PRODUCT MANUAL

IonPac® AS11
IonPac® AG11

DIONEX

IC | HPLC | MS | EXTRACTION | PROCESS | AUTOMATION

PRODUCT MANUAL

for the

IONPAC® AG11 GUARD COLUMN

(2 x 50 mm, P/N 044079)
(4 x 50 mm, P/N 044078)

IONPAC® AS11 ANALYTICAL COLUMN

(2 x 250 mm, P/N 044077)
(4 x 250 mm, P/N 044076)

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SECTION 1 - INTRODUCTION

The lonPac® AS11 2-mm (P/N 044077) and 4-mm (P/N 044076) Analytical Columns are specifically designed to resolve a large number of inorganic anions and organic acid anions from a single sample injection in one gradient run using hydroxide eluent systems. Strongly retained trivalent ions, such as phosphate and citrate, are efficiently eluted in the same run that also gives baseline resolution of the weakly retained monovalent anions fluoride, acetate, formate, and pyruvate. Another benefit of using the AS11 column is the ability to easily change the order of elution of ions with different valences simply by changing the gradient profile. For example, if chromate is present in high enough concentration to interfere with citrate, the citrate peak can be moved ahead of the chromate peak by using a slightly different gradient. The AS11 column provides rapid elution of strongly retained ions such as iodide, thiocyanate, thiosulfate, and chromate using hydroxide/methanol eluents. The selectivity of the AS11 column makes it possible to rapidly elute strongly retained species such as iodide and thiocyanate in brines without interference from the large chloride peak typical of such samples. Hydroxide is normally used for gradient elution to minimize background shift. Because of high background conductance, sodium carbonate/bicarbonate eluents are not appropriate for gradient analysis but can be used for isocratic applications. AS11 columns are stable between pH 0 and 14 and are compatible with eluents containing 0-100% organic solvents.

Table 1
IonPac AS11/AG11 Packing Specifications

Column	Particle Diameter µm	Substrate[a] X-linking %	Latex Diameter nm	Latex[b] X-Linking %	Column Capacity µeq/column	Functional Group	Hydrophobicity
AS11 4 x 250 mm	13	55	85	6	45	Alkanol quaternary ammonium	Very Low
AG11 4 x 50 mm	13	55	85	6	9	Alkanol quaternary ammonium	Very Low
AS11 2 x 250 mm	13	55	85	6	11	Alkanol quaternary ammonium	Very Low
AG11 2 x 50 mm	13	55	85	6	2.2	Alkanol quaternary ammonium	Very Low

[a] macroporous (2,000 Å) divinylbenzene/ethylvinylbenzene polymer
[b] microporous polyvinylbenzylammonium polymer cross-linked with divinylbenzene

Table 2
AS11/AG11 Operating Parameters

Column	Typical Back Pressure psi (MPa)	Standard Flow Rate mL/min	Maximum Flow Rate mL/min
AS11 4-mm Analytical	≤ 800 (5.51)	1.0	3.0
AG11 4-mm Guard	≤ 300 (2.07)	1.0	3.0
AS11 + AG11 4-mm columns	**≤ 1,100 (7.58)**	**1.0**	**3.0**
AS11 2-mm Analytical	≤ 800 (5.51)	0.25	0.75
AG11 2-mm Guard	≤ 300 (2.07)	0.25	0.75
AS11 + AG11 2-mm columns	**≤ 1,100 (7.58)**	**0.25**	**0.75**

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

SECTION 2 - COMPARISON OF 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 applications.

- 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 B, "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.

NOTE: Do not operate suppressors over 40 C. If application requires a higher temperature, place the suppressor outside of the chromatographic oven. Use of an EG40 (P/N 053920) or EG50 (P/N 060585) with an EGC II KOH (P/N 058900) or EGC II NaOH (P/N 058908) cartridge for gradient applications is highly recommended for minimum baseline change when performing eluent step changes or gradients.

SECTION 3 - INSTALLATION

3.1 System Requirements

3.1.1 System Requirements for 2-mm Operation

The IonPac AS11 2-mm Guard and Analytical Columns are designed to be run on any Dionex Ion Chromatograph equipped with suppressed conductivity detection. Gradient or isocratic methods should be performed on a gradient pump configured for narrow bore operation.

3.1.2 System Requirements for 4-mm Operation

The IonPac AS11 4-mm Guard and Analytical Columns are designed to be run on any Dionex Ion Chromatograph equipped with suppressed conductivity detection. Gradient or isocratic methods should be performed on a system having a gradient pump configured for standard bore operation.

3.2 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 void volume in a standard 4-mm system. For best performance, all of the tubing installed between the injection valve and detector should be 0.005" (P/N 044221) ID PEEK tubing. 0.010" ID PEEK tubing (P/N 042260) or 0.012" Tefzel tubing (see, “Dionex Product Selection Guide”) may be used but peak efficiency will be compromised which may also result in decreased peak resolution. Minimize the lengths of all connecting tubing and remove all unnecessary switching valves and couplers. If you need assistance in properly configuring your system contact the nearest Dionex Office (see, “Dionex Worldwide Offices”).

3.3 The AG11 Guard Columns

An IonPac AG11 Guard Column is normally used with the IonPac AS11 Analytical Column. Retention times will increase by approximately 20% when a guard column is placed in-line prior to the analytical column. A guard is placed prior to the analytical column to prevent sample contaminants from eluting onto the analytical column. It is easier to clean or replace a guard column than it is an analytical column. Replacing the AG11 Guard Column at the first sign of peak efficiency loss or decreased retention time will prolong the life of the AS11 Analytical Column.

3.4 The Sample Concentrator

The Low Pressure Trace Anion Concentrator Column (TAC-LP1, P/N 046026), Ultra Low Pressure Trace Anion Concetrator Column (TAC-ULP1, P/N 061400), or the IonPac AG11 Guard Column can be used for trace anion concentration work. 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 function of the TAC-LP1, TAC-ULP1, UTAC-LP1, UTAC-ULP1, UTAC-XLP1, or the AG11 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 AG11 leading to a lowering of detection limits by 2-5 orders of magnitude. 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. Concentrating an excessive amount of sample can result in inaccurate results being obtained. It is possible during the concentration step for the polyvalent anions such as phosphate and sulfate to elute the weakly retained anions such as fluoride and acetate off the concentrator column. The unique advantage to the analytical chemist of the TAC-LP1, TAC-ULP1, UTAC-LP1, UTAC-ULP1, UTAC-XLP1, or the AG11 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 more detailed information on sample concentration techniques for high sensitivity work refer to Section 3, “Operation,” of theTAC-LP1 and TAC-ULP1 Column Product Manual (Document No. 034972) or Section 3, “Operation,” of the UTAC-LP1, 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 AS11. Use the TAC-LP1, TAC-ULP1, UTAC-LP1, UTAC-ULP1, UTAC-XLP1, or AG11 guard columns.

3.5 Installing the CR-ATC Anion Trap Column for Use with EGC II KOH Cartridge

For IonPac AS11 applications using the EG40 or EG50 with EGC II KOH 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 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 3.6.

3.6 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 gradient pump and the injection valve. The ATC-3 column is used when performing sodium hydroxide gradient anion exchange applications using hand-prepared bottled eluents. The ATC-3 Trap Column will require off-line regeneration.

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).

3.7 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

3.7.1 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. Dionex recommends that a 2.5 µL injection loop be used to avoid overloading the AS11 2-mm Analytical Column. Generally, you should not inject more than 2.5 nanomoles (25–50 ppm) of any one analyte onto a 2-mm analytical column. Injecting larger volumes of samples can result in overloading the column which can affect the detection linearity. The AS11 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 (see Section 2, “Comparison of Ion Chromatography Systems”).

3.7.2 The 4-mm System Injection Loop, 10–50 µL

For most applications on a 4-mm analytical system, a 10–50 µL injection loop will be sufficient. Dionex recommends that a 10 µL injection loop be used to avoid overloading the AS11 4-mm Analytical Column. Generally, do not inject more than 10 nanomoles (100–200 ppm) of any one analyte onto the 4-mm analytical column. Injecting larger volumes of samples can result in overloading the column which can affect the detection linearity. This phenomenon will be more prevalent at higher concentrations of the analytes of interest.

3.8 Eluent Storage

IonPac AS11 columns are designed to be used with sodium 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 IonPac AS11 column performance.

3.9 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® modes of operation.

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

If you are installing an IonPac AS11 4-mm Analytical Column, use an ASRS ULTRA (4-mm, P/N 053946).

If you are installing an IonPac AS11 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, the ASRS ULTRA."

3.10 Anion MicroMembrane Suppressor Requirements

An Anion MicroMembrane Suppressor (AMMS® III) may be used instead of an ASRS ULTRA for applications that require suppressed conductivity detection. Use an AMMS III (P/N 057750) with the IonPac AS11 4-mm Analytical Column and an AMMS III 2-mm (P/N 057751) with the IonPac AS11 2-mm Analytical Column. It is compatible with all solvents and concentrations with which the systems and columns are compatible. 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."

3.11 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.

3.12 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 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).

3.13 Detector Requirements

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

3.14 Using the EG50 or EG40 with AS11

Please refer to the EG50 Product Manual (Document No. 031908) or to 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: AS11 2-mm Analytical Column + AG11 2-mm Guard Column 4-mm: AS11 4-mm Analytical Column + AG11 4-mm Guard Column
Eluent:	12 mM NaOH
Eluent Flow Rate:	2-mm: 0.25 mL/min 4-mm: 1.0 mL/min
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS ULTRA (4-mm or 2-mm) AutoSuppression Recycle Mode
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III (4-mm or 2-mm)
MMS Regenerant:	50 mN H_2SO_4
Expected Background Conductivity:	$\leq$ 8 µS
Storage Solution:	Eluent (12 mM NaOH)

4.2 IonPac AS11 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
0.75 mL/min Maximum Flow Rate for 2-mm Columns

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 Solvents

The AS11 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. 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. As Figure 1 shows, 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 AS11 columns is 4,000 psi (27.57 MPa).

CAUTION

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

4.4.1 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 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.

SAFETY

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

Table 5
HPLC Solvents for Use with IonPac AS11 Columns

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

4.5 Making Eluents

4.5.1 Making Sodium Hydroxide Eluents

Sodium Hydroxide Eluents

Dilute the amount of 50% (w/w) NaOH (in water) specified in Table 6, "Dilution of 50% (w/w) NaOH to Make Standard AS11 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 or the deionized water being used to make the eluent. Do not shake the 50% (w/w) NaOH or pipette the required aliquot from the top of the solution where sodium carbonate may have formed.

When formulating eluents from 50% sodium hydroxide, Dionex recommends weighing out the required amount of 50% sodium hydroxide.

Example: To make 1 L of 12 mM NaOH use 0.96 g of 50% sodium hydroxide:
(as used in Section 5.1, "Production Test Chromatogram")

$$\text{For 12 mM:} \quad \frac{0.012 \text{ mole/L} \times 40.01 \text{ g/mole}}{50\%} = 0.96 \text{ g diluted to 1 L}$$

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)
ρ = % purity of the concentrated solution

Example: To make 1 L of 12 mM NaOH use 0.63 mL of 50% sodium hydroxide:
(as used in Section 5.1, "Production Test Chromatogram")

$$\text{For 12 mM:} \quad \frac{0.012 \text{ mole/L} \times 40.01 \text{ g/mole}}{50\% \times 1.53 \text{ g/mL}} = 0.63 \text{ mL diluted to 1 L}$$

The sodium hydroxide eluents that can be used with the IonPac AS11 columns will readily absorb carbon dioxide, producing carbonate. Precautions must be taken during eluent preparation to minimize contamination with carbon dioxide from the air. These precautions, if taken, ensure smooth, reproducible ramps, with 1 to 5 µS total change in background conductivity.

The eluents can be prepared either volumetrically using a syringe or by weighing. Using a syringe is more effective in preventing carbonate contamination while preparing the eluents. If you decide to use the weighing method, pipette, do not pour, the 50% sodium hydroxide into the weighing dish. Minimize the time that the solution is exposed to air.

Table 6

Dilution of 50% (w/w) NaOH to Make Standard AS11 Eluents

50% (w/w) NaOH g (mL)	Concentration of NaOH Eluent (mM)
0.08 (0.0525)	1
0.40 (0.262)	5
0.96 (0.63)	12
8.00 (5.25)	100
16.00 (10.5)	200

4.6 Regenerant Preparation for the AMMS III

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 (ASRS ULTRA) see Document No. 034449-02, the "Product Manual for the Anion MicroMembrane Suppressor III, the AMMS III."

SECTION 5 - EXAMPLE APPLICATIONS

The chromatograms in this section were obtained using columns that reproduced the Production test Chromatogram (see Section 5.1, "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 AS11 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.

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."

The addition of chromate to the sample will help stabilize organic acids. If your sample or standard contains organic acids, adding chromate (about 10 mg/L) will help stabilize them from bacterial degradation at room temperature. The sample chromatograms in Sections 5.2, "Gradient Elution of a Large Number of Inorganic and Organic Acid Anions," (see Figure 2, "Separation of Mono-, Di-, and Trivalent Anions in One Sample Run").

Install an Anion Trap Column, ATC-3 (4-mm) or the ATC-3 (2-mm) in the system. See Section 3.6, "The Anion Trap Column," to guarantee reproducible retention times of analytes when doing gradient chromatography.

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 3.4, "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.

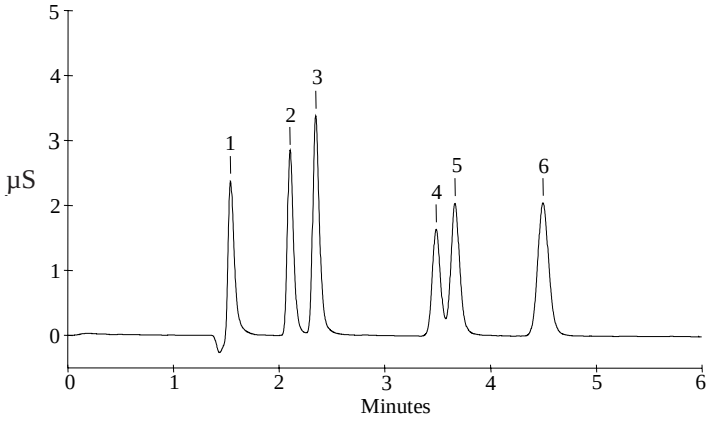
5.1 Production Test Chromatogram

Isocratic elution of anions on the IonPac AS11 Analytical Column has been optimized utilizing an hydroxide eluent. By using this eluent, common inorganic anions can be isocratically separated and quantitated in a single injection. To guarantee that all IonPac AS11 2-mm and 4-mm Analytical Columns meet high quality and reproducible performance specification standards, all columns undergo the following production control test.

Sample Loop Volume:	2-mm: 2.5 µL 4-mm: 10 µL
Analytical Column:	IonPac AS11 Analytical Column
Eluent:	12 mM Sodium Hydroxide
Eluent Flow Rate:	2-mm: 0.25 mL/min 4-mm: 1.0 mL/min
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS ULTRA (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:	≤ 2 µS
Expected System Operating Back Pressure:	< 800 psi (5.51 MPa)
Storage Solution:	Eluent, 12 mM Sodium Hydroxide

	Analyte	mg/L
1.	Fluoride	0.5
2.	Chloride	1.0
3.	Nitrite	2.0
4.	Bromide	2.0
5.	Nitrate	2.0
6.	Sulfate	2.0

where 1 mg/L = 1 ppm



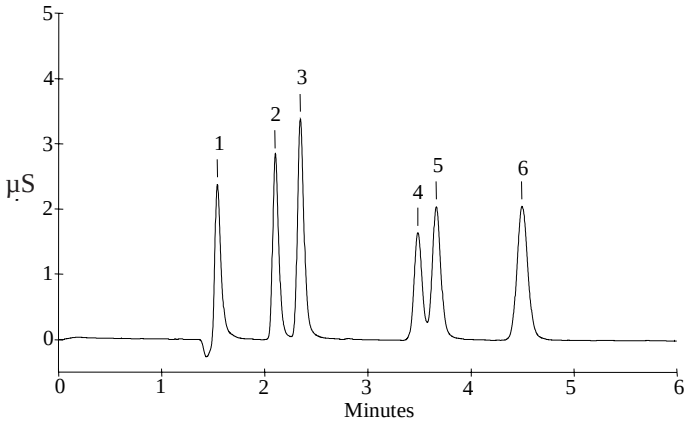


Figure 1
IonPac AS11 Production Test Chromatogram

5.2 Gradient Elution of a Large Number of Inorganic Anions and Organic Acid Anions

A large number of inorganic anions and organic acid anions can be separated on the IonPac AS11 using gradient elution. The sodium hydroxide concentration in Eluent 1 (E1) is weak enough that fluoride elutes after the void volume. E1 will also separate several weakly retained monovalent organic acids. The sodium hydroxide concentration in Eluent 2 will elute polyvalent ions such as trivalent phosphate, citrate, and cis- and trans-aconitate. See Section 4.5, "Making Eluents," for eluent preparation instructions.

One of the unique features of the AS11 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 3 to 7 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.

The gradient shown in the example can be adjusted to improve resolution or to adjust retention times either by changing the gradient timing or by changing the gradient eluent proportions.

NOTE

If an injection is made before the column is fully equilibrated with E1, the early-eluting peaks (fluoride and the monoprotic organic acids) will elute too soon and resolution may be impaired. Furthermore, retention times will not be reproducible.

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 that changing the gradient can affect the elution order of ions of different charge. For example, increasing the gradient ramp slope will cause citrate to elute earlier than chromate.

Sample Loop Volume:	2-mm: 2.5 µL 4-mm: 10 µL	
Trap Column:	Anion Trap Column (ATC-3)	
Guard Column:	IonPac AG11 Guard Column	
Analytical Column:	IonPac AS11Analytical Column	
Eluents	E1:	Type I Deionized Water
	E2:	5.0 mM NaOH
	E3:	100 mM NaOH
Eluent Flow Rate:	2-mm: 0.5 mL/min 4-mm: 2.0 mL/min	
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS ULTRA (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:	0.5 mM NaOH: ≤ 1 µS 35 mM NaOH: ≤ 3.5 µS	
Expected System Operating Back Pressure:	Without Guard: 1,600 psi (11.03 MPa) With Guard: 1,850 psi (12.75 MPa)	

The addition of the AG11 to the AS11 increases the column capacity by 20%. Retention times in isocratic runs will increase by approximately 20%. All times in gradient schedules for analytical columns must be increased by 20% when the AG11 is added to the system. Figure 2, "Separation of Mono-, Di-, and Trivalent Anions in One Sample Run," demonstrates how gradient run schedules must be changed to adjust for the increase in capacity of the column set when the AG11 Guard column is added to the system.

Gradient Conditions With Guard

Time (min)	%E1	%E2	%E3	Comments
Equilibration				
0	90	10	0	0.5 mM NaOH for 7 min
7.0	90	10	0	
Analysis				
0.0	90	10	0	0.5 mM NaOH, Inject
0.2	90	10	0	Inject Valve to Load Position
2.5	90	10	0	0.5-5.0 mM NaOH in 3.5 min
6.0	0	100	0	5.0-38.25 mM NaOH in 12 min
18.0	0	65	35	

Gradient Conditions Without Guard

TIME (min)	%E1	%E2	%E3	Comments
Equilibration				
0	90	10	0	0.5 mM NaOH for 7 min
7.0	90	10	0	
Analysis				
0.0	90	10	0	0.5 mM NaOH, Inject
0.2	90	10	0	Inject Valve to Load Position
2.0	90	10	0	0.5-5.0 mM NaOH in 3 min
5.0	0	100	0	5.0-38.25 mM NaOH in 10 min
15.0	0	65	35	

NOTE

Seven minutes are required at the beginning of this program for equilibration of the AS11 with E1 prior to injecting the next sample. If the system is not used continuously, that is, the run program (equilibration plus analysis) is not started exactly every 22 minutes (without AG11) or 25 minutes (with AG11), the run program can be modified to start with 2 minutes of the highest eluent concentration for regeneration and then to equilibrate with E1 for 7 minutes with the next injection 9 minutes into the program.

All anion concentrations are 5 mg/L unless noted

		mg/L
1.	Isopropyl methylphosphonate	
2.	Quinate	
3.	Fluoride	1
4.	Acetate	
5.	Propionate	
6.	Formate	
7.	Methylsulfonate	
8.	Pyruvate	
9.	Chlorite	
10.	Valerate	
11.	Monochloroacetate	
12.	Bromate	
13.	Chloride	2
14.	Nitrite	
15.	Trifluoroacetate	
16.	Bromide	3
17.	Nitrate	3
18.	Chlorate	3
19.	Selenite	
20.	Carbonate	trace
21.	Malonate	
22.	Maleate	
23.	Sulfate	
24.	Oxalate	
25.	Ketomalonate	
26.	Tungstate	10
27.	Phthalate	10
28.	Phosphate	10
29.	Chromate	10
30.	Citrate	10
31.	Tricarballylate	10
32.	Isocitrate	10
33.	cis-Aconitate	10
34.	trans-Aconitate	

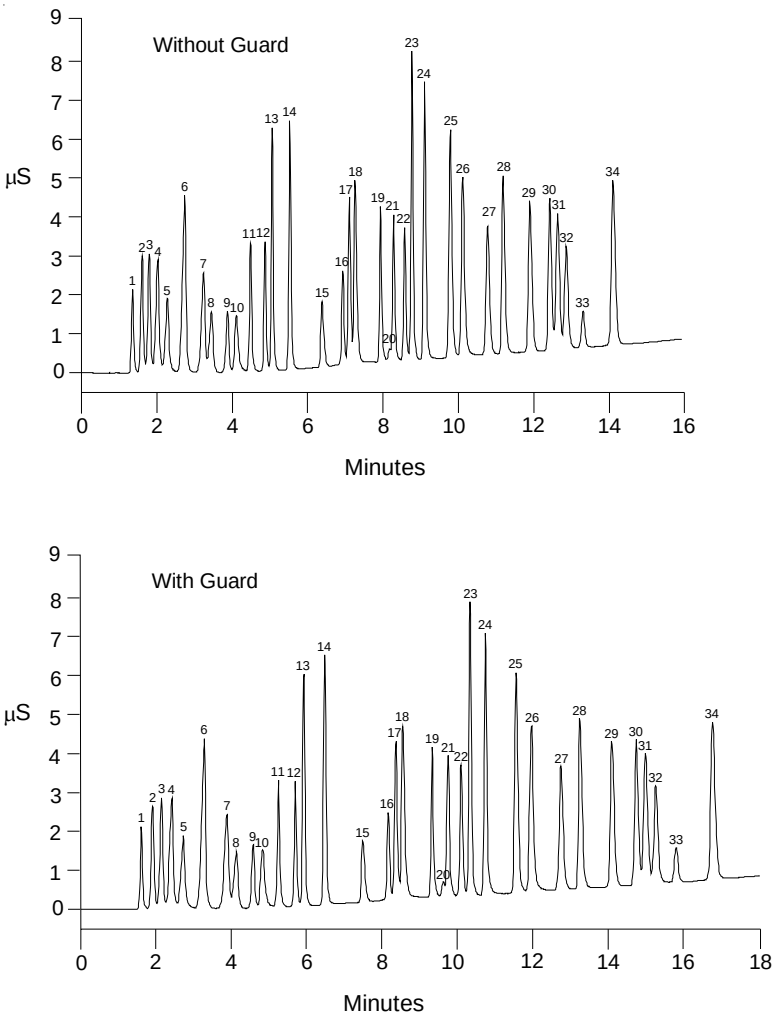


Figure 2
Separation of Mono-, Di-, and Trivalent Anions in One Sample Run

If resolution is a problem, consider these possibilities before changing the gradient to improve resolution:

A. Make sure that eluents E1 and E2 have been prepared correctly.

B. Check the eluent flow rate.

C. The column capacity may be different from that of the column used to obtain the sample chromatogram. In this case it may be necessary to adjust the gradient to provide the desired resolution.

5.3 Using the EluGen EGC-KOH Cartridge for IonPac AS11 Hydroxide Gradients

This application demonstrates the comparison of an IonPac AS11 gradient separation using conventional gradient pump delivery with the gradient separation using EG40 gradient delivery. Figure 4, "Conventional Hydroxide Gradient on the IonPac AS11," illustrates the use of a conventional pump method. Since the EG40 is located close to the injection valve, the gradient reaches the head of the column more quickly resulting in a shift in the gradient as shown in Figure 5, "EluGen EGC-KOH Gradient on the IonPac AS11." Figure 5 illustrates the use of the EG40 with an identical gradient program using the default OFFSET VOLUME of 0 µL. In Figure 6, "EluGen EGC-KOH Gradient on the IonPac AS11 (OFFSET VOLUME = 400 µL)," the default value for the OFFSET VOLUME (400 µL) is used. The Software uses this value to delay the EG40 gradient program by 0.2 minutes (0.400 mL/2 mL per minute). Note that the baseline shift using the gradient pump is approximately 1.5 µS. Using the EG40 to generate carbonate-free hydroxide reduces the baseline shift to approximately 50 nS.

Trap Column:	ATC-3, (Located between pump and injection valve)
Sample Volume:	10 µL
Column:	IonPac AS11 Analytical and AG11 Guard (4-mm)
Eluent:	See table of conditions
Eluent Flow Rate:	2.0 mL/min
Suppressor:	Anion Self-Regenerating Suppressor, ASRS ULTRA (4-mm) AutoSuppression® Recycle Mode (300 mA)
Expected Background Conductivity:	
(GP40 or GP50)	0.5 mM NaOH: ≤ 1 µS 35 mM NaOH: ≤ 2.5 µS
(EG40)	0.5 mM NaOH: ≤ 0.7 µS 35 mM NaOH: ≤ 0.75 µS
Typical Operating Back Pressure:	
(GP40 or GP50)	1850 psi (12.75 MPa)
(EG40)	2200 psi (15.15 MPa) Pressure Restrictor, (P/N 53762) was used with the EG40

	Analyte	mg/L(ppm)
1.	Quinate	5
2.	Fluoride	1
3.	Acetate	5
4.	Propionate	5
5.	Formate	5
6.	Methylsulfonate	5
7.	Pyruvate	5
8.	Valerate	5
9.	Monochloroacetate	5
10.	Bromate	5
11.	Chloride	2
12.	Nitrite	5
13.	Trifluoroacetate	5
14.	Bromide	3
15.	Nitrate	3
16.	Chlorate	3
17.	Selenite	5
18.	Carbonate	trace
19.	Malonate	5
20.	Maleate	5
21.	Sulfate	5
22.	Oxalate	5
23.	Tungstate	10
24.	Phthalate	10
25.	Phosphate	10
26.	Chromate	10
27.	Citrate	10
28.	Tricarballylate	10
29.	Isocitrate	10
30.	cis-Aconitate	10
31.	trans-Aconitate	

GP40 Conditions

E1: Deionized water
E2: 5.0 mM NaOH
E3: 100 mM NaOH

Time (min)	%E1	%E2	%E3	Comments
Equilibration				
0	90	10	0	0.5 mM NaOH for 7 min
7.0	90	10	0	
Analysis				
0.0	90	10	0	0.5 mM NaOH, Inject
0.2	90	10	0	Inject Valve to Load Position
2.5	90	10	0	0.5-5.0 mM NaOH in 3.5 min
6.0	0	100	0	5.0-38.25 mM NaOH in 12 min
18.0	0	65	35	

EG40 Conditions

Eluent: Deionized water

Time (min)	Eluent Conc. (mM)	Comments
Equilibration		
0	0.5	0.5 mM KOH for 7 min
7.0	0.5	
Analysis		
0.0	0.5	0.5 mM KOH, Inject
0.2	0.5	Inject Valve to Load Position
2.5	0.5	0.5-5.0 mM KOH in 3.5 min
6.0	5.0	5.0-38.3 mM KOH in 12 min
18.0	38.3	

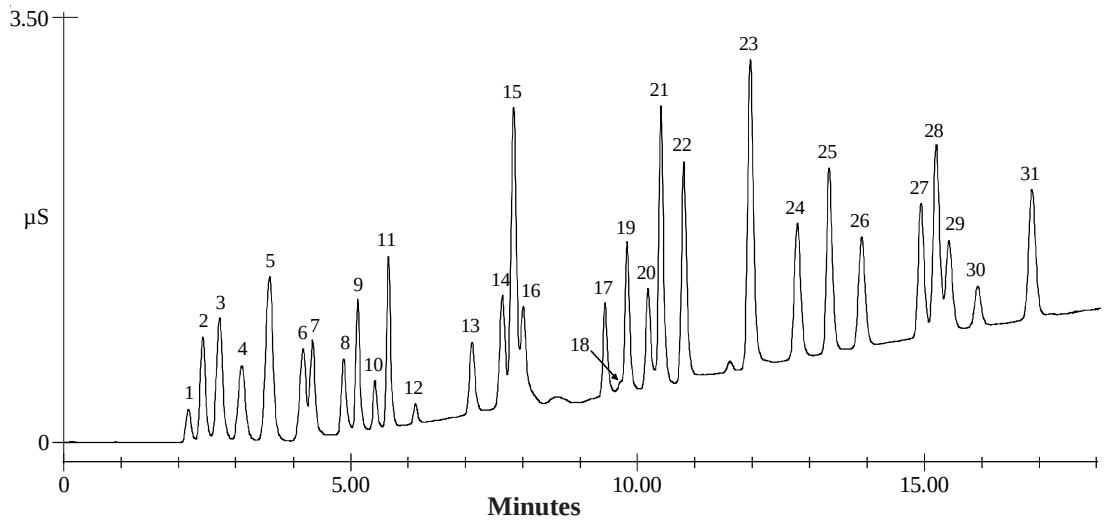


Figure 4
Conventional Hydroxide Gradient on the IonPac AS11

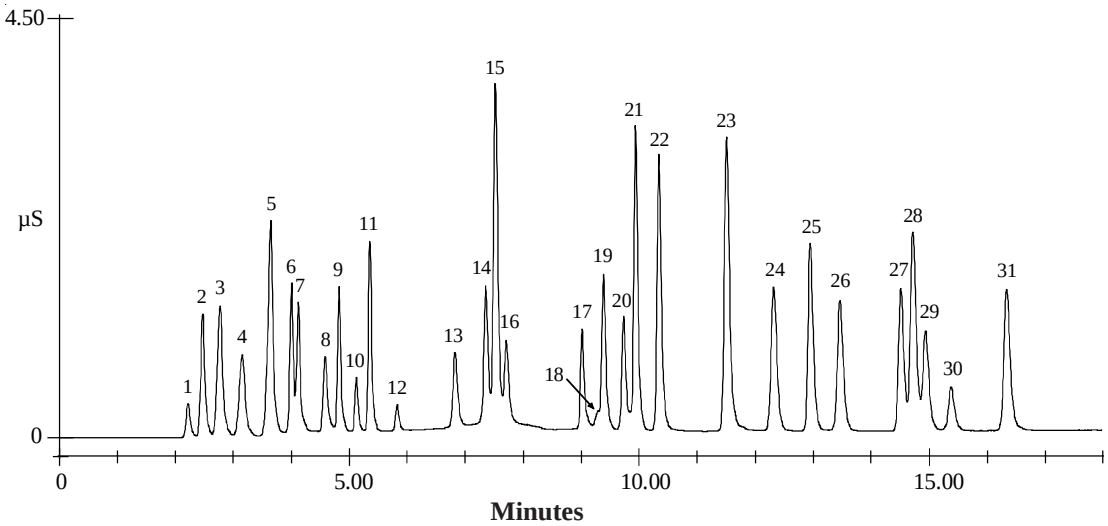


Figure 5
EluGen EGC-KOH Gradient on the IonPac AS11 (OFFSET VOLUME = 0 µL)

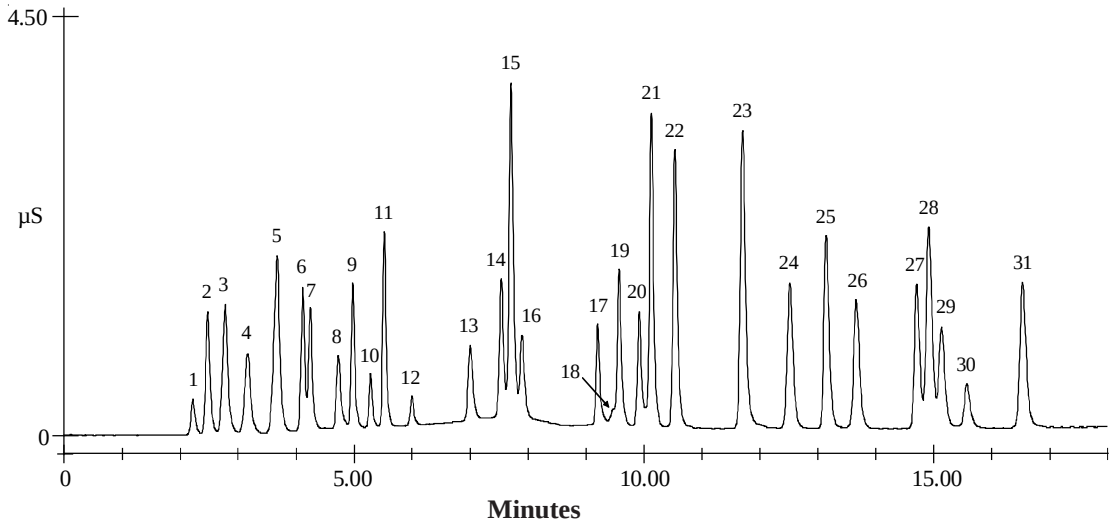


Figure 6
EluGen EGC-KOH Gradient on the IonPac AS11 (OFFSET VOLUME = 400 µL)

5.4 How System Void Volume Affects the Length of Time Required for Equilibration to the First Eluent

The following application demonstrates the affect of the system void volume on the time required to equilibrate the column to the first eluent. The following three 2-mm chromatograms use the same method used in Section 5.3, "Gradient Elution of a Large Number of Inorganic and Organic Acid Anions." Compare them to the 4-mm chromatogram ("Without Guard") in Figure 2, "Separation of Mono-, Di, and Trivalent Anions in One Sample Run." All three of the following 2-mm chromatograms use 2-mm columns and hardware and the same Analysis Gradient Program. The equilibration time to the first eluent must be varied depending on the system void volume.

NOTE

If an injection is made before the column is fully equilibrated with E1, the early-eluting peaks (fluoride and the monoprotic organic acids) will elute too soon and resolution may be impaired. Furthermore, retention times will not be reproducible.

The top chromatogram is performed on a Dionex Microbore System (1/16" pistons) having minimized void volume. The time of equilibration to the first eluent is 7 min. The Analysis Gradient Program in the method is the same for all three chromatograms. This chromatogram demonstrates equivalent performance to the AS11 4-mm Analytical Column when run on a Standard-Bore system with 1/8" pistons at 2.0 mL/min.

The middle chromatogram is performed on a Dionex Standard-Bore System (1/8" pistons) having a significant larger void volume. The time of equilibration to the first eluent is 7 min. The Analysis Gradient Program in the method is the same for all three chromatograms. Note that there is a significant loss of resolution of the early-eluting anions compared to the first chromatogram.

The bottom chromatogram is performed on the same Dionex Standard-Bore System (1/8" pistons) used to perform the second chromatogram. The time of equilibration to the first eluent is extended to 20 min. The Analysis Gradient Program in the method is the same for all three chromatograms. The extended equilibration time restored the resolution of the early-eluting anions.

Sample Loop Volume:	2-mm: 2.5 µL
Trap Column:	Anion Trap Column (ATC-3, 2-mm)
Analytical Column:	IonPac AS11
Eluents	E1: Type I Deionized Water E2: 5.0 mM NaOH E3: 100 mM NaOH
Eluent Flow Rate:	2-mm: 0.5 mL/min
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS ULTRA (2-mm) AutoSuppression Recycle Mode
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III (2-mm)
MMS Regenerant:	50 mN H_2SO_4
Expected Background Conductivity:	0.5 mM NaOH: ≤ 1 µS 35 mM NaOH: ≤ 3.5 µS
Expected System Operating Back Pressure:	1,600 psi (11.03 MPa)

Gradient Conditions with a 7-Minute Equilibration Time

TIME (min)	%E1	%E2	%E3	Comments
Equilibration				
0	90	10	0	0.5 mM NaOH for 7 min
7.0	**90**	**10**	**0**	
Analysis				
0.0	90	10	0	0.5 mM NaOH, Inject
0.2	90	10	0	Inject Valve to Load Position
2.0	90	10	0	0.5-5.0 mM NaOH in 3 min
5.0	0	100	0	5.0-38.25 mM NaOH in 10 min
15.0	0	65	35	

Gradient Conditions with a 20-Minute Equilibration Time

TIME (min)	%E1	%E2	%E3	Comments
Equilibration				
0	90	10	0	0.5 mM NaOH for 7 min
20.0	**90**	**10**	**0**	
Analysis				
0.0	90	10	0	0.5 mM NaOH, Inject
0.2	90	10	0	Inject Valve to Load Position
2.5	90	10	0	0.5-5.0 mM NaOH in 3.5 min
6.0	0	100	0	5.0-38.25 mM NaOH in 12 min
18.0	0	65	35	

All anion concentrations are 5 mg/L unless noted

		mg/L
1.	Isopropyl methylphosphonate	
2.	Quinate	
3.	Fluoride	1
4.	Acetate	
5.	Propionate	
6.	Formate	
7.	Methylsulfonate	
8.	Pyruvate	
9.	Chlorite	
10.	Valerate	
11.	Monochloroacetate	
12.	Bromate	
13.	Chloride	2
14.	Nitrite	
15.	Trifluoroacetate	
16.	Bromide	3
17.	Nitrate	3
18.	Chlorate	3
19.	Selenite	
20.	Carbonate	trace
21.	Malonate	
22.	Maleate	
23.	Sulfate	
24.	Oxalate	
25.	Ketomalonate	
26.	Tungstate	10
27.	Phthalate	10
28.	Phosphate	10
29.	Chromate	10
30.	Citrate	10
31.	Tricarballylate	10
32.	Isocitrate	10
33.	cis-Aconitate	10
34.	trans-Aconitate	

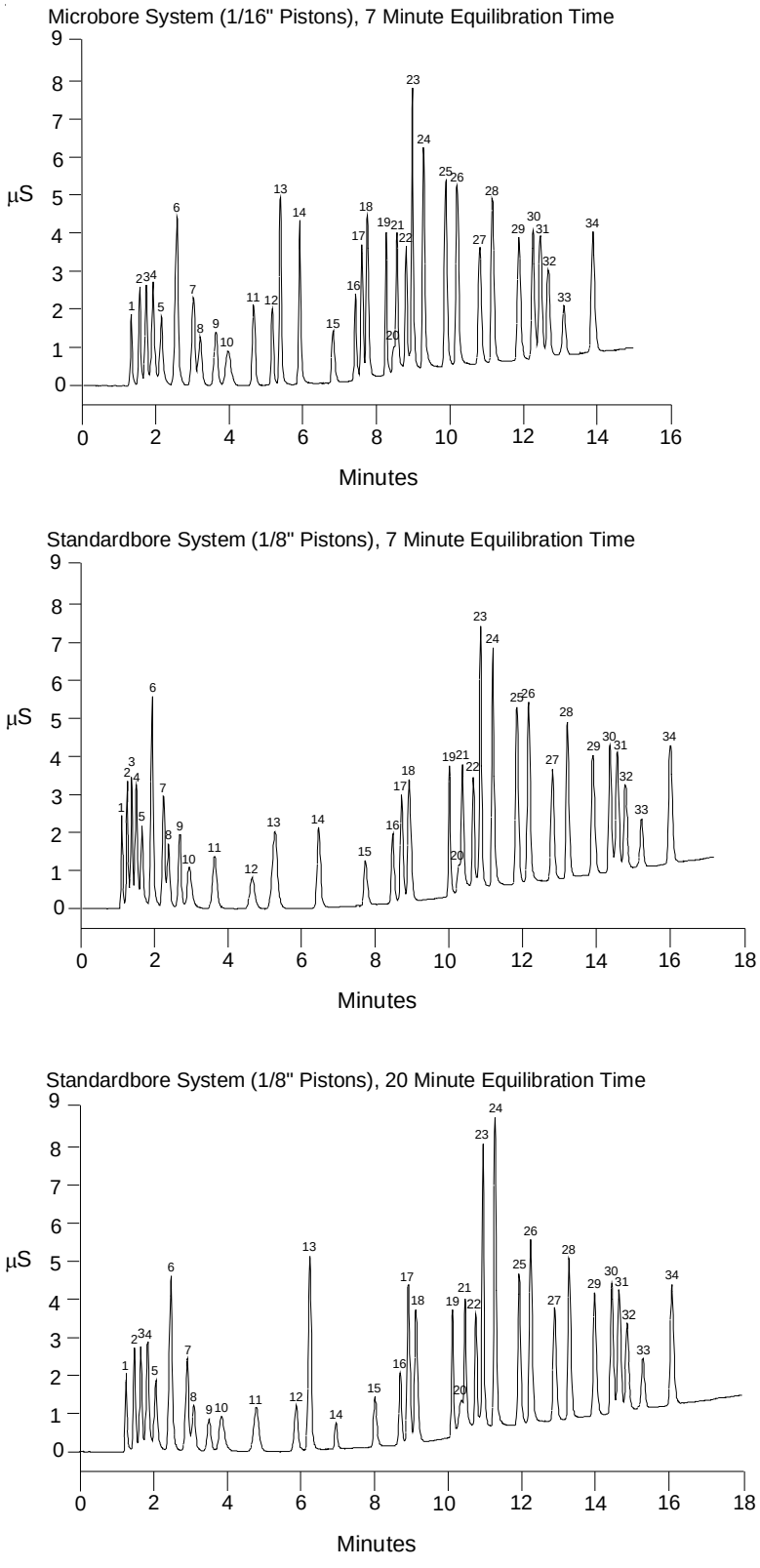
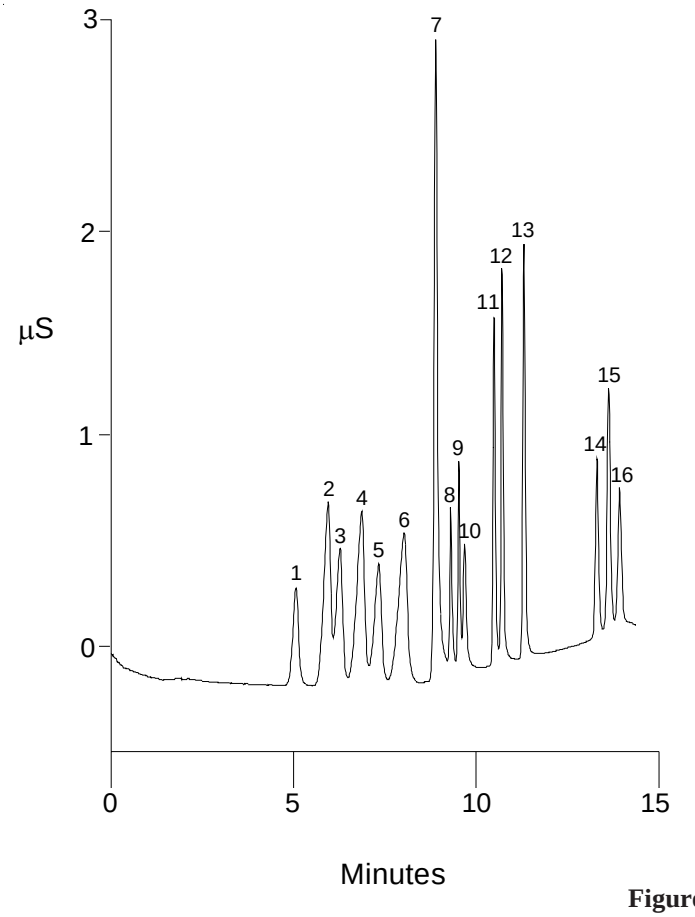


Figure 7
How System Void Volume Affects the
Length of Time Required for Equilibration to the First Eluent

5.5 Resolution of Short-Chain Monovalent Organic Acid Anions

Quantification of short-chain organic acids and inorganic anions in a single run is an important application for the biotech, chemical and power industries. For applications requiring quantification of early eluting anions such as fluoride, acetate, lactate and formate, resolution of these early eluting peaks can be improved by using 0.1-0.2 mM sodium hydroxide as the initial eluent concentration. Retention of more hydrophilic anions may be improved by eliminating organic solvent from the eluent. Resolution is improved especially for sub-ppm quantification of acetate in the presence of high amounts of lactate. A step change or gradient can be used to quickly purge concentrations of phosphate, sulfate or other highly retained anions from the column. When using low initial eluent concentrations, this high eluent concentration is important to regenerate the column in order to ensure consistent run times and a stable baseline.

Sample Loop Volume:	2-mm: 2.5 µL 4-mm: 10 µL
Analytical Column:	IonPac AS11
Eluents	
E1:	Degassed Type I Reagent Grade Water
E2:	1.0 mM NaOH
E3:	100 mM NaOH
Eluent Flow Rate:	2-mm: 0.25 mL/min 4-mm: 1.0 mL/min
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS ULTRA (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:	0.2 mM NaOH: ≤ 0.2 µS 15 mM NaOH: ≤ 0.6 µS
Storage Solution:	12 mM NaOH



Gradient Conditions

Time (min)	%E1	%E2	%E3	Comments
Equilibration				
0	80	20	0	0.2 mM NaOH for 5 min
10.0	80	20	0	
Analysis				
0.0	80	20	0	0.2 mM NaOH, Inject
0.2	80	20	0	Inject Valve to Load Position
5.0	80	20	0	0.2 - 15 mM NaOH in 10 min
15.0	85	0	15	
Regeneration				
15.1	65	0	35	35 mM NaOH regeneration
25.0	65	0	35	for 10 min

	Analyte	mg/L
1.	Quinate	3.0
2.	Fluoride	0.5
3.	Lactate	2.0
4.	Acetate	2.0
5.	Iodate	3.0
6.	Propionate	3.0
7.	Butyrate, Formate	2.0, 1.0
8.	Pyruvate	1.0
9.	Chlorite	1.0
10.	Valerate	1.0
11.	Bromate	2.0
12.	Chloride	0.5
13.	Nitrite	1.0
14.	Bromide	1.0
15.	Nitrate	1.0
16.	Chlorate	1.0

where 1 mg/L = 1 ppm

Figure 8

Short-Chain Monovalent Organic Acid Anions

5.6 Effect of Methanol on IonPac AS11 Selectivity

These two examples illustrate the use of organic solvent to control the selectivity of the IonPac AS11 for hydrophobic ions. The upper chromatogram uses gradient conditions of 0.5 mM to 38 mM NaOH aqueous sodium hydroxide (containing no solvents) to elute 17 anions in 15 minutes. However, these conditions result in the co-elution of the following pairs of ions: succinate/malate, tartrate/maleate and fumarate/oxalate. As demonstrated in the second chromatogram, the addition of 16% methanol to the eluent decreases the retention times of the more hydrophobic member of each unresolved pair in the top chromatogram and resolves all of the anions in 17 minutes. The retention time of the more hydrophilic anion may in some instances actually increase.

The Anion Self-Regenerating Suppressor was used in the AutoSuppression Recycle Mode for the upper chromatogram which uses an aqueous eluent (containing no solvent) and in the AutoSuppression External Water Mode to generate the second chromatogram that uses an eluent containing solvent.

Sample Loop Volume:	2-mm: 2.5 µL 4-mm: 10 µL
Analytical Column:	IonPac AS11
Eluents	E1: Degassed Type I Reagent Grade Water E2: 1.0 mM NaOH E3: 100 mM NaOH E4: 100% Methanol
Eluent Flow Rate:	2-mm: 0.5 mL/min 4-mm: 2.0 mL/min
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS ULTRA (2-mm or 4-mm) See Chromatogram for Mode
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III (2-mm or 4-mm)
MMS Regenerant:	50 mN H_2SO_4
Expected Background Conductivity:	0.5 mM NaOH: ≤ 1 µS 38 mM NaOH: ≤ 3 µS
Storage Solution:	12 mM NaOH

Solvent Added Gradient Conditions (Lower Chromatogram)

TIME (min)	%E1	%E2	%E3	%E4	Comments
Equilibration					
0	74	10	0	16	0.5 mM NaOH/16% CH_3OH for 7 min
7.0	74	10	0	16	
Analysis					
0.0	74	10	0	16	0.5 mM NaOH/16% CH_3OH, Inject
0.2	74	10	0	16	Inject Valve to Load Position
2.0	74	10	0	16	0.5-4.2 mM NaOH/16% CH_3OH in 3 min
5.0	0	84	0	16	4.2-37.45 mM NaOH/16% CH_3OH in 10 min
15.0	0	49	35	16	

Aqueous Gradient Conditions (Upper Chromatogram)

TIME (min)	%E1	%E2	%E3	%E4	Comments
Equilibration					
0	90	10	0	0	0.5 mM NaOH for 7 min
7.0	90	10	0	0	
Analysis					
0.0	90	10	0	0	0.5 mM NaOH, Inject
0.2	90	10	0	0	Inject Valve to Load Position
2.0	90	10	0	0	0.5-5.0 mM NaOH in 3 min
5.0	0	100	0	0	5.0-38.25 mM NaOH in 10 min
15.0	0	65	35	0	

NOTE

Seven minutes are required at the beginning of this program for equilibration of the AS11 with E1 prior to injecting the next sample. If the system is not used continuously, that is, the run program (equilibration plus analysis) is not started exactly every 22 minutes (without AG11), the run program can be modified to start with 2 minutes of the highest eluent concentration for regeneration and then to equilibrate with E1 for 7 minutes with the next injection 9 minutes into the program.

All anion concentrations are 5 mg/L (5 ppm)

1. Acetate
2. Chloride
3. Nitrate
4. Glutarate
5. Succinate
6. Malate
7. Malonate
8. Tartrate
9. Maleate
10. Fumarate
11. Sulfate
12. Oxalate
13. Phosphate
14. Citrate
15. Isocitrate
16. cis-Aconitate
17. trans-Aconitate

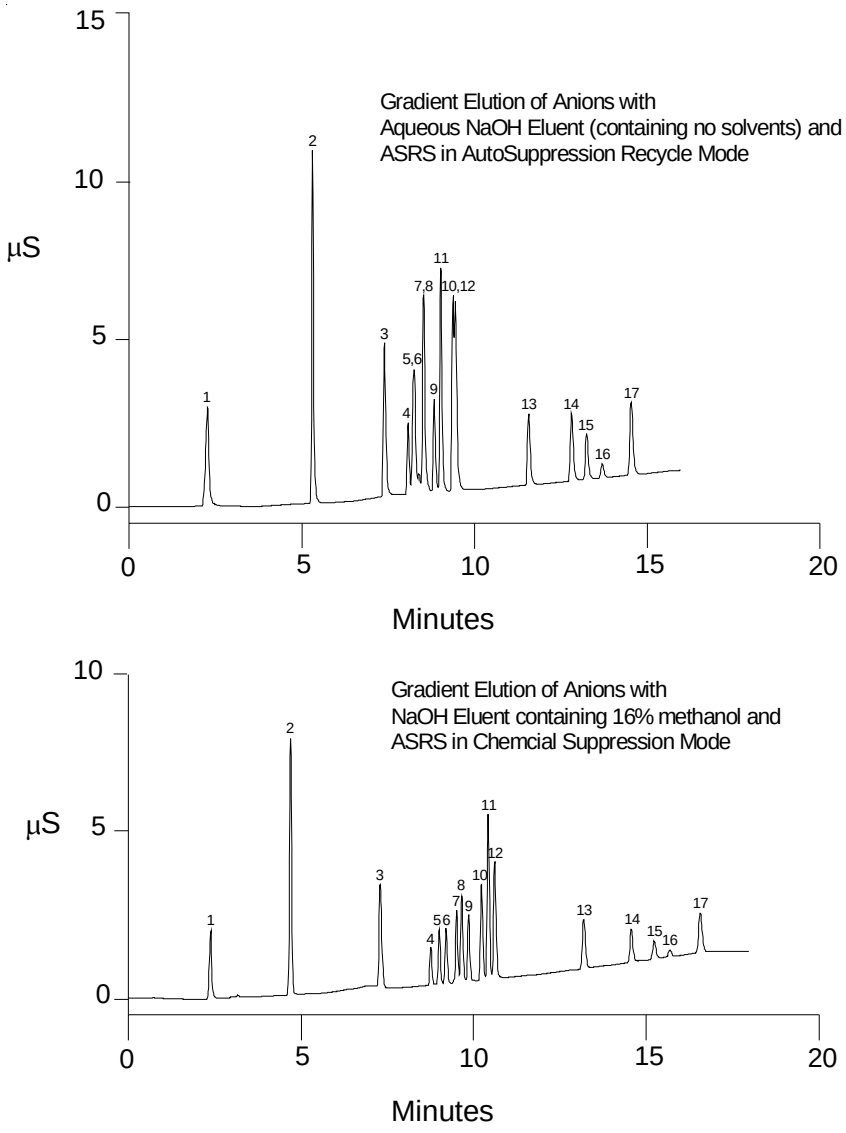


Figure 9
Effect of Methanol on IonPac AS11 Selectivity

5.7 Gradient Separation of Krebs Cycle Acids

The Krebs Cycle is the major mechanism of oxidative degradation of carbohydrates. One molecule of oxaloacetate can bring about the oxidation of an unlimited number of acetate molecules. Also called the tricarboxylic acid cycle or the citric acid cycle, this group of organic acids is a good example of how organic acids can be determined quantitatively with the IonPac AS11 Analytical Column using gradient elution.

Generally, once a gradient is optimized for a given column for a large number of anions (see Section 5.3, "Gradient Elution of a Large Number of Inorganic Anions and Organic Acid Anions"), the same gradient can be used without modification for the analysis of the Krebs Cycle acids.

Although malonate and lactate are not part of the tricarboxylic acid cycle, they can be present in samples. Malonate has been found to inhibit the oxidation of pyruvate by any of the catalytically active di- and tricarboxylic acids of the cycle. Under the gradient conditions shown, malonate co-elutes with malate and lactate co-elutes with acetate. See Figure 8, "Short-Chain Monovalent Organic Acid Anions," for the optimized chromatography required to resolve lactate from acetate.

Inorganic anions such as chloride, sulfate and phosphate may also be present in this type of sample. These are resolved from the Krebs Cycle acids as shown in the example chromatogram.

Sample Loop Volume:	2-mm: 2.5 µL 4-mm: 10 µL
Analytical Column:	IonPac AS11
Eluents	E1: Degassed Type I Reagent Grade Water E2: 5.0 mM NaOH E3: 100 mM NaOH E4: 100% Methanol (CH_3OH)
Eluent Flow Rate:	2-mm: 0.5 mL/min 4-mm: 2.0 mL/min
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS ULTRA (2-mm or 4-mm) AutoSuppression External Water Mode
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III (2-mm or 4-mm)
MMS Regenerant:	50 mN H_2SO_4
Expected Background Conductivity:	2.5 mM NaOH: ≤ 1 µS 45 mM NaOH/18% CH_3OH: ≤ 3 µS
Storage Solution:	12 mM NaOH

Gradient Conditions

TIME (min)	%E1	%E2	%E3	%E4	Comments
Equilibration					
0	32	50	0	18	2.5 mM NaOH/18% CH_3OH for 7 min
7.0	32	50	0	18	
Analysis					
0.0	32	50	0	18	2.5 mM NaOH/18% CH_3OH, Inject
0.1	32	50	0	18	Inject Valve to Load Position
1.1	32	50	0	18	2.5 mM NaOH/18% CH_3OH to
14.0	37	0	45	18	45 mM NaOH/18% CH_3OH in 13 min

NOTE

Seven minutes are required at the beginning of this program for equilibration of the AS11 with E1 prior to injecting the next sample. If the system is not used continuously, that is, the run program (equilibration plus analysis) is not started exactly every 20 minutes (without AG11), the run program can be modified to start with 2 minutes of the highest eluent concentration for regeneration and then to equilibrate with E1 for 7 minutes with the next injection 9 minutes into the program.

All anion concentrations are 5 mg/L unless noted

		mg/L
1.	Acetate	
2.	Pyruvate	
3.	Chloride	2.5
4.	Nitrate	
5.	Succinate	
6.	Malate	
7.	Carbonate	trace
8.	a-Ketoglutarate	
9.	Fumarate	
10.	Sulfate	
11.	Oxaloacetate	10
12.	Phosphate	10
13.	Citrate	10
14.	Isocitrate	10
15.	cis-Aconitate	} 10
16.	trans-Aconitate	

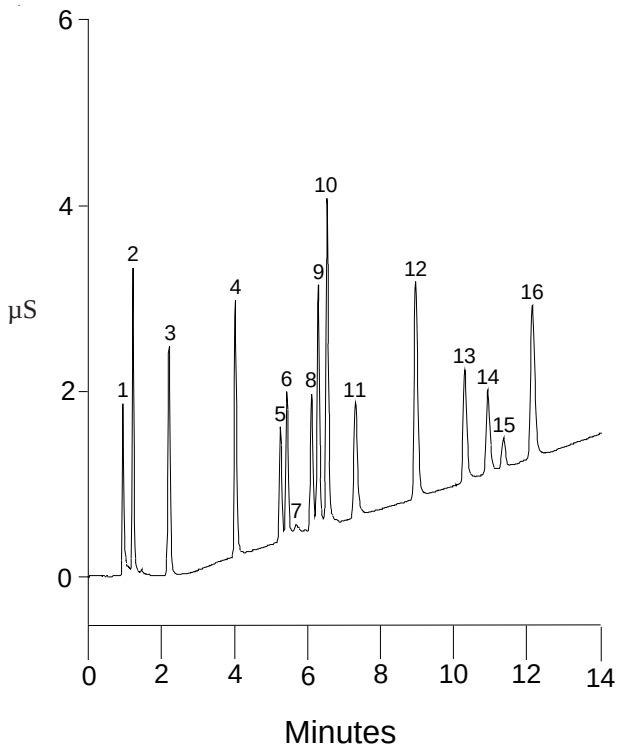


Figure 10
Separation of Krebs Cycle Acids

5.8 Optimized Separation of Organic Acids in Orange Juice

The following application illustrates the chromatography conditions optimized for the carboxylic acids and inorganic anions commonly encountered in juices. The resolution of lactate and acetate is optimized at the front end of the chromatogram by using low organic solvent (10% methanol) and low concentration of sodium hydroxide (0.2 mM). The divalent and trivalent acids are eluted efficiently and quickly by running dual solvent and hydroxide gradients to 45 mM sodium hydroxide and 20% methanol in 15 minutes.

The Anion Self-Regenerating Suppressor was used in the AutoSuppression External Water Mode.

Sample Loop Volume:	2-mm: 2.5 µL 4-mm: 10 µL
Sample Dilution:	1:100 with degassed Type I Reagent Grade Water
Analytical Column:	IonPac AS11
Eluents	E1: Degassed Type I Reagent Grade Water E2: 1.0 mM NaOH E3: 100 mM NaOH E4: 100% Methanol (CH_3OH)
Eluent Flow Rate:	2-mm: 0.5 mL/min 4-mm: 2.0 mL/min
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS ULTRA (2-mm or 4-mm) AutoSuppression External Water Mode
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III (2-mm or 4-mm)
MMS Regenerant:	50 mN H_2SO_4
Expected Background Conductivity:	0.2 mM NaOH/10% CH_3OH: ≤ 1 µS 45.35 mM NaOH/20% CH_3OH: ≤ 3 µS

Gradient Conditions

TIME (min)	%E1	%E2	%E3	%E4	Comments
Equilibration					
0	70	20	0	10	0.2 mM NaOH/10% CH_3OH for 7 min
7.0	70	20	0	10	
Analysis					
0.0	70	20	0	10	0.2 mM NaOH/10% CH_3OH, Inject
0.2	70	20	0	10	Inject Valve to Load Position
5.0	70	20	0	10	0.2 mM NaOH/10% CH_3OH to
15.0	0	35	45	20	45.35 mM NaOH/20% CH_3OH in 10 min

NOTE

Seven minutes are required at the beginning of this program for equilibration of the AS11 with E1 prior to injecting the next sample. If the system is not used continuously, that is, the run program (equilibration plus analysis) is not started exactly every 22 minutes (without AG11), the run program can be modified to start with 2 minutes of the highest eluent concentration for regeneration and then to equilibrate with E1 for 7 minutes with the next injection 9 minutes into the program.

1. Quinate
2. Lactate
3. Acetate
4. Glycolate
5. Formate
6. D-Galacturonate
7. Chloride
8. Nitrate
9. Glutarate
10. Succinate
11. Malate
12. Sulfate
13. Oxalate
14. Phosphate
15. Citrate
16. Isocitrate
17. trans-Aconitate

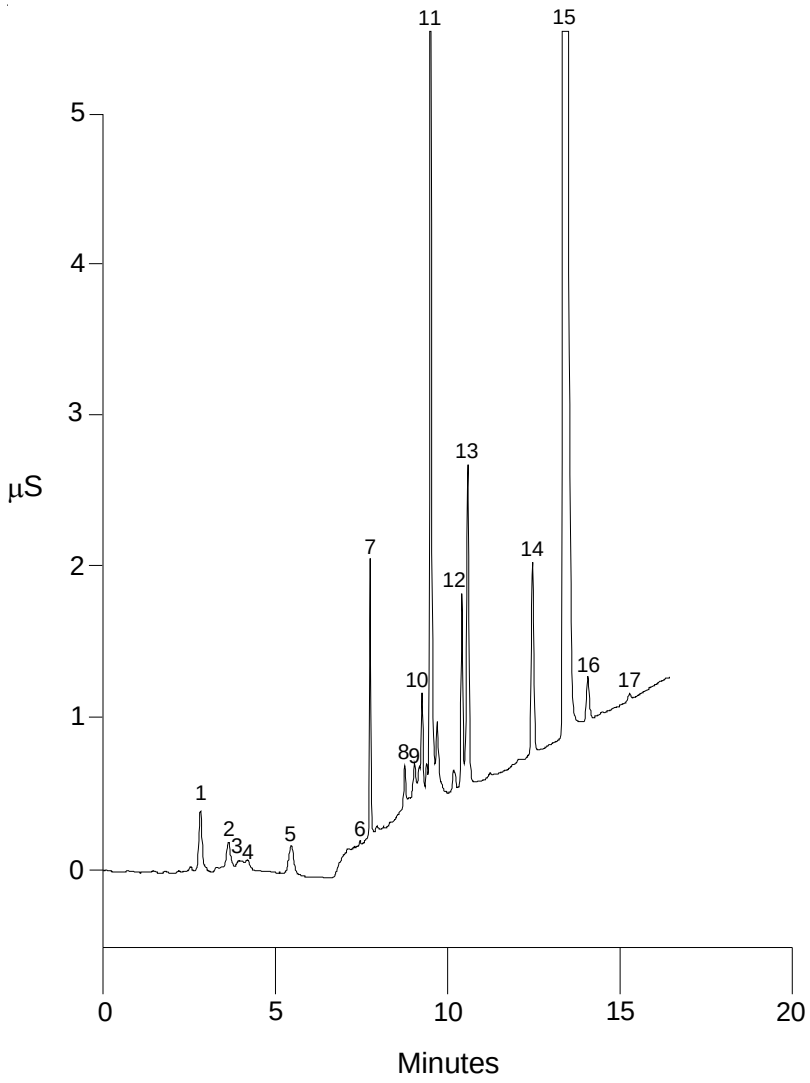


Figure 11
Gradient Analysis of Orange Juice

If resolution is a problem, consider these possibilities before changing the gradient to improve resolution:

A. Make sure that eluents E2 and E3 have been prepared correctly.

B. Check the eluent flow rate.

C. Adjust the gradient to provide the desired resolution. The column capacity may be slightly different from that of the column used to obtain the sample chromatogram.

5.9 Effect of Solvent on Highly retained Inorganic Anions Including Iodide, Thiocyanate, Thiosulfate and Perchlorate

Since the IonPac AS11 is 100% HPLC solvent compatible, organic solvents can be used to modify ion exchange selectivity. For highly retained surface active anions such as iodide and thiocyanate, retention times are significantly decreased and peak efficiency improved by using methanol or acetonitrile. The retention times of hydrophilic anions may increase. Sulfate and phosphate co-elute at 20% methanol but are well resolved at 40%. See the IonPac AS16 manual for an alternate method that does not require solvent in the eluent.

The Anion Self-Regenerating Suppressor was used in the AutoSuppression External Water Mode.

Sample Loop Volume:	2-mm: 2.5 µL 4-mm: 10 µL
Analytical Column:	IonPac AS11
Eluent:	45 mM Sodium hydroxide in 20% methanol/water **or** 45 mM Sodium hydroxide in 40% methanol/water
Eluent Flow Rate:	2-mm: 0.25 mL/min 4-mm: 1.0 mL/min
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS ULTRA (2-mm or 4-mm) AutoSuppression External Water Mode
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III (2-mm or 4-mm)
MMS Regenerant:	50 mN H_2SO_4
Expected Background Conductivity:	45 mM NaOH/20% CH_3OH: ≤ 3 µS 45 mM NaOH/40% CH_3OH: ≤ 3 µS

Proportioned Isocratic Eluents

Eluent 1: Degassed Type I Reagent Grade Water

Eluent 2: 100 mM NaOH Eluent Flow Rate: 1.0 mL/min

Eluent 3: 100% Methanol (CH_3OH)

%E1	%E2	%E3	Comments
35	45	20	45 mM NaOH/20% Methanol*
15	45	40	45 mM NaOH/40% Methanol*

Or

45 mM NaOH/20% Methanol = 3.6 g (2.34 mL) 50% NaOH + 798 mL Type I Deionized Water + 200 mL CH_3OH

45 mM NaOH/40% Methanol: = 3.6 g (2.34 mL) 50% NaOH + 599 mL Type I Deionized Water + 400 mL CH_3OH

	Analyte	mg/L
1.	Fluoride	2
2.	Chloride	2
3.	Nitrate	5
4.	Sulfate	5
5.	Phosphate	10
6.	Iodide	20
7.	Thiocyanate	20
8.	Thiosulfate	20
9.	Perchlorate	20

where 1 mg/L = 1 ppm

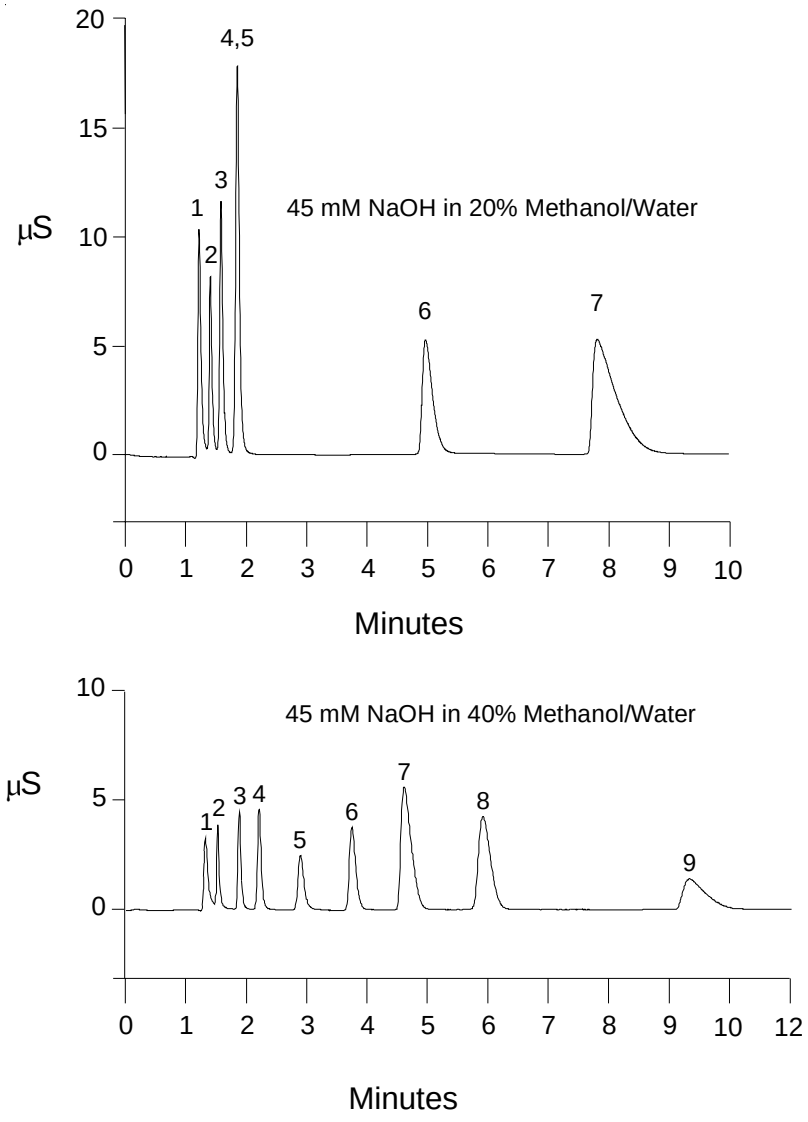


Figure 12
Effect of Solvent on Highly Retained Inorganic Anions
Including Iodide, Thiocyanate, Thiosulfate and Perchlorate

5.10 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 20 mM to 80 mM aqueous sodium hydroxide (containing no solvents) at a flow rate of 2.0 mL/min to elute 9 anions in 8 minutes. Note that tripolyphosphate is eluted with 50 mM sodium hydroxide compared to approximately 120 mM NaOH when using the OmniPac® PAX-100. See the IonPac AS16 Product Manual for an alternate method.

Since organic solvent is not used in the eluent, the Anion Self-Regenerating Suppressor, ASRS ULTRA, can be used in the AutoSuppression Recycle Mode.

Sample Loop Volume:	2-mm: 2.5 µL 4-mm: 10 µL
Analytical Column:	IonPac AS11
Eluents	E1: Degassed Type I Reagent Grade Water E2: 200 mM NaOH
Eluent Flow Rate:	2-mm: 0.5 mL/min 4-mm: 2.0 mL/min
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS ULTRA (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:	20 mM NaOH: ≤ 2.0 µS 80 mM NaOH: ≤ 4.0 µS

Gradient Conditions

TIME (min)	%E1	%E2	Comments
Analysis			
0	90	10	20 mM NaOH, Inject
0.2	90	10	20-80 mM NaOH in 10 min
10.0	60	40	

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

where 1 mg/L = 1 ppm

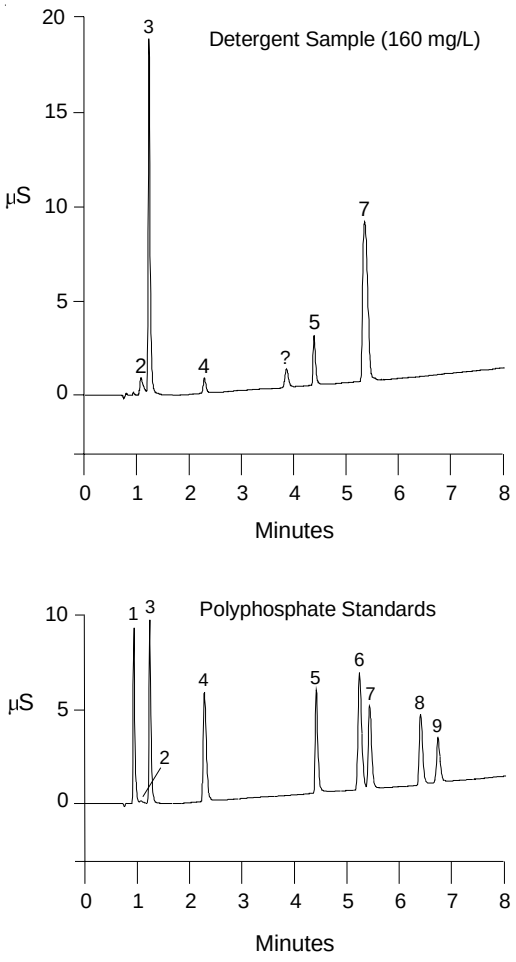


Figure 13
Separation of Polyphosphate Anions

5.11 Separation of High Valency Polycarboxylic Aromatic Acids

The IonPac AS11 has the selectivity required to resolve aromatic carboxylate ions with very similar structures. Aromatic carboxylate ions are monitored in chemical process solutions and as impurities in precursors in the polymer industry. For example, the compound 1,3-benzenedicarboxylic acid is a trace contaminate in 1,2-benzenedicarboxylic acid. The highly charged benzenehexacarboxylate ion is eluted with a gradient to 100 mM hydroxide using a flow rate of 2 mL/min. The improved suppression capacity of the ASRS can easily suppress this eluent concentration.

Since organic solvent is not used in the eluent, the Anion Self-Regenerating Suppressor, ASRS ULTRA, can be used in the AutoSuppression Recycle Mode.

Sample Loop Volume:	2-mm: 2.5 µL 4-mm: 10 µL
Analytical Column:	IonPac AS11
Eluents	E1: Degassed Type I Reagent Grade Water E2: 200 mM NaOH
Eluent Flow Rate:	2-mm: 0.5 mL/min 4-mm: 2.0 mL/min
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS ULTRA (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:	20 mM NaOH: ≤ 2.0 µS 100 mM NaOH: ≤ 4.0 µS

Gradient Conditions

TIME (min)	%E1	%E2	Comments
Analysis			
0	90	10	20 mM NaOH, Inject
0.2	90	10	20-80 mM NaOH in 10 min
10.0	60	40	

All anion concentrations are 20 mg/L

1. Benzoic Acid
2. 1,2-Benzenedicarboxylic Acid (Phthalic)
3. 1,4-Benzenedicarboxylic Acid (Terephthalic)
4. 1,3-Benzenedicarboxylic Acid (Isophthalic)
5. 1,2,3-Benzenetricarboxylic Acid
6. 1,3,5-Benzenetricarboxylic Acid
7. 1,2,4,5-Benzenetetracarboxylic Acid
8. Benzenepentacarboxylic Acid
9. Benzenehexacarboxylic Acid

where 1 mg/L = 1 ppm

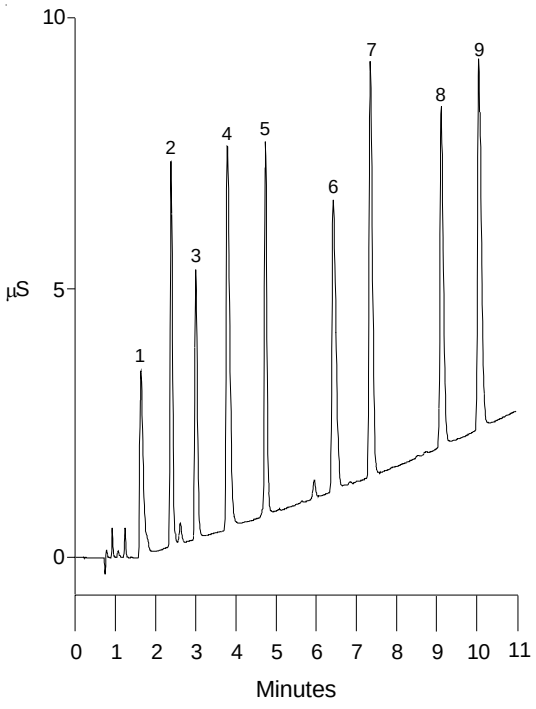


Figure 14
Separation of High Valency Polycarboxylic Aromatic Acids

5.12 Separation of Polygalacturonic Acid Oligomers

The IonPac AS11 is ideally suited for the analysis of very complex mixtures of highly charged polycarboxylic anions such as polygalaturonic acid. Oligomers of this type are found in cell wall extracts analyzed in basic research, in pharmaceutical preparations and in health care products.

A convex curvilinear gradient is used in this application. The very high hydroxide selectivity of the AS11 resin permits elution of these ions at lower hydroxide concentrations than were possible on the OmniPac PAX-100 thus permitting the elution of highly charged anions on either the AS11 2-mm or 4-mm Analytical Column. In this application, a gradient to 86 mM hydroxide at 2.0 mL/min can easily be suppressed using the ASRS ULTRA (4-mm).

Since organic solvent is not used in the eluent, the Anion Self-Regenerating Suppressor, ASRS ULTRA, can be used in the AutoSuppression Recycle Mode.

Sample Loop Volume:	2-mm: 2.5 µL 4-mm: 10 µL
Analytical Column:	IonPac AS11
Eluents	E1: Degassed Type I Reagent Grade Water E2: 200 mM NaOH
Eluent Flow Rate:	2-mm: 0.50 mL/min 4-mm: 2.0 mL/min
Gradient:	Convex curvilinear
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS ULTRA (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:	20 mM NaOH: ≤ 2.0 µS 86 mM NaOH: ≤ 4.0 µS

Gradient Conditions

TIME (min)	%E1	%E2	Comments
Analysis			
0	90	10	20 mM NaOH, Inject
0.2	90	10	20-86 mM NaOH in 10 min
10.0	57	43	

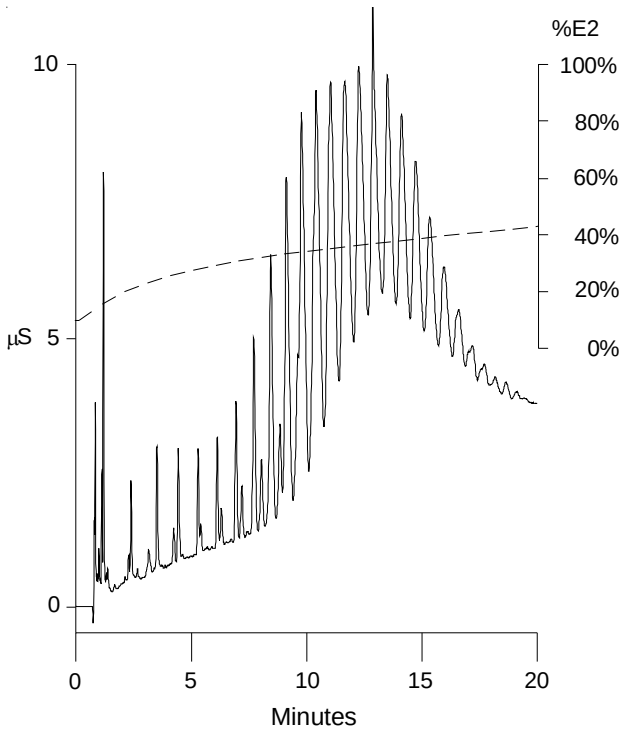


Figure 15
Separation of Polygalacturonic Acid Oligomers

SECTION 6 - TROUBLESHOOTING GUIDE

The purpose of the Troubleshooting Guide is to help you solve operating problems that may arise while using IonPac AS11 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, contact the nearest Dionex Office (see, "Dionex Worldwide Offices").

Table 7
AS11/AG11 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	Disconnect, Replace	Component Manual
High Background Conductivity	Bad Eluents	Remake Eluents	6.2, 6.2.1
	Contaminated Columns	Clean Column	6.2.2, 6.2.3, Column Care
	Contaminated ASRS or AMMS	Clean Suppressor	6.2.5, Component Manual
	Contaminated Hardware	Clean Component	Component Manual
Poor Resolution	Poor Efficiency Due to Large System Void Volumes	Replumb System	6.3.1.A, 6.3.3.D Component Manual
	Column Headspace	Replace Column	6.3.1.B
Short Retention Times	Unequilibrated System	Lengthen First Eluent Time before Inject	6.3.3.C
	Flow Rate Too fast	Recalibrate Pump	6.3.2.A
	Bad Eluents	Remake Eluents	6.3.2.B
	Column Contamination	Clean Column	6.3.2.C, 6.3.2.D, Column Care
Poor Front End Resolution	Bad 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 Contamination	Pretreat Samples	6.4.A, 6.4.B, Column Care
	Sluggish Injection Valve	Service Valve	6.4.C, Component Manual

6.1 HIGH BACK PRESSURE

6.1.1 Finding the Source of High System Pressure

Total system pressure when using the IonPac AG11 (2-mm) Guard and AS11 (2-mm) Analytical Columns at 0.50 mL/min should be less than 1,600 psi (11.03 MPa) when using the eluent used to generate the test chromatogram. Total system pressure when using the IonPac AG11 (4-mm) Guard and AS11 (4-mm) Analytical Columns at 2.0 mL/min should also be less than 1,600 psi (11.03 MPa) when using the eluent used to generate the test chromatogram. Refer to Section 4.4, "Solvents," to see how solvent concentration can affect the column operating pressure. If the system pressure is higher than 1,600 psi (11.03 MPa), it is advisable to find out what is causing 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. Find out what part of the system is causing the high pressure. It could be a piece of tubing that has plugged or whose walls are collapsed, an injection valve with a plugged port, a column with particulates plugging the bed support, a plugged High-Pressure In-Line Filter, the suppressor, or the detector cell.

To find out which part of the chromatographic system is causing the problem, disconnect the pump eluent line from the injection valve and turn the pump on. Watch the pressure; it should not exceed 50 psi (0.34 MPa). Continue adding the system's components (injection valve, column(s), ASRS or AMMS and the detector) one by one, while watching the system pressure. The pressure should increase up to a maximum when the Guard and Analytical columns are connected (see Table 8, "Typical AS11/AG11 Operating Back Pressures").

The ASRS or AMMS 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 8
Typical AS11/AG11 Operating Back Pressures

Column	Typical Back Pressure psi (MPa)	Standard Flow Rate mL/min
AS11 4-mm Analytical	≤ 800 (5.51)	1.0
AG11 4-mm Guard	≤ 300 (2.07)	1.0
AS11 + AG11 4-mm columns	**≤ 1,100 (7.58)**	**1.0**
AS11 2-mm Analytical	≤ 800 (5.51)	0.25
AG11 2-mm Guard	≤ 300 (2.07)	0.25
AS11 + AG11 2-mm columns	**≤ 1,100 (7.58)**	**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 using two open end wrenches.

C. Remove the old 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 AS11 Columns	
	2-mm	**4-mm**
Part	**(P/N)**	**(P/N)**
Analytical Column	044077	044076
Guard Column	044079	044078
Bed Support Assembly	044689	042955
End Fitting	043278	052809

CAUTION

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

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

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

NOTE

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

6.2 HIGH BACKGROUND OR NOISE

In a properly working system, the background conductivity levels expected for several eluent systems are shown below:

ELUENT	**EXPECTED BACKGROUND CONDUCTIVITY**
0.5 mM NaOH	≤ 1.5 µS
35 mM NaOH	≤ 3.5 µS
50 mM NaOH	≤ 4.0 µS
45 mM NaOH/40% CH_3OH	≤ 3.5 µS

6.2.1 Preparation of Eluents

A. Make sure that all eluents and regenerants are made correctly. Were the proper precautions taken to prepare the sodium hydroxide eluent? If carbonate was present in the eluent, the Anion Trap column will eventually be spent and the background level will increase.

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

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

6.2.2 A Contaminated Anion Trap Column, the ATC-3

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

Determine if the ATC is the source of high background conductivity. Remove the ATC. If the background conductivity remains high, then the ATC is not the problem. If the background conductivity decreases, the ATC is the source of the high background conductivity.

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

B. Flush the ATC-3 (4-mm) **with 100 mL of 200 mM NaOH or 50 mL of 200 mM NaOH for 2-mm ATC-3.** Use a flow rate of 0.5 mL/min on a 2-mm system or a flow rate of 2.0 mL/min on a 4-mm system.

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

D. If the problem persists, replace the ATC.

6.2.3 A Contaminated Guard or Analytical Column

Remove the IonPac AG11 Guard and AS11 Analytical Columns from the system. If the background conductivity decreases, then one (or both) of these columns is (or are) the cause of the high background conductivity, clean the column as instructed in, "Column Cleanup" in "Column Care."

6.2.4 Contaminated Hardware

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

6.2.5 A Contaminated Suppressor

If the above items have been checked and the problem persists, the Anion Self-Regenerating Suppressor or the Anion MicroMembrane Suppressor is probably causing the problem. For details on Anion Self-Regenerating Suppressor operation, refer to the Anion Self-Regenerating Suppressor ULTRA Product Manual (Document No. 031367). For details on Anion Membrane Suppressor III operation, refer to the Product Manual (Document No. 031727) for assistance.

A. Check the power level and alarms on the SRS Control.

B. Check the regenerant flow rate at the REGEN OUT port of the ASRS if operating in the AutoSuppression External Waster mode or the Chemical Suppression mode or the AMMS.

C. Check the eluent flow rate.

D. If you are using an AutoRegen Accessory with the ASRS in the Chemical Suppression Mode or the AMMS, 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 ASRS or AMMS.

2. **If the background conductivity is low when freshly prepared regenerant is run through the ASRS or AMMS 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

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

6.3.1 Loss of Column Efficiency

A. Ensure that system void volumes have been minimized. Extra-column system effects can result in sample band dispersion and decreasing peak efficiencies. Make sure you are using PEEK tubing with an ID of no greater than 0.010" to make all eluent liquid line connections between the injection valve and the detector cell inlet on 4-mm systems. Use 0.005" ID tubing on 2-mm systems. Make all tubing lengths are as short as possible. Check for leaks.

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

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 eluent flow rate. If it is different than the flow rate specified by the analytical protocol, recalibrate the pump. 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.

For isocratic analysis, an eluent that is too strong will cause the peaks to elute faster. Prepare fresh eluent. If you are using a gradient pump to proportion the final eluent from concentrated eluents in two or three different eluent reservoirs, the composition of the final eluent 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%.

For gradient analysis, remake the eluents or adjust the times in the gradient program to obtain the required peak resolutions.

C. Column contamination can lead to a loss of column capacity because fewer of the anion exchange sites will be available for the sample ions. Polyvalent anions or metal ions might be concentrating on the column. Refer to, "Column Cleanup" in "Column Care," for recommended column cleanup procedures.

Possible sources of column contamination are impurities in chemicals, in the deionized water or from the sample matrix being used. 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. 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 nearest (see, "Dionex Worldwide Offices").

6.3.3 Loss of Front End Resolution

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

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

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

C. The column may not be equilibrated to the first eluent. Increase the amount of time that the first eluent runs through the columns before injection. See Section 5.5, "Resolution of Short Chain Monovalent Organic Acid Anions," for an example of an analysis requiring both regeneration and equilibration.

D. 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.

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

6.4 Spurious Peaks

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

B. If you need assistance in determining the best way to clean strongly retained solutes in your specific sample matrix from the IonPac AS11 columns, contact the nearest Dionex Office (see, "Dionex Worldwide Offices").

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

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

For DX-300 systems equipped with a Rheodyne Microinjection Valve, Model 9126 (Dionex P/N 044697), consult the accompanying manual for service instructions. See Section 3.4, "The Injection Loop," for injection valve and loop requirements for 2-mm operation.

6.5 Small Analyte Peak Areas Observed when Using an ASRS

The ASRS ULTRA is installed in the column compartment of the chromatography module right after the analytical column and before the conductivity detector cell. On the DX-500, DX-120, and DX-100 instruments, the ASRS ULTRA mounts on tabs on the component panel. Orient the ASRS ULTRA with the **ELUENT IN** port and the cable at the top; align the slots on the back of the ASRS ULTRA with the tabs on the panel. Press in, and then down, to lock the ASRS ULTRA in place. Lift up and pull out to remove the ASRS ULTRA. Make sure the ASRS ULTRA is plumbed properly, according to the selected mode of operation. Refer to Section 2, "Installation," for complete installation instructions.

CAUTION

The membranes and screens in the ASRS ULTRA must be completely hydrated to maintain liquid seals and chromatographic performance. This requirement is achieved by maintaining the regenerant chambers full of the appropriate regenerant solution or water. This will ensure that the membranes and screens remain properly hydrated.

CAUTION

The correct amount of back pressure for optimum operation is 40 psi. Connect the back pressure coil(s) appropriate for your column ID and flow rate. Back pressures over 125 psi after the ASRS ULTRA can cause irreversible damage!

CAUTION

DO NOT CAP THE WASTE RESERVOIR!

The very small amount of hydrogen gas generated by the ASRS ULTRA is not dangerous unless the gas is trapped in a closed container and allowed to concentrate. The Gas Separator Waste Tube must be open to the atmosphere and not in a confined space to operate properly.

A. Using a disposable plastic syringe, push approximately 3 mL of 200 mN H_2SO_4 into the **ELUENT OUT**.

B. Using a disposable plastic syringe, push approximately 5 mL of 200 mN H_2SO_4 through the **REGEN IN** port.

C. Allow the suppressor to sit for approximately 20 minutes to fully hydrate the suppressor screens and membranes.

D. Mount the suppressor and plug it in. Plumb it according to the mode of operation. See Sections 3.3 - 3.6.

E. Turn on the power to the ASRS ULTRA and establish eluent flow through the ASRS ULTRA. Do this by turning on the Self-Regenerating Suppressor Control power and the pump flow at the same time.

CAUTION

Always turn the pump and the SRS Control on and off at the same time! Eluent flow through the ASRS ULTRA is required for proper operation. However, without current, the membranes and screens in the ASRS ULTRA will become expended by the flowing eluent resulting in small analyte peak areas. If this should occur, perform the procedure outlined in section 4.2, "Small Analyte Peak Areas."

F. Start operation. Allow the system to equilibrate before beginning analysis.

APPENDIX A - COLUMN CARE

A.1 Recommended Operation Pressures

Operating a column above its recommended pressure limit can cause irreversible loss of column performance. The maximum recommended operating pressure for IonPac AS11 columns is 3,000 psi (20.68 MPa).

A.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.

A.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.

A.4 Column Cleanup

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

A.4.1 Choosing the Appropriate Cleanup Solution

A. **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.

B. **Concentrated acid solutions** such as 1 to 3 M HCl, 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 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.

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 AS11 Columns.

E. 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. The organic solvent then removes the subsequent nonionic and hydrophobic contamination. See Section D above.

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.

F. Regardless of the cleanup solution chosen, use the following cleanup procedure in, "Column Cleanup Procedure", to clean the AG11 and AS11.

A.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 AS11 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 AS11 4-mm Analytical or Guard Column or set the pump flow rate to 0.25 mL/min for an AS11 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 AS11 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 B - 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)
AAE Suppressor	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, 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) 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