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Carbomix H-NP and Ca-NP Phases

Column Information

Carbomix H-NP and Carbomix Ca-NP columns have been specifically designed for high resolution separation of carbohydrates, organic acids, peptides, and nucleic acids. These novel packing materials are based on low crosslinked polystyrene / divinylbenzene (PS/DVB) particles with the surface modified with sulfonic acid (-SO_3H) for Carbomix H-NP resins, followed by chelating of calcium ions (Ca^{+2}) for synthesis of Carbomix Ca-NP resins. Their narrow particle size distribution offers high efficiency and high resolution separations. The low cross-linking generates swelling for the resin in the mobile phase, resulting in reasonable surface area and capacity. Figure 1 is a typical test chromatogram for the separation of carbohydrates by a Carbomix Ca-NP5 column.

Separation Mechanism

The separation mechanism for the Carbomix Ca-NP phases includes ion-exchange and hydrophilic interactions with the analytes, such as carbohydrates and organic acids. The separation mechanism could also be due to size exclusion, ion exclusion, and ligand exchange. These multiple modes of interaction enable a unique capability to separate a variety of water soluble compounds. Resin cross-linking degree is an important parameter in the separation. Styrene divinylbenzene resin is a relatively rigid gel-type media. The lower the cross-linking, the more open the pore structure, and the more permeable it is to higher molecular weight substances. A 5% crosslinked Carbomix resin can resolve higher oligosaccharides compared to 10% cross-linked resin. For smaller molecular weight compounds an 8% crosslinked resin is used.

Column Configuration

Carbomix resins can be packed into wide range of column dimensions with ID from 75 µm to 21.2 mm and the length from 5 cm to 30 cm. A custom-made column is also available. Column length and diameter affect resolution and analysis time. The selection of the column is to use only as much resin as necessary to achieve the desired separation. If the compound is strongly retained by the resin and analysis time is long on a 300 x 7.8 mm column, a shorter column, such as 150 mm long can significantly decrease the analysis time.

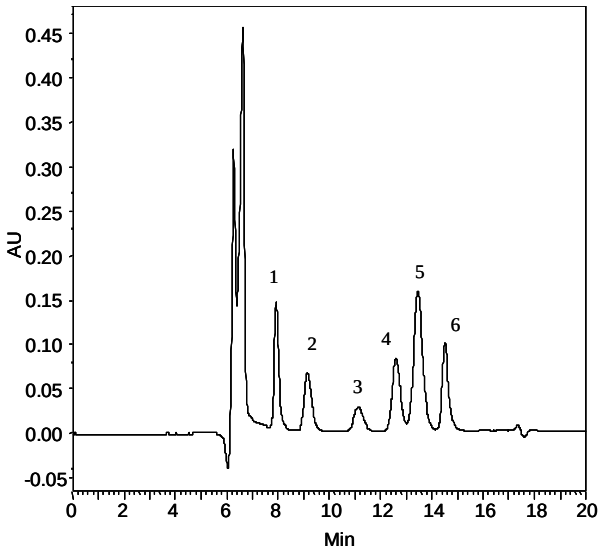

Figure 1. Separation of carbohydrate mixture by a Carbomix Ca-NP5 column (5µm, 8% cross-linking, 7.8x300mm)

Mobile phase: nanopure water
Flow rate: 0.6 mL/min
Backpressure: 708 psi
Column Temperature: 85 °C
Injection Volume: 10 µL
Detector: UV 192 nm
Sample (50 mM in H_2O for each)

1. Melezitose
2. Maltose
3. Glucose
4. Mannose
5. Fructose
6. Adonitol (N=64,200/m)

Column Operation

Sample Preparation The samples are commended to be prepared by filtering through a 0.45 µm filter.

Solvent These columns allow the use of simple isocratic methods, eluting with water or dilute acid (for Carbomix H-NP columns). Simplified solvent selection is a major advantage of Carbomix columns. Most carbohydrate separations can be carried out with deionized water as the mobile phase. The addition of an organic solvent, such as acetonitrile can improve the resolution of some special molecules, such as sugar alcohols. In the other aspect, the addition of organic solvent to the eluant as an organic modifier would decrease adsorption of organic compounds to the column matrix. Organic modifiers are recommended up to 30% acetonitrile or less than 5% tert-butanol

or isopropanol in the eluant. This is particularly useful in the separation of aromatic acids. Organic modifiers can be used to reduce analysis time. However, there is a possibility that the organic modifier penetrates and swells the PS/DVB resin to change the resin volume. Ethanol and isopropanol are similar to acetonitrile. Methanol, THF, DMF and other non-polar solvents are not recommended due to the possibility for bed shrinkage or bed swelling. *It is highly recommended that the mobile phase is on-line degassed when the column is in use.*

Pressure The Carbomix resins exhibit high pressure stability as well as pH stability over a wide range. Column back pressure decreases when temperature increases.

Temperature Temperature has a great impact on the separation with Carbomix columns. The retention time and separation efficiency are affected by column temperature. Even though the effect of temperature on a given analysis depends on the individual chemistry, the type of column packing, and the mobile phase, for most applications increasing the column temperature decreases retention time, and increases column efficiency. High temperature can optimize efficiency by minimizing the band spreading from slow mass transfer in the stationary phase. Higher temperature also decreases the viscosity of the eluant and allows deeper penetration of samples into the interior of the resin, resulting in higher resolution. So control of the temperature is crucial for accurate, quantitative and qualitative analysis. For carbohydrate analysis, the columns are usually heated to 85 °C to optimize the separation efficiency and resolution.

Flow rate Due to low cross-linking for synthesizing Carbomix media, the Carbomix resin is more like a soft gel that would generate huge backpressure at high flow rate. Carbomix columns typically operate at low flow rates. For 7.8x300 mm and 4.6x300 mm columns, the typical flow rate is no more than 1.0 mL/min, and 0.35 mL/min, respectively. For routine analysis, flow rates of 0.4–1.0 ml/min and 0.12-0.35 mL/min are recommended for a 7.8x300 mm and 4.6x300 mm Carbomix column, respectively for optimized separation efficiency and retention time. Even though low flow rate (e.g. <0.6 mL/min for a 7.8x300 mm column) increase the analysis time, it could increase efficiency. For some special applications, a low flow rate combined with two or three columns in series offers the ability to isolate and examine compounds within a complex sample matrix.

pH The optimum performance and operation for longest lifetime are at pH range (5-9) for Carbomix Ca-NP and (1-3) for Carbomix H-NP columns.

Precolumn filter or guard column It is highly recommended to use a pre-column filter or a guard column to prevent the column fouling when the column is in use.

Safety Precaution

The Carbomix columns are normally operated under moderate pressure. Loose connections will cause leaking of organic solvents and injected samples, all of which should be considered as the hazards. In the case of leaking, proper gloves should be worn for handling the leaked columns. When open the columns, proper protections should be used to avoid inhalation of the small polymer particles.

Column Installation and Operation

When column is shipped or not in use, it is always capped at both ends. When install the column to the system, first remove the end caps. Make the flow direction as marked on the column. Unless a user has special purpose to reverse the flow direction, for example, removal of the inlet pluggage, follow the flow direction as labeled. Column connections are an integral part of the chromatographic process. If ferrules are over tightened, not set properly, or are not specific for the fitting, leakage can occur. Set the ferrules for column installation to the HPLC system as follows:

(a) Place the male nut and ferrule, in order, onto a 1/16" o.d. piece of tubing. Be certain that the wider end of the ferrule is against the nut.

(b) Press tubing firmly into the column end fitting. Slide the nut and ferrule forward, engage the threads, and fingertighten the nut.

(c) While continuing to press the tube firmly into the endfitting, use a 1/4" wrench to tighten the nut 90 degrees past fingertightness.

(d) Repeat this coupling procedure for the other end of the column.

Column Care

Shipping Solvent New Carbomix H-NP and Ca-NP columns are shipped in 5.0 mM H_2SO_4 and pure water (pH 5-7), separately. During stocking and shipping, the packing could be dried out. It is recommended that 10-20 column volumes of the stocking solvent be purged to activate the column. Flush the column with your mobile phase with gradual increasing the flow rate from 0.1 mL/min to your operation condition, until the baseline is stable.

Storage When not in use for extended time, store the new Carbomix H-NP and Ca-NP columns in 5.0 mM H_2SO_4 and pure water, respectively. Each column is shipped with two removable end plugs. To prevent the drying of the column bed, seal both ends of the column with the end plugs provided.

Typical Applications

The Carbomix resins and columns offer many advantages for the analysis of carbohydrates, alcohols, and organic acids in food, beverage, biochemical, biomedical, and biotechnology applications.

Organic acid and alcohol analysis include sugars with organic acids, alcohol, glycol, and fermentation products.

Carbohydrate analysis includes samples of beet sugars, molasses, corn syrup, pentose sugars, cellulose hydrolysates, oligosaccharides, glucose, galactose, sucrose, and fructose.