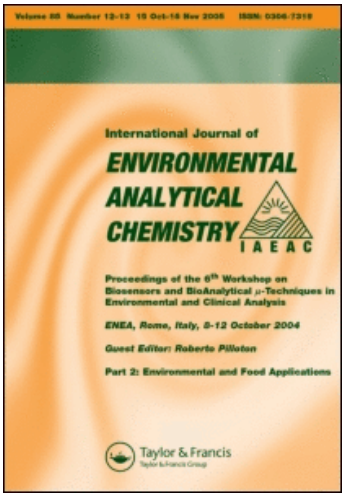

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METHODS FOR THE DETERMINATION OF DIMETHYLETHYLAMINE AND DIMETHYLETHYLAMINE-N-OXIDE IN AIR, PLASMA AND URINE SAMPLES

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Sensitive routine gas-liquid chromatographic methods for the determination of dimethylethylamine (DMEA) in air and of DMEA and dimethylethylamine-N-oxide (DMEAO) in plasma and urine, have been developed. DMEA was extracted in di-n-butyl ether and analysis was performed on an alkali-treated packing material (Carbowax 20M/0.8% KOH) with a nitrogen-sensitive detector. The detection limit for DMEA in air was 0.01 mg/m^3 (151 sample). DMEAO in blood and urine was determined as difference of DMEA after and before reduction of the sample. The standard curves were linear for DMEA levels up to 400 mg/l (5.5 mmol/l) and the detection limit was 0.01 mg/l (0.1 μmol/l). Urinary DMEA and DMEAO concentrations could be determined with a precision of 1–3 and 2–4%, respectively. The precisions for DMEA and DMEAO in plasma were 6 and 4–6%, respectively. The methods were validated to exposure levels of DMEA of up to 100 mg/m^3 and corresponding plasma levels and urinary excretion of DMEA and DMEAO.

KEY WORDS: Dimethylethylamine, dimethylethylamine-N-oxide, air sample, plasma, urine, analysis.

1. INTRODUCTION

Amines are of great importance in many processes in chemical and pharmaceutical industries. A number of tertiary aliphatic amines are used as catalysts in polymer production, in synthesis of pharmaceuticals, and as pH adjusters. In foundries, dimethylethylamine (DMEA) is often used as a catalyst in the core-making process (the Cold-box technique; Isocure process). DMEA has a high vapor pressure at normal workroom temperature, which may result in high air concentrations in the inhalation zone of the workers,[1–4] and cause irritation of mucous membranes of eyes, nose, and throat. Visual disturbances, such as mydriasis, cycloplegia[5–7] and haze (due to corneal edema)[8–11] have been reported after exposure to tertiary aliphatic amines.

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Occupational exposures are measured by collection of amines on solid sorbents[12,13] or in an acidic absorption solution. Analysis is performed by gas chromatography (GC)[14,15] or isotachophoresis.[16,17] Since workers' exposure to industrial amines is intermittent, this makes the evaluation of exposure and risk by air sampling difficult and expensive. Also, any absorption through the skin would be disregarded. Thus, there is a need for biological monitoring of occupational exposure to DMEA.

Tertiary, aliphatic amines, e.g. trimethylamine (TMA) and triethylamine (TEA), which appear in plasma, are excreted in urine quantitatively as the unchanged amine and, to a large extent, as compared to amine drugs, as their N-oxide (trimethylamine-N-oxide, TMAO;[18] triethylamine-N-oxide, TEAO).[19] The average half-life of TMA and TEA in plasma and urine is about 3 h, for both amines. Studies on the metabolism of DMEA in man show the same metabolic pattern as TEA.[20] No methods for the determination of DMEA and DMEAO in plasma or urine samples have been reported.

We here report data on the determination of DMEA in air and of DMEA and DMEAO in plasma and urine samples.

MATERIALS AND METHODS

Chemical

DMEA (99%) and di-n-butyl ether p.a. (99%) were obtained from Janssen Chimica (Beerse, Belgium). DMEAO was synthesized by treatment of DMEA with hydrogen peroxide according to a method of Dunstan and Goulding.[21] The identity of the synthesized DMEAO was confirmed as its amine-N-oxide picric salt by infrared spectrophotometry and nuclear magnetic resonance spectroscopy analysis, respectively. Potassium hydroxide p.a. (KOH), hydrochloric acid p.a. (HCl, 37%), and ammonia p.a. (NH_3, 25%) were obtained from Merck (Darmstadt, Germany) and tin (Sn, fine grain 20 mesh) from Fisher Sci. (NJ, USA).

Sample Preparation

Air samples were bubbled (1 l/min) through impinger vessels containing 10 ml of absorption solution (0.1 M HCl). Two ml were used for analysis. *Venous blood* was sampled in heparinized tubes (Venoject). After cooling of the blood samples for 0.5 h, and centrifugation (1,500 g, 15 min), 2 ml plasma were acidified with 0.5 ml 1 M HCl are stored at 4 °C. Aliquots of 2.5 ml were used for analysis.

Urine samples were collected in polyethylene bottles, acidified with concentrated HCl (37%; 2 ml per 100 ml urine) and stored at 4 °C until analysis. Two ml were used for analysis.

Determination of Dimethylethylamine

Analytical procedure. For the determination of DMEA, aliquots of absorption solution, plasma, or urine were extracted with 2 ml di-n-butyl ether and 4 ml

12 M KOH containing 0.25% NH_3. After shaking (10 min) and centrifuging (1,500 g, 10 min), 1 µl (plasma: 4 µl) of the ether phase was injected into the injection port (200 °C) of the GC (Varian 3700; autosampler 8035). A 3 m × 2 mm i.d. stainless-steel column packed with 4% Carbowax 20M/0.8% KOH on Carbopack B, 60/80 mesh (Supelco, Bellefonte, PA, USA) was used. Carrier gas was nitrogen (30 ml/min) and the column temperature program was first 5 min at 120 °C, programmed from 120 to 220 °C at 50 °C/min, and then 7 min at 220 °C. The amines were detected with a Nitrogen Phosphorus Detector (NPD; Varian TSD; 250 °C with flow rates for hydrogen and air of 4.2 and 180 ml/min, respectively) and the peak were evaluated by integration (Shimadzu C-R3A Chromatopac integrator). Aliquots were analyzed with and without addition of DMEA. The concentrations of DMEA in the samples were calculated according to the formula

$$C_0 = C_\Delta[a_0/(a_\Delta - a_0)], \quad (1)$$

where C_0 = concentration of DMEA in the sample, a_0 = integrated area of the sample, C_Δ = concentration of the added DMEA in the sample, and a_Δ = integrated area of the sample with addition of DMEA.

Relevant additions of DMEA to the absorption solution aliquots (air samples) were 0.060 mg (0.040 ml of 1.5 mg DMEA/ml 1 M HCl), plasma aliquots 0.40 µg (0.040 ml 0.010 mg/ml 1 M HCl), and urine aliquots 0.40 mg (0.040 ml of 10 mg/ml 1 M HCl).

Determination of Dimethylethylamine-N-Oxide

Analytical procedure. DMEAO was determined in plasma and urine as the difference of DMEA content after and before reduction. The sample aliquots were added with 1 ml concentrated HCl and 200 mg Sn, heated for 1 h at 95 °C (shaken every 10 min). After cooling in ice water, 2 ml di-n-butyl ether and 4 ml 12 M KOH, containing 0.25% NH_3 were added to the samples. After shaking (10 min) and centrifugation (1,500 g, 10 min), the ether phase was analyzed with the same GC method as for DMEA.

RESULTS AND DISCUSSION

Absorption Efficiency and Sample Stability

The absorption efficiency for DMEA into the absorption solution was tested by connecting two impingers in series, glass to glass. Samples were collected during 15 min at a generated DMEA level of 20 mg/m^3 ($n = 10$) and during 1 h ($n = 6$) in a foundry plant, at a level of 1.4 (mean; range 0.6–2.2) mg/m^3. The absorption efficiency test for DMEA showed a median relative amount (of the summed amount in both impingers) of 0.1% in second impinger (not detectable—1.9%) at the generated DMEA level and 0.3 (median; range 0.1–0.6)% in the plant. Samples

Table 1 Extraction ability of added DMEA in di-n-butyl ether for absorption solution, and plasma and urine samples

Sample ($n=5$)	*Concentration* (*mg/l*)	*Extraction ability* (%)	
		Mean	*Range*
Absorption solution (air sampling)	60	99	97–101
Plasma	0.10	98	96–99
Reduced plasma[a]	0.10	88	85–89
Urine	1.00	100	99–102
Reduced urine[a]	10.0	94	92–95

[a]See text.

of absorption solution and acidified plasma and urine, analyzed repeatedly during a 4-month period, showed no systematical trends with respect to the levels of DMEA and DMEAO. There are some disadvantages from a practical point of view when sampling into impingers. However, the excellent absorption efficiency and sample stability makes the sampling into acidic absorption solution preferable.

Extraction Ability

The alkali concentration of the aqueous phase affected the extraction ability. Addition of 1 ml 12 M KOH, instead of 4 ml, decreased the ability by a factor of two. The presence of 0.25% NH_3 in the sample extraction procedure minimized the adverse effect of adsorption onto the glass walls. This is in accordance with findings of Audunsson and Mathiasson.[14] The extraction ability of DMEA into di-n-butyl ether from spiked absorption solution samples ($n=5$) and plasma and urine of five individuals are shown in Table 1. The extraction ability was good. The ability was somewhat less for reduced samples. The difference was caused by a larger amount of acid in this analytical procedure.

Chromatography

Excellent separation was achieved of DMEA, a series of amines, and di-n-butyl ether. Chromatograms recorded for urine spiked with 0.1 mmol DMEA/l and 0.9 mmol DMEAO/l before and after reduction of the urine sample are shown in Figure 1. Our recommended methods, with addition of DMEA to the samples, eliminates systematically changes of the sensitivity of the NPD detector during a series of analyses. However, the quantitative determination of DMEA and DMEAO has successfully been performed by calculation on standard curve.

The good chromatographic separation in combination with the high selectivity of the NPD for nitrogen-containing compounds, compared to hydrocarbons, probably makes the methods applicable, without modifications, for the determination of other aliphatic tertiary amines and amine-N-oxides.

Linearity

Spiked absorption solution and urine samples, varying in concentration over the

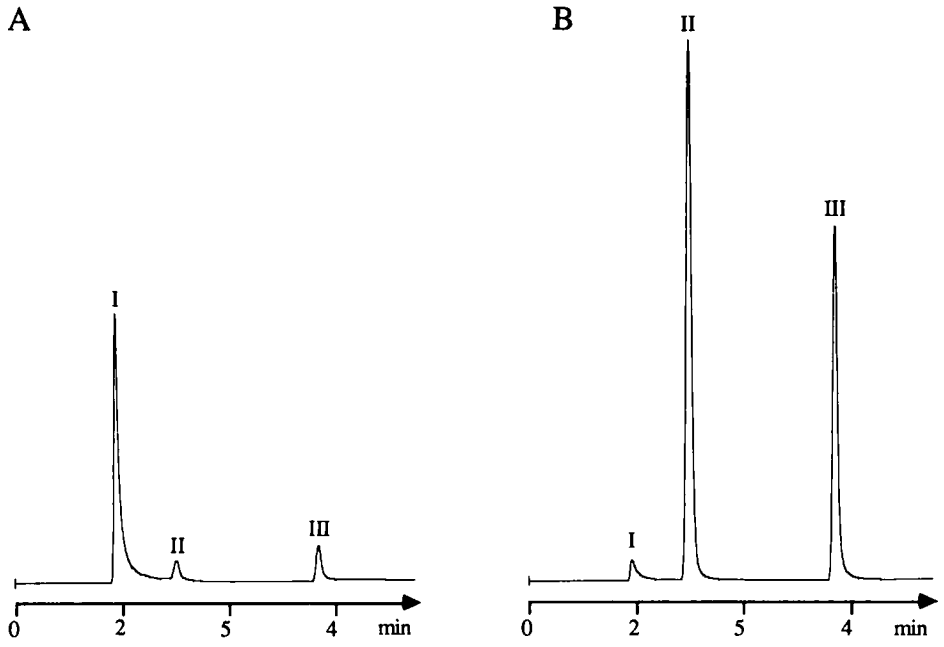

Figure 1 Chromatograms of urine spiked with dimethylethylamine (DMEA; 0.3 mmol/l) and dimethylethylamine-N-oxide (DMEAO; 2.7 mmol/l) in urine before (A) and after (B) reduction of the urine with tin. Dimethylamine (DMA; I), trimethylamine (TMA; 2), and DMEA (3). The lower extraction ability of DMA (chromatogram B) was caused by the smaller excess of KOH, when the urine sample was reduced.

range of interest, showed linearity for DMEA up to 400 mg/l. In practice, for plasma and urine samples the possibility to dilute the samples eliminates this upper limit. For air samples, the upper limit of the method is dependent on the absorption capacity of the 10 ml 0.1 M HCl solution in the impinger. This absorption capacity corresponds to a level of about 5000 mg/m^3 at a 15 l air sample. With long sampling periods, the volume of the absorption solution must be compensated for water evaporation. The limit of detection of DMEA in absorption solution and urine samples was 0.01 mg/l (0.1 μmol/l). For air samples, this corresponds to <0.01 mg/m^3 with a 15 liter air sample. For plasma (4 μl injection volume) the limit was 0.003 mg/l (0.04 μmol/l).

Recovery and Precision

The recovery and precision of the methods were determined by analysis (consecutively) of ten spiked samples by duplicate injections (Table 2). Inhaled DMEA is excreted to an extent of about 10% as DMEA and 90% as DMEAO.[20] Thus, in order to simulate plasma and urine sampled for exposed subjects, plasma and urine were spiked with 10% DMEA and 90% DMEAO. The recovery of the added DMEA to plasma and urine samples was high and the precision was good. The recovery of DMEAO after 1 h reduction of the plasma and the urine at 95 °C

Table 2 The recovery and precision (SD/mean × 100) of DMEA and DMEAO of spiked plasma and urine samples

Sample ($n = 10$)	*Concentration DMEA + DMEAO* (*mmol/l*)	*DMEA*		*DMEAO*	
		Recovery (%)	*Precision* (%)	*Recovery* (%)	*Precision* (%)
Plasma	1.00×10^{-3}	105	6	105	6
	2.00×10^{-3}	100	6	98	4
	3.00×10^{-3}	95	6	95	5
Urine	1.00	100	1	98	4
	2.00	101	1	104	2
	3.00	99	3	98	3

was about 100%. However, when the reduction of DMEAO was performed at a lower temperature (90 °C, 1 h), or during a shorter time (30 min, 95 °C), the recoveries were only 70 and 50%, respectively.

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