

TheReporter

Reprinted from Volume 14, No. 1, 1995

T295031

© 1999 Sigma-Aldrich Co.

For more information, or current prices, contact your nearest Supelco subsidiary listed below. To obtain further contact information, visit our website (www.sigma-aldrich.com), see the Supelco catalog, or contact Supelco, Bellefonte, PA 16823-0048 USA.

ARGENTINA • Sigma-Aldrich de Argentina, S.A. • Av. Pueyrredon 2446/50 • Piso 5-B • Buenos Aires 1119
AUSTRALIA • Sigma-Aldrich Pty. Ltd. • Unit #2, 14 Anella Avenue • Castle Hill NSW 2154
AUSTRIA • Sigma-Aldrich Handels GmbH • Hebbelplatz 7 • A-1110 Wien
BELGIUM • Sigma-Aldrich N.V./S.A. • K. Cardijnplein 8 • B-2880 Bornem
BRAZIL • Sigma-Aldrich Quimica Brasil Ltda. • Rua Sabara, 566-Conj. 53 • 01239-010 São Paulo, SP
CANADA • Sigma-Aldrich Canada, Ltd. • 2149 Winston Park Dr., Oakville, ON L6H 6J8
CZECH REPUBLIC • Sigma-Aldrich s.r.o. • Pobrezni 46 • 186 21 Praha 8
DENMARK • Sigma-Aldrich Denmark A/S • Vejlegaardsvej 65B • DK-2665 Vallensbaek Strand
FINLAND • Sigma-Aldrich Finland/YA-Kemia Oy • Teerisuonkuja 4 • FIN-00700 Helsinki
FRANCE • Sigma-Aldrich Chimie • Chromatographie Supelco • L'Isle d'Abeau Chesnes - B.P. 701 • 38297 Saint-Quentin Fallavier Cedex
GERMANY • Sigma-Aldrich Chemie GmbH • Geschäftsbereich Supelco • Grünwalder Weg 30 • D-82041 Deisenhofen
GREECE • Sigma-Aldrich (o.m.) Ltd. • 72 Argonafton Str. • 16346 Ilioupoli, Athens
HUNGARY • Sigma-Aldrich Kft. • Nagy Diófa u. 7., IV fl. • H-1067 Budapest
INDIA • Sigma-Aldrich Co. • Survey No. 31/1, Sitharamapalaya • Mahadevapura P.O. • Bangalore 560 048
IRELAND • Sigma-Aldrich Ireland Ltd. • Airton Road • Tallaght • Dublin 24
ISRAEL • Sigma Israel Chemicals Ltd. • Park Rabin • Rohovot 76100
ITALY • Sigma-Aldrich s.r.l. • Via Gallarate, 154 • 20151 Milano
JAPAN • Sigma-Aldrich Japan K.K. • Division Supelco • JL Nihonbashi Building • 1-10-15 Nihonbashi Horidome-cho, Chuo-ku • Tokyo 103
KOREA • Sigma-Aldrich Korea • Samhan Camus Annex, 10th Floor • 17-26 Yoido-dong, Yungdeungpo-ku • Seoul
MALAYSIA • Sigma-Aldrich (M) Sdn. Bhd. • 9-2, Jalan 2/128, Taman Gembira • Off Jalan Kuchai Lama • 58200 Kuala Lumpur • Selangor
MEXICO • Sigma-Aldrich Quimica S.A. de C.V. • Calle 6 North No. 107 • Parque Industrial Toluca 2000 • 50200 Toluca
NETHERLANDS • Sigma-Aldrich Chemie BV • Postbus 27 • 3330 AA Zwijndrecht
NORWAY • Sigma-Aldrich Norway • Sandakerveien 102 • N-0483 Oslo
POLAND • Sigma-Aldrich Sp. z o.o. • Szelagowska 30 • 61-626 Poznań
PORTUGAL • Sigma-Aldrich Quimica, S.A. • P.O. Box 131 • Sintra 2710
RUSSIA • Sigma-Aldrich Russia • TOO Techmedbiochem • Makarenko Str. 2/21 • Building 1, Flat 22 • Moscow 103062
SINGAPORE • Sigma-Aldrich Pte. Ltd. • 102E Pasir Panjang Road • #08-01 Citilink Warehouse • Singapore 118529
SOUTH AFRICA • Sigma-Aldrich (pty) Ltd. • CNR Kelly & Ackerman Streets • Southern Life Industrial Park Unit • Unit 16/17 • Jet Park 1459
SPAIN • Sigma-Aldrich Quimica, S.A. • Apt. Correos 161 • 28100 Alcobendas, Madrid
SWEDEN • Sigma-Aldrich Sweden AB • Solkraftsvägen 14C • 135 70 Stockholm
SWITZERLAND • Supelco Switzerland • Industriestrasse 25 • P.O. Box 260 • CH-9471 Buchs
UNITED KINGDOM • Sigma-Aldrich Company Ltd. • Supelco UK • Fancy Road, Poole • Dorset BH12 4QH
UNITED STATES • Supelco • Supelco Park • Bellefonte, PA 16823-0048 • Phone 800-247-6628 or 814-359-3441 • Fax 800-447-3044 or 814-359-3044 • email:supelco@sial.com

H

This article is archived from a past issue of The Supelco Reporter. Information in the article was appropriate at the time of publication, but product specifications, catalog numbers, and availability may have changed over time.

If you have questions about applying methodology described in this article to a current application, please contact our technical service chemists.



Supelco is a member of the Sigma-Aldrich family. Supelco products are sold through Sigma-Aldrich, Inc. Sigma-Aldrich warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product for a particular use. Additional terms and conditions may apply. Please see the reverse side of the invoice or packing slip.

Choosing an HPLC Column for Carbohydrate Analyses

E.I. Doughty

Because carbohydrates are chemically and physically similar, they are more difficult to analyze than most other classes of compounds. Advantages and limitations of each of five columns specifically prepared for various carbohydrate analyses are summarized here.

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 carbohydrate analyses. Advantages and limitations of each column are summarized here.

SUPELCOSIL™ LC-NH₂ (amino bonded silica)

This column provides good separations of a variety of mono, di-, and some trisaccharides found in foods. Disaccharide separations are difficult to obtain with resin-based column packings. Separation is in order of increasing molecular weight, with monosaccharides eluting first. 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 (e.g., 75:25 or 80:20). Sugar retention decreases as the proportion of water is increased.

SUPELCOGEL™ Ca (calcium-form resin)

This column separates oligo-, tri-, and disaccharides by class by 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 by using only water as the mobile phase. Separations are best at elevated temperatures (e.g., 80°C).

SUPELCOGEL C-610H (hydrogen-form resin)

This column is ideal for separating mixtures of organic acids, fermentation products (e.g., alcohols), and carbohydrates such as commonly are present in fruits, vegetables, and beverages. Larger and acidic analytes elute before smaller analytes. Results are best at low pH. A simple mobile phase containing 0.1% H₃PO₄ is suitable for a wide variety of analyses.

Table 1. Column Operating Conditions

Column	Max. Pressure (psi)	Max. Flow Rate (mL/min)	pH Range	Max. Temp. (°C)
SUPELCOGEL Ca	1000 •	1.5	1-13	90
SUPELCOGEL C-610H	1000	1.5	1-13	60
SUPELCOGEL C-611	1000	1.5	1-13	85
SUPELCOGEL Ag2	1000	1.5	1-13	90
SUPELCOSIL LC-NH ₂	6000	2.0	2-7	30

•1000psi = 6.89 MPa

SUPELCOGEL C-611 (calcium- and magnesium-form resin)

The unique packing in this column contains two divalent cations, rather than one. The separation mechanism is a combination of size exclusion and ion exchange modes. The pores in the resin exclude polysaccharides and larger oligosaccharides, which elute first. Smaller di- and monosaccharides enter the pores, interact with the counterions, and are more strongly retained. 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 (10⁻⁴ N sodium hydroxide) at 60°C.

SUPELCOGEL Ag2 (silver-form resin)

This column was developed specifically for separating the oligosaccharides typically present in corn syrup and fermentation products, such as beer, and also can be used to monitor starch hydrolysis. It provides rapid separations of oligomers up to DP7 and resolves glycerol and ethanol. Larger analytes elute first.

Detection

Because the UV wavelengths required to detect carbohydrates also will be strongly absorbed by impurities in the samples, 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.

Making a Decision

To choose the best column for a particular analysis, find the compounds of interest in Table 2 and note their retention times on each of the five columns. Select the column that will separate the

compounds of interest with at least 1 minute between any pair. We also strongly recommend using a guard column to protect the analytical column. Guard columns are listed under Ordering Information.

Table 2. Typical Retention Times on Supelco™ Carbohydrate Columns

Column: Cat. No.:	SUPELCOGEL Ca 59305-U	SUPELCOGEL C-610H 59320-U	SUPELCOGEL C-611 59310-U	SUPELCOGEL Ag2 59315	SUPELCOGEL LC-NH ₂ 58338
Dimensions:	30cm x 7.8mm	30cm x 7.8mm	30cm x 7.8mm	30cm x 7.8mm	25cm x 4.6mm
Support:	resin	resin	resin	resin	silica
Active Group:	calcium	hydrogen	calcium/magnesium	silver	amino
Temp.:	80°C	30°C	60°C	85°C	ambient
Mobile Phase:	deionized water	0.1% H ₃ PO ₄	10 ⁻⁴ N NaOH	deionized water	acetonitrile:water (75:25)
Flow Rate:	0.5mL/min	0.5mL/min	0.5mL/min	0.5mL/min	1.0mL/min
Compound	Retention Time (min)				
Arabinose	15.3	13.9	19.6	17.1	7.5
Arabitol	19.8	14.1	22.8	16.0	7.2
Dulcitol	22.3	13.4	25.7	15.9	9.0
Erythritol	17.7	15.0	20.2	16.1	5.9
Ethanol	19.4	25.6	21.0	18.4	not retained
Fructose	14.9	13.1	20.7	16.0	8.3
Galactose	13.4	12.9	17.6	15.8	10.3
Glucose	12.0	12.1	15.8	14.6	9.8
Glycerol	18.7	16.8	20.9	17.1	4.9
Inositol	14.9	12.6	20.1	17.4	13.3
Isomaltose	9.6	10.3	13.8	11.6	19.4
Isomaltotriose	8.5	9.5	12.6	9.8	not recommended
Lactose	10.2	10.8	14.3	11.8	19.5
Maltitol	13.6	11.0	17.7	15.0	15.5
Maltoheptose	7.5	8.8	11.6	7.3	not recommended
Maltohexose	7.7	8.9	12.0	7.6	not recommended
Maltopentose	7.9	9.1	12.6	8.1	not recommended
Maltose	9.8	10.5	14.2	11.5	17.4
Maltotetrose	8.3	9.3	13.2	8.8	not recommended
Maltotriose	8.8	9.7	13.6	9.8	31.0
Mannitol	19.2	13.2	22.1	15.2	9.2
Mannose	13.7	12.8	18.9	15.9	9.1
Melezitose	8.7	9.7	12.4	9.3	24.5
Psicose	22.5	13.4	32.9	17.2	6.6
Raffinose	8.7	9.7	12.6	9.6	29.7
Ribitol	16.7	13.7	19.5	15.3	6.8
Ribose	24.3	14.2	34.6	19.1	6.0
Sorbitol	23.4	13.4	28.3	16.3	9.0
Stachyose	8.1	9.3	11.9	8.5	67.3
Sucrose	9.8	10.6	13.6	11.2	14.0
Xylitol	23.3	14.4	28.0	17.1	7.3
Xylose	13.2	12.8	17.2	15.6	6.8

Ordering Information: Carbohydrate Columns and Guard Columns

Analytical Column	Cat. No.	Guard Column	Cat. No.
SUPELCOGEL Ca	59305-U	Supelguard™ Ca (5cm x 4.6mm)	59306-U
SUPELCOGEL C-610H	59320-U	Supelguard H (5cm x 4.6mm)	59319
SUPELCOGEL C-611	59310-U	Supelguard Ca (5cm x 4.6mm)	59306-U
SUPELCOGEL Ag2	59315	Supelguard Ag2 (5cm x 4.6mm)	59316
SUPELCOGEL NH ₂	58338	Supelguard LC-NH ₂ (2cm x 4.6mm)	
		Kit (column & holder)	59558
		Two Replacement columns	59568

For applications and additional information about these columns for carbohydrate analyses, request Bulletin 887.

Supelco, SUPELCOGEL, SUPELCOGEL, and Supelguard are trademarks of Sigma-Aldrich Co.

