Characterization and application of acridine-9-*N*-acetyl-*N*-hydroxysuccinimide as a pre-column derivatization agent for fluorimetric detection of amino acids in liquid chromatography

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A simple and sensitive LC method that rapidly labels amino compounds including amino acids, using acridine-9-*N*-acetyl-*N*-hydroxysuccinimide (AAHS) which was synthesized by the reaction of acridine-9-*N*-acetic acid with benzenedisulfonyl-*N*-hydroxysuccinimide, was developed. A mixture of amines is treated with AAHS in the presence of triethylamine in non-aqueous acetonitrile or in 0.2 mol l^{-1} borate buffer at pH 8.0–9.0 in 40% v/v acetonitrile solution to give quantitative yields of amides. The emission maximum for the derivatized amines is 435 nm (λ_{ex} = 404 nm). The labeled derivatives are very stable; no significant decomposition is observed after heating in 50% acetonitrile at 40 °C for 24 h. Studies on the derivatization conditions indicate that amines or amino acids react very rapidly with AAHS under the proposed conditions. The method, in conjunction with a multi-step gradient, offers baseline resolution of common amine or amino acid derivatives on a reversed-phase C_{18} column. This method is more convenient and more efficient than previous methods which require prior conversion of carboxylic acids to acyl chlorides, which are unstable to moisture. The LC separation of amine or amino acid derivatives has good reproducibility. The established method is also suitable for the determination of other amine compounds in various biological fluids.

Various aliphatic amines are of environmental interest owing to their toxicity, reactivity and likely occurrence as a result of the decarboxylation of their precursor amino acids.[1] In the last two decades, the determination of the content of amines in food products and in environmental samples has become more and more important. However, most aliphatic amines show neither natural UV absorption nor fluorescence. The main difficulty with the chromatography of these substances is their detection, and chemical derivatization is necessary to increase the detection sensitivity and improve selectivity. Derivatizing reagents commonly used include 5-dimethylaminonaphthalene-1-sulfonyl chloride (dansyl chloride),[2–4] *o*-phthaldialdehyde (OPA),[5,6] 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) and NBD-F,[7] 4-(2-phthalimidyl)benzoyl chloride (PIB-Cl),[8] 3,4-dihydro-6,7-dimethoxy-4-methyl-3-oxoquinoxaline-2-carbonyl chloride,[9–11] 2-methylanilinonaphthalene-6-sulfonyl chloride,[12] 4-(*N*-phthalimidyl)benzenesulfonyl chloride,[13] 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC)[14,15] and 9-fluorenylmethyl chloroformate (FMOC).[16] Among these reagents, the common OPA method offers greater sensitivity and more selectivity, but is limited to primary amines. The instability of the OPA derivatives makes manual derivatization difficult to reproduce. The PIB-Cl method offers greater sensitivity, but is not appropriate to aromatic amines. NBD-Cl shows *ca.* 50% decomposition in methanol–water solution within 25 min when exposed to daylight. The AQC method is rapid, convenient and yields stable derivatives. However, the detection limits for the early-eluted amino acids, such as Asp, Ser, Glu and Gly, are 8–10-fold higher than those for the finally eluted acids, such as Phe, Leu, Ile and Met.[14] The FMOC method has good UV absorption and very high sensitivity with laser induced fluorescence detection, but the derivatized solution must be extracted with pentane because excess of derivatizing reagent interferes with the separation of amino acid derivatives and is detrimental to column performance.[17,18]

With the development of current techniques for the separation of amines and amino acids by LC, new reagents and approaches are constantly being developed. In a previous paper, we described the synthesis of acridine-9-*N*-acetic acid and its application to the determination of common amino acids after their conversion to the corresponding acid chlorides.[19] The ultimate aim of this study was to develop a new condensation agent (activated ester) for the rapid determination of amines including amino acids by the reaction of corresponding acid with benzenedisulfonyl-*N*-hydroxysuccinimide in the presence of triethylamine. This activated ester can rapidly label most amino compounds with little matrix interference. The detection sensitivity for labeled amines or amino acids was significantly improved. Optimum reaction conditions for the preparation of this activated ester and the analytical parameters for the determination of amines or amino acid derivatives were investigated. Complete and reproducible separation of a multi-component amine mixture, in conjunction with gradient elution, was obtained with satisfactory results.

Experimental

Instrumentation

A Model 655 liquid chromatograph equipped with a Model 650-10 S spectrofluorimeter (Hitachi Seisakusho, Tokyo, Japan), a Model 7125 injection valve (Rheodyne, Cotati, CA, USA), a Model 655 proportioning valve and a Model 644-61 integrator (Hitachi Seisakusho) were used. Fluorescence excitation and emission spectra were also obtained on a Model 650-10

S spectrophotometer fluorimeter (Hitachi Seisakusho). The excitation and emission bandpasses are both at 15 nm. Amine derivatives were separated on a 200 × 4.6 mm id, 5 μm Spherisorb column (Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, China). A Paratherm U$_2$ electronic water-bath (Hitachi, Tokyo, Japan) was used to control the column temperature. All mobile phases were treated ultrasonically for 15 min in order to remove gas bubbles prior to use.

Reagents

Ammonium dihydrogenorthophosphate was obtained from Jining Chemical Reagent Co. (Shandong, China). Triethylamine, pyridine, 2-methylpyridine and 4-dimethylaminopyridine were treated with molecular sieve and potassium hydroxide pellets, then redistilled prior to use. Dichloromethane, chloroform and other reagents were of analytical-reagent grade. Ammonium dihydrogenorthophosphate (4.0 mol l^{-1}) and triethylamine (0.36 mol l^{-1}) stock standard solutions, used for the preparation of LC eluents, were adjusted to pH 6.5 with ammonia solution and 4 mol l^{-1} hydrochloric acid. The quenching reagent was acetonitrile–water–acetic acid (20 + 3 + 2, v/v).

Acridine-9-*N*-acetic acid was prepared according to the procedure described previously.[19] A 5.0 × 10^{-3} mol l^{-1} solution of acridine-9-*N*-acetic acid was prepared by dissolving 126.5 mg of acridine-9-*N*-acetic acid in 100 ml of LC grade acetonitrile. Working standard solutions were obtained by appropriate dilution with acetonitrile. Benzenedisulfonyl chloride was also prepared according to a method described previously.[20]

Benzene-disulfonyl-*N*-hydroxysuccinimide (BDHS) was synthesized by the reaction of benzenedisulfonyl chloride (137.5 mg) with *N*-hydroxysuccinimide (115 mg) in a 1:1 molar ratio under the conditions of the Schotten–Baumann reaction in 100 ml of pyridine at 88 °C for 2 h. A 5.0 × 10^{-3} mol l^{-1} stock standard solution was prepared and working standard solutions were obtained by appropriate dilution with pyridine.

Chromatographic method

LC separation of amine derivatives was performed on a Spherisorb C$_{18}$ column with a binary gradient. The elution conditions for aliphatic amines were A = 0.005 mol l^{-1} ammonium dihydrogenorthophosphate + 0.005 mol l^{-1} triethylamine (pH 6.5)–acetonitrile (36 + 65 v/v) and B acetonitrile–water (95 + 5 v/v) and those for amino acids were A = 0.005 mol l^{-1} ammonium dihydrogenorthophosphate + 0.005 mol l^{-1} triethylamine (pH 6.5)–acetonitrile (91 + 9 v/v) and B acetonitrile–water (95 + 5 v/v). The flow rate was constant at 1.0 ml min^{-1} and the column temperature was kept at 30 °C. The gradient conditions used for the separation of amine and amino acid derivatives are indicated in the Figure captions.

Derivatization

(1) Preparation of acridine-9-*N*-acetyl-*N*-hydroxysuccinimide (AAHS). Acridine-9-*N*-acetyl-*N*-hydroxysuccinimide (AAHS) was prepared by the reaction of acridine-9-*N*-acetic acid with benzenedisulfonyl-*N*-hydroxysuccinimide in a 1:1 molar ratio within 30 min in the presence of triethylamine catalyst in acetonitrile. A 5.0 × 10^{-5} mol l^{-1} solution was prepared and AAHS was used directly without further treatment, after appropriate dilution with acetonitrile. The reaction scheme is shown in Fig. 1 (procedure A).

(2) Derivatization in non-aqueous solvents (method B or D). *(a) Method B.* To a mixture of an appropriate amount of amines and 25 μl of triethylamine in 50 μl of acetonitrile were added 20 μl of acridine-9-*N*-acetyl-*N*-hydroxysuccinimide acetonitrile solution (*ca.* 3 mg ml^{-1}) and the mixture was brought to a final volume of 100 μl with acetonitrile. After the mixture had been mechanically shaken for 15 min at 25–30 °C, 20 μl of this mixture were taken and evaporated to dryness under a stream of nitrogen and the residue was dissolved in 400 μl of chromatographic grade acetonitrile, followed by LC analysis.

(b) Method D. To a solution of 20 μl of acridine-9-*N*-acetyl acid (3.0 mg ml^{-1}) and 5 μl of triethylamine in 50 μl of acetonitrile were added 20 μl of acetonitrile solution containing an appropriate amount of amines. To this mixture were added 20 μl of benzenedisulfonyl-*N*-hydroxysuccinimide solution in acetonitrile (*ca.* 8.7 mg ml^{-1}), then 20 μl of triethylamine were immediately added and the mixture was brought to a final volume of 100 μl with acetonitrile. After the reaction had proceeded at 25–30 °C for 15 min, 20 μl of derivatizing solution were taken and evaporated to dryness under a stream of nitrogen, and the residue was dissolved in 400 μl of acetonitrile, followed by LC analysis.

(3) Derivatization in aqueous solution (method C). A 10–20 μl volume of amine standard was pipetted into glass tube (6 × 50 mm) followed by addition of 30 μl of borate buffer (pH 8.8, 0.2 M) and 40 μl of acetonitrile. Then 10–20 μl of acridine-9-*N*-acetyl-*N*-hydroxysuccinimide solution in acetonitrile (*ca.* 3.0 mg ml^{-1}) were added. After the glass tube had been agitated in a water-bath at 50 °C for 10 min, the reaction was stopped by the addition of 30 μl of quenching reagent. A 10–20 μl volume of this derivatizing solution was removed and diluted to 100 μl with acetonitrile for LC analysis.

Results and discussion

Fluorescence spectra and stability of derivatives

For many of the other fluorescence derivatives formed, such as with AQC and NBD-Cl, there are usually significant solvent effects upon their fluorescence parameters. In pure aqueous solution, only 10% of the fluorescence intensity relative to that in acetonitrile solution is observed for the AQC derivatives.[14] Hence the detection sensitivities for the early-eluted amino acid derivatives are lower than for those eluted later. The high fluorescence quantum efficiency in medium-polarity solvents makes fluorescence detection for NBD derivatives in those solvents favorable. However, the 40–80-fold decrease in quantum efficiency on changing from medium-polarity solvents to water has already been observed.[7]

In this study, no shift in the excitation or emission spectra in the range 0–100% acetonitrile or methanol is observed for AAHS derivatives. The maximum emission remains unchanged in acetonitrile or methanol aqueous solution, possibly owing to the isolation of the reactive site and fluorophoric moiety of the reagent. The detector is therefore operated at these excitation and emission wavelengths. In pure aqueous solution, only a 25.6% decrease in fluorescence intensity relative to that in acetonitrile and methanol solutions is observed. Hence the detection sensitivities of AAHS for the early-eluted amino acid derivatives are better than those of AQC or NBD.

The stabilities of the amine derivatives were investigated by the analysis of representative butylamine and glutamic acid derivatives at 6 h intervals. The results indicate that derivatives are sufficiently stable to allow further analysis of the derivatized samples for at least 24 h at room temperature.

Evaluation of derivatization methods

(1) Derivatization in non-aqueous solvents. Method B is carried out in non-aqueous acetonitrile in the presence of triethylamine or pyridine catalyst at 25–30 °C (Fig. 1). When a 5–10-fold excess of derivatization reagent is added, the derivatization yields determined for butylamine derivatives with method B by LC are $>80\%$. The yield of method D is about 5–10% lower than that of method B. No side-reactions are observed for derivatizing amines using methods B and D in acetonitrile under the proposed conditions. However, methods B and D are not suitable for amino acid analysis.

(2) Derivatization in aqueous solution. Method C is carried out by the reaction of AAHS with amines in borate buffer at pH 8.0–9.0 in 40% v/v acetonitrile solution; the addition of an appropriate amount of acetonitrile is to avoid the precipitation of hydrophobic derivatives. The derivatization yields for most amines including amino acids in a 5–10-fold excess of reagent are $>90\%$. This method is more suitable for derivatizing high molecular weight amines, such as polyamines, as it avoids the problem of precipitation of aliphatic amines. Method C is more suitable for amino acid analysis and superior to the AQC method as it has high sensitivity for the detection of early-eluted amino acids, such as Asp, Ser, Glu and Gly. In addition, the reaction reactivities for derivatizing primary and secondary amines were investigated. The results indicate that the reactivities of most primary amines are at least one order of magnitude greater than that of secondary amines. On comparison of diethylamine and ethylamine, the latter is nearly 50–100 times more reactive than the former. Common secondary amines with two alkyl groups have more spatially demanding configurations than those of primary amines.

Temperature conditions

The optimum temperature for derivatizing amines by methods B and D was investigated in the temperature range 0–40 °C in 5 °C increments. The results indicated that the optimum temperature range was 25–30 °C; above 40 °C, HPLC of the reaction products showed a small amount of benzenedisulfonamide, which not only interfered with the separation but also led to a risk of incomplete derivatization; below 25 °C, the rate of reaction decreased significantly and resulted in a long derivatization time.

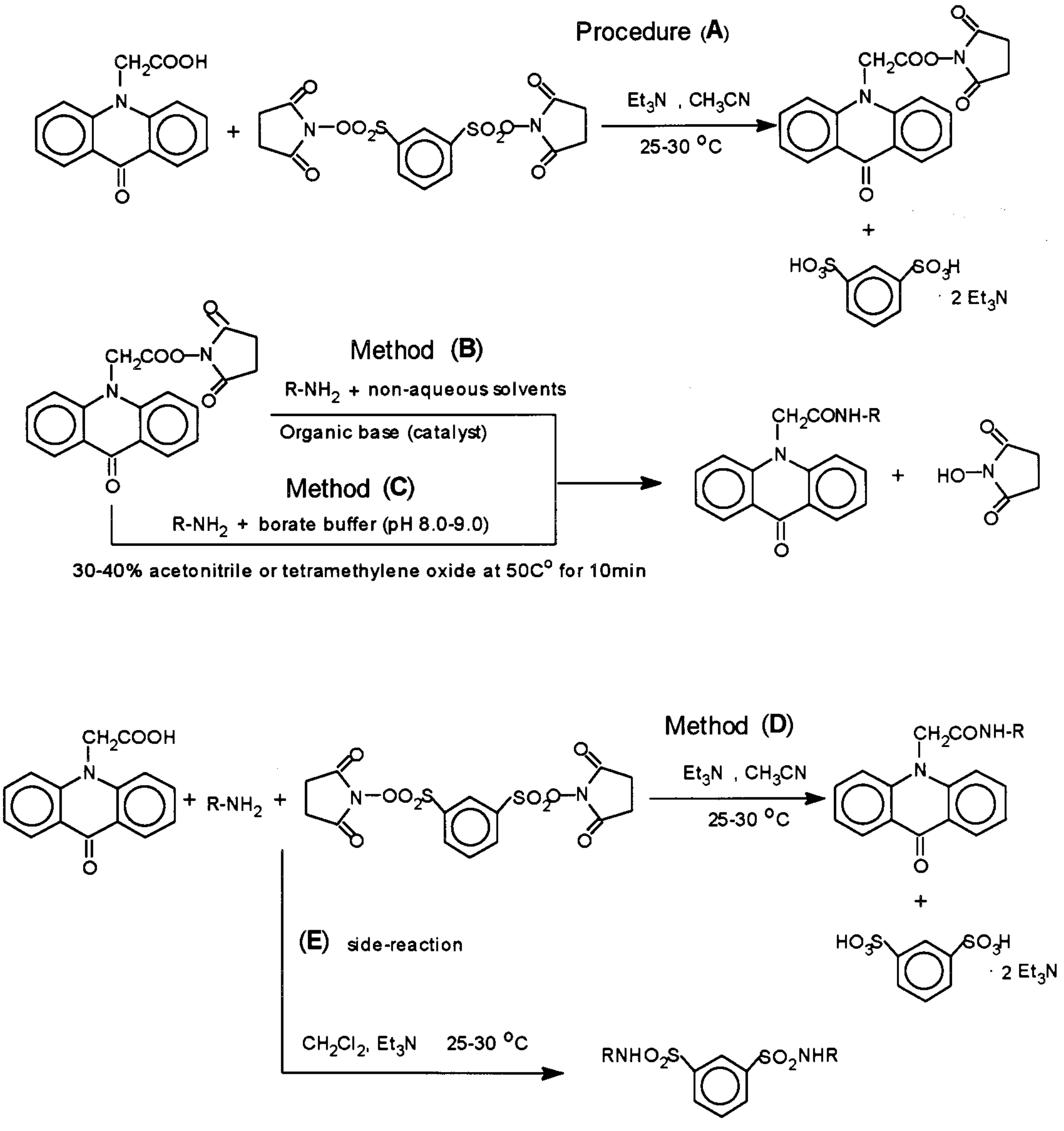

Fig. 1 Scheme of derivatization reaction using acridine-9-*N*-acetic acid and benzenedisulfonyl chloride as condensation agent for derivatizing amines.

The optimum temperature for derivatizing amino acids with method C was also investigated in the temperature range 10–80 °C in 10 °C increments. The results indicated that the optimum temperature range was 45–50 °C; above this range derivatives were partly hydrolyzed in the pH range 8.0–9.0; below 25 °C, the rate of reaction decreased and there was serious interference from the excess of derivatizing reagent, especially for the elution of Ala. Generally, the derivatization temperature selected with method C was 50 °C and the reaction time was 10 min.

Optimization of derivatization conditions

(1) Derivatization in non-aqueous solvents. It was important to assess the influence of the AAHS concentration and reaction time for effective derivatization. A solution containing ethylamine, butylamine and heptylamine (0.002 mol l^{-1} of each amine) was prepared, which was mixed with AAHS at concentrations from 0 to 0.01 mol l^{-1} in the presence of 0.1 mol l^{-1} triethylamine in acetonitrile at 25 °C. A 30 min reaction was carried out. The results indicated that the fluorescence intensities of the corresponding amine derivatives increased with increasing reagent concentration from 0 to 0.008 mol l^{-1}, then remained constant. It was also found that if the reagent concentrations were insufficient to obtain the maximum yield, addition of more reagent could reproducibly increase the yield to the maximum. Incomplete derivatization was also observed for non-linear alkylamines; this problem could be solved by heating the derivatized solution at 40 °C for 10 min, after which most linear alkylamines were derivatized. The effect of reaction time on the fluorescence intensities of representative ethylamine, butylamine and heptylamine derivatives was also investigated. The AAHS concentration was kept at 0.01 mol l^{-1} and all other conditions were similar to those mentioned above. The reaction time varied from 0 to 30 min. The intensity increased steadily from 0 to 15 min and then remained constant. Since increasing the reaction time to more than 15 min did not significantly alter the yields, this time was adopted throughout the study under non-aqueous conditions.

Dichloromethane, chloroform and acetonitrile were investigated as non-aqueous reaction solvents for derivatization. It was found that the products obtained with method D were not the only products using chloroform or dichloromethane as solvents in the presence of triethylamine catalyst. Evaluation of LC data (UV detection, not shown) indicated that small amounts of by-products were formed, possibly due to amines reacting directly with benzenedisulfonyl-*N*-hydroxysuccinimide to form the corresponding benzenedisulfonamides. However, the side-reaction (Fig. 1, procedure E) could be completely suppressed by using acetonitrile instead of dichloromethane or chloroform. Acetonitrile used as the reaction co-solvent was preferable to dichloromethane or chloroform as it not only accelerated the reaction rate but also restrained the side-reaction. A chromatogram of amines derivatized with method B in non-aqueous acetonitrile is in Fig. 2. The same chromatogram was also observed for the separation of amines derivatized with method D.

The alkaline catalysts triethylamine, pyridine, 2-methylpyridine and 4-dimethylaminopyridine were investigated as reaction catalysts. Triethylamine, pyridine and 4-dimethylaminopyridine gave satisfactory results, but the catalytic activity of 2-methylpyridine was lower, possibly owing to a significant increase in steric hindrance. Most subsequent derivatizations were carried out in the presence of triethylamine catalyst. Further study indicated that the final triethylamine concentration in the derivatized solution should be 0.05–0.1 mol l^{-1} to give complete the derivatization. In addition, triethylamine in the derivatized solution in excess of 0.1 mol l^{-1} did not significantly increase the reaction yields.

(2) Derivatization in aqueous solution. Optimum derivatization of AAHS with representative amines was investigated with different solvent compositions, buffer type, pH and concentration. Acetonitrile, tetrahydrofuran and acetone as the reaction co-solvents for derivatizing amines in aqueous solution were preferable to acetone as they avoided the problem of precipitation of hydrophobic derivatives, but a separation of phases at high buffer concentration was also observed using acetonitrile as co-solvent. This can be avoided in two ways: either the buffer concentration is controlled at < 0.2 mol l^{-1} or the acetonitrile concentration in the derivatized solution is kept at $< 50\%$ v/v. Subsequent derivatizing reactions were carried out using 40% v/v of acetonitrile with 0.2 mol l^{-1} borate buffer.

Borate, phosphate and bicarbonate as buffers were investigated for derivatization. Both borate and bicarbonate were satisfactory, but phosphate proved unacceptable as it produced many interfering peaks. Borate buffer concentrations from 0.1 to 0.2 mol l^{-1} had little effect on the yields as long provided that the reaction pH was maintained at 8.0–9.0, but outside this range, particularly in more acidic solution, decreased responses were observed and higher pH resulted in partial hydrolysis of derivatives. Although bicarbonate was also an effective buffer, some contaminants from the buffer in the derivatization blanks were observed. Generally, most subsequent derivatizations were carried out using 0.2 mol l^{-1} borate buffer at pH 8.0–9.0.

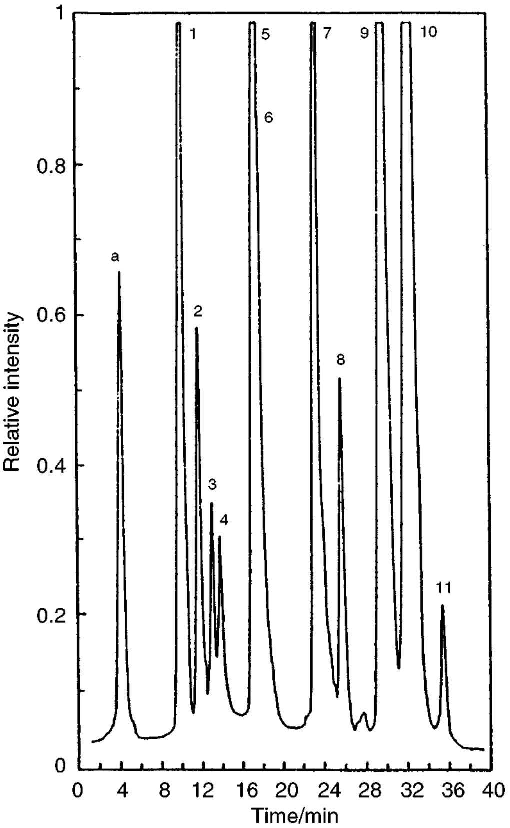

Fig. 2 Chromatogram of amine standard derivatized with method B in non-aqueous acetonitrile solution. Column, 200 × 4.6 mm id Spherisorb (5 μm). Eluents: A = 0.005 mol l^{-1} ammonium dihydrogenorthophosphate + 0.005 mol l^{-1} triethylamine (pH 6.5)–acetonitrile (36 + 65 v/v) and B acetonitrile–water (95 + 5, v/v); flow rate = 1.0 ml min^{-1}; column temperature = 30 °C; injection volume = 10 μl. Elution conditions: 0–5 min, 95–90% A; 10 min, 80% A; 20 min, 70% A; 25 min, 60% A; 30 min, 40% A; 40 min, 20% A; 50 min, 100% B. Peaks: a = acridine-9-*N*-acetic acid; 1 = methylamine; 2 = ethylamine; 3 = isopropylamine; 4 = propylamine; 5 = acridine-9-acetyl-*N*-hydroxysuccinimide; 6 = butylamine; 7 = heptylamine; 8 = 2-octylamine; 9 = decylamine; 10 = undecylamine; 11 = spermine.

Table 1 Concentrations of volatile amines in environmental samples (average values, $n = 5$)

Sample No.[a]	Amine	Waste water/ µmol l^{-1} (determined)	Added/µmol l^{-1}	Found/ µmol l^{-1}	RSD (%)	Recovery (%)
1	Methylamine	140.3	20.0	158.7	3.7	98.8
	Ethylamine	12.1	20.0	31.8	3.2	97.5
	Isopropylamine	11.4	20.0	29.7	3.0	115
	Propylamine	8.4	20.0	28.2	3.4	97.6
	Butylamine	24.6	20.0	46.2	4.3	106
2	Methylamine	32.8	20.0	50.8	4.0	93.9
	Ethylamine	16.2	20.0	37.0	3.7	105
	Isopropylamine	—	20.0	—	—	—
	Propylamine	—	20.0	—	—	—
	Butylamine	6.8	20.0	27.2	4.3	106
3	Methylamine	16.7	20.0	35.8	4.0	94.6
	Ethylamine	7.7	20.0	28.2	3.6	106
	Isopropylamine	23.1	20.0	44.8	3.4	107
	Propylamine	3.6	20.0	23.5	3.5	97.2
	Butylamine	67.4	20.0	89.6	4.0	103

[a] Samples 1 and 3: waste water from Qufu Normal University and Qufu city, respectively (Qufu, China); sample 2: snow water from Qufu city (China).

Analytical reproducibility and linearity

Standard samples containing C_1–C_4 amines were derivatized and analyzed according to the procedures described with method C; 50 pmol were injected and the relative standard deviation was less than 4.8%. Linearity was established by the analysis of amine standard solutions. For calibration, working standard solutions in the concentration range 0–200 µmol l^{-1} were prepared in acetonitrile. The standards were derivatized and each standard was analyzed in triplicate. Linear regression analysis was performed by plotting the observed peak height (Y cm) *versus* concentration of each amine in µmol l^{-1}. It was found that C_1–C_4 amines gave linear derivatization over the range 2.0–160 µmol l^{-1} with the correlation coefficients > 0.998; other amines were not detected.

Detection limits

The detection limit depends upon different detector and different elution conditions. The detection limits were determined, using derivatized amine standard solutions of successively lower concentration, by 10 µl injections until a signal-to-noise ratio of 3:1 was achieved. The detection limits for methylamine, ethylamine, propylamine and butylamine are 17–87 fmol and those for heptylamine, 2-octylamine, decylamine, undecylamine and spermine are 0.16–0.87 pmol. The detection limits for most amino acids are 4.6–36 fmol.

Analysis of amines in environmental water samples

With the optimized method C, an application was developed for the direct determination of amines in environmental water samples. Compounds were confirmed by spiking with portions of the corresponding pure compounds and the contents were determined using the standard addition method. Table 1 summarizes the results obtained with the proposed method.

LC separation of amino acid derivatives

LC separation of a standard solution of an amino acid mixture was carried out using a reversed-phase Spherisorb-C_{18} column. The amino acids were derivatized with method C. The elution order of amino acid derivatives at pH 6.5 is shown in Fig. 3.

Conclusions

In comparison with currently available methods for the determination of amines or amino acids, the proposed method offers a number of advantages: the derivatizing reagent is easily prepared from commercially available reagents (only acridine-

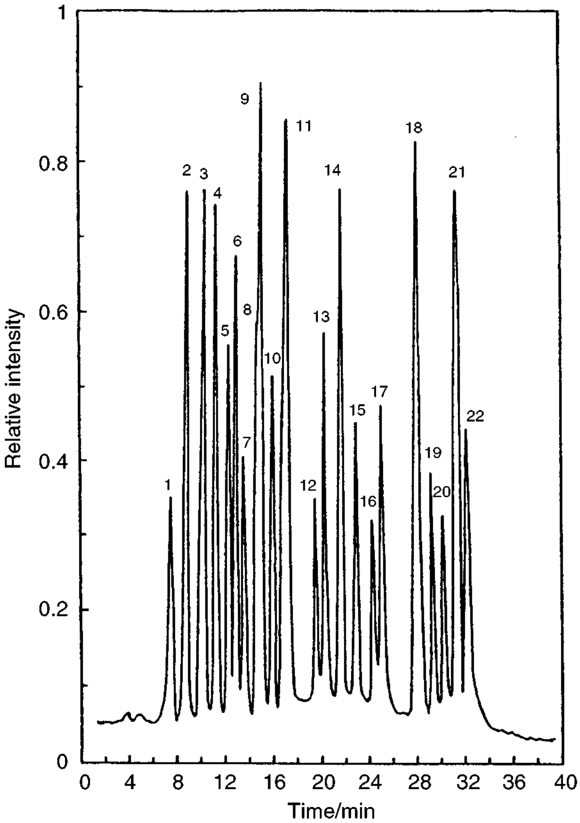

Fig. 3 Chromatogram of a standard mixture of amino acids (50 pmol of each amino acid) derivatized using AAHS with method C. Excitation wavelength λ_{ex} = 404 nm, emission wavelength λ_{em} = 435 nm. Column, 200 × 4.6 mm id Spherisorb (5 µm); flow rate = 1.0 ml min^{-1}; column temperature = 30 °C; injection volume = 10µl. Eluent A = 0.005 mol l^{-1} ammonium dihydrogenorthophosphate + 0.005 mol l^{-1} triethylamine; B = acetonitrile–water (95 + 5 v/v). Gradient conditions: 0–6 min, 95% A; 16 min, 85% A; 25 min, 75% A; 30 min, 70% A; 35 min, 60% A; 40 min, 50% A; 45 min, 30% A; 50 min, 100% B. Peaks: 1 = acridine-9-*N*-acetic acid; 2 = Asp; 3 = Glu; 4 = Ser; 5 = His; 6 = Gly; 7 = Thr; 8 = acridine-9-*N*-acetyl-*N*-hydroxysuccinimide; 9 = Ala; 10 = Pro; 11 = NH_3; 12 = Cys; 13 = Tyr; 14 = Arg; 15 = Trp; 16 = Val; 17 = Met; 18 = Ile; 14 = Leu; 20 = Orn; 21 = Phe; 22 = Lys.

9-*N*-acetic acid was synthesized according to the procedure described previously); the method is facile, inexpensive and reproducible; the reactive functional group of AAHS is relatively selective for amines or amino acids under the conditions described; and the reagents and the derivatized samples are stable. Comparing the molecular structure with current derivatizing reagents, the acridine-9-*N*-acetic acid molecule simultaneously contains nitrogen and oxygen atoms, which can provide lone-pair electrons leading to a large conjugated system and significantly increases the detection sensitivity. The selective determination of aliphatic amines or amino acids using the proposed method is possible. Methods B and D are suitable only for aliphatic amines and method C is more suitable for the simultaneous determination of amines and amino acids. Comparing AAHS with AQC and NBD-Cl, the reaction rate of AAHS with amines and amino acids is relatively slow, but the detection sensitivities for the early-eluted amino acids are good. A possible disadvantage of the proposed method is that the reagent can only be used in the pre-column mode.

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