PRODUCT MANUAL

IONPAC® AG5A-5µ GUARD COLUMN

(4 x 35 mm, P/N 037134)

IONPAC® AS5A-5µ ANALYTICAL COLUMN

(4 x 150 mm, P/N 037131)

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

1. See Section 4, “Operation”. Note operation precautions and chemical purity requirements. Make the required eluents.

2. See “Quality Assurance Reports”. Run the Production Test Chromatogram as a system check.

3. See Section 5, "Example Applications" for example applications.

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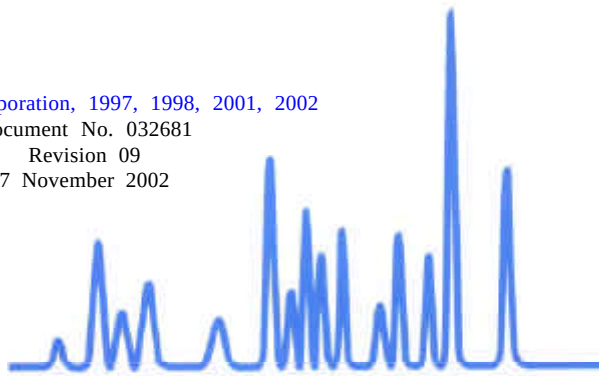

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SECTION 1 - INTRODUCTION

The IonPac AS5A-5µ Analytical Column (P/N 037131) is packed with a 5 micron substrate with a surface coating of anion exchange latex.

The IonPac AS5A-5µ (P/N 037131) Analytical Column is 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, gluconate, formate, and pyruvate. Another benefit of using the AS5A-5µ 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 nitrate is present in high enough concentration to interfere with malate, the malate peak can be moved ahead of the nitrate peak by using a slightly different gradient. 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. The AS5A-5µ column is stable between pH 0 and 14 and is compatible with eluents containing 0-5% organic solvents.

Table 1
IonPac AS5A-5µ/AG5A-5µ Packing Specification

Column	Particle Diameter µm	Substrate X-Linking %	Latex Diameter nm	Latex X-Linking %	Column Capacity µeq/column	Functional Group	Hydrophobicity
AS5A-5µ (4 x 150 mm)	5.0	2%	60	4%	35	Alkanol quaternary ammonium	Low
AG5A-5µ (4 x 35 mm)	5.0	2%	60	4%	7	Alkanol quaternary ammonium	Low

Table 2
IonPac AS5A-5µ/AG5A-5µ Operating Parameters

Column	Typical Back Pressure psi (MPa)	Standard Flow Rate mL/min	Maximum Flow Rate mL/min
AS5A-5µ Analytical	<1,700 (11.72)	1.0 mL/min	2.0 mL/min
AG5A-5µ Guard	< 500 (3.45)	1.0 mL/min	2.0 mL/min
AS5A-5µ Analytical + Guard	**<2,200 (15.17)**	**1.0 mL/min**	**2.0 mL/min**

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

SECTION 2 - COMPARISON OF ION CHROMATOGRAPHY SYSTEMS

CONDITION	4-mm
Eluent Flow Rate	1.0 mL/min
SRS Suppressor	ASRS-ULTRA (4-mm) (P/N 053946)
MMS Suppressor	AMMS III (4-mm) (P/N 056750)
NOTE Do not run a suppressor over 40°C. If an application requires a higher temperature, place the suppressor outside the chromatographic oven.	
Injection Loop	10 µL
System Void Volume	Minimize dead volumes. Switching valves, couplers can be used. Use the GM-2, GM-3 or recommended gradient mixers.
Pumps	Use the GP40/GP50/IP20/IP25 in Standard-Bore Configuration. The GM-3 Gradient Mixer should be used for gradient analysis on systems other than the GP40/GP50/IP20/IP25 and the DX-300 HPLC Pump.
Detectors	AD20/AD25 Cell (10-mm, 9 µL, P/N 049393) VDM-2 Cell (6-mm, 10 µL P/N 043113) CD20, CD25, CD25A, ED40, ED50, or ED50A Conductivity Cell with DS3 (P/N 044130) or with shield (P/N 044132) CDM-2/CDM-3 Cell P/N 042770 Either the TS-1 with the TS-2 can be used with the CDM-2 or the CDM-3. Do not use the TS-2 or the TS-1 with the ED40/ED50/ED50A or the CD20/CD25/CD25A. DIONEX Back Pressure Regulator 75 psi rating (P/N 039760, 046480) or Tubing (see Table 3) Ensure 50-75 psi back pressure.

Table 2
Tubing Back Pressure

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

SECTION 3 - INSTALLATION

3.1 System Requirements

The IonPac AS5A-5µ Analytical Column may be run on any DIONEX Chromatograph (IC) equipped with the Gradient Pump Module, a high pressure (4000 psi) injection valve, a Conductivity Detector, an Anion Self-Regenerating Suppressor-ULTRA (ASRS-ULTRA, P/N 053946) or an Anion MicroMembrane™ Suppressor (AMMS III, P/N 056750). Install a high pressure in-line filter (P/N 035331) before the injection valve to prolong the life of the column.

3.1.1 System Requirements for 4-mm Operation

The AS5A-5µ Analytical Column and Guard are designed to be run on any DIONEX Ion Chromatograph equipped with suppressed conductivity detection. Gradient methods and methods requiring solvent containing eluents should be performed on a system having a Gradient Pump Module (GPM-2) or an Advanced Gradient Pump (AGP) or a GP40/GP50/GS50 with standard 1/8" pump heads. Isocratic analysis can also be performed on an IP20/IP25 with standard bore.

3.2 The Anion Trap Column

When performing an anion exchange application that involves a hydroxide gradient, an IonPac Anion Trap Column (ATC-3, (4-mm) P/N 059660) should be installed in place of the high pressure Gradient Mixer between the Gradient Pump Module (GPM-2), the Advanced Gradient Pump (AGP), or GP40/GP50/GS50 and the injection valve. The ATC-3 is filled with high capacity anion exchange resin which helps to minimize the baseline shift caused by increasing anionic contaminant levels in the eluent as the ionic concentration of the eluent is increased over the course of the gradient analysis.

To install the ATC-3 (4-mm), 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.

D. Pump 20 mL of eluent through the 4-mm ATC-3.

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

3.3 The Sample Concentrator

Use the IonPac AG5A-5µ Guard Column or the TAC-LP1 for trace anion concentration work. The function of AG5A-5µ 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 AG5A-5µ Guard Column leading to a lowering of detection limits by 2-5 orders of magnitude. The unique advantage of using the AG5A-5µ Guard Column in these applications is the capability of performing routine trace analyses of sample matrix ions at µg/L (ppb) levels without extensive and laborious sample pretreatment.

For a detailed discussion of anion concentration techniques, refer to Section 3, "Operation," of the Low Pressure Trace Anion Concentrator (TAC-LP1) Column Product Manual (Document No. 034972).

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 AS5A-5µ. Use the AG5A-5µ 4-mm guard.

3.4 The Injection Loop

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 AS5A-5µ 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.

Table 5
The Smallest Injectable Volume

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

3.5 The IonPac AG5A Guard Column

An IonPac AG5A-5µ Guard Column is normally used with the IonPac AS5A-5µ 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 AG5A-5µ Guard Column at the first sign of peak efficiency loss or decreased retention time will prolong the life of the AS5A-5µ Analytical Column.

3.6 Eluent Storage

Always degas and store all eluents in glass eluent bottles pressurized with helium. Only helium can be used to sparge and degas ionic eluents and solvents, since nitrogen is soluble in eluents. This ensures contamination free operation and proper pump performance (nitrogen can be used if eluents do not contain solvents).

3.7 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.

CAUTION
Solvent containing eluents should be used in the AutoSuppression™ External Water Mode.
If you are installing an IonPac AS5A-5µ 4-mm Analytical Column, use an ASRS-ULTRA (4-mm, P/N 053946).

For detailed information on the operation of the Anion Self-Regenerating Suppressor, see Document No. 034650, the "Product Manual for the Anion Self-Regenerating Suppressor, the ASRS-ULTRA."

3.8 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 AS5A-5µ 4-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. 034449-02, the "Product Manual for the Anion MicroMembrane Suppressor III, the AMMS III."

3.9 Using Displacement Chemical Regeneration (DCR) with 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.

WARNING
Use proper safety precautions in handling acids and bases.

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

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

3.11 Detector Requirements

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

SECTION 4 - OPERATION

4.1 General Operating Conditions

Sample Loop Volume:	10 µL
Trap Column:	Anion Trap Column (ATC-3, P/N 059660)
Analytical Column:	IonPac AS5A-5µ Analytical Column
Eluents,	Eluent 1: 0.75 mM NaOH
	Eluent 2: 200 mM NaOH
Eluent Flow Rate:	1.0 mL/min
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS-ULTRA (4-mm)
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III (4-mm)
MMS Regenerant:	20 mN H_2SO_4
Expected Background Conductivity:	0.75 mM NaOH; 2-4 µS
	50 mM NaOH; 4-7 µS
Expected System Operating Backpressure:	2200-2500 psi

CAUTION
Using a sample loop larger than 10 µL will reduce peak efficiency.

4.2 IonPac AS5A Operation Precautions

CAUTION
Filter and Degas Eluents
Filter Samples
Eluent pH between 0 and 14
Sample pH between 0 and 14
2.0 mL/min Maximum Flow Rate for 4-mm Columns

4.3 Chemical Purity Requirements

It is very important for the eluent to be as free of impurities as possible. For this reason the chemicals and water used to prepare them should be of the highest purity available.

Use certified Sodium Hydroxide Solution, 50% w/w, Fisher Scientific Co. Use reasonably fresh bottles of 50% solution. Discard if sodium carbonate precipitate is evident.

Use DIONEX Anion Regenerant Concentrate, (0.50 N sulfuric acid solution, P/N 037164).

Use deionized water having a specific resistance of 18.2 megohm-cm.

4.4 Eluent Preparation

Because the sodium hydroxide eluents used with the IonPac AS5A-5µ Analytical Column 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 gradient ramps, with 1 to 3 µs total change in the background.

4.4.1 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.

4.4.2 Eluent Preparation

Sodium Hydroxide Eluent Concentration

Weight Method

When formulating eluents from 50% sodium hydroxide, DIONEX recommends weighing out the required amount of 50% sodium hydroxide. Use Fisher Grade 50% sodium hydroxide. Do not use pellets.

Example: To make 1 L of 0.75 mM NaOH use 0.06 g of 50% sodium hydroxide:

$$\text{For 0.75 mM:} \quad \frac{0.00075 \text{ mole/L} \times 40.01 \text{ g/mole}}{50\%} = 0.06 \text{ g diluted to 1 L}$$

Example: To make 1 L of 200 mM NaOH use 16 g of 50% sodium hydroxide:

$$\text{For 200 mM:} \quad \frac{0.2 \text{ mole/L} \times 40.01 \text{ g/mole}}{50\%} = 16 \text{ g diluted to 1 L}$$

Volume Method

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

$$\mathbf{g = dvr}$$

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

Example: To make 1 L of 0.75 mM NaOH use 0.04 mL of 50% sodium hydroxide:

$$\text{For 0.75 mM:} \quad \frac{0.00075 \text{ mole/L} \times 40.01 \text{ g/mole}}{50\% \times 1.53 \text{ g/mL}} = 0.04 \text{ mL diluted to 1 L}$$

Example: To make 1 L of 200 mM NaOH use 10.4 mL of 50% sodium hydroxide:

$$\text{For 200 mM:} \quad \frac{0.2 \text{ mole/L} \times 40.01 \text{ g/mole}}{50\% \times 1.53 \text{ g/mL}} = 10.4 \text{ mL diluted to 1 L}$$

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

4.5 Regenerant Preparation

20 mN sulfuric acid

Dilute 40 mL (about 40 grams) of the Anion Regenerant Concentrate (0.50 N H_2SO_4) to 1 liter using deionized water having a specific resistance of 18.2 megohm-cm.

CAUTION
If you are not using the AutoRegen Accessory (P/N 039594), prepare several liters of the regenerant.

4.6 Initial Operation

After installing the IonPac AS5A-5µ Analytical Column, test it by reproducing the chromatogram shown in Section 4.1. This gradient program has been optimized for maximum resolution of a large number of anions. Variations between systems (eluent concentrations, flow rate, tubing lengths, columns) may make it necessary to perform minor adjustments to this gradient to obtain the chromatographic performance shown.

4.7 Sample Concentration

Detection limits can be enhanced by concentrating the sample onto a concentrator column and using this column in place of the sample loop. The sample must be pumped into the concentrator in the **OPPOSITE** direction of the eluent flow to prevent the chromatography from being compromised.

When concentrating samples, it is important that the sample solution is not basic. Otherwise, the sample concentration will be ineffective for the weakly retained anions. To ensure that the sample is not basic, add high purity boric acid to the sample to make a final concentration of approximately 5 mM boric acid. High purity boric acid (99.999% purity, Gold Label) is available from Aldrich Chemical. Before adding the boric acid, concentrate a sample blank and analyze it to be sure that it will not add contaminant ions to the sample.

If possible, degas your sample before you load it onto the concentrator column. This will reduce the size of the carbonate peak, which typically elutes under phosphite through nitrate.

The following columns can be used for sample concentration with the IonPac AS5A-5µ Analytical Column: the IonPac AG5A-5µ Guard Column (P/N 037134), the IonPac AG5 Guard Column (P/N 035396), or the TAC-LP1 (P/N 046026).

A. When using the IonPac AG5A-5µ Guard Column for sample concentration the system backpressure will increase by approximately 300 psi when the injection valve is switched to INJECT, placing the IonPac AG5A-5µ Guard Column in the eluent flow path. This pressure change can cause a baseline upset at the beginning of the chromatogram. Make sure your method program does not switch the injection valve back to the LOAD position until all the peaks have eluted to prevent baseline disturbances where peaks are eluting, as well as to ensure that all the peaks have been eluted from the concentrator column.

B. The IonPac AG5 Guard Column will provide the least baseline disturbance when the sample is injected; this is due to its lower backpressure contribution.

SECTION 5 - EXAMPLE APPLICATIONS

Although the IonPac AS5A-5µ Analytical Column may be used isocratically for chromatographing a limited number of anions, its principal applications are to perform the analyses of a large number of anions through gradient elution, or to elute anions of different valencies in one run.

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.

If your sample or standard contains organic acids, adding chromate will help stabilize them. The sample chromatograms in Sections 5.2 (see Figure 2, “Gradient Separation of a Few Organic and Inorganic Anions”) and 5.3 (see Figure 3, “Gradient Elution of a Large Number of Anions”) show where chromate elutes in the chromatogram.

5.1 Production Test Chromatogram

Sample Volume:	10 µL
Column:	IonPac AS5A-5µ Analytical Column
Eluent:	3.9 mM $NaHCO_3$/3.1 mM Na_2CO_3
Eluent Flow Rate:	1.0 mL/min
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS-ULTRA (4-mm) AutoSuppression Recycle Mode
Detection:	30 µS FS
Storage Solution:	100 mM NaOH

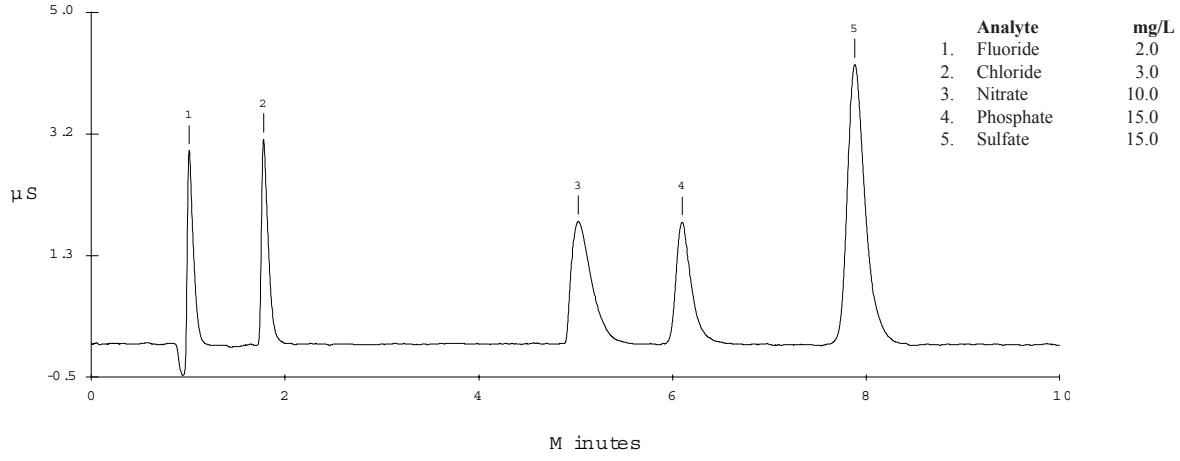


Figure 1
Production Test Chromatogram

5.2 Gradient Separation of a Few Organic and Inorganic Anions

The standard used to generate the sample chromatogram is a simplified version of the one used in Section 5.3. It can be used to test the standard gradient conditions in your system and, if necessary, optimize the gradient slope and/or composition.

Sample Volume:	10 µL
Trap Column:	Anion Trap Column (ATC-3, P/N 059660)
Column:	IonPac AS5A-5µ Analytical Column
Eluents:	Eluent 1: 0.75 mM NaOH Eluent 2: 200 mM NaOH
Eluent Flow Rate:	1.0 mL/min
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS-ULTRA (4-mm) AutoSuppression Recycle Mode
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III (4-mm)
MMS Regenerant:	20 mN H_2SO_4
Expected Background Conductivity:	0.75 mM NaOH; 2-4 µS 50 mM NaOH; 4-7 µS
Expected System Operating Backpressure:	2200-2500 psi

GRADIENT PROGRAM

TIME (Minutes)	% E1	% E2	INJECTION VALVE
0	100	0	inject
5	100	0	
15	85	15	
30	57	43	
30.1	100	0	load
43	100	0	

NOTE

Thirteen minutes are required at the end of the program for reequilibration of the column with E1 prior to injecting the next sample. If the system is not used continuously, that is, the gradient program is not started every 43 minutes exactly, the gradient program can be modified to start with 2 minutes of the highest eluent concentration (57% E1, 43% E2), reequilibrate with E1 for 13 minutes, then make the next injection 15 minutes into the program.

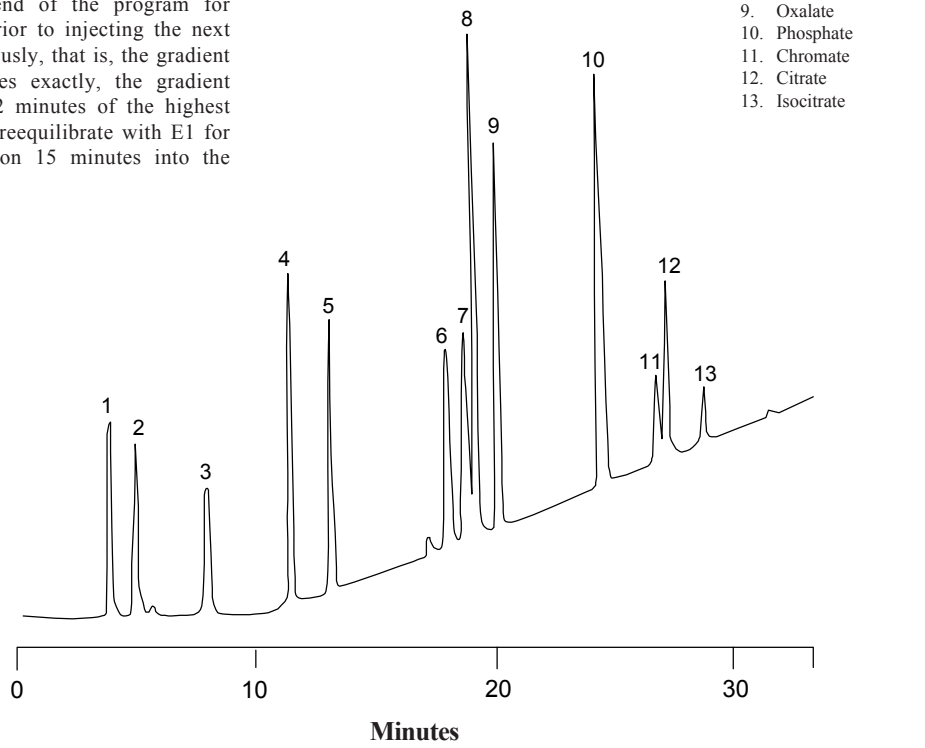


Figure 2
Gradient Separation of a Few Organic and Inorganic Anions

5.3 Gradient Elution of a Large Number of Anions

The sodium hydroxide concentration in Eluent 1 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 3.3 for eluent preparation instructions.

Equilibrate the column thoroughly with Eluent 1 (0.75 mN NaOH) before injecting the sample. Equilibration time is typically 13 minutes. If the final eluent concentration used in a gradient is stronger than the one shown in the example chromatogram (i.e., 43% of E2, 57% of E1), the time required for equilibration will probably be longer.

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.

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

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

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

   For example, if nitrate and sulfate are well resolved but phosphite and bromide are not, multiply the gradient times by a factor less than 1 (e.g., 0.90) to increase the gradient slope. On the other hand, if nitrate and sulfate are coeluting, multiply gradient times by a factor greater than 1 (e.g., 1.1) to make the gradient slope less steep.

   To reduce the total gradient time, and if resolution allows it (i.e., not all the peaks shown the sample chromatogram are present in the sample), multiply the gradient times by a factor less than 1.

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 sulfate to elute earlier than nitrate.

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

1. Make sure that eluents E1 and E2 have been prepared correctly. Too low a hydroxide concentration in one or both eluents will result in poor resolution of phosphite and bromide. Too high a concentration in one or both eluents will result in poor resolution of nitrate and sulfate.

2. Check the eluent flow rate. If the flow rate is greater than 1.0 mL/min, resolution of phosphite and bromide may suffer. If the flow rate is less than 1.0 mL/min, resolution of nitrate and sulfate may improve.

3. 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.

SampleVolume:	10 µL
Trap Column:	ATC-3 (P/N 059660)
Column:	IonPac AS5A-5µ Analytical Column
Eluents:	Eluent 1: 0.75 mM NaOH
	Eluent 2: 200 mM NaOH
Eluent Flow Rate:	1.0 mL/min
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS-ULTRA (4-mm) AutoSuppression Recycle Mode
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III (4-mm)
MMS Regenerant:	20 mN H_2SO_4
Expected Background Conductivity:	0.75 mM NaOH; < 4 µS
	50 mM NaOH; < 7 µS
Expected System Operating Backpressure:	2200-2500 psi

TIME (minutes)	% E1	% E2	INJECTION VALVE
0	100	0	inject
5	100	0	
15	85	15	
30	57	43	
30.1	100	0	load
43	100	0	

NOTE

Thirteen minutes are required at the end of the program for reequilibration of the column with E1 prior to injecting the next sample. If the system is not used continuously, that is, the gradient program is not started every 43 minutes exactly, the gradient program can be modified to start with 2 minutes of the highest eluent concentration (57% E1, 43% E2), reequilibrate with E1 for 13 minutes, then make the next injection 15 minutes into the program.

All anion concentrations are 10 mg/L unless noted

	Analyte	mg/L
1.	Fluoride	1.5
2.	a-Hydroxybutyrate	
3.	Acetate	
4.	Glycolate	
5.	Butyrate	
6.	Gluconate	
7.	a-Hydroxyvalerate	
8.	Formate	5
9.	Valerate	
10.	Pyruvate	
11.	Monochloroacetate	
12.	Bromate	
13.	Chloride	3
14.	Galacturonate	
15.	Nitrite	5
16.	Glucuronate	
17.	Dichloracetate	
18.	Trifluoroacetate	
19.	Phosphite	
20.	Selenite	
21.	Bromide	
22.	Nitrate	
23.	Sulfate	
24.	Oxalate	
25.	Selenate	
26.	a-Ketoglutarate	
27.	Fumarate	
28.	Phthalate	
29.	Oxalacetate	
30.	Phosphate	
31.	Arsenate	
32.	Chromate	
33.	Citrate	
34.	Isocitrate	
35.	cis-Aconitate	
36.	trans-Aconitate	

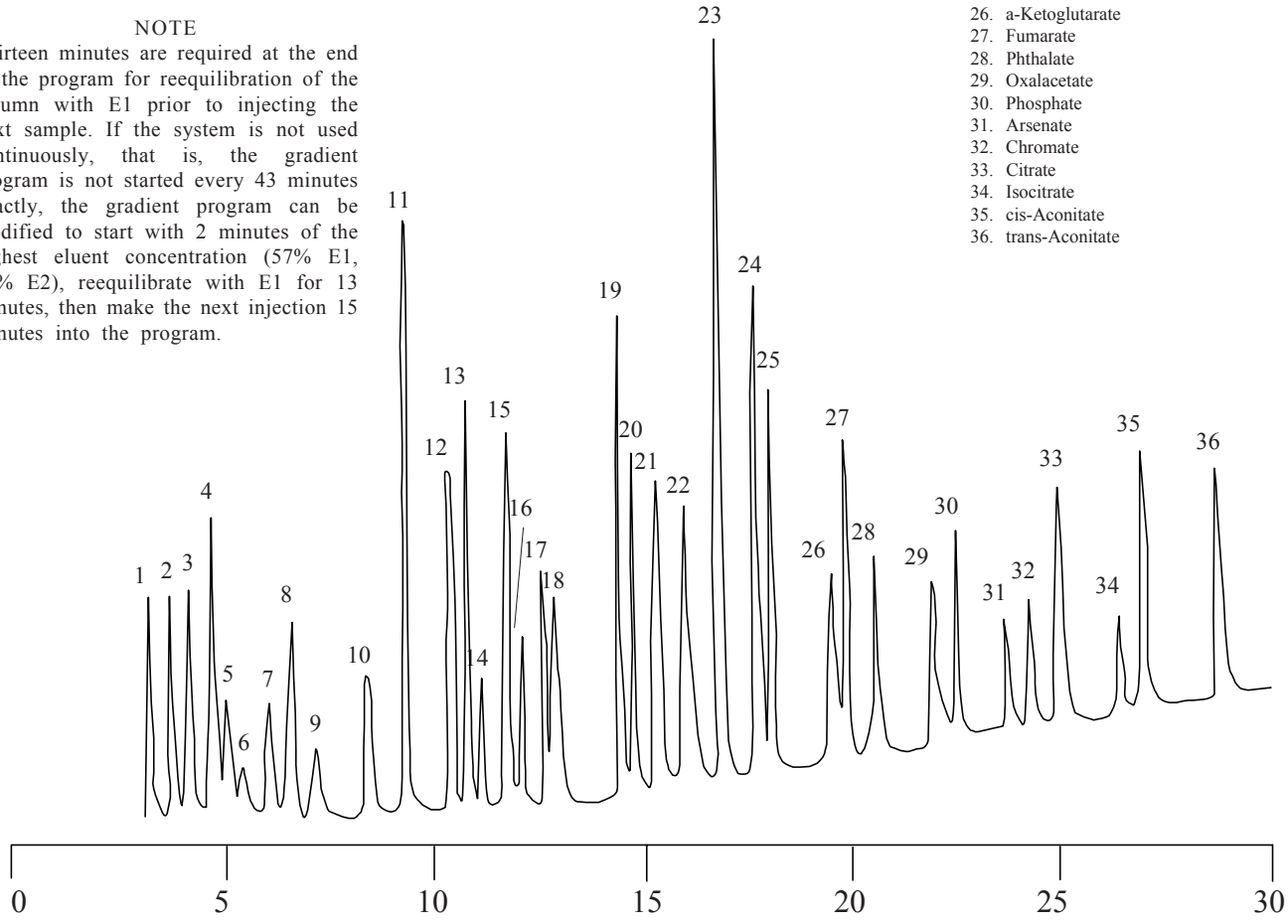


Figure 3
Gradient Elution of a Large Number of Anions

5.4 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 AS5A-5µ 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), 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. Malonate has been found to inhibit the oxidation of pyruvate by any of the catalytically active di- and tri-carboxylic acids of the cycle. Under the gradient conditions shown, malonate coelutes with malate and lactate coelutes with acetate.

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

SampleVolume:	10 µL
Trap Column:	ATC-3 (P/N 059660)
Column:	IonPac AS5A-5µ Analytical Column
Eluents:	Eluent 1: 0.75 mM NaOH Eluent 2: 200 mM NaOH
Eluent Flow Rate:	1.0 mL/min
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS-ULTRA (4-mm) AutoSuppression Recycle Mode
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III (4-mm)
MMS Regenerant:	20 mN H_2SO_4
Expected Background Conductivity:	0.75 mM NaOH; < 4 µS 50 mM NaOH; < 7 µS
Expected System Operating Backpressure:	2200-2500 psi

NOTE
Thirteen minutes are required at the end of the program for reequilibration of the column with E1 prior to injecting the next sample. If the system is not used continuously, that is, the gradient program is not started every 43 minutes exactly, the gradient program can be modified to start with 2 minutes of the highest eluent concentration (57% E1, 43% E2), reequilibrate with E1 for 13 minutes, then make the next injection 15 minutes into the program.

TIME (minutes)	% E1	% E2	INJECTION VALVE
0	100	0	Inject
5	100	0	
15	85	15	
30	57	43	
32	57	43	
32.1	100	0	Load
45	100	0	

1. Acetate
2. Pyruvate
3. Chloride
4. Succinate
5. Malate
6. Sulfate
7. a-Ketoglutarate
8. Fumarate
9. Oxaloacetate
10. Phosphate
11. Citrate
12. Isocitrate
13. cis-Aconitate
14. trans-Aconitate

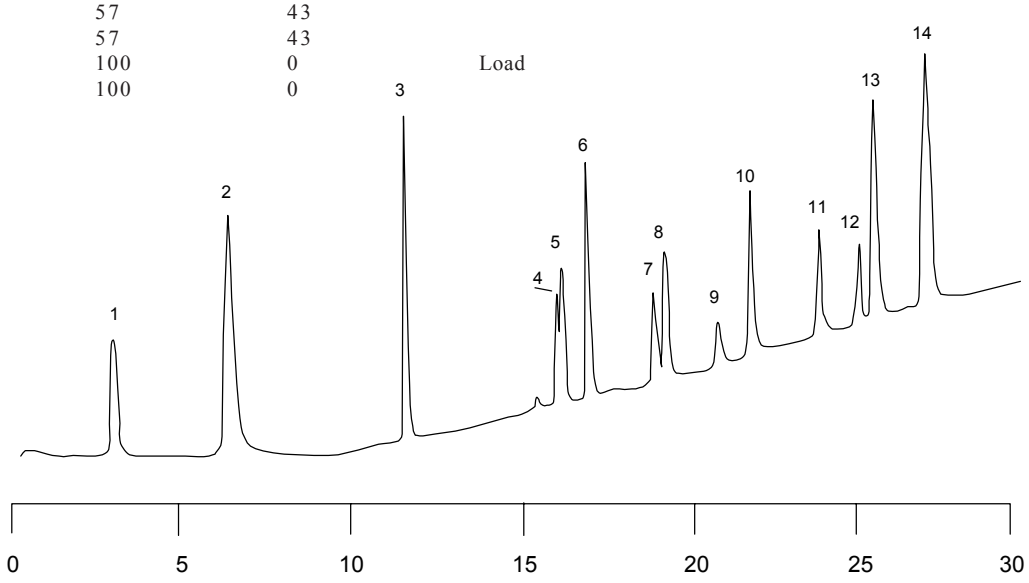


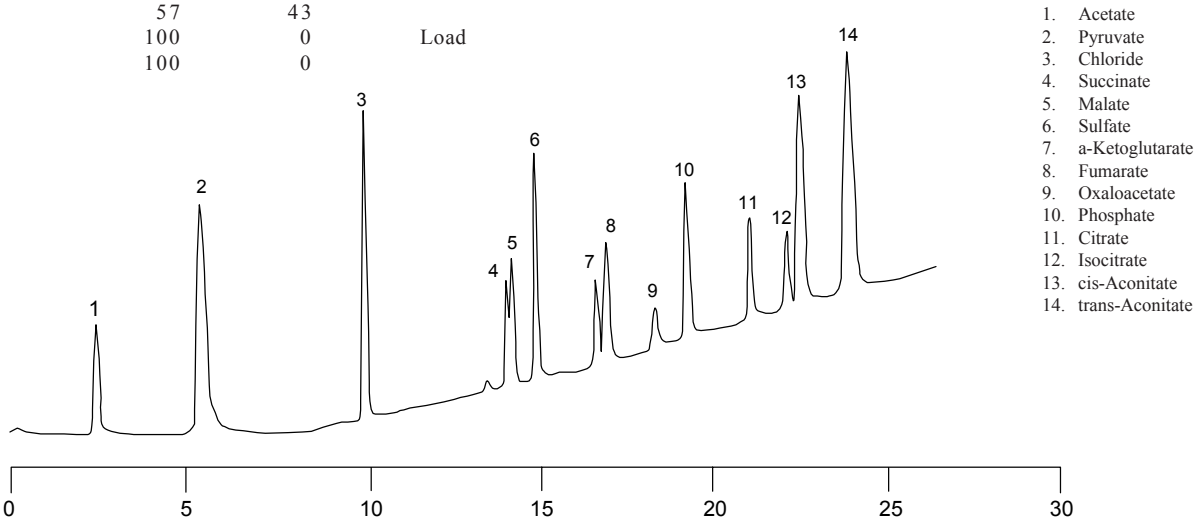
Figure 4
Gradient Separation of Krebs Cycle Acids

5.5 Fast Separation of Krebs Cycle Acids

The sample chromatogram shown in Figure 5 was generated using a steeper gradient than the one shown in Section 5.4 (see Figure 4, "Gradient Separation of Krebs Cycle Acids"). The gradient times were multiplied by a factor of 0.8, resulting in a 20% reduction in the total gradient run time.

Sample Loop Volume:	10 µL
Trap Column:	Anion Trap Column (ATC-3, P/N 059660)
Analytical Column:	IonPac AS5A-5µ Analytical Column
Eluents:	E1: 0.75 mM NaOH E2: 200 mM NaOH
Eluent Flow Rate:	1.0 mL/min
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS-ULTRA (4-mm) AutoSuppression Recycle Mode
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III (4-mm)
MMS Regenerant:	50 mN H_2SO_4
Expected Background Conductivity:	0.75 mM NaOH: < 4 µS 50 mM NaOH: < 7 µS
Expected System Operating Back Pressure:	2200 - 2500 psi

TIME (minutes)	% E1	% E2	INJECTION VALVE
0	100	0	Inject
4	100	0	
12	85	15	
24	57	43	
26	57	43	
26.1	100	0	Load
39	100	0	



1. Acetate
2. Pyruvate
3. Chloride
4. Succinate
5. Malate
6. Sulfate
7. a-Ketoglutarate
8. Fumarate
9. Oxaloacetate
10. Phosphate
11. Citrate
12. Isocitrate
13. cis-Aconitate
14. trans-Aconitate

Minutes

Figure 5
Fast Separation of Krebs Cycle

5.6 Isocratic Separation of Variuos Anions - F^-, Cl^-, SO_4^{2-}, Br^-, $N0_3^-$, and PO_4^{3-}

Depending on the type of anions of interest, it may be possible to find isocratic eluent conditions. Be aware that coelution may occur if other anions with similar selectivities are present in the sample. See Figure 6, "Isocratic Separation of Various Anions - F^-, Cl^-, SO_4^{2-}, Br^-, $N0_3^-$, and PO_4^{3-} " and the example in Section 5.7.

Fluoride, chloride, bromide, nitrate, sulfate and phosphate can be eluted isocratically in less than 8 minutes with 40 mM NaOH eluent. Be aware that coelution may occur if other anions with similar selectivities are present.

Sample Loop Volume:	10 µL
Trap Column:	Anion Trap Column (ATC-3, P/N 059660)
Analytical Column:	IonPac AS5A-5µ Analytical Column
Eluent:	40 mM NaOH
Eluent Flow Rate:	1.0 mL/min
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS-ULTRA (4-mm) AutoSuppression Recycle Mode
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III (4-mm)
MMS Regenerant:	50 mN H_2SO_4

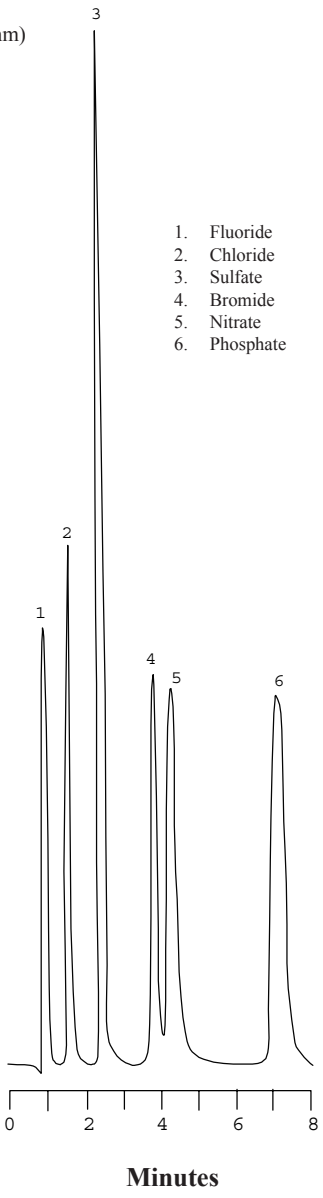


Figure 6
Isocratic Separation of Various Anions - F^-, Cl^-, SO_4^{2-}, Br^-, $N0_3^-$, and PO_4^{3-}

5.7 Isocratic Separation of Fluoride, Acetate, Gluconate, Formate and Pyruvate

Fluoride, acetate, gluconate, formate and pyruvate can be eluted isocratically in less than 7 minutes with 2.0 mN NaOH eluent. Be aware that coelution may occur if other anions with similar selectivities are present. If decreasing retention times or coelutions are observed, clean the column between runs by increasing the strength of the hydroxide eluent to elute carbonate, sulfate and other strongly retained ions (see "Column Care").

Sample Loop Volume:	10 µL
Trap Column:	Anion Trap Column (ATC-3,P/N 059660)
Analytical Column:	IonPac AS5A-5µ Analytical Column
Eluent:	2.0 mN NaOH
Eluent Flow Rate:	1.0 mL/min
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS-ULTRA (4-mm) AutoSuppression Recycle Mode
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III (4-mm)
MMS Regenerant:	50 mN H_2SO_4

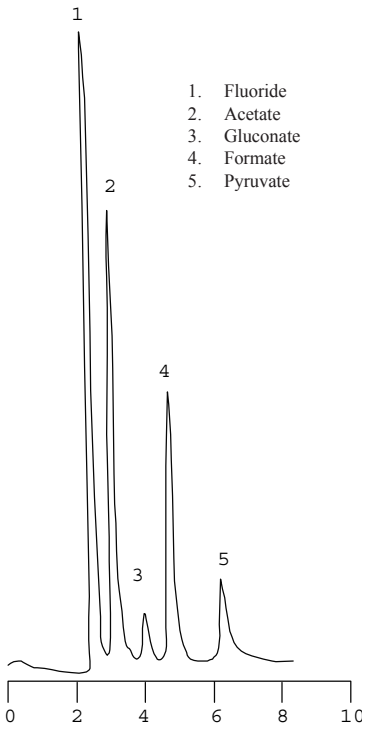


Figure 7
Elution of Fluoride, Acetate, Gluconate, Formate and Pyruvate

SECTION 6 - TROUBLESHOOTING GUIDE

The purpose of the Troubleshooting Guide is to help you solve operating problems that may arise while using IonPac AS5A-5µ column. For more information on problems that originate with the Ion Chromatograph (IC), refer to the Troubleshooting Guide in the appropriate operator's manual. If you cannot solve the problem on your own, contact the DIONEX North America Technical Call Center at 1-800-DIONEX-0 (1-800-346-6390) or the nearest DIONEX Office (see, "DIONEX Worldwide Offices").

Table 6
AS5A-5µ/AG5A-5µ Troubleshooting Summary

Observation	Cause	Action	Reference Section
High Back Pressure	Unknown	Isolate Blocked Component	6.1.1
	Plugged Column Inlet Bed Support	Replace Inlet Bed Support	6.1.2
	Other System Components	Unplug, Replace	Component Manual
High Background Conductivity	Contaminated Eluents	Remake Eluents Check Chemical Source	6.2, 6.2.1
	Contaminated Columns	Clean Column	6.2.2, 6.2.3
	Contaminated Suppressor	Clean Suppressor	6.2.5, Component Manual
	Contaminated Hardware	Clean Component	6.2.4, Component Manual
Poor Resolution	Poor Efficiency Due to Large System Void Volumes	Replumb System	6.3.1.B, Component Manual
	Column Headspace	Replace Column	6.3.1.A
	Column Overloading	Reduce Sample Size	6.3.3.B, 3.4
Short Retention Times	Flow Rate Too Fast	Recalibrate Pump	6.3.2.A
	Conc. Incorrect Eluents	Remake Eluents	6.3.2.B
	Column Contamination	Clean Column	6.3.2.C, 6.3.2.D,
Poor Front End Resolution	Conc. Incorrect Eluents	Remake Eluents	6.3.3.A
	Column Overloading	Reduce Sample Size	6.3.3.B, 3.4
	Sluggish Injection Valve	Service Valve	6.3.3.C, Component Manual
	Large System Void Volumes	Replumb System	6.3.3.D, Component Manual
Spurious Peaks	Sample Contaminated	Pretreat Samples	6.3.4.A, 6.3.4.B
	Sluggish Injection Valve	Service Valve	6.3.4.B, Component Manual

6.1 High Back Pressure

6.1.1 Finding the Source of High System Pressure

Total system pressure for the 4-mm AG5A-5µ Guard Column plus the 4-mm AS5A-5µ Analytical Column when using the test chromatogram conditions should be equal or less than 2,000 psi.

If the system pressure is higher than 2,000 psi for 4-mm system, it is advisable to determine the cause of the high system pressure. The system should be operated with a High-Pressure In-Line Filter (P/N 044105) which is positioned between the Gradient Pump pressure transducer and the injection valve. Make sure you have one in place and that it is not contaminated.

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

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

To determine which part of the chromatographic system is causing the problem, disconnect the pump eluent line from the injection valve and turn the pump on. Watch the pressure; it should not exceed 50 psi. Continue adding system components (injection valve and detector) one by one, while monitoring the system pressure. The pressure should increase up to a maximum when the Guard and Analytical columns are connected (see Table 7, "Typical AS5A-5µ/AG5A-5µ Operating Back Pressures").

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

Table 7
Typical AS5A-5µ/AG5A-5µ Operating Back Pressures

Column	Typical Back Pressure psi (MPa)	Flow Rate mL/min
IonPac AS5A-5µ Analytical	< 1,700 (11.72)	1.0
IonPac AG5A-5µ Guard	< 500 (3.45)	1.0
IonPac AS5A-5µ + AS5G-5µ columns	< 2,200 (15.17)	1.0

6.1.2 Replacing Column Bed Support Assemblies

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

A. Disconnect the column from the system.

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

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

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

	AS5A-5µ 4-mm Columns (P/N)
Analytical Column	037131
Guard Column	037134
Bed Support Assembly	056823
End Fitting	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-lb). Tighten further only if leaks are observed.

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

6.2 High Background or Noise

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

ELUENT	EXPECTED BACKGROUND CONDUCTIVITY
10 mM Hydroxide	2 - 3 µS
50 mM Hydroxide	2 - 5 µS

6.2.1 Preparation of Eluents

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

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

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

6.2.2 A Contaminated Trap Column

High background may be caused by contamination of the ATC-3 with carbonate or other anions from the eluent. Clean the ATC-3 with 100 mL of 2.0 M NaOH. Rinse the ATC-3 immediately with 20 mL of eluent into a beaker prior to use.

6.2.3 A Contaminated Guard or Analytical Column

Remove the AG5A-5µ Guard and AS5A-5µ Analytical Columns from the system. If the background conductivity decreases, the column(s) is (are) the cause of the high background conductivity. Clean or replace the AG5A-5µ at the first sign of column performance degradation (compared to your original installation chromatogram or to the original test chromatogram) to eliminate downtime. Clean the column(s) as instructed in, “Column Cleanup” (See “Column Care”).

6.2.4 Contaminated Hardware

To eliminate the hardware as the source of the high background conductivity, bypass the columns and the Anion Self-Regenerating Suppressor. 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 detector manual for details.

6.2.5 A Contaminated Suppressor

A. The Anion Self-Regenerating Suppressor (ASRS-ULTRA) Cleanup

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

Metal Contaminants or Precipitates

CAUTION

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

1. Turn off the SRS Control unit.

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

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

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

5. Connect a temporary line from the priming block or the low-pressure tee on the isocratic or gradient pump to a container with a solution of 0.2 M oxalic acid. Pump this solution through the ASRS-ULTRA (4-mm) at 1-2 mL/min for 30 minutes.

CAUTION

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

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

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

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

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

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

B. The Anion MicroMembrane Suppressor (AMMS) Cleanup

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

Metal Contaminants or Precipitates

CAUTION

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

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

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

3. Disconnect the liquid line from the AMMS III **ELUENT OUT** port to the cell at the cell fitting and reconnect it to the **REGEN IN** port.

4. Connect a temporary line from the priming block or the low-pressure tee on the isocratic or gradient pump to a container with a solution of 0.2 M oxalic acid. Pump this solution through the AMMS III (4-mm) at 1-2 mL/min for 30 minutes.

CAUTION

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

5. Flush the AMMS III with deionized water for 10 minutes.

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

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

8. Flush the AMMS III with eluent for 10 minutes.

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

6.3 Poor Peak Resolution

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

6.3.1 Loss of Column Efficiency

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

B. Extra-column effects can result in sample band dispersion, making the peaks' elution less efficient. Make sure you are using PEEK tubing with an ID of no greater than 0.010" for 4-mm systems or no greater than 0.005" for 3-mm and 2-mm systems to make all eluent liquid line connections between the injection valve and the detector cell inlet. Cut the tubing lengths as short as possible. Check for leaks.

6.3.2 Poor Resolution Due to Shortened Retention Times

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

A. Check the flow rate. See if the eluent flow rate is equivalent to the flow rate specified by the analytical protocol. Measure the eluent flow rate after the column using a stopwatch and a balance to weigh the collected amount.

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

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

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

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

After cleaning the column, reinstall it in the system and let it equilibrate with eluent for about 30 minutes. No water wash is necessary. The column is equilibrated when consecutive injections of the standard give reproducible retention times. The original column capacity should be restored by this treatment, since the contaminants should be eluted from the column. If you need assistance in solving resolution problems, contact the DIONEX North America Technical Call Center at 1-800-DIONEX-0 (1-800-346-6390) or the nearest DIONEX Office (see, "DIONEX Worldwide Offices").

6.3.3 Loss of Front End Resolution

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

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

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

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

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

6.3.4 Spurious Peaks

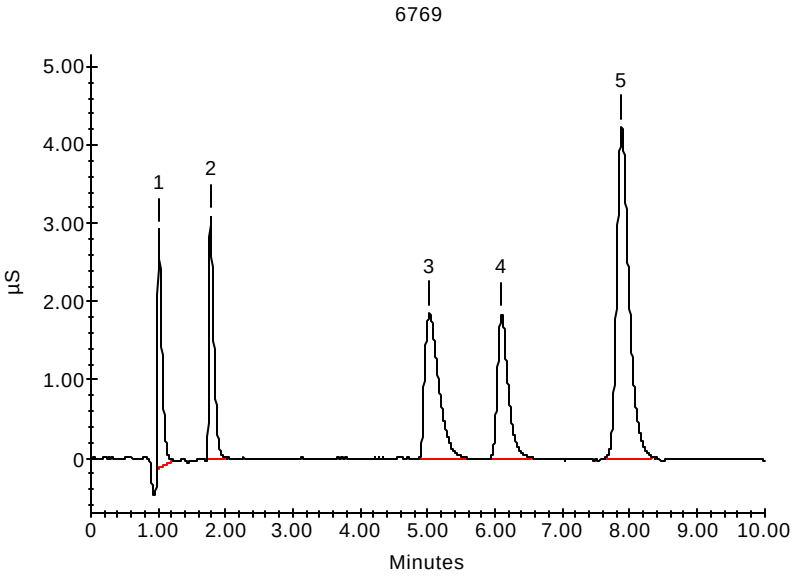
A. **The columns may be contaminated.** If the samples contain an appreciable level of polyvalent ions and the column is used with a weak eluent system, the retention times for the analytes will then decrease and be spurious, inefficient (broad) peaks that can show up at unexpected times. Clean the column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

If you need assistance in determining the best way to clean strongly retained solutes in your specific sample matrix from the AS5A-5µ column, contact the North America Technical Call Center at 1-800-DIONEX-0 (1-800-346-6390) or the nearest DIONEX Office (see, "DIONEX Worldwide Offices").

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

IonPac® AS5A-5µ
Analytical (4 x 150 mm)
Product No. 37131

Serial No. : 6769 **Pressure (PSI) : 1610** **Date : 6/29/00 3:43:50 PM**



Eluent: 3.9 mM $NaHCO_3$ / 3.1 mM Na_2CO_3
Flow Rate: 1.0 mL/min
Detection: Suppressed Conductivity at 30 µSFS
ASRS®-ULTRA
AutoSuppression® Recycle Mode

Injection Volume: 10 µL

Storage Solution: 100 mM NaOH

Peak Information : Found Components

Peak No.	Retention Time	Name	(mg/L)	Efficiency	Asymmetry (2%)	Resolution
1	1.01	Fluoride	2.0	1153	n/a	6.19
2	1.78	Chloride	3.0	2986	3.5	12.78
3	5.03	Nitrate	10.0	2810	3.4	3.24
4	6.10	Phosphate	15.0	7421	2.4	5.83
5	7.88	Sulfate	15.0	9203	2.0	n/a

File Name : C:\PEAKNET\DATA\EXAMPLES\37131 AS5A4MM_010.DXD