ELSEVIER

Journal of Chromatography A, 729 (1996) 393–398

JOURNAL OF CHROMATOGRAPHY A

High-performance liquid chromatography with electrochemical detection of aspartame with a post-column photochemical reactor

Guido C. Galletti[a,*], Paola Bocchini[b]

[a]*Istituto di Microbiologia e Tecnologia Agraria e Forestale, Università di Reggio Calabria, p.zza S. Francesco 4, I-89061 Gallina (RC), Italy*

[b]*Centro di Studio per la Conservazione dei Foraggi, Consiglio Nazionale delle Ricerche, via F. Re 8, I-40126 Bologna, Italy*

Abstract

Aspartame (N-L-α-aspartyl-L-phenylalanine 1-methyl ester) was determined by high-performance liquid chromatography with electrochemical detection. Aspartame, which is electrochemically inactive, was made oxidizable in the range 0.1–1.1 V after post-column irradiation at 254 nm. A detection limit of 0.5 mg/l (signal-to-noise ratio 3:1) was attained using a coulometric detector with the working cell set at 0.8 V and a C_6 column (150 × 4.6 mm I.D.) operated under isocratic conditions with 0.1% perchloric acid–methanol (85:15, v/v) as the eluent at a flow-rate of 1 ml/min. A linear response for aqueous solutions of aspartame in the range 1–20 mg/l and a 5% standard deviation for five replicate injections were obtained. The method was applied to the determination of aspartame in two diet colas and a pharmaceutical product. Chromatograms and a hydrodynamic voltammogram are shown.

Keywords: Post-column reactors; Electrochemical detector; Detectors, LC; Photochemical reactor; Soft drinks; Artificial sweeteners; Aspartame

1. Introduction

High-performance liquid chromatography (HPLC) with UV detection is the primary tool for the determination of aspartame (N-L-α-aspartyl-L-phenylalanine 1-methyl ester), the best known of the artificial sweeteners [1–4]. Such a molecule is used as a sugar substitute in many dietary and pharmaceutical products addressed to consumers who want to avoid sugar. Recently, we have studied the thermal degradation products of aspartame by pyrolysis–gas chromatography–mass spectrometry, a technique which might be useful for analytical purposes [5].

Aromatic and sulphur-containing amino acids, peptides and proteins have been determined by HPLC with electrochemical detection after post-column photolytic derivatization (HPLC–$h\nu$–ED) [6]. By continuous, on-line UV irradiation, non-electrochemically active amino acids such as phenylalanine have shown an oxidative current [6]. Consequently, the selectivity and sensitivity of electrochemical detection, as well as the possible differences in lamp on/lamp off chromatograms, can be exploited for such a particular class of amino acids [6].

* Corresponding author.

0021-9673/96/$15.00
SSDI 0021-9673(95)00901-9

Aspartame is a dipeptide methyl ester containing the aromatic phenylalanine moiety (Fig. 1). It was reasonable to expect that post-column UV irradiation could be exploited to make this molecule electrochemically detectable, in spite of the lack of typical oxidizable aromatic substituents. However, to our knowledge, no paper has been published on the HPLC determination of aspartame by electrochemical detection after post-column photochemical reaction.

This present paper reports on the HPLC–$h\nu$–ED determination of aspartame. Lamp on/lamp off chromatograms, hydrodynamic voltammograms, the detection limit and the quantitative results of the determination of aspartame in three commercial products are provided. Although aspartame is routinely determined using UV detection, introducing the use of electrochemical detection after a photochemical reaction is important because it offers a further, unequivocal identification tool and extends the range of applications of such a detector.

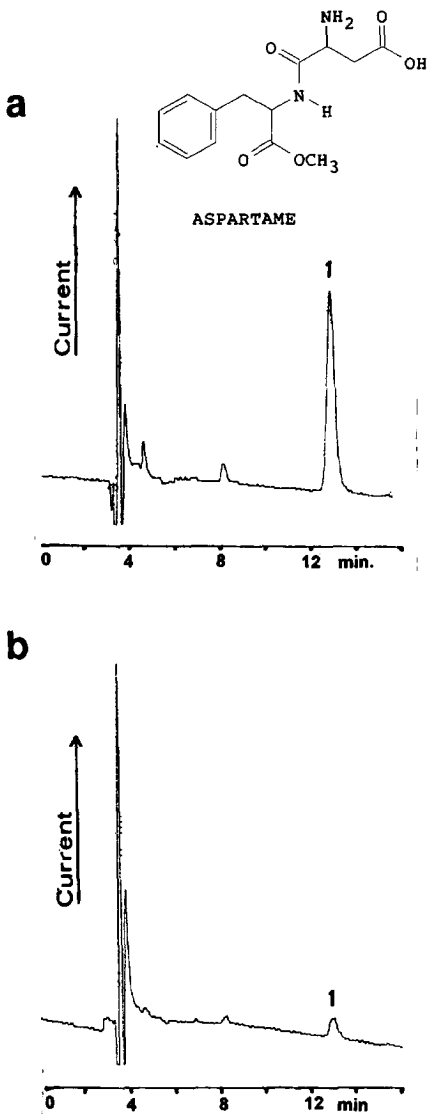

Fig. 1. HPLC–$h\nu$–ED of standard aspartame at (a) 10 and (b) 1 mg/l. Peak 1 = aspartame. Detector sensitivity full-scale, 10 μA; chromatogram full-scale, 300 nA.

2. Experimental

2.1. HPLC conditions

The liquid chromatographic system consisted of the following components connected in series: a Waters (Milford, MA, USA) Model 590 pump, a Rheodyne (Cotati, CA, USA) Model 7725i injector, a reversed-phase column (150 × 4.6 mm I.D., 5-μm particle size) of Spherisorb Hexyl (Phase Separation, Clwyd, UK), an ICT Beam Boost (ICT, Frankfurt, Germany) D-6808 photochemical reactor unit equipped with a 254-nm UV lamp and a 20 m × 0.3 mm I.D. Teflon reaction coil and an ESA (Bedford, MA, USA) Coulochem Model 5100A electrochemical detector.

The column was operated isocratically with methanol–0.1% perchloric acid (15:85, v/v) (corresponding to pH 2.8) at a flow-rate of 1 ml/min, corresponding to 120 s of irradiation in the photochemical reaction unit. Before use, the mobile phase was filtered through a 0.22-μm filter under water pump suction. Unless specified otherwise, the electrochemical detector was operated with the first electrode (V_1) set at +0.10 V acting in a screen mode, all the determinations being made on the second electrode (V_2) set at +0.80 V.

2.2. Standard aspartame

Pure aspartame was purchased from Sigma (Milan, Italy). A stock standard solution (100 mg/l) was prepared by dissolving 5 mg of aspartame in 50 ml of deionized water previously filtered through a 0.45-μm filter. Working standard solutions containing 0.5, 1, 5, 10 and 20 mg/l of aspartame were prepared by appropriate dilutions of aliquots of the stock standard solution in water.

2.2. Real samples

The following two dietary soft drinks and one pharmaceutical product were analysed: (a) a diet cola (label indicating 20 mg of aspartame per 100 ml of product); (b) a light cola of a different brand (label indicating 58 mg of aspartame per 100 ml of product); and (c) a pharmaceutical product for bronchitis containing 25 mg of aspartame per dose. The products were diluted with water as follows: (a) 5 ml to 100 ml; (b) 1 ml to 20 ml; and (c) 1 dose in 1 l of water. Hence the theoretical (according to the label figures) aspartame concentrations obtained for each product were (a) 10, (b) 29 and (c) 25 mg/l.

3. Results and discussion

3.1. Method set-up

Initially, the mobile phase conditions used by Dou and Krull [6] to determine peptides by HPLC–$h\nu$–ED, namely methanol–phosphate buffer (pH 6.8) (1:99, v/v), and those of Di Pietra et al. [3] for aspartame and other artificial sweeteners and decomposition products, i.e., methanol–0.08 *M* triethylammonium phosphate (pH 3) (35:65), were compared with the mobile phase described in the Experimental section, i.e., aqueous perchloric acid–methanol. We adopted the latter mobile phase with appropriate modifications of the relative ratios for other HPLC separations, including phenolic and sulphonylurea analyses [7,8]. For the present work, it was chosen because it offered the lowest background current at the electrochemical detector with inherent baseline stability.

For aspartame analysis, the present mobile phase kept the retention time within 15 min whilst at the same time allowing its separation from caffeine, a compound present in some cola soft drinks which might interfere (see below). The retention time of aspartame was dependent on the perchloric acid concentration, increasing with diminishing acid concentration. The linear regression equation for $y = \log[(t - t_0)/t_0]$ versus $x = \mathrm{pH}$ (0.1, 0.05 and 0.01% perchloric acid concentrations in water, corresponding to pH 2.8, 3.1 and 3.8, respectively, were tested) was $y = 0.2487x$, with a linear correlation coefficient of 0.99.

Injections of aspartame solutions (160 mg/l) gave no signal at the electrochemical detector (V_2 in the range +0.75 to 1.20 V) when the photochemical reactor was off, whereas a regular response was observed at the UV detector set at 220 nm (chromatogram not shown). This is in accord with the behaviour of phenylalanine as tested by Dou and Krull [6], which was not oxidizable up to an applied potential of 1.20 V. When the photochemical reactor was turned on, aspartame responded at the electrochemical detector with a symmetrical peak (see Fig. 1 for the response of 1 and 10 mg/l aspartame solutions).

A hydrodynamic voltammogram was recorded in order to check both the electrochemical behaviour of the irradiated aspartame and the optimum voltage for its detection (Fig. 2). Aspartame (16 mg/l) was injected at V_2 voltages in the range 0 to +1.2 V. V_1 was kept at +0.02 V. A two-step curve was obtained with a first plateau from +0.2 to +0.7 V and a second plateau in the range +0.1 to 1.1 V. This behaviour is consistent with a plateau observed for phenylalanine [6]. Higher potentials could not be explored because of the mobile phase oxidation. It is apparent that two sites of the irradiated molecule with different oxidation potentials are responsible for the two-step hydrodynamic voltammogram. However, we are unable to indicate which moieties of the molecule have electrochemical activity. So far, it

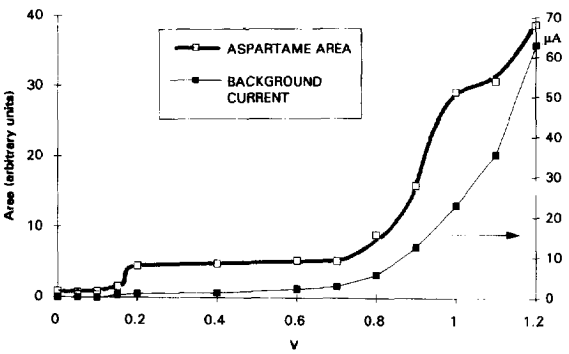

Fig. 2. Hydrodynamic voltammogram of aspartame and background current recorded by HPLC–$h\nu$–ED.

is unknown which molecule is responsible for such a dramatic change in redox properties. It is apparent that the excited state reached by aspartame during the irradiation (ca. 120 s according to the manufacturer at a mobile phase flow-rate of 1 ml/min for an irradiation coil of 20 m × 0.3 mm I.D. at room temperature) yields a product with different redox properties. A possibility is that aspartame is converted into a diketopiperazine, namely 5-benzyl-3,6-dioxo-2-piperazineacetic acid, a well known decomposition product of aspartame under certain conditions of temperature and pH [1]. By analogy with the behaviour of phenylalanine, it may be inferred that, whichever species is involved in the oxidation, it should contain the phenylalanine moiety. However, it is difficult to explain the basic chemistry of such a reaction, owing to the difficulty in identifying the reaction products. Therefore, all the above interpretations must be considered pure speculations. Further experiments by HPLC–electrospray mass spectrometry are in progress in an attempt to identify the products of aspartame irradiation.

A V_2 potential of +0.8 was used throughout the rest of the experiments because it maximized the signal-to-noise ratio and was a relatively low potential, thus maximizing the selectivity of the detection. Under such conditions, five replicate injections of a 20 mg/l aspartame solution yielded a relative standard deviation of 5.3%, the detection limit (signal-to-noise ratio 3:1) was 0.5 mg/l and the response was linear in the concentration range 1–20 mg/l with a linear regression equation of $y = 48.45 \cdot 10^3 x$, where y = area in arbitrary units and x concentration in mg/l, with a linear correlation coefficient of 1.00.

3.2. Real sample analysis

The chromatograms obtained by HPLC–$h\nu$–ED of the three commercial products containing aspartame are shown in Fig. 3. In general, the concentrations of aspartame declared by the manufacturer are in the tens or hundreds of mg/l so that a detection limit problem never occurred, but it was necessary to dilute the original sample. The selectivity of the conditions with the photochemical reactor on/off (Fig. 3a) can be used to confirm the identification of aspartame. Moreover, caffeine (peak 2 in Fig. 3, identified by injection of the pure compound) was a small peak compared with aspartame whereas in the corresponding UV profile it was the major peak in the aspartame–caffeine doublet. This is due to the fact that caffeine is electrochemically active, but the working potential suggested for such a compound is +0.95 V [9]. While a reduced response of caffeine in the HPLC–$h\nu$–ED system is useful to overcome possible problems in the separation and quantification of aspartame, the comparison of the different characteristics of the ED and UV chromatograms could be of further help in assigning the peaks.

Table 1 summarizes the quantitative results obtained by comparing the aspartame areas in the samples with the calibration graph (three replicate injections). The relative standard deviations ranged from about 2% for sample A (a diet cola) to 9% for sample C (the pharmaceutical product). Compared with the values declared on the label, acceptable agreement was obtained for all samples.

4. Conclusion

The use of a post-column photochemical reactor in HPLC allowed electrochemical detection to be extended to the unequivocal identification of the artificial sweetener aspartame, a molecule

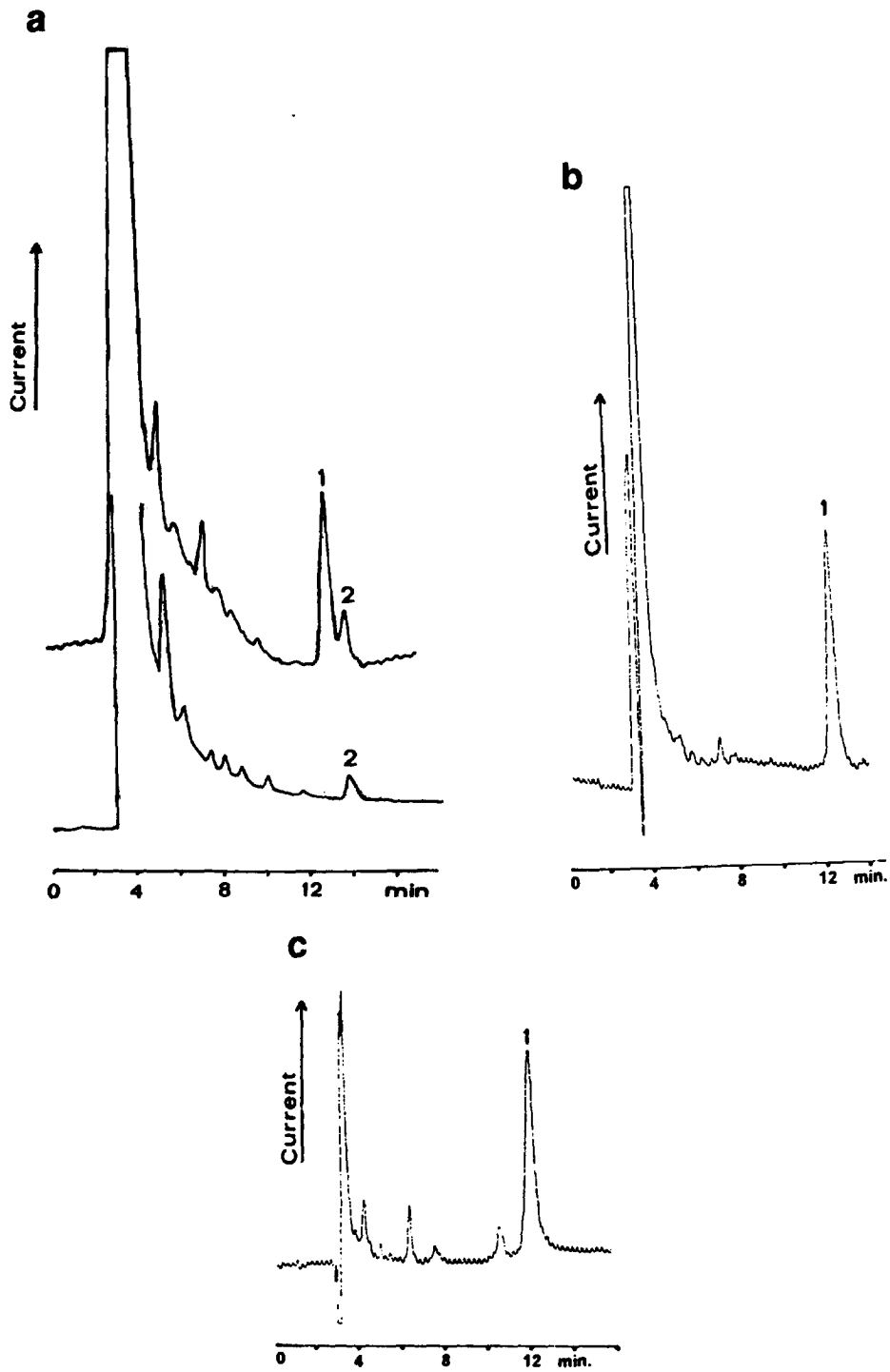

Fig. 3. HPLC–$h\nu$–ED (0.80 V) of (a) a diet cola [photochemical reactor on (upper trace) and off (lower trace)], (b) a diet cola of different brand and (c) a pharmaceutical product. Peaks: 1 = aspartame; 2 = caffeine; other peaks are unknown. Detector sensitivity full-scale, 10 μA; chromatogram full-scale, 300 nA.

Table 1
Quantitative results of triplicate HPLC–$h\nu$–ED determination of aspartame in three commercial products: (A) and (B) diet colas and (C) a pharmaceutical product

Sample	Label concentration (mg/l)	Found (mg/l)	R.S.D. (%)
A	200	202	1.9
B	580	540	7.0
C	25[a]	30	8.0

[a] Declared as mg per dose, which was dissolved in 1 l of water.

so far determined essentially by UV detection. While this finding would be interesting per se, the selectivity of both electrochemical detection and photochemical reactor on/off conditions are additional tools for the identification of aspartame and the improvement of possibly critical separations such as that of aspartame from caffeine. The linearity and repeatability of the whole HPLC–$h\nu$–ED system are suitable for the detection of aspartame in commercial products.

References

[1] J. Prodolliet and M. Bruelhart, J. Assoc. Off. Anal. Chem., 76 (1993) 275.
[2] B.A. Bidlingmeyer, J. Chem. Educ., 68 (1991) A195.
[3] A.M. Di Pietra, V. Cavrini, D. Bonazzi and L. Benfenati, Chromatographia, 30 (1990) 215.
[4] J.F. Lawrence and C. Charbonneau, J. Assoc. Off. Anal. Chem., 71 (1988) 934.
[5] G.C. Galletti, G. Chiavari and P. Bocchini, J. Anal. Appl. Pyrol., 32 (1995) 137.
[6] L. Dou and I.S. Krull, Anal. Chem., 62 (1990) 2599.
[7] G.C. Galletti, R. Piccaglia and V. Concialini, J. Chromatogr., 507 (1990) 439.
[8] G.C. Galletti, A. Bonetti and G. Dinelli, J. Chromatogr., A, 692 (1995) 27.
[9] The Model 5100A Coulochem Detector, ESA, Bedford, MA, Rev. 87/02, Section 5, p. 6.