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Carbomix H-NP Phases

Column Information

Carbomix H-NP columns have been specifically designed for high resolution separations of carbohydrates, alcohols, etc. These novel packing materials are based on low cross-linked polystyrene/divinylbenzene (PS/DVB) particles with sulfonic acid (-SO_3H) surface modifications for Carbomix H-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 H-NP10 column.

Separation Mechanism

The separation mechanisms for the Carbomix H-NP phases include ion exchange and hydrophilic interactions with the analytes. 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% cross-linked Carbomix resin can resolve higher oligosaccharides compared to 10% cross-linked resin. For smaller molecular weight compounds an 8% cross-linked resin is used.

Column Configuration

Carbomix resins can be packed into wide range of column dimensions with IDs ranging from 75 µm to 21.2 mm and lengths 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 7.8 x 300 mm column, a shorter column, such as a 150 mm length can significantly decrease the analysis time.

Column Operation

Sample Preparation Carbomix H-NP phases are designed for acidic or neutral substances separation, such as organic acids, sugars, sugar alcohols and their derivates. Basic samples are not suitable to be analyzed on Carbomix-H resins. The samples should be prepared by filtering through a 0.45 µm filter.

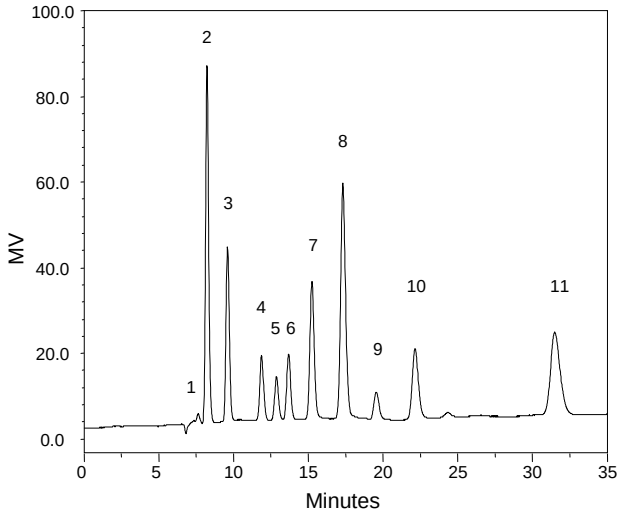

Figure 1. Separation of carbohydrate mixture by a Carbomix H-NP10 column (10 µm, 8% cross-linking, 7.8 x 300 mm)
Mobile phase: 2.5 mM H_2SO_4
Flow rate: 0.6 mL/min
Column temperature: 55 ^oC
Injection volume: 10 µL
Detector: RI
Samples:

1. Maltotriose
2. Maltose
3. Glucose
4. Succinic acid
5. Lactic acid
6. Glycerol
7. Acetic acid
8. 1,2-Propylene glycol
9. Methanol
10. Ethanol
11. Sec-Butanol

Solvent Carbomix H-NP phases allow the use of simple isocratic methods, eluting with dilute acid (e.g. 2.5 mM H_2SO_4 or 0.1% H_3PO_4). Work pH range for this resin is 1-3. Simplified solvent selection is a major advantage of Carbomix columns. Most carbohydrate separations can be carried out with aqueous solution 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 mobile phase as an organic modifier would decrease adsorption of organic compounds to the column matrix. Organic modifiers can be used to reduce analysis time. However, there is a possibility that the organic modifier may penetrate and swell 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 H-NP resins exhibit high pressure stability as well as pH stability over a wide range (pH 1-3). Column backpressure decreases when temperature increases.

Temperature Temperature has a great impact on the separation of the Carbomix columns. The retention time and separation efficiency are both affected by column temperature. Although 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 eluent and allows deeper penetration of samples into the interior of the resin, resulting in higher resolution. Therefore control of the temperature is crucial for accurate, quantitative and qualitative analysis.

Attention: Recommended temperature for Carbomix H-NP phase is 55 ℃. To improve the separation efficiency and resolution at special conditions, users can optimize the column temperature at the range of 35-85 ℃. Any operation beyond this temperature limit would cause column damage.

Flow rate Due to low cross-linking of 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 7.8 x 300 mm and 4.6 x 300 mm columns, the typical flow rate is no more than 1.0 mL/min and 0.35 mL/min, respectively. For routine analysis optimized separation efficiency and retention time, flow rates of 0.4–1.0 mL/min and 0.12-0.35 mL/min are recommended for a 7.8 x 300 mm and 4.6 x 300 mm Carbomix column, respectively. Even though low flow rates (e.g. < 0.6 mL/min for a 7.8 x 300 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 is at pH range (1-3) for Carbomix H-NP columns. Any operation beyond this pH limit would cause column damage.

Pre-column filter or guard column It is highly recommended to use a pre-column filter or a guard column to prevent 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 hazards. In the case of leaking, proper gloves should be worn for handling the leaked columns. When opening the column, proper protection should be used to avoid inhalation of the small polymer particles.

Column Installation and Operation

When column is shipped or not in use, it should be capped at both ends. When installing the column to the system, first remove the end caps. Make the flow direction is as marked on the column. Only reverse the flow direction when some special cases occur such as removal of the inlet blockage. 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" outer diameter piece of tubing. Make certain that the wider end of the ferrule is against the nut.

(b) Press the tubing firmly into the column's end fitting. Slide the nut and ferrule forward, engage the threads, and finger-tighten the nut.

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

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

(e) Once the Carbomix H-NP column is properly installed on the HPLC system, please keep the flow rate at 0.1 mL/min and make sure the column temperature rises to set value, which is strongly recommended at above 35℃, and then increase the flow rate gradually.

(f) When finished using, make sure to cool down the column to below 40 ℃ at a lower flow rate 0.1 mL/min, then stop the flow and remove the column.

Column Care

Shipping Solvent New Carbomix H-NP columns are shipped in 2.5 mM H_2SO_4. During stocking and shipping, the packing could become 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 while gradually increasing the flow rate from 0.1 mL/min to your operating condition until the baseline is stable.

Storage When not in use for an extended amount of time, store the new Carbomix H-NP columns in 2.5-5.0 mM H_2SO_4. 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 acids and alcohols analyses include sugars with organic acid, alcohol, glycol, and fermentation products.

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