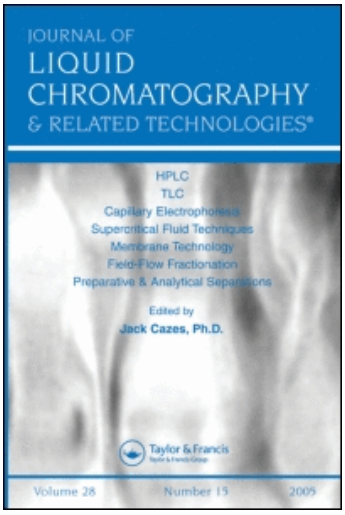

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CARBAZOLE-9-N-ACETYL-N-HYDROXYSUCCINIMIDE (CAHS) AS PRE-COLUMN DERIVATIZATION AGENT FOR FLUORIMETRIC DETECTION OF AMINO COMPOUNDS WITH LIQUID CHROMATOGRAPHY

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ABSTRACT

A simple and sensitive LC method for the determination of amino compounds, using carbazole-N-acetyl-N-hydroxy-succinimide (CAHS) as condensation agent, has been developed. A mixture of amines is treated with CAHS, in the presence of triethylamine catalyst in non-aqueous acetonitrile or in the presence of 0.2 M borate buffer at pH 8.0-9.0 in 40% (v/v) of acetonitrile solution, to give quantitative yields of amides. Emission maximum for the derivatized amines is 365 nm (λ_{ex} 335 nm). Studies on derivatization conditions indicate that amines react very fast with CAHS under proposed conditions. The method, in conjunction with a multi-step gradient, offers baseline resolution of common amine derivatives on a reversed-phase C_{18} column.

Derivatization reaction is more convenient and more efficient than previous methods which require prior conversion of carboxylic acids to acyl chlorides. The LC separation of amine derivatives has good reproducibility. The established method is also suitable for the determination of other amines in various biological fluids.

INTRODUCTION

Various aliphatic amines are of environmental interest due to their toxicity, reactivity, and likely occurrence as a result of the decarboxylation of their precursor amino acids.[1] The determination of the content of amines in food products or 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. Therefore, chemical derivatization is necessary to increase detection sensitivity and improve selectivity. Gas chromatography is frequently used to determine amines using various derivatizing reagents.[2] Many other methods including enzymatic,[3,4] spectrophotometric,[5,6] and ion-exchange chromatographic detection[7] have been described for the determination of amines in various matrices. These methods are usually limited due to low sensitivity.

Determination of amines with UV-absorption or fluorescence tagging reagents by liquid chromatography (LC) is based on amidation of amino groups with acyl chlorides from corresponding UV-absorbing or fluorescent carboxylic acids. Dansyl chloride (5-dimethylaminonaphthalene-1-sulfonyl-chloride) is probably the most familiar[8-10] along with related compounds such as dabsyl and debsyl chloride.[11] Other reagents commonly used include benzoyl chloride,[12] m-toluoyl chloride,[13,14] fluorescamine,[15] phenyl isothiocyanate (PITC) and p-toluenesulfonyl chloride,[16] o-phthaldialdehyde (OPA),[17-19] 3,5-dinitrobenzoyl chloride,[20] 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) along with NBD-F,[21] 4-(2-phthalimidyl)-benzoyl chloride (PIB-Cl),[22] phthalimidylbenzoyl chloride,[23] 3,4-dihydro-6,7-dimethoxy-4-methyl-3-oxoquinoxaline-2-carbonyl chloride,[24-26] 4-(N,N-dimethyl-aminosulfonyl) - 7- (2-chloroformyl-pyrrolidin-1-yl)-2,1,3-benz-oxazole,[27] 2-(1-pyrenyl)ethyl-chloro-formate,[28] 7-dimethyl-aminocoumarin-3-carbonyl-fluoride,[29] 2-methyl-anilinonaphthalene-6-sulfonyl chloride,[30] and 4-(N-phthalimidyl)-benzene-sulfonyl chloride.[31]

Among these methods, the common PITC method has two disadvantages: the derivatization procedure is lengthy; excess derivatization agent is required to be removed before analysis. The OPA method offers greater sensitivity and more selectivity, but it is limited only to primary amine. The instability of the OPA derivatives makes manual derivatization difficult to reproduce. The PIB-Cl method offers greater sensitivity, but is not appropriate for aromatic amines, although NBD-F is more reactive than NBD-Cl for derivatizing amino compounds. Data previously reported indicated that the two reagents

themselves have about 30-50% decomposition in methanol-water solution exposed to daylight for 25 min. In addition, the application of sulfonate-type coupling reagents for derivatizing amino compounds has also been reported.[32] However, the reaction requires generally 6 to 8 h in chloroform or EtOAc, and 2 to 3 h in acetonitrile. The AQC method is rapid, convenient, and yields stable derivatives. But, in pure aqueous solution, only 10% of the fluorescence intensity relative to that in acetonitrile solution is observed for its derivatives. Thus, the detection limits for the early eluted amine derivatives are usually higher than those eluted later.[33, 34,35]

In a previous paper,[36] we have already described the synthesis and analytical application of carbazole-9-N-acetyl acid for the determination of common amino acids after it is converted to corresponding acid chlorides. In this study, the principal goal is to develop a new condensation agent for rapid determination of amino compounds. Carbazole-9-N-acetyl acid reacts with benzene-disulfonyl-N-hydroxysuccinimide in the presence of triethylamine or pyridine catalyst to give an activated ester which can rapidly label most amino compounds with little matrix interference. The optimal reaction conditions for the preparation of this activated ester and analytical parameters for the determination of amines are investigated. Complete and reproducible separation of a multi-component amine mixture, in conjunction with a multi-step gradient, is obtained. At the same time, the amines from an environmental water sample are determined with satisfactory results.

EXPERIMENTAL

Apparatus

A model 655 liquid chromatograph equipped with 650-10 S fluorescence spectrophotometer (Hitachi Seisakusho, Tokyo, Japan) and 644-61 integrator were used in the experiments. Fluorescence excitation and emission spectra were also obtained on 650-10 S fluorescence spectrophotometer. Excitation and emission bandpass are both at 15 nm. Amine derivatives were separated on a 200 x 4.6mm Spherisorb column, 5 μm (Dalian Institute of Chemical Physics, Chinese Academy of Sciences). A Paratherm U_2 electronic water-bath (Hitachi, Ltd. Tokyo, Japan) was used to control column temperature. All mobile phases were treated ultrasonically for 15 min to remove gas bubbles prior to use.

Reagents

Ammonium dihydrogenorthophosphate, analytical grade, was from Jining Chemical Reagent Co. Triethylamine, pyridine, 2-methylpyridine and 4-dimethylaminopyridine were treated with molecular sieve and potassium hydroxide pellets, respectively, then was redistilled prior to use. Double

distilled water was used throughout. Dichloromethane, chloroform, and other reagents were of analytical reagent grade. Acetonitrile was chromatographic grade. Ammonium dihydrogenorthophosphate stock solution (2.67M), used for preparation of LC eluents, was adjusted to pH 6.5 with ammonia solution. Triethylamine (0.36M) stock solution used for preparation of LC eluent was adjusted to pH 6.5 with 4M hydrochloric acid. Carbazole-9-N-acetyl acid was prepared according to the method we previously published.[36] Benzene-disulfonic acid and its chloride were also prepared according to a previous method.[37] The quenching reagent was acetonitrile-water-acetic acid (20:3:2, v/v/v).

Chromatographic Method

HPLC separation of amine derivatives was performed on a Spherisorb C_{18} column with a binary gradient. Eluent A consisted of 10 mM ammonium dihydrogenorthophosphate and 10 mM triethylamine (pH6.5)/acetonitrile (35:65, v/v) and B was 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 derivatives have been indicated in the legend of each Figure.

Preparation of Carbazole-9-N-acetyl-N-hydroxysuccinimide Acetonitrile Solution

Carbazole-9-N-acetyl-N-hydroxysuccinimide is prepared by the reaction of carbazole-9-N-acetyl acid with benzene-disulfonyl-N-hydroxysuccinimide (as shown in Figure 1, procedure A). To a solution containing 8.2 mg (0.0364 mmol) of carbazole-9-N-acetyl acid and 10 μL of triethylamine in 1.0 mL acetonitrile, was added a solution containing ca. 7.9 mg (0.0182 mmol) benzene-disulfonyl-N-hydroxysuccinimide in 1.0 mL acetonitrile. The mixture was allowed to stand at room temperature for 30 min to form carbazole-9-N-acetyl-N-hydroxysuccinimide (CAHS). Corresponding concentration of CAHS was ca. 3.0 mg/mL and directly used without further treatment.

Derivatization of Amines

Derivatization in Non-Aqueous Solvents

(a) Method **B**: To a mixture of appropriate amount of amines and 25 μL of triethylamine in 50 μL acetonitrile, was added 20 μL CAHS acetonitrile solution, and the final volume was brought up to 100 μL with acetonitrile.

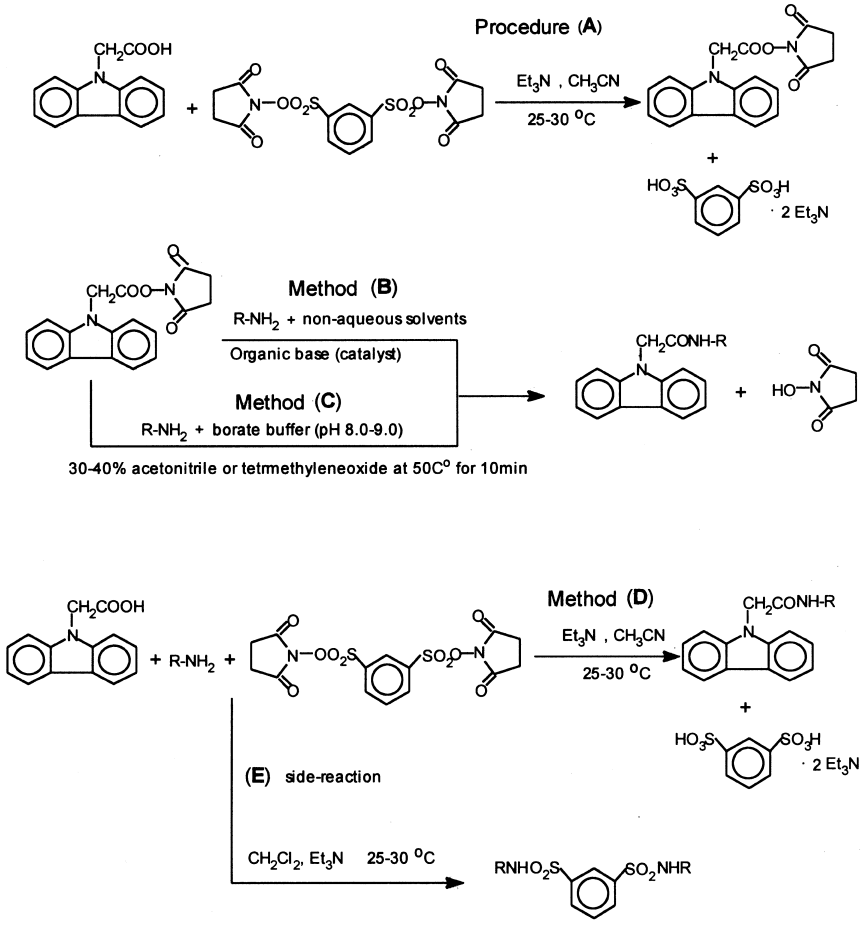

Figure 1. The scheme of condensation reactions.

After the mixture was mechanically shaken for 15 min at room temperature, 20 μL of the reaction solution was taken and evaporated under a stream of nitrogen, and the residue redissolved in 100 μL of chromatographic grade acetonitrile, followed by LC analysis.

(b) Method **D**: To a solution containing 20 μL carbazole-9-N-acetyl acid (3.0 mg/mL) and 10 μL of triethylamine in 50 μL of acetonitrile, was added a mixture of appropriate amount of amines and 5 μL of triethylamine in 50 μL of acetonitrile. The mixture was immediately added to 10 μL triethylamine and 20 μL of benzene-disulfonyl-N-hydroxysuccinimide acetonitrile solution.

Derivatization reaction was allowed to proceed for 15 min at room temperature, and then 20 μL of the derivatizing solution was taken and evaporated under a stream of nitrogen, and the residue redissolved in 100 μL acetonitrile, followed by LC analysis.

Derivatization in Aqueous Solution (Method C)

10-20 μL volume of amine standard was pipetted into glass tube (6 x 50 mm) followed by addition of 30 μL of borate buffer (pH = 8.8, 0.2 M) and 40 μL of acetonitrile. 10-20 μL of CAHS acetonitrile solution was then added. After the glass tube was agitated in water-bath at 50°C for 10 min, derivatizing reaction was stopped by adding 30 μL of quenching reagent. 10-20 μL of derivatizing solution was removed followed by dilution with acetonitrile to bring the volume up to 100 μL directly for HPLC analysis.

Preparation of Carbazole-N-Acetyl-Butylamine

0.5 g carbazole-N-acetic acid was dissolved in 5 mL of dichloromethane and placed in a 10-mL rounded bottom flask. To this flask, 1.5 mL of $SOCl_2$ was added and the mixture was allowed to reflux for 30 min. After the reaction was finished, the solvent and excess of $SOCl_2$ were removed by a rotary evaporator.

The residue was redissolved in 2 mL of dichloromethane, and immediately added a solution containing 0.30 g n-butylamine in 2 mL dichloromethane, followed by the addition of appropriate amount of triethylamine (final concentration 0.05-0.1M). The mixture was well mixed and allowed to stand for 10 min on a stirrer plate with micro stirrer.

After reaction was completed, the mixture was shaken successively with 2 mL each of 2 M hydrochloric acid, water, 2 M sodium hydroxide and deionized water. Dichloromethane layer was then dried with anhydrous sodium sulphate. The supernatant was removed and evaporated at a stream of nitrogen. The residue was redissolved in 2 mL of dry acetonitrile. Corresponding low concentration of carbazole-N-acetyl-butylamine was obtained by appropriate dilution with acetonitrile and directly used to investigate the influences of various organic solvents and different temperature on the fluorescence spectra.

RESULTS AND DISCUSSION

Stabilities of Reagents and Amine Derivatives

Benzene-disulfo-chloride, which is a solid with more moisture-resistance, is an active reagent and has been widely used as condensation reagent especially in the synthesis of β-cylcodextrin derivatives.[38] Benzene-disulfonyl-N-hydroxysuccinimide (BDHS) was easily prepared by the reaction of benzene-disulfo-chloride with N-hydroxysuccinimide according to the Schotten-Baumann reaction in pyridine at 88°C for 2 h. BDHS solution, which was directly used as reaction solution without further treatment, is very stable allowing it to be used for a week. The prepared CAHS acetonitrile solution is also very stable and sufficient to allow further application for a week at room temperature. During this time, derivatization yields of CAHS with amines show no remarkable differences.

The stabilities of amine derivatives at room temperature were investigated by the analysis of corresponding derivatives at 6 h intervals. As expected, daylight had no effect on the stability. The labeled derivatives are very stable; no remarkable decomposition was observed after heating in 50% acetonitrile solution (pH 6.5) at 40°C for 24 h.

Effect of Solvents on the Fluorescence Intensity of Representative N-Butylamine Derivative

The effects of various solvents on the fluorescence spectra of n-butylamine derivative are investigated. Using the following classes of solvents: group 1 = methanol, ethanol, 1-propanol, n-butanol, n-pentanol, 1-hexanol, n-heptanol and 1-octanol; group 2 = methylamine, methylamine, n-propylamine and n-butylamine; group 3 = formic acid, glacial acetic acid, propionic acid, butyric acid and pentoic acid; group 4 = acetonitrile/water mixture (acetonitrile concentration from 10 to 100 % v/v). The fluorescence intensities of n-butylamine derivatives in various alcohols increase with increasing carbon chain length of alcohols (see Figure 2). Such an observation is new as far as we know. It is probably due to the fact that hydrogen bond forces between derivative and various alcoholic molecules decrease with increasing solvent viscosity. Note that, if the carbon number of alcohol is >8, the fluorescence intensity of n-butylamine derivative decreases with increasing carbon number of the alcohol, this may be attributed to the progressively decreasing solubility in corresponding alcoholic solvents.

Fluorescence intensities of n-butylamine derivatives in solvents from group 2 increase with the increasing carbon chain length of amines. Note that, the fluorescence intensity of n-butylamine derivatives in methylamine is near three times less relative to that in n-butylamine.

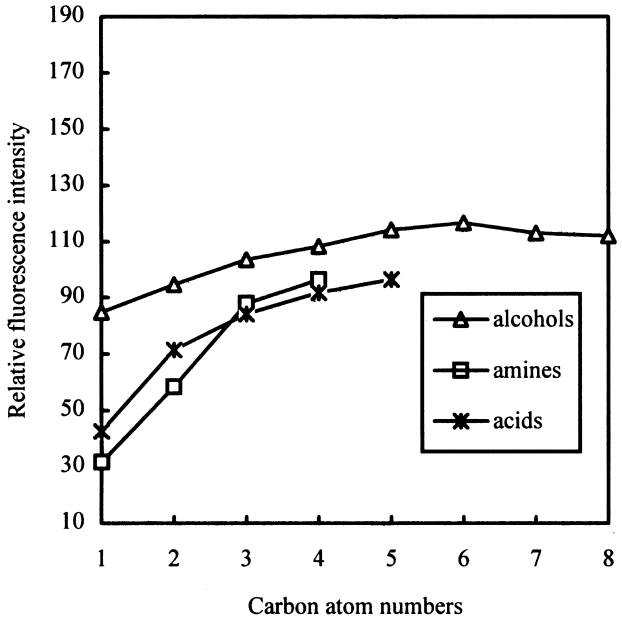

Figure 2. The relative fluorescence intensity of representative n-butylamine derivative in various alcohols, amines and acids; the values of relative fluorescence intensity are calculated using tetrahydrofuran as 100% at the same conditions. Alcohols: methanol, ethanol, 1-propanol, n-butylamine, n-pentanol 1-hexanol, 1-heptanol, and 1-octanol. Amines: methylamine, ethylamine, n-propylamine, n-butylamine. Acids: formic acid, glacial acetic acid, propionic acid, butyric acid, pentanoic acid.

This is probably due to the fact that the hydrogen bond force is stronger in methylamine than that in n-butylamine. With the solvents from group 3, it is also found that the fluorescence intensities of n-butylamine derivatives increase with increasing carbon chain lengths of acids, which is also similar to that observed in the cases of alcohols. Note that, the fluorescence intensity in formic acid is nearly half that relative to glacial acetic acid or propionic acid or butyric acid. It is probably due to the fact that n-butylamine derivative is partially protonated in relatively strong formic acid, resulting in corresponding weak fluorescence emission. When other derivatives are tested in different medium polar solvents, similar maximum wavelengths are obtained. No shift in excitation or emission spectra in the range of 10%-100% acetonitrile or methanol is observed. Solvent polarity shows, in all cases, little effect on the maximum emission wavelength. The maximum emission remains unchanged in acetonitrile or methanol aqueous solution possibly due to the isolation of the reactive site and fluorophoric moiety of reagent.

With the solvents from group 4, the emission intensity of representative n-butylamine derivatives in various concentrations of acetonitrile are studied. Emission intensity decreases with decreasing acetonitrile concentrations. There is a 18.4% difference between 100% and 10% (v/v) of acetonitrile solution.

Evaluation of Various Derivatization Methods

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 (see Figure 1). When a five- to ten-fold excess of derivatization reagent is added, derivatization yields determined for n-butylamine derivatives with method **B** by LC is >80%. Derivatization yields of stored CAHS with n-butylamine show no remarkable differences for a week. The yields of method **D** can achieve 75% or above and it is simple in operation. At the same time, no side-reactions are observed for derivatizing amines using methods **B** and **D** in non-aqueous acetonitrile under proposed conditions in the presence of triethylamine or pyridine catalyst. But, methods **B** and **D** are not suitable for derivatizing amino acids.

Derivatization in Aqueous Solution

Method **C** is carried out by the reaction of CAHS with amines in borate buffer at pH 8.0-9.0 containing 40% (v/v) of acetonitrile; the addition of appropriate amounts of acetonitrile or tetrahydrofuran is to avoid the precipitation of hydrophobic derivatives. The derivatization yields for most amines in a five- to ten-fold excess of CAHS are > 90 %. This method is more suitable for derivatizing high carbon chains of amines as it avoids the problem of precipitation of aliphatic amines, such as polyamines, which are partially dissolved in non-aqueous acetonitrile or dichloromethane. In addition, a slightly decrease (ca. 14%) in quantum efficiency for amines derivatives when changing from methanol and acetonitrile solvents to water is observed. Thus, with a gradient elution, the detection sensitivity of CAHS for the early eluted amine derivatives are better than that of AQC .

Temperature Condition

The optimum temperature for derivatizing amines with methods **B** and **D** were investigated at the temperature range from 0-40°C in 10°C increments. Results indicated that the optimal temperature range was 25-30°C, above which the equilibrium position reduced the proportion of CAHS reacting with amines. When derivatization temperature was >40°C, a small amount of benzene-disulfonamide was formed, which not only interfered with separation but there was also a risk of incomplete derivatization; while below 25°C the reaction rate was significantly decreased and resulted in a long derivatization time.

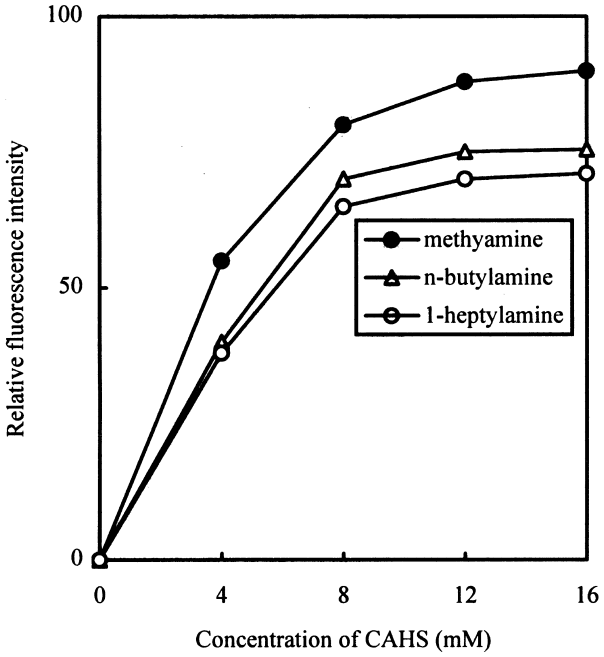

Figure 3. Effects of CAHS concentrations on fluorescent signals of representative methylamine, n-butylamine, and 1-heptylamine (each amine, 2 mM).

Therefore, most subsequent derivatization temperature selected in experiments was 25-30°C. An optimal temperature for derivatizing amines in aqueous solution with method **C** was also investigated at the temperature range from 10-90°C in 10°C increments. It was found that the optimal temperature range was 45-50°C; above this range the derivatives were partially hydrolysated in the pH 8.0 to 9.0 range; while below 40°C, slower reaction rate was also observed. Moreover, excess CAHS can not be completely hydrolysated; a serious interference especially for the elution of n-butylamine derivatives was observed. Therefore, the temperature selected for method **C** was usually at 45-50°C, and the reaction time was 10 min.

Optimization of Derivatization Conditions

Derivatization in Non-Aqueous Solvents

Figure 3 demonstrates the effects of CAHS concentrations on the fluorescence intensities for derivatizing methylamine, n-butylamine and 1-

heptylamine (2 mM each amine) with method **B**. CAHS at concentration ranges from 0-10 mM was well mixed with amines in non-aqueous acetonitrile in the presence of 0.05-0.1 M triethylamine catalyst at 25°C. A 30 min reaction was carried out. As shown in the plot, fluorescent intensities of amine derivatives increased with increasing derivatization agent concentrations from 0-8 mM, above which fluorescence intensities were constants. It was also found that if reagent concentration was insufficient to obtained maximal yields, addition of more reagent could reproducibly increase the yield to the maximum. The occurrence of incomplete reaction was also observed for non-linear alkylamines; this could easily be solved by heating the derivatized solution to 40°C for 20 min after most linear alkylamines were derivatized.

The effects of reaction time on fluorescence intensity for amine derivatives were also investigated. The concentration of CAHS was kept at 10 mM; all other conditions were similar to those of Figure 3. The reaction time varied from 0-30 min. The fluorescence intensities steadily increased from 0-15 min and were constant after 15 min.

Dichloromethane, chloroform, and acetonitrile were investigated as reaction non-aqueous solvents for derivatization. Dichloromethane and chloroform were easily evaporated under a steam of nitrogen. But evaluation of the LC data (UV detection, not shown) indicated that a small amount of by-products were formed when chloroform or dichloromethane was used as the reaction solvent. It was possibly due to the fact that a small amount of amines directly reacted with benzene-disulfonyl-N-hydroxysuccinimide to form corresponding benzene-disulfonamides. However, the side-reaction (Figure 1, procedure E) could be completely suppressed by the use of acetonitrile giving the desired amides in good yield.

Comparison of these products derivatized from the method **D** with those derivatized from the method **B** confirmed that derivatization products of method **D** were also the expected amides. Acetonitrile used as the reaction solvent is preferable to dichloromethane or chloroform as it not only accelerated the reaction rate but also restrained the side-reaction. The chromatogram of amines derivatized in non-aqueous acetonitrile was shown in Figure 4 (a and b).

Alkaline catalysts: triethylamine, pyridine, 2-methylpyridine, and 4-dimethylaminopyridine were investigated as reaction catalysts. Triethylamine, pyridine and 4-dimethylaminopyridine as catalysts gave satisfactory results. But catalytic activity of 2-methylpyridine was lower than that of triethylamine, pyridine, and 4-dimethylaminopyridine possibly due to a significant increase by steric hindrance. Most subsequent derivatization was carried out in the presence of the triethylamine catalyst. Further study indicated that final triethylamine concentrations in derivatized solution should be 0.05-0.10 M to give effective derivatization. In addition, triethylamine in derivatized solution in excess of 0.1 M does not significantly increase the reaction yields.

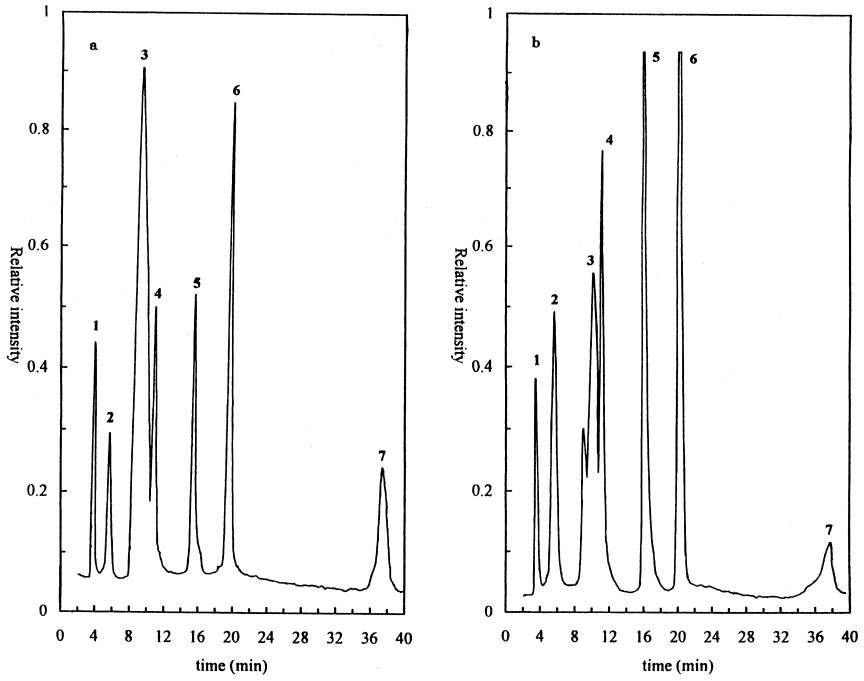

Figure 4. Chromatogram of amine standard derivatized with CAHS using various derivatization methods (a. derivatized with method B; b. derivatized with method D). Chromatographic conditions: column, 2004.6 mm I.D. Spherisorb 5 •m; flow rate = 1.0 mL min^{-1}; column temperature 30. Gradient conditions: 0-5 min = 95 - 90% A; 10 min = 80% A; 20 min = 70% A; 25 min = 60% A; 30 min = 50% A; 40 min = 30% A; 50 min = 100% B; peaks: 1 = carbazole-9-N-acetyl acid; 2 = methylamine; 3 = carbazole-9-N-acetyl-N-hydroxysuccinimide; 4 = n-propylamine; 5 = n-butylamine; 6 = 1-heptylamine; 7 = spermine.

Derivatization in Aqueous Solution

Optimal derivatization of CAHS with representative amines was investigated at different solvent composition, buffer type, pH, and concentration. Acetonitrile, tetrahydrofuran, and acetone as reaction cosolvents for derivatizing amines were investigated. The results indicated that acetonitrile and tetrahydrofuran as reaction cosolvents for derivatizing amines in aqueous solution were preferable to other solvents as they avoided the problem of precipitation of hydrophobic derivatives, but a separation of phases at high buffer concentration is also observed using acetonitrile as cosolvent.

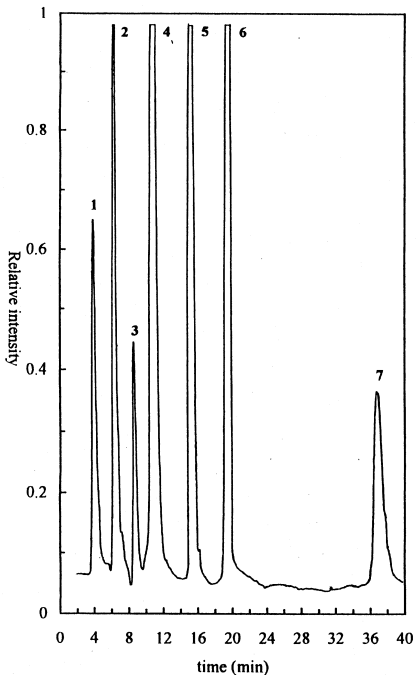

Figure 5. Chromatogram of amine standard derivatized with CAHS using method C. Chromatographic conditions: column, 2004.6 mm I.D. Spherisorb 5 μm; fluorescence (excitation 335 nm, emission 365 nm). flow rate = 1.0 mL min^{1}; column temperature 30. Gradient conditions and peaks as Figure 4.

This can be avoided by two ways: (1) the buffer concentration is controlled <0.2 M; (2) acetonitrile concentration in derivatized solution is <50% (v/v). Most subsequent derivatization was carried out in 0.2 M borate buffer containing 40% (v/v) of acetonitrile. Separation of amines derivatized with method **C** is shown in Figure 5.

Borate, phosphate, and bicarbonate buffers were investigated for derivatizing amines. Both borate and bicarbonate were satisfactory, but phosphate as a buffer proved unacceptable as it produced many interfering peaks. Derivatization yields for representative methylamine, n-butylamine, and 1-heptylamine were studied using borate buffer in the pH range from 7.5-10.0. It was found that there was little effect of buffer pH on derivatization yields in the pH range from 8.5-9.5, but outside this range, particularly in more acidic solution, decreased responses were observed, while above this range, resulting in partial hydrolysis of derivatives.

Buffer concentrations from 0.1-0.2 M had little effect on derivatization yields as long as the reaction pH was maintained in the optimal range. Generally, most subsequent derivatization is carried out using 0.2 M borate buffer at pH 8.0-9.0.

Effect of Other Substances

Generally, 50- to 100-fold excess of secondary alcohols did not disturb the derivatization of most amino compounds. However, when derivatization reaction was carried out in non-aqueous dichloromethane or acetonitrile with method **B** or **D**, primary alcohols interfered seriously with derivatization of amines under proposed conditions. This problem can be solved by the use of derivatization method **C**, in which most alcohols show no interference.

Linearity of Derivatization

The linearities were established by derivatizing amine standards. For calibration, working standards, to have different levels in the range of 0-400 μmol/L were prepared in acetonitrile. Standards were derivatized and each standard was analyzed in triplicate. Linear regression was performed by plotting the observed peak height (Y cm) versus concentration in μmol/L of each amine. It was found that methylamine, n-propylamine, and n-butylamine gave linear derivatization over 0.5-120 μmol/L with the correlation coefficients > 0.997; 1-heptylamine and spermine gave linear derivatization over 15-400 μmol/L and 60-500 μmol/L, respectively, with the correlation coefficients > 0.998.

Detection Limits

Detection limit depends upon different detector and different elution conditions. The detection limits are estimated, using amine standards, by injecting successively lower concentrations until a signal-to-noise of 3:1 was obtained. The detection limits of methylamine, n-propylamine, and n-butylamine are 35, 64, and 105 fmol, respectively. The detection limits of 1-heptylamine and spermine are 0.36 and 12.6 pmol, respectively.

LC Separation of Amine Derivatives from Environmental Sample

LC separation of amines from environmental water samples which were derivatized with method **C** is shown in Figure 6. It is found that excess reagent does not disturb the separation because most carbazole-N-acetyl-N-hydroxysuccinimide has been hydrolysated under proposed conditions.

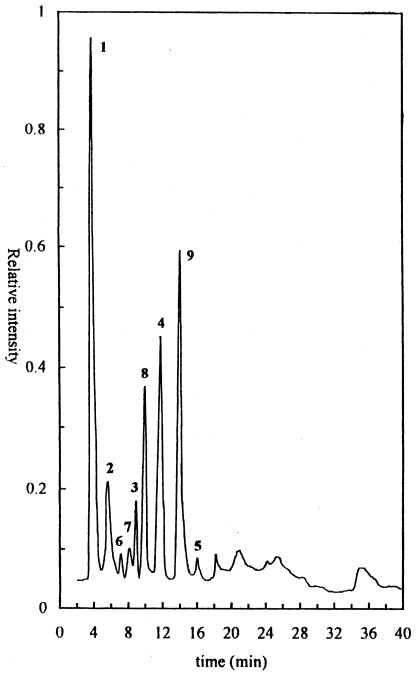

Figure 6. Chromatogram of amines from environmental water sample water sample was directly derivatized using method C fluorescence (excitation 335 nm, emission 365 nm). Chromatographic conditions: column, 2004.6 mm I.D. Spherisorb 5 •m; flow rate = 1.0 mL min^{-1}; column temperature 30; Elution conditions as Figure 4. Peaks: 1 = carbazole-9-N-acetyl acid; 2 = methylamine; 3 = carbazole-9-N-acetyl-N-hydroxysuccinimide; 4 = n-propylamine; 5 = n-butylamine; 6 = ethylamine; 7 = unknown; 8 = 2-aminopropane; 9 = diethylamine.

CONCLUSIONS

Carbazole-9-N-acetyl-N-hydroxy-succinimide (CAHS) has high reactivity for derivatizing amines with method **B** or **C**. Method **C** is more suitable for amines analysis as it has little interference from other substances. Yields of method **D** is slightly low relative to that of method **B** or **C**. But, its derivatization procedure is only a mixing of several reagents which exhibits a more simple operation. These methods are superior to previous approaches that require prior conversion of carboxylic acid to acid chloride or acid anhydride. At the same time, CAHS also exhibits high sensitivity and reproducibility for the analysis of amine derivatives. The proposed method is also suitable for the determination of other amines in different matrices.

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