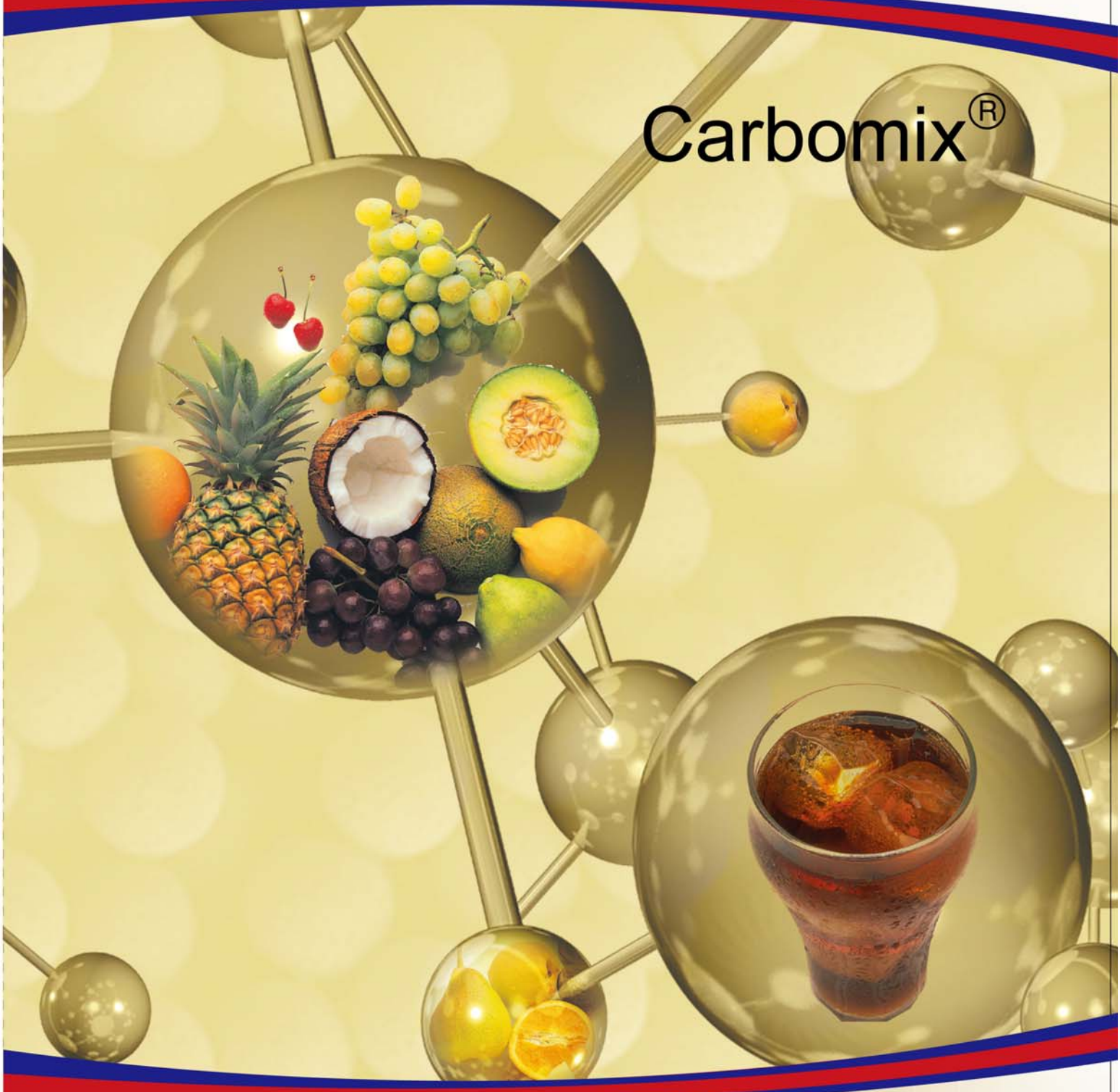
Carbohydrate and Organic Acid Separation

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.

A 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 the separation challenges in biological area.

Bioseparation Products

Size Exclusion

SRT®

Nanofilm®

Zenix™

Ion-exchange

Proteomix®

Antibody Separation

Antibodix™

Carbohydrate Separation

Carbomix®

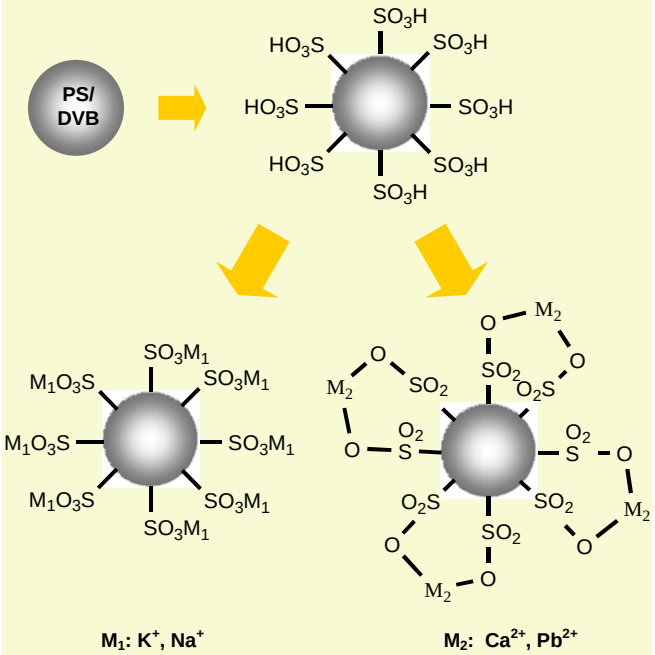
Analytical, Semi-prep and Preparative

Carbomix® Phases

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, 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).

Figure 1. Chemical processing of Carbomix phases



Highlights of Carbomix Resins

- Uniform particle size for high resolution and efficiency separation
- 5%, 8% and 10% crosslinking
- 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 ℃)

Characters 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 to offer high efficiency and high resolution separations. Figure 3 shows that a Carbomix Ca-NP5 column provides higher efficiencies for separating monosaccharides as a comparison to other brands.

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

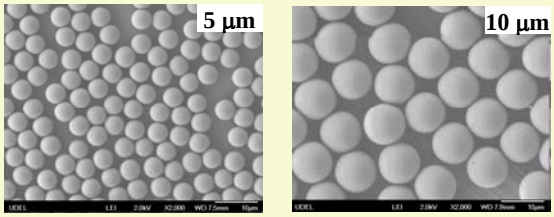
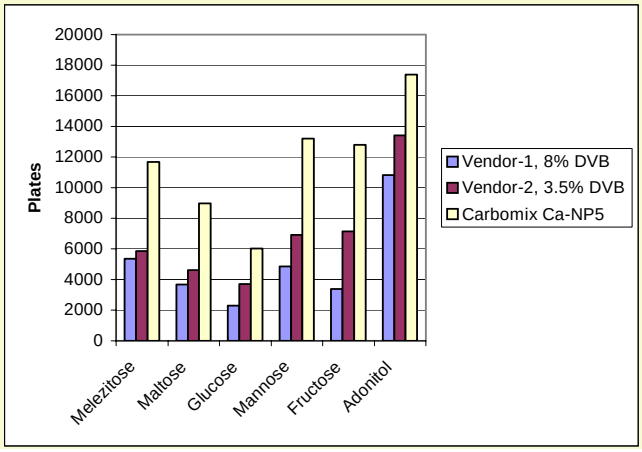


Figure 3. Comparison of efficiencies for 6 monosacchrides on a Carbomix Ca-NP5 (5 μm, 8% crosslinking, 7.8 × 300 mm) and same dimension Calcium columns from other vendors at same separation conditions (mobile phase: H_2O; flow rate: 0.60 mL/min; temperature: 85 °C; injection volume: 20 μL; detection: RI).



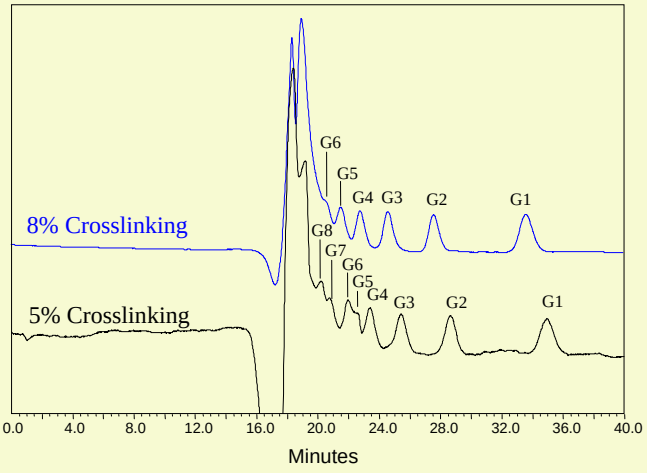
Various Ionic Forms. The wide range ionic forms of Carbomix phases available allows for most suitable column choices for achieving the best separation for different kinds of sugar molecules, sugar alcohols and other water soluble compounds from different sources (Table 1). For instance, arabinose, ribitol and arabitol are difficult to be separated on an H-form phase but well resolved on a 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 tetrasaccharide 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

Figure 4. Elution profiles of glucidex and glycerol on Carbomix Ca-NP5 (5um, 7.8 × 300 mm) columns with different crosslinkage.

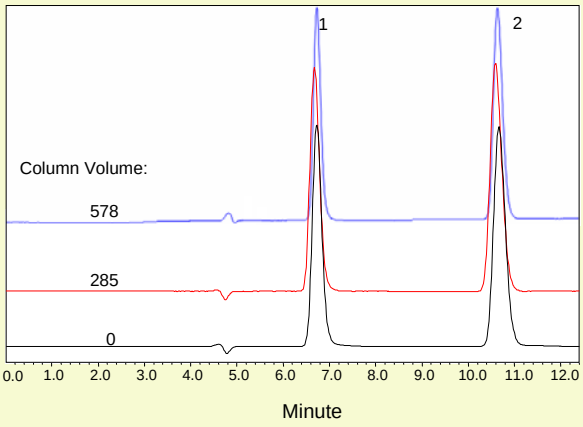


Columns: Carbomix Ca-NP5
Mobile phase: Water
Flow Rate: 0.20 mL/min
Temperature: 85 °C
Injection volume: 20 µL
Detection: 192 nm
Sample: Glucidex (100mg/mL) and glycerol (10 mg/mL) (G3 to G7 refer to oligosaccharides)

Low Crosslinkage. The low crosslinking property of Carbomix resins allows for proper swell in the mobile, especially at high temperature that is 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 separation. The lower the crosslinkage of PS/DVB beads, the more open the phase structure and the permeability it is to sample like larger oligosaccharides. Figure 4 shows that hexasaccharide (G6) is well separated from the sample matrix on a 5% Carbomix Ca-NP5 column accompanied by two more peaks, heptasaccharide (G7) and octasaccharide (G8); however, on an 8% Carbomix Ca-NP5 column, not only G7 and G8 peaks cannot be observed, but also G6 peak is hard to be distinguished.

Stability. Carbomix HPLC 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 temperature. As shown in Figure 5, the retention time of the acids changed only 0.1% after continuously washed with 578 column volumes at 55℃ for 6 days.

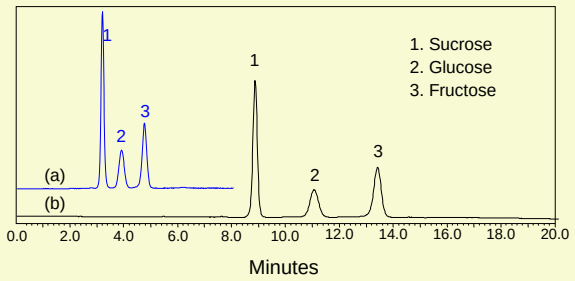
Figure 5. Chromatograms of organic acids on a Carbomix H-NP5 (5um, 8% crosslinking) column after the column being continuously flushed with 5 mM H_2SO_4 at 55 ℃.



Column: 4.6 × 250 mm
Mobile Phase: 5 mM H_2SO_4
Flow Rate: 0.30 mL/min
Temperature: 55 °C
Injection Volume: 20 µL
Detection: RI
Sample: 1, Citric acid (50mM); 2, Acetic acid (100mM)

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. Custom columns are also available. 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 6, by using a 7.8 × 100 mm Carbomix Ca-NP5 column, the analysis time for an orange juice sample is only 1/3 of that using a 7.8 × 300 mm.

Figure 6. Separating profiles of orange juice by a 7.8 × 300 mm and a 7.8 × 100 mm Carbomix Ca-NP5 column (5um, 8% cross-linking) column.



Column:	(a) 7.8 × 100 mm; (b) 7.8 × 300 mm
Mobile phase:	Water
Flow rate:	0.6 mL/min
Temperature:	85℃
Injection volume:	10 μL
Detector:	RI
Sample:	Sucrose, glucose and fructose (15mM/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 mechanism, including size-exclusion, ion-exclusion, ion-exchange, ligand-exchange, reversed-phase and normal-phase, are involved.

For carbohydrate sample, size-exclusion mechanism is the primary one for oligosaccharides, as shown in Figure 4. However, ligand-exchange is the primary one for separating monosaccharides, e.g., the separation of α- and β-anomers of glucose on a 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 effect 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 mechanism. Hydrophobicity and pK_a together impact the retention time of a component in the sample. It is due to this property, it has turned Carbomix-H phase into an ideal choice for monitoring changing components in the process of fermentation.

Technical Specifications

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% and 10%	5%, 8% and 10%	5%, 8% and 10%	5%, 8% and 10%	5%, 8% and 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) (1)	10% crosslinking	10 μm	1,500	1,500	1,500	1,500	1,500
		5 μm	1,000	1,000	1,000	1,000	1,000
	8% crosslinking	10 μm	1,000	1,000	1,000	1,000	1,000
		5 μm	800	800	800	800	800
	5% crosslinking	10 μm	600	600	600	600	600
		5 μm	400	400	400	400	400
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)(2)	A 7.8x300mm column		0.4-0.8	0.4-0.8	0.4-0.8	0.4-0.8	0.4-0.8
	A 4.6x300mm column		0.1-0.30	0.1-0.30	0.1-0.30	0.1-0.30	0.1-0.30
Maximum temperature (°C)			85	85	85	85	85

(1) For a 7.8 × 300 mm column at maximum temperature, and (2) the back pressure from the instrument being subtracted.

(2) For 5μm, 5% crosslinking resin, the flow rate for a 7.8x300mm column is 0.1 to 0.15 mL/min.

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

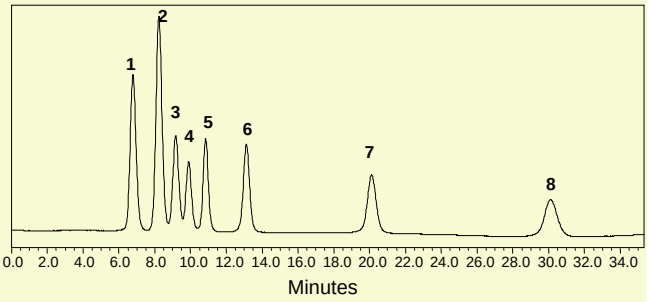
Carbohydrate analyses include samples of beet sugars, molasses, corn syrup, pentose sugars, cellulose hydrolysates, oligosaccharides, glucose, galactose, sucrose, and fructose.

Aspects of 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

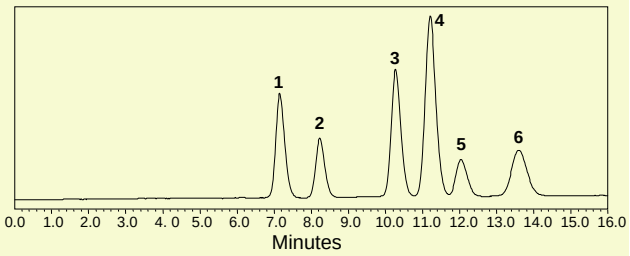
Detection of Carbohydrates on Different Type of Carbomix Columns

Figure 7. Separation of carbohydrates and sugar alcohols on a Carbomix Pb-NP5 column (5 µm, 5% crosslinking).



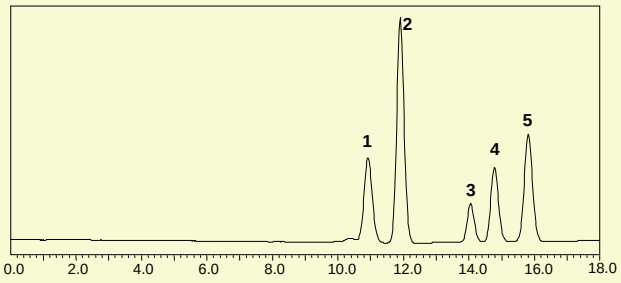
Column: 7.8 × 100 mm
Mobile phase: Water
Flow rate: 0.3 mL/min
Temperature: 75℃
Injection volume: 20 µL
Detector: RI
Sample: Stachyose (1), Maltose (2), Glucose (3), Xylose (4), Galactose (5), Fructose (6), Mannitol (7), Sorbitol (8)

Figure 8. Separation of carbohydrates on a Carbomix Na-NP10 column (10 µm, 8% crosslinking).



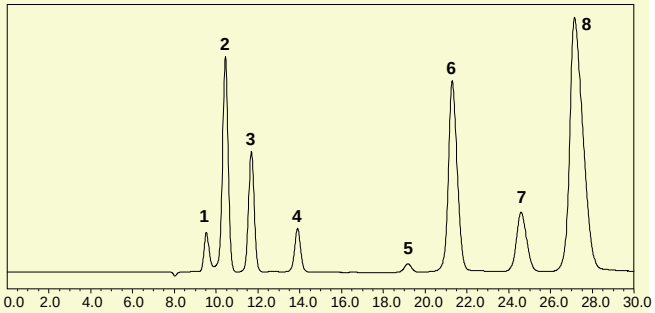
Column: 7.8 × 300 mm
Mobile phase: Water
Flow rate: 0.6 mL/min
Temperature: 75℃
Injection volume: 10 µL
Detector: RI
Sample: Stachyose (1), Cellobiose (2), Glucose (3), Fructose (4), Arabinose (5), Ribose (6)

Figure 9. Separation of carbohydrates on a Carbomix K-NP5 column (5 µm, 10% crosslinking).



Column: 7.8 × 300 mm
Mobile phase: Water
Flow rate: 0.4 mL/min
Temperature: 85℃
Injection volume: 5 µL
Detector: RI
Sample: Maltotriose (1), Maltose (2), Glucose (3), Mannose (4), Fructose (5)

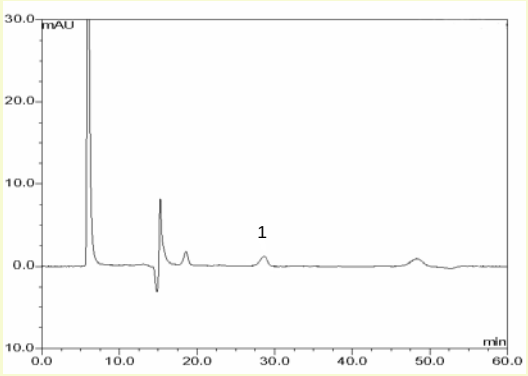
Figure 10. Separation of main components of the broth for ethanol production on a Carbomix H-NP10 column (10 µm, 5% crosslinking).



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

Detection of Acrylamide in Oliver Oil

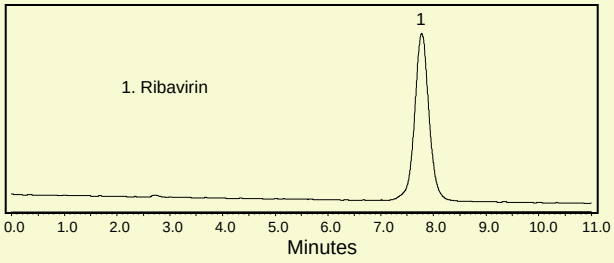
Figure 11. Separation profile of acrylamide in Oliver oil by a Carbomix H-NP5 column (5 μm, 8% crosslinking).



Column: 4.6 × 250 mm
Mobile phase: 3.5mM H_2SO_4: ACN = 93: 7 (v/v)
Flow rate: 0.25 mL/min
Temperature: 55℃
Injection volume: 20 μL
Detector: 202 nm
Sample: Oliver oil (1, acrylamide)

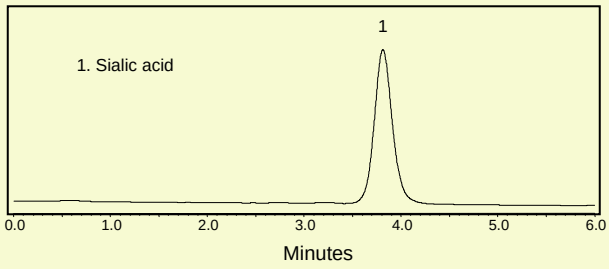
QC for Biomedical and Pharmaceutical

Figure 12. Chromatogram of Ribavirin on a Carbomix H-NP5 column (5 μm, 8% crosslinking).



Column: 7.8 × 300 mm
Mobile phase: H_2SO_4 solution (pH: 2.5)
Flow rate: 0.6 mL/min
Temperature: 55℃
Injection volume: 10 μL
Detector: 207 nm
Sample: Ribavirin (50μg/mL)

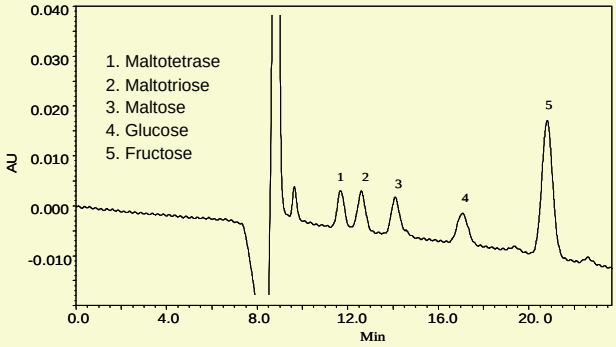
Figure 13. Chromatogram of Sialic acid by a Carbomix H-NP5 column (5 μm, 8% crosslinking).



Column: 7.8 × 100 mm
Mobile phase: 2.5 mM H_2SO_4 solution
Flow rate: 0.6 mL/min
Temperature: 55℃
Injection volume: 10 μL
Detector: 192 nm
Sample: Sialic acid (1mg/mL)

Separation of Carbohydrates in Beer

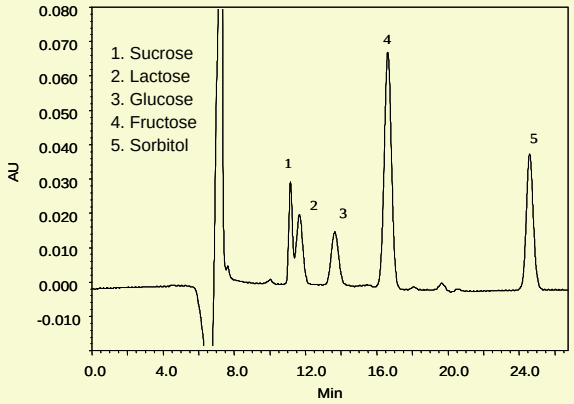
Figure 14. Separation of carbohydrates in beer by a Carbomix Ca-NP5 column (5um, 8% crosslinking).



Column: 7.8 × 300 mm
Mobile phase: Water
Flow rate: 0.4 mL/min
Temperature: 85℃
Injection volume: 2 μL
Detector: 192 nm
Sample: Mixture of carbohydrates. (6mg/mL of each)

Separation of Carbohydrates in Food

Figure 15. Separation of carbohydrates in food by a Carbomix Ca-NP5 column (5 μm, 8% crosslinking).

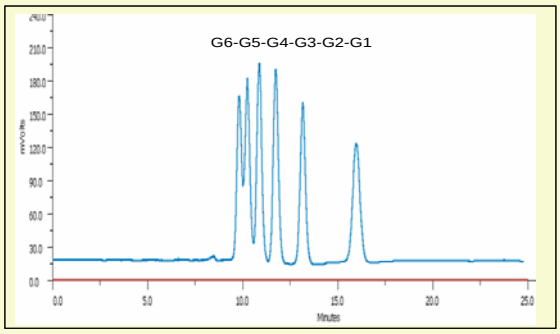


Column: 7.8 × 300 mm
Mobile phase: Water

Flow rate:	0.5 mL/min
Temperature:	85℃
Injection volume:	2 µL
Detector:	192 nm
Sample:	Mixture of carbohydrates (6 mg/mL for each)

Separation of Glucose and its Oligomers

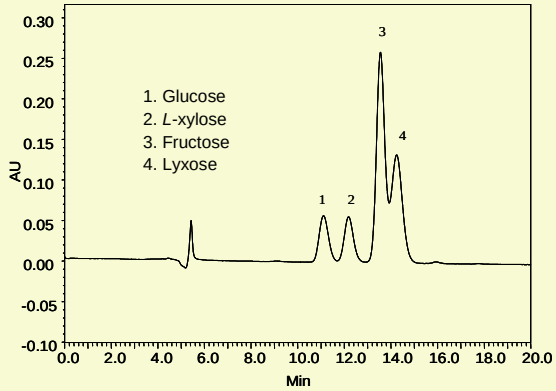
Figure 16. Separation of glucose and its oligomers by a Carbomix Ca-NP5 column (5 µm, 8% crosslinking). (Courtesy of Miyako Kawakatsu, M&S Instruments, Inc)



Column:	4.6 × 300 mm
Mobile phase:	Water
Flow rate:	0.15 mL/min
Temperature:	85℃
Injection volume:	10 µL
Detector:	192 nm
Sample:	Glucose (G1) and its oligomers (G2 to G6)

Separation of Monosacchrides

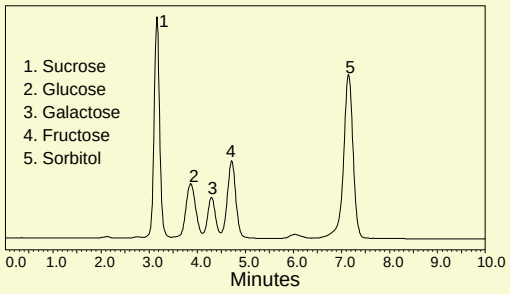
Figure 17. Separation of monosacchrides by a Carbomix Ca-NP5 column (5 µm, 8% crosslinking, 7.8 × 300 mm).



Column:	7.8 × 300 mm
Mobile phase:	Water (pH: 6.0)
Flow rate:	0.6 mL/min
Temperature:	85℃
Injection volume:	2 µL
Detector:	192 nm
Sample:	Glucose, *L*-xylose, fructose and lyxose (50 mM/each in water)

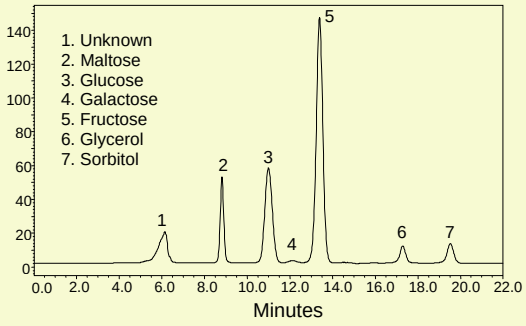
Separation of Carbohydrates and Sugar Alcohols in Beverage

Figure 18. Separation of carbohydrate and sugar alcohol in apple juice on a Carbomix Ca-NP5 column (5 µm, 8% crosslinking).



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

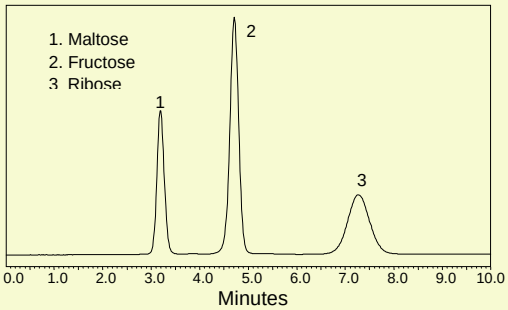
Figure 19. Profile of carbohydrates and alcohols in a Martinelli's Sparkling Apple-Cranberry juice on a Carbomix Ca-NP5 column (5 µm, 8% crosslinking).



Column:	7.8 × 300 mm
Mobile phase:	Water
Flow rate:	0.6 mL/min
Temperature:	85℃
Injection volume:	5 µL
Detector:	RI
Sample:	Martinelli's Sparkling Apple-Cranberry juice

Separation of Carbohydrate in Corn Syrup

Figure 20. Separation of carbohydrates on a Carbomix Ca-NP5 column (5 µm, 8% crosslinking).

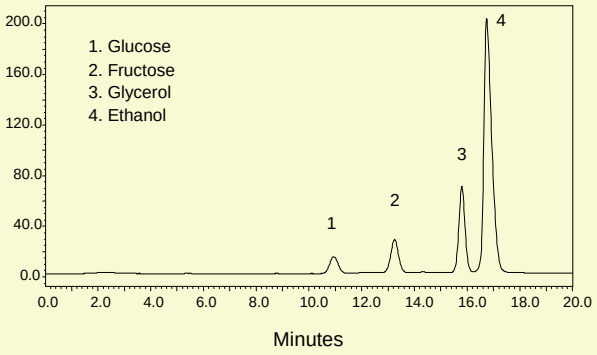


Column:	7.8 × 100 mm

Mobile phase:	Water
Flow rate:	0.5 mL/min
Temperature:	85℃
Injection volume:	2 µL
Detector:	RI
Sample:	Mixture of maltose, fructose and ribose (50 mM/each in water)

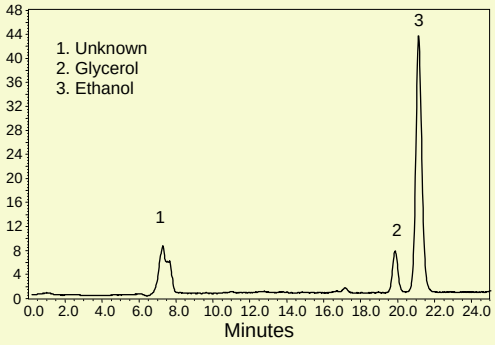
Analysis of Carbohydrate and Alcohol in Wine

Figure 21. Profile of carbohydrate and alcohols in a Cabernet Sauvignon wine on a Carbomix Ca-NP5 column (5 µm, 8% crosslinking).



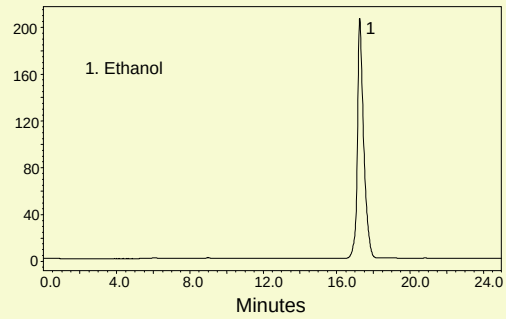
Column:	7.8 × 300 mm
Mobile phase:	Water
Flow rate:	0.6 mL/min
Temperature:	85℃
Injection volume:	10 µL
Detector:	RI
Sample:	Cabernet Sauvignon

Figure 22. Profile of main components in a Beaujolais-Villages wine on a Carbomix Ca-NP5 column (5 µm, 8% crosslinking).



Column:	7.8 × 300 mm
Mobile phase:	Water
Flow rate:	0.6 mL/min
Temperature:	85℃
Injection volume:	2.5 µL
Detector:	RI
Sample:	Beaujolais-Villages (Louis Jadot 2007)

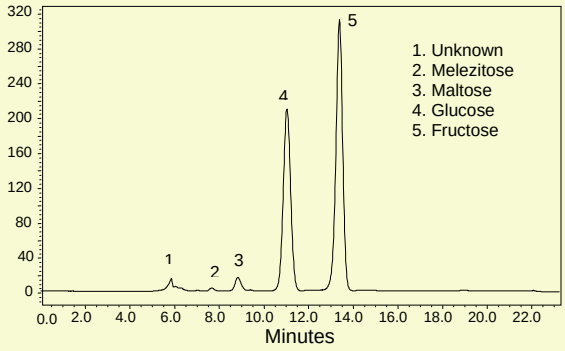
Figure 22. Profile of a Chinese white wine on a Carbomix Ca-NP5 column (5 µm, 8% crosslinking).



Column:	7.8 × 300 mm
Mobile phase:	Water
Flow rate:	0.6 mL/min
Temperature:	85℃
Injection volume:	2.5 µL
Detector:	RI
Sample:	Fen-Jiu (Apricot Blossom Village)

Separation of Carbohydrate in Corn Syrup

Figure 24. Profile of carbohydrates in Sprite on a Carbomix Ca-NP5 column (5 µm, 8% crosslinking).



Column:	7.8 × 300 mm
Mobile phase:	Water
Flow rate:	0.6 mL/min
Temperature:	85℃
Injection volume:	10 µL
Detector:	RI
Sample:	Sprite

Price list of Carbomix Columns

Carbomix H-NP Columns

ID × Length (mm)	P/N	Price	P/N			Price
	5 μm		10 μm			
	Crosslinkage		*Crosslinkage*			
	8%		5%	8%	10%	
4.6 × 50	260508-4605	$ 275.00	261005-4605	261008-4605	261010-4605	$235.00
4.6 × 250	260508-4625	$575.00	261005-4625	261008-4625	261010-4625	$485.00
4.6 × 300	260508-4630	$635.00	261005-4630	261008-4630	261010-4630	$540.00
7.8 × 50	260508-7805	$375.00	261005-7805	261008-7805	261010-7805	$325.00
7.8 × 100	260508-7810	$675.00	261005-7810	261008-7810	261010-7810	$575.00
7.8 × 300	260508-7830	$835.00	261005-7830	261008-7830	261010-7830	$710.00
10 × 300	260508-10030	$1285.00	261005-10030	261008-10030	261010-10030	$1090.00
21.2 × 250	260508-21225	Inquire	261005-21225	261008-21225	261010-21225	Inquire

Carbomix Ca-NP Columns

ID × Length (mm)	P/N	Price	P/N			Price
	5 μm		10 μm			
	Crosslinkage		*Crosslinkage*			
	8%		5%	8%	10%	
4.6 × 50	250508-4605	$ 275.00	251005-4605	251008-4605	251010-4605	$235.00
4.6 × 250	250508-4625	$575.00	251005-4625	251008-4625	251010-4625	$485.00
4.6 × 300	250508-4630	$635.00	251005-4630	251008-4630	251010-4630	$540.00
7.8 × 50	250508-7805	$375.00	251005-7805	251008-7805	251010-7805	$325.00
7.8 × 100	250508-7810	$675.00	251005-7810	251008-7810	251010-7810	$575.00
7.8 × 300	250508-7830	$835.00	251005-7830	251008-7830	251010-7830	$710.00
10 × 300	250508-10030	$1285.00	251005-10030	251008-10030	251010-10030	$1090.00
21.2 × 250	250508-21225	Inquire	251005-21225	251008-21225	251010-21225	Inquire

Carbomix Pb-NP Columns

ID × Length (mm)	P/N	Price	P/N			Price
	5 μm		10 μm			
	Crosslinkage		*Crosslinkage*			
	8%		5%	8%	10%	
4.6 × 50	240508-4605	$ 275.00	241005-4605	241008-4605	241010-4605	$235.00
4.6 × 250	240508-4625	$575.00	241005-4625	241008-4625	241010-4625	$485.00
4.6 × 300	240508-4630	$635.00	241005-4630	241008-4630	241010-4630	$540.00
7.8 × 50	240508-7805	$375.00	241005-7805	241008-7805	241010-7805	$325.00
7.8 × 100	240508-7810	$675.00	241005-7810	241008-7810	241010-7810	$575.00
7.8 × 300	240508-7830	$835.00	241005-7830	241008-7830	241010-7830	$710.00
10 × 300	240508-10030	$1285.00	241005-10030	241008-10030	241010-10030	$1090.00
21.2 × 250	240508-21225	Inquire	241005-21225	241008-21225	241010-21225	Inquire

Carbomix K-NP Columns (ID × Length mm)

ID × Length (mm)	P/N	Price	P/N			Price
	5 μm		10 μm			
	Crosslinkage		*Crosslinkage*			
	8%		5%	8%	10%	
4.6 × 50	230508-4605	$ 275.00	231005-4605	231008-4605	231010-4605	$235.00
4.6 × 250	230508-4625	$575.00	231005-4625	231008-4625	231010-4625	$485.00
4.6 × 300	230508-4630	$635.00	231005-4630	231008-4630	231010-4630	$540.00
7.8 × 50	230508-7805	$375.00	231005-7805	231008-7805	231010-7805	$325.00
7.8 × 100	230508-7810	$675.00	231005-7810	231008-7810	231010-7810	$575.00
7.8 × 300	230508-7830	$835.00	231005-7830	231008-7830	231010-7830	$710.00
10 × 300	230508-10030	$1285.00	231005-10030	231008-10030	231010-10030	$1090.00
21.2 × 250	230508-21225	Inquire	231005-21225	231008-21225	231010-21225	Inquire

Carbomix Na-NP Columns

ID × Length (mm)	P/N	Price	P/N			Price
	5 μm		10 μm			
	Crosslinkage		*Crosslinkage*			
	8%		5%	8%	10%	
4.6 × 50	220508-4605	$ 275.00	221005-4605	221008-4605	221010-4605	$235.00
4.6 × 250	220508-4625	$575.00	221005-4625	221008-4625	221010-4625	$485.00
4.6 × 300	220508-4630	$635.00	221005-4630	221008-4630	221010-4630	$540.00
7.8 × 50	220508-7805	$375.00	221005-7805	221008-7805	221010-7805	$325.00
7.8 × 100	220508-7810	$675.00	221005-7810	221008-7810	221010-7810	$575.00
7.8 × 300	220508-7830	$835.00	221005-7830	221008-7830	221010-7830	$710.00
10 × 300	220508-10030	$1285.00	221005-10030	221008-10030	221010-10030	$1090.00
21.2 × 250	220508-21225	Inquire	221005-21225	221008-21225	221010-21225	Inquire

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