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ORIGINAL

Fluorescence Probe of 10-Phenyl-acridone-2-sulfonyl Chloride and Its Application for Determination of Free Aliphatic Amines in Environmental Samples by HPLC with Fluorescence Detection and APCI-MS

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Abstract A simple and sensitive method for the determination of free aliphatic amines using 10-phenyl-acridone-2-sulfonyl chloride (PASC) as a labeling reagent by high-performance liquid chromatography with fluorescence detection and online mass spectrometry identification (HPLC-FLD-MS) has been developed. Derivatization conditions including reagent concentration, buffer pH, reaction time and temperature were optimized. PASC reacted with aliphatic amines at 50 °C for 4 min in aqueous acetonitrile (ACN) in the presence of sodiumtetraborate–NaOH buffer (0.10 mol L^{-1}, pH 9.0) to give high yields of PASC-amine derivatives. Derivatives exhibited intense fluorescence with an excitation maximum at λ_{ex} 265 nm and an emission maximum at λ_{em} 418 nm. The separation of derivatives was performed by a reversed-phase Hypersil BDS C8 column in combination with a gradient elution. The identification of derivatives was carried out by online post-column mass spectrometry with atmospheric pressure chemical ionization (APCI) source in positive-ion detection mode. Excellent linear responses were observed with the correlation coefficients of larger than 0.9997, and detection limits (at a signal-to-noise of 3:1) were from 3.0 to 24.3 fmol. Comparing with 10-ethyl-acridine-2-sulfonyl chloride (EASC), PASC exhibited more intense fluorescence and ultraviolet absorbance. The proposed method is sensitive and reproducible for the determination of aliphatic amines from water and soil samples.

Keywords HPLC/MS · Aliphatic amines · 10-Phenyl-acridone-2-sulfonyl chloride · Pre-column derivatization

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Introduction

Amines are naturally occurring compounds formed as metabolic products in microorganisms, plants and animals, in which the principal routes of amine formation include the decarboxylation of amino acids, amination of carbonyl compounds and degradation of nitrogen-containing compounds [1–3]. Aliphatic amines are also important raw materials and intermediates in the manufacture, chemical and pharmaceutical industries and so on. Most of them are widely distributed in the nature. However, volatile amines have an unpleasant smell and are hazardous to health [4, 5]. Amines are not only toxic themselves, but can also become more toxic N-nitrosamines through chemical reactions with nitrosating agents, such as nitrite or nitrate [6–8]. Therefore, it is important to determine aliphatic amines in real environmental samples. Most aliphatic amines show neither natural UV absorption nor fluorescence, thus the analysis of amines has been traditionally difficult at trace levels in environmental samples. In the recent years, gas chromatography is frequently used to determine amines using various derivatization reagents [9–11]. Other methods including enzymatic [12, 13] and TLC [14, 15] have been described for the determination of amines in various matrices. These methods also suffer from some limitations to their applications owing to low sensitivity with UV–visible

spectrophotometer detection. Therefore, chemical derivatization is necessary to reduce detection limits [16, 17]. Recently, 9-fluorenylmethyl chloroformate (FMOC) [18], 4-chloro-7-nitrobenzofurazan (NBD-Cl) [19], and 2-(9-anthryl)-ethyl chloroformate (AEOC) [20] have been used for the derivatization of amino compounds. However, the derivatized solution using these reagents must be extracted with pentane to remove an excess of reagent [21], because it is detrimental to column performance. 6-Aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) method gives stable fluorescent derivatives, but detection limits for the early eluted amines are usually higher than those for later ones [22]. Therefore, development of high sensitivity and convenient fluorescent reagents for determination of amines is very necessary.

In our previous study [23], a simple and sensitive method for the determination of amino compounds using the synthesized 10-ethyl-acridine-2-sulfonyl chloride (EASC) was described. On the basis of the fluorescence properties of EASC, the ethyl functional group in EASC molecules was replaced by phenyl group to give a novel fluorescence reagent of 10-phenyl-acridone-2-sulfonyl chloride (PASC). PASC and its derivatives exhibited more intense fluorescence than EASC. In this study, the optimal derivatization conditions, such as reagent concentration, buffer pH, reaction time and temperature 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 PASC probe and its further application for the determination of amino compounds from environmental water and soil samples has been reported.

Experimental

Apparatus

Experiments were performed using a 1100 Series LC/MSD-Trap-SL liquid chromatograph–mass spectrometer. All the HPLC system devices were from the HP 1100 series and consisted of a vacuum degasser (model G1379A), a quaternary pump (model G1311A), an autosampler (model G1313A), a thermostated column compartment (model G1316A), a fluorescence detector (model G1321A), and a diode array detector (model G1315B). Derivatives were separated on a Hypersil BDS C8 column (200 × 4.6 mm, 5 μm, Dalian Yilite, China). The HPLC system was controlled by HP Chemstation software. The mass spectrometer 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. Fluorescence excitation and emission spectra were obtained on an F-7000 fluorescence spectrophotometer (Hitachi, Japan). Excitation and emission slit are both set at 5 nm. Scan speed is set at 12,000 nm min^{-1}. UV detection was carried out by CARY 300 Bio UV/vis spectrophotometer (Varian, USA). The mobile phase was filtered through a 0.2-μm nylon membrane filter (Alltech, Deerfiled, IL, USA); melting point was measured on a PHMK 79/2289 micro-melting point apparatus (Shaihai Precision and Scientific Instrument Co., LTD).

Chemicals

All aliphatic amine standards were purchased from Sigma (St. Louis, MO, USA). HPLC grade ACN was purchased from Yucheng Chemical Reagent (Shandong Province, China). Water was purified on a Milli-Q system (Millipore, Bedford, MA, USA). Other chemicals for the synthesis of PASC were analytical grade from Jining Chemical Reagent (Shandong, China). Soil sample was collected from the garden of Qufu Normal University (Shandong China). Environmental water sample was collected from the Yi river of Qufu.

Synthesis of 10-Phenyl-acridone-2-sulfonyl Chloride (PASC)

Synthesis of 2-(Diphenylamino)benzoic Acid

To a 250-mL three-necked bottom flask, 2-(phenylamino)-benzoic acid (5 g), spherical copper powder (0.1 g), potassium carbonate (5 g), 50 mL bromobenzene and 30 mL nitrobenzene were successively added. After the reaction content was stirred for 15 min at room temperature, the mixture was heated to reflux for 8 h. Then nitrobenzene was removed by steam distillation. After distillation, the insolvable residue was quickly filtered to remove the copper powder. After cooling, the mixture was carefully transferred into 15 mL hydrochloric acid solution and stirred for 30 min. The precipitated solid was recovered by filtration, washed with water and vacuum dried overnight at room temperature. The crude product was recrystallized three times from ethanol to afford mercury bulk crystal (yield, 75.3 %), and then analyzed by HPLC, purity 98.5 %. IR (KBr):3417 (ν_{O-H}); 1693 ($\nu_{C=O}$); 1590, 1490 ($\nu_{C=C}$); 1404 (ν_{C-N}); 748,695 (δ_{Ar-H}).APCI/MS: *m/z*: 289.1 $[M + H]^+$. m.p.: 206.6–206.8 °C.

Synthesis of 10-Phenyl-acridone

To a solution containing 50 mL sulfuric acid (95–98 %) in 100 mL round-bottom flask, 2-(diphenylamino)benzoic acid (2.5 g) was slowly added. The mixture was heated to 80 °C and stirred for 1 h. After cooling, the solution was transferred into 250 mL of water with vigorous stirring for 15 min. The excess sulfuric acid was neutralized by the

addition of the appropriate amount of an aqueous solution of ammonia. The precipitated solid was recovered by filtration and successively washed with 0.5 mol L^{-1} NaOH solution and water. The cure product was vacuum dried overnight at room temperature to give yellow green color solid 1.35 g (yield 72 %). The purity of object product was 99.5 % (HPLC).IR (KBr): 1634 ($\nu_{C=O}$); 1598, 1486, 1455 ($\nu_{C=C}$); 1356 (ν_{C-N}); 748,703 (δ_{Ar-H}). °APCI/MS: *m/z*: 271.2 $[M + H]^+$. m.p. > 250 °C.

Synthesis of PASC

Chlorosulfonic acid (20 mL) was added into a 100-mL round-bottom flask and rapidly cooled to 0 °C in an ice water bath with stirring by a magnetic stirrer device. 10-Phenyl-acridone (2 g) was then added and vortex-mixed carefully for 1.0 min. This mixture was allowed to stand for 24 h at ambient temperature with stirring. The reaction mixture was then heated to 50 °C for 15 min. After cooling, the mixture was poured into 400 mL of ice water with vigorous stirring for 10 min. The precipitated solid was recovered by filtration, and washed with ice water. The crude product was recrystallized three times from ACN/acetone (v/v, 1:1) to afford a yellow crystal (yield 94.4 %, purity 99.8 %). IR (KBr): 1785 ($\nu_{C=O}$); 1453, 1428; 1386 ($\nu_{C=C}$); 1590, 1173 (ν_{C-N}); 1185, 1142 ($\nu_{O=S=O}$), 775,861 cm^{-1} (δ_{C-H}). LC/APCI/MS: *m/z*: 369.6 $[M + H]^+$. m.p. > 250 °C. The synthesis of PASC is shown in Fig. 1.

HPLC and MS Conditions

Derivatives were separated on a Hypersil BDS C8 column (200 × 4.6 mm, 5 μm, Dalian Yilite, China) by a gradient elution. Mobile phase component (A) was water (containing 1 % formic acid, v/v) and (B) was ACN (100 %). Gradient conditions: initial = 50 % B; 10 min 80 % B, 20 %A; 20 min 100 % B. Before injection of the next sample, the column was equilibrated with 50 % B 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 $\lambda_{ex}/\lambda_{em}$ = 265/418 nm. The identification of PASC-amine derivatives were performed by online post-column APCI-MS in positive-ion detection mode. Ion source type, atmospheric pressure chemical ionization (APCI) detection in positive-ion mode; nebulizer pressure 60 psi; dry gas temperature, 350 °C; dry gas flow, 5.0 L min^{-1}. APCI Vap temperature 450 °C; Corona Current (nA) 4,000 (pos); Capillary voltage 3,500 V.

Preparation of Standard Solution

The derivatizing reagent solution (5.0×10^{-3} mol L^{-1}) was prepared by dissolving 18.5 mg (PASC) in 10 mL of anhydrous ACN prepared by distilling HPLC grade ACN from P_2O_5. Individual stock solution of aliphatic amines was prepared in ACN. The standard aliphatic amines for HPLC analysis at individual concentration of 5.0×10^{-4} mol L^{-1} were prepared by diluting the corresponding stock solution (1.0×10^{-2} mol L^{-1}) of each amine with ACN. When not in use, all standards were stored at 4 °C.

Extraction of Free Aliphatic Amines from Soil and Environmental Water

Pretreatment of Environmental Water Sample

To a solution containing 400 mL of environmental water in 500 mL beaker, an appropriate amount of hydrochloric acid (1.0 mol L^{-1}) was added to adjust the pH value close to 3.0. The content of the beaker was vortexed for 15 min and filtrated to remove the fine particles. The mixture was then evaporated to dryness under reduced pressure with a filtration pump. The residue was re-dissolved in 5 mL of 80 % ACN/H_2O (v/v) solution, and stored at 4 °C until HPLC analysis.

Pretreatment of Soil Sample

Soil (150 g) was taken into a 250-mL round-bottom flask, subsequently 100 mL chloroform was added. The contents of the flask were vortexed for 0.5 min, and then sonicated for 30 min. The slurry was filtered through analytical filter paper to remove fine particles, and the residues were again

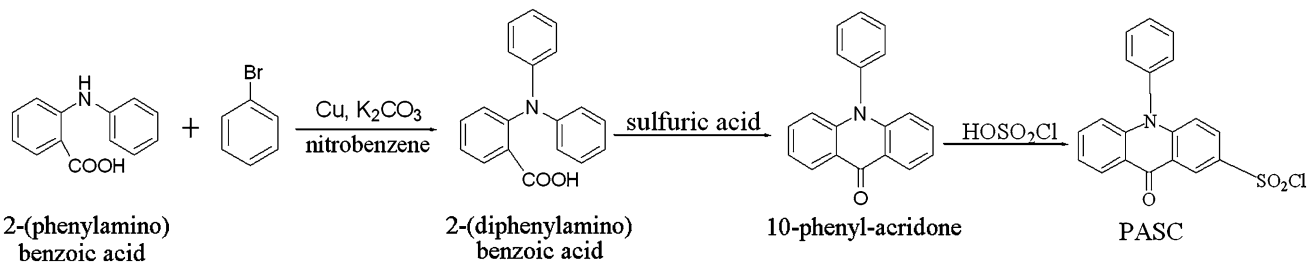

Fig. 1 The synthetic route of PASC

extracted with another 100 mL of chloroform. After the chloroform was pooled, a 3.0 mL of formic acid was added and then sonicated for 30 s. The result solution was then evaporated to dryness under vacuum. The residue was redissolved in 3 mL of aqueous ACN (80 %, ACN/H_2O, v/v), and stored at 4 °C until HPLC analysis.

Derivatization Procedure

The derivatization process is shown in Fig. 2. To 50 μL of mixed amine standard in a 2 mL vial, labeling reagent solution (70 μL), sodiumtetraborate-NaOH buffer (200 μL, 0.1 mol L^{-1}, pH 9.0) and ACN (170 μL) were successively added. The vial was then sealed and heated for 4 min in a thermostatic water bath at 50 °C. After cooling at room temperature, 20 μL of aqueous ACN (50 %, 1:1, v/v) and 20 μL of 50 % acetic acid solution were added. The solution was adjusted to neutral pH. A 10 μL volume of the reaction solution was injected into the chromatograph directly.

Preparation of Representative PASC-Dodecylamine (PASC-C12) and EASC-Dodecylamine (EASC-C12) Derivatives to Evaluate Their Fluorescence Properties

The preparation of PASC-dodecylamine (PASC-C12) was carried out as follows: to a solution of dodecylamine (1.0 mL, 5×10^{-3} mol L^{-1}) in 10 mL centrifuge tube, 2.0 mL sodiumtetraborate–NaOH buffer (0.1 mol L^{-1}, pH 9.0) and 1.2 mL PASC solution (5×10^{-3} mol L^{-1}) were added, respectively. The tube was then sealed and heated in a thermostatic water bath at 50 °C for 4 min. After the reaction was completed, the solution was neutralized to near neutral conditions (pH 7.0) with acetic acid (50 %, v/v). The neutralized solution was passed through a pre-conditioned ODS-C18 column with 4.0 mL methanol and 5.0 mL water. The desired PASC-C12 was eluted with 4.2 mL of 50 % ACN solution. The eluted solution was evaporated to dryness by a stream of nitrogen gas. The residue was redissolved with aqueous ACN (70 %, v/v) and made up to a total volume of 5.0 mL. The corresponding obtained PASC-C12 concentration was about 1.0×10^{-3} mol L^{-1}. The preparation of EASC-C12 derivative was similar to the method described above with a little modification, the desired EASC-C12 derivative was eluted with 13 mL of a 50 % ACN solution. The low concentrations of PASC-C12 and EASC-C12 used to test fluorescence quantum efficiencies were prepared by diluting the stock solution (1.0×10^{-3} mol L^{-1}) with aqueous ACN (70 %, v/v). When not in use, all solutions were stored at 4 °C.

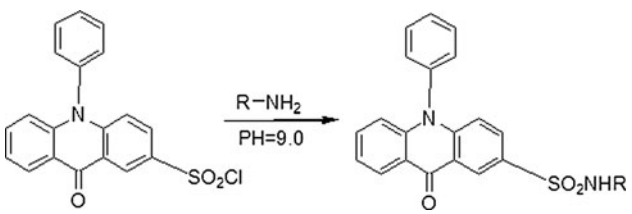

Fig. 2 Derivatization scheme of PASC with aliphatic amines

Quantitative Analysis

Quantitative conversion of amines from the wastewater and soil samples to their PASC-amine derivatives was guaranteed using an excess of PASC. All amines were quantified in samples using the external standard method with detection at 418 nm. The calibration curves for each PASC-amine were obtained by linear regression plotting peak area versus concentration.

Results and Discussion

Fluorescence Excitation and Emission of PASC and Its Derivatives

To evaluate the fluorescence property, PASC-C12 derivative was prepared by the reaction of dodecylamine with PASC. The effects of ACN concentration and solvent (acetonitrile, methanol, *N*,*N*-dimethylformamide on the fluorescence intensity of the PASC-C12 derivative were also evaluated (as shown in Fig. 3a, b). PASC-C12 derivative in aqueous ACN (40 %, v/v) showed the maximum fluorescence intensity with an excitation maximum at λ_{ex} 265 nm and an emission maximum at λ_{em} 418 nm. An increase in solvent polarity resulted in obvious red shift in fluorescent emission spectra. The emission intensities in pure methanol, acetonitrile and *N*,*N*-dimethylformamide exhibited 9.59, 3.55 and 3.24 times stronger than that in pure water with peak blue-shift about 14, 25 and 25 nm, respectively. The quenching in *N*,*N*-dimethylformamide, aqueous acetonitrile and methanol for PASC-C12 derivative should be attributed to the formation of quaternary ammonium ion (c) by the protonation reaction with H_2O as described in Fig. 4. The quaternary ammonium ion (c) was very stable in an aqueous or acidic media.

Comparison of PASC and EASC

PASC and EASC were compared from the aspects of ultraviolet spectra, fluorescence spectra and solubility, and the results are as follows:

PASC exhibits more intense UV absorption and fluorescence properties than EASC. This should be attributed to the introduction of one phenyl functional group into

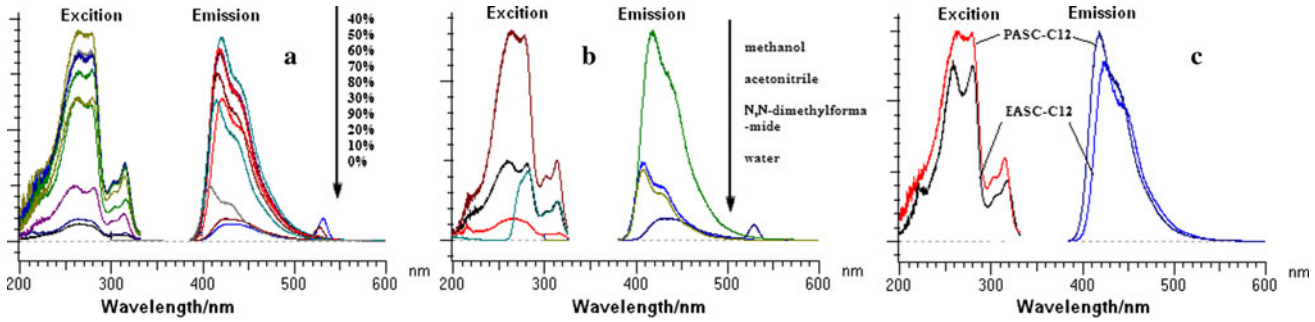

Fig. 3 Fluorescence intensity of PASC-C12 derivative in the presence of varying amount of acetonitrile (**a**): the effects of solvent on the fluorescence intensity of the PASC-C12 derivative (**b**): fluorescence spectra of PASC-C12 ($\lambda_{ex}/\lambda_{em}$ = 265/418 nm, PASC-C12 concentration at 2×10^{-5} mol L^{-1}; Solvent: 70 % ACN) and EASC-C12 ($\lambda_{ex}/\lambda_{em}$ = 259/424 nm, PASC-C12 concentration at 2×10^{-5} mol L^{-1}; solvent: 70 % ACN) (**c**)

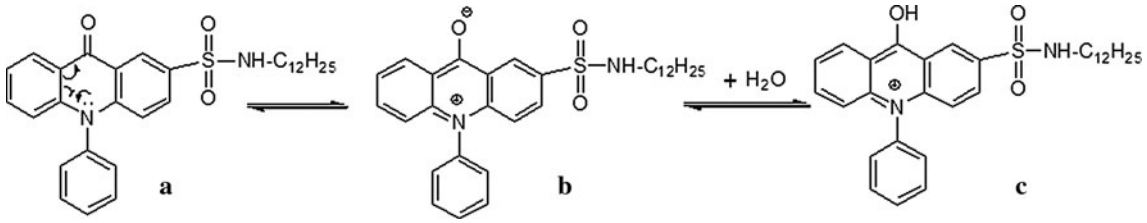

Fig. 4 The scheme of intramolecular keto-enol isomerization of PASC-C12 molecule

acridone core backbone resulting in an intense conjugation system for PASC molecule. Maximum ultraviolet absorption responses are observed at the wavelengths of 252 nm with the molar absorption coefficient (ε) of 3.85×10^4 (L mol^{-1} cm^{-1}). The maximum UV absorption of EASC in acetonitrile solvent was 238 nm (ε: 2.5×10^4, L mol^{-1} cm^{-1}). Obviously, PASC exhibits high UV absorption than that of EASC. The maximum molar absorption coefficient (at 252 nm) for PASC was about 1.54 times stronger than that of EASC (at 238 nm).

To make a quantitative comparison between PASC and EASC with respect to fluorescence quantum efficiency, individual standard solution at the concentration of 2×10^{-5} mol L^{-1} for each PASC-C12 and EASC-C12 was prepared in aqueous ACN (70 %, v/v). The fluorescence intensities were, respectively, detected using their maximum excitation and emission wavelengths (here, PASC-C12: $\lambda_{ex}/\lambda_{em}$ = 265/418 nm; EASC-C12: $\lambda_{ex}/\lambda_{em}$ = 259/424 nm). The fluorescence spectra are shown in Fig. 3c. The relative fluorescence quantum efficiency (Q_E) was calculated according to the formulas of (Eq. 1) and (Eq. 2) [26].

$$I_{f(PASC-C12)} K' \varphi_{f(PASC-C12)} I_o \left(1 - 10^{-A_{(PASC-C12)}}\right) \quad (1)$$

$$Q_E = \frac{\phi_{f(PASC-C12)}}{\phi_{f(EASC-C12)}} = \frac{I_{f(PASC-C12)} \times \left(1 - 10^{-A_{(EASC-C12)}}\right)}{I_{f(EASC-C12)} \times \left(1 - 10^{-A_{(PASC-C12)}}\right)} \quad (2)$$

(I_f: relative fluorescence intensity ϕ_f: fluorescence quantum efficiency, K': correlative constant; I_o: incident light intensity, A: absorbance). ($I_{f(PASC-C12)}$ and $I_{f(EASC-C12)}$ are relative fluorescence intensity of PASC-C12 and EASC-C12, respectively; $A_{(PASC-C12)}$ and $A_{(EASC-C12)}$ are UV absorption of PASC-C12 and EASC-C12 at their maximum excitation wavelengths, respectively). The calculated value of Q_E was 1.3. Obviously, PASC exhibited more intense fluorescence than EASC. It should be attributed to the introduction of a phenyl functional group into molecular core resulting in an intense conjugation system in molecular core.

In addition, the solubility of PASC in ACN was far better than EASC. 5.0×10^{-3} mol L^{-1} PASC standard solution can be prepared at room temperature. However, 5.0×10^{-3} mol L^{-1} EASC standard solution cannot be prepared at room temperature. 1.0×10^{-3} mol L^{-1} EASC needed to heat and vortex to dissolve completely. In the same derivatization conditions, the derivatization reaction of PASC was better than EASC. It is favorable to get high derivatization yields using high concentration of reagent in derivatization procedure.

Optimization Derivatization

With ethylamine, butylamine, octylamine and undecylamine as representative amines, the effects of PASC concentrations on the derivatization yields are evaluated. The results indicate that fluorescence responses of PASC-amine derivatives increase with the increasing amounts of PASC.

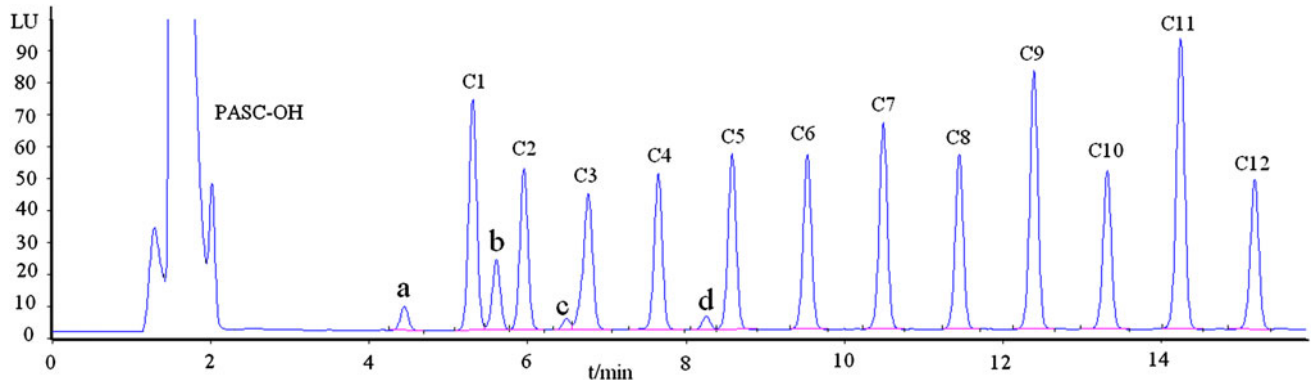

Fig. 5 Chromatogram for the separation of standard aliphatic amines derivatized with PASC. Column temperature is set at 30 °C; flow rate = 1.0 mL min^{-1} *C1* methylamine, *C2* ethylamine, *C3* propylamine, *C4* butylamine, *C5* pentylamine, *C6* hexylamine, *C7* heptylamine, *C8* octylamine, *C9* nonylamine, *C10* decylamine, *C11* undecylamine, *C12* dodecylamine, **a**, **b**, **c** and **d** (unidentified), *PASC-OH* PASC-sulfonyl acid

Constant fluorescence intensity is achieved with the addition of three- to fourfold molar reagent excess to total molar amines, further increasing the excess of reagent beyond this level has no significant effect on yields. The derivatization of PASC with amines is usually accelerated in the presence of basic catalyst. To obtain high derivatization yields, several types of basic catalysts including Na_2HPO_4–K_2HPO_4, NaOH and sodium tetraborate are evaluated. The results show that sodiumtetraborate–NaOH buffer (0.10 mol L^{-1}, pH 9.0) is found to be the best choice. With pH > 9.5, an obviously low detector response was observed and it may be attribute to the fast hydrolysis reactions of products. Therefore, the derivatized solution must be neutralized to near neutral pH (7.0) by the addition of an appropriate amount of acetic acid. The effect of temperature on yield is also tested in the range of 30–70 °C. The results indicate that derivatization temperature of 50 °C gives the highest derivatization yields. The effect of the reaction time on the derivatization was also evaluated. The results indicated that the maximum and constant of peak heights for four representative amines were obtained within 4 min. Based on these results, derivatization occurred for 4 min at 50 °C with an optimal pH in the range of 9.0–9.5.

Chromatographic Separation and MS Analysis

For the simultaneous separation of standard aliphatic amine derivatives, a Hypersil BDS C8 column was selected and eluted under the chromatographic conditions proposed. As observed, no significant influence of mobile phase pH on resolution for 12 aliphatic amine derivatives was observed at pH 6–8. In comparison with the neutral medium solution, elution at low pH (<5.0) resulted in a slight decrease in retention value for all derivatives. With pH 9.0, all aliphatic amine derivatives were separated within 15.5 min with a good baseline resolution (see Fig. 5).

As expected, all amine derivatives exhibited protonated molecular ion corresponding at *m/z* $[M + H]^+$. The collision-induced dissociation spectra for representative hexylamine derivative produced the specific fragment ions at *m/z* 289.2, *m/z* 271.2 and *m/z* 242.1. These ions were mainly produced by the cleavage of the molecular core of PASC. The specific fragment ion at *m/z* 289.1 was occurred by losing of CO molecule to form an unstable intermediate of *N*-hexyl-9-phenyl-9*H*-fluorene-3-sulfonamide, and further elimination of H_2O and $C_6H_{13}NH$ to produce the specific fragment ion at *m/z* 289.1. The characteristic fragment ion at *m/z* 334.6 was also produced by S–N bond cleavage by losing of neutral molecule of $C_6H_{13}NH$. The ion of *m/z* 334.6 was further eliminated one molecule of SO_2 to produce the fragment ion at *m/z* 271.1. The ion of *m/z* 271.1 was further eliminated one molecular of CO to give the specific ion at *m/z* 242.3. The MS spectra and cleavage mode of the representative PASC-hexylamine derivative is shown in Fig. 6(a: MS, b: MS/MS; c: cleavage mode). The MS/MS data for all amine derivatives are shown in Table 1.

Interference

To eliminate the interference of amino acids, some strongly retained amino acid derivatives, such as ornithine, histidine and lysine with relatively long retention under the chromatographic conditions were investigated. It was found that under the proposed chromatographic conditions, these amino acid derivatives were eluted at a very short retention time compared with aliphatic amine derivatives; therefore they did not interfere with the analysis.

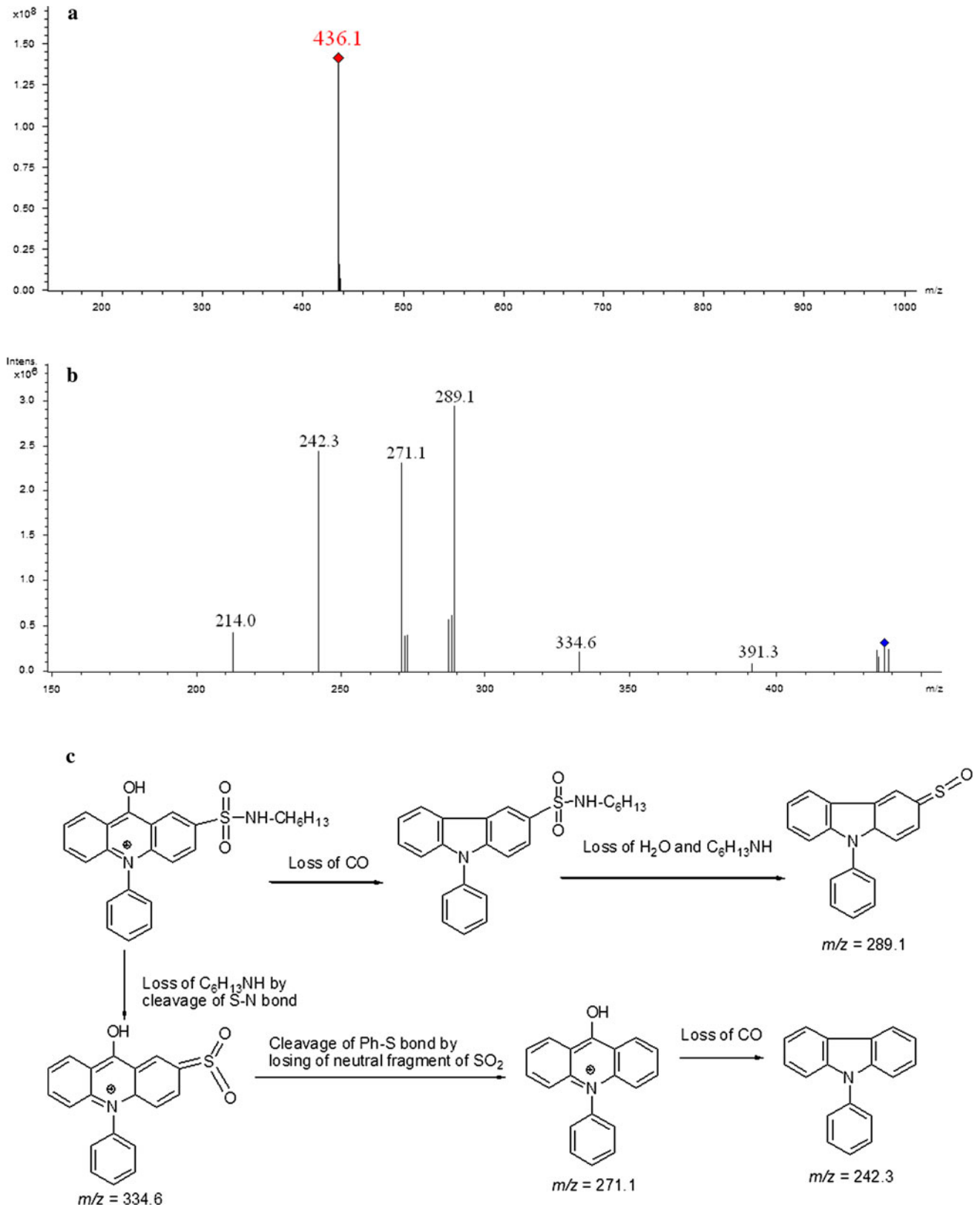

Fig. 6 **a** MS, **b** MS/MS and **c** cleavage mode for representative PASC-hexylamine derivative

Table 1 MS and MS/MS data for PASC-amine derivatives

Amine derivatives	Molecular weight	Molecular ion $[M + H]^+$	MS/MS fragmentation
C1	364	365.6	242.4, 271.5, 289.0
C2	378	379.5	242.4, 271.6, 289.0
C3	392	393.5	242.1, 271.2, 289.2
C4	406	407.6	242.1, 271.2, 289.2
C5	420	421.5	242.4, 271.0, 289.1
C6	434	435.5	242.3, 271.1, 289.1
C7	448	449.5	242.0, 271.2, 289.0
C8	462	463.5	241.4, 271.4, 289.1
C9	476	477.6	242.1, 270.8, 289.4
C10	490	491.6	241.8, 270.9, 289.3
C11	504	505.6	242.1, 270.9, 289.3
C12	518	519.6	242.6, 271.2, 289.3

Table 2 Linear regression equations, correlation coefficients, detection limits, RSD of retention and peak area of aliphatic amine derivatives

Amine derivatives	$Y = A \times X + BX$: (injected amount pmol) Y: (peak area)	r	Detection limits (fmol)	RSD of retention time (%)	RSD of peak area (%)
C1	$Y = 44.69X + 2.23$	0.99982	11.7	0.062	0.67
C2	$Y = 28.95X + 2.27$	0.99981	19.3	0.059	0.28
C3	$Y = 28.88X + 2.31$	0.99980	22.5	0.052	0.39
C4	$Y = 28.30X + 2.00$	0.99988	20.8	0.046	0.48
C5	$Y = 31.56X + 7.86$	0.99975	3.0	0.047	0.59
C6	$Y = 31.44X + 2.56$	0.99977	16.9	0.042	0.36
C7	$Y = 37.21X + 2.65$	0.99981	18.0	0.033	0.54
C8	$Y = 31.44X + 2.30$	0.99981	16.9	0.035	0.73
C9	$Y = 46.69X + 3.48$	0.99977	12.8	0.043	0.50
C10	$Y = 28.89X + 2.31$	0.99978	24.3	0.041	0.55
C11	$Y = 54.02X + 4.08$	0.99977	11.2	0.035	0.32
C12	$Y = 28.38X + 2.15$	0.99986	18.0	0.038	0.30

Table 3 Content of aliphatic amines from environmental water and soil samples and recoveries

Amine derivatives	Water sample from Yi river ($\mu g\ L^{-1}$)	Recovery R (%)	Soil sample ($ng\ g^{-1}$)	Recovery R (%)
C1	29.4	100.2	1.92	100.1
C2	2.88	100.1	0.34	100.3
C3	*	99.7	*	100.1
C4	2.09	103.1	3.34	101.2
C5	2.50	102.1	0.82	100.7
C6	5.59	101.5	0.20	102.4
C7	0.22	99.8	0.56	103.2
C8	*	103.4	0.16	101.6
C9	*	100.5	*	102.4
C10	*	102.9	*	101.8
C11	*	100.6	*	102.7
C12	*	101.8	*	103.1

*C3, *C8, *C9, *C10, *C11, *C12 was not determined owing to co-eluting with unknown components

Detection Limits, Linearity and Recovery for Derivatized Amines

Based on the optimum derivatization conditions, the calibration graphs are established with the peak area (Y) versus aliphatic amines quantities (X, pmol). Linear regression equations, correlation coefficients and detection limits for all aliphatic amine derivatives are shown in Table 2. All aliphatic amines are found to give excellent linear responses with correlation coefficients of larger than

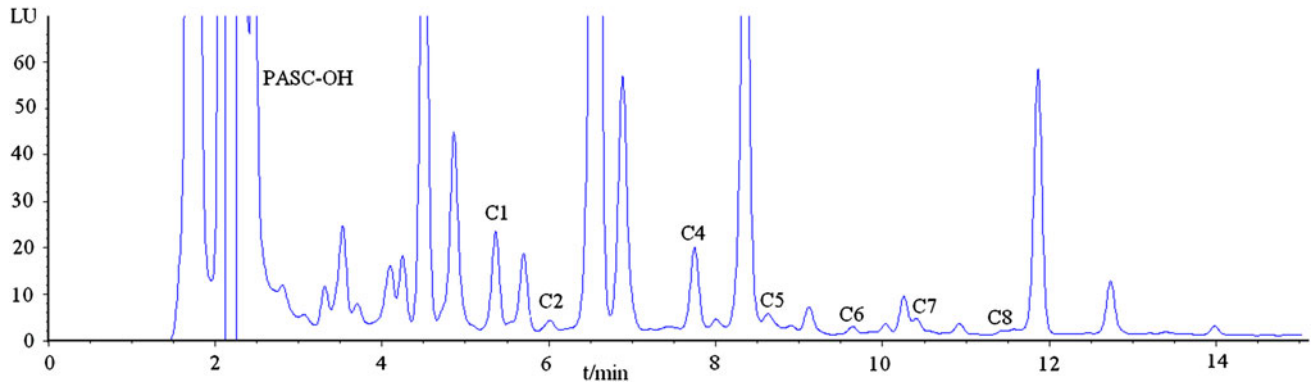

Fig. 7 Chromatogram of aliphatic amines from soil sample (peaks as in Fig. 5)

0.9997 (corresponding injected amount from 50.0 pmol to 24.38 fmol). The calculated detection limits with a 24.4 fmol injection for each aliphatic amine (at a signal-to-noise ratio of 3:1) are from 3.0 to 24.3 fmol. Under the optimum chromatographic conditions, a standard stock solution containing 20 pmol of each amine was prepared to examine the method repeatability. The relative standard deviations (RSDs) of the peak areas and retention times are shown in Table 2.

The total concentrations of the amine analytes in the spiked sample and the endogenous concentrations in the non-spiked sample are determined and used to calculate the recovery. A known amount of aliphatic amines was added into environmental water or soil sample in which the contents of amines had been determined, while the extraction and derivatization were the same as described above. The analyses were carried out thrice, and the recoveries for 12 aliphatic amines were 100.1–103.2 % (Table 3).

Analysis of Samples

Detailed knowledge of the chemical composition of environmental water and soil samples is very important for quality control and for evaluating the effects on consumers' health. The toxicity associated with volatile amines is quite often related to nausea, vomiting, sweating, confusion, and rapid heartbeat and hangover headaches. Figure 7 shows the application of this method for the determination of amines present in an environmental soil sample. The quantitative analysis is carried out by fluorescence; corresponding components are simultaneously identified by online APCI-MS. Generally, the amine compositions depend on the type of samples. The contents of C1–C6 (except C3) amines in environmental water sample (chromatogram is not shown) are higher than that obtained in soil sample. The data for all amines in soil and environmental water samples are listed in Table 3.

Conclusions

The labeling reagent PASC, which contains one sulfonyl chloride functional group for the derivatization of amines exhibits superior properties including convenient derivatization and excellent sensitivity. One of the most attractive features of this method is that labeling reaction can easily react with amines to give stable fluorescence derivatives. Detection limits are in the femtomole range. Current studies should further explore the derivatization of different amine-containing compounds such as aromatic amines, catecholamines and polyamines. The HPLC separation for the derivatized amines shows good repeatability. A possible disadvantage of the proposed method is that the reagent PASC can be only be used in the pre-column derivatization.

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