TRANSGENOMIC

transforming the world

2032 Concourse Drive San Jose, CA 95131
Re-order Phone: 888-813-7253 Tech Support Phone: 800-605-0267
Re-order Fax: 402-452-5401 Tech Support Fax: 408-432-3231

USER GUIDE

ICSep CN2
CATION/TRANSITION METAL
ANALYSIS COLUMN

CATALOG NO. CTX-99-5250

WARNING. THE TRANSGENOMIC ICSEP CN2 CATION/TRANSITION METAL ANALYSIS COLUMN IS PACKED WITH A POLYMERIC MATERIAL THAT REQUIRES SPECIAL CARE. INTRODUCTION OF ORGANIC SOLVENTS INTO THE COLUMN EXCEPT AS DESCRIBED BELOW WILL CAUSE THE POLYMER TO SWELL AND THE COLUMN WILL OVERPRESSURE. CONSEQUENTLY, PRIOR TO COLUMN INSTALLATION, YOU SHOULD FAMILIARIZE YOURSELF WITH THE CONTENTS OF THIS USER GUIDE. IMPROPER USE WILL INVALIDATE THE WARRANTY. IF YOU HAVE ANY QUESTIONS AFTER READING THIS MANUAL, PLEASE CALL OUR APPLICATIONS LAB PRIOR TO USE OF THE COLUMN.

DESCRIPTION

The Transgenomic metals column contains a .32 x 10-cm bed of Macronex MC210 5 μm cation-exchange polymer with a nominal capacity of 500 μeqv/g. This unique polymer permits separation of common mono- and divalent cations as well as many transition metals. The hydrophilic polymer is stable from pH 0 to 14 and is compatible with virtually any aqueous mobile phase. The CN2 is designed for single column applications. The column can be used for determination of common mono- and divalent cations (PART ONE) or for detection of transition metals (PART TWO). The following sections deal with general column use and apply to either application.

PRE-COLUMN FILTER

Pre-column filters containing 0.5-2.0 μm porosity passivated stainless steel or titanium frits should be used between the sample injector and the column to remove particulates from the mobile phase stream. This will help prevent excessive pressure through the analytical column and will prolong column life.

GUARD COLUMNS

Guard columns should be used with your polymeric column because sample and mobile phase contamination can result in excessive pressures. Contaminants such as salts and proteins can alter column performance and should always be removed from samples prior to injection onto the column. We recommend a Transgenomic ICSepCN2 Guard (cat. no. CTX-99-2050). This guard column contains a cartridge packed with a similar polymer used in the ICSep CN2 analytical column. Cartridge replacement is required when increased column pressure and/or loss of resolution is observed. Replacement cartridges are available (cat. no. CTX-99-1350).

SAMPLE PREPARATION

The key to long column life is proper treatment of sample prior to injection onto the polymer bed. You should avoid introduction into the column of fats, oils, proteinaceous materials, and particulates that may originate in either mobile phases or samples. These will ultimately cause an increase in operating pressure and can be difficult or impossible to remove. Numerous methods of sample purification are in the literature; but sample preparation schemes such as those employing solid phase extraction tubes, e.g., Transgenomic POLYSorb, work well. If you do not have a particular scheme, we suggest centrifugation followed by membrane filtration of your samples.

Note that biological samples should be de-proteinized before injection. The preferred de-proteinizing agent is sulfosalicylic acid. Prepared samples should match the mobile phase matrix whenever possible.

SAMPLE VOLUME

The low bed volume of the CN2 column enhances sensitivity and reduces analysis time. The column will not tolerate large injection volumes. You must determine empirically the maximum injection volume tolerated t the column for your particular sample. Generally, no problems occur with sample volumes of 50 µl or less. Injections of 100 µL or more can cause peaks to broaden or merge with nearby peaks.

PART ONE: DETERMINATION OF MONO- AND DIVALET CATIONS

The CN2 column can be used for determination of mono- and divalent cations. The following sections describe mobile phases and detectors used for such applications with typical chromatograms shown:

MOBILE PHASE

The column is shipped equilibrated with 3.2-m*M* ethylenediamine and 10 m*M* citric acid. The preferred mobile phase for cation separation is cerium (III). To convert the column to the cerium ionic form, follow these steps: Pump 10-m*M* cerium (III) sulfate through the column at 1.0 mL/min for an hour. Then pump the desired concentration of cerium sulfate through the column until the baseline stabilizes (about 0.5 hour).

Cerium (III) Sulfate

Prepare the mobile phase as follows dissolve 7.125 g Ce (III) sulfate octahydrate 99.99% (Aldrich Chemical Company, Milwaukee, WI or equivalent) in one liter de-ionized, distilled water to make the 10 m*M* stock solution. Filter through a 0.45 µm membrane and store in a clean, brown glass bottle.

For general use, we recommend using a 0.1-m*M* Ce (III) solution that can be prepared by diluting 10 mL stock solution into one liter of high purity water, then filtering. Sodium determination can be achieved using 0.05 m*M* Ce (III) at the cost of increased k' values for other ions (Sherman, J and Danielson, W., *Anal. Chem*. 59, 490 (1987)).

DETECTION

A variety of detectors can be used with the CN2 column. The original work in ion chromatography was performed with conductivity detectors. Electrochemical detectors were used for readily oxidized ions. UV detectors have been used in the Indirect

Photometric Chromatography method (Small, H., and Miller, T.E. Jr., *Anal. Chem*. 54 462 (1982)).

Detection sensitivity of ions is determined by several factors, the most important of which is type of detector utilized. Manufacturers' specifications for each type of detectors vary, so some models may provide more sensitivity than others. All other factors being equal, UV detectors using the indirect photometric chromatography technique can detect in the low *ppb* range.

Electrochemical detectors provide the highest sensitivity available for oxidizable species. Detection in low *ppb* range is common with these detectors. Sample concentration will, of course, enhance sensitivity of any detector.

Note: Use of ultraviolet detector for determination of these ions (indirect photometric chromatography) is a method patented by The Dow Chemical company in the United States and other countries. Transgenomic customers wishing to practice the method should contact Dow Chemical Company, 2030 W.H. Dow Center, Midland, MI 48640 for a license to practice under the patent.

PART TWO: DETECTION OF TRANSITION METALS

The CN2 column can be used for separation of transition metals. The following sections describe mobile phases and detectors used for such applications:

MOBILE PHASES

According to Sevenich and Fritz (1983, *Anal. Chem.*, 55, 12), selectivity and peak sharpness are enhanced by utilizing mobile phases containing complexing agents. Mobile phases are composed of two parts. The first is the ion-exchange component; the second, the complexing component. Optimization of these components for your particular sample is required.

Although metal ions exist in solution primarily as cations, partial complexation will permit rapid separations that would not otherwise occur. Ionic strength of mobile phase should be kept low if conductivity detection is used. Greater flexibility in mobile phase selection with concomitant improvement is selectivity is possible with photometric detection involving post column reaction with 4-(2-pyridylazo)-resorcinol (PAR) reagent to form a colored complex that absorbs at visible wavelengths.

MOBILE PHASE FOR CONDUCTIVITY DETECTION

The stock solutions are 35 m*M* ethylenediamine (EDA) and 100 m*M* citric acid. Citric acid stock: dissolve 19.2 g reagent grade citric acid and dilute to one liter with distilled water. Filter through 0.45 μm membrane.

The mobile phase is prepared by combining 100 mL each EDA and citric acid stock solutions and diluting to one liter with distilled water. This is filtered through 0.45 μm membrane and degassed prior to use. This solution is 3.5 m*M* EDA and 10 m*M* citric acid.

MOBILE PHASES FOR PHOTOMETRIC DETECTION

The stock solutions are 35 m*M* EDA and 100 m*M* citric acid prepared as described above. Mobile phase A is 66 mL of EDA stock solution plus 100 mL of citric acid stock solution diluted to one liter with distilled water. This mobile phase is 32.3 m*M* EDA and 10 m*M* citric acid. Mobile phase B is 100 mL each of EDA and citric acid stock solutions diluted to one liter with distilled water. The mobile phase is 3.5 m*M* EDA and 10 m*M* citric acid. Both mobile phases should be filtered through 0.45 μm filter and degassed prior to use.

POST COLUMN REACTION WITH PAR REAGENT

Prepare the PAR reagent by combining 0.026 g 4-(2-pyridylzo)-resorcinol monosodium monohydrate, 77.08 g ammonium acetate, 117 mL concentrated ammonium hydroxide and dilute to one liter with distilled water. This solution is filtered through 0.45 μm membrane and degassed prior to use.

The post column system for determination of transition metals with PAR requires a pump for delivering PAR reagent, a mixing tee and a specrophotometer capable of monitoring 520 *nm*. Higher detection sensitivities are possible if the PAR reagent is delivered pulse-free. The PAR reagent reacts with metals rapidly at room temperature. Flow rate of the PAR reagent should equal that of the analytical pump.

MOBILE PHASE FLOW RATE

The recommended mobile phase flow rates for the Transgenomic ICSep CN2 column are 0.1-1.0 ml/min. Do not exceed 2. mL/min. High flow rates accelerate analysis at the expense of resolution; lower flow rates result in improved resolution but slightly longer analysis time. Maximum column separation efficiencies are achieved at the lowest flow rates.

MOBILE PHASE FLOW DIRECTION

An arrow may appear on the column body. This arrow is for reference purposes only and indicates the flow direction used during testing. The column can be operated with mobile phase flowing in either direction.

COLUMN TEMPERATURE

The ICSep CN2 column can be used between 20° - 90° C. A particular characteristic of the packing material is the reduced sample retention, higher separation efficiency, and lower column pressure when used in conjunction with a column heater. Since column temperature influences sample retention it must be carefully controlled to ensure repeatable results. Certain separations are particularly sensitive to temperature, so it may be necessary to carefully manipulate your column heating device in order to optimize separation. If it is necessary to use the column at room temperature, eluent flow rate should be adjusted to keep pressure below 150 atm (2200 psi).

NOTE. When the column is used above 80° C, care must be taken in disposing of column waste mobile phase. Acids, even at low concentration, are much more corrosive at high temperatures than at room temperature. Severe burns may occur if hot column waste mobile phase comes in contact with skin.

COLUMN PRESSURE

Remember that the pump pressure required to deliver mobile phase through the column is a *consequence* of mobile phase flow rate, column temperature, mobile phase viscosity, etc. The maximum recommended column pressure is 150 atm (2200 psi). Under normal operating conditions, a flow rate of 1.0 mL/min should not require pump pressures greater than 150 atm (2200 psi). If high pressures result from use of the column at normal flow rates, this usually indicates that some contaminants have become deposited on the packing material and corrective action must be taken (see TROUBLE SHOOTING). To prevent irreversible damage to the column; however, you must exercise care in preparing mobile phases and samples.

COLUMN STORAGE

The column as supplied is equilibrated with 3.5 m*M* ethylenediamine and 10 m*M* citric acid. This is also the recommended mobile phase for storage. Retain the compression nuts used to seal the column end fittings. These should be used to seal the column when it is disconnected from the liquid chromatograph. This is necessary to prevent the polymeric packing material from drying.

POSSIBLE CAUSES OF PERFORMANCE LOSS IN THE TRANSGENOMIC ICSEP CN2 CATION/TRANSITION METALS COLUMN

The following outline is intended as an aid in locating sources of performance loss. Because of the nature of polymeric materials and the manufacturing procedures employed by Transgenomic, it is highly unusual for a column to lose performance due to manufacturing problems. In our experience, nearly all column failures are a result of the

introduction of contaminants onto the polymer bed. All Transgenomic columns are thoroughly tested prior to shipment and are supplied with a sample chromatogram illustrating performance of that particular column. Due to the nature of polymeric materials, column lifetime should be long and column regeneration unnecessary.

1. Post-column mixing and /or diffusion-keep tubing length and I.D. to a minimum

2. Improper column temperature

3. Improper mobile phase flow rate

4. Insufficient equilibration time with mobile phase

5. Improper pH or ionic strength of mobile phase

6. Improper mobile phase cation (e.g. use of K^+ mobile phase when column equilibrated with EDA)

7. Polymer contamination
   a. High column pressure accompanies performance loss
      (1) particulate accumulation on inlet frit or polymer bed
         (a) sample origin – filter or centrifuge samples
         (b) mobile phase origin – filter mobile phase; enclose mobile phase reservoirs
         (c) system origin – flush all lines and pump; install in-line filter system

      (2) proteinaceous material accumulation
         (a) microbial growth in samples
         (b) microbial growth in mobile phase

   b. Normal column pressure accompanies performance loss
      (1) heavy metal ion contamination
         (a) inappropriate steel alloy present in LC system
         (b) excessive halide containing mobile phase
         (c) mobile phase contaminated with heavy metal ions during preparation or transfer

      (1) organic contamination
         (a) fats, oils, lipids in sample – polymer surface becomes coated
         (b) non-specific organics from improperly prepared mobile phase or source material
         (c) non-specific organics introduced into mobile phase after preparation (e.g. from atmosphere, during transfer, etc.)

8. Bed compression (voids)
   a. Excessive mobile phase flow rate
   b. Use of inappropriate organic modifier or excessive concentration of modifier.

OPERATIONS DESIGNED TO CORRECT PERFORMANCE LOSSES RESULTING FROM POLYMER CONTAMINATION OR BED COMPRESSION

The procedures outlined below will in some cases restore performance by removing contaminants from the polymer bed. It is important, however, to attempt to locate the source of the problem before again using a column for analysis of samples.

1. **Prepare Fresh Mobile Phase**. In some cases performance loss is traced to mobile phase contamination. Therefore, prepare fresh mobile phase and flush all liquid lines before using column; mobile phase should be filtered through 0.2 – 0.45 μm membranes prior to use.

2. **Invert Column**. If performance problems persist, column should be inverted and operated under standard analytical conditions. If performance returns to normal, continue operation in this configuration. If performance does not improve, polymer may be permanently contaminated and column may required replacement.

3. **Column Regeneration**. Pump de-ionized water through the column for 20 minutes at 0.7 mL/min. Then pump 0.01 *N* NaOH through column for 1 hour at same flow rate. Pump de-ionized water through the column for 20 minutes. If contamination by fats, oils, or similar organics is suspected, pump 80% ACN/water for 1 hr, followed by de-ionized water for 30 minutes at 0.3 mL/min. Adjust flow rate if necessary to keep pressure below 150 atm (2200 psi)

   If using Ce (III) mobile phases, re-equilibrate the column as described under "Mobile Phases" using 10 m*M* Ce (III) sulfate solution. Then convert to desired cerium concentration.

   If using EDA/citrate mobile phases, re-equilibrate with 3.5 m*M* ethylenediamine plus 10 m*M* citric acid.

4. **Column Checking**. Return column temperature to normal. Operate column in normal analytical mode with normal mobile phase, but in the inverted position. If performance does not return, orient column in normal direction and repeat. Note that it may take some time for the baseline to stabilize.

5. **Column Replacement**. Above procedure will restore performance only in certain cases. Heavy metal contamination and certain organic contaminants are particularly refractory and may not respond to treatment. Under these circumstances, column replacement is necessary. It is highly advisable to locate the cause of the problem before installing a new column. Consult manufacturer of your LC system for aid in this matter.

COLUMN LIFETIME

To extend column lifetime, please keep in mind the following:

1. All mobile phases should be freshly made, filtered through a 0.2 – 0.45 μm membrane and degassed.

2. Keep flow rate in the range of 0.1 – 1.0 mL/min. Do not exceed 2.0 mL/min.

3. Use recommended guard column and in line pre-column filter.

4. Adjust flow rate to keep column pressure below 170 atm (2500 psi).

5. When the column is not to be used for extended periods, flush with 3.5 m*M* ethylenediamine and 10 m*M* citric acid. Use this mobile phase as the storage liquid.

6. Filter samples through 0.2 – 0.45 μm membrane before injection.

7. Use analytical grade or better reagents and HPLC grade solvent for all work. Discard any solutions that show evidence of bacterial growth.