PRODUCT MANUAL

for

IonPac® AS7
IonPac® AG7

DIONEX

IC | HPLC | MS | EXTRACTION | PROCESS | AUTOMATION

PRODUCT MANUAL

for the

IONPAC® AG7 GUARD COLUMN, 4-mm
(4 x 50 mm, P/N 035394)

IONPAC® AS7 ANALYTICAL COLUMN, 4-mm
(4 x 250 mm, P/N 035393)

IONPAC® AG7 GUARD COLUMN, 2-mm
(2 x 50 mm, P/N 063099)

IONPAC® AS7 ANALYTICAL COLUMN, 2-mm
(2 x 250 mm, P/N 063097)

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SECTION 1 - INTRODUCTION TO IONPAC AS7/AG7 CHROMATOGRAPHY

The IonPac® AS7 Analytical Column in combination with the AG7 Guard Column is designed for the analysis of a wide range of polyvalent anions. The selectivity of the IonPac AS7 Analytical and Guard Column set is designed to determine a wide variety of applications, including polyphosphates, polyphosphonates, cyanide, and iodide. The AS7 is compatible with pH 0-14 eluents and eluents containing organic solvents from 0 - 5% in concentration. The AS7 can be used with a wide range of eluents and detectors.

CAUTION
Eluents must contain less than 5% organic solvents.

Table 1
IonPac AS7/AG7 Packing Specifications

Column	Particle Diameter µm	Substrate X-linking %	Column Capacity µeq/column	Functional Group	Hydrophobicity
AS7 4 x 250 mm	10.0	2	100	Alkyl quaternary ammonium	Medium-High
AG7 4 x 50 mm	10.0	2	25	Alkyl quaternary ammonium	Medium-High
AS7 2 x 250 mm	10.0	2	25	Alkyl quaternary ammonium	Medium-High
AG7 2 x 50 mm	10.0	2	6.25	Alkyl quaternary ammonium	Medium-High

Table 2
IonPac AS7/AG7 Operating Parameters

Column	Typical Back Pressure psi (MPa)	Standard Flow Rate mL/min	Maximum Flow Rate mL/min
AS7 4-mm Analytical Column	≤ 900 (6.20)	0.5	3.0
AG7 4-mm Guard Column	≤ 225 (1.55)	0.5	3.0
AS7 + AG7 4-mm columns	≤ 1125 (7.75)	**0.5**	**3.0**
AS7 2-mm Analytical Column	≤ 900 (6.20)	0.12	0.75
AG7 2-mm Guard Column	≤ 225 (1.55)	0.12	0.75
AS7 + AG7 2-mm columns	≤ 1125 (7.75)	**0.12**	**0.75**

Assistance is available for any problem encountered during the shipment or operation of DIONEX instrumentation and columns through the North America Technical Call Center at 1-800-DIONEX-0 (1-800-346-6390) or the nearest DIONEX Office (see, "DIONEX Worldwide Offices" on the Dionex Reference Library CD-ROM (P/N 053891).

SECTION 2 - ION CHROMATOGRAPHY SYSTEMS

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

- For an ICS in 2-mm format, Dionex recommends a microbore isocratic pump, standard bore isocratic pump, microbore gradient pump, or standard bore gradient pump .
- For an ICS in 4-mm format, Dionex recommends a standard bore isocratic pump or standard bore gradient pump.

See Appendix C, Comparison of Ion Chromatography Systems for specific recommended settings and parts including pumps, eluent flow rate, Self-Regenerating Suppressor (SRS), MicroMembrane Suppressor (MMS), injection loop, system void volume, detectors, and tubing back pressure.

SECTION 3 - INSTALLATION

3.1 System Requirements

3.1.1 System Requirements for 2-mm Operation

The IonPac AS7 2-mm Guard and Analytical Columns are designed to run on Dionex Ion Chromatographs equipped with suppressed conductivity detection. Isocratic analyses at flow rates of 0.5 mL/min or greater can be performed on a pump with standard (1/8" pistons) pump heads. For isocratic analyses at flow rates below 0.5 mL/min and gradient analyses, a microbore pump (1/16" pistons) is recommended.

3.1.2 System Requirements for 4-mm Operation

The IonPac AS7 4-mm Guard and Analytical Columns are designed to 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 pump with a standard pump heads (1/8" pistons). Isocratic analysis can also be performed on a pump with standard bore pump heads (1/8" pistons).

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"ID PEEK tubing (P/N 044221). 0.010" ID PEEK tubing (P/N 042260) may be used but peak efficiency will be compromised which may also result in decreased peak resolution. Minimize the lengths of all connecting tubing and remove all unnecessary switching valves and couplers.

3.2 The Sample Concentrator

For 2-mm or 4-mm concentrator work, use the IonPac AG7 Guard Column when a single piston pump is used for sample delivery. Use the Trace Anion Concentrator Low Pressure Column (TAC-LP1, P/N 046026) or Trace Anion Concentrator Ultra Low Pressure Column (TAC-ULP1, P/N 061400) when the sample is delivered with a syringe or with an autosampler. Alternatively, use the Ultra Trace Anion Concentrator Low Pressure Column (UTAC-LP1, P/N 063079), Ultra Trace Anion Concentrator Ultra Low Pressure Column (UTAC-ULP1, P/N 063475), or Ultra Trace Anion Concentrator Extremely Low Pressure Column (UTAC-XLP1, P/N 063459). The TAC-LP1, TAC-ULP1, UTAC-LP1, UTAC-ULP1, UTAC-XLP1, or the IonPac AG7 Guard Column can be used for trace anion concentration work. The concentrator column is used in lieu of the sample loop. Pump the sample onto the concentrator column in the **OPPOSITE** direction of the eluent flow.

When using concentration techniques, do not overload the concentrator column by concentrating an excessive amount of sample as this 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.

The function of the TAC-LP1, TAC-ULP1, UTAC-LP1, UTAC-ULP1, UTAC-XLP1, or the AG7 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 TAC-LP1, TAC-ULP1, UTAC-LP1, UTAC-ULP1, UTAC-XLP1, or the AG7 leading to a lowering of detection limits by 2–5 orders of magnitude. The unique advantage to the analytical chemist of the TAC-LP1, TAC-ULP1, UTAC-LP1, UTAC-ULP1, UTAC-XLP1, or the AG7 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.

For a detailed discussion of anion concentration techniques, refer to Section 3, “Operation,” of the Trace Anion Concentrator (TAC-LP1 and TAC-ULP1) Column Product Manual (Document No. 034972) or Section 3, “Operation,” of the Ultra Trace Anion Concentrator (UTAC-LP1, UTAC-ULP1, and UTAC-XLP1) Column Product Manual (Document No. 065091).

3.3 The Injection Loop

3.3.1 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 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 AS7 2-mm requires a microbore HPLC system configuration. Install an injection loop one-fourth or less (<15 µL) of the loop volume used with a 4-mm analytical system (Section 2, "Comparison of Ion Chromatography Systems").

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

For most applications on a 4-mm analytical system, a 10 - 50 µL injection loop 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.

3.4 The IonPac AG7 Guard Column

An IonPac AG7 Guard Column is normally used with the IonPac AS7 Analytical Column. Retention times will increase by approximately 4% 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. Replacing the AG7 Guard Column at the first sign of peak efficiency loss or decreased retention time will prolong the life of the AS7 Analytical Column.

3.5 Eluent Storage

IonPac AS7 columns are designed to be used with bicarbonate/carbonate 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).

3.6 Anion Self-Regenerating Suppressor (ASRS ULTRA II) Requirements

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 compatible with the systems and columns. Aqueous ionic eluents can be used in all ASRS ULTRA II modes of operation.

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

If you are installing an IonPac AS7 4-mm Analytical Column, use an ASRS ULTRA II (4-mm, P/N 053946).
If you are installing an IonPac AS7 2-mm Analytical Column, use an ASRS ULTRA II (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 II (ASRS ULTRA II.)"

3.7 Anion Atlas Electrolytic Suppressor (AAES) Requirements

An Atlas Anion Electrolytic Suppressor (AAES) may be used instead of an ASRS ULTRA II for applications that require suppressed conductivity detection. The AAES (P/N 056116) can be used for AS7 2-mm and 4-mm applications using eluents up to 25 µeq/min.

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

3.8 Anion MicroMembrane Suppressor (AMMS III) Requirements

An Anion MicroMembrane Suppressor (AMMS III) may be used instead of an ASRS ULTRA II (4-mm) for applications that require suppressed conductivity detection. Use an AMMS III (P/N 056750) with the IonPac AS7 4-mm Analytical Column. It is compatible with all solvents and concentrations with which the systems and columns are compatible. For 2-mm operation, use the AMMS III (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 AutoRegen with the ASRS ULTRA II or the AMMS III in the Chemical Suppression Mode

To save regenerant preparation time and reduce regenerant consumption and waste, Dionex recommends using an AutoRegen® Accessory (P/N 039594). For more detailed information on the use of the AutoRegen Accessory see the AutoRegen Accessory manual (Document No. 032853). For more detailed information on the use of AutoRegen Regenerant Cartridges, see the "Product Manual for the AutoRegen Regenerant Cartridge Refills" (Document No. 032852).

3.10 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 (H_2SO_4) and the Anion MicroMembrane Suppressor (AMMS III). See the DCR Kit Manual (Document No. 031664) for details.

SAFETY

Use proper safety precautions in handling acids and bases.

3.11 Detector Requirements

See Appendix C, "Comparison of 2-mm and 4-mm Ion Chromatography Systems," for 2-mm and 4-mm system detector, cell, and thermal stabilizer requirements.

SECTION 4 - OPERATION

4.1 General Operating Conditions

Sample Volume:	4-mm: 10 µL Loop + 0.8 µL Injection valve dead volume
Column:	4-mm: AS7 4-mm Analytical Column + AG7 4-mm Guard Column
Eluent:	30 mN Nitric acid
Eluent Flow Rate:	0.5 mL/min
Post Column Reagent:	1 g/L Ferric nitrate, 2% Perchloric acid
PCR Flow Rate:	0.5 mL/min
Detection:	UV, 330 nm
Storage Solution:	Long Term Storage Solution as Shipped from Dionex: Test Eluent (50 mM HNO_3) Alternate Long Term Storage Solution: 100 mM NaOH

4.2 Ionpac AS7 Operation Precautions

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

4.3 Chemical Purity Requirements

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

4.3.1 Inorganic Chemicals

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

4.3.2 Deionized Water

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

4.3.3 Solvents

Solvents, up to 5%, can be added to the ionic eluents used with IonPac AS7 columns to modify the ion exchange process or improve sample solubility. The solvents used must be free of ionic impurities. However, since most manufacturers of solvents do not test for ionic impurities, it is important that the highest grade of solvents available be used. Currently, several manufacturers are making ultrahigh purity solvents that are compatible for HPLC and spectrophotometric applications. These ultrahigh purity solvents will usually ensure that your chromatography is not affected by ionic impurities in the solvent. Currently at DIONEX, we have obtained consistent results using High Purity Solvents manufactured by Burdick and Jackson and Optima® Solvents by Fisher Scientific.

When using a solvent in an ionic eluent, column generated back pressures will depend on the solvent used, concentration of the solvent, the ionic strength of the eluent and the flow rate used. The column back pressure will vary as the composition of water-methanol and water-acetonitrile mixture varies. The practical back pressure limit for the IonPac AS7 columns is 4,000 psi (27.57 MPa).

The AS7 can withstand common HPLC solvents in a concentration range of 0 - 5%. Solvents and water should be premixed in concentrations which allow proper mixing by the gradient pump and to minimize outgassing. Ensure that all of the inorganic chemicals are soluble in the highest solvent concentration to be used during the analysis.

Table 5
HPLC Solvents for Use with IonPac AS7 Columns

Solvent	Maximum Operating Concentration
Acetonitrile	5%
Methanol	5%
2-Propanol	5%
Tetrahydrofuran	5%

4.4 Making Eluents that Contain Solvents

When mixing solvents with water remember to mix solvent with water on a volume to volume basis. If a procedure requires an eluent of 5% acetonitrile, prepare the eluent by adding 5 mL of acetonitrile to an eluent reservoir. Then add 995 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.

CAUTION

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

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

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.

CAUTION

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

SECTION 5 - EXAMPLE APPLICATIONS

The chromatograms in this section were obtained using columns that reproduced the Production Test Chromatogram (see Section 5.2, "Production Test Chromatogram") on optimized Ion Chromatographs (see Section 3, "Installation"). Systems may vary slightly in performance due to different column sets, system void volumes, liquid sweep-out times of components, and laboratory temperatures.

Before attempting any of the following example applications, take the time to ensure that your system is properly configured. Ensure that all of the eluents have been made from high purity reagents and deionized water. All water used in the preparation of eluents should be degassed, deionized water. For chemical purity requirements, see Section 4.3, "Chemical Purity Requirements." 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, "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.

5.1 Preparation of Eluent Stock Solution Concentrates

Dilute 63 mL (or 90 g) of concentrated nitric acid (Ultrex grade, 70% HNO_3) to one liter with deionized water to make a 1.0 M solution. Ultrex Grade is strongly recommended and can be obtained from VWR P/N JT6901-05.

5.2 Eluent and Post-column Reagent

A. Eluent: 50 mM HNO_3

Dilute 50 mL of 1.0 M HNO_3 stock solution to 1 L with deionized water.

B. Post Column Reagent

Dilute 1.0 g of iron nitrate ($Fe(NO_3)_3 \cdot 9\ H_2O$) and 20 mL of concentrated perchloric acid ($HClO_4$, 70-72%) to 1 L with deionized water.

C. Long Term Storage Solution when shipped from Dionex: Test Eluent (50 mM HNO_3)

D. Alternate Long Term Storage Solution: 100 mM NaOH

Dilute 5.2 mL of 50% Sodium Hydroxide to 1 L with deionized water.

Weight Method

Example: To make 1 L of 0.05 M HNO_3 use 4.50 g of 70% nitric acid:

$$g = \frac{(M) \times (V) \times (MW)}{\rho}$$

Where: g = g of 70% nitric acid needed
V = volume (L)
MW = molecular weight of nitric acid
M = final molarity
ρ = fractional purity of concentrated solution

$$\frac{0.050 \text{ moles/L} \times 1 \text{ L} \times 63.02 \text{ g/mole}}{0.7} = 4.50 \text{ g}$$

Volume Method

Example: To make 1 L of 0.050 M HNO_3 use 3.17 mL of 70% nitric acid:

$$v = \frac{(M) \times (V) \times (MW)}{(d) \times (\rho)}$$

Where: M = Final Molarity
d = density of the concentrated solution (g/mL)
v = volume of the concentrated solution (mL)
V = volume of final solution (L)
ρ = fractional purity of the concentrated solution
MW = molecular weight (g/mole)

$$\frac{0.050 \text{ moles/L} \times 1 \text{ L} \times 63.02 \text{ g/mole}}{1.419 \text{ g/mL} \times 0.7} = 3.17 \text{ mL of 70\% } HNO_3 \text{ (diluted to 1 L)}$$

DIONEX recommends using the Weight Method for preparing HNO_3 eluents. Note that either the 50 mM HNO_3 or the 100 mM NaOH is acceptable as a storage solution.

5.3 Production Test Chromatogram

5.3.1 Production Test Chromatogram (4 x 250 mm)

Isocratic elution of anions on the IonPac AS7 Analytical Column has been optimized utilizing a nitric acid eluent. By using this eluent, chelating anions can be isocratically separated and quantitated in a single injection. The IonPac AS7 Analytical Column should always be used with the IonPac AG7 Guard Column. To guarantee that all IonPac AS7 Analytical Columns meet high quality and reproducible performance specification standards, all columns undergo the following production control test.

Column:	IonPac AS7 (4-mm) Analytical Column
Eluent:	50 mM HNO_3
Eluent Flow Rate:	0.5 mL/min
Post Column Reagent:	1 g $Fe(NO_3)_3$ • 9 H_2O and 20 mL $HClO_4$ (70-72%)/L deionized water
PCR Flow Rate:	0.5 mL/min
Injection Volume:	25 µL
Detection:	UV, 330 nm
Storage Solution:	Long Term Storage Solution as Shipped from Dionex: Test Eluent (50 mM HNO_3) Alternate Long Term Storage Solution: 100 mM NaOH

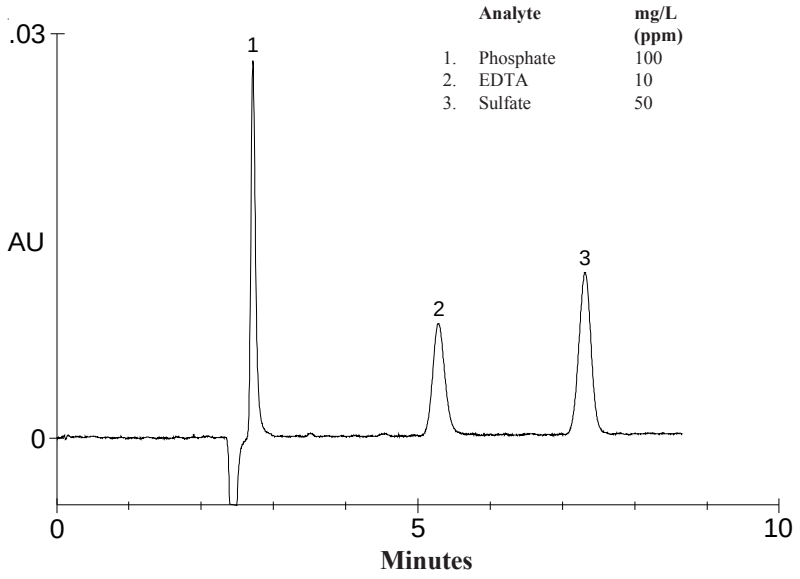
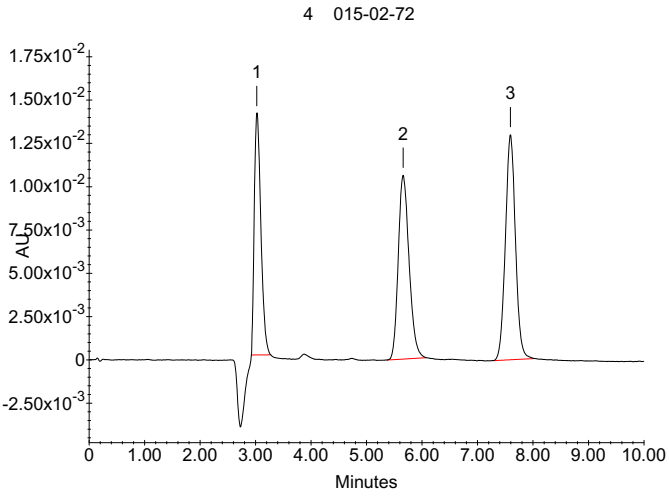


Figure 2a
IonPac AS7 4-mm Production Test Chromatogram

5.3.2 Production Test Chromatogram (2 x 250 mm)

IonPac® AS7
Analytical (2 x 250 mm)
Product No. 063097

Serial No. : 4 015-02-72 **Pressure (PSI) : 525** **Date : 10/22/2004 4:53:44 PM**



Eluent: 50 mM HNO_3
Eluent Flow Rate: 0.12 mL/min
Detection: Absorbance at 330 nm
Range: 0.05 AUFS

Post Column Reagent: 1.0g $Fe(NO_3)_3 \cdot 9\ H_20$
20 mL 70-72% $HClO_4$
per liter

PCR Flow Rate: 0.12 mL/min.
Injection Volume: 5 µL
Storage Solution: Eluent

Peak Information : Found Components

Peak No.	Retention Time	Name	(mg/L)	Efficiency	Asymmetry (10%)	Resolution
1	3.02	Phosphate	100.0	2705	2.0	8.99
2	5.66	EDTA	10.0	4024	1.4	5.61
3	7.59	Sulfate	50.0	8259	1.1	n/a

Figure 2b
IonPac AS7 2-mm Production Test Chromatogram

5.4 Analysis of Chelating Agents with Postcolumn Ferric Nitrate

The following chromatogram demonstrates the elution of chelating agents using the AS7 column with postcolumn addition of ferric nitrate coupled with UV detection at 330 nm.

Column:	IonPac AS7 (4-mm) Analytical Column
Eluent:	24 mM Nitric acid
Flow Rate:	0.5 mL/min
Postcolumn Reagent:	1 g/L $Fe(NO_3)_3 \cdot 9\ H_2O$, 2% (v/v) $HClO_4$/L deionized water
PCR Flow Rate:	0.5 mL/min
Injection Volume:	25 µL
Detection:	UV, 330 nm

Peaks:	**mg/L (ppm)**
1. Phosphate	100
2. NTA	20
3. EDTA	10

NTA=nitrilotriacetic acid
EDTA=ethylenediamine tetraacetic acid

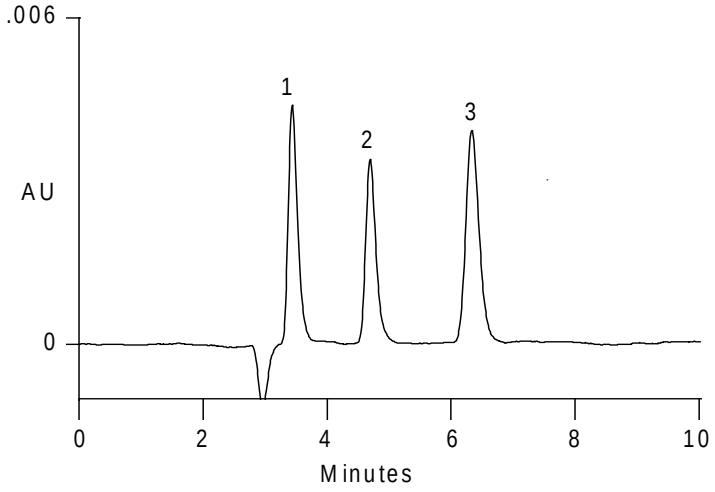
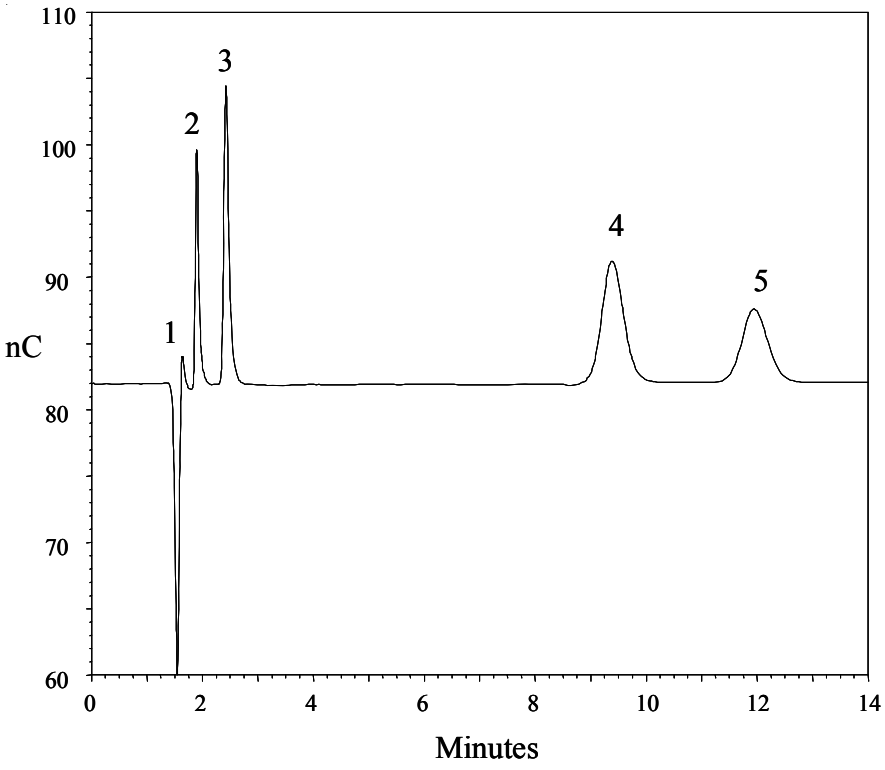


Figure 3
Chelating Agents with Postcolumn Ferric Nitrate

5.5 Analysis of Chelating Agents with Amperometric Detection

The following chromatogram shows the elution of chelating agents using the AS7 column with amperometric detection and a disposable platinum electrode.



Column:	IonPac® AS7 (2 x 250 mm)
Temp.:	30 °C
Eluent:	100 mM MSA
Flow Rate:	0.20 mL/min
Inj. Vol. :	25 µL
Det. Met.:	(Int. at E2) see document number 065040 for details
Electrode:	Disposable Pt
Samples:	NTA, EDTA, DTPA and EGTA (10 µM each)

Peaks:

1. Exclusion Volume
2. NTA
3. EDTA
4. DTPA
5. EGTA

Figure 4
Analysis of Chelating Agents

5.6 Analysis of Polyphosphonate Scale Inhibitors

The following chromatogram demonstrates the elution of polyphosphonate scale inhibitors using the AS7 column with postcolumn addition of ferric nitrate coupled with UV detection at 330 nm.

Column:	IonPac AS7 (4-mm) Analytical Column
Eluent:	30 mM Nitric acid
Flow Rate:	0.8 mL/min
Postcolumn Reagent:	1 g/L $Fe(NO_3)_3 \cdot 9\ H_2O$, 2% (v/v) $HClO_4$/L deionized water
PCR Flow Rate:	0.8 mL/min
Injection Volume:	25 µL
Detection:	UV, 330 nm

Peaks:	**mg/L (ppm)**
1. Phosphate	–
2. Dequest 2010	50
3. Dequest 2051	50
4. Dequest 2000	50
5. Dequest 2041	50

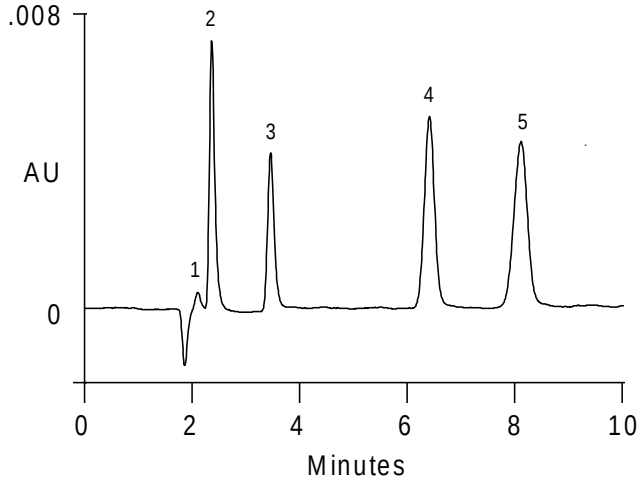


Figure 5
Polyphosphonate Scale Inhibitors

5.7 Analysis of Polyphosphates in Detergents

The following chromatograms demonstrate the elution of polyphosphates in different detergents using the AS7 column with postcolumn addition of ferric nitrate coupled with UV detection at 330 nm. The NG1 Guard Column is used to remove surfactants and organic additives in the detergent samples.

Columns:	IonPac® AS7 (4-mm) Analytical Column, AG7 (4-mm) Guard Column, NG1Guard column
Eluent:	30 - 70 mM Nitric acid
Flow Rate:	0.5 mL/min.
Injection Vol:	50 µL
Post Column Reagent:	1 g/1 $Fe(NO_3)_3 \cdot 9$ H2O, 2% (V/V) $HCLO_4$/L deionized water
PCR Flow:	
Detection:	UV, 330 nm

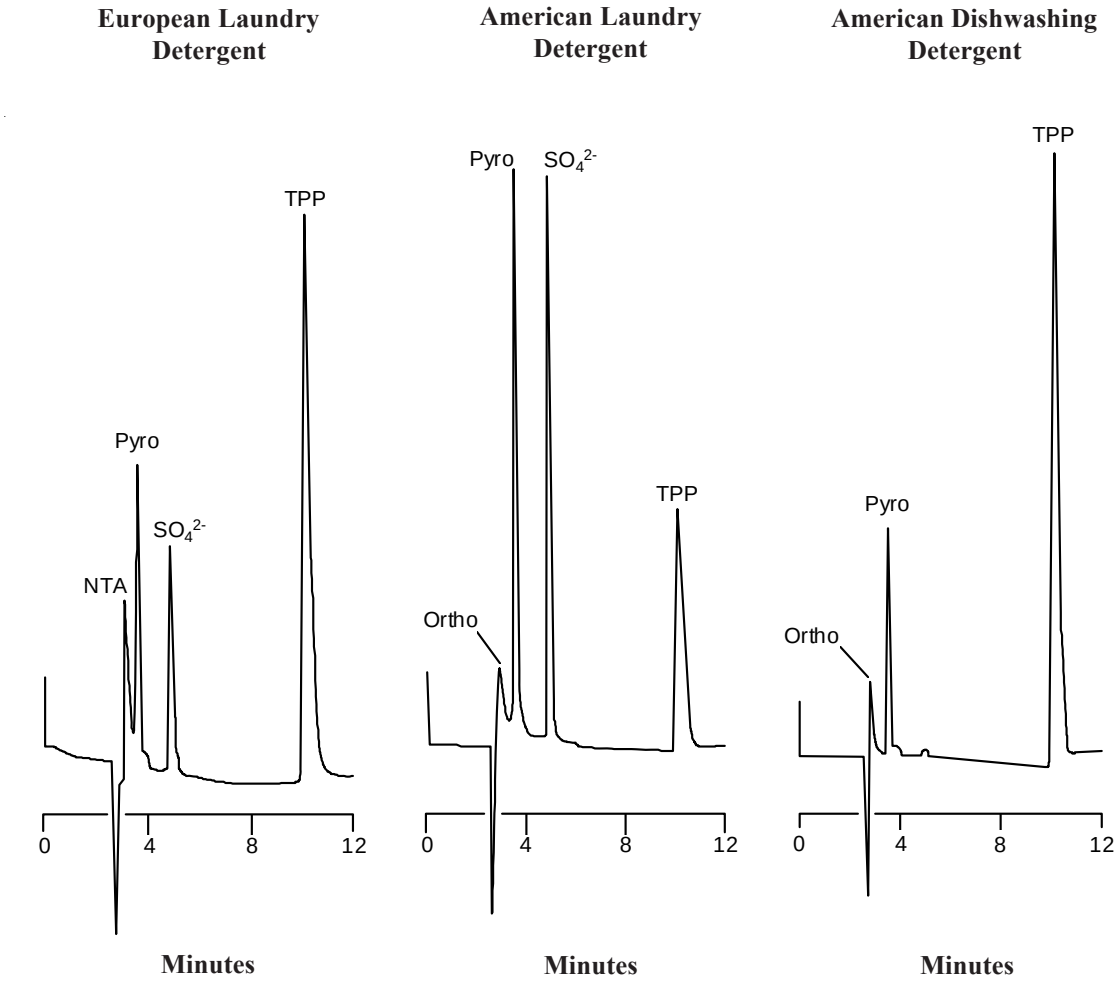
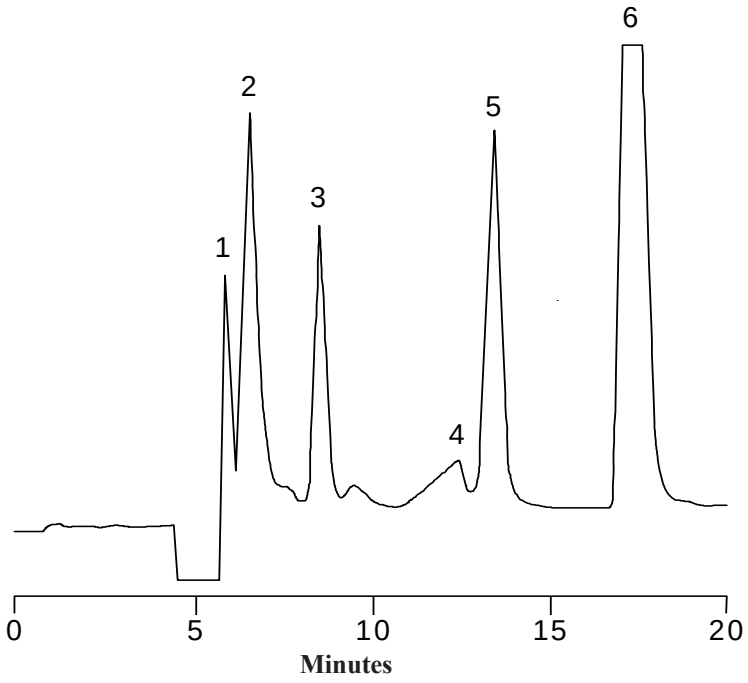


Figure 6
Analysis of Polyphosphates in Detergents

5.8 Analysis of Corrosion Inhibitors in Secondary Cooling Waters

The following chromatogram demonstrates the elution of corrosion inhibitors in secondary cooling waters using the AS7.

Column:	IonPac AS7 (4-mm) Analytical Column
Eluent:	18 mM Nitric Acid
Flow Rate:	0.5 mL/min.
Post Column Reagent:	1 g/L Fe $(NO_3)_3 \cdot 9\ H_2O$, 2% (V/V) $HCLO_4$/L deionized water
PCR Flow Rate:	0.5 mL/min
Detection:	UV, 310 nm, 0.1 AUFS



	Peaks:	mg/L
1.	Phosphate	5
2.	HEDP	5
3.	PBTC	5
4.	Chloride	760
5.	AMP	5
6.	Sulfate	190

HEDP = Hydroxyethylene diphosphonic acid (Dequest® 2010)
PBTC = Phosphonobutanetricarboxylic acid (Bayhibit AM™)
AMP = Aminomethylene phosphonic acid (Dequest 2000)

Figure 7
Analysis of Corrosion Inhibitors

5.9 Analysis of Iodide Using Amperometric Detection

The following chromatograms demonstrate the elution of iodide using the AS7 column with amperometric detection.

Column:	IonPac AG7 (4-mm) Guard Column, AS7 (4-mm) Analytical Column
Eluent:	200 mM Nitric acid
Flow Rate:	1.5 mL/min
Injection Volume:	See Chromatogram
Detection:	DC Amperometry, Pt electrode, 0.8 V

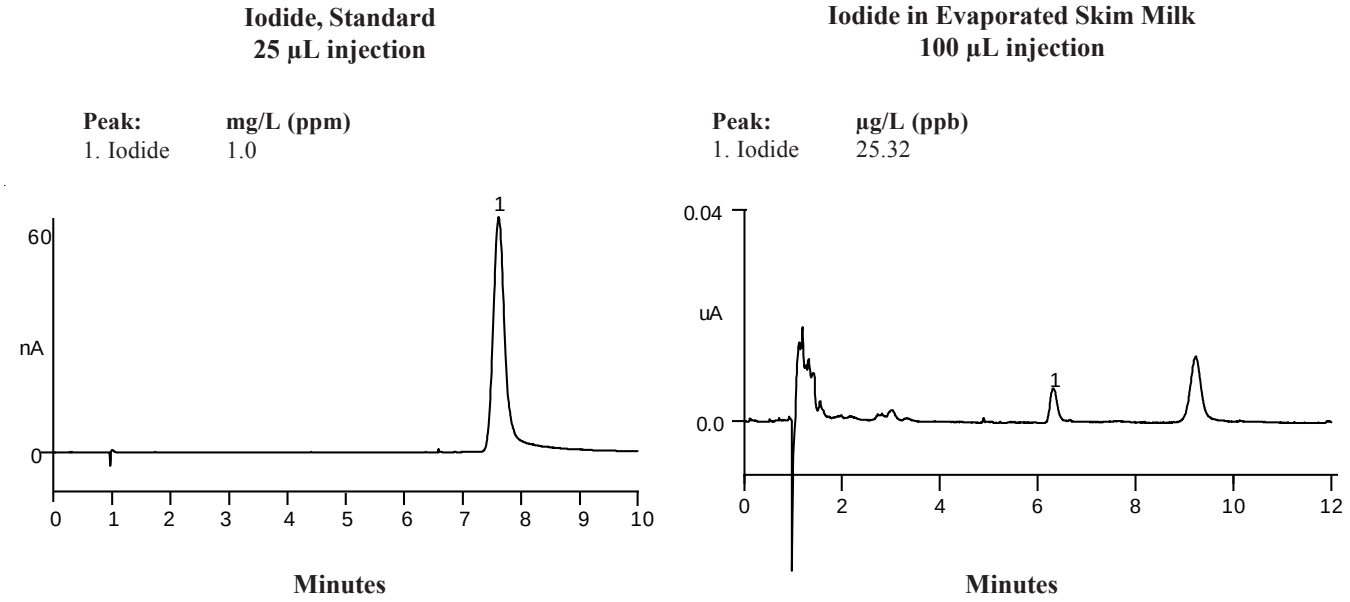


Figure 8
Amperometric Detection of Iodide

5.10 Analysis of Cyanide

5.10.1 IonPac AS7 (4-mm) Application Chromatogram

The following chromatogram demonstrates the elution of cyanide in sodium hydroxide and sodium acetate for aqueous standards using the AS7 (4-mm) column with pulsed amperometric detection.

Injection Volume:	10 µL	Peaks:
Sample Conc.:	See chromatogram	1. Cyanide
Column:	IonPac AS7 (4 x 250 mm)	2. Oxygen
	Guard (4 x 50 mm)	
Eluent:	75 mM NaOH, 100 mM NaAc	
	7.5 mM or 0.05% (v:v) Ethylenediamine (EDA)	
Flow Rate:	1.00 mL/min	
	Detection: Integrated amperometry	
	Waveform of Table 8	
	Disposable Ag electrode	
Temp.:	30°C	

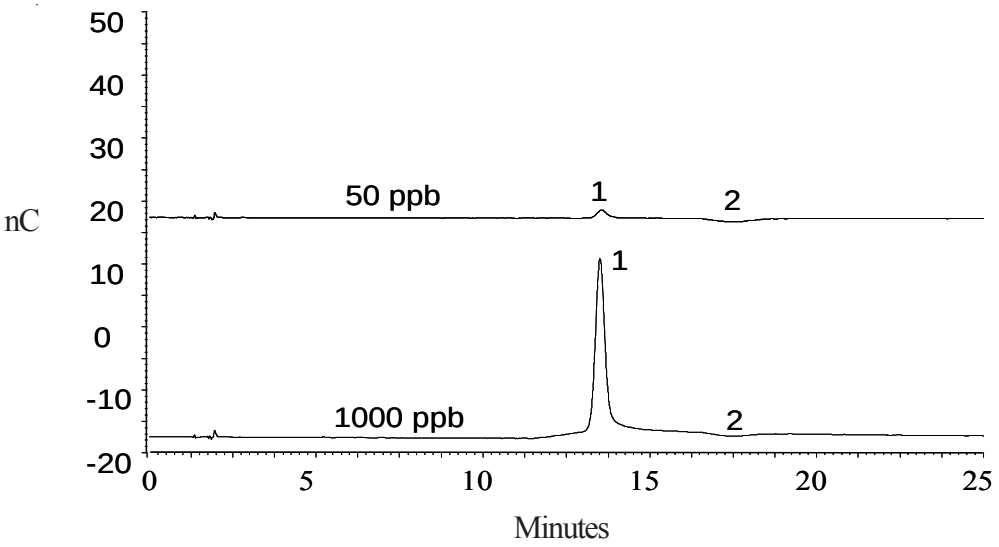


Figure 9
Analysis of Cyanide

See Section 6.3 for qualification of detection performance and troubleshooting.

5.10.2 IonPac AS7 (2-mm) Application Chromatogram

The following chromatogram demonstrates the elution of cyanide and sulfide in sodium hydroxide and sodium acetate for aqueous standards using the AS7 (2-mm) column with pulsed amperometric detection and a disposable silver electrode.

There are several waveforms that can be used for the analysis of cyanide, sulfide, bromide and thiosulfate. Choice of waveform depends upon concentration of sulfide in the sample and the goal of the analysis. The waveform in Table 8 is the best choice when comparatively low concentrations of sulfide are present, as long as the sulfide concentrate is not more than about 10 ppm.

Injection Volume:	10 µL	Peaks:
Sample Conc.:	See chromatogram	1. Cyanide
Column:	IonPac AS7 (2 x 250 mm) Guard (2 x 50 mm)	2. Sulfide
Eluent:	100 mM sodium hydroxide, 200 mM sodium acetate 7.5 mM ethylenediamine (EDA)	
Flow Rate:	0.25 mL/min	
Detection:	Integrated amperometry, Disposable silver electrode	

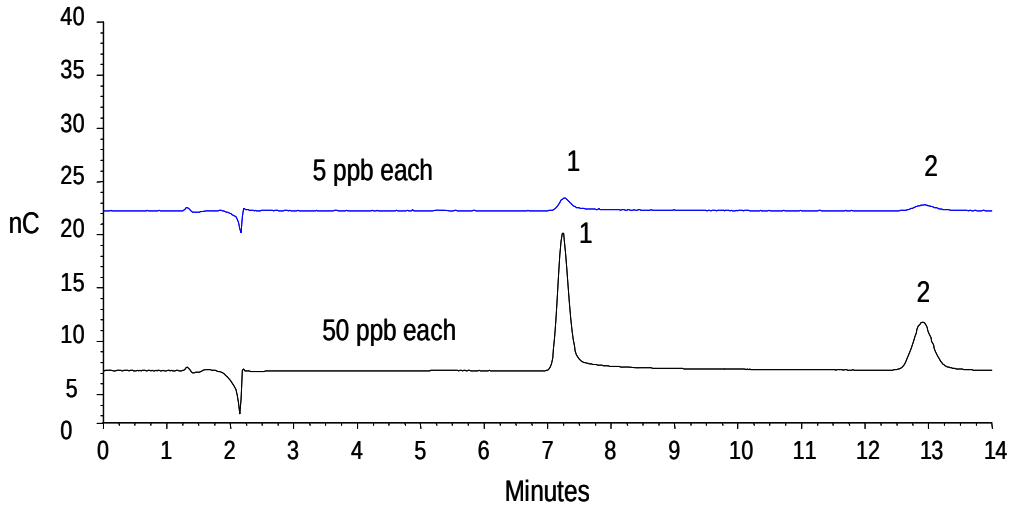


Figure 10
IonPac 2-mm Application Diagram

See Section 6.3 for qualification of detection performance and troubleshooting.

5.11 Analysis of Hexavalent Chromium

The following chromatograms demonstrate the elution of hexavalent chromium using the AS7 column with postcolumn addition of diphenylcarbazide coupled with Visible detection.

Column:	IonPac AS7 (4-mm) Analytical Column, AG7 (4-mm) Guard Column
Eluent:	250 mM $(NH_4)_2$ SO_4/100 mM NH_4OH
Flow Rate:	1.5 mL/min.
Postcolumn Reagent:	2 mM Diphenylcarbizide 10% CH_3OH, 1 N H_2SO_4
PCR Flow Rate:	0.5 mL/min.
Detector:	VIS, 530 nm

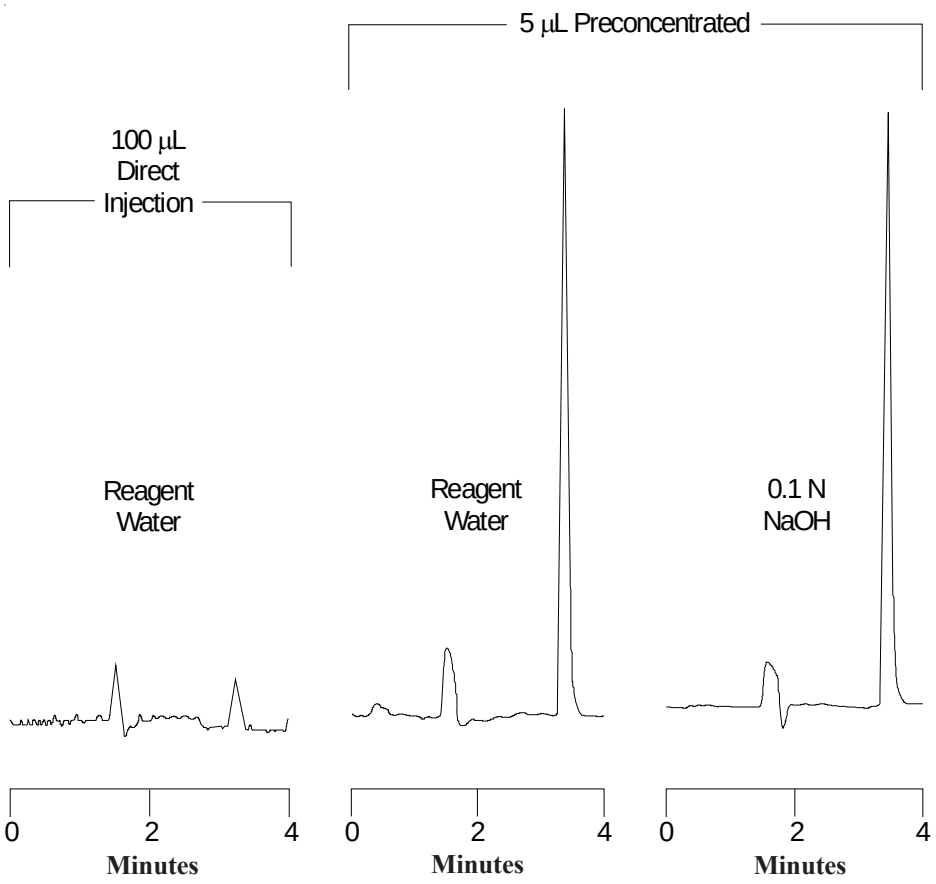


Figure 11
Analysis of Hexavalent Chromium
by Direct Injection and Preconcentration

5.12 Arsenic Speciation Using Ion Chromatography Coupled with ICP-MS

The following chromatogram demonstrates the separation of arsenic speciation using ion chromatography coupled with ICP-MS detection.

Column:	IonPac AS7 (4-mm) Analytical Column, AG7 (4-mm) Guard Column
Eluent:	E1 Type I Deionized Water
	E2 30 mM Ammonium Borate (pH 9.5)
	E3 200 mM Ammonium Hydroxide
	E4 30 mM Ammonium Dihydrogen Phosphate
Flow Rate:	1.5 mL/min.
Sample Volume:	25 µL
Detection:	Sciex Elan 500 ICP-MS
Sample:	100 ppb in 1% NaCl

Gradient Conditions

TIME (min)	%E1	%E2	%E3	%E4	Comments
0	70	30	0	0	
0.1	70	30	0	0	Inject
3.0	70	30	0	0	
3.1	0	0	0	100	
7.0	0	0	0	100	
7.1	25	0	25	50	
17.0	25	0	25	50	
17.1	0	100	0	0	
22.0	0	100	0	0	
22.1	70	30	0	0	
27.0	70	30	0	0	End Run

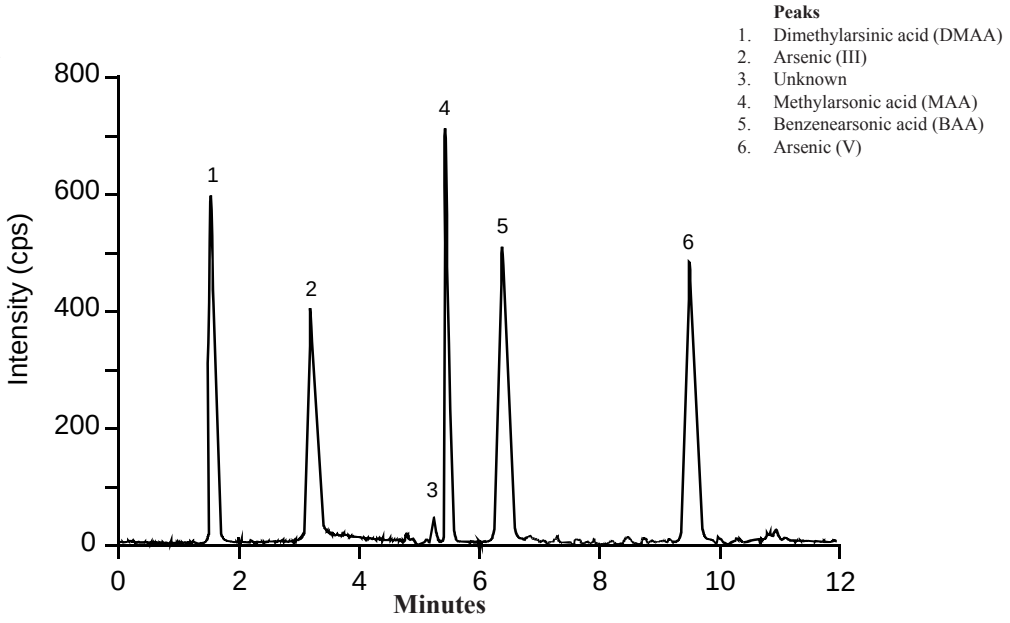


Figure 12
Arsenic Speciation using Ion Chromatography Coupled with ICP-MS

SECTION 6 - TROUBLESHOOTING GUIDE

The purpose of the Troubleshooting Guide is to help you solve operating problems that may arise while using IonPac AS7 column. For more information on problems that originate with the Ion Chromatograph (IC), refer to the Troubleshooting Guide in the appropriate operator's manual. If you cannot solve the problem on your own, contact the North America Technical Call Center at 1-800-DIONEX-0 (1-800-346-6390) or the nearest DIONEX Office (see, "DIONEX Worldwide Offices").

Table 6
AS7/AG7 Troubleshooting Summary

Observation	Cause	Action	Reference Section
High Back Pressure	Unknown	Isolate Blocked Component	6.1.1
	Plugged Column Bed Supports	Replace Bed Supports	6.1.2
	Other System Components	Unplug, Replace	Component Manual
Poor Resolution	Poor Efficiency Due to Large System Void Volumes	Replumb System	6.2.1.B, Component Manual
	Column Headspace	Replace Column	6.2.1.A
Short Retention Times	Flow Rate Too Fast	Recalibrate Pump	6.2.2.A
	Conc. Incorrect Eluents	Remake Eluents	6.2.2.B
	Column Contamination	Clean Column	6.2.2.C, 6.2.2 D
Poor Front End Resolution	Conc. Incorrect Eluents	Remake Eluents	6.2.3.A
	Column Overloading	Reduce Sample Size	6.2.3.B, 3.1.1, 3.1.2
	Sluggish Injection Valve	Service Valve	6.2.3.C, Component Manual
	Large System Void Volumes	Replumb System	6.2.3.D, Component Manual
Spurious Peaks	Sample Contaminated	Pretreat Samples	6.2.4.A, 6.2.4.B
	Sluggish Injection Valve	Service Valve	6.2.3.C, Component Manual

6.1 High Back Pressure

6.1.1 Finding the Source of High System Pressure

Total system pressure for the IonPac AG7 (4-mm) Guard Column plus the AS7 (4-mm) Analytical Column when using the test chromatogram conditions should be equal to or less than 2,000 psi. If the system pressure is higher than 2,000 psi, it is advisable to determine the cause of the high system pressure. The system should be operated with a High-Pressure In-Line Filter (P/N 044105) which is positioned between the Gradient Pump pressure transducer and the injection valve. Make sure you have one in place and that it is not contaminated.

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

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

To determine which part of the chromatographic system is causing the problem, disconnect the pump eluent line from the injection valve and turn the pump on. Watch the pressure; it should not exceed 50 psi. Continue adding system components (injection valve, column(s), detector cell, and waste line) one by one, while monitoring the system pressure. The pressure should increase up to a maximum when the Guard and Analytical columns are connected (see Table 7, "Typical AS7/AG7 Operating Back Pressures").

No other components should add more than 100 psi (0.69 MPa) of pressure. Refer to the appropriate manual for cleanup or replacement of the problem component.

Table 7
Typical AS7/AG7 Operating Back Pressures

Column	Typical Back Pressure psi (MPa)	Flow Rate mL/min
AS7 4-mm Analytical	≤ 900 (6.20)	0.5
AG7 4-mm Guard	≤ 225 (1.55)	0.5
AS7 + AG7 4-mm columns	**≤ 1125 (7.75)**	**0.5**

6.1.2 Replacing Column Bed Support Assemblies

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

A. Disconnect the column from the system.

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

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

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

	IonPac AS7 4-mm Columns (P/N)	IonPac AS7 2-mm Columns (P/N)
Analytical Column	035393	063097
Guard Column	035394	063099
Bed Support Assembly	042955	044689
End Fitting	052809	043278

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 Poor Peak Resolution

Poor peak resolution can be due to any or all of the following factors.

6.2.1 Loss of Column Efficiency

A. Check to see if headspace has developed in the guard or analytical column. This is usually due to improper use of the column such as submitting it to high pressures or to solvent concentrations greater than 5%. Remove the column's top end fitting (see Section 6.1.2, "Replacing Column Bed Support Assemblies"). If the resin does not fill the column body all the way to the top, it means that the resin bed has collapsed, creating a headspace. The column must be replaced.

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

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

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

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

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

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

After cleaning the column, reinstall it in the system and let it equilibrate with eluent for about 30 minutes. No water wash is necessary. The column is equilibrated when consecutive injections of the standard give reproducible retention times. The original column capacity should be restored by this treatment, since the contaminants should be eluted from the column. If you need assistance in solving resolution problems, contact the nearest DIONEX Office (see, "DIONEX Worldwide Offices")

6.2.3 Loss of Front End Resolution

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

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

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

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

D. **Improperly swept out volumes anywhere in the system prior to the guard and analytical columns may be the problem.** Swap components, one at a time, in the system prior to the analytical column and test for front-end resolution after every system change. Be very careful that tubing is cut at a perfect 90° angle. Slide the tubing into the fitting firmly before tightening the fitting.

6.2.4 Spurious Peaks

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

If you need assistance in determining the best way to clean strongly retained solutes in your specific sample matrix from the IonPac AS7 columns, contact the North America Technical Call Center at 1-800-DIONEX-0 (1-800-346-6390) or the nearest DIONEX Office (see, "DIONEX Worldwide Offices").

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

6.3 Missing or excessively tailing cyanide peaks with Disposable or Conventional Silver Working Electrodes.

The cause of this is transition metal contamination in the eluent. Review the following recommendations in the order listed below.

6.3.1 Performance qualification and rinsing with an eluent containing ethylenediamine (EDA). EDA is used for preventing interference by transition metals with the detection of cyanide on silver electrodes. The EDA concentration should be kept at the optimal concentration value of 7.5 mM or 0.05% (v:v). Too high concentration of EDA can shorten the lifetime of disposable silver electrodes and cause significantly higher noise with both conventional and disposable silver electrodes. On the other hand, too low concentration of EDA may not be effective enough for preventing interference by transition metals.

A. Install an IonPac AS7 column and IonPac AG7 Guard (2-mm or 4-mm). Select 30ºC for the column temperature.

B. Install a freshly polished conventional or new disposable silver electrode. Select 25ºC for the detector cell heater temperature if using an ICS-3000 system configured with a DC module. Otherwise, maintain the cell at the same temperature as the column.

C. Prepare an eluent solution containing 75 mM sodium hydroxide (NaOH), 250 mM sodium acetate (NaOAc) and 7.5 mM or 0.05% (v:v) EDA. The next section C.1.D. details the eluent preparation procedure.

ATTENTION
Read the Material Safety Data Sheet before handling EDA.

D. Eluent Preparation:
- i. Weigh 20.50 g of anhydrous NaOAc and dissolve it in 500- 600 g of 18 MΩ-cm water.
- ii. Fill up to ~980 g with 18MΩ-cm water.
- iii. Stir thoroughly and filter through a 0.2 µm Nylon filter.
- iv. Add 5.97 g (3.9 mL) of 50% NaOH and 0.4495 g (0.50 mL) of EDA.
- v. Fill up to 1,015 g (1.0 L) with 18 MΩ-cm water in the bottom container of the filtration unit.
- vi. Transfer the solution immediately to eluent container A, which is connected to nitrogen. Adjust the flow rate at 0.25 mL/min (2-mm ID) or 1.00 mL/min (4-mm ID)

E. Apply the waveform from Table 8. below. A 10 µL injection of 50 ppb standard of cyanide should result in a well defined peak with an area **> 1.0 nC min** and with **asymmetry in the range of 0.9 to 2.0** for 2-mm ID column set. With a 4-mm ID column set, a 50 µL injection of the same standard should generate a peak area > 0.8 nC min in the same range of asymmetry values.

F. Preparation of 50-ppb CN standard:

DANGER
Sodium cyanide may be harmful or fatal if swallowed, inhaled or adsorbed through skin. Contact with acids liberates poisonous gas. Causes burns to skin, eyes, and respiratory tract. Affects blood, cardiovascular system, central nervous system and thyroid. Prepare all solutions in a laboratory fume hood whenever possible and follow all safety precautions for the handling of this reagent (safety glasses, gloves, laboratory coat).

- i. **Stock standard:** Weigh 0.1885 g of sodium cyanide into a plastic scintillation vial and dissolve the whole amount with 10 g of 10 mM NaOH to obtain a 1% cyanide (10,000 ppm) solution {Add 80 mg (52 µL) of 50 % NaOH to 100 g of DI water to make a 10 mM NaOH solution}. The 1% standard can be stored for up to 3 months.
- ii. **Intermediate standard (10 ppm):** Transfer 0.10 g of 1% cyanide into a tared, new disposable container, dilute with 100 g of 10 mM NaOH to obtain a 10 ppm cyanide standard. The 10 ppm standard can be stored for up to 3 months.
- iii. **50-ppb cyanide standard:** In a tared new disposable container, dilute 0.50 g of 10 ppm cyanide standard with 100 g of 10 mM NaOH to obtain a 50 ppb cyanide standard. This standard has to be prepared fresh daily.

NOTE
Refrigerate all prepared standards.

Table 8
Waveform for Silver Electrodes

Time (sec)	Potential (V) vs. Ag/AgCl, 3 M KCl	Integration
0.00	-0.10	
0.20	-0.10	Start
0.90	-0.10	End
0.91	-1.00	
0.93	-0.30	
1.00	-0.30	

G. If there is a discernible peak but with a peak area < 1.0 nC min (< 0.8 nC min with 4-mm ID) and/or asymmetry value > 2.0, the performance is usually improved by pumping the EDA-containing eluent at 0.25 mL/min (1.00 mL/min with 4-mm ID) for about 1-2 hours.

6.3.2 Clean-up Protocol. If the rinse with the EDA-containing eluent did not improve the peak parameters (or if there was no discernible peak), rinse the column with ~2 M nitric acid (HNO_3) following the steps described below.

Preparation of 500 mL of ~2 M HNO_3: In a laboratory fume hood, slowly add 92 g (65 mL) of concentrated HNO_3 (68-70%) into a 1 or 2 L glass eluent container containing ~300 g of filtered (0.2 µm Nylon filter) DI water, stir, then dilute to 533 g (500 mL) with additional filtered DI water.

CAUTION
Concentrated nitric acid is corrosive and causes severe burns! Always wear safety glasses, laboratory coat and gloves when handling acids.

A. Disconnect the detection cell from the column.
B. Rinse the column set with DI water to replace the eluent.
C. Pump ~2M nitric acid for ca. 30 min at 0.25 mL/min (1mL/min with 4 mm ID columns set).
D. Rinse with water until pH becomes neutral (check with pH indicator strip).
E. Condition the column with at least 5 mL (20 mL with 4-mm ID) of the EDA-containing eluent.
F. Reconnect the ED cell to the column outlet and perform **Steps (B-D) from Section 6.3.1**.

An asymmetry value > 2.0 after the nitric acid clean up indicates a need for replacement of either the guard column or the analytical column, or both. Perform an injection of the 50 ppb cyanide standard without the guard column to determine the status of the analytical column.

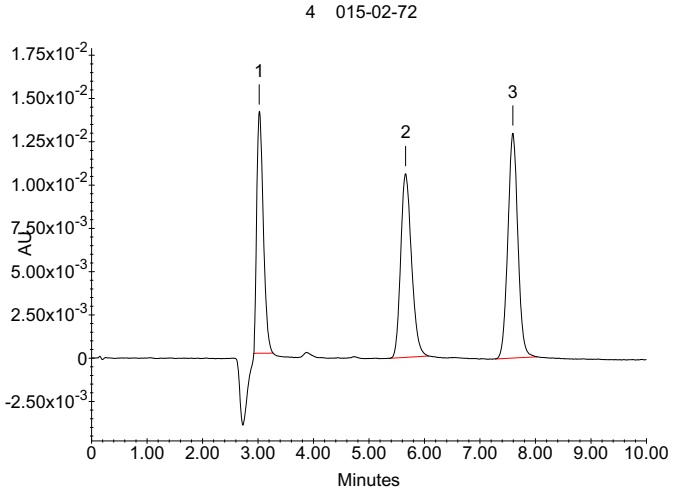
Table 9
Materials/Chemicals to Order

Description	Source	Part Number
Sodium hydroxide, 50% w/w (certified)	Fisher Scientific	SS254-500
Anhydrous Sodium Acetate	Dionex	059326
Ethylenediamine	Aldrich	240729
Sodium Cyanide	Aldrich	380970
Nitric Acid (68 – 70%)	Aldrich	438073
Filtration Unit with 0.2 µm Nylon Filter	VWR	28198-154

APPENDIX A
A.1 Quality Assurance Report

IonPac® AS7
Analytical (2 x 250 mm)
Product No. 063097

Serial No. : 4 015-02-72 **Pressure (PSI) : 525** **Date : 10/22/2004 4:53:44 PM**



Eluent: 50 mM HNO_3
Eluent Flow Rate: 0.12 mL/min
Detection: Absorbance at 330 nm
Range: 0.05 AUFS

Post Column Reagent: 1.0g $Fe(NO_3)_3 \cdot 9\ H_20$
20 mL 70-72% $HClO_4$
per liter

PCR Flow Rate: 0.12 mL/min.
Injection Volume: 5 µL
Storage Solution: Eluent

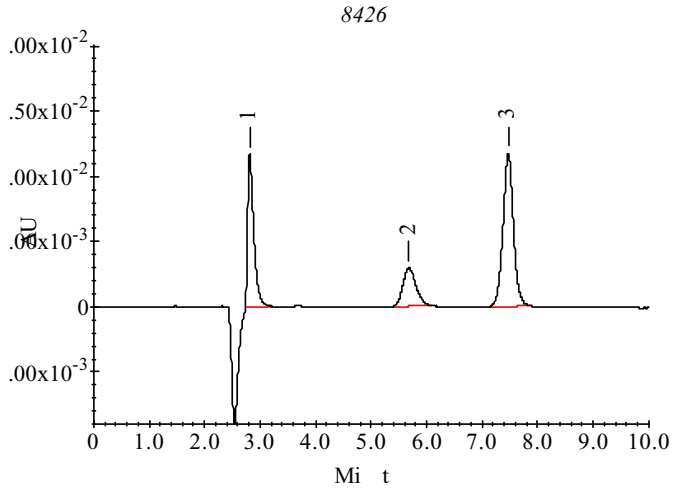
Peak Information : Found Components

Peak No.	Retention Time	Name	(mg/L)	Efficiency	Asymmetry (10%)	Resolution
1	3.02	Phosphate	100.0	2705	2.0	8.99
2	5.66	EDTA	10.0	4024	1.4	5.61
3	7.59	Sulfate	50.0	8259	1.1	n/a

A.2 Quality Assurance Report

IonPac® AS7
Analytical (4 x 250 mm)
Product No. 035393

Serial No. : 8426 **Pressure (PSI) : 530** **Date : 5/9/00 8:45:32 AM**



Eluent: 50 mM HNO_3
Eluent Flow Rate: 0.5 mL/min
Detection: Absorbance at 330 nm
Range: 0.05 AUFS

Post Column Reagent: 1.0g $Fe(NO_3)_3 \cdot 9\ H_20$
20 mL 70-72% $HClO_4$
per liter

PCR Flow Rate: 0.5 mL/min.
Injection Volume: 25 µL
Storage Solution: Eluent

Peak Information : Found Components

Peak No.	Retention Time	Name	(mg/L)	Efficiency	Asymmetry (10%)	Resolution
1	2.81	Phosphate	100.0	3406	2.8	9.12
2	5.67	EDTA	10.0	2704	1.6	4.65
3	7.47	Sulfate	50.0	7728	1.1	n/a

APPENDIX B - COLUMN CARE

B.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 IonPac AS7 columns is 3,000 psi (20.68 MPa).

B.2 Column Start-Up

The column is shipped using the column test eluent as the 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.

B.3 Column Storage

For both short-term and long-term storage, use column test eluent for the column storage solution. Flush the column for a minimum of 10 minutes with the storage solution (eluent). Cap both ends securely, using the plugs supplied with the column.

B.4 Column Cleanup

The following column cleanup protocols have been divided into three general isocratic protocols to remove acid-soluble, base-soluble or organic contaminants. They can be combined into one gradient protocol if desired 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 zones in the column. High pressure zones can disrupt the uniformity of the packing of the column bed and irreversibly damage the performance of the column. High pressure zones in the column can be created by pumping successive eluents through the column that are not miscible, that have eluent components in one eluent that will precipitate out in the other eluent or by using an acid eluent followed by a base eluent which may create a neutralization pressure band. The precipitation of the salts in solvents during column rinses can result in very high pressure zones. High viscosity mixing zones can be created between two eluents having solvents with a very high energy of mixing.

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

B.4.1 Choosing the Appropriate Cleanup Solution

A. **Concentrated eluent solutions** such as a 10X concentrate of the most concentrated eluent used in the application is sufficient to remove hydrophilic contamination of low valence.

B. **Concentrated acid solutions** such as 1 to 3 M HNO_3, remove high valence hydrophilic ions by ion suppression and elution by the chloride ion.

C. **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 HNO_3, 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.

D. **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 listed in Table 3, HPLC Solvents for Use with IonPac AS7 Columns.

E. **Concentrated acid solutions such as 1 to 3 M HNO_3 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. The organic solvent then removes the subsequent nonionic and hydrophobic contamination. See Section D above.

A frequently used cleanup solution is 200 mM HNO_3 in 5% acetonitrile. This solution must be made immediately before use because the acetonitrile will decompose in the acid solution during long term storage.

F. **Regardless of the cleanup solution chosen, use the following cleanup procedure in, "Column Cleanup Procedure", to clean the AG7 and AS7.**

B.4.2 Column Cleanup Procedure

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

B. **Disconnect the ASRS ULTRA II or AMMS III** from the IonPac AS7 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.

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 1.0 mL/min for an AS7 4-mm Analytical** or Guard Column or set the pump flow rate to 0.25 mL/min for an AS7 2-mm Analytical or Guard Column.

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

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

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

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

H. **Reconnect the ASRS ULTRA II or AMMS III** to the AS7 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.

APPENDIX C - CONFIGURATION

Table 1
Configuration

CONFIGURATION	2-mm	4-mm
Eluent Flow Rate	0.12 mL/min	0.5 mL/min
SRS Suppressor	ASRS ULTRA II (2-mm) (P/N 061562)	ASRS ULTRA II (4-mm) (P/N 061561)
MMS Suppressor	AMMS III (2-mm) (P/N 056751)	AMMS III (4-mm) (P/N 056750)
AAES uppressor	AAES (P/N 056116)	AAES (P/N 056116)
Injection Loop	2 - 15 µL	10-50 µL
	Rheodyne Microinjection Valve (P/N 044697) for full loop injections <15 µL.	
System Void Volume	Eliminate switching valves, couplers and the GM-3 Gradient Mixer. Use only the 2-mm GM-4 Mixer (P/N 049135).	Minimize dead volume. Switching valves, couplers can be used. Use the GM-2 , GM-3 or recommended gradient mixers.
Pumps	Use the DP/SP/GS50/GP50/GP40/IP20/IP25 in Microbore Configuration with a Microbore GM-4 (2-mm) Gradient Mixer. The GPM-2 can be used for 2-mm isocratic chromatography at flow rates of 0.5 mL/min or greater but cannot be used for 2-mm gradient chromatography.	Use the DP/SP/GP40/GP50/IP20/IP25 in Standard-Bore Configuration. The GM-3 Gradient Mixer should be used for gradient analysis on systems other than the GP50. Note: The GP40 has an active mixer.
Detectors	AD20 Cell (6-mm, 7.5 µL, P/N 046423) VDM-2 Cell (3-mm, 2.0 µL) (P/N 043120) DC/CD20, CD25, CD25A, ED40, ED50, ED50A and ED ICS-3000 Conductivity Cell with DS3 (P/N 044130) or Conductivity Cell with Shield (P/N 044132) CDM-2/CDM-3 Cell (P/N 042770) Do not use the TS-1 or TS-2 with ED40/ED50/ED50A or CD20/CD25/CD25A. The TS-2 (P/N 043117) is optimized for 2-mm operation on CDM-2 or CDM-3. Recommended back pressure: 30–40 psi	AD25 Cell (10-mm, 9 µL, P/N 049393) VDM-2 Cell (6-mm, 10 µL) (P/N 043113) DC/CD20, CD25, CD25A, 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) Do not use the TS-1 or TS-2 with ED40/ED50/ED50A or CD20/CD25/CD25A. The TS-1 or TS-2 (P/N 043117) is optimized for 4-mm operation on CDM-2 or CDM-3. Recommended back pressure: 30–40 psi

Table 2
Tubing Back Pressures

Color	Dionex P/N	ID Inches	ID cm	Volume mL/cm	Back Presure psi/ft at 1 mL/min	Back Presure psi/ft at 0.25 mL/min	Back Presure psi/cm at 1 mL/min
Green	044777	0.030	0.076	4.560	0.086	0.021	0.003
Orange	042855	0.020	0.051	2.027	0.435	0.109	0.015
Blue	049714	0.013	0.033	0.856	2.437	0.609	0.081
Black	042690	0.010	0.025	0.507	6.960	1.740	0.232
Red	044221	0.005	0.013	0.127	111.360	27.840	3.712
Yellow	049715	0.003	0.008	0.046	859.259	214.815	28.642