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Short communication

Analysis of carbohydrates in drinks by high-performance liquid chromatography with a dynamically modified amino column and evaporative light scattering detection

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Abstract

A high-performance liquid chromatographic method with a dynamically modified amino column and evaporative light-scattering detector (ELSD) was established for the direct analysis of the carbohydrates in some drinks. A separation column (Zorbax Rx-SIL, 250 mm×4.6 mm I.D., 5 μm, Hewlett-Packard, USA) which was modified by ethylenediamine and a guard column (Zorbax Rx-SIL, 12.5 mm×4.6 mm I.D., 5 μm) were used. The mobile phase was a mixture of water–acetonitrile (1:2.6, v/v) containing 0.03% (v/v) ethylenediamine. Regression equations revealed linear relationship (correlation coefficients=0.996–0.999) between the mass of carbohydrates injected and the carbohydrates peak areas detected by ELSD. The detection limits of ELSD ($S/N=3$) were between 0.2 and 1.2 μg for different carbohydrates. This method is simple and sensitive.

Keywords: Fruit juices; Wine; Food analysis; Carbohydrates

1. Introduction

The analysis of carbohydrates is generally carried out by high-performance liquid chromatography (HPLC), which can provide not only the qualitative analysis but also the quantitative determination.

The main chromatographic systems used for the separation of underivatized carbohydrates can be generalized as follows: (1) anion-exchange column with water containing bases or salts as the eluent [1]; (2) cation-exchange column with water as the eluent [2]; (3) alkyl-bonded silica gel column with water as the eluent [3]; (4) amine-bonded silica gel column with water–acetonitrile as the eluent [2,4–6]. Of these systems, an amine-bonded silica gel column was the one mostly used. But the separation of carbohydrates on the amine-bonded silica gel column is not always quantitative. This is due to the possible interaction between reducing carbohydrates and the amino group of the ligate (formation of a Schiff's base) and to a self-hydrolysis of the basic material. Some succedaneums for the amine-bonded silica gel column appeared more recently, such as diol-bonded [7] and cyclodextrin-bonded [8] silica gel columns. It is also possible to get a dynamically modified amino column. Various techniques have been developed which use silica columns [9,10] and octadecyl-silica columns [11] for separation of carbohydrates by adding small amounts of amines and *n*-alkylamines

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to the eluent, respectively. With the same separation efficiency, the dynamically modified amino columns are more stable than the chemically-bonded amino columns during the separation, since they are continuously generated.

Generally, carbohydrates lack chromophoric and fluorophoric groups which are necessary for UV and fluorescence detection. Refractive index (RI) [2,3,6,9] measurement is the most popular detection method for carbohydrates. However, it has many disadvantages, such as lacking sensitivity, depending on temperature and flow-rate, and incompatibility with gradient elution. A evaporative light scattering detection (ELSD) [12] is widely used as a semiuniversal mass detector for HPLC now. It is based on the detection of solute molecules by light scattering after nebulization and evaporation of the mobile phase, so it is suitable to detect the nonvolatile compounds such as lipids [13,14] and carbohydrates [2,4,5].

This paper describes a simple, direct analysis technique for some common carbohydrates in drinks with a dynamically modified amino column and ELSD.

2. Experimental

2.1. Apparatus

HPLC analysis was performed with a HP 1100 HPLC system (Hewlett-Packard, USA), consisting of a quaternary pump, a vacuum degasser, a thermostatted column compartment, a 20-μl manual injector and a HP Chemstation for data analysis. Detection was carried out using a Alltech 500 ELSD system (Alltech, USA).

2.2. Chemicals and samples

All chemicals used in this study were of analytical-reagent grade and deionized water was used to prepare all solutions. Fructose was purchased from Beijing Xizhong Chemical Plant (China), glucose from Yinghai Fine Chemical Plant (China), sucrose from Beijing Chemical Plant (China), maltose and raffinose from Fluka (Switzerland), and lactose from the Chinese Academy of Military Medical Science (China). Acetonitrile (HPLC-grade) was purchased from Fisher Scientific (USA), ethylenediamine from the Chinese Academy of Military Medical Science (China), and ammonium hydroxide from Beijing Jingzhan Chemical Plant (China).

Stock solutions of each carbohydrate were prepared as an aqueous solution with a concentration of 2000 mg/l (6000 mg/l for lactose). The standard mixture was prepared using these stock solutions.

The samples, apple juice, pineapple juice, and orange juice (Parmalat Dairy, Tianjin, China); grape wine (Weilong grape wine, China); liquor (Shanxi Xinghua Cun Brewery, China), were purchased from local markets. All the samples were filtered through a 0.45-μm membrane filter and diluted 50-fold with water. The sample solutions were injected into the HPLC system using a 20-μl loop injector.

2.3. LC conditions

A separation column (Zorbax Rx-SIL, 250 mm×4.6 mm I.D., 5 μm, Hewlett-Packard, USA) and a guard column (Zorbax Rx-SIL, 12.5 mm×4.6 mm I.D., 5 μm) were used. The column temperature was 25°C. The mobile phase for isocratic elution was a mixture of water–acetonitrile (1:2.6, v/v) containing 0.03% (v/v) ethylenediamine as a modifier and ammonium hydroxide (0.05%, v/v), which was used to adjust the pH to 9~10. The flow-rate was 1.0 ml/min. The temperature of the heated drift tube was 85°C and the gas flow-rate was 2.0 l/min for the ELSD. Before analysis, a mixture of water–acetonitrile (1:2.6, v/v) containing 0.3% (v/v) ethylenediamine was run through the column forming a dynamic coating layer on the silica surface.

3. Results and discussion

3.1. Separation of the carbohydrates

Di- or polyamines in the eluent can be adsorbed to the silica surface to form a dense monolayer via a hydrogen bond between one of the amino groups of the amine modifiers and the silanol group of the silica surface, then carbohydrates can be retained on this dynamically modified amino stationary phase via

hydrogen bonding between another amino group of the amine modifiers and the hydroxyl groups of the carbohydrates. Researchers have used some di- or polyamines such as piperazin [9] or tetraethylenepentamine [10] to modify the silica column successfully. But they were not suitable for ELSD because of their low volatility. For the same reason, the *n*-alkylamine with a long carbon-chain [11], which was used to modify the octadecyl-silica column, was not suitable for ELSD either. Ethylenediamine was chosen as modifier because it was the most volatile diamine. Before analysis, a mobile phase containing 0.3% (v/v) ethylenediamine was used so that the silica surface could be completely coated by ethylenediamine. In analysis, it was proved that there was little effect on the separation when the concentration of ethylenediamine in the mobile phase was changed from 0.03 to 0.1%. It showed that 0.03% (v/v) of ethylenediamine in the mobile phase was enough to dynamically replace the ethylenediamine already adsorbed on the silica surface. In the acidic situation, the amino groups of the ethylenediamine would be partly ionized, and the hydrogen bond between the amine modifiers and silica surface or carbohydrates would be destroyed. It was found that when the pH was lower than 8, the carbohydrates were hardly retain on the column. But at high pH, the silica would dissolve. Ammonium hydroxide (0.05%, v/v) was used to adjust the pH to 9~10, under these conditions good separation for six carbohydrates was be obtained by adjusting the ratio of water and acetonitrile, and the column was stable during the analysis.

Different ratios of water and acetonitrile as the mobile phase were studied. The content of water showed a great effect on the retention of the carbohydrates, and on increasing the content of water, the carbohydrates would be eluted more quickly. The difference between the retention time of fructose and

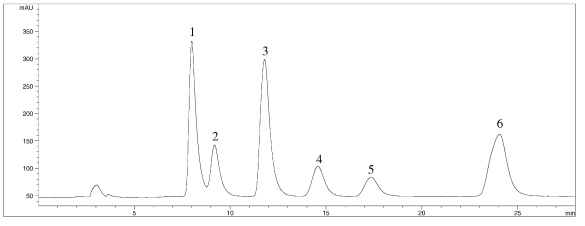

Fig. 1. LC–ELSD chromatogram of a standard mixture of six carbohydrates. Column: Zorbax Rx-SIL (250 mm×4.6 mm I.D., 5 μm); column temperature: 25°C; mobile phase: water–acetonitrile (1:2.6, v/v) containing 0.03% (v/v) ethylenediamine and 0.05% (v/v) ammonium hydroxide; flow-rate: 1.0 ml/min; heated drift tube temperature of ELSD: 85°C; gas flow-rate of ELSD: 2.0 l/min. Peak identification: 1=fructose (30 μg), 2=glucose (30 μg), 3=sucrose (30 μg), 4=maltose (30 μg), 5=lactose (90 μg), 6=raffinose (30 μg).

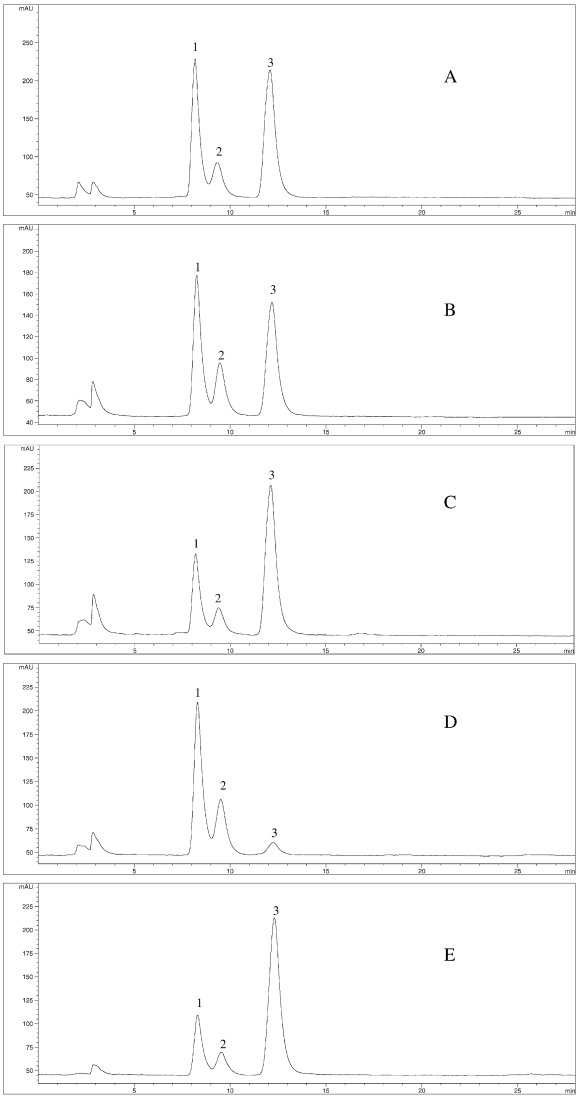

Fig. 2. LC–ELSD chromatogram of drinks sample. (A) Apple juice, (B) pineapple juice, (C) orange juice, (D) grape wine, (E) liquor. The chromatographic conditions were as in Fig. 1. Peak identification: 1=fructose, 2=glucose, 3=sucrose.

Table 1
Parameters of quantitative analysis for six carbohydrates

Carbohydrate	Linear range (μg)	Calibration curve equation	Calibration coefficient	Detection limit (μg)
Fructose	1~30	$A = -70.20 + 267.68C$	0.999	0.2
Glucose	1~30	$A = -38.06 + 113.50C$	0.999	0.4
Sucrose	1~30	$A = -199.94 + 310.50C$	0.997	0.2
Maltose	1~30	$A = -51.18 + 91.00C$	0.997	0.4
Lactose	3~90	$A = -56.62 + 24.50C$	0.996	1.2
Raffinose	1~30	$A = -175.84 + 284.50C$	0.998	0.2

glucose was not so marked as the other four carbohydrates. Using water–acetonitrile in a ratio of 1:2.6 (v/v) as the mobile phase, the analysis time was the shortest, and was able to give a good separation between fructose and glucose. The chromatograms of a standard mixture and the drink samples at optimum conditions are shown in Figs. 1 and 2. A good separation of the six common carbohydrates was obtained.

3.2. Parameters for quantitative analysis

Eleven aqueous solutions of carbohydrates of different concentrations in the range of 30–2000 mg/l (90–6000 mg/l for lactose) were prepared for the calibration curve, which was equal to 0.6–40 μg of carbohydrates injected (1.8–120 μg for lactose). The detection limits ($S/N=3$) and linear range for the six carbohydrates were investigated which are showed in Table 1. Many studies reported that the ELSD response usually increased exponential by increasing the mass of sample injected, the linearity between peak area and the mass of the analyte injected could be obtained in double logarithmic coordinate, which could be demonstrated with the equation: $\log A = b \log C + \log a$, where A was the peak area measured by ELSD, C was the mass of analyte injected and a and b were constants. From our experimental results, it was found that calibration curves between peak areas and the mass of analyte injected were linear for these six carbohydrates, following the equation $A = bC + a$, with a correlation coefficient of 0.996–0.999 in the range of 1–30 μg (3–90 μg for lactose). The detection limits of ELSD ($S/N=3$) were between 0.2 and 1.2 μg for different carbohydrates. In ELSD, the amount of light scattered is dependent on not only the number but also on the size and shape of particles, which are influenced by a lot of factors such as the physical and chemical properties of the carbohydrates and the mobile phase, the heated drift tube temperature, the gas flow-rate and so on, until now no theory models could predict the ELSD response perfectly.

3.3. Analysis of drinks

The linear calibration equations must be used in the indicated range, so the samples: three kinds of fruit juices (apple juice, pineapple juice, and orange juice), grape wine, and liquor were diluted 50-fold before analysis for the carbohydrate content. All of these samples only had three kinds of carbohydrates: fructose, glucose, and sucrose. The results are shown in Table 2. Suitable amounts of the carbohydrate

Table 2
Determination results of carbohydrates in 50-fold diluted drinks ($N=5$)

Samples	Fructose content (mg/l)	Glucose content (mg/l)	Sucrose content (mg/l)
Apple juice	983.0±16	870.0±17	1121.0±15
Pineapple juice	772.0±11	873.0±15	742.0±9
Orange juice	499.0±6	531.0±8	1091.0±6
Grape wine	959.0±14	1066.0±17	121.0±4
Liquor	380.0±5	464.0±8	1169.0±12

standards were added to the drink samples of known carbohydrate content, the mixtures were analyzed using the proposed procedure. Recovery was expressed for each component as the mean percentage ratio between the measured amounts and the added ones. The standard addition recoveries of carbohydrates were between 95.8 and 103.0%.

It was shown that the proportions of the three kinds of carbohydrates were different for different drinks. Although no pre-dialysis was done, the carbohydrates in the drinks were separated successfully from each other without any interference by other substances existing in the drinks. In conclusion, this HPLC method is simple and useful for the direct analysis of common carbohydrates in drinks.

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