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Carbomix K-NP Phases

Column Information

Carbomix K-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, followed by the chelating of Potassium ions (K^+) for the synthesis of Carbomix K-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 K-NP10 column.

Separation Mechanism

The separation mechanisms for the Carbomix K-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% crosslinked 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.

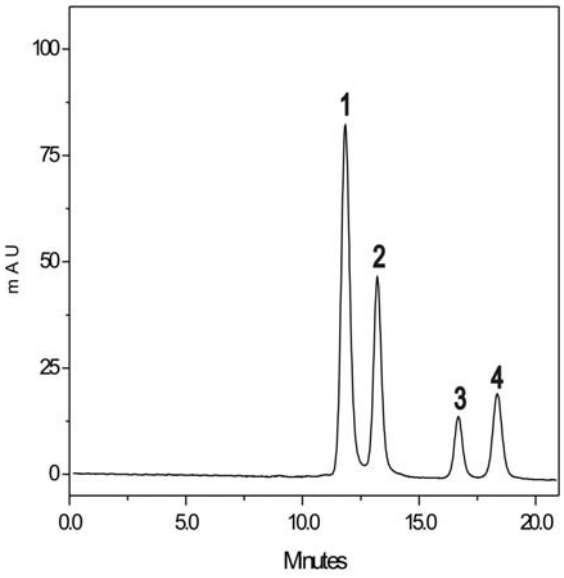

Figure 1. Separation of carbohydrate mixture by a Carbomix K-NP10 column (10 µm, 8% cross-linking, 7.8 x 300 mm)
Mobile phase: Millipore water
Flow rate: 0.4 mL/min
Column temperature: 85 ℃
Injection volume: 5 µL
Detector: RI
Samples:
1. Maltotriose 2. Maltose 3. Glucose
4. Mannose

Column Operation

Sample Preparation All analytes should be neutralized to pH 5-9 and filtered through a 0.45 µm filter before injection as Carbomix K-NP phases are designed for neutral substances separation, such as sugars, sugar alcohols and their derivatives.

Solvent These columns allow the use of simple isocratic methods, eluting with water. 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 mobile phase 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% t-butanol or

isopropanol in the eluent. 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 K-NP resins exhibit high pressure stability as well as pH stability over a wide range (pH 5-9). 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 K-NP phase is 85 °C. To improve the separation efficiency and resolution at special conditions, users can optimize the column temperature at the range of 50-85 °C. 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 and 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 columns, 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 (5-9) for Carbomix K-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 leaking 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 K-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 50 ℃, 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 K-NP columns are shipped in pure water (pH 5-7). 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 K-NP columns in pure water. 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 in food, beverage, biochemical, biomedical, and biotechnology applications.

Alcohol analyses include sugars with 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.

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Carbomix K-NP 柱
使用手册

色谱柱信息

Carbomix K-NP 柱专为糖类、醇类的高效分离而设计。该新型填料是在低交联度聚苯乙烯/二乙烯苯(PS/DVB)颗粒的表面修饰磺酸基团(-SO_3H)得到 Carbomix H-NP，并在此基础上进一步螯合钾离子（K^+）得到的。极窄的粒径分布确保了高的分离效率，而较低的交联度则保证其可以在流动相中发生溶胀，从而具有合适的比表面积和柱容量。图 1 是使用 Carbomix K-NP10 柱分离糖及糖醇混合物得到的一张色谱图。

分离机理

Carbomix K-NP 固定相的分离机理包括离子交换作用以及与待测物亲水相互作用。此外还涉及了体积排阻、离子排斥、配体交换等机理。多种相互作用模式使 Carbomix K-NP 柱具备分离多种水溶性化合物的独特能力。在分离中树脂交联度是一个重要参数。PS/DVB 树脂是一种相对坚硬的凝胶介质。交联度越低，孔结构就越容易溶胀，也就越利于大分子化合物的渗透。因此，交联度 5%的 Carbomix 树脂比 10%交联度的树脂更适合于分离大分子量的低聚糖。小分子量的化合物可用 8%交联度的 Carbomix 树脂进行分离。

色谱柱参数

我们可提供多种规格的 Carbomix K-NP 柱，内径从 75 μm 到 21.2 mm，长度从 5 cm 到 30 cm 不等。此外还可根据客户的要求定制色谱柱。色谱柱的长度和内径会影响分离的效果以及分析所需的时间。因此，在选择 Carbomix 柱的规格时，一定要保证选用的色谱柱内的树脂量恰好能够达到所需要的分离效果。如果化合物在树脂上保留过强，例如，在 7.8×300 mm 柱子上保留时间太长时，可选用短的柱子如 150 mm 色谱柱来缩短分析的时间。

色谱柱操作

样品制备 Carbomix K-NP 柱适合分离中性样品，请务必将待分析样品的 pH 控制在 5-9 范围内，并经 0.45 μm 滤膜过滤后，再进样。

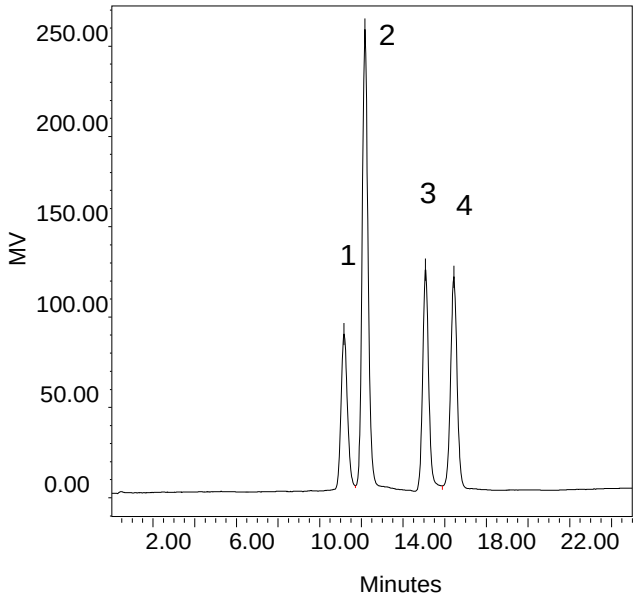

图 1 在 Carbomix K-NP 5 （5 μm, 8%交联度, 7.8×300 mm）柱上进行糖及糖醇混合物的分离
流动相：超纯水
流速：0.4 mL/min
柱温：85 ℃
进样量：10 μL
检测器：示差折光检测器
样品：
1. 麦芽三糖 (Maltotriose)
2. 麦芽糖 (Maltose)
3. 葡萄糖 (Glucose)
4. 甘露糖 (Mannose)

溶剂 简单的溶剂选择和等度洗脱方式是使用 Carbomix 柱的一个主要特色。Carbomix K-NP 柱建议流动相为 18 MΩ 超纯水，其适用的 pH 范围为 5-9。大多数糖类化合物在 Carbomix K-NP 柱上的分离都可以用超纯水作为流动相。在某些特殊情况下， 流动相中添加有机溶剂如乙腈等能够改善糖醇等化合物的分离度。另一方面，流动相中加入有机溶剂也可作为改性剂减少有机化合物在固定相上的保留。建议流动相中有机改性剂的含量应不高于 30%乙腈或低于 5%叔丁醇（或异丙醇）。加入改性剂在芳香酸类化合物的分离中特别有效。有机改性剂可以用于减少分析的时间，但也可能因渗入 PS/DVB 使其溶胀从而改变树脂的容积。乙醇和异丙

醇与乙腈的性质相似。不推荐使用甲醇、四氢呋喃、二甲基甲酰胺以及其它非极性溶剂，因为它们可能会使柱床发生收缩或溶胀。在色谱柱的使用过程中，强烈建议采用在线脱气的方法。

温度 温度对 Carbomix K-NP 柱的分离性能有着显著影响。柱温会影响保留时间和分离效率。对于某一特定分析而言，温度所产生的影响取决于该分析所涉及的化学特性、填料类型、流动相等。但对大多数应用而言，随着柱温的升高保留时间将会缩短，柱效将会提高，同时柱压会随之降低。升高温度可减少固定相中慢传质过程所引起的谱带展宽效应，从而可提高分离的效率。此外，升高温度还能减小流动相的粘度，使溶质分子更容易地渗入树脂内部，从而可以获得高的分辨率。因此，控制温度对于精确的定性及定量分析都非常重要。**注意：Carbomix K-NP 柱的建议柱温为 85 ℃。用户可根据分离情况对柱温进行优化，但需保证在 Carbomix K-NP 柱的工作温度范围内，超出温度上限（85℃）或低于下限（50 ℃）都将可能对色谱柱造成损伤。**

流速 由于合成的 Carbomix 介质交联度较低，Carbomix 树脂更像软凝胶，在高流速下会产生高柱压（Carbomix 柱工作压力上限请参见技术参数表）。Carbomix K-NP 通常在低流速下使用。尽管低流速会增加分析的时间，但这将有助于提高色谱柱的柱效。在某些应用中， 低的操作流速还允许将 2-3 根色谱柱串联起来，可用于分离和检测复杂样品中的组分。在常规分析中， 对规格为 7.8×300 mm 和 4.6×300 mm 的 Carbomix 色谱柱而言，其建议使用流速分别为 0.4-0.8 mL/min 和 0.1-0.3 mL/min 流速（取决于交联度），以获得最佳的分离效果和保留时间.

pH 为了获得最佳的分离效果和延长柱的使用寿命，Carbomix K-NP 柱的 pH 适用范围是 5-9。如使用了超出上述 pH 范围的流动相，将可能对色谱柱造成不可修复的损伤。如特殊情况下需要使用 Carbomix K-NP 柱来分析酸性样品，则务必在进样前对样品进行预处理，以保证其 pH 在 5-9 的中性范围内，方可进样。

安全注意事项

Carbomix 柱通常在中等压力下运行。如果管路连接不紧，将会导致有机溶剂和注入样品的泄漏，从而对操作人员的健康产生影响。一旦发生泄漏，应佩戴适当的手套进行处理。另外当打开色谱柱时还应采取适当的保护措施，以防止微小的填料颗粒进入呼吸道。

色谱柱安装与操作

色谱柱在运输过程中或在没有使用时，它的两端总是用堵头进行密封。当将色谱柱接入色谱仪器系统时，首先移去两端的堵头。请注意将流动相流动的方向与柱上标记的方向保持一致。除非出于特殊考虑，例如为了清除堵在色谱柱入口端的脏污等而需要将色谱柱反接以进行冲洗时，建议用户在接上色谱柱时一定要遵循柱上标记的方向。由于色谱柱的连接是整个色谱操作过程的一部分，如果密封卡套过紧，或安装不合适，或者密封卡套与色谱柱端口不匹配，都有可能导致溶液的泄漏。请按照下面步骤将色谱柱与密封卡套相连接，从而将色谱柱接入 HPLC 系统：

（a）第一次使用的管线，请依次将管线接头和密封卡套装在外径 1/16”的管线上。密封卡套的宽口端应朝向管线接头。由于 Carbomix 色谱柱的使用温度通常较高，因此色谱柱前的连接管线推荐使用 50 cm 以上长度的金属管线，以保证流动相在进入色谱柱前被充分预热。

（b）将管线紧紧插入色谱柱的接口，向前滑动密封卡套和管线接头，并使管线接头的螺纹与色谱柱端口的螺纹相互衔接，然后拧紧管线接头。如果管线为高分子材料，请转到步骤（d）；如果是金属管线，请继续（c）。

（c）在用力将管线压入柱端接口之后，用 1/4”扳手将已拧紧的螺帽再进一步紧固。

（d）对色谱柱的另一端采用上述方法进行操作。

（e）连接好管线后，先设定流速为 0.1 mL/min，待柱温升至设定温度后再逐渐升高流速至设定值。

（f）使用结束后，先将流速设定为 0.1 mL/min，再降低柱温至 40 ℃以下后，方可取下色谱柱。

色谱柱保养

运输溶剂 新的Carbomix K-NP柱保存在超纯水中。在储存和运输过程中，柱填料可能会干涸。这时推荐用10-20倍柱体积的保存溶剂进行冲洗以活化色谱柱。接着可用用户自己选择的流动相冲洗色谱柱。流速由0.1 mL/min逐渐升至所需的操作条件，直至基线稳定为止。（建议整个操作过程均待温度升至50 ℃以上、压力稳定时进行）。

储存 长期不用时，Carbomix K-NP 柱保存在超纯水中。每根色谱柱在运输过程中均会附有两个可拆卸的堵头。为了防止柱床干涸，请用堵头塞紧色谱柱的两端。

典型应用

Carbomix K-NP 树脂基质色谱柱为食品和饮料中糖类、醇类等的分离，以及生物化学、生物医药、生物技术等方面的应用提供了许多便利。

醇类分析涉及糖类与乙醇、乙二醇和发酵产品等的分离。

糖类分析样品包括甜菜糖、糖蜜、玉米糖浆、戊糖、纤维素水解物、寡糖、葡萄糖、半乳糖、蔗糖、果糖等。

Table1. Specification Parameters for Carbomix K-NP Columns dimension 7.8 x 300 mm.

Matrix		Sulfonated Poly-Styrene Divinyl Benzene Copolymer				
Ionic Form		Potassium				
Particle Size (μm)		10			5	
Cross Linking		10%	8%	5%	10%	8%
Max. Pressure (psi, 75°C)		1200	1000	800	1100	900
Max Temperature (°C)		85				
Typical Mobile Phase		Ultra-pure water				
pH Range		5 - 9				
Organic Modifiers (Max)		5% ethanol, or isopropyl alcohol, or 30% acetonitrile				
Avoid		Methanol, THF, bases, acids, non-potassium salts/metal ions				
Cleaning	Solvent	20% acetonitrile water				
	Flow Rate (mL/min)	0.2 mL/min				
	Temperature (°C)	50				
	Duration (hrs)	12				
Regeneration	Mobile Phase	100 mM KOH				
	Flow Rate (mL/min)	0.2 mL/min				
	Temperature (°C)	85				
	Duration (hrs)	16				
Ship/Storage Solvent		Ultra-pure water				

产品规格

Carbomix K-NP

内径×长度 (mm×mm）	交联度	粒径	孔径	型号
4.6×250	5%	5 μm	NP	230505-4625
7.8×300	5%	5 μm	NP	230505-7830
4.6×250	8%	5 μm	NP	230508-4625
7.8×300	8%	5 μm	NP	230508-7830
4.6×250	10%	5 μm	NP	230510-4625
7.8×300	10%	5 μm	NP	230510-7830
4.6×250	5%	10 μm	NP	231005-4625
7.8×300	5%	10 μm	NP	231005-7830
4.6×250	8%	10 μm	NP	231008-4625
7.8×300	8%	10 μm	NP	231008-7830
4.6×250	10%	10 μm	NP	231010-4625
7.8×300	10%	10 μm	NP	231010-7830