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IC-Pak Column and Guard Column

CONTENTS

I. INTRODUCTION

Waters IC-Pak™ anion (A) and cation (C) columns are designed for use in Ion Chromatography (IC) applications to separate ions.

a. Anion Columns

The stainless steel anion columns separate and quantify many anions, such as F^-, Cl^-, NO_2^-, Br^-, NO_3^-, HPO_4^{2-}, and SO_4^{2-} anions at ppb levels. Table 1 lists the characteristics of IC-Pak anion columns.

Table 1: Anion Column Characteristics

Anion Column	Dimensions	Particle Size	Capacity
IC-Pak A	4.6 x 50 mm	10 µm	30 ± 3 µeq/mL
IC-Pak A HC (High Capacity)	4.6 x 150 mm	10 µm	30 ± 3 µeq/mL
IC-Pak A HR (High Resolution)	4.6 x 75 mm	6 µm	30 ± 3 µeq/mL

The packing material is a polymethacrylate resin with a quaternary ammonium functional group. The columns are shipped in 1.3 mM gluconic acid/1.3 mM boric acid (pH 8.5).

b. Cation Columns

The non-metallic cation columns separate and quantify Li^+, Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+} cations at ppb levels. The Waters cation guard column, placed in the solvent stream before the sample injector, is used only to aid monovalent cation detection by removing polyvalent cations from the solvent stream.

Table 2 lists the characteristics of IC-Pak cation and cation guard columns.

Table 2: Cation Column Characteristics

Cation Column	Dimensions	Particle Size	Capacity
IC-Pak C	4.6 x 50 mm	10 µm	12.0 ± 0.2 µeg/mL
Guard	4.6 x 50 mm	n/a	2.0 ± 0.2 meg/mL

The packing material is a styrene divinylbenzene resin with a sulfonic acid functional group. The columns are shipped in 2 mM nitric acid.

II. INSTALLATION

Before attaching the column in the flow path:

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

a. Installing a Stainless Steel Column

Remove the end plugs from the column and save them for use when the column is removed from the system and stored. The column outlet is indicated by an arrow on the label (showing the direction solvent should flow).

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. Do not over-tighten. Over-tightening will damage the connection. A properly prepared and assembled compression fitting in good condition is all that is required.

Prepare a new tubing/ferrule connection (Figure 1) when a new column is connected or when a damaged compression screw or worn ferrule are removed.

Note: The tubing distance beyond the ferrule may differ for different column types. Re-size the tubing to the correct distance by replacing the ferrule.

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 the 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. Other wise, dead volume could result in sample band spreading.

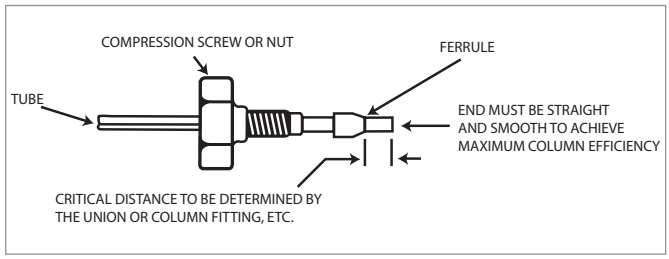

Figure 1: Ferrule and Compression Assembly (Stainless Steel Column).

b. Installing Non-Metallic Columns

Remove the end plugs from your column and save them for use when the column is removed from the system and stored. The column outlet is indicated by an arrow on the label (showing the direction solvent should flow).

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. Use caution with non-metallic columns. Over-tightening these fittings may cause damage which results in a leaking connection. A properly prepared and assembled compression fitting in good condition is all that is required.

To replace a fitting:

1. Use a sharp razor blade to make a long tapering cut in the tubing as shown in Figure 2. Alternately, if tubing is not in short supply, stretch the tubing (after warming over low heat) between two sets of pliers and cut the tube at thinnest point. The resulting taper will slip through the fittings more easily.
2. Pass the tubing through the compression screw and ferrule. Be sure the stainless steel side of the ferrule is facing the screw. Pull the tubing with pliers to ensure the bevel or taper is completely past the ferrule.
3. Hold the ferrule with a second set of pliers and give the tubing a half twist to lock the ferrule. Cut the tubing flush with the end of the ferrule.

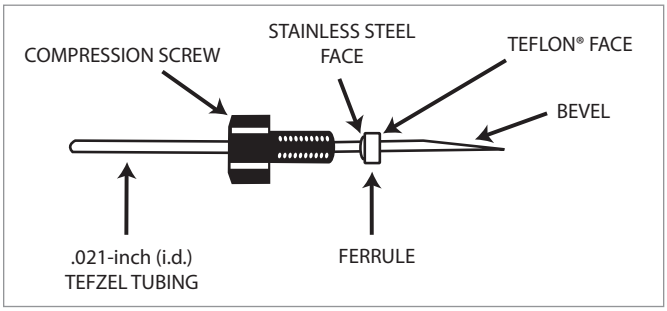

Figure 2: Ferrule and Compression Assembly (Non-Metallic Column).

c. Configuring the Column in the System

Install stainless steel or non-metallic anion columns relative to the other major components as shown in Figure 3.

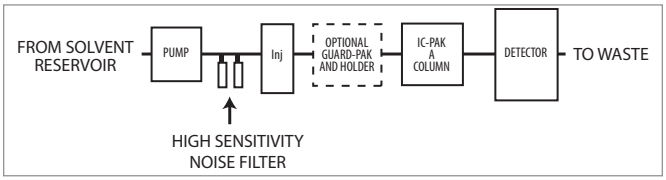

Figure 3: IC System for Anion Analysis.

Install cation columns with a guard column for detecting monovalent cations (Figure 4) or without a guard column for polyvalent cations (Figure 5).

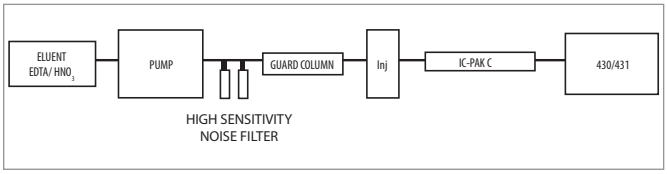

Figure 4: IC Systems for Monovalent Cation Analysis.

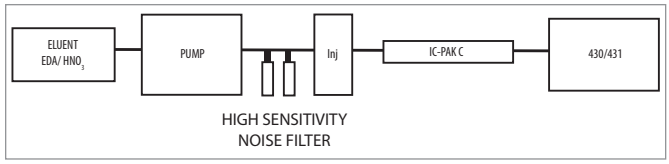

Figure 5: IC Systems for Divalent Cation Analysis.

III. ELUENT AND SAMPLE GUIDELINES

a. Preparing the Eluent Requirements

Water-miscible organic solvents, which may be used as modifiers to reduce hydrophobic interaction between sample and packing, must not exceed the concentrations listed below.

IC-Pak A Columns	12% acetonitrile in water
IC-Pak C and Guard Columns	10% acetonitrile in water

Use high performance liquid chromatography (HPLC) grade solvents that have been filtered to remove microparticulate matter greater than 0.45 µm in size. Filtering will ensure optimum long-term performance of the column. Vacuum filtration, sonication, or sparging may be used to remove dissolved gas which could affect results.

Before filtering or clarifying any solvents, flush the filters with 100 mL of the eluent. Discard the filtered eluent. Surfactants used to treat some filters may otherwise affect analysis.

An in-line filter may be used to remove particulates in the solvent and contaminants which the 0.45 µm filter does not remove.

Note: Do not use tetrahydrofuran (THF) or methanol with IC-Pak A columns.

i. Solvents Containing Salts

Changeover between an organic solvent and water containing salts should be performed gradually using 18 megohm water as the intermediate solvent. Use care when adding organic solvents to aqueous buffer solutions as salt precipitation may occur. As a general rule, do not exceed salt concentrations of 0.1 M.

ii. High pH Mobile Phase

If a high pH mobile phase (greater than 9) is to be used, a soda-lime or AscariteTM absorbing trap is recommended. Otherwise, CO_2 absorption from air contact with the reservoir may cause changes in pH and ionic strength and impair reproducibility. Sparging with helium is another recommended method to avoid this problem.

iv. Equilibration

The shipping solvent maintains equilibrium. Flush the newly attached column with fresh mobile phase before performing the first separation.

v. Preparing Eluent for Efficiency Testing

For general use and for efficiency testing (Section IV, b.), prepare the eluent specified in Table 3.

Table 3: Eluents for Efficiency Testing

Column Name	Eluent	Section
IC-Pak A IC-Pak A HC IC-Pak A HR	Lithium Borate/Gluconate or Sodium Borate/Gluconate*	Section III. b) Section III, c)
IC-Pak C (Monovalent)	EDTA/Nitric Acid	Section III, d)
IC-Pak C (Divalent)	EDA/Nitric Acid	Section III, e)

*Sodium borate gluconate may be used in place of lithium borate gluconate if desired. The chromatographic effects of the two eluents are essentially identical.

b. Lithium Borate/Gluconate Eluent

All reagents should be of the highest purity available.

To prepare Lithium Borate/Gluconate concentrate:

1. To a one liter volumetric flask add:
   34 g boric acid (H3BO3)
   23.5 mL d-gluconic acid*
   8.6 g lithium hydroxide monohydrate

* The compound d-gluconic acid is available as a 50 wt. percent aqueous solution (Aldrich or Eastman Kodak).

Note: Sep-Pak® C_{18} clean-up of this reagent is recommended. Clean-up is complete when a clear solution is obtained.

2. Add approximately 500 mL of Milli-Q® water and mix thoroughly until dissolved. Then add 250 mL glycerin.
3. Fill to the mark with Milli-Q water and mix thoroughly. Concentrate may be stored for up to six months before requiring replacement.

To prepare Lithium Borate Gluconate Eluent, conductivity 240 µS:

1. Place approximately 500 mL of Milli-Q water into a one liter volumetric flask. To this add:
   20 mL borate gluconate concentrate
   20 mL n-butanol
   120 mL acetonitrile
2. Fill the flask to the mark with Milli-Q water and mix thoroughly. Filter through a 0.22 µm GHP membrane.

c. Sodium Borate/Gluconate Eluent

All reagents should be of the highest purity available.

To prepare Sodium Borate/Gluconate concentrate:

1. To a one liter volumetric flask add:
   16 g sodium gluconate
   18 g boric acid
   25 g sodium tetraborate decahydrate
2. Add approximately 500 mL of Milli-Q water and mix thoroughly until dissolved. Then add 250 mL glycerin.
3. Fill to the mark with Milli-Q water and mix thoroughly. Concentrate may be stored for up to six months before requiring replacement.

To prepare Sodium Borate/Gluconate Eluent, conductivity 270 RS:

1. Place approximately 500 mL of Milli-Q water into a one liter volumetric flask. To this add:
   20 mL borate gluconate concentrate 20 mL n-butanol
   120 mL acetonitrile
2. Fill the flask to the mark with Milli-Q water and mix thoroughly. Filter through a 0.22 µm GHP membrane.

d. Monovalent Cation Eluent

All reagents should be of the highest purity available.

1. To approximately 500 mL of Milli-Q water, add 120 ELL of concentrated nitric acid (Ultrex® grade).

2. Accurately weigh 18.6 mg of disodium EDTA and add this to the nitric acid solution.
3. Dilute the solution to one liter with Milli-Q water.
4. Filter particulate matter from the solution with one of the following Pall Life Science membranes (refer to Section VI for part numbers):
   Aqueous replacement filters, GHP (0.45 µm, 47 mm)
   GHP filters (0.22 µm, 47 mm)
5. Degas the eluent.

e. Divalent Cation Eluent

All reagents should be of the highest purity available.

1. To prepare the stock HNO_3 solution, add 2.00 mL Ultrex HNO_3 (nitric acid) to a 100 mL volumetric flask and dilute to the mark with Milli-Q water. Store in a refrigerator for no longer than six months.
2. To a one liter volumetric flask filled with approximately 500 mL of deionized water, add 35 µL anhydrous ethylene diamine (EDA).
3. Add 3.25 mL of stock HNO3 prepared in Step 1 above.
4. Fill to the mark with Milli-Q water and mix thoroughly.
5. Filter particulate matter from eluent with GHP filters, (0.45 µm, 47 mm, refer to Section VI for part number).

f. Sample Preparation and Filtration

Filter prepared samples using a GHP membrane filter to prevent excessive pressure buildup due to particulate matter. Rinse the filter with 10 mL of 18 megohm water then filter the first few drops of sample through the filter to waste.

IV. OPERATION

a. Chromatography Guidelines

Liquid chromatography columns have a finite life which is directly related to the care and use they receive. Column life is reduced by contamination from samples and solvents, frequent solvent changeovers, and improper handling and storage.

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

Follow generally accepted procedures for quality control and methods development when using these columns.

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

I. Precautions

Maximum pressure should not exceed:

IC-Pak A, IC-Pak C and Guard Column	6.7 MPa (67 atm or 1000 psi)
IC-Pak A HR	13 MPa (130 atm or 2000 psi)
IC-Pak A HC	20 MPa (200 atm or 3000 psi)

Recommended Flow Rate:

IC-Pak A	1.2 mL/min
IC-Pak A HC	2.0 mL/min*
IC-Pak A HR	1.0 mL/min
IC-Pak C	1.2 mL/min

pH Range for all columns: 1-12

Temperature:

Normal operation	25 °C
Limits	10 °C - 50 °C

* Do not exceed a flow rate of 1.0 mL/min for the IC-Pak A HR column. Flow rates higher than this may damage the column and will void the warranty.

i. General Considerations:

- Sodium silicate leaching from glass vials will cause artifacts when analyzing anions or cations. Plastic containers should be used for all solutions.
- Dedicate columns to one eluent only, if possible. Multiple eluent changes may result in shortened column life. Dedicated columns should be used for either monovalent or divalent cation analysis.
- Filter all aqueous buffers. Do not use turbid or cloudy buffers.
- Remove aliquots from the sample container for pH readings, and then dispose of the contaminated aliquots.
- Use a pH meter to measure the pH of eluents.
- Protect columns from vibration, mechanical shock, and rapid changes in pressure, flow rate or solvent composition. Any thermal, physical or chemical shock (such as changing solvents rapidly or at high flow rates) can cause a loss of efficiency.

- When using water, treat with a Milli-Q water system capable of delivering 18 megohm water. Neither deionized water nor HPLC grade bottled water is acceptable because each may contain organic compounds which alter column selectivity.
- DO NOT inject concentrated samples directly into the eluent. Direct injection may cause precipitation of the salts in the sample. Dissolve (or dilute) samples in an appropriate volume of the eluent first. If other solvents must be used, be sure no precipitation occurs upon injection into the eluent. Always filter samples before use.
- Highly concentrated samples (greater than 100 ppm per ion) may yield poor peak shape due to overloading the column. Dilute the sample before injection. When analyzing an unknown, prepare a 1:100 dilution as a first step in optimizing the method.

b. Efficiency Testing

Waters columns are tested for adherence to Waters specifications. Slight variations in results will occur depending on:

- Equipment used
- Test sample makeup
- Equipment settings and conditions

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

The initial efficiency test is performed by:

1. Preparing the eluent (Section III, a)
2. Preparing the calibration standards (Section IV, b to Section IV, d)
3. Running the calibration standard and determining column efficiency (Section IV, e)

If problems occur during normal operation of the column, repeat the conditions for the initial efficiency test and compare the results. Differences in the results may indicate the source of the problem.

c. Anion Calibration Standard Preparation

1. To prepare individual 1000 ppm (mg/L) stock standards, refer to Table 4 for the weight of salt required. Select the highest purity salt available, weigh the specified amount, and add to a 1 liter volumetric flask.

Table 4: Salt Weight for Anion Stock Solution Preparation

Anion (expressed as compound listed)	Compound	Weight in grams
F^-	NaF	2.2101
Cl^-	NaCl	1.6485
Br^-	NaBr	1.2877
NO_3^-	$NaNO_3$	1.3708
HPO_4^{-2}	KH_2PO_4	1.4179
SO_4^{-2}	Na_2SO_4	1.4787

The following sample equation shows how these weights were determined:

1 g Cl -/L x 58.44 g NaCl/35.45 g Cl - x 1 L = 1.6485 g NaCl

2. Fill the flask to the mark with 18 megohm water. Store the stock solutions in clean plasticware for up to one month.
3. Prepare a working standard containing all seven anions by combining volumes of the stock standards as follows in a 100 mL volumetric flask:

Table 5: Stock Standard Volumes

Anion Stock Solution	Volume (µL)
Fluoride	100
Chloride	200
Nitrite	400
Bromide	400
Nitrate	400
Phosphate	600
Sulfate	400

Fill the flask to the mark with 18 megohm water. Table 6 contains the species concentrations in the working standard.

Table 6: Anion Working Standard Concentrations

Anion Stock Solution	ppm
Fluoride	1
Chloride	2
Nitrite	4
Bromide	4
Nitrate	4
Phosphate	6
Sulfate	4

d. Cation Calibration Standard Preparation

Cation standard concentrates may be purchased from most major chemical suppliers. Use the highest purity solutions available. A number of anionic species can cause the precipitation of alkali and alkaline earth metals. Consult solubility tables to avoid these species. Also, avoid hygroscopic salts.

1. To prepare individual 1000 ppm (mg/L) stock standards, refer to Table 7 for the weight of salt required. Select the highest purity salt available (atomic absorption standards if possible), weigh the specified amount, and add to a 1 liter volumetric flask.

Table 7: Salt Weight for Cation Stock Solution Preparation

Cation (expressed as compound listed)	Compound	Weight in grams
Li^+	$LiOH \bullet H_2O$	6.0476
Na^+	NaCl	2.5421
NH_4^+	NH_4Cl	2.9640
	KCl	1.9067
Mg^{+2}	$Mg(NO_3)_2 \bullet 6H_2O$	10.5466
Ca^{+2}	$Ca(NO)_2 \bullet 4H_2O$	5.8919
Sr^{+2}	$Sr(NO_3)_2 \bullet 4H_2O$	3.2377
Ba^{+2}	$BaCl_2 \bullet 2H_2O$	1.7786

The following sample equation shows how these weights were determined:

1 g K+/L x 74.553 g KCl/39.100 g K+ x 1 L = 1.9067 g KCl

2. Fill the flask to the mark with 18 megohm water.
3. Store the stock solutions in clean plasticware for up to one month.
4. Prepare a working standard for monovalent or divalent cations. Take volumes of the stock standards as follows and combine in a 100 mL volumetric flask.

For **monovalent cation** use the following volume of the appropriate stock solutions:

Lithium	10 µL
Sodium	50 µL
Ammonium	100 µL
Potassium	100 µL

For **divalent cation** use the following volume of the appropriate stock solutions:

Magnesium	200 µL
Calcium	400 µL
Strontium	600 µL
Barium	1600 µL

5. Fill the flask to the mark with 18 megohm water. Tables 8 and 9 contain the species concentrations in the monovalent and divalent cation working standards.

Table 8: Monovalent Cation Working Standard Concentrations

Monovalent Cation Stock Solution	ppm
Lithium	0.1
Sodium	0.5
Ammonium	1.0
Potassium	1.0

Table 9: Divalent Cation Working Standard Concentrations

Divalent Cation Stock Solution	ppm
Magnesium	2
Calcium	4
Strontium	6
Barium	16

e. Running the Calibration Standard and Determining Column Efficiency

Refer to Table 10 for the parameters to run the calibration standard.

Table 10: Calibration Standard Parameters

Column Name	Optimal Flow Rate (mL/min)	Test Sample	Efficiency (Plates)*	Peak for Plate Measurement
IC-Pak A	1.2	Anion	800	sulfate
IC-Pak C	1.2	Cation	1100	sodium
IC-Pak A HC	2.0	Anion	1500	sulfate
IC-Pak A HR	1.0**	Anion	2500	sulfate

* By half-height method on a low dispersion Waters ion chromatograph

** Do not exceed a flow rate of 1.0 mL/min for the IC-Pak A HR column. Flow rates higher than this may damage the column and void the warranty.

f. Calculating Efficiency

Refer to Figure 6 for the appropriate peak to measure for your anion or cation column. Use the equation in the figure below to compute column efficiency for comparison to the value in the table.

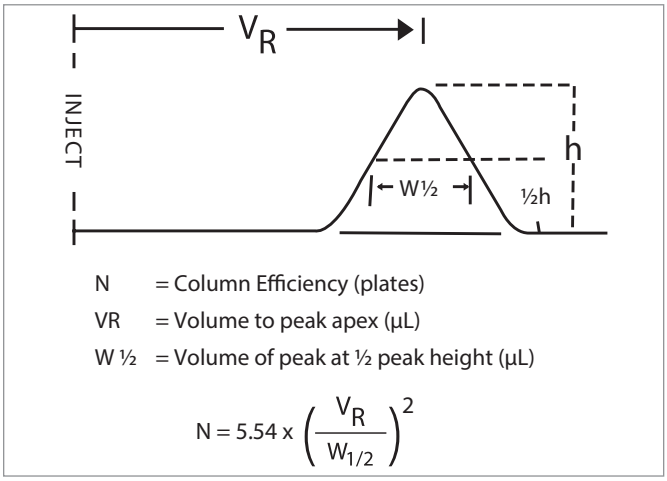

Figure 6: Half Height Method Test Calculations.

V. CARE AND MAINTENANCE

a. Troubleshooting

Table 11: Column Problems and Solutions

Symptom	Conditions	Corrective Action
Excess pressure buildup	Filters plugged with particulates. Check for injector and pump seal shedding	Back flush column at 0.1 mL/min with eluent. Always filter eluents and samples. Use an in-line precolumn filter between the pump and injector.
	Sample precipitates on column (sample no soluble in eluent)	Black flush column at 0.1 mL/min with eluent. Use a solvent that is compatible with the sample.
	Clogged tubing	Unclog or replace tubing.
Loss of resolution, broad peaks, low plate counts	Polyvalent electrolyte accumulation	**IC-Pak A** – Slowly purge (below 1 mL/min) with 100 mL of the following solution:
		In a 1 liter volumetric flask combine 50 mL eluent concentrate, 120 mL Acetonitrile, and 20 mL Butanol. Fill to the mark with 18 megohm water and filter.
		IC-Pak C – Slowly purge (below 1 mL/min) with 50 mL of 100 mM HNO_3^-
	Failing injector	Repair injector.
	Contaminated column	Slowly purge with strong solvent.
	Insufficient equilibration	Continue equilibration.
	Incorrect 0.040-inch stainless steel tubing	Install correct 0.009-inch stainless steel tubing.

b. Column Storage

Remove columns not in use for over 72 hours and store:

- Anion columns in Borate Gluconate Eluent
- Cation columns in the eluent used for analysis (either EDTA/HNO_3 or EDA/HNO_3)

Eluent may be left in the columns with the columns connected to the system for short-term storage.

When storing columns:

- DO NOT store columns in water. To prevent growth of bacteria store the columns in the eluent being used. Fill the column with eluent, 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. Eluent may be left in the columns with the columns connected to the system for short-term storage.
- Store at 15 °C to 35 °C. Freezing during storage will cause performance degradation.

c. Cation Guard Column Preparation and Regeneration

Monovalent cation analysis may require frequent changing or regeneration of the guard column due to interference from polyvalent cations. Change or regenerate the column when:

- k' changes or there is a noticeable decrease in retention time (greater than 15%)
- Fused peaks are seen
- Resolution is abnormal

Changing or regeneration of the guard column may be frequent, depending on the particular analysis. Monovalent detection using a sample matrix containing heavy metals may load the column easily.

To regenerate the column, flush the column with 200 mL 0.1 M HNO_3 at a flow rate of 1.2 mL/min.

VI. ORDERING INFORMATION

Table 12: Recommended Spare Parts

Item	Quantity	Part No.
IC-Pak A Column – 0.46 x 5.0 cm	1	WAT007355
IC-Pak A HC Column – 0.46 x 15.0 cm	1	WAT026770
IC-Pak A HR Column – 0.46 x 7.5 cm	1	WAT026765
IC-Pak C Column – 0.46 x 5.0 cm	1	WAT007354
Cation Guard Column – 0.46 x 5.0 cm	1	WAT007356
Compression Screws and Ferrules (for anion column only)	5/box	WAT025604
Sample Clarification Kit (Aqueous)	1	WAT026865
Filters:		
Solvent Clarification Kit w/pump and Filters (110 V. 60 Hz)	1	WAT085113
Acrodisc GHP 25 mm, 0.45 µm	100/box	WAT200514
Solvent Filters GHP 47 mm, 0.22 µm	100/box	186003527
In-Line Precolumn Filter	–	WAT084560
Guard-Pak™ Precolumn Module:		
Module (no cartridges)	1	WAT088141
IC-Pak Anion Guard-Pak Inserts	5/box	WAT010551
IC-Pak Anion Concentrator Inserts	5/box	WAT007358
IC-Pak Cation Concentrator Inserts	5/box	WAT010565
Sep-Pak C_{18} Cartridges	50/box	WAT051910
Sep-Pak C_{18} Plus Cartridges	50/box	WAT011191
Polypropylene Vials 12 mm x 32 mm	100/box	186004112

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