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Journal of Pharmaceutical and Biomedical Analysis 37 (2005) 165–170
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Simultaneous determination of *N*-hydroxymethyl-*N*-methylformamide, *N*-methylformamide and *N*-acetyl-*S*-(*N*-methylcarbamoyl)cystein in urine samples from workers exposed to *N*,*N*-dimethylformamide by liquid chromatography–tandem mass spectrometry

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Received 1 September 2004; received in revised form 30 September 2004; accepted 2 October 2004

Abstract

N-Hydroxymethyl-*N*-methylformamide (HMMF) and *N*-methylformamide (NMF) in urine samples from workers exposed to *N*,*N*-dimethylformamide (DMF) cannot be distinguished by a gas chromatographic method because HMMF is converted to NMF at the injection port of gas chromatography (GC). Total NMF (HMMF + NMF) has been measured instead. Also, the determination of *N*-acetyl-*S*-(*N*-methylcarbamoyl)cystein (AMCC), which is supposed to be related to the toxicity of DMF, needs multiple treatments to convert to a volatile compound before GC analysis. There is no previous report of a simultaneous determination of three major metabolites of DMF in urine. The aim of this study is to develop a simple and selective method for the determination of DMF metabolite in urine. By using a liquid chromatography–tandem mass spectrometry, we can directly distinguish these three major metabolites of DMF in a single run. The diluted urine samples were analyzed on Capcell Pak MF SG80 column with the mobile phase of methanol in 2 mM formic acid (10:90, v/v). The analytes were detected by an electrospray ionization tandem mass spectrometry in the multiple-reaction-monitoring mode. The standard curves were linear ($r > 0.999$) over the concentration ranges of 0.004–8 μg/mL. The precision and accuracy of quality control samples for inter-batch ($n = 6$) analyses were in the range of 1.3–9.8% and 94.7–116.8, respectively. The sum of each HMMF and NMF concentration determined by LC-MS/MS method shows high correlation ($r = 0.9927$ with the slope of 1.0415, $p < 0.0001$) with NMF included HMMF concentration determined by GC method for 13 urine samples taken from workers exposed to DMF. The excretion ratio of HMMF:NMF:AMCC is approximately 4:1:1 in molar concentration.

Keywords: *N*,*N*-Dimethylformamide; *N*-Hydroxymethyl-*N*-methylformamide; *N*-methylformamide; *N*-acetyl-*S*-(*N*-methylcarbamoyl)cystein; LC-MS/MS

1. Introduction

N,*N*-Dimethylformamide (DMF) has been widely used in synthetic leather and polyacrylonitrile fiber industries as a washing solvent. Because of its high miscibility with both water and organic solvents, DMF has been important in these industries in washing off impurities, despite its toxicity. DMF has been reported to increase the chance of getting pancreatic disorders, liver dysfunction, testicular carcinoma, etc. [1–3]. The occupational exposure limit of DMF was set as 10 ppm in most of countries [4,5]. DMF is absorbed through both inhalation of the vapor and dermal contacts such as submerging hands under the DMF fluid. Therefore, ambient air monitoring by itself cannot effectively reflect the amount of real intake.

The major metabolites include *N*-hydroxymethyl-*N*-methylformamide (HMMF) and *N*-methylformamide (NMF)

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doi:10.1016/j.jpba.2004.10.001

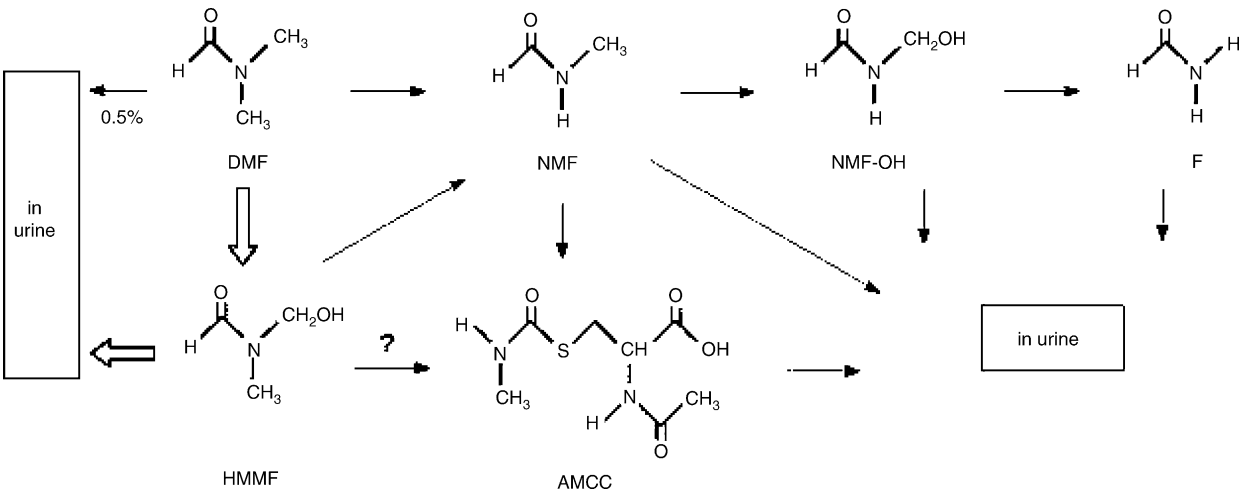

Fig. 1. Hypothetical breakdown of DMF in human, copied with a written permission from Ref. [6].

and *N*-acetyl-*S*-(*N*-methylcarbamoyl)cystein (AMCC). The American Conference of Governmental Industrial Hygienists (ACGIH) set the Biological Exposure Indices (BEI) for DMF as 15 mg of NMF and 40 mg of AMCC in one liter urine [5]. Fig. 1 shows the hypothetical breakdown of DMF in human [6]. The determination of each metabolite of DMF is important in understanding the metabolic pathway of DMF and assessing the exact amount of intake in body after a DMF exposure.

A number of methods for the analysis of the metabolites of DMF in urine were reported using gas chromatography (GC). HMMF, which is the major metabolite of DMF, was detected as the total NMF in urine because HMMF was converted to NMF during the analytical procedure by GC [6–10]. AMCC, which is supposed to be related to the toxicity of DMF and has prolonged half-life, has been focused for the field study of biological monitoring for workers [11–14]. Quantitation of AMCC in urine by GC needed multiple treatments to convert AMCC to a volatile compound. Kafferlein et al. [15] compared the suitability and accuracy of four GC methods developed for determination of urinary metabolites of DMF. Two methods were able to measure only the total NMF, and the other two methods measured both the total NMF and AMCC. The two tested methods for the determination of AMCC showed high correlation but differed in the values significantly. So, there was still a need to develop a reliable, simple and selective method for the determination of AMCC for the further application of AMCC to the field study. High performance liquid chromatographic methods were tried but under the UV detector, it was hard to surpass numerous background peaks in urine [16,17]. No method was available for simultaneous determination of three major metabolites of DMF, i.e., HMMF, NMF and AMCC in urine in a single run.

The purpose of this study is to develop a simple, reliable and selective method for simultaneous determination of the major metabolites of DMF, that is, HMMF, NMF and AMCC in urine by using a liquid chromatography–tandem mass spectrometry (LC-MS/MS). The presented method has been successfully applied to the evaluation of HMMF, NMF and AMCC in urine samples from workers exposed to DMF.

2. Experimental

2.1. Chemicals

NMF, DMF (99.9%, HPLC grade) and formic acid (96%, A.C.S. reagent) were purchased from Sigma-Aldrich Korea Chemical Co. (Seoul, Korea). Deionized water was produced by the Milipore Q system, and methanol (HPLC grade) was purchased from J.T. Baker (Phillipsburg, NJ, USA). HMMF was purchased from Research Institute for Pharmaceutical Institute of Ewha University (>95%, Seoul, Korea). AMCC (>95%) was kindly donated from Dr. Mraz from Czech [11].

2.2. Preparation of standard solutions and quality control samples

Ten milligrams of AMCC, HMMF and NMF were accurately weighed into a 10 ml volumetric flask, and dissolved in methanol to make standard stock solution (1 mg/ml). Aliquot amount of 1 mg/ml stock solution was diluted with water to make each working standard solutions. All solutions were stored at 4 °C in the dark when not in use. Blank urine was collected from non-exposed volunteers and stored at −20 °C in a deep freezer. Human urine calibration standards of HMMF, NMF and AMCC (0.004, 0.02, 0.04, 0.20, 1.0, 2.0, 4.0 and 8.0 μg/ml) were prepared by spiking appropriate amount of the working standard solutions into human blank urine. Quality control (QC) samples at 0.004, 0.2, 1.0 and 8.0 μg/ml were prepared in bulk. The QC samples were aliquoted (1 ml) into polypropylene tubes and stored −20 °C until analysis.