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Research Article

Dispersive liquid–liquid microextraction coupled with capillary electrophoresis for simultaneous determination of sulfonamides with the aid of experimental design

A novel method for the simultaneous determination of sulfonamides (SAs) in water samples has been developed by using dispersive liquid–liquid microextraction (DLLME) coupled with CE. Orthogonal and Box–Behnken designs were employed together to assist the optimization of DLLME parameters, including volumes of extraction and disperser solvents, ionic strength, extraction time, and centrifugation time and speed as variable factors. Under the optimum extraction and detection conditions, successful separation of the five SAs was achieved within 5 min, and excellent analytical performances were attained, such as good linear relationships ($R > 0.980$) between peak area and concentration for each SA from 0.5 to 50 μg/mL, low limits of detection for the five SAs between 0.020 and 0.570 μg/mL and the intra-day precisions of migration time below 0.80%. The method recoveries obtained at fortified 10 μg/mL for three water samples ranged from 53.6 to 94.0% with precisions of 1.23–5.60%. The proposed method proved highly sensitive and selective, rapid, convenient and cost-effective, showing great potential for the simultaneous determination of SAs in water samples.

Keywords:
CE / Dispersive liquid–liquid microextraction / Experimental design / Sulfonamides / Water sample
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1 Introduction

Sulfonamides (SAs), also known as 'sulfa drugs', derived from sulfanilamide (*p*-aminobenzenesulfonamide) are widely used in both veterinary and human medicine [1] for the treatment of many human and animal diseases, such as infectious diseases of digestive and respiratory tracts. Their metabolites have been often found in aquatic environment, which are likely due to the inadequate treatment of human and animal excretions or insufficient waste disposal measures. They can be present in the environment (soils, ground and surface waters) for a long period of time, leading to the appearance of antimicrobial resistance [2]. It is therefore important to develop efficient methods for separation and determination of SAs.

So far, various detection methods including HPLC [3, 4], CE [5–7], gas chromatography–mass spectrometry (GC-MS) [8] and electrochemical methods [9, 10] have been widely used for analysis of SAs. And, more remarkable, numerous classic extraction methods such as dissolution [11, 12], liquid–liquid extraction (LLE) [13], solid-phase extraction (SPE) [14] and LLE followed by SPE [15] have also been developed for sample treatment of SAs. Nowadays, off-line methods such as hollow fiber renewal liquid membrane extraction [4] and molecularly imprinted polymer extraction [16], and on-line methods such as on-fiber derivatization and large-volume sample stacking of CE [6] are also used to obtain high-sensitive determination of SAs. In 2011, selective extraction of SAs from water samples was realized by dispersive liquid–liquid microextraction (DLLME) using

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Abbreviations: DLLME, dispersive liquid–liquid microextraction; **SAs,** sulfonamides; **SDM,** sulfadinidin; **SDX,** sulfadoxin; **SDZ,** sulfadiazine; **SMR,** sulfamerazin; **SPD,** sulfapyridine

Colour Online: See the article online to view Fig. 1 in colour.

functionalized ionic liquids [17]. The method of DLLME was first proposed in 2006 by Rezaee et al. [18] and receives increasing attentions based on its advantages of simplicity of operation, rapidity, low cost, high-recovery, high enrichment factor and environmental benignity, with wide application prospects in trace analysis [19].

DLLME is a novel miniaturized sample pre-treatment technique which requires smaller amounts of organic solvents and reduces the analysis cost more compared with other methods, such as LLE and SPE which have involved drawbacks (e.g. complicated, time-consuming procedures, large amounts of sample and organic solvents and difficulty in automation). In DLLME, the appropriate mixture of extraction and disperser solvents is rapidly injected by syringe into an aqueous sample containing the analytes of interest. The fine particle of extracting solvent, which is dispersed into aqueous phase, allows its interaction with the analyte. In the latest known SA determination [17], functionalized ionic liquids were investigated as extraction solvents, and single factor alternate method was employed for the optimization of extraction conditions. All the analyses were carried out using a LC-20A liquid chromatograph with UV detector. However, DLLME coupled with CE has not been reported for the determination of SAs.

One of the most important objectives of modern analytical chemistry is miniaturization, simplification and automation of the whole analytical procedure, especially to speed up sample treatment, which is currently the bottleneck of analysis. Although traditional solvent extraction has been used for many years as the basic and powerful method of concentration, it requires large amounts of organic solvents, easily causing pollution. And some phase extraction methods used are time and elution chemicals consumptive, usually including several procedures which cost much time [20, 21]. Petersson et al. [20] successfully developed a miniaturized on-line SPE method to enhance the concentration sensitivity in CE for terbutaline, but the on-line connection needs several time-consuming steps. Guzman [21] tactfully designed a solid-phase microextraction (SPME) device in the form of a four-part cross-shaped configuration, including a large-bore tube to transport samples and washing buffers and a small-bore fused-silica capillary for the separation of analytes. But these methods need construction of the enrichment capillary including washing, wetting, conditioning, sorption, washing, filling and desorption which takes much time. For smaller id capillaries about this method, containing longer packed sections of the analyte concentrator, it may take up to more elution chemicals and 1 h or more to complete the entire process [21]. On the other hand, the designs of the analyte concentrator are also time-consuming. Therefore, DLLME as one simpler and faster extraction method receives special attentions. The whole process of DLLME can be schematically illustrated in Fig. 1. Briefly, a cloudy solution is formed when an appropriate mixture of extraction and dispersive solvents is injected into an aqueous sample containing the analytes of interest. The extraction solvent must be a high-density water-immiscible solvent, such as chlorobenzene, carbon tetrachloride and tetrachloroethylene, whereas the disperser solvent must be a water miscible, polar solvent, such as acetone, methanol and ACN. After centrifugation, extraction solvent is normally sedimented at the bottom of the tube and taken with a microsyringe for its later analysis like HPLC [22, 23], GC [24, 25], GC-MS [26], electrothermal atomic absorption spectrometry (ETAAS) [27, 28] and flame atomic absorption spectrometry (FAAS) [29, 30]. Dadfarnia et al. [31], Herrera-Herrera et al. [32] and Sarafraz-Yazdi et al. [33] have already reviewed the theory and application of DLLME in the determination of various analytes.

Compared with other extraction methods, there are more conditions to be optimized, such as types and volumes of extraction and disperser solvents, ionic strength and sample pH, and extraction and centrifugation time, all of which can significantly affect the extraction efficiency. On the other hand, in many cases, it is difficult to quickly find suitable extraction conditions for a given task. To solve the

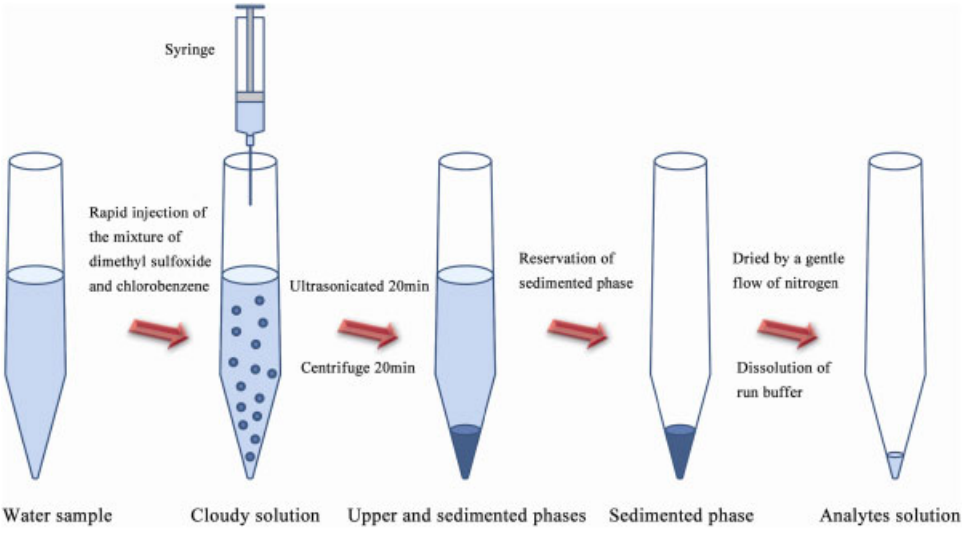

Figure 1. Scheme of the DLLME procedure.

problem, various types of experimental designs have been employed, such as Plackett–Burman design [34], central composite design [35], orthogonal design [36] and Box–Behnken design [37]. Lately in 2010, experimental design was adopted to optimize the DLLME conditions and then followed by nonaqueous CE procedure for the determination of fluoroquinolone antibiotics in waters [38].

In this work, orthogonal design was used to assist to find the major extraction factors as well as Box–Behnken design for the optimum extraction conditions of DLLME for five SAs. For the first time, the experimental design assisted DLLME coupled with CE method was developed and successfully applied for simultaneous determination of the several SAs in water samples.

2 Materials and methods

2.1 Chemicals and samples

Five SAs standards of sulfapyridine (SPD), sulfadimidin (SDM), sulfadoxin (SDX), sulfadiazine (SDZ) and sulfamerazin (SMR) were purchased from Sigma-Aldrich (Steinheim, Germany), and their structures are shown in Fig. 2. Chromatographic grade ACN, dimethyl sulfoxide (DMSO) and chlorobenzene were purchased from J&K Chemical (Beijing, China). Milli-Q water was used throughout the work. All chemicals such as sodium dihydrogen phosphate, phosphoric acid and sodium hydroxide were all of analytical grade.

Standard stock solutions containing 1000 μg/mL of each SA were prepared by dissolving the required amounts of the standard in DMSO. They were stored in a refrigerator at 4°C. Less concentrated standard solutions were prepared from the stock solutions by dilution with Milli-Q water.

Lake water was collected from an artificial lake located in Laishan District of Yantai City (China); pond water was collected near freshwater fisheries in Yantai. Lake water and pond water were stored in the dark at 4°C for use. Tap water was obtained in the laboratory when needed. Before use, the samples were passed through microporous nylon filters with the pore sizes of 0.45 μm in diameter. Several aliquots from 5 mL filtered water samples were spiked with the SA standard with different concentrations and followed by the DLLME procedure.

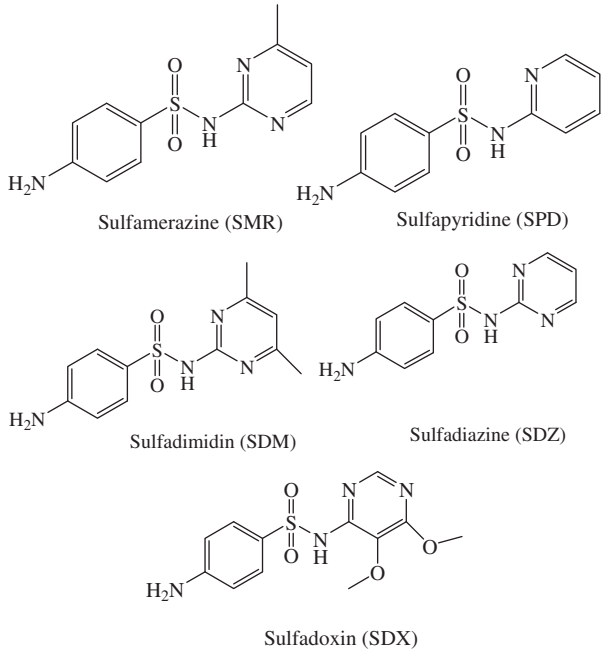

Figure 2. Molecular structures of the SAs analyzed in this work.

2.2 Apparatus and software

All experiments were performed on a P/ACE MDQ CE system (Beckman Coulter, Fullerton, CA, USA) in conjunction with a diode-array detector (DAD) monitoring at 254 nm. Separation was performed at 25°C, using an applied voltage of 25 kV for 5 min. The run buffer was prepared by freshly mixing 20 mM sodium dihydrogen phosphate and 10% ACN adjusted to pH 8.5 with 1 M phosphoric acid. Bare fused-silica capillaries (Yongnian Photoconductive Fiber Factory, Hebei, China) were used for SAs separations, with 75 μm id, 375 μm od, total length of 50.2 cm and effective length of 40 cm. An Ion 510 pH meter (Ayer Rajah Crescent, Singapore) was used to monitor pH adjustment. New capillaries were initialized by flushing with water (10 min), 1.0 M NaOH (40 min), water (10 min) and run buffer (30 min) before use. Between analyses the capillary was rinsed with run buffer (2 min). All the samples were passed through microporous nylon filters of 0.45 μm pore sizes in diameter.

SPSS software was used to construct the regression analysis of standard peak-area against conditions of DLLME. Lingo software was used to obtain the optimum conditions of DLLME.

2.3 DLLME procedure

For the DLLME, 5.00 mL of aqueous sample was placed in a 10-mL of screw cap glass test tube with conical bottom and spiked with five SAs individual at 10 μg/mL. Eight hundred microliters of DMSO (as disperser solvent) containing 400 μL chlorobenzene (as extraction solvent) was rapidly injected into the sample solution with a 2.00-mL glass syringe and the mixture was shaken gently and then ultrasonicated for 20 min. In this step, a cloudy solution was formed and the SAs in the water samples were extracted into fine droplets. Then, the mixture was centrifuged for 20 min at 2500 rpm. Finally, the sedimented phase (chlorobenzene) was dried under a gentle flow of nitrogen. And the residue was dissolved using 50 μL of run buffer for CE analysis. The extraction steps are illustrated in Fig. 1.

3 Results and discussion

3.1 Preliminary experiments

Several factors that have major influences on the DLLME efficiency have been analyzed in many publications [22–30]. During a series of experiments, preliminary attempts were made to identify those factors having the most significant influence on the extraction efficiency of DLLME in order to evaluate them more thoroughly later in an experimental design. In the experiments, six factors were selected, including the volumes of extraction solvent (V_{ext}, μL) and disperser solvent (V_{dis}, μL), ionic strength (NaCl, %), extraction time (t_{ext}, min), and centrifugation time (t_{cen}, min) and speed (r_{cen}, rpm).

The first step in the optimization procedure was to select an appropriate extraction solvent. As dispersive solvents, acetone, methanol and ethanol were usually tested. But for SAs, their solubility in these solvents was very small. Therefore, DMSO was selected as the disperser solvent based on the rule of similarity. Chloroform, carbon tetrachloride and chlorobenzene were often used as extraction solvents. For chloroform, a cloudy solution was not formed after injected with the disperser into the sample solution. And for carbon tetrachloride, the extraction efficiency was very low. Therefore, the results have demonstrated that DMSO as dispersive solvent and chlorobenzene as extraction solvent displayed the highest extraction efficiencies of the SAs in combination with cleaning-up the extracts.

The effect of the ionic strength on the extraction performance was assessed with samples containing five different concentrations of sodium chloride (0–5%, w/v). Finally, the run of CE cannot obtain any obvious fine peak and baseline separation of the SAs. Therefore, the concentration of sodium chloride was 0.

3.2 Orthogonal design

A four-level five-factor orthogonal design was built for the determination of the main factors affecting the extraction efficiency and a total of 16 experiments were performed as shown in Table 1. And the obtained peak areas of the five SAs were also listed in Table 1. The aim was to find which variable had predominant influence on peak area of SAs (A). The dependence of A on a certain parameter can be expressed by the following formula:

$$\Delta A = \frac{(\sum A)_{max}}{N} - \frac{(\sum A)_{min}}{N} \quad (1)$$

Summation of the first term is the maximum value of A with the labels (1), (2), (3) and (4) (as shown in Table 1), and the second summation term is the minimum value, while N

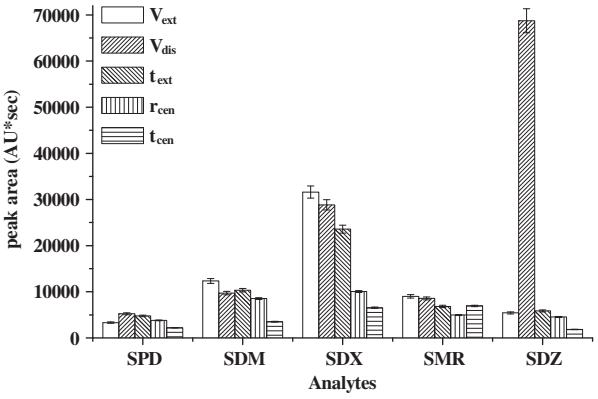

Figure 3. Dependence of peak area on each parameter influencing DLLME efficiency. The SAs standards were at 10 μg/mL. Optimum CE separation conditions: run buffer, 20 mM sodium dihydrogen phosphate (pH 8.5) and 10% ACN; applied voltage, 25 kV; wavelength, 254 nm.

Table 1. Experimental design chart including five variables for four levels orthogonal design and peak area of the five SAs

Experimental no.	V_{ext} (μL)	V_{dis} (μL)	t_{ext} (min)	r_{cen} (rmp)	t_{cen} (min)	A_{SPD} (AU × s)	A_{SDM} (AU × sec)	A_{SDX} (AU × s)	A_{SMR} (AU × s)	A_{SDZ} (AU × s)
1	200 (1)	600 (1)	10 (1)	1500 (1)	5 (1)	9088	17 971	48 847	17 349	14 774
2	200 (1)	700 (2)	20 (2)	2000 (2)	10 (2)	6453	13 504	37 864	12 259	10 004
3	200 (1)	800 (3)	30 (3)	2500 (3)	20 (3)	6711	12 971	38 362	12 063	9876
4	200 (1)	900 (4)	40 (4)	3000 (4)	30 (4)	4540	10 075	29 061	10 392	8659
5	300 (2)	600 (1)	20 (2)	2500 (3)	30 (4)	10 084	20 414	49 424	18 503	15 317
6	300 (2)	700 (2)	10 (1)	3000 (4)	20 (3)	6567	14 047	45 742	12 979	10 465
7	300 (2)	800 (3)	40 (4)	1500 (1)	10 (2)	8251	17 680	58 258	16 790	13 759
8	300 (2)	900 (4)	30 (3)	2000 (2)	5 (1)	3559	12 150	37 880	11 531	9243
9	400 (3)	600 (1)	30 (3)	3000 (4)	10 (2)	6283	21 924	62 543	17 226	11 807
10	400 (3)	700 (2)	40 (4)	2500 (3)	5 (1)	11 438	39 162	93 982	22 886	16 640
11	400 (3)	800 (3)	10 (1)	2000 (2)	30 (4)	7904	21 949	61 778	18 937	13 145
12	400 (3)	900 (4)	20 (2)	1500 (1)	20 (3)	3938	10 568	35 709	97 70	6953
13	500 (4)	600 (1)	40 (4)	2000 (2)	20 (3)	17 756	35 335	89 104	32 358	27 151
14	500 (4)	700 (2)	30 (3)	1500 (1)	30 (4)	6314	28 283	89 442	21 701	13 857
15	500 (4)	800 (3)	20 (2)	3000 (4)	5 (1)	5843	16 333	53 094	14 604	10 401
16	500 (4)	900 (4)	10 (1)	2500 (3)	10 (2)	10 194	23 947	48 969	19 425	13 672

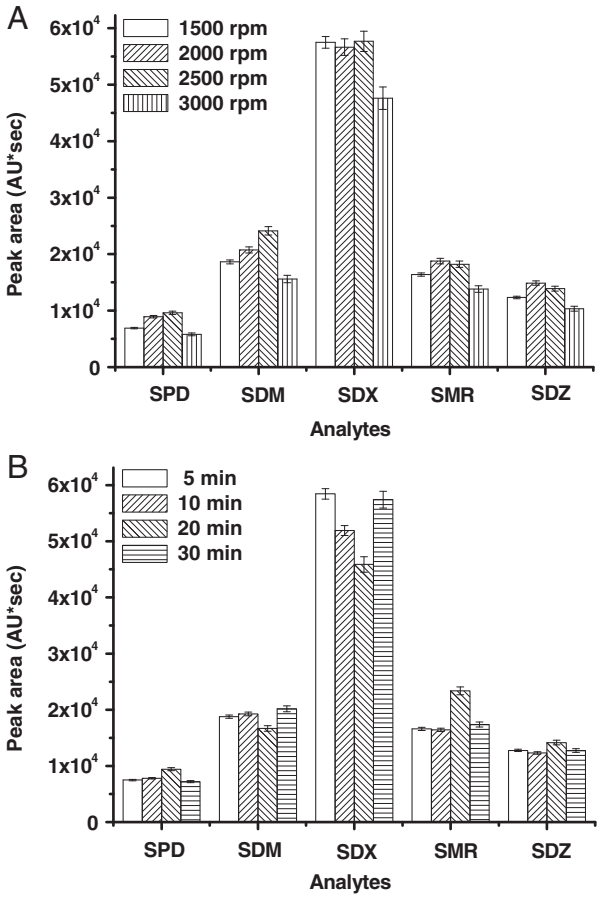

Figure 4. Dependence of peak area on (A) centrifugation speed and (B) centrifugation time. The SAs standards were at 10 μg/mL. The CE separation conditions were the same as those described in Fig. 3.

is the number of levels of each parameter. For each variable, the level that gives the maximum sum of A, i.e. $(\sum A)_{max}$, is its optimum operation conditions [39]. The dependence of A on each parameter is shown in Fig. 3. From the figure, it can be seen the volumes of extraction and disperser solvents and extraction time are the most important three factors affecting the extraction efficiency of SMR, SDM, SDX and SDZ except SPD. Although the first three factors affecting the extraction efficiency of SPD were the volume of disperser solvent, extraction time and centrifugation speed, the influences of centrifugation speed and the volume of extraction solvent were more or less. In order to simultaneously extract the five SAs, the volumes of extraction and disperser solvents and extraction time were selected as the factors in the next step. According to the obtained results of orthogonal design, centrifugation time and speed had no significant impact on the extraction efficiency. The best conditions of the two factors for the extraction of the five SAs were shown in Fig. 4. Although the conditions were not identical, the highest extraction efficiency can be obtained for most of the SAs at 2500 rpm for 20 min. Therefore, 2500 rpm and 20 min were selected as the centrifugation speed and time, respectively.

Finally, the factors that were considered in Box–Behnken design were volumes of disperser (DMSO) and extraction solvent (chlorobenzene) and extraction time.

3.3 Box–Behnken design

The next step in our research was to optimize the analytical method according to the chosen factors by the employment

Table 2. Box–Behnken design chart including factors, levels and matrix with three factors

Variable		Factor		Level		
			−1	0	+1
X_1		V_{ext} (μL)	200	300	400
X_2		V_{dis} (μL)	600	700	800
X_3		t_{ext} (min)	20	30	40

Experimental no.	X_1	X_2	X_3	A_{SPD} (AU × s)	A_{SDM} (AU × s)	A_{SDX} (AU × s)	A_{SMR} (AU × s)	A_{SDZ} (AU × s)
1	−1	−1	0	10 443	9232	28 322	7346	4828
2	−1	1	0	8844	9643	30 543	9118	6865
3	1	−1	0	10 223	28 107	76 808	23 656	16 206
4	1	1	0	6394	18 225	51 373	14 635	9960
5	−1	0	−1	5735	13 448	38 416	11 944	9245
6	−1	0	1	3618	12 085	43 185	10 241	7350
7	1	0	−1	16 882	40 011	111 944	37 622	30 431
8	1	0	1	7689	27 217	100 282	20 159	13 324
9	0	−1	−1	8613	21 598	72 163	18 133	14 056
10	0	−1	1	6167	17 475	60 183	14 547	9652
11	0	1	1	5346	16 515	58 724	14 004	10 556
12	0	1	1	4255	15 818	57 104	10 833	7847
13	0	0	0	5952	16 537	57 167	13 541	9586
14	0	0	0	6817	20 015	61 858	15 979	11 007
15	0	0	0	5278	18 968	72 213	13 003	9178

of a Box–Behnken design. The low, medium and high levels of each variable were coded as −1, 0 and +1, respectively, shown in Table 2. The Box–Behnken design matrix, i.e. the extraction conditions for each of the 15 experimental runs, and the *A* values of five SAs are also shown in Table 2. The optimum extraction conditions of SAs obtained from Table 2 and Lingo software are that all of the SAs can obtain the highest extraction efficiency when using 400 μL chlorobenzene and 800 μL DMSO to extract the SAs for 20 min except SPD using 200 μL chlorobenzene. For simultaneous extraction of the five SAs, the optimum separation conditions are obtained as follows: 400 μL of chlorobenzene, 800 μL of DMSO, 20 min of extraction time and 2500 rpm of centrifugation speed for 20 min. The typical electropherograms before and after DLLME are shown in Fig. 5, in which all the five SAs were baseline-separated within 5 min, except that SDX and SMR were not completely separated after DLLME. Peak area value was attained by automatic integration, which might eliminate the systematic and accidental errors especially for the incomplete separation of SDX and SMR. We believe the quantification of SDX and SMR is acceptable. Data analysis permitted to obtain regressions of peak area (*A*) to factors for each SA is given below in Supporting Information Table S1. From the statistic data (*R* and *F* value), it therefore concludes that the final models are considered to be satisfactory.

3.4 Evaluation and application of the method

Method performance of the optimized DLLME was evaluated by CE. The method was applied to several water samples from lake, pond and tap. Before the spiking procedure, the samples were analyzed and were found to be free of SAs contamination. Typical electropherograms of the three water samples before and after DLLME are shown in Fig. 6. Linear correlation coefficients (*R*) assessed using samples fortified at six different concentration levels were obtained between peak area, and the corresponding concentrations of SAs in the range from 0.5 to 50 μg/mL were listed in Table 3. Limits of detection (LODs) for all the five SAs in tap, lake and pond water samples, calculated as the analyte concentration for which the peak height was three times the background noise (3 S/N), were attained within 0.038–0.570, 0.020–0.266 and 0.035–0.485 μg/mL, respectively (Table 3). The recoveries obtained in different samples spiked at 10 μg/mL were from 53.6 to 94.0% with the relative standard deviations (RSDs) of 1.23–5.60%

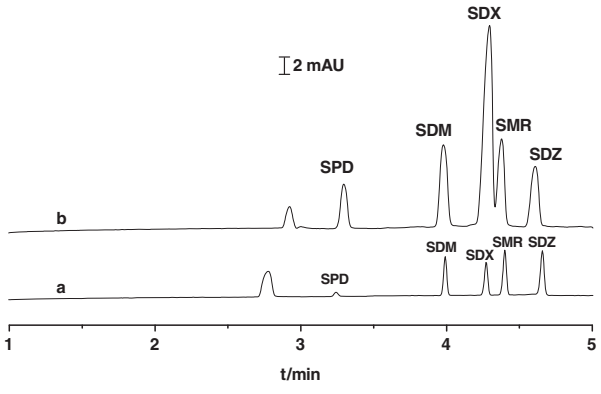

Figure 5. Typical electropherograms of SAs standards at 10 μg/mL (a) before and (b) after DLLME. The CE separation conditions were the same as those described in Fig. 3.

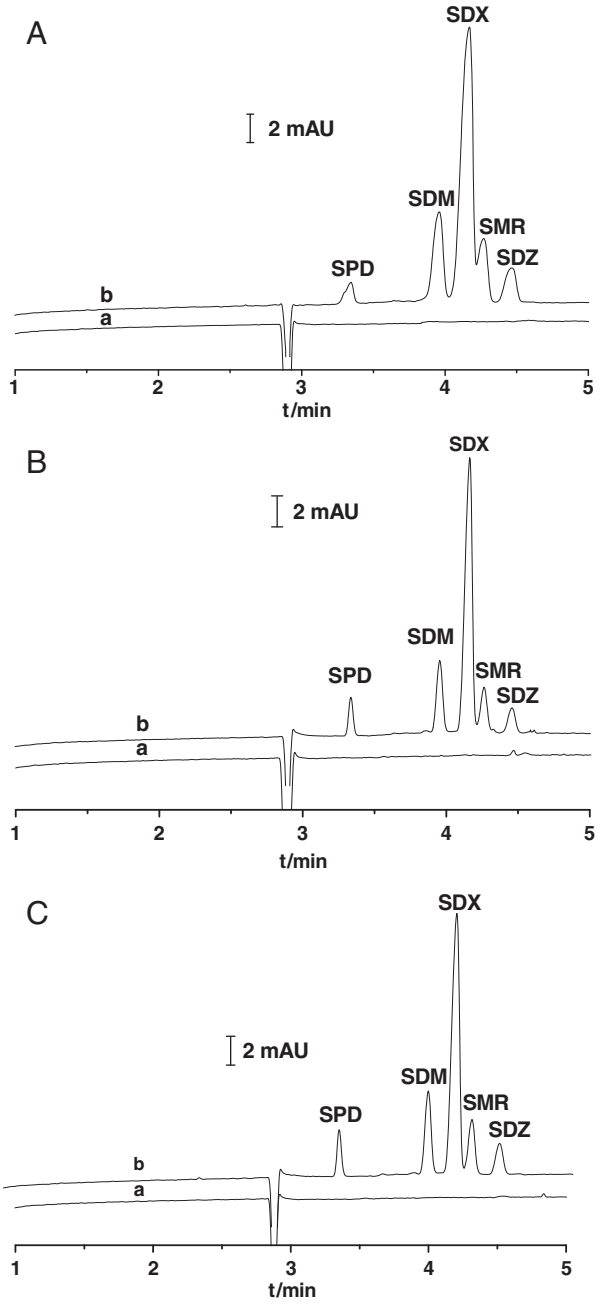

Figure 6. Typical electropherograms of (a) blank and (b) spiked water samples after DLLME. (A) Tap water (B) Lake water and (C) Pond water. The spiked concentration of SAs standards was 10 μg/mL. The CE separation conditions were the same as those described in Fig. 3.

Table 3. Linear relations and detection limits of SAs for tap, lake and pond water samples

Sample	SAs	Linear range (μg/mL)	Slope ± error	Interception	*R*	LOD (μg/mL)
Tap water	SPD	0.5–50	362.8 ± 2.8	−312.5	0.999	0.038
	SDM	0.5–50	737.3 ± 7.2	−990.8	0.989	0.202
	SDX	0.5–50	371.6 ± 2.7	−585.2	0.998	0.042
	SMR	0.5–50	401.5 ± 10.4	−539.3	0.991	0.387
	SDZ	0.5–50	270.7 ± 14.8	−263.0	0.996	0.570
Lake water	SPD	0.5–50	800.3 ± 8.0	−1074	0.997	0.020
	SDM	0.5–50	936.3 ± 12.3	−857.3	0.990	0.164
	SDX	0.5–50	493.1 ± 3.3	−203.0	0.987	0.033
	SMR	0.5–50	693.6 ± 5.1	−612.8	0.999	0.207
	SDZ	0.5–50	612.0 ± 4.2	−710.9	0.990	0.266
Pond water	SPD	0.5–50	308.6 ± 2.9	+209.7	0.997	0.035
	SDM	0.5–50	549.4 ± 6.9	+485.1	0.999	0.238
	SDX	0.5–50	222.2 ± 2.0	+540.7	0.989	0.064
	SMR	0.5–50	303.2 ± 3.5	+577.1	0.983	0.412
	SDZ	0.5–50	325.0 ± 2.6	−117.7	0.994	0.485

Table 4. Method recoveries for SAs in tap, lake and pond water samples

SAs	Tap water		Pond water		Lake water	
	Recovery[a)] (%)	RSD[a)] (%)	Recovery[a)] (%)	RSD[a)] (%)	Recovery[a)] (%)	RSD[a)] (%)
SPD	84.8	1.42	82.4	2.10	77.6	2.90
SDM	64.4	3.29	66.4	1.39	54.5	3.28
SDX	89.6	1.74	77.5	4.03	86.7	3.28
SMR	63.3	5.60	57.4	1.23	94.0	3.42
SDZ	93.5	2.19	80.4	4.40	53.6	4.84

a) $n = 6$.

Table 5. Intra-day and inter-day precision of migration time and peak area for the DLLME-CE determination of SAs[a)]

SAs	RSD (%)			
	Intra-day ($n = 6$)		Inter-day ($n = 6$)	
	Migration time	Peak area	Migration time	Peak area
SPD	0.47	2.04	2.49	6.17
SDM	0.62	4.02	3.36	5.43
SDX	0.74	4.17	3.72	10.08
SMR	0.72	5.09	3.78	7.68
SDZ	0.80	6.98	3.99	11.15

a) Spiking 10 μg/mL.

(Table 4). On the other hand, the RSDs obtained under repeatability (intra-day precision) conditions in terms of migration time and peak area were less than 0.80 and 6.98%, respectively, while under reproducibility (inter-day precision) conditions remained under 3.99 and 11.15% (Table 5), respectively. The method was demonstrated potentially applicable for the simultaneous separation and determination of five SAs in water samples.

4 Concluding remarks

The proposed DLLME with the aid of experimental design coupled to CE method was demonstrated a simple, fast and economic option for simultaneous determination of SAs in water samples compared with some reported methods of SPE, LLE and SPME. To the best of our knowledge, this is the first time that SAs are analyzed from water samples by the coupling of experimental design assisted DLLME with CE-UV. The method performance was mainly influenced by the volumes of extractant and disperser solvent, and the extraction time. Precision, LODs and linearity obtained under optimized conditions were suitable for the determination of SAs in surface water samples. The combination of experimental design was found to be a powerful tool for optimizing the best extraction conditions from a small number of experiments. Further exploration into the experimental design and DLLME for high-efficient sample treatment prior to CE-UV detection of SAs and/or other typical environmental pollutants will be performed.

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The authors have declared no conflict of interest.

5 References

[1] Evanthia, P. T., Victoria, F. S., Ioannis, N. P., *Curr. Pharm. Anal.* 2010, *6*, 198–212.

[2] Díaz-Cruz, M. S., López de Alda, M. J., Barceló, D., *Trends Anal. Chem.* 2003, *22*, 340–351.

[3] Costi, E. M., Sicilia, M. D., Rubio, S., *J. Chromatogr. A* 2010, *1217*, 6250–6257.

[4] Bedendo, G. C., Jardim, I. C. S. F., Carasek, E., *J. Chromatogr. A* 2010, *1217*, 6449–6454.

[5] Soto-Chinchilla, J. J., García-Campana, A. M., Gámiz-Gracia, L., *Electrophoresis* 2007, *28*, 4164–4172.

[6] Soto-Chinchilla, J. J., García-Campa, A. M., Gámiz-Gracia, L., Cruces-Blanco, C., *Electrophoresis* 2006, *27*, 4060–4068.

[7] Lin, Y. T., Liu, Y. W., Cheng, Y. J., Huang, H. Y., *Electrophoresis* 2010, *31*, 2260–2266.

[8] Stanway, S. J., Purohit, A., Reed, M. J., *Anticancer Res.* 2007, *27*, 2765–2767.

[9] Msagati, T. A. M., Ngila, J. C., *Talanta* 2002, *58*, 605–610.

[10] Campestrini, I., de Braga, O. C., Vieira, I. C., Spinelli, A., *Electrochim. Acta* 2010, *55*, 4970–4975.

[11] Caballero, R. D., Torres-Lapasió, J. R., Baeza-Baeza, J. J., García-Alvarez-Coque, M. C., *J. Liq. Chrom. Rel. Technol.* 2001, *24*, 117–131.

[12] Vinas, P., Erroz, C. L., Canals, A. H., Córdoba, M. H., *Chromatographia* 1995, *40*, 382–386.

[13] Schwaiger, I., Schuch, R., *Dtsch. Lebensm.-Rundsch* 2000, *96*, 93–98.

[14] Posyniak, A., Sniegocki, T., Zmudzki, J., *Bull. Vet. Inst. Pulawy* 2002, *46*, 111–117.

[15] Maudens, K. E., Zhang, G. F., Lambert, W. E., *J. Chromatogr. A* 2004, *1047*, 85–92.

[16] Gao, R. X., Zhang, J. J., He, X. W., Chen, L. X., Zhang, Y. K., *Anal. Bioanal. Chem.* 2010, *398*, 451–461.

[17] Yao, C., Li, T. H., Twu, P., Pitner, W. R., Anderson, J. L., *J. Chromatogr. A* 2011, DOI: 10.1016/j.chroma.2011.01.035.

[18] Rezaee, M., Assadi, Y., Hosseini, M. R. M., Aghaee, E., Ahmadi, F., Berijani, S., *J. Chromatogr. A* 2006, *1116*, 1–9.

[19] Zang, X. H., Wu, Q. H., Zhang, M. Y., Xi, G. H., Wang, Z., *Chin. J. Anal. Chem.*, 2009, *37*, 161–168.

[20] Petersson, M., Wahlund, K. G., Nilsson, S., *J. Chromatogr. A* 1999, *841*, 249–261.

[21] Guzman, N. A., *Electrophoresis* 2003, *24*, 3718–3727.

[22] Wu, Q., Wang, C., Liu, Z., Wu, C., Wang, Z., *J. Chromatogr. A* 2009, *1216*, 5504–5510.

[23] Wu, Q., Li, Y., Wang, C., Liu, Z., Zang, X., Xin, Z., Wang, Z., *Anal. Chim. Acta* 2009, *638*, 139–145.

[24] Bidari, A., Hemmatkhah, P., Jafarvand, S., Hosseini, M. R. M., Assadi, Y., *Microchim. Acta* 2008, *163*, 243–249.

[25] Birjandi, A. P., Bidari, A., Rezaei, F., Hosseini, M. R. M., Assadi, Y., *J. Chromatogr. A* 2008, *1193*, 19–25.

[26] Tsai, W. C., Huang, S. D., *J. Chromatogr. A* 2009, *1216*, 5171–5175.

[27] Shamsipur, M., Ramezani, M., *Talanta* 2008, *75*, 294–300.

[28] Liang, P., Zhao, E., Li, F., *Talanta* 2009, *77*, 1854–1857.

[29] Farajzadeh, M. A., Bahram, M., Mehr, B. G., Jonsson, J. A., *Talanta* 2008, *75*, 832–840.

[30] Naseria, M. T., Hemmatkhaha, P., Milani Hosseinia, M. R., Assadi, Y., *Anal. Chim. Acta* 2008, *610*, 135–141.

[31] Dadfarnia, S., Shabani, A. M. H., *Anal. Chim. Acta* 2010, *658*, 107–119.

[32] Herrera-Herrera, A. V., Asensio-Ramos, M., Hernandez-Borges, J., Rodrỳguez-Delgado, M. A., *Trends Anal. Chem.* 2010, *29*, 728–751.

[33] Sarafraz-Yazdi, A., Amiri, A., *Trends Anal. Chem.* 2010, *29*, 1–14.

[34] Oita, I., Halewyck, H., Pieters, S., Dejaegher, B., Thys, B., Rombaut, B., Heyden, Y. V., *J. Pharm. Biomed. Anal.* 2009, *50*, 655–663.

[35] Panagiotou, A. N., Sakkas, V. A., Albanis, T. A., *Anal. Chim. Acta* 2009, *649*, 135–140.

[36] Wen, Y. Y., Liu, H. T., Tian, L., Han, P., Luan, F., *Acta Chromatogr.* 2010, *22*, 445–457.

[37] Kuila, A., Singh, A., Mukhopadhyay, M. R., Banerjee, L. W. T., *J. Food Sci. Technol* 2011, *44*, 62–66.

[38] Herrera-Herrera, A. V., Hernández-Borges, J., Borges-Miquel, T. M., Rodríguez-Delgado, M. Á., *Electrophoresis* 2010, *31*, 3457–3465.

[39] Liu, H. T., Wen, Y. Y., Luan, F., Gao, Y., *Anal. Chim. Acta* 2009, *638*, 88–93.

