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Short communication

High-performance liquid chromatographic determination of *n*-methylformamide, a biological index for occupational exposure to dimethylformamide

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Abstract

This report describes an analytical method for the biological monitoring of workers exposed to *N*,*N*-dimethylformamide (DMF), a solvent widely used in the chemical industry. The human main metabolites of DMF are *N*-hydroxymethyl-*N*-methylformamide (HMMF) and the minor metabolites *N*-methylformamide (NMF) and *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine. The metabolite selected by the American Conference of Governmental Hygienists for occupational biomonitoring purposes, is NMF measured by gas chromatographic analysis, as during it HMMF may be converted to the minor metabolite NMF. HMMF and NFM can be measured independently using HPLC analysis. The procedure proposed here involves the thermal transformation of the primary metabolite HMMF into the minor metabolite NMF, which is then determined by HPLC. This method makes it possible to determine, using HPLC, both metabolites of DMF by measuring only one peak, thus offering two major advantages: (i) it increases the sensitivity of the test and (ii) it deploys only one reference standard.

Keywords: Dimethylformamide; Hydroxymethylmethylformamide

1. Introduction

N,*N*-Dimethylformamide (DMF) is widely used in the chemical industry as a solvent, especially for the production of synthetic leather and polymeric fibres: in man, it exerts its main toxic action on the liver and on the first gastrointestinal tract [1]. It is listed as a Group 2B substance ('possible human carcinogen') by the IARC [2]. Moreover, increases in sister chromatid exchange rates have been associated with exposure to DMF [3]. Placental transfer of DMF has also been documented in rats [4].

Exposure to DMF may occur through inhalation and dermal absorption [5,6], therefore biological monitoring of exposed workers is required to assess the extent of individual exposure dose. DMF biotransformation, which occurs mainly in the liver of humans, results in the formation of the major metabolite *N*-hydroxymethyl-*N*-methylformamide (HMMF) and the minor metabolite *N*-methylformamide (NMF) [7] (Fig. 1). The presence of these metabolites provides an index of recent exposure to DMF.

The mercapturic acid, *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine (AMCC), is excreted in urine after DMF exposures. Measurement of this metabolite has been shown to serve as an index of the average exposure to DMF during several working days [8].

The concentration of NMF in post-shift urine,

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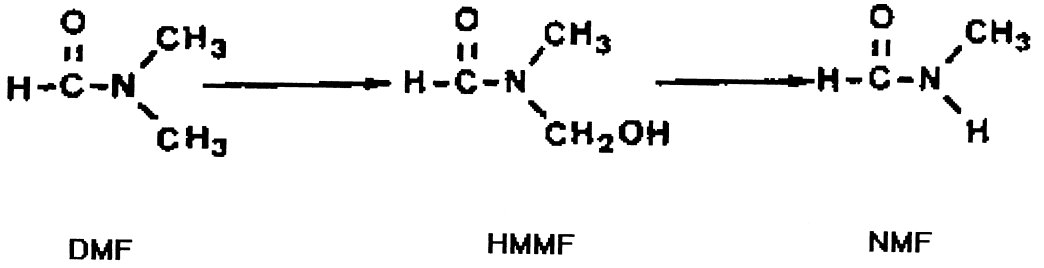

Fig. 1. Metabolic pathways of *N,N*-dimethylformamide.

measured by gas chromatography [9], has been validated as the biomarker for occupational exposure to DMF. In this analytical system, HMMF is thermally transformed into NMF during injection where direct injections are performed at 250°C and splitless injections at 150°C [10]. Here, the final concentration of NMF represents the sum of the concentrations of the two metabolites.

The adopted biological exposure index (BEI), that is the threshold limit value for the resulting concentration, is 40 mg of urinary NMF per g of creatinine (8 h TWA; i.e., time weighted average on 8 h) [11].

HPLC methods developed to monitor occupational exposure to DMF [12,13] must measure both HMMF and NMF concentrations. The sum of these values is then compared with the BEI value. Reference standards of both metabolites are required for the analysis.

The aim of the present work was to develop an analytical method in which only the peak relating to NMF, the reference standard of which is commercially available, needs to be quantified. This aim is achieved by thermal transformation of HMMF to NMF prior to HPLC analysis.

2. Experimental

2.1. Reagents and chemicals

NMF was supplied by Fluka (Buchs, Switzerland). HMMF was synthesised as described in Ref. [12]. Identity and purity have been confirmed by HPLC. Residual NMF was 10%. Water (HPLC grade) was produced by a Millipore Milli-Q system. Methanol (HPLC grade), sulphuric acid (96%), hydrochloric acid (37%), anhydrous potassium carbonate (analytical reagent grade) and paraformaldehyde (synthesis grade) were purchased from Bracco (Milan, Italy). Oasis HLB extraction cartridges, 60 mg, were obtained from Waters (USA). Anotop 10, 0.2-μm alumina membrane filters were purchased from Bracco.

2.2. Instrumentation

The instrument used was a Perkin-Elmer HPLC system equipped with a Series 200 LC pump and 785A UV–Vis detector. The injection system was a Rheodyne valve incorporating a 20-μl injection loop.

The column was a stainless steel 300×7.8 mm I.D. Aminex Ion Exclusion HPX-87, 9-μm particle size (Bio-Rad, Richmond, USA). The mobile phase was 7.5×10^{-4} *M* sulphuric acid maintained at 40°C with a flow-rate of 0.7 ml/min. The detector wavelength was set at 196 nm.

2.3. Sample preparation and thermal treatment

Urine samples from non-exposed subjects were collected from volunteers in this Institute. End-of-shift urine samples were collected from workers in a factory manufacturing synthetic leather.

Solid-phase extraction (SPE) of the urine samples was performed as follows: in the conditioning step, Oasis HLB 60-mg extraction cartridges were loaded with 3 ml of methanol and subsequently with 3 ml of 1 *M* HCl. One ml of urine was loaded and then washed off with 2 ml of water. The first 1-ml elution fraction was discarded and the second 1-ml eluate was collected.

The collected fraction was heated at 120°C for 2 h, in order to transform HMMF into NMF, and then centrifuged at 1200 rpm (at room temperature) for 5 min. The supernatant was collected, filtered on a

0.2-μm alumina membrane filter, and finally 20 μl were injected into the HPLC system.

2.4. Standard solutions and calibration curves

An aqueous standard solution of 1.0 mg/ml NMF was prepared by accurately weighing the reference substance, from which three dilutions were prepared, at the following concentrations: 5.0, 1.0 and 0.1 mg/ml. Ten μl from each dilution were added to 1 ml of a non-exposed subject urine (blank), thus obtaining the following concentrations: 50, 10 and 1 mg/l. These mixtures were treated as described in Section 2.3, and analysed by HPLC, in order to construct a calibration curve.

3. Results and discussion

The peak of NMF was identified by comparison

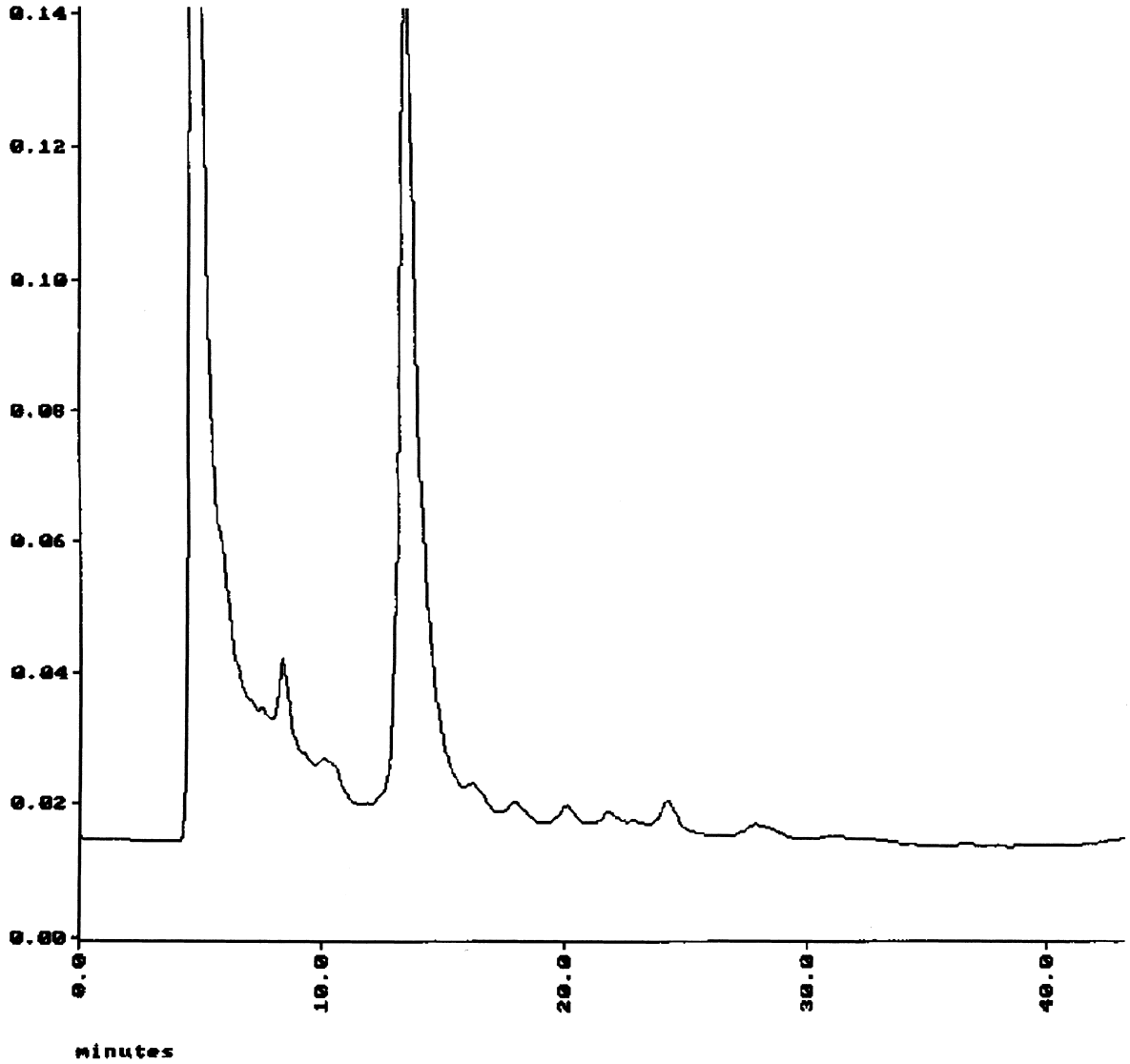

Fig. 2. Chromatographic profile of a blank urine.

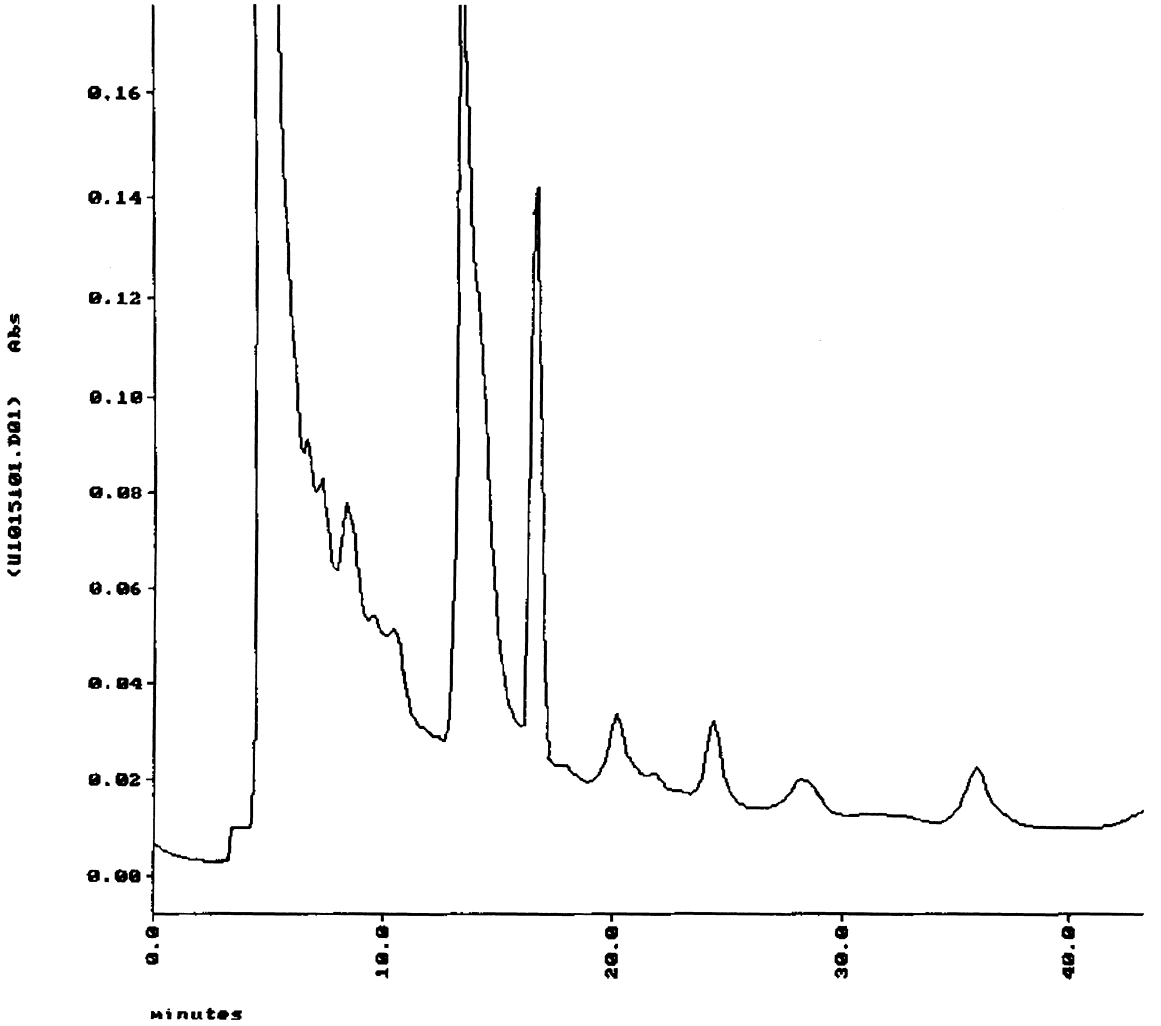

Fig. 3. Chromatographic profile of a sample containing HMMF and NMF, before thermal treatment.

with a chromatogram of a solution of the reference material. The retention time of the NMF peak was 35.5 min, using the operating conditions described.

A chromatogram of a blank urine, treated as described in Section 2.3 and analysed by HPLC, did not show any interfering peak at the retention time of NMF (Fig. 2).

The method response linearity has been verified in the range 1–50 mg/l, which exceeds the requirement as determined by the American Conference of Governmental Hygienists (ACGIH) limit value (BEI). The detection limit, at a signal to noise ratio of 3, was 0.5 mg/l, that is, about 1/80 of the BEI. The reproducibility of the method, calculated by analysing six replicates of a calibration solution (10 mg/l), and expressed as a coefficient of variation, was 2%.

Blank urine samples were spiked with HMMF in order to obtain the following concentrations: 50, 10 and 5 mg/l. The mixtures were treated as described in Section 2.3 and analysed three times each by HPLC to determine the concentration of NMF.

The accuracy of the method, calculated on the results of the nine determinations and expressed as the average recovery of NMF, was 97% (the 10% of

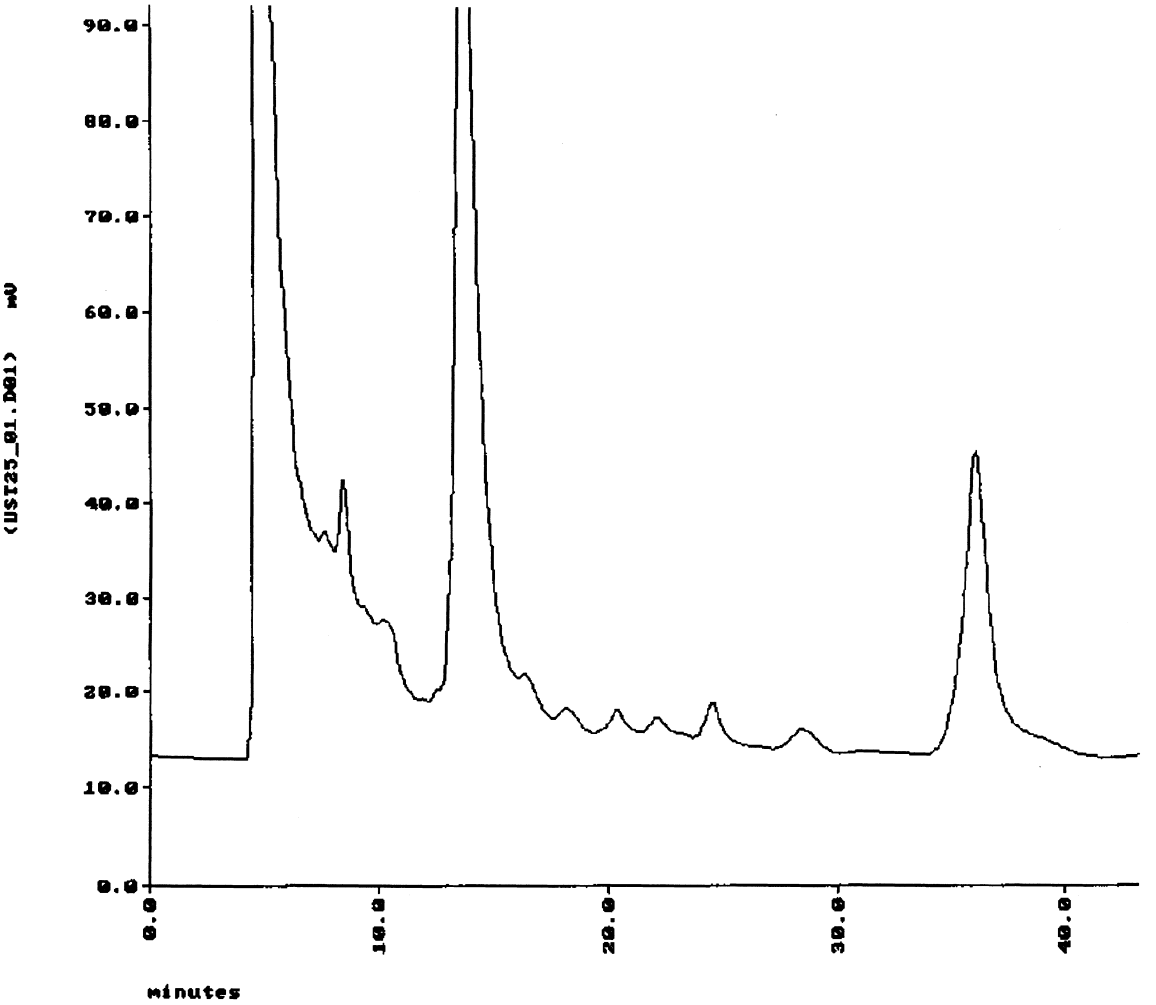

Fig. 4. Chromatographic profile of a sample containing HMMF and NMF, after thermal treatment.

NMF already present in HMMF needs to be considered). Assay precision was 5.1 % (CRSD).

Figs. 3 and 4 show the chromatographic profile of a sample containing 25 mg/l of NMF before and after the thermal treatment.

4. Conclusions

The performance of the thermal transformation/HPLC method described renders it a suitable tool for biological monitoring studies of workers exposed to DMF.

This method, compared with previously published HPLC methods, offers the following advantages: Where both metabolites of DMF are determined independently it is necessary to deploy two reference standards, i.e., HMMF and NMF. The latter material alone is available commercially and, therefore, HMMF would require to be synthesised for the analysis. The thermal transformation step devised in this project obviates the need of the chemical synthesis of HMMF.

The identification and quantitation of only one HPLC peak reduces analysis time and increases assay sensitivity.

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