PRODUCT MANUAL

OMNIPAC® PAX-100 GUARD COLUMN
(4 x 50 mm, P/N 042151)
OMNIPAC® PAX-500 GUARD COLUMN
(4 x 50 mm, P/N 042153)

OMNIPAC® PAX-100 ANALYTICAL COLUMN
(4 x 250 mm, P/N 042150)
OMNIPAC® PAX-500 ANALYTICAL COLUMN
(4 x 250 mm, P/N 042152)

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

1. The standard test eluent for the OmniPac PAX-100 is 5.0 mM NaOH/1.0 mM Na_2CO_3/ 30% CH_3CN and the standared test eluent for the OmniPac PAX-500 is 3.6 mM Na2CO_3/ 3.4 mM $NaHCO_3$/25% Methanol.

   See Section 3.3, "Sodium Hydroxide Eluent Preparation." Make the required stock and working solutions for eluents. See Section 3, "Operation," for details.

2. Run the Production Test Chromatogram as a system check.
   See Quality Assurance Report for details.

3. See Section 4, "Example Applications" for example applications.

4. See "Column Care" for column cleanup and long-term storage recommendations.

Now sold under the Thermo Scientific brand

Thermo SCIENTIFIC

Document No. 034217
Revision 09
21 May 2003

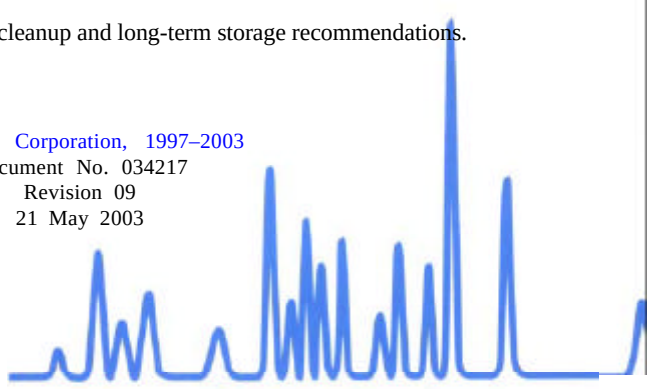


TABLE OF CONTENTS

SECTION 1 - INTRODUCTION

The OmniPac columns are a unique family of columns developed to expand the chromatographic selectivity available to the analytical chemist. The OmniPac PAX-100 is an ion exchange column, while the OmniPac PAX-500 is a mixed mode column; it exhibits both reversed phase and ion exchange characteristics. Using the OmniPac columns it is possible to separate neutral and ionic compounds simultaneously or independently, with the addition of solvents. The addition of different solvents at different concentrations allows a broader scope of ionic analytes to be determined on a single column. The ability to use solvents on an ion exchange column also results in longer column life and less sample preparation since strongly retained hydrophobic contaminants can be eluted using solvent. The OmniPac columns are compatible with solvents, acids and bases from pH 0–14.

1.1 Resin Structure

The substrate for the OmniPac columns is an 8.5 µm diameter particle composed of ethylvinylbenzene highly cross linked with divinylbenzene. The OmniPac PAX-100 is manufactured using microporous substrate beads with very low surface area. The OmniPac PAX-500, in contrast, is manufactured using macroporous substrate beads with a surface area of approximately 300 m^2/g and pore size of 60Å. The macroporous structure provides an accessible hydrophobic core where reversed phase retention occurs.

The polymeric core is coated with a polymeric colloid to create a solvent compatible ion exchange substrate, as shown in Figure 1. The polymeric colloid is actually a layer of MicroBead latex particles, which are functionalized with a quaternary ammonium base and carry the actual anion exchange sites. The extremely small particle size of the pellicular layer results in excellent mass transfer characteristics and consequently very high efficiency.

Since the support material is polymer-based, ionic eluents in the pH range of 0 to 14 can be used to affect selectivity and convert molecular species into ionic compounds. However, to elute ions such as alkyl and aryl amines, carboxylates, and sulfonates efficiently, it is necessary to add organic solvents to the ionic eluent to prevent the organic analytes from being absorbed by the ion exchange phase and ensure the major retention mechanism is ion exchange. The highly crosslinked polymeric substrate allows the use of common HPLC solvents as eluent modifiers in ion exchange separations. The OmniPac columns increase the flexibility of the analytical system since the user can tailor the separations to the requirements of the sample.

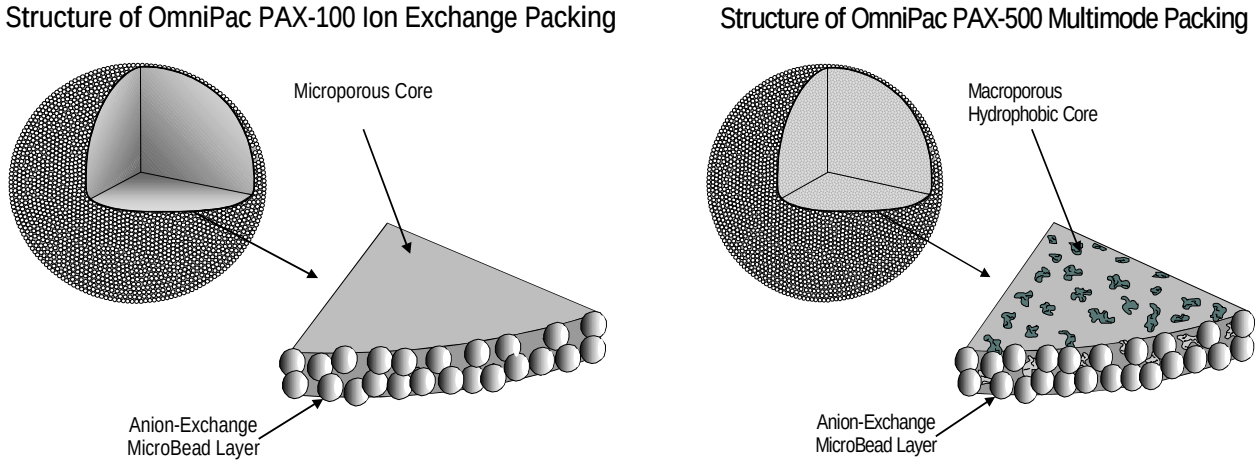


Figure 1
OmniPac Resin Structure

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."

1.2 Column Selection Guide

Table 1
OmniPac Column Selection

Column	PAX-100	PAX-500	PCX-100	PCX-500
Retention Mechanism	Anion Exchange	Anion Exchange and/or Reversed Phase	Cation Exchange	Cation Exchange and/or Reversed Phase
Applications	Inorganic anions Organic anions	Neutral organics Anions Ion suppressible weak bases	Inorganic cations Organic cations	Neutral organics Cations Ion suppressible weak acids
Special Selectivity Applications	Very hydrophobic anions Halogenated anions (eliminates hydrophobic retention of PAX-500)	Separation of organic and inorganic anions by hydrophobic and ion exchange differences (separates neutrals and anions in a single run)	Very hydrophobic cations Halogenated cations (eliminates hydrophobic retention of PCX-500)	Separation of organic and inorganic cations by hydrophobic and ion exchange differences (separates neutrals and cations in a single run)
On-Column Sample Prep	All neutral matrix components are eluted in the void	Selectively elutes neutral or anionic matrix interferences	All neutral matrix components are eluted in the void	Selectively elutes neutral or cationic matrix interferences.

1.3 Column Specifications

Table 2
OmniPac PAX-500 Packing Specifications

Column	Particle Diameter µm	Substrate X-Linking %	Latex Diameter nm	%	Latex X-Linking µeq/column	Column Capacity	Functional Group	Hydrophobicity
PAX-100 Analytical (4 x 250 mm)	8.5	55	60		4	40	Alkanol quaternary amine	Hydrophilic
PAX-100 Guard (4 x 50 mm)	8.5	55	60		4	8	Alkanol quaternary amine	Hydrophilic
PAX-500 Analytical (4 x 250 mm)	8.5	55	10		4	40	Quaternary ammonium	Hydrophilic
PAX-500 Guard (4 x 250 mm)	8.5	55	10		4	8	Quaternary ammonium	Hydrophilic

1.4 Applications Guide

Table 3
Applications and Preferred Columns

Application	Column
Adrenergics	PCX-500
Alcohols	PAX-500 or PCX-500
Alkylbenzene sulfonates	PAX-100
Alkanolamines	PAX-500
Anilines	PCX-500 or PCX-100
Antidepressants	PCX-500 or PCX-100
Antihistamines	PAX-500
Anti-inflammatories	PAX-500 or PCX-500
Aromatic Acids	PAX-500 or PAX-100
Aromatic Amines	PCX-100
Barbiturates	PAX-500 or PAX-100
Benzidines	PCX-500
Brighteners (plating baths)	PCX-500
Carboxylic acids	PAX-500 or PAX-100
Cephalosporins	PCX-500
Dyes	PCX-500
Ephedrines	PCX-500 or PCX-100
Herbicides	PCX-500
Inorganic Anions	PAX-500 or PAX-100
Inorganic Cations	PCX-500 or PCX-100
Methotrexate, Folic acids	PCX-100
Nitrogen-containing organics	PCX-500 or PCX-100
Nucleic acid constituents	PCX-500 or PCX-100
Nucleotides and nucleosides	PCX-500
Peptides	PAX-500 or PCX-500
Purines and pyrimidines	PAX-500
Sulfonamides	PAX-500 or PCX-500
Sulfonated anionic surfactants	PAX-500 or PAX-100
Thiocyante	PAX-100
Thiosulfate	PAX-100
Vitamins	PAX-500 or PCX-500

SECTION 2 - INSTALLATION

2.1 System Requirements

The OmniPac PAX columns are designed for use with any liquid chromatograph capable of withstanding high pH and high salt conditions. Dionex recommends using a PEEK (Polyetheretherketone) system. For 2-mm OmniPac columns, the pump should be configured for narrow-bore operation. For 4-mm OmniPac PAX columns the pump should be configured for standard bore operation.

It is always important to minimize the system void volume to ensure maximum efficiencies and reproducible chromatography. For best performance, all of the tubing installed between the injection valve and detector should be 0.010" i.d. PEEK tubing (P/N 042260) for 4-mm systems or smaller for 2-mm systems. PEEK has excellent chemical resistance to most organic and inorganic liquids. However, concentrated sulfuric acid, concentrated nitric acid and methylene chloride will attack PEEK, so should always be diluted. In addition, tetrahydrofuran is not compatible with OmniPac columns at concentrations of greater than 10%. Tefzel tubing (0.012" i.d.) may be used but peak efficiency may be compromised which may also result in decreased peak resolution. Minimize the lengths of all connecting tubing and remove all unnecessary switching valves and couplers. If you need assistance in configuring your system properly, contact the Dionex Regional Office nearest you.

NOTE

Tetrahydrofuran is not compatible with OmniPac columns at concentrations of greater than 10%.

2.1.1 Detection Requirements

Any detector can be configured with a Dionex ion chromatograph, depending on your application. For OmniPac PAX separations detection typically will be either suppressed conductivity or UV-Visible spectrometry. If you will be using suppressed conductivity detection you will also need to purchase a suppressor.

Suppressor Requirements

The ASRS ULTRA suppressor is the suppressor of choice for straightforward ion exchange applications that do not require the use of solvent. The ASRS ULTRA is a suppressor that can be operated in the electrolytic or chemical regeneration modes. Please see the ASRS suppressor manual for installation instructions.

For applications requiring the use of solvent, an AMMS III should be used, since the ASRS ULTRA is only compatible with solvents up to 40% solvent, and only in the external water or chemical regeneration modes. The AMMS III is operated in the chemical regeneration mode, and provides the lowest noise and fastest start-up of all the suppressors that are compatible with the OmniPac columns. The AMMS III is compatible with all solvents and at all concentrations. Please see the AMMS III suppressor manual for installation instructions.

The AMMS-ICE II is the required suppressor for use with OmniPac columns used for ion-exclusion or ion-suppression applications using conductivity detection. The AMMS-ICE II is ideally suited to ion-exclusion chromatography of samples such as organic acids and alcohols in complex or high-ionic strength samples, including food and beverage products, biological samples, fermentation processes, industrial process liquors, and treated waste waters. Please see the AMMS-ICE II suppressor manual for installation instructions.

This manual assumes that you are familiar with the installation and operation of your Ion Chromatograph and suppressor. If you do not understand the operation of your system, please take the time to familiarize yourself with the operator’s manuals for the products before beginning an analysis.

CAUTION

Do operate any suppressor over 40 °C; if an application requires a higher temperature, place suppressor outside of the chromatography oven.

Using AutoRegen and Eluents Containing Solvent

To minimize the baseline shift when performing a hydroxide gradient, a high regenerant flow rate (10-15 mL/min) is required. To save regenerant preparation time and reduce regenerant consumption and waste, Dionex recommends using an AutoRegen® Accessory (P/N 039594).

During normal operation, both sodium ions and solvents from the eluents will diffuse through the membrane in the suppressor, from the eluent channel into the regenerant stream. Unlike the sodium ions which are exchanged for hydronium ions by the AutoRegen Regenerant Cartridge and thus removed from the regenerant stream, the solvent is not removed from the recycled regenerant and continues to accumulate. Eventually the concentration of solvent in the recycled regenerant can cause the background conductivity to increase which can result in a noisy background. Although the noise may increase, solvent has no affect on the AutoRegen Anion Regenerant Cartridge lifetime, which continues to remove sodium ions. The ionic strength of the eluent determines the lifetime of the AutoRegen Cartridge.

When using an AutoRegen System, it is still necessary to replace the sulfuric acid regenerant solution, though the frequency is significantly less than without the AutoRegen system. How often the regenerant is replaced will depend on the application and the concentration of the solvent in the eluent. Minimally, the regenerant should be replaced once a week. When replacing the recycled regenerant, the first 200 mL of the regenerant should be pumped to waste before recycling of the regenerant is started. It is not necessary to change the AutoRegen cartridge until it is completely expended.

2.1.2 Injection Requirements

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

2.2 Consumables Requirements

2.2.1 Guard Column

Dionex recommends the use of guard columns prior to the analytical column. A guard is placed prior to the analytical column to prevent sample contaminants from being eluted onto the analytical column. Once a guard has become contaminated, it is easier to clean and more cost-effective to replace than the longer analytical column. Retention times will increase by approximately 20% when a guard is added to the analytical column.

The OmniPac PAX-500 Guard Column can be used for trace anion concentration work for example in high purity water analysis. The function of PAX-500 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 PAX-500 Guard Column leading to a lowering of detection limits by 2–5 orders of magnitude. The unique advantage of using the PAX-500 Guard Column in these applications is the capability of performing routine trace analyses of sample matrix ions at µg/L levels without extensive and laborious sample pretreatment.

CAUTION

The 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 OmniPac PAX-500. Use the PAX-500 Guard.

2.2.2 Anion Trap Column

When performing applications that include a hydroxide gradient, an IonPac Anion Trap Column (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.

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

IMPORTANT

When using the ATC-3 Trap Column with eluents containing solvents, it is very important to regenerate the ATC-3 on a daily basis.

SECTION 3 - OPERATION

3.1 General Operating Conditions

The OmniPac PAX columns are compatible with eluents ranging in pH from 0 to 14. They are also compatible with organic solvents as a result of the high crosslinking of the polymeric packing material.

Table 4
OmniPac PAX Column Operating Specifications

Operating Parameter	OmniPac PAX-100	OmniPac PAX-500
Backpressure (psi)	4,000	3,000
Eluent Compatibility- typical eluents including:	Hydroxide, chloride	Hydroxide, carbonate, chloride, TRIS
Solvent Compatibility- typical HPLC solvents:	ACN, MeOH, EtOH, IPA	ACN, MeOH, EtOH, IPA
Solvent Concentration Limits	1–100%	1–100%
pH Compatibility	0–14	0–14
Temperature (^{0}C)		
Ion Pair Reagent Compatibility	N/A	Typical Ion Pair reagents
Ion Pair Reagent Concentration (mM)	N/A	$\leq$ 10

Table 5
OmniPac PAX Typical Operating Conditions

Operating Parameter	OmniPac PAX-100	OmniPac PAX-500
Flow Rate (mL/min)	1.0	1.0
Backpressure with guard @ 30^{o}C (psi)	2,270	2,450
Typical eluents	NaOH	NaOH, Na_2CO_3, KCl, NaCl
Typical Solvents	ACN, MeOH, EtOH, IPA	CAN, MeOH
Solvent Concentration	5–40%	10–80%
pH	11–14	8.5–13
Temperature (^{0}C)	30 ^{o}C	30 ^{o}C
Ion Pair Reagent Compatibility	N/A	$TBAPO_4$
Ion Pair Reagent Concentration (mM)	N/A	$\leq$ 10

3.1.1 Eluent Storage

When using hydroxide eluents, it is essential to keep the eluent under a helium atmosphere at all times to avoid the adsorption of carbon dioxide into the eluents. Carbon dioxide readily dissolves in dilute basic solutions producing carbonate. The presence of carbonate in the eluent will increase the baseline shift during a gradient analysis and may even affect selectivity during an analysis. Eluents should be kept in glass reservoirs, because plastic reservoirs are permeable to carbon dioxide.

3.1.2 Solvents

Due to the high degree of cross-linking of the polymeric core, the OmniPac PAX columns are compatible with typical HPLC solvents such as acetonitrile and methanol. These modifiers can be used to control the ion exchange selectivity of the columns. In fact, it is essential that at least 1% organic solvent is used at all times with the OmniPac PAX columns, regardless of the application, since the core particle has a neutral hydrophobic internal surface. The 1% organic solvent will ensure that the substrate

remains 'wetted' and maximum column performance is maintained. The OmniPac PAX columns are compatible with up to 100% solvent but for practical reasons, that is, to ensure proper mixing and minimize outgassing, it is recommended that 95% solvent not be exceeded.

The OmniPac PAX columns can be used with any suppressible ionic eluent that does not exceed the capacity of the suppressor, but they have been specifically optimized for use with hydroxide eluents. When adding the minimum 1% solvent to hydroxide eluents, do not add acetonitrile directly to the hydroxide, but instead proportion the two liquids with the pump. The major concern is that at sufficiently high concentrations of acetonitrile in hydroxide, the acetonitrile will hydrolyze forming acetate and ammonia. This issue is of no concern when hydroxide is to be used with alcohol, and premixing hydroxide and alcohol is acceptable. Another concern to be aware of is the limited solubility of hydroxide in acetonitrile. If you do not have a gradient pump and cannot proportion your eluents, make sure to prepare fresh eluents daily. Premixing hydroxide and alcohols does not present the same concerns.

When proportioning a solvent gradient, the backpressure experienced by the column will increase with increasing solvent to a maximum at about 40% methanol or about 20% acetonitrile. Backpressure is also affected by temperature, salt concentration and flowrate. The backpressure limit of the OmniPac PAX-100 column is 4,000 psi, while that of the OmniPac PAX-500 is 3,000 psi, so it is important to keep all these factors in mind when developing a new method.

NOTE

The operating backpressure will vary as the eluent-solvent composition varies. Any eluent-solvent composition from 1% solvent to 95% solvent can be used as long as the backpressure remains below the backpressure limit of the column, that is, 4,000 psi for the OmniPac PAX-100 and 3,000 psi for the OmniPac PAX-500.

Table 6
HPLC Solvents for Use with OmniPac PAX-500 Columns

Solvent	Maximum Operating Concentration
Acetonitrile	100%
Methanol	100%
2-Propanol	100%
Tetrahydrofuran	10%

3.1.3 Gradients

Applications requiring solvent gradients are best performed when organic solvents are premixed with deionized water. This allows proper mixing by the gradient pump to give the required gradient ramp for your chromatography. For example, if you want to run a solvent gradient from 10% solvent to 90% solvent, make the following eluents and run from 100% Eluent 1 to 100% Eluent 2:

Eluent 1: 10% solvent/90% water
Eluent 2: 90% solvent/10% water

Premixing small quantities of solvents and water will prevent outgassing and refractive index problems associated with proportioning neat solvents and water with a pump.

3.1.4 Ion Pair Reagents

As a result of its reversed phase characteristics, the OmniPac PAX-500 column can be used for ion pair applications. All ion pair reagents can be used and buffering these reagents for column stability is not necessary. Ion pair reagents are usually used at concentrations up to 10 mM and generally do not benefit chromatographic system performance at concentrations greater than 10 mM in the eluent. In fact, at concentrations higher than 10 mM, the eluent may be difficult to suppress resulting in higher background response with absorbance or conductivity detection.

3.2 Chemicals Purity Requirements

Obtaining reliable, consistent and accurate results requires eluents that are free from ionic and spectrophotometric impurities. Chemicals, solvents and deionized water used to prepare eluents must be of the highest purity available. Maintaining low trace impurities and low particle levels in eluents also help to protect your analytical column 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.

3.2.1 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 (the universally accepted standard for reagents) should be used. These inorganic chemicals will detail the purity by having an actual lot analysis on each label.

NOTE

Sodium hydroxide eluents should always be prepared from a reasonably fresh bottle of certified 50% sodium hydroxide solution that is low in carbonate (do not use the 50% sodium hydroxide solution if a large amount of a white, sodium carbonate precipitate is present). Do not use sodium hydroxide pellets to prepare eluents since these pellets readily absorb carbon dioxide from the air.

3.2.2 Solvents

When solvents are added to eluents to enhance the ion exchange process, it is important that the solvents used do not introduce ionic impurities that will adversely affect the separation. Since most manufacturers of solvents do not test for ionic impurities, it is important that the highest grade of solvents available be used. Currently, several manufacturers are making ultrahigh purity solvents that are compatible with HPLC and spectrophotometric applications. Use of these ultrahigh purity solvents will usually ensure that your chromatography is not affected by ionic impurities in your solvent. Currently at Dionex, we have obtained consistent results using High Purity Solvents manufactured by Burdick and Jackson and Optima Solvents by Fisher Scientific.

3.2.3 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 from ionic impurities, organics, microorganisms and particulate matter larger than 0.2 µm. Bottled HPLC-Grade Water should not be used since most bottled water contains an unacceptable level of ionic impurities. Finally, thoroughly degas all deionized water prior to preparing any eluents.

3.3 Sodium Hydroxide Eluent Preparation

Sodium hydroxide eluents will readily absorb carbon dioxide, producing carbonate. Thus, precautions must be taken during eluent preparation to minimize contamination with carbon dioxide from the air. These precautions, if taken, ensure smooth, reproducible ramps, with 1 to 3 µS total change in background conductivity.

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

NOTE

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.

3.3.1 Vacuum Degassing Type I Reagent Grade Water

Vacuum degassing the Type I Reagent Grade Water is an effective way to remove carbon dioxide. Vacuum degas the water by placing the eluent reservoir in a sonicator and drawing a vacuum on the filled reservoir with a vacuum pump. Vacuum degas the reservoir of water for 5-10 minutes. Cap each bottle and minimize the length of time the bottle is opened to the atmosphere.

3.3.2 Calculating Mass of Hydroxide Required for Weight Method of Preparation

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

Example: To make 1 L of 20 mM NaOH use 1.60 g of 50% sodium hydroxide:
(as used in Section 4.1.2, “Production Test Chromatogram”)

$$\text{For 20 mM:} \quad \frac{0.02 \text{ mole/L} \times 40.01 \text{ g/mole}}{50\%} = 1.60 \text{ g diluted to 1 L}$$

3.3.3 Calculating Volume of Hydroxide Required for Volume Method of Preparation

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 = % purity of the concentrated solution

Example: To make 1 L of 20 mM NaOH use 1.05 mL of 50% sodium hydroxide:
(as used in Section 4.1.2, “Production Test Chromatogram”)

$$\text{For 20 mM:} \quad \frac{0.02 \text{ mole/L} \times 40.01 \text{ g/mole}}{50\% \times 1.53 \text{ g/mL}} = 1.05 \text{ mL diluted to 1 L}$$

* 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.

3.3.4 Preparing Sodium Hydroxide Eluent

Determine the amount of 50% (w/w) NaOH required either by calculating it according to the equations above, or by using Table 7 below. From this, calculate the amount of deionized water required to make up 1L of eluent. Measure the required amount of water into a glass reservoir and degas according to the instructions in section 3.3.1. Measure out the required amount of 50% (w/w) NaOH and add it to the glass reservoir containing the degassed, deionized water. Avoid introducing carbon dioxide from the air into the eluent by adding the hydroxide directly into the water with the tip of the pipette below the surface of the water. 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 7
Dilution of 50% (w/w) NaOH to Make Sodium Hydroxide Eluents

50% (w/w) NaOH		Concentration of NaOH Eluent
g	(mL)	(mM)
0.08	(0.05)	1
0.80	(0.52)	10
3.20	(2.09)	40
8.00	(5.25)	100
16.00	(10.5)	200

3.4 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 to the acetonitrile in the reservoir. Using this procedure to mix solvents with water will ensure that a true volume/ volume eluent is obtained.

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

3.5 Regenerant Preparation

The regenerant is sulfuric acid. If you are not using the AutoRegen Accessory (P/N 039594), prepare several liters of the regenerant. Refer to the suppressor manual for instructions on the regenerant concentration and preparation.

3.6 Column Preparation

The OmniPac PAX columns are shipped filled with a long-term storage solution. For the OmniPac PAX-100 this long term solution is 50 mM NaOH/ 5% MeOH. For the OmniPac PAX-500 the long term storage solution is 25% MeOH/ 3.6 mM Na_2CO_3/ 3.4 mM $NaHCO_3$. If the eluent for your application contains methanol, then go to Section 3.6.1 for instructions on installing a new column. If your eluent system contains a solvent other than methanol, refer to Section 3.6.2 for instructions on how to install your new column. Always ensure that any eluent passing through the OmniPac PAX columns contain at least 1% solvent.

3.6.1 Installing a New Column

Equilibrate the OmniPac PAX columns with the initial application eluent for 30 minutes at the flow rate required by the application. The column is now ready for the initial standard injection and is fully equilibrated when two successive injections of the standard display retention times within 2%.

3.6.2 Changing Solvents

When changing from a method containing a given solvent, such as methanol, to a method containing a different solvent, such as acetonitrile, care should be taken to avoid creating high viscosity pressure fronts that could damage the column. The safest way to change solvents is to

A. Equilibrate the column for approximately 10 minutes with the original eluent containing only 5% of the original solvent.

B. Switch to the new method, containing only 5% of the new solvent and equilibrate for another 10 minutes.

C. Next run a 15 minute gradient from 5% of the new solvent to the highest percentage that will be used in the method.

D. Finally re-equilibrate the column under the starting conditions for the new method.

CAUTION

The OmniPac PAX columns are shipped in methanol. You must follow the procedure above when preparing a new OmniPac PAX column for use with acetonitrile.

3.6.3 Cleaning the Column for Applications Requiring UV-Visible Detection

If you intend to use a UV detector with either methanol-based or acetonitrile-based eluent systems, then a cleanup to remove the UV-active leachables is recommended in order to ensure a low background absorbance. To clean your column, prepare the following solutions:

Eluent 1:	DI water
Eluent 2:	90% solvent/10% DI water
Eluent 3:	Strong eluent (e.g. 200 mM NaOH)

Then run the following gradient:

Eluent Flow Rate: 1.0 mL/min

Time (min)	%1	%2	%3	%4
0.0	90	5	5	0
15.0	45	5	50	0
30.0	45	5	50	0
35.0	25	50	25	0
45.0	25	50	25	0
50.0	75	5	20	0
55.0	75	2	20	0

The column will now be ready for your eluent.

SECTION 4 - EXAMPLE APPLICATIONS

4.1 Production Test Chromatograms

The OmniPac PAX Analytical Columns are tested prior to shipment to ensure that they perform as designed. Each column is subsequently shipped with its quality assurance report (QAR). An example of the QAR is given below.

4.1.1 OmniPac PAX-100

The test mixture for the OmniPac PAX-100 is designed strictly to test the ion exchange properties of the column. Included in the text mixture are 4-hydroxybenzamide, benzenesulfonate, nitrate, 4-cyanophenol, 4-bromophenol and phenylphosphonate. Benzenesulfonate and nitrate are in the test matrix to ensure the OmniPac PAX-100 is able to resolve two species with similar ionic retention mechanisms.

Column:	OmniPac PAX-100 Analytical Column
Eluent:	5.0 mM NaOH/ 1.0 mM Na_2CO_3/ 30% CH_3CN
Flow Rate:	1.0 mL/min
Detection:	UV at 220 nm, 0.05 AUFS
Injection Volume:	10 µL
Storage Solution:	50 mM NaOH / 5% CH_3OH

	Analyte	mg/L
1	4-Hydroxybenzamide	1.5
2	Benzenesulfonate	3.0
3	Nitrate	1.5
4	4-Cyanophenol	2.0
5	4-Bromophenol	3.0
6	Phenylphosphonate	3.0

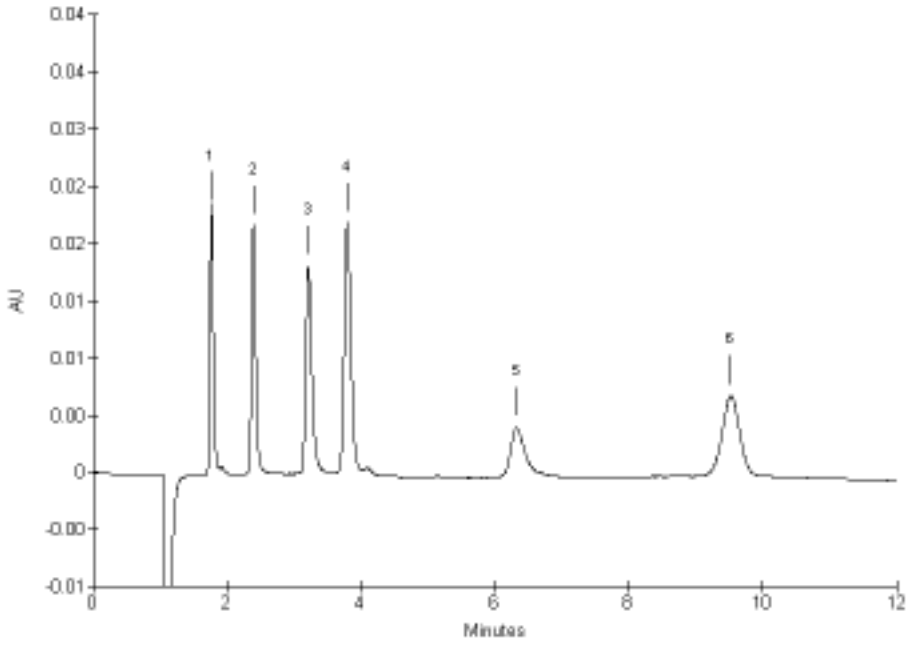


Figure 2
OmniPac PAX-100 Test Chromatogram

4.1.2 OmniPac PAX-500 Production Test Chromatogram

The test mixture for the OmniPac PAX-500 contains chloride, nitrate, benzenesulfonate and sulfate. Benzenesulfonate and nitrate are in the test matrix to ensure the OmniPac PAX-500 is able to resolve two species with similar ionic retention mechanisms. The presence of chloride demonstrates that the column elutes inorganic anions efficiently. To ensure divalent anions are eluted efficiently and the column has the proper capacity, sulfate is added to the test matrix.

Injection Volume:	10 µL
Column:	OmniPac PAX-500 4-mm
Eluent:	3.6 mM Na2CO3 3.4 mM NaHCO3 25% CH3OH
Flow Rate:	1.0 mL/min
Detection:	Conductivity 5 µS full scale
Storage Solution:	Eluent

	Analyte	mg/L
1.	Chloride	4.0
2.	Nitrate	10.0
3.	Benzenesulfate	25.0
4.	Sulfate	15.0

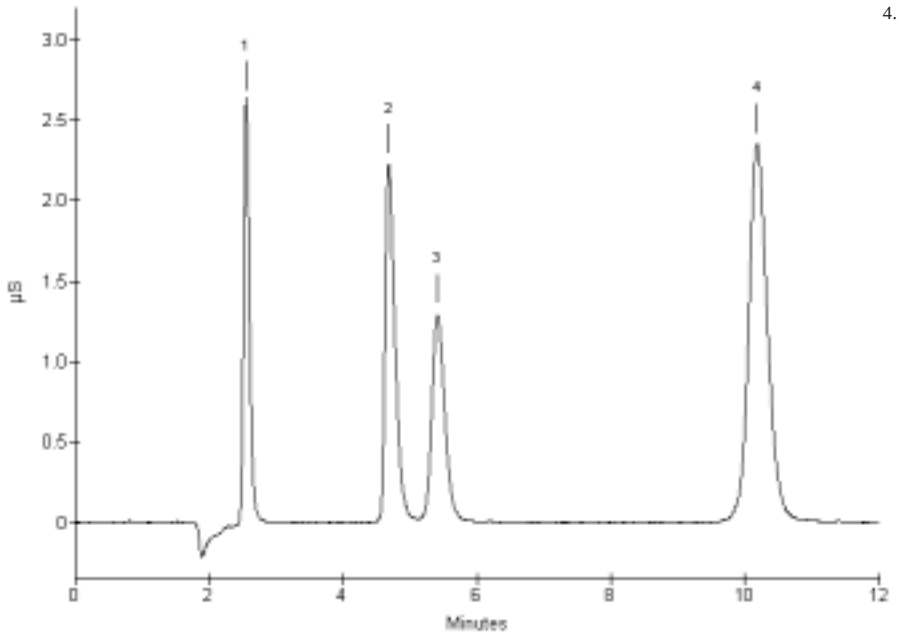


Figure 3
OmniPac PAX-500 Test Chromatogram

4.2 OmniPac PAX-100 Examples

4.2.1 Inorganic Anions

Sample Loop Volume:	10 µL
Guard Column:	OmniPac PAX-100 Guard Column
Analytical Column:	OmniPac PAX-100 Analytical Column
Eluent:	40 mM NaOH/5% CH_3OH or 40 mM NaOH/2.5% CH_3CN *
Eluent Flow Rate:	1.0 mL/min
MMS Suppressor:	Anion MicroMembrane Suppressor
MMS Regenerant:	20 mN H_2SO_4
Expected Background Conductivity:	2-4 µS
Expected System Operating Backpressure:	1500 - 1800 psi

* Eluent should be prepared daily or prepare 2 eluents and proportion together to minimize eluent preparation.

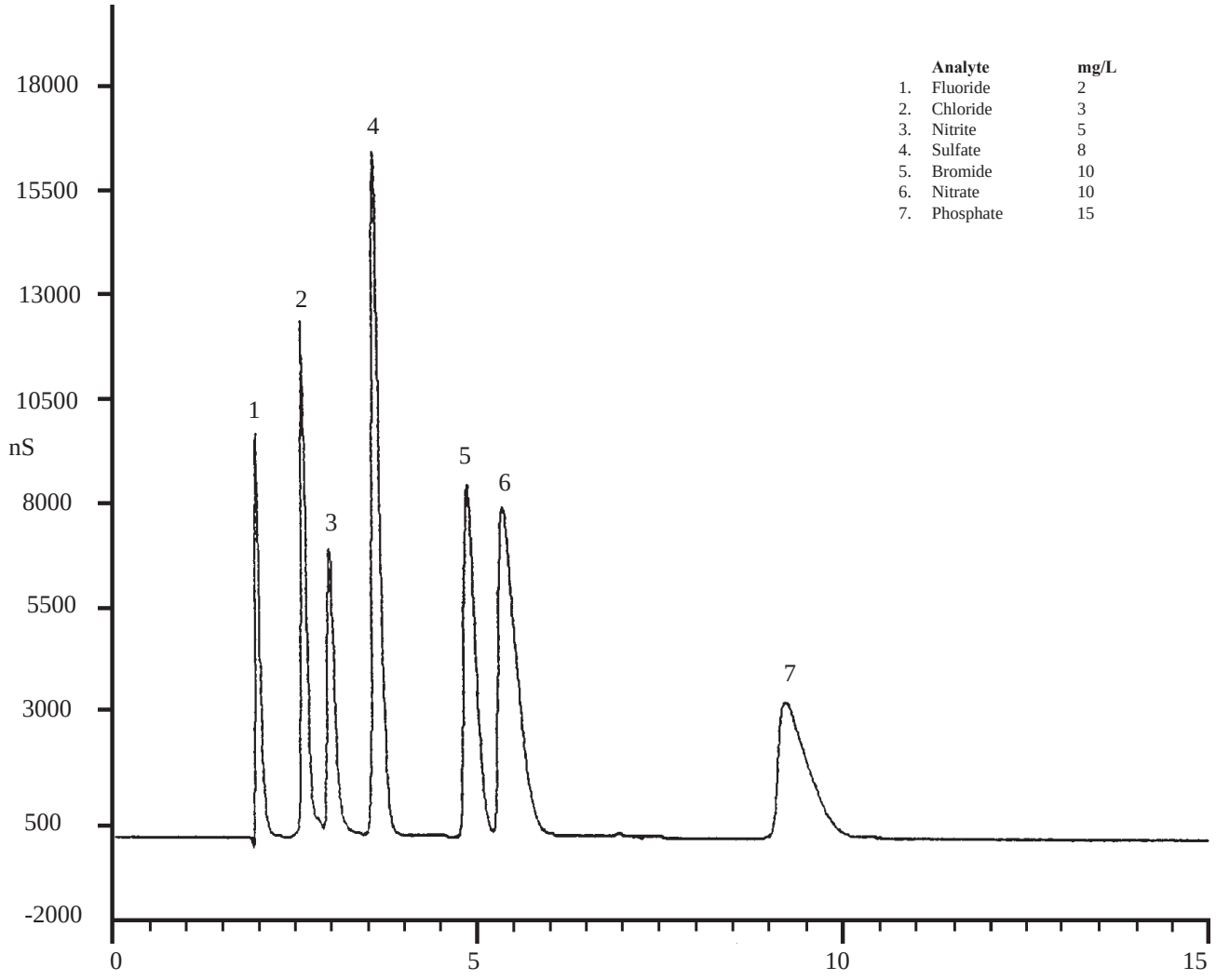


Figure 4
Inorganic Anions

4.2.2 Carbonate Eluent

Sample Loop Volume:	10 µL
Guard Column:	OmniPac PAX-100 Guard Column
Analytical Column:	OmniPac PAX-100 Analytical Column
Eluent:	3.9 mM $NaHCO_3$/3.1 mM Na_2CO_3/5% CH_3OH
Eluent Flow Rate:	1.0 mL/min
MMS Suppressor:	Anion MicroMembrane Suppressor
MMS Regenerant:	25 mN H_2SO_4
Expected Background Conductivity:	18 - 23 µS
Expected System Operating Backpressure:	1500 - 1800 psi

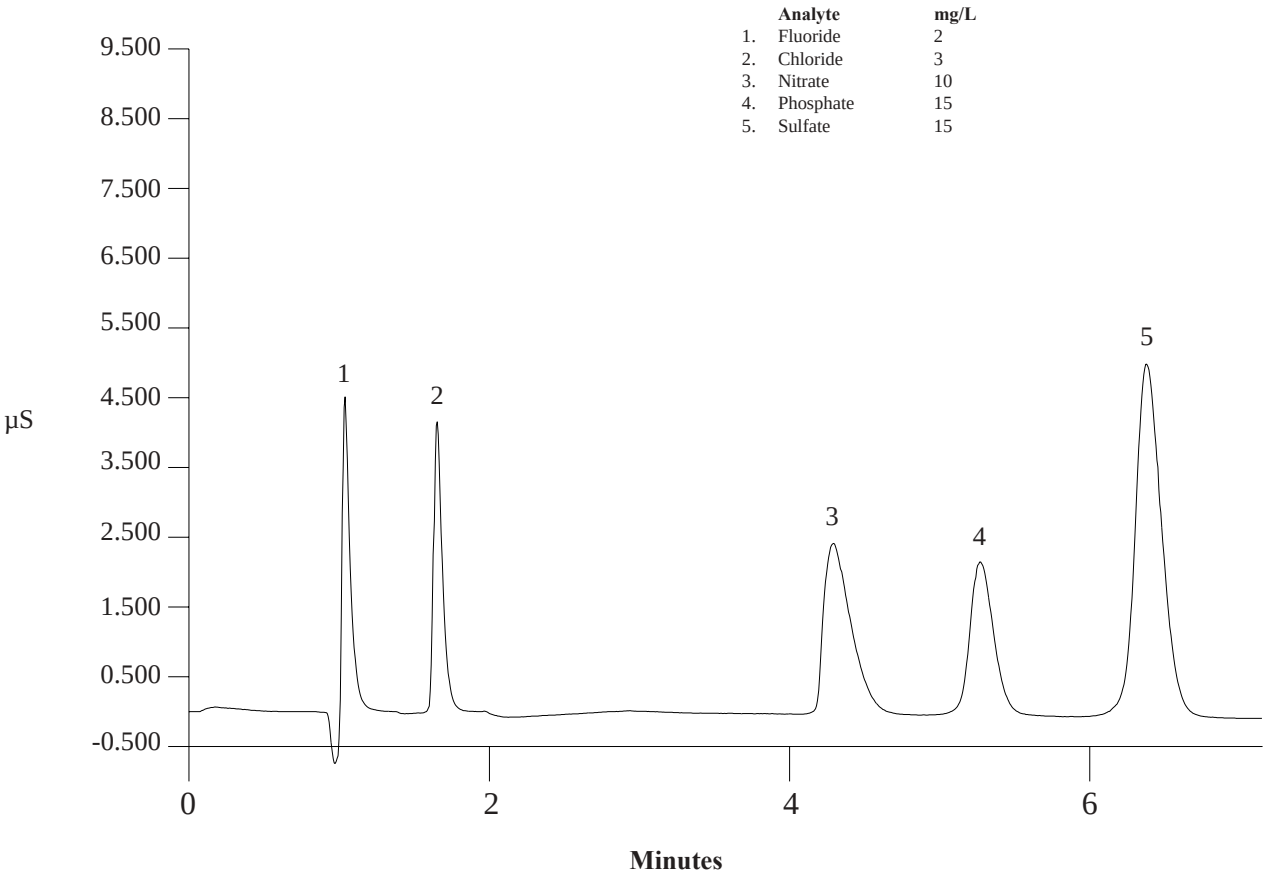


Figure 5
Carbonate Eluent

4.2.3 Gradient Elution of Inorganic and Organic Anions

The sodium hydroxide concentration of the eluent at the beginning of the gradient program is weak enough that fluoride elutes after the void volume. The weak ionic eluent will also separate several weakly retained monovalent organic acids. The sodium hydroxide concentration is increased to elute polyvalent ions such as trivalent phosphate and citrate. See Section 3 – Operation, for eluent preparation instructions.

Equilibrate the column thoroughly with 0.25 mM NaOH/12% methanol/16% ethanol before injecting the sample. Equilibration time is typically 15 minutes. If the final eluent concentration used in a gradient is stronger than the one shown in the example chromatogram (i.e. 60% mM NaOH/28% ethanol) , the time required to equilibrate the OmniPac PAX-100 should be increased.

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

If better resolution is needed for the first eluting peaks, dilute eluent E1, 1 mM NaOH, since this part of the chromatogram is run isocratically at an eluent concentration of 0.25 mM NaOH.

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 eluent gradient proportions.

A. Keep the concentrations of Eluent 1 and Eluent 2 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 phosphate and bromide are not, multiply the gradient program 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 the gradient program times by a factor greater than 1 (e.g, 1.1) to have a gradient slope that is less steep.

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

B. Change the proportions of Eluent 1 and Eluent 2 and adjust the gradient program times. This approach requires more time to develop and more knowledge in methods development work. The advantage of this approach is that a method can be tailored for a particular application, where selectivity, resolution, and the 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 eluent results in poor resolution of phosphate and bromide. Too high a concentration in one or both eluents results 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 phosphate 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 differ slightly 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.

4. The concentration of CH_3OH can be varied during the analysis to affect resolution. By increasing the concentration of CH_3OH, it is possible to obtain better resolution of weakly retained anions. However, some loss in resolution of the later eluting anions may be seen.

Sample Loop Volume:	10 µL
Guard Column:	OmniPac PAX-100 Guard Column
Analytical Column:	OmniPac PAX-100 Analytical Column
Eluents,	
	Eluent 1: 0.25 mM NaOH/12% methanol/16% ethanol
	Eluent 2: 60 mM NaOH/28% ethanol
Eluent Flow Rate:	1.0 mL/min
MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III
MMS Regenerant:	20 mN H_2SO_4
Expected Background Conductivity:	0.25 mM NaOH; 2-4µS 60 mM NaOH; 4-7 µS
Expected System Operating Backpressure:	2000 - 2300 psi

Analyte

1. Fluoride
2. Acetate
3. Propionate
4. Lactate
5. Quinate
6. Formate
7. Pyruvate
8. Monochloroacetate
9. Chloride
10. Nitrite
11. Dichloroacetate
12. Mannuronate
13. Nitrate
14. Selenite
15. Succinate
16. Sulfite
17. Malate
18. Tartrate
19. Sulfate
20. Phthalate
21. Phosphate
22. Chromate
23. Citrate

Gradient Program

Time (min)	%1	%2	%3	%4	V5	V6	Comments
0.0	100	0	0	0	0	0	Equilibration
10.0	100	0	0	0	1	0	load
10.1	100	0	0	0	1	0	inject
13.1	100	0	0	0	1	0	isocratic condition
30.0	70	30	0	0	1	0	gradient
45.0	0	100	0	0	1	0	end gradient
50.0	0	100	0	0	1	0	
50.1	100	0	0	0	1	0	gradient reset

Note that 10 minutes are required for equilibration of the column with the weak eluent prior to injecting the next sample.

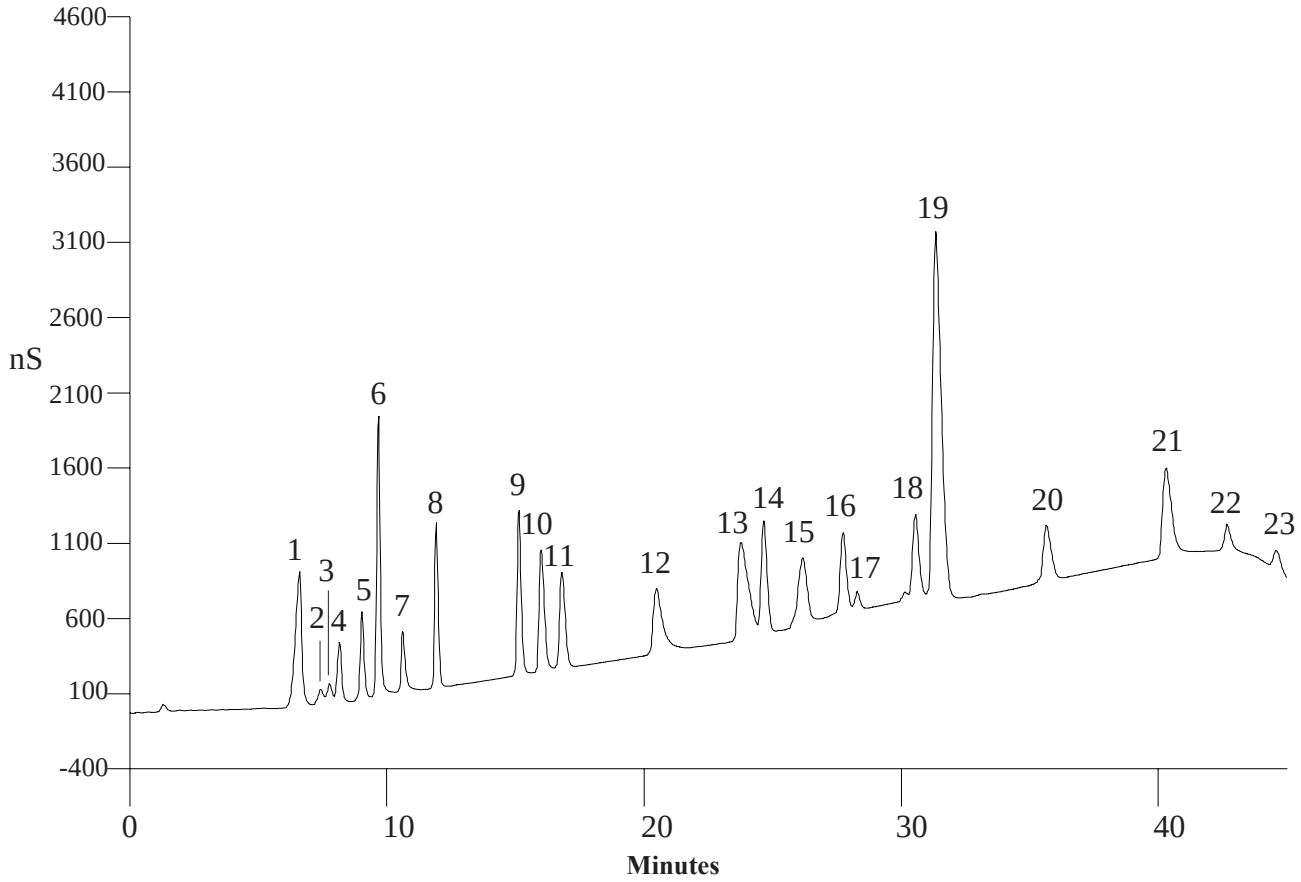


Figure 6
Gradient Elution of Inorganic and Organic Anions

4.2.4 Separation of Aromatic Acids

Using UV detection and the OmniPac PAX-100 Analytical Column, it is possible to separate, by ion exchange, a wide variety of aromatic acids which cannot be separated by ion suppression or ion pair chromatography. The uniform peak width and uniform peak asymmetry gives an indication of the excellent focusing properties using the ionic gradient.

Sample Loop Volume:	10 µL
Guard Column:	OmniPac PAX-100 Guard Column
Analytical Column:	OmniPac PAX-100 Analytical Column
Eluents:	Eluent 1: Deionized Water (specific resistance of 18.2 megohm-cm) Eluent 2: 80% CH_3CN Eluent 3: 1 M NaCl/4 mM NaOH
Eluent Flow Rate:	1.0 mL/min
Detection:	UV at 254 nm
Expected System Operating Backpressure:	1800 - 2000 psi

Gradient Program

Time (min)	%1	%2	%3	Comments
0.0	70	25	5	Equilibration with weak eluent
10.1	70	25	5	Injection of Sample
30.0	35	25	40	Gradient Ramp

	Analyte
1.	Benzoate
2.	Benzenesulfonate
3.	Toluenesulfonate
4.	p-Chlorobenzenesulfonate
5.	p-Bromobenzoate
6.	3,4-Dinitrobenzoate
7.	Phthalate
8.	Terephthalate
9.	p-Hydroxybenzenoate
10.	p-Hydroxybenzenesulfonate
11.	Gentisate
12.	Trimesate
13.	Pyromellitate

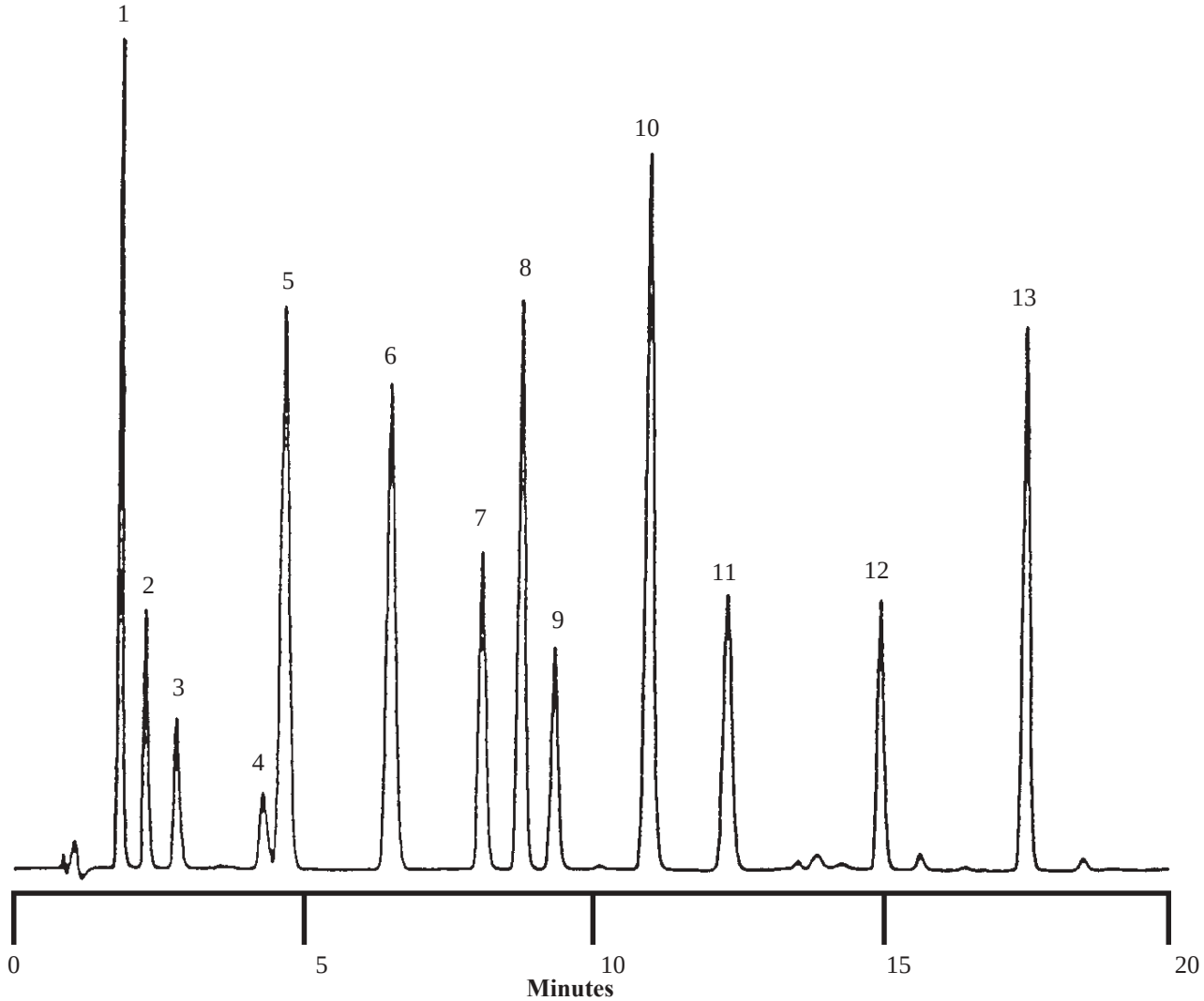


Figure 6
Separation of Aromatic Acids

4.3 OmniPac PAX-500 Examples

4.3.1 The Effect of Increasing Acetonitrile on Retention Times

As with any reversed phase column, increasing the concentration of solvent in the eluent will cause the peaks to be eluted earlier. The effect acetonitrile has on the selectivity of inorganic anions injected on to the OmniPac PAX-500 column is shown in Figure 8.

When 5% CH_3CN is added to an eluent of 40 mM NaOH, selectivity is essentially the same selectivity as if the eluent was completely aqueous with a 3.5% cross linked latex phase. Remember that a minimum of 1% solvent must be maintained in any eluent used with the OmniPac PAX-500. When the concentration of acetonitrile is increased to 20%, the retention times are radically reduced, with loss of resolution between chloride and nitrite and also between bromide and nitrate. The selectivity now approximates an ion exchange phase with 1% effective cross-linking. When the concentration of acetonitrile is further increased to 40%, the effective cross-link of the ion exchange phase is decreased to approximately 1/4%, causing analytes to be eluted one on top of the other. This figure illustrates that by increasing the solvent in the eluent, strongly retained analytes can be eluted much faster than if base alone were used.

Sample Loop Volume:	10 µL
Guard Column:	OmniPac PAX-500 Guard Column
Analytical Column:	OmniPac PAX-500 Analytical Column
Eluent:	See Chromatogram
Eluent Flow Rate:	1.0 mL/min
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS ULTRA
SRS Mode:	AutoSuppression External Water Mode
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III
MMS Regenerant:	50 mN H_2SO_4
Expected Background Conductivity:	2-4 µS
Expected System Operating Back Pressure:	800 - 1,200 psi

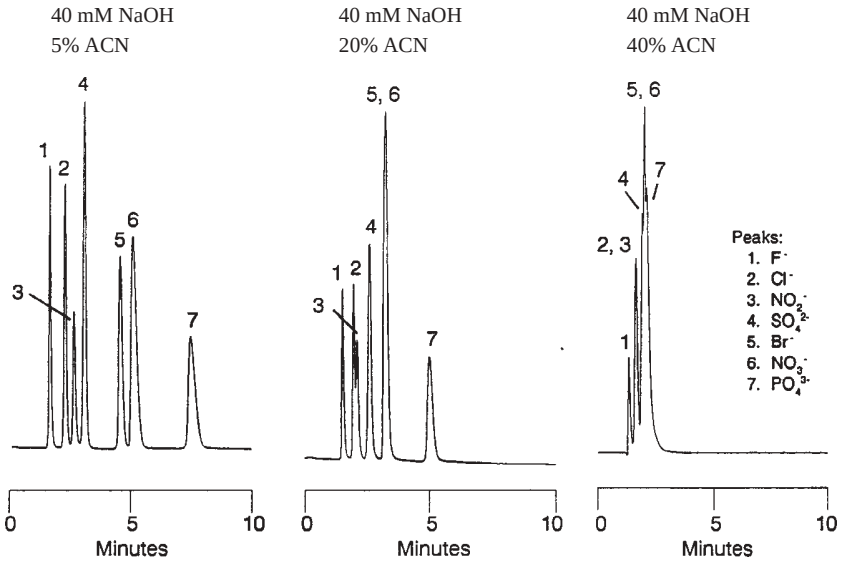


Figure 8
The Effect of Acetonitrile on Times with the Omnipac PAX-500

4.3.2 Isocratic Elution of Seven Common Anions

The following example demonstrates the separation of seven common anions using the ion exchange mode of separation with suppressed conductivity detection. Note that sulfate elutes before bromide and nitrate in this example. Contrast this with the subsequent gradient example (see Section 4.3.3, "Gradient Elution of Inorganic and Organic Anions") in which sulfate elutes after bromide and nitrate.

Sample Loop Volume:	10 µL
Guard Column:	OmniPac PAX-500 Guard Column
Analytical Column:	OmniPac PAX-500 Analytical Column
Eluent:	40 mM NaOH/5% CH_3OH*
Eluent Flow Rate:	1.0 mL/min
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS ULTRA
SRS Mode:	AutoSuppression External Water Mode
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III
MMS Regenerant:	50 mN H_2SO_4
Expected Background Conductivity:	2-4 µS
Expected System Operating Back Pressure:	800 - 1,200 psi

*2.5% CH_3CN can be used instead of 5% CH_3OH however, prepare two eluents (El) 25% CH_3CN and (E2) 44 mM NaOH, then proportion together at 10% (E1) and 90% (E2) to minimize eluent preparation and to minimize the possibility of hydrolysis of the acetonitrile (see Section 3.9, "Preparation of Eluents Containing Solvents").

	Analyte	mg/L
1.	Fluoride	2
2.	Chloride	3
3.	Nitrite	5
4.	Sulfate	8
5.	Bromide	10
6.	Nitrate	10
7.	Phosphate	15

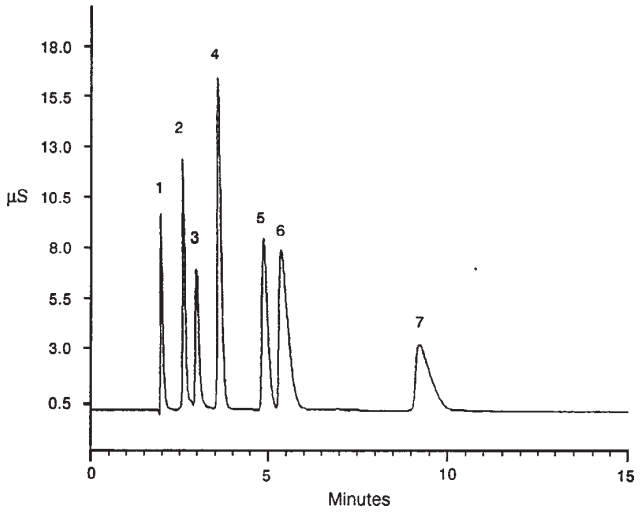


Figure 9
Isocratic Elution of Seven Common Anions

4.3.3 Gradient Elution of Inorganic and Organic Anions

The sodium hydroxide concentration of the eluent at the beginning of the gradient program, is weak enough that fluoride elutes after the void volume. The weak ionic eluent will also separate several weakly retained monovalent organic acids. The sodium hydroxide concentration is increased to elute polyvalent ions such as trivalent phosphate, citrate, and cis- and transaconitate. See Section 3, "Operation," for eluent preparation instructions.

Equilibrate the column thoroughly with 0.75 mM NaOH before injecting the sample. Equilibration time is typically 15 minutes. If the final eluent concentration used in a gradient is stronger than the one shown in the example chromatogram (i.e. 35% of El, 40% of E2), the time required to equilibrate the OmniPac PAX-500 should be increased.

If an injection is made before the column is fully equilibrated with the weak eluent, the early eluting peaks (fluoride and the monoprotic organic acids) will elute too soon and resolution will be impaired. Furthermore, retention times will not be reproducible. If better resolution is needed for the first eluting peaks, dilute eluent El, 1 mM NaOH, since this part of the chromatogram is run isocratically at an eluent concentration of 0.75 mM NaOH.

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 eluent gradient proportions.

A. Keep the concentrations of Eluent 1 and Eluent 2 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 phosphate and bromide are not, multiply the gradient program 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 the gradient program times by a factor greater than 1 (e.g., 1.1) to have a gradient slope that is less steep.

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

B. Change the proportions of Eluent 1 and Eluent 2 and adjust the gradient program times. This approach requires more time to develop and more knowledge in methods development work. The advantage of this approach is that a method can be tailored for a particular application, where selectivity, resolution, and the 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 El 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 differ slightly 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.

   4. The concentration of CH_3OH can be varied during the analysis to affect resolution. By increasing the concentration of CH_3OH, it is possible to obtain better resolution of weakly retained anions. However, some loss in resolution of the later eluting anions may be seen.

   5. The Test/Storage Eluent contains carbonate/bicarbonate. If the ion exchange sites are not completely converted to the hydroxide form before the application is tried, resolution will be poor and all retention times will be too short. The baseline shift during the gradient and the initial background conductivity will be too high.

Sample Loop Volume:	10 µL
Guard Column:	OmniPac PAX-500 Guard Column
Analytical Column:	OmniPac PAX-500 Analytical Column
Eluents:	El: 1.0 mM NaOH E2: 200 mM NaOH E3: 5% CH_3OH
Eluent Flow Rate:	1.0 mL/min
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS ULTRA
SRS Mode:	AutoSuppression External Water Mode
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III
MMS Regenerant:	50 mN H_2SO_4
Expected Background Conductivity:	0.75 mM NaOH; 2-4 µS 50 mM NaOH; 4-7 µS
Expected System Operating Back Pressure:	800 - 1,000 psi

Gradient Program

Time (min)	%E1	%E2	%E3	Comments
0.0	75	0	25	Equilibration with weak eluent
15.0	75	0	25	Inject sample
15.1	75	0	25	Elution of weakly retained anions
20.0	75	0	25	Start of gradient ramp 1
35.0	60	15	25	Start of gradient ramp 2
55.0	35	40	25	End of Analysis

NOTE
15 minutes are required for equilibration of the column with the weak eluent prior to injecting the next sample.

	Analyte	mg/L
1.	Fluoride	1.5
2.	α-Hydroxybutyrate	10
3.	Acetate	10
4.	Glycolate	10
5.	Gluconate	10
6.	α-Hydroxyvalerate	10
7.	Formate	5.0
8.	Valerate	10
9.	Pyruvate	10
10.	Monochloroacetate	10
11.	Bromate	10
12.	Chloride	3.0
13.	Nitrite	5.0
14.	Dichloroacetate	10
15.	Selenite	10
16.	Bromide	10
17.	Nitrate	10
18.	Sulfate	10
19.	Oxalate	10
20.	Selenate	10
21.	α-Ketoglutarate	10
22.	Fumarate	10
23.	Phthalate	10
24.	Oxalacetate	10
25.	Phosphate	10
26.	Arsenate	10
27.	Chromate	10
28.	Citrate	10
29.	Isocitrate	10
30.	cis-Aconitate	10
31.	trans-Aconitate	10

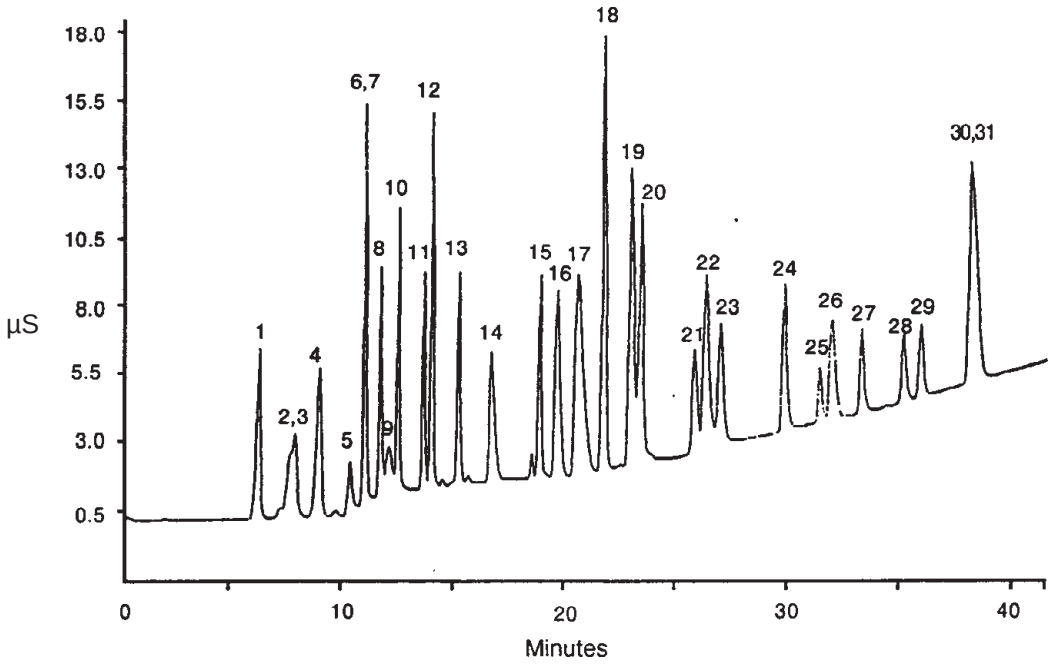


Figure 10
Gradient Elution of Inorganic and Organic Anions

4.3.4 Ion Exchange/Ion Pair Chromatography

When resolution using the anion exchange mechanism alone is insufficient to resolve all the peaks, depending upon the nature of the analytes the ion pair mechanism can be added for increased resolution. Ion pair chromatography is a technique which approximates a dynamic ion exchange mechanism, as a result of the addition of a hydrophobic ion to the eluent. The hydrophobic ion is typically tetrabutyl ammonium hydroxide (TBAOH) for anion separations and this bulky ion adsorbs at the interface of the non-polar stationary phase and the eluent, forming a charged layer for increased ion exchange.

Figure 8 (Section 4.3.1) demonstrated that when 20% acetonitrile was added to the eluent, resolution was lost between chloride and nitrite and also between bromide and nitrate. Figure 11A shows the effect of adding 1.0 mM TBAOH to the eluent. Despite the fact that the sample contains succinate, oxalate and chlorate in addition to the standard seven anions and the eluent has not been optimized, there is now baseline resolution between chloride and nitrite and significant resolution between bromide and nitrate. When the eluent is somewhat optimized by decreasing the solvent concentration, which effectively increases the contribution of the ion pair reagent, all ten analytes are resolved, most of them baseline resolved. (Figure 11B)

Sample Loop Volume:	10 µL
Guard Column:	OmniPac PAX-500 Guard Column
Analytical Column:	OmniPac PAX-500 Analytical Column
Eluent:	See Chromatograms
Eluent Flow Rate:	1.0 mL/min
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS ULTRA AutoSuppression External Water Mode
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III
MMS Regenerant:	50 mN H_2SO_4
Expected Background Conductivity:	3 - 5 µS
Expected System Operating Back Pressure:	1,100 - 1,400 psi

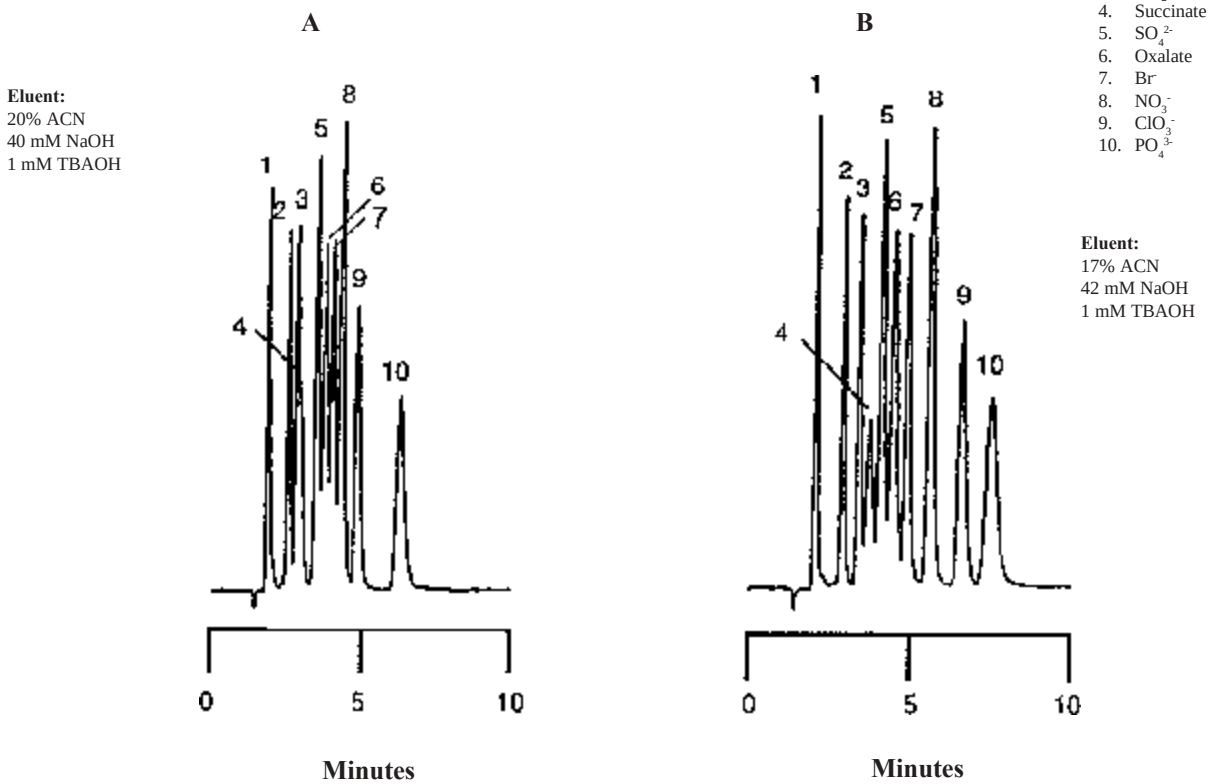


Figure 11
Combined Ion Pair/Ion Exchange Separation on the OmniPac PAX-500

Figure 12 shows a comparison of the separation of the same ten analytes by ion exchange (Figure 12A), Ion Pair (Figure 12B) and Ion exchange/Ion Pair. In Figure 12A, the substrate is a non porous, highly cross-linked particle to which the anion exchange MicroBeads are agglomerated. Since the substrate is non-porous, there is no hydrophobic core available for the ion pair reagent, and the ion pair mechanism is not in effect. In Figure 12B, the substrate is a macroporous, highly cross-linked particle with no anion exchange MicroBeads attached to the surface, so there is no ion exchange mechanism in effect, only ion pair. Figure 12C shows the combined ion exchange/ion pair effects of the OmniPac PAX-500.

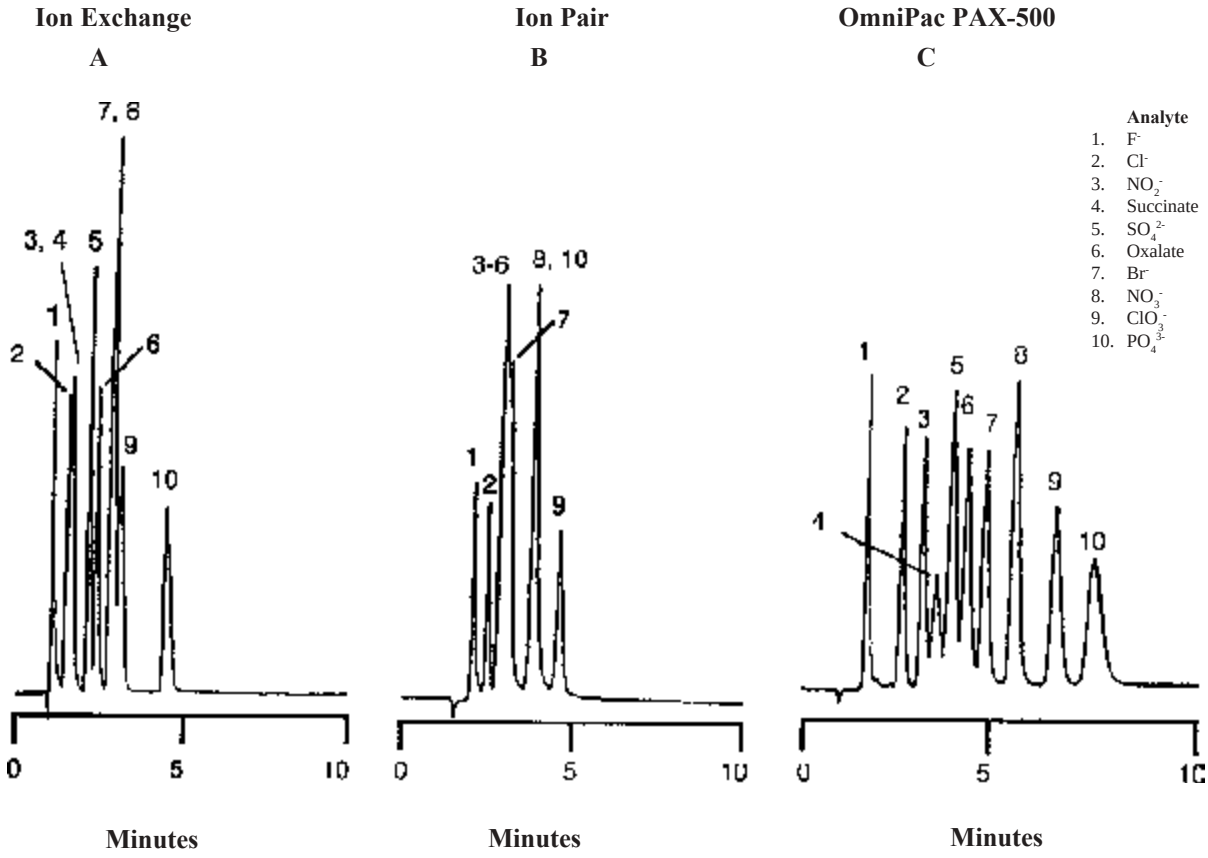


Figure 12
Comparison of Ion Exchange, Ion Pair and Multi-mode Selectivity

4.3.5 Multi-Mode Chromatographic Separation of Ionic and Nonionic Aromatic Compounds

The multiphase material used in the OmniPac PAX-500 greatly increases the flexibility of the analytical system. By choosing the right eluent conditions, neutral compounds can be separated from ionic components. The freedom to choose between ion exchange and multiphase retention is demonstrated in the separation of ionic and nonionic aromatic compounds. For ten minutes of the separation the eluent is 80% CH_3CN with no ionic characteristics. Using solvent only at the beginning of the chromatographic run allows the elution of neutral components such as benzyl alcohol and benzene without any interference from ionic components. The ionic components can be eluted by adjusting the organic modifier in the eluent and at the same time increasing the ionic strength of the eluent. In principle, this multi-mode approach can be performed in either order depending on the requirements of the sample.

Eluent #1: Degassed, Type I Reagent Grade Water
Eluent #2: 80% CH_3CN
Eluent #3: 1 M NaCl/4 mM NaOH

Gradient Program

Time (min)	%E1	%E2	%E3	Comments
0.0	0	100	0	Equilibration
10.1	0	100	0	Inject sample
13.9	0	100	0	End reversed-phase
14.0	75	25	0	Begin low CH_3CN equilibration
16.9	75	25	0	End low CH_3CN equilibration
37.0	35	25	40	Ionic gradient

Sample Loop Volume:	10 µL
Guard Column:	OmniPac PAX-500 Guard Column
Analytical Column:	OmniPac PAX-500 Analytical Column
Eluents:	El: Degassed, Type I Reagent Grade Water
	E2: 80% CH_3CN
	E3: 1 M NaCl/4 mM NaOH
Eluent Flow Rate:	1.0 mL/min
Detection:	UV at 254 nm
Expected System Operating Back Pressure:	1,000 - 1,500 psi

	Analyte	mg/L
1.	BenzylaJoohol	100
2.	Dethyttoluarnide	50
3.	Benzene	200
4.	Benzoic Acid	40
5.	Benzenesulfonic Acid	40
6.	Toluenesulfonic Acid	40
7.	p-ChlorobenzenesulfcnicAcid	50
8.	p-Bromobenzoic Acid	20
9.	Phthalic Acid	30
10.	Terephthalic Acid	2
11.	p-Hydroxybenzenesulfonic Acid	2
12.	1,3,5 Benzenetricarboxylic Acid	20
13.	1,2,4,5 Benzenetetracarboxylic Acid	10

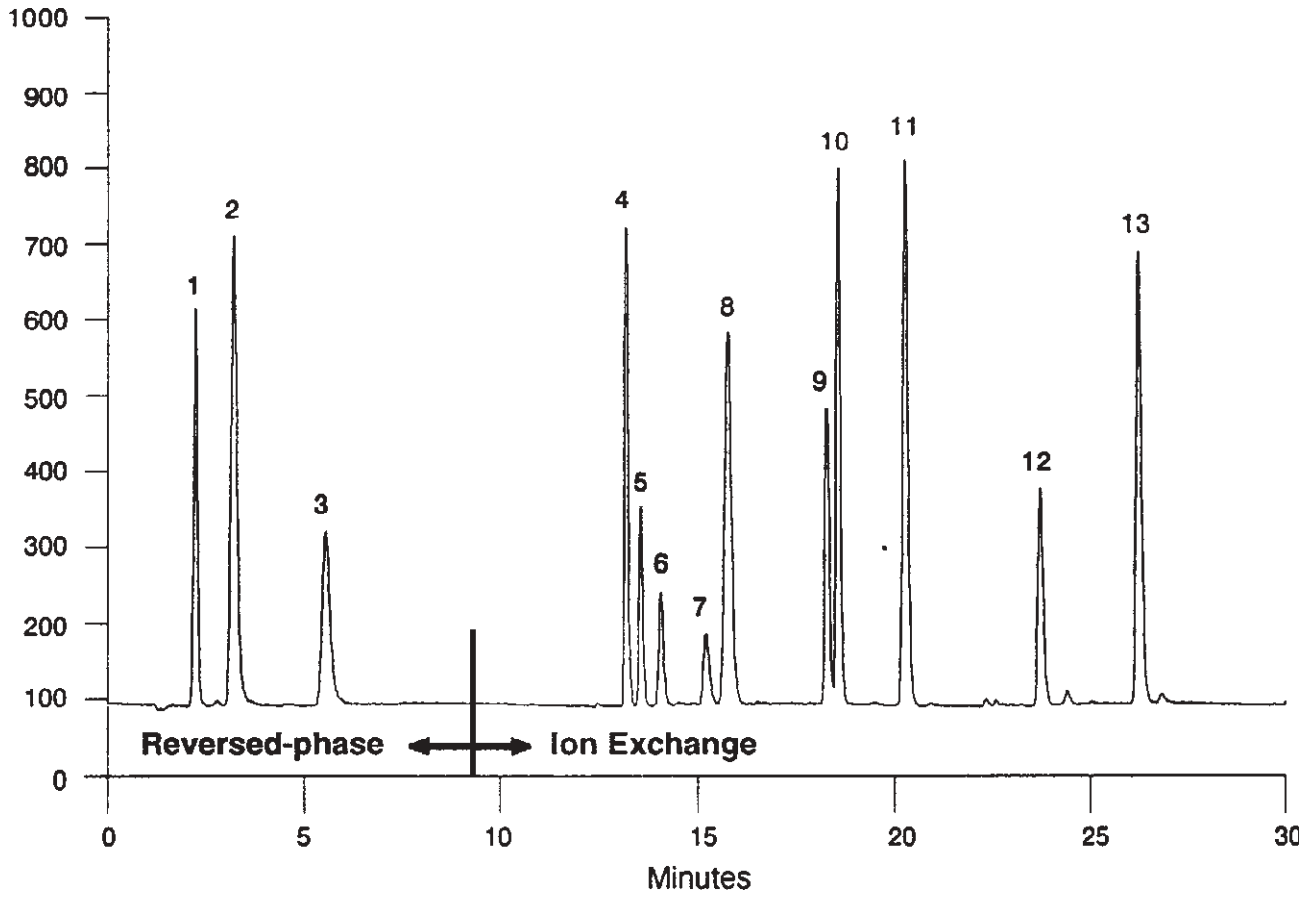
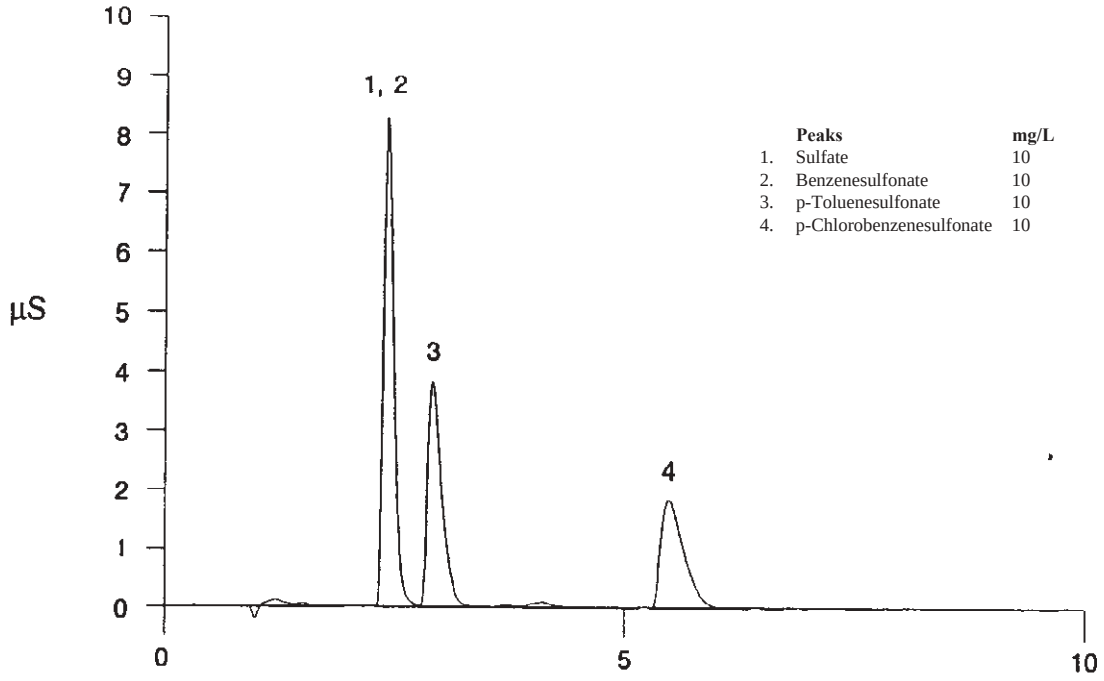


Figure 13
Multi-mode Chromatographic Separation of Ionic and Nonionic Aromatic Compounds

4.3.6 Combined Adsorption and Ion Exchange Separation of Inorganic and Organic Compounds

The OmniPac PAX-500 was developed to allow the analytical chemist to use solvents as a mobile phase modifier in ion exchange separations and enhance ion exchange separations. For example, with an eluent of 40 mM NaOH/20% CH_3CN, sulfate and benzenesulfonate coelute on an equivalent ion exchange column without adsorption retention (multi-phase) character. Resolving sulfate and benzenesulfonate can be accomplished by switching to the OmniPac PAX-500 Analytical Column. Without changing the eluent, the OmniPac PAX-500 separates these two anions by utilizing two retention modes - ion exchange and reversed-phase, both contributing to the increased retention of the aromatic suffonate analytes. Note that sulfate, retained only by ion exchange, has the same retention on both columns.

Sample Loop Volume:	10 µL
Guard Column:	OmniPac PAX-500 Guard Column
Analytical Column:	OmniPac PAX-500 Analytical Column
Eluent:	40 mM NaOH/20% CH_3CN
Eluent Flow Rate:	1.0 mL/min
SRS Suppressor:	Anion Self-Regenerating Suppressor, ASRS ULTRA AutoSuppression External Water Mode
or MMS Suppressor:	Anion MicroMembrane Suppressor, AMMS III
MMS Regenerant:	50 mN H_2SO_4
Expected Background Conductivity:	2 - 5 µS
Expected System Operating Back Pressure:	1,100 - 1,500 psi



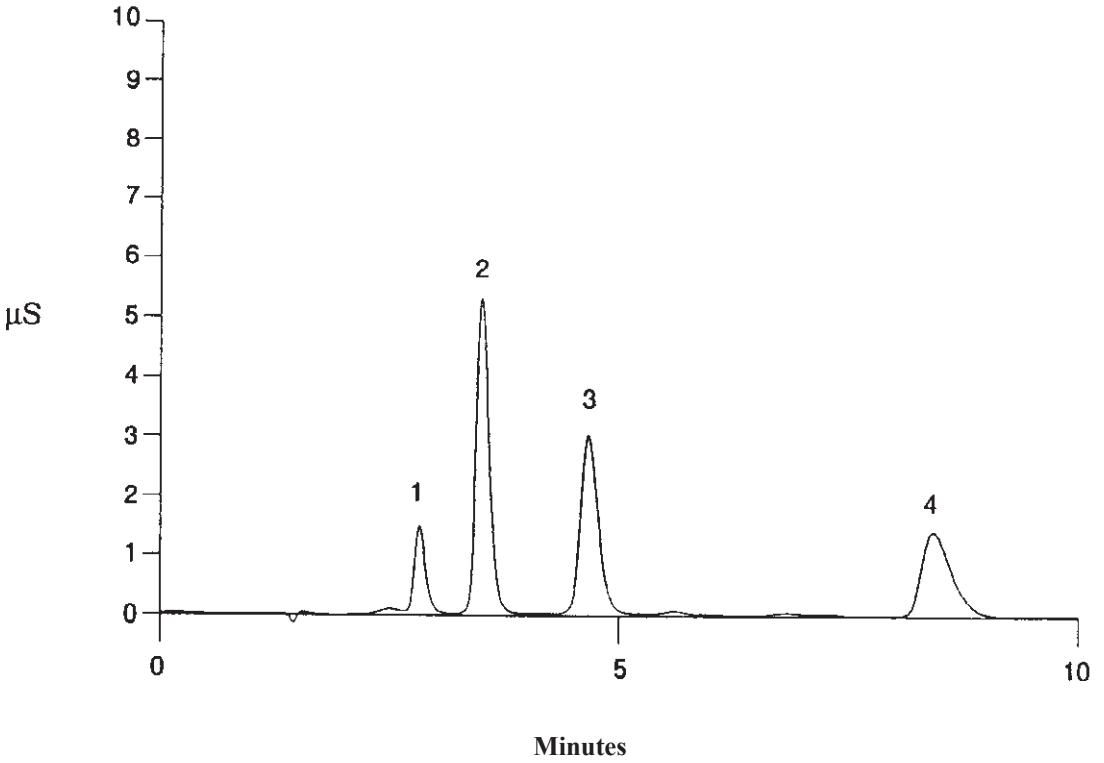


Figure 14
Combined Adsorption and Ion Exchange Retention of One Analyte

SECTION 5 - TROUBLESHOOTING GUIDE

The purpose of the Troubleshooting Guide is to help you solve operating problems that may arise while using the OmniPac PAX Analytical Columns. For more information on problems that originate with the instrument hardware or the suppressor, refer to the Troubleshooting Guide in the appropriate Product Manual. If you cannot solve the problem on your own, call the Dionex Regional Office nearest you (see Dionex Worldwide Offices).

5.1 High Backpressure

Total system backpressure when using the OmniPac PAX columns at 1.0 mL/min should be less than 3000 psi. Refer to Section 3.1.2 Solvents, to see how solvent concentration can affect the column operating backpressure. If the backpressure is higher than 3000 psi, it is advisable to find out what is causing the high pressure. The system should be used with an in-line filter for the eluents. Make sure you have one in place and that it is not contaminated.

5.1.1 Incorrect Flow Rate

Make sure that the pump is set to 1.0 mL/min. Higher flow rates will cause higher pressure. Measure the pump flow rate if necessary.

5.1.2 Plugged Tubing

Find out what part of the system is causing the high backpressure. It could be a piece of tubing that has plugged or whose walls are collapsed, an injection valve with a plugged port, a column with particulates plugging the bed support, a plugged high-pressure in-line filter, the MicroMembrane Suppressor or the detector.

To find out which part of the chromatographic system is causing the problem, disconnect the pump eluent line from the injection valve and turn the pump on. Watch the pressure; it should not exceed 50 psi. Continue adding the system's components (injection valve, column, suppressor, detector) one by one, while watching the pressure. The pressure should increase up to a maximum of 3000 psi when the column is connected. The MicroMembrane Suppressor will add up to 100 psi. No other components should add more than 100 psi of pressure. Refer to the appropriate manual for cleanup or replacement of the problem component.

5.1.3 Contaminated Bed Support

If the analytical column is the cause of high backpressure, its inlet bed support may be contaminated. To change the bed support, follow the instructions below using one of the two spare bed supports included in the Ship Kit.

A. Disconnect the column from the system.

B. Using two open-end wrenches, carefully unscrew the inlet (top) column end fitting.

C. Turn the end fitting over and tap it against a bench top or other hard, flat surface to remove the bed support and seal assembly. Discard the old assembly.

D. Place a new bed support assembly into the end fitting. Use the end of the column to carefully push the bed support assembly into the end fitting.

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

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

NOTE

If any of the column packing becomes lodged between the end of the column and the bed support washer assembly, no amount of tightening will seal the column. Make sure that the washer and the end of the column are clean before screwing the end fitting back onto the column.

Part	PAX-100 (P/N)	PAX-500 (P/N)
Analytical Column	042150	042152
Guard Column	042151	042153
Bed Support Assembly	042955	042955
End Fitting	052809	052809

5.2 High Background Signal

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

Eluent	Expected Background Conductivity
0.75 mM NaOH/ 2% CH_3OH	1-3 µS
80 mM NaOH/ 2% CH_3OH	4-7 µS
40 mM NaOH/ 5% CH_3OH	2-4 µS
3.6 mM Na_2CO_3/ 3.4 mM $NaHCO_3$/ 25% CH_3OH	15-19 µS

The background conductivity typically increases between 1 and 3 µS when running a gradient as described in Section 4.

One of the most common reasons for high background, noise and drifting baselines when using methanol based eluents is using the ASRS ULTRA in the wrong suppression mode or having the current set incorrectly. Refer to the Anion Self-Regenerating Suppressor Product Manual (Document No. 031367) for details on choosing the proper suppression mode and current setting for eluent.

NOTE

Solvent-based eluent systems, below 40% solvent, can be used in the AutoSuppression External Water Mode. Often the cure for high background, noise and drifting baselines is lowering the current setting by one level.

If acetonitrile and hydroxide are left in the system overnight, the acetonitrile will decompose to acetate and ammonia. Initial background conductivity during the next start-up of the system will flush out of the system will be very high but the decomposed acetonitrile will flush out of the system within 30 minutes and normal background conductivity will be observe.

5.2.1 Eluent Considerations

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

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

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

5.2.2 Anion Trap Considerations

A. Do you have an Anion Trap Column (ATC-3) installed? If it has not, install one according to the directions in the ATC-3 manual and watch the background conductivity. If the background conductivity is now low, this means that the ATC-3 is trapping contaminants from the eluent. The eluents probably have too many impurities and should be remade with purer chemicals and DI water.

B. If an ATC-3 is already installed, remove it. Is the background conductivity still high? If the background conductivity decreases, the ATC-3 is the source of the high background conductivity.

1. Disconnect the ATC-3 from the analytical column and direct the outlet to waste. Clean according to the directions in the ATC-3 manual.

2. If the problem persists, replace the ATC-3.

5.2.3 Column Considerations

Remove the OmniPac PAX column from the system. Is the background conductivity still high? If the column is the cause of the high background conductivity, clean the column as instructed in Column Care Section in Appendix B.

5.2.4 Suppressor Considerations

If the above items have been checked and the problem persists, the suppressor is probably causing the problem. See the ASRS ULTRA or AMMS III manuals for operating details.

A. If using chemical regernation, check the regenerant flow rate at the **REGEN OUT** port of the suppressor. This flow should be greater or equal to 3 to 5 mL/min.

B. Check the eluent flow rate. It should be 1.0 mL/min for 4-mm systems.

C. Prepare fresh regenerant solution. Bypass the Anion AutoRegen Regenerant Cartridge (if you are using the AutoRegen Accessory). If the background conductivity is high, you probably need to clean or replace your suppressor. Refer to the suppressor Product Manual for assistance.

D. If you are using an AutoRegen Accessory, connect the freshly prepared regenerant to the Anion AutoRegen Regenerant Cartridge. Pump approximately 200 mL of regenerant through the Anion AutoRegen Regenerant Cartridge to waste before recycling the regenerant back to the regenerant reservoir. If the background conductivity is now high, you probably need to replace the Anion AutoRegen Regenerant Cartridge (P/N 039564). Refer to the AutoRegen Regenerant Cartridge Refill Product Manual for assistance.

5.2.5 Hardware Considerations

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

5.3 Poor Peak Resolution

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

5.3.1 Extra Column Effects

Extra-column effects can result in sample band dispersion, making the peaks' elution less efficient. Make sure you are using tubing with an i.d. of no greater than 0.012 inch, in all cases, between the injection valve and the detector cell inlet, and that the tubing lengths are as short as possible. Check for leaks.

5.3.2 Loss of Efficiency

Replace each suspect component (guard and analytical columns, suppressor and detector cell), one at a time, with a “known good” reference column, suppressor or cell. This will help isolate the location of the efficiency problem.

A. If the tests indicate that the efficiency problem is due to the suppressor, refer to the Anion Self-Regenerating Suppressor Product Manual (Document No. 031367) or the Anion MicroMembrane Suppressor Product Manual (Document No. 031727) for assistance in troubleshooting the efficiency problem.

B. If the tests indicate that the efficiency problem is due to the column check to see if headspace has developed in the analytical column (e.g., due to improper use of the column such as using the column without 1% organic solvent in the eluent or submitting it to high pressures). Remove the column's top end fitting (see Section 5.1). 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.

C. If the tests indicate that the efficiency problem is due to the detector cell, look for any signs of leaks from the cell, excessive baseline noise or spurious peaks. Consult your detector manual or your nearest Dionex Regional Office (see "Dionex Worldwide Offices") for cell troubleshooting procedures.

5.3.3 Shorter Retention Times

A. Check to see if eluent flow rate is faster than expected. Measure the eluent flow rate after the column using a stopwatch and a graduated cylinder.

B. Check to see if the eluents' composition and concentration are correct. An eluent that is too strong will cause the peaks to elute sooner. Prepare fresh eluent. Check the pump programming or the operation of the proportioning valve. Avoid proportioning less than 5% from any given line for best accuracy. To test the proportioning valve, place the eluent bottles in lines 3 and 4 and see of the problem persists.

C. Column contamination can lead to a loss of column capacity because all of the anion exchange sites will no longer be available for the sample ions. Polyvalent anions might be concentrating on the column. Refer to Column Care (Appendix B) for recommended column cleanup procedures. Possible sources of column contamination are impurities in chemicals and in the deionized water used. Be especially careful to make sure that the recommended chemicals are used. The deionized water should have a specific resistance of at least 18.2 megohm-cm.

5.3.4. Loss of Front End Resolution During a Gradient

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

A. Improper eluent E1 concentration may be the problem. Remake the eluent as described in Section 3.

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. Improperly swept out volumes anywhere in the system prior to the analytical column may be the problem. See item A above.

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

5.4 Spurious Peaks

5.4.1. Column Contamination

Run the gradient program without making an injection. Examine the baseline. If you see spurious peaks, the column may be contaminated.

If the samples contain an appreciable concentration of polyvalent ions and the column is used with a weak eluent system, polyvalent anions may be contaminating the analytical column. The retention times for the analytes will then decrease and spurious, inefficient (broad) peaks can show up at unexpected times. Clean the column as indicated in Column Care (Appendix B). Using the recommended eluent will ensure that strongly retained polyvalent anions are eluted before the next injection.

5.4.2 Injection Valve Contamination

Check for column contamination first. Then run the gradient program again, this time switching the injection valve but not injecting sample or standard (make sure that the sample loop contains either deionized water or eluent). If you see a baseline upset, especially at the beginning of the chromatogram, it is probably due to the injection valve.

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. It will happen when the injection valve needs to be cleaned or retorqued (see valve manual). Check to see that there are no restrictions in the tubing connected to the valve. Also check the valve port faces for blockage and replace them if necessary. Refer to the Valve Manual for troubleshooting and service procedures. Small baseline disturbances at the beginning or at the end of the chromatogram can be overlooked as long as they do not interfere with the quantitation of the peaks of interest.

5.5 Small Analyte Peak Areas When Using an ASRS ULTRA

This problem is usually caused by running eluent through the ASRS ULTRA using the AutoSuppression Recycle Mode or the AutoSuppression External Water Mode, with the power off. The problem may also occur in the Chemical Suppression Mode by not running acid through the regenerant chambers.

A. Disconnect the eluent line from the analytical column attached to the **ELUENT IN** port of the ASRS ULTRA at the analytical column end of the line. Direct this line to a separate waste beaker.

B. Disconnect the eluent line from the **ELUENT OUT** port of the ASRS ULTRA to the detector conductivity cell at the detector conductivity cell end of the line and install a 10-32 to 1/4-28 union (P/N 042806) on this line.

C. Install a plastic syringe with a Luer adaptor in the **ELUENT OUT** port and inject 5 mL of 0.5 N H_2SO_4 through the ASRS ULTRA in the reverse direction to normal flow so that the waste comes out of the **ELUENT IN** port.

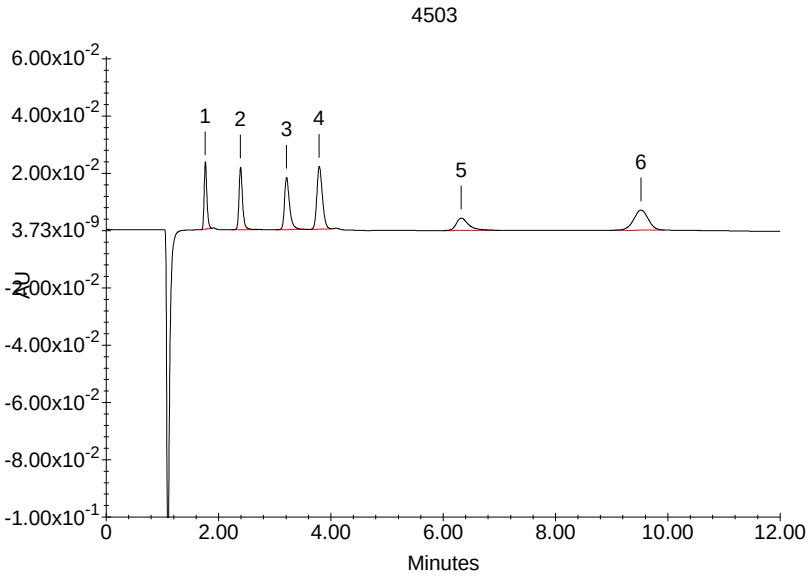
D. Reconnect the eluent line from the **ELUENT IN** port of the ASRS ULTRA to the analytical column and the eluent line from the **ELUENT OUT** port of the ASRS ULTRA to the conductivity detector cell.

E. If you are in the AutoSuppression Recycle Mode of operation, turn on the power and then begin pumping eluent. If you are in the AutoSuppression External Water Mode of operation, establish water flow through the regenerant chambers, turn on the power and then begin pumping eluent. If you are in the Chemical Suppression Mode of operation, establish acid regenerant flow through the regenerant chambers and then begin pumping eluent. Power is not used in this mode of operation.

F If the correct peak areas are not observed following two injections of a standard test solution, contact the nearest Dionex Regional Office (see “Dionex Worldwide Offices”).

OmniPac® PAX-100
Analytical (4 x 250 mm)
Product No. 42150

Serial No. : 4503 **Pressure (PSI) : 1850** **Date : 4/3/01 1:06:25 PM**



Eluent: 5.0 mM NaOH
1.0 mM Na_2CO_3
30% CH_3CN

Flow Rate: 1.0 mL/min

Detection: UV at 220 nm, 0.05 AUFS

Injection Volume: 10 µL

Storage Solution: 50 mM NaOH / 5% CH_3OH

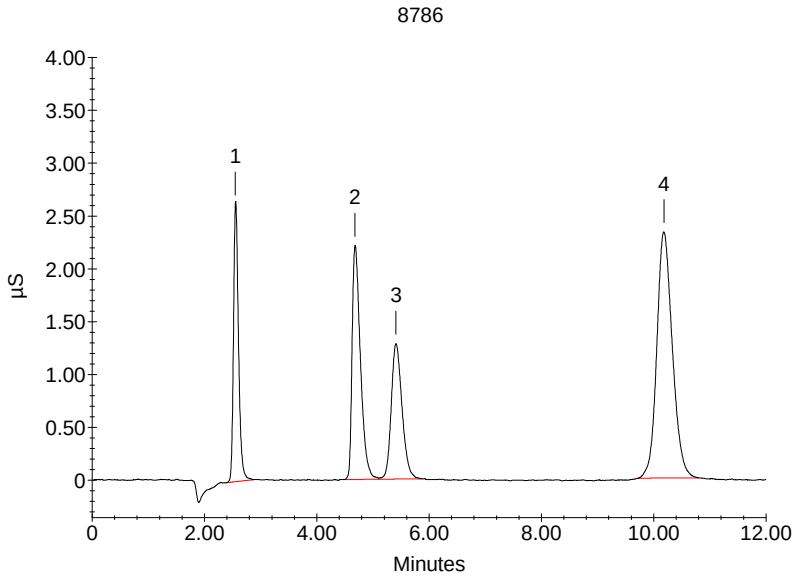
Peak Information : Found Components

Peak No.	Retention Time	Name		Efficiency	Asymmetry (10%)	Resolution
1	1.76	4-Hydroxybenzamide	1.5	6033	1.9	6.19
2	2.39	Benzenesulfonate	3.0	7120	1.5	6.05
3	3.21	Nitrate	1.5	6553	1.7	3.42
4	3.79	4-Cyanophenol	2.0	6994	1.5	9.05
5	6.32	4-Bromophenol	3.0	4436	1.9	7.50
6	9.52	Phenylphosphonate	3.0	6404	1.0	n/a

File Name : C:\PEAKNET\DATA\EXAMPLES\42150 PAX-100 4MM_004.DXD

OmniPac® PAX-500
Analytical (4 x 250 mm)
Product No. 42152

Serial No. : 8786 **Pressure (PSI) : 1400** **Date : 12/6/00 11:48:43 AM**



Eluent: 3.6 mM Na_2CO_3
3.4 mM $NaHCO_3$
25% CH_3OH

Flow Rate: 1.0 mL/min

Detection: Conductivity 5 µS full scale

Injection Volume: 10 µL

Storage Solution: Eluent

Peak Information : Found Components

Peak No.	Retention Time	Name	(mg/L)	Efficiency	Asymmetry (10%)	Resolution
1	2.55	Chloride	4.0	4135	1.7	10.05
2	4.68	Nitrate	10.0	4944	2.2	2.35
3	5.41	Benzenesulfate	25.0	3677	1.4	11.18
4	10.18	Sulfate	15.0	6672	1.3	n/a

File Name : C:\PEAKNET\DATA\EXAMPLES\42152 PAX-500 4MM_003.DXD

COLUMN CARE

Recommended Operating Pressures

Operating a column above its recommended pressure limit can cause irreversible loss of column performance. The maximum recommended operating pressure for OmniPac PAX-100 columns is 4,000 psi and for OmniPac PAX-500 columns is 3,000 psi.

Column Start-up

The OmniPac PAX columns are shipped filled with a long-term storage solution. For the OmniPac PAX-100, this long term solution is 50 mM NaOH/5% MeOH. For the OmniPac PAX-500, the long term storage solution is 25% MeOH/ 3.6 mM Na_2CO_3/ 3.4 mM $NaHCO_3$.

CAUTION

Upon receiving a new column or removing a column from long term storage you must perform the gradient listed in Section 3.6, Column Preparation, to ensure that the column is properly cleaned of any contaminants that might interfere with the reproducibility of subsequent analyses.

After the column has been cleaned according to Section 3.6, 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. Let the column equilibrate with eluent for a few minutes. Equilibration is complete when consecutive injections of the standard give reproducible retention times.

Column Storage

The column's short-term storage solution should be eluent. If the column will not be used for one week or more, prepare it for long-term storage by first cleaning it according to Column Cleanup, then flushing the column for 10 minutes with the long term storage solution described in Column Start-up, before plugging it.

Column Cleanup

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 with 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.

Section 3.6 describes how to change from an eluent containing one solvent to an eluent containing a second solvent, without harming the column. When in doubt, always include short column rinse steps to reduce the solvent content of the eluent to 5% levels and the ionic strength of the eluent to 50 mM levels to avoid creating high pressure zones in the column that may disrupt the uniformity of the column packing.

Choosing the Appropriate Cleanup Solution

A. In general, concentrated hydroxide cleanup solutions such as a 10X concentrate of the most concentrated eluent used in the application is sufficient to remove hydrophilic contamination of low valence.

B. Concentrated acid solutions such as 1 to 3 M HCl (the solution must contain at least 1% solvent), remove hydrophilic contamination of higher valence by ion suppression and elution by the chloride ion.

C. Concentrated acid solutions such as 1 to 3 M HCl (the solution must contain at least 1% solvent) also remove a variety of metals. If after acid treatment, the chromatography still reveals metal contamination, treatment with chelating acids

such as oxalic acid (0.1 M) is recommended.

D. Organic solvents can be used alone if the contamination is nonionic and hydrophobic. The degree of nonpolar character of the solvent should be increased in direct proportion to the hydrophobicity of the contamination. Acceptable solvents include typical HPLC solvents, such as acetonitrile, methanol, ethanol, isopropyl alcohol, etc.

E. Concentrated acid solutions such as 1 to 3 M HCl can be used with compatible organic solvents to remove contamination that is ionic and hydrophobic. The acid suppresses ionization and ion exchange interactions of the contamination with the resin. The organic solvent then removes the subsequent nonionic and hydrophobic contamination. A solution of 0.2 M HCl and 80% acetonitrile is very effective at removing many organic contaminants. This solution must be made immediately before use because acetonitrile will decompose in the acid solution.

Having chosen the most appropriate cleanup solution for your sample matrix, use the cleanup procedure described in Column Cleanup Procedure, to clean the PAX Analytical and Guard Columns.

Column Cleanup Procedure

A. Prepare a 500 mL solution of the chosen cleanup solution

B. Disconnect the suppressor from the OmniPac PAX column. If your system is configured with both a guard column and an analytical column, reverse the order of the guard and analytical column in the eluent flow path. Double check that the eluent flows in the direction designated on each of the column labels. Direct the effluent from the outlet line of the PAX Guard Column to a separate waste container.

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 2.0 mL/min (4-mm systems).

D. If your eluent contains a solvent or a salt that is not compatible with the chosen cleanup solution, slowly decrease the solvent concentration to 5%, then rinse the column for 15 minutes with 5% MeOH/95% deionized water before pumping the cleanup solution over the column.

E. Pump the cleanup solution through the column for 30–60 minutes. The cleaning solution must contain at least 1% solvent.

F. If your eluent (or long-term storage solution) contains a solvent or a salt that is incompatible with the cleanup solution, rinse the column for 15 minutes with 5% MeOH/95% deionized water before pumping eluent (or long-term storage solution) over the column, again.

G. Reconnect the suppressor to the OmniPac PAX 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.

H. Equilibrate the column(s) with eluent before resuming normal operation.

Carbonate Removal

Carbonate should not really be considered a column contaminant, but the presence of carbonate on the OmniPac PAX columns or in the eluent when using hydroxide eluents to perform chromatography can cause shifts in retention times, irreproducible results, high background conductivity and large baseline shifts. Following the recommended procedures for eluent preparation and storage will minimize the chances of hydroxide eluents absorbing carbon dioxide, thereby producing carbonate and affecting chromatography. The following steps can be used to remove carbonate from the OmniPac PAX columns.

A. Connect the OmniPac PAX Guard or Analytical Column directly to the pump, bypassing the Gradient Mixer or

the Anion Trap Column. Direct the column effluent directly to a separate waste container bypassing the suppressor.

B. Prepare an eluent of 200 mM NaOH/5% CH_3OH, taking the necessary precautions to minimize contamination with carbon dioxide. See Section 3.3.4, Sodium Hydroxide Eluent Preparation, for complete details.

C. Pump 250 mL of the 200 mM NaOH/5% CH_3OH through the column at 1.0 mL/min on 4-mm systems.

E. Reconnect the suppressor to the OmniPac PAX 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.

F. Rinse the guard and analytical column with eluent for 30 minutes. If performing a gradient application, rinse the column(s) with the strongest eluent used during the gradient ramp.

G. Equilibrate the column(s) with the starting eluent. Continue by pumping eluent through the column until the background conductivity stabilizes. The column is fully equilibrated when successive injections of a standard give reproducible retention times.