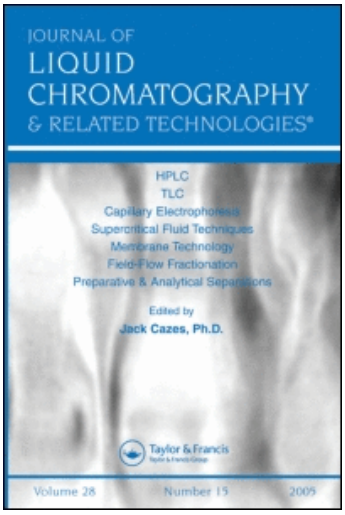

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ETHANOLAMINE AS MODIFIER FOR ANALYSIS OF CARBOHYDRATES IN FOODS BY HPLC AND EVAPORATIVE LIGHT SCATTERING DETECTION

Yang Wei[a]; Ming-Yu Ding[a]
[a] Department of Chemistry, Tsinghua University, Beijing, P. R. China

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ETHANOLAMINE AS MODIFIER FOR ANALYSIS OF CARBOHYDRATES IN FOODS BY HPLC AND EVAPORATIVE LIGHT SCATTERING DETECTION

Yang Wei and Ming-Yu Ding*

Department of Chemistry, Tsinghua University,
Beijing 100084, P. R. China

ABSTRACT

A high performance liquid chromatographic method with evaporative light scattering detector (ELSD) was established for the direct analysis of the carbohydrates in some foods. Ethanolamine and triethylamine were used as modifier and pH adjuster to dynamically modify a silica gel column for their low nucleophilicity. The mobile phase was a mixture of water/acetonitrile containing 0.05% (V/V) ethanolamine and 0.05% (V/V) triethylamine. Regression equations revealed linear relationships (correlation coefficients: 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 almost the same (0.16–0.20 μg) for different carbohydrates. The low nucleophilicity of ethanolamine and triethylamine would help to decrease the detection limits and improve the accuracy of the quantitative

*Corresponding author. E-mail: dingmy@chem.tsinghua.edu.cn

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result of the reducing carbohydrates. This system could be useful for routine analysis of simple carbohydrates in foods, especially for the analysis of reducing carbohydrates.

INTRODUCTION

The analysis of carbohydrates is an area of high interest, because of their abundant content in nature and their important role in a wide range of biological processes. High performance liquid chromatography (HPLC) is the most common method for carbohydrate analysis. In 1975, Linden and Lawhead reported the first application of HPLC to carbohydrate analysis.[1] Since then, the use of the HPLC technique expanded rapidly; various chromatographic modes were used to analyze underivatized carbohydrates, such as anion-exchange chromatography,[2,3] cation-exchange chromatography,[4,5] chromatography with alkyl-bonded silica gel column,[6,7] chromatography with amine-bonded silica gel column.[4,8,9] The amine-bonded silica gel column was the most used one of these modes, but it had two major drawbacks in that it had a short column life and the amino group tended to react with reducing carbohydrates to form Schiff's base.

In a previous paper,[10] we established a HPLC method with a dynamically modified amino column for the analysis of carbohydrates in drinks. With ethylenediamine as modifier and ammonium hydroxide as pH adjuster, using the mixture of acetonitrile/water as mobile phase, six carbohydrates (fructose, glucose, sucrose, maltose, lactose, and reffinose) were separated entirely in 25 min on a silica column. During the separation, the dynamically modified amino column was very stable since the surface of the stationary phase was constantly renewed by ethylenediamine. But, the reducing carbohydrates like glucose, maltose, and lactose had higher detection limits than non-reducing carbohydrates; that was due to the on-column reaction between the reducing carbohydrates and the nucleophilic agents ethylenediamine and ammonium hydroxide. In order to improve the detection sensitivity for reducing carbohydrates, modifier and pH adjuster with low nucleophilicity should be used.

The present study reported on the utility of a dynamically modified silica gel column modified by ethanolamine and triethylamine as pH adjuster to directly analyze the carbohydrates in some foods. Detection was carried out by evaporative light scattering detection (ELSD), which was suitable to detect nonvolatile compounds.

EXPERIMENTAL

Apparatus

A HP 1100 HPLC system (Hewlett-Packard, USA) was used to perform the analysis, which was equipped with a quaternary pump, a vacuum degasser, a column thermostat, a 20 μL manual injector, and a HP Chemstation for instrument control, data collection, and data handling. Detection was carried out using an Alltech 500 evaporative light scattering detector (Alltech, USA).

Chemicals

All chemicals used in this study were of analytical purity. Fructose was purchased from Beijing Xizhong Chemical Plant (China), glucose from Yinghai Fine Chemical Plant (China), sucrose from Beijing Chemical Plant (China), maltose and reffinose from Fluka Chemical Co. (Switzerland), lactose from the Chinese Academy of Military Medical Science (China). Acetonitrile (HPLC grade) was purchased from Scharlau Chemie S. A. (Spain), methanol (HPLC grade) from Tianjin Shield Co. (China), ethanolamine from the Beijing Hongxing Chemical Plant (China), triethylamine and chloroform from Beijing Chemical Plant (China), *n*-hexane from Beijing Yili Fine Chemical Company (China), and *n*-butyl alcohol from Beijing Yatai Fine Chemical Plant (China). Water was deionized and doubly distilled. The mobile phase was filtered through a 0.45 μm membrane filter.

A stock solution of a mixture of six carbohydrates was prepared as aqueous solution at a concentration of 10,000 mg/L for each carbohydrate. Standard mixtures were diluted from the stock solution with methanol.

Sample Preparation

The samples: pineapple juice and instant full cream milk powder (Parmalat Dairy Co. Ltd, Tianjin, China); pineapple flavored candy (Beijing Biwang Food Co. Ltd, China); honey (Beijing Bee Products Co., China), were purchased from local markets.

1.0 g of milk powder was weighed, totally 24 mL *n*-hexane was used to extract the fat from milk powder for three times. Subsequently, the Sevag sample clean-up method was performed to remove proteins. Defatted milk powder was dissolved in 25 mL water, to which 5 mL chloroform and 1 mL *n*-butyl alcohol were added, the resulting mixture was shaken heavily, the precipitate was filtered off. The same procedure was repeated until the solution became clear. The clear

solution was filtered through a 0.45 μm membrane filter and transferred into a 50 mL flask, adding methanol to the mark. Then 3.5 mL of the solution was diluted to 25 mL with methanol. The final sample solution was injected to HPLC.

Honey (0.5 g) was weighed and then dissolved in 25 mL water. The aqueous solution (2.0 mL) was diluted to 25 mL with methanol and then filtered through a 0.45 μm membrane filter before being injected into the HPLC.

Candy (2.1 g) was weighed and then dissolved in 50 mL water. Aqueous solution (4 mL) was diluted in 25 mL with methanol and then filtered through a 0.45 μm membrane filter before injection to the HPLC.

Pineapple juice was filtered through a 0.45 μm membrane filter and diluted 50-fold with methanol.

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 mixture of water/acetonitrile containing 0.05% (V/V) ethanolamine and 0.05% (V/V) triethylamine was used as mobile phase, with flow rate of 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 detection with ELSD. Samples were injected using a 20 μL loop injector.

RESULTS AND DISCUSSION

Glycol and Glycerol as Modifier

In our previous paper,[10] ethylenediamine was used to modify the silica gel column via forming the hydrogen bond between one of the amino groups of the ethylenediamine and the silanol group of the silica surface. The attempt at modifying the silica gel column to form a "dynamically modified hydroxyl column" using glycol and glycerol failed. It could be concluded, that glycol and glycerol had much stronger intermolecular hydrogen bonds than ethylenediamine, by comparing their boiling points: glycol 197°C, glycerol 290°C, and ethylenediamine 116.5°C. Modifiers adsorbed to the silica surface to form a dense monolayer, should overcome their own intermolecular hydrogen bond. It was the reason for the failure in forming a "dynamically modified hydroxyl column". Ethanolamine was then used as a modifier in this work. There was an amido in the molecule of ethanolamine. Some hydrogen bonds could be formed between the amido and silanol group of the silica surface.

Concentration of Ethanolamine

The concentration of ethanolamine in the eluent should be thick enough so that the silica surface is completely covered by ethanolamine. The retention of the carbohydrates increased with the augment of the ethanolamine concentration, before the concentration reached saturation. This trend was shown in Fig. 1(A) and Fig. 1(B).

There was only a little effect on the separation when the concentration of ethanolamine in the mobile phase was increased from 0.05% to 0.15%. It showed that 0.05% (V/V) of ethylenediamine in the mobile phase was thick enough. Triethylamine 0.05% (V/V) was used as pH adjuster for two reasons. First, if not

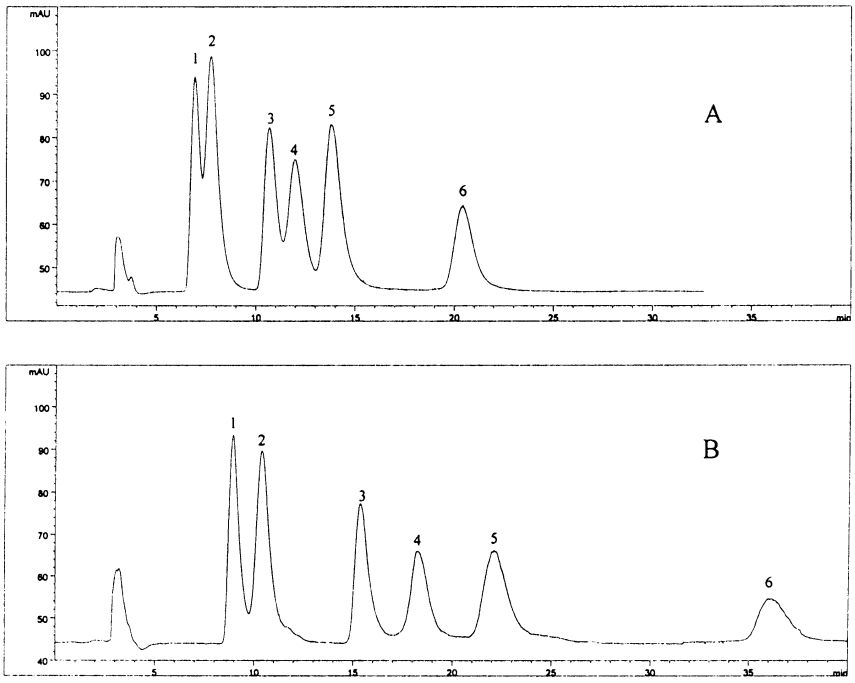

Figure 1. LC-ELSD chromatogram of a standard mixture of six carbohydrates. Column: Zorbax Rx-SIL(250 mm × 4.6 mm I.D., 5 μm); column temperature: 25°C; mobile phase: water/acetonitrile (1 : 3.8, V/V) containing 0.05% (V/V) triethylamine and 0.01% (V/V) ethanolamine (Fig. 1A) or 0.05% (V/V) ethanolamine (Fig. 1B); flow rate: 1.0 mL/min. Heated drift tube temperature of ELSD: 95°; gas flow-rate of ELSD: 2.5 L/min. Peak identification: 1. fructose (12 μg), 2. glucose (12 μg), 3. sucrose (12 μg) , 4. maltose (12 μg), 5. lactose (12 μg), 6. reffinose (12 μg).

used, the amino groups of the ethanolamine would be partly ionized in the acidic situation and the hydrogen bond between the modifiers and silica surface would be destroyed. Secondly, peaks would be broadened or eluted as a doublet, owing to the multirotation of the carbohydrates.

Separation of Carbohydrates

Compared with the ethylenediamine dynamically modified column in our previous paper,[10] the retention of carbohydrates on the ethanolamine dynamically modified column was weaker, with the same retention order. A higher content of acetonitrile should be used for an ethanolamine dynamically modified column than for an ethylenediamine dynamically modified column.

Using acetonitrile/water in a ratio of 83 : 17 (V/V) as the mobile phase, baseline separation could be obtained between the fructose, glucose, sucrose, maltose, and lactose; but, reffinose eluted as a broad peak at 82 min. A linear gradient elution was adopted: 0–10 min, the ratio of acetonitrile/water maintained at 83 : 17; 10–15 min, the ratio changed from 83 : 17 to 75 : 25; then this ratio maintained from 15 to 22 min; in 22–30 min, the ratio changed from 75 : 25 to 70 : 30; 30–35 the ratio changed from 70 : 30 back to 83 : 17. With gradient elution, six carbohydrates could elute as sharp peaks in 30 min, but the whole analytical time was 40 min because of the fluctuated baseline. The chromatograms of a standard mixture with isocratic and gradient elution at the above conditions were shown in Figs. 2 and 3.

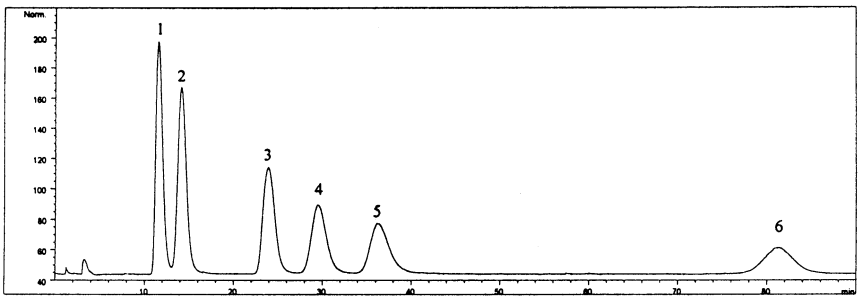

Figure 2. LC-ELSD chromatogram of a standard mixture of six carbohydrates with isocratic elution. Column: Zorbax Rx-SIL(250 mm × 4.6 mm I.D., 5 μm); column temperature: 25°C; mobile phase: water/acetonitrile (1 : 3.8, V/V) containing 0.05% (V/V) ethanolamine and 0.05% (V/V) triethylamine; flow rate: 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 (32 μg), 2. glucose (32 μg), 3. sucrose (32 μg), 4. maltose (32 μg), 5. lactose (32 μg), 6. reffinose (32 μg).

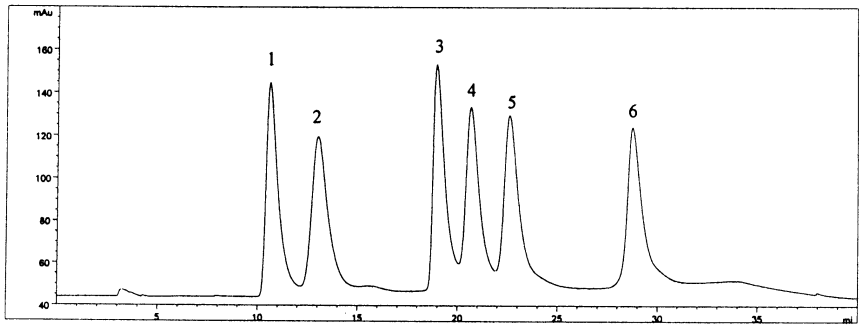

Figure 3. LC-ELSD chromatogram of a standard mixture of six carbohydrates with gradient elution. Gradient procedure: 0–10 min, the ratio of acetonitrile/water maintained at 83 : 17; 10–15 min, the ratio changed from 83 : 17 to 75 : 25; then this ratio maintained from 15 to 22min; 22–30 min, the ratio changed from 75 : 25 to 70 : 30; 30–35 min, the ratio changed from 70 : 30 back to 83 : 17. Other conditions were the same as Fig. 1.

Parameters for Quantitative Analysis

Eight standard mixtures with different concentrations (40–2000 mg/L) were used for the calibration curves of each carbohydrate, which was equal to 0.8–40 μg of carbohydrates injected. It was found, that the calibration curves between peak areas and the masses of these six carbohydrate injected were linear in the range 1.6–32 μg, following the equation $A = bC + a$, where A was the peak area measured by ELSD, C was the mass of carbohydrate injected, and a and b were constants, with a correlation coefficient of 0.999 for each carbohydrate.

The detection limits of ELSD (S/N = 3) were investigated. Compared with the ethylenediamine dynamically modified column in our previous paper,[10] the detection limits of the reducing carbohydrates, such as glucose, maltose, and

Table 1. Parameters of Quantitative Analysis for Six Carbohydrates

Carbohydrate	Linear Range (μg)	Calibration Curve Equation	Correlation Coefficient	Detection Limit (μg)
Fructose	1.6 ~ 32	$A = -192.98 + 131.79C$	0.999	0.16
Glucose	1.6 ~ 32	$A = -181.95 + 126.82C$	0.999	0.20
Sucrose	1.6 ~ 32	$A = -132.15 + 122.83C$	0.999	0.16
Maltose	1.6 ~ 32	$A = -148.22 + 102.90C$	0.999	0.20
Lactose	1.6 ~ 32	$A = -196.97 + 119.87C$	0.999	0.20
Reffinose	1.6 ~ 32	$A = -160.26 + 129.32C$	0.999	0.20

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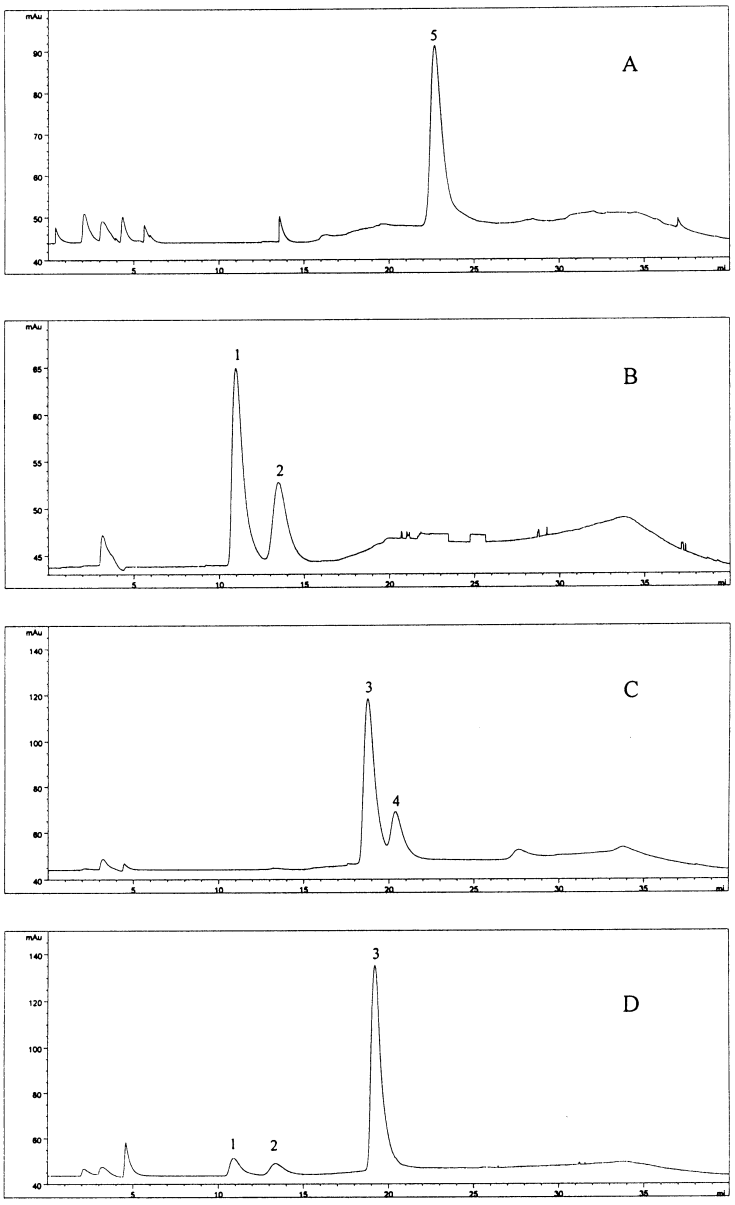

Figure 4. LC-ELSD chromatogram of food samples. A: milk powder, B: honey, C: candy, D: pineapple juice. The chromatographic conditions were as same as the Fig. 2. Peak identification: 1. fructose, 2. glucose, 3. sucrose 4. maltose, 5. lactose.

lactose, were decreased, especially for lactose; its detection limit decreased from 1.2 μg to 0.2 μg, since ethanolamine and triethylamine had relatively low nucleophilicity. Forming a Schiff's base could almost be avoided in the current chromatographic system.

The quantitative parameters obtained were shown in Table 1.

Analysis of Food Samples

The method developed in this work was used for the determination of the carbohydrates in several food samples. The chromatograms of four kinds of food samples were shown in Fig. 4. Different kinds of carbohydrates existed in different food samples. Only lactose was detected in milk powder; fructose, and glucose were detected in honey, sucrose and maltose were detected in candy. Three kinds of carbohydrates (fructose, glucose, and sucrose) were detected in pineapple juice. Chromatographic precision, expressed as relative standard deviation (RSD), was calculated by injecting five replicates of the samples. RSD values were between 2.20% and 3.83%. Suitable amounts of the carbohydrate standards were added to the food samples of known carbohydrate content; the mixtures were analyzed using the proposed procedure. Recovery was investigated by the above ways, which 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 95.0 and 104.2%. The determination results were shown in Table 2. The RSD and recovery values showed that this method had satisfactory accuracy and repeatability.

Table 2. Determination Results of Carbohydrates in Food Samples

Samples	Carbohydrates	Content (mg/g)	RSD (%) ($n = 5$)	Recovery (%)
Milk powder	Lactose	380.0	2.74	95.0
Honey	Fructose	275.6	3.16	102.5
	Glucose	168.1	3.69	95.4
Candy	Sucrose	183.7	2.61	97.9
	Maltose	59.0	2.20	96.2
Pineapple juice[a]	Fructose	10.5	3.62	103.5
	Glucose	9.9	3.83	104.2
	Sucrose	77.8	2.44	98.7

[a]In pineapple juice, the carbohydrates content were expressed as mg/mL.

CONCLUSION

Compared with the ethylenediamine dynamically modified column, the retention of carbohydrates on a ethanolamine dynamically modified column was weaker, but the ethanolamine dynamically modified column had enough efficiency to separate the common carbohydrates existing in foods within a suitable time. The modifier ethanolamine and pH adjuster triethylamine used in this paper, had much lower nucleophilicity than ethylenediamine and ammonium hydroxide used in a previous study, which could almost avoid the formation of a Schiff's base between strong nucleophile and reducing carbohydrates. This advantage would help to decrease the detection limits and improve the accuracy of the quantitative result of the reducing carbohydrates.

This system could be useful for routine analysis of simple carbohydrates in foods, especially for the analysis of reducing carbohydrates.

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