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Separation and identification of aromatic acids in soil and the Everglades sediment samples using solid-phase microextraction followed by capillary zone electrophoresis

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Abstract

The separation and identification of aromatic acids in soil and the Everglades sediment samples was carried out using solid-phase microextraction (SPME) followed by capillary zone electrophoresis (CZE). The soil and sediment samples were subject to a series of sample treatments including oxidative hydrolysis with molecular oxygen in a sodium hydroxide solution, acidification and filtration. The aromatic acids in the sample filtrate were extracted using SPME with a polyacrylate-coated fiber. The acids adsorbed on the fiber were subsequently desorbed in methanol. The desorbed acids were then separated by CZE. Several aromatic acids (e.g., salicylic acid, *p*-coumaric acid, ferulic acid and vanillic acid) in both soil and sediment samples were separated, identified and quantified. The results of this study show that the combination of SPME with CZE is promising for environmental analysis.

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1. Introduction

Lignin compounds are phenolic polymers that are produced solely by vascular land plants as structural units of their cell walls [1]. Their high natural abundance on land, exclusive terrestrial source, resistance to microbial degradation and wide distribution in soils and sediments render lignins attractive molecular tracers for terrestrial organic matter in aquatic environments [1–6]. The most important components of lignin polymers are vanillic, syringic and cinnamic units [3,4,6]. On oxidation by cupric oxide (CuO), lignins of all vascular plant tissues produce vanillyl phenols, which can be used as general tracers of terrestrially derived materials [3,5,6]. In addition, syringl phenols produced only from angiosperm (flowering vascular plants) lignin and cinnamyl phenols produced only from non-woody vascular plant tissues [3]. Besides cupric oxide, trace amounts of oxygen were also used to oxidize lignin compounds in alkaline media, yielding a group of aromatic acids with a hydroxyl functional group such as vanillic acid, *p*-coumaric and ferulic acids [7]. The analysis of these phenolic compounds present in lignin generally requires preliminary oxidative hydrolysis [5,7]. The lignin oxidation products can subsequently be separated by high-performance liquid chromatography (HPLC) [7] or gas chromatography (GC) [5]. Analysis of the lignin oxidation products by GC requires an additional step

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of derivatization to convert the oxidation products to volatile forms prior to the separation.

In recent years, capillary zone electrophoresis (CZE) appears to be an attractive complementary technique to HPLC and GC with promising features such as high separation efficiency, simplicity, low injection volume and short analytical time. Because of the ionic nature of aromatic acids, it is possible to analyze these compounds directly using CZE [8,9]. Isolation and quantification of aromatic acids in soil and sediment samples, however, is difficult because of the great variety of species present and the wide variations in their levels. Thus, it is essential to devise a sample preparation stage, which ensures reliable identification and quantification. Traditional extraction methods such as liquid–liquid extraction are usually tedious and generate a large amount of organic solvent waste. Solid phase extraction (SPE) requires less solvent, but the device is easily clogged by dirty samples. Solid-phase microextraction (SPME) is a new, simple, solvent-waste free and selective extraction, which has recently become a popular technique for isolation of various organic compounds (e.g., phenols) in the environment samples [10]. Several previous studies have shown that a SPME fiber coated with a relatively polar polyacrylate (PA) polymer can be used to extract phenols from water and soil samples [10–13]. The phenols adsorbed on the fiber were then thermally disorbed in the port of a gas chromatograph and analyzed by GC [10–13]. The separation and identification of aromatic acids degraded from soil and wetland sediment samples using SPME coupled with CZE have not yet been reported. The purpose of this work was to assess the feasibility of using SPME coupled with CZE for separation, identification and quantitation of aromatic acids degraded from soil and wetland sediment samples.

2. Experimental

2.1. Reagents

4-Hydroxybenzoic acid and 4-hydroxyphenylacetic acid were obtained from Aldrich (Milwaukee, WI, USA). 3,4,5-trimethoxybenzoic acid was obtained from Sigma (St. Louis, MO, USA). Other chemicals were obtained from Fisher Scientific (Pittsburgh, PA, USA).

All solutions were prepared using deionized water from a Milli-Q Water Purification System (Millipore, Bedford, MA, USA) and filtered through a 0.4-μm pore size biphenol polycarbonate (PB) filter (Millipore). Sodium tetraborate ($Na_2B_4O_7 \cdot 10H_2O$) was used to prepare a run buffer solution. The pH values of the buffer were adjusted using either a 1.0 *M* NaOH or a 1.0 *M* HCl solution. The stock solutions of the aromatic acids (5.0×10^{-3} *M* for each acid) were prepared by dissolving appropriate amounts of the acids in a 0.01 *M* NaOH solution.

2.2. Instrumentation and optimization

Electrophoretic analyses were carried out using a Beckman P/AC 5500 capillary electrophoresis system 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 computer. Prior to their first use, all capillary columns were conditioned by rinsing in sequence with 1 *M* HCl for 5 min, deionized water for 2 min, 1% (w/v) NaOH for 10 min, deionized water for 2 min and finally run buffer for 5 min. The capillary column was rinsed with the run buffer for 2 min before each injection. The samples were introduced by pressure sample injection at the inlet end and the direct UV detection was performed at a wavelength of 214 nm. The cathode was placed at the outlet buffer and the anode at the inlet buffer.

The effect of injection time on peak area was investigated by injecting a mixture of the acids (5.0 μ*M* for each acid) at 10, 15, 20, 25 and 30 s. The mixture was injected twice into the CE system and the acids were separated under the optimized condition described in our previous study [8]. The values of peak area of the analytes were averaged and the mean value for each concentration of a single compound was plotted against injection time. The optimized injection time was 25 s for the acids tested.

2.3. SPME procedure

SPME device and fibers coated with PA polymer

(Bellefonte, PA, USA) were used to extract the aromatic acids. Prior to the extraction, each PA fiber with a thickness of 85 μm was preconditioned with a solution of methanol–water (50:50) for 30 min. The PA fiber was then immersed vertically in a sample solution with a pH value of 2 to extract the aromatic acids at 20 °C. The relative acidic condition is necessary for the extraction of aromatic acids with pK_a values between 4 and 9.5 because the acids need to be kept in their undissociated forms to ensure the effective extraction [11,13]. After the extraction equilibrium was reached between the coating of a fiber and the analytes, the fiber was withdrawn from the solution. The extracted analytes on the fiber were then desorbed by immersing the fiber into 10 μl of methanol solvent at pH 7 in a PTFE tube (5 cm × 1.5 mm I.D.) that stood horizontally for desorption [14]. The methanol solution along with the analytes desorbed was then transferred to a small vial and injected into the CE system for separation. After each extraction–desorption operation was complete, the fiber was cleaned by immersing it in a methanol–water (50:50) solution for 10 min and dried in the air for 1 min.

To ensure effective extraction of the acids, both extraction and desorption times need to be optimized. To determine the optimized extraction time, a PA fiber was immersed into 4 ml of a standard solution of the acids (10.0 μ*M* for each acid, pH 2.0 ± 0.2) for an extraction time ranging from 5 to 30 min. A fresh standard solution was used for each extraction time period. The extracted acids on the fiber were then desorbed in 10 μl of methanol for 10 min. The resulting solution was transferred to an injection vial for CZE analysis. The optimized extraction time was 20 min. To determine the optimized desorption time, a PA fiber was immersed into 4 ml of a standard solution of the acids (10.0 μ*M* for each acid, pH 2.0 ± 0.2) for 20 min. The extracted acids on the fiber were then desorbed in 10 μl of methanol from 5 to 20 min. A fresh 10 μl of methanol was used for each desorption time period.

2.4. Quantitation

The stock standard solutions were diluted to make mixtures of the acids at a concentration range of 0.50–100 μ*M* for each compound. The mixtures were adjusted to a pH value of 2.0. The acids in each solution were extracted by a PA fiber for 20 min and the extracted acids on the fiber were subsequently desorbed in 10 μl of methanol in a PTFE tube for 10 min. The acids desorbed in methanol were then injected three times onto the CE system. The values of peak area of each compound were then averaged and the mean value for each concentration of a compound was used for linear regression calculations. Repeatability was examined by eight replicate injections of a mixture of the acids with the lowest concentration (0.50 μ*M* for each compound) used within the standard series.

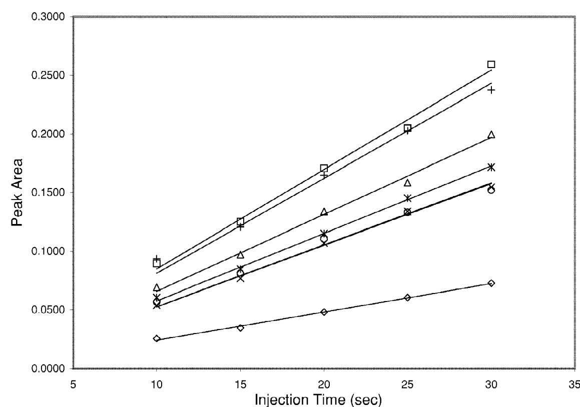
2.5. Analysis of soil and sediment samples

Soil (25.0 g) and sediment (25.0 g) samples were taken from the campus of Florida International University (FIU) and the Everglades (Miami, FL, USA), respectively. The extraction of the analytes from the solid samples was performed using the procedure reported previously [7]. A solution (40 ml) of 1.0 *M* NaOH was added to each sample. Trace amounts of oxygen were introduced to the sample suspensions by bubbling nitrogen gas containing 5 ppm of oxygen at 20 °C for 20 h. The suspensions were centrifuged and filtered using a Whatman filter paper to remove solid particles. A 10-ml filtrate of each sample was acidified with 12 *M* HCl to pH 2.0. The acidified samples were centrifuged and filtered using a 0.4-μm pore size BP filter (Millipore). A 4-ml filtrate was then subjected to the optimized SPME procedure followed by CZE analysis.

3. Results and discussion

3.1. Effect of sample injection time

As reported in our previous study, the best separation was achieved using the following conditions: a run buffer containing 13 m*M* sodium borate at pH 9.68 ± 0.02, 20 kV run voltage, 22 °C operation temperature and 10 s hydrodynamic injection time [8]. To improve the sensitivity of the method, a larger sample volume was injected by employing longer injection time using a solution with low concentrations of the acids. The effect of injection time on peak area was investigated by injecting a



□ 3,4,5-trimethoxybenzoic ◇ 4-hydroxtphenylacetic △ Salicylic ✱ Ferulic ○ p-coumaric + Vanillic × 4-hydrxybenoic

Fig. 1. Effect of sample injection on peak area for the aromatic acids tested. Experimental conditions: pH of run buffer, 9.68; concentration of run buffer, 13 m*M*; concentration of the acids, 5.0 μ*M* each; UV detection, 214 nm; run voltage, 20 kV; capillary, fused-silica 57 cm × 57 μm I.D.; and operation temperature, 22 °C.

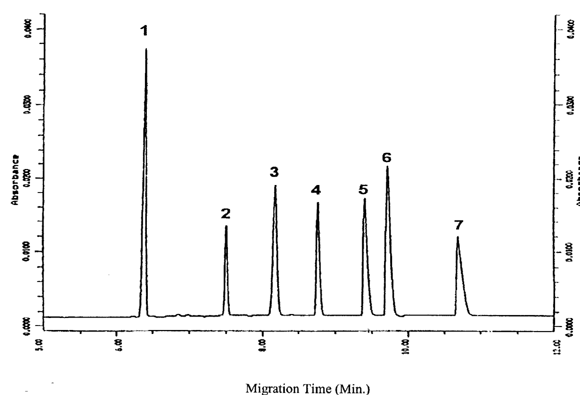


Fig. 2. An electropherogram of the acids (5.0 μ*M* each). CZE conditions were: pH of run buffer, 9.68; concentration of run buffer, 13 m*M*; injection time, 25 s; UV detection, 214 nm; run voltage, 20 kV; capillary, fused-silica 57 cm × 57 μm I.D.; and operation temperature, 22 °C. Peaks: (1) 3,4,5-trimethoxybenzoic acid; (2) 4-hydroxyphenylacetic acid; (3) salicylic acid; (4) ferulic acid; (5) *p*-coumaric acid; (6) vanillic acid; and (7) 4-hydroxybenzoic acid.

standard solution (5.0 μ*M* for each compound) at 10, 15, 20, 25 and 30 s into the CE system. Fig. 1 shows the correlation between peak area and injection time for the acids tested. A linear relationship up to 30-s injection time was observed. However, the separation of the acids became deteriorated when the injection time was 30 s. Thus, 25 s was chosen as the optimized injection time. A representative electropherogram of the acids under the optimized CZE separation conditions shows that all compounds tested are well separated within 12 min (Fig. 2).

3.2. Quantitation

The data of calibration, repeatability and limit of detection (LOD) of the method developed in this study are summarized in Table 1. The calibration data were obtained by plotting peak area versus concentration of the acids. A good linearity was observed for the acids tested in a wide range of 0.50 to 1.0×10^{2} μ*M*. The repeatability for peak area was examined by eight replicate injections of a mixture containing 0.50 μ*M* for each acid and the relative standard deviations (RSDs) were between 2.7 and 7.0%. The limit of detection was in a range of 0.13–0.25 μ*M* and calculated using a signal-to-noise ratio of 3.

3.3. Extraction and desorption time profiles

To ensure extraction efficiency of analytes from a sample, one of the important steps in the development of a SPME method is to determine the time needed when an extraction process reaches equilibrium between analytes and coating of a fiber. Fig. 3 shows the extraction time profiles of the analytes (10.0 μ*M* for each acid) for extraction times of 5, 10, 20 and 30 min. The amounts of the analytes extracted increased rapidly within the first 10 min and tended to reach constant values after 20 min. Thus, the optimized extraction time was 20 min.

Desorption experiments were carried out for 5, 10 and 20 min. The desorption processes occurred rapidly for the first 5 min, and more than 80% of the extracted analytes were desorbed within 10 min (Fig. 4). A compromise was made between the analytical time and the efficiency of desorption by choosing 10 min as the optimized desorption time.

3.4. Identification and quantitation of aromatic acids in soil and wetland sediment samples

To evaluate the performance of the optimized SPME–CZE method developed in this study, we performed analysis on two natural samples. One was

Table 1
Calibration data, repeatability and detection limits for the acids studied

Compound	Linear range (μ*M*)	Slope (A)	R^2	Repeatability[a] (RSD%; $n=8$)	LOD[b] (μ*M*)
3,4,5-Trimethoxybenzoic acid	$0.50–1.0\times10^2$ ($1.1\times10^{-1}-21$ mg/l)	2.2×10^{-2}	1.00	2.7	0.13
4-Hydroxyphenylacetic acid	$0.50–1.0\times10^2$ ($7.6\times10^{-2}-15$ mg/l)	6.5×10^{-3}	1.00	7.0	0.25
Salicylic acid	$0.50–1.0\times10^2$ ($6.9\times10^{-2}-14$ mg/l)	1.7×10^{-2}	1.00	5.0	0.18
Ferulic acid	$0.50–1.0\times10^2$ ($9.7\times10^{-2}-19$ mg/l)	1.1×10^{-2}	1.00	4.5	0.18
p-Coumaric acid	$0.50–1.0\times10^2$ ($8.2\times10^{-2}-16$ mg/l)	1.4×10^{-2}	1.00	4.7	0.23
Vanillic acid	$0.50–1.0\times10^2$ ($8.4\times10^{-2}-17$ mg/l)	2.2×10^{-2}	1.00	2.9	0.16
4-Hydroxybenzoic acid	$0.50–1.0\times10^2$ ($6.9\times10^{-2}-14$ mg/l)	1.4×10^{-2}	0.99	3.8	0.21

[a] Relative standard deviations were calculated based on the peak areas of the acids obtained by making eight replicate injections of a solution containing seven acids (0.50 μ*M* for each compound).
[b] The limits of detection were determined based a signal-to-noise of 3.

the soil sample taken from the University Park campus of FIU and the other was the sediment sample taken from the Everglades. Fig. 5 shows the electropherogram of the soil sample analyzed by the SPME–CZE method. It was found that it took longer time for all test compounds in the soil sample to migrate along the capillary compare to the corresponding compounds in the standard solution (Fig. 2). This may due to the difference in sample matrix between the soil sample and the standard solution. The method we used to identify the compounds was to spike one test compound into the soil sample at a time and observed either appearance of a new peak or an increase in peak area for the existing compound in an electropherogram. Five aromatic acids, including salicylic acid, ferulic acid, *p*-coumaric acid, vanillic acid and 4-hydroxybenzoic acid, were identified by spiking the standards into the sample. Among these acids, *p*-coumaric acid and 4-hydroxybenzoic acid were the predominant acids in the soil sample. 4-Hydroxybenzoic acid is known to have important nonlignin sources that sometimes limit its use as a geochemical tracer [15]. Fig. 6 shows the electropherogram of the sediment sample taken from the Everglades. Five acids found in the soil sample were also identified in the sediment sample by means of spiking the standards into the sediment sample. Among the compounds identified, *p*-cumaric acids and ferulic acid were the predominant acids in the sediment sample. The concentrations of the acids

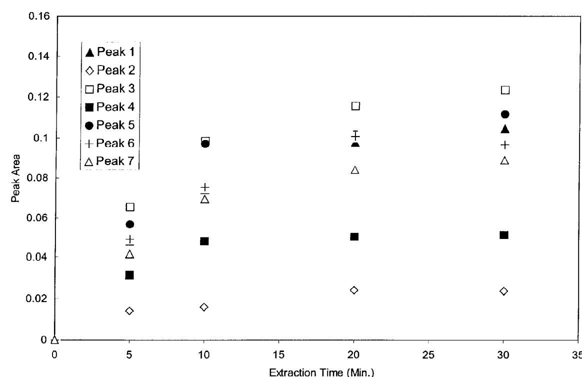


Fig. 3. Extraction time profile for the acids tested using a PA (polyacrylate) fiber. Extraction conditions: a PA fiber immersed in a solution containing the seven acids (10 μ*M* each, pH 2) for a time range of 5–30 min. The absorbed compounds were then desorbed in 10 μl methanol for 10 min. Twenty minutes was chosen as the optimized extraction time. CZE conditions and peak notation as in Fig. 2.

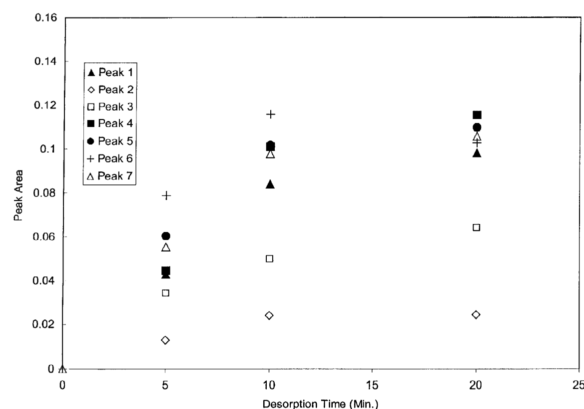


Fig. 4. Desorption time profile for the acids tested using a PA fiber. Extraction conditions as in Fig. 3. The extraction time was 20 min. Ten minutes was chosen as the optimized desorption time. CZE conditions and peak notation as in Fig. 2.

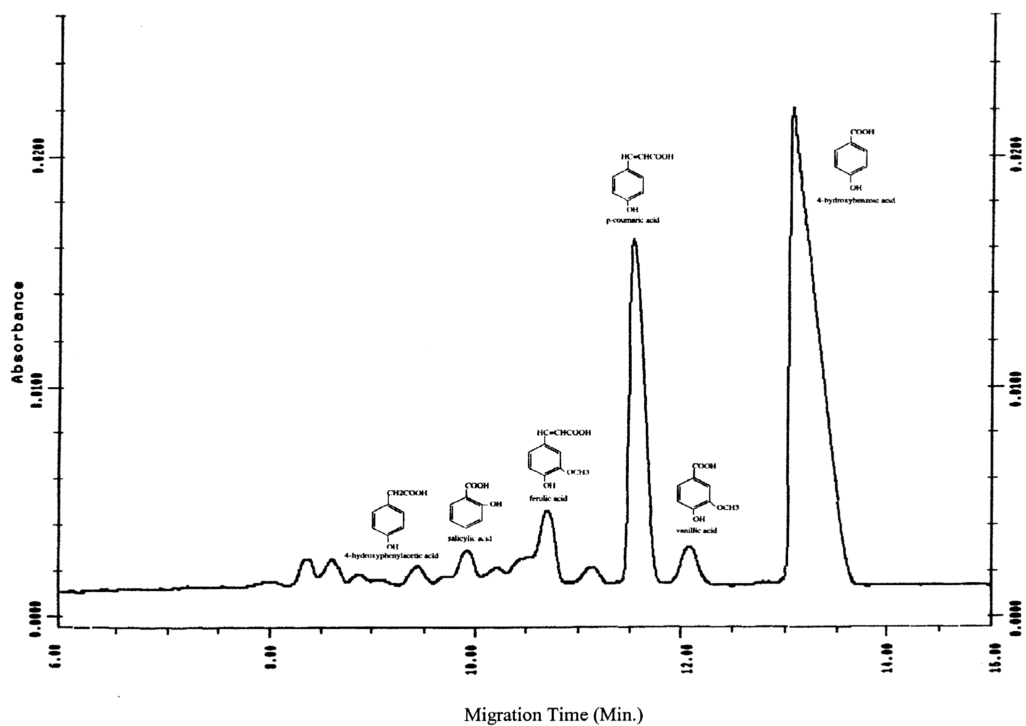


Fig. 5. An electropherogram of the soil sample. The acids in the soil sample were extracted under the optimized SPME conditions followed by CZE separation. CZE conditions as in Fig. 2.

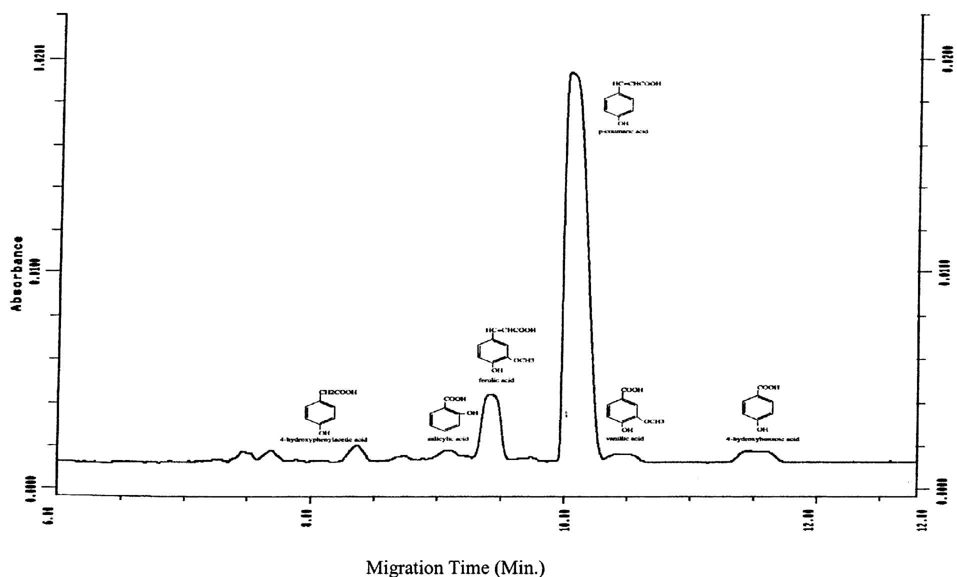


Fig. 6. An electropherogram of the Everglades sediment sample. The acids in the sediment sample were extracted under the optimized SPME conditions followed by CZE separation. CZE conditions as in Fig. 2.

Table 2
Concentrations of the acids in the soil and the Everglades sediment samples

Compound	Soil		Sediment	
	μmol/l[a]	μmol/g wet solid sample[b]	μmol/l[a]	μmol/g wet solid sample[b]
3,4,5-Trimethoxybenzoic acid	ND[c]	ND	ND	ND
4-Hydroxyphenylacetic acid	<ND	<ND	<ND	<ND
Salicylic acid	2.1	3.4×10^{-3}	3.0	4.8×10^{-3}
Ferulic acid	49	7.8×10^{-2}	46	7.3×10^{-2}
p-Coumaric acid	1.4×10^{2}	2.2×10^{-1}	2.6×10^{2}[d]	4.2×10^{-1}
Vanillic acid	12	1.9×10^{-2}	3.5	1.0×10^{-2}
4-Hydroxybenzoic acid	3.5×10^{2}[d]	5.6×10^{-1}	12	1.9×10^{-2}

[a] The concentrations of the acids in a NaOH (1.0 *M*) solution as an extraction agent.
[b] The amounts of the acids extracted from 1 g of the wet solid sample.
[c] ND, not detected.
[d] The concentrations were beyond the linear concentration range of the standard curves, and estimated by assuming that the linear concentration range could be extended to 3.5×10^{2} μ*M* for the acids tested.

identified in both soil and sediment samples are estimated based on the calibration data and listed in Table 2. The amounts of the acids identified are in the range of 3.4×10^{-3} to 5.6×10^{-1} (μmol/g wet solid sample) in the soil sample, and 4.8×10^{-3} to 4.2×10^{-1} (μmol/g wet solid sample) in the Everglades sediment sample. The relative abundance of cinnamyl compounds (e.g., *p*-coumaric and ferulic acid) over vanillyl compounds (e.g., vanillic acid) is useful information for distinguishing between woody (e.g., red cedar, slash pine) and nonwoody (e.g., black mangrove, cord grass) vascular plant tissues [3]. Only the nonwoody vascular plant tissues typically produce significant amounts of two cinnamyl compounds (i.e., *p*-coumaric and ferulic acid) and lower yields of vanillyl and syringyl compounds [3,4]. Our results suggest that the soil and the Everglades sediment samples tested in this study contain essentially nonwoody vascular plant tissues.

Aromatic acids degraded from lignin polymers in various soil and sediment samples have been analyzed not only by the SPME–CZE method developed in this study, but also by the GC and HPLC methods reported previously [5,7]. Table 3 lists the concentrations of several aromatic acids degraded from soil and sediment samples determined by the SPME–CZE, GC and HPLC methods. Useful comparison among the data listed in Table 3 may be limited as the samples collected from various locations were analyzed by different procedures and methods, among which there were many variants that could greatly affect the final measurements. Nevertheless, striking similarities exist among these data as *p*-coumaric acid and ferulic acid have been identified as two of the major lignin degradation products in a concentration range of 15–69 μg/g solid sample. The concentrations of vanillic acid in the soil and the Everglades sediment samples are generally low (1–

Table 3
Comparison of the concentrations of aromatic acids in soil and sediment samples analyzed by SPME–CZE, GC and HPLC

Sample	Compound (μmol/g solid sample)[a]				Method	Refs.
	Ferulic acid	*p*-Coumaric acid	Vanillic acid	4-Hydroxybenzoic acid		
Soil	7.8×10^{-2} (15[b])	2.3×10^{-1} (37)	1.9×10^{-2} (3.2)	5.6×10^{-1} (78)	SPME–CZE	This study
Everglades sediment	7.3×10^{-2} (14)	4.2×10^{-1} (69)	5.6×10^{-3} (0.94)	1.9×10^{-2} (2.7)	SPME–CZE	This study
Freshwater sediment	2.8×10^{-1} (55.2)	2.2×10^{-1} (36.3)	7.3×10^{-1} (123)	5.6×10^{-1} (76.7)	GC	[5][c]
Soil	1.2×10^{-1} (23.84)	2.8×10^{-1} (46.72)	5.4×10^{-2} (9.1)	1.1 (152)	HPLC	[7]

[a] The concentrations of the acids obtained from this study are expressed as μmol/g wet solid sample, whereas the concentrations of the acids reported in Refs. [5,7] were expressed as μmol/g dry solid sample.
[b] The unit for data in parenthesis is μg/g solid sample.
[c] The data reported were obtained from the Lake Washington sediment sample 0.06.

10 μg/g solid sample) with the exception of the freshwater sediment sample (123 μg/g solid sample) collected from Lake Washington. The compositional characteristics of these samples indicates that the soil and the Everglades sediment samples contain likely high contents of nonwoody vascular plant materials, whereas the freshwater sediment collected from Lake Washington may contain a significant fraction of woody vascular plant tissues.

4. Conclusions

An analytical method using SPME coupled with CZE was developed for the separation, identification and quantitation of seven aromatic acids. A good linearity was observed for the acids tested over a concentration range of 0.50 to 1.0×10^2 μ*M* with detection limits of 0.13 to 0.25 μ*M*. The method was applied to separate, identify and quantify aromatic acids degraded from soil and the Everglades sediment samples. Among five acids identified, *p*-coumaric acid and ferulic acid were two major lignin degradation products in the samples. The relative abundance of *p*-coumaric acid and ferulic acid over vanillic acid suggests that the soil and the Everglades sediment samples contain likely high contents of nonwoody vascular plant tissues. This work has demonstrated that the combination of SPME with CZE is suitable for the analysis of aromatic acids in soil and sediment samples.

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