WATERS IC-PAK™ ION EXCLUSION COLUMNS

CONTENTS

I. INTRODUCTION

Waters IC-Pak™ Ion Exclusion columns provide a durable column bed for repetitive analyses at high sensitivities. These columns are nominal 7 µm, spherical, fully sulfonated resin.

The Ion Exclusion columns are available in 7.8 mm diameter, in both the 150 mm and the 300 mm formats. The columns are shipped in a solution of 10 parts methanol and 90 parts water.

Please take a few moments to read this manual carefully. The recommendations contained here will help you maximize column lifetime and help you obtain the most reproducible chromatographic results.

II. INSTALLING AND EQUILIBRATING THE COLUMN

a. Preparation

Before attaching a new column in the flow path:

1. Directly connect the HPLC injector to the detector by replacing the of column with a zero-dead-volume union.
2. Flush the lines to remove any microparticulates and old solvents. Flush the injector loop, if applicable.
3. Remove the union.

b. Installing the Column

Remove the compression screws from your column with a 5/16-inch wrench and save them for use when storing the column. Initially attach the column so that flow follows the direction of the arrow on the column label.

Note: The IC-Pak Ion Exclusion column actually can be used in either flow direction.

Procedure

To install the column, thread the inlet and outlet fittings into the column until finger tight, and then tighten the fittings 1/4 to 1/2 turn. A properly prepared and assembled compression fitting will seal if these instructions are followed.

Note: Do not over-tighten; this will damage the connection.

Note: The critical distance between the end of the ferrule and the end of the tubing may differ for different column types. If this is the case, make up a new full (Figure 1) using tubing with an internal diameter of 0.009-inch. Tubing of this diameter should be used for all lines between the injector and detector for the best analytical chromatography.

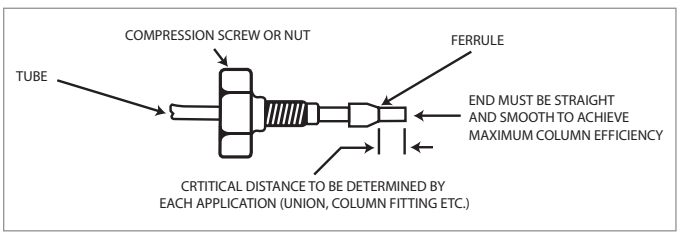

Figure 1. Ferrule and Compression Screw Assembly

When the Seal is Poor

Occassionally, a ferrule may not form an effective seal. Do not try to over-tighten the fitting. To replace a worn reffule or damaged screw, use the following procedure:

1. Using file with a cutting edge, or a tubing cutter, 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 tubing surface), and gently work the tubing back and forth until it separates.

   Note: Ensure that the tubing end is straight, open, and free of burrs.
3. Slide the compression screw fitting, followed by the ferrule (large end of the taper first) over the tube (Figure 1).

   Note: Properly bottom the tubing in the fitting seat. If the tubing is not completely seated, the resulting dead volume can lead to poor chromatographic results.

c. Equilibration

The Ion Exclusion column is shipped in 10% methanol and requires equilibration with the test eluent or mobile phase prior to use.

Procedure

To equilibrate the column:

1. Connect the column to your system.
2. Equilibrate with the desired eluent for 10 minutes at 0.5 mL/min to remove most of the methanol.
3. Ramp to 1.0 mL/min in 60 seconds, allowing the pump pressure to stabilize between 0.1 mL/min increments.
4. Maintain the flow at 1.0 mL/min for 20 minutes, or until the baseline is stable, and proceed with your analysis. If the baseline does not stabilize, check the other components of your LC system.

III. PREPARATION OF ELUENTS AN SAMPLES

a. Preparing the Eluent

Follow these guidelines when preparing eluents:

- Recommended eluents are dilute aqueous solutions of acids.

  Note: The high concentrations (more than 20%) of organic solvents in the mobile phase will impair column performance. Also avoid amines, metals, and corrosives such as hydrochloric acid (HCl).

- Filter eluents to remove microparticulate matter using a filter of 0.45 µm porosity, or smaller. Use ultrapure water (18 megohm resistivity), such as that supplied by the Milli-Q® reagent grade water system.
- Use vacuum filtration and/or sonication to remove dissolved gases which could affect your pump.
- Use a Waters in-line precolumn filter to capture any particulates that may have entered the system.

b. Sample Preparation and Filtration

If the sample contains dissolved contaminants or particulates that may bind irreversibly to the column, follow one of these procedures:

- Use a Waters Sample Clarification Kit, to filter samples and prevent the high back pressures that result from blocked column inlets.
- Use Sep-Pak® cartridges to remove contaminants from the sample that may adsorb on the packing material surface, causing changes in chromatographic performance and reduced column lifetime.
- Use a Waters Guard-Pak™ holder, along with IC-Pak Ion Exclusion Guard-Pak inserts, to adsorb both chemical and physical contaminants.

IV. OPERATION

a. Chromatorgraphy guidelines

Liquid chromatography columns have a finite lifetime which ss directly related to the care and use they receive. Column life is reduced by contamination from samples and eluents, frequent eluent changeover and improper handling and storage.

Adopting some of the simple procedures outlined in this and the previous section can extend column lifetime.

If you observe a change in peak shape, retention of a particular compound, or resolution between two compounds, take immediate steps to determine the reason. Until the cause of the change is determined, do not rely upon the results of the analyses.

Note: Before running the first analysis on your new column, perform the test sample separation given in Section IV. b.

Guidelines

The following operating guidelines will help you obtain the best performance from the Waters analytical HPLC column:

- Do not exceed an operating pressure of 13 MPa (130 atm or 2,000 psi).
- Filter all eluents. Never use turbid or cloudy mobile phases.
- Protect the column from vibration, mechanical shock, and rapid changes in pressure, which can result from rapidly changing the composition of the eluent.
- When using water as a mobile phase component, use water which has been purified with a Milli-Q water system capable of delivering 18 megohm water. Neither deionized water nor bottled HPLC-grade water are acceptable, because they contain organic compounds which may after column selectivity.

b. Efficiency Testing

Waters columns are tested for adherence to our specifications using the sample, mobile phase, and flow rate detailed in Table 1. Slight variations in your results will occur depending on:

- Condition of the equipment used
- Test sample makeup
- Equipment settings and conditions (such as flow rate or composition).

Initial Efficiency Test

Before attempting the first analysis, perform an initial efficiency test. To do this, run the test sample using the following procedure and record the results and instrument settings:

1. Equilibrate the column with the appropriate mobile phase at the desired flow rate.
2. Record the retention time, the instrument settings, and the system configuration so these conditions can be reproduced exactly for future comparison.

Measuring Efficiency

Waters uses the 5 sigma method, shown in Figure 2, to measure column efficiency. Unlike the tangent method, this more stringent method considers naturally occurring peak asymmetry.

If problems occur during normal operation of the column, repeat the efficiency test and compare the results. This may help identify the source of the problem.

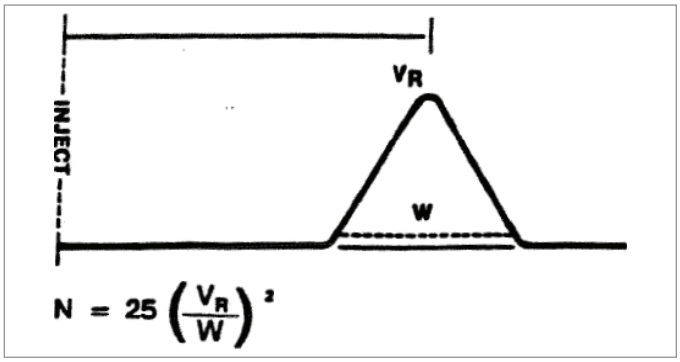

Figure 2. 5 Sigma Test Method

N = Column efficiency (plates)

VR = Volume to peak apex (ml)

W = Volume at 4.4% of peak height (ml)

Test Conditions

Table 1 lists the conditions Waters uses to check the efficiency of IC-Pak Ion Exclusion columns. Figure 3 is a representative chromatogram of flouride and short-chain weak organic acids.

Table 1. Column Test Conditions

Mobile Phase	Flow Rate	Sample
1 mM octanesulfonic acid	1.0 mL/min	see standard preparation

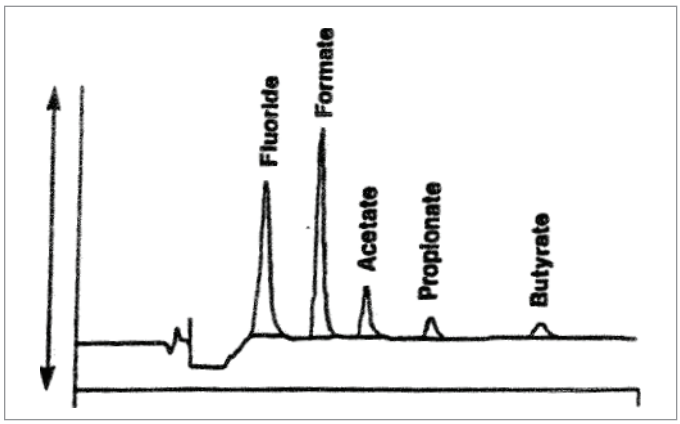

Figure 3. Chromatogram of Fluoride and Short-chain Weak Organic Acids

Working Standard

Fluoride	1 ppm
Acetate	5 ppm
Formate	5 ppm
Propionate	5 ppm
Butyrate	5 ppm

Eluent:	1 mM Octanesulfonic Acid
Pump:	590 Solvent Delivery Module
Injector:	710B WISP
Column:	Ion Exclusion (300 mm X 7.8 mm)
Data:	840 Data System
Flow Rate:	1.0 mL/min
Injection:	100 µL of Working Standard
Detection:	430 Conductivity
Range:	500 µS
Temperature:	On
Polarity:	+
Background:	320 µS

c. Eluent Preparation

10 mM Octanesulfonic Acid Concentrate

1. To a 254 mL beaker add 2.163 g of the sodium salt of octane sulfonic acid (98% Aldrich) and dissolve in 100 mL Milli-Q water.
2. Add 100 mL of precleaqned cation exchange resin in the H+ form (BloRad AG 50W-X12, 200-400 mesh, or equivalent) and stir in the resulting slurry for 10 minutes.
3. Filter the resin, rinsing with approximately 800 mL of Milli-Q water, into a one liter volumetric flask. Dilute up to the mark with Milli-Q water.

Note: This 10 mM oc tanesuffonic acid solution istable for at least one month.

1 mM Octanesulfonic Acid Eluent (pH3)

1. Into a one-liter volumetric flask add 100 mL of the octanesulfonic acid concentrate.
2. Fill the flask to the mark with Milli-Q water and mix thoroughly.
3. Filter and degas through a 0.45 µm HA filter.

The H+ ion from the acid influences the separation. The acid's counter-anion has no effect but to after the background conductivity. Thus, other strong acids can be substituted for octanesulfonic acid.

Note: Acid Normality should be kept constant.

Weak organic acids can also be detected by direct UV absorption in the 205 nm to 215 nm range.

d. Standard Preparation

1. Prepare 1000 ppm stock standards from their sodium salts
2. For this working standard, dilute:

0.1 mL of 1000 ppm Fluoride
0.5 mL of 1000 ppm Formate
1.0 mL of 1000 ppm Acetate
1.0 mL of 1000 ppm Propionate
1.0 mL of 1000 pnm Butyrate
to 100 mL with MHH-Q water.

Note: This working standard should be prepared weekly, since the formate concentration decreases with time.

Table 2. Problems and Corrective Actions

Symptom	Cause	Corrective Action	Prevention
Buildup in HPLC system operating pressure	Inlet frit plugged with particulates	Clean and regenerate column (see Section V. b.). Check for injector and pump seal shedding.	Always filter the eluent and sample prior to use. Use an in-line filter between the pump and injector.
	Guard-Pak insert clogged with particulates or adsorbed materials	Replace the spent Guard-Pak insert. Replace the	Guard-Pak inserts more frequently.
Fluctuating backpressure	Gas in the eluent	Check degassing procedure, Displace gas from the pump.	Always degas the eluent prior to use.
Spurious peaks	Weakly dissociated inoganic anions can sometimes coellute with carboxylic acids	Analysze your sample for carbonate, sulfite, or other anions using an IC-Pak anionn column.	Modify sample preparation.
Variable elution times	Variation in flow rate or eluent composition	Check flow rate being delivered. Check eluent composition.	Always throughly mix eluents prior to use.
Band broadening or loss of resolution	Guard-Pak insert fouled	Replace spend Guard-Pak insert. Replace	Guard-Pak inserts more frequently.
	Column fouled or old	Clean and regenerate the column (see Section V. b.). If column does not recover, use a new column.	Isolate the source of the contamination and use a Guard-Pak insert to protect the column from contaminants in the sample.

V. CARE & MAINTENANCE

a. Troobleshooting

Table 2 lists problems that can occur when using IC-Pak Ion Exclusion columns, and describes corrective actions for these problems.

Loss of efficiency can often be due to bed compression over an extended period of time. If this occurs:

1. Replace the inlet filter.
2. Reverse the column and flow the eluent at 1.0 mL/min for 15 minutes.
3. Recheck the column efficiency.

These steps can also be used if the chromatographer suspects column contamination from a fouled or old column or if the system back pressure has increased from the initial column efficiency

Removing Adsorbed Contaminants

Although Guard-Pak inserts can be relied upon to protect the contaminants column from chemically adsorbed compounds and any stray particulate matter, it may still be necessary to wash the column with solutions designed to remove chemically adsorbed contaminants.

Cleaning agents, such as 0.1 N phosphoric acid (H_3PO_4), 0.1 N nitric sold (HNO_3), or 0.1 M sulfuric acid (H_2SO_4) are ideal for washing the column.

Organs adsorbed to the resin can be removed using a wash of 90 parts water and 10 parts methanol. The cleaning agents should initially be introduced at a low flow rate, and the flow gradually increased. Do not exceed 20 % organic content in any mobile phase or eluent.

The column can be stored in either fresh eluent or in 10% methanol in water. Remove the column (filled with the aqueous/methanol solvent) from the system and replace the compression screws. Re-equilibrate and test the column to establish its condition before putting it back into service.

Considerations

When storing the column, keep these considerations in mind:

- DO NOT store the column in water alone, as this may result in bacterial growth in the column. Storing the column in a 10% aqueous solution of methanol hinders bacterial
- DO NOT leave a column at elevated temperatures without eluent flow.
- Return the column to its box with the compression screws firmly in place for storage. Allowing the column to dry out may result in poor chromatographic performance.

VI. ORDERING INFORMATION

Table 3. Waters HPLC Product Part Numbers

HPLC Accessories	Part Number
Waters IC-Pak Ion Exchange Columns:	
7.8 mm x 150 mm	WAT010295
7.8 mm x 300 mm	WAT010290
Waters IC-Pak Anion Column	WAT007355
Waters IC-Pak Ion Exclusion Guard-Pak Inserts	WAT020770
Replacable Frit	WAT086591
Compression Screws and Ferrules (5 pk)	WAT025604
Milli-Q Reagent Grade Water System (110 V)	WAT098697
In-line Precolumn Filter Kit	WAT084560
Sample Clarification Kit	WAT026865
Filter Reorder Kit, which contains:	WAT026872
400 MF Millipore filters (HATF 012 00)	
400 Aqueous Prefilters (AP25 010 00)	
1 O-Ring	
1 Flat Gasket	
1 Support Screen	
Millex HV4 Low Dead Volume Filters	WAT082680
Tube Cutter	WAT022384

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