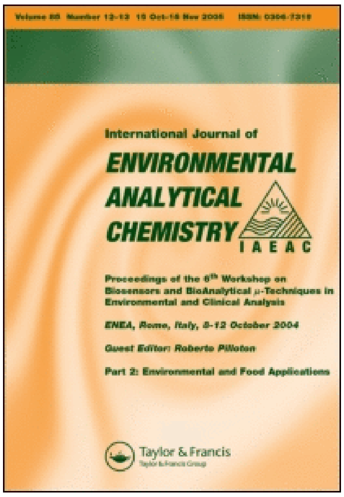

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APPLICATION OF CAPILLARY ZONE ELECTROPHORESIS FOR THE ANALYSIS OF LOW MOLECULAR WEIGHT ORGANIC ACIDS IN ENVIRONMENTAL SAMPLES

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The applicability of a recently developed capillary zone electrophoretic (CZE) method for the determination of low molecular weight (LMW) organic acids in water was tested on five types of environmental samples (rainwater, water extract from peat, and soil water from two polluted sites). A full baseline resolved separation of fourteen commonly found LMW carboxylic acids in natural waters (malonic, oxalic, fumaric, maleic, formic, succinic, tartaric, glutaric, adipic, acetic, propionic, butyric, valeric and citric acids), was achieved within eight minutes. The limits of detection (2 × noise) were in the ranges 90–200 µg/l and 0.5 – 5 µg/l for hydrodynamic and electrokinetic injection, respectively. Two different CZE systems, a Waters *Quanta 4000* and a Hewlett Packard HP ^{3D}CE system, were used and their performance compared.

Keywords: Capillary zone electrophoresis, carboxylic acids, environmental samples.

INTRODUCTION

Separation of organic ionic species such as low molecular weight (LMW) carboxylic acids is a relative new application of capillary zone electrophoresis (CZE) [1–5]. These organic compounds are of particular interest in environmental chemistry due to their role in the carbon cycle (e.g. microbial production [6], transport processes and bioavailability of associated compounds [7], microbial and photo induced degradation [8]) as well as in various other fields such as food

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chemistry, agricultural chemistry and biomedical science. Carboxylic acids have usually been analysed with well-established techniques such as gas chromatography (GC) and high performance liquid chromatography (HPLC), but these techniques have some limitations. For instance, derivatization is often necessary in GC and an incomplete baseline separation is frequently observed in several HPLC methods. The separation mechanism of the CZE technique makes it complementary to chromatographic methods and it provides several promising features like high separation efficiency, fast analysis and low consumption of chemicals.

In CZE, LMW organic acids are frequently separated as anions in a co-electroosmotic mode with indirect UV detection [5, 9, 10]. In order to establish this mode of detection the electroosmotic flow must be reversed and directed towards the anode, which is achieved by the addition of an electroosmotic flow modifier like tetradecyltrimethylammonium bromide (TTAB) to the electrolyte. For the detection of LMW organic acids that lack aromatic features the addition of a compound with UV-absorbing properties to the electrolyte provides suitable conditions for indirect UV detection. Several suitable UV-absorbing electrolytes for indirect detection have been reported, for example chromate [11, 12], phthalate [3, 13], 4-hydroxybenzoic acid [4], pyromellitic acid [14, 15], trimellitic acid, sorbate and benzoate [9]. If the UV-absorbing probe does not provide a stable pH a buffering component is added. Improved selectivity of the separation of organic acids can for example be achieved through the addition of divalent cations to the carrier electrolyte [4, 12, 16].

The aim of this study was to investigate the suitability of a previously developed CZE procedure that in its first design separated fourteen LMW carboxylic acids in relatively simple sample matrices. In the present study, the applicability of the method is demonstrated by the analysis of rainwater, water extracts from peat and soil water from sites polluted with heavy metals as well as anthropogenic organic carbon compounds.

EXPERIMENTAL

Instrumentation and procedures

Analyses were made on two different CZE systems.

System I: A Waters *Quanta 4000* (from 1989) equipped with a 75 µm (i.d.) fused silica capillary (J&W Scientific) and a mercury lamp operated at 254 nm. Data were collected and evaluated by the software Millenium 2.0 (Waters). The effective and total lengths of the fused silica capillary were 75 and 83 cm, respectively. Injection was made hydrostatically at 10 cm for 30 seconds or electrokinetically at –5 kV for 45 seconds. The applied separation voltage was –30 kV (anode at the detection side), which gave a current of 10 µA. Detection was made by indirect UV at 254 nm.

System II: A *HP ^{3D}CE* system (from 1998) equipped with a diode-array detector which was interfaced to a HP ChemStation data acquisition system (Hewlett Packard). A 75 µm (i.d.) fused silica capillary (Chromtech) with an effective and total length of 76 and 83 cm, respectively. Injection was made hydrostatically at 10 mbar for 30 seconds or electrokinetically at –5 kV for 45 seconds. An applied voltage of –30 kV gave a current of 9.6 µA through the capillary. Indirect UV detection at 254 nm was used.

Octanesulfonate was added to samples and standards to a concentration of 0.07 mM, in order to establish an isotachophoretic steady state during the sample introduction when employing the elektrokinetic injection mode [17]. Prior to analysis the capillary was conditioned by purging with 0.1 M NaOH (5 minutes) followed by Milli-Q (5 minutes) and carrier electrolyte (20 minutes). The capillary was flushed with carrier electrolyte for 2 minutes before each injection. All solutions were prepared in Milli-Q water (Millipore). Samples were filtered through 0.50 µm teflon filters (PTFE; Micro Filtration Systems, MFS) for removal of particulate matter.

Carrier electrolytes

The carrier electrolyte consisted of 5.0 mM Tris(hydroxymethyl)aminomethane (TRIS), 2.0 mM trimellitic acid (TMA), 0.2 mM tetradecyltrimethylammonium bromide (TTAB) (system I) or 0.6 mM TTAB (system II) and 0.6 mM calcium hydroxide with pH adjusted to 8.5 by dropwise addition of NaOH. The electrolyte was filtered through 0.5 µm teflon filters (PTFE; MSF) and degassed by sonication during 5 minutes. Tris(hydroxymethyl)aminomethane (analytical reagent grade), tetradecyltrimethylammonium bromide (TTAB; 99 %), 1,2,4-benzenetricarboxylic acid (i.e. trimellitic acid); 99+ %), and 1-octanesulfonic acid sodium salt monohydrate (>99 %) were obtained from Merck, Sigma, Janssen and Fluka, respectively. Calcium hydroxide, sodium molybdate dihydrate and sodium hydroxide of analytical reagent grade were obtained from Merck.

Carboxylic acids

The following organic acids, obtained from Merck with some exceptions, were used: sodium salts of formic acid (99 %), acetic acid (analytical reagent grade),

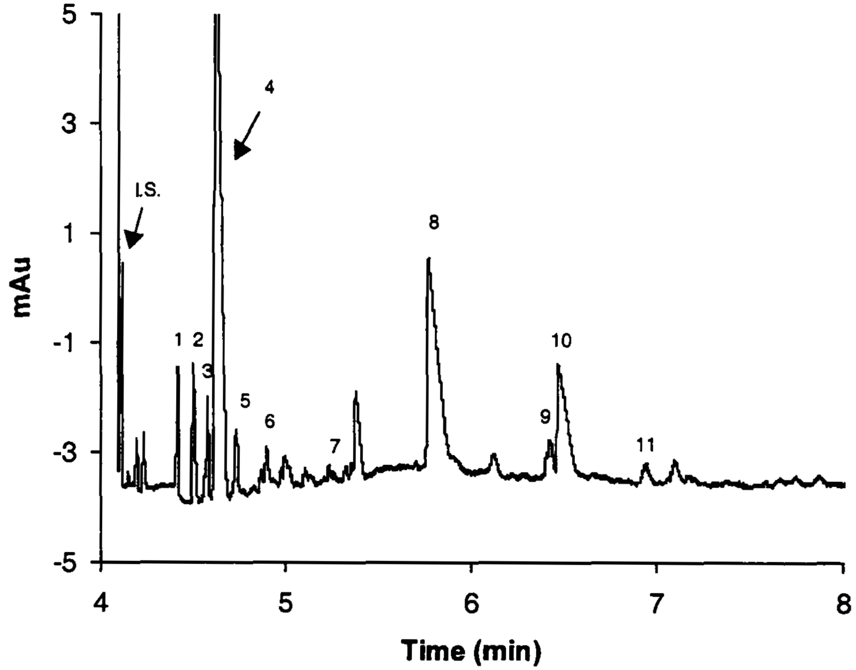

FIGURE 1 Electropherogram of a rainwater sample from the outskirts of Örebro, obtained with system II. Peaks were tentatively identified by migration times and spiking. Peaks: (I.S.) internal standard (molybdate), (1) malonic acid, (2) oxalic acid, (3) maleic acid, (4) formic acid, (5) succinic acid, (6) tartaric acid, (7) adipic acid, (8) acetic acid, (9) propionic acid, (10) tentatively lactic acid; (11) butyric acid. Injection: electrokinetically at –5 kV for 45 s. Carrier electrolyte: 5.0 mM TRIS, 2.0 mM TMA, 0.6 mM TTAB, 0.6 mM Ca^{2+}, pH 8.5. Capillary: total length 83 cm, effective length 76 cm, internal diameter 75 μ m. Voltage: –30 kV. Current: 9.6 μA. Detection: indirect UV at 254 nm

butyric acid (>98 %), valeric acid (>98 %), oxalic acid (analytical reagent grade); the acid forms of malonic acid (>99 %), maleic acid (>99 %), succinic acid (analytical reagent grade), glutaric acid (99 %), adipic acid (>99 %), fumaric acid (>99 %), citric acid (analytical reagent grade). The sodium salt of propionic acid (99 %) and the acid form of L-tartaric acid (99.5 %) were obtained from Aldrich.

Samples

Samples were collected in the Örebro area, southern Sweden, from various environments in order to evaluate the possible impact of sample matrices on the separation and detection of the carboxylic acids. Rainwater samples were collected in the rural outskirts of Örebro and at the site of a waste incineration plant. A sample of unpolluted peat (Sphagnum) was taken from the Skagershultsmosse, while a metal (mainly lead) contaminated bog peat sample was collected from a skeet/trap shooting range. A soil sample, contaminated with organic pollutants and mercury, was taken from the site of a chloralkali factory.

Rainwater samples were collected in well-rinsed polyethene funnels and bottles. The water extracts were prepared in well-rinsed polyethene beakers. Sample of 0.5 g peat or 1.0 g of bog peat and contaminated soil were added to a volume of 300 ml alkaline Milli-Q water (pH 9; NaOH). The sample solutions were stirred for 2 hours. All samples, including extracts, were filtered trough 0.50 µm teflon filters (PTFE; MFS) prior to analysis. Since LMW organic acids are known to be decomposed due to microbial activity quite rapidly [18], analyses were made within 20 minutes after sampling. The identity of the acids was determined by migration times and by spiking.

RESULTS AND DISCUSSION

Analysis of environmental samples

A total of five samples were analysed; two rainwater samples and three soil extracts, see above.

Rainwater samples

Figure 1 illustrates the LMW organic acid content in rainwater from the outskirts of Örebro. High concentrations of formic and acetic acid are observed along with lower amounts of malonic, oxalic and succinic acid. The other rainwater sample, taken in the vicinity of a waste incineration plant shows lower levels and a different composition of carboxylic acids (Figure 2). The carboxylic acid content corresponds well with other studies [10].

Peat and bog peat samples

The water extracts from uncontaminated peat is given in Figure 3. Acetic acid and bicarbonate are the major constituents of this sample. Only a few carboxylic acids can be identified. This could of course point at a poor extraction efficiency or a too short extraction time.

In the metal contaminated bog peat the amount of carboxylic acids are even lower (Figure 4). Oxalic, formic and acetic acid, which are very commonly

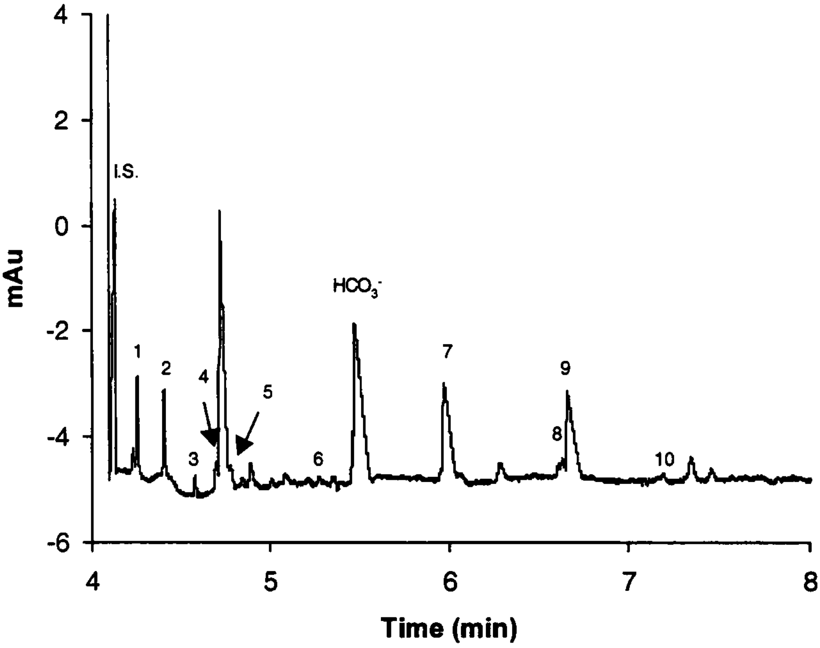

FIGURE 2 Electropherogram of a rainwater sample from the site of a waste incineration plant, obtained with system II. Peaks were tentatively identified by migration times and spiking. Peaks: (I.S.) internal standard (molybdate); (1) oxalic acid, (2) fumaric acid, (3) maleic acid, (4) formic acid, (5) succinic acid, (6) adipic acid, (7) acetic acid, (8) propionic acid, (9) tentatively lactic acid; (10) butyric acid. Injection: electrokinetically at −5 kV for 45 s. Other conditions as in Fig. 1

found as products of biological activity, are the principal components in this sample besides the large peak representing bicarbonate.

Contaminated soil sample

This sample contains only few components that can be identified as LMW carboxylic acids (Figure 5). There are minor levels of oxalic, formic and acetic acid present, along with two unknown peaks.

All samples were analysed with the electrokinetic injection mode. A problem with this technique is that the injected amount of analyte depends on the conductivity of the sample [19]. When the sample has a high conductivity the field strength of the sample plug will be low, resulting in a reduced amount of analyte being injected. As a consequence, the peak area is a function of the sample matrix, and the calibration curves should therefore be used with care. Experi-

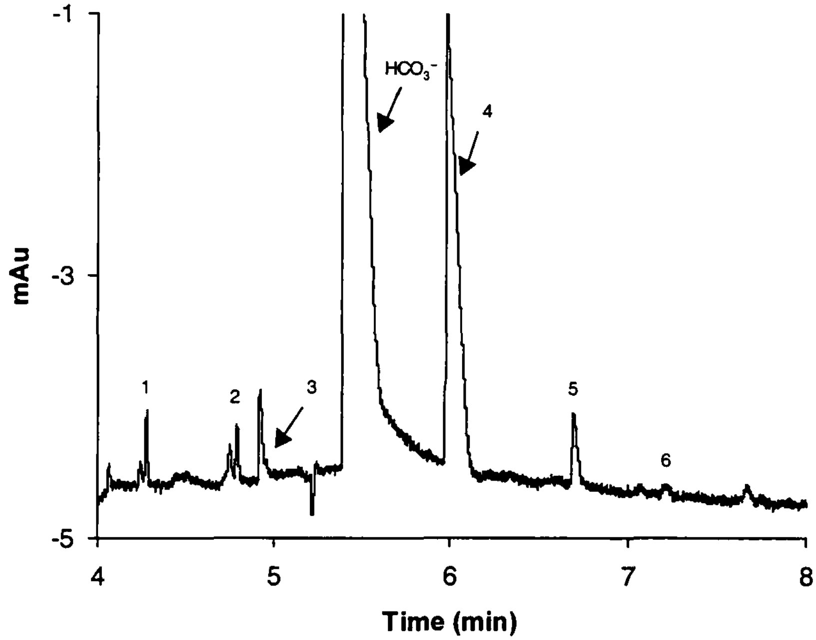

FIGURE 3 Electropherogram of a water extract from peat, obtained with system II. Peaks were tentatively identified by migration times and spiking. Peaks: (1) oxalic acid, (2) formic acid, (3) tartaric acid, (4) acetic acid, (5) propionic acid, (6) butyric acid. Injection: electrokinetically at −5 kV for 45 s. Other conditions as in Fig. 1

ments showed that especially the water extracts in this study do suffer from severe sample matrix effects. The use of internal standards or a standard addition procedure [20] can however surmount this obstacle. In addition, the electrokinetic injection mode discriminates ions with low mobilities, which also influences the amount of analyte being injected [10]. This does not, however, affect the quantification since sample and standard are influenced in the same way.

A slight shift in the migration times for the acids can be seen in the electropherograms (Figure 1 – 2). The acids numbered 8–11 in Figure 1 correspond to the acids 7–10 in Figure 2, but their migration times differ. This problem can also be attributed to the sample matrix. The shift is probably due to differences in the samples ionic strength, which is a well known phenomena [21]. Another possible explanation is that the presence of cations in the sample influence the selectivity of the acids and thereby affect their migration times. This effect is often utilised when manipulating the selectivity of analytes in CZE [4, 12, 16].

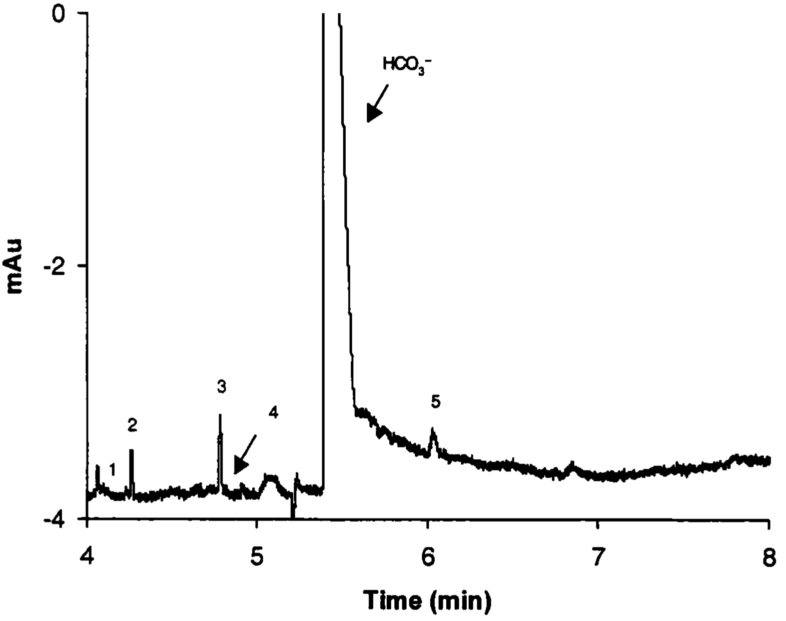

FIGURE 4 Electropherogram of a water extract from a metal contaminated bog peat, obtained with system II. Peaks were tentatively identified by migration times and spiking. Peaks: (1) malonic acid, (2) oxalic acid, (3) formic acid, (4) succinic acid, (5) acetic acid. Injection: electrokinetically at −5 kV for 45 s. Other conditions as in Fig. 1

Comparison between two different CZE systems

System I (Waters *Quanta 4000*) was used in an previous study when the method was developed [22]. For system II (*HP* ^{3D}*CE*) (used in the present work), the original procedure was modified by increasing the concentration of TTAB in the electrolyte. This modification decreased the analysis time by approximately three minutes, due to an increased EOF. This alteration of the electrolyte would not significantly influence the repeatability and limit of detection (LOD), which makes the comparison between the two CZE-systems possible.

Hydrodynamic injection mode

Linearity (r^2; 0–10 mg/l) showed a good agreement between the two CZE systems (i.e. 0,9993–0,9999 and 0,9991–1,000 for system I and II, respectively, with exception for citric acid which had a correlation coefficient of 0,9878 and 0,9975

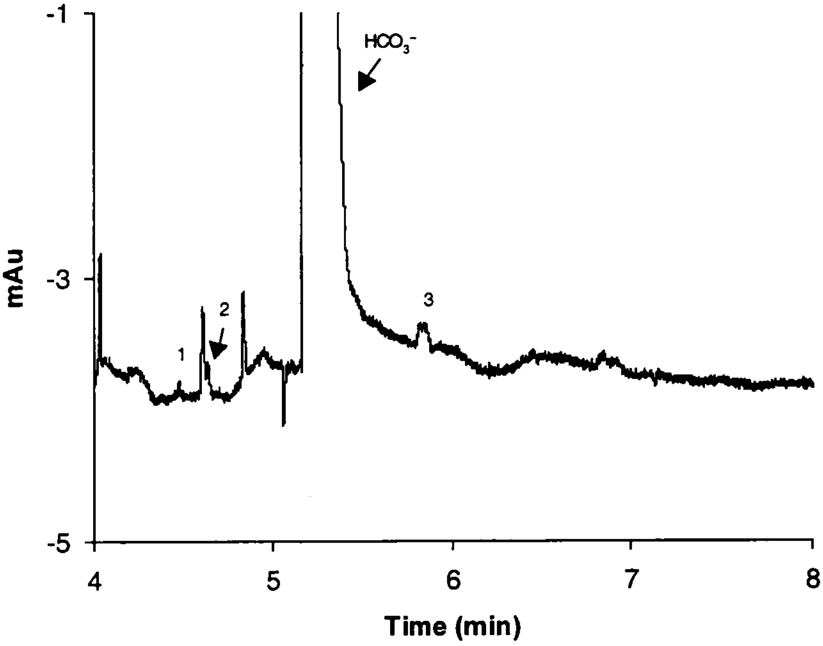

FIGURE 5 Electropherogram of a water extract from a soil from the site of a chloralkali plant, obtained with system II. Peaks were tentatively identified by migration times and spiking. Peaks: (1) oxalic acid, (2) formic acid, (3) acetic acid. Injection: electrokinetically at −5 kV for 45 s. Other conditions as in Fig. 1

in the two systems), as well as limit of detection (LOD; 2 × noise) and repeatability (n=9), expressed as relative standard deviation (RSD). An exception is citric acid where an improved analytical performance was found in system II (Table I). The reason for this is unknown, although it could be attributed to the modification of the carrier electrolyte rather than to system II.

Electrokinetic injection mode

In general, system II showed better LOD and RSD (n=9) (Table I). The linearity (r^2; 0–0,050 mg/l) was similar for the two instruments (i.e. 0,9988–1,000 and 0,9966–0,9999 for system I and II, respectively), with the exception of formic and acetic acid. There is, however, a substantial contribution of these acids from the Milli-Q equipment utilised in the present study. The use of the area/internal standard (IS) quotient seems not to be essential for good repeatability in system

II as it was in system I (Table I). The use of internal standards is, however, essential since it corrects for differences in the amount of introduced sample, that occurs as a consequence of varying sample conductivity. The LODs was significantly improved for most of the acids when using system II.

TABLE I Comparison of hydrodynamic and electrokinetic injection modes for two CZE systems, Waters *Quanta 4000* (system I) and Hewlett Packards *HP* ^{3D}*CE* system (system II). Hydrodynamic injection: LOD (2×noise) and repeatability (RSD) (5 mg/l; n=9) for the LMW carboxylic acids when employing hydrostatic injection (10 cm for 30 s; system I and 10 mbar for 30 s; system II). Electrokinetic injection: LOD (2×noise) and repeatability (0.1 mg/l; n=9) for the LMW carboxylic acids when employing electrokinetic injection (45 s at –5 kV). Analytical conditions in system I and II as described under "Experimental". Corrected area = peak area/migration time

Compound	*Hydrodynamic injection*						*Electrokinetic injection*					
	LOD (mg/L)		*RSD (%) Area*		*RSD(%) Corrected Area*		*LOD (mg/L)*		*RSD (%) Area*		*RSD(%) Area / IS*	
	I	*II*	*I*	*II*	*I*	*II*	*I*	*II*	*I*	*II*	*I*	*II*
Malonic acid	0,1	0,1	1,4	1,1	0,4	1,1	0,002	0,0005	11,1	1,1	1,5	1,1
Oxalic acid	0,1	0,1	1,1	1,5	0,9	1,4	0,001	0,0005	10,7	1,7	1,7	0,5
Fumaric acid	0,2	0,15	1,2	1,0	0,6	0,9	0,003	0,001	11,4	0,8	1,8	0,9
Maleic acid	0,15	0,2	1,6	1,1	0,6	1,1	0,003	0,002	11,1	1,5	1,9	0,8
Formic acid	0,15	0,2	1,6	1,8	0,8	1,7	0,001	0,001	12,2	4,3	5,6	4,0
Succinic acid	0,15	0,09	1,7	1,1	0,5	1,0	0,002	0,0005	11,5	1,5	1,8	0,9
Tartaric acid	0,2	0,2	0,9	1,3	1,9	1,1	0,004	0,002	11,9	1,9	2,5	0,9
Glutaric acid	0,15	0,1	0,2	0,6	0,7	0,6	0,003	0,001	9,7	1,7	2,1	0,9
Adipic acid	0,15	0,09	0,9	0,6	0,9	0,5	0,001	0,0005	11,7	2,1	3,1	1,9
Acetic acid	0,15	0,2	2,7	1,1	1,4	1,1	0,006	0,004	13,4	14,6	4,1	14,4
Propionic acid	0,15	0,15	2,9	0,8	1,4	0,8	0,005	0,0005	13,4	1,7	3,9	1,1
Butyric acid	0,2	0,15	2,9	0,9	2,1	0,9	0,007	0,002	11,9	1,6	2,4	1,1
Valeric acid	0,2	0,2	2,4	1,0	2,0	1,0	0,007	0,005	12,2	2,2	4,0	1,8
Citric acid	1,0	0,5	10,4	4,5	9,5	4,5	0,020	0,004	11,8	3,0	4,9	2,1

CONCLUSIONS

The applicability of a CZE method for the determination of LMW carboxylic acids have been demonstrated by the analysis of five different environmental samples. The sample types include rainwater, water extracts of peat, bog peat and contaminated soil. Several organic acids, including malonic, oxalic, fumaric, maleic, formic, succinic, tartaric, glutaric, adipic, acetic, propionic and butyric acid could be found in the various samples. The method is fast and an analysis is feasible within eight minutes. Use of electrokinetic injection mode allows for detection at 0.5–5 μg/l-levels, which are relevant concentrations when analysing this type of compounds in environmental samples. Hence, the method is useful for fast determination of organic acids in a wide range of environmental samples.

The comparison between the two CZE systems, i.e. a Waters *Quanta 4000*(system I) and a *HP* ^{3D}*CE* system (system II), showed similar performances for the hydrodynamic injection mode. However, the *HP* ^{3D}*CE* system showed better results in LOD and in RSD of the peak area for the electrokinetic injection mode, than the Waters system. This better performance can possible be related to more modern equipment with improved voltage control and intense UV-lamp, the Waters-system being about 10 years older than the HP-system.

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Mr. Mattias Bäckström (MSc), Ms. Pascal Suèr (MSc) and Mr. Thomas Lifvergren (MSc) are acknowledged for providing some of the samples. The use of facilities at SAKAB (Waste Management International) is gratefully acknowledged, as well as financial contribution from the Foundation of Knowledge and Competence Development and the Swedish Natural Science Research Council.

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