Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1026 (2004) 31–39
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Determination of free sulfite in wine by zone electrophoresis with isotachophoresis sample pretreatment on a column-coupling chip

Marián Masár[a], Mariana Danková[a], Eva Ölvecká[a], Adela Stachurová[a], Dušan Kaniansky[a,*], Bernd Stanislawski[b]

[a] *Department of Analytical Chemistry, Faculty of Natural Sciences, Comenius University, Mlynská Dolina CH-2, SK-84215 Bratislava, Slovak Republic*
[b] *Merck KGaA, Frankfurter Strasse 250, D-64293 Darmstadt, Germany*

Received 16 September 2003; received in revised form 7 November 2003; accepted 10 November 2003

Abstract

This work deals with the determination of free sulfite in wine by zone electrophoresis (ZE) with on-line isotachophoresis (ITP) sample pretreatment on a column-coupling (CC) chip with conductivity detection. A rapid pre-column conversion of sulfite to hydroxymethanesulfonate (HMS), to minimize oxidation losses of the analyte, was included into the developed analytical procedure, while ITP and ZE were responsible for specific analytical tasks in the separations performed on the CC chip. ITP, for example, eliminated the sample matrix from the separation compartment and, at the same time, provided a selective concentration of HMS before its transfer to the ZE stage of the separation. On the other hand, ZE served as a final separation (destacking) method and it was used under the separating conditions favoring a sensitive conductivity detection of HMS. In this way, ITP and ZE cooperatively contributed to a 900 μg/l concentration detectability for sulfite as attained for a 60 nl load of wine (a 15-fold wine dilution and the use of a 0.9 μl sample injection channel of the chip) and, consequently, to the determination of free sulfite when this was present in wine at the concentrations as low as 3 mg/l. The separations were carried out in a closed separation compartment of the chip with suppressed hydrodynamic and electroosmotic flows. Such transport conditions, minimizing fluctuations of the migration velocities of the separated constituents, made a frame for precise migration and quantitation data as achieved for HMS in both the model and wine samples. Ninety percent recoveries, as typically obtained for free sulfite in wine samples, indicate promising potentialities of the present method as far as the accuracies of the provided analytical results are concerned.

Keywords: Wine; Food analysis; Chip technology; Isotachophoresis; Column-coupling electrophoresis chip; Sample preparation; Sulfite

1. Introduction

Sulfur dioxide and various oxoanions of S(IV) are widely used in food processing and preservation. Products formed by reactions of sulfite with natural food constituents and food preservatives are numerous (for details see, e.g., references [1–3]). Of these, for example, adducts of hydrogensulfite anion with carbonyl groups of some food constituents are responsible for the fact that a part of the sulfur additive is present in food in a reversibly bound form [1]. The use of sulfiting agents in the vinification process is essential [4,5] and contents of sulfite in wines have close links with their use in this process. Adverse effects of the sulfiting agents are known [1,2,6,7] and an acceptable daily intake of sulfite advised [6]. Therefore, the determination of free and/or total sulfite is common in wine laboratories and these analytical tasks are included into a winery laboratory proficiency testing as well [8].

Several authors reviewed analytical methods and procedures applicable to the determination of sulfite in wine (see, e.g., references [9,10]). Although wine matrices are multicomponent with relatively high concentrations of organic ionic constituents, it appears that this is less important when Monier–Williams distillation method and its modifications [11,12], preferred sample preparation procedures in the determination of sulfite in foods and beverages, are employed. Here, sulfite present in the sample is converted to an equivalent amount of sulfuric acid and this is usually determined

* Corresponding author. Tel.: +421-2-60296-379; fax: +421-2-60296-706.
E-mail address: kaniansky@fns.uniba.sk (D. Kaniansky).

0021-9673/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2003.11.038

by titrimetry. Titrimetry is non-selective and it was found responsible for significant positive biases in the determination of sulfite in some food matrices [13,14]. Its replacement is a logical step and, for example, ion chromatography [15] and capillary electrophoresis (CE) [14] were shown to offer means effective in alleviating this quantitation problem. One of the modified Monier–Williams distillation procedures can be employed in the quantitation of both free and reversibly bound sulfite in wine [15]. In this instance, different temperatures at which sulfur dioxide is released from the acidified wine sample provide differentiation means for these species.

The distillation step is considered a bottleneck of the analytical methods used in the determination of sulfite and attempts to eliminate this sample pretreatment step are apparent (see, e.g., references [6,9,16–18]). When wine is alkalized the reversibly bound sulfite is released and, subsequently, sulfite can be oxidized to sulfate, for example, by hydrogen peroxide. This approach eliminates the distillation pretreatment and, for example, in combination with ion chromatography separation and quantitation of sulfate makes possible the determination of total sulfite in wine [6].

Although ethanol present in wine reduces the rate of air oxidation of sulfite [6], it seems reasonable to expect that a chemical conversion (derivatization) of this anion into a more stable constituent can enhance reliability of its quantitation in comparison to the direct determination. Isotachophoresis (ITP) determination of free sulfite in wine [19], based on formation of a stable sulfite–formaldehyde complex (hydroxymethanesulfonate, HMS), may serve as an example of the use of such an approach. This method, requiring only simple sample handling (addition of formaldehyde to wine and a proper dilution of the sample loaded onto the column), apparently keeps a pre-analysis sample manipulation at minimum. Fundamental studies, performed in a context with behavior of sulfur dioxide in atmosphere (see, e.g., references [20,21] and references given therein), indirectly support a general use of this reaction in analytical methods intended to the determination of free sulfite. Obviously, this requires that HMS is separated from the sample matrix and, besides ITP, ion chromatography [20] and capillary zone electrophoresis (CZE) [21] were proved to be convenient column separation methods for this purpose.

Currently, capillary electrophoresis becomes a subject of a broader interest in wine analysis. This is apparently due to the fact that it offers simple and rapid procedures for several groups of wine analytes (e.g., organic acids [19,22–28], amines and amino acids [29], preservatives [14,26,30,31]). Many of these CE procedures can be very likely transferred into miniaturized CE systems. Not considering a recent work dealing with the ITP separation and determination of some organic acids on a poly(methyl methacrylate) (PMMA) chip [32], research activities along this line are, however, still very limited. This situation seems reflect the fact that many of the chip based CE equipment do not provide adequate concentration sensitivities (for a current status in miniaturized CE see, e.g., references [33–36] and references given therein).

High sample loadabilities along with well-defined concentration capabilities are characteristic features of the ITP separations [37–39]. Therefore, ITP makes possible the separations of μl sample volumes on the CE chips [32,40–45] and, consequently, this method contribute to favorable concentration detectabilities of the analytes also with the aid of less sensitive CE detectors. Here, especially, the use of ITP on-line combined with ZE in the column-coupling (CC) separation compartment is analytically very beneficial [40–43,45]. This is due to the fact that the column-coupling, conceptually following an approach as proposed for conventional capillary ITP by Everaerts et al. [46], allows to perform the CE run in two stages in which specific advantages of different electrophoresis methods (e.g., ITP and ZE [47–49]) can be effectively combined.

A transfer of the CC separation technology to a chip format is a subject of our current research interest [32,34,40–45] and this work was aimed at investigating its applicability to the ITP–ZE separation and determination of free sulfite in wine. Although sulfite migrates electrophoretically we preferred, for reasons mentioned above, its determination after conversion to HMS. Choice of suitable working conditions and assessments of performance of ITP–ZE on a CC PMMA chip to the determination of free sulfite in wine in this way were main tasks of this feasibility study.

2. Experimental

2.1. Instrumentation

A CC PMMA chip employed in this work (see, Fig. 1) was manufactured by a procedure described elsewhere [50]. The separations in this miniaturized device were performed with the aid of a laboratory constructed CE equipment. This equipment includes two units (Fig. 2):

(1) An electrolyte and sample management unit (E&SMU, in Fig. 2), provided with peristaltic micropumps (P-ITP, P-ZE, P-S, P-TE, in Fig. 2) and membrane driving electrodes (E1, E2, E3, in Fig. 2). Mutual connections of these devices and their connections to the inputs to the chip channels are apparent from a scheme shown in Fig. 2. Here, the rollers of a particular pump automatically close the corresponding inlet to the chip channel when the solution pumping is stopped (they act as a valve). Excesses of the solutions pumped through the chip channels in the preparation of the run are trapped into a container (W, in Fig. 2) connected to a permanently opened outlet channel of the chip. The membrane driving electrodes are used to eliminate disturbances due to the bubble formation during the separation (a concept of their design can be found elsewhere [34]).
(2) An electronic and control unit (E&CU, in Fig. 2) delivers the driving current either to the counter-electrode of the ITP channel (E1, in Fig. 2) or to the counter-electrode of the ZE channel (E2, in Fig. 2). The change of the

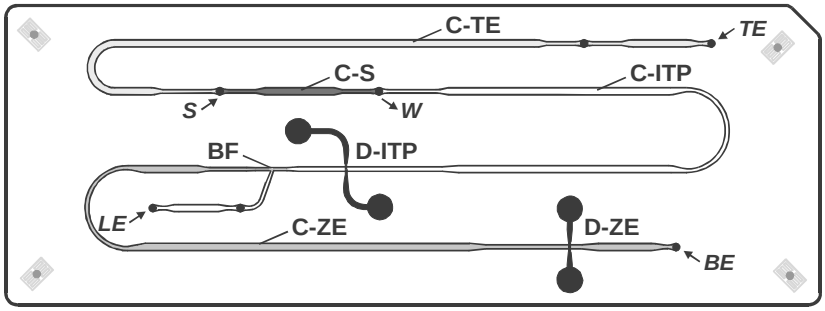

Fig. 1. An arrangement of the channels on a PMMA CC chip and their dimensions. C-TE: terminating electrolyte channel [a 9.8 μl volume; 60 × 0.2–0.5 × 0.2–0.38 (length × width × depth)]; C-S: sample injection channel (a 0.9 μl volume; 12 × 0.2–0.5 × 0.2 mm); C-ITP: ITP separation channel (a 4.5 μl volume; 59 × 0.2–0.5 × 0.14–0.2 mm) with a platinum conductivity sensor, D-ITP; C-ZE: ZE separation channel (a 4.3 μl volume; 56 × 0.2–0.5 × 0.14–0.2 mm) with a platinum conductivity sensor, D-ZE; BF: bifurcation section. BE, LE, TE, S: inlets for the background, leading, terminating and sample solutions to the chip channels, respectively. W: outlet for the solutions from the chip channels.

direction of the driving current (the column-switching) is actuated via a relay (HVR, in Fig. 2).

The E&CU includes the measuring electronics of the ac contact conductivity detectors. The measuring electronics is galvanically decoupled from the platinum conductivity sensors on the chip (sputtered on the cover of the channels of the chip [50]) by transformers [37]. The E&CU drives the peristaltic pumps of the E&SMU in the preparation step of the run. Its control unit (CU, in Fig. 2) also interfaces the CE equipment to a PC.

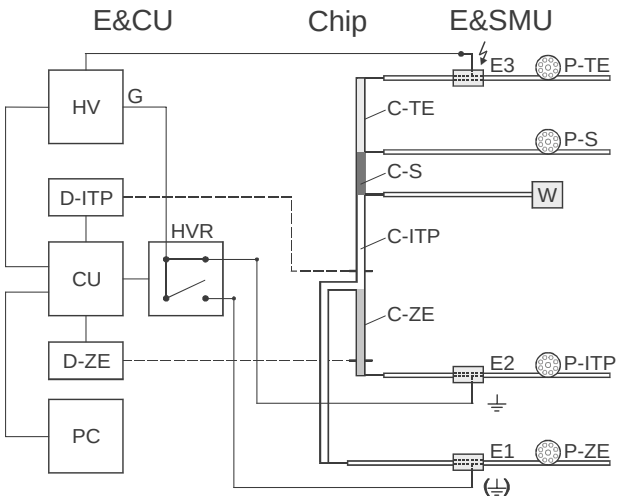

Fig. 2. A scheme of the CE equipment provided with the CC chip. Electronic and control unit (E&CU): CU: control unit; HV: high-voltage power supply (0–50 μA, 0–7 kV); D-ITP, D-ZE: conductivity detectors for the ITP and ZE separation channels, respectively; HVR: high-voltage relay switching the direction of the driving current in the separation compartment (moving reeds of this relay connect to the ground pole, G, of HV either E1 or E2). Electrolyte and sample management unit (E&SMU): P-ITP, P-ZE, P-S, P-TE: peristaltic pumps for filling the ITP (C-ITP), ZE (C-ZE), terminating (C-TE) and sample (S) channels with the electrolyte and sample solutions, respectively. W: waste container connected to the outlet hole on the chip. E1, E2: driving electrodes for the ITP and ZE separation channels, respectively; E3: terminating driving electrode connected to a high-voltage pole of HV.

MicroCE Win software (version 2.4), written in the laboratory, was used for automated preparations of the runs (filling of the chip channels with the corresponding solutions in a required sequence), provided a time-programmed control of the ITP–ZE runs (including the column-switching operation during the run derived from the signal of the conductivity detector in the ITP channel), acquired the detection data and provided their processing.

2.2. Chemicals, electrolyte solutions and samples

Chemicals used for the preparation of the electrolyte solutions and the solutions of model samples were obtained from Merck (Darmstadt, Germany), Sigma–Aldrich (Seelze, Germany), Serva (Heidelberg, Germany) and Lachema (Brno, Czech Republic).

Methylhydroxyethylcellulose 30000 (Serva), purified on a mixed-bed ion exchanger (Amberlite MB-1, Merck), was used as a suppressor of electroosmotic flow. It was added to the electrolyte solutions or it was applied as a coating of the inner walls of the separation channels [51]. Compositions of the electrolyte solutions employed in the ITP–ZE separations on the chip are given in Table 1.

Water demineralized by a Pro-PS water purification system (Labconco, Kansas City, KS, USA), and kept highly demineralized by a circulation in a Simplicity deionization unit (Millipore, Molsheim, France), was used for the preparation of the electrolyte and sample solutions. The electrolyte solutions were filtered by disposable syringe membrane filters of 0.8 μm pore sizes (Millipore) before the use.

A stock aqueous solution of sodium sulfite (Merck) was prepared fresh daily, while the stock solution of its complex with formaldehyde (HMS), corresponding to a 1000 mg/l concentration of sulfite in a 10 mmol/l formaldehyde, was stable, at least, for 1 week when stored in the refrigerator at +4 °C.

Test samples of white and red wines employed in this work are listed in Table 2. They were analyzed immediately after bottle (storage container) opening. A particular wine sample was diluted in a 10 ml volumetric flask in an

Table 1
Electrolyte system

ITP		ZE	
Leading anion	Chloride	Carrier anion	Succinate
Concentration (mmol/l)	10	Concentration (mmol/l)	15
Counter-ion	β-Alanine	Counter-ion	β-Alanine
Additive	MHEC	Additive	MHEC
Concentration (%, w/v)	0.05	Concentration (%, w/v)	0.2
pH	3.0	pH	4.0
Terminating anion	Tartrate		
Concentration (mmol/l)	20		
Counter-ion	β-Alanine		
Additive	MHEC		
Concentration (%, w/v)	0.05		
pH	3.9		

MHEC: methylhydroxyethylcellulose.

aqueous solution containing formaldehyde (corresponding to a 10 mmol/l final concentration) and the terminating electrolyte solution (corresponding to a 1 mmol/l final concentration of the terminating anion). The solution was made up to the mark with freshly demineralized water. This step gave 15-fold dilutions of the wine samples before their ITP–ZE analyses. To guaranty a full conversion of free sulfite to HMS, the sample was analyzed ca. 60 min after the preparation. Comparative samples (without formaldehyde) were prepared, in parallel, in the same way. To minimize oxidation losses of free sulfite, these samples were analyzed immediately after their preparations. Parts of the original samples, transferred to 1.5 ml Eppendorf tubes (with or without formaldehyde), were stored at −20 °C for further analyses.

3. Results and discussion

3.1. Separation and quantitation of sulfite present in sulfite–formaldehyde complex (HMS)

As already mentioned in the above sections we preferred conversion of free sulfite into hydroxymethanesulfonic acid (HMS) using the reaction:

$$SO_2 + HCOH + H_2O \rightarrow HOCH_2SO_3H \quad (1)$$

ITP–ZE experiments performed with model solutions containing sodium sulfite at concentrations to be expected in wines [6] revealed that already slight excesses of formaldehyde, relative to stoichiometry of the reaction, led to quantitative conversions of the preservative to HMS.

HMS is a strong acid [21] and it is stable at low pH values [1]. Such properties of HMS made possible to perform the anionic ITP–ZE separations at low pH (see the composition of the electrolyte system in Table 1) and, consequently, reduce the number of anionically migrating wine constituents. In addition, the use of a relatively mobile terminating anion (tartrate) a priori kept the number of sample constituents that could migrate in the ITP stack in the first separation stage at a minimum (an in-column sample clean-up [52]). On the other hand, the background (carrier) electrolyte employed in the ZE stage of the separation was chosen to provide, besides the resolution of HMS from the matrix constituents accompanying the analyte in the fraction provided by the ITP sample pretreatment, also an adequate detectability for HMS (corresponding to a low mg/l concentration level of free sulfite in wine) by the conductivity detector [53,54].

Repeated runs with test samples of HMS, carried out under the favored separating conditions (Table 1), characterized very good repeatabilities of both the migration time and peak area data (Table 3). Beside this, the peak area data provide a clear indication of chemical stability of this constituent under the working conditions employed on the CC chip. Small differences of mean values of the migration times of HMS when this was present in the loaded samples at different concentrations are apparent from the data presented in Table 3. ITP–ZE experiments aimed at explaining their origins showed their links with the electromigration dispersion of the HMS peak [53] (higher concentrations of HMS in the loaded sample were accompanied by proportionally larger time shifts of its peak apex). Here, we should note that this is a typical feature of the separations performed in low ionic strength background electrolytes as favored in ZE with conductivity detection [53–56].

Recommendations regarding an estimation of the concentration limit of detection (cLOD) in elution chromatography

Table 2
A list of test wine samples

Sample no.	Brand (trade name)	Specification	Producer
1	Muscat Ottonel	White wine, vintage: 2000	Boranal (Kiskoros, Hungary)
2	Frankovka modrá	Red wine, vintage: 2000	Vinársky závod (Banská Bystrica, Slovakia)
3	Heppenheimer Centgericht, Spätburgunder	Red wine, vintage: 1999	Staatsweingut Bergstrasse (Bensheim, Germany)
4	German wine standard	White wine	Deutsche Weinanalytiker (Oestrich-Winkel, Germany)
5	Titrivin, reference AA1	Red wine (lot no. A.01011205 1)	Chambre d'Agriculture de la Gironde (Blanquefort, France)
6	Titrivin, reference AA2	Red wine (lot no. A.01011205 2)	Chambre d'Agriculture de la Gironde
7	Titrivin, reference AA3	Red wine (lot no. A.01011205 3)	Chambre d'Agriculture de la Gironde
8	Titrivin, reference AA4	White wine (lot no. A.01011205 4)	Chambre d'Agriculture de la Gironde

Table 3
Repeatabilities of the migration times and peak areas of HMS present in model samples

Concentration (mg/l)	Total migration time		Peak area		n
	Mean (s)	R.S.D. (%)	Mean (mV s)	R.S.D. (%)	
1[a]	698	0.3	57.2	3.6	10
4[b]	706	1.1	222.4	4.4	25
8[a]	712	0.7	445.7	0.8	10

The separations were carried out in the electrolyte system given in Table 1. The driving currents were 30 and 15 μA in the ITP and ZE separation channel, respectively.
[a] ITP–ZE runs were performed in 1 day.
[b] ITP–ZE runs were performed in 5 days (a freshly prepared test sample of HMS was employed each day).

[57] were followed in this work. An actual cLOD value for sulfite was calculated from the detection data as obtained in the CZE stage of the runs with model samples. The following relationship was used for the calculation:

$$\mathrm{cLOD} = \frac{3N_{p-p}}{5S} \quad (2)$$

where N_{p-p} is the peak-to-peak noise of the detector on the baseline during the run in the CZE stage; S the slope of the dependence of the peak height on the concentration of the analyte for a 0.9 μl injection volume (in this work it was estimated from the data obtained for 0.2–1.0 mg/l concentrations of the analyte). This procedure gave for sulfite the cLOD value of 60 μg/l (ca. 1 μmol/l).

The calibration graph was determined for 0.2–5.0 mg/l concentrations of sulfite (loaded onto the chip in the calibration samples in which it was converted into HMS in the way as described in Section 2.2) to cover its concentration span expected in wine samples. It was described by a linear regression equation: $y = 3.6 + 48.33x$ (y: peak area in mV s units; x: concentration of sulfite in mg/l) with a correlation coefficient of 0.9992 for 18 data points.

3.2. ITP sample pretreatment and ZE quantitation of HMS in wine matrices

In introductory experiments we preferred a scheme of the ITP–ZE run as shown in Fig. 3A. Here, HMS was focused

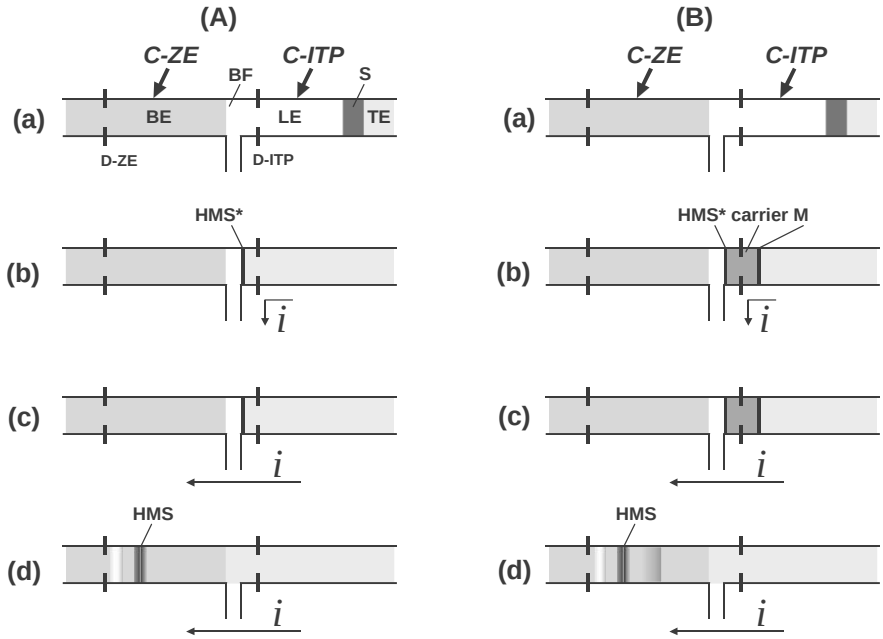

Fig. 3. Schemes of the ITP–ZE separations as employed in the analysis of wine on the CC chip. (A) a scheme of the separation with a transfer of the constituents stacked in one band (HMS*) to the ZE channel. (a) An initial arrangement of the solutions in the chip channels; (b) end of the run in the ITP channel; (c) a transfer of the stacked sample constituents (HMS*) to the ZE channel by switching the direction of the driving current; (d) the separation and detection of the transferred sample constituents in the ZE channel. (B) a scheme of the separation with an enhanced ITP sample clean-up via the carrier and spacing effect of dichloroacetate. Operational steps (a–d) have identical functions as in the scheme A (meanings of the relevant symbols are the following: HMS*: HMS containing band stacked between the leading anion and dichloroacetate; carrier: dichloroacetate zone; M: a band of the matrix constituents stacked between dichloroacetate and the terminating anion). C-ITP, C-ZE: the ITP and ZE separation channels on the CC chip, respectively; BF: bifurcation section; LE, TE, BE: the leading, terminating and background electrolyte solutions, respectively; S: sample; D-ITP, D-ZE: detection sensors in the ITP and ZE separation channels, respectively; i: direction of the driving current.

between the chloride and tartrate zones in the ITP stage of the run (a HMS*band, in Fig. 3A). As could be expected [52], a narrow span of the effective mobilities, determined by these leading and terminating constituents, restricted the number of anionic wine constituents accompanying HMS in the ITP stack and, in fact, led to a selective ITP concentration of the analyte. A majority of the anionic wine constituents migrated zone electrophoretically in the terminating zone in this stage and their effective mobilities corresponded to the steady-state composition of the terminating zone [37] as this acted as the background electrolyte for these sample constituents.

In screening runs we found that for a majority of wine samples the above sample pretreatment scheme was not sufficient as one of the matrix constituents, transferred into the ZE stage in the HMS* fraction (the step c, in Fig. 3A), was not resolved from HMS. ITP–ZE experiments carried out with model samples containing organic acids currently present in wine [19,22–26,28,32] revealed that this occurred in instances when the concentration of pyruvate in wine was not negligible relative to that of free sulfite (see an electropherogram in Fig. 4a). This pyruvate disturbance was eliminated by the use of dichloroacetate in the function of discrete spacer [37,52]. Added to the sample, this anion spaced some of the wine constituents into the boundary layer formed by the dichloroacetate and terminating (tartrate) zones (constituents present in the band M, in Fig. 3B). For

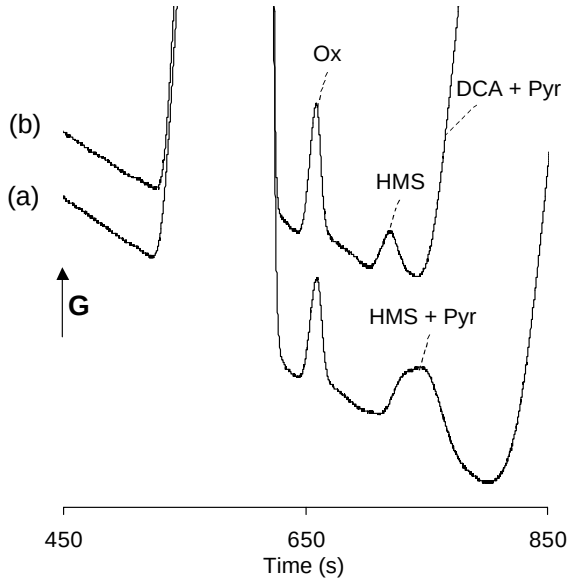

Fig. 4. An impact of dichloroacetate (DCA) on a purity of the HMS peak in the presence of pyruvate (Pyr) and oxalate in the loaded sample. (a) An electropherogram from the separation of a model sample containing HMS (its concentration corresponded to a 1 mg/l concentration of sulfite), pyruvate (5 mg/l) and oxalate (1 mg/l, Ox) using a scheme as shown in Fig. 3A; (b) the same sample as in (a) separated with the aid of the scheme as shown in Fig. 3B (the loaded sample contained, in addition, DCA at a 200 mg/l concentration). The separations were carried out in the electrolyte system given in Table 1 with 30 and 15 μA driving currents in the ITP and ZE separation channels, respectively. G: increasing conductance.

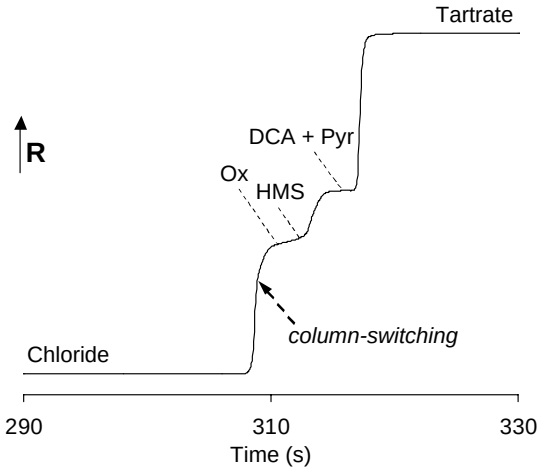

Fig. 5. An ITP migration configuration of HMS, oxalate, pyruvate and dichloroacetate. The sample loaded onto the chip contained HMS (corresponding to a 20 mg/l concentration of sulfite), pyruvate (30 mg/l, Pyr), oxalate (20 mg/l, Ox) and dichloroacetate (DCA) at a 30 mg/l concentration. The ITP separation was carried out in the electrolyte system given in Table 1 with a 30 μA driving current. A position of an arrow corresponds to a front edge of the HMS* band transferred to the ZE stage (steps b and c, in Fig. 3B). R: increasing resistance.

pyruvate it acted as a carrier anion [37] and proportionally diluted pyruvate in the ITP stack (see an isotachopherogram in Fig. 5). From the point of view of the separation in the ZE stage it is apparent that such a migration configuration, in fact, selectively increased the injection dispersion of pyruvate linked with its transfer to this stage. An analytical benefit attributable to the use of dichloroacetate documents electropherograms in Fig. 4. We found that dichloroacetate present in the loaded sample at a 400 mg/l concentration was still effective and prevented overlaps of the HMS and pyruvate peaks also in instances when the concentration of the latter constituent was 20 mg/l (a 300 mg/l concentration in wine when its 15-fold dilution is taken into account).

3.3. ITP–ZE determination of free sulfite in wine

For reasons apparent from the above discussion the scheme shown in Fig. 3B was preferred in the ITP–ZE runs performed in a context with evaluations of quantitative aspects of the determination of sulfite. Repeatabilities of the migration and quantitation data as achieved by ITP–ZE on the CC chip for HMS, formed from free sulfite in wine samples taken into a detail study (Table 2), are summarized in Table 4. Here, we can see that the mean values of both the ITP pretreatment and ZE migration times exhibited only very small fluctuations. Analogous conclusions can be drawn from the peak area data. In addition, electropherograms shown in Figs. 6 and 7, obtained for the Chambre d'Agriculture de la Gironde and Deutsche Weinanalytiker reference wine samples, clearly document a high selectivity of the elaborated ITP–ZE procedure. These electropherograms also demonstrate advantages of the free sulfite

Table 4
Reproducibilities of the migration times and peak areas of HMS present in wine samples

Sample no.	ITP pretreatment time[a]		Migration time in the ZE stage[b]		Total migration time[c]		Peak area		*n*
	Mean (s)	R.S.D. (%)	Mean (s)	R.S.D. (%)	Mean (s)	R.S.D. (%)	Mean (mV s)	R.S.D. (%)	
3	321	0.3	403	0.6	724	0.4	73.2	2.1	4
4	327	0.6	401	0.9	728	0.7	57.2	4.3	3
6	326	0.7	400	0.3	726	0.3	14.3	6.8	4
7	324	1.5	399	0.3	723	0.8	78.4	2.8	4
8	323	0.4	393	0.8	716	0.4	45.9	3.4	4

For the sample assignments see the list in Table 2. The separations were carried out in the electrolyte system described in Table 1. The driving currents were 30 and 15 μA in the ITP and ZE separation channels, respectively.

[a] A time of the entrance of the HMS fraction to the bifurcation region of the CC chip (BF, in Fig. 3).

[b] A migration time of the analyte in ZE stage.

[c] Time in which at which the peak apex of HMS of the analyte in ITP and ZE stages.

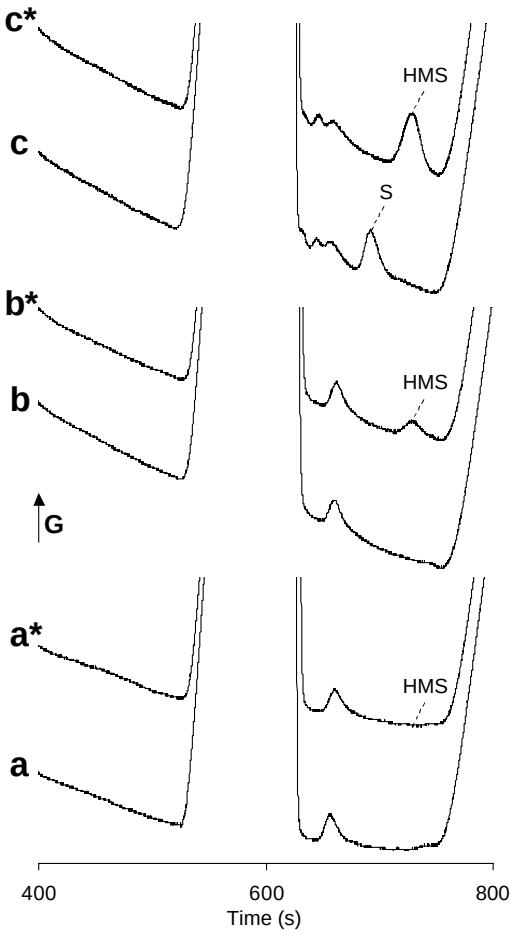

Fig. 6. Electropherograms from the ITP–ZE determination of free sulfite in Titrivin reference wine samples on the CC chip. The loaded samples were handled in the way described in Section 2.2. (a) A comparative run (sulfite was not converted to HMS) with the sample no. 5 (Table 2); (a*) the same sample as in (a) with free sulfite converted to HMS. (b) A comparative run (sulfite was not converted to HMS) with the sample no. 6 (Table 2); (b*) the same sample as in (b) with free sulfite converted to HMS. (c) A comparative run (sulfite was not converted to HMS) with the sample no. 7 (Table 2); (c*) the same sample as in (c) with free sulfite converted to HMS. The separations were carried out in the electrolyte system given in Table 1 with 30 and 15 μA driving currents in the ITP and ZE separation channels, respectively. S: sulfite, HMS: hydroxymethanesulfonate, G: increasing conductance.

determination based on its conversion to HMS. These, for example, include possibilities of confirmation of a "purity" of the migration position of HMS when the run with the comparative (original) sample is performed (a–c, in Fig. 6 and, a, in Fig. 7) and a higher detection response for HMS, when this is related to the one obtained for the corresponding response for sulfite (Figs. 6 and 7).

With respect to a 60 μg/l concentration detection limit (see Section 3.1) we could quantify on the CC chip a 3 mg/l concentration of free sulfite in wine (for an illustration see, the electropherograms b and b*, in Fig. 6). Undoubtedly, such a limit of quantification competes very well with the values reported for ZE in conventional CE equipment (see,

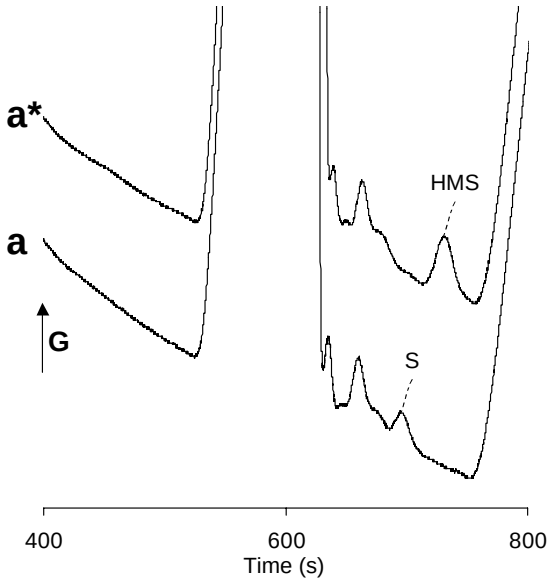

Fig. 7. Electropherograms from the ITP–ZE determination of free sulfite in a German reference wine sample on the CC chip. The loaded samples were handled in the way described in Section 2.2. (a) A comparative run (sulfite was not converted to HMS) with the sample no. 4 (Table 2); (a*) the same sample as in (a) with free sulfite converted to HMS. The separations were carried out in the electrolyte system given in Table 1 with 30 and 15 μA driving currents in the ITP and ZE separation channels, respectively. S: sulfite, HMS: hydroxymethanesulfonate, G: increasing conductance.

Table 5
ITP–ZE determination of free sulfite in wine samples

Sample no[a].	Sample dilution	Calibration curve		Standard addition[b] determined (mg/l)	Free sulfite content[c] (mg/l)	Recovery (%)
		Determined (mg/l)	S.D. (mg/l)			
1	1:15	<2	–	<2	–	–
2	1:15	<2	–	<2	–	88
3	1:15	21.6	0.3	24.1	–	88
4	1:15	16.6	1.0	18.4	27.1	90
5	1:15	<2	–	<2	–	–
6	1:15	3.3	0.4	–	–	–
7	1:15	23.2	0.7	–	–	–
8	1:15	13.1	0.9	14.8	–	89

S.D.: standard deviation.
[a] For the sample assignments see the list in Table 2.
[b] The value obtained from two parallel determinations.
[c] The value reported for a particular sample.

e.g., references [14,26]) and ion chromatography [6]. The contents of sulfite were estimated from the calibration graph (see the regression equation in Section 3.1) and for some samples also by the standard addition method (Table 5). With the exception of the sample no. 3 (the quantitations were performed for the sample taken from two different bottles) both methods provided good agreements of the results.

The content of free sulfite was available only for one of the samples taken into our study (sample no. 4, in Table 5). Our results, as obtained for this sample, were ca. 50% lower in comparison to the content claimed. Such a difference may originate in various sources of systematic errors currently associated with the determination of sulfite in wine (see, e.g., references [6,13–15]). However, a missing specification of the analytical method with which free sulfite in the reference sample was determined made its critical assessment impossible. On the other hand, the recovery values as determined for free sulfite in this work (Table 5) indicate a very good performance of the present ITP–ZE method from the point of view of accuracy of the provided analytical results.

4. Conclusions

This feasibility study showed that ZE with on-line ITP sample pretreatment on the CC chip with conductivity detection offers simple and, at the same time, sensitive and reproducible procedure to the determination of free sulfite (after its conversion to HMS) in wine. Here, both ITP and ZE, performing specific analytical tasks, contributed to a 900 μg/l concentration detectability for sulfite as attained for a 60 nl load of wine (a 15-fold wine dilution and the use of a 0.9 μl sample injection channel of the chip) and, consequently, to the determination of free sulfite when this was present in wine at a 3 mg/l concentration. In this context we should note that this value is not a minimum attainable by ITP–ZE on the present CC chip under the particular separating conditions, as a maximum sample load for wine (the volume of wine loaded onto the chip that still provides a full recovery of HMS in the ITP stage of the run) was not reached in our experiments.

The ITP–ZE separations were carried out in a hydrodynamically closed separation compartment of the chip with suppressed hydrodynamic and electroosmotic flows. Such an approach is apparently advantageous when reproducible migration velocities of the separated constituents are of a major concern [34]. Therefore, it seems logical to ascribe high precisions as typically achieved for HMS in both the model and practical samples (see Tables 3 and 4), at least, in part to the use of such transport conditions. The same arguments are applicable also for precisions as attained for the ITP sample pretreatment (see Table 4).

Pyruvate may be present in wines at concentrations that positively bias the ITP–ZE determination of HMS under the electrolyte conditions as employed in this work. The use of the carrier effect of dichloroacetate for pyruvate (dilution of pyruvate by dichloroacetate) in the ITP stage of the separation eliminated this problem. This simple solution, undoubtedly, demonstrates a flexibility of the tools available for the ITP sample pretreatment. From the analytical point of view it is also important that the ITP clean-up was accompanied by a highly selective concentration of HMS before its transfer to the ZE stage of the separation.

Recoveries of free sulfite as determined for some of the wine samples (Table 5) indicate promising potentialities of the present method as far as the accuracy of the provided analytical results is concerned. On the other hand, however, the content of free sulfite as determined by this method in one of the samples taken into our study (see, Table 5) was ca. 50% lower in comparison to the value reported for this sample (the only sample for which the data regarding the sulfite content were available). As discussed in the previous section such a difference may originate from various sources of systematic errors as currently encountered in the determination of free sulfite in wine and, therefore, it seems inappropriate to be conclusive in an interpretation of the difference found. Here, only the quantitations performed in parallel, preferably on a larger number of samples, by both

the present procedure and procedures currently employed for this purpose may lead to justified conclusions.

Acknowledgements

This work was supported by Merck (Darmstadt, Germany) and, in part, by a grant from the Slovak Grant Agency for Science (project no. 1/0087/03). The authors thank to Dr. Dieter Tanzer (Merck, Darmstadt) for valuable discussions and suggestions.

References

[1] B.L. Wedzicha, Chem. Br. 27 (1991) 1030.
[2] B.L. Wedzicha, Chemistry of Sulphur Dioxide in Foods, Elsevier Applied Science, London, 1984.
[3] J.B. Adams, Food Chem. 59 (1997) 401.
[4] J. Farkaš, Technologie a Biochemie Vína, SNTL/ALFA, Prague, 1980.
[5] T. Stevenson, Svetová Encyklopédia Vín, Ikar, Bratislava, 1999.
[6] L.M. Edmond, E.A. Magee, J.H. Cummings, LC–GC Eur. 16 (2003) 88.
[7] R. Walker, Food Addit. Contam. 2 (1985) 5.
[8] C.E. Butzke, Am. J. Enol. Viticult. 53 (2002) 163.
[9] J.M.C. Pavon, J.G. Plaza, E.V. Alonso, A.G. de Torres, M.E.U. Pozo, A.R. Molina, Quim. Anal. 16 (1997) 235.
[10] J. Sádecká, J. Polonský, J. Chromatogr. A 834 (1999) 401.
[11] G.W. Monier-Williams, Analyst 52 (1927) 415.
[12] S. Williams, Official Methods of the AOAC, Method 20.123, Association of Official Analytical Chemists, Arlington, VA, 1984.
[13] Y.C. Su, S.L. Taylor, Food Addit. Contam. 12 (1995) 153.
[14] V.C. Trenerry, Food Chem. 55 (1996) 299.
[15] L. Pizzoferrato, G. Di Lullo, E. Quattrucci, Food Chem. 63 (1998) 275.
[16] T.J. Cardwell, R.W. Cattrall, G.N. Chen, G.R. Scollary, I.C. Hamilton, Analyst 116 (1991) 253.
[17] F. Kvasnička, K. Miková, J. Food Compos. Anal. 15 (2002) 585.
[18] M. Situmorang, D.B. Hibbert, J.J. Gooding, D. Barnett, Analyst 124 (1999) 1775.
[19] J.C. Reijenga, T.P.E.M. Verheggen, F.M. Everaerts, J. Chromatogr. 245 (1982) 120.
[20] R.W. Dixon, H. Aasen, Atmos. Environ. 33 (1999) 2023.
[21] E. Dabek-Zlotorzynska, M. Piechowski, K. Keppel-Jones, R. Aranda-Rodriguez, J. Sep. Sci. 25 (2002) 1123.
[22] A. Castineira, R.M. Pena, C. Herrero, S. Garcia-Martin, J. High Resolut. Chromatogr. 23 (2000) 647.
[23] A. Castineira, R.M. Pena, C. Herrero, S. Garcia-Martin, J. Food Compos. Anal. 15 (2002) 319.
[24] V. Galli, A. Garcia, L. Saavedra, C. Barbas, Electrophoresis 24 (2003) 1951.
[25] R.S. Monson, T.S. Collins, A.L. Waterhouse, Anal. Lett. 30 (1997) 1753.
[26] L. Saavedra, C. Barbas, Electrophoresis 24 (2003) 2235.
[27] J. Sádecká, J. Polonský, J. Chromatogr. A 880 (2000) 243.
[28] M. Arellano, F. Couderc, P. Puig, Am. J. Enol. Viticult. 48 (1997) 408.
[29] G. Nouadje, N. Simeon, F. Dedieu, M. Nertz, P. Puig, F. Couderc, J. Chromatogr. A 765 (1997) 337.
[30] G. Jankovskiene, Z. Daunoravicius, A. Padarauskas, J. Chromatogr. A 934 (2001) 67.
[31] D. Kaniansky, M. Masár, V. Madajová, J. Marák, J. Chromatogr. A 677 (1994) 179.
[32] M. Masár, D. Kaniansky, R. Bodor, M. Jöhnck, B. Stanislawski, J. Chromatogr. A 916 (2001) 167.
[33] P.A. Auroux, D. Iossifidis, D.R. Reyes, A. Manz, Anal. Chem. 74 (2002) 2637.
[34] D. Kaniansky, M. Masár, R. Bodor, M. Žúborová, E. Ölvecká, M. Jöhnck, B. Stanislawski, Electrophoresis 24 (2003) 2208.
[35] D.R. Reyes, D. Iossifidis, P.A. Auroux, A. Manz, Anal. Chem. 74 (2002) 2623.
[36] H.D. Willauer, G.E. Collins, Electrophoresis 24 (2003) 2193.
[37] F.M. Everaerts, J.L. Beckers, T.P.E.M. Verheggen, Isotachophoresis—Theory, Instrumentation and Applications, Elsevier, Amsterdam, 1976.
[38] P. Boček, M. Deml, B. Kaplanová, J. Janák, J. Chromatogr. 160 (1978) 1.
[39] F.E.P. Mikkers, F.M. Everaerts, J.A.F. Peek, J. Chromatogr. 168 (1979) 293.
[40] R. Bodor, M. Žúborová, E. Ölvecká, V. Madajová, M. Masár, D. Kaniansky, B. Stanislawski, J. Sep. Sci. 24 (2001) 802.
[41] R. Bodor, V. Madajová, D. Kaniansky, M. Masár, M. Jöhnck, B. Stanislawski, J. Chromatogr. A 916 (2001) 155.
[42] R. Bodor, D. Kaniansky, M. Masár, K. Silleová, B. Stanislawski, Electrophoresis 23 (2002) 3630.
[43] D. Kaniansky, M. Masár, J. Bielčíková, F. Iványi, F. Eisenbeiss, B. Stanislawski, B. Grass, A. Neyer, M. Jöhnck, Anal. Chem. 72 (2000) 3596.
[44] E. Ölvecká, M. Masár, D. Kaniansky, M. Jöhnck, B. Stanislawski, Electrophoresis 22 (2001) 3347.
[45] E. Ölvecká, M. Koníková, N. Grobuschek, D. Kaniansky, B. Stanislawski, J. Sep. Sci. 26 (2003) 693.
[46] F.M. Everaerts, T.P.E.M. Verheggen, F.E.P. Mikkers, J. Chromatogr. 169 (1979) 21.
[47] D. Kaniansky, J. Marák, J. Chromatogr. 498 (1990) 191.
[48] D.S. Stegehuis, H. Irth, U.R. Tjaden, J. Vandergreef, J. Chromatogr. 538 (1991) 393.
[49] L. Křivánková, F. Foret, P. Boček, J. Chromatogr. 545 (1991) 307.
[50] B. Grass, A. Neyer, M. Jöhnck, D. Siepe, F. Eisenbeiss, G. Weber, R. Hergenröder, Sens. Actuators B: Chem. 72 (2001) 249.
[51] D. Kaniansky, M. Masár, J. Bielčíková, J. Chromatogr. A 792 (1997) 483.
[52] D. Kaniansky, J. Marák, J. Laštinec, J.C. Reijenga, F.I. Onuska, J. Microcolumn Sep. 11 (1999) 141.
[53] F.E.P. Mikkers, F.M. Everaerts, T.P.E.M. Verheggen, J. Chromatogr. 169 (1979) 1.
[54] P. Gebauer, J. Caslavska, W. Thormann, P. Boček, J. Chromatogr. A 772 (1997) 63.
[55] F. Foret, L. Křivánková, P. Boček, Capillary Zone Electrophoresis, VCH, Weinheim, 1993.
[56] F.E.P. Mikkers, F.M. Everaerts, T.P.E.M. Verheggen, J. Chromatogr. 169 (1979) 11.
[57] J.P. Foley, J.G. Dorsey, Chromatographia 18 (1984) 503.