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Short communication

Separation of alditols of interest in food products by high-performance anion-exchange chromatography with pulsed amperometric detection

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Abstract

High-performance anion-exchange chromatography (HPAEC)–pulsed amperometric detection (PAD) employing a CarboPac MA 1 column was investigated with respect to mobile phase composition, linear response characteristics, repeatability, reproducibility and sensitivity with different alditols used as sugar substitutes in food and confectionery products. The energy-reduced bulk sweeteners isomalt and maltitol were well resolved in less than 25 min by isocratic elution with 600 m*M* sodium hydroxide solution. HPAEC–PAD was also successfully applied to the determination of alditols in sugar-free products and a low-calorie sweetener containing sorbitol, mannitol and fructose at different levels. © 1997 Elsevier Science B.V.

Keywords: Food analysis; Alditols; Sugar alcohols; Artificial sweeteners

1. Introduction

Sugar alcohols, also known as alditols or polyols, are polyhydric compounds obtained by reduction of the corresponding aldoses and ketoses. Xylitol, sorbitol and mannitol are alditols gaining popularity for the replacement of sucrose in confectionery products, as high intensity sweeteners without the calories associated with sugars [1]. Furthermore, disaccharide alditols such as isomalt and maltitol are used as sweetening agents for the formulation of sugar-free products, where they provide both sweetness and bulking properties [2]. The importance of these sweeteners is based on their reduced cariogenic properties and a postulated lower calorie value. Furthermore, in the small intestine, isomalt and maltitol are only partially hydrolyzed and absorbed. Therefore, they do not cause significant increase in blood sugar or insulin levels. Hence, they may be useful as sweeteners for diabetics [3].

Alditols have been separated by high-performance liquid chromatography (HPLC) on aminopropyl columns [4,5] and cation-exchange resins in various ionic forms [6–8], with either low-wavelength UV or refractive index detection. However, these modes of HPLC for carbohydrates can suffer several drawbacks such as instability and a short lifetime of the column and lack of sensitivity of direct detection.

Since its introduction, pulsed amperometric detection (PAD) has considerably improved the sensitivity and selectivity of the separation of carbohydrates by high-performance anion-exchange chroma-

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tography (HPAEC) using alkaline mobile phases [9]. AEC is not a technique commonly associated with analysis of neutral carbohydrates. However, most carbohydrates are weak acids with pK_a values in the range of 12–14 and, consequently at high pH values their hydroxyl groups are partially or totally transformed into oxyanions, enabling this class of compounds to be chromatographed as anions by HPAEC. Under alkaline conditions, carbohydrates are readily separated by highly efficient anion-exchange columns and the order of increasing retention is correlated with decreasing pK_a values. While separation of most mono- and disaccharides on HPAEC columns in aqueous sodium hydroxide is adequate [10–14], the ionization of alditols is weak and their separation is poor. Recently, another anion exchanger, specifically designed by Dionex for the separation of weakly ionizable carbohydrates such as alditols, has been introduced [15]. This column has a capacity 45-times greater than that of other columns usually employed, allowing the use of higher sodium hydroxide concentrations and promoting greater ionization and stronger retention of carbohydrates.

In order to select the optimum chromatographic conditions to perform the rapid and reproducible separations and quantitation of neutral carbohydrates and sugar alcohols in dietetic sweeteners as well as in processed foods, the effect of mobile phase composition on the retention time and selectivity was examined using a CarboPac MA 1 column, specifically tailored for alditol analyses.

2. Experimental

2.1. Materials

Glycerol, alditol and monosaccharide standards were obtained from Sigma (St. Louis, MO, USA). Isomalt and malbit syrup were kindly provided by Gazzoni 1907 SpA (Bologna, Italy). Isomaltitol was from Seikagaku (Tokyo, Japan). Sodium hydroxide solution (50%, w/w) and sodium acetate were purchased from J.T. Baker (Deventer, Netherlands). Chewing gums, candies and the low-caloric sweetener were purchased from a local market. HPLC-grade water was from Carlo Erba (Milan, Italy).

2.2. Equipment

The experiments were performed on a Dionex (Sunnyvale, CA, USA) Model 4000i gradient pump module equipped with a pulsed electrochemical detection (PED) system consisting of an amperometric flow-through cell with a gold working electrode and a silver–silver chloride reference electrode. The time and voltage parameters for the detector were provided by Dionex [16] and were set as follows: $E_1=0.05$ V, $t_1=450$ ms, integration from 200 to 450 ms; $E_2=0.65$ V, $t_2=180$ ms; $E_3=-0.15$ V, $t_3=350$ ms. Samples were injected using a Rheodyne Model 9125 non-metal polyether ether ketone (PEEK) injection valve with a PEEK sample loop of 10 μl (Cotati, CA, USA). The Dionex eluent degas module was employed to sparge and pressurize the eluent with helium to degas and to prevent adsorption of carbon dioxide and subsequent production of carbonate which would act as displacing ion and shorten retention times.

Chromatographic data were collected and plotted using the Dionex AI-450 chromatography workstation. Peak heights and peak areas were measured with internal standardization. Eluents were prepared by suitable dilution of sodium hydroxide solution (50%) with HPLC-grade water. The HPAEC CarboPac MA1 column (250 mm×4 mm I.D.), with associated guard column used for sample separation was also supplied by Dionex.

2.3. Standard solutions and sample preparation

A stock solution of the analytes was prepared prior to use at a concentration of 15 mg/ml. Internal standard aqueous solutions containing 10 mg/ml of arabinose or myo-inositol were also freshly prepared. Appropriate amounts of the stock solution were diluted with water to produce working standard solutions at five different concentrations within the required range. The appropriate volume of internal standard solution was added to each working solution to give a final concentration of 75 μg/ml of arabinose or myo-inositol, respectively. Calibration graphs were plotted based on the linear regression analysis of both the peak-height and the peak-area ratios.

Chewing gum samples were prepared for analysis

by a modification of the method proposed by Kiba et al. [17], according to the following procedure: 0.5–0.8 g (depending on expected concentrations) of a stick of chewing gum was cut into small pieces and placed in a beaker with 30–40 ml of HPLC-grade water. The sample was subjected to ultrasonic treatment for 10 min, then heated at 60°C (±2°C) under stirring for 1 h. After cooling, the extract was filtered through a sinter glass filter and diluted with water in a volumetric flask to 250 ml. To remove anions, 5 ml of the sample were passed through a pre-wetted OnGuard A cartridge (Dionex). The first 3 ml of the sample were discarded, then 1 ml was subjected to a further 10-fold dilution after the addition of the appropriate volume of internal standard solution to give a concentration of 75 μg/ml of arabinose. The use of a OnGuard A cartridge in chewing gum sample preparation is recommended in an application note published by Dionex [18]. However, this step could be avoided in samples which do not contain inorganic anions such as chloride or other potential column contaminants.

Aqueous solutions of tabletop low calorie sweetener (4.8 mg/ml) were filtered through a 0.22 μm single use membrane filter (Millipore, Bedford, MA, USA), then diluted 1:5 with water after the addition of the appropriate volume of internal standard solution to give a concentration of 75 μg/ml of myo-inositol.

All aqueous solutions were made using HPLC-grade water, filtered through a Type HA 0.22 μm single use membrane filter, and degassed prior to use by sparging with helium.

3. Results and discussion

In the initial phase of our investigation, we were concerned with optimizing eluent composition in order to achieve maximum separation of sugars and alditols of food interest in a reasonable time, using a CarboPac MA1 column. Disaccharide alditols isomalt and maltitol are saccharose substitutes employed as sweeteners in dietetic or sugarless products. Isomalt, also marketed with commercial name of Palatinit, is an equimolecular mixture of the isomers α-D-glucopyranosyl-1,6-sorbitol (GPS) and α-D-glucopyranosyl-1,6-mannitol (GPM). Maltitol (α-D-glucopyranosyl-1,4-sorbitol) is obtained by the hydrogenation of maltose, which is produced from starch, and the final product is a syrup, marketed also with the commercial name malbit, contains maltitol as the major component.

A standard mixture of isomalt and malbit was eluted isocratically with mobile phases containing sodium hydroxide at various concentrations ranging from 420 to 675 m*M*. The retention times of the isomalt isomers GPS and GPM, maltitol and sorbitol, present as a minor component of malbit, decreased with increasing sodium hydroxide concentration. Sorbitol was eluted first, while disaccharide alcohols exhibited longer retention times and eluted in the following order: GPS, GPM, maltitol. Decreasing sodium hydroxide concentration below 540 m*M*, disaccharide alditols eluted as rather broad peaks.

As expected, GPS and GPM follow the elution order of monosaccharide alditols sorbitol and mannitol, whereas maltitol and GPS (corresponding to isomaltitol), are disaccharide isomers differing only in α-1,4 and α-1,6 linkages and their elution order is similar to that of glucobiose maltose and isomaltose obtained with other HPAEC columns [12,19].

Sodium acetate solutions ranging from 5 to 25 m*M* were added to various levels of sodium hydroxide (between 480 to 600 m*M*). The retention order was always the same, but the retention times were reduced. Although the resolution often was not altered significantly, sorbitol was poorly resolved from the other components. A satisfactory separation of isomalt and malbit components was obtained using 600 m*M* sodium hydroxide delivered at 0.4 ml/min, as indicated in Fig. 1.

3.1. Determination of alditols in chewing gum extract

We next investigated the separation and quantitation of alditols derived from reduced monosaccharide aldoses, usually employed as artificial sweeteners in food products. Elution conditions were adapted for the rapid determination of alditols usually present as sweeteners in sugar-free chewing gums. Increasing sodium hydroxide concentration from 450 to 600 m*M*, baseline separation of glycerol, xylitol, sorbitol and mannitol from a chewing gum extract was obtained. The quantification of these alditols was

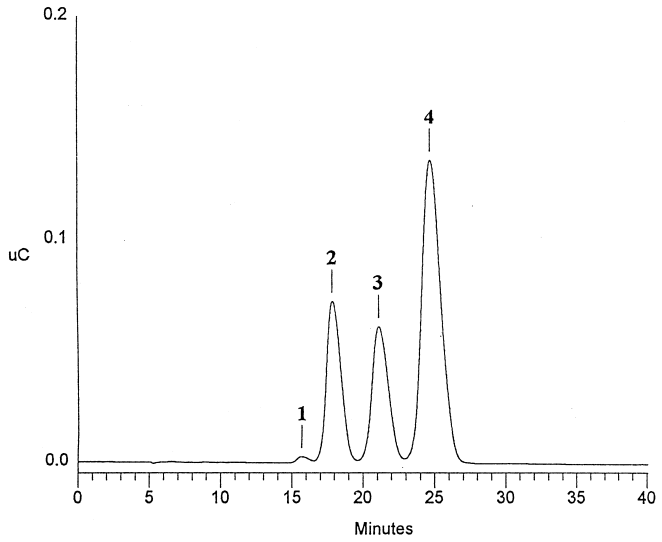

Fig. 1. Typical HPAEC–PAD chromatogram obtained for a standard mixture of isomalt (0.23 mg/ml) and malbit (0.19 mg/ml). Chromatographic conditions: column, CarboPac MA 1; mobile phase, 600 m*M* sodium hydroxide; flow-rate, 0.4 ml/min at room temperature, detector, PED operating in pulse amperometric mode as reported in Section 2.2. Peaks: 1 sorbitol, 2 GPS, 3 GPM, 4 maltitol.

performed using arabinose as an internal standard; arabinose is not present in the chewing gum extract, is completely resolved in the chromatogram from the other carbohydrates and is eluted near the peaks of interest.

Over the course of the experiments, the relative standard deviations (R.S.D.s) of the retention times of alditols (optimized conditions, $n=12$) ranged from 0.2 to 0.4%. The peak-area ratio (R.S.D.s less than 2.3%) gave a higher repeatability than the peak-height ratio and for this reason was selected as the method of analysis.

The detector response (peak area) for a given alditol must be linear over a large concentration range in order to analyze samples containing different levels of alditols in one chromatographic run. An example of such a sample is a chewing gum extract, which may contain up to 165 μg/ml of sorbitol and only 3–4.5 μg/ml of glycerol. All calibration curves (five different concentrations) for each analyte were linear over the concentration range studied. The regression equations for xylitol and mannitol (2–32 μg/ml), were $y=1.73x-0.28$ ($r=0.9999$); and $y=1.62x+0.14$ ($r=0.9999$), respectively. For glycerol (0.5–15 μg/ml), the equation was $y=1.76x+0.20$ ($r=0.9999$), whereas for sorbitol (60–240 μg/ml) the regression equation was $y=1.56x-0.13$ ($r=0.9998$). The detection limits were 2 ng for glycerol xylitol and mannitol, and 3 ng for sorbitol, respectively at a signal-to-noise ratio of 3 in an injection volume of 10 μl. It is evident that with an increased injection loop volume the detection of even lower concentrations would be feasible. Furthermore, under these chromatographic conditions, it was possible to separate glucose, fructose and sucrose from the above alditols (data not shown). Results of triplicate determinations of three sugar-free chewing gum extracts from different sources (A, B, C), are summarized in Table 1 and a typical chromatogram is shown in Fig. 2.

3.2. Determination of sorbitol, mannitol and fructose in a tabletop sweetener

We have also developed a HPAEC–PAD method for the analysis of a sugar substitute which contains sorbitol as the major component with small amounts of mannitol and fructose. As expected, using the CarboPac MA 1 column the monosaccharide fructose is highly retained even at sodium hydroxide concentrations up to 600 m*M*. To allow good resolution of sorbitol, mannitol and fructose, and reduce the

Table 1
Glycerol, xylitol, sorbitol and mannitol content in sugar-free chewing gums

Source	Content[a] (values expressed in g/100 g of product)			
	Glycerol	Xylitol	Sorbitol	Mannitol
A	1.5±0.02	4.20±0.05	54.78±1.4	4.52±0.1
B	1.9±0.03	5.31±0.1	61.13±1.5	5.47±0.1
C	1.7±0.03	4.78±0.1	58.27±1.3	4.95±0.1

[a] Mean±S.D. (n=3).

analysis time it was necessary to add sodium acetate to the eluent to force elution of the strongly retained fructose. Sodium acetate gradients can allow faster elution of strongly retained carbohydrates without compromising selectivity and PAD. However, the isocratic mode was preferred in order to avoid the time required to restore the initial conditions of the analytical column.

The best operating conditions were obtained using a solution of 25 m*M* sodium acetate in 600 m*M* sodium hydroxide as the mobile phase. Sample components were eluted within 20 min, and in spite of the high level of sorbitol present in the sample, the adjacent mannitol peak is well resolved. The sample was directly injected onto the column without any further pretreatment, except it was diluted with water to a concentration range that would ensure no significant loss of resolution due to overloading of the column. Arabinose, which was selected as internal standard in the determination of alditols in chewing gum extracts, at these optimized chromatographic conditions partially coeluted with mannitol. Consequently we have selected a different compound to be used as the internal standard: myo-inositol was found suitable because it elutes in an area of the chromatogram which is free of sample peaks. A typical chromatogram of 0.92 mg/ml of the tabletop sweetener sample with myo-inositol as internal standard is shown in Fig. 3.

Regression analysis on the plots of area response ratio versus mass ratio over internal standard yielded correlation coefficients of 0.9998, 0.9988 and 0.9987 for the three compounds, respectively, and nearly passed through the origin. The minimum detectable concentration of alditols was in agreement with results above reported and a little higher, 6 ng, for fructose. These results are similar to those reported elsewhere [18]. The analysis in triplicate resulted in an average dosage of 0.925 g of sorbitol (82.77%), 0.105 g of mannitol (9.39%), and 0.0875 g of fructose (7.83%), with repeatability of data better than 1.75% (R.S.D.).

In order to determine the accuracy of the method, a recovery study was carried out. Three different

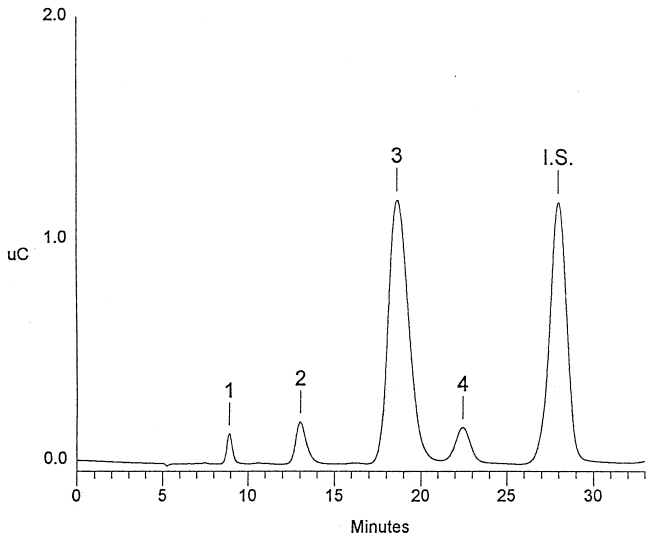

Fig. 2. Separation of alditols in a sugar-free chewing gum extract. Chromatographic conditions: mobile phase, 450 m*M* sodium hydroxide. Other conditions as in Fig. 1. Peaks: 1=glycerol, 2=xylitol, 3=sorbitol, 4=mannitol, 5=internal standard (arabinose).

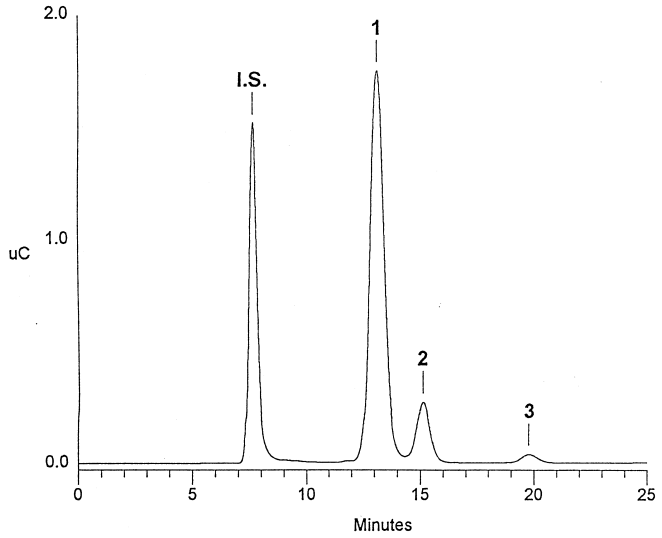

Fig. 3. Separation of (1) sorbitol, (2) mannitol, (3) fructose and internal standard myo-inositol in a tabletop low calorie sweetner. Chromatographic conditions: mobile phase, 25 m*M* sodium acetate in 600 m*M* sodium hydroxide. Other conditions as in Fig. 1.

amounts of standard sorbitol, mannitol and fructose were added to the sample which was subjected to the entire analytical method. The recovery was calculated based on the difference between the total concentrations determined in the spiked samples and the concentration dosed in the not spiked samples. Results with the R.S.D.s are reported in Table 2. The R.S.D. of the determinations for each concentration was better than 1.9% and the mean recovery ranged from 98 to 103%, indicating a high degree of accuracy.

Table 2
Recovery study of the analyzed carbohydrates added to a commercial tabletop low-calorie sweetener

Compound	Added (mg/ml)	Recovered (mg/ml)	Recovery (%)	R.S.D. (%)
Sorbitol	0.12	0.84	98	1.5
	0.24	0.99	101	1.1
	0.36	1.12	102	1.1
Mannitol	0.02	1.104	98	1.6
	0.04	0.127	98	1.8
	0.07	0.150	99	1.8
Fructose	0.01	0.079	99	1.9
	0.02	0.089	98	1.8
	0.03	0.104	103	1.8

Results are the mean for three samples that underwent the whole analytical procedure.

4. Conclusions

The proposed methods combine, under isocratic conditions, simple, rapid analysis times and ease of sample preparation. The CarboPac MA1 column permits appropriate separation and quantitation of the most important sugars and sugar alcohols present as sweeteners in food products. Elution of disaccharide and monosaccharide alditols, and sugars such as glucose, fructose and sucrose can be controlled by varying the composition of the eluent. When the mobile phase of 600 m*M* sodium hydroxide was employed, isomalt and malbit were completely separated at shorter retention times, whereas the best separation of tabletop sweetener components was achieved using as the mobile phase 600 m*M* sodium hydroxide containing 25 m*M* sodium acetate.

Furthermore, the proposed methods have a considerable potential for simplifying comparison studies of dietetic foods containing various amounts of sugars and alditols.

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