

Errata

Product Manual for Dionex IonPac™ Cryptand Columns

031852-03

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

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



PRODUCT MANUAL

IONPAC® CRYPTAND G1 GUARD COLUMN (3 x 30 mm, P/N 059900)

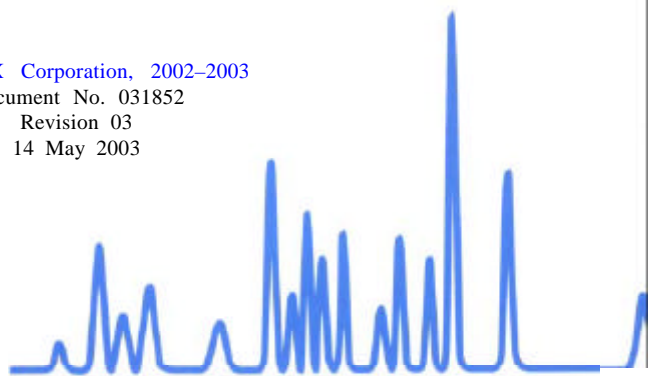
IONPAC® CRYPTAND A1 ANALYTICAL COLUMN (3 x 150 mm, P/N 059898)

QUICKSTART STEPS AND LINKS

Click on the blue text below to get started.

1. See [Section 4, "Operation."](#) Note operation precautions and chemical purity requirements.
2. See [Section 4.4, "Eluent Preparation."](#) Make the required stock and working solutions for eluents.
3. See ["Quality Assurance Report."](#) Run the Production Test Chromatogram as a system check.
4. See [Section 5, "Example Applications,"](#) for example operating conditions and applications.
5. See ["Column Care,"](#) for column cleanup and long-term storage recommendations.

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SECTION 1 - INTRODUCTION

The IonPac Cryptand A1 (3 x 150 mm) is an adjustable capacity anion-exchange column. The Cryptand A1 column is a new advancement in Dionex resin technology which allows column capacity control by changing the cation component of the hydroxide eluent. The IonPac Cryptand A1 column uses a unique resin technology based on a cryptand molecule, covalently attached to a macroporous, styrene divinylbenzene resin as illustrated in Figure 1. A cryptand is a bi-cyclic compound capable of complexing metal cations such as sodium, lithium or potassium. In the presence of metal cations, the cryptand molecule generates a positively charged anion-exchange site.

The Cryptand A1 column uses lithium, sodium or potassium hydroxide eluents with each eluent producing a different column capacity range. The capacity of the column is related to the binding constant of the complexed cation for the cryptand functional group. The higher the binding constant, the higher the capacity resulting from the higher number of ion-exchange sites created. Table 1 is a table of binding constants for various cations. As can be seen, lithium has a very low binding constant, and hence very few ion-exchange sites are generated resulting in near zero capacity. Potassium, however, has a very high binding constant which gives a very high capacity. Sodium gives an intermediate capacity, resulting from a binding constant between that of lithium and potassium. The capacity of the column is also related to the concentration of the eluent cation, low concentration resulting in low capacity and high concentration resulting in higher capacity.

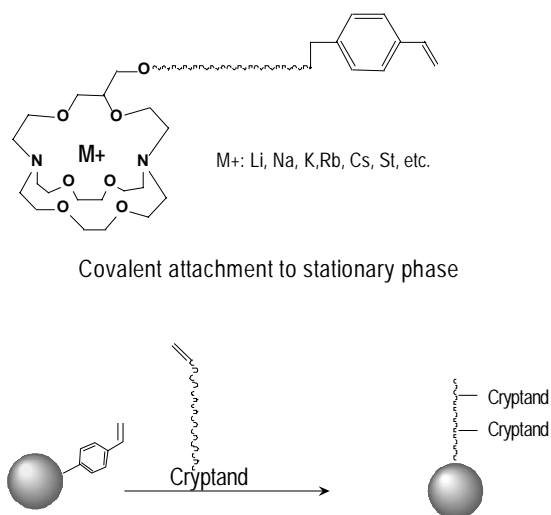
Sodium hydroxide and lithium hydroxide can be used individually or together in capacity gradients for most inorganic anion separations. For applications such as the separation of quinate, glycolate, acetate, and formate, increased retention is required. For this application, a potassium hydroxide eluent (binding constant ~ 5.4) can be used.

Table 1
Binding Constants for 2,2,2 Cryptand

Cation	Log K
Li ⁺	~1.0
Cs ⁺	<2.0
Na ⁺	3.9
Rb ⁺	4.35
Ca ²⁺	4.4
NH ₄ ⁺	4.5
K ⁺	5.4
Sr ²⁺	8.0
Ba ²⁺	9.5
Cu ²⁺	6.8
Ag ⁺	9.6

1.1 Advantages of Using Capacity Gradients

What is a capacity gradient? A capacity gradient starts with the column in one form and at some point during the run, a step change to a different cation changes the capacity of the column during the run. An example of a capacity gradient starts a run using sodium hydroxide as the starting eluent. The column will exhibit an intermediate capacity as long as the sodium cation is passing through the column. A step change to lithium hydroxide, for example, will result in a gradual change in the column capacity to a very low capacity. The result is shorter run times and peak focusing. "Capacity gradients" are the recommended mode of operation for the Cryptand A1 column. Shorter run times and improved peak focusing can be achieved using capacity gradients. Typically, the gradient starts with an initial eluent of 10 mM sodium hydroxide, producing a moderate capacity anion-exchange surface. The column is converted to a low capacity anion exchange surface with a step change to 10 mM lithium hydroxide at 0.1 minutes. For cryptand columns, capacity gradients can be used to dramatically shorten run times, rather than using an eluent concentration gradient, which is used with conventional anion exchange columns. In addition, the Cryptand A1 can be used in the conventional mode with an eluent concentration gradient.



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Figure 1
Cryptand Resin Structure Using 2,2,2 Cryptand Monomer

Table 2
Cryptand Column Specifications

Column	Particle Diameter μm	Substrate* X-linking %	Column Capacity μeq/column	Functional Group	Hydrophobicity
Cryptand A1 3 x 150 mm	5.0	55	85	Cryptand	Variable (eluent dependent)
Cryptand G1 3 x 30 mm	5.0	55	17	Cryptand	Variable (eluent dependent)

* macroporous (100 Å) divinylbenzene/ethylvinylbenzene polymer

Table 3
Cryptand Operating Parameters

Column	Typical Back Pressure psi (MPa)	Standard Flow Rate mL/min	Maximum Flow Rate mL/min
Cryptand A1 3-mm Analytical	≤ 1,500 (10.34)	0.5	1.5
Cryptand G1 3-mm Guard	≤ 350 (3.08)	0.5	1.5
Cryptand A1 + Cryptand G1	≤ 1,850 (13.42)	0.5	1.5

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 - ION CHROMATOGRAPHY SYSTEM

CONDITION	3-mm System
Eluent Flow Rate	Typically 0.5 mL/min
Self-Regenerating Suppressor	ASRS-ULTRA (2-mm) (P/N 053947)
MicroMembrane Suppressor	AMMS III (2-mm) (P/N 057751)
Atlas Suppressor	AAES (P/N 056116)
NOTE Do not operate suppressors over 40°C. If an application requires a higher temperature, place the suppressor outside the chromatographic oven.	
Regenerant Flow Rate	Typically 3 - 8 mL/min
Injection Loop	Typically 5 - 25 µL
System Void Volume	Eliminate switching valves, couplers and the Gradient Mixer since an ATC-3 will be installed (See Section 3.2, "Installing the Anion Trap Column for Eluent Step Change or Gradient Operation.")
Pumps	Use the GS50/GP50/GP40/IP25/IP20 in Microbore Configuration with a Microbore GM-4 (2-mm) Gradient Mixer. No External Gradient Mixer is required for the GS50/GP50/GP40 Pump when performing gradient analysis. 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.

CONDITION	3-mm System
Detectors	<p>AD20/AD25 Cell (6-mm, 7.5 µL, P/N 046423) (10-mm, 9 µL, P/N 049393)</p> <p>VDM-2 Cell (3-mm, 2.0 µL, P/N 043120) (6-mm, 10 µL, P/N 043113)</p> <p>CD20, CD25, CD25A, ED40, ED50, or ED50A Conductivity Cell with DS3 (P/N 044130) or Conductivity Cell with shield (P/N 044132)</p> <p>CDM-2/CDM-3 Cell (P/N 042770)</p> <p>Replace the TS-1 with the TS-2 (P/N 043117) on the CDM-2 or the CDM-3. The TS-2 has been optimized for 2-mm operation. Do not use the TS-2 or the TS-1 with the ED40/ED50/ED50A or the CD20/CD25/CD25A.</p> <p>Ensure 30-40 psi back pressure after the cell.</p>

Table 4
Tubing Back Pressures

Color	Dionex P/N	ID inches	ID cm	Volume mL/ft	Back pressure Psi/ft at 1 mL/min	Back pressure Psi/ft at 0.25 mL/min	Back pressure Psi/cm at 1 mL/min
Green	044777	0.030	0.076	0.137	0.086	0.021	0.003
Orange	042855	0.020	0.051	0.061	0.435	0.109	0.015
Blue	049714	0.013	0.033	0.026	2.437	0.609	0.081
Black	042690	0.010	0.025	0.015	6.960	1.740	0.232
Red	044221	0.005	0.013	0.004	111.360	27.840	3.712
Yellow	049715	0.003	0.008	0.001	859.259	214.815	28.642

SECTION 3 - INSTALLATION

3.1 System Requirements

3.1.1 System Requirements for 3-mm Operation

The Cryptand A1 3-mm Guard and Analytical Columns are designed to be run on the Dionex Ion Chromatographs equipped with a microbore pump and suppressed conductivity detection. For analyses at flow rates below 0.5 mL/min and gradient analyses, a microbore gradient pump (1/16" pistons) must be employed.

See Section 2, "3-mm Ion Chromatography System," for 3-mm system detector, cell and thermal stabilizer requirements.

3.2 Installing the Anion Trap Column for Eluent Step Change or Gradient Operation

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

The Cryptand A1 3-mm can be used with either ATC-3 column. Using the ATC-3 (4-mm), results in a slightly longer delay time for step changes or gradients due to a slightly higher void volume than the ATC-3 (2-mm). The 4-mm ATC-3 has the advantage however of lasting longer before regeneration is required since it has a larger volume of resin relative to the 2-mm ATC-3.

As suggested in the ATC-3 manual, regenerate the ATC-3 with 100 mL of 2 M sodium hydroxide. Then, rinse with 20 mL of eluent into a waste beaker prior to use. For details of regenerating the ATC-3 column, refer to the ATC-3 Product Manual (Document No. 032697).

3.3 The Injection Loop

3.3.1 The 3-mm System Injection Loop, 5 μ L

For most applications on a 3-mm analytical system, a 5 μ L injection loop is sufficient. Generally, you should not inject more than a total of 25 nanomoles of all analytes onto a 3-mm analytical column. Injecting larger number of moles of analyte can result in overloading the column which can affect the detection linearity. For low concentrations of analytes, larger injection loops can be used to increase sensitivity. The Cryptand A1 3-mm requires a microbore HPLC system configuration. Keep all tubing lengths between modules, columns, suppressors, etc., to a minimum. This will ensure peak efficiencies are optimum. Use either 0.010" (black) or 0.005" (red) tubing to minimize system void volume. Note that for the 0.005" PEEK tubing, the volume per unit length is 0.322 μ L/in, or 0.127 μ L/cm. For the 0.010" PEEK tubing, the volume is 1.287 μ L/in or 0.507 μ L/cm.

3.4 Cryptand G1 Guard Column

A Cryptand A1 Guard Column is normally used with the Cryptand A1 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. The guard column should be cleaned or replaced on a regular basis. Replacing the Cryptand G1 Guard Column at the first sign of peak efficiency loss or decreased retention time will prolong the life of the Cryptand A1 Analytical Column.

3.5 Eluent Storage

Cryptand A1 columns are designed to be used with lithium, sodium, or potassium 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 Cryptand A1 column performance.

It is also very important to use the recommended sources for all eluents. See section 5.2, “Eluent Preparation,” for all recommended sources.

3.6 Suppressor Requirements

3.6.1 Anion Self-Regenerating Suppressor Requirements

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

NOTE

Use the AutoSuppression External Water Mode for eluents containing solvents.

If you are installing a Cryptand A1 3-mm Analytical Column, use an ASRS ULTRA (2-mm, P/N 053947). Use a current setting consistent with the recommendations of the Anion Self-Regenerating Suppressor, “Installation.”

For detailed information on the operation of the Anion Self-Regenerating Suppressor, see Document No. 031367, the “Product Manual for the Anion Self-Regenerating Suppressor ULTRA, the ASRS ULTRA.”

3.6.2 Anion Atlas Electrolytic Suppressor Requirements

An Atlas® Anion Electrolytic Suppressor (AAES) may be used instead of an ASRS ULTRA for applications that require suppressed conductivity detection. The AAES™ (P/N 056116) can be used for 3-mm IonPac Cryptand A1 applications using eluents up to 25 µeq/min.

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

3.6.3 Anion MicroMembrane Suppressor Requirements

An Anion MicroMembrane Suppressor AMMS® III 2-mm (P/N 056571) may be used instead of an ASRS ULTRA (2-mm). 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.”

3.6.4 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).

SECTION 4 - OPERATION

4.1 General Operating Conditions

Sample Volume:	5 μ L Loop
Column:	Cryptand A1 3-mm Analytical Column + Cryptand G1 3-mm Guard Column
Test Chromatogram Eluent:	15 mM NaOH
Eluent Flow Rate:	0.5 mL/min
Temperature:	35°C
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS ULTRA (2-mm) in either External Water Mode or AutoSuppression Recycle Mode
or AES Suppressor:	Anion Atlas Electrolytic Suppressor, AAES if eluent suppression required is less than 25 μ eq/min.
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III (2-mm)
MMS Regenerant:	50 mN H ₂ SO ₄ and the Regenerant Flow Rate is 3 - 8 mL/min.
Expected Background	
Conductivity:	2-3 μ S
Storage Solution:	Eluent

4.2 Cryptand A1 Operation Precautions

CAUTIONS

Filter and Degas Eluents

Filter Samples

Eluent pH between 0 and 14

Sample pH between 0 and 14

1.5 mL/min Maximum Flow Rate for 3-mm Columns

Maximum Operating Pressure = 3,000 psi (20.68 MPa)

4.3 Chemical Purity Requirements

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

4.3.1 Inorganic Chemicals

Reagent Grade inorganic chemicals should always be used to prepare ionic eluents. Whenever possible, inorganic chemicals that meet or surpass the latest American Chemical Society standard for purity should be used.

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 μ m. Bottled HPLC-Grade Water (with the exception of Burdick & Jackson) should not be used since most bottled water contains an unacceptable level of ionic impurities.

4.4 Eluent Preparation

NOTE

Dionex Strongly Recommends that you use the following sources for all hydroxides used in eluent preparation:

Lithium Hydroxide

Aldrich, Lithium Hydroxide monohydrate, 99.95%, 50g (P/N: 25,427-4)

Sodium Hydroxide:

Fisher Chemicals, Sodium Hydroxide Solution, 50% w/w, 500 mL (P/N SS254-500)

Potassium Hydroxide:

Fisher Chemicals, Potassium Hydroxide, 45% w/w, 500 mL (P/N SP236-500)

4.4.1 Hydroxide Eluent Preparation

Weight Method

When formulating eluents from 50% sodium hydroxide, or from 45% potassium hydroxide, Dionex suggests that you use a balance to weigh the required amount.

Example: To make 1 L of 15 mM NaOH use 1.20 g of 50% sodium hydroxide:

$$\text{For 15 mM: } \frac{0.015 \text{ mole/L} \times 40.01 \text{ g/mole}}{50\%} = 1.20 \text{ g diluted to 1 L}$$

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.

$$g = dvr$$

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 = % concentration of the concentrated solution

Example: To make 1 L of 15 mM NaOH, use 0.79 mL of 50% sodium hydroxide:
(as used in Section 5.3, "Production Test Chromatogram")

$$\text{For 15 mM: } \frac{0.015 \text{ mole/L} \times 40.01 \text{ g/mole}}{0.50 \times 1.53 \text{ g/mL}} = 0.79 \text{ mL diluted to 1 L}$$

* 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.

Hydroxide Eluents

Dilute the amount of hydroxide specified in Table 5, "Preparation of Standard Cryptand A1 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.

Table 5
Preparation of Standard Cryptand A1 Eluents

Eluent Concentration (mM)	50% (w/w) NaOH* g (mL)	45% KOH** g (mL)	100% (solid) LiOH·H₂O g
10	0.80 (0.52)	1.25 (0.83)	0.42
15	1.20 (0.79)	1.87 (0.79)	0.63
25	2.00 (1.31)	3.12 (2.06)	1.05
50	4.00 (2.62)	6.23 (4.12)	2.10
100	8.00 (5.24)	12.47 (8.23)	4.20
200	16.00 (10.48)	24.93 (16.47)	8.40
	*d=1.526g/mL FW=40.00	**d=1.514g/mL FW=56.10	FW=41.96

4.5 Solvents

Solvents can be added to the ionic eluents used with Cryptand A1 columns to modify the ion exchange process or improve sample solubility. The solvents used must be free of ionic impurities. However, since most manufacturers of solvents do not test for ionic impurities, it is important that the highest grade of solvents available be used. Currently, several manufacturers are making ultra high purity solvents that are compatible for HPLC and spectrophotometric applications. These ultra high purity solvents will usually ensure that your chromatography is not affected by ionic impurities in the solvent. At Dionex, we have obtained consistent results using High Purity Solvents manufactured by Burdick and Jackson and Optima® Solvents by Fisher Scientific.

When using a solvent in an ionic eluent, column generated back pressures will depend on the solvent used, concentration of the solvent, the ionic strength of the eluent and the flow rate used. The column back pressure will vary as the composition of water-methanol and water-acetonitrile mixture varies. The practical back pressure limit for the Cryptand A1 columns is 4,000 psi (27.57 MPa).

The Cryptand A1 can withstand common HPLC solvents in a concentration range of 0 - 100%. Solvents and water should be premixed in concentrations which allow proper mixing by the gradient pump and to minimize outgassing. Ensure that all of the inorganic chemicals are soluble in the highest solvent concentration to be used during the analysis.

Table 6
HPLC Solvents for Use with Cryptand A1 Columns

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

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

CAUTION

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

4.5.1 Making Eluents Containing Solvents

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

NOTE

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

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

Acetonitrile (ACN) hydrolyzes to ammonia and acetate when left exposed to basic solutions. To prevent eluent contamination from acetonitrile hydrolysis, always add acetonitrile to basic aqueous eluents by proportioning the acetonitrile into the basic eluent with the gradient pump. Keep the acetonitrile in a separate eluent bottle containing only acetonitrile and water. Never add the acetonitrile directly to the basic carbonate or hydroxide eluent bottle.

4.6 Regenerant Preparation for 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 ULTRA (ASRS ULTRA) see Document No. 031727, the “Product Manual for the Anion MicroMembrane Suppressor III.”

SECTION 5 - EXAMPLE APPLICATIONS

5.1 Recommendations for Optimum Performance

The chromatograms in this section were obtained using columns that reproduced the Production Test Chromatogram (see Section 5.2, "Production Test Chromatograms") 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 Cryptand A1 is designed for the determination of polyvalent anions including polyphosphates and polysulfonates. 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, lithium, or potassium hydroxide is converted to water in the suppressor, they are the best choices for eluents. 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 10 mM NaOH and end at 100 mM NaOH, with only a resulting 1 to 3 μ S total baseline change.

Ensure that your system is properly configured.

It is very important that applications run on 3-mm columns utilize the proper pump configuration (see Section 2, "The 3-mm Ion Chromatography System") and have all system void volumes minimized. Fluctuations in operating temperature can affect the retention time and resolution of analytes and should be controlled.

Ensure that adequate equilibration time is allowed between runs.

If downward shift in baseline is observed during the isocratic section of the chromatogram, increase the equilibration time.

Ensure that all of the eluents have been made from high purity reagents and deionized water.

All water used in the preparation of eluents should be degassed, deionized water. For chemical purity requirements see Section 4.3, "Chemical Purity Requirements."

Install an Anion Trap Column, ATC-3 (2-mm).

See Section 3.2 of "Product Manual for the Anion Trap Column." Install the ATC-3 Trap Column to minimize the baseline shift and to improve retention time reproducibility of analytes when doing gradient chromatography and to keep baseline shift to a minimum. (Refer to the ATC-3 column cleanup protocol in Section 6.2.2, "A Contaminated Trap Column.")

Use a guard column to protect the analytical column.

If column performance deteriorates and it is determined that the guard and analytical columns have been fouled, refer to the column cleanup protocols in section 6.2.3, "Column Care."

NOTE

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

5.2 Production Test Chromatogram

Isocratic elution of anions on the Cryptand A1 Analytical Column has been optimized utilizing a sodium hydroxide eluent. By using this eluent, mono- and divalent anions can be isocratically separated and quantitated in a single injection. The Cryptand A1 Analytical Column should always be used with the Cryptand G1 Guard Column. To guarantee that all Cryptand A1 Analytical Columns meet high quality and reproducible performance specification standards, all columns undergo the following production control test. The Cryptand A1 column should be operated at elevated temperature to ensure reproducible retention times.

Sample Volume: 5 μ L Loop
Column: Cryptand A1 3-mm Analytical Column
Trap Column: ATC-3 (2-mm)
Test Chromatogram Eluent: 15 mM NaOH
Eluent Flow Rate: 0.5 mL/min
Temperature: 35°C
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 H₂SO₄ and the Regenerant Flow Rate is 3 - 8 mL/min
or AES Suppressor: Anion Atlas Electrolytic Suppressor, AAES
Expected Background Conductivity: 2-3 μ S
Storage Solution: Eluent

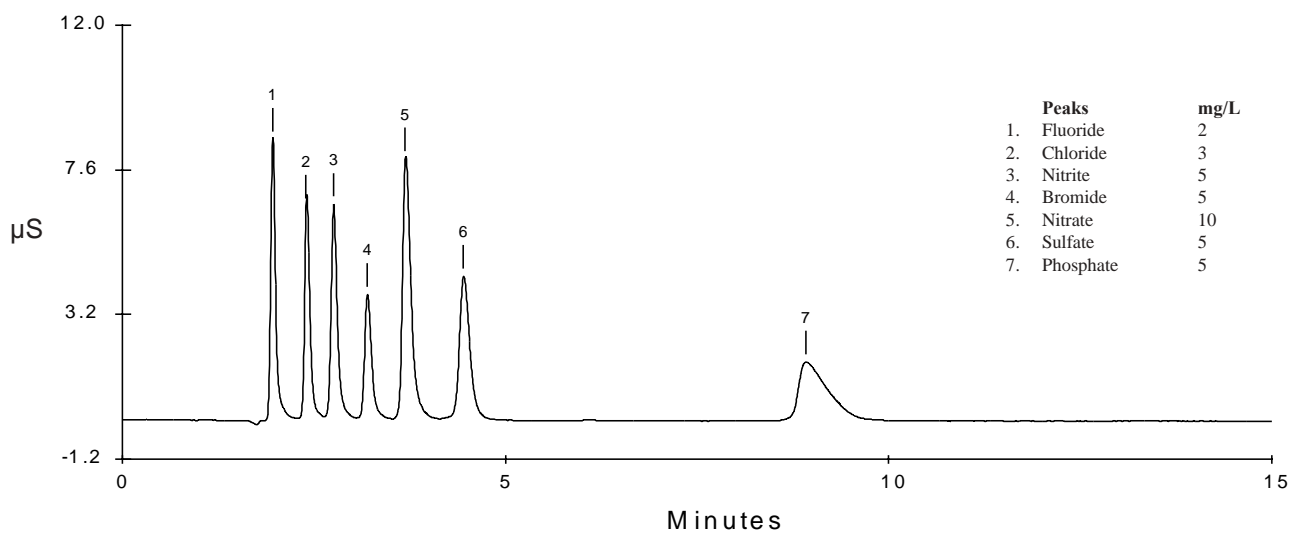


Figure 3
Production Test Chromatogram

5.3 Capacity Dependence Versus Cation Form

The capacity of the Cryptand based stationary phase is determined by the cation with which it is complexed. This figure shows a comparison of the effect on capacity when using different cationic eluents. 70 mM eluent concentration was used in this example. Potassium hydroxide provides the highest capacity and sodium provides moderate capacity. Lithium provides almost no capacity as is observed in the bottom chromatogram. The lower the binding constant, the lower the capacity.

Sample Volume:	5 μ L Loop
Trap Column:	ATC-3 (2-mm)
Column:	Cryptand A1 3-mm Analytical Column
Test Chromatogram Eluent:	See Chromatogram, 30 mM each
Eluent Flow Rate:	0.5 mL/min
Temperature:	35°C
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS ULTRA (2-mm) External Water Mode
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III (2-mm)
MMS Regenerant:	50 mN H ₂ SO ₄ and the Regenerant Flow Rate is 3 - 8 mL/min
or AES Suppressor	Anion Atlas Electrolytic Suppressor, AAES
Expected Background Conductivity:	2-3 μ S
Storage Solution:	Eluent

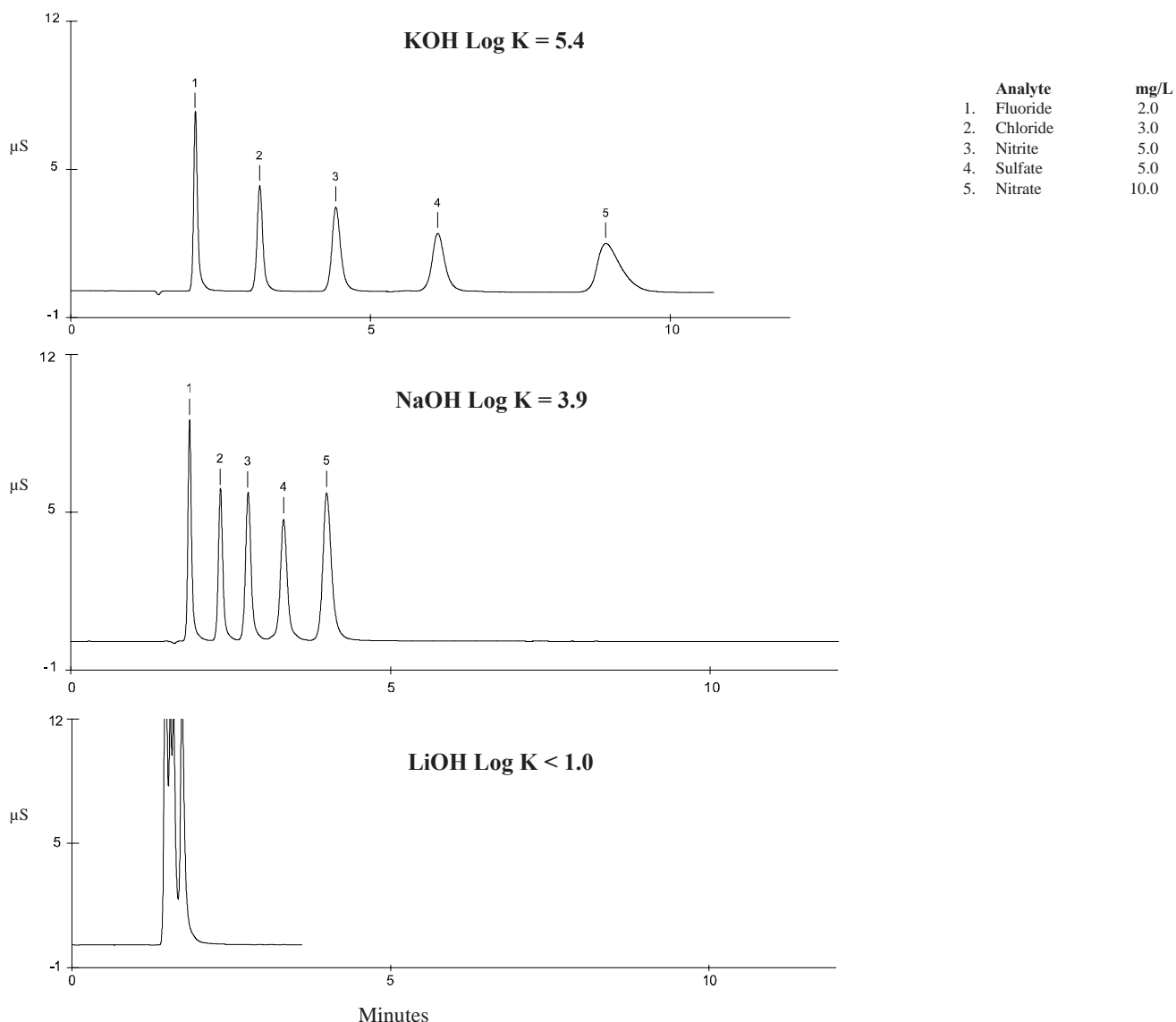


Figure 4
Capacity Dependence vs Cation Form

5.4 Capacity Gradient

“Capacity gradients” are the recommended mode of operation for the Cryptand A1 column. Shorter run times and improved peak focusing can be achieved using capacity gradients. The gradient starts with an initial eluent of 10 mM sodium hydroxide, producing a moderate capacity anion-exchange surface. The column is converted to a low capacity anion exchange surface with a step change to 10 mM lithium hydroxide at 0.1 minutes. For cryptand columns, capacity gradients can be used to dramatically shorten run times, rather than using an eluent concentration gradient, which is used with conventional anion exchange columns. In addition, the Cryptand A1 can be used in the conventional mode with an eluent concentration gradient.

Using capacity gradients allows control of the capacity of the column during the run to control efficiency and method run time. This chromatograph illustrates the use of a capacity gradient for the determination of inorganic anions and polarizable anions. This separation can be accomplished in less than 10 minutes with excellent separation of the common anions along with excellent peak shape for the hydrophobic anions including iodide, thiocyanate, thiosulfate, and perchlorate. By using a capacity gradient, highly retained anions can be eluted at low eluent concentrations, thus providing lower noise and improved detection limits.

Column:	Cryptand A1 3-mm Analytical Column	Peaks	mg/L
Trap Column:	ATC-3 (2-mm)	1. Fluoride	2
Eluent:	10 mM NaOH, step at 0.1 min. to 10 mM LiOH	2. Chloride	3
Flow Rate:	0.5 mL/min.	3. Nitrite	5
Inj. Volume:	5 μ L	4. Bromide	10
Temperature:	35°C	5. Nitrate	10
Detection:	Suppressed Conductivity	6. Sulfate	5
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS ULTRA (2-mm) Recycle Mode	7. Thiosulfate	10
or AES Suppressor:	Anion Atlas Electrolytic Suppressor, AAES	8. Phosphate	15
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III (2-mm)	9. Iodide	10
MMS Regenerant:	50 mN H ₂ SO ₄	10. Thiocyanate	10
		11. Perchlorate	15

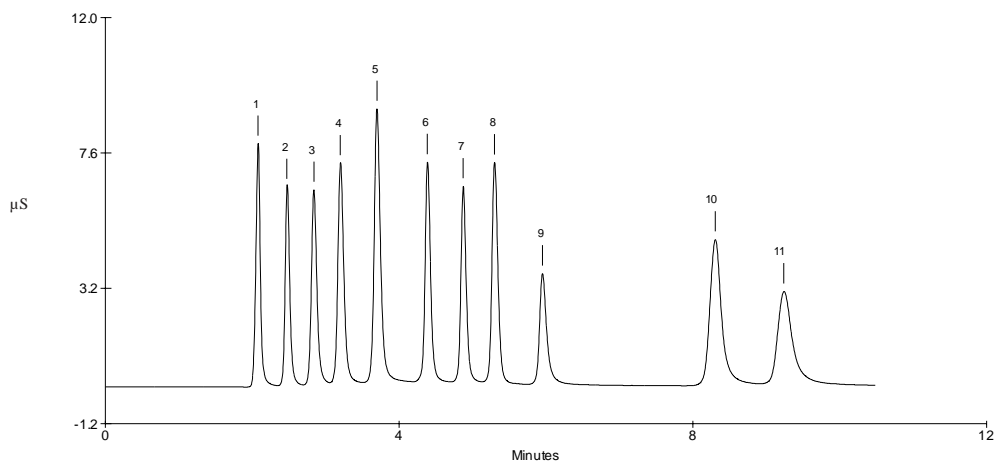


Figure 5
Capacity Gradient

5.5 Isocratic vs. Capacity Gradients

This figure shows a comparison of isocratic eluents vs. capacity gradients. Capacity gradients are the recommended mode of operation for the Cryptand A1 column. In the top chromatogram, a 10 mM sodium hydroxide eluent is used to separate the common inorganic anions. The bottom chromatogram demonstrates the use of a capacity gradient. The gradient starts with an initial eluent of 10 mM sodium hydroxide, producing a moderate capacity anion-exchange surface. The column is converted to a low capacity anion exchange surface with a step change to 10 mM lithium hydroxide at 0.1 minutes. Using capacity gradients allows control of the capacity of the column during the run to control efficiency and method run time. For Cryptand columns, capacity gradients can be used to dramatically shorten run times, rather than using an eluent concentration gradient, which is used with conventional anion exchange columns. In addition, the Cryptand A1 can be used in the conventional mode with an eluent concentration gradient. By using a high capacity gradient, highly retained anions can be eluted at low eluent concentrations, thus providing lower noise and improved detection limits.

Column:	Cryptand A1 3-mm Analytical Column		
Trap Column:	ATC-3 (2-mm)		
Eluent:	A) 10 mM NaOH B) 10 mM NaOH, step at 0.1 min to 10 mM LiOH		
Flow Rate:	0.5 mL/min.		
Inj. Volume:	5 µL		
Temperature:	35°C		
Detection:	Suppressed Conductivity		
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS ULTRA (2-mm) External Water Mode		
or AES Suppressor:	Anion Atlas Electrolytic Suppressor, AAES		
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III (2-mm)		
MMS Regenerant:	50 mN H ₂ SO ₄		

Peaks	mg/L
1. Fluoride	2
2. Chloride	3
3. Nitrite	5
4. Bromide	10
5. Nitrate	10
6. Sulfate	5
7. Phosphate	15

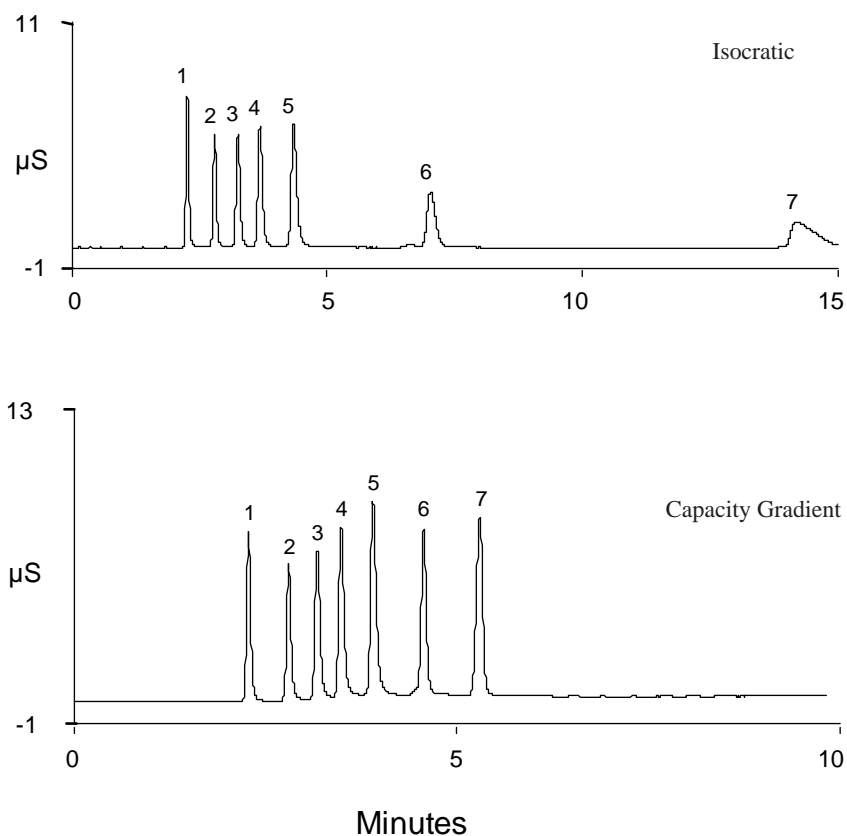


Figure 6
Isocratic vs. Capacity Gradients

5.6 Effect Of Expended ATC-3

How do you know when its time to regenerate the ATC-3? The example below illustrates the baseline you will see when your ATC-3 is beginning to expend. Note the hump appearing underneath the peaks at three to six minutes. As the ATC-3 gets closer and closer to being completely expended, this hump will continue to increase in size. As soon as you begin to see this phenomenon, regenerate the ATC-3. Regenerate the ATC-3 by running 100 mL of 2 M NaOH at 2 mL/min through the ATC-3 with the effluent going to waste. Before using again, run at least 10 mL of eluent through the column to rinse out the excess NaOH. After installation, you will see an initial high background that will decrease down to normal in approximately 15 minutes.

NOTE

If you are running isocratically with a nearly expended ATC-3 you will not see a hump, but rather an increasing background with time. If this occurs, regenerate the ATC-3.

Column:	Cryptand A1 3-mm Analytical Column		
Trap Column:	ATC-3 (2-mm)		
Eluent:	10 mM NaOH, step at 0.1 min. to 10 mM LiOH		
Flow Rate:	0.5 mL/min.		
Inj. Volume:	5 μ L		
Temperature:	35°C		
Detection:	Suppressed Conductivity		
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS ULTRA (2-mm) Recycle Mode		
or AES Suppressor:	Anion Atlas Electrolytic Suppressor, AAES		
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III (2-mm)		
MMS Regenerant:	50 mN H ₂ SO ₄		
		Peaks	mg/L
		1. Fluoride	2
		2. Chloride	3
		3. Nitrite	5
		4. Bromide	10
		5. Nitrate	10
		6. Sulfate	5
		7. Thiosulfate	10
		8. Phosphate	15
		9. Iodide	10
		10. Thiocyanate	10
		11. Perchlorate	15

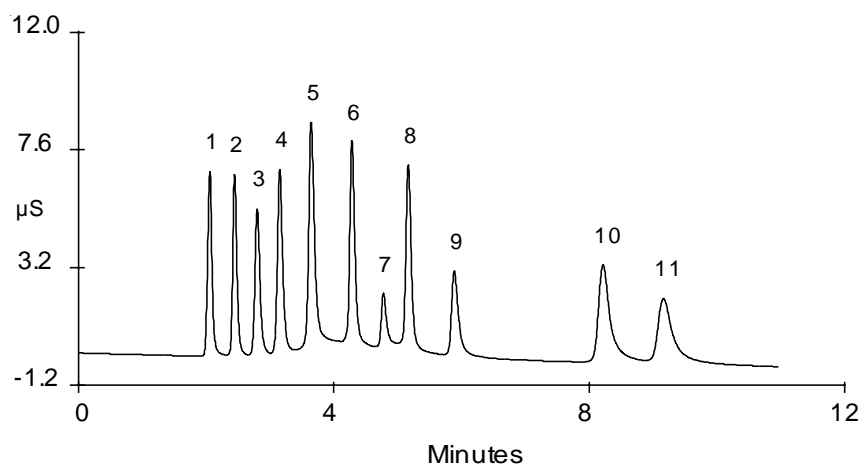


Figure 7
Effect of Expended ATC-3

5.7 Chromic Acid Plating Bath

This example shows the determination of organic additives in a chromic acid plating bath. The sample contains over 30% chromic acid, 14% iron, and other anions at high concentration. The organic additives, methane disulfonic acid (MDSA) and ethane disulfonic acid (EDSA), can easily be separated with the Cryptand A1 column. With most quaternary ammonium anion exchange columns, these two peaks are not resolved. With the Cryptand A1 column, using a delayed capacity gradient, the two peaks can easily be separated. A contaminant, present within methanesulfonic acid, is also well separated. Note also they are well resolved from the huge chromic acid peak.

Column:	Cryptand A1 3-mm Analytical Column
Trap Column:	ATC-3 (2-mm)
Eluent:	25 mM NaOH, step at 2.1 min. to 25 mM LiOH
Flow Rate:	0.5 mL/min.
Inj. Volume:	5 μ L
Temperature:	30 $^{\circ}$ C
Detection:	Suppressed Conductivity
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS ULTRA (2-mm) Recycle Mode
or AES Suppressor:	Anion Atlas Electrolytic Suppressor, AAES
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III (2-mm)
MMS Regenerant:	50 mN H_2SO_4

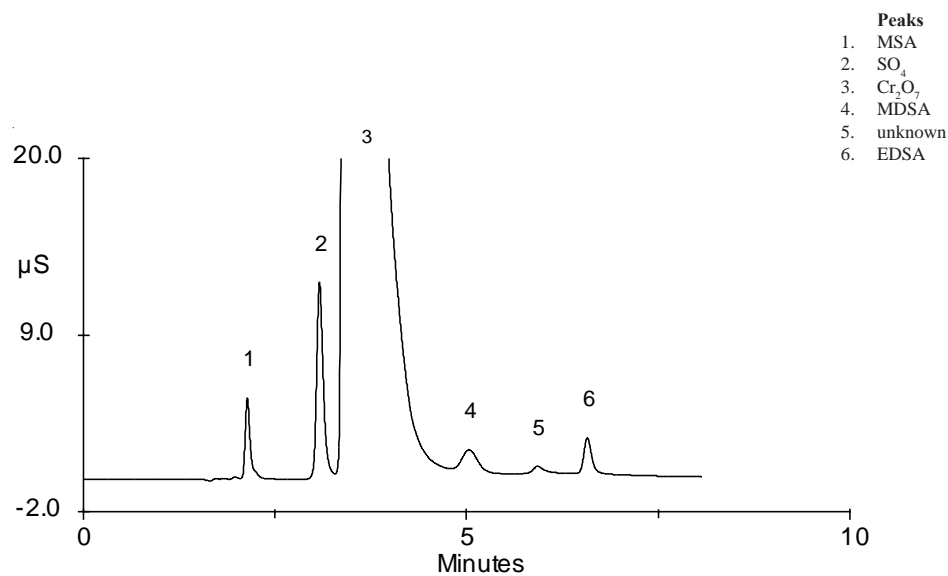


Figure 8
Chromic Acid Plating Bath

5.8 Polyphosphate Application

Capacity gradients allow the analysis of polyvalent samples such as polyphosphates using relatively low concentrations of mobile phases. In this application, normally a gradient up to approximately 200 mM hydroxide would be required to elute the polyphosphates. With the Cryptand A1 column, polyphosphates can be separated in a shorter time using only 50 mM hydroxide. The run starts with NaOH and then steps to LiOH. As the capacity of the column decreases the polyvalents elute without increasing the eluent strength.

Column:	Cryptand A1 3-mm Analytical Column + Cryptand G1 3-mm Guard Column
Trap Column	ATC-3 (2-mm)
Eluent:	50 mM NaOH step at 0.1 minutes to 50 mM LiOH
Eluent Flow Rate:	0.5 mL/min
Inj. Volume:	5 μ L
Temperature:	30°C
Detection:	Suppressed Conductivity
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS ULTRA (2-mm) Recycle Mode
or AES Suppressor:	Anion Atlas Electrolytic Suppressor, AAES
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III (2-mm)
MMS Regenerant:	50 mN H ₂ SO ₄

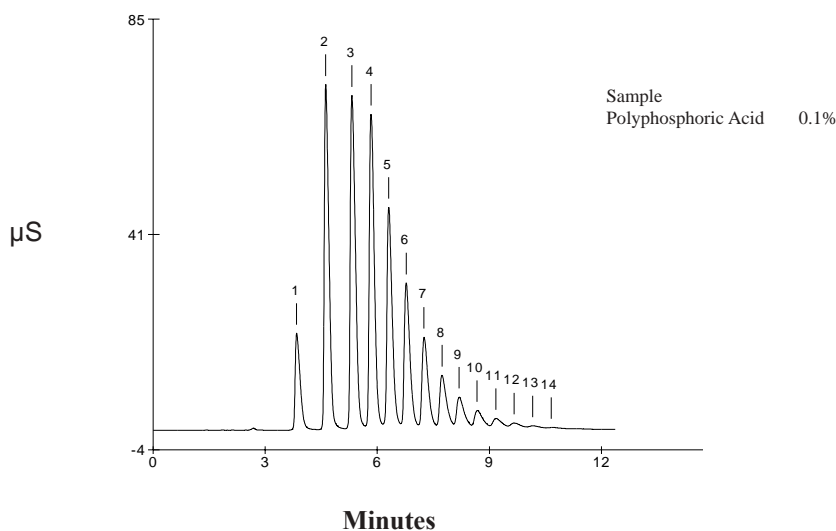


Figure 9
Polyphosphate Application

5.9 Polyvinylsulfonic Acid Application

This example shows the separation of polyvinylsulfonic oligomers. The high molecular weight oligomers elute in less than 20 minutes. With quaternary ammonium functionalized anion exchange columns, these oligomers would not elute even with a high hydroxide concentration gradient.

Column:	Cryptand A1 3-mm Analytical Column
Trap Column:	ATC-3 (2-mm)
Eluent:	10 mM NaOH step at 0.1 min. to 10 mM LiOH
Eluent Flow Rate:	0.5 mL/min
Inj. Volume:	5 μ L
Temperature:	35°C
Detection:	Suppressed Conductivity
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS ULTRA (2-mm) Recycle Mode
or AES Suppressor:	Anion Atlas Electrolytic Suppressor, AAES
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III (2-mm)
MMS Regenerant:	50 mN H ₂ SO ₄

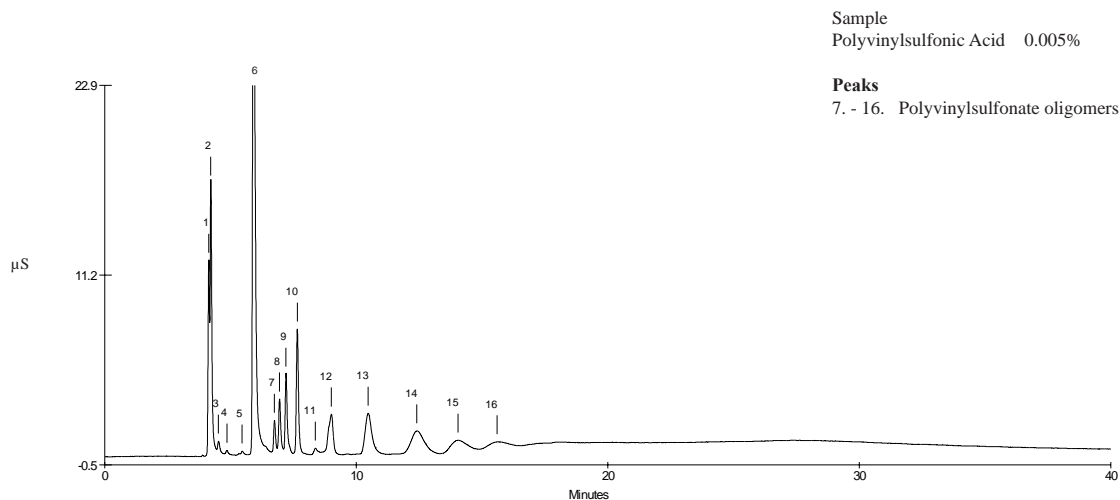


Figure 10
Polyvinylsulfonic Acid Application

SECTION 6 - TROUBLESHOOTING GUIDE

The purpose of the Troubleshooting Guide is to help you solve operating problems that may arise while using Cryptand A1 columns. 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 7
CRYPTAND A1/G1 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	6.2.4, Component Manual
	Contaminated Hardware	Clean Component	Component Manual
Poor Resolution	Poor Efficiency Due to Large System Void Volumes	Replumb System	6.3.1.A, Component Manual
	Column Headspace	Replace Column	6.3.1.B
	Column Overloading	Reduce Sample Size	6.3.3.B
Short Retention Times	Flow Rate Too Fast	Recalibrate Pump	6.3.2.A
	Conc. Incorrect Eluents	Remake Eluents	6.3.2.B
	Column Contamination	Clean Column	6.3.2.C, 6.3.2.D
Poor Front End Resolution	Conc. Incorrect Eluents	Remake Eluents	6.3.3.A
	Column Overloading	Reduce Sample Size	6.3.3.B, 3.3.1, 3.3.2
	Sluggish Injection Valve	Service Valve	6.3.3.C, Component Manual
	Large System Void Volumes	Replumb System	6.3.3.D, Component Manual
Spurious Peaks	Sample Contaminated	Pretreat Samples	6.3.4.A, 6.3.4.B,
	Sluggish Injection Valve	Service Valve	6.3.3.C, Component Manual

6.1 High Back Pressure

6.1.1 Finding the Source of High System Pressure

Total system pressure for the 3-mm Cryptand A1 Guard Column plus the 3-mm Cryptand A1 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 3-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 Cryptand A1/G1 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 8
Typical Cryptand A1/G1 Operating Back Pressures

Column	Typical Back Pressure psi (MPa)	Flow Rate mL/min
Cryptand A1 3-mm Analytical	≤ 1,500 (10.34)	0.5
Cryptand G1 3-mm Guard	≤ 350 (3.08)	0.5
Cryptand A1 + Cryptand G1 3-mm columns	≤ 1,850 (13.42)	0.5

6.1.2 Replacing Column Bed Support Assemblies

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

- A. Disconnect the column from the system.**
- B. Carefully unscrew the inlet (top) column fitting.** Use two open-end wrenches.
- C. Remove the bed support.** Turn the end fitting over and tap it against a benchtop or other hard, flat surface to remove the bed support and seal assembly. If the bed support must be pried out of the end fitting, use a sharp pointed object such as a pair of tweezers, but be careful that you **DO NOT SCRATCH THE WALLS OF THE END FITTING**. Discard the old bed support assembly.
- D. Place a new bed support assembly into the end fitting.** Make sure that the end of the column tube is clean and free of any particulate matter so that it will properly seal against the bed support assembly. Use the end of the column to carefully start the bed support assembly into the end fitting.

Cryptand A1

3-mm Columns (P/N)

Part	(P/N)
Analytical Column	059898
Guard Column	059900
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 μ S
50 mM Hydroxide	2 - 5 μ S

NOTE

Lithium hydroxide tends to contain more carbonate than sodium hydroxide, and sodium hydroxide more than potassium hydroxide. So, depending on which hydroxide eluent system you use, you will see slight differences in the background depending on the cation counter ion.

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

The ATC-3 needs to be regenerated periodically. You will know when this is the case when you see one of two things. When running in isocratic mode, you will begin to see higher and higher background conductivity. If you are running capacity gradients, you will see an increasing, large hump in the middle of the run. The magnitude of this hump will simply continue to increase with each injection. This is the carbonate from the eluent coming through the expended ATC-3. Regenerate the ATC-3 by running 100 mL of 2.0 M NaOH through the ATC-3. Immediately, rinse with 20 mL of eluent prior to use.

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 Cryptand G1 Guard and Cryptand A1 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 Cryptand G1 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 μ S. 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

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

NOTE

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

- A. Turn off the SRS Control unit.
- B. Disconnect the analytical (and guard) column(s) from the injection valve and the ASRS ULTRA. Refer to the specific analytical column Product Manual for column cleanup procedures.
- C. If you are running in the **AutoSuppression External Water Mode**, turn off the external water and disconnect the external water line from the ASRS ULTRA **REGEN IN** port.
- D. Disconnect the liquid line from the ASRS ULTRA **ELUENT OUT** port to the cell at the cell fitting and reconnect it to the **REGEN IN** port.
- E. Connect a temporary line from the priming block or the low-pressure tee on the isocratic or gradient pump to a container with a solution of 0.2 M oxalic acid. Pump this solution through the ASRS-ULTRA (2-mm) at 0.25-0.50 mL/min for 30 minutes.

NOTE

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

- F. Flush the ASRS ULTRA with deionized water for 10 minutes.
- G. Perform steps A - D of the procedure in Section 4.1, "Small Analyte Peak Areas."
- H. Turn on the SRS Control unit for the **AutoSuppression Recycle or External Water Modes** of operation. Ensure that the SRS Control unit is **off** for the **Chemical Suppression Mode** of operation.
- I. Flush the ASRS ULTRA with eluent for 10 minutes.
- J. Reinstall the analytical (and guard) column(s). Begin pumping eluent through the system at the flow rate required for your analysis and equilibrate the system.

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

NOTE

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

- A. Turn off the SRS Control unit.
- B. 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.
- C. 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.
- D. 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.
- E. 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 (2-mm) at 0.25-0.50 mL/min for 30 minutes.

NOTE

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

- F. Flush the AMMS III with deionized water for 10 minutes.
- G. Perform steps A - D of the procedure in Section 4.1, "Small Analyte Peak Areas."
- H. Turn on the SRS Control unit for the **AutoSuppression Recycle or External Water Modes** of operation. Ensure that the SRS Control unit is **off** for the **Chemical Suppression Mode** of operation.
- I. Flush the AMMS III with eluent for 10 minutes.
- J. Reinstall the analytical (and guard) column(s). Begin pumping eluent through the system at the flow rate required for your analysis and equilibrate the system.

The Anion Atlas Electrolytic Suppressor (AAES) Cleanup

Metal Contaminants or Precipitates

- A. Turn off the power to the AAES.
 - B. Disconnect the analytical (and guard) column(s) from the injection valve and the AAES. Refer to the specific analytical column Product Manual for column cleanup procedures.
 - C. If you are running in the **AutoSuppression External Water Mode**, turn off the external water and disconnect the external water line from the AAES **REGEN IN** port.
 - D. Disconnect the liquid line from the AAES **ELUENT OUT** port to the cell at the cell fitting and reconnect it to the **REGEN IN** port.
 - E. Connect a temporary line from the priming block or the low-pressure tee on the isocratic or gradient pump to a container with a solution of 0.5 M oxalic acid. Pass 60 mL of this solution through the AAES using the Trap
-

Column / Suppressor Clean-up Kit (P/N 059649) or pump this solution through the AAES at 2.0 mL/min for 30 minutes.

NOTE

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

- F. Flush the AAES with deionized water at 2 mL/min for 30 minutes.
- G. Reinstall the AAES according to procedures in Section 4.2.1, “Eluent and Regenerant Flow Path Connections in the AutoSuppression Recycle Mode Operation” or Section 4.3.1, “Eluent Flow Path Connections in the AutoSuppression External Water Mode Operation” and resume operation.

Organic Contaminants

- A. Turn off the power to the AAES.
- B. Disconnect the analytical (and guard) column(s) from the injection valve and the AAES. Refer to the specific analytical column Product Manual for column cleanup procedures.
- C. If you are running in the **AutoSuppression External Water Mode**, turn off the external water and disconnect the external water line from the AAES REGEN IN port. If you are running in the **AutoSuppression Recycle Mode**, proceed to D.
- D. Disconnect the liquid line from the AAES **ELUENT OUT** port to the cell at the cell fitting and reconnect it to the **REGEN IN** port.
- E. Connect a temporary line from the priming block or the low-pressure tee on the isocratic or gradient pump to a container with a solution of freshly prepared 10% 1.0 M H₂SO₄/90% acetonitrile. H₂SO₄/acetonitrile solutions are not stable during long term storage so this cleanup solution must be made immediately before each column cleanup. Alternatively, it can be proportioned from 1 bottle containing 1.0 M H₂SO₄ and another bottle containing 100% acetonitrile. Pass 60 mL of this solution through the AAES using the Trap Column / Suppressor Clean-up Kit (P/N 059649) or pump this solution through the AAES at 1.0 mL/min for 60 minutes.

NOTE

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

- F. Flush the AAES with deionized water at 2 mL/min for 30 minutes.
- G. Reinstall the AAES according to procedures in Section 4.2.1, “Eluent and Regenerant Flow Path Connections in the AutoSuppression Recycle Mode Operation” or Section 4.3.1, “Eluent Flow Path Connections in the AutoSuppression External Water Mode Operation” and resume operation.

6.3 Poor Peak Resolution

Poor peak resolution can be due 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.
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- 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 i.d. 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”).

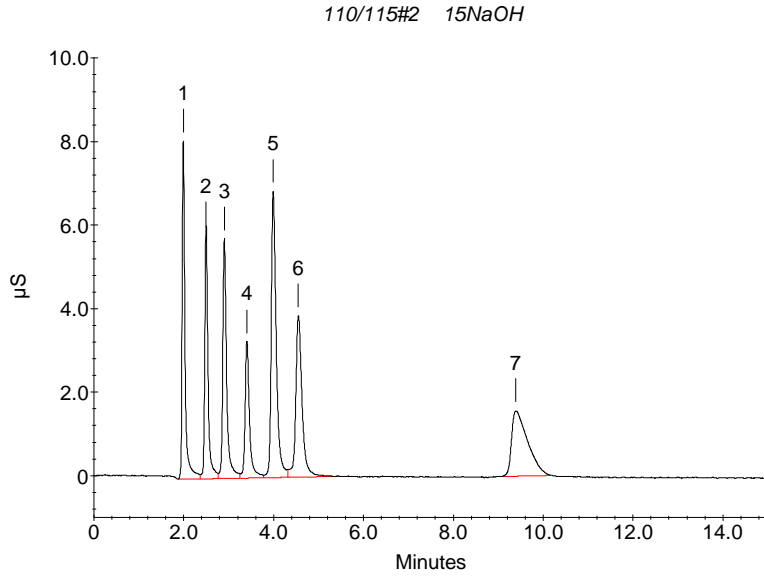
If you need assistance in determining the best way to clean strongly retained solutes in your specific sample matrix from the Cryptand A1 columns, contact the North America Technical Call Center at 1-800-DIONEX-0 (1-800-346-6390) or the nearest Dionex Office (see, “Dionex Worldwide Offices”).

- B. The injection valve may need maintenance.** When an injection valve is actuated, the possibility of creating a baseline disturbance exists. This baseline upset can show up as a peak of varying size and shape. This will occur when the injection valve needs to be cleaned or retorqued (see valve manual). Check to see that there are no restrictions in the tubing connected to the valve. Also check the valve port faces for blockage and replace them if necessary. Refer to the Valve Manual for troubleshooting and service procedures. Small baseline disturbances at the beginning or at the end of the chromatogram can be overlooked as long as they do not interfere with the quantification of the peaks of interest.
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IonPac® Cryptand A1
Analytical (3 x 150 mm)
Product No. 59898

Serial No. : 110/115#2 15NaOH

Pressure (PSI) : 110/115#2: 2.9us,1377psi... Date : 7/2/02 10:43:54 AM



Eluent: 15 mM NaOH

Trap Column: ATC-3 2mm

Eluent Flow Rate: 0.50 mL/min

Temperature: 35°C

Detection: Suppressed Conductivity
 ASRS®-ULTRA 2-mm, 50 mA
 AutoSuppression® Recycle Mode

Background Conductivity: 2-3 µS

Injection Volume: 5 µL

Storage Solution: Eluent

Peak Information : Found Components

Peak No.	Retention Time	Name	(mg/L)	Efficiency	Asymmetry (10%)	Resolution
1	1.99	Fluoride	2.0	6094	1.8	4.42
2	2.49	Chloride	3.0	6387	2.1	3.02
3	2.90	Nitrite	5.0	6203	1.8	3.28
4	3.40	Bromide	5.0	7352	1.9	3.37
5	3.99	Nitrate	10.0	6867	1.8	2.64
6	4.55	Sulfate	5.0	6141	1.6	10.91
7	9.39	Phosphate	5.0	3267	3.4	n/a

COLUMN CARE

Recommended Operation Pressures

Operating a column above its recommended pressure limit can cause irreversible loss of column performance. The maximum recommended operating pressure for Cryptand A1 column is 3,000 psi (20.68 MPa).

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.

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). Plug both ends securely, using the plugs supplied with the column.

Column Cleanup

The following column cleanup protocols have been divided into three general isocratic protocols to remove polyvalent anions, metals, or organic contaminants.

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 ≤ 50 mM levels to avoid creating high pressure zones in the column that may disrupt the uniformity of the column packing.

Choosing the Appropriate Cleanup Solution

- A. **Concentrated lithium hydroxide solutions** such as a 100 mM LiOH is sufficient to remove polyvalent anion contamination.
- B. **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 fluoride and phosphate recoveries.

Concentrated acid solutions such as 0.1 to 1 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.

- C. **Organic solvent solutions** can be used 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 4, HPLC Solvents for Use with Cryptand A1 Columns.
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- D. Regardless of the cleanup solution chosen, use the following cleanup procedure in, “Column Cleanup Procedure,” to clean the Cryptand A1 and G1.**

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 suppressor** from the Cryptand A1 Analytical Column. If your system is configured with both a guard column and an analytical column, move the guard after the 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 0.5 mL/min for a Cryptand A1 3-mm Analytical or Guard Column.**
- D. **Pump the cleanup solution through the column for at least 60 minutes.**
- E. Rinse the column with eluent for 5 minutes.
- F. **Reconnect the suppressor** to the Cryptand A1 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.
- G. **Equilibrate the column(s) with eluent** for at least 60 minutes before resuming normal operation.
- H. In the case of metal contamination, if contamination levels are very high and the above cleanup does not restore column performance, run the column overnight at 0.4 mL/min with 0.2 M oxalic acid.
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