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Separation and determination of aromatic acids in natural water with preconcentration by capillary zone electrophoresis

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Abstract

A method was developed for the determination of aromatic acids by capillary zone electrophoresis (CZE) in combination with UV detection using hydrodynamic sample injection. The electrophoretic behavior of aromatic acids was investigated to optimize their separation as a function of buffer pH, buffer concentration, applied voltage and sample injection time. A good linearity was observed for all analytes over a wide concentration range (5–100 μM). The relative standard deviation was less than 7% for all the aromatic acids tested. The method was applied to the analysis of aromatic acids in natural waters. The aromatic acids in the natural water sample were preconcentrated by means of solid-phase extraction (SPE). The recovery using the CZE in combination with the SPE procedure for the determination of aromatic acids was also investigated.

Keywords: Water analysis; Organic acids; Aromatic compounds

1. Introduction

Aromatic acids such as vanillic acid, *p*-coumaric acid, ferulic acid and salicylic acid are important compounds in the aquatic environment. They have been identified in several rivers as the degradation products of plant matter such as lignins (polyphenolic substances) that are present in vascular plants [1–3]. The aromatic acids as lignin degradation products have been used as indicators of dissolved organic matter from terrestrial sources [4,5]. Distribution of aromatic acids in the aquatic environment can provide basic information on the different sources of the overall dissolved organic matter [1,4,5].

Because of the ionic nature of aromatic acids, it is possible to determine these compounds using capillary zone electrophoresis (CZE). CZE appears to be an attractive complementary technique to ion chromatography (IC) and high-performance liquid chromatography (HPLC) with promising features such as high separation efficiency, short analytical time and simplicity. A number of studies have demonstrated that aromatic acids can be separated by CZE [6–11]. Most of them focused on optimizing conditions (e.g., buffer pH, run voltage) for separation of the acids. pH was found as a critical parameter in the separation of the aromatic acids with a hydroxyl group (–OH) attached to the benzene ring [6,10]. Because the extent of dissociation of the –OH group, which determines the overall electric charge of the aromatic acids, is governed by the pH of the buffer, the selection of buffer pH can greatly influence the separation of mixture of aromatic acids. Beside pH,

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other parameters such as buffer concentration, run voltage and sample injection time also influence the separation of the aromatic acids and can be optimized to obtain a desirable separation and quantitation [6,9].

The concentrations of aromatic acids in some rivers were found at the level of μg/l ([2,12] and references therein). The isolation and quantitation of aromatic acids in natural waters is difficult due to the chemical complexity of natural waters. The low concentrations of aromatic acids in natural waters and chemical complexity of natural waters create challenging requirements for isolation and quantitative analysis of the acids using CZE. The extraction and recovery of aromatic acids present difficulties, due to their wide range of polarity. In recent years, solid-phase extraction (SPE) has become a popular procedure used for isolation and preconcentration of organic compounds in food and biological materials [13,14]. SPE with C_{18} cartridge has been used to separate and preconcentrate polyphenols in apple musts and ciders with the recovery between 84 and 111% [13].

The objective of this study was to investigate the electrophoretic behavior of the seven aromatic acids and to establish a simple analytical method for determination of aromatic acids in natural waters using CZE. In this work, the effect of pH, buffer concentration, applied voltage and injection time on separation and quantitation of the aromatic acids was investigated. Under optimized conditions separation of the seven acids can be accomplished in less than 12 min and a satisfactory linearity is obtained for all the acids between 5 and 100 μ*M*. The CZE method combined with the SPE can be applied to the determination of aromatic acids in natural waters.

2. Experimental

2.1. Reagents and solutions

The structures of seven aromatic acids used in this study are listed in Fig. 1. 3,4,5-Trimethoxybenzoic acid was obtained from Sigma (St. Louis, MO, USA). 4-Hydroxyphenylacetic acid and 4-hydroxybenzoic acid were from Aldrich (Milwaukee, WI, USA). Other chemicals were obtained from Fisher

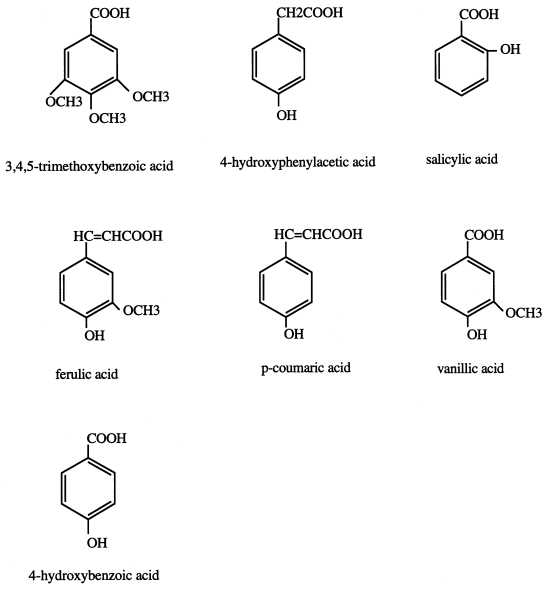

Fig. 1. Structures of the seven aromatic acids.

(Pittsburgh, PA, USA). All chemicals were used without further purification. All solutions were prepared using 18 MΩ water generated by a Milli-Q Water Purification System (Millipore, Bedford, MA, USA) and filtered through a 0.4-μm pore size biphenol polycarbonate filter (Millipore).

Stock solutions of the seven acids ($5.0 \cdot 10^{-3}$ *M*) were prepared by dissolving appropriate amounts of the acids in a 0.01 *M* NaOH solution. The mixtures of the acids in the concentration range of $5.0 \cdot 10^{-6}$ *M* to $2.0 \cdot 10^{-4}$ *M* were obtained by appropriate dilution of the stock solutions with deionized water.

Sodium tetraborate ($Na_2B_4O_7 \cdot 10H_2O$) was used as a carrier electrolyte (running buffer). The pH values of the buffer were adjusted using either 1.0 *M* sodium hydroxide or 1.0 *M* hydrochloric acid solution.

2.2. Instrumentation and electrophoretic conditions

The capillary electrophoresis system employed was a Beckman P/AC 5500 equipped with a UV detector and a fused-silica capillary (57 cm×75 μm I.D.) (Beckman Instruments, Fullerton, CA, USA). Data acquisition was carried out using the System Gold software (Beckman Instruments) on an IBM personal computer.

For all experiments, the samples were introduced by hydrodynamic sample injection at the inlet end and direct UV detection was performed at wavelength of 214 nm through the capillary at 50 cm from the inlet. The cathode was placed at the outlet side and the anode at the inlet side. Unless otherwise specified, the standard conditions used for individual parameters of the system are: 20 kV applied voltage, 10 s injection time, 22°C operating temperature, and a carried electrolyte consisting of sodium tetraborate (13 m*M*) at pH 9.68±0.02. All new capillary columns were conditioned by rinsing in sequence with 1 *M* HCl (5 min), deionized water (2 min), 1% (w/v) NaOH (10 min), deionized water (2 min) and finally running buffer (5 min). The capillary was rinsed with the buffer before each injection for 2 min.

2.3. SPE procedure and recovery study

The SPE was basically followed the procedure suggested by Suarez et al. [13]. Sep-Pak C_{18} PLUS cartridges (Millipore) were used for extracting aromatic acids from aqueous solutions. The packing material in the C_{18} PLUS has the polymer structure of $-Si(CH_3)_2C_{18}H_{37}$. The cartridges were preconditioned by sequentially passing 5 ml methanol and 4 ml of 0.01 *M* HCl at flow-rate of 4 ml/min. A sample solution was adjusted to pH 2 with HCl, loaded onto the preconditioned C_{18} cartridge at a flow-rate of 0.5–1 ml/min. The adsorbed aromatic acids were then eluted with 12 ml methanol at a flow-rate 1 ml/min. and evaporated till dryness below 35°C. The residue was redissolved in 5 ml of $2 \cdot 10^{-4}$ *M* NaOH. The resulting solution was filtered through a 0.4-μm pore size membrane and injected into the CE system.

To establish the efficiency of the applied SPE method, a recovery test was performed using two standard solutions containing seven aromatic acids. The concentration of each compound in one of the solutions (S1) was 50 μ*M* and the other (S2) was 5 μ*M*. The solutions were adjusted to pH 2 with 12 *M* HCl, loaded separately onto the preconditioned C_{18} PLUS cartridge. The loaded volumes were 5 ml and 1000 ml for S1 and S2 solutions, respectively. The elution of the absorbed aromatic acids for these two samples was followed the procedure described above.

2.4. Preparation of a natural water sample

A natural water sample was taken from Lake Fairy, Canada. The sample (1000 ml) was adjusted to pH 2 with HCl and filtered through a 0.4-μm pore size membrane. The filtrate was then loaded onto the preconditioned C_{18} PLUS cartridge. The elution of the absorbed aromatic acids for sample was followed the procedure described in Section 2.3.

3. Results and discussion

3.1. Selection of buffer pH and concentration

The effect of pH on the separation of seven aromatic acids was investigated with 17 m*M* sodium borate buffers at pH 7.8, 8.7, 9.2, 9.4, 9.6, 9.7 and 9.8. Among these seven sets of conditions, migration at pH 9.6–9.7 provided the best resolution and selectivity of the seven acids. Thus pH 9.68±0.02 was selected as the optimized pH value for the separation of the acids.

The concentration of buffer can influence the CZE separation in several ways. High buffer concentrations can minimize solute adsorption on the wall of capillary, but may produce large amounts of heat due to the Joule effect. Low buffer concentrations, on the other hand, can increase the electroosmotic flow, resulting in a shorter analytical time. In general, a certain minimum buffer concentration is necessary for uniform distribution of the electrical field and for adequate buffer capacity. The optimum buffer concentration has to be determined experimentally.

The influence of buffer concentration on the migration time of seven acids were investigated from 10 m*M* to 17 m*M* sodium borate buffer at pH 9.68±0.02. The result indicates that the peaks of all seven acids can be well resolved between 12 m*M* and 13 m*M* of the buffer solution (Fig. 2). As the buffer concentration further decreases, the peaks of salicylic acid and ferulic acid become overlapped. Based on the results in Fig. 2, 13 m*M* was chosen as the optimized concentration of the buffer.

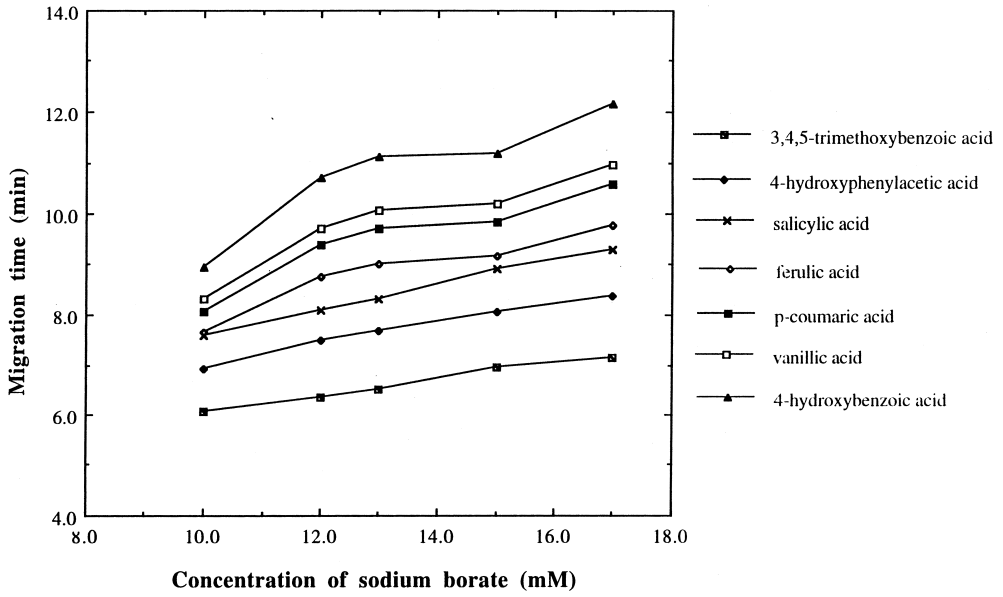

Fig. 2. Effect of sodium tetraborate (buffer) concentration on migration time of the seven aromatic acids. Experimental conditions: pH of run buffer: 9.68±0.02, concentrations of the seven acids: 100 μ*M* each, hydrodynamic sample injection: 10 s, indirect UV detection: wavelength (λ)=214 nm; run voltage: 20 kV, capillary: fused-silica 57 cm×75 μm I.D.; operating temperature: 22°C. The concentration of sodium tetraborate varied from 10 to 17 m*M*. Based on the data shown in this Figure, 13 m*M* of sodium tetraborate was chosen as the run buffer concentration for the rest of the experiments.

3.2. Effect of run voltage

In principle the ideal separation is generally obtained by applying a voltage as high as possible because the plate number in CE is proportional to the applied voltage [15]. However, this benefit is limited by the Joule heat generated during the electrophoretic process. The Joule heat results in broadening of peaks and decrease of separation efficiency. In this study, a better separation seems to be achieved in the voltage range of 18–20 kV (Fig. 3). The peaks of *p*-coumaric acid and vallinic acid tend to be overlapped as the run voltage further increases. Thus, the voltage of 20 kV was selected as the optimized run voltage.

3.3. Effect of sample injection time

Sample injection time (or loading time) has been proven to be proportional to the volume of a sample introduced into the capillary, and can be optimized in order to yield analyte peaks large enough for quantitation [16,17]. The effect of loading time on peak area was investigated by hydrodynamic sample injecting the standard solution (100 μ*M* for each compound) at 5, 8, 10, 12 and 15 s injection time. The standard was injected twice into the CE system under the conditions described in Section 2.2. The resulting peak area values were then averaged, and the mean value for each compound was plotted as a function of injection time (Fig. 4). A linear relationship up to a 15-s injection time is observed for all seven compounds. However the peaks of ferulic acid and salicylic acid tend to be overlapped when the injection time is ≥12 s. Thus, 10 s was chosen as the optimized injection time.

3.4. Quantitation

The stock standard solutions of seven aromatic acids were diluted to make mixtures of the analytes at concentrations of 5, 8, 10, 20, 30, 40, 50, 60, 80, 100 μ*M* for each compound. Each of these solutions was then injected three times on the CE system under the conditions described in Section 2.2. A representative electropherogram is shown in Fig. 5.

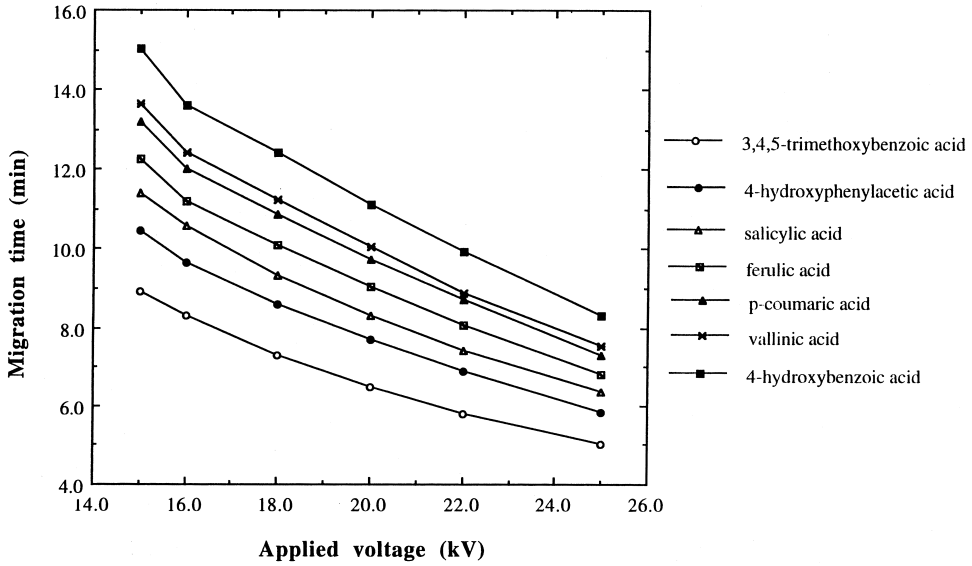

Fig. 3. Effect of run voltage on migration time of the seven aromatic acids. Experimental conditions as in Fig. 2.

The values of peak area of compounds were then averaged and the mean value for each concentration of a single compound was used in the linear regression calculation. A good linearity was observed for all analytes in a wide concentration range of 5–100 μ*M*. Repeatability was examined by 10 replicate injections of mixture of seven compounds with the lowest concentration (5 μ*M* for each compound) used within the standard series. The relative standard deviations (R.S.D.s) of the seven acids fell below

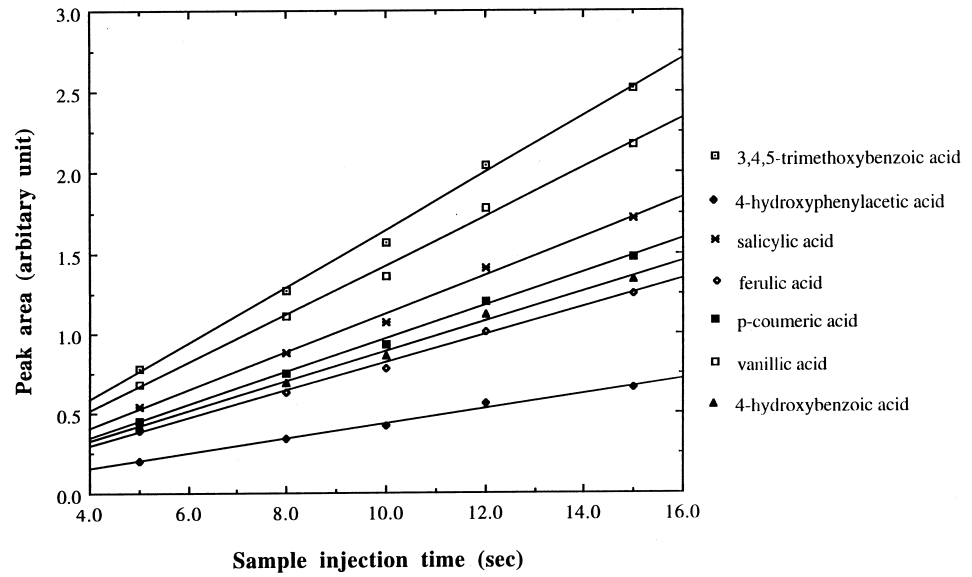

Fig. 4. Effect of sample injection time on the peak area for the seven aromatic acids. Experimental conditions as in Fig. 2.

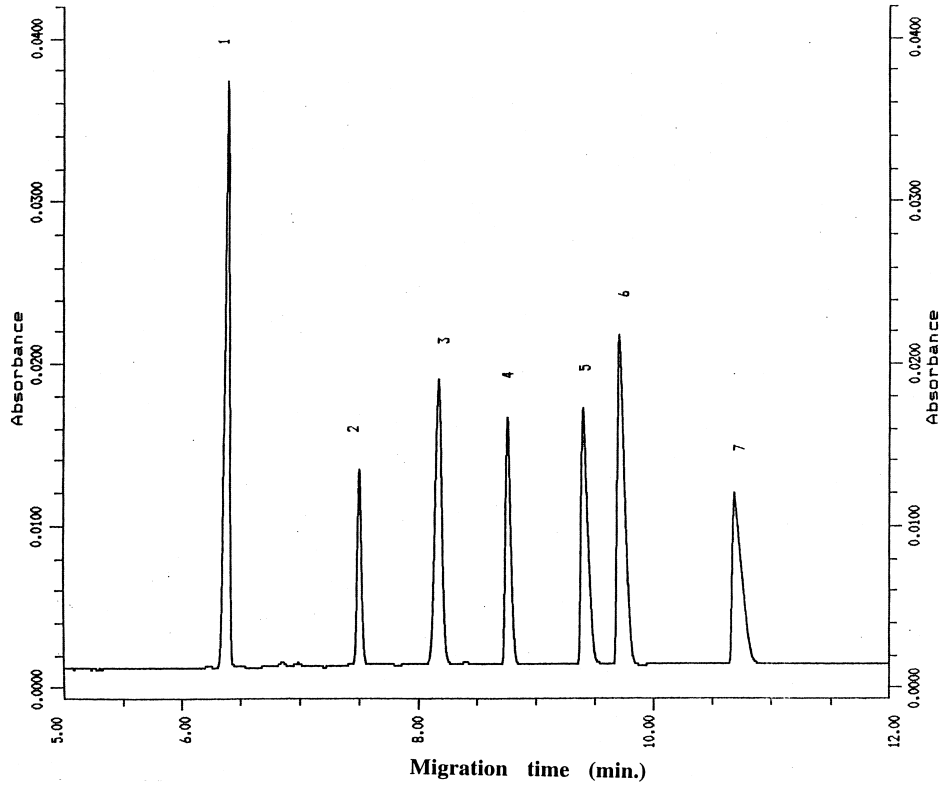

Fig. 5. A representative electropherogram of the seven acids. Experimental conditions as in Fig. 2. Peaks: 1=3,4,5,- trimethoxybenzoic acid, 2=4-hydroxyphenylacetic acid, 3=salicylic acid, 4=ferulic acid, 5=*p*-coumaric acid, 6=vanillic acid and 7=4-hydroxybenzoic acid.

7%. The results of the linear regression calculations and the relative standard deviation for the seven aromatic acids are summarized in Table 1.

Two solutions containing seven aromatic acids were used for the recovery study. The solutions were separately loaded onto the individual preconditioned C_{18} PLUS cartridge and subjected to entire SPE procedure and analytical sequence. In the case of the solution (S1) containing 50 μM of each compound, the recoveries varied from 98 to 108% except for salicylic acid (58%). The low recovery observed for the salicylic acid may be attributed to the high

Table 1
Calibration data for the seven aromatic acids

Compound	Linear range (μM)	Slope (A)	Intercept (B)	r[a]	R.S.D. (%)[b]
3,4,5-Trimethoxybenzoic acid	$5.0–1.0\cdot10^{2}$ (1.1–21 ppm)	$1.6\cdot10^{-2}$	$1.7\cdot10^{-2}$	0.998	3.25
4-Hydroxyphenylacetic acid	$5.0–1.0\cdot10^{2}$ (0.76–15 ppm)	$4.2\cdot10^{-3}$	$5.3\cdot10^{-3}$	0.999	6.83
Salicylic acid	$5.0–1.0\cdot10^{2}$ (0.69–14 ppm)	$1.1\cdot10^{-2}$	$1.1\cdot10^{-2}$	0.999	5.28
Ferulic acid	$5.0–1.0\cdot10^{2}$ (0.97–19 ppm)	$7.8\cdot10^{-3}$	$1.4\cdot10^{-2}$	0.998	6.95
p-Coumaric acid	$5.0–1.0\cdot10^{2}$ (0.82–16 ppm)	$9.4\cdot10^{-3}$	$1.4\cdot10^{-2}$	0.998	4.05
Vanillic acid	$5.0–1.0\cdot10^{2}$ (0.84–17 ppm)	$1.4\cdot10^{-2}$	$1.8\cdot10^{-2}$	0.998	3.08
4-Hydroxybenzoic acid	$5.0–1.0\cdot10^{2}$ (0.69–14 ppm)	$8.5\cdot10^{-3}$	$1.3\cdot10^{-2}$	0.999	2.82

[a] r is the linear regression coefficient. The concentration (y) of the aromatic acids can be calculated using $y=Ax+B$, where x is the peak area of a compound.
[b] R.S.D.=Relative standard deviation.

polarity and low affinity for the non-polar packing material in the C_{18} PLUS column [18]. In the case of the solution (S2) containing 5 μM of each compound, the recoveries of the compounds tested were generally low (below 30%) except for 3,4,5-trimethoxybenzoic acid (75%) which is the least polar compound among the seven acids. These more polar compounds could not be highly retained in the column particularly when a large sample volume (e.g., 1000 ml) was used. This results in the loss of the compounds and therefore the low recoveries [18].

3.5. Determination of aromatic acids in natural waters

CZE coupled with SPE was applied to the natural water analysis. Fig. 6 shows the electropherogram of the natural water sample taken from the Lake Fairy, Canada. Comparing the electropherograms in Figs. 5 and 6, the first peak (migration time 8.1 min) is identified as salicylic acid based on the migration time. The second peak (migration time 11.1 min) cannot be positively identified based merely on the migration time of the seven aromatic acids tested. However, a very broad ''hump'' is a typical pattern of electropherograms of humic and fulvic acids [19,20].

4. Conclusions

A method using CE is optimized in terms of buffer pH, buffer concentration, run voltage and injection time to achieve desirable separation efficiency, reproducibility and adequate linear dynamic range for the analysis of seven aromatic acids in aqueous matrices. A good linearity was observed for all analytes over a wide concentration range (5–100

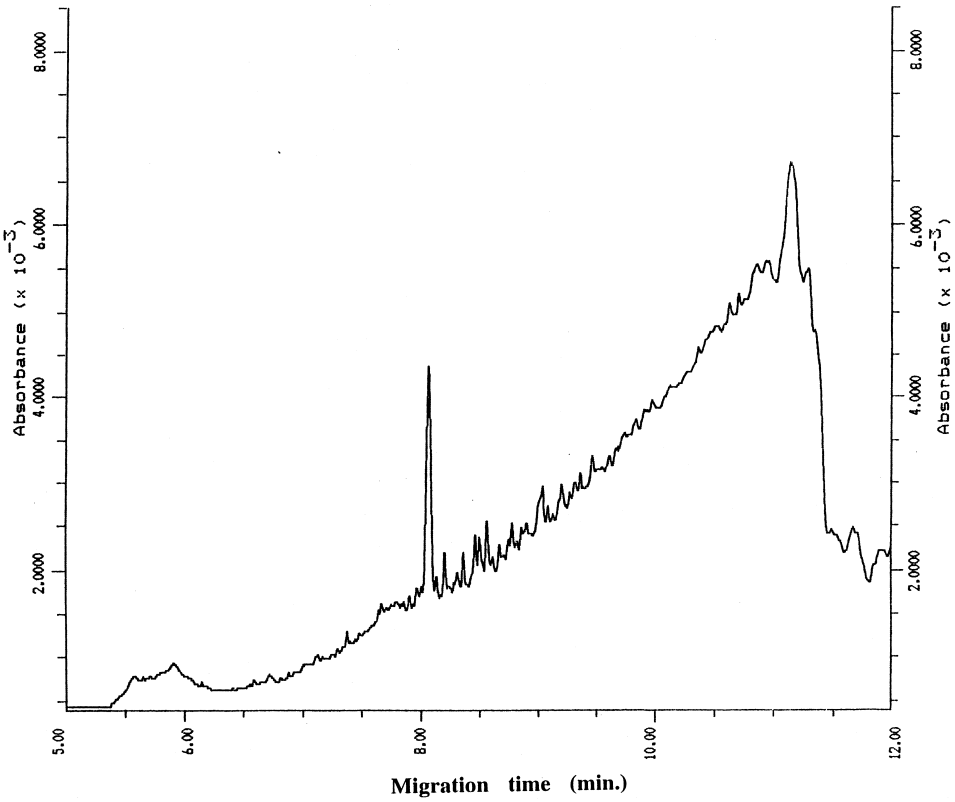

Fig. 6. An electropherogram of the water sample taken from Lake Fairy, Canada. Experimental conditions as in Fig. 2. The dominant peak at about 8 min migration time is salicylic acid.

μ*M*). The R.S.D. was less than 7% for all the aromatic acid tested. The optimized method in combination with SPE is simple and could be used for analysis of aromatic acids in natural waters.

Acknowledgements

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