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Short communication

Determination of aspartame by high-performance capillary electrophoresis

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Abstract

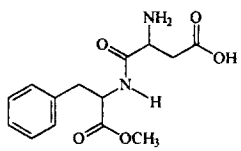
A rapid method for the analysis of the artificial sweetener aspartame in food products using high-performance capillary electrophoresis with a bare capillary, a pH 2.14 buffer and detection at 211 nm has been developed. The analysis time is faster than that reported for HPLC methods. A linear calibration curve between 25 and 150 μg/ml for the analyte solution is established which can be used for quantitative determinations of aspartame in typical food and beverage products. Six commercial samples are analyzed and one diet cola with a known aspartame concentration gives an R.S.D. of 2.6% from the manufacturer's value. No other sample constituents are detected in this high-performance capillary electrophoresis (HPCE) method. © 1997 Elsevier Science B.V.

Keywords: Aspartame; Artificial sweeteners; Food analysis

1. Introduction

Aspartame (N-L-α-aspartyl-L-phenylalanine 1-methyl ester) is one of the most frequently used artificial sweeteners and can be found in a variety of food and pharmaceutical products. The presence of sugar substitutes is increasing because of the demand for more products which can be used in weight control and diabetes management. Therefore, good analytical methods to assure quality control and product integrity are essential in meeting the needs of this growing market. In recent years the use of high-performance liquid chromatography has become the method of choice for aspartame analysis because it is rapid and relatively simple while generally providing good qualitative and quantitative results for many types of samples [1–4]. Another approach is an analysis based on the thermal degradation products provided by pyrolysis–gas chromatography–mass spectrometry [5]. The latter is a more complex and costly method which does not appear to offer any advantages over HPLC. Because of the two types of chromophores in aspartame (an aromatic group and a peptide bond), detection can be readily done in HPLC by UV absorption [1–4]. Another choice with high sensitivity is through electrochemical detection after a post-column photochemical reaction [6]. The typical analysis time by HPLC is about 14 min.

Another technique which might offer some advantages in aspartame analysis is high-performance capillary electrophoresis (HPCE). The structure of aspartame (shown below) is amenable to analysis by HPCE because of its basic nature (the primary amine) which means under acidic conditions the



ASPARTAME

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molecule will be positively charged. Therefore, migration toward the cathode will be facilitated by both electrophoretic mobility and electroosmotic flow. The major complicating factor would be the possible adsorption of the cationic aspartame molecule on the wall (negatively charged from $Si-O^-$ groups) as it migrates through the capillary. The effects of solute adsorption could be minimized by proper choice of pH and buffer. As in HPLC, UV absorption would be the most convenient type of detection. This paper presents an approach to the analysis of aspartame by HPCE which offers some advantages to the HPLC determinations previously reported. The method could provide a fast, convenient and cost-effective alternative for quality control and other analytical measurements which are necessary in food, beverage and pharmaceutical products.

2. Experimental

2.1. Materials

Aspartame was purchased from Aldrich (Milwaukee, WI, USA) and used without further purification as the primary standard. A stock solution was made at 1 mg/ml and working standard solutions for the calibration curve were made at concentrations of 25, 50, 75, 100 and 150 μg/ml. Caffeine (Sigma, St. Louis, MO, USA) was used as received. The pH 2.14 buffer used for all standards and real samples consisted of 30 m*M* phosphate (phosphoric acid) and 19 m*M* Tris. The working buffer was prepared by a 10-fold dilution of a stock solution. The food and beverage samples were obtained from local supermarkets and were analyzed as purchased. Water for the buffer was prepared on a Milli-Q apparatus (Millipore, Milford, MA, USA).

2.2. Instrumentation

All HPCE experiments were done on a Perkin–Elmer/Applied Biosystems Model 270A-HT capillary electrophoresis system at 30°C. Injections were made in both the hydrodynamic and electrokinetic modes. Detection was at 211 nm. The buffer and samples were first sonicated and then degassed with helium.

2.3. Sample preparation

The working standard solutions of aspartame were prepared by placing measured volumes of stock solution and Milli-Q water (sum of stock+water=1 ml) in a 5 ml volumetric flask and diluting to the mark with buffer. A typical preparation of a real liquid sample that would work for all the products tested in this study is as follows: add 600 μl of sample and 400 μl of Milli-Q water to a 5 ml volumetric flask and dilute to the mark with buffer. A typical preparation of a solid sample that would work for the two products tested in this study is as follows: dissolve 250 mg of solid in 10 ml of water, then place 500 μl of this solution plus 500 μl of water in a 5 ml volumetric flask and dilute to the mark with buffer. Other samples may require a different procedure in order to make the analyte solution compatible with the calibration curve developed.

The capillary (Polymicro Technologies, Phoenix, AZ, USA) was conditioned at the beginning of each day with a 5 min rinse using 0.10 *M* NaOH, followed by a 5 min rinse with Milli-Q water and finally a 5 min rinse with the buffer. After one sample run, the column was rinsed for 2 min with the running buffer. After the second sample run, the column was rinsed using the following protocol: 2 min with 0.10 *M* NaOH; 2 min with Milli-Q water and 2 min with the running buffer.

3. Results and discussion

In order to make HPCE competitive with HPLC for the determination of aspartame in real commercial products, the sample preparation must be uncomplicated and the analysis time must be comparable or better. In a recent HPLC study [5], liquid samples were simply diluted in water and the elution time for aspartame under the optimum eluent conditions in the reversed-phase mode with a C_{18} column was approximately 14 min. In this study liquid samples were diluted to the appropriate concentration range with water and buffer; certainly similar in simplicity to the HPLC method. For solid samples, both the HPLC method [5] and the HPCE analysis described in this study require that the

requisite amount of product material be dissolved directly in water for the chromatographic procedure and in buffer for the capillary electrophoresis determination. Therefore, it can be concluded that sample preparation for both analytical methods is comparable. With respect to analysis time, a typical electropherogram for aspartame is shown in Fig. 1a. Under the buffer and voltage conditions selected, the analysis time of less than 4 min is considerably faster than that obtained in the HPLC method.

Some consideration must be given to the process of selecting or optimizing the experimental conditions in order to achieve the results reported in Fig. 1a. First, it is known that as the pH is raised the number of ionized silanol groups on the capillary wall increases. This would lead to a greater likelihood of solute adsorption due to attraction between the $Si-O^-$ group on the capillary surface and the cationic analyte. Therefore a relatively low pH of 2.14 was chosen so that virtually all of the silanols are not ionized [7]. Since the aspartame is most probably singly charged at this pH, migration through the column is primarily by electrophoretic mobility with only a very small contribution by the very low electroosmotic flow. This situation does not lead to an unnecessarily long migration time. On the contrary, the migration time observed (<4 min) is very fast compared with HPLC elution. Another factor in the analysis time is the applied voltage. In this case, the maximum voltage available (30 kV)

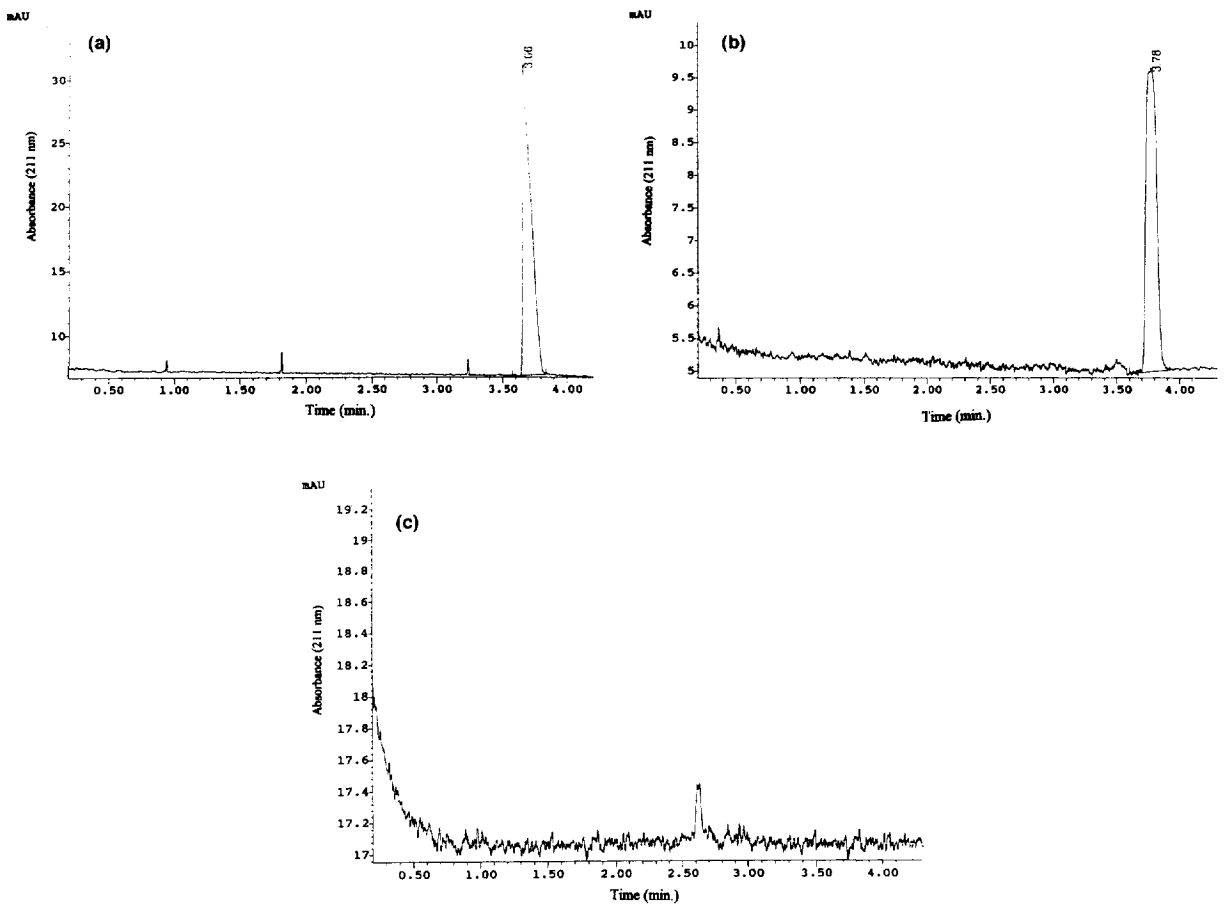

Fig. 1. Electropherograms for various aspartame samples: (a) aspartame standard at 50 μg/ml; (b) diet cola sample (diluted 1:10) ≅51 μg/ml; (c) caffeine at 150 μg/ml. Conditions: 50 μm I.D. bare capillary, pH 2.14 buffer, 30 kV applied voltage, detection at 211 nm and injection for 3 s at 12.5 cm Hg vacuum (1 mmHg=133.322 Pa), L=44 cm, l=25 cm.

was used in order to minimize the migration time. In contrast to HPLC where peak broadening occurs at higher flow rates, there is very little loss in efficiency in HPCE when migration through the capillary is increased at higher applied voltages. Some degradation in peak width can occur due to Joule heating but this is expected to be minimal in a 50 μm capillary at 30 kV. Finally, it is necessary to have sufficient sensitivity so that those products at the lowest aspartame concentrations in liquids and smallest amounts in solids can be readily analyzed. Because of the presence of the peptide bond and an aromatic group in aspartame, detection near 250 or 210 nm is possible. From comparisons between these wavelength regions for the same sample, it was determined that detection at 211 nm provided the best sensitivity. One HPLC study reported detection at 230 nm [4].

To test the viability of this method on a real sample, a diet cola of known aspartame concentration was diluted and prepared according to the procedure described above so that its concentration in the analyte solution was about 50 μg/ml. The electropherogram of this cola sample is shown in Fig. 1b and can be compared to the aspartame sample of approximately the same concentration shown in Fig. 1a. One aspect to note about the electropherogram in Fig. 1b is the absence of any other peaks which could be associated with other constituents in the product. Of particular interest is caffeine, which can be present in colas and has been shown to be a potential problem in HPLC analysis [5]. In order to determine if caffeine is an unresolved component in cola analysis, a sample at a concentration of 150 μg/ml was run under identical experimental conditions. As shown in Fig. 1c, caffeine gives a relatively small peak near 2.5 min. Therefore, caffeine (λ_{max}=235 nm) is not an interference in aspartame analysis by HPCE because of the large differences in migration times and detection sensitivity at 211 nm. It is not surprising then that no caffeine peak is seen in the diet cola electropherogram because its concentration in this sample as provided by the manufacturer is approximately one-fourth that of aspartame.

Another option available in the HPCE method is the choice of injection mode. The electropherograms shown in Fig. 1 were all obtained with hydrodynamic injection. However, electrokinetic injection is also possible. The most important aspect of aspartame analysis by HPCE is an assessment of the viability of quantitative determinations. Fig. 2 is the calibration curve obtained for both hydrodynamic injection and electrokinetic injection over the concentration range of 25 to 150 μg/ml. The correlation coefficients are high in both cases, 0.99 and 0.98, respectively, indicating a good linear fit. Multiple determinations (n=10) of the same standard (50 μg/ml) gave an R.S.D. of ±2.6%. The concentrations used for the plot shown in Fig. 3 approxi-

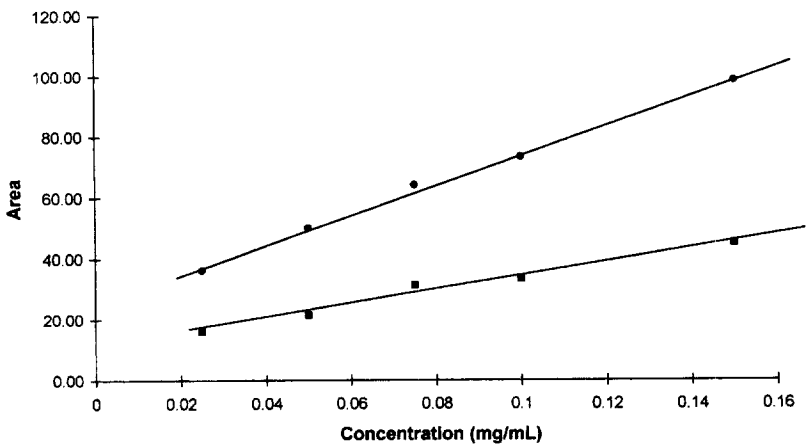

Fig. 2. Calibration curves for aspartame analysis with both hydrodynamic and electrokinetic injection. (●) Injection 3 s, 12 cm Hg vacuum; (■) 8 s, 10 kV; r=0.998 and 0.989, respectively.

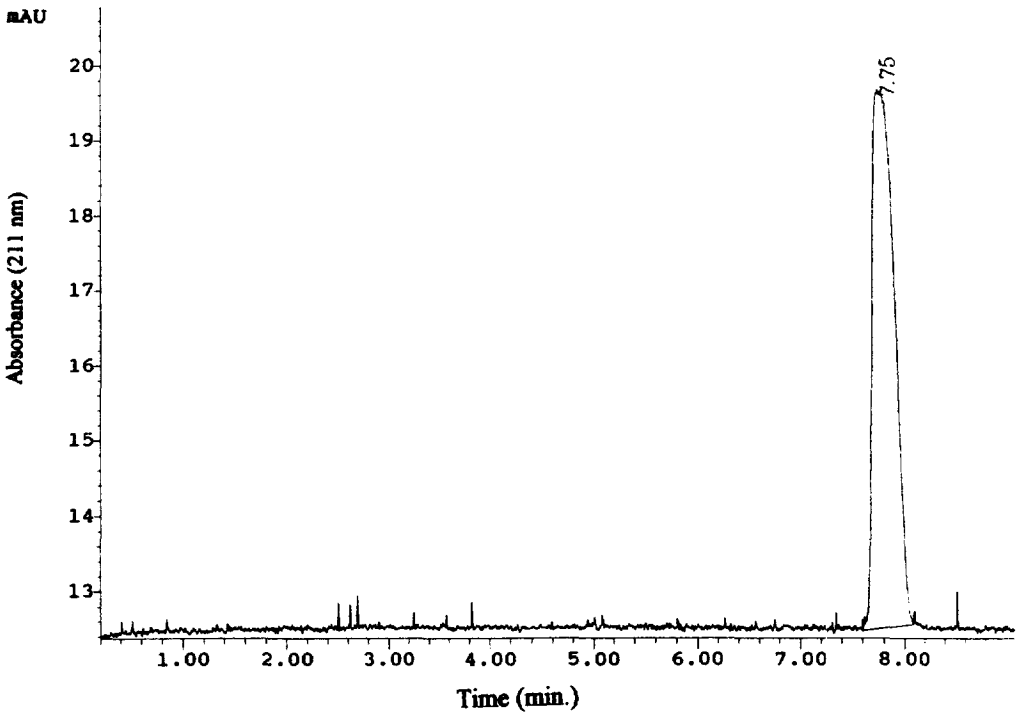

Fig. 3. Electrochromatogram of 50 μg/ml aspartame standard using a diol modified etched capillary at 25 kV applied voltage. All other conditions are the same as in Fig. 1.

mately encompass the complete linear range. Below 25 μg/ml the response is lower than expected. This may be due to some adsorption of the analyte on the capillary walls even though at pH 2.14 very few unprotonated silanols remain. As indicated in the procedure above, the column was flushed with 0.10 *M* NaOH after every two runs to make the surface more uniform for each sample. With this procedure, retention times were reproducible to about ±3%. Without the NaOH wash retention was observed to slowly decrease, which would agree with the assumption that a small amount of analyte adsorption is occurring on the capillary wall. Above 150 μg/ml column overload is evident for hydrodynamic injection as indicated by nonsymmetrical peaks (results not shown) and thus the calibration curve is no longer linear beyond this point.

The results for the quantitative determination of aspartame in six commercial products are shown in Table 1. The aspartame peak was easily identified in all the samples and like the electropherogram for the diet cola shown in Fig. 1b, no other constituents were detected during the run time for any of the products. The agreement between the experimentally measured amount of aspartame and that reported by the manufacturer for one type of diet cola is very good (2.7%). This value is better than the HPLC determination (R.S.D.=7.0%) for a comparable beverage in the same concentration range and approaches the best agreement obtained on any product (R.S.D.=1.9%) in that study [5]. The linear range determined in the calibration curve is quite compatible with at least the six commercial products tested

Table 1
Aspartame analysis of commercial products

Sample	Amount found
Diet cola A	507 mg/l[a]
Diet cola B	426 mg/l
Diet ginger ale	207 mg/l
Diet iced tea	143 mg/l
Solid sweetener	3.6% (w/w)
Diet dessert	2.6% (w/w)

Reported by manufacturer 521 mg/l, R.S.D.=2.7%.

which are presumably representative of the concentrations encountered in other food or pharmaceutical samples. All liquid samples required dilution and relatively small amounts of material were needed for the solid samples.

Another approach which was tested for the analysis of aspartame utilized capillary electrochromatography (CEC). The format of CEC chosen was a 50 μm capillary whose inner walls have been etched and subsequently modified [8–10] in this case by attachment of a diol moiety, 7-octene-1,2-diol. A typical electrochromatogram is shown in Fig. 3. It can be seen that the peak shape is very good for aspartame, and the elution time under the same buffer conditions used for CE, but at 25 kV, is just under 8 min. The lower applied voltage was necessary because at 30 kV the baseline was not stable. The analysis time is longer than the CE method but still shorter than in the HPLC method. However, while qualitative measurements are possible under these conditions peak area determinations were not reproducible enough to construct a good linear calibration curve in the same concentration range used for the CE experiments. Because the etched surface consisted of a bonded organic group, it was not possible to wash the capillary regularly with 0.10 *M* NaOH. Washing with methanol lessened the variation in peak area but still did not result in an acceptable calibration curve. Further testing is underway on the diol and other chemically modified etched capillaries as well as packed capillaries for the analysis of aspartame and other basic compounds by CEC.

4. Conclusions

HPCE appears to be a viable method for the determination of aspartame in real commercial products. The analysis time is significantly faster than that reported for HPLC methods and no interferences were detected in the samples tested. Caffeine has a different migration time from aspartame and its low sensitivity at the detection wavelength precludes the appearance of a peak in the electropherogram. The linear calibration curve developed is compatible with the range of aspartame concentrations or amounts in the products tested. It appears that a small amount of aspartame adsorption occurs on the capillary walls, but this effects quantitative determinations only well below the useful range for typical commercial samples.

Acknowledgments

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