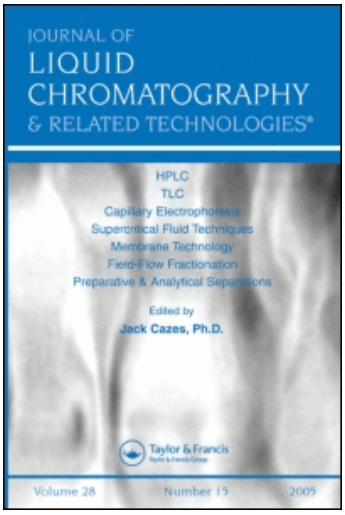

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4-(1-METHYL-1H-PHENANTHRO[9,10-d]IMIDAZOLE-2-) BENZOIC ACID (MPIBA) AND ITS APPLICATION FOR DETERMINATION OF AMINES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH FLUORESCENCE DETECTION AND IDENTIFICATION WITH MASS SPECTROSCOPY/ATMOSPHERIC PRESSURE CHEMICAL IONIZATION

Xia Lian[abc]; Sun Zhiwei[ac]; Suo Yourui[a]; You Jinmao[ab]
[a] Northwest Plateau Institute of Biology, Chinese Academy of Sciences, Xining, P. R. China [b] Key Laboratory of Life-Organic Analysis of Shan Dong Province, College of Chemistry Science, Qufu Normal University, Qufu, P. R. China [c] Graduate University of Chinese Academy of Sciences, Beijing, P. R. China

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4-(1-METHYL-1H-PHENANTHRO[9,10-d]IMIDAZOLE-2-) BENZOIC ACID (MPIBA) AND ITS APPLICATION FOR DETERMINATION OF AMINES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH FLUORESCENCE DETECTION AND IDENTIFICATION WITH MASS SPECTROSCOPY/ATMOSPHERIC PRESSURE CHEMICAL IONIZATION

Xia Lian,[1,2,3] Sun Zhiwei,[1,3] Suo Yourui,[1] and You Jinmao[1,2]

[1]*Northwest Plateau Institute of Biology, Chinese Academy of Sciences, Xining, P. R. China*
[2]*Key Laboratory of Life-Organic Analysis of Shan Dong Province, College of Chemistry Science, Qufu Normal University, Qufu, P. R. China*
[3]*Graduate University of Chinese Academy of Sciences, Beijing, P. R. China*

□ *A simple, sensitive, and mild method for the determination of amines based on a condensation reaction with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC· HCl) as the dehydrant with fluorescence detection has been developed. Amines were derivatized to their acid amides with labeling reagent 4-s(1-methyl-1H-phenanthro[9,10-d]imidazole-2-) benzoic acid (MPIBA). Studies on derivatization conditions indicated that the coupling reaction proceeded rapidly and smoothly in acetonitrile to give the corresponding sensitively fluorescent derivatives with a maximum excitation at λex 260 nm and a maximum emission at λem 446 nm. The labeled derivatives exhibited high stability and were enough to be efficiently analyzed by high-performance liquid chromatography. Identification of derivatives was carried out by online post-column mass spectrometry (LC/APCI–MS/MS) and showed an intense protonated molecular ion corresponding* m/z *$[MH]^+$ under APCI in positive-ion mode. At the same time, the fluorescence properties of derivatives in various solvents were investigated. The method, in conjunction with a gradient elution, offered a baseline resolution of the common amine derivatives on a reversed-phase Eclipse XDB-C8 column. Liquid chromatography separation for the derivatized amines showed good reproducibility with acetonitrile–water as mobile phase. Detection limits calculated from 90.00 pmol to 88 fmol injection, at a signal-to-noise ratio of 3, were 10.5–53.4 fmol. Excellent linear responses were observed with coefficients of >0.9996. The established method for the determination of aliphatic amines from real wastewater was satisfactory.*

Keywords 4-(1-methyl-1H-phenanthro[9,10-d]imidazole-2-) benzoic acid (MPIBA), amines, determination, fluorescence detection, HPLC, mass spectrometry

Correspondence: You Jinmao, College of Chemistry Science, Qufu Normal University, Qufu Shandong 273165, P. R. China. E-mail: jmyou6304@163.com

INTRODUCTION

Aliphatic amines are widely distributed in nature and are paid much attention for their toxicity and reaction activity. Most amine compounds including aliphatic amines may occur as biodegradation products of organic matter such as proteins, amino acids, and other nitrogen-containing organic compounds. Volatile amines may react with nitrosating agents, leading to the formation of potentially carcinogenic N-nitrosamine compounds.[1–5] Therefore, it is important to determine certain amino compounds 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.[6] Other methods including enzymatic[7,8] and ion-exchange chromatographic analysis[9] have been described for the determination of amines in various matrices. These methods are usually limited due to low sensitivity.

Most amine compounds show neither natural UV absorption nor fluorescence. At present, one of the most popular techniques used to improve detection limits is pre-column or post-column derivatization. A large number of derivatization reagents have been developed for the labeling of amine compounds, but at the same time, some shortcomings of these derivatization reagents in the determination of amines were also reported. For example, orthophthalaldehyde(OPA) method had poor reproducibility and stability of derivatives was dissatisfying[10]; 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole(NBD-Cl) had poor stability and underwent about 30–50% decomposition in methanol-water solution within 25 min when exposed to daylight[11]; 6-Aminoquinolyl-N-hydroxycuccinimidyl carbamate (AQC)[12] was developed as a popular pre-column derevatization reagent for the determination of amines with satisfactory results. However, only 10% of the fluorescent intensity in aqueous solution compared with that in pure acetonitrile solution was observed for its derivatives. Thus, the detection limits for early-eluted amine derivatives were usually higher; 9-Fluorenylmethyl chloroformate (FMOC)[13,14] and 1,2-benzo-3,4-dihydrocarbazole-9-ethyl chloroformate[15] have been used for the derivatization of amines, amino acids, and peptides. For the effective derivatization, an excess reagent must be used, and derivatized solution must be extract with pentane to remove excess of reagent because it interfered with the separation of derivatives, as a result, sometimes it resulted in some loss of their hydrophobic derivatives. Furthermore, these reagents were difficult to synthesize and easy to decompose. Based on the fact that fluorescence derivatization reagent with hydrazine group could lable fatty acids sensitively with EDC as condensing reagent[16] and previous works of authors,[17] 4-(1-methyl-1H-phenanthro[9,10-d]imidazole-2-) benzoic acid (MPIBA)was synthesized, which was more sensitive and more easily protonized

in MS detection. Aliphatic amine derivatives were separated with a good baseline resolution in conjunction with a gradient elution and identified by post-column online mass spectrometry with APCI source in positive ion mode. The sensitivity, stability, simple derivatization reaction of MPIBA with amines, and its applications for the determination of aliphatic amines from real samples were satisfactory.

EXPERIMENTAL

Instruments

Experiments were performed using the LC/MSD-Trap-SL electrospray ion trap liquid chromatography/mass spectrometry (1100 Series LC/MSD Trap, a complete LC/MS/MS). All the HPLC system devices were from the HP 1100 series and consisted of 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). Ion source type, APCI (positive mode); nebulizer pressure 60 psi; dry gas temperature, 350°C; dry gas flow, 5.0 L/min. APCI Vap temperature 450°C; corona current (nA) 4000(pos); capillary voltage 3500 V. Derivatives were separated on a semi-prepatation chromatograph (waters 600, USA). The HPLC system was controlled by HP Chemstation software. The mass spectrometer from Bruker Daltonik (Bremen, Germany) was equipped with an atmospheric pressure chemical ionization (APCI) source. The mass spectrometer system was controlled by Esquire-LCNT software, version 4.1. Fluorescence excitation and emission spectra were obtained at a F7000 fluorescence spectrophotometer (Hitachi, Japan). Excitation and emission bandpass are both set at 5 nm. The mobile phase was filtered through a 0.2 μm nylon membrane filter (Alltech, Deerfiled, IL). Semi-preparative HPLC separation was used to obtain the single MPIBA-nonylamine derivative, The semi-preparative HPLC system was Waters Delta 600 (Waters, Japan) and consisted of an online degasser (AF), a Waters 600 controller with Waters 2489 UV/visible detector, an auto-fraction collector III. Reverse-phase semi-preparative HPLC-separation was performed on a SunFire^{TM} Prep-C18 column (10 × 150 mm, 10 μm, Made in Ireland) with Zorbax PrepHT guard cartridge columns (21.2 mm).

Chemicals

All aliphatic amine standards and N-ethyl-N′-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were purchased from Sigma Co. (St. Louis, MO, USA). HPLC grade acetonitrile 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).

Synthesis of Derivatization Reagent (MPIBA)

Synthesis of 4-(1H-phenanthro[9,10] imidazole-2-yl) Benzoic Acid (PIBA)

4-(1H-phenanthro[9,10] imidazole-2-yl) benzoic acid was synthesized as follows: 9,10-phenanthroquinone (8 g), 4-formaxyl-benzoic acid (8 g) and ammonium acetate (60 g) were fully mixed in 250 ml of round-bottom flask. After the addition of 150 glacial acetic acid, the contents were rapidly heated to 80–90°C with vigorous stirring for 3 h. After cooling, the solution was added to 150 ml water, and pH of the solution was adjusted to 7–8 with ammonia water. The precipitated solid was attained by filtration, then washed with water, and dried at room temperature for 48 h. The crude product was recrystallized twice from DMF/benzene mixed solvent (DMF/benzene = 1:5, v/v) to afford a yellow crystal, yield 90%.

Synthesis of 4-(1-methyl-1H-phenanthro[9,10] imidozole-2) Benzoic Acid (MPIBA)

4-(1-Methyl-1H-phenanthro[9,10] imidozole-2) benzoic acid was synthesized as follows: 4-(1H-phenanthro[9,10] imidazole-2-yl) benzoic acid (8 g), KOH (2.6 g) and dimethylsulfoxide (50 ml) were mixed in a 100 ml round-bottom flask and rapidly heated with vigorous stirring in order to the solids dissolved. The dimethyl sulfate (7.0 ml) was added dropwise within 30 min with vigorous stirring at room temperature. After cooling, 150 ml water and 10 g KOH were added and continuously heated on boiling for 30 min, after the solution was cooled to ambient temperature, the mixture was filtrated, the result solution was neutralized to pH 3.0 with 3.0 mol/L HCl solution. The precipitated solid was recovered by filtration, washed twice with water, the crude product was dried at room temperature for 48 h and recrystallized twice from DMF to afford the slight yellow crystal. Yield 80%. m.p > 300°C, found: C 78.39; H 4.58; O 9.08; N 7.95. Calculated: C 78.41; H 4.55; O 9.09; N 7.95. IR(KBr): 1692.40(−C=O), 1679.23, 1610.48(ph−C=N-), 1467.15, 1424.87, 1408.37(ph), 1264.44(C−H), 1107.87, 781.79, 756.04, 719.62, MS: M/Z$[M+H]^+$ 353.1.

Preparation of Standard Solution

The derivatization reagent solution $5.0 \times 10^{-3}\,\mathrm{mol \cdot L^{-1}}$ was prepared by dissolving 17.6 mg 4-(1-methyl-1H-phenanthro[9,10] imidazole-2-yl) benzoic acid (MPIBA) in 10 ml of tetrahydrofuran. Individual stock solutions of the amines ($1.0 \times 10^{-2}\,\mathrm{mol \cdot L^{-1}}$) were prepared in acetonitrile.

The standard amines for HPLC analysis at individual concentrations of 1.0×10^{-4} moL^{-1} were prepared by dilution of the corresponding stock solutions (1.0×10^{-2} mol · L^{-1}) of each amine with acetonitrile. The nonylamine solution (1.0×10^{-3} mol · L^{-1}) were prepared by dilution of the corresponding stock solution (1.0×10^{-2} mol · L) of nonylamine with acetonitrile. Standard solution of 0.1 mol · L^{-1} condensing reagent was prepared by dissolving 0.192 g EDC · HCl in 10 ml of anhydrous acetonitrile. When not in use, all standards were stored at 4°C.

Derivatization Procedure

The solution of amine (30 μl) was placed in a 2-ml vial, and EDC · HCl acetonitrile solution (30 μl) and solution of derivatization reagent(60 μl) were added successively. The vial was then sealed and heated at 80°C for 10 min in a thermostatic water-bath and then left to cool at room temperature. After the addition of 1080 μl acetonitrile, a 10 μl volume of the crude reaction solution was injected into the chromatograph directly. The derivatization process is shown in Fig. 1.

Preparation of MPIBA-Nonylamine

MPIBA-nonylamine was prepared by the reaction of MPIBA with nonylamine as follows: To 600 μl of a standard solution of nonylamine (1.0×10^{-3} mol/L) in acetonitrile was successively added 600 μl EDC · HCl acetonitrile solution (0.1 mol/L) and 1200 μl derivatization reagent solution (5×10^{-3} mol/L) into a 2 ml-vial. The vial was then sealed and heated at 80°C for 10 min in a thermostatic water-bath. After the reaction was completed, the mixture was cooled to room temperature, The MPIBA-nonylamine solution (1000 μL, 2.5×10^{-4} M) was injected into the semi-preparative HPLC system. The mobile phase consisted of (A) 30% aqueous acetonitrile and (B) 100% acetonitrile. The flow rate was 2 mL/min. The gradient started at 100% A and linearly increased from 100% A to 100% B in 30 min. The derivatized MPIBA-nonylamine fraction was time separated from 23 to 25 min automatically. The collected MPIBA-nonylamine fraction was made up to total volume of 12 mL with acetonitrile. The corresponding MPIBA-nonylamine concentration was 5.0×10^{-5} M. This solution was used to evaluate the fluorescence properties of the representative amine derivatives.

High-Performance Liquid Chromatography

Derivatives were separated on a reversed-phase Eclipse XDB-C8 column (150 mm × 4.6 mm,5 μm,Agilent) by a gradient elution. Eluen A was 30% of

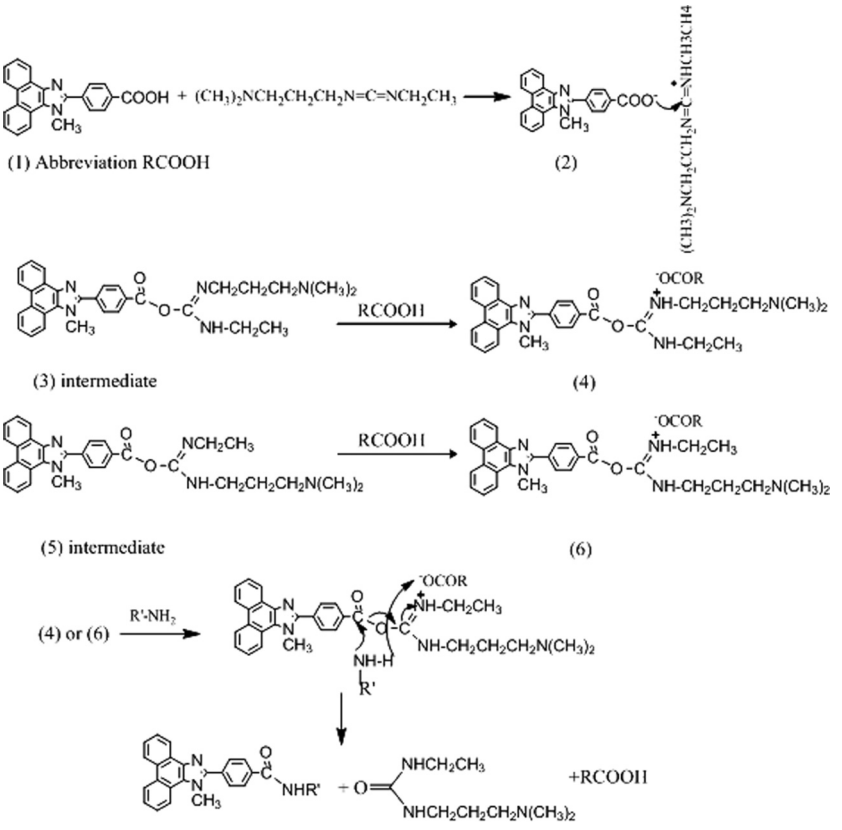


FIGURE 1 Derivatization scheme of 4-(1-methyl-1H-phenanthro[9,10] imidozole-2) benzoic acid (MPIBA) with amines.

acetonitrile consisting of 30 mM ammonium formate (pH 3.55); B was pure acetonitrile. Gradient conditions: initial = 100%A, 35 min = 100%B (kept for 5 min, injection 10 µl). Before injection of the next sample, the column was equilibrated with mobile phase A for 10 min. The flow rate was constant at 1.0 ml/min and the column temperature was set at 30°C. The fluorescence excitation and emission wavelengths were set at $\lambda ex = 260$ nm and $\lambda em = 446$ nm, respectively. The detection and identification of MPIBA derivatives were performed by online post-column fluorescence and MS/APCI at positive ion detection mode.

RESULTS AND DISCUSSION

Stability of MPIBA

MPIBA was synthesized in dimethylsulfoxide solvent; after washing with water and recrystallization in DMF, it was stable in water or in common

organic solvents. When an anhydrous solution of MPIBA in acetonitrile was stored under room temperature for two weeks, the derivatization yields for amines were not obviously different.

Ultraviolet Absorption of MPIBA

For the determination of λ-max and molar absorptivity (ε) of MPIBA, 1.0×10^{-5} mol/l solvent solutions (methanol, acetonitrile, dioxane, and tetrahydrofuran, acetonitrile/water, methanol/water) were used. The ultraviolet absorption of MPIBA was investigated in four solvent systems. Maximum ultraviolet absorption responses were observed at the wavelengths of 254 nm and 257 nm respectively. See Fig. 2a. Maximum ultraviolet responses

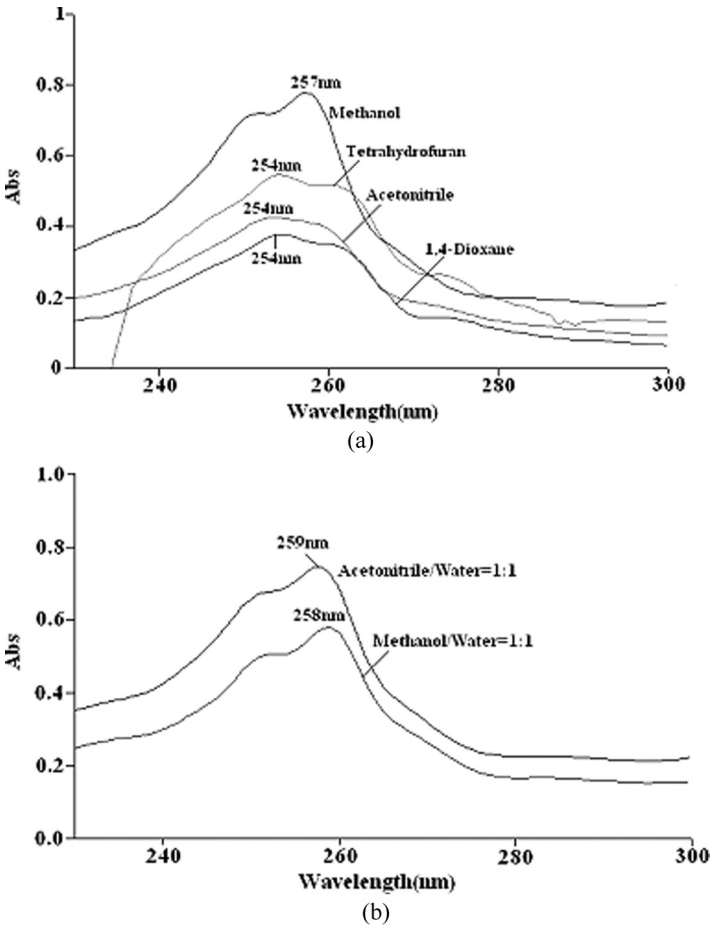


FIGURE 2 Ultraviolet absorption (UV) of MPIBA in various solvent systems. The concentration of MPIBA in each solvent system was 1.0×10^{-5} M.

did not exhibit obviously blue- or red-shift in four solvent systems. The molar absorption coefficients (ε) were $7.79 \times 10^4\,L\,mol^{-1}\,cm^{-1}$ (methanol), $5.47 \times 10^4\,L\,mol^{-1}\,cm^{-1}$ (tedrahydrofuran), $4.27 \times 10^4\,L\,mol^{-1}\,cm^{-1}$ (acetonitrile), $3.78 \times 10^4\,L\,mol^{-1}\,cm^{-1}$ (1, 4-Dioxane), respectively. However, The maximum ultraviolet responses exhibit red-shift in methanol/water and acetonitrile/water solution, and the maximum ultraviolet absorption wavelengths was 258 nm and 259 nm respectively, and the molar absorption coefficient was $7.46 \times 10^4\,L\ mol^{-1}cm^{-1}$ (acetonitrile/water $= 4\,L\,mol^{-1}\,cm^{-1}$ (methanol/water = 1:1, v/v). See Fig. 2b.

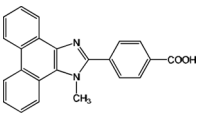
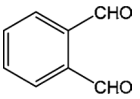
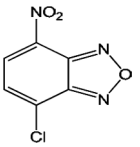
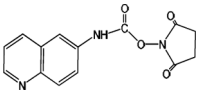
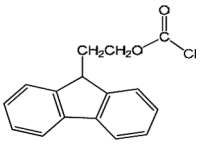
Fluorescence Excitation and Emission

The excitation and emission spectra of the representative MPIBA-nonylamine derivative were obtained at a F7000 fluorescence spectrophotometer (single derivative was obtained according to the derivatization method and purified by a semi-preparative HPLC system as previously described in experimental section). Maximum fluorescence responses of MPIBA-nonylamine derivative were achieved at the excitation wavelength of 260 nm and emission wavelength of 446 nm (no correction). The excitation and emission wavelengths in acetonitrile solution (0–100%) exhibited no obvious blue- or red-shift. Fluorescence intensities of derivatives were minimally quenched by inorganic anions such as sulfate, nitrate, and phosphate, organic anions such as acetate, formate and citrate. However, the emission intensity of MPIBA-nonylamine was dramatically decreased with the increasing of acidity. The fluorescence intensity of MPIBA derivatives in neutral solution was 10–12 times of that in acidity solution. The probably reason is that in the strong acidity solution, the nitrogen atom in imidazole ring of MPIBA-nonylamine accepts a H^+, which lead to the nitrogen atom partly protonized, and the whole conjugated degree was decreased correspondingly. In 10–30 mM formic acid solution, the fluorescence intensity of MPIBA-nonylamine exhibits no obviously quenched. So the solution of acetonitrile participated of 30 mM ammonium formate can adopted as mobile phase, which didn't interferes to detection of derivatives.

The exitation and emission wavelengths and molecular structure of MPIBA and four fluorescence derivatization reagents (OPA[18], NBD-Cl[19], AQC,[20] FMOC[21]) are shown in Table 1.

The more red-shift the exitation and emission wavelengths exhibit, the less interference for fluorescence signal, and it was higher sensitivity for fluorescence detection. And in Table 1, the molecular conjugated degree and the exitation and emission wavelengths of MPIBA were all the maximal besides NBD-Cl, but NBD-Cl had poor stability and underwent about 30–50% decomposition in methanol-water solution within 25 min when

TABLE 1 Comparison of Molar Absorptivity for MPIBA with the Other Four Fluorescence Derivatization Reagents

Derivatization Reagents	Molecular Structure	Exitation and Emission Wavelengths	Detection Limits	The Detected Compounds	Ref.
MPIBA		λex = 260 nm λem = 446 nm	1.76 μg/L	Amines	This work
OPA		λex = 335 nm λem = 440 nm	8.26 mg/L	Amino acids	[18]
NBD-Cl		λex = 469 nm λem = 529 nm	25ppb	Domic acid	[19]
AQC		λex = 250 nm λem = 395 nm	0.02 mg/L	**1**-deoxyn-ojirimycin	[20]
FMOC		λex = 254 nm λem = 322 nm	0.03 mg/L	Amino acids	[21]

exposed to daylight. The detection limits of the MPIBA among the dirivatization reagent in Table 1 is the lowest.

Optimization of the Derivatization Conditions

The derivatization yields for MPIBA and aimines were obviously different at various derivatization time periods, temperature, and concentration of MPIBA and EDC. The optimal derivatization conditions were as follows: Maximum of derivatization yield was found at 80°C, above which there were some by-products and the derivatization yield decreased. Maximum and constant peak intensities could be attained with derivatization time at 10 min, with further long derivatization time, the detector responses didn't significantly increase. The constant fluorescence intensity was achieved with the addition of derivatization reagent being four-fold molar excess over the amines, increasing the excess of reagent beyond this level, it had no significant effect on yield. For the condensing reagent EDC, 30 μl

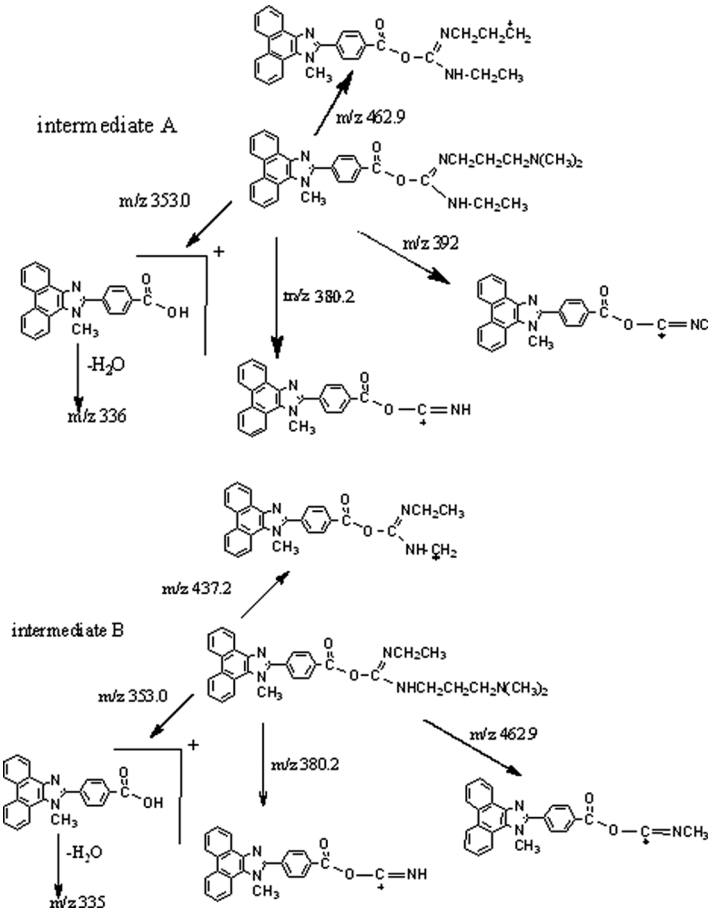


FIGURE 3 Scheme of MS cleavage mode of intermediates A and B.

EDC · HCl (0.1 M,10-fold molar excess to MPIBA) was enough for complete derivatization. The effect of DMAP as catalyst on derivatization yield was tested in this study. The results showed that the derivatization reaction can also proceeded smoothly without DMAP as catalyst, and all subsequent derivatization was, therefore, performed without catalyst.

Derivatization Mechanism

Based on the derivatization mechanism of fluorescence labling reagent containing hydrazine with fatty acids,[16] the derivatization mechanism of MPIBA and amines was guessed theoretically as follows: first, MPIBA react with EDC · HCl to create active intermediate, and second, amines react with the active intermediate to give stable fluorescence derivatives. Peak A and B

in the Fig. 4 of standard amine derivatives were identified by on-line post-column mass spectrometry, the MS and MS/MS information about specific fragment ions about peak A and B were analyzed and shown in Fig. 4, and peak A and B were confirmed as the active intermediates. MS spectrometry regression mode of intermediate A and B was shown in Fig. 3 and the derivatization mechanism of MPIBA with amines was shown in Fig. 1.

HPLC Separation and MS Analysis for Derivatized Amines

A good baseline separation was achieved by adjusting the gradient elution procedure and the pH of elution A. Because of the two alkalescence

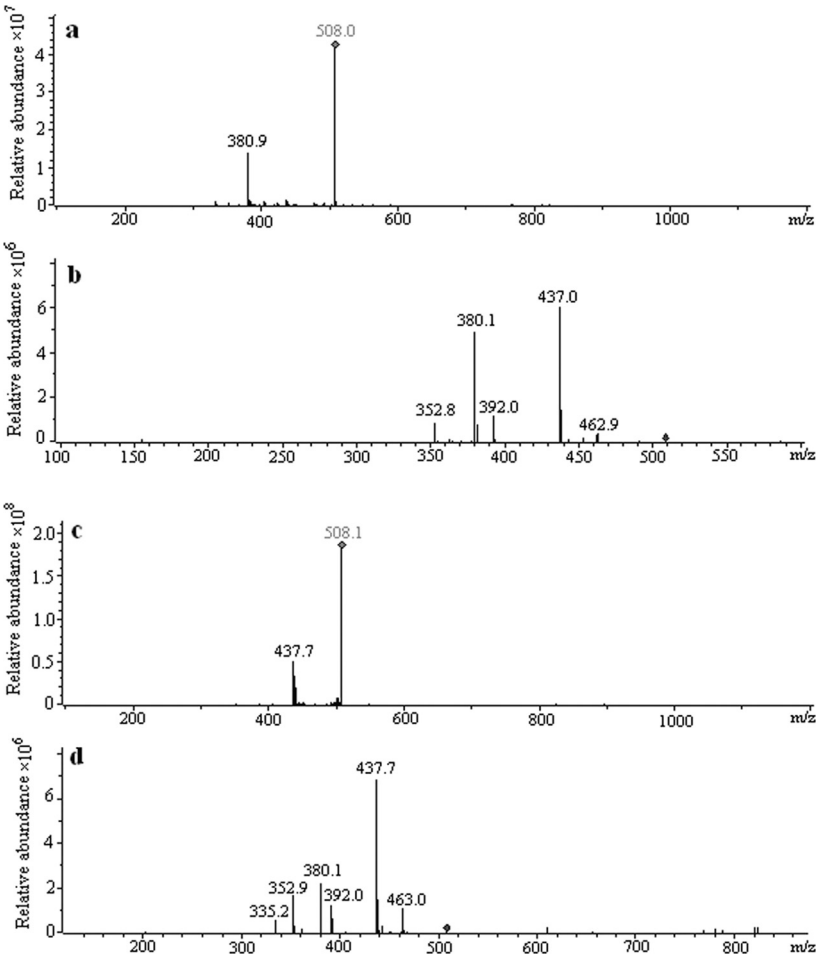


FIGURE 4 Mass spectrum of intermediates A and B (a and b is molecular ion MS and MS/MS belonging to intermediate A; c and d is molecular ion MS and MS/MS belonging to intermediate B).

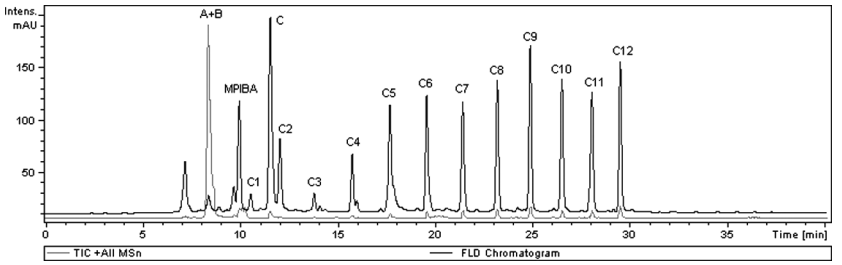


FIGURE 5 Chromatogram and MS spectrum of total ion current for standard amines derivatived with MPIBA Chromatographic conditions as described in experimental section. C1 (methylamine); C2 (ethylamine); C3 (propylamine); C4 (butylamine); C5 (pentylamine); C6 (hexylamine); C7 (heptylamine); C8 (octylamine); C9 (nonylamine); C10 (decylamine); C11 (undecylamine); C12 (dodecylamine); A and B (intermediates); C (2-phenyl-1H-phenanthro-[9,10] imidazole).

nitrogen atoms in the molecular core structure of MPIBA, by controlling the pH of eluent A at 3.55 with formic acid, the 12 amine derivatives were simultaneously separated with the shortest retention times and the sharpest peaks. Additionally, an acidic mobile phase would be useful to the ionization of MPIBA-amines.

Chromatogram and MS spectrum of total ion current for standard amines derivatized with MPIBA were shown in Fig. 5. MPIBA-amine derivatives were identified by online post-column mass spectrometry with APCI source in positive ion mode. The MS data are shown in Table 2, and the MS and MS/MS spectra of representative C11-amine derivative are shown in Figs. 6b and Fig. 6c. The cleavage mode is shown in Fig. 6a.

TABLE 2 Linear Regression Equations, Correlation Coefficients, Detection Limits, MS of Aliphatic Amine Derivatives, and Repeatability for Peak Area and Retention Time (n = 6)

Amine	Y = AX + BX: Injected Amount(pmol), Y: Peak Area	Correlation Coefficient r	Detection Limits (fmol)	MS $[M+1]^+$	Retention Time (RSD) (%)	Peak Area (RSD) (%)
C1	Y = 12.49X + 3.62	0.9999	53.4	366.0	0.096	2.12
C2	Y = 42.03X + 13.15	0.9999	17.5	380.3	0.093	2.19
C3	Y = 20.05X − 8.81	0.9996	36.9	394.4	0.091	2.31
C4	Y = 39.99X + 0.057	0.9997	30.1	408.4	0.063	1.83
C5	Y = 62.19X − 11.25	0.9996	29.4	422.4	0.071	1.79
C6	Y = 59.80X + 12.19	0.9998	23.8	436.4	0.062	1.18
C7	Y = 48.59X − 8.24	0.9999	23.8	450.5	0.056	0.77
C8	Y = 68.70X − 14.00	0.9998	18.7	464.5	0.061	0.72
C9	Y = 94.34X − 7.79	0.9999	16.4	478.4	0.054	0.59
C10	Y = 71.50X − 26.41	0.9999	13.8	492.4	0.052	0.60
C11	Y = 68.63X − 19.04	0.9999	10.5	506.5	0.051	0.61
C12	Y = 79.20X − 14.94	0.9999	10.5	520.5	0.043	0.58

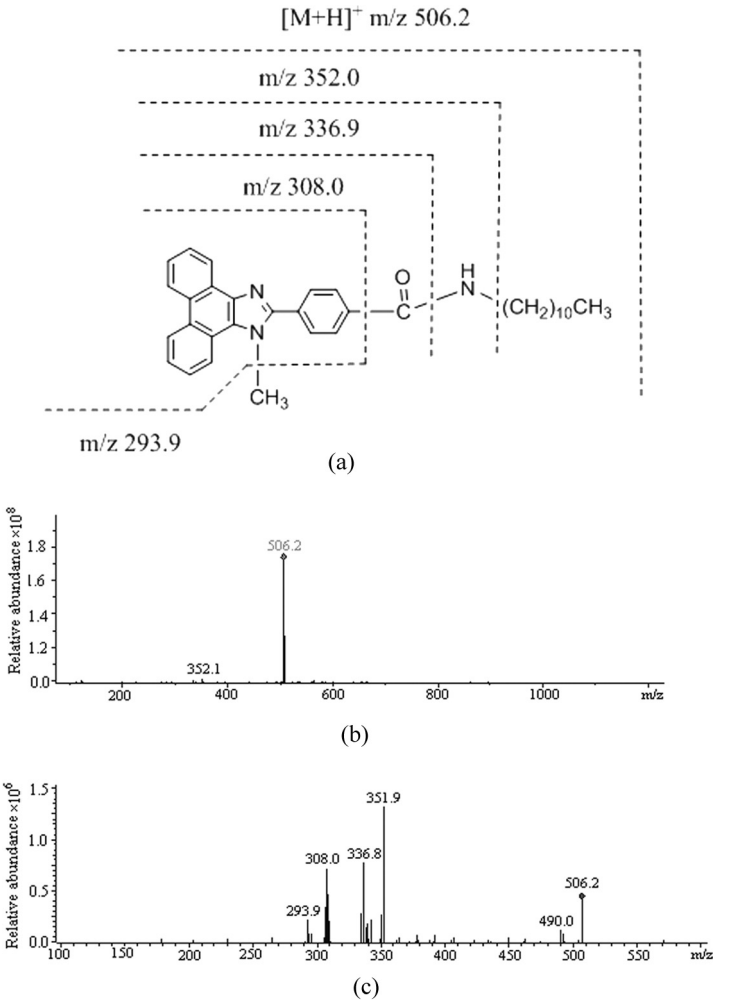


FIGURE 6 The profile of cleavage mode and ion mass spectra for the scanning of the derivatized C11-amine derivative. Typical MS chromatogram of C11-amine derivative from full scanning range from 200 to 1000 amu under APCI in positive-ion mode; (a: cleavage mode; b: molecular ion MS; c: MS/MS).

Repeatability, Linear Regression Equations and Detection Limits

Under the same optimum chromatographic conditions, a standard solution consisting 60 pmol C_1-C_{12} amine derivatives was prepared for the examination of the method repeatability. The relative standard deviations (RSDs) of the peak areas and retention times are shown in Table 2. RSDs of retention time were less than 0.096%, and RSDs of peak area were less than 2.31%.

Based on the optimum derivatization conditions, the calibration graphs were established with the peak area (Y) versus amines quantities (X, pmol, corresponding injected amount from 90.00 pmol to 88 fmol). Linear regression equations, correlation coefficients, and detection limits for all amine derivatives were shown in Table 2. All amine derivatives were found to give excellent linear responses over this range with correlation coefficients of 0.9996–0.9999. The calculated detection limits of each amine (at signal-to-noise ratio of 3:1) were 10.5–53.4 fmol

Analysis of Samples

Pretreatment of Wastewater

To a solution containing 50 ml of wastewater of pharmaceutical factory in 50 ml roundbottom flask, 2.0 ml hydrochloric acid (3.0 M) was added. The contents of the flask were vortexed for 2 min and filtrated. The result solution was evaporated to dryness under reduced pressure in nitrogen atmosphere. The residue was re-dissolved with 50% acetonitrile solution consisted of 0.2 M borate buffer (pH 9.0) to a total volume of 5 ml and stored at 4°C until HPLC analysis.

Determination of Samples

Derivatization and chromatographic separation conditions were according to the optimum conditions above, and the derivatives of amines in real samples were identified by online post column mass spectrometry. Chromatogram of aliphatic amines from waste water of pharmaceutical factory were shown in Fig. 7 and the contents of amines from them are shown in Table 3.

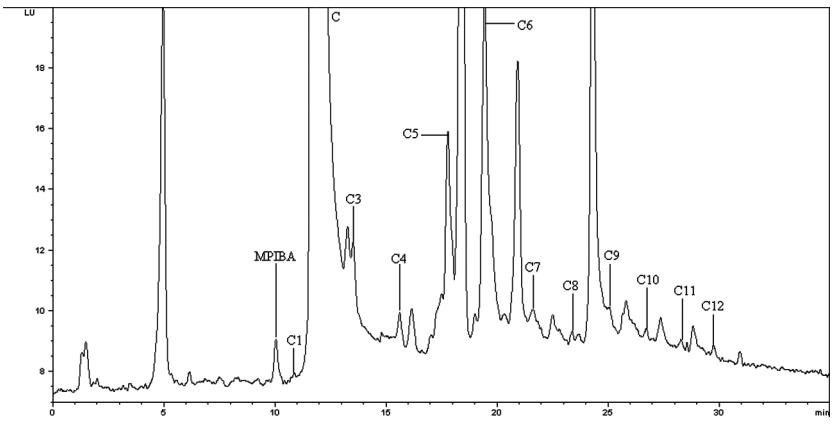


FIGURE 7 Chromatogram of free aliphatic amines from waste water of pharmaceutical factory.

TABLE 3 Contents of Aliphatic Amines from Real Samples and Recoveries (n = 3)

Amine	Waster Water of Pharmaceutical Factory (μg/L)	Recoveries (%) of Waste Water
CH_3NH_2	0.382	82.6
$CH_3CH_2NH_2$	0	93.2
$CH_3(CH_2)_2NH_2$	0.577	88.5
$CH_3(CH_2)_3NH_2$	0.344	91.4
$CH_3(CH_2)_4NH_2$	1.152	102.4
$CH_3(CH_2)_5NH_2$	3.591	104.8
$CH_3(CH_2)_6NH_2$	0.386	97.6
$CH_3(CH_2)_7NH_2$	0.333	85.9
$CH_3(CH_2)_8NH_2$	0.176	96.2
$CH_3(CH_2)_9NH_2$	0.681	91.9
$CH_3(CH_2)_{10}NH_2$	0.561	100.2
$CH_3(CH_2)_{11}NH_2$	0.440	103.1

Recovery

A known amount of aliphatic amines(10 μl, 1.0×10^{-4} M) was added into 50 ml wastewater of pharmaceutical factory in which the contents of amines had been determined, while the extraction and dereivatization were the same as described above. The analyses were carried out thrice, and the recoveries for 12 aliphatic amines were 82.6–104.8% (Table 3).

CONCLUSIONS

The present paper introduces a new reagent MPIBA for derivatizing amines with superior properties including convenient derivatization and excellent sensitivity. The improved performance of the reagent MPIBA for quantitative analysis of amines has been demonstrated in detail. One of the most attractive features of this method exhibits its simpleness for the preparation of amine derivatives. Detection limits are in the femtomole range. Current studies should further explore the derivatization of different amine containing compounds such as alkylamines, catecholamines and polyamines. The HPLC separation for the derivatized amines shows good repeatability. A possible disadvantage of the proposed method is that the reagent MPIBA can be only be used in the pre-column derivatization.

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