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USER GUIDE

ICSep ICE-COREGEL 64H
ORGANIC ACIDS COLUMN

CATALOG NO. ICE-99-9860

WARNING. THE TRANSGENOMIC ICSEP ICE-COREGEL 64H COLUMN IS PACKED WITH A POLYMERIC MATERIAL THAT REQUIRES SPECIAL CARE. INTRODUCTION OF ORGANIC SOLVENTS INTO THE ANALYTICAL 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 MANUAL. 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 ICE COREGEL-64H organic acids column contains a 0.78 x 30-cm bed packed with a cation-exchange resin in the hydrogen form. It is designed specifically for separating weak organic and inorganic acids that are contained in wine, fruit juices, dairy products, physiological samples, industrial formulations and environmental samples. Only an aqueous mobile phase is required to achieve separation of a variety of acids. The primary mechanism for separation of these acids is ion exclusion, although other types of interactions such as steric exclusion and partitioning have been observed. The column's physical size (3/8 in. O.D. x 30-cm) is compatible with most commercially available heaters found in modular and dedicated liquid chromatographs.

PRE-COLUMN FILTER

Pre-column filters containing 0.5 – 2.0 micron 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 from developing 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 column 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 ICSep ICE-COREGEL 64H Guard Kit (cat. no. ICE-99-2361). This holder contains a cartridge packed with the same polymer used in the ICSep ICE-COREGEL-64H analytical column. Cartridge replacement is required when increased column pressure and/or loss of resolution is observed. Replacement cartridges are available (cat. no. ICE-99-2371). Silica guard columns are <u>not</u> recommended due to degradation and eventual leakage into the analytical column.

SAMPLE PREPARATION

Properly prepared samples will extend column lifetime. Avoid introduction into the column of fats, oils, proteinaceous materials, heavy metal ions 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's Polysorb, work well. If you do not have a particular scheme, we suggest centrifugation followed by membrane

filtration of your samples. Biological samples should be deproteinized before injection. The preferred deproteinizing agent is sulfosalicylic acid. Prepared samples should match the mobile phase composition whenever possible.

SAMPLE VOLUME

Ion-exclusion and steric-exclusion separation modes require small sample volumes to produce the highest separation efficiencies. We recommend sample volumes in the 10-50 µL range. Injection of 100 µL or more can cause peaks to broaden or merge with nearly peaks.

MOBILE PHASES

The recommended mobile phase is dilute sulfuric acid at a concentration between 0.001 and 0.05 *N*. Most analyses can be successfully achieved with 0.01 *N* H_2SO_4 (0.29 mL concentrated sulfuric acid per liter, approximate pH 2.1). Other strong acids, such as perchloric and nitric, can be used; but halide-containing acids, such as hydrochloric acid, are not recommended due to their corrosive effect on stainless steel. Mobile phases, which contain cations other than H^+, will damage the column.

When strong acids are used as mobile phases, the column is self-regenerating; therefore, special regeneration steps are not usually required. Stronger mobile phases usually increase retention times for most weak organic acids.

The use of an organic modifier is not recommended. Once an organic modifier is introduced onto the column, the column retains the chromatographic characteristics of the mobile phase with modifier and will not perform as originally shipped even if the mobile phase has been returned to the original 100% aqueous composition. Consequently, if your application involves aromatic or unsaturated acids that exhibit long retention times, we recommend use of an Transgenomic ICSep ICE-COREGEL-87H1 (0.78 x 10-cm) column (cat. no ICE-99-5861) which is especially designed for those compounds.

All mobile phases should be filtered through 0.45 µm membranes and degassed prior to use. To avoid problems associated with bubble formation in detector flow cells; it is good laboratory practice to thoroughly degas your mobile phase daily. This is particularly important when optimum separation requires high column temperatures. In these circumstances, it is advised to purge your mobile phase with helium.

MOBILE PHASE FLOW RATE

Recommended mobile phase flow rates for the ICSep ICE-COREGEL 64H column are 0.1-1.0 mL/min. Do not exceed 1.0 mL/min. High flow rates accelerate analysis at the expense of resolution; lower flow rate results 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 PRESSURE

Remember that the pump pressure required to deliver mobile phases through the column is a ***consequence*** of mobile phase flow rate, column temperature, mobile phase viscosity, *etc*. The maximum recommended column pressure is 70 atm (1000 psi). Under normal operating conditions, a flow rate of 1.0 mL/min at 35° C should not require pump pressures greater than 70 atm (1000 psi). If high pressures result from use of the column at normal flow rates, this usually indicates that some contaminants have became 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 TEMPERATURE

The Transgenomic ICSep ICE-COREGEL-64H column can be used at ambient temperature and up to 90° C. In general, higher column temperatures result in reduced sample retention, higher separation efficiency, and lower column pressure. Since column temperature influences sample retention, it must be carefully controlled to ensure repeatable results. If it is necessary to use the column at room temperature, mobile phase flow rate should be adjusted to keep pressure below 70 atm (1000 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.

DETECTION AND SENSITIVTY

A variety of detectors can be used with the ICSep-ICE-COREGEL-64H column. The simplicity of the mobile phase and requirement of only isocratic conditions enable chromatographers to choose among spectrophotometers, refractometers, conductivity detectors, and electrochemical detectors. A limiting factor when using conductivity detectors is background conductivity of the mobile phase. The recommended mobile phase concentrations for conductivity detection are 0.001 to 0.005 *N*. If electrochemical

detectors are used, note that high temperature may be incompatible with some working electrodes. Electrode selection should be made with this limitation in mind.

COLUMN STORAGE

The column as supplied is equilibrated with 0.001 *N* sulfuric 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-COREGEL-64H HYDROGEN FORM COLUMN

The following outline is intended as an aid in locating sources of performance loss. 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. In the unlikely event of performance loss, the following outline is intended as an aid in locating the potential source.

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 Na+ instead of H+ mobile phase)

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 sample
         (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) metal ion contamination
   (a) inappropriate steel alloy present in LC system
   (b) halide containing mobile phase
   (c) mobile phase contaminated with metal ions during preparation or transfer
(2) 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 problem before again using a column for analysis of samples.

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

2. **"Loosening" the Polymer Bed**. Many polymers lack the rigidity associated with silica materials, and can compress or collapse if inappropriately high mobile phase flow rates are used. Polymers are resilient, and compression is reversible except in severe cases. To correct collapsed beds, shut off the pump and allow the polymer to "relax" for approximately 30 minutes. Invert column and pump mobile phase at 0.1mL/min overnight at 65° C. Return column to normal operating conditions.

3. **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 require replacement.

4. **Column Cleaning and Regeneration**. Prepare 0.05 *N* H_2SO_4. Set column temperature to 65° C and pump acid solution through (inverted) column at normal mobile phase flow rates. Watch column pressure carefully to guard against overpressure. Do not allow pressure to exceed 70 atm (1000 psi); adjust flow rate

if necessary. Pump this solution through the column for two hours (overnight for severely contaminated columns).

5. **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.

6. **Column Replacement**. Above procedures 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 1.0 mL/min.

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

4. Adjust flow rate to keep column pressure below 70 atm (1000 psi).

5. When the column is not to be used for extended periods, flush analytical column with 0.001 *N* H_2SO_4. 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 solvents for all work. Discard any solutions that show evidence of bacterial growth.