Errata

Product Manual for Dionex IonPac™ AS4A-SC and AG4A-SC Columns
034528-07

For new orders of the following parts discussed in this manual, please use the updated part numbers listed below.

Part	Old Part Number in this manual	Updated Part Number to use for new orders
PROD,COL,IP,ATC-3,4X35MM	*059661*	*079932*

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PRODUCT MANUAL

IONPAC® AG4A-SC GUARD COLUMN

(4 x 50 mm, P/N 043175)
(2 x 50 mm, P/N 043126)

IONPAC® AS4A-SC ANALYTICAL COLUMN

(4 x 250 mm, P/N 043174)
(2 x 250 mm, P/N 043125)

QUICKSTART STEPS AND LINKS
Click blue text below to get started.

1. See Section 4, “Operation”. Note operation precautions and chemical purity requirements.
2. See Section 4.3, “Eluent Preparation". Make the required eluents.
3. See “Quality Assurance Report”. Run the Production Test Chromatogram as a system check.
4. See Section 5, "Example Applications" for example applications.
5. See “Column Care" for column cleanup and long-term storage recommendations.

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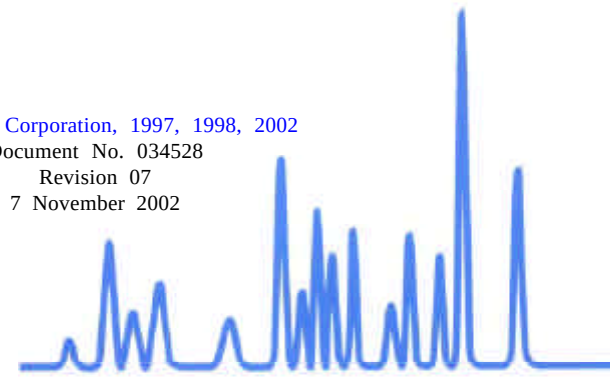
Revision 07
7 November 2002



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SECTION 1 - INTRODUCTION TO IONPAC AS4A-SC ION CHROMATOGRAPHY

The IonPac® AS4A-SC 2-mm (P/N 043125) and 4-mm (P/N 043174) Analytical Columns are designed for the analysis of anions and organic acids using carbonate/bicarbonate or borate eluent systems. For many isocratic applications sodium carbonate/bicarbonate eluent systems are used. Borate eluents are used instead of carbonate/bicarbonate eluents for gradient applications because the lower background conductivity of the suppressed borate eluents produces shallower gradient ramps. The advantage of both of these eluent systems is that much less care must be taken to prevent contamination from carbon dioxide in the air compared to hydroxide eluent systems.

The AS4A-SC 2-mm can be operated at flow rates up to 0.75 mL/min with eluents containing 0 - 100% organic solvents. The AS4A-SC 4-mm can be operated at flow rates up to 3.0 mL/min with eluents containing 0 - 100% organic solvents. The columns are stable between pH 0 and 14. PEEK (polyetheretherketone) is used to make column hardware. PEEK has excellent chemical resistance to most organic solvents and inorganic solutions. Concentrated sulfuric acid and concentrated nitric acid will attack PEEK. Tetrahydrofuran at concentrations of greater than 20% is not compatible with PEEK systems. The AS4A-SC 2-mm and 4-mm Analytical Columns have minimum efficiencies of 4,000 plates/column for sulfate, under standard operating conditions. The AS4A-SC 2-mm operates at a backpressure between 1,000 - 1,400 psi at 0.50 mL/min with standard eluent. The AS4A-SC 4-mm operates at a backpressure between 1,000 - 1,400 psi at 2.0 mL/min with standard eluent. However, both columns are capable of operating at backpressures up to 4,000 psi.

The AS4A-SC is composed of a 13 µm highly cross-linked polyethylvinylbenzene/divinylbenzene substrate agglomerated with anion exchange latex that has been completely aminated. The ion exchange capacity of the 2 x 250 mm analytical column is 5 µeq/column. The ion exchange capacity of the 4 x 250 mm analytical column is 20 µeq/column. The latex particles are permanently bonded to the substrate surface by electrostatic and van der Waals interactions.

The selectivity of the IonPac AS4A-SC 2-mm Analytical Column is the same as the IonPac AS4A-SC 4-mm Analytical Column. Both columns have been designed to separate F^-, Cl^-, NO_2^-, Br^-, NO_3^-, HPO_4^{2-} and SO_4^{2-} isocratically in less than 8 minutes. Use the same eluent concentrations with the AS4A-SC 2-mm that are used with the AS4A-SC 4-mm but reduce the flow rate to one-fourth of the flow rate used on the 4-mm system. The AS4A-SC columns have a highly cross-linked (55%), microporous, hydrophobic core resin that has been agglomerated with totally permeable latex particles that are completely aminated. The latex particles carry the actual ion exchange function - an alkanol quaternary ammonium group. The polymeric structure of the packing material makes the AS4A-SC compatible with pH 0-14 eluents and organic solvents. The AS4A-SC can be used with any suppressible ionic eluent that does not exceed the capacity of the suppressor.

This manual assumes that you are familiar with the installation and operation of the 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 IonPac AS4A-SC Analytical and AG4A-SC Guard Columns have 10-32 threaded PEEK end fittings for use with ferrule/bolt liquid line fittings. If your system is otherwise configured, refer to - DIONEX Liquid Line Fittings.

Table 1
AS4A-SC/AG4A-SC Packing Specifications

Column	Particle Diameter µm	Substrate X-Linking %	Latex Diameter nm	Latex X-Linking %	Column Capacity µeq/column	Functional Group	Hydrophobicity
AS4A-SC 4 x 250 mm	13	55	160	0.5	20 µeq	Alkanol quaternary ammonium	Medium - low
AG4A-SC 4 x 50 mm	13	55	160	0.5	4 µeq	Alkanol quaternary ammonium	Medium - low
AS4A-SC 2 x 250 mm	13	55	160	0.5	5 µeq	Alkanol quaternary ammonium	Medium - low
AG4A-SC 2 x 50 mm	13	55	160	0.5	1 µeq	Alkanol quaternary ammonium	Medium - low

Table 2
AS4A-SC/AG4A-SC Operating Parameters

Column	Typical Back Pressure psi (MPa) at 30°C	Standard Flow Rate mL/min	Maximum Flow Rate mL/min
AS4A-SC Analytical (4-mm)	< 1,000 (6.89)	2.0	3.0
AG4A-SC Guard (4-mm)	< 200 (1.38)	2.0	3.0
AS4A-SC Analytical + Guard (4-mm)	**<1,200 (8.27)**	**2.0**	**3.0**
AS4A-SC Analytical (2-mm)	<1,000 (6.89)	0.50	0.75
AG4A-SC Guard (2-mm)	< 200 (1.38)	0.50	0.75
AS4A-SC Analytical + Guard (2-mm)	**<1,200 (8.27)**	**0.50**	**0.75**

Always remember that assistance is available for any problem that may be encountered during the shipment or operation of DIONEX instrumentation and columns through the DIONEX North America Technical Call Center at 1-800-DIONEX-0 (1-800-346-6390) or through any of the DIONEX Offices listed in, "DIONEX Worldwide Offices."

SECTION 2 - COMPARISON OF ION CHROMATOGRAPHY SYSTEMS

CONFIGURATION	2-mm	4-mm
Eluent Flow Rate	0.5 mL/min	2.0 mL/min
SRS	ASRS-ULTRA (2-mm) (P/N 053947)	ASRS-ULTRA (4-mm) (P/N 053946)
MMS	AMMS III (2-mm) (P/N 056751)	AMMS III (4-mm) (P/N 056750)
AES	AAES (P/N 056116)	AAES (P/N 056116)
NOTE Do not run suppressors over 40°C. If application requires a higher temperature, place suppressor outside of chromatographic oven.		
Regenerant Flow Rate	25 - 100% of 4-mm system	Typically 10 - 15 mL/min. See suppressor manual
Injection Loop	2 - 15 µL Use the Rheodyne Microinjection Valve, Model No. 9126 DIONEX P/N 044697) for full loop injections <15 µL.	10-50 µL
System Void Volume	Eliminate switching valves, couplers and tubing with long lengths or Ids greater than 0.005".	Minimize dead volumes. Switching valves, couplers can be used. Use the GM-2, GM-3, GM-5 or recommended gradient mixers.
Pumps	Use the GP40/GP50/GS50/IP20/IP25 in Microbore Configuration with a Microbore GM-4 (2-mm) Gradient Mixer. The older GPM-2 has a large delay volume. Gradient applications in this manual can not be reproduced without method modifications.	Use the GP40/GS50/GP50/IP20/IP25 in Standard-Bore Configuration. Use the GM-5 Gradient Mixer The GM-3 Gradient Mixer should be used for gradient analysis on systems other than the GP40/GP50/IP20/IP25 and the DX-300 HPLC Pump.

CONFIGURATION	2-mm	4-mm
Detectors	AD20/AD25 Cell (6-mm, 7.5 µL, P/N 046423) VDM-2 Cell (3-mm, 2.0 µL, P/N 043120) CD20, CD25, CD 25A, ED40, ED50, or ED50A Conductivity Cell with DS3 P/N 044130 or Conductivity Cell with shield P/N 044132 CDM-2/CDM-3 Cell P/N 042770 Replace the TS-1 with the TS-2 (P/N 043117) on the CDM-2 or the CDM-3. The TS-2 has been optimized for 2-mm operation. Do not use the TS-2 or the TS-1 with the ED40/ED50/ED50A or the CD20/CD25/25A. DIONEX Back Pressure Regulator 75 psi rating (P/N 039760, 046480) or Tubing (see Table 3) Ensure 50-75 psi back pressure.	AD20/AD25 Cell (10-mm, 9 µL, P/N 049393) VDM-2 Cell (6-mm, 10 µL P/N 043113) CD20, CD25, CD25A, ED40, ED50, or ED50A Conductivity Cell with DS3 P/N 044130 or with shield P/N 044132 CDM-2/CDM-3 Cell P/N 042770 Either the TS-1 with the TS-2 can be used with the CDM-2 or the CDM-3. Do not use the TS-2 or the TS-1 with the ED40/ED50/ED50A or the CD20/CD25/25A. DIONEX Back Pressure Regulator 75 psi rating (P/N 039760, 046480) or Tubing (see Table 3) Ensure 50-75 psi back pressure.

Table 1
Tubing Backpressures

Tubing ID in	H_20 Back Pressure Psi/ft at 1 mL/min
0.005	111.4
0.007	29.0
0.010	7.0
0.012	3.4

SECTION 3 - INSTALLATION

3.1 System Requirements

3.1.1 System Requirements for 2-mm Operation

The IonPac AS4A-SC 2-mm Guard and Analytical Columns are designed to be run on any DIONEX Ion Chromatograph equipped with suppressed conductivity detection. Isocratic analyses at flow rates of 0.5 mL/min or greater can be performed on a Gradient Pump Module (GPM-2), an Advanced Gradient Pump (AGP), or a GP40/GP50/GS50 with standard (1/8" pistons) pump heads. For isocratic analyses at flow rates below 0.5 mL/min and gradient analyses, a microbore Advanced Gradient Pump or a microbore IP20/IP25 (1/16" pistons) must be employed.

NOTE

The Gradient Pump Module (GPM-2) can be used for 2-mm isocratic chromatography down to 0.5 mL/min but CANNOT be used for 2-mm gradient chromatography.

Use the same eluent concentration used for the standard 4-mm system but reduce the flow rate to one-fourth of the flow rate used on the 4-mm column system. The typical flow rate of the AS4A-SC 2-mm is 0.50 mL/min.

3.1.2 System Requirements for 4-mm Operation

The IonPac AS4A-SC 4-mm Guard and Analytical Columns are designed to be run on any DIONEX Ion Chromatograph equipped with suppressed conductivity detection. Gradient methods and methods requiring solvent containing eluents should be performed on a system having a Gradient Pump Module (GPM-2), or an Advanced Gradient Pump (AGP), or a GP40/GP50/GS50 with standard 1/8" pump heads. Isocratic analysis can also be performed on an IP20/IP25 with standard bore.

3.1.3 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" (P/N 044221) ID PEEK tubing. 0.010" ID PEEK tubing (P/N 042260) or 0.012" Tefzel tubing (see, DIONEX Product Selection Guide) may be used but peak efficiency will be compromised which may also result in decreased peak resolution. Minimize the lengths of all connecting tubing and remove all unnecessary switching valves and couplers. If you need assistance in properly configuring your system contact the nearest DIONEX Regional Office (see, DIONEX Worldwide Offices).

Ensure that all tubing installed between the injection valve and the detector has an internal diameter of 0.012" (Tefzel) or less. 2-mm systems are shipped with 0.005" ID PEEK tubing. Do not use a high pressure Gradient Mixer (GM-3) between the Gradient Pump Module (GPM) and the injection valve if performing gradients.

3.2 Installing the Anion Trap Column, ATC-3

When performing a gradient anion exchange application, a borate eluent system should be used instead of a carbonate system because of its low background conductivity. An IonPac Anion Trap Column, ATC-3 (2-mm), P/N 059661 or ATC-3 (4-mm), P/N 059660, should be installed between the Gradient Pump and the injection valve. Remove the high pressure Gradient Mixer if present. The ATC is filled with high capacity anion exchange resin which helps to minimize the baseline shift caused by increasing anionic contaminant levels in the eluent as the ionic concentration of the eluent is increased over the course of the gradient analysis.

To install the ATC-3 (2-mm) or ATC-3 (4-mm), complete the following steps:

A. Remove the Gradient Mixer, if installed between the gradient pump pressure transducer and the injection valve.

B. Connect the gradient pump directly to the ATC. Connect a waste line to the ATC outlet and direct the line to a waste

container.

C. Flush the ATC with 200 mL of 70 mM $Na_2B_4O_7$ at a flow rate of 0.5 mL/min when using the ATC-3 (2-mm) or 2.0 mL/min when using the ATC-3 (4-mm).

D. Rinse the ATC with the strongest eluent that will be used during the gradient analysis.

E. After flushing the ATC with eluent, connect the ATC to the eluent line that is connected to the injection valve.

The background conductivity of your system should be less than 7 µS when $Na_2B_4O_7$ is being pumped through the chromatographic system with the AMMS in-line and properly functioning. The baseline shift should be no greater than 10 µS during a borate gradient eluent concentration ramp from 0 to 70 mM $Na_2B_4O_7$. If the baseline shifts are greater than 10 µS, the ATC should be cleaned using steps A - E above.

The ATC can be flushed, at the end of each operating day, to remove any impurities that may have accumulated on it. This will minimize periodic maintenance and lost data.

A. Flush the ATC with 30 mL of 70 mM $Na_2B_4O_7$.

B. On the next day, prior to use of the chromatographic system, flush the ATC with 30 mL of the strongest eluent used in the gradient program.

See the Product Manual for the IonPac ATC-3 (P/N 032697) for instructions on cleaning a contaminated Anion Trap Column

3.3 Sample Concentration

A. The IonPac AG4A-SC 2-mm or 4-mm Guard Column can be used for trace anion concentration work primarily in high purity water analysis. The function of AG4A-SC Guard Column in these applications is to strip ions from a measured volume of a relatively clean aqueous sample matrix. This process concentrates all anionic analyte species onto the AG4A-SC Guard Column leading to a lowering of detection limits by 2-5 orders of magnitude. The unique advantage of the AG4A-SC Guard Column for the analytical chemist in these applications is the capability of performing routine trace analyses of sample matrix ions at µg/L levels without extensive and laborious sample pretreatment.

B. A Trace Anion Concentrator (TAC-2 provides the least baseline disturbance when the concentrator is switched into the eluent flow path. This is due to its low void volume that must be filled and pressurized when the column is switched into the eluent flow path. the TAC-2 column should only be used when using carbonate eluents. For a detailed discussion of anion concentration techniques, refer to Section 3, “Operation,” of the Product Manual for the Trace Anion Concentrator (TAC-2) Column (Document No. 034467).

CAUTION

IonPac Trace Anion Concentrator (TAC-2) Column (P/N 043101) is designed for 4-mm systems. Although the principles of sample concentration are the same for both the AG4A-SC 2-mm and the TAC-2, the large void volume in the TAC-2 makes it unacceptable for use with 2-mm systems.

C. An Anion MicroConcentrator (AMC-1, P/N 051760) can also be used for sample concentration. For further information see the AMC-1 Product Manual (Document No. 031262).

D. The TAC-LP1 (P/N 046026) can be used with hydroxide and carbonate eluents, with or without solvent, to concentrate samples on either 4-mm or 2-mm analytical systems. For further information, see the TAC-LP1 Product Manual (Document No. 034972).

3.4 The Injection Loop

Table 2

Smallest Injectable Volumes (µL)

Valve Type	Using 0.012" ID Tefzel Tubing	Using 0.007" ID Tefzel Tubing	Using 0.010" ID PEEK Tubing	Using 0.005" ID PEEK Tubing
DIONEX BF2 Valve (8 µL Internal Volume) (10 cm Loop)	15.2	10.5	13.1	9.2
DIONEX MicroInject Valve (10.5 µL Internal Volume) (14 cm Loop)	20.5	14.0	17.6	12.2
Rheodyne Microinjection Valve Model 9126 (0.8 µL Internal Volume) (10 cm Loop)	8.0	3.3	5.9	2.0

3.4.1 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 2.5 nanomoles (25 - 50 ppm) of any one analyte onto a 2-mm analytical column. Injecting larger volumes of samples can result in overloading the column which can affect the detection linearity. The AS4A-SC 2-mm requires a microbore HPLC system configuration. Install an injection loop one-fourth or less (< 15µL) of the loop volume used with a 4-mm analytical system (see Section 3.10, Detector, requirements for 2-mm operation for more details). For injection volumes < 15 µL, the Rheodyne Microinjection Valve, Model No. 9126 (DIONEX P/N 044697) is required. See Table 2, Smallest Injectable Volumes (µL).

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

For most applications on a 4-mm analytical system, a 10 - 50 µL injection loop will be sufficient. Generally, do not inject more than 10 nanomoles (100 - 200 ppm) of any one analyte onto the 4-mm analytical column. Injecting larger volumes of samples can result in overloading the column which can affect the detection linearity. This phenomenon will be more prevalent at higher concentrations of the analytes of interest.

3.5 IonPac AG4A-SC Guard Column

An IonPac AG4A-SC Guard Column is normally used with the IonPac AS4A-SC Analytical Column. Retention times will increase by approximately 20% when a guard column is placed in-line prior to the analytical column. A guard is placed prior to the analytical column to prevent sample contaminants from eluting onto the analytical column. It is easier to clean or replace a guard column than it is an analytical column.

3.6 Anion Self-Regenerating Suppressor

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

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

If you are installing an IonPac AS4A-SC 4-mm Analytical Column, use an ASRS-ULTRA (4-mm, P/N 053946).
If you are installing an IonPac AS4A-SC 2-mm Analytical Column, use an ASRS-ULTRA (2-mm, P/N 053947).

For detailed information on the operation of the Anion Self-Regenerating Suppressor, see Document No. 031367, the "Product Manual for the Anion Self-Regenerating Suppressor-ULTRA, the ASRS-ULTRA (4-mm) and the ASRS-ULTRA (2-mm)."

3.7 Anion Atlas Electrolytic Suppressor

An Anion Atlas Electrolytic Suppressor (AAES) may be used instead of an ASRS-ULTRA for applications that require suppressed conductivity detection. The AAES (P/N 056116) can be used for AS4A-SC 2-mm and 4-mm applications using eluents up to 25 µeq/min.

For detailed information on the operation of the Anion Atlas Electrolytic Suppressor, see Document No. 031770, the "Product Manual for the Anion Atlas Electrolytic Suppressor."

3.8 Anion MicroMembrane Suppressor

An Anion MicroMembrane Suppressor should be used for applications that require suppressed conductivity detection. It is compatible with all solvents and concentrations with which the systems and columns are compatible.

If you are installing an IonPac AS4A-SC 4-mm Analytical Column, use an AMMS III (4-mm) (P/N 056750).
If you are installing an IonPac AS4A-SC 2-mm Analytical Column, use an AMMS III (2-mm) (P/N 056751).

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

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

DIONEX recommends using the Displacement Chemical Regeneration (DCR) Mode for chemical suppression using sulfuric acid and the Anion MicroMembrane Suppressor (AMMS III). See the DCR kit manual, Document P/N 031664, for details.

SAFETY
Use proper safety precautions in handling acids and bases.

3.10 Using AutoRegen with the ASRS-ULTRA or the AMMS III in the Chemical Suppression Mode

To minimize the baseline shift when performing an analysis that requires a hydroxide or borate gradient, a **high regenerant flow rate (10 - 15 mL/min)** is required. To save regenerant preparation time and reduce regenerant consumption and waste, DIONEX recommends using an AutoRegen® Accessory (P/N 039594).

When using an AutoRegen System, specific contaminants are continuously removed from the regenerant solution to restore it to the correct ionic state. It is necessary however to replace the regenerant on a regular basis. If solvents are used in the eluent, ionic contaminants from the solvent component of the eluent which are not removed by the Anion AutoRegen Regenerant Cartridge may slowly accumulate in the regenerant. This results in slowly increasing background conductivity. The rate at which the background conductivity increases versus the required analysis sensitivity will determine how often the regenerant must be changed.

It is not necessary to change the Anion AutoRegen Regenerant Cartridge until it is completely expended and a sudden jump to very high background conductivity is observed. For more detailed information on the use of AutoRegen Regenerant Cartridges, see the Product Manual for the AutoRegen Regenerant Cartridge Refills (Document No. 032852).

3.11 Detector Requirements

Consult Section 2, “Comparison of Ion Chromatography Systems,”for system detector, cell and thermal stabilizer requirements.

3.12 Eluent Storage

IonPac AS4A-SC columns are designed to be used with borate (gradient or isocratic analysis) or bicarbonate/carbonate (isocratic analysis only) eluent systems. Storage under a helium atmosphere ensures contamination free operation and proper pump performance (nitrogen can be used if eluents do not contain solvents).

SECTION 4 - OPERATION

This section describes the performance comparison of 2-mm ID analytical columns and systems to 4-mm ID analytical columns and systems. Note the similarities and differences listed below for the two column and system types.

4.1 Comparison of 2-mm and 4-mm Systems

Sample Loop Volume:	10 µL (Figure A) 2.5 µL (Figure B)
Analytical Column:	IonPac AS4A-SC 4-mm Analytical Column (Figure A) IonPac AS4A-SC 2-mm Analytical Column (Figure B)
Eluent:	1.8 mM Na_2CO_3/1.7 mM $NaHCO_3$
Eluent Flow Rate:	2.0 mL/min (Figure A) 0.5 mL/min (Figure B)
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS-ULTRA
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III
MMS Regenerant:	50 mN H_2SO_4
or AES Suppressor:	Anion Atlas Electrolytic Suppressor, AAES
Expected Background Conductivity:	15 - 20 µS

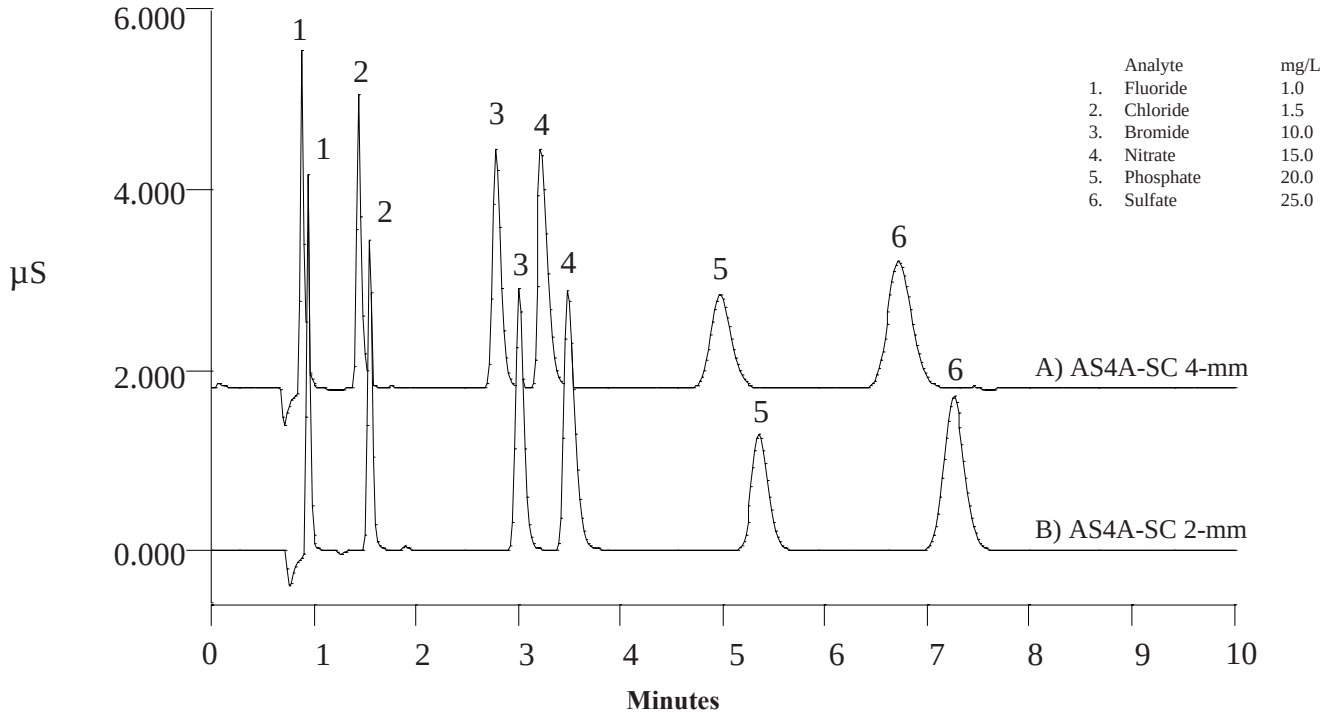


Figure 1
Comparison of 2-mm and 4-mm Operation

4.1.1 IonPac AS4A-SC Operation Precaution

CAUTION
Filter and Degas Eluents
Filter Samples
0.75 mL/min Maximum Flow Rate for AS4A-SC 2-mm
3.00 mL/min Maximum Flow Rate for AS4A-SC 4-mm

4.1.2 Solvents

The AS4A-SC can withstand all common HPLC solvents in a concentration range of 0 - 100%. However, solvents and water should be premixed in concentrations to 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.

One of the benefits of the IonPac AS4A-SC Analytical Column is the ability to use most common HPLC solvents from concentrations of 0% to 100% as mobile phase modifiers in ion exchange separations. When using a solvent in an ionic eluent, column generated backpressures 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 backpressure limit for the IonPac AS4A-SC columns is 4,000 psi. Therefore, any combination of the above contributions to operating backpressure that totals up to 4,000 psi can be used.

Table 3
HPLC Solvents for Use with IonPac AS4A-SC Columns

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

4.2 Chemical Requirements

Obtaining reliable, consistent and accurate results requires eluents that are free of ionic and spectrophotometric 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. DIONEX cannot guarantee proper column performance when the quality of the chemicals, solvents and water used to prepare eluents has been compromised.

4.2.1 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.

4.2.2 Solvents

Since solvents used with the IonPac AS4A-SC columns are added to ionic eluents 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 DIONEX, we have obtained consistent results using High Purity Solvents manufactured by Burdick and Jackson and Optima Solvents by Fisher Scientific.

4.2.3 Deionized Water

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

4.3 Eluent Preparation

NOTE

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

The above precautions, if taken when making eluents, ensure smooth, reproducible ramps, with minimum total change in background conductivity when using sodium carbonate/bicarbonate, borate or hydroxide eluents with the AS4A-SC columns.

The following table details the use of the above eluent types:

Eluent: 1.8 mM Carbonate/1.7 mM Bicarbonate

Pipette 10.0 mL of the AS4A Combined Carbonate/Bicarbonate Eluent Concentrate, (100X concentrate = 180 mM Na_2CO_3/170 mM $NaHCO_3$, P/N 039513) into a 1 L volumetric flask. Use degassed, deionized water with a specific resistance of 18.2 megohm-cm to dilute to a final volume of 1,000 mL.

or

The 100X concentrate (180 mM Na_2CO_3/170 mM $NaHCO_3$) can be prepared by thoroughly dissolving 19.078 g of sodium carbonate (MW 106.00 g/mole) plus 14.282 g sodium bicarbonate (MW 84.00 g/mole) in 700 mL degassed, 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.

Make the final eluent by pipetting 10.0 mL of the eluent concentrate, (100X concentrate = 180 mM Na_2CO_3/170 mM $NaHCO_3$) prepared above into a 1 L volumetric flask. Use degassed, deionized water with a specific resistance of 18.2 megohm-cm to dilute to a final volume of 1,000 mL.

Eluent: 5 mM Sodium Borate

A. Thoroughly dissolve 1.90 g sodium borate, tetrahydrate (MW 381.42 g/mole) in 700 mL degassed, 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.

Regeneration:

A. During daily operation, it may be necessary to elute the polyvalent anions that concentrate on the column. The best way to remove these anions from the concentrator, guard, and analytical column is to pump 50 mM sodium borate (10X eluent strength) through the system for 10 minutes. The system should be flushed with these high-strength eluents at the end of the day. After cleaning the column, equilibrate it for 20 minutes with the operating eluent. During this cleanup, it is not necessary to disconnect the column from the suppressor.

4.4 Eluents Containing Solvents

Table 4
Eluent Type Selection

Eluent	Application
Bicarbonate/Carbonate	Isocratic Analysis Only
Borate	Isocratic and Gradient Analysis
Hydroxide	Eluent pH adjustment and Column Cleanup only

When mixing solvents with water remember to mix solvent with water on a volume to volume basis. If a procedure requires an eluent of 90% acetonitrile, prepare the eluent by adding 900 mL of acetonitrile to an eluent reservoir. Then add 100 mL of deionized water 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.

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

4.5 Regenerant Preparation

The regenerant is 50 mN sulfuric acid. Dilute 100 mL (about 100 g) of 0.50 N sulfuric acid (P/N 037164 or P/N 039601) to 1 L using deionized water. If you are not using the AutoRegen Accessory (P/N 039594), prepare several liters of the regenerant.

For 2-mm operation, use the same regenerant flow rate as used in standard 4-mm column applications. For many analyses, the regenerant flow rate can be reduced by a factor of 4 times to conserve regenerant. This is especially useful if a pressurized vessel is being used rather than an AutoRegen Accessory Unit.

For a guide to properly adjusting the regenerant flow rate, see Document No. 031727, the "Product Manual for the Anion MicroMembrane Suppressor III, the AMMS III."

4.6 Sample Concentration

The IonPac AG4A-SC Guard Column can be used for trace anion concentration work primarily in high purity water analysis. The function of AG4A-SC Guard 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 AG4A-SC Guard Column, lowering detection limits by 2-5 orders of magnitude. The AG4A-SC is used in lieu of the sample loop. Pump the sample onto the AG4A-SC 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 with IonPac AS4A-SC columns refer to Section 3, Operation, of the Product Manual for theTrace Anion Concentrator (TAC-2) Column (Document No. 034467). An Anion MicroConcentrator (AMC-1, P/N 051760) can also be used for sample concentration. For further information see the AMC-1 Product Manual (Document No. 031262).

CAUTION

IonPac Trace Anion Concentrator (TAC-2) Column (P/N 043101) is designed for 4-mm systems. Although the principles of sample concentration are the same for both the AG4A-SC and the TAC-2, the large void volume in the TAC-2 makes it unacceptable for use with 2-mm systems.

SECTION 5 - EXAMPLE APPLICATIONS

Before attempting any of the following example applications, take the time to ensure that your system is properly configured. It is very important that applications run on 2-mm columns utilize the proper pump configuration (see Section 2, Comparison of Ion Chromatography Systems) and have all system void volumes minimized (see Section 3.13, 2-mm Column Operation Summary).

Ensure that all of the eluents have been made from high purity reagents and deionized water. All water used in the preparation of eluents should be degassed, deionized water. For chemical purity requirements see Section 4.2, Chemicals Required.

In order to guarantee reproducible retention times of analytes when doing gradient chromatography, it is important to install an Anion Trap Column, ATC-3 (4-mm) or the ATC-3 (2-mm) in the system (see Section 3.2, Installing the Anion Trap Column).

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 and analytical columns has been fouled, refer to the column cleanup protocols in “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 (see Section 3.3, Sample Concentration).

5.1 Production Test Chromatogram

Isocratic elution of anions on the IonPac AS4A-SC Analytical 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. To guarantee that all IonPac AS4A-SC 2-mm and 4-mm Analytical Columns meet high quality and reproducible performance specification standards, all columns undergo the following production control test.

Sample Loop Volume:	12.5 µL (2-mm), 50 µL (4-mm)
Analytical Column:	IonPac AS4A-SC Analytical Column
Eluent:	1.8 mM Na_2CO_3/1.7 mM $NaHCO_3$
Eluent Flow Rate:	0.5 mL/min (2-mm), 2.0 mL/min (4-mm)
MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III
MMS Regenerant:	50 mN H_2SO_4
or SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS-ULTRA
or AES Suppressor:	Anion Atlas Electrolytic Suppressor, AAES
Expected Background Conductivity:	15 to 17 µS
Storage Solution:	100 mM NaOH

	Analyte	mg/L
1.	Fluoride	2.0
2.	Chloride	3.0
3.	Nitrite	5.0
4.	Bromide	10.0
5.	Nitrate	10.0
6.	Phosphate	15.0
7.	Sulfate	15.0

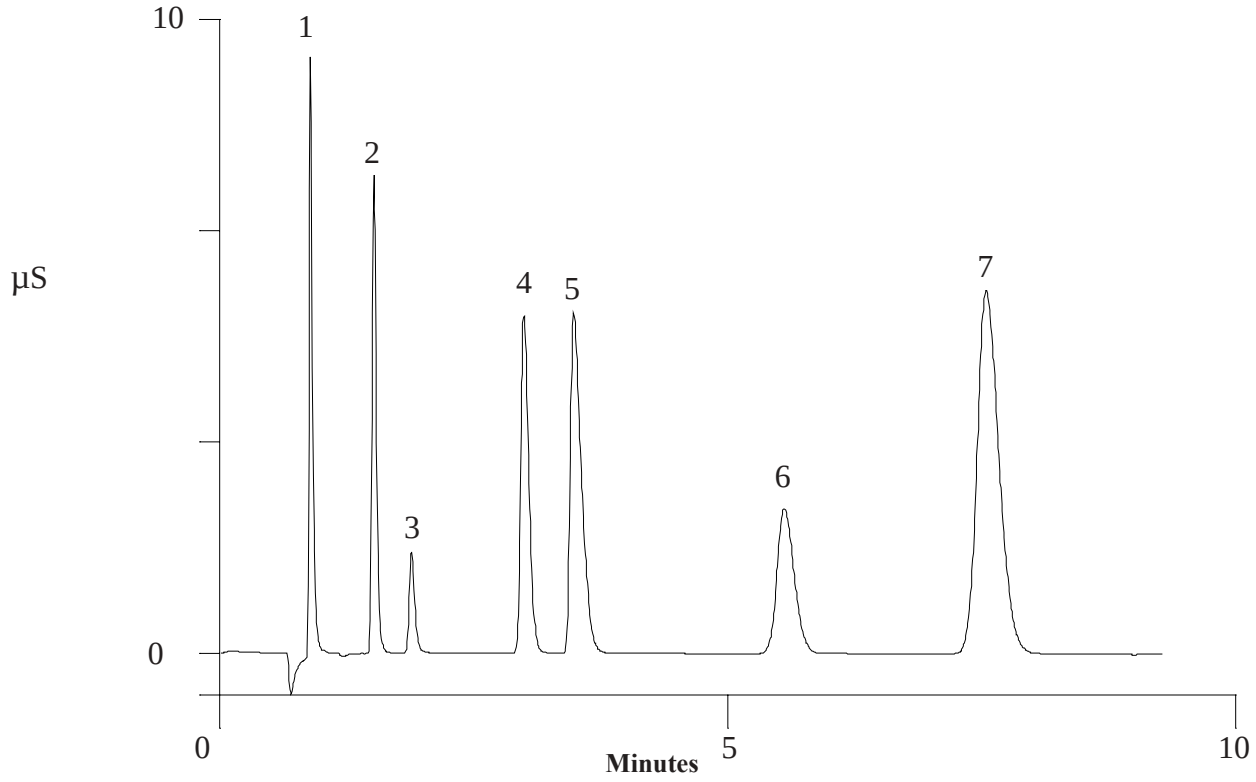


Figure 3
IonPac AS4A-SC Production Test Chromatogram

5.2 Isocratic Elution of Inorganic Anions Plus Oxalate

Separation and elution of inorganic anions plus oxalate on the IonPac AS4A-SC Analytical Column has been optimized utilizing a carbonate/bicarbonate eluent. By using this eluent, monovalent and divalent anions can be isocratically eluted and quantitated in a single injection. The carbonate/bicarbonate mixture allows the strength of the eluent, and thus the selectivity of the system, to be changed by varying the HCO_3^-/CO_3^{2-} ratio. The suppressor reaction product is carbonic acid (H_2CO_3), which results in a low background conductivity (15 - 17 µS).

Sample Loop Volume:	5 µL (2-mm), 20 µL (4-mm)
Guard Column:	IonPac AG4A-SC Guard Column
Analytical Column:	IonPac AS4A-SC Analytical Column
Eluent:	1.8 mM Na_2CO_3/1.7 mM $NaHCO_3$
Eluent Flow Rate:	0.5 mL/min (2-mm), 2.0 mL/min (4-mm)
MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III
MMS Regenerant:	50 mN H_2SO_4
or SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS-ULTRA
or AES Suppressor:	Anion Atlas Electrolytic Suppressor, AAES
Expected Background Conductivity:	15 - 20 µS
Concentrator for Trace Level Analysis:	IonPac AG4A-SC 2-mm Guard Column (2-mm) IonPac TAC-2 (4-mm) or IonPac AMC-1
Sample Volume for Trace Level Analysis:	4 - 6 mL*
Column Storage Solution:	100 mM NaOH

***** The practical anionic loading capacity for any sample matrix must be determined using the procedures outlined in Document No. 034467, the Product Manual for the IonPac® Trace Anion Concentrator (TAC-2) Column.

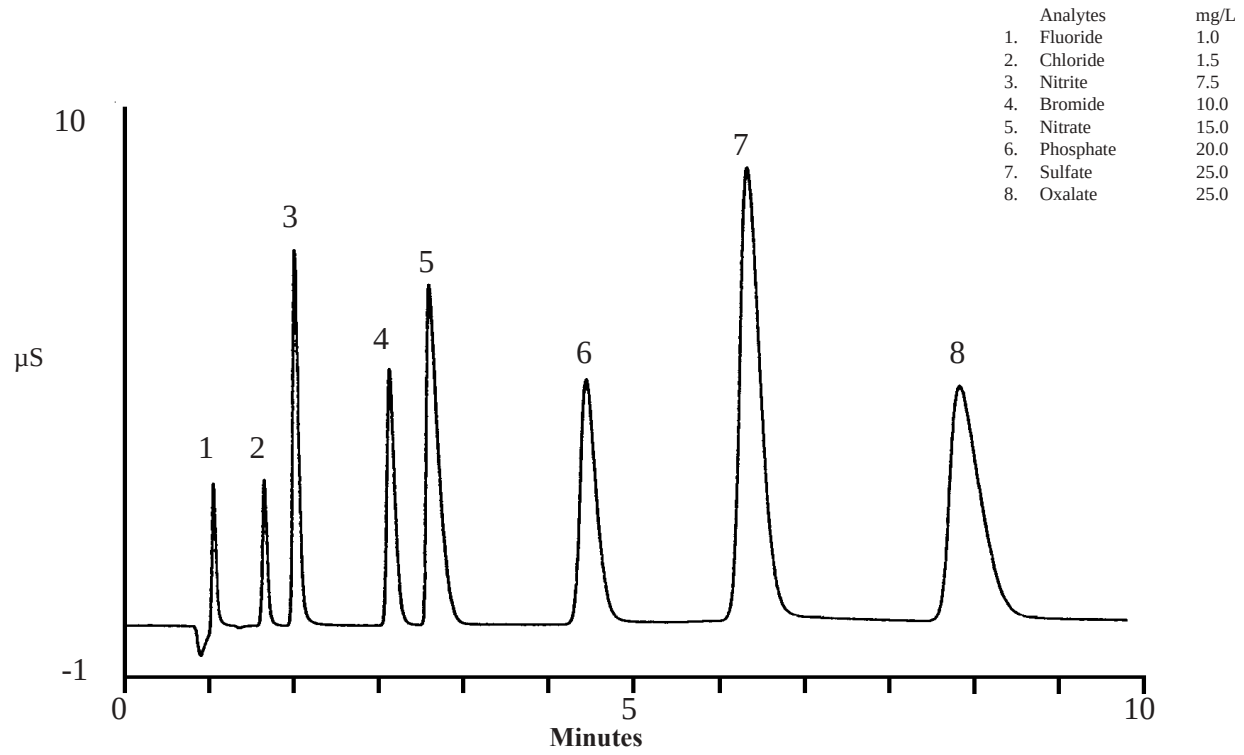


Figure 4
Isocratic Elution of Inorganic Anions plus Oxalate

5.3 Isocratic Elution of Weakly Retained Inorganic Anions and Organic Acids

When using a carbonate/bicarbonate eluent for the isocratic elution of inorganic anions and organic acids, small aliphatic organic acids such as acetate and formate are weakly retained and tend to elute close to the void volume of the column. They often coelute with fluoride during the analysis. If quantification of acetate, fluoride, and formate is required, an eluent with a weak eluting ion must be used. In this case, sodium borate can be used as the eluent since the borate ion ($B_4O_7^{2-}$) is a very weak eluting ion.

Sample Volume:	5 µL (2-mm), 20 µL (4-mm)
Guard Column:	IonPac AG4A-SC Guard Column
Analytical Column:	IonPac AS4A-SC Analytical Column
Eluent:	5.0 mM $Na_2B_4O_7$, isocratic
Eluent Flow Rate:	0.5 mL/min (2-mm), 2.0 mL/min (4-mm)
MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III
MMS Regenerant:	50 mN H_2SO_4
or SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS-ULTRA
or AES Suppressor:	Anion Atlas Electrolytic Suppressor, AAES
Expected Background Conductivity:	2-4 µS
Concentrator Column for Trace Level Analysis:	IonPac AG4A-SC 2-mm Guard Column (2-mm) IonPac TAC-2 (4-mm) or IonPac AMC-1
Sample Volume Required for Trace Level Analysis:	2 - 4 mL*
Column Storage Solution:	100 mM NaOH

***** The practical anionic loading capacity for any sample matrix must be determined using the procedures outlined in Document No. 034467, the Product Manual for the IonPac® Trace Anion Concentrator (TAC-2) Column.

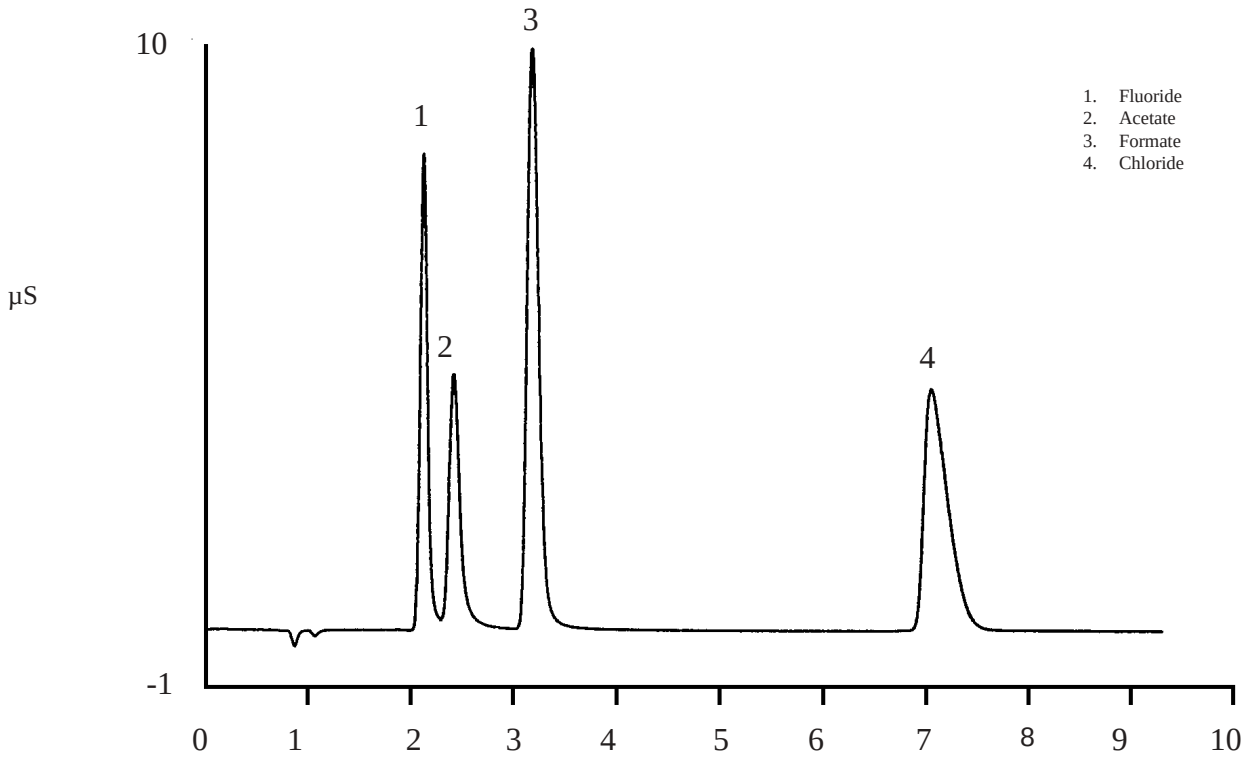


Figure 5
Isocratic Elution of Weakly Retained Inorganic Anions and Organic Acids

5.4 Gradient Elution of Inorganic Anions and Organic Acids

The determination of low MW small aliphatic organic acids, monovalent and divalent anions in one injection requires a gradient change in the eluent strength during the run. By using this technique it is possible to resolve and quantitate fluoride, formate, and acetate, and at the same time to elute and quantitate sulfate in a 15 minute run time. Sodium borate is converted to the weakly ionized boric acid in the suppressor resulting in a much lower background conductivity and baseline shift from the beginning to the end of the run than is observed with carbonate/bicarbonate eluent systems. The initial 4.9 mM sodium borate eluent is weak enough to elute fluoride beyond the void volume and separate formate and acetate. The final 28 mM sodium borate eluent concentration is capable of eluting phosphate and sulfate. Prior to an injection, the column should be equilibrated with the weak eluent for 10 minutes. During this time, the sample can be loaded onto the IonPac AG4A-SC (2-mm) guard column or TAC-2 (4-mm) or AMC-1 if concentration of the sample is required. If an injection is made before the column has been equilibrated with weak eluent, resolution of fluoride and acetate will suffer and the retention times of all peaks may not be reproducible. Prior to equilibrating the system with the weak eluent, deionized water should be pumped through the entire system for 2 minutes. This step helps to equilibrate the system more quickly with the weak eluent.

Eluent 1: Deionized water, with a specific resistance of 18.2 megohm-cm

Eluent 2: 70 mM Sodium Borate

A. Thoroughly dissolve 26.696 g $Na_2B_4O_7 \cdot 10\ H_2O$ (MW 381.42 g/mole) in 700 mL degassed, 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.

Sample Volume:	See Concentration Volume Below
Guard Column:	IonPac AG4A-SC Guard Column
Analytical Column:	IonPac AS4A-SC Analytical Column
Eluent #1:	Deionized water (Specific Resistance 18.2 megohm-cm)
Eluent #2:	70 mM $Na_2B_4O_7$
Eluent Flow Rate:	0.5 mL/min (2-mm), 2.0 mL/min (4-mm)
MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III
MMS Regenerant:	50 mN H_2SO_4
or SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS-ULTRA
or AES Suppressor:	Anion Atlas Electrolytic Suppressor, AAES
Expected Background Conductivity:	
Weak Eluent (4.9 mM $Na_2B_4O_7$):	2 - 4 µS
Strong Eluent (28.0 mM $Na_2B_4O_7$):	10 - 12 µS
Anion Trap Column:	ATC-3 (2-mm) or ATC-3 (4-mm)
Concentrator Column for Trace Level Analysis:	IonPac AG4A-SC 2-mm Guard Column (2-mm) IonPac TAC-2 (4-mm) or IonPac AMC-1
Sample Volume for Trace Level Analysis:	3 - 5 mL*

* The practical anionic loading capacity for any sample matrix must be determined using the procedures outlined in Document No. 034467, the Product Manual for the IonPac® Trace Anion Concentrator (TAC-2) Column.

Gradient Program

TIME (min.)	%E1	%E2	INJECTION VALVE	COMMENT
0.0	100	0	Inject	Start of system rinse.
2.0	100	0	Inject	End of system rinse.
2.1	93	7	Load	System equilibrated with weak eluent. Start sample concentration.
12.0	93	7	Load	Stop sample concentrate.
12.1	93	7	Inject	Inject sample. Start gradient.
20.1	60	40	Inject	Hold gradient.
25.0	60	40	Inject	End analysis.

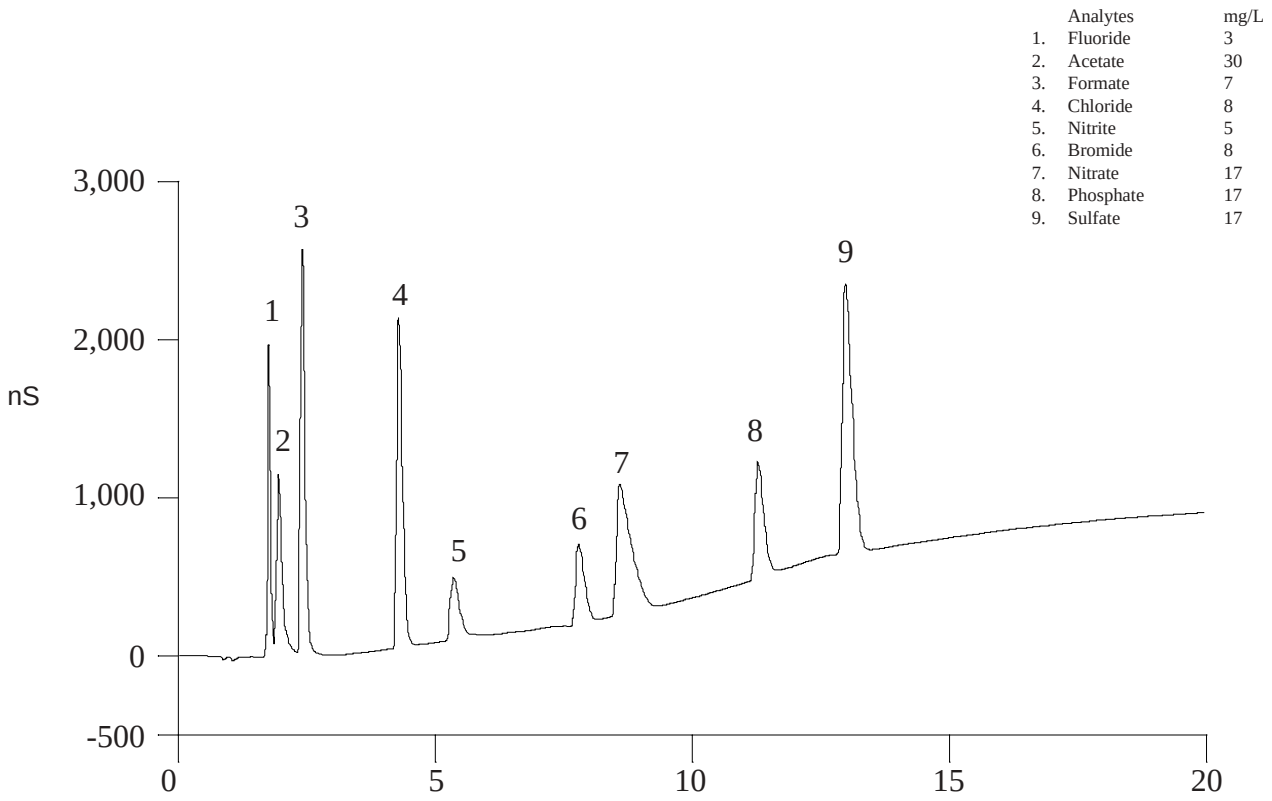


Figure 6
Gradient Elution of Inorganic Anions and Organic Acids

SECTION 6 - TROUBLESHOOTING GUIDE

The purpose of the Troubleshooting Guide is to help you solve operating problems that may arise while using IonPac AS4A-SC 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. If you cannot solve the problem on your own, call the DIONEX Regional Office nearest you (see, DIONEX Worldwide Offices).

6.1 High Backpressure

6.1.1 Finding the Source of High System Pressure

Total system pressure when using the IonPac AG4A-SC (2-mm) Guard and AS4A-SC (2-mm) Analytical Columns at 0.50 mL/min should less than 1,500 psi when using the eluent used to generate the test chromatogram. Total system pressure when using the IonPac AG4A-SC (4-mm) Guard and AS4A-SC (4-mm) Analytical Columns at 2.0 mL/min should also be less than 1,500 psi when using the eluent used to generate the test chromatogram. Refer to Section 4.1.3, Solvents, to see how solvent concentration can affect the column operating pressure. If the system pressure is higher than 1,500 psi, it is advisable to find out what is causing the high system pressure. The system should be used with a High-Pressure In-Line Filter (P/N 035331) for the eluents which is positioned between the 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. Find out what part of the system is causing the high pressure. It could be a piece of tubing that has plugged or whose walls are collapsed, an injection valve with a plugged port, a column with particulates plugging the bed support, a plugged High-Pressure In-Line Filter, the suppressor or the detector cell.

To find out which part of the chromatographic system is causing the problem, disconnect the pump eluent line from the injection valve and turn the pump on. Watch the pressure; it should not exceed 50 psi. Continue adding the system's components (injection valve, column(s), suppressor and detector) one by one, while watching the system pressure. The pressure should increase up to a maximum of 1,500 psi when the column(s) are connected. The suppressor may add up to 100 psi. No other components should add more than 100 psi of pressure. Refer to the appropriate manual for cleanup or replacement of the problem component.

6.1.2 Replacing Column Bed Support Assemblies

If the column inlet bed support is determined to be the cause of the high backpressure, 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. Using two open end wrenches, carefully unscrew the inlet (top) column fitting.

C. 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.

	IonPac AS4A-SC	
	2-mm Columns (P/N)	4-mm Columns (P/N)
Analytical Column	043125	043174
Guard Column	043126	043175
Bed Support Assembly	044689	042955
End Fitting	043278	052809

CAUTION

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

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

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

NOTE

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

6.2 High Background or Noise

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

ELUENT	EXPECTED BACKGROUND CONDUCTIVITY
1.8 mM Na_2CO_3/1.7 mM $NaHCO_3$	14 - 18 µS
4.9 mM $Na_2B_4O_7$	2 - 4 µS
28 mM $Na_2B_4O_7$	12 - 14 µS

The background conductivity typically increases between 1 and 10 µS when running a gradient as described in Section 5.4, “Gradient Elution of Inorganic Anions and Organic Acids.”

6.2.1 Preparation of Eluents

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

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

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

6.2.2 A Contaminated Anion Trap Column, ATC-3

When doing gradient analysis, has the Anion Trap Column, the ATC-3 (2-mm) or the ATC-3 (4-mm) been installed correctly? If it has not, install one as directed in Section 3.2, Installing the Anion Trap Column, and watch the background conductivity. If the background conductivity is now low, this means that the ATC is trapping contaminants from the eluent. The eluents probably have too many impurities (see items 1 - 3 above).

If the ATC is already installed, remove it. Is the background conductivity still high? If the background conductivity decreases,

the ATC is the source of the high background conductivity.

A. Disconnect either the ATC-3 (2-mm) or the ATC-3 (4-mm) from the injection valve and direct the outlet to waste.

B. Flush the ATC with 200 mL of 70 mM $Na_2B_4O_7$. Use a flow rate of 0.5 mL/min on a 2-mm system or a flow rate of 2.0 mL/min on a 4-mm system.

C. Equilibrate the ATC with the strongest eluent used during the gradient run. Use a flow rate of 0.5 mL/min on a 2-mm system or a flow rate of 2.0 mL/min on a 4-mm system.

D. If the problem persists, replace the ATC.

6.2.3 A Contaminated Guard or Analytical Column

Remove the IonPac AG4A-SC Guard and AS4A-SC Analytical Columns from the system. Is the background conductivity still high? If the column is the cause of the high background conductivity, clean the column as instructed in "Column Care."

6.2.4 Contaminated Hardware

To eliminate the hardware as the source of the high background conductivity, bypass the suppressor and pump deionized water with a specific resistance of 18.2 megohm-cm through the system. The background conductivity should be less than 2 µS. If it is not, check the detector/conductivity cell calibration by injecting deionized water directly into it. See the appropriate manual for details.

6.2.5 A Contaminated Anion Suppressor

A. A Contaminated ASRS-ULTRA

This section describes routine cleanup procedures for the Anion Self-Regenerating Suppressors (ASRS-ULTRA) in the case of contamination. Consult the Troubleshooting Guide (see Section 4, "Troubleshooting Guide") to first determine that the system is operating properly. If the ASRS-ULTRA is determined to be the source of higher than normal back pressure, higher than anticipated conductivity, decreased suppression capacity or decreased sensitivity, cleaning the membrane may restore the performance of the system. Use the following procedures to clean the membrane.

1. Metal Contaminants or Precipitates

NOTE

The suppressor voltage is a good indicator of the resistance across the suppressor. Higher resistance may indicate contamination of the suppressor. For more information regarding monitoring the voltage, see Document No. 031814-02, "Removal of Iron Contamination from Electrolytic Suppressors."

A. Turn off the SRS power.

B. Disconnect the analytical (and guard) column(s) from the injection valve and the ASRS-ULTRA. Refer to the specific analytical column Product Manual for column cleanup procedures.

C. If you are running in the **AutoSuppression External Water Mode**, turn off the external water and disconnect the external water line from the ASRS-ULTRA **REGEN IN** port.

D. Disconnect the liquid line from the ASRS-ULTRA **ELUENT OUT** port to the cell at the cell fitting and reconnect it to the **REGEN IN** port.

E. Connect a temporary line from the priming block or the low-pressure tee on the isocratic or gradient pump to a

container of 0.2 M oxalic acid. Pump this solution through the ASRS-ULTRA (4-mm) at 1-2 mL/min for 30 minutes. For 2-mm systems pump this solution through the ASRS-ULTRA (2-mm) at 0.25-0.50 mL/min for 30 minutes.

NOTE

Bypassing internal pump manifolds when temporarily pumping high concentration cleaning solutions significantly reduces the time required to reequilibrate the system to low concentration eluents.

F. Flush the ASRS-ULTRA with deionized water for 10 minutes.

G. Perform steps A - D of the procedure in Section 4.1, "Small Analyte Peak Areas."

H. Turn on the SRS Control unit for the **AutoSuppression Recycle or External Water Modes** of operation. Ensure that the SRS Control unit is **off** for the **Chemical Suppression Mode** of operation.

I. Flush the ASRS-ULTRA with eluent for 10 minutes.

J. Reinstall the analytical (and guard) column(s). Begin pumping eluent through the system at the flow rate required for your analysis and equilibrate the system.

B. A Contaminated Anion MicroMembrane Suppressor, AMMS III.

A. **Check the regenerant flow rate at the REGEN OUT port of the AMMS. For the example of isocratic applications, this flow rate should be 3 - 5 mL/min.**

B. Check the eluent flow rate. In general, the eluent flow rate for 2-mm applications should be 0.50 mL/min and for 4-mm applications, it should be 2.0 mL/min. Refer to the Product Manual for the Anion MicroMembrane Suppressor (Document No. 031727) for assistance in determining that the eluent is within suppressible limits.

C. Prepare fresh regenerant solution. Bypass the Anion AutoRegen Regenerant Cartridge (if you are using the AutoRegen Accessory). If the background conductivity is high, you probably need to clean or replace your Anion MicroMembrane Suppressor. Refer to the Product Manual for the Anion MicroMembrane Suppressor (Document No. 031727) for assistance.

D. If you are using an AutoRegen Accessory, connect the freshly prepared regenerant to the Anion AutoRegen Regenerant Cartridge. Pump approximately 200 mL of regenerant through the Anion AutoRegen Regenerant Cartridge to waste before recycling the regenerant back to the regenerant reservoir. If the background conductivity is now high, you probably need to replace the Anion AutoRegen Regenerant Cartridge (P/N 039564). Refer to the AutoRegen Regenerant Cartridge Refill Product Manual (Document No. 032852) for assistance.

C. A Contaminated Anion Atlas Electrolytic Suppressor (AAES)

Metal Contaminants or Precipitates

1. Turn off the power to the AAES.

2. Disconnect the analytical (and guard) column(s) from the injection valve and the AAES. Refer to the specific analytical column Product Manual for column cleanup procedures.

3. If you are running in the **AutoSuppression External Water Mode**, turn off the external water and disconnect the external water line from the AAES **REGEN IN** port.

4. Disconnect the liquid line from the AAES **ELUENT OUT** port to the cell at the cell fitting and reconnect it to the **REGEN IN** port.

5. Connect a temporary line from the priming block or the low-pressure tee on the isocratic or gradient pump to a container with a solution of 0.5 M oxalic acid. Pass 60 mL of this solution through the AAES using the Trap Column / Suppressor Clean-up Kit (P/N 059649) or pump this solution through the AAES at 2.0 mL/min for 30 minutes.

NOTE

Bypassing internal pump manifolds when temporarily pumping high concentration cleaning solutions significantly reduces the time required to re-equilibrate the system to low concentration eluents.

6. Flush the AAES with deionized water at 2 mL/min for 30 minutes.

7. Reinstall the AAES according to procedures in Section 4.2.1, "Eluent and Regenerant Flow Path Connections in the AutoSuppression Recycle Mode Operation" or Section 4.3.1, "Eluent Flow Path Connections in the AutoSuppression External Water Mode Operation" and resume operation.

Organic Contaminants

1. Turn off the power to the AAES.

2. Disconnect the analytical (and guard) column(s) from the injection valve and the AAES. Refer to the specific analytical column Product Manual for column cleanup procedures.

3. If you are running in the **AutoSuppression External Water Mode**, turn off the external water and disconnect the external water line from the AAES **REGEN IN** port. If you are running in the **AutoSuppression Recycle Mode**, proceed to D.

4. Disconnect the liquid line from the AAES **ELUENT OUT** port to the cell at the cell fitting and reconnect it to the **REGEN IN** port.

5. Connect a temporary line from the priming block or the low-pressure tee on the isocratic or gradient pump to a container with a solution of freshly prepared 10% 1.0 M H_2SO_4/90% acetonitrile. H_2SO_4/acetonitrile solutions are not stable during long term storage so this cleanup solution must be made immediately before each column cleanup. Alternatively, it can be proportioned from 1 bottle containing 1.0 M H_2SO_4 and another bottle containing 100% acetonitrile. Pass 60 mL of this solution through the AAES using the Trap Column / Suppressor Clean-up Kit (P/N 059649) or pump this solution through the AAES at 1.0 mL/min for 60 minutes.

NOTE

Bypassing internal pump manifolds when temporarily pumping high concentration cleaning solutions significantly reduces the time required to re-equilibrate the system to low concentration eluents.

6. Flush the AAES with deionized water at 2 mL/min for 30 minutes.

7. Reinstall the AAES according to procedures in Section 4.2.1, "Eluent and Regenerant Flow Path Connections in the AutoSuppression Recycle Mode Operation" or Section 4.3.1, "Eluent Flow Path Connections in the AutoSuppression External Water Mode Operation" and resume operation.

6.3 Poor Peak Resolution

Poor peak resolution can be due 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 (e.g., 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 columns or 0.005" for 2-mm columns to make all eluent liquid line connections between the injection valve and the detector cell inlet, and that the tubing lengths are as short as possible. Check for leaks.

6.3.2 Poor Resolution Due to Shortened Retention Times

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

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

C. Column contamination can lead to a loss of column capacity because all of the anion exchange sites will no longer be available for the sample ions. Polyvalent anions might be concentrating on the column. Refer to "Column Care," for recommended column cleanup procedures.

   Possible sources of column contamination are impurities in chemicals and in the deionized water being used. Be especially careful to make sure that the recommended chemicals are used. The deionized water should have a specific resistance of at least 18.2 megohm-cm.

D. Diluting the eluent will improve peak resolution, but will also increase the analytes' retention times. If a 10% dilution of the eluent is not sufficient to obtain the desired peak resolution, or if the resulting increase in retention times is unacceptable, clean the column (see "Column Care").

After cleaning the column, reinstall it in the system and let it equilibrate with eluent for about 30 minutes. 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. If you need assistance in solving resolution problems, contact the nearest DIONEX Regional Office (see, DIONEX Worldwide Offices)

6.3.3 Loss of Front End Resolution

If poor resolutions and efficiencies are observed for the very early eluting peaks near the system void volume compared to the later eluting peaks, check the following:

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

B. Column overloading may be the problem. Reduce the amount of sample ions being injected onto the analytical column

by either diluting the sample or injecting a smaller volume onto the column.

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

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

6.4 Spurious Peaks

A. If the samples contain an appreciable level of polyvalent ions and the column is used with a weak eluent system, polyvalent anions may contaminate the analytical column. The retention times for the analytes will then decrease and spurious, inefficient (broad) peaks can show up at unexpected times. Clean the column as indicated in "Column Care."

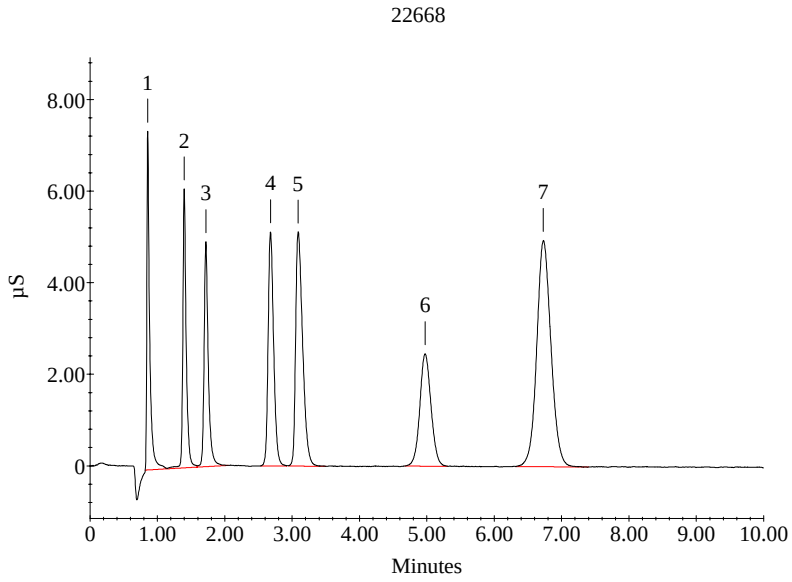
B. If you need assistance in determining the best way to clean strongly retained solutes in your specific sample matrix from the IonPac AS4A-SC columns, contact the nearest DIONEX Regional Office (see, DIONEX Worldwide Offices).

C. 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. It will happen 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.

If cleaning and retorquing the valve does not help, replace the valve. Use a DIONEX High Pressure Injection Valve (P/N 037142) or a DIONEX High Pressure Inert Valve (P/N 037143) as required.

IonPac® AS4A-SC
Analytical (4 x 250 mm)
Product No. 43174

Serial No. : 22668 **Pressure (PSI) : 990** **Date : 8/15/00 11:07:48 AM**



Eluent: 1.8 mM Na_2CO_3 / 1.7 mM $NaHCO_3$
Flow Rate: 2.0 mL/min
Detection: Suppressed Conductivity
ASRS®-ULTRA
AutoSuppression® Recycle Mode

Injection Volume: 25 µL

Storage Solution: 0.1 M NaOH

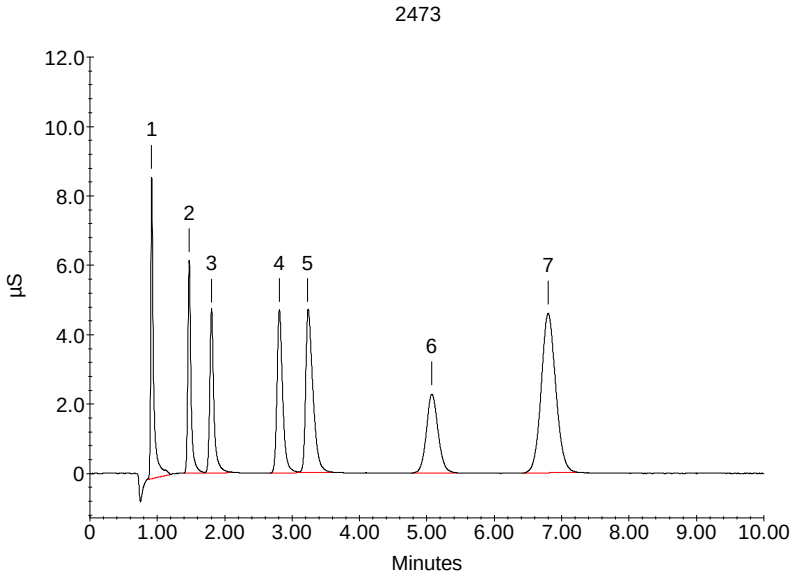
Peak Information : Found Components

Peak No.	Retention Time	Name		Efficiency	Asymmetry (10%)	Resolution
1	0.86	Fluoride	2.0	2541	2.6	7.08
2	1.40	Chloride	3.0	4323	1.8	3.37
3	1.72	Nitrite	5.0	4086	1.6	7.39
4	2.68	Bromide	10.0	4912	1.4	2.36
5	3.09	Nitrate	10.0	4109	2.2	7.59
6	4.97	Phosphate	15.0	4229	1.1	5.13
7	6.73	Sulfate	15.0	5026	1.2	n/a

File Name : c:\PEAKNET\DATA\EXAMPLES\43174 as4asc 4mm_aa009.dxd

IonPac® AS4A-SC
Analytical (2 x 250 mm)
Product No. 43125

Serial No. : 2473 **Pressure (PSI) : 850** **Date : 3/2/01 3:37:09 PM**



Eluent: 1.8 mM Na_2CO_3 / 1.7 mM $NaHCO_3$
Flow Rate: 0.5 mL/min
Detection: Suppressed Conductivity
ASRS®-ULTRA, 2-mm
AutoSuppression® Recycle Mode

Injection Volume: 5 µL

Storage Solution: 0.1 M NaOH

Peak Information : Found Components

Peak No.	Retention Time	Name	(mg/L)	Efficiency	Asymmetry (10%)	Resolution
1	0.92	Fluoride	2.0	3800	2.5	8.24
2	1.48	Chloride	3.0	5952	1.4	3.65
3	1.80	Nitrite	5.0	4986	1.9	8.11
4	2.81	Bromide	10.0	5817	1.6	2.51
5	3.23	Nitrate	10.0	4509	2.5	7.33
6	5.08	Phosphate	15.0	4245	1.2	4.92
7	6.80	Sulfate	15.0	4843	1.2	n/a

File Name : I:\LBURHANUDIN\QA REPORT BOOK _PDF_DXD_FILES\43125_AS4ASC_2MM_A014.DXD

COLUMN CARE

RECOMMENDED OPERATION PRESSURES

Operating a column above its recommended pressure limit can cause irreversible loss of column performance. The maximum recommended operating pressure for IonPac AS4A-SC columns is 4,000 psi.

COLUMN START-UP

The column is shipped in 100 mM NaOH storage solution.

Prepare the eluent shown on the test chromatogram, install the column in the chromatography module and test the column performance under the conditions described in the test chromatogram. 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.

COLUMN STORAGE

For short-term storage, the strongest eluent in use can be used as the storage solution. For long-term storage, 100 mM NaOH should be used as the 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.

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 but the following precautions should be observed.

Always ensure that the cleanup protocol used does not switch between eluents which may create high pressure eluent interface bands in the column. High pressure bands can disrupt the uniformity of the packing of the column bed and irreversibly damage the performance of the column. High pressure bands 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 with may create a neutralization pressure band. The precipitation of the salts in solvents during column rinses can result in very high pressure bands. High viscosity mixing bands can be created between two eluents having solvents with a very high energy of mixing.

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

BASE-SOLUBLE CONTAMINANTS

A. Prepare a 500 mL solution of 500 mM NaOH in deionized water having a specific resistance of 18.2 megohm-cm.

B. Disconnect the AMMS III (2mm) or AMMS III (4mm) from the IonPac AS4A-SC Analytical Column. 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 direction designated on each of the column labels. Direct the effluent from the outlet line of the AS4A-SC Guard Column to a separate waste container.

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. Set the pump flow rate to 0.50 mL/min (2mm systems) or 2.0 mL/min (4mm systems).

D. If your eluent contains a solvent that is not compatible with 500 mM NaOH, rinse the column for 15 minutes with deionized water before pumping the 500 mM NaOH over the column.

E. Pump 500 mM NaOH solution through the column for 30-60 minutes

F. If your eluent contains a solvent that is not compatible with 500 mM NaOH, rinse the column for 15 minutes with deionized water before pumping eluent over the column.

G. Reconnect the AMMS III (2mm) or AMMS III (4mm) to the AS4A-SC Analytical Column and place the guard column in line between the injection valve and the analytical column if your system was originally configured with a guard column.

H. Equilibrate the column(s) with eluent before resuming normal operation.

NOTE

It may not be necessary to use 500 mM sodium hydroxide in the above cleanup procedure. You may find it more beneficial, depending on you sample matrix, to use a 10X concentrate of your sodium carbonate/bicarbonate or sodium borate eluent. Note that the maximum solubility of sodium borate is 300 mM.

ACID-SOLUBLE CONTAMINANTS

A. Prepare a 500 mL solution of 1 M HCl in deionized water having a specific resistance of 18.2 megohm-cm.

B. Disconnect the AMMS III (2mm) or AMMS III (4mm) from the IonPac AS4A-SC Analytical Column. 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 direction designated on each of the column labels. Direct the effluent from the outlet line of the AS4A-SC Guard Column to a separate waste container.

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. Set the pump flow rate to 0.50 mL/min (2mm systems) or 2.0 mL/min (4mm systems).

D. Rinse the column for 15 minutes with deionized water before pumping the 1 M HCl over the column.

E. Pump 1 M HCl solution through the column for 30-60 minutes.

NOTE

Nitric acid should not be used instead of hydrochloric acid since nitric acid will not efficiently remove iron contaminants.

F. Rinse the column for 15 minutes with deionized water before pumping the 100 mM NaOH over the column.

G. Pump 100 mM NaOH through the column for 15 minutes.

H. If your eluent contains a solvent that is not compatible with 100 mM NaOH, rinse the column for 15 minutes with deionized water before pumping eluent over the column.

I. Reconnect the AMMS III (2mm) or AMMS III (4mm) to the AS4A-SC Analytical Column and place the guard column in line between the injection valve and the analytical column if your system was originally configured with a guard column.

J. Equilibrate the column(s) with eluent before resuming normal operation.

ORGANIC CONTAMINANTS

A. Prepare a 500 mL solution of 90% acetonitrile in deionized water having a specific resistance of 18.2 megohm-cm.

B. Disconnect the AMMS III (2mm) or AMMS III (4mm) from the IonPac AS4A-SC Analytical Column. 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 direction designated on each of the column labels. Direct the effluent from the outlet line of the AS4A-SC Guard Column to a separate waste container.

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. Set the pump flow rate to 0.50 mL/min (2mm systems) or 2.0 mL/min (4mm systems).

D. If your eluent contains a solvent that is not compatible with 90% acetonitrile, rinse the column for 15 minutes with deionized water before pumping the 90% acetonitrile over the column.

E. Pump a 90% acetonitrile solution through the column for 30-60 minutes.

F. If your eluent contains a solvent that is not compatible with 90% acetonitrile, rinse the column for 15 minutes with deionized water before pumping eluent over the column.

G. Reconnect the AMMS III (2mm) or AMMS III (4mm) to the AS4A-SC Analytical Column and place the guard column in line between the injection valve and the analytical column if your system was originally configured with a guard column.

H. Equilibrate the column(s) with eluent before resuming normal operation.

NOTE

It is not necessary to use 90% acetonitrile in the above cleanup procedure. You may find it more beneficial, depending on you sample matrix, to use a different organic solvent such as methanol or isopropanol.