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Non-suppressed conductivity and indirect UV detection of carboxylic acids in environmental samples by ion-exclusion chromatography using 2,6-pyridinedicarboxylic acid eluent

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Abstract

2,6-Pyridinedicarboxylic acid (PDCA) was evaluated as an eluent for indirect UV and non-suppressed conductivity detection of carboxylic acids in ion-exclusion chromatography. The effect of PDCA concentration on the separation and detection sensitivity was investigated. The reasonable resolutions between carboxylic acids were achieved using 1 mM PDCA eluent. Detection limits were 1.0–7.0 μM for conductivity detection and 8–30 μM for UV detection. Compared to the eluent containing 1 mM sulfuric acid, the method offers a high resolution and high detection sensitivity for both detectors due to its high molar absorptivity and low background conductance. The proposed method was demonstrated to be useful for the determination of carboxylic acids in environmental samples with direct sample injection. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Ion-exclusion chromatography (IEC) is a useful technique for the analysis of carboxylic acids. The separation of carboxylic acids is performed using a column packed with a high capacity of polystyrene–divinylbenzene (PS–DVB) exchange resin in the H^+ form with an eluent containing a strong or a weak acid [1]. The eluent containing a strong or weak acid can press the ionization of carboxylic acids in the

neutral form and allow them to be retained on the column. Such acidic eluents as sulfuric [2], aliphatic sulfonic [3], phosphoric [4], perfluorobutric [5], succinic and aromatic acids [6] have been used in IEC for the separation of organic acids with various detection modes. Detection modes include refractive index (RI) [7], potentiometry [8], direct UV [9] and conductivity [10]. Refractive index detection offers a universal response but is characterized by low detection sensitivity and thermal instability [7]. Potentiometry with ion selective electrode is too selective to be widely used in many applications [8]. Direct UV detection is most commonly used in the analysis of carboxylic acids with sulfuric acidic eluent at low wavelength (200–220 nm). However, since carboxy-

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lic acids lack the chromophore, detection sensitivity is limited and the sample matrix may cause serious interference [2]. Conductivity is frequently employed in ICE, but it lacks sensitivity due to dissociation of acids and is additionally depressed by the buffer [9].

Aromatic acids can be used as the eluents for the separation of anions in ion chromatography, and indirect UV or direct conductivity modes are frequently employed for the detection of anions. Because an aromatic acidic eluent has a low conductance and strong absorbance in UV region, it enables both detection modes to be used simultaneously. One approach is the use of indirect UV detection because it is universal detection, and is based on increase or decrease in the concentration of the chromophore in the analyte zone, resulting in either an increase or a decrease in the background signal [10]. However, as detection modes applicable to IEC are limited, new detection schemes need to be explored. So far, there are few reports on indirect UV employed in IEC. Our previous studies [11,12] have shown that a range of aromatic acids used as eluents for conductivity and indirect UV detection of organic acids offer reasonable resolution and detection sensitivity. As a subsequent study, it is therefore of interest to determine whether direct conductivity and indirect UV detection can be employed simultaneously in IEC for the separation of carboxylic acids.

In this paper, PDCA was evaluated as the eluent. Since PDCA has a high molar absorptivity and low conductance, it is possible to detect carboxylic acids by simultaneous use of both indirect UV and conductivity. The effects of PDCA concentration in eluent on the separation and on detection sensitivity were examined to find an optimal condition. Finally, the application of the proposed method used for the determination of the carboxylic acids in environmental samples was demonstrated.

2. Experimental

2.1. Instrumentation

All experiments utilized a fully automated Waters liquid chromatographic system including an auto-sampler (717), a temperature controller (626), an UV detector (486), conductivity (431) and computer-based Millennium³² software. A Bio-Rad cation

guard column and an HPX-87 organic acid column (300×7.8 mm I.D., Richmond, CA, USA) were used for separation of carboxylic acids. The HPLC system was fully controlled by the Millennium³² for data collection and processing.

2.2. Chemicals

All reagents were of analytical grade and dissolved in Milli-Q water. Standard solutions of the aliphatic acids were prepared using analytical grade chemicals without further purification. Standards of the aliphatic acids tested were prepared daily from a 10 mM stock in Milli-Q water and diluted to the required concentrations before use. The mobile phase required for ion-exclusion chromatography was prepared by dissolution of an appropriate amount of aromatic acid solution in Milli-Q water. All eluents were filtered through a Millipore 0.45 μm membrane filter and degassed in an ultrasonic bath prior to use.

2.3. Procedure

The mobile phase was pumped through the column and UV detector at a flow rate of 0.6 ml/min. The column temperature was maintained at 40°C and equilibrated for 30 min prior to use. The injected volume of aliphatic acid solution ranged from 10 to 30 μl. The UV detector was operated at 200 or 290 nm with a sensitivity setting of 0.002 a.u.f.s.

3. Results and discussion

3.1. Separation and detection of carboxylic acids

In UV detection mode in IEC, the change in absorbance (ΔA) is given by [1]

$$\Delta A = \epsilon_A^- C_A I_A + \epsilon_{HA} C_A (1 - I_A) \quad (1)$$

where ϵ_A^- and ϵ_{HA} are the molar absorptivities of the two forms of solute, C_A is the total concentration of the solute, and I_A is the degree of the dissociation of the solute. Eq. (1) predicts that indirect UV detection results when a negative value is obtained. Therefore, change in absorbance depends on the degree of ionization of the solutes and their molar absorptivities, as well as the concentration of solute.

For conductivity detection, the change in conductance (ΔG), accompanying solute elution, is given simply by [6,12]

$$\Delta G = G_{\text{analyte}} - G_{\text{eluent}} \quad (2)$$

where G_{analyte} and G_{eluent} represent the conductance of carboxylic acid and the conductance of eluent, respectively. Eq. (2) shows that a high conductimetric response should be obtained using an eluent with a low conductance, and that a linear relationship

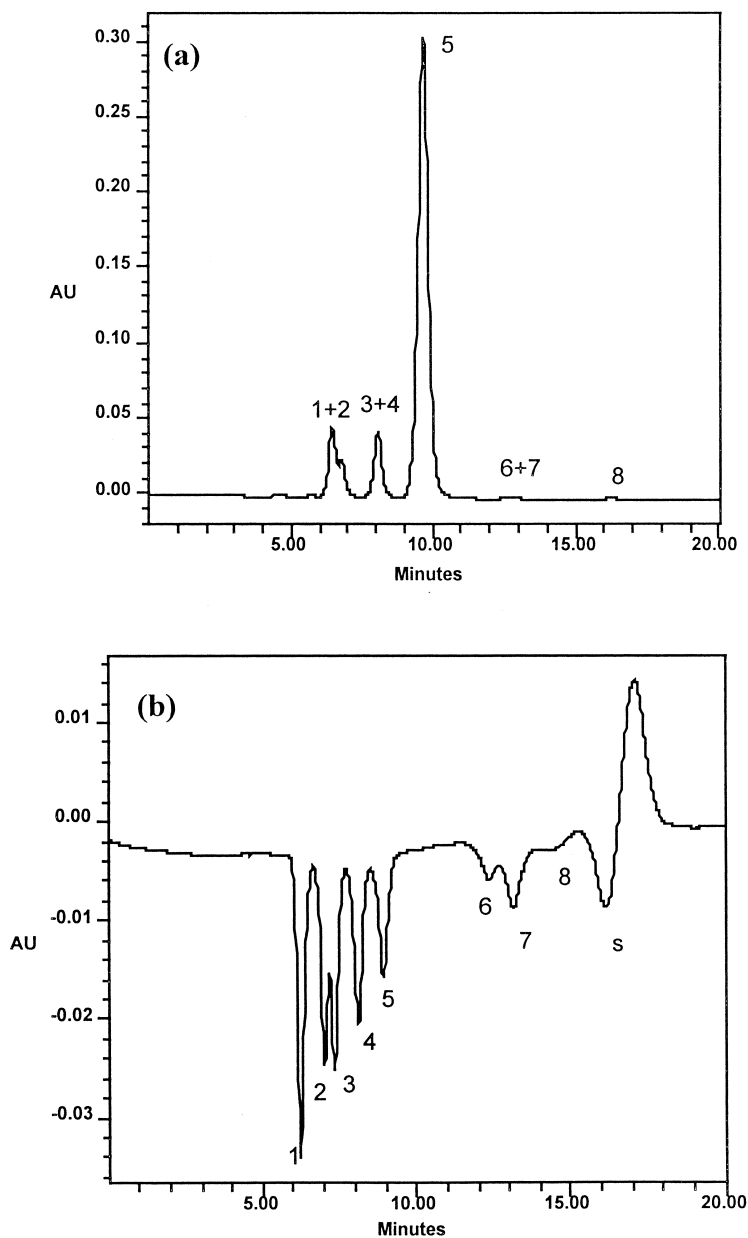


Fig. 1. (a) Ion-exclusion chromatography of carboxylic acids eluted by 1 mM sulfuric acid eluent with direct UV detection at 200 nm. (b) Ion-exclusion chromatography of carboxylic acids with 0.75 mM PDCA eluent with indirect UV detection at 290 nm. (1) oxalic (0.5 mM), (2) pyruvic (0.25 mM), (3) citric (0.25 mM), (4) T-aconitic (0.5 mM), (5) malic (0.5 mM), (6) formic (0.5 mM), (7) succinic (0.5 mM), (8) acetic acid (0.5 mM). Conditions: flow rate: 0.6 ml/min; column temperature 40°C; injected volume: 10 μ l.

between conductimetric response and the solute concentration should be obtained when the concentration of aromatic acid in the eluent is kept constant [6,12].

Fig. 1 (a) shows the IEC separation of the carboxylic acids using 1 mM sulfuric acid as the eluent with direct UV and conductivity detection. It is clearly demonstrated that a poor resolution was obtained with the sulfuric acidic eluent. The co-elution of oxalic and pyruvic acids, and t-aconitic and malic acids was observed due to their similarity of pK_a . A lower detection sensitivity was obtained using both detectors. Low sensitivity of direct UV for the test solutes could be attributed to the fact that the carboxylic acids have weak or no UV absorbance. In addition, the sensitivity of conductivity was reduced since sulfuric acidic eluent has a high background conductance [6].

PDCA is a weak acid with a high molar absorptivity and low conductance. On the basis of the Eqs. (1) and (2), it is possible to use PDCA as an eluent for the separation of carboxylic acids in IEC with indirect UV and direct conductivity detection. Fur-

ther, the pK_a of PDCA falls between those of oxalic acid and acetic acid, which would reduce the ionization of the carboxylic acids. Thus the separation of carboxylic acids is possible. Fig. 1(b) presents a chromatogram using 1 mM PDCA eluent. Reasonable resolutions with the symmetric peak for the carboxylic acids were achieved. Both indirect UV and conductivity responded to the solutes with high detection sensitivity. However, a system peak was observed due to the strong adsorption of PDCA onto the PS–DVB resin [14]. Similar results were obtained in our previous studies [11,12]. Compared to sulfuric acidic eluent, the resolution and peak sharpness were improved significantly using PDCA eluent. The reason could be the buffering action of the PDCA eluent, which maintains the eluting band of analyte at a constant level of ionization, and thus the analyte molecules moved at the same speed down the column to give a symmetrical peak. In addition, the detection sensitivity using PDCA eluent was higher than that obtained with sulfuric acidic eluent due to the high molar absorptivity and low background conductance of the latter.

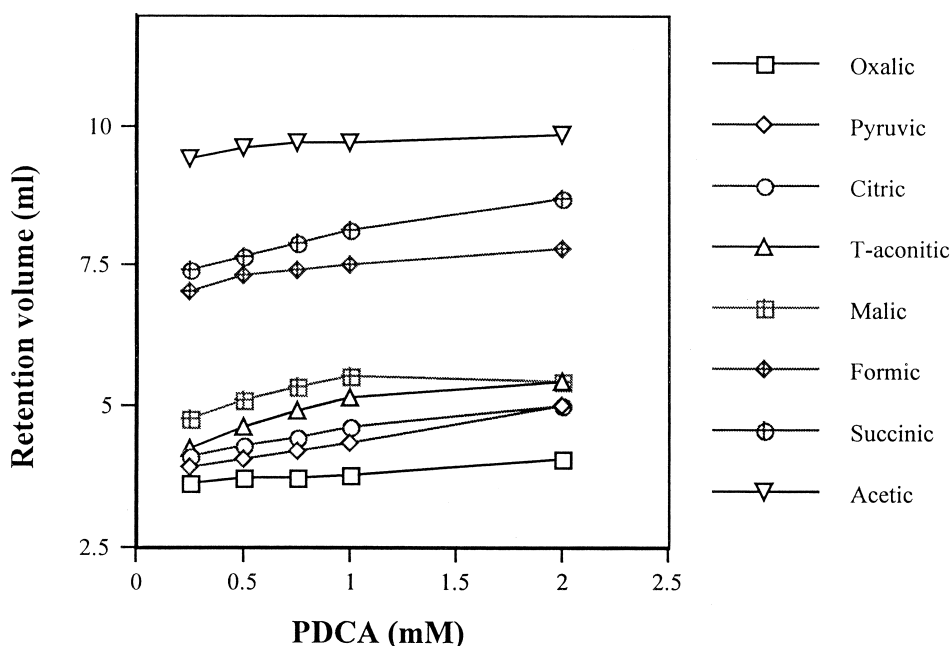


Fig. 2. The effect of the concentration of PDCA in the eluent on the retention of the carboxylic acids. Conditions: flow rate: 0.6 ml/min; column temperature 40°C; injected volume: 10 μ l.

3.2. Effect of PDCA concentration on separation and detection

Fig. 2 shows the effect of the concentration of PDCA in the eluent on retention. In general, the retention of the solutes slightly increases with increasing PDCA concentration in the eluent. The poor resolution and peak tailing were observed using the eluents with PDCA concentration below 0.75 mM. The reason for this was that the ionization of the carboxylic acids increased when PDCA concentration was low, resulting in a decreased retention of the carboxylic acids on the column [11,13]. However, co-elution of solutes such as pyruvic and citric acids, and formic and succinic acids appeared when PDCA concentration was above 2 mM. In this case, increased PDCA concentration in the eluent resulted in a decreased retention because the elution of the carboxylic acids was enhanced.

Fig. 3 shows that the effect of PDCA concentration in the eluent on indirect UV response. It exhibited that indirect UV response decreased with increasing PDCA concentration. The changes in

absorbance were inversely correlated to the dissociation constant and PDCA concentration in the eluent. Moreover, the proportional changes of absorbance to the dissociation constant of a solute were also noted. For instance, indirect UV response was greatest for oxalic acid (pK_a : 1.27) and was lowest for acetic acid (pK_a : 4.76). However, increasing PDCA concentration increased the baseline noise due to a high background absorbance of the eluent [12].

The effect of PDCA concentration on the sensitivity of conductivity is shown in Fig. 4. For direct conductivity, it was expected from Eq. (2) that the detection sensitivity increased with decreasing the background conductance. The result shown in Fig. 4 is in agreement with Eq. (2). Increased PDCA concentration in the eluent led to rise the background conductance, resulting in decreased detection sensitivity. But in our case, the sensitivity was only reduced slightly due to small changes in concentration. Despite that fact that low PDCA concentration (below 0.5 mM) in the eluent offered high detection sensitivity, it becomes necessary to compromise the sensitivity with a desired resolution.

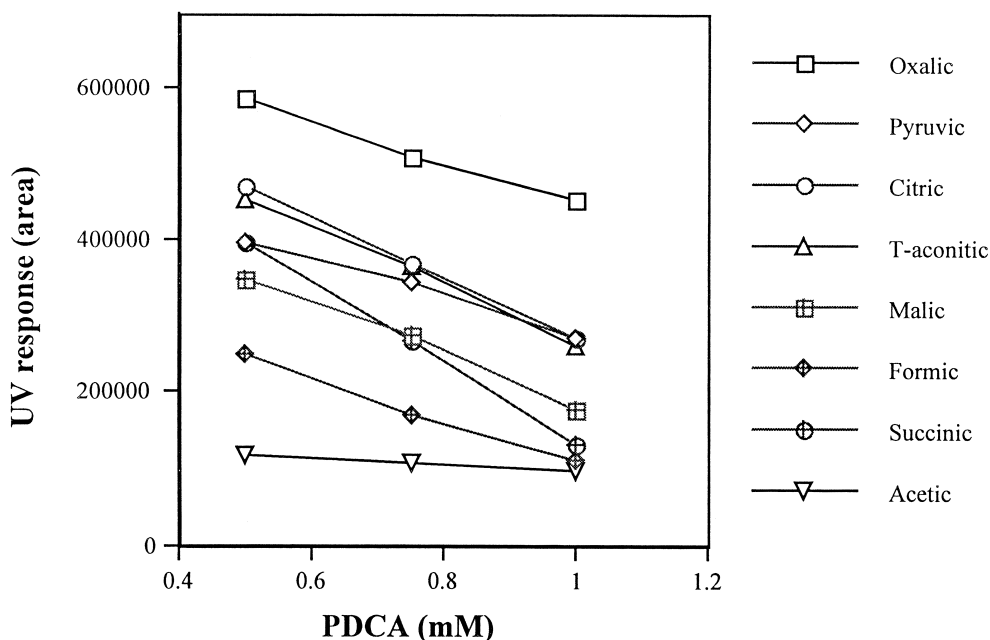


Fig. 3. The effect of the concentration of PDCA on the detection sensitivity of indirect UV. Conditions: flow rate: 0.6 ml/min; column temperature 40°C; injected volume: 10 μ l.

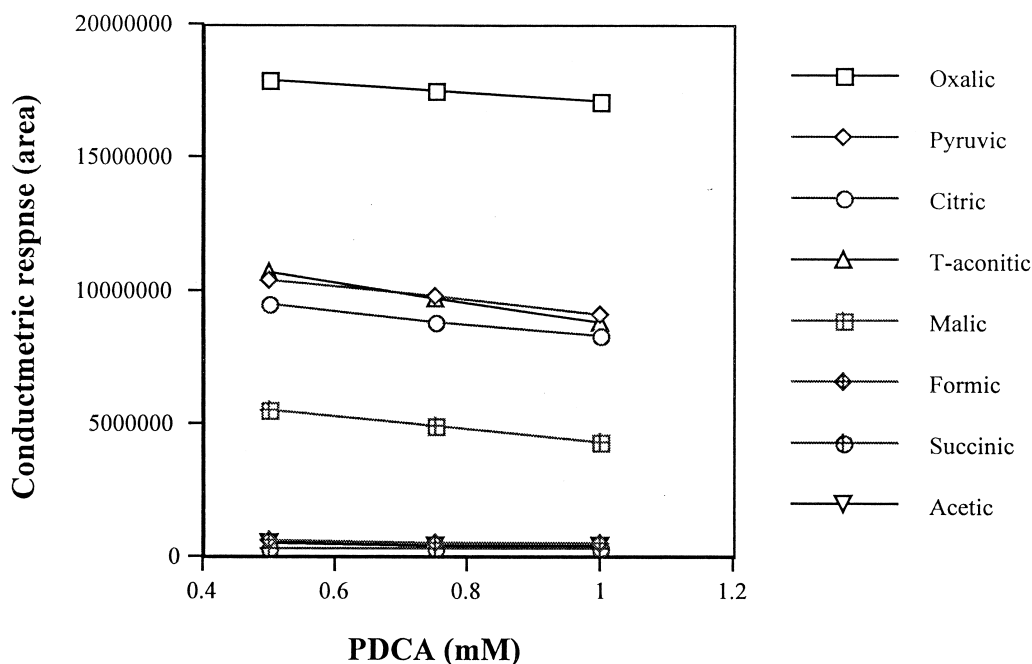


Fig. 4. The effect of the concentration of PDCA on the detection sensitivity of conductivity. Conditions: flow rate: 0.6 ml/min; column temperature 40°C; injected volume: 10 μ l.

Considering both the separation and detection sensitivities, 1 mM PDCA in the eluent appeared to provide optimal conditions.

3.3. Analytical characteristics

Fig. 5 represents a typical chromatogram obtained using 1 mM PDCA eluent, where the carboxylic acids were separated by IEC and detected by both indirect UV at 290 nm (Fig. 5(a)) and non-suppressed conductivity (Fig. 5(b)). The carboxylic acids were eluted in the order of oxalic, pyruvic, citric, malic, *trans*-aconitic, formic, succinic and acetic acids. Reasonable resolution, symmetrical peaks and sensitive detection were achieved under the chosen experimental conditions, indicating that the eluent containing PDCA depressed the ionization of the carboxylic acids, and hence increased the retention. Moreover, PDCA offered a high UV absorptivity and a low background conductance, which allowed the detection of the carboxylic acids in IEC using indirect UV and conductivity. By plotting peak area vs. the concentration of the acids, the linearity was

obtained in the concentration range of 0.01 to 1 mM with the correlation coefficients (r^2) of 0.993–1.00. Detection limits (signal/noise=3) were in the concentration range of 1.0–7.0 μ M for conductivity detection and 8–30 μ M for UV detection. The reproducibility (relative standard derivation, $n=5$) from injecting a solution containing 1 mM test acid solution ranged from 1.1 to 3.1%. The analytical characteristics for the proposed method are listed in Table 1. The detection limits obtained in conductivity were lower than those of indirect UV detection.

3.4. Analysis of carboxylic acids in various samples

The proposed method was used to determine the carboxylic acids in a wastewater (Fig. 6(a)), a soil extract (Fig. 6(b)) and a plant tissue extract (Fig. 6(c)). Samples were diluted ten-fold prior to injection where necessary and was filtered through a Millipore 0.45- μ m membrane. Reasonable resolution for the carboxylic acids was obtained with an exception of

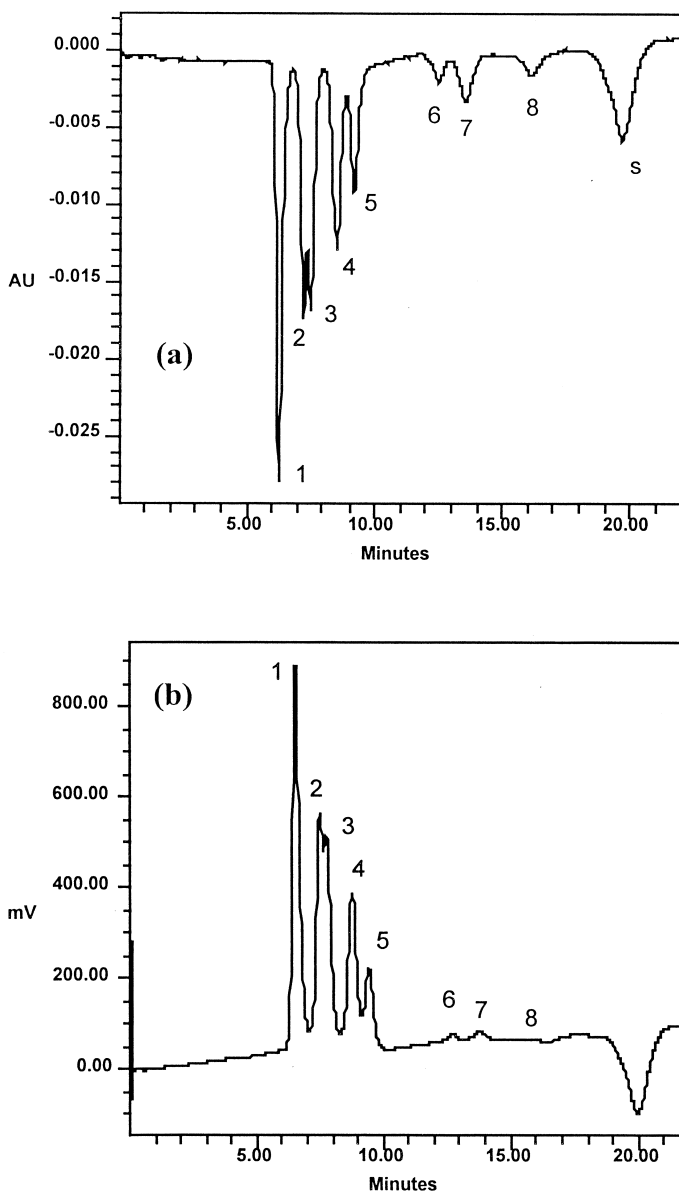


Fig. 5. Ion-exclusion chromatography of the carboxylic acids using 1 mM PDCA in the eluent. (a) Indirect UV detection at 290 nm. (b) Conductivity detection: (1) oxalic (0.5 mM), (2) pyruvic (0.25 mM), (3) citric (0.25 mM), (4) T-aconitic (0.5 mM), (5) malic (0.5 mM), (6) formic (0.5 mM), (7) succinic (0.5 mM), (8) acetic acid (0.5 mM). Other conditions as described in Fig. 1.

oxalic acid. The concentrations of carboxylic acids in various samples, together with spiked carboxylic acids and their recoveries for the proposed method, are listed in Table 2. The results have shown that the proposed method with direct injection is useful for the determination of carboxylic acids in environmen-

tal samples. However, the problem of co-elution of oxalic acid with inorganic anions such as Cl^- and NO_3^- [12,14] was found. An extraction procedure to eliminate anion interference is required in our further work for determination of oxalic acid in real samples.

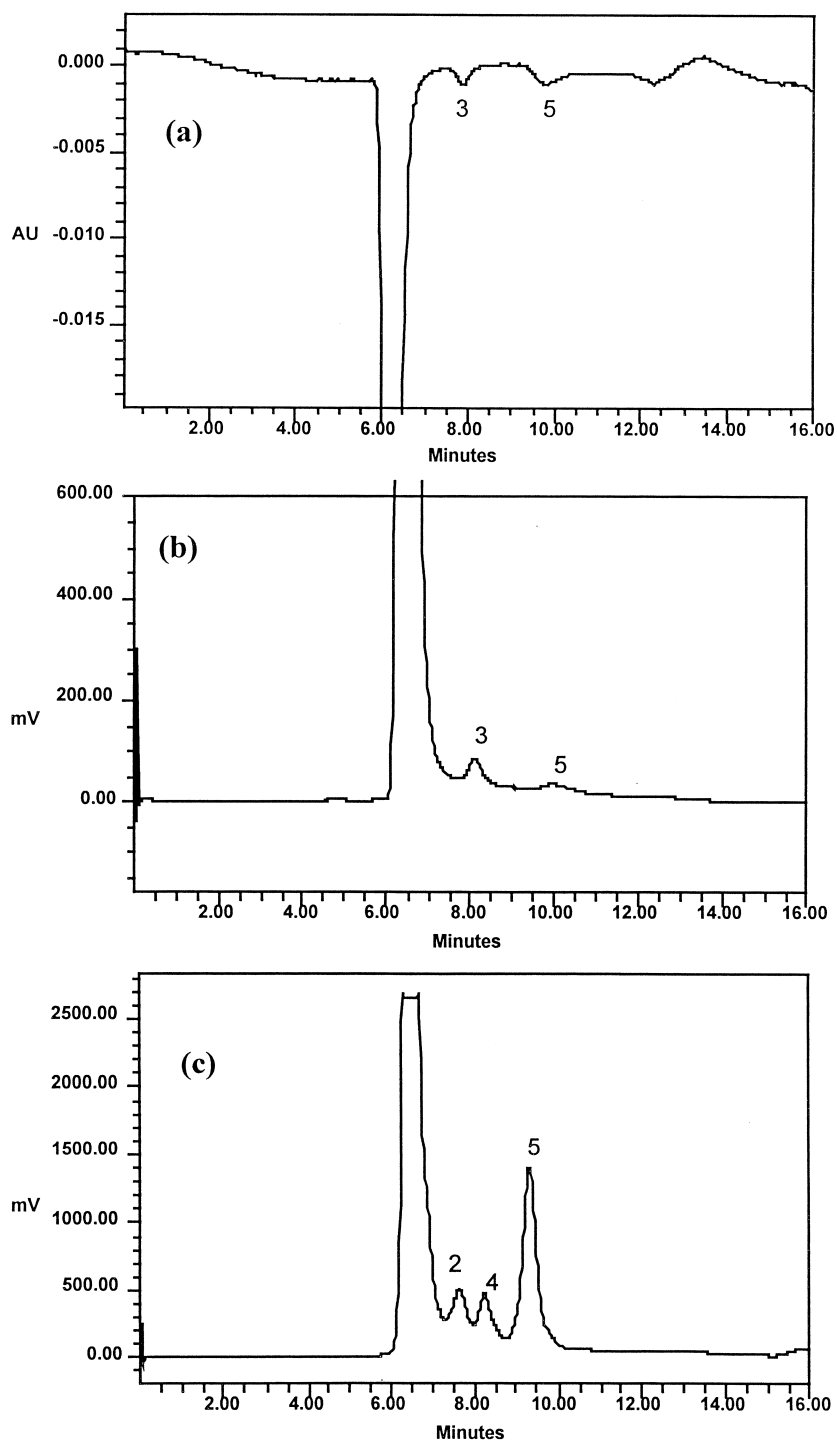


Fig. 6. Chromatograms obtained from environmental samples using 1 mM PDCA in the eluent: (a) water samples with indirect UV detection; (b) soil extract with conductivity detection; (c) plant tissue extract with conductivity detection. Other conditions as described in Fig. 1.

Table 1

Dissociation constant (pK_a), retention volume (V_R), detection limit (D_L) with relative standard deviation (RSD%, $n=5$) of the carboxylic acids with indirect UV and conductivity detection in IEC^a

| Acids | pK_a | Retention V_R (ml) | Indirect UV | | Conductivity | |
|------------|--------|-------------------------|-------------------|------|-------------------|------|
| | | | D_L (μM) | RSD% | D_L (μM) | RSD% |
| Oxalic | 1.27 | 3.76 | 8 | 1.5 | 1.0 | 1.8 |
| Pyruvic | 2.49 | 4.35 | 10 | 1.9 | 1.5 | 1.1 |
| Citric | 3.13 | 4.51 | 12 | 1.6 | 2.0 | 1.2 |
| T-aconitic | 2.80 | 5.12 | 15 | 1.9 | 2.0 | 1.4 |
| Malic | 3.40 | 5.51 | 20 | 2.0 | 3.0 | 1.8 |
| Formic | 3.75 | 7.50 | 25 | 2.4 | 3.5 | 1.9 |
| Succinic | 4.21 | 8.13 | 20 | 1.9 | 4.0 | 2.5 |
| Acetic | 4.76 | 9.69 | 30 | 2.8 | 7.0 | 3.3 |

^a Conditions: flow rate: 0.6 ml/min; column temperature 40°C; injected volume: 10 μ l; 1 mM 2,6-PDCA in the eluent.

Table 2

Concentration of the carboxylic acids in waste water, soil and plant extracts determined by the proposed method^a

| Sample | Citric (mM) | Malic (mM) | Pyruvic (mM) | Formic (mM) |
|-----------------|----------------|---------------|-----------------|----------------|
| 1. Waste water | 0.040 | 0.060 | n.d. | 0.020 |
| Spiked | 0.100 | 0.100 | – | 0.100 |
| Found | 0.138 | 0.156 | – | 0.119 |
| 2. Soil | 0.051 | 0.042 | n.d. | n.d. |
| Spiked | 0.100 | 0.100 | – | – |
| Found | 0.148 | 0.137 | – | – |
| 3. Plant tissue | 0.260 | 1.420 | 0.230 | n.d. |
| Spiked | 0.500 | 0.500 | 0.500 | – |
| Found | 0.762 | 1.930 | 0.740 | – |

^a Conditions as described in Table 1; n.d.: not detected, – not spiked.

4. Conclusion

The work has shown that PDCA can be used as an eluent for the separation of the carboxylic acids in IEC, which enabled both indirect UV and direct conductivity detection modes to be employed simultaneously. However, the low detection limit was obtained using conductivity detection. In comparison with sulfuric acidic eluent, reasonable resolution and high detection sensitivity were given using 1 mM PDCA in the eluent. The proposed method was demonstrated for the analysis of carboxylic acids in environmental samples with direct injection.

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