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Note

Determination of aliphatic amines in air by reversed-phase high-performance liquid chromatography using 1-naphthyl isocyanate derivatives

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Aliphatic amines are widely used as chemical intermediates in the production of polymers, pharmaceuticals and ion-exchange resins, and as corrosion inhibitors. As such, they are of considerable industrial and commercial importance. The need for a sensitive and rapid trace determination of aliphatic amines in environmental samples has thus become increasingly important. Amines are usually determined by gas chromatography (GC) after a derivatization step[1]. Determination of underivatized amines using GC is severely hindered by their strong adsorption in the chromatographic system. This problem can be partly solved by incorporating potassium hydroxide in the column packings[2,3] or using fused-silica columns with *in situ* cross-linking of the silicone-type stationary phases[4]. Recently, GC methods have also been developed for direct analysis of free amines at the nanogram level[2,3,5]. Adsorption and rather low sensitivity can be overcome by an appropriate derivatization procedure prior to the GC analysis. Typically, fluorine-containing derivatives, together with electron capture detection, have been used for sensitive determination of amines[1,6–8]. However, derivatives of this type are often relatively unstable and are more suitable for immediate use. An interesting reagent for amines in serum and urine is dimethylthiophosphinic chloride, which forms stable derivatives with good GC properties[9,10].

In high-performance liquid chromatography (HPLC), precolumn and postcolumn derivatization with a variety of fluorescent reagents have both been used frequently to enhance detection sensitivity for aliphatic amines[11]. The use of 2-naphthylchloroformate[12], dabsyl chloride[13], *m*-toluoyl chloride[14] and 3,5-dinitrobenzoyl chloride[15] has recently been reported. Jandera *et al.* investigated a new fluorescent reagent, ten times as sensitive for aliphatic amines, compared with the corresponding dansyl derivatives[16]. Björkqvist used phenyl isocyanate for precolumn derivatization of aliphatic amines prior to HPLC analysis[17].

NIOSH recommends silica gel for the collection of vapours of aliphatic amines in a workplace area[18]. Charcoal tubes[19], acidified silica[20] or Tenax[21] and Porasil A[22] in a sorbent test-tube have also been used. Böhm *et al.* reported a new method for the determination of dimethylamine in air using an impinger with diluted sulphuric acid[23]. None of these sampling methods utilizes derivatization to stabilize the

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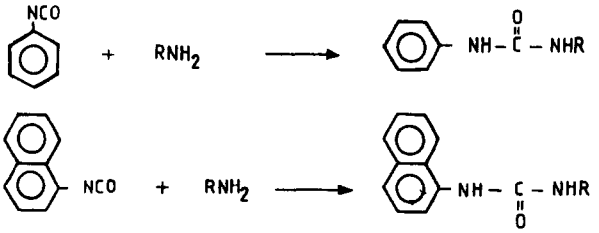
amines or to enhance detector sensitivity. This study was undertaken to examine whether phenyl isocyanate or 1-naphthyl isocyanate could be used for sample collection of primary aliphatic amines in air at low mg/m³ level.



EXPERIMENTAL

Materials

Chemicals. Amines of *pro analysi* quality were obtained from Fluka (*n*-propylamine), Merck (allylamine), BDH (*n*-butylamine) and Aldrich (di-*n*-butylamine). Phenyl isocyanate (p.a.) and 1-naphthyl isocyanate were purchased from Merck.

Solvents. Acetonitrile (Rathburn Chemicals, HPLC grade), dioxane (Fluka, für HPLC), N,N-dimethylformamide (Burdick & Jackson, distilled in glass) and *n*-hexane (Merck, p.a.) were used without further purification. Water for the HPLC runs was purified in a Millipore Milli R/Q water purifier.

Equipment

The chromatographic runs were performed with a Waters high-performance liquid chromatograph consisting of an M-710 B autosampler, an M6000 A solvent delivery system, an M440 UV-absorbance detector and an M-720 data module. The instrument was equipped with a Waters radial compression separation system with a C_{18} reversed-phase column (Waters Radial Pak A, 100 × 8 mm I.D., 5 μm) operated at a flow-rate of 1.5 ml/min [pressure *ca.* 600 p.s.i. ($4.1 \cdot 10^4$ Pa)]. The chromatographic eluent, consisting of 60% (v/v) aqueous acetonitrile, was continuously degassed with helium. The detector was operated at 280 nm. UV spectra were run on a Pye Unicam SP 1700 ultraviolet spectrophotometer and recorded on a Unicam AR 25 linear recorder. Melting points, determined with a Büchi SMP-20 melting point apparatus, are uncorrected.

Methods

Derivatization of amines with arylisocyanates. N-Phenyl-N′-alkylurea and N-1-naphthyl-N′-alkylurea standards were prepared by dissolving 55 mmol of each amine in 200 ml of *n*-hexane. The solutions were cooled in an ice-bath and 50 mmol of phenyl isocyanate or 1-naphthyl isocyanate in 50 ml of *n*-hexane was added dropwise with stirring. The reaction is instantaneous and quantitative. The voluminous precipitate was collected by suction filtration, washed repeatedly with cold hexane, dried and finally recrystallized until a sharp melting point was obtained (Table I).

The alkylnaphthylureas were characterized by mass spectrometry. A Finnigan 4021 mass spectrometer was used. During electron impact (70 eV) the naphthylureas

of allylamine, propylamine and butylamine all showed molecular ions at *m/e* 226 (21%), *m/e* 228 (25%) and *m/e* 298 (10%) respectively. An other characteristic fragment was *m/e* 143 (parent peak in all spectra). In the positive chemical ionisation mode, with methane as reagent gas, M + 1, and in the negative chemical ionisation mode the M − 1, was parent peak for all three derivatives.

Preparation of standard solutions of amines and ureas. Standard solutions of the amines were prepared by dissolving accurately weighed amounts (*ca.* 5 g) in 50 ml of *n*-hexane. These solutions were further diluted with *n*-hexane to contain 2.25, 22.5, and 112.5 mg/ml. The standard amine solutions were freshly prepared on each occasion. Reference standards for qualitative and quantitative analyses were prepared by dissolving exact amounts of the alkylphenyl- or alkylnaphthylureas in dioxane. The standard urea solutions could be stored for at least one month at room temperature without deterioration.

Reagent solution. 1-Naphthyl isocyanate (0.5 ml) was dissolved in 500 ml of dioxane and 10.0 ml of this solution were added to the fritted-glass bubbler. Then 2 ml of di-*n*-butylamine was diluted to 100 ml with dioxane. This solution was used to destroy excess reagents.

Sample collection. Aliquots (10 μl) of the amine standard solutions were injected into a 10-cm glass tube equipped with glass-wool plugs at both ends. Pressurized air, with known flow and humidity, was used in the recovery experiments. The system enables small adjustments to be made to both flow and humidity[24]. The amine vapour was passed through the fritted-glass bubbler containing the reagent solution. Sampling was performed at 20% and 85% relative humidity with an air flow of 1 l/min. After a sampling time of 15 min, the bubbler was disconnected and the remaining solution transferred to a graduated glass test-tube. The volume was made up to 10.0 ml with a minimum of 1 ml of a 2% solution of di-*n*-butylamine in dioxane so as to destroy excess reagent. (This would otherwise cause the slow deterioration of the column.) The test-tubes were sealed with PTFE-lined screwcaps and stored in the dark until analysed. The samples need not be concentrated prior to analysis.

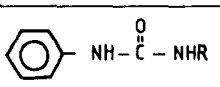
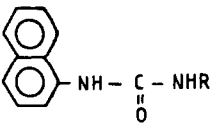
HPLC analysis. Samples (10 μl), free from particulate matter, were automatically injected. If necessary, the samples were filtered through a Millex SR (0.5 μm) filter prior to analysis. Recoveries were determined by the external standard method. The minimum detectable amount (corresponding to the peak height equal to four times the noise level) was *ca.* 1 ng for a pure standard solution of alkylnaphthylureas. The practical detection limit for *n*-propylamine, *n*-butylamine and allylamine in workroom air is 0.5 mg/m^3 based on an air sample of 15 l.

RESULTS AND DISCUSSION

The physicochemical properties of the phenyl isocyanate and 1-naphthyl isocyanate derivatives of the investigated amines, including melting points, electronic absorption maxima and molar absorptivities, are shown in Table I. The two types of derivative show similar molar absorptivities, but their absorption maxima differ. Interfering impurities were formed with the phenyl isocyanate reagent, especially when N,N-dimethylformamide was used as a solvent in the reaction, as recommended by Björkqvist[17]. It has been suggested that DMF catalyses the formation of alkylarylureas[17]. However, in this work it was observed that the reaction is as fast and

TABLE I

PHYSICOCHEMICAL PROPERTIES OF PHENYL- AND 1-NAPHTHYLUREAS

Amine	*R*	*M.p.* (°C)	*Litt. m.p.* (°C, *ref.*)	λ_{max} *(dioxan)* (nm)*	*Molar absorptivity* (10^{-3} mol^{-1} cm^{-1})
					
n-Propyl	n-C_3H_7	115–116**	114–115 (25)	252	6.23
n-Butyl	n-C_4H_9	129–130**	130 (25)	252	6.94
					
n-Propyl	n-C_3H_7	180–181***	196 (25)	250, 300	6.54
n-Butyl	n-C_4H_9	155–156***	149 (25)	250, 300	6.55
Allyl	$CH_2=CH-CH_2-$	204–205	203–204*** (26)	250, 300	5.21

* Dominant absorption maximum underlined.
** Recrystallized from 50% aqueous ethanol.
*** Recrystallized from 95% ethanol.

quantitative when dioxane is used as a solvent. The formation of interfering by-products could thus be minimized by substituting dioxane for DMF.

In the preparation of 1-naphthyl-di-*n*-butylamine, we failed to recrystallize the product according to the description given by Suggit and Wright[27]. For a qualitative analysis of the product formed from the quenched reagent, we therefore used the crude reaction product from di-*n*-butylamine and 1-naphthyl isocyanate.

Like alkylphenylureas[17], alkylnaphthylureas were found to be excellent chromatographic agents. Fig. 1 illustrates a situation where a mixture of *n*-propylamine, *n*-butylamine and allylamine has been sampled at a concentration equal to one-tenth of the current threshold limit value in Sweden (15 mg/m^3). Excess reagent has been treated with an excess of di-*n*-butylamine. The resultant reaction product elutes long after the amine derivatives.

1-Naphthyl isocyanate, like phenyl isocyanate, may react with water; it forms N,N'-dinaphthylurea[17]. In fact, an unknown component appears in the HPLC chromatogram, the amount of which increases with increasing relative humidity and/or if the sample is allowed to stand for some time prior to destruction of excess reagent (Fig. 2). Final identification of the unknown peak in the chromatogram still remains to be performed. The analysis of the amine derivatives is not disturbed even when sampling is carried out at 85% relative humidity, owing to excellent chromatographic separation. In order to suppress this side reaction, excess regent should be quenched with di-*n*-butylamine as soon as the sample has been collected, preferably when the sample solution is transferred into transport bottles.

Table II shows the recovery of *n*-propylamine, *n*-butylamine and allylamine sampled at different levels. The recoveries are generally high, mostly higher than

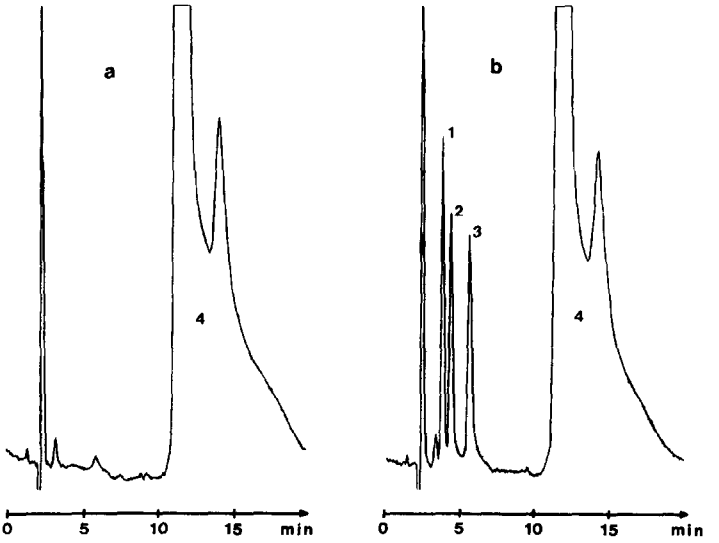


Fig. 1. HPLC chromatogram showing blank (a) and a mixture of allylamine (1), *n*-propylamine (2) and *n*-butylamine (3) eluting as their 1-naphthylurea derivtives at a concentration equal to 1.5 mg/m^3 each in a 15-l air sample. Component 4 is the 1-naphthylurea derivative of di-*n*-butylamine formed by the quenching of excess reagent.

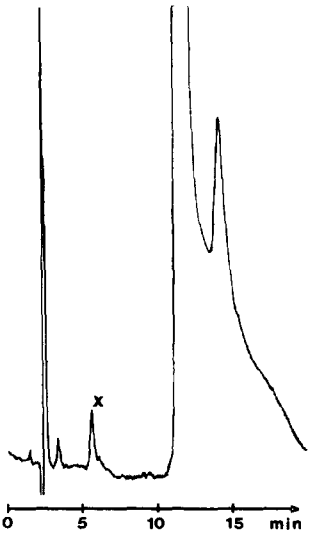


Fig. 2. HPLC chromatogram showing an unidentified component (×), which appears when the sample has been stored for 24 h prior to destruction of excess 1-naphthyl isocyanate.

95%, even at a relatively high amine concentration and at a high relative humidity. Storing the quenched samples for a week does not significantly influence the result. The alkylnaphthylureas show very strong UV absorption, with maximum at *ca.* 290 nm, in acetonitrile–water (60:40). The molar coefficient is *ca.* 5000 l mol^{-1} cm^{-1}, which allows detection down to the 1 ng level.

TABLE II

RECOVERY OF *n*-PROPYLAMINE, *n*-BUTYL AMINE AND ALLYLAMINE FROM A GAS BUBBLER CONTAINING 5% 1-NAPHTHYL ISOCYANATE IN DIOXANE

Collection of amine vapours was performed at two different relative humidities, 20% and 85% (values in parentheses). Number of experiments, n = 6. R.S.D. = Relative standard deviation.

Amine	*Amount added (μg)*	*Air level 15-l sample (mg/m³)*	*Recovery (%)*	*R.S.D. (%)*
n-Propyl	22.5	1.5	97 (100)	2 (5)
	225	15.0	96 (97)	5 (3)
	1125	75.0	93 (95)	2 (4)
n-Butyl	22.5	1.5	87 (86)	4 (4)
	225	15.0	97 (97)	4 (3)
	1125	75.0	95 (98)	2 (2)
Allyl	21.0	1.4	98 (96)	3 (3)
	210	14.0	95 (97)	1 (4)
	1050	70.0	100 (100)	4 (4)

CONCLUSION

A new sensitive and selective derivative has been examined for determining trace amounts of *n*-propylamine, *n*-butylamine and allylamine in air. A major advantage of this procedure is that the amines can be rendered non-volatile —and hence stable— in the field by reaction, and can be transported and stored prior to analysis. Increased sensitivity is attained since the derivatives have excellent chromatographic and UV spectrophotometric properties.

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