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Ultrahydrogel Columns

CONTENTS

I. INTRODUCTION

The Waters Ultrahydrogel® Column you have purchased is a sophisticated aqueous-compatible gel column for analytical and preparative separations of water-soluble polymers. The gel is a cross-linked hydroxylated polymer and contains some residual carboxyl functionality. Ultrahydrogel DP contains some residual amine functionality. Compounds normally characterized include oligomers and biological substances such as polysaccharides, nucleic acids, proteins, and peptides.

This Ultrahydrogel Column provides:

- High resolution
- Low adsorption
- A wide range of molecular weight separations
- Stability in a pH range of 2 to 12
- A temperature range of 10 °C to 80 °C

Please take a few moments to read this manual carefully. Use the procedures it contains to ensure that you obtain quality results and take full advantage of the features your Waters column offers.

II. INSTALLATION

a. Solvents

Ultrahydrogel Columns are packed in distilled water for shipping. Table 1 shows the maximum solvent replacement flow rate for each Ultrahydrogel Column. Perform solvent changeover at a lower flow rate than that shown in Table 2 for analysis.

Caution: Ultrahydrogel Columns are flow (pressure) sensitive and extreme care must be taken not to exceed maximum flow rate on start-up.

b. Column

Table 1. Flow rates for replacing solvents

Column	Column size	Max. flow rate during solvent change
Ultrahydrogel (all pore sizes)	7.8 mm I.D. x 30 cm	0.3 mL/min
Ultrahydrogel DP	7.8 mm I.D. x 30 cm	0.3 mL/min
Ultrahydrogel DNA	7.8 mm I.D.. x 30 cm	0.3 mL/min
Ultrahydrogel Linear	7.8 mm I.D. x 30 cm	0.3 mL/min

Note: Change solvents as seldom as possible. Frequent solvent exchanges accelerate the degradation of column efficiency.

c. Organic solvents

Ultrahydrogel Columns are physically and chemically stable in ordinary water-soluble organic solvent mixtures. Aqueous solutions of methanol, ethanol, acetonitrile, formic acid, and dimethylsulfoxide also provide dependable separations. Organic solvent concentration should not exceed 20%. It is possible however, to use organic solvents up to 50% concentration by gradual introduction via gradient method.

d. Aqueous salt and buffer solution

Although some non-ionic samples can be analyzed in distilled water, most water soluble polymers require aqueous salt or buffer solutions.

Some typical aqueous salt solutions:

- Sodium sulfate
- Sodium acetate
- Sodium dihydrogenphosphate
- Ammonium acetate
- Ammonium formate

Compatible buffer solutions:

- Phosphate
- Tris phosphate
- Tris hydrochloric acid
- Citrate
- Tris acetate
- Acetate

e. Flow rate

For best resolution and maximum column life the flow rate should not exceed the maximum flow rate listed below. Use Table 2 below to determine recommended flow rates and maximum pressures.

Table 2. Recommended flow rates

Column	Typical operating flow rate (mL/min)	Maximum flow rate (mL/min)
Ultrahydrogel 120	0.5–0.8	1.0
Ultrahydrogel 250	0.5–0.8	1.0
Ultrahydrogel 500	0.3–0.6	1.0
Ultrahydrogel 1000	0.3–0.6	1.0
Ultrahydrogel 2000	0.3–0.6	1.0
Ultrahydrogel Linear	0.3–0.6	1.0
Ultrahydrogel DP	0.5–0.8	1.0

f. Solvent and sample preparation

- Use LC grade solvents, filtered through a GHP (or other suitable) membrane to remove microparticulate matter above 0.45 µm. Use of the Waters Solvent Clarification Kit (p/n WAT085113) is recommended.
- Particulate-free solvents reduce the problem of plugged filters and column beds and preserve column life.
- Vacuum filtration or sonication of solvents may be used to remove dissolved gases which could affect the solvent delivery system.
- Always filter prepared sample solutions to prevent excessive pressure buildup due to particulate matter (Acrodisc® Filters are available for this purpose).
- Use of a Waters In-Line Precolumn Filter (p/n WAT084560) is recommended to obtain maximum column life.

g. Guard Columns

While guard columns are not generally used for organic GPC, they will remove and/or filter many impurities found in aqueous soluable samples such as saccharides, peptides and hydrolyzates that might adsorb irreversibly on the Ultrahydrogel Column. Guard columns are also used to remove particulates and strongly retained sample components which, ultimately decrease column life from the sample and solvent. Guard columns will aid in obtaining consistently reliable and reproducible results with your Ultrahydrogel Column. Ultrahydrogel Guard Columns are available in both Ultrahydrogel (p/n WAT011565) and Ultrahydrogel DP (p/n WAT011570) configurations.

Guard columns have a finite capacity for impurities. Either the number of sample injections or an observed change in backpressure will indicate when a change of guard column is required. If the capacity of the guard column is exceeded, undesirable contaminants will elute onto the analytical column.

While guard columns will help lengthen column life, they perform a specific function for a short period of time and must be changed or regenerated often. It is important to use good laboratory practices to increase column life. Ultrahydrogel Guard Columns can be regenerated by flushing with 6–10 column volumes of one or more of the following solvents:

- Salt buffer (0.5M–1.0M)
- High pH buffer (pH 9–12)
- Urea, SDS or other surfactants
- Low pH buffer (pH 2–3)
- Methanol or acetonitrile

h. Column Installation

Remove the end cap fittings from your steel column and save them to recap the column when it is removed from the system. The column outlet is indicated by an arrow on the label showing the direction that the solvent should flow. If solvent is not visible when the end cap is removed, connect the outlet side of the column to the system and slowly pump solvent through the column to expel the air. Rapid pressurization or solvent flow may cause degradation of the column. When solvent flow is visible at the inlet side of the column, connect the column in the direction of normal flow and attach the inlet side of the column to the injector or guard column. Follow the next four steps of this procedure to cut tubing to connect a new steel column, or to improve the end connections on your existing fittings.

Note: When changing columns, a new ferrule must be carefully reseated to ensure that no dead volume is present.

1. Using a three-cornered file with a cutting edge scribe the circumference of the tubing at the desired break.
2. Grasp the tubing on both sides of the scribe mark with cloth-covered pliers (to prevent marring the tube surface) and gently work the tube back and forth until it separates.
3. Slide the compression fitting, followed by the ferrule (large end of the taper first) over the tube. Be certain to bottom the tube in the fitting seat to assure a leak-free connection.

Note: Attach a union in place of the column and flush the lines free of previous solvents before attaching the column.

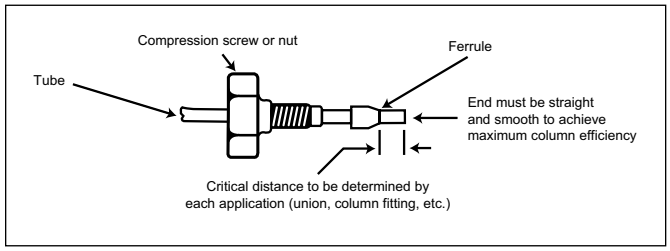


Figure 1. Ferrule and compression screw assembly

If you wish to use Ultrahydrogel Columns in series:

1. Connect the columns in order of decreasing pore size (e.g. Ultra 500 before Ultra 250).
2. To minimize dead volume insert interconnecting tubing into the compression fittings before tightening the fittings.
3. Connect the outlet end of the last column to the detector.

II. OPERATING TIPS

- Use the column only in the direction indicated by the arrow. Flow in the reverse direction may cause degradation in column performance.
- Be careful not to introduce air into the column during installation or removal. Air bubbles will cause voids in the column packing.
- Slowly increase flow rates and change solvents.
- Continue to pump solvent through the column after separation is complete. Air may be sucked into the column by contraction of the solvent if the pump is turned off while the column is still hot.
- Maintain a slow flow rate (below 0.2 mL/min) through the column to prevent precipitation of buffer salts during daily operation. If halides are being used, replace the buffer with distilled water for overnight storage.
- Ultrahydrogel Columns maybe operated at a temperature range of 10 °C to 80 °C. High temperature analysis reduces viscosity, increases theoretical plate count and resolution and reduces adsorptivity.

a. Column efficiency

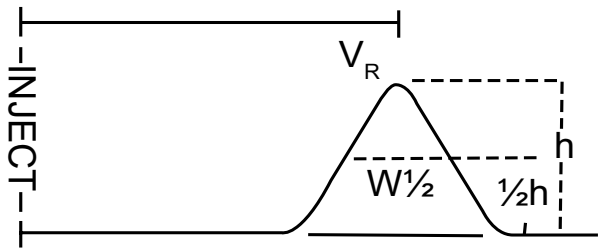
Liquid chromatography columns have a finite life that is directly related to the care and use they receive. Column life is influenced by the number of injections, sample and solvent cleanliness, frequency of solvent changeover, and handling and storage procedures.

To perform a plate count to check for column efficiency, proceed as follows:

Flow rate:	0.8 mL/min
Detector:	Refractive Index
Eluent:	Water
Temperature:	Ambient
Injection vol.:	20 µL
Marker:	Ethylene glycol

Test standard conc.: 0.0025 mL/mL

Calculate the plates (MN) at the half height using the calculations shown in Figure 2.



N = Column efficiency (plates)
V_R = Volume to peak apex (µL)
W½= Volume of peak at ½ peak height (µL)

$$N = 5.54 \times \left(\frac{V_R}{W½}\right)^2$$

Figure 2. Half height method test calculations

b. System efficiency

Column degeneration may not always be the cause of unacceptable sample resolution values. Troubleshooting the entire system may be necessary to determine the exact cause of low performance. In order to isolate the cause of poor system efficiency, always measure bandspreading before installing a column. If loss of resolution is observed after column use, repeat the test for system bandspreading and compare with the initial bandspreading results. If bandspreading has increased, focus troubleshooting efforts on the system hardware (such as the injector or an in-line filter). Isolating or eliminating the column as the source of reduced efficiency in an LC system allows the operator to perform maintenance procedures to restore system efficiency with minimal downtime.

IV. COLUMN STORAGE

a. Short term (several days)

- If the solvent does not contain buffers or other harmful salts, (e.g. halogens), remove the column from the system and seal both ends with the end cap fittings provided.
- If the solvent does contain buffers, etc., rinse the column with distilled water, remove the column from the system and seal with the end cap fittings provided.
- Store at room temperature.
- Avoid direct sunlight.
- Keep away from corrosive gases.

b. Long term (more than three days)

- Replace the solvent with distilled water. Remove the column from the system and seal both ends with the end cap fittings provided.
- Adding sodium azide (0.05%) to the water is recommended for storage periods of 72 hours or more.
- Store at room temperature.
- Avoid direct sunlight.
- Keep away from corrosive gases.

V. WARRANTY

Waters Corporation warrants its high performance liquid chromatography columns in accordance with the following terms and conditions:

Waters will repack or replace (at our discretion) without cost any column that fails to perform satisfactorily if notified within 90 days from your receipt of a steel column. Any column that is returned must have a return authorization number granted by the Waters Customer Service Department. Approval is subject to the following exclusions:

- Physical damage to the column because of misuse or abuse.
- Chemical damage to the packing material because of use with incompatible solvents or buffers, or at an incorrect pH.
- Physical damage to the packing material because of operation at incorrect temperatures or pressures.
- Particulate buildup or precipitation in the column or end fittings causing high internal pressure which has occurred because of improper solvent or sample filtration practices.

VI. ORDERING INFORMATION

Item	Part number
Ultrahydrogel 120	WAT011520
Ultrahydrogel 250	WAT011525
Ultrahydrogel 500	WAT011530
Ultrahydrogel 1000	WAT011535
Ultrahydrogel 2000	WAT011540
Ultrahydrogel Linear	WAT011545
Ultrahydrogel DP	WAT011550
Guard Columns	
Ultrahydrogel (for Ultrahydrogel 120–2000 and Linear)	WAT011565
Ultrahydrogel DP (for Ultrahydrogel DP)	WAT011570

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