TRANSGENOMIC

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USER GUIDE

ICSep ICE-ION-300
ORGANIC ACIDS COLUMN

CATALOG NO. ICE-99-9850

WARNING. THE TRANSGENOMIC ICSEP ICE-ION-300 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 THOROUGHLY 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 DEPARTMENT PRIOR TO USE OF THE COLUMN.

DESCRIPTION

The Transgenomic ICSep ICE-ION-300 column is a high performance column designed for separation of a variety of organic species including organic acids, carbohydrates and some alcohols. It is especially useful in separating organic acids found in the Krebs cycle (tricarboxylic acid cycle). Separation of acids, alcohols and carbohydrates contained in wine, fruit juice and other beverages are possible. In addition, the column can be used for separation of similar compounds found in dairy products, physiological fluids, industrial formulations, and environmental samples. The column has other special properties and can be used for separation of certain inorganic ions.

The Transgenomic ICSep ICE-ION-300 column (300 x 7.8 mm) contains a cation-exchange polymer in the hydrogen ionic form. Only an aqueous mobile phase containing dilute acid is required to achieve separation of organic acids. The primary mechanism for separation of acids is ion exclusion. Steric exclusion and partitioning are reported in separating other types of molecules. 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. It is highly recommended that the column be used in conjunction with a column heating device.

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 ICSep-ICE-GC-801 Guard Column (cat. no. ICE-99-2354). This guard column contains a cartridge packed with a similar polymer used in the ICSep ICE-ION-300 analytical column. Cartridge replacement is required when increased column pressure and/or loss of resolution is observed. Replacement cartridges are available (cat. no. ICE-99-2364). Silica guard columns are <u>not</u> recommended due to degradation and eventual leakage into the analytical column.

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, 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 literature; but sample preparation schemes such as those employing solid phase extraction tubes, e.g., Transgenomic POLYSorb^{TM} ACT-1, work well. If you do not have a particular scheme, we suggest centrifugation followed by membrane filtration of your samples.

Special consideration must be made for metabolic studies involving living organisms. Krebs cycle acids must be isolated from the media before injection of sample onto the column. Furthermore, all basic elements of the sample must be thoroughly eliminated from the injectable material. 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

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. Injections of 100 μL or more can cause peaks to broaden or merge with nearby peaks.

MOBILE PHASE

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 0.005 *N* H_2SO_4 (0.145 mL concentrated sulfuric acid per liter). 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 acids. By manipulating mobile phase strength and column temperature, it is possible to achieve excellent separations of mixtures containing both organic acids and carbohydrates.

The use of organic modifiers is not recommended. Once an organic modifier is introduced to 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 acids or unsaturated acids that exhibit long retention times,

we recommend use of an Transgenomic ICSep ICE-ARH-601 column for Aromatic acids. (cat. no. ICE-99-5753) 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

It is good practice to limit mobile phase flow rates such that pump pressure does not exceed 70 atm (1025 psi). The recommended mobile phase flow rates for the Transgenomic ICSep ICE-ION-300 column are 0.1-0.6 mL/min. Do not exceed 0.6 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 ICE-ION-300 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. 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 70 atm (1025 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 70 atm (1025 psi). Under normal operating conditions, a flow rate of 0.4 mL/min at 70° C should not require pump pressures greater than 70 atm (1025 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.

DETECTION AND SENSITIVITY

A variety of detectors can be used with the Transgenomic ICSep ICE-ION-300 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 ICE-ION-300 H+ FORM 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 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 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) metal ion contamination

            (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 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. "**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 the 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 required replacement.

4. **Column Regeneration**. Prepare 0.05 *N* H_2SO_4. Set column temperature to 65°C and pump acid solution through (inverted) column at normal mobile phases flow rates. Watch column pressure carefully to guard against overpressure. Do not allow pressure to exceed 70 atm (1025 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 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 – 0.6 mL/min. Do not exceed 0.6 mL/min.

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

4. Adjust flow rate to keep column pressure below 70 atm (1025 psi).

5. When the column is not to be used for extended periods, flush 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 solvent for all work. Discard any solutions that show evidence of bacterial growth.