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Determination of three phenoxyacid herbicides in environmental water samples by the application of dispersive liquid-liquid microextraction coupled with micellar electrokinetic chromatography

Research Article

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Abstract: An efficient method based on dispersive liquid-liquid microextraction coupled with micellar electrokinetic chromatography has been developed for determination of three phenoxyacid herbicides (PAs) of 2,4-dichlorophenoxybutyric acid (2,4-DB), dicamba and 2,4-dichlorophenoxyacetic acid (2,4-D), in environmental water samples. The types and volumes of extracting and dispersing solvents, ionic strength, extraction and centrifugation time and centrifugation speed were investigated. Successful separation of the three PAs was achieved within 7 min, by using the background electrolyte solution consisting of 10 mmol L^{-1} sodium tetraborate, 25 mmol L^{-1} sodium dodecyl sulfate and 15% (v/v) methanol, at pH 9.75. Excellent analytical performances were attained, such as good linear relationships ($R \geq 0.9993$) between peak area and concentration for each PAs from 10–1000 ng mL^{-1}, limits of detection of 1.56–1.91 ng mL^{-1}, and intra-day precisions at two spiked levels in terms of migration time and peak area within the range of 0.22–0.42% and 3.88–6.39%, respectively. Enrichment factors of 2,4-DB, dicamba and 2,4-D were 180, 151 and 216, respectively. The method recoveries obtained at fortified 20.0, 50.0 and 100.0 ng mL^{-1} for lake, river and reservoir water samples varied from 67.91 to 119.07% with the relative standard deviation of 1.47–6.89%.

Keywords: *Dispersive liquid-liquid microextraction • Micellar electrokinetic chromatography • Phenoxyacid herbicides • Water samples*

1. Introduction

Phenoxyacid herbicides (PAs) have been most widely used for controlling the growth of weed and other vegetation. They are relatively cheap and very efficient even at low concentration; therefore they are extensively applied to be against grass and broad leaf weeds in many cereal crops. Due to their strong polarity, PAs can easily dissolve and diffuse in waters. By moving in agricultural ecosystems, they can lead to the contamination of the environmental surface and underground waters. It was confirmed that they can accumulate in river and lake sediments [1,2]. 2,4-dichlorophenoxybutyric acid (2,4-DB), dicamba, 2,4-dichlorophenoxyacetic acid (2,4-D) are some of the main members of the PAs. They show moderate toxicity, while their metabolites, especially chloride present placenta toxicities to human and aquatic organisms. Talking about their main hazards, the PAs can cause soft tissue carcinoma in humans [3] and embryotoxicity in animals [4]. The maximum contaminant

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level (MCL) of 2,4-D in drinking water is set at 70 μg L^{-1} both in Taiwan EPA drinking water quality standards and US drinking water regulations [5]. Therefore, it is important to develop efficient methods for the separation and determination of PAs.

To date, gas chromatography (GC) [6–8], liquid chromatography (LC) [9–13] and capillary electrophoresis (CE) [14,15] have been widely used for separation and determination of PAs. However, as the volatility of these types of compounds is comparatively low, there is the need to increase the volatility by various derivatization prior to GC, which is cumbersome, time consuming, and usually involves the use of hazardous reagents. Meanwhile, LC consumes much organic reagent, the injection amount is fairly large and separation time is relatively long. Compared with these two analysis methods, CE can provide some distinct advantages, such as stronger resolution power and higher separation efficiency, much smaller sample and reagent requirement, and shorter analysis time [16–18].

On account of the complexity of matrix and low concentrations of these compounds in environmental water samples, various procedures need to be developed for the extraction and preconcentration of PAs from water samples. So far, a number of classic extraction methods such as liquid-liquid extraction (LLE) [19], solid-phase extraction (SPE) [11], liquid-liquid-liquid microextraction (LLLME) [12,20,21] and dispersive liquid-liquid microextraction (DLLME) [22] have been developed for sample pretreatment of PAs. DLLME, as a relatively new sample preparation technique, showing remarkable advantages in small amount of organic solvent, short extraction time, low cost, simplicity of operation, high enrichment factor and environmental benignity, has been increasingly developed and applied for trace analysis [23–26]. In DLLME, the proper mixture of extracting and dispersing solvents is rapidly injected into an aqueous sample containing the analytes by a syringe, and a cloudy solution is formed and the fine particle of extracting solvent facilitates fast extraction of analytes from the aqueous sample. After centrifugation, extracting solvent containing analytes is normally sedimented at the bottom of the tube and taken for its following analysis such as GC [27], GC-MS [28], HPLC [29] and electrothermal atomic absorption spectrometry (ETAAS) [30]. The general aspects and applications of DLLME have been summarized in some recent reviews [31–33]. Moreover, DLLME has found particular applications as an analytical pretreatment process for the determination of pesticides besides PAs in diverse matrices, for examples, N-methylcarbamates in water [34] and in vegetables [35], sulfonamides [26] and carbamate pesticides [36–38] in water samples, and carbamate pesticide in soils [39]. Recently, a DLLME-LC method was developed for the determination of two PAs of 2,4-D and 4-chloro-2-methyl phenoxyacetic acid in tap and well water samples [22], in which chlorobenzene was investigated as extracting solvent while acetone as dispersing solvent, and single factor alternate method was employed for the optimization of extraction conditions. However, to the best of our knowledge, DLLME coupled with CE has not been reported for the determination of PAs.

In this work, DLLME coupled with micellar electrokinetic chromatography (MEKC) has been investigated for the simultaneous determination of three PAs (2,4-DB, dicamba, 2,4-D) in environmental water samples. The types and volumes of extracting and dispersing solvent, ionic strength, extraction and centrifugation time and centrifugation speed, all of which can significantly affect extraction efficiency were studied and optimized. The optimal method was applied for the simultaneous determination of the three PAs in lake, river and reservoir water samples.

2. Experimental procedure

2.1. Chemicals and samples

Three PAs standards of 2,4-DB, dicamba and 2,4-D were purchased from Sigma-Aldrich (Steinheim, Germany), and their structures are shown in Fig. 1. Chromatographic grade acetonitrile (ACN), methanol (MeOH), dimethyl sulfoxide (DMSO), chlorobenzene, bromobenzene were purchased from J&K Technology Limited (Beijing, China). Sodium dodecyl sulfate (SDS), sodium tetraborate ($Na_2B_4O_7$) and carbon tetrachloride (CCl_4) were purchased from Aladdin (Shanghai, China). Sodium hydroxide (NaOH), sodium chloride (NaCl), hydrochloric acid (HCl) and ethanol (EtOH) were purchased from SCRC (Shanghai, China). All the chemicals were of analytical grade. Deionized water used throughout the work was produced by a Milli-Q Ultrapure water system (Millipore, Bedford, MA, USA).

Standard stock solutions containing 1000 μg mL^{-1} of individual PAs were prepared by dissolving the required amounts of the standards in methanol. They were stored in a refrigerator at 4°C. Working solutions were prepared by diluting the stock solutions with methanol–water (50/50, v/v).

Lake water was collected from an artificial lake; river water was collected near freshwater fisheries; and reservoir water was collected near a piece of farmland, all of which were located in Laishan District of Yantai City (China). All the collected water samples were stored in the dark at 4°C for use. Before use, the samples were

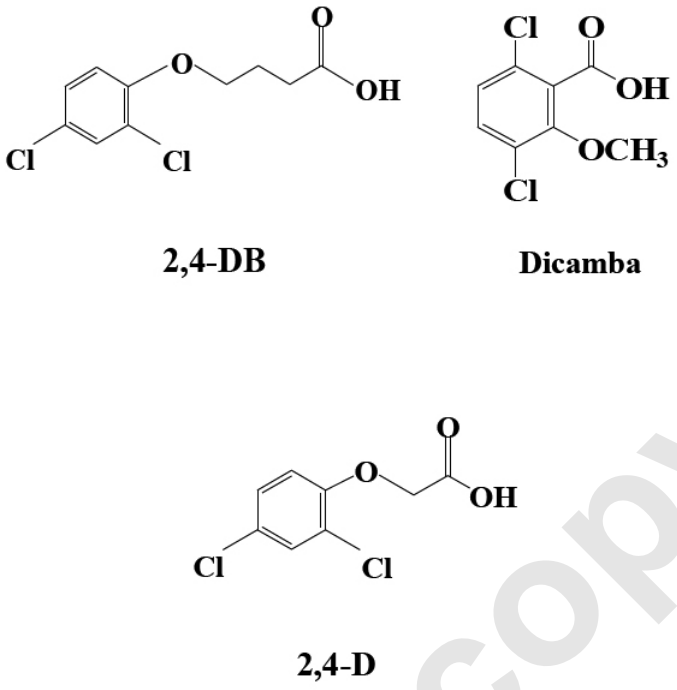

Figure 1. Molecular structures of the PAs analyzed in this work.

filtered through a 0.45-μm microporous nylon membrane to eliminate particulate matter. Several aliquots from 5 mL filtered water samples were spiked with the PAs standards with different concentrations and followed by the DLLME procedure.

2.2. Apparatus and software

A Beckman-Coulter P/ACE MDQ CE system (Fullerton, CA, USA) equipped with a diode-array detector (DAD) and bare fused-silica capillary (Yongnian Photoconductive Fiber Factory, Hebei, China) with 75 μm i.d., 375 μm o.d., total length of 50.2 cm, and effective length of 40 cm were utilized in all the experiments. An Ion 510 pH meter (Ayer Rajah Crescent, Singapore) was used for adjusting pH. Data acquisition was performed using Karat 32 software (Beckman-Coulter, Fullerton, CA, USA).

2.3. MEKC separation

Samples were hydrodynamically (5 s, 0.5 psi) injected at the anode and subsequent separation was performed at 25°C, using an applied voltage of 28 kV for 7 min with a wavelength of 230 nm. The running buffer was prepared by freshly mixing 10 mmoL L^{-1} $Na_2B_4O_7$, 25 mmol L^{-1} SDS and 15% (v/v) MeOH, adjusted to pH 9.75 with 1 mol L^{-1} NaOH.

Before the first usage, new capillary was conditioned by rinsing in order, with water (10 min), 0.1 mol L^{-1} NaOH (40 min), water (20 min) and running buffer (30 min). Each buffer solution was prepared daily and degassed for 10 min prior to use. The capillary was conditioned daily by flushing with water, 0.1 mol L^{-1} NaOH, water and running buffer for 5, 10, 5 and 10 min, respectively. Between the two separation analyses, it should be rinsed with running buffer for 2 min. At the end of daily usage, the capillary was rinsed sequentially with water (5 min), MeOH (10 min), water (5 min), 0.1 mol L^{-1} HCl (10 min), water (5 min), 0.1 mol L^{-1} NaOH (10 min) and water (5 min). All the aqueous solutions were passed through a 0.45-μm membrane before use.

2.4. DLLME procedure

For the DLLME, 5.00 mL aqueous sample containing the analytes (pH adjusted to 2.00 with 1 mol L^{-1} HCl) was placed in a 10-mL glass test tube with conical bottom, in which the spiked three PAs individual content is 1 μg mL^{-1}. ACN of 1.0 mL as dispersing solvent, containing 180 μL chlorobenzene as extracting solvent, was promptly injected into the aqueous sample, and then the mixture was gently shaken. A cloudy solution of fine droplets of extracting solvent was formed, with the PAs extracted into the fine droplets. After centrifugation for 15 min at 4000 rpm, the extracting solvent containing the analytes was sedimented at the bottom of the glass test tube. Then the sedimentation was removed with a syringe and dried under a gentle flow of nitrogen. At last, the evaporation residue was redissolved using 20 μL running buffer for further MEKC analysis.

3. Results and discussions

3.1. Optimization of MEKC separation conditions

For MEKC analysis, the separation of the three PAs were greatly influenced by the concentration of buffer and SDS, pH of buffer solution and the concentration of the adding organic solvent as well as the applied voltage by affecting electroosmotic flow. In order to optimize the separation conditions, each factor was studied. Herein, we employed hydrodynamic injection (0.5 psi) for 5 s at the anode to introduce samples into capillary, referring to previous work such as [40,41].

Selection of the buffer used as background electrolyte has a great influence on the migration behavior. For this system, three kinds of buffers including sodium tetraborate, ammonium acetate and phosphate buffers were tested. Finally, sodium tetraborate buffer was selected as the optimized background electrolyte owing to its better separation effect. Six levels (5, 10, 15, 20, 25 and 30 mmol L^{-1}) of borate solution, with 25 mmol L^{-1} SDS, at pH 9.75 and 15% (v/v) MeOH were used. Migration time of the analytes increased with increasing $Na_2B_4O_7$ concentration. In order to obtain a good separation resolution in a short time, 5 mmol L^{-1} and 10 mmol L^{-1} $Na_2B_4O_7$ solution were selected and the migration time was within 7 min. Because better separation of dicamba and 2,4-D was achieved, 10 mmol L^{-1} was chosen as the optimal $Na_2B_4O_7$ concentration for the three PAs separation.

To determine the effect of the concentration of SDS, experiments were implemented using different concentration of SDS (10, 15, 20, 25, 30 and 35 mmol L^{-1}), with 10 mmol L^{-1} $Na_2B_4O_7$ solution, 15% (v/v) MeOH and pH adjustment to 9.75. Good separation of dicamba and 2,4-D was achieved at the concentration both of 25 and 30 mmol L^{-1} and for its shorter analysis time, 25 mmol L^{-1} SDS was selected as the optimum concentration.

To determine the effect of buffer pH on migration behavior, experiments were performed by using a background electrolyte solution consisting of 10 mmol L^{-1} $Na_2B_4O_7$, 25 mmol L^{-1} SDS and 15% (v/v) MeOH with the pH in the range of 7.0–11.0. When the buffer pH was 9.75, the electrophoretic resolution and sensitivity were the best, so a pH value of 9.75 was chosen as the optimum pH for the three PAs separation.

It has been reported that it can improve separation effect by adding organic modifier to the running buffer solution. In this study, MeOH and ACN were studied. Poor resolution was obtained with ACN, while the presence of MeOH produced an improvement in the separation of the three PAs. Therefore, MeOH was selected as the organic modifier and then its concentration were varied from 5% to 30% (v/v). The best separation with shorter analytical time, high sensitivity and baseline separation was acquired at 15% (v/v) MeOH.

To determine the influence of applied voltage on migration behavior, the separation voltage of 25, 28 and 30 kV were checked using a background electrolyte solution consisting of 10 mmol L^{-1} $Na_2B_4O_7$, 25 mmol L^{-1} SDS and 15% (v/v) MeOH, at a pH value of 9.75. For 25 kV, the analysis time was longer and for 30 kV, there wasn't a good separation between dicamba and 2,4-D. Therefore, an applied voltage of 28 kV was chosen because of its good separation effect and shorter analysis time.

On the basis of the above described optimum separation conditions, the background electrolyte solution consisting of 10 mmol L^{-1} $Na_2B_4O_7$, 25 mmol L^{-1} SDS, 15% (v/v) MeOH, at pH 9.75 and an applied voltage of 28 kV at 25°C with a wavelength of 230 nm was used to achieve baseline separation of the three PAs within 7 min.

3.2. Optimization of DLLME

In DLLME, all of these parameters including types and volumes of extracting and dispersing solvents, ionic strength, extraction and centrifugation time and centrifugation speed, can significantly affect extraction efficiency. In order to obtain the optimized extraction conditions, all the mentioned factors were investigated. In doing that, enrichment factor (EF) was employed to evaluate the extraction efficiency according to Eq. 1 as follows:

$$EF = \frac{C_{sed}}{C_0} \quad (1)$$

where, the C_{sed} and C_0 are the concentration of analyte redissolved in the running buffer after the sedimentation evaporation and the initial analyte concentration in the aqueous samples, respectively.

3.2.1. Selection of extracting solvent

For the DLLME process, the selection of extracting solvent is important. In this work, different extracting solvents such as chlorobenzene, carbon tetrachloride (CCl_4) and bromobenzene were studied with the use of ACN as dispersing solvent. For bromobenzene, the electrophoretic peak cannot be separated from the analytes' peaks. For CCl_4, the EF was much lower than that chlorobenzene as extracting solvent. Moreover, using chlorobenzene, a two-phase system formed and it can achieve a high EF extraction procedure, therefore, chlorobenzene was confirmed to be the extracting solvent.

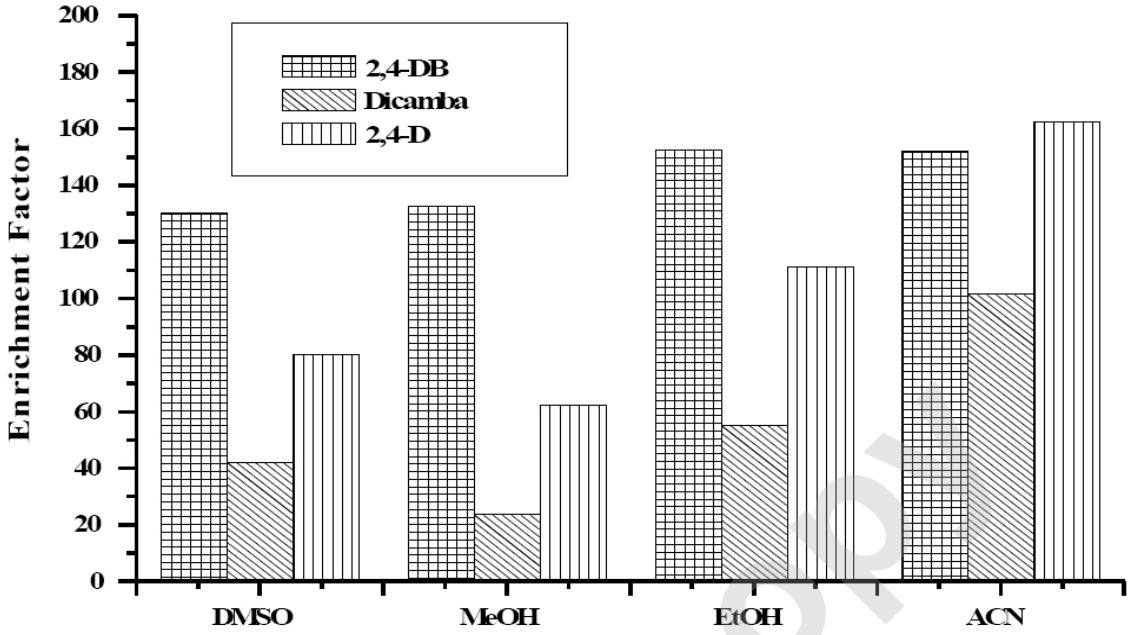

Figure 2. Selection of different dispersing solvent for the DLLME of PAs. Extraction conditions: sample volume, 5.0 mL; extracting solvent, 180 μL chlorobenzene; dispersing solvent, 1000 μL ACN. Optimum MEKC separation conditions: running buffer, 10 mmol L^{-1} $Na_2B_4O_7$, 25 mmol L^{-1} SDS, 15% (v/v) MeOH, at pH 9.75; applied voltage, 28 kV at 25°C; wavelength, 230 nm.

3.2.2. Selection of dispersing solvent

With the use of chlorobenzene as extracting solvent, different dispersing solvents such as DMSO, MeOH, EtOH and ACN were investigated. The effects of these four dispersing solvents on EF is presented in Fig. 2. As can be seen, ACN showed much higher EF of dicamba and 2,4-D compared to that of the other three dispersing solvents, but only a little bit lower for 2,4-DB. After considering all the factors, ACN was chosen as the dispersing solvent for this work.

3.2.3. Effect of extracting solvent volume

For the sake of evaluating the effect of the extracting solvent volume on the extraction efficiency, different volumes of chlorobenzene contained in 1000 µL ACN, which varied in the range of 100–220 µL in 20 µL intervals, were tested to find the best performance of the DLLME procedure. Fig. 3a displays the different EF values with different extracting volumes. As can be perceived, with increasing of the extracting solvent volume until 180 µL, the EF of 2,4-DB, dicamba and 2,4-D increased from 111–125, 68–93 and 108–139, respectively. On increasing the volume of extracting solvent, the EF decreased on the contrary. Thus, 180 µL chlorobenzene was selected for the subsequent experiments.

3.2.4. Effect of dispersing solvent volume

To investigate the influence of different dispersing solvent volumes on the extraction efficiency, the ACN volumes of 600, 800, 1000, 1200, 1400 µL were studied. As can be seen in Fig. 3b, EF increased with increasing the volume of ACN in the range of 600–1000 µL, however, a reduction was obtained when the volume of ACN exceeded 1000 µL for all the three PAs. The reason may be that at a low volume, a cloudy solution can not form completely because low content of ACN was insufficient to disperse chlorobenzene, and at a high volume, the dissolving distribution of PAs in water increased. In conclusion, the ACN volume of 1000 µL was selected as the optimum dispersing solvent volume.

3.2.5. Effect of centrifugation time

In order to observe the effect of centrifugation time on the extraction efficiency, different centrifugation time of 5, 10, 15, 20 and 25 min were tested. According to the results in Fig. 3c, 15 min was chosen to be as the optimum centrifugation time.

3.2.6. Effect of centrifugation speed

The centrifugation speed may affect the extraction efficiency. To study the influence of centrifugation speed on the DLLME procedure, various experiments were implemented by changing different centrifugation speed in the range of 2000–5000 rpm in 500 rpm intervals. Different EF values were gained at different centrifugation speeds as displayed in Fig. 3d. When the centrifugation speed was below 4000 rpm, the EF increased as the analytes were completely sedimented with the extracting solvent, while, with the speed up,

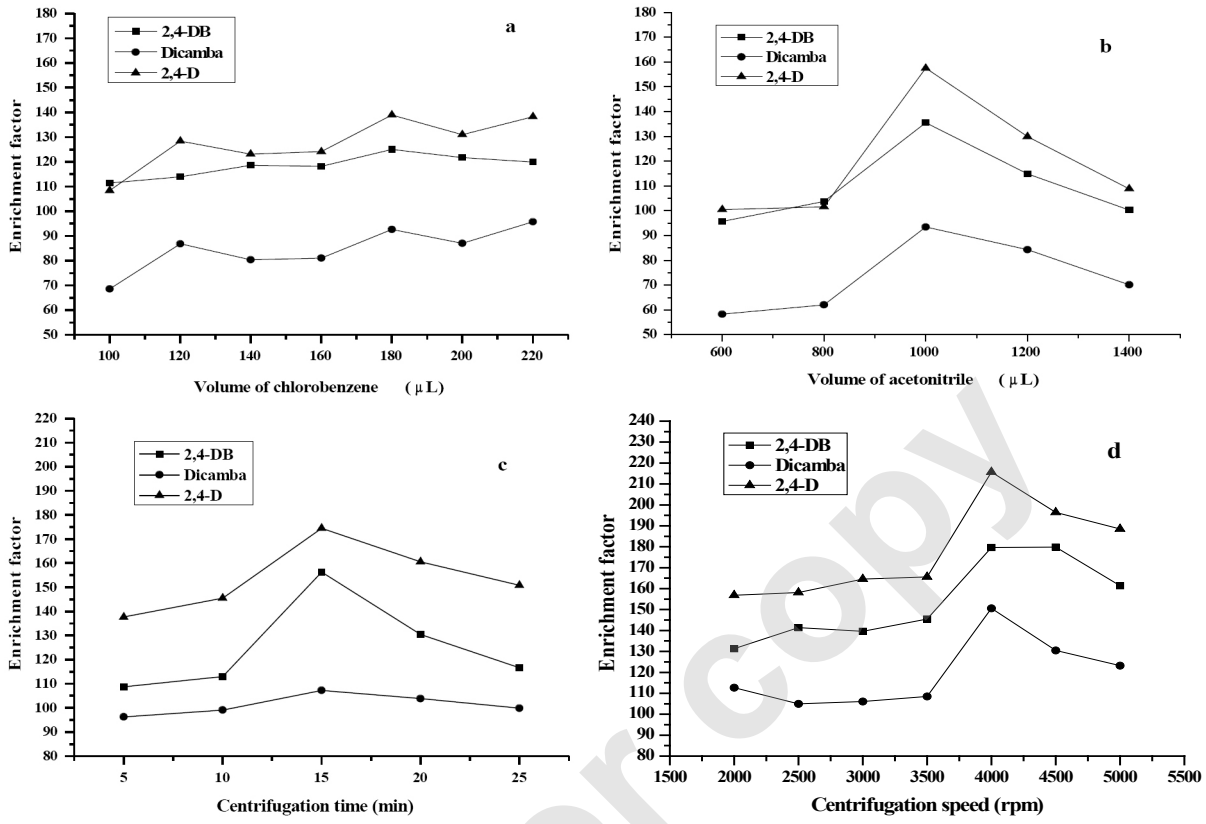

Figure 3. Effect of (a) extracting solvent volume, (b) dispersing solvent volume, (c) centrifugation time and (d) centrifugation speed on the EFs of DLLME for PAs. Extraction conditions: sample volume, 5.0 mL; (a) extracting solvent, chlorobenzene; dispersing solvent, 1000 μL ACN; centrifugation time, 15 min; centrifugation speed, 4000 rpm; (b) extracting solvent, 180 μL chlorobenzene; dispersing solvent, ACN; centrifugation time, 15 min; centrifugation speed, 4000 rpm; (c) extracting solvent, 180 μL chlorobenzene; dispersing solvent, 1000 μL ACN; centrifugation speed, 4000 rpm; (d) extracting solvent, 180 μL chlorobenzene; dispersing solvent, 1000 μL ACN; centrifugation time, 15 min. The MEKC separation conditions were the same as those described in Fig. 2.

PAs redissolved back into water because of the heat generated in the process of centrifugation, leading to the decrease of the EF. So, 4000 rpm was selected as the optimum centrifugation speed.

3.2.7. Effect of extraction time

In DLLME, the extraction time is defined as an interval time between the injection of the mixture of the dispersing solvent (ACN) and the extracting solvent (chlorobenzene) to the aqueous sample and the beginning of centrifugation. For the evaluation of this factor, extraction time ranging from 0–30 min was examined. It was revealed that there was no significant effect of extraction time on the extraction efficiency. The reason was that the equilibrium state was achieved promptly. A very short extraction time is also the remarkable advantage of DLLME technique.

3.2.8. Effect of ionic strength

There may be some effects on the extraction efficiency created by the salt addition. To investigate the impact of the ionic strength on the performance of DLLME, different NaCl contents (0–5%, w/v) were tested to find the optimum amount of salt addition. It turned out that with the increase of the NaCl amount, the separation efficiency decreased dramatically. On the other hand, without salt addition, high EF values and good peak shapes were achieved. Therefore, all the extraction experiments were carried out with no addition of salt.

3.3. Evaluation of the DLLME method

3.3.1. Features of DLLME method

A series of working solutions containing each of 2,4-DB, dicamba and 2,4-D at eight concentration levels of 5.0, 10.0, 20.0, 50.0, 100.0, 200.0, 500.0 and 1000.0 ng mL^{-1} were used for the determination of the calibration curves. For each level, the solutions were pretreated with the aid of DLLME procedure as described in Section 2.4. Under the optimum extraction and separation conditions, linear ranges, linear equations, correlation coefficients, limits of detection (LODs), limits of quantification (LOQs) and EFs of the DLLME method in couple with MEKC for the three PAs were obtained and displayed in Table 1.

Table 1. Linear range, regression data, concentration limits and enrichment factor for the PAs.

PAs	Linear range (ng mL^{-1})	a (RSD [a], %)	b (RSD, %)	Correlation coefficient □R□	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	Enrichment factor
2,4-DB	10.0–1000.0	+19.49(3.4)	+12.16(4.2)	0.9995	1.65	5.50	180
Dicamba	10.0–1000.0	+16.86(3.6)	-1.66(6.7)	0.9998	1.91	6.37	151
2,4-D	10.0–1000.0	+20.60(1.9)	-267.04(1.2)	0.9993	1.56	5.20	216

Calibration equation: A (Peak area) = ac (Concentration, ng mL^{-1}) + b.
[a]Relative standard deviation, n = 3.

Table 2. Intra-day and inter-day precision of migration time and peak area for the DLLME-MEKC determination of PAs.

PAs	Spiked (ng mL^{-1})	RSD (%)			
		Intra-day (n = 6)		Inter-day (n = 6)	
		Migration time	Peak area	Migration time	Peak area
2,4-DB	50	0.35	4.77	2.04	6.92
	1000	0.22	3.88	1.13	4.67
Dicamba	50	0.42	5.16	1.11	7.64
	1000	0.23	6.39	1.02	8.62
2,4-D	50	0.41	6.08	1.49	5.47
	1000	0.32	4.75	0.72	7.10

As can be seen, it observed good linearity for all the three PAs, with the correlation coefficients (R) over 0.9993. The LODs for 2,4-DB, dicamba and 2,4-D, calculated as the analyte concentration for which the peak height was three times the background noise (S/N=3), were obtained as 1.65, 1.91 and 1.56 ng mL^{-1}, respectively. Moreover, on the basis of the peak height being ten times the background noise (S/N=10), the LOQs of 2,4-DB, dicamba and 2,4-D were 5.50, 6.37 and 5.20 ng mL^{-1} , respectively (Table 1). Although the presented LOD for 2,4-D is higher than that reported (0.16 ng mL^{-1}) [22], the LODs for all the three PAs are lower than the MCLs formulated by Taiwan EPA drinking water quality standards and US drinking water regulations. Meanwhile, the LOQs for all the three PAs are also lower than the MCLs formulated by Taiwan EPA drinking water quality standards and US drinking water regulations. Thus, this developed method could meet the requirements of MCLs detection of PAs [5].

On the other hand, the intra-day and inter-day precisions in terms of migration time and peak area obtained on the basis of 6 consecutive injections were shown in Table 2. As can be seen, the RSDs of migration time and peak area obtained of a working solution containing each of the three PAs at a concentration level of 1000 ng mL^{-1} based on intra-day precision were less than 0.32% and 6.39%, respectively, while the inter-day remained under 1.13% and 8.62%, respectively. Meanwhile, for a lower concentration level of 50 ng mL^{-1}, the RSDs of migration time and peak area based on intra-day precision were below 0.42% and 6.08%, and the inter-day precisions were less than 2.04% and 7.64%, respectively. Moreover, it is found that the intra-day and inter-day precisions for 2,4-D are lower than that reported (7.08%) [22]. It revealed that this DLLME-MEKC method was potentially applicable for the accurately quantitative determination of the three PAs.

3.3.2. Analysis of real environmental water samples

Three kinds of practical water samples of lake, river and reservoir water were analyzed using the developed method. Recoveries at three concentration levels of 20.0, 50.0, 100.0 ng.mL^{-1} were obtained and shown in Table 3. Meanwhile, electropherograms achieved for the three water samples with and without standards addition after DLLME were displayed in Fig. 4. As can be seen, the concentration of dicamba determined was 14.94 ng mL^{-1} in lake water and 2,4-D of 12.87 ng mL^{-1} in river water. Recoveries varied from 67.91 to 119.07%, with RSD in the range of 1.47–6.89%. These results indicated that the DLLME-MEKC had the feasibility for the determination of PAs in water samples.

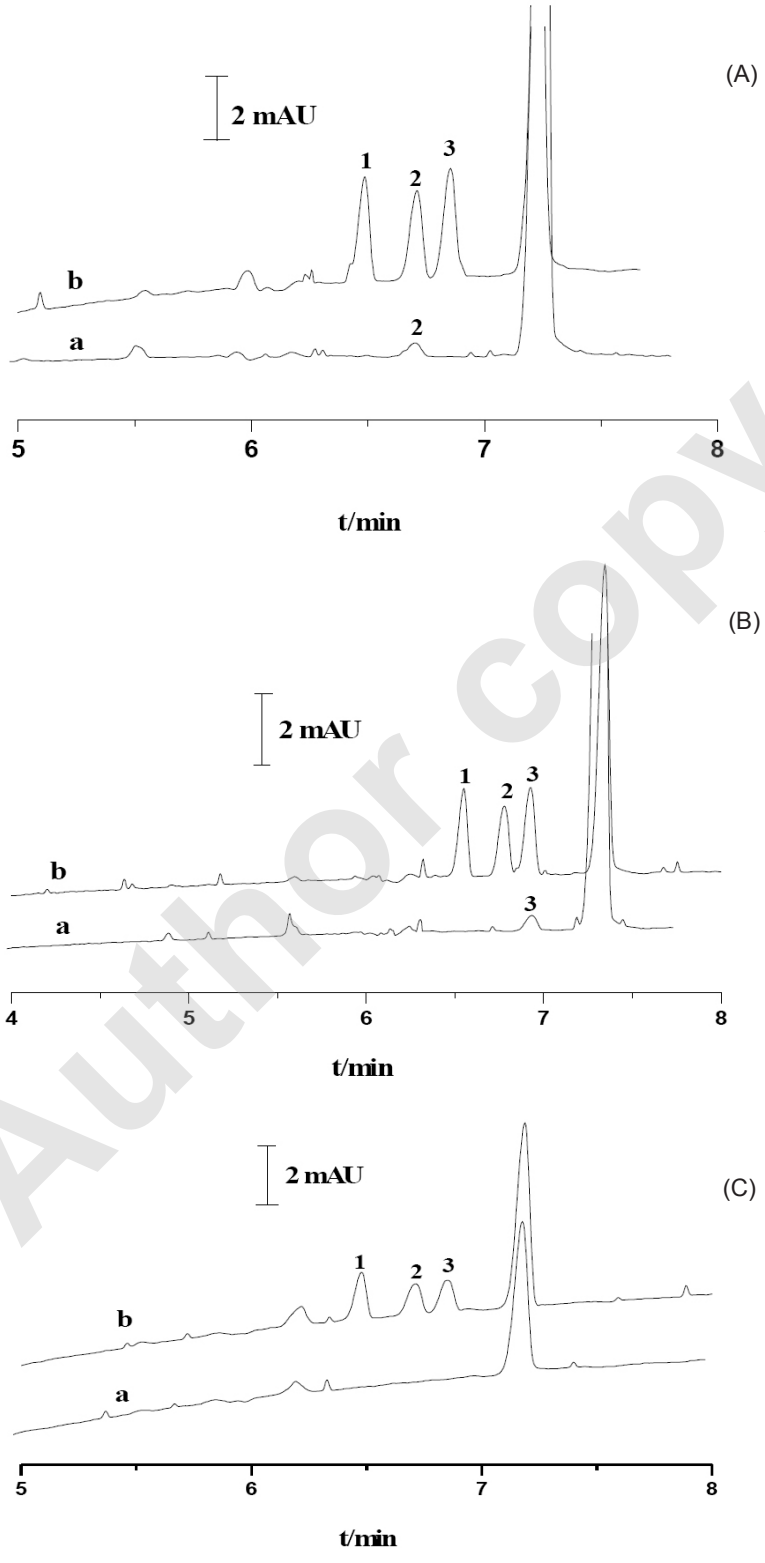

Figure 4. Typical electropherograms of (A) lake water (B) river water and (C) reservoir water samples without (a) and with (b) standards addition after DLLME extraction under the optimum conditions. The spiked concentration of PAs standards was 100.0 ng mL^{-1}. Peaks: 1: 2,4-DB; 2: Dicamba; 3: 2,4-D.

Table 3. Determination results of PAs and method recoveries in real water samples.

PAs	Spiked	Lake water		River water		Reservoir water	
	(ng mL^{-1})	Found (ng mL^{-1})	Recovery (%±RSD, n =3)	Found (ng mL^{-1})	Recovery (%±RSD, n =3)	Found (ng mL^{-1})	Recovery (%±RSD, n =3)
2,4-DB	0	nd[a)]		nd		nd	
	20.0	18.48	92.40±3.45	13.58	67.91±6.14	16.82	84.10±4.12
	50.0	50.87	101.76±2.13	47.25	94.49±3.41	51.19	102.39±3.73
	100.0	92.81	92.81±3.28	108.28	108.28±3.17	89.52	89.52±3.04
Dicamba	0	14.94		nd		nd	
	20.0	16.75	83.77±6.89	18.88	94.40±4.13	18.71	93.55±2.96
	50.0	50.27	100.54±4.78	50.72	101.45±1.98	47.61	95.22±1.47
	100.0	92.85	92.85±2.56	96.09	96.09±2.55	97.90	97.90±1.73
2,4-D	0	nd		12.87		nd	
	20.0	18.12	90.59±4.77	17.27	86.34±5.91	23.81	119.07±5.08
	50.0	49.75	99.50±3.01	44.57	89.15±4.73	19.82	99.10±4.33
	100.0	106.61	106.61±2.71	88.58	88.58±3.29	92.67	92.67±4.59

a) Not detected.

4. Conclusion

This work has developed and evaluated a simple, rapid and efficient DLLME sample pretreatment coupled with MEKC separation method for the simultaneous separation and determination of 2,4-DB, dicamba and 2,4-D in environmental water samples. As far as we know, this is the first time that PAs are analyzed by DLLME-MEKC. It has provided a method of high EF and good recovery with a short analysis time. In addition, the low consumption of organic solvent helped to achieve an environmentally benign approach. All the analytical results confirm that it is feasible to conduct routine analysis of PAs in water samples. More work on the applications of DLLME-MEKC for simultaneous separation and determination of a higher number of PAs and improving the method sensitivity is currently in progress.

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