DETERMINATION OF ADDITIVES IN BEVERAGES BY ELECTROMIGRATIVE METHODS

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Abstract: *The advantageous combination of isotachophoresis and capillary zone electrophoresis using a commercial instrument with two coupled capillaries is presented.*
Sorbic and ascorbic acid were chosen as model components since they are widely used additives in food and beverage industry and need to be controlled according the national regulations in Germany and other countries as well.
The determination of these food additives is demonstrated in matrix-free model solutions and real samples as well. As a real sample a so called "alco-pop"-drink has been used.

Keywords: *Capillary zone electrophoresis, Isotachophoresis, ITP, ITP-CZE, Food analysis.*

Introduction

Capillary zone electrophoresis (CZE) seems to be a promising tool in the analysis of ionic sample components [Jandik, 1993]. However, the restricted sample load capacity and the short optical pathlength using normal capillaries with I.D. from 25 – 100 µm are limiting factors with respect to the attainable limits of detection (LODs) [Reinhoud, 1993; Engelhardt, 1994].

An on-line combination of isotachophoresis (ITP) and CZE in a column-coupling separation system offers a convenient way to overcome the above mentioned restrictions [Everaerts, 1979; Bocek, 1988]. Using the inherent concentration capability during the ITP step in the first capillary is probably the main benefit of this setup. A defined segment of concentrated analytes are transferred into the final CZE separation in the second capillary. Consequently, the second capillary is made of a greater I.D. (300 µm) than in classical CZE thus providing a more effective optical pathlength for the final UV detection.

Our experiments were aimed at the investigation of possible applications concerning the quality and process control of preservatives and antioxidants used in food and beverages. Sorbic acid and ascorbic acid are widely used additives in food and beverage industry and need to be controlled according the national regulations in Germany and other countries as well.

At present both analytes are usually subject of classical analytical methods like acid-base titration and/or photometric methods. After extraction with oxalic acid and reaction with 2-6 dichlorophenolindophenol, ascorbic acid can be determined by titration [Matissek, 1992]. For sorbic acid the photometric detection consists of the following steps; oxidation with potassium chromate, reaction with 2-thiobarbituric acid and final detection at 532 nm [Matissek, 1992].

An Alco-Pop-drink (Bacardi Rigo) seems to be a convenient example of a food product of current interest, high control requirements in a rather huge amount of

sugar, ethanol, citric acid and carbon dioxide as a difficult matrix.

Experimental

Chemicals

All electrolyte solutions were obtained from JH-Analytik (Aalen, Germany). Additionally, the following chemicals were used: Hydrochloric acid (1 mol*L^{-1}, p.a.), histidine (for biochemical purposes), *N*-morpholino-ethane-sulfonic acid mono-hydrate (MES), all from Merck (Darmstadt, Germany), purified methylhydroxyethylcellulose 1% stock solution (JH-Analytik, Aalen, Germany). The standard substances ascorbic acid (p.a.) and benzoic acid (p.a.) were obtained from Merck (Darmstadt, Germany); potassium sorbate (purum p.a.) was obtained from Fluka (Buchs, Switzerland). 10 mmol * L^{-1} stock solutions of the standard substances were prepared by dissolving the standards in distilled water. Working solutions were obtained by diluting the stock solutions in distilled water. Distilled water with a conductivity of less than 0.1 µS * cm^{-1} was obtained from a Milli-Q RG water purifier (Millipore, Bedford, USA).

Experimental setup

ITP was carried out with a column coupling instrument (ItaChrom II-M, JH-Analytik, Aalen, Germany). A preseparation column (capillary: 90 mm length, 0.8 mm inner diameter, 1.2 mm outer diameter) and an analytical column (capillary: 160 mm length, 0.3 mm inner diameter, 0.7 mm outer diameter) were used, both made of fluorinated ethylene-propylene copolymer. The capillary tube was placed in a compartment made of plexiglas, allowing heat dissipation produced on the passage of current. The preseparation column was equipped with an on-column contact conductivity detector, the analytical column additionally with an UV-detector (deuterium lamp with a 254 nm filter). The samples were injected with the aid of an 30 µL and 0.2 µL injection valve, respectively. For data evaluation and processing the ITPPro32-software was used.

Further experimental conditions are given in Table 1.

Table 1 Electrolyte systems and measurement conditions

	ITP	Single CZE	ITP-CZE
Injection volume	30 µL	0.2 µL	30 µL
Applied current in preseparation column	250 µA	Not used	250 µA
Applied current in analytical column	50 µA	100 µA	100 µA
Electrolyte systems:			
Leading electrolyte	10 mmol * L^{-1} HCl, 0.1% MHEC, histidine (pH 6)	-	10 mmol * L^{-1} HCl, 0.1% MHEC, Histidine (pH 6)
Terminating electrolyte	5 mmol * L^{-1} MES, histidine (pH ≈ 5)	-	5 mmol * L^{-1} MES, histidine (pH ≈ 5)
Background electrolyte	-	100 mmol * L^{-1} MES, histidine (pH 5.4)	100 mmol * L^{-1} MES, histidine (pH 5.4)
Detection	Conductivity	UV (254 nm)	UV (254 nm)

Results and Discussion

ITP, CZE and ITP-CZE analysis of sorbate, benzoate and ascorbate

With regard to the potential problems in the determination of sorbate, benzoate and ascorbate in heavy matrices it was our aim to develop a ITP and ITP-CZE separation as an alternative to the above mentioned classical methods.

Recording calibration lines offers a convenient way to verify the desired working range. Table 2 shows the measured calibration lines illustrating the working range.The separation efficiency of ITP using a pH 6 electrolyte system (Table 1) is sufficient for the determination of the analytes. The calibration curves for single ITP measurements show a good linearity with a weak sensitivity (Table 2). The inherent concentration effect of ITP leads to short analyte zone lengths and thus decreasing the sensitivity of the method. Single CZE experiments using a 0.2 µL injection valve and a pH 5.4 electrolyte system (Table 1) show lower limits of detection (Table 2), but the main reason is the use of UV detection instead of conductivity detection. The ITP-CZE coupling combines the advantages of both methods. In the ITP step performed in the preseparation column a high sample volume of a low concentrated sample can be injected. During this step the analytes are preseparated and concentrated and thus transferred in a small volume into the final CZE stage performed in the analytical column. In order to confirm the limits of detection calculated according to DIN 32645 (Table 2) a model mixture in the concentration range of the detection limit was measured. The obtained electropherogram is shown in Fig. 1. The migration order of ascorbate and sorbate in ITP using a pH 6 electrolyte system is reversed to the CZE experiments using a pH 5.4 electrolyte system due to the pH dependance of the electrophoretic mobilities [Hirokawa, 1983; Shamsi, 1994; Bocek, 1978].

Table 2 Calibration data for sorbate and ascorbate

	ITP	Single CZE	ITP-CZE	ITP-CZE	ITP-CZE
Analyte	**Sorbate**	**Sorbate**	**Sorbate**	**Sorbate**	**Ascorbate**
Data evaluation by	**Zone length (conductivity detector)**	**Peak area (UV-detector)**	**Peak area (UV-detector)**	**Peak area (UV-detector)**	**Peak area (UV-detector)**
Calibration range [µmol * L^{-1}]	100 – 300	3.0 – 9.0	1.0 – 3.0	0.10 – 1.0	0.10 – 1.0
Number of calibration points (repeats)	5 (2)	5 (2)	5 (2)	10 (1)	10 (1)
Coefficient of variation [%]	2.0	2.2	1.6	1.6	3.8
Slope[a]	0.107	27.5	9407	7565	4184
Intercept[b]	0.589	25.1	125	118	- 119
Coefficient of correlation	0.9991	0.9989	0.9994	0.9996	0.9979
Limit of detection (DIN 32645) [µmol * L^{-1}]	9.9	0.33	0.08	0.02	0.05
Limit of quantification (DIN 32645) [µmol * L^{-1}]	35	1.2	0.29	0.07	0.17
Critical value (DIN 32645)[b]	1.65	34.1	892	268	79.3

[a] Units: [s * L * µmol^{-1}] for zone length evaluation, [mV * s * L * µmol^{-1}] for peak area evaluation

[b] Units: [s] for zone length evaluation, [mV * s] for peak area evaluation

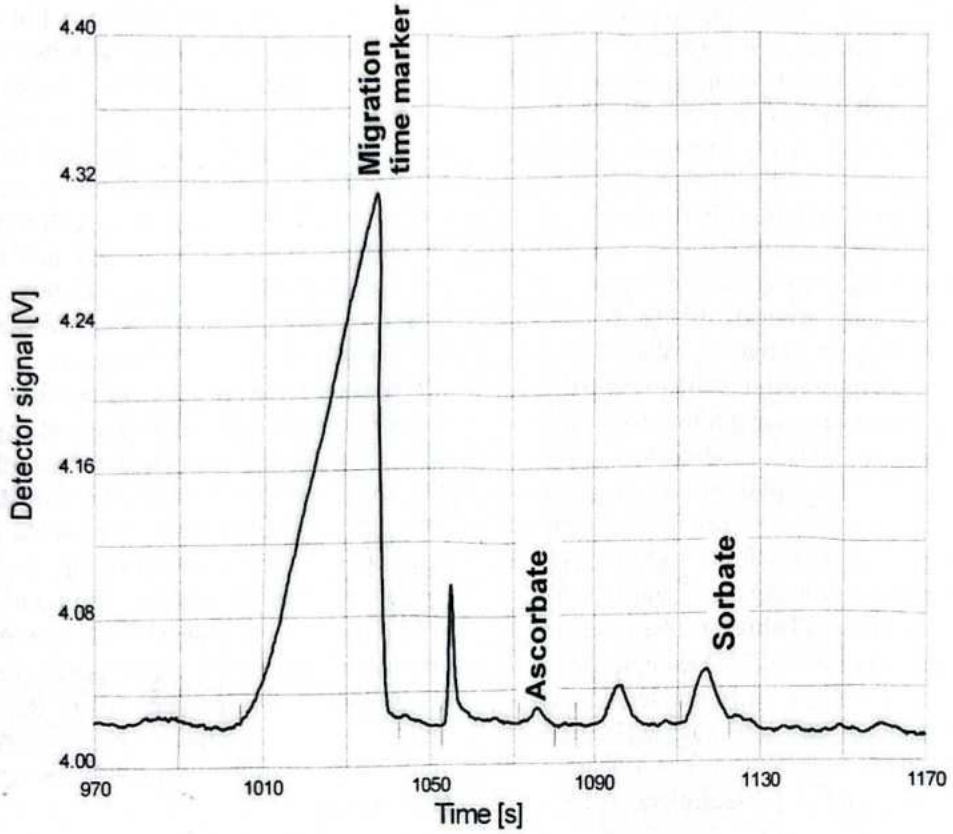

Figure 1 ITP-CZE separation of 20 nmol * L^{-1} ascorbate and sorbate. Benzoate was used as a migration time marker.

ITP and ITP-CZE analysis of real samples

Identification of the analytes in single ITP mode was executed by comparing the RSHs (relative step heights) in the sample with the RSHs in the standard solution and, for confirmation, by adding the appropriate ion to the sample.

For quantification, the mean value of three independent measurements was put in the recorded calibration equation.

Fig. 2 shows two isotachopherograms of a 1:10 diluted Bacardi Rigo® sample.

The large front migrating zone results from the high content of citrate in the sample (1).

The overlaid isotachopherogram (2) was obtained from a measurement without transferring all isotachophoretic zones into the analytical column.

It shows a much shorter citrate zone length but the same zone lengths for the analytes benzoate, sorbate and ascorbate. Thus cutting out the citrate as an excess component without affecting the analytes of interest provides high separation efficiency [Hirokawa, 1991].

In ITP-CZE mode the analytes were identified by comparing the migration times of the analytes in the sample with the migration times obtained from the measurement of a standard solution and, for confirmation, by adding the appropriate ion to the sample.

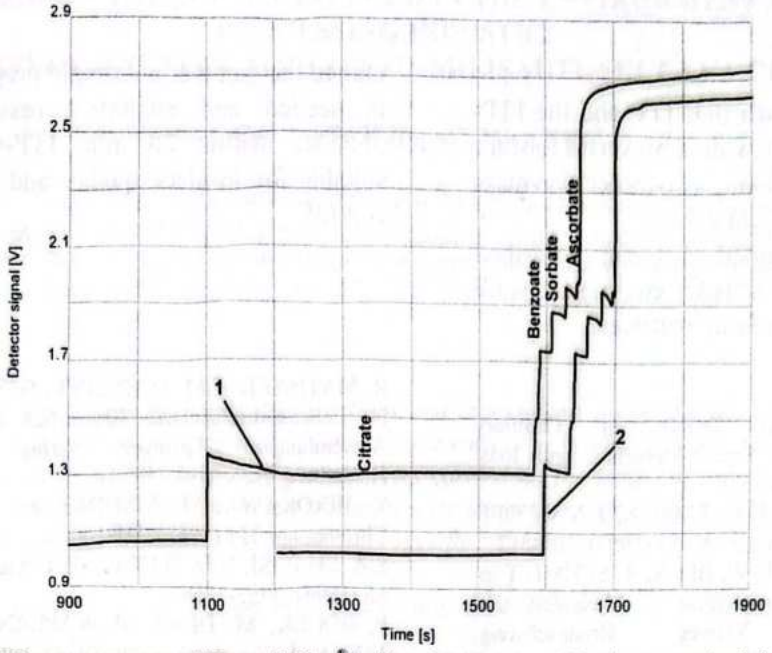

Figure 2 ITP measurements of "Bacardi Rigo®", diluted 1:10, trace of the lower conductivity detector.

Quantification was performed by the standard addition method (1:1000 diluted sample; number of calibration points: 4; concentration range of standards for spiking: 1.0 – 3.0 µmol * L^{-1}). The obtained calibration line shows a good linearity with a coefficient of correlation of 0.9985. As a result the sample in Fig. 3 contained 1.91 mmol * L^{-1} (214 mg * L^{-1}) sorbic acid. The ascorbic acid in this dilution was below the limit of quantification (Table 2).

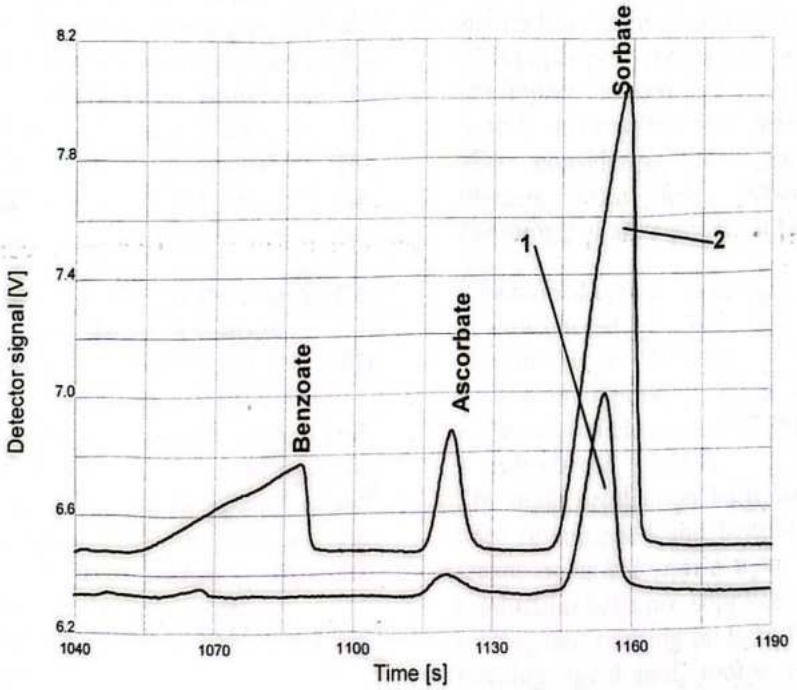

Figure 3 ITP-CZE measurement of "Bacardi Rigo®", diluted 1:1000, trace of the UV-detector (1); overlay: spiked with 1 µmol * L^{-1} ascorbate and sorbate, 50 µmol * L^{-1} benzoate (2).

Conclusions

It has been shown that ITP and the ITP-CZE coupling provide a powerful tool for the analysis of ionic low-molecular-mass analytes in heavy matrices.

It can be assumed that the described method can be transferred to nearly all neutral and even ionic matrices.

Due to the fact that no sample preparation is needed and analytical results are obtained within 20 min, ITP-CZE is suitable for in-place quality and process control.

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