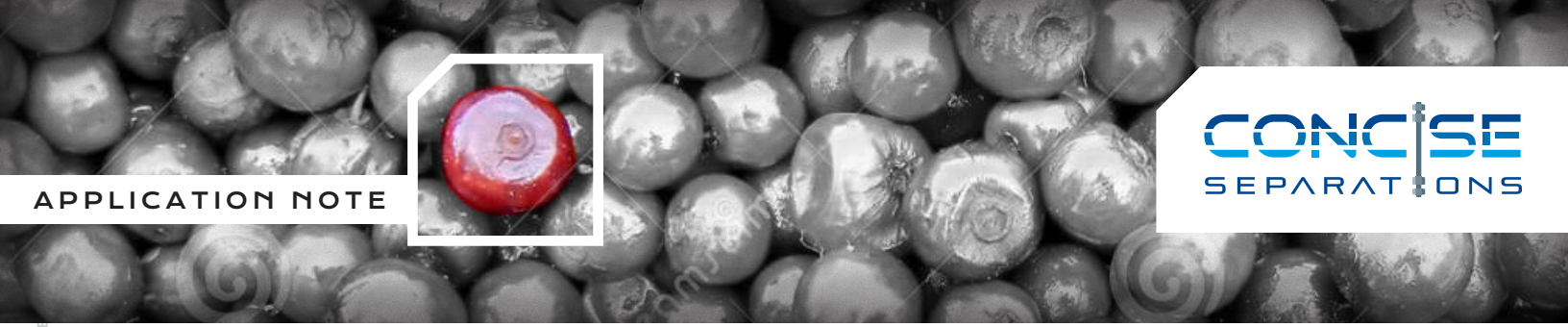


APPLICATION NOTE

CONCISE SEPARATIONS

CARBOHYDRATE ANALYSIS HPLC COLUMNS

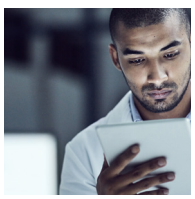
For over 30 years, Concise Separations has supplied the world with superior HPLC products and columns, providing customers with solutions for all types of carbohydrate separations. In this issue, we highlight some exciting new applications of our products, developed for customers using our Carbohydrate Analysis HPLC columns.

Ligand exchange is the preferred method for the separation of many sugars and sugar alcohols due to the simple water eluent. In ligand exchange, the negatively charged hydroxyl groups on the carbohydrate molecule interact with the positively charged, metal-loaded groups on the chromatography substrates. The carbohydrates are eluted by the polar water eluent mobile phase which competes for the sites on the metal ion.

Besides the ligand exchange mechanism, several secondary mechanisms' processes are also involved in the separation of the carbohydrates including size exclusion and normal phase partitioning. HPLC columns packed with low cross-linked polymers (gels) serve as the primary packings for carbohydrate analysis columns, and are available from a number of suppliers. In order to maximize the separation of a wide variety of samples, Concise Separations has developed the most complete line of carbohydrate analysis columns available on the market by combining ligand exchange (metals), size exclusion and partitioning (cross- linkage of polymer), particle size (column efficiency) and column size (speed versus resolution).

// FEATURES & BENEFITS //

- Packed with chemically stable polymeric polystyrene divinylbenzene copolymers, varying in percent cross-linkage and particle sizes
- Stable at high temperatures up to 95°C
- Display consistent performance from column to column, polymer batch to polymer batch
- Utilize the simplest and safest eluent of all– water
- Offer more choices through combinations of cross-linkage (porosity), particle size, metal ligands, and column formats to meet your separation needs



30 years

CHROMATOGRAPHY
SEPARATIONS
REPRODUCIBILITY
PRECISION

CPS analitica | Calibre SCIENTIFIC

CARBOHYDRATE ANALYSIS HPLC COLUMNS //

Table 1 illustrates the number of Concise Separations carbohydrate columns available to maximize your separation needs compared to the other leading companies in the industry. The chart does not include ion exclusion columns (ligand in the hydrogen form). Please refer to the Chromatography Application Notes on organic acids analysis to see the Concise Separations difference for organic acids analysis columns.

Table 1

	4% XL	6% XL	7% XL	8% XL	Column Size (mm)	Ionic Form	Particle Size (µM)
Concise Separations							
CarboSep CHO 411	X				300x7.8	Sodium	20
CarboSep CHO 682		X			300x7.8	Lead	7
CarboSep CHO 611		X			300x6.5	Sodium	10
CarboSep CHO 611OH		X			150x6.5	Sodium	10
CarboSep CHO 620		X			300x6.5	Calcium	10
CarboSep CHO 782			X		300x7.8	Lead	7
CarboSep CHO 882				X	300x7.8	Lead	7
CarboSep CHO 882 FA				X	150x7.8	Lead	7
CarboSep CHO 87C FA				X	100x7.8	Calcium	8
CarboSep CHO 87MM				X	300x7.8	Sodium/Calcium	8
CarboSep USP L19				X	250x4.0	Calcium	8
CarboSep CHO 820				X	300x7.8	Calcium	9
CarboSep CHO 87C FA				X	100x7.8	Calcium	9
CarboSep CHO 87C				X	300x7.8	Calcium	9
CarboSep CHO 87K				X	300x7.8	Potassium	9
CarboSep CHO 87N				X	300x7.8	Sodium	9
CarboSep CHO 87P				X	300x7.8	Lead	9
Bio-Rad							
Aminex™ HPX-42C Column	X				300x7.8	Calcium	25
Aminex™ HPX-87C Column				X	300x7.8	Calcium	9
Aminex™ HPX-87C Column (USP L19)				X	300x7.8	Calcium	9
Aminex™ HPX-87K Column				X	300x7.8	Potassium	9
Aminex™ HPX-87N Column				X	300x7.8	Sodium	9
Aminex™ HPX-87P Column				X	300x7.8	Lead	9
Fast Carbohydrate Column				X	100x7.8	Calcium	9
Shodex							
Sugar SC1011				N/A	300x8.0	Calcium	6
Sugar SC1821				N/A	300x8.0	Calcium	6
Sugar SP0810				N/A	300x8.0	Lead	7
Sugar SC1211				N/A	250x6.0	Calcium	6
Sugar SZ5532				N/A	150x6.0	Zinc	6
MN-431				N/A	250x4.0	Calcium	N/A

Table 1 (Cont.)

	4% XL	6% XL	7% XL	8% XL	Column Size (mm)	Ionic Form	Particle Size (µM)
Phenomenex							
Rezex™ RNO-Oligosaccharide	X				200x10.0	Sodium	12
Rezex™ RAM-Carbohydrate Ag+				X	300x7.8	Silver	8
Rezex™ RCM-Monosaccharide Ca+				X	300x7.8	Calcium	8
Rezex™ RCU-USP Sugar Alchohols				X	250x4.0	Calcium	8
Rezex™ RKP-Potassium K+				X	300x7.8	Potassium	8
Rezex™ RNM-Carbohydrate Na+				X	300x7.8	Sodium	8
Rezex™ RPM-Monosaccharide Pb++				X	300x7.8	Lead	8
Rezex™ RPM-Monosaccharide Pb++ USP				X	100x7.8	Lead	8
RPM-Monosaccharide Pb++ (Fast Analysis)				X	100x7.8	Lead	8
Supelco							
Supelcogel™ C-611				N/A	300x7.8	Mixed	9
Supelcogel™ Ca				N/A	300x7.8	Calcium	9
Supelcogel™ K				N/A	300x7.8	Potassium	9

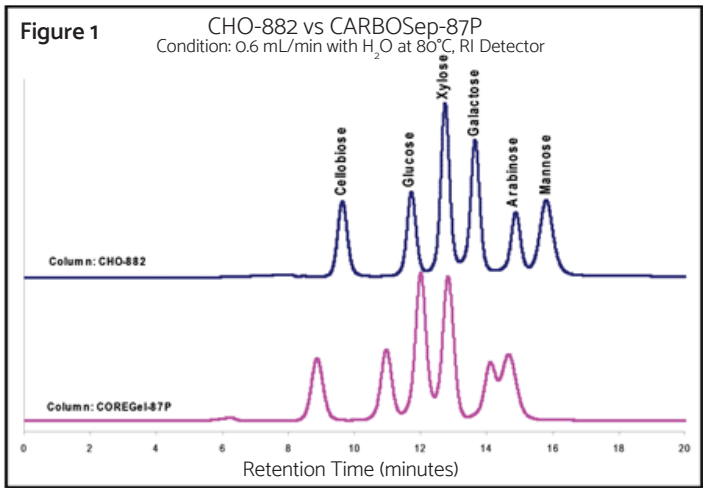
Choosing a column for your sample

By combining polymer cross-linkage, particle size, and metal ligand in a variety of column formats, Concise Separations offers more column choices, maximizing our ability to meet your separation needs.

// COLUMN CONSIDERATIONS //

- Resolution of peaks of interest
- Analysis time
- Selectivity (Elution order of peaks)
- Durability

Figure 1



Resolution and Particle Size Effect

Using a smaller particle size (7 µM) in the CHO 882 column can improve the separation compared to the same type of ligand exchange column, CHO 87P, using a 9 µM particle, as seen in Figure 1.

Figure 2 CARBOSep-87C-8%

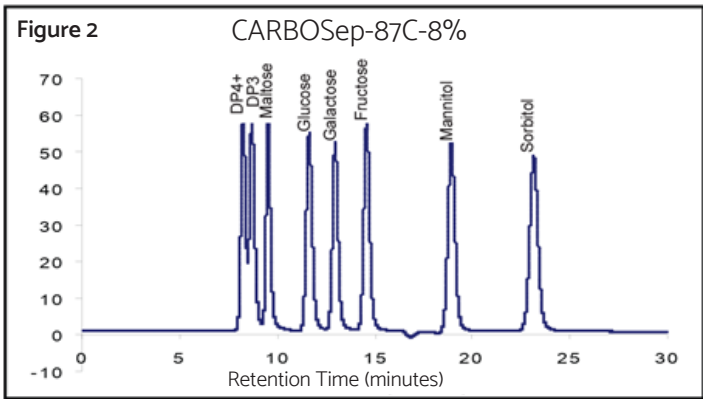


Figure 3 CHO-620 - 6%

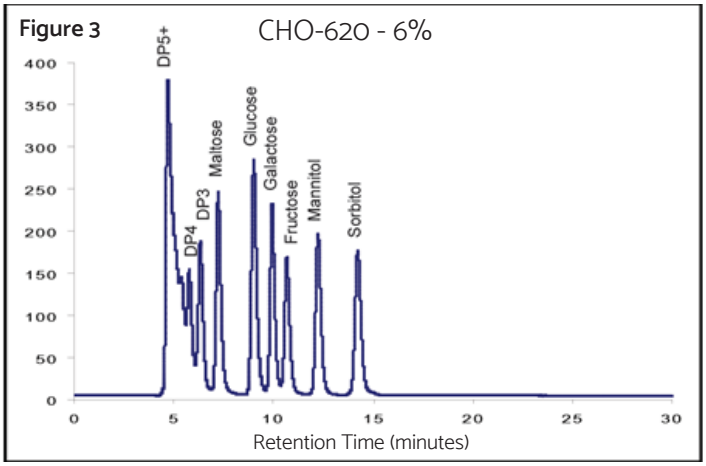
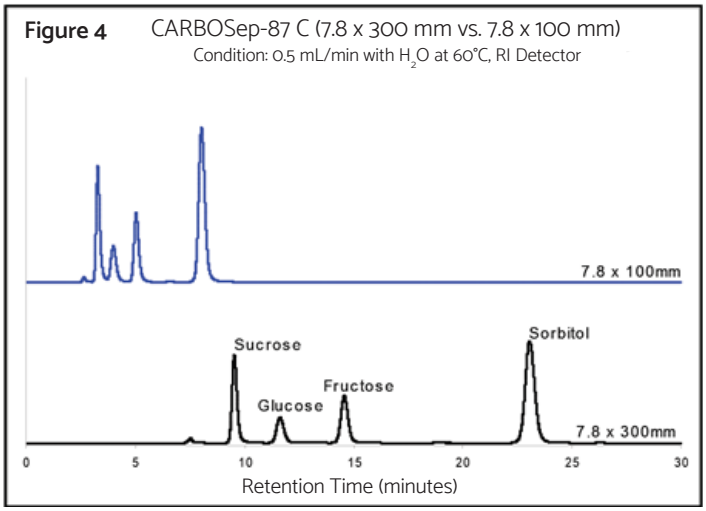


Figure 4 CARBOSep-87 C (7.8 x 300 mm vs. 7.8 x 100 mm)
Condition: 0.5 mL/min with H_2O at 60°C, RI Detector



Resolution and Cross-Linkage Effect

The lower the cross-linkage, the larger the pore size. For samples containing larger sugar polymers, the industry standard 8% cross-linked (xl) polymer may not adequately resolve your sample. This effect is shown in Figures 2 and 3.

Analysis Time and Column Size

Since polymeric gels are sensitive to pressure, the higher the polymeric cross-linkage, the higher the flow rate you can use. This will shorten your analysis time; however, lower cross-linked polymers will generally give better resolution. The easiest way to shorten analysis time for simple sugar samples is to use a smaller column.

// ANALYSIS TIME INFLUENCES //

- / Flow rate
- / Temperature
- / Column size
- / Polymer cross-linkage

Figure 5

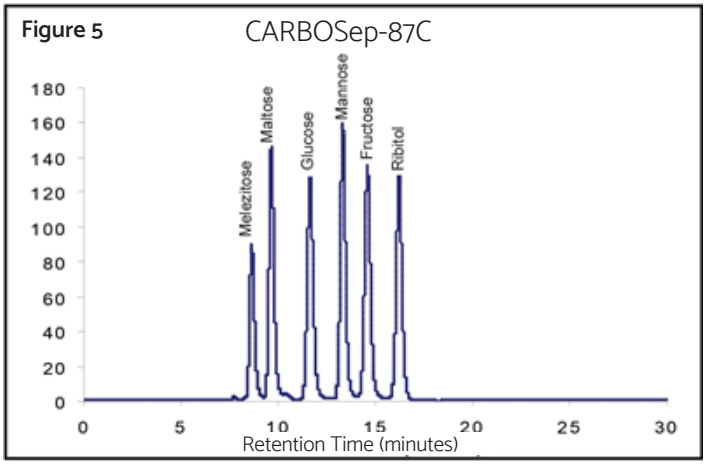


Figure 6

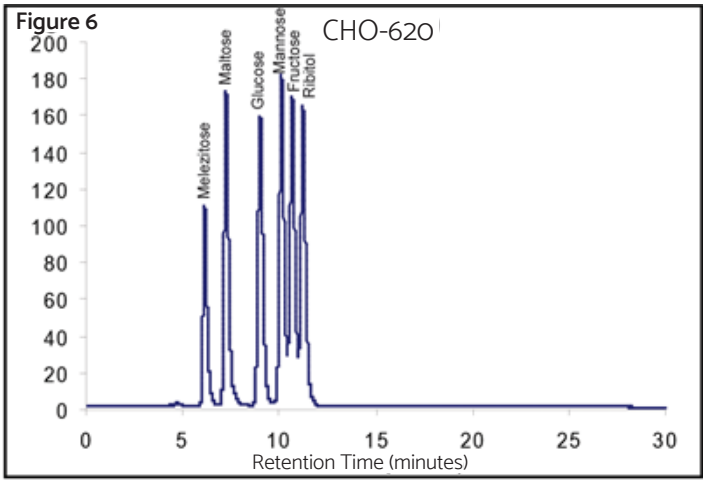
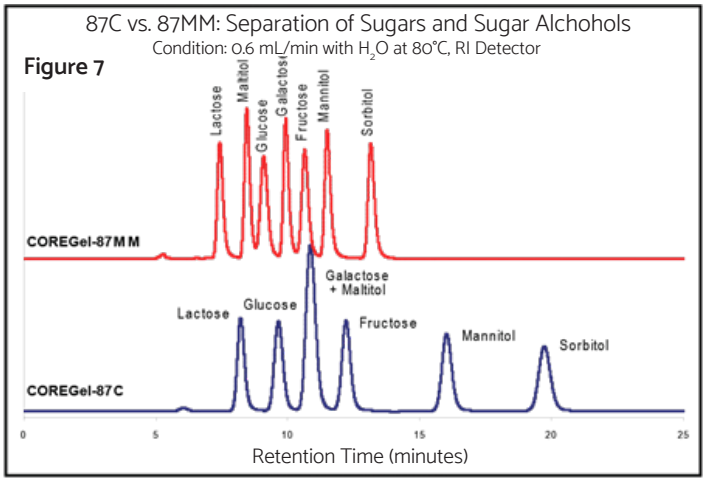


Figure 7



Selectivity and Ligand Exchange

The type of metal attached to the polymeric material has a dramatic effect on both selectivity and analysis time of the sample. The industry standard for separating sugar and sugar alcohols is an ion exclusion column in the calcium form.

Figures 5 and 6 illustrate how the CHO 87C with the calcium form 8%xl polymer can separate a sample more effectively than the CHO 620 column packed with calcium form 6%xl polymer.

Figure 7 shows how a mixed-mode column packed with calcium and sodium-form polymer can greatly improve a separation, compared to the CHO 87C, with the same column dimension and bead size.

// ANOTHER COMPARISON //

Refer to Resolution and Cross-linkage Effect for a separation comparison using another type of sample.

Durability

As long as the columns are used within the operating parameters, they last a long time, since polymers are chemically stable. When using polymeric gels, the key to extending your column's lifespan is to keep the column below the pressure maximum at all times. Temperature is a key component of pressure, along with flow rate, so it is extremely important to allow the column to reach the proper temperature before starting the flow. The columns are also sensitive to water quality, so water purity is essential (minimum purity requirements 18MΩ). Sample preparation and the use of guards and filters will extend column lifetime by reducing risk of column contamination. In general, the higher the cross-linkage of the polymer and the larger the particle size, the greater the flow rate you can use before reaching the maximum allowable pressure.

Table 2 is a summary of properties for Concise Separations columns, to aid in the choice of column for your sample.

Table 2

Concise Separations Column	Cross-Linkage	Ionic Form	Particle Size (µM)	Key Samples	Comments
CHO 411	4	Sodum	20	Oligosaccharides through DP11	Easier to regenerate than Ag+ form
CHO 682	6	Lead	7	High resolution column, including sucrose/maltose/lactose	Pressure sensitive, low flow rates
CHO 611	6	Sodium	10	Oligosaccharides through DP5	
CHO 611OH	6	Sodium	10	Fast analysis of simple sugars	PAD detector compatible
CHO 620	6	Calcium	10	Versatile analysis of corn syrup, sugars, sugar alcohol	
CHO 782	7	Lead	7	Biomass sugar analysis	Flow rate limited
CHO 882	8	Lead	7	Monosacchardies and cellulose products	Higher speed, lower resolution than CHO 682
CHO 882 FA	8	Lead	7	Fast analysis of monosaccharides	
CHO 87C FA	8	Calcium	8	Fast analysis of simple sugars	
CHO 87MM	8	Sodium/ Calcium	8	Fast analysis of sugar alochols	
USP L19	8	Calcium	8	Mannitol and Sorbitol - USP approved	
CHO 820	8	Calcium	9	General sugar analysis	Higher efficiency version of CHO 87C
CHO 87C	8	Calcium	9	Industry standard for analysis of general sweeteners	
CHO 87K	8	Potassium	9	Sugar samples such as brewing wort, betaine analysis	Use with samples containing potassium
CHO 87N	8	Sodium	9	Molasses and other sugars high salt samples	Easy to regenerate sodium form, low selectivity
CHO 87P	8	Lead	9	Monosaccharides and cellulose products	Less resolution than CHO 882, high flow rate

Retention

Retention charts are another useful tool when choosing the best column for your sample. Compounds with at least a one-minute difference in retention time should be adequately separated; however, the wide variety of carbohydrates precludes developing a comprehensive chart for all compounds. Also, the selectivity of the column can be altered to enhance the separation of the compounds, using different temperatures and flow rates. If your compound does not appear in a retention chart, or the ability of a column to separate your compounds is in question, please contact Concise Separations technical support. In addition to the retention charts, we have many chromatograms of a variety of applications, which show separations using different test methods.

All columns in Table 3 were tested using the recommended QC test conditions of flow rate and temperature.

Table 3

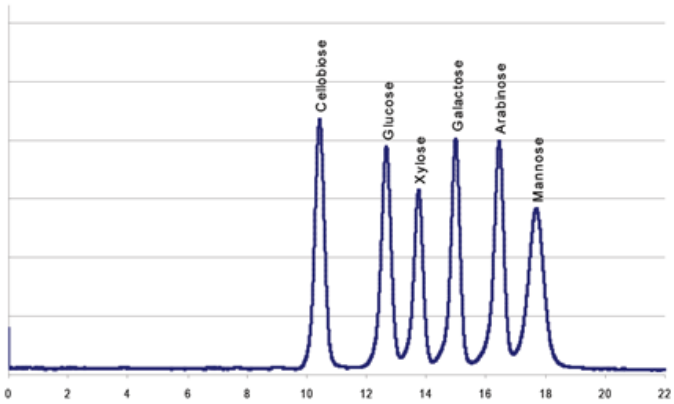
Compound	CHO 620	CHO 87N	CHO 87C & CHO 820	CHO 87P & CHO 882	CHO 682	CHO 87K
Nitrate	4.50	5.70	7.37	8.40	10.37	6.40
Maltoheptose	4.66	5.84	7.35	8.52	11.81	6.61
Maltohexose	4.78	5.94	7.45	8.80	13.31	6.74
Maltopentose	5.00	6.11	7.60	9.34	13.15	7.02
Amiprylose	N/A	5.74	7.75	9.46	N/A	6.42
Stachyose	5.94	6.33	7.85	11.84	13.48	6.32
Maltotetrose	5.37	6.42	7.87	9.84	14.14	7.02
Melezitose	5.78	6.81	8.27	13.08	13.92	7.82
Raffinose	6.56	6.88	8.31	10.22	14.47	7.92
Maltotriose	6.68	6.98	8.35	10.54	15.24	8.16
Cellobiose	7.36	7.90	9.01	10.98	15.65	9.26
Trehalose	7.32	7.85	9.14	11.20	16.05	9.02
Sucrose	7.48	7.99	9.18	11.10	15.77	9.11
Maltose	7.59	8.08	9.24	11.54	16.68	9.48
Melibiose	7.67	8.19	9.43	11.74	17.70	9.72
Lactose	7.84	8.18	9.51	11.84	17.44	9.63
Lactulose	8.53	8.48	10.24	13.24	20.77	10.08
Glucose	9.36	10.72	11.24	13.38	19.21	12.55
Lactitol	9.16	8.45	12.24	19.50	33.30	9.34
Xylose	10.31	11.77	12.39	14.42	20.71	13.69
Maltitol	9.15	8.28	12.29	17.76	30.45	9.06
Galactose	10.29	11.44	13.89	15.16	22.39	13.36
Sorbose	10.22	11.08	12.93	15.24	22.45	12.66
Mannose	10.51	11.57	12.83	16.76	25.57	13.74
Rhamnose	10.41	11.08	12.93	15.26	22.63	12.83
Fructose	11.40	11.61	13.70	16.96	25.91	13.31
Fucose	11.33	12.34	13.89	16.44	24.23	14.39

Table 3 (Cont.)

Compound	CHO 620	CHO 87N	CHO 87C & CHO 820	CHO 87P & CHO 882	CHO 682	CHO 87K
Arabinose	11.63	12.64	14.00	16.32	24.02	14.72
Myo-inositol	11.83	12.48	14.34	20.06	35.65	14.08
Digitoxose	N/A	12.41	14.27	N/A	21.02	N/A
Ribitol	11.95	11.26	15.62	20.44	30.79	11.84
Tagatose	N/A	11.86	16.53	N/A	N/A	N/A
Mannitol	12.76	10.81	17.89	24.98	40.10	11.42
Arabitol	13.23	11.64	18.43	25.24	39.89	12.10
Xylitol	14.61	12.16	22.00	31.10	51.22	12.64
Galactitol	14.41	11.15	20.52	31.60	52.50	11.61
Sorbitol	14.91	11.32	21.41	33.40	56.63	11.86
Ribose	16.46	11.52	21.99	28.59	55.00	14.16

New CARBOSep Column Applications

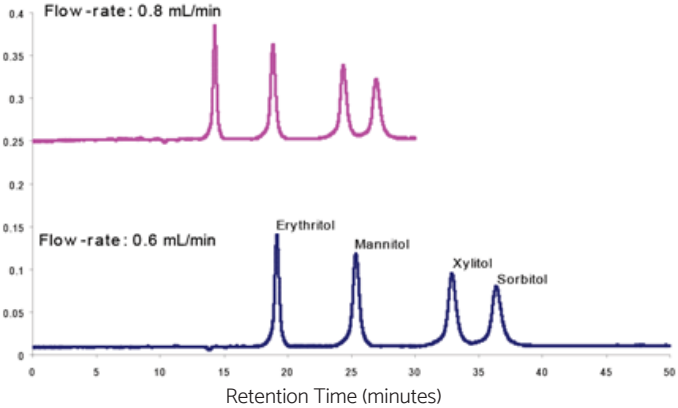
Figure 8 Separation of Sugars on CHO-782 Column
Condition: H_2O with 0.6 mL/min at 70°C, RI Detector



Biomass Analysis

One of the most important emerging areas of research is the development of alternative fuels. In response to the need for increased resolution and faster analysis of key carbohydrate compounds generated during ethanol production, Concise Separations developed the CHO 782 column. The CHO 782 column rapidly separates key carbohydrates such as arabinose and mannose without loss of resolution (see Figure 8).

Figure 9 Separation of Alcohols on CHO-882 Column
Condition: 0.8 mL or 0.6 mL/min with H_2O at 85°C, RI Detector



Sugar Alcohols Analysis

Many sugar alcohols need a strong ligand exchange metal such as lead to separate properly. The sugar alcohols are strongly retained and separated, however the strong affinity for the lead metal can result in long analysis times. The CHO 882 column combines the mechanical strength of a higher cross-linked material with the high efficiency of a smaller particle size to separate a sugar alcohol sample effectively and rapidly (see Figure 9).

Tips on Maintaining the Performance of CARBOSep Columns

The most important fact to remember when using CARBOSep columns is that the polystyrene divinylbenzene copolymer is a low cross-linked material: this polymeric packing has a limited resistance to flow rate and pressure, and will irreversibly compact and overpressure at a certain level. Unlike polymers, silica-based materials are not flow-rate sensitive, and the relation between pressure and flow rate remains relatively constant; therefore, CARBOSep columns should be carefully monitored for pressure, and operated within the recommended flow rates and pressure specifications.

Customer Support

Please do not hesitate to contact us with any questions about our products, or for help with your application needs. By assisting you with new sample separations, we not only provide solutions for our valued customers, we discover new column methods and technologies that can benefit others in the industry. We encourage you to visit our website periodically for updates on new products and applications; we are continuously upgrading the website with improved accessibility options and new support information. Please feel free to contact us with website suggestions. Your opinion matters!

// ADDITIONAL TIPS //

- Use column ovens to increase column efficiency and lower column back pressure.
- Set the pressure shut off for the analytical test system at or slightly below the recommended pressure maximum for the column used, to prevent irreversible damage to the column.
- When installing, allow column to warm up in column over for 15 minutes, then start the flow rate below your target flow rate. After another 15 minutes, increase the flower rate to the target flow rate and confirm that the column is operating at the expected back pressure.
- To increase the lifetime of your analytical column, we recommend proper use of guard columns or cartridges. How frequently you change your guard column depends on pretreated or purity of the sample.
- Filter and remove potentially harmful organics from samples to decrease the need to change guard columns. Carefully monitor the guard columns for pressure increase and monitor the chromatograms for changes in retention and efficiecy to determine the approximate useful lifetime of the guard columns.

CARBOSep Catalogue Numbers

Catalogue #	Part Name	Length (mm)
CHO-99-9850	CARBOSep CHO 411 Na Form Oligosaccharides	300
CHO-99-9854	CARBOSep CHO 682 Pb Form Carbohydrate	300
CHO-99-9751	CARBOSep CHO 611 Na Form Corn Syrup	300
CHO-99-7752	CARBOSep CHO 611 OH Na Form Carbohydrate	150
CHO-99-9753	CARBOSep CHO 620 Ca Form Carbohydrate	300
CHO-99-7770	CARBOSep CHO 782 Pb Form Carbo & Biomass Analysis	300
CHO-99-8770	CARBOSep CHO 882 Pb Form Carbohydrate	300
CHO-99-5882	CARBOSep CHO 882 7.8 x 150 mm Pb Form Carbohydrate	150
CHO-99-5860	CARBOSep CHO 87C FA Ca Form Fast Analysis Column	100
CHO-99-9865	CARBOSep CHO 87MM Ca/Na Form Carbohydrate	300
CHO-99-8453	CARBOSep USP L19 Ca Form Mannitol/Sorbitol Analysis	250
CHO-99-9855	CARBOSep CHO 820 Ca Form Carbohydrate	300
CHO-99-9860	CARBOSep CHO 87C Ca Form	300
CHO-99-9862	CARBOSep CHO 87K K Form Carbohydrate	300
CHO-99-9863	CARBOSep CHO 87N Na Form Carbohydrate	300
CHO-99-9864	CARBOSep CHO 87P Pb Form Carbohydrate	300

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