

# WATERS IC-PAK C M/D COLUMN

## I. INTRODUCTION

This manual describes procedures for using the Waters IC-Pak™ C M/D Ion Chromatography separation of monovalent and divalent cations. Please take a few moments to read this manual carefully. Follow its recommendations to obtain reproducible chromatography and column stability.

### a. Waters IC-Pak C M/D Column Description

Waters IC-Pak C M/D column is designed for ion chromatography applications. Use the M/D column to simultaneously analyze monovalent and divalent cations isocratically. The column separates and allows quantitation of Li<sup>+</sup>, Na<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, <sup>+</sup>, Mg<sub>2</sub><sup>+</sup> Ca<sup>+</sup>, Sr<sub>2</sub><sup>+</sup>, and Ba<sub>2</sub><sup>+</sup> cations at ppb levels.

### b. Specifications

Column	IC-Pak C M/D
Dimensions	3.9 x 150 mm
Particle size	5 μm
Capacity	1.5 ± 0.2 meq/g
Packing material	Silica base coated with polybutadiene/maleic acid copolymer
Shipped in	20/80 (v/v) acetonitrile/water

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## II. INSTALLING THE COLUMN

### a. Preparing the Ion-Exchange System

To prepare the system, you need two 3/8-inch wrenches and a zero dead-volume union. Before installing the column in the flow path:

1. Directly connect the injector to the detector by replacing the old column with a zero dead-volume union.
2. Flush the lines and the detector of microparticulates and previous solvents. Use fresh eluent at a flow rate of 1.0 mL/min. Flush the injector loop if applicable.
3. Remove the union.
4. Install the column between the injector and detector (see Section II. b).

### b. Installing the Column

Remove the end plugs from the column and save them for use when you store the column. To install the column:

1. Thread the inlet and outlet fittings into the column until finger-tight. An arrow on the label indicates the direction of flow.
2. Use the wrenches to tighten the fittings 1/4 to 1/2 turn. Do not overtighten. Overtightening damages the connection.
3. Prepare a new tubing-ferrule connection (Figure 1) when connecting a new column or when removing a damaged compression screw or worn ferrule.

*Note: The tubing distance beyond the ferrule may differ for each column type. Resize the tubing to the correct distance by replacing the ferrule.*

### Preparing a New Connection

To prepare a new tubing-ferrule connection:

1. Scribe the circumference of the tubing at the desired break using either a file with a cutting edge or a tube cutter.
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.

*Note: Ensure that tubing end is straight, open, and free of burrs.*

3. Slide the compression fitting, followed by the ferrule (large end of the taper first) over the tube.

*Note: Properly bottom the tubing in the fitting seat. Otherwise, dead volume could result in sample band spreading.*

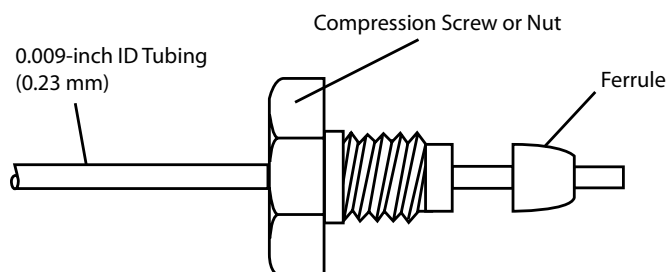


Figure 1: Ferrule and Compression Screw Assembly

### c. Equilibrating the Column

1. Precondition new columns by flushing with 100% acetonitrile for 30 minutes at 0.5 mL/min.
2. Flush the column with mobile phase for 5 minutes at 0.5 mL/min, and then for 30 minutes at 1.0 mL/min before performing the first separation.

## III. PREPARING MOBILE PHASE AND SAMPLES

### a. Preparing the Mobile Phase

#### Chemical requirements

The IC-Pak C M/D uses 0.1 mM EDTA/3.0 mM HNO<sup>3</sup> as a mobile phase. Store mobile phase at room temperature for up to one month.

- ACS Reagent grade or equivalent quality free-acid EDTA
- Quality nitric acid
- Quality 18 megohm water
- Plasticware to prepare and store all mobile phases, standards, and samples

## Filtration

Before filtering the mobile phase, flush the filters with 100 mL of mobile phase. Discard the filtered mobile phase. Filter and degas mobile phases with a compatible Millex® 0.45 µ filter for optimum long-term performance of the column.

Use an in-line precolumn filter to remove particulates in the mobile phase and contaminants which the 0.45 µm filter does not remove.

## Glass Containers

Sodium leaching from glass vials causes artifacts when analyzing cations. Use plastic containers for all solutions.

## Organic Mobile Phases

Samples containing organic amines may exhibit hydrophobic interaction between the mobile phase and packing. You may use a water-miscible organic mobile phase, such as acetonitrile, as a modifier to reduce this. Do not use methanol; over time it causes a significant decrease in cation retention time. Pretreat the sample with a C1<sup>8</sup> Sep-Pak® cartridge to remove hydrophobic compounds.

## Changeover to Mobile Phases Containing Salts

Perform changeover between on organic solvent and water containing salts gradually to avoid salt precipitation. Use 18 megohm water as the intermediate solvent. Do not exceed salt concentrations of 0.1 M.

## Preparing the Mobile Phase

1. Put 800 mL of Milli-Q™ water into a one-liter volumetric flask.
2. Add 0.0292 g EDTA, free acid (reagent grade). Stir or place in ultrasonic bath for 15 minutes.
3. Add 189 µL Nitric Acid (Ultrex).
4. Dilute the solution to one liter with Milli-Q water and mix thoroughly.
5. Filter any undissolved EDTA from the solution and degas with one of the following Millipore membranes (refer to Section ??? for part numbers):

Aqueous Replacement Filters (0.45 µm, 47 mm)

Durapore® Filters (0.22 µm, 47 mm)

## b. Preparing the Sample Preparing

Sample cleanup with Sep-Pak cartridges prevents alteration of the column chemistry by strongly adsorbing or precipitating sample components. Pass the first few drops of sample to waste.

Filter prepared samples with a microporous filter to prevent excessive pressure buildup due to particulate matter. Rinse the filter with 10 mL of 18 megohm water. Pass the first few drops of Sample through the filter to waste.

## IV. USING THE COLUMN

### a. Chromatography Guidelines

The life of a column is directly related to its care and use. Contamination from samples and mobile phases and improper handling and storage reduce column life.

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

### Physical Limits

Maximum Pressure	27 MPa (27 atm or 4000 psi)
Flow Rate	1 mL/min
pH Range	2 to 7
Temperature:	
Normal operation	25 °C
Limits	≤ 50 °C (do not freeze column)

## General considerations

- Filter all aqueous buffers. Do not use turbid or cloudy buffers.
- Do not reuse aliquots taken from the sample container; dispose of them.
- Protect the column from vibration, mechanical shock, and rapid changes in pressure, flow rate or mobile phase composition. Any thermal, physical or chemical shock (such as changing mobile phases rapidly) can cause a loss of efficiency.
- When using water, treat it with a water system capable of delivering 18 megohm water. Neither deionized water nor HPLC-grade bottled water are acceptable because they may contain organic compounds which alter column selectivity.
- Highly concentrated samples (greater than 10 ppm per ion) can overload the packing material, resulting in poor peak shape. Dilute the sample before injection. When analyzing an unknown, start with a 1:100 dilution.
- **DO NOT** inject concentrated samples directly into the mobile phase. Direct injection may cause precipitation of the salts in the sample. Dissolve (or dilute) samples in an appropriate volume of the mobile phase first. If you must use other solvents, watch for precipitation upon injection into the eluent. Always filter samples before use.

## b. Efficiency Testing

Perform an efficiency test before attempting the first analysis. Run the test sample using the calibration standards detailed in the following pages. Record the retention time and the settings used

There are four parts to performing the efficiency test:

1. Preparing the mobile phase
2. Preparing the stock calibration standards
3. Running the calibration (working) standard
4. Calculating column efficiency

If you experience problems during normal operation, repeat the conditions for the initial efficiency test and compare the results. Differences may indicate a problem with the column.

## Preparing Mobile Phase

The IC-Pak C M/D column uses 0.1 mM EDTA/3.0 mM HNO<sup>3</sup> as a mobile phase. See phase Section III. a, Preparing the Mobile Phase, for procedures for mobile phase preparation.

## Guidelines for the Calibration Standard

Standard concentrates are available from most major chemical suppliers. Use reagent-grade or analytical standard-grade solutions. Several anionic species can cause the precipitation of alkali and alkaline earth metals. Consult solubility tables to avoid these species. Avoid hygroscopic salts. Select the highest purity salt available. Certain atomic absorption standards are made from ammonium salts and must not be used to prepare calibration standard.

## Preparing Individual 1000 ppm Stock Standards

1. Weigh the specified amount of salt (refer to Table 1).
2. Add the salt to a plastic 1 liter volumetric flask.
3. Fill the flask to the mark with 18 megohm water.
4. Store the stock solutions in clean plasticware at room temperature for up to 6 months.

Table 1: Salt Weight for Stock Solution Preparation

Cation	Compound	Weight (g)
Li <sup>+</sup> (Lithium)	UOH • H <sub>2</sub> O	6.0476
Na <sup>+</sup> (Sodium)	NaCl	2.5421
NH <sub>4</sub> <sup>+</sup> (Ammonium)	NH <sub>4</sub> Cl	2.9640
K <sup>+</sup> (Potassium)	KCl	1.9067
Mg <sup>2+</sup> (Magnesium)	Mg(NO <sub>3</sub> ) <sub>2</sub> • 6H <sub>2</sub> O	10.5466
Ca <sup>2+</sup> (Calcium)	Ca(NO <sub>3</sub> ) <sub>2</sub> • 4H <sub>2</sub> O	5.8919
Sr <sup>2+</sup> (Strontium)	Sr(NO <sub>3</sub> ) <sub>2</sub> • 4H <sub>2</sub> O	3.2377
Ba <sup>2+</sup> (Barium)	BaCl <sub>2</sub> • 2H <sub>2</sub> O	1.7786

The following equation is an example of how these weights were determined:

$$1 \text{ g K}^+/\text{L} \times 74.553 \text{ g KCl}/39.100 \text{ g K}^+ \times 1 \text{ L} = 1.9067 \text{ g KCl}$$

To prepare 1 liter of working standard:

1. Measure the appropriate quantity of stock standard (refer to Table 2).
2. Add the stock standard to a plastic 1000 ml volumetric flask.
3. Fill the flask to the mark with 18 megohm water. Table 2 shows the concentrations for the monovalent and divalent cation working standards.
4. Store working standards in clean plasticware at room temperature for up to one month.

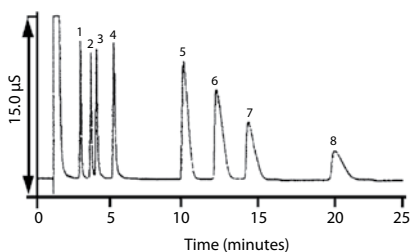
Table 2: Working Standard Solutions and Final Concentrations

Cation	Use This Volume of Stock Standard	To Yield This Concentration of Working Standard (ppm)
Li <sup>+</sup> (Lithium)	0.250 ml	0.25
Na <sup>+</sup> (Sodium)	1.000 ml	1.00
NH <sub>4</sub> <sup>+</sup> Ammonium)	1.000 ml	1.00
K <sup>+</sup> (Potassium)	3.000 ml	3.00
Mg <sub>2</sub> <sup>+</sup> agnesium)	2.000 ml	2.00
Ca <sub>2</sub> <sup>+</sup> (Calcium	3.000 ml	3.00
Sr <sub>2</sub> <sup>+</sup> (Strontium	5.000 ml	5.00
Ba <sup>+</sup> (Barium)	5.000 ml	5.00

### Determining Column Efficiency

Use a flow rate of 1.0 ml/min. The peak for potassium should yield >2,000 plates by the half-height method on a low dispersion Waters ion chromatograph.

Figure 2 shows the conditions and resulting chromatogram for monovalent and divalent cation standards.



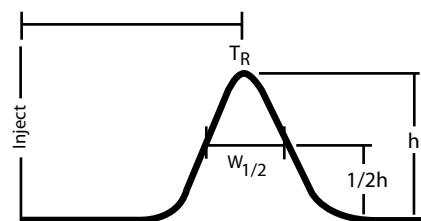
- |              |          |              |         |
|--------------|----------|--------------|---------|
| 1. Lithium   | 0.25 ppm | 5. Magnesium | 2.0 ppm |
| 2. Sodium    | 1.0 ppm  | 6. Calcium   | 3.0 ppm |
| 3. Ammonium  | 1.0 ppm  | 7. Strontium | 5.0 ppm |
| 4. Potassium | 3.0 ppm  | 8. Barium    | 5.0 ppm |

Conditions	
Column	IC-Pak C M/D
Eluent	0.1 mM EDTA/ 3 mM HNO <sub>3</sub>
Detector	Waters 431 Conductivity
Flow Rate	1.0 mL/min
Injection Volume	100 µL

Figure 2: IC-Pak C M/D Standard Cation Chromatogram

### Calculating Efficiency

Use the potassium peak (#4) to measure your column efficiency with the half-height method equation in Figure 3.



N = Column efficiency (plates)

T<sub>R</sub> = Time to peak apex (minutes)

W<sub>1/2</sub> = Width of peak at 1/2 peak height (minutes)

$$N = 5.54 \times \left( \frac{T_R}{W_{1/2}} \right)^2$$

Figure 3: Half-Height Method Test Calculations

## V. CARE & MAINTENANCE

### a. Storing the Column

For short-term storage, leave the mobile phase in the column with the column connected to the system.

If the column will not be used for more than 72 hours, store it in 20/80 acetonitrile/water (v/v).

When storing columns:

**DO NOT** store the column in methanol or methanol-containing solutions. Over time, methanol causes significant decreases in cation retention times. To prevent growth of bacteria, fill with 20/80 acetonitrile/water (v/v), replace the end plugs, and return the column to its box.

**DO NOT** allow the column to dry out. Allowing the column packing to dry out can result in poor chromatographic performance.

Store at 15 °C to 35 °C. Freezing during storage degrades performance.

**b. Troubleshooting**

Table 3: Column Problems and Solutions

Symptom	Conditions	Corrective Action
Excess pressure buildup	Filters plugged with particulates. Check for injector and pump seal shedding.	Replace filter.
	Clogged tubing	Unclog or replace tubing.
	Failing injector	Repair the injector.
Sample precipitates on column	Sample not soluble in eluent	Backflush column with mobile phase for 30 minutes at 0.1 mL/min.
		Use a compatible mobile phase.
Loss of resolution, broad peaks, low plate counts	Insufficient equilibration	Continue equilibration
	Incorrect diameter stainless steel tubing	Install 0.0009-inch stainless steel tubing.
	Contaminated column	Flush column with 100% acetonitrile at 0.5 mL/min for 30 minutes.

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**VI. ORDERING INFORMATION**

To order these parts, contact the nearest subsidiary (see back cover).

Table 4: Spare Parts and Accessories

Part	Number
IC-Pak C M/D Column, 3.9 x 150 mm	WAT036570
Replacement frit for 3.9 mm column	WAT015931
IC-Pak C M/D Guard-Pak™ Inserts, 10/pkg	WAT098801



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November 2007 WAT036549 Rev 4 VW-PDF

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