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Qualitative analysis of some carboxylic acids by ion-exclusion chromatography with atmospheric pressure chemical ionization mass spectrometric detection

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Abstract

A simple, selective and sensitive method for the determination of carboxylic acids has been developed. A mixture of formic, acetic, propionic, valeric, isovaleric, isobutyric, and isocaproic acids has been separated on a polymethacrylate-based weak acidic cation-exchange resin (TSK gel OA pak-A) based on an ion-exclusion chromatographic mechanism with detection using UV-photodiode array, conductivity and atmospheric pressure chemical ionization mass spectrometry (APCI-MS). A mobile phase consisting of 0.85 m*M* benzoic acid in 10% aqueous methanol (pH 3.89) was used to separate the above carboxylic acids in about 40 min. For LC–MS, the APCI interface was used in the negative ionization mode. Linear plots of peak area versus concentration were obtained over the range 1–30 m*M* (r^2=0.9982) and 1–30 m*M* (r^2=0.9958) for conductimetric and MS detection, respectively. The detection limits of the target carboxylic acids calculated at S/N=3 ranged from 0.078 to 2.3 μ*M* for conductimetric and photometric detection and from 0.66 to 3.82 μ*M* for ion-exclusion chromatography–APCI-MS. The reproducibility of retention times was 0.12–0.16% relative standard deviation for ion-exclusion chromatography and 1.21–2.5% for ion-exclusion chromatography–APCI-MS. The method was applied to the determination of carboxylic acids in red wine, white wine, apple vinegar, and Japanese rice wine.

Keywords: Carboxylic acids

1. Introduction

Numerous applications for the rapid and sensitive determination of carboxylic acids in environmental and biological samples have been developed. Much of this attention has been directed towards the study of components involved in the acidification of environmental water, such as rain and snow [1,2]. Ion-exclusion chromatography is a useful and widely accepted technique for the determination of organic anions, with inorganic anions being excluded from the resin pores due to ionic repulsion and are completely separated from the weak organic anions which can partially or entirely permeate into the pores [3–7]. UV absorbance and conductivity have been used routinely in ion-exclusion chromatog-

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raphy, but mass spectrometry (MS) offers major advantages for specific applications due to its potential to combine high sensitivity with mass selectivity. Electrospray (ESI) MS or atmospheric pressure chemical ionization (APCI) MS are particularly attractive techniques which allow the soft ionization of a wide range of substances in positive and negative modes.

Gas chromatography–mass spectrometry (GC–MS) is the most commonly used method for the determination of carboxylic acids. Detection limits for GC–MS are approximately 1 ppm [8], but the analysis is time consuming since it requires a derivatization step to extract the analyte from the sample matrix. Few papers have been published on the application of MS detection of carboxylic acids after their separation by liquid chromatography (LC) [9,10]. Modest detection limits of 10 ppm were obtained for glycolic, acetic, propionic, and butyric acids [10], although Johnson et al. [11] have described the separation of a mixture of formic, glyoxylic, oxalic, 2-hydroxyisobutyric, and maleic acids with detection limits of 40–200 ppb and 2–8 ppm being obtained for direct infusion of the sample into ESI-MS and for ion-exclusion chromatography–ESI-MS, respectively.

The main intention of the present work was to develop a rapid analytical method to determine low-molecular-mass carboxylic acids. This study focuses on the determination of hydrochloric acid, formic acid, acetic acid, propionic acid, isobutyric acid, isovaleric acid, valeric acid, and isocaproic acid by ion-exclusion chromatography–LC and ion-exclusion chromatography–APCI-MS with little sample preparation. The sample solution is injected directly onto the ion-exclusion chromatography column, which is coupled directly to the mass spectrometer. A polymethacrylate-based weak acidic cation-exchange resin (TSK gel OA pak-A) is used in this study, with benzoic acid as mobile phase for both conductivity and MS detection. APCI conditions are examined, calibration curve and deflector voltage are discussed. Two analytical ion-exclusion chromatography systems, differing in the detection mode, and operated under two sets of chromatographic conditions are applied to enhance the chromatographic versatility and certainty of analyte identification.

2. Experimental

2.1. Reagents and samples

All materials were of analytical-reagent grade. Water was distilled with an Aquarius water distillation apparatus (Advantec GC-500). The mobile phase was 0.85 m*M* benzoic acid (HBZ) in 10% aqueous methanol at a pH of 3.89. The analytes used were: hydrochloric acid, formic acid (99%), acetic acid (99.7%), propionic acid (98%), isovaleric acid (98%), valeric acid (95%), isobutyric acid (98%), isocaproic acid (98%) and benzoic acid (99.5%) and were purchased from Wako (Osaka, Japan). A 1.0 m*M* stock solution of each carboxylic acid was prepared and aliquots of these stock solutions were frozen to avoid sample decomposition. Working solutions of each individual analyte were diluted with mobile phase.

2.2. Apparatus

A Shimadzu (Kyoto, Japan) quadrupole LC–MS instrument was used, comprising an LC-10AD VP liquid chromatograph, a CTO-10A VP column oven, an SPD-M10A VP photodiode array detector, a CDD-6A conductivity detector, an SCL-10A VP system controller, a DGU-12AM degasser, and a LCMS-QP8000 mass spectrometer. Acquired data were processed by Shimadzu class-8000 software, which was also used to control the LC system, interface, vacuum system and quadrupole MS hardware. Tuning standards used for adjusting resolution, sensitivity and mass calibration were poly(ethylene glycol) (PEG) 400 (M_r 200–600), PEG 600 (M_r 400–1000), PEG 1000 (M_r 700–1500) and PEG 200+600+1000 (M_r 200–1500). Formic acid was detected at m/z 45, acetic acid at m/z 59, propionic acid at m/z 73, isobutyric acid at m/z 87, isovaleric acid and valeric acid at m/z 101 and isocaproic acid at m/z 15.

Instrumental operating conditions for APCI-MS are summarized in Table 1. Some values were adjusted daily to produce a good ion signal and chromatographic quality.

Table 1
APCI-MS operating conditions

Ionization source	APCI (−ve)
Nebulizer gas flow-rate	2.5 l/min
Sampling rate	0.64 s
Time constant	0.64 s
APCI temperature	400 °C
CDL* temperature	230 °C
Detector voltage	1.7 kV
Probe high voltage	−4.0 kV
CDL* voltage	0.0 V
Deflector voltage upper	−50.0 V
Lower	−50.0 V
Left	−50.0 V
Right	−50.0 V
Interval	1.0 s
Microscan	0.5 u
m/z value monitored	*m/z* 49 (formic acid)
	m/z 59 (acetic acid)
	m/z 73 (propionic acid)
	m/z 87 (isobutyric acid)
	m/z 101 (isovaleric and valeric acids)
	m/z 115 (isocaproic acid)

* Curved Disolvation Line.

2.3. Chromatographic conditions

Ion-exclusion chromatography separation of carboxylic acids was carried out using a 30 cm×7.8 mm I.D. TSK gel OA pak-A (Tosoh, Tokyo, Japan) weak acidic cation-exchange column. This column was packed with polymethacrylate-based weak acidic cation-exchange resin in the H^+-form (5 μm particle size, 0.1 mequiv./ml) and was operated at 40 °C. Details of the chromatographic conditions used are summarized in Table 2.

3. Results and discussion

3.1. Optimization of mobile phase composition for ion-exclusion chromatographic separation of carboxylic acids

The effectiveness of acidic mobile phases in ion-exclusion chromatography for improvement of the peak shape has been reported previously [12,13], with dilute sulfuric acid typically being employed as mobile phase. While such an mobile phase provides good resolution and peak shape for the analytes under study, it is not compatible with MS detection due to potential damage to the surface of the interface. For this reason, weak acid mobile phases were investigated and when benzoic acid was employed as the mobile phase, appropriate separation and detection of carboxylic acids was obtained. Fig. 1 shows the separation of carboxylic acids using 0.85 m*M* benzoic acid in 10% aqueous methanol, with conductimetric detection. The buffering capacity of this mobile phase is sufficient to maintain the degree of ionization of the analytes at a constant value, thereby ensuring that peak fronting caused by variable ionization to be avoided [3,14]. Variation of the benzoic acid concentration over the range 0.05–1.0 m*M* gave virtually no change in the retention volumes for the analyte acids. The working concentration of 0.85 m*M* benzoic acid was selected on the basis that this concentration gave the highest sensitivity with MS detection.

Methanol was added to the mobile phase in order to modify secondary hydrophobic interactions between the analytes and the stationary phase and

Table 2
Operating conditions for ion-exclusion chromatography

Column	TSK gel OA pak-A polymethacrylate-based weakly acidic cation-exchange resin
Mobile phase	0.85 m*M* benzoic acid+10% aqueous methanol (pH 3.89)
Column temperature	40 °C
Sample injection	0.1 ml
Flow-rate	1.2 ml/min
Pressure	52 kgf/cm^2
LC start time–stop time	0–40 min
Detection	(1) UV, $^2H_2$, 210–215 nm,
	(2) conductivity

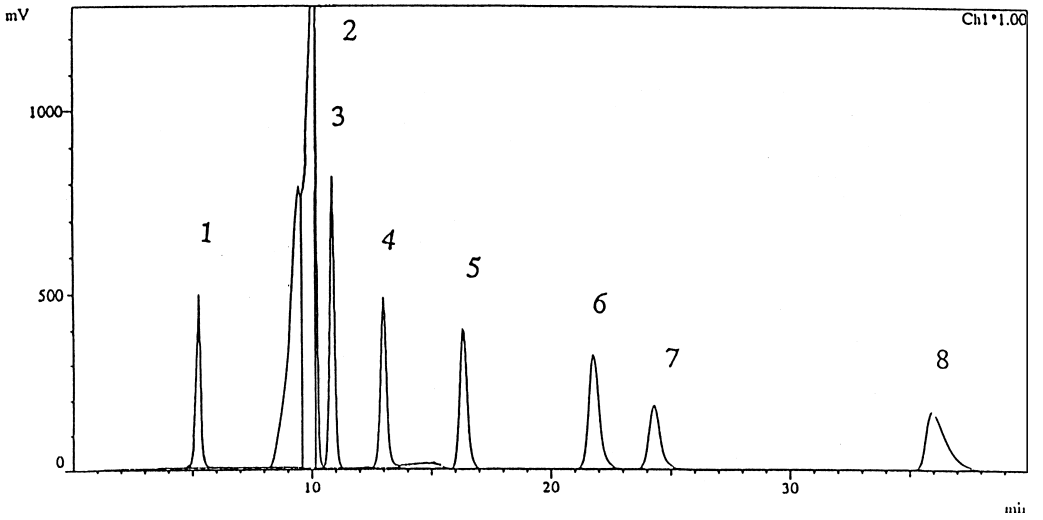

Fig. 1. Ion-exclusion chromatogram of carboxylic acids by elution with 0.85 m*M* benzoic acid+10% aqueous methanol at pH 3.98. Column: TSKgel OA pak-A polymethacrylate-based weakly acidic cation-exchange resin; column temperature: 40 °C; flow-rate 1.2 ml/min; detection: conductivity: injection volume: 0.1 ml; sample concentration: 20 m*M* for all aliphatic carboxylic acids. Peaks: 1=hydrochloric acid, 2=formic acid, 3=acetic acid, 4=propionic acid, 5=isobutyric acid, 6=isovaleric acid, 7=valeric acid, 8=isocaproic acid.

therefore to manipulate retention. The effects of adding methanol in the range 0–15% to a 0.85 m*M* benzoic acid mobile phase are shown in Fig. 2, which indicates that with increasing methanol concentration, V_R values for hydrochloric acid, acetic acid, formic acid, propionic acid and isobutyric acid were not altered, but those of valeric acid and isocaproic acid decreased gradually. The intensity of the MS signal increased for all analyte carboxylic acids with increasing methanol content, but because

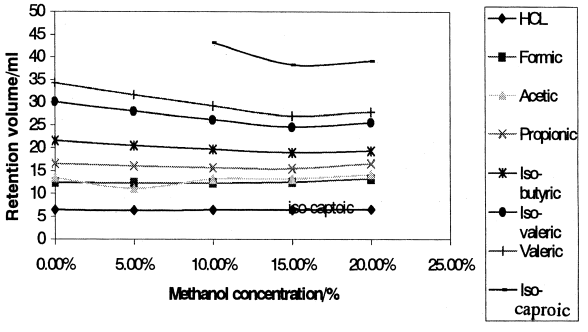

Fig. 2. Effect of methanol concentration in the eluent on retention volumes (V_R) of HCl and carboxylic acids. Eluent 0.85 m*M* benzoic acid containing 0–15% methanol. Other conditions as in Fig. 1.

the separation column could not tolerate a higher methanol content than 12%, the optimal mobile phase consisted of 0.85 m*M* benzoic acid solution at pH 3.89 and 10% (v/v) methanol. Fig. 3 shows an example for a typical total ion chromatogram of the carboxylic acids using APCI-MS detection. The same separation monitored with UV detection at 210–215 nm (in this case, indirect detection due to the presence of absorbing benzoic acid in the mobile phase) is shown in Fig. 4 and reveals that much poorer sensitivity was obtained using this approach.

3.2. Effect of deflector voltage

Careful adjustment of voltage at the exit of the transfer capillary of the APCI interface could be used to enhance or inhibit fragmentation, offering the possibility to increase sensitivity and to obtain structural data for the analytes. For unequivocal identification of analytes, both molecular and fragment ions must be monitored. The effect of the deflector voltage on the detection sensitivity of acetic acid was investigated in the range from −30 to −70 kV and the relationship between peak area and the deflector voltage is shown in Fig. 5. This shows that a deflector voltage of −50.0 kV gave the highest

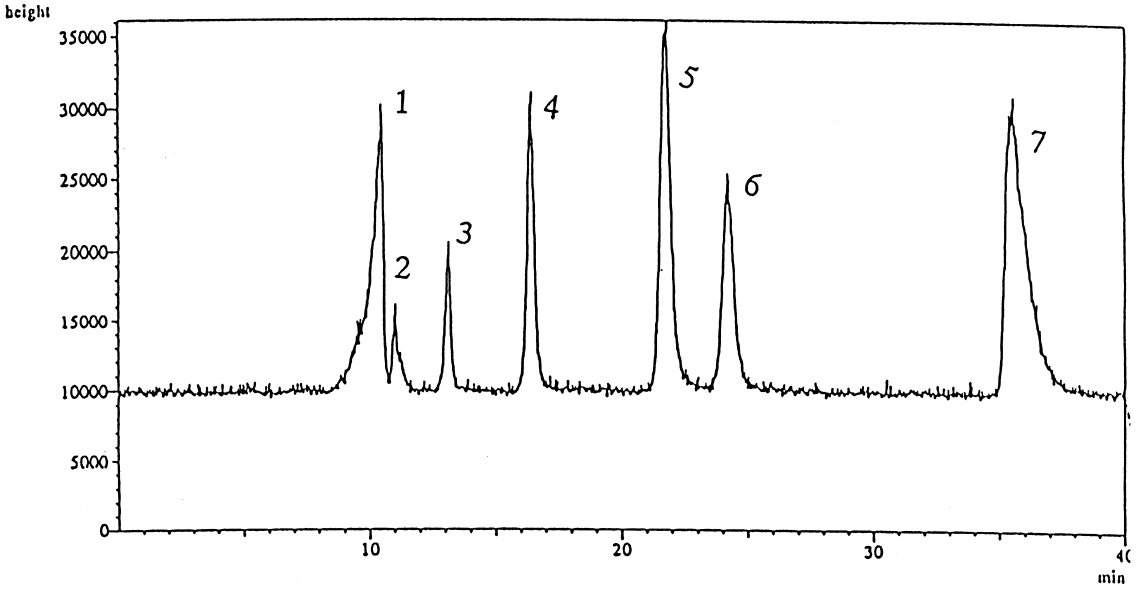

Fig. 3. Total ion chromatogram for the separation of 25 m*M* carboxylic acids elution with 0.85 m*M* benzoic acid+10% aqueous methanol at pH 3.98. Column: TSKgel OA pak-A polymethacrylate-based weakly acidic cation-exchange resin; column temperature: 40 °C; flow-rate 1.2 ml/min; detection: MS detection, using APCI-MS interface in the negative form. Deflector voltage=−50.0 kV. Sample concentration: 25 m*M* for all aliphatic carboxylic acids. Peaks: 1=formic acid, 2=acetic acid, 3=propionic acid, 4=isobutryic acid, 5=isovaleric acid, 6=valeric acid, 7=isocaproic acid.

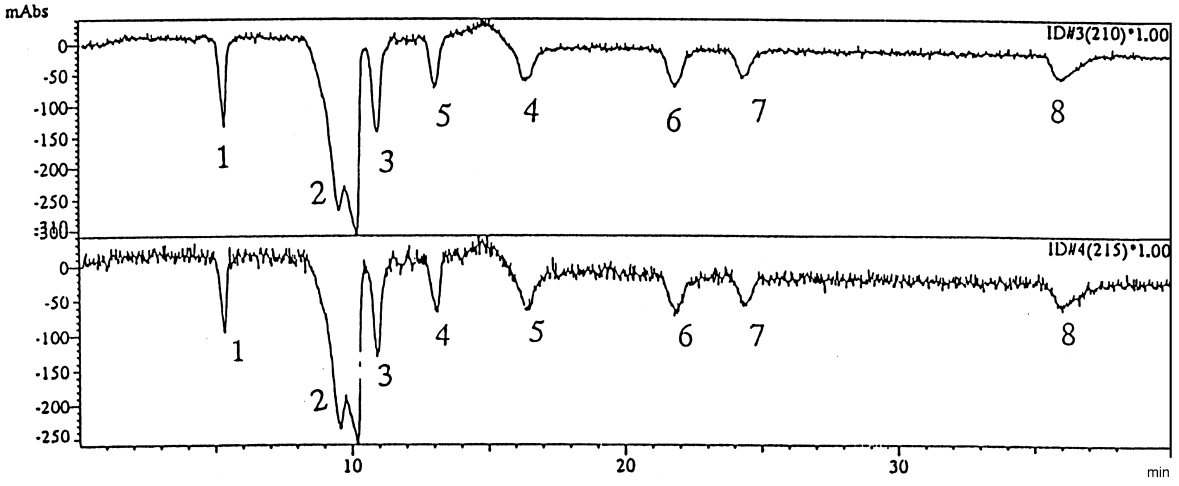

Fig. 4. Ion-exclusion chromatogram of carboxylic acids by elution with 0.85 m*M* benzoic acid+10% aqueous methanol at pH 3.98. Column: TSKgel OA pak-A polymethacrylate-based weakly acidic cation-exchange resin; column temperature: 40 °C; flow-rate 1.2 ml/min; detection: UV (210 nm and 215 nm): injection volume: 0.1 ml; sample concentration: 15 m*M* for all aliphatic carboxylic acids. Peaks: 1=hydrochloric acid, 2=formic acid, 3=acetic acid, 4=propionic acid, 5=isobutylic acid, 6=isovaleric acid, 7=valeric acid, 8=isocaproic acid.

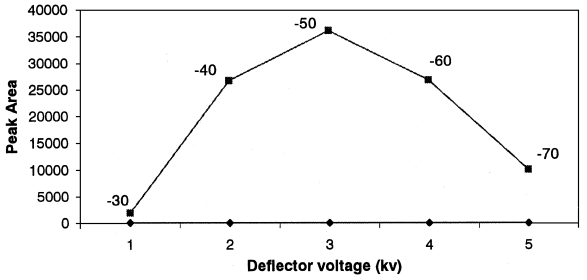

Fig. 5. Effect of deflector voltage on the sensitivity. Acetic acid (20 m*M*) was used to examine the deflector voltage value.

sensitivity and was also found to provide a good calibration linearity for the entire range of carboxylic acids studied.

3.3. Analytical performance characteristics

Calibration curves for the carboxylic acids were constructed by plotting the relationship between peak areas and concentration, using both conductimetric and MS detection. The results obtained for both detection systems are summarized in Tables 3 and 4. The correlation coefficients ranged from 0.9946 to 0.9998 and from 0.9915 to 0.9993 for conductimetric and MS detection, respectively.

Detection limits calculated at a signal-to-noise ratio of 3 for both conductimetric and MS detection are also shown in Tables 3 and 4. The detection limits for ion-exclusion chromatography–APCI-MS were obtained by selective ion monitoring (SIM) of the parent anions of the carboxylic acids studied and are subject to change when more m/z values are included in the SIM program or if the mass detection is scanned to determine unknown acids at other m/z values. Tables 3 and 4 show that the two detection methods gave similar detection limits, but the MS approach also provides analyte identification.

The reproducibility of the retention time values of the carboxylic acids was 0.12–0.16% RSD for conductivity detection and 1.21–2.5% for APCI–MS detection. Therefore, the proposed method is quick, highly sensitive and reproducible.

3.4. Applications

The determination of carboxylic acids in several samples was performed using the methods described

Table 3
Calibration data for the carboxylic acids eluted with 0.85% benzoic acid+10% aqueous methanol, using conductivity detection

Analyte	Concentration range (m*M*)	Regression equation	Correlation coefficient (r^2)	Detection limit ($S/N=3$) (μM)
Formic acid	1–25	$y=2\cdot10^6x-675\,563$	0.9996	0.078
Acetic acid	5–25	$y=58\,799x+4\cdot10^6$	0.9985	0.62
Propionic acid	1–30	$y=56\,306x+878\,216$	0.9994	0.84
Isobutyric acid	1–30	$y=56\,865x+811\,265$	0.9995	0.98
Isovaleric acid	1–30	$y=618\,653x+1\cdot10^6$	0.9998	1.26
Valeric acid	1–30	$y=557\,323x+555\,528$	0.9946	1.5
Isocaproic acid	1–30	$y=616\,286x-47\,614$	0.9957	2.3

Table 4
Calibration data for the carboxylic acids eluted with 0.85% benzoic acid+10% aqueous methanol, using APCI-MS detection

Carboxylic acid	Concentration range (m*M*)	Regression equation	Correlation coefficient (r^2)	Detection limit ($S/N=3$) (μM)
Formic acid	1–25	$y=6677.6x-13\,569$	0.9993	0.81
Acetic acid	1–20	$y=1367.2x+1526.6$	0.9993	0.79
Propionic acid	1–20	$y=2103.6x+18\,630$	0.9991	3.82
Isobutyric acid	5–30	$y=9819.5x-51\,356$	0.9991	1.91
Isovaleric acid	1–30	$y=26\,037x-53\,970$	0.9959	0.66
Valeric acid	5–25	$y=5657.3x-32\,501$	0.9925	1.31
Isocaproic acid	5–25	$y=1712.8x-1668.7$	0.9915	1.7

above and without any sample pretreatment except for dilution. The method was found to be applicable for the detection of a number of carboxylic acids in several beverage samples, including red wine, white wine, Japanese rice wine, and apple vinegar. As an example of the typical results obtained, Fig. 6 shows the chromatograms obtained for the analysis of white wine.

4. Conclusion

APCI-MS has been shown to be a viable method of detection for ion-exclusion chromatography of low-molecular-mass carboxylic acids. Careful optimization of ion-exclusion chromatography and MS conditions is very important to obtain high sensitivity, but detection limits of less than 1 μ*M* could be

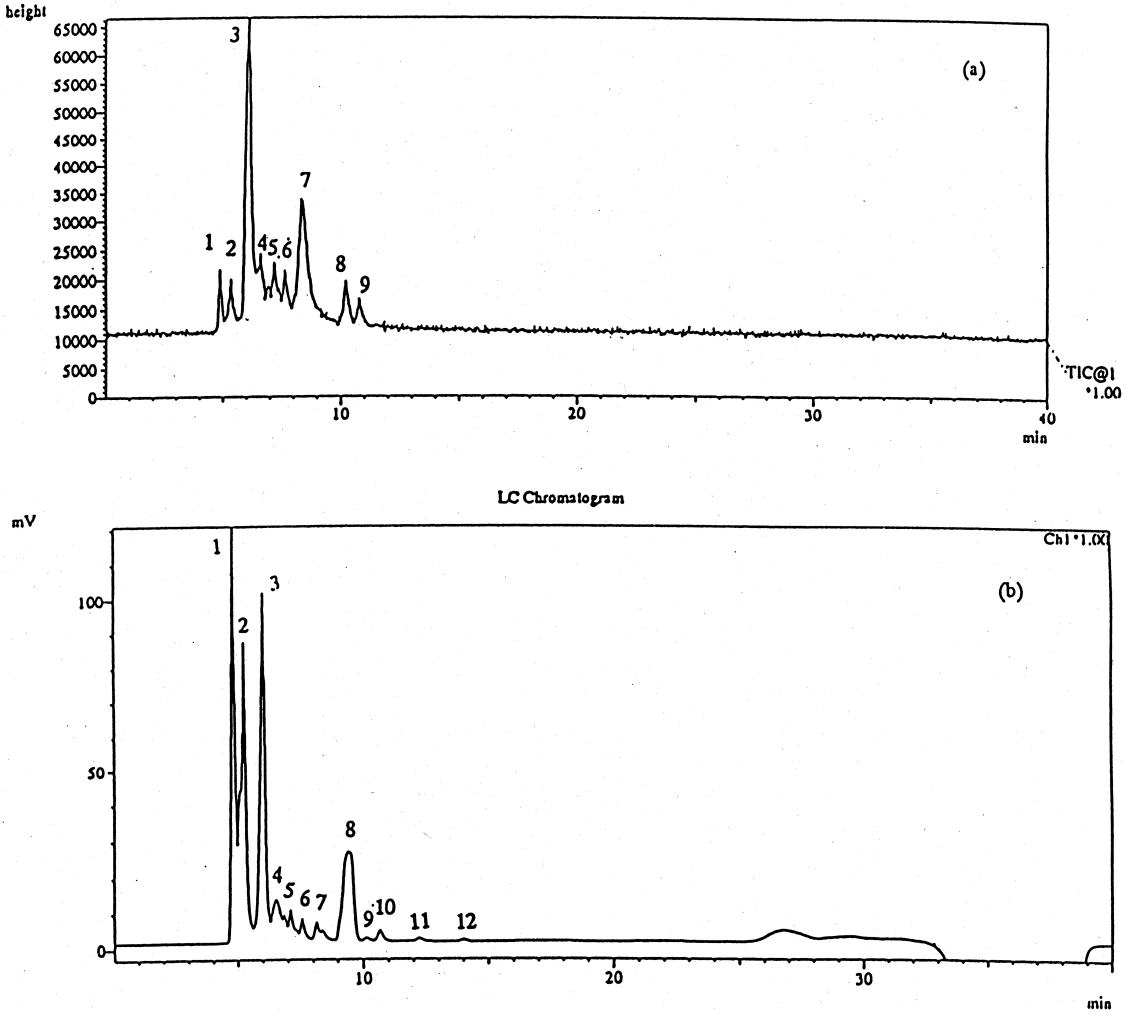

Fig. 6. (a) Total ion chromatogram for the separation of carboxylic acids in white wine (Chile). Eluent: 0.85 m*M* benzoic acid+10% aqueous methanol; other chromatographic conditions were as described in Fig. 3. Sample: white wine (Chile) diluted 100-fold with eluent. Peaks: 1–7=unknown, 8=formic acid, 9=acetic acid. (b) Determination of carboxylic acids in white wine (Chile) using ion-exclusion chromatography. Eluent: 0.85 m*M* benzoic acid+10% aqueous methanol; other chromatographic conditions were as described in Fig. 1. Sample: white wine (Chile) diluted 100-fold with eluent. Peaks: 1=unknown, 2=hydrochloric acid, 3–8=unknown, 9=formic acid, 10=acetic acid, 11=unknown, 12=propionic acid.

achieved for some carboxylic acids and this provides encouragement to further develop this approach.

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