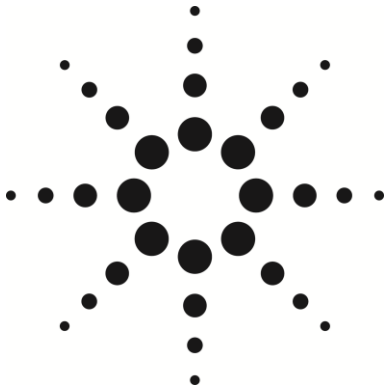

# Agilent MetaCarb USP L19 Carbohydrate Column $Ca^{++}$ Form User Manual

Part No. A5092

**Warning:** The Agilent MetaCarb USP L19 calcium 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, prior to installing the column you should thoroughly flush the lines of your LC with iso-propanol followed by de-ionized water to for at least one hour to remove any organic solvents.

You should thoroughly familiarize yourself with the contents of this manual before using your column. Improper use will invalidate the warranty.

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**Description**

The Agilent MetaCarb USP L19 carbohydrate column contains a 0.4 x 25 cm bed packed with an Agilent cation-exchange resin in the $Ca^{++}$ ionic form. It is specifically designed for the separation of polysaccharides, monosaccharides and sugar alcohols in a single chromatographic profile. The polymer is designed to meet the official USP L-19 method requirements. Only distilled, de-ionized water is required as the mobile phase. The primary mode of separation is steric exclusion although partitioning and ligand exchange mechanisms may be involved in the separation of certain sugars. Optimum performance is obtained at elevated temperatures; consequently, a column-heating device is required. The column's physical dimensions (¼ in. od x 25 cm) are compatible with most commercially available heaters.

**Mobile phase**

The only recommended mobile phase is water, de-gassed, de-ionized (especially free of metal ions and halides) and bacteria-free (filtered through 0.2 micron membrane). Alternatively, "HPLC grade" water is available commercially and is satisfactory for use with this column. The preferred method for degassing the mobile phase is by vigorous heating (boiling for at least 15 minutes). Alternatively, the mobile phase can be degassed by helium sparging or ultrasonically for at least 15 minutes 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 68 atm (1000 psi). The recommended mobile phase flow rate for the Agilent MetaCarb USP L19 calcium form column is 0.2 mL/min. Do not exceed 0.5 mL/min. It is highly recommended that the column be flushed for 30 minutes prior to each installation before attaching it to the inlet of the detector. This allows for any air remaining in the column to be flushed out of the column prior to being trapped in the column flow cell and causing baseline disturbance.

**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 0.2 mL/min at 30 °C should require pump pressures less than 54 atm (800 psi). It is inadvisable to utilize mobile phase flow rates that produce pump pressures in excess of 68 atm (1000 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 result from improper use of the column. Use of a guard column or guard cartridges (see below) will usually prevent contaminants from accumulating on the analytical column. It is highly recommended to allow the column to adjust to the column oven temperature before starting the flow (5-10 minutes) to prevent any

spikes to the column pressure. Alternatively, the column flow can be reduced initially to 0.2 mL/minute until the column is brought up to the proper operating pressure. It is recommended to flush the column before attaching to the inlet of the detector (see **Column pressure**).

**Mobile phase flow direction**

For the best results the column should be operated in the same flow direction as indicated by the arrow on the column body. However, the column can be reversed as needed to flush out contaminants or any particulates that may build up on the inlet end of the column.

**Column temperature**

The Agilent MetaCarb USP L19 calcium form column should 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 30-90 °C has been determined to be the optimum for the Agilent MetaCarb USP L19 calcium form column. If you are trying to duplicate a USP L19 method, use the recommended test conditions. Temperatures below 30 °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 rates should be adjusted to keep pump pressures below 68 atm (1000 psi).

**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 cartridges should be used with polymeric carbohydrate 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 MetaCarb USP L19 guard cartridges (Cat. No. A5092-GC) for this column. The purchase of a reusable Agilent Guard Holder (Cat. No. A5204) is required in conjunction with the guard cartridges. Cartridge replacement is required when increased column pressure and/or loss of resolution is observed. Silica guard columns are not recommended due to

degradation and eventual leakage into the analytical column. Use of guard cartridges 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 the 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. Use of guard cartridges also helps prevent this occurrence, providing 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. Numerous methods of sample purification can be found in literature, but sample preparation schemes such as those employing solid phase extraction tubes are the most common.

**Sample volume**

The Agilent MetaCarb USP L19 calcium form column contains a bed of 0.4 x 25 cm polymer. Although this 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 100 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 requirements for Agilent MetaCarb USP L19 calcium form column enables chromatographers to use a variety of detectors. Spectrophotometers, refractometers, universal, 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 electrode with desired temperature and mobile phase 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 simply to separate the compounds of interest.

**Column storage**

The column as supplied is equilibrated with 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 de-ionized water at a flow rate of 0.1 mL/min at 85 °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 Agilent MetaCarb USP L19 calcium 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 Agilent, 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 or high column pressure beyond recommended limits. Use of guard cartridges will help prevent these problems, as will sample pretreatment (see above). All Agilent 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 id are kept to a minimum

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 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 phase after preparation (e.g. from atmosphere, 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 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 phases 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 in less 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 0.1 mL/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 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.

   If metal contamination is the suspected cause of column failure, or loss of performance (indications would be broad glucose or fructose peaks, shift in retention times), an EDTA wash may return column performance. Prepare a 0.01N calcium disodium EDTA solution and flush the column for one hour. Equilibrate the column for at least one hour and check performance. Wash times can be extended to overnight washes if the metal contamination is severe.

   If high pressure is experienced, it may simply be a build-up of particulates on the column inlet. Reverse the flow of the direction for 15 minutes. If the cause is particulate build-up the pressure should begin to drop immediately. The source of the particulate build-up should be determined to prevent reoccurrence.

   If you suspect the source of contamination is organic in nature and the steps above did not return column performance, an organic wash may be required. However, this should be used as a last step since the polymer in the column will swell in organic and can permanently disturb the column bed. Prepare an aqueous solution of 5% acetonitrile. Set column temperature to 65 °C and pump solution through (inverted) column at 0.2 mL/min overnight. If necessary, adjust mobile phase flow rate such that pressure does not exceed 68 atm. You may see a dark-colored material eluting from the column.

   Next day, replace acetonitrile solution with de-ionized water, and continue pumping at 0.2 mL/min to determine if high pressure has subsided. If pressure is low, return column temperature to 85 °C and gradually increase mobile phase flow rate to 0.5 mL/min. Test column under normal conditions but if performance remains inadequate, repeat procedure described above. If pressure does not return to normal column may be permanently damaged and require replacement.

4. **Column Replacement**. The procedures above 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. The only recommended mobile phase is distilled, de-ionized water. It should be filtered through a 0.2-0.45 micron membrane and degassed prior to use.

2. The recommended flow rate is 0.2 mL/min. Do not exceed 0.5 mL/min.

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

4. Adjust flow rate to keep column back pressures below 68 atm (1000 psi).

5. When the column is not to be used for extended periods, store equilibrated in de-ionized water.

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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