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USER GUIDE

ICSep ION-120
ANION ANALYSIS COLUMN

CATALOG NO. ANX-99-6550

WARNING. THE TRANSGENOMIC™ ICSEP ANION 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 ICSep ION-120 anion chromatography column is intended for single column applications. "Suppressor" devices (post-column modification of mobile phases) are not required. The 0.46 x 12-cm bed contains a macroporous polymeric anion exchange material with capacity of approximately 100 μeqv/g. The column frits are titanium. The ICSep ION-120 column has been specifically configured for analyzing samples containing low levels of one ion in the presence of high levels of another, or for analyzing particularly complex samples. The higher capacity of the ICSep ION-120 permits analysis of samples intractable on many other ion chromatography columns

The polymer used in the ICSep ION-120 is stable from pH 0 to 14. Consequently, virtually any aqueous mobile phase (except strong oxidants) can be used without fear of damaging the column material. It may be inappropriate; however, to use very low pH acids such as 1 N HCl, as these may attack certain steel alloys in your chromatograph. Thus, column degradation should not be expected when using samples of high pH or mobile phases having high pH values.

PRE-COLUMN FILTER

Pre-column filters containing 0.5-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 an Transgenomic™ ICSep ION-120 Guard (cat. no. ANX-99-2350). This holder contains a cartridge packed with a similar polymer used in the ICSep ION-120 analytical column. Cartridge replacement is required when increased column pressure and/or loss of resolution is observed. Replacement cartridges are available (cat. no. ANX-99-0090).

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 ICSEP ION-120 column enhances sensitivity and reduces analysis time of common anions. The column will not tolerate large injection volumes. You must determine empirically the maximum injection volume for your particular sample. Generally, no problems occur with sample volumes of 200 µl or less. Injections of 200 µL or more can cause peaks to broaden or merge with nearby peaks. If the injection volume is too great, fluoride merges with excluded ions and will not be observed as a distinct peak. This problem can sometimes be corrected by adjusting other chromatographic parameters. For example, lowering mobile phase pH or ionic strength results in longer retention of fluoride and may permit successful separation.

MOBILE PHASES

Each ICSEP ION-120 column is individually tested for separation of fluoride, chloride, nitrite, nitrate, bromide, phosphate and sulfate. The chromatogram illustrating performance is provided. Salicylic acid mobile phase systems are most commonly used with this column. Several suggested formulations are summarized below.

4 mM Salicylate, pH 7.8

Weigh 0.276 g salicylic acid (99% and place in a one liter Erlenmeyer flask. Add 5 mL methanol (HPLC) grade. Swirl flask for several minutes until acid is dissolved. Weigh 0.320 g sodium salicylate (99%) and add to flask. Add 950 mL distilled water to flask. Stir solution with magnetic stirrer for 10 minutes, taking care not to draw air into solution while mixing. Transfer solution to a one-liter beaker. While stirring the solution, adjust pH to 7.80 by adding drop wise, a 5% solution of tris(hydroxymethyl)aminomethane (99%). Prepare Tris solution by dissolving 1.25 g Tris in 25 mL distilled water. Transfer the pH 7.8 solution into a one-liter volumetric flask and add distilled water to the mark. Mix well. This soulut9ion is the final mobile phase, 4 mM salicylate at pH 7.8, adjusted with Tris.

4 mM Salicylic Acid

Weigh 0.552 g salicylic acid (99%) and place in a one-liter Erlenmeyer flask. Add 5 mL methanol (HPLC grade). Swirl flask for several minutes until acid is dissolved. Add 950

mL distilled water to flask. Stir solution on a magnetic stirrer for 10 minutes. Transfer the solution into a one-liter volumetric flask and add distilled water to the mark. Mix well. This solution is 4 mM salicylic acid.

4 mM Sodium Salicylate

Weigh 0.640 g sodium salicylate (99%) and place in a one-liter Erlenmeyer flask. Add 900 mL distilled water to the flask and stir for 10 minutes. Transfer the solution to a one-liter volumetric flask and add distilled water to the mark. Mix well. This solution is 4 mM sodium salicylate

Selectivity

Changes in concentration of salicylate mobile phase affect retention of multivalent anions more severely than monovalent anions. As an example, sulfate retention more than doubles with a decrease in salicylate mobile phase concentration from 3 to 2 mM. Nitrate retention increases by only 50% with the same change in mobile phase concentration.

Separation selectivity for common anions can be altered by changes in mobile phase pH. Lowering the pH of a salicylate mobile phase from 7.8 to 2.7 causes a decrease in relative phosphate retention and a large increase in sulfate retention.

Note: All solutions must be prepared fresh daily. They should be protected from excessive exposure to light and air. Pas mobile phase through a 0.45 um nylon membrane filter if necessary. Dissolved CO2 in mobile phases is particularly undesirable. Growth of microorganisms could lead to plugged column frits, resulting in high column pressures.

DETECTION

A variety of detectors can be used with the ICSep ION-120 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)).

Carbonate interference is more pronounced with UV detectors. The sensitivity of carbonate relative to other peaks with indirect photometric chromatography is increased approximately ten times over conductivity detection. Carbonate displays relatively low conductivity.

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 may. All other

factors being equal, UV detectors using the indirect photometric chromatography technique can detect in the 1 – 10 ppb range without sample concentration.

Conductivity detectors generally exhibit slightly less sensitivity; typically, detection is possible only at the very low PPM 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.

MOBILE PHASE FLOW RATE

The recommended mobile phase flow rates for the Transgenomic™ ICSep ION-120 column are 0.1 - 1.5 ml/min. Do not exceed 2.0 mL/min. Most separations can be satisfactorily achieved at 1.0 mL/min. High flow rates accelerate analysis at the expense of resolution; lower flow rate 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 ION-120 column can be used at ambient or elevated temperatures. Column efficiency has been observed to vary slightly with changes in column temperature. Retention of common anions has been observed to increase with an increase in column temperature. Pronounced increase in retention has been observed for multivalent anions such as sulfate and phosphate at elevated temperatures. Elevated temperature results in lower column pressure due to decreased mobile phase viscosity.

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 1.3 mL/min at ambient temperature should not require pump pressures greater than 100 atm (1500 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 4.0 mM salicylate, pH 7.8. This is also the recommended eluent 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 ION120 ANION ANALYSIS 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 anion (e.g. use of carbonate mobile phase instead of salicylate mobile phases)

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) 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 organic modifier (not recommended)

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. **Invert Column**. Invert the column and pump 0.1 M EDTA through it for 60 minutes at 1.0 mL/min. Adjust the flow rate if necessary to keep the pump pressure below 150 atm (2200 psi). Pump de-ionized water through the column for 30 minutes at 1.0 mL/min. Then pump 0.05 N H2SO4 for 60 minutes at 1.0 mL/min.

   Equilibration. Re-orient column in normal direction and equilibrate in your normal mobile phase at normal flow rates until the baseline stabilizes. In some cases, it may take several hours of equilibration before the baseline stabilizes. If the column does not return to normal performance, remove the column end fitting from the original inlet and examine the packed bed. If the polymer bed is discolored, contaminants have been introduce that may be responsible for the column's failure. It may be necessary to replace the column.

2. **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.

2. Keep flow rate in the range of 0.1 – 1.5 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 150 atm (2200 psi).

5. When the column is not to be used for extended periods, flush with 4 m*M* salicylate, pH 7.8. 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.