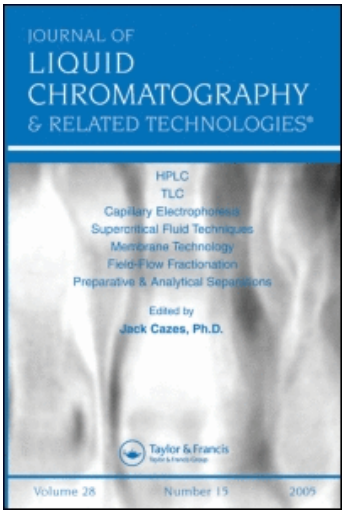

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Improved Separation of Polyols and Carbohydrates by High Performance Liquid Chromatography

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IMPROVED SEPARATION OF POLYOLS AND CARBOHYDRATES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A reliable High Performance Liquid Chromatographic analysis of glucose-fructose-sucrose-sorbitol and mannitol is developed. A Sugar Pak I column at 85ºC is used employing water as the mobile phase. The separation is completed within 20 minutes, and the resolution is very acceptable.

INTRODUCTION

The application of high performance liquid chromatography (HPLC) to the analysis of carbohydrates has signified a great improvement in their qualitative and quantitative determination (1-4).

Polyhydric alcohols, such as mannitol and sorbitol, usually employed as artificial sweeteners in food products, have been determined by different authors (5).

Nonetheless, the simultaneous presence of sugars and polyols in fruits and dietetic foodstuffs

poses a difficult analytical problem due to difficulty of resolution of these compounds by HPLC.

Various attempts have been carried out in this regard. Brandao et al. (6) and Richmond et al.(7) achieved the separation of polyols and carbohydrates by HPLC, using two columns joined in tandem, which increased the cost of the analyses. Gordy et al. (8) and Dokladalova et al. (9) give some chromatographic constants for carbohydrates and polyols.

EXPERIMENTAL

High performance liquid chromatography was carried out in an ALC/GPC (Model 201) equipped with model 6000 A pump dual reciprocating piston heads, model U 6K septumless injector, Sugar Pak I column 30cm x 6.5 mm i.d., water jacketed at 85ºC, pre-column filter, and model R-401 differential refractometer detector optical deflection type, maintained at 30 C (Waters Associates,Milford,Mass, USA). The detector signal was recorded on a Houston Instrument Omniscribe recorder.

Mobile phase: Bidistilled water was degassed by immersion in an ultrasonic bath and filtered through a Millipore HA (0.45µm) membrane (Millipore Corp., Bedford, Mass., USA).

Standard solution: Various amounts of sucrose, glucose, fructose, sorbitol and mannitol (Merck) were dissolved in bidistilled water and filtered through a Millipore HA (0.45µm) membrane.

RESULTS AND DISCUSSION

The performance of the Sugar Pak I column for a mixture of sucrose, glucose, fructose, mannitol

TABLE 1

Standardization of Waters Sugar Pak I High Performance Liquid Chromatographic Column

	RT_S	Sucrose	Glucose	Fructose	Mannitol	Sorbitol	k'	N
		Separation factor (α)						
Sucrose	0.472	\	1.923	2.846	3.615	4.692	0.433	1183
Glucose	0.604	2.182	\	1.480	1.880	2.440	0.833	1344
Fructose	0.736	4.000	1.846	\	1.270	1.649	1.233	1466
Mannitol	0.846	6.182	3.666	1.539	\	1.298	1.567	2635
Sorbitol	1.000	8.727	6.000	3.692	2.545	\	2.033	3680
		Resolution (R)						

RT_S = relative retention time to sorbitol; Separation factor $(\alpha) = k'_2/k'_1$;
$W \cong$ peak width; Resolution $(R) = V_2 - V_1 / \frac{1}{2}(W_2 + W_1)$; V = retention volume;
V_0 = void volume; Capacity factor $(k') = V - V_0/V_0$;
Number of theoretical plates $(N) = 16\ (V/W)^2$

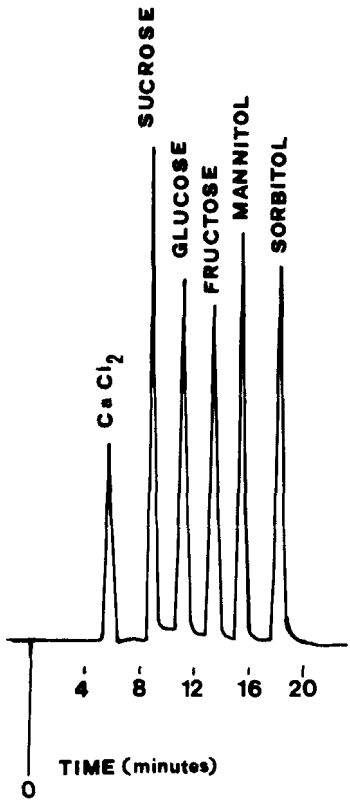

Fig.1: Flow rate: 0.4ml/min.; Attenuation 32x

and sorbitol, was characterized by the following numerical values (see Table 1): retention time to sorbitol (RT_s), separation factor (α), resolution (R), capacity factor (k'), and theoretical plates (N). The void volume used to calculate (k') was determined using the retention time of $CaCl_2$.

A typical chromatogram of the standard solution is shown in Fig.1.

The response of the detector was a rectilinear response between peak height (cm) and weight,(25,

50,75 and 100 µg in 50 µl) of the carbohydrates and polyols. The equations y=a+bx of the 5 lines are:

for sucrose	a=0.050	b=0.082	r=0.999
for glucose	a=0.063	b=0.066	r=0.999
for fructose	a=0.063	b=0.061	r=0.999
for mannitol	a=0.025	b=0.071	r=0.999
for sorbitol	a=0.062	b=0.064	r=0.999

The number of theoretical plates and the capacity factor for the polyhidric alcohols, as well as for the mono- and disaccharides analyzed were substantially greater than those obtained previously (8).

Dokladalova et al. (9) indicate only the retention times of the products separated, which are similar to those reported here, though ours, especially those corresponding to sucrose and glucose, are more adequate for the achievement of complete resolution of a mixture of them.

In general, it can be concluded that the chromatographic conditions established here are appropiate for the separation and quantitation of a mixture of sucrose, glucose, fructose, mannitol and sorbitol.

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