Determination of ammonia and aliphatic amines in environmental aqueous samples utilizing pre-column derivatization to their phenylthioureas and high performance liquid chromatography

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Pre-column conversion of ammonia and a number of aliphatic amines into phenylthiourea or its derivatives by reaction with phenyl isothiocyanate, followed by HPLC, has been used for their determination in environmental waters. Optimum conversion was found when the reaction was carried out in sodium hydrogencarbonate–carbonate medium at 40 °C for 15 min. Well separated peaks were obtained on a C_{18} column with an acetonitrile–water gradient (1 ml min^{-1}) of 30% acetonitrile for an initial 5 min which was increased linearly to 100% over 15 min and then maintained isocratic for 5 min, the acetonitrile ratio finally being returned to 30% in 5 min. The derivatized analytes were subjected to off-line solid phase extraction on C_{18} sorbent. A linear calibration graph was obtained for 0.01–10 mg l^{-1} analytes with a correlation coefficient of 0.9954 for ammonia and in the range 0.9982–0.9996 for amines. The limit of detection for ammonia was 0.2 μg l^{-1} and for amines in the range 0.3–0.6 μg l^{-1}. The method was applied to tap, underground, river and aquarium waters, the recovery being in the range 97–106% (RSD 1.8–4.5%). Many of the samples were found to contain more than the permissible limit of ammonia. Phenyl isothiocyanate is stable for long periods in aqueous medium over wide ranges of pH and temperature, and the resulting phenylthioureas have adequate retention on C_{18} sorbent and strong UV absorption, making this reagent suitable for the determination of amines in water.

Most aquatic species excrete ammonia and urea, the rate of excretion and level of efflux being dependent upon the individual physiological status of the species and the modifying influences of their environment.[1] Ammonia is more toxic and water soluble than urea and this has important implications for the successful husbandry of commercially important, cultured aquatic species, or any that are held in fixed volumes of water. The quantitative determination of the principal metabolite end-products excreted to the external medium are therefore important analytical procedures.

Short-chain aliphatic amines are emitted into atmosphere from anthropogenic sources such as cattle feedlot operations, waste incineration, sewage treatment and various industries.[2–4] Amines are also emitted in car exhausts.[5] A natural background level of aliphatic amines can also be assumed to originate from animal wastes and microbiological activities.[3] Aliphatic amines are industrial chemicals with a wide range of applications. They are used as raw materials or as chemical intermediates in the production of other chemicals, pharmaceuticals, polymers, pesticides, dyestuffs and corrosion inhibitors. Aliphatic amines and polyamines are well known as odorous substances and as precursors of *N*-nitrosamines, which are carcinogenic.[6,7] Dimethylamine is present in untreated waste water discharges from aramide polymer manufacturing facilities, where it is produced by the decomposition of *N*,*N*-dimethylacetamide in the solvent stripping step. Environmental protection authorities demand analytical monitoring of unconverted dimethylamine in the aquatic environment close to waste treatment facilities. The monitoring of alkylamines is of considerable interest as most of them are toxic, sensitizers of and irritants to the skin, mucous membrane and respiratory tract, through all routes of exposure, *i.e.*, inhalation, ingestion and contact. The American Conference of Government Industrial Hygienists (ACGIH) has adopted threshold limit values–time weighted average (TLV–TWA) in the range 5–10 mg l^{-1} for various alkylamines and 0.5 mg l^{-1} for ammonia.[8]

Most primary and secondary amines exhibit poor chromatographic performance *via* direct HPLC approaches, making quantitative analysis difficult.[9] Methods for their determination require a high degree of specificity and sensitivity as they do not exhibit any structural feature that allows detection without derivatization. All existing liquid chromatographic methods for amine determination require at least two steps, separation from potential interferents in the sample and pre- or post-column formation of derivatives with better detectability. Chemical derivatization in solution has long been accepted as an effective modification technique in HPLC, improving the overall specificity, chromatographic performance and sensitivity for trace analysis.[10–12] The diverse reagents and conditions for derivatization of ammonia and aliphatic amines, as available in the literature, are summarized in Table 1. With secondary amines there is no reaction of *o*-phthalaldehyde, fluorescamine gives a non-fluorescent product and Lumarin 1 has a long reaction time. Many reagents require high temperatures and a prolonged period for derivatization, and the volatility of aliphatic amines may require special handling.

Phenyl isothiocyanate has been utilized for the determination of dimethylamine in waste waters but the method was reported to show poor linearity and a poor limit of detection.[29]

In this work, phenyl isothiocyanate was used for the determination of ammonia and a number of aliphatic amines in environmental waters, involving their conversion into phenylthioureas and HPLC. Phenyl isothiocyanate is stable for long periods in aqueous medium over a wide range of pH and temperature and the resulting phenylthioureas have adequate retention on C_{18} sorbent and strong UV absorption, making this reagent suitable for the determination of amines in water.

Experimental

Equipment

The chromatographic system consisted of a Beckman System Gold 127 binary gradient pump and Model 166 UV-Vis spectrophotometric detector (8 μl flow-through cell) (Beckman, Fullerton, CA, USA). Detection was carried out at 240 nm. A Rheodyne Model 7010 valve (Alltech, Deerfield, IL, USA) equipped with a 10 μl sample loop was used for sample injection. Data processing was carried out with an HP 3395 integrator (Hewlett-Packard, Palo Alto, CA, USA). The analytical column was 25 cm × 4.6 mm id ODS2 (5 μm particle size) (Anachem, Luton, UK). Solid-phase extraction cartridges (2.8 ml) containing 500 mg of C_{18} sorbent were obtained from Alltech. Before analysis, all environmental aqueous samples were filtered through a 0.45 μm membrane filter (Millipore-India, Mumbai, India).

Quantification was effected by measuring both peak height and area; peak height measurements gave better results.

Reagents and standard solutions

Phenyl isothiocyanate (PITC) was obtained from Merck (Darmstadt, Germany) and a standard solution was prepared by dissolving 4 ml of PITC in 100 ml of acetonitrile. Acetonitrile and HPLC-grade water were obtained from Merck (Mumbai, India).

Stock standard solutions (1000 mg l^{-1}), of ammonia (as ammonium chloride; Qualigens, Mumbai, India), methylamine, ethylamine (BDH, Poole, Dorset, UK), dimethylamine, isopropylamine, and diethylamine (Merck) were prepared in methanol, and standardized by titration with acid or by the dithiocarbamic acid formation method.[30] The stock standard solutions were stored refrigerated when not in use. Test samples were prepared freshly by spiking with known aliquots of suitably diluted stock standard solution before analysis.

Solutions of sodium hydrogencarbonate and sodium carbonate (5%) were prepared in water.

Mobile phase and HPLC gradient programme

Acetonitrile–water at a flow rate of 1 ml min^{-1} was used for elution. The optimum gradient programme consisted of an initial 30% acetonitrile for 5 min that was increased linearly to 100% over 15 min and maintained isocratic for 5 min. Finally, the acetonitrile concentration was returned to 30% in 5 min.

Table 1 Conditions for the derivatization of ammonia and aliphatic amines

Reagent[a]	NH_3/amine[b]	Time	Temperature/°C	Medium[c]	Ref.
OPA	1°	1 min	18	pH 9.5	13,14
	2°	OPA does not react with 2° amines			14
	1°	On-line	40	pH 9	15
	1°	Post-column	23	pH 10.5	16
FMOC	1°/2°	40 s	18	pH 7.7	10
FMOC	NH_3	2 min	61	pH 6.8	17
FMOC	2°	2 min	18	pH 8.0	18
FMOC-tagged silica	1°/2°	10–15 min	60	MeCN/Py	19
Polymer activated FMOC	1°/2°	On-line	60	pH 10	20
Dansyl chloride	1°/2°	10 min	40	pH 8.5	13
4-Chloro-7-nitrobenzo-1,2,5-oxadiazole	1°/2°	60 min	55	pH 8.5	13
Lumarin 1	1°	20 min	50	THF–DMSO	21
	2°	180 min	70	THF–DMSO	21
Fluorescamine	1°	5–30 min	18	pH 8–8.5	10
	1°	1 min	18	pH 10	22
	2°	Non-fluorescent derivative is formed			14
3-Toluoyl chloride	1°/2°	10 min	18	MeCN–NaOH	23
2-Naphthyloxycarbonyl chloride	1°/2°	3 min	18	pH 9	24
8-Quinolinesulfonyl chloride	1°/2°	20 min	65	pH 8.5	25
1-Fluoro-2,4-dinitrobenzene	1°/2°	?	20	pH 10.5	26
1-Naphthyl isocyanate	1°/2°	Immediate	Ice-bath	Hexane	27
1-Naphthyl isothiocyanate	1°/2°	Exposure of impregnated reagent to air			28
Phenyl isothiocyanate	1°/2°	15 min	40	pH 8.5	This work

[a] OPA = *o*-phthalaldehyde; FMOC = 9-fluorenylmethyl chloroformate. [b] Amines: 1° = primary; 2° = secondary. [c] THF = tetrahydrofuran; DMSO = dimethyl sulfoxide; MeCN = acetonitrile; Py = pyridine.

Table 2 Calibration and other statistical data for the determination of ammonia and aliphatic amines (range 0.01–10 mg l^{-1} each) after derivatization with phenyl isothiocyanate

Analyte	r[a]	Intercept (IU)[b]	Slope (IU)[b]	Conversion[c] (%)	LOD[d]/ μg l^{-1}	Recovery (%) SPE[e]
Ammonia	0.9874	6564	514035	98.7	0.2	80
Methylamine	0.9994	−86	1144255	—	0.3	90
Dimethylamine	0.9992	2257	436408	—	0.6	93
Ethylamine	0.9996	−99	790337	99.0	0.4	88
Isopropylamine	0.9982	3786	801363	—	0.5	94
Diethylamine	0.9988	5530	304008	99.6	0.5	71

[a] Average of six replicate analyses. [b] Integrator units; AFS = 0.008. [c] Conversion to thiourea derivative takes into account the peak area produced by equimolar amount of corresponding authentic compound; a dash indicates that conversion was not determined. [d] LOD = limit of detection.[31] [e] Average of three replicate analyses. Recovery of analytes from 25 ml of derivatization mixture after SPE on C_{18} sorbent.

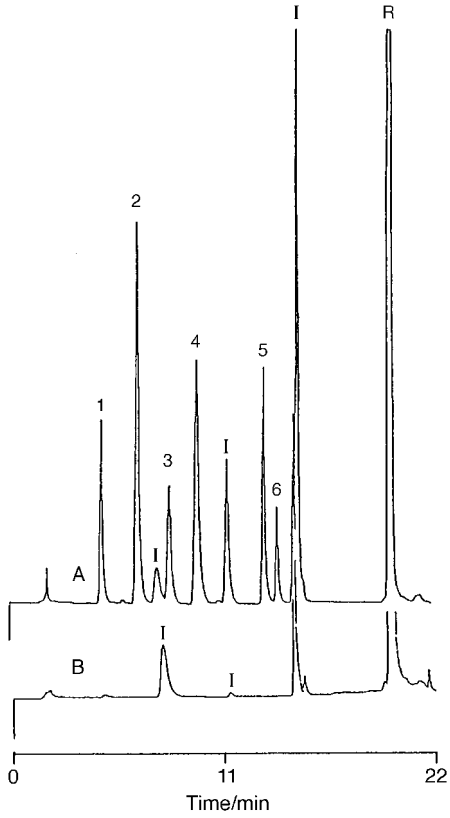

Fig. 1 Chromatogram obtained for (A) standard solution (1 mg l^{-1}) of ammonia and five aliphatic amines derivatized with phenyl isothiocyanate and (B) reagent blank. Peaks (as phenylthiourea and its derivatives): 1 = ammonia; 2 = methylamine; 3 = dimethylamine; 4 = ethylamine; 5 = isopropylamine; 6 = diethylamine; R = phenyl isothiocyanate reagent; I = unknown impurity. Column, C_{18}, 25 cm × 4.6 mm id (5 μm particle size); detection wavelength, 240 nm; mobile phase, acetonitrile–water, gradient elution; absorbance full-scale (AFS), 0.05; flow rate, 1 ml min^{-1}.

All compounds were eluted within 20 min. A 20 min flushing of the column with 30% acetonitrile is recommended before the next injection.

Sampling

The performance of the method was tested with unspiked and spiked domestic, surface and river water samples. Once environmental water samples had been collected, they were immediately acidified with 1 ml of 0.1 M hydrochloric acid to avoid volatilization of amines and filtered through a 0.45 μm membrane filter. A 4 ml portion of real water sample, unspiked and spiked with amine standard, was subjected to derivatization and analysis by HPLC.

Derivatization procedure and analysis

A 0.1–2 ml aliquot of sample solution was mixed with 0.5 ml of 4% phenyl isothiocyanate and 0.5 ml of 5% sodium hydrogencarbonate in a 10 ml calibrated flask and the flask was capped, shaken well, and heated at 40 °C in a water-bath for 10 min. Then, 0.5 ml of 5% sodium carbonate solution was added and the flask again heated at the same temperature for 5 min. Subsequently, one of the following methods was used. (1) The contents were cooled to room temperature, diluted to the mark with acetonitrile–water (30 + 70 v/v) and a 10 μl aliquot of derivatized amine mixture was injected into the chromatographic system. (2) The derivatized amine solution, after cooling to room temperature, was passed through a C_{18} cartridge that had previously been activated with 2 ml of acetonitrile and equilibrated with 2 ml of de-ionized, distilled water. The sorbent was washed with 1 ml of distilled water and the retained derivatives were eluted with 2 ml of acetonitrile. A 10 μl aliquot of eluate was injected into the liquid chromatograph.

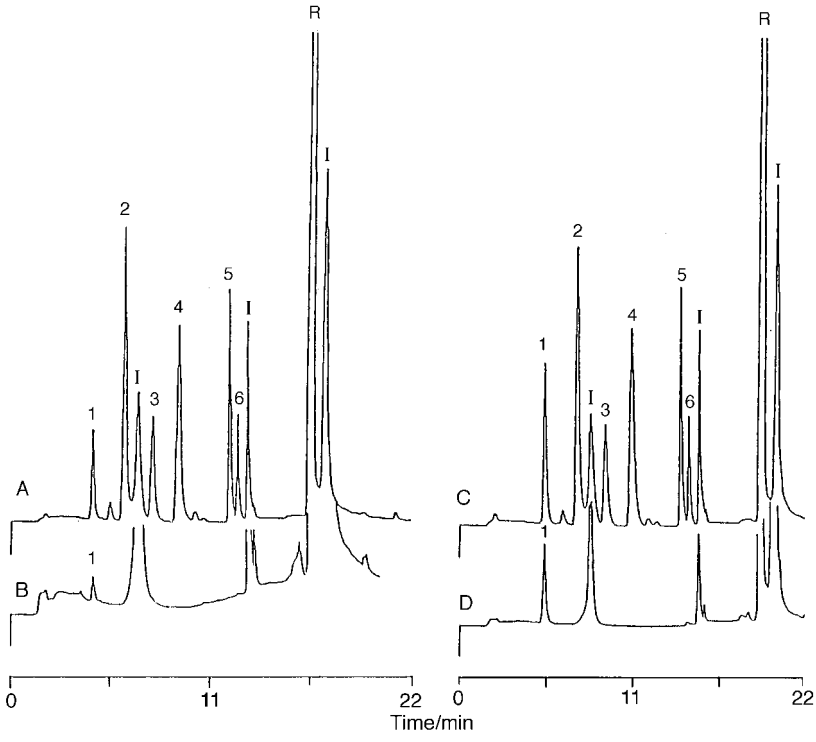

Fig. 2 Chromatograms obtained for aliphatic amines spiked at the 2 mg l^{-1} level in two river water samples: Narmada water, (A) spiked and (B) unspiked; and Ganga water, (C) spiked and (D) unspiked. C_{18} column, 25 cm × 4.6 mm id (5 μm particle size); detection wavelength, 240 nm; mobile phase, acetonitrile–water, gradient elution; AFS, 0.05; flow rate, 1 ml min^{-1}. Peak designation as for Fig. 1.

Results and discussion

Optimization of the chromatographic separation

Conventional HPLC with aqueous organic mobile phases was initially tested for the separation of ammonia and different amine derivatives. Isocratic elutions were unsuccessful because the peaks remained almost unresolved and the reagent peak eluted 10 min after all peaks of derivatives. The latter problem resulted in an unnecessarily long analysis time. Studies were performed with different ratios of methanol–water and acetonitrile–water as eluents but without success. Adjustment of the mobile phase pH also did not improve the resolution. These observations indicated the necessity for gradient elution. Well defined, sharp and reproducible peaks were obtained when acetonitrile–water was used as the mobile phase, the flow rate being 1.0 ml min^{-1}. For the initial 5 min of the chromatographic run, acetonitrile–water (30 + 70, v/v) was used, to acetonitrile concentration being increased linearly to 100% over 15 min and then maintained isocratic for another 5 min. Finally, the acetonitrile concentration was returned to 30% in 5 min, and the column was flushed for about 20 min before the next injection. The detector was set at 240 nm as at this wavelength all thioureas absorbed strongly. Under these optimum conditions of HPLC, all peaks were baseline separated (Fig. 1).

Amino acids are also known to react with phenyl isothiocyanate;[10] however, glycine and alanine did not produce any interfering peaks in the working chromatogram for amines.

Optimization of derivatization reaction

In acidic solutions, the derivatization reaction was incomplete since the protonated amines are only weak nucleophiles towards their addition to the thiocyanato group of the derivatizing agent. To increase the percentage conversion, attempts were made to carry the reaction at higher temperatures and in the presence of buffering agents. All amines responded to higher conversion in sodium acetate or hydrogencarbonate medium but reaction with ammonia was still incomplete even after allowing the reaction mixture to stand for 1 h at room temperature. Reaction in the presence of sodium hydrogencarbonate at elevated temperatures (the range tested was 35–60 °C) for 10 min served to increase the conversion for all amines but the effect levelled off at 40 °C except for ammonia, which had an optimum peak height at 60 °C. The effect was almost the same in sodium carbonate for all amines but ammonia showed a significantly different behaviour, a lower levelling off temperature (40 °C), and poor precision (RSD 5–10%). In sodium carbonate medium a pale yellow colour developed during heating and some additional peaks appeared especially close to the derivative of dimethylamine and with which it merged at lower amine concentrations. It appeared that all amines had optimum conversion in hydrogencarbonate but ammonia required a higher pH (carbonate medium). Optimum peak heights and the best precision were obtained when the derivatization reaction was carried out at 40–45 °C first in the presence of hydrogencarbonate for 10 min, and then sodium carbonate for an additional 5 min. Under these conditions, the reaction mixture

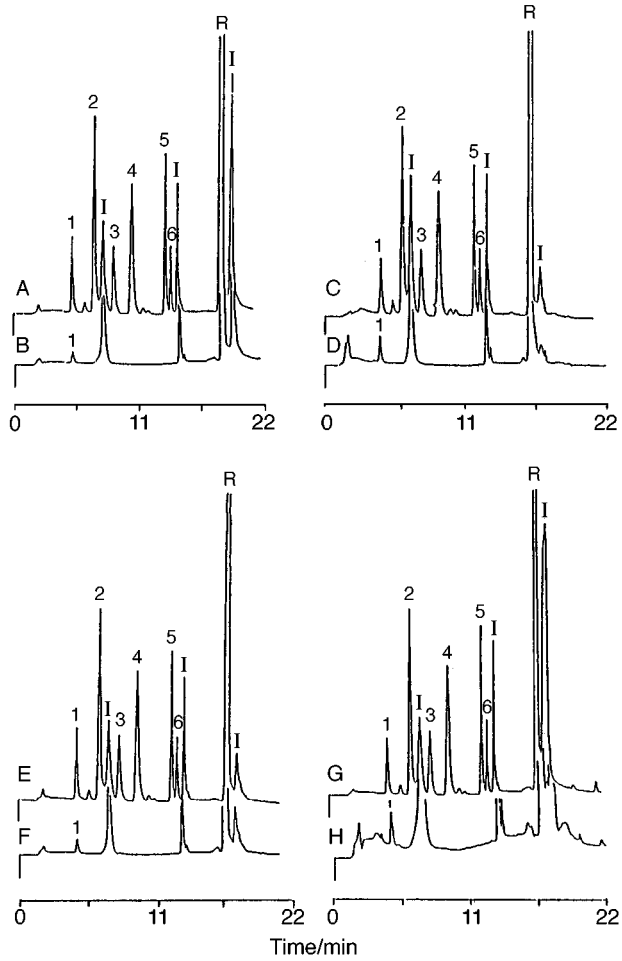

Fig. 3 Chromatograms obtained for aliphatic amines spiked at the 2 mg l^{-1} level in different environmental water matrices. Laboratory tap water, (A) spiked and (B) unspiked; aquarium water, (C) spiked and (D) unspiked; underground water, (E) spiked and (F) unspiked; and city tap water, (G) spiked and (H) unspiked. C_{18} column, 25 cm × 4.6 mm id (5 μm particle size); detection wavelength, 240 nm; mobile phase, acetonitrile–water, gradient elution; AFS, 0.05; flow rate, 1 ml min^{-1}; sample volume, 10 ml. Peak designation as for Fig. 1.

Table 3 Determination of ammonia and aliphatic amines in real samples

Sample	Ammonia found[a]/ mg l^{-1}	Recovery of 2 mg l^{-1} spike (%)[b]					
		1	2	3	4	5	6
Narmada river water	0.17	98.2	104.8	105.6	100.9	97.9	96.9
Ganga river water	1.96	97.8	101.6	100.7	101.9	98.7	97.9
Underground water no. 1	0.53	104.4	103.9	101.2	100.7	97.9	97.6
Underground water no. 2	0.95						
Tap water no. 1	0.42	106.0	107.9	103.9	104.4	100.0	97.8
Tap water no. 2	0.44						
Aquarium water	1.01	101.6	99.3	98.4	99.0	102.5	103.0
Average RSD of recovery (%)		4.1	3.7	3.8	3.3	3.6	3.9

[a] The results are averages of three determinations; RSD = 1.2–3.5%. [b] The results are the average of three determinations; RSD = 1.8–4.5%. 1 = Ammonia; 2 = methylamine; 3 = dimethylamine; 4 = ethylamine; 5 = isopropylamine; 6 = diethylamine. The recovery for ammonia takes into account the concentration already present in the real sample. No aliphatic amine was found in any of the samples analysed.

did not turn yellow, nor were there unwanted additional peaks.

The optimum concentrations of reagents for derivatization were 0.5 ml each of 5% sodium hydrogencarbonate, 5% sodium carbonate and 4% phenyl isothiocyanate.

Calibration data

Calibration graphs were obtained using a series of six standard solutions of ammonia and amines. Three replicate derivatizations at each concentration level were performed and their average response was plotted against the concentration of the corresponding analyte. Rectilinear calibration graphs were obtained over the range 0.01–10 mg l^{-1} of analytes. Calibration and other statistical data for the determination of ammonia and aliphatic amines are given in Table 2.

Analysis of real samples

The method was validated by spiking natural samples with known amounts of ammonia and amines (range sub-μg l^{-1} to low mg l^{-1} level) and evaluating the recovery. All chromatographic peaks of interest were well separated from peaks due to extraneous matter. Typical chromatograms for a 2 mg l^{-1} spike are given in Fig. 2 and 3. Ammonia was found in all samples but none of the samples analysed showed the presence of any amine. The results for real water samples are presented in Table 3. From the recovery results we observe that there is no significant matrix effect and the recoveries are within acceptable limits. Hence, this method can be used for real water samples. Narmada river water and Jabalpur city tap water showed acceptable ammonia levels, but Ganga water (collected from Kanpur) and both underground (well) waters of Jabalpur city exceeded the prescribed limit for ammonia. The use of untreated river and underground (well) waters for drinking purposes is a common practice in India, and therefore caution is advisable.

Conclusions

Conversion of ammonia and aliphatic amines into their corresponding thiourea derivatives followed by HPLC with gradient elution with acetonitrile–water and UV detection at 240 nm is an elegant method for their determination in environmental waters.

In comparison with other reagents available for the synthesis of derivatives of amines, phenyl isothiocyanate is a fast reacting and stable reagent over a wide range of pH, and it can react with both primary and secondary amines. Sample clean-up and analyte enrichment by solid-phase extraction on C_{18} sorbent were feasible, and this technique has potential for still better detection when used on-line with HPLC. Although not tested, the reducing property of the thiourea group makes possible the detection of derivatives by an electrochemical method.

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