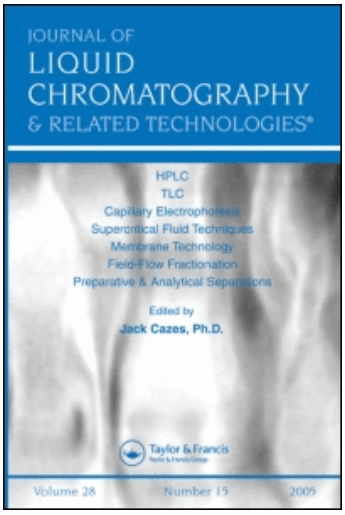

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THE DETERMINATION OF ASPARTAME IN DIET SOFT DRINKS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY*

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ABSTRACT

A simple procedure for the qualitative and quantitative determination of aspartame (Nutrasweet) in diet soft drinks is described. A high performance liquid chromatography method is used which requires a 250 x 4.6 mm β-cyclodextrin bonded silica gel column and a mobile phase of methanol/1% triethyl ammonium acetate (pH 4.5). The effluent was monitored at 214 nm. The method was applied for the analysis of aspartame in diet Coke, diet Pepsi, diet 7-Up and the sweetner Equal. Determination of sodium benzoate caramel coloring and caffeine is also possible by this procedure.

INTRODUCTION

The use of artifical sweetners in soft drinks and food is an established fact. Recently, aspartame, a dipeptide which is the methyl ester of aspartyl-phenylalanine (Nutrasweet), replaced saccharin as the sweetner of choice in diet foods and drinks. It is found among others in diet 7-Up, diet Pepsi, diet Coke, iced tea, lemonade, breakfast cereals, chewing gum

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and desserts. The methods used for the analysis of aspartame in foods and drinks are chromatographic and spectroscopic. Aspartame has been quantified by a fluorimetric method (1), gas chromatography (2), amino acid analysis (3), and high performance liquid chromatography (4-12). The HPLC procedures used strong cation exchange column (8) with a 0.1 M $NH_4H_2PO_4$ solution as the mobile phase or a reversed phase C_{18} bonded column, normally 4.6 x 300 mm column, with either a buffer or a buffer with an organic modifier. For example, while Tsang et al (12) used 0.0125 M KH_2PH_4, pH 3.5/CH_3CN (90:10) as the mobile phase, Webb and Beckman (5) used a solution of water, acetic acid and isopropanol, which was prepared as follows: 10% acetic acid (v/v) buffered to pH 3.0 with saturated sodium acetate solution, modified with 3% isopropanol. Add 100 ml glacial acetic acid to ca 750-800 ml water. Adjust pH to 3.0 with saturated sodium acetate solution. Add 30 ml isopropanol and dilute to 1 L with water. Solution is stable for 2-3 days. Both (5, 12) used the same reversed phase column, μ-Bondapak, to achieve relatively good results with a retention time for aspartame of approximately 7.5 and 15 minutes, respectively.

Recently we developed an HPLC method for the separation of dipeptides and some of their isomers which employs a β-cyclodextrin bonded silica gel column and methanol/H_2O (13).

In this study, the application of the above procedure, with slight modification, to the analysis of aspartame in diet soft drinks was explored. The soft drinks selected for this study were diet Coke, diet 7-Up and diet Pepsi. Also, the analysis for aspartame in the sweetner Equal is described.

EXPERIMENTAL

Materials

The aspartame used in this study was purchased from Aldrich Chemical Co. (Milwakee, WI) and used without further purification. Methanol was glass

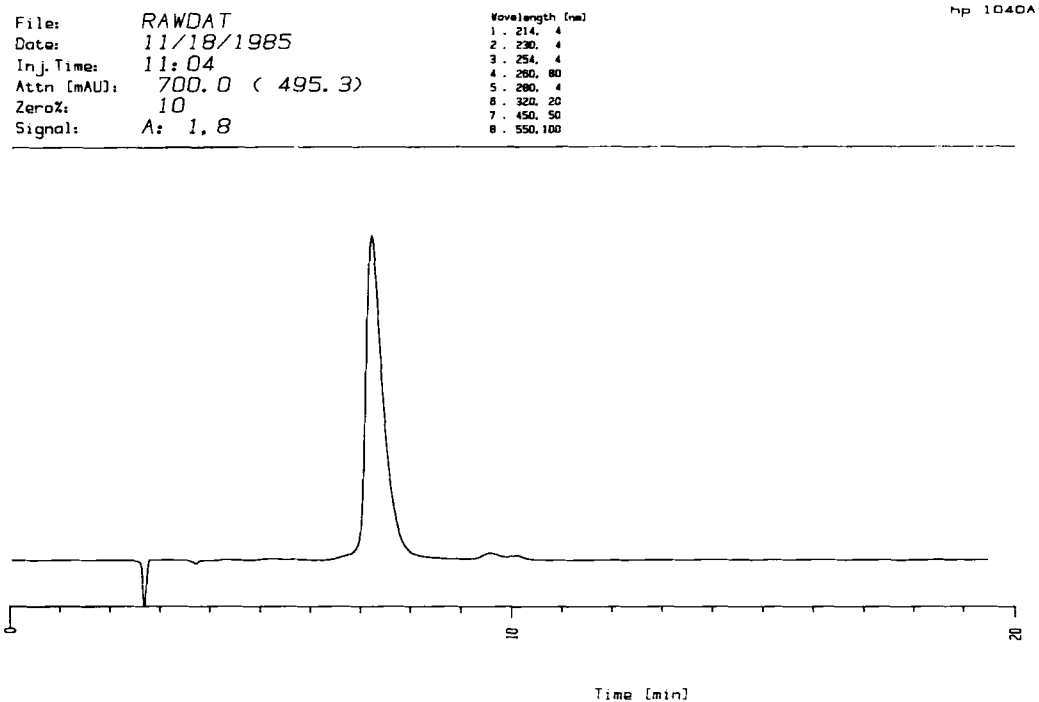
distilled uv grade (Burdick and Jackson, Muskegon, MI). Triethylamine and glacial acetic acid were from Fisher Scientific (Fair Lawn, NJ). The β-cyclodextrin column (Cyclobond I) was purchased from Advanced Separations Technology, Inc. (Whippany, NJ).

Apparatus

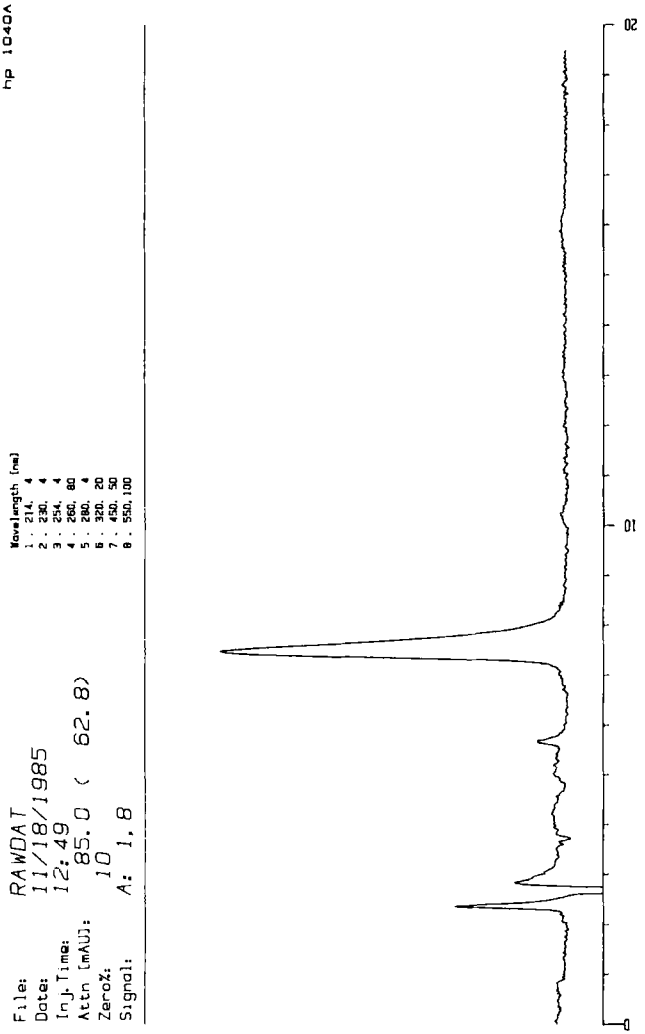
A Hewlett-Packard Model 1090 Liquid Chromatograph equipped with a photo-diode array detector, an automatic injector, a strip chart recorder, a Hewlett-Packard Model 3392A integrator and a Hewlett-Packard Model 85 computer/controller was used. A Cyclobond I column, 250 mm x 4.6 mm, pre-packed with β-cyclodextrin bonded to 5 micron irregular silica gel particles was used. Five µl of solution (Coke, Pepsi, 7-Up, Equal) were injected and the absorption was monitored at 214 nm. The mobile phase was methanol/1% (v/v) triethylammonium acetate pH 4.5. The mobile phase was filtered and degassed before use and maintained under helium throughout the experiment. Pepsi, Coke and 7-Up were degassed before injection, by sonication under vacuum for 15 minutes. Equal was dissolved in 0.1 N HCl and filtered through a 0.2 µm millipore filter.

RESULTS AND DISCUSSION

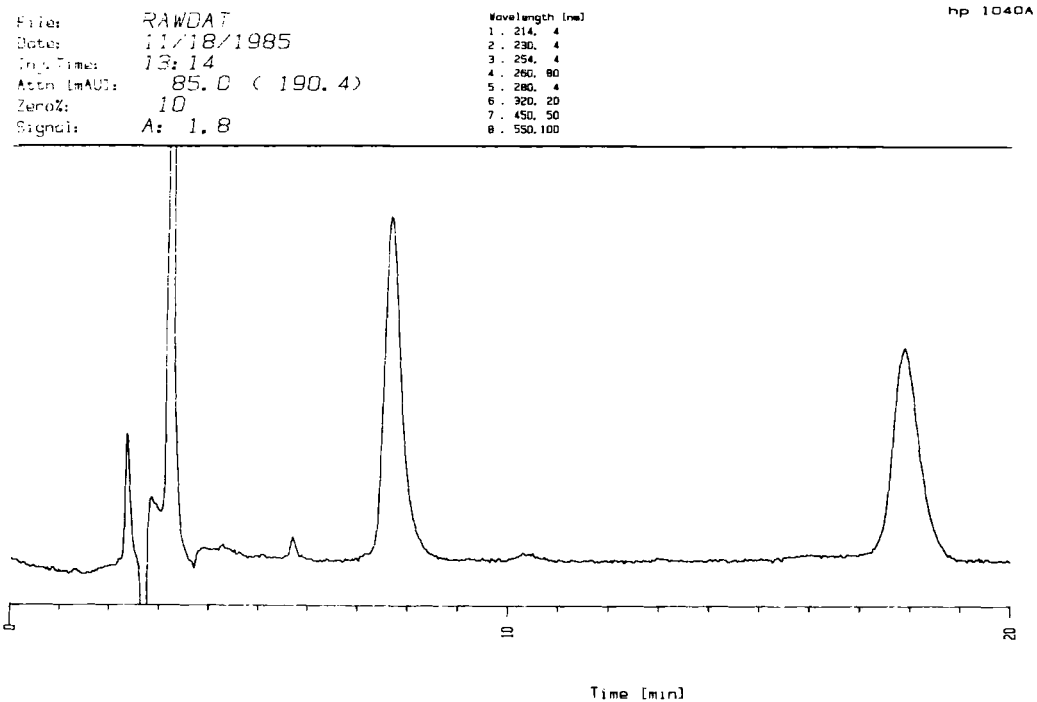
Figure 1 shows the chromatogram obtained by injecting an aqueous solution of the sweetner Equal which contains aspartame. The Rt of aspartame was 7.3 minutes when 25% methanol in 1% triethylammonium acetate pH 4.5 was used as the mobile phase, and a Rt of 5.7 when the volume of methanol was increased to 40%. Figures 2 and 3 are the chromatograms obtained by injecting diet Pepsi free and diet Pepsi, respectively. Note that the peaks at Rt 3.5 and 17.6 minutes which corresponds to caffeine and sodium benzoate were absent from Figure 2, the diet Pepsi free which contains no caffeine and potassium citrate in place of sodium benzoate. Figure 4 is a chromatogram of diet 7-Up using a mobile phase of 40% methanol/triethyl ammonium acetate. Note that only two peaks were observed at 214 nm, those



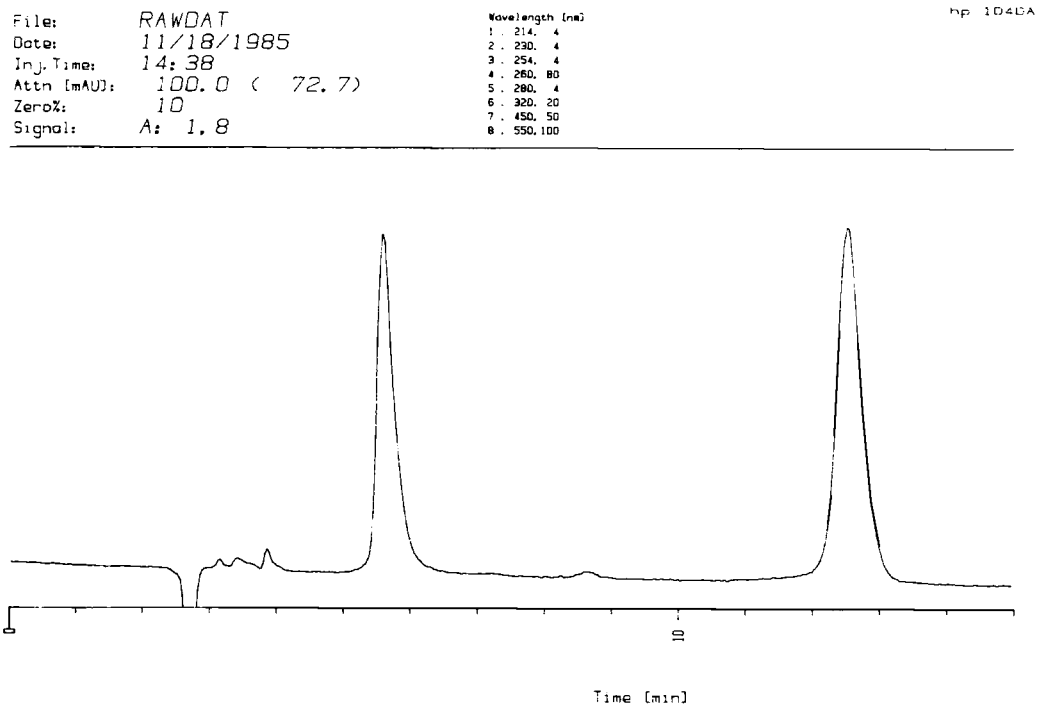
1. Chromatogram of 5 µl solution of the sweetner Equal using a 5 µ Cyclobond I (4.6 x 250 mm) column and a mobile phase of 25% methanol in 1% triethylammonium acetate pH 4.5 at a flow rate of 1 ml/min and detection at 214 nm.



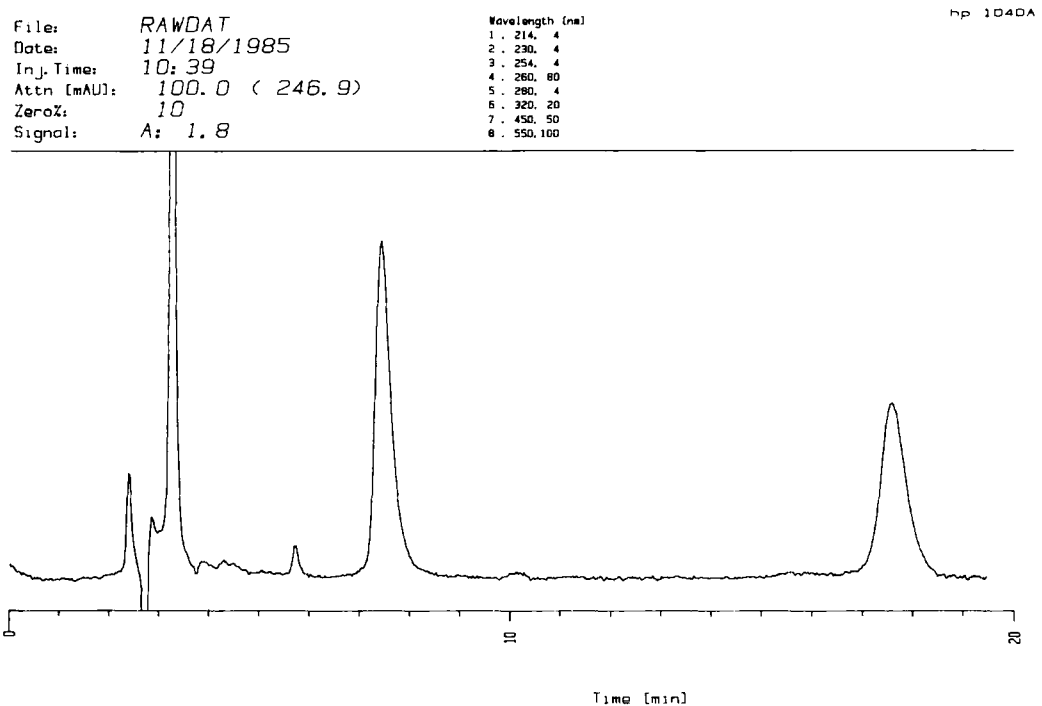
2. Chromatogram of 5 µl of diet Pepsi free. Chromatographic conditions as in Figure 1.



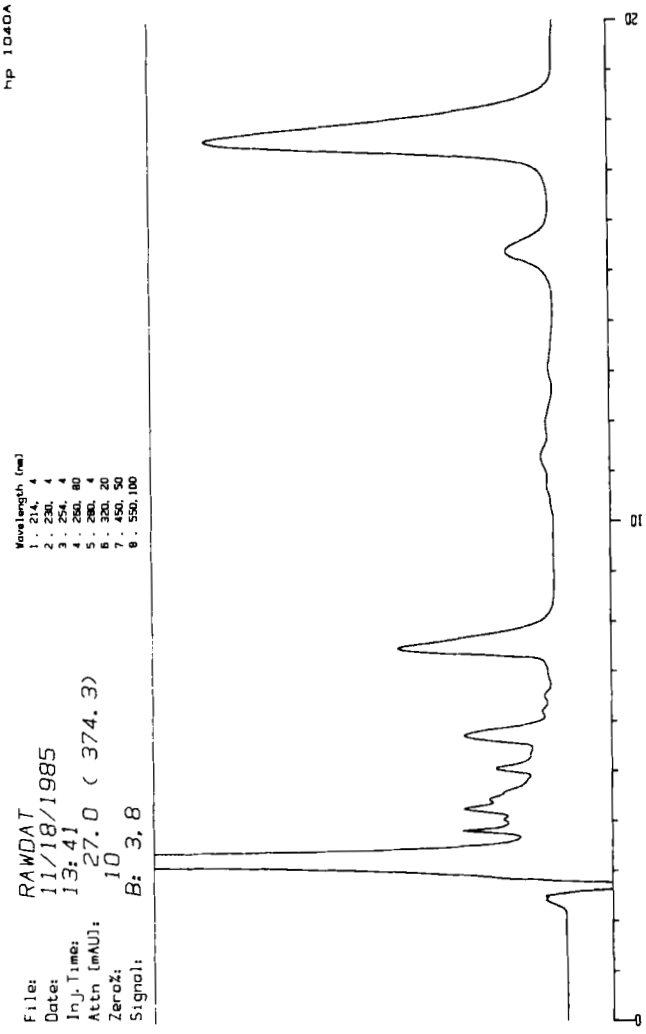
3. Chromatogram of diet Pepsi. Chromatographic conditions as in Figure 1.



4. Chromatogram of diet 7-Up. Chromatographic conditions as in Figure 1.



5. Chromatogram of diet Coke. Chromatographic conditions as in Figure 1.



6. Chromatogram of diet Coke monitored at 254 nm. Other conditions are the same as in Figure 5.

which correspond to aspartame (Rt 5.7) and sodium benzoate (Rt 12.4). Figure 5 is a chromatogram of diet Coke which shows three main peaks when the absorption is monitored at 214 nm. The peaks at 3.5, 7.3 and 17.6 correspond to caffeine, asparatame and sodium benzoate, respectively. When the absorption was monitored at 254 nm (Figure 6), a major peak was observed at 15.1 minutes which corresponds to caramel coloring. In addition, a few small peaks were observed, which were not detected at 214 nm.

The above results show that by using the Cyclobond I column and a mobile phase of methanol/1% triethylammonium acetate at a pH of 4-5, it is possible to analyze for not only aspartame but caffeine, sodium benzoate and caramel coloring. Other components can also be analyzed for. In order to be able to detect all the components in the diet soft drinks, it is recommended that the absorption be monitored at 214 nm (for aspartame, caffeine, and sodium benzoate) and at 254 nm (for caramel coloring and other components). The resolutions obtained, using this simple chromatographic procedure, for carmel coloring, aspartame, caffeine and sodium benzoate exceeded 1.5, which gave baseline separations.

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