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Determination of organic acids and inorganic anions in wine by isotachophoresis on a planar chip

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Abstract

Isotachophoretic (ITP) separation and determination of a group of 13 organic and inorganic acids, currently present in wines, on a poly(methyl methacrylate) chip provided with on-column conductivity detection was a subject of a detailed study performed in this work. Experiments with the ITP electrolyte systems proposed to the separation of anionic constituents present in wine revealed that their separation at a low pH (2.9) provides the best results in terms of the resolution. Using a 94 mm long separation channel of the chip, the acids could be resolved within 10–15 min also in instances when their concentrations corresponded to those at which they typically occur in wines. A procedure suitable to the ITP determination of organic acids responsible for some important organoleptic characteristics of wines (tartaric, lactic, malic and citric acids) was developed. Concentrations of 2–10 mg/l of these acids represented their limits of quantitation for a 0.9 μl volume sample loop on the chip. A maximum sample load on the chip, under the preferred separating conditions, was set by the resolution of malate and citrate. A complete resolution of these constituents in wine samples was reached when their molar concentration ratio was 20:1 or less. ITP analyses of a large series of model and wine samples on the chip showed that qualitative indices [RSH (relative step height) values] of the acids, based on the response of the conductivity detector, reproduced with RSD better than 2% while reproducibilities of the determination of the acids of our interest characterized RSD values better than 3.5%.

Keywords: Isotactophoresis; Chip technology; Wine; Food analysis; Coupled columns; Organic acids; Inorganic anions

1. Introduction

Organoleptic characteristics of wines are closely linked with contents of some organic acids. Therefore, α-hydroxy acids (tartaric, malic, lactic and citric acids), mainly, responsible for these characteristics are routinely determined not only in wines but also in various phases of the production process [1,2]. Other acids or anionic constituents to be determined in wines in some specific situations include acetate, ascorbate, sulfite, sulphate, phosphate, malonate, gluconate and sorbate [1,2].

At present, the use of spectrophotometry [2–5], enzymatic methods [2] and various column chromatography techniques [2,6–11] dominates in the determination of acidic constituents of wine. The ionogenic nature of these analytes makes capillary

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electrophoresis (CE) techniques very convenient alternatives for these analytical tasks. Their capabilities in the separation and/or determination of organic acids, inorganic anions and preservatives in white and red wines were already demonstrated (see, e.g., Refs. [10–25]). In this respect the use of capillary isotachophoresis (ITP) with conductivity [12–17], thermometric [18] and UV absorption [12,18] detections should be mentioned. Recently, however, capillary zone electrophoresis (CZE) with direct or indirect photometric [10,11,19–24] and conductivity [24–25] detections attracts more attention in wine analysis. None or only minimum sample preparation and short analysis times are apparent advantages of both CE techniques in the analysis of wine.

A poly(methyl methacrylate) planar chip applicable to various modes of ITP and ITP–CZE separations provided with the column-coupling (CC) configuration of the separation channels and on-column conductivity detection sensors was developed recently in our laboratories [26]. It was already shown to offer analytical advantages of the CC technology in CE separations performed in a miniaturized format [27]. The present work was aimed at investigating a potential applicability of the CC chip to the ITP separations and determination of the above acids in wine. Various electrolyte systems proposed for the analysis of wines in conventional ITP equipment were assessed from the point of view of their applications to ITP wine analysis on the chip. An ITP method suitable to the determination of acids responsible for some organoleptic characteristics of wines (tartaric, malic, lactic and citric acids) was elaborated. Its practical utility in the analysis of wine samples was demonstrated.

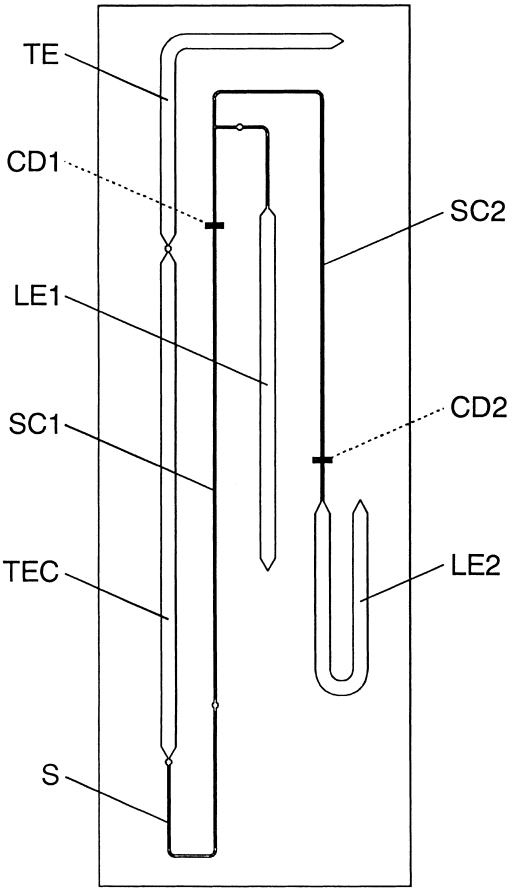

Fig. 1. A schematic of the CC chip used in this work. TE, terminating buffer reservoir (8.8 μl); TEC, terminating channel (9 μl); S, a 0.9 μl sample injection channel [21.8×0.26×0.2 mm (length×width×depth)]; SC1, the first separation channel (a 2.1 μl volume; 51.8×0.26×0.2 mm) with a platinum conductivity sensor (CD1); SC2, the second separation channel (a 2.6 μl volume; 64.0×0.26×0.2 mm) with a platinum conductivity sensor (CD2); LE1, LE2, leading buffer reservoirs (each 8.8 μl); W, waste outlet.

2. Experimental

2.1. Instrumentation

A schematic arrangement of the channels of a poly(methyl methacrylate) (PMMA) CC chip used in this work is given in Fig. 1 (for further details see design No. 2 in [26]). The ITP separations on the chip were performed on laboratory constructed CE equipment [27]. This equipment includes two units:

(1) An electrolyte and sample management unit (E&SMU, in Fig. 2), connected via 300 μm I.D. FEP (fluorinated ethylene–propylene copolymer) capillary tubes to the inlets of the channels on the chip. Valves of this unit (V1, V2, VT and VS, in Fig. 2) serve to open these inlets on filling the channels and they are closed during the CE runs. Pumping syringes (P1,

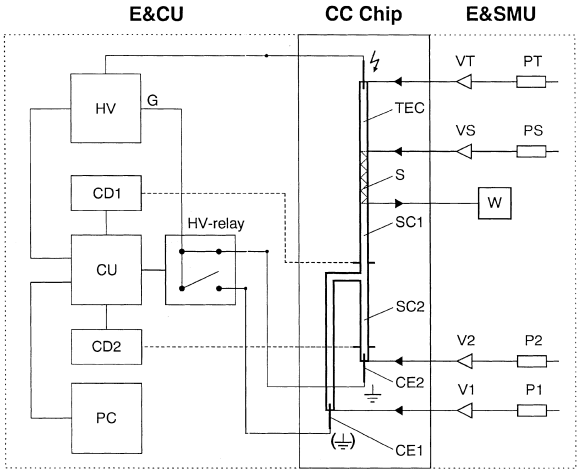

Fig. 2. A block scheme of the CE equipment with the closed separation compartment of the chip. E&CU, electronic and control unit; HV, high-voltage power supply [its high-voltage pole is connected to the driving electrode in the high voltage (terminating) channel of the chip, TEC]; CD1, CD2, conductivity detectors for the first and second separation channels, respectively; HV-relay, a 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 CE1 or CE2). CU, control unit; PC, personal computer; S, sample injection channel; SC1, the first separation channel with a platinum conductivity sensor (connected to CD1); SC2, the second separation channel with a platinum conductivity sensor (connected to CD2); CE1, CE2, counter-electrodes for the first and second separation channels, respectively. E&SMU, electrolyte and sample management unit; V1, V2, VT, needle valves for the inlets of the separation and terminating channels; VS, a pinch valve for the inlet of the sample injection channel; W, waste container. P1, P2, PS, PT, syringes for filling the first, second, sample injection and terminating channels with the electrolyte and sample solutions, respectively.

P2, PS, PT, in Fig. 2), connected to the inlets of the corresponding valves, deliver appropriate electrolyte solutions and the sample to the channels before the separation. An outlet channel of the chip, connected to a waste container (W, in Fig. 2), is permanently opened.

(2) An electronic and control unit (E&CU, in Fig. 2) delivers the driving current, measures conductivity using platinum detection sensors sputtered on the channels of the chip and interfaces the CE equipment with a personal computer. This unit includes the following modules: (i) High-voltage power supply (HV, in Fig. 2), delivering the stabilized driving current in the range of 0–50 μA with a maximum voltage of 5 kV connected to the chip; (ii) High-voltage-relay (HV-relay, in Fig. 2), for the column-switching operation of the equipment; (iii) Two conductivity detectors (CD1 and CD2, in Fig. 2), decoupled from the detection sensors on the chip by transformers with PTFE insulated coils. The detector for the first channel (CD1) is provided with a comparator circuit to identify a front boundary of the ITP zone of a selected effective mobility (needed in a control of the column-switching operation of the equipment); (iv) Control unit (CU, in Fig. 2), connecting the CE unit to a Pentium personal computer.

ITP Win software (version 2.31) obtained from Kascomp (Bratislava, Slovak Republic) was used for a time-programmed control of the CE runs and for the acquisition of the detection data and their processing.

2.2. Chemicals

Analytical grade chemicals used for the preparation of electrolyte solutions were obtained from Merck (Darmstadt, Germany) and Serva (Heidelberg, Germany). Some were purified by conventional purification methods. Methylhydroxyethylcellulose 30 000 (Serva) in the leading electrolyte solutions served as a suppressor of electroosmotic flow. Demineralized water from Milli Q (Millipore, Molsheim, France) was used for the preparation of solutions. Electrolyte solutions were filtered by 0.8 μm pore size membrane filters (Millipore) and stored in the refrigerator at +4°C.

2.3. Samples

Stock solutions of the analytes were prepared at 600–2100 mg/l concentrations from analytical grade chemicals (Merck). Red and white wine samples were obtained from a vineyard near Darmstadt (Germany). They were diluted 20–100 times by demineralized water before the analysis. The sample solutions were filtered by a 0.8 μm pore size membrane filter (Millipore) and then stored in a refrigerator at +4°C.

3. Results and discussion

3.1. Separating conditions

A detail assessment of ITP separabilities of organic acids and other anionic constituents to be determined in wine was performed with model samples resembling the composition of wine. The electrolyte systems employed in the ITP analysis of wine in conventional equipment [12–18] were used in our assessment. Covering a pH range of 2.9–6, the leading electrolytes used with the ITP separations resulted in various separation mechanisms. The electrolyte system in the composition identical to that proposed by Reijenga et al. [12] provided the best results in terms of the resolution of the acids of our interest. With the exception of sulfate (migrating with the effective mobility close to that of chloride), it resolved all analytes of interest in one ITP run (see Fig. 3). Sulfite, also present in the injected sample, was probably oxidized to sulfate under these separating conditions. Its ITP determination in wine, however, requires a different approach [12].

Other electrolyte systems tested in this work failed to resolve some of the acids (e.g., the resolution of malate and citrate was usually critical) in a 94 mm long tandem coupled separation system [27] of the CC chip (a sequential ITP separation in both separation channels; SC1 and SC2 in Fig. 1).

3.2. Quantitation of organic acids

In the analysis of anionic wine constituents a top priority is attributed to the determination of α-hydroxy acids (tartaric, lactic, malic and citric acids), as these are mainly responsible for a total acidity of wine and some of its key organoleptic characteristics [2]. For obvious reasons, we paid attention to their ITP determination on the CC chip. This part of our study was carried out under separating conditions (Table 1) providing a complete resolution of the acids to be expected in wine samples at concentrations detectable by the conductivity detector on

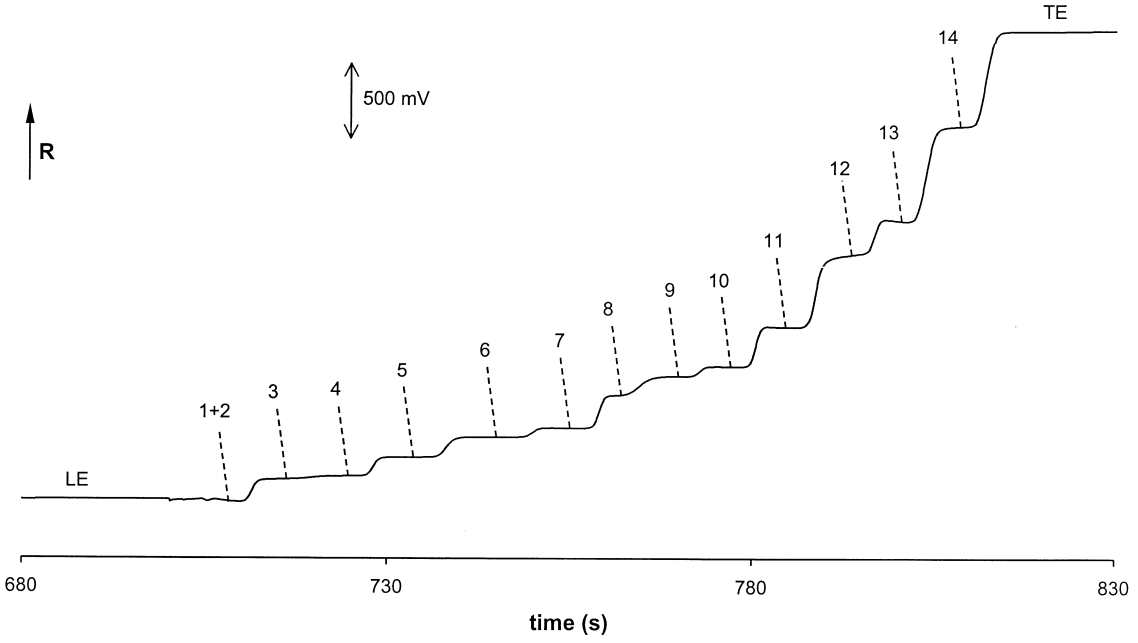

Fig. 3. Isotachophoretic separation of organic acids and inorganic anions present in a model mixture on the CC chip. Zone assignments: LE, leading anion (chloride); 1*, migration position of sulphate; 2, sulphite; 3, phosphate; 4, malonate; 5, tartrate; 6, citrate; 7, malate; 8, lactate; 9, gluconate; 10, aspartate; 11, succinate; 12, ascorbate; 13, acetate; 14, sorbate; TE, terminating anion (capronate). The separation was carried out in the electrolyte system (Table 1) using capronate as a terminating ion in both separation channels. The driving current was 10 μA. The concentrations of the analytes in the injected model sample were 12–42 mg/l. R, increasing resistance.

Table 1
Electrolyte systems

Parameter	
Solvent	Water
Leading ion	Chloride
Concentration (m*M*)	10
Counter-ion	β-Alanine
pH	2.9
Suppressor of electroosmotic flow	Methylhydroxyethylcellulose
Concentration (%, w/v)	0.1
Terminating ion	Glutamate or capronate[a]
Concentration (m*M*)	5.0
Counter-ion	Histidine
pH	5.0

[a] The use of an actual constituent is given in the text.

the chip. We found that under these separating conditions the lowest concentrations at which the acids of interest could still be quantified were in the range of 2–10 mg/l.

A mutual resolution of citrate and malate was found to be critical in reaching a complete ITP resolution of the acids present in wine samples. Under the preferred working conditions we could resolve them when a 20 mg/l concentration of citrate was accompanied in the sample (injected by a 0.9 μl volume sample loop on the chip) by a 270 mg/l concentration of malate. These amounts, in fact, determined a maximum loadability of an actual wine sample on the chip.

Reproducibilities of qualitative indices of the acids in the ITP separations {RSH (relative step height) values, using the leading and terminating anions as Ref. [28]}, expressed via RSD values, were better than 2% for both the model and wine samples (see Table 2). The zone lengths for the four acids reproduced with the RSD values 3.5% or better when their concentrations in the injected model samples corresponded to those currently expected in wine samples (Table 2). Parameters of the regression equations for the calibration graphs used in the quantitation of the acids in wine samples are given in Table 3.

3.3. Analysis of wines

Typical isotachophoreograms as obtained from the analyses of white and red wines performed in this work are given in Figs. 4 and 5, respectively. Relevant data characterizing the quantitation of the acids of interest in these particular samples are summarized in Table 4. From these data and from those obtained for model samples (Table 2) it is apparent that the reproducibilities attained in the analysis of practical samples did not deviate from those characterizing the quantitation of these acids in model samples.

4. Conclusions

Isotachophoresis performed on the CC chip with a 94 mm total length of the separation channel and the

Table 2
Reproducibilities of the RSH values and zone lengths of the studied organic acids present in the model and real samples[a]

Organic acid	Concentration (mg/l)	RSD of RSH (%)	RSD of zone length (%)	*n*	Concentration (mg/l)	RSD of RSH (%)	RSD of zone length (%)	*n*
	Model samples							
Tartrate	22.5	1.37	2.47	7	60.0	1.85	2.22	9
Citrate	12.6	1.19	3.10	7	33.6	1.88	2.80	9
Malate	60.3	0.99	1.99	7	160.9	1.72	2.13	9
Lactate	13.5	0.47	1.13	7	36.0	1.32	2.65	9
	White wine				Red wine			
Tartrate	53.6	1.32	1.70	5	35.3	3.05	2.91	5
Citrate	24.4	1.53	1.66	5	41.1	1.65	3.55	5
Malate	178.9	0.75	1.65	5	198.8	1.97	2.39	5
Lactate	27.4	0.73	1.38	5	40.8	0.53	2.62	5

[a] RSD, relative standard deviation; RSH, relative step height calculated for glutamate as a reference; *n*, number of parallel measurements.

Table 3
Parameters of the regression equations ($y = a + bx$) for the calibration graphs of organic acids[a]

Organic acid	a (s)	b (s.l/mg)	r	n	Δx (mg/l)
Tartrate	−0.55	0.370	0.9980	20	15.0–105.1
Citrate	0.16	0.315	0.9988	20	8.4–58.8
Malate	−3.16	0.341	0.9981	20	40.2–281.6
Lactate	1.82	0.240	0.9975	20	9.0–63.1

[a] a, intercept; b, slope of the calibration line; n, number of data points; x, concentration of the analyte; y, zone length; Δx, concentration interval for which the calibration data were measured.

conductivity detection of the zones was found suitable in the separation of 13 anionic constituents (organic acids and inorganic anions), currently occurring in wines. Using an optimum electrolyte system, in this respect pH 2.9, separation of these constituents via their differences in pK_a values, provided the analysis times of 10–15 min.

Although a sample loadability of the present chip is significantly lower in comparison to conventional ITP equipment, it was sufficient to separate anionic constituents present in 0.9 μl volumes of 20–100 times diluted wine samples. A maximum sample loadability in the analysis of wine samples was set by the resolution of citrate and malate. Nevertheless, this pair of the analytes could be still resolved and quantified when a citrate to malate molar concentration ratio was 1:20.

Reproducible ITP determination of tartaric, lactic, malic and citric acids (the acids responsible for some organoleptic characteristics of wines and their qual-

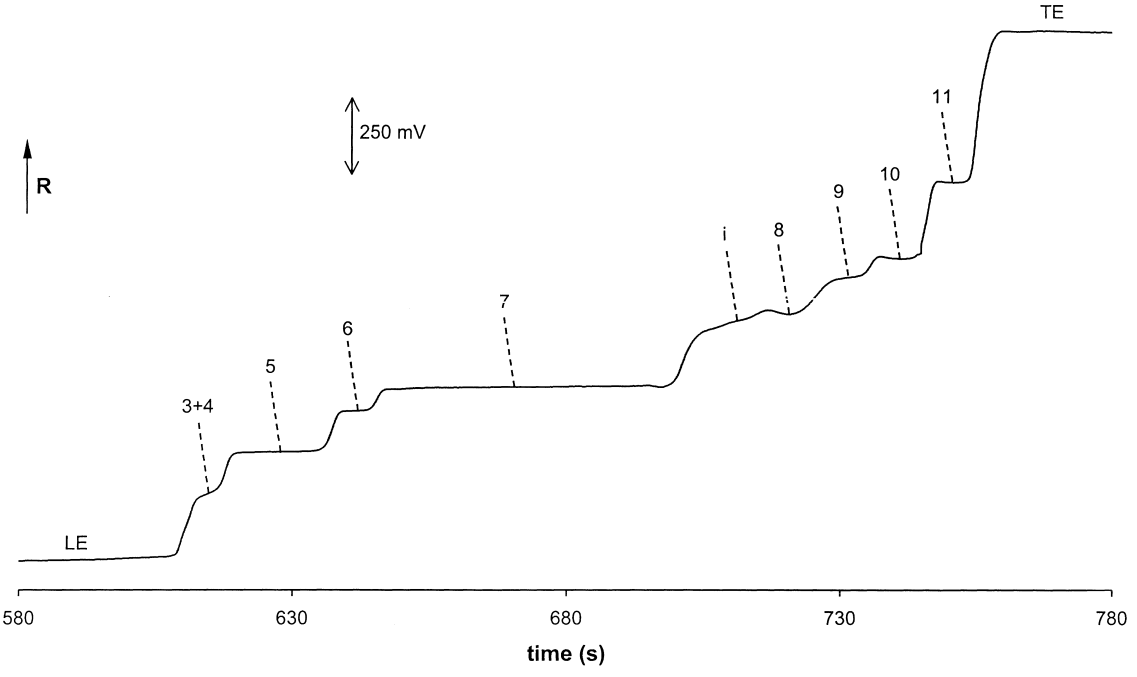

Fig. 4. An isotachophoreogram from the ITP separation of organic acids and inorganic anions present in a white wine sample (20 times diluted) on the CC chip. The separation was carried out in the electrolyte system (Table 1) using glutamate as a terminating ion in both separation channels. The driving current was 20 μA in the first separation channel, in the second channel it was 10 μA. LE, leading anion (chloride); TE, terminating anion (glutamate); i, unidentified wine constituents. For the zone assignments, see the legend to Fig. 3.

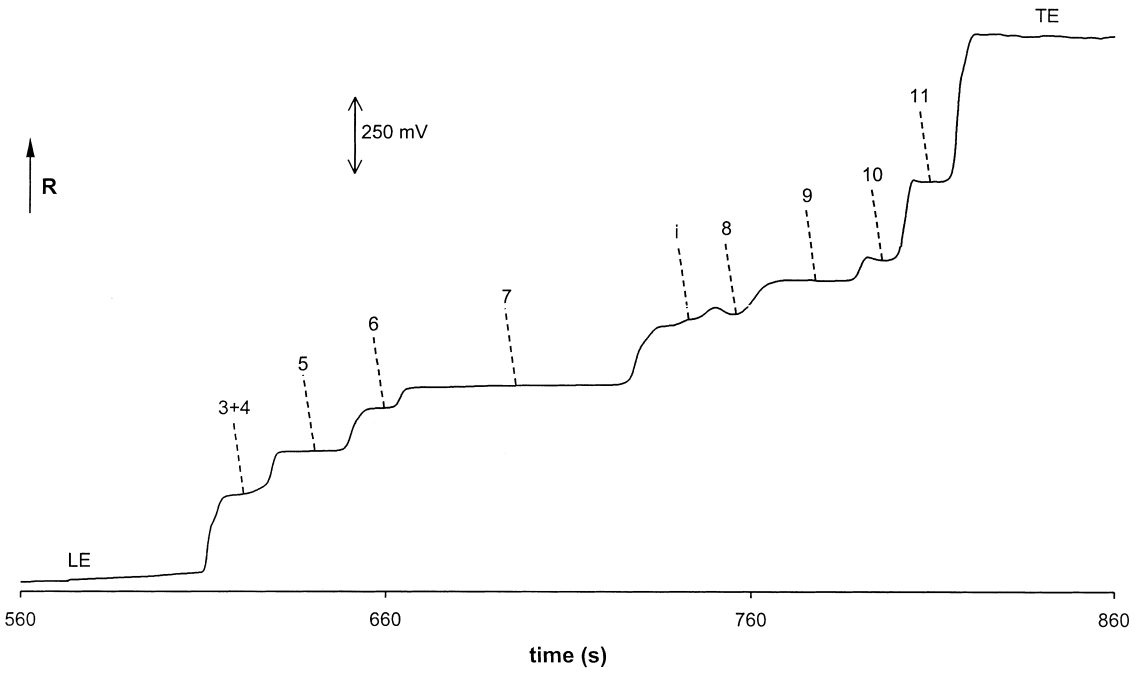

Fig. 5. An isotachophoreogram from the separation of organic acids and inorganic anions present in a red wine sample (20 times diluted) on the CC chip. The separation was carried out in the electrolyte system (Table 1) using glutamate as a terminating ion in both separation channels. The driving current was 20 μA in the first separation channel, in the second channel it was 10 μA. LE, leading anion (chloride); TE, terminating anion (glutamate); i, unidentified anionic constituents from the sample. For the zone assignments, see the legend to Fig. 3.

Table 4
Determination of organic acids in wines[a]

Organic acid	White wine			Red wine		
	Determined (mg/l)	RSD (%)	*n*	Determined (mg/l)	RSD (%)	*n*
Tartrate	1072.7	1.65	5	1243.6	2.84	5
Citrate	488.2	1.69	5	821.3	3.59	5
Malate	3576.9	1.57	5	3976.8	2.28	5
Lactate	547.4	1.76	5	814.9	2.08	5

[a] RSD, relative standard deviation; *n*, number of parallel determinations.

ity) was typical under our working conditions. It should be noted that the reproducibilities attained for wine samples did not deviate from those determining the acids in model samples resembling that of compositions of tested wines.

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