Thermo Scientific

Dionex IonPac AS16 Column

Product Manual

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Thermo SCIENTIFIC

Product Manual

for

Thermo Scientific Dionex IonPac AG16 Guard Column
(4 × 50 mm, P/N 055377)
(2 × 50 mm, P/N 055379)

Thermo Scientific Dionex IonPac AG16 Capillary Guard Column
(0.4 × 50 mm, P/N 082316)

Thermo Scientific Dionex IonPac AS16 Analytical Column
(4 × 250 mm, P/N 055376)
(2 × 250 mm, P/N 055378)

Thermo Scientific Dionex IonPac AS16 Capillary Column
(0.4 × 250 mm, P/N 082315)

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Revision 07, December, 2012, Rebranded and formatted for Thermo Fisher Scientific. Added information for capillary columns.

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Safety and special notices include the following:

SAFETY	***Indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.***
WARNING	***Indicates a potentially hazardous situation which, if not avoided, could result in damage to equipment.***
CAUTION	***Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. Also used to identify a situation or practice that may seriously damage the instrument, but will not cause injury.***
NOTE	***Indicates information of general interest.***
IMPORTANT	***Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.***
Tip	***Highlights helpful information that can make a task easier.***

Contents

1. Introduction

The Thermo Scientific™ Dionex™ IonPac™ AS16 Analytical and Capillary Columns are high capacity, hydroxide selective anion exchange columns designed for the isocratic separation of polarizable anions including iodide, thiocyanate, thiosulfate, and perchlorate in a variety of sample matrices. The Dionex IonPac™ AS16 column (4 × 250 mm) has a capacity of approximately 170 µeq/column which allows large loop injections without column overloading. Under isocratic conditions, the polarizable anions can easily be separated in approximately 20 minutes. Trace concentrations of perchlorate in drinking water, surface water and ground water matrices can easily be determined using a large loop injection. With 50 mM sodium hydroxide eluent, perchlorate can be determined in approximately 10 minutes. The Dionex IonPac AS16 column is available in 0.4 × 250 mm, 2 × 250 mm and 4 × 250 mm formats, thus supporting flow rates from 0.010 mL/min to 3.0 mL/min. The Dionex IonPac AS16 column is stable between pH 0 and 14 and is compatible with eluents containing 0-100% organic solvents. The Dionex IonPac AG16 guard column is packed with a microporous resin with a lower capacity. The microporous resin ensures optimum long term performance of the guard column.

The Dionex IonPac AS16 Capillary Column (0.4 × 250 mm) is packed with the same material as the equivalent standard bore version (producing the same performance as a 4 mm column), but requires only one-hundredth (1/100) the eluent flow rate. The capillary format has the advantage of less eluent consumption, providing reduced costs.

Table 1 Dionex IonPac AS16/AG16 Packing Specifications

Column	Particle Diameter µm	Substrate X-linking %	Column Capacity µeq/column	Functional Group	Hydrophobicity
Dionex IonPac AS16* Column 4 x 250 mm	9.0	55	170	Alkanol quaternary ammonium	Ultra-low
Dionex IonPac AG16** Guard Column 4 x 50 mm	13.0	55	3.5	Alkanol quaternary ammonium	Ultra-low
Dionex IonPac AS16* Column 2 x 250 mm	9.0	55	42.5	Alkanol quaternary ammonium	Ultra-low
Dionex IonPac AG16** Guard Column 2 x 50 mm	13.0	55	0.875	Alkanol quaternary ammonium	Ultra-low
Dionex IonPac AS16* Capillary Column 0.4 x 250 mm	9.0	55	1.7	Alkanol quaternary ammonium	Ultra-low
Dionex IonPac AG16** Capillary Guard Column 0.4 x 50 mm	13.0	55	0.035	Alkanol quaternary ammonium	Ultra-low

* macroporous (2000 Å) divinylbenzene/ethylvinylbenzene polymer
** microporous divinylbenzene/ethylvinylbenzene polymer

Table 2 Dionex IonPac AS16/AG16 Column Operating Parameters

Column	Typical Backpressure psi (MPa)	Standard Flow Rate mL/min	Maximum Flow Rate mL/min
Dionex IonPac AS16 4 mm Analytical column	$\leq$ 1400 (9.64)	1.0	3.0
Dionex IonPac AG16 4 mm Guard column	$\leq$ 150 (1.03)	1.0	3.0
Dionex IonPac AS16 + AG16 4 mm columns	$\leq$ 1550 (10.67)	1.0	3.0
Dionex IonPac AS16 2 mm Analytical column	$\leq$ 1400 (9.64)	0.25	0.75
Dionex IonPac AG16 2 mm Guard column	$\leq$ 150 (1.03)	0.25	0.75
Dionex IonPac AS16 + AG16 2 mm columns	$\leq$ 1550 (10.67)	0.25	0.75
Dionex IonPac AS16 0.4 mm Capillary column	$\leq$ 1400 (9.64)	0.01	0.03
Dionex IonPac AG16 0.4 mm Capillary Guard column	$\leq$ 150 (1.03)	0.01	0.03
Dionex IonPac AS16 + AG16 0.4 mm columns	$\leq$ 1550 (10.67)	0.01	0.03

NOTE

For assistance, contact Technical Support for Dionex Products. In the U.S., call 1-800-346-6390. Outside the U.S., call the nearest Thermo Fisher Scientific office.

2. Ion Chromatography Systems

The proper configuration of an Ion Chromatography System (ICS) in 2 mm or 4 mm format is based on the ratio of the 2 mm to 4 mm column cross-sectional area (a factor of 1/4). The selected format will affect the type of pump recommended. A gradient pump is designed to blend and pump isocratic, linear, or gradient mixtures of up to four mobile phase components at precisely controlled flow rates. An isocratic pump is for applications not requiring gradient and multi-eluent proportioning capabilities. Both are offered in either standard bore or microbore options.

- For an ICS in 2 mm format, a microbore isocratic pump, standard bore isocratic pump, microbore gradient pump, or standard bore gradient pump is recommended.
- For an ICS in 4 mm format, a standard bore isocratic pump or standard bore gradient pump is recommended.
- For an ICS in 0.4 mm format, a Capillary IC system such as the Thermo Scientific Dionex ICS-5000 system is recommended.

See Appendix C, "System Configuration" for specific recommended settings and parts including pumps, eluent flow rate, Thermo Scientific Dionex Self-Regenerating Suppressor (Dionex SRS), Thermo Scientific Dionex MicroMembrane Suppressor (Dionex MMS), Thermo Scientific Dionex Capillary Electrolytic Suppressor (Dionex CES), injection loop, system void volume, detectors, and tubing back pressure.

NOTE

Do not operate suppressors over 40 °C. If application requires a higher temperature, place the suppressor outside of the chromatographic oven. Use of a Dionex EG with a Dionex EGC III KOH (P/N 074532 or 072076) cartridge for gradient applications is highly recommended for minimum baseline change when performing eluent step changes or gradients.

3. Installation

3.1 Column Start-Up

The column is shipped using 100 mM Sodium Borate as the storage solution. Prepare the eluent shown on the Quality Assurance Report (QAR), install the column in the chromatography module and direct the column effluent to waste for 30 minutes, and then connect to the suppressor. Test the column performance under the conditions described in the QAR. Continue making injections of the test standard until consecutive injections of the standard give reproducible retention times. Equilibration is complete when consecutive injections of the standard give reproducible retention times.

If peak efficiencies or resolution on the capillary column are poorer than the QAR, see Sections 3.13 "Installation of the Capillary Column" and 6.3.5 "Poor Efficiency using Capillary Columns" for information regarding proper connections.

3.2 Column Storage

For short-term storage (< 1 week), use Eluent, for long-term storage (> 1 week), use 100 mM Sodium Borate for the column storage solution. Flush the column for a minimum of 10 minutes with the storage solution. Cap both ends securely, using the plugs supplied with the column.

3.3 System Requirements

3.3.1 System Requirements for 0.4 mm Operation

The Dionex IonPac AS16 0.4 mm Capillary Guard and Capillary Columns are designed to be run on a capillary ion chromatograph system equipped with suppressed conductivity detection. It is recommended to run the capillary column on a capillary system such as the Dionex ICS-5000 capillary system for best performance.

3.3.2 System Requirements for 2 mm Operation

The Dionex IonPac AS16 2 mm Guard and Analytical Columns are designed to be run on Dionex Ion Chromatographs equipped with suppressed conductivity detection. Isocratic analyses at flow rates of 0.5 mL/min or greater can be performed on a pump with standard (1/8" pistons) pump heads. For isocratic analyses at flow rates below 0.5 mL/min and gradient analyses, a microbore pump (1/16" pistons) must be employed.

3.3.3 System Requirements for 4 mm Operation

The Dionex IonPac AS16 4 mm Guard and Analytical Columns are designed to be run on any Dionex Ion Chromatograph equipped with suppressed conductivity detection. Gradient methods and methods requiring solvent containing eluents should be performed on a system having a standard 1/8" pump heads. Isocratic analysis can also be performed on a standard bore pump.

3.3.4 System Void Volume

When using 2 mm columns, it is particularly important to minimize system void volume. The system void volume should be scaled down to at least 1/4 of the system volume in a standard 4 mm system. For best performance, all of the tubing installed between the injection valve and detector should be 0.005" (P/N 044221) i.d. PEEK tubing. Peek tubing with an i.d. of 0.010" (P/N 042260) may be used but peak efficiency will be compromised which may also result in decreased peak resolution. Minimize the lengths of all connecting tubing and remove all unnecessary switching valves and couplers.

3.4 The Sample Concentrator

The function of a concentrator column in these applications is to strip ions from a measured volume of a relatively clean aqueous sample matrix. This process “concentrates” the desired analyte species onto the concentrator column, lowering detection limits by 2- 5 orders of magnitude. The concentrator column is used in lieu of the sample loop.

The Dionex IonPac Trace Anion Concentrator Low Pressure Column (Dionex IonPac TAC-LP1, P/N 046026), the Dionex IonPac Trace Anion Concentrator Ultra Low Pressure Column (Dionex IonPac TAC-ULP1, P/N 061400), the Dionex IonPac Ultra Trace Anion Concentrator Low Pressure Column (Dionex IonPac UTAC-LP1, P/N 063079) or (Dionex IonPac UTAC-LP2, P/N 079917), the Dionex IonPac Ultra Trace Anion Concentrator Ultra Low Pressure Column (Dionex IonPac UTAC-ULP1, P/N 063475) or (Dionex IonPac UTAC-ULP2, P/N 079918), the Dionex IonPac Ultra Trace Anion Concentrator Extremely Low Pressure Column (Dionex IonPac UTAC-XLP1, P/N 063459) or (Dionex IonPac UTAC-XLP2, P/N 072781), or the Dionex IonPac AG16 Guard Column can be used for trace anion concentration work with the 2 mm and 4 mm Dionex IonPac AS16 columns. For trace anion concentration work with the 0.4 mm Dionex IonPac AS16 column, use the Dionex IonSwift MAC-100 Concentrator Column.

Pump the sample onto the concentrator column in the OPPOSITE direction of the eluent flow. When using concentration techniques, do not overload the concentrator column by concentrating an excessive amount of sample. Concentrating an excessive amount of sample can result in inaccurate results being obtained. It is possible during the concentration step for the polyvalent anions such as phosphate and sulfate to elute the weakly retained anions such as fluoride and acetate off the concentrator column. For more detailed information on sample concentration techniques for high sensitivity work and a detailed discussion of anion concentration techniques refer to:

- Section 3, “Operation,” of the Dionex IonPac Trace Anion Concentrator Low Pressure (Dionex IonPac TAC-LP1) and Dionex IonPac Ultra Low Pressure (Dionex IonPac TAC-ULP1) Column Product Manual (Document No. 034972).
- Section 3, “Operation,” of the Dionex IonPac Ultra Trace Anion Concentrator Low Pressure (Dionex IonPac UTAC-LP1), Dionex IonPac Ultra Low Pressure (Dionex IonPac UTAC-ULP1), and Dionex IonPac Extremely Low Pressure (Dionex IonPac UTAC-XLP1) Column Product Manual (Document No. 065091.)
- Section 4, “Operation,” of the Dionex IonPac Ultra Trace Anion Concentrator 2 Low Pressure (Dionex IonPac UTAC-LP2), Dionex IonPac Ultra Low Pressure (Dionex IonPac UTAC-ULP2), and Dionex IonPac Extremely Low Pressure (Dionex IonPac UTAC-XLP2) Column Product Manual (Document No. 065376.)
- Section 3, “Operation” of the IonSwift Monolith Anion Concentrator (MAC) Column Product Manual (Document No. 065387).

CAUTION

Dionex IonPac Trace Anion Concentrator (Dionex IonPac TAC-2) Column (P/N 043101) is not optimized for use with hydroxide eluents and should not be used for concentrator work with the Dionex IonPac AS16 column. Use the Dionex IonPac TAC-LP1, TAC-ULP1, UTAC-LP1, UTAC-ULP1, UTAC-XLP1, the Dionex IonPac AG16 4 mm guard column, or the Dionex IonPac AG16 2 mm guard column.

3.5 The Injection Loop

3.5.1 The 0.4 mm System Injection Loop, 0.4 µL Internal Loop

For most applications on a 0.4 mm capillary system, a 0.4 µL injection loop is sufficient. Generally, do not inject more than 0.5 nanomoles of any one analyte into a 0.4 mm capillary column. Injecting larger numbers of moles of a sample can result in overloading the column, which can affect the detection linearity. For samples containing low concentrations of analytes, larger injection loops can be used to increase sensitivity.

3.5.2 The 2 mm System Injection Loop, 2 - 15 µL

For most applications on a 2 mm analytical system, a 2 - 15 µL injection loop is sufficient. Generally, you should not inject more than 10 nanomoles of any one analyte onto a 2 mm analytical column. Injecting larger number of moles of a sample can result in overloading the column which can affect the detection linearity. For low concentrations of analytes, larger injection loops can be used to increase sensitivity. The Dionex IonPac AS16 2 mm requires a microbore HPLC system configuration. Install an injection loop one-fourth or less (<15 µL) of the loop volume used with a 4 mm analytical system

3.5.3 The 4 mm System Injection Loop, 10 - 50 µL

For most applications on a 4 mm analytical system, a 10 - 50 µL injection loop is sufficient. Generally, you should not inject more than 40 nanomoles of any one analyte onto the 4 mm analytical column. Injecting larger number of moles of a sample can result in overloading the column which can affect the detection linearity. For low concentrations of analytes, larger injection loops can be used to increase sensitivity.

3.6 The Dionex IonPac AG16 Guard Column

A Dionex IonPac AG16 Guard/Capillary Guard Column is normally used with the Dionex IonPac AS16 Analytical/Capillary Column. Retention times will increase by approximately 1.5% when a guard/capillary guard column is placed in-line prior to the analytical/capillary column. A guard/capillary guard column is placed prior to the analytical/capillary column to prevent sample contaminants from eluting onto the analytical/capillary column. It is easier to clean or replace a guard/capillary guard column than it is an analytical/capillary column. Replacing the Dionex IonPac AG16 Guard/Capillary Guard Column at the first sign of peak efficiency loss or decreased retention time will prolong the life of the Dionex IonPac AS16 Analytical/Capillary Column.

3.7 Eluent Storage

Dionex IonPac AS16 columns are designed to be used with hydroxide eluent systems. Storage under a helium atmosphere ensures contamination free operation and proper pump performance. (Nitrogen can be used if eluents do not contain solvents).

CAUTION

Do Not Use Glass bottles for either stock solution bottles or eluent bottles! Base slowly dissolves glass, releasing impurities that adversely affect the Dionex IonPac AS16 column performance.

3.8 Installing the Thermo Scientific Dionex CR-ATC Continuously Regenerated Anion Trap Column for Use with Dionex EGC III KOH Cartridge

For Dionex IonPac AS16 applications using the Dionex EGC KOH cartridge, a Dionex CR-ATC Continuously Regenerated Trap Column (P/N 060477 or 072078) should be installed at the Dionex EGC eluent outlet to remove trace level anionic contaminants from the carrier deionized water. See the Dionex CR-TC Product Manual (Document No. 031910) for instructions. As an alternative for 2 mm and 4 mm columns, the Dionex ATC-HC Trap Column (P/N 059604) should be installed between the pump outlet and the inlet of the Dionex EluGen Cartridge to remove anionic contaminants from the carrier deionized water. See the Dionex IonPac ATC-HC Product Manual (Document No. 032697) for instructions.

If the lower capacity Dionex IonPac ATC-3 Trap Column (P/N 059660 and 079932) is used with 2 mm and 4 mm columns, it should be installed between the gradient pump and the injection valve to remove anionic contaminants from the eluent. The Dionex IonPac ATC-3 column is used when performing sodium hydroxide gradient anion exchange applications using manually prepared eluents. See the Dionex IonPac ATC-3 Product Manual (Document No. 032697) for instructions.

The Dionex IonPac ATC-HC (P/N 059604) and Dionex IonPac ATC-3 Trap Columns will require off-line regeneration. To use the Dionex IonPac ATC-HC or Dionex IonPac ATC- 3 Anion Trap Columns, refer to the Product Manuals.

3.9 Dionex Anion Self-Regenerating Suppressor Requirements

A Dionex Anion Self-Regenerating Suppressor should be used for applications that require suppressed conductivity detection. It is compatible with solvent containing eluents and aqueous ionic eluents of all concentrations with which the systems and columns are compatible. Aqueous ionic eluents can be used in all Thermo Scientific Dionex ASRS 300 Anion Self-Regenerating Suppressor modes of operation.

NOTE

Solvent containing eluents should be used in the AutoSuppression External Water Mode.

For Dionex IonPac AS16 4 mm Analytical Column, use a Dionex ASRS™ 300 suppressor (4 mm, P/N 061561).

For Dionex IonPac AS16 2 mm Analytical Column, use a Dionex ASRS 300 suppressor (2 mm, P/N 061562).

For Dionex IonPac AS16 0.4 mm Capillary Column, use a Dionex ACES 300 suppressor (0.4 mm, P/N 072052).

For detailed information on the operation of the Dionex Anion Self-Regenerating Suppressor, see Document No. 031956, the "Product Manual for the Dionex Anion Self-Regenerating Suppressor 300"

For detailed information on the operation of the Dionex Anion Capillary Electrolytic Suppressor 300, see Document No. 065386, the "Product Manual for the Dionex Anion Capillary Electrolytic Suppressor 300, the Dionex ACES 300".

3.10 Dionex Anion MicroMembrane Suppressor Requirements

A Thermo Scientific Dionex AMMS 300 may be used instead of a Dionex ASRS 300 Anion Self-Regenerating Suppressor (4 mm) for applications that require suppressed conductivity detection. Use a Dionex AMMS™ 300 (4 mm) (P/N 064558) with the Dionex IonPac AS16 4 mm Analytical Column. It is compatible with all solvents and concentrations with which the systems and columns are compatible. For 2 mm operation, use the Dionex AMMS 300 2 mm (P/N 064559).

For detailed information on the operation of the Dionex Anion MicroMembrane Suppressor, see Document No. 031727, the "Product Manual for the Anion MicroMembrane Suppressor 300 the Dionex AMMS 300.

3.11 Using Displacement Chemical Regeneration (DCR) with the Chemical Suppression Mode

The Dionex Displacement Chemical Regeneration (Dionex DCR) Mode is recommended for chemical suppression using sulfuric acid and the Dionex Anion MicroMembrane Suppressor (Dionex AMMS 300). See the Dionex DCR kit manual, Document P/N 031664, for details.

SAFETY

Use proper safety precautions in handling acids and bases.

3.12 Using the Dionex EGC-KOH with Dionex IonPac AS16 Column

The Dionex IonPac AS16 column is recommended for use with Thermo Scientific Dionex ICS-2100, or Dionex ICS-5000 IC Systems equipped with a Dionex Eluent Generator. The Dionex Eluent Generator is used to automatically produce potassium hydroxide gradients from deionized water. The Dionex IonPac AS16 column can be used with older Dionex IC Systems equipped with an Eluent Generator or a Dionex RFC-30 Reagent Free Controller. Please refer to the Thermo Scientific Dionex EG40 Eluent Generator manual, Document No. 031373, for information on the operation of the Dionex EG40 Eluent Generator. Please refer to the Thermo Scientific Dionex EG50 Product Manual, Document No. 031908, for information on the operation of the Dionex EG50 Eluent Generator.

3.13 Installation of the Capillary Column

1. Before installing the new separator column, cut off the column label and slide it into the holder on the front of the cartridge (see Figure 6).
2. For reference, Figure 1 shows the column cartridge after installation of both a capillary guard column and a capillary separator column. Figure 2 shows the column cartridge after installation of only a capillary separator column.

Figure 1 Separator and Guard Columns Installed in Column Cartridge

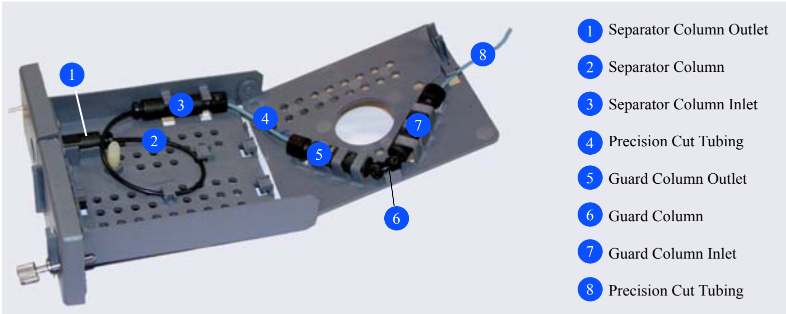
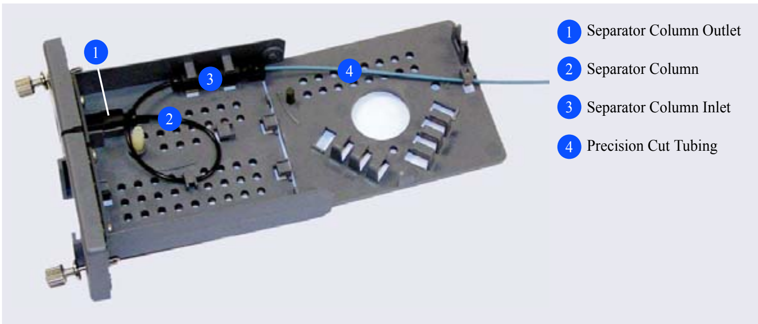


Figure 2 Separator Column Only Installed in Column Cartridge



3. Locate the Dionex IC Cube Tubing Kit (P/N 072186) that is shipped with the Dionex IC Cube. The tubing kit includes the following items:

Table 3 Contents of the Dionex IC Cube Tubing Kit (P/N 072186)

Part	Length / Quantity	Part Number	Used To Connect
Precision cut 0.062 mm (0.0025-in) ID PEEK tubing, blue	65 mm (2.56 in)	072188	50 mm guard column outlet to 250 mm separator column inlet
Precision cut 0.062 mm (0.0025-in) ID PEEK tubing, blue, labeled VALVE PORT 3	115 mm (4.53 in)	072189	Guard column inlet to injection valve
Precision cut 0.062 mm (0.0025-in) ID PEEK tubing, blue	75 mm (2.93 in)	074603	35 mm guard column outlet to 150 mm separator column inlet
Precision cut 0.062 mm (0.0025-in) ID PEEK tubing, blue, labeled VALVE PORT 3	210 mm (8.27 in)	072187	Separator column inlet to injection valve (if a guard column is not present)
0.25 mm (0.010-in) ID PEEK tubing, black	610 mm (24 in)	042690	EG degas cartridge REGEN OUT to waste (if an EG is not present)
Fitting bolt, 10-32 hex double-cone (smaller), black	3	072949	Connect precision cut 0.062 mm (0.0025-in) ID PEEK tubing
Fitting bolt, 10-32 double-cone (larger), black	1	043275	Connect 0.25 mm (0.010-in) ID PEEK tubing (black)
Ferrule fitting, 10-32 double-cone, tan	4	043276	Use with both sizes of fitting bolts

4. Refer to the following figures for the precision cut tubing required for your configuration:

Figure 3 Tubing Connections for 250 mm Separator Column and 50 mm Guard Column

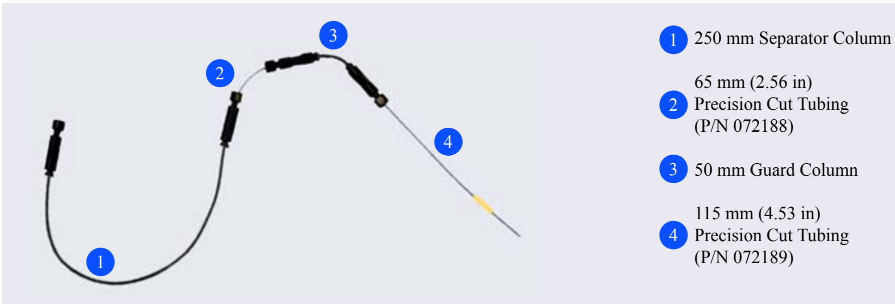
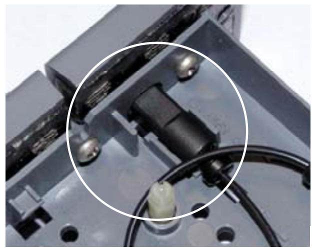


Figure 4 Tubing Connections for Separator Column Only

5. Lift up the lid of the column cartridge to open it.
6. Remove the fitting plug from the outlet fitting on the separator column. Orient the fitting with a flat side up (see Figure 5) and push the fitting into the opening at the front of the column cartridge until it stops.

Figure 5 Column Outlet Fitting Installed in Column Cartridge



7. Coil the separator column tubing inside the cartridge as shown in Figure 1 or Figure 2. Secure the column tubing and the inlet fitting in the clips on the column cartridge.
8. Secure the inlet and outlet fittings on the guard column (if used) in the column clips on the lid of the column cartridge.
9. Route the guard column inlet tubing (if used) or the separator column inlet tubing through the clip on the top edge of the column cartridge lid.
10. Close the lid (you should hear a click) and route the tubing into the slot on the front of the column cartridge (see Figure 6).

NOTE

If the columns are installed correctly, the cartridge lid snaps closed easily. If the lid does not close easily, do not force it. Open the lid and verify that the columns and tubing are installed correctly and secured in the clips.

Figure 6 Column Cartridge Closed

1. Separator Column Outlet
2. Column Inlet Tubing

4. Operation

4.1 General Operating Conditions

Sample Volume:	0.4 mm:	0.4 µL loop
	2 mm:	2.5 µL Loop + 0.8 µL Injection valve dead volume
	4 mm:	10 µL Loop + 0.8 µL Injection valve dead volume
Column:	0.4 mm:	Dionex IonPac AS16 0.4 mm Capillary Column + Dionex IonPac AG16 0.4 mm Capillary Guard column
	2 mm:	Dionex IonPac AS16 2 mm Analytical Column + Dionex IonPac AG16 2 mm Guard Column
	4 mm:	Dionex IonPac AS16 4 mm Analytical Column + Dionex IonPac AG16 4 mm Guard Column
Eluent:	35 mM KOH (for test chromatogram)	
Eluent Source:	Dionex EGC III KOH cartridge	
Eluent Flow Rate:	0.4 mm:	0.010 mL/min
	2 mm:	0.25 mL/min
	4 mm:	1.0 mL/min
SRS Suppressor:	Dionex Anion Self-Regenerating Suppressor, Dionex ASRS 300 (2 or 4 mm)	
	Dionex Anion Capillary Electrolytic Suppression, ACES 300 AutoSuppression Recycle Mode	
or MMS Suppressor:	Dionex Anion MicroMembrane Suppressor, Dionex AMMS 300 (2 mm or 4 mm)	
MMS Regenerant:	50 mN H_2SO_4	

Expected Background Conductivity:	< 3 µS
Long-term Storage Solution (> 1 week):	100 mM Sodium Borate
Short-term Storage Solution (< 1 week):	Eluent

4.2 Dionex IonPac AS16 Column Operation Precautions

CAUTION

- ***Filter and Degas Eluents***
- ***Filter Samples***
- ***Eluent pH between 0 and 14***
- ***Sample pH between 0 and 14***
- ***0.75 mL/min Maximum Flow Rate for 2 mm Columns***
- ***3.0 mL/min Maximum Flow Rate for 4 mm Columns***
- ***0.030 mL/min Maximum Flow Rate for 0.4 mm Columns***
- ***Maximum Operating Pressure = 4,000 psi (27.57 MPa)***

4.3 Chemical Purity Requirements

Obtaining reliable, consistent and accurate results requires eluents that are free of ionic impurities. Chemicals, solvents and deionized water used to prepare eluents must be of the highest purity available. Low trace impurities and low particle levels in eluents also help to protect your ion exchange columns and system components. Thermo Fisher Scientific cannot guarantee proper column performance when the quality of the chemicals, solvents and water used to prepare eluents has been compromised.

4.3.1 Inorganic Chemicals

Reagent Grade inorganic chemicals should always be used to prepare ionic eluents. Whenever possible, inorganic chemicals that meet or surpass the latest American Chemical Society standard for purity should be used. These inorganic chemicals will detail the purity by having an actual lot analysis on each label.

4.3.2 Deionized Water

The deionized water used to prepare eluents should be Type I Reagent Grade Water with a specific resistance of 18.2 megohm-cm. The deionized water should be free of ionized impurities, organics, microorganisms and particulate matter larger than 0.2 µm. Bottled HPLC-Grade Water (with the exception of Burdick & Jackson) should not be used since most bottled water contains an unacceptable level of ionic impurities.

4.3.3 Solvents

Solvents can be added to the ionic eluents used with Dionex IonPac AS16 columns to modify the ion exchange process or improve sample solubility. The solvents used must be free of ionic impurities. However, since most manufacturers of solvents do not test for ionic impurities, it is important that the highest grade of solvents available be used. Currently, several manufacturers are making ultrahigh purity solvents that are compatible for HPLC and spectrophotometric applications. These ultrahigh purity solvents will usually ensure that your chromatography is not affected by ionic impurities in the solvent. Currently at Thermo Fisher Scientific, we have obtained consistent results using Optima™ Solvents by Fisher Scientific.

When using a solvent in an ionic eluent, column generated back pressures will depend on the solvent used, concentration of the solvent, the ionic strength of the eluent and the flow rate used. The column back pressure will vary as the composition of water-methanol and water-acetonitrile mixture varies. The practical back pressure limit for the Dionex IonPac AS16 columns is 4,000 psi (27.57 MPa).

The Dionex IonPac AS16 column can withstand common HPLC solvents in a concentration range of 0 - 100%. Solvents and water should be premixed in concentrations which allow proper mixing by the gradient pump and to minimize outgassing. Ensure that all of the inorganic chemicals are soluble in the highest solvent concentration to be used during the analysis.

Table 4 HPLC Solvents for Use with Dionex IonPac AS16 Columns

Solvent	Maximum Operating Concentration
Acetonitrile	100 %
Methanol	100 %
2-Propanol	100 %
Tetrahydrofuran	20 %*

* Higher concentrations may only be used for limited duration applications such as column clean-up at pressures < 2000 psi.

CAUTION

The Dionex ASRS 300 and the Dionex ACES 300 must be operated in the AutoSuppression External Water Mode when using eluents containing solvents. Do not use > 40% solvent with the Dionex ASRS 300 and the Dionex ACES 300 in the electrolytic mode (power on).

4.4 Making Eluents that Contain Solvents

When mixing solvents with water, remember to mix the solvent with the water on a volume to volume basis. If a procedure requires an eluent of 90% acetonitrile, prepare the eluent by adding 900 mL of acetonitrile to an eluent reservoir. Then add 100 mL of deionized water or eluent concentrate to the acetonitrile in the reservoir. Using this procedure to mix solvents with water will ensure that a consistent true volume/volume eluent is obtained. Premixing water with solvent will minimize the possibility of outgassing.

NOTE

When purging or degassing eluents containing solvents, do not purge or degas the eluent excessively since it is possible that a volatile solvent can be "boiled" off from the solution.

Always degas and store all eluents in plastic eluent bottles pressurized with helium. Only helium can be used to purge and degas ionic eluents containing solvents, since nitrogen is soluble in solvent containing eluents.

Acetonitrile (ACN) hydrolyzes to ammonia and acetate when left exposed to basic solutions. To prevent eluent contamination from acetonitrile hydrolysis, always add acetonitrile to basic aqueous eluents by proportioning the acetonitrile into the basic eluent with the gradient pump. Keep the acetonitrile in a separate eluent bottle containing only acetonitrile and water.

SAFETY

Never add the acetonitrile directly to the basic carbonate or hydroxide eluent bottle.

4.5 Eluent Preparation

4.5.1 Sodium Hydroxide Eluent Concentration

Weight Method

When formulating eluents from 50% sodium hydroxide, it is recommended to weigh out the required amount of 50% sodium hydroxide. Use the assayed concentration value from the sodium hydroxide bottle.

Example: To make 1 L of 35 mM NaOH use 2.8 g of 50% sodium hydroxide:

$$\text{For 35 mM:} \quad \frac{0.035 \text{ mole/L} \times 40.01 \text{ g/mole}}{50\%} = 2.8 \text{ g diluted to 1 L}$$

Volume Method

Although it is more difficult to make precise carbonate-free eluents for gradient analysis volumetrically, you may choose to use the following formula to determine the correct volume of 50% sodium hydroxide to be diluted.

$g = dvr$

Where: g = weight of sodium hydroxide required (g)
d = density of the concentrated solution (g/mL)
v = volume of the 50% sodium hydroxide required (mL)
r = % purity of the concentrated solution

Example: To make 1 L of 35 mM NaOH use 1.83 mL of 50% sodium hydroxide:

$$\text{For 35 mM:} \quad \frac{0.035 \text{ mole/L} \times 40.01 \text{ g/mole}}{50\% \times 1.53^{*} \text{ g/mL}} = 1.83 \text{ mL diluted to 1 L}$$

* This density applies to 50% NaOH. If the concentration of the NaOH solution is significantly different from 50%, the upper (weight method) calculation should be used instead.

4.5.2 Sodium Hydroxide Eluents

Dilute the amount of 50% (w/w) NaOH Reagent specified in Table 5, "Dilution of 50% (w/w) NaOH to Make Standard Dionex IonPac AS16 Eluents" with degassed, deionized water (having a specific resistance of 18.2 megohm-cm) to a final volume of 1,000 mL using a volumetric flask. Avoid the introduction of carbon dioxide from the air into the aliquot of 50% (w/w) NaOH bottle or the deionized water being used to make the eluent. Do not shake the 50% (w/w) NaOH bottle or pipette the required aliquot from the top of the solution where sodium carbonate may have formed.

Table 5 Dilution of 50% (w/w) NaOH to Make Standard Dionex IonPac AS16 Eluents

50% (w/w) NaOH g (mL)	Concentration of NaOH Eluent (mM)
0.40 (0.262)	5
2.80 (1.83)	35
8.00 (5.23)	100
40.00 (26.15)	500

4.6 Regenerant Preparation for the Dionex AMMS

The Dionex Anion MicroMembrane Suppressor (Dionex AMMS 300) requires the use of a regenerant solution. If you are using the Dionex AMMS 300 instead of the Dionex ASRS 300 Anion Self-Regenerating Suppressor see Document No. 031727, the "Product Manual for the Anion MicroMembrane Suppressor, (Dionex AMMS) 300."

5. Example Applications

5.1 Recommendations for Optimum System Performance

The chromatograms in this section were obtained using columns that reproduced the Quality Assurance Report on an optimized Ion Chromatograph. Different systems will differ slightly in performance due to slight differences in column sets, system void volumes, liquid sweep-out times of different components and laboratory temperatures.

The Dionex IonPac AS16 column is designed to perform analyses of large numbers of anions of varying valencies through gradient elution. In any type of gradient elution system it is important to use eluents that produce a minimum shift in baseline conductivity during the run, as well as a fast equilibration time from one run to the next. Because potassium hydroxide is converted to water in the suppressor, it is the best choice for an eluent. As long as the capacity of the suppressor is not exceeded, the eluent hydroxide concentration has little effect on background conductivity. For example, a gradient run could begin at a few mM KOH and end at 100 mM KOH, with only a resulting 1 to 3 µS total baseline change.

Ensure that your system is properly configured. It is very important that applications run on 2 mm columns utilize the proper pump configuration (see Section 2, "Ion Chromatography Systems") and have all system void volumes minimized. Fluctuations in operating temperature can affect the retention time and resolution of analytes and should be controlled.

Ensure that adequate equilibration time is allowed between runs. If downward shift in baseline is observed during the isocratic section of the chromatogram, increase the equilibration time.

Ensure that all of the eluents have been made from high purity reagents and deionized water. All water used in the preparation of eluents should be degassed, deionized water. For chemical purity requirements see Section 4.3, "Chemical Purity Requirements."

Use a guard column to protect the analytical column. If column performance deteriorates and it is determined that the guard and analytical columns has been fouled, refer to the column cleanup protocols in Column Care in the Appendix B.

You can increase the sensitivity of your system by using sample concentration techniques (see Section 3.4, "The Sample Concentrator").

NOTE

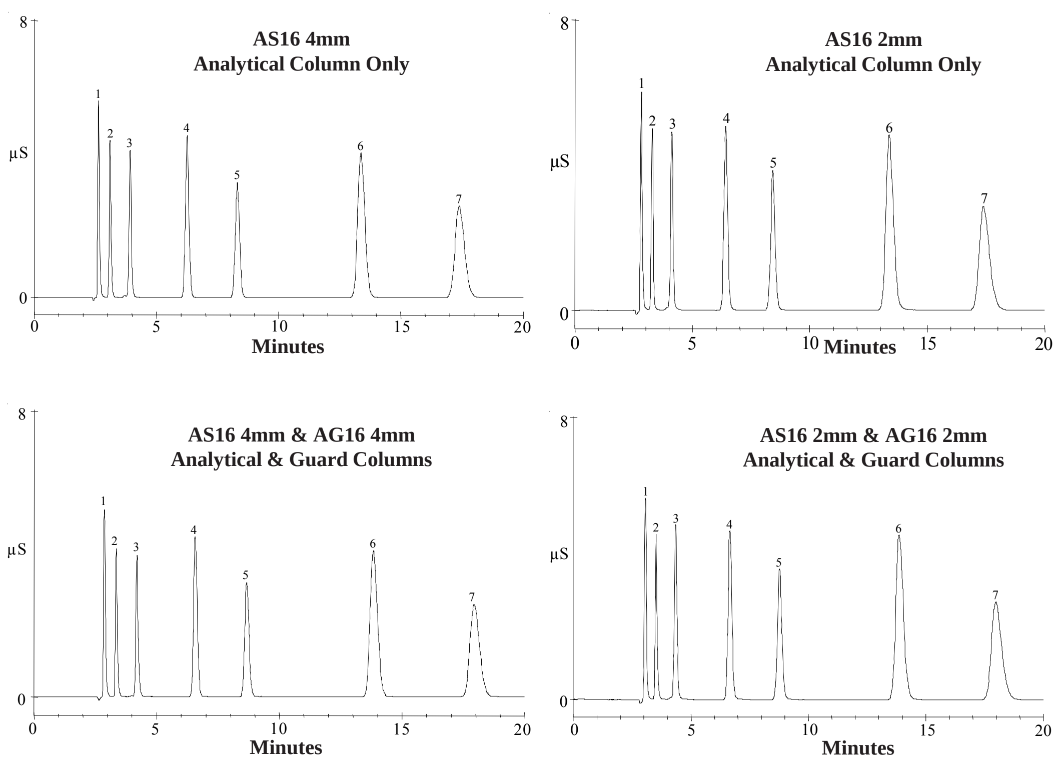
Carbon dioxide readily dissolves in dilute basic solutions, forming carbonate. Carbonate contamination of eluents can affect the retention times of the anions being analyzed. Eluents should be maintained under an inert helium atmosphere to avoid carbonate contamination.

5.2 Isocratic Elution With and Without a Guard

Isocratic elution of inorganic anions including polarizable anions using the Dionex IonPac AS16 Analytical/Capillary Column has been optimized utilizing a hydroxide eluent. By using this eluent, common inorganic anions including polarizable anions can be used to test the performance of the Dionex IonPac AS16 Column. The Dionex IonPac AS16 Analytical/Capillary Column should always be used with the Dionex IonPac AG16 Guard/Capillary Guard Column. An operating temperature of 30°C is used to ensure reproducible resolution and retention. Note that the Dionex IonPac AG16 Guard/Capillary Guard is packed with a microporous resin of proportionally lower capacity and contributes approximately 1.5% increase in retention times when a guard column is placed in-line prior to the analytical/capillary column.

Figure 7A Dionex IonPac AS16 Analytical Columns With and Without a Guard Column

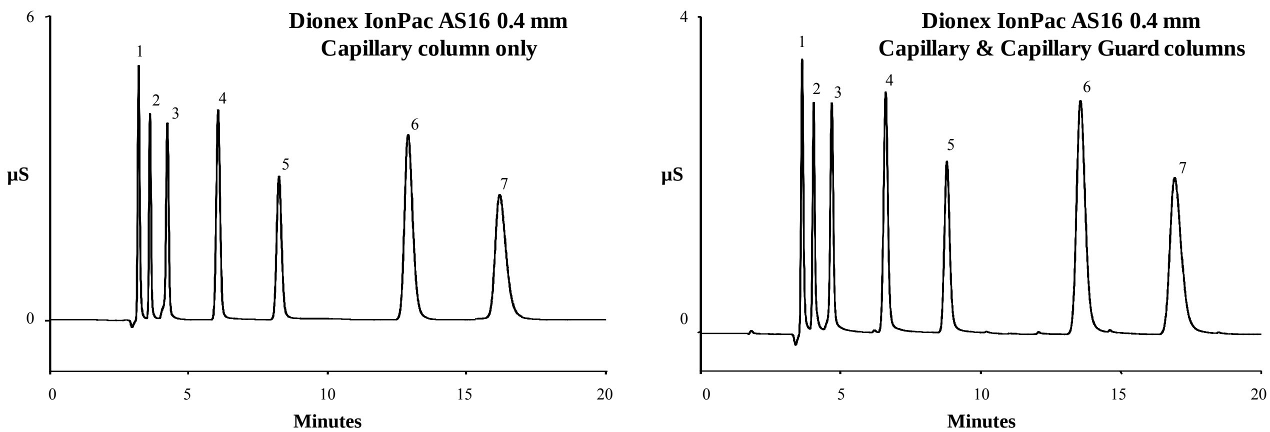
Sample Volume:	2 mm: 2.5 µL Loop + 0.8 µL Injection valve dead volume 4 mm: 10 µL Loop + 0.8 µL Injection valve dead volume
Column:	See chromatogram
Eluent:	35 mM NaOH
Eluent Flow Rate:	0.25 mL/min (2 mm), 1.0 mL/min (4 mm)
Operating Temperature:	30°C
SRS Suppressor:	Dionex Anion Self-Regenerating Suppressor, Dionex ASRS ULTRA II (2 mm or 4 mm) AutoSuppression Recycle Mode
or MMS Suppressor:	Dionex Anion MicroMembrane Suppressor, Dionex AMMS III (2 mm or 4 mm)
MMS Regenerant:	50 mN H_2SO_4.
Expected Background Conductivity:	≤ 3 µS
Long-term Storage Solution (> 1 week):	100 mM Sodium Borate
Short-term Storage Solution (< 1 week):	Eluent



Analyte	mg/L (ppm)
1. Fluoride	2.0
2. Chloride	3.0
3. Sulfate	5.0
4. Thiosulfate	10.0
5. Iodide	20.0
6. Thiocyanate	20.0
7. Perchlorate	30.0

Figure 7B Dionex IonPac AS16 Capillary Column With and Without a Capillary Guard Column

Column:	See chromatograms
Eluent Source:	Dionex EGC KOH (Capillary) Cartridge
Eluent:	35mM KOH
Flow Rate:	0.010 mL/min
Inj. Volume:	0.4 µL
Temperature:	30 °C
Detection:	Suppressed conductivity, Dionex ACES 300, recycle mode



Peaks	mg/L (ppm)
1. Fluoride	0.5
2. Chloride	0.75
3. Sulfate	1.25
4. Thiosulfate	2.5
5. Iodide	5.0
6. Thiocyanate	5.0
7. Perchlorate	7.5

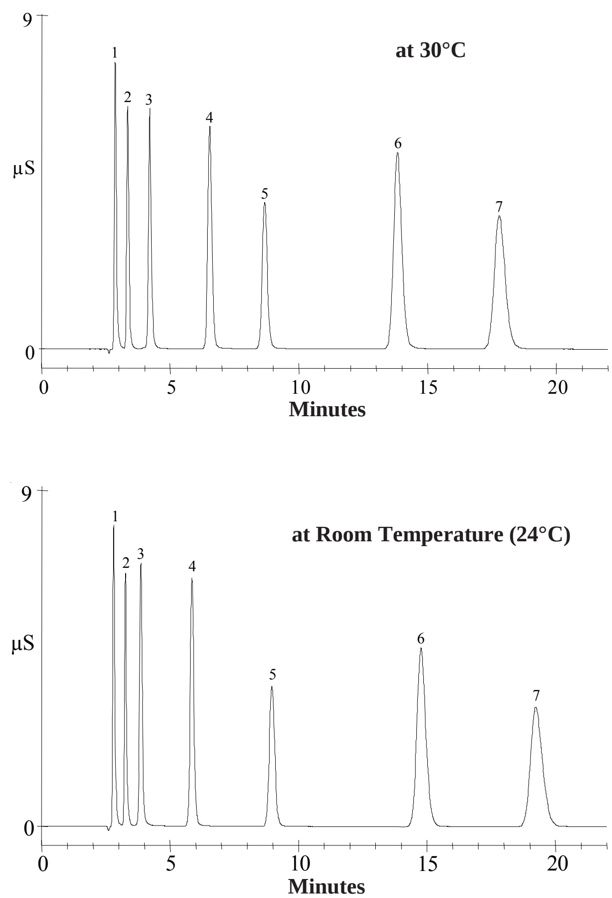
Peaks	mg/L (ppm)
1. Fluoride	0.5
2. Chloride	0.5
3. Sulfate	0.5
4. Thiosulfate	2.5
5. Iodide	5.0
6. Thiocyanate	5.0
7. Perchlorate	7.5

5.3 Comparison of Isocratic Elution Using the Dionex IonPac AS16 Analytical Column at Room Temperature and 30°C

Isocratic elution of inorganic anions including polarizable anions on the Dionex IonPac AS16 Analytical Column has been optimized at 30 °C. However, the column can be operated at room temperature. Notice that at room temperature (24 °C) the divalent ions sulfate and thiosulfate have shorter retention times with 35 mM NaOH eluent. For optimum retention time reproducibility, the temperature should be controlled.

Figure 8 Comparison of Isocratic Elution using the Dionex IonPac AS16 Analytical Column at 30 °C and at Room Temperature

Sample Volume:	10 µL Loop + 0.8 µL Injection valve dead volume
Column:	Dionex IonPac AG16/AS16 (4 × 250 mm)
Eluent:	35.0 mM NaOH
Eluent Flow Rate:	1.0 mL/min
Operating Temperature:	See Chromatogram
SRS Suppressor:	Dionex Anion Self-Regenerating Suppressor, Dionex ASRS ULTRA II (4 mm) AutoSuppression Recycle Mode
or MMS Suppressor:	Dionex Anion MicroMembrane Suppressor, Dionex AMMS III (4 mm)
MMS Regenerant:	50 mN H_2SO_4.
Expected Background Conductivity:	≤ 3 µS
Long-term Storage Solution (> 1 week):	100 mM Sodium Borate
Short-term Storage Solution (< 1 week):	Eluent



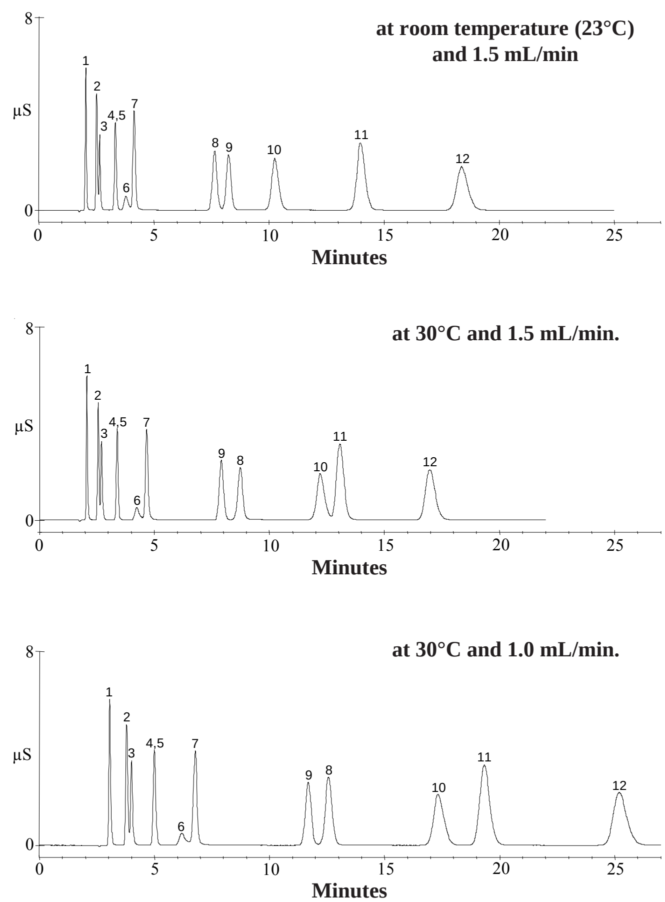
Peaks	mg/L (ppm)
1. Fluoride	2.0
2. Chloride	3.0
3. Sulfate	5.0
4. Thiosulfate	10.0
5. Iodide	20.0
6. Thiocyanate	20.0
7. Perchlorate	30.0

5.4 Isocratic Separation of 7 Anions and 4 Polarizable Anions

Figure 9 illustrates the isocratic separation of 7 common anions and 4 polarizable anions in a single run. With the standard eluent (35 mM NaOH) phosphate elutes too close to the thiosulfate peak. This eluent (22 mM NaOH) is optimized for the separation of phosphate from thiosulfate. These chromatograms also demonstrate the effect of temperature and flow rate on the separation. Notice that in order to achieve good peak shape and peak efficiency for polarizable anions, the resolution of bromide and nitrate is compromised.

Figure 9 Separation of 7 Anions and 4 Polarizable Anions

Sample Volume:	10 µL Loop + 0.8 µL Injection valve dead volume
Column:	Dionex IonPac AG16/AS16 (4 × 250 mm)
Eluent:	22.0 mM NaOH
Eluent Flow Rate:	See chromatogram
Operating Temperature:	See chromatogram
SRS Suppressor:	Dionex Anion Self-Regenerating Suppressor, Dionex ASRS ULTRA II (4 mm) AutoSuppression Recycle Mode
or MMS Suppressor:	Dionex Anion MicroMembrane Suppressor, Dionex AMMS III (4 mm)
MMS Regenerant:	50 mN H_2SO_4.
Expected Background Conductivity:	≤ 3 µS
Long-term Storage Solution (> 1 week):	100 mM Sodium Borate
Short-term Storage Solution (< 1 week):	Eluent



Peaks	mg/L (ppm)
1. Fluoride	2.0
2. Chloride	3.0
3. Nitrite	3.0
4. Bromide	3.0
5. Nitrate	3.0
6. Carbonate	20.0
7. Sulfate	5.0
8. Thiosulfate	10.0
9. Iodide	20.0
10. Phosphate	20.0
11. Thiocyanate	20.0
12. Perchlorate	30.0

5.5 Separation of Polarizable Anions and Inorganic Anions Using Gradient Elution

Figure 10 illustrates the separation of a wide variety of inorganic anions including polarizable anions. Weakly retained anions such as acetate, propionate, and formate are resolved using an isocratic hydroxide eluent and the highly retained anions such as thiosulfate, thiocyanate, and perchlorate are eluted with a hydroxide gradient. Peak shape and efficiency are greatly improved for the polarizable anions using the Dionex IonPac AS16 column.

The following example also illustrates a comparison of a gradient delivered using a manually prepared eluent system and using the Dionex EG40 Eluent Generator system. When using the conventional manually prepared eluent delivery, dissolved carbonate causes a baseline shift of approximately 1 µS. The carbonate free potassium hydroxide gradient produced by the Dionex EG40 system results in a very low baseline shift (<0.3 µS). This low baseline shift allows easy integration of trace components.

Trap Column:	Manually Prepared Eluent System: Dionex IonPac ATC-3 located after pump Dionex EG40 system: Dionex IonPac ATC-3 (2), 1 located after pump; 1 located between Dionex EG40 degas module and injector
Sample Volume:	10 µL
Column:	Dionex IonPac AG16/AS16 (4 × 250 mm)
Eluent:	E1: 5.0 mM NaOH E2: Deionized water E3: 100 mM NaOH
Eluent Flow Rate:	1.5 mL/min
Operating Temperature:	30°C
SRS Suppressor:	Dionex Anion Self-Regenerating Suppressor, Dionex ASRS ULTRA II (4 mm) AutoSuppression Recycle Mode
or MMS Suppressor:	Dionex Anion MicroMembrane Suppressor, Dionex AMMS III (4 mm)
MMS Regenerant:	50 mN H_2SO_4.
Expected Background Conductivity:	1.5 mM NaOH: $\leq$ 1 µS 55 mM NaOH: $\leq$ 3.5 µS
Typical Operating Back Pressure:	2,300 psi (15.15 MPa)

Gradient Conditions with Manually Prepared Eluent System

TIME (min)	%E1	%E2	%E3	Comments
Equilibration				
0	30	70	0	1.5 mM NaOH for 7 min.
7.0	30	70	0	
Analysis				
7.1	30	70	0	Start isocratic analysis
7.5	30	70	0	Inject Valve to Load Position
14.0	30	70	0	End Isocratic analysis, Begin Gradient analysis
20.0	0	90	10	
30.0	0	45	55	

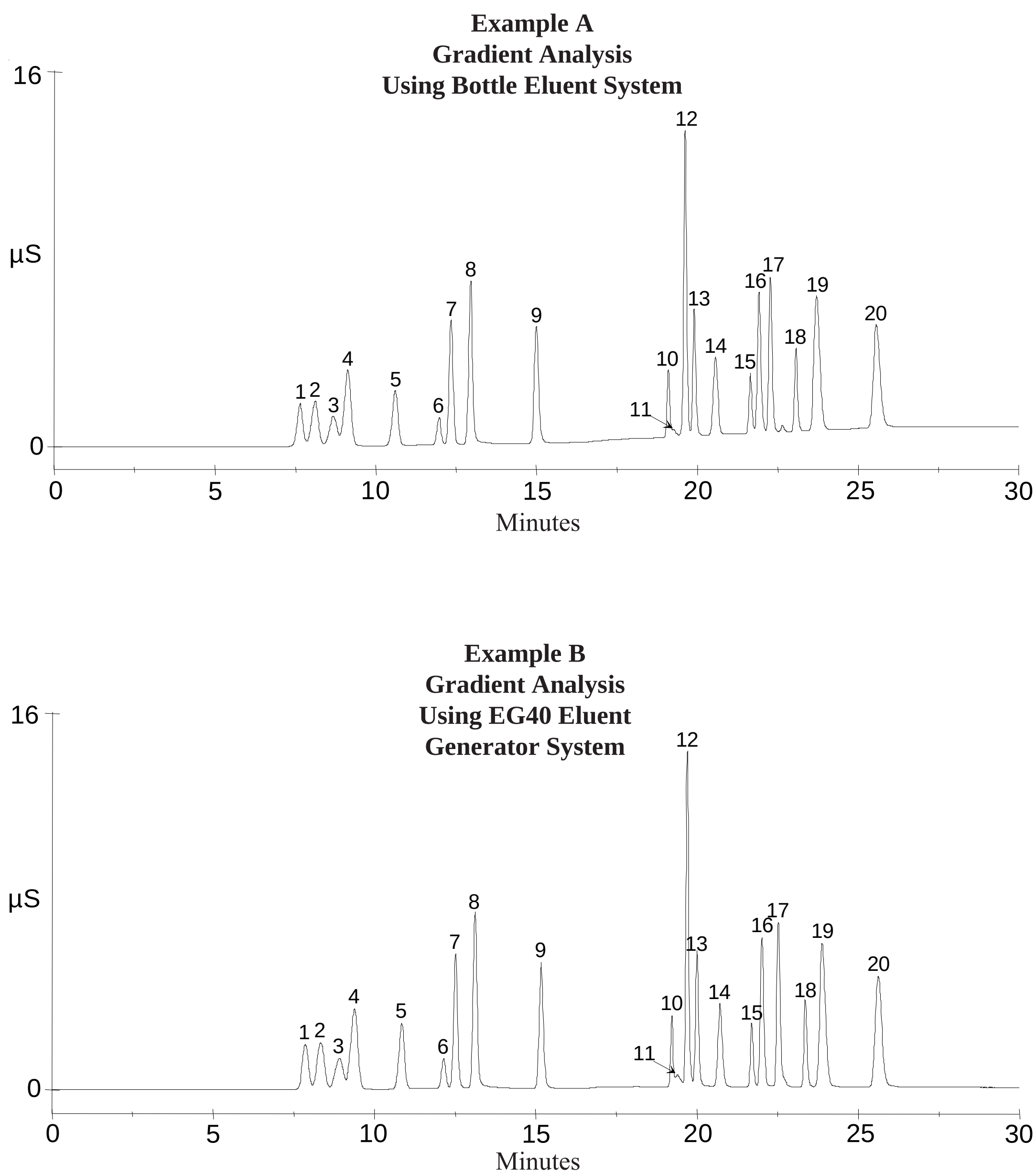
Dionex EG40 Conditions

Eluent: Deionized water
Offset volume = 0.0 µL

TIME (min)	Eluent Concentration	Comments
Equilibration		
0	1.5	1.5 mM KOH for 7 min.
7.0	1.5	
Analysis		
7.1	1.5	Start isocratic analysis
7.5	1.5	Inject Valve to Load Position
15.3	1.5	End Isocratic analysis, Begin Gradient analysis
21.3	10.0	
31.3	55.0	

Peaks	mg/L (ppm)
1. Fluoride	2.0
2. Acetate	10.0
3. Propionate	10.0
4. Formate	10.0
5. Chlorite	10.0
6. Bromate	10.0
7. Chloride	5.0
8. Nitrite	10.0
9. Nitrate	10.0
10. Selenite	10.0
11. Carbonate	20.0
12. Sulfate	10.0
13. Selenate	10.0
14. Iodide	20.0
15. Thiosulfate	10.0
16. Chromate	20.0
17. Phosphate	20.0
18. Arsenate	20.0
19. Thiocyanate	20.0
20. Perchlorate	30.0

Figure 10 Separation of Polarizable Anions and Inorganic Anions Using Gradient Elution



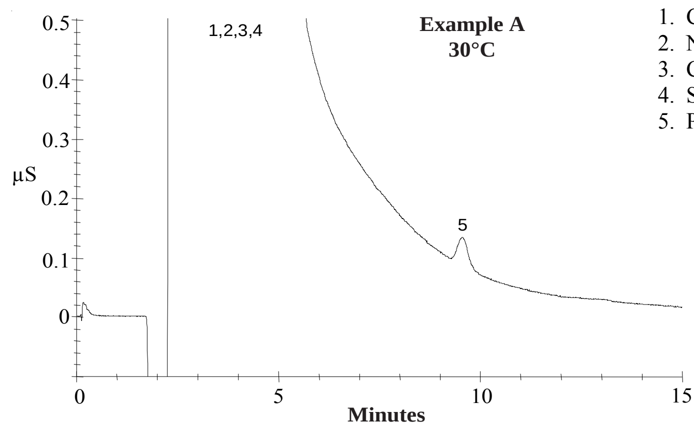
5.6 Determination of Trace Perchlorate using a Large Loop Injection with a Dionex IonPac AS16 Analytical Column (4 × 250 mm)

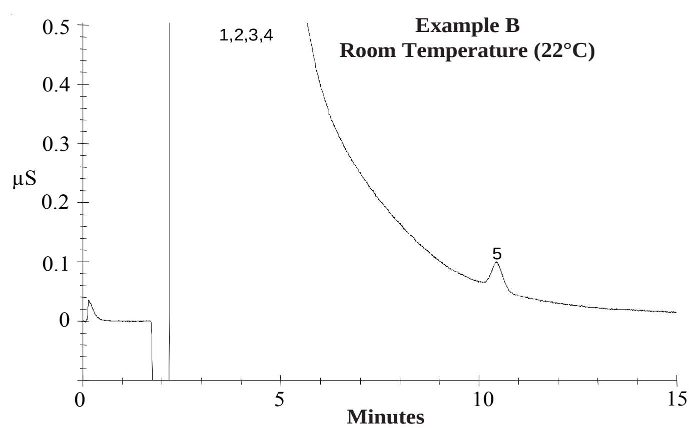
Trace concentrations of perchlorate in drinking water, surface water, and ground water matrices can easily be determined using a large loop injection. With 50 mM sodium hydroxide eluent at a controlled temperature of 30 °C, perchlorate can be determined in approximately 10 minutes. This application can be done at room temperature, as in example B, however, for optimum retention time reproducibility, the temperature should be controlled.

Figure 11 Determination of Trace Perchlorate Using a Large Loop Injection with the Dionex IonPac AS16 Analytical Column

Sample Volume:	1.0 mL
Column:	Dionex IonPac AG16/AS16 (4 × 250 mm)
Eluent:	50 mM NaOH
Eluent Flow Rate:	1.5 mL/min
Operating Temperature:	See Chromatogram
SRS Suppressor:	Dionex Anion Self-Regenerating Suppressor, Dionex ASRS ULTRA II (4 mm) AutoSuppression Recycle Mode
or MMS Suppressor:	Dionex Anion MicroMembrane Suppressor, Dionex AMMS III (4 mm)
MMS Regenerant:	50 mN H_2SO_4.
Expected Background Conductivity:	≤ 3 µS
Long-term Storage Solution (> 1 week):	100 mM Sodium Borate
Short-term Storage Solution (< 1 week):	Eluent

Peaks	mg/L (ppm)
1. Chloride	200
2. Nitrate	50
3. Carbonate	200
4. Sulfate	1000
5. Perchlorate	0.005





5.7 Determination of Trace Perchlorate using a Large Loop Injection with the Dionex IonPac AS16 Capillary Column (0.4 × 250 mm)

Trace concentrations of perchlorate in drinking water, surface water, and ground water matrices can easily be determined using a large loop injection on the capillary system. With 50 mM sodium hydroxide eluent at a controlled temperature of 30 °C, perchlorate can be determined in approximately 10 minutes. For optimum retention time reproducibility, the temperature is controlled at 30 º C.

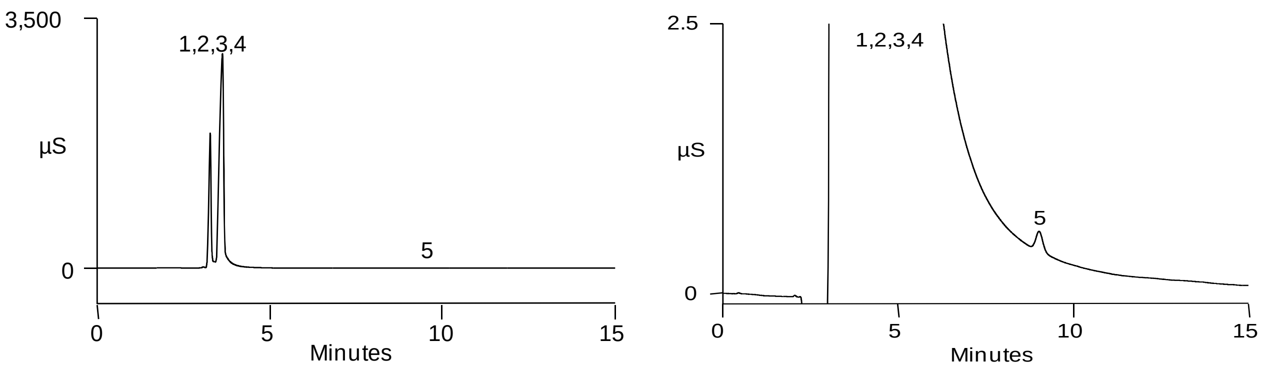
NOTE

The more efficient sweep-out characteristics of the analytical system in comparison to the capillary system allows for the analysis of higher ionic strength matrices when using an analytical system in comparison to the capillary system. In Figure 12 below, using a capillary system, the sample matrix is 10 times less in ionic strength than the sample matrix shown in Figure 11 ***above, using an analytical system. Equivalent injection volumes are shown in the figures. The sample in*** Figure 11 ***requires dilution prior to analysis using the capillary system resulting in higher detection limits for perchlorate.***

Figure 12 Determination of Trace Perchlorate using a Large Loop Injection with the Dionex IonPac AS16 Capillary Column (0.4 x 250 mm)

Column:	Dionex IonPac AG16/AS16 (0.4 × 250 mm)
Eluent Source:	Dionex EGC KOH (Capillary) Cartridge
Eluent:	50mM KOH
Flow Rate:	0.015 mL/min
Inj. Volume:	10 µL
Temperature:	30 º C
Detection:	Suppressed conductivity, Dionex ACES 300, recycle mode

Peaks:	**mg/L (ppm)**
1. Chloride	20
2. Nitrate	5
3. Carbonate	20
4. Sulfate	100
5. Perchlorate	0.005



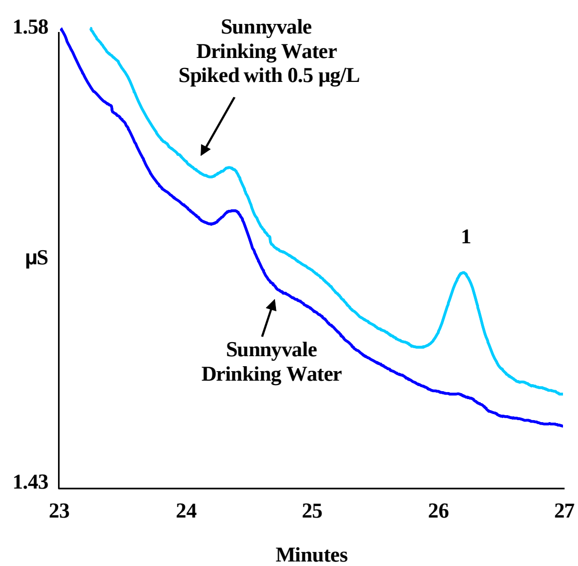
5.8 Determination of Trace Perchlorate in Drinking Water Using the Dionex IonPac AS16 Analytical Column and the Dionex IonPac Cryptand C1 Concentrator Column

Perchlorate, initially ammonium perchlorate, widely used in the manufacture of rocket propellants, munitions, fireworks, and road flares, has been found in drinking water in areas where aerospace materials and munitions have been manufactured and tested. Perchlorate is a potential health concern because it interferes with the production of thyroid hormones. The Dionex IonPac AS16 column was designed to determine trace perchlorate in groundwater and drinking water matrices. Figure 13 shows the determination of trace perchlorate in a drinking water sample using sample preconcentration with the Dionex IonPac Cryptand C1 Concentrator Column and a sodium hydroxide eluent coupled with suppressed conductivity detection. The Dionex IonPac Cryptand C1 Concentrator Column is used with sodium hydroxide eluent to allow optimum concentrator capacity control. At high concentrations of sodium, the Dionex IonPac Cryptand C1column has high capacity, but at lower concentrations the capacity decreases and the analytes can be eluted. Figure 14 shows the system flow path for the determination of trace perchlorate according to U.S. EPA Method 314.1.

Low-µg/L (ppb) levels of perchlorate can easily be quantified using the Dionex IonPac AS16 column and a 2-mL sample pre-concentration, as shown in Figure 13.

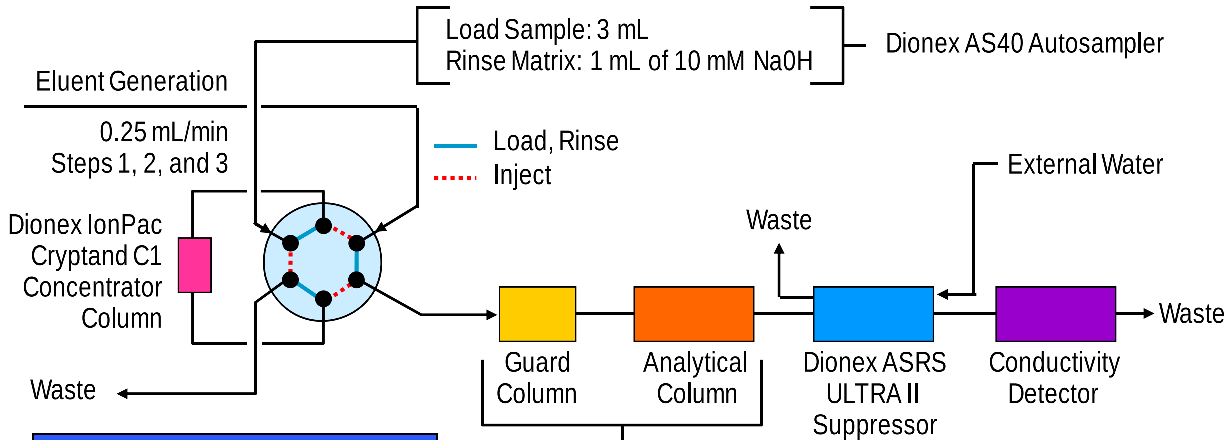
Figure 13 Determination of Trace Perchlorate in Drinking Water Using the AS16 Column and the Cryptand C1 Concentrator Column

Column:	Dionex IonPac AG16/AS16, (2 × 250 mm)
Concentrator Column:	Dionex IonPac Cryptand C1, 4x35 mm
Eluent:	Sodium hydroxide: 0.5 mM from 0–12 min, 65 mM from 12.1–28 min, 100 mM from 28.1–30 min.
Eluent Source:	EGC II NaOH Cartridge with Dionex CR-ATC Continuously Regenerated Anion Trap Column
Temperature:	35 °C
Flow Rate:	0.25 mL/min
Inj. Volume:	2 mL
Rinse Volume:	1 mL (10 mM NaOH)
Detection:	Suppressed conductivity, Dionex ASRS ULTRA II, 2 mm, AutoSuppression external water mode, external water flow rate, 1-3 mL/min, 100 mA



Peaks	µg/L (ppb)
1. Perchlorate	0.5

Figure 14 Perchlorate Analysis Using RFIC with Pre-concentration and Matrix Rinse—EPA Method 314.1



NaOH Eluent Generation			
Steps	Function	Conc.	Time
1.	Perchlorate Transfer	0.5 mM	12 Min
2.	Analysis	65 mM	13 Min
3.	Column Cleanup	100 mM	5 Min

Primary Method—Dionex IonPac AG16/AS16, 2 x 250 mm

Confirmatory Method—Dionex IonPac AG20/AS20, 2 x 250 mm

Method Report Limit = 0.5 ppb

NOTE

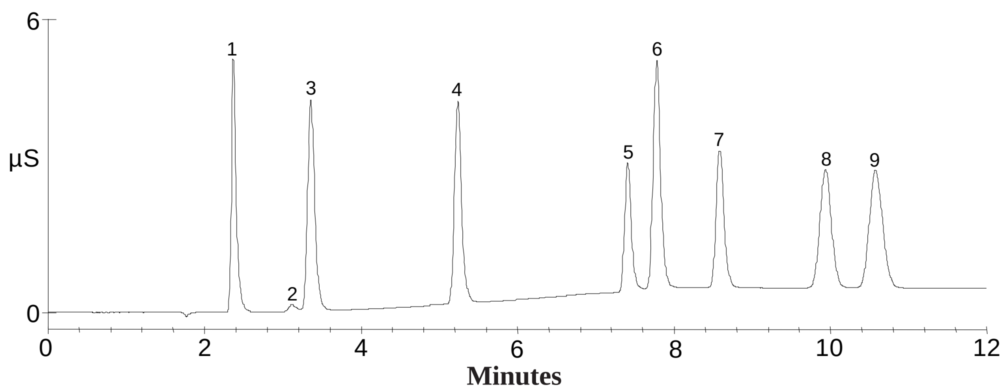
Autosampler must be capable of loading concentrator columns.

5.9 Separation of Polyphosphate Anions

Monitoring polyphosphates is an important environmental concern. Polyphosphates are commonly found in processed foods, hard water treatment products and personal care products. The determination of polyvalent phosphates uses gradient conditions of 30 mM to 60 mM aqueous sodium hydroxide (containing no solvents) at a flow rate of 1.5 mL/min to elute 8 anions in 10 minutes.

Figure 15 Determination of Polyphosphate Anions

Trap Column:	Dionex IonPac ATC-3 (Located at pump outlet)
Sample Volume:	10 µL
Column:	Dionex IonPac AG16/AS16 (4 x 250 mm)
Eluent:	E1: Deionized water E2: 100 mM NaOH
Eluent Flow Rate:	1.5 mL/min
Operating Temperature:	30°C
SRS Suppressor:	Dionex Anion Self-Regenerating Suppressor, Dionex ASRS ULTRA II (4 mm) AutoSuppression Recycle Mode
or MMS Suppressor:	Dionex Anion MicroMembrane Suppressor, Dionex AMMS III (4 mm)
MMS Regenerant:	50 mN H_2SO_4.
Expected Background Conductivity:	30 mM NaOH: $\leq$ 2 µS 60 mM NaOH: $\leq$ 3.5 µS
Typical Operating Back Pressure:	2,300 psi (15.15 MPa)



Gradient Conditions Without Solvent

TIME (min)	%E1	%E2	Comments
Equilibration			
0	70	30	30 mM NaOH for 7 min.
7.0	70	30	
Analysis			
7.1	70	30	Start isocratic analysis
7.5	70	30	Inject Valve to Load Position begin gradient analysis
12.0	40	60	

Peaks (ppm)	mg/L
1. Chloride (Cl^-)	3.0
2. Carbonate (CO_3)	--
3. Sulfate (SO_4^{2-})	5.0
4. Phosphate (PO_4^{3-})	10.0
5. Pyrophosphate ($P_2O_7^{4-}$)	10.0
6. Trimetaphosphate ($P_3O_9^{3-}$)	10.0
7 Tripolyphosphate ($P_3O_{10}^{5-}$)	10.0
8. Tetrametaphosphate ($P_4O_{12}^{4-}$)	10.0
9. Tetrapolyphosphate ($P_4O_{13}^{6-}$)	10.0

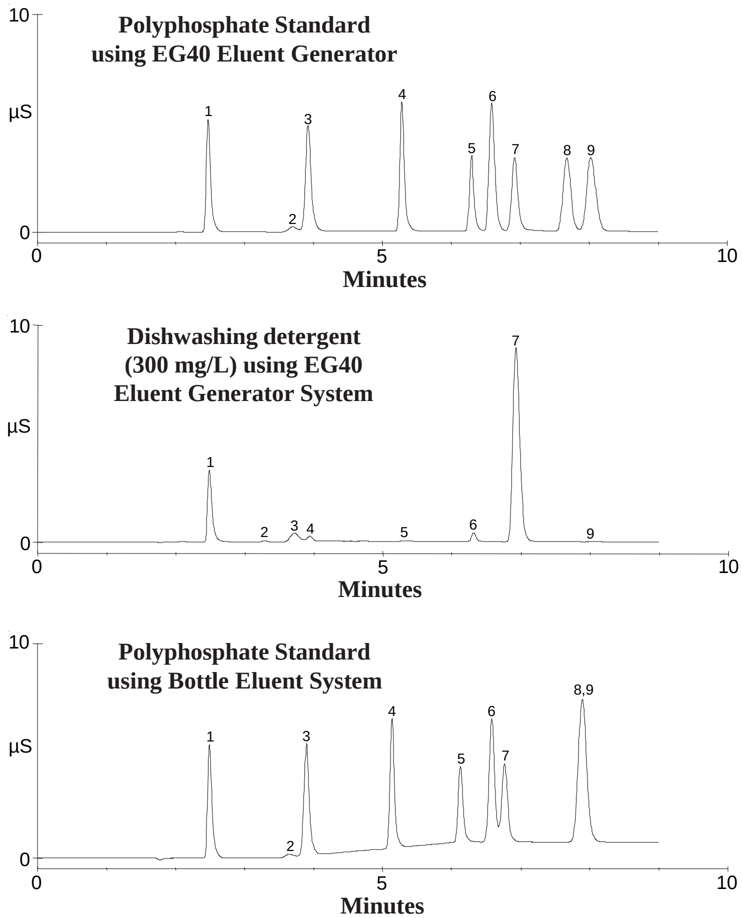
5.10 Separation of Polyphosphate Anions Using the Dionex EG40 Eluent Generator System

Figure 16 shows the separation of polyvalent phosphates using the Dionex EG40 Eluent Generator System. Notice the excellent separation of polyvalent phosphates using the Dionex EG40 gradient from 25 mM KOH to 65 mM KOH. In spite of the steep gradient, a minimum baseline shift is observed which facilitates quantitation of trace components as demonstrated in the dishwasher detergent chromatogram.

The following example also shows a comparison of a gradient delivered using the Dionex EG40 Eluent Generator System and a manually prepared eluent system. Due to the carbonate contamination of the manually prepared eluent system, polyvalent phosphates such as tetrametaphosphate and tetrapolyphosphate have less retention and coelute. For optimum manually prepared eluent system conditions, see Figure 15.

Trap Columns:	Dionex IonPac ATC-3 (2), 1 located at pump outlet; 1 located between Dionex EG40 degas module and injector
Sample Volume:	10 µL
Column:	Dionex IonPac AG16/AS16 (4 × 250 mm)
Eluent:	E1: Deionized water
	E2: 100 mM NaOH
Eluent Flow Rate:	1.5 mL/min
Operating Temperature:	30 °C
SRS Suppressor:	Dionex Anion Self-Regenerating Suppressor, Dionex ASRS ULTRA II (4 mm) AutoSuppression Recycle Mode
or MMS Suppressor:	Dionex Anion MicroMembrane Suppressor, Dionex AMMS III (4 mm)
MMS Regenerant:	50 mN H_2SO_4.
Expected Background Conductivity:	25 mM NaOH: $\leq$ 2 µS
	60 mM NaOH: $\leq$ 3.5 µS
Typical Operating Back Pressure:	2,300 psi (15.15 MPa)

Figure 16 Determination of Polyphosphate Anions



Peaks (ppm)	mg/L
1. Chloride (Cl^-)	3.0
2. Carbonate (CO_3)	—
3. Sulfate (SO_4^{2-})	5.0
4. Phosphate (PO_4^{3-})	10.0
5. Pyrophosphate ($P_2O_7^{4-}$)	10.0
6. Trimetaphosphate ($P_3O_9^{3-}$)	10.0
7 Tripolyphosphate ($P_3O_{10}^{5-}$)	10.0
8. Tetrametaphosphate ($P_4O_{12}^{4-}$)	10.0
9. Tetrapolyphosphate ($P_4O_{13}^{6-}$)	10.0

EG40 Conditions

Eluent: Deionized water
Offset volume = 0.0 µL

TIME (min)	Eluent Concentration	Comments
Equilibration		
0	25	25 mM KOH for 7 min
7.0	25	
Analysis		
7.1	25	Start isocratic analysis
7.5	25	Inject Valve to Load Position
8.8	25	Begin gradient Analysis
11.3	65	

Gradient Conditions Without Bottle Eluent

TIME (min)	%E1	%E2	Comments
Equilibration			
0	75	25	
7.0	75	25	
Analysis			
7.1	75	25	Start isocratic analysis
7.5	75	25	Inject Valve to Load Position
10.0	35	65	Begin gradient Analysis

5.11 Separation of Polyphosphate Anions using the Dionex IonPac AS16 Capillary Column (0.4 x 250 mm)

Figure 17 shows the separation of polyvalent phosphates using the Dionex ICS 5000 Capillary System and the Dionex IonPac AS16 Capillary Column. Notice the excellent separation of polyvalent phosphates using the Dionex EGC KOH gradient from 25 mM KOH to 65 mM KOH. In spite of the steep gradient, a minimum baseline shift is observed which facilitates quantitation of trace components.

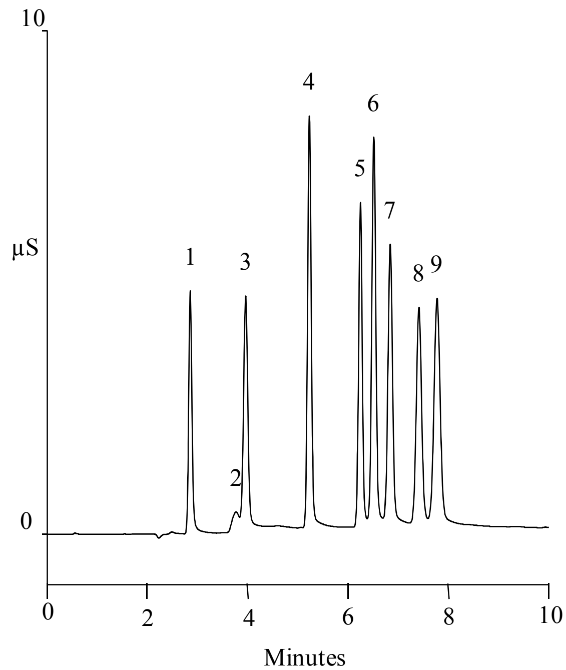
NOTE

Due to the difference in system delay volume between the analytical and capillary systems, the isocratic portion of the gradient was reduced by one minute for the capillary method in comparison to the analytical method shown in Figure 15**.**

Figure 17 Separation of Polyphosphate Anions using the Dionex IonPac AS16 Capillary Column (0.4 × 250 mm)

Column:	Dionex IonPac AG16/AS16 (0.4 x 250 mm)
Eluent Source:	Dionex EGC- KOH (Capillary) Cartridge
Eluent:	KOH Gradient 25 mM from 0 to 0.5 min, 25 mM to 65 mM from 0.5 to 3 min
Flow Rate:	0.015mL/min
Inj. Volume:	0.4 µL
Temperature:	30 ° C
Detection:	Suppressed conductivity, Dionex ACES 300, recycle mode

Peaks:	**mg/L**
1.Chloride	3.0
2. Carbonate	---
3. Sulfate	5.0
4. Phosphate	10.0
5. Pyrophosphate	10.0
6. Trimetaphosphate	10.0
7. Tripolyphosphate	10.0
8. Tetrametaphosphate	10.0
9. Tetrapolyphosphate	10.0



5.12 Analysis of Trace Perchlorate in High Ionic Strength Matrices using the Dionex IonPac AS16 Capillary Column with 2 Dimensional Matrix Elimination Ion Chromatography (2D-MEIC)

Trace perchlorate (sub ppb level) in the presence of high ionic strength matrices can be analyzed using a 2D-MEIC instrument configuration with the Dionex IonPac AS20 analytical column and the Dionex IonPac AS16 capillary column. There are several advantages of using the 2-D matrix elimination approach. First, initial sample loading onto the analytical column allows a large sample injection volume (large amount of sample) due to the high capacity of the analytical column and higher selectivity for analytes of interest relative to the matrix ions. Second, it is possible to focus the analyte peak that is partially resolved in the first dimension onto a concentrator column, the Dionex IonSwift MAC-200, in the second dimension. The hydroxide eluent is suppressed to water, which provides the ideal environment for ion-exchange retention and focusing. Third, the second dimension column has a smaller cross-sectional area relative to the first dimension, thereby enhancing the detection sensitivity. Finally, this approach allows the potential of combining two different chemistries in two column dimensions, which enables analyte selectivity not possible when using a single chemistry and dimension.

Figure 18 below shows the system configuration for the 2D-MEIC method for the analysis of low level perchlorate in the presence of high ionic strength matrices.

Figure 19 below shows the chromatogram from the first dimension using the Dionex IonPac AG20/AS20 analytical column (2 × 250 mm). An aliquot of the column effluent between 18.3 minutes to 22.3 minutes, where the perchlorate elutes, is directed to the Dionex IonSwift MAC-200 concentrator column in the second dimension. This concentrated aliquot is then separated using the Dionex IonPac AG16/AS16 capillary column (0.4 x 250 mm) as shown in the lower chromatogram.

Figure 18 2D-MEIC System Configuration for the Analysis of Trace Perchlorate in High Ionic Strength Matrices

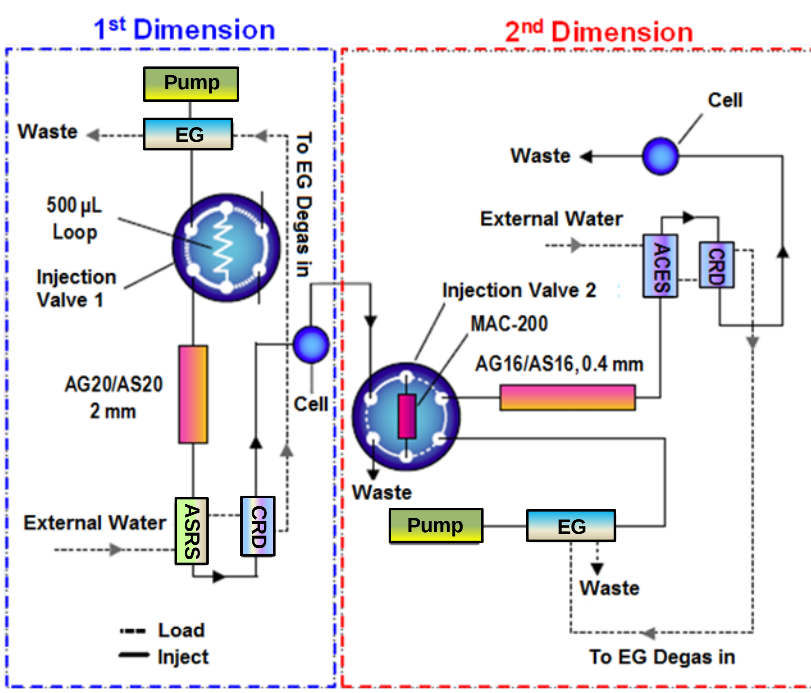
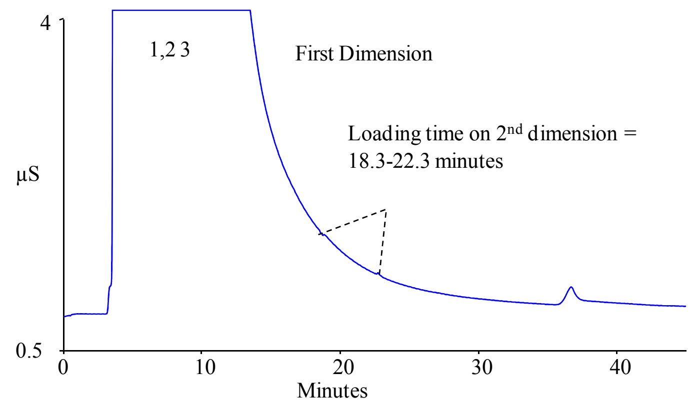


Figure 19 2D-MEIC Analysis of Trace Perchlorate using the Dionex IonPac AS20 Analytical Column in the First Dimension (top chromatogram) and Dionex IonPac AS16 Capillary Column in the Second Dimension (bottom chromatogram).

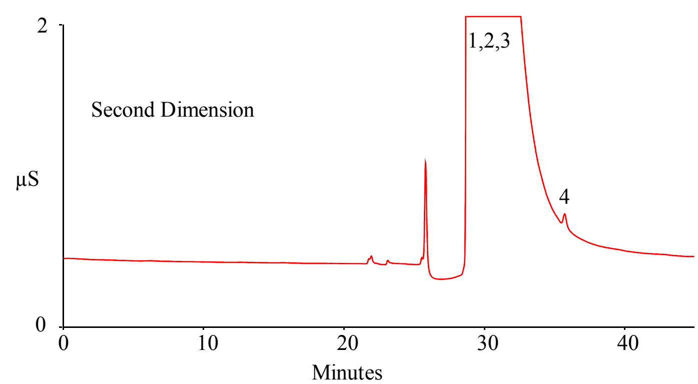
First Dimension

Column:	Dionex IonPac AG20/AS20 (2 x 250 mm)
Eluent Source:	Dionex EGC III KOH Cartridge
Eluent:	Potassium hydroxide 0-30 min, 35 mM 30-40 min, 60 mM 40-45 min, 35 mM
Flow Rate:	0.25 mL/min
Inj. Volume:	500 µL
Temperature:	30 ºC
Detection:	Suppressed conductivity, Dionex ASRS 300, External water mode
Peaks:	**mg/L (ppm)**
1. Chloride	1000
2. Carbonate	1000
3. Sulfate	1000
4. Perchlorate	---



Second Dimension

Column:	Dionex IonPac AG16/AS16 (0.4 x 250 mm)
Eluent Source:	Dionex EGC KOH (Capillary) Cartridge
Eluent:	Potassium hydroxide 65 mM
Flow Rate:	0.010 mL/min
Inj. Volume:	1000 µL
Temperature:	30 ºC
Concentrator:	Dionex IonSwift MAC-200
Detection:	Suppressed conductivity, Dionex ACES 300, External water mode
Peaks:	**µg/L (ppb)**
1. Chloride	--
2. Carbonate	--
3. Sulfate	--
4. Perchlorate	0.02

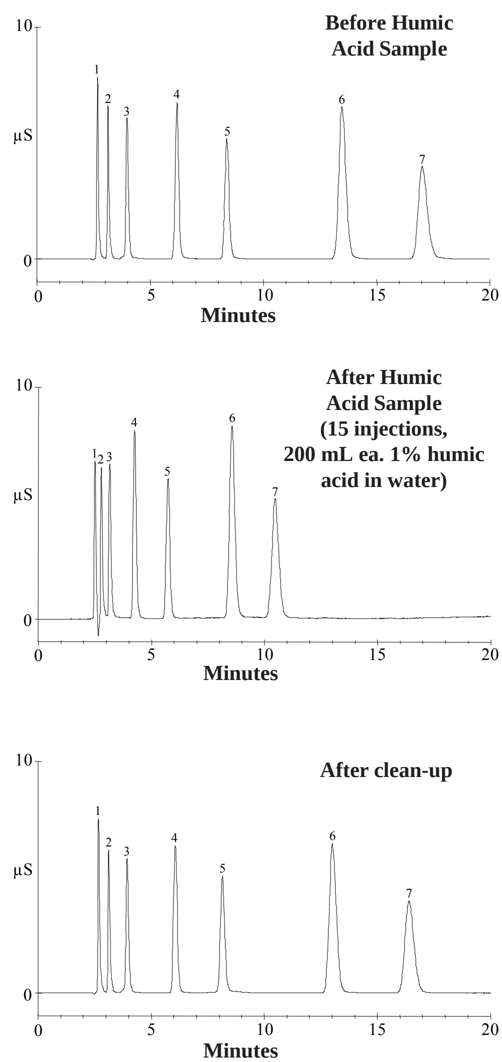


5.13 Clean-up After Humic Acid Samples

Solvent compatibility of the Dionex IonPac AS16 permits the use of organic solvents to effectively remove organic contaminates from the column. A Dionex IonPac AS16 column, after losing over 45% of its original capacity due to fouling with humic acid samples, can easily be restored to 95% of its original performance by cleaning for 4 hours with 80% tetrahydrofuran (THF)/20% 1.0 M HCl. Longer cleaning is required due to the high capacity of the Dionex IonPac AS16 column.

Column:	Dionex IonPac AS16 Column (4 × 250 mm)
Eluent:	35 mM Sodium hydroxide
Temperature:	30 ºC
Flow Rate:	1.0 mL/min
Inj. Volume:	10 µL
Detection:	Suppressed conductivity, Dionex ASRS ULTRA II, AutoSuppression, recycle mode

Figure 20 Clean-up after Humic Acid Samples



Peaks	mg/L (ppm)
1. Fluoride	2.0
2. Chloride	3.0
3. Sulfate	5.0
4. Thiosulfate	10.0
5. Iodide	20.0
6. Thiocyanate	20.0
7. Perchlorate	30.0

6. Troubleshooting Guide

The purpose of the Troubleshooting Guide is to help you solve operating problems that may arise while using the Dionex IonPac AS16 column. For more information on problems that originate with the Ion Chromatograph (IC) or the suppressor, refer to the Troubleshooting Guide in the appropriate operator's manual.

NOTE

For assistance, contact Technical Support for Dionex Products. In the U.S., call 1-800-346-6390. Outside the U.S., call the nearest Thermo Fisher Scientific office.

Table 6 Dionex IonPac AS16/AG16 Troubleshooting Summary

Observation	Cause	Action	Reference Section
High Back Pressure	Unknown	Isolate Blocked Component	6.1.1
	Plugged Column Bed Supports	Filter Eluents, and Filter Samples	6.1.2, 6.1.3, 6.1.4
	Other System Components	Unplug, Replace	Component Manual
High Background Conductivity	Contaminated Eluents	Remake Eluents	6.2, 6.2.1
	Contaminated Trap Column	Clean Trap Column	6.2.2, 6.2.3
	Contaminated Guard or Analytical/Capillary Column	Clean Guard and Analytical/Capillary Column	6.2.4
	Contaminated Suppressor	Clean Suppressor	6.2.6, Component Manual
	Contaminated Hardware	Clean Component	6.2.5, Component Manual
Poor Resolution	Method Not Optimized	Optimize Method	6.3.1.B, Component Manual
Poor Efficiency	Large System Void Volumes	Replumb System	6.3.1.A
	Column Headspace	Replace Column	6.3.1.B
Short Retention Times	Flow Rate Too fast	Recalibrate Pump	6.3.2.A
	Conc. Incorrect Eluents	Remake Eluents	6.3.2.B
	Column Contamination	Clean Column	6.3.2.C, 6.3.2.D
Poor Front End Resolution	Conc. Incorrect Eluents	Remake Eluents	6.3.3.A
	Column Overloading	Reduce Sample Size	6.3.3.B, 3.5
	Sluggish Injection Valve	Service Valve	6.3.3.C, Component Manual
	Large System Void Volumes	Replumb System	6.3.3.D, Component Manual
Spurious Peaks	Column Contaminated	Pretreat Samples	6.3.4.A,
	Sluggish Injection Valve	Service Valve	6.3.3.B, Component Manual

6.1 High Back Pressure

6.1.1 Finding the Source of High System Pressure

Total system pressure for the Dionex IonPac AG16 Guard/Capillary Guard Column plus the Dionex IonPac AS16 Analytical/Capillary Column when using the test chromatogram conditions should be equal or less than 1,650 psi. If the system pressure is higher than 1,650 psi, it is advisable to determine the cause of the high system pressure. The system should be operated with a Thermo Scientific Dionex High-Pressure In-Line Filter (P/N 044105) which is positioned between the Gradient Pump pressure transducer and the injection valve. Make sure you have one in place and that it is not contaminated.

A. **Make sure that the pump is set to the correct eluent flow rate.** Higher than recommended eluent flow rates will cause higher pressure. Measure the pump flow rate if necessary with a stop watch and graduated cylinder.

B. **Determine which part of the system is causing the high pressure.** High pressure could be due to a plugged tubing or tubing with collapsed walls, an injection valve with a clogged port, a column with particulates clogging the bed support, a clogged High-Pressure In-Line Filter, the suppressor or the detector cell.

   To determine which part of the chromatographic system is causing the problem, disconnect the pump eluent line from the injection valve and turn the pump on. Watch the pressure; it should not exceed 50 psi. Continue adding system components (injection valve, column(s), suppressor and detector) one by one, while monitoring the system pressure. The pressure should increase up to a maximum when the Guard and Analytical columns are connected (see Table 7, "Typical Dionex IonPac AS16/AG16 Operating Back Pressures").

   The Dionex Anion Self-Regenerating Suppressor, Dionex ASRS 300 with backpressure loops may add up to 100 psi (0.69 MPa). No other components should add more than 100 psi (0.69 MPa) of pressure. Refer to the appropriate manual for cleanup or replacement of the problem component.

Table 7 Typical Dionex IonPac AS16/AG16 Column Operating Back Pressures

Column	Typical Back Pressure with Aqueous eluents psi (MPa) at 30°C	Flow Rate mL/min
Dionex IonPac AS16 4 mm Analytical Column	1400 (9.64)	1.0
Dionex IonPac AG16 4 mm Guard Column	150 (1.03)	1.0
Dionex IonPac AS16 + AG16 4 mm Columns	**1550 (10.67)**	**1.0**
Dionex IonPac AS16 2 mm Analytical Column	1400 (9.64)	0.25
Dionex IonPac AG16 2 mm Guard Column	150 (1.03)	0.25
Dionex IonPac AS16 + AG16 2 mm Columns	**1550 (10.67)**	**0.25**
Dionex IonPac AS16 0.4 mm Capillary Column	1400 (9.64)	0.010
Dionex IonPac AG16 0.4 mm Capillary Guard Column	150 (1.03)	0.010
Dionex IonPac AS16 + AG16 0.4 mm Columns	**1550 (10.67)**	**0.010**

6.1.2 Replacing Column Bed Support Assemblies

If the column inlet bed support is determined to be the cause of the high back pressure, it should be replaced. To change the inlet bed support assembly, refer to the following instructions, using one of the two spare inlet bed support assemblies included in the Ship Kit.

A. **Disconnect the column from the system.**

B. **Carefully unscrew the inlet (top) column fitting.** Use two open-end wrenches.

C. **Remove the bed support.** Turn the end fitting over and tap it against a benchtop or other hard, flat surface to remove the bed support and seal assembly. If the bed support must be pried out of the end fitting, use a sharp pointed object such as a pair of tweezers, but be careful that you **DO NOT SCRATCH THE WALLS OF THE END FITTING.** Discard the old bed support assembly.

D. **Place a new bed support assembly into the end fitting.** Make sure that the end of the column tube is clean and free of any particulate matter so that it will properly seal against the bed support assembly. Use the end of the column to carefully start the bed support assembly into the end fitting.

Table 8 Ordering Information

Product	Dionex IonPac AS16 4 mm Columns (P/N)	Dionex IonPac AS16 2 mm Columns (P/N)	Dionex IonPac AS16 0.4 mm Columns (P/N)
Analytical Column	052960	052961	082315
Guard Column	052962	052963	082316
Bed Support Assembly	042955	044689	N/A
End Fitting	052809	043278	N/A

CAUTION

If the column tube end is not clean when inserted into the end fitting, particulate matter may obstruct a proper seal between the end of the column tube and the bed support assembly. If this is the case, additional tightening may not seal the column but instead damage the column tube or the end fitting.

E. **Screw the end fitting back onto the column.** Tighten it fingertight, then an additional 1/4 turn (25 in x lb). Tighten further only if leaks are observed.

F. **Reconnect the column to the system and resume operation.**

NOTE

Replace the outlet bed support ONLY if high pressure persists after replacement of the inlet fitting.

6.1.3 Filter Eluent

Eluents containing particulate material or bacteria may clog the column inlet bed support. Filter water used for eluents through a 0.45 µm filter.

6.1.4 Filter Samples

Samples containing particulate material may clog the column inlet bed support. Filter samples through a 0.45 µm filter prior to injection.

6.2 High Background

In a properly working system, the background conductivity level for the standard eluent system is shown below:

MANUALLY PREPARED ELUENT SYSTEM	EXPECTED BACKGROUND CONDUCTIVITY
1.5 mM NaOH	≤ 1.0 µS
35 mM NaOH	≤ 2.5 µS
55 mM NaOH	≤ 3.5 µS

6.2.1 Preparation of Eluents

A. Make sure that the eluents and the regenerant are made correctly.

B. Make sure that the eluents are made from chemicals with the recommended purity.

C. Make sure that the deionized water used to prepare the reagents has a specific resistance of 18.2 megohm-cm.

6.2.2 A Contaminated Trap Column

High background may be caused by contamination of the Dionex IonPac ATC-HC or Dionex IonPac ATC-3 with carbonate or other anions from the eluent.

A. Clean the Dionex IonPac ATC-HC or Dionex IonPac ATC-3 (4 mm) with 100 mL of 2.0 M NaOH or 50 mL for the 2 mm Dionex IonPac ATC-3.

B. Rinse the Dionex IonPac ATC-HC or Dionex IonPac ATC-3(4 mm) immediately with 20 mL of eluent or 10 mL of eluent for the 2 mm Dionex IonPac ATC-3 into a beaker prior to use.

6.2.3 Contaminated Dionex CR-ATC Continuously Regenerated Anion Trap Column

Install a Dionex CR-TC Anion Trap Column (P/N 060488 or 072078) if using an Eluent Generator with Dionex EGC KOH cartridge.

If the Dionex CR-ATC Continuously Regenerated Anion Trap Column becomes contaminated, please refer to Section 6, Clean-Up, in the Dionex CR-ATC Continuously Regenerated Anion Trap Column Product Manual (Document No. 031910).

6.2.4 A Contaminated Guard or Analytical Column

A. Remove the Dionex IonPac AG16 Guard and Dionex IonPac AS16 Analytical/Capillary Columns from the system.

B. Install a back pressure coil that generates approximately 1,500 psi and continue to pump eluent.

- If the background conductivity decreases, the column(s) is (are) the cause of the high background conductivity.

C. To eliminate downtime, clean or replace the Dionex IonPac AG16 at the first sign of column performance degradation. Clean the column as instructed in, "Column Cleanup" (See "Column Care").

6.2.5 Contaminated Hardware

Eliminate the hardware as the source of the high background conductivity.

A. Bypass the columns and the suppressor.

B. Install a back pressure coil that generates approximately 1,500 psi and continue to pump eluent.

C. Pump deionized water with a specific resistance of 18.2 megohm-cm through the system.

D. The background conductivity should be less than 2 µS. If it is not, check the detector/conductivity cell calibration by injecting deionized water directly into it. See the appropriate manual for details.

6.2.6 A Contaminated Dionex ASRS 300, Dionex ACES 300 or Dionex AMMS 300 Suppressor

If the above items have been checked and the problem persists, the Dionex Anion Self-Regenerating Suppressor, the Dionex Anion Capillary Electrolytic Suppressor or the Dionex Anion MicroMembrane Suppressor is probably causing the problem. For details on Dionex Anion Self-Regenerating Suppressor operation, refer to the Dionex Anion Self-Regenerating Suppressor 300 Product Manual (Document No. 031956). For details on Dionex Anion Membrane Suppressor 300 operation, refer to the Product Manual (Document No. 031727) for assistance. For details on the Dionex Anion Capillary Electrolytic Suppressor 300 (Dionex ACES 300) operation, refer to the product manual (Document No. 065388) for assistance.

A. Check the power level and alarms on the Dionex SRS Control.

B. Check the regenerant flow rate at the REGEN OUT port of the Dionex ASRS if operating in the AutoSuppression External Waster mode or the Chemical Suppression mode or the Dionex AMMS.

C. Check the eluent flow rate.

D. If you are using a Dionex AutoRegen accessory with the Dionex ASRS in the Chemical Suppression Mode or the Dionex AMMS, prepare fresh regenerant solution.

E. Test both the suppressor and the Dionex AutoRegen Regenerant Cartridge for contamination. If the background conductivity is high after preparing fresh regenerant and bypassing the Dionex AutoRegen Regenerant Cartridge, you probably need to clean or replace your Dionex ASRS, Dionex ACES or Dionex AMMS.

If the background conductivity is low when freshly prepared regenerant is run through the Dionex ASRS or Dionex AMMS without an AutoRegen accessory in-line, test the Dionex AutoRegen Regenerant Cartridge to see if it is expended.

A. Connect the freshly prepared regenerant to the Dionex AutoRegen Regenerant Cartridge.

B. Pump approximately 200 mL of regenerant through the Dionex AutoRegen Regenerant Cartridge to waste before recycling the regenerant back to the regenerant reservoir. If the background conductivity is high after placing the Dionex AutoRegen accessory in-line, you probably need to replace the Dionex AutoRegen Regenerant Cartridge. Refer to the "Dionex AutoRegen Regenerant Cartridge Refill Product Manual" (Document No. 032852) for assistance.

6.3 Poor Peak Resolution

One of the unique features of the Dionex IonPac AS16 is fast equilibration time in gradient applications from the last eluent (high ionic strength) to the first eluent (low ionic strength). The actual equilibration time depends on the ratio of the strongest eluent concentration to the weakest eluent concentration. Typically equilibration times range from 7 to 10 minutes.

If increased separation is needed for the first group of peaks, dilute eluent E1. This part of the chromatogram is run isocratically with E1.

NOTE

Due to different system configurations, the gradient profile may not match the gradient shown in the example. The gradient conditions can be adjusted to improve resolution or to adjust retention times either by changing the gradient timing or by changing the gradient eluent proportions.

A. **Keep the concentrations of E1 and E2 constant and adjust the gradient time.** This is the simplest way to compensate for total system differences if resolution is the problem.

B. **Change the proportions of E1 and E2 and adjust the gradient time.** This approach requires more time to develop and more knowledge in methods development work. Its advantage is that it allows a method to be tailored for a particular application, where selectivity, resolution, and total run time are optimized. Be aware poor peak resolution can be due to any or all of the following factors.

6.3.1 Loss of Column Efficiency

A. **Check to see if headspace has developed in the guard or analytical column.** This is usually due to improper use of the column such as subjecting it to high pressures. Remove the column's top end fitting (see Section 6.1.2, "Replacing Column Bed Support Assemblies"). If the resin does not fill the column body all the way to the top, it means that the resin bed has collapsed, creating a headspace. The column must be replaced.

B. **Extra-column effects can result in sample band dispersion, making the peaks'** elution less efficient. Make sure you are using PEEK tubing with an ID of no greater than 0.010" for 4 mm systems or no greater than 0.005" for 2 mm systems to make all eluent liquid line connections between the injection valve and the detector cell inlet. Cut the tubing lengths as short as possible. Check for leaks.

6.3.2 Poor Resolution Due to Shortened Retention Times

NOTE

Even with adequate system and column efficiency, resolution of peaks will be compromised if analytes elute too fast.

A. **Check the flow rate.** See if the eluent flow rate is equivalent to the flow rate specified by the analytical protocol. Measure the eluent flow rate after the column using a stopwatch and graduated cylinder.

B. **Check to see if the eluent compositions and concentrations are correct.** An eluent that is too concentrated will cause the peaks to elute faster. Prepare fresh eluent.

NOTE

If you are using a gradient pump to proportion the eluent, components from two or three different eluent reservoirs, the resulting eluent composition may not be accurate enough for the application. Use one reservoir containing the correct eluent composition to see if this is the problem. This may be a problem when one of the proportioned eluents is less than 5%.

C. **Column contamination can lead to a loss of column capacity.** This is because all of the anion exchange sites will no longer be available for the sample ions. For example, polyvalent anions from the sample or metals may concentrate on the column. Refer to "Column Cleanup" (see "Column Care"), for recommended column cleanup procedures.

NOTE

Possible sources of column contamination are impurities in chemicals and in the deionized water used for eluents or components of the sample matrix. Be especially careful to make sure that the recommended chemicals are used. The deionized water should have a specific resistance of 18.2 megohm-cm.

D. **Diluting the eluent will improve peak resolution, but will also increase the analytes' retention times.** If a 10% dilution of the eluent is not sufficient to obtain the desired peak resolution, or if the resulting increase in retention times is unacceptable, clean the column (see "Column Cleanup").
After cleaning the column, reinstall it in the system and let it equilibrate with eluent for about 60 minutes. No water wash is necessary. The column is equilibrated when consecutive injections of the standard give reproducible retention times. The original column capacity should be restored by this treatment, since the contaminants should be eluted from the column.

NOTE

For assistance, contact Technical Support for Dionex Products. In the U.S., call 1-800-346-6390. Outside the U.S., call the nearest Thermo Fisher Scientific office.

6.3.3 Loss of Front End Resolution

If poor resolution or efficiency is observed for the peaks eluting near the system void volume compared to the later eluting peaks, check the following:

A. **Improper eluent concentration may be the problem.** Remake the eluent as required for your application. Ensure that the water and chemicals used are of the required purity.

B. **Column overloading may be the problem.** Reduce the amount of sample ions being injected onto the analytical column by either diluting the sample or injecting a smaller volume onto the column.

C. **Sluggish operation of the injection valve may be the problem.** Check the air pressure and make sure there are no gas leaks or partially plugged port faces. Refer to the valve manual for instructions.

D. **Improperly swept out volumes anywhere in the system prior to the guard and analytical columns may be the problem.** Swap components, one at a time, in the system prior to the analytical column and test for front-end resolution after every system change.

6.3.4 Spurious Peaks

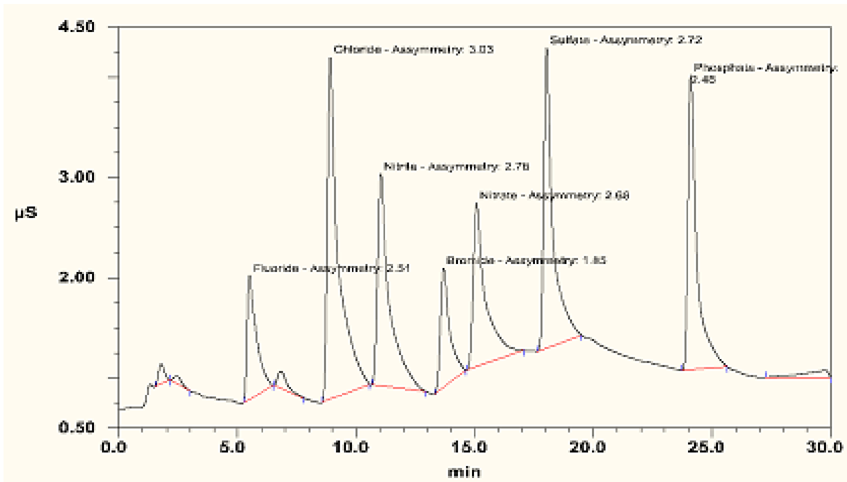
A. **The columns may be contaminated.** If the samples contain an appreciable level of polyvalent ions and the column is used with a weak eluent system, the retention times for the analytes will then decrease and be spurious, inefficient (broad) peaks that can show up at unexpected times. Clean the column as indicated in "Column Cleanup" (see Appendix B).

B. **The injection valve may need maintenance.** When an injection valve is actuated, the possibility of creating a baseline disturbance exists. This baseline upset can show up as a peak of varying size and shape. This will occur when the injection valve needs to be cleaned or retorqued (see valve manual). Check to see that there are no restrictions in the tubing connected to the valve. Also check the valve port faces for blockage and replace them if necessary. Refer to the Valve Manual for troubleshooting and service procedures. Small baseline disturbances at the beginning or at the end of the chromatogram can be overlooked as long as they do not interfere with the quantification of the peaks of interest.

6.3.5 Poor Efficiency Using Capillary Columns

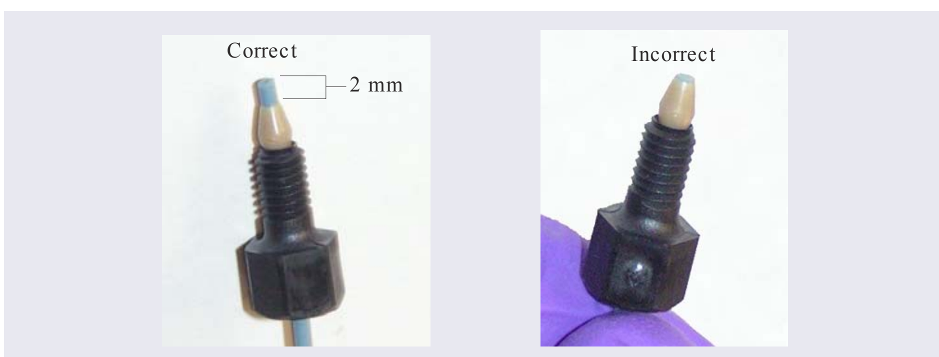
Incorrectly installed fittings on capillary tubing can increase void volumes, causing chromatograms with tailing peaks.

Figure 21 Tailing Peaks Caused by Incorrectly Installed Capillary Tubing Fittings



When connecting a capillary tube fitting, make sure that the ferrule and fitting bolt are at least 2 mm (0.1 in) from the end of the tubing before you insert the tubing into the port. Do not place the ferrule and fitting bolt flush with the end of the tubing. Insert the tubing hard and hold it in place while tightening the fitting. Figure 22 illustrates the correct and incorrect placement of the ferrule and fitting bolt on the tubing. If necessary to hold the ferrule and nut securely, turn the pump off while making capillary connections.

Figure 22 Correct and Incorrect Ferrule and Fitting Bolt Placement for Capillary Tubing Connections



Appendix A – Quality Assurance Report

Quality Assurance Report - Dionex IonPac AS16 Analytical Column - 2 mm

Quality Assurance Report - Dionex IonPac AS16 Analytical Column - 4 mm

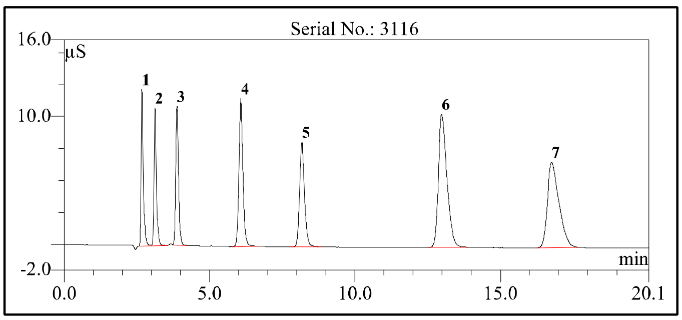
Quality Assurance Report - Dionex IonPac AS16 Capillary Column – 0.4 mm

A.1 Quality Assurance Report - Dionex IonPac AS16 Analytical Column (2 x 250 mm)

Dionex IonPac™ AS16
Analytical (2 x 250 mm)
Product No. 055378

Date: 08-May-12 14:32
Serial No. : 003116
Lot No. : 011-33-092

Eluent:	35 mM NaOH
Flow Rate:	0.25 mL/min
Temperature:	30 °C
Detection:	Suppressed Conductivity
Suppressor:	Dionex Anion Self-Regenerating Suppressor (Dionex ASRS™ 300 2mm) AutoSuppression™ Recycle Mode
Applied Current:	22 mA
Injection Volume:	2.5 µL
Storage Solution:	100 mM Sodium Tetraborate



No.	Peak Name	Ret.Time (min)	Asymmetry (AIA)	Resolution (EP)	Efficiency (EP)	Concentration (mg/L)
1	Fluoride	2.68	2.1	3.59	7692	2.0
2	Chloride	3.13	1.9	5.20	9336	2.0
3	Sulfate	3.88	1.4	10.86	9160	5.0
4	Thiosulfate	6.08	1.2	7.72	9992	10.0
5	Iodide	8.18	1.3	11.59	11756	20.0
6	Thiocyanate	12.98	1.8	6.09	9614	20.0
7	Perchlorate	16.76	2.0	n.a.	8811	30.0

QA Results:

Analyte	Parameter	Specification	Results
Iodide	Efficiency	>=6300	Passed
Iodide	Asymmetry	1.0-1.7	Passed
Iodide	Retention Time	7.75-8.85	Passed
	Pressure	<=1650	1253

Production Reference:

Datasource: Column
Directory CPF\CPF_4
Sequence: 1466083_AS16_2MM _HG
Sample No.: 48

6.80 SR11 Build 3160 (183147) (Demo-Installation)

Chromeleon™ Dionex 1994-2012

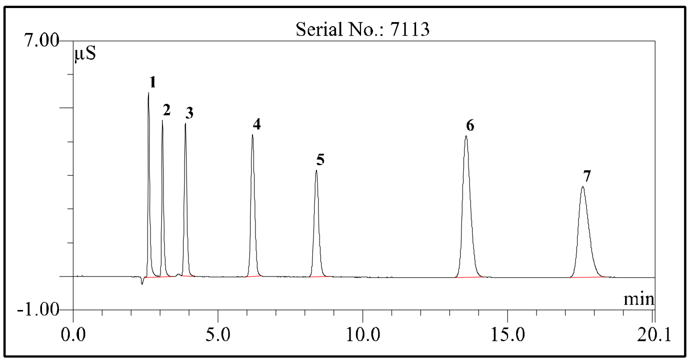
066728-04 (QAR)

A.2 Quality Assurance Report - Dionex IonPac AS16 Analytical Column (4 x 250 mm)

Dionex IonPac™ AS16
Analytical (4 x 250 mm)
Product No. 055376

Date: 01-Jun-12 14:26
Serial No. : 007113
Lot No. : 011-15-112

Eluent:	35 mM NaOH
Flow Rate:	1.0 mL/min
Temperature:	30 °C
Detection:	Suppressed Conductivity
Suppressor:	Dionex Anion Self-Regenerating Suppressor (Dionex ASRS™ 300 4mm) AutoSuppression™ Recycle Mode
Applied Current:	87 mA
Injection Volume:	10 µL
Storage Solution:	100 mM Sodium Tetraborate



No.	Peak Name	Ret.Time (min)	Asymmetry (AIA)	Resolution (EP)	Efficiency (EP)	Concentration (mg/L)
1	Fluoride	2.60	1.8	4.30	9665	2.0
2	Chloride	3.08	1.7	5.83	11256	3.0
3	Sulfate	3.87	1.3	11.72	9785	5.0
4	Thiosulfate	6.19	1.2	8.27	10614	10.0
5	Iodide	8.39	1.2	13.15	13142	20.0
6	Thiocyanate	13.56	1.4	6.99	11981	20.0
7	Perchlorate	17.60	1.5	n.a.	11298	30.0

QA Results:

Analyte	Parameter	Specification	Results
Iodide	Efficiency	>=6300	Passed
Iodide	Asymmetry	1.0-1.7	Passed
Iodide	Retention Time	7.75-8.85	Passed
	Pressure	<=1650	1253

Production Reference:

Datasource: Column
Directory CPF\CPF_5
Sequence: 1467381_AS16_4mm_HG
Sample No.: 48

6.80 SR11 Build 3160 (183147) (Demo-Installation)

Chromeleon™ Dionex 1994-2012

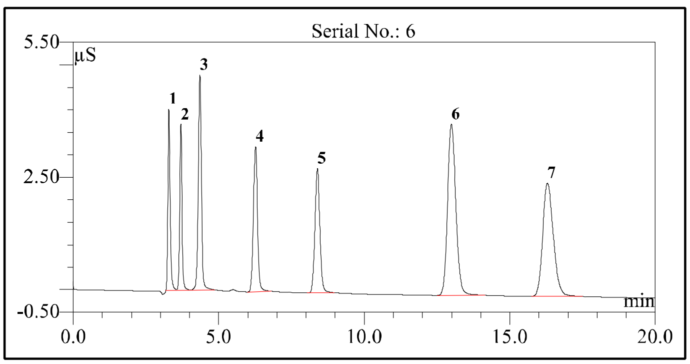
066731-04 (QAR)

A.3 Quality Assurance Report - Dionex IonPac AS16 Analytical Column (0.4 x 250 mm)

Dionex IonPac™ AS16
Capillary (0.4 x 250 mm)
Product No. 082315

Date: 22-Jun-12 13:51
Serial No. : 6
Lot No. : 2011-038-127

Eluent:	35 mM KOH
Eluent Source:	Dionex EGC III Capillary Cartridge
Flow Rate:	0.010 mL/min
Temperature:	30 °C
Detection:	Suppressed Conductivity
Suppressor:	Dionex Anion Capillary Electrolytic Suppressor (ACES™ 300) AutoSuppression™ Recycle Mode
Applied Current:	12 mA
Injection Volume:	0.4 µL
Storage Solution:	100 mM Sodium Tetraborate



No.	Peak Name	Ret.Time (min)	Asymmetry (AIA)	Resolution (EP)	Efficiency (EP)	Concentration (mg/L)
1	Fluoride	3.28	1.3	2.88	8234	0.50
2	Chloride	3.69	1.2	4.05	11014	0.75
3	Sulfate	4.35	1.0	8.72	8905	1.25
4	Thiosulfate	6.26	1.0	7.60	9649	2.50
5	Iodide	8.39	1.1	11.28	12027	5.00
6	Thiocyanate	12.99	1.2	5.64	10255	5.00
7	Perchlorate	16.29	1.4	n.a.	9728	7.50

QA Results:

Analyte	Parameter	Specification	Results
Iodide	Efficiency	>=6300	Passed
Iodide	Asymmetry	1.0-1.7	Passed
Iodide	Retention Time	7.75-8.85	Passed
	Pressure	<=1650	785

Production Reference:

Datasource: Archive-Con
Directory Con-2012\COLUMN\CAPILLARY\CAP_ANION-2
Sequence: AS16_0P4X250_VAL_6-22-2012
Sample No.: 11 6.80 SR10 Build 2818 (166959)

Chromeleon™ Thermo Fisher Scientific

078993-02 (QAR)

Appendix B – Column Care

B.1 Recommended Operating Pressure

Operating a column above its recommended pressure limit can cause irreversible loss of column performance. The maximum recommended operating pressure for Dionex IonPac AS16 columns is 4,000 psi (27.57 MPa).

B.2 Column Start-Up

The column is shipped using 100 mM Sodium Borate as the storage solution. Prepare the eluent shown on the Quality Assurance Report (QAR). Install the column in the chromatography module and direct the column effluent to waste for 30 minutes, then connect to the suppressor. Test the column performance under the conditions described in the QAR. Continue making injections of the test standard until consecutive injections of the standard give reproducible retention times. Equilibration is complete when consecutive injections of the standard give reproducible retention times.

B.3 Column Storage

For short-term storage (< 1 week), use Eluent, for long-term storage (> 1 week), use 100 mM Sodium Borate for the column storage solution. Flush the column for a minimum of 10 minutes with the storage solution. Cap both ends securely, using the plugs supplied with the column.

B.4 Column Cleanup

The following column cleanup protocols have been divided into three general isocratic protocols to remove acid-soluble, base-soluble, or organic contaminants. They can be combined into one gradient protocol if desired; however, the following precautions should be observed.

WARNING

- ***Always ensure that the cleanup protocol used does not switch between eluents which may create high pressure eluent interface zones in the column.***
- ***High pressure zones can disrupt the uniformity of the packing of the column bed and irreversibly damage the performance of the column.***
- ***High pressure zones in the column can be created by pumping successive eluents through the column that are not miscible, that have eluent components in one eluent that will precipitate out in the other eluent or by using an acid eluent followed by a base eluent which may create a neutralization pressure band.***
- ***The precipitation of the salts in solvents during column rinses can result in very high pressure zones. High viscosity mixing zones can be created between two eluents having solvents with a very high energy of mixing.***

When in doubt, always include short column rinse steps to reduce the solvent content of the eluent to ≤ 5% levels and the ionic strength of the eluent to ≤ 50 mM levels to avoid creating high pressure zones in the column that may disrupt the uniformity of the column packing.

B.4.1 Choosing the Appropriate Cleanup Solution

Contamination	Solution
Hydrophilic Contamination of Low Valence	Concentrated hydroxide solutions such as a 10X concentrate of the most concentrated eluent used in the application is sufficient to remove hydrophilic contamination of low valence.
High Valence Hydrophilic Ions Contamination	Concentrated acid solutions such as 1 to 3 M HCl will remove high valence hydrophilic ions by ion suppression and elution by the chloride ion.
Metal Contamination	Metal contamination often results in asymmetric peak shapes and/or variable analyte recoveries. For example, iron or aluminum contamination often results in tailing of sulfate and phosphate. Aluminum contamination can also result in low phosphate recoveries.
	Concentrated acid solutions such as 1 to 3 M HCl remove a variety of metals. If after acid treatment, the chromatography still suggests metal contamination, treatment with chelating acids such as 0.2 M oxalic acid is recommended.
Nonionic and Hydrophobic Contamination	Organic solvents can be used alone if the contamination is nonionic and hydrophobic. The degree of nonpolar character of the solvent should be increased as the degree of hydrophobicity of the contamination within the range of acceptable solvents.
Ionic and Hydrophobic Contamination	Concentrated acid solutions such as 1 to 3 M HCl can be used with compatible organic solvents to remove contamination that is ionic and hydrophobic. The acid suppresses ionization and ion exchange interactions of the contamination with the resin.
	A frequently used cleanup solution is 200 mM HCl in 80% acetonitrile. This solution must be made immediately before use because the acetonitrile will decompose in the acid solution during long term storage.

B.4.2 Column Cleanup Procedure

A. Prepare a 500 mL solution of the appropriate cleanup solution using the guidelines in, "Choosing the Appropriate Cleanup Solution".

B. Disconnect the Dionex ASRS 300 Anion Self-Regenerating Suppressor or Dionex AMMS 300 or Dionex ACES 300 from the Dionex IonPac AS16 Analytical/Capillary Column.

C. If your system is configured with both a guard column and an analytical column, reverse the order of the guard and analytical column in the eluent flow path.

D. Double check that the eluent flows in the direction designated on each of the column labels.

CAUTION

When cleaning an analytical column and a guard column in series, ensure that the guard column is placed after the analytical column in the eluent flow path. If not, the contaminants that have accumulated on the guard column can be eluted onto the analytical column and irreversibly damage it. If in doubt, clean each column separately.

E. Set the pump flow rate to 1.0 mL/min for a Dionex IonPac AS16 4 mm Analytical or Guard Column or set the pump flow rate to 0.25 mL/min for an Dionex IonPac AS16 2 mm Analytical or Guard Column. Set the pump flow rate to 0.010 mL/min for a Dionex IonPac AS16 Capillary or Capillary Guard column.

F. Rinse the column for 10 minutes with deionized water before pumping the chosen cleanup solution over the column.

G. Pump the cleanup solution through the column for at least 60 minutes.

H. Rinse the column for 10 minutes with deionized water before pumping eluent over the column.

I. Equilibrate the column(s) with eluent for at least 60 minutes before resuming normal operation.

J. Reconnect the Dionex ASRS 300 Anion Self-Regenerating Suppressor or Dionex AMMS or Dionex ACES 300 to the Dionex IonPac AS16 Analytical/Capillary Column.

K. Place the guard column in line between the injection valve and the analytical column if your system was originally configured with a guard column.

Appendix C – System Configuration

Table C1 System Configuration

CONFIGURATION	2 mm	4 mm	0.4 mm
Eluent Flow Rate	0.25 mL/min	1.0 mL/min	0.010 mL/min
SRS Suppressor	Dionex ASRS 300 (2 mm) (P/N 061562)	Dionex ASRS 300 (4 mm) (P/N 061561)	N/A
MMS Suppressor	Dionex AMMS (2 mm) (P/N 056751)	Dionex AMMS (4 mm) (P/N 056750)	N/A
ACES Suppressor	N/A	N/A	Dionex ACES 300 (P/N 072052)
Injection Loop	2 - 15 µL	10-50 µL	0.4 µL (typical)
	Rheodyne Microinjection Valve (P/N 044697) for full loop injections <15 µL.		
System Void Volume	Eliminate switching valves, couplers and the Dionex GM-3 Gradient Mixer. Use only the 2 mm Dionex GM-4 Mixer (P/N 049135).	Minimize dead volume. Switching valves, couplers can be used. Use the Dionex GM-2, GM-3 or recommended gradient mixers.	Use only in an IC system equipped for capillary analysis.
Pumps	Use the Dionex ICS 2100/5000, or Dionex GS50/GP50/GP40/ IP20/IP25 in Microbore Configuration with a Microbore Dionex GM-4 (2 mm) Gradient Mixer. The Dionex GPM-2 can be used for 2 mm isocratic chromatography at flow rates of 0.5 mL/min or greater. Note: The GPM-2 should not be used for 2 mm gradient chromatography.	Use the Dionex ICS 2100/5000, or Dionex GP40/GP50/IP20/ IP25 in Standard-Bore Configuration. The Dionex GM-3 Gradient Mixer should be used for gradient analysis on systems other than the Dionex GP50. Note: The Dionex GP40 has an active mixer.	Use only a pump designed for capillary flow rates such as the Dionex ICS-5000 capillary pump.
Note:* *Use of a Dionex EGC-KOH cartridge (P/N 074532 or 072076 in conjunction with a Dionex CR-ATC P/N 060477 or 072078) for gradient applications is highly recommended for minimum baseline change when performing eluent step changes or gradients.			
Chromatographic Module	A thermally controlled column oven such as the Dionex LC25,LC30,ICS-10,11,15,16,20,2100,3000,5000 DC	A thermally controlled column oven such as the Dionex LC25,LC30,ICS-10,11,15,16,20,2100,3000,5000 DC	A thermally controlled column compartment such as the Dionex ICS-5000 DC or Dionex IC-Cube.

CONFIGURATION	2 mm	4 mm	0.4 mm
Detectors	Dionex Conductivity Detector P/N 061830 Dionex AD20/AD25 Cell (6 mm, 7.5 µL, P/N 046423) Dionex VDM-2 Cell (3 mm, 2.0 µL) (P/N 043120) Dionex CD20, CD25, CD25A, ED40, ED50, or ED50A Dionex Conductivity Cell with Dionex DS3 P/N 044130 or Dionex Conductivity Cell with shield P/N 044132 Dionex CDM-2/CDM-3 Cell P/N 042770 Replace the Dionex TS-1 with the Dionex TS-2 (P/N 043117) on the Dionex CDM-2 or the Dionex CDM-3. The Dionex TS-2 has been optimized for 2 mm operation. Do not use the Dionex TS-2 or the Dionex TS-1 with the Dionex ED40/ED50/ED50A or the Dionex CD20/CD25/CD25A. Ensure 30–40 psi back pressure.	Dionex Conductivity Detector P/N 061830 Dionex AD20/AD25 Cell (10 mm, 9 µL, P/N 049393) Dionex VDM-2 Cell (6 mm, 10 µL) P/N 043113 Dionex CD20, CD25, CD25A, ED40, ED50, or ED50A Dionex Conductivity Cell with Dionex DS3 P/N 044130 or Dionex Conductivity Cell with shield P/N 044132 Dionex CDM-2/CDM-3 Cell P/N 042770 Either the Dionex TS-1 with the Dionex TS-2 can be used with the Dionex CDM-2 or the Dionex CDM-3. Do not use the Dionex TS-2 or the Dionex TS-1 with the Dionex ED40/ED50/ED50A or the Dionex CD20/CD25/CD25A. Ensure 30–40 psi back pressure.	Use only a conductivity detector designed for capillary flow rates such as the Dionex ICS-5000 Capillary CD.

Table C2 Tubing Back Pressures

Color	Part Number	I.D. inch	I.D. cm	Volume mL/ft	Back Pressure Psi/ft. at 1 mL/min	Back Pressure Psi/ft. at 0.25 mL/min	Back Pressure Psi/cm. at 1 mL/min
Green	044777	0.030	0.076	0.137	0.086	0.021	0.003
Orange	042855	0.020	0.051	0.061	0.435	0.109	0.015
Blue	049714	0.013	0.033	0.026	2.437	0.609	0.081
Black	042690	0.010	0.025	0.015	6.960	1.740	0.232
Red	044221	0.005	0.013	0.004	111.360	27.840	3.712
Yellow	049715	0.003	0.008	0.001	859.259	214.815	28.642
Light Blue	071870	0.0025	0.006	0.0009	1766.0	441.0	58.0