Errata

Product Manual for Dionex IonPac™ AS16 and AG16 Columns
031475-06

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*

PRODUCT MANUAL

for

IonPac® AG16
IonPac® AS16

Now sold under the
Thermo Scientific brand

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IC | HPLC | MS | EXTRACTION | PROCESS | AUTOMATION

PRODUCT MANUAL

for the

IONPAC® AG16 GUARD COLUMN

(4 x 50 mm, P/N 055377)

(2 x 50 mm, P/N 055379)

IONPAC® AS16 ANALYTICAL COLUMN

(4 x 250 mm, P/N 055376)

(2 x 250 mm, P/N 055378)

Document No. 031475
Revision 06
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SECTION 1 - INTRODUCTION

The IonPac® AS16 2-mm (P/N 055378) and 4-mm (P/N 055376) Analytical Columns are high capacity, hydroxide selective anion exchange columns designed for the isocratic separation of polarizable anions including iodide, thiocyanate, thiosulfate, and perchlorate in a variety of sample matrices. The AS16 column has a capacity of approximately 170 µeq/column which allows large loop injections without column overloading. Under isocratic conditions, the polarizable anions can easily be separated in approximately 20 minutes. Trace concentrations of perchlorate in drinking water, surface water and ground water matrices can easily be determined using a large loop injection. With 50 mM sodium hydroxide eluent, perchlorate can be determined in approximately 10 minutes. The AG16 guard column is packed with a microporous resin with a lower capacity. The microporous resin ensures optimum long term performance of the guard column.

Table 1
IonPac AS16/AG16 Packing Specifications

Column	Particle Diameter µm	Substrate X-linking %	Column Capacity µeq/column	Functional Group	Hydrophobicity
AS16[a] 4 x 250 mm	9.0	55	170	Alkanol quaternary ammonium	Ultra-low
AG16[b] 4 x 50 mm	13.0	55	3.5	Alkanol quaternary ammonium	Ultra-low
AS16[a] 2 x 250 mm	9.0	55	42.5	Alkanol quaternary ammonium	Ultra-low
AG16[b] 2 x 50 mm	13.0	55	0.875	Alkanol quaternary ammonium	Ultra-low

[a] macroporous (2000 Å) divinylbenzene/ethylvinylbenzene polymer
[b] microporous divinylbenzene/ethylvinylbenzene polymer

Table 2
AS16/AG16 Operating Parameters

Column	Typical Back Pressure with Aqueous Eluents psi (MPa) at 30 °C	Standard Flow Rate mL/min	Maximum Flow Rate mL/min
AS16 4-mm Analytical	≤ 1400 (9.64)	1.0	3.0
AG16 4-mm Guard	≤ 150 (1.03)	1.0	3.0
AS16 + AG16 4-mm columns	**≤ 1550 (10.67)**	**1.0**	**3.0**
AS16 2-mm Analytical	≤ 1400 (9.64)	0.25	0.75
AG16 2-mm Guard	≤ 150 (1.03)	0.25	0.75
AS16 + AG16 2-mm columns	**≤ 1550 (10.67)**	**0.25**	**0.75**

Assistance is available for any problem 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" on the Dionex Reference Library CD-ROM.

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

3.1.2 System Requirements for 4-mm Operation

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

3.1.3 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 Dionex 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 CD-ROM.

3.2 The Sample Concentrator

For 2-mm or 4-mm concentrator work, use the IonPac AG16 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 AG16 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 AG16 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 AG16 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 AG16 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-XP1, UTAC-ULP1, and UTAC-XLP1) Column Product Manual (Document No. 065091).

CAUTION

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

3.3 The Injection Loop

Table 4
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

3.3.1 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 10 nanomoles of any one analyte onto a 2-mm analytical column. Injecting larger number of moles of a sample can result in overloading the column which can affect the detection linearity. For low concentrations of analytes, larger injection loops can be used to increase sensitivity. The AS16 2-mm requires a microbore HPLC system configuration. Install an injection loop one-fourth or less (<15 µL) of the loop volume used with a 4-mm analytical system (Section 2, “Comparison of 2-mm and 4-mm 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 40 nanomoles of any one analyte onto the 4-mm analytical column. Injecting larger number of moles of a sample can result in overloading the column which can affect the detection linearity. For low concentrations of analytes, larger injection loops can be used to increase sensitivity.

3.4 The IonPac AG16 Guard Column

An IonPac AG16 Guard Column is normally used with the IonPac AS16 Analytical Column. Retention times will increase by approximately 1.5% 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 AG16 Guard Column at the first sign of peak efficiency loss or decreased retention time will prolong the life of the AS16 Analytical Column.

3.5 Eluent Storage

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

CAUTION

DO NOT USE GLASS BOTTLES for either stock solution bottles or eluent bottles! Base slowly dissolves glass, releasing impurities that adversely effect the AS16 column performance.

3.6 Installing the CR-ATC Anion Trap Column for Use with EGC II KOH or EGC II NaOH Cartridge

For IonPac AS16 applications using the EG40 or EG50 with EGC II KOH or EGC II NaOH cartridge, a CR-ATC Continuously Regenerated Anion Trap Column (P/N 060477) should be installed at the EGC eluent outlet to remove trace level anionic contaminants such as carbonate from the carrier deionized water. See the CR-TC Product Manual (Document No. 031910) for instructions.

As an alternative, the ATC-HC Trap Column (P/N 059604) should be installed between the pump outlet and the inlet of the EluGen Cartridge in the EG40 or EG50 Module to remove anionic contaminants from the carrier deionized water. The ATC-HC is for use with EGC II KOH or EGC II NaOH cartridge in the EG40 and EG50 Eluent Generators. See the ATC-HC Product Manual (Document No. 032697) for instructions.

Alternatively, the ATC-3 Trap Column (P/N 059660 and 059661) may be used. The ATC-HC (P/N 059604) and ATC-3 Trap Columns will require off-line regeneration. To use the ATC- 3 Anion Trap Columns, see Section 3.7.

3.7 The Anion Trap Column, ATC-3

When performing an anion exchange application that involves a hydroxide gradient, an IonPac Anion Trap Column (ATC-3, (4-mm) P/N 059660 or ATC-3 (2-mm), P/N 059661) should be installed in place of the high pressure Gradient Mixer between the pump and the injection valve. The ATC-3 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 (4-mm) or ATC-3 (2-mm), complete the following steps:

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

B. Connect the gradient pump directly to the ATC-3. Connect a waste line to the ATC-3 outlet and direct the line to a waste container.

C. Flush the ATC-3 with 100 mL of 2.0 M NaOH through the 4-mm ATC-3 Column or 50 mL for the 2-mm ATC-3 Column.

D. Pump 20 mL of eluent through the 4-mm ATC-3 or 10 mL for the 2-mm ATC-3 Column.

E. Reconnect the ATC-3 after flushing it with eluent. Connect the ATC-3 to the eluent line that is connected to the injection valve.

The background conductivity of your system should be between 1.5 µS and 2.5 µS when 0.75 mM NaOH is being pumped through the chromatographic system. The baseline shift should be no greater than 5 µS during a gradient eluent concentration ramp from 0 to 80 mM NaOH. If the baseline shifts are greater than 5 µS, the ATC-3 should be cleaned using steps B - E above.

At the end of each operating day, the ATC-3 should be flushed to remove any impurities that may have accumulated on it.

Under normal operating conditions, the ATC-3 column should be regenerated at the end of each operational day to remove any contaminants that may have collected on it, including carbonate. The daily regeneration of the ATC-3 column ensures that the IC system is systematically equilibrated for the most reproducible determinations of those anions being eluted by the weak eluents.

See the conditioning procedure above for regeneration of ATC-3 columns. For detailed information refer to the ATC-3 Product Manual (Document No. 032697).

3.8 Anion Self-Regenerating Suppressor 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 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 AS16 4-mm Analytical Column, use an ASRS ULTRA (4-mm, P/N 053946).
If you are installing an IonPac AS16 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.”

3.9 Anion MicroMembrane Suppressor Requirements

An Anion MicroMembrane Suppressor (AMMS® III) may be used instead of an ASRS ULTRA (4-mm) for applications that require suppressed conductivity detection. Use an AMMS III 4-mm (P/N 056750) with the IonPac AS16 4-mm Analytical Column. It is compatible with all solvents and concentrations with which the systems and columns are compatible. For 2-mm operation, use the 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.

3.10 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 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.

3.11 Using AutoRegen

To save regenerant preparation time and reduce regenerant consumption and waste, Dionex recommends using an AutoRegen® Accessory (P/N 039594) with the ASRS ULTRA in the Chemical Suppression Mode or with the AMMS III. 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).

3.12 Using the EG40 or EG50 with AS16

Please refer to the EG50 Product Manual (Document No. 031908) or the EG40 Product Manual (Document No. 031373), for information on the operation of these Eluent Generators and the modules available for them.

SECTION 4 - OPERATION

4.1 General Operating Conditions

Sample Volume:	2-mm: 2.5 µL Loop + 0.8 µL Injection valve dead volume 4-mm: 10 µL Loop + 0.8 µL Injection valve dead volume
Column:	2-mm: AS16 2-mm Analytical Column + AG16 2-mm Guard Column 4-mm: AS16 4-mm Analytical Column + AG16 4-mm Guard Column
Eluent:	35 mM NaOH (for test chromatogram)
Eluent Flow Rate:	2-mm: 0.25 mL/min 4-mm: 1.0 mL/min
SRS Suppressor:	Anion Self-Regenerating Suppressor ULTRA II (2-mm or 4-mm) AutoSuppression Recycle Mode for aqueous gradients AutoSuppression External Water Mode for eluents with solvent
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III (2-mm or 4-mm)
MMS Regenerant:	50 mN H_2SO_4
Expected Background Conductivity:	≤ 3 µS
Long-term Storage Solution (> 1 week):	100 mM Sodium Borate
Short-term Storage Solution (< 1 week):	Eluent

4.2 IonPac AS16 Operation Precautions

CAUTIONS
Filter and Degas Eluents
Filter Samples
Eluent pH between 0 and 14
Sample pH between 0 and 14
0.75 mL/min Maximum Flow Rate for 2-mm Columns
3.0 mL/min Maximum Flow Rate for 4-mm Columns
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 by having an actual lot analysis on each label.

4.3.2 Deionized Water

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

4.4 Eluent Preparation

Sodium Hydroxide Eluent Concentration

Weight Method

When formulating eluents from 50% sodium hydroxide, Dionex recommends weighing out the required amount of 50% sodium hydroxide. Use the assayed concentration value from the sodium hydroxide bottle.

Example: To make 1 L of 35 mM NaOH use 2.8 g of 50% sodium hydroxide:
(as used in Section 5.3, "Production Test Chromatogram")

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

Volume Method

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

$$\mathbf{g = dvr}$$

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

Example: To make 1 L of 35 mM NaOH use 1.83 mL of 50% sodium hydroxide:
(as used in Section 5.3, "Production Test Chromatogram")

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

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

Sodium Hydroxide Eluents

Dilute the amount of 50% (w/w) NaOH Reagent specified in Table 6, "Dilution of 50% (w/w) NaOH to Make Standard AS16 Eluents" with degassed, deionized water (having a specific resistance of 18.2 megohm-cm) to a final volume of 1,000 mL using a volumetric flask. Avoid the introduction of carbon dioxide from the air into the aliquot of 50% (w/w) NaOH bottle or the deionized water being used to make the eluent. Do not shake the 50% (w/w) NaOH bottle or pipette the required aliquot from the top of the solution where sodium carbonate may have formed.

Table 6
Dilution of 50% (w/w) NaOH to Make Standard AS16 Eluents

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

4.5 Solvents

Solvents can be added to the ionic eluents used with IonPac AS16 columns to modify the ion exchange process or improve sample solubility. The solvents used must be free of ionic impurities. However, since most manufacturers of solvents do not test for ionic impurities, it is important that the highest grade of solvents available be used. Currently, several manufacturers are making ultrahigh purity solvents that are compatible for HPLC and spectrophotometric applications. These ultrahigh purity solvents will usually ensure that your chromatography is not affected by ionic impurities in the solvent. Currently at 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 AS16 columns is 4,000 psi (27.57 MPa).

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

Table 5
HPLC Solvents for Use with IonPac AS16 Columns

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

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

CAUTION

The Anion Self-Regenerating Anion Suppressor (ASRS ULTRA) must be operated in the AutoSuppression External Water Mode when using eluents containing solvents.

4.5.1 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 90% acetonitrile, prepare the eluent by adding 900 mL of acetonitrile to an eluent reservoir. Then add 100 mL of deionized water or eluent concentrate to the acetonitrile in the reservoir. Using this procedure to mix solvents with water will ensure that a consistent true volume/volume eluent is obtained. Premixing water with solvent will minimize the possibility of outgassing.

NOTE

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

Always degas and store all eluents in 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. Never add the acetonitrile directly to the basic carbonate or hydroxide eluent bottle.

4.6 Regenerant Preparation for the AMMS III or ASRS ULTRA II

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

SECTION 5 - EXAMPLE APPLICATIONS

5.1 Recommendations for Optimum System Performance

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”). Different systems will differ slightly in performance due to slight differences in column sets, system void volumes, liquid sweep-out times of different components and laboratory temperatures.

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

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

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

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

Install an Anion Trap Column CR-ATC, ATC-HC, or an ATC-3 in the system. See Section 3 of the product manual for the CR-TC (Document No. 031910-01) and Section 2 of the product manual for the ATC-HC and ATC-3 (Document No. 032697-08) to minimize the baseline shift and to improve retention time reproducibility of analytes when doing gradient chromatography and to keep baseline shift to a minimum. (Refer to the column cleanup protocol of an ATC-3 in Section 6.2.2, "A Contaminated Trap Column.")

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

You can increase the sensitivity of your system by using sample concentration techniques (see Section 3.2, “The Sample Concentrator”).

NOTE

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

5.2 Production Test Chromatograms

Isocratic elution of inorganic anions including polarizable anions on the IonPac AS16 Analytical Column has been optimized utilizing a hydroxide eluent. By using this eluent, common inorganic anions including polarizable anions can be used to test the performance of the AS16 Column. The IonPac AS16 Analytical Column should always be used with the IonPac AG16 Guard Column. To guarantee that all IonPac AS16 Analytical Columns meet high quality and reproducible performance specification standards, all columns undergo the following production control test. An operating temperature of 30°C is used to ensure reproducible resolution and retention. Note that the AG16 Guard is packed with a microporous resin of proportionally lower capacity and contributes approximately 1.5% increase in retention times when a guard column is placed in-line prior to the analytical column.

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

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

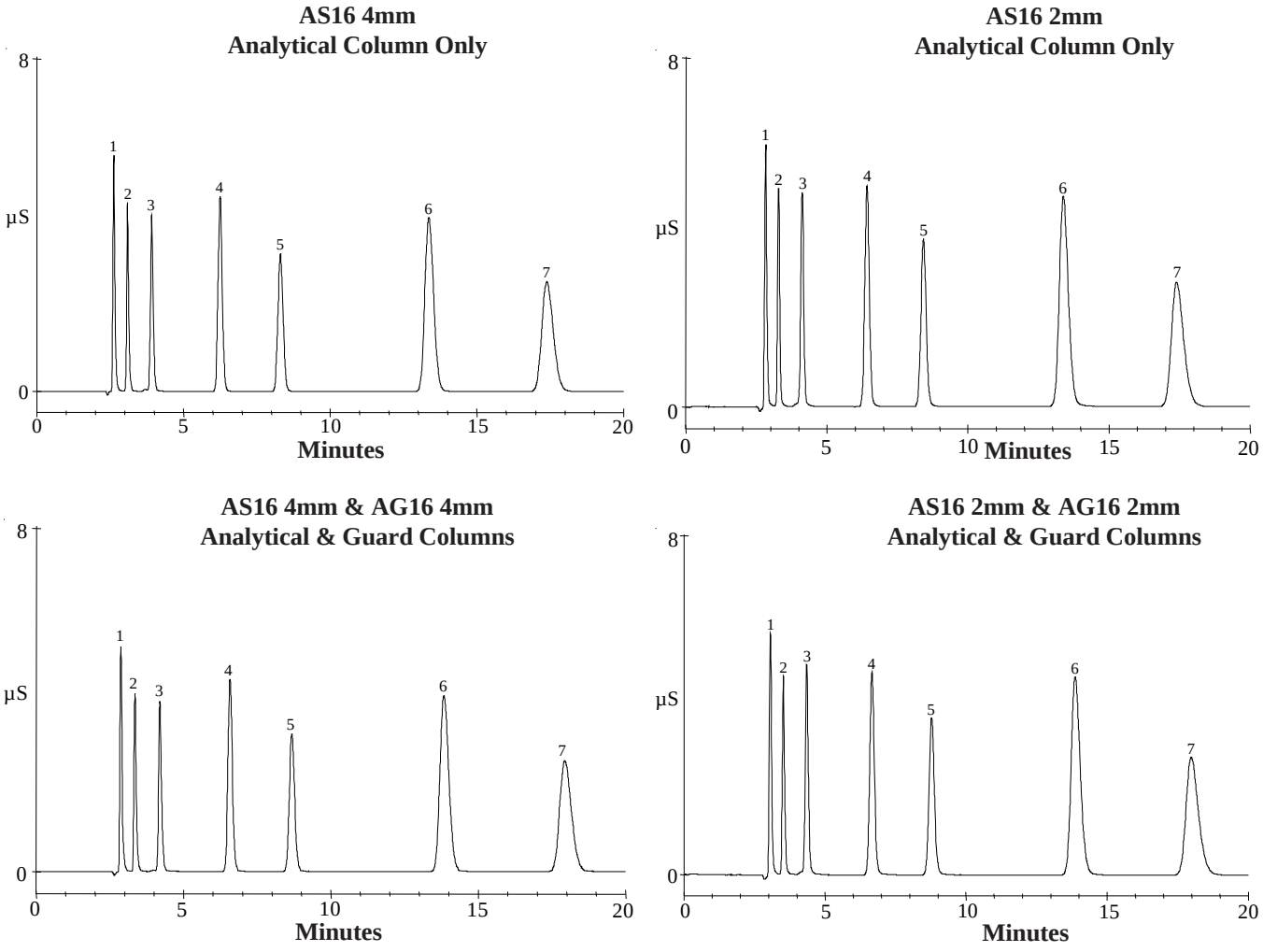


Figure 2
IonPac AS16 Production Test Chromatograms

5.3 Comparison of Test Chromatogram at Room Temperature and 30°C

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

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

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

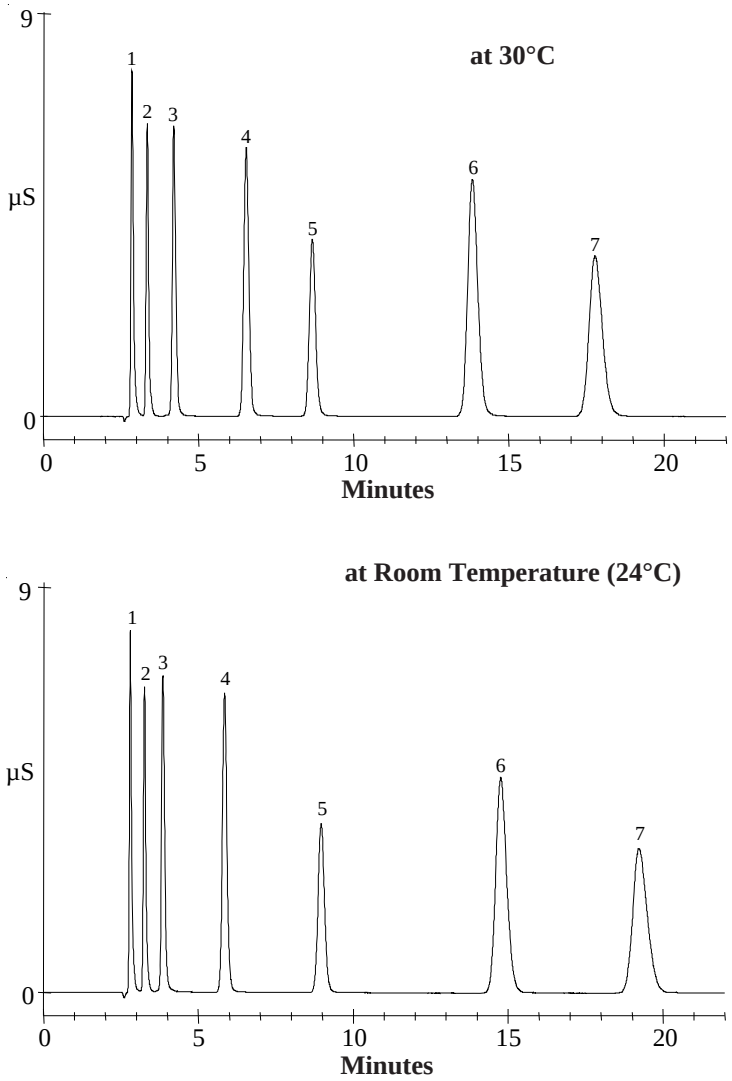


Figure 3
IonPac AS16 Test Chromatogram at 30°C and at Room Temperature

5.4 Isocratic Separation of 7 Anions and 4 Polarizable Anions

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

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

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

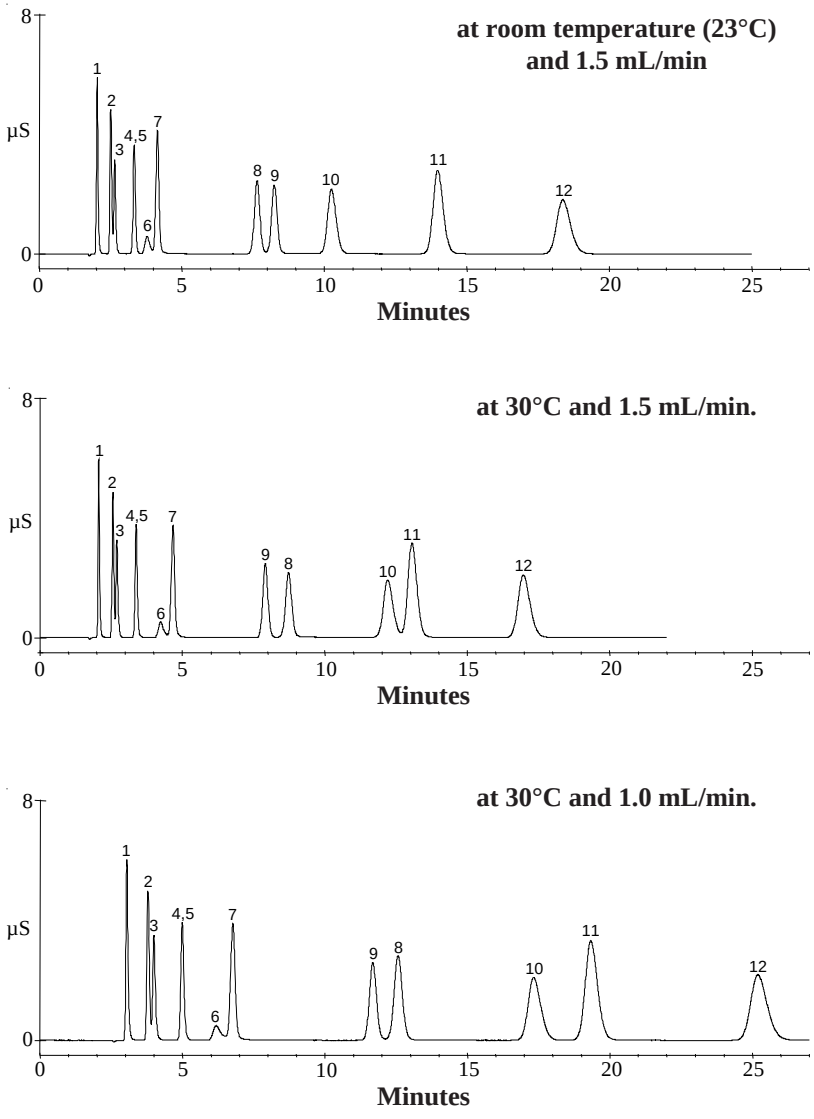


Figure 4
Separation of 7 Anions and 4 Polarizable Anions

5.5 Separation of Polarizable Anions and Inorganic Anions Using Gradient Elution

Figure 5 illustrates the separation of a wide variety of inorganic anions including polarizable anions. Weakly retained anions such as acetate, propionate, and formate are resolved using an isocratic hydroxide eluent and the highly retained anions such as thiosulfate, thiocyanate, and perchlorate are eluted with a hydroxide gradient. Peak shape and efficiency are greatly improved for the polarizable anions on the IonPac AS16 column.

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

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

Gradient Conditions with Bottle Eluent System

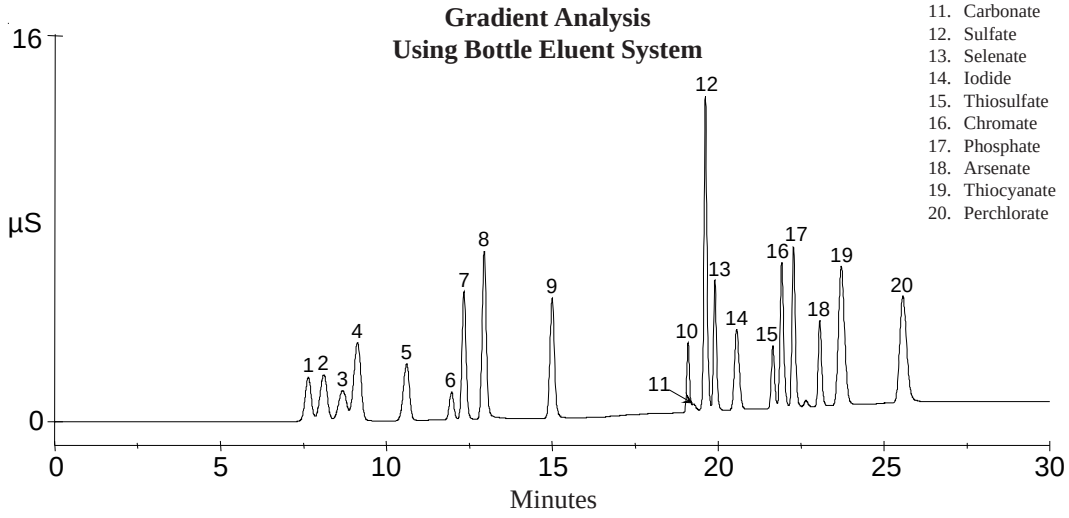
TIME (min)	%E1	%E2	%E3	Comments
Equilibration				
0	30	70	0	1.5 mM NaOH for 7 min.
7.0	30	70	0	
Analysis				
7.1	30	70	0	Start isocratic analysis
7.5	30	70	0	Inject Valve to Load Position
14.0	30	70	0	End Isocratic analysis, Begin Gradient analysis
20.0	0	90	10	
30.0	0	45	55	

EG40 Conditions

Eluent: Deionized water
Offset volume = 0.0 µL

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

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



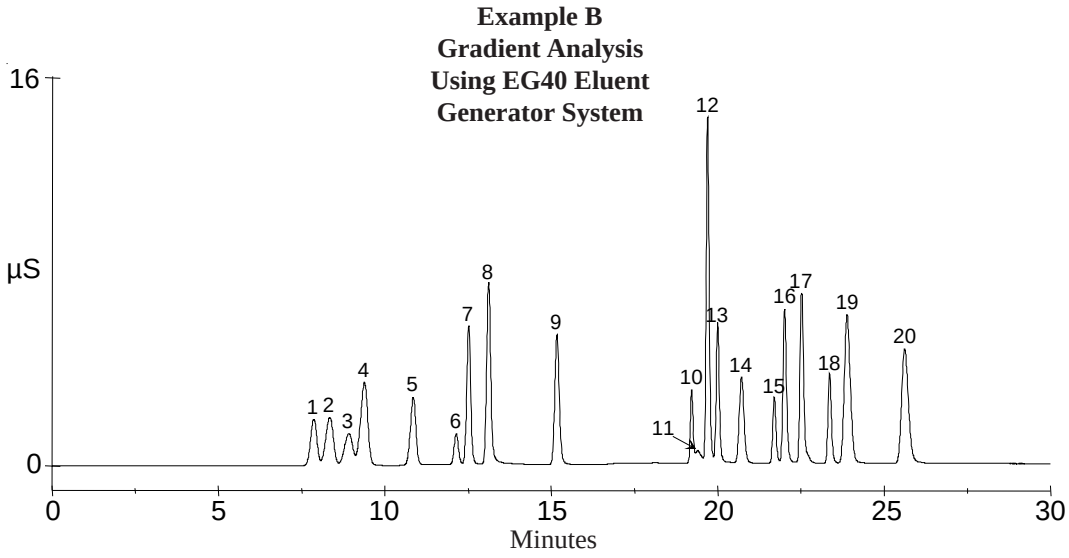


Figure 5
Separation of Polarizable Anions and Inorganic Anions Using Gradient Elution

5.6 Determination of Trace Perchlorate Using a Large Loop Injection

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

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

	Analyte	mg/L (ppm)
1.	Chloride	200.0
2.	Nitrate	50.0
3.	Carbonate	200.0
4.	Sulfate	1000.0
5.	Perchlorate	0.005

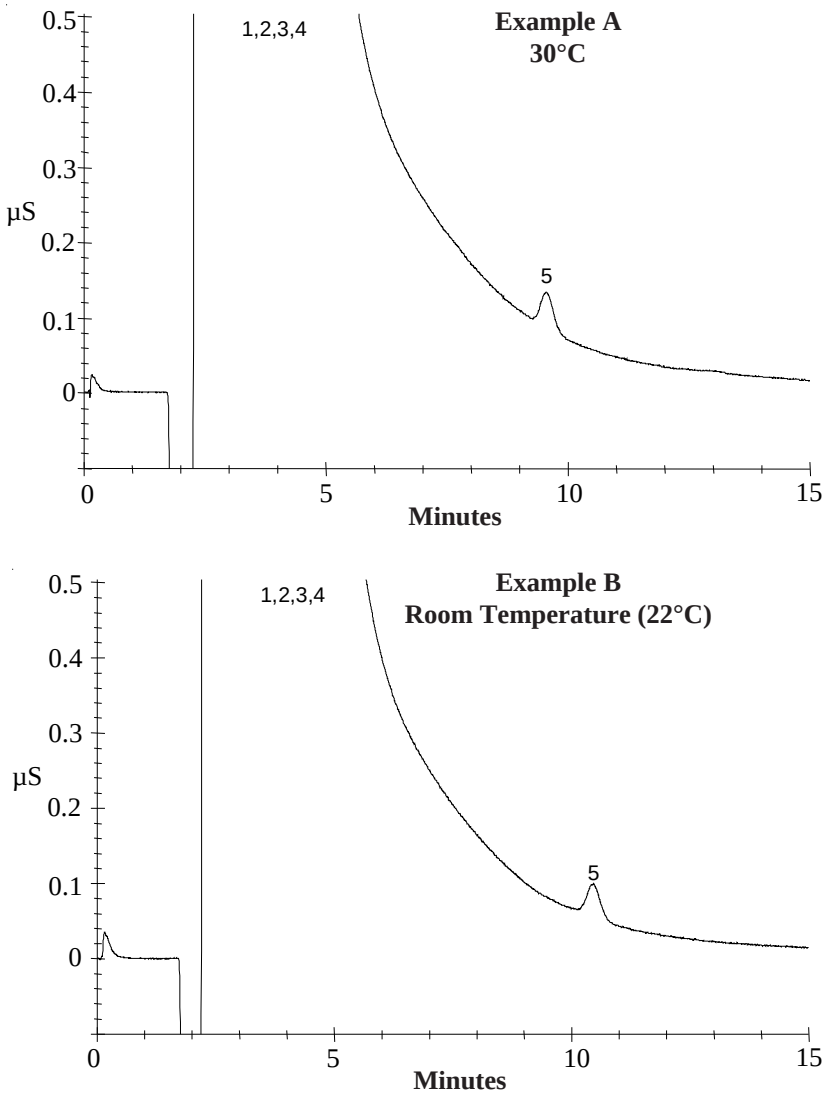


Figure 6
Determination of Trace Perchlorate Using a Large Loop Injection on the IonPac AS16

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

Perchlorate, initially ammonium perchlorate, widely used in the manufacture of rocket propellants, munitions, fireworks, and road flares, has been found in drinking water in areas where aerospace materials and munitions have been manufactured and tested. Perchlorate is a potential health concern because it interferes with the production of thyroid hormones. The IonPac AS16 column was designed to determine trace perchlorate in groundwater and drinking water matrices. Figure 7 shows the determination of trace perchlorate in a drinking water sample using sample preconcentration with the Cryptand C1 Concentrator Column and a sodium hydroxide eluent coupled with suppressed conductivity detection. The Cryptand C1 Concentrator Column is used with sodium hydroxide eluent to allow optimum concentrator capacity control. At high concentrations of sodium, the Cryptand C1 has high capacity, but at lower concentrations the capacity decreases and the analytes can be eluted. Figure 8 shows the system flow path for the determination of trace perchlorate according to U.S. EPA Method 314.1.

Low-µg/L (ppb) levels of perchlorate can easily be quantified using the AS16 column and a 2-mL sample preconcentration, as shown in Figure 7.

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

Peak #	Analyte	µg/L (ppb)
1	Perchlorate	0.5

Recovery - 116.5%

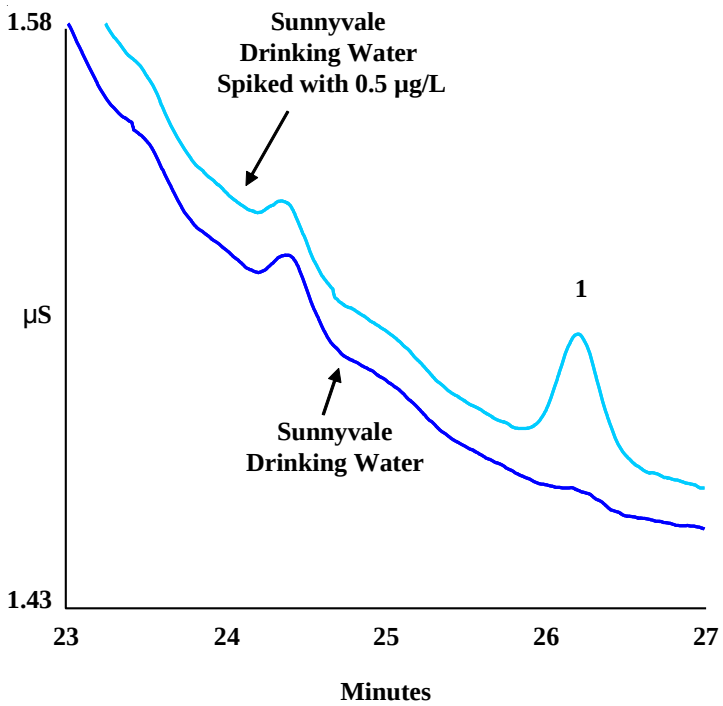
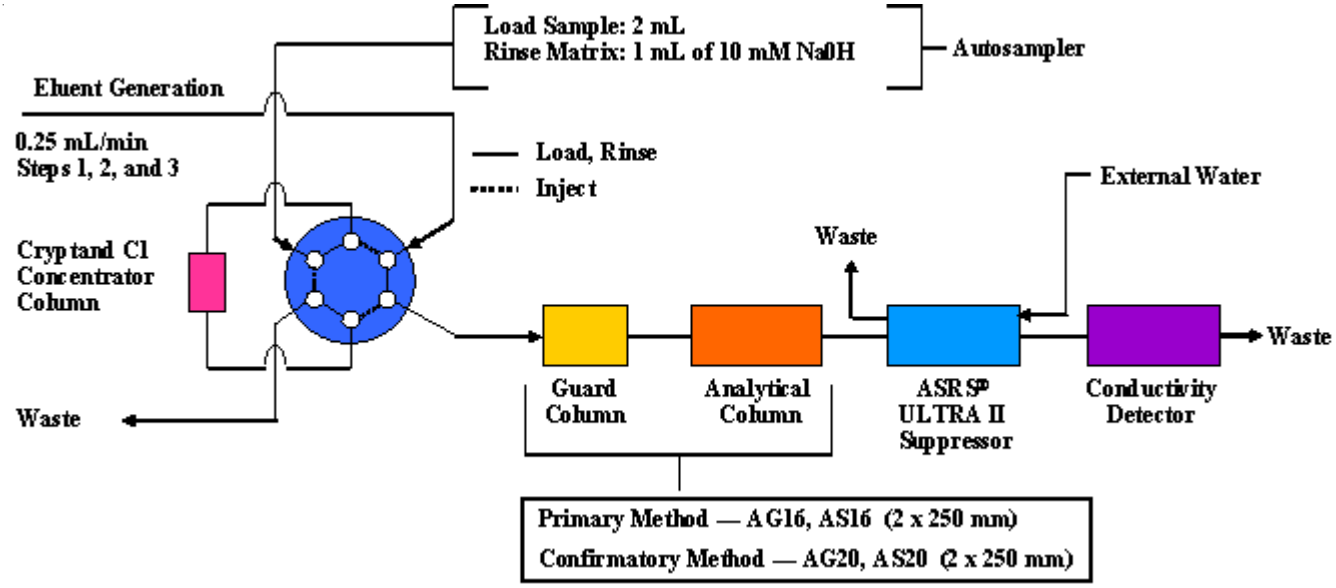


Figure 7
Determination of Trace Perchlorate in Drinking Water Using the AS16 Column and the Cryptand C1 Concentrator Column

NaOH Eluent Generation			
Steps	**Function**	**Conc.**	**Time**
1	Perchlorate Transfer	0.5 mM	12 min
2	Analysis	65 mM	16 min
3	Column Cleanup	100 mM	2 min



NOTE ***Autosampler must be capable of loading concentrator columns.***

Figure 8
Perchlorate Analysis Using RFIC with Preconcentration and Matrix Rinse—EPA Method 314.1

5.8 Separation of Polyphosphate Anions

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

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

Gradient Conditions
Without Solvent

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

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

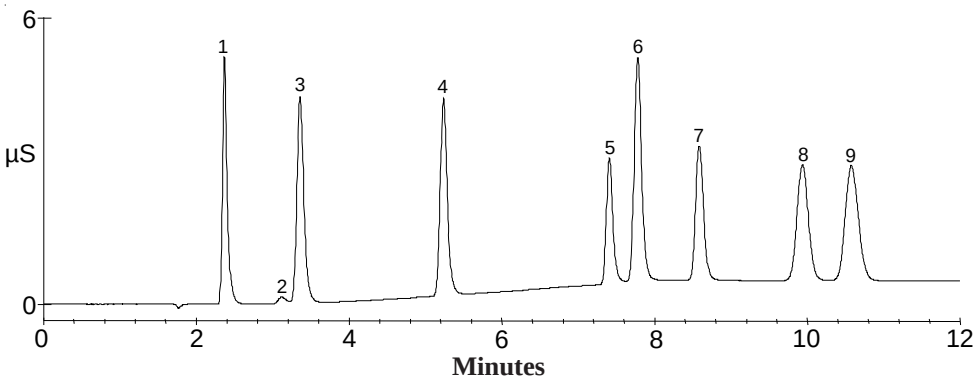


Figure 9
Determination of Polyphosphate Anions

5.9 Separation of Polyphosphate Anions Using EG40

Figure 10 shows the separation of polyvalent phosphates using the EG40 Eluent Generator System. Notice the excellent separation of polyvalent phosphates using an EG40 gradient from 25 mM KOH to 65 mM KOH. In spite of the steep gradient, a minimum baseline shift is observed which facilitates quantitation of trace components as demonstrated in the dishwasher detergent chromatogram.

The following example also shows a comparison of a gradient delivered using the EG40 Eluent Generator System and a bottle eluent system. Due to the carbonate contamination of the bottle eluent system, polyvalent phosphates such as tetrametaphosphate and tetrapolyphosphate have less retention and coelute. For optimum bottle eluent system conditions, see Figure 9.

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

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

EG40 Conditions

Eluent: Deionized water
Offset volume = 0.0 µL

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

Gradient Conditions With Bottle Eluent

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

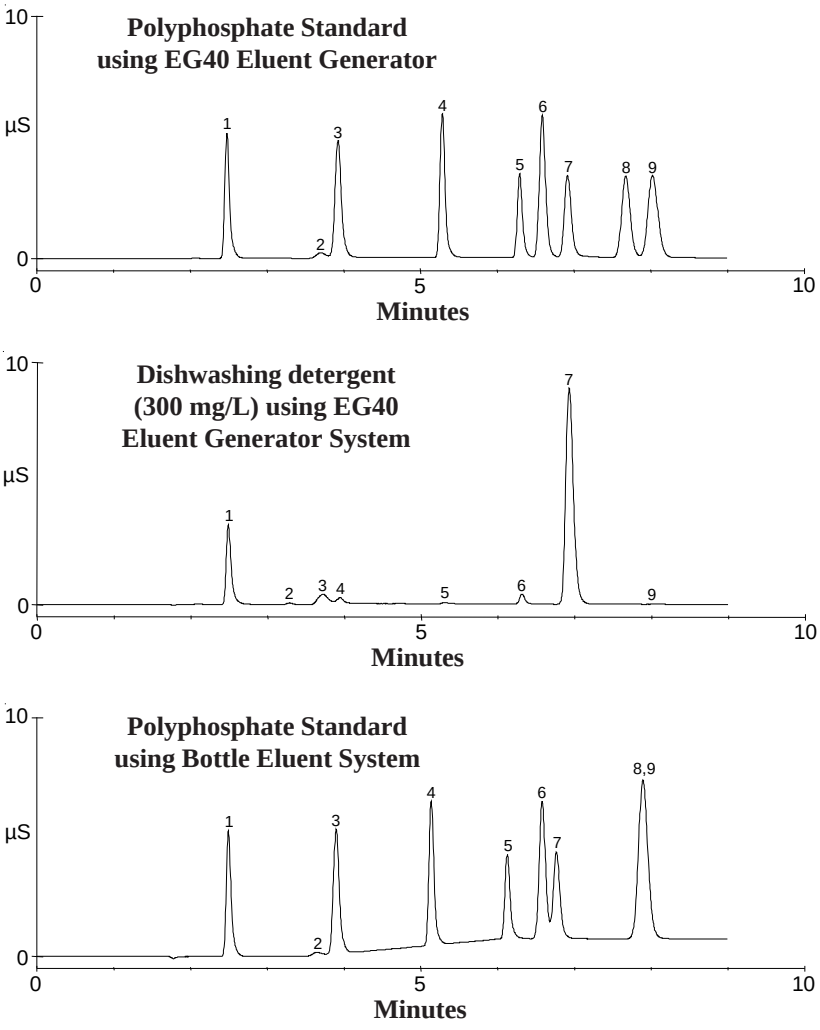


Figure 10
Determination of Polyphosphate Anions

5.10 Clean-up After Humic Acid Samples

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

Column:	IonPac, AS16 4- mm
Eluent:	35 mM Sodium hydroxide
Temperature:	30 °C
Flow Rate:	1.0 mL/min
Inj. Volume:	10 µL
Detection:	Suppressed conductivity, ASRS® ULTRA II, AutoSuppression recycle mode

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

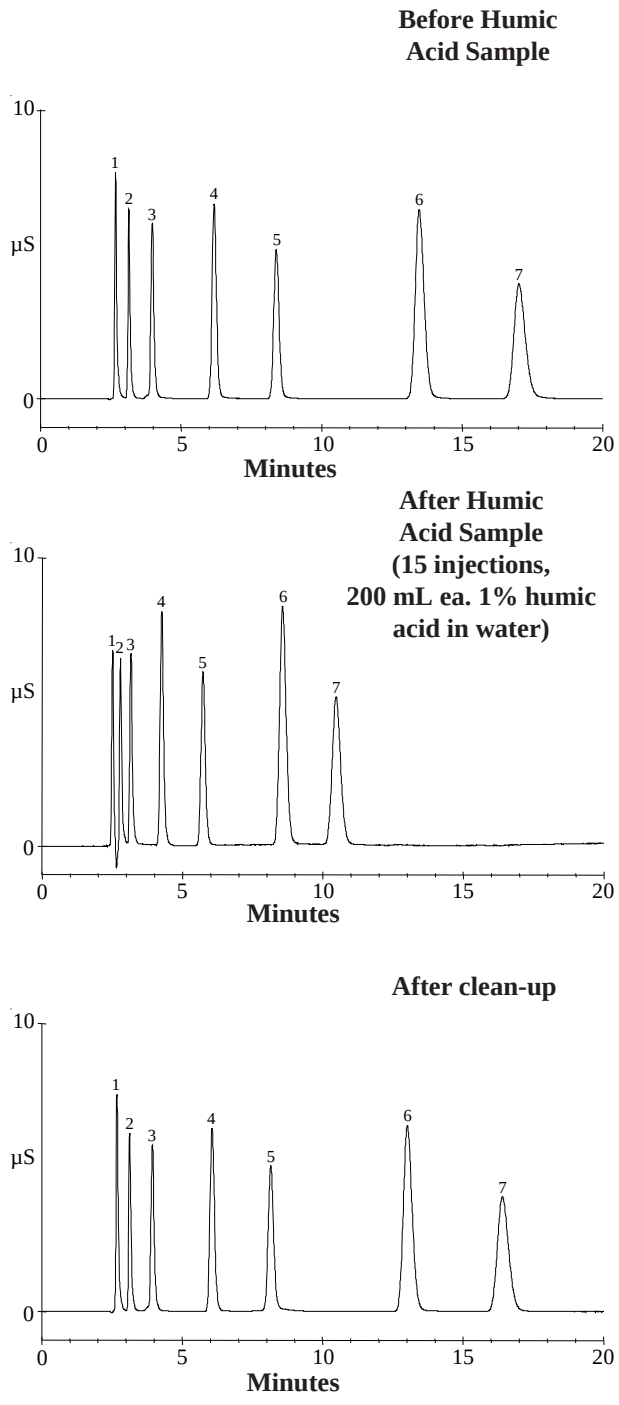


Figure 11
Clean-up after Humic Acid Samples

SECTION 6 - TROUBLESHOOTING GUIDE

The purpose of the Troubleshooting Guide is to help you solve operating problems that may arise while using IonPac AS16 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 North America Technical Call Center at 1-800-DIONEX-0 (1-800-346-6390) or the Dionex Office nearest you (see "Dionex Worldwide Offices").

Table 6
AS16/AG16 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
High Background Conductivity	Contaminated ATC-3	Clean Column	6.2.2
	Contaminated Eluents	Remake Eluents	6.2, 6.2.1
	Contaminated Columns	Clean Column	6.2.3
	Contaminated ASRS or AMMS	Clean Suppressor	6.2.4, Component Manual
	Contaminated Hardware	Clean Component	Component Manual
Poor Resolution	Poor Efficiency Due to Large System Void Volumes	Replumb System	6.3.1.A, Component Manual
	Column Headspace	Replace Column	6.3.1.B
Short Retention Times	Flow Rate Too fast	Recalibrate Pump	6.3.2.A
	Conc. Incorrect Eluents	Remake Eluents	6.3.2.B
	Column Contamination	Clean Column	6.3.2.C, 6.3.2.D
Poor Front End Resolution	Conc. Incorrect Eluents	Remake Eluents	6.3.3.A
	Column Overloading	Reduce Sample Size	6.3.3.B, 3.3.1, 3.3.2
	Sluggish Injection Valve	Service Valve	6.3.3.C, Component Manual
	Large System Void Volumes	Replumb System	6.3.3.D, Component Manual
Spurious Peaks	Sample Contaminated	Pretreat Samples	6.3.4.A, 6.3.4.B
	Sluggish Injection Valve	Service Valve	6.3.3.C, Component Manual

6.1 High Back Pressure

6.1.1 Finding the Source of High System Pressure

Total system pressure for the IonPac AG16 (4-mm) Guard Column plus the AS16 (4-mm) Analytical Column when using the test chromatogram conditions should be equal or less than 1,650 psi. If the system pressure is higher than 1,650 psi, it is advisable to determine the cause of the high system pressure. The system should be operated with a High-Pressure In-Line Filter (P/N 044105) which is positioned between the Gradient Pump pressure transducer and the injection valve. Make sure you have one in place and that it is not contaminated.

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

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

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

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

Table 7
Typical AS16/AG16 Operating Back Pressures

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

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.

Part	IonPac AS16 4-mm Columns (P/N)	IonPac AS16 2-mm Columns (P/N)
Analytical Column	055376	055378
Guard Column	055377	055379
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 High Background

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

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

6.2.1 Preparation of Eluents

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

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

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

6.2.2 A Contaminated Trap Column

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

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

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

6.2.3 Contaminated CR-ATC Column

Install a CR-TC Anion Trap Column (P/N 060477) if using an Eluent Generator with EGC II KOH or EGC II NaOH cartridge.

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

6.2.4 A Contaminated Guard or Analytical Column

A. Remove the IonPac AG16 Guard and AS16 Analytical Columns from the system.
B. Install a back pressure coil that generates approximately 1,500 psi and continue to pump eluent.

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

C. To eliminate downtime, clean or replace the AG16 at the first sign of column performance degradation.

• Clean the column as instructed in, “Column Cleanup” (See “Column Care”).

6.2.5 Contaminated Hardware

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

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

6.2.6 A Contaminated Suppressor

If the above items have been checked and the problem persists, the suppressor is probably causing the problem.

A. Check the eluent flow rate. In general, the eluent flow rate for 4-mm applications should be 1.5 mL/min. Refer to the Anion Self-Regenerating Suppressor Product Manual (Document No. 031367) for assistance in determining that the eluent is within supp ressible limits.

B. If the background is very high, (>1,000 µS) or the baseline noise is very high, the ASRS may have failed to suppress the eluent. You may need to replace the ASRS ULTRA suppressor.

C. If you are using eluents containing solvents, use the ASRS ULTRA in external water mode and flow rate should be 7–10 mL/min.

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

E. If you are using an AutoRegen Accessory with the SRS (in the Chemical Suppression Mode) or the MMS, prepare fresh regenerant solution. Test both the suppressor and the AutoRegen Regenerant Cartridge for contamination.

1. If the background conductivity is high after preparing fresh regenerant and bypassing the AutoRegen Regenerant Cartridge, you probably need to clean or replace your SRS or MMS.

2. If the background conductivity is low when freshly prepared regenerant is run through the SRS or MMS

without an AutoRegen Accessory in-line, test the AutoRegen Regenerant Cartridge to see if it is expended. Connect the freshly prepared regenerant to the AutoRegen Regenerant Cartridge. Pump approximately 200 mL of regenerant through the AutoRegen Regenerant Cartridge to waste before recycling the regenerant back to the regenerant reservoir. If the background conductivity is high after placing the AutoRegen Accessory in-line, you probably need to replace the AutoRegen Regenerant Cartridge. Refer to the "AutoRegen Regenerant Cartridge Refill Product Manual" (Document No. 032852) for assistance.

6.3 Poor Peak Resolution

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

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

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

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

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

6.3.1 Loss of Column Efficiency

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

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

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 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").

After cleaning the column, reinstall it in the system and let it equilibrate with eluent for about 60 minutes. No water wash is necessary. The column is equilibrated when consecutive injections of the standard give reproducible retention times. The original column capacity should be restored by this treatment, since the contaminants should be eluted from the column. If you need assistance in solving resolution problems, 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").

6.3.3 Loss of Front End Resolution

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

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

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

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

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

6.3.4 Spurious Peaks

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

If you need assistance in determining the best way to clean strongly retained solutes in your specific sample matrix from the IonPac AS16 columns, contact the North America Technical Call Center at 1-800-DIONEX-0 (1-800-346-6390) or the nearest Dionex Office ("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.

For DX-300 systems equipped with a Rheodyne Microinjection Valve, Model 9126 (Dionex P/N 044697), consult the accompanying manual for service instructions.

APPENDIX A - QUALITY ASSURANCE REPORT

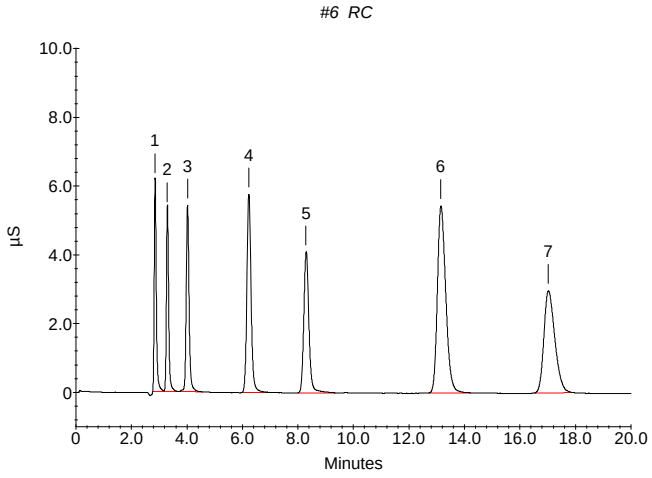
Quality Assurance Report - IonPac AS16 Analytical Column - 2-mm

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

A.1 Quality Assurance Report - IonPac AS16 Analytical and Guard Columns

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

Serial No. : #6 RC **Pressure (PSI) : 850** **Date : 5/16/03 7:40:42 AM**



Eluent: 35 mM NaOH
Flow Rate: 0.25 mL/min
Operating Temp: 30° C
Detection: Suppressed Conductivity
ASRS®-ULTRA, 2-mm
AutoSuppression® Recycle Mode

Injection Volume: 2.5 µL

Long-term Storage Solution (> 1 week): 100 mM Sodium Borate
Short-term Storage Solution (< 1 week): Eluent

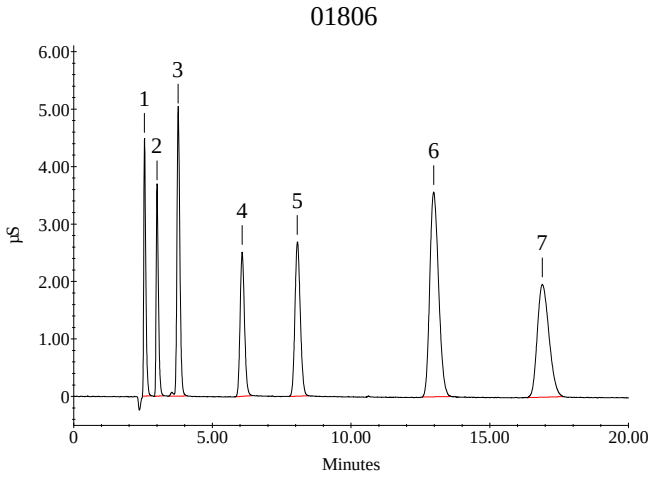
Peak Information : Found Components

Peak No.	Retention Time	Name	(mg/L)	Efficiency	Asymmetry (10%)	Resolution
1	2.85	Fluoride	2.0	6958	1.6	3.18
2	3.29	Chloride	3.0	7697	1.6	4.53
3	4.02	Sulfate	5.0	7980	1.3	9.66
4	6.23	Thiosulfate	10.0	8312	1.2	6.88
5	8.29	Iodide	20.0	10097	1.3	11.17
6	13.15	Thiocyanate	20.0	8616	1.5	6.12
7	17.02	Perchlorate	30.0	8095	1.6	n/a

A.2 Quality Assurance Report - IonPac AS16 Analytical and Guard Columns

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

Serial No. : 01806 **Pressure (PSI) : 1393** **Date : 3/13/01 11:55:37 AM**



Eluent: 35 mM NaOH
Flow Rate: 1.0 mL/min
Operating Temp: 30° C
Detection: Suppressed Conductivity
ASRS®-ULTRA
AutoSuppression® Recycle Mode

Injection Volume: 10 µL

Long-term Storage Solution (> 1 week): 100 mM Sodium Borate
Short-term Storage Solution (< 1 week): Eluent

Peak Information : Found Components

Peak No.	Retention Time	Name	(mg/L)	Efficiency	Asymmetry (10%)	Resolution
1	2.55	Fluoride	2.0	5308	2.1	3.19
2	3.00	Chloride	3.0	5398	2.0	4.48
3	3.76	Sulfate	5.0	5129	1.7	9.51
4	6.07	Thiosulfate	10.0	6851	1.3	6.13
5	8.06	Iodide	20.0	7874	1.3	10.37
6	12.98	Thiocyanate	20.0	7744	1.4	5.67
7	16.89	Perchlorate	30.0	7073	1.5	n/a

APPENDIX B - COLUMN CARE

B.1 Recommended Operating Pressure

Operating a column above its recommended pressure limit can cause irreversible loss of column performance. The maximum recommended operating pressure for IonPac AS16 columns is 4,000 psi (27.57 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 short-term storage (< 1 week), use Eluent, for long-term storage (> 1 week), use 100 mM Sodium Borate for the column storage solution. Flush the column for a minimum of 10 minutes with the storage solution. Cap both ends securely, using the plugs supplied with the column.

B.4 Column Cleanup

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

WARNING

•Always ensure that the cleanup protocol used does not switch between eluents which may create high pressure eluent interface zones in the column.

•High pressure zones can disrupt the uniformity of the packing of the column bed and irreversibly damage the performance of the column.

•High pressure zones in the column can be created by pumping successive eluents through the column that are not miscible, that have eluent components in one eluent that will precipitate out in the other eluent or by using an acid eluent followed by a base eluent which may create a neutralization pressure band.

•The precipitation of the salts in solvents during column rinses can result in very high pressure zones. High viscosity mixing zones can be created between two eluents having solvents with a very high energy of mixing.

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

B.4.1 Choosing the Appropriate Cleanup Solution

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

B.4.2 Column Cleanup Procedure

Use the following cleanup procedures to clean the AG16 and AS16.

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 AS16 Analytical Column.
c) If your system is configured with both a guard column and an analytical column, reverse the order of the guard and analytical column in the eluent flow path.
d) Double check that the eluent flows in the direction designated on each of the column labels.

CAUTION

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

e) Set the pump flow rate to 1.0 mL/min for an AS16 4-mm Analytical or Guard Column or set the pump flow rate to 0.25 mL/min for an AS16 2-mm Analytical or Guard Column.
f) Rinse the column for 10 minutes with deionized water before pumping the chosen cleanup solution over the column.
g) Pump the cleanup solution through the column for at least 60 minutes.
h) Rinse the column for 10 minutes with deionized water before pumping eluent over the column.
i) Equilibrate the column(s) with eluent for at least 60 minutes before resuming normal operation.
j) Reconnect the ASRS ULTRA II or AMMS III to the AS16 Analytical Column.
k) Place the guard column in line between the injection valve and the analytical column if your system was originally configured with a guard column.

APPENDIX C - CONFIGURATION

Table 1
Configuration

CONFIGURATION	2-mm	4-mm
Eluent Flow Rate	0.25 mL/min	1.0 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)
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 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. Note: The GPM-2 should not be used for 2-mm gradient chromatography.	Use the 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) 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-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) 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) can be used with CDM-2 or CDM-3 for 4-mm operation. Recommended back pressure: 30–40 psi

Table 2
Tubing Back Pressures

Color	Dionex P/N	ID Inches	ID cm	Volume mL/ft	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	0.137	0.086	0.021	0.003
Orange	042855	0.020	0.051	0.061	0.435	0.109	0.015
Blue	049714	0.013	0.033	0.026	2.437	0.609	0.081
Black	042690	0.010	0.025	0.015	6.960	1.740	0.232
Red	044221	0.005	0.013	0.004	111.360	27.840	3.712
Yellow	049715	0.003	0.008	0.001	859.259	214.815	28.642