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Note

Analysis of the sweetener aspartame by capillary isotachophoresis

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In this work the development of a simple, sensitive and rapid capillary isotachophoresis (ITP) method for determination of aspartame in beverages, yoghurt and black coffee is reported.

Aspartame is the dipeptide L-aspartyl-L-phenylalanine methyl ester and has $pK_1 = 3.18$, $pK_2 = 7.82$, $pI = 5.2$ at 20°C. In Czechoslovakia it is produced under trade-mark USAL as aspartame hydrochloride. Aspartame is used as a low-calorie artificial sweetener for soft drinks. So far a number of methods have been reported for its determination in foods and/or purity check analysis namely thin-layer chromatography[1,3], high-performance liquid chromatography[2,4–7] and capillary isotachophoresis. In purity check analysis of aspartame, Boček[8] used cationic separation at low pH. The leading electrolyte consisted of 0.05 *M* ammonium hydroxide + 0.1 *M* acetic acid, and 0.05 *M* acetic acid served as the terminating electrolyte. If this system is used for the determination of aspartame in beverages, the samples have to be concentrated. For this reason in the present work the anionic separation at intermediate pH was used and no sample concentration was necessary.

EXPERIMENTAL

Isotachophoresis

The isotachophoretic analyser used was a ZKI-001 (URVJT, Spišská Nová Ves, Czechoslovakia) with column coupling. The separations were performed in a PTFE pre-separation capillary (200 mm × 0.8 mm I.D.) which was coupled with a PTFE separation capillary (200 mm × 0.3 mm I.D.). Zones were detected by a conductivity detector. The isotachopherograms were recorded by a line recorder TZ 4200 (Laboratorní přístroje, Prague, Czechoslovakia). The driving currents applied to the pre-separation capillary and separation capillary were 200 and 30 μA, respectively. Anionic analysis of the sweetener was performed with a leading electrolyte comprising of 5 m*M* acetic acid + Tris, pH 7.7 and a terminating electrolyte of 5 m*M* L-histidine + Tris, pH 7.8. Each analysis required 15–20 min. Deionized water containing a very low concentration of bicarbonate was used for the preparation of electrolytes. The calibration analysis (calibration curve goes through zero) and the method of standard addition showed that the addition of barium hydroxide to the terminating electrolyte is not necessary. The terminating electrolyte was freshly prepared every day.

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Reagents

Aspartame hydrochloride (USAL), $C_{14}H_{18}N_2O_5 \cdot HCl \cdot 2\ H_2O$, MW 366.8, was obtained from the Research Institute of Pharmacy and Biochemistry (Prague, Czechoslovakia). It was not further purified. Acetic acid and Tris were provided by Lachema (Brno, Czechoslovakia) and L-histidine was obtained from Reanal (Budapest, Hungary). These chemicals were purified by conventional methods.

Calibration

The external standard method was used. Aspartame was injected (three replicates) into the ITP analyser from the 5 m*M* stock solution at six different levels (1–15 nmol) by use of a 10-μl Hamilton syringe.

Preparation of samples

Samples were obtained at the local market, except for Dia-Orange. Carbonated beverages were decarbonated by using a supersonic bath. Into a 100-ml volumetric flask containing a known amount of sweetener, 80 ml of the sample were placed and made up to volume with distilled water. After mixing, 5 μl were injected into the ITP analyser by means of a 10-μl syringe or sampling valve (fixed volume 23.6 μl). Black coffee was treated in the same way as beverages.

A 40-g amount of yoghurt was weighted into a 200-ml volumetric flask containing aspartame and made up to volume with distilled water. After mixing and filtering through filter paper (pore diameter 2 μm), 5 μl were injected into the ITP analyser.

The beverage Dia-Orange is produced by Fruta (Brno, Czechoslovakia) from orange concentrate (fy Arvanitis) containing 250 mg of aspartame per litre, *i.e.*, 311 mg of $C_{14}H_{18}N_2O_5 \cdot HCl \cdot 2H_2O$ per litre, according to the supplier. A 5-μl volume of Dia-Orange was injected directly into the ITP analyser without any pre-treatment.

TABLE I

RESULTS OF CALIBRATION

Leading electrolyte: 5 m*M* acetic acid + Tris, pH 7.7. Terminating electrolyte: 5 m*M* L-histidine + Tris, pH 7.8. Driving currents: pre-separation capillary, 200 μA; separation capillary, 30 μA. Chart speed: 6 cm/min. Linear relationship: $Y = 8.73\ X + 0.9$, where Y = step length in mm, X = amount of $C_{14}H_{18}N_2O_5 \cdot HCl \cdot 2H_2O$ in nmol.

Amount of $C_{14}H_{18}N_2O_5 \cdot HCl \cdot 2H_2O$ injected		*Step length** (*mm*)
nmol	*μg*	
1.25	0.46	11
2,50	0.92	21.5
5.00	1.83	44.5
6.00	2.20	55.5
10.00	3.67	88
15.00	5.50	131

* Average of three replices.

TABLE II

DETERMINATION OF ASPARTAME IN SAMPLES

Conditions as in Table I.

Sample	*Amount of $C_{14}H_{18}N_2O_5 \cdot HCl \cdot 2H_2O$ (mg/l)*		*Recovery (%)*
	Added	*Determined**	
Black coffee	250	245	98.0
Beverage Grepus	138	133	96.4
Beverage Višeň	459	463	101.0
Coca Cola	459	461	100.5
Juice Dia-Orange	311	305	98.0
Yoghurt**	1150	1146	99.5

* Average from five determinations; relative standard deviation was less than 3%.
** In mg/kg.

RESULTS AND DISCUSSION

The calibration data are given in Table I. The content of aspartame (see Table II) was calculated by means of the calibration equation (Table I). The correlation coefficient of the straight-line graph was 0.999.

In Fig. 1 isotachopherograms of a sample of Coca Cola without aspartame (A) and with aspartame (B) are given. It is clear that no compound interfering with aspartame was found in this sample (and in the others analysed). The evidence that the step denoted as aspartame in Fig. 1 corresponds to the aspartame contents was made by the method of standard addition.

The recovery was in range 96–101% at all levels of addition of aspartame. The

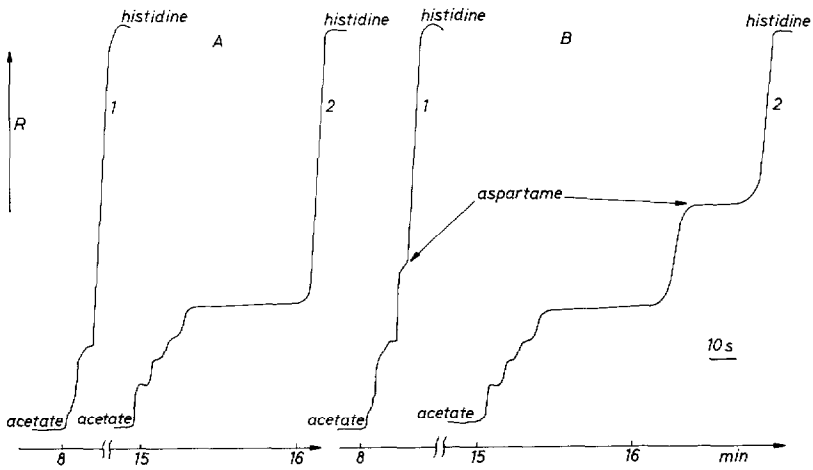

Fig. 1. Isotachopherograms of Coca Cola. Analysis conditions as in Table I. 1 = pre-separation capillary, 2 = separation capillary. A 5-μl mixture of 80 ml of sample + 20 ml of water (A) or a 5-μl mixture of 80 ml of sample + 20 ml of 5 m*M* $C_{14}H_{18}N_2O_5 \cdot HCl \cdot 2H_2O$ (B) was injected.

TABLE III

EXPERIMENTAL VALUES OF RELATIVE STEP HEIGHTS (RSHs)

Conditions of analysis as in Table I.

Species	*RSH*
Acetic acid	0.00
L-Aspartyl-L-phenylalanine	0.08
Dioxopiperazine	0.28
Aspartame	0.55
β-Aspartame	0.85
L-Histidine	1.00

detection limit for $C_{14}H_{18}N_2O_5 \cdot HCl \cdot 2H_2O$ was found 1.8 μg/ml at a chart speed of 6 cm/min.

For increased sensitivity of this method it is possible to use an operational system with two leading electrolytes. The pre-separation capillary was filled with a leading electrolyte consisting of 5-m*M* acetic acid + Tris, pH 7.7 and the separation capillary with 1 m*M* acetic acid + Tris, pH 7.7. The driving currents applied to the pre-separation and the separation capillary were 200 and 7.5 μA, respectively. At a chart speed of 15 cm/min, the detection limit of $C_{14}H_{18}N_2O_5 \cdot HCl \cdot 2H_2O$ was 0.2 μg/ml.

It is possible to use this method for purity check analysis of aspartame. Some impurities from its synthesis, dioxopiperazine, L-aspartyl-L-phenylalanine and β-aspartame, can be determined in one anionic experiment. The experimental values of the relative step heights (RSHs) of these components are given in Table III.

The results obtained clearly show that the method is suitable for the determination of aspartame in beverage, yoghurt and black coffee.

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