Thermo Scientific

Dionex IonPac AS22-Fast-4µm

Column Product Manual

P/N: 065604-01 August 2014

Thermo SCIENTIFIC

Product Manual

for

Dionex IonPac AS22-Fast-4µm Analytical Columns

4 × 150 mm (088486)
2 × 150 mm (088488)

Dionex IonPac AS22-Fast-4µm Capillary Column

0.4 × 150 mm (088490)

Dionex IonPac AG22-Fast-4µm Guard Columns

4 × 30 mm (088487)
2 × 30 mm (088489)

Dionex IonPac AG22-Fast-4µm Capillary Guard Column

0.4 × 35 mm (088491)

For Research Use Only. Not for use in diagnostic procedures.

Revision History:

Revision 01, August, 2014; 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.***

Contents

1. Introduction

The Thermo Scientific™ Dionex™ IonPac™ AS22-Fast-4µm column is designed for compliance monitoring of inorganic anions in accordance with U.S. EPA Methods 300.0 (A) and 300.1. The common inorganic anions and low molecular weight organic acids including fluoride, acetate, formate, chloride, nitrite, chlorate, bromide, nitrate, phosphate and sulfate can be easily separated in a variety of sample matrices including drinking water, wastewater, process streams and scrubber solutions. The selectivity of the Dionex IonPac AS22-Fast-4µm column has been designed to retain fluoride well out of the water dip (system dip) and to isocratically separate common anions including carbonate. In comparison to the Thermo Scientific Dionex IonPac AS22-Fast column, the Dionex IonPac AS22-Fast-4µm column exhibits higher peak efficiency while maintaining the same selectivity. This is due to the fact that the same functionality is attached to the smaller resin particles used in the Dionex IonPac AS22-Fast-4µm column. The Dionex IonPac AS22-Fast-4µm column is available in standard bore (4 mm i.d.), microbore (2 mm i.d.) and capillary (0.4 mm i.d.) formats by 150 mm long for fast analysis of samples with moderate ionic strength. The Dionex IonPac AS22-Fast-4µm column is compatible with pH 0-14 eluents and eluents containing organic solvents from 0–100% in concentration. The Dionex IonPac AS22-Fast-4µm column can be used with any suppressible ionic eluent that does not exceed the capacity of the Thermo Scientific Dionex Anion Electrolytically Regenerated Suppressor 500 (Dionex AERS 500) or the Thermo Scientific Dionex Anion Capillary Electrolytic Suppressor 300 (Dionex ACES 300). The Dionex IonPac AS22-Fast-4µm column has nominal efficiency for sulfate of at least 9, 000 plates/column using standard operating conditions.

The Dionex IonPac AS22-Fast-4µm Capillary Column (0.4 × 150 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 AS22-Fast-4µm/Dionex IonPac AG22-Fast-4µm Column Packing Specifications

Resin Characteristics:

Nominal Particle Size:	4 µm (Analytical/Capillary column*)
Nominal Particle Size:	10 µm (Guard/Capillary Guard column**)
Particle Cross-linking:	55%
Ion exchange capacity:	126 µeq per 4 × 150 mm column
	31.5 µeq per 2 × 150 mm column
	1.26 µeq per 0.4 × 150 mm column
	4.0 µeq per 4 × 30 mm column
	1.0 µeq per 2 × 30 mm column
	0.04 µeq per 0.4 × 35 mm column

Functional Characteristics:

Functional Group:	Alkanol quaternary ammonium ion
Hydrophobicity:	Ultralow

*Analytical/Capillary Column resin composition: supermacroporous polyvinylbenzyl ammonium polymer cross-linked with divinylbenzene

**Guard/Capillary Guard Column resin composition: microporous polyvinylbenzyl ammonium polymer cross-linked with divinylbenzene

Table 1 Dionex IonPac AS22-4µm/Dionex IonPac AG22-4µm Operating Parameters

Column	Typical Back Pressure psi (MPa[a]), 30°C[b]	Standard Flow Rate, mL/min	Maximum Flow Rate, mL/min[c, d]
Dionex IonPac AS22-Fast-4µm, 4 mm Analytical column	~ 2100 (14.48)	1.20	2.0
Dionex IonPac AG22-Fast-4µm, 4 mm Guard column	~ 200 (1.38)	1.20	2.0
Dionex IonPac AS22-Fast-4µm and AG22-Fast-4µm, 4 mm columns	~ 2300 (15.86)	1.20	2.0
Dionex IonPac AS22-Fast-4µm, 2 mm Analytical column	~ 2100 (14.48)	0.30	0.50
Dionex IonPac AG22-Fast-4µm, 2 mm Guard column	~ 200 (1.38)	0.30	0.50
Dionex IonPac AS22-Fast-4µm and AG22-Fast-4µm, 2 mm columns	~ 2300 (15.86)	0.30	0.50
Dionex IonPac AS22-Fast-4µm, 0.4 mm Capillary column	~ 2100 (14.48)	0.012	0.02
Dionex IonPac AG22-Fast-4µm, 0.4 mm Capillary Guard column	~ 200 (1.38)	0.012	0.02
Dionex IonPac AS22-Fast-4µm and AG22-Fast-4µm, 0.4 mm columns	~ 2300 (15.86)	0.012	0.02

[a] Note: 1MPa = 145.04 psi

[b] Total backpressure at standard flow rates

[c] In all cases, flow rate should not result in pressures over 5,000 psi

[d] When developing a method for multiple column formats or long term, wide spread use, it is important to operate within the maximum flow rate listed above to ensure a reproducible method.

NOTE

For assistance, visit Unity Lab Services online at www.unitylabservices.com.
From the U.S., call the Customer Care Center for Dionex Products at 1-800-346-6390.
Outside the U.S., call the nearest Thermo Fisher Scientific office.

WARNING

Exceeding the maximum flow rates listed in the above table, can disrupt the uniformity of the packing of the column bed and irreversibly damage the performance of the column.

2. Installation

NOTE

Read the instrument manuals. This manual assumes that you are using Thermo Scientific Dionex instrumentation and are familiar with the installation and operation of the Thermo Scientific Dionex Ion Chromatograph (IC). If you do not understand the operation of the system, take the time to familiarize yourself with the various system components before beginning an analysis.

The proper configuration of an Ion Chromatography System (ICS) is dependent on the column format. Although they can typically use the same system, the use of a 2 mm or 4 mm column requires different set up to ensure optimum performance. The selected format and analysis type 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 or multi-eluent proportioning capabilities. For high pressure applications (over 3000 psi), the use of high pressure consumables, components and fittings are required.

A Dionex Capillary IC system should be used for capillary applications and the use of precut tubing and Dionex high pressure fittings is required for best performance. Please refer to the instrument manual to ensure column and component installation is correct and all fittings are made correctly.

2.1 The Thermo Scientific Dionex High Pressure Ion Chromatography Systems

A minimum of a Dionex High Pressure Ion Chromatography System (HPIC) is recommended when running Dionex IonPac 4µm columns due to the higher backpressures generated at typical operational flow rates. Systems should have the capability to operate up to at least 5000 psi. Standard IC systems, with an upper limit of 3000 psi, are insufficient for proper column operation.

All systems should allow 100% metal-free operation to prevent column damage. This includes pump heads and all flow paths.

WARNING

Care should always be taken not to exceed the maximum operating pressure of the system components. ICS systems with lower backpressure capabilities are not recommended as reduced flow rates may result in loss of performance.

NOTE

Contact your local representative for information on how to customize your system to your application needs.

CAUTION

Dionex ICS 5000 capillary systems shipped <u>before</u> October 2011 may require the installation of a high pressure upgrade kit to enable operation at 5000 psi. For more information please contact your local representative or call the Customer Care Center for Dionex Products at 1-800-346-6390 from inside the US.

2.2 System Requirements

The Dionex IonPac Columns are designed to run on Dionex Ion Chromatographs equipped with suppressed conductivity detection. We recommend the use of ferrules and fittings rated with a pressure of >5000 psi. The use of precut tubing, complete with high pressure fitting and ferrules, is recommended for easier installation, and required for capillary systems.

2.3 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" i.d. PEEK tubing. In order to reduce system backpressure at higher flow rates, 0.010" i.d. PEEK tubing may be used for connections *before* the injection valve but peak efficiency will be compromised if used for post injector connections, which may also result in decreased peak resolution. Minimize the lengths of all connecting tubing and remove all unnecessary switching valves and couplers.

With capillary systems correct tubing connections become of greater importance. Precut tubing, complete with high pressure fitting and ferrules, is required for easier installation and optimal performance. It should also be noted that due to system configuration differences, the system void volume in the capillary system will result in longer void time than observed with the analytical system at the same linear velocity. Slight modifications of the method may be required to ensure equivalent retention time and peak resolution.

2.4 Column Start-Up

The column is shipped using 100 mM sodium bicarbonate as the storage solution.

Prepare the eluent shown on the Quality Assurance Report. To remove the storage solution, flush the column to waste with the QAR eluent for at least 30 minutes before attaching the column outlet to the suppressor. Install the column in the column module and 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.

IMPORTANT

When making any tubing connections, first turn off the pump. This will avoid any slippage of the ferrule under high pressure conditions. For capillary connections, inject deionized 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.

2.5 Installation of the Capillary Column

Correct installation of the capillary column is vital for good column performance. Please refer to the Instrument Manual for instructions. A quick guide can be found in Section 8.2, “Installation of the Capillary Column”.

2.6 Dionex IonPac Guard Columns

A Dionex IonPac Guard Column is normally used with the Dionex IonPac Analytical/Capillary Column. A guard is placed in front of the analytical/capillary column to prevent sample contaminants from damaging the analytical/capillary column. It is easier to clean or replace the guard column than it is the analytical/capillary column. Placing a guard column in front of the analytical/capillary column will cause retention times to increase by approximately 5% under isocratic test conditions. Replacing the guard column at the first sign of peak efficiency loss or decreased retention time will prolong the life of the analytical/capillary column.

2.7 Sample Concentrators

The function of a concentrator column is to strip ions of interest 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 at the start of the analysis.

Dionex Concentrator columns or the Dionex IonPac Guard Column can be used for trace anion concentration work with Dionex IonPac columns. A pump is used to load the sample onto the concentrator column in the OPPOSITE direction of the eluent flow. Once concentration is complete the eluent flow is then directed through the concentrator to the analytical column. When using concentration techniques, care should be taken not to overload the concentrator column by concentrating an excessive amount of sample. If an excessive amount of sample is used inaccurate results may be obtained. It is possible during the concentration step for the stronger binding polyvalent ions to elute the weakly retained ions from the concentrator column. For more detailed information on sample concentration techniques for high sensitivity work and a detailed discussion of concentration techniques refer to the appropriate concentrator manual for your application.

CAUTION

Dionex IonPac Concentrator Columns are designed for use with specific eluent systems. Use only concentrator columns designed for the eluent system you are using.

2.8 The Injection Loop

2.8.1.1 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.

2.8.1.2 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 12.5 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 AS22-Fast-4µm 2 mm columns require 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.

2.8.1.3 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 50 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.

2.9 Thermo Scientific Dionex Anion Electrolytically Regenerated Suppressor (Dionex AERS 500) and Dionex Anion Capillary Electrolytic Suppressor Requirements

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

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

2.10 Using Dionex 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. Operation

3.1 General Operating Conditions

The following conditions should be used as a starting point towards method development.

Table 2 General Operating Conditions for the Dionex IonPac AS22-Fast-4µm Column

Sample Volume:	0.4 mm: 2 mm: 4 mm:	0.4 µL Loop 2.5 µL Loop 10 µL Loop
Column:	0.4 mm: 2 mm: 4 mm:	Dionex IonPac AS22-Fast-4µm 0.4 mm Capillary Column +Dionex IonPac AG22-Fast-4µm 0.4 mm Capillary Guard Column Dionex IonPac AS22-Fast-4µm 2 mm Analytical Column + Dionex IonPac AG22-Fast-4µm 2 mm Guard Column Dionex IonPac AS22-Fast-4µm 4 mm Analytical Column + Dionex IonPac AG22-Fast-4µm 4 mm Guard Column
Eluent:	4.5 mM Na_2CO_3/1.4 mM $NaHCO_3$ (QAR eluent)	
Eluent Flow Rate:	0.4 mm: 2 mm: 4 mm:	12 µL/min 0.30 mL/min 1.2 mL/min
ERS/CES Suppressor: **or MMS Suppressor:**	0.4 mm: 2 mm and 4 mm:	Dionex Anion Capillary Electrolytic Suppressor, Dionex ACES 300 Dionex Anion Electrolytically Regenerated Suppressor, Dionex AERS 500, AutoSuppression Recycle Mode Dionex Anion MicroMembrane Suppressor, Dionex AMMS 300 (2 or 4 mm only)
MMS Regenerant:	50 mN H_2SO_4	
Expected Background Conductivity:	13-20 µS	
Long-term Storage Solution (> 1 week):	100 mM Sodium bicarbonate	
Short-term Storage Solution (< 1 week):	Eluent	

NOTE

For assistance, visit Unity Lab Services online at www.unitylabservices.com.
From the U.S., call the Customer Care Center for Dionex Products at 1-800-346-6390.
Outside the U.S., call the nearest Thermo Fisher Scientific office.

3.2 General Operational Precautions

The following precautions should always be adhered to when using Dionex IonPac columns.

- Samples and manually prepared eluents should always be filtered and degassed to protect the system and column from particulates and ensure a stable background.
- Eluents and samples used should be used within the allowable limits for the column.
- Do not exceed the operational pressure of the system.
- Take care not to exceed the maximum operational flow rate and pressure of the column. If the pressure approached the maximum allowed pressure, reduce the operational flow rate.

Table 3 Operational Limits for the Dionex IonPac AS22-Fast-4µm Columns

Eluent pH	Between 0 and 14
Sample pH	Between 0 and 14
Maximum Flow Rate for 0.4 mm Columns	20 µL/min
Maximum Flow Rate for 2 mm Columns	0.50 mL/min
Maximum Flow Rate for 4 mm Columns	2.0 mL/min
Maximum Operating Pressure	5,000 psi (34.47MPa)

3.3 Quality Assurance Reports

Each column is qualified to ensure it meets specifications. Example copies of these Quality Assurance Reports (QARs) can be found in Appendix B – Quality Assurance Reports. The QAR supplied with the column should be used as a guide to ensure system performance.

3.4 Chemical Requirements and Solvent Compatibility

Chemical purity can influence separation performance. Only chemicals of the highest purity should be used. Refer to Section 6.4, "Chemical Purity Requirements" for more details.

Some solvents may be used for cleaning or eluent modification.

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 Typical HPLC Solvents for Cleaning and Use with Dionex IonPac 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 < 4000 psi.

CAUTION

The Dionex AERS and ACES must be operated in the AutoSuppression External Water Mode when using eluents containing organic solvents. Refer to the Suppressor Product Manual for operation limitations in the electrolytic mode (power on).

3.5 Eluent Preparation

3.5.1 Eluent Generation

It is recommended that Dionex IonPac columns are used with Dionex HPIC Systems equipped with a Thermo Scientific Dionex Eluent Generator Cartridge. The use of eluent generation provides a more stable baseline, improved performance and increased reproducibility over the use of manually prepared eluents.

The Dionex Eluent Generator is used to automatically produce eluents either isocratically or as gradients from deionized water. Please refer to the Dionex EG manual for information on the operation of the Dionex eluent generators.

CAUTION

Only Dionex EG Cartridges (Dionex EGCs) rated for 5,000 psi or higher should be used when running Dionex IonPac 4µm columns due to the higher backpressures generated at typical operational flow rates.

3.5.2 Making Eluents that Contain Solvents

Mixing solvents with water should be done on a volume to volume basis. For example, if a procedure requires an eluent of 40% acetonitrile, prepare the eluent by adding 400 mL of acetonitrile to an eluent reservoir. Then add 600 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 will evaporate or can be "boiled" off from the solution under reduced pressure.

NOTE

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.

NOTE

Acetonitrile (ACN) hydrolyzes to ammonia and acetate when 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.

3.5.3 Preparation of Eluent Stock Solution Concentrates

A. Sodium Carbonate/Bicarbonate Eluent Concentrate: The Dionex IonPac AS22 Sodium Carbonate/Bicarbonate Eluent Concentrate (0.45 M Na_2CO_3/0.14 M $NaHCO_3$), P/N 063965, can be used to prepare eluents for the Dionex IonPac AS22-Fast-4µm column.

   To make the eluent concentrate from reagents, thoroughly dissolve 47.7 g of sodium carbonate (MW 106.00 g/mole) plus 11.76 g sodium bicarbonate (MW 84.00 g/mole) in 700 L of deionized water with a specific resistance of 18.2 megohm-cm in a 1 L volumetric flask. Dilute to a final volume of 1,000 mL.

B. 0.5 M Sodium Carbonate (Na_2CO_3) Concentrate: The Dionex Carbonate Concentrate, 0.5M, 500 mL is available by ordering P/N 037162.

   To make this eluent concentrate from reagents, thoroughly dissolve 26.49 g of Na_2CO_3 in 400 mL of deionized water with a specific resistance of 18.2 megohm-cm. Dilute to a final volume of 500 mL.

   Occasionally, batches of sodium carbonate are produced with low concentrations of residual hydroxide impurity. Use of such reagent can adversely affect the resolution of phosphate and sulfate. Use of Dionex 0.5 molar Sodium Carbonate Concentrate is recommended in order to avoid this problem. Otherwise, use of a high purity grade of sodium carbonate to prepare eluents will generally prevent the problem. We recommend EMD Chemicals sodium carbonate (P/N SX0395) for this purpose. Do not dry sodium carbonate at excessive temperatures (> 110 °C) as this will increase the pH of the salt.

C. 0.5 M Sodium Bicarbonate ($NaHCO_3$) Concentrate: The Dionex Bicarbonate Concentrate, 0.5M, 500 mL is available by ordering P/N 037163.

To make this eluent concentrate from reagents, thoroughly dissolve 21.00 g of $NaHCO_3$ in 400 mL of deionized water with a specific resistance of 18.2 megohm-cm. Dilute to a final volume of 500 mL.

3.5.4 Manually Prepared Eluents

Eluents should be stored in plastic bottles under a helium atmosphere to ensure contamination free operation and proper pump performance (nitrogen can be used if eluents do not contain solvents). Contamination from carbon dioxide when basic eluents are exposed to the air can cause performance variability such as retention time shifts.

3.5.4.1 Eluent: 4.5 mM Sodium Carbonate/1.4 mM Sodium Bicarbonate

A. Using Dionex IonPac AS22 Eluent Concentrate:
By Weight: Weigh 988.0 g of deionized water and add 10.5 g of the Dionex IonPac AS22 Eluent Concentrate.
By Volume: To make 1 liter of eluent, pipette 10 mL of the Dionex IonPac AS22 Eluent Concentrate into a 1 L volumetric flask and dilute to a final volume of 1 L using deionized water.

B. Using 0.5 M Na_2CO_3 and 0.5 M $NaHCO_3$ Concentrates:
By Weight: Weigh 986.2 g of deionized water and add 9.45 g of 0.5 M Na_2CO_3 plus 2.94 g of 0.5 M $NaHCO_3$.
By Volume: Prepare the eluent by pipetting 9.0 mL of 0.5 M Na_2CO_3 plus 2.8 mL of 0.5 M $NaHCO_3$ into a 1 L volumetric flask. Use degassed, deionized water with a specific resistance of 18.2 megohm-cm to dilute the concentrate to a final volume of 1,000 mL.

NOTE

It is highly recommended to pressurize the eluent with nitrogen or helium to maintain the pH, as any change in pH due to absorption of CO_2 will affect retention times and selectivity. This is particularly important for Capillary IC as a single batch of eluent can last up to 3 months.

4. Example Applications

NOTE

For assistance, visit Unity Lab Services online at www.unitylabservices.com.
From the U.S., call the Customer Care Center for Dionex Products at 1-800-346-6390.
Outside the U.S., call the nearest Thermo Fisher Scientific office.

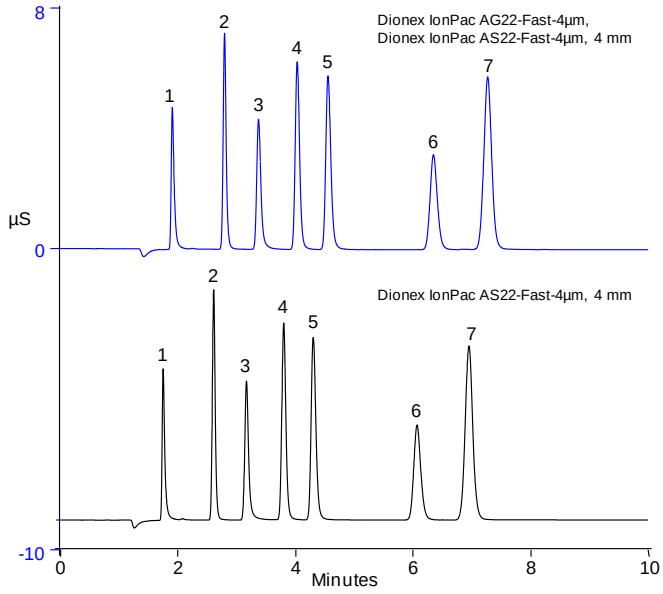
The chromatograms in this section were obtained using columns that reproduced the Quality Assurance Report on an optimized Ion Chromatograph. Different systems will differ slightly in performance due to slight differences in column sets, system void volumes, liquid sweep-out times of different components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

Ensure that your system is properly configured and 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 with a specific resistance of 18.2 megohm-cm. For chemical purity requirements, see Section 6.4, "Chemical Purity Requirements". After running synthetic standards to calibrate your system, you may find that real sample matrices foul your columns. For this reason it is always advisable to use a guard column to protect the analytical column. If column performance deteriorates and it is determined that the guard or the analytical column has been fouled, refer to the column cleanup protocols in Appendix A – Column Care". If your sample matrices are relatively low in ionic concentration, you may be able to increase the sensitivity of your system by using sample concentration techniques.

4.1 Isocratic Elution With and Without a Guard

Isocratic elution of anions on the Dionex IonPac AS22-Fast-4µm Analytical/Capillary Column has been optimized utilizing a carbonate/bicarbonate eluent. By using this eluent, mono- and divalent anions can be isocratically separated and quantitated in a single injection. The Dionex IonPac AS22-Fast-4µm Analytical Column should always be used with the Dionex IonPac AG22-Fast-4µm Guard Column. Note that the Dionex IonPac AG22-Fast-4µm Guard Column is packed with a microporous resin of proportionally lower capacity and retention times will increase by approximately 5% when a guard column is placed in-line prior to the analytical column.

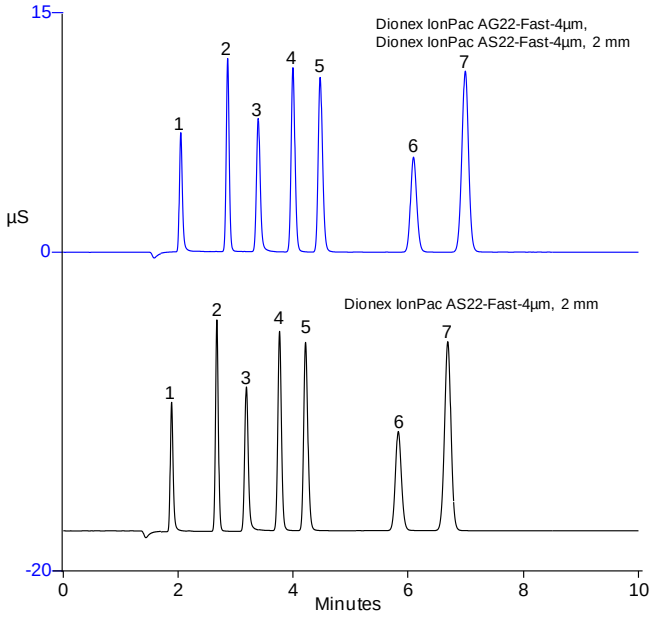
Figure 1 Dionex IonPac AS22-Fast-4µm Column (4×150 mm) With and Without Guard Column



Column:	See Chromatogram
Eluent :	4.5 mM Na_2CO_3/1.4mM $NaHCO_3$
Flow Rate:	1.2 mL/min
Inj. Volume:	10 µL
Temperature:	30 ˚C
Detection:	Suppressed Conductivity, Dionex AERS 500 Suppressor, 4 mm AutoSuppression, recycle mode

Peaks:	mg/L
1. Fluoride	5.0
2. Chloride	10.0
3. Nitrite	15.0
4. Bromide	25.0
5. Nitrate	25.0
6. Phosphate	40.0
7. Sulfate	30.0

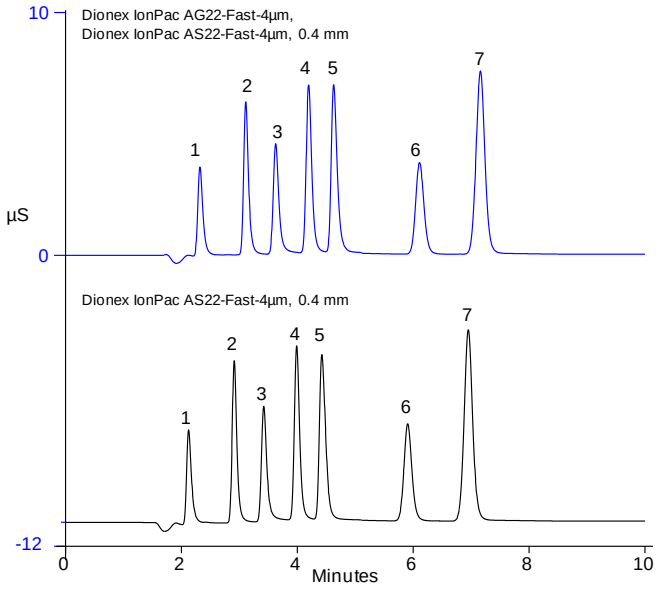
Figure 2 Dionex IonPac AS22-Fast-4µm Column (2×150 mm) With and Without Guard Column



Column:	See Chromatogram
Eluent:	4.5 mM Na_2CO_3/1.4mM $NaHCO_3$
Flow Rate:	0.3 mL/min
Inj. Volume:	2.5 µL
Temperature:	30 ˚C
Detection:	Suppressed Conductivity, Dionex AERS 500 Suppressor, 2 mm AutoSuppression, recycle mode

Peaks:	mg/L
1. Fluoride	5.0
2. Chloride	10.0
3. Nitrite	15.0
4. Bromide	25.0
5. Nitrate	25.0
6. Phosphate	40.0
7. Sulfate	30.0

Figure 3 Dionex IonPac AS22-Fast-4µm Column (0.4×150 mm) With and Without Guard Column



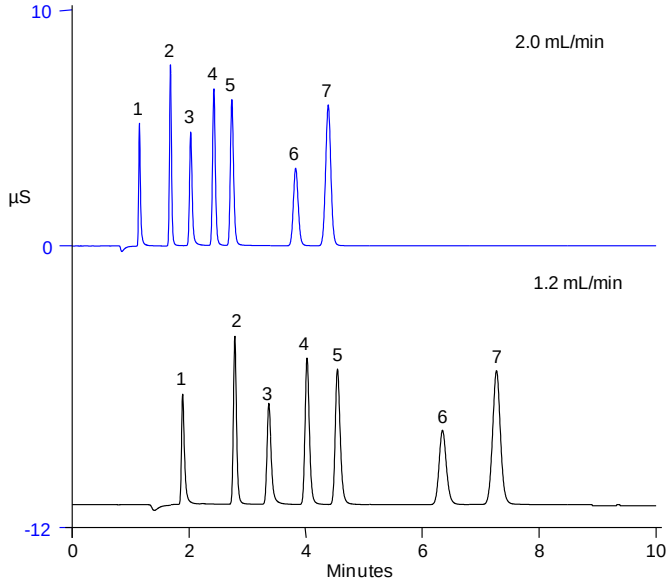
Column:	See Chromatogram
Eluent:	4.5 mM Na_2CO_3/1.4mM $NaHCO_3$
Flow Rate:	12 µL/min
Inj. Volume:	0.4 µL
Temperature:	30 ˚C
Detection:	Suppressed Conductivity, Dionex ACES 300 Suppressor, AutoSuppression, recycle mode

Peaks:	mg/L
1. Fluoride	1.3
2. Chloride	2.5
3. Nitrite	3.8
4. Bromide	6.3
5. Nitrate	6.3
6. Phosphate	10.0
7. Sulfate	7.5

4.2 Fast Analysis of the Common Inorganic Anions using the Dionex IonPac AS22-Fast-4µm Column with an Optimized Flow Rate

The following chromatograms demonstrate the use of a high flow rate for the fast analysis of the common inorganic anions. Smaller particles used in the Dionex IonPac AS22-Fast-4µm column, produce very sharp, well-resolved peaks making fast runs easier to integrate. Note that analysis time can be reduced below 5 minutes while still maintaining good resolution of the common inorganic anions. When developing a method for multiple column formats or long term, wide spread use, it is important to operate within the maximum flow rate to ensure a reproducible method. ***Note the maximum flow rate for the various formats for the Dionex IonPac AS22-Fast-4µm column is listed in Table 1.***

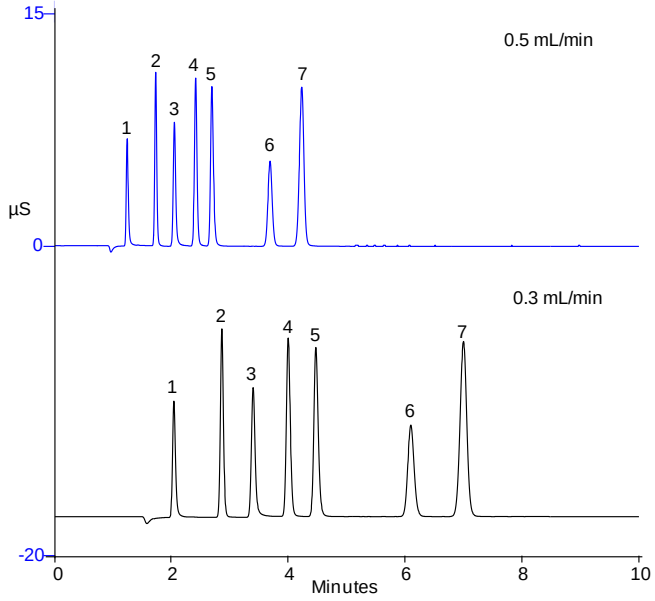
Figure 4 Fast Analysis of the Common Inorganic Anions using the Dionex IonPac AS22-Fast-4µm Column Using Optimized Flow Rate (4×150 mm)



Column: Dionex IonPac AG22-Fast-4µm (4×30 mm)/AS22-Fast-4µm (4×150 mm)
Eluent: 4.5 mM Na_2CO_3/1.4mM $NaHCO_3$
Flow Rate: See Chromatogram
Inj. Volume: 10 µL
Temperature: 30 °C
Detection: Suppressed conductivity, Dionex AERS 500 Suppressor, 4 mm, AutoSuppression, recycle mode

Peaks:	mg/L
1. Fluoride	5.0
2. Chloride	10.0
3. Nitrite	15.0
4. Bromide	25.0
5. Nitrate	25.0
6. Phosphate	40.0
7. Sulfate	30.0

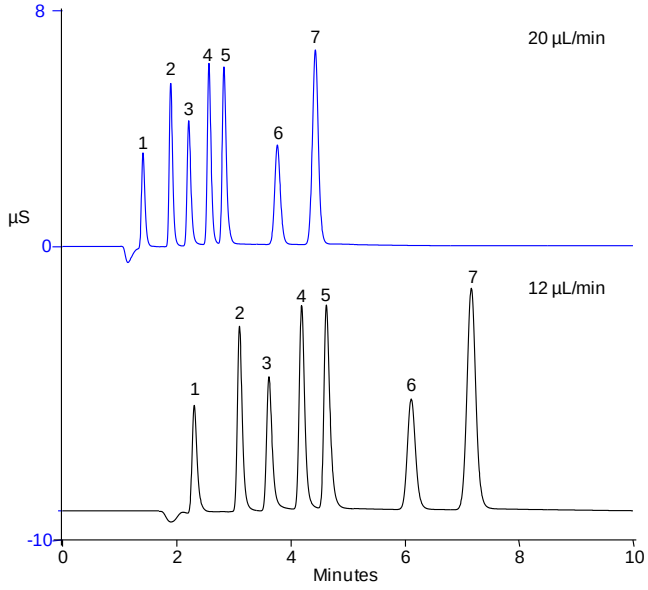
Figure 5 Fast Analysis of the Common Inorganic Anions using the Dionex IonPac AS22-Fast-4µm Column Using Optimized Flow Rate (2×150 mm)



Column: Dionex IonPac AG22-Fast-4µm (2×30 mm)/AS22-Fast-4µm (2×150 mm)
Eluent: 4.5 mM Na_2CO_3/1.4mM $NaHCO_3$
Flow Rate: See Chromatogram
Inj. Volume: 2.5 µL
Temperature: 30 °C
Detection: Suppressed conductivity, Dionex AERS 500 Suppressor, 2 mm, AutoSuppression, recycle mode

Peaks:	mg/L
1. Fluoride	5.0
2. Chloride	10.0
3. Nitrite	15.0
4. Bromide	25.0
5. Nitrate	25.0
6. Phosphate	40.0
7. Sulfate	30.0

Figure 6 Fast Analysis of the Common Inorganic Anions using the Dionex IonPac AS22-Fast-4µm Column Using Optimized Flow Rate (0.4×150 mm)



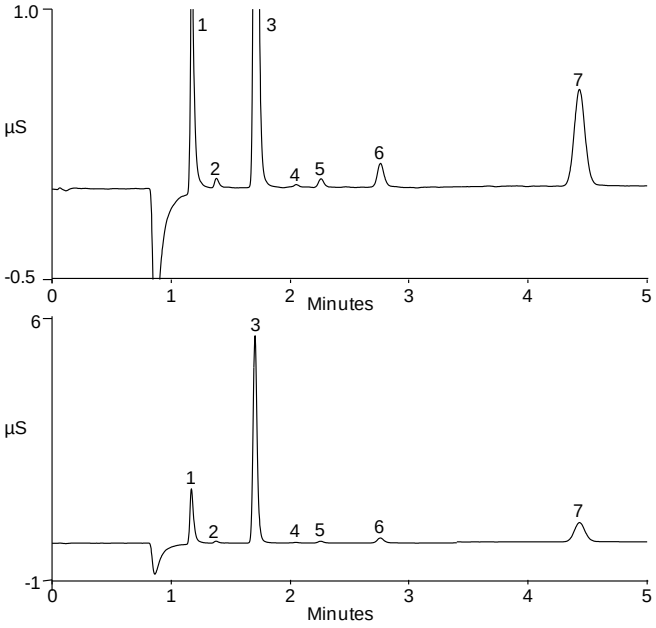
Column: Dionex IonPac AG22-Fast-4µm (0.4×35 mm)/AS22-Fast-4µm (0.4×150 mm)
Eluent: 4.5 mM Na_2CO_3/1.4mM $NaHCO_3$
Flow Rate: See Chromatogram
Inj. Volume: 0.4 µL
Temperature: 30 °C
Detection: Suppressed conductivity, Dionex ACES 300 Suppressor, AutoSuppression, recycle mode

Peaks:	mg/L
1. Fluoride	1.3
2. Chloride	2.5
3. Nitrite	3.8
4. Bromide	6.3
5. Nitrate	6.3
6. Phosphate	10.0
7. Sulfate	7.5

4.3 Analysis of Municipal Drinking Water using the Dionex IonPac AS22-Fast-4µm Column

The fast analysis of municipal drinking water is demonstrated in the Figures 7, 8, and 9 using each of the column formats. The eluent flow rate is increased to reduce the run time to less than 5 minutes while the column maintains excellent resolution of the seven anions.

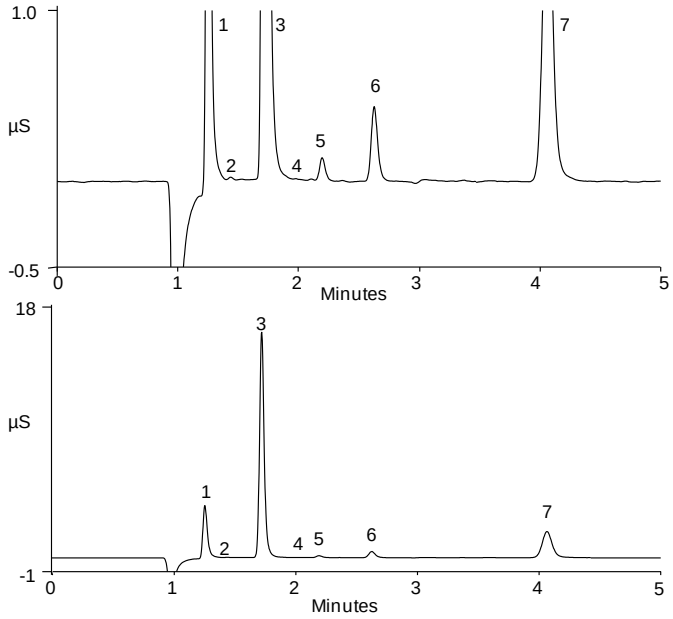
Figure 7 Fast Analysis of Municipal Drinking Water using the Dionex IonPac AS22-Fast-4µm Column (4×150 mm)



Column:	Dionex IonPac AG22-Fast-4µm (4×30 mm)/AS22-Fast-4µm (4×150 mm)
Eluent:	4.5 mM Na_2CO_3/1.4mM $NaHCO_3$
Flow Rate:	2.0 mL/min
Inj. Volume:	40 µL
Temperature:	30 °C
Detection:	Suppressed conductivity, Dionex AERS 500 Suppressor, 4 mm, AutoSuppression, recycle mode
Sample:	Municipal Drinking Water

Peaks:	mg/L
1. Fluoride	0.81
2. Formate	NQ
3. Chloride	3.14
4. Nitrite	0.02
5. Chlorate	0.15
6. Nitrate	0.31
7. Sulfate	1.42

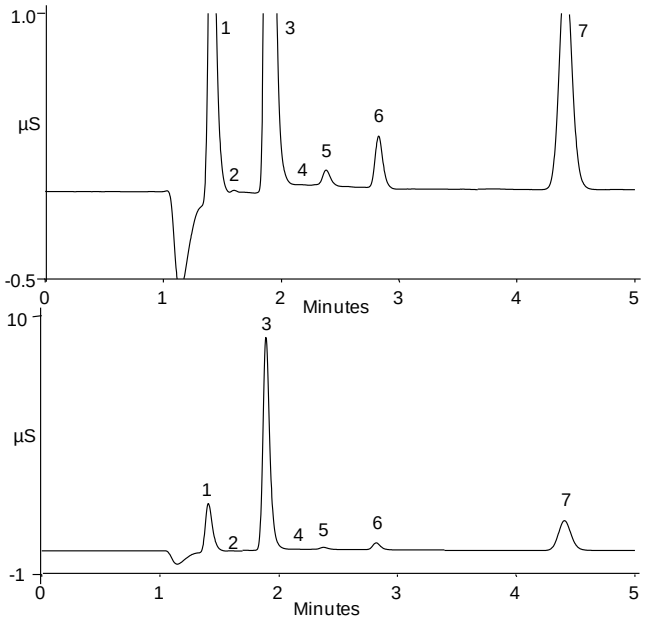
Figure 8 Fast Analysis of Municipal Drinking Water using the Dionex IonPac AS22-Fast-4µm Column (2×150 mm)



Column:	Dionex IonPac AG22-Fast-4µm (2×30 mm)/AS22-Fast-4µm (2×150 mm)
Eluent:	4.5 mM Na_2CO_3/1.4mM $NaHCO_3$
Flow Rate:	0.5 mL/min
Inj. Volume:	10 µL
Temperature:	30 °C
Detection:	Suppressed conductivity, Dionex AERS 500 Suppressor, 2 mm, AutoSuppression, recycle mode
Sample:	Municipal Drinking Water

Peaks:	mg/L
1. Fluoride	0.87
2. Formate	NQ
3. Chloride	3.35
4. Nitrite	ND
5. Chlorate	0.14
6. Nitrate	0.34
7. Sulfate	1.42

Figure 9 Fast Analysis of Municipal Drinking Water using the Dionex IonPac AS22-Fast-4µm Column (0.4×150 mm)



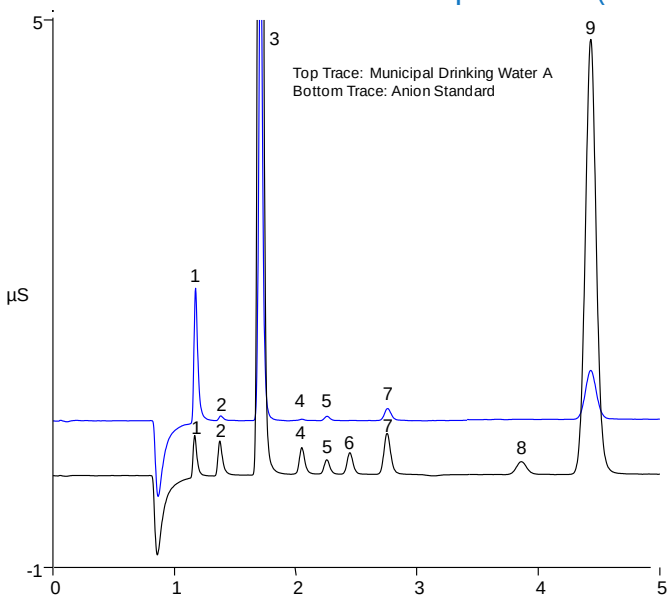
Column:	Dionex IonPac AG22-Fast-4µm (0.4×35 mm)/AS22-Fast-4µm (0.4×150 mm)
Eluent:	4.5 mM Na_2CO_3/1.4mM $NaHCO_3$
Flow Rate:	20 µL/min
Inj. Volume:	0.4 µL
Temperature:	30 °C
Detection:	Suppressed conductivity, Dionex ACES 300 Suppressor, AutoSuppression, recycle mode
Sample:	Municipal Drinking Water

Peaks:	mg/L
1. Fluoride	0.83
2. Formate	NQ
3. Chloride	3.14
4. Nitrite	ND
5. Chlorate	0.18
6. Nitrate	0.29
7. Sulfate	1.35

4.4 A Demonstration of High Separation Power of the Dionex IonPac AS22-Fast-4µm Column Using Common Anion Standard and Municipal Drinking Water

Figure 10 demonstrates the resolution power of the Dionex IonPac AS22-Fast-4µm column using a common anion standard and a municipal drinking water. Note that 9 different common anions have baseline resolution in less than 5 minutes.

Figure 10 Comparison of an Anion Standard and Municipal Drinking Water A using the Dionex IonPac AS22-Fast-4µm Column (4×150 mm)



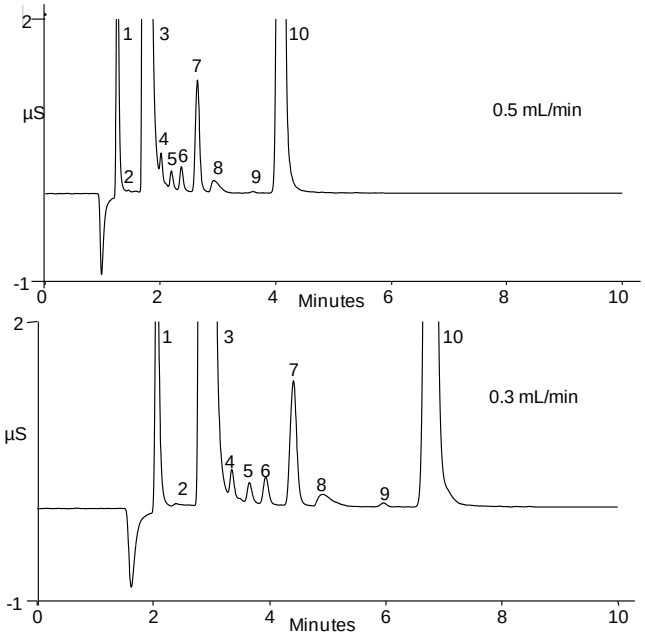
Column:	Dionex IonPac AG22-Fast-4µm (4×30 mm)/AS22-Fast-4µm (4×150 mm)
Eluent:	4.5 mM Na_2CO_3/1.4mM $NaHCO_3$
Flow Rate:	2.0 mL/min
Inj. Volume:	40 µL
Temperature:	30 °C
Detection:	Suppressed conductivity, Dionex AERS 500 Suppressor, 4 mm, AutoSuppression, recycle mode
Sample:	See Chromatogram

Peaks:	Standard mg/L
1. Fluoride	0.25
2. Formate	1.0
3. Chloride	12.5
4. Nitrite	0.5
5. Chlorate	0.5
6. Bromide	0.5
7. Nitrate	1.0
8. Phosphate	1.0
9. Sulfate	12.5

4.5 Fast versus Standard Flow Rate When Analyzing Municipal Drinking Water with High Ionic Strength using the Dionex IonPac AS22-Fast-4µm Column

The selection of optimum analysis parameters is important when dealing with high ionic strength samples. As demonstrated in Figure 11, using a fast flow rate is not optimum as the resolution for chloride, peak 3 and nitrite, peak 4 is compromised. When dealing with a high ionic strength sample, one can reduce the injection volume or dilute the sample or reduce the analysis flow rate to improve the peak resolution. Some higher ionic strength samples may require the use of the Dionex IonPac AS22 Column, which is 250 mm in length, to get the best resolution of all the peaks.

Figure 11 Effect of Over Loading on the Peak Resolution using Fast versus Standard Flow Rate with the Dionex IonPac AS22-Fast-4µm Column (2×150 mm)



Column:	Dionex IonPac AG22-Fast-4µm (2×30 mm)/AS22-Fast-4µm (2×150 mm)
Eluent:	4.5 mM Na_2CO_3/1.4mM $NaHCO_3$
Flow Rate:	See chromatograms
Inj. Volume:	10 µL
Temperature:	30 °C
Detection:	Suppressed conductivity, Dionex AERS 500 Suppressor, 4 mm, AutoSuppression, recycle mode
Sample:	Municipal Drinking Water B

Peaks:	mg/L
1. Fluoride	0.88
2. Formate	NQ
3. Chloride	142.4
4. Nitrite	0.29
5. Chlorate	0.21
6. Bromide	0.22
7. Nitrate	1.31
8. Carbonate	NQ
9. Phosphate	0.08
10. Sulfate	26.97

5. Troubleshooting

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

NOTE

For assistance, visit Unity Lab Services online at www.unitylabservices.com
Or call the Customer Care Center for Dionex Products at 1-800-346-6390
Outside the U.S., call the nearest Thermo Fisher Scientific office.

Table 5 Troubleshooting Summary

Observation	Cause	Action	Reference Section
High Back Pressure	Unknown	Isolate Blocked Component	5.1.1
	Plugged Column Bed Supports	Replace Bed Supports, Filter Eluents, and Filter Samples	5.1.2, 5.1.3, 5.1.4
	Other System Components	Unplug, Replace, Filter Eluents and Samples	Component Manual
High Background Conductivity	Contaminated Eluents	Remake Eluents	5.2.1
	Contaminated Trap Column	Clean or Replace Trap Column	5.2.2,5.2.3
	Contaminated Guard or Analytical Column	Clean or Replace Guard and Analytical Column	5.2.4
	Contaminated Suppressor	Clean or Replace Suppressor	5.2.6, Component Manual
	Contaminated Hardware	Clean Component	5.2.5, Component Manual
Poor Resolution	Gradient method not optimized	Optimize method	5.4
Poor Efficiency	Large System Void Volumes	Replumb System	Component Manual, 5.4.1B
	Column Headspace	Replace Column	5.1.2, 5.4.1A
	Improper connections	Replumb system	5.4.1B, 5.4.1C
	Leaks in the system	Check for leaks, Replumb system	5.4.1D, 5.4.1C
	Contaminated Suppressor	Clean or Replace Suppressor	5.4.1E, Component Manual
Short Retention Times	Flow Rate Too fast	Check Flow Rate, Recalibrate Pump	5.4.3B
	Conc. Incorrect Eluents	Remake Eluents	5.2.1, 5.4.3C
	Column Contamination	Clean Column	5.2.4
	Insufficient Equilibration	Extend Equilibration Time at the Start of the Gradient Run	5.4.3A

Observation	Cause	Action	Reference Section
Retention Time Drift	Contaminated sample or eluent, Poorly mixed eluent	Remake Sample/Eluents	5.2.1, 5.3.1A, E, 5.3.2A
	Temperature variability	Use a thermostatted oven, check oven operation	5.3.1C,D, 5.3.2D, Component Manual
	Poor pump priming or loss of prime	Prime Pump	5.3.1B, 5.3.2B, Component Manual
	Inconsistent flow due to leaking pump	Repair pump	5.3.2C,Component Manual
Oscillating Retention Time	Pump Problems	Recalibrate/Repair Pump	5.3.3A, Component Manual
	Temperature variability	Use a thermostatted oven, check oven operation	5.3.3B, Component Manual
	Sluggish Injection valve	Service Valve	5.3.3C, Component Manual
Poor Front End Resolution	Conc. Incorrect Eluents	Remake Eluents	5.2.1, 5.4.4A
	Column Overloading	Reduce Sample Size	5.4.4B
	Large System Void Volumes	Replumb System	Component Manual, 5.4.4C
Spurious Peaks	Sample Contaminated	Pretreat Samples	5.5A
	Sluggish Injection Valve	Service Valve	5.5B, Component Manual
Analyte Specific Efficiency Loss	Column Contamination from sample or system	Purge contamination, employ a trap or guard column, clean or replace column	5.4.2

5.1 High Back Pressure

5.1.1 Finding the Source of High System Pressure

Total system pressure for the Dionex IonPac 4µm Guard Column plus the Dionex IonPac 4µm Analytical Column when using the test chromatogram conditions should be less than 4000 psi. If the system pressure at the standard flow rate is higher than the maximum operational pressure for the system, it is advisable to determine the cause of the high system pressure.

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 to confirm flow rate is correct. Pre-weigh the graduated cylinder and calculate the weight of eluent collected to obtain a more accurate measure of flow.

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

C. 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 be at its lowest (<50 psi) with everything disconnected. The pressure with the eluent generator components (EGC, EPM and degasser) connected should be <400 psi at 1.0 mL/min. 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.

D. Measure the system back pressure by attaching a short piece of new 0.010” tubing in place of the column.

E. The Dionex Electrolytically Regenerated Suppressor 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.

F. A Dionex High-Pressure In-Line Filter positioned between the Pump and Eluent Generator (or injection valve if and EGC is not installed) should be installed to prevent particulates from blocking the system.

5.1.2 Replacing Column Bed Support Assemblies (4 mm and 2 mm columns only)

If the column or guard 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.

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.

NOTE

Replacement of the 2 or 4 mm outlet bed support is not recommended.

NOTE

Replacement of capillary column bed supports is not supported.

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 bench top 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.

E. Screw the end fitting back onto the column. Tighten it finger-tight, 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.

5.1.3 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.

5.1.4 Filter Samples

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

5.2 High Background

5.2.1 Preparation of Eluents

A. Make sure that the eluents prepared manually 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.

5.2.2 A Contaminated Trap Column

Please refer to the Product Manual for the Trap column in use.

5.2.3 Contaminated Dionex CR-TC Column

A. A Dionex CR-TC Trap Column should be installed if using a Dionex Eluent Generator KOH Cartridge.

B. If the Dionex CR-TC becomes contaminated, please refer to the Clean-Up Procedure, in the Dionex CR-TC Product Manual.

5.2.4 A Contaminated Guard or Analytical Column

A. Remove the Dionex IonPac Guard and Dionex IonPac 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.

To eliminate downtime caused by fouling, a guard column should be used. Clean or replace the Dionex IonPac Guard Column at the first sign of column performance degradation. The columns can be cleaned as instructed in Section 6.5.2, “Column Cleanup Procedure”.

5.2.5 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.

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.

5.2.6 A Contaminated Suppressor

If the above items have been checked and the problem persists, the Dionex Electrolytically Regenerated Suppressor, the Dionex Capillary Electrolytic Suppressor or the Dionex MicroMembrane Suppressor is probably causing the problem. For details on Dionex Electrolytically Regenerated Suppressor operation, refer to the Dionex Electrolytically Regenerating Suppressor Product Manual. For details on Dionex Membrane Suppressor operation, refer to the Product Manual.

5.3 Inconsistent Retention Times

Inconsistent or shifting retention time could be due to one of several different factors. These should be checked to determine the cause and address the issue.

5.3.1 Drifting to Shorter Retention Time

A. Contamination of the sample or eluent. Poorly mixed eluent. Remake sample and/or eluent.

B. Insufficient pump priming. Prime the pump. Refer to the Product Manual for correct pump operation.

C. Changes in temperature will also cause peaks to drift. Place the column in a thermally controlled column compartment. Check the temperature control of the column compartment, calibrate/service as needed. Refer to the Product Manual for correct operation of the column compartment.

D. Excessive temperatures may cause column degradation. Check the temperature of the column compartment. Reduce the operational temperature. Refer to the Product Manual for correct operation of the column compartment. Replace the column.

E. Oxidizing eluent may cause hydrolysis or degradation of the column resulting in decreased analyte retention. Remove oxidizing agent, remake eluents, and replace the column.

5.3.2 Drifting to Longer Retention Time

A. Poorly mixed eluent. Remake eluent.

B. A pump losing prime will cause retention time drift. Prime the pump. Refer to the Product Manual for correct pump operation.

C. A leaking pump will cause longer retention times. Check the pump flow. Refer to the Product Manual for correct pump operation.

D. Changes in temperature will also cause peaks to drift. Place the column in a thermally controlled column compartment. Check the temperature control of the column compartment, calibrate/service as needed. Refer to the Product Manual for correct operation of the column compartment.

5.3.3 Oscillating Retention Times

A. Pump problems can cause retention time to shift to longer and shorter time, run to run. Check pump flow rate, prime the pump. Refer to the Product Manual for correct pump operation.

B. Temperature fluctuation will also cause peaks to shift. Place the column in a thermally controlled column compartment. Check the temperature control of the column compartment, calibrate/service as needed. Refer to the Product Manual for correct operation of the column compartment.

C. A sluggish injection valve will cause peaks to shift if the injection time varies. The injection valve may need maintenance. When an injection valve is actuated, the timing is critical for consistent retention times. This will occur when the injection valve needs to be cleaned or retorqued. Refer to the valve manual for troubleshooting and service procedures.

5.4 Poor Peak Resolution

When carrying out separations using gradient analysis the column must be sufficiently equilibrated with the eluent concentration used at the start of the analysis. The actual equilibration time depends on the ratio of the strongest eluent concentration to the weakest eluent concentration. Typically equilibration takes place in 3-5 column volumes of eluent. Depending on flow rate and concentration change, equilibration times range from 3 to 10 minutes.

A. If increased separation is needed for the first group of peaks, reduce the concentration of the starting eluent (E1). This part of the chromatogram is run isocratically with E1.

B. Due to different system configurations, the observed 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.

C. Keep the concentrations of E1 and E2 (final eluent concentration) constant and adjust the gradient time. This is the simplest way to compensate for total system differences if resolution is the problem.

D. 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. The 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.

5.4.1 Loss of Column Efficiency

When chromatographic efficiency is lost, peak resolution may decrease to an unacceptable level as the peaks broaden.

A. Check to see if headspace (2 and 4 mm columns only) has developed in the guard or analytical column. This is usually due to improper use of the column such as exposing it to high pressures. Remove the column's top end fitting according to Section 5.1.2, "Replacing Column Bed Support Assemblies (4 mm and 2 mm columns only)". 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 elution of the peaks broader resulting in reduced efficiency. Make sure connections are made correctly with 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. Cut the tubing lengths as short as possible, checking to ensure a smooth, 90^{o} cut.

C. If tubing is not connected properly at the column inlet and outlet, it can cause dispersion resulting in low efficiency numbers.

    a. When installing the columns, it is recommended to turn off the pump while making connections. This will avoid any slippage of the ferrule under high pressure conditions which can result in a void in the fitting. Dispersion, particularly evident in capillary systems results in chromatograms with tailing peaks as shown in Figure 12 below.

    b. Before connecting the fittings, it is recommended to inject water into the cavities of the fluidic system using a syringe or a micropipette while the flow is off. This will result in faster equilibration by preventing air from entering the system.

    c. When making capillary connections, make sure the ferrule and fitting bolt are at least 2 mm (0.1 inch) 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 13 illustrates the correct and incorrect placement of the ferrule and fitting bolt on the tubing.

Figure 12 Tailing Peaks Caused by Incorrectly Installed Capillary Tubing Fittings

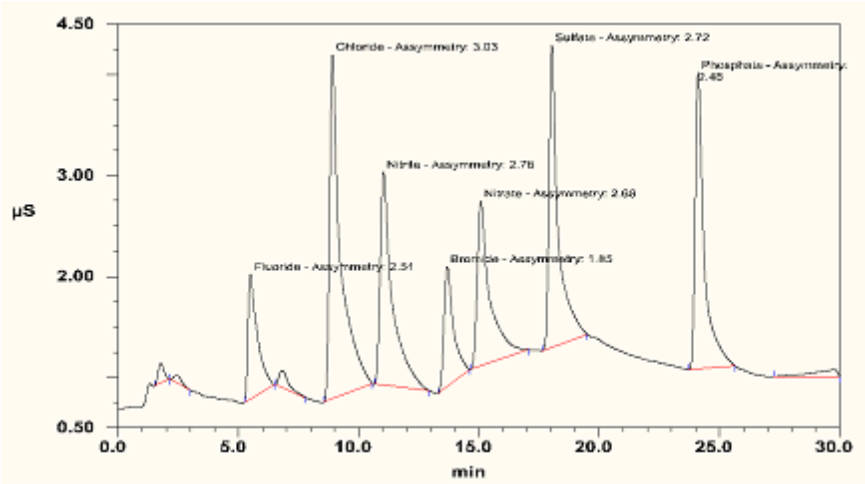
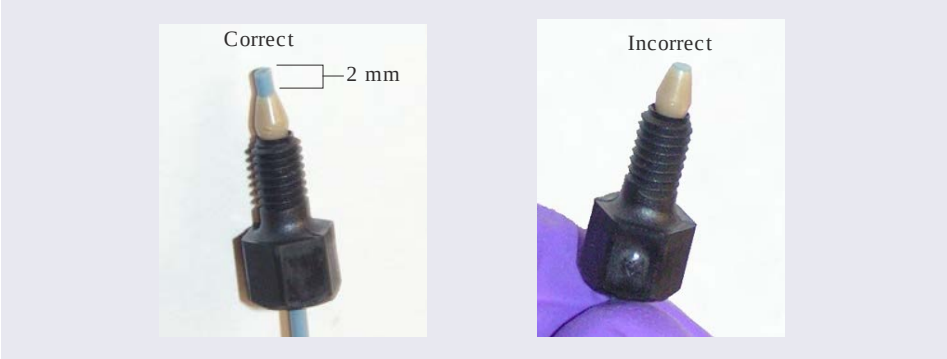


Figure 13 Correct and Incorrect Ferrule and Fitting Bolt Placement for Capillary Tubing Connections



D. Check for leaks. This can be done by visual inspection at elevated flow rates. Use of a paper towel or KimWipe™ touched to connection will show noticeable wetness even with micro liter volume leaks.

E. Suppressor contamination may be the cause of efficiency loss. Typically such contamination is analyte dependant so if all analytes are equally effected it is most likely due to one of the causes above. Refer to the Suppressor Product Manual for cleaning protocols.

5.4.2 Analyte Specific Efficiency Loss

If a specific analyte exhibits an efficiency problem this could be due to contamination. For example, iron oxide causes sulfate and phosphate to tail; aluminum causes poor recovery and peak shape for phosphate; magnesium and calcium hydroxide precipitation in the suppressor cause poor recovery and peak shape for magnesium and calcium (refer to the Product Manual for the suppressor for product specific troubleshooting); nonionic surfactants can cause sulfate to tail.

A. Purge system of suspected contamination. Refer to the Product Manual for the system for details.

B. Check sample as source of contamination.

C. Employ the use of an appropriate guard or trap column to remove contaminates. Contact your local representative for current product specific information.

5.4.3 Shortened Retention Times

Shortened retention times will cause peaks to elute closer together and may be due to one or more factors. Fast eluent flow will cause quicker elution of analytes. Higher eluent concentration or a contaminated column (which results in loss of capacity) will reduce the analyte retention causing peaks to elute early.

NOTE

Even with adequate system and column efficiency, resolution of peaks will be compromised if analytes elute too fast due to elevated flow rate or eluent concentration.

A. During gradient analysis the column must be equilibrated with the starting eluent prior to analysis. The time required is dependent on the difference in concentration between the start and the end of the gradient. If the ion concentration on the column is higher than the stating eluent concentration then peaks may elute early or with inconsistent retention time. Typically 3-5 column volumes of eluent are suggested for equilibration. Increase the length of time the column is in the starting eluent prior to injection to ensure adequate equilibration.

B. Check the flow rate. Ensure the eluent flow rate is equivalent to the flow rate specified by the analytical protocol. Confirm the eluent flow rate after the column using a stopwatch and graduated cylinder.

C. Ensure the eluent compositions and concentrations are correct. An eluent that is too concentrated will cause the peaks to elute sooner. Prepare fresh eluent.

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, Section Appendix A – Column Care").

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 resolves the problem. There may be a problem when one of the proportioned eluents is less than 5%

Column contamination can lead to a loss of column capacity. This is because all of the ion exchange sites will no longer be available for the sample ions. For example, polyvalent ions or metals from the sample may concentrate on the column. Refer to Section 6.5, Column Cleanup 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.

E. After cleaning the column, reinstall it in the system and let it equilibrate with eluent for about 30 minutes. The column is sufficiently 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.

5.4.4 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. Eluent concentration: Incorrect 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. The eluent concentration maybe incorrect if the flow delivered to the eluent generator cartridge is incorrect. Check pump flow rate.

B. Sample concentration and injection volume: 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. System plumbing: Improperly swept out volumes anywhere in the system prior to the guard and analytical columns may be the problem. Reconnect components, one at a time, in the system prior to the columns and test for front-end resolution after every system change.

5.5 Spurious Peaks

In a system where peaks are observed where none are expected both the column and the system should be checked.

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 Section 6.5, “Column Cleanup”.

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. 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 so long as they do not interfere with the quantification of the peaks of interest.

6. Appendix A – Column Care

6.1 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 Column is listed in Sections 1, "Introduction" and 3.1, "General Operating Conditions".

6.2 Column Start-Up

The column is shipped using 100mM sodium bicarbonate as the storage solution.

Prepare the eluent shown on the Quality Assurance Report. To remove the storage solution, flush the column to waste with the QAR eluent for at least 30 minutes before attaching the column outlet to the suppressor. Install the column in the column module and 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.

IMPORTANT

When making any tubing connections, first turn off the pump. This will avoid any slippage of the ferrule under high pressure conditions. For capillary connections, inject deionized 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

6.3 Column Storage

For short-term storage (< 1 week), use eluent; for long-term storage (> 1 week), store in eluent in a refrigerator or use 100 mM Sodium bicarbonate for the column storage solution when stored at room temperature. With the column outlet to waste, flush the column for a minimum of 10 minutes with the storage solution. Cap both ends securely, using the plugs supplied with the column.

6.4 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.

Currently at Thermo Fisher Scientific, we have obtained consistent results using Optima® Solvents by Fisher Scientific. We have found that bottled HPLC-grade water from Burdick & Jackson contains acceptably low levels of impurities.

6.4.1 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.

6.4.2 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.

6.4.3 Solvents

In most cases, unless otherwise noted in Section 3.2, "General Operational Precautions", solvents can be added to the ionic eluents used with Dionex IonPac 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.

When using a solvent in an ionic eluent, the back pressure generated 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. Do not exceed the maximum operating backpressure of the Dionex IonPac column.

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.

6.5 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 viscosity mixing zones can be created between two eluents having solvents with a very high energy of mixing.***
- ***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 by solvents during column rinses can result in very high pressure zones.***

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.

6.5.1 Choosing the Appropriate Cleanup Solution

Table 6 Cleaning Solutions for Anion Exchange Columns

Contamination	Anion Solutions
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.
Hydrophilic Contamination of High Valence	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.
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.
Nonionic and Hydrophobic Contamination	Although this is extremely rare, nonionic and hydrophobic contaminants can contaminate an ion exchange column. The symptoms include reduced chromatographic efficiency, elevated pressure or tailing for a subset of analyte ions. Retention is unaffected by this type of 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.

6.5.2 Column Cleanup Procedure

A. Prepare a 500 mL solution of the appropriate cleanup solution using the guidelines in the table above.

B. Disconnect the outlet of the Dionex IonPac Column from the Dionex Suppressor. 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. Double check that the eluent flows in the correct direction as 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. 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

C. For aqueous cleaning solutions; set the pump flow rate to 1.0 mL/min for a Dionex IonPac 4 mm Analytical or Guard Column or 0.25 mL/min for a Dionex IonPac 2 mm Analytical or Guard Column. Set the pump flow rate to 10 µL/min for a Dionex IonPac Capillary or Capillary Guard Column.

D. For cleaning solutions containing organic solvents; set the pump flow rate to 0.5 mL/min for a Dionex IonPac 4 mm Analytical or Guard Column or 0.12 mL/min for a Dionex IonPac 2 mm Analytical or Guard Column. Set the pump flow rate to 5 µL/min for a Dionex IonPac Capillary or Capillary Guard Column.

E. Rinse the column for 10 minutes with deionized water before pumping the chosen cleanup solution over the column or before switching cleanup solutions.

F. Unless otherwise noted, pump the cleanup solution through the column for at least 60 minutes (pump for longer time if column is highly contaminated). A step gradient is used for column cleanup when switching between solutions.

G. Rinse the column for at least 10 minutes with deionized water before pumping eluent over the column.

H. Equilibrate the column(s) with eluent, still directing the effluent to waste, for at least 30 minutes before resuming normal operation.

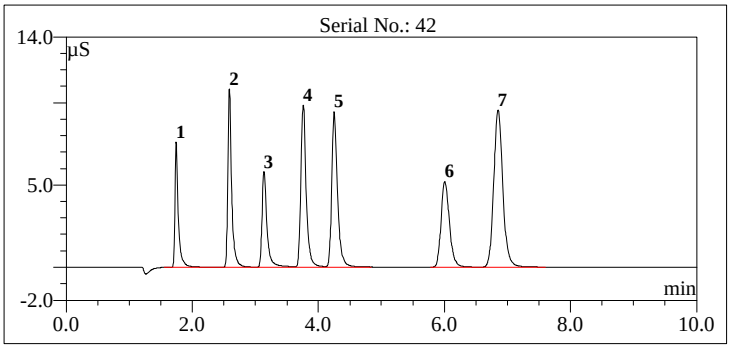
I. Reconnect the Dionex IonPac Analytical Column to the Dionex Suppressor and if your system was originally configured with a guard column, place the guard column in line between the injection valve and the analytical column.

7. Appendix B – Quality Assurance Reports

Dionex IonPac™ AS22-Fast-4µm
Capillary (0.4 x 150 mm)
Product No. 088490

Date: 16-Jul-14 11:58
Serial No. : 000042
Lot No. : 2014-10

Eluent: 4.5 mM Na2CO3/ 1.4 mM NaHCO3
Flow Rate: 12 µL/min
Temperature: 30 °C
Detection: Suppressed Conductivity
Suppressor: Dionex Anion Capillary Electrolytic Suppressor (ACES™ 300) AutoSuppression™ Recycle Mode
Applied Current: 7 mA
Injection Volume: 0.4 µL
Storage Solution: 100 mM Sodium bicarbonate



No.	Peak Name	Ret.Time (min)	Asymmetry (AIA)	Resolution (EP)	Efficiency (EP)	Concentration (mg/L)
1	Fluoride	1.74	2.2	9.15	6559	1.25
2	Chloride	2.59	1.7	4.88	10859	2.50
3	Nitrite	3.14	1.8	4.69	10044	3.75
4	Bromide	3.76	1.6	3.29	11534	6.25
5	Nitrate	4.25	1.6	8.87	11127	6.25
6	Phosphate	6.01	1.4	3.47	10370	10.00
7	Sulfate	6.85	1.2	n.a.	11868	7.50

QA Results:

Analyte	Parameter	Specification	Results
Sulfate	Efficiency	>=8100	Passed
Sulfate	Asymmetry	0.9-1.7	Passed
Sulfate	Retention Time	6.24-7.56	Passed
	Pressure	<=2750	1520

Production Reference:
Datasource: QAR
Directory: Cap\AS22-Fast-4µm
Sequence: AS22-4µm_p4X150MM
Sample No.: 1

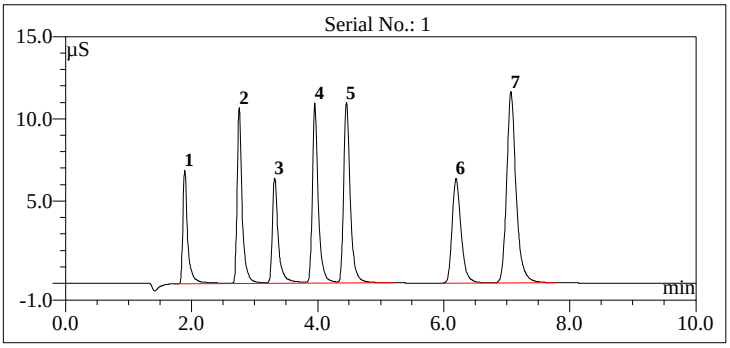
6.80 SR11 Build 3161 (184582) (Demo-Installation)

Chromeleon™ Thermo Fisher Scientific

Dionex IonPac™ AS22-Fast-4µm
Analytical (2 x 150 mm)
Product No. 088488

Date: 16-Jul-14 11:58
Serial No. : 000001
Lot No. : 2014-10

Eluent: 4.5 mM Na2CO3/ 1.4 mM NaHCO3
Flow Rate: 0.30 mL/min
Temperature: 30 °C
Detection: Suppressed Conductivity
Suppressor: Dionex Anion Electrolytically-Regenerated Suppressor (Dionex AERS™ 500 2mm) AutoSuppression™ Recycle Mode
Applied Current: 8 mA
Injection Volume: 2.5 µL
Storage Solution: 100 mM Sodium bicarbonate



No.	Peak Name	Ret.Time (min)	Asymmetry (AIA)	Resolution (EP)	Efficiency (EP)	Concentration (mg/L)
1	Fluoride	1.89	2.12	6.92	3853	5.0
2	Chloride	2.76	1.80	4.01	7257	10.0
3	Nitrite	3.32	1.85	4.06	7595	15.0
4	Bromide	3.96	1.65	2.93	9576	25.0
5	Nitrate	4.46	1.63	8.19	9762	25.0
6	Phosphate	6.20	1.48	3.48	10165	40.0
7	Sulfate	7.07	1.34	n.a.	12065	30.0

QA Results:

Analyte	Parameter	Specification	Results
Sulfate	Efficiency	>=8100	Passed
Sulfate	Asymmetry	0.95-1.76	Passed
Sulfate	Retention Time	6.24-7.56	Passed
	Pressure	<=2750	2228

Production Reference:
Datasource: QAR
Directory: Anion\AS22-Fast-4µm
Sequence: AS22-FAST-4µM_2X150MM
Sample No.: 1

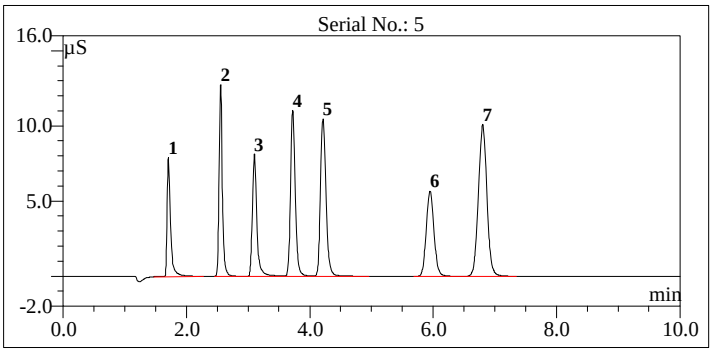
6.80 SR11 Build 3161 (184582) (Demo-Installation)

Chromeleon™ Thermo Fisher Scientific

Dionex IonPac™ AS22-Fast-4 µm Analytical (4 x 150 mm)
Product No. 088486

Date: 31-Jul-14 14:52
Serial No. : 000005
Lot No. : 2014-10-42

Eluent: 4.5 mM Na2CO3/ 1.4 mM NaHCO3
Flow Rate: 1.2 mL/min
Temperature: 30 ºC
Detection: Suppressed Conductivity
Suppressor: Dionex Anion Electrol ytically Regenerated Su ppressor (Dionex AERS™ 500 4mm) AutoSu ppression™ Rec ycle Mode
Applied Current: 31 mA
Injection Volume: 10 µL
Storage Solution: 100 mM Sodium bicarbonate



No.	Peak Name	Ret.Time (min)	Asymmetry (AIA)	Resolution (EP)	Efficiency (EP)	Concentration (mg/L)
1	Fluoride	1.71	2.11	9.32	6084	5.0
2	Chloride	2.55	1.26	5.06	11656	5.0
3	Nitrite	3.10	1.50	4.87	10318	15.0
4	Bromide	3.72	1.30	3.37	12203	25.0
5	Nitrate	4.21	1.36	9.08	11619	25.0
6	Phosphate	5.95	1.14	3.59	10977	40.0
7	Sulfate	6.81	0.99	n.a.	11692	30.0

QA Results:

Analyte	Parameter	Specification	Results
Sulfate	Efficiency	>=8100	Passed
Sulfate	Asymmetry	0.95-1.76	Passed
Sulfate	Retention Time	6.2-7.6	Passed
	Pressure	<=2750	2258

Production Reference:
Datasource: QAR
Directory: Anion\AS22-Fast-4µm
Sequence: AS22-Fast-4µm_4X150MM
Sample No.: 1

6.80 SR11 Build 3161 (184582) (Demo-Installation)
Chromeleon™ Thermo Fisher Scientific

8. Appendix C – Additional Information

NOTE

For assistance, visit Unity Lab Services online at www.unitylabservices.com.
From the U.S. call the Customer Care Center for Dionex Products at 1-800-346-6390
Outside the U.S., call the nearest Thermo Fisher Scientific office.

8.1 General Information on PEEK™ Tubing

Dionex brand PEEK™ tubing is available. Note that tubing from different suppliers varies with respect to color and i.d. Care should be taken to ensure the correct i.d. is being used to avoid problems caused by sample dispersion.

Table 7 Tubing Back Pressures.

Color	Part Number	I.D. inch	I.D. cm	Volume mL/ft	Back Pressure, Psi/ft. at 1mL/min	Back Pressure, Psi/ft. at 0.25mL/min	Back Pressure, Psi/cm. at 1mL/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

Note: Blue and Light blue are very similar in color. Additionally, tubing from different suppliers may differ to that stated above.

8.2 Installation of the Capillary Column

The following information has been adapted from document # 065446-01 and is correct at the time of print. To ensure information is correct and up to date, please refer to the applicable manual for your system.

Correct installation of the capillary column is vital for good column performance.

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 19).
2. For reference, Figure 14 shows the column cartridge after installation of both a capillary guard column and a capillary separator column. Figure 15 shows the column cartridge after installation of only a capillary separator column.

Figure 14 Separator and Guard Columns Installed in Column Cartridge

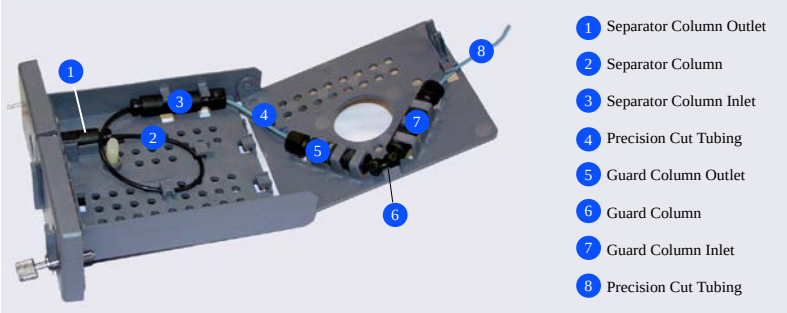
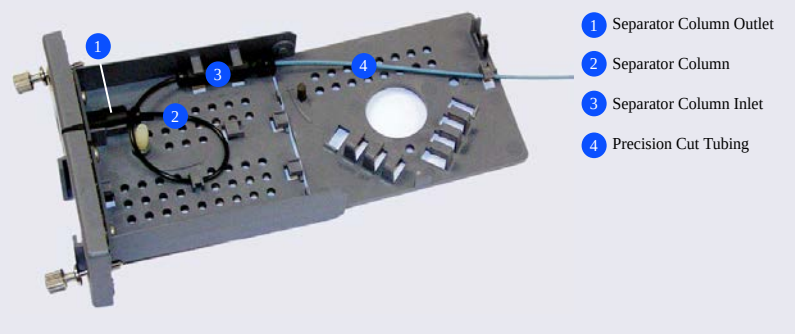


Figure 15 Separator Column Only Installed in Column Cartridge



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 8 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

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

Figure 16 Tubing Connections for 250 mm Separator Column and 50 mm Guard Column

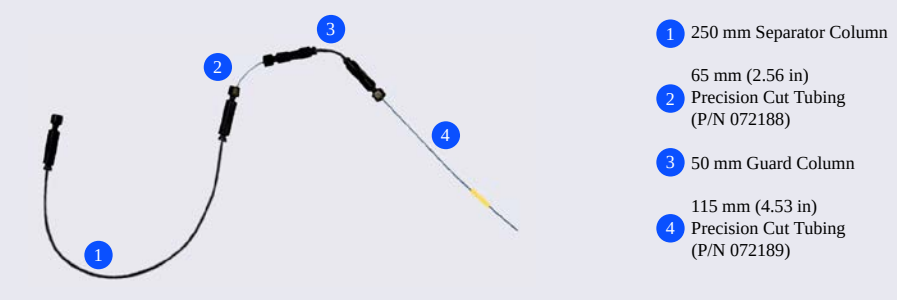
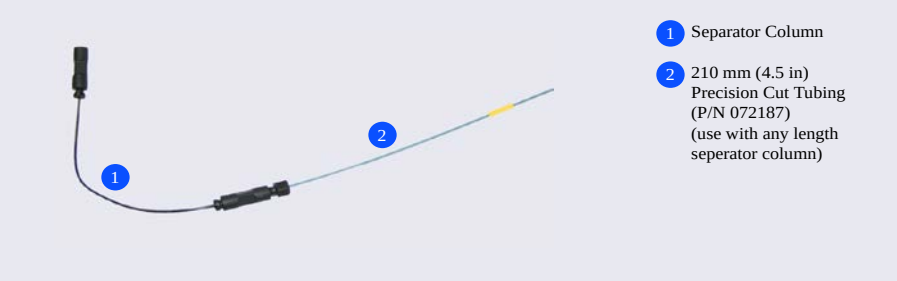
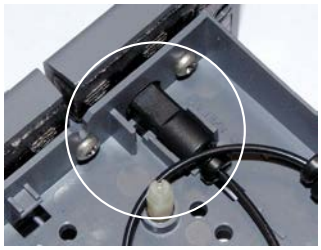


Figure 17 Tubing Connections for Separator Column Only



4. Lift up the lid of the column cartridge to open it.
5. Remove the fitting plug from the outlet fitting on the separator column. Orient the fitting with a flat side up (see Figure 18) and push the fitting into the opening at the front of the column cartridge until it stops.

Figure 18 Column Outlet Fitting Installed in Column Cartridge



6. Coil the separator column tubing inside the cartridge as shown in Figure 14 or Figure 15. Secure the column tubing and the inlet fitting in the clips on the column cartridge.
7. Secure the inlet and outlet fittings on the guard column (if used) in the column clips on the lid of the column cartridge.
8. 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.
9. Close the lid (you should hear a click) and route the tubing into the slot on the front of the column cartridge (see Figure 19).

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 19 Column Cartridge Closed