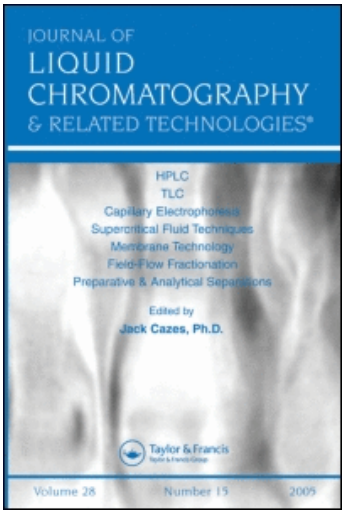

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DETERMINATION AND IDENTIFICATION OF ALIPHATIC AMINES FROM ENVIRONMENTAL WATER WITH HPLC-FLD AND APCI/MS USING 1-[1,2,5,6-DIBENZOCARBAZOL-9-YL]PROPAN-2-YL CHLOROFORMATE (DBCPC-CL) AS NOVEL LABELING REAGENT

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DETERMINATION AND IDENTIFICATION OF ALIPHATIC AMINES FROM ENVIRONMENTAL WATER WITH HPLC-FLD AND APCI/MS USING 1-[1,2,5,6-DIBENZOCARBAZOL-9-YL]PROPAN-2-YL CHLOROFORMATE (DBCPC-CL) AS NOVEL LABELING REAGENT

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□ *A novel fluorescent probe, 1-[1,2,5,6-dibenzocarbazol-9-yl]propan-2-yl chloroformate (DBCPC-Cl) has been designed and synthesized for amine labeling in HPLC. Using 12 aliphatic amines as the models, the derivatization conditions were optimized. In 0.2 mol/L borate buffer (pH 9.0), amines reacted with DBCPC-Cl at 30°C to form the derivatives in 3 min, and the derivatives were stable enough to be efficiently analyzed by HPLC. The separation of these amine derivatives was achieved with a C_8 column and gradient elution by using 30% acetonitrile (containing 20 mmol/L formic acid) and 100% acetonitrile, and online APCI/MS identification of the derivatives was carried out in positive-ion mode. The fluorescence responses for DBCPC-derivatives were higher relative to those obtained using previously reported reagents. With fluorescence detection at an emission wavelength of 390 nm and an excitation wavelength of 300 nm, the detection limits of aliphatic amines were 0.3 – 5.1 fmol (at a signal-to-noise ratio of 3:1). The relative standard deviations for within-day determination ($n = 12$) were 0.051–0.079% for retention time and 0.84–1.12% for peak area for the tested aliphatic amines. The mean intra- and inter-assay precision for all amines levels were $< 2.52\%$ and 3.74%, respectively. The mean recoveries ranged from 86.9 to 104.7%. Excellent linear responses were observed with coefficients > 0.9991.*

Keywords 1-[1,2,5,6-dibenzocarbazol-9-yl]propan-2-yl chloroformate (DBCPC-Cl), aliphatic amines, derivatization, fluorescent probe, HPLC

INTRODUCTION

Amino compounds are a group of important substances that widely exist in nature and have a lot of functions in biological and environmental systems, such as amino acids,[1] peptides, proteins, and biogenic amines[2]

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in organisms, short-chain aliphatic amines,[3] and aromatic amines[4] in the environment. Their determination is almost a routine task for biological, medical, and environmental interest. Various methods for the separation, identification, and determination of amines have been published throughout during the last decade or so.[5] However, analysis of amines has been traditionally difficult due to their particular physicochemical properties, i.e., high volatility and polarity, basic character, and high solubility in water. Gas chromatography is frequently used to determine amines using various derivatization reagents.[6] Other methods including enzymatic[7] and ion-exchange chromatographic detection[8] have been described for the determination of amines in various matrices. These methods are usually limited due to low sensitivity. At present, precolumn derivatization in conjunction with reversed phase liquid chromatography is the most used technique for the determination of amines. Great efforts have been devoted to exploiting the more appropriate fluorescent tagging reagents for amine derivatization and developing new analytical methods with high sensitivity and selectivity. Various fluorescent reagents were used for amines labeling including orthophthalaldehyde (OPA),[9,10] 4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-F),[11] 9-fluorenylmethyl chloroformate (FMOC),[12] 1-(9-fluorenyl)ethyl chloroformate (FLEC),[13] and 2-(9-anthyl)ethyl chloroformate (AEOC),[14] 6-aminoquinolyl-*N*-hydroxysuccinimidyl- carbamate (AQC),[15] and so on. These reagents have also been reported to have some various shortcomings in their application, such as short detection wavelengths, poor stability, and serious interference for the determination of real biological samples.

In our previous studies,[16–19] we had described chloroformate reagents derived from the core structure of carbozole and benzocarbozole, such as CEOC-Cl, BCEOC-Cl, and BCEC-Cl, which exhibited interesting spectral properties and high sensitivities in the application of analyzing amino compounds. In this work, 1-[1,2,5,6-dibenzocarbazol-9-yl]propan-2-yl chloroformate (DBCPC-Cl), a novel reagent with dibenzocarbozole as core structure, are synthesized and studied. DBCPC-Cl has been found to be very stable in its crystal state, and exhibits relatively high fluorescence response and high molar absorbance. DBCPC-Cl reacts readily with amines and is successfully used as a precolumn labeling reagent for the determination of amines in combination with HPLC. DBCPC-amine derivatives exhibit not only excellent fluorescence but also high MS ionizable potential with APCI detection in positive-ion mode. In this study, the optimal derivatization conditions such as buffer pH, reaction time, and solvent system are investigated. Linearity, detection limits, and precision of the procedure are also determined. To the best of our knowledge, this is the first time that DBCPC-Cl fluorescent probe and its applications for the determination of amines have been reported.

EXPERIMENTAL

Instrumentation

Experiments were performed using a LC/MSD-Trap-SL ion trap liquid chromatography/mass spectrometry (Agilent 1100 Series LC/MSD Trap, USA). All the HPLC system devices were from the HP 1100 series and contained a vacuum degasser (model G1322A), a quaternary pump (model G1311A), an autosampler (model G1329A), a thermostated column compartment (model G1316A), a fluorescence detector (FLD) (model G1321A), and a diode array detector (DAD) (model G1315A). The mass spectrometer from Bruker Daltonik (Bremen, Germany) was equipped with an atmospheric pressure chemical ionization (APCI) source. APCI conditions: positive mode; nebulizer pressure 60 psi; dry gas temperature, 350°; dry gas flow 5.0 L min^{-1}; APCI Vap temperature 450; Corona Current (nA) 4000 (pos); Capillary voltage 3500 V. Derivatives were separated on reversed phase Eclipse XDB-C8 column (150 × 4.6 mm, 5 μm, Agilent, USA). The HPLC system was controlled by HP Chemstation software. The mass spectrometer system was controlled by Esquire-LC NT software, version 4.1. Fluorescence excitation and emission spectra were obtained on a F7000 fluorescence spectrophotometer (Hitachi). The mobile phase was filtered through a 0.2 μm nylon membrane filter (Alltech, Deerfiled, IL).

Chemicals

All aliphatic amine standards were purchased from Sigma Co (St. Louis, MO, USA). HPLC grade acetonitrile (ACN) was purchased from Yucheng Chemical Reagent Co. (Shandong Province, China). Formic acid was analytical grade from Shanghai Chemical Reagent Co. (Shanghai, China). Water was purified on a Milli-Q system (Millipore, Bedford, MA). Borate buffer was prepared from 0.2 mol L^{-1} boric acid solution adjusted to pH 9.0 using 4 mol L^{-1} sodium hydroxide solution prepared from sodium hydroxide pellets.

Synthesis of Derivatization Reagent (DBCPC-Cl)

Synthesis of 1-[1,2,5,6-dibenzocarbazol-9-yl] Propanol (DBCPC-OH)

The novel reagent was synthesized according to the reaction route in Figure 1. Dibenzocarbazole (19.5 g),[20] KOH (6 g), and 200 mL 2-butanone were mixed and rapidly cooled to 0° with ice water with vigorous stirring. A cooled mixture of 1,2-epoxypropane (5.8 g) in 50 mL of 2-butanone solution was added dropwise within 1 h. The contents were kept at ambient temperature for another 2 h with stirring. The solution was heated to 50°C

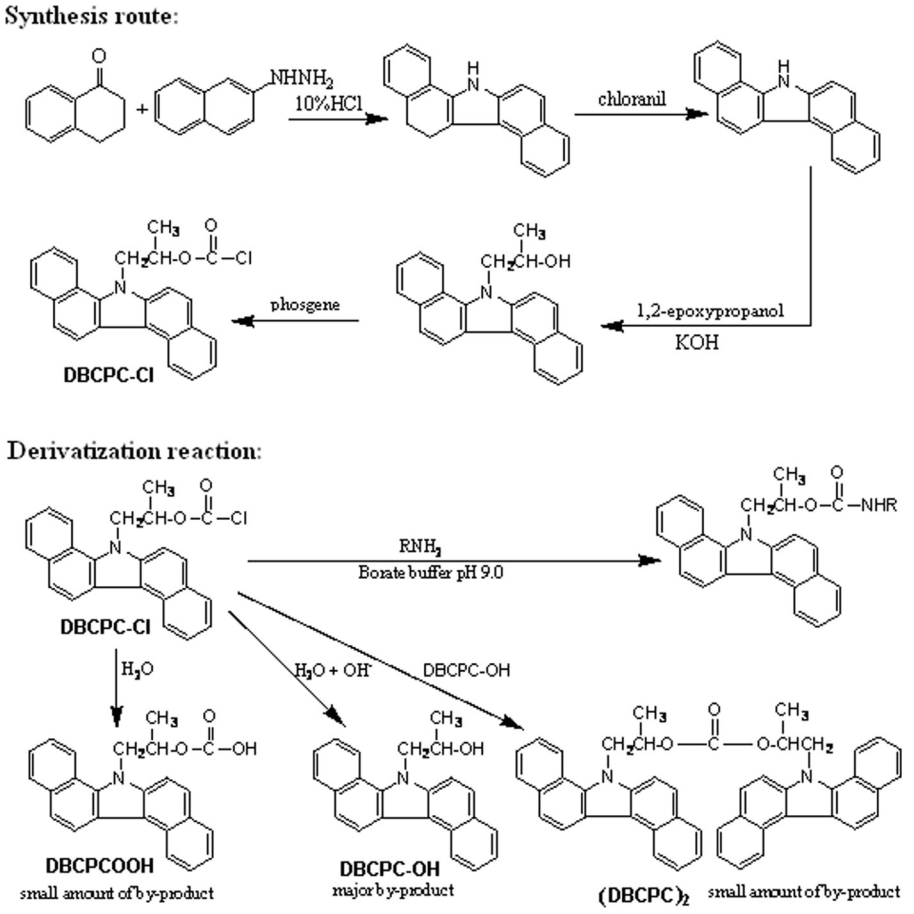


FIGURE 1 Synthesis route of 1-[1,2,5,6-dibenzocarbazol-9-yl]propan-2-yl chloroformate (DBCPC-Cl) and derivatization scheme of DBCPC-Cl with aliphatic amines.

for 1 h and concentrated by a rotary evaporator. After cooling, the residue was transferred into 200 mL of ice water with vigorous stirring for 0.5 h, the precipitated solid was recovered by filtration, washed with water, 75% ethanol solution, and dried at room temperature for 48 h. The crude product was recrystallized three times from methanol (100 mL × 3) to afford a white crystal, yield (84%). m.p.141.6 ~ 142.1; APCI/MS: *m/z*: 326.5 (in positive ion mode).

Preparation of 1-[1,2,5,6-dibenzocarbazol-9-yl]propan-2-yl Chloroformate (DBCPC-Cl)

To a solution containing 10.0 g solid phosgene and 100 mL dichloromethane (0°C) in a 500 mL round-bottom flask, a mixture of DBCPC-OH

(6.2 g) and pyridine (1.0 g catalyst) in 200 mL dichloromethane solution was added dropwise within 2 h with stirring. After stirring at 0°C for 4 h, the contents were kept at ambient temperature for another 6 h period with vigorous stirring, the solution was then concentrated by a rotary evaporator. The residue was extracted four times with warm ether; the combined ether layers were concentrated in *vacuum* to yield a white crystal. The crude products were recrystallized twice from ether to give the white crystal (70.0%, yield), m.p. 59.3–60.0°C. Found: C 74.33%, H 4.67%, N 3.64%, Cl 9.16%, O 8.23%. Calculated: C 74.32%, H 4.68%, N 3.61%, Cl 9.14%, O 8.25%. IR (KBr): 3040.18, 2973.80, 2922.62, (Ar), 1764.45 (−C=O), 1619.34, 1444.94, 1408.92 (C−N), 807.05, 737.96.

Preparation of Standard Solutions

The derivatization reagent solution 1.0×10^{-3} mol L^{-1} was prepared by dissolving 41.75 mg DBCPC-Cl in 10 mL of anhydrous acetonitrile prepared by distilling the dried HPLC grade acetonitrile with P_2O_5. Individual stock solutions of the amines were prepared in acetonitrile. The standard amines for HPLC analysis at individual concentrations of 5.0×10^{-5} mol L^{-1} were prepared by diluting the corresponding stock solutions (1.0×10^{-3} mol L^{-1}) of each amine with acetonitrile. When not in use, all standards were stored at 4°C.

Extraction of Amines from River Water

The tested water samples were taken from River Si (located at the borders of Qufu, Shandong province, China). To a solution containing 60 mL of water sample in a 50 mL round bottom flask, 2.0 mL hydrochloric acid (1.0 M) was added. The contents of the flask were vortexed for 2 min and filtrated. The resulting solution was evaporated to dryness under reduced pressure in a nitrogen atmosphere. The resulting residue was redissolved in 3 mL aqueous acetonitrile (80:20, *v*/*v*) to a total volume of 5.0 mL and stored at 4°C until analysis.

Derivatization Procedure

The DBCPC amine derivatization was carried out in aqueous acetonitrile in a basic medium. One hundred μL aqueous of amines was added in a vial, to which 150 μL borate buffer (0.2 mol L^{-1}, pH 9.0) and 50 μL DBCPC-Cl acetonitrile solution were then added. The solution was shaken for 30 s and allowed to stay at room temperature for 3–4 min, a 10 μL acetic acid (36%, *w*/*w*) was then added until the final pH range of 6.0–6.5. The

derivatized sample was directly injected into the HPLC system, and then into the online mass spectrometry with APCI ion source. The derivatization process is shown in Figure 1.

High-Performance Liquid Chromatography

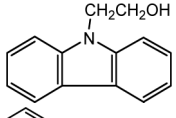
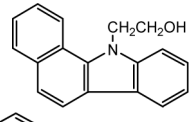
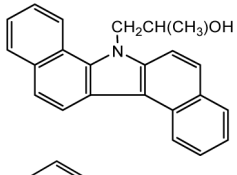
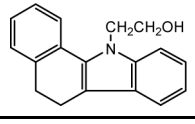
Eluent A was 30% of acetonitrile consisting of 20 mmol L^{-1} formic acid/ammonia buffer (pH 3.5); B was acetonitrile (100%). Derivatives were separated on a reversed phase Eclipse XDB-C8 column in conjunction with a gradient elution. Gradient conditions: initial = 70% A and 30% B; 35 min = 100% B (kept for 5 min). Before injection of the next sample, the column was equilibrated with the initial elution condition for 5 min. The flow rate was constant at 1.0 mL min^{-1} and the column temperature was set at 30°C. The fluorescence excitation and emission wavelengths were set at λ_{ex} 300 and λ_{em} 395 nm, respectively. The detection and identification of the DBCPC derivatives was performed by FLD and APCI/MS.

RESULTS AND DISCUSSION

Ultraviolet and Fluorescence Spectral Properties

Carbazole derivatives are one of the most studied and important classes of photochromic molecules, and they exhibit interesting photochromic properties. In this work, the spectral properties of DBCPC-Cl were investigated in comparison with CEOC-Cl, BCEOC-Cl, and BCEC-Cl. To eliminate the heavy atom effect of chlorine on the fluorescence response, the solutions (1.0×10^{-5} mol L^{-1} ACN/H_2O, 80:20, v/v) of hydroxyl precursor for each chloroformate reagent were individually prepared and used to determine the molar absorbance (ε) and relative fluorescence intensity (I_f), respectively. (Table 1 shows the structure of hydroxyl precursors for each chloroformate reagent: CEOC-OH, BCEOC-OH, BCEC-OH, DBCPC-OH; it had been proven that spectra of these alcohol compounds can well represent those of amine derivatives.[19]) And the obtained ultraviolet and fluorescent data were presented in Table 1. For BCEC-OH and DBCPC-OH, their core structure were formed by fusing one or two benzo groups to carbazole; therefore, BCEC-OH and DBCPC-OH, as well as CEOC-OH can be deemed to a series of carbazole derivatives. For BCEOC-OH, no complete carbazole moiety can be found in its core structure, and its core structure was more like a benzo substituted indole. In the series of CEOC-OH, BCEC-OH, and DBCPC-OH, as the augment of $\pi - \pi$ conjugation system in core structure by fusing benzo groups to carbazole, the maximum absorption wavelengths exhibited an obvious red shit and

TABLE 1 Ultraviolet and Fluorescence Properties of CEOC-OH, BCEOC-OH, BCEC-OH and DBCPC-OH

Hydroxyl Precursors for Chloroformats	Chemical Structure	Ultraviolet Absorption*		Fluorescence*	
		Maximum Absorption Wavelengths (nm)	ε ($L mol L^{-1} cm^{-1}$)	Maximum Excitation/ Maximum Emission λ_{ex}(nm)/λ_{em} (nm)	Relative Fluorescence Intensity**
CEOC-OH		235 260 294	4.38×10^4 2.43×10^4 1.87×10^4	249/360	567
BCEC-OH		256 279 309	4.44×10^4 4.58×10^4 2.63×10^4	279/380	724
DBCPC-OH		243 290 300	3.21×10^4 5.20×10^4 $5.30 \times 10_4$	300/390	895
BCEOC-OH		249 323	2.36×10^4 2.15×10^4	333/390	643

*DBCPC-OH, CEOC-OH, BCEOC-OH and BCEC-OH solutions were individually prepared at concentration of $1.0 \times 10^{-5} mol L^{-1}$ (used to test ultraviolet absorption) and $1.0 \times 10^{-7} mol L^{-1}$(used to test fluorescence properties) in aqueous acetonitrile (ACN/H_2O, 80:20, v/v).

**Fluorescence intensities were obtained under respective maximum λex and maximum λem; Excitation and emission slit are both set at 5 nm; PMT voltage were 350 V; Scan speed was set at 1200 nm min^{-1}.

corresponding molar absorption coefficients (ε) remarkable increase. DBCPC-OH has the highest ultraviolet absorption among the tested four compounds with the molar absorption coefficients of $5.20 \times 10^4\,\mathrm{L\,moL^{-1}\,cm^{-1}}$ (λ_{max} 290 nm). The fluorescence spectra of DBCPC-OH exhibited the maximum excitation and emission at 300 and 395 nm, respectively. Compared with fluorescence intensity of CEOC-OH (λex/λem: 249/360 nm), BCEOC-OH (λex/λem: 333/390 nm) and BCEC-OH (λex/λem: 279/380 nm), DBCPC-OH (λex/λem: 300/390 nm) exhibited the highest fluorescence intensity under the tested conditions (see Table 1). This intense fluorescence was probably due to the fact that DBCPC-OH has the biggest rigid structural plane with a highly conjugated $\pi - \pi$ system, which would make DBCPC-Cl more sensitive for derivatizing amines relative to that of BCEC-Cl, BCEOC-Cl, and CEOC-Cl.

Optimization for Derivatization

DBCPC-Cl has the same chloroformate reaction with primary and secondary amino compounds as does CEOC-Cl and BCEC-Cl.[19,20] Derivatization of DBCPC-Cl with amines could be accomplished within 3–4 min at room temperature. With pH > 10, the fluorescence intensities of the tested standard amines decreased with increasing the reaction times. This was probably due to the fact that the DBCPC-amine derivatives rapidly resulted in the decomposition in high basic medium. Therefore, the derivatization solution should immediately be neutralized to pH 6.0–6.5 with 36% acetic acid solution when derivatization was accomplished. The fluorescence intensity of DBCPC-derivatives increased with increasing the amounts of derivatization reagent. A constant fluorescence intensity was achieved with the addition of 4 fold molar reagent excess to total molar amines, increasing the excess of reagent beyond this level had no significant effect on yields. With as little as a 2.0 fold molar excess of derivatization reagent, the derivatization of amines was incomplete and it obviously resulted in low detection responses. Two side reactions were also observed, the formation of the by-products should be attributed to the reagent hydrolysis. The by-products were, respectively, 1-[1,2,5,6-dibenzocarbazol-9-yl]propanol (DBCPC-OH) and bis-(1-[1,2,5,6-dibenzocarbazol-9-yl]propanol)-carbonate $(DBCPC)_2$. The di-substituted by-product $(DBCPC)_2$ was formed by the reaction of the hydrolysated DBCPC-OH with the excess reagent DBCPC-Cl. The presence of DBCPC-OH and $(DBCPC)_2$ did not interfere with the separation of amine derivatives. The derivatized amines were found to be stable for more than 48 h at room temperature when the derivatization solution was neutralized to pH 6.0–6.5 with 36% acetic acid solution.

HPLC Separation and MS Identification for Derivatized Amines

For the separation of derivatized amines, several mobile phase compositions were tested. For the rapidly simultaneous separation of 12 amine derivatives, a reversed phase Eclipse XDB-C8 column was selected and eluted with (A) 30% acetonitrile containing 20 mmol/L formic acid and (B) 100% acetonitrile. The gradient elution from 70% A + 30% B to 100% B within 35 min was used to give the best resolution with the shortest retention times and the sharpest peaks. As observed, to achieve optimal separation, the pH value of mobile phase **A** can significantly affect the resolution of all amine derivatives. Separation of the derivatized amine standards can be accomplished at acidic condition with pH 3.5. In comparison with the acidic conditions (pH 3.5), operation at pH > 7.0 resulted in an obvious increase in retention value for most amine derivatives. Subsequently, all separation was carried out with acidic eluent of pH 3.5. The complete separation for the derivatized amine standards was shown in Figure 2.

As expected, the DBCPC amine derivatives also exhibit excellent ionizable efficiency under the acidic elution conditions. Derivatives show intense protonated molecular ion corresponding m/z $[M+H]^+$ in positive-ion mode and regular fragment ions. The MS and MS/MS spectra of representative C11 amine derivative are shown in Figures 3a and 3b. The cleavage mode is also shown in Figure b. The characteristic fragment ion at m/z 307.7 comes from the cleavage of $RNCH_2CH(CH_3)$-OCONHR′ (RN: molecular core structure; R′: alkyl group) bond, and the fragment ion at m/z 325.6 comes from the cleavage of $RNCH_2CH(CH_3)O-CONHR'$ bond.

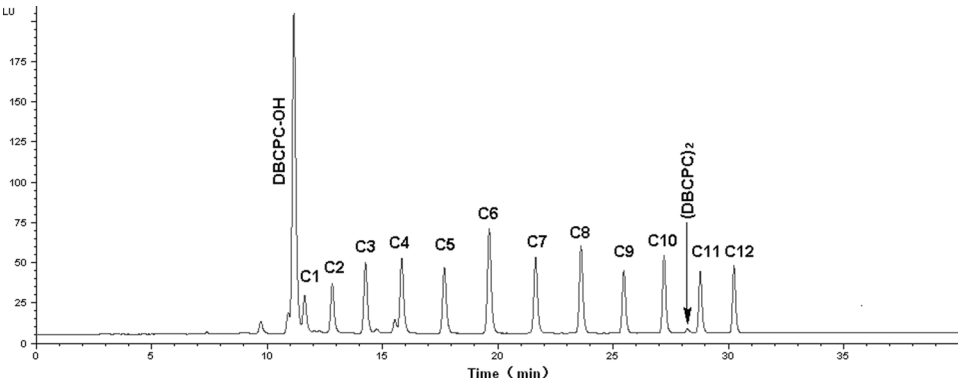


FIGURE 2 Chromatogram for DBCPC-amine derivatives (FLD detection: $\lambda_{ex}/\lambda_{em}$ 300 nm/390 nm) C1. methylamine; C2. ethylamine; C3. propylamine; C4. butylamine; C5. pentylamine; C6. hexylamine; C7. heptylamine); C8. octylamine; C9. nonylamine; C10. decylamine; C11. undecylamine; C12. dodecylamine; DBCPC-OH: 1-[1,2,5,6-dibenzocarbazol-9-yl] propanol; $(DBCPC)_2$: bis(1-[1,2,5,6-dibenzocarbazol-9-yl] propanol)-carbonate.

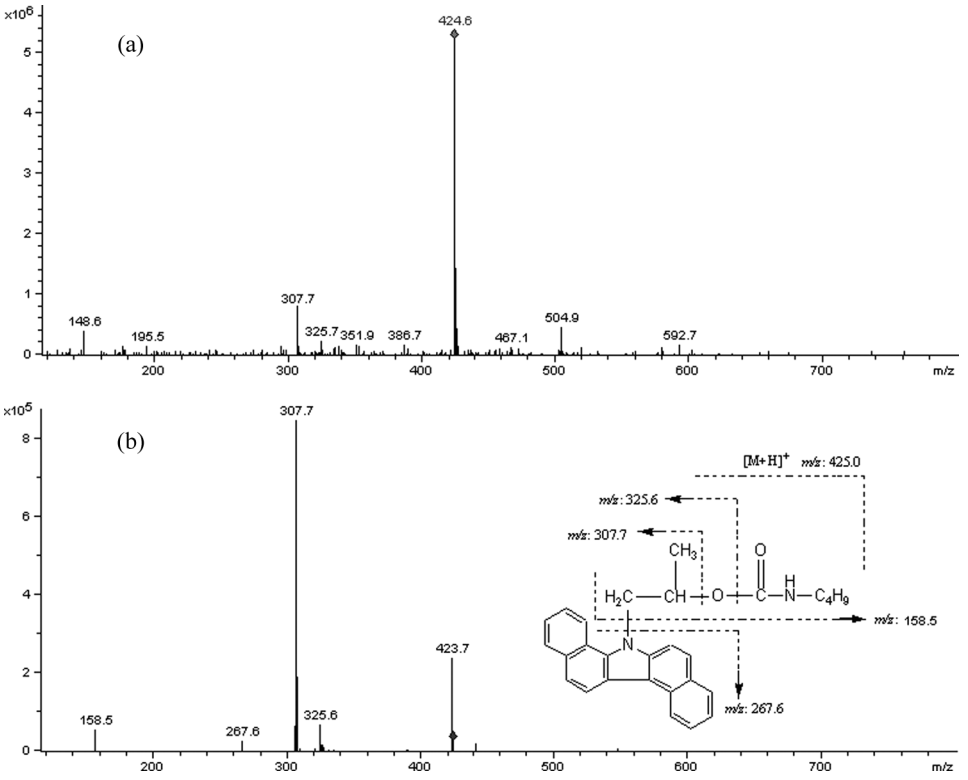


FIGURE 3 The profile of mass spectra and cleavage mode of representative C4 amine derivative, (a) MS; (b) MS/MS and cleavage mode.

The selected reaction monitoring, based on the $m/z\ [M+H]^+ \rightarrow m/z\ 307.7$ and $m/z\ 325.6$ transitions, was specific for amine derivatives. There was no detectable signal from the blank water sample using this transition. Although other endogenous basic compounds present in the natural environmental sample were presumably coextracted and derivatized by DBCPC-Cl reagent, no interference was observed due to the highly specific parent mass-to-charge ratio and the characteristic product ions in the $m/z\ [M+H]^+ \rightarrow m/z\ 307.7$ and $m/z\ 325.6$ transitions. To reduce the disturbance to a minimum, the gradient elution with HPLC for the separation and determination of derivatized DBCPC-amines was an efficient method.

Method Validity

Detection Limits and Linearity for Derivatized Amines

The linear regressions of amines were established over a concentration range from 1.0×10^{-3} to $2.5\,\mu mol\,L^{-1}$(The corresponding injected

amounts of amines were from 25 pmol to 0.010 pmol). All of the amines were found to give excellent linear responses over this range, with correlation coefficients of >0.9991. The linear regression analysis for higher concentrations of amines was not tested due to all the peaks with large overruns. The linear regression equations, correlation coefficients (r) and detection limits (LOD) are shown in Table 2. The calculated detection limits with an injection of 0.025 pmol for each derivatized amine were from 0.3–5.1 fmol (at a signal-to-noise ratio = 3:1).

Repeatability, Precision, and Accuracy

A standard containing 50 pmol DBCPC amine derivatives was prepared to examine the method repeatability. For within-day successive determination ($n = 12$), the relative standard deviations (RSD) of the tested aliphatic amines were 0.051–0.079% for retention time and 0.84–1.12% for peak area. The precisions were examined by using three water samples, which were respectively spiked with 0.05, 0.1, and 0.2 μmol/L of amine. The RSD for intra-assay determination ($n = 6$) were 1.68–2.52% for the tested amines, and the inter-assay precision ($n = 5$) for all amines were <3.74%.

The accuracy was evaluated with recoveries of amines from real water samples. In the tested water samples, the known amount of the twelve above mentioned amines was added. The samples were treated according to the method as described in the text and derivatized with DBCPC-Cl, and analyses were carried out ($n = 5$). The experimental recoveries are in the range of 86.9–104.7% with their standard deviations in the range of 1.80–2.56.

Comparison of Fluorescence Responses of 12 Amines Derivatized by DBCPC-Cl, BCEC-Cl, BCEOC-Cl, CEOC-Cl and FMOC-Cl

Relative fluorescence responses for 12 amines derivatized by DBCPC-Cl (λex/λem: 300/395 nm), BCEC-Cl (λex/λem: 279/380 nm), BCEOC-Cl (λex/λem: 333/390 nm), CEOC-Cl (λex/λem: 293/360 nm), and FMOC-Cl (λex/λem: 265/310 nm) were investigated under the same chromatographic conditions. The results indicated that fluorescence intensity for derivatized amines using DBCPC-Cl as labeling reagents were, respectively, 1.02–1.60-fold, 1.30–2.76-fold, 2.54–4.67-fold, and 2.20–4.12-fold greater compared with those using BCEC-Cl, BCEOC-Cl, CEOC-Cl, and FMOC-Cl as labeling reagents. A comparison of the proposed method (using DBCPC-Cl as labeling reagent) with those reported methods (using FMOC-Cl, CEOC-Cl, BCEOC-Cl, and BCEC-Cl as labeling reagent, respectively) was carried out in respect of detection limits (Table 2), and the results show that the proposed method exhibit highest detection sensitivities for amines with the lowest LODs of 0.3–5.1 fmol. This was probably

TABLE 2 Linear Regression Equations, Correlation Coefficients, Detection Limits and MS Data for 12 Aliphatic Amine Derivatives

Amine Derivatives	MS Identification of DBCPC-amine					LOD of Amines Labeled with Previous Reagents (fmol)			
	MS	MS/MS	$Y = AX + B^*$	R	LOD (fmol)	FMOC [12]**	CEOC [16]	BCEOC [18]	BCEC [19]
C_1	382.6	307.7, 325.7	$Y = 5.78 \times 10^{-2}X + 27.42$	0.9996	5.1	162	–	29.77	14.4
C_2	396.7	307.7, 325.7, 129.6, 267.0	$Y = 6.81 \times 10^{-2}X + 52.56$	0.9991	0.56	56	–	37.68	5.75
C_3	410.8	307.7, 325.6, 143.6, 266.8	$Y = 6.02 \times 10^{-2}X + 29.26$	0.9996	0.69	85	–	25.56	6.27
C_4	424.6	307.7, 325.6, 158.5, 267.6	$Y = 7.64 \times 10^{-2}X + 43.24$	0.9995	0.86	137	–	18.65	3.28
C_5	438.7	307.7, 325.6, 171.6	$Y = 7.53 \times 10^{-2}X + 39.75$	0.9996	0.87	575	–	27.95	4.30
C_6	453.0	307.7, 325.8, 185.7, 267.8	$Y = 1.89 \times 10^{-2}X + 85.34$	0.9995	0.44	248	–	35.58	3.63
C_7	466.9	307.7, 325.7, 199.7	$Y = 8.56 \times 10^{-2}X + 49.56$	0.9995	0.62	–	132	38.82	4.90
C_8	480.9	307.7, 325.6, 213.7, 267.5	$Y = 1.32 \times 10^{-2}X + 41.21$	0.9995	0.46	–	521	32.26	3.45
C_9	494.6	307.7, 325.8, 227.5, 266.8	$Y = 7.89 \times 10^{-2}X + 55.16$	0.9995	0.30	–	–	26.40	2.02
C_{10}	508.9	307.7, 325.2, 241.7, 266.5	$Y = 9.57 \times 10^{-2}X + 47.83$	0.9996	0.31	–	504	21.72	2.30
C_{11}	522.6	307.7, 325.8, 255.7, 267.8	$Y = 8.04 \times 10^{-2}X + 40.89$	0.9996	0.43	–	–	36.57	1.92
C_{12}	536.6	307.7, 325.7, 269.8	$Y = 9.01 \times 10^{-2}X + 45.69$	0.9995	0.51	–	–	28.80	1.77

*X: injected amount (pmol); Y: peak area.

**LOD (fmol) of FMOC-amine was conversed from LOD (μg/L) in literature [12].

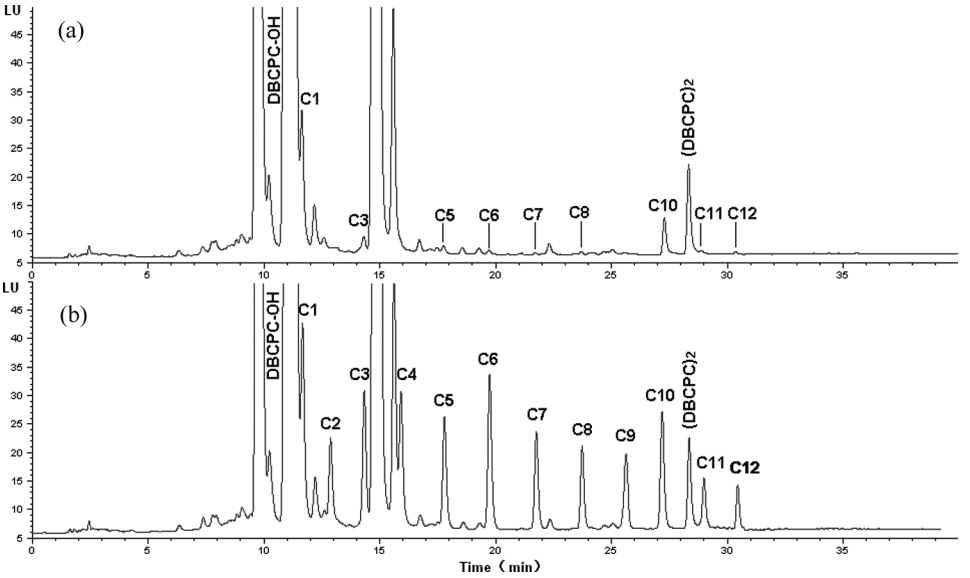


FIGURE 4 Chromatograms of primary aliphatic amines from river water sample (a) and river water spiked with standard amines (b). (Chromatographic conditions and peaks as Figure 2).

due to the fact that DBCPC-Cl had the larger molar absorbance coefficients that made it more sensitive for derivatizing amines. The difference in molar absorbance coefficient might be attributed to the introduction of two benzo functional group to the carbozole molecular structure, in which an $n-\pi$ conjugation system was obviously augmented. This made DBCPC-Cl the most sensitive labeling reagent among the compared fives.

TABLE 3 Contents of Fatty Amines from Environmental Water Sample and Recoveries

Amines	Original Value (ng/mL)	Added Value (ng/mL)	Total Found Value (ng/mL)	Recovery (%, $n=5$)
C1	2.17	2.00	3.91	86.9 ± 3.08
C2	*	0.50	0.469	93.9 ± 2.47
C3	0.342	0.50	0.821	95.8 ± 2.08
C4	*	0.50	0.490	97.9 ± 3.46
C5	0.236	0.50	0.728	98.4 ± 2.16
C6	0.143	0.10	0.245	101.7 ± 1.69
C7	0.108	0.10	0.209	100.7 ± 3.04
C8	0.077	0.10	0.179	102.1 ± 2.02
C9	*	0.10	0.105	104.7 ± 2.47
C10	#	2.00	2.07	103.3 ± 3.54
C11	0.072	0.10	0.175	102.8 ± 2.34
C12	0.087	0.10	0.190	102.6 ± 1.96

*Not detection or below LOQ; #Not quantified because of serious interference.

Analysis of Samples

The chromatogram for the analysis of free amines from a water sample with fluorescence detection was shown in Figure 4. Amine compositional data of water samples were shown in Table 3. As can be seen, the established method was suitable for the determination of the aliphatic amine composition from water with satisfactory results. The facile DBCPC-Cl derivatization coupled with mass spectrometry identification allowed the development of a highly sensitive and specific method for the quantitative analysis of trace levels of amines from food or natural environmental samples.

CONCLUSIONS

A new sensitive fluorescent labeling reagent, 1-[1,2,5,6-dibenzocarbazol-9-yl]propan-2-yl chloroformate (DBCPC-Cl) was developed for the determination of amines by HPLC. The UV absorption intensity of the DBCPC-Cl reagent at the wavelengths of 290 and 301 nm was clearly enhanced. Compared to amine derivatives obtained using BCEC-Cl, BCEOC-Cl, CEOC-Cl, and FMOC-Cl as labeling reagents, DBCPC amine derivatives exhibited relatively high fluorescence intensity and excellent MS ionizable potential. The improved reagent for quantitative analysis of amines had been demonstrated in detail. One of the most attractive features of this method exhibits its simplicity for the preparation of amine derivatives. It takes less than 4 minutes to perform derivatization under mild conditions. Detection limits are in the femtomole range (0.3–5.1 fmol). Current studies should further explore the derivatization of different amino containing compounds such as aromatic amines, polyamines and amino acids.

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