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Short communication

Determination of sugar alcohols in confectioneries by high-performance liquid chromatography after nitrobenzoylation

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Abstract

A method was developed for the determination of sugar alcohols, *meso*-erythritol, xylitol, D-glucitol, D-mannitol, maltitol and parachinit by high-performance liquid chromatography (HPLC). The sugar alcohols were converted into strong ultraviolet (UV)-absorbing derivatives with *p*-nitrobenzoyl chloride. HPLC was performed on a phenyl column, using acetonitrile–water (67:33) as mobile phase and UV detection (260 nm). The calibration curves for all sugar alcohols tested were linear in the 10–250 μg/ml range. The average recoveries of the sugar alcohols from four sugarless confectioneries spiked at 5 and 10% levels of six sugar alcohol standards ranged from 73.2 to 109.0% with relative standard deviations ranging from 0.7 to 9.0%. The detection limit of the developed method was 0.1% for the above sugar alcohols contained in the samples.

Keywords: Confectioneries; Food analysis; Derivatization, LC; Sugar alcohols; Nitrobenzoyl chloride

1. Introduction

Sugar alcohols such as xylitol, D-glucitol and D-mannitol, are widely used in pharmaceuticals, confectionery products, chewing gums, and mixed juices. Xylitol, D-glucitol and D-mannitol are regulated as a food additive according to the Food Sanitation Law in Japan. Since xylitol was admitted as a sweetener in April 1997, the use of sugar alcohols has dramatically increased as a sugar substitute, reflecting a health diet boom. Even though the acceptable daily intake (ADI) is not specified by JECFA [1], these sugar alcohols have a laxation side effect when taken in excess.

Various analytical methods have been reported for the determination of sugar alcohols by HPLC [2–5]. An ion-exchange chromatographic method with pulsed amperometric detection has been used to determine sugar alcohols without prior derivatization and is a sensitive and peculiar method [6,7]. However, pulsed amperometric detection is not available in every laboratory.

Pre-column derivatization of sugar alcohols with a chromophoric reagent, such as phenyl isocyanate [8], benzoate [9,10] and 2,4-dinitrobenzoate [11] allows the use of inexpensive UV detectors. *p*-Nitrobenzoyl chloride (PNBC) was found to be a rapid and quantitative derivatizing agent for sugar alcohols [12–15]. In a previous study, we developed a new method to separate these sugar alcohols in foodstuffs by HPLC on an ODS column after derivatization to esters with PNBC [16]. However, this method was

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found to be unsuitable for the simultaneous determination of the disaccharides, parachinit and maltitol. This is because maltitol (4-α-glucopyranosylglucitol) and one of the components of parachinit, 6-α-glucopyranosylglucitol, result in overlapping peaks by our previous method. In the present study, we modified the previous method for routine analysis of *meso*-erythritol, xylitol, D-glucitol, D-mannitol, maltitol and parachinit in various sugarless confectioneries.

2. Experimental

2.1. Chemicals

The sugar alcohol standards, *meso*-erythritol, xylitol, D-glucitol, D-mannitol, D-glucose, D-mannose and D-sucrose were purchased in highly pure grades from Wako (Tokyo, Japan). Maltitol and parachinit (a 1:1 mixture of 6-α-glucopyranosylglucitol and 6-α-glucopyranosylmannitol) were provided by Nikken Kagaku (Tokyo, Japan). Sucralose was obtained from San Eigen FFI (Tokyo, Japan). PNBC was purchased from Tokyo Kasei (Tokyo, Japan). Pyridine was analytical grade and stored over sodium hydroxide pellets. Mobile phases were all of HPLC grade. All other chemicals were of analytical grade.

2.2. Sample preparation and standard solutions

Approximately 1 g of confectionery was accurately weighed and homogenised with 30% ethanol (100 ml). The homogenate was centrifuged at 3000 *g* for 10 min and the supernatant was diluted to 10 times with 30% ethanol. In this case, the concentration of the sample solution should be adjusted to the appropriate concentration by dilution. 1 ml of sample and standard solutions were evaporated in a rotary evaporator for the derivatization process.

The combined standard contained *meso*-erythritol, xylitol, D-glucitiol, D-mannitol, maltitol and parachinit (10 mg/ml) was diluted to 10, 50, 100, 150, 200 and 250 μg/ml solutions, respectively with 30% ethanol, respectively.

2.3. Spiked samples

To evaluate the effect of a typical sample matrix on the accuracy and precision of the analysis, recovery experiments were carried out by spiking 5 and 10 ml quantities of 10 mg/ml sugar alcohol standard solutions to 1 g sample homogenate which were taken through the complete analysis procedure.

2.4. Derivatization

A 2 ml volume of 10% PNBC pyridine solution was added to both standard and sample residues. The mixture was incubated at 50°C for 90 min. After reaction completion, several drops of methanol were added to destroy excess reagent and then the reactant was evaporated in a rotary evaporator. Throughout derivatization, it is desirable to maintain conditions as anhydrous as possible. Pipettes and other equipment were kept in an oven and dried just prior to use. Sample containers were kept tightly closed during this process.

2.5. Extraction

After derivatization, sample residues were dissolved in 2 ml of chloroform, and the mixture was applied to a silica Sep-Pak cartridge (1 g; Waters, Milford, MA, USA) conditioned with *n*-hexane. The column was washed with 10 ml of 10% ethyl acetate in hexane and the PNBC derivatives were eluted with 25 ml of ethyl acetate. After the solvent was evaporated in a rotary evaporator, the residues were dissolved in 10 ml acetonitrile for HPLC analysis.

2.6. Chromatography

A moderate-cost modular HPLC system was assembled for the present study. This unit contained a Jasco PU-980 pump (Jasco, Tokyo, Japan), a Rheodyne Model 7125 loop injector with a 10 μl loop (Rheodyne, Berkeley, CA, USA), a Jasco UV-970 detector (Jasco, Tokyo, Japan) at 260 nm, which is the extinction maximum of *p*-nitrobenzoyl derivatives of sugar alcohols, and a Shimadzu C-R6A Chromatopac (Shimadzu, Tokyo, Japan) operated at 1 cm/min chart speed. Stainless-steel columns (250×4.5 mm I.D.) were packed with Inertsil Ph-3

(Phenyl column, GL Sciences, Tokyo, Japan). All separations were performed isocratically at room temperature. The acetonitrile–water (67:33) as mobile phase gave the best resolution with the mixtures studied. The flow-rate was maintained at 1 ml/min.

3. Results and discussion

3.1. Reaction time and temperature

Modification to the previous method [16] was necessary for the determination of maltitol and parachinit. The derivatives formed from alditols, *meso*-erythritol, xylitol, D-glucitol and D-mannitol, were rapidly destroyed at 60°C, but showed stability at 50°C for 1 h. A longer derivatization time for maltitol and parachinit was required. In order to find an appropriate reaction condition, time courses at several temperatures (40°C, 50°C, 60°C, 80°C) were investigated. At 40°C, derivatization had not been completed within several hours. At reaction temperatures of more than 60°C, a decrease in derivative peaks and other additional peaks on HPLC profiles was observed, and it appeared that the derivatives were destroyed by the high reaction temperature. Fig. 1 shows that it takes approximately 60 min at 50°C to derivatize these sugar alcohols which then remain stable for 150 min under these conditions. Thus, we standardized the reaction time as 90 min at 50°C. This resulted in a single peak for each derivative except the reagent on the HPLC profile. ^{1}H-NMR spectra of the maltitol derivative showed that all hydroxyl groups were converted into *p*-nitrobenzoate[1].

3.2. Sep-Pak extraction

In the previous method [16], it was necessary to apply the silica Sep-Pak to eliminate excess amounts of reagent. However, as the derivatives themselves are insoluble in the wash solution (10% ethyl acetate in hexane), this could have caused worsening of RSD values. Therefore, we modified our previous method, by dissolving derivatives in 2 ml chloroform and applying to the silica Sep-Pak. We were thus able to minimize RSDs successfully.

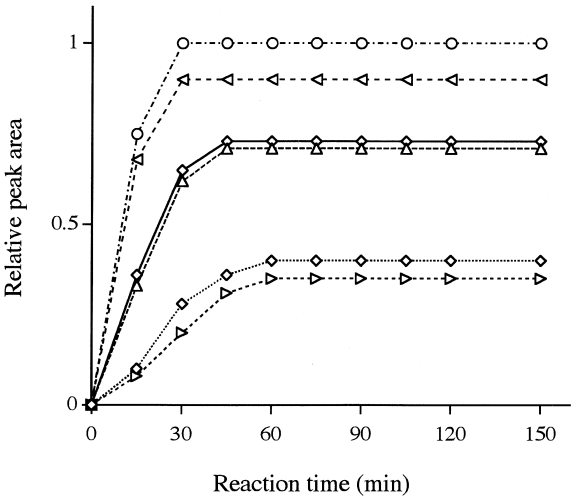

Fig. 1. Time course of nitrobenzoylation. Standards of sugar alcohols were derivatized for various times at 50°C and then subjected to HPLC analysis. Peak area is expressed as relative peak area. Marks on curves represent *p*-nitobenzoates of: (···○-···) *meso*-erythritol, (––◁––) xylitol, (--△--) D-glucitol, (—◇—) D-mannitol, (--▷--) maltitol, and (···◇···) parachinit. HPLC conditions: 150 mm×4.6 mm I.D. Inertsil Ph-3 (GL Sciences); mobile phase, acetonitrile–water (67:33); flow-rate, 1 ml/min; UV monitor at 260 nm; sample volume, 10 μl.

3.3. Chromatography

An investigation of the chromatographic behavior of the derivatives was carried out. Fig. 2 shows the chromatograms obtained from the separation of the derivatives. In our previous method [16], martitol and peaks overlapped. An attempt to change columns was made to separate maltitol and 6-α-glucopyranosylglucitol. Separation is not achieved with ODS, C_8, NH_2 and CN columns, whereas it successfully takes place when a phenyl column with acetonitrile–water (67:33) is employed, as shown in Fig. 2b. Hanai and Hubert suggested that the selectivities within the polyaromatic hydrocarbons were slightly different between phenyl-bonded silica gel and ODS

[1] ^{1}H-NMR measurement: The derivatives of maltitol were prepared according to Section 2.5 and dissolved in C_2HCl_3 after recrystallization with ethyl acetate–*n*-hexane (1:9). ^{1}H-NMR measurements were carried out with a JEOL JMN-A500 instrument (500 MHz) (JEOL, Tokyo, Japan). The proton signals of δ 4.2–6.2 ppm (15H, 9 methine and 3 methylene bonded hydroxyl group of maltitol) and δ 7.8–8.4 ppm (36H,9 *p*-nitrobenzoyl groups) were observed.

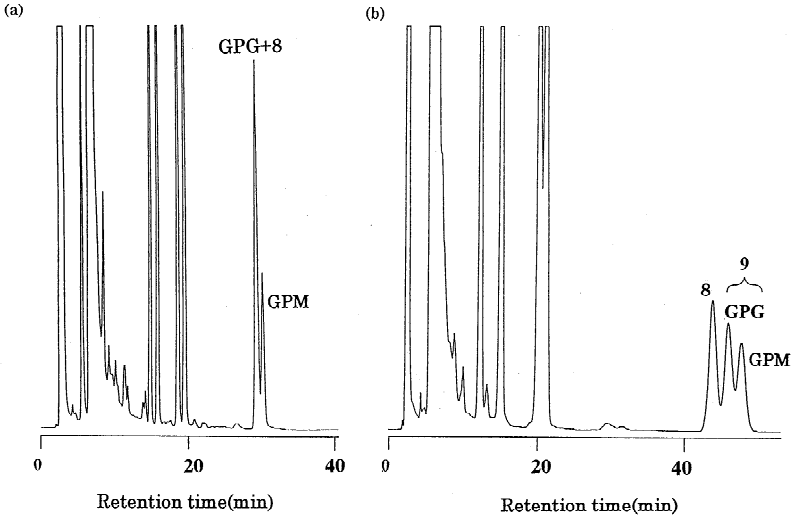

Fig. 2. The separation of *p*-nitrobenzoates by reversed-phase chromatography: comparison between (a) ODS column and (b) Phenyl column. GPM=6-α-glucopyranocylmannitol, GPG=6-α-glucopyranocylglucitol, 8=maltitol. 9=parachinit. HPLC conditions: (a) Cosmosil $5C_{18}$AR (250×4.6 mm I.D., Nakarai Tesque); mobile phase, acetonitrile–water (65:35); flow-rate, 1 ml/min; UV monitor at 260 nm; sample volume, 10 μl. (b) HPLC conditions as in Fig. 2.

columns, owing to the π energy effect derived from the Van der Waals volume and the delocalization energy [17]. In addition, Melander et al. showed that polar interactions with the stationary phase contribute significantly to polar benzene derivatives [18]. Good separation for GPG and maltitol may be attributed to the interaction between phenyl groups in the column and nitrobenzoyl groups in the derivatives.

Plots of solvent combination rate vs. retention time indicates that a relatively small range of 65–70% acetonitrile is feasible for application to the sugar alcohols (Fig. 3).

Typical separations of a mixed standard (Fig. 4) show retention times of 12.2, 15.3, 16.7, 21.1, 22.2, 29.8, 39.9, 48.9, 51.0 and 53.0 min for *meso*-erythritol, glucose and mannose (overlapping), xylitol, D-glucitol, sucralose, sucrose, D-mannitol, maltitol and parachinit, respectively.

3.4. Calibration and precision

The derivatization of *meso*-erythritol, xylitol, D-glucitol, D-mannitol, maltitol and parachinit were found to be linear in the range of concentrations from 10 to 250 μg/ml (r=0.999). The detection limit was 0.1% for the sugar alcohols contained in the samples.

With regard to the quantitative accuracy of the sugar alcohol content using this method, the average recoveries of the sugar alcohols spiked at 5 and 10%

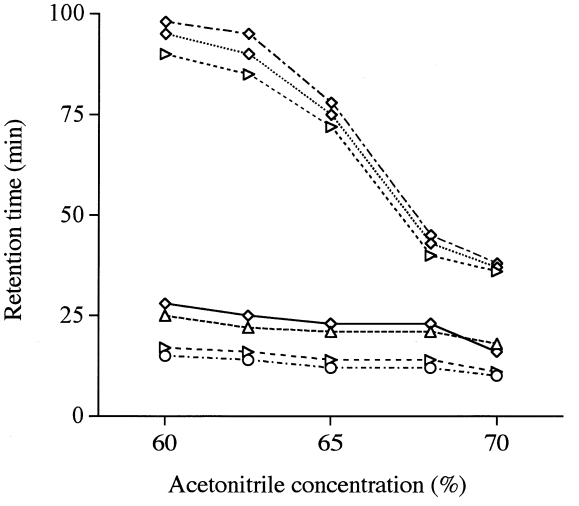

Fig. 3. Plots of retention time on phenyl column vs. acetonitrile content (%). HPLC conditions as in Fig. 2. Marks on curves represent *p*-nitrobenzolates of: (·–·○·–·) *meso*-erythritol, (––◁––) xylitol, (--△--) D-glucitol, (—◇—) D-mannitol, (---▷---) maltitol, (···◇···) parachinit (GPG) and (---◇---) parachinit (GPM).

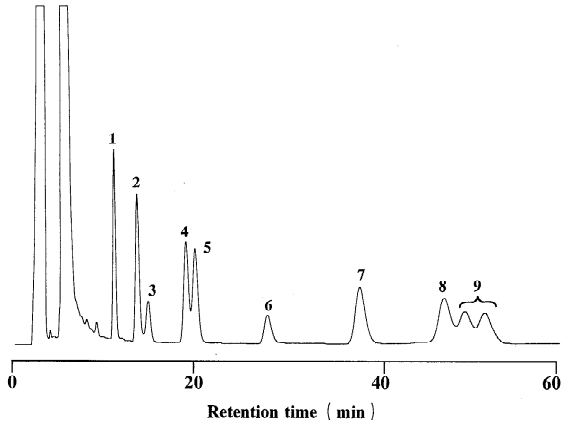

Fig. 4. Liquid chromatographic separation of *p*-nitrobenzoates of mixed sugar alcohol standards. Peak: 1=*meso*-erythritol, 2=xylitol, 3=D-mannose, glucose, 4=D-glucitol, 5=D-mannitol, 6=sucralose(trichlorogalactosucrose), 7=sucrose, 8=maltitol and 9=parachinit. HPLC conditions as in Fig. 2 legend.

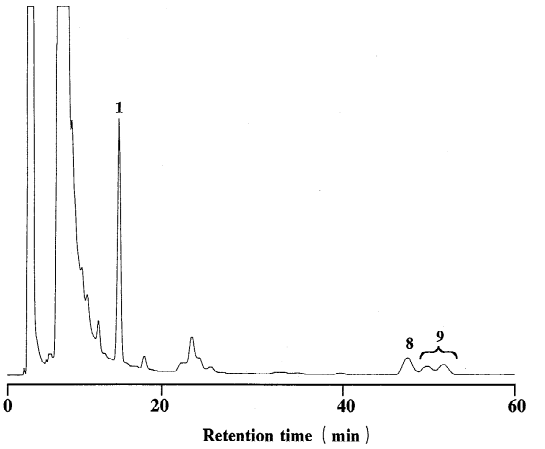

Fig. 5. Liquid chromatogram of sugar alcohols in a sugarless chewing gum. Peaks: 1=*meso*-erythritol, 8=maltitol and 9=parachinit. HPLC conditions as in Fig. 2.

levels of six sugar alcohol standards to four sugarless confectioneries ranged from 73.2 to 109.0% with the RSDs ranging from 0.7 to 9.0% as shown in Table 1. Peppermint, chewing gum, gummy candy and candy showed no interferences under the components peaks of interest.

3.5. Applications

Xylitol, especially, has an anti-caries effect, and this sweetener has become increasingly used in chewing gum. Other sugar alcohols are also added to dietary food products as sugar substitutes. A variety

Table 1
Analytical results of recovery test[a]

Products	Added standard	Recovery (%)						
		Erythritol	Xylitol	Glucitol	Mannitol	Maritol	Parachinit	Matrix
Gumi candy	Standard 100 mg	88.1(1.6)	82.3(2.5)	97.0(8.3)	94.2(2.6)	88.4(2.9)	87.2(3.7)	Citric acid
	Standard 50 mg	80.0(1.0)	88.9(1.6)	96.6(1.9)	90.4(3.4)	87.6(1.7)	87.4(0.6)	color agent
Gum	Standard 100 mg	97.9(6.3)	104.1(1.5)	94.5(7.5)	102.7(3.5)	98.7(1.5)	95.8(9.0)	Glutinous
	Standard 50 mg	90.6(3.5)	105.6(1.5)	108.0(2.5)	90.1(4.5)	87.5(4.6)	88.2(3.0)	syrup emulsifier dextrin stevioside, gel
Candy	Standard 100 mg	73.2(1.3)	82.4(3.7)	78.1(5.3)	105.2(3.3)	74.0(4.3)	107.0(7.8)	Aspartame
	Standard 50 mg	84.8(1.8)	82.6(1.6)	90.3(8.4)	105.0(4.6)	76.6(5.2)	109.0(0.7)	essence emulsifier gum, base
Pepper mint	Standard 100 mg	95.1(4.9)	78.6(5.7)	90.8(2.4)	90.0(2.4)	94.4(1.6)	80.0(1.1)	Aspartame
	Standard 50 mg	84.6(1.9)	88.0(5.6)	92.4(0.8)	96.7(2.8)	91.0(3.2)	87.4(2.0)	menthol gelatin essence plant wax

[a] (): RSD, $n=3$.

Table 2
Analysis of sugar alcohols in various confectioneries

Sample		Content (%)					
		Erythitol	Xrylitol	Glucitol	Mannitol	Maritol	Parachinit
Domestic foods (Japan)							
Candy	1						14.7
Gummy candy	1			2.3			
	2			2.6			
	3	57.8					
Chewing gum	1						2.7
	2					5.3	
	3		29.4				
	4				5.8		23.9
	5		50.0				
	6						21.5
Pepper mint	1		51.3				
	2			58.7			
Imported foods							
Candy	1						24.4
Gummy candy	1			3.3			
Pepper mint	1			38.8			
	2			59.4			

of commercially available sugarless confectioneries were analyzed by the present method. Typical separation of the sugar alcohol derivatives in a sugarless chewing gum sample is shown in Fig. 5. The results of the analysis of sugarless confectioneries are summarized in Table 2. It was found that the sugar alcohol content of samples investigated varied between 2.3 and 59.4%. In general, in terms of sugarless foodstuffs, other alternative sweeteners include aspartame, stevioside, rebaudioside A and saccharin, which are permitted for use in Japan. However, there is no interference on the HPLC profiles in this condition.

In the present paper, we describe a method for the determination of six sugar alcohols, *meso*-erythritol, xylitol, D-glucitol, D-mannitol, maltitol and parachinit, using a pre-column derivatization technique. Sugar alcohols in mainly sugarless confectioneries have also been determined by this method.

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