

Waters

Lab Highlights

CARBOHYDRATE ANALYSIS ON AMINE-MODIFIED RADIAL-PAK™ SI CARTRIDGE

Several methodologies are available for the LC analysis of carbohydrates and polyhydric alcohols: size exclusion/calcium complexation (Sugar-PAK™ I), C₁₈ reverse phase (Dextro-PAK Radial-PAK™) and bonded amine normal phase chromatography (Carbohydrate Analysis Column). Each offers its own unique applications.

Another approach to carbohydrate analysis is the use of silica columns modified with an amine, producing a separation system similar to that of the Carbohydrate Analysis Column. The research group of Dr. John Baust of the University of Houston, with the collaboration of Howie James of Waters, has studied the Radial-PAK™ Si Cartridge in the RCM-100^R Radial Compression Module as the column in this system and found that it gave excellent performance - low cost, relatively long column life (months) despite a high operating pH (8.9 - 9.2), high sample capacity, and high resolution. This work has been published in the Journal of Chromatography, (1) and is abstracted here.

This method is an alternative means to carry out a carbohydrate analysis with RCSS technology. The particular advantages of RCSS are the relatively low cost of the cartridge and the enhanced column lifetime obtained by use of the RCM-100^R Radial Compression Module.

Figure 1 is a chromatogram of several standards. Elution is generally in order of molecular size, smaller first, although there are exceptions.

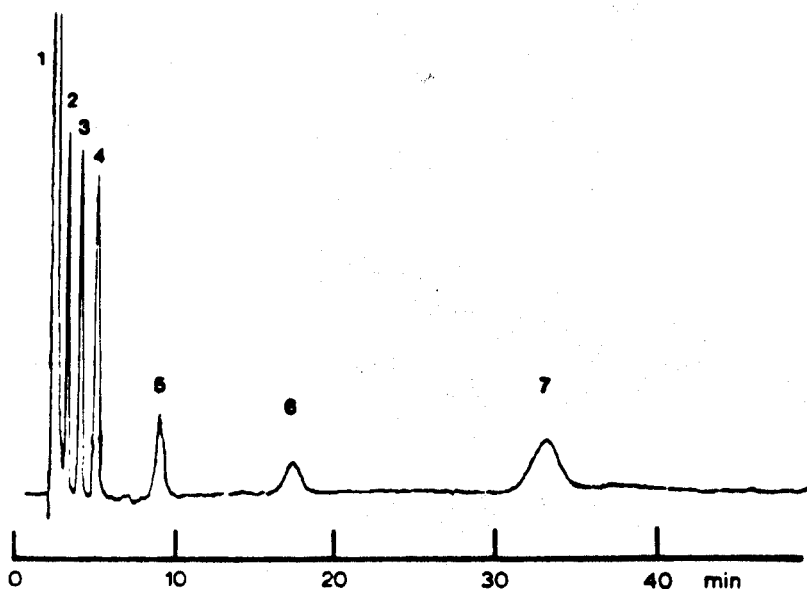


Fig. 1. Chromatogram of elution of polyol-saccharide mixture by a hydraulically compressed Radial-Pak silica column (10 cm x 8 mm I.D.) modified with TEPA. Elution solvent: acetonitrile-water (81:19), pH 8.9, containing 0.02% TEPA. Eluent flow-rate, 2 ml/min, pressure, 2.34 MPa (340 p.s.i.), 26°C. Injected sample contained, in 50 µl, 250 µg of each of the following: 1 = solvent "front"; 2 = ethylene glycol; 3 = glycerol; 4 = erythritol; 5 = glucose; 6 = maltose; and 7 = raffinose.

Table 1 presents data for a variety of samples, including efficiencies (ranging as high as 14,700 theoretical plates per meter) and resolution between adjacent members. Resolution may be adjusted by varying the concentration of acetonitrile in the mobile phase. This affects α as well as k' , as higher molecular weight substances show much greater increases in k' as the concentration of acetonitrile is increased.

TABLE I
ELUTION OF CARBOHYDRATES AND POLYOLS FROM A RADIAL-PAK SILICA COLUMN
Conditions of elution as in Fig. 1.

Compound	Molecular weight	k'	$N/10\text{ cm}$	R_s^*
Ethylene glycol	62.07	0.497	634	1.889
Glycerol	92.09	1.017	657	1.355
Rhamnose	164.16	1.451	903	0.417
Erythritol	122.12	1.594	834	0.136
Threitol	122.12	1.640	1125	0.610
Fucose	164.16	1.880	596	0.515
Xylose	150.13	2.091	1259	0.480
Ribitol (adonitol)	152.15	2.286	812	0.010
Sedoheptulose anhydride	210.20	2.331	924	0.355
Arabitol	152.15	2.497	800	0.321
Fructose	180.16	2.903	837	1.038
Sorbitol	180.16	3.451	1185	0.270
Dulcitol (galactitol)	182.16	3.594	1160	0.035
Mannitol	182.16	3.617	583	0.620
Glucose	182.16	4.046	1066	3.100
Sucrose	342.30	6.520	946	1.680
Inositol	180.16	8.257	1153	0.131
Cellobiose	324.30	8.400	1189	0.100
Maltose	360.31	8.617	1270	0.581
Trehalose	378.33	9.286	1132	0.424
Lactose	342.30	9.829	1049	5.091
Raffinose	594.52	17.640	1470	

* Calculated resolution between adjacent members of the table.

The k' 's are found to decrease with decreasing pH, but large, negative water peaks appear in the acid pH range. The optimum pH (8.9) is normally not preferred for HPLC due to etching of the silica. However, the active radial compression of the RCM-100^R Radial Compression Module is able to maintain a uniform bed and a relatively long column life despite the undesirable pH.

The cartridge is prepared by pumping a 0.1 % (w/v) solution of tetraethylenepentamine (TEPA) in acetonitrile:water (70:30) through the cartridge. The analytical eluent is 0.02% TEPA in acetonitrile:water (81:19). The column was operated at 2 ml/min at a back pressure of 400 psi. Although this flow rate is optimum, the low back pressure of the cartridge permits flow rates as high as 10 ml/min. The mobile phase was recirculated using a stirred 1 litre reservoir.

1. D. L. Hendrix, R. E. Lee, Jr., J. G. Baust, and H. James, J. Chromatogr. 210 (1981) 45-53.