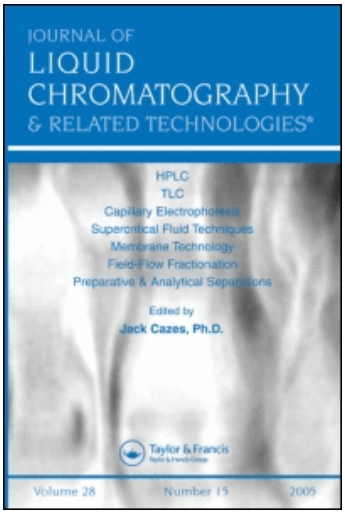

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Abstract: A pre-column derivatization method for the sensitive determination of aliphatic amines using the labeling reagent 1,2-benzo-3,4-dihydrocarbazole-9-ethyl chloroformate (BCEOC) followed by HPLC with fluorescence detection and APCI/MS identification in positive-ion mode has been developed. The chromophore of 2-(9-carbazole)-ethyl chloroformate (CEOC) reagent was replaced by the 1,2-benzo-3,4-dihydrocarbazole functional group, which resulted in a sensitive fluorescence derivatizing reagent, BCEOC, that could easily and quickly label amines. Derivatives were stable enough to be efficiently analyzed by HPLC and showed an intense protonated molecular ion corresponding m/z $[M+H]^+$ with APCI/MS in positive-ion mode. The collision induced dissociation of the protonated molecular ion formed characteristic fragment ions at m/z 264.1, m/z 246.0 and m/z 218.1, corresponding to the cleavages of CH_2CH_2O-CO, CH_2CH_2-OCO, and N-CH_2CH_2O bonds. Studies on derivatization conditions demonstrated that excellent derivatization yields close to 100% were observed with a 3 to 4-fold molar reagent excess in acetonitrile solvent, in the presence of borate buffer (pH 9.0) at 40°C for 10 min. In addition, the detection responses for BCEOC derivatives were compared with those obtained with CEOC

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and FMOC as labeling reagents. The ratios I_{BCEOC}/I_{CEOC} and I_{BCEOC}/I_{FMOC} were, respectively, 1.40–2.76 and 1.36–2.92 for fluorescence responses (here, I was the relative fluorescence intensity). Separation of the amine derivatives had been optimized on an Eclipse XDB-C_8 column. Detection limits calculated from an 0.10 pmol injection, at a signal-to-noise ratio of 3, were 18.65–38.82 fmol (injection volume 10 μL) for fluorescence detection. The relative standard deviations for intraday determination ($n = 6$) of standard amine derivatives (50 pmol) were 0.0063–0.037% for retention times and 3.36-6.93% for peak areas. The mean intra- and inter-assay precision for all amines were $<5.4\%$ and 5.8%, respectively. The recoveries of amines ranged from 96 to 113%. Excellent linear responses were observed with correlation coefficients of >0.9994. The established method provided a simple and highly sensitive technique for the quantitative analysis of trace amounts of aliphatic amines from biological and natural environmental samples.

Keywords: Derivatization, Aliphatic amines, HPLC, APCI/MS, 1,2-Benzo-3,4-dihydrocarbazole-9-ethyl chloroformate (BCEOC), Fluorescence detection

INTRODUCTION

Pre-column derivatization in conjunction with reversed-phase liquid chromatography is one of the most used techniques for the determination of amines. In order to achieve high sensitivity in the determination, fluorescence probes are extensively used in chemical and biological sciences for investigating the compositions of significant environmental and biological samples.[1,2] It is well known that most aliphatic and aromatic amines may occur as biodegradation products of organic matter like proteins, amino acids, and other nitrogen containing organic compounds. Volatile amines not only have an unpleasant smell but also possess heat hazards. Moreover, they may react with nitrosating reagents, leading to the formation of potentially carcinogenic N-nitrosamine compounds.[3–7] In addition, amines are often used as raw material or intermediates in the manufacture of a wide range of industrial products and chemical reagents. Therefore, it is important to determine amines in real environmental samples. 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.[8] Other methods, including an enzymatic method,[9,10] have been described for the determination of amines in various matrices. These methods are usually limited due to low sensitivity.

Nowadays, popular methods for the determination of amino compounds are pre-column and post-column derivatization with fluorescence detection. Various fluorescent derivatization reagents were used for labeling amines, including ortho-phthalaldehyde (OPA),[11,12] 4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-F),[13] 9-fluorenylmethyl chloroformate (FMOC-Cl),[14]

1-(9-fluorenyl)ethyl chloroformate (FLEC),[15] 2-(9-anthyl)ethyl chloroformate (AEOC),[16] and 6-aminoquinolyl-*N*-hydroxysuccinimidyl-carbamate (AQC),[17] etc. These labeling reagents have also reported some various shortcomings in their application, such as short detection wavelengths, bad reproducibility, poor stability, and serious interference for the determination of real samples.

In our previous studies,[18–20] we described the synthesis of some fluorescence labeling reagents and its applications for the analysis of amines and amino acids. On the basis of the fluorescence characteristics of carbazole moiety as previously described,[20] we synthesized a sensitive fluorescence labeling reagent 1,2-benzo-3,4-dihydrocarbazole-9-ethyl chloroformate (BCEOC)[18] and described its application for the analysis of 20 amino acids. In this study, we described the determination of aliphatic amines in the wastewater of paper mills and soil, using BCEOC as pre-column labeling reagents by HPLC and MS/MS identification. BCEOC has been found to be very stable in its crystal state and exhibit a stronger fluorescence response and higher MS ionization efficiency in APCI positive-ion mode by comparison with FMOC and CEOC as labeling reagents. The optimal derivatization conditions such as buffer pH, reaction time, reaction temperature, and solvent system were investigated. Linearity, reproducibility, detection limits, and precision of the procedure were also determined. The suitability of the developed method for the analysis of amines from real samples was satisfactory.

EXPERIMENTAL

Instrumentation

Experiments were performed using an Agilent HP 1100 Series liquid chromatograph and a tandem mass spectrometer (LC-MSD Trap SL). All the HPLC system devices were from the Agilent HP 1100 series and consisted of a online vacuum degasser (model G1322A), a quaternary pump (model G1311A), an autosampler (model G1329A), a thermostated column compartment (model G1316A), and a fluorescence detector (FLD) (model G1321A). Derivatives were separated on a reversed-phase Eclipse XDB-C_8 column (150 × 4.6 mm, 5 μm, Agilent Co.) by a gradient elution. The HPLC system was controlled by HP Chemstation software. The mass spectrometer 1100 Series LC/MSD Trap-SL (ion trap) from Bruker Daltonik (Bremen, Germany) was equipped with an APCI source. The mass spectrometer system was controlled by Esquire-LC NT software, version 4.1. Ion source conditions: APCI in positive-ion mode; nebulizer pressure 413.69 KPa; dry gas temperature, 350°C; dry gas flow, 5.0 L/min. APCI Vap temperature 450°C; corona current (nA) 4000 (pos); capillary voltage 3500V. Fluorescence excitation and emission spectra were obtained on a 650-10 S

fluorescence spectrophotometer (Hitachi). Excitation and emission bandpass are both set at 10 nm. A Paratherm U2 electronic water bath (Hitachi, Tokyo, Japan) was used to control temperature. The mobile phase was filtered through a 0.2 μm nylon membrane filter (Alltech, Deerfiled, IL).

Prior to its use, the instrument was checked to meet the sensitivity defined by the manufacturer. The FL was calibrated and tested using the FL diagnosis procedure of the ChemStation software for HP1100 system. The HP1100 LC/MSD Trap SL was calibrated with APCI tuning solution obtained from Agilent Technology (Palo Alto, CA). The mass spectrometer was calibrated so that mass accuracy specification and sensitivity were achieved over the entire mass range. APCI source and instrument parameters were optimized by infusing the BCEOC derivatives that were isolated from an HPLC column using fluorescence detection into the online post-column mass spectrometry.

Chemicals

All aliphatic amine standards were purchased from Sigma Co (St. Louis, MO). Spectroscopically pure acetonitrile was purchased from Merck Co. (Germany). Formic acid and acetic acid were analytical grade from Shanghai Chemical Reagent Co. Water was purified on a Milli-Q system (Millipore, Bedford, MA, USA). Wastewater of a paper mill was obtained from Jin Zhuang paper mill (Si Shui, Shandong Province, China). Borate buffer was prepared from 0.2 mol/L boric acid solution, adjusted to pH 9.0, with 4 mol/L sodium hydroxide solution prepared from sodium hydroxide pellets. The quenching reagent was 50% acetic acid solution. 1,2-Benzo-3,4-dihydrocarbazole-9-ethyl chloroformate (BCEOC)[18] and carbazole-9-ethyl chloroformate (CEOC)[20] were synthesized in our laboratory, 9-Fluorenylmethyl chloroformate (FMOC-Cl) was purchased from Sigma Co for comparison with the MS ion current signals with BCEOC and CEOC.

Preparation of Standard and Sample Solutions

The derivatizing reagent solution, 2.5×10^{-3} mol/L, was prepared by dissolving 8.15 mg 1,2-benzo-3,4-dihydrocarbazole-9-ethyl chloroformate in 10 mL of anhydrous acetonitrile. The individual stock solution of the aliphatic amines was prepared in acetonitrile. The standard amines for HPLC analysis at zindividual concentrations of 5.0×10^{-5} mol/L were prepared by dilution of the corresponding stock solutions (1.0×10^{-2} mol/L) of each amine with acetonitrile. When not in use, all standards were stored at -20°C in a refrigerator.

To a 250 mL round bottom flask, the powered soil (100 g) and 50 mL of chloroform were added, the mixture was vortexed for 1 min and immersed in a sonicator water bath and sonicated for 10 min in 3 min intervals, and then

allowed to stand for another 10 min at room temperature. The suspended organic layer was collected. The residue was again extracted with another 50 mL chloroform, the combined chloroform was added to 1.0 mL of hydrochloric acid, and then the mixture was evaporated to dryness under a stream of nitrogen. The residue was redissolved in 2.0 mL of 80% acetonitrile until HPLC analysis.

Collected wastewater samples from the paper mill were immediately cooled to 0–5°C to avoid volatilization of amines, and then samples were filtered through a 0.45 μm membrane filter, and stored in glass bottles in a refrigerator before extraction. To a 250 mL round-bottom flask, 50 mL of filtered wastewater was added, the contents of the flask were adjusted to pH 3.0 with 6 mol/L HCl, and the water sample was concentrated at 60°C in vacuum to 5 mL. After cooling, the water phase was transferred to another volumetric flask, which was made up to a total volume of 10 mL with 80% acetonitrile. All analyses were immediately performed by derivatization and HPLC analysis within one day.

Derivatization of Standard and Sample

The BCEOC amines derivatization proceeded in a water/acetonitrile (1:1, *v*/*v*) solution in a basic medium. A 100 μL of amine was added in a vial, to which 200 μL borate buffer (0.2 mol/L, pH 9.0), 150 μL acetonitrile, and 75 μL BCEOC solution were consecutively added. The solution was shaken for 0.5 min and allowed to heat at 40°C in a water bath for 10 min. After the reaction was completed, the mixture was cooled at room temperature. Acetic acid of 5 μL (50%, *v*/*v*) was added until the solution pH range of 6.0–6.5, and then a 300 μL mixture solution of acetonitrile and water (ACN/H_2O, 1:1, *v*/*v*) was added to dilute the derivatization solution. The diluted solution (10 μL, 60 pmol) was injected directly for chromatographic analysis. The derivatization process is shown in Figure 1. The derivatization of the extracted sample solutions was the same as above.

Chromatographic Conditions

HPLC separation of amine derivatives was carried out on Hypersil BDS C_{18} column in conjunction with a gradient elution. Eluent A was 30% of acetonitrile containing 10 mmol/L acetic acid (pH 3.7); B was acetonitrile (100%). Gradient conditions: initial = 80% A and 20% B; 40 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 $\lambda_{ex} = 333$ and $\lambda_{em} = 390$ nm, respectively.

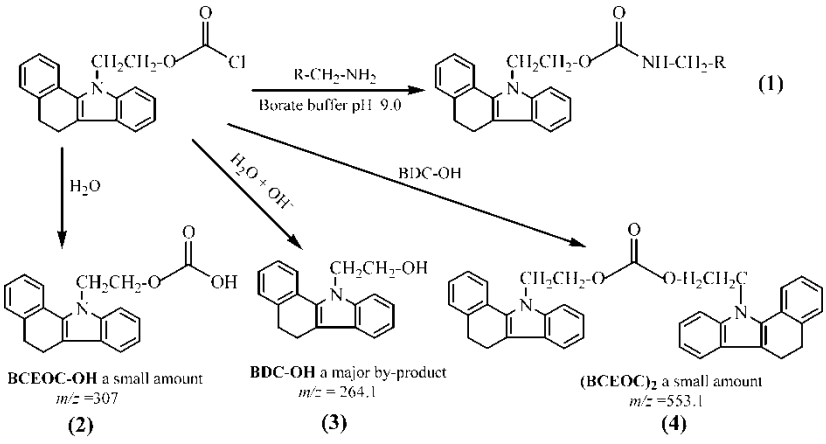

Figure 1. Derivatization scheme of 1,2-benzo-3,4-dihydrocarbazole-ethyl chloroformate (BCEOC) with aliphatic amines (**1**), hydrolysis of BCEOC in basic aqueous media to form BCEOC-OH (**2**), and BDC-OH (**3**), and the reaction of BCEOC and BDC-OH to form $(BCEOC)_2$ (**4**).

RESULTS AND DISCUSSION

Ultraviolet and Fluorescence Spectrum of BCEOC

The absorption wavelength of BCEOC was obtained with the scanning range of 200 to 400 nm. Maximum ultraviolet absorption responses were observed at the wavelengths of 249 nm and 320 nm, respectively. The molar absorption coefficients (ε) were 2.54×10^4 L mol^{-1} cm^{-1} (249 nm) and 2.40×10^4 L mol^{-1} cm^{-1} (320 nm), respectively. The fluorescence excitation and emission spectra of BCEOC were scanned on a 650-10S fluorescence spectrophotometer (Hitachi) with the excitation and emission bandpass both at 10 nm. The maximum fluorescence excitation and emission wavelengths of BCEOC in acetonitrile were at $\lambda ex = 333$ and $\lambda em = 390$ nm, respectively. The spectra of fluorescence excitation and emission were shown in Figure 2.

Optimal Derivatization

The effect of the reaction temperature on the derivatization yields was evaluated from 30°C to 90°C. As observed, fluorescence responses reached the maximum at 40°C within 10 min, indicating that BCEOC reacted rapidly with amines under mild conditions to form fluorescence derivatives. Several types of basic media were tested in this study for derivatization, including carbonate buffers, phosphate buffers, and borate buffers. The

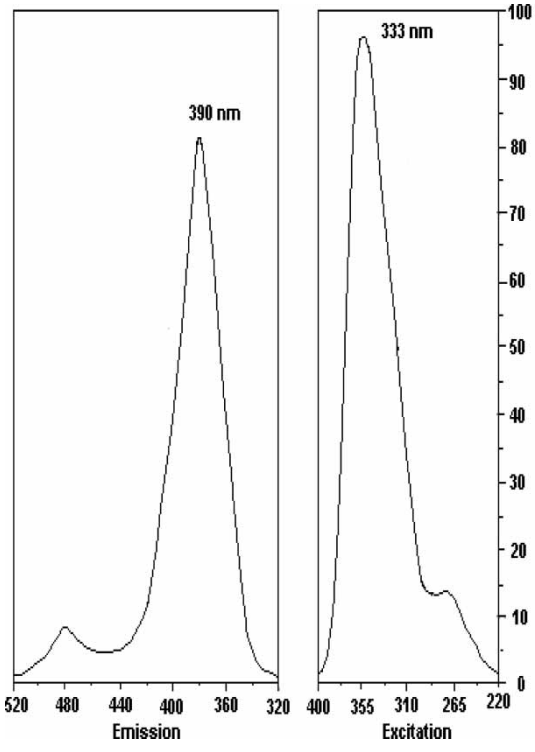

Figure 2. Fluorescence excitation and emission spectra of BCEOC in acetonitrile.

maximum fluorescence response was obtained with borate buffer in aqueous acetonitrile. Acetonitrile was used to avoid the problem of precipitation of hydrophobic amine derivatives. However, high concentration buffers (>0.3 mol/L) yielded a slight reduction of derivatization yields, which could be avoided by adjusting borate buffer concentration to ≤ 0.2 mol/L. To achieve optimal derivatization of amines, the buffers must be within the suitable pH range and provide adequate buffering capacity. The effect of pH on the derivatization reaction was then investigated with borate buffer (0.2 mol/L) in the pH range of 8.0–10.5. The maximum derivatization yields were achieved with 0.2 mol/L borate buffer at pH = 9.0. In addition, the effect of BCEOC concentrations on the derivatization yields was investigated for amine derivatives. The fluorescence intensity of amine derivatives increased along with the increasing amounts of reagent. A constant fluorescence intensity was achieved with the addition of 3-fold molar reagent excess to total molar aliphatic amines, and with a further excess of BCEOC reagent beyond this level, there was no significant effect on yields. To an unknown concentration of sample, such as the extracted wastewater and soil samples, complete derivatization was guaranteed by using an excess of BCEOC until constant peak intensity for detector responses.

HPLC Separation and MS/MS Identification

An Eclipse XDB-C_8 column was selected in conjunction with a gradient elution, several programs were investigated to ensure satisfactory HPLC separation within the shortest time. The optimal gradient elution was carried out as described above. In addition, the choice of pH value of mobile phase **A** was also tested. Complete separation of the derivatized amines could be accomplished at pH = 3.7 with 10 mmol/L acetic acid. With acetic acid >15 mmol/L, most of the amine derivatives were resolved with the exception being $(BCEOC)_2$/nonylamine partially coeluted. In comparison with the acidic conditions (acetic acid >10 mmol/L), operation at acetic acid <10 mmol/L resulted in an obvious increase in retention time for most of amines derivatives. The complete separation of standard mixtures consisting of 12 amines on an Eclipse XDB-C_8 column is shown in Figure 3. A side reaction was that the reagent reacted with its hydrolysis product (BCEOC-OH) to give the bis-(1,2-benzo-3,4-dihydrocarbazole-9-ethyl)-carbonate $(BCEOC)_2$ (*m/z:* 553.1, see Figure 1). Another two side reactions came from its hydrolysis of BCEOC reagent and resulted in two by-products 1,2-benzo-3,4-dihydrocarbazole-9-ethanol (BDC-OH) (*m/z:* 246) and mono-(1,2-benzo-3,4-dihydrocarbazole-9-ethyl)carbonnate (BCEOC-OH) (*m/z*: 307, see Figure 1). The presence of $(BCEOC)_2$ and BCD-OH (major by-product) did not interfere with the separation of other amine compounds by the adjusting of the pH of eluent A and gradient elution procedure.

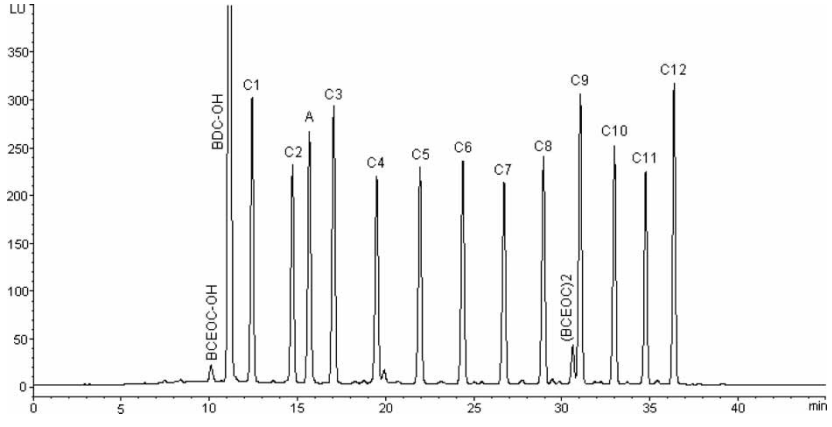

Figure 3. Chromatogram for 60 pmol of 12 standard amines derivatized with BCEOC. Chromatographic conditions as in text. C1 methylamine; C2 ethylamine; C3 propylamine; C4 butylamine; C5 pentylamine; C6 hexylamine; C7 heptylamine; C8 octylamine; C9 nonylamine; C10 decylamine; C11 undecylamine; C12 dodecylamine; A: unidentified. BCEOC-OH mono-(1,2-benzo-3,4- dihydrocarbazole-9-ethyl)-carbonate; BDC-OH (1,2-benzo-3,4-dihydrocarbazole-9-ethanol); $(BCEOC)_2$ (bis-(1,2-benzo-3,4-dihydrocarbazole-9-ethyl) carbonate).

The ionization and fragmentation of the isolated BCEOC amine derivatives was studied by mass spectrometry with atmospheric pressure chemical ionization (APCI) source in positive-ion mode. As expected, the amine derivatives produced an intense molecular ion peak at m/z $[M + H]^+$ (Figure 4 A). With MS/MS analysis of amine derivatives, the collision induced dissociation spectra of m/z $[M + H]^+$ produced the specific fragment ions at m/z 264.1, m/z 246.0, and m/z 218.1 corresponding to the cleavages of CH_2CH_2O-CO, CH_2CH_2-OCO, and N-CH_2CH_2O bonds. With APCI/MS in positive-ion mode, intense ion current signals should be attributed to the introduction of a weak basic nitrogen atom in BCEOC molecular core structure resulting in highly ionizable efficiency. The MS/MS analysis and cleavage mode for a representative BCEOC-C_7 derivative is shown in Figure 4 (A, B, C). All molecular ions $[M + H]^+$ and specific fragment ions for 12 BCEOC amine derivatives are shown in Table 1.

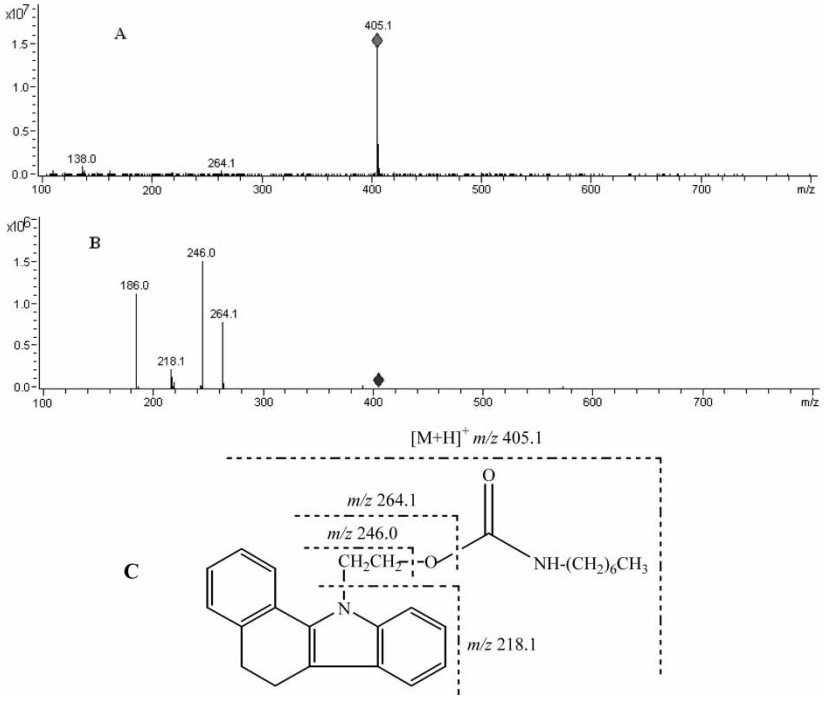

Figure 4. The profile of molecular ion chromatogram and MS/MS scanning of the isolated representative heptylamine derivatives (BCEOC-C_7). (A) Typical HPLC-MS chromatogram of heptylamine derivatives from full scanning range from 100 to 800 amu with APCI in positive-ion mode. (B) Typical MS/MS chromatogram of heptylamine derivative from full scanning range from 100 to 800 amu with APCI in positive-ion mode. (C) The MS/MS cleavage mode of BCEOC-C_7 derivative.

Table 1. Comparison of fluorescence sensitivity and MS ionization for BCEOC-, CEOC-, and FMOC-amine derivatives

	Ratio of FL intensity		$[M+H]^+$			MS/MS		
Amines	I_{BCEOC}/I_{CEOC}	I_{BCEOC}/I_{FMOC}	BCEOC	CEOC	FMOC	BCEOC	CEOC	FMOC
C1	1.62	1.47	321.1	[a]	[a]	264.1, 246.0	[a]	[a]
C2	1.48	1.53	335.2	283.1	[a]	264.1, 246.0	194, 116	[a]
C3	1.76	1.36	349.2	297.1	[a]	264.0, 246.0	212, 194	[a]
C4	1.40	1.84	363.2	311.0	[a]	264.1, 246.0	212, 194	[a]
C5	2.14	2.09	377.2	325.1	[a]	264.0, 246.0	212, 194	[a]
C6	1.96	2.38	391.2	339.0	[a]	264.0, 246.0	212, 194	[a]
C7	2.52	2.05	405.2	353.1	[a]	264.1, 246.0	212, 194	[a]
C8	2.37	2.26	419.2	367.1	[a]	264.0, 246.0	212, 194	[a]
C9	2.11	2.34	433.2	381.1	[a]	264.0, 246.0	212, 194	[a]
C10	1.95	2.92	447.1	395.1	[a]	264.0, 246.1	212, 194	[a]
C11	2.76	2.78	461.2	409.0	[a]	263.9, 246.0	212, 194	[a]
C12	2.61	2.63	475.3	423.2	[a]	264.1, 246.0	212, 194	[a]

[a]no signal.

Comparison of Fluorescence Responses and MS Ionization of BCEOC, CEOC, and FMOC

Relative fluorescence responses for BCEOC ($\lambda_{ex}/\lambda_{em}$: 333/390 nm), CEOC ($\lambda_{ex}/\lambda_{em}$: 335/360 nm), and FMOC ($\lambda_{ex}/\lambda_{em}$: 263/313 nm) for amine derivatives were investigated under the same chromatographic conditions. The results indicated that fluorescence intensity for derivatized amines using BCEOC as labeling reagents were, respectively, 1.40–2.76-fold and 1.36–2.92-fold greater compared with that using CEOC and FMOC as labeling reagents (Table 1). This was probably due to the fact that BCEOC had the larger molar absorbance coefficients that made it more sensitive for derivatizing amines (BCEOC: $\varepsilon = 2.54 \times 10^4$ L mol^{-1} cm^{-1} (249 nm); CEOC: $\varepsilon = 2.34 \times 10^4$ L mol^{-1} cm^{-1} (256 nm); FMOC: 1.7×10^4 L mol^{-1} cm^{-1} (254 nm).[20] The difference in molar absorbance coefficient might be attributed to the introduction of a 1,2-benzo functional group to the BCEOC molecular structure, in which an n-π conjugation system was obviously augmented.

As described above, BCEOC amine derivatives produced an intense molecular ion peak at m/z $[M + H]^+$ (Figure 5 A) and specific fragment ions at m/z 264.1, m/z 246.0, and m/z 218.1. In addition, the ionization efficiency of CEOC[20] was also investigated. As expected, the CEOC amine derivatives could also produce a molecular ion peak at m/z $[M + H]^+$ in positive ion mode (Figure 5 B). The collision induced dissociation of molecular ion for CEOC derivatives generated fragment ions, respectively, at m/z 194.0 and m/z 212.0. All molecular ions $[M + H]^+$ and specific fragment ions for 12 amine derivatives are shown in Table 1. However, total ion current intensity of molecular ions for BCEOC-amine derivatives was 10-15-fold stronger compared with that of molecular ions for CEOC-amine derivatives. This was probably, in part, due to the fact that the BCEOC molecule had a basic nitrogen atom and obviously augmented n-π conjugation system, which resulted in a more stable molecular ion and higher ionization efficiency. However, in contrast with BCEOC and CEOC, no detectable molecular ion signal from FMOC-amine derivatives was observed with APCI/MS detection in positive or negative ion mode (Figure 5 C). This was probably due to the fact that the FMOC molecule did not form a stable molecular ion owing to having no conjugated basic nitrogen atom in its molecular core structure.

Reproducibility, Precision, Linearity, and Detection Limits

A standard solution containing C_1-C_{12} aliphatic amines (5×10^{-5}mol/L) was prepared, and the method reproducibility was examined by injecting quantitative amine derivatives six times (corresponding injected amount 60 pmol, 10 μL). The relative standard deviations (R.S.D.s) of the peak areas and retention times are from 3.36 to 6.93% and from 0.0063 to 0.037% (shown

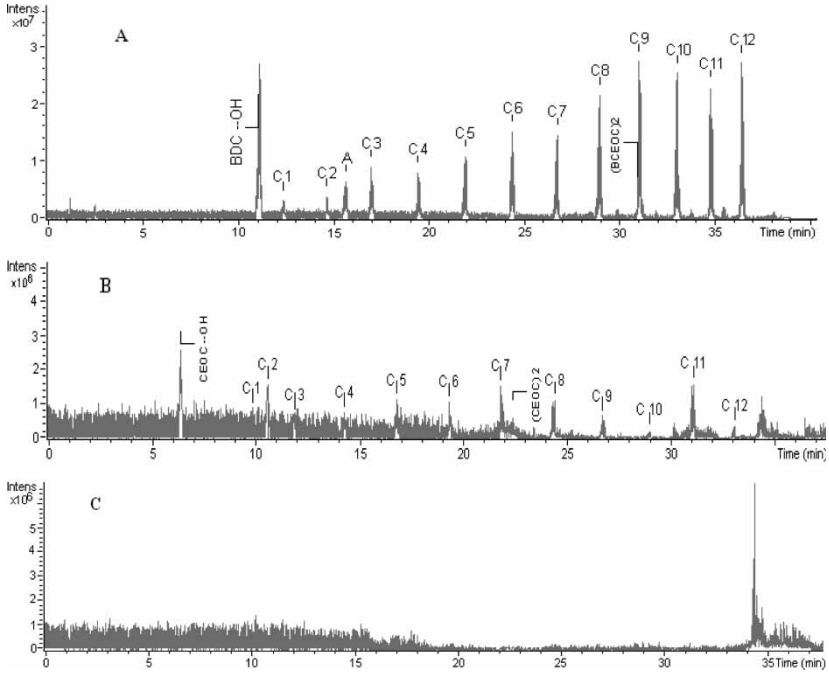

Figure 5. The profile of total ion current (TIC) MS chromatogram for 60 pmol aliphatic amine derivatives using BCEOC (A), CEOC (B), FMOC (C) as labeling reagent, respectively. (A) TIC MS chromatogram of 60 pmol BCEOCamine derivatives in positive ion mode. (B) TIC MS chromatogram of 60 pmol CEOC amine derivatives in positive ion mode. (C) TIC MS chromatogram of 60 pmol FMOC-amine derivatives with no signal in positive or negtive ion mode.

in Table 2), respectively. Precision and accuracy: Six replicates ($n = 6$) at 0.1, 1.0, and 5.0 μmol/L of 12 amines were used to make the low to high range concentrations. The mean inter-day accuracy ranged from 95.2 to 105.4% with the largest mean coefficients of variation (R.S.D.) $<5.8\%$. The mean intra-day precision for all standards was $<5.4\%$ of the expected concentration.

Based on the optimum derivatization conditions, the linearities of 12 amines ($C_1 \sim C_{12}$) were evaluated in the range of 6.25×10^{-3} to 18 μmol/L (injection volume 10 μL, injected amount from 180.0 pmol to 62.5 fmol with a 2880-fold concentration range). The calibration graph was established with the peak area (y) versus amine concentration (x: pmol, injected amount). The linear regression equations were shown in Table 2. All amines were found to give excellent linear responses over this range with correlation coefficients of 0.9994∼0.9998. The linear relationships for higher concentrations were not tested because they were out of the linearity range. With 1.0 pmol injection for each derivatized amine, the calculated detection limits (at signal-to-noise of 3:1, $S/N = 3:1$) are from 18.65 to 38.82 fmol (injection volume 10 μL, shown in Table 2).

Table 2. Linearity, correlation coefficients, detection limits and reproducibility of retention time and peak area (n = 6) for amine derivatives

Amines	Y = A * X + B X: Injected amount (pmol) Y: Peak area	Correlation coefficients	Detection limits (fmol)	Retention time RSD (%)	Peak area RSD (%)
C1	Y = 54.09X + 37.07	0.9996	29.77	0.037	3.88
C2	Y = 43.56X + 15.41	0.9998	37.68	0.033	3.83
C3	Y = 55.77X + 9.606	0.9995	25.56	0.034	3.86
C4	Y = 42.98X + 23.21	0.9994	18.65	0.028	3.81
C5	Y = 45.81X + 8.41	0.9995	27.95	0.020	3.88
C6	Y = 46.48X + 3.67	0.9995	35.58	0.016	3.76
C7	Y = 42.04X + 1.78	0.9997	38.82	0.015	3.89
C8	Y = 46.58X + 1.71	0.9997	32.26	0.014	3.91
C9	Y = 58.06X − 1.26	0.9997	26.40	0.011	4.09
C10	Y = 46.23X + 2.97	0.9997	21.72	0.015	3.36
C11	Y = 39.84X − 5.25	0.9997	36.57	0.015	5.49
C12	Y = 53.68X − 12.14	0.9996	28.80	0.0063	6.93

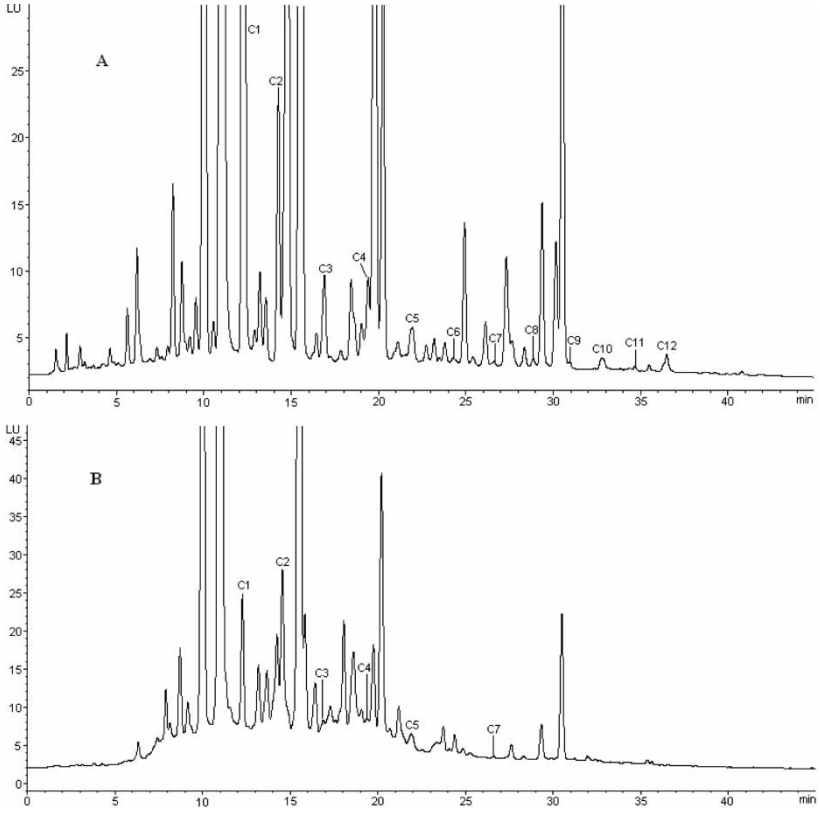

Figure 6. Chromatogram of derivatized amines from extracted soil (A) and wastewater of paper mill (B). Chromatographic conditions and peaks as Figure 3.

Analysis of Samples and Recovery

The chromatogram for the analysis of aliphatic amines extracted from soil (A) and wastewater (B) of paper mills with fluorescence detection are shown in Figure 6 (A, B), respectively. Chromatographic peaks were identified by contrasting chromatograms of real samples with that of amine standards by retention time, and simultaneously confirmed by post-column mass spectrometry identification. Quantitative derivatization of amines in the extracts of soil and wastewater of paper mills to their BCEOC derivatives was guaranteed by using an excess of the BCEOC labeling reagent. All amines were quantified by linear regression equations. Aliphatic amines compositional data from extracted soil and wastewater of paper mills are shown in Table 3.

The recoveries of 12 amines were investigated by the addition of known amounts of 12 amines standard solution (10 μL, 5.0×10^{-5} mol/L) into the wastewater of paper mills, in which the contents of amines were in accordance

Table 3. Compositional analysis of free amines from soil and wastewater of paper mill and recoveries

Amines	Wastewater of paper mill (μg/L)	Soil (ng/g)	Recovery (%)
C1	4.15	14.59	113
C2	9.19	0.29	104
C3	0.68	0.83	101
C4	0.72	1.01	103
C5	0.94	0.70	109
C6	0	0.02	100
C7	0.63	0.08	96
C8	0	0.13	106
C9	0	0.18	97
C10	0	0.56	104
C11	0	0.32	98
C12	0	1.36	102

with the calculation from linear regression equations. The experimental recoveries were in the range of 96∼113% (Table 3).

CONCLUSION

In this study, simultaneous determination of 12 aliphatic amines extracted from wastewater of paper mills and soil using BCEOC as pre-column derivatization with HPLC fluorescence detection and APCI/MS identification could be successfully achieved. The derivatization and separation conditions for the labeled 12 amines were evaluated. Although the labeling reagent BCEOC for amines showed good similarity with CEOC and FMOC, it had higher sensitivity and the detection limits were in the femtomol level. Complete derivatization in basic medium at 40°C took less than 10 min, and derivatives were stable for at least 24 h in neutral solution stored at 4°C in a refrigerator. Hydrolysates of BCEOC reagents did not interfere with the separation by the adjusting of the composition of mobile phase and the gradient elution program. The established method could also be applied to the determination of aliphatic amines from various foods, environmental, and biochemical samples.

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