Journal of Chromatography, 245 (1982) 120–125
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14.930

Note

Simultaneous determination of organic and inorganic acids and additives in wines by capillary isotachophoresis using UV and a.c. conductivity detection

J. C. REIJENGA, Th. P. E. M. VERHEGGEN and F. M. EVERAERTS*
Eindhoven University of Technology, Laboratory of Instrumental Analysis, P.O. Box 513, 5600 MB Eindhoven (The Netherlands)

(Received March 26th, 1982)

In cooperation with an international organization for quality control of various food products (Monde Selection, Brussels, Belgium), a wide variety of wines were analysed. The main purpose of the analyses was to check for any serious violations of the EEC Legislation on Consumer Goods, especially with respect to the addition of preservatives.

About 100 wines from 13, mainly non-European, countries were analysed by capillary isotachophoresis. Sorbic, tartaric, ascorbic, citric and sulphurous acid could simultaneously be analysed. Several other inorganic and organic acids (Krebs cycle), were determined in the same run. The simultaneous determination of these ionic compounds with other analytical techniques has been performed in the past. Of these, the application of gas chromatography is hampered by the need for extensive sample pre-treatment (derivatisation) procedures, and in high-performance liquid chromatography the detection of these mostly non-UV-absorbing compounds is a limiting factor. Some applications of isotachophoresis with thermometric detection in food analysis have been reviewed[1]. Capillary isotachophoresis with universal (conductivity) and specific (UV, 254 nm) detection has proved a valuable analytical tool in this respect, with a minimum of sample pre-treatment and a relatively short analysis time.

EXPERIMENTAL

Capillary isotachophoresis was performed with home-made equipment as described elsewhere[2]. The dimensions of the capillary were I.D. 0.2 mm, O.D. 0.35 mm and length *ca.* 25 cm. The operational system is specified in Table I. This system was chosen because a low pH is favourable for sharp zone boundaries and thus a low limit of detection can be achieved using both UV and conductivity detectors. The separation capacity at this pH is increased because many of the acids under investigation have p*K* values in this region. The pH of the leading electrolyte, however, is critical because sorbic acid migrates as an enforced zone in front of the propionate terminator with a conductivity equal to that of the terminator, so that sorbate can be detected and determined only by the UV detector.

0021-9673/82 0000–0000/$02.75

TABLE I

OPERATIONAL SYSTEM FOR ANIONIC SEPARATIONS AT LOW pH

Parameter	*Leading electrolyte**	*Terminating electrolyte*
Anion	Chloride	Propionate
Concentration	0.01 *M*	*ca.* 0.005 *M*
Counter ion	β-Alanine	Sodium
pH	2.90	*ca.* 7
Additive	0.05% Mowiol 0.2% HEC	–

* Mowiol = poly(vinyl alcohol) (Hoechst, Frankfurt, G.F.R.); HEC = hydroxyethylcellulose (Polyscience, Warrington, PA, U.S.A.).

Initially the separation current was 45 μA. This current was automatically reduced to 25 μA (during detection) by an electronic device developed for coupled column isotachophoresis[3]. Home-made UV (254 nm) and conductivity detectors were used. One of the advantages of conductivity detection is the high resolution obtained, so that only a small amount of sample is needed. This reduces the capillary volume and time of analysis considerably compared with thermometric detection[1]. With a conductivity detector cell volume of 3 nl in a 0.2 mm I.D. capillary the limit of detection approaches the theoretical limit of 30 pmole. The steady-state isotachophoretic velocity (*ca.* 1 mm/sec) corresponded to approximately 90 pmole/sec.

Wine samples were analysed by the external standard calibration method. Immediately after opening the bottle, 200 μl of the wine were removed with a fixed-volume pipette (Finnpipette, Helsinki, Finland) and mixed with 2 ml of 0.01 *M* formaldehyde solution. Of this mixture 2 μl were injected with a 10-μl microsyringe (Hamilton, Bonaduz, Switzerland) equipped with a fixed-volume accessory. The wine was diluted with formaldehyde in order to prevent oxidation of sulphite during the

TABLE II

AVERAGE CONCENTRATIONS (RANGE IN PARENTHESES) OF POSSIBLE ADDITIVES FOUND IN WINES, COMPARED WITH THE CONCENTRATIONS ALLOWED BY EEC LEGISLATION FOR ORDINARY TABLE WINES

Additive	*Red (n = 50)*	*White/rosé (n = 50)*	*EEC requirement*	
	($M \times 10^{-3}$)	*($M \times 10^{-3}$)*	*$M \times 10^{-3}$*	*mg/l*
Sulphite	1.3 (0.1–3.1)	1.5 (0.1–3.0)	2.7*	175*
Tartrate	11.3 (5.6–19.2)	14.4 (6.4–24.7)	0.67	100
Citrate	1.5 (0.1–7.7)	2.6 (0.5–15.3)	5.2	1000
Ascorbate**	0.2 (0.1–1.1)	1.2 (0.1–2.3)	0.85	150
Sorbate	0.3 (0.1–3.2)	0.5 (0.1–3.1)	1.8	200

* $3.5 \cdot 10^{-3}$ *M* or 225 mg/l for white/rosé wines.

** Mainly as dehydroascorbic acid.

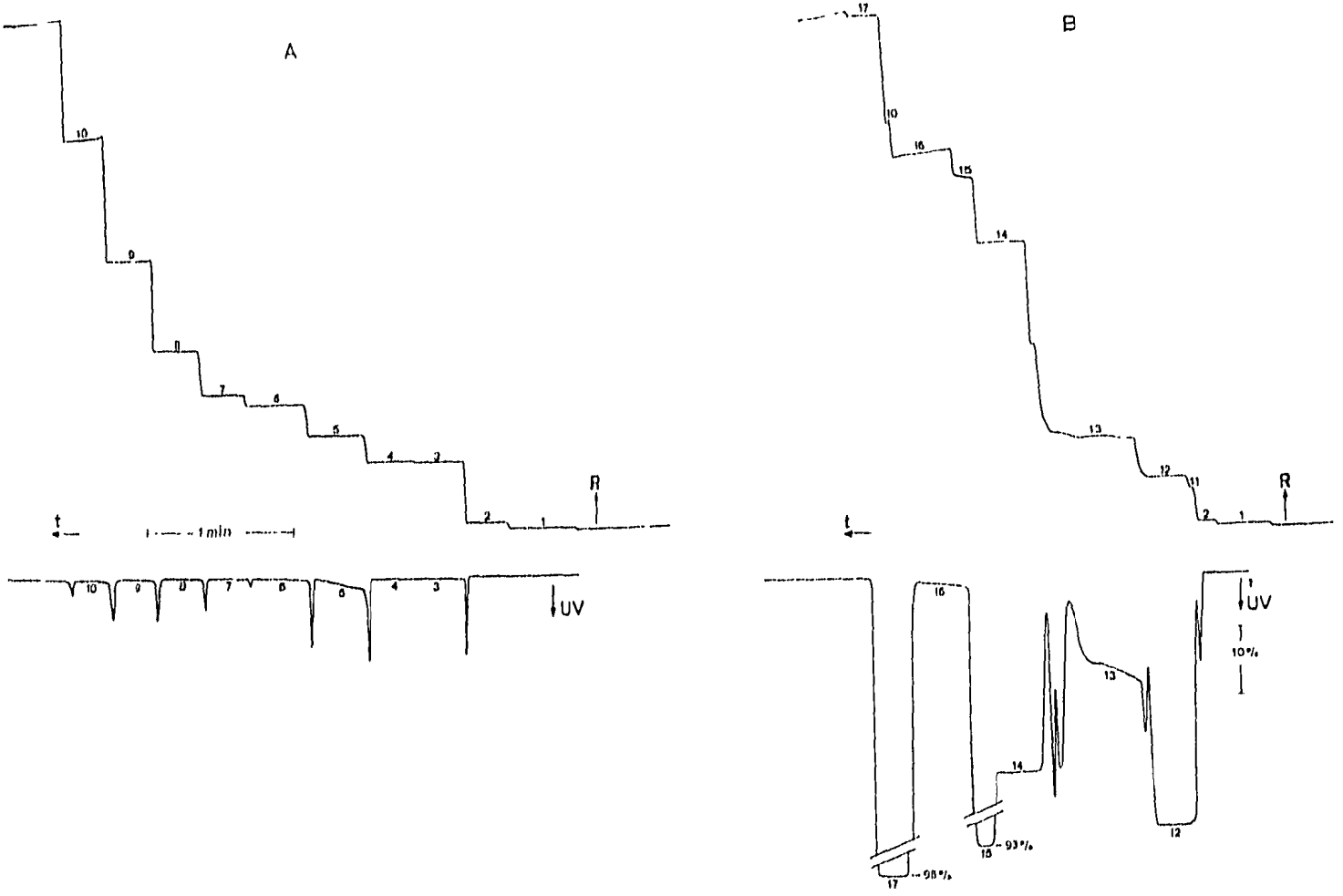

Fig. 1. Conductivity (R) and UV signals for the analysis of standards A (acids) and B (food additives). 1 = Sulphate; 2 = chlorate; 3 = phosphate; 4 = malonate; 5 = tartrate; 6 = citrate; 7 = malate; 8 = lactate; 9 = succinate; 10 = acetate; 11 = sulphite; 12 = salicylate; 13 = saccharin; 14 = benzoate; 15 = ascorbate; 16 = glutamate; 17 = sorbate.

sample pre-treatment procedure. The same procedure was used for the two standard solutions. A calibration run on standards A (acids) and B (additives) was performed each day. The day-to-day reproducibility of the response in mmole/l/mm zone length of the sample constituents was determined over a period of 18 days, and varied around 5% without the use of an internal standard. Fig. 1 shows the isotachopherograms of standards A and B.

RESULTS AND DISCUSSION

Using capillary isotachophoresis, 50 red, 37 white and 13 rosé wines were analysed. Typical isotachopherograms are shown in Figs. 2 and 3.

With the operational system used (Table I), sorbic, tartaric, ascorbic, citric and sulphurous acid could be selected and determined simultaneously. A large number of wines contained sulphite and sorbate was found in a number of mainly white wines. The average concentrations of these additives in the wines analysed are summarized in Table II. The maximal allowed concentrations of these additives according to EEC

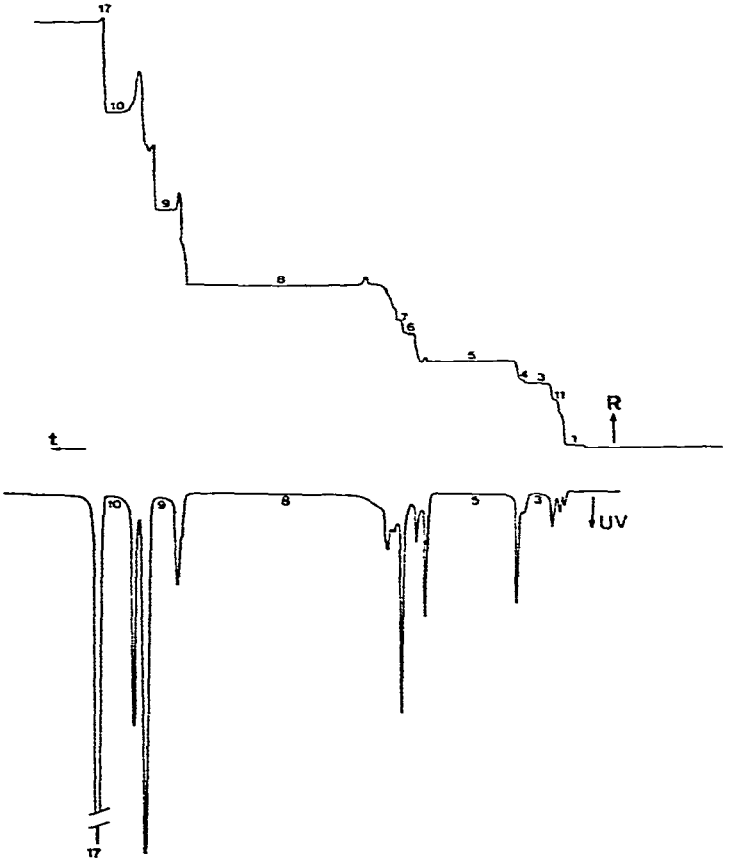

Fig. 2. Conductivity (R) and UV signals for the analysis of a red wine. The zone numbers correspond to those in Fig. 1.

TABLE III

AVERAGE CONCENTRATIONS OF SOME OF THE ACIDS IDENTIFIED IN WINES

Values in parentheses are standard deviations.

Anion	*Concentration ($M \times 10^{-3}$)*		
	Red (n = 50)	*White (n = 37)*	*Rosé (n = 13)*
Sulphate	4.1 (1.1)	4.0 (1.1)	3.1 (1.1)
Phosphate	3.7 (1.1)	4.5 (5.5)	2.8 (0.7)
Malonate	1.6 (0.6)	1.2 (0.5)	1.4 (0.3)
Malate	2.0 (1.5)	14.5 (10)	5.5 (11)
Lactate	21.1 (9.1)	11.0 (10)	14.3 (6.2)
Gluconate	0.6 (0.9)	1.5 (2.2)	1.1 (0.8)
Succinate	5.3 (1.1)	3.8 (1.6)	5.2 (1.2)
Acetate	9.3 (3.0)	6.0 (2.3)	8.2 (2.6)

legislation are given. As can be seen, with the method described, at a lower limit of detection of 10^{-4} *M*, the maximal acceptable concentration of these additives can easily be determined in a single run, using as the only simple sample pre-treatment step dilution with formaldehyde solution.

The reproducibility of the method was determined by six replicate injections. The relative standard deviations ranged from 4 to 5% without and were around 2% with the use of chlorate as an internal standard, depending on the sample ion. The day-to-day reproducibility had a relative standard deviation of 3–9% without the use of an internal standard.

An additional advantage of the method is the simultaneous determination of various other inorganic and organic acids (Krebs cycle). The average concentrations of the identified components are listed in Table III. From the isotachopherograms (Figs. 2 and 3) it can be easily seen that many unidentified UV and non-UV absorbing anions were also detected, even at this low pH of the leading electrolyte. Most of the acids identified were present in all wines, although in varying concentrations. For the determination of any significant deviations from the average composition, standard deviations were calculated for all concentrations (Table III).

A brief summary will be given of some major compounds determined in the wines. Of the possible additives, sulphite and sorbate can easily be recognized as they do not occur naturally in wines. Allowed concentrations of both compounds can be measured, but the sulphite concentration will decrease during storage owing to oxidation to sulphate. The same applies to ascorbic acid, most of which appeared to be oxidized to dehydroascorbic acid. The effective mobility of these compounds is the same but the UV signal gives positive evidence as only the unoxidized form shows strong UV absorption at 254 nm. Addition of tartrate and citrate is not easily determined as both occur in the natural product. High citrate levels were measured in some of the white wines. Relatively low concentrations of phosphate and malonate were found in most wines. Several compounds were present in a large concentration range in white and rosé wines in contrast to red wines, as can be seen from the standard deviations in Table III. This is especially true of phosphate, malate and gluconate.

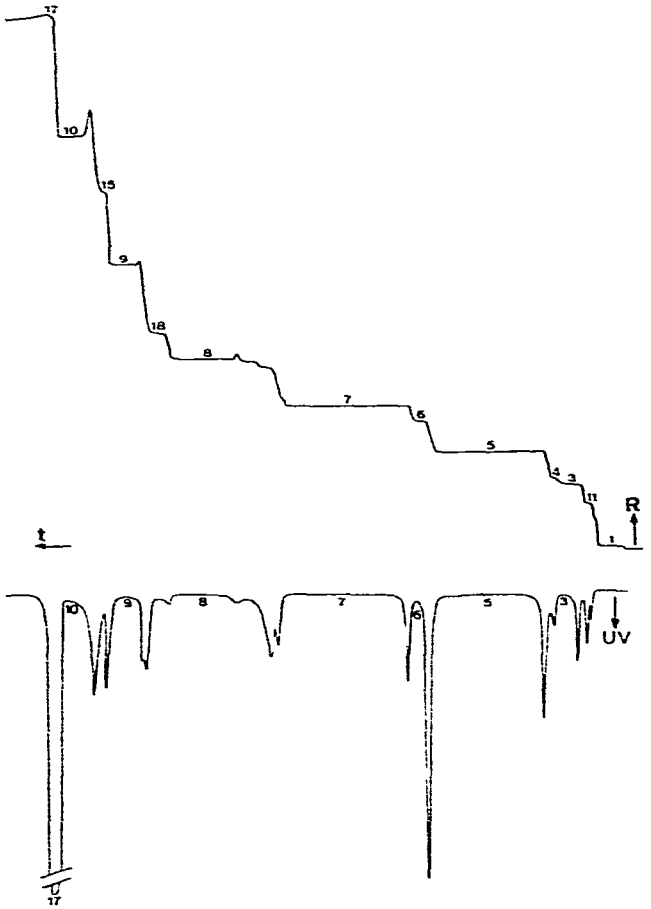

Fig. 3. Conductivity (R) and UV signals of the analysis of a white wine. The zone numbers correspond to those in Fig. 1; 18 = gluconate.

In conclusion, capillary isotachophoresis with conductivity detection is a useful technique for determining rapidly both major and minor constituents and additives in food products such as wines.

REFERENCES

1 K.-P. Kaiser and H. Hupf, *Deut. Lebensm.-Rundsch.*, 75 (1979) 346–349.
2 F. M. Everaerts, J. L. Beckers and Th. P. E. M. Verheggen, *Isotachophoresis, Theory, Instrumentation and Applications*, Elsevier, Amsterdam, Oxford, New York, 1976.
3 Th. P. E. M. Verheggen, F. E. P. Mikkers, D. M. J. Kroonenberg and F. M. Everaerts, in A. Adam and C. Schots (Editors), *Biochemical and Biological Applications in Isotachophoresis*, Elsevier, Amsterdam, Oxford, New York, 1980, pp. 41–46.