



Dionex IonPac AS16-4 μ m

065723 Revision 01 • August 2017

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Product Manual

for

Dionex IonPac AS16-4 μ m Capillary Column

(0.4 \times 250 mm, Item # 302757)

Dionex IonPac AS16-4 μ m Analytical Column

(2 \times 250 mm, Item # 302755)

(4 \times 250 mm, Item # 302753)

Dionex IonPac AG16-4 μ m Capillary Guard Column

(0.4 \times 50 mm, Item # 302758)

Dionex IonPac AG16-4 μ m Guard Column

(2 \times 50 mm, Item # 302756)

(4 \times 50 mm, Item # 302754)

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Revision History:

Revision 01, August, 2017, Original Publication.

Safety and Special Notices

Make sure you follow the precautionary statements presented in this guide. The safety and other special notices appear in boxes.

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.

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1. Introduction

The Thermo Scientific™ Dionex™ IonPac™ AS16-4µm Analytical and Capillary Columns are high resolution and 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 high resolution provides better peak identification and high capacity allows injection of more concentrated samples without overloading the column. Dionex IonPac AS16-4µm column capacity and selectivity is similar to the Thermo Scientific Dionex IonPac AS16 column. The Dionex IonPac AS16-4µm 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 AS16-4µm 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 1.5 mL/min. The Dionex IonPac AS16-4µm 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 AS16-4µm 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.

1.1 Dionex IonPac AS16-4µm/Dionex IonPac AG16-4µm Column Packing Specifications

Resin Characteristics:

Particle Size:	4 µm (Analytical/Capillary column*)
Particle Size:	10 µm (Guard/Capillary Guard column**)
Particle Cross-linking:	55%
Ion exchange capacity:	170 µeq per 4 × 250 mm column
	42.5 µeq per 2 × 250 mm column
	1.7 µeq per 0.4 × 250 mm column
	3.5 µeq per 4 × 50 mm column
	0.88 µeq per 2 × 50 mm column
	0.04 µeq per 0.4 × 50 mm column

Latex Characteristics:

Functional Group:	Alkanol quaternary ammonium ion
Hydrophobicity:	Ultra-Low

* Analytical and Capillary Column resin composition: supermacroporous ethylvinylbenzene polymer cross-linked with divinylbenzene.

** Guard Column resin composition: microporous ethylvinylbenzene polymer cross-linked with divinylbenzene.

Table 1 Dionex IonPac AS16-4 μ m/Dionex IonPac AG16-4 μ m Column Operating Parameters

Column	Typical Back Pressure psi (MPa)	Standard Flow Rate mL/min	Maximum Flow Rate mL/min
Dionex IonPac AS16-4 μ m 0.4 mm Capillary Column	\leq 2900 (19.99)	0.010	0.015
Dionex IonPac AG16-4 μ m 0.4 mm Capillary Guard Column	\leq 250 (1.72)	0.010	0.015
Dionex IonPac AS16-4 μ m and AG16-4 μ m 0.4 mm columns	\leq 3150 (21.72)	0.010	0.015
Dionex IonPac AS16-4 μ m 2 mm Analytical Column	$<$ 2900 (19.99)	0.25	0.38
Dionex IonPac AG16-4 μ m 2 mm Guard Column	\leq 250 (1.72)	0.25	0.38
Dionex IonPac AS16-4 μ m and AG16-4 μ m 2 mm columns	\leq 3150 (21.72)	0.25	0.38
Dionex IonPac AS16-4 μ m 4 mm Analytical Column	\leq 2900 (19.99)	1.00	1.5
Dionex IonPac AG16-4 μ m 4 mm Guard Column	\leq 250 (1.72)	1.00	1.5
Dionex IonPac AS16-4 μ m and AG16-4 μ m 4 mm columns	\leq 3150 (21.72)	1.00	1.5

**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 Dionex IonPac AS16-4 μ m Analytical/Capillary Column can only be operated using Ion Chromatographs capable of operating at 5000 psi or higher such as the Thermo Scientific Dionex ICS-5000⁺, Thermo Scientific Dionex Integrion™ HPIC™, or Thermo Scientific Dionex ICS-4000 Integrated Capillary HPIC Systems. These systems are capable of operating up to 5000 psi to support the back pressure generated by the Dionex IonPac AS16-4 μ m Column under standard operating conditions.

See Appendix B, "System Configuration" for specific recommendations including pumps, eluent flow rate, Thermo Scientific Dionex AERS™ 500 Anion Electrolytically Regenerated Suppressor™, Thermo Scientific Dionex ACES™ 300 Anion Capillary Electrolytic Suppressor, injection loop, system void volume, and tubing back pressure.



NOTE

Do not operate suppressors over 40°C. It is highly recommended to use Dionex ACES 300 at lower temperature (15°C) for optimum performance. Use of a Thermo Scientific Dionex EGC 500 KOH (Item # 075778) for the analytical set-up and Thermo Scientific Dionex EGC-KOH (Capillary) (Item # 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 Tetraborate 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 are poorer than the QAR, see Sections 3.15 [Installation of the Capillary Column](#), Section 6.3.1 [Loss of Column Efficiency](#) for information regarding proper connections.

IMPORTANT

When making any tubing connections (column installation, replacing tubing, etc.), it is recommended to make these connections with the pump turned off. This will avoid any slippage of the ferrule under high pressure conditions. For capillary connections, it is recommended to inject water into the cavities of the fluidic system using a syringe or a micropipette with the flow off before joining two components together. This will prevent air from entering the system and result in a faster equilibration.

3.2 Column Storage

For short-term storage (< 1 week), use Eluent, for long-term storage (> 1 week), use 100 mM Sodium Tetraborate 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 for 0.4 mm Operation

The Dionex IonPac AS16-4 μ m Capillary Guard and Capillary Columns are designed to be run on a capillary ion chromatograph system equipped with suppressed conductivity detection with the capability of operating continuously at 5000 psi or higher. For best performance, it is recommended to run the capillary column only on the Dionex ICS-5000⁺ HPIC system or the Dionex ICS-4000 Integrated Capillary HPIC system.

3.4 System Requirements for 2 mm and 4 mm Operation

The Dionex IonPac AS16-4 μ m Guard and Analytical Columns are designed to be run on an Ion Chromatograph equipped with suppressed conductivity detection with the capability of operating continuously at 5000 psi or higher. For best performance, it is recommended to run the analytical column on a system rated 5000 psi or higher such as Dionex ICS-5000⁺ HPIC system or the Dionex Integrion HPIC system.

3.5 System Void Volume

The Dionex ICS-5000⁺ HPIC system, Dionex Integrion HPIC system, and the Dionex ICS-4000 Integrated Capillary HPIC system have preconfigured tubing to minimize the system void volume. The capillary tubing should only be replaced with precut tubing of the same type. It should also be noted that due to system configuration differences, the system void time in the capillary system will typically be longer than that observed with the analytical system at the same linear velocity. Slight modification of the method may be required to ensure equivalent retention time and peak resolution.

3.6 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 Thermo Scientific Dionex Trace Anion Concentrator Low Pressure Column (Dionex TAC-LP1, Item # 046026), the Dionex Trace Anion Concentrator Ultra Low Pressure Column (Dionex TAC-ULP1, Item # 061400), the Dionex Ultra Trace Anion Concentrator Low Pressure Column (Dionex UTAC-LP1, Item # 063079) or (Dionex UTAC-LP2, Item # 079917), the Dionex Ultra Trace Anion Concentrator Ultra Low Pressure Column (Dionex UTAC-ULP1, Item # 063475) or (Dionex UTAC-ULP2, Item # 079918), the Dionex Ultra Trace Anion Concentrator Extremely Low Pressure Column (Dionex UTAC-XLP1, Item # 063459) or (Dionex UTAC-XLP2, Item # 072781), or the Dionex IonPac AG16-4 μ m Guard Column can be used for trace anion concentration work with the 2 mm and 4 mm Dionex IonPac AS16-4 μ m columns. For trace anion concentration work with the 0.4 mm Dionex IonPac AS16-4 μ m column, use the Thermo Scientific 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 Thermo Scientific Dionex Trace Anion Concentrator Low Pressure (Dionex TAC-LP1) and Dionex Ultra Low Pressure (Dionex TAC-ULP1) Column Product Manual (Document No. 034972).
- Section 3, “Operation,” of the Thermo Scientific Dionex Ultra Trace Anion Concentrator Low Pressure (Dionex UTAC-LP1), Dionex Ultra Low Pressure (Dionex UTAC-ULP1), and Dionex Extremely Low Pressure (Dionex UTAC-XLP1) Column Product Manual (Document No. 065091.)
- Section 4, “Operation,” of the Thermo Scientific Dionex Ultra Trace Anion Concentrator 2 Low Pressure (Dionex UTAC-LP2), Dionex Ultra Low Pressure (Dionex UTAC-ULP2), and Dionex Extremely Low Pressure (Dionex UTAC-XLP2) Column Product Manual (Document No. 065376.)



CAUTION

Thermo Scientific Dionex IonPac Trace Anion Concentrator Column, Dionex TAC-2 (Item # 043101), is not optimized for use with hydroxide eluents and should not be used for concentrator work with the Dionex IonPac AS16-4 μ m column. Instead, Concentrators (Dionex TAC-LP1, TAC-ULP1, UTAC 1, UTAC 2 or Dionex IonSwift MAC-100) or Guards (Dionex IonPac AG16-4 μ m 4 mm or Dionex IonPac AG16-4 μ m 2 mm) should be used.

3.7 The Injection Loop

3.7.1 The 0.4 mm System Injection Loop, 0.4 μ L Internal Loop

For most applications on a 0.4 mm capillary system, a 0.4 μ L 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.

3.7.2 The 2 mm System Injection Loop, 2 – 15 μ L

For most applications on a 2 mm analytical system, a 2 – 15 μ L 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. As a general rule, install an injection loop one-fourth or less (<15 μ L) of the loop volume used with a 4 mm analytical system.

3.7.3 The 4 mm System Injection Loop, 10 – 50 μ L

For most applications on a 4 mm analytical system, a 10 – 50 μ L 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.

3.8 The Dionex IonPac AG16-4 μ m Guard/Capillary Guard Column

A Dionex IonPac AG16-4 μ m Guard/Capillary Guard Column is normally used with the Dionex IonPac AS16-4 μ m Analytical/Capillary Column. Retention times will increase by approximately 4.5% when a guard column is placed in-line prior to the analytical/capillary column under isocratic test conditions. A 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 column than it is an analytical/capillary column. Replacing the Dionex IonPac AG16-4 μ m Guard/Capillary Guard Column at the first sign of peak efficiency loss or decreased retention time will prolong the life of the Dionex IonPac AS16-4 μ m Analytical/Capillary Column.

3.9 Installing the Dionex CR-ATC Trap Column for Use with Dionex EGC

For Dionex IonPac AS16-4 μ m 2mm and 4mm applications using eluent generation, a Dionex CR-ATC 500 (Item # 075550) should be installed at the EGC eluent outlet to remove trace level anionic contaminants from the carrier deionized water. A Dionex CR-ATC 600 (Item # 088662) should be used in Dionex Integrion HPIC systems. For capillary applications, use the Dionex CR-ATC (Capillary) (Item # 072078). See the Dionex CR-TC Product Manual (Document No. 079684) for instructions.

3.10 Eluent Storage

Dionex IonPac AS16-4 μ m columns are designed to be used with hydroxide eluent systems. Deionized water storage under a helium atmosphere ensures contamination free operation and proper pump performance (nitrogen can be used if eluents do not contain solvents).

3.11 Dionex Anion Electrolytically Regenerated Suppressor Requirements

A Dionex Anion Electrolytically Regenerated 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 Dionex AERS 500 modes of operation.



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

For Dionex IonPac AS16-4 μ m 4 mm Analytical Column, use a Dionex AERS 500 (4 mm, Item # 082540).

For Dionex IonPac AS16-4 μ m 2 mm Analytical Column, use a Dionex AERS 500 (2 mm, Item # 082541).

For Dionex IonPac AS16-4 μ m 0.4 mm Capillary Column, use a Dionex ACES 300 (Item # 072052).

For detailed information on the operation of the Dionex Anion Electrolytically Regenerated Suppressor, see Document No. 031956, the “Product Manual for the Dionex Electrolytically Regenerated Suppressor 500, the Dionex AERS 500”.

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”.

3.12 Dionex Chemically Regenerated Suppressor Requirements

A Thermo Scientific Dionex Anion Chemically Regenerated Suppressor (Dionex ACRS 500) may be used instead of a Dionex AERS 500 for applications that require suppressed conductivity detection. Use a Dionex ACRS 500 (4 mm) (Item # 085090) with the Dionex IonPac AS16-4 μ m 4 mm Analytical Column. For 2 mm operation, use the Dionex ACRS 500 (2 mm) (Item # 085091). They are compatible with all solvents and concentrations with which the systems and columns are compatible.

For detailed information on the operation of the Dionex Anion Chemically Regenerated Suppressor, see Document No. 031727, the “Product Manual for the Dionex Anion Chemically Regenerated Suppressor 500, the Dionex ACRS 500”.

3.13 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 Chemically Regenerated Suppressor (Dionex ACRS 500). See the DCR kit manual, Document Item # 031664, for details.



SAFETY

Use proper safety precautions in handling acids and bases.

3.14 Dionex EGC-KOH Cartridge with Dionex IonPac AS16-4 μ m Column

The Dionex IonPac AS16-4 μ m column is recommended for use with ion chromatographs equipped with a Thermo Scientific Dionex Eluent Generator Cartridge (Dionex EGC 500 KOH Cartridge for analytical systems (Item # 075778) or Dionex EGC-KOH (Capillary) Cartridge (Item # 072076) for capillary systems). The Dionex Eluent Generator is used to automatically produce potassium hydroxide gradients from deionized water. For detailed information on the operation of the Dionex EGC Cartridges, see Document No. 065018, the "Product Manual for the Dionex Eluent Generator Cartridges".

3.15 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

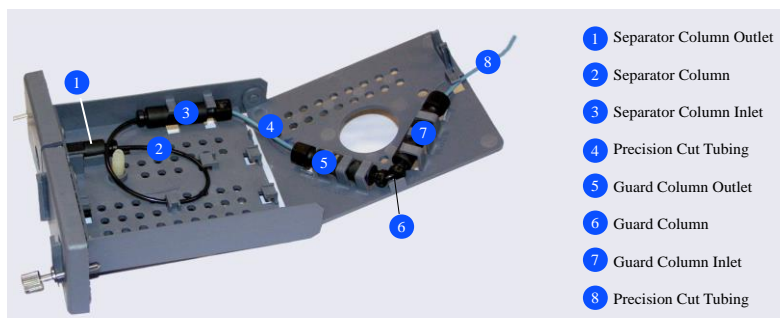
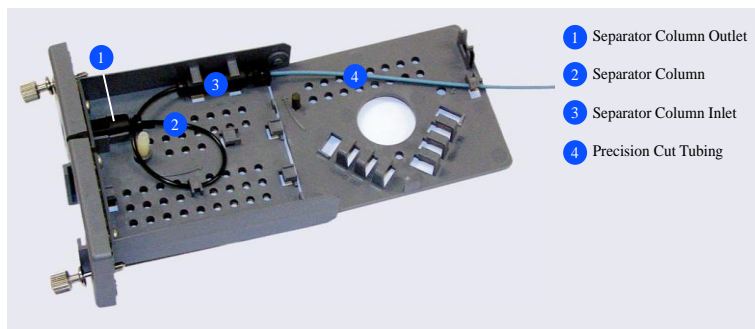


Figure 2 Separator Column Only Installed in Column Cartridge



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

Table 2 Contents of the Dionex IC Cube Tubing Kit (Item # 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	125 mm (4.92 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)
0.125 mm (0.005-in) ID PEEK tubing, red	610 mm (24 in)	044221	Injection valve to waste
Fitting bolt, 10-32 double-cone, blue	7	074449	Connect precision cut 0.062 mm (0.0025-in) ID PEEK tubing
Ferrule fitting, 10-32 double-cone, blue	7	074373	Use with fitting bolt

3 – Installation

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

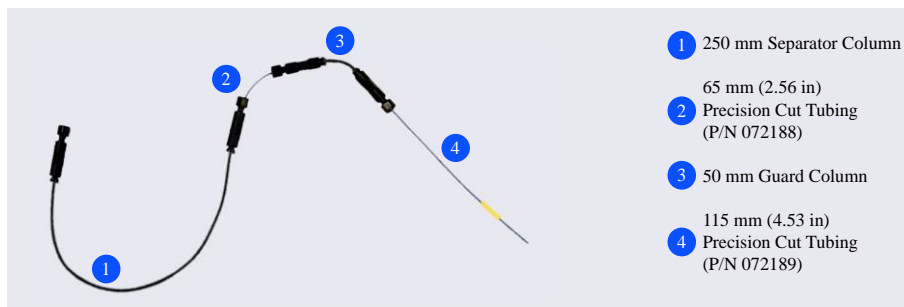
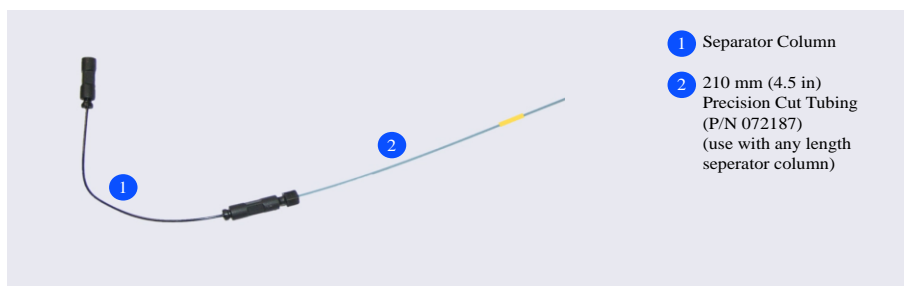
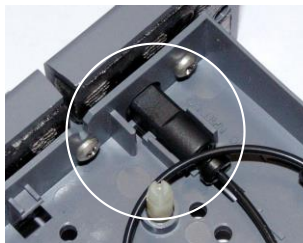


Figure 4 Tubing Connections for Separator Column Only



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).



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 μ L Loop 2 mm: 2.5 μ L Loop + 0.8 μ L Injection valve dead volume 4 mm: 10 μ L Loop + 0.8 μ L Injection valve dead volume
Column:	0.4 mm: Dionex IonPac AS16-4 μ m 0.4 mm Capillary Column + Dionex IonPacAG16-4 μ m 0.4 mm Capillary Guard Column 2 mm: Dionex IonPac AS16-4 μ m 2 mm Analytical Column + Dionex IonPac AG16-4 μ m 2 mm Guard Column 4 mm: Dionex IonPac AS16-4 μ m 4 mm Analytical Column + Dionex IonPac AG16-4 μ m 4 mm Guard Column
Eluent:	35 mM KOH (for Quality Assurance Report)
Eluent Source:	0.4 mm: Dionex EGC-KOH (Capillary) cartridge 2 mm and 4 mm: Dionex EGC 500 KOH cartridge
Eluent Flow Rate:	0.4 mm: 0.010 mL/min 2 mm: 0.25 mL/min 4 mm: 1.0 mL/min
ERS Suppressor:	Dionex Anion Electrolytically Regenerated Suppressor, Dionex AERS 500 (2 mm or 4 mm) Dionex Anion Capillary Electrolytic Suppression, Dionex ACES 300 (0.4 mm) AutoSuppression Recycle Mode
Expected Background Conductivity:	< 2 μ S
Long-term Storage Solution (> 1 week):	100 mM Sodium Tetraborate
Short-term Storage Solution (< 1 week):	Eluent

4.2 Dionex IonPac AS16-4 μ m Column Operating Precautions

Table 3 Operating Precautions

Filter and Degas Eluents and Samples if Necessary	
Eluent pH	Between 0 and 14
Sample pH	Between 0 and 14
Maximum Flow Rate for 0.4 mm Columns	0.015 mL/min
Maximum Flow Rate for 2 mm Columns	0.38 mL/min
Maximum Flow Rate for 4 mm Columns	1.5 mL/min
Maximum Operating Pressure	5,000 psi (34.47MPa)

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 ionic 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 AS16-4 μm column 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® LC/MS Grade 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 AS16-4 μm column is 5,000 psi (34.47MPa).

The Dionex IonPac AS16-4 μm 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.



NOTE

Adding solvent to the aqueous eluent can reduce the peak response by up to half due to increased eluent viscosity, decreased ionization of organic acids and lower peak efficiencies. Therefore, only use solvent in the eluent when needed for improved resolution of analytes of interest.

Table 4 HPLC Solvents for Use with Dionex IonPac AS16-4 μ m Column

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 < 4000 psi.

**CAUTION**

The Dionex AERS 500 and Dionex ACES 300 suppressors must be operated in the AutoSuppression External Water Mode when using eluents containing solvents. Do not use > 40% solvent with the Dionex AERS 500 and Dionex ACES 300 suppressors in the electrolytic mode (power on).

4.4 Making Eluents that Contain Solvents

When mixing solvents with water, remember to mix solvent with water on a volume to volume basis. For example, 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.

**NOTE**

Always degas and store all eluents in glass or 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.

**NOTE**

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 solutions.

4.5 Eluent Preparation

The Dionex Eluent Generator Cartridge (Dionex EGC 500 KOH cartridge or Dionex EGC-KOH (Capillary) cartridge) is used to automatically produce potassium hydroxide gradients from deionized water. Please refer to the Dionex ICS-5000⁺ HPIC system (Document No. 065446), or the Dionex ICS-4000 Capillary HPIC system (Document No. 065468) manual for information on the operation of the Eluent Generator.

For detailed information on the operation of the Dionex EGC Cartridges, see Document No. 065018, the "Product Manual for the Dionex Eluent Generator Cartridges".

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 AS16-4 μ m 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 2 μ S total baseline change.

Ensure that your system is properly configured. 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.

The addition of chromate to the sample will help stabilize organic acids. If your sample or standard contains organic acids, adding chromate (about 10 mg/L) will help stabilize them from bacterial degradation at room temperature. See the sample chromatogram in Section 5.3, "[Separation of Polarizable and Inorganic Anions Using Gradient Elution](#)".

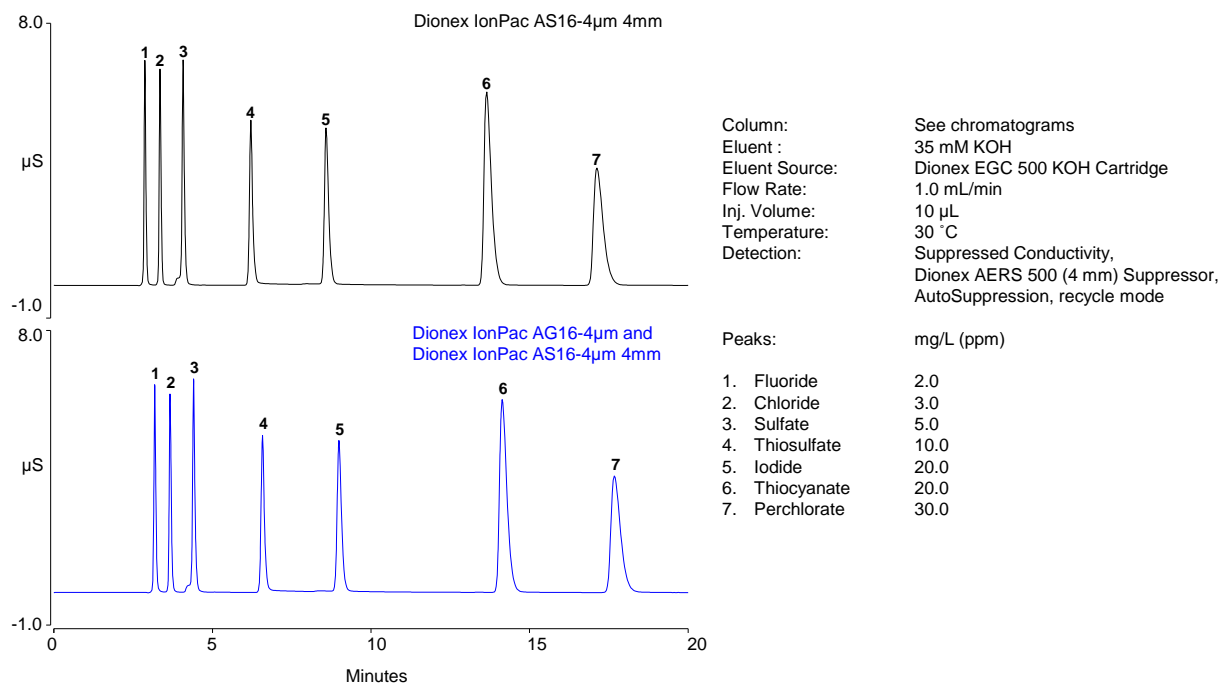
Use a guard/capillary guard column to protect the analytical/capillary column. If column performance deteriorates and it is determined that the guard/capillary guard and analytical/capillary columns have been fouled, refer to the column cleanup protocols in Appendix A, "[Column Care](#)."

The sensitivity of the IC system can be increased by using sample concentration techniques (see Section 3.6, "[The Sample Concentrator](#)").

5.2 Dionex IonPac AS16-4 μ m Column With and Without Guard Column

Isocratic elution of common anions using the Dionex IonPac AS16-4 μ m Analytical/Capillary Column has been optimized utilizing a hydroxide eluent. By using this eluent, common inorganic anions can be used to test the performance of the Dionex IonPac AS16-4 μ m Analytical/Capillary Column. The Dionex IonPac AS16-4 μ m Analytical/Capillary Column should always be used with the Dionex IonPac AG16-4 μ m Guard/Capillary Guard Column. An operating temperature of 30°C is used to ensure reproducible resolution and retention of analytes. Note that the Dionex IonPac AG16-4 μ m Guard/Capillary Guard column is packed with a microporous resin of proportionally lower capacity and increases retention times by approximately 4.5% when used in-line prior to the Analytical/Capillary column under isocratic test conditions as shown in Figures 7, 8, and 9.

Figure 7 Dionex IonPac AS16-4 μ m Column (4 × 250 mm) With and Without Guard Column



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Figure 8 Dionex IonPac AS16-4 μ m Column (2 \times 250 mm) With and Without Guard Column

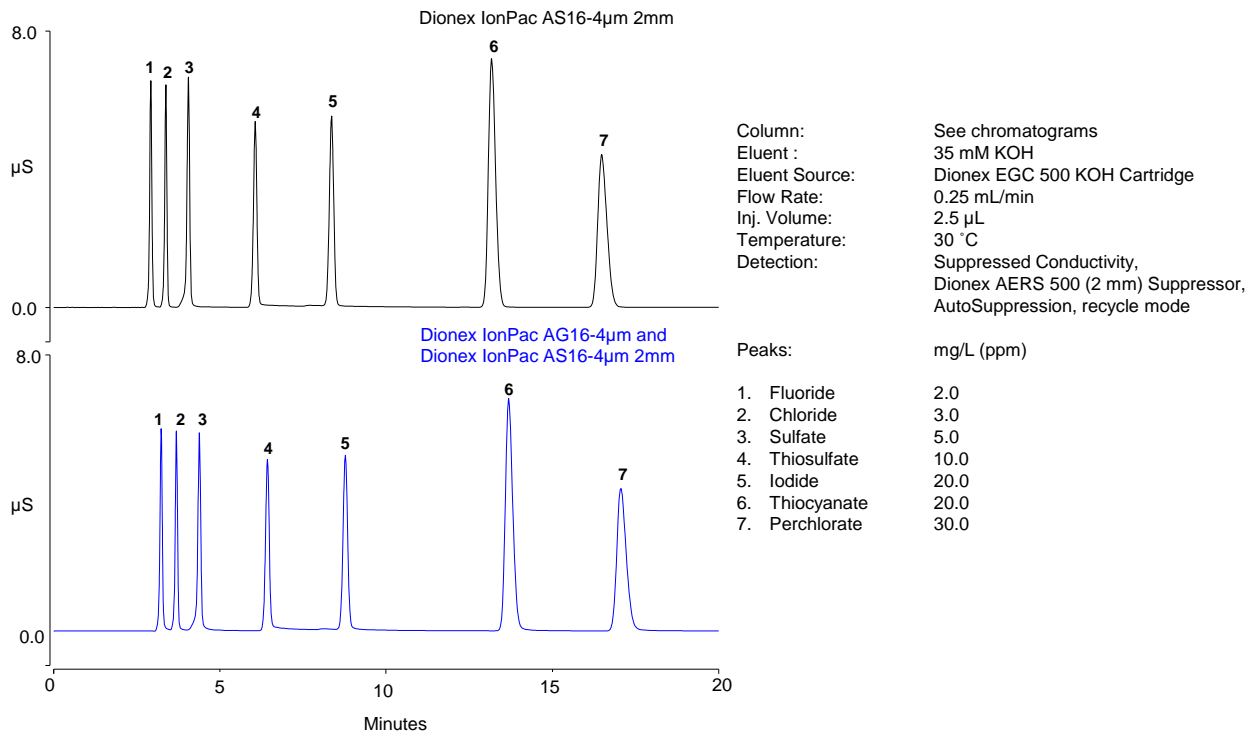
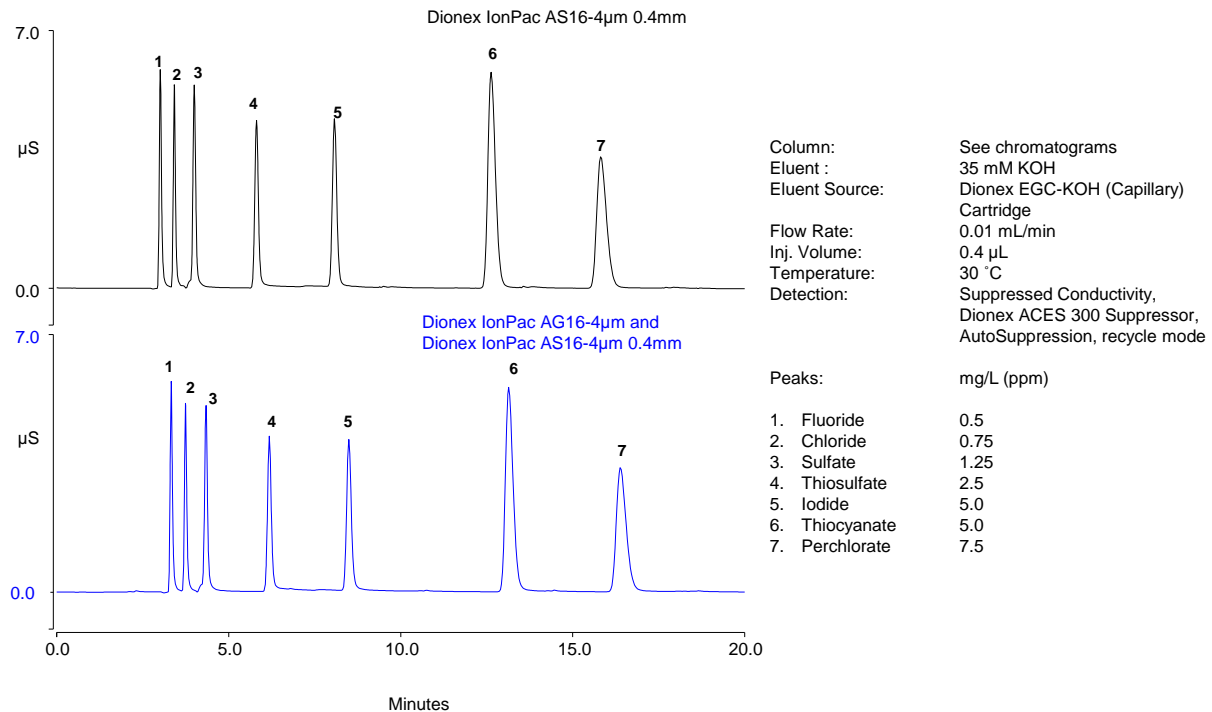


Figure 9 Dionex IonPac AS16-4 μ m Capillary Column (0.4 \times 250 mm) With and Without Capillary Guard Column

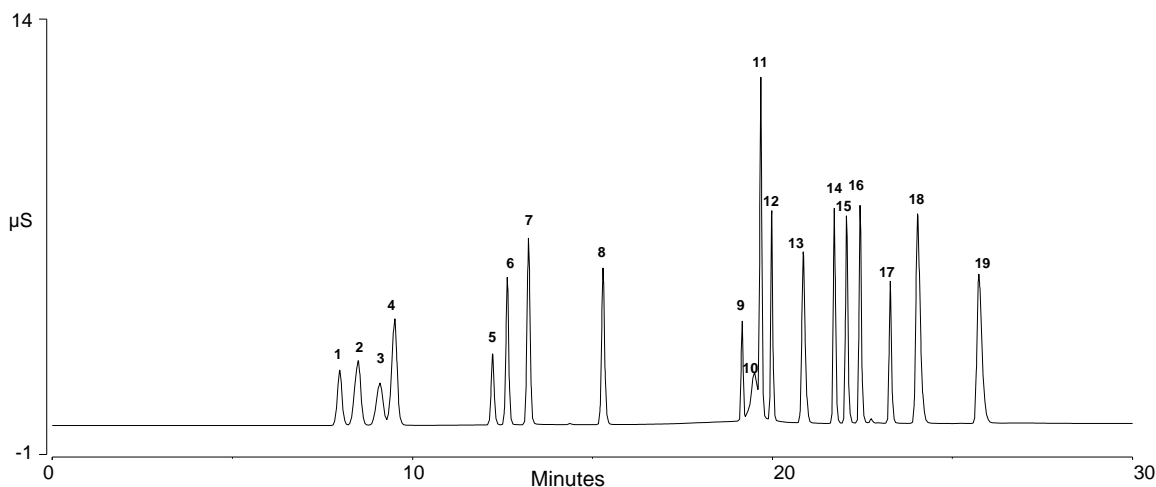


5.3 Separation of Polarizable and Inorganic Anions using Gradient Elution

Figures 10-12 illustrate 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 and inorganic anions using the Dionex IonPac AS16-4 μ m column.

Figure 10 Separation of Polarizable and Inorganic Anions Using Gradient Elution (4 \times 250 mm)

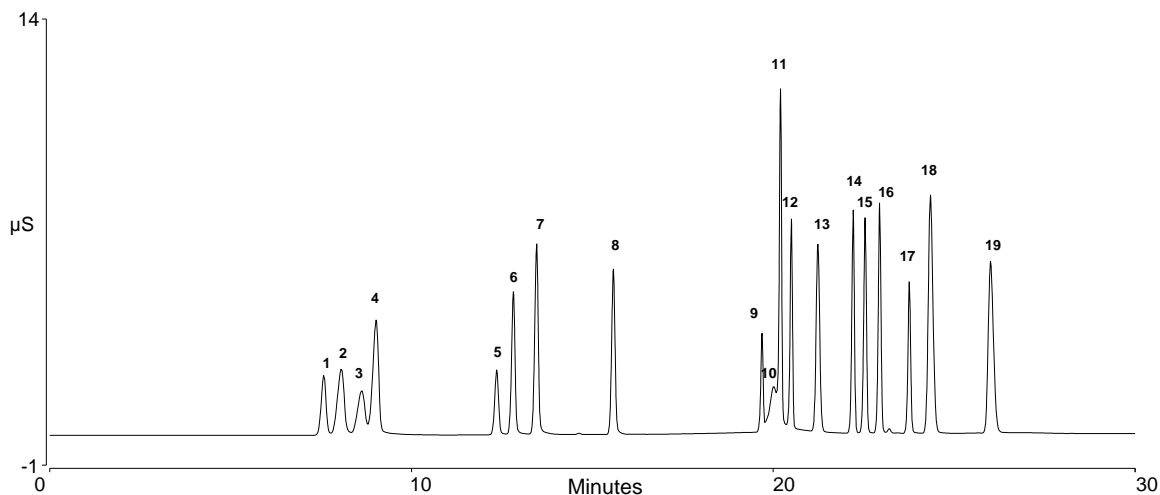
Column:	Dionex IonPac AG16-4 μ m / AS16-4 μ m (4 x 250 mm)	Peaks:	mg/L (ppm)	Peaks:	mg/L (ppm)
Eluent :	1.5 mM KOH from 0 to 8 minutes, 1.5-10 mM KOH from 8 to 14 minutes, 10-55 mM KOH from 14 to 24 minutes, 55 mM KOH from 24 to 30 minutes	1. Fluoride	2.0	11. Sulfate	10.0
Eluent Source:	Dionex EGC 500 KOH Cartridge	2. Acetate	10.0	12. Selenate	10.0
Flow Rate:	1.5 mL/min	3. Propionate	10.0	13. Iodide	20.0
Inj. Volume:	10 μ L	4. Formate	10.0	14. Thiosulfate	10.0
Temperature:	30 $^{\circ}$ C	5. Bromate	10.0	15. Chromate	20.0
Detection:	Suppressed Conductivity, Dionex AERS 500 (4 mm) Suppressor AutoSuppression, recycle mode	6. Chloride	5.0	16. Phosphate	20.0
		7. Nitrite	10.0	17. Arsenate	20.0
		8. Nitrate	10.0	18. Thiocyanate	20.0
		9. Selenite	10.0	19. Perchlorate	30.0
		10. Carbonate	20.0		



5 – Example Applications

Figure 11 Separation of Polarizable and Inorganic Anions Using Gradient Elution
(2 × 250 mm)

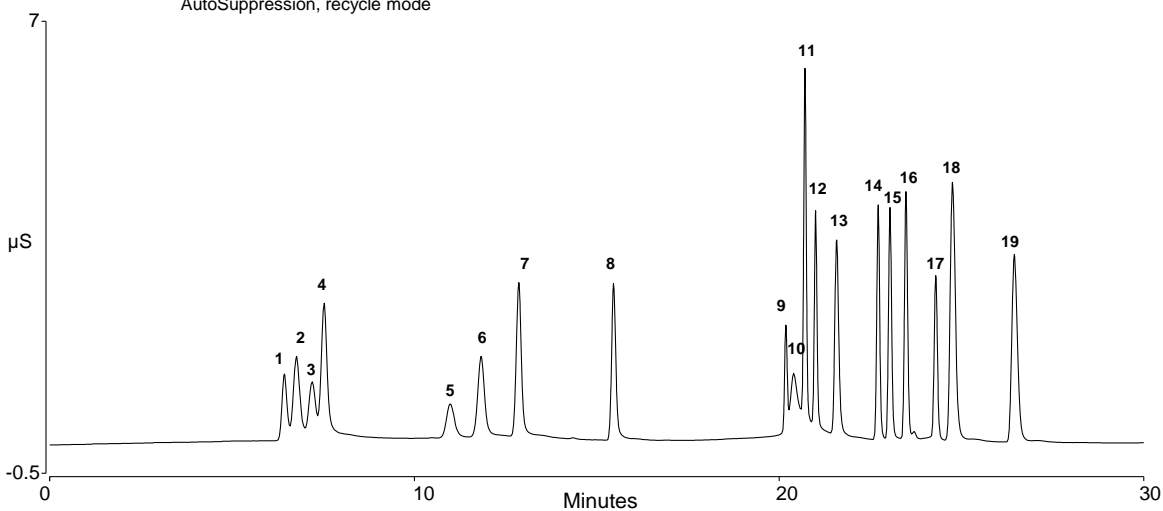
Column:	Dionex IonPac AG16-4 μ m / AS16-4 μ m (2 x 250 mm)	Peaks:	mg/L (ppm)	Peaks:	mg/L (ppm)
Eluent :	1.5 mM KOH from 0 to 8 minutes, 1.5-10 mM KOH from 8 to 14 minutes, 10-55 mM KOH from 14 to 24 minutes, 55 mM KOH from 24 to 30 minutes	1. Fluoride	2.0	11. Sulfate	10.0
Eluent Source:	Dionex EGC 500 KOH Cartridge	2. Acetate	10.0	12. Selenate	10.0
Flow Rate:	0.38 mL/min	3. Propionate	10.0	13. Iodide	20.0
Inj. Volume:	2.5 μ L	4. Formate	10.0	14. Thiosulfate	10.0
Temperature:	30 °C	5. Bromate	10.0	15. Chromate	20.0
Detection:	Suppressed Conductivity, Dionex AERS 500 (2 mm) Suppressor AutoSuppression, recycle mode	6. Chloride	5.0	16. Phosphate	20.0
		7. Nitrite	10.0	17. Arsenate	20.0
		8. Nitrate	10.0	18. Thiocyanate	20.0
		9. Selenite	10.0	19. Perchlorate	30.0
		10. Carbonate	20.0		



5 – Example Applications

Figure 12 Separation of Polarizable and Inorganic Anions Using Gradient Elution (0.4 × 250 mm)

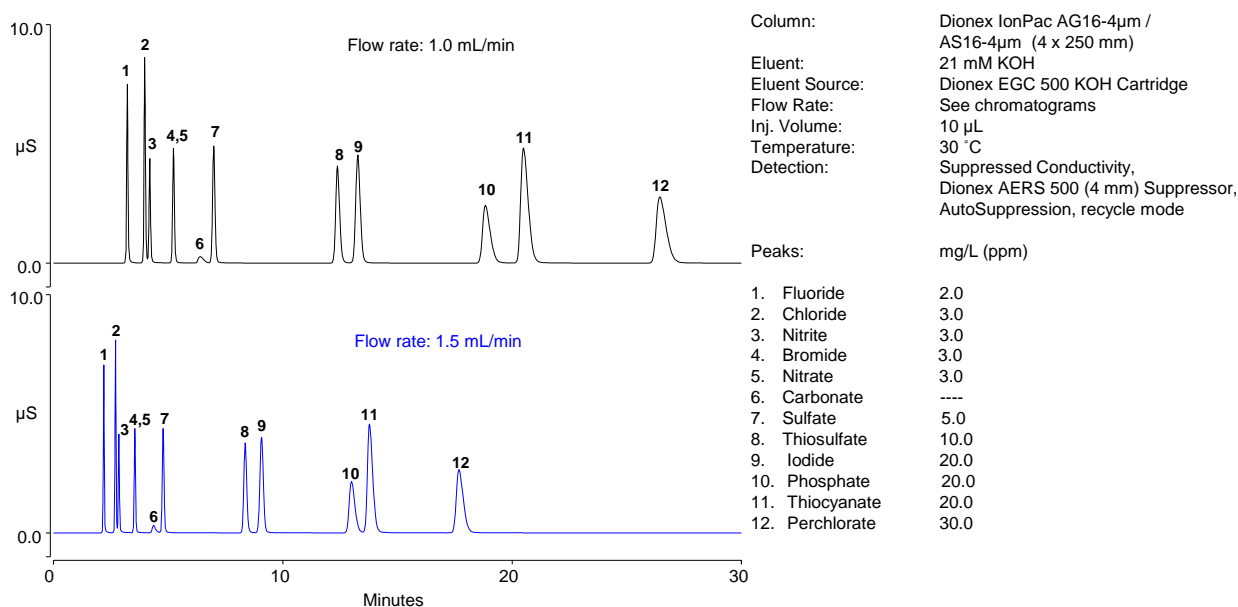
Column:	Dionex IonPac AG16-4 μ m / AS16-4 μ m (0.4 x 250 mm)	Peaks:	mg/L (ppm)	Peaks:	mg/L (ppm)
Eluent :	1.5 mM KOH from 0 to 8 minutes, 1.5-10 mM KOH from 8 to 14 minutes, 10-55 mM KOH from 14 to 24 minutes, 55 mM KOH from 24 to 30 minutes	1. Fluoride	0.5	11. Sulfate	2.5
Eluent Source:	Dionex EGC-KOH (Capillary) Cartridge	2. Acetate	2.5	12. Selenate	2.5
Flow Rate:	0.015 mL/min	3. Propionate	2.5	13. Iodide	5.0
Inj. Volume:	0.4 μ L	4. Formate	2.5	14. Thiosulfate	2.5
Temperature:	30 °C	5. Bromate	2.5	15. Chromate	5.0
Detection:	Suppressed Conductivity, Dionex ACES 300 (Capillary) Suppressor AutoSuppression, recycle mode	6. Chloride	1.25	16. Phosphate	5.0
		7. Nitrite	2.5	17. Arsenate	5.0
		8. Nitrate	2.5	18. Thiocyanate	5.0
		9. Selenite	2.5	19. Perchlorate	7.5
		10. Carbonate	5.0		



5.4 Isocratic Separation of 7 Anions and 4 Polarizable Anions

Figures 13-15 illustrate the isocratic separation of 7 common anions and 4 polarizable anions in a single run. With the standard eluent (35 mM KOH), phosphate elutes too close to the thiosulfate peak. The eluent concentration of 21 mM KOH is optimized for the separation of phosphate and thiosulfate when using 4/2mm column formats. However, the optimum eluent concentration for the Dionex IonPac AS16-4 μ m capillary column is 20 mM KOH. These chromatograms also demonstrate the effect of 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 13 Isocratic Separation of 7 Anions and 4 Polarizable Anions (4 × 250 mm)



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Figure 14 Isocratic Separation of 7 Anions and 4 Polarizable Anions (2 × 250 mm)

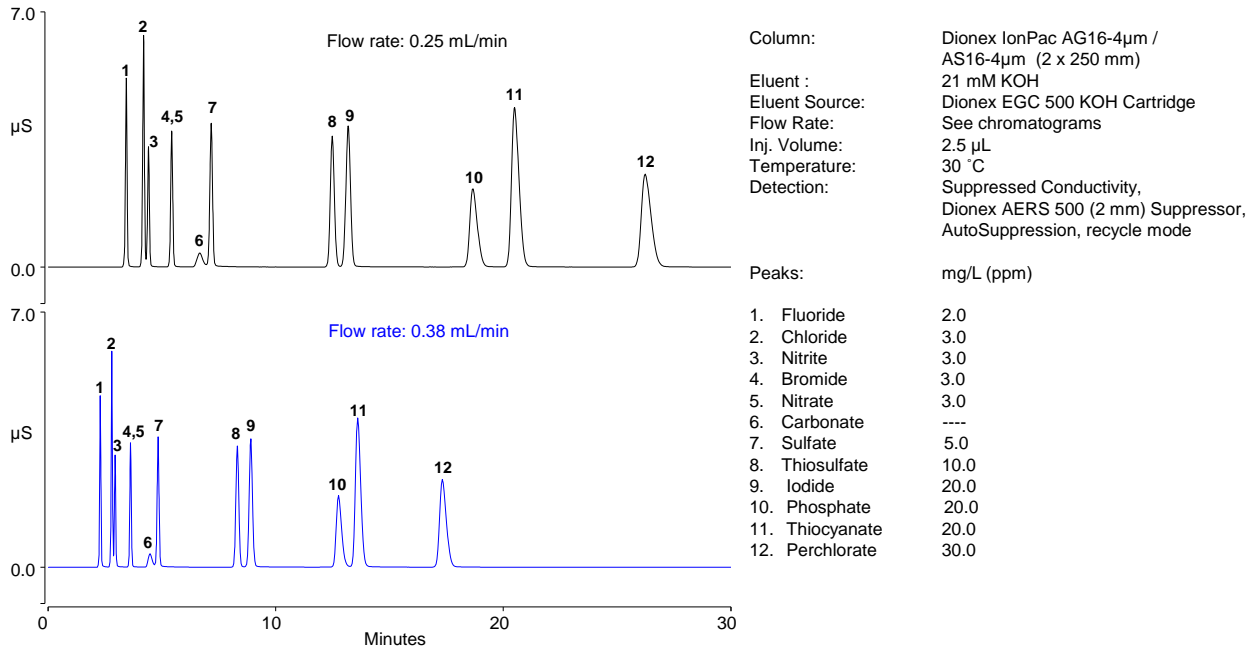
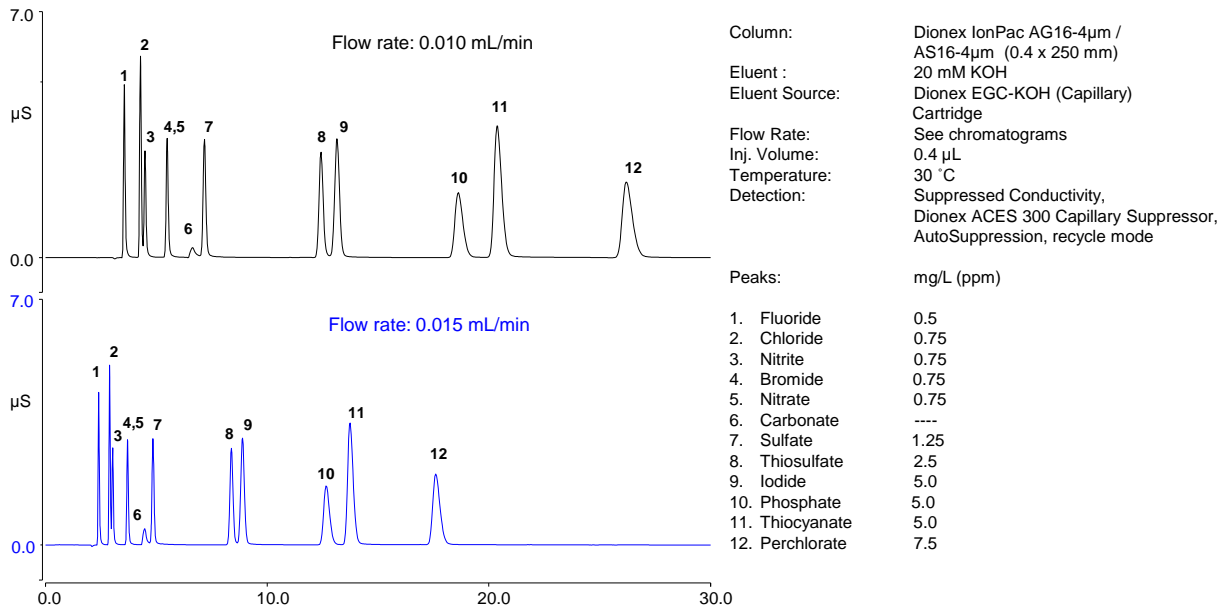


Figure 15 Isocratic Separation of 7 Anions and 4 Polarizable Anions (0.4 × 250 mm)



5.5 Determination of Trace Perchlorate using a Large Loop Injection

Trace concentrations of perchlorate in drinking water, surface water, and ground water matrices can easily be determined using a large loop injection. With a 50 mM potassium hydroxide eluent at a controlled temperature of 30°C, perchlorate can be determined in approximately 10 minutes (Figures 16-18). Note that the perchlorate concentration is 5 ppb for 4/2mm column formats and 10 ppb for 0.4mm capillary format due to matrix overloading of the Dionex IonPac AS16-4µm capillary column. The presence of high matrix can make it difficult to use a large loop injection (10 µL) on the Dionex IonPac AS16-4µm capillary column. Initial screening can be done using the Dionex IonPac AS16-4µm capillary column and a standard injection loop (0.4 µL) for traces of perchlorate (20 ppb) as shown in Figure 19. To further improve detection limits of perchlorate in drinking water sample, the use of a 2D-IC method is highly recommended (see Thermo Scientific [Application Note 1024](#), *Improved Determination of Trace Perchlorate in Drinking Water Using 2D-IC*).

Figure 16 Determination of Trace Perchlorate Using a Large Loop Injection (4 × 250 mm)

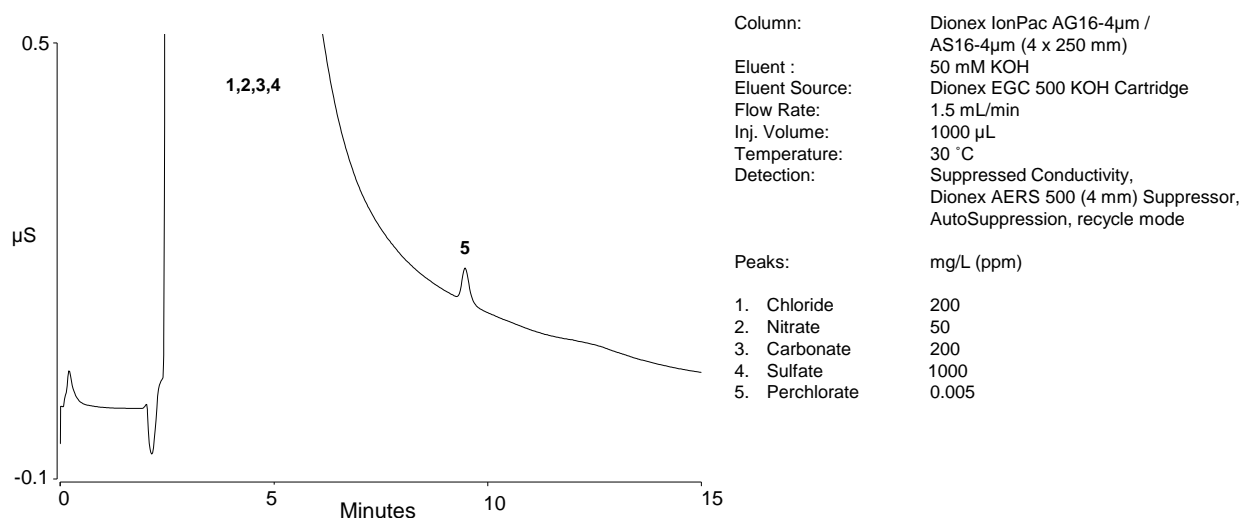


Figure 17 Determination of Trace Perchlorate Using a Large Loop Injection (2 × 250 mm)

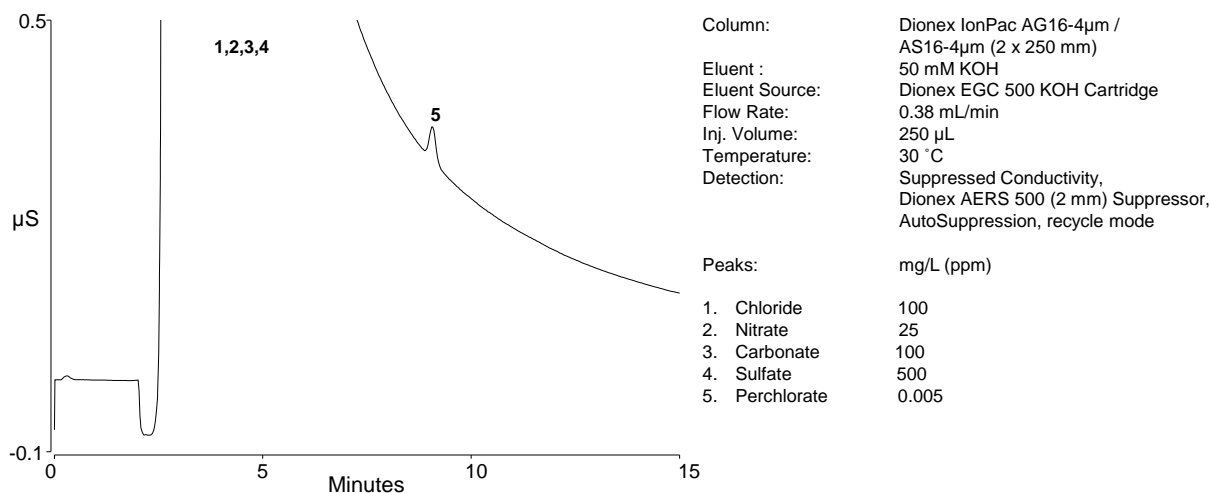


Figure 18 Determination of Trace Perchlorate Using a Large Loop Injection (0.4 × 250 mm)

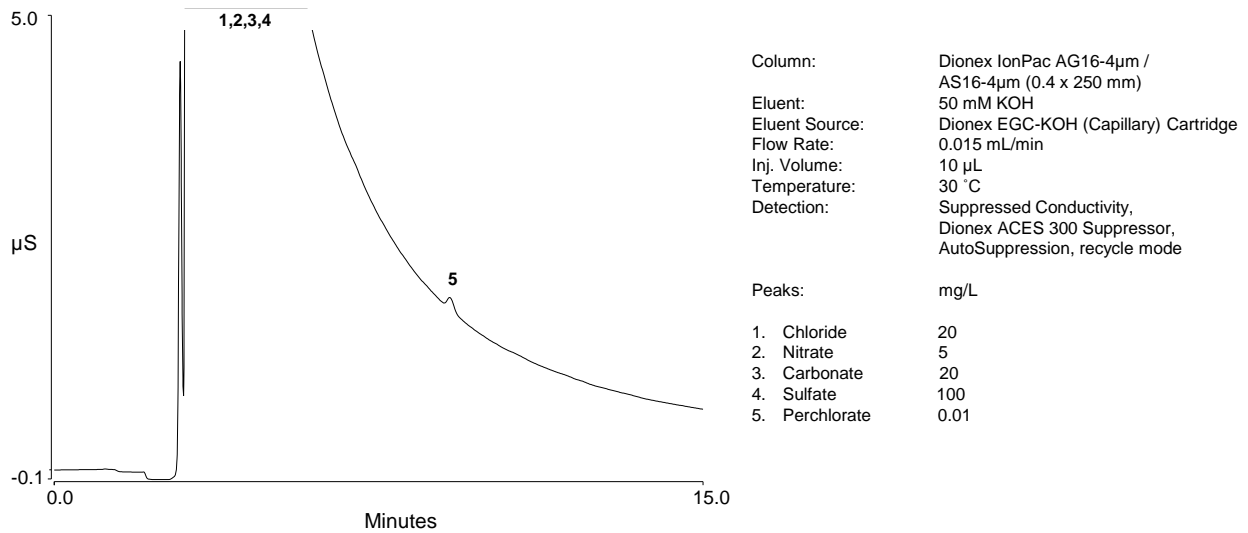
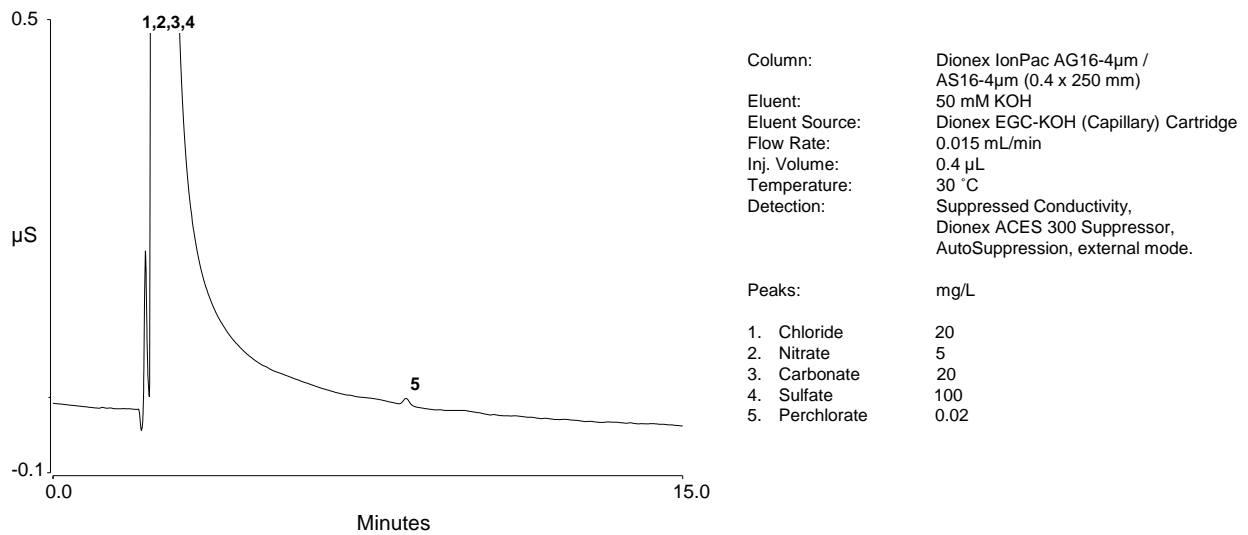


Figure 19 Determination of Trace Perchlorate Using a Standard Loop Injection (0.4 × 250 mm)



5.6 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 25 mM to 65 mM aqueous potassium hydroxide (containing no solvents) at a flow rate of 1.5 mL/min for 4mm column format to elute 9 anions in 10 minutes. In spite of the steep gradient, a minimum baseline shift is observed which facilitates quantitation of trace components.

Figure 20 Gradient Separation of Polyphosphate Anions (4 × 250 mm)

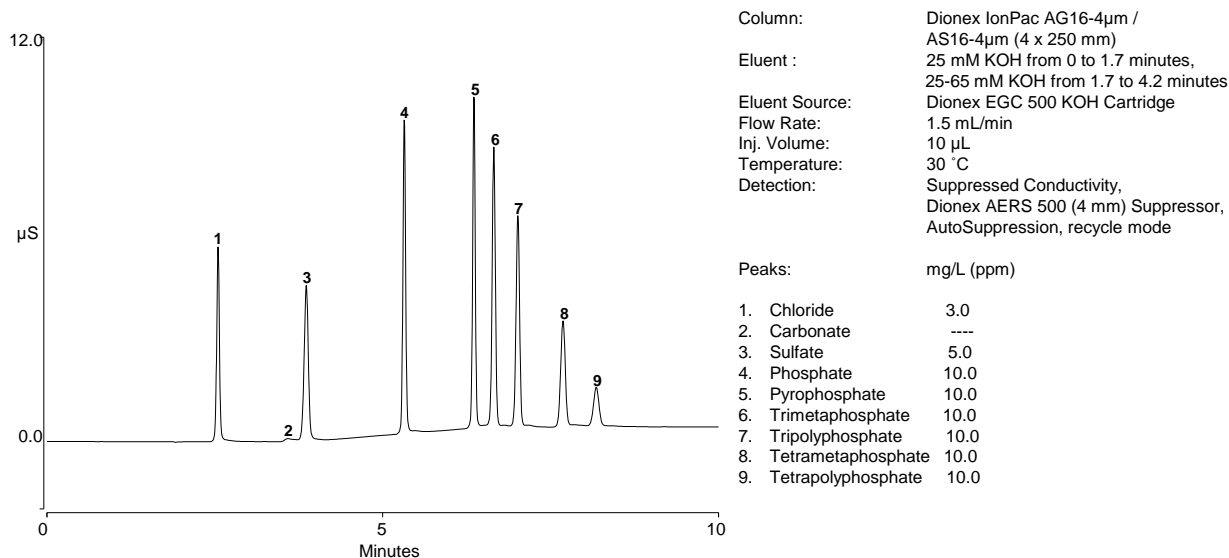


Figure 21 Gradient Separation of Polyphosphate Anions (2 × 250 mm)

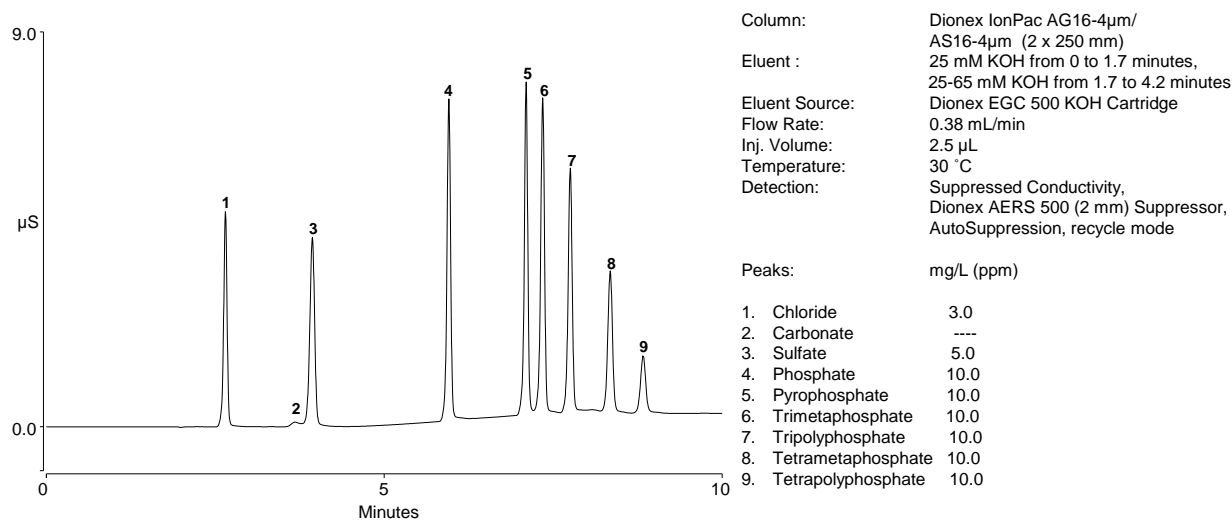
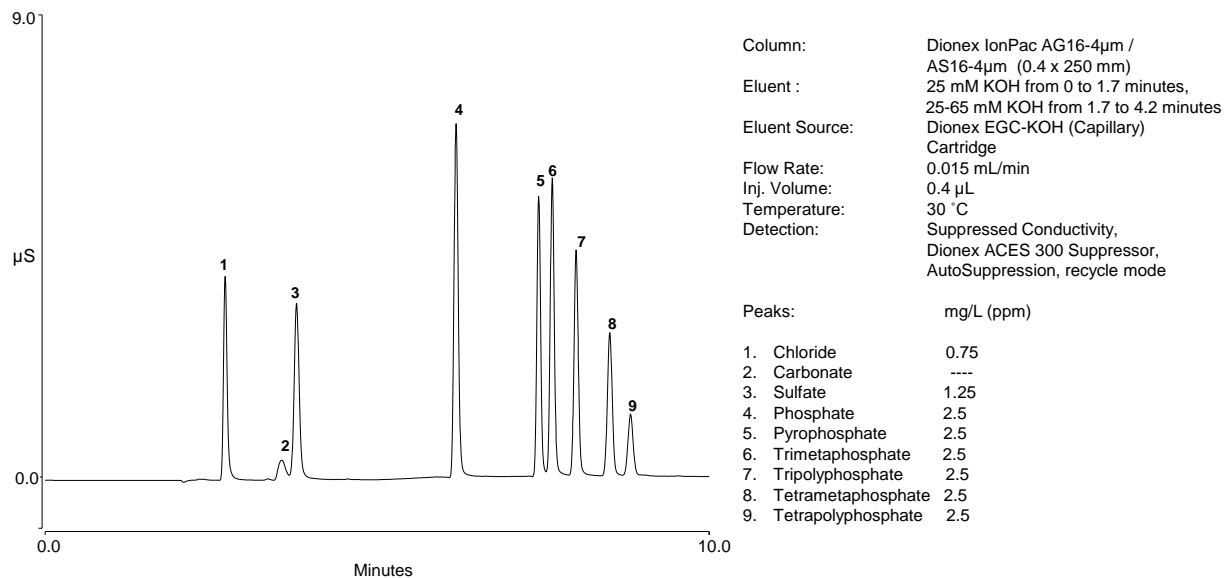


Figure 22 Gradient Separation of Polyphosphate Anions (0.4 × 250 mm)



6. Troubleshooting Guide

The purpose of the Troubleshooting Guide is to help you solve operating problems that may arise while using Dionex IonPac AS16-4 μ m columns. 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. *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 5 Dionex IonPac AS16-4 μ m/Dionex IonPac AG16-4 μ m Troubleshooting Summary

Observation	Cause	Action	Reference Section
High Back Pressure	Unknown	Isolate Blocked Component	6.1.1
	Plugged Column Bed Supports	Replace Bed Supports, Filter Eluents, and Filter Samples	6.1.2
	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
	Contaminated Guard or Analyte Column	Clean Guard and Analytical/Capillary Column	6.2.3
	Contaminated Suppressor	Clean Suppressor	6.2.5, Component Manual
	Contaminated Hardware	Clean Component	6.2.4 Component Manual
Poor Resolution	Method Not Optimized	Optimize Method	6.3.A, B
Poor Efficiency	Large System Void Volumes	Replumb System	6.3.1.B, Component Manual
	Column Headspace	Replace Column	6.3.1.A
	Improper Connections	Remake Connections	6.3.1.C
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, 3.3.1, 3.3.2
	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	Sample 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 AG16-4 μ m Guard/Capillary Guard Column plus the Dionex IonPac AS16-4 μ m Analytical/Capillary Column when using the test chromatogram conditions should be equal or less than 3300 psi. If the system pressure is higher than 3300 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 (Item # 074505) 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 or pinched walls, an injection valve with a clogged port, a column with particulates clogging the bed support, a clogged high-pressure inline 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 500 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/Capillary Guard and Analytical/Capillary columns are connected (see Table 6, “Typical Dionex IonPac AS16-4 μ m/Dionex IonPac AG16-4 μ m Operating Back Pressures”).

The suppressor may add up to 100 psi (0.69 MPa). The EGC 500 KOH can add up to 500 psi at 1.5 mL/min. 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 6 Typical Dionex IonPac AS16-4 μ m/Dionex IonPac AG16-4 μ m Operating Back Pressures

Column	Typical Back Pressure psi (MPa)	Standard Flow Rate mL/min	Maximum Flow Rate mL/min
Dionex IonPac AS16-4 μ m 0.4 mm Capillary Column	\leq 2900 (19.99)	0.010	0.015
Dionex IonPac AG16-4 μ m 0.4 mm Capillary Guard Column	\leq 250 (1.72)	0.010	0.015
Dionex IonPac AS16-4 μ m and AG16-4 μ m 0.4 mm columns	\leq 3150 (21.72)	0.010	0.015
Dionex IonPac AS16-4 μ m 2 mm Analytical Column	$<$ 2900 (19.99)	0.25	0.38
Dionex IonPac AG16-4 μ m 2 mm Guard Column	\leq 250 (1.72)	0.25	0.38
Dionex IonPac AS16-4 μ m and AG16-4 μ m 2 mm columns	\leq 3150 (21.72)	0.25	0.38
Dionex IonPac AS16-4 μ m 4 mm Analytical Column	\leq 2900 (19.99)	1.00	1.5
Dionex IonPac AG16-4 μ m 4 mm Guard Column	\leq 250 (1.72)	1.00	1.5
Dionex IonPac AS16-4 μ m and AG16-4 μ m 4 mm columns	\leq 3150 (21.72)	1.00	1.5

6.1.2 Replacing Column Bed Support Assemblies for 2 mm and 4 mm columns

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 7 Product Information

Product	Dionex IonPac AS16-4µm 4 mm Columns (Item #)	Dionex IonPac AS16-4µm 2 mm Columns (Item #)	Dionex IonPac AS16-4µm 0.4 mm Columns (Item #)
Analytical Column	302753	302755	302757
Guard Column	302754	302756	302758
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 finger-tight, then an additional 1/4 turn (25 in × lb). Tighten further only if leaks are observed.
- F. Reconnect the column to the system and resume operation.

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:

Table 8 Background Conductivity

Eluent	Expected Background Conductivity
1.5 mM KOH	0.2 – 0.8 μ S
65 mM KOH	0.6 – 1.5 μ S

6.2.1 Preparation of Eluents

- Make sure that the eluents and the regenerant are made correctly.
- Make sure that the eluents are made from chemicals with the recommended purity.
- Make sure that the deionized water used to prepare the reagents has a specific resistance of 18.2 megohm-cm.

6.2.2 Contaminated Dionex CR-ATC Column

- Install a Dionex CR-ATC Anion Trap Column (Item # 075550 or 072078) if using a Dionex Eluent Generator with Dionex EGC 500 KOH or Dionex EGC-KOH (Capillary) cartridge.
- If the Dionex CR-ATC becomes contaminated, please refer to Section 6, Clean-Up, in the Dionex CR-ATC Product Manual (Document No. 079684).

6.2.3 A Contaminated Guard/Capillary Guard or Analytical/Capillary Column

- Remove the columns from the system.
- 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.
- To eliminate downtime, clean or replace the analytical/capillary column at the first sign of column performance degradation. Clean the column as instructed in, “Column Cleanup” (See Appendix A “Column Care”).

6.2.4 Contaminated Hardware

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

- Bypass the columns and the suppressor.
- Install a back pressure coil that generates approximately 1,500 psi and continue to pump eluent.
- Pump deionized water with a specific resistance of 18.2 megohm-cm through the system.
- The background conductivity should be less than 2 μ S. If it is not, check the detector/conductivity cell calibration by injecting deionized water directly into it. See the appropriate manual for details.

6.2.5 A Contaminated Suppressor

If the above items have been checked and the problem persists, the suppressor is probably causing the problem. For details on Dionex Anion Electrolytically Regenerated Suppressor operation, refer to the Dionex Electrolytically Regenerated Suppressor 500 Product Manual (Document No. 031956). For details on Dionex Anion Chemically Regenerated Suppressor 500 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. 065386) for assistance.

6.3 Poor Peak Resolution

One of the unique features of the Dionex IonPac AS16-4 μ m 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, reduce the initial eluent concentration.

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 initial and/or final eluent concentration.

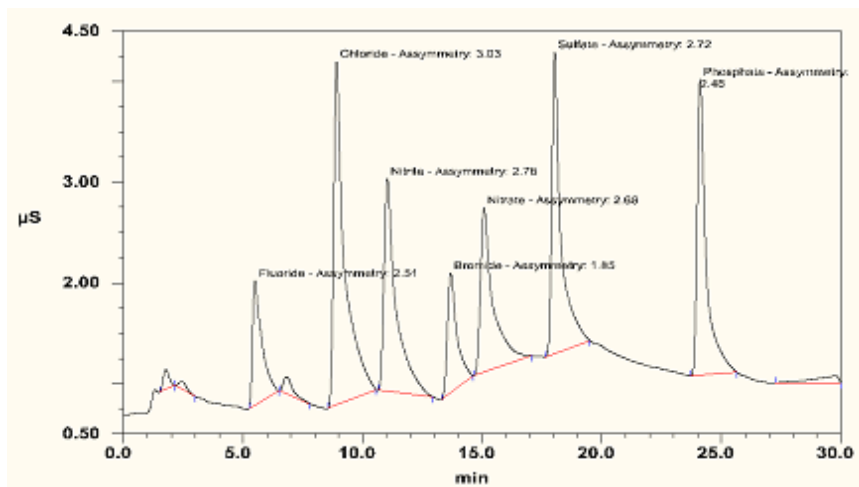
- A. Keep the eluent concentrations constant and adjust the gradient time. This is the simplest way to compensate for total system differences if resolution is the problem.
- B. Change the initial and/or final eluent concentration and adjust the gradient time. This approach requires more time to develop 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 cannot be checked on capillary columns). This is usually due to improper use of the column such as submitting 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. For capillary systems, only use precut tubing of the same type.
- C. If tubing is not connected properly from the inlet and outlet of the column, it can cause low efficiency. When installing AS16-4 μ m columns, it is recommended to turn off the pump while connecting the column inlet and the column outlet to the suppressor. This will avoid any slippage of the ferrule under high pressure conditions which can cause low peak efficiencies.

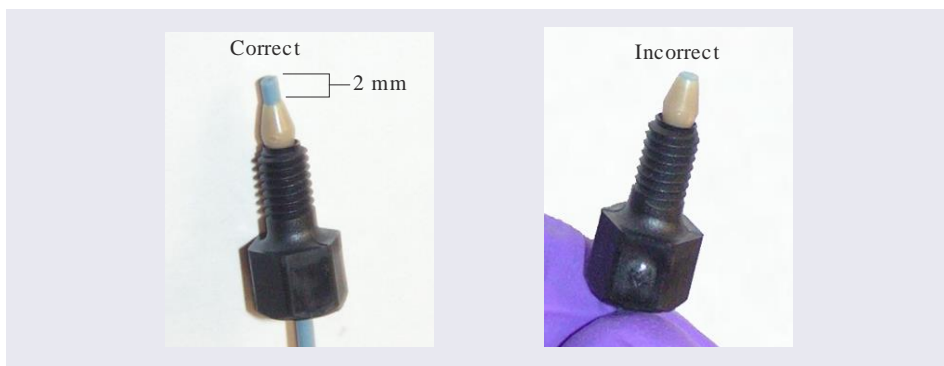
Incorrectly installed fittings on capillary tubing can increase void volumes, causing chromatograms with tailing peaks.

Figure 23 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. Figure 24 illustrates the correct and incorrect placement of the ferrule and fitting bolt on the tubing.

Figure 24 Correct and Incorrect Ferrule and Fitting Bolt Placement for Capillary Tubing Connections



6.3.2 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 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 Appendix A “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, Appendix A, “Column Care”).

After cleaning the column, reinstall it in the system and let it equilibrate with eluent for about 30 minutes directing the column effluent to waste. Then connect the column to the suppressor. 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. If manually prepared eluent is used, remake the eluent as required for your application and ensure that the water and chemicals used are of the required purity. If Dionex eluent generator is used to generate the eluent; check the flow rate, as pump flow rate will affect the eluent concentration.
- B. Column overloading may be the problem. Reduce the amount of sample ions being injected onto the analytical/capillary 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/capillary columns may be the problem. Swap components, one at a time, in the system prior to the analytical/capillary 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 Appendix A “Column Care”.



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.

- 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.

Appendix A – Column Care

A.1 Recommended Operation Pressures

Operating a column above its recommended pressure limit can cause irreversible loss of column performance. The maximum recommended operating pressure for Dionex IonPac AS16-4 μ m columns is 5,000 psi (34.47 MPa).

A.2 Column Start-Up

The column is shipped using 100 mM Sodium Tetraborate 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 are poorer than the QAR, see Sections 3.15 [Installation of the Capillary Column](#), Section 6.3 [Poor Peak Resolution](#) and Section 6.3.1 [Loss of Column Efficiency](#).

IMPORTANT

When making any tubing connections (column installation, replacing tubing, etc.), it is recommended to make these connections with the pump turned off. This will avoid any slippage of the ferrule under high pressure conditions. For capillary connections, it is recommended to inject water into the cavities of the fluidic system using a syringe or a micropipette with the flow off before joining two components together. This will prevent air from entering the system and result in a faster equilibration.

A.3 Column Storage

For short-term storage (< 1 week), use Eluent, for long-term storage (> 1 week), use 100 mM Sodium Tetraborate 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.

A.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. 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.



- *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.*

A.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.

A.4.2 Column Cleanup Procedure

- A. Prepare a 500 mL solution of the appropriate cleanup solution using the guidelines in Section A.4.1, "[Choosing the Appropriate Cleanup Solution](#)."
- B. Disconnect the suppressor from the outlet end of the column and direct the column effluent to waste. If your system is configured with both a guard column and an analytical/capillary column, reverse the order of the guard and analytical/capillary column in the eluent flow path. Double check that the eluent flows in the direction designated on each of the column labels.



CAUTION

When cleaning an analytical/capillary column and a guard column in series, ensure that the guard column is placed after the analytical/capillary column in the eluent flow path. Contaminants that have accumulated on the guard column can be eluted onto the analytical/capillary column and irreversibly damage it. If in doubt, clean each column separately.

- C. Set the pump flow rate to 1.0 mL/min for a 4 mm analytical and/or guard column, 0.25 mL/min for a 2 mm analytical and/or guard column and 0.010 mL/min for 0.4 mm capillary and/or capillary guard column.
- D. Pump DI water at setup flow rate (see section A.4.2.C) for 10 minutes, to rinse the column.
- E. Pump the cleanup solution through the column for at least 60 minutes at setup flow rate (see section A.4.2.C). If column is contaminated heavily, then clean the column for four hours to overnight.
- F. Pump DI water at setup flow rate (see section A.4.2.C) for 10 minutes, to rinse the column again.
- G. Equilibrate the column with eluent still directing the column effluent to waste for at least 60 minutes before resuming normal operation.
- H. Reinstall the guard/capillary guard column in line between the injection valve and the analytical/capillary column and reconnect the analytical/capillary column to the suppressor

Appendix B – System Configuration

Table B1 Configuration

CONFIGURATION	2 mm	4 mm	0.4 mm
Eluent Flow Rate	0.25 mL/min	1.0 mL/min	0.01 mL/min
AERS Suppressor	Dionex AERS 500 (Item # 082541)	Dionex AERS 500 (Item # 082540)	N/A
ACRS Suppressor	Dionex ACRS 500 (Item # 085091)	Dionex ACRS 500 (Item # 085090)	N/A
ACES Suppressor	N/A	N/A	Dionex ACES 300 (Item # 072052)
NOTE: <i>Do not run suppressors over 40°C. If application requires a higher temperature, place suppressor outside of chromatographic oven.</i>			
Injection Loop	2 – 15 µL	10 – 50 µL	0.4 µL
System Void Volume	Eliminate switching valves, couplers and use only the 2 mm Dionex GM-4 Mixer (Item # 049135).	Eliminate switching valves, couplers and use the Dionex GM-2, GM-3 or recommended gradient mixers.	Use only an IC system equipped for capillary analysis where dead volumes are fixed such as the Dionex ICS-5000 ⁺ HPIC capillary system.
Pumps	IC single or dual pump capable of operating up to 5000 psi or higher such as the Dionex ICS-5000 ⁺ HPIC pump.	IC single or dual pump capable of operating up to 5000 psi or higher such as the Dionex ICS-5000 ⁺ HPIC pump.	Use only a pump designed for capillary flow rates such as the Dionex ICS-5000 ⁺ HPIC capillary pump.
NOTE: <i>Use of a Dionex EGC 500 KOH cartridge (Item # 075778 or 072076 in conjunction with a Dionex CR-ATC 500 (Item # 075550 or 072078) for gradient applications is highly recommended for minimum baseline change when performing eluent step changes or gradients.</i>			
Chromatographic Module	A thermally controlled column oven such as the Dionex ICS-5000 ⁺ DC	A thermally controlled column oven such as the Dionex ICS-5000 ⁺ DC	A thermally controlled column compartment such as the Dionex ICS-5000 ⁺ DC equipped with Dionex IC-Cube.

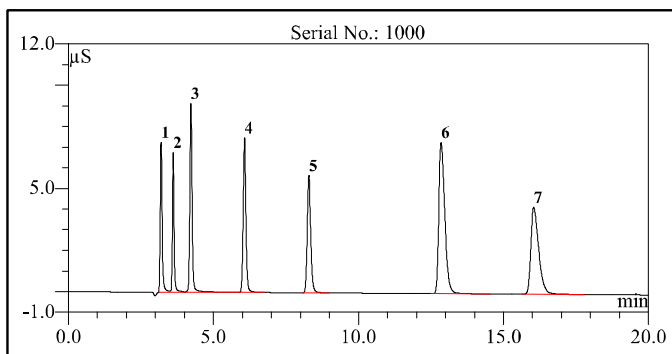
Table B2 Tubing Back Pressures

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

Appendix C – Quality Assurance Reports

Dionex IonPac™ AS16-4µm **Date:** 09-Aug-17 08:46
Capillary (0.4 x 250 mm) **Serial No. :** 001000
Product No. 302757 **Lot No. :** 01329110

Eluent: 35 mM KOH
Eluent Source: Dionex EGC-KOH (Capillary)
Flow Rate: 0.010 mL/min
Temperature: 30 °C
Detection: Suppressed Conductivity
Suppressor: Dionex Anion Capillary Electrolytic Suppressor (ACESTM 300)
 AutoSuppression™ Recycle Mode
Applied Current: 9 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	3.20	1.3	3.88	13678	0.50
2	Chloride	3.61	1.3	5.24	19089	0.75
3	Sulfate	4.22	1.1	12.14	16812	1.25
4	Thiosulfate	6.07	1.1	11.32	19303	2.50
5	Iodide	8.29	1.3	15.49	23159	5.00
6	Thiocyanate	12.85	1.8	7.48	18987	5.00
7	Perchlorate	16.04	1.9	n.a.	17679	7.50

QA Results:

Analyte	Parameter	Specification	Results
Iodide	Efficiency	>=13500	Passed
Iodide	Asymmetry	1.0-1.7	Passed
Iodide	Retention Time	7.75-8.85	Passed
	Pressure	<=3190	2703

Production Reference:

Datasource: QAR
 Directory: Cap\AS16-4µm
 Sequence: AS16-4µM_0P4X250
 Sample No.: 1

6.80 SR15 Build 4656 (243203) (Demo-Installation)

Chromleon™ Thermo Fisher Scientific

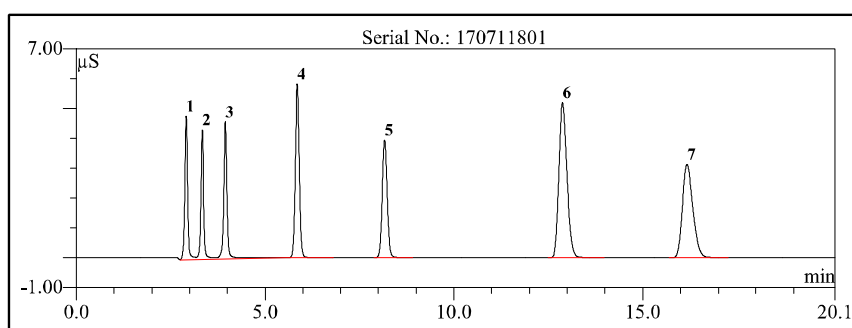
Appendix C – Quality Assurance Reports

Device Monitoring Enabled
and Viper Fitting Ready

Dionex IonPac™ AS16-4µm
Analytical (2 x 250 mm)
Product No. 302755

Date: 11-Jul-17 09:48
Serial No. : 170711801
Lot No. : 01706021

Eluent: 35 mM KOH
Flow Rate: 0.25 mL/min
Temperature: 30 °C
Detection: Suppressed Conductivity
Suppressor: Dionex Anion Electrolytically Regenerated Suppressor (Dionex AERS™ 500 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	2.91	1.1	3.52	9029	2.0
2	Chloride	3.33	1.0	4.63	12154	3.0
3	Sulfate	3.94	1.0	11.57	12113	5.0
4	Thiosulfate	5.85	1.0	10.92	15621	10.0
5	Iodide	8.16	1.1	15.06	18881	20.0
6	Thiocyanate	12.88	1.3	7.40	17440	20.0
7	Perchlorate	16.17	1.4	n.a.	16521	30.0

QA Results:

Analyte	Parameter	Specification	Results
Iodide	Efficiency	>=13500	Passed
Iodide	Asymmetry	1.0-1.7	Passed
Iodide	Retention Time	7.75-8.85	Passed
	Pressure	<=3190	2368

Production Reference:

Datasource: QAR
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Sequence: AS16-4µm_2X250
Sample No.: 1

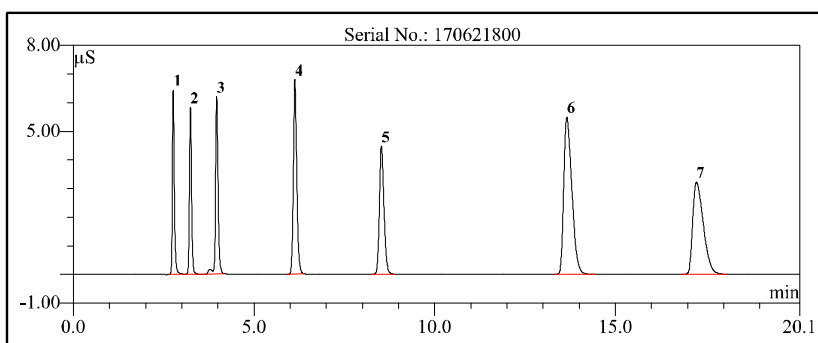
6.80 SR15 Build 4656 (243203) (Demo-Installation)

Chromleon™ Thermo Fisher Scientific

Appendix C – Quality Assurance Reports

Dionex IonPac™ AS16-4µm **Date:** 21-Jun-17 07:34
 Device Monitoring Enabled **Analytical (4 x 250 mm)** **Serial No. :** 170621800
 and Viper Fitting Ready **Product No. 302753** **Lot No. :** 01706021

Eluent: 35 mM KOH
Flow Rate: 1.0 mL/min
Temperature: 30 °C
Detection: Suppressed Conductivity
Suppressor: Dionex Anion Electrolytically Regenerated Suppressor (Dionex AERS™ 500 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	2.76	1.3	4.81	12648	2.0
2	Chloride	3.24	1.3	6.73	17154	3.0
3	Sulfate	3.97	1.3	14.94	17875	5.0
4	Thiosulfate	6.13	1.2	11.93	20295	10.0
5	Iodide	8.53	1.3	16.05	21992	20.0
6	Thiocyanate	13.66	1.9	7.55	17667	20.0
7	Perchlorate	17.25	2.1	n.a.	16143	30.0

QA Results:

Analyte	Parameter	Specification	Results
Iodide	Efficiency	>=13500	Passed
Iodide	Asymmetry	1.0-1.7	Passed
Iodide	Retention Time	7.75-8.85	Passed
	Pressure	<=3190	2787

Production Reference:

Datasource: QAR
 Directory: _RFID\Anion\AS16-4µm
 Sequence: AS16-4µm_4X250
 Sample No.: 1

6.80 SR15 Build 4656 (243203) (Demo-Installation)

Chromleon™ Thermo Fisher Scientific