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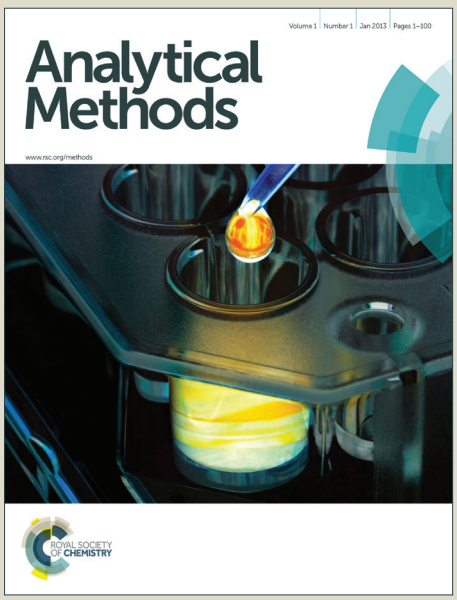

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Simultaneous determination of four aliphatic amines in aquatic products by ultrasound-assisted dispersive liquid-liquid microextraction coupled with high performance capillary electrophoresis

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Abstract

A simple, rapid, inexpensive and efficient method based on ultrasound-assisted dispersive liquid–liquid microextraction (UDLLME) after pre-column derivatization coupled with high performance capillary electrophoresis (HPCE) has been developed for determination of methylamine (MA), ethylamine (EA), dimethylamine (DMA) and diethylamine (DEA) in aquatic products. The aliphatic amines were derivatized with 9-fluorenyl methylchloroformate (FMOC-Cl) in alkaline aqueous solution and then the derivatives were extracted by ultrasound-assisted liquid–liquid microextraction (UDLLME) with trichloromethane. The factors affecting the derivatization and extraction efficiencies were investigated in detail. The electrophoresis separation was performed in an uncoated fused-silica capillary (50 cm × 50 μm *i.d*) with an effective length of 41 cm; 25 mmol L^{-1} sodium tetraborate containing 15 mmol L^{-1} sodium dodecyl sulfate (SDS) was used as running buffer; the applied voltage was 20 kV; the separation temperature was set to 25 ^{o}C; the UV detection wavelength was set at 265 nm. Four derivatized products of aliphatic amines could be completely separated within 7 min. The calibration curves were linear in the range of 0.5 to 12.5 mg L^{-1} for MA and DMA, and 1.0 to 25 mg L^{-1} for EA and DEA with the correlation coefficients ranging from 0.9984 to 0.9994. The limits of detection (LODs) and limits of quantification (LOQs) of the method were in the ranges of 0.028-0.16 mg kg^{-1} and 0.095–0.53 mg kg^{-1}, respectively. The enrichment factors ranged from 42

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to 68 for the four derivatives. The recoveries of the method were in the range of 72.8 %–97.0 %, with the intraday relative standard deviations (RSDs) of peak area in the range of 2.80 %–4.61 %. This method has been successfully applied to analysis of the four aliphatic amines in aquatic products.

Key words: aliphatic amine; derivatization; ultrasound-assisted dispersive liquid–liquid microextraction (UDLLME); capillary electrophoresis; aquatic product

1 Introduction

Aliphatic amines are amines in the molecules of which there are no aromatic rings directly on the nitrogen atom. Primary and secondary aliphatic amines such as methylamine, dimethylamine, ethylamine, diethylamine are important raw materials and intermediates in the chemical and pharmaceutical industry, mainly used to synthetize polymers, dyestuffs, corrosion inhibitors, pharmaceuticals, and so on [1]. Aliphatic amines are also degradation products of such organic materials as proteins and amino acids, and other nitrogen-containing organic compounds [2] and can be found in biological tissues, usually at trace levels. Researches have shown that aliphatic amines with less than eight carbon atoms are toxic to human's body, especially methylamine, dimethylamine, ethylamine, diethylamine, propyl amine, morpholine, piperidine.[3,4] Most of them are toxic sensitizers and irritants to the skin, mucous membrane, and respiratory tract and also notorious for their easy formation of carcinogenic nitrosamines when reacting with nitrite. [5-7] Since the freshness of food is associated with the contents of these aliphatic amines, the monitoring of the aliphatic amines is crucial for quality control in the food storage and distribution process. [8-11] Thus, it is very important to establish a reliable analytical method for the quantification of aliphatic amines in food products.

There are many methods reported for the determination of aliphatic amines, including

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UV-spectrophotometry,[12] thin-layer chromatography (TLC),[13] gas chromatography (GC),[14] high performance liquid chromatography (HPLC),[15-17] ion chromatography (IC),[18,19] gas chromatography- mass spectrometry (GC-MS),[3,20] and capillary electrophoresis (CE). [21-23]

Due to the lack of intrinsic chromophores or fluorescence, aliphatic amines have no ultraviolet absorption or fluorescence. One of the effective methods to improve their detection sensitivity is chemical derivatization.

There are various derivatization reagents for chemical derivatization, including 9-fluorenylmethoxycarbonyl chloride (FMOC-Cl) [24], o-phthalaldehyde (OPA) [25], dansyl chloride (DNS-Cl) [26], fluorescein isothiocyanate isomer I (FITC) [21,27], and some newly synthetic fluorescent reagents such as 1,2-benzo-3,4- dihydrocarbazole-9-aceticacid (BCAA) [28] and 4-(1-methyl-1*H*-and [9,10-d]imidazole)-2-benzoic acid (MPIBA).[29] However, OPA doesn't react with secondary amine and it cannot exhibit satisfactory reproducibility, stability and detection sensitivity; Nor is DNS-Cl an ideal derivative reagent since it is not stable and the derivatized products have fluorescence quenching phenomenon, thereby reducing the detection sensitivity; FITC is a small micromolecule fluorescein and the efficiency of derivatization is affected greatly by the pH of medium; BCAA and MPIBA both are highly sensitive derivatization agents, but they have not yet been commercialized. FMOC-Cl is the most widely used derivatization reagent on account of its reaction speediness, stability of the derivative, operation simplicity and mild reaction conditions. Our previous research [30] has also proved that FMOC-Cl is a suitable derivatization reagent for UV-derivatization of aliphatic amines.

In recent years, CE has emerged as a useful analytical method in separation and determination of various chemicals. Compared to the widely used methods such as HPLC or GC, CE has many advantages including low sample volume requirement, high resolution, cost-effectiveness, and time-savingness.

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Recently, there have been some reports on the determination of low-molecular aliphatic amines by capillary electrophoresis coupled with indirect ultraviolet detection without derivatization [21-23] or with laser-induced fluorescence detection after derivatization with fluorescein isothiocyanate isomer I (FITC). [27]

Due to the complicated matrix of biological samples, as well as the low contents of aliphatic amines, an effective sample extraction and purification technique is necessary for accurate determination of the target analytes. Dispersive liquid-liquid microextraction is a newly emerged sample pretreatment technique and has attracted much attention in recent years. [31,32] There have been some reports on the extraction of primary short-chain aliphatic amines such as methylamine, ethylamine, propylamine, dipropylamine, dibutylamine in water samples using DLLME based on solidification of floating organic droplet,[33] dynamic hollow fiber liquid-phase microextraction[34] or ultrasonic-mixed water-ionic liquid two-phase system.[35] After extraction, the amines were derivatized with pentafluorobenzaldehyde (PFBAY), phenylisothiocyanate (FITC), or dansyl chloride (DNS-Cl) and the derivatives were analyzed with GC-MS or HPLC. However, it has not been found so far any work on CE analysis of aliphatic amines in aquatic products after derivatization using FMOC-Cl and extraction by UDLLME. In the study, a CE method with UV detection after derivatization using FMOC-Cl and ultrasound-assisted dispersive liquid-liquid microextraction has been established for the determination of aliphatic amines including MA, DMA, EA and DEA and the proposed method has been applied to the analysis of the aforementioned four aliphatic amines in aquatic products with satisfactory results.

2 Experimental

2.1 Chemicals and reagents

The standards of aliphatic amines including methylamine hydrochloride (MA), dimethylamine hydrochloride (DMA), ethylamine hydrochloride (EA) and diethylamine hydrochloride (DEA) were

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purchased from Best Reagent Co., Ltd. (Chengdu, China). The purity of all the chemicals was greater than 99.0%. All of the aliphatic amines were separately dissolved in water to obtain a stock solution with a concentration of 1000 mg L^{-1} and stored in a refrigerator at 4 ^{o}C prior to use. A series of mixed standard solutions were prepared by diluting the stock solution with water before use.

The derivatization agent 9-fluorenylmethoxycarbonyl chloride (FMOC-Cl) was purchased from GL Biochem (Shanghai) Ltd. (Shanghai, China) and was prepared by dissolving FMOC-Cl in anhydrous acetonitrile before use (2 g L^{-1}).

The running buffer solution consisted of 25 mmol L^{-1} sodium tetraborate and 15 mmol L^{-1} sodium dodecyl sulfate (SDS), which was kept in the refrigerator at 4 ^{o}C and ultrasonicated for 10 min before use.

All the reagents were analytical grade unless otherwise stated. HPLC-grade trichloromethane was from Kermel Chemical Reagent Co., Ltd., Tianjin, China; HPLC-grade methanol was from Damao Chemical Reagent Factory, Tianjin, China; HPLC-grade acetonitrile was from Tedia Chemicals, USA; trichloroacetic acid (TCA), sodium hydroxide, sodium dodecyl sulfate (SDS) and sodium tetraborate were from Kelong Chemical Reagent Factory, Chengdu, China. Ultrapure water (18.2 MΩ•cm) was obtained from a water purification system (Ultrapure Technology Co., Ltd., China).

2.2 Apparatus and electrophoresis conditions

The separation and detection were performed on a CL1020 capillary electrophoresis system equipped with ultraviolet detector (Huayang Limin Instrument Co., Beijing, China). An uncoated fused-silica capillary (50 cm × 50 μm *i.d.*, Yongnian Ruifeng Chromatographic Device Co. Ltd, Hebei, China) with an effective length of 41 cm was employed in this study. Before separation, the capillary was flushed with ultrapure water, followed by 1 mol L^{-1} sodium hydroxide solution, and ultrapure water again for 3 min. The temperature was set at 25 ^{o}C; a voltage of 20 kV was applied with the positive polarity; the samples were

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injected for 10 s with gravity injection mode; the detection wavelength was set to 265 nm. Throughout the experiment, between runs of different samples, the capillary was flushed with the running buffer for 3 min.

2.3 Sample preparation

The samples of aquatic products including cuttlefish, navodon septentrionalis, shrimp, tentacles of squid, sleeve-fish, hairtail, yellow croaker and black carp were purchased from local markets (Chengdu, China) and frozen in a refrigerator（-20 ^{o}C）before analysis. Edible parts of the samples were chopped and homogenated. One gram of homogenated sample was accurately weighed and put in a mortar on an ice bath and 4.00 mL at 7.5 % trichloroacetic acid (TCA) was added and fully ground. Finally, a portion of the homogenated sample was transferred to a centrifuge tube and centrifuged at 15,000 rpm for 5 min. Then 1.00 mL of supernatant was taken and neutralized with 0.10mL of 1.0 mol L^{-1} sodium hydroxide, which served as sample solution for further derivatization.

2.4 Derivatization and UDLLME procedure

Derivatization was carried out by mixing 100 μL at 0.10 mol L^{-1} sodium borate solution and 100 μL at 2 mg ml^{-1} FMOC-Cl with 200 μL of the mixed standard solutions with the concentration of 0.50 μg mL^{-1}, 3.13 μg mL^{-1}, 6.25 μg mL^{-1} and 12.5 μg mL^{-1} for MA and DMA and 1.00 μg mL^{-1}, 6.25 μg mL^{-1}, 12.5 μg mL^{-1} and 25.0 μg mL^{-1} for EA and DEA in a series of centrifuge tubes, respectively. The solutions were vortex mixed for 2 min, and kept for 10 min at room temperature, and 75 μL trichloromethane was added quickly. The solutions were ultrasonicated for 5 min and cloudy emulsions would form. The cloudy solutions were centrifuged for 5 min at 10,000 rpm, and the trichloromethane droplet would aggregate at the bottom of the centrifuge tube. From each tube, 50 μL trichloromethane was pipetted into a 100 μL EP tube and the trichloromethane was dried-up under gentle nitrogen flow. The residue was reconstituted with

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20 μL running buffer solution, then vortex mixed and centrifuged, the supernatant was used for CE analysis. The sample solution was experienced the same derivatization and extraction procedure as described above.

2.5 Quantification analysis

The aliphatic amines were identified by comparison of their electrophoretograms and migration time with those of the standards. Standard calibration curves were used for the quantification of the aliphatic amines. The contents of the aliphatic amines in samples were calculated by the following formula:

$$C(mg/\mathrm{kg}) = \frac{5 \times 1.1a}{W} F$$

Where C is the content of the aliphatic amine (mg/kg); a is the amine content of sample calculated through the regression equation of the standard curve (μg/mL); W is the sample weight (g); F is the corrected factor for recovery. For MA and DMA, F=1; for EA, F=1.28; and for DEA, F= 1.37.

3. Results and discussion

3.1 Selection of the detection wavelength

The experimental results showed that the four primary and secondary aliphatic amines could react with FMOC-Cl to form the derivatives with ultraviolet absorption at 265 nm in alkaline medium. Therefore, 265 nm was used as the absorption wavelength throughout the study. The experimental results also showed that the derivatives could be stable for at least 90 min at room temperature. Fig. 1 presented the reaction mechanism of the aliphatic amines with FMOC-Cl.

3.2 Optimization of electrophoresis conditions

3.2.1 Selection and optimization of the running buffer Running buffer plays a key role in the whole procedure of CE analysis. In CZE mode, the migration rate or mobility of a solute ion is governed largely by its charge/mass ratio. To improve the symmetry of peaks and their resolution, the composition and concentration of the running buffer and other affecting factors were optimized in detail.

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The effect of borax concentration (10-40 mmol L^{-1}) on separation was investigated. The experimental results indicated that, with the increase of borax concentration, the separation efficiency was improved. However, high concentration of borax would lead to a higher noise level, the broad peaks, as well as much longer migration time.

SDS, an anionic surface-active agent, has played a critical role in the separation of the aliphatic amines derivatives in the experiment. The effect of SDS concentration (5-25 mmol L^{-1}) on the separation was studied. As shown in Fig. 2, the concentration of SDS exhibited a significant influence on the separation efficiency but an unobvious impact on the peak area. When the SDS concentration was low, the separation efficiency was acceptable but the migration time was rather long, and the peaks tailed slightly, especially for the derivatives of ethylamine and diethylamine. With the increase of SDS concentration, the migration time would decrease, but when its concentration was increased to 25 mmol L^{-1}, the peaks would deteriorate and split.

Therefore, in view of the separation efficiency and the analysis time, 25 mmol L^{-1} of borax buffer and 15 mmol L^{-1} of SDS were used as running buffer solution in the experiment.

3.2.2 Optimization of pH of the running buffer The pH of the running buffer affects the electroosmotic flow (EOF) and the migration rate, thereby affecting the separation efficiency. The influence of the pH of running buffer in the range of 7.5 - 11.0 on the separation was investigated and the results showed that the four target amines derivatives could be well separated when the pH was greater than 8.0. With the increase of the pH, the resolution and their peak areas increased as well. When pH was in the range of 9.0-10.0, good peak shapes and maximum peak area were obtained. When pH was greater than 10.5, the baseline noise increased tremendously and the resolution declined. Since the pH of sodium tetraborate buffer was 9.5, it was not necessary to adjust the pH in the study.

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3.2.3 Optimization of the applied voltage The influences of the separation voltage (15-27 kV) on peak area and resolution were investigated. The voltage influenced the peak area and migration time greatly; with the voltage increasing, the peak area as well as the baseline noise increased, but the migration time decreased. Especially when voltage was increased to 27 kV, the baseline was quite unstable. Therefore, 20 kV was used as the separation voltage in the study.

3.2.4 Optimization of the separation temperature The temperature affects the separation efficiency. The experimental results indicated that with the increase of temperature, the migration time increased and the derivative peaks would broaden, especially the baseline noise showed a significant increase. So the separation temperature was controlled at 25 □ in the experiment. Fig.3 showed the electropherogram of mixed standard solution under the optimum conditions.

3.3 Optimization of the extraction conditions

Since the contents of the aliphatic amines in aquatic products were generally very low, furthermore, the sample matrix was very complex. In order to effectively extract the target components from the samples, eliminate the interference as far as possible, and improve the sensitivity of the method, the ultrasound-assisted dispersive liquid-liquid microextraction (UDLLME) was investigated. UDLLME is a simple, rapid, and environmental friendly pretreatment approach with high enrichment factor and low consumption of organic solvents. The factors affecting UDLLME procedure were optimized and satisfactory extraction efficiencies were obtained.

3.3.1 Selection of the extraction solvent The extraction solvent should be similar with the derivatives in the physicochemical properties. The derivatives are liposoluble, which means the extraction solvent is hydrophobic and unsoluble in water. Carbon disulfide, methylene chloride, trichloromethane, ethyl acetate were compared for extraction of the derivatives. From Fig. 4, we can see that among all the solvents, ethyl

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acetate showed the highest enrichment factors, while carbon disulfide showed the lowest. The enrichment factors of ethyl acetate were 49, 48, 67 and 76 for MA, EA, DMA and DEA, respectively. The enrichment factors of trichloromethane were 44, 42, 52 and 68, respectively. The enrichment factors of other two extraction solvents are much lower than those of ethyl acetate and trichloromethane. Because of its high volatility, ethyl acetate could not give satisfactory precision; trichloromethane was selected as extraction solvent in the experiment.

3.3.2 Effect of the extraction solvent volume Volume of extraction solvent affects the sensitivity significantly. With the increase of volume of extraction solvent, the sensitivity of the method also increased, but the longer equilibrium time was needed. [36]

To compare the effect of the extraction solvent volume on the extraction efficiency, different volumes (20, 50, 75, 100 and 150 μL) of trichloromethane were investigated under the same UDLLME conditions. The results indicated that when the volume of trichloromethane was increased to 75 μL, emulsified effect became obvious and the satisfactory recoveries (90.0, 78.3, 97.0 and 72.8% for MA, EA, DMA and DEA, respectively) were obtained. Therefore, 75μL trichloromethane was used for extraction of the derivatives in the subsequent experiments.

3.3.3 Selection of the disperser Generally，the dispersive liquid-liquid microextraction procedure needs a dispersant which not only is easily soluble in extraction solvent, but also is miscible with water. Commonly used dispersants include methanol, ethanol, acetone, acetonitrile and tetrahydrofuran.[37] In our study, acetonitrile was used as solvent for FOMC-Cl, so it could play a role of disperser. Furthermore, the ultrasonication was also beneficial to the emulsion formation. The experimental results showed that the emulsification effect was remarkable and the extraction efficiencies were satisfactory even without addition of any other dispersant.

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3.3.4 Effect of the sonification time The effect of sonification time (1, 3, 5, 10 and 15 min) on extraction efficiencies were investigated. The experimental results showed that when the sonification time was longer than 5 min, the maximum and constant peak areas of the four derivatives were obtained. So the ultrasonic extraction time of 5 min was selected in the experiment.

3.3.5 Effect of salt addition Because the partition coefficients of analytes between the organic solvent and sample solution are affected by sample matrix, they will change as the sample matrix changes. The usual approach is to add some inorganic salts (NaCl, KCl, Na_2SO_4 etc.) into sample solution to increase its ionic strength. The effect of different amount of NaCl on the extraction efficiencies was studied, but no obvious improvement on the extraction efficiencies was observed. Therefore, the addition of salt into the sample solution was not employed during UDLLME procedure. Fig.5 showed the electropherogram of the mixed standard solution before and after UDLLME procedure under the optimum conditions.

3.4 Method validation

3.4.1 Performance of the method Table 1 listed the linear ranges, linearity equations and correlation coefficients of the proposed method. Within the linear ranges, the correlation coefficients of the method were greater than 0.998. The limits of detection (LODs) and the limits of quantification (LOQs) were calculated as three times and ten times of the signal-to-noise ratio (S/N=3, S/N=10), respectively. The LODs and LOQs of the method were in the range of 0.028-0.16 and 0.095-0.53 mg kg^{-1}, respectively.

The enrichment factor (EF) is defined as the ratio of analyte concentration in the organic phase to the initial analyte concentration in aqueous phase.[38] Table 1 shows that the EFs of the method ranged from 42 to 68 for all the target analytes.

The repeatability of the method was evaluated by intra-day precision. The relative standard deviations (RSDs) were calculated according to the peak areas and migration times of six measurements within a day

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(Table 2). Accuracy of the method was evaluated by addition of known amounts of MA, DMA, EA and DEA into the sample (navodon septentrionalis) and then pretreatment and analysis with this method. The recoveries of the method ranged from 72.8 to 97.0 % (Table 3). For MA and DMA, the recoveries were in the range of 90.0%-97.0%, but the recoveries of EA and DEA were in the range of 72.8%-78.3%, which indicated that there was systematic error for EA and DEA. To reduce the error, a correction factor (the reciprocal of the recovery of EA or DEA) was introduced in the calculation formula.

The comparison of the proposed method with HPLC method[30] showed that the proposed method is superior to HPLC method in respect of LODs, the total analysis time, as well as the cost.

Table 1 the linear ranges, limits of detection (LODs) , limits of quantifications (LOQs) and enrichment factors (EFs) of the method

Analyte	Linear ranges ($\mu g\ mL^{-1}$)	Regression equations	correlation coefficients (r)	LOD (mg kg^{-1})	LOQ (mg kg^{-1})	Enrichment factor (EF)
MA	0.5-12.5	y = 2890x + 549.5	0.9987	0.028	0.095	44
EA	1.0-25.0	y = 3914x + 660.4	0.9986	0.054	0.18	42
DMA	0.5-12.5	y = 1610x + 400.3	0.9984	0.054	0.18	52
DEA	1.0-25.0	y = 1565x + 73.46	0.9994	0.16	0.53	68

Table 2 Intra-day precision of the method (n=6)

Analyte	Migration time			Peak area		
	Range	Mean (min)	RSD (%)	Range	Mean	RSD (%)
MA	3.56-3.81	3.62	2.74	1.25×10^4-1.37×10^4	1.30×10^4	3.42
EA	4.02-4.30	4.08	2.70	1.15×10^4-1.24×10^4	1.20×10^4	2.80
DMA	4.61-4.94	4.71	2.48	1.74×10^4-1.91×10^4	1.82×10^4	3.16
DEA	5.44-5.75	5.51	2.38	2.21×10^4-2.53×10^4	2.43×10^4	4.61

Table 3 Recoveries of the method (n=3)

Analyte	Background (mg kg^{-1})	Added (mg kg^{-1})	Found (mg kg^{-1})	Recovery (%)	Average Recovery (%)
MA	14.4	5.00	18.7	86.0	90.0
		10.0	23.9	95.0	
		20.0	32.2	89.0	

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		5.00	3.70	74.0	
EA	ND	10.0	8.90	89.0	78.3
		20.0	14.4	72.0	
		5.00	8.66	97.4	
DMA	3.79	10.0	14.4	106	97.0
		20.0	21.3	87.6	
		5.00	3.60	72.0	
DEA	ND	10.0	7.90	79.0	72.8
		20.0	13.5	67.5	

*ND: not detected.

*a,b : corrected recoveries

3.4.2 Method application The proposed method was applied to the determination of MA, EA, DMA and DEA in frozen aquatic products. Fig. 6 presents the electropherograms of samples including navodon septentrionalis, cuttlefish and shrimp. The contents of the aliphatic amines detected in the aquatic products were listed in Table 4.

Table 4 The contents of the aliphatic amines in aquatic samples (mg kg^{-1})

Samples	MA（mg kg^{-1}）	EA(mg kg^{-1}）	DMA (mg kg^{-1}）	DEA (mg kg^{-1}）
Cuttlefish 1	23.8	3.88	0.78	ND
Cuttlefish 2	19.9	2.97	1.89	ND
Sleeve-fish 1	18.7	ND	7.18	ND
Sleeve-fish 2	14.3	ND	3.41	ND
Sleeve-fish 3	20.5	ND	4.84	ND
Tentacles of squid 1	6.50	ND	0.81	ND
Tentacles of squid 2	9.83	ND	1.07	ND
Navodon Septentrionalis 1	22.5	4.10	3.37	39.6
Navodon Septentrionalis 2	26.4	7.73	ND	7.56
Navodon Septentrionalis 3	18.1	5.32	5.61	8.85
Hairtail 1	13.2	0.32	3.09	ND
Hairtail 2	11.6	0.65	3.6	ND
Shrimp (raw)1	204	ND	28.4	ND
Shrimp (raw)2	183	ND	22.0	ND
Shrimp (cooked)	5.37	ND	2.95	ND
Yellow croaker	9.12	ND	0.33	ND
Yellow croaker 2	11.0	ND	0.20	ND

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Black carp 1	1.84	ND	ND	ND
Black carp 2	0.13	ND	0.01	ND

*ND: not detected.

4. Conclusion

In this experiment, a novel method combined UDLLME with HPCE was established for the simultaneous determination of four aliphatic amines in aquatic products. The proposed method is reliable, inexpensive, time-saving and environmental friendly, thereby suitable for routine analysis of these four aliphatic amines in aquatic products.

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Figure Captions：

Fig.1 Reaction mechanism of aliphatic amine with 9-fluorenyl methylchloroformate

Fig.2 The influence of SDS concentration on the electropherogram

The SDS concentration: a. 5 mmol L^{-1}; b.10 mmol L^{-1}; c. 15 mmol L^{-1}; d. 20 mmol L^{-1}; e. 25 mmol L^{-1}

Fig. 3 The electropherogram of a mixed standard solution. Peak identifications：1. MA; 2.EA; 3. DMA; 4. DEA（The concentration were 12.5 μg mL^{-1}, 25 μg mL^{-1}, 12.5 μg $mL^{-1}$and 25 μg mL^{-1} ， respectively）

Fig. 4 The comparison of enrichment factors of extraction solvents

Fig. 5 The electropherograms of a mixed standard solution before (a) and after (b) UDLLME procedure. Peak identifications are same as those in Fig.3

Fig.6 The typical electropherograms of samples: a. navodon septentrionalis; b.cuttlefish; c.shrimp. Peak identifications are same as those in Fig.3

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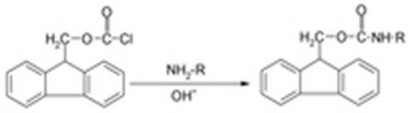

Fig.1 Reaction mechanism of aliphatic amine with 9-fluorenyl methylchloroformate
17x4mm (300 x 300 DPI)

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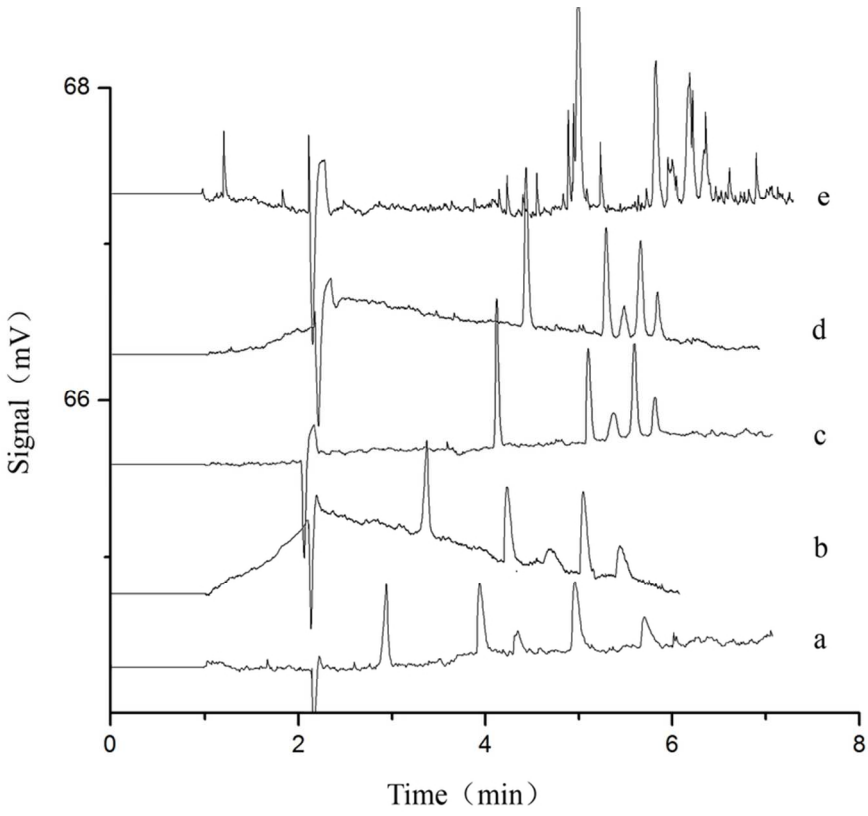

Fig.2 The influence of SDS concentration on the electropherogram
The SDS concentration: a. 5 mmol L-1; b.10 mmol L-1; c. 15 mmol L-1; d. 20 mmol L-1; e. 25 mmol L-1

83x71mm (300 x 300 DPI)

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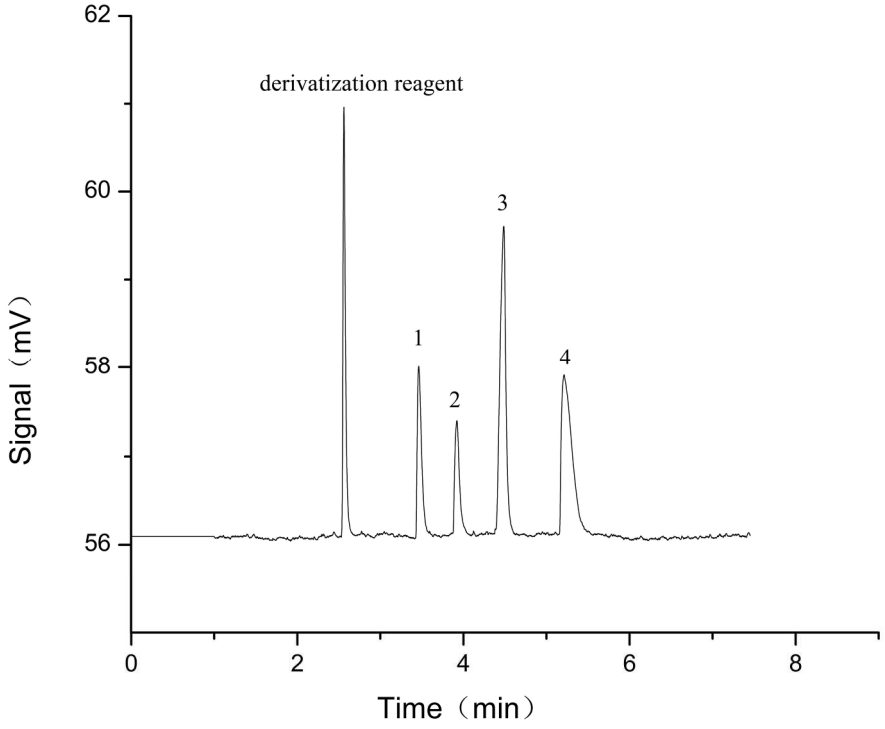

Fig. 3 Capillary electropherogram of a mixed standard solution. Peak identifications：1. MA； 2.EA; 3. DMA； 4. DEA（The concentration were 12.5μg mL-1, 25μg mL-1, 12.5μg mL-1and 25μg mL-1，respectively）
208x177mm (300 x 300 DPI)

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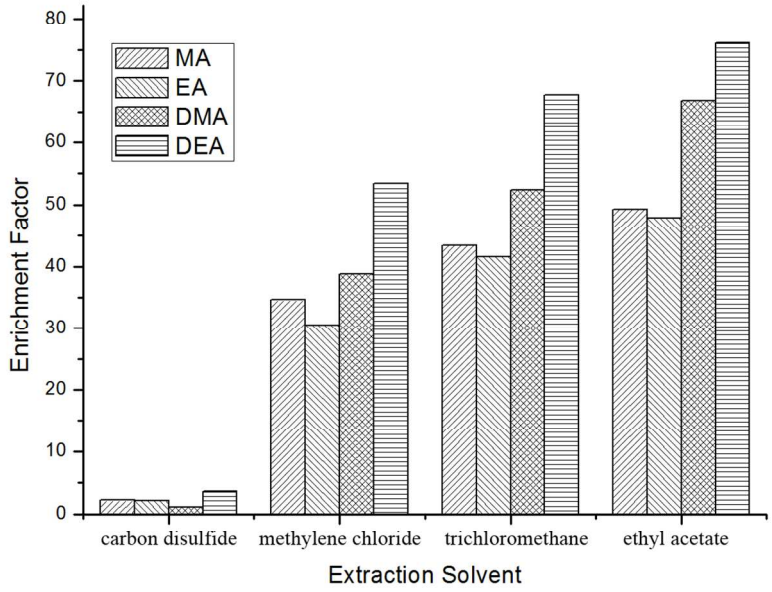

Fig.4 The comparison of enrichment factors of extraction solvents
118x84mm (300 x 300 DPI)

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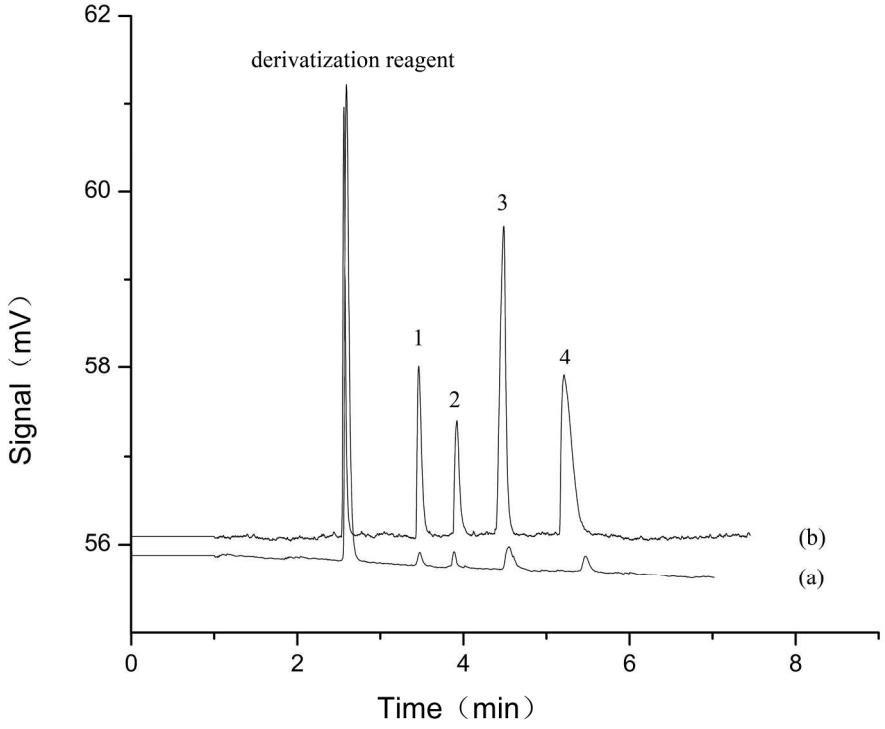

Fig. 5 The electropherograms of a mixed standard solution before (a) and after (b) UDLLME procedure. Peak identifications are same as those in Fig.3
208x177mm (300 x 300 DPI)

Analytical Methods Accepted Manuscript

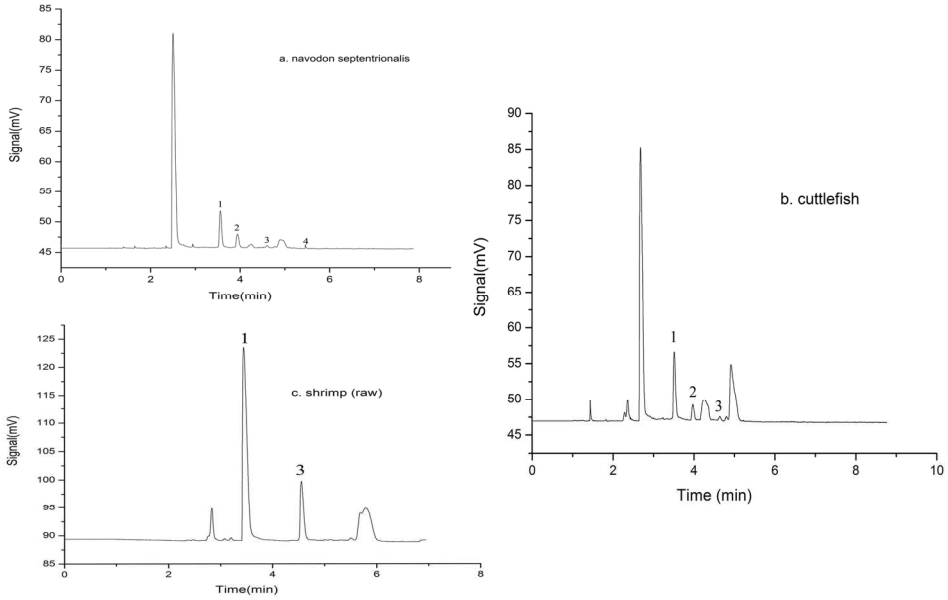

Fig.6 Typical electropherograms of samples: a. navodon septentrionalis; b.cuttlefish; c.shrimp. Peak identifications are same as those in Fig.3
162x102mm (300 x 300 DPI)

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