Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography B, 828 (2005) 103–107
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

Development of a solid phase microextraction–gas chromatography method to determine *N*-hydroxymethyl-*N*-methylformamide and *N*-methylformamide in urine

Vagner Fernandes Knupp, Edna Maria Alvarez Leite, Zenilda de Lourdes Cardeal *

Chemistry Department, ICEx, Federal University of Minas Gerais, Av. Antonio Carlos 6627, C.P. 702, 31270901 Belo Horizonte, M.G., Brazil

Available online 10 October 2005

Abstract

A headspace solid phase microextraction (SPME) method has been developed to determine metabolites of dimethylformamide, *N*-hydroxymethyl-*N*-methylformamide, and *N*-methylformamide (NMF) as NMF in urine by gas chromatography with nitrogen–phosphorus detector (GC-NPD). An SPME holder with a 65-μm PDMS/DVB fiber coating was used. Optimal desorption conditions were 250 °C for 1 min, adsorption at 80 °C for 15 min, and 3.00 mL of sample in the headspace vial. The method presented good resolution, repeatability, recovery, detection limit, ruggedness and response linearity.

Keywords: Dimethylformamide; *N*-Methylformamide; *N*-Hydroxymethyl-*N*-methylformamide; GC-SPME

1. Introduction

N,*N*-Dimethylformamide (DMF) is an organic polar solvent extensively produced throughout the world and widely used in the chemical industry, especially in the manufacture of polyacrynitrile fibers and synthetic leather; and as an intermediate and an additive in the manufacture of pharmaceutical products. DMF is classified by the Danish Work Environmental Protection Agency [1] as having a neurotoxicity risk index of 4 out of 10, and it may induce severe chronic damage to the nervous system over long time work place exposition [2]. Some articles show the relation between DMF exposition and its effects, such as hepatic damage and alcohol intolerance [1]. The International Agency for Research on Cancer (IARC) had classified it as a 2B carcinogen [3], but has changed its classification to 3 after revision of current toxicological and epidemiological information [1].

Occupational exposure to DMF occurs through inhalation of vapors, and especially by direct skin contact with the liquid and vapor [1]. Dermal exposition leads to acute effects, such as very strong abdominal cramps, alcohol intolerance, skin damage, nausea, and vomiting [1]. Long exposition can induce loss of appetite, anxiety, hepatic and neurological problems, and headache [1,3,4]. After absorption, DMF is rapidly distributed to the body through the blood stream and metabolized. Many studies [1] reported that workers exposed to low concentrations of DMF in air present strong intoxication symptoms. In these cases, called over exposition, workers in fact had direct skin contact with DMF during its manipulation. This shows that air monitoring is not sufficient yet to assure workers' safety and demonstrates the need of biological monitoring [1].

Usually, metabolite analysis is employed in biologic monitoring of DMF because it is quickly transformed into *N*-hydroximethyl-*N*-methylformamide (HODMF) by the action of enzymes of the P450 group. This is the metabolite with the highest concentration in urine in the first hours after exposition. Another important metabolite is *N*′-acetyl-*S*-(*N*′-methylcarbomyl) cysteine (AMCC), but its origin has not been totally elucidated. It is known that HODMF can be demethylated to *N*-methylformamide (NMF) and then oxidized to an intermediate metabolite, presumably methylisocyanate, which in turn possibly reacts with glutathione to originate AMCC [1].

The elimination of DMF and its metabolites occurs through urine excretion. Metabolite analysis is the most commonly used biological monitoring method of human exposition to DMF. DMF and HODMF excretion reaches a maximum 6–8 h after the initial exposition, NMF after 8–14 h, and AMCC after 24–48 h.

* Corresponding author. Fax: +55 31 34995700.
E-mail address: zenilda@ufmg.br (Z. de Lourdes Cardeal).

1570-0232/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2005.09.030

Elimination half times of these compounds occurs after approximately 2, 4, 7, and 23 h, respectively [1]. The total concentration of HODMF and NMF (expressed as NMF concentration) is a good indicator of short time exposition to DMF because it rapidly builds up in the organism and is totally eliminated after 24 h. In contrast, the concentration of AMCC is a good indicator of long time exposition to DMF because of its slow build-up in the organism and its long elimination time [1].

However, HODMF undergoes thermolytic transformation into NMF at the injection port during analysis using gas chromatography. NMF concentration in urine represents the sum of the concentrations of these two metabolites [5–10].

The biological exposure index (BEI) adopted in the USA and Germany is 15 mg/L of urinary NMF [5]. In Brazil, BEI is 40 mg of urinary MF per gram of creatinine [11].

Quantification of HODMF and NMF is performed by GC and HPLC methods using liquid–liquid extraction (LLE), solid phase extraction and clean up procedures [4–10]. Angerer and Käfferlein [5] developed a method of simultaneous analysis of NMF and AMCC. In this study, they classify other existing methods [6–10] as "having several disadvantages in regard to sample preparation and clean-up procedures". The Angerer Käfferlein method has the advantage of simultaneous analysis of two DMF metabolites, but it is not better than others in its preparation because it has many steps as well. An alternative analysis technique is solid phase microextraction (SPME) [12]. SPME has the advantages of being selective, sensitive, solvent-free, and having a single extraction step.

The aim of the present work was to develop a solid phase microextraction method in headspace mode to analyze HODMF and NMF in urine by GC with nitrogen–phosphorus detection. The analysis of urine of workers exposed to DMF is described.

2. Experimental

2.1. Reagents, solvents and synthesized compounds

All reagents were of analytical grade: *N*-methylformamide (99.8%) and chloroform (99.5%) were obtained from Aldrich Chemical (Bellefonte, PA). Paraformaldehyde (99%) and dry potassium carbonate (99.8%) were purchased from Merck (Darmstadt, Germany). Water was purified with a Milli-Q water purification system from Millipore (Milford, MA, USA).

2.2. Synthesis of N-hydroxymethyl-N-methylformamide

HODMF was synthesized as described by Santoni et al. [6]. A mixture of NMF (23.60 g), paraformaldehyde (11.20 g) and anhydrous potassium carbonate (0.40 g) was heated to 90 °C for 1 h under magnetic stirring. After cooling to room temperature, chloroform was added, and after filtration, the solvent was removed under reduced pressure. The crude product was purified by distillation (boiling point 136 °C) under atmospheric pressure. Compound purity was determined by HPLC with a calibration curve at the concentration range from 6.00 to 30.00 mg/L NMF and showed that the product contained 88.3% HODMF and 11.7% NMF.

2.3. Instrumentation

The purity of the HODMF synthesized was evaluated by a Shimadzu HPLC system equipped with an LC-10AD pump and an SPD-10A UV–vis detector (Kyoto, Japan). The injection system used was a Rheodyne valve incorporating a 20.00-μL injection loop. The detector wavelength was set at 196 nm. A stainless steel column Shim-Pack Perp ODS, 5-μm particle size (Shimadzu, Kyoto, Japan) of 250 mm × 4.6 mm I.D was used. The mobile phase was water with a flow rate of 0.8 mL/min.

GC analyses were performed on a Varian 3800 system equipped with a nitrogen–phosphorus detector (NPD) and a split/splitless injector operating in splitless mode at 250 °C (Walnut Creek, CA). The detector was operated with hydrogen at 4.2 mL/min and air at 175 mL/min. For analyte separation, a Supelcowax fused-silica capillary column 30 m long, 0.25 mm I.D., 0.25-μm film thickness (Supelco-Bellefonte, PA) was used at 100 °C. Helium was used as a carrier gas with a constant flow of 1.0 mL/min.

Extractions were performed on a manual SPME holder with 65-μm polydimethylsiloxane/divinilbenzene (PDMS/DVB) fiber purchased from Supelco (Bellefonte, PA, USA). For the SPME procedure, an aliquot of 3.00 mL of urine was placed into 22-mL Pyrex vials and immediately sealed with Teflon-lined rubber septum aluminum caps. Samples were heated to 80 °C for 15 min on a sample vial aluminum rack in a stirrer/heater before extraction.

2.4. Standard solutions and sample preparation

The standard solution of HODMF and NMF was prepared in pooled urine from non-exposed workers with concentration ranging from 6.00 to 120.00 mg/L. Solutions were prepared by accurately weighing 7.53 g/L HODMF and 1.00 g/L NMF of the synthesized substance. Method reliability was verified by the recovery of spiked pooled urine from workers.

3. Results and discussion

The development of an SPME method requires a study to establish optimal analyte extraction conditions. For this purpose, some SPME-HS parameters were studied: fiber type, desorption time, sample volume in the vial, injection time and temperature. Desorption temperature was not optimized because we used 250 °C as it was demonstrated in literature that at this temperature thermolytic transformation from HODMF to NMF is complete [4–10]. The solution with 37.71 mg/L of HODMF and 5.485 mg/L of NMF prepared with pooled urine was used for method optimization.

The HS sorption profile of different fibers exposed to HODMF and NMF was tested with 2.00 mL of solution for 15 min at 65 °C. Three fibers with different polarities and coating thicknesses were tested: polyacrilate 85 μm (PA), polydimethylsiloxane 100 μm (PDMS), and polydimethylsiloxane/divinilbenzene 65 μm (PDMS/DVB). The results showed that the PDMS/DVB phase had the highest sorption capacity.

Desorption time at 250 °C was studied by monitoring the peak area at 0.10, 0.25, 0.50, 1.0 and 2.0 min. During this procedure, adsorption was kept at 65 °C for 15 min with 2.00 mL of sample in the vial. It was observed that equilibrium was achieved in 1 min. Therefore, 1 min was considered the ideal desorption time of HODMF and NMF from a PDMS/DVB fiber.

The effect of sample volume on the sorption of NMF and HODMF was evaluated with 0.25, 0.50, 1.00, 2.00, 3.00, 4.00, and 5.00 mL in a headspace 22-mL vial. During this procedure, adsorption was kept at 65 °C for 15 min, with 1.00 min desorption time. The results obtained indicated that volumes higher than 3.00 mL did not interfere with fiber mass uptake. This volume was used as the best for analysis.

Extraction temperatures between 60 and 95 °C were investigated to determine fiber coating concentration efficiency. These experiments were carried out with 3.00 mL of sample, desorption time of 1 min, and adsorption time of 15 min. The results obtained showed that temperatures from 60 to 80 °C increased fiber analyte sorption. Concentration decreased with the increase in temperature (at 95 °C) because of exothermic effects of the sorption process. Consequently, the temperature of 80 °C was selected as adequate for the extraction procedure.

The adsorption time profile was investigated by varying time from 5 to 20 min with desorption time of 1 min; temperature extraction of 80 °C, sample volume of 3.00 mL. The best absorption time was 15 min. With times shorter than 15 min, the signal was insufficient, because maximum adsorption was not achieved. After 15 min, analyte signals decrease as there is competitive adsorption of other substances by the fiber phase. This time produces the best signal areas for analytes.

After optimal extraction and desorption conditions had been established, method validation was carried out. Chromatograms of a worker's urine, spiked and non-spiked, and a blank from the urine pool are shown in Fig. 1.

For construction of analytical curves, standard solutions of HODMF and NMF prepared in pooled urine were analyzed in triplicate under the conditions optimized for the SPME HS method. The linearity study is summarized in Table 1. The correlation coefficient $R = 0.996$ and variance analysis indicated that there was a linear correlation between concentration and curve area. It was the most straight line through the calibration graph point because the correlation between the regression average square (QM_{reg}) and the residue average square (QM_r) is larger than the tabulated test F value for 1, 14 degree of freedom ($F_{1,14} = 4.60$ with 95% confidence).

Table 1
Linear regression analysis parameters

	Area MF (mV)		
	Average	S.D.	R.S.D. (%)
Concentration NMF + HODMF (mg/L)			
6.00 ± 0.02	100366	6516	6.5
17.97 ± 0.04	296336	20229	6.8
47.49 ± 0.08	1116104	49782	4.5
71.91 ± 0.09	1589762	116524	7.3
119.8 ± 0.2	2781878	155351	5.6
Equation	$Y = 23582\ (\pm 542)$ $X - 63881(\pm 34982)$		
R	0.996		
QM_{reg}/QM_r	1894.86		

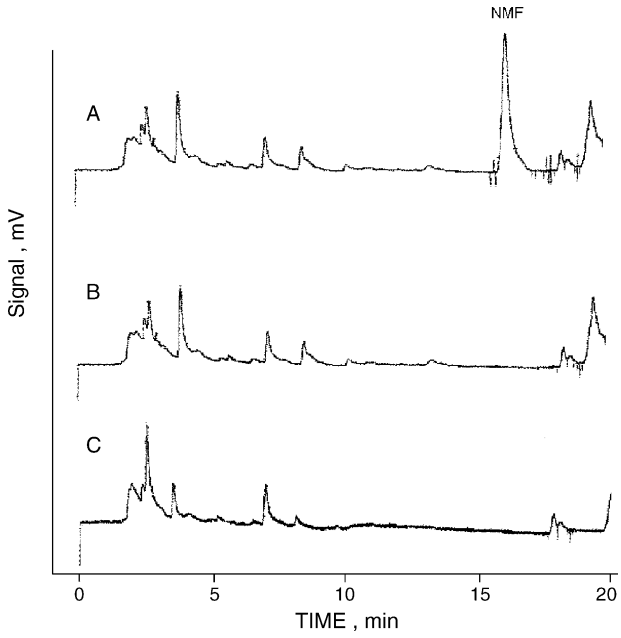

Fig. 1. Chromatogram of urine samples. Chromatography conditions: injector in splitless mode at 250 °C, detector NPD at 290 °C, column of 30 m × 0.25 mm I.D. × 0.25 μm (polyethylenglycol) at 100 °C with He at 1.0 mL/min. Extractions were performed on a manual SPME holder with PDMS/DVB of 65 μm, urine sample volume of 3.00 mL, sealed 22-mL Pyrex vials, heated to 80 °C for 15 min under stirring. (A) Chromatogram of worker urine spiked with HODMF + MF (19.00 mg/L) with retention time of 18.625 min. (B) Chromatogram of non-spiked worker urine. (C) Chromatogram of the blank pool urine used to calculate LD and LQ.

Within-day and between-day repeatability, and recovery tests were carried out through analysis of urine samples spiked with NMF and HODMF in two levels of concentrations. Seven injections were made for within-day test, and fourteen injections for between-day test. The results shown in Table 2 (%R.S.D. 4.4–10.4) indicate that this method is precise, since according to Huber [13] for biological samples, analysis precision can be up to 15% R.S.D. at concentration limits, and 10% R.S.D. at other concentration levels.

Limits of detection (LD) and quantification (LQ) were determined according to IUPAC recommendations [14,15] through the analysis of twenty urine samples (blank). Analysis data were substituted in the equations:

$$LD = 3.52\sigma_B$$

$$LQ = 16.67\sigma_B$$

where σ_B represents the relative standard deviation (s_B) of the blank. The results obtained were LD = 0.29 mg/L and LQ = 1.08 mg/L.

In the ruggedness study, small method changes were introduced and examined by screening with a Plackett–Burman design as described by Heyden [16]. The factors selected for investigation were those of GC: injection temperature (249 and

Table 2
Repeatability and recovery data of spiked workers' urine (NMF + HODMF)[a]

	Sample 1 (spiked 19.0 mg/L of NMF)	Sample 2 (spiked 19.0 mg/L of NMF)	Sample 1 (spiked 74.0 mg/L of NMF)	Sample 2 (spiked 74.0 mg/L of NMF)
Within-day, average ($n = 7$)	19.5	17.6	71.9	75.6
S.D.	1.7	1.8	4.0	3.3
%R.S.D.	8.9	10.2	5.5	4.4
Between-day, average ($n = 14$)	18.6		73.3	
S.D.	1.8		3.8	
%R.S.D.	9.6		5.1	
%Recovery	102.7	88.5	97.2	100.9

[a] NMF concentration in urine of two workers (samples 1 and 2) was <LD.

251 °C), desorption time (0.99 and 1.01 min), column temperature (99 and 101 °C), and carrier gas rate (0.9 and 1.1 mL/min). Extraction conditions, extraction temperature (75 and 85 °C), adsorption time (14 and 16 min), sample volume (2.99 and 3.02 mL), and other factors were evaluated by analysis in two different days (1st and 2nd). The effect of these factors on response retention time and area measurement was determined by a t test with 95% confidence. The results allowed the conclusion that the method yields the same results in the presence of small changes in experimental conditions, such as might occur during actual use. Recovery of spiked samples of workers' urine showed that the method is rugged for matrix changes.

Application of the SPME method to real samples was carried out through the analysis of four urine samples of workers from a metallurgic industry where a previous study showed atmospheric contamination by DMF, and nine urine samples of workers from a shoe industry that uses DMF as a cleaning solvent. Results of triplicate analysis are presented in Table 3. NMF was not detected in any sample from the metallurgic industry, perhaps because the value of ambient DMF was too low. Analysis results of urine of the shoe industry workers ranged from 7.5 to 13.7 mg/L, which shows that exposition to DMF is below BEI adopted in the USA and Germany (15 mg/L of urinary NMF [5]). We observed with two samples used in the recovery experiment that urine samples can be stored in freezer for analysis for 2 weeks.

Table 3
Analysis of NMF in 13 different urine samples of workers exposed to DMF

Sample[a]	Concentration (mg/L of NMF)	
	Average ($n = 3$)	S.D.
1	<LD	–
2	<LD	–
3	<LD	–
4	<LD	–
5	<LD	–
6	12.4	0.7
7	7.5	0.9
8	<LD	–
9	10.2	0.8
10	8.4	0.6
11	<LD	–
12	13.7	0.5
13	<LD	–

[a] Samples 1–4, metallurgic industry workers. Samples 5–13, shoe industry workers.

4. Conclusions

This paper proposes an SPME HS method for the determination of NMF as a sum of HODMF and NMF in urine. This procedure was validated and found to be precise, sensitive, linear and rugged in the range of interest. SPME has the added advantage of not requiring organic solvent and allowing analyte extraction in a single step. There are other methods with few extraction steps, such as the method of Angerer and Käfferlein [5], but the author himself call attention to several disadvantages with regard to sample preparation and clean up procedures. The present method does not require clean up procedures. Therefore, because of these advantages, SPME HS could to be a good alternative method for the quantitative analysis of DMF metabolites in urine by gas chromatography.

Acknowledgement

This study has been supported by FAPEMIG (Fundação de Amparo a Pesquisa do Estado de Minas Gerais) and CNPq (Conselho Nacional de Pesquisa e Desenvolvimento).

References

[1] G.L. Kenedy Jr., Crit. Rev. Toxicol. 31 (2001) 139.
[2] L. Simonsen, S.P. Laund, Am. J. Ind. Med. 21 (1992) 773.
[3] A. Bainova, Dimethylformamide—Environmental Health Criteria 114, World Health Organization, Geneva, 1991.
[4] R. Lauwerys, Biological indicators for the assessment of human exposure to industrial chemicals, Joint Reserch Centre Ispra Establishment, EUR 10704 EN, 1986.
[5] J. Angerer, H.U. Käfferlein, J. Chromatogr. B 734 (1999) 285.
[6] P.G. Santoni, P. Bavazzano, A. Perico, A. Colzi, S. Benassi, A. Medica, R. La Morgia, G. Giuliano, J. Chromatogr. 581 (1992) 287.
[7] M. Ikeda, T. Kawai, T. Yasugi, K. Mizunuma, T. Watanabe, S. Cai, M. Huang, L. Xi, J. Qu, B. Yao, Int. Arch. Occup. Environ. Health 63 (1992) 455.
[8] M.T. Rosseel, F.M. Belpaire, N. Samijn, P. Wijnant, J. Chromatogr. 615 (1993) 154.

[9] A.C. Lareo, A. Perico, P. Bavazzano, C. Soave, L. Perbelline, Int. Arch. Occup. Environ. Health 67 (1995) 41.
[10] G. Tranfo, C. Plebani, A. Salerno, A.S. Panebianco, A.M. Marcelloni, J. Chromatogr. A 847 (1999) 19.
[11] "Programa de Controle Médico de Saúde Ocupacional" (PCMOS), Brazilian Law 6514-NR 7, 6th August1978, Annex I, Table I.
[12] J. Pawliszyn, Solid Fase Microextration—Theory and Practice, Wiley-VCH Inc., New York, 1997.
[13] L. Huber, LC–GC Int. (1998) 96.
[14] L.A. Currie, W. Horwitz, Analusis Mag. 22 (1994) M24.
[15] L.A. Currie, Anal. Chem. ACTA 391 (1999) 127.
[16] Y.V. Heyden, Analusis Mag. 22 (1994) M27.