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Sensitive, accurate and rapid detection of trace aliphatic amines in environmental samples with ultrasonic-assisted derivatization microextraction using a new fluorescent reagent for high performance liquid chromatography

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ABSTRACT

A new fluorescent reagent, 1-(1*H*-imidazol-1-yl)-2-(2-phenyl-1*H*-phenanthro[9,10-*d*]imidazol-1-yl)ethanone (IPPIE), is synthesized, and a simple pretreatment based on ultrasonic-assisted derivatization microextraction (UDME) with IPPIE is proposed for the selective derivatization of 12 aliphatic amines (C_1: methylamine–C_{12}: dodecylamine) in complex matrix samples (irrigation water, river water, waste water, cultivated soil, riverbank soil and riverbed soil). Under the optimal experimental conditions (solvent: ACN–HCl, catalyst: none, molar ratio: 4.3, time: 8 min and temperature: 80 °C), micro amount of sample (40 μL; 5 mg) can be pretreated in only 10 min, with no preconcentration, evaporation or other additional manual operations required. The interfering substances (aromatic amines, aliphatic alcohols and phenols) get the derivatization yields of <5%, causing insignificant matrix effects (<4%). IPPIE–analyte derivatives are separated by high performance liquid chromatography (HPLC) and quantified by fluorescence detection (FD). The very low instrumental detection limits (IDL: 0.66–4.02 ng/L) and method detection limits (MDL: 0.04–0.33 ng/g; 5.96–45.61 ng/L) are achieved. Analytes are further identified from adjacent peaks by on-line ion trap mass spectrometry (MS), thereby avoiding additional operations for impurities. With this UDME–HPLC–FD–MS method, the accuracy (−0.73–2.12%), precision (intra-day: 0.87–3.39%; inter-day: 0.16–4.12%), recovery (97.01–104.10%) and sensitivity were significantly improved. Successful applications in environmental samples demonstrate the superiority of this method in the sensitive, accurate and rapid determination of trace aliphatic amines in micro amount of complex samples.

1. Introduction

Aliphatic amines, as raw materials for chemical industry and biodegradation products in ecological cycle, are widely distributed in the environment and can be easily transferred through soil or water [1]. Unfortunately, aliphatic amines tend to react with nitroso compounds, producing the carcinogenic *N*-nitrosamine compounds [2], which implies that aliphatic amines in ecological cycle will pose a serious threat to human health. Therefore, it is significant to detect aliphatic amines in environmental samples [3].

However, there are many difficulties in the determination of aliphatic amines in environmental samples [4]; due to degradation and dilution effect, aliphatic amine content is rather low; without chromophore moieties, trace aliphatic amines cannot be directly determined; strong basicity, polarity and reactivity of aliphatic amines are unfavorable to both extraction and chromatographic analysis [5]; environmental samples are much more complex and thereby difficult to pretreat. To overcome these difficulties, some methods utilizing different techniques have been established [6–10]. The high analytical

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sensitivities are achieved mainly by applying the pre-column derivatization technique [11] with excellent reagents, such as OPA-NAC (*o*-phthaldialdehyde-*N*-acetylcysteine) [12], NBD-Cl (7-chloro-4-nitro-2,1,3-benzoxadiazole) [13], BCEC-Cl (2-(11*H*-benzo[*a*]carbazol-11-yl) ethyl chloroformate) [14] and Dns-Cl (dansyl chloride) [15] etc. In spite of being successfully applied to various samples, these preferred reagents are reported to show relatively low sensitivity, unsatisfactory stability of derivatives, short detection wavelengths and serious interferences [16,17]. Preconcentration can be used to lower the detection limits, but unfortunately, the matrix components will be also concentrated together with the amines, leading to low accuracy [8]. Moreover, pretreatment of complex sample usually requires multiple manual operations involving preconcentration, evaporation and redissolution [4,10,18–24], causing the high loss of low-molecular-weight aliphatic amines (with high volatility) even at ambient temperature. This may account for the fact that some methods with complex procedure of pretreatment provided low recoveries for aliphatic amines [7,15]. Besides, the multiple operations with long run-time are beneficial neither to micro sample analysis nor to batch analysis. Consequently, though a lot of work has been done, there are still challenging tasks left to be performed in the sensitive, accurate and rapid determination of trace aliphatic amines in environmental samples.

In this work, a new derivatization reagent 1-(1*H*-imidazol-1-yl)-2-(2-phenyl-1*H*-phenanthro[9,10-*d*]imidazol-1-yl)ethanone (IPPIE) for labeling aliphatic amines is designed with both intense fluorescence response and strong mass spectrometry signals. An efficient pretreatment, the ultrasonic-assisted derivatization microextraction (UDME) with IPPIE is developed to enhance the analytical sensitivity, avoid multiple manual operations and reduce errors. The optimal conditions for UDME are achieved by applying two robust multivariate methods (artificial neural network (ANN [25]) and response surface methodology (RSM [26])). Under optimized conditions, aliphatic amines rather than their analogs (aromatic amines, aniline, phenol and alcohol) can be selectively derivatized in a very short time. The obtained derivatives are separated by high performance liquid chromatography (HPLC) and quantified by fluorescence detection (FD). On-line MS (mass spectrometry) technique is introduced to further identify the analytes, which can practically avoid the additional operations for impurities. The established UDME–HPLC–FD–MS method is applied to the simultaneous determination of 12 aliphatic amines in water samples and soil samples. To the best of our knowledge, this method provided the lowest detection limit for aliphatic amines. The improved sensitivity, accuracy and recovery demonstrate that the established method is a superior alternative for the selective determination of trace aliphatic amines in environmental samples.

2. Experimental

2.1. Instrumentation and conditions

HPLC was performed using an Agilent HP 1100 system (Waldbron, Germany) equipped with a vacuum degasser (G1322A), a quaternary pump (G1311A), a thermostated column compartment (G1316A), an autosampler (G1329A) and a fluorescence detector (G1321A). Mass spectrometer 1100 Series LC–MSD Trap SL (ion trap) (Bruker Daltonik, Bremen, Germany) was equipped with an atmospheric pressure chemical ionization (APCI) source (positive ion detection mode). Ion source conditions were: nebulizer pressure 60 psi; dry gas temperature 350 °C; dry gas flow 5.0 L/min; corona current (nA) 4000 (pos); capillary voltage 350 V. The semi-preparative HPLC column (Sun Fire C_{18}, 10 μm, 10 × 150 mm) was applied to prepare the single IPPIE–amine derivative. Paratherm U2 electronic water bath (Hitachi, Tokyo, Japan) was used to control temperature. Ultrasonic cleaner (SB-5200DTD, 40 kHz, Xinzhi Biotech Co., Ningbo, China) was used for the ultrasonic-assisted derivatization microextraction. Eluent A was 30% of acetonitrile solution containing formic acid/ammonia buffer (1000/7.5, v/v) (pH 3.7, 10 mmol/L); eluent B was 100% of acetonitrile. Eluents were filtered through a 0.20 μm nylon membrane (Alltech, Deerfiled, IL), respectively. Elution gradient was: 0–22 min, 90% to 55% of A; 22–26 min, 55% to 15% of A; 26–33 min, 15% to 0% of A. Flow rate was constant at 1.0 mL/min and column temperature was set at 30 °C. The labeled analytes were separated with a reversed phase C_{18} column (Eclipse Hypersil BDS, 4.6 mm × 150 mm, 5 μm) and then detected by FD at excitation wavelength λ_{ex} 260 nm and emission wavelength λ_{em} 380 nm. PHMK 79/2289 micro-melting point apparatus (Radebeul, Germany), Carlo-Erba 1106 elemental analyzer (Rodano, Italy), Nicolet 1ODX FTIR spectrometer (KBr, tablet, USA), and mass spectrometer 1100 Series LC–MSD Trap SL (ion trap) (Bruker Daltonik, Bremen, Germany) were used to characterize the synthesized IPPIE.

2.2. Chemical and samples

Standard aliphatic amines (C_1: methylamine; C_2: ethylamine; C_3: propylamine; C_4: butylamine; C_5: pentylamine; C_6: hexylamine; C_7: heptylamine; C_8: octylamine; C_9: nonylamine; C_{10}: decylamine; C_{11}: undecylamine; C_{12}: dodecylamine) were purchased from Shanghai Chemical Reagent Co. (Shanghai, China). Water was prepared by Milli–Q water system (Millipore, Bedford, MA, USA). Other reagents used were analytically pure and purchased from Tianjin Damao Chemical Reagent Co., Ltd. (Tianjin, China).

Six environmental samples, irrigation water (pH: 7.6, EC (electrical conductivity): 0.032 mS/cm), river water (pH: 7.6, EC: 0.058 mS/cm), waste water (pH: 4.1, EC: 0.047 mS/cm), cultivated soil (pH: 6.7, EC: 0.204 mS/cm), riverbank soil (pH: 7.8, EC: 0.295 mS/cm) and riverbed soil (pH: 8.2, EC: 0.271 mS/cm) were collected from a ecosphere near to the Sihe river in Qufu (Shandong, China) and hermetically stored at −20 °C in darkness until use.

2.3. Synthesis of IPPIE

An amount of 2 g of *N*,*N*′-carbonyldiimidazole and 3 g of intermediate 2-(2-phenyl-1*H*-phenanthro-[9,10-*d*]imidazole-l-yl)-acetic acid (PPIA) [27] were successively added into 100 mL of mixed solvents (acetonitrile/DMSO, v/v = 1:2). The obtained mixture was heated under reflux for 1 h and then cooled to room temperature. The obtained solution was transferred to a stirring ice–water bath (300 mL), remaining immersed for 10 min. The precipitated solid was filtered, then washed with water and dried at room temperature for 48 h. The crude product was recrystallized three times with acetonitrile, yielding a white crystal (78%). *M.p*: 266.6–268.2 °C (decomposition). Found: C: 77.59, H: 4.51, N: 13.92; calculated: 77.54, H: 4.49, N: 13.85. IR (KBr): 1733.26 (—C=O), 1475.68, 1455.48, 1467.19, 1493.01 (Ar), 1235.41, 1178.38, 773.73, 745.65, 703.15, 724.70. LC–APCI–MS: *m*/*z* 403.1 $[M-H]^+$; MS/MS: *m*/*z* 295.7 (molecular core moiety). The synthetic route is illustrated in Fig. 1.

2.4. Preparation of solutions

A volume of 10 mL of IPPIE solution (4.05×10^6 ng/mL) was prepared by dissolving 40.5 mg of IPPIE in DMF and then diluted with ACN (acetonitrile) to low-concentration solutions. The 20 mL of stock solution (C_1: 3.11×10^4 ng/mL; C_2: 4.52×10^4 ng/mL; C_3: