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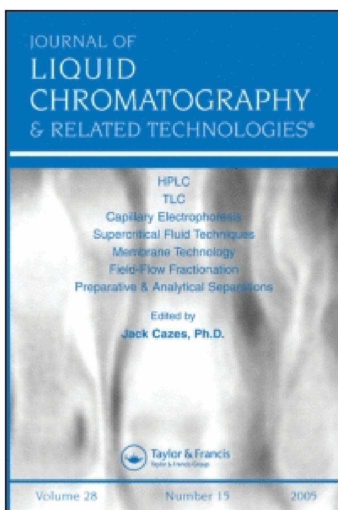

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DETERMINATION OF ALDITOLS AND CARBOHYDRATES OF FOOD INTEREST USING A SULFONATED MONODISPERSE RESIN-BASED COLUMN, COUPLED WITH PULSED AMPEROMETRIC DETECTION (PAD) AND POSTCOLUMN pH ADJUSTMENT

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DETERMINATION OF ALDITOLS AND CARBOHYDRATES OF FOOD INTEREST USING A SULFONATED MONODISPERSE RESIN-BASED COLUMN, COUPLED WITH PULSED AMPEROMETRIC DETECTION (PAD) AND POSTCOLUMN pH ADJUSTMENT

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ABSTRACT

Carbohydrates such as glucose, fructose, sucrose, and sugar alcohols mannitol, sorbitol, and xylitol are widely distributed in various foods and beverages.

In this work we developed a HPLC method for the determination of sugars and alditols, using a sulfonated monodisperse resin-based column in hydrogen form (PL Hi-Plex , 300 × 7.7 mm, from Polymer Laboratories), coupled with pulsed amperometric detection (PAD). Performing isocratic separation using water as the mobile phase, post column addition of 0.5 M sodium hydroxide was necessary to detect sugars and alditols by PAD. After the optimization of the operating conditions, the method was characterized and validated in terms of selectivity, repeatability, sensitivity,

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and accuracy. Furthermore, quantification of lactose, glucose, and galactose in lactose-hydrolyzed milk products was found to be highly accurate.

INTRODUCTION

The presence and the quantity of the major carbohydrates present in most foods such as glucose, fructose, and sucrose can indicate the quality and the properties of a product, and can also be used to detect adulteration in food components. Moreover, separation and quantification of lactose, glucose, and galactose is of interest to determine lactose content in lactose-hydrolyzed milk. Sugar alcohols, also known as alditols or polyols, mannitol, sorbitol, xylitol, and disaccharide alditols lactitol, and maltitol, can be substituted for sugar in a wide variety of food applications, where they provide both sweetness and bulking properties.[1]

High-performance liquid chromatography (HPLC) has been widely used for the separation and quantitation of reducing sugars and alditols in foods. However, many of the developed HPLC methods suffer from some serious limitations in terms of selectivity, sensitivity, ease of use, and applicability. In the past few years, much work has been published on the application of high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD), which offers a powerful alternative to traditional HPLC methods.[2–3]

The basis of this technique is the high powerful resolution capacity of the strong anion-exchange stationary phase, combined with the sensitivity, specificity, and reliability of pulsed amperometric detection (PAD). This specific detector has been developed to achieve the highly sensitive and reproducible detection of alditols, monosaccharides, and oligosaccharides at Au electrodes.[4]

Most of the applications of HPAEC-PAd for the determination of mono disaccharides and alditols in food products, have been performed using specifically designed columns.[5–8] However, PAD which provides much more sensitive and specific detection of carbohydrates than RI or UV, is usually not associated to other traditional columns employed in HPLC analysis of carbohydrates.

In this work, we developed a HPLC method for the determination of sugars and alditols, using a sulfonated monodisperse resin-based column (PL Hi-Plex, 300 × 7.7 mm, from Polymer Laboratories), especially useful for profiling both carbohydrates and organic acids by combining the mechanisms of ion exclusion and partition. This column allows the use of simple isocratic methods, eluting either with water or dilute acid, in which the measurement of refractive index (RI) is the conventional means of detection. Here, detection of sugars and alditols was

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performed by pulsed amperometric detection, adding 0.5 M sodium hydroxide to the column effluent through a tee connection before the cell of the detector during elution with water.

EXPERIMENTAL

Materials

All saccharide standards were purchased from Sigma (St. Louis, MO, USA). Maltotriitol (purity > 99%) and maltotetraitol (purity > 97%) were purchased from Seikagaku Corporation, Tokyo, Japan). Fifty percent (w/w) sodium hydroxide solution and sodium acetate anhydrous were purchased from J. T. Baker (Deventer, The Netherlands). HPLC-grade water was from Carlo Erba (Milan, Italy).

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Instrumentation and Chromatographic Conditions

HPAEC-PAD analyses were performed on a Dionex (Sunnyvale, CA, USA) Model 4000i gradient pump module, connected to a Rheodyne Model 9125 non metal (peek) injection valve with a peek sample loop of 20 µL (Cotati, CA, USA). Separations were performed at ambient temperature (23 ± 1°C), on a sulfonate monodisperse resin-based column (PL Hi- Plex™, 300 × 7.7 mm I.D., from Polymers Laboratories, LabService Analytica, Bologna, Italy). Some experiments took place on a CarboPac MA1 column and matching guard column.

Carbohydrates were detected by a Dionex Pulsed Electrochemical Detector (PED). Detection was by triple-pulsed amperometry with a gold working electrode. The following working pulse potentials and durations were used for detection of alditols: E_1 = 0.05 V, E_2 = 0.65 V, E_3 = −0.15 V; t_1, 0–0.45 s; t_2, 0.46–0.64 s; t_3, 0.65–1.00 s. Integration of the signal occurred between 0.20–0.45 s.

Mono- and disaccharide detection was carried out with the following pulse potentials and durations. E_1= +0.10 V, E_2=+0.60 V and E_3 = −0.60 V, with the assigned pulse durations t_1, 0–0.50 s; t_2, 0.51–0.59 s; t_3, 0.60–0.65 s. Integration of the signal occurred between 0.30–0.50 s.

A Dionex DXP single piston pump was used to add strong base to the eluent stream post-column through a tee connection, providing the alkaline environment necessary for carbohydrate detection at the Au working electrode.

For all work, instrument control and data collection were performed with a personal computer and the Dionex AI-450 chromatography workstation.

Standard Solutions and Sample Preparation

A stock solution of analytes was prepared prior to use by dissolving 10 mg of each standard carbohydrate in 5 mL of HPLC water. Appropriate amounts of the stock solution were diluted with water to produce working standard solutions at five different concentrations within the range 20 – 480 μg/mL for maltitol, lactitol, sucrose, glucose, fructose, galactose, lactose, mannitol, sorbitol, xylitol, and glycerol, and within the range 15 –360 μg/mL for maltotriitol and maltotetraitol.

Preparation of Standard Solutions for Calibration Curves to Determine Glucose, Galactose, and Lactose in Lactose-Hydrolyzed Milk

Stock standard solutions were prepared by dissolving known amounts of glucose and galactose in water to obtain 2.5 mg/mL solutions. The same procedure was used to obtain 1.5 mg/mL for lactose stock standard solutions. An internal standard aqueous solution containing 750 μg/mL of fucose was also freshly prepared. Appropriate amounts of the stock solutions were diluted to produce working standard solutions at five different concentrations within the range 100 – 300 μg/mL for glucose and galactose, and within the range 50 – 150 μg/mL for lactose (which are all within 50 – 150% of nominal content in 100-fold diluted lactose-hydrolyzed milk). The appropriate volume of internal standard solution was added to each working solution to give a final concentration of 75 μg/mL of fucose.

Calibration graphs were plotted based on the linear regression analysis of the peak-area ratio. Triplicate determinations were performed for each sample and the results were reported as the average.

Sample Preparation

The apple and pineapple concentrates were prepared for analysis by dissolving, in water, the appropriate amount of the thawed sample, conditioned at room temperature for 60 minutes. A billberry fruit juice sample was prepared for the analysis by a 50-fold dilution with water. Milk samples were prepared for analysis by a first 50-fold dilution with water, followed by centrifugal ultrafiltration through 30 KDa cutoff filter to remove fat and proteins, and then a further 1:1 dilution with water after the addition of the appropriate amount of internal standard solution, to give a concentration of 75 μg/mL of fucose.

All sample solutions were made with HPLC-grade water and filtered through a Type HA 0.22 μm single-use membrane filter (Millipore, Bedford, USA).

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Sensitivity Factors, Detection, and Quantitation Limits

The precision of the chromatographic system was determined using the relative standard deviation of the response factors for the different peaks in the injections of the standard solutions. Response factor was calculated as RF = DR/C, where DR is the detector response (peak area) and C is the concentration of the analyte.

As limit of detection, a signal-to-noise ratio 3:1 was accepted. The limit of quantitation (LOQ) was set at the concentration of the lowest calibration standard with a R.S.D. better than 5% and a signal-to-noise ratio of at least 10:1.

RESULTS AND DISCUSSION

PL-Hi Plex column is a sulfonated monodisperse resin-based column in protonated form, usually employed to separate organic acids alone or in combination with carbohydrates. In this work, we show the usefulness of such a column to separate alditols, as well as sugars of food interest, by HPLC coupled with PAD. Using water as the mobile phase at room temperature, both reducing sugars and alditols are eluted as a single peak. The triple-pulse amperometric detector at a gold electrode requires high pH conditions for direct detection of sugars and alditols. This prerequisite to detection is accomplished by the addition of 0.5 M sodium hydroxide solution to the column effluent through a tee connection before the cell of the detector during elution with water, which was used as the mobile phase at a flow rate of 0.5 mL/min. It was necessary to establish the most suitable post-column conditions required using the sodium hydroxide solution which would provide the best pulsed amperometric detection of analytes.

The concentration of NaOH affected the peak responses of carbohydrates. The effect of concentration of sodium hydroxide on peak areas of some sugars and alditols is depicted in Figure 1. Pulsed amperometric response was examined by measuring the chromatographic peak areas carried out by 20 μL injection of 0.25 mg/mL each of maltotriitol, maltitol, sucrose, glucose, fructose, sorbitol, and xylitol. Maximum peak areas were achieved at NaOH concentration between 0.4 – 0.5 M. The post-column flow rate also altered the detector response. The baseline noise increased dramatically at a flow rate of less than 0.3 mL/min. This was due to the low back-pressure on the post-column pump. The optimum flow rate was 0.4 ÷ 0.6 mL/min. In this study, a NaOH concentration of 0.5 M and a flow rate of 0.5 mL/min were adapted as the optimum condition for post-column pH adjustment.

The relative standard deviations (R.S.D.s) for amperometric response stability, which was determined by peak areas measured after repeated injections (n = 8) of the same sample solution, were better than 3.5% for all analyzed carbohy-

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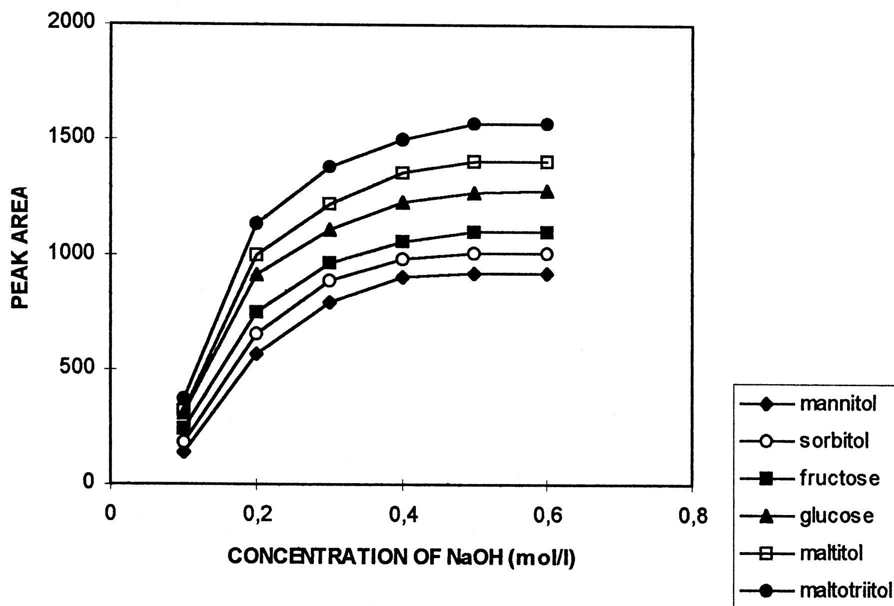

Figure 1. Dependence of peak response of some carbohydrates on sodium hydroxide concentration. Chromatographic conditions: column, Polymer PL Hi-Plex in protonated form (300 × 7.7 mm I.D.); eluent, water at flow rate of 0.5 mL/min; temperature, ambient (23°C ± 1°C); detection, PAD. Post column addition of sodium hydroxide at concentration ranging from 0.1 to 0.6 M was delivered at a flow rate of 0.5 mL/min.

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drates. The precision of the chromatographic system was determined using the relative standard deviation of the response factors. Furthermore, chromatographic parameters, including t_R, working range of linearity, correlation coefficient, and detection limit, were investigated for all analytes listed in Table 1. Relationship between peak areas and concentrations was linear over the whole range of concentrations with correlation coefficients (r^2) higher than 0.997. The detection limits of sugars and alditols, are in the range of 20 – 37 picomol.

To demonstrate the capability of the proposed HPLC procedure to efficiently perform separation and detection of sugars and alditols of food interest, three specific examples are reported below. Figure 2 (panel A and B), shows the chromatographic profiles of a maltitol syrup, which is a saccharose substitute, usually employed as a sweetener in dietetic or sugarless products. This bulk sweetener is obtained by the hydrogenation of maltose, produced from starch. Usually, maltitol syrups are commercially available with a range of maltitol content from 55% to 87%, corresponding to the parent maltose syrups before hydro-

Table 1. Retention Time, Response Factor, Range of Linearity, and Detection Limit of Some Sugars and Alditols

Carbohydrate	t_R (min)	Response Factor	Range of Linearity (μg/mL)	Correlation Coefficient (r^2)	Detection Limit (pM)
Maltotetraitol	9.95	24465	15 - 360	0.9972	20
Maltotriitol	10.70	26212	15 - 360	0.9983	20
Maltitol	12.20	63387	20 - 480	0.9992	27
Lactitol	12.81	47856	20 - 480	0.9983	35
Sucrose	11.25	21590	20 - 480	0.9973	35
Lactose	11.60	42429	20 - 480	0.9986	30
Glucose	12.35	69759	20 - 480	0.9984	25
Galactose	13.31	47122	20 - 480	0.9986	22
Fructose	14.30	24492	20 - 480	0.9988	37
Mannitol	14.98	52048	20 - 480	0.9994	20
Sorbitol	16.45	55290	20 - 480	0.9993	22
Xylitol	17.60	59781	20 - 480	0.9991	20
Glycerol	18.50	58662	20 - 480	0.9987	20

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genation. They could contain sorbitol and other sugar alcohols including maltotriitol and maltotetraitol.

Panel A shows the chromatogram of a malbit syrup solution at a concentration of 1.5 mg/mL which was chromatographed by connecting the PL Hi-Plex column to a Dionex HPLC system, using water as the mobile phase at room temperature, at a flow rate of 0.5 mL/min, where detection of the analytes was accomplished by means of a pulsed electrochemical detector in pulsed amperometric mode, as reported in the experimental part. Post column addition of base was necessary, therefore, 0.5 M NaOH was delivered at 0.5 mL/min.

An optimized separation of malbit syrup by HPAEC-PAD, eluting isocratically with 0.6 M sodium hydroxide, is shown in panel B. The separation was obtained employing a Dionex CarboPac MA1 column, which is an anion-exchange column especially designed for the determination of sugar alcohols in confectionery products and fruit juices by HPAEC-PAD. Comparing the chromatographic profile of panel A with that reported in panel B, it is evident that the opposite elution order of the analytes was achieved by the two chromatographic methods. Employing the PL Hi Plex column, maltotetraitol and maltotriitol, which are the reduced form of maltotetraose and maltotriose, are well separated and both eluted before maltitol, whereas sorbitol is the last eluted peak.

On the other hand, the elution order using the CarboPac MA1 column is determined by pK_a value for mono- and disaccharides, whereas for linear

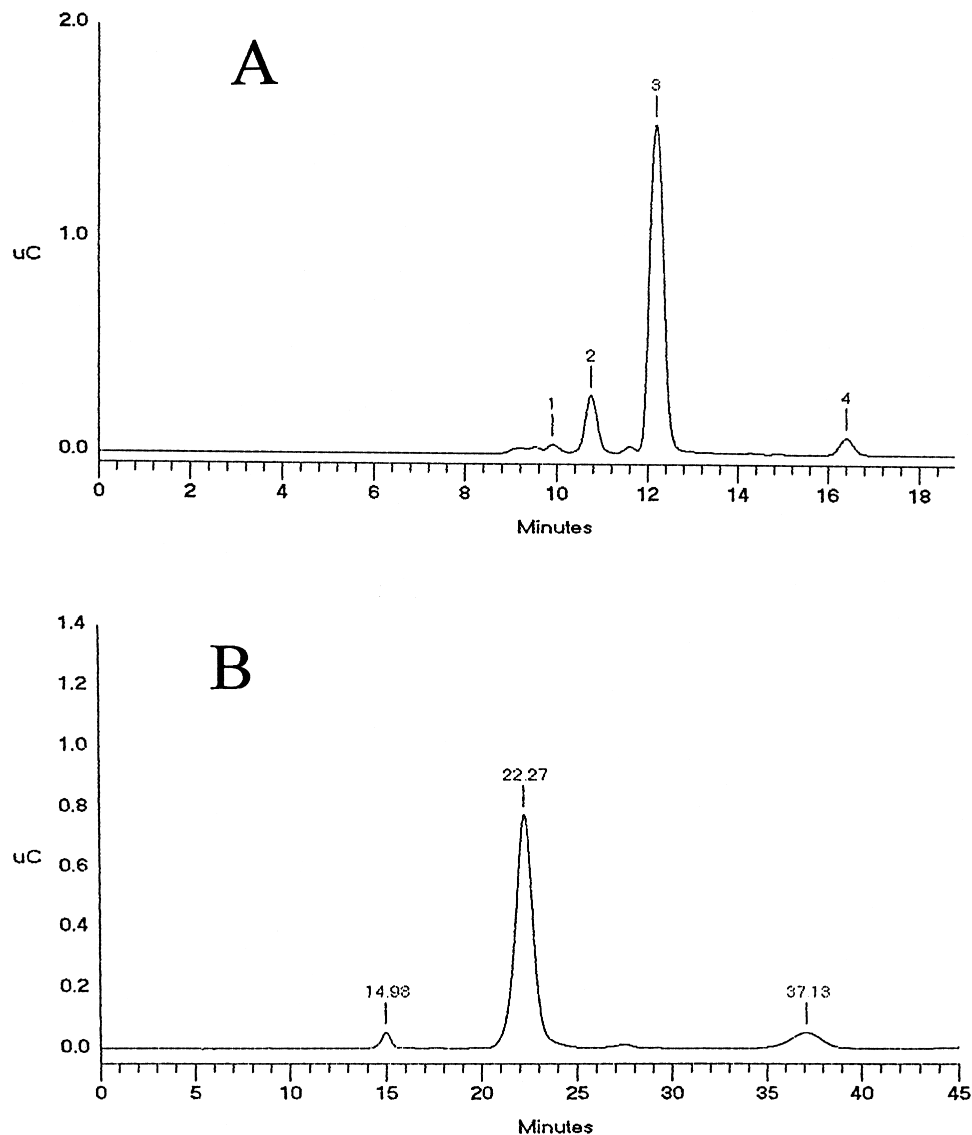

Figure 2. Chromatographic profiles of a maltitol syrup eluted on the Polymer PL Hi-Plex column (Panel A) and on a Dionex CarboPac MA1 column (Panel B). Chromatographic conditions as reported in the text. Peak identification: Panel A, 1) maltotetraitol, 2) maltotriitol, 3) maltitol, 4) sorbitol. Panel B, sorbitol (t_r = 14.98), maltitol (t_r = 22.27), maltotriitol (t_r = 37.13).

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oligosaccharides, retention increases in a regular manner with chain length. The sugar alcohol sorbitol with higher pK_a value elutes before maltitol, whereas maltotriitol and maltotetraitol are more retained. However, under the proposed conditions, the trisaccharise alcohol maltotriitol eluted with a retention time of 37.13 min, and the tetrasaccharide alcohol maltotetraitol eluted as a very broad peak at a retention time higher than 50 min and was not detected. To elute maltotritol and maltotetraitol from the column in a reasonable time, addition of considerable amounts of sodium acetate (until 200 mM) to the eluent was tried, either by isocratic or gradient elution mode.

Nevertheless, in all cases maltotetraitol, which is present only in small amount in the analyzed maltitol syrup, was not well visualized in the chromatogram and maltotritol was eluted as a rather broad, not very quantifiable peak. Furthermore, sodium acetate gradients are not recommended for the CarboPac MA1 column, because column regeneration between runs to remove the excess of acetate can require several hours.[9]

On other hand, it is worthy to note, that separation of maltotriitol, maltitol, and sorbitol on the PL Hi-Plex column was accomplished in less than 17 min of chromatographic run, which is considerably shorter than that required with the column expressly tailored for the separation of alditols by HPAEC-PAD, using strong alkaline eluents.[10]

A sulfonated resin-based column in protonated form, coupled with pulsed amperometric detection, can also be a useful technique for the analysis and quantification of sucrose, glucose, and fructose in fruit juices. Figure 3 shows the chromatographic profiles of fruit juice concentrates of apple [Panel A] and pineapple [Panel B], obtained under the same condition employed to separate the maltitol syrup, except that the PED detector was programmed with the waveform for mono and disaccharides, as reported in the experimental part.

The suitability to separate sorbitol from sucrose, glucose, and fructose and the high sensibility of PAD detection, can be used as a specific tool for authenticity assessment. Sorbitol, present in apples, pears, and other fruits, is virtually absent in berry fruits, such as raspberry, strawberry, blueberry, and can be an excellent tracer to verify addition of cheap apples or pear juice to expensive berry juices/pulps or fruit preparations. Furthermore, it was possible to separate mannitol, sorbitol, and xylitol, which are usually present as sweeteners in sugar-free chewing gums and confectionery products, from the above carbohydrates.

In addition, the proposed chromatographic method was applied to the determination of the major sugars in lactose-hydrolyzed milks, which are characterized by a low value of lactose. These commercially available milks are usually dietetic products directed towards consumers, which are lactose intolerant.

The quantitation of lactose, glucose, and galactose was performed by an internal standard method. The monosaccharide fucose was selected as the internal standard because it is not naturally present in milk, is completely resolved in

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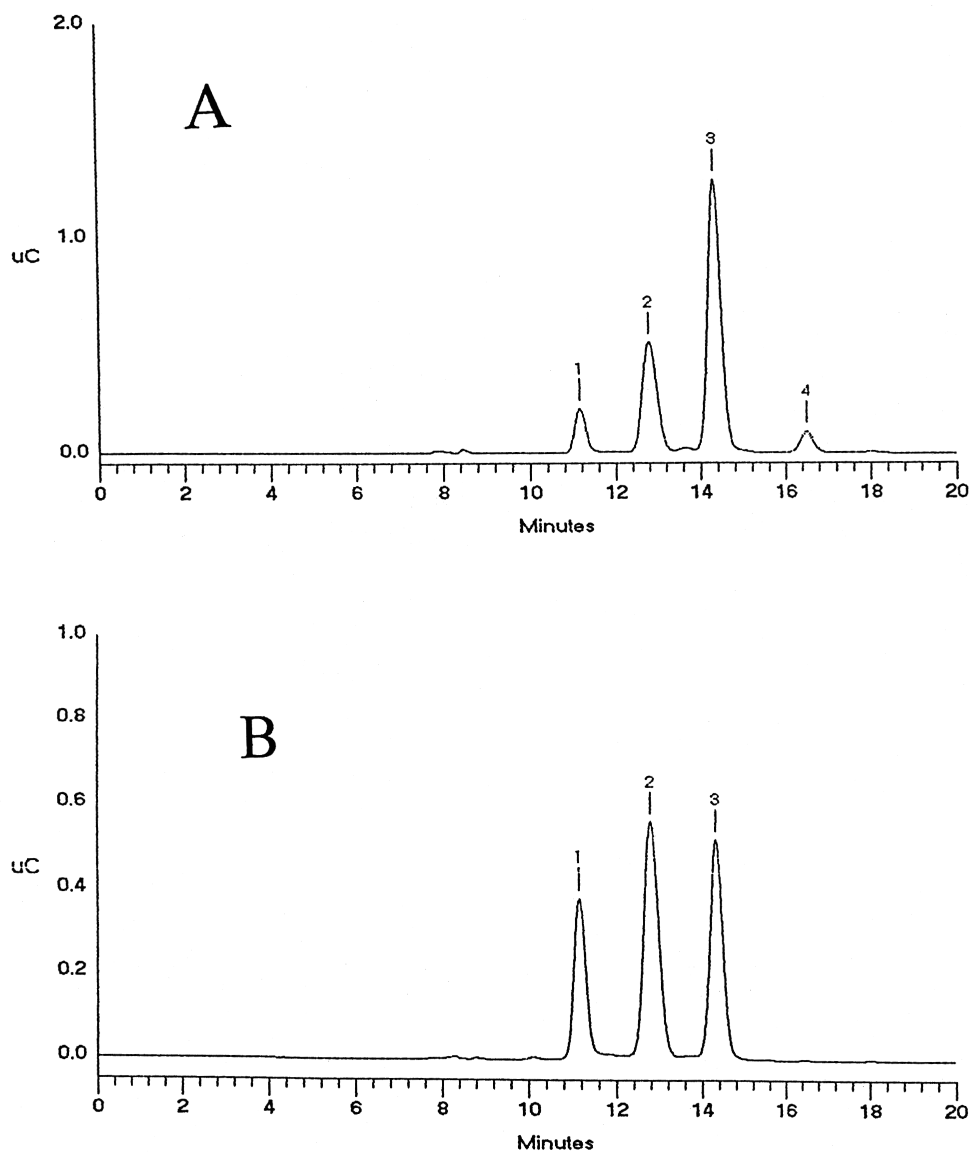

Figure 3. Chromatographic profiles of concentrated fruit juices of apple (Panel A) and pineapple (Panel B). Chromatographic conditions as reported in the text. Peak identification: Panel A, 1) sucrose, 2) glucose, 3) fructose, 4) sorbitol. Panel B, 1) sucrose, 2) glucose, 3) fructose.

the chromatogram from the other sugars, and is eluted near the peaks of interest. The chromatogram of a lactose-hydrolyzed milk added with the internal standard fucose is shown in Figure 4. The sugars of interest were determined, without interferences, eluting with water at a flow rate of 0.4 mL/min and detected by PAD placed in-line after 0.5 M NaOH addition at a flow rate of 0.5 mL/min.

The repeatability of the retention times of the above sugars was investigated by repeated injection (n = 15) of an equimolar sample solution containing the internal standard. Results are summarized in Table 2 and show that the R.S.D.s were better than 1.9% for the four compounds. The same retention times with the above R.S.D.s were found in the milk samples, either with or without the addition of known amounts of sugars, ensuring sufficient peak identification.

Calibration graphs were based on peak-area ratio to the internal standard fucose against the sugars concentration. The correlation coefficients of the linear regression curves were 0.99986, 0.99975 over the concentration range between 100 – 300 μg/mL for glucose and galactose, and 0.99967 over the concentration range between 50 – 150 μg/mL for lactose. Results of five replicate analyses of lactose, glucose, and galactose content in lactose-hydrolyzed milk samples, are summarized in Table 3. The reported data demonstrate the usefulness of the proposed method for the rapid routine analysis of these sugars in lactose-hydrolyzed

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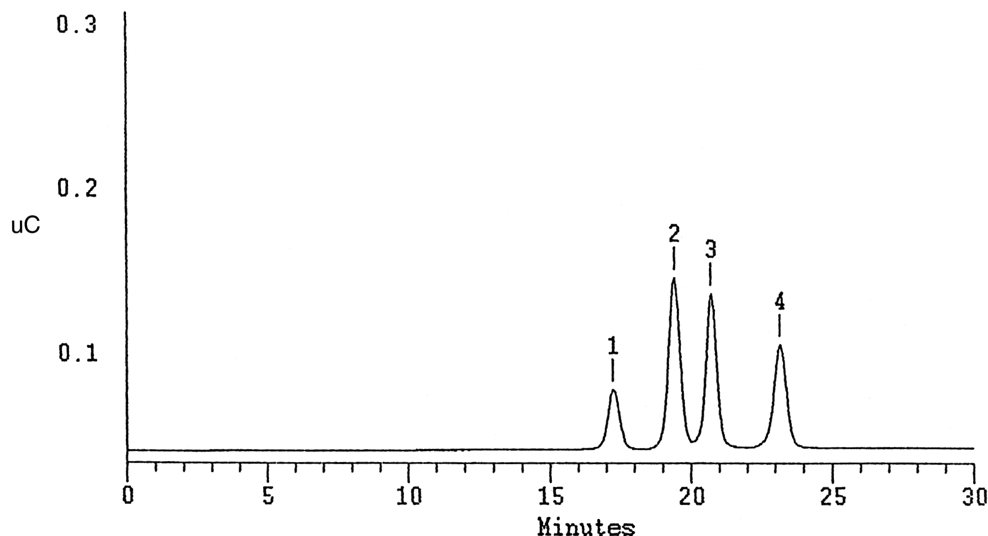

Figure 4. Separation of sugars in a lactose-hydrolyzed milk. Chromatographic conditions as in the text. Peak identification: 1) lactose, 2) glucose, 3) galactose, 4) fucose (as internal standard).

Table 2. Repeatability of Retention Times

Sugar	Mean Value* (min)	S.D. (min)	R.S.D. (%)
Lactose	17.85	0.270	1.51
Glucose	19.92	0.362	1.82
Galactose	23.78	0.438	1.84

*Mean value of 15 repeat injections.
S.D. = Standard Deviation, R.S.D. = Relative Standard Deviation.
Chromatographic conditions as in the text.

milks, which were in good agreement with those reported on the label of the analyzed products.

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Validation

Validation parameters, such as specificity and linearity, have been discussed above and documented. The limit of detection values (LOD), were estimated to be 10 ng for lactose and 5 ng for glucose and galactose, respectively. These values were in agreement with those found in a previous work, in which the separation of glucose and galactose was achieved using either a Carbopac PA10 or a Carbopac PA100 column.[11] The limit of quantitation (LOQ) appeared to be 4 μg/mL for lactose and 2.5 μg/mL for glucose and galactose, respectively.

Table 3. Results of the Quantitative Determination of Lactose, Glucose, and Galactose in Lactose-Hydrolyzed Milk Samples

	Sample A	Sample B	Sample C
Lactose*	0.82	0.94	1.19
R.S.D. %	1.37	1.41	1.77
Labeled*	0.80		1.20
Glucose*	1.97	1.92	1.78
R.S.D. %	1.56	1.49	1.58
Labeled*	2.00		1.80
Galactose*	1.95	1.89	1.75
R.S.D. %	1.63	1.79	1.65
Labeled*	2.00		1.80

*Expressed as g/100 mL of milk.

In order to determine the suitability of the proposed method to determine sugars in lactose-hydrolyzed milk, aqueous solutions of standard lactose, glucose, and galactose at five different concentrations were prepared on three different days and analyzed ten times each day with the HPLC procedure. The relative standard deviations for the measured peak areas for the three days are reported in Tables 4 – 6 and show that there is a little variability in the instrumental response.

Recovery experiments were carried out by spiking the lactose-hydrolyzed milk samples with each sugar at three different concentrations and analyzing these solutions in triplicate, according to the proposed method. Typical recoveries, determined by comparing the found and calculated amounts of sugars after spiking, is reported in Table 7. The R.S.D.s of the results for each concentration ranged from 1.42 to 2.24 and the average recoveries lay between 97.41 to 102.6, indicating that the method has an adequate degree of accuracy for the determination of these sugars in the analyzed samples.

To evaluate the precision of the method, within-run and between-run relative standard deviations were calculated in all the three analyzed milk samples. The intra-day data were based on six repeated analyses of aliquots of the same milk. Results for inter-day precision, were obtained from analyses of the same sample repeated on five subsequent days. As shown in Table 8, the precision of the method for lactose, glucose, and galactose in a lactose-hydrolyzed milk sample (sample A) are less than 3% R.S.D. for both intra-day and inter-day analyses.

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Table 4. Intra-Day and Inter-Day Peak Area Repeatability of Lactose Standard Solution at Five Different Concentrations

Concentration (μg/mL)	25	35	50	65	90
Day 1	4319[a]	5961	8490	11071	15360
S.D.	130.2	175.8	221.6	237.1	287.2
R.S.D. %	3.01	2.95	2.61	2.14	1.87
Day 2	4361	6077	8612	11135	15417
S.D.	130.1	185.3	227.8	263.9	296.1
R.S.D. %	2.98	3.05	2.64	2.37	1.92
Day 3	4305	6019	8532	11042	15478
S.D.	122.6	161.9	209.9	218.6	283.3
R.S.D. %	2.80	2.69	2.46	1.98	1.83
Mean	4328	6019	8545	11083	15418
S.D.	29.14	58.02	61.98	47.58	59.01
R.S.D. %	0.67	0.96	0.73	0.43	0.38

[a] Entries in the upper part of the table are mean values of ten repeated injections. Summary statistics are included in the lower part of the table.

Table 5. Intra-Day and Inter-Day Peak Area Repeatability of Glucose Standard Solution at Five Different Concentrations

Concentration (μg/mL)	25	50	75	100	150
Day 1	12245[a]	24476	36750	49698	73153
S.D.	253.2	448.3	624.7	834.5	1051.8
R.S.D. %	2.07	1.83	1.70	1.68	1.44
Day 2	12164	24605	37008	49972	73812
S.D.	268.5	521.6	688.4	899.5	1097.6
R.S.D. %	2.18	2.12	1.86	1.80	1.49
Day 3	12164	24299	36519	49125	72811
S.D.	244.5	401.3	607.7	771.2	1027.6
R.S.D. %	2.01	1.65	1.66	1.57	1.41
Mean	12243	24460	36759	49598	73265
S.D.	78.51	153.63	244.21	432.21	500.05
R.S.D. %	0.64	0.62	0.66	0.87	0.68

[a] Entries in the upper part of the table are mean values of ten repeated injections. Summary statistics are included in the lower part of the table.

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Table 6. Intra-Day and Inter-Day Peak Area Repeatability of Galactose Standard Solution at Five Different Concentrations

Concentration (μg/mL)	25	50	75	100	150
Day 1	9109[a]	17922	27345	36391	54225
S.D.	179.4	361.7	543.2	701.6	995.8
R.S.D. %	1.97	2.02	1.99	1.93	1.83
Day 2	9178	18221	27465	36511	54523
S.D.	168.7	371.8	558.1	687.4	989.8
R.S.D. %	1.84	2.04	2.03	1.88	1.81
Day 3	9220	17988	27378	37001	55780
S.D.	187.5	341.7	553.1	699.5	1065.1
R.S.D. %	2.03	1.9	2.02	1.89	1.90
Mean	9169	18044	27496	36634	54842
S.D.	56.04	157.1	168.65	323.16	825.31
R.S.D. %	0.61	0.87	0.61	0.88	1.50

[a] Entries in the upper part of the table are mean values of ten repeated injections. Summary statistics are included in the lower part of the table.

Table 7. Recovery Study of the Analyzed Sugars Added to a Commercial Lactose-Hydrolyzed Milk

Sugar	Amount in Milk g/100 mL	Amount Added g/100 mL	Found g/100 mL	Recovery %	R.S.D. %
Lactose	0.820	0.120	0.913	97.1	2.24
		0.240	1.032	97.4	1.81
		0.360	1.213	102.8	1.43
Glucose	1.970	0.150	2.158	101.8	1.88
		0.300	2.209	97.3	1.64
		0.450	2.483	102.6	1.47
Galactose	1.950	0.150	2.045	97.4	1.59
		0.300	2.187	97.2	1.42
		0.450	2.453	102.2	1.44

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Table 8. Intra-Day and Inter-Day Precision for the Determination of Lactose, Glucose, and Galactose in a Lactose-Hydrolyzed Milk (Sample A)

Sugar (g/100 mL)*	Intra-Day (n = 6)	R.S.D. (%)	Inter-Day (n = 5)	R.S.D. (%)
Lactose	1.193 ± 0.034	2.85	1.196 ± 0.0025	0.65
Glucose	1.783 ± 0.026	1.46	1.783 ± 0.0041	0.63
Galactose	1.772 ± 0.025	1.41	1.775 ± 0.0032	0.87

* Expressed as grams of sugar in 100 mL of milk.

Similar results were obtained with sample B and sample C. In conclusion, we have shown that a sulfonated monodisperse resin-based column in hydrogen form, combined with the ease and the sensibility of PAD detection, provide a valuable methodology for compositional analysis of sugars and alditols in some food matrices.

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