PRODUCT MANUAL

OMNIPAC® PCX-100 GUARD COLUMN
(PCX-100 GUARD, P/N 042193

OMNIPAC® PCX-100 ANALYTICAL COLUMN
(PCX-100 ANALYTICAL, P/N 042189)

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

1. The standard test eluent for the OmniPac PCX-100 is 100 mM KCl/50 mM HCl/ 32% CH_3CN. See Section 3.2, “Column Preparation.” Make the required stock and working solutions for eluents. See Section 3, “Operation,” for details. Note operation precautions and chemical purity requirements.

2. Run the Production Test Chromatogram as a system check. See Section 4.1, “Production Test Chromatogram,” for details.

3. See Section 4, “Example Applications” for example applications.

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

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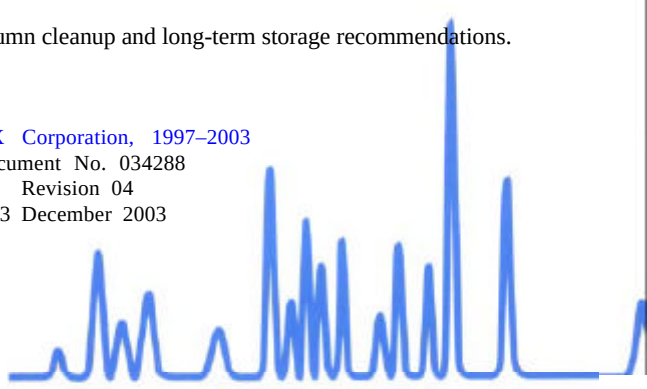

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SECTION 1 - INTRODUCTION

The OmniPac PCX-100 Analytical Column (P/N 042189) was developed to permit the use of organic solvents in eluents for polymer-based ion exchange separations.

The column packing of the OmniPac PCX-100 consists of a highly cross-linked microporous (low surface area) core covered with a pellicular layer of cation exchange latex. The ethylvinylbenzene/divinylbenzene polymeric core is produced in a manner so that the core has a reactive exterior surface to which a polymeric latex can be attached. The latex particles are functionalized to create acidic sulfonate groups, the cation exchange sites. The active cation exchange latex is permanently attached to the microporous core of the column packing with an anchor latex.

Since the support material is polymer-based, ionic eluents in the pH range of 0–14 can be used to effect selectivity and convert molecular species into ionic compounds. The highly cross-linked polymeric microporous substrate also allows the use of common HPLC solvents as eluent modifiers. HPLC solvents can often be used to advantage in eluent systems to elute hydrophobic species, to dissolve certain sample matrices and to clean columns contaminated with organic matrix components.

This manual assumes that you are familiar with the installation and operation of the Ion Chromatograph (IC). If you do not understand the operation of your system, take the time to familiarize yourself with the various system component manuals before beginning an analysis.

The OmniPac PCX-100 Analytical Column has 10-32 PEEK end fittings. If your chromatograph is not outfitted with PEEK tubing, it will be necessary to make one or more Tefzel liquid line connections with a 10-32 PEEK bolt and ferrule fitting on one end and a 1/4-28 ThermoFlare fitting on the other end. See “Dionex Liquid Line Fittings” for instructions on assembling these end fittings.

Table 1
OmniPac PCX-100 Packing Specifications

Column	Particle Diameter µm	Substrate X-Linking %	Latex Diameter nm	Latex X-Linking %	Column Capacity µeq/column	Functional Group	Hydrophobicity
OmniPac PCX-100 (4 x 250)	9	55	200	5%	120	Sulfonic Acid	Hydrophilic
OP PCX-100 Guard (4 x 50)	9	55	200	5%	24	Sulfonic Acid	Hydrophilic

Table 2
OmniPac PCX-100 Operating Parameters

Column	Typical Back Pressure psi (MPa) at 30°C	Standard Flow Rate mL/min
OmniPac PCX 100 Analytical (4 x 250 mm)	< 2,500 (17.23)	1.0
OmniPac PCX 100 Guard (4 x 50 mm)	< 750 (5.17)	1.0
OmniPac PCX 100 Column + Guard	< 3,250 (22.40)	1.0

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 - INSTALLATION

The majority of the applications developed for the OmniPac PCX-100 Analytical Column use UV/Vis or electrochemical detection. Additional system requirements for those applications that are best performed using suppressed conductivity detection are in Section 2.3.

2.1 HPLC Compatibility

The OmniPac PCX-100 Analytical Column should be run on any Dionex Chromatographic system. However, concentrated sulfuric acid, concentrated nitric acid and methylene chloride will attack PEEK, so care should be taken to prevent this. Tetrahydrofuran at concentrations greater than 10% is not compatible with OmniPac Columns.

2.2 Guard Columns

To prolong the life of the OmniPac PCX-100 Analytical Column, it is recommended that an OmniPac PCX-100 Guard Column (P/N 042193) be used. Retention times will increase by approximately 20% when a guard column is placed in-line prior to the analytical column. A guard is placed prior to the analytical column to prevent sample contaminants from eluting onto the analytical column. It is less expensive to clean or replace a guard column than an analytical column.

2.3 System Requirements for Suppressed Conductivity

The following system requirements are for those applications developed for the OmniPac PCX-100 Analytical Column that are best performed using suppressed conductivity detection.

2.3.1 Micromembrane Suppressor Requirements

When performing applications that require suppressed conductivity detection, a Cation MicroMembrane Suppressor (CMMS III, P/N 056750) must be used. The CMMS III is completely compatible with ionic eluents that contain organic solvents. This manual assumes that you are familiar with the installation and operation of the CMMS III. If you are not, take the time to review the Installation Instructions for the CMMS III before beginning an analysis.

2.3.2 Installing the IonPac Cation Trap Column (CTC-I)

When performing ion exchange applications that involve a gradient and suppressed conductivity, an IonPac Cation Trap Column (CTC-1, P/N 040192) should be installed instead of a Gradient Mixer between the gradient pump and the injection valve. The CTC-1 is filled with high capacity cation exchange resin and helps to minimize the baseline change due to cationic contaminants in the eluent as the ionic concentration of the eluent is increased during a gradient analysis.

When used properly the CTC-1 will minimize baseline shifts during the gradient analysis by preventing trace cationic contaminants from entering the eluent stream.

A. The CTC-1 is installed between the Gradient Pump Module (GPM) and the injection valve.

   1. Remove the Gradient Mixer (GM-2) installed between the GPM and the injection valve.

   2. Connect the GPM directly to the CTC-1. Connect a waste line to the CTC-1 outlet and direct the line to a waste container.

   3. Rinse the CTC-1 with 30 mL of a 10X concentrate of the strongest eluent that will be used during the gradient analysis at the flow rate used in the application (e.g., 2mL/min for 15 minutes).

   4. After flushing the CTC-1 with eluent, connect the CTC-1 to the eluent line that is connected to the injection valve.

B. The background conductivity of your system should be 2–5 µS. The baseline shift should be no greater than 3 µS during the gradient ramp. If the baseline shifts are greater than 3 µS, flush the CTC-1 with 30 mL of a 2X to 3X concentrate of the strongest eluent used in the gradient. At the end of each operation day the CTC-1 should be rinsed of any accumulated impurities by flushing with 30 mL of the strongest eluent of the gradient program.

2.4 Using the AutoRegen Accessory and Eluents with Solvents

To minimize the baseline shift when performing an analysis that requires a 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).

In the course of using an AutoRegen Accessory equipped with an AutoRegen Cation Regenerant Cartridge and the Cation MicroMembrane Suppressor (CMMS III), it is necessary to replace the regenerant on a regular basis. The frequency of replacement 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 250 mL of the regenerant should be pumped to waste before recycling of the regenerant is started. It is not necessary to change the AutoRegen Cation Regenerant Cartridge until it is completely expended.

2.4.1 Using Solvents with the AutoRegen

Solvents in the eluent continuously diffuse through the membrane in the Cation MicroMembrane Suppressor from the eluent channel into the regenerant stream and build up in the regenerant solution. However, the solvent is not removed from the recycled regenerant by the AutoRegen Cation Regenerant Cartridge and continues to accumulate. Eventually the acetonitrile hydrolyzes to acetate and ammonium, fouling the AutoRegen cartridge. Solvents other than acetonitrile have no effect on the cartridge lifetime. The ionic strength of the eluent and acetonitrile concentration determines the lifetime of the AutoRegen Cation Regenerant Cartridge.

CAUTION

Acetonitrile decomposes to ammonium acetate when subjected to basic solutions such as the 0.1 M tetrabutylammmonium hydroxide (TBAOH) used as the regenerant solution in the AutoRegen Cation Regenerant Cartridge. The acetate ion exchanges onto the AutoRegen Cation Regenerant Cartridge. If high levels of acetonitrile are used in the eluents, the cartridge can become expended within a few hours. A pressurized regenerant delivery system should be used as an alterative.

SECTION 3 - OPERATION

3.1 Chemicals Required

Obtaining reliable, consistent and accurate results requires eluents that are free of ionic and spectrophotometric impurities. Chemicals, solvents and deionized water used to prepare the eluents must be of the highest purity available. Dionex cannot guarantee proper column performance when the quality of the chemicals, solvents or water used to prepare eluents has been compromised.

3.1.1 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 with an actual lot analysis.

3.1.2 Solvents

Since the solvents used with OmniPac PCX-100 Analytical Column are added to aqueous ionic eluents to enhance the ion exchange process, 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 be used. Currently, several manufacturers produce ultrahigh purity solvents that are compatible with HPLC and spectrophotometric applications. These ultrahigh purity solvents will usually ensure that your chromatography is not affected due to ionic impurities in your solvent. At Dionex, we have obtained consistent results using High Purity Solvents manufactured by Burdick and Jackson, and Optima Solvents manufactured by Fisher Scientific.

3.1.3 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 of ionized 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. All deionized water should be thoroughly degassed prior to eluent preparation.

3.1.4 Regenerant For Suppressed Conductivity Applications

For those applications performed with suppressed conductivity, use Dionex Cation Regenerant Solution (TBAOH, 0.1 M tetrabutylammonium hydroxide, P/N 039602) to ensure maximum system performance. If you are using the AutoRegen Accessory (P/N 039594) equipped with an AutoRegen Cation Regenerant Cartridge (P/N 039563), prepare 0.5 to 1.0 liter of the regenerant. If you plan to use a pressurized vessel, prepare several liters.

NOTE

Acetonitrile is not compatible with the Cation MicroMembrane Suppressor when using an AutoRegen System. The acetonitrile diffuses into the TBAOH regenerant, concentrates during recirculation and eventually hydrolyzes to acetate and ammonia, fouling the AutoRegen System. If acetonitrile is used with suppressed conductivity, a pressurized vessel rather than the AutoRegen must be used.

3.2 Column Preparation

The OmniPac PCX-100 Analytical Column is tested to ensure that the column will meet performance specifications for retention time, peak efficiency and peak symmetry of the test mixture specified on the test chromatogram. The OmniPac PCX-100 is shipped in 100 mM KCl/50 mM HCl/32% acetonitrile.

Prior to using the OmniPac PCX-100 Analytical Column for the first time or after long term storage, the column should be flushed using the following gradient program. Direct the column effluent to waste.

CAUTION

The OmniPac PCX-100 Analytical and Guard Columns require a minimum of 1% solvent in the eluent to ensure that the column packing is properly wetted and to maintain the integrity of the packed column.

When changing the eluent solvent type or the ion species in the eluent, a 10 minute (or longer) gradient from the old eluent to the new eluent must be performed.

Lo Pressure Limit = 300
Hi Pressure Limit = 3000

Flow Rate 1.0 mL/min

Eluent 1: 95% Acetonitrile/Deionized Water
Eluent 2: 0.5 M HCl/1.0 M KCl/1% Acetonitrile
Eluent 3: 1% Acetonitrile/Deionized Water
Eluent 4: None

Time (min)	%1	%2	%3	%4
0.0	0	0	100	0
15.0	0	0	100	0
30.0	0	10	90	0
40.0	0	10	90	0
45.0	90	10	0	0
60.0	90	10	0	0

The OmniPac PCX-100 Analytical Column is then equilibrated with eluent for approximately 30 minutes prior to use. The column is fully equilibrated when two consecutive injections of the standard produce chromatograms with identical retention times.

3.3 Sample Injection

For most applications, a 10 µL to 25 µL injection loop will be sufficient. Generally, do not inject more than 10 nanomoles of any one analyte onto the column. Injecting larger volumes or higher concentrations of samples can result in overloading the column which can affect the detection linearity, peak efficiency and peak symmetry.

Whenever practical, the chemical matrix of the sample should be matched with the eluent. Matching the sample matrix to the eluent will minimize baseline upsets due to refractive index and pH differences between the sample and the eluent. When matrix matching, always run a blank to ensure that no impurities are added to your sample.

3.4 Column Selection

Before starting an analysis, look at the structures of the analytes to determine if they can be ionized to form cations. The OmniPac PCX-100 Analytical Column should be chosen when cation exchange will be the only mechanism for the separation of the sample analytes. The OmniPac PCX-100 should also be chosen when the cationic analytes are so hydrophobic that they are retained too long on the OmniPac PCX-500 Analytical Column (P/N 042191) or when the sample matrix contains hydrophobic neutral or anionic species that are highly retained on the hydrophobic surface of the OmniPac PCX-500.

Often a separation can be achieved by taking advantage of the hydrophobic differences of the sample analytes. If the sample contains cations and neutral molecules with a significant range of hydrophobic character, the OmniPac PCX-500 Analytical Column should be chosen for application development.

The IonPac CS10 Analytical Column (P/N 043015) should be chosen for separations of inorganic Group I & Group II cations when the separation can be accomplished without solvents.

3.5 Selection of Detection Method

3.5.1 Absorbance Detection

After selecting the analytical column, determine the mode of detection by examining the structures of the sample analytes. Due to the nature of the eluent systems which can be used with the OmniPac PCX-100 Analytical Columns, UV/Visible detectors are ideal. If the analytes absorb UV or visible light, determine the absorbance maxima which occurs at an acidic pH.

3.5.2 Electrochemical Detection

If the analytes do not absorb in the UV or visible regions, or if they have a low extinction coefficient, DC amperometry or pulsed electrochemical detector (PED) should be considered for detection. Note that Dionex ED40, ED50 and ED50A can also be used for conductivity. PED is used to detect alcohols, carbohydrates, aldehydes, sugar alcohols, monosaccharides, oligosaccharides, glycols, amino acids, amines and sulfur-containing compounds. When using an acidic eluent for amine detection with a gold working electrode, post-column addition of base is necessary (final pH at the detector must be approximately 13).

3.5.3 Conductivity and Other Detection Modes

Other detectors that can be used with the OmniPac PCX-series of analytical columns are the Conductivity Detector Module (CDM-II, 039698), the pulsed electrochemical detector (PED, 042038) in conductivity mode and the Fluorescence Detector Module (FDM-II, 042095). For conductivity detection, check the specific conductance of the analyte or experimentally determine if the analyte is conductive enough to give the desired limit of detection. If the compound is polyvalent, it may require unsuppressible amounts of acid to be eluted from the analytical column; therefore, a suppressible divalent cation such as DL-2,3-diaminopropionic acid (DAP, P/N 039602) must be used. Refer to the Cation MicroMembrane Suppressor (CMMS III) Product Manual (Document No. 031728) for details on using eluents that contain DAP.

3.5.4 Multiple Detectors in Series

Because the OmniPac PCX-100 Analytical Column can separate a large variety of analytes bycation exchange, the run time of multiple assays can be significantly reduced by using the analytical column with two detectors in series. For example, one might need to analyze compounds that absorb in the UV simultaneously with compounds that do not absorb in the UV but that can be electrochemically oxidized. In this case, a UV Detector can be connected in series with an Electrochemical Detector.

3.6 Eluent Systems

3.6.1 The Role of Solvents in Eluent Systems

CAUTION

The OmniPac PCX-100 Analytical and Guard Columns require a minimum of 1% solvent in the eluent to ensure that the column packing is properly wetted and to maintain the integrity of the packed column.

When changing the eluent solvent type or the ion species in the eluent, a 10 minute (or longer) gradient from the old eluent to the new eluent must be performed.

Eluting cations stronger than hydrogen, such as sodium and potassium, cannot be used in the eluent with suppressed conductivity detection since these ions have high specific conductance and cannot be suppressed in the CMMS III. As a result, the background will be too high and detection limits will be dramatically increased. See Section 2.7, "Using the AutoRegen Accessory and Eluents with Solvents," in this manual if your eluent system contains acetonitrile.

Since the polymeric core has an electrostatically neutral hydrophobic internal surface, it is essential that the OmniPac PCX-100 Analytical Column is operated so that any aqueous eluent pumped through the column has minimally 1% organic solvent. The 1% organic solvent in the eluent will ensure that the hydrophobic surface of the substrate is "wetted" and maximum column performance is maintained. In order to ensure this, it is advisable to have at least 1% organic solvent in the deionized water eluent bottle. This precaution can prevent loss of column performance due to mistakes in setting eluent proportioning which otherwise may result in pumping pure aqueous eluents over the column for long periods of time.

The OmniPac PCX-100 Analytical Column packing can withstand all common reversed phase HPLC solvents in a concentration range of 1% to 100%. The two most commonly used solvents are acetonitrile and methanol. Acetonitrile is a stronger solvent than methanol, and has lower absorbance at low UV wavelengths than many other solvents. It is electrochemically clean, and delivers low (PED) backgrounds for amine and thiol detection on gold electrodes.

NOTE

Acetonitrile is not compatible with the Cation MicroMembrane Suppressor when using an AutoRegen System. The acetonitrile diffuses into the TBAOH regenerant, concentrates during recirculation and eventually hydrolyzes to acetate and ammonia, fouling the AutoRegen System. If acetonitrile is used with suppressed conductivity, a pressurized vessel rather than the AutoRegen must be used.

Acetonitrile plays several roles as an eluent component.

A. It can be added to swell the cation exchange latex which lowers the charge density in the latex and thus can change the cation exchange selectivity.

B. As the concentration of the acetonitrile in the eluent is increased, less water is available to hydrate the sample analytes which often changes the cation exchange selectivity observed in the separation.

The OmniPac PCX-100 Analytical Column can be used with any suppressible ionic eluent that does not exceed the capacity of the Cation MicroMembrane Suppressor (CMMS III). Even when performing ion exchange applications that do not normally require solvent, at least 1% organic solvent must be maintained in the eluent being used to maintain column packing integrity.

Observed system back pressures will depend on the type of solvent used in the eluent, the concentration of that solvent, the ionic strength of the eluent and the flow rate. The system back pressure, which is very dependent on eluent viscosity, will vary as the composition of water-methanol and water-acetonitrile mixture changes.

The practical column operational back pressure limit of the OmniPac PCX-100 Analytical and Guard Columns is 5000 psi.

Therefore, any combination of eluent formulations whose contributions to the back pressure total less than 5000 psi can be used.

All water used for the formulation of all eluents including those with solvents should have a specific resistance of 18.2 megohm-cm. The eluent should be thoroughly degassed.

CAUTION

In gradient methods that include salts, always be careful to determine the aqueous salt solubility at the maximum solvent concentration before running the gradient to prevent precipitation of the salt in the system during the gradient run.

The salt solubility under the desired eluent solvent concentration can be empirically determined by making small samples of gradually increasing levels of salt in the solvent containing eluent and observing when precipitation occurs.

Applications utilizing solvent gradients are best performed when organic solvents used in the eluents are premixed with the aqueous components. This will eliminate incomplete mixing of solvents with water and excessive pressure fronts traveling down the column due to the high viscosities of mixing neat solvents with other solvents or with water. Premixing solvent containing aqueous eluents will also prevent outgassing and refractive index problems commonly associated with mixing neat solvents and water with proportioning valves. For these reasons, the practical range of solvents in aqueous eluents used in gradient applications is from 1% to 95% solvent. This precaution will improve the reproducibility of the solvent gradient ramp for your chromatography. For example, if you plan to run a gradient from 10% solvent to 90% solvent, make the following eluents:

Eluent A: 10% solvent/90% water
Eluent B: 90% solvent/10% water

By programming the gradient pump properly, you can change from 100% Eluent A to 100% Eluent B in a prescribed time.

3.6.2 The Role of Acids in Eluent Systems

For weak bases, such as amines, that can be separated by cation exchange the mobile phase should be kept at least two pH units below the pKa values of the analytes to ensure that they are fully protonated. Additional amounts of acid in the eluent will help to elute the analytes. If the analyte is polyvalent, complete protonation may cause excessively long retention times. Retention can be reduced by increasing the pH of the eluent to two pH units above the lower pKa.

Acids commonly used in OmniPac PCX eluents are hydrochloric acid (HCl) and perchloric acid ($HClO_4$). Acetic acid is a weaker acid than HCl or $HClO_4$ and can be used with ammonium acetate buffer to control eluent pH between 4 and 5. These acids have low UV absorbance at wavelengths most commonly monitored. If the system is to be used for pulsed electrochemical detection (PED) with a gold working electrode, then a nonoxidizable acid such as perchloric acid should be chosen as the acid component in the eluent. The chloride in the hydrochloric acid is strongly adsorbed onto the gold electrode and after a short time will hinder analyte response. In most other cases, hydrochloric acid should be used to simplify the retention mechanism, thus avoiding introduction of more hydrophobic complexing anions in the eluent.

3.6.3 The Role of Salts in Eluent Systems

When the column affinity for the acid in the eluent is too low to elute sample analytes, when two analytes co-elute or when peak efficiency is low, salts can be added to the eluent to rectify the problem. Cation exchange selectivity of sulfonate cation exchange sites is dependent to a great extent on the hydration of the eluting cations. The effective hydration of the common cations used to elute sample analytes is in the decreasing order of $H^+ > Li^+ > Na^+ > K^+$. Compared with carboxylic acid exchange sites, the sulfonic acid exchange sites are less hydrated. Cations with low hydration are better able to enter the more hydrophobic stationary phase than more highly hydrated cations which prefer to partition into aqueous eluents. Consequently, highly hydrated, hydrophilic analytes will elute first.

Salts that are commonly used with the OmniPac PCX-100 Analytical Column include LiCl, NaCl, KCl, CsCl, sodium acetate, ammonium acetate and sodium perchlorate. As explained above, lithium will be a more efficient eluting cation for hydrophilic analytes, while potassium or cesium will be a more efficient eluting cation for hydrophobic compounds.

Before actually proportioning the salt in the Gradient Pump Module (GPM), it is advisable to check the salt's solubility under the desired solvent concentration. For a rough guide, the salt solubility limits in 54% acetonitrile are as follows:

Table 3
Maximum Acid and Salt Solubility in Acetonitrile by Cation Type

Acid	Lithium	Sodium	Potassium
<0.2 M HCl	0.4 M LiCl	0.3 M NaCl <0.8 M $NaClO_4$ 0.1 M Na_2SO_4 0.2 M sodium acetate	0.3 M KCl

3.6.4 The Role of the Counter Ion in Eluent Systems

The counterion of the eluent cation (i.e., the eluent anion) can also have an effect on resolving sample analytes. This occurs by changing the solubility parameters of the analyte cation due to the formation of an ion pair complex between it and the eluent anion. Highly hydrated salts such as sodium acetate can enhance salting-out effects and change the selectivity of the column stationary phase. Depending on the particular ion pair complex, additional changes in selectivity may take place if the pH or the ionic strength of the eluent is changed.

Perchlorate anions in the eluent can form ion pairs with monovalent cations having low hydration energy and with surface active cations. Since perchlorate anions tend to form ion pairs having increased hydrophobicity compared to the free analyte cations, the effect of this ion pair complex is longer retention times.

Nonoxidizable anions should be used in the eluent if the pulsed electrochemical detection with a gold working electrode is carried out. Chloride can corrode the gold electrode and is not recommended for PED.

Ion pairing reagents, such as hexane sulfonic acid are commonly used in applications for the separation of small cations using suppressed conductivity detection. Ion pairing reagents are used at concentrations below 10 mM depending on the specific reagent. At higher concentrations, these reagents may cause high background conductivity.

3.6.5 Preliminary Eluent Systems

Select the appropriate detection method (see Section 3.5, "Selection of Detection Method") then select a compatible eluent system. Start with the following general conditions:

A. The eluent flow rate should be 1.0 mL/minute.

B. The high pressure limit on the Gradient Pump Module (GPM) should be set at 3500 psi.

C. Prepare individual eluent bottles containing

1. Acid in deionized water with 1% solvent
2. Salt in deionized water with 1% solvent
3. 90% solvent in deionized water

Using a gradient pump, proportion different amounts from each eluent bottle to develop the isocratic or gradient eluent system which will accomplish the desired separation.

For example, if the UV/Visible Detection Method is selected, the four eluent bottles might contain:

A. 0.5M HCl in deionized water with 1% acetonitrile

B. 1 M NaCl in deionized water with 1% acetonitrile

C. 90% Acetonitrile in deionized water

D. Deionized water with 1% acetonitrile.

If pulsed electrochemical detection (PED) is selected, the eluent system and the post column reagent might consist of:

A. 0.5 M $HClO_4$ in deionized water with 1% acetonitrile

B. 1 M $NaClO_4$ in deionized water with 1% acetonitrile

C. 90% acetonitrile in deionized water

D. 1% acetonitrile in deionized water

E. Post column addition of 0.3 M sodium hydroxide at 1 mL/min. is necessary when using the gold working electrode for the detection of amines. The post column additon of sodium hydroxide is not required for the detection of thiols (including aminothiols).

If the conductivity detection is selected, the eluent system might consist of:

A. 500 mM HCl in deionized water with 1% acetonitrile

B. 4 mM DAP in deionized water with 1% acetonitrile

C. 90% acetonitrile in deionized water

D. 1% acetonitrile in deionized water (Do not use AutoRegen when using acetonitrile. See Section 3.6, “Eluent Systems”).

CAUTION

Cation exchange and solvent exchange gradients can be run simultaneously to manipulate the cation exchange solubility of the OmniPac PCX-100 Analytical Column. When designing wide concentration gradients it is very important that the solubility of the salts and acids in the particular solvent being used are not exceeded. It is also wise to determine experimentally in a test tube the salt and acid solubility limits under your chosen eluent system conditions.

For a "starter gradient," study the chromatograms and conditions shown in Section 4, "Example Applications." If your analytes resemble any of the groups of compounds chromatographed with the OmniPac PCX-100 Analytical Column, start with the gradient conditions for that particular application. Check first that the eluent system is compatible with the detection method you wish to use.

If your sample analytes do no resemble any of the example applications shown, you may want to start with the following suggested gradients:

A. When using the UV/Visible detector:
A dual gradient of: 50 mM HCl with 100 to 300 mM NaCl and 10% to 60% acetonitrile in 10 minutes. Hold at the upper end until all peaks elute.

B. When using PED detection:
A dual gradient of: 50 mM $HClO_4$ with 100 to 300 mM $NaClO_4$ and 10% to 60% acetonitrile in 10 minutes. Hold at the upper end until all peaks elute. Post-column addition of 0.3M NaOH at 1.0 mL/min required to increase the final post column eluent to pH 13.

C. When using conductivity detection:
A triple gradient of: 10 mM to 50 mM HCl with 10% to 60% acetonitrile and 1 mM to 5 mM DAP in 10 minutes. Hold at the upper end until all peaks elute.

3.6.6 Eluent System Development Process

If initial attempts at developing a preliminary eluent system as described in Section 3.6.5 have not yielded complete resolution of all sample analytes, proceed with the following eluent system development process.

Start the eluent system development process by ramping only one component of the mobile phase at a time so that the chromatographic behavior of the analytes and matrix components can be clearly understood. Neutral and anionic compounds will be eluted in the void volume. Cationic analytes will be eluted by a cation exchange gradient in which either the acid or salt concentration of the eluent is increased. The eluent can then be modified so that the ionization of weak bases are either enhanced or suppressed by adjusting the eluent's pH according to the pKa values of the different analytes. Study the structural features of the analytes and their pKa values so that different separation strategies can be developed. Resolution is proportional to the selectivity and elution efficiency. Sensitivity is directly dependent on peak elution efficiency.

Peaks sharpen as the slope of the gradient is increased. This is accomplished by reducing the overall time of the gradient or by increasing the eluent concentrations at the end of the gradient. Gradients should be designed so that all peaks elute within the time of the gradient. Peaks which elute within the gradient will have better efficiency than those which do not. Remember that there is usually 2 - 3 minutes of delay time between changing the gradient at the pump and observing the effect of the change by the detector.

Acid is often used in the eluent systems to set the pH in a useful range. Long reequilibration times may be encountered for applications run at fixed pH with varying salt concentrations. Reequilibration of an ion exchange gradient is fastest if the ratio of counterions in the eluent remains constant throughout the gradient. Applications that separate sample analytes based on differences in their pKa values often require pH gradient ramps.

The best peak efficiencies are obtained when the eluting ions are as similar as possible to the sample analyte ions. Ionic strength or concentration gradients allow the elution of ions of widely different charge. Higher solvent concentrations have the effect of lowering the charge density in the latex due to swelling, and thus cations of higher valence will elute earlier.

CAUTION

The OmniPac PCX-100 Analytical and Guard Columns require a minimum of 1% solvent in the eluent to ensure that the column packing is properly wetted and to maintain the integrity of the packed column.

When changing the eluent solvent type or the nature of the ionic strength in the eluent, a 10 minute (or longer) gradient from the old eluent to the new eluent must be performed.

Always check the salt solubility in the solvent/aqueous mixtures outside of the system before actually modifying the eluent system.

SECTION 4 - EXAMPLE APPLICATIONS

The OmniPac PCX-100 Analytical Column is a powerful ion exchange column that is solvent compatible.

The OmniPac PCX-100 key operating parameters are listed below:

Maximum Operating Pressure:	5000 psi
Organic Solvent Concentration:	1 to 100%
Eluent pH Range:	0 to 14
Maximum Eluent Flow Rate:	3.0 mL/min

The OmniPac PCX-100 must always be operated so that at least 1% organic solvent is maintained in the eluent. Maintaining 1% organic solvent in the eluent ensures that the highly cross-linked hydrophobic core is "wetted" and maximum column performance is maintained.

4.1 Production Test Chromatogram

The OmniPac PCX-100 Analytical Column is tested as described here to ensure proper performance. The retention of methylpseudoephedrine, pseudoephedrine, benzylamine and phenylethylamine are reported on the Certificate of Performance shipped with the column.

Sample Loop Volume:	10 µL
Analytical Column:	OmniPac PCX-100 Analytical Column
Eluent:	100 mM KCl/50 mM HCl/32% CH_3CN
Eluent Flow Rate:	1.0 mL/min
Detection:	UV at 220 nm, 0.02 AUFS
Expected System Operating Back pressure:	1,600 - 2,200 psi

	Analyte	ppm
1.	Methylpseudoephedrine	5
2.	Pseudoephedrine	5
3.	Benzylamine	5
4.	Phenylethylamine	5

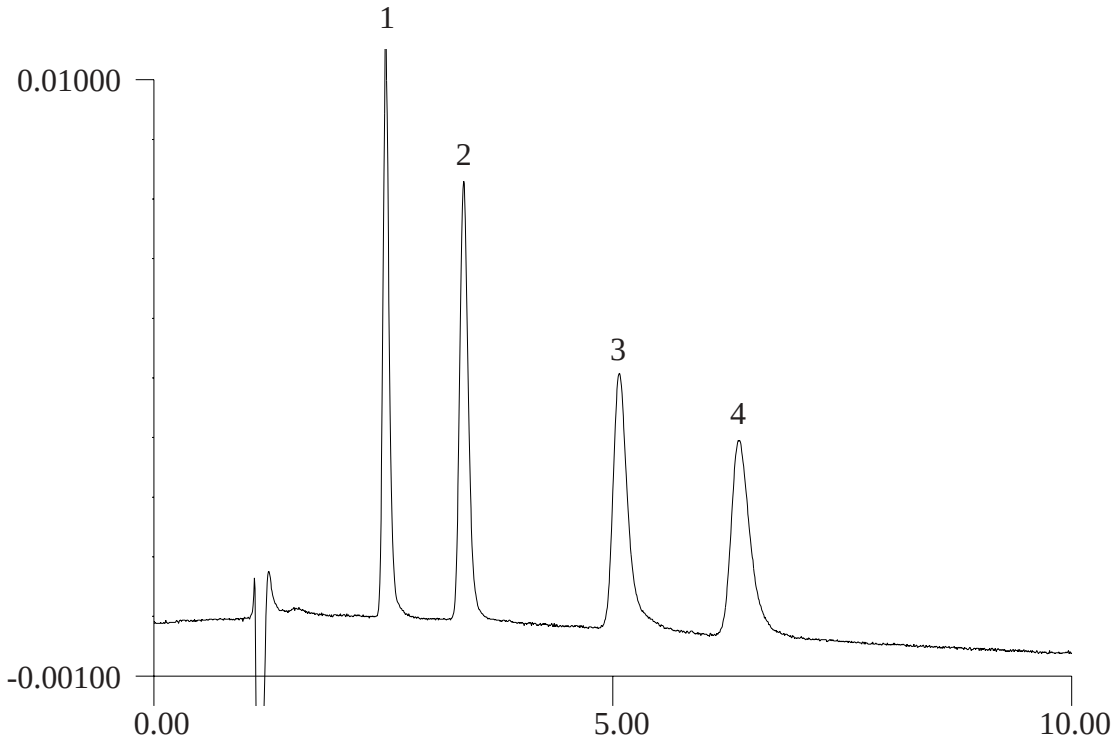
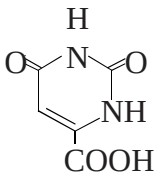


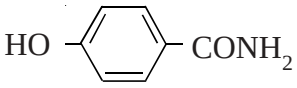
Figure 1
PCX-100 Production Test Chromatogram

4.2 Nitrogen-containing Aromatic Compounds

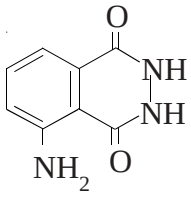
1. Orotic Acid
   pKa_1 1.96
   pKa_2 9.34



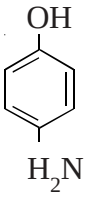
2. 4-Hydroxybenzamide



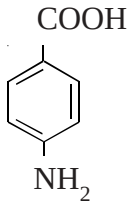
3. Luminol



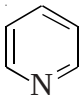
4. p-Aminophenol
   pKa_1 8.5
   pKa_2 10.3



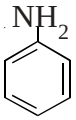
5. p-Aminobenzoic Acid
   pKa_1 2.4
   pKa_2 4.85



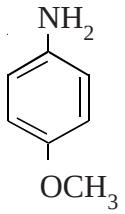
6. Pyridine
   pKa 5.2



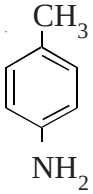
7. Aniline
   pKa 4.6



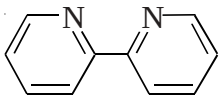
8. p-Anisidine
   pKa 5.3



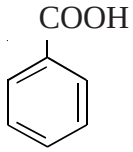
9. p-Toluidine
   pKa 5.1



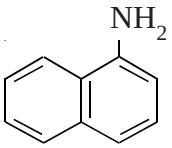
10. 2,2'-Bipyridine



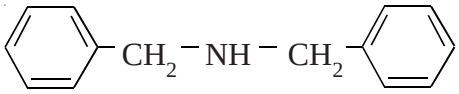
11. Benzoic Acid
    pKa 4.2



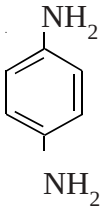
12. Naphthylamine
    pKa 4.0 (1-,3.9;2-,4.1)



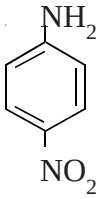
13. Dibenzylamine
    pKa 8.5



14. p-Phenylenediamine
    pKa_1 3.3
    pKa_2 6.1



15. p-Nitroaniline
    pKa 1.0



16. Benzylpiperazine

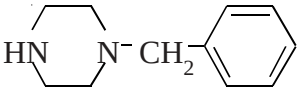


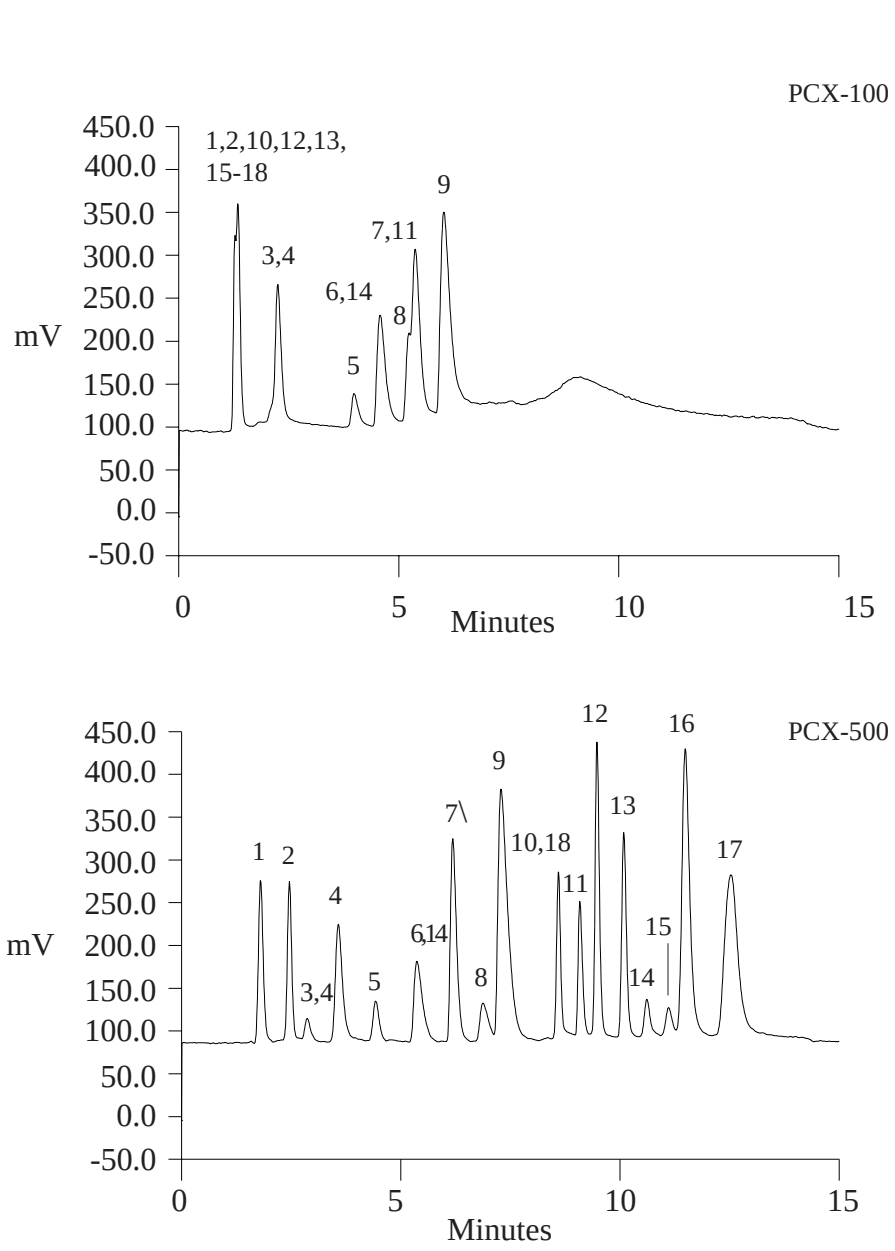
Figure 2
Structures of the Nitrogen-containing Aromatic Compounds

Sample Loop Volume:	25 µL
Analytical Column:	OmniPac PCX-100 Analytical Column
Eluents,	Eluent 1: 500 mM HCl Eluent 2: 1000 mM NaCl Eluent 3: 90% acetonitrile in Deionized Water (having a specific resistance of 18.2 megohm-cm) Eluent 4: 5% acetonitrile in Deionized Water
Eluent Flow Rate:	1.0 mL/min
Detection:	UV at 254 nm

Gradient Program

Time (min)	%1	%2	%3	%4	Comments
0.0	10	10	30	50	
0.1	10	10	30	50	Injection
0.2	10	10	30	50	
4.0	10	45	45	0	
7.0	10	0	90	0	
11.0	10	0	90	0	
11.1	10	10	30	50	

Figure 3 compares the retention properties of the OmniPac PCX-100 and PCX-500 for a broad range of neutral and cationic compounds. Using the chromatographic conditions shown above on a PCX-500, the neutral components are retained by reversed phase mechanism while the cation components are retained by reversed phase and cation exchange. The chromatography of this same group of compounds on the PCX-100 illustrates the use of this column for on-column sample preparation. Since the PCX-100 does not have reversed phase retention, the neutrals elute in the void volume. The cation exchange gradient can then be optimized for separation of the cationic components strictly by cation exchange using a shallower gradient.



1. Orotic acid
2. 4-Hydroxybenzamide
3. Luminol Impurity
4. Luminol
5. Pyridine
6. p-Aminobenzoic acid
7. 2,2'-Bipyridine
8. p-Phenylenediamine
9. Naphthylamine
10. Nitrobenzoic acid
11. Tribenzylamine
12. p-Nitroaniline
13. 2,4-Dinitroaniline
14. Dibenzylamine
15. N-Methyl-N-nitrosoaniline
16. 4-Chloro-2-nitroaniline
17. 2,6-Dichloro-4-nitroaniline
18. Benzoic Acid

Figure 3
Nitrogen-containing Aromatic Compounds

4.3 Nucleic Acid Constituents

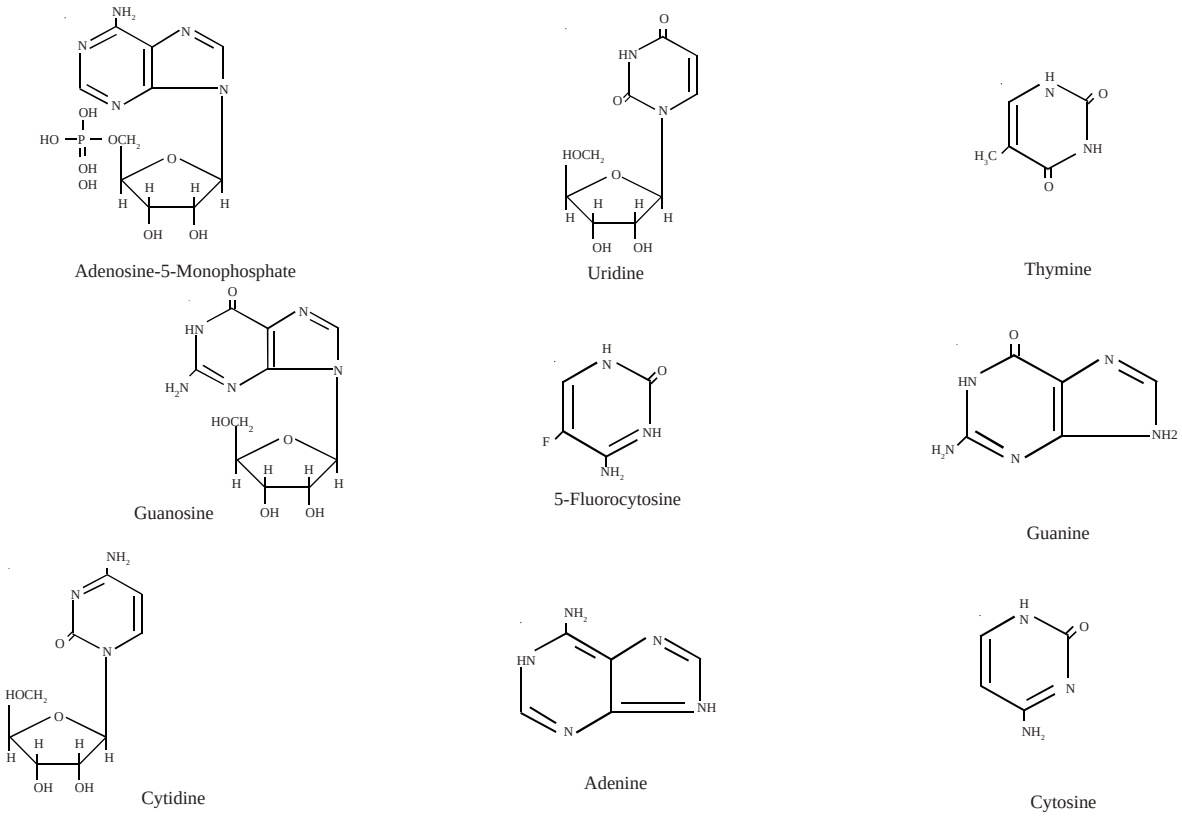


Figure 4
Structures of the Nucleic Acid Constituents

Sample Loop Volume:	25 µL
Analytical Column:	OmniPac PCX-100 Analytical Column
Eluents,	
Eluent 1:	0.5 M HCl
Eluent 2:	0.4 M sodium acetate
Eluent 3:	90% acetonitrile in Deionized Water (having a specific resistance of 18.2 megohm-cm)
Eluent 4:	5% acetonitrile in Deionized Water
Eluent Flow Rate:	1.0 mL/min.
Detection:	UV at 254 nm

Gradient Program

Time (min)	Flow Rate	%1	%2	%3	%4	Comments
0.0	1.0	5	5	5	85	
0.1	1.0	5	5	5	85	Inject
0.2	1.0	5	5	5	85	Start Gradient
5.0	1.0	20	30	50	0	
6.0	1.0	20	30	50	0	
6.1	1.0	5	5	5	85	Reequilibrate

4.3.1 Sample Chromatogram of Nucleic Acid Constituents

This comparison of the OmniPac PCX-500 and PCX-100 Analytical Column separations illustrates the reversed phase selectivity of the OmniPac PCX-500 Analytical Column. Note the elution reversal between guanine and adenosine as well as the retention of the phosphated nucleotides out of the void volume on the OmniPac PCX-500 Analytical Column due to the added reversed phase adsorption properties of the column.

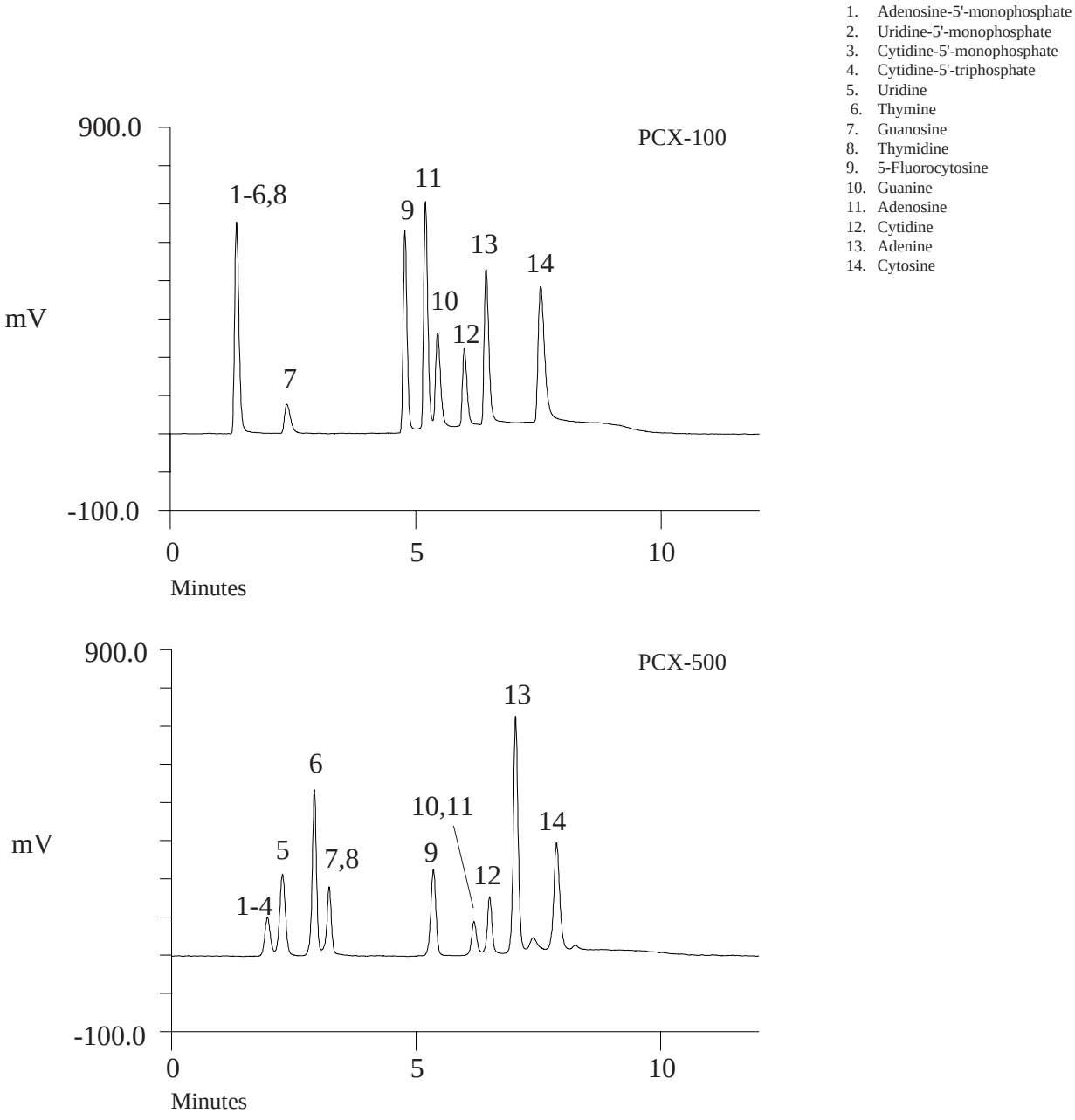


Figure 5

4.4 Tricyclic Antidepressants

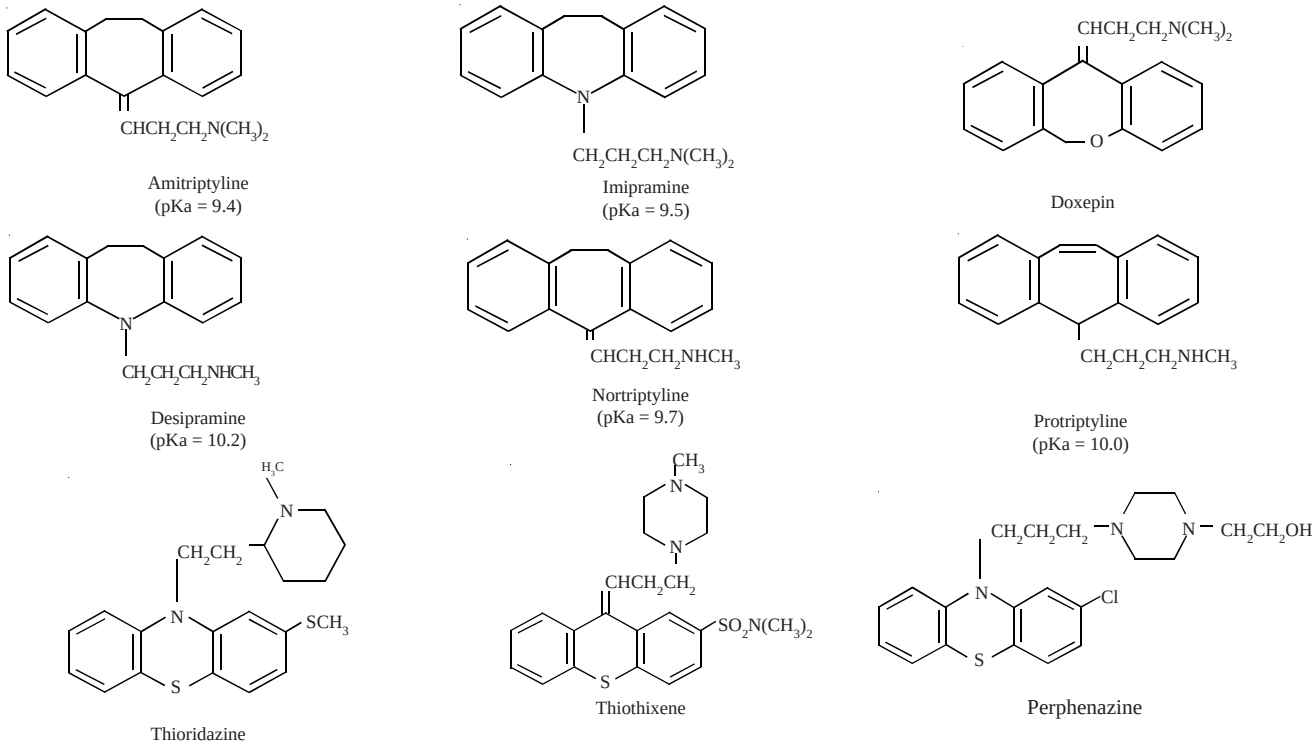


Figure 6
Structures of the Tricyclic Antidepressants

Sample Loop Volume:	25 µL
Analytical Column:	OmniPac PCX-100 Analytical Column
Eluents,	Eluent 1: 5 mM HCl
	Eluent 2: 1 M KCl
	Eluent 3: 90% acetonitrile in Deionized Water having a specific resistance of 18.2 megohm-cm.
	Eluent 4: 5 % acetonitrile in Deionized Water
Eluent Flow Rate:	1.0 mL/min
Detection:	UV at 254 nm

Gradient Program

Time (min)	%1	%2	%3	%4	Comments
0.0	2	1	70	27	
0.1	2	1	70	27	Injection
0.2	2	1	70	27	
3.0	2	4	70	24	
10.0	2	20	70	8	
13.0	2	20	70	8	
13.1	2	1	70	27	

4.4.1 Sample Chromatogram of Tricyclic Antidepressants

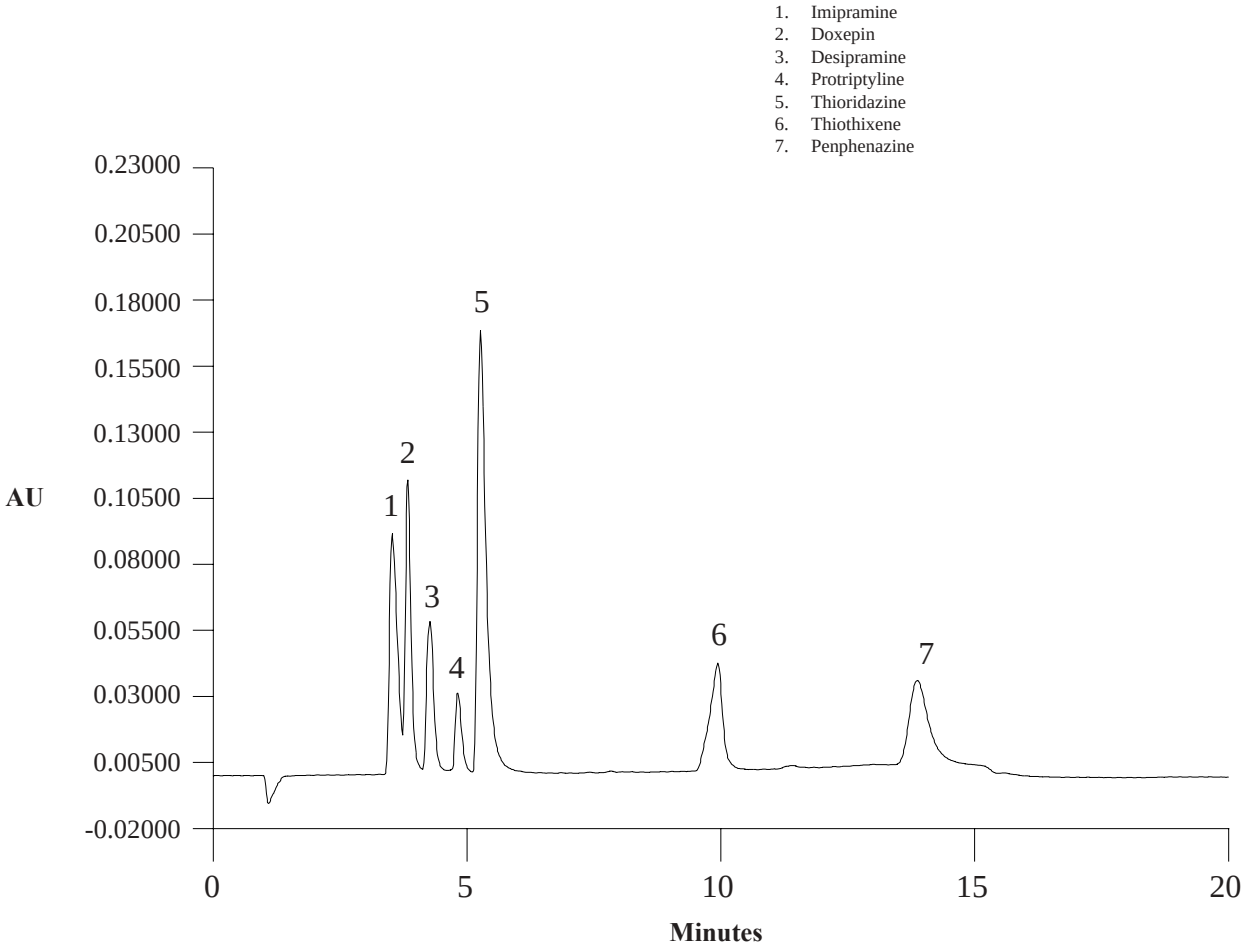
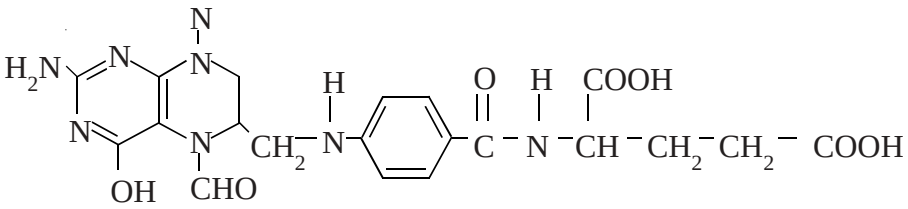
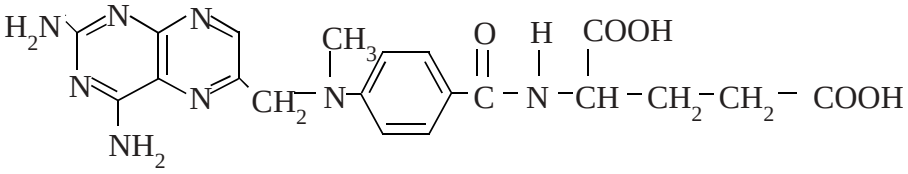


Figure 7
Tricyclic Antidepressants

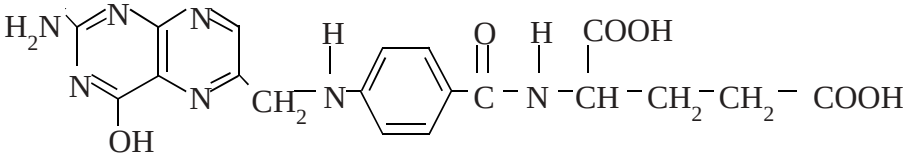
4.5 Folinic Acid, Methotrexate and Folic Acid in Serum



Folinic Acid



Methotrexate



Folic Acid

Figure 8
Structures of Folinic Acid, Methotrexate and Folic Acid

4.5.1 Sample Chromatograms of Folinic Acid, Methotrexate and Folic Acid

Sample Loop Volume:	25 µL
Analytical Column:	OmniPac PCX-100 Analytical Column
Isocratic Eluent:	50 mM HCl/50 mM NaCl/45% Acetonitrile
Eluent Flow Rate:	1.0 mL/min
Detection:	UV at 285 nm

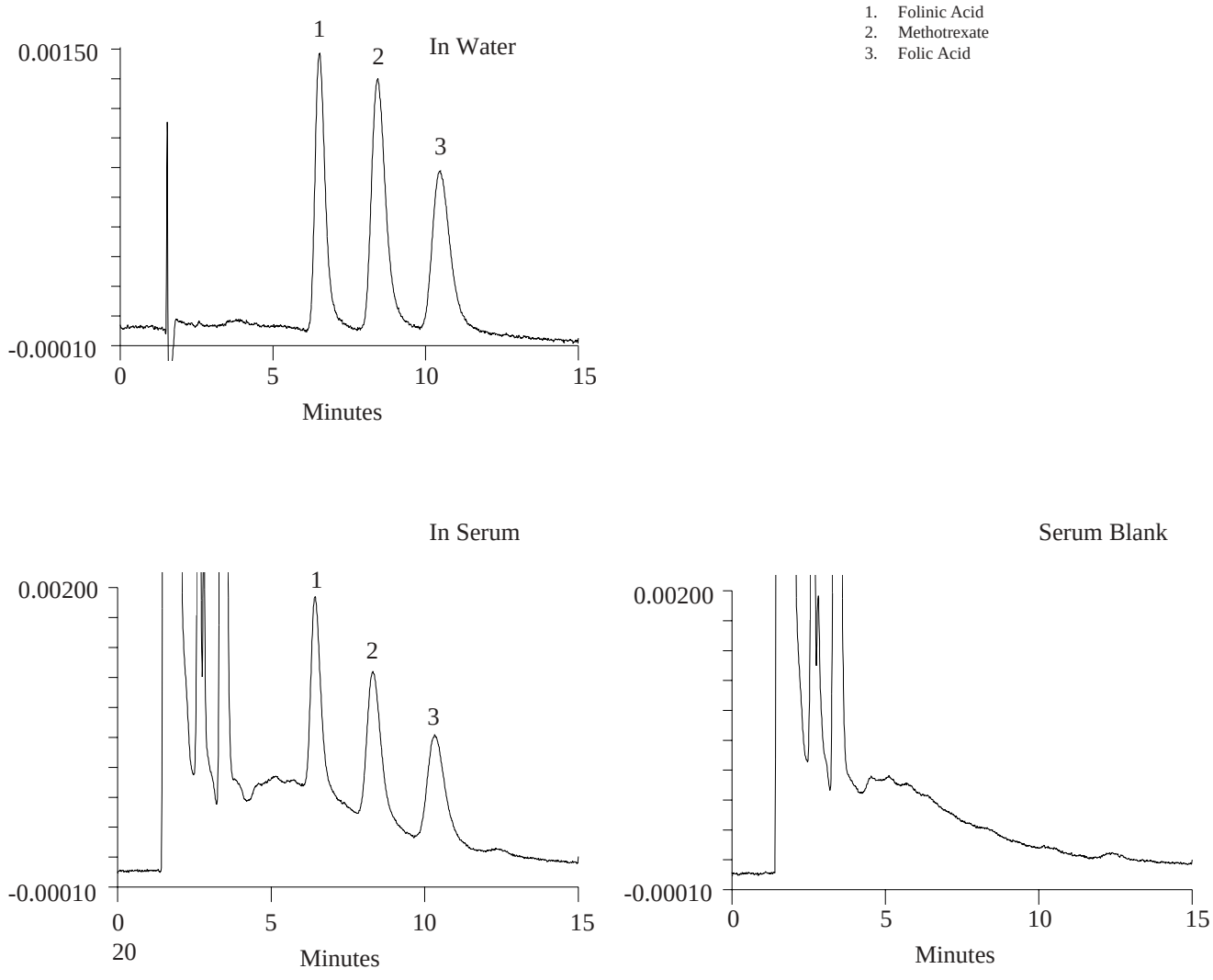


Figure 9
Sample Chromatograms of Folinic Acid, Methotrexate and Folic Acid

SECTION 5 - TROUBLESHOOTING GUIDE

The purpose of the Troubleshooting Guide is to help solve operating problems that may arise while using the OmniPac PCX-100 Analytical Column. For more information on problems that originate with the Liquid Chromatograph, the detectors or the suppressor, refer to the Troubleshooting Guide in the appropriate Installation Instructions. If you cannot solve the problem, call the nearest Dionex Regional Office.

5.1 High Back pressure

Total system back pressure when using the OmniPac PCX-100 Analytical Column at 1.0 mL/min should be less than 2500 psi. Solvent concentration can affect the column operating back pressure. If the back pressure 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.

A. Make sure that the pump is set to 1.0 mL/min. Higher flow rates will cause higher pressure. If necessary, measure the pump flow rate with a graduated cylinder and stopwatch.

B. Determine which part of the system is causing the high back pressure. It could be a piece of tubing that has plugged or whose walls are collapsed, an injection valve with a plugged port, a column with particulates plugging the bed support, a plugged high-pressure in-line filter, the suppressor or the detector.

   To locate 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 components (injection valve, guard, column, or detection device) one by one, while watching the pressure. The pressure should increase up to a maximum of 2500 psi when the column is connected. The suppressor will add up to 100 psi. No other components should add more than 100 psi of pressure. Refer to the appropriate Installation Instructions and Troubleshooting Guide for cleanup or replacement of the problem component.

C. If the column is the cause of high back pressure, 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.

   1. Disconnect the column from the system.

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

   3. 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.

   4. 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.

OmniPac PAX-100

Part	(P/N)
Bed Support Assembly	042955
Seal Washer	042956
Bed Support	041375
End Fitting	052809

   5. 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.

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.

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

5.2 High Background Noise When Using Conductivity Detection

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

ELUENT	EXPECTED BACKGROUND CONDUCTIVITY
40 mM HCl/5% ACN/4 mM DAP	2-5 µS

The background conductivity typically increases between 2 and 5 µS when running a gradient.

A. Ensure that the eluents and the regenerant are made correctly.

B. Ensure that the eluents are made from chemicals with the recommended purity (see Section 3.1, Chemicals Required).

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

D. For applications using suppressed conductivity detection, the standard system configuration includes replacing the Gradient Mixer that is normally positioned after the Gradient Pump Module and in front of the injection valve with a Cation Trap Column (CTC-1, P/N 040192). Most of the applications using UV detection do not require the installation of a CTC-1.

Is the CTC-1 installed before the injection valve? If it is not and you are performing an application with suppressed conductivity detection, install one as directed in in this section and observe the background conductivity. If the background conductivity is now low, this means that the CTC-1 is trapping contaminants from the eluent. The eluents probably have too many impurities (see items A - C above).

If the CTC-1 is installed, remove it. Is the background conductivity still high? If the background conductivity decreases, the CTC-1 is the source of the high background conductivity. Clean the CTC-1 as instructed in A above.

1. Connect a waste line to the CTC-1 outlet and direct the line to a waste container.

2. Rinse the CTC-1 with 30 mL of a 10X concentrate of the strongest eluent that will be used during the gradient analysis at the flow rate used in the application (e.g., 1 mL/min for 30 minutes).

3. After flushing the CTC-1 with eluent, connect the CTC-1 to the eluent line that is connected to the injection valve.

E. Remove the OmniPac PCX-100 Analytical 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.”

F. To eliminate the hardware as the source of the high background conductivity, bypass the MicroMembrane Suppressor and pump deionized water having 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.

G. If the above items have been checked and the problem persists, the MicroMembrane Suppressor is probably causing the problem.

1. Check the regenerant flow rate using a graduated cylinder and a stopwatch at the **REGEN OUT** port of the MicroMembrane Suppressor. This flow should be 5–10 mL/min or greater. See the CMMS III Manual (Document No. 031728) for details on determining optimum flow rates.

2. Check the eluent flow rate using a graduated cylinder and a stopwatch. It should be 1.0 mL/min.

3. Prepare fresh regenerant solution. Bypass the Cation AutoRegen Regenerant Cartridge (if you are using the AutoRegen Accessory). If the background conductivity is high, you probably need to clean or replace your MicroMembrane Suppressor. Refer to the MicroMembrane Suppressor Installation Instructions and Troubleshooting Guide for assistance.

4. If you are using an AutoRegen Accessory, connect the regenerant to the Anion AutoRegen Regenerant Cartridge. Pump approximately 250 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 Cation AutoRegen Regenerant Cartridge (P/N 039563). Refer to the AutoRegen Regenerant Cartridge Refills Installation Instructions and Troubleshooting Guide for assistance.

5.3 Poor Peak Resolution

Poor peak resolution can be due to:

A. Loss of Column Efficiency:

1. Check to see if headspace has developed in the 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 pressure pulses). Remove the column's top end fitting (see Section 5.1, Step C). If the resin does not fill the column body completely to the top, it means that the resin bed has collapsed, creating a headspace. The column must be replaced.

2. Extra-column effects can result in sample band dispersion, reducing the peaks' efficiencies. 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.

B. Shorter Retention Times - peaks elute too fast, compromising resolution:

1. Check to see if eluent flow rate is greater than 1.0 mL/min. Check the eluent flow rate after the column using a graduated cylinder and a stopwatch.

2. Check to see if the eluent composition and concentration is correct. An eluent that is too strong will cause the peaks to elute sooner. 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%.

3. Column contamination can lead to a loss of column capacity because all of the cation exchange sites will no longer be available for the sample ions. Polyvalent cations might be concentrating on the column. This may occur if a metallic HPLC system is used. Refer to "Column Care" for recommended column cleanup procedures.

Possible sources of column contamination are impurities in chemicals or in the deionized water used. Be especially careful to make sure that the recommended chemicals are used. Deionized water should have a specific resistance of at least 18.2 megohm-cm.

C. 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 using a suitably strong eluent composition (see Appendix B, "Column Care").

After cleaning the column, reinstall it in the system and let it equilibrate with eluent for about 30 minutes. The column is equilibrated when consecutive injections of the standard give reproducible retention times. The original column capacity should be restored by this treatment, since the contaminants should be eluted from the column.

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

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

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

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

4. 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

A. 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 level of polyvalent ions and the column is used with a weak eluent system, polyvalent anions may be contaminating the 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 Appendix B, “Column Care.” Modify the eluent to ensure that strongly retained polyvalent cations are eluted before the next injection.

B. 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. 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 quantitation of the peaks of interest.

If cleaning and retorquing the injection valve does not help, replace the valve.

5.5 Application Development Troubleshooting Guide

The purpose of the Application Development Troubleshooting Guide is to help you solve problems that may arise while developing methods for use with the OmniPac PCX-100 Analytical Column. For more information on problems that originate with the Chromatograph, the detectors or the suppressor, refer to the Troubleshooting Guide in the appropriate set of Installation Instructions. If you cannot solve the problem on your own, call the Dionex Regional Office nearest you (see “Dionex Worldwide Offices”) for assistance.

5.5.1 Peaks That Elute in the Void Volume

A. Make sure the system hardware is functioning properly by chromatographing a well-characterized application.

B. If you are proportioning concentrated eluent components with the pump to form a weak eluent for the initial eluent in a gradient ramp, mix the eluent components for the weak eluent in one eluent reservoir and pump this eluent isocratically through the analytical column. An example of such an eluent system is 5% acetonitrile/10 mM acid with no salt. Make sure the column is well equilibrated with this weak eluent before obtaining a "final" chromatogram of the analyte of interest. The column is fully equilibrated when successive injections of a test standard containing the analytes of interest give reproducible retention times.

C. Study the structure of the analyte and find its pKa value. If the analyte can be protonated, make sure that the pH of the eluent is at least 2 pH units below the analyte's pKa value. This will ensure that the analyte will be fully protonated and retained on the column by cation exchange. If the analyte is already fully protonated, further lowering the pH of the eluent may elute the analyte off the column even more rapidly with the increased concentration of hydronium ion. In this case, lowering the acid concentration may prove beneficial.

D. If the analyte still elutes in the void volume after varying the amount of acid in the eluent, the analyte is not a cation and therefore can not be retained on the column by cation exchange. If the analyte is hydrophobic, the OmniPac PCX-500 Analytical Column or the IonPac NS1 Analytical Column will retain the analyte through adsorption. If the analyte is an anion, method development should employ the OmniPac PAX-500 or the OmniPac PAX-100 Analytical Columns.

5.5.2 Peaks That Do Not Elute or Have Very Long Elution Times

A. Make sure the analyte type and concentration is detectable in the eluent being used. Dilute a test sample of the analyte in the eluent and inject it directly into the detector.

B. Assuming that the analyte is retained by the column, the eluent system must be modified. Study the structure of the analyte and find its pKa to determine if it is a cation under the eluent pH being used.

1. Prepare an isocratic eluent with the highest practical amount of salt in it, taking care not to precipitate any salt due to the solvent that may be present.

2. If there is no change in the retention time of the analyte, prepare a new eluent with the same amount of salt and half the amount of solvent.

3. If there is no change in the retention time of the analyte, prepare a new eluent with the minimum 1% solvent in it so that the salt concentration can be increased.

4. The above steps assumed that the analyte was a monovalent cation and that it was retained by ion exchange. Some polyvalent cations can be very strongly retained on the analytical column. In this case the pH can be raised somewhat to partially deprotonate some cationic sites on the analyte and effectively lower its total positive charge. If the analyte is still retained too long on the column, then change the type and concentration of the eluting cation in the eluent. Remember that like ions will elute the analyte with the highest efficiency. A more hydrophobic eluting cation like potassium can elute a more hydrophobic analyte cation better than a hydrophilic eluting cation.

5.5.3 Poor Peak Symmetry or Efficiency

A. Determine that the system is functioning properly by running a standard application which you know gives good analyte separation and peak shapes with the OmniPac PCX-100 Analytical Column under the same detection mode.

B. The analyte concentration may be too high. A small peak does not necessarily mean that the analyte is present in low concentration. The particular analyte of concern might have a low extinction coefficient at the particular UV wavelength being used. Even though the peak height is small, the concentration might be large enough to be overloading the column. Dilute the sample to see if peak shape improves. Find the UV maximum of the analyte at the pH of the eluent. You may have to use a different detection system if detection is the problem.

C. The poor peak shape may actually be caused by a matrix interference in the sample which is only partially resolved from the analyte of interest.

D. When the pH of the sample is very different from the eluent pH, the analyte peak can show poor symmetry and/or efficiency. Dilute the sample with the initial eluent.

E. If you are running a gradient, the gradient ramp may need to be modified. Remember that peak efficiency increases as the slope of the gradient ramp is increased. The slope of the gradient ramp is increased by increasing the overall concentration change per unit time.

F. The eluent components may need to be changed. Remember that like ions elute the sample analyte with the highest efficiency. A more hydrophobic eluting cation like potassium can elute a more hydrophobic analyte cation better than a hydrophilic eluting cation.

5.5.4 Co-elution of Sample Peaks

A. Verify that the system is functioning properly by checking the efficiency of sample analytes in a well characterized separation (see Section 4, “Example Applications”) using the same detection mode.

B. Modify the gradient or eluent components.

1. Ramp the eluent pH (i.e., the acid concentration) to separate the analytes on the basis of their pKa values.

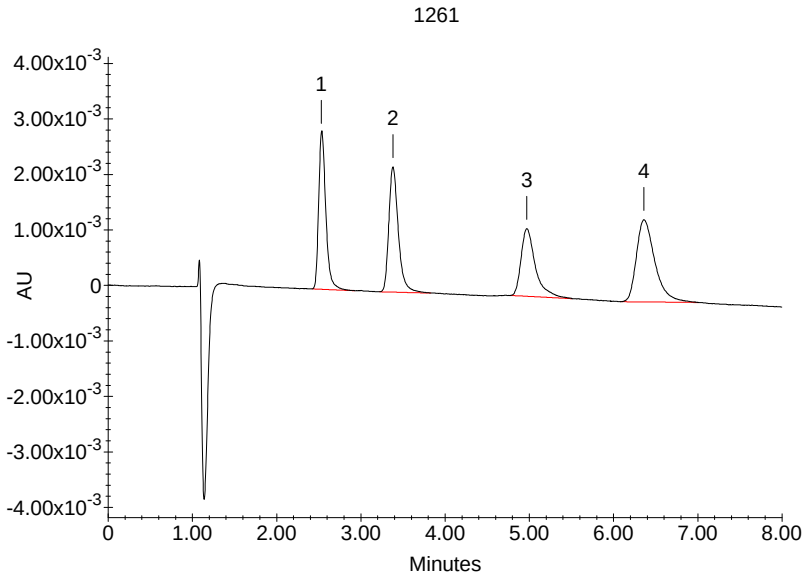
2. Cationic analytes of higher valence respond more to increases in the eluting salt than cations of lower valence so that the separation of cationic analytes of different valence is easily accomplished by reducing the eluting cation concentration until resolution occurs.

3. Change the eluting cation used in the eluent. For example, the elution order of norephedrine and methylephedrine can be reversed depending on whether lithium or potassium are used as the eluting cation in the eluent system (which also contains acetonitrile and acid). Norephedrine is less hydrophobic than the methyl- substituted methylephedrine. Potassium which is a more hydrophobic cation than lithium, will elute the more hydrophobic methylephedrine more efficiently than lithium will. Methylephedrine elutes before norephedrine when the eluting cation in the eluent is potassium. Conversely, if lithium is the eluting cation in the eluent, norephedrine will elute before methylephedrine.

4. The affinity of the analyte for the solution phase can be altered by changing the counter ion the eluent eluting cation. For example, change from HCl to $HClO_4$ or use sodium acetate to replace sodium chloride.

OmniPac® PCX-100
Analytical (4 x 250 mm)
Product No. 042189

Serial No. : 1261 **Pressure (PSI) : 1790** **Date : 1/25/01 10:24:58 AM**



Eluent: 5 mM HCl
50 mM KCl
40% CH_3CN

Flow Rate: 1.0 mL/min

Detection: Absorbance at 220 nm, 0.02 AUFS

Injection Volume: 10 µL

Storage Solution: Eluent

Peak Information : Found Components

Peak No.	Retention Time	Name	(mg / L)	Efficiency	Asymmetry (10%)	Resolution
1	2.53	Methylpseudoephedrine	5.0	4071	1.7	4.78
2	3.38	Pseudoephedrine	5.0	4650	1.5	6.54
3	4.97	Benzylamine	5.0	4735	1.7	4.10
4	6.36	Phenylethylamine	5.0	4236	1.7	n/a

File Name : C:\PEAKNET\DATA\EXAMPLES\42189 PCX-100 4MM_A022.DXD

APPENDIX B - COLUMN CARE

B.1 Recommended Operation Pressures

Operating a column above its recommended pressure limit can cause irreversible loss of column performance. The maximum recommended operating pressure for the OmniPac PCX-100 Analytical Column is 5000 psi.

B.2 Column Start Up

CAUTION

Immediately after the installation of an OmniPac PCX-100 Analytical Column, the column must be regenerated and equilibrated as described in Section 3.2, "Column Preparation."

You MUST prepare the eluents listed below and run the accompanying gradient.

Eluent 1: 95% Acetonitrile/Deionized Water
Eluent 2: 0.5 M HCl/1.0 M KCl/1% Acetonitrile in Deionized Water
Eluent 3: 1% Acetonitrile/Deionized Water
Eluent 4: None

Time (min)	%1	%2	%3	%4	V5	V6
0.0	0	0	100	0	0	0
15.0	0	0	100	0	0	0
30.0	0	10	90	0	0	0
40.0	0	10	90	0	0	0
45.0	90	10	0	0	0	0
60.0	90	10	0	0	0	0

Avoid creating high viscosity pressure fronts that may disrupt the column packing when the eluent solvent component is changed. To do this, equilibrate the column for approximately 10 minutes with an eluent containing only 5% of the current solvent type (e.g., methanol). Exchange this eluent for an eluent with 5% of the new solvent type (e.g., acetonitrile) and then equilibrate the column for approximately 10 minutes. Next run a 15 minute gradient from 5% of the new solvent type to the highest percentage that will be used during the new analysis protocol.

B.3 Column Storage Solution

The column is shipped in 100 mM KCl/50 mM HCl/32% ACN 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. Let the column equilibrate with eluent for a few minutes. Equilibration is complete when consecutive injections of the standard give reproducible retention times.

B.4 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. Flush the column using the base-soluble/organic contaminants gradient program described in Section B.5.2. Then flush the column for 10 minutes with 100 mM KCl/50 mM HCl/32% ACN. Cap both ends securely, using the plugs supplied with the column.

B.5 Column Cleanup

CAUTION

The OmniPac PCX-100 Analytical and Guard Columns require a minimum of 1% solvent in the eluent to ensure that the column packing is properly wetted and to maintain the integrity of the packed column.

When changing the eluent solvent type or the nature of the ionic strength in the eluent, a 10 minute (or longer) gradient from the old eluent to the new eluent must be performed.

B.5.1 Polyvalent Anions and Acid-soluble Contaminants

A. Prepare a 500 mL solution of 1 M HCl/0.1 M KCl/5% solvent (CH_3OH or ACN).

B. Disconnect the analytical column from the injection valve and the MicroMembrane Suppressor. Disconnect the Gradient Mixer or the CTC-1 from the gradient pump. Connect the OmniPac PCX-100 Analytical Column directly to the GPM. Direct the effluent from the analytical column directly to waste.

C. Set the pump flow rate to 1.0 mL/min.

D. Run a 10 minute gradient from the current eluent to the 1 M HCl/0.1 M KCl/5% solvent (CH_3OH or ACN) cleanup solution.

E. Pump 1 M HCl/0.1 M KCl/5% solvent (CH_3OH or ACN) solution through the column for 30–60 minutes.

F. Run a 10 minute gradient from the cleanup solution to the current eluent.

NOTE

Nitric acid should not be used instead of hydrochloric acid since nitric acid will not efficiently remove iron contaminants.

G. Reconnect the MicroMembrane Suppressor to the analytical column.

H. Equilibrate the column with eluent before resuming normal operation.

B.5.2 Base-soluble or Organic Contaminants

A. Disconnect the MicroMembrane Suppressor from the analytical column. Direct the analytical column outlet line to waste.

B. Set the pump flow rate to 1 mL/min. Use the following gradient program to remove base-soluble or organic contaminants from the OmniPac PCX-100 Analytical Column.

Eluent 1: Deionized Water Eluent 2: 90% Acetonitrile
Eluent 3: 100 mM NaOH Eluent 4: Current Eluent
Eluent Flow Rate: 1.0 mL/min

Time (min)	%1	%2	%3	%4	Comments
0.0	0	0	0	100	
10.0	55	5	40	0	
25.0	0	5	95	0	
35.0	0	5	50	0	
40.0	0	50	50	0	
50.0	0	50	50	0	
55.0	55	5	40	0	Hold for 5 minutes
65.0	0	0	0	100	

C. Reconnect the MicroMembrane Suppressor to the analytical column.

D. Equilibrate the column with eluent before resuming normal operation.

NOTE

It is not necessary to use sodium hydroxide and acetonitrile in the above cleanup procedure. You may find it more beneficial, depending on you sample matrix, to use a different ionic eluent such as sodium chloride and a different solvent such as isopropyl alcohol.