

CarboPac™ MA1 Column for Carbohydrate Alditol and Aldose Analysis



HPLC Columns for the Analysis of Weakly Ionizable Carbohydrates

- *Simple methods with pulsed amperometric detection*
- *Reduced mono- and disaccharides in food products and physiological samples*
- *Unique selectivity*
- *Higher sensitivity than refractive index detection*
- *No sample derivatization required*
- *Ambient temperature separations*

Simple Methods with Pulsed Amperometric Detection

The CarboPac MA1 column is specifically designed to separate reduced monosaccharides and disaccharides commonly found in food products, as well as those found in physiological samples. Using a simple hydroxide eluent, analytes can be separated isocratically or with gradient elution. The low flow rate ensures low eluent consumption and reduces errors associated with eluent preparation.

The CarboPac MA1 is also well suited to the separation of reduced glycoconjugate samples after β -elimination. Designed to retain and separate reduced carbohydrates, the CarboPac MA1 separates sugar alcohols as their anions by high performance anion-exchange chromatography (HPAE). Detection of these complex compounds has been optimized with the use of pulsed amperometric detection (PAD).

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Reduced Mono- and Disaccharides in Food Products

Several alditols, including sorbitol and mannitol, occur naturally in foods. These alditols can be separated isocratically and detected using pulsed amperometry (see Figure 1). Mannose, glucose, and galactose were added to the sample to demonstrate that aldoses exhibit greater retention than their reduced alditol forms. Gradient elution can be used, allowing fast run times of selected analytes.

Commercial Sweeteners

Isomaltitol and glucopyranosyl-mannitol are found in commercial sweeteners. In Figure 2, lactitol is centered between isomaltitol and glucopyranosylmannitol. Quantification of lactitol and isomaltitol is improved and total run time is reduced by a step change to 600 mM sodium hydroxide.

Sucralose

Sucralose, developed jointly by Tate and Lyle and McNeil Specialty Products, is a high-intensity sweetener with 400–800 times the sweetness of sucrose. Sucralose is manufactured by selective chlorination of sucrose; it is currently the only non-nutritive sweetener based on sucrose. The product was approved in Canada in September 1991 for use in a variety of food products. Figure 3 shows an analysis of trace impurities in a Sucralose sample using HPAE-PAD.

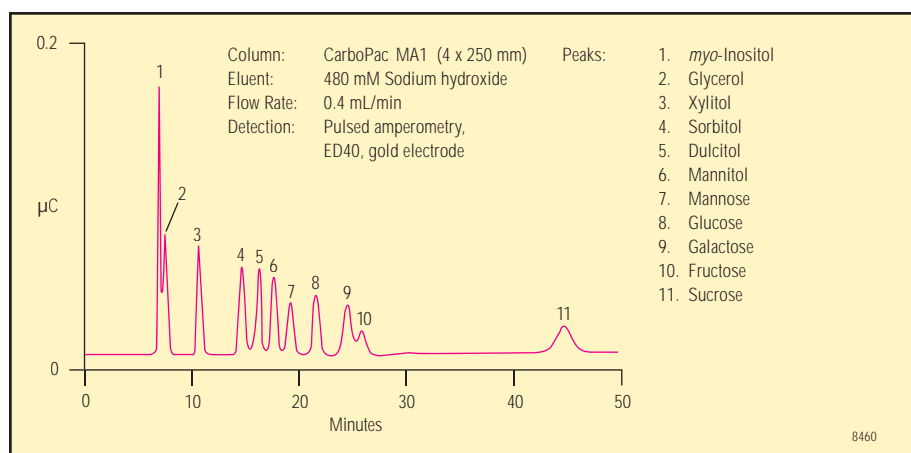


Figure 1. Isocratic separation and pulsed amperometric detection of common, naturally occurring food alditols and aldoses. Mannose, glucose, and galactose were added to the sample to demonstrate that aldoses exhibit greater retention than their reduced alditol forms.

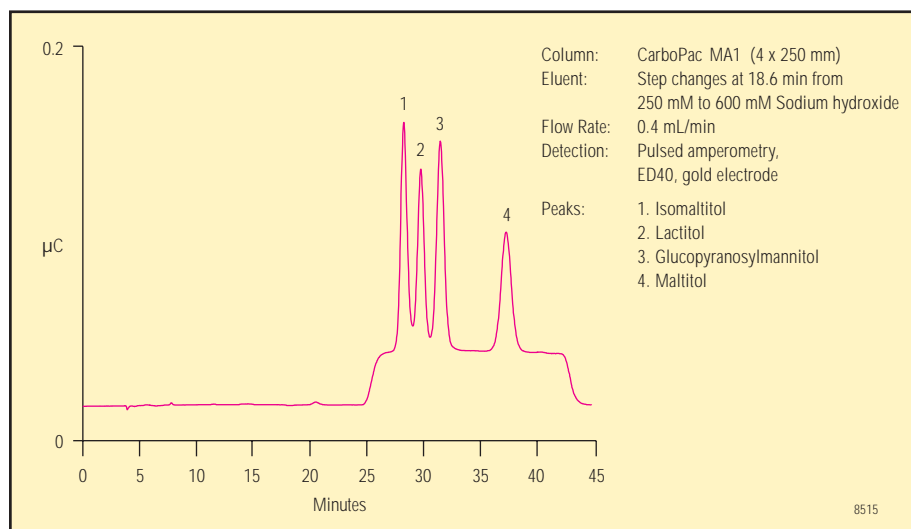


Figure 2. Separation of compounds found in commercial sweeteners.

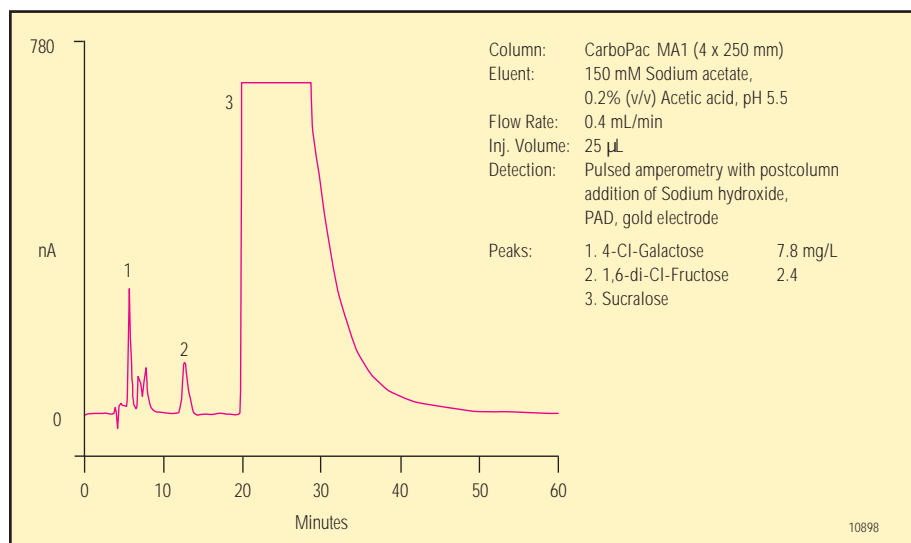


Figure 3. Analysis of Sucralose.

Vanillin

Vanillin is the most widely used flavoring in food products. Adulteration of natural vanillin by synthetic products is a major concern. Vanillin is an aromatic aldehyde and can be detected by pulsed amperometric detection with high sensitivity and specificity. This is particularly advantageous when determinations are made in complex food matrices since clean-up procedures can be minimized. Figure 4 shows the determination of vanillin in a sample of yogurt. Sample preparation involved a 100-fold weight/volume dilution and filtration (0.45 µg/L).

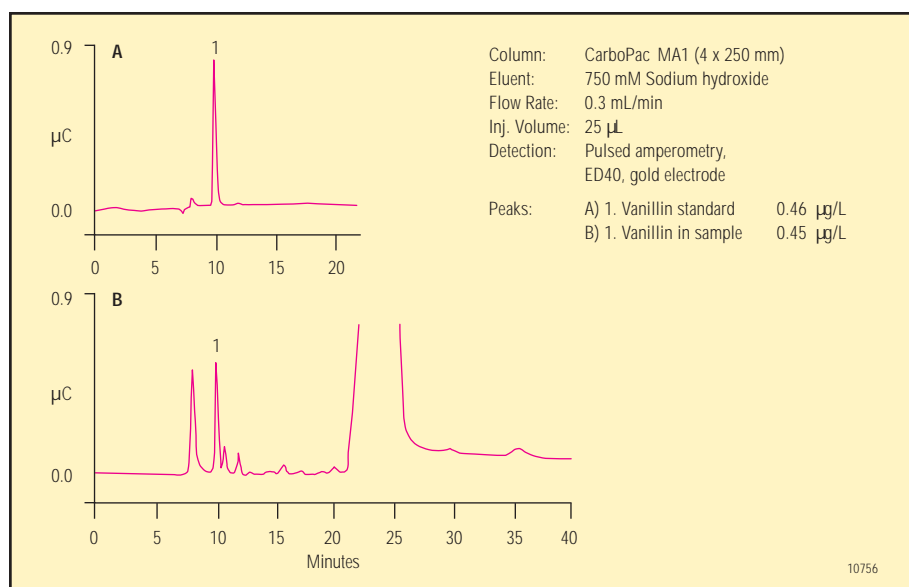


Figure 4. Determination of vanillin in yogurt.

Sugar Alcohols in Candy

Sugar alcohols are often used as sweeteners in dietetic confectionery products for diabetics. They are also non-cariogenic (i.e., they do not cause tooth decay) since they are not fermented by bacteria in the mouth. However, the use of sugar alcohols in foods is regulated because they sometimes exhibit laxative or diuretic properties. As shown in Figure 5, sorbitol and mannitol are easily determined, interference-free, in a hard candy (boiled sweets) sample after dissolution and dilution.

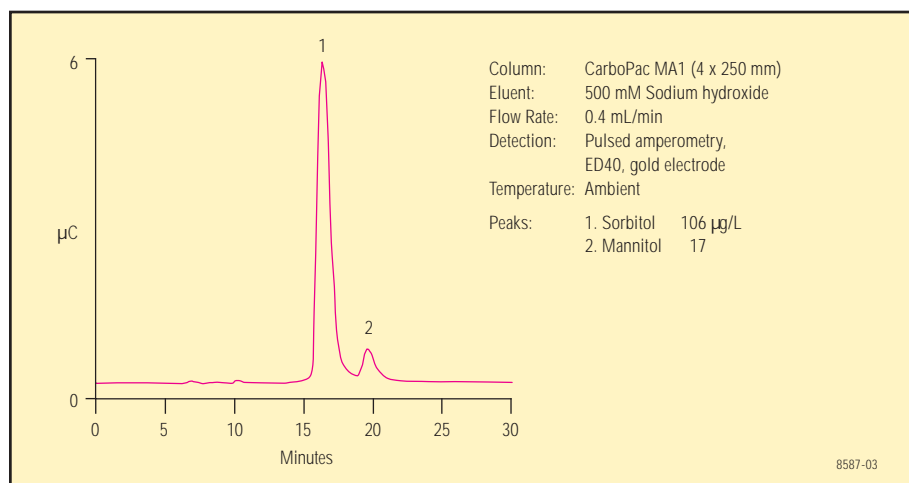


Figure 5. Determination of sugar alcohols in sugarless hard candy.

Carbohydrates in Chewing Gum

Figure 6 shows an analysis of chewing gum. Glycerol, sorbitol, mannitol, and glucose were determined directly in the chewing gum sample after sonication with deionized water, followed by passage through an OnGuard®-A cartridge and filtration through a 0.45-µm filter.

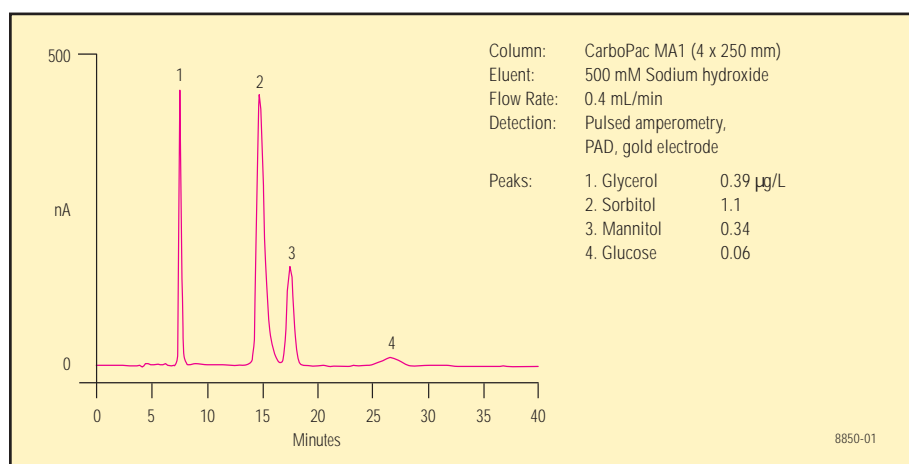


Figure 6. Determination of carbohydrates in chewing gum.

Sugars in Fruit Juices

The CarboPac MA1 column is specifically tailored for the determination of sugar alcohols, but mono- and disaccharides can also be determined in the same run. Sorbitol is present in high levels in apple juice relative to other fruit juices. It is a useful marker for the detection of adulteration of more costly juices with apple juice. The only sample preparation required is dilution and filtration (Figure 7).

Reduced Mono- and Disaccharides in Physiological Fluids

Inositol derivatives are ubiquitous in living tissues and physiological fluids. The CarboPac MA1 separates *myo*-inositol from *scyllo*-inositol under isocratic conditions. Separation of inositols from other alditols such as erythritol, arabitol, and sorbitol can be achieved under isocratic conditions, but sample throughput can be enhanced by gradient elution (Figure 8).

Glycoconjugates

The carbohydrate moieties of glycoproteins and glycolipids can be analyzed by HPAE-PAD. Figure 9 illustrates the identification of monosaccharides derived from glycoprotein oligosaccharides. Oligosaccharides are released by reductive β -elimination, which converts only the terminal peptide-linked sugar to the alditol form. The terminally reduced oligosaccharide is chemically or enzymatically digested into a mixture containing one alditol and one or more non-reduced monosaccharides.

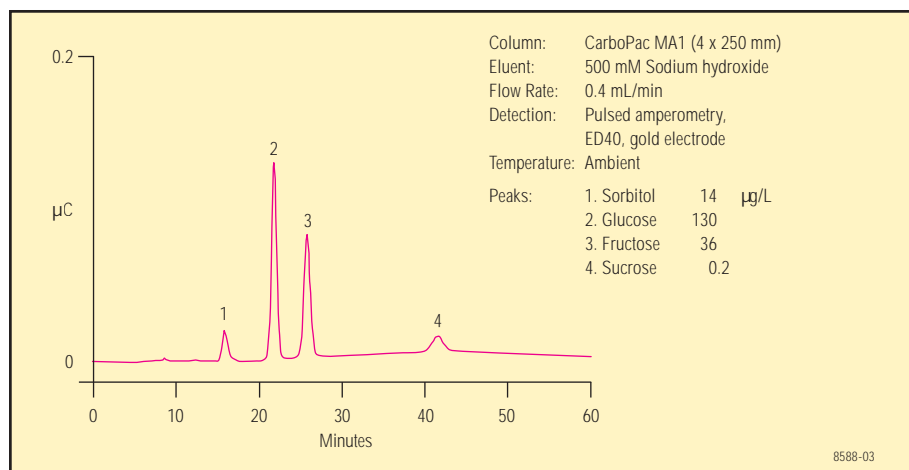


Figure 7. Determination of sugars and sorbitol in fruit juice.

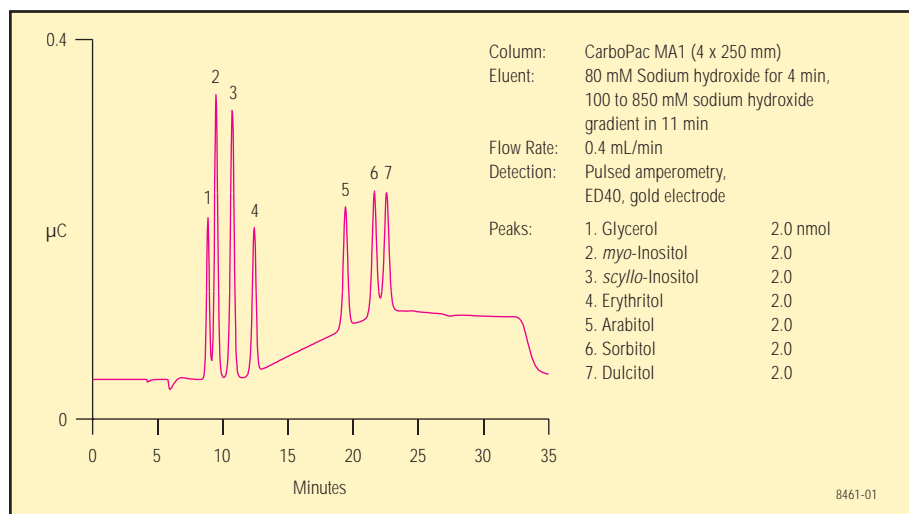


Figure 8. Separation of alditols typically found in living tissues and physiological fluids.

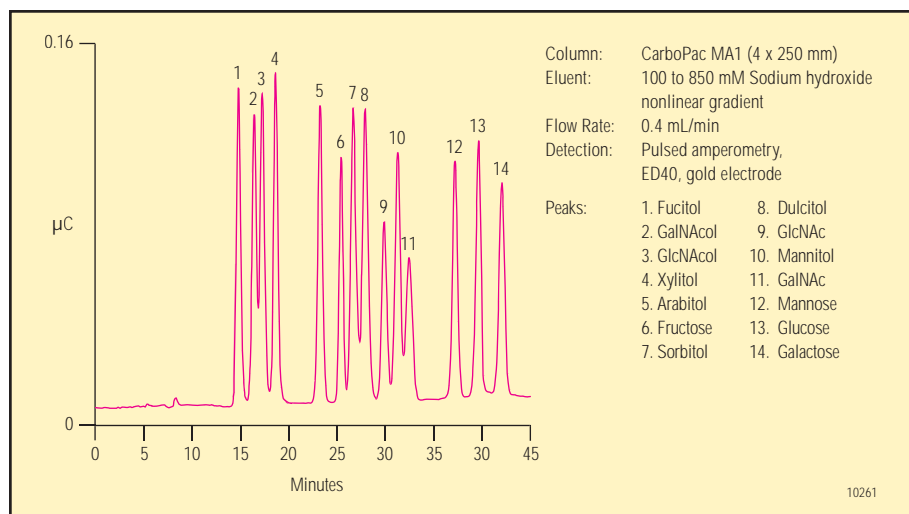


Figure 9. Gradient separation of alditols and aldoses potentially released by hydrolysis of β -eliminated oligosaccharides. Arabitol serves as an internal standard.

No Sample Derivatization Required

The use of sugar alcohols (also known as polyols) as alternative sweeteners is increasing rapidly for dietetic foods and products such as chewing gum because of their non-cariogenic properties. Nutritional labeling requirements for sugar alcohols are presently optional in the U.S., but as with sugars, total sugar alcohol content is listed. Therefore, minor sugar alcohols have to be determined (see Figure 10). GC methods for sugar alcohols have been developed but are complicated by the requirement for derivatization. The only official method in existence is AOAC 973.28, a GC method for sorbitol.

A simpler, direct approach that does not require derivatization is shown in Figure 10. The selectivity of the column was designed to allow sugar alcohols as a group to be eluted before sugars; therefore sugar alcohols and sugars can be determined in the same run. Sorbitol, mannitol, and xylitol are monosaccharide polyols in widespread use. Erythritol is expected to receive regulatory approval as a commercial sweetener in the near future. It has all the advantages of other polyols with the added benefit that only 10% or less is metabolized, giving it only 10% of the caloric value of sucrose.

Rugged, Reliable Analyses with Guaranteed Performance

The entire manufacturing process (including resin synthesis, amination, and packing of the chromatography columns) is carefully controlled to ensure that every Dionex CarboPac MA1 column delivers reproducible performance. CarboPac MA1 columns are tested with a representative set of alditols and aldoses (Figure 11) to ensure lot-to-lot consistency. Even with challenging samples, the CarboPac MA1 delivers run-to-run reproducibility and a long useful lifetime (Figure 12).

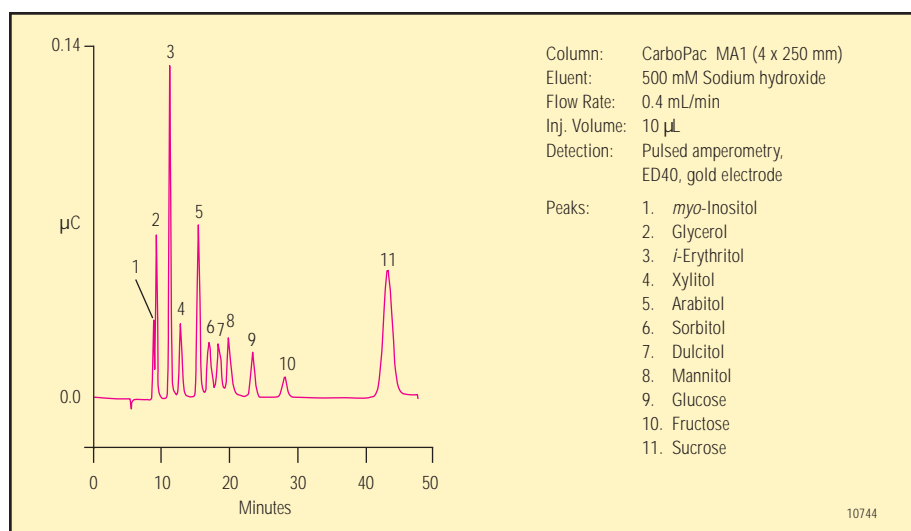


Figure 10. HPAE separation of sugars and sugar alcohols without the need for derivatization.

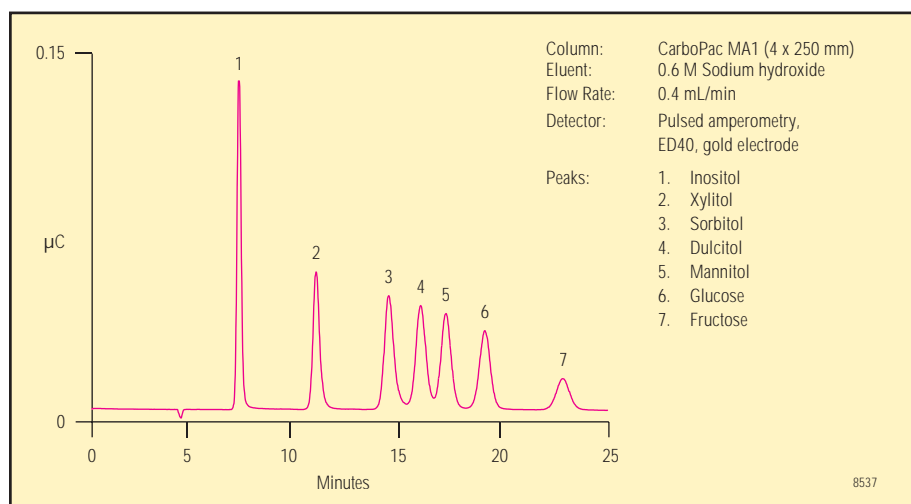


Figure 11. Isocratic separation of a representative set of alditols and aldoses ensures lot-to-lot reproducibility in the manufacturing of the CarboPac MA1 column.

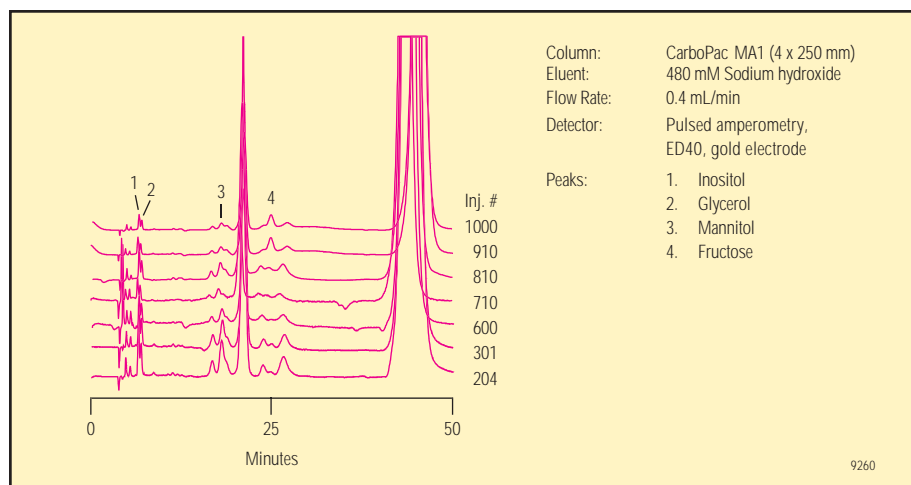


Figure 12. Separation of carbohydrates from instant coffee. In this run-to-run reproducibility study, an instant coffee sample was injected repeatedly onto the CarboPac MA1. Retention time RSDs for all components were less than 0.8% over a one month period using multiple eluent preparations.

Easy Isocratic Operation

Retention of carbohydrates on the CarboPac MA1 can be varied with eluent concentration (see Table 1). Note that the elution order for several sugars changes with the sodium hydroxide concentration. Table 1 is a guide for the development of separations of specific sets of analytes.

TABLE 1 K' VALUES OF SELECTED ANALYTES

Analyte	Eluent Concentration (M NaOH)				
	0.05	0.14	0.25	0.38	0.50
Glycerol	1.13	0.99	0.89	0.80	0.72
<i>myo</i> -Inositol	1.32	1.08	0.86	0.69	0.56
<i>scyllo</i> -Inositol	1.63	1.30	1.02	0.81	0.64
GlcNol	1.81	1.40	1.09	0.89	0.75
Fucitol	1.94	1.63	1.40	1.18	1.05
Erythritol	2.02	1.71	1.44	1.25	1.13
GalNol	2.29	1.81	1.39	1.13	0.95
GalNAcol	2.35	1.81	1.38	1.12	0.95
GlcNAcol	2.61	1.96	1.48	1.18	0.95
Xylitol	3.09	2.48	1.95	1.59	1.35
Arabitol	4.69	3.62	2.76	2.24	1.92
Sorbitol	6.43	4.72	3.33	2.55	2.06
Dulcitol	6.52	5.04	3.73	2.87	2.26
Adonitol	7.09	5.31	3.83	2.96	2.43
Mannitol	8.98	6.38	4.37	3.28	2.63
Fucose	10.34	4.72	2.52	1.69	1.25
Isomaltitol	12.22	8.15	4.89	3.30	2.43
Lactitol	14.97	9.61	5.49	3.57	2.43
<i>gp</i> -Mannitol	15.66	10.36	6.18	4.15	3.05
GalN	18.56	7.16	3.39	2.13	1.48
GlcN	20.88	7.71	3.61	2.24	1.55
Maltitol	31.21	17.25	8.80	5.44	3.67
Glucose	> 32	15.70	7.19	4.31	2.91
Mannose	> 32	13.55	6.15	3.72	2.53
Galactose	> 32	17.82	8.25	4.99	3.43

SPECIFICATIONS

Resin Composition:

7.5- μ m diameter vinylbenzyl chloride/
divinylbenzene macroporous substrate
fully functionalized with an alkyl
quaternary ammonium group (15%
crosslinked)

Chemical Compatibility:

pH 0–14; incompatible with solvents
and anionic detergents

Typical Operating Conditions:

1400 psi at 0.4 mL/min (guard and
analytical columns)

Anion-Exchange Capacity:

1450 μ eq/column (4 x 250 mm
analytical column)

Temperature Range:

4–50 °C

Recommended Operating Temperature:

Ambient

Maximum Operating Pressure:

2000 psi (14 MPa)

Test Procedure:

Baseline resolution of inositol, xylitol,
sorbitol, dulcitol,
mannitol, glucose, and fructose

Recommended Flow Rate:

0.2–0.5 mL/min

Eluents:

Acetate or hydroxide only

ORDERING INFORMATION

In the U.S., call 1-800-346-6390 or contact the Dionex regional office nearest you. Outside the U.S., order through your local Dionex office or distributor. Refer to the following part numbers. Dionex can also make special-order CarboPac columns to your specifications; call for more information.

Description	Part Number
CarboPac MA1 Analytical Column (4 x 250 mm)	044066
CarboPac MA1 Guard Column (4 x 50 mm)	044067



CarboPac is a trademark and OnGuard is a registered trademark of Dionex Corporation.

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* Designed, developed, and manufactured under an NSAI registered ISO 9001 Quality System.



LPN 034796-02 6M 01/01
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