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

1. See Section 3, “Operation”. Note operation precautions and chemical purity requirements.

2. See Section 4.1, “Eluent Preparation”. Make the required stock and working solutions for eluents.

3. See “Quality Assurance Report”. Run the Production Test Chromatogram as a system check.

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

5. See “Column Care” for column cleanup and long-term storage recommendations.
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- 2.5 Eluent Storage
- 2.6 IonPac CG5 Guard Columns

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SECTION 1 - INTRODUCTION TO IONPAC CS5 ION CHROMATOGRAPHY

The IonPac® CS5 Analytical Column (P/N 037028) is designed for the analysis of transition metals and lanthanides. The IonPac CG5 Guard Column (P/N 037029) is placed prior to the CS5 to prevent possible sample contaminants from fouling the analytical column. The versatility of the CS5 makes it possible to obtain two different separations simply by changing the eluent. A combination anion-cation exchange separation of transition metal such as Pb²⁺, Cu²⁺, Cd²⁺, Mn²⁺, Co²⁺, Zn²⁺, and Ni²⁺ is achieved by using an oxalic acid eluent. The use of a pyridine-2,6-dicarboxylate (PDCA) eluent allows speciation and quantification of Fe²⁺ and Fe³⁺. 4-(2-pyridylazo) resorcinol (PAR) is used as the post-column reagent for visible absorbance detection.

CAUTION
Eluents with greater than 5% solvent will damage the column.

Table 1
IonPac CS5/CG5 Packing Specifications

<table>
<thead>
<tr>
<th>Column</th>
<th>Particle Diameter</th>
<th>Substrate X-linking</th>
<th>Latex Diameter</th>
<th>Latex X-Linking</th>
<th>Column Capacity µeq/column</th>
<th>Functional Group</th>
<th>Hydophobicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS5</td>
<td>13.0 µm</td>
<td>2%</td>
<td>110 nm</td>
<td>2%</td>
<td>150 µeq/column</td>
<td>Sulfonic Alkanol quaternary ammonium</td>
<td>Low</td>
</tr>
<tr>
<td>4 x 250 mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CG5</td>
<td>13.0 µm</td>
<td>2%</td>
<td>110 nm</td>
<td>2%</td>
<td>30 µeq/column</td>
<td>Alkanol quaternary ammonium</td>
<td>Low</td>
</tr>
<tr>
<td>4 x 50 mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a microporous divinylbenzene/styrene polymer
b microporous polyvinylbenzylammonium polymer cross-linked with divinylbenzene

Table 2
CS5/CG5 Operating Parameters

<table>
<thead>
<tr>
<th>Column</th>
<th>Typical Back Pressure psi (MPa)</th>
<th>Standard Flow Rate mL/min</th>
<th>Maximum Flow Rate mL/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS5 Analytical Column</td>
<td>≤ 850 (5.86)</td>
<td>1.0</td>
<td>3.0</td>
</tr>
<tr>
<td>CG5 Guard Column</td>
<td>≤ 300 (2.06)</td>
<td>1.0</td>
<td>3.0</td>
</tr>
<tr>
<td>CS5 Analytical + CG5 Guard columns</td>
<td>≤ 1,150 (7.92)</td>
<td>1.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>
The CS5 can be operated at flow rates up to 3.0 mL/min. PEEK (polyetheretherketone) is used to make column hardware. PEEK has excellent chemical resistance to most organic solvents and inorganic solutions. Concentrated sulfuric acid and concentrated nitric acid will attack PEEK. Tetrahydrofuran at concentrations of greater than 20% is not compatible with PEEK systems. The CS5 Analytical Columns have minimum efficiencies for Cobalt of 3,000 plates/column and Cadmium of 1,200 plates/column, under standard operating conditions. The CS5 operates at a back pressure between 850 psi at 1.0 mL/min with the test eluent. However, CS5 columns are capable of operating at back pressures up to 4,000 psi.

The IonPac CS5 Analytical and CG5 Guard Columns have 10-32 threaded PEEK end fittings for use with ferrule/bolt liquid line fittings. If your system is otherwise configured, refer to, “DIONEX Liquid Line Fittings.”

This manual assumes that you are familiar with the installation and operation of the DIONEX Ion Chromatograph (IC). If you do not understand the operation of the system, take the time to familiarize yourself with the various system components before beginning an analysis.
SECTION 2 - INSTALLATION

2.1 System Requirements for 4-mm Operation

The IonPac CS5 Guard and Analytical Columns are designed to be run on any DIONEX Ion Chromatograph equipped with a variable wavelength detector, a Reagent Delivery Module (RDM, P/N 039582) and an IonPac Membrane Reactor (P/N 035354).

2.2 System Void Volume

For best performance, all of the tubing installed between the injection valve and detector should be 0.010” ID PEEK tubing (P/N 042260) or 0.012” Tefzel tubing (see, “DIONEX Product Selection Guide”). Minimize the lengths of all connecting tubing and remove all unnecessary switching valves and couplers. If you need assistance in properly configuring your system contact the nearest DIONEX Office (see, “DIONEX Worldwide Offices”).

2.3 The Injection Loop

<table>
<thead>
<tr>
<th>Valve Type</th>
<th>Using 0.012” ID Tefzel Tubing</th>
<th>Using 0.007” ID Tefzel Tubing</th>
<th>Using 0.010” ID PEEK Tubing</th>
<th>Using 0.005” ID PEEK Tubing</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIONEX BF2 Valve</td>
<td>15.2</td>
<td>10.5</td>
<td>13.1</td>
<td>9.2</td>
</tr>
<tr>
<td>(8 µL Internal Volume)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(10 cm Loop)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIONEX MicrolInject Valve</td>
<td>20.5</td>
<td>14.0</td>
<td>17.6</td>
<td>12.2</td>
</tr>
<tr>
<td>(10.5 µL Internal Volume)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(14 cm Loop)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rheodyne Microinjection Valve</td>
<td>8.0</td>
<td>3.3</td>
<td>5.9</td>
<td>2.0</td>
</tr>
<tr>
<td>Model 9126</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(0.8 µL Internal Volume)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(10 cm Loop)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For most applications on a 4-mm analytical system, a 10 - 50 µL injection loop will be sufficient. DIONEX recommends that a 10 µL injection loop be used to avoid overloading the CS5 Analytical Column. Generally, do not inject more than 10 nanomoles (100 - 200 ppm) of any one analyte onto the 4-mm analytical column. Injecting larger volumes of samples can result in overloading the column which can affect the detection linearity. This phenomenon will be more prevalent at higher concentrations of the analytes of interest.
2.4 Sample Concentration

The IonPac Trace Cation Concentrator (TCC-2) Column (P/N 043103) can be used for trace metal concentration work in high purity water analysis. If additional capacity is required, an IonPac CG2 Guard Column (P/N 035370) can be used. The function of the concentrator column in these applications is to strip ions from a measured volume of a relatively clean aqueous sample matrix. This process “concentrates” all cationic analyte species onto the concentrator column leading to a lowering of detection limits by 2-5 orders of magnitude. The unique advantage of using a concentrator column in these applications is the capability of performing routine trace analyses of sample matrix ions at μg/L (ppb) levels without extensive and laborious sample pretreatment.

For a detailed discussion of cation concentration techniques, refer to Section 3, “Operation,” of the Trace Cation Concentrator (TCC-2) Column Installation Instructions and Troubleshooting Guide (Document No. 034466).

2.5 Eluent Storage

IonPac CS5 columns are designed to be used with chelating eluent systems. Storage under a nitrogen or helium atmosphere ensures contamination free operation and proper pump performance.

2.6 IonPac CG5 Guard Columns

An IonPac CG5 Guard Column is normally used with the IonPac CG5 Analytical Column. The guard column is placed prior to the analytical column to prevent sample contaminants from eluting onto the analytical column. It is easier to clean or replace a guard column than it is an analytical column.

The addition of the CG5 to the CS5 increases the column capacity by 20%. Retention times in isocratic runs will increase by approximately 20%.

As a general rule, the guard column needs to be cleaned or replaced when a 10 to 15% reduction in retention times is observed compared to when it was originally placed on the system.
SECTION 3 - OPERATION

3.1 General Operating Conditions

The IonPac CS5 has been designed to separate a broad range of chelated metal complexes by anion chromatography. The CS5 columns are 2% cross-linked, microporous, hydrophobic resin core that has been agglomerated with totally permeable latex particles that are completely aminated. The latex particles carry the actual anion exchange function, an alkanol quaternary ammonium group. The nature of the cross-linked polymeric structure of the packing material makes CS5 columns compatible with pH 0-14 eluents.

3.2 IonPac CS5 Operation Precautions

CAUTION
Filter and Degas Eluents
Filter Samples
2% Solvent Maximum
3 mL/min Maximum Flow Rate

3.3 Chemical Purity Requirements

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

3.3.1 Inorganic Chemicals

Reagent Grade inorganic chemicals should always be used to prepare ionic eluents. Whenever possible, inorganic chemicals that meet or surpass the latest American Chemical Society standard for purity should be used. These inorganic chemicals will detail the purity by having an actual lot analysis on each label.

3.3.2 Deionized Water

The deionized water used to prepare eluents should be Type I Reagent Grade Water with a specific resistance of 18.2 megohm-cm. The deionized water should be free of ionized impurities, organics, microorganisms and particulate matter larger than 0.2 µm. Bottled HPLC-Grade Water (with the exception of Burdick & Jackson) should not be used since most bottled water contains an unacceptable level of ionic impurities.
3.4 Reagents for Eluents

**Oxalate Eluent: 50 mM oxalic acid/95 mM lithium hydroxide (pH 4.80)**
You must use reagents of extremely high purity. DIONEX recommends the following high purity reagents.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithium hydroxide monohydrate</td>
<td>FLUKA #62530</td>
</tr>
<tr>
<td>Oxalic acid dihydrate</td>
<td>FLUKA #75700</td>
</tr>
</tbody>
</table>

**PDCA Eluent: 6 mM PDCA/8.6 mM lithium hydroxide**
You must use reagents of extremely high purity. DIONEX recommends the following high purity reagents.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithium hydroxide monohydrate</td>
<td>FLUKA #62530</td>
</tr>
<tr>
<td>Pyridine-2,6-dicarboxylic acid (PDCA)</td>
<td>DIONEX #39671</td>
</tr>
</tbody>
</table>

3.5 Post-Column Reagent Preparation

The post-column reagent for both eluent systems is a solution of 4-(2-pyridylazo)resorcinol (PAR) in trace metal grade ammonium hydroxide and acetic acid. The recommended PAR flow rate is 0.6 to 0.8 mL/min. Because both oxalate and PDCA compete with PAR for the metal ion, it is important to maintain the recommended flow rate to ensure a reproducible derivatization reaction.

PAR is readily oxidized by oxygen. Therefore, always pressurize the reservoir with either nitrogen or helium when using a Reagent Delivery Module and/or Membrane Reactor to add PAR to the eluent stream. To prevent system contamination, rinse the analytical pump and flow cell with deionized water at the end of each day.

A. In a well-ventilated fume hood, thoroughly dissolve PAR in 400 mL of 7.4 M NH₄OH.

NOTE
The recommended PAR concentration is 3 x 10⁻⁴ M. Higher concentrations, such as 4 x 10⁻⁴ M, can be used to extend the linear range of the post-column reaction.

B. Add 600 mL of 1.7 M acetic acid to the PAR solution.

WARNING
Add the acetic acid slowly; this reaction is highly exothermic.

C. To prevent oxidation, store the reagent under nitrogen. PAR has a shelf life of only about 2 weeks, so do not prepare more PAR than you can use within that time.
SECTION 4 - EXAMPLE APPLICATIONS

Before attempting any of the following example applications, take the time to ensure that your system is properly configured.

Ensure that all of the eluents have been made from high purity reagents and deionized water. All water used in the preparation of eluents should be degassed, deionized water. For chemical purity requirements see Section 3.3, “Chemical Purity Requirements.”

In order to guarantee reproducible retention times of analytes and minimum baselines changes when doing gradient chromatography, it is important to install a Cation Trap Column, the CTC.

After running synthetic standards to calibrate your system, you may find that real sample matrices foul your columns. For this reason it is always advisable to use a guard column to protect the analytical column. If column performance deteriorates and it is determined that the guard and analytical columns have been fouled, refer to the column cleanup protocols in, “Column Care.”

If your sample matrices are relatively low in ionic concentration, you may be able to increase the sensitivity of your system by using sample concentration techniques. See Section 2.4, “Sample Concentration,” for details on sample concentration techniques.

4.1 Eluent Preparation

**Isocratic Eluent: 50 mM Oxalic acid/95 mM LiOH (pH 4.8)**

Add 6.30 g oxalic acid·2H₂O (MW 126.07) to a 1 L volumetric flask. Add 3.6 g of LiOH·H₂O (MW 14.96) to the volumetric flask. Dilute the salts to 1 L using degassed Type I Reagent Grade water with a specific resistance of 18.2 megohm-cm. Stir for 5 minutes. Calibrate a pH meter to 4.00 pH ± 0.03 and check the eluent pH. It will be lower than 4.80. Gradually add just enough 1 M LiOH to adjust the pH to 4.80 ± 0.02.

If the pH goes higher than 4.82, DO NOT lower the pH by backtitrating the solution with HCl. Remake the eluent.

**Isocratic Eluent: 6 mM PDCA/8.6 mM LiOH**

Add approximately 500 mL degassed Type I Reagent Grade water with a specific resistance of 18.2 megohm-cm to a 1 L volumetric flask. Add 1.00 g pyridine-2,6-dicarboxylic acid (PDCA, MW 167.12) to the volumetric flask. Add 0.361 g of LiOH·H₂O (MW 41.96) to the volumetric flask. After the PDCA is thoroughly dissolved (in about 45 minutes). Using degassed Type I Reagent Grade water with a specific resistance of 18.2 megohm-cm dilute to the 1 L graduation and stir thoroughly.

Because Fe²⁺ is readily oxidized by molecular oxygen to Fe³⁺, you must be sure to remove oxygen from the eluent before beginning the analysis. To do this, purge the eluent with nitrogen for 5 minutes and then degas it, using a vacuum with ultrasonication. Store the degassed eluent in a pressurized glass reservoir, under nitrogen.

**Gradient Eluent: 6 mM PDCA/50 mM Sodium Acetate/50 mM Acetic Acid**

Add approximately 500 mL degassed Type I Reagent Grade water with a specific resistance of 18.2 megohm-cm to a 1 L volumetric flask. Add 1.00 g pyridine-2,6-dicarboxylic acid (PDCA, MW 167.12) to the volumetric flask. Add 4.10 g of sodium acetate (MW 82.03) to the volumetric flask. Add 3.00 g of glacial acetic acid (MW 60.05) to the volumetric flask. Using degassed Type I Reagent Grade water with a specific resistance of 18.2 megohm-cm dilute to the 1 L graduation and stir thoroughly (about 45 minutes).

Because Fe²⁺ is readily oxidized by molecular oxygen to Fe³⁺, you must be sure to remove oxygen from the eluent before beginning the analysis. To do this, purge the eluent with nitrogen for 5 minutes and then degas it, using a vacuum with ultrasonication. Store the degassed eluent in a pressurized glass reservoir, under nitrogen or helium.
Gradient Eluent: 100 mM Oxalic Acid/190 mM LiOH

Add approximately 500 mL degassed Type I Reagent Grade water with a specific resistance of 18.2 megohm-cm to a 1 L volumetric flask. Add 12.60 g oxalic acid·2H₂O (MW 126.07) to a 1 L volumetric flask. Add 7.97 g of LiOH·H₂O (MW 41.96) to the volumetric flask. Dilute the salts to 1 L using degassed Type I Reagent Grade water with a specific resistance of 18.2 megohm-cm. Stir for 5 minutes.

Gradient Eluent: 100 mM Diglycolic Acid/190 mM LiOH

Add approximately 500 mL degassed Type I Reagent Grade water with a specific resistance of 18.2 megohm-cm to a 1 L volumetric flask. Add 13.41 g diglycolic acid (MW 134.09) to a 1 L volumetric flask. Add 7.97 g of LiOH·H₂O (MW 41.96) to the volumetric flask. Dilute the salts to 1 L using degassed Type I Reagent Grade water with a specific resistance of 18.2 megohm-cm. Stir for 5 minutes.

Post Column Reagent: 0.2 mM 4-(2-pryidylazo)resorcinol (PAR, P/N 039672)/1 M Acetic Acid/3 M ammonium hydroxide

Add approximately 500 mL degassed Type I Reagent Grade water with a specific resistance of 18.2 megohm-cm to a 1 L volumetric flask. Add 200 mL of 30% ammonium hydroxide. Dissolve 0.05 g of PAR in the ammonium hydroxide solution. Add 57 mL of glacial acetic acid. Dilute the salts to 1 L using degassed Type I Reagent Grade water with a specific resistance of 18.2 megohm-cm. Stir for 5 minutes.
4.2 Production Test Chromatogram

Isocratic elution of transition metals on the IonPac CS5 Analytical Column has been optimized utilizing oxalic acid. To guarantee that all IonPac CS5 Analytical Columns meet high quality and reproducible performance specification standards, all columns undergo the following production control test.

Sample Loop Volume: 50 µL
Analytical Column: IonPac CS5 Analytical Column
Eluent: 50 mM Oxalic acid/95 mM LiOH (pH 4.8)
Eluent Flow Rate: 1.0 mL/min

Detection: Using a Reagent Delivery Module and Membrane Reactor
Reagent: $4 \times 10^{-4}$ M 4-(2-pyridylazo)resorcinol (PAR) in 3 M ammonium hydroxide and 1 M acetic acid
Reagent Flow Rate: 0.6 mL/min
Detector: VDM-2, Vis at 520 nm
Detector Sensitivity: 0.5 AUFS

![Figure 1: IonPac CS5 Production Test Chromatogram](image)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb$^{2+}$</td>
<td>4.0</td>
</tr>
<tr>
<td>Cu$^{2+}$</td>
<td>0.5</td>
</tr>
<tr>
<td>Cd$^{2+}$</td>
<td>4.0</td>
</tr>
<tr>
<td>Co$^{2+}$</td>
<td>2.0</td>
</tr>
</tbody>
</table>

1 mg/L = 1 ppm
4.3 Separation of Transition Metals using an Oxalate Eluent

Before beginning operation, equilibrate both the analytical column and the guard column (P/N 035370) with oxalate eluent for 15 minutes at 1.0 mL/min. (When switching from PDCA to oxalate eluent, allow 30 minutes for column equilibration.)

Sample Loop Volume: 50 µL
Analytical Column: IonPac CS5 Analytical Column
Eluent: 50 mM Oxalic acid/95 mM LiOH (pH 4.8)
Eluent Flow Rate: 1.0 mL/min
Detection: Using a Reagent Delivery Module and Membrane Reactor
Reagent: 4 x 10^{-4} M 4-(2-pyridylazo)resorcinol (PAR)
3 M ammonium hydroxide and 1 M acetic acid
Reagent Flow Rate: 0.6 mL/min
Detector: VDM-2, Vis at 520 nm
Detector Sensitivity: 0.2 AUFS

![Figure 2](image)

**Oxalate Separation of Transition Metals**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Pb^{2+}</td>
<td>4.0</td>
</tr>
<tr>
<td>2. Cu^{2+}</td>
<td>0.5</td>
</tr>
<tr>
<td>3. Cd^{2+}</td>
<td>4.0</td>
</tr>
<tr>
<td>4. Co^{2+}</td>
<td>2.0</td>
</tr>
<tr>
<td>5. Zn^{2+}</td>
<td>2.0</td>
</tr>
<tr>
<td>6. Ni^{2+}</td>
<td>4.0</td>
</tr>
</tbody>
</table>

1 mg/L = 1 ppm

Chromatography Troubleshooting

A. A decrease in the eluent pH increases the retention of the analytes.

B. Adding sodium sulfate to the eluent decreases the retention of Co^{2+}, Zn^{2+}, and Ni^{2+}, while maintaining most of the resolution of Pb^{2+}, Cu^{2+}, and Cd^{2+}.

C. The efficiency of Pb^{2+} decreases if the PAR flow rate is less than 0.5 mL/min.

D. Fe^{2+} and Fe^{3+} do not elute when using oxalate eluent.

E. Under these chromatographic conditions, Cd^{2+} and Mn^{2+} co-elute.
4.3 Separation of Transition Metals using a PDCA Eluent

Before beginning operation, condition both the analytical column and the guard column (P/N 035370).

A. Pump 100 mM Na₂SO₃ through the column at 1.0 mL/min for about one hour. This removes oxygen from the column.

B. Next, equilibrate the column with nitrogen or helium purged, degassed eluent for at least 5 minutes.

C. Complete the equilibration by injecting the standard. This first injection facilitates the elution of any impurities adsorbed onto the column.

D. To eliminate any oxygen buildup on the column, periodically condition the column with 100 mM sodium sulfite.

NOTE

Oxygen buildup on the column is indicated by a change in the Peak height ratio of Fe²⁺ to Fe³⁺. As oxygen builds up, Fe²⁺ is oxidized to Fe³⁺, resulting in an increase in the Fe³⁺ peak and a decrease in the Fe²⁺ peak.

Sample Loop Volume: 50 µL
Analytical Column: IonPac CS5 Analytical Column
Eluent: 6 mM PDCA/8.5 mM LiOH (pH 4.8)
Eluent Flow Rate: 1.0 mL/min

Detection: Using a Reagent Delivery Module and Membrane Reactor
Reagent:
- 4 x 10⁻⁴ M 4-(2-pyridylazo)resorcinal (PAR)
- 3 M ammonium hydroxide and 1 M acetic acid
Reagent Flow Rate: 0.6 mL/min
Detector: VDM-2, Vis at 520 nm
Detector Sensitivity: 0.2 AUFS

<table>
<thead>
<tr>
<th>Analyte</th>
<th>mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Fe³⁺</td>
<td>1.0</td>
</tr>
<tr>
<td>2. Cu²⁺</td>
<td>1.0</td>
</tr>
<tr>
<td>3. Ni²⁺</td>
<td>3.0</td>
</tr>
<tr>
<td>4. Zn²⁺</td>
<td>4.0</td>
</tr>
<tr>
<td>5. Co²⁺</td>
<td>2.0</td>
</tr>
<tr>
<td>6. Fe²⁺</td>
<td>3.0</td>
</tr>
</tbody>
</table>

1 mg/L = 1 ppm

Figure 3
PDCA Separation of Transition Metals

Chromatogram Troubleshooting

A. An increase in the eluent pH decreases the retention of the analytes.

B. The ratio of k's of Fe³⁺/Cu²⁺ increases if the eluent pH increases.

C. Upon switching from oxalate to PDCA eluent, iron buildup on the column causes a large, off-scale peak to elute.
4.5 Simultaneous Separation of Transition Metals and Lanthanides

Pyridine-2,6-dicarboxylic acid (PDCA) forms stable mono- and divalent anionic complexes with transition metals and stable trivalent complexes with lanthanides. Lanthanides are retained on the column during the elution of the transition metals with the PDCA eluent and then are eluted with an oxalate/diglycolate eluent.

Sample Loop Volume: 50µL
Analytical Column: IonPac CS5 Analytical Column
Eluent:
E1 6 mM PDCA/50 mM Sodium Acetate/50 mM Acetic Acid
E2 100 mM Oxalic Acid/190 mM LiOH
E3 100 mM Diglycolic Acid/190 mM LiOH
E4 18 megohm-cm deionized water
Eluent Flow Rate: 1.0 mL/min
Detection: Using a Reagent Delivery Module and Membrane Reactor
Reagent: 0.2 mM 4-(2-pyridylazo)resorcinal (PAR)/1 M acetic acid/3 M NH₄OH
Reagent Flow Rate: 0.7 mL/min
Detector: VDM-2, Vis at 520 nm
Detector Sensitivity: 0.2 AUFS

Gradient Program

<table>
<thead>
<tr>
<th>Time</th>
<th>%E1</th>
<th>%E2</th>
<th>%E3</th>
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<td>0</td>
<td>40</td>
</tr>
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<td>21</td>
<td>0</td>
<td>60</td>
<td>0</td>
<td>40</td>
</tr>
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<td>21.1</td>
<td>0</td>
<td>80</td>
<td>0</td>
<td>20</td>
</tr>
</tbody>
</table>

1 mg/L = 1 ppm

Figure 4
Simultaneous Separation of Transition Metals and Lanthanides
SECTION 5 - TROUBLESHOOTING GUIDE

The purpose of the Troubleshooting Guide is to help you solve operating problems that may arise while using the IonPac CS5/CG5 column. For more information on problems that originate with the Ion Chromatograph (IC), refer to the Troubleshooting Guide in the appropriate operator’s manual. If you cannot solve the problem on your own, contact the nearest DIONEX Office (see, “DIONEX Worldwide Offices”).

5.1 High Back Pressure

5.1.1 Finding the Source of High System Pressure

Total system pressure for the IonPac CG5 Guard Column plus the CS5 Analytical Column when using the test chromatogram conditions should be equal or less than 1,150 psi (7.92 MPa). If the system pressure is higher than 1,200 psi (8.27 MPa), it is advisable to determine the cause of the high system pressure. The system should be operated with a High-Pressure In-Line Filter (P/N 044105) which is positioned between the pump pressure transducer and the injection valve. Make sure you have one in place and that it is not contaminated.

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

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

To determine which part of the chromatographic system is causing the problem, disconnect the pump eluent line from the injection valve and turn the pump on. Watch the pressure; it should not exceed 50 psi (0.34 MPa). Continue adding system components (injection valve, column(s) and detector) one by one, while monitoring the system pressure. The pressure should increase up to a maximum when the Guard and Analytical columns are connected (see Table 4, “Typical CS5/CG5 Operating Back Pressures”). No other components should add more than 100 psi (0.69 MPa) of pressure. Refer to the appropriate manual for cleanup or replacement of the problem component.

### Table 4

<table>
<thead>
<tr>
<th>Column</th>
<th>Typical Back Pressure</th>
<th>Flow Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS5 Analytical</td>
<td>≤ 850 (5.86)</td>
<td>1.0</td>
</tr>
<tr>
<td>CG5 Guard</td>
<td>≤ 300 (2.06)</td>
<td>1.0</td>
</tr>
<tr>
<td>CS5 + CG5 columns</td>
<td>≤ 1,150 (7.92)</td>
<td>1.0</td>
</tr>
</tbody>
</table>
5.1.2 Replacing Column Bed Support Assemblies

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

A. **Disconnect the column from the system.**

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

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

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

<table>
<thead>
<tr>
<th>Part</th>
<th>IonPac CS5/CG5 (P/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical Column</td>
<td>037028</td>
</tr>
<tr>
<td>Guard Column</td>
<td>037029</td>
</tr>
<tr>
<td>Bed Support Assembly</td>
<td>042955</td>
</tr>
<tr>
<td>End Fitting</td>
<td>052809</td>
</tr>
</tbody>
</table>

**CAUTION**

If the column tube end is not clean when inserted into the end fitting, particulate matter may obstruct a proper seal between the end of the column tube and the bed support assembly. If this is the case, additional tightening may not seal the column but instead damage the column tube or the end fitting.

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

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

**NOTE**

Replace the outlet bed support **ONLY** if high pressure persists after replacement of the inlet fitting.
5.2 High Background or Noise

A. Ground the recorder.
B. Reprime the pump.
C. Clean the inlet and outlet check valves on the pump.
D. Prepare fresh PAR solution. This solution has a lifetime of only 2 weeks.

5.2.1 Preparation of Eluents

A. Make sure that the eluent and the post column reagent are made correctly.
B. Make sure that the eluent and the post column reagent are made from chemicals with the recommended purity.
C. Make sure that the deionized water used to prepare the eluent and post column reagent has a specific resistance of 18.2 megohm-cm.

5.2.2 A Contaminated Guard or Analytical Column

Remove the IonPac CG5 Guard and CS5 Analytical Columns from the system. If the background conductivity decreases, then one (or both) of these columns is (or are) the cause of the high background conductivity, clean the column as instructed in “Column Cleanup” (see, “Column Care”).

5.3 Poor Peak Resolution

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

5.3.1 Loss of Column Efficiency

A. Check to see if headspace has developed in the guard or analytical column. This may be due to improper use of the column such as submitting it to high pressures. Remove the column’s top end fitting (see Section 5.1.2, “Replacing Column Bed Support Assemblies”). If the resin does not fill the column body all the way to the top, it means that the resin bed has collapsed, creating a headspace. The column must be replaced.
B. Extra-column system effects can result in sample band dispersion, decreasing peak efficiencies. Make sure you are using tubing with an ID of no greater than 0.012” to make all eluent liquid line connections between the injection valve and the detector cell inlet. Make all tubing lengths are as short as possible. Check for leaks.
5.3.2 Poor Resolution Due to Shortened Retention Times

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

A. **Check the flowrate.** See if the eluent flow rate is different than the flow rate specified by the analytical protocol. Measure the eluent flow rate after the column using a stopwatch and graduated cylinder.

*Long Retention Time for Co$^{2+}$ is an indication that the eluent flow rate is too low.* Increase the flow rate to 1.0 mL/min.

B. **Check to see if the eluent compositions and concentrations are correct.** For isocratic analysis, an eluent that is too strong will cause the peaks to elute faster. Prepare fresh eluent. If you are using a gradient pump to proportion the final eluent from concentrated eluents in two or three different eluent reservoirs, the composition of the final eluent 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%.

For gradient analysis, remake the eluents or adjust the times in the gradient program to obtain the required peak resolutions.

*Short retention time for Co$^{2+}$ may be due to the eluent pH being too high.* Remake the eluent.

C. **Column contamination can lead to a loss of column capacity.** This is because fewer of the ion exchange sites will be available for the sample ions. Polyvalent anions or metal ions might be concentrating on the column. Refer to “Column Cleanup” (see, “Column Care”), for recommended column cleanup procedures.

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

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

After cleaning the column, reinstall it in the system and let it equilibrate with eluent for about 30 minutes. The column is equilibrated when consecutive injections of the standard give reproducible retention times. The original column capacity should be restored by this treatment, since the contaminants should be eluted from the column. If you need assistance in solving resolution problems, contact the nearest DIONEX Office (see, “DIONEX Worldwide Offices”).

5.3.3 Loss of Front End Resolution

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

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

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

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

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

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

If you need assistance in determining the best way to clean strongly retained solutes in your specific sample matrix from the IonPac CS5 columns, contact the nearest DIONEX Office (see, “DIONEX Worldwide Offices”).

B. The detector may be adjusted to the wrong wavelength. If so, large, irregular peaks may appear immediately after injection. Make sure the detector is set to 520 nm.

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

If cleaning and retorquing the valve does not help, replace the valve. Use a DIONEX High Pressure Injection Valve (P/N 037142) or a DIONEX High Pressure Inert Valve (P/N 037143) as required.

For DX-300 systems equipped with a Rheodyne Microinjection Valve, Model 9126 (DIONEX P/N 044697), consult the accompanying manual for service instructions.
IonPac® CS5
Analytical (4 x 250 mm)
Product No. 37028

Serial No. : 8904  Pressure (PSI) : 620  Date : 11/21/00 2:14:27 PM

Eluent:  50.0 mM Oxalic acid, pH 4.80 w/LiOH

Eluent Flow Rate:  1.0 mL/min

Post-Column Reagent:  4x10^-4 M 4-(2-pyridylazo) resorcinol on 3.0 M NH₄OH/1.0 M CH₃COOH

PCR Flow Rate:  0.5 mL/min.

Detection:  Absorbance at 520 nm, 0.5 AUFS

Injection Volume:  50 µL

Storage Solution:  0.1 M NaOH

Peak Information : Found Components

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<th>Peak No.</th>
<th>Retention Time</th>
<th>Name</th>
<th>Efficiency</th>
<th>Asymmetry (10%)</th>
<th>Resolution</th>
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</thead>
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<td>733</td>
<td>3.7</td>
</tr>
<tr>
<td>2</td>
<td>2.94</td>
<td>Copper</td>
<td>0.5</td>
<td>3728</td>
<td>1.9</td>
</tr>
<tr>
<td>3</td>
<td>4.88</td>
<td>Cadmium</td>
<td>4.0</td>
<td>2355</td>
<td>1.6</td>
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<tr>
<td>4</td>
<td>7.56</td>
<td>Cobalt</td>
<td>1.0</td>
<td>3598</td>
<td>1.6</td>
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</tbody>
</table>

File Name : C:\PEAKNET\DATA\EXAMPLES\37028 CS5 4MM_A012.DXD
COLUMN CARE

Recommended Operation Pressures

Operating a column above its recommended pressure limit can cause irreversible loss of column performance. The maximum recommended operating pressure for IonPac CS5 columns is 4,000 psi (27.57 MPa).

Column Start-Up

The column is shipped in 0.5 M NaOH storage solution.

Prepare the eluent shown on the test chromatogram, install the column in the chromatography module and test the column performance under the conditions described in the test chromatogram. Continue making injections of the test standard until consecutive injections of the standard give reproducible retention times. Equilibration is complete when consecutive injections of the standard give reproducible retention times.

Column Storage

For short-term storage, the strongest eluent in use can be used as the storage solution. For long-term storage, 0.5 M NaOH should be used as the storage solution. Flush the column for a minimum of 10 minutes with the storage solution. Cap both ends securely, using the plugs supplied with the column.

Column Cleanup

Always ensure that the cleanup protocol used does not switch between eluents which may create high pressure eluent interface zones in the column. High pressure zones can disrupt the uniformity of the packing of the column bed and irreversibly damage the performance of the column. High pressure zones in the column can be created by pumping successive eluents through the column that are not miscible, that have eluent components in one eluent that will precipitate out in the other eluent or by using an acid eluent followed by a base eluent with may create a neutralization pressure band.

When in doubt, always include short column rinse steps to reduce the ionic strength of the eluent to 50 mM levels to avoid creating high pressure zones in the column that may disrupt the uniformity of the column packing.

CAUTION
Eluents with greater than 5% solvent will damage the column.

Choosing the Appropriate Cleanup Solution

A. A 10X concentrate of the most concentrated PDCA or oxalate eluent used in the application will remove most metals. Iron will not be eluted with oxalate eluents.

B. Concentrated acid solutions such as 1 to 3 M HCl remove a variety of metals including iron.

C. The IonPac CS5 can only tolerate up to 2% solvent in the eluent. Organic solvents can be used alone if the contamination is nonionic and hydrophobic.
Column Cleanup Procedure

A. **Prepare a 500 mL solution of cleanup solution.** Use the guidelines in Section 6.5, "Choosing the Appropriate Cleanup Solution."

B. **Disconnect the analytical column from the Membrane Reactor.** If your system is configured with both a guard column and an analytical column, reverse the order of the guard and analytical column in the eluent flow path. Double check that the eluent flows in the direction designated on each of the column labels. Direct the effluent from the outlet line of the CG5 Guard Column to a separate waste container.

    **CAUTION**
    When cleaning an analytical column and a guard column in series, ensure that the guard column is placed after the analytical column in the eluent flow path. Contaminants that have accumulated on the guard column can be eluted onto the analytical column and irreversibly damage it. If in doubt, clean each column separately.

C. **Set the pump flow rate to 2.0 mL/min.**

    If your eluent contains a solvent or a salt that is not compatible with the chosen cleanup solution, rinse the column for 15 minutes with deionized water before pumping the cleanup solution over the column.

D. **Pump the cleanup solution through the column for 60 minutes.**

    If your cleanup solution contains a solvent or a salt that is not compatible with the eluent, rinse the column for 15 minutes with deionized water before pumping eluent over the column.

E. **Reconnect the analytical column to the Membrane Reactor.** Place the CG5 Guard Column in line between the injection valve and the analytical column if your system was originally configured with a guard column.

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