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USER GUIDE

ICSep ICE-ORH-801
ORGANIC ACIDS COLUMN

H+ Form

CATALOG NUMBER ICE-99-9754

WARNING. THE TRANSGENOMIC ICSep ICE-ORH-801 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. THE COLUMN WARRANTY IS INVALIDATED IF THIS OCCURS. CONSEQUENTLY, YOU SHOULD THOROUGHLY FLUSH THE LINES OF YOUR LC WITH ISO-PROPANOL FOLLOWED BY DE-IONIZED WATER TO REMOVE ANY ORGANIC SOLVENTS PRIOR TO INSTALLATION OF THIS COLUMN.

YOU SHOULD THOROUGHLY FAMILIARIZE YOURSELF WITH THE CONTENTS OF THIS MANUAL BEFORE USING YOUR COLUMN. IMPROPER USE WILL INVALIDATE THE WARRANTY.

DESCRIPTION

The Transgenomic ICSep ICE-ORH-801 column contains a 0.65 x 30-cm bed packed with a Transgenomic cation - exchange resin in the H^+ ionic form. It is specifically designed for the separation of 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 mode of separation is ion exclusion although steric exclusion and partitioning mechanisms may be involved. Optimum performance is obtained at elevated temperatures; consequently, a column heating device is required. The column's physical dimensions (3/8 in. O.D. x 30-cm) are compatible with most commercially available heaters found in modular and dedicated liquid chromatographs.

MOBILE PHASE

The recommended mobile phase is dilute sulfuric acid at a concentration between 0.0001 and 0.05 *N*. Most analysis can be successfully achieved with 0.01 *N* H_2SO_4 (0.29 mL concentrated sulfuric acid per liter, approximately 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 that contain cations other than H+ will damage the column.

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

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 percent aqueous composition. Consequently, if your application involves aromatic or unsaturated acids that exhibit long retention times, we recommend the use of an Transgenomic ICSep ICE-ARH-601 column (catalog number ICE-99-5753) which is especially designed for those compounds.

All mobile phases should be filtered through 0.45-micron membranes and degassed prior to use. To avoid problems associated with bubble formation in detector flow cells, it is good 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 helium.

MOBILE PHASE FLOW RATE

It is good practice to limit mobile phase flow rates such that pump pressure does not exceed 150 atm (2200). The recommended mobile phase flow rate for the Transgenomic ICSep ICE-ORH-801 is 0.1 - 0.8 mL/min. Do not exceed 0.8 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.

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. Under normal operating conditions, a flow rate of 0.8 mL/min at 35° C should require pump pressures less than 150 atm(2200 psi). It is inadvisable to utilize mobile phase flow rates that produce pump pressures in excess of 150 atm(2200 psi). If high pressures result from use of the column at nominal flow rates, this usually indicates that some contaminants have become deposited on the packing material and corrective action must be taken (see section below on "Causes of Performance Loss"). To prevent irreversible damage to the column, however, you must exercise care in preparing mobile phases and samples. High column pressures nearly always results from improper use of the column. Use of a guard column (see below) will usually prevent contaminants from accumulating on the analytical column.

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 Transgenomic ICSep ICE-ORH-801 column can be used at ambient temperature and with a heating device up to 90° C. A particular characteristic of the packing material is the improved efficiency that results from use of the column at elevated temperatures. However, column temperature also influences sample retention and therefore must be carefully maintained to insure repeatable results. Although temperature can be used to influence certain separations, the temperature range of 85° - 90° C has been determined to be the optimum for the Transgenomic ICSep ICE-ORH-801 hydrogen form column. Temperatures below 80° C can be used for some applications but mobile phase pump pressures may be excessive unless flow rates are reduced. If it is necessary to use the column at lower than recommended temperatures, mobile phase flow rate should be adjusted to keep pump pressures below 150 atm (2200 psi).

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

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 polymeric columns 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 GC-801 Guard Column (cat. no. ICE-99-2354). This holder contains a cartridge packed with the same polymer used in the ICSep ICE-ORH-801 hydrogen form analytical column. Cartridge replacement is required when increased column pressure and/or loss of resolution is observed. Replacement cartridges are available (cat. no. ICSep ICE-99-2364). Silica guard columns are <u>not</u> recommended due to degradation and eventual leakage into the analytical column. Use of guard columns should dramatically extend column lifetime and column cleaning or regeneration (described below) should not be required.

SAMPLE PREPARATION

The key to long column life is proper treatment of sample prior to injection onto the polymer bed. You should avoid introduction of fats, oils, proteinaceous materials and heavy metal ions into the column by either mobile phases or samples. If possible, you should avoid introduction of particulate matter onto the column. These will ultimately cause an increase in operating pressure and may be difficult or impossible to remove.
Alternately, the sample can be cleaned off line using one of the numerous methods of sample purification found in literature. Numerous methods of sample purification can also be found in literature, but sample preparation schemes such as those employing solid phase extraction tubes, e.g., Transgenomic's POLYSorb SPE tubes work quite will. 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 sulfosalycylic acid. Prepared samples should match the mobile phase composition whenever possible

SAMPLE VOLUME

Ion-exclusion and steric exclusions separation modes requires 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.

DETECTION AND SENSITIVTY

The mobile phase requirement for Transgenomic ICSep ICE-ORH-801 column enables chromatographers to use a variety of detectors. The simplicity of the mobile phase and requirement of only isocratic conditions allows spectrophotometers, refractometers and conductivity detectors to be used successfully. 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. Remember that the type of detector chosen ultimately determines sensitivity of detection; the responsibility of the column is simply to separate the compounds of interest.

COLUMN STORAGE

The column as supplied is equilibrated with 0.001 *N* H_2SO_4. This is also the recommended mobile phase for storage. When the column is stored, be sure the end fittings are tightly sealed using the end plugs provided. Long term storage without these seals can result in partial drying of packing material and high pressures can ensue. Under these circumstances invert the column and pump de-ionized water at a flow rate of 0.1 mL/min at 90° C. Gradually increase the flow rate to 0.5 mL/min. Normal pressure should be observed and the column can be used in either direction. If this does not correct the problem, the column may have become contaminated with particulates or other material.

POSSIBLE CAUSES OF PERFORMANCE LOSS IN THE TRANSGENOMIC ICSep ICE-ORH-801 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 introduction of contaminants onto the polymer bed. Use of a guard column will help prevent these problems, as will sample pretreatment (see above). All Transgenomic columns are thoroughly tested prior to shipment and are supplied with a sample chromatogram illustrating performance of the particular column.

1. Post-column mixing and or diffusion – make sure the tubing length and tubing I.D. are kept to a minimum

2. Improper column temperature

3. Improper mobile phase flow rate

4. Insufficient equilibration time with mobile phase

5. Improper mobile phase pH or ionic strength of mobile phase

6. Presence of improper cation in mobile phase – (Na^+ instead of H^+)

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
            (b) mobile phase origin — filter mobile phase; enclose mobile phase reservoirs
            (c) system origin — flush all lines and pump; install in-line system filter

        (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 phases
            (c) mobile phases contaminated with metal ions during preparation or transfer
        (2) organic contamination
            (a) fats, oils, lipids in sample – polymer surface becomes coated
            (b) non-specific organic 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 to 0.45-micron membranes and de-gassed 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 utilized. They are resilient, however, and the 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 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. **Cleaning of Polymer and Regeneration**. If performance problems persist and particularly if high column pressures remain, an attempt should be made to clean the column to remove built up contaminants. Cleaning and regeneration 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 the column for analysis of samples.

   Prepare an aqueous solution of 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. Monitor column backpressure carefully to guard against overpressure. If necessary, adjust mobile phase flow rate such that pressure does not exceed 150 atm. 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 the 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. It should be filtered through a 0.2-0.45 micron membrane and degassed prior to use.

2. The recommended flow rate is 0.1 – 0.8 mL/min. Do not exceed 0.8 mL/min.

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

4. Adjust flow rate to keep column back pressures below 150 atm (2200 psi).

5. When the column is not to be used for extended periods, store equilibrated in 0.001 *N* H_2SO_4

6. Filter samples through 0.2-0.45 micron 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.

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