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Determination of sorbic acid in food products by capillary zone electrophoresis in a hydrodynamically closed separation compartment

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Abstract

A simple, selective and rapid capillary zone electrophoresis method for the determination of sorbic acid in food products is described. The determination was carried out in a hydrodynamically closed separation compartment at pH 5.2 and for the samples taken into this study (soft drinks, wine, fruit and juice concentrates, margarine and marmalade) only simple sample preparation procedures were needed. A very good reproducibility in the migration time of the analyte (R.S.D. = 0.61%) was achieved and as could be expected no negative effect of matrix constituents on this performance parameter was detected. The recoveries of sorbic acid in the spiked samples ranged from 98–102% and 2–3% R.S.D.s in parallel determinations of the acid were typical.

1. Introduction

Sorbic acid is a widely used preservative in food products and, therefore, numerous methods were proposed for its determination in various food matrices [1]. At present, however, high-performance liquid chromatography (HPLC) [2–4], thin-layer chromatography (TLC) [5,6], and gas chromatography (GC) [7,8] combined with suitable sample preparation procedures are dominantly used.

A considerable reduced sample preparation is typical when sorbic acid is analyzed by capillary isotachophoresis (ITP) as demonstrated for various food matrices [2,9–12]. Although the time for an ITP analysis (15–20 min) compares favourably with the above chromatography techniques (see, e.g., ref. [2]) it is less favourable when large series of samples are to be analyzed in a routine control laboratory.

Under ITP separating conditions sorbic acid has a large light absorptivity at a 254 nm wavelength [2,9–12]. In addition, from the quoted works it is also apparent that ITP-migrating and UV-light-absorbing constituents of food matrices typically represent only smaller parts of the total signals as obtained from the UV detector for the ITP separands. These facts imply that capillary electrophoresis techniques handling smaller sam-

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ple loads than ITP with the quantitation based on the zone length measurements [13] should be considered as suitable candidates for a rapid electrophoretic determination of sorbic acid in food products. ITP in the spike mode of detection (see, e.g., ref. [14]) and capillary zone electrophoresis (CZE) [15–19] meet such requirements and the latter of these techniques was investigated in this work.

Recent reviews of the CZE instruments show [20,21] that at present CZE is almost exclusively carried out in hydrodynamically opened separation compartments as proposed by Jorgenson and Lukacs [19]. This arrangement of the separation compartment is usually employed with non-zero nett electroosmotic flow. A simultaneous analysis of both positively and negatively charged ions in one run and a negligible band broadening due to electroosmosis are apparent advantages of this approach. On the other hand, any change of ζ potential in the opened separation compartment (e.g., due to adsorption of the sample constituents) is influencing the migration velocities of the separands and, consequently, time based peak area measurements. In the analysis of complex biological fluids even very careful sample pretreatment need not eliminate this problem [22]. Although it can be diminished via appropriate normalization procedures [22,23] irreproducibilities in migration velocities due to changes of the electroosmotic flow during the run can be corrected for only with difficulties. As some food matrices can cause similar problems we preferred the use of hydrodynamically closed separation compartment [15–18]. The main reasons for this preference were in high reproducibilities of the migration times as reported for some constituents in serum [18] and highly proteinous tissue extracts [24]. However, in this context it is necessary to stress that in the closed systems the electroosmotic flow has a negative impact on the band broadening [16,25,26]. Here, appropriate measures, such as coated inner walls of the capillary tubes and/or the use of suitable additives in the carrier electrolyte [15,17,18] must be taken to achieve acceptable separation efficiencies.

2. Experimental

2.1. Instrumentation

A CS isotachophoretic analyser (Villa-Labeco, Spišská Nová Ves, Slovak Republic) was assembled in the single-column mode. The separation unit of the analyser consisted of a laboratory-developed CZE injection valve (90-nl sample loop), a column provided with a 0.30 mm I.D. capillary tube (O.D. $\approx$ 0.65 mm) made of fluorinated ethylene–propylene (FEP) copolymer (Villa-Labeco). The length of the capillary tube was 250 mm (200 mm to the detector) and the column was provided with a UVD 2 on-column photometric detector (Villa-Labeco).

The signal from the photometric detector was registered by a TZ 4200 line recorder (Laboratorní Přístroje, Prague, Czech Republic) and in parallel by an HP 3390A reporting integrator (Hewlett-Packard, Avondale, PA, USA). The data provided by the integrator (peak area and peak area/peak height ratio) served for the evaluation of the quantitation and for the calculation of the column efficiency as described in the literature [27].

2.2. Chemicals

Chemicals used for the preparation of the electrolyte solutions were obtained from Serva (Heidelberg, Germany), Sigma (St. Louis, MO, USA) and Lachema (Brno, Czech Republic). Polyethyleneglycol 5 000 000 (PEG) obtained from Serva was used as an anticonvective additive in the carrier electrolyte solutions.

Water from an Aqualabo two-stage demineralization unit (Aqualabo, Brno, Czech Republic) was further purified by circulation through laboratory-made polytetrafluoroethylene (PTFE) cartridges packed with Amberlite MB-1 mixed-bed ion exchanger (Serva). The electrolyte solutions were prepared from freshly recirculated water and filtered through a 0.45-μm syringe filter (Gelman, Ann Arbor, MI, USA).

2.3. Samples

Fanta and Sprite (soft drinks), Diplomat (wine) and Halvarine (margarine) were bought in a local supermarket. Athena Orange, Athena Lemon, Exotic Zeus, Lesná Zmes and Cherry Sukus (juice and fruit concentrates) and Apricot Spread (marmalade) were kindly provided by NOKO (Nové Mesto nad Váhom, Slovak Republic).

Soft drinks and wine were diluted with an aqueous solution of Na_2SO_4 in required ratios so that the final concentrations of sorbic acid were within the concentration span for which the calibration graphs were measured. The final concentrations of Na_2SO_4 were 10^{-3} *M* (this electrolyte in the sample solution was present to minimize adsorption of sorbic acid on the walls of sample containers made of polyethylene).

Margarine (weighed amount of 0.35–0.40 g) was dissolved in 15 ml of *n*-heptane and 10 ml of a 1 m*M* aqueous solution of Bis-Tris (see Table 1) was added to extract sorbic acid from the organic phase. The aqueous phase was separated in a separatory funnel and the organic phase was extracted repeatedly with a fresh portion of the aqueous extractant. The extracts were made up to 25 ml with the extractant. Also in this instance the aqueous phase contained Na_2SO_4 at a 10^{-3} *M* concentration.

Weighed amounts of juice and fruit concentrates and marmalade (0.2–0.5 g) were diluted in 10-ml volumes of 10^{-3} *M* aqueous solution of Na_2SO_4 and sonicated for 5 min. The solutions were filtered and the filtrates analyzed by CZE.

Table 1
Electrolyte system

Solvent	Water
Anion	MES[a]
Concentration (m*M*)	100
Counter-ion	Bis-Tris[b]
Concentration (m*M*)	10
pH	5.2
Additive	PEG[c]
Concentration (%, w/v)	0.2

[a] MES = 2-(N-Morpholino)-ethanesulfonic acid.
[b] Bis-Tris = 2,2 - Bis(hydroxymethyl) - 2,2',2'' - nitrilotriethanol.
[c] PEG = Polyethyleneglycol 5 000 000.

3. Results and discussion

3.1. Separating conditions and some performance characteristics of the separation system

The composition of the carrier electrolyte used in this work is given in Table 1. Sorbic acid ($pK_a = 4.77$) is ionized from 75% in this carrier electrolyte. Such a distribution into the ionic forms is less favourable as far as the separation efficiency is concerned because a maximum efficiency requires a full ionization of the separand [28], i.e., in this particular instance a higher pH. On the other hand, reduced effective mobilities of the anionic matrix constituents (favoured by a low pH of the carrier electrolyte) were desired to achieve a high selectivity of the analysis via a reduced number of migrating sample constituents. No detailed search for an optimum electrolyte system in terms of the resolutions of the separands was carried out as various food matrices differing not only in the concentrations of the matrix constituents but also qualitatively (e.g., refs. [1,29]) make such a search cumbersome and only of a limited general validity.

Benzoic and ascorbic acids are probably the only among current food additives [1,12] which could interfere in the analysis of sorbate by CZE. We found that under our separating conditions they migrated with higher effective mobilities (lower pK_a values than that of sorbic acid) and did not disturb the analysis of the preservative (see Fig. 2). Electropherograms in Fig. 1 show that for most of the samples the carrier electrolyte provided high analytical selectivities with acceptable separation efficiencies for the analyte (see Table 2). The same applies for the samples of somewhat higher complexities (Fig. 2) as the matrix constituents unresolved

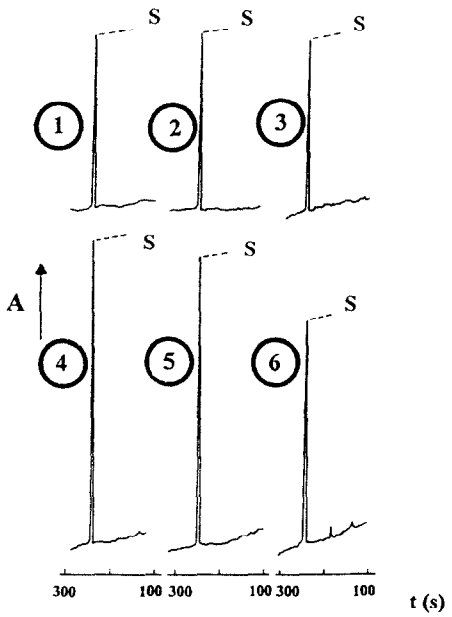

Fig. 1. Determination of sorbic acid (S) in food products. 1 = Halvarine; 2 = Apricot Spread; 3 = Diplomat wine; 4 = Fanta soft drink; 5 = Sprite soft drink; 6 = Lesná Zmes fruit lemonade concentrate. The samples were pretreated as described under Experimental. The composition of the electrolyte system is given in Table 1. The driving current was 150 μA. A = Increasing light absorption.

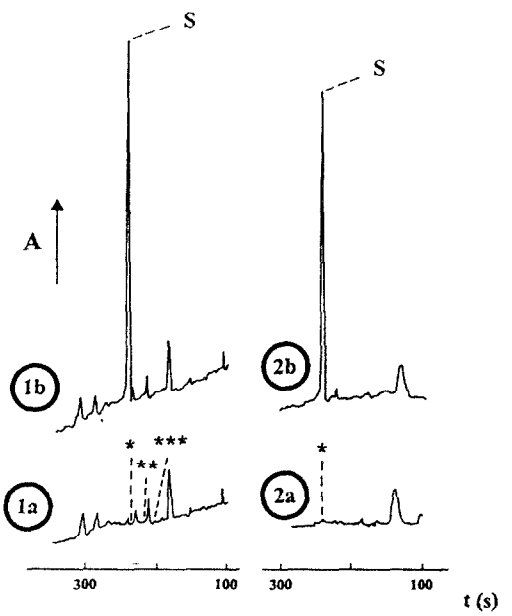

Fig. 2. CZE of sorbic acid (S) in fruit concentrates. 1 = Cherry Sukus; 2 = Athena Orange (a = fruit concentrates without the preservative; b = the same fruit concentrates as in a containing sorbic acid). The samples were pretreated in a way described under Experimental. The separations were carried out in the electrolyte system given in Table 1 with a 150 μA driving current. *, **, *** = Migration positions of sorbate, ascorbate and benzoate, respectively.

Table 2
Separation performance characteristics for sorbic acid (n = 72)

Parameter[a]	
Migration time (s)	246.1
R.S.D. (%)	0.61
Column efficiency (N/m)[b]	175 000
R.S.D. (%)	10
Concentration span of the analyte (μM)	5–70

R.S.D. = Relative standard deviation; N/m = number of theoretical plates per metre; n = number of data points.

[a] The average values calculated from the data obtained in 72 runs within 7 days in three (separately prepared) solutions of the carrier electrolyte. The data obtained in the runs with both model and practical samples (listed in Table 5) are included.

[b] Calculated from the data provided by the integrator (see Experimental).

from the analyte introduced only negligible systematic errors into its quantification.

As mentioned in the Introduction the separations in a hydrodynamically closed separation compartment were preferred to minimize uncontrolled fluctuations in the migration time of the analyte due to unpredictable changes of ζ potential. The relevant data in Table 2 show that the migration time of sorbate was very reproducible also when random errors due to the use of different solutions of the carrier electrolyte were included.

The I.D. of the capillary tube as used in this work is less suitable from the point of view of thermal dispersion [16,25,26]. However, the use of a less conductive carrier electrolyte enabled us to apply a field strength of ca. 400 V/cm (for a 150 μA driving current) to achieve rapid and high-efficiency CZE separations. In this context it is necessary to stress that an overall dispersion

included also a contribution due to electroosmosis [16,25,26]. Although fluoroplastic surfaces have lower ζ potentials than, e.g., of fused silica [30,31] the use of a high-molecular-mass polyethyleneglycol (Table 1) in the carrier electrolyte was essential in minimizing the electroosmotic dispersion. This anticonvective additive was preferred as it gave the highest separation efficiencies for a model group of ionic separands when high-molecular-mass water-soluble polymers such as hydroxyethylcellulose, methylhydroxyethylcellulose, poly(vinyl alcohol), poly(vinylpyrrolidone) and polyethyleneglycol were compared under otherwise identical working conditions [32].

In the CZE analysis of complex ionic mixtures capillary tubes of preferred dimensions are overloaded easily by sample macroconstituents (see, e.g., ref. [33]). The use of the capillary tube of a higher I.D. provides one way in solving these problems as the load capacity of the column is proportional to its cross-section. Therefore, in our instance the sample load could be increased up to 90 nl also for less diluted highly ionic fruit concentrates (see, e.g., Fig. 2) without any indication of the column overloading [18]. In addition, a 0.3 mm pathlength of the detection cell enabled us to achieve for sorbic acid the limit of detection at a $5 \cdot 10^{-7}$ M concentration (254 nm detection wavelength and a 90-nl injection volume) with a simple on-column photometric detector.

3.2. Quantitation

Parameters of the regression equations related to the calibration graphs are given in Table 3. The concentration range for which the data were measured covered the concentrations of the preservative in appropriately diluted food products and/or in their extracts.

The reproducibilities of the determinations of sorbic acid were assessed for five concentrations (the lowest concentration corresponded to the limit of determination [34]) by using the data provided by the integrator (peak areas and peak heights) and those evaluated from the electropherograms as registered by the line recorder (peak heights). The relative standard deviations presented in Table 4 show that the peak area measurements were, in general, more scattered. This problem was associated with the detector noise which prevented reproducible assignments of the start and end points of the analyte peak and, consequently, reduced reproducibilities in the peak integration. Undoubtedly, this also explains a lower correlation coefficient for the regression equation: peak area vs. sorbic acid concentration (Table 3). It was shown recently by Wanders [35] that analogous problems are

Table 3
Parameters of the regression equations ($y = a + bx$) and correlation coefficients for sorbic acid at 5–70 μM concentrations ($n = 19$)

Parameter	Peak area[a], integrator	Peak height[a]	
		Integrator	Recorder
a (mV min)	−274.00	–	–
b (mV min/μmol)	154	–	–
r	0.9964	–	–
a (mV)	–	0.471	1.12
b (mV/μmol)	–	2.497	2.739
r	–	0.9996	0.9995

y = Peak area (peak height); a = intercept; b = slope; x = concentration (μM); n = number of data points; r = correlation coefficient.

[a] The data were obtained in the same runs.

Table 4
Reproducibilities of the determinations of sorbic acid at different concentrations for various data evaluations

Concentration (μM)	Peak area[a], integrator		Peak height[a]			
			Integrator		Recorder	
	R.S.D. (%)	n	R.S.D. (%)	n	R.S.D. (%)	n
5[b]	2.3	3	0.9	3	2.1	3
10	6.9	4	2.4	4	1.0	4
30	2.8	4	3.6	4	3.2	4
50	5.3	4	1.0	4	1.0	4
70	5.1	4	1.0	4	0.9	4

[a] The data were obtained from the same runs.
[b]One of the four analysis was excluded by a test.

typical when current chromatography software packages are used to data analysis in CZE, especially while working with a low signal-to-noise ratio.

The concentrations of sorbic acid were determined in some food products which were claimed by the manufacturers to contain this preservative. Mean values of the parallel determinations are given in Table 5. The results of the parallels deviated by less than 5% with 2–3% R.S.D.s being dominant. Tabulated results (Table 5) also show differences due to the use of different alternatives in the peak height measurements in the same CZE runs.

A current preparative of sorbic acid served as a reference analyte in our measurements. As in none of the samples an actual ("true") concentration of the preservative was known, accuracies of the analyses could not be assessed directly. Nevertheless, 98–102% recoveries of sorbic acid in the studied food products (evaluated from the spiked samples) and negligible systematic errors due to matrix constituents (experiments with samples without the preserva-

Table 5
Examples of the determination of sorbic acid in various food products

Food products	Determined (mg/kg)		Maximum permitted (mg/kg)	Sample preparation[a]
	A	B		
Fanta (soft drink)	203.5	206.2	200	Dilution (1:25, v/w), filtration
Sprite (soft drink)	195.6	194.8	200	As for Fanta
Diplomat (wine)	146.2	146.1	200	Dilution (3:100, v/v), filtration
Athena Orange (juice concentrate)	137.0	136.9	300	Dilution (3.9:100,w/v), filtration
Athena Lemon (juice concentrate)	2.4	2.5	–	Filtration
Apricot (marmalade)	118.5	119.4	400	Extraction (1:25, w/v), filtration
Halvarine (margarine)	180.6	178.9	200	Extraction (3.9:150, w/v)

A, B = Peak height measurements from the integrator and the recorder, respectively.
[a]For details, see Experimental section.

tive (Fig. 2)) indicate that also this performance parameter was met.

In conclusion we can state that CZE performed in a hydrodynamically closed separation compartment offers a rapid and reproducible alternative to the determination of sorbic acid in food products. When necessary a 4-min analysis time as achieved in this work can be further reduced by relevant means (e.g., length of the column, a higher field strength combined with an appropriate carrier electrolyte solution).

Simple sample preparation procedures used for food products investigated in this work probably need not be of a general applicability. Then an extraction procedure as described by Eichler and Rubach [12] for ITP seems a convenient alternative also for CZE. On-line ITP pretreatment providing a powerful sample clean-up also for very complex ionic matrices [36] should be also considered in such situations.

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