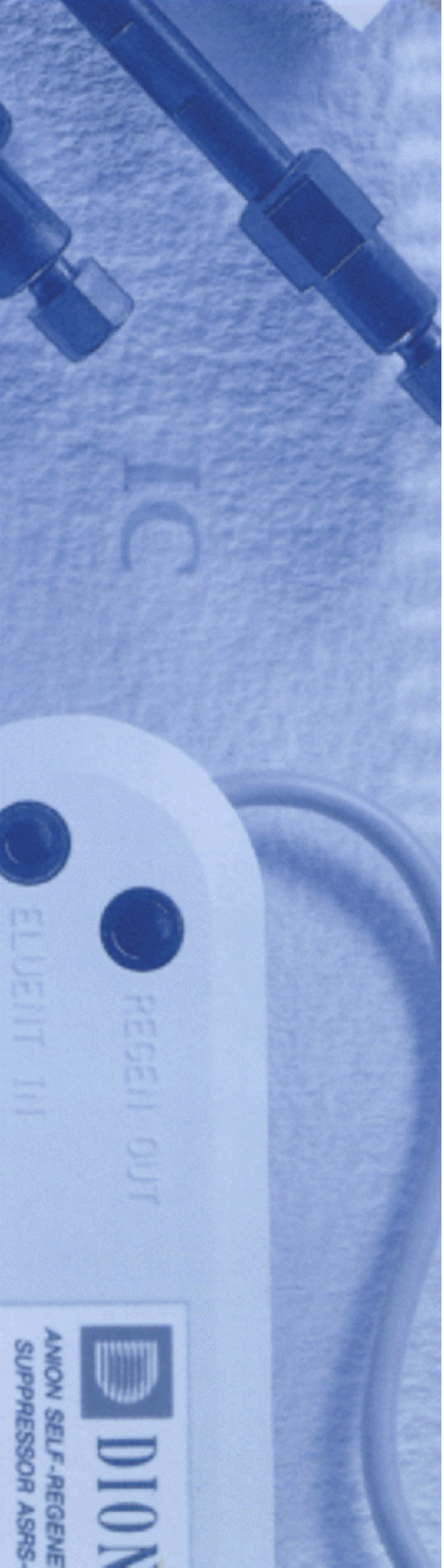

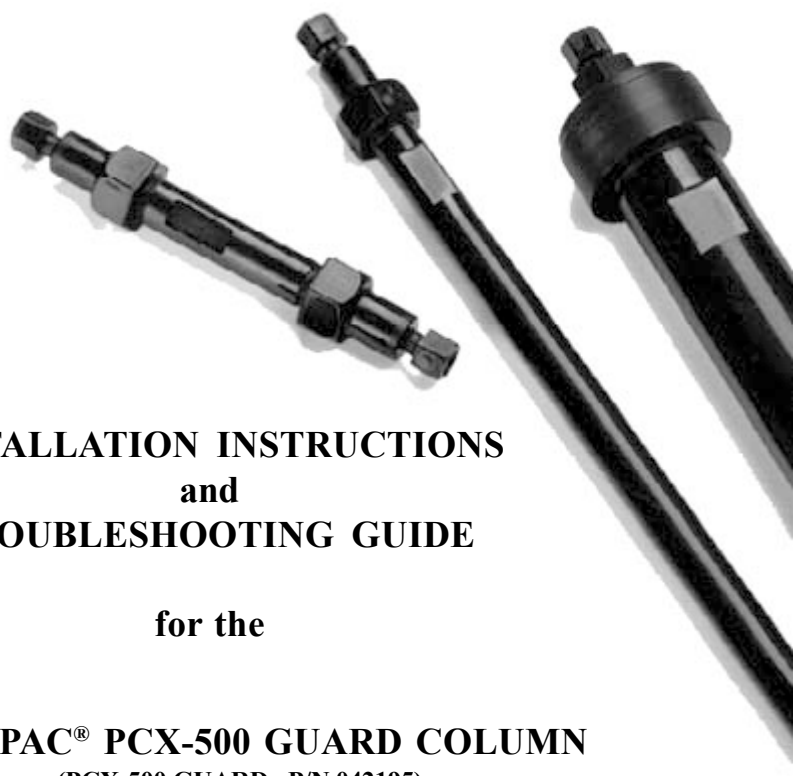

INSTALLATION INSTRUCTIONS and TROUBLESHOOTING GUIDE

for the

OMNIPAC® PCX-500 GUARD COLUMN

(PCX-500 GUARD, P/N 042195)

OMNIPAC® PCX-500 ANALYTICAL COLUMN

(PCX-500 ANALYTICAL, P/N 042191)

QUICKSTART STEPS AND LINKS

Click blue text below to get started.

1. The test eluent and storage solution for the OmniPac PCX-500 is 50 mM HCl, 100mM KCl, 32% Acetonitrile. See Section 3.2, "Column Preparation", for column preparation details.

2. Run the Production Test Chromatogram as a system check.
   See Section 5.2, "OmniPac PCX-500 Analytical Column Test Mixture", for details.

3. See Section 5, "Example Applications Done on the OmniPac PCX-500 Analytical Column" for example applications.

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

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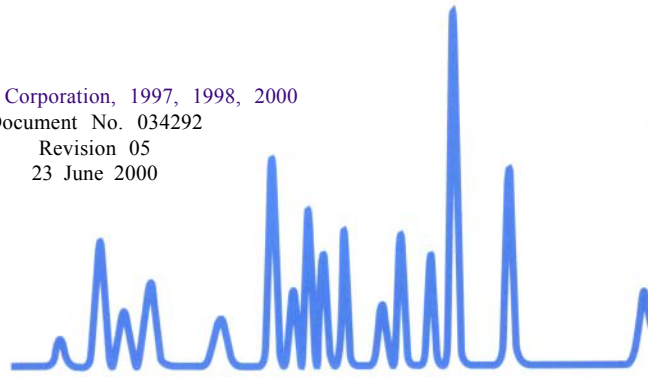

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SECTION I - INTRODUCTION

The OmniPac PCX-500 Analytical Column (P/N 042191) was developed to greatly expand the selectivity available to the analytical Chemist. The OmniPac PCX-500 allows the analytical chemist to utilize ion exchange, ion suppression, ion pair, and reversed phase techniques on the same column. These techniques can be performed in almost any conceivable combination, either simultaneously or in series.

The OmniPac PCX-500 utilizes a multi-phase column packing to combine ion exchange and reversed phase mechanisms in a single packing. The column packing of the OmniPac PCX-500 consists of a highly cross-linked macroporous (high surface area) core with a neutral hydrophobic internal surface. The ethylvinylbenzene/divinylbenzene polymeric core is produced in a manner so that the core has a reactive exterior surface to which a polymeric colloid can be attached. This polymeric colloid, in the form of latex particles, is functionalized to create acidic sulfonate groups, the cation exchange sites. The active cation exchange latex is permanently anchored to the macroporous 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 affect selectivity and convert molecular species into ionic compounds. However, to efficiently elute ions such as alkyl and aryl amines, it is necessary to add organic solvents to the ionic eluent to prevent the organic analytes from being adsorbed by the ion exchange phase. The highly cross-linked polymeric macroporous substrate allows the use of common HPLC solvents as eluent modifiers in multimode separations.

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-500 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.

For any problems that have been encountered in shipping or that are not covered by the appropriate Installation Instructions and Troubleshooting Guides, additional support can be obtained through any of the DIONEX Regional Offices listed in "DIONEX Worldwide offices".

SECTION 2 - INSTALLATION

The majority of the applications developed for the OmniPac PCX-500 Analytical Column use UV/Vis or electrochemical detection. Section 2.1, “General System Requirements”, lists system requirements for all applications using the OmniPac PCX-500. Additional system requirements for those applications that are best performed using suppressed conductivity detection are in Section 6, “Installation of Suppressed Conductivity Detection Accessories”.

2.1 GENERAL SYSTEM REQUIREMENTS

2.2 HPLC COMPATIBILITY

The OmniPac PCX-500 Analytical Column should be run on a DIONEX gradient Ion Chromatograph. PEEK (polyetheretherketone) has excellent chemical resistance to most organic and inorganic liquids. Concentrated sulfuric acid, concentrated nitric acid and methylene chloride will attack PEEK. Tetrahydrofuran at concentrations greater than 10% is not compatible with OmniPac Columns.

2.3 GUARD COLUMNS

An OmniPac PCX-500 Guard Column (P/N 042195) can be used with the OmniPac PCX-500 Analytical Column. Retention times will increase by approximately 20% when a guard column is placed in-line prior to the analytical column. A guard is placed prior to the analytical column to prevent sample contaminants from eluting onto the analytical column. It is easier to clean or replace a guard column than it is a analytical column.

SECTION 3 - GENERAL OPERATION AND START-UP

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 by having an actual lot analysis on each label.

3.1.2 Solvents

Since the solvents used with OmniPac PCX-500 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 are making “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. Currently 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 17.8 megohm-cm or greater. The deionized water should be free of ionized impurities, organics, microorganisms and particulate matter larger than 0.2 gm. 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.

3.1.4 Regenerant for Suppressed Conductivity Applications

For those applications being 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 not using the AutoRegen Accessory (P/N 039594) equipped with an AutoRegen Cation Regenerant Cartridge (P/N 039563), prepare several liters of the regenerant.

3.2 COLUMN PREPARATION

The OmniPac PCX-500 Analytical Column is tested to assure that the column will perform ion exchange and reversed phase chromatography simultaneously. The OmniPac PCX-500 is shipped in 0.1 M KC1/0.05 M HC1/32% acetonitrile.

Prior to using the OmniPac PCX-500 Analytical Column for the first time or after long term storage, the column should be pumped down using the following gradient program. Direct the column effluent to waste.

CAUTION

The OmniPac PCX-500 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.

Lo Pressure Limit = 300
Hi Pressure Limit = 3,000

Eluent 1: 95% Acetonitrile/Deionized Water
Eluent 2: 0.5 M HC1/1.0 M KC1/1% Acetonitrile in deionized Water
Eluent 3: 1% Acetonitrile/Deionized Water
Eluent 4: None

Time (min)	Flow (mL/min)	%1	%2	%3	%4	V5	V6	Comments
0.0	1.0	0	0	100	0	0	0	
15.0	1.0	0	0	100	0	0	0	
30.0	1.0	0	10	90	0	0	0	
40.0	1.0	0	10	90	0	0	0	
45.0	1.0	90	10	0	0	0	0	
60.0	1.0	90	10	0	0	0	0	

The OmniPac PCX-500 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 of samples can result in overloading the column which can affect the detection linearity and column efficiency.

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.

SECTION 4 - APPLICATION DEVELOPMENT USING THE OMNIPAC PCX-500 ANALYTICAL COLUMN

The OmniPac PCX-500 Analytical Column is a pellicular cation exchange column with a relatively high degree of hydrophobicity. The column is pH stable from 0 to 14 and is solvent compatible. The OmniPac PCX-500 Analytical Column can perform separations based either cation exchange and reversed phase mechanisms or on a combination of both.

4.1 COLUMN SELECTION

Before starting an analysis, look at the structures of the analytes to first determine if they can be ionized to form cations. The OmniPac PCX-500 Analytical Column should be chosen when it is determined that cation exchange that is enhanced with reverse phase will be the major mechanisms for the separation of the sample analytes.

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 CS12A Analytical Column (P/N 046073) should be chosen for separations of inorganic Group I & Group II cations when using aqueous eluents without solvents.

4.2 DETECTOR SELECTION

4.2.1 Absorbance Detectors

After selecting the analytical column, determine the mode of detection. To do this, look at the structures of the sample analytes. Due to the nature of the eluent systems which can be used with the OmniPac PCX-500 Analytical Columns, the simplest detector is the UV/Visible detector (AD25, P/N 056965). Determine if the analytes absorb UV or Visible light. If they do, find the wavelength at an acidic pH where the maximum absorbance occurs.

4.2.2 Electrochemical Detectors

If the analytes do not absorb in the UV or in the Visible, or if they have a low extinction coefficient, the Pulsed Electrochemical Detector (ED-50, 056963) is the second detector to consider. The ED-50 is used to detect alcohols, carbohydrates, aldehydes, sugar alcohols, monosaccharides, oligosaccharides, glycols, amino acids, amines and sulfur-containing compounds. When using an acidic eluent and the ED-50 with a gold working electrode, post-column addition of base is necessary (final pH must be around 13).

4.2.3 Conductivity and Other Detectors

Other detectors that can be used with the OmniPac PCX-series of analytical columns are the Conductivity Detector Module (CD-25, 056961) and the Fluorescence Detector Module (RF2000, 056101). 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 will probably require unsuppressible amounts of acid to elute it from the analytical column so 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-II) Installation Instructions and Troubleshooting Guide (Document No. 034359) for details on using eluents that contain DAP. Eluting cations stronger than hydrogen, such as sodium and potassium, cannot be used in the eluent with suppressed conductivity detection as the background will be too high and detection limits will dramatically increase.

4.2.4 Multiple Detectors Used in Series

Because the OmniPac PCX-500 Analytical Column can separate such a large variety of analytes through cation exchange and/or reversed phase, the time required for analysis of many sample matrices can be significantly reduced by using the analytical column with two detectors in series. For example, one might need to simultaneously analyze compounds that absorb in the UV along with compounds that do not absorb in the UV but that can be electrochemically oxidized. In this case, a UV/Visible Detector (AD25, P/N 056925) might be connected in series with a Pulsed Electrochemical Detector (ED-50, P/N 056963).

4.3 ELUENT SYSTEMS

4.3.1 The Role of Solvents in Eluent Systems

CAUTION

The OmniPac PCX-500 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.

Since the porous core has an electrostatically neutral hydrophobic internal surface, it is essential that the OmniPac PCX-500 Analytical Column is operated so that any aqueous eluent being pumped through the column has minimally 1% organic solvent in aqueous eluents. The 1% organic solvent in the eluent will ensure that the hydrophobic surface of the substrate is "wetted" and maximum column performance is maintained. The OmniPac PCX-500 Analytical Column packing can withstand all common HPLC solvents in a concentration range of 1% to 100%. 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 might result in pumping pure aqueous eluents over the column for long periods of time.

The two most common solvents are acetonitrile and methanol. Acetonitrile is a stronger solvent than methanol, and is the reversed phase eluent solvent of choice for use with the OmniPac PCX-500 Analytical Columns. Acetonitrile has lower UV absorption at useful wavelengths than many other solvents. It is electrochemically clean, producing low Pulsed Electrochemical Detector backgrounds, and does not interfere with the pulsed electrochemical detection of the species of interest.

Acetonitrile plays several roles as an eluent component.

A. It serves to elute analytes with reversed phase retention mechanisms.

B. 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.

C. 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-500 Analytical Column can be used with any suppressible ionic eluent that does not exceed the capacity of the Cation MicroMembrane Suppressor (CMMS-II). 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. As Figure 1, "Variation of Solvent Viscosity with Changing Temperature and Composition of Water-Solvent Mixtures", shows, 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-500 Analytical and Guard Columns is 5000 psi. Therefore, any variation of eluent formulations whose contributions to the back pressure total less than 5000 psi can be used.

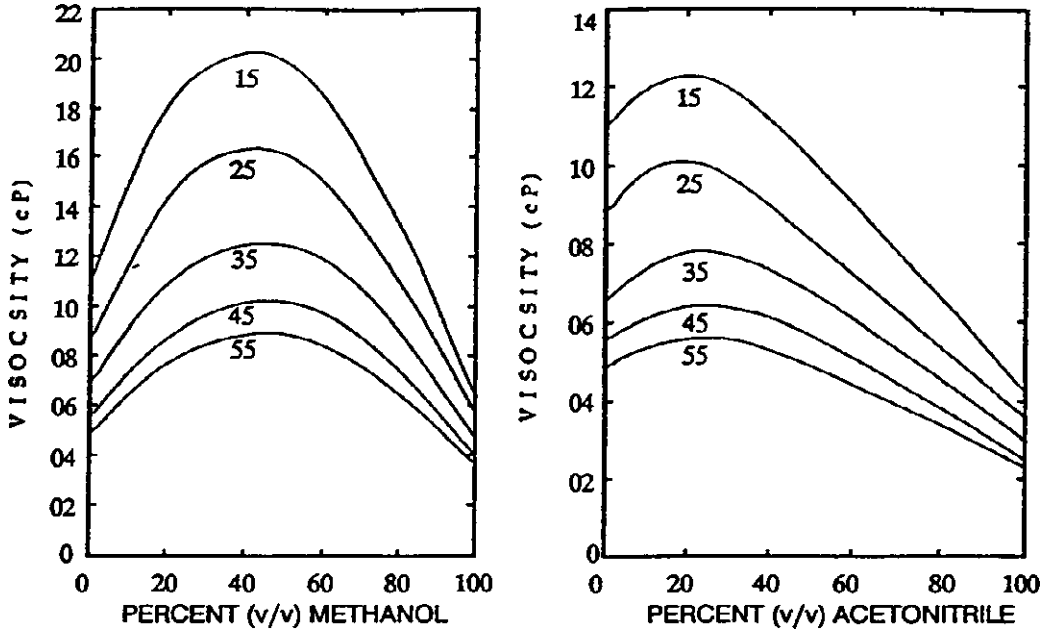


Figure 1

Variation of Solvent Viscosity with Changing Temperature and Composition of Water-Solvent Mixtures

The composition is expressed as percent (v/v) at 20.5°C and the viscosity is in centipoise.
The temperature at which the viscosity was measured is indicated below each curve.*

* C. Horvath, Ed., "High Performance Liquid Chromatography. Advances and Gradient applications utilizing solvent gradients are best performed when organic solvents chosen for use in the eluents are premixed with the aqueous components of the eluent to facilitate optimum mixing by the gradient pump. This precaution will improve the reproducibility of the required solvent gradient ramp for your chromatography. For example, if you want 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.

All water used for the formulation of all eluents including those with solvents should have a specific resistance of 17.8 megohm-cm or greater and be thoroughly degassed before formulating the eluent. In order to avoid 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, never use eluent protocols that call for the on-line dilution of neat solvents with other neat solvents or pure deionized water by the Gradient Pump Module. 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.

CAUTION

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

The salt's 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.

4.3.2 The Role of Acids in Eluent Systems

For analytes which are weak bases, such as amines, that can be separated by cation exchange based on the difference in their pKa values, the mobile phase should be kept at least 2 pH units below the pKa values of the analytes to ensure that they are fully protonated. Additional amounts of acid in the eluent will elute analytes retained on the OmniPac PCX-500 Analytical Column by cation exchange. Weakly acidic analytes, such as organic acids, can be protonated to suppress their ionization by the addition of acid to the eluent so that they are retained on the OmniPac PCX-500 Analytical Column by adsorption. They can then be eluted by the addition of a reversed phase solvent to the eluent.

Acids commonly used in OmniPac PCX eluents are hydrochloric acid (HCl) and perchloric acid (HC104). Both acids have low UV absorbance at common analytes' absorbance maxima wavelengths. If the system is to be used with the Pulsed Electrochemical Detector (ED-50) equipped with a gold (Au) working electrode, then 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 the analytes' response on the ED-50. In most other cases, hydrochloric acid should be used to simplify the retention mechanism as it will avoid the introduction of more hydrophobic complexing anions in the eluent.

4.3.3 The Role of Salts in Eluent Systems

When the concentration of acid in the eluent is not high enough to elute sample analytes retained on OmniPac PCX-500 Analytical Column by cation exchange, when two analytes co-elute or when peak elution efficiency might be improved, salts can be added to the eluent. 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 to packing with carboxylic acid exchange sites, the sulfonic acid exchange sites are not well hydrated. Less hydrated cations are better able to enter the more hydrophobic stationary phase than more

highly hydrated cations which prefer to partition into aqueous eluents. Conversely, when the eluting cation in the eluent is highly hydrated, the elution of more hydrophilic analytes will take place first.

Salts that are commonly used with the OmniPac PCX-500 Analytical Column include LiCl, NaCl, KC1, sodium acetate and sodium perchlorate. As explained above, lithium will be a more efficient eluting cation for more hydrophilic analytes, while potassium will be a more efficient eluting cation for more hydrophobic compounds.

Before actually proportioning the salt in the gradient pump , it is advisable to check on the salt's solubility under the desired solvent concentration. For a rough guide, the solubility limits in 54% acetonitrile for the salts are as follows:

>0.2 M HC1	0.4 M LiCl	0.3 M NaCl > 0.8 M NaC104 0.1 M Na2SO4 0.2 M sodium acetate	0.3 M KC1

4.3.4 The Role of Ion Pair Complexes in Eluent Systems

The counterion of the eluting eluent cation (the eluent anion) can also have an effect on resolving sample analytes. This occurs by changing the solubility parameters of the sample analyte cation by making an ion pair complex with 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 also cause some analyte cations to form ion pairs. Since perchlorate anions tend to form ion pairs with increased hydrophobicity compared to the free analyte cations., the effect of this ion pair complex is usually seen as analyte cations moving toward longer retention times. The two most common categories of cations that will ion pair with the perchlorate anion are monovalent cations with low hydration energy and surface active analytes.

Remember that if the Pulsed Electrochemical Detector equipped with a gold (Au) working electrode is selected for the detector, then the eluent should be made with perchloric acid and a perchlorate salt. Hydrochloric acid can corrode the gold electrode. The chloride is strongly adsorbed onto the gold working electrode making the electrochemistry of the analytes of interest unreliable.

Ion pair reagents, such as hexane sulfonic acid are commonly used in applications for the separation of small cations using suppressed conductivity as the detection method. Ion pair reagents are usually used at concentrations of 2 to 10 mM depending on the ion pair reagent. At higher concentrations ion pair reagents may give high background conductivity.

4.3.5 Preliminary Eluent Systems

Before selecting the eluent system, select the appropriate detector (see Section 4.2, "Detector Selection"). Start with the following initial conditions:

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

B. The high pressure limit on the gradient pump should be set at 3,500 psi.

C. Prepare individual eluent bottles containing

   1. 90% solvent in deionized water

2. Acid in deionized water with 5% solvent
3. Salt in deionized water with 1% solvent
4. Deionized water with 5% solvent

Using the gradient pump, proportion different amounts from each eluent bottle to develop the eluent system or gradient which will accomplish the desired separation using the OmniPac PCX-500 Analytical Column.

If the UV/Visible Detector Module (VDM-II) has been selected, then the four eluent bottles should contain:

A. 0.5 HCI in deionized water with 1% solvent

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

C. 90% Acetonitrile in Deionized water

D. Deionized water with 5 % acetonitrile

E. Post column addition of sodium hydroxide is necessary when using the gold working electrode. The ED-50 requires that the final eluent pH is 13.

If the Conductivity Detector (ED-40) is selected, then the eluent system should consist of:

A. 0.5 M HC1 in deionized water with 1% acetonitrile

B. 90% acetonitrile in deionized water

C. 5% acetonitrile in deionized water

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

CAUTION

Cation exchange and solvent exchange gradients can be run simultaneously to take advantage of the multi-phase character of the OmniPac PCX-500 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. Before actually proportioning the eluents through the gradient pump, check the solubility information given above for rough estimates. It is also vise 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 example applications chromatograms and conditions shown below. If your analytes resemble any of the group of compounds chromatographed with the OmniPac PCX-500 Analytical Column, use the gradient conditions for the particular application of interest. Check to make sure first that the eluent system is compatible with the detection system you wish to use.

If your sample analytes do not particularly resemble any of the example applications shown below, you may want to start with the following suggested gradients:

A. When using the UV/Visible detector: 50 mM HC1 with 0.1 to 0.3 M NaCl and 10% to 60% acetonitrile in 10 minutes. Hold at the upper end until all peaks elute.

B. When using PED detection: 50 mM HC104 with 0.1 to 0.3 M NaC104 and 10% to 60% acetonitrile in 10 minutes. Hold at the upper end until all peaks elute. Post-column addition of 0.3M NaOH.

C. When using conductivity detection: 10 mM to 50 mM HC1 with 10% to 60% acetonitrile and 1 mM to 5 mM DAP in 10 minutes. Hold at the upper end until all peaks elute.

4.3.6 Eluent System Development Process

If initial attempts at developing a preliminary eluent system as described in Section 4.3.5, "Preliminary Eluent Systems", have not yielded complete resolution of all of the desired 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 compounds will be eluted by a solvent gradient only. Cationic analytes will be eluted by a cation exchange gradient in which the acid or salt concentrations of the eluent are changed. The eluent can then be modified so that weak base ionization can be either enhanced or suppressed. Study the structural features of the analytes so that different separation strategies can be developed and then verified by actual chromatography. Resolution is proportional to the selectivity and elution efficiency. Sensitivity is directly dependent on peak elution efficiency.

Peak elution efficiency is improved as the slope of the gradient is made steeper. 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 between 2 and 3 minutes of delay time between changing the gradient at the Gradient Pump Module 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 for the separation problem at hand. Many applications can be run at a fixed pH with varying salt levels but they can then require as long as 5 to 10 minutes to equilibrate to the initial starting conditions after the end of the gradient. Ion exchange gradients equilibrate faster to the initial starting conditions after the end of the run 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-500 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.

4.4 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-500 Analytical Column. For more information on problems that originate with the Ion Chromatograph (IC), the detectors or the Cation MicroMembrane Suppressor (CMMS-II), 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.

4.4.1 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 gradient 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 use the Gradient pump to 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 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. At the same time, the analyte is not being retained by adsorption because of the very low- amount of solvent in the eluent. It probably is too hydrophilic and a different column may have to be used.

4.4.2 Peaks that Do Not Elute or Have Very Long Elution Times

A. Make sure the sample 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 sample 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. If the sample analyte is not a cation, then it is being retained on the OmniPac PCX-500 Analytical Column by adsorption. The acid component in the eluent serves no purpose and can be removed from the eluent.

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

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

    c. If there is no change in the retention time of the sample analyte, prepare a new eluent with no salt in it so that the solvent concentration can be increased to over 90%.

    d. If the sample analyte still has a long retention time, change the type of solvent used in the eluent to a solvent with more eluting power. When selecting solvents for inclusion in the eluent make sure that the eluent is water miscible and compatible with PEEK tubing at the temperature and pressure being used.

2. If the sample analyte is a cation, then it is being retained on the OmniPac PCX-500 Analytical Column by cation exchange. Using an isocratic eluent increase the ionic strength until the sample analyte elutes rapidly off the column.

    a. 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.

    b. If there is no change in the retention time of the sample analyte, prepare a new eluent with the same

amount of salt solvent and half of the solvent.

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

d. The above steps assumed that if the sample analyte was a cation that it was monovalent. Sometimes a polyvalent cation can be very strongly ion exchanged onto the analytical column. In this case the pH can be raised somewhat to partially deprotonate some cationic sites on the sample analyte and effectively lower its total positive charge. If the sample 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 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.

4.4.3 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-500 Analytical Column under the same detection mode.

B. The sample 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 at the pH of the eluent. You may have to use a different detection system if detection is the problem.

C. There may be sample matrix interferences. The poor peak shape might actually be caused by a component in the sample which is only partially resolved from the sample 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. 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 over all concentration change per unit time.

F. The eluent components may need to be changed. Remember that like ions elute will 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.

4.4.4 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 5, “Example Applications Done on the OmniPac PCX-500 Analytical Column”) using the same detection mode.

B. Modify the gradient or eluent components.

1. Lower the concentration of the mobile phase component which is the strongest eluent component by 20%. Remember that the reversed phase and the cation exchange retention mechanisms operate independently on the OmniPac PCX-500 Analytical Column. Neutral analytes are eluted differently than cationic analytes. Often they can be separated by adjusting the eluent components appropriately.

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

SECTION 5 - EXAMPLE APPLICATIONS DONE ON THE OMNIPAC PCX-500 ANALYTICAL COLUMN

5.1 INTRODUCTION

The OmniPac PCX-500 Analytical Column expands the selectivity control available to the analytical chemist by allowing a broad range of separation techniques to be used with a single column. Ion exchange, ion pair, reversed phase and ion suppression can be performed on the OmniPac PCX-500. These techniques can be performed in any combination, either simultaneously or in series. It is possible to perform either ion exchange gradients or reversed phase gradients simultaneously or in series with both separation modes acting independently in order to achieve the desired separation.

5.1.1 OmniPac PCX-500 Key Operating Parameters

Maximum Operating Pressure:	5,000 psi
Organic Solvent Concentration:	1 to 100%
Eluant pH Range:	0 to 14
Maximum Eluant Flow Rate:	3. 0 mL/min
Ion Pair Reagent Concentration:	Up to 30.0 mM

The OmniPac PCX-500 must always be operated so that at least 1% organic solvent is maintained in the eluant. Maintaining 1% organic solvent in the eluant ensures that the highly cross-linked hydrophobic core is “wetted” and maximum column performance is maintained.

5.2 OMNIPAC PCX-500 ANALYTICAL COLUMN TEST MIXTURE

The OmniPac PCX-500 Analytical Column is tested as described here to ensure proper performance in both the ion exchange and adsorption retention modes. The retention of pyridine, retained by cation exchange and benzyl alcohol, retained by adsorption are reported on the column Certificate of Performance received with the column. The chromatogram in Figure 2, "OmniPac PCX-500 Analytical Column Test Mixture", is an example of the test chromatogram.

Column:	OmniPac PCX-500 Analytical Column
Eluent:	50 mM HCl 100 mM KCl 32% Acetonitrile
Flow Rate:	1.0 mL/min
Detection:	Absorbance at 220 nm, 0.02 AUFS
Injection Volume:	10 µL
Storage Solution:	Eluent

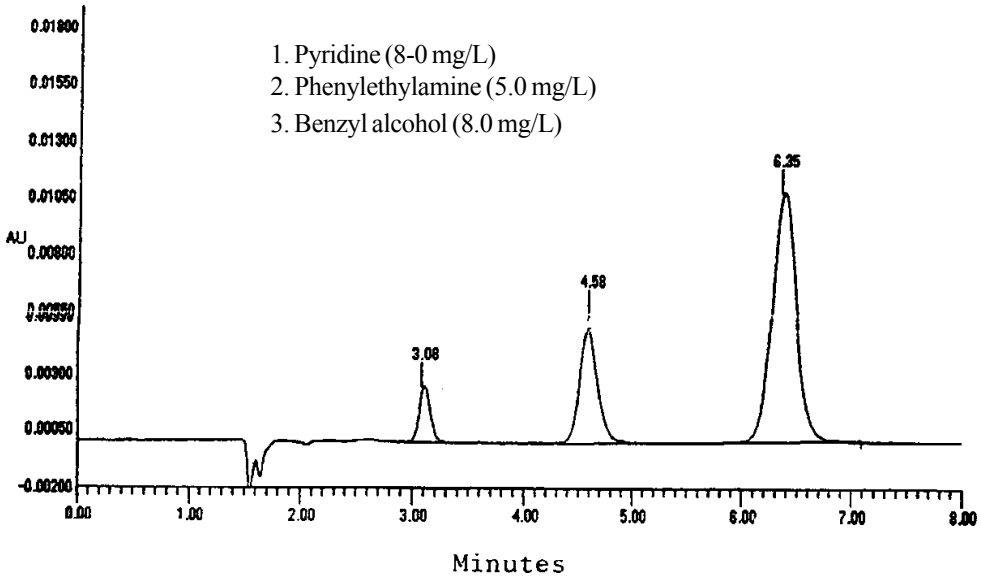


Figure 2

OmniPac PCX-500 Analytical Column Test Mixture

5.3 GRADIENT SEPARATION OF AMINES AND INORGANIC CATIONS

Sample Loop Volume:	10 µL
Guard Column:	OmniPac PCX-500 Guard Column
Analytical Column:	OmniPac PCX-500 Analytical Column
Eluents, Eluent 1:	200 mM HCl
Eluent 2:	95% ACN/Deionized Water
Eluent 3:	Deionized Water (having a specific resistance of 17.8 megohm-cm or greater
Eluent 4:	20 mM DAP
Eluent Flow Rate:	1.0 mL/Min
Suppressor:	Cation MicroMembrane Suppressor (CMMS-II)
Regenerant:	0.1 M Tetrabutylammonium hydroxide
Regenerant Flow Rate:	10 mL/Min
Expected Background Conductivity:	2 - 4 µS
Expected System Operating Back Pressure:	1,600 - 2,200 psi

Gradient Program:

Time (min)	%1	%2	%3	%4
0.0	2	5	88	5
15.0	2	5	88	5
15.5	2	5	88	5
40.5	40	15	0	45
40.6	2	5	88	5

1.	Lithium	(1.0 mg/L)
2.	Sodium	(5.0 mg/L)
3.	Ammonium	(5.0 mg/L)
4.	Potassium	(5.0 mg/L)
5.	Methylamine	(5.0 mg/L)
6.	Trimethylamine	(5.0 mg/L)
7.	Diethylamine	(10.0 mg/L)
8.	Triethylamine	(10.0 mg/L)
9.	Tetraethylamine	(10.0 mg/L)
10.	Pyrrolidine	(10.0 mg/L)
11.	Piperidine	(10.0 mg/L)
12.	Cyclohexylamine	(15.0 mg/L)
13.	Magnesium	(5.0 mg/L)
14.	Calcium	(10.0 mg/L)

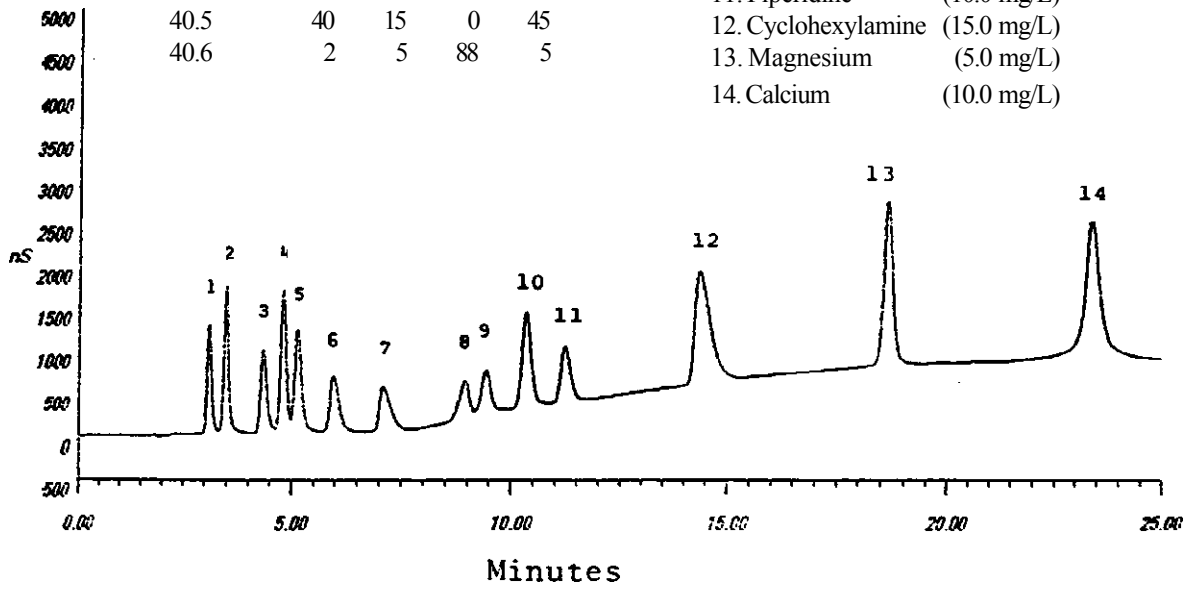


Figure 3

Gradient Separation of Amines and Inorganic Cations

5.4 SULFONAMIDE ANTIBACTERIALS

Sample Loop Volume:	25 µL
Analytical Column:	OmniPac PCX-500 Analytical Column
Eluents, Eluent 1:	60 mM $HClO_4$
Eluent 2:	400 mM $NaC_2H_3O_2$
Eluent 3:	90% ACN/Deionized Water (having a specific resistance of 17.8 megohm-cm or greater)
Eluent 4:	5% ACN/Deionized Water
Eluent Flow Rate:	1.0 mL/Min
Detection:	UV at 254 nm

Gradient Program:

Time (min)	%1	%2	%3	%4	V5	Comments
0.0	18	5	30	47	0	
0.1	18	5	30	47	1	Injection
0.2	18	5	30	47	0	
5.0	18	26	56	0	0	
7.0	18	26	56	0	0	
7.1	18	5	30	47	0	

1. Sulfanilic Acid
2. Sulfanilamide
3. Sulfathiazole
4. Sulfamethizole + Sulfadiazine
5. Sulfamerazine
6. Sulfamethazine
7. Sulfisoxazole
8. Sulfamethoxazole + Trimethoprim
9. Sulfadimethoxine

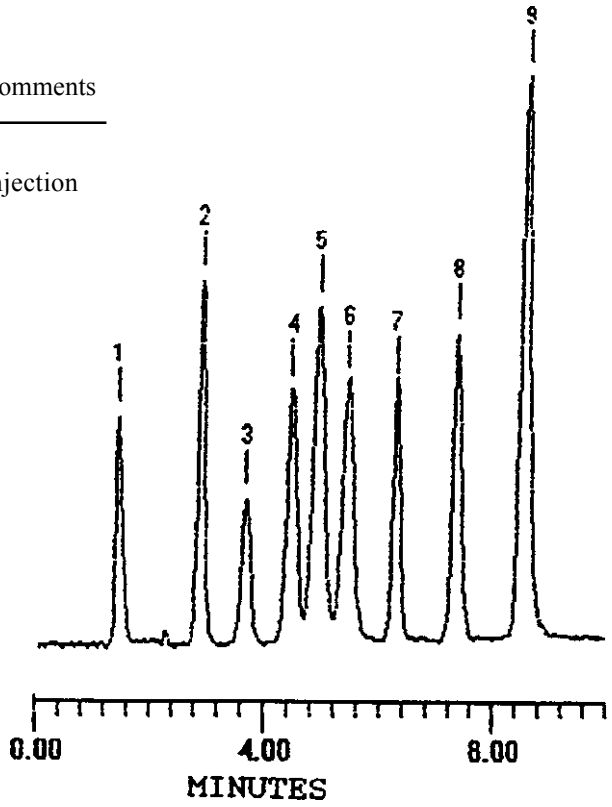


Figure 4

Sulfonamide Antibacterials

5.5 WATER SOLUBLE VITAMINS

Sample Loop Volume:	25 µL
Analytical Column:	OmniPac PCX-500 Analytical Column
Eluents, Eluent 1:	600 mM $HClO_4$
Eluent 2:	1,000 mM $NaClO_4$
Eluent 3:	90% ACN/Deionized Water (having a specific resistance of 17.8 megohm-cm or greater)
Eluent 4:	5% ACN/Deionized Water
Eluent Flow Rate:	1.0 mL/Min
Detection:	UV at 254 nm

Gradient Program:

Time (min)	%1	%2	%3	%4	V5	Comments
0.0	5	5	5	85	0	
0.1	5	5	5	85	1	Injection
0.2	5	5	5	85	0	
5.0	5	6	30	59	0	
5.1	5	15	80	0	0	
10.1	5	5	5	85	0	

1. Ascorbic Acid
2. Pantothenic Acid
3. Carnitine
4. Riboflavin
5. Biotin
6. Pyridoxine
7. PABA
8. Adenine
9. Folic Acid
10. Thiamine
11. Pyrridoxamine

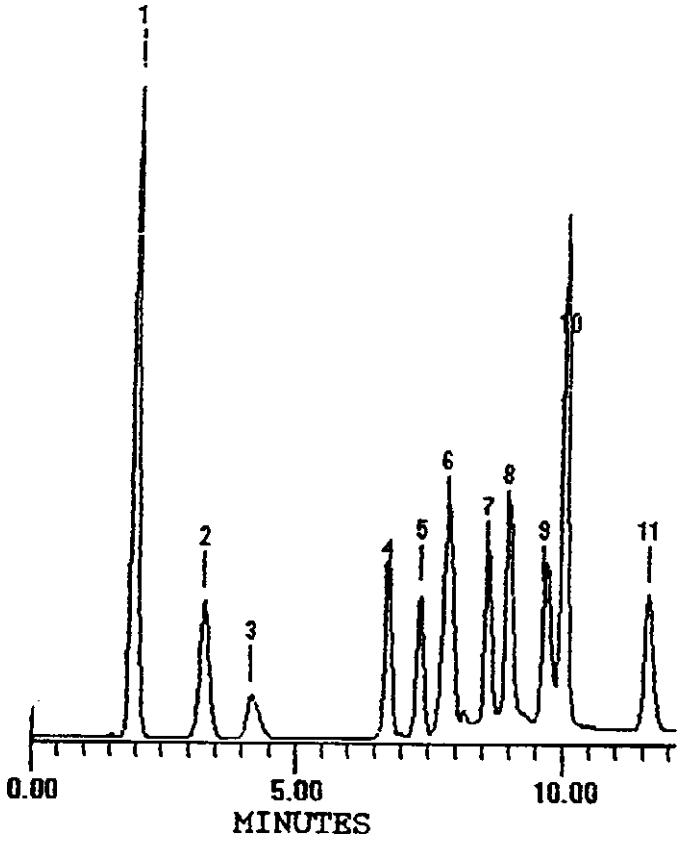


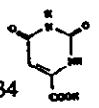
Figure 5

Water Soluble Vitamins

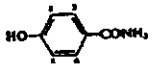
5.6 N-CONTAINING AROMATIC COMPOUNDS

5.6.1 Structures of the N-Containing Aromatic Compounds

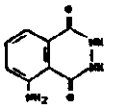
1. Orotic Acid
pKa 1.96 9.34



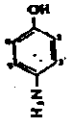
2. 4-Hydroxybenzamide



3. Luminol



4. p-Aminophenol
pKa 8.5 10.3



5. p-Aminobenzoic Acid
pKa 2.4 4.85

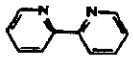
6. Pyridine
pKa 5.2

7. Aniline
pKa 4.6

8. p-Anisidine
pKa 5.3

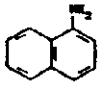
9. p-Toluidine
pKa 5.1

10. 2,2'-Bipyridine

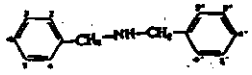


11. Benzoic Acid
pka 4.2

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



13. Dibenzylamine
pKa 8.5



14. p-Phenylenediamine
pKa 3.3 6.1

15. p-Nitroaniline
pKa 1.0

16. 1-Benzylpiperazine

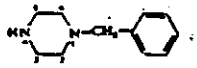


Figure 6

Structures of the N-Containing Aromatic Compounds

5.6.2 Chromatography of the N-Containing Aromatic Compounds

Sample Loop Volume:	25 µL
Analytical Column:	OmniPac PCX-500 Analytical Column
Eluents, Eluent 1:	500 mM HCl
Eluent 2:	1,000 mM NaCl
Eluent 3:	90% ACN/Deionized Water (having a specific resistance of 17.8 megohm-cm or greater)
Eluent 4:	5% ACN/Deionized Water
Eluent Flow Rate:	1.0 mL/Min
Dection:	UV at 254 nm

Gradient Program:

Time (min)	%1	%2	%3	%4	V5	Comments
0.0	10	10	30	50	0	
0.1	10	10	30	50	1	Injection
0.2	10	10	30	50	0	
4.0	10	45	45	0	0	
7.0	10	0	90	0	0	
11.0	10	0	90	0	0	
11.1	10	10	30	50	0	

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

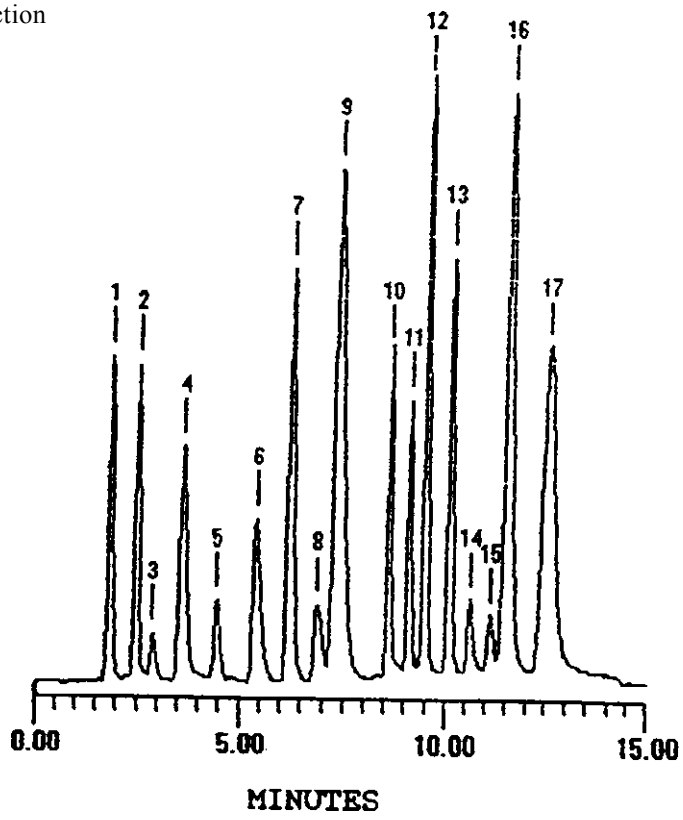


Figure 7

N-Containing Aromatic Compounds

5.6.4 Sample Chromatograms Demonstrating Selectivity Changes Obtained by Changing the Eluting Cation

The following two chromatograms demonstrate the selectivity changes that occur when the eluting cation in the eluent is changed from sodium (see Figure 7, "N-Containing Aromatic Compounds") to lithium and then to potassium. The operating conditions used are the same as those in Section 5.6.2, "Chromatography of the N-Containing Aromatic Compounds". The sample analytes are also the same except for the addition of 18. Benzoic acid.

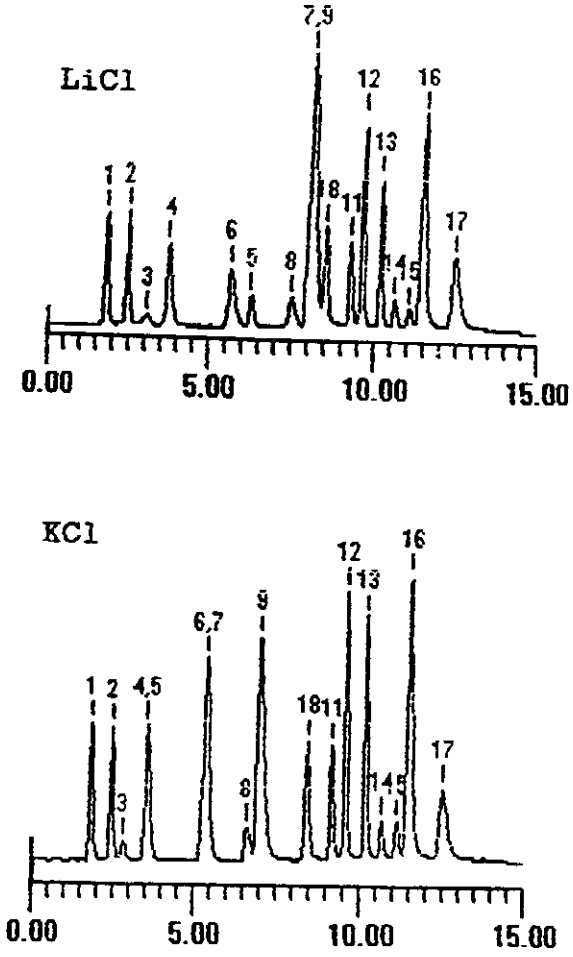


Figure 8

Selectivity Changes Obtained by Changing the Eluting Cation

5.6.5 Sample Chromatograms Demonstrating Selectivity Changes Obtained by Changing the Counterion of the Eluting Cation (The Eluent Anion)

The following two chromatograms demonstrate the selectivity changes that occur when the counterion of the eluting cation in the eluent is changed from chloride to perchlorate. The operating conditions used are the same as those in Section 5.6.2, "Chromatography of the N-Containing Aromatic Compounds". The sample analytes are also the same except for the addition of 18. Benzoic acid.

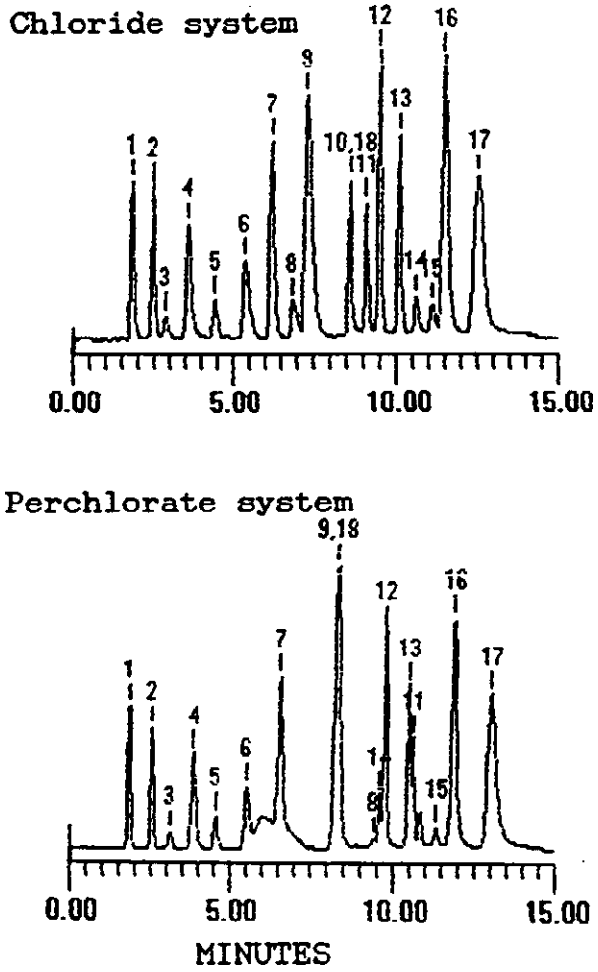


Figure 9

Selectivity Changes Obtained by Changing the Counterion of the Eluting Cation (The Eluent Anion)

5.7 BIOLOGICAL STAINS AND DYES

5.7.1 Structures of Selected Biological Stains and Dyes

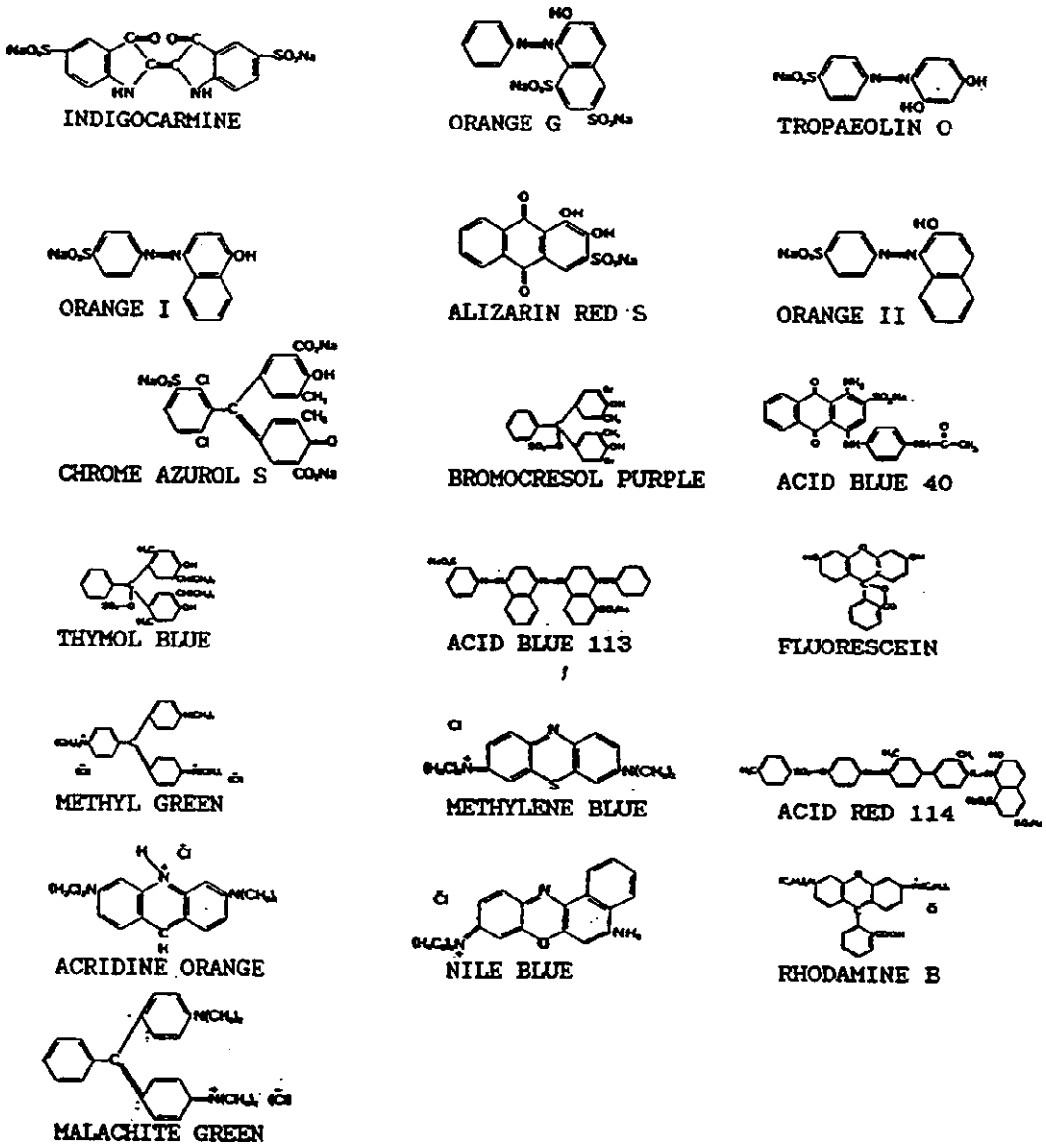


Figure 10

Structures of Selected Biological Stains and Dyes

5.7.2 Chromatography of Biological Stains and Dyes

Sample Loop Volume:	25 µL
Analytical Column:	OmniPac PCX-500 Analytical Column
Eluents, Eluent 1:	600 mM $HClO_4$
Eluent 2:	1,000 mM $NaClO_4$
Eluent 3:	90% ACN/Deionized Water (having a specific resistance of 17.8 megohm-cm or greater)
Eluent 4:	5% ACN/Deionized Water
Eluent Flow Rate:	1.0 mL/Min
Dection:	UV at 254 nm

Gradient Program:

Time (min)	%1	%2	%3	%4	V5	Comments
0.0	2	10	30	58	0	
0.1	2	10	30	58	1	Injection
0.2	2	10	30	58	0	
4.0	2	15	40	43	0	
6.0	2	38	60	0	0	
10.0	2	38	60	0	0	
10.1	2	8	90	0	0	
15.0	2	8	90	0	0	
15.1	2	10	30	58	0	

1. Indigo Carmine
2. Orange G
3. Tropaeolin 0
4. Orange 1
5. Alizarin Red S
6. Orange II
7. Chrome Azurol S
8. Bromocresol purple
9. Thymol Blue
10. Acid Blue 11310
11. Fluorescein
12. Methyl Green
13. Methylene Blue
14. Acridine
15. Nile Blue
16. Rhodamine B
17. Malachite Green

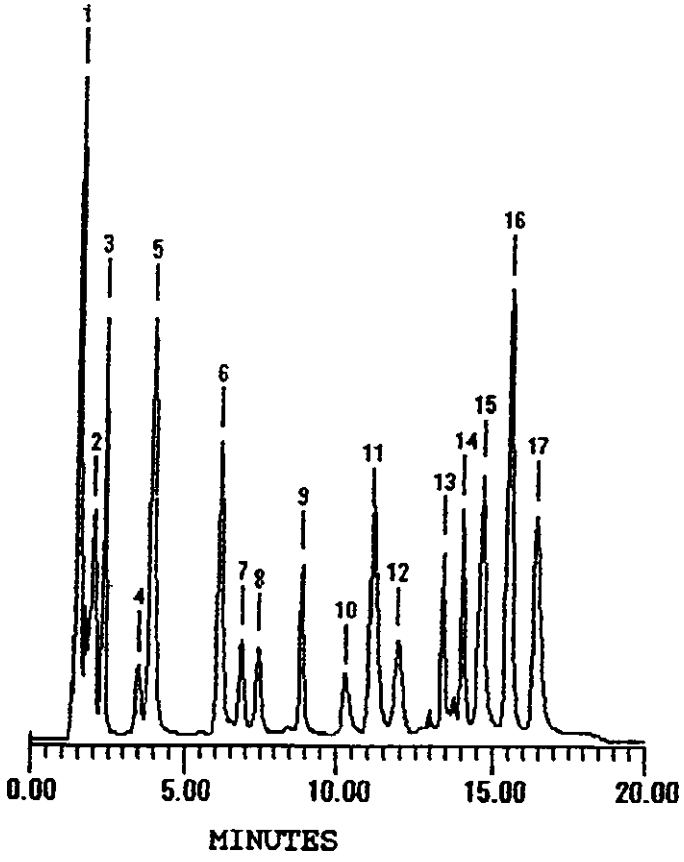


Figure 11

Biological Stains and Dyes

SECTION 6 - INSTALLATION OF SUPPRESSED CONDUCTIVITY DETECTION ACCESSORIES

6.1 SYSTEM REQUIREMENTS FOR SUPPRESSED CONDUCTIVITY

The following system requirements are for those applications developed for the OmniPac PCX-500 Analytical Column that are best performed using suppressed conductivity detection.

6.2 INSTALLING THE IONPAC® CATION TRAP COLUMN (CTC-1)

When performing ion exchange applications that involve a gradient and suppressed conductivity, a 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 and the injection valve.

B. Connect the gradient pump directly to the CTC-1. Connect a waste line to the CTC-1 outlet and direct the line to a waste container.

C. Rinse the CTC-1 with 30 mL of a 10 X 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).

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

E. The background conductivity of your system should be between 2 µS to 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, the CTC-1 should be flushed using the initial operating conditions. At the end of each operating day, the CTC-1 should be rinsed of any impurities that may have accumulated onto the CTC-1.

F. Flush the CTC-1 with 30 mL of a 2X to 3X concentrate of the strongest eluent used in the gradient.

G. Prior to the next day use of the chromatographic system, flush the CTC-1 with 30 mL of the strongest eluent of the gradient program.

6.3 MICROMEMBRANE™ SUPPRESSOR REQUIREMENTS

When performing applications that require suppressed conductivity detection, a Cation MicroMembrane Suppressor (CMMS-II, P/N 043021) must be used. The CMMS-II 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-II. If you do not, take the time to review the Installation Instructions for the CMMS-II before beginning an analysis.

6.4 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-II), it is necessary to replace the regenerant on a regular basis. 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. It is not necessary to change the AutoRegen Cation Regenerant Cartridge until it is completely

expended.

The Cation MicroMembrane Suppressor (CMMS-II) continuously exchanges all of the eluent counter anions for hydroxide ions in the regenerant solution before the eluent stream enters the Conductivity Detector (suppressed conductivity detection). These eluent counter anions are exchanged out of the regenerant solution and replaced with hydroxide ions by the AutoRegen Cation Regenerant Cartridge. The level of anions other than hydroxide in the regenerant solution is very low until the AutoRegen Cation Regenerant Cartridge expires.

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 in the recycling regenerant solution stream- - Eventually ally the concentration of the solvent in the recycled regenerant can cause the background conductivity to increase which can result in a noisy background. Solvent has no effect on the cartridge lifetime. The ionic strength of the eluent determines the lifetime of the Autoregen Cation Regenerant Cartridge.

CAUTION

When the solvent is acetonitrile, the AutoRegen Accessory with the Cation AutoRegen Regenerant Cartridge can be used but the regenerant should not be recycled through the Cation MicroMembrane Suppressor (CMMS-II) because acetonitrile breakdown products resulting from prolonged exposure to the strong base in the AutoRegen Accessory can affect system performance.

When replacing the recycled regenerant, the first 250 mL of the regenerant should be pumped to waste before recycling of the regenerant is started. Utilizing AutoRegen Accessory in this manner will allow the use of high regenerant flow rates with the minimum of consumption and waste.

SECTION 7 - TROUBLESHOOTING GUIDE

The purpose of the Troubleshooting Guide is to help you solve operating problems that may arise while using the OmniPac PCX-500 Analytical Column. For more information on problems that originate with the Ion Chromatograph (IC), the detectors or the Cation MicroMembrane Suppressor (CMMS-II), 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.

7.1 HIGH BACK PRESSURE

Total system back pressure when- using the OmniPac PCX-500 Analytical Column at 1.0 mL/min should be less than 2500 psi. Refer to Section 4.3.1, "The Role of Solvents in Eluent Systems", to see how 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. Measure the pump flow rate after the analytical column with a stopwatch and graduated cylinder if necessary.

B. Find out what 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 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, MicroMembrane Suppressor, detector) one by one, while watching the pressure. The pressure should increase up to a maximum of 2500 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 clean-up 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 (P/N 042955 which consists of a seal washer, P/N 042956 and a frit P/N 041375) into the end fitting (P/N 042367). Use the end of the column to carefully push the bed support assembly into the end fitting.
   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.
   6. Reconnect the column to the system and resume operation.

7.2 HIGH BACKGROUND OR 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 as described in Section 5.3, "Gradient Separation of Amines and Inorganic Cations".

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

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

C. Make sure that the deionized water used to prepare the reagents has a specific resistance of 17.8 megohm-cm or greater.

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.

1. Is CTC-1 the installed before the injection valve? If it is not and you are doing an application with suppressed conductivity detection, install one as directed in Section 6.2, "Installing the IonPac Cation Trap Column (CTC-1)", and watch 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).

2. If the CTC-1 is already 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.

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

   b. Rinse the CTC-1 with 30 mL of a 10 X 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).

   c. 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-500 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 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 suppressor is probably causing the problem.

1 . Check the regenerant flow rate at the REGEN OUT Port of the MicroMembrane Suppressor. This flow should be greater or equal to 10 mL/min.

2. Check the eluent flow rate. 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 Manual for instructions.

4. If you are using an AutoRegen Accessory, connect the regenerant to the AutoRegen Regenerant Cartridge. Pump approximately 250 mL of regenerant through the Anion AutoRegen 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 039564). Refer to the AutoRegen Cartridge Manual for Instructions.

7.3 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 pressures). Remove the column's top end fitting (see Section 7.1, "High Back Pressure", Step C). 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.

2. Extra-column effects can result in sample band dispersion, making the peaks elution less efficient. Make sure you are using tubing with an ID 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 faster than 1.0 mL/min. Check the eluent flow rate after the column.

2. Check to see if the eluent composition and concentration is correct. An eluent that is too strong will make the peaks 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 might not be accurate enough for this application. Use one reservoir containing the correct eluent composition to see if this is the problem.

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. Refer to "Column Care" 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 17.8 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 (see "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 the very early eluting peaks near the system void volume compared to the later eluting peaks, check the following:

1. Improper eluent El concentration may be the problem. Remake the eluent. Make sure that the all chemicals meet the purity requirements described in Section 3, "General Operation and Start-up".

2. Column overloading may be the problem. Reduce the amount of sample ions being injected onto the column 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.

7.4 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 cations may be contaminating the column. The retention times for the analytes will then decrease and spurious, inefficient peaks can show up at unexpected times. Clean the column as indicated in "Column Care". Using the recommended eluent will 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. It will happen when the injection valve needs to be cleaned or retorqued (see system 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 is 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 baseline disturbances still occur after the valve has been cleaned, reassembled and torqued (see manual), replace the valve with a DIONEX MicroInjection Valve as required.