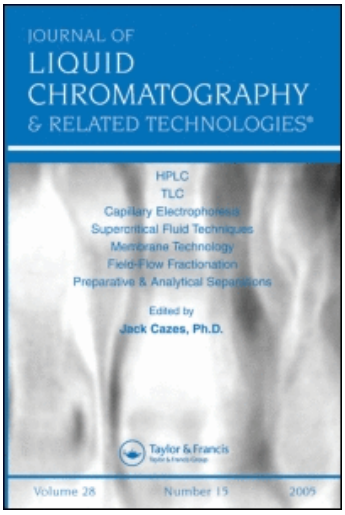

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Reliable Separation of Xylitol from Some Carbohydrates and Polyols by High Performance Liquid Chromatography

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RELIABLE SEPARATION OF XYLITOL FROM SOME CARBOHYDRATES AND POLYOLS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

An acceptable separation of xylitol from a mixture of sucrose, glucose, fructose, mannitol and sorbitol was carried out by High Performance Liquid Chromatography. A Sugar Pak I column at 80ºC was used employing acetonitrile/water (25/75) (v/v) as the mobile phase.

INTRODUCTION

Xylitol is a polyol that has been proposed recently as a sugar substitute for oral use in diabetic foods due to its retarded release of glucose which avoids blood sugar peak (1). Moreover, it presents the advantage of its relative sweetness, approximately equal to that of sucrose, and it is the only sweetener available to the food technologist which permits a 1:1 replacement of sucrose in his formulation (2), thus making it recommendable for use in products considered cariogenics, such as chewing gums, lozenges, candies and similar sweets (3).

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The analysis of a carbohydrate mixture by conventional means is dreary and time consuming task. The high performance liquid chromatographic (HPLC) technique has greatly facilitated this endeavour, allowing not only the identification, but the quantification of these mixtures. The technique has been widely used in the field of nutrition (4-8).

The determination of soluble carbohydrates in dietetic foods for diabetic subjects poses the problem of the simultaneous determination of sugars and polyols since the latter have been used as sweeteners.

The information already published on this point is scarce. Olano has described(9) a gas liquid chromatographic (GLC) method to determine carbohydrates and polyols in wines. The GLC technique presents in our opinion, a complex picture since each sugar originates two or more peaks, thus complicating the interpretation of the chromatograms. Samarco et al. (10) separated sorbitol, mannitol and xylitol by HPLC but did not report on the presence of sugars. Dokladalova et al. (11) and Vidal-Valverde et al. (12), acceptably separated carbohydrates and polyols, but xylitol and sorbitol eluted simultaneously. We have found no previous references on the analysis of xylitol in the presence of sorbitol, mannitol, sucrose, glucose and fructose.

There are indications that xylitol will be the sweetener of choice in the near future. It is important, then, to have a method of separating and quantifiyng xylitol in the presence of other sugars and polyols which may be present in dietetic foods.

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 6.5mm i.d., with a column temperature control accessory at 75ºC, or 80ºC, pre-column filter, and model R-401 differential refractometer detector optical deflection type, maintained at 30ºC (Waters Associates, Milford, Mass. USA). The detector signal was recorded on a M 730 data module. Chart recorder speed: .25cm/min.Attenuation x16. Injection volume : 25µl.

Mobile phase: acetonitrile/water 25/75, filtered through a Millipore FH(.5µm) membrane, degassed by immersion in an ultrasonic bath. The flows rates were .4 ml/min. and .5 ml/min.

Standard solution: various amounts of sucrose, glucose, fructose, mannitol, xylitol and sorbitol (Merck) were dissolved in the mobile phase.

The use of acetonitrile/water (25/75)(v/v) as the mobile phase and the Sugar PakI column has meant a considerable improvement in the separation of mixtures containing xylitol. The operating conditions were somewhat different from the ordinary ones, but with no adverse effects on the life span of the column The operating procedure was as follows: After ten analyses (generally a day's work), the column pressure rose to 2500 psi. At this point, the column was placed tail first and bidistilled water (previously filtered and degassed) was pumped through it overnight at 90ºC. In this way, the operating pressure dropped from 2500 psi to normal values and the column was ready for a new set of analyses. Approximately once a week the

column was regenerated by passing through it, tail first, a 0.001M solution of calcium acetate during 3.5 hours at .5ml/min. flux. The column was kept at 90ºC during the process. This treatment was followed by the pass of bidistilled water (filtered and degassed) during 30 minutes at the flux previously indicated.

RESULTS AND DISCUSSION

The analysis of xylitol, in the presence of sugars and polyols, which may be present in dietetic foods, could be done by thin layer chromatography (13), but, in practice, this is not feasible since some of these compounds are present in very large amounts while some others are only present in minute amounts. HPLC technique constitutes a great improvement for this type of analyses. In this work, the aim has been to set up appropiate HPLC conditions to detect and quantify xylitol in the presence of polyols, such as mannitol and sorbitol, and carbohydrates, such as sucrose, glucose and fructose.

Fig. 1 represent two chromatograms obtained with a standard solution of sucrose, glucose, fructose, mannitol, xylitol and sorbitol, under two different set of conditions.

Table 1 lists the chromatographic constants obtained from the standard curve (Fig 1 A). The void volume was determined using the retention time of Cl_2Ca. The response factor of the detector was a rectilinear response between peak height (cm) and weight (25-200µg) of the carbohydrates and polyols. The equations y=a+bx of the six lines were:

for sucrose a=-0.15 b=0.037 r=0.999

for glucose	a=-0.15	b=0.044	r=0.999
for fructose	a=-0.10	b=0.038	r=0.999
for mannitol	a=-0.25	b=0.038	r=0.999
for xylitol	a=-0.12	b=0.033	r=0.999
for sorbitol	a=-0.07	b=0.032	r=0.999

The conditions established, both in Fig 1A and Fig. 1B, were adequate to quantify xylitol on the

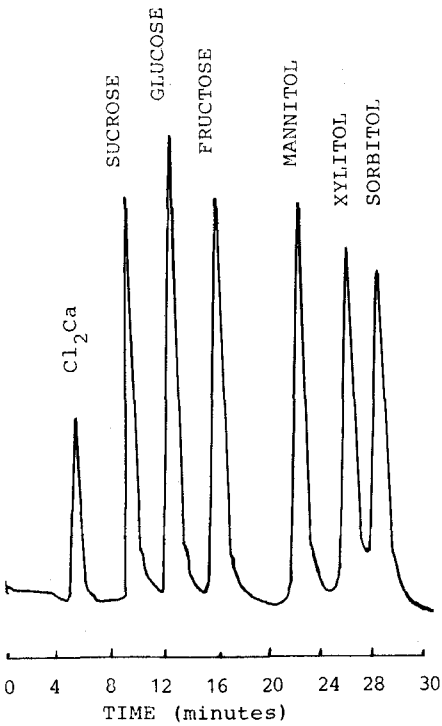

FIG. 1 A.-

Mobile phase:
acetonitrile /water, 25/75

Flow rate: 0.4 ml/min.

Temperature column: 75ºC

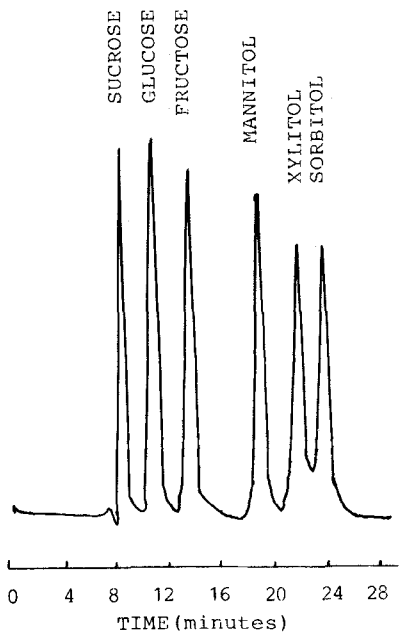

FIG. 1 B.-

mobile phase:
acetonitrile/water, 25/75

Flow rate: 0.5 ml/min.

Temperature column: 80ºC

TABLE 1

Standardization of Waters Sugar Pak I High Performance Liquid Chromatographic Column

	RT_S	Sucrose	Glucose	Fructose	Mannitol	Xylitol	Sorbitol	K'	N
		Separation factor (α)							
Sucrose	0.338	\	1.867	2.779	4.406	5.360	5.954	0.655	958
Glucose	0.454	2.056	\	1.488	2.360	2.870	3.189	1.223	1287
Fructose	0.576	3.750	1.922	\	1.586	1.929	2.142	1.820	1045
Mannitol	0.793	7.600	5.665	3.250	\	1.217	1.351	2.886	1778
Xylitol	0.921	9.187	7.361	4.895	1.905	\	1.111	3.511	1672
Sorbitol	1.000	10.438	8.611	6.020	3.089	1.125	\	3.900	1816
		Resolution (R)							

RT_S = relative retention time to sorbitol; Separation factor $(\alpha) = k'_2/k'_1$;
W = 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 $(N) = 16\ (V/W)^2$

presence of the sugars and polyols indicated above. Acetonitrile/water, 35/65 (v/v), with a flow rate of 0.5 ml/min. also separates the standard mixture but cannot be recommended as a mobile phase, since it would produce a rapid build up of the column pressure.

The other components of the mixture could also be quantified since the separation between them was acceptable and the correlation coefficients of the curves were adequate. Nonetheless, it has been observed that occasionally the sucrose peak splits in two, fact which could interfere with the quantitation of it.

It can be concluded that the chromatographic conditions established here are appropriate for the separation and quantification of a mixture of xylitol, mannitol, sorbitol, sucrose, glucose and fructose, compounds which may be present in dietetics foods.

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