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ION-EXCLUSION CHROMATOGRAPHY OF CARBOXYLIC ACIDS WITH CONDUCTIVITY DETECTION

PEAK ENHANCEMENT USING A CATION-EXCHANGE HOLLOW-FIBRE MEMBRANE AND AN ALKALINE SOLUTION

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SUMMARY

A sensitive method for the determination of carboxylic acids is described. They were separated using a dilute aqueous solution of sulphuric acid as an eluent on a strong cation-exchange column, passed through a cation-exchange hollow-fibre membrane surrounded by an alkaline solution and detected by electrical conductivity. They gave positive and/or negative chromatographic peaks, depending on the concentration of the alkaline solution used. For most monocarboxylic acids, the response was enhanced 16 to 93 times as both the positive and negative peaks, while the latter gave 1.8 to 3 times higher responses compared with the former. The quantification of carboxylic acids in a wine sample as their negative peaks is also described.

INTRODUCTION

Carboxylic acids are separated usually by three different liquid chromatographic methods: anion exchange[1,2], ion-exclusion[2–5] and reversed-phase[6] chromatography, while the detection methods most commonly used are UV absorbance and conductivity. Due to the lack of selectivity and sensitivity, UV detection is inferior to conductivity detection. A chromatographic method using a dilute acid solution as an eluent on a strong cation-exchange column (that is, ion-exclusion chromatography) and conductivity detection was used for the determination of carboxylic acids. This technique has the advantage that inorganic anions which are completely dissociated, such as chloride, nitrate and sulphate, are excluded from the column. A disadvantage is the low sensitivity towards weak carboxylic acids because of their elution as unionized forms. In order to improve the sensitivity, the background con-

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ductivity of the dilute acid eluant must be reduced prior to detection. A peak enhancement system has been used: the eluent was passed through a cation-exchange membrane (inserted between the column and the detector) surrounded by alkaline solutions[2,7] or neutral salt solutions[6]. Thus, the complete dissociation of carboxylic acids occurred and they were sensitively detected by conductivity.

In this paper, we describe a peak enhancement system using a cation-exchange hollow-fibre membrane and a strong alkaline solution for the detection of carboxylic acids separated by ion-exclusion chromatography. The present system gave negative (decreasing conducitivity) peaks proportional to the amounts of carboxylic acids. The method was successfully applied to the determination of the carboxylic acids in a wine sample.

EXPERIMENTAL

Reagents and materials

All carboxylic acids (free form) and other chemicals of analytical reagent grade were obtained from Nakarai Chemicals (Kyoto, Japan), and used without further purification.

A cation-exchange hollow-fibre membrane (AFS-2) was obtained from Dionex (Sunnyvale, CA, U.S.A.) and used at the desired length.

Water prepared with Nanopure unit (Barnstead, Boston, MA, U.S.A.) was used for the preparation of the eluent and sample solutions.

Chromatography

The QIC analyzer (Dionex) was used for the separation and detection of carboxylic acids. It consisted of a pump, a 50-μl sample loop, a separation column, a cation-exchange hollow-fibre membrane and a conductivity detector. A separation column packed with an high capacity, fully sulphonated styrene–divinylbenzene cation-exchange resin, HPICE-AS1 (250 mm × 9 mm I.D., Dionex), was used. The eluents used were 1 and 2 m*M* sulphuric acid for the separation of carboxylic acids in standard and wine samples, respectively, at a flow-rate of 0.8 ml/min. A cation-exchange hollow-fibre membrane (length 50 cm) was inserted between the column and the detector. The enhancers, which were delivered on the outside of the membrane at a flow-rate of 1.5 ml/min, were 600 and 700 m*M* sodium hydroxide solution for the detection of carboxylic acids in standard and wine samples, respectively. A 50-μl aliquot of the sample solution was loaded onto a column. All the separations and detection were performed at ambient temperature.

Comparison of detection methods

The detection methods were compared as follows: method A, direct conductivity detection without the peak enhancement system; B, conductivity detection with the peak enhancement system using a cation-exchange hollow-fibre membrane (length 250 cm) and a 10 m*M* sodium hydroxide solution; C, conductivity detection with the peak enhancement system using a cation-exchange hollow-fibre membrane (length 50 cm) and a 600 m*M* sodium hydroxide solution. The other chromatographic conditions were the same as those for the separation of the standard carboxylic acids.

Sample preparation

Tamba white wine (Kyoto, Japan) was diluted by twenty-fold in water, filtered with a 0.45-μm microfilter (Gelman Science Japan, Tokyo, Japan) and loaded onto the column.

RESULTS AND DISCUSSION

Separation

It has been reported that carboxylic acids are well separated from strong inorganic anions, *e.g.*, chloride, sulphate, etc., on a strong cation-exchange resin (H^+) using a dilute solution of acids, *e.g.*, hydrochloric acid, sulphuric acid, perchloric acid, octanesulphonic acid, phosphoric acid, benzoic acid[2–5]. The retention times of carboxylic acids were compared by using hydrochloric acid, sulphuric acid or octanesulphonic acid as the eluent. There were almost no differences in their retention times and the column efficiency among the eluents studied. Thus, 1 and 2 m*M* sulphuric acid were used as the eluents for the separation of carboxylic acids in standard and wine samples, respectively.

Detection

Since weak carboxylic acids are only partially ionized in the dilute strong acid eluent, they are not sensitively detected by conductivity. Therefore, it is necessary to enhance the detector response by accelerating the dissociation of the acids and re-

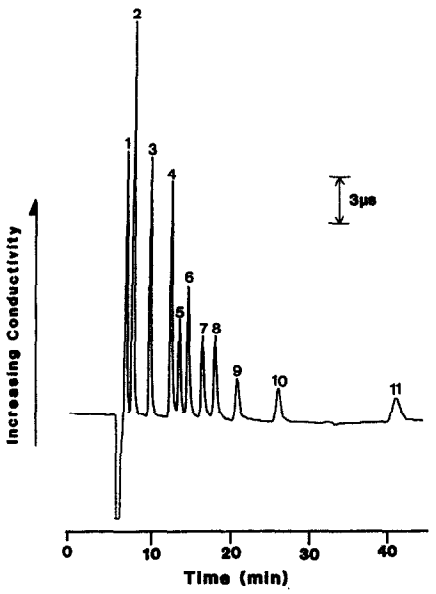

Fig. 1. Chromatogram of carboxylic acids detected at increasing conductivity. Conditions: column, HPICE-AS1 (250 mm × 9 mm I.D.); eluent, 1 m*M* sulphuric acid; flow-rate, 0.8 ml/min; cation-exchange hollow-fibre membrane, 250 cm; enhancer, 10 m*M* sodium hydroxide; flow-rate, 1.5 ml/min; injection volume, 50 μl. Peaks: 1 = citric acid; 2 = malonic acid; 3 = succinic acid; 4 = acetic acid; 5 = levulinic acid; 6 = propionic acid; 7 = isobutyric acid; 8 = *n*-butyric acid; 9 = isovaleric acid; 10 = *n*-valeric acid; 11 = *n*-caproic acid.

ducing the background conductivity. A peak enhancement system using a cation-exchange membrane and an alkaline solution was proposed by Rocklin *et al.*[2] and Slingsby[7]. The concentration of the alkaline solution (tetrabutylammonium hydroxide or potassium hydroxide) used as the enhancer was 2.5–10 m*M*. We tried to detect carboxylic acids more sensitively by using more concentrated alkaline solutions as the enhancers.

Fig. 1 shows a chromatogram of carboxylic acids obtained by conductivity detection after peak enhancement using a cation-exchange hollow-fibre membrane (length 250 cm) and a 10 m*M* sodium hydroxide solution. Carboxylic acids were sensitively detected as their ionic forms. The results obtained were almost the same as those described by Rocklin *et al.*[2] who used octanesulphonic acid as the eluent and tetrabutylammonium hydroxide as the enhancer. Figs. 2A, B and 3 show chromatograms of carboxylic acids obtained by conductivity detection after peak enhancement using the sodium hydroxide solutions of 300, 350 and 600 m*M*, respectively, and a cation-exchange hollow-fibre membrane (length 50 cm). As seen in Fig. 2A and B, the response is observed at increasing and decreasing conductivity, respectively, to give a complicated peak shape. These phenomena may be due to a water molecule produced by the reaction of an hydrogen ion with an hydroxide ion[5]. When a 500 m*M* sodium hydroxide solution was used, the response was observed at decreasing conductivity with a sharp single peak (Fig. 3). The negative peaks in the chromatographic separation of carboxylic acids are due to the lack of hydroxide ion, caused by the greater equivalent conductance of an hydroxide ion compared with that of a carboxylic acid ion.

In line with the main object of this study, that is, the sensitive determination of carboxylic acids in the negative detection mode, the factors affecting the response

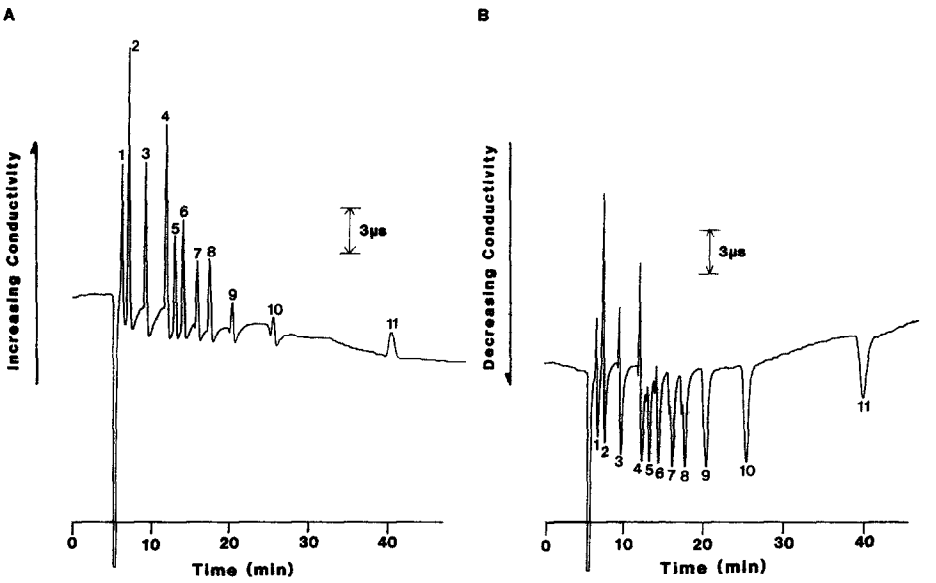

Fig. 2. Chromatograms of carboxylic acids detected at increasing and decreasing conductivity. Conditions as in Fig. 1 except that the length of the cation-exchange hollow-fibre membrane was 50 cm and the sodium hydroxide concentration was 300 (A) and 350 m*M* (B). Peaks as in Fig. 1.

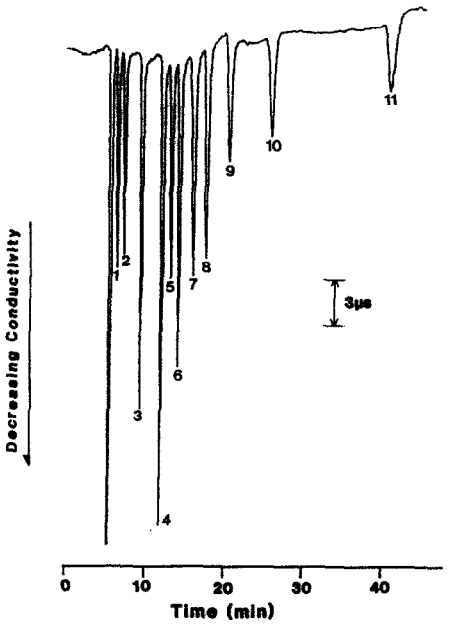

Fig. 3. Chromatogram of carboxylic acids detected at decreasing conductivity. Conditions as in Fig. 1 except that the length of the cation-exchange hollow-fibre membrane was 50 cm and the sodium hydroxide concentration was 600 m*M*. Peaks as in Fig. 1.

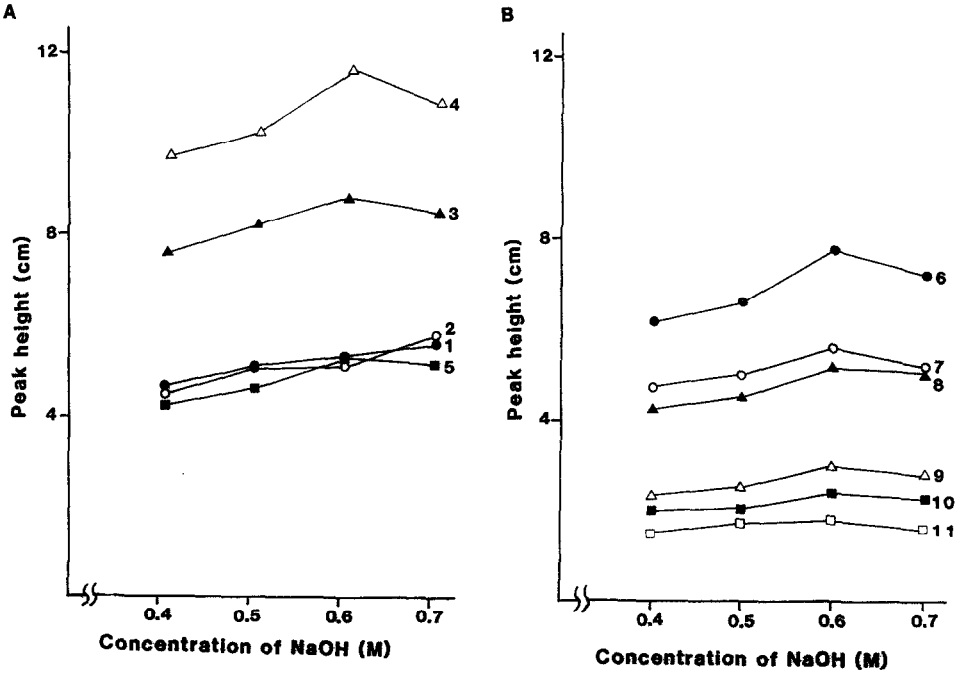

Fig. 4. Effect of the sodium hydroxide concentration on the detector response (decreased conductivity). Chromatographic conditions as in Fig. 1 except that the length of the cation-exchange hollow-fibre membrane was 50 cm. Peak numbers as in Fig. 1.

(decreased conductivity), the length of a cation-exchange hollow-fibre membrane and enhancer concentration, were examined. Lithium, sodium and potassium hydroxides were tested as the enhancers. With a cation-exchange hollow-fibre membrane of 50 cm, all carboxylic acids were observed only as negative chromatographic peaks using lithium, sodium and potassium hydroxides at concentrations of 300–500, 400–700 and 600–900 m*M*, respectively. The enhancer concentration needed for negative peaks increased in the following order of counter cations: lithium < sodium < potassium. These observations are in accord with the fact that the permeability of the hydroxide ion in the cation-exchange hollow-fibre membrane increases with an increase in its concentration in the enhancer and also with a decrease in the radius of the counter cation[5]. When lithium and potassium hydroxides were used as the enhancers, the response obtained changed depending on their concentrations. When sodium hydroxide was used as the enhancer, there was almost no concentration dependence. Thus, sodium hydroxide was used as the enhancer in subsequent experiments.

The length of the cation-exchange hollow-fibre membrane was changed from 25 to 250 cm. Figs. 4 and 5 show the peak heights of carboxylic acids obtained with the various concentrations of enhancer and fibre lengths of 50 and 100 cm, respectively. As the length was increased, the enhancer concentration needed for the negative peaks was decreased. At 250 cm, the peak heights obtained were lower than that with a length of 100 cm. Much the same peak heights were obtained with lengths of 25 and 50 cm. However, the baseline noise at 25 cm was higher than that at 50 cm. Thus, the peak enhancement system selected for the routine assay of carboxylic acids was a cation-exchange hollow-fibre membrane of length 50 cm and a 600 m*M* sodium hydroxide solution.

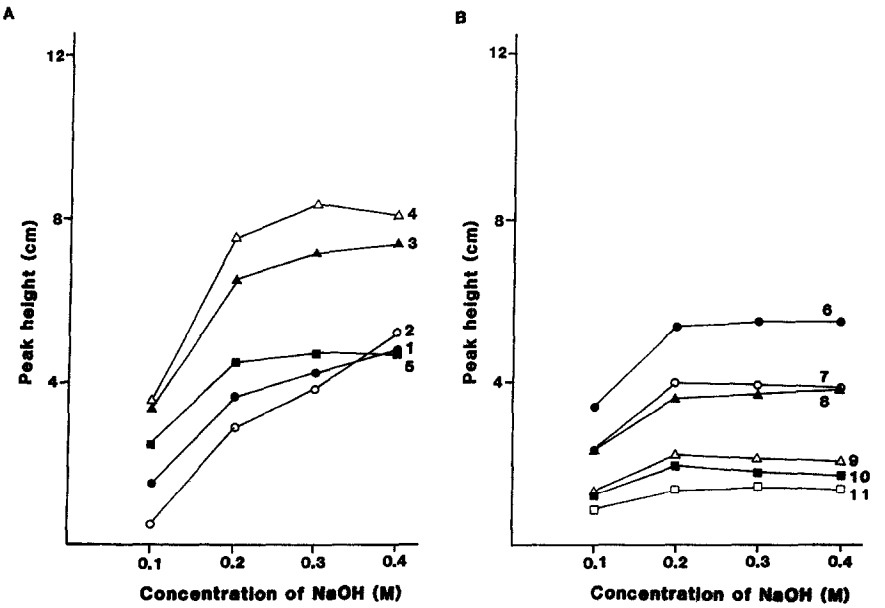

Fig. 5. Effect of the sodium hydroxide concentration on the detector response (decreased conductivity), conditions as in Fig. 4 except that the length of the cation-exchange hollow-fibre membrane was 100 cm.

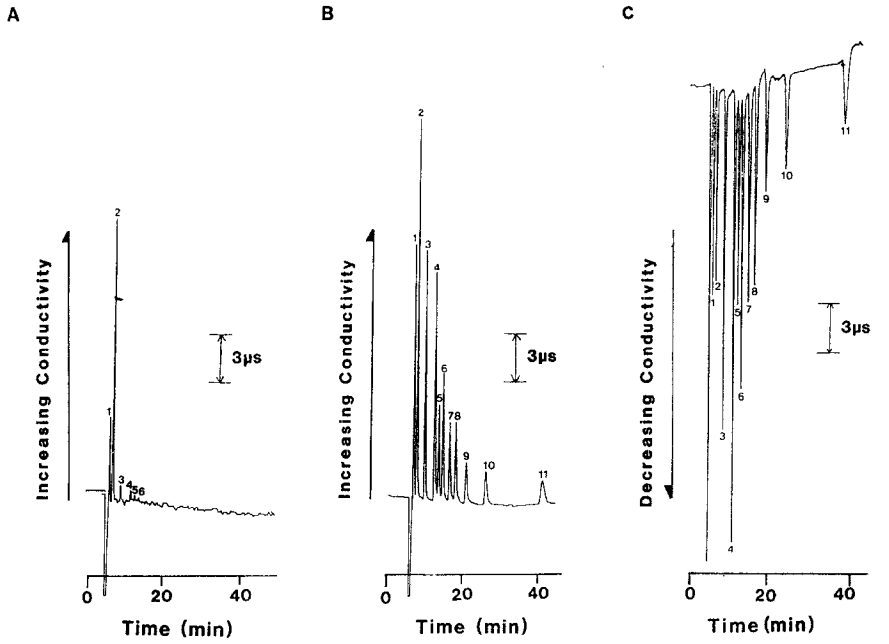

Fig. 6. Comparison of detection methods for carboxylic acids. Methods: (A) direct detection without the peak enhancement system; (B) detection with the peak enhancement system using a cation-exchange hollow-fibre membrane of length 250 cm and a 10 m*M* sodium hydroxide solution; (C) detection with the peak enhancement system using a cation-exchange hollow-fibre membrane of length 50 cm and a 600 m*M* sodium hydroxide solution. Peaks as in Fig. 1.

TABLE I

COMPARISON OF ENHANCEMENT FACTORS FOR CARBOXYLIC ACIDS

Carboxylic acid	*Enhancement factor**	
	Method B	*Method C*
Citric acid	3.1	2.3
Malonic acid	1.4	0.7
Succinic acid	21	27
Acetic acid	28	50
Levulinic acid	16	34
Propionic acid	41	93
Isobutyric acid	25	64
n-Butyric acid	29	69
Isovaleric acid	23	61
n-Valeric acid	32	89
n-Caproic acid	29	86

* The peak enhancement factor is the ratio of the peak areas obtained with and without the peak enhancement system.

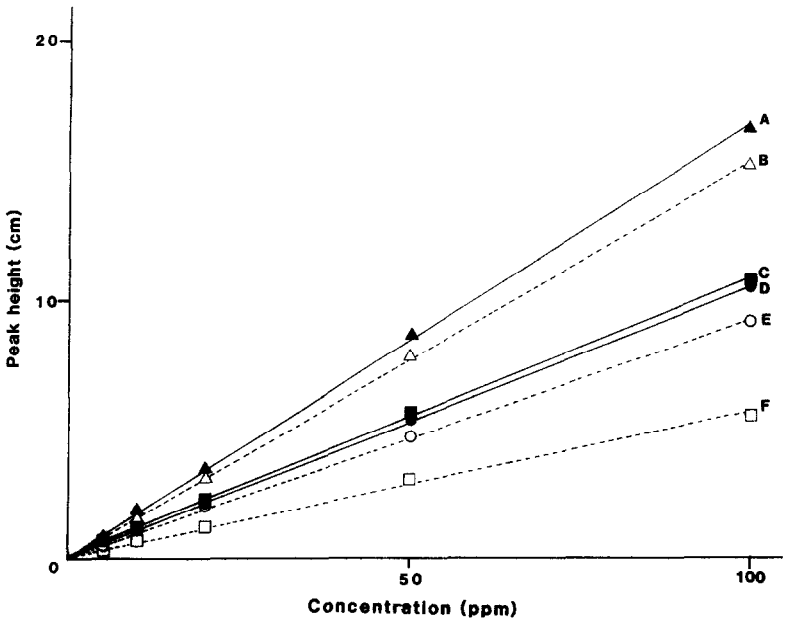

Fig. 7. Typical calibration graphs for carboxylic acids: A, succinic acid; B, propionic acid; C, isobutyric acid; D, citric acid; E, malonic acid; F, isovaleric acid. Chromatographic conditions as in Fig. 3.

Fig. 6 shows a comparison of the detection methods: (A), (B) and (C) correspond to methods A, B and C, respectively. Table I illustrates the peak enhancement factors of methods B (Fig. 6B) and C (Fig. 6C) compared with that of method A (Fig. 6A). This result shows that the present method is 1.8–3-fold more sensitive for

TABLE II

REPRODUCIBILITY AND DETECTION LIMITS FOR CARBOXYLIC ACIDS

The concentration of each carboxylic acid was 20 μg/ml.

Carboxylic acid	*C.V.** (%)	*Detection limit*** (ng)
Citric acid	1.1	90
Malonic acid	1.4	100
Succinic acid	1.8	60
Acetic acid	1.1	40
Levulinic acid	4.1	90
Propionic acid	3.1	70
Isobutyric acid	1.9	90
n-Butyric acid	4.0	100
Isovaleric acid	4.0	160
n-Valeric acid	6.5	200
n-Caproic acid	5.3	280

* Coefficient of variation for five analyses.

** The detection limit was based on a signal-to-noise ratio of 3.

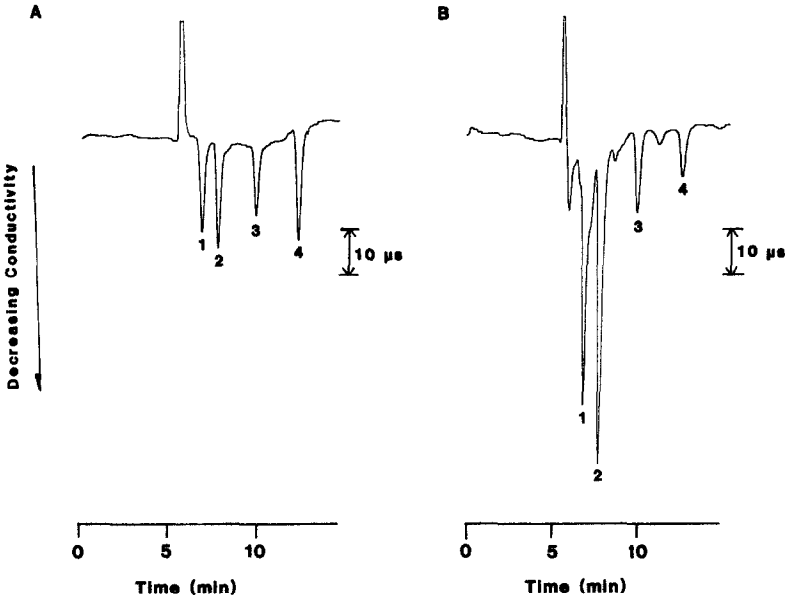

Fig. 8. Chromatograms of carboxylic acids in standard (A) and wine (B) samples. Conditions: column, HPICE-AS1 (250 mm × 9 mm I.D.); eluent, 2 m*M* sulphuric acid; flow-rate, 0.8 ml/min; cation-exchange hollow-fibre membrane, 50 cm; enhancer, 700 m*M* sodium hydroxide; flow-rate, 1.5 ml/min; injection volume, 50 µl. A wine sample was diluted twenty-fold. Peaks: 1 = lactic acid; 2 = tartaric acid; 3 = malic acid; 4 = acetic acid. Concentrations: (A), 50 µg/ml each; (B), peaks 1, 2, 3 and 4 were estimated to contain 97, 139, 63 and 23 µg/ml, respectively.

the detection of monovalent carboxylic acids (acetic acid through *n*-caproic acid) compared with the previously reported one[2].

Calibration graph

Fig. 7 shows typical calibration graphs of peak height *versus* concentration for carboxylic acids. The graphs were linear over the concentration ranges of 5–100 µg/ml with a correlation coefficient >0.99, and passed through the origin.

Reproducibility and detection limits

Table II shows the reproducibility (coefficient of variation for five analyses) and detection limits at a signal-to-noise ratio of 3.

Application

On the basis of the aforementioned results, we attempted to apply the present method to the determination of carboxylic acids in a wine sample. Fig. 8A shows the chromatogram of a standard mixture of carboxylic acids; a chromatogram of a wine sample after twenty-fold dilution is shown in Fig. 8B.

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