptide Separation Technology OBD Columns

BEH130 and BEH300 C_{18} OBD Prep Columns, 5 µm and 10 µm

Inner Diameter	Length	µmoles of a Single Peptide	Weight of a Single Peptide (mg)	Typical Flow Rate (mL/min)
10 mm	50 mm	0.25-5	0.5-10	4.5-9
10 mm	100 mm	0.25-5	0.5-10	4.5-9
10 mm	150 mm	0.25-5	0.5-10	4.5-9
10 mm	250 mm	0.25-5	0.5-10	4.5-9
19 mm	50 mm	1-18	2.0-36	16-32
19 mm	100 mm	1-18	2.0-36	16-32
19 mm	150 mm	1-18	2.0-36	16-32
19 mm	250 mm	1-18	2.0-36	16-32

BEH130 and BEH300 C_{18} OBD Prep Columns, 10 µm

Inner Diameter	Length	µmoles of a Single Peptide	Weight of a Single Peptide (mg)	Typical Flow Rate (mL/min)
30	50	2.5-25	5-100	40-80
30	100	2.5-25	5-100	40-80
30	150	2.5-25	5-100	40-80
30	250	2.5-25	5-100	40-80

Literature References

Peptide Separation Technology Brochure, Literature Reference 720001835EN

UPLC Peptide Analysis Solution Brochure, Literature Reference 720001836EN

Optimum Bed Density (OBD) Preparative Column Technology Brochure, Literature Reference 720002336EN

Purification and Isolation Cartridge Holders

Description	Qty.	Part No.
10 x 10 mm Cartridge Holder		289000779
19 x 10 mm Cartridge Holder		186000709
Replacement o-ring 7.8 mm	2/pkg	700001019
Replacement o-ring 10 mm	2/pkg	700001436
Replacement o-ring 19 mm	2/pkg	700001020

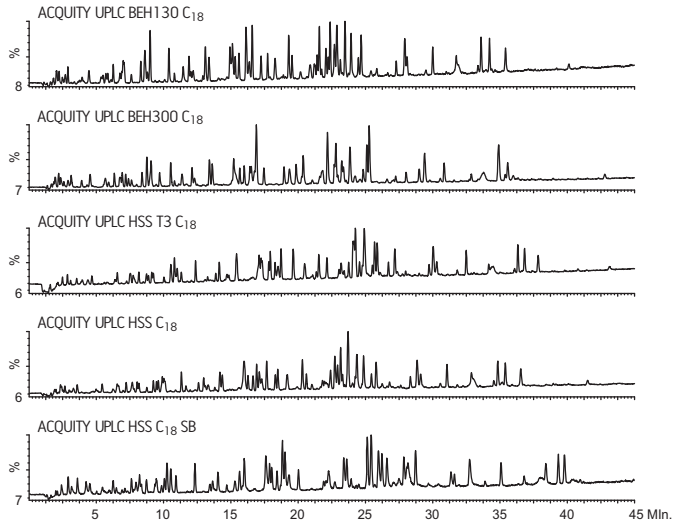
Waters Atlantis UPLC and HPLC Columns for Peptide Separations

The Peptide Separation Technology family of reversed-phase columns was developed to provide good resolution for both the analysis and lab-scale isolation of peptides. In addition to these offerings, Waters Atlantis® family of UPLC and HPLC offerings are useful when alternative column chemistries that exhibit different peptide separation selectivities are required for a particular application.

The Atlantis T3 series of analytical and preparative columns, available in 3-10 mm particles, are well suited for HPLC-based peptide analysis and purification needs. Waters ACQUITY® UPLC HSS C_{18}, HSS Shield RP18, HSS C_{18} SB and HSS T3 columns, when combined with the UPLC Instrumentation provide peptide component resolution and speed of analysis similar to that obtained with our BEH130 or BEH300, C_{18} UPLC columns.

Comparative Peptide Separations on Waters BEH and Silica-Based C_{18} Column Offerings

Columns:	2.1 x 100 mm				
Sample:	30 pmoles MassPREP phosphorylase digestion standard				
Detection:	ESI+; Waters Q-Tof Premier™				
Run Time:	70 min				
Temperature:	40 °C				
Solvent A:	0.1% Formic Acid in Water				
Solvent A:	0.1% Formic Acid in Acetonitrile				
Gradient:	Time(min)	Flow Rate	%A	%B	Curve
	Initial	0.200	95.0	5.0	
	1.00	0.200	95.0	5.0	6
	59.00	0.200	50.0	50.0	6
	60.00	0.200	30.0	70.0	6
	61.00	0.200	95.0	5.0	6



Atlantis T3 HPLC and ACQUITY HSS UPLC Analytical Columns

Inner Diameter	Length	Type	Atlantis T3, 3 µm	Atlantis T3, 5 µm	ACQUITY HSS T3, 1.8 µm	ACQUITY HSS C_{18}, 1.8 µm	ACQUITY HSS C_{18} SB, 1.8 µm
1.0 mm	50 mm	Column	186003713	186003730	186003535	186003529	186004114
1.0 mm	100 mm	Column	—	—	186003536	186003530	186004115
1.0 mm	150 mm	Column	186003714	186003731	186003537	186003531	186004116
2.1 mm	10 mm	Guard	186003756[1]	186003759[1]	—	—	—
2.1 mm	30 mm	Column	186003716	186003733	186003944	186003987	186004117
2.1 mm	50 mm	Column	186003717	186003734	186003538	186003532	186004118
2.1 mm	100 mm	Column	186003718	186003735	186003539	186003533	186004119
2.1 mm	150 mm	Column	186003719	186003736	186003540	186003534	186004120
3.0 mm	50 mm	Column	186003721	186003738	—	—	—
3.0 mm	100 mm	Column	186003722	186003739	—	—	—
3.0 mm	150 mm	Column	186003723	186003740	—	—	—
3.0 mm	250 mm	Column	—	186003741	—	—	—
3.9 mm	20 mm	Guard	186003757[2]	186003760[2]	—	—	—
4.6 mm	20 mm	Guard	186003758[2]	186003761[2]	—	—	—
4.6 mm	30 mm	Column	186003725	186003743	—	—	—
4.6 mm	50 mm	Column	186003726	186003744	—	—	—
4.6 mm	75 mm	Column	186003727	186003745	—	—	—
4.6 mm	100 mm	Column	186003728	186003746	—	—	—
4.6 mm	150 mm	Column	186003729	186003747	—	—	—
4.6 mm	250 mm	Column	—	186003748	—	—	—

[1] Requires Sentry Guard Holder WAT097958
[2] Requires Sentry Guard Holder WAT046910

Atlantis Prep Columns for Peptides

Inner Diameter	Length	Configuration Style	Particle Size	Part No. T3
10 mm	10 mm	Guard	5 µm	186003695[1]
10 mm	50 mm	Column	5 µm	186003691
10 mm	100 mm	Column	5 µm	186003692
10 mm	150 mm	Column	5 µm	186003693
10 mm	250 mm	Column	5 µm	186003694
19 mm	10 mm	Guard	5 µm	186003699[2]
19 mm	50 mm	OBD Column	5 µm	186003696
19 mm	100 mm	OBD Column	5 µm	186003697
19 mm	150 mm	OBD Column	5 µm	186003698
19 mm	250 mm	OBD Column	5 µm	186004026
30 mm	50 mm	OBD Column	5 µm	186003700
30 mm	75 mm	OBD Column	5 µm	186003701
30 mm	100 mm	OBD Column	5 µm	186003702
30 mm	150 mm	OBD Column	5 µm	186003703
50 mm	50 mm	OBD Column	5 µm	186004080
50 mm	100 mm	OBD Column	5 µm	186004081
50 mm	150 mm	OBD Column	5 µm	186004082
10 mm	10 mm	Guard	10 µm	186003706[1]
10 mm	150 mm	Column	10 µm	186003704
10 mm	250 mm	Column	10 µm	186003705
19 mm	10 mm	Guard	10 µm	186003710[2]
19 mm	50 mm	OBD Column	10 µm	186003707
19 mm	150 mm	OBD Column	10 µm	186003708
19 mm	250 mm	OBD Column	10 µm	186003709
30 mm	150 mm	OBD Column	10 µm	186003711
30 mm	250 mm	OBD Column	10 µm	186003712
50 mm	50 mm	OBD Column	10 µm	186004083
50 mm	100 mm	OBD Column	10 µm	186004084
50 mm	150 mm	OBD Column	10 µm	186004085
50 mm	250 mm	OBD Column	10 µm	186004086

[1] Requires 10 x 10 mm Prep Guard Holder 289000779
[2] Requires 19 x 10 mm Prep Guard Holder 186000709

Purification and Isolation Cartridge Holders

Description	Qty.	Part No.
10 x 10 mm Cartridge Holder		289000779
19 x 10 mm Cartridge Holder		186000709
Replacement o-ring 7.8 mm	2/pkg	700001019
Replacement o-ring 10 mm	2/pkg	700001436
Replacement o-ring 19 mm	2/pkg	700001020

Prep OBD Columns—Ideal for Purification Labs

Column lifetime of a preparative column is no longer a random event

- Developed in 2003, Waters has shipped over 10,000 prep columns without a single OBD column bed failure due to voiding. No other column manufacturer has been able to match Waters performance.

The critical difference

- Waters proprietary OBD Technology ensures excellent scalability and equivalent performance across a range of dimensions.

The right size to get the job done

- Waters offers an extensive set of Prep OBD columns including 19, 30, and 50 mm i.d.

Long, predictable lifetimes

- Customers have documented consistently achieving over 3,000 injections with their Waters Prep OBD columns, increasing productivity and saving money.

An Exploded View of the Elements of an Empty OBD Column

(Actual Prep OBD column lifetime depends on the quality of injected samples and operating conditions throughout the use of the column. For maximum column lifetime for peptide separations, injection of soluble, particulate-free peptides is required. Please refer to the OBD Care and Use Instructions or contact your Waters Representative for more information.)

BioSuite HPLC Peptide Analysis Columns

- Two HPLC column chemistries that provide alternative chemistries for peptide separations
- Designed for maximum resolution of complex digests
- Available in various configurations for LC or LC/MS applications
- Excellent batch-to-batch reproducibility for consistent results
- Uniquely QC-tested specifically for peptide mapping

BioSuite Peptide Analysis Series

BioSuite PA Series consists of two of Waters premier reversed-phase column chemistries specifically optimized for peptide mapping from simple to complicated digests.

BioSuite C_{18} 3 µm PA-A

BioSuite C_{18} 3 µm PA-A is a100Å, difunctional bonded, low ligand density, silica-based column.

- Specifically designed for excellent retention of polar peptides
- Ideal choice for LC/MS applications using formic acid (FA) that minimizes ion-suppression
- Excellent performance for traditional HPLC separations using low TFA concentrations (e.g., 0.025% TFA)

BioSuite C_{18} 3.5 µm PA-B

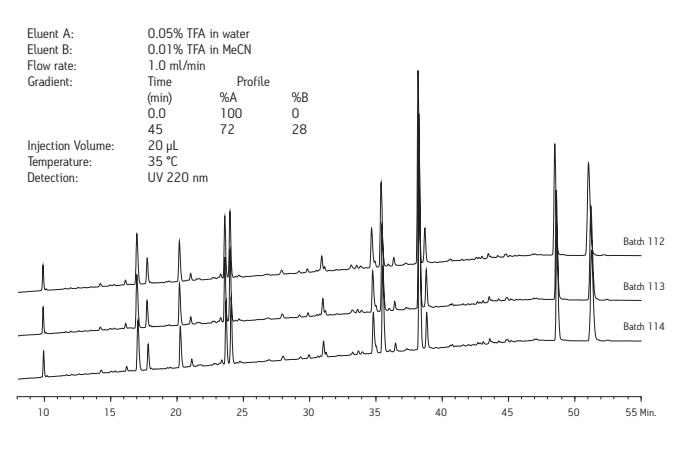
BioSuite C_{18} 3.5 µm PA-B is a 300Å, high ligand density, monofunctional, silica-based column.

- Outstanding performance when separating complex digests containing hydrophilic, hydrophobic, and basic peptides
- Superior peak shape and capacity for peptide separations using TFA containing eluents (e.g., 0.1% TFA)
- Good choice for the separation of larger peptide fragments generated by some endoproteases (e.g., Lys-C)

Consistent Results Due to Superior Batch-to-Batch Reproducibility

Waters batch release protocol includes a tryptic map of cytochrome *c* which is used to test for reproducibility to retention times and resolution. The three test chromatograms below show the results of the protein digest test for different batches of PA-B material.

Cytochrome *c* Tryptic Map Q.C. Test

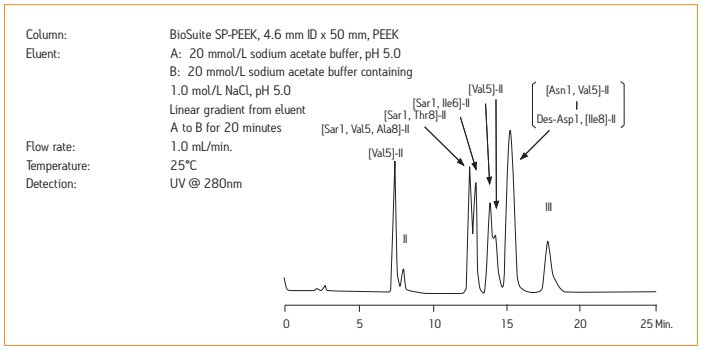


Inner Diameter	Length	BioSuite C_{18} 3 µm PA-A	BioSuite C_{18} 3.5 µm PA-B
2.1 mm	50 mm	186002425	186002433
2.1 mm	100 mm	186002426	186002434
2.1 mm	150 mm	186002427	186002435
2.1 mm	250 mm	186002428	186002436
4.6 mm	50 mm	186002429	186002437
4.6 mm	100 mm	186002430	186002438
4.6 mm	150 mm	186002431	186002439
4.6 mm	250 mm	186002432	186002440

BioSuite Cation-Exchange HPLC Columns

BioSuite SP NP, SP-PEEK, and SP cation-exchange chemistries (CXC) consists of the "strong" sulfopropyl ligand bonded to a pH stable (i.e., pH 2–12), methacrylic ester-based polymeric resin. The availability of different pore and particle size materials provides chromatographers with the flexibility required to isolate and or characterize peptides based upon minor charge differences. Non-porous (NP) and porous IEX columns are also available to meet various separations requirements. Speed and superior chromatographic resolution are possible using the non-porous IEX offerings while porous BioSuite offerings are available for applications requiring greater peptide binding capacity. In addition, BioSuite SP material is offered in PEEK™ hardware as well as in 21.5 mm i.d. stainless steel "lab-scale" preparative column dimensions.

Separation of Angiotensins on BioSuite SP-PEEK Cation-Exchange HPLC Column



BioSuite Q-PEEK and SP-PEEK columns are available in 4.6 x 50 mm

Description	Matrix	Pore Size	Exclusion Limit (Daltons) against Polyethylene Glycol	Inner Diameter	Length	Column Volume (mL)	# Approx Protein Binding Capacity Per Pre-Packed Column	Part No.
BioSuite SP-PEEK 7 µm CXC	Polymer	1300Å	>4,000,000	4.6 mm	50 mm	0.83	58 mg*	186002182
BioSuite SP 2.5 µm NP CXC	Polymer	n/a	500	4.6 mm	35 mm	0.58	2.9 mg**	186002183
BioSuite SP 10 µm CXC	Polymer	1000Å	1,000,000	7.5 mm	75 mm	3.31	132 mg**	186002184
BioSuite SP 13 µm CXC	Polymer	1000Å	1,000,000	21.5 mm	150 mm	54.45	2,178 mg**	186002185

* = Data generated with Gamma Globulin ** = Data generated with Hemoglobin
# Note: For best resolution of complex samples, do not exceed 20% of the column's protein binding capacity

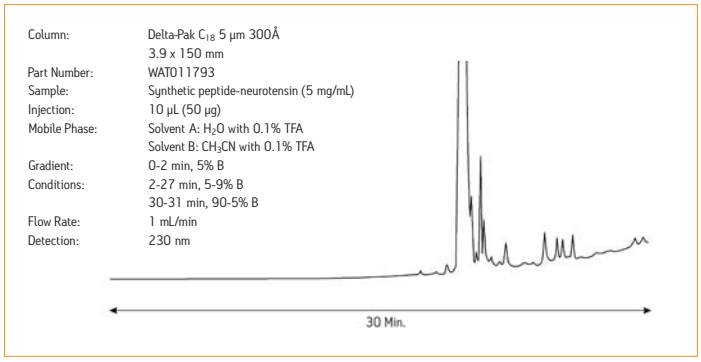
Delta-Pak HPLC Columns

Delta-Pak™ packings, ideal for the separation of peptides, proteins, and natural products, are based on a highly stable, bonded, endcapped 5 µm or 15 µm spherical silica. Delta-Pak is available in two different pore size materials (100Å and 300Å) with a C_{18} or C_4 bonded phase. For more information and part numbers, visit waters.com/biosep.

Physical Characteristics

Packing	Chemistry	Particle Size	Particle Shape	Pore Size	Carbon Load	End-capped
Delta-Pak	C_{18}	5 µm	Spherical	100Å	17%	Yes
	C_{18}	5 µm	Spherical	300Å	7%	Yes
	C_4	5 µm	Spherical	100Å	7%	Yes
	C_4	5 µm	Spherical	300Å	3%	Yes
	C_{18}	15 µm	Spherical	100Å	17%	Yes
	C_{18}	15 µm	Spherical	300Å	7%	Yes
	C_4	15 µm	Spherical	100Å	7%	Yes
	C_4	15 µm	Spherical	300Å	3%	Yes

Synthetic Peptide Separation on Delta-Pak C_{18} HPLC Column



Waters Insulin HMWP HPLC Column

The Waters Insulin HMWP column is specifically designed for use in the manufacture and quality control of insulin products. This column is tested for performance in the analysis of impurities with molecular masses greater than that of insulin.

Description	Dimensions	Part No.
Waters Insulin HMWP Column	7.8 x 300 mm	WAT201549
Protein-Pak 125 Sentry Guard Column, 2/pkg (requires holder)	3.9 x 20 mm	186000926
Sentry™ Universal Guard Column Holder		WAT046910

Tested to perform in the method published in PharmaEuropa Vol. 8, No 3, pages 359-360, September 1996.

Symmetry HPLC Columns

Waters Symmetry®, reversed-phase, silica-based particles are synthesized using ultrapure organic reagents, resulting in high purity material with very low silanol activity. When combined with the high surface coverage of the bonded phase, outstanding peptide separations and recoveries are possible.

- Superior manufacturing control for consistent batch-to-batch and column-to-column results
- 100Å and 300Å pore size offerings for small or larger size peptides
- SymmetryShield™ column chemistry offers complementary selectivity to Symmetry column offerings
- SymmetryPrep™ columns provide direct scale up while maintaining resolution

Symmetry300 Columns: The First Columns Specifically Engineered for the Discovery and Development of New Biopharmaceuticals

As a scientist, you know that tremendous time and resources are required when developing HPLC assays for well-characterized biopharmaceuticals. You also know that column-to-column variability is the "Achilles heel" of this demanding process.

In the past you've had no choice but to deal with variability by finding costly and time-consuming ways around this problem. The regulatory guidelines being adopted worldwide as a result of the International Conference on Harmonization (ICH) and the U.S. Food and Drug Administration's specified biological products initiatives are placing increasingly stringent demands on this process. Full analytical characterization is becoming more important for regulatory filing, making column variability an unacceptable risk.

Now, regardless of whether you're developing HPLC assays for purity, stability, or identity, you can have confidence in the long-term compliance of your methods, batch-to-batch, column-after-column, year-after-year. That's because Waters has developed Symmetry300™ columns, specifically engineered for the discovery and development of new biopharmaceuticals.

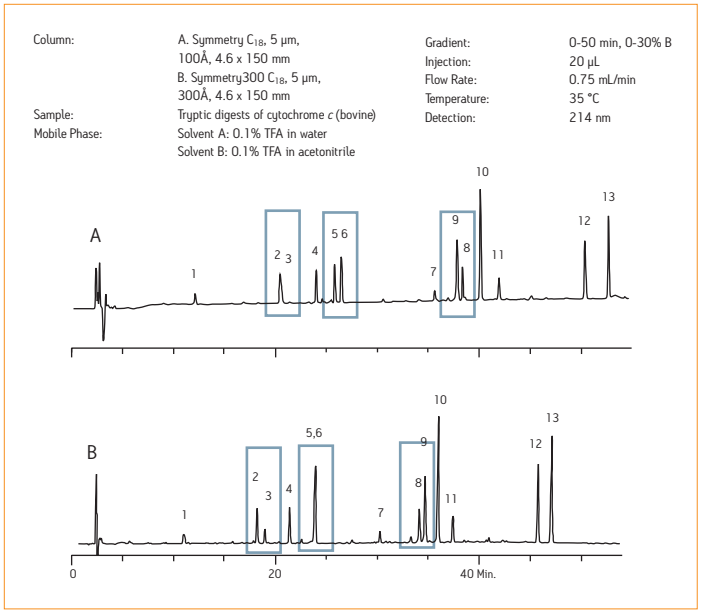
Symmetry300 columns are a 300Å reversed-phase addition to the existing Symmetry family of columns. They have been specifically designed to provide maximum batch-to-batch and column-to-column performance consistency and recovery of protein and peptide applications. Symmetry300 columns are offered in two particle sizes (3.5 µm and 5 µm) and in two chemistries (C_4 for large peptides and proteins and C_{18} for smaller peptides) to address various needs.

High Recoveries of Peptides and Proteins

The heart of the column is high purity-based deactivated silica. Waters dedicated chromatography chemistry manufacturing plant operates under the stringent standards of cGMP supplemented with FDA registration for Class 1 medical devices and ISO 9002. The silica used in the manufacture of our Symmetry300 columns is synthesized using ultrapure organic reagents that yields high purity particles with very low silanol activity. These particles when combined with innovative ligand (i.e., C_4 and C_{18}) bonding techniques helps produce reversed-phase columns with minimal non-desired secondary interactions between bound ligand and biomolecules.

There are several factors to assess when developing a reversed-phase HPLC method for peptides. Batch-to-batch reproducibility of the column is an important consideration when developing a validated and transferable method.Symmetry300 columns have outstanding batch-to-batch and column-to-column reproducibility.

Pore Size Effects on Peptide Selectivity: Comparative Results on Symmetry 100Å vs. Symmetry300 Columns

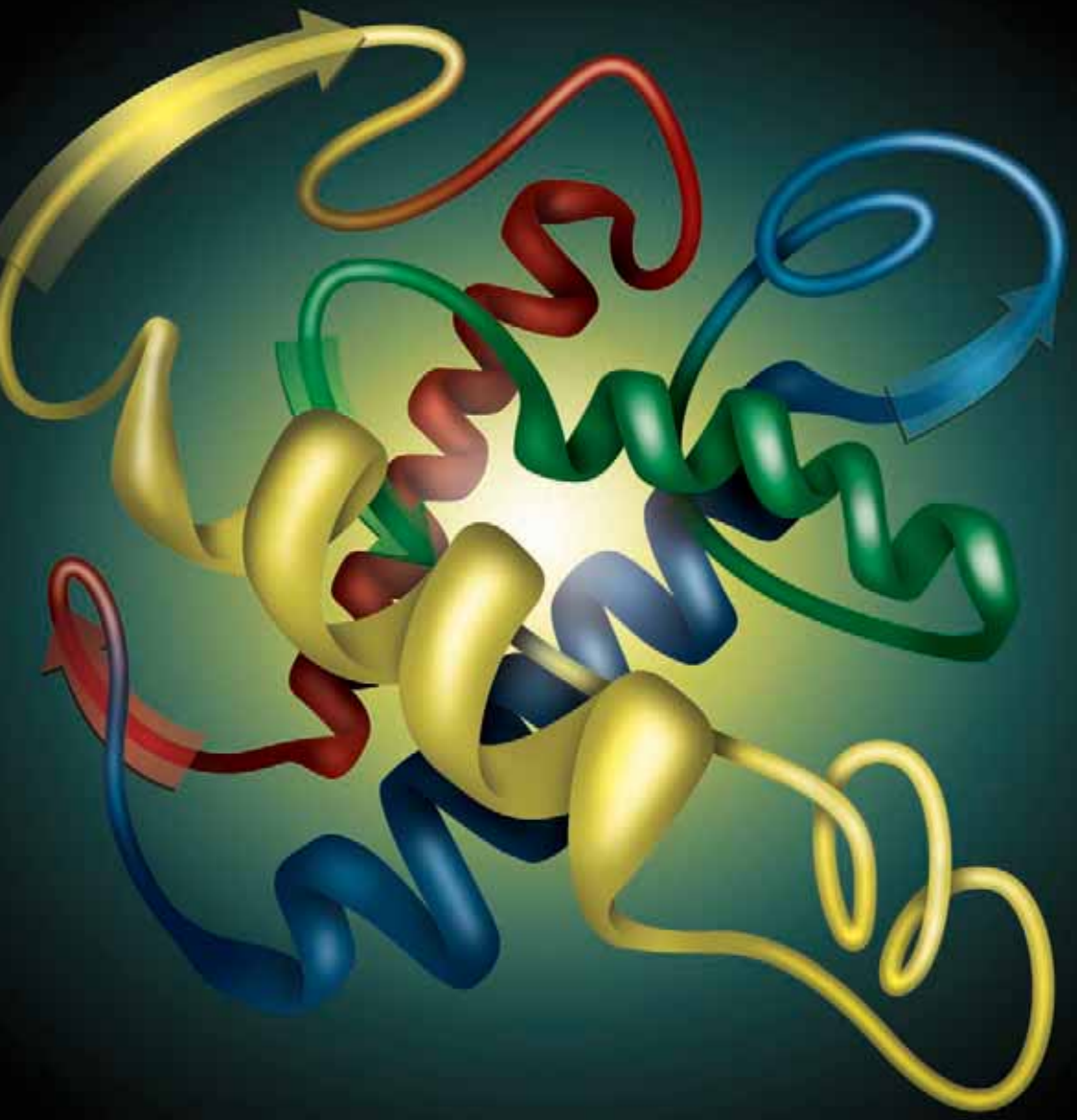


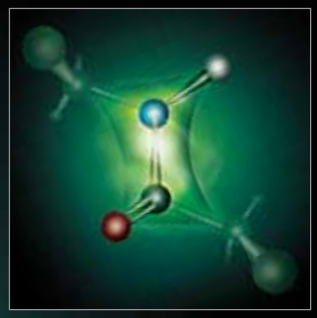
The key to a successful separation is the selection of a column that gives the highest chemistry resolution with maximum peak capacity and recovery.

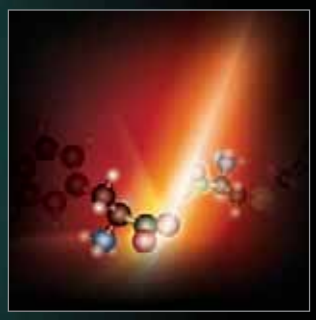
Symmetry300 Columns

Inner Diameter	Length	Particle Size	C_{18}	C_4
1.0 mm	150 mm	3.5 µm	186000185	186000276
2.1 mm	50 mm	3.5 µm	186000187	186000277
2.1 mm	100 mm	3.5 µm	186000188	186000278
2.1 mm	150 mm	3.5 µm	186000200	186000279
4.6 mm	50 mm	3.5 µm	186000201	186000280
4.6 mm	75 mm	3.5 µm	186000189	186000281
4.6 mm	100 mm	3.5 µm	186000190	186000282
4.6 mm	150 mm	3.5 µm	186000197	186000283
2.1 mm	150 mm	5 µm	WAT106172	186000285
3.9 mm	150 mm	5 µm	WAT106154	186000286
4.6 mm	50 mm	5 µm	WAT106209	186000287
4.6 mm	150 mm	5 µm	WAT106157	186000288
4.6 mm	250 mm	5 µm	WAT106151	186000289
19 mm	10 mm	5 µm	186001847	—
19 mm	50 mm	5 µm	186001848	—
19 mm	100 mm	5 µm	186001849	—

For more complete listings, go to www.waters.com







[BIOMOLECULES]

Waters Hybrid Particle Technology has helped chromatographers better separate and analyze amino acids, peptides, and oligonucleotides. Now see how Waters new BEH300 C_4 UPLC® and HPLC columns can improve your protein characterizations.

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PROTEINS

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Protein Isolation and Characterization

Selecting the Separation Mode

There are five principals of chromatography used for protein purification; ion exchange, size exclusion, reversed phase, affinity, and hydrophobic interaction.

A protein purification protocol will always include several steps. The techniques should be used in a sequence that reflects different selectivity mechanisms. The order of steps should be chosen to move from high capacity and high speed to higher resolution.

Ion-Exchange Chromatography

In ion-exchange separations, the distribution and net charge on the protein's surface determines the interaction of the protein with the charged groups on the packing material's surface. The charges on the protein and the packing material must be opposite for interaction to occur. The variables that can be manipulated, such as buffer pH, buffer composition, gradient slope, and gradient forming salt, offer a wide range of options for optimizing a separation.

Size-Exclusion Chromatography

The apparent size and shape of proteins in solution is the basis for separation in size-exclusion chromatography. This simple separation mechanism relies on protein size differences, packing material, pore size, and particle size as well as the operating conditions, such as sample concentration and volume for the successful resolution of protein mixtures. Size-exclusion packing materials have evolved from the large particle polysaccharide soft gels to small, rigid packings that offer enhanced resolution and significantly faster separations.

Size exclusion has a wide range of applicability in protein isolations. It is often used to assay fractions, as run times are relatively short, and the technique relatively straightforward. It can also be used for buffer exchange, as the low molecular weight salt molecules are easily separated from the much larger proteins.

Reversed-Phase Chromatography

Reversed phase is an alternative hydrophobic-separation technique that relies on the interaction between the protein's non-polar amino acid side chains with the packing surface and ligands. This mechanism provides another high resolution dimension for protein chromatography that can yield good recovery of biological activity with lower molecular weight proteins and peptides. It is an excellent tool for assessing fraction purity and for preparing proteins for sequencing or structural analysis.

Affinity Chromatography

Affinity is the only chromatographic mode in which proteins are isolated on the basis of their biospecific interaction with an immobilized ligand. Proteins can be recovered with retention of bioactivity and high purity. Modern affinity packings take advantage of rigid base materials that combine enhanced bed stability for crude sample processing with high throughput for rapid microgram to gram scale affinity purifications. Many separations can be achieved in a single step resulting in more cost-effective separations.

Hydrophobic-Interaction Chromatography

In this mode, proteins are bound to a moderately hydrophobic surface at high ionic strength. The protein's solubility is lowered under these conditions. The protein is then eluted with a gradient of decreasing ionic strength. Hydrophobic-interaction chromatography often has good resolution and retains biological activity. Aqueous buffer systems are used in hydrophobic interaction. Many different factors affect resolution, including buffer salt concentration and type, buffer pH, surfactant, modifiers, column temperature, and packing material functionality. Since proteins may also interact with one another under conditions of high ionic strength, better resolution is achieved when the starting material is already partially purified. It is also inconvenient to load large volumes of sample at high ionic strength. This would suggest that hydrophobic interaction would normally be a good second or subsequent step in an isolation scheme, such as when an ammonium sulfate precipitation occurs prior to the chromatographic protocol.

Particle Considerations for Lab-Scale Purifications

High resolution traditional HPLC packings for protein chromatography offer familiar chemical functionalities bonded onto rigid, spherical polymeric or silica supports. These packings provide improved chemical and physical stability, better reproducibility and dramatically faster separations than traditional soft gels. Columns containing lab-scale preparative packings (8, 15, or 40 µm) produce more concentrated fractions. The hydrophilic polymer with familiar functional groups result in high recovery of biological activity.

Pore Size Considerations

Good peak shape and recovery for proteins had been observed on the 100-130Å pore size materials; however, the bias is toward the 300Å packings for protein samples. The best advice is to test both 130Å and larger pore sizes (>300Å) as a step in the development of an optimized protein separation. Purification and analysis media is available in pore sizes ranging from 130Å to 4000Å.

Sample Preparation

Protein samples derived from tissue homogenates, cell lysis, or physiologic fluids frequently require removal of particulate contaminants prior to LC, MS, or LC/MS analysis. In some situations, use of an on-line protein desalting device (e.g., MassPREP on-line desalting cartridge) can effectively be used to remove ion-suppressing compounds prior to mass determination by LC/MS techniques. Other situations exist where simple filtration or centrifugation can be effectively used to remove insoluble components prior to analysis. Glycoproteins offer additional challenges that can be addressed via the use of specialty reagents such as Waters *Rapi*Gest SF surfactant that has been shown to significantly enhance protein deglycosylation. In addition, solid-phase extraction techniques, such as those offered in Waters MassPREP Deglycosylation Kit and Plate, can effectively isolate and concentrate glycans, cleaved from gylcoproteins, prior to analysis. Accell™ Plus ion-exchanger cartridges are also available for solid-phase extraction of proteins from complex matrices or for isolation or sample concentration.

Strengths of Different Modes of Chromatography

Ion Exchange	Hydrophobic Interaction	Affinity	Reversed Phase	Size Exclusion
Concentrates sample	Concentrates sample	Concentrates sample	Concentrates sample	Rapid
Column can be cleaned using NaOH	Column can be cleaned using NaOH	Superb selectivity	Good resolution for proteins and peptides	Good recovery of activity
Moderate to high resolution	Moderate resolution	Outstanding resolution	Simple technique	Reliable medium to high resolution technique
High capacity	High capacity	High capacity	Moderate capacity	Can be used to buffer exchange
Good recovery of activity	Good recovery of activity	Good recovery of activity		Compatible with detergents
Widespread applicability		Significant purification in a single step		

Limitations of Different Modes of Chromatography

Ion Exchange	Hydrophobic Interaction	Affinity	Reversed Phase	Size Exclusion
Fractions may need to be desalted prior to the next purification step	Fractions may need to be desalted prior to the next purification step	Ligand leakage	Limited applicability with high MW proteins	Moderate resolution
		Column cannot be cleaned using aggressive chemicals	Column cannot be cleaned using aggressive chemicals	Non concentrating
		Stationary phases can be expensive	Organic mobile phase may denature protein	Moderate capacity

Planning a Protein Isolation Protocol

The design of an overall chromatography protocol depends on many factors, such as the sample volume, the cleanliness of the starting material, whether economic affinity supports are available, and what the final use of the purified protein might be.

For instance, many of the initial feed streams for protein isolations are relatively dilute protein solutions. Chromatographic modes that concentrate samples (ion exchange, hydrophobic interaction, and affinity) are good starting points for these solutions.

For samples such as cell lysates and homogenates or physiologic fluids, there are additional considerations. These mixtures contain cell fragments, lipids, and nucleic acid fragments, which will bind strongly to a column. Columns becoming contaminated in this way are typically cleaned using 0.1 M -1.0 M NaOH prior to re-equilibration with the starting buffers. This would destroy a peptide or protein ligand on an affinity column and would irreversibly damage a silica-based, reversed-phase column. Therefore, ion exchange or hydrophobic interaction would be appropriate modes for a first purification step.

In reversed-phase chromatography, the sample is loaded onto the column in an aqueous environment and then eluted using an increasing gradient of an organic solvent, such as acetonitrile or propanol. The major drawback to this mode of chromatography is that some proteins will denature (causing loss of biological activity) in the presence of the organic solvents at the concentration required to elute the protein. As a general rule, the likelihood of denaturation increases with increasing molecular weight of the protein.

Typical Isolation Protocol

Taking all these facts into consideration, a typical purification protocol would start with an ion-exchange step, as the resolving power is good where a dilute feed stream can be concentrated prior to elution and the packing materials can be cleaned using aggressive agents. As the protein is usually eluted using an increasing salt gradient, the active fractions often contain high concentrations of salt. It is sometimes necessary to desalt the fractions prior to the next step. This can be avoided if the next step is hydrophobic interaction, where the protein is loaded onto the column in a concentrated salt solution. In some instances, especially where the feedstream comes from an ammonium sulfate precipitation, hydrophobic interaction may be a good first purification step, followed by ion exchange.

The eluate from this ion-exchange and/or hydrophobic-interaction step is usually free of lipids and other contaminants that may cause problems with an affinity column. It can now be put onto an affinity column should one be available. The use of an affinity column is possible, if available, once lipids and other contaminants have been removed by the ion-exchange and/or hydrophobic-interaction step. Alternatively, reversed-phase chromatography or a second ion-exchange step under different conditions or with higher resolving power (smaller particle size or longer column) can be used. Both of these approaches result in high resolution and sample concentration.

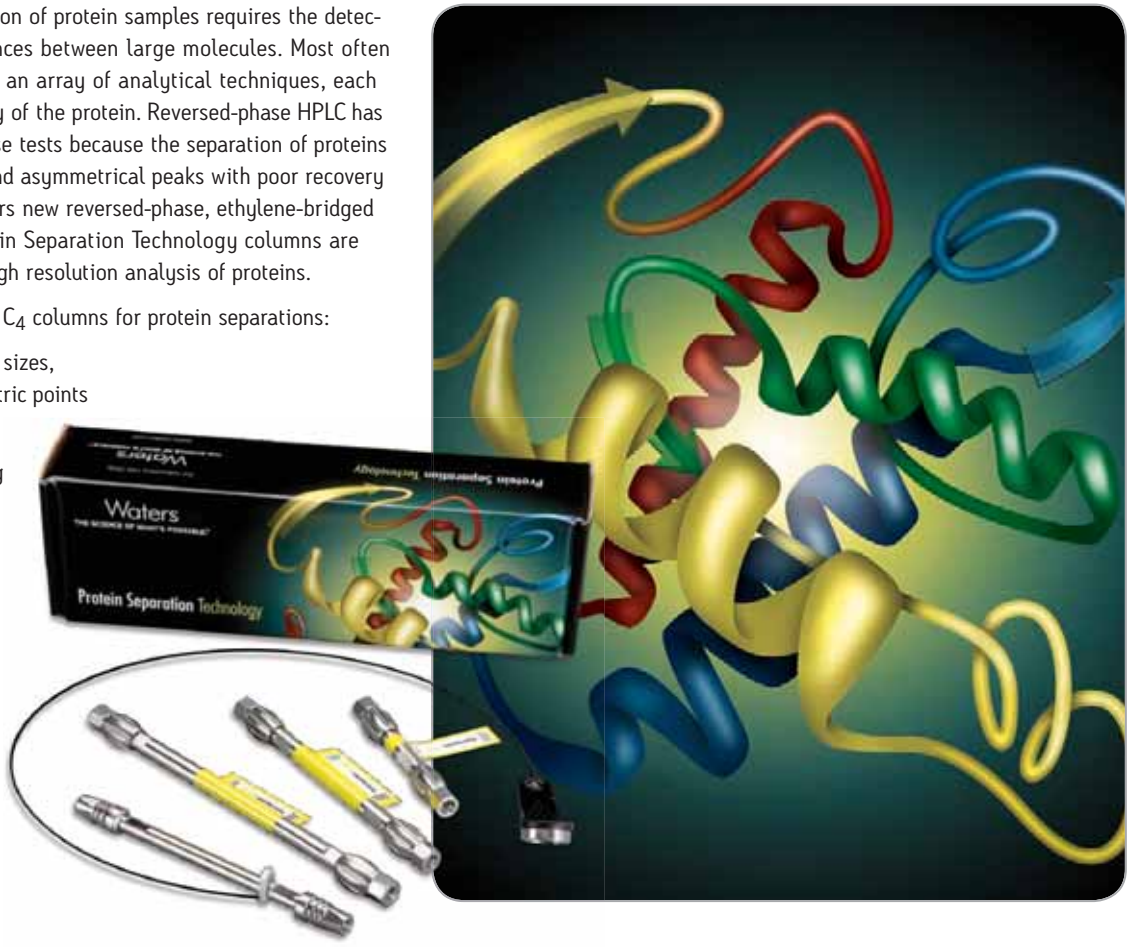
The final step in the purification is often size exclusion, which will separate out dimers and polymers of the protein of interest, and potentially desalt the protein.

Protein Separation Technology

The analysis and characterization of protein samples requires the detection of small chemical differences between large molecules. Most often these analyses have employed an array of analytical techniques, each sensitive to a different property of the protein. Reversed-phase HPLC has not been fully exploited in these tests because the separation of proteins often yields relatively broad and asymmetrical peaks with poor recovery and significant carryover. Waters new reversed-phase, ethylene-bridged hybrid (BEH Technology) Protein Separation Technology columns are specifically designed for the high resolution analysis of proteins.

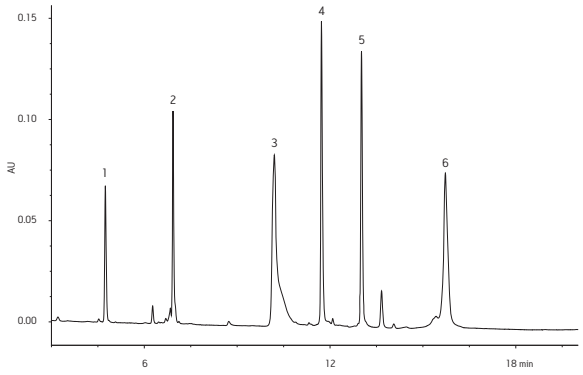
Waters New Family of BEH300 C_4 columns for protein separations:

- Separates proteins of various sizes, hydrophobicities, and isoelectric points
- Available as 3.5 µm packing for HPLC and 1.7 µm packing for UPLC methods
- Maximizes recovery and minimizes protein carryover
- Tolerates extreme pH and temperature
- Quality-control tested with protein mixture
- Couples directly to ESI-MS for protein identification



300Å C4 Columns Developed for Protein Chromatography

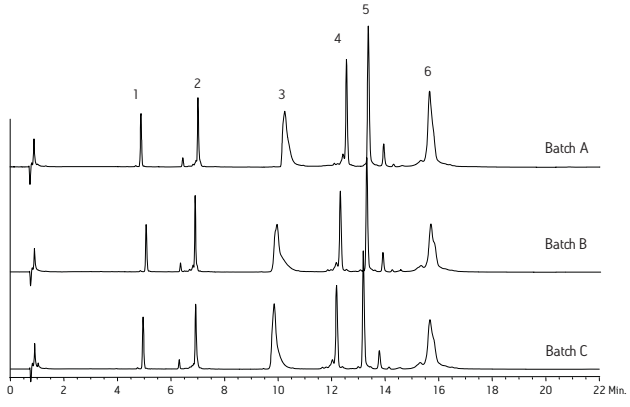
Column:	XBridge™ BEH300 C_4, 3.5 µm, 2.1 x 50 mm	Sample: Protein mixture prepared in
Mobile Phase A:	0.1% TFA in water	0.1% TFA in 5% acetonitrile
Mobile Phase B:	0.075% TFA in 71.4% acetonitrile	1. Ribonuclease A (0.04 mg/mL)
Flow Rate:	0.2 mL/min	2. Cytochrome c (0.06 mg/mL)
Gradient:	28-100% B in 25 min	3. Bovine Serum Albumin (0.20 mg/mL)
Column Temp.:	40 °C	4. Myoglobin (0.13 mg/mL)
Injection:	5 µL	5. Enolase (0.22 mg/mL)
Detection λ:	220 nm	6. Phosphorylase b (0.59 mg/mL)



BEH300 C_4 columns can be used with proteins that have a wide range of properties. This protein mix was chosen to represent a range of isoelectric points, molecular weights, and hydrophobicities.

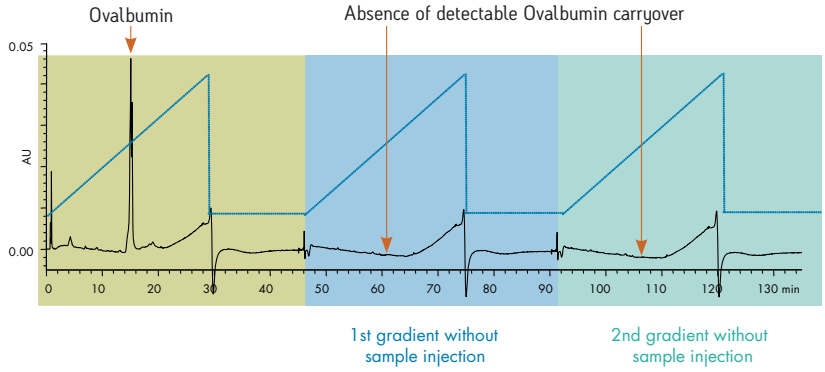
Batch-to-Batch Reproducibility

Column:	ACQUITY UPLC BEH300 C_4, 1.7 µm, 2.1 mm x 50 mm	Sample: Protein mixture prepared in
Eluent A:	0.1% TFA in 100% HPLC grade water	0.1% TFA in 5% acetonitrile
Eluent B:	0.075% TFA in 71.4% acetonitrile	1. Ribonuclease
Flow Rate:	0.2 mL/min	2. Cytochrome *c*
Gradient:	28-100% B in 25 min	3. BSA
Column Temp.:	40 °C	4. β-Lactoglobulin
Detection λ:	220 nm	5. Enolase
		6. Phosphorylase b



This comparison shows the consistent batch-to-batch performance with a protein separation.

Minimal Protein Carryover

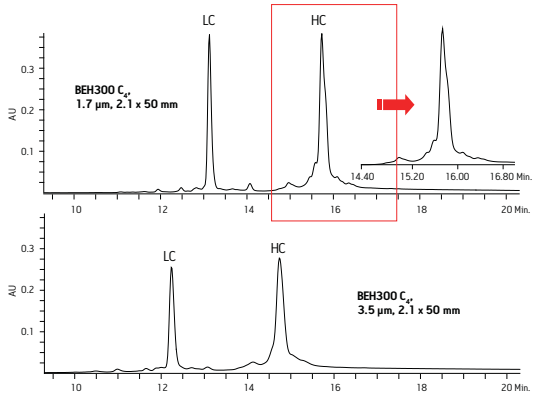


Sample:	1.3 µg Ovalbumin injected
Columns:	ACQUITY UPLC BEH300 C4, 1.7 µm, 2.1 mm x 50 mm
Eluent A:	0.1% TFA in 100% HPLC grade water
Eluent B:	0.075% TFA in 71.4% acetonitrile
Flow Rate:	0.2 mL/min
Gradient:	28-100% B in 25 min Hold at 100 %B for 2 min then re-equilibrate column at 28% B for 18 min Gradient restarted at 45 min and at 90 min
Column Temp.:	40 °C
Detection λ:	220 nm

Column carryover was tested by running multiple gradients following a single injection. Protein peaks observed during the first gradient are not found in subsequent gradients.

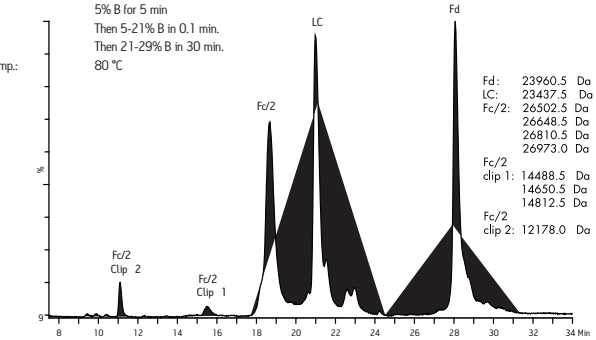
LC/UV Analysis of Reduced Monoclonal Antibody

Sample:	Reduced monoclonal antibody (IgG1), 0.5 µg/µl	Flow Rate:	0.2 mL/min
		Gradient:	20-50% B in 20 min
Column:	ACQUITY UPLC BEH 300 C_4, 2.1 x 50 mm, 1.7 µm (top), 3.5 µm (bottom)	Column Temp:	80 °C
		Injection:	5 µl
Mobile Phase A:	0.03%TFA in Water	Detection:	215 nm
Mobile Phase A:	0.03%TFA in ACN		



BEH300 C_4 Columns for Protein Characterization with UPLC / MS

Sample:	Lys-C digested (limited) reduced monoclonal antibody	Waters LCT Premier™ XE MS Detection	
		Capillary Voltage:	3200 V
Column:	ACQUITY UPLC BEH300 C_4, 1.7 µm, 2.1 x 50 mm	Cone Voltage:	45 V
		Source Temp.:	150 °C
Eluent A:	0.1% FA in 100% HPLC grade water	Desolvation Temp.:	350 °C
Eluent B:	0.1% FA in 100% acetonitrile	Desolvation Gas:	000 L/h
Flow Rate:	0.2 mL/min		
Gradient:	5% B for 5 min Then 5-21% B in 0.1 min. Then 21-29% B in 30 min.		
Column Temp.:	80 °C		



The large fragments obtained through LysC digestion of a monoclonal antibody can be separated on the UPLC BEH300 C_4 column coupled directly to ESI/Tof MS for identification of the individual peptide products.

Ordering information for Waters BEH300 C_4 offerings for traditional HPLC and advanced UPLC protein separations are shown below.

UPLC Columns	Particle Size	Dimensions	Part No.
ACQUITY UPLC BEH300 C_4	1.7 µm	2.1 x 50 mm	186004495
ACQUITY UPLC BEH300 C_4	1.7 µm	2.1 x 100 mm	186004496
ACQUITY UPLC BEH300 C_4	1.7 µm	2.1 x 150 mm	186004497
ACQUITY UPLC BEH300 C_4	1.7 µm	VanGuard™ Pre-Column	186004623

Note: ACQUITY UPLC BEH300 C_4, 1.7 µm columns are designed for use with the ACQUITY UPLC system. The benefits of the small particle packing in ACQUITY UPLC BEH300 C_4, 1.7 µm columns are only realized with the low system volume and low detector dispersion of an ACQUITY UPLC system.

nanoACQUITY UPLC Columns (10,000 psi)	Particle Size	Dimensions	Part No.
nanoACQUITY UPLC BEH300 C_4	1.7 µm	75 µm x 100 mm	186004639
nanoACQUITY UPLC BEH300 C_4	1.7 µm	100 µm x 100 mm	186004640
nanoACQUITY UPLC BEH300 C_4	1.7 µm	150 µm x 100 mm	186004641

For use with nanoACQUITY UPLC systems rated to 10,000 psi only. Not for use with nanoACQUITY UPLC systems rated to 5,000 psi.

HPLC Columns	Particle Size	Dimensions	Part No.
XBridge BEH300 C_4	3.5 µm	2.1 x 50 mm	186004498
XBridge BEH300 C_4	3.5 µm	2.1 x 100 mm	186004499
XBridge BEH300 C_4	3.5 µm	2.1 x 150 mm	186004500
XBridge BEH300 C_4	3.5 µm	2.1 x 250 mm	186004501
XBridge BEH300 C_4	3.5 µm	4.6 x 50 mm	186004502
XBridge BEH300 C_4	3.5 µm	4.6 x 100 mm	186004503
XBridge BEH300 C_4	3.5 µm	4.6 x 150 mm	186004504
XBridge BEH300 C_4	3.5 µm	4.6 x 250 mm	186004505
Custom BEH300 C_4			186004506

BioSuite HPLC Columns for Protein Separations

BioSuite™ Columns

- Ion-exchange, size-exclusion, hydrophobic-interaction and reversed-phase column offerings
- Excellent resolution and recovery of proteins and peptides
- Available in different particle and pore sizes
- Scalable from analytical to "lab-scale" preparative applications

Waters BioSuite HPLC columns for protein and peptide separations contain new high-performance chemistries dedicated to the isolation, analysis, and characterization of biomolecules. Separation offerings include ion-exchange, size-exclusion, hydrophobic-interaction, and reversed-phase columns and support Waters array of LC and LC/MS systems.

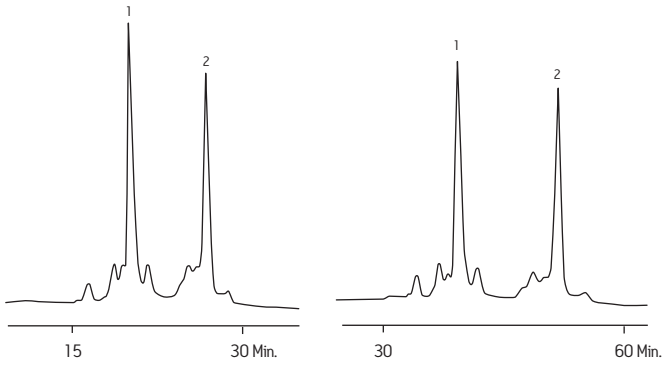
Waters Alliance® Bioseparations System

The latest information on the BioSuite family of application-driven solutions that address separations needs in the areas of well characterized biopharmaceuticals, genomics, proteomics, and well characterized biotherapeutics are available online at www.waters.com/biosep.

Predictable Ion-Exchange Chromatography on BioSuite Analytical and "Lab-Scale" Preparative DEAE AXC Columns

A) BioSuite DEAE 10 µm, 7.5 mm i.d. x 75 mm

B) BioSuite DEAE 13 µm, 21.5 mm i.d. x 150 mm

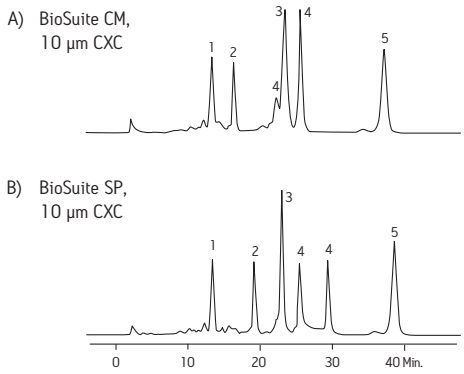


Sample:	Mixture of Ovalbumin (peak 1) and Trypsin Inhibitor (peak 2) A: 0.2 mg each in 0.1 mL Eluent A B: 2 mg each in 1 mL Eluent A
Eluent A:	20 mM Tris-HCl, pH 8.0
Eluent B:	20 mM Tris-HCl, pH 8.0 containing 0.5 M NaCl
Flow Rate:	A: 1.0 mL/min B: 6.0 mL/min
Gradient:	A: Linear gradient from 0 to 100%B in 60 min. B: Linear gradient from 0 to 100%B in 120 min.
Temperature:	25 °C
Detection:	280 nm

BioSuite Ion-Exchange HPLC Columns

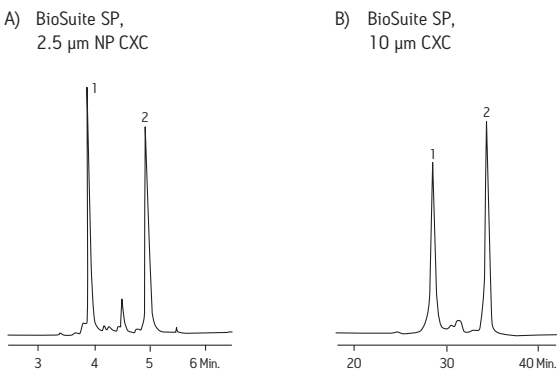
BioSuite ion-exchange column offerings include strong and weak, cation- (CXC) and anion exchangers (AXC) bonded to a pH stable (i.e., pH 2-12), methacrylic ester-based polymeric resin. The availability of four separation chemistries provides chromatographers with the flexibility required to develop methods that separate proteins or peptides based upon minor charge differences. Non-porous (NP) and porous IEX columns are also available. Speed and superior chromatographic resolution are possible using the NP IEX offerings. Waters porous ion exchangers are available for applications requiring greater protein or peptide binding capacity. In addition, selected BioSuite ion-exchange columns are offered in PEEK hardware as well as in 21.5 mm i.d. preparative column sizes.

Protein Selectivity Differences on BioSuite CM (Weak Cation-Exchange) vs. SP (Strong Cation-Exchange) Columns



Sample:	Trypsinogen (Peak 1), Ribonuclease (Peak 2), Alpha-Chymotrypsinogen A (Peak 3), Cytochrome *c* (Peak 4), and Lysozyme (Peak 5)
Columns:	A: BioSuite CM, 10 µm CXC (7.5 x 75 mm) B: BioSuite SP, 10 µm CXC (7.5 x 75 mm)
Eluent: A:	20 mM Sodium Phosphate, pH 7.0
Eluent B:	20 mM Sodium Phosphate, pH 7.0 containing 0.5 M NaCl
Flow Rate:	1.0 mL/min
Gradient:	Linear gradient from 0 to 100% B in 60 min.
Temperature:	25 °C
Detection:	280 nm

Enhanced Compound Resolution by Ion-Exchange Chromatography on BioSuite SP Non-Porous (NP) vs. Porous CXC Columns



Sample:	Alpha-Chymotrypsinogen A (Peak 1) and Ribonuclease (Peak 2)
Columns:	A: BioSuite SP, 2.5 µm NP CXC (4.6 x 35 mm) B: BioSuite SP, 10 µm CXC (7.5 x 75 mm)
Eluent: A:	20 mM MES [2[N-morpholino]ethanesulfonic acid], pH 6.0
Eluent B:	20 mM MES, pH 6.0 containing 0.5 M NaCl
Flow Rate:	A: 1.5 mL/min B: 1.0 mL/min
Gradient:	A: Linear gradient from 0 to 100% B in 10 min. B: Linear gradient from 0 to 100% B in 60 min.
Temperature:	25 °C
Detection:	280 nm

BioSuite Q-PEEK and SP-PEEK columns are available in 4.6 x 50 mm

Description	Matrix	Pore Size	Exclusion Limit (Daltons) against Polyethylene Glycol	Inner Diameter	Length	Column Volume (mL)	# Approx Protein Binding Capacity Per Pre-Packed Column	Part No.
BioSuite Q-PEEK 10 µm AXC	Polymer	4000Å	>5,000,000	4.6 mm	50 mm	0.83	58 mg*	186002176
BioSuite SP-PEEK 7 µm CXC	Polymer	1300Å	>4,000,000	4.6 mm	50 mm	0.83	58 mg**	186002182
BioSuite DEAE 2.5 µm NP AXC	Polymer	n/a	500	4.6 mm	35 mm	0.58	2.9 mg*	186002179
BioSuite SP 2.5 µm NP CXC	Polymer	n/a	500	4.6 mm	35 mm	0.58	2.9 mg***	186002183
BioSuite Q 10 µm AXC	Polymer	1000Å	1,000,000	7.5 mm	75 mm	3.31	331 mg*	186002177
BioSuite Q 13 µm AXC	Polymer	1000Å	1,000,000	21.5 mm	150 mm	54.45	5,445 mg*	186002178
BioSuite DEAE 10 µm AXC	Polymer	1000Å	1,000,000	7.5 mm	75 mm	3.31	99 mg*	186002180
BioSuite DEAE 13 µm AXC	Polymer	1000Å	1,000,000	21.5 mm	150 mm	54.45	1633 mg*	186002181
BioSuite SP 10 µm CXC	Polymer	1000Å	1,000,000	7.5 mm	75 mm	3.31	132 mg***	186002184
BioSuite SP 13 µm CXC	Polymer	1000Å	1,000,000	21.5 mm	150 mm	54.45	2,178 mg***	186002185
BioSuite CM 10 µm CXC	Polymer	1000Å	1,000,000	7.5 mm	75 mm	3.31	149 mg***	186002186
BioSuite CM 13 µm CXC	Polymer	1000Å	1,000,000	21.5 mm	150 mm	54.45	2,450 mg***	186002187

* Data generated with BSA ** Data generated with Gamma Globulin *** Data generated with Hemoglobin

Note: For best resolution of complex samples, do not exceed 20% of the column's protein binding capacity

Protein-Pak HR Ion-Exchange Glass Columns

The Protein-Pak HR 8 µm and 15 µm packing materials are available prepacked in Waters Advanced Purification (AP) glass columns in a choice of 5 mm i.d. (minicolumn) or 10 mm i.d. by 100 mm in length. The 5 mm i.d. column is also available in a 50 mm length. These columns are compatible with any HPLC and FPLC system with the use of an adapter kit.

Waters Protein-Pak HR packing materials are based on rigid, hydrophilic, polymethacrylate particles with large 1000Å pores. The naturally hydrophilic polymer reduces non-specific adsorption, resulting in quantitative recovery of protein mass and bioactivity. These packings are compatible with buffers in the pH range 2-12, and will withstand exposure to caustic solutions, such as 0.1-1.0 M sodium hydroxide and acetic solutions, such as 20% acetic acid, for cleaning purposes.

Protein-Pak HR ion exchangers are available with a Q functional group, a strong anion exchanger; DEAE, a weak anion exchanger; SP a strong cation exchanger; and CM, a weak cation exchanger. The principle difference between a weak and strong ion exchanger does not lie in the protein binding capacity, but in the pH range of operation. Weak ion exchangers tend to have a more restricted useful pH range of operation.

Ion-Exchange Packing	Particle Type	Pore Size	Size	Dimensions	Part No.
Protein-Pak Q 8HR	Polymeric strong anion exchanger	8 µm	1000Å	5 x 50 mm 5 x 100 mm 10 x 100 mm	WAT039575 WAT039630 WAT035980
Protein-Pak Q 15HR	Polymeric strong anion exchanger	15 µm	1000Å	5 x 50 mm 10 x 100 mm	WAT039782 WAT037663
Protein-Pak DEAE 8HR	Polymeric weak anion exchanger	8 µm	1000Å	5 x 50 mm 5 x 100 mm 10 x 100 mm	WAT039791 WAT039783 WAT035650
Protein-Pak DEAE 15HR	Polymeric weak anion exchanger	15 µm	1000Å	5 x 50 mm 5 x 100 mm 10 x 100 mm	WAT039780 WAT039786 WAT038564
Protein-Pak SP 8HR	Polymeric strong cation exchanger	8 µm	1000Å	5 x 50 mm 5 x 100 mm 10 x 100 mm	WAT039570 WAT039625 WAT035655
Protein-Pak SP 15HR	Polymeric strong cation exchanger	15 µm	1000Å	10 x 100 mm	WAT038567
Protein-Pak CM 8HR	Polymeric weak cation exchanger	8 µm	1000Å	5 x 50 mm 5 x 100 mm 10 x 100 mm	WAT039790 WAT039785 WAT035970
Protein-Pak CM 15HR	Polymeric weak cation exchanger	15 µm	1000Å	5 x 50 mm	WAT039787

Note: Additional custom column dimensions are available.

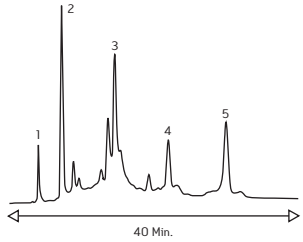
Approximate Protein Binding Capacity per Prepacked Column*

Prepacked Column	Protein-Pak HR Packing Q**	DEAE**	SP***	CM***
5 x 50 mm	60 mg	40 mg	40 mg	25 mg
5 x 100 mm	130 mg	150 mg	80 mg	45 mg
10 x 100 mm	500 mg	300 mg	300 mg	180 mg

Protein Resolution on Protein-Pak DEAE 15HR Anion-Exchange Column

Column: AP-1 Glass Column, 10 x 100 mm
Buffer A: 20 mM Tris-HCl pH 8.2
Buffer B: Buffer A with 1M Sodium Chloride
Flow Rate: 1.56 mL/min
Gradient: 0 to 25% Buffer B over 38 min
Load: 0.5 mg protein
Detection: 280 nm

1. Adenosine
2. Carbonic anhydrase
3. Human transferrin
4. Ovalbumin
5. Soybean trypsin inhibitor



Protein-Pak PW Series Columns

Waters also offers a line of 10 µm polymer-based ion-exchangers prepacked in steel or glass columns. The Protein-Pak™ 5PW columns are available as DEAE and SP ion-exchangers. These columns can be used on HPLC and FPLC systems in both analytical and preparative configurations.

Description	Dimensions	Part No.
Polymeric Weak Anion-Exchanger	7.5 x 75 mm	WAT088044
Protein-Pak™ DEAE 5PW Glass Column	8 x 75 mm	WAT011783
Protein-Pak™ DEAE 5PW Steel Column	21.5 x 150 mm	WAT010640
Polymeric Strong Cation-Exchanger	7.5 x 75 mm	WAT088043
Protein-Pak™ SP 5PW Glass Column	8 x 75 mm	WAT011784

Properties of Protein-Pak HR* Columns

Protein-Pak	Protein-Pak Q HR **	Protein-Pak DEAE HR **	Protein-Pak CM HR ***	SP HR ****
Type of material	Polymer	Polymer	Polymer	Polymer
Protein binding capacity	60 mg/mL	40 mg/mL	25 mg/mL	40 mg/mL
Ion-exchange capacity	200 µeq/mL	250 µeq/mL	175 µeq/mL	225 µeq/mL
Nominal pK	11.7	9.0	5.7	2.2
Typical protein recovery	>95%	>95%	>95%	>95%
Typical recovery of biological activity	>90%	>90%	>90%	>90%
pH stability	2-12	2-12	2-12	2-12

* For best resolution do not exceed 20% of the protein binding capacity.

** Bovine serum albumin in 20mM Tris/Cl pH 8.2 was used to measure protein binding capacity of Protein-Pak Q and DEAE HR.

*** Cytochrome c in 25mM MES pH 5.0 was used to measure protein binding capacity of Protein-Pak SP and CM HR.

**** Same conditions as CM. Protein binding capacity of Protein-Pak SP 40 HR is 20 mg/mL.

Advanced Purification (AP) Glass Columns

Waters Advanced Purification (AP) series of glass columns are constructed of biocompatible glass and polymeric materials and can be easily used with silica, polymer, or soft gel packings. To optimize flow and ensure uniform sample distribution onto the packed bed, each column incorporates a distributor. A replaceable filter protects the packing from large particulate contaminants. Empty AP glass columns are available in a variety of sizes and utilize the same design to ensure predictable methods transfer among them. AP glass columns are compatible with both analytical and preparative HPLC and FPLC systems.

Dimensions	Bed Volume (mL)	Flow Rate (mL/min)	Pressure Rating (psi/MPa)	Part No.
5 x 50 mm	0.8-1.2	0-4	1500 psi/10 MPa	WAT064-01
5 x 100 mm	1.8-2.2	0-4	1500 psi/10 MPa	WAT064-02
10 x 100 mm	5-8	0-4	1500 psi/10 MPa	WAT021901
10 x 200 mm	13-16	0-4	1500 psi/10 MPa	WAT021902
10 x 300 mm	21-24	0-4	1500 psi/10 MPa	WAT021903
10 x 600 mm	45-48	0-4	1500 psi/10 MPa	WAT021906
20 x 100 mm	22-31	4-16	1000 psi/6.8 MPa	WAT027501
20 x 200 mm	53-62	4-16	1000 psi/6.8 MPa	WAT027502
20 x 300 mm	85-94	4-16	1000 psi/6.8 MPa	WAT027503
20 x 600 mm	179-188	4-16	1000 psi/6.8 MPa	WAT027506
50 x 100 mm	137-196	16-100	500 psi/3.4 MPa	WAT023331
50 x 200 mm	333-392	16-100	500 psi/3.4 MPa	WAT023332
50 x 300 mm	530-589	16-100	500 psi/3.4 MPa	WAT023333
50 x 600 mm	1118-1177	16-100	500 psi/3.4 MPa	WAT023336

Additional connectors and fitting are available.

Advanced Purification (AP) Glass Column Accessories and Spare Parts

Waters Advanced Purification (AP) glass columns feature non-metallic construction and adjustable bed height with easy-to-use coarse and fine adjustments. The AP glass columns are available in a variety of dimensions.

AP Minicolumn Accessories and Spare Parts

Description	Dimensions	Part No.
Glass Tube	5 x 50 mm	WAT038802
	5 x 100 mm	WAT038803
Column Jacket	5 x 50 mm	WAT038804
	5 x 100 mm	WAT038805
Filters, 10/pkg		WAT038806
O-rings, 13/pkg (includes 10 inlet/outlet and 3 funnel)		WAT038807

AP-1 Column Accessories and Spare Parts

Description	Dimensions	Part No.
Glass tube	10 x 100 mm	WAT021992
	10 x 200 mm	WAT022033
	10 x 300 mm	WAT022034
	10 x 600 mm	WAT022035
Plastic shield	10 x 100 mm	WAT021927
	10 x 200 mm	WAT021945
	10 x 300 mm	WAT021946
	10 x 600 mm	WAT021947
O-rings, 5/pkg		WAT021907
Filters, 10/pkg		WAT021910
Replacement tubing (Tefzel) 1/16 inch o.d. x 0.009 inch i.d. x 10 feet (1.6 mm o.d. x 0.23 mm i.d. x 3 m)		WAT021950

AP-2 Column Accessories and Spare Parts

Description	Dimensions	Part No.
Glass Tube	20 x 100 mm	WAT019891
	20 x 200 mm	WAT019892
	20 x 300 mm	WAT019893
Plastic Shield	20 x 100 mm	WAT027542
	20 x 200 mm	WAT027543
	20 x 300 mm	WAT027544
	20 x 600 mm	WAT027545
O-rings, 5/pkg		WAT027528
Filters, 2/pkg		WAT027530
Replacement Tubing (Tefzel) 1/8 inch o.d. x 0.040 inch i.d. x 10 feet (3.2 mm o.d. x 1.02 mm i.d. x 3 m)		WAT023344

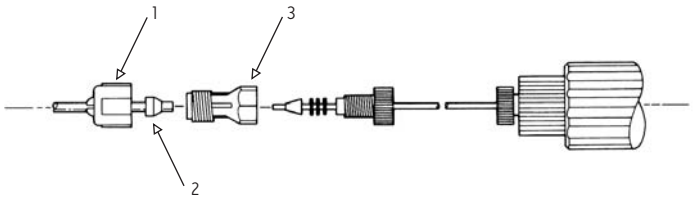
AP-5 Column Accessories and Spare Parts

Description	Dimensions	Part No.
Glass Tube	50 x 100 mm	WAT019876
	50 x 200 mm	WAT019877
	50 x 300 mm	WAT019878
Plastic Shield	50 x 100 mm	WAT023370
	50 x 200 mm	WAT023371
	50 x 300 mm	WAT023372
	50 x 600 mm	WAT023373
O-rings, 5/pkg		WAT023345
Filter, 2/pkg		WAT023343
Replacement Tubing (Tefzel) 1/8 inch o.d. x 0.040 inch i.d. x 10 feet) (3.2 mm o.d. x 1.02 mm i.d. x 3 m)		WAT023344

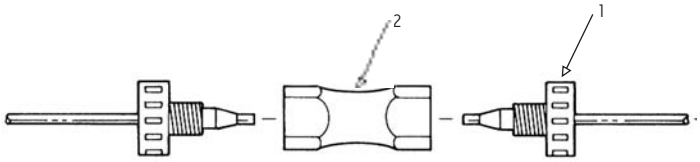
Consult your appropriate Care and Use Manual for additional spare parts information at www.waters.com/chemcu

Fittings and Connectors for Advanced Purification (AP) Glass Columns

Connection of an AP Minicolumn and an AP-1 Column to 1/8" o.d. Tubing

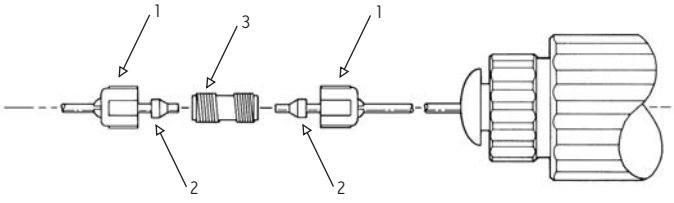


Description	Qty.	Part No.
1 Collet and nut assembly (3/8-24)	10/pkg	WAT005138
2 Ferrule 1/8" tube	10/pkg	WAT005136
3 Union 3/8-24 x 'Z' fitting	5/pkg	WAT005137



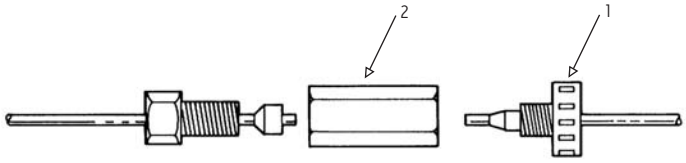
Description	Qty.	Part No.
1 Compression screw and ferrule 'Z' fitting, plastic	1/pkg	WAT082708
2 Union 'Z' fitting to 'Z' fitting, plastic	1/pkg	WAT082745

Connection of an AP-2 and an AP-5 Column to 1/8" o.d. Tubing



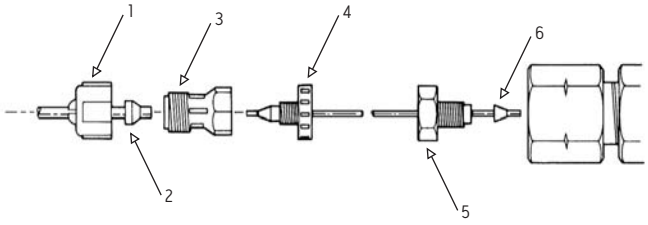
Description	Qty.	Part No.
1 Collet and nut assembly (3/8-24)	10/pkg	WAT005138
2 Ferrule 1/8" tube	10/pkg	WAT005136
3 Union 3/8-24 x 3/8-24	1/pkg	WAT082734

Connection of Pharmacia Fitting to 1/16" o.d. Tubing



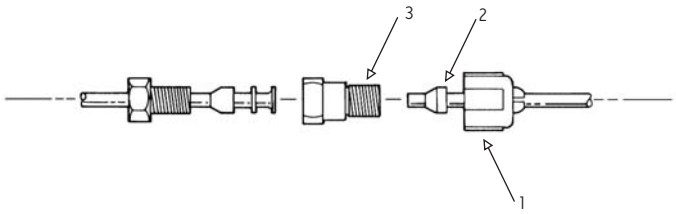
Description	Qty.	Part No.
1 Compression screw and ferrule 'Z' fitting, plastic	1/pkg	WAT082708
2 Union, plastic	1/pkg	WAT021951

Connection of a Protein-Pak™ Steel Column to 1/16" and 1/8" o.d. Tubing



Description	Qty.	Part No.
1 Collet and nut assembly (3/8-24)	10/pkg	WAT005138
2 Ferrule 1/8" tube	10/pkg	WAT005136
3 Union 3/8-24 x 'Z' fitting	5/pkg	WAT005137
4 Compression screw and ferrule 'Z' fitting, plastic	1/pkg	WAT082708
5 Compression screw 'Z' fitting, steel	10/pkg	WAT005070
6 Ferrule 1/16" steel	10/pkg	WAT005063

Connection of 1/8" or 1/16" Flanged Type Fitting to 1/8" o.d. Tubing



Description	Qty.	Part No.
1 Collet and nut assembly (3/8-24)	10/pkg	WAT005138
2 Ferrule 1/8" tube	10/pkg	WAT005136
3 Adapter 3/8-24 x 1/4-28	1/pkg	WAT082735

Accell Plus Ion-Exchange Packings

Solid-phase extraction for protein sample preparation

Waters Accell Plus ion-exchange packings are 40 µm, 300Å polymer-coated, silica-based materials for both lab- and process-scale chromatography. Accell Plus, available as a QMA strong anion exchanger or CM, a weak cation exchanger, is easy to pack and is excellent for the purification of proteins, enzymes, and immunoglobulins. The rigid silica-based packing material will withstand very high flow rates during cleaning and re-equilibration cycles. Normal flow rates are used during sample loading and elution to obtain the best possible resolution.

Accell Plus bulk material may be packed into our Advanced Purification (AP) glass columns. Prepacked 0.7 mL Sep-Pak® cartridges can be used for rapid method screening or solid phase extraction applications.

To estimate packed bed volume for a known amount of Accell Plus:

Accell Plus used (g) x 2 = packed bed volume (mL).

Accell Plus PrepPak Cartridges* (47 x 300 mm)

Economical, convenient preparative separations in the 500 mg to 10 g range. For a complete listing of Waters products for preparative chromatography, go to www.waters.com.

Particle Description	Particle Size	Pore Size	Part No.
Accell Plus CM *	40 µm	300Å	WAT036545
PrepPak™ 1000 Module			WAT089592

* Requires PrepPak 1000 Module

Protein Binding Capacity* of Accell Plus

Accell Plus QMA**	Accell Plus CM***
200 mg BSA/g packing	175 mg Cytochrome c/g packing

* For best resolution do not exceed 20% of the protein binding capacity.

** Bovine serum albumin in 20 mM Tris/Cl pH 7.0 was used to measure protein binding capacity of Accell Plus QMA.

*** Cytochrome c in 20 mM sodium phosphate pH 6.3 was used to measure protein binding capacity of Accell Plus CM.

Accell Plus Ion-Exchange Bulk Packings

For all preparative isolations based on ionic interactions, particularly proteins, enzymes, and immunoglobulins.

Particle Description	Particle Size	Pore Size	Qty.	Part No.
Accell Plus QMA	40 µm	300Å	100 g	WAT010742
Anion Exchanger			500 g	WAT010741
Accell Plus CM	40 µm	300Å	100 g	WAT010740
Cation Exchanger			500 g	WAT010739

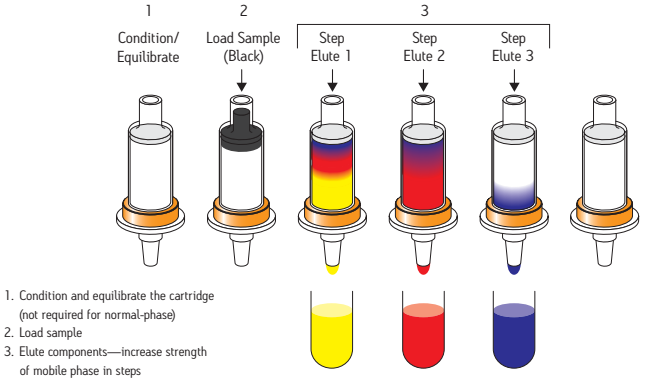
Ion-Exchange Sample Preparation with Sep-Pak Cartridges

To perform ion-exchange sample preparation with Sep-Pak cartridges, use a gradient of pH or ionic strength with Accell Plus CM, Accell Plus QMA or NH_2 as a sorbent.

- Condition the cartridge with six to ten hold-up volumes of deionized water or weak buffer
- Load the sample dissolved in a solution of deionized water or buffer
- Elute unwanted weakly bound components with a weak buffer
- Elute the first component of interest with a stronger buffer (change the pH or ionic strength)
- Elute other components of interest with progressively stronger buffers
- When you recover all of your components, discard the used cartridge in an appropriate manner

General Elution Protocol for Ion-Exchange Chromatography on Sep-Pak Cartridges (NH_2, Accell Plus QMA, Accell Plus CM)

Basic Steps for SPE



1. Condition and equilibrate the cartridge (not required for normal-phase)
2. Load sample
3. Elute components—increase strength of mobile phase in steps

Accell Plus Sep-Pak Cartridges

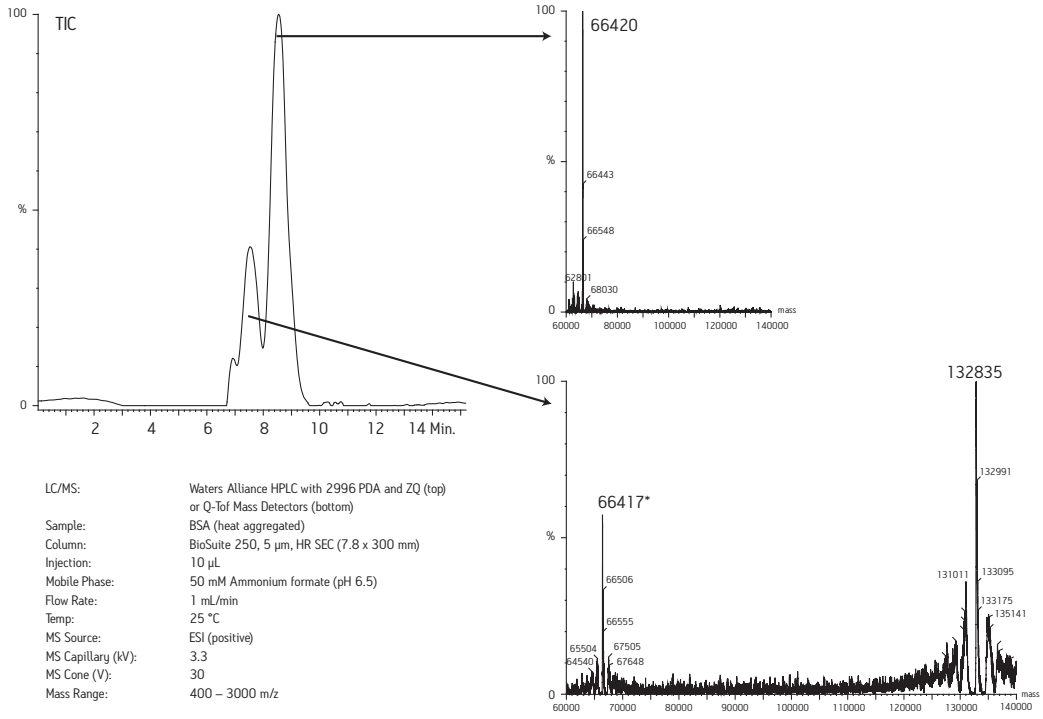
Sep-Pak Plus cartridges packed with Accell Plus ion exchangers provide a rapid, economical means to clean up heavily contaminated samples that would damage a high resolution column. They can also be used to rapidly screen chromatographic conditions. These are also available in a variety of configurations.

Description	Ion-Exchange Type	Part No.
Accell Plus CM	Weak Cation Exchanger	WAT020550
Accell Plus QMA	Strong Anion Exchanger	WAT020545

BioSuite Size-Exclusion HPLC Columns (SEC)

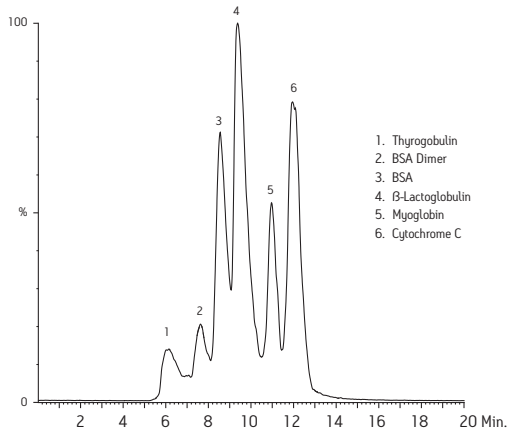
BioSuite Ultra High Resolution (UHR), High Resolution (HR), and Standard size-Exclusion Column packings use a rigid yet "wettable" silica-based media that is stable from pH 2.5-7.5. As indicated in the calibration curve tables, the exclusion limits of BioSuite SEC packings are determined by the particle and pore size of the silica-based material. Particle size of the SEC packing media as well as column length are important parameters that determine separation efficiency. BioSuite 4 µm particle size, UHR (Ultra-High Resolution) columns provide maximum separation efficiency, followed by BioSuite HR (High Resolution) columns and BioSuite Standard SEC columns. To maximize column life of analytical (i.e., 4.6 mm or 7.8 mm i.d.) or preparative (i.e., 21.5 mm i.d.) SEC columns, use of BioSuite Guard columns is recommended.

LC/MS Analysis of BSA Aggregation Using BioSuite 250, 5 µm HR SEC Column



LC/MS:	Waters Alliance HPLC with 2996 PDA and ZQ (top) or Q-Tof Mass Detectors (bottom)
Sample:	BSA (heat aggregated)
Column:	BioSuite 250, 5 µm, HR SEC (7.8 x 300 mm)
Injection:	10 µL
Mobile Phase:	50 mM Ammonium formate (pH 6.5)
Flow Rate:	1 mL/min
Temp:	25 °C
MS Source:	ESI (positive)
MS Capillary (kV):	3.3
MS Cone (V):	30
Mass Range:	400 – 3000 m/z

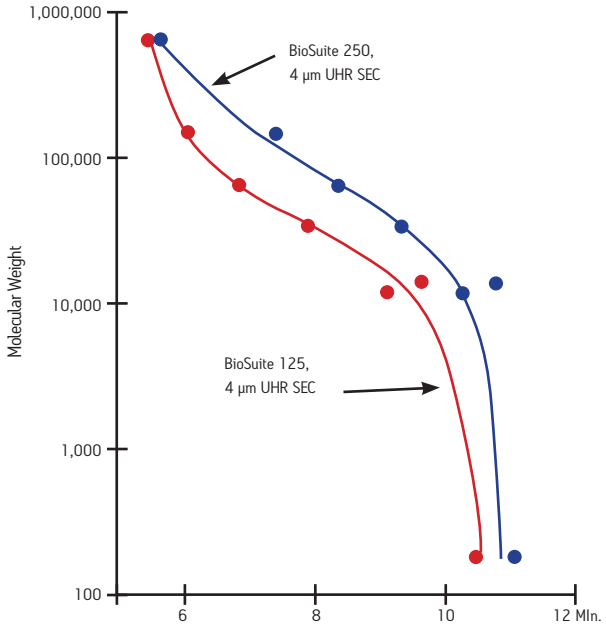
LC/MS Analysis of Protein Standards Using BioSuite 250, 5 µm High Resolution (HR) SEC Column (LC/MS conditions as above)



1.	Thyrogobulin	669 KDa
2.	BSA Dimer	136 KDa
3.	BSA	66 KDa
4.	ß-Lactoglobulin	36 KDa
5.	Myoglobin	16 KDa
6.	Cytochrome C	12 KDa

BioSuite SEC Reference: SEC-MS Analysis of Aggregates in Protein Mixtures. Application Book Supplement of LC/GC Europe. Sept. 2003. (Waters Literature Code Number: 720000743EN)

Protein Calibration Curves for BioSuite Ultra-High Resolution (UHR) SEC Columns

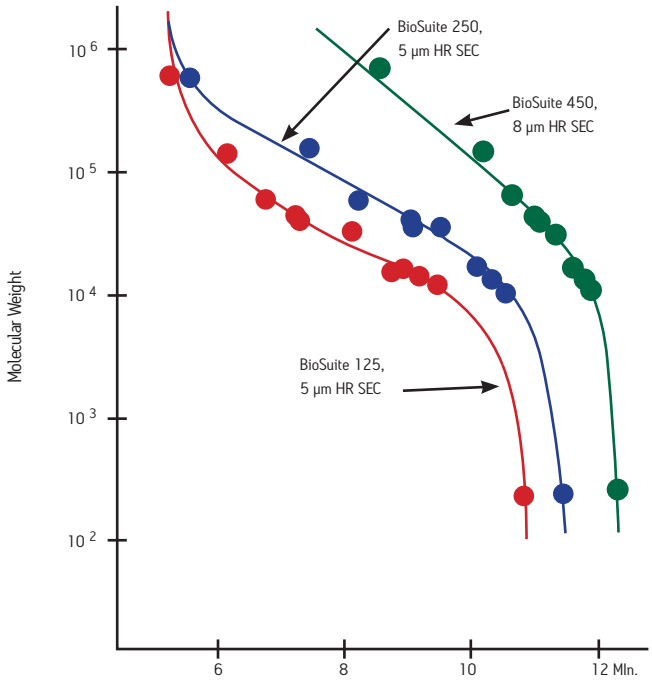


Sample: Thyroglobulin (MW 670,000 Da), Gamma globulin (MW 155,000 Da), Bovine Serum Albumin (66,330Da), Beta Lactoglobulin (MW18,400 Da), Lysozyme (14,300 Da), Cytochrome *c* (12,400 Da),Triglycine (189 Da)
Columns: BioSuite 250, 4 µm UHR SEC (4.6 x 300 mm)
BioSuite 125, 4 µm UHR SEC (4.6 x 300 mm)
Eluent: 0.15 M Sodium Phosphate, pH 6.8
Flow rate: 0.35 mL/min
Temperature: 25 °C
Detection: 280 nm (220 nm for triglycine)

Column	Globular Protein Mol. Wt. Range	Branched Dextrans	Linear PEG/PEO
BioSuite 125	*5,000-150,000*	*1,000-30,000*	*500-15,000*
BioSuite 250	*10,000-500,000*	*2,000-70,000*	*1,000-35,000*

Note: Operating flow rates for BioSuite Ultra-High Resolution (UHR) SEC columns (4.6 mm i.d.) are from 0.1 - 0.4 mL/min. Use of an HPLC system (e.g. Waters Alliance HPLC System) capable of operating at these flows is essential for optimal UHR SEC column performance.

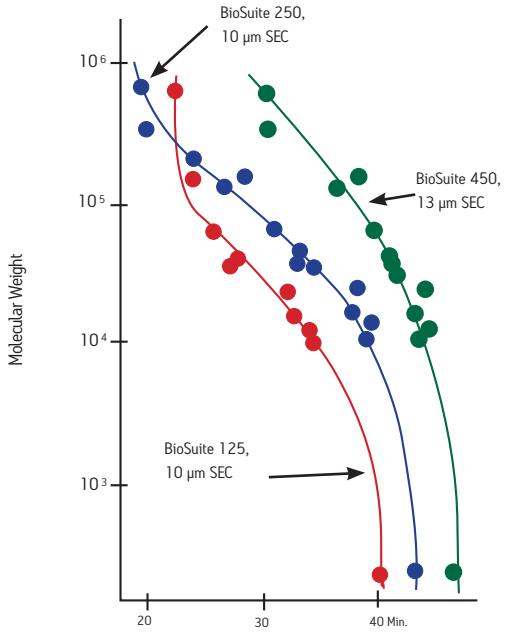
Protein Calibration Curves for BioSuite High Resolution (HR) SEC Columns



Sample: Thyroglobulin (MW 670,000 Da), IgG (MW 156,000 Da), BSA (66,330Da), Ovalbumin (MW 43,000 Da), Peroxidase (40,200 Da),Beta Lactoglobulin (MW18,400 Da), Myoglobin (MW 16,900 Da), Ribonuclease A (MW 13,700 Da), Cytochrome C (12,400 Da), Glycine tetramer (246 Da)
Columns: BioSuite 450, 8 µm HR SEC (7.8 x 300 mm)
BioSuite 250, 5 µm HR SEC (7.8 x 300 mm)
BioSuite 125, 5 µm HR SEC (7.8 x 300 mm)
Eluent: 0.1 M Sodium Phosphate, pH 7.0 containing 0.3M Sodium Chloride
Flow rate: 1.0 mL/min
Temperature: 25 °C
Detection: 220 nm

Column	Globular Protein Mol. Wt. Range	Branched Dextrans	Linear PEG/PEO
BioSuite 125	*5,000-150,000*	*1,000-30,000*	*500-15,000*
BioSuite 250	*10,000-500,000*	*2,000-70,000*	*1,000-35,000*
BioSuite 450	*20,000-7,000,000*	*4,000-500,000*	*2,000-250,000*

Protein Calibration Curves for BioSuite Standard SEC Columns



Sample: Thyroglobulin (MW 670,000 Da), IgG (MW 156,000 Da), BSA (66,330Da), Ovalbumin (MW 43,000 Da), Peroxidase (40,200 Da),Beta Lactoglobulin (MW18,400 Da), Myoglobin (MW 16,900 Da), Ribonuclease A (MW 13,700 Da), Cytochrome C (12,400 Da), Glycine tetramer (246 Da)
Columns: BioSuite 450, 13 µm SEC (7.5 x 300 mm); BioSuite 250, 10 µm SEC (7.5 x 300 mm); BioSuite 125, 10 µm SEC (7.5 x 300 mm)
Eluent: 0.1M Sodium Phosphate, pH 7.0 containing 0.3M Sodium Chloride
Flow rate: 1.0 mL/min
Temperature: 25 °C
Detection: 220 nm

Column	Globular Protein Mol. Wt. Range	Branched Dextrans	Linear PEG/PEO
BioSuite 125	*5,000-100,000*	*1,000-30,000*	*500-15,000*
BioSuite 250	*10,000-500,000*	*2,000-70,000*	*1,000-35,000*
BioSuite 450	*20,000-7,000,000*	*4,000-500,000*	*2,000-250,000*

BioSuite 4.6 mm, 7.8 mm, and 7.5 mm i.d. Analytical or 21.5 mm i.d. Preparative Columns are Available.

Description	Matrix	Inner Diameter	Length	Column Volume	Globular Protein Mol. Wt. Range	Suggested Protein Mass Load for Maximum Multicomponent Resolution*	Suggested Volume Load for Maximum Multicomponent Resolution*	Part No.
BioSuite 125, 4 µm UHR SEC	Silica	4.6 mm	300 mm	4.98 mL	5,000-150,000	Less than 8 mg/mL	Less than 40 µL	186002161
BioSuite 250, 4 µm UHR SEC	Silica	4.6 mm	300 mm	4.98 mL	10,000 - 500,000	Less than 8 mg/mL	Less than 80 µL	186002162
BioSuite UHR Guard SEC	Silica	4.6 mm	35 mm					186002163
BioSuite 125, 5 µm HR SEC	Silica	7.8 mm	300 mm	14.33 mL	5,000-150,000	Less than 8 mg/mL	Less than 200 µL	186002164
BioSuite 250, 5 µm HR SEC	Silica	7.8 mm	300 mm	14.33 mL	10,000 - 500,000	Less than 8 mg/mL	Less than 200 µL	186002165
BioSuite 450, 8 µm HR SEC	Silica	7.8 mm	300 mm	14.33 mL	20,000-7,000,000	Less than 8 mg/mL	Less than 200 µL	186002166
BioSuite HR Guard SEC	Silica	6 mm	40 mm					186002167
BioSuite 125, 10 µm SEC	Silica	7.5 mm	300 mm	13.25 mL	5,000-150,000	Less than 8 mg/mL	Less than 200 µL	186002168
BioSuite 125, 13 µm SEC	Silica	21.5 mm	300 mm	108.9 mL	5,000-100,000	Less than 8 mg/mL	Less than 1.6 mLs	186002169
BioSuite 250, 10 µm SEC	Silica	7.5 mm	300 mm	13.25 mL	10,000 - 500,000	Less than 8 mg/mL	Less than 200 µL	186002170
BioSuite 250, 13 µm SEC	Silica	21.5 mm	300 mm	108.9 mL	10,000 - 500,000	Less than 8 mg/mL	Less than 1.6 mLs	186002171
BioSuite 450, 13 µm SEC	Silica	7.5 mm	300 mm	13.25 mL	20,000-7,000,000	Less than 8 mg/mL	Less than 200 µL	186002172
BioSuite 450, 17 µm SEC	Silica	21.5 mm	300 mm	108.9 mL	20,000-7,000,000	Less than 8 mg/mL	Less than 1.6 mLs	186002173
BioSuite Guard SEC	Silica	7.5 mm	75 mm					186002174
BioSuite Guard SEC	Silica	21.5 mm	75 mm					186002175

* Using a BSA protein standard in a 50 mM phosphate buffer containing salt (either 0.1 M NaCl or 0.1 M Na_2SO_4) eluent. Useful protein mass loads will vary depending upon separation eluent, complexity of sample, and on the type of proteins contained in mixture. In general, maximum component resolution is obtained by injecting the smallest possible volume of a dilute protein solution.

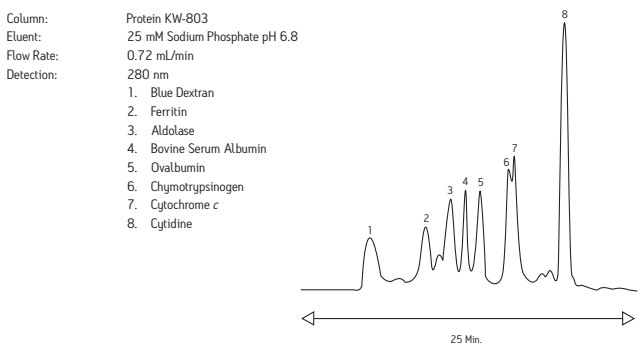
* Note: Operating flow rates for BioSuite Ultra-High Resolution (UHR) SEC columns (4.6 mm i.d.) are from 0.1-0.4 mL/min. Use of an HPLC system (e.g. Waters Alliance HPLC System) capable of operating at these flows is essential for optimal UHR SEC column performance.

Protein-Pak and Shodex Size-Exclusion HPLC Columns

Waters offers two families of packings for size-exclusion chromatography. Protein-Pak packings are based on a 10 µm diol-bonded silica and are available in a selection of pore sizes and column configurations. In addition, Waters offers a series of Shodex™ 7 µm high-resolution, gel filtration packings.

The Protein-Pak size-exclusion columns can be expected to resolve proteins that differ in molecular weight by a factor of two and to distinguish proteins differing by as little as 15% in molecular weight. The degree of resolution is more dependent on the sample mass and volume than the interaction between the sample and the stationary phase. Ideally, there should be no interaction between the sample and the stationary phase and the sample molecules. Secondary interactions are most often ionic and can, therefore, be reduced by increasing the ionic strength of the mobile phase. Typical, salt concentrations range to 0.2-0.5M NaCl. It may also be useful in some cases to consider adding 10-20% methanol to eliminate hydrophobic and other hydrogen-bonding interactions.

Standard Protein Mix on KW-803 Column



This gel filtration separation of protein standards demonstrates the ability to separate proteins in a wide range of molecular weights in minutes for high sensitivity analysis or protein isolation up to the milligram scale.

Protein-Pak Columns and Packings

Steel Column	Dimensions	MW Range	Part No.
Protein-Pak 60	7.8 x 300 mm	1,000-20,000	WAT085250
Protein-Pak 125	7.8 x 300 mm	2,000-80,000	WAT084601
Protein-Pak 300SW	7.5 x 300 mm	10,000-300,000	WAT080013
Protein-Pak 125 Sentry Guard Column 3.9 x 20 mm, 2/pkg (requires holder)			186000926
Sentry Universal Guard Column Holder			WAT046910
Glass Column			
Protein-Pak 200SW	8 x 300 mm	500-60,000	WAT011786
Protein-Pak 300SW	8 x 300 mm	10,000-300,000	WAT011787

Inquire for additional offerings including prep.

Shodex Size-Exclusion Columns

Column	Dimensions	Particle Size	MW Range	Part No.
Protein KW-802.5	8 x 300 mm	7 µm	100-50,000	WAT035943
Protein KW-803	8 x 300 mm	7 µm	100-150,000	WAT035946
Protein KW-804	8 x 300 mm	7 µm	500-600,000	WAT036613
Protein-Pak 125 Sentry Guard Column 3.9 x 20 mm, 2/pkg (requires holder)				186000926
Sentry Universal Guard Column Holder				WAT046910

Ultrahydrogel HPLC Columns

Packed with hydroxylated polymethacrylate-based gel, Waters Ultrahydrogel™ SEC columns are ideal for the analysis of aqueous-soluble samples, such as oligomers; oligosaccharides; polysaccharides; and cationic, anionic, and amphoteric polymers. Measuring 7.8 x 300 mm, these high-resolution columns offer many advantages over conventional aqueous SEC columns, such as:

- A wide pH range (2-12)
- Compatibility with high concentrations of organic solvents (up to 20% organic, 50% organic if the mobile phase is introduced by gradient)
- Greater flexibility for the mobile phase
- Minimal non-size-exclusion effects

Ultrahydrogel Columns (7.8 x 300 mm)

Column	Pore Size	Exclusion Limit	Part No.
Ultrahydrogel 120	120 Å	5×10^3	WAT011520
Ultrahydrogel 250	250 Å	8×10^4	WAT011525
Ultrahydrogel 500	500 Å	4×10^5	WAT011530
Ultrahydrogel 1000	1000 Å	1×10^6	WAT011535
Ultrahydrogel 2000	2000 Å	7×10^6	WAT011540
Ultrahydrogel Linear	Blend	7×10^6	WAT011545
Ultrahydrogel DP*	120 Å	5×10^3	WAT011550
Ultrahydrogel Guard Column	N/A	N/A	WAT011565
Ultrahydrogel Guard Column DP*	N/A	N/A	WAT011570

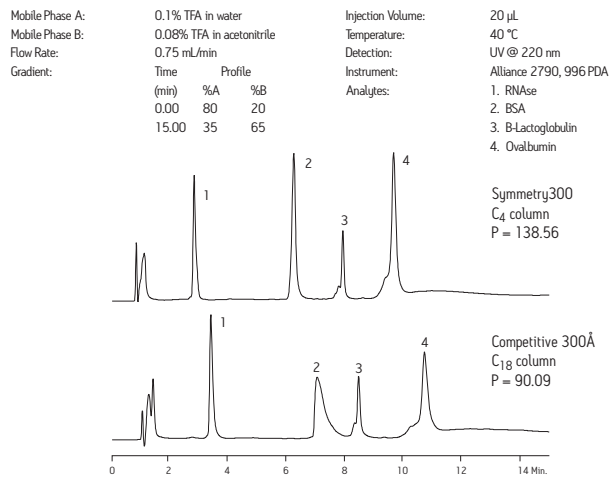
* DP = Degree of Polymerization, choice of column when working with glucose oligomers.

Symmetry300 C_4 HPLC Columns

Compared to our BEH300 C_4 offerings, Symmetry300 C_4 particles are 100% silica-based and are synthesized using ultrapure organic reagents resulting in high purity material with very low silanol activity for outstanding peptide and protein separations and recoveries.

- 300Å pore for peptide and protein applications
- Fully end-capped to minimize undersired secondary interactions
- Alternative separation selectivity compared to Waters BEH300 C_4 hybrid material
- QC tested with peptide samples to help ensure excellent batch-to-batch consistency

Protein: Symmetry300 C_4 Versus Competitors



Symmetry300 C_4 Columns

Description	Inner Diameter	Length	Particle Size	Part No.
Symmetry300 C_4	1.0 mm	150 mm	3.5 µm	186000276
Symmetry300 C_4	2.1 mm	50 mm	3.5 µm	186000277
Symmetry300 C_4	2.1 mm	100 mm	3.5 µm	186000278
Symmetry300 C_4	2.1 mm	150 mm	3.5 µm	186000279
Symmetry300 C_4	4.6 mm	50 mm	3.5 µm	186000280
Symmetry300 C_4	4.6 mm	75 mm	3.5 µm	186000281
Symmetry300 C_4	4.6 mm	100 mm	3.5 µm	186000282
Symmetry300 C_4	4.6 mm	150 mm	3.5 µm	186000283
Symmetry300 C_4	2.1 mm	150 mm	5 µm	186000285
Symmetry300 C_4	3.9 mm	150 mm	5 µm	186000286
Symmetry300 C_4	4.6 mm	50 mm	5 µm	186000287
Symmetry300 C_4	4.6 mm	150 mm	5 µm	186000288
Symmetry300 C_4	4.6 mm	250 mm	5 µm	186000289

Protein-Pak Affinity Columns

The Protein-Pak™ Affinity Epoxy-Activated packing consists of 40 µm, 500Å pore size particles having a hydrophilic bonding layer with a glycidoxypropyl functionality resulting in a seven atom spacer arm. The epoxy-activated surface can immobilize a wide range of ligands via a covalent linkage with amino, hydroxyl or sulfhydryl groups using simple coupling procedures. For method screening or small scale separation, choose the convenience of prepacked microcolumns. Larger-scale separations are easily achieved by packing bulk material in our Advanced Purification (AP) glass column.

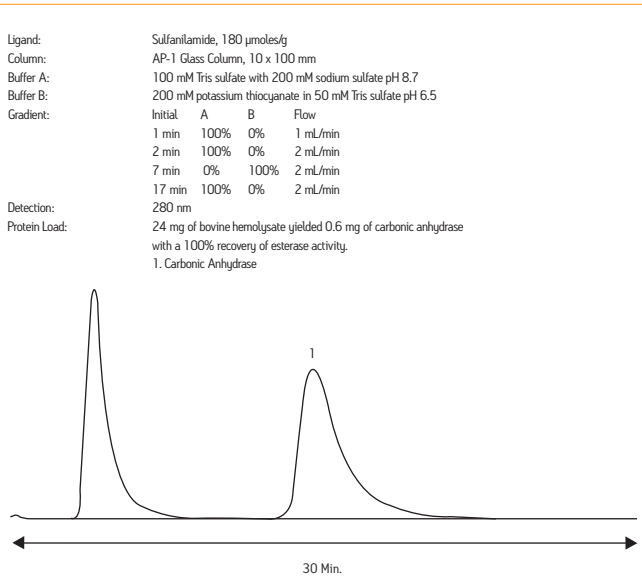
To estimate packed bed volume for a known amount of Protein-Pak™ Affinity Epoxy-Activated packing:

Protein-Pak Affinity Epoxy-Activated used (g) x 2 = packed bed volume (mL).

Particle Packing	Particle Size	Pore Size	Qty.	Part No.
Protein-Pak Affinity Epoxy-Activated Packing	40 µm	500Å	25 g	WAT030653
			100 g	WAT030654
Protein-Pak Affinity Epoxy-Activated Microcolumn (500 mg of material in a 3 cc syringe barrel)	40 µm	500Å	10/box	WAT035955

Inquire for additional offerings.

Purification of Carbonic Anhydrase



BioSuite pC_{18} and pPhenyl Reversed-Phase Chromatography (RPC) HPLC Columns

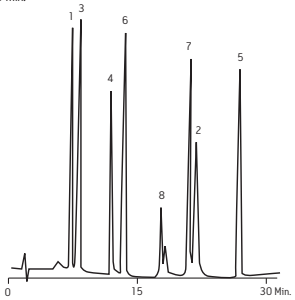
Reversed-phase chromatography (RPC) has become a widely accepted tool for the separation of proteins, peptides, synthetic oligonucleotides, and other biomolecules. For many applications, Symmetry and Symmetry300, Atlantis, T3, or XBridge BEH130 and BEH300 chemistries can be successfully used for the isolation and analyses of these biocompounds. However for some applications, the large pore size and high chemical stability of BioSuite pC_{18} and pPhenyl resin-based packings may be preferred. BioSuite RPC column offerings include a C_{18} (pC_{18}) and a phenyl (pPhenyl) chemistry bonded to a pH stable, methacrylic ester-based polymeric resin. The 500Å pore size of the pC_{18} base matrix accommodates proteins up to 2,500,000 Daltons while the 1,000Å pore size of the pPhenyl base matrix accommodates proteins up to 5,000,000 Daltons.

The BioSuite pC_{18}, 2.5 µm, NP column contains a non-porous chemistry that yields superior chromatographic resolution in less time compared to chromatography performed on the porous, pC_{18}, 500Å, 7 µm RPC selection. Waters porous, pC_{18}, 500Å, 7 µm RPC column is available for applications requiring greater binding capacity. The pC_{18} and pPhenyl RPC chemistries are available in 21.5 x 150 mm columns for "lab-scale" isolations while a 2.0 x 75 mm column is well suited for narrow-bore HPLC and LC/MS applications.

Reversed-phase Chromatography at Elevated pH on BioSuite™ pC_{18} RP Column Possible on Polymer Based Material

BioSuite™ pC_{18}, 500Å, 7 µm RPC

Sample:	Met-enkephalin (Peak 1), bradykinin (Peak 2), leu-enkephalin (Peak 3), Neurotensin (Peak 4), bombesin (Peak 5), Angiotensin I (Peak 6), Somatostatin (Peak 7), Insulin (Peak 8)
Column:	BioSuite pC_{18}, 500Å, 7 µm RPC (4.6 x 150 mm)
Eluent: A:	0.2 M NH_4OH, pH 10.8
Eluent B:	0.2 M NH_4OH, pH 10.8 (20%) / Acetonitrile (80%)
Flow rate:	1.0 mL/min
Gradient:	Linear gradient from 0 to 80% B in 50 min.
Temperature:	25 °C
Detection:	220 nm

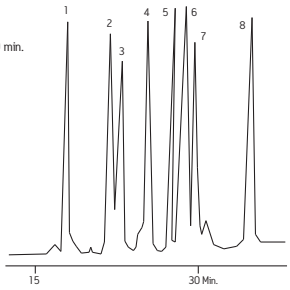


Note: The BioSuite pPhenyl, 1000Å RPC columns have a higher ligand density compared to the BioSuite Phenyl, 1000Å HIC columns and are not recommended for hydrophobic-interaction separations.

Hydrophobic Proteins are Well Resolved by Reversed-phase Chromatography on BioSuite™ pPhenyl RP Column

BioSuite™ pPhenyl, 1000Å, 10 µm RPC

Sample:	Ribonuclease (Peak 1), bovine Insulin (Peak 2bovine), Cytochrome-C (Peak 3), Lysozyme (Peak 4), Transferrin (Peak 5), Bovine serum albumin (Peak 6), Myoglobin (Peak 7), Ovalbumin (Peak 8)
Column:	BioSuite™ pPhenyl, 1000Å, 10 µm RPC (4.6 x 75 mm)
Eluent: A:	HPLC grade water with 0.05% TFA
Eluent B:	Acetonitrile with 0.05% TFA
Flow rate:	1.0 mL/min
Gradient:	Linear gradient from 5 to 80% B in 60 min.
Temperature:	25 °C
Detection:	220 nm

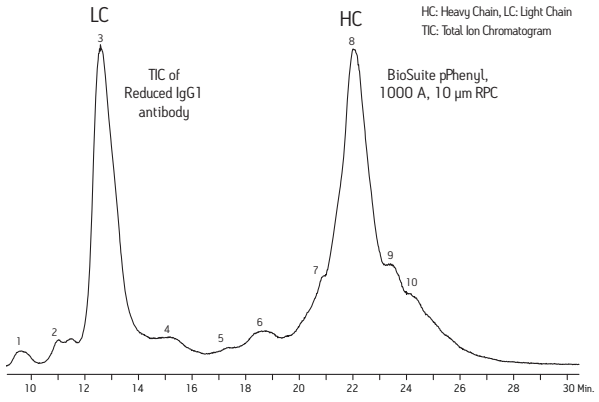


LC/MS Analysis of a Reduced Monoclonal IgG1 Antibody on a BioSuite pPhenyl RPC Column

Sample:	Reduced monoclonal IgG1 antibody
Column:	BioSuite pPhenyl, 1000 A, 10 µm RPC (2.0 x 75 mm)
Eluent A:	Water with 0.1% Formic Acid
Eluent B:	Acetonitrile with 0.1% Formic Acid
Flow Rate:	0.2 mL/min
Gradient:	10% B for 6 min (waste), then 20-35% B in 30 min
Temperature:	80°C
Detection:	Waters LCT Premier™ ESI-TOFMS

Peak No.	Identity
1	HC Clip1 (with glycan variants)
2	LC (1-2 S-S Intact)
3	LC
4	LC+1 Da
5	HC Clip 2 (w/o glycan variants)
6	HC Clip 3 (w/o glycan variants)
7	Antibody Half mer
8	HC (with glycan variants)
9	HC Fragment (w/o glycan variants)
10	HC Fragment-18 Da (w/o glycan variants)

HC: Heavy Chain, LC: Light Chain
TIC: Total Ion Chromatogram



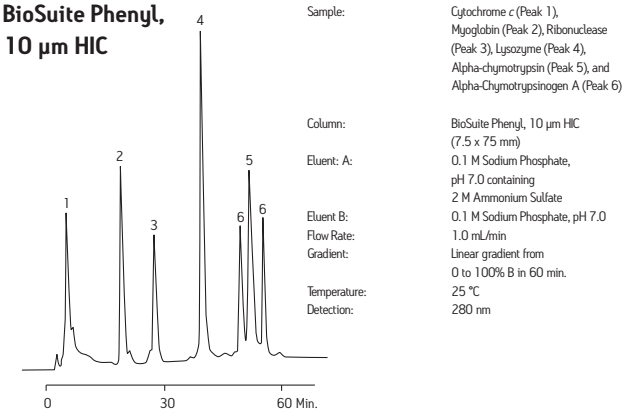
Description	Matrix	Inner Diameter	Length	Part No.
BioSuite pC_{18}, 2.5 µm NP RPC	Polymer	4.6 mm	35 mm	186002152
BioSuite pC_{18}, 500, 7 µm RPC	Polymer	2.0 mm	150 mm	186002153
BioSuite pC_{18}, 500, 7 µm RPC	Polymer	4.6 mm	150 mm	186002154
BioSuite pC_{18}, 500, 13 µm RPC	Polymer	21.5 mm	150 mm	186002155
BioSuite pPhenyl, 1000, 10 µm RPC	Polymer	2.0 mm	75 mm	186002156
BioSuite pPhenyl, 1000, 10 µm RPC	Polymer	4.6 mm	75 mm	186002157
BioSuite pPhenyl, 1000, 13 µm RPC	Polymer	21.5 mm	150 mm	186002158

BioSuite Hydrophobic-Interaction Chromatography (HIC) HPLC Columns

The separation of proteins and peptides based upon hydrophobic characteristics is a powerful chromatographic technique. However, some proteins denature at elevated organic solvent concentrations making reversed-phase chromatography (RPC) difficult. BioSuite Phenyl HIC columns provide a viable separation alternative to RPC. HIC is characterized by the adsorption of compounds to a weakly hydrophobic surface at high salt concentrations, followed by elution with a decreasing salt gradient. HIC combines the non-denaturing characteristics of salt precipitation with the precision of HPLC to yield excellent separation of biologically active material. BioSuite Phenyl, 1000Å, 10 µm HIC column media consists of a phenyl group bonded to a methacrylic ester-based polymeric resin. The large 1000Å pore size accommodates proteins up to 5,000,000 Daltons. A 21.5 mm i.d. x 150 mm column is also available for "lab-scale" isolations.

Note: The BioSuite Phenyl, 1000Å HIC columns have a lower ligand density compared to the BioSuite pPhenyl, 1000Å RPC columns and are not recommended for reversed-phase separations.

Hydrophobic-Interaction Chromatography on BioSuite™ Phenyl HIC Column is an Excellent Alternative to Reversed-phase Methods.



Description	Matrix	Inner Diameter	Length	Part No.
BioSuite Phenyl 10 µm HIC	Polymer	7.5 mm	75 mm	186002159
BioSuite Phenyl 13 µm HIC	Polymer	21.5 mm	150 mm	186002160

Protein HIC and Protein-Pak Phenyl HIC HPLC Columns

Waters Protein HIC and Protein-Pak Phenyl hydrophobic-interaction columns are packed with rigid, 10 µm polymethacrylate packing materials with 500Å pore size to ensure rapid protein diffusion. A low density bonding with phenyl groups results in a hydrophobic surface that allows for protein purification with a high recovery of both mass and biological activity.

HIC Packing Material Capacities

Protein HIC PH-814	40 mg/mL *
Protein-Pak Phenyl 5PW	25 mg/mL *

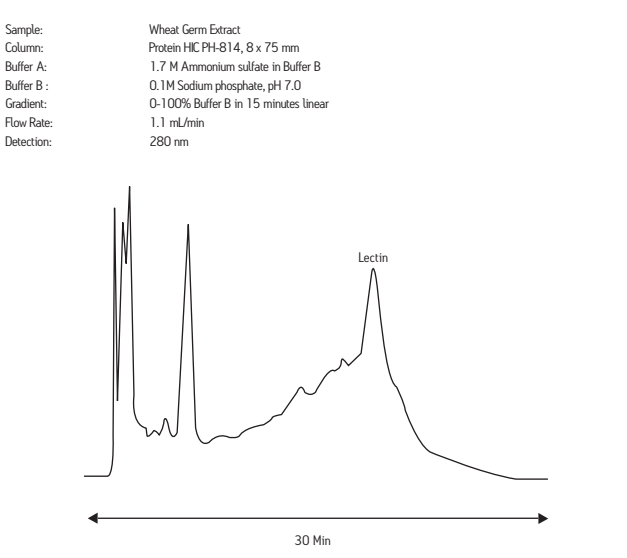
* Ovalbumin in 1.8 M ammonium sulfate, 0.1 M sodium phosphate pH.

Hydrophobic-Interaction Columns

Description	Dimensions	Part No.
Protein HIC PH-814 Steel Column	8 x 75 mm	WAT035520
Protein-Pak Phenyl-5PW Glass Column	8 x 75 mm	WAT011785

Inquire for additional offerings.

Protein Purification by Hydrophobic-Interaction Chromatography



Unique selectivity of hydrophobic interaction packings provides resolution and recovery of biologically active proteins with little denaturation.

Amino Acid Analysis

Overview

Amino Acid Analysis (AAA) is required for many applications. Amino acids are the constituents of proteins and are intermediates in many metabolic pathways. Bound amino acids are measured following hydrolysis of the protein to determine the concentration of protein, to identify a protein, and to detect structural variants. Amino acid composition is a critical component of the nutritional value of foods and feeds. Free amino acids are measured without hydrolysis using the same analytical tools used to monitor cell culture and fermentation processes. Similar assays are applied in the food industry to recognize the origin of the natural products based on the free amino acids released by extraction. AAA is also used to assess the status of metabolic pathways in clinical research laboratories. Each of these applications requires specific sample-handling and some modification to the method.

Sample Preparation

Samples for AAA can be divided into two groups. Where the protein in the sample is the ultimate object of the analysis, the sample must be hydrolyzed to release the amino acids. In contrast, where the amino acids are measured as metabolic indicators, no hydrolysis is required. However, the amino acids may need to be separated from other sample constituents that could bind amino acids or otherwise interfere with the analysis.

Purified proteins and peptides are hydrolyzed with acid at high temperature, commonly 6N HCl at 100-105 °C for 24 hours. Higher temperatures for shorter times are sometimes used for specific purposes but with reduced recovery and increased side reactions. Vapor-phase hydrolysis is useful for sensitive analysis of small amounts of pure proteins.

There are artifacts of hydrolysis. The amine, asparagine, and glutamine, are converted to the corresponding acids. Tryptophan is completely lost in hydrolysis, and significant amounts of the sulfur-containing amino acids are also destroyed. Some amino acids, e.g., threonine, serine, and tyrosine may be oxidized while the bonds between hydrophobic residues are hydrolyzed slowly, leading to underestimates in both cases. Tryptophan may be preserved with alkaline hydrolysis or with the use of methanesulfonic acid. The sulfur amino acids may be quantitatively oxidized with performic acid to the very stable methionine sulfone and cysteic acid. Oxidation degrades other amino acids, including tyrosine, serine, and threonine, so reduction and alkylation of cysteine residues and carefully excluding oxygen from the hydrolysis to preserve methionine is more useful. To correct for gradual destruction or release, data from three hydrolysis times, for example, 24, 48, and 72 hours, are extrapolated to constant values.

Food and feed samples for assessing nutritional content are much more complex. Hydrolysis requires large volumes of 6N HCl to ensure both dispersal of the sample and a sufficient excess of acid. The sulfur-containing amino acids are particularly important in these assays because they are growth-limiting. Performic acid oxidation is preferred for accurate determination of methionine and cysteine in these complex samples. The most complete profile is obtained by analyzing the sample with and without oxidation.

In cell culture and fermentations, free amino acids are supplied in the media as nutrients. A larger group of amino acids, including especially glutamine and tryptophan must be included in the measurement for assessing the health of the cell culture and supporting a feeding schedule.

Analysis of Amino Acids

After hydrolysis or other sample preparation, the amino acids are ready for analysis. This is a challenging analytical problem because the set of compounds covers a wide range of chemical properties but with pairs of components that are very similar. They also lack common chemical features that can be used for convenient detection. Several suitable solutions have been developed to address these concerns.

Amino acids were first separated by cation-exchange chromatography. Decades of development and continual improvements have produced methods that give complete resolution in less than 60 minutes. Ion-exchange methods are not compatible with direct detection. An added post-column reaction system uses a colorimetric reagent like ninhydrin or a fluorescent reactant like o-pthalaldehyde.

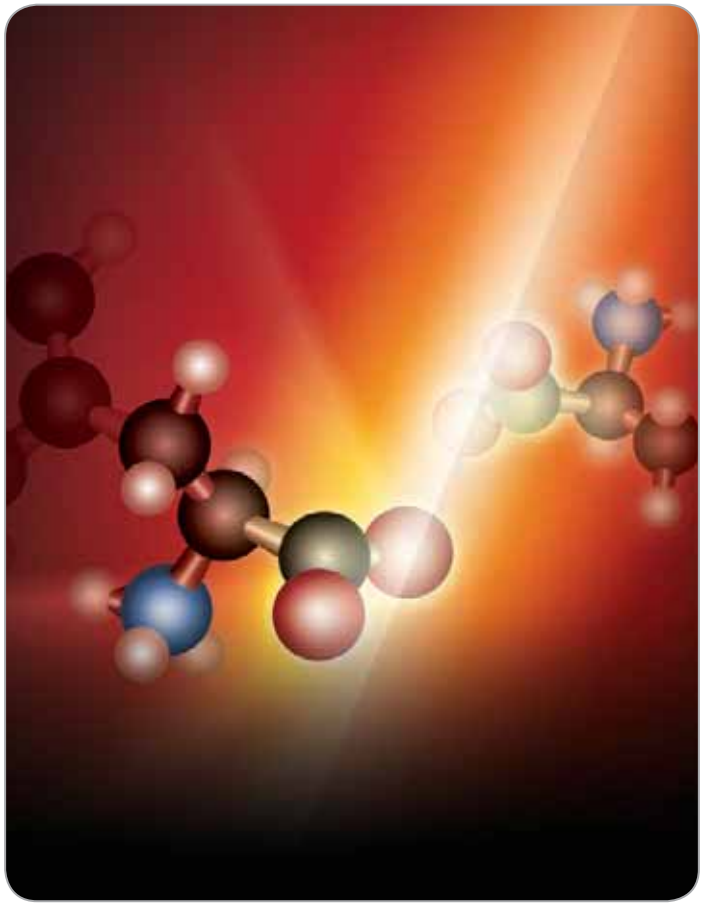
The differences between the amino acids are more suitable for reversed-phase chromatography. Because most of the compounds are polar, reversed-phase separations incorporate an ion-pairing reagent like a perfluorinated acid. These separations may be coupled to an electrospray mass spectrometer for detection.

A more general approach to reversed-phase AAA is pre-column derivatization. The derivatized amino acids retain better on the reversed-phase column and can be more easily separated. Most common derivatization reagents react with the amines. Some reagents react only with primary amines, but the generally useful ones also react with secondary amines so that proline is detected. In addition to improving chromatography, derivatization can make the amino acids readily detectable. The detection may be UV absorbance or fluorescence. Several reagents have been used, and two of the most thoroughly developed and best understood are described below.

Both approaches to AAA yield satisfactory results. Ion exchange with post-column detection is the oldest and has the larger knowledge base about unusual amino acids. Reversed-phase methods with pre-column derivatization are faster, more sensitive, and do not require specialized equipment.

UPLC® Amino Acid Analysis Solution

AccQ•Tag™ Ultra
UPLC® Amino Acid Analysis



The UPLC Amino Acid Analysis Solution

Over the years, Waters has provided three distinct products utilizing pre-column derivatization and reversed-phase HPLC: Auto•Tag™, Pico•Tag, and AccQ•Tag. Today, Waters continues to lead the way by bringing together its most ground-breaking and popular technologies: ACQUITY UPLC and AccQ•Tag Ultra, the newest addition to the AccQ•Tag family of products.

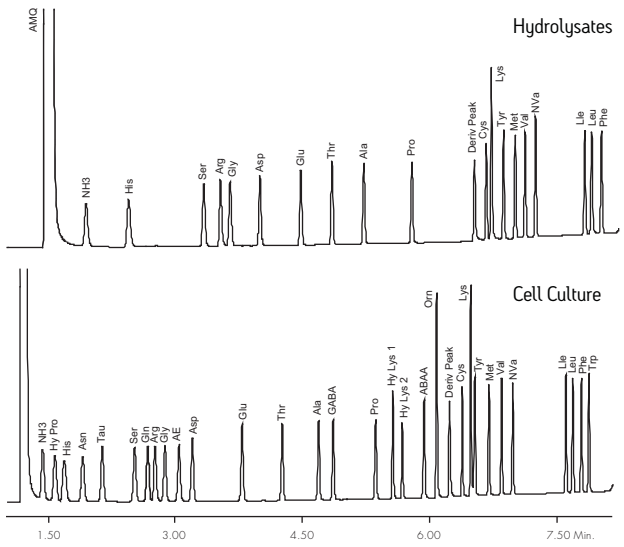
The UPLC Amino Acid Analysis Solution is holistically designed and optimized specifically for amino acid analyses. Derivatized amino acids are separated using the ACQUITY UPLC System, enhancing resolution to ensure accurate and precise qualitative and quantitative results. Just as important, our solution provides performance-qualified methodologies which are designed to be rugged and reliable, assuring reproducible results day-to-day, instrument-to-instrument, lab-to-lab, around the world—with the expert support that scientists have come to expect from Waters.

The UPLC Amino Acid Analysis Solution leverages Waters experience in separation science, derivatization chemistries, and information management. It is a total application solution optimized for accurate, reliable, and reproducible analysis of amino acids. Based on Waters AccQ•Tag Ultra chemistry, combined with our award winning ACQUITY UPLC separation technology, users can feel confident with assured performance in the areas of protein characterization, cell culture monitoring, and nutritional analysis of foods and feeds.

The UPLC Amino Acid Analysis Solution consists of:

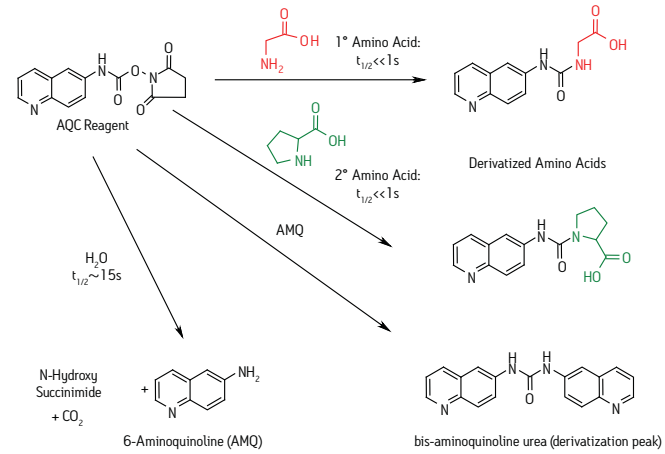
- Waters ACQUITY UPLC System and tunable UV detector (Optional fluorescence and PDA detectors are also fully supported)
- AccQ•Tag Ultra derivatization chemistries including column, reagents, and eluents (all quality control tested)
- Empower™ 2 pre-configured projects, methods, and report templates
- Installation and application training and support included
- Application-specific Performance Qualification
- Connections INSIGHT™ Intelligent Services

Amino Acid Analysis Methods for the Analysis of Hydrolysates and of Cell Culture Media



The UPLC Amino Acid Analysis solution can be used for the analysis of protein hydrolysates or for the larger set of amino acids found in cell culture media. The only differences in the methods are a different dilution of the supplied eluent A concentrate and operation at a different temperature. No method development or buffer reformulation is required.

Chemistry of the AccQ•Tag Derivatization Reaction



AccQ•Tag Ultra Chemistry

The AccQ•Tag Ultra chemistry products are a comprehensive, fully-tested set of reagents, columns, and eluents optimized for use with the UPLC Amino Acid Analysis Solution. This chemistry is based on Waters widely-used and well-understood AccQ•Tag derivatization method. Primary and secondary amino acids are derivatized before separation with a single reagent, AccQ•Fluor™, in a high-throughput batch process resulting in exceptionally stable derivatives. High resolution separations are achieved using the pre-qualified AccQ•Tag Ultra UPLC columns and mobile phases. Derivatized amino acids are quantified to sub-pmol levels with single wavelength UV detection.

AccQ•Tag Ultra Chemistry

Description	Part No.
UPLC AAA Application Add-on Kit*	176001279
AccQ•Tag Ultra Refill Package**	176001235
Refill Package Includes:	
AccQ•Tag Ultra Derivatization Kit, 250 Analyses	186003836
AccQ•Tag Ultra Column. 2.1 x 100 mm	186003837
AccQ•Tag Ultra Eluent A, Concentrate (950 mL)	186003838
AccQ•Tag Ultra Eluent B (950 mL)	186003839
Sample Tubes, 4 x 72/pkg	WAT007571
Amino Acid Standard, Hydrolysate, 10 x 1 mL ampules	WAT088122
Total Recovery Vials, 3 x 100 vial/pkg	186000384C

* This kit is intended to enable existing ACQUITY UPLC systems for AAA applications. The Add on Kit contains the AccQ•Tag Ultra Chemistries and Column, Documentation and additional hardware accessories needed for AAA applications.

** The Refill Kit is intended to recharge the AccQ•Tag Ultra chemistries that are a part of the Application Add on Kit. This kit should not be purchased as part of an initial system.

AccQ•Tag Derivatization Reaction

- Utilizes AccQ•Tag Ultra Reagent Powder
  - 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate
  - US Patent 5,296,599 and European Patent EP 0 533 200 B1
- Reacts rapidly with both primary and secondary amines
- Excess reagent reacts more slowly with water to form a product that can be separated chromatographically from the derivatized amino acids
- Requires no vacuum drying, sample prep, or extraction

Literature References

HPLC and UPLC Amino Acid Analysis Brochure, Literature Reference 720001946EN

UPLC Amino Acid Analysis Solution Brochure, Literature Reference 720001837EN

Amino Acid Analysis for Monitoring Cell Culture Media and Protein Structure Poster, Literature Reference 720002035EN

Amino Acid Analysis for Monitoring Protein Structure and for Measuring Protein Concentration Poster, Literature Reference 720001993EN

Amino Acid Analysis of Pure Protein Hydrolysates with Application of Waters UPLC Amino Acid Analysis Application, Literature Reference 720002404EN

Monitoring Cell Culture Media with the Waters Amino Acid Analysis Solution, Literature Reference 720002381EN

UPLC Amino Acid Analysis Solution, Literature Reference 720001683EN

ACQUITY UPLC for the Rapid Analysis of Amino Acids in Wine, Literature Reference 720002044EN

Determination of Amino Acids in Beers Using the UPLC Amino Acid Analysis Solution, Literature Reference 7200002158EN

Application Solutions for Biopharmaceuticals, Literature Reference 720002487EN

AccQ•Tag Amino Acid Analysis Using HPLC

The HPLC AccQ•Tag method utilizes pre-column derivatized reagents that yield easily detected fluorescence adducts. The AccQ•Fluor reagent, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate derivatizes primary and secondary amines in a simple, single-step reaction to yield highly stable, fluorescent adducts. We offer the AccQ•Tag method as a system package consisting of prepackaged reagents and extensive documentation.

The AccQ•Tag Chemistry Package contains the items you need for up to 250 analyses of protein and peptide hydrolysate amino acids.

AccQ•Fluor Reagent Kit (Five Bottles Each)–

- AccQ•Fluor Borate Buffer
- AccQ•Fluor Reagent Powder
- AccQ•Fluor Reagent Diluent

AccQ•Tag Amino Acid Analysis Column–

Separates the amino acid derivatives produced by the AccQ•Fluor derivatization reaction. The AccQ•Tag column is a high-efficiency Column specifically certified for use with the AccQ•Tag method. Care and use of the column is described in the Waters AccQ•Tag Amino Acid Analysis Column Care and Use.

AccQ•Tag Eluent A Concentrate–

A premixed concentrated aqueous buffer.

Amino Acid Hydrolysate Standard–

Ten 1 mL ampules of the Amino Acid Hydrolysate Standard. Each ampules contains a 2.5 mM mixture of the 17 hydrolysate amino acids with the exception of cystine (1.25 mM).

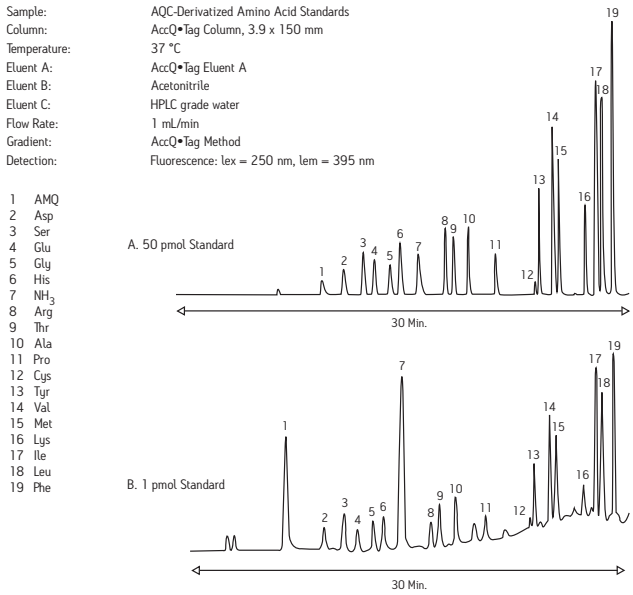
6 x 50 mm Sample Tubes–

Used for preparing samples and standards.

AccQ•Tag Chemistry Package Instruction Manual–

Describes the AccQ•Tag Amino Acid Analysis Method.

AccQ•Tag Analysis of Hydrolysate Amino Acid



Application of the AccQ•Tag Method to the analysis of hydrolysate amino acids is illustrated. The high purity reagents provided in the AccQ•Tag Chemistry Package enable high sensitivity analysis by minimizing background amino acid content.

** AQC-6-aminoquinolyl-N-hydroxysuccinimidyl, NHS-N-hydroxysuccinimidyl, AMQ-6-aminoquinoline*

Injection Volume Guidelines

Sample	Estimated Sample Quantity*	Injection Volume
Proteins	0.1 to 1.0 µg 4 to 40 picomole	10 to 20 µL
	0.1 to 5.0 µg 4 to 200 picomole	5 to 10 µL
Peptides	0.02 to 0.20 µg 20 to 200 picomole	10 to 20 µL
	0.02 to 1.0 µg 200 to 1000 picomole	5 to 10 µL
Standard	50 picomole	5 µL

*Based on protein average molecular weight=25,000, and peptide average molecular weight=1,000.

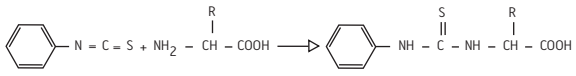
Column and Accessories	Dimensions/Qty.	Part No.
AccQ•Tag Chemistry Package for up to 250 analyses. Includes:		WAT052875
AccQ•Fluor Reagent 1	5 x 6 mL vials	
AccQ•Fluor Reagent 2A	5 x 3 mg vials	
AccQ•Fluor Reagent 2B	5 x 3 mL vials	
AccQ•Tag Column	3.9 x 150 mm	
AccQ•Tag Eluent A, Concentrate	2 x 1 liter	
Sample tubes	4 x 72/pkg	
Amino Acid Standard, Hydrolysate	10 x 1 mL ampules	WAT088122
AccQ•Tag User Guide		WAT052874
AccQ•Fluor Reagent Kit* Includes: AccQ•Fluor Reagent 1, 5 x 6 mL vials, AccQ•Fluor Reagent 2A, 5 x 3 mg vials, AccQ•Fluor Reagent 2B, 5 x 4 mL vials		WAT052880
AccQ•Tag Column	3.9 x 150 mm	WAT052885
AccQ•Tag Eluent A, Concentrate	1 x 1 liter	WAT052890
AccQ•Tag Eluent B**	1 x 1 liter	WAT052895

* The components of this kit are not available separately.
** For use with multi-pump gradient systems.

Pico•Tag HPLC Method

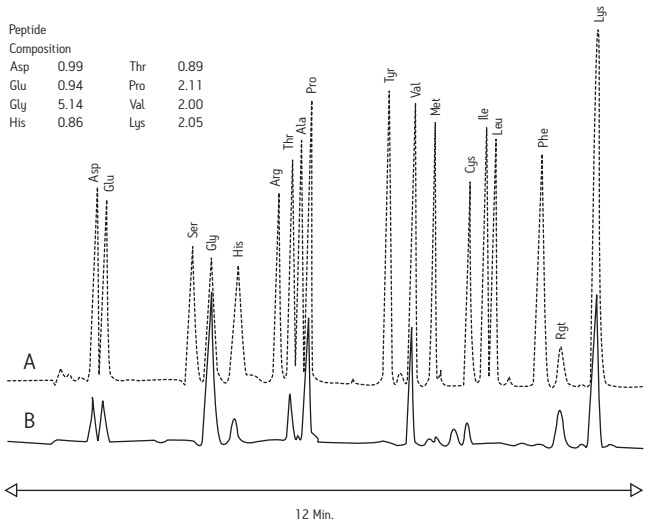
A widely used technique for HPLC amino acid analysis is the Waters Pico•Tag method. Based on an optimized system configuration, prepackaged reagents and extensive documentation, the Pico•Tag method provides a turnkey, guaranteed approach to modern HPLC amino acid analysis. Precolumn derivatization relies on the coupling reaction of the well known Edman Degradation, the reaction of phenylisothiocyanate (PITC) with both primary and secondary amino acids to form phenylthiocarbamyl (PTC) derivatives. The method is applicable to any sample including protein hydrolysates, physiologic fluids, feeds, foods, and pharmaceutical preparations.

Pico•Tag Derivatization Reaction



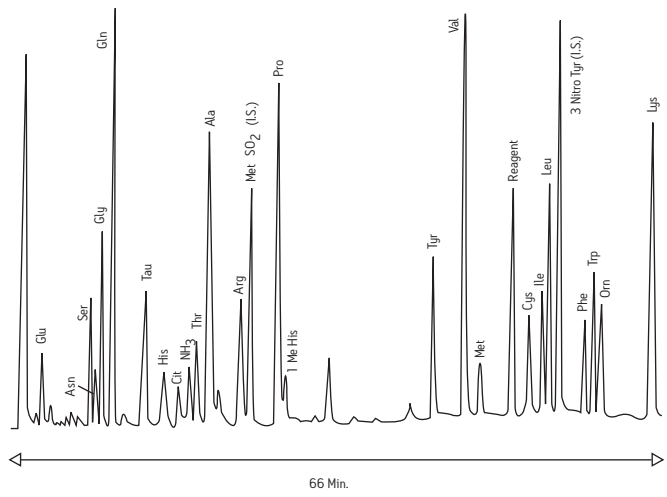
The derivatization of amino acids with PITC is the first step of the well-known Edman degradation reaction. The PTC-amino acid adducts are stable and easily separated by reversed-phase HPLC. A single product is formed for each amino acid. Most reaction by-products and all derivatization reagents are volatile, so they may be removed from the sample by vacuum drying.

Peptide Hydrolysate Amino Acid Analysis



This 12 minute analysis using Waters Pico•Tag Amino Acid Analysis Method provides identification and accurate quantitation of the amino acid composition.

Plasma Amino Acid Profile Using the Pico•Tag Method



Reproducible and reliable plasma amino acid profiles are obtained in 66 minutes using Waters Pico•Tag Method. In this analysis, 100 µL plasma was diluted with an internal standard, deproteinized by centrifugal ultrafiltration, and derivatized. The methionine sulfone (internal standard) peak represents 25 picomoles.

Courtesy of A.S. Feste, R.W. Drummond, and S.J. Dudrich, Nutritional Support Service, St. Luke Episcopal Hospital, Houston, Texas.

Pico•Tag Amino Acid Analysis of Protein Hydrolysates

Column and Accessories	Dimensions/Qty.	Part No.
Pico•Tag Chemistry Package (includes column, reagent kit, eluents, diluent, manual, and column heater inserts)		WAT007360
Pico•Tag Column	3.9 x 150 mm	WAT088131
Reagent Kit (contains PITC, TEA, and Standards)		WAT088123
Pico•Tag Eluent A	4 x 1 liter	WAT088108
Pico•Tag Eluent B	4 x 1 liter	WAT088112
Pico•Tag Diluent	100 mL bottle	WAT088119

Pico•Tag Amino Acid Analysis of Physiologic Amino Acids

Column and Accessories	Dimensions/Qty.	Part No.
Free Amino Acid Chemistry Package (includes column, reagent kit, eluents, diluent, manual, column heater inserts, and sample tubes)		WAT091681
Free Amino Acid Analysis column	3.9 x 300 mm	WAT010950
Reagent Kit (contains PITC, TEA and Standards A/N and B)		WAT010947
Pico•Tag Eluent 1	4 x 1 liter	WAT010960
Pico•Tag Eluent 2	4 x 1 liter	WAT010965
Pico•Tag Diluent	100 mL bottle	WAT088119

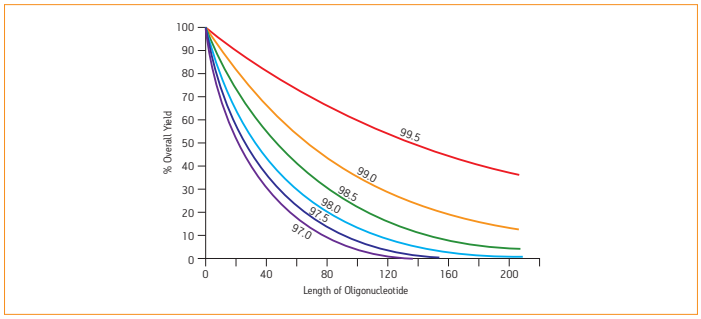
Oligonucleotide Purification and Analysis

Synthesis

Oligonucleotides are employed in an ever-expanding host of applications, including use as primers or hybridization probes, to adoption as therapeutic agents. In brief, their synthesis is frequently performed via a series of step-wise reactions that result in the addition of specific nucleotides (protected at their 5' hydroxyl end with a dimethoxytrityl group [DMT]) to the solid phase support containing the growing chain. Variations in choice of incoming nucleotide monomers (both protected ribose and deoxyribose nucleotides), as well as modifications after synthesis, can yield a final product containing atypical bases, sugars, or backbone composition. Some common modifications include substitution of sulfur atoms or methyl groups for oxygen on the phosphate backbone (creating phosphorothioates and methylphosphonates) or post-synthesis labeling to create molecular probes used for various diagnostic investigations.

To keep pace with the ever increasing demand for high-quality oligonucleotides, automated synthesizers capable of producing 0.05 to thousands of micromoles of product in a single synthesis exist. These instruments and methods frequently possess high coupling-efficiency capability with multi-strand synthesis flexibility. Yet, even with constant technology advancements, the coupling efficiency in each synthesis cycle is less than 100% resulting in contamination of the target oligonucleotide product (e.g., 60 mers) with undesired impurities. The total yield of full length product is a function of the length of the desired sequence and the degree of synthesis coupling efficiency as shown (right). Most "failure products" (typically labeled N-1, N-2..., N-x) are shorter sequences generated by a premature coupling failure of an incoming nucleotide monomer to the growing oligonucleotide chain. Some failures contain a missing nucleotide(s) in the middle of sequence, rather than at the end. "Mismatched failure sequences" having a greater molecular weight (often labeled N+x) than the desired oligonucleotide product can also be produced from either incomplete post-synthesis deprotection or from branching of the growing oligonucleotide chain during synthesis.

Synthetic Oligonucleotide Length Compared to Theoretical Yield at Various Coupling Efficiencies



Isolation

A variety of methods exist for the lab-scale purification (25-500 nmole) of oligonucleotides. The advantages as well as disadvantages of techniques are outlined in the table below.

Analysis

While polyacrylamide slab gel techniques can resolve long oligonucleotide sequences, impurity detection of analyzed samples frequently involves use of radio labeled nucleotides or gel staining techniques. The advent of capillary gel electrophoresis (CGE) with on-line detection helped overcome some post-run detection issues. However, precise chemical composition determination of each separated oligonucleotide species cannot to determined using UV absorption detection. Furthermore, in some environments the routine use of CGE for oligonucleotide analysis has been problematic due to method robustness and ruggedness concerns. HPLC, Waters UltraPerformance LC® Technology, and Mass Spectrometry have overcome many challenges related to the separation, quantitation, and identification of synthetic oligonucleotides. Rugged and robust separation and analysis methods can frequently be performed in minutes rather than in hours. In addition, quantitation with oligonucleotide composition confirmation is possible using MS or hyphenated-MS methods.

	Advantages	Disadvantages
Polyacrylamide Gel Electrophoresis (PAGE)	Well-established and efficient method. It separates long oligonucleotides (>50-60 mer).	Low mass-loading capacity. Gels are typically overloaded for purification and the resolution is compromised. PAGE does not separate N+x sequences. Manual band cutting. Excision is based on markers without detailed knowledge of target oligo retention. Samples need to be extracted from the gel and desalted, recovery of target oligonucleotides is low. Method is laborious; it is typically utilized only when no other technique is suitable for the task.
Ion-Exchange Liquid Chromatography (IEX-LC)	Trityl-off method. Separation of failure sequences is due to the backbone charge.	IEX-LC is efficient only for relatively short oligos (<20-25 mers); longer oligos are poorly resolved. Sample is contaminated with high concentration of salts; further desalting is required. IEX columns packed with non-porous sorbent offer improved resolution, but suffer with low mass load capacity. When loading exceeds 10-20 nmoles (for 4.6 mm i.d. columns), the resolution is compromised. IEX-LC does not separate N+x sequences.
Trityl-on Liquid Chromatography (Trytil-on LC; DMT-on LC)	Elegant, fast, and universal method for oligos of various length and sequence. RP columns used with this method have sufficient mass load capacity.	Does not adequately remove mismatch failure sequences (similarly as the target oligo they carry DMT group). DMT group is labile, part of the product may be lost due to the spontaneous detritylation. DMT residue and remaining acid have to removed after the detritylation.
Trityl-off Liquid Chromatography (Trityl-off LC; DMT-off LC)	Effectively removes practically all types of failure products. Uses volatile solvents; samples do not have to be further desalted. Collected fractions are simply lyophilized and ready for use. RP columns used with this method have sufficient mass load capacity. Labeled and dually-labeled oligonucleotide probes can be also purified. Method is suitable for LC-MS analysis (with MS compatible ion-pairing buffers).	Method requires efficient columns packed with small particle size sorbent. Oligonucleotide retention and resolution partially depends on the sequence. Method development for different oligonucleotide sequence and length probes is necessary.

Oligonucleotide Separation Technology

Waters Oligonucleotide Separation Technology (OST) columns contain second-generation hybrid silica BEH Technology particles functionalized with C_{18}. The separation of detritylated synthetic oligonucleotide samples is based on the well-established method of ion-pair, reversed-phase chromatography. The availability of 1.7 µm UPLC particles or 2.5 µm HPLC particles in various column dimensions provides flexibility to meet various lab-scale isolation or analysis needs and delivers exceptional sample resolution and superior column life. In addition, Waters manufacturing and quality control testing procedures help ensure consistent batch-to-batch and column-to-column performance regardless of application demands.

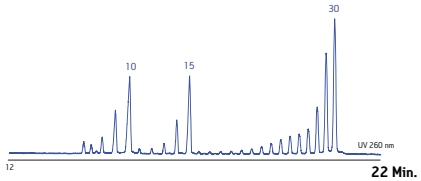
- Separation efficiencies equivalent or better than PAGE, CGE, or ion-exchange HPLC methods
- Resolve failure sequences from detritylated full length products
- Scaleable column offerings for lab-scale isolation needs
- Exceptional column life for reduced cost per analysis
- QC Tested with MassPREP
- QC Tested with MassPREP OST Standard to help ensure performance consistency

Exceptional Resolution of Oligonucleotide Mixtures

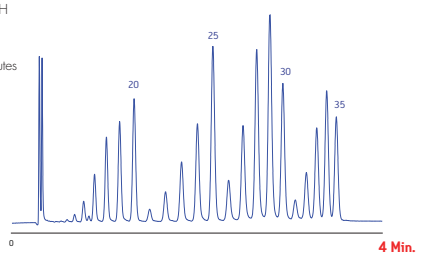
ACQUITY UPLC OST C_{18}, 1.7 µm (designed or use with an ACQUITY UPLC System) and XBridge OST C_{18}, 2.5 µm columns are well suited for the analysis of detritylated oligonucleotides using ion-pair, reversed-phase chromatography. As indicated (see figure on right), separations are comparable to that obtained by capillary gel electrophoresis (CGE) in terms of component resolution yet analyses times are significantly decreased using Waters UPLC Technology. The ability to resolve large oligonucleotide sequences (e.g., N from N-1) is possible due to the enhanced resolving power obtained using sub-3 µm, BEH Technology particles. In addition, quantitation with molecular weight characterization of the separated target oligonucleotide product from failure sequences is possible using Waters OST columns with hyphenated-Mass Spectrometry methods and MS friendly eluents.

Separation of Detritylated Oligodeoxythymidine Ladders by Capillary Gel Electrophoresis (CGE) vs. Ion-Pair, Reversed-Phase Chromatography

System:	Capillary Gel Electrophoresis System
CGE Column:	PEG sieving matrix (BioCap 75 µm x 27.5 (to detector) / 34.5 cm (total length)
Injection:	45 injection at 5 kV
Running:	15 kV
Temp:	30 °C

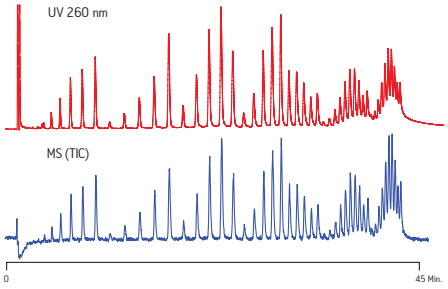


LC system:	Waters ACQUITY UPLC System
Column:	ACQUITY OST C_{18}, 1.7 µm (2.1 x 50 mm)
Mobile Phase:	A: 15 mM TEA, 400 mM HFIP, pH 7.9
	B: 50% A, 50% MeOH
Flow Rate:	0.4 mL/min
Column Temp:	60 °C
Gradient:	40 to 48% B in 4 minutes (20-24% MeOH)
Detection:	260 nm

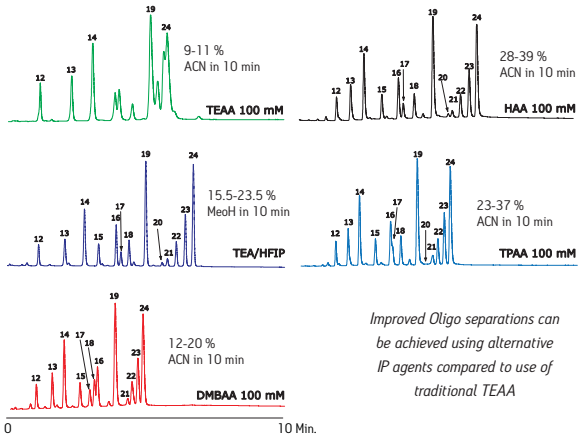


Separation of a 15-60 mer Detritylated Oligodeoxythymidine Ladder

LC System:	Waters ACQUITY UPLC System with PDA and QTof micro MS	Column Temp.:	60 °C
Column:	ACQUITY UPLC OST C_{18}, 1.7 µm (2.1 x 50 mm)	Gradient:	19 to 26.5.5% B in 60 minutes
Mobile Phase:	A: 15 mM TEA - 400 mM HFIP, B: Methanol	Detection:	UV: 260 nm, 10 scans per second MS: 1 scan per second, 0.1 sec interscan
Flow Rate:	0.1 mL/min		



Impact of Different Ion-Pairing Agents on Varying Oligonucleotide Sequence Separations



Improved Oligo separations can be achieved using alternative IP agents compared to use of traditional TEAA

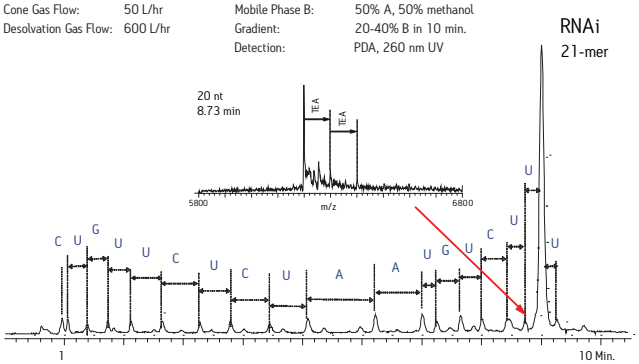
UPLC/MS Analysis Of Interfering RNA Oligonucleotides

Discovery of the RNA interference (RNAi) mechanism now broadly used for silencing of target gene expression has prompted a need for the analysis of small interfering RNAs (siRNA) molecules. To satisfy the need for a robust, fast, and sensitive analysis of 20-25 nucleotides of small interfering RNA (siRNA), a UPLC/MS method has been developed utilizing UPLC OST columns and Synapt HDMS mass spectrometer.

The acquisition of the accurate masses allowed for an assignment of the peaks of 5'-truncated oligomers (failed sequences generated during oligonucleotide synthesis), as well as some other impurities. The mass of each peak in the MS chromatogram was deconvoluted using MaxEnt 1 software. The tentative 5'-end failure products are assigned in Figure 2. Nearly the entire sequence of the parent oligonucleotide was elucidated. MS analysis also revealed a presence of an extra uridine mononucleotide added to the target 21-mer RNAi sequence.

LC/MS Analysis of RNA (21 mer)

TIC MS:	Synapt MS	LC System:	Waters ACQUITY UPLC
Capillary:	2000 V	Column:	ACQUITY UPLC OST C_{18}, 2.1 x 50 mm, 1.7 µm
Sample Cone:	35 V	Column Temp.:	60 °C
Extraction Cone:	3 V	Sample Injected:	2.5 µL (100 pmole of 21 nt RNAi)
Desolvation Temp.:	200 °C	Flow Rate:	0.2 mL/min
Source Temp.:	120 °C	Mobile Phase A:	15 mM TEA. 400 mM HFIP
Cone Gas Flow:	50 L/hr	Mobile Phase B:	50% A, 50% methanol
Desolvation Gas Flow:	600 L/hr	Gradient:	20-40% B in 10 min.
		Detection:	PDA, 260 nm UV

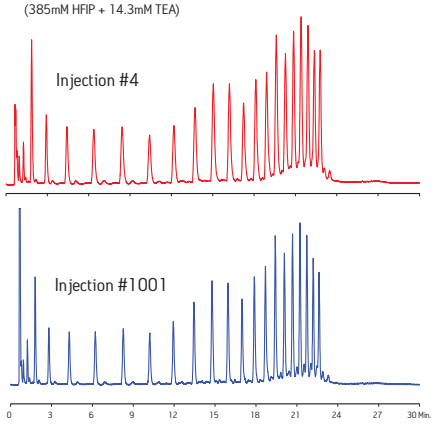


Outstanding Column Life

Waters OST columns packed with BEH Technology particles have shown remarkable column longevity, under these demanding separation conditions, while maintaining outstanding separation performance. By comparison, significantly reduced column life results when traditional silica-based columns are used under these same demanding separation conditions.

Separation of 5-25 mer Detritylated Oligodeoxythymidine Ladder

HPLC System:	Alliance Bio 2796 with PDA	Column temp:	60° C
Column:	XBridge OST C_{18}, 2.5 µm (2.1 x 50 mm)	Gradient:	0 – 100% B in 30 min (10-25 % MeOH).
Mobile Phase:	A: 10% MeOH / 90% (385mM HFIP + 14.3mM TEA) B: 25% meOH / 75% (385mM HFIP + 14.3mM TEA)	Flow Rate:	1.0 mL/min
		Detection:	260 nm, 5 scans per second



Description	Particle Size	Pore Size	Dimension	Part No.
ACQUITY UPLC OST C_{18} *	1.7 µm	135Å	2.1 x 50 mm	186003949
ACQUITY UPLC OST C_{18} *	1.7 µm	135Å	2.1 x 100 mm	186003950
Custom ACQUITY UPLC OST C_{18} *	—	—	—	186003951
XBridge OST C_{18}	2.5 µm	135Å	2.1 x 50 mm	186003952
XBridge OST C_{18}	2.5 µm	135Å	4.6 x 50 mm	186003953
XBridge OST C_{18}	2.5 µm	135Å	10 x 50 mm	186003954
Custom XBridge OST C_{18}	—	—	—	186003955

* For use on Waters ACQUITY UPLC Systems

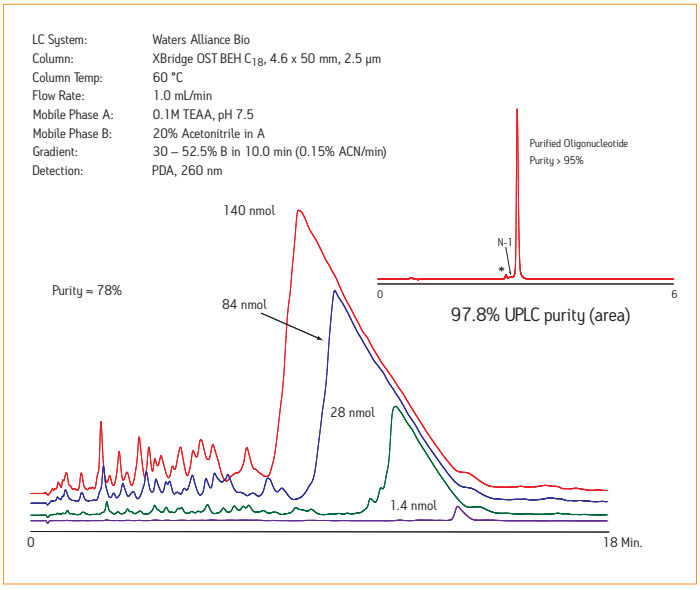
Scalable DNA and RNAi Separations with Good Product Recovery

XBridge OST C_{18} columns are the preferred offering for detritylated oligonucleotide purifications due to the availability of column sizes designed to meet lab-scale isolation requirements. As indicated in the table below, the choice of XBridge OST C_{18} column dimension and operating flow rate depends primarily on the scale of the synthesis reaction mixture. For example, a 4.6 x 50 mm column containing XBridge OST C_{18}, 2.5 µm material is an excellent selection when oligonucleotide mass loads are less than or equal to 0.2 µmol. Selection of the appropriate column size for the amount of oligonucletide sample loaded is recommended to maximize component resolution and recovery of the target product from non-desired failure sequences.

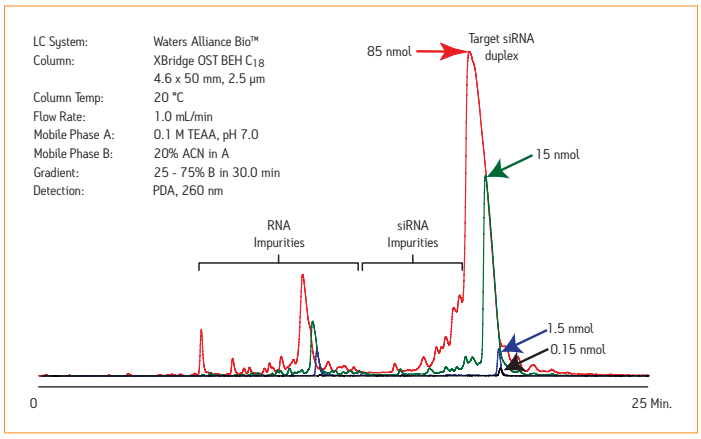
For researchers involved in gene silencing it is often necessary to work with RNA of high purity. Crude synthetic oligonucleotides used for gene knockout are typically purified. The figure below illustrates a a lab-scale purification of 21 mer RNA at various column loads. Using OST column chemistry and a Alliance System, large quantities of crude single stranded RNA can be successfully purified yielding material of high purity, ca. 95%, with an estimated yield of 55% based on collected peak area to the total peak area of the sample.

In addition, OST columns are well suited for analysis and purification of siRNA. As shown in the figure to the right siRNA is well resolved from single stranded RNA and truncated duplexes.

Purification of Single Stranded RNA



Purification of siRNA Duplex from Impurities



XBridge OST C_{18} Column Selection Guide for Detritylated Oligonucleotide Purification

Dimensions	Approx Mass Load**	mg***	Flow Rate
2.1 x 50 mm	0.04 µmoles	0.2 mg	0.2 mL/min
4.6 x 50 mm	0.20 µmoles	1.0 mg	1.0 mL/min
10 x 50 mm	1.00 µmoles	4.5 mg	4.5 mL/min
19 x 50 mm*	4.00 µmoles	16.0 mg	16.0 mL/min
30 x 50 mm*	9.00 µmoles	40.0 mg	40.0 mL/min
50 x 50 mm*	25.00 µmoles	110.0 mg	110.0 mL/min

* OST Custom Column

** Values are only approximates and vary depending on oligonucleotide length, base composition, and "heart-cutting" fraction collection method used.

*** Estimated for average oligonucleotide MW and synthesis yield.

Literature References

RNAi Duplex Analysis and Purification Application Note, Literature Reference 720002800EN

Optimization of LCT Premier XE MS Settings for Oligonucleotide Analysis Application Note, Literature Reference 70002798EN

UPLC Separation of DNA Duplexes Application Note, Literature Reference 720002741EN

UPLC/UV-MS Analysis of Phosphorothioate Oligonucleotides Application Note, Literature Reference 720002621EN

Semi-Preparative Scale Single-Stranded RNA Purification Application Note, Literature Reference 720002602EN

Real-Time Analysis of RNAi Duplexes Application Note, Literature Reference 720002573EN

UPLC/UV-MS Analysis of Application Note Oligonucleotides, Literature Reference 720002413EN

UPLC/MS Analysis of Interfering RNA Oligonucleotides Application Note, Literature Reference 720002412EN

UPLC Analysis of Phosphorothioate Oligonucleotides: Method Development Application Note, Literature Reference 720002405EN

UPLC/MS Separation of Oligonucleotides in Less than Five Minutes: Method Development Application Note, Literature Reference 720002387EN

Oligonucleotide Separation Technology: Synthesis Challenges and HPLC Isolation Options Application Note, Literature Reference 720002386EN

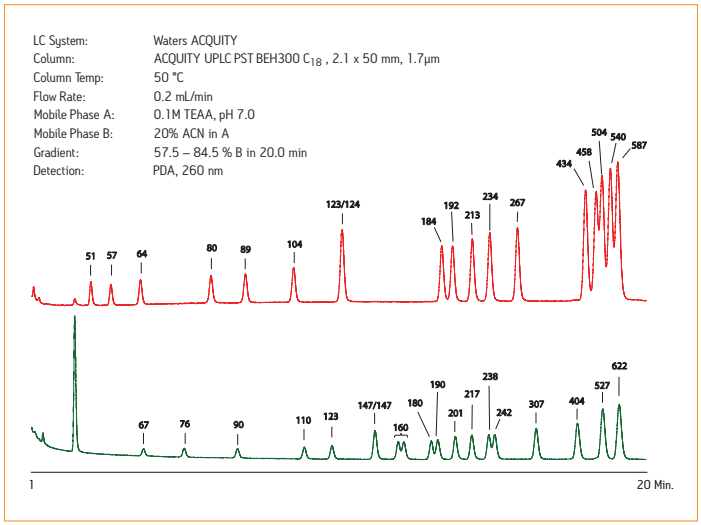
UPLC Separation of Oligonucleotides: Method Development Application Note, Literature Reference 720002383EN

HPLC and UPLC Columns for the Analysis of Oligonucleotides Application Note, Literature Reference 720002376EN

Columns for Large DNA/RNA Species

In general, molecular biology methods for manipulation of DNA rely on restriction enzymes, polymerase-chain reaction (PCR), and sequencing techniques. Using these methods, genomic DNA is typically converted into shorter double stranded (ds)DNA sequences, typically 100-1000 base pairs (bp) in length. The shorter dsDNA molecules are often analyzed or isolated by methods such as slab gel or capillary electrophoresis. Use of Waters ACQUITY BEH300 C_{18} reversed-phase or GenPak FAX anion-exchange columns offer alternatives to more traditional electrophoretic methods and are particularly well suited for various analytical and small-scale purification applications.

Separation of Duplex DNA Fragments: HaeIII and MspI Restriction Enzyme Digests of pBR322 Plasmid

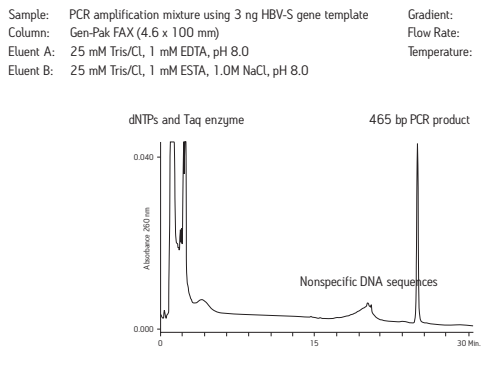


Description	Dimension	Particle Size	Part No.
ACQUITY UPLC BEH300 C_{18}	2.1 x 50 mm	1.7 µm	186003685

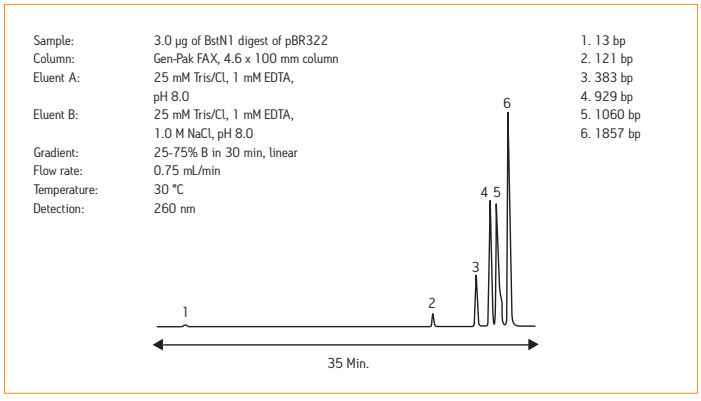
Gen-Pak FAX Anion-Exchange Column

Waters Gen-Pak FAX columns offer the highest resolution available in anion-exchange HPLC of nucleic acids. The Gen-Pak FAX column contains a weak anion exchanger based on DEAE functionalized non-porous resin. It contains 2.5 µm particles and is well suited for analytical and micro-preparative applications.

Chromatography of a PCR Amplification Mixture Generated using 3 ng and 1 fg of HBV S-gene Template



Separation of DNA Restriction Fragments



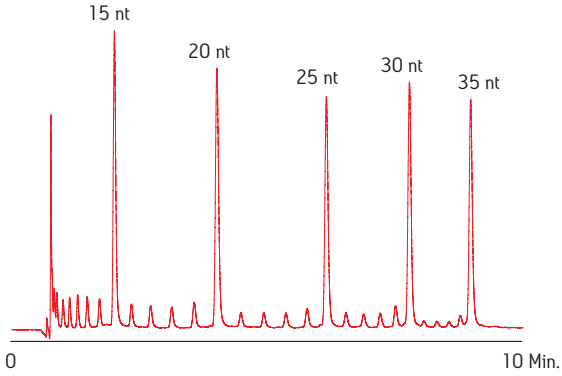
Description	Dimensions	Part No.
Gen-Pak FAX Column	4.6 x 100 mm	WAT015490

MassPREP OST Standard

- Contains a carefully defined mixture of synthesized oligodeoxythymidine fragments
- Useful in testing and confirming HPLC/UPLC, LC/MS, and column performance for oligo applications
- Each QC tested and shipped with a certificate of analysis

The pre-packaged MassPREP Oligonucleotide Separation Technology (OST) Standard is designed for verification of HPLC/UPLC instrument and column performance for analysis of synthetic oligonucleotides. Approximately equimolar amounts of 15, 20, 25, 30, and 35 nucleo-tide (nt) long oligodeoxythymidines are lyophilized and packaged in 1.5 ml LC vials. These vials are vacuum-sealed in foil pouches to reduce degradation that can occur by excessive exposure to light and air. Approximately 1 nmole of each oligonucleotide is present in the vial.

Description	Qty.	Part No.
MassPREP OST Standard	1/pk	186004135



Waters ACQUITY UPLC analysis of MassPREP OST Standard on an ACQUITY UPLC OST C_{18}, 1.7 µm column. The main components are labeled. Small peaks eluting between labeled oligonucleotides are N-1, N-2, etc. failure sequences generated during the oligonucleotide syntheses. The ACQUITY UPLC system is equipped with 50 µL standard mixer and PDA detector (260 nm).

Oasis µElution Plates

Oligonucleotide Desalting by Solid-Phase Extraction

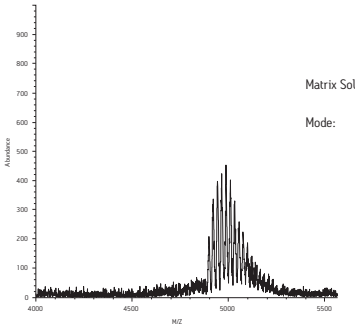
- Removes salt prior to MS analysis
- Low elution volumes
- High sensitivity
- Sample concentrating
- High throughput

Desalting of synthetic oligonucleotides is essential for MS analysis (QC, genotyping applications and SNP analysis). Waters new µElution plate is an excellent choice for high throughput analysis with minimal amount of sample. The Oasis® µElution plate combines patented plate design, proven Oasis chemistries, and generic protocols enabling elution volumes as low as 25 µL. Now for the first time you can perform SPE clean-up and concentration of very small sample volumes. The Oasis HLB sample extraction products incorporate a patented copolymer made from a balanced ratio of two monomers; the lipophilic divinylbenzene and the hydrophilic N-vinylpyrolodone that is ideally suited for this application.

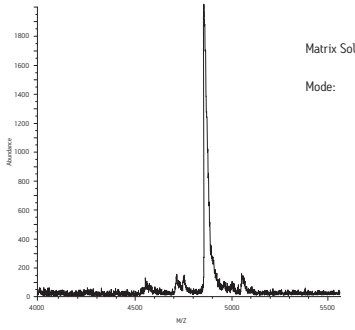
Description	Part No.
Oasis HLB µElution Plate (for oligonucleotides)	186001828BA

Effective Use of Oassis HLB for Oligonucleotide Desalting Prior to MALDI-TOF MS

MALDI-TOF Spectrum of 16 mer Prior to Desalting



MALDI-TOF Spectrum of 16 mer After Desalting using Oasis HLB µElution Plate



[EXCELLENCE]

BETTER RESULTS FROM YOUR
MS OR LC/MS SYSTEM

GREATER ACCURACY
INCREASED SENSITIVITY
REPRODUCIBLE DATA

MassPREP™

MASSPREP™ SAMPLE PREP FOR MASS SPECTROMETRY AND LC/MS

You need high quality results from your research and have made a significant investment in your mass spectrometry equipment. Obtain the best possible results by using the highest quality MS consumables from Waters.

The MassPREP™ family of products includes conveniently packaged standards, sample prep plates, and application specific kits.

To discover how Waters consumables can help you, visit www.waters.com/biosep

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

MS and LC/MS Consumables for Biomolecules

The use of mass spectrometry is increasing in laboratories involved with proteomics and the characterization of biopharmaceuticals. Sample preparation, the testing of system performance with simple standards, the use high quality reagents and matrices, and the LC process are critical to the quality of results.

Only when all these elements are considered can the highest quality results can be obtained. Waters has developed MS and LC/MS consumables specifically designed to ensure the best results from MS and LC/MS systems.

The MassPREP family of products includes conveniently packaged, highly-purified standards, specialty plates, and kits and reagents.

LC/MS

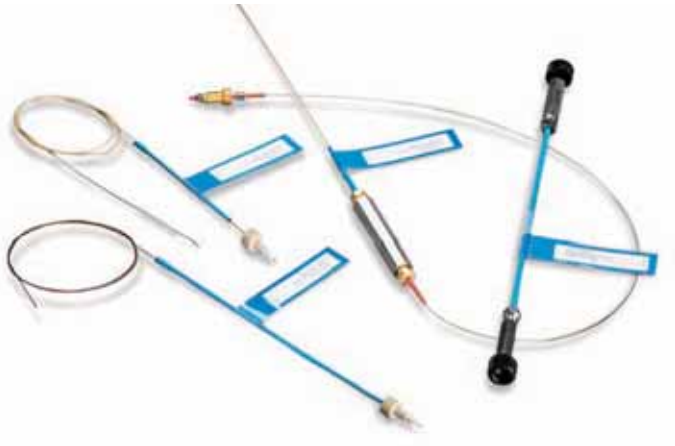
Proteomics

- RapiGest SF
- MassPREP Protein Digestion Standards
- MassPREP Digestion Standards Mixtures
- MassPREP Peptide Reference Standards
- MassPREP Phosphopeptide Standards
- NanoEase Traps, Capillary, and Nano Columns
- MassPREP Phosphopeptide Enrichment Kit
- nanoACQUITY UPLC Traps, Capillary, and Nano Column and 2D Kit

Protein Characterization/QC

- RapiGest SF
- MassPREP OST Standard
- MassPREP Protein Digestion Standards
- MassPREP Peptide Reference Standards
- MassPREP Desalting Cartridges
- NanoEase Traps, Capillary, and Nano Columns
- nanoACQUITY UPLC Traps, Capillary, and Nano Column and 2D Kit

The NanoEase family contains high performance capillary, nano-scale, and trapping columns.



nanoACQUITY UPLC columns consist of a complete line of columns specifically designed for the nanoACQUITY UPLC system.

*Rapi*Gest SF and MassPREP Oligonucleotide, Peptide, and Protein Digest Standards

Prepackaged MassPREP synthetic oligonucleotide peptide and protein digest standards eliminate the need to prepare, test, and store these materials for use in LC or LC/MS applications. In addition, *Rapi*Gest™ SF Surfactant is valuable in the enzymatic digestion of proteins for faster, higher quality, and more reproducible sample preparation.

MassPREP Peptide Standards

The MassPREP peptide standard mixture contains a void volume (V_O) column marker and nine carefully selected peptides with a broad range of polarities and isoelectric points. The MassPREP standard is useful to test UPLC and HPLC columns and systems dedicated to peptide separations.

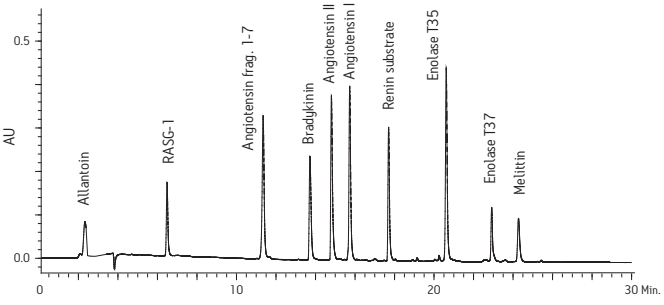
Components contained in MassPREP Peptide Standard Mixture

Peak #	Component name	Molecular weight ** (g/mol)	pK_a	Peptide sequence
1	Allantoin (V_0 marker)	158.0440	–	–
2	RASG-1	1000.4938	9.34	RGDSPASSKP
3	Angiotensin frag.1-7	898.4661	7.35	DRVYIHP
4	Bradykinin	1059.5613	12.00	RPPGFSPFR
5	Angiotensin II.	1045.5345	7.35	DRVYIHPF
6	Angiotensin I.	1295.6775	7.51	DRVYIHPFHL
7	Renin substrate	1757.9253	7.61	DRVYIHPFHLLVYS
8	Enolase T35	1871.9604	7.34	WLTGPQLADLYHSLMK
9	Enolase T37	2827.2806	3.97	YPIVSIEDPFAEDDWEAWSHFFK
10	Melittin	2845.7381	12.06	GIGAVLKVLTTGLPALISWIKRKRQQ

* BioSuite C_{18} 3 µm, Peptide Analysis - A Column (2.1 x 150 mm). Eluent A: 0.02% TFA in water, Eluent B: 0.016% TFA in acetonitrile. Gradient: 0-50% B in 30 minutes. Flow: 0.20 mL/min. Temp: 40 °C.

** Monoisotopic molecular weight

Baseline HPLC Resolution of Nine Peptides Contained in MassPREP Standard Mixture



Chromatography of MassPREP Peptide Standard Mixture obtained on a BioSuite C_{18}, 3 µm Peptide Analysis – A Column (2.1 x 150 mm) performed on a Waters Alliance HPLC System.

Description	Qty./Pack	Part No.
MassPREP Peptide Standards	1 vial	186002337
MassPREP Peptide Standards	5 vials	186002338

Note: The peptide mixture contains approximately 1.5 µg (~1 nmole) of each peptide

MassPREP Protein Digestion Standards

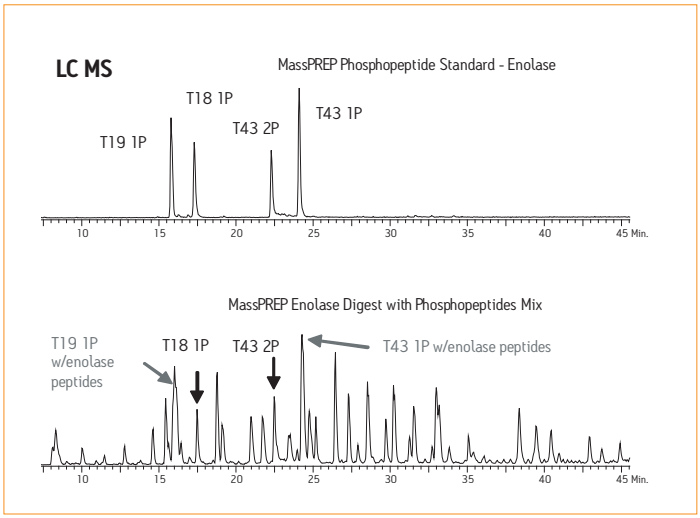
The MassPREP protein digestion sandards are prepared under strict quality control and contain no undigested standard proteins, trypsin, or other hydrophilic components. Test results from each batch of digestion standards are provided on the included Certificate of Analysis report.

Description	Qty./Pack	Part No.
MassPREP Digestion Standard Kit	1 vial from each digestion	186002330
Standard (5 vials total) Kit Consists of:		
MassPREP Phosphorylase b Digestion Standard	1 vial	186002326
MassPREP Bovine Hemoglobin Digestion Standard	1 vial	186002327
MassPREP ADH Digestion Standard	1 vial	186002328
MassPREP BSA Digestion Standard	1 vial	186002329
MassPREP Enolase Digestion Standard	1 vial	186002325

MassPREP Phosphopeptide Standards

- Contains phosphoserince, phosphotyrosine, and phosphothreonine peptides
- Use to optimize phosphopeptide detection in LC/MS, LC/UV, and MALDI-MS
- Can be used as control samples

Phosphorylated protein and peptides are difficult to detect and characterize due to their low abundance and low ionization efficiency. Using these standards, scientists have greater control over sample preparation, with the option to use pure peptides or to define phosphopeptides to unmodified peptide ratios.



The MassPREP phosphopeptide standard-enolase contains four phosphopeptides based on tryptic yeast enolase peptides. The MassPREP enolase digest with phosphopeptide mix contains equimolar mixture of enolase digest and four phoshopeptides.

Each new phosphopeptide standard contains the following four purified peptides:

Synthetic Phosphopeptides	Sequence	Amino Acid Residue	$[M + H]^+$	$[M + 2H]^{2+}$
Enolase T18 1P	NVPL(pY) K	126-131	813.3912	407.1995
Enolase T19 1P	HLADL (pS)K	132-138	863.4028	432.2053
Enolase T43 1P	VNQIG (pT)LSES IK	346-357	1368.6776	684.8428
Enolase T43 2P	VNQIG TL(pS)E(pS) IK	346-357	1448.6439	724.8259

These standards can be used on many Waters instrument systems, including the MALDI micro MX™, ACQUITY UPLC, Alliance , ZQ™, Q-Tof™, LCT Premier, and nanoACQUITY UPLC systems. Either UV or MS detection is possible.

MassPREP Phosphopeptide Standards

Description	Qty./Pack	Part No.
MassPREP Phosphopeptide Standard Enolase		186003285
MassPREP Enolase Digest with Phosphopeptides Mix		186003286
MassPREP Phosphopeptide Sample Kit - Enolase	2 vials -	186003287
Kit Consists of:		
MassPREP Phosphopeptide Standard Enolase	1 nmol/vial, 1 vial	186003285
MassPREP Enolase Digestion Standard	1 vial	186002325

MassPREP Digestion Standard Mixtures

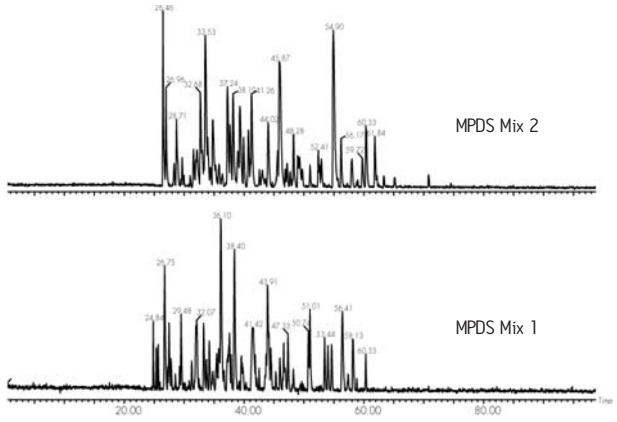
- Used to ensure an analytical system's ability to provide both qualitative protein analysis and relative protein quantification
- Yields high confidence and coverage during expression profiling of complex protein samples (by spiking the standards)
- Designed as standards for the Waters Protein Expression System

MassPREP Digestion Standards consist of two standard mixtures (MPDS Mix 1 and MPDS Mix 2), which were prepared by individually digesting Yeast Alcohol Dehydrogenase (ADH, SwissProt P00330), Rabbit Glycogen Phosphorylase b (GPB, SwissProt P00489), Bovine Serum Albumin (BSA, SwissProt P02769), and Yeast Enolase I (ENO, SwissProt P00924) with sequencing grade trypsin, and producing mixtures in the indicated molar ratios. The MPDS mixtures are purified, and do not contain undigested target protein, trypsin, other hydrophilic components or salts.

MPDS Mix 1 and Mix 2 Contents

Protein	MPDS Mix 1 [Molar ratio, Amount (pmol)]	MPDS Mix 2 [Molar ratio, Amount (pmol)]
ADH	1.0, 50 pmol	1.0, 50 pmol
GPB	1.0, 50 pmol	0.5, 25 pmol
ENO	1.0, 50 pmol	2.0, 100 pmol
BSA	1.0, 50 pmol	8.0, 400 pmol

MPDS Mix 1 and MPDS Mix 2 Separation Using ACQUITY UPLC BEH C_{18}, 1.7 µm, 75 µm x 100 mm nanoACQUITY UPLC Column



Sample:	Column:	nanoACQUITY UPLC BEH C_{18}, 1.7 µm, 75 µm x 100 mm
MPDS Mix 1: Alcohol Dehydrogenase 25 fmol/µL, PhosphorylaseB 25 fmol/µL; Enolase 25 fmol/µL; Bovine Serum Albumin 25 fmol/µL MPDS Mix 2: Alcohol Dehydrogenase 25 fmol/µL; Phosphorylase B 12.5 fmol/µL; Enolase 50 fmol/µL; Bovine Serum Albumin 200 fmol/µL	Mobile Phase A:	0.1% FA in H_2O
	Mobile Phase B:	0.1% FA in ACN
	Gradient:	Linear Gradient from 3 to 40% B in 90 min.
	Injection Volume:	2 µL
	Detection:	MS on Q-Tof Premier (m/z 50-1990)
	Ionization: Mode:	ESI Positive

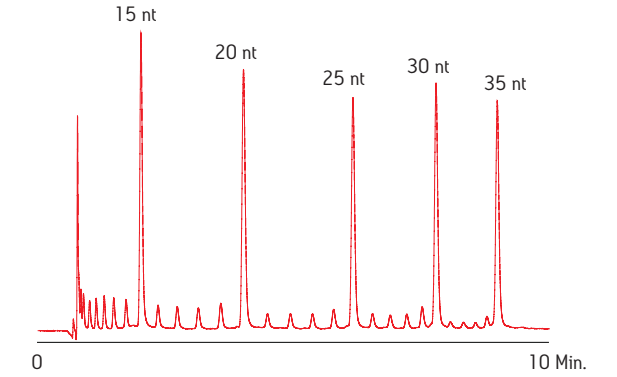
Description	Qty.	Part No.
MassPREP Digestion Standard Mix 1	1/pk	186002865
MassPREP Digestion Standard Mix 2	1/pk	186002866

MassPREP OST Standard

- Contains a carefully defined mixture of synthesized oligodeoxythymidine fragments
- Useful in testing and confirming HPLC/UPLC, LC/MS, and column performance for oligo applications
- Each QC tested and shipped with a certificate of analysis

The pre-packaged MassPREP Oligonucleotide Separation Technology (OST) Standard is designed for verification of HPLC/UPLC instrument and column performance for analysis of synthetic oligonucleotides. Approximately equimolar amounts of 15, 20, 25, 30, and 35 nucleotide (nt) long oligodeoxythymidines are lyophilized and packaged in 1.5 ml LC vials. These vials are vacuum-sealed in foil pouches to reduce degradation that can occur by excessive exposure to light and air. Approximately 1 nmole of each oligonucleotide is present in the vial.

Description	Qty./Pack	Part No.
MassPREP OST Standard	1 vial	186004135



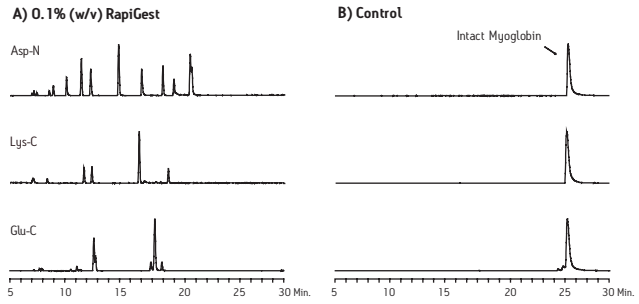
Waters ACQUITY UPLC analysis of MassPREP OST Standard on an ACQUITY UPLC OST C_{18} 1.7 µm column. The main components are labeled. Small peaks eluting between labeled oligonucleotides are N-1, N-2, etc. failure sequences generated during the oligonucleotide syntheses. The ACQUITY UPLC system is equipped with 50 µL standard mixer and PDA detector (260 nm).

*Rapi*Gest SF Protein Digestion Surfactant

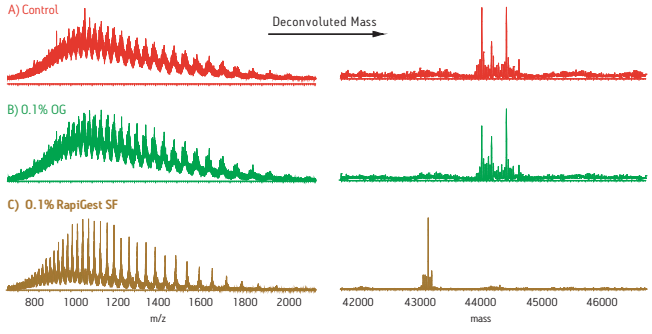
*Rapi*Gest SF, a novel Surfactant improves protein enzymatic digestion in terms of speed and peptide recovery. The unique features of this product are:

- Improves solubility of hydrophobic proteins for improved enzymatic digest
- Compatible with various enzymes
- Unlike conventional denaturants, *Rapi*Gest SF does not inhibit enzyme activity
- Reduces the digestion time, requires less enzyme to achieve optimum digestion
- Improves the digestion of enzyme resistant proteins such as membrane proteins
- Compatible with current protein sample prep protocols.
- Decomposes at low pH and degradation products do not interfere with LC/MS or MALDI MS analysis
- Does not cause protein modifications

1 hour Proteolysis of Myoglobin Using Various Endoproteases



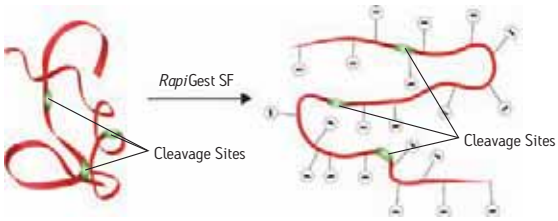
Use of *Rapi*Gest SF to Assist in Protein Deglycosylation



LC/MS spectra of deglycosylated ovalbumin are shown. A) Ovalbumin was solubilzed without the use of denaturant, and was not deglycosylated. B) Ovalbumin was denatured using 0.1% n-octyl-b-glycopyranoside (OG) and deglycosylated. C) Ovalbumin was denatrued in 0.1% RapiGest SF solution and deglycosylated. The MS scans were deconvoluted to the MW of the protein. Complete deglycosylated was observed after 2 hours deglycosylation for the RapiGest SF solubilized ovalbumin.

Numerous published scientific articles and presentations have documented the benefits of *Rapi*Gest SF use to improve the protein sequence coverage and reduce the sample preparation time. The areas of application are diverse, ranging from proteomic research to therapeutic protein characterization and is effective for in-solution digestion protocols.

How *Rapi*Gest SF works

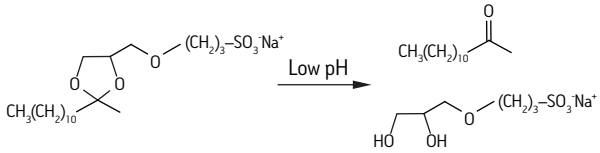


RapiGest shows significant advantage over other denaturants since it is not disruptive to endoprotease activity.

Trypsin * Solution	Trypsin Activity (%) **	Trypsin * Solution	Trypsin Activity (%) **
No additive	100	0.1% SDS/0.1% *Rapi*Gest	67
0.1% *Rapi*Gest	100	50% Methanol	29
0.5% *Rapi*Gest	100	50% Acetonitrile	87
0.1% SDS	24	1M Urea	97
0.5% SDS	1	2M Urea	83

* .5 µg of trypsin in 50 mM ammonium bicarbonate, pH 7.9; 0.2 mM of BEAA

** Measured as delta BEAA absorbance @ 253 nm (slope within 5 min)



RapiGest hydrolyzes under acidic condition (half life time = 7.6 minutes at pH 2). Therefore, it is compatible with LC/MS and MALDI MS analysis.

Description	Part No.
*Rapi*Gest SF 1 mg vial	186001860
*Rapi*Gest SF 1 mg vial (5 pack)	186001861
*Rapi*Gest SF 10 mg vial	186002123
*Rapi*Gest SF 50 mg vial	186002122
*Rapi*Gest SF Custom	186002118

Literature References

Enzyme-Friendly, Mass Spectrometry-Compatible Surfactant for In-Solution Enzymatic Digestion of Proteins, *Yu YQ, Gilar M, Lee PJ, Bouvier ES, Gebler JC, Anal Chem.* ***2003*** *Nov 1; 75(21):6023-8.*

A complete peptide mapping of membrane proteins: a novel surfactant aiding the enzymatic digestion of bacteriorhodopsin, *Yu YQ, Gilar M, Gebler JC, Rapid Commun Mass Spectrom.* ***2004****; 18 (6):711-5.*

A rapid sample preparation method for mass spectrometric characterization of N-linked glycans, *Ying Qing Yu, Martin Gilar, Jennifer Kaska and John C. Gebler, Rapid Commun Mass Spectrom.* ***2005****; 19: 2331-2336.*

MassPREP On-Line Desalting Devices

- Effectively desalts proteins yielding improved LC/MS results
- Fast on-line method for high throughput applications
- Excellent protein recoveries and no detectable carryover
- >100 injections from a single cartridge

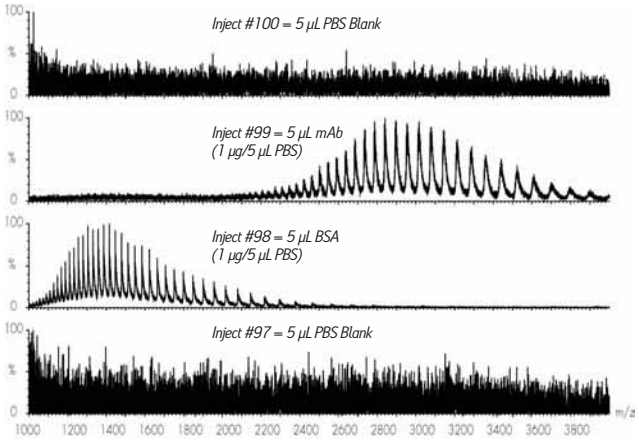
The MassPREP on-line desalting column can effectively desalt proteins prior to LC/MS analyses. Because non-volatile salts (e.g., NaCl) can suppress ionization of intact proteins leading to poor detection sensitivity, it is important to remove or significantly minimize the introduction of these compounds into the mass analyzer. The reversed-phase, phenyl material contained in MassPREP on-line column successfully "traps" proteins, allowing the salts to be washed to waste prior to protein elution into the mass spectrometer. With an optimized LC/MS method, cycle times as low as 4 minutes (for intact antibody) and 10 minutes (for reduced antibody) are achievable.

Description	Qty.	Part No.
MassPREP Micro Desalting Column	1/pk	186004032
MassPREP On-line Desalting Cartridge (2.1 x 10 mm)*	2/pk	186002785
UPLC Intact Mass Analysis Application Kit** (Includes MassPREP Micro Desalting Column and ACQUITY Tubing Kit)	1/pk	176001519
Sentry 2.1 x 10 mm Guard Cartridge Holder.* Required for use of MassPREP On-line DesaltingCartridge	1/pk	WAT097958

** See: UPLC Intact Mass Analysis Application Kit Manual (715001664)

Waters MassPREP On-Line Desalting Cartridge (2.1 x 10 mm)

Experimental
LC System: Alliance 2796 Separation Module
MS System: Q-Tof micro™, ESI Positive
Eluent A: 0.1%Formic Acid (H_2O) Eluent B: 0.1% Formic Acid (ACN)

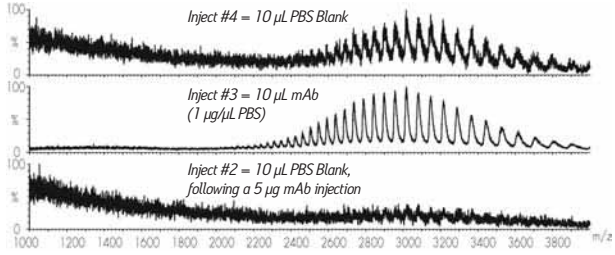


Over a series of 100 injections, satisfactory results were obtained for BSA and a mAb, as shown for injections #97-100 on a MassPREP on-line desalting cartridge.

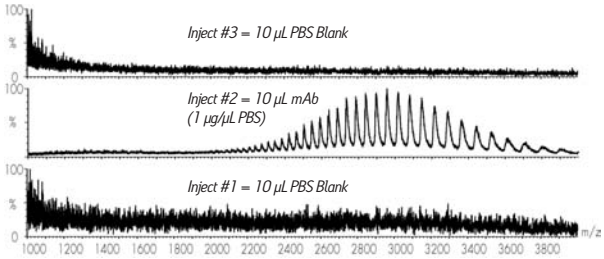
Reference Desalting of Proteins Using MassPREP On-line Desalting Cartridges Prior to Mass Spectrometry. 2005 Waters Applications Note 720001077EN

Excellent Recovery with No Detectable Carryover

Competitor's On-line Desalting Cartridge



Waters MassPREP On-line Desalting Cartridge

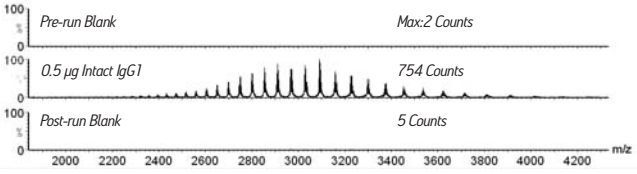


"Column-related carryover" from previous protein sample injections can compromise the integrity of collected LC/MS data. Compared to results obtained on a competitive on-line desalting cartridge (top), excellent sample recovery is obtained with Waters MassPREP on-line desalting cartridge (bottom).

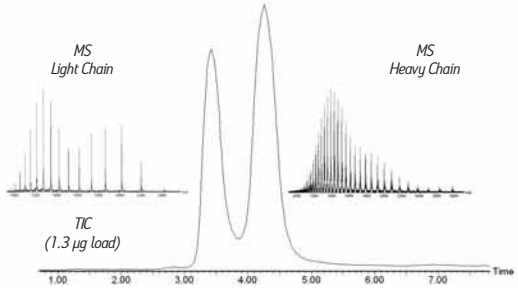
Waters MassPREP Micro Desalting Column (2.1 x 5 mm)

Experimental

LC System:	Waters ACQUITY UPLC system
MS System:	Waters LCT Premier™ ESI-TOF MS/Synapt HDMS
Ionization Mode:	ESI Positive, V mode
Eluent A:	0.1% Formic Acid (H_2O)
Eluent B:	0.1% Formic Acid (ACN)
Column temp:	80 °C



Combined ESI-TOF mass spectra of an intact IgG1 antibody from a 4 minutes LC/MS analysis. The results reveal no detectable carryover following a 0.5 µg injection of the antibody.



Total ion chromatogram (TIC) from UPLC/MS analysis of light and heavy chains from a reduced IgG1 antibody. A 10 min LC/MS run largely resolved the earlier eluting light chain from the later eluting glycosylated heavy chains.

NanoEase Trap, Capillary, and Nano Columns

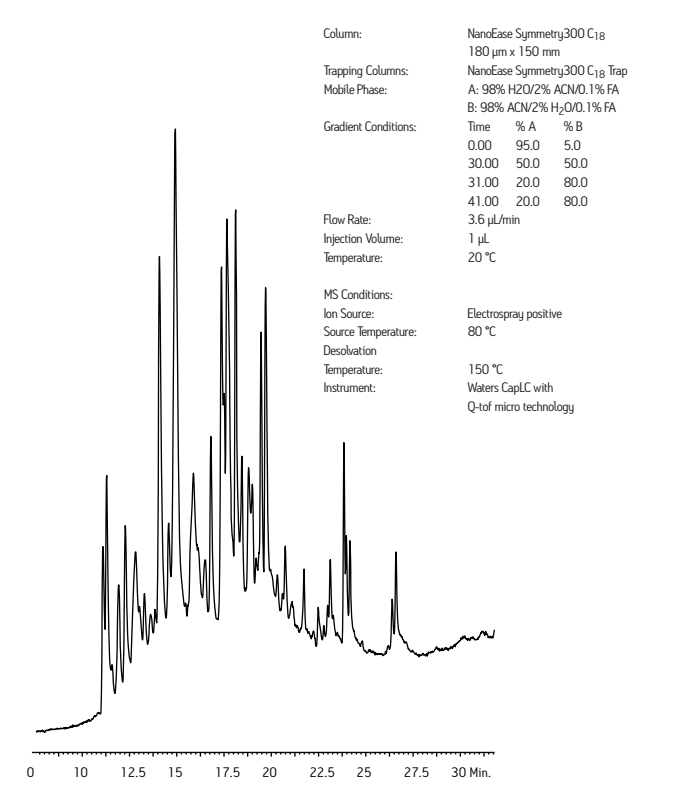
Waters NanoEase Trapping, Capillary, and Nano columns, contain carefully-selected, reversed-phase and ion-exchange chemistries for bioseparations. The column offerings are developed and intended for use on various traditional low pressure, capillary, and nano flow LC Systems.

NanoEase Trapping Columns

- NanoEase trap "direct connect" design minimizes band broadening
- SCX offering for 2D applications
- RP offerings for sample desalting or concentration

Symmetry300 C_{18}, Atlantis dC_{18}, and strong cation-exchange (SCX) NanoEase trap columns are robust devices that can effectively separate complex samples or remove buffers and high concentrations of salts through many injections. The reversed-phase trap design minimizes peak band broadening while the design of the SCX device provides increased binding capacity. These devices generate low backpressure and yield good recovery to the subfemtomole peptide level. In addition, NanoEase trap column "direct connect" design allows for easy configuration and replacement in a six or a ten-port valve for either one-dimensional or two-dimensional LC/MS analyses.

BSA Digest Using NanoEase Symmetry300 C_{18} Trap Columns



Description	Particle Size	Dimension	Part No.
Symmetry300 C_{18} Trap Column 5/pack	5 µm	0.18 x 23.5 mm	186002622
Atlantis dC_{18} Trap Column 5/pack	5 µm	0.18 x 23.5 mm	186002574
SCX 300Å Trap Column 5/pack	5 µm	0.50 x 23.5 mm	186002623
Symmetry C_{18} Trap Column 5/pack	5 µm	0.18 x 23.5 mm	186002808
0.18 i.d. Custom Trap Column 5/pack	Custom	0.18 x 23.5 mm	186002687
0.50 i.d. Custom Trap Column 5/pack	Custom	0.50 x 23.5 mm	186002806

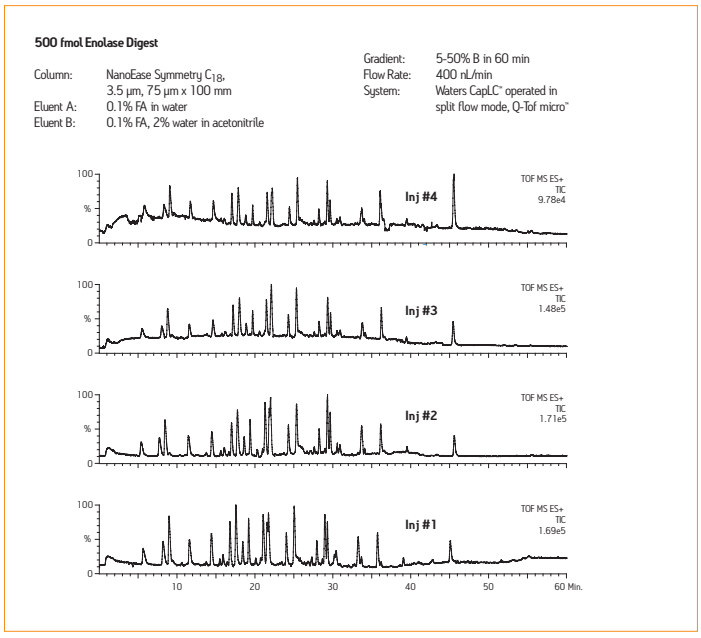
NanoEase Trap Column hardware obtained from Optimize Technologies Inc., Oregon City, OR, 97045-0009, USA

NanoEase Nano and Capillary Columns

NanoEase columns contain high performance, small particle sorbents. Exacting manufacturing and QC production procedures ensure consistent column performance. Superior column longevity is ensured by a novel frit technology that minimizes bed disturbance throughout the life of the column.

- Superior chromatographic performance
- Available in variety of Waters stationary phases
- Robust and easy to handle
- Can be used with any capillary LC system

NanoEase LC/MS Nano Column Reproducibility



Description	Inner Diameter	Length	Particle Size	Part No.
Symmetry C_{18}	75 µm	50 mm	3.5 µm	186002188
Symmetry C_{18}	75 µm	100 mm	3.5 µm	186002189
Symmetry C_{18}	75 µm	150 mm	3.5 µm	186002190
Symmetry300 C_{18}	75 µm	50 mm	3.5 µm	186002191
Symmetry300 C_{18}	75 µm	100 mm	3.5 µm	186002192
Symmetry300 C_{18}	75 µm	150 mm	3.5 µm	186002193
Atlantis dC_{18}	75 µm	50 mm	3 µm	186002194
Atlantis dC_{18}	75 µm	100 mm	3 µm	186002195
Atlantis dC_{18}	75 µm	150 mm	3 µm	186002197
Symmetry C_{18}	100 µm	50 mm	3.5 µm	186002201
Symmetry C_{18}	100 µm	100 mm	3.5 µm	186002202
Symmetry C_{18}	100 µm	150 mm	3.5 µm	186002203
Symmetry300 C_{18}	100 µm	50 mm	3.5 µm	186002204
Symmetry300 C_{18}	100 µm	100 mm	3.5 µm	186002205
Symmetry300 C_{18}	100 µm	150 mm	3.5 µm	186002206
Atlantis dC_{18}	100 µm	50 mm	3 µm	186002207
Atlantis dC_{18}	100 µm	100 mm	3 µm	186002208
Atlantis dC_{18}	100 µm	150 mm	3 µm	186002209
Symmetry C_{18}	150 µm	50 mm	3.5 µm	186002459
Symmetry C_{18}	150 µm	100 mm	3.5 µm	186002460
Symmetry C_{18}	150 µm	150 mm	3.5 µm	186002461
Symmetry300 C_{18}	150 µm	50 mm	3.5 µm	186002462
Symmetry300 C_{18}	150 µm	100 mm	3.5 µm	186002463
Symmetry300 C_{18}	150 µm	150 mm	3.5 µm	186002464
Atlantis dC_{18}	150 µm	50 mm	3 µm	186002466
Atlantis dC_{18}	150 µm	100 mm	3 µm	186002467
Atlantis dC_{18}	150 µm	150 mm	3 µm	186002468

Description	Inner Diameter	Length	Particle Size	Part No.
Custom	75 µm	Custom	Custom	186002213
Custom	100 µm	Custom	Custom	186002214
Custom	150 µm	Custom	Custom	186002472
Symmetry C_{18}	300 µm	50 mm	3.5 µm	186002581
Symmetry C_{18}	300 µm	100 mm	3.5 µm	186002582
Symmetry C_{18}	300 µm	150 mm	3.5 µm	186002583
Symmetry C_{18}	300 µm	50 mm	5 µm	186002584
Symmetry C_{18}	300 µm	100 mm	5 µm	186002585
Symmetry C_{18}	300 µm	150 mm	5 µm	186002586
Symmetry300 C_{18}	300 µm	50 mm	3.5 µm	186002587
Symmetry300 C_{18}	300 µm	100 mm	3.5 µm	186002588
Symmetry300 C_{18}	300 µm	150 mm	3.5 µm	186002589
Symmetry300 C_{18}	300 µm	50 mm	5 µm	186002590
Symmetry300 C_{18}	300 µm	100 mm	5 µm	186002591
Symmetry300 C_{18}	300 µm	150 mm	5 µm	186002592
Atlantis dC_{18}	300 µm	50 mm	3 µm	186002593
Atlantis dC_{18}	300 µm	100 mm	3 µm	186002594
Atlantis dC_{18}	300 µm	150 mm	3 µm	186002595
Atlantis dC_{18}	300 µm	50 mm	5 µm	186002596
Atlantis dC_{18}	300 µm	100 mm	5 µm	186002597
Atlantis dC_{18}	300 µm	150 mm	5 µm	186002598
XBridge BEH130 C_{18}	300 µm	50 mm	5 µm	186003682
Custom	300µm	Custom	Custom	186002605

For Peptide Separation Technology NanoEase and Capillary Columns, See Page 192

nanoACQUITY UltraPerformance LC Columns

- Novel frit technology and design for extended column life
- Capable of operation up to 10,000 PSI
- Easily interfaces with LC/MS/MS systems
- Available with ACQUITY UPLC C_{18}, 1.7 µm BEH Technology particles

nanoACQUITY UPLC System

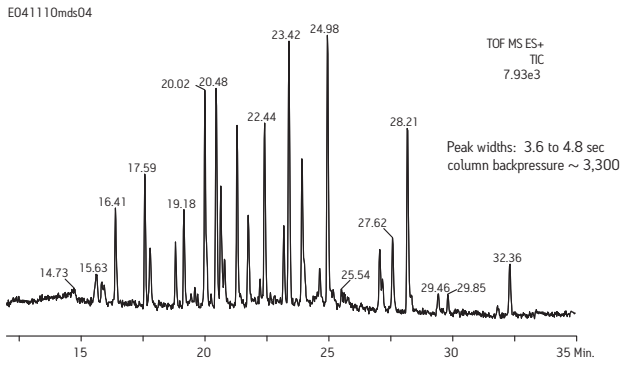
Waters nanoACQUITY Trapping, Capillary, and Nano columns are designed specifically for use on Waters nanoACQUITY UPLC System. As with our line of NanoEase columns, these offerings containing carefully selected reversed-phase and ion-exchange chemistries for bioseparations.

Column i.d.	Flow Rate
300 µm	4 µL/min
150 µm	1 µL/min
75 µm	250 nL/min

Flow Rates and Column Internal Diameters for Capillary and Nanoflow. Capillary-scale separations are done in a flow rate range of 1 µL/min to 10 µL/min on columns with internal diameters (i.d.) ranging from 300 µm to 100 µm. Nanoflow is <1 µL/min on columns with i.d. ≥ 75 µm.

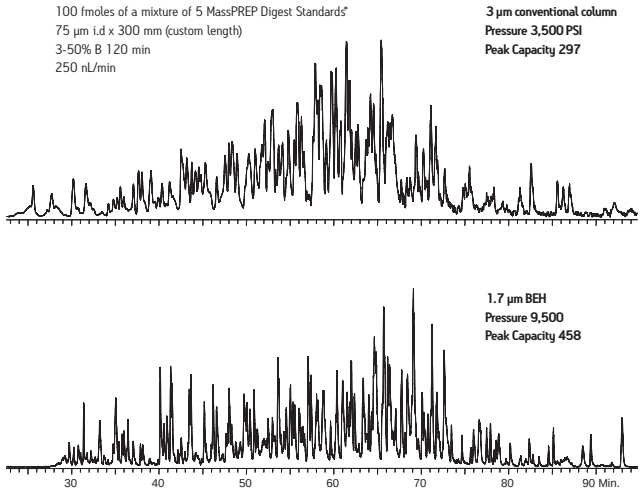
Separation of Enolose Digest

200 fmol enolase digest, 5% B - 60% B in 30 minutes, 250 nL/min flow rate



The ability to operate at higher pressures enables the use of longer columns (up to and including 250 mm in length) and further leverage the separation power of 1.7 µm particles. Thus, the nanoACQUITY UPLC chemistry offering now includes the Peptide Separation Technology nanoACQUITY UPLC columns, which incorporate the 1.7 µm BEH130 material.

Increased Peak Capacity Separations



* Mixture includes:

	Part Number
MassPREP Enolase Digestion Standard	186002325
MassPREP Phosphorylase b Digestion Standard	186002326
MassPREP Bovine Hemoglobin Digestion Standard	186002327
MassPREP ADH Digestion Standard	186002328
MassPREP BSH Digestion Standard	186002329

nanoACQUITY UPLC Columns (10,000 psi)

Description	Particle Size	Inner Diameter	Length	Part No.
Symmetry C_{18}	3.5 µm	75 µm	100 mm	186003491
Symmetry C_{18}	3.5 µm	75 µm	150 mm	186003492
Symmetry C_{18}	3.5 µm	100 µm	100 mm	186003493
Symmetry C_{18}	3.5 µm	100 µm	150 mm	186003494
Symmetry C_{18}	3.5 µm	150 µm	100 mm	186003495
Symmetry C_{18}	3.5 µm	150 µm	150 mm	186003496
Symmetry C_{18}	3.5 µm	300 µm	100 mm	186003497
Symmetry C_{18}	3.5 µm	300 µm	150 mm	186003498
Atlantis dC_{18}	3 µm	75 µm	100 mm	186003499
Atlantis dC_{18}	3 µm	75 µm	150 mm	186003500
Atlantis dC_{18}	3 µm	100 µm	100 mm	186003501
Atlantis dC_{18}	3 µm	100 µm	150 mm	186003502
Atlantis dC_{18}	3 µm	150 µm	100 mm	186003503
Atlantis dC_{18}	3 µm	150 µm	150 mm	186003504
Atlantis dC_{18}	3 µm	300 µm	100 mm	186003505
Atlantis dC_{18}	3 µm	300 µm	150 mm	186003506
nanoACQUITY UPLC Trap, SCX (std fitting)		180 µm	20 mm	186003507
nanoACQUITY UPLC Trap, Symmetry C_{18}, (std fitting)	5µm	180 µm	20 mm	186003514
nanoACQUITY UPLC Trap Symmetry C_{18}, (w/v fitting)	5µm	180µm	20mm	186004630
Custom				186003513

Peptide Separation Technology Columns

BEH130 C_{18}	1.7 µm	75 µm	100 mm	186003542
BEH130 C_{18}	1.7 µm	75 µm	150 mm	186003543
BEH130 C_{18}	1.7 µm	75 µm	200 mm	186003544
BEH130 C_{18}	1.7 µm	75 µm	250 mm	186003545
BEH130 C_{18}	1.7 µm	100 µm	100 mm	186003546
BEH130 C_{18}	1.7 µm	150 µm	100 mm	186003550
BEH300 C_4	1.7 µm	75 µm	100 mm	186004639
BEH300 C_4	1.7 µm	100 µm	100 mm	186004640
BEH300 C_4	1.7 µm	150 µm	100 mm	186004641

For use with nanoACQUITY UPLC systems rated to 10,000 psi only.Not for use with nanoACQUITY UPLC systems rated to 5,000 psi.

nanoACQUITY UPLC Columns (5,000 psi)

Description	Particle Size	Inner Diameter	Length	Part No.
Symmetry C_{18}	3.5 µm	75 µm	100 mm	186002821
Symmetry C_{18}	3.5 µm	75 µm	150 mm	186002822
Symmetry C_{18}	3.5 µm	100 µm	100 mm	186002823
Symmetry C_{18}	3.5 µm	100 µm	150 mm	186002824
Symmetry C_{18}	3.5 µm	150 µm	100 mm	186002825
Symmetry C_{18}	3.5 µm	150 µm	150 mm	186002826
Symmetry C_{18}	3.5 µm	300 µm	100 mm	186002827
Symmetry C_{18}	3.5 µm	300 µm	150 mm	186002828
Atlantis dC_{18}	3 µm	75 µm	100 mm	186002829
Atlantis dC_{18}	3 µm	75 µm	150 mm	186002830
Atlantis dC_{18}	3 µm	100 µm	100 mm	186002831
Atlantis dC_{18}	3 µm	100 µm	150 mm	186002832
Atlantis dC_{18}	3 µm	150 µm	100 mm	186002833
Atlantis dC_{18}	3 µm	150 µm	150 mm	186002834
Atlantis dC_{18}	3 µm	300 µm	100 mm	186002835
Atlantis dC_{18}	3 µm	300 µm	150 mm	186002836
BEH130 C_{18}	1.7 µm	75 µm	100 mm	186002837
BEH130 C_{18}	1.7 µm	100 µm	100 mm	186002838
BEH130 C_{18}	1.7 µm	150 µm	100 mm	186002839
Custom				186002840
Symmetry C_{18} Trap Column 5 µm		180 µm	20 mm	186002841

For use with nanoACQUITY UPLC systems rated to 5,000 psi only. Not for use with nanoACQUITY UPLC systems rated to 10,000 psi.

nanoACQUITY UPLC 2D Kit

The two-dimensional (2D) LC/MS Kit for the nanoACQUITY UPLC System provides high efficiency on-line 2D-LC/MS analysis for complex proteomics samples, such as protein global digests.

The first and second dimensions are strong cation exchange (SCX) and reversed phase (RP), respectively.

The nanoACQUITY UPLC on-line 2D-LC/MS kit contains the tubing to configure the system for 2-pump traping in conjuction with online 2D operations, the nanoACQUITY UPLC SCX, trapping and RP analytical columns, and MassPREP Protein digest standards.

First-Dimension Separation

The first-dimension separation employs the Waters nanoACQUITY UPLC 180 µm x 23 mm SCX column. Two step gradients, a salt gradient and then an organic gradient, are applied to the SCX column. While the salt gradient separates peptides that have different charge properties, the organic gradient gradually elutes the hydrophobic peptides that remain on the SCX column after the salt gradient is applied. A nanoACQUITY UPLC trapping column collects the peptides that elute.

Second-Dimension Separation

The second-dimension separation employs a RP gradient to separated each SCX fraction with a nanoACQUITY UPLC 75 µm x 100 mm reversed-phase analytical column. The reversed-phase analytical column is used to separate the peptides collected on the trapping column. The separated peptides are then directed to an on-line MS system for characterization

nanoACQUITY UPLC 10,000 psi On-Line 2D Kit

Description	Part No.
nanoACQUITY UPLC (10K psi) on-line 2D-LC/MS kit	176001367
Kit consists of:	
Assembly, 25 µm, capillary, BSM (binary solvent manager) to trap valve	430001575
Assembly, 40 µm capillary, ASM (auxiliary solvent manager) to injection valve	430001576
Assembly, capillary tubing, injection valve to trap valve	430001577
Assembly, capillary tubing, injection to trap valve	430001629
nanoACQUITY UPLC SCX column, 5 µm, 180 µm x 23 mm	186003507
nanoACQUITY UPLC trapping column, 5 µm Symmetry C_{18}, 180 µm x 20 mm	186003514
nanoACQUITY UPLC analytical column, 1.7 µm BEH C_{18}, 75 µm x 100 mm	186003542
MassPREP 5 Protein Digest Standard Kit	186002330
nanoACQUITY UPLC on-line 2D-LS/MS User's Guide	715001357

MassPREP Kits

Water MassPREP Gylcoanalysis, Phosphopeptide Enrichment, and Protein Expression Kits were developed to address important sample preparation needs required prior to subsequent LC, MS, or LC/MS analyses. Each application specific kit contains a comprehensive, fully-tested set of reagents and documentation regarding effective use.

- Simple protocols and easy to use
- Enables high-throughput sample preparation
- Highly reproducible and consistent sample prep results

MassPREP Glycoanalysis Kit

- Optimize protein deglycosylation reactions with *Rapi*Gest SF surfactant
- Minimize sample manipulations
- Effective desalting /sample clean-up method
- Compatible with MALDI MS and other glycan analysis techniques
- Assist in isolation of 2-AB labeled glycans

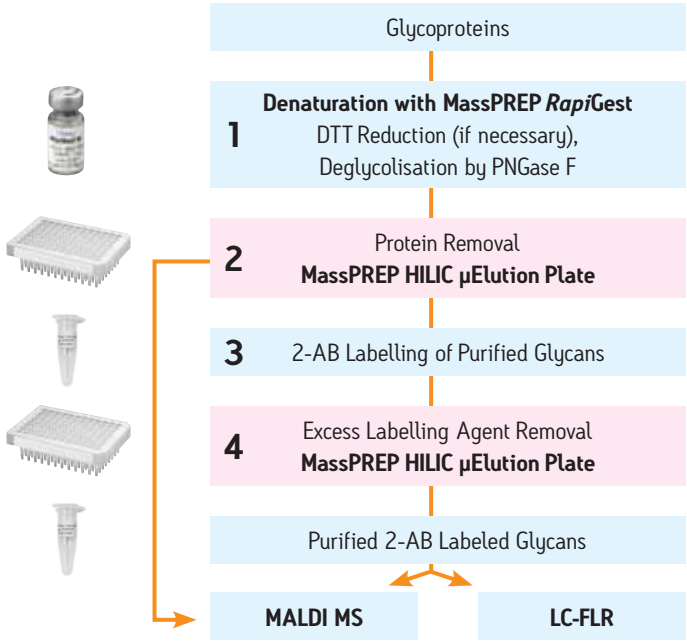
Glycosylation is one of the most important types of post-translational modification (PTM) in eukaryotic proteins. Efficient sample deglycosylation and sample preparation is a key requirement for successful and sensitive glycan analysis. In addition, the preparation and purification 2-AB-labeled glycans released from glycoproteins can also be an important step in their successful analyses.

The MassPREP Glycoanalysis kit provides simple and robust sample preparation without compromising sample recovery. As shown (right), unlabeled or 2-AB labeled glycans can be successully analyzed by MALDI-MS or by LC with fluorescence detection following MassPREP Glycoanalysis Kit use.

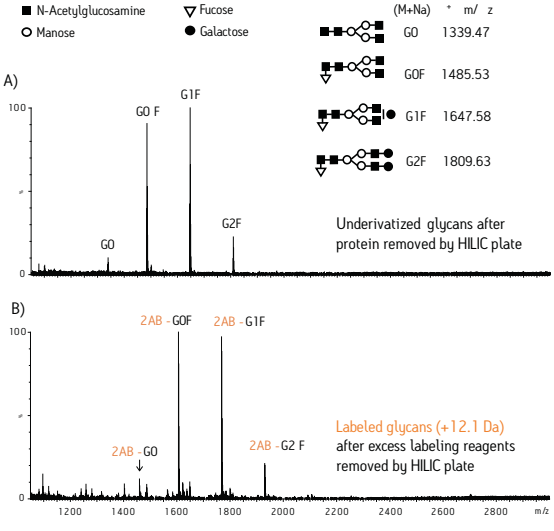
Description	Dimensions/Qty.	Part No.
MassPREP Glycoanalysis Kit (includes, MassPREP HILIC µElution Plate, *Rapi*Gest SF, and MassPREP MALDI Matrix DHB)		186002817
Kit Consists of:		
MassPREP HILIC uElution Plate	96-well	186002780
*Rapi*Gest SF	1 mg vial	186001860
MassPREP MALDI Matrix DHB	10 mg vial 5/pk	186002333

For more information see *Rapi*Gest SF Page 233

Sample Preparation from Glycoprotein to Purified 2-AB Labeled Glycans



MALDI MS Mass Profiling of Native and 2AB-Labeled IgG Glycans



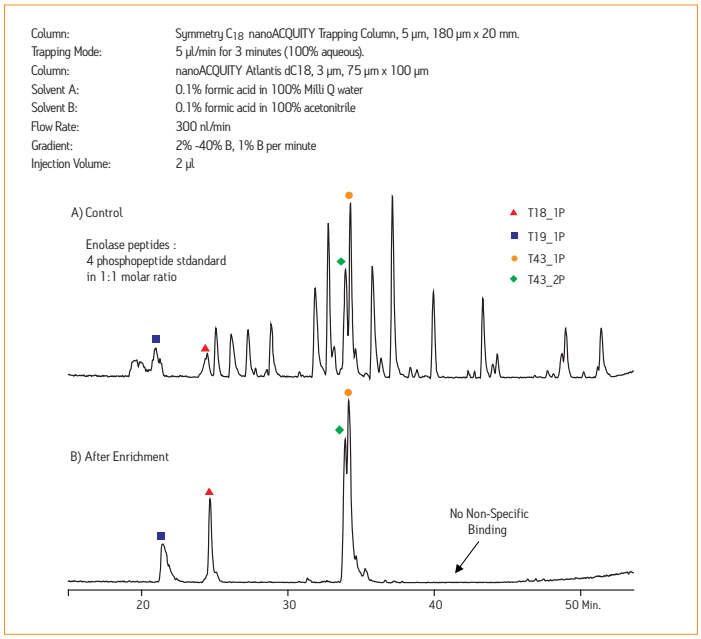
MALDI-TOF MS spectra of: (A) 8 pmol of the unlabeled IgG1glycans after first HILIC cleanup prior to labeling. (B) 3 pmol of the 2-AB-labeled IgG1 glycans after second HILIC cleanup. Each spectrum shows well purified glycans with no inferences from impurities.

MassPREP Phosphopeptide Enrichment Kit

- Selective enrichment of phosphopeptides from complex proteomic samples
- Highly efficient and reproducible affinity-based enrichment
- Easy to use
- Application in high-throughput analysis

MassPREP Phosphopeptide Enrichment Kit, developed for selective enrichment of phosphopeptides from complex samples, includes a 96-well microscale, solid-phase extraction (SPE) plate packed with an affinity sorbent. The kit also includes a unique chemical (Enhancer™) that can be added to further improve the selectivity of phosphopeptides. In contrast to currently popular immobilized metal affinity chromatography (IMAC) technology, this isolation method does not require pre-loading the sorbent with metal chelator. This phosphopeptide enrichment protocol is simplified. The robustness of this method is provided by the sorbent that has high native affinity towards phosphopeptides and the Enhancer which yields superior selectivity of the sorbent towards phosphopeptides.

Phosphopeptide Analysis without (top) and with (bottom) MassPREP Phosphopeptide Kit Enrichment



Description	Units/Kit	Amt/Unit	Part No.
MassPREP Phosphopeptide Enrichment Kit			186003864
Kit Consists of:			
MassPREP Phosphopeptide Enrichment µElution Plate	1	1 plate	186003820
MassPREP Enolase/Phosphopeptide Standard	1	1 nmole	186003286
MassPREP Enhancer	10	500 mg	186003821
MassPREP Enhancer	5	500 mg	186003863

For more information see Phosphopeptide Standards on page 231

Waters Protein Expression System Kits

The Protein Expression System consists of nanoACQUITY UPLC system, Q-Tof Premier, MassLynx™ ProteinLynx™ Global Server bioinformatics software, and Waters MassPREP chemistry consumables and kits.

MassPREP Chemistry Consumable Kits for the Protein Express System include nanoACQUITY UPLC columns, MassPREP Protein Digestion Standards Mix 1 and 2, MassPREP Enolase Digest Standard, and *Rapi*Gest SF Surfactant. Both Microscale and a Nanoscale Kits are available.

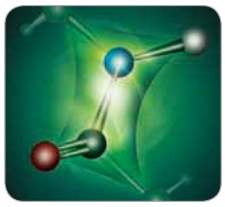
Microscale Kit

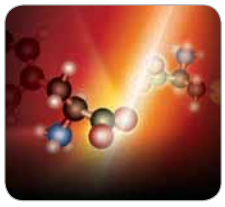
Description	Qty.	Part No.
Microscale Kit		186003903
Kit Contains :		
nanoACQUITY UPLC 300 µm x 150 mm		
Atlantis dC_{18} 3 µm Column	1	186003506
MPDS Mix 1	1	186002865
MPDS Mix 2	1	186002866
MPDS Enolase	1	186002325
*Rapi*Gest SF, 1 mg	1	186001860

Nanoscale Kit

Description	Qty.	Part No.
Nanoscale Kit		186003904
Kit Contains :		
nanoACQUITY UPLC 75 µm x 150 mm		
Atlantis dC_{18} 3 µm Column	1	186003500
nanoACQUITY UPLC 180 µm x 20 mm		
Symmetry C_{18} 5 µm Trap Column	1	186003514
MPDS Mix 1	1	186002865
MPDS Mix 2	1	186002866
MPDS Enolase	1	186002325
*Rapi*Gest SF, 1 mg	1	186001860

UPLC Technology for Biomolecules





Waters offers a collection of powerful tools for characterizing biopharmaceuticals. These solutions are designed specifically to provide more accurate, reliable, and robust results, simplify your analyses and thereby increase productivity. Our system solutions combine Waters market-leading instrumentation, column chemistries, and software and informatics products, that leverage the sensitivity and resolution of UltraPerformance LC (UPLC) Technology.

UPLC Technology was introduced by Waters as a novel chromatographic technique that takes full advantage of sub-2 µm column chemistries used in a system optimized for pressures necessary to produce best-in-class separations. By packing columns with very small particles, band-broadening during separation is minimized. In addition, since distances are reduced in small particles, separations can be optimized using higher linear velocities (i.e., flow rates), while maintaining chromatographic equilibrium. The result of these chromatographic developments is separations with dramatically increased resolution, sensitivity, and speed.

The properties of these columns places a new level of demand on the chromatographic system, and Waters has holistically developed the ACQUITY UPLC and nanoACQUITY UPLC systems to take advantage of these chromatographic principles.

To gain the benefits of the small particles columns, the system must generate exceptionally accurate and precise gradients at relatively high operation pressures. The sample manager must provide accurate, small-volume injections with minimum cycle time. Furthermore, the detectors must truly minimize band-broadening while providing high signal-to-noise ratios for peaks that are narrow in both time and volume.

Based on chromatographic principles, Waters synergistically developed the columns and the instrumentation that combine to make UPLC technology a reality–and now a proven success in laboratories around the world. While the first successful application of UPLC technology was in small molecule pharmaceuticals, Waters dedication to developing impactful laboratory innovations quickly saw the benefits of UPLC technology applied to the separation of biological and biochemically-significant macromolecules.

UPLC technology brings a new level of separation and detection performance to the analysis of biomolecules, whether from complex peptide mixtures or from a potentially life saving monoclonal antibody biotherapeutic. Today, Waters UPLC technology provides improvements in the resolution, sensitivity, separation, and detection efficiency for amino acids, peptides, proteins, and synthetic DNA and RNA oligonucleotides that help scientists and chromatographers achieve desired objectives.

Peptide Mapping

Waters UPLC Peptide Analysis Solution, including UPLC Peptide Separation Technology columns, delivers maximum resolution and sensitivity. UPLC chromatographic improvements have lead to greater confidence in protein identification with improved determination of modified peptides, including glycopeptides. In the comparison of separations using 3.5 µm particles and 1.7 µm particles, peaks are clearly sharper, and more small peaks are resolved using UPLC technology.

Synthetic Oligonucleotides Analysis

The UPLC Oligonucleotide Analysis Solution and UPLC Oligonculeotide Separation Technology (OST) columns provide higher resolution in less time of synthetic oligonucleotide target products from contaminating failure sequences. This Waters technology leads to better resolution and increased sample throughput compared to use of traditional HPLC methods.

Protein Analysis

UPLC/MS technology is available for rapid, efficient mass profiling of protein samples, including intact antibodies and their associated variants. Samples can be successfully desalted on-line using the UPLC Intact Mass Analysis Kit, which ensures a high resolution spectrum with good signal-to-noise. The resulting total ion chromatograms (TICs), combined ESI-TOF mass spectra, and MaxEnt1 deconvolution mass spectrum of the intact IgG1 quickly confirm the identity of the molecule while providing a measure of the degree of modification of the protein in this sample.

In addition, reversed-phase separations of proteins are improved with UPLC Protein Separation Technology columns, used with ACQUITY UPLC. The BEH300 C_4 column gives good peak shape for wide range of proteins with minimized carryover. This column chemistry is available in 3.5 and 1.7 µm particles for UPLC separations and can be effectively used to analyze reduced and alkylated monoclonal antibodies.

Amino Acid Analysis

The UPLC Amino Acid Analysis Solution is a total application solution optimized for an accurate, reliable, and reproducible analysis of amino acids. Combining Waters new AccQ•Tag Ultra chemistry and ACQUITY UPLC system, performance is assured in the areas of protein characterization, cell culture monitoring, and the nutritional analysis of foods and feeds. The enhanced resolution and sensitivity of the separation ensures that the analysis yields accurate and precise qualitative and quantitative results. This complete solution includes the instrument, derivitization and separation chemistry, software, and comprehensive support for a true amino acid analyzer solution.

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Supplies and Hardware

Column and Cartridge Fittings and Accessories

ACQUITY Column Replacement Parts

ACQUITY UPLC® Column In-Line Filter Unit

Description	Part No.
In-line filter holder and six 0.2 µm stainless steel replacement filters	205000343
Five 0.2 µm stainless steel replacement filters and End Nuts for 205000343	700002775

ACQUITY UPLC Column Replacement Parts

Description	Part No.
Three 0.2 µm Inlet/Outlet Frits for 2.1 mm i.d. UPLC Columns	700003776
Three 0.2 µm Inlet/Outlet Frits for 1.0 mm i.d. UPLC Columns	700003775
One Inlet End Nut for 2.1 mm i.d. UPLC Column	700003779
One Outlet End Nut for 2.1 mm i.d. UPLC Column	700003780
One Inlet End Nut for 1.0 mm i.d. UPLC Column	700003777
One Outlet End Nut for 1.0 mm i.d. UPLC Column	700003778

Waters Column and Cartridge Replacement Parts

End Connector Kit

(endfittings for cartridge columns)

Description	Part No.
End connector kit (contains 1 pair of endfittings, c-clips and coupling)	WAT037525
Replacement o-ring 2/pkg	WAT023401
Replacement c-clip 1/pkg	WAT037560

Replacement Filter Assemblies for Columns

Column i.d.	Porosity	Part No.
1 mm	2 µm	600000175
1 mm	0.5 µm	600000176
2.1 mm	2 µm	600000177
2.1 mm	0.5 µm	600000178
3.0, 3.9, 4.6 mm	2 µm	600000179
3.0, 3.9, 4.6 mm	0.5 µm	600000180
7.8 mm	2 µm	600000181
7.8 mm	5 µm	600000182
19 mm	2 µm	600000183
30 mm	2 µm	600000184

Parker Style Cartridge Fittings and Accessories

These endfittings and accessories can be used with 4.6 mm, 4.0 mm, or 3.0 mm i.d. cartridges.

Description	Qty.	Part No.
Removable Column Endfitting	2/pk	PSS614100
Frit Assembly (2 µm)	5/pk	PSS614103
Frit Assembly (0.5 µm)	5/pk	PSS614104
Column Coupler	2/pk	PSS614102
Long Tail Endfitting	2/pk	PSS614101
Extended Endfitting for use with 10 mm Integral Guard	1/pk	PSS614108
Nylon Column Plugs for Storage of Complete Column	1/pk	WAT015674
Nylon Column Caps for Storage of Replacement Cartridge Column	10/pk	PSS614113
Inline 10 mm Guard Cartridge Holder Kit for use with above items		PSS830008

[1] 30 mm Stand Alone Guard/Column (endfittings not included)
[2] Extended endfitting for use with 10 mm Integral Guard: PSS614108
[3] 10 mm Integral Guard Column
[4] Column Coupler : PSS614102

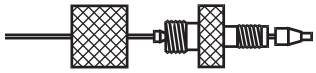
Tubing and Fittings

SLIPFREE Connectors

New Generation HPLC Column Connector

- Guarantees a void-free connection because it pushes the tubing into the end-fitting; This design must come installed on the tubing.
- Fingertight to 10,000 psi–Never need wrenches.
- Readjusts to all column end-fittings; Compatible with all commercially available end-fittings that we have tried.
- Stainless stell tread for good stability and no particle generation.
- Unique design separates tube holding function from sealing function.

Single SLIPFREE



Double SLIPFREE

SLIPFREE Fittings	Part No.
Single SLIPFREE, 6 cm long, 0.005 in. i.d.	PSL618000
Single SLIPFREE, 20 cm long, 0.005 in. i.d.	PSL618004
Single SLIPFREE, 6 cm long, 0.010 in. i.d.	PSL618006
Single SLIPFREE, 10 cm long, 0.010 in. i.d.	PSL618008
Single SLIPFREE, 20 cm long, 0.010 in. i.d.	PSL618010
Double SLIPFREE, 6 cm long, 0.005 in. i.d.	PSL618001
Double SLIPFREE, 10 cm long, 0.005 in. i.d.	PSL618003
Double SLIPFREE, 20 cm long, 0.005 in. i.d.	PSL618005
Double SLIPFREE, 6 cm long, 0.010 in. i.d.	PSL618007
Double SLIPFREE, 10 cm long, 0.010 in. i.d.	PSL618009
Double SLIPFREE, 20 cm long, 0.010 in. i.d.	PSL618011

The SLIPFREE connector comes standard with a replaceable Vespel ferrule.

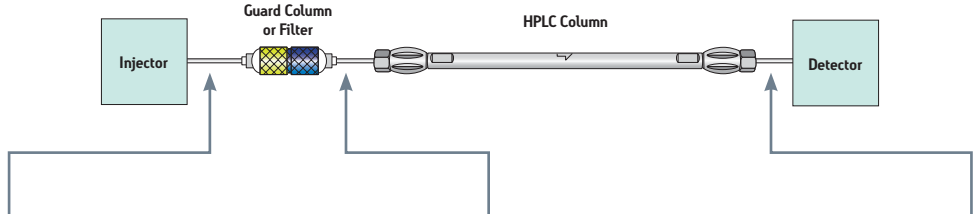
0.010 inch i.d. is recommended for routine work.

0.005 inch i.d. is recommended for column connection to short 4.6 mm i.d. and for small-bore or microbore connections.

0.020 inch i.d. is recommended for prep or semi-prep connections, or for connections ahead of the injector.

How to Use a SLIPFREE Connector

Place a SLIPFREE® connector at any location in the HPLC system where fittings must be frequently made or broke. Choose the single for installation to the injector or at any other fitting with conventional nuts and ferrules that would seldom need to be removed. Choose the double SLIPFREE connector for column coupling or places where both ends must be loosened frequently.



Single SLIPFREE (Length as needed)

A single SLIPFREE Connector has the SLIPFREE design only on one end. This end should be placed where connections and disconnections are going to be made frequently, such as the end-fitting of a column or detector. In this example, the other end-fitting is seated into the injector using a stainless steel nut and ferrule that is compatible with the brand of injector.

Double SLIPFREE (60 mm Length)

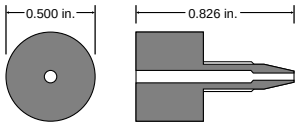
A Double SLIPFREE Connector has a SLIPFREE design on both ends. It should be placed where both connections and disconnections are going to be made frequently, such as between a column and guard column. Very short (6 cm), small i.d. connectors are available to minimize extra dead-volume. Because SLIPFREE Connectors fit any column manufacturer's end-fitting, the two items being connected do not have to be from the same manufacturer.

Single SLIPFREE (Length as needed)

A Single SLIPFREE Connector has the SLIPFREE design only on one end. This end should be placed where connections and disconnections are going to be made frequently, such as the end-fitting of a column or detector. In this example, the other end of the tubing is seated into the detector using a stainless steel nut and ferrule (one that is compatible with the brand of detector. If there is not a convenient way to attach it to the detector, a union can be used.

PEEK One-Piece Fingertight Fitting, 1/16 in., 10-32 Thread

This high performance fingertight is recommended for the most demanding applications and will resist pressures up to 6000 psi (420 Bar). The diameter of the head of the nuts has been increased to facilitate tightening without tools—it is still a genuine fingertight. Nut and ferrule are made from a single piece of PEEK™.

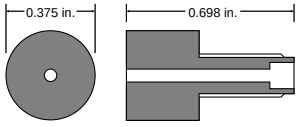


PSL613315

PEEK Fingertight One-Piece Fitting	Part No.
PEEK Fingertight One-Piece Fitting	PSL613315

PEEK Two-Piece Fingertight Fittings, 1/16 in., 10-32 Thread

These fittings allow connections to be made by hand with a pressure rating of 4000 psi (280 Bar). The inexpensive PEEK ferrules will last for at least 50 connections before it has to be replaced. The nuts may be reused again and again. The PEEK ferrule is highly chemically inert and may be used with any mobile phase. This fitting provides a budget alternative for HPLC plumbing—which will fit virtually all existing HPLC fittings including Swagelok, Parker, Rheodyne, Beckman, Valco, Waters, etc—all having 10-32 female threads.



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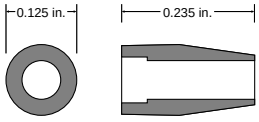


PSL613316

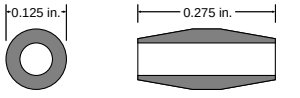
PEEK Fingertight Two-Piece Fittings	Part No.
PEEK Fingertight Two-Piece Nut	PSL613322
PEEK Single Ferrule	PSL613316

PEEK Fittings with Double-Ferrules, 1/16 in., 10-32 Thread

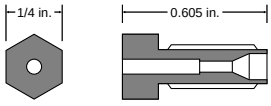
These fittings use a DOUBLE-FERRULE made of PEEK which grips the tubing in two places for unmatched reliability. These ferrules actually provide twice the holding power of single-ferrule fittings, and are ideal for use with PEEK and Tefzel tubing, which will often slip when used with single-ferrule fittings. When used with stainless steel or titanium tubing, Double-Ferrule Fittings grip tighter for a more reliable connection at high pressure.

We offer both fingertight and hex-head nuts for use with Double-Ferrules. The fingertight version may be hand-tightened for operation at up to 6,000 psi. Use the hex-head version for connections that are difficult to reach or closely spaced.

These fittings fit virtually any female 1/16 in. fitting—Parker, Swagelok, Waters, Valco, Rheodyne, Upchurch, etc—all having 10-32 threads.



PSL613302



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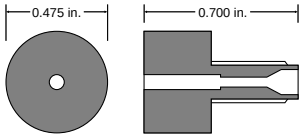

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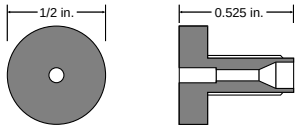


PSL613325

PEEK Fittings With Double-Ferrules	Part No.
PEEK Double-Ferrule	PSL613302
PEEK Hex-Head Nut	PSL613324
PEEK Fingertight Nut	PSL613301
Stainless Steel Fingertight Nut	PSL613325

PTFE/ETFE Tubing and Fittings

o.d. Inches (mm)	i.d. Inches (mm)	Length/Material	Part No.
0.125 (3.2)	0.062 (1.57)	25 ft. (7.6 m), PTFE	WAT026808
0.149 (3.8)	0.119 (30.0)	25 ft. (7.6 m), PTFE	WAT026809
0.250 (6.3)	0.190 (4.8)	10 ft. (3 m), PTFE	WAT026810
0.080 (2.0)	0.058 (1.5)	25 ft. (7.6 m), PTFE	WAT026974
0.178 (4.52)	0.148 (3.76)	25 ft. (7.6 m), PTFE	WAT051041
0.149 (3.8)	0.119 (30.0)	20 ft. (6 m), PTFE	WAT051052
0.125 (3.2)	0.020 (0.508)	10 ft. (3 m), PTFE	WAT088430
0.125 (3.2)	0.009 (0.228)	10 ft. (3 m), PTFE	WAT088431
0.125 (3.2)	0.040 (1.0)	10 ft. (3 m) , PTFE	WAT088432
0.062 (1.57)	0.009 (0.228)	36 in. (1 m), ETFE	WAT088561
0.062 (1.57)	0.040 (1.0)	36 in. (1 m), PTFE	WAT088563
0.0625 (1.57)		25 ft Black Teflon	WAT077045
PTFE Adapter, 0.125 (3.2) to 0.065 (1.6) 5/pkg			WAT005137

Compression Screws & Ferrules

Description	Part No.
Ferrule, 01, Stainless Steel, 10/pk	WAT005063
Compression Screw, 0.0625 in., 10/pk	WAT005070
Compression Fitting Plug, Stainless Steel, 5/pk	WAT005079
Rheodyne Ferrule, 10/pk	WAT007020
Ferrule, Stainless Steel	WAT022330
Ferrule, 1/16 in. o.d., PEEK	WAT021817
Compression Screw, Stainless Steel	WAT025313
Compression Fitting Plug, Stainless Steel	WAT025566
Compression Screws & Ferrules, 0.166 in., 5/pk	WAT025604
Compression Screws, 0.125 in., PEEK, 2/pk	WAT046-12
Compression Screw, Long, 1/16 in.	WAT021812
Compression Screw, Short, PEEK 1/16 in.	WAT021815
Extra Long Compression Screw, Stainless Steel, 10/pk	WAT060051
Finger Tight Poly Knob used with compression screws plus PEEK ferrules	WAT021816
Tee, 0.0625 in. Compression Screw, Stainless Steel	WAT075215
Tubing Cap, Hex Stainless Steel	WAT084078
Union, 0.0625 in. Stainless Steel	WAT097332

Stainless Steel Tubing and Fittings

o.d. Inches (mm)	i.d. Inches (mm)	Length/Material	Part No.
0.0625 (1.6)	0.005 (0.127)	10 ft. (3 m), SS	WAT241039
0.0625 (1.6)	0.020 (0.508)	10 ft. (3 m), SS	WAT026804
0.0625 (1.6)	0.030 (0.762)	10 ft. (3 m), SS	430000366
0.0625 (1.6)	0.040 (1.020)	10 ft. (3 m), SS	WAT026805
0.125 (3.2)	0.062 (1.57)	10 ft. (3 m), SS	WAT026806
0.125 (3.2)	0.093 (2.36)	10 ft. (3 m), SS	WAT026807
0.0625 (1.6)	0.009 (0.228)	10 ft. (3 m), SS	WAT026973
0.0625 inch o.d. Stainless Steel Tubing Cutter with 3 Blades			WAT022384
Replacement Blades for WAT022384, 3/pk			WAT022385

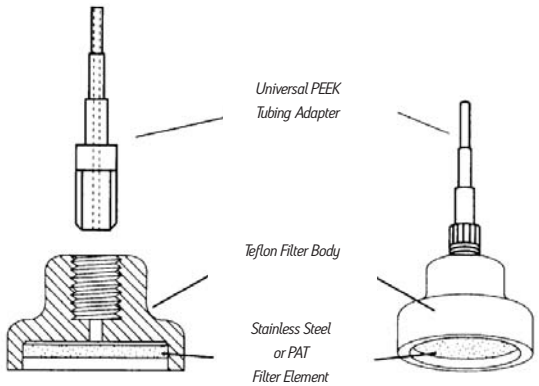
PEEK Tubing and Fittings

o.d. Inches (mm)	i.d. Inches (mm)	Length/Material	Part No.
0.0625 (1.6)	0.005 (0.127)	5 ft. (1.5 m), PEEK	WAT022995
0.0625 (1.6)	0.010 (0.254)	5 ft. (1.5 m), PEEK	WAT022996
0.0625 (1.6)	0.015 (0.381)	5 ft. (1.5 m), PEEK	WAT022997
0.0625 (1.6)	0.020 (0.508)	5 ft. (1.5 m), PEEK	WAT022998
0.0625 (1.6)	0.005 (0.127)	2 ft. (0.609 m), PEEK	WAT033390
0.0625 (1.6)	0.010 (0.254)	2 ft. (0.609 m), PEEK	WAT033391
0.0625 (1.6)	0.020 (0.508)	2 ft. (0.609 m), PEEK	WAT033392
PEEK Tubing Cutter			WAT031795
PEEK Tubing and Fitting Kit			WAT022999
PEEK Union, 0.0625 in.			WAT026-04

Last Drop Mobile Phase Filters

The Last Drop™ Mobile Phase Filter utilizes a flat filter element which sits parallel to the bottom of the reservoir. This design allows the filter to draw all but the last 2% of the mobile phase from the reservoir without drawing air into the system. Last Drop Filters are available with 316L Stainless Steel or PAT™ (PEEK alloyed with Teflon) filter elements in inert Teflon housings. The top of the housing has a PEEK tripod which fits into 1.5, 2.2 or 3.5 mm id pump inlet lines.

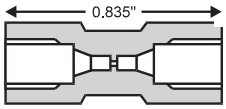
Last Drop Mobile Phase Filter	Part No.
with 2 µm Stainless Steel Filter	PSL901290
with 10 µm Stainless Steel Filter	PSL901291
with 2 µm PAT Filter	PSL901292
with 5 µm PAT Filter	PSL901294



PEEK Unions, Tees, and Crosses

The PEEK union allows you to connect two pieces of 1/16 in. tubing quickly and reliably and can be used up to 6000 psi. Because this is a PEEK union, it is absolutely inert and biocompatible. PEEK Tees and Crosses may be used up to 10,000 psi.

- Connects any 1/16 in. Tubing PEEK, SS, Ti, Tefzel)
- Low Dead Volume
- 10-32 Thread

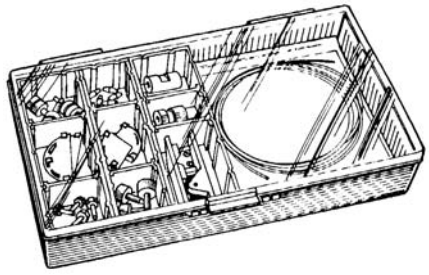


PEEK union

Description	Part No.
PEEK Union with 2 PEEK Fingertight Nuts and Double Ferrules 1/16 in.	PSL613312
PEEK Union without Nuts and Ferrules 1/16 in.	PSL613313
PEEK TEE with One Piece Fingertight Fitting	PSL613317
PEEK CROSS with One Piece Fingertight Fitting	PSL613319
PEEK TEE Without Fittings	PSL613318
PEEK CROSS Without Fittings	PSL613320
PEEK One Piece Fingertight Fitting	PSL613315

PEEK Starter Kit

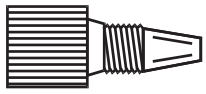
By replacing stainless steel parts, such as tubings, fittings, ferrules, mobile phase filters, in-line filters, etc. you are creating a biocompatible and metal-free environment for your samples and mobile phase. The PEEK Starter Kit is a collection of items which we believe is a good start for everybody working in the field of Biochromatography. Each kit is supplied in a sturdy plastic case. There is an approximate saving of 25% in buying this kit compared to the individual components.



Description	Part No.
PEEK Starter Kit	PSL613321
Contains the following :	
PEEK Fingertight One-Piece (6), PEEK Handtight Nut (4), PEEK Hex-Head Nut (4), PEEK Double Ferrules (20), PEEK Tubing 1/16 in. x 0.25 mm (1 x 3 m), PEEK Tubing 1/16 in. x 0.50 mm (1 x 3 m), PEEK Union (1), Elbow 90 Degrees (2), Elbow 180 Degrees (2), Guillotine Cutter (1), PAT Mobile Phase Filter - "Last Drop" (1)	

Handilok CTFE Fittings

Handilok™ fittings can replace conventional compression fittings used with 1/16 in. tubing without the need for tools and are compatible with all internal fittings having a 10-32 thread. They meet rigid high pressure requirements and will stand pressures in excess of 4000 psi.

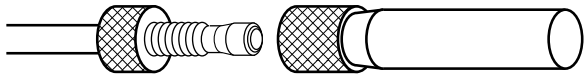


Handilok CTFE Fitting
Part No.: PSL618021

Handilok Fittings	Qty.	Part No.
1/16 in. fitting	1/pk	PSL618021
1/16 in. fitting	10/pk	PSL618022

PEEK Biocompatible Mobile Phase Filter

The mobile phase filter protects your HPLC pumping system against small particles in the mobile phase. Many macromolecules are fairly labile and require not only biocompatible chromatographs, but also mobile phase filters which are totally inert. These filters are designed from inert polymeric components, which effectively eliminate metal from the fluid path. With a porosity of 5 µm all fittings, including the inlet tube, are manufactured in totally inert PEEK.

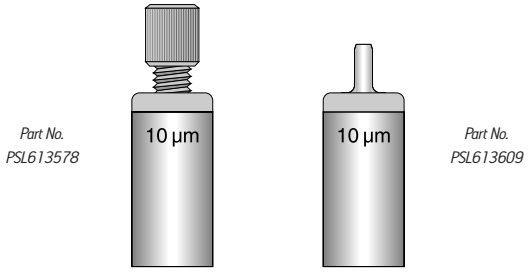


Description	Part No.
Biocompatible Mobile Phase Filter	PSL901282

Solvent Inlet Filters

It is good practice to always filter your solvents to prevent any pump damage. These 10 µm filters provide the pump protection required. Their large surface area means long life without pump cavitation.

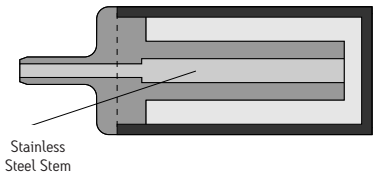
Filters should be changed periodically depending on usage and mobile phase. Replacing the filter is easy; no tools are needed. The unique Plastictight male nut is screwed into the filter and tightened by hand. Finger tightening is all that is necessary; the Plastictight fitting holds without flanging.



Description	Part No.
Solvent Inlet Filter Kits	
Solvent Inlet Filter Kit - Contains one 3 in. length of Teflon Tubing, 1 Plastictight Fitting, 5 Replacement 10 µm Filters	PSL613600
Plastictight Fitting with Teflon Tubing 1/16 in. i.d. x 1/8 in. o.d. x 3 ft.	PSL613602
Replacement Filter 10 µm 5/pk	PSL613604
Solvent Inlet Filters for General Use	
Solvent Inlet Filter 10 µm with 1/16 in. o.d. stem for 1/8 in. o.d. tubing	PSL613570
Solvent Inlet Filter 10 µm with flangeless fittings for 1/8 in. o.d. tubing	PSL613578
Solvent Inlet Filters for Preparative HPLC	
Solvent Inlet Filter 10 µm with 1/16 in. o.d. stem for 1/8 in. o.d. tubing	PSL613607
Solvent Inlet Filter 10 µm with flangeless fittings for 1/8 in. o.d. tubing	PSL613608
Solvent Inlet Filters for Waters HPLC Systems	
Solvent Inlet Filter 10 µm with 1/8 in. o.d. stem for 3/16 in. o.d. tubing	PSL613609

Bottom-of-the-Bottle Solvent Filters

This filter has been designed after the original Bottom-of-the-Bottle™ replaceable filters. This unique filter has a stainless steel stem on top to accommodate 1/16 in. i.d. tubings. The lower stem which goes directly into the 10 µm filter reaches to within 0.06 in. of the Bottom-of-the-Bottle filters. The Bottom-of-the-Bottle Solvent Filter has a 10 µm filter that can easily handle flow rates up to 10 mL/min.



Bottom-of-the-Bottle Solvent Filter	Part No.
Stainless Steel Filter Assembly	PSL613457

Click anywhere on page. Type desired page number. Click OK.

Certified Reference Materials From ERA

Environmental Resource Associates

An organization committed to customer satisfaction.

We want to thank you for all of your loyalty and business year after year. It is the privilege of being able to work for each of you that keeps us coming back every day and enjoying what we do. We want to make certain that working with ERA is valuable to you, and therefore we continue to push our customer and technical service to be the benchmark in the industry. When you reach out to us, we want to answer every call with a positive attitude and a desire to help that is tangible. We want you to feel the difference in our level of customer service.

As you may know, ERA was recently acquired by Waters Corporation, and we want to assure all of our customers that each of us will remain true to the core values that have made us the leading provider of quality assurance, validation and certified reference materials (CRM) in the industry. We are the same people that you have seen in our catalogs year after year and we are excited about this new phase in the life of ERA. We are also committed to customer satisfaction and we believe it shows.

ERA's Certified Reference Materials

The word that best describes ERA's Certified Reference Materials (CRM) standards is "true". We prepare every standard with starting materials traced to NIST or the highest possible metrological authority, and we verify the preparation, production and packaging via exhaustive in-house analyses. We analyze and test every Lot for stability, accuracy and precision. We scrutinize analytical data to make certain there are no unexpected problems with the standards, and then, only after all of that, we sell them to you. When you purchase Certified Reference Standards from ERA, you are looking for the truth. We guarantee that is what you will find.

Wastewater Inorganics CRM

UP TO 12
UNIQUE LOTS AVAILABLE PER YEAR

The industry standard for 30 years! ERA Wastewater Inorganics CRM standards provide you the easiest way to verify the accuracy of all your water and Wastewater analyses. Use these "known" CRM standards any time to compare your results against ERA's certified values and acceptance limits. Our acceptance limits, derived from over two million data points, will let you know with absolute confidence whether your analytical performance is where you need it to be.

Minerals/Solids CRMs

Minerals 186004350

One 500 mL Whole-Volume bottle is ready to analyze.

Total alkalinity as $CaCO_3$	10-120 mg/L
Chloride	35-275 mg/L
Fluoride	0.3-4 mg/L
Potassium	4-40 mg/L
Sodium	6-100 mg/L
Specific conductance at 25°C	200-930 µmhos/cm
Sulfate	5-125 mg/L
Total dissolved solids at 180°C	140-650 mg/L
Total solids at 105°C	140-675 mg/L

Hardness 186004351

One 500 mL Whole-Volume bottle is ready to analyze.

Calcium	3.5-110 mg/L
Calcium hardness as $CaCO_3$	8.7-275 mg/L
Total hardness as $CaCO_3$	17-440 mg/L
Magnesium	2-40 mg/L
Non-filterable residue (TSS)	23-100 mg/L

pH 186004381

One 250 mL Whole-Volume bottle is ready to analyze.
Use with electrometric methods.

pH	5-10 units

Settleable Solids 186004375

One 60 mL poly bottle with a solid concentrate yields 1 liter after dilution.

Settleable solids	5-100 mL/L

Volatile Solids 186004376

One 12 mL screw-top vial with a solid concentrate yields 1 liter after dilution.

Volatile solids	100-500 mg/L

Trace Metals CRMs

Trace Metals 186004345

One 15 mL screw-top vial yields up to 1 liter of sample after dilution.
Use with AA, ICP-OES or ICP-MS and selected colorimetric methods.

Aluminum	200-4,000 µg/L
Antimony	95-900 µg/L
Arsenic	70-900 µg/L
Barium	100-2,500 µg/L
Beryllium	8-900 µg/L
Boron	800-2,000 µg/L
Cadmium	8-750 µg/L
Chromium	17-1,000 µg/L
Cobalt	28-1,000 µg/L
Copper	40-900 µg/L
Iron	200-4,000 µg/L
Lead	70-3,000 µg/L
Manganese	70-4,000 µg/L
Molybdenum	60-600 µg/L
Nickel	80-3,000 µg/L
Selenium	90-2,000 µg/L
Silver	26-600 µg/L
Strontium	30-300 µg/L
Thallium	60-900 µg/L
Vanadium	55-2,000 µg/L
Zinc	100-2,000 µg/L

Mercury 186004354

One 15 mL screw-top vial yields up to 1 liter after dilution. Contains both inorganic and organic mercury to test both digestion and analysis procedures. Use with CVAA, ICP-MS or CVAFS methods.

Mercury, total	2-30 µg/L

Low-Level Mercury 186004380

Designed for ng/L level testing. One 5 mL flame-sealed ampule yields up to 4 liters after dilution. Contains organic and inorganic mercury to test both digestion and analysis procedures. Use with sensitive CVAA methods.

Mercury, total	1-100 ng/L

Hexavalent Chromium 186004202

One 15 mL screw-top vial yields up to 2 liters after dilution.
Use with colorimetric or IC methods.

Hexavalent chromium	45-880 µg/L

Tin and Titanium 186004357

One 15 mL screw-top vial yields up to 1 liter after dilution.
Use with AA, ICP-OES or ICP-MS methods.

Tin	1,000-5,000 µg/L
Titanium	80-300 µg/L

Wastewater Inorganics CRM

Demand CRMs

Demand	186004356
One 15 mL screw-top vial yields up to 2 liters after dilution.	
5-day BOD	15-250 mg/L
Carbonaceous BOD	15-250 mg/L
COD	30-250 mg/L
TOC	6-100 mg/L

Nutrient CRMs

Simple Nutrients	186004349
One 15 mL screw-top vial yields up to 2 liters after dilution. Use with colorimetric, ion selective electrode or ion chromatography methods.	
Ammonia as N	0.65-19 mg/L
Nitrate as N	0.25-40 mg/L
Nitrate plus nitrite as N	0.25-40 mg/L
Orthophosphate as P	0.5-5.5 mg/L

Complex Nutrients	186004361
One 15 mL screw-top vial yields up to 2 liters after dilution. Use with digestion followed by colorimetric, ISE or ICP methods.	
Total Kjeldahl-nitrogen as N	1.5-35 mg/L
Total phosphorus as P	0.5-10 mg/L

Nitrite	186004370
One 2 mL flame-sealed ampule yields up to 2 liters after dilution. Use with ion chromatography or colorimetric methods.	
Nitrite as N	0.4-4 mg/L

Wastewater Inorganics CRM Set

Includes the Minerals (186004350), Hardness (186004351), pH (186004381), Trace Metals (186004345), Mercury (186004354), Demand (186004356), Simple Nutrients (186004349), Complex Nutrients (186004361), Oil and Grease (186004348), Total Residual Chlorine (186004346) and Cyanide and Phenol (186004347) CRM standards.

Single Set Purchase	186004342

ERA CRM standards help you ensure the quality of your everyday data.

Wastewater Inorganics CRM

Oil and Grease CRMs

Oil and Grease 186004348

One 250 mL Whole-Volume bottle is ready to analyze.
Certified values are provided for IR and gravimetric methods.

Oil and Grease ..... 20-100 mg/bottle

1 liter Oil and Grease 186004358

One liter Whole-Volume glass bottle with a 33-430 thread is ready to analyze.

Oil and Grease ..... 20-100 mg/L

1 liter Boston Round Oil and Grease 186004374

One liter Whole-Volume bottle is ready to analyze. Designed for SPE equipment with Boston Round glass bottles with a 33-400 thread.

Oil and Grease ..... 20-100 mg/L

HEM / SGT-HEM 186004359

One 5 mL flame-sealed ampule yields up to 2 liters after dilution.
Contains both hexadecane and stearic acid.

HEM ..... 5-100 mg/L
SGT-HEM ..... 5-100 mg/L

Total Petroleum Hydrocarbons (TPH) in Water # 1 186004363

One liter Whole-Volume bottle is ready to analyze for Total Petroleum Hydrocarbons without interfering fatty acids.

Total Petroleum Hydrocarbons ..... 20-170 mg/L

Total Petroleum Hydrocarbons (TPH) in Water # 2 186004364

One liter Whole-Volume bottle is ready to analyze for Total Petroleum Hydrocarbons in the presence of interfering fatty acids.

Total Petroleum Hydrocarbons ..... 20-170 mg/L

Microbiology CRMs

All ERA microbiology standards are lyophilized and require re-hydration before analysis–sterile fluid provided. This ensures stability and provides flexibility when the samples can be analyzed!

Wastewater Coliforms 186004384

Each set contains two lyophilized samples, one quantitative positive and one negative. Use with all CWA quantitative methods – MF and MPN. Each set can be used for total coliforms and/or fecal coliforms as E.coli, which are present in the range 20–2,400 CFU/100 mL or MPN/100 mL.

Enterococci 186004383

Each set contains two lyophilized samples, one quantitative positive and one negative, for Enterococci and/or Fecal Streptococci, MF or MPN, in the range 20–1,000 CFU/ 100 mL or MPN/100 mL.
Note that a hazardous materials shipping charge will apply.

Physical Property CRMs

Color 186004340

One 125 mL Whole-Volume bottle is ready to analyze.

Color ..... 10-75 PC units

Turbidity 186004373

One 15 mL screw-top vial yields up to 1 liter after dilution.
Use with nephelometric methods.

Turbidity ..... 1-20 NTU

Chemical CRMs

Acidity 186004377
One 250 mL Whole-Volume bottle is ready to analyze as received.
Designed for use with titrimetric methods to a pH endpoint of 8.3.
Acidity as $CaCO_3$ ..... 650-1,800 mg/L

Boron 186004379
One unpreserved 60 mL poly bottle yields in excess of 2 liters after dilution.
Designed for colorimetric methods.
Boron ..... 0.8-2 mg/L

Bromide 186004369
One 2 mL flame-sealed ampule yields up to 1 liter after dilution.
Use with ion chromatography or colorimetric methods.
Bromide ..... 1-10 mg/L

For bromate/chlorate/chlorite CRMs see page 267.

Total Residual Chlorine 186004346
One 2 mL flame-sealed ampule yields up to 2 liters after dilution.
Use with titrimetric or colorimetric methods.
Total Residual Chlorine ..... 0.5-3 mg/L

Low-Level Total Residual Chlorine 186004378
Designed for testing at low µg/L levels. One 2 mL flame-sealed ampule yields up to 2 liters after dilution. Use with sensitive titrimetric or colorimetric methods.
Total Residual Chlorine ..... 20-250 µg/L

Cyanide and Phenol 186004347
One 15 mL screw-top vial yields up to 2 liters after dilution. As appropriate for each analyte use with digestion or distillation followed by colorimetric, titrimetric or ISE methods.
Total Cyanide ..... 0.1-1 mg/L
Phenol ..... 0.06-5 mg/L

Total Organic Halides (TOX) 186004242
One 2 mL flame-sealed ampule yields up to 2 liters of TOX standard after dilution. Use with adsorption pyrolysis titrimetric methods.
TOX ..... 300-1,500 µg/L

For perchlorate CRMs see page 246.

Total Phenolics (4-AAP) 186004355
One 2 mL flame-sealed ampule yields up to 2 liters after dilution.
Analyze for a mixture of phenolic compounds using 4-AAP methods.
Total phenolics by 4-AAP ..... 0.06-5 mg/L

Silica 186004371
One unpreserved 60 mL poly bottle yields up to 1 liter after dilution.
Use with colorimetric or ICP methods.
Silica as SiO_2 ..... 50-250 mg/L

Sulfide 186004341
One 10 mL flame-sealed ampule yields up to 1 liter after dilution.
Use with titrimetric or colorimetric methods. Guaranteed stable for one year.
Sulfide ..... 1-10 mg/L

Surfactants-MBAS 186004372
One 10 mL flame-sealed ampule yields up to 2 liters after dilution.
Surfactants-MBAS ..... 0.2-1 mg/L

Small Lab Wastewater CRM

4
UNIQUE LOTS AVAILABLE PER YEAR

Each ERA Small Lab Wastewater CRM standard is a "known" that comes with certified values and acceptance limits so you can get immediate feedback about the accuracy of your water and Wastewater analyses. Use our CRM standards routinely for staff training and periodic evaluation or to troubleshoot problems.

Whole-Volume CRMs

The following Whole-Volume standards are ready to use as provided and require no dilution before analysis.

Small Lab Minerals 186004353

One 500 mL Whole-Volume bottle. The concentration of all solids analytes is designed to mimic the samples commonly found in treatment plant labs.

pH	5-10 units
Total solids at 105°C	500-2,500 mg/L
Total dissolved solids at 180°C	500-2,000 mg/L
Non-filterable residue (TSS)	20-120 mg/L

Oil and Grease 186004348

One 250 mL Whole-Volume bottle. Certified values are provided for IR and gravimetric methods.

Oil and Grease	20-100 mg/bottle

ERA Whole-Volume Minerals and Oil and Grease standards are easier to use and have proven for thirty years to be more reliable!

Concentrate CRMs

The following standards are provided as concentrates that require dilution before analysis.

Demand 186004356

One 15 mL screw-top vial yields up to 2 liters after dilution.

5-day BOD	15-250 mg/L
Carbonaceous BOD	15-250 mg/L
COD	30-250 mg/L
TOC	6-100 mg/L

Simple Nutrients 186004349

One 15 mL screw-top vial yields up to 2 liters after dilution.
Use with colorimetric, ion selective electrode or ion chromatography methods.

Ammonia as N	0.65-19 mg/L
Nitrate as N	0.25-40 mg/L
Nitrate plus nitrite as N	0.25-40 mg/L
Orthophosphate as P	0.5-5.5 mg/L

Complex Nutrients 186004361

One 15 mL screw-top vial yields up to 2 liters after dilution. Use with digestion followed by colorimetric, ISE or ICP methods.

Total Kjeldahl-nitrogen as N	1.5-35 mg/L
Total Phosphorus as P	0.5-10 mg/L

Small Lab Wastewater CRM Set

Includes one of each CRM standard listed on this page.

Single Set Purchase 186004343

Ready-to-Use Wastewater CRM

6
UNIQUE LOTS AVAILABLE PER YEAR

ERA Ready-to-Use Wastewater are Whole-Volume CRM standards that require no dilution before analysis. Each CRM standard comes with certified values and acceptance limits so you can get immediate feedback on the quality of your results. The Ready-to-Use standards are guaranteed stable for a minimum of one month after receipt at your facility. Just order, open and analyze!

Whole-Volume CRMs

The following Whole-Volume standards are ready to use as provided and require no dilution before analysis.

Minerals	186004350
One 500 mL bottle to be analyzed for alkalinity as $CaCO_3$, chloride, conductivity at 25 C, fluoride, potassium, sodium, sulfate, total dissolved solids at 180 C and total solids at 105 C.	
Hardness	186004351
One 500 mL bottle to be analyzed for calcium, magnesium, total hardness as $CaCO_3$, calcium hardness as $CaCO_3$ and non-filterable residue or total suspended solids (TSS).	
pH	186004381
One 250 mL bottle to be analyzed for pH. Use with electrometric methods.	
Oil and Grease	186004348
One 250 mL Whole-Volume bottle. Certified values are provided for IR and gravimetric methods. For additional Oil and Grease CRMs see page 241.	
Trace Metals	186004366
One 500 mL bottle to be analyzed for aluminum, antimony, arsenic, barium, beryllium, boron, cadmium, chromium, cobalt, copper, iron, lead, manganese, molybdenum, nickel, selenium, silver, strontium, thallium, vanadium and zinc. Use with AA, ICP-OES or ICP-MS methods.	
Demand	186004368
One 500 mL bottle to be analyzed for 5-day BOD, carbonaceous BOD, COD and TOC.	
Simple Nutrients	186004365
One 500 mL bottle to be analyzed for ammonia as N, nitrate as N, nitrate plus nitrite as N and orthophosphate as P. Use with colorimetric, ion selective electrode or ion chromatography methods.	
Complex Nutrients	186004367
One 500 mL bottle to be analyzed for total Kjeldahl-nitrogen as N and total phosphorus as P. Use with digestion followed by colorimetric, ISE or ICP methods.	

Ready-to-Use Wastewater CRM Set

Includes one of each CRM standard listed on this page.

Set Purchase	186004344
Just open and analyze!	

Wastewater Organics CRM

UP TO 12
UNIQUE LOTS AVAILABLE PER YEAR

All ERA CRM standards are provided with certified values and acceptance limits, which are derived from over two million data points. You can rely on them with absolute confidence to identify whether your analytical performance is where you need it to be. Use ERA's Organics standards to help you make your every-day CRM program even more effective.

Volatiles CRMs

Volatiles 186004389

One 2 mL flame-sealed ampule yields in excess of 200 mL after dilution. Each standard contains at least 27 VOA analytes, randomly selected from the list below, at 5-300 µg/L after dilution. All unspiked analytes are certified at <5 µg/L.

- Acetone
- Acetonitrile
- Acrylonitrile
- Acrolein
- Benzene
- Bromodichloromethane
- Bromoform
- Bromomethane
- 2-Butanone (MEK)
- Carbon disulfide
- Carbon tetrachloride
- Chlorobenzene
- Chlorodibromomethane
- Chloroethane
- 2-Chloroethyl vinyl ether
- Chloroform
- Chloromethane
- 1,2-Dibromo-3-chloropropane (DBCP)
- 1,2-Dibromoethane (EDB)
- Dibromomethane
- 1,2-Dichlorobenzene
- 1,3-Dichlorobenzene
- 1,4-Dichlorobenzene
- Dichlorodifluoromethane
- 1,1-Dichloroethane
- 1,2-Dichloroethane
- 1,1-Dichloroethene
- cis-1,2-Dichloroethene
- trans-1,2-Dichloroethene
- 1,2-Dichloropropane
- cis-1,3-Dichloropropene
- trans-1,3-Dichloropropene
- Ethylbenzene
- Hexachlorobutadiene
- 2-Hexanone
- Methylene chloride
- Methyl tert-butyl ether (MTBE)
- 4-Methyl-2-pentanone (MIBK)
- Naphthalene
- Styrene
- 1,1,1,2-Tetrachloroethane
- 1,1,2,2-Tetrachloroethane
- Tetrachloroethene
- Toluene
- 1,2,4-Trichlorobenzene
- 1,1,1-Trichloroethane
- 1,1,2-Trichloroethane
- Trichloroethene
- Trichlorofluoromethane
- 1,2,3-Trichloropropane
- Vinyl acetate
- Vinyl chloride
- Xylenes, total

BTEX and MTBE in Water 186004399

One 2 mL flame-sealed ampule yields in excess of 200 mL after dilution. Contains all BTEX analytes below and Methyl tert-butyl ether (MTBE) all at 7-300 µg/L after dilution.

- Benzene
- Ethylbenzene
- Methyl tert-butyl ether (MTBE)
- Toluene
- Xylenes, total

For Gasoline Additives CRMs see page 247.

Gasoline Range Organics (GRO) in Water 186004400

One 2 mL flame-sealed ampule yields up to 2 liters after dilution. Contains unleaded regular gasoline in the range of 200-4,000 µg/L after dilution. Also certified for all BTEX analytes.

We provide performance acceptance limits with our CRM standards. They allow you to reliably compare your performance to other experienced labs!

Wastewater Organics CRM

Semivolatiles CRMs

Base/Neutrals 186004390

One 2 mL flame-sealed ampule yields up to 2 liters after dilution. Each standard contains at least 31 analytes, randomly selected from the list below, at 10-225 µg/L (except Benzidine at 200-1,000 µg/L) after dilution. All unspiked analytes are certified at <10 µg/L.

- Acenaphthylene
- 2-Amino-1-methylbenzene (o-Toluidine)
- Aniline
- Anthracene
- Benzidine
- Benzo(a)anthracene
- Benzo(b)fluoranthene
- Benzo(k)fluoranthene
- Benzo(g,h,i)perylene
- Benzo(a)pyrene
- Benzyl alcohol
- 4-Bromophenyl -phenylether
- Butylbenzylphthalate
- Carbazole
- 4-Chloroaniline
- bis(2-Chloroethoxy) methane
- bis(2-Chloroethyl)ether
- bis(2-Chloroisopropyl) ether
- 1-Chloronaphthalene
- 2-Chloronaphthalene
- 4-Chlorophenyl -phenylether
- Chrysene
- Dibenz(a,h)anthracene
- Dibenzofuran
- 1,2-Dichlorobenzene
- 1,3-Dichlorobenzene
- 1,4-Dichlorobenzene
- 3,3'-Dichlorobenzidine
- Diethyl phthalate
- Dimethyl phthalate
- Di-n-butylphthalate
- 2,4-Dinitrotoluene
- 2,6-Dinitrotoluene
- Di-n-octylphthalate
- bis(2-Ethylhexyl)phthalate
- Fluoranthene
- Fluorene
- Hexachlorobenzene
- Hexachlorobutadiene
- Hexachlorocyclo-pentadiene
- Hexachloroethane
- Indeno(1,2,3-cd)pyrene
- Isophorone
- 2-Methylnaphthalene
- Naphthalene
- 2-Nitroaniline
- 3-Nitroaniline
- 4-Nitroaniline
- Nitrobenzene
- N-Nitrosodiethylamine
- N-Nitrosodimethylamine
- N-Nitroso-di-n-propylamine
- N-Nitrosodiphenylamine
- Pentachlorobenzene
- Phenanthrene
- Pyrene
- Pyridine
- 1,2,4,5-Tetrachlorobenzene
- 1,2,4-Trichlorobenzene

Acids

186004391

One 2 mL flame-sealed ampule yields up to 2 liters after dilution. Each standard contains at least 13 analytes, randomly selected from the list below, at 30-200 µg/L after dilution. All unspiked analytes are certified at <30 µg/L.

- Benzoic Acid
- 4-Chloro-3-methylphenol
- 2-Chlorophenol
- 2,4-Dichlorophenol
- 2,6-Dichlorophenol
- 2,4-Dimethylphenol
- 2,4-Dinitrophenol
- 2-Methyl-4,6-dinitrophenol
- 2-Methylphenol
- 3 & 4-Methylphenol
- 2-Nitrophenol
- 4-Nitrophenol
- Pentachlorophenol
- Phenol
- 2,3,4,6-Tetrachlorophenol
- 2,4,5-Trichlorophenol
- 2,4,6-Trichlorophenol

Low-Level Nitroaromatics and Nitramines 186004388

One 2 mL flame-sealed ampule yields up to 2 liters after dilution. Each standard contains at least 12 analytes, randomly selected from the list below, at 1-20 µg/L after dilution.

- 4-Amino-2,6-dinitrotoluene
- 2-Amino-4,6-dinitrotoluene
- 1,3-Dinitrobenzene
- 2,4-Dinitrotoluene
- 2,6-Dinitrotoluene
- HMX
- Nitrobenzene
- 2-Nitrotoluene
- 3-Nitrotoluene
- 4-Nitrotoluene
- RDX
- Tetryl
- 1,3,5-Trinitrobenzene
- 2,4,6-Trinitrotoluene

Diesel Range Organics (DRO) in Water 186004401

One 2 mL flame-sealed ampule yields up to 2 liters after dilution. Contains No. 2 Diesel for use with modified EPA 8015 methods. DRO is in the concentration range of 500-4,000 µg/L after dilution.

Low-Level PAHs 186004393

One 2 mL flame-sealed ampule yields up to 2 liters of sample after dilution. Each standard includes at least 13 analytes, randomly selected from the list below, at 0.3-10 µg/L after dilution. The UV absorbing and fluorescent analytes are present at 2-10 and 0.3-2 µg/L, respectively.

- Acenaphthene
- Acenaphthylene
- Anthracene
- Benzo(a)anthracene
- Benzo(b)fluoranthene
- Benzo(k)fluoranthene
- Benzo(g,h,i)perylene
- Benzo(a)pyrene
- Chrysene
- Dibenz(a,h)anthracene
- Fluoranthene
- Fluorene
- Indeno(1,2,3-cd)pyrene
- Naphthalene
- Phenanthrene
- Pyrene

PCBs CRMs

PCBs in Water 186004398

One 2 mL flame-sealed ampule yields up to 2 liters after dilution. Each standard includes a different Aroclor, randomly selected from the list below, at 1-15 µg/L after dilution. All unspiked Aroclors are certified at <1 µg/L.

- Aroclor 1016
- Aroclor 1221
- Aroclor 1232
- Aroclor 1242
- Aroclor 1248
- Aroclor 1254
- Aroclor 1260

PCBs in Oil 186004397

One 10 mL flame-sealed ampule is ready to analyze. Each standard contains a different Aroclor, randomly selected, at 12-50 mg/kg.

Wastewater Organics CRM Set

Includes the Volatiles (186004389), Base/Neutrals (186004390), Acids (186004391), PCBs in Water (186004398) and Organochlorine Pesticides (186004392) CRM standards.

Set Purchase 186004304

Wastewater Organics CRM

Pesticides CRMs

Organochlorine Pesticides — 186004392

One 2 mL flame-sealed ampule yields up to 2 liters after dilution. Each standard contains at least 16 analytes, randomly selected from the list below, at 0.5-20 µg/L after dilution. All unspiked analytes are certified at <0.5 µg/L.

- Aldrin
- alpha-BHC
- beta-BHC
- delta-BHC
- gamma-BHC (Lindane)
- alpha-Chlordane
- gamma-Chlordane
- 4,4'-DDD
- 4,4'-DDE
- 4,4'-DDT
- Dieldrin
- Endosulfan I
- Endosulfan II
- Endosulfan sulfate
- Endrin
- Endrin aldehyde
- Endrin ketone
- Heptachlor
- Heptachlor epoxide (beta)
- Methoxychlor

Carbamate Pesticides — 186004409

One 2 mL flame-sealed ampule yields up to 2 liters after dilution. Each standard now contains at least 10 analytes, randomly selected from the list below, at 5-200 µg/L after dilution. All unspiked analytes are certified at <5 µg/L.

- Aldicarb
- Aldicarb sulfone
- Aldicarb sulfoxide
- Baygon
- Carbaryl
- Carbofuran
- Diuron
- 3-Hydroxycarbofuran
- Methiocarb
- Methomyl
- Oxamyl (Vydate)
- Propham

Nitrogen Pesticides — 186004387

One 2 mL flame-sealed ampule yields up to 2 liters after dilution. Each standard contains at least 8 analytes, randomly selected from the list below, at 2-20 µg/L after dilution. All unspiked analytes are certified at <2 µg/L.

- Alachlor
- Ametryn
- Anilazine
- Atraton
- Atrazine
- Bromacil
- Butachlor
- Butylate
- Cyanazine
- Deethyl atrazine
- Deisopropyl atrazine
- Diaminoatrazine
- EPTC (Eptam)
- Hexazinone
- Metolachlor
- Metribuzin
- Napropamide
- Prometon
- Prometryn
- Pronamide
- Propachlor
- Propazine
- Simazine
- Terbacil
- Trifluralin

Chlordane — 186004394

One 2 mL flame-sealed ampule yields up to 2 liters after dilution. Each standard contains technical chlordane at 3-25 µg/L after dilution.

Toxaphene — 186004395

One 2 mL flame-sealed ampule yields up to 2 liters after dilution. Each standard contains toxaphene at 20-100 µg/L after dilution.

Organophosphorus Pesticides (OPP) — 186004386

One 2 mL flame-sealed ampule yields up to 2 liters after dilution. Each standard contains Diazinon, Disulfoton, Ethyl Parathion, Malathion and Azinphos-methyl and at least 4 additional OPP analytes, randomly selected from the list below, at 2-20 µg/L after dilution.

- Azinophos-methyl (Guthion)
- Carbophenothion
- Chlorpyrifos
- Demeton O and S
- Diazinon
- Dichlorvos (DDVP)
- Dimethoate
- Dioxathion
- Disulfoton
- Ethion
- Ethoprop
- Ethyl Parathion (Parathion)
- Famphur
- Fonofos
- Malathion
- Methyl Parathion
- Phorate
- Phosmet
- Ronnel
- Stirophos (Tetrachlorovinphos)
- Terbufos

Herbicides CRM

Chlorinated Acid Herbicides — 186004396

One 2 mL flame-sealed ampule yields up to 2 liters after dilution. Four herbicides – 2,4-D, Dicamba, 2,4,5-T, and 2,4,5-TP (Silvex) – are included in every standard at 2-10 µg/L after dilution. At least 6 additional analytes, randomly selected from the list shown below, are included in every standard at 2-10 µg/L, except MCPA and MCPP, which if spiked, are included at 10-100 µg/L after dilution. All unspiked analytes are certified at <2 µg/L.

- Acifluorfen
- Bentazon
- Chloramben
- 2,4-D
- 2,4-DB
- Dacthal diacid (DCPA)
- Dalapon
- Dicamba
- 3,5-Dichlorobenzoic acid
- Dichlorprop
- Dinoseb
- MCPA
- MCPP
- 4-Nitrophenol
- Pentachlorophenol
- Picloram
- 2,4,5-T
- 2,4,5-TP (Silvex)

ERA Cal 1000 mg/L Standards

ERA 1000 mg/L standards can be used for primary calibration or to prepare second source calibration check standards. They are traceable to NIST Standard Reference Materials, where available, and are guaranteed stable for one year. The certification documentation includes manufacturing uncertainties, traceability summaries and densities to aid in performing quantitative dilutions. The documentation for metal standards includes impurities.

Inorganics - 1000 mg/L

Chemical Oxygen Demand (COD)	186004225 (500 mL Bottle) 186004216 (125 mL Bottle)
One 1,000 mg/L standard preserved with HCl in an amber glass bottle.	
Total Kjeldahl-Nitrogen (TKN)	186004230 (500 mL Bottle) 186004217 (125 mL Bottle)
One 1,000 mg/L standard preserved with HCl in a poly bottle.	
MBAS/LAS Surfactants	186004226
One 10 mL flame-sealed ampule containing 1,000 mg/L LAS preserved with H_2SO_4.	
Total Organic Carbon (TOC)	186004228
One 500 mL 1,000 mg/L amber glass bottle preserved with H_2SO_4.	
Total Organic Halides (TOX)	186004227
One 2 mL flame-sealed ampule at 1,000 mg/L in MeOH.	
Phenol	186004229
A 500 mL 1,000 mg/L amber glass bottle preserved with H_2SO_4.	
Sulfide	186004233
One 10 mL flame-sealed ampule with 1,000 mg/L sulfide preserved with NaOH and zinc acetate.	

Ions – 1000 mg/L Parameter	Matrix	500 mL Bottle Part No.	125 mL Bottle Part No.
Ammonia as NH_3	H_2O	186004157	186004139
Ammonia as N	H_2O	186004156	186004140
Bromate	H_2O	–	186004152
Bromide	H_2O	186004158	186004141
Chlorate	H_2O	–	186004153
Chloride	H_2O	186004159	186004142
Chlorite	H_2O	–	186004154
Free cyanide	NaOH	186004231	186004143
Complex cyanide	NaOH	186004232	186004144
Fluoride	H_2O	186004160	186004145
Nitrate as NO_3	H_2O	186004163	186004146
Nitrate as N	H_2O	186004162	186004147
Nitrite as N	H_2O	186004161	186004148
Perchlorate	H_2O	–	186004155
Phosphate as PO_4	H_2O	186004165	186004149
Phosphate as P	H_2O	186004164	186004150
Sulfate	H_2O	186004166	186004151

Metals – 1000 mg/L Parameter	Matrix	125 mL Bottle Part No.	
Aluminum	HNO_3	186004170	–
Antimony	HNO_3	186004171	–
Arsenic	HNO_3	186004172	–
Barium	HNO_3	186004173	–
Beryllium	HNO_3	186004174	–
Bismuth	HNO_3	186004203	–
Boron	HNO_3	186004175	–
Cadmium	HNO_3	186004176	–
Calcium	HNO_3	186004177	–
Cerium	HNO_3	186004211	–
Chromium VI	H_2O	186004178	–
Total chromium	HNO_3	186004179	–
Cobalt	HNO_3	186004180	–
Copper	HNO_3	186004181	–
Holmium	HNO_3	186004204	–
Indium	HNO_3	186004205	–
Iron	HNO_3	186004182	–
Lead	HNO_3	186004183	–
Lithium	HNO_3	186004206	–
Magnesium	HNO_3	186004184	–
Manganese	HNO_3	186004185	–
Mercury	HNO_3	186004186	–
Molybdenum	HNO_3	186004187	–
Nickel	HNO_3	186004188	–
Phosphorus	HNO_3	186004200	–
Potassium	HNO_3	186004189	–
Rhodium	HCl	186004207	–
Scandium	HNO_3	186004208	–
Selenium	HNO_3	186004190	–
Silica	H_2O	186004201	–
Silicon	HNO_3	186004191	–
Silver	HNO_3	186004192	–
Sodium	HNO_3	186004193	–
Strontium	HNO_3	186004194	–
Terbium	HNO_3	186004209	–
Thallium	HNO_3	186004195	–
Tin	HCl	186004196	–
Titanium	HCl	186004197	–
Vanadium	HNO_3	186004198	–
Yttrium	HNO_3	186004210	–
Zinc	HNO_3	186004199	–

ERA Cal Metals, Anions, and pH Buffer Standards

ICP-MS Metals

These standards come with a Certificate of Traceability and Uncertainty. Use for initial as well as continuing calibration and tuning verification. Provided as convenient concentrates with densities allowing you to easily perform gravimetric dilutions.

ICP-MS Trace Metals 186004212

One 125 mL concentrate is preserved with HNO_3 and tartaric acid. Contains aluminum, antimony, arsenic, barium, beryllium, cadmium, chromium, cobalt, copper, iron, lead, manganese, molybdenum, nickel, selenium, silver, thallium, thorium, uranium, vanadium and zinc, each at 10.0 mg/L.

ICP-MS Major Cations 186004213

One 125 mL concentrate is preserved with HNO_3. Contains calcium, magnesium, potassium and sodium, each at 50.0 mg/L.

ICP-MS Tuning Standard 186004215

One 125 mL concentrate is preserved with HNO_3 and HCl. Contains barium, beryllium, cerium, cobalt, indium, lead, lithium, magnesium, rhodium, thallium, uranium and yttrium, each at 10.0 mg/L.

ICP-MS Calibration/CRM Set

Includes the ICP-MS Trace Metals (186004212) and Cations (186004213) standards.

Set Purchase 186004214

Anions

Ion Chromatography 186004382

One 15 mL screw-top vial yields up to 200 mL after dilution. Designed to calibrate or verify IC calibrations. Comes with a Certificate of NIST Traceability. Call for anion standards at lower levels.

- Bromide ..... 0.2-20 mg/L
- Chloride ..... 0.2-20 mg/L
- Fluoride ..... 0.1-10 mg/L
- Nitrate as N ..... 0.2-20 mg/L
- Phosphate as P ..... 0.5-30 mg/L
- Sulfate ..... 0.5-30 mg/L

pH Buffers

ERA Cal pH Buffers are directly traceable to NIST SRMs, mercury free, guaranteed stable for at least one year after your receipt, and are supplied with a full certificate of analysis. Choose single bottles or convenient 6-bottle cases.

Value	Volume	Single Bottles Part No.	Case of 6 Bottles Part No.
pH 4.00	1 pint	186004218	186004219
pH 7.00	1 pint	186004220	186004221
pH 10.00	1 pint	186004222	186004223
2 each of pH 4, 7 and 10	1 pint	–	186004224

AA/ICP Metals

All metals standards come with a Certificate of Traceability. The ICP Trace Metals standard also includes uncertainties. Use as initial as well as continuing calibration verification.

Flame AA Trace Metals 186004352

One 20 mL screw-top vial, preserved with HNO_3, yields up to 500 mL after dilution. Designed for flame AA. Provided with a Certificate of NIST Traceability. Includes aluminum, antimony, arsenic, barium, beryllium, boron, cadmium, chromium, cobalt, copper, iron, lead, manganese, molybdenum, nickel, selenium, silver, strontium, thallium, vanadium and zinc.

Flame AA/ICP Cations 186004362

One 15 mL screw-top vial, preserved with HNO_3, yields up to 250 mL after dilution. Use with ICP and AA methods.

- Calcium ..... 10-200 mg/L
- Magnesium ..... 10-200 mg/L
- Potassium ..... 5-100 mg/L
- Sodium ..... 10-250 mg/L

ICP Trace Metals 186004360

Designed for radial and axial-view ICP. One 500 mL Whole-Volume standard, preserved with HNO_3 and HCl, is ready to use. Includes antimony, arsenic, barium, beryllium, bismuth, boron, cadmium, chromium, cobalt, copper, lead, manganese, molybdenum, nickel, phosphorus, strontium, tin, vanadium and zinc, each at 1.0 mg/L, plus aluminum, calcium, iron, potassium, lanthanum, magnesium, selenium and sodium, each at 10.0 mg/L.

Whole Effluent Toxicity (WET) CRM

ERA recognizes that Whole Effluent Toxicity (WET) testing has its own unique characteristics. That's why we derive the expected performance on our WET CRM standards directly from the results of our historical proficiency testing studies. They include the largest pool of participant toxicity testing laboratories available. ERA's acceptance limits, which are provided for each WET standard, are derived from these data.

ERA WET CRM standards allow you to assess the consistency and quality of your routine aquatic toxicology analyses. All toxicants are supplied as ready-to-use concentrates – simply dilute and test.

Whole Effluent Toxicity (WET) Testing CRMs

Reference Toxicant for Test Organism and Conditions	EPA Test Code	EPA Method Code	Part No.
Fathead minnow (Pimephales promelas) 48-hour acute, non-renewal, 20°C, MHSF	11	2000.0	186004410
Fathead minnow (Pimephales promelas) 48-hour acute, non-renewal, 25°C, MHSF	13	2000.0	186004411
Fathead minnow (Pimephales promelas) 48-hour acute, non-renewal, 25°C, 20% DMW	14	2000.0	186004412
Fathead minnow (Pimephales promelas) 7-day short-term chronic, daily renewal, 25°C, MHSF	15	1000.0	186004413
Fathead minnow (Pimephales promelas) 7-day short-term chronic, daily renewal, 25°C, 20% DMW	16	1000.0	186004414
Ceriodaphnia dubia 48-hour acute, renewal, 20°C, MHSF	17	2002.0	186004415
Ceriodaphnia dubia 48-hour acute, renewal, 20°C, 20% DMW	18	2002.0	186004416
Ceriodaphnia dubia 48-hour acute, renewal, 25°C, MHSF	19	2002.0	186004417
Ceriodaphnia dubia 48-hour acute, renewal, 25°C, 20% DMW	20	2002.0	186004418
Ceriodaphnia dubia 7-day short-term chronic, daily renewal, 25°C, MHSF	21	1002.0	186004419
Ceriodaphnia dubia 7-day short-term chronic, daily renewal, 25°C, 20% DMW	22	1002.0	186004420
Daphnia magna 48-hour acute, non-renewal, 20°C, MHSF	32	2021.0	186004421
Daphnia pulex 48-hour acute, non-renewal, 20°C, MHSF	36	2021.0	186004422
Daphnia pulex 48-hour acute, non-renewal, 25°C, MHSF	38	2021.0	186004423
Mysid (Mysidopsis bahia) 48-hour acute, non-renewal, 20°C, 40 fathoms seawater	42	2007.0	186004424
Mysid (Mysidopsis bahia) 7-day short-term chronic, daily renewal, 26°C, 40 fathoms seawater	43	1007.0	186004425
Inland silverside (Menidia beryllina) 48-hour acute, non-renewal, 20°C, 40 fathoms seawater	44	2006.0	186004426
Sheepshead minnow (Cyprinodon variegatus) 48-hour acute, non-renewal, 20°C, 40 fathoms seawater	46	2004.0	186004427
Sheepshead minnow (Cyprinodon variegatus) 7-day short-term chronic, daily renewal, 25°C, 40 fathoms seawater	47	1004.0	186004428

Microbiology CRM

UP TO 12
UNIQUE LOTS AVAILABLE PER YEAR

ERA standards provide you the easiest way to evaluate and improve every aspect of your microbiology analyses from dilution technique to viability of your media. Use these "known" CRM standards any time to compare your results against ERA's certified values and acceptance limits, which will let you know with absolute confidence whether your performance is where you need it to be.

All ERA Microbiology standards are lyophilized and require re-hydration before analysis–sterile fluid provided. This ensures stability and provides maximum flexibility when the samples can be analyzed!

Wastewater CRMs

Wastewater Coliforms 186004384

Each set contains two lyophilized samples, one quantitative positive and one negative. Use with all CWA quantitative methods – MF and MPN. Each set can be used for total coliforms and/or fecal coliforms as E.coli, which are present in the range 20–2,400 CFU/100 mL or MPN/100 mL.

Enterococci 186004383

Each set contains two lyophilized samples, one quantitative positive and one negative, which after re-hydration can be analyzed for Enterococci and/or Fecal Streptococci, MF or MPN in the range 20–1,000 CFU/100 mL or MPN/100 mL. Note that a hazardous materials shipping charge will apply.

Drinking Water CRMs

Source Water E.coli 186004257

One quantitative lyophilized sample containing E.coli. Formulated for all SDWA quantitative methods. Also use for CRM for the Long Term 2 Enhanced Surface Water Treatment Rule. Each standard contains E.coli in the range 10-300 CFU/100 mL.

Drinking Water Coliforms 186004259

Each set now contains five lyophilized samples including one total and fecal positive (Escherichia coli), one total positive and fecal negative (Enterobacter cloacae), two total and fecal negative (Proteus mirablis and Pseudomonas aeruginosa), and one blank. Use with all SDWA methods - MF, MPN, presence/absence and ONPG-MUG. Each set can be used for total coliforms and/or fecal coliforms as E.coli.

Heterotrophic Plate Count 186004258

One quantitative lyophilized sample containing a Heterotrophic bacteria present in the range 5-500 CFU/mL. Use to CRM your recreational, drinking and Wastewater analyses. Use with Standard Method 9215B-Pour Plate and Most Probable Number (MPN) Method (Simplate).

Drinking Water Inorganics CRM

UP TO 12
UNIQUE LOTS AVAILABLE PER YEAR

ERA Drinking Water Inorganics CRM standards provide you the simplest way to verify the accuracy of your analyses of drinking and ground water samples. Use these "known" CRM standards any time to compare your results against ERA's certified values and acceptance limits. Our acceptance limits, derived from over two million data points, will let you know with absolute confidence whether your analytical performance is where you need it to be.

Minerals/Solids CRMs

Hardness 186004244

One 250 mL Whole-Volume bottle is ready to analyze. Use with AA, ICP-OES, ICP-MS or titrimetric methods.

Calcium	30-90 mg/L
Calcium hardness as $CaCO_3$	75-375 mg/L
Total hardness as $CaCO_3$	83-307 mg/L
Magnesium	2-20 mg/L
Sodium	12-24 mg/L

Inorganics 186004248

One 500 mL Whole-Volume bottle is ready to analyze. Also includes sodium at an intentionally higher range than in the Hardness standard.

Alkalinity as $CaCO_3$	25-200 mg/L
Chloride	5-100 mg/L
Fluoride	1-8 mg/L
Nitrate as N	3-10 mg/L
Nitrate plus Nitrite as N	3.5-9 mg/L
Potassium	10-40 mg/L
Sodium	10-400 mg/L
Specific Conductance at 25°C	250-2,500 µmhos/cm
Sulfate	5-500 mg/L
Total filterable residue (TDS) at 180°C	200-450 mg/L

pH 186004250

One 250 mL Whole-Volume bottle is ready to analyze. Use with electrometric methods.

pH	5-10 units

Trace Metals CRMs

Metals 186004247

One 15 mL screw-top vial yields up to 2 liters after dilution. Use with AA, ICP-OES or ICP-MS methods.

Aluminum	130-2,500 µg/L
Antimony	6-50 µg/L
Arsenic	5-50 µg/L
Barium	500-3,000 µg/L
Beryllium	1-10 µg/L
Boron	800-2,000 µg/L
Cadmium	2-50 µg/L
Chromium	10-200 µg/L
Copper	50-2,000 µg/L
Iron	100-1,800 µg/L
Lead	5-100 µg/L
Manganese	40-900 µg/L
Molybdenum	15-130 µg/L
Nickel	10-500 µg/L
Selenium	10-100 µg/L
Silver	20-300 µg/L
Thallium	2-10 µg/L
Vanadium	315-2,500 µg/L
Zinc	400- 2,500 µg/L

Mercury 186004239

One 15 mL screw-top vial yields up to 1 liter after dilution. Contains both organic and inorganic mercury to test both digestion and analysis procedures. Use with CVAA, ICP-MS or CVAFS methods. For a ng/L level mercury standard see page 239.

Mercury, total	0.5-10 µg/L

Hexavalent Chromium 186004236

One 15 mL screw-top vial yields up to 2 liters after dilution. Use with colorimetric or IC methods.

Hexavalent Chromium	5-50 µg/L

Uranium 186004254

One 15 mL screw-top vial yields up to 1 liter after dilution. Use with ICP-MS methods. For uranium CRMs in different matrices.

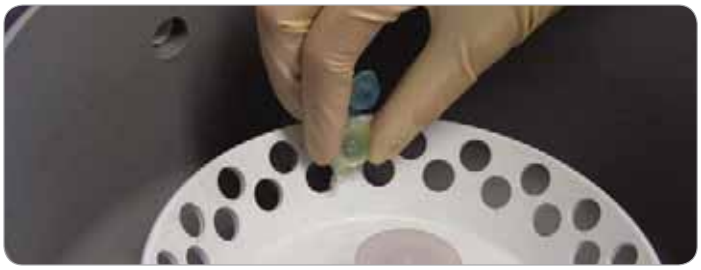
Uranium	3-104 µg/L

Vanadium 186004237

One 15 mL screw-top vial yields up to 2 liters after dilution. Use with AA, ICP-OES or ICP-MS methods.

Vanadium	5-50 µg/L

Drinking Water Inorganics CRM



Drinking Water Inorganics CRM Set

Includes the Hardness (186004244), Inorganics (186004248), pH (186004250), Metals (186004247), Mercury (186004239), Drinking Water Coliforms (186004259), Nitrite (186004245), Residual Chlorine (186004246) and Turbidity (186004249) CRM standards.

Single Set Purchase — 186004235 Single

Inorganic Disinfection By-Products CRMs

Bromide, Bromate and Chlorate — 186004243

One 2 mL flame-sealed ampule yields up to 2 liters after dilution.
Use with colorimetric, ion chromatography or ISE methods.

- Bromate ..... 7-50 µg/L
- Bromide ..... 75-500 µg/L
- Chlorate ..... 60-180 µg/L

Chlorite — 186004234

One 2 mL flame-sealed ampule yields up to 2 liters after dilution.
Use with ion chromatography methods.

- Chlorite ..... 100-1,000 µg/L

Nutrients CRMs

Nitrite

186004245

One 2 mL flame-sealed ampule yields up to 2 liters after dilution.
Use with colorimetric or ISE methods.

- Nitrite as N ..... 0.4-2 mg/L

o-Phosphate Nutrients — 186004240

One 15 mL screw-top vial yields up to 2 liters after dilution.
Use with colorimetric or IC methods.

- ortho-Phosphate as P ..... 0.5-5.5 mg/L

Microbiology CRMs

All ERA microbiology standards are lyophilized and require re-hydration before analysis–sterile fluid provided. This ensures stability and provides maximum flexibility when the samples can be analyzed!

Drinking Water Coliforms — 186004259

Each set now includes five lyophilized standards: one total and fecal positive (Escherichia coli), one total positive and fecal negative (Enterobacter cloacae), two total and fecal negative (Proteus mirablis and Pseudomonas aeruginosa), and one blank. Use for all SDWA methods-MF, MPN, presence/absence and ONPG-MUG. Can be used for total coliforms and/or fecal coliforms as E. coli.

Heterotrophic Plate Count — 186004258

One quantitative lyophilized sample containing a Heterotrophic bacteria present in the range 5-500 CFU/mL. Use to CRM your recreational, drinking and Wastewater analyses. Use with Standard Method 9215B-Pour Plate and Most Probable Number (MPN) Method (Simplate).

Source Water E.coli — 186004257

One quantitative lyophilized standard containing E.coli is formulated for all SDWA quantitative methods. Also use for CRM under proposed monitoring for the Long Term 2 Enhanced Surface Water Treatment Rule. Each standard contains E.coli in the range 10-300 CFU/100 mL.

Drinking Water Inorganics CRM

Additional Inorganic CRMs

Residual Chlorine 186004246

One 2 mL flame-sealed ampule yields up to 2 liters after dilution.
Use with titrimetric or colorimetric methods. For µg/L level residual chlorine CRMs see page 242.

- Total residual chlorine ..... 0.5-3 mg/L
- Free residual chlorine ..... 0.5-3 mg/L

Cyanide 186004256

One 15 mL screw-top vial yields up to 2 liters after dilution.
Use with digestion and/or colorimetric, titrimetric or ISE methods.

- Cyanide ..... 0.1-0.5 mg/L

Organic Carbon 186004241

One 15 mL screw-top vial yields up to 1 liter after dilution.
Use for total (TOC) and dissolved (DOC) organic carbon with combustion or persulfate oxidation procedures.

- Total Organic Carbon ..... 1.2-4.9 mg/L
- Dissolved Organic Carbon ..... 1.2-4.9 mg/L

Perchlorate 186004253

One 15 mL screw-top vial yields up to 2 liters after dilution.
Use with IC or IC-MS methods. Call for ng/L level Perchlorate CRM standards.

- Perchlorate ..... 4-20 µg/L

Silica 186004252

One 60 mL poly bottle yields 1 liter after dilution.
Use with colorimetric or ICP methods.

- Silica as SiO_2 ..... 5-50 mg/L

Surfactants - MBAS 186004251

One 10 mL flame-sealed ampule yields up to 2 liters after dilution.

- Surfactants – MBAS ..... 0.05-1 mg/L

For total organic halides (TOX) CRMs see page 242.

Physical Property CRMs

Corrosivity 186004255

One 500 mL Whole-Volume bottle is ready to use. Use for corrosivity, calcium carbonate saturation and Langelier saturation index.

- Corrosivity ..... -4 to +4 SI units

Turbidity 186004249

One 15 mL screw-top vial yields up to 1 liter after dilution.
Use with nephelometric methods.

- Turbidity ..... 0.5-8 NTU

UV 254 Absorbance 186004238

One 15 mL screw-top vial yields up to 1 liter after dilution.
Use with Standard Method 5910B.

- UV 254 Absorbance 0.02-0.7 cm-1

Drinking Water Organics CRM

UP TO 12
UNIQUE LOTS AVAILABLE PER YEAR

ERA Drinking Water Organics CRM standards provide you the simplest way to verify the accuracy of your analyses of drinking and ground waters as well as other clean water samples. Use these "known" CRM standards any time to compare your results against ERA's certified values and acceptance limits. Our acceptance limits are derived from over two million data points. They will let you know with absolute confidence whether your analytical performance is where you need it to be.

Volatile Organics CRMs

Halomethanes (THMs) 186004273

One 2 mL flame-sealed ampule yields in excess of 200 mL after dilution.
Each standard is certified for all analytes below at 10-50 µg/L after dilution.

- Bromodichloromethane
- Bromoform
- Chlorodibromomethane
- Chloroform

Regulated Volatiles 186004274

One 2 mL flame-sealed ampule yields in excess of 200 mL after dilution.
Each standard contains all the analytes below at 1-50 µg/L after dilution.

- Benzene
- Carbon tetrachloride
- Chlorobenzene
- 1,2-Dichlorobenzene
- 1,4-Dichlorobenzene
- 1,2-Dichloroethane
- 1,1-Dichloroethene
- cis-1,2-Dichloroethene
- trans-1,2-Dichloroethene
- 1,2-Dichloropropane
- Ethylbenzene
- Methylene Chloride
- Styrene
- Tetrachloroethylene
- Toluene
- 1,2,4-Trichlorobenzene
- 1,1,1-Trichloroethane
- 1,1,2-Trichloroethane
- Trichloroethylene
- Vinyl chloride
- Xylenes, total

Gasoline Additives 186004281

One 2 mL flame-sealed ampule yields in excess of 200 mL after dilution.
Each standard contains all the analytes below at 5-50 µg/L after dilution.

- tert-Amylmethylether (TAME)
- tert-Butyl Alcohol
- Di-isopropylether (DIPE)
- Ethyl tert-butyl ether (ETBE)
- Methyl tert-butyl ether (MTBE)
- Trichlorofluoromethane (Freon® 11)
- Trichlorotrifluoroethane (Freon® 113)

Unregulated Volatiles 186004268

One 2 mL flame-sealed ampule yields in excess of 200 mL after dilution.
Each standard contains at least 20 analytes, randomly selected from the list below, at 5-50 µg/L, except naphthalene, which if spiked, is included at 2-50 µg/L after dilution. All unspiked analytes are certified at <5 µg/L.

- Bromobenzene
- Bromochloromethane
- Bromomethane
- n-Butylbenzene
- sec-Butylbenzene
- tert-Butylbenzene
- Chloroethane
- Chloromethane
- 2-Chlorotoluene
- 4-Chlorotoluene
- Dibromomethane
- 1,3-Dichlorobenzene
- Dichlorodifluoromethane
- 1,1-Dichloroethane
- 1,3-Dichloropropane
- 2,2-Dichloropropane
- 1,1-Dichloropropene
- cis-1,3-Dichloropropene
- trans-1,3-Dichloropropene
- Fluorotrichloromethane
- Hexachlorobutadiene
- Isopropylbenzene
- 4-Isopropyltoluene
- Methyl tert-butyl ether (MTBE)
- Naphthalene
- n-Propylbenzene
- 1,1,1,2-Tetrachloroethane
- 1,1,2,2-Tetrachloroethane
- 1,2,3-Trichlorobenzene
- 1,2,3-Trichloropropane
- 1,2,4-Trimethylbenzene
- 1,3,5-Trimethylbenzene

Semivolatile Organics CRMs

Dioxin

186004266
One 2 mL flame-sealed ampule yields up to 2 liters after dilution.
Each standard contains 2,3,7,8-TCDD at 25-80 pg/L after dilution.

PCBs as Decachlorobiphenyl 186004279

One 2 mL flame-sealed ampule yields up to 2 liters after dilution. This standard can also be used for Aroclor identification and quantitation.
Each standard includes a different Aroclor, randomly selected from the list below, at 0.5-5 µg/L as decachlorobiphenyl after dilution.

- Aroclor 1016
- Aroclor 1221
- Aroclor 1232
- Aroclor 1242
- Aroclor 1248
- Aroclor 1254
- Aroclor 1260

Semivolatiles # 1 186004270

Includes PAHs, phthalates and adipates. One 2 mL flame-sealed ampule yields up to 2 liters after dilution. Each lot contains Benzo(a)pyrene, bis(2-Ethylhexyl)adipate, and bis(2-Ethylhexyl)phthalate plus at least 13 additional analytes, selected from the list below, at 0.2-50 µg/L after dilution.

- Acenaphthene
- Acenaphthylene
- Anthracene
- Benzo(a)anthracene
- Benzo(b)fluoranthene
- Benzo(k)fluoranthene
- Benzo(g,h,i)perylene
- Benzo(a)pyrene
- Butylbenzylphthalate
- Chyrsene
- Dibenz(a,h)anthracene
- Di-n-butylphthalate
- Diethylphthalate
- Dimethylphthalate
- Di-n-octylphthalate
- bis(2-Ethylhexyl)adipate
- bis(2-Ethylhexyl)phthalate
- Fluoranthene
- Fluorene
- Indeno(1,2,3-cd)pyrene
- Naphthalene
- Phenanthrene
- Pyrene

For Regulated Semivolatiles # 2 Herbicides CRMs see page 248.

Disinfection-by-Products CRMs

Chloral Hydrate 186004267

One 2 mL flame-sealed ampule yields in excess of 200 mL after dilution.
Each standard contains Chloral Hydrate at 4-30 µg/L after dilution.

Haloacetic Acids (HAA) 186004269

One 2 mL flame-sealed ampule yields up to 2 liters after dilution.
Each standard contains all six analytes listed below at 10-50 µg/L after dilution.

- Bromochloroacetic Acid
- Dibromoacetic Acid
- Dichloroacetic Acid
- Monobromoacetic Acid
- Monochloroacetic Acid
- Trichloroacetic Acid

Drinking Water Organics CRM

Our CRM acceptance limits will let you know with absolute confidence whether your analytical performance is where you need it to be!

Pesticides CRMs

Pesticides — 186004280

One 2 mL flame-sealed ampule yields up to 2 liters after dilution.
Each standard contains at least 16 analytes, randomly selected from the list below, at 0.1-100 µg/L after dilution. All unspiked analytes are certified at <0.1 µg/L. Includes organochlorine, nitrogen and organophosphorus pesticides.

Alachlor	Heptachlor	Molinate (Ordram)
Aldrin	Heptachlor epoxide (beta)	Prometon
Atrazine	Hexachlorobenzene	Propachlor
Bromacil	Hexachlorocyclopentadiene	Simazine
Butachlor	Lindane (gamma-BHC)	Thiobencarb
Diazinon	Methoxychlor	Trifluralin
Dieldrin	Metolachlor	
Endrin	Metribuzin	

Carbamate/Carbamoxyloxime Pesticides — 186004278

One 2 mL flame-sealed ampule yields up to 2 liters after dilution.
Each standard contains all analytes below at 15-150 µg/L after dilution.

Aldicarb	Carbaryl	Methiocarb
Aldicarb sulfone	Carbofuran	Methomyl
Aldicarb sulfoxide	3-Hydroxycarbofuran	Oxamyl (Vydate)
Baygon		

Chlordane — 186004276

One 2 mL flame-sealed ampule yields up to 2 liters after dilution.
Each standard contains technical chlordane at 2-20 µg/L after dilution.

Toxaphene — 186004272

One 2 mL flame-sealed ampule yields up to 2 liters after dilution.
Each standard contains toxaphene at 3-20 µg/L after dilution.

EDB/DBCP/TCP — 186004277

One 2 mL flame-sealed ampule yields in excess of 200 mL after dilution.
Each lot contains all analytes below at 0.1-2 µg/L after dilution.

1,2-Dibromo-3-chloropropane (DBCP)
Ethylene Dibromide (EDB)
1,2,3-Trichloropropane (1,2,3-TCP)

Herbicides CRMs

Chlorinated Acid Herbicides — 186004275

One 2 mL flame-sealed ampule yields up to 2 liters after dilution. Each standard contains at least 13 analytes, randomly selected from the list below, at 1-150 µg/L after dilution. All unspiked analytes are certified at <1 µg/L.

Acifluorfen	Dalapon	Pentachlorophenol
Bentazon	Dicamba	Picloram
Chloramben	3,5-Dichlorobenzoic acid	2,4,5-T
2,4-D	Dichlorprop	2,4,5-TP (Silvex)
2,4-DB	Dinoseb	
Dacthal diacid (DCPA)	4-Nitrophenol	

Semivolatiles #2 Herbicides — 186004271

One 2 mL flame-sealed ampule yields up to 2 liters after dilution.
Each standard contains all the analytes below at 8-800 µg/L after dilution.

Diquat	Glyphosate
Endothall	Paraquat

Drinking Water Organics CRM Set

Includes the Halomethanes (186004273), Regulated Volatiles (186004274), Unregulated Volatiles (186004268), Pesticides (186004280), Carbamate/Carbamoxyloxime Pesticides (186004278), Chlordane (186004276), Toxaphene (186004272), EDB/DBCP/TCP (186004277) and Chlorinated Acid Herbicides (186004275) CRM standards.

Set Purchase — 186004265

Unregulated Contaminant Monitoring Rule 2 (UCMR 2) CRM

ERA is a provider of standards for UCMR 2. We are making these CRM standards available to laboratories looking to prepare for analysis of these new contaminants that are soon to become regular test analytes. Call us if you have any questions about analyzing these compounds.

Unregulated Contaminant Monitoring Rule 2 (UCMR 2) Drinking Water CRMs

UCMR 2 Pesticides and Flame Retardants in Water 186004260

One 2 mL flame-sealed ampule yields in excess of 2 liters after dilution.
Each standard contains all analytes below at 0.5-10 µg/L after dilution.

- Dimethoate
- 2,2',4,4',5-Pentabromodiphenyl ether (BDE-99)
- 2,2',4,4',6-Pentabromodiphenyl ether (BDE-100)
- 2,2',4,4',5,5'-Hexabromobiphenyl (245-HBB)
- 2,2',4,4',5,5'-Hexabromodiphenyl ether (BDE-153)
- Terbufos sulfone
- 2,2',4,4'-Tetrabromodiphenyl ether (BDE-47)

UCMR 2 Nitrosamines in Water 186004262

One 2 mL flame-sealed ampule yields in excess of 2 liters after dilution.
Each standard contains all analytes below at 5-100 ng/L after dilution.

- N-Nitrosodiethylamine (NDEA)
- N-Nitrosodimethylamine (NDMA)
- N-Nitrosodi-n-butylamine (NDBA)
- N-Nitrosodi-n-propylamine (NDPA)
- N-Nitrosomethylethylamine (NMEA)
- N-Nitrosopyrollidine (NPYR)

UCMR 2 Explosives in Water 186004261

One 2 mL flame-sealed ampule yields in excess of 2 liters after dilution.
Each standard contains all analytes below at 1-15 µg/L after dilution.

- 1,3-Dinitrobenzene
- Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)
- 2,4,6-Trinitrotoluene (TNT)

UCMR 2 Chlorinated Pesticides in Water 186004263

One 2 mL flame-sealed ampule yields in excess of 2 liters after dilution.
Each standard contains all analytes below at 1-20 µg/L after dilution.

- Acetochlor
- Alachlor
- Metolachlor

UCMR 2 Herbicide Degradates in Water 186004264

One 2 mL flame-sealed ampule yields in excess of 2 liters after dilution.
Each standard contains all analytes below at 1-20 µg/L after dilution.

- Acetochlor ethane sulfonic acid (ESA)
- Acetochlor oxanilic acid (OA)
- Alachlor ethane sulfonic acid (ESA)
- Alachlor oxanilic acid (OA)
- Metolachlor ethane sulfonic acid (ESA)
- Metolachlor oxanilic acid (OA)

Blank Soil CRM

Certified clean ERA Blank Soils allow you to evaluate any potential contamination during sample collection, preparation and analysis. They provide the perfect way to comply with all CRM program requirements. For volatile and inorganic analyses you can select between sand and soil matrices, whichever is most appropriate to your specific need.

Volatile Blank Sand	186004302

One 40 g clean sand sample in a VOA vial. The certified concentrations of all analytes are below the lowest NELAC required spiking concentration level of <20 µg/kg.

Volatile Blank Soil	186004301

One 40 g clean soil sample in a VOA vial. The certified concentrations of all analytes are below the lowest NELAC required spiking concentration levels of <20 µg/kg, except acetone at <150 µg/kg, and MEK, 2-hexanone and MIBK at <50 µg/kg.

Semivolatile Blank Soil	186004303

One 60 g certified-clean soil sample in a screw-top bottle. The certified concentration of all analytes are below the lowest NELAC required spiking concentration levels of <500 µg/kg for BNAs and PCBs, <100 µg/kg for chlordane and toxaphene, <5 µg/kg for pesticides and <10 µg/kg for herbicides. In addition, the concentration of total petroleum hydrocarbons (TPH), diesel range organics (DRO) and gasoline range organics (GRO) are certified to be <20 mg/kg.

Metals and Cyanide Blank Sand	186004283

One 40 g sand sample in a screw-top bottle. The concentrations of all EPA/NELAC including the Priority Pollutant metal and cyanide analytes are below the CLP Required Detection Limits (CRDLs) except iron, which is <250 mg/kg.

Metals and Cyanide Blank Soil	186004282

One 40 g soil sample in a screw-top bottle. The concentrations of all of the following analytes are below the CLP CRDL's: antimony, arsenic, beryllium, cadmium, cobalt, mercury, nickel, selenium, silver, sodium, thallium and cyanide. The concentrations of the following analytes are below 10X the CLP CRDL's: barium, chromium, copper, lead, magnesium, potassium and vanadium. The concentrations of manganese and zinc are <750 mg/kg. The concentration range for aluminum, calcium and iron is 3,000-25,000 mg/kg.

Inorganics in Soil CRM

4
UNIQUE LOTS AVAILABLE PER YEAR

In order to ensure the quality and long-term consistency of our Inorganics in Soil CRM standards, ERA uses very carefully selected and prepared substrates along with systematic fortification, homogenization and packaging processes. We verify the accuracy, homogeneity and stability of every analyte in every standard.

Metals CRMs

Metals in Soil 186004288

Use for all ICP and AA, RCRA and Superfund methods. One 40 g soil standard in a screw-top bottle designed and certified for use with digestion methods 3050 hot plate and 3051 microwave. Certified values provided for the hot plate and microwave digestion procedures.

Aluminum	1,000-50,000 mg/kg
Antimony	80-300 mg/kg
Arsenic	50-400 mg/kg
Barium	80-3,000 mg/kg
Beryllium	30-200 mg/kg
Boron	80-200 mg/kg
Cadmium	40-300 mg/kg
Calcium	1,500-25,000 mg/kg
Chromium	40-300 mg/kg
Cobalt	30-200 mg/kg
Copper	40-200 mg/kg
Iron	1,000-22,000 mg/kg
Lead	50-250 mg/kg
Magnesium	1,200-25,000 mg/kg
Manganese	150-2,000 mg/kg
Mercury	1-50 mg/kg
Molybdenum	5-250 mg/kg
Nickel	40-250 mg/kg
Potassium	1,400-25,000 mg/kg
Selenium	50-250 mg/kg
Silver	50-250 mg/kg
Sodium	150-15,000 mg/kg
Strontium	5-250 mg/kg
Thallium	50-250 mg/kg
Tin	75-250 mg/kg
Titanium	10-2,000 mg/kg
Vanadium	50-250 mg/kg
Zinc	70-1,500 mg/kg

Inorganics in Soil CRM Set

Includes the Metals (186004288), Hexavalent Chromium (186004336) and Cyanide in Soil (186004289) CRM standards.

Set Purchase 186004284

Hexavalent Chromium in Soil 186004336

One 40 g soil standard in a screw-top bottle for use with all promulgated hexavalent chromium methods.

Hexavalent chromium	40-300 mg/kg

TCLP Metals in Soil 186004292

One 105 g ready-to-extract soil standard in a screw-top bottle designed specifically to verify the quality of TCLP metals analysis methods. Certified concentrations are provided for antimony, arsenic, barium, beryllium, cadmium, chromium, lead, mercury, nickel, selenium, silver, and zinc.

Metals in Sewage Sludge 186004285

One 40 g sludge standard in a screw-top bottle that is ideal for quality control in Wastewater treatment plant laboratories.

Aluminum	1,000-50,000 mg/kg
Antimony	80-300 mg/kg
Arsenic	50-400 mg/kg
Barium	250-2,000 mg/kg
Beryllium	30-200 mg/kg
Cadmium	40-300 mg/kg
Calcium	5,000-70,000 mg/kg
Chromium	40-300 mg/kg
Cobalt	5 50 mg/kg
Copper	40-1,000 mg/kg
Iron	1,000-50,000 mg/kg
Lead	50-250 mg/kg
Magnesium	1,200-25,000 mg/kg
Manganese	100-2,000 mg/kg
Mercury	1-50 mg/kg
Molybdenum	5-250 mg/kg
Nickel	40-250 mg/kg
Potassium	1,400-25,000 mg/kg
Selenium	50-250 mg/kg
Silver	50-250 mg/kg
Sodium	150-15,000 mg/kg
Strontium	200-2,000 mg/kg
Thallium	50-250 mg/kg
Vanadium	5-250 mg/kg
Zinc	70-1,500 mg/kg

Inorganics in Soil CRM

The reliability of our "new" perchlorate CRM standards has been confirmed over the past several years in extensive round-robin testing.

Inorganics CRMs

Anions in Soil 186004291

CRM all major anions in soil. One 40 g soil standard designed for use with DI water extraction procedures.

- Bromide ..... 10–200 mg/kg
- Chloride ..... 25–500 mg/kg
- Fluoride ..... 25–500 mg/kg
- Nitrate as N ..... 25–500 mg/kg
- Phosphate as P ..... 10–200 mg/kg
- Sulfate ..... 25–1,000 mg/kg

Cyanide in Soil 186004289

One 40 g soil standard in a screw-top bottle for use with distillation/colorimetric methods.

- Total cyanide ..... 25-500 mg/kg

Nutrients in Soil 186004290

One 40 g soil standard in a screw-top bottle is ready for analysis.

- Ammonia as N ..... 50-1,000 mg/kg
- Total Kjeldahl-nitrogen as N ..... 100-2,000 mg/kg
- Total organic carbon (TOC) ..... 2,000-15,000 mg/kg
- Total phosphorus as P ..... 100-2,000 mg/kg

Nutrients in Sludge 186004293

One 40 g sludge standard in a screw-top bottle is ready for analysis.

- Ammonia as N ..... 0.5-3% (w/w)
- Total Kjeldahl-nitrogen as N ..... 2-10% (w/w)
- Total organic carbon (TOC) ..... 20-40% (w/w)
- Total phosphorus as P ..... 2-10% (w/w)

Physical Parameters in Soil CRMs

Corrosivity/pH in Soil 186004299

One 100 g soil standard in a screw-top bottle.

Ignitability/Flash Point 186004300

One standard in a 125 mL bottle. Note that a hazardous materials shipping charge will apply.

Perchlorate CRMs

All perchlorate standards are certified at a specific concentration in the µg/kg range.

Perchlorate in Soil 186004294

One screw-top bottle containing 40 g of soil suitable for deionized water leach and perchlorate analysis using any of the currently available methodologies.

Perchlorate in Sludge 186004295

One screw-top bottle containing 40 g of sludge suitable for deionized water leach and perchlorate analysis using any of the currently available methodologies.

Perchlorate in Vegetation 186004296

One screw-top bottle containing 30 g of freeze-dried vegetable tissue suitable for deionized water leach and perchlorate analysis methods.

TPH in Soil CRMs

Total Petroleum Hydrocarbons (TPH) in Soil # 1 186004297

One screw-top bottle contains 50 g of soil that contains TPH without interfering fatty acids. TPH is present in the range of 250-3,000 mg/kg.

Total Petroleum Hydrocarbons (TPH) in Soil # 2 186004298

One screw-top bottle contains 50 g of soil with TPH in the presence of interfering fatty acids. TPH is present in the range of 250-3,000 mg/kg.

Organics in Soil CRM

4
UNIQUE LOTS AVAILABLE PER YEAR

Over the past 15 years, ERA has refined the selection and preparation of our soil substrates for all our organic standards to ensure consistent extraction and analyte recovery. The accuracy, homogeneity and stability of every analyte in every standard is verified according to NELAC and ISO protocols.

ERA's Organics in Soil CRM standards provide the simplest way to verify the accuracy of your analyses from extraction through analysis. Use these "known" CRM standards any time to compare your results against ERA's certified values and acceptance limits.

Volatiles in Soil CRMs

Volatiles in Soil — 186004316

One 2 mL flame-sealed ampule that requires spiking onto 10 g of provided solid matrix. By altering the amount of concentrate, this can be used for both low and medium level methods. Each standard contains at least 22 of the analytes, randomly selected from the list below at 20-200 µg/kg for low level and 1,000-10,000 µg/kg for medium level. All unspiked analytes are certified at <20 µg/kg for low level and <1,000 µg/kg for medium level.

- Acetone
- Acetonitrile
- Acrolein
- Benzene
- Bromodichloromethane
- Bromoform
- Bromomethane
- 2-Butanone (MEK)
- Carbon disulfide
- Carbon tetrachloride
- Chlorobenzene
- Chlorodibromomethane
- Chloroethane
- 2-Chloroethylvinylether
- Chloroform
- Chloromethane
- 1,2-Dibromo-3-chloropropane (DBCP)
- 1,2-Dibromoethane (EDB)
- Dibromomethane
- 1,2-Dichlorobenzene
- 1,3-Dichlorobenzene
- 1,4-Dichlorobenzene
- Dichlorodifluoromethane
- 1,1-Dichloroethane
- 1,2-Dichloroethane
- 1,1-Dichloroethylene
- cis-1,2-Dichloroethylene
- trans-1,2-Dichloroethylene
- 1,2-Dichloropropane
- cis-1,3-Dichloropropylene
- trans-1,3-Dichloropropylene
- Ethylbenzene
- 2-Hexanone
- Methylene chloride
- Methyl tert-butyl ether (MTBE)
- 4-Methyl-2-pentanone (MIBK)
- Styrene
- 1,1,1,2-Tetrachloroethane
- 1,1,2,2-Tetrachloroethane
- Tetrachloroethene
- Toluene
- 1,1,1-Trichloroethane
- 1,1,2-Trichloroethane
- Trichloroethene
- Trichlorofluoromethane
- 1,2,3-Trichloropropane
- Vinyl acetate
- Vinyl chloride
- Xylenes, total

Ready-to-Use VOAs in Soil — 186004337

One 20 mL flame-sealed ampule with 10 g of soil and 10 mL of methanol is ready to analyze. Each standard contains at least 22 analytes, randomly selected from the list below, at 500-13,000 µg/kg. All unspiked analytes are certified at <1,000 µg/kg.

- Acetone
- Acetonitrile
- Acrolein
- Benzene
- Bromobenzene
- Bromodichloromethane
- Bromoform
- Bromomethane
- 2-Butanone (MEK)
- Carbon disulfide
- Carbon tetrachloride
- Chlorobenzene
- Chlorodibromomethane
- Chloroethane
- 2-Chloroethylvinylether
- Chloroform
- Chloromethane
- 1,2-Dibromo-3-chloropropane (DBCP)
- 1,2-Dibromoethane (EDB)
- Dibromomethane
- 1,2-Dichlorobenzene
- 1,3-Dichlorobenzene
- 1,4-Dichlorobenzene
- Dichlorodifluoromethane
- 1,1-Dichloroethane
- 1,2-Dichloroethane
- 1,1-Dichloroethene
- cis-1,2-Dichloroethylene
- trans-1,2-Dichloroethylene
- 1,2-Dichloropropane
- cis-1,3-Dichloropropylene
- trans-1,3-Dichloropropylene
- Ethylbenzene
- 2-Hexanone
- Hexachlorobutadiene
- Hexachloroethane
- Isopropylbenzene
- Methylene chloride
- Methyl tert-butyl ether (MTBE)
- 4-Methyl-2-pentanone (MIBK)
- Naphthalene
- Nitrobenzene
- Styrene
- 1,1,1,2-Tetrachloroethane
- 1,1,2,2-Tetrachloroethane
- Tetrachloroethene
- Toluene
- 1,2,4-Trichlorobenzene
- 1,1,1-Trichloroethane
- 1,1,2-Trichloroethane
- Trichloroethene
- Trichlorofluoromethane
- 1,2,3-Trichloropropane
- Vinyl acetate
- Vinyl chloride
- Xylenes, total

Organics in Soil CRM

With decades of experience performing environmental analyses, we know what it takes to make soil standards that work for you!

Semivolatiles in Soil CRMs

Nitroaromatics and Nitramines in Soil 186004335

Two flame-sealed ampules, each with 30 g of soil are ready to analyze. Each standard contains at least 9 analytes, randomly selected from the list below, at 500-15,000 µg/kg. All unspiked analytes are certified at <500 µg/kg.

- 4-Amino-2,6-dinitrotoluene
- 2-Amino-4,6-dinitrotoluene
- 1,3-Dinitrobenzene
- 2,4-Dinitrotoluene
- 2,6-Dinitrotoluene
- HMX
- Nitrobenzene
- 2-Nitrotoluene
- 3-Nitrotoluene
- 4-Nitrotoluene
- RDX
- Tetryl
- 1,3,5-Trinitrobenzene
- 2,4,6-Trinitrotoluene

Base/Neutrals and Acids in Soil 186004322

Two 30 g flame-sealed ampules are ready to analyze. Each standard contains at least 36 Base/Neutral and Acid analytes at 500-16,000 µg/kg. All unspiked analytes are certified at <500 µg/kg.

- Acenaphthene
- Acenaphthylene
- 2-Amino-1-methylbenzene (o-Toluidine)
- Aniline
- Anthracene
- Benzidine
- Benzoic acid
- Benzo(a)anthracene
- Benzo(b)fluoranthene
- Benzo(k)fluoranthene
- Benzo(g,h,i)perylene
- Benzo(a)pyrene
- Benzyl alcohol
- 4-Bromophenyl-phenylether
- Butylbenzylphthalate
- Carbazole
- 4-Chloroaniline
- bis(2-Chloroethoxy) methane
- bis(2-Chloroethyl)ether
- bis(2-Chloroisopropyl)ether
- 4-Chloro-3-methylphenol
- 1-Chloronaphthalene
- 2-Chloronaphthalene
- 2-Chlorophenol
- 4-Chlorophenyl-phenylether
- Chrysene
- Dibenz(a,h)anthracene
- Dibenzofuran
- Di-n-butylphthalate
- 1,2-Dichlorobenzene
- 1,3-Dichlorobenzene
- 1,4-Dichlorobenzene
- 3,3'-Dichlorobenzidine
- 2,4-Dichlorophenol
- 2,6-Dichlorophenol
- Diethylphthalate
- 2,4-Dimethylphenol
- Dimethylphthalate
- 2,4-Dinitrophenol
- 2,4-Dinitrotoluene
- 2,6-Dinitrotoluene
- Di-n-octylphthalate
- bis(2-Ethylhexyl)phthalate
- Fluoranthene
- Fluorene
- Hexachlorobenzene
- Hexachlorobutadiene
- Hexachlorocyclopentadiene
- Hexachloroethane
- Indeno(1,2,3-cd)pyrene
- Isophorone
- 2-Methyl-4,6-dinitrophenol
- 2-Methylnaphthalene
- 2-Methylphenol
- 3 & 4-Methylphenol
- Naphthalene
- 2-Nitroaniline
- 3-Nitroaniline
- 4-Nitroaniline
- Nitrobenzene
- 2-Nitrophenol
- 4-Nitrophenol
- N-Nitrosodiethylamine
- N-Nitrosodimethylamine
- N-Nitrosodiphenylamine
- N-Nitroso-di-n-propylamine
- Pentachlorobenzene
- Pentachlorophenol
- Phenanthrene
- Phenol
- Pyrene
- Pyridine
- 1,2,4,5-Tetrachlorobenzene
- 2,3,4,6-Tetrachlorophenol
- 1,2,4-Trichlorobenzene
- 2,4,5-Trichlorophenol
- 2,4,6-Trichlorophenol

Low-Level PAHs in Soil 186004317

Two flame-sealed ampules each with 30 g of soil are ready to analyze. Includes at least 13 analytes, randomly selected from the list below, at 50-1,000 µg/kg. Includes both UV absorbing PAHs at 100-1,000 µg/kg and fluorescent PAHs at 50-200 µg/kg.

- Acenaphthene
- Acenaphthylene
- Anthracene
- Benzo(a)anthracene
- Benzo(b)fluoranthene
- Benzo(k)fluoranthene
- Benzo(g,h,i)perylene
- Benzo(a)pyrene
- Chrysene
- Dibenz(a,h)anthracene
- Fluoranthene
- Fluorene
- Indeno(1,2,3-cd)pyrene
- Naphthalene
- Phenanthrene
- Pyrene

Base/Neutrals and Acids and Pesticides in Soil CRM Set

Includes the Base/Neutrals and Acids (186004322) and Organochlorine Pesticides in Soil (186004323) CRM standards.

Set Purchase 186004315

Organics in Soil CRM Set

Includes the Volatiles (186004316), Base/Neutrals and Acids (186004322) and Organochlorine Pesticides in Soil (186004323) CRM standards.

Set Purchase 186004305

Organics in Soil CRM

Pesticides in Soil CRMs

Organochlorine Pesticides in Soil — 186004323

Two 30 g flame-sealed ampules are ready to analyze. Each standard includes at least 17 pesticides, randomly selected from the list below at 5-500 µg/kg. All unspiked analytes are certified at <5 µg/kg.

- Aldrin
- alpha-BHC
- beta-BHC
- delta-BHC
- gamma-BHC (Lindane)
- alpha-Chlordane
- gamma-Chlordane
- 4,4'-DDD
- 4,4'-DDE
- 4,4'-DDT
- Dieldrin
- Endosulfan I
- Endosulfan II
- Endosulfan sulfate
- Endrin
- Endrin aldehyde
- Endrin ketone
- Heptachlor
- Heptachlor epoxide
- Methoxychlor

Chlordane in Soil — 186004320

One screw-top bottle containing 50 g of soil is ready to analyze. Certified for technical chlordane at 100-500 µg/kg.

Toxaphene in Soil — 186004319

One screw-top bottle containing 50 g of soil is ready to analyze. Certified for toxaphene at 100-500 µg/kg.

Carbamate Pesticides in Soil — 186004339

Two flame-sealed ampules, each with 30 g of soil are ready to analyze. Each standard includes at least 7 analytes, randomly selected from the list below, at 250–2,500 µg/kg. All unspiked analytes are certified at <250 µg/kg.

- Aldicarb sulfone
- Aldicarb sulfoxide
- Carbaryl
- Carbofuran
- Dioxacarb
- Diuron
- 3-Hydroxycarbofuran
- Methiocarb
- Methomyl
- Oxamyl
- Promecarb
- Propham
- Propoxur

Organophosphorus Pesticides (OPP) in Soil — 186004338

Two flame-sealed ampules, each with 30 g of soil are ready to analyze. Each standard includes Disulfoton at 5-500 µg/kg and at least 8 additional analytes, randomly selected from the list below, at 250-2,500 µg/kg. All unspiked analytes are certified at <250 µg/kg.

- Azinophos-methyl (Guthion)
- Chlorpyrifos
- Demeton O & S
- Diazinon
- Dichlorvos (DDVP)
- Disulfoton
- Ethyl Parathion (Parathion)
- Malathion
- Methyl Parathion
- Phorate
- Ronnel
- Stirophos (tetrachlorovinphos)
- Terbufos

Herbicides in Soil CRMs

Chlorinated Acid Herbicides in Soil — 186004318

Two flame-sealed ampules, each with 30 g of soil are ready to analyze. Each standard includes 2,4-D, Dicamba, 4-Nitrophenol, Pentachlorophenol, 2,4,5-T and 2,4,5-TP (Silvex) at 5-10,000 µg/kg and at least 6 additional analytes, randomly selected from the list shown below at 250-2,500 µg/kg. All unspiked analytes are certified at <250 µg/kg.

- Acifluorfen
- Bentazon
- Chloramben
- 2,4-D
- 2,4-DB
- Dacthal diacid (DCPA)
- Dalapon
- Dicamba
- 3,5-Dichlorobenzoic acid
- Dichlorprop
- Dinoseb
- MCPA
- MCPP
- 4-Nitrophenol
- Pentachlorophenol
- Picloram
- 2,4,5-T
- 2,4,5-TP (Silvex)

PCBs in Soil CRMs

PCBs in Soil — 186004321

One screw-top bottle containing 50 grams of sample is ready to analyze. Each standard includes a different Aroclor, randomly selected from the list below, at 500-50,000 µg/kg.

- Aroclor 1016
- Aroclor 1221
- Aroclor 1232
- Aroclor 1242
- Aroclor 1248
- Aroclor 1254
- Aroclor 1260

PCBs in Soil/Oil/Water CRM

ERA PCBs in Soil/Oil/Water CRM standards provide you the simplest and most reliable way to verify the accuracy of your PCB analyses including extraction, clean-up and calibration. Over the past 15 years, ERA has refined the selection and preparation of our substrates to ensure consistent extraction and analyte recovery. Use these "known" CRM standards any time to compare your results against ERA's certified values and acceptance limits.

Soil

PCBs in Soil

PCBs in soil standards are sold individually in screw-top bottles containing 50 g of soil. LOW LEVEL standards contain an Aroclor in the range 0.5-50 ppm. HIGH LEVEL standards contain an Aroclor in the range 51-500 ppm.

Part No.	Concentration	Aroclor
186004307	LOW	1242
186004308	HIGH	1242
186004313	LOW	1248
186004314	HIGH	1248
186004309	LOW	1254
186004310	HIGH	1254
186004311	LOW	1260
186004312	HIGH	1260

Oil

PCBs in Oil

PCBs in oil standards are sold individually in ready-to-use flame-sealed ampules with 5 g of oil. LOW LEVEL standards contain an Aroclor in the range 10-50 ppm. HIGH LEVEL standards contain an Aroclor in the range 51-500 ppm.

Part No.	Concentration	Aroclor
186004327	LOW	1242
186004328	HIGH	1242
186004333	LOW	1248
186004334	HIGH	1248
186004329	LOW	1254
186004330	HIGH	1254
186004331	LOW	1260
186004332	HIGH	1260

Water

PCBs in Water

PCBs in water standards are sold individually in 2 mL flame-sealed ampules that yield 1 liter after dilution. Each standard contains an Aroclor at 1-15 µg/L after dilution.

Part No.	Aroclor
186004402	1016
186004403	1221
186004404	1232
186004405	1242
186004406	1248
186004407	1254
186004408	1260

Hydrocarbon Fuels in Water/Soil CRM

4
UNIQUE LOTS AVAILABLE PER YEAR

ERA Hydrocarbon Fuels in Water and Soil CRM standards provide you the simplest and most reliable way to verify your GC/FID hydrocarbon analyses. See the following pages for state-specific UST standards. Use these "known" CRM standards any time to compare your results against ERA's certified values and acceptance limits. Our acceptance limits are derived from over two million data points. They will let you know with absolute confidence whether your analytical performance is where you need it to be.

Hydrocarbon Fuels in Water CRMs

BTEX and MTBE in Water 186004399

One 2 mL flame-sealed ampule yields in excess of 200 mL after dilution. Each standard includes all analytes below at 7-300 µg/L after dilution.

Benzene	Methyl tert-butyl ether (MTBE)	Toluene
Ethylbenzene		Xylenes, total

Gasoline Range Organics (GRO) in Water 186004400

One 2 mL flame-sealed ampule yields up to 2 liters after dilution. Use with purge and trap methods. Contains unleaded regular gasoline at 200-4,000 µg/L after dilution. Also certified for all BTEX compounds.

Diesel Range Organics (DRO) in Water 186004401

One 2 mL flame-sealed ampule yields up to 2 liters after dilution. Contains No. 2 Diesel. DRO is at 500-4,000 µg/L after dilution.

Designed for GC/FID hydrocarbon methods!

Hydrocarbon Fuels in Water CRM Set

Includes the BTEX and MTBE (186004399), GRO (186004400) and DRO (186004401) in Water CRM standards.

Set Purchase 186004385

Hydrocarbon Fuels in Soil CRM Set

Includes the BTEX and MTBE (186004324), GRO (186004325) and DRO (186004326) in Soil CRM standards.

Set Purchase 186004306

Designed for GC/FID Hydrocarbon methods!

Hydrocarbon Fuels in Soil CRMs

BTEX and MTBE in Soil 186004324

Includes one 2 mL flame-sealed ampule with all analytes below to be spiked onto 10 g of provided soil matrix. All analytes are at 7-500 µg/kg after spiking onto the soil.

Benzene	Methyl tert-butyl ether (MTBE)	Toluene
Ethylbenzene		Xylenes, total

Gasoline Range Organics (GRO) in Soil 186004325

One flame-sealed ampule with 20 g of soil spiked with unleaded regular gasoline at 100-2,000 mg/kg. Use with purge and trap methods. Also certified for all BTEX compounds.

Diesel Range Organics (DRO) in Soil 186004326

One flame-sealed ampule with 20 g of soil spiked with No. 2 Diesel fuel at 100-5,000 mg/kg.

Total Petroleum Hydrocarbons in Water/Soil CRM

4
UNIQUE LOTS AVAILABLE PER YEAR

For both TPH in Water and Soil, standard #1 contains TPH only and standard #2 contains TPH plus interfering fatty acids. These standards are designed specifically to evaluate both your clean-up and analysis techniques and to work with all IR and gravimetric TPH methods.

TPH in Water CRMs

Total Petroleum Hydrocarbons (TPH) in Water # 1	186004363
One liter Whole-Volume bottle is ready to analyze for TPH in water without interfering fatty acids.	
TPH	20-170 mg/L
Total Petroleum Hydrocarbons (TPH) in Water # 2	186004364
One liter Whole-Volume bottle is ready to analyze for TPH in water in the presence of interfering fatty acids.	
TPH	20-170 mg/L

TPH in Water CRM Set

Includes the TPH # 1 (186004363) and TPH # 2 (186004364) in Water CRM standards.

Set Purchase	186004287

Designed to work with all IR and gravimetric TPH methods!

TPH in Soil CRMs

Total Petroleum Hydrocarbons (TPH) in Soil #1	186004297
One screw-top bottle contains 50 g of soil that contains total petroleum hydrocarbons without interfering fatty acids. TPH is present in the range of 250-3,000 mg/kg.	
Total Petroleum Hydrocarbons (TPH) in Soil #2	186004298
One screw-top bottle contains 50 g of soil with total petroleum hydrocarbons in the presence of interfering fatty acids. TPH is present in the range of 250-3,000 mg/kg.	

TPH in Soil CRM Set

Includes the TPH # 1 (186004297) and TPH # 2 (186004298) in Soil CRM standards.

Set Purchase	186004286

Air and Emissions CRM

Volatiles Standards

Volatiles in Gas Cylinder 186004508

One pressurized gas cylinder for use with EPA Methods TO-14 and TO-15. Contains at least 10 analytes, randomly selected from the list below, at 1-25 ppb.

- Benzene
- Bromodichloromethane
- Bromoform
- Bromomethane
- 2-Butanone (MEK)
- tert-Butyl methyl ether (MTBE)
- Carbon tetrachloride
- Chlorobenzene
- Chlorodibromomethane
- Chloroethane
- Chloroform
- Chloromethane
- Cyclohexane
- 1,2-Dibromoethane (EDB)
- 1,2-Dichlorobenzene
- 1,4-Dichlorobenzene
- Dichlorodifluoromethane (Freon 12)
- 1,1-Dichloroethane
- 1,2-Dichloroethane
- 1,1-Dichloroethylene
- cis-1,2-Dichloroethylene
- 1,2-Dichloropropane
- cis-1,3-Dichloropropylene
- trans-1,3-Dichloropropylene
- 1,2-Dichlorotetrafluoroethane (Freon 114)
- Ethylbenzene
- p-Ethyltoluene
- n-Heptane
- Hexachlorobutadiene
- n-Hexane
- 2-Hexanone
- 4-Methyl-2-pentanone (MIBK)
- Propylene
- 1,1,1,2-Tetrachloroethane
- 1,1,2,2-Tetrachloroethane
- Tetrachloroethylene
- Toluene
- 1,1,1-Trichloroethane
- 1,1,2-Trichloroethane
- Trichlorotrifluoromethane (Freon 11)
- Trichlorofluoromethane (Freon 113)
- 1,2,4-Trimethylbenzene
- 1,3,5-Trimethylbenzene
- Vinyl bromide
- Vinyl chloride
- Xylenes, total

Volatiles on Sorbent 186004509

One 2 mL flame-sealed ampule for spiking client-specific sorbent. Use with EPA Methods TO-17, 0030, and 0031. Contains at least 24 analytes, randomly selected from the list below, at 50-2,000 ng/sample (200-3,000 ng/sample for Total Xylenes) after preparation.

- Acetone
- Acetonitrile
- Acrolein
- Acrylonitrile
- Benzene
- Bromodichloromethane
- Bromoform
- Bromomethane
- 2-Butanone (MEK)
- tert-Butyl methyl ether (MTBE)
- Carbon disulfide
- Carbon tetrachloride
- Chlorobenzene
- Chlorodibromomethane
- Chloroethane
- 2-Chloroethylvinylether
- Chloroform
- Chloromethane
- 1,2-Dibromo-3-chloropropane (DBCP)
- 1,2-Dibromoethane (EDB)
- Dibromomethane
- 1,2-Dichlorobenzene
- 1,3-Dichlorobenzene
- 1,4-Dichlorobenzene
- Dichlorodifluoromethane (Freon 12)
- 1,1-Dichloroethane
- 1,2-Dichloroethane
- 1,1-Dichloroethene
- cis-1,2-Dichloroethene
- trans-1,2-Dichloroethene
- 1,2-Dichloropropane
- cis-1,3-Dichloropropene
- trans-1,3-Dichloropropene
- Ethylbenzene
- Hexachlorobutadiene
- 2-Hexanone
- Methylene Chloride
- 4-Methyl-2-pentanone (MIBK)
- Naphthalene
- Styrene
- 1,1,1,2-Tetrachloroethane
- 1,1,2,2-Tetrachloroethane
- Tetrachloroethene
- Toluene
- 1,2,4-Trichlorobenzene
- 1,1,1-Trichloroethane
- 1,1,2-Trichloroethane
- Trichloroethlyene
- Trichlorofluoromethane
- 1,2,3-Trichloropropane
- Vinyl acetate
- Vinyl chloride
- Xylenes, total

Semivolatiles Standards

Semivolatiles on PUF 186004510

Two 2 mL flame-sealed ampules plus one polyurethane foam (PUF). Use with EPA Method 0010. Contains at least 42 analytes, randomly selected from the list below, at 10-225 µg/sample (200-1,000 µg/sample for Benzidine) after preparation.

- Acenaphthene
- Acenaphthylene
- Aniline
- Anthracene
- Benzidine
- Benzo(a)anthracene
- Benzo(b)fluoranthene
- Benzo(k)fluoranthene
- Benzo(g,h,i)perylene
- Benzo(a)pyrene
- Benzyl alcohol
- 4-Bromophenyl-phenylether
- Butylbenzylphthalate
- Carbazole
- 4-Chloroaniline
- Bis(2-chloroethoxy) methane
- Bis(2-chloroethyl)ether
- Bis(2-chloroisopropyl) ether
- Bis(2-ethylhexyl) phthalate
- 1-Chloronaphthalene
- 2-Chloronaphthalene
- 4-Chlorophenyl-phenylether
- Chrysene
- Dibenz(a,h)anthracene
- Dibenzofuran
- Di-n-butylphthalate
- 1,2-Dichlorobenzene
- 1,3-Dichlorobenzene
- 1,4-Dichlorobenzene
- 3,3'-Dichlorobenzidine
- Diethyl phthalate
- Dimethyl phthalate
- 2,4-Dinitrotoluene
- 2,6-Dinitrotoluene
- Di-n-octylphthalate
- Fluoranthene
- Fluorene
- Hexachlorobenzene
- Hexachlorobutadiene
- Hexachlorocyclo-pentadiene
- Hexachloroethane
- Indeno(1,2,3-cd)pyrene
- Isophorone
- 2-Methylnaphthalene
- Naphthalene
- 2-Nitroaniline
- 3-Nitroaniline
- 4-Nitroaniline
- Nitrobenzene
- N-Nitrosodiethylamine
- N-Nitrosodimethylamine (NDMA)
- N-Nitrosodiphenylamine
- N-Nitroso-di-n-propylamine
- Pentachlorobenzene
- Phenanthrene
- Pyrene
- Pyridine
- o-Toluidine
- 1,2,4,5-Tetrachlorobenzene
- 1,2,4-Trichlorobenzene
- Benzoic Acid
- 4-Chloro-3-methylphenol
- 2-Chlorophenol
- 2,4-Dichlorophenol
- 2,6-Dichlorophenol
- 2,4-Dimethylphenol
- 2,4-Dinitrophenol
- 2-Methyl-4,6-dinitrophenol
- 2-Methylphenol (o-Cresol)
- 4-Methylphenol (p-Cresol)
- 2-Nitrophenol
- 4-Nitrophenol
- Pentachlorophenol
- Phenol
- 2,3,4,6-Tetrachlorophenol
- 2,4,5-Trichlorophenol
- 2,4,6-Trichlorophenol

PCBs on PUF 186004512

One 2 mL flame-sealed ampule plus one polyurethane foam (PUF). Use with EPA Methods TO-04A and TO-10A. Contains one Aroclor, randomly selected from the list below, at 1-15 µg/sample after preparation.

- Aroclor 1016
- Aroclor 1221
- Aroclor 1232
- Aroclor 1242
- Aroclor 1248
- Aroclor 1254
- Aroclor 1260

Organochlorine Pesticides on PUF 186004511

One 2 mL flame-sealed ampule plus one polyurethane foam (PUF). Use with EPA Methods TO-04A and TO-10A. Contains at least 16 analytes, randomly selected from the list below, at 0.5-20 µg/sample after preparation.

- aldrin
- Alpha-BHC
- beta-BHC
- delta-BHC
- gamma-BHC (Lindane)
- alpha-Chlordane
- gamma-Chlordane
- 4,4'-DDD
- 4,4'-DDE
- 4,4'-DDT
- Dieldrin
- Endosulfan I
- Endosulfan II
- Endosulfan sulfate
- Endrin
- Endrin aldehyde
- Endrin ketone
- Heptachlor
- Heptachlor Epoxide (beta)
- Methoxychlor

PAHs on PUF 186004513

One 2 mL flame-sealed ampule plus one polyurethane foam (PUF). Use with EPA Method TO-13A. Contains at least 13 analytes, randomly selected from the list below, at 10-200 µg/sample after preparation.

- Acenaphthene
- Acenaphthylene
- Anthracene
- Benzo(a)anthracene
- Benzo(b)fluoranthene
- Benzo(k)fluoranthene
- Benzo(g,h,i)perylene
- Benzo(a)pyrene
- Chrysene
- Dibenz(a,h)anthracene
- Fluoranthene
- Fluorene
- Indeno(1,2,3-cd)pyrene
- Naphthalene
- Phenanthrene
- Pyrene

Aldehydes and Ketones on Sorbent 186004514

One 2 mL flame-sealed ampule to be spiked onto Sorbent. Use with EPA method TO-11A. Contains at least 4 analytes, randomly selected from the list below, at 0.5-10 µg/sample after preparation.

- Acetaldehyde
- Acetone
- Benzaldehyde
- Butyraldehyde (butanal)
- Crotonaldehyde
- 2,5-Dimethylbenzaldehyde
- Formaldehyde
- Hexaldehyde (hexanal)
- Isovaleraldehyde
- Methyl Ethyl Ketone
- Propionaldehyde (propanal)
- o-Tolualdehyde
- m-Tolualdehyde
- p-Tolualdehyde
- Valeraldehyde (pentanal)

Air and Emissions CRM

Metals Standards

Metals on Filter Paper 186004515

One filter paper sample ready for use with EPA method 29.
Contains the metals listed below at 30-1,200 µg/filter.

Antimony	Cobalt	Phosphorus
Arsenic	Copper	Selenium
Barium	Lead	Silver
Beryllium	Manganese	Thallium
Cadmium	Nickel	Zinc
Chromium		

Metals in Impinger Solution 186004516

One impinger solution sample for use with EPA method 29.
Contains the metals listed below at 0.1-10 µg/mL after dilution.

Antimony	Cobalt	Phosphorus
Arsenic	Copper	Selenium
Barium	Lead	Silver
Beryllium	Manganese	Thallium
Cadmium	Nickel	Zinc
Chromium		

Mercury on Filter Paper 186004517

One filter paper sample ready for use with EPA method 29.
Contains Mercury at 0.3-9 µg/filter.

Mercury in Impinger Solution 186004518

One impinger solution sample for use with EPA methods 29 and 101a.
Contains Mercury at 1-30 ng/mL after dilution.

Lead on Filter Paper 186004519

One filter paper sample ready for use with EPA method 12.
Contains Lead at 25-750 µg/filter.

Lead in Impinger Solution 186004520

One impinger solution sample for use with EPA method 12.
Contains Lead at 0.1-3 µg/mL after dilution.

Chromium on Filter Paper 186004521

One filter paper sample for use with CARB method 425.
Contains Total and Hexavalent Chromium each at 1-20 µg/filter.

Hexavalent Chromium in Impinger Solution 186004522

One impinger solution sample for use with EPA method 0061/7199.
Contains Hexavalent Chromium at 45-880 µg/L after dilution.

Inorganic Standards

Hydrogen Halides and Halogens in Impinger Solution 186004523

Two impinger solution samples for use with EPA Methods 26 and 26a.
Contains Total Halides and Total Halogens each at 5-100 mg/L after dilution.

Fluoride in Impinger Solution 186004524

One impinger solution sample for use with EPA Methods 13a,
13b and 14. Contains Fluoride at 1-50 µg/mL after dilution.

Nitrogen Oxide in Impinger Solution 186004525

One impinger solution sample for use with EPA Method 7.
Contains Nitrogen Oxide at 2-400 mg/dscm after dilution.

Sulfur Dioxide in Impinger Solution 186004526

One impinger solution sample for use with EPA Method 6.
Contains Sulfur Dioxide at 200-2,400 mg/dscm after dilution.

Sulfuric Acid and Sulfur Dioxide in Impinger Solution 186004527

One impinger solution sample for use with EPA Method 8. Contains
Sulfuric Acid and Sulfur Dioxide each at 1-120 mg/dscm after dilution.

Ammonia in Impinger Solution 186004528

One impinger solution sample for use with EPA CTM 027.
Contains Ammonium at 0.1-10 mg/L after dilution.

Particulate Matter on Filter Paper 186004529

One filter paper sample ready for use with EPA Methods
5, 5A, 5B, 5D, and 5F. Contains Particulate Matter at 50-600 mg/filter.

Particulate Matter in Impinger Solution 186004530

One impinger solution sample ready for use with EPA Methods
5, 5A, 5B, 5D, and 5F. Contains Particulate Matter at 140-675 mg/L.

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Indices

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Florisil	US Silica Corporation.	Synergi	Thermo Fisher Scientific
FPLC	Pharmacia LKB	TaqMan	Roche Molecular Systems, Inc
Gemini	Phenomenex, Inc.	VICAM	VICAM
Inertsil	GL SCiences	Z-Spin	Pall Life Sciences
Inter-Seal	Integrated Liner Technologies, Inc.	ZipTIP	Millipore Corporation
Luna	Phenomenex, Inc.	Zorbax	Agilent Technologies
OptiPak	Optimize Technologies	Zorbax Extend	Agilent Technologies
µPage	J & W		

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