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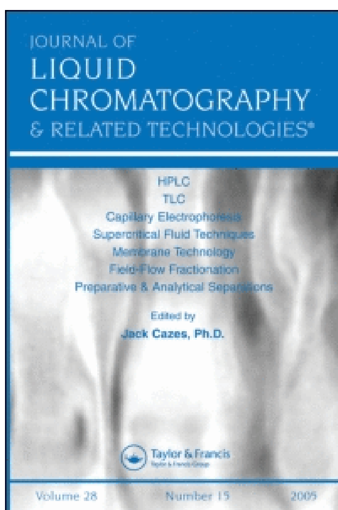

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COMPARATIVE STUDY OF THE SEPARATION AND DETERMINATION OF ASPARTAME AND ITS DECOMPOSITION PRODUCTS IN BULK MATERIAL AND DIET SOFT DRINKS BY HPLC AND CE

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ABSTRACT

The direct separation of aspartame (α-L-aspartyl-L-phenylalanine methyl ester) LL-α-APM, and several decomposition products namely LL-β-aspartame (LL-β-APM), L-α-aspartyl-L-phenylalanine (α-AP), L-β-aspartyl-L-phenyl-alanine (β-AP), and diketopiperazine (DKP) was accomplished by high performance liquid chromatography (HPLC) using a Chirobiotic T (Teicoplanin) column, and capillary zone electrophoretic (CZE) methods. The presence of any of these decomposition products in diet soft drinks labeled to contain the sweetener, also in coffee containing the artificial sweetener LL-α-APM, were investigated.

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INTRODUCTION

Aspartame (LL-α-APM) is widely used as an artificial sweetener in both dry powder form and in aqueous solution, as in the case of various diet soft drinks and other beverages. In dry powder LL-α-APM is relatively stable while, in aqueous solution it undergoes decomposition and racemization forming a variety of degradation products which is reported to be related to the length of storage, temperature and pH of the diet foods and beverages containing the sweetener.[1,2,3]

Witt[4] indicated that the analysis of a diet beverage (pH 2.55), after 50 weeks of storage at 20°C, had approximately 20% of the original LL-α-APM converted to DKP, another 20% was converted to α-AP, and 15% was converted to β-AP and LL-β-APM. Gaine and Bada[5] also identified six diastereomeric products after heating LL-α-APM sample for several hours at pH 8.8 - 9.8.

To help in evaluating the health significance of these decomposition products, various methods for monitoring the concentrations of aspartame and these products has been developed, including fluorescence,[6] gas chromatography[7] and the most frequently used high performance liquid chromatography,[8,9,10,11] and recently capillary zone electrophoresis.[12] In this study, an HPLC method using Chirobiotic T (Teicoplanin) column, and a capillary zone electrophoretic (CZE) method were used for the separation of aspartame and four of its decomposition products in a direct single run. A calibration curve for each of these compounds was individually constructed, and the presence of these products in diet soft drink and coffee sweetened with aspartame, were investigated.

EXPERIMENTAL

HPLC Apparatus

The HPLC system consisted of the following (all were products of Waters, Milford, MA, USA): a 501 solvent delivery pump, a Lambda Max 481 variable wavelength detector, a 746 data module, and a U6K injector. Chirobiotic T (Teicoplanin) column serial no. 8615 (250 x 4.6 mm I.D., particle type 5μm spherical) was obtained from Advanced Separation Technologies, Inc., Whippany, New Jersey, USA.

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Capillary Electrophoresis Apparatus

The work was carried out on Waters Quanta 4000E Capillary Electrophoresis System equipped with a UV detector (214 nm), and a positive power supply deliver upto 30KV (Waters Corporation, Milford, MA, USA). All analyses were performed on Accusep polyamide fused-silica capillaries (60cm x 75 μm I.D.)obtained from (Waters, Milford, MA, USA). The detection time constant was set at 0.3 second, all analyses were for 20 second injections with hydrostatic mode. A constant applied voltage of +15 KV was used in all experiments. Electrophoretic conditions and data acquisition were controlled by the Millennium 2010 Chromatographic Manager (Waters, Milford, MA, USA).

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Chemicals

LL-α-Aspartame (Lot:E-403240), LL-β-aspartame (Lot:672-45), L-α-aspartyl-L-phenylalanine (Lot:N941-122A), L-β-aspartyl-L-phenylalanine (Lot:490-135-A), and DKP (Lot: 7R-1) were kindly supplied by NutraSweet AG, Zug, Switzerland. Ethanol was purchased from Merck (Darmstadt, Germany), di-sodium hydrogen orthophosphate and di-sodium tetraborate was obtained from BDH Chemicals (Dorset, England).

Buffer and Solutions

Stock solutions of 4 mg/mL of all compounds (except for DKP, for which a stock solution of 1 mg/mL was used was made due to the limited solubility); all were prepared daily with Milli-Q purified water. To establish a calibration curve for each compound, five concentrations in the range of 5 -100 μg/mL for HPLC and in the range of 250 - 4000 μg/mL for CZE (except for DKP for which the range was 50 - 1000 μg/mL); all preparations were made in Milli-Q purified water.

Sample Preparations

Diet Seven-Up® soft drink was degassed in an ultrasonic bath then diluted 5 and 20 fold in Milli-Q-purified water and filtered through a 0.45 μm Millipore filter for the determination by CZE and HPLC respectively. To a 100mL of warm coffee , 500 mg of aspartame (commercially known as Equal® NutraSweet, Deerfield, Il, USA) was added.

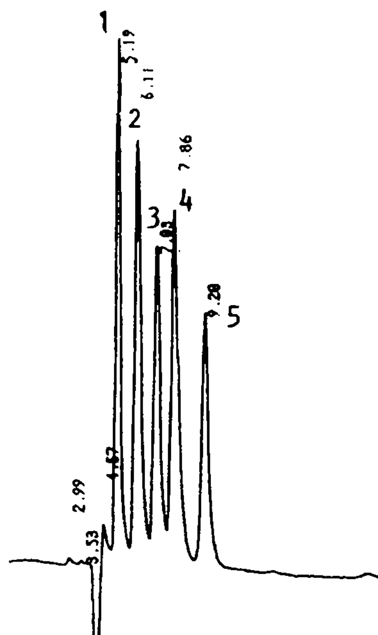

Figure 1. Chromatogram of a mixture containing aspartame and decomposition products (100 μg/each) Peaks: 1=DKP; 2=L-β-AP; 3=LL-β-APM; 4=L-α-AP; 5=LL-α-APM. Chromatographic conditions: column: Chirobiotic T (Teicoplanin) 250 x 4.6 mm I.D. Mobile phase: Ethanol:H_2O (55:45 V/V) pH 3.85. Flow rate: 0.6 mL/min. Detection: 215 nm, sensitivity=0.01 a.u.f.s.; attenuation: 32.

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The solution was heated to a temperature of 70°C for 10 minutes, immediately diluted 10 and 50 fold in Milli-Q purified water then filtered through a 0.45 μm Millipore filter for the determination of CZE and HPLC respectively.

RESULTS AND DISCUSSION

The chromatographic baseline separation of LL-α-APM and four of its degradation products which includes LL-β-APM, L-α-AP, L-β-AP, and DKP were achieved in a single run using an HPLC method with Chirobiotic T (Teicoplanin) column in less than 10 min (Fig. 1). The separation of the same compounds were also accomplished by capillary zone electrophoresis method in less than 18 min run (Fig. 2).

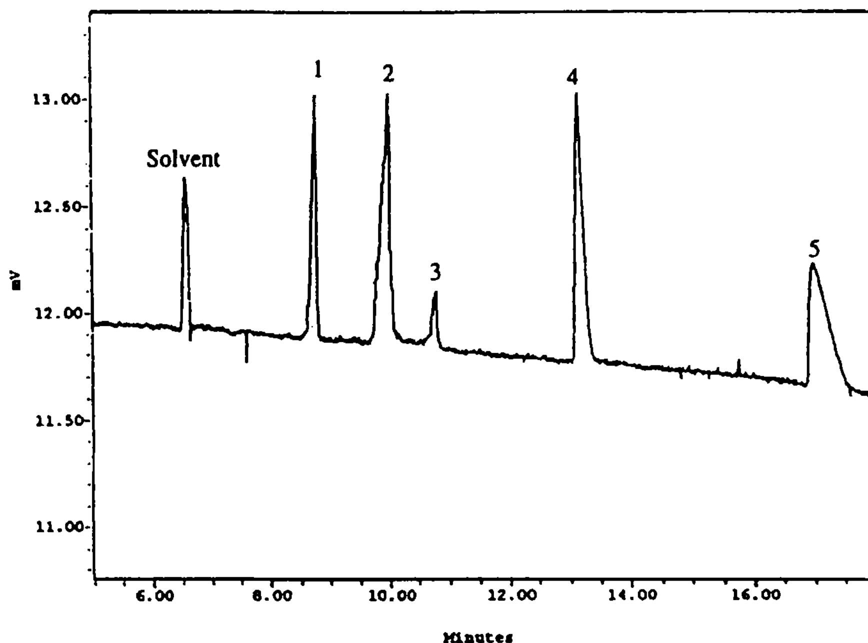

Figure 2. Electropherogram (2 mg mixture), Peaks: 1=LL-β-APM; 2=LL-α-APM; 3=DKP; 4=L-β-AP; L-α-AP. Condition: Buffer=25mM phosphate/25 mM Borate (1:1) pH 9.0; run voltage=15 Kv; injection mode=hydrostatic for 20 sec.

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Standard curves for the quantitative determination of each of these compounds were constructed individually using both methods. The regression analysis of these curves (Table 1) indicated a linear relationship between the peak area (Y), and the concentration (C) over the range of 5-100 μg/mL for all the compounds when the HPLC method was used (Fig. 3), while a higher linear range of 250-4000 μg/mL was obtained by CZE method. (Fig. 4) Except for DKP, the linear range was 100-1000 μg/mL owing to the lower detection limit of HPLC.

These methods were also applied to the analysis of LL-α-APM and the degradation products in Diet Seven-Up® soft drink, and after the addition of commercially available aspartame dry powder (Equal NutraSweet) to coffee. The results of the analyses of diet soft drink samples by both methods indicated the presence of 4-5% DKP of the total amount of LL-a-APM present in the

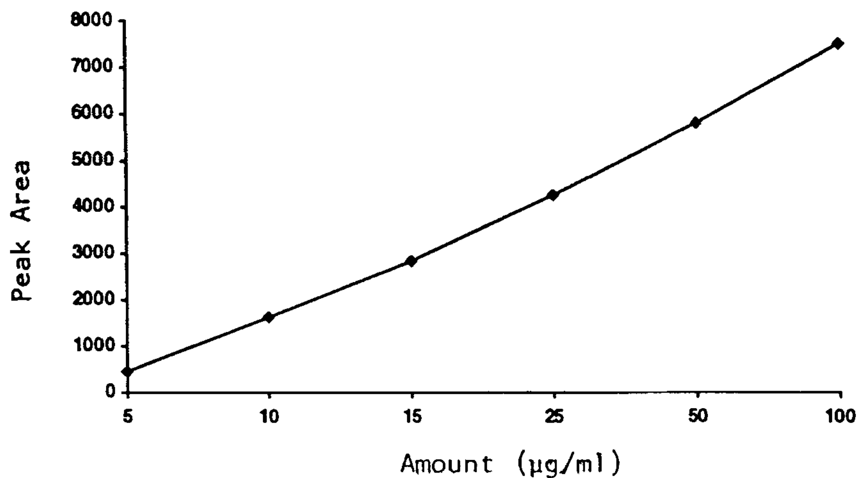

Figure 3. Sample standard curve for the quantitative determination of LL-α-APM in a bulk powder form by HPLC method.

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Table 1

Results of Standard Curves Regression Analysis for LL-α-APM and Degradation Products

Compound	Method	Slope	r
LL-α-APM	HPLC	113.395	0.998
	CZE	0.566	0.998
LL-β-APM	HPLC	117.670	0.999
	CZE	0.568	0.999
α-AP	HPLC	142.206	0.998
	CZE	549.7 + 3.5x	0.998
β-AP	HPLC	96.750	0.998
	CZE	1.067	0.999
DKP	HPLC	142.056	0.997
	CZE	9.929	0.999

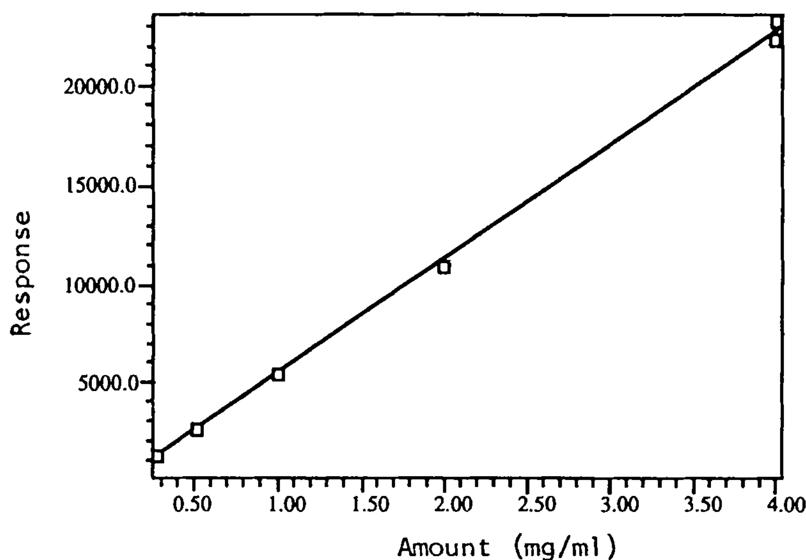

Figure 4. Sample standard curve for the quantitative determination of LL-α-APM in a bulk powder form using capillary zone electrophoresis.

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sample. The analysis did not show any of the other degradation products which were analyzed in our standard samples. On the other hand, the analyses of the coffee samples showed only LL-α-APM with mean recovery of 99.6±1.2 and no degradation products were detected.

The HPLC method appears to have some advantage when compared with CZE method. The limit of detection (the injection amount which give 3 times of the baseline to noise ratio) was lower by a factor of 20, and injections reproducibility was better for all the compounds used in this work, while the separation efficiency (N) of these compounds was higher in CZE method. Both methods gave good linearity. These findings were in agreement with the previous work done by Jimidar et al.[12]

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