

HPLC Carbohydrate Column Selection Guide

Because carbohydrates exhibit a significant degree of chemical and physical similarity, they are more difficult to analyze than most other classes of compounds. No single HPLC column or method is capable of separating all carbohydrates. Advantages, limitations, and applications for SUPELCOGEL and SUPELCOSIL columns specifically prepared for various carbohydrate analyses are summarized here.

Key Words

- carbohydrates ● sugars ● saccharides
- monosaccharides ● disaccharides ● oligosaccharides

Carbohydrate analyses are a challenge for the chromatographer. Diversity among compounds classified as carbohydrates (Table 1) is far greater than among other classes of biochemicals. The potential for complexity in the structure of carbohydrate molecules is summarized by the fact that three amino acids can combine in a total of six different ways, while three monosaccharides can form more than 1000 distinct trisaccharides. To further complicate the situation, chemical and physical properties among monosaccharides, disaccharides, and trisaccharides differ only slightly. Hence, HPLC separations of carbohydrates depend on differences in conformation, configuration, and bonding mode, and are more difficult than analyses of other classes of compounds. No single HPLC column or method is capable of separating all carbohydrates. For this reason, we offer a selection of columns specifically prepared for various carbohydrate analyses. Advantages and limitations of each column are summarized in this bulletin. Table 2 summarizes applications for these columns; Table 3 shows retention times for an extensive number of carbohydrates, under typical operating conditions.

Table 1. Types of Carbohydrates

Monosaccharides

Pentoses (arabinose, ribose)

Hexaoses (glucose, galactose, fructose)

Disaccharides (sucrose, lactose, maltose)

Trisaccharides (raffinose, melezitose)

Tetrasaccharides (stachyose)

Oligosaccharides (DP3 to DP10*)

Polysaccharides (100–1000 units)

Polyhydric Alcohols/Sugar Alcohols

(mannitol, sorbitol, polyols)

*DP (degree of polymerization) = number of monosaccharide units in the molecule.

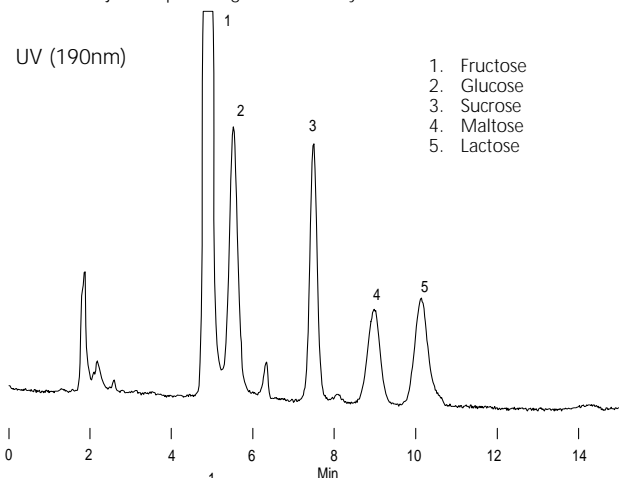
Detection

Because the UV wavelengths required to detect carbohydrates also will be strongly absorbed by impurities in the samples (Figure A), refractive index (RI) detection is most commonly used in analyses of carbohydrates.

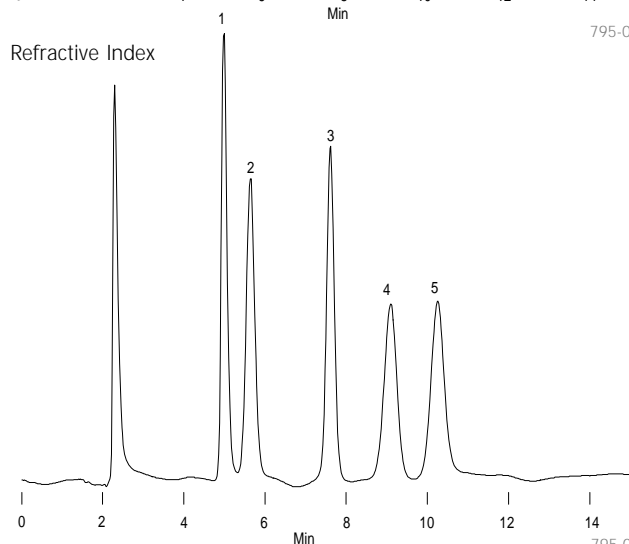
The major advantage of RI detection is the universal response over a fairly wide linear range of analyte concentrations. Major disadvantages are poor sensitivity and baseline instability caused by temperature, solvent, or pressure changes (RI detection is impractical with gradient elution). Both positive and negative peaks can be present in a chromatogram. The abundance of carbohydrates in food and other samples, however, makes RI detection a suitable tool for these analyses.

Figure A. Refractive Index Affords Superior Detection for Carbohydrates

Column: SUPELCOSIL LC-NH₂, 25cm x 4.6mm, 5µm particles
 Cat. No.: 58338
 Mobile Phase: acetonitrile:water, 75:25
 Flow Rate: 1.5mL/min
 Temp.: ambient
 Inj.: 20µL, 10mg/mL each analyte in acetonitrile:water, 25:75



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Table 2. Carbohydrate Column Applications and Operating Parameters

SUPELCOGEL™ columns are resin-based; SUPELCOFIL™ LC-NH₂ column is silica-based.

Column	Application	Form	Typical Mobile Phase	Maximum Flow (mL/min)	Sample pH Range	Max. Temp. (°C)
SUPELCOGEL K	beet sugar, cane sugar, molasses, corn syrup	potassium	10mM K ₂ HPO ₄	1.5	1-13	90
SUPELCOGEL Pb	monosaccharides, xylose/galactose/mannose	lead	deionized water	1.5	1-13	90
SUPELCOGEL Ca	high fructose corn syrup, monosaccharides, sugar alcohols, oligosaccharides	calcium	deionized water	1.5	1-13	90
SUPELCOGEL C-610H	organic acids, acid/carbohydrate/ alcohol mixes	hydrogen	0.1% H ₃ PO ₄ or H ₂ SO ₄	1.5	1-13	60
SUPELCOGEL H	organic acids, acid/carbohydrate/ alcohol mixes	hydrogen	0.1% H ₃ PO ₄ or H ₂ SO ₄	1.5	1-13	90
SUPELCOGEL C-611	mono-, di-, and trisaccharides, galactose/mannose	2 divalent cations	10 ⁻⁴ N NaOH	1.5	1-13	85
SUPELCOGEL Ag	oligosaccharides, glycerol/ethanol, beer, corn syrup, hydrolyzed starch	silver	deionized water	1.5	1-13	90
SUPELCOFIL LC-NH ₂	mono-, di-, some trisaccharides	aminopropyl silica	75% CH ₃ CN in water	2.0	2-7	70

Table 3. Typical Retention Times on Supelco Carbohydrate Columns

Cat. No.:	SUPELCOGEL Columns								SUPELCOFIL LC-NH ₂ 58338
	Ca 59305-U	C-610H 59320-U	H 59304-U	H 59346	Pb 59343	C-611 59310-U	K 59342	Ag2 59315	
Dimens. (mm):	300 x 7.8	300 x 7.8	300 x 7.8	250 x 4.6	300 x 7.8	300 x 7.8	300 x 7.8	300 x 7.8	250 x 4.6
Temp.:	80°C	30°C	30°C	30°C	85°C	60°C	85°C	85°C	ambient
Mobile Phase:	DH ₂ O	0.1% H ₃ PO ₄	0.1% H ₃ PO ₄	0.1% H ₃ PO ₄	DH ₂ O	10 ⁻⁴ N NaOH	15mM K ₂ HPO ₄	DH ₂ O	ACN: DH ₂ O (3:1)
Flow Rate (mL/min):	0.5	0.5	0.5	0.17	0.5	0.5	0.5	0.5	1.0
Det.:	refractive index								
Compound	Retention Times (min)								
Arabinose	15.3	13.9	14.3	13.8	19.2	19.6	16.8	17.1	7.5
Arabitol	19.8	14.1	14.9	14.3	32.3	22.8	13.5	16.0	7.2
Betaine	ND	ND	ND	ND	NR	ND	13.0	ND	ND
Dulcitol	22.3	13.4	14.2	13.7	43.4	25.7	12.9	15.9	9.0
Erythritol	17.7	15.0	15.6	14.8	24.5	20.2	14.0	16.1	5.9
Ethanol	19.4	25.6	ND	ND	ND	21.0	ND	18.4	NR
Fructose	14.9	13.1	13.3	12.9	20.8	20.7	15.2	16.0	8.3
Galactose	13.4	12.9	13.0	12.6	17.6	17.6	15.1	15.8	10.3
Glucose	12.0	12.1	11.9	11.7	14.9	15.8	14.0	14.6	9.8
Glycerol	18.7	16.8	17.6	16.6	23.8	20.9	15.2	17.1	NR
Inositol	14.9	12.6	12.7	12.4	24.5	20.1	15.7	17.4	ND
Isomaltose	9.6	10.3	ND	ND	ND	13.8	ND	11.6	19.4
Isomaltotriose	8.5	9.5	ND	ND	ND	12.6	ND	9.8	NR
Lactitol	ND	ND	11.1	11.0	26.5	ND	10.6	ND	ND
Lactose	10.2	10.8	10.2	10.2	13.5	14.3	10.9	11.8	19.5
Maltitol	13.6	11.0	10.7	10.7	23.8	17.7	10.2	15.0	15.5
Maltoheptaose	7.5	8.8	7.6	7.9	9.2	11.6	7.2	7.3	NR
Maltohexaose	7.7	8.9	7.7	8.1	9.7	12.0	7.4	7.6	NR
Maltopentaose	7.9	9.1	7.9	8.2	10.5	12.6	7.8	8.1	NR
Maltose	9.8	10.5	9.9	9.9	13.0	14.2	10.7	11.5	17.4
Maltotetraose	8.3	9.3	8.2	8.5	11.2	13.2	8.4	8.8	NR
Maltotriose	8.8	9.7	8.8	9.0	12.0	13.6	9.2	9.8	31.0
Mannitol	19.2	13.2	13.7	13.2	32.5	22.1	12.6	15.2	9.2
Mannose	13.7	12.8	12.9	12.5	19.8	18.9	15.6	15.9	9.1
Melezitose	8.7	9.7	8.8	9.0	10.8	12.4	8.6	9.3	24.5
Psicose	22.5	13.4	14.5	13.9	36.5	32.9	15.5	17.2	6.6
Raffinose	8.7	9.7	8.7	8.9	11.2	12.6	8.7	9.6	29.7
Ribitol	16.7	13.7	14.2	13.6	25.1	19.5	13.1	15.3	ND
Ribose	24.3	14.2	15.8	15.0	40.7	34.6	17.7	19.1	6.0
Sorbitol	23.4	13.4	14.4	13.9	46.9	28.3	13.3	16.3	9.0
Stachyose	8.1	9.3	8.1	8.4	10.4	11.9	7.9	8.5	67.3
Sucrose	9.8	10.6	9.9	9.9	12.2	13.6	10.1	11.2	14.0
Xylitol	23.3	14.4	15.7	15.0	42.1	28.0	14.2	17.1	7.3
Xylose	13.2	12.8	12.8	12.6	16.1	17.2	15.3	15.6	6.8

NR - not recommended

ND - no data available

For optimal separations, allow at least 1 minute between compounds.

SUPELCO SIL LC-NH₂ Column

This column, containing a silica-based, aminopropyl-modified phase packing, provides good separations of a variety of mono-, di-, and some trisaccharides found in foods. Separation is in order of increasing molecular weight, with monosaccharides eluting first (Figures B and C). Oligosaccharides are strongly retained and should not be analyzed on this column. Sample preparation is minimal. The sugars do not require derivatization and most samples require only dilution with deionized water, extraction of the sugars, and filtration (0.45µm filter). Many juices and soft drinks can be analyzed undiluted, after degassing and filtration. Mobile phases typically contain acetonitrile and water in various proportions (75:25 or 80:20 mixtures are recommended). Note that the disaccharides sucrose and lactose are best separated on the SUPELCO SIL LC-NH₂ column.

Figure B. Amino Column Test Mix

Column: SUPELCO SIL LC-NH₂, 25cm x 4.6mm, 5µm particles
Cat. No.: 58338
Mobile Phase: acetonitrile:water, 75:25
Flow Rate: 1.0mL/min
Temp.: ambient
Det.: RI
Inj.: 10µL, 10mg/mL each analyte in acetonitrile:water, 25:75

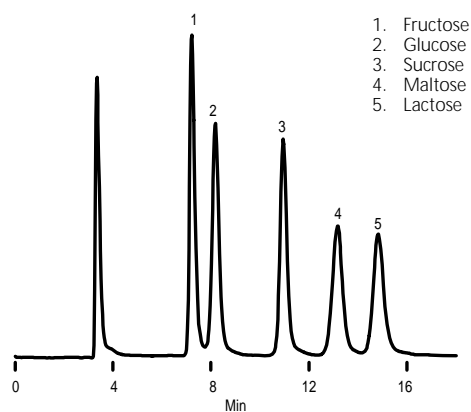


Figure C. Carbohydrate Standards on an Amino Column

Column: SUPELCO SIL LC-NH₂, 25cm x 4.6mm, 5µm particles
Cat. No.: 58338
Mobile Phase: acetonitrile:water, 75:25
Flow Rate: 1.0mL/min
Temp.: ambient
Det.: RI
Inj.: 10µL, 10mg/mL each analyte in water

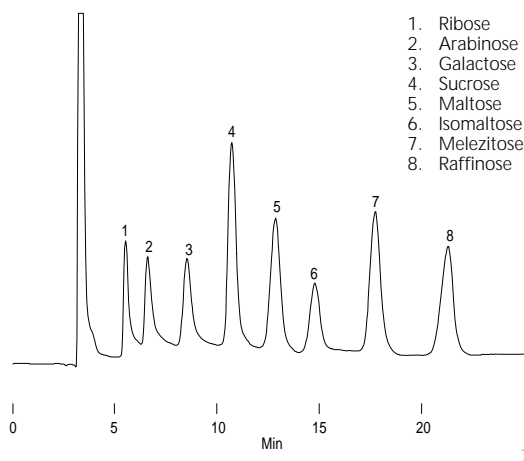


Figure B shows an analysis of the test mix used to evaluate the performance of SUPELCO SIL LC-NH₂ columns. The mix is available (Cat. No. 58424) for routine monitoring of the column in the laboratory. Figure C shows the variety of sugars that can be separated by using a SUPELCO SIL LC-NH₂ column. Resolution between each pair of analytes is good.

SUPELCO GEL Columns

In contrast to their elution order on SUPELCO SIL LC-NH₂ columns, carbohydrates elute in descending order of molecular size, monosaccharides last, from the resin-based SUPELCO GEL columns described below. The pores in the resins exclude polysaccharides and larger oligosaccharides, which elute first. Smaller di- and monosaccharides enter the pores, interact with the counterions, and are more strongly retained. Figures D and E show comparable separations on five columns. In addition to the differences in elution order and resolution, note the negative peak, a common characteristic of refractive index detection, in both figures. Also note that in Figure E the disaccharides sucrose and lactose are separated only on the SUPELCO SIL LC-NH₂ column. Disaccharide separations are difficult to obtain with resin-based column packings.

SUPELCO GEL Ca Column

The SUPELCO GEL Ca column contains a polystyrene-divinylbenzene cross-linked resin in the calcium form. It separates oligo-, tri-, and disaccharides by class, using a mixed size exclusion/ion exchange mode, with the largest molecules eluting first. Its true separating power, however, is in its chromatography of monosaccharides and sugar alcohols – a variety of monosaccharides can be separated, using only water as the mobile phase. The column can be operated at low temperatures, but separations are best at elevated temperatures. Figures F and G show a separation of carbohydrate standards and an analysis of high fructose corn syrup, respectively, on a SUPELCO GEL Ca column. Both figures were obtained using a temperature of 80°C and a mobile phase consisting solely of deionized water.

SUPELCO GEL C-611 Column

The unique polystyrene-divinylbenzene resin-based packing in this column contains two divalent cations, strontium and barium, rather than one. As with the other SUPELCO GEL columns, the separation mechanism is a combination of size exclusion and ion exchange modes. Disaccharides elute before monosaccharides, usually as a single peak. Sugar alcohols are strongly retained and elute with the monosaccharides. The column is compatible with many inorganic salts and can be used with dilute bases. Separations normally are carried out using a very weak basic solution, such as 10⁻⁴ N sodium hydroxide, at 60°C. Analyses on a SUPELCO GEL C-611 column are temperature dependent. Figure I shows that resolution is improved as the temperature is increased.

SUPELCO GEL Ag Columns

These columns contain a silver-form styrene-divinylbenzene resin. Larger analytes elute before smaller analytes. The Ag1 column was developed specifically for separating the oligosaccharides in beer and corn syrup, and provides rapid separations of oligomers up to DP7. It will separate ethanol and glycerol. The Ag2 column is better suited for larger oligosaccharides, resolving up to and including DP12. Hydrolysis products and oligosaccharides can be analyzed on either an Ag1 or an Ag2 column. Figure J shows the carbohydrate profile of a domestic beer; Figures K and L show separations of corn syrup on SUPELCO GEL Ag1 and Ag2 columns, respectively.

Figure D. Carbohydrate Standards

Flow Rate (SUPELCOSIL LC-NH₂ column): 1.5mL/min
 Inj.: 10µL, 2.5mg/mL each analyte in water
 Other conditions listed in Table 3

1. Maltotriose
2. Maltose
3. Glucose
4. Xylose
5. Arabinose
6. Ribitol
7. Arabitol
8. Xylitol

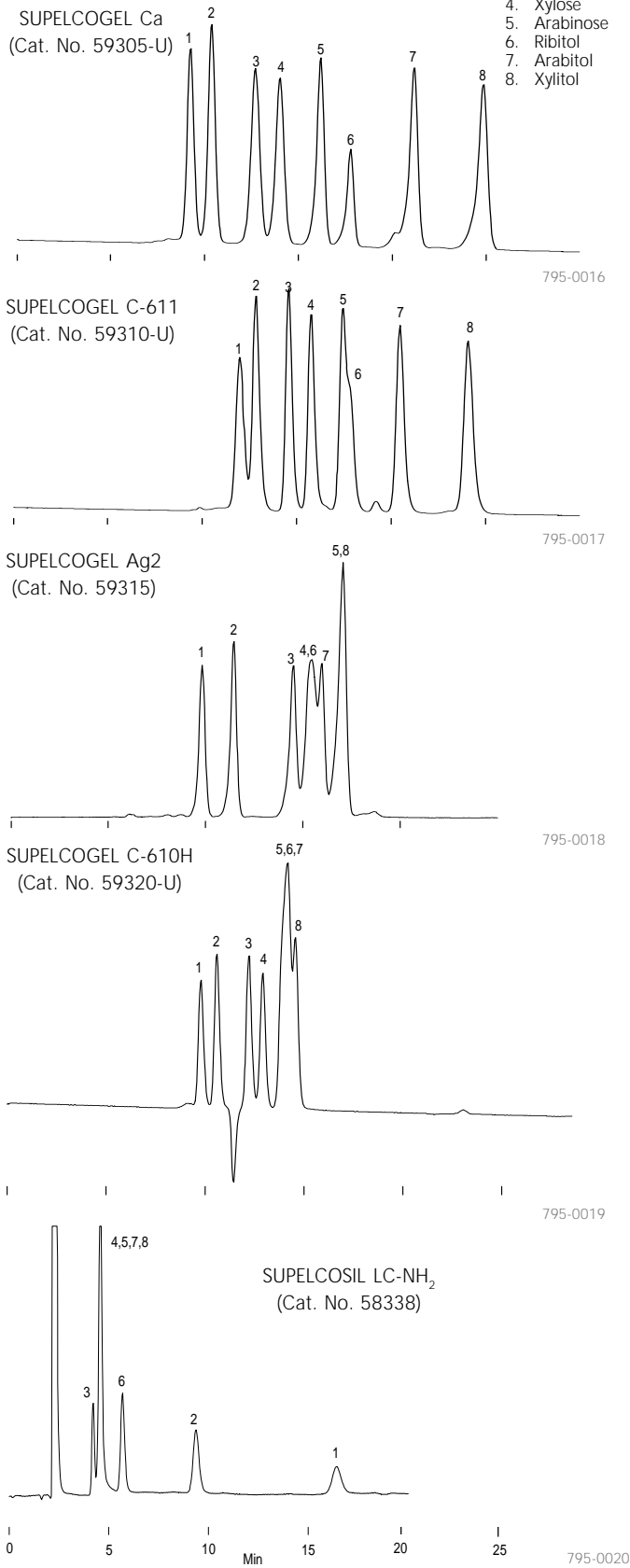


Figure E. Carbohydrates in Fruit Yogurt

Flow Rate (SUPELCOSIL LC-NH₂ column): 1.5mL/min
 Inj.: 10µL of 10g yogurt/100mL DI water, filtered (0.20µm filter)
 Other conditions listed in Table 3

1. Oligosaccharides
2. Sucrose
3. Lactose
4. Glucose
5. Fructose

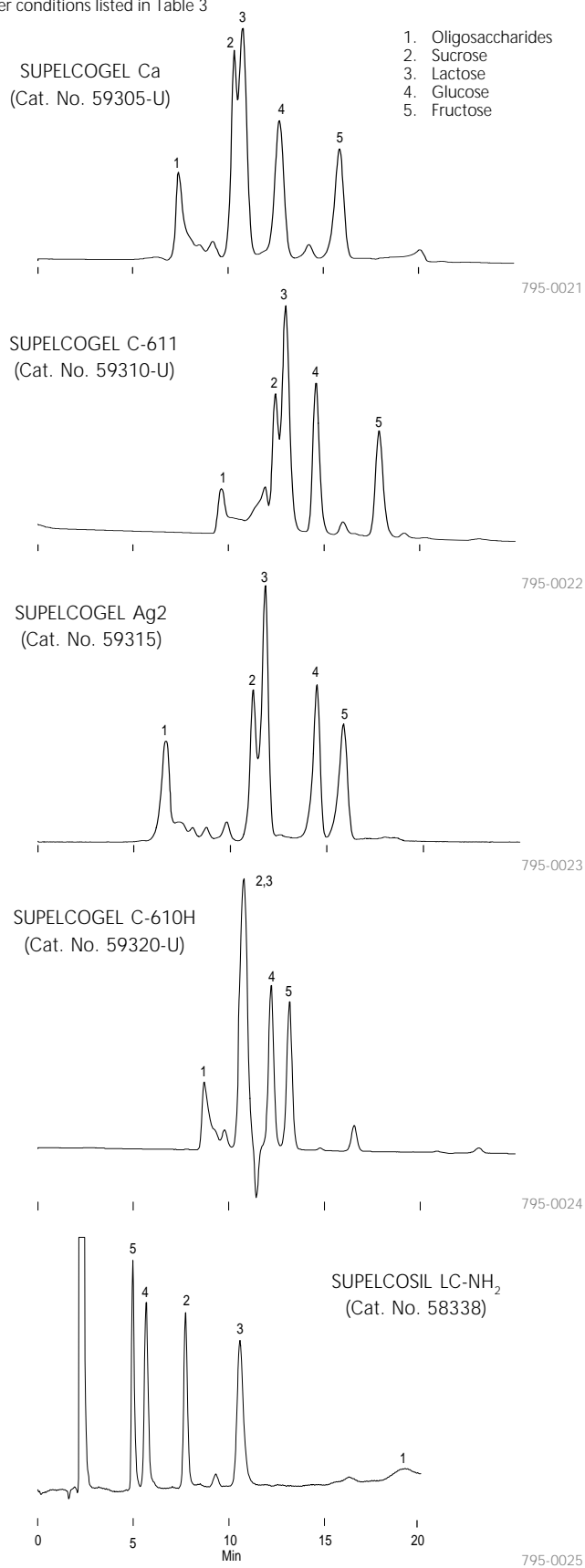
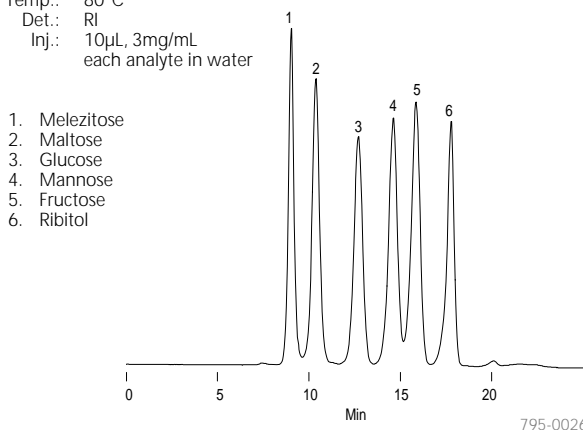


Figure F. Carbohydrate Standards on a SUPELCOGEL Ca Column

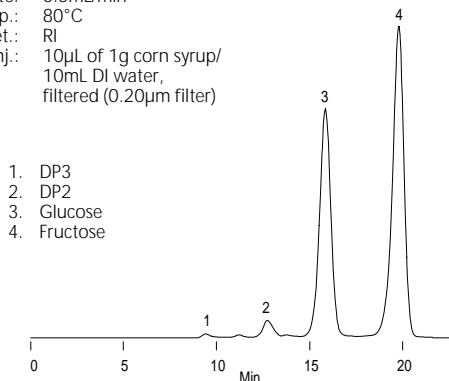
Column: SUPELCOGEL Ca, 30cm x 7.8mm
 Cat. No.: 59305-U
 Mobile Phase: water
 Flow Rate: 0.5mL/min
 Temp.: 80°C
 Det.: RI
 Inj.: 10µL, 3mg/mL each analyte in water



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Figure G. High Fructose Corn Syrup

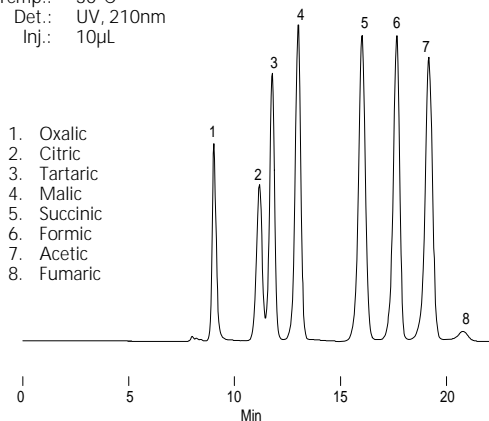
Column: SUPELCOGEL Ca, 30cm x 7.8mm
 Cat. No.: 59305-U
 Mobile Phase: water
 Flow Rate: 0.5mL/min
 Temp.: 80°C
 Det.: RI
 Inj.: 10µL of 1g corn syrup/
 10mL DI water,
 filtered (0.20µm filter)



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Figure H. Organic Acid Standards

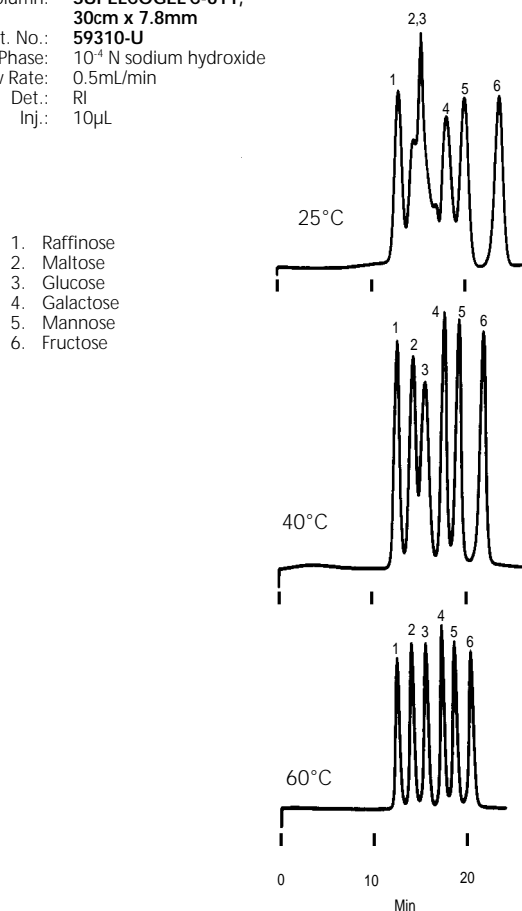
Column: SUPELCOGEL C-610H, 30cm x 7.8mm
 Cat. No.: 59320-U
 Mobile Phase: 0.1% phosphoric acid
 Flow Rate: 0.5mL/min
 Temp.: 30°C
 Det.: UV, 210nm
 Inj.: 10µL



794-0231

Figure I. Temperature Affects Carbohydrate Analyses on SUPELCOGEL Columns

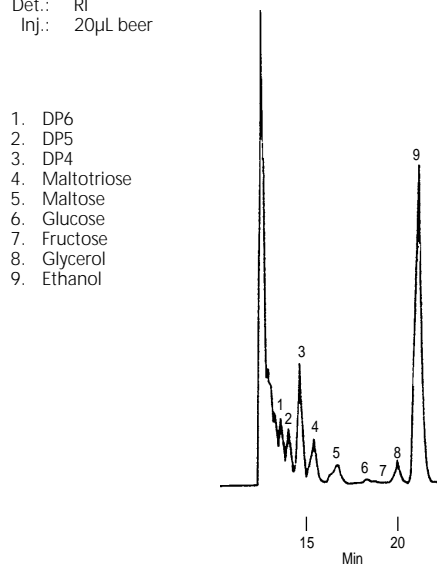
Column: SUPELCOGEL C-611,
 30cm x 7.8mm
 Cat. No.: 59310-U
 Mobile Phase: 10⁻⁴ N sodium hydroxide
 Flow Rate: 0.5mL/min
 Det.: RI
 Inj.: 10µL



713-0519

Figure J. Domestic Beer

Column: SUPELCOGEL Ag1, 30cm x 7.8mm
 Cat. No.: 59318-U
 Mobile Phase: water
 Flow Rate: 0.3mL/min
 Temp.: 90°C
 Det.: RI
 Inj.: 20µL beer



G000994

Figure K. Dark Corn Syrup

Column: **SUPELCOGEL Ag1, 30cm x 7.8mm**
Cat. No.: **59318-U**
Mobile Phase: water
Flow Rate: 0.5mL/min
Temp.: 90°C
Det.: RI
Inj.: 10µL of 1g syrup/10mL DI water, filtered (0.20µm filter)

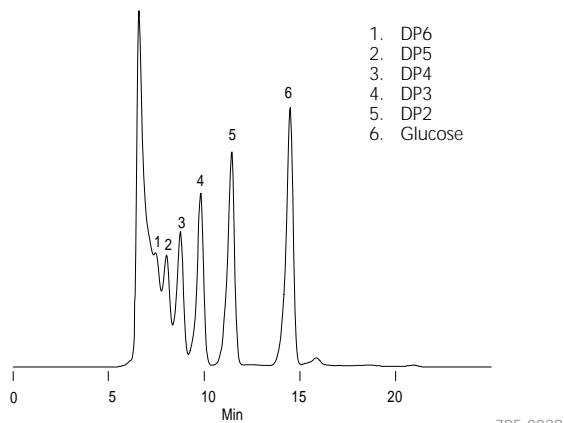
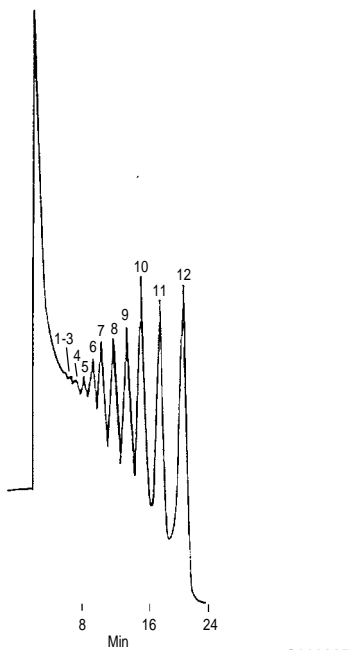


Figure L. Corn Syrup

Column: **SUPELCOGEL Ag2, 30mm x 7.8mm**
Cat. No.: **59315**
Mobile Phase: water
Flow Rate: 0.4mL/min
Temp.: 90°C
Det.: RI
Inj.: 20µL

- 1-3. DP12-10
- 4. DP9
- 5. DP8
- 6. DP7
- 7. DP6
- 8. DP5
- 9. DP4
- 10. Maltotriose
- 11. Maltose
- 12. Glucose



SUPELCOGEL C-610H Column

This column contains a polystyrene resin in the hydrogen form. It is ideal for separating mixtures of organic acids (Figure H), fermentation products (e.g., alcohols), and carbohydrates. Such mixtures commonly occur in fruits, vegetables, and beverages. Larger and acidic analytes elute before smaller analytes. The column is stable between pH 1 and 13, but results are best at low pH. A simple mobile phase containing 0.1% H₃PO₄ is suitable for a wide variety of analyses.

SUPELCOGEL K Column

A SUPELCOGEL K column separates raffinose, sucrose, glucose, fructose, and betaine, a trimethylammonium zwitterionic compound found in beet and cane sugars and widely distributed in other plants.

SUPELCOGEL Pb Column

The lead-form resin in SUPELCOGEL Pb columns provides the highest resolution and best selectivity for monosaccharides. SUPELCOGEL Pb columns provide excellent separation of xylose, galactose, and mannose, which are not completely resolved on calcium-form resin columns.

SUPELCOGEL H Columns

SUPELCOGEL H columns have the same particle composition, retention mechanism, performance, sensitivity, and applications as SUPELCOGEL C-610H columns. However, particle improvements have made it possible to pack the SUPELCOGEL H packing material efficiently into conventional 4.6mm ID columns, to improve detection and reduce solvent consumption relative to 7.8mm ID columns.

Making a Decision

To choose the best column for your particular carbohydrates analysis, we suggest you find the compounds of interest in Table 3 and note their retention times on each column. Using this information, select the column that will separate the compounds of interest with at least 1 minute between any pair.

Guard Columns

Although filtration removes particulate matter from a sample, food, beverages, and other samples often contain soluble components that can be strongly retained by the analytical columns described here. We strongly recommend using a guard column to protect the analytical column from these potentially damaging sample components. We offer 5cm x 4.6mm Supelguard™ guard columns that are compatible with our SUPELCOGEL resin-based analytical columns and a 2cm x 4.6mm Supelguard guard column for use with the SUPELCOGEL LC-NH₂ silica-based column. Guard columns are listed under Ordering Information. Note that SUPELCOGEL Ca columns and SUPELCOGEL C-611 columns use the same guard column (Supelguard Ca).

Suggested Reading

- Knight, P. *Biotechnology*, **7**: 35 (1989).
- Parriot, D. *A Practical Guide to HPLC Detection* (Chapter 2) Academic Press (1992).
- Lehninger, A. *Biochemistry* (Chapter 10) Worth Publishers (1975).
- Nollet, L. *Food Analysis by HPLC* (Chapter 8) Marcel Dekker (1992).

Ordering Information:

SUPELCOGEL and SUPELCOFIL Carbohydrate Columns and Guard Columns

Column	Length (cm)	ID (mm)	Cat. No.	Supelguard Guard Column	Cat. No.
SUPELCOGEL K	30	7.8	59342	K	59344
SUPELCOGEL Pb	30	7.8	59343	Pb	59345
SUPELCOGEL Ca	30	7.8	59305-U	Ca	59306-U
SUPELCOGEL C-610H	30	7.8	59320-U	H	59319
SUPELCOGEL H	30	7.8	59304-U	H	59319
SUPELCOGEL H	25	4.6	59346	H	59319
SUPELCOGEL C-611	30	7.8	59310-U	Ca	59306-U
SUPELCOGEL Ag1	30	7.8	59318-U	Ag1	59317-U
SUPELCOGEL Ag2	30	7.8	59315	Ag2	59316
SUPELCOFIL LC-NH ₂	25	4.6	58338	LC-NH ₂ (kit)	59558
				LC-NH ₂ (pk. 2)	59568

Carbohydrate/Organic Acid/Sugar Alcohol Reference Standards

Prepared, tested, and packaged using rigorous manufacturing procedures.

Description	CAS No.	Qty.	Cat. No.
Monosaccharides			
D-(-)Arabinose	28697-53-2	500mg	47246-U
D-(-)Fructose	57-48-7	500mg	47247
D-(+)Galactose	59-23-4	500mg	47248
D-(+)Glucose (mixed anomers)	50-99-7	500mg	47249
D-(+)Mannose (mixed anomers)	3458-28-4	500mg	47250
D-Psicose (mixed anomers)	551-68-8	100mg	47251
D-(-)Ribose	50-69-1	500mg	47252
D-(+)Xylose	58-86-6	500mg	47253
Disaccharides			
α-Lactose	5989-81-1	500mg	47287-U
Maltose	6363-53-7	500mg	47288
Sucrose	57-50-1	500mg	47289
Oligosaccharides			
Maltoheptaose (DP7)	34620-78-5	100mg	47872
Maltohexaose (DP6)	34620-77-4	100mg	47873
Maltopentaose (DP5)	34620-76-3	100mg	47876
Maltotetraose (DP4)	34612-38-9	100mg	47877
Stachyose (DP4)	10094-58-3	100mg	47879
Maltotriose (DP3)	1109-28-0	100mg	47878
D-(+)Melezitose (DP3)	10030-67-8	100mg	47882-U
D-(+)Raffinose (DP3)	17629-30-0	100mg	47883
Isomaltotriose (DP3)	3371-50-4	100mg	47884
Organic Acids			
Acetic acid	64-19-7	500mg	46928
Adipic acid	124-04-9	500mg	46929
L-Ascorbic acid	50-81-7	500mg	46930-U
Benzoic acid	65-85-0	500mg	46931
Butyric acid	107-92-6	500mg	46932
Citric acid	77-92-9	500mg	46933
Formic acid	64-18-6	500mg	46934-U
Fumaric acid	110-17-8	500mg	46948
Isobutyric acid	79-31-2	500mg	46935
DL-Isocitric acid	1637-73-6	100mg	46936
L-(+)Lactic acid	79-33-4	100mg	46937
Maleic acid	110-16-7	500mg	46939
D-Malic acid	636-61-3	100mg	46940-U
Malonic acid	141-82-2	500mg	46938
Oxalic acid	144-62-7	500mg	46941-U
Phytic acid	123408-98-0	500mg	46942-U
Propionic acid	79-09-4	500mg	46943-U
(-)Quinic acid	77-95-2	500mg	46944-U
Shikimic acid	138-59-0	100mg	46945-U
Succinic acid	110-15-6	500mg	46946-U
D-Tartaric acid	147-71-7	500mg	46947-U

Description	CAS No.	Qty.	Cat.
No. Sugar Alcohols			
D-(+)Arabitol	488-82-4	500mg	46919-U
Dulcitol (Galactitol)	608-66-2	500mg	46920-U
iso-Erythritol	149-32-6	500mg	46921
Glycerol	56-81-5	500mg	46922
Maltitol	585-88-6	500mg	46923-U
D-Mannitol	69-65-8	500mg	46924-U
Ribitol (Adonitol)	488-81-3	500mg	46925-U
D-Sorbitol	50-70-4	500mg	46926-U
Xylitol	87-99-0	500mg	46927

Kits

Description	Cat. No.
Monosaccharides Kit: each monosaccharide standard listed	47267
Disaccharides Kit: each disaccharide standard listed	47268-U
Oligosaccharides Kit: each oligosaccharide standard listed	47265
Organic Acids Kit: each organic acid standard listed	47264
Sugar Alcohols Kit: each sugar alcohol standard listed	47266

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