Carbohydrate and Organic Acid Separation

Sepax Technologies

Carbomix®

Better Surface Chemistry for Better Separation

Sepax Technologies, Inc.

Sepax Technologies, Inc. develops and manufactures products in the area of chemical and biological separations, biosurfaces and proteomics. Sepax product portfolio includes 1) liquid chromatography columns and media, 2) SPE and Flash chromatography columns and tubes, 3) bulk resin for preparative separation and process chromatography, and 4) natural product and Chinese traditional medicine separation and purification.

Leader in Biological Separations

Sepax develops and manufactures wide range of biological separation products using both silica and polymeric resins as the support. The selection of particle size is from 1 µm to 100 µm and pore size from non-porous to 2000 Å. Unique and proprietary resin synthesis and surface technologies have been developed for solving separation challenges in biological area.

Bioseparation Products

Size Exclusion

SRT®, SRT®-C
Nanofilm®
Zenix™, Zenix™-C

Ion-exchange

Proteomix®
Glycomix™

Antibody Separation

Antibodix™

Carbohydrate Separation

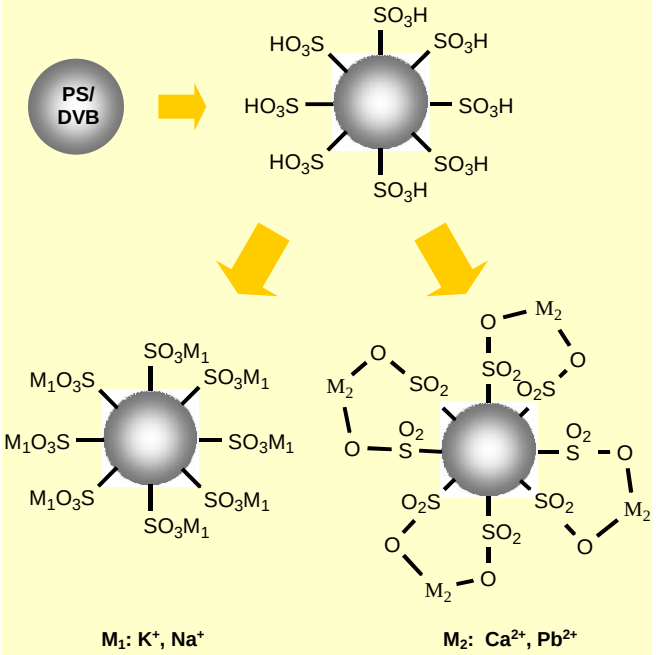
Carbomix®

Analytical, Semi-prep and Preparative

General Description

Carbomix lines of columns have been specifically designed for high resolution separation of water soluble and partially water soluble organic compounds, including carbohydrates, organic acids, peptides, and small bio organic molecules involved in cell metabolism. These novel packing materials are based on low crosslinked (5%, 8% and 10%) polystyrene/divinylbenzene (PS/DVB) particles (5 and 10 µm) with surface modification of sulfonic acid (-SO_3H) for Carbomix H-NP resins, followed by chelating of various metal ions for example, calcium ions (Ca^{2+}) for Carbomix Ca-NP, lead ion (Pb^{2+}) for Carbomix Pb-NP, potassium ion (K^+) for Carbomix K-NP, and sodium ion (Na^+) for Carbomix Na-NP resins (Figure 1).

Fig. 1. Chemical processing of Carbomix phases



Highlights of Carbomix Resins

- Uniform 5 and 10 µm particle choices for high resolution and efficiency separation
- 5%, 8% and 10% crosslinkage
- Compatibility with most aqueous mobile phases, including pure water as the eluent
- Wide selection on ionic forms: H^+, Ca^{2+}, Pb^{2+}, K^+, and Na^+
- Wide operating-temperature range (20 – 85 °C)
- pH range (1-3) for Carbomix H-NP and (5-9) for the other types of Carbomix phase
- Analytical and preparative columns

Characteristics of Carbomix Resins

Uniform Particle size. The particle size distribution of Carbomix phases is very narrow, 5.0 ± 0.2 for 5 µm and 10.0 ± 0.2 for 10 µm respectively, as shown in Figure 2. This mono-dispersed particle size distribution guarantees high efficiency and high resolution separations. Figure 3 shows higher separation efficiencies of monosaccharides with Carbomix Ca-NP5 columns as compared to other brands.

Fig. 2. SEM images of 5 and 10 µm Carbomix resins (× 2000).

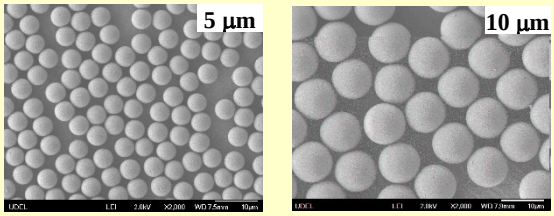
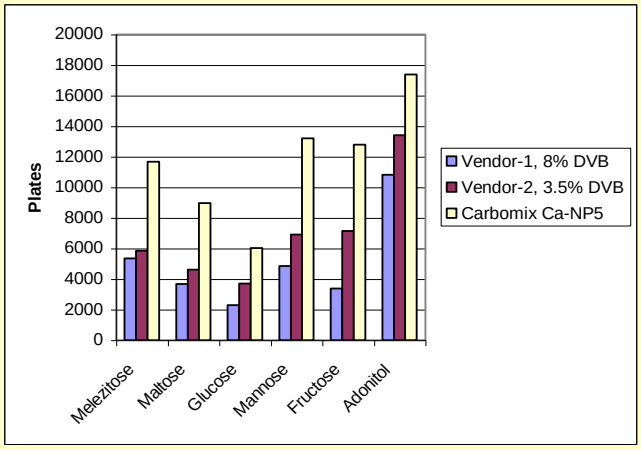


Fig. 3. Efficiency comparison of 6 monosacchrides on Carbomix Ca-NP5 (5 µm, 8% crosslinkage) and calcium columns (7.8×300 mm) from other vendors. The separation conditions: mobile phase, H_2O; flow rate, 0.60 mL/min; temperature, 85 °C; injection volume, 20 µL; detection, RI.



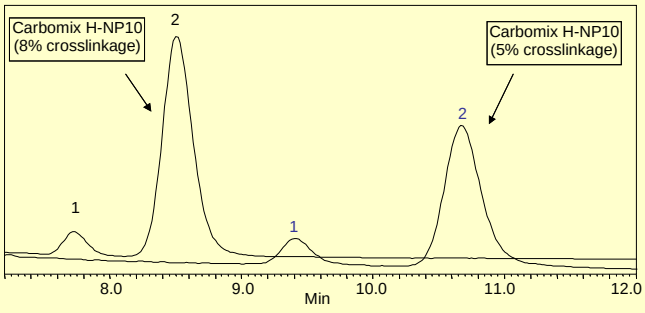
Variety of Ionic Forms. The wide range of ionic forms available for Carbomix phases allow for the best possible separation for different kinds of sugar molecules, sugar alcohols, and other water soluble compounds from different sources (Table 1). For instance, it is difficult to separate arabinose, ribitol and arabitol on H-form phase but well resolved on Ca-form column; the peaks of xylose, galactose, and mannose on Ca-form column merge to one but elutes separately one after another on a Pb-form column.

Table 1. Selection of Ionic-form for Different Applications

Ionic form	Applications
H	Fermentation products, fruit juices containing organic acids, alcohols and carbohydrates
Ca	Carbohydrate in high fructose corn syrup; excellent for mono-, di-, tri- and tetra saccharides and sugar alcohols
Pb	Pentoses and hexoses in wood products Dairy products containing sucrose, lactose, etc
K	Cane sugar, molasses, corn syrup, beet sugar and other plant products containing carbohydrates in the presence of betaine, and trimethylammonium zwitterionic compounds; Glyphosate
Na	Oligosaccharides, especially in the presence of high concentration of inorganic sodium, e.g. molasses

Low Crosslinkage. The low cross-linking property of Carbomix resins allows for proper swell in the mobile, especially at high temperatures, a typical operation for fulfilling most separation tasks. Such swelling effect results in optimized surface area, permeability, capacity, selectivity, and response to changes in ionic strength for high resolution separation. The lower the crosslinkage of PS/DVB beads, the more open the phase structure and permeability is to samples like larger oligosaccharides. Fig. 4 shows that NGNA and NANA is separated with higher resolution on 5% crosslinkage Carbomix H-NP10 column than 8% cross-linkage column.

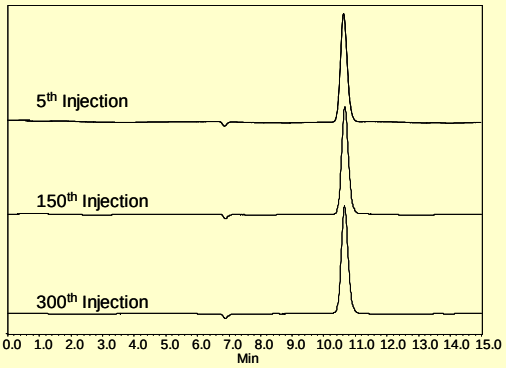
Fig. 4. Separation of NGNA and NANA on Carbomix H-NP10 columns with different crosslinkage.



Columns:	Carbomix H-NP10 (10 µm, 7.8x300 mm)
Mobile phase:	Water with acid
Flow rate:	0.60 mL/min
Temperature:	70 ºC
Injection volume:	20 µL
Detection:	206 nm
Sample:	1) NGNA (0.01 mg/mL) 2) NANA (0.1 mg/mL)

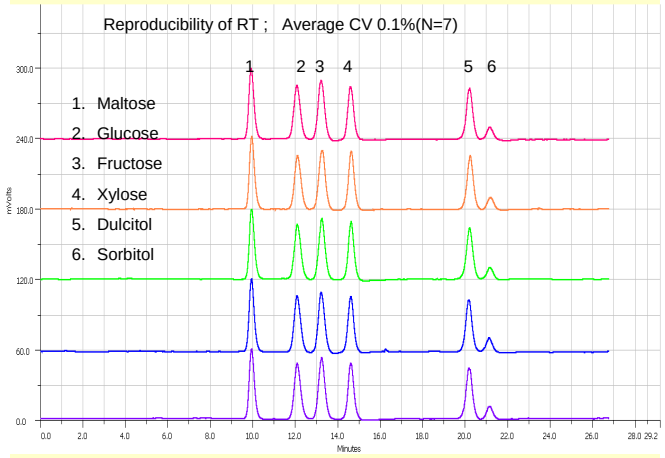
Stability. Carbomix columns are well manufactured with proprietary packing technique to guarantee high stability. Carbomix resins are stable in pure water and other aqueous buffers at elevated temperatures. As shown in Fig. 5, the variation of the retention time of galactitol is less than 0.3% within 300 injections at 80 °C. The robustness of Carbomix columns is further confirmed by its run-to-run reproducibility as Figure 6 shows consistent retention time with only 0.1% variation for Carbomix Ca-NP5 column.

Fig. 5. Robustness of Carbomix H-NP10 column after 300 injections.



Column:	Carbomix H-NP10 (10 µm, 8%, 7.8×300 mm)
Mobile phase:	5 mM H_2SO_4
Flow rate:	0.60 mL/min
Temperature:	80 ºC
Injection volume:	10 µL
Detection:	RI
Sample:	Galactitol

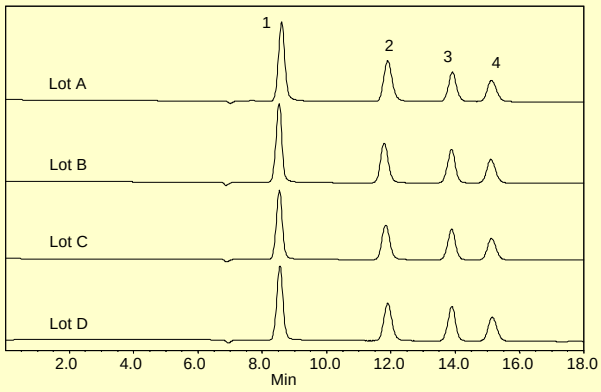
Fig. 6. Separation of carbohydrates by Carbomix Ca-NP5 column (Courtesy of Miyako Kawakatsu, M&S Instruments, Inc.).



Column:	Carbomix Ca-NP5 (5 µm, 8%, 4.6 × 300 mm)
Mobile phase:	Water
Flow rate:	0.2 mL/min
Temperature:	85 ºC
Injection volume:	10 µL
Detector:	ELSD
Sample:	Mixture of maltose, glucose, fructose, xylose, dulcitol and sorbitol (6 mg/mL of each in H_2O)

Lot-to-lot reproducibility. With well controlled polymer resin production, surface chemistry and column packing, the manufacturing of Carbomix columns is highly reproducible. As shown in Figure 7, typical variation of retention time is less than 3% from batch to batch.

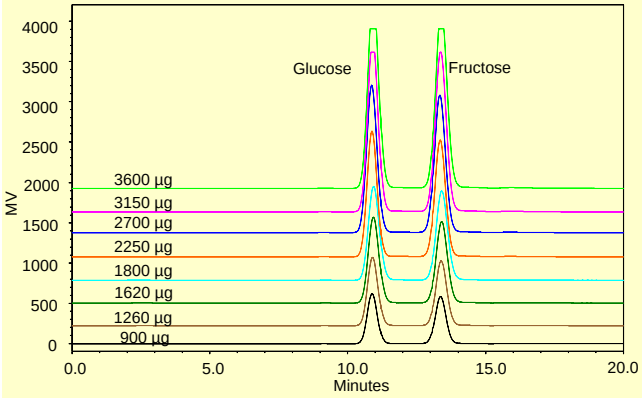
Fig. 7. QC of Carbomix H-NP10 columns from 4 different lots.



Column: Carbomix H-NP10 (10 µm, 8%, 7.8×300 mm)
Mobile phase: 2.5 mM H_2SO_4
Flow rate: 0.6 mL/min
Temperature: 55 ºC
Injection volume: 10 µL
Detector: RI
Sample: 1) Citric acid, 2) Succinic acid, 3) Formic acid, 4) Acetic acid.

Loading Capacity. Figure 8 shows a loading capacity study for glucose and fructose separation on a Carbomix Ca-NP10:8% column. At 3.6 mg of each standard injection, both peaks are resolved with baseline separation.

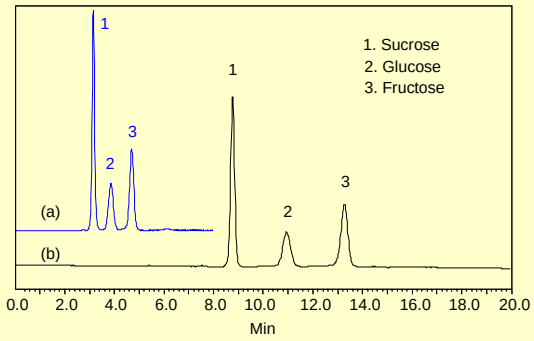
Fig. 8. Loading capacity test on Carbomix Ca NP10 column.



Column: Carbomix Ca-NP10 (10 µm, 8%, 7.8x300 mm)
Mobile phase: H_2O
Flow rate: 0.6 mL/min
Temperature: 85 °C
Detection: RI
Injection amount: 0.9, 1.26, 1.62, 1.8, 2.25, 2.7, 3.25, and 3.6 mg each
Samples: Glucose and Fructose

Column Configuration. Carbomix resins can be packed into a wide range of column dimensions with ID from 2.1mm to 50 mm and the length from 5 cm to 30 cm. Custom-made columns are also available upon request. Column length and diameter affect resolution and analysis time. The principle for choosing a suitable column is to use only as much resin as needed to achieve the desired separation. As shown in Figure 8, by using 7.8×100 mm Carbomix Ca-NP5 column, the analysis time for orange juice sample is only 1/3 of that using 7.8×300 mm.

Fig. 8. Separating profiles of orange juice on two different dimensions of Carbomix Ca-NP5 column.



Column: Carbomix Ca-NP5 (5 µm, 8% a) 7.8x300 mm b) 7.8x100 mm)
Mobile phase: Water
Flow rate: 0.6 mL/min
Temperature: 85 ºC
Injection volume: 10 µL
Detector: RI
Sample: Sucrose, glucose and fructose (15 mM/each)

Separation Mechanisms

The partition process on Carbomix phases is moderated by the counterion (H^+, Ca^{2+}, Pb^{2+}, K^+ and Na^+) bounded to the surface. Usually, at least two or more mechanisms, including size-exclusion, ion-exclusion, ion-exchange, ligand-exchange, reversed-phase and normal-phase, are involved.

As shown in Fig. 4, a size-exclusion mechanism is the primary mechanism for separation of NGNA and NANA. However, ligand-exchange is the primary mechanism for separating monosaccharides, e.g., separation of α- and β-anomers of glucose on Ca-form phase.

To fulfill the separation of a molasses sample, the first primary mechanism involved is ion-exclusion, which allows inorganic sodium to be eluted near the void volume. And then size-exclusion and ligand-exchange take affect one after the other for oligosaccharides and monosaccharides. Ion-exclusion can still play a role for sugar alcohols and carboxylic acids in the matrix.

As to Carbomix-H-form phase for the separation of organic acids, both reversed-phase and ion-exclusion are the primary mechanisms. Hydrophobicity and pK_a together impact the retention time of a component in sample. Due to this property, Carbomix-H phase is the ideal choice for monitoring changing components in the process of fermentation.

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 includes carbohydrates with organic acids, alcohols, glycols, and fermentation products.

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

Phase			**Carbomix H-NP**	**Carbomix Ca-NP**	**Carbomix Pb-NP**	**Carbomix K-NP**	**Carbomix Na-NP**
Support			Non-porous PS/DVB	Non-porous PS/DVB	Non-porous PS/DVB	Non-porous PS/DVB	Non-porous PS/DVB
Particle size (µm)			5, 10	5, 10	5, 10	5, 10	5, 10
Crosslinkage			5%, 8%, 10%	5%, 8%, 10%	5%, 8%, 10%	5%, 8%, 10%	5%, 8%, 10%
Stationary phase			$-SO_3H$	$-(SO_3)_2Ca$	$-(SO_3)_2Pb$	$-SO_3K$	$-SO_3Na$
pH stability			1 – 3	5 – 9	5 – 9	5 – 9	5 – 9
Maximum backpressure (psi)	10%	10 µm	1,200	1,200	1,200	1,200	1,200
	8%	10 µm	1,000	1,000	1,000	1,000	1,000
		5 µm	1,000	1,000	1,000	1,000	1,000
	5%	10 µm	1,000	1,000	1,000	1,000	1,000
Typical mobile phase			2.5 mM H_2SO_4 or 0.1% H_3PO_4	H_2O	H_2O	H_2O	H_2O
Typical flow rate (mL/min)	7.8x300mm		0.4-0.8	0.4-0.8	0.4-0.8	0.4-0.8	0.4-0.8
	4.6x300mm		0.1-0.3	0.1-0.3	0.1-0.3	0.1-0.3	0.1-0.3
Maximum temperature (°C)			85	85	85	85	85

"/" means not suitable for analysis at the operation conditions or not available.

Technical Specifications

Retention timetable for Carbohydrates and Sugar Alcohols

Compound	Carbonix H-NP5	Carbonix H-NP10			Carbonix Ca-NP5	Carbonix Ca-NP10			Carbonix Pb-NP5	Carbonix Pb-NP10	
	8%	5%	8%	10%	8%	5%	8%	10%	8%	5%	8%
Acetic acid	14.11	17.94	15.33	13.7	/	/	/	/	/	/	/
Adonitol	10.39	13.73	11.76	9.97	14.706	16.7625	14.654	13.67	19.84	21.93	20.07
Arabinitol	/	/	/	/	/	/	/	16.06	/	/	/
D-(-)-Arabinose	10.36	13.76	11.71	9.89	13.6045	15.5905	13.3615	12.52	16.03	17.61	16.08
L-(+)-Arabinose	10.35	13.76	11.71	9.89	13.552	15.5945	13.379	12.53	16.02	17.58	16.12
1,4-Butanediol	19.72	23.35	20.63	19.57	15.428	17.9025	15.412	14.48	16.67	17.66	16.64
n-Butanol	33.74	35.76	33.79	34.38	24.583	27.14	25.19	/	27.93	28.34	28.09
t-Butanol	23.21	26.46	23.8	23.43	16.2755	18.6255	16.243	/	16.97	17.71	16.95
sec-butyl alcohol	28.61	31.29	28.81	28.89	19.8715	22.497	20.095	19.01	21.26	22.35	21.37
D-(+)-Cellobiose	8.34	9.87	9.55	7.87	9.92	10.355	9.055	8.96	11.72	11.92	11.47
Citric acid	8.6	10.6	9.9	8.17	/	/	/	/	/	/	/
Erythritol	11.27	14.77	12.62	10.86	15.368	17.3185	15.35	14.47	19.62	21.26	19.77
Ethanol	20.66	22.82	21.22	20.63	16.515	18.1795	16.394	15.89	17.08		17.09
Formic acid	13.08	16.46	14.3	12.67	/	/	/	/	/	/	/
D-Fructose	9.81	12.9	11.17	9.36	13.5535	15.4645	13.347	12.45	17.06	18.82	17.22
Fumaric acid	12.39	17.07	13.77	11.79	/	/	/	/	/	/	/
Galactitol	10.07	13.31	11.45	9.64	19.624	22.5455	20.343	18.05	31.46	35.88	32.43
D-(+)-Galactose	9.7	12.77	11.05	9.25	12.1835	13.963	11.809	11.2	14.85	16.35	14.88
D-Glucose	9.36	12	10.64	8.87	11.274	12.554	10.714	10.32	13.29	14.04	13.18
Glycerol	12.68	16.26	13.98	12.29	15.9965	17.8425	15.974	15.12	19.34	20.59	19.42
2-Propanol	22.29	24.87	22.87	22.38	16.434	18.447	16.374	15.88	17.16	17.72	17.11
Lactic acid	11.93	15.69	13.27	11.51	/	/	/	/	/	/	/
B-Lactose	8.44	10.41	9.71	8.02	10.221	11.092	9.452	9.22	12.35	13.34	12.21
D-Lyxose	10.07	13.25	11.43	9.61	14.02775	16.167	13.922	13.02	16.5	18.04	16.61
Maleic acid	8.75	9.32	9.96	8.39	/	/	/	/	/	/	/
Malic acid	9.59	12.52	10.93	9.13	/	/	/	/	/	/	/
Maltotetraose	/	/	/	/	/	/	/	/	/	/	/
Maltitol	8.55	10.72	9.87	8.14	12.4125	15.0435	12.274	11.24	18.16	22.19	18.65
D-(+)-Maltose	8.37	10.15	9.65	7.94	10.064	10.665	9.268	9.09	12.19	12.8	12.01
Maltotriose	/	/	/	/	/	/	/	8.57	/	/	/
D-Mannitol	9.95	13.14	11.34	9.52	16.92	19.563	17.2445	15.6	24.23	27.79	24.86
D-(+)-Mannose	9.68	12.68	11.02	9.22	12.422	14.238	12.093	11.45	16.23	18.04	16.37
D-(+)-Melezitose	8.05	9.03	9.26	7.63	9.493	9.404	8.51	8.49	10.92	10.9	10.6
Methanol	18.45	20.61	19.14	18.36	16.325	17.7215	16.13	15.64	16.79	17.16	16.69
Oxalic acid dihydrate	7.95	7.53	9.12	7.63	/	/	/	/	/	/	/
1-Propanol	25.24	27.48	25.63	25.57	19.332	21.2605	19.403	18.95	20.75	21.33	20.76
1,2-Propylene glycol	15.89	19.45	17	15.6	17.307	18.8945	17.274	/	20.45	20.97	20.45
Pyruvic acid	/	/	/	/	/	/	/	/	/	/	/
D-(-)-Ribose	10.6	14.04	11.9	10.08	20.842	24.489	21.913	19.23	30.21	34.62	31.1
D-Sorbitol	10.05	13.3	11.43	9.61	20.632	23.786	21.2095	18.71	33.51	39.07	34.72
Succinic acid	11.07	15.33	12.49	10.57	/	/	/	/	/	/	/
D-(+)-Sucrose	/	/	/	/	9.9875	10.594	9.175	9.03	11.74	12.12	11.51
Tartaric Acid	8.93	11.06	10.23	8.49	/	/	/	/	/	/	/
Triethylene glycol	/	/	/	/	/	/	/	17.21	/	/	/
Xylitol	10.75	14.27	12.15	10.33	20.0725	23.067	20.79	18.66	30.7	35.15	31.54
D-Xylose	9.75	12.74	11.11	9.31	12.1335	13.6325	11.692	11.19	14.17	14.94	14.1
Rhamnose	10.09	13.41	11.46	9.64	12.4475	14.172	12.088		14.75	15.76	14.79
Trehalose	8.37	10.14	9.64	7.95	9.9815	10.5605	9.158		11.8	12.25	11.58
Xylulose	/	/	/	/		15.6985			/	/	/
Propanoic acid	16.04	20.68	17.27	15.65					/	/	/
n-butyric acid	18.87	24.7	20.17	18.51					/	/	/
Isobutyric acid	17.52	23.21	18.83	17.14					/	/	/
Butane tetracarboxylic acid	8.43	10.6	9.73	8					/	/	/
Sodium acetate	14.11	17.95	15.34	13.7					/	/	/
Sodium nitrite	/	/	/	/					/	/	/
Sodium Pyrosulfite	12.37	12.07	12.93	/					/	/	/

Conditions

Column Size
7.8 x 300 mm

Carbomix H-NP10
Mobile phase : 2.5 mM H_2SO_4
Flow rate : 0.6 mL/min
Detector : RID
Column Tem. : 80°C
Injection Volume : 10 µL
(3 injections)

Carbomix H-NP5
Mobile phase : 2.5 mM H_2SO_4
Flow rate : 0.6 mL/min
Detector : RID
Column Tem. : 55°C
Injection Volume : 10 µL
(3 injections)

Carbomix Ca-NP10
Mobile phase : H_2O
Flow rate : 0.6 mL/min
Detector : RID
Column Tem. : 85°C
Injection Volume : 10 µL
(3 injections)

Carbomix Ca-NP5
Mobile phase : H_2O
Flow rate : 0.6 mL/min
Detector : RID
Column Tem. : 85°C
Injection Volume : 10 µL
(3 injections)

Carbomix Pb-NP10
Mobile phase : H_2O
Flow rate : 0.6 mL/min
Detector : RID
Column Tem. : 75°C
Injection Volume : 10 µL
(3 injections)

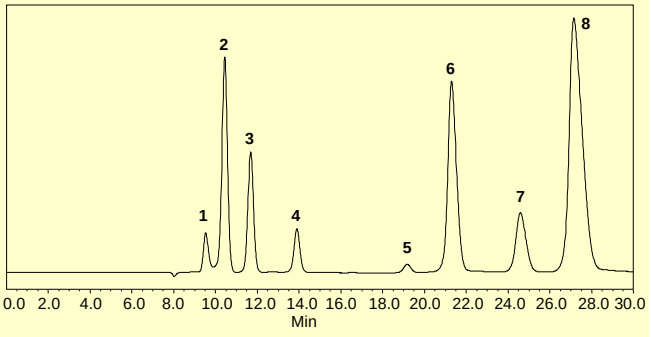
Carbomix Pb-NP5
Mobile phase : H_2O
Flow rate : 0.6 mL/min
Detector : RID
Column Tem. : 75°C
Injection Volume : 10 µL
(3 injections)

Typical industrial applications:

- Food and Beverage
- Fruits and Vegetables
- Wine and Beer
- Clinical Applications
- Oligosaccharides Analysis
- Cellulose and Wood
- Plant Biochemistry
- Fermentation Monitoring
- Metabolite Analysis
- Bacteria and Yeast Analysis
- Glycoproteins and Glycoconjugates
- Nucleic Acids

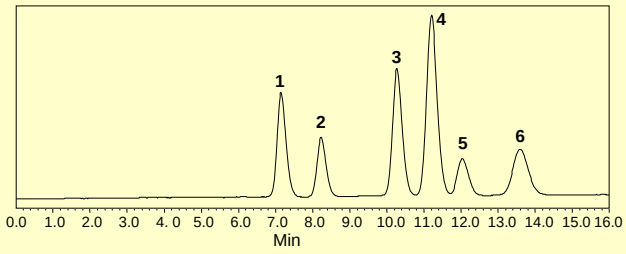
Analysis of Carbohydrates

Fig. 10. Analysis of main components of ethanol production broth on Carbomix H-NP10 column.



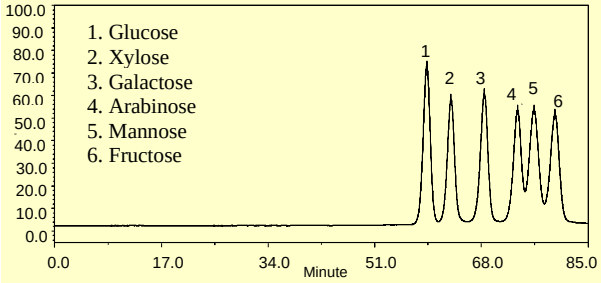
Column:	Carbomix H-NP10 (10 µm, 5%, 7.8×300 mm)
Mobile phase:	2.5 mM H_2SO_4 solution
Flow rate:	0.6 mL/min
Temperature:	55 °C
Injection volume:	20 µL
Detector:	RI
Sample:	1) Stachyose, 2) Maltotriose, 3) Maltose, 4) Glucose, 5) Glycerol, 6) Acetic acid, 7) Methanol, 8) Ethanol

Fig. 11. Separation of carbohydrates on Carbomix Na-NP10 column.



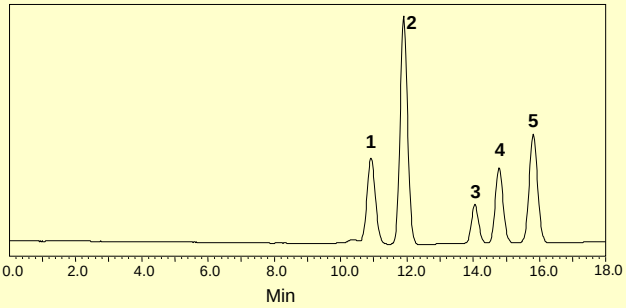
Column:	Carbomix Na-NP10 (10 µm, 8%, 7.8×300 mm)
Mobile phase:	Water
Flow rate:	0.6 mL/min
Temperature:	75 °C
Injection volume:	10 µL
Detector:	RI
Sample:	1) Stachyose, 2) Cellobiose, 3) Glucose, 4) Fructose, 5) Arabinose, 6) Ribose

Fig. 12. Separation of Carbohydrates on two Carbomix Pb-NP10 columns in tandem.



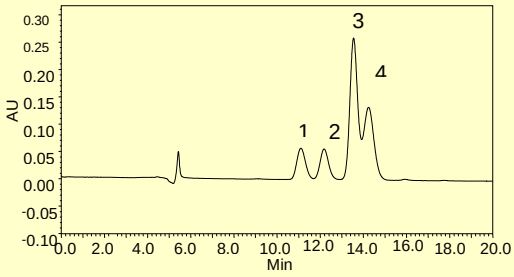
Column:	2 x Carbomix Pb-NP10 (10 µm, 5%, 7.8x300 mm)
Mobile phase:	water
Temperature:	75°C
Flow rate:	0.3 mL/min
Detection:	RI
Sample Injection:	10 µL
Samples:	1) Glucose, 2) Xylose, 3) Galactose, 4) L-Arabinose, 5) Mannose, 6) Fructose (0.5 M each)

Fig. 13. Separation of carbohydrates on Carbomix K-NP5 column.



Column:	Carbomix K-NP5 (5 µm, 8%, 7.8×300 mm)
Mobile phase:	Water
Flow rate:	0.4 mL/min
Temperature:	85 °C
Injection volume:	5 µL
Detector:	RI
Sample:	1) Maltotriose, 2) Maltose, 3) Glucose, 4) Mannose, 5) Fructose

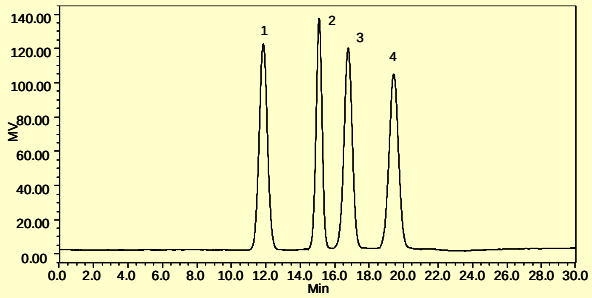
Fig. 14. Separation of monosacchrides by Carbomix Ca-NP5 column.



Column:	Carbomix Ca-NP5 (5 µm, 8%, 7.8×300 mm)
Mobile phase:	Water
Flow rate:	0.6 mL/min
Temperature:	85 °C
Injection volume:	2 µL
Detector:	192 nm
Sample:	1) Glucose, 2) *L*-xylose, 3) Fructose and 4) Lyxose (50 mM/each in water)

Analysis of Sugar Alcohols

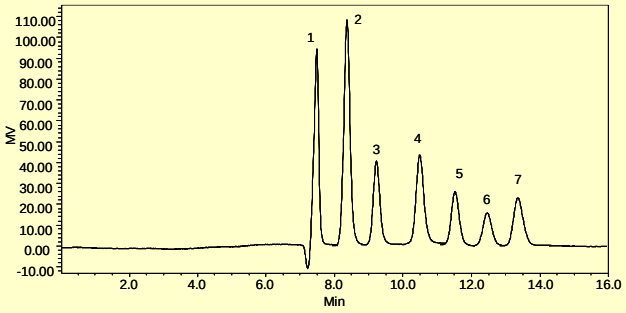
Fig. 15. Separation of sugar alcohols by Carbomix Ca-NP10 column.



Column:	Carbomix Ca-NP10 (10 µm, 8%, 7.8×300 mm)
Mobile phase:	Water
Flow rate:	0.6 mL/min
Temperature:	85 ºC
Injection volume:	10 µL
Detector:	RID
Sample:	1) Maltitol, 2) Erythritol, 3) Mannitol and 4) Galactitol

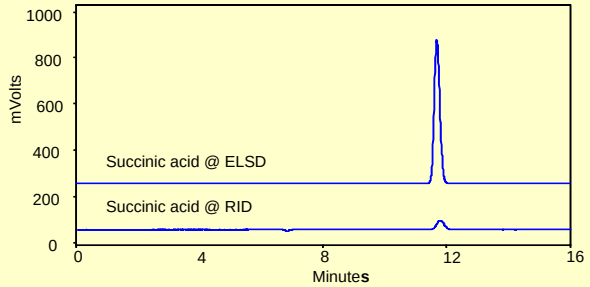
Analysis of Organic Acids

Fig. 16. Separation of organic acid mixture by Carbomix H-NP10 column.



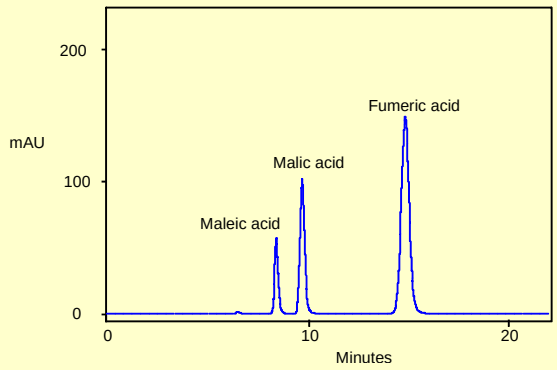
Column:	Carbomix H-NP10 (10 µm, 10%, 7.8×300 mm)
Mobile phase:	2.5 mM H_2SO_4 solution
Flow rate:	0.6 mL/min
Temperature:	80 ℃
Injection volume:	10 µL
Detector:	RI
Sample:	1) Fumaric acid, 2) Citric acid, 3) Malic acid, 4) Succinic acid, 5) Lactic acid, 6) Formic acid, 7) Acetic acid

Fig. 17. Analysis of succinic acid on Carbomix H-NP10 column.



Column:	Carbomix H-NP10 (10 µm, 8%, 7.8x300mm)
Mobile phase:	ELSD - TFA solution, pH 2.5 RID - 2.5 mM H_2SO_4
Flow rate:	0.6 mL/min
Temperature:	55 °C
Detection:	ELSD (drift tube temp.: 65°C; gas flow rate: 2.0 L/min) and RI (30 °C)
Injection volume:	10 µL
Samples:	succinic acid, 0.05 M

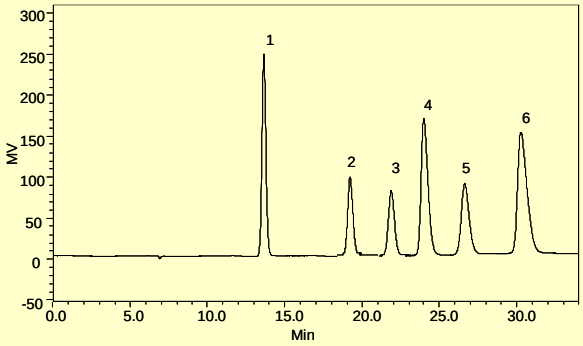
Fig. 18 Analysis of malic acid on Carbomix H-NP10 column.



Column:	Carbomix H-NP10 (10 µm, 8%, 7.8x300 mm)
Mobile phase:	5 mM H_2SO_4
Flow rate:	0.6 mL/min
Temperature:	37 °C
Detection:	UV 210 nm
Injection volume:	20 µL
Samples:	1) maleic acid, 2) malic acid, 3) fumeric acid

Analysis of Alcohols

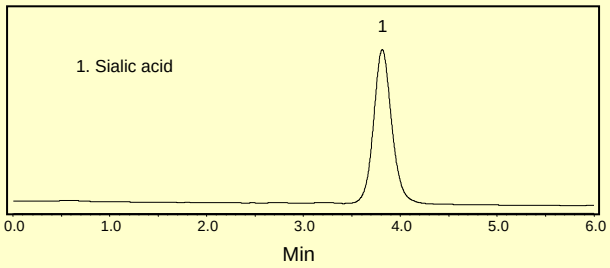
Fig. 19. Separation of alcohol mixture by Carbomix H-NP10 column.



Column:	Carbomix H-NP10 (10 µm, 8%, 7.8×300 mm)
Mobile phase:	2.5 mM H_2SO_4 solution
Flow rate:	0.6 mL/min
Temperature:	80 ºC
Injection volume:	10 µL
Detector:	RI
Sample:	1) Glycerol, 2) Methanol, 3) Ethanol, 4) Iso-propanol, 5) 1-Propanol, 6) sec-Butyl alcohol

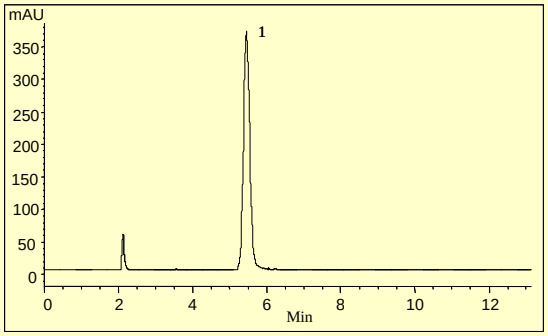
QC of Pharmaceuticals

Fig. 20. Analysis of Sialic acid by Carbomix H-NP5 column.



Column:	Carbomix H-NP5 (5 µm, 7.8 × 100 mm)
Mobile phase:	2.5 mM H_2SO_4 solution
Flow rate:	0.6 mL/min
Temperature:	55 °C
Injection volume:	10 µL
Detector:	192 nm
Sample:	Sialic acid (1mg/mL)

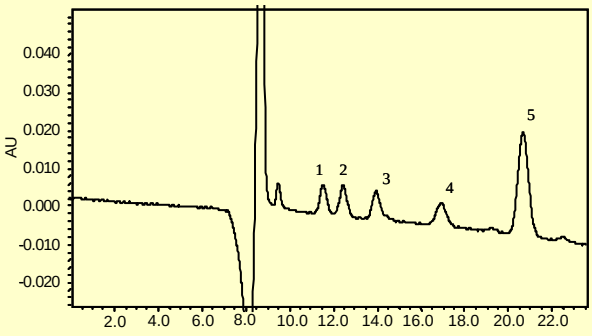
Fig. 21. Analysis of Ribavirin by Carbomix H-NP5 column.



Column:	Carbomix H-NP5 (5 µm, 8%, 7.8×300 mm)
Mobile phase:	2.5 mM H_2SO_4 solution
Flow rate:	0.6 mL/min
Temperature:	55 °C
Injection volume:	10 µL
Detector:	207 nm
Sample:	Ribavirin (50 µg/mL)

Separation of Carbohydrates in Beer

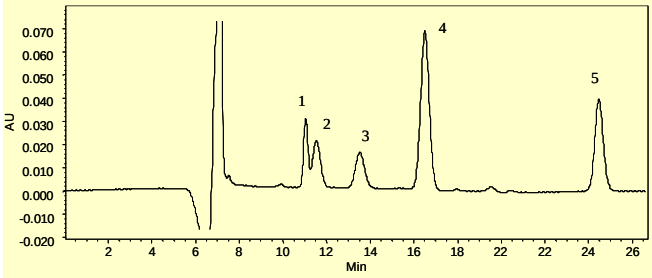
Fig. 22. Separation of carbohydrates in beer by a Carbomix Ca-NP5 column .



Column:	Carbomix H-NP5 (5 µm, 8%, 7.8×300 mm)
Mobile phase:	Water
Flow rate:	0.4 mL/min
Temperature:	85 °C
Injection volume:	2 µL
Detector:	192 nm
Sample:	1) Maltotetrase, 2) Maltotriose, 3) Maltose, 4) Glucose, 5) Fructose (6 mg/mL of each)

Separation of Carbohydrates in Food

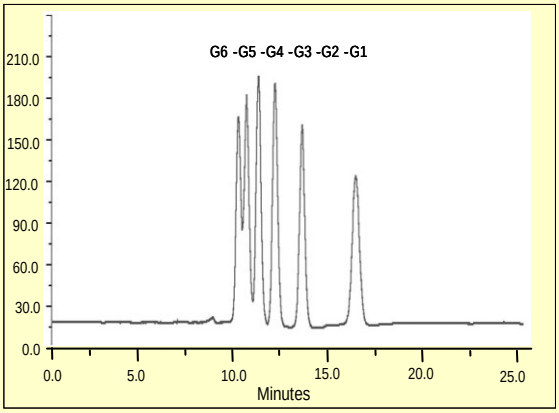
Fig. 23. Separation of carbohydrates in food by a Carbomix Ca-NP5 column.



Column:	Carbomix Ca-NP5 (5 µm, 8%, 7.8×300 mm)
Mobile phase:	Water
Flow rate:	0.5 mL/min
Temperature:	85 °C
Injection volume:	2 µL
Detector:	192 nm
Sample:	1) Surcose, 2) Lactose, 3) Glucose, 4) Fructose, 5) Sorbitol (6 mg/mL in H_2O for each)

Separation of Glucose and its Oligomers

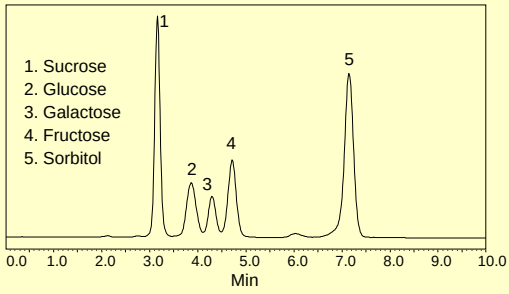
Fig. 24. Separation of glucose and its oligomers by a Carbomix Ca-NP5 column. (Courtesy of Miyako Kawakatsu, M&S Instruments, Inc)



Column:	Carbomix Ca-NP5 (5 µm, 8%, 4.6 × 300 mm)
Mobile phase:	Water
Flow rate:	0.15 mL/min
Temperature:	85 ºC
Injection volume:	10 µL
Detector:	192 nm
Sample:	Glucose (G1) and its oligomers (G2 to G6)

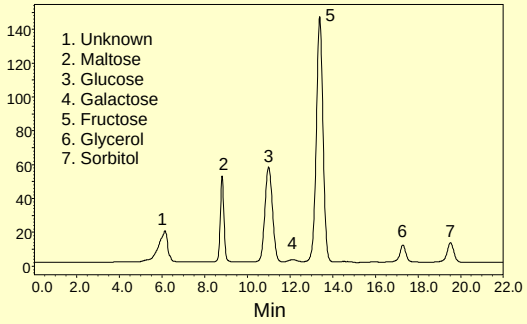
Separation of Carbohydrates and Sugar Alcohols in Beverage

Fig. 25. Separation of carbohydrate and sugar alcohol in apple juice on a Carbomix Ca-NP5 column.



Column:	7.8 × 100 mm
Mobile phase:	Water
Flow rate:	0.6 mL/min
Temperature:	85 ºC
Injection volume:	2 µL
Detector:	RI
Sample:	Mixture of sucrose, glucose, galactose, fructose and sorbitol (50 mM/each in water)

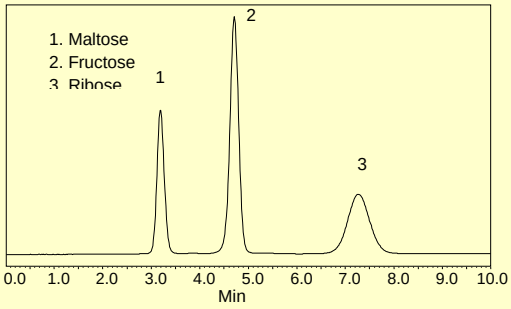
Fig. 26. Profile of carbohydrates and alcohols in a Martinelli's Sparkling Apple-Cranberry juice on a Carbomix Ca-NP5 column.



Column:	Carbomix Ca-NP5 (5 µm, 8%, 7.8×300 mm)
Mobile phase:	Water
Flow rate:	0.6 mL/min
Temperature:	85 ºC
Injection volume:	5 µL
Detector:	RI
Sample:	Martinelli's Sparkling Apple-Cranberry juice

Separation of Carbohydrate in Corn Syrup

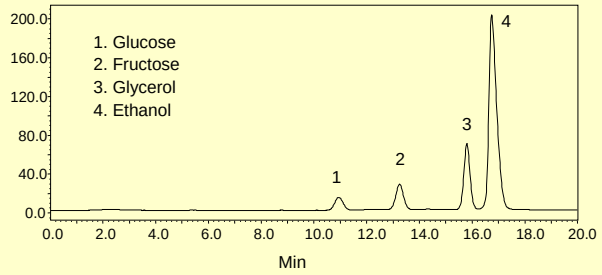
Fig. 27. Separation of carbohydrates on a Carbomix Ca-NP5 column.



Column:	Carbomix Ca-NP5 (5 µm, 8%, 7.8×100 mm)
Mobile phase:	Water
Flow rate:	0.5 mL/min
Temperature:	85 ºC
Injection volume:	2 µL
Detector:	RI
Sample:	Mixture of maltose, fructose and ribose (50 mM/each in water)

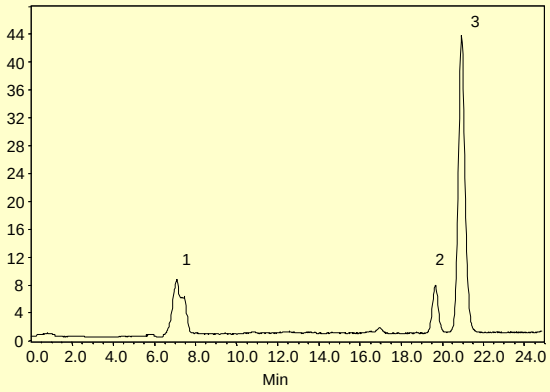
Analysis of Carbohydrate and Alcohol in Wine

Fig. 28. Profile of carbohydrate and alcohols in a Cabernet Sauvignon wine on a Carbomix Ca-NP5 column.



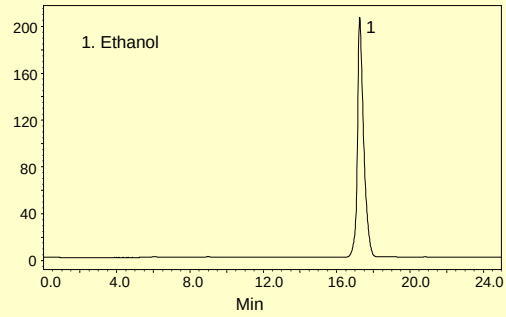
Column:	Carbomix Ca-NP5 (5 µm, 8%, 7.8×300 mm)
Mobile phase:	Water
Flow rate:	0.6 mL/min
Temperature:	85 ºC
Injection volume:	10 µL
Detector:	RI
Sample:	Cabernet Sauvignon

Fig. 29. Profile of main components in a Beaujolais-Villages wine on a Carbomix Ca-NP5 column.



Column:	Carbomix Ca-NP5 (5 µm, 8%, 7.8x300 mm)
Mobile phase:	Water
Flow rate:	0.6 mL/min
Temperature:	85 ºC
Injection volume:	2.5 µL
Detector:	RI
Sample:	Beaujolais-Villages (Louis Jadot 2007)

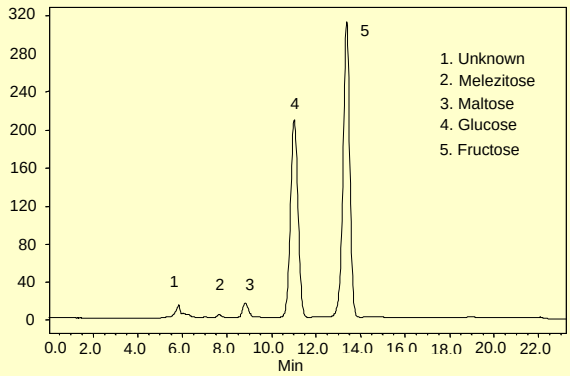
Fig. 30. Profile of a Chinese white wine on a Carbomix Ca-NP5 column.



Column:	Carbomix Ca-NP5 (5 µm, 8%, 7.8×300 mm)
Mobile phase:	Water
Flow rate:	0.6 mL/min
Temperature:	85 ºC
Injection volume:	2.5 µL
Detector:	RI
Sample:	Fen-Jiu (Apricot Blossom Village)

Separation of Carbohydrate in Beverage

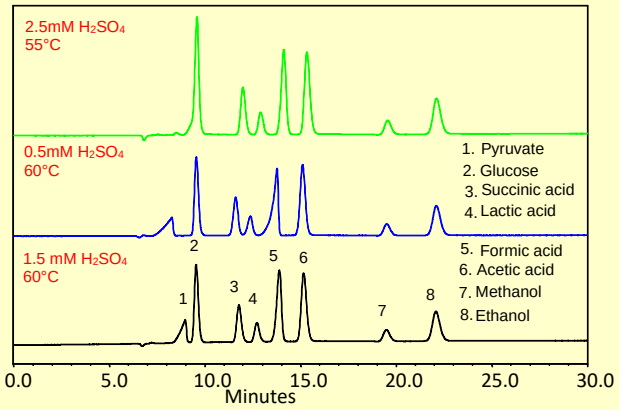
Fig. 31. Profile of carbohydrates in Sprite on a Carbomix Ca-NP5 column.



Column:	Carbomix Ca-NP5 (5 µm, 8%, 7.8x300 mm)
Mobile phase:	Water
Flow rate:	0.6 mL/min
Temperature:	85 ºC
Injection volume:	10 µL
Detector:	RI
Sample:	Sprite

Mobile Phase Optimization

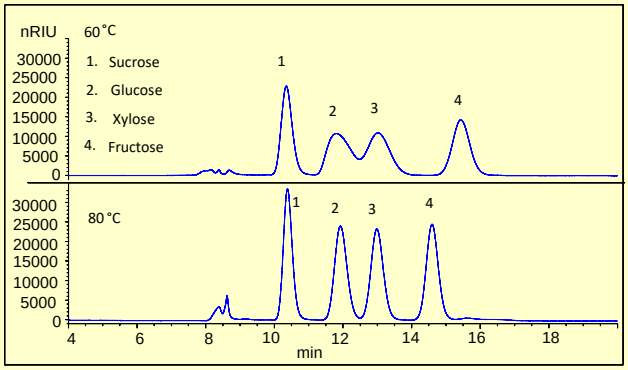
Fig. 32. Analysis of an acid, sugar, and alcohol mixture with different mobile phase concentrations on Carbomix H-NP10.



Column: Carbomix H-NP10 (10 µm, 8%, 7.8x300 mm)
Mobile phase: on chromatogram
Flow rate: 0.6 mL/min
Temperature: on chromatogram
Injection volume: 5 µL
Samples: 1) Pyruvate (0.5 M), 2) Glucose (0.5 M), 3) Succinic Acid (0.5 M), 4) Lactate (0.5 M), 5) Formate (20%), 6) Acetate (20%), 7) Methanol (20%), 8) Ethanol (20%)

Effect of Column Temperature on Sugar Separations

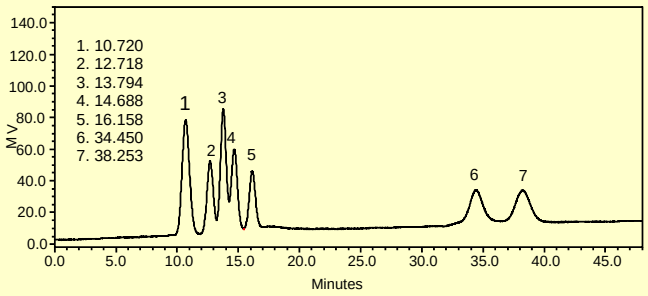
Fig. 33. Effect of column Temperature on sugar separations on a Carbomix Ca-NP5 column.



Column: Carbomix Ca-NP5 (5 µm, 8%, 7.8x300 mm)
Mobile phase: water
Temperature: on chromatogram
Flow rate: 0.5 mL/min
Detection: RI
Injection volume: 2.5 µL
Samples: 1) Sucrose, 2) Glucose, 3) Xylose, 4) Fructose (20mg/mL each)

Analysis of Sugar Mixture

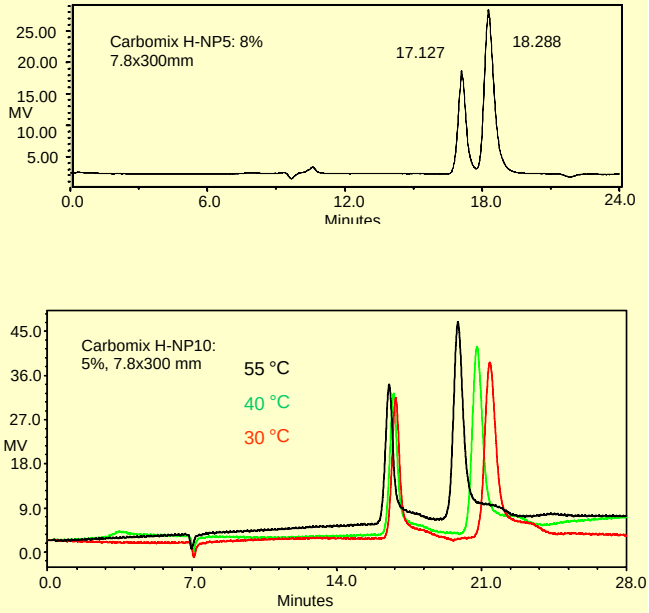
Fig. 34. Analysis of sugar mixture on Carbomix Pb-NP10 column .



Column: Carbomix Pb-NP10 (10 µm, 8%, 7.8x300 mm)
Mobile phase: water
Temperature: 80 °C
Flow rate: 0.6 mL/min
Detection: RI
Sample Injection: 10 µL
Samples: 1) Cellobiose, 2) Glucose, 3) Xylose, 4) Galactose, 5) Arabinose, 6) Xylitol, 7) Sorbitol

Effects of Crosslinkage and Column Temperature

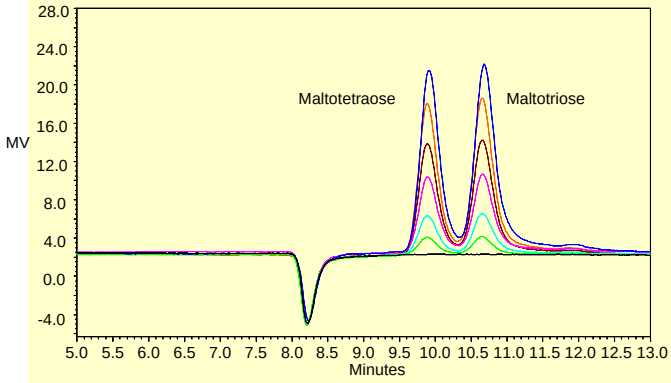
Fig. 35. Effects of crosslinkage and temperature on the separation of formic and levulinic acid on Carbomix H-NP5 and NP10 column .



Mobile phase: 2.5 mM H_2SO_4
Flow rate: 0.5 mL/min
Temperature: 55 °C
Detection: RI
Injection volume: 10 µL
Samples: 1) Formic acid and 2) Levulinic acid (1% each in water)

Analysis of Maltotriose and Maltotetraose

Fig. 36. Analysis Maltotriose and Maltotetraose on Carbomix H-NP10 column.



Column: Carbomix H-NP10 (10 µm, 5%, 7.8x300 mm)
Mobile phase: 2.5 mM H_2SO_4
Flow rate: 0.5 mL/min
Temperature: 55 °C
Detection: RI
Injection volume: 20 µL
Samples: Maltotriose and Maltotetraose (0.1, 0.2, 0.4, 0.6, and 0.8 mg/ml in water)

Maltotetraose/(mg/mL)	Area
0.0	0
0.1	30677
0.2	70406
0.4	144197
0.6	217613
0.8	292733
1.0	356482

Maltotriose/(mg/mL)	Area
0.0	0
0.1	30614
0.2	74714
0.4	148368
0.6	223655
0.8	308321
1.0	383919

Fig. 37. Calibration curve of Maltotetraose on Carbomix H-NP10 column.

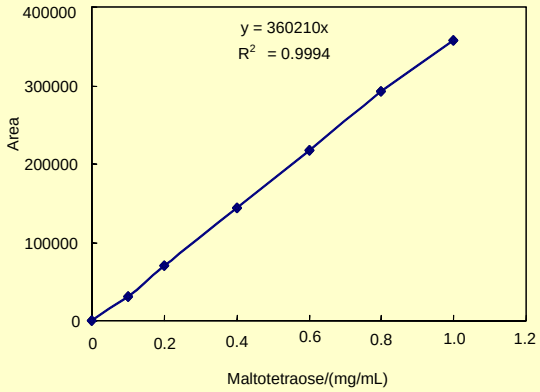
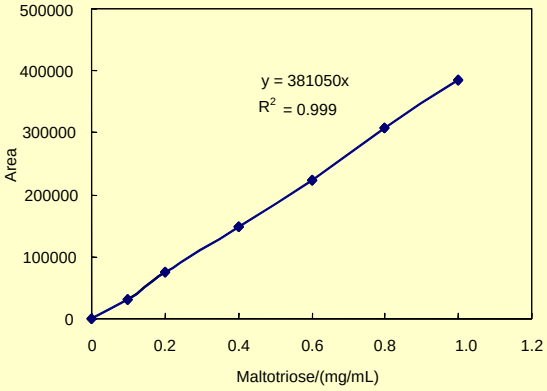


Fig. 38. Calibration curve of Maltotriose on Carbomix H-NP10 column.



Ordering Information

Carbomix resin	**5 µm, 8% Crosslinkage**				
Column	P/N				
ID x Length (mm)	Carbomix H-NP5	Carbomix Ca-NP5	Carbomix Pb-NP5	Carbomix Na-NP5	Carbomix K-NP5
4.6x300	260508-4630	250508-4630	240508-4630	230508-4630	220508-4630
4.6x250	260508-4625	250508-4625	240508-4625	230508-4625	220508-4625
4.6x50 (Guard)	260508-4605	250508-4605	240508-4605	230508-4605	220508-4605
7.8x300	260508-7830	250508-7830	240508-7830	230508-7830	220508-7830
7.8x250	260508-7825	250508-7825	240508-7825	230508-7825	220508-7825
7.8x100	260508-7810	250508-7810	240508-7810	230508-7810	220508-7810
7.8x50 (Guard)	260508-7805	250508-7805	240508-7805	230508-7805	220508-7805
10x300	260508-10030	250508-10030	240508-10030	230508-10030	220508-10030
21.2x300	260508-21230	250508-21230	240508-21230	230508-21230	220508-21230
30x300	260508-30030	250508-30030	240508-30030	230508-30030	220508-30030

Carbomix resin	**10 µm, 5% Crosslinkage**				
Column	P/N				
ID x Length (mm)	Carbomix H-NP10	Carbomix Ca-NP10	Carbomix Pb-NP10	Carbomix Na-NP10	Carbomix K-NP10
4.6x300	261005-4630	251005-4630	241005-4630	231005-4630	221005-4630
4.6x250	261005-4625	251005-4625	241005-4625	231005-4625	221005-4625
4.6x50 (Guard)	261005-4605	251005-4605	241005-4605	231005-4605	221005-4605
7.8x300	261005-7830	251005-7830	241005-7830	231005-7830	221005-7830
7.8x250	261005-7825	251005-7825	241005-7825	231005-7825	221005-7825
7.8x100	261005-7810	251005-7810	241005-7810	231005-7810	221005-7810
7.8x50 (Guard)	261005-7805	251005-7805	241005-7805	231005-7805	221005-7805
10x300	261005-10030	251005-10030	241005-10030	231005-10030	221005-10030
21.2x300	261005-21230	251005-21230	241005-21230	231005-21230	221005-21230
30x300	261005-30030	251005-30030	241005-30030	231005-30030	221005-30030

Carbomix resin	**10 µm, 8% Crosslinkage**				
Column	P/N				
ID x Length (mm)	Carbomix H-NP10	Carbomix Ca-NP10	Carbomix Pb-NP10	Carbomix Na-NP10	Carbomix K-NP10
4.6x300	261008-4630	251008-4630	241008-4630	231008-4630	221008-4630
4.6x250	261008-4625	251008-4625	241008-4625	231008-4625	221008-4625
4.6x50 (Guard)	261008-4605	251008-4605	241008-4605	231008-4605	221008-4605
7.8x300	261008-7830	251008-7830	241008-7830	231008-7830	221008-7830
7.8x250	261008-7825	251008-7825	241008-7825	231008-7825	221008-7825
7.8x100	261008-7810	251008-7810	241008-7810	231008-7810	221008-7810
7.8x50 (Guard)	261008-7805	251008-7805	241008-7805	231008-7805	221008-7805
10x300	261008-10030	251008-10030	241008-10030	231008-10030	221008-10030
21.2x300	261008-21230	251008-21230	241008-21230	231008-21230	221008-21230
30x300	261008-30030	251008-30030	241008-30030	231008-30030	221008-30030

Carbomix resin	**10 µm, 10% Crosslinkage**				
Column	P/N				
ID x Length (mm)	Carbomix H-NP10	Carbomix Ca-NP10	Carbomix Pb-NP10	Carbomix Na-NP10	Carbomix K-NP10
4.6x300	261010-4630	251010-4630	241010-4630	231010-4630	221010-4630
4.6x250	261010-4625	251010-4625	241010-4625	231010-4625	2210101-4625
4.6x50 (Guard)	261010-4605	251010-4605	241010-4605	231010-4605	221010-4605
7.8x300	261010-7830	251010-7830	241010-7830	231010-7830	221010-7830
7.8x250	261010-7825	251010-7825	241010-7825	231010-7825	221010-7825
7.8x100	261010-7810	251010-7810	241010-7810	231010-7810	221010-7810
7.8x50 (Guard)	261010-7805	251010-7805	241010-7805	231010-7805	221010-7805
10x300	261010-10030	251010-10030	241010-10030	231010-10030	221010-10030
21.2x300	261010-21230	251010-21230	241010-21230	231010-21230	221010-21230
30x300	261010-30030	251010-30030	241010-30030	231010-30030	221010-30030

How to Order

www.sepax-tech.com

Contact Sepax Sales Department by
Phone: (302) 366-1101
Fax: (302) 366-1151
E-mail: sales@sepax-tech.com

5 Innovation Way, Suite 100
Delaware Technology Park
Newark, Delaware 19711
USA

Discounts

Sepax Technologies offers best discounts determined by the volume of the purchase. Please contact the Sepax Sales Department for your maximum discount.

Opening a Sepax account

Call the Sepax Sales Department and supply your business information, and billing and shipping address to set up a Sepax account. Open account terms are subject to credit approval.

Payment Terms

Terms of payment are net 30 days. Mastercard®, Visa®, and American Express® are accepted. There is no minimum order.

Shipping

If items are damaged in transit, simply follow these instructions:
• If shipment is visibly damaged on arrival, do not accept it until the delivery person has endorsed it with a statement for the extent of damage.
• Notify us immediately of the damaged shipment in order for us to make the appropriate adjustment and/or provide you with return instructions.

Returns

Returns are accepted only with prior authorization. Call Sepax Technical Support to describe the problem that happened. Please provide us with the sales order number, product number, and quantity damaged. Sepax Technical Support will give you instructions for returns. All claims must be made within 15 business days after receipt of product. A 10% charge will be made on cancelled orders or customer order errors.

Warranty

Sepax Technologies warrants its products to be free from manufacturing defects for 90 days after the shipment. Sepax will accept for return or replacement any product which fails to meet the stated specifications. This warranty shall not apply to any defect, failure or damage caused by improper use or improper or in-adequate maintenance and care. This warranty is exclusive and no other warranty, whether written or oral is expressed or implied. Sepax specifically disclaims the implied warranties of merchant-stability and fitness for a particular purpose. Under no circumstance shall Sepax be liable for direct, indirect or consequential damages arising from the use of its products. The maximum liability that Sepax will assume should be no more than the invoice price of the product.