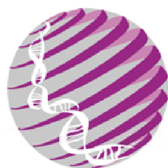

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USER GUIDE

CARBOSep CHO882
CARBOHYDRATE COLUMN

LEAD Form

CATALOG NUMBER CHO-99-8770

WARNING. THE TRANSGENOMIC CARBOSep CHO882 LEAD FORM COLUMN IS PACKED WITH A POLYMERIC MATERIAL THAT REQUIRES SPECIAL CARE. INTRODUCTION OF ORGANIC SOLVENTS ONTO 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 FAMILIARIZE YOURSELF WITH THE CONTENTS OF THIS MANUAL BEFORE USING YOUR COLUMN. 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 CARBOSep CHO882 carbohydrate column contains a 0.78 x 30-cm bed packed with an Transgenomic cation - exchange resin in the Pb^{++} ionic form. It is specifically designed for the separation of monosaccharides and disaccharides found in food products and physiological samples. Only distilled, de-ionized water is required as the mobile phase. 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.

A particular feature of the column is its ability to distinguish maltose and sucrose. This is useful in analyzing food samples containing sweeteners from corn syrup and cane sugar.

MOBILE PHASE

The only recommended mobile phase is water, de-ionized (especially free of metal ions and halides) and bacteria-free (filtered through 0.45 micron membrane). Alternatively, "HPLC grade" water is available commercially and is satisfactory for use with this column. The mobile phase should be de-gassed by vigorous boiling prior to use and kept in a container that precludes introduction of airborne bacterial and fungal contamination. Fresh mobile phase should be prepared every 24 hours.

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 rate for the Transgenomic, CARBOSep CHO882 column is between 0.4-0.7 mL/min. Do not exceed 0.7 mL/min.

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. Under normal operating conditions, a flow rate of 0.5 mL/min at 85°C should require pump pressures less than 50 atm (700 psi). It is inadvisable to utilize mobile phase flow rates that produce pump pressures in excess of 70 atm (1025 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 pressure 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 CARBOSep CHO882 lead form column must always be used with a heating device. 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 80° - 90° C has been determined to be the optimum for the Transgenomic CARBOSep CHO882 lead 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 pressure below 70 atm (1025 psi).

PRE-COLUMN FILTER

Pre-column filters containing 0.5 – 2.0 micron porosity stainless steel frits should be used between sample injector and 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 carbohydrate columns because sample and eluent 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. The recommended guard column is a Transgenomic CARBOSep 87P Guard Kit (cat. no. CHO-99-2364). This kit included a stainlees steel holder and two 20x4.0mm cartridges packed with the same material. Cartridge replacement is required when increased column pressure and/or loss of resolution is observed. Replacement cartridges are available (cat. no. CHO-99-1364). Silica guard columns are not 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.

Furthermore, certain samples found in the sweetener industry may contain organic matter that is soluble in the sweetener sample, but not in the column environment. Build up of these compounds in the column will lead to plugging and eventual column overpressure. The column can sometimes be regenerated if this occurs (see below), but it is best to avoid deposition of these matters on the polymer bed. Uses of guard cartridges are replaced frequently. Alternately, the sample can be cleaned off line using one of the numerous methods of sample purification found in literature. Fast sample treatment packages have been introduced by Waters Associates (Waters Sep-Pak) and Analytichem International (Bond-Elut). These have been evaluated by our laboratory and usually found to be satisfactory for use with our columns. We recommend sample treatment with these products in order to prolong column life.

SAMPLE VOLUME

The Transgenomic CARBOSep CHO882 lead form column contains a polymer bed of 0.78 x 30 cm. Although this large bed should tolerate large injection volumes, remember that one of the separation modes is steric exclusion. Under these circumstances, smaller sample volumes usually promote higher separation efficiencies. Consequently, you must determine empirically the maximum injection volume tolerated by the column for your particular sample. Although we generally use sample volumes in the 10 – 50 µL range, problems should not occur with sample volumes of as high as 70 µL Injections of 500 µL or more may cause problems, depending on sample content. If injection volume is too great, peaks may broaden or merge with nearby peaks.

DETECTION AND SENSITIVTY

The mobile phase requirement for Transgenomic CARBOSep CHO882 lead form column enables chromatographers to use a variety of detectors. Spectrophotometers, refractometers and electrochemical detectors can all be used successfully. If electrochemical detectors are used, note that high temperatures may be incompatible with some working electrodes. Selection of the right electrodes with desired temperature and eluent strength is the key to successful applications. If higher sensitivity is required, post-column reactions followed by the appropriate detector for the reaction product (e.g. fluorometer, photometer) can be utilized. Remember that sensitivity of detection is ultimately determined by the type of detector chosen; the responsibility of the column is simple to separate compounds of interest.

STORAGE

The column as supplied is equilibrated with de-gassed, de-ionized water. 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 degassed, de-ionized water at a flow rate of 0.2 mL/min at 90° C. Adjust the flow rate if necessary to keep pump pressure below 70 atm (1025 psi). 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.

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POSSIBLE CAUSES OF PERFORMANCE LOSS IN THE TRANSGENOMIC CARBOSep CHO882 LEAD 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 is a result of introduction of contaminants to 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 (ref. 2).

2. Improper column temperature

3. Improper mobile phase flow rate

4. Insufficient equilibration time with mobile phase

5. Presence of cations in mobile phase – (e.g., Na^+ or H^+)

6. Polymer contamination

   a. High column pressure accompanies performance loss
      (1) particulate accumulation on column 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

(3) refractory organic component from sweetener samples

b. Normal column pressure accompanies performance loss

(1) metal ion contamination
(a) inappropriate steel alloy present in LC system
(b) samples contaminated with metal ions
(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 phases after preparation (e.g. from atmoshphere, during transfer, etc.)

7. 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 th source of the problem before again using a column for analysis of samples.

1. **Prepare Fresh Eluent**. 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 pump mobile phase at 0.1mL/min overnight at 90 °C. Return column to normal operating conditions.

3. **Cleaning of Polymer**. If performance problems persist and particularly if high column pressures remain, an attempt should be made to clean the column to remove build up contaminants. Cleaning and regeneration procedures outlined below will in some cases restore performance by removing contaminants from 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 20% acetonitrile, 0.1M Pb(NO3)2 solution. Set column temperature to 65 °C and pump solution through (inverted) column at 0.1 mL/min overnight. If necessary, adjust mobile phase flow rate such that pressure does not exceed 70 atm (1025 psi). You may see a dark – colored material eluting from the column.

   The following day, replace acetonitrile, lead nitrate solution with degassed, de-ionized water, and continue pumping at 0.1 mL/min to determine if high pressure has subsided. If pressure is low, return column temperature to 90° C and gradually increase mobile phase flow rate to 0.5 ml/min. Test column under normal operating conditions. If pressure has returned to normal but performance remains inadequate, attempt regeneration procedure described below. If pressure does not return to normal column may be permanently damaged and require replacement.

4. **Column Regeneration**. Prepare an aqueous 0.1 *M* solution of lead nitrate. Filter this solution through a 0.45 micron membrane and degas. With the column in the inverted direction, pump at 0.1 ml/min at 90° C overnight. Retest the column under normal operating conditions. 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 cause of problem before installing 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. The only recommended mobile phase is distilled, de-ionized water. It should be filtered through a 0.2-0.45 um membrane and degassed.

2. Keep flow rate between 0.4 and 0.7 mL/min **and** pressure below 70 atm (1025psi). Do not exceed 0.7 mL/min.

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

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, store in de-ionized, degassed water

6. Filter samples through 0.2-.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.

REFERENCES

(1) Benson, J.R. and Woo, D.J. (1984) J. Chrom. Sci. 22 (9) 386-399

(2) Majors, R. (1983), LC 1 (Nov), 464

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