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Liquid membrane enrichment for the ion chromatographic determination of carboxylic acids in soil samples

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Abstract

The studies of low-molecular-mass carboxylic acids in complex matrices as root exudates and soil liquids are important for the evaluation of their ecological significance and role in pedological processes and chemical reactions. A supported liquid membrane, impregnated with 10% tri-*n*-octylphosphine oxide in di-*n*-hexyl ether, was used for selective enrichment of the carboxylic acids in soil samples. The acids were determined by an anion-exchange chromatograph coupled to the enrichment system. A cation-exchange precolumn was found to improve the resolution of the peaks. Two short ion-exchange columns were incorporated into the automatic flow system to remove interfering inorganic ions. Extraction efficiencies in the range 14–75%, depending on the polarity and acidic strength of the analytes, were achieved. The system permits the determination of low-molecular-mass carboxylic acids at micromolar level in the presence of interfering ions such as nitrite, chloride, sulphate, iron and aluminium at the concentration found in authentic samples.

Keywords: Soil; Membranes; Sample enrichment; Carboxylic acids

1. Introduction

Plant root exudates contain large quantities of low-molecular-mass carboxylic acids, e.g. formic, malic, oxalic, citric and acetic acid or their salts [1–3]. It has been suggested that some acids may regulate the ability of plants to grow in particular soils, in solubilizing soil mineral nutrients and in modifying soil chemical properties [4]. Furthermore, the molecular size of humic substances has been shown to be modulated by carboxylic acids in root exudates [5].

Ion chromatography is widely used for the determination of free carboxylic acids in aqueous samples. A variety of organic acids in root exudates was determined with ion-exclusion chromatography and UV detection [2,6,7]. This technique is generally not as sensitive and selective as ion chromatography with conductivity detection [4]. Gas chromatographic methods usually involve derivatization due to the low FID response and high polarity of low molecular carboxylic acids [8]. Thin-layer chromatography has been used for carboxylic acids in root and seed exudates [3,9].

Sample matrices such as soil liquids normally demand a clean-up step prior to the detection of the analytes of interest. Several solutions to this problem has been suggested e.g. filtration [2,4], freeze–drying [6] and ion-exchange precolumns [3]. Preconcentration of carboxylic acids in air samples by a

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supported liquid membrane technique has been recently presented [10]. The technique allows simultaneous clean-up and enrichment of the sample and on-line transfer to the ion chromatograph.

The objectives of this work have been to develop and modify an automated ion chromatographic method for analysis of low concentrations of carboxylic acids in the presence of high concentration of inorganic anions such as nitrite, chloride, sulphate and phosphate as well as metal ions such as iron and aluminium. The method involves on-line sample pretreatment by means of three precolumns and a supported liquid membrane. The results of an ecological investigation using this method are published elsewhere [11].

2. Experimental

2.1. Equipment

Analyses were performed on an ion chromatograph connected on-line to a liquid membrane enrichment flow system. An AS11 anion-exchange column (4 mm × 250 mm, Dionex corporation, Sunnyvale, CA, USA) with an AG11 guard column, an anion self-regenerating suppressor (ASRS) and a NaOH gradient was used. The chromatograms were collected and handled by a Borwin Chromatography Data System (version 1.20, JMBS Developpements, La Fontanil, France) which also controlled the time sequence for the operation of the valves in the flow system.

The flow system is shown in Fig. 1. Two pneumatic valves (VA and VB, type 5701, Rheodyne, Cotati, CA, USA) and another six-port rotary valve (VC, type A60, VICI, Houston, TX, USA) were employed together with three three-port slider valves (V1, V2 and V3, model 5300, Rheodyne) for the flow system which also comprised two cation-exchange resin precolumns (b and h, 100 μl, Dowex 50 × 8, 50–100 mesh) and an anion-exchange resin precolumn (d, 100 μl, Dowex 1 × 8, 100–200 mesh).

A peristaltic pump (type IPN-16, Ismatec, Labinett, Zürich, Switzerland) with PVC pump tubes

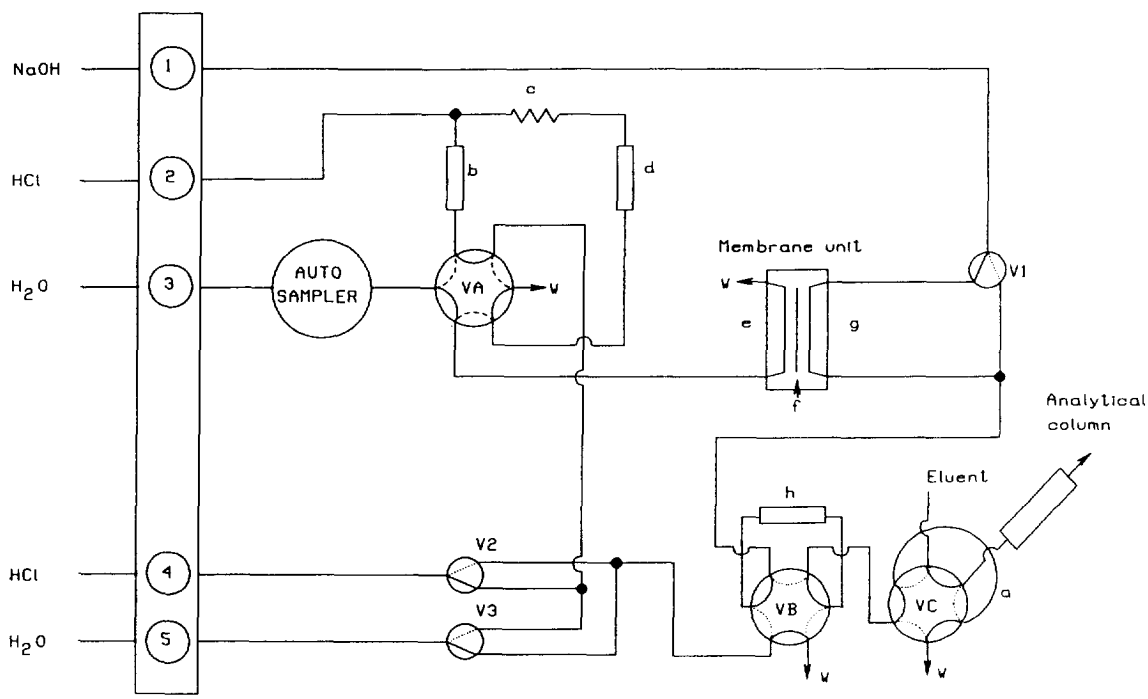

Fig. 1. Flow system.

(Ismatec) was used. The confluences, where the channels meet at a 60° angle, were made of PTFE. The donor reagents were mixed in a knotted tube reactor (c, 0.5 mm I.D., six knots). PTFE tubing, flange-free fittings (Alltech, Deerfield, IL, USA) and Dionex standard fittings were used to connect the various parts of the flow system.

The membrane used was a porous PTFE membrane (TE 35 Membrane filter, Schleicher and Schuell GmbH, Dassel, Germany) with a pore size of 0.2 μm and cut to a diameter of 55 mm. The membrane was held between two blocks of PTFE, with spiral channels facing each other. The channel was 0.15 mm deep, 2.0 mm wide and 500 mm long, giving a calculated volume of 150 μl. The PTFE blocks were backed up with aluminium blocks to ensure rigidity.

The solution for impregnating the membrane was prepared by adding 10 ml di-*n*-hexyl ether to 0.88 g of tri-*n*-octylphosphine oxide and sonicating it for about 1 h. The membrane was immersed in the organic liquid in a petri dish for a few minutes (time not critical). After mounting the membrane, excess solvent was washed out from the separator by pumping acceptor and donor solutions through the channels for at least 30 min.

Iron and aluminium were determined with an atomic absorption spectrometer (SpectrAA 400, Varian, Sunnyvale, CA, USA).

2.2. Operation of the liquid membrane enrichment system

Referring to Fig. 1, the operation of the flow system can be described as follows. The sample was introduced through a 2-ml loop into the aqueous carrier stream (channel 3 of the peristaltic pump, 0.14 ml/min) by a autosampler (HPLC 360, Kontron Instruments, Zürich, Switzerland) coupled with a cooling system to keep the temperature of the sample vial at 5°C. The sample passed through a cation-exchange column (b) in order to trap metal ions. The pH was lowered to about 1 by mixing with 1 *M* HCl (channel 2, 0.02 ml/min) in a knitted tube reactor (c). Unwanted anions were removed in an anion-exchange column (d) before the sample reached the donor side of the membrane separator (e), while the carboxylic acids were uncharged at the prevailing pH. In the membrane unit, the carboxylic acids were extracted into the liquid membrane (f) and trapped in a stagnant NaOH solution on the other side of the membrane (acceptor phase, g). After the whole sample had passed the donor phase of the separator, the acceptor valve (V1) was switched. The enriched sample plug was pushed by the acceptor solution (channel 1) through a second cation-exchange column (h). A portion, centred on the peak maximum of the plug profile, was caught in a 50-μl injection loop (a) and finally injected into the anion-separation column by switching the six-port valve (VC).

A typical enrichment cycle was 18 min. The membrane donor and acceptor phases were washed with the corresponding solution for about 20 min to reduce memory effects between every sample enrichment cycle; at the same time, the precolumns were regenerated with 3 *M* HCl (channel 4, 0.2 ml/min) and subsequently washed with water (channel 5, 0.20 ml/min). The total procedure took 38 min and during 25 min of the time a previously extracted sample was chromatographed.

2.3. Chromatographic conditions

The eluent gradient program recommended by the manufacturers [12] for separation of a variety of inorganic anions and carboxylic acid anions with the AS11 column was used for the present application as shown in Table 1. Three solutions: E1 (water), E2 (5 m*M* NaOH) and E3 (100 m*M* NaOH) in different percentage of volume were used as eluent.

2.4. Determination of enrichment factors

The concentration enrichment factor, E_c, is defined as $E_c = C_A/C_D$, where C_A and C_D are the concentrations of analyte in the acceptor and donor phases, respectively.

Enrichment factors were determined using 5-μM standard solutions of the carboxylic acids; 2 ml were enriched and injected as described above. The same solution was manually introduced into the 50-μl loop without passing the membrane unit and injected. The enrichment factor was then obtained as the ratio of the peak areas.

The extraction efficiency, E, is defined by $E = n_A/n_D$ where n_A is the number of analyte moles col-

Table 1
Gradient program

Time (min)	0.0	2.5	6.0	18.0	18.1	25.0
%E1 (water)	90	90	0	0	90	90
%E2 (5 m*M* NaOH)	10	10	100	65	10	10
%E3 (100 m*M* NaOH)	0	0	0	35	0	0
[OH^-] (m*M*)	0.5	0.5	5	38.25	0.5	0.5

lected in the acceptor phase and n_D is the number of moles pumped into the donor channel. E can be calculated from E_c by the equation $E = E_c \cdot V_A/V_S$, where V_A and V_S are the volume of the acceptor channel and of the total sample pumped into the donor channel, respectively.

2.5. Chemicals

Formic, acetic, oxalic, tartaric, citric acids, iron nitrate and aluminium nitrate were obtained from Merck (Darmstadt, Germany), lactic acid from BDH (Poole, UK), malic acid and di-*n*-hexyl ether from Sigma (St. Louis, MO, USA), sodium hydroxide from EKA (Bohus, Sweden), pyruvic, isocitric, *cis*-aconitic, *trans*-aconitic acids and tri-*n*-octylphosphine oxide (TOPO) from Fluka (Buchs, Switzerland) P.a. qualities were used when available. The water was purified with Milli-Q/RO-4 unit (Millipore, Bedford, MA, USA).

Stock solutions of the acids (20 m*M*) were prepared in water and stored in a refrigerator. Fresh standard solutions were prepared every day. The 50% w/v NaOH stock solutions (for eluent and acceptor) was prepared by dissolving NaOH pellets in degassed water. The acceptor solution (10 m*M*) was kept in a eluent bottle under nitrogen pressure in order to minimise the production of carbonate ions from dissolution of atmospheric carbon dioxide.

3. Results and discussion

3.1. Sample enrichment

Enrichment factors and extraction efficiency for some studied acids are presented in Table 2. From our previous work [13], it was known that the extraction efficiency in this system was reasonably high for the monocarboxylic acids but lower for oxo, di- and tricarboxylic acids which have relatively higher polarity.

The time needed for the enriched sample to reach the injection loop (transfer time) was determined by plotting curves of peak area versus the time interval between switching valve V1 and valve VC. Based on this curve a suitable time was selected.

3.2. Removal of disturbing anions

In typical soil water samples, the concentrations of the carboxylic acids are in the low μM range, while the concentrations of inorganic anions are considerably higher (500–1000 μM NO_3^-, 200–600 μM Cl^- and 100–200 μM SO_4^{2-}). As can be seen in Fig. 2, which shows a chromatogram from a direct injection of a soil liquid sample, this excess of inorganic ions severely disturbs the chromatographic run, both by masking parts of the chromatogram and by influencing the peak shapes of the early eluting peaks. The latter effect is due to overloading of the column by the inorganic ions.

By liquid membrane extraction, the carboxylic acid anions can be enriched and the sample can be purified to a certain extent. However, the inorganic anions, especially nitrate, are also enriched by the TOPO-containing membrane, which results in even

Table 2

Carboxylic acid	Enrichment factor	Extraction efficiency (%)
Lactic	3.8	34
Acetic	5.6	51
Formic	8.4	76
Malic	3.2	29
Oxalic	2.8	25
Pyruvic	1.8	16
Citric	1.6	14

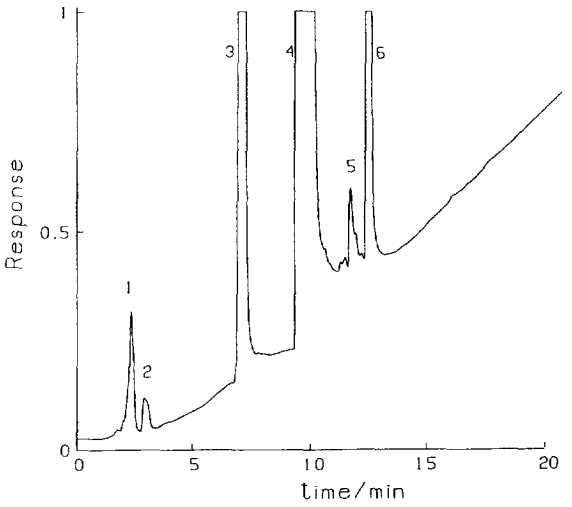

Fig. 2. Chromatogram of a soil sample. Peak identities: 1, lactic/acetic; 2, formic; 3, Cl^-; 4, NO_3^-; 5, malic and CO_3^{2-}; 6, SO_4^{2-}.

more serious overloading of the analytical column and overlap with the peak of malic acid. To solve this problem, an anion precolumn (d) was introduced into the flow system and the pH of the sample was lowered to about 1 by on-line mixing with 1 *M* HCl in the coil (c). The major part of the NO_3^-, and SO_4^{2-} were trapped by the precolumn and exchanged to Cl^-, while the carboxylic acids were protonated and not trapped. After this column, the sample was passed to the membrane unit, where the protonated carboxylic acids were extracted as described above.

The Cl^- is extracted less efficiently than NO_3^- by the liquid membrane and does not overlap interesting peaks in the chromatogram, as can be seen in Fig. 3. The small fraction of remaining NO_3^- and SO_4^{2-} does not cause problems in the chromatogram. The same is the case with PO_4^{3-} which normally does not exist in high concentration in soil samples.

3.3. Removal of metal ions

Two catex precolumns which contain strong cation-exchange resin were introduced into the system to remove metal ions from the soil solution. The existence of some metals such as iron and aluminium in high concentration will seriously affect the determination of such acids as oxalic, citric and aconitic acids, since the metals ions can form stable

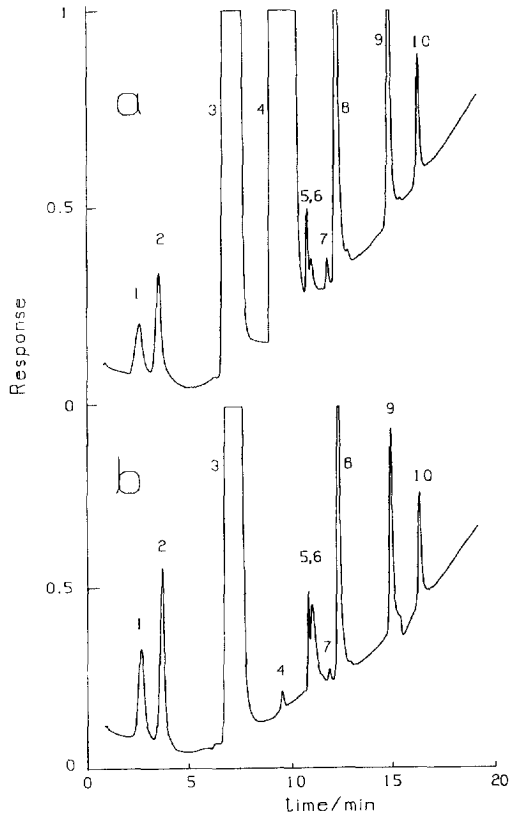

Fig. 3. Chromatograms of a standard solution: 2-μM carboxylic acids in 1 m*M* NO_3^-, 0.2 m*M* SO_4^{2-} and 0.2 m*M* PO_4^{3-} after enrichment (a) without and (b) with the anion precolumn. Peak identities: 1, lactic and acetic; 2, formic; 3, Cl^-; 4, NO_3^-; 5, malic; 6, CO_3^{2-}; 7, SO_4^{2-}; 8, oxalic; 9, PO_4^{3-}; 10, citric.

complexes with the acids. Additionally, the metal will contaminate the analytical column and result in asymmetric peak shapes and/or variable analyte recoveries [12].

Standard acids solutions (10 μM) which contained different concentrations of Fe^{3+} and Al^{3+} from 0 μM to 60 μM were analysed by the system without the catex precolumns. The relative recovery of oxalic acid (based on an assumption of 100% recovery in the solution without the metals) dramatically decreases as the concentration of the metals increase. This can be seen in Fig. 4. Similar phenomena can also be found for some other acids. This provides strong evidence that the metals have to be removed.

The same experiments were done in the system with the precolumns (b and h). Only pyruvic acid

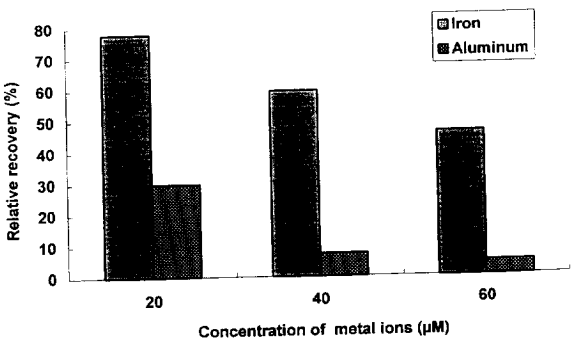

Fig. 4. Influence of the concentration of Fe^{3+} and Al^{3+} on the relative recovery of oxalic acid without catex precolumns. Sample: 20 μM carboxylic acids in 1 mM NO_3^- + 0.2 mM SO_4^{2-}.

shows somewhat lower relatively recovery with high concentration of aluminium. No significant decreases of the recoveries were found with the acids when the samples contained as much as 80 μM Fe^{3+} or Al^{3+} (Fig. 5 and Fig. 6), indicating that the catex precolumns are essential to the system.

Table 3 lists the trapped fractions of Al^{3+} and Fe^{3+} ions by the catex precolumn (b) from standard solutions with different pH. No evident differences are observed with or without the acids in the solutions, and there are only slight changes with pH. After treatment the pH always turns to 3 for the pH 5 and pH 10 samples.

The extraction efficiencies of the metal ions by the membrane were determined by the same procedure as used for the acids. The disadvantage that the precolumn has a lower efficiency of removal of Al^{3+} is compensated by the lower membrane-extraction efficiency (Table 4). After the membrane, the remains of metals were further removed by the second catex precolumn (h). Thus an efficient clean-up of the metals was obtained by a combination of the precolumns and the liquid membrane.

3.4. The effect of the second catex precolumn on chromatographic resolution

It was observed that the resolution of early peaks, especially those of lactic and acetic acids, was impaired when the injected sample originated from liquid membrane extraction compared with direct injection of a standard solution of carboxylic acids. Also, when a solution contains inorganic anions in high concentrations, a similar degradation of the resolution is observed. It has been found that the inclusion of a H^+-saturated cation-exchanger column before the analytical column corrected these problems (see Fig. 7).

A tentative explanation to this effect is the following. The acceptor liquid into which the carboxylic acids are extracted is NaOH (10 mM), partly neutralised by co-extracted HCl. When this is injected into the column it will act as a relatively strong eluent, stronger than the 0.5 mM initial eluent. This causes the acid ions to be applied to the column as a wide band. The cation exchanger exchanges Na^+ ions for

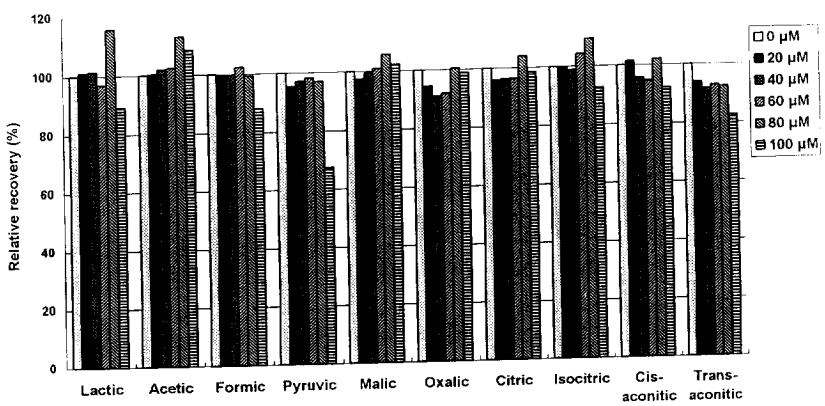

Fig. 5. Influence of the concentration of Fe^{3+} on the relative recovery of the target acids with catex precolumns (b and h). Other conditions same as Fig. 4.

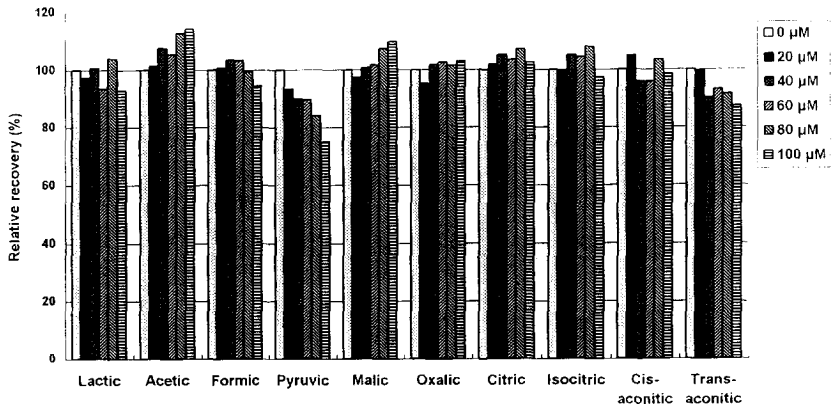

Fig. 6. Influence of the concentration of Al^{3+} on the relative recovery of the target acids. The conditions same as Fig. 5.

Table 3
Trapped fractions (%) of the metals with the catex precolumn (b)

	pH 2		pH 5		pH 10	
	With acids	Without acids	With acids	Without acids	With acids	Without acids
Al^{3+}	83.1	82.7	85.2	85.8	87.9	86.3
Fe^{3+}	96.0	95.4	94.6	94.6	96.8	96.8

Sample: Fe^{3+} (50 μM), Al^{3+} (100 μM) in 1 mM $NaNO_3$ with or without acids (formic, acetic, malic, oxalic, citric and aconitic acids, 10 μM).

H^+ ions, thus neutralising OH^- ions which will lead to a focusing of the sample anions on the column.

However, the peaks of lactic and acetic acids are still only partly separated due to the close elution. A fronting or a tailing peak will be seen in the case where one of these acids is in relatively low concentration.

3.5. Loss of the analytes in precolumns

A certain loss of the analytes due to their adsorption on the precolumns was found. The adsorbed fractions of some target acids on the precolumns are shown in Fig. 8. It is very interesting to see that the existence of some amount of salts in the samples, as is the case with soil solutions, significantly decreased the adsorption. The major parts of the acids were trapped by the anion-exchange precolumn as seen in

Table 4
The extraction efficiency of the metals by 10% TOPO membrane

Sample	Fe^{3+}	Al^{3+}
50 μM	12.1%	3.0%
100 μM	11.4%	2.5%

Sample: Metals ion in the acids solution as in Table 3.

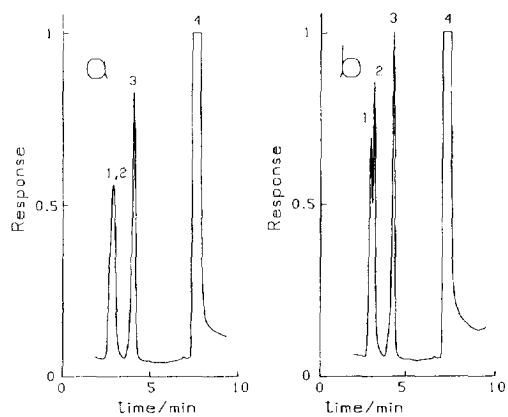

Fig. 7. Part of the chromatograms of a standard solution: 5 μM carboxylic acids in 1 mM inorganic anions after enrichment and (a) without and (b) with the catex precolumn (h). Peak identities: 1, lactic; 2, acetic; 3, formic; 4, Cl^-.

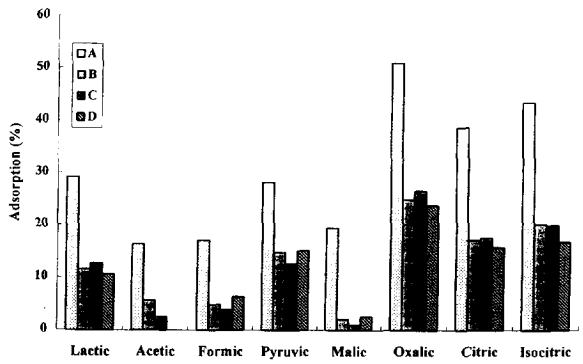

Fig. 8. Adsorption percentage of the acids on the precolumns. 20 μM acids in (A) no salt, (B) 2 mM $NaNO_3$, (C) 2 mM Na_3PO_4, (D) 1 mM $NaNO_3$ + 0.2 mM Na_2SO_4.

Table 5, obviously indicating the adsorption of the anionic forms. To minimize the adsorption, an imitation of the major inorganic salt composition of the soil solution was made by adding 1 mM $NaNO_3$ and 0.2 mM Na_2SO_4 to the standard solution.

3.6. Quantitation

Precision

The relative standard deviation (R.S.D.) values of peak area for 10-μM standard samples ($n = 5$) ranged from 1% to 5% for all the acids.

Calibration curves

Calibration curves for eight acids in 1 mM $NaNO_3$ are listed in Table 6, which shows that the curves were linear for all acids and that the intercepts were in most cases insignificant (95% confidence intervals).

Detection limits

The detection limits with enrichment of 2 ml of sample were 60 nM for oxalic acid and 100 nM for formic acid. For pyruvic, citric, isocitric and aconitic acids, a practical detection limit was about 200 nM partly due to their low enrichment factor. Because of the interference from the carbonate peak, as seen in Fig. 3, the detection limit for malic acid was 250 nM. Lactic and acetic acids had individually about the same detection limit as formic acid, but due to poor peak resolution, it was difficult to attain such low detection limits simultaneously.

Recovery of the analytes

A mixture of 10 μM of the studied acids was added into some soil solutions and the recovery of the acids (concentration found/concentration expected) were calculated. The results are presented in Ref. [11], showing that none of these recoveries are significantly less than 100%. Therefore, the analysis of the soil solution is satisfactory.

3.7. Measurements of the acids in the soil samples

To demonstrate the applicability of the described technique, some soil solutions were analysed giving the results presented in Table 7. The samples were collected with centrifugation of 130 g soil closest to the roots of different plant species at 12 000 rev./min for 60 min, then filtered through a 0.2-μm filter. All plant species were planted in an intermediate soil originating from the 0–10 cm top layer of a loamy Eutric Cambisol ($pH(_{H_2O}) = 5.3$) from Scania, south Sweden (Fyledalen). This is not intended as a detailed investigation of the role in pedological processes and soil chemical reactions of the acids. More results can be seen in Ref. [11] which presents the application of the technique to real soil liquid samples.

Table 5
The adsorption (%) of the acids on the anion and cation exchange precolumns

	Lactic	Formic	Pyruvic	Malic	Oxalic	Citric	Isocitric
Cation exchanger	5.6	0	5.9	0.9	9.1	5.0	3.6
Anion exchanger	5.0	6.3	9.3	1.7	14.7	10.8	13.2
Sum	10.6	6.3	15.2	2.6	23.8	15.8	16.8

The same condition as Fig. 8 (D).

Table 6
Calibration curves for various acids

Acid	Concentration range (μM)	Slope[a]	Intercept[a]	r
Lactic	4–32	14.3±3.7	40±69	0.996
Acetic	2–32	16.2±2.8	−2±48	0.996
Formic	2–20	23.5±7.3	26±96	0.986
Pyruvic	2–40	12.6±2.3	−8±49	0.988
Malic	2–40	9.2±1.0	12±21	0.996
Oxalic	2–40	38.8±3.6	57±89	0.999
Citric	2–40	14.4±1.7	26±40	0.996
Isocitric	2–40	13.5±1.4	24±31	0.996

[a] Arbitrary units, 95% confidence interval.

Table 7
Low-molecular-mass carboxylic acids (nmol/100 g soil) collected from an Eutri Cambisol soil closest to the roots of the plants. Mean of two determinations

Species	Lactic + acetic	Formic	Malic/succinic	Oxalic	Citric
D. flexuosa	395.9	54.7	–[a]	164.1	115.6
R. acetosella	402.4	192.4	–	85.3	1929.4
R. acetosella	242.4	398.8	–	208.4	1617.9
G. sanguineum	507.0	649.2	–	93.5	36.6
S. minor	277.8	162.2	57.1	102.8	36.2

[a] No peak identified at appropriate retention time.

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