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Waters IC-Pak Ion Exclusion Columns

CONTENTS

I. INTRODUCTION

II. INSTALLATION AND EQUILIBRATING THE COLUMN

- A. Preparation
- B. Installing the Column
- C. Equilibration

III. PREPARATION OF ELUENTS AND SAMPLES

- A. Preparing the Eluent
- B. Sample Preparation and Filtration

IV. OPERATION

- A. Chromatography Guidelines
- B. Efficiency Testing
- C. Eluent Preparation
- D. Standard Preparation

V. CARE AND MAINTENANCE

- A. Troubleshooting
- B. Removing Adsorbed Contaminants
- C. Storage Considerations

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.

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 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

a. Column Connectors and System Tubing Considerations

Tools needed:

3/8 inch wrench
5/16 inch wrench

Handle the column with care. Do not drop or hit the column on a hard surface as it may disturb the bed and affect its performance.

1. Correct connection of 1/16 inch outer diameter stainless steel tubing leading to and from the column is essential for high quality chromatographic results.
2. When using standard stainless steel compression screw fittings, it is important to ensure proper fit of the 1/16 inch outer diameter stainless steel tubing. When tightening or loosening the compression screw, place a 5/16 inch wrench on the compression screw and a 3/8 inch wrench on the hex head of the column endfitting.

Note: If one of the wrenches is placed on the column tube flat during this process, the endfitting will be loosened and leak.

3. If a leak occurs between the stainless steel compression screw fitting and the column endfitting, a new compression screw fitting, tubing, and ferrule must be assembled.
4. An arrow on the column identification label indicates correct direction of solvent flow.

Correct connection of 1/16 inch outer diameter stainless steel tubing leading to and from the column is essential for high-quality chromatographic results. Tubing touches the bottom of the column endfitting, with no void between them. It is important to realize that extra column peak broadening can destroy a successful separation. The choice of appropriate column connectors and system tubing is discussed in detail below.

Due to the absence of an industry standard, various column manufacturers used different types of chromatographic column connectors. The chromatographic performance of the separation can be negatively affected if the style of the column endfittings does not match the existing tubing ferrule settings. This section explains the differences between Waters style and Parker style ferrules and endfittings (Figure 1). Each endfitting style varies in the required length of the tubing protruding from the ferrule. The Waters IC-Pak Ion Exclusion Column is equipped with Waters style endfittings that require a 0.130 inch ferrule. If a non-Waters style column is presently being used, it is critical that ferrule depth be reset for optimal performance prior to installing the column.

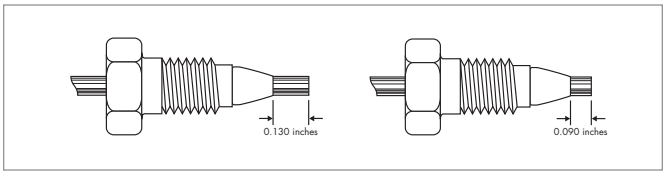

Figure 1. Waters and Parker style ferrule types.

In a proper tubing/column connection (Figure 2), the tubing touches the bottom of the column endfitting, with no void between them.

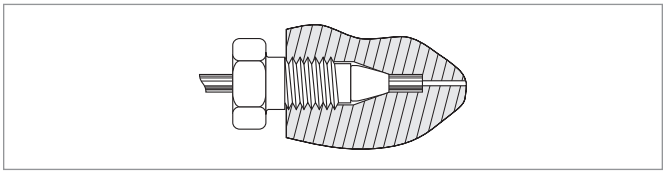

Figure 2. Proper tubing/column connection.

The presence of a void in the flow stream reduces column performance. This can occur if a Parker style ferrule is connected to a Waters endfitting (Figure 3).

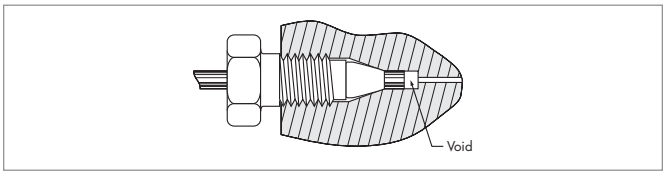

Figure 3. Parker ferrule in a Waters style endfitting.

Note: A void appears if tubing with a Parker style ferrule is connected to a Waters style column.

There is only one way to fix this problem: Cut the end of the tubing with the ferrule, place a new ferrule on the tubing, and make a new connection. Before tightening the screw, make sure that the tubing bottoms out in the endfitting of the column.

Conversely, if tubing with a Waters ferrule is connected to a column with Parker style endfitting, the end of the tubing will bottom out before the ferrule reaches its proper sealing position. This will leave a gap and create a leak (Figure 4).

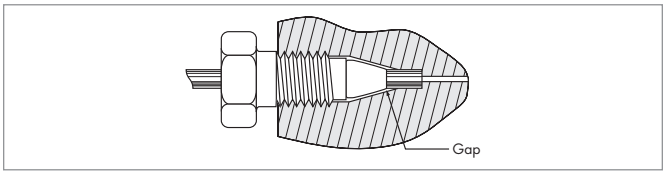

Figure 4. Waters ferrule in a Parker style endfittings.

Note: The connection leaks if a Waters ferrule is connected to a column with a Parker style endfitting.

There are two ways to fix the problem:

5. Tighten the screw a bit more. The ferrule moves forward, and reaches the sealing surface. Do not overtighten since this may break the screw.
6. Cut the tubing, replace the ferrule, and make a new connection. Alternatively, replace the conventional compression screw fitting with an all-in-one PEEK® fitting that allows resetting of the ferrule depth. Another approach is to use a Thermo Corporation SLIPFREE® connector to always ensure the correct fit.

The fingertight SLIPFREE connectors automatically adjust to fit all compression screw type fittings without the use of tools (Figure 5).

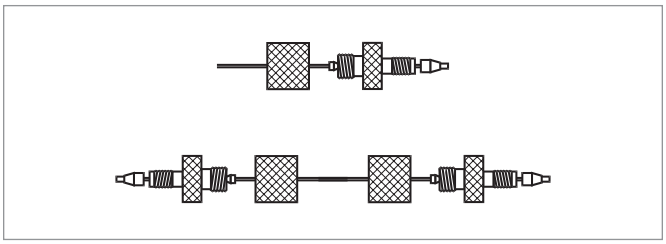

Figure 5. Single and double SLIPFREE connectors.

b. Band Spreading Minimization

Figure 6 shows the influence of tubing internal diameter on system band spreading and peak shape. As can be seen, the larger tubing diameter causes excessive peak broadening and lower sensitivity.

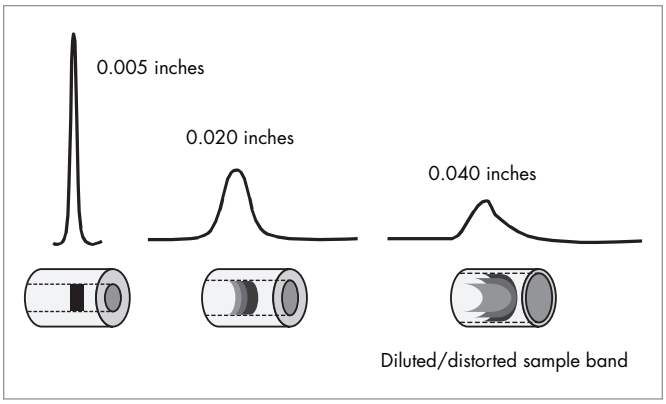

Figure 6. Effect of connecting tubing on system.

c. Measuring System Bandspreading Volume and System Variance

This test should be performed on an HPLC system with a single wavelength UV detector (not a Photodiode Array [PDA]).

1. Disconnect column from system and replace with a zero dead volume union.
2. Set flow rate to 1 mL/min.
3. Dilute a test mix in mobile phase to give a detector sensitivity of 0.5–1.0 AUFS.
4. Inject 2 to 5 µL of this solution.
5. Measure the peak width at 4.4% of peak height (5-sigma method):

   5-sigma Bandspreading (µL) = Peak Width (min) Flow Rate (mL/min x (1000 µL/1 mL)System Variance (µL^2) = (5-sigma bandspreading)^2/25

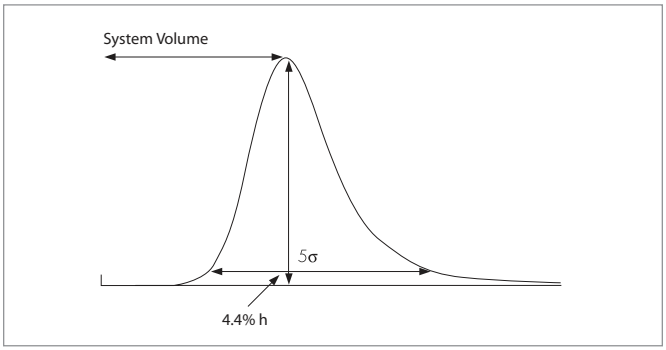

Figure 7. Determination of system bandspreading volume using 5-sigma method.

In a typical HPLC system, the bandspreading volume should be 100 µL ± 30µL (or variance of 400 µL^2 ± 36 µL^2).

d. Measuring System Volume

System volume is important in scaling separations because it creates an isocratic hold at the start of every run. This hold is often several column volumes on a small scale, but a fraction of the volume of a prep column. Compensation for this volume must be included in planning a scaling experiment to avoid distorting the chromatography (Figure 8).

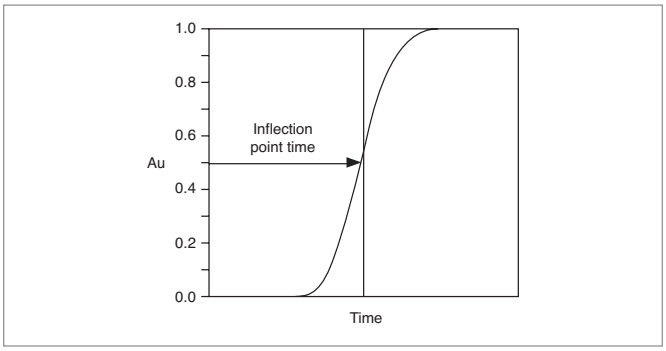

Figure 8. Determination of gradient delay volume.

1. Remove column.
2. Use acetonitrile as mobile phase A, and acetonitrile with 0.05 mg/mL uracil as mobile phase B (eliminates non-additive mixing and viscosity problems).
3. Set UV detector at 254 nm.
4. Use the flow rate in the original method and the intended flow rate on the target instrument.
5. Collect 100% A baseline for 5 minutes.
6. Program a step change at 5 minutes to 100% B, and collect data for an additional 5 minutes.
7. Measure absorbance difference between 100% A and 100% B.
8. Measure time at 50% of that absorbance difference.
9. Calculate time difference between start of step and 50% point.
10. Multiply time difference by flow rate.

C. EQUILIBRATION

IC-Pak Ion Exclusion Columns are shipped in 10 parts methanol and 90 parts water 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 AND 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.
- Filter eluents to remove microparticulate matter using a 0.45 µm filter, or smaller. Use ultrapure water (18 megohm resistivity), such as that supplied by the Milli-Q® reagent gradewater 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:

1. Use a Waters sample clarification kit, to prevent the high back pressures that result from blocked column inlets.
2. Use Sep-Pak® Cartridges to remove contaminants from the sample that may adsorb on the packing material surface.
3. 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. CHROMATOGRAPHY GUIDELINES

Liquid chromatography columns have a finite lifetime which is 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.

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 IVb.

Guidelines

The following operating guidelines will help you obtain the best performance from an 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 nor bottled HPLC-grade water are acceptable, because they contain organic compounds which may alter 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 9, 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.

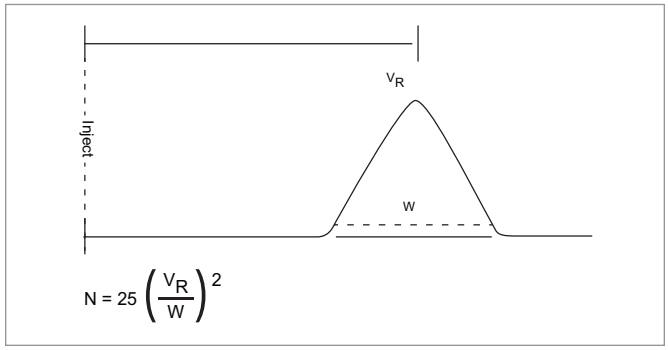

Figure 9. 5 sigma test method.

N = Column efficiency (plates)

V_R = 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 10 is a representative chromatogram of fluoride 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

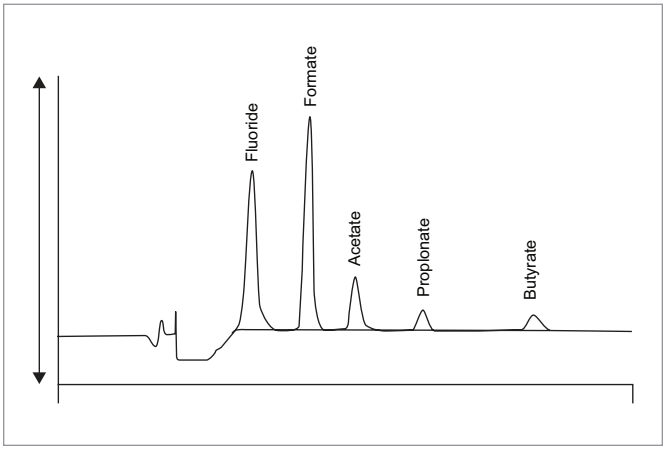

Figure 10. Chromatogram of fluoride and short-chain weak organic acids.

Working Standard

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

Eluent:	1 mM octanesulfonic acid
Column:	IC-Pak Ion Exclusion
Flow rate:	1.0 mL/min
Injection:	100 µL of working standard
Detection:	conductivity
Background:	320 µS

C. ELUENT PREPARATION

10 mM Octanesulfonic Acid Concentrate

1. To a 250 mL beaker add 2.163 g of sodium salt sulfonic acid (98% purity) and dissolve in 100 mL Milli-Q water.
2. Add 100 mL of precleaned cation exchange resin in the H^+ form (BioRad 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 octanesulfonic acid solution is stable 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.

Note: This 10 mM octanesulfonic acid solution is stable for at least one month.

The H^+ ion from the acid influences the separation. The acid's counter-anion has no effect but to alter 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 1,000 ppm stock standards from their sodium salts
2. For this working standard, dilute:

0.1 mL of 1,000 ppm Fluoride

0.5 mL of 1,000 ppm Formate

1.0 mL of 1,000 ppm Acetate

1.0 mL of 1,000 ppm Propionate

1.0 mL of 1,000 ppm Butyrate

to 100 mL with Milli-Q water.

V. CARE & MAINTENANCE

A. TROUBLESHOOTING

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.

B. 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, 0.1 N nitric acid, or 0.1 M sulfuric acid are ideal for washing the column.

Organic contaminants 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.

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 Vb.). 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 inorganic anions can sometimes coelute with carboxylic acids	Analyze your sample for carbonate, sulfite, or other anions using an IC-Pak Anion Column.	Modify sample preparation.
Variable elution times	Variation in flow rate or eluent composition	Check flow rate being delivered. Check eluent composition.	Always thoroughly 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 Vb). 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.

C. STORAGE 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 growth.
- 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.

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