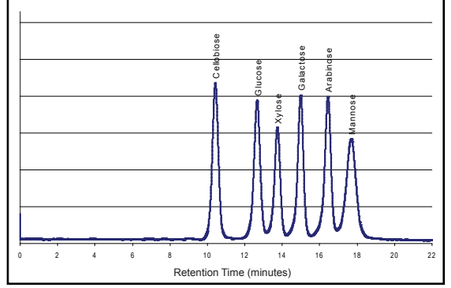
New CARBOSep Column Applications

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 speed of analysis of key carbohydrate compounds generated during ethanol production, Transgenomic developed the **CHO-782** column. The **CHO-782** column rapidly separates key carbohydrates such as arabinose and mannose without loss of resolution.

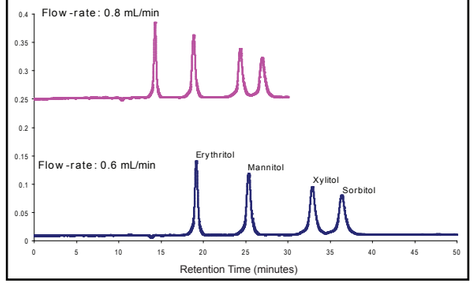
Figure 8. Separation of Sugars on Transgenomic CHO-782 column
Condition: H_2O with 0.6 mL/min at 70 °C RI detector



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.

Figure 9. Separation of Alcohols on Transgenomic's CHO-882 Column
Condition: 0.8 mL or 0.6 mL/min with H_2O at 85 °C RI detection



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, the **CARBOSep** columns should be carefully monitored for pressure, and should be operated within the recommended flow rates and pressure specifications.

- Use column ovens to serve the dual purpose of increasing column efficiency and lowering column back pressure.
- Set the pressure shut off for the analytical test system at or slightly below the recommended pressure maximum for the column to prevent irreversible damage to the column.
- When installing, allow the column to warm up in the column oven for 15 minutes, and then start the flow rate below your target flow rate. After 15 minutes, increase the flow 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 the proper use of guard columns or cartridges. How frequently you change your guard column depends on pretreatment or purity of the sample.
- Filter and remove potentially harmful organics from the samples to decrease the need to change guard columns. Carefully monitor the guard columns for pressure increase and the chromatograms for changes in retention and efficiency to determine the approximate useful lifetime of the guard columns.

CARBOSep Catalog Numbers

Catalog #	Part Name	Length
CHO-99-9850	CARBOSep CHO-411 Na Form Oligosaccharides	300 mm
CHO-99-8500	CARBOSep CHO-511 Na Form Oligosaccharides	300 mm
CHO-99-9854	CARBOSep CHO-682 Pb Form Carbohydrate	300 mm
CHO-99-9751	CARBOSep CHO-611 Na Form Corn Syrup	300 mm
CHO-99-7752	CARBOSep CHO-611 OH Na Form Carbohydrate	150 mm
CHO-99-9753	CARBOSep CHO-620 Ca Form Carbohydrate	300 mm
CHO-99-7770	CARBOSep CHO-782 Pb Form Carbo & Biomass Analysis	300 mm
CHO-99-8770	CARBOSep CHO-882 Pb Form Carbohydrate	300 mm
CHO-99-5882	CARBOSep CHO-882 7.8 x 150 mm Pb Form Carbohydrate	150 mm
CHO-99-5860	CARBOSep COREGel-87C Ca Form Fast Analysis Column	100 mm
CHO-99-9865	CARBOSep COREGel-87MM Ca/Na Form Carbohydrate	300 mm
CHO-99-8453	CARBOSep USP L-19 Ca Form Mannitol/Sorbitol Analysis	250 mm
CHO-99-9855	CARBOSep CHO-820 Ca Form Carbohydrate	300 mm
CHO-99-9860	CARBOSep COREGel-87C Ca Form	300 mm
CHO-99-9862	CARBOSep COREGel-87K K Form Carbohydrate	300 mm
CHO-99-9863	CARBOSep COREGel-87N Na Form Carbohydrate	300 mm
CHO-99-9864	CARBOSep COREGel-87P Pb Form Carbohydrate	300 mm

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Chromatography Application Notes
Carbohydrate Analysis HPLC Columns

For over 25 years, Transgenomic 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 developed for customers using our Carbohydrate Analysis HPLC columns.

Introduction

CARBOSep Columns for Carbohydrate Analysis

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, Transgenomic 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

CARBOSep columns are packed with chemically stable polymeric polystyrene-divinylbenzene copolymers varying in percent cross-linkage and particle sizes.

- Stable at high temperatures up to 95 ºC
- Consistent performance from column to column, and polymer batch to polymer batch
- The simplest and safest eluent of all—water
- More choices of columns utilizing combinations of cross-linkage (porosity), particle size, metal ligands and column formats to maximize your separation

The Transgenomic Difference

Table 1 illustrates the number of Transgenomic 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—Organic Acids Analysis HPLC Columns* on organic acids analysis to see the Transgenomic difference for organic acids analysis columns.

Table 1. Column Comparison

Transgenomic	4%xl	5%xl	6% XL	7%xl	8%xl	Column Size (mm)	Ionic Form	Particle Size (uM)
Carbo Sep CHO411	x					300x7.8	Sodium	20
Carbo Sep CHO511		x				300x7.8	Sodium	12
Carbo Sep CHO682			x			300x7.8	Lead	7
Carbo Sep CHO611			x			300x6.5	Sodium	10
Carbo Sep CHO611OH			x			150x6.5	Sodium	10
Carbo Sep CHO620			x			300x6.5	Calcium	10
Carbo Sep CHO782				x		300x7.8	Lead	7
Carbo Sep CHO882					x	300x7.8	Lead	7
Carbo Sep CHO882 Fast Analysis					x	150x7.8	Lead	7
Carbo Sep Coregel 87C Fast Analysis					x	100x7.8	Calcium	8
Carbo Sep Coregel 87MM					x	300x7.8	Sodium/Calcium	8
Carbo Sep USP L19					x	250x4.0	Calcium	8
Carbo Sep CHO820					x	300x7.8	Calcium	9
Carbo Sep Coregel 87C					x	100x7.8	Calcium	9
Carbo Sep Coregel 87C					x	300x7.8	Calcium	9
Carbo Sep Coregel 87K					x	300x7.8	Potassium	9
Carbo Sep Coregel 87N					x	300x7.8	Sodium	9
Carbo Sep Coregel 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
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 Alcohols					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
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
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

How to Choose a Column for Your Sample

In choosing the best column for your application, there are several factors to consider:

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

By combining polymer cross-linkage, particle size and metal ligand in a variety of column formats, Transgenomic offers more column choices to maximize your separation needs.

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 polymer may not adequately resolve your sample.

Figure 1. COREGel-87C–8%

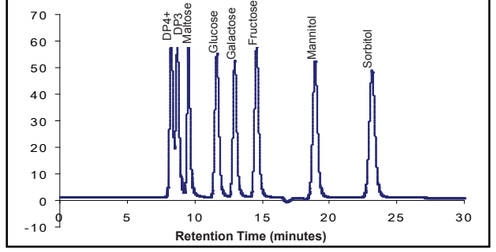
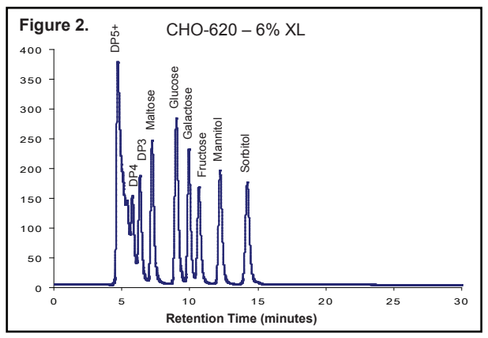


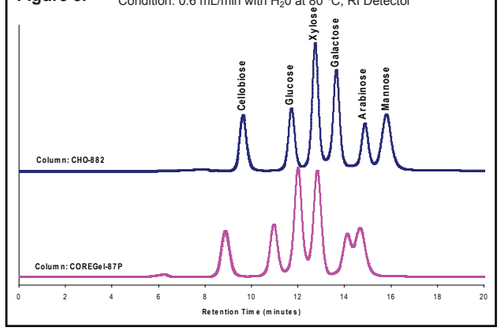
Figure 2. CHO-620 – 6% XL



Resolution and Particle Size Effect

The separation below illustrates how using a smaller particle size (7µm) in the **CHO-882** column can improve the separation compared to the same type of ligand exchange column, **COREGel-87P** using a 9 µm particle:

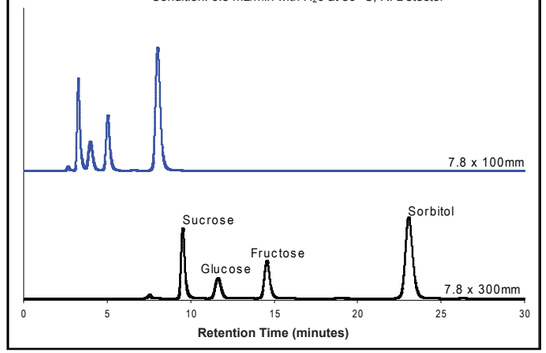
Figure 3. CHO-882 vs. COREGel-87P
Condition: 0.6 mL/min with H_20 at 80 ºC, RI Detector



Analysis Time and Column Size

The analysis time is influenced by a number of factors including flow rate, temperature, column size and polymer cross-linkage. Since polymeric gels are sensitive to pressure, the higher the polymeric cross-linkage, the higher the flow rate you can use to shorten your analysis time. However, lower cross-linked polymers in general give better resolution. The easiest way to shorten analysis time for simple sugar samples is to use a smaller column.

Figure 4. COREGel-87C (7.8 x 300 mm vs. 7.8 x 100 mm)
Condition: 0.5 mL/min with H_20 at 80 ºC, RI Detector



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 calcium form 8%xl cross-linked material adequately separates a wide array of sugars and sugar alcohols. The chromatogram below illustrates how the **COREGel-87C** with the calcium form 8%xl polymer can better separate a sample compared to the **CHO-620** column packed with calcium form 6%xl polymer. Please refer to the *Resolution and Cross-linkage Effect* section that shows a superior separation when using the CHO-620 column versus the **COREGel-87C** column for another type of sample:

Figure 5. COREGel-87C

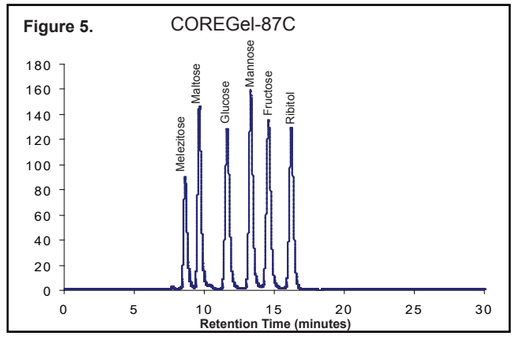
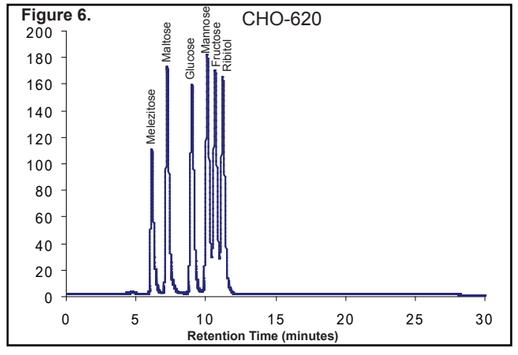


Figure 6. CHO-620



Using the same column format and polymer, but by altering the metal ligand, Figure 7 shows how a mixed mode column packed with calcium and sodium form polymer, can greatly improve a separation compared to the industry standard calcium form 8%xl polymer.

Durability

Since polymers are chemically stable, as long as the columns are used within the operating parameters, they last a long time. The key to long column lifetime when using polymeric gels is to keep the column at all times below the pressure maximum. Since temperature is a key component of pressure along with flow rate, it is extremely important to allow the column to reach temperature before starting the flow. The columns are also sensitive to water quality, so water purity is essential (minimum purity requirements 18MΩ). To reduce the column from being contaminated, sample preparation, as well as the use of guards and filters, extends column lifetime. In general, the higher the cross-linkage of the polymer and the larger the particle size, the greater the flow rate that can be used before reaching the maximum allowable pressure. Table 2 is a summary of properties for Transgenomic columns to aid in the choice of column for your sample.

87C vs. 87MM; Separation of Sugars and Sugar Alcohols
Condition: 0.6 mL/min with H_20 at 80 ºC, RI Detector

Figure 7.

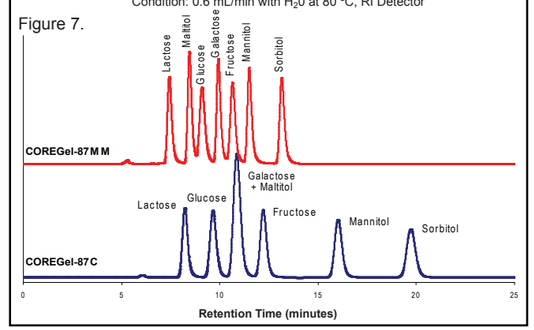


Table 2. Retention Table for CARBOSep Colums

Transgenomic	Cross-linkage	Ionic Form	Particle Size (uM)	Key Samples	Comments
CHO411	4	**Sodium**	**20**	Oligosaccharides through DP11	Easier to regenerate than Ag+ form
CHO511	5	**Sodium**	**12**	Oligosaccharides through DP8	
CHO682	6	**Lead**	**7**	High resolution column, including sucrose/maltose/lactose	Pressure sensitive, low flow rates
CHO611	6	**Sodium**	**10**	Oligosaccharides through DP5	
CHO611OH	6	**Sodium**	**10**	Fast analysis of simple sugars	PAD detector compatible
CHO620	6	**Calcium**	**10**	Versatile analysis of corn syrup, sugars, sugar alcohols	
CHO782	7	**Lead**	**7**	Biomass sugar analysis	Flow rate limited
CHO882	8	**Lead**	**7**	Monosaccharides and cellulose products	Higher speed, lower resolution than CHO682
CHO882 Fast Analysis	8	**Lead**	**7**	Fast analysis of monosaccharides	
Coregel 87C Fast Analysis	8	**Calcium**	**8**	Fast analysis of simple sugars	
Coregel 87MM	8	**Sodium/Calcium**	**8**	Fast analysis of sugar alcohols	
USP L19	8	**Calcium**	**8**	Mannitol and Sorbitol - USP approved	
CHO820	8	**Calcium**	**9**	General sugar analysis	Higher efficiency version of Coregel 87C
Coregel 87C Fast Analysis	8	**Calcium**	**9**	Fast analysis of simple sugars	
Coregel 87C	8	**Calcium**	**9**	Industry standard for analysis of general sweetners	
Coregel 87K	8	**Potassium**	**9**	Sugar samples such as brewing wort, betaine analysis	Use with samples containing potassium
Coregel 87N	8	**Sodium**	**9**	Molasses and other sugars high salt samples	Easy to regenerate sodium form, low selectivity
Coregel 87P	8	**Lead**	**9**	Monosaccharides and cellulose products	Less resolution than CHO882, high flow rate

Retention Charts

Another useful tool in choosing the best column for your sample is the use of retention charts. Compounds with at least 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, by using different temperatures and flow rates, the selectivity of the column can be altered to enhance the separation of the compounds. 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 Transgenomic technical support. In addition to the retention charts, we have many chromatograms of a variety of applications showing 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. CARBOSep Column Comparison Chart

Compound	CHO-620	COREGel-87N	COREGel-87C & CHO-820	COREGel-87P & CHO-882	CHO-682	COREGel-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
Maltotpentose	5.00	6.11	7.60	9.34	13.15	7.02
Amiprylose	NA	5.74	7.75	9.46	NA	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
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	NA	12.41	14.27	NA	21.02	NA
Ribitol	11.95	11.26	15.62	20.44	30.79	11.84
Tagatose	NA	11.86	16.53	NA	NA	NA
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	20.53	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

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