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ION-PAIR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF ASPARTAME AND RELATED PRODUCTS

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SUMMARY

A simple and accurate quantitative determination of aspartame (L-α-aspartyl-L-phenylalanine methyl ester), a new artificial sweetener, is described. The method, which is based on ion-pair high-performance liquid chromatography, allows the determination of aspartame in finished bulk and dosage forms, and the detection of a few related products at levels down to 0.1%.

INTRODUCTION

Aspartame (I) is a new synthetic sweetener[1], which is now very widely used all over the world and is obtained by synthesis from two amino acids, L-phenylalanine and L-aspartic acid.

Several workers have described high-performance liquid chromatographic (HPLC) methods[2–6] for aspartame, with different procedures for the complete analysis of related products. In a previous paper[7] we described a very rapid and efficient HPLC procedure for the determination of I in the finished bulk form and of some of the more common related products (III, VI, VII, VIII and IX) (see Scheme I).

In order to extend the quantitative analysis of I to commercial formulated products, such as table-top sweeteners, and to gain further knowledge about related impurities obtained during the synthesis, we have investigated a new method, which allows the complete separation of I from nine related compounds and its determination in dosage forms. It also makes it possible to detect potential impurities in final products at levels as low as 0.1%. The method is based on ion-pair formation[8] associated with reversed-phase chromatography. As the retention time of ionized components is increased, the selectivity of the chromatographic column is enhanced and this method is useful alternative to ion-exchange chromatography.

EXPERIMENTAL

Materials

Standards of aspartame and all aspartame derivatives (III–IX) were obtained from our Chemical Development Laboratories and tested for purity by HPLC, IR, PMR and elemental analyses. They were found to be not less than 99.0% pure on

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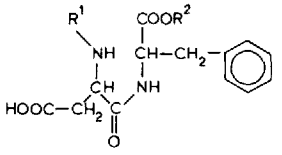
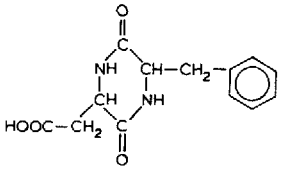


$R^1 = H; R^2 = CH_3$ (I)

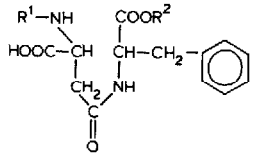
$R^1 = CHO; R^2 = H$ L-α-[(N-Formyl)aspartyl]-L-phenylalanine (IV)

$R^1 = H; R^2 = H$ L-α-Aspartyl-L-phenylalanine (VI)

$R^1 = CHO; R^2 = CH_3$ L-α-[(N-Formyl)aspartyl]-L-phenylalanine methyl ester (VIII)



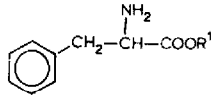
5-Benzyl-3,6-dioxo-2-piperazineacetic acid (III)



$R^1 = H; R^2 = CH_3$ L-β-Aspartyl-L-phenylalanine methyl ester (IX)

$R^1 = H; R^2 = H$ L-β-Aspartyl-L-phenylalanine (V)

$R^1 = CHO; R^2 = CH_3$ L-β-[(N-Formyl)aspartyl]-L-phenylalanine methyl ester (VII)



$R^1 = H$ L-Phenylalanine (II)

$R^1 = CH_3$ L-Phenylalanine methyl ester (X)

Scheme I.

a dry basis. L-Phenylalanine (II), L-phenylalanine methyl ester (X) and sodium hexanesulphonate were of analytical-reagent grade from Fluka (Buchs, Switzerland). Potassium monohydrogen phosphate, 85% phosphoric acid and acetonitrile (LiChrosolv) were obtained from Merck (Darmstadt, F.R.G.). Table-top sweeteners, such as Aspartina and Mini D, were Pierrel's and Vantaggio was obtained from Chiari and Forti (Treviso, Italy).

Apparatus

A Perkin-Elmer Series 3B high-performance liquid chromatograph, equipped with an LC-75 variable-wavelength UV detector, operating at 220 nm, a Series Σ 10 electronic calculating system and a 20-μl loop-valve automatic injector from Rheodyne (Berkeley, CA, U.S.A.) were used. The analytical column was a pre-packed RP-8, 10 μm, 250 × 4 mm I.D. column (Hibar-RP8) from Merck, kept at room temperature.

Mobile phase

Eluent A was prepared by dissolving in 1 l of distilled water 2.062 g of sodium hexanesulphonate monohydrate and 0.450 g of anhydrous potassium monohydrogen phosphate. The pH of the solution was then adjusted to 2.5 with dilute phosphoric acid.

Eluent B was prepared by dissolving sodium hexanesulphonate monohydrate (2.062 g) in 1 l of water–acetonitrile (2:3); the apparent pH was then adjusted to 3.0 with dilute phosphoric acid.

The eluents were filtered through a 0.45-μm membrane and degassed by applying a moderate vacuum in an ultrasonic bath.

Chromatographic conditions

The mobile phase was obtained mixing eluents A and B in the ratio 90:10 (v/v). After 5 min the concentration of eluent B began to increase linearly to 30% at 25 min. The flow-rate was 2 ml/min.

Sample preparation

Analysis of finished bulk. To approximately 200 mg of a weighed sample of I in a 100-ml volumetric flask 6 ml of acetonitrile are added and the powder is dissolved in a solution prepared by weighing 2.062 g of sodium hexanesulphonate into 1 l of water and adjusting the pH to 4.5 with dilute phosphoric acid.

Analysis of table-top sweetener. An amount of powder or tablets containing about 200 mg of I is exactly weighed and dissolved as above. If necessary, the solutions are filtered.

Reference standard. Aspartame (I) is weighed and dissolved as in the case of finished bulk in the range 1–3 mg/ml, and related products (II–X) are dissolved to give solutions with concentrations in the range 0.01–0.03 mg/ml.

The quantitative determination is performed by comparing the chromatographic peak areas of samples in triplicate with those of a known standard solution.

RESULTS AND DISCUSSION

Aspartame (I) is relatively unstable in aqueous medium, giving rise to a dipeptide (VI) and mainly the corresponding diketopiperazine (III), which is devoid of sweetness[1]. The same occurs during heating of the product[9]. Because in the preparation of table-top sweeteners there is a granulation step and the drying operation involves heating of the mixture, it is necessary to monitor the levels of III and VI in the final formulations. Moreover (see Scheme I), a number of related products are formed during the synthesis, and each of them must be carefully checked in the finished bulk.

Only a few HPLC methods[3,4] separate I from III and VI and also from VIII[3], a major intermediate of the synthesis[10], in carbonated soft drinks[4] or the finished bulk form[3]. We have reported[7] a very fast HPLC procedure that also separates VII and VIII. The use of ion-pair chromatography on a reversed-phase column allows the separation of related compounds with very similar chemical structures having very close pK_a values in a relatively short time.

Fig. 1 is a chromatogram showing the separation of a mixture of I–X in equal

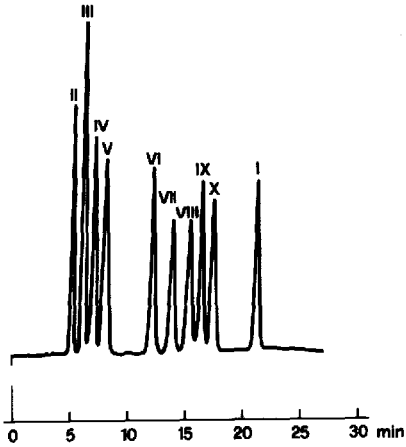
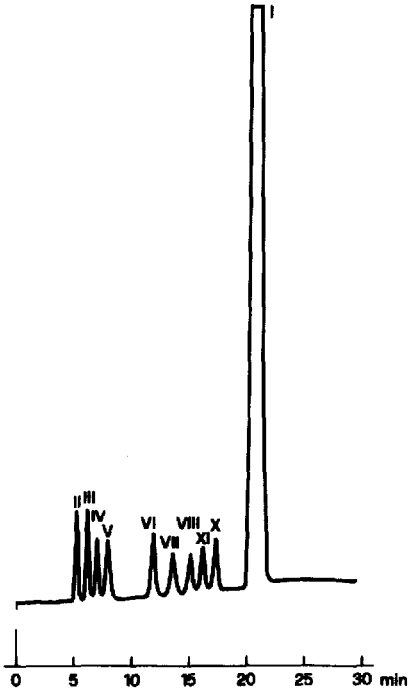


Fig. 1. Chromatogram of a mixture of I–X in equal amounts.

amounts. Baseline separation of these is accomplished in less than 25 min. Fig. 2 shows the separation of another artificial mixture, where each of the related products amount to only 1% with respect to I.

The limit of detectability of this method for the analysis of aspartame is evident from Fig. 3 and is about 0.1%.



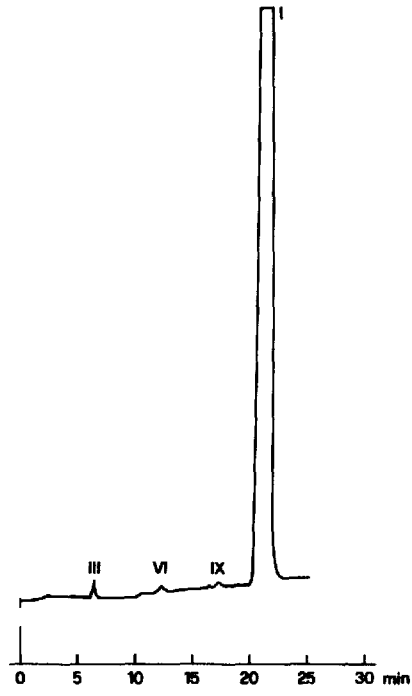


Fig. 2. Chromatogram of an artificial mixture of I and II–X, each 1% with respect to I.

Fig. 3. Chromatogram of aspartame standard, in which the level of III is 0.2%, VI is about 0.05% and IX is 0.1% with respect to aspartame.

TABLE I

VALIDATION OF ASPARTAME ANALYSIS BY PEAK-AREA MEASUREMENT

Results given are peak-area units.

Run No.	*Aspartame concentration (mg/ml)*		
	1.0	*2.0*	*3.0*
1	160 982	321 320	476 494
2	161 781	320 889	473 476
3	164 431	323 325	475 696
Average	162 368	321 845	475 222
Linear regression:	A (area units) = mC (mg/ml) + q, where m = 156 430 and q = 6960; correlation coefficient r = 0.99994.		

TABLE II

REPRODUCIBILITY OF ASPARTAME ANALYSIS

Injection No.	*Peak area*
1	317 626
2	318 947
3	325 524
4	320 070
5	321 842
6	315 660
Average	319 945
S.D.	3449
Precision (t = 0.05; n = 6)	±2.77%

TABLE III

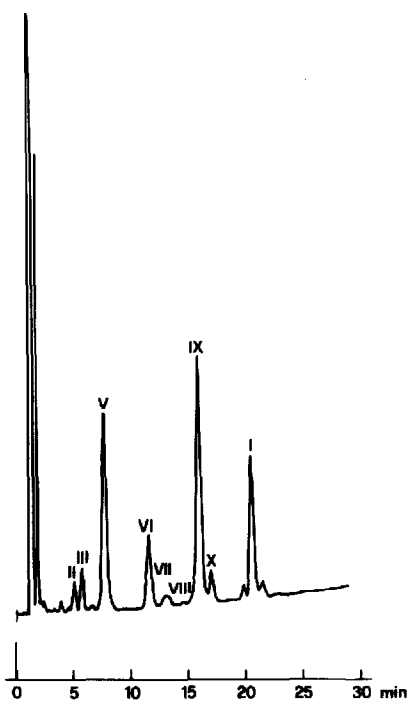
LINEAR REGRESSION FOR RELATED PRODUCTS (II–X)

A (area counts) = mC (mg/ml)* + q

Component	m	q	r**
II	160.7	0.085	0.9990
III	278.0	−0.25	0.9996
IV	192.2	−0.165	0.9997
V	215.4	0.068	0.9996
VI	246.5	0.16	0.9995
VII	149.2	0.172	0.9998
VIII	151.5	0.07	0.9999
IX	191.5	−0.04	0.9999
X	187.15	−0.03	0.9999

* From 0.01 to 0.03 mg/ml.

** Correlation coefficient.



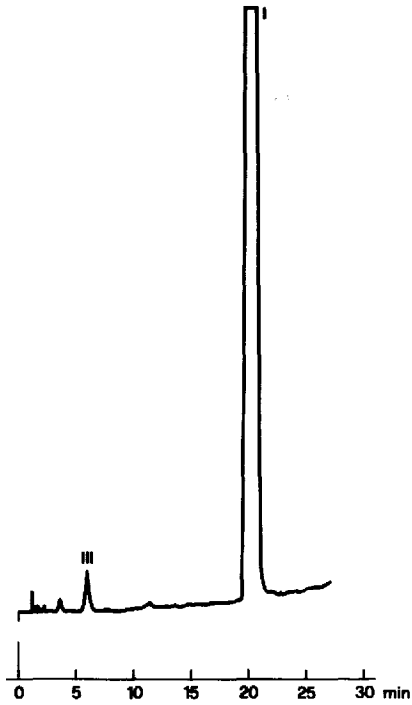


Fig. 4. Chromatogram of a mother liquor from aspartame crystallization.

Fig. 5. Chromatogram of Aspartina tablets analysis.

TABLE IV

DETERMINATION OF ASPARTAME IN COMMERCIAL DOSAGE FORMS

Brand name	*Manufacturer*	*Theoretical content*	*Found**	*S.D.*	*Precision (t = 0.05)*
Mini D**	Pierrel	3.0%	2.99%	0.06%	5%
Vantaggio**	Chiari & Forti	2.8%	2.78%	0.05%	5%
Aspartina***	Pierrel	20 mg/tablet	20.4 mg/tablet	0.45 mg/tablet	6%

* Mean of five determinations.
** Free-flowing powder.
*** Tablet.

Table I reports data for the validation of the quantitative determination of aspartame in the range 1–3 mg/ml by peak-area measurement, based on triplicate analyses of each solution. The reproducibility of the analytical results is shown in Table II. It was determined by six replicate injections of the same solution of aspartame at a concentration of 2 mg/ml. The validity of the analysis of related compounds II–X is shown in Table III, where the linear regression and correlation coefficient are presented for each component in the range 0.01–0.03 mg/ml.

The method was applied routinely for the control of the synthesis, for purity checks of the final finished bulk and for the analysis of complex mixtures, *e.g.*, mother liquors (Fig. 4). Dosage forms (both free-flowing powder and tablets) were tested mainly for assay of the active ingredient and for the incidental increase of degradation products III and VI (Fig. 5). III, which represents the main impurity, can easily be detected at levels down to about 0.3%. Table IV gives results for the quantitative determination of aspartame obtained in the analysis of three dosage forms of different composition.

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