Separate Determination by Gas-Chromatography of Dimethylformamide, Dimethylacetamide, Monomethylformamide and Monomethylacetamide in Urine for Biological Monitoring*

Toshio KAWAI1, Kazunori MIZUNUMA1, Yoko OKADA1, Takanori ODACHI1, Shun’ichi HORIGUCHI1, Hiroshi IGUCHI2 and Masayuki IKEDA2

1Osaka Occupational Health Service Center and
2Department of Public Health, Kyoto University Faculty of Medicine

Abstract: Separate Determination by Gas-Chromatography of Dimethylformamide, Dimethylacetamide, Monomethylformamide and Monomethylacetamide in Urine for Biological Monitoring: Toshio KAWAI, et al. Osaka Occupational Health Service Center—A gaschromatographic method is developed, which allows simultaneous determination of N,N-dimethylformamide (DMF) and N,N-dimethylacetamide (DMA), as well as their metabolites of N-monomethylformamide (MMF) and N-monomethylacetamide (MMA), respectively. Quantitative heat decomposition of N-hydroxymethyl-N-methylacetamide to N-monomethylacetamide required an injection port temperature of 225°C or above, similar to the case of conversion from N-hydroxymethyl-N-methylformamide to N-monomethylformamide. Analysis of urine samples from workers simultaneously exposed to DMF and DMA showed that the separation of small peaks for unidentified materials (which were detected in some urine samples) from that of DMF and DMA was achieved only on a 60 m-long DB-1071 column (and not on a 30 m-long one). The analysis of urine samples from 27 exposed workers showed that both DMF and DMA, in addition to MMF and MMA, were excreted in urine at measurable concentrations. There was a significant correlation between MMF and DMF, and between MMA and DMA; the ratio was about 0.02 to 0.03 for both DMF/MMF and DMA/MMA. (J Occup Health 1997; 39: 113–118)

Key words: Biological monitoring, Dimethylacetamide, Dimethylformamide, Monomethylacetamide, Monomethylformamide, Occupational health, Urinalysis

Both N,N-dimethylformamide (DMF) and N,N-dimethylacetamide (DMA) are potentially hepatotoxic industrial solvents1–5. Although they are seldom mixed for use, mixed exposure sometimes takes place in a synthetic fiber plant in certain working situations. For example, some production lines use DMF as a solvent whereas remaining lines in the same room employ DMA for production of other types of fiber so that the workroom air is polluted by both DMF and DMA, and workers in the rooms are resultingly exposed to the two solvent vapors simultaneously. The proportions of DMF and DMA in air may vary as a function of the location in the workroom. In addition, both solvents may penetrate through intact skin as suggested by experimental exposure studies2. For biological monitoring of workers exposed to DMF and DMA under such conditions, it is important to make separate determinations of N-monomethylformamide (MMF) and N-monomethylacetamide (MMA) in urine.

Trials were made in the present study to achieve separation of MMF and MMA on a single gas-chromatographic (GC) column, and successful results are presented in this article. Under the same analytical conditions, two parent compounds of DMF and DMA were also detected in urine of workers exposed to these solvents. It is well known that DMF is hydroxylated in vivo when absorbed, and that N-hydroxymethyl-N-methylformamide thus formed is heat-decomposed at the injection port to be measured as MMF2,5 in GC analysis. Accordingly, efforts were made to establish the optimum conditions for heat-decomposition of N-hydroxymethyl-N-methylacetamide to MMA at the GC injection port.

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Correspondence to: M. Ikeda. Present address: Kyoto Industrial Health Association, 67 Nishinokyo-Kitatsuboicho, Nakagyo-ku, Kyoto 604, Japan
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Materials and Methods

Urine samples and pretreatment

Urine samples from 27 male workers exposed to a mixed vapor of DMF and DMA in a synthetic fiber plant were subjected to analysis. Female Wistar rats (weighing about 200 g) were used in animal experiments; urine samples were collected separately from feces by means of stainless steel-made metabolic cages (Natsume Instruments., Tokyo, Japan). Each urine sample, either human or murine, was mixed with an equal volume of methanol (spiked with aniline at 100 ng/ml as an internal standard), and the mixture was spun at 3,000 rpm for 10 min in a clinical centrifuge. The supernatant solution, 1 µl/injection, was introduced to GC.

Gaschromatographic analysis

A gaschromatograph (GC; Shimadzu Model 15 A) equipped with FTD (flame thermionic detectors) and connected to an automatic liquid sample injector (Shimadzu AOC-9) and a data processor (Shimadzu Chromatopac CR4A) was employed. Under standard conditions, a 60 m-long DB-1701 megabore column (J&W, U.S.A., 0.53 mm in inner diameter and 1.0 µm in film thickness) was used. Both the injection port and the detectors were heated at 250°C. Helium, a carrier gas, was allowed to flow at a rate of 20 ml/min. The supply of hydrogen gas and air to the detectors was at 0.5 kg/cm² and 0.6 kg/cm², respectively. The sample was introduced to the column in a splitless mode. The column temperature was kept at 95°C for 18 min followed by a cleaning step with an increase at a rate of 40°C/min to 220°C and then keeping at this temperature for 20 min. The column was cooled down to the original temperature of 95°C so that one analysis was terminated in 50 min after injection. When indicated, two megabore columns (DB-WAX and DB-1701, both from J&W, U.S.A., being 30 m in length, 0.53 mm in inner diameter and 1.0 µm in film thickness) were also tested under the same GC conditions.

Authentic chemicals

Authentic DMF, DMA, MMF and MMA were purchased from Nacalai Tesque (Kyoto, Japan).

Statistical analysis

For statistical analysis, Student’s t-test was employed when necessary.

Results

Separation of MMF and MMA on a GC column

Solutions of the authentic DMF, DMA, MMF and MMA as well as workers’ urine samples were applied to the GC under two column conditions, i.e., with a DB-WAX column (a polar column) and with a DB-1701 column (a semi-polar one). Separation of MMF and MMA dissolved in water was achieved when a 60 m-long DB-1701 column was employed (Fig. 1A), but it was not possible to separate MMF from MMA on a DB-WAX column (Fig. 1C).

When urine samples from workers exposed to a combination of DMF and DMA were analyzed, small peaks sometimes appeared immediately after peaks for MMF and MMA (Fig. 1B). These peaks could not be separated from MMF and MMA peaks on a 30 m-long DB 1701 column (Fig. 1D). A clear separation was achieved only when the length of the DB-1701 column was extended to 60 m (Fig. 1B). It was essential to analyze at a rather low temperature of 95°C because a large peak due to unidentified components in urine (present also in urine samples from nonexposed subjects) disturbed isolation of peaks of MMF and MMA. It should be noted in Fig. 1A and B that, under the same analytical conditions with a 60 m-long DB-1701 column heated at 95°C, peaks of two parent compounds, DMF and DMA, could also be detected.

Injection port temperature for quantitative heat-decomposition

It is known that DMF is hydroxylated in the body to form N-hydroxymethyl-N-methylformamide to be excreted into urine, and this metabolite is heat-decomposed in the injection port of a GC to be measured as MMF7). It was considered quite likely by analogy that such should also be the case in DMA metabolite analysis. It is further known that a high temperature of 250°C or above is necessary for maximum decomposition8). Accordingly, a urine sample from an exposed worker was introduced to the GC at various injection port temperatures.

It was found that heat decomposition of MMF was saturated at 250°C or above in confirmation of the previous observation8,9). Saturation of MMA detection occurred at a slightly lower temperature of 225°C (Fig. 2). For simultaneous determination of MMF and MMA, an injection port temperature of 250°C was considered therefore to be appropriate.

Precision and accuracy of the determination

When the urine samples from nonexposed subjects were spiked with authentic DMF, DMA, MMF and MMA at the concentrations up to 1,000 µmoles/l and subjected to the analysis with an injection port temperature of 250°C and on the DB-1701 column, it was found that the relative peak area (i.e., the ratio of the peak area for DMF, DMA, MMF or MMA over the peak area of the internal standard) was linearly related to the added amount of either DMF, DMA, MMF or MMA. The regression lines were identical to each other when compared on an equimolar basis between MMF and DMA, and between MMA and DMA.

The results of triplicate analyses of DMF, MMF, DMA and MMA in water and in urine samples are summarized in Table 1. It is evident from the table that the measurement is highly reproducible with coefficients of variation of well below 10% especially when the internal standard is added,
Fig. 1. GC gas chromatograms. (A) Separation of authentic DMF, DMA, MMF and MMA on a 60 m-long DB-1701 column, (B) Separation of DMF, DMA, MMF and MMA in the urine from a DMF+DMA-exposed worker on a 60 m-long DB-1701 column, (C) No separation of the four compounds in the urine on a 30 m-long DB-WAX column, and (D) Difficulty in separating a small peak and that of DMF on a 30 m-long DB-1701 column when the urine sample was analyzed. Fig. 1 (B), (C) and (D) show the results of the analyses of the same urine sample from a DMF+DMA-exposed worker. Concentrations of DMF, MMF, DMA and MMA in Fig. 1 (A) were 4.66 mg/l (64 µmoles/l), 10.06 mg/l (170 µmoles/l), 4.61 mg/l (52.9 µmoles/l) and 9.48 mg/l (129.7 µmoles/l), respectively, and those in Fig. 1 (B, C and D) were 7.1 mg/l (97.1 µmoles/l), 44.3 mg/l (749.6 µmoles/l), 1.2 mg/l (13.8 µmoles/l) and 10.1 mg/l (138.2 µmoles/l), respectively.

and that the recovery from urine samples is sufficient (i.e., 91 to 104% when the internal standard is added).

Application of the established method for monitoring of combined exposure of rats to DMF and DMA

Female Wistar rats, eight animals per group, were given i.p. either 1.37 mmoles DMF/kg body weight (diluted with corn oil to a volume of 1 ml/kg; the DMF group), 1.15 mmoles DMA/kg body weight (ibid.; the DMA group), or the combination (ibid.; the DMF+DMA group). Two animals were housed together in a metabolic cage to obtain a sufficient amount (i.e., 10 ml or more) of urine, and accordingly, four urine samples were obtained per group.

Fig. 2. Changes in the amount of MMF and MMA detected as a function of the injection port temperature. Each spot shows one determination, and the lines connect the means. The maximum amounts are taken as 100%. Circles connected with solid lines show MMF formation, and triangles with broken lines show MMA formation.
The results of the analysis of urine samples collected over a 24-h period are summarized in Table 2. The results showed that it was possible to determine MMF and MMA separately, in addition to DMF and DMA when DMF and DMA were given in combination (i.e., the DMF+DMA group). It was probable that the metabolic interaction between DMF and DMA was not extensive under the experimental conditions employed, because there were no significant (p>0.05) changes in DMF and DMA excretion after combined administration as compared with single administration, and DMF excretion did not change either. Only MMA excretion appeared to be reduced, but this observation apparently needs further study for confirmation because the number of determinations in the experiment was still small.

### Table 1. Accuracy and precision of the method

<table>
<thead>
<tr>
<th>Item</th>
<th>Conc.</th>
<th>CV (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMF/IS</td>
<td>127</td>
<td>1.6</td>
<td>95.5</td>
</tr>
<tr>
<td>MMF/IS</td>
<td>170</td>
<td>5.2</td>
<td>98.2</td>
</tr>
<tr>
<td>DMA/IS</td>
<td>106</td>
<td>1.3</td>
<td>102.6</td>
</tr>
<tr>
<td>MMA/IS</td>
<td>130</td>
<td>4.2</td>
<td>103.8</td>
</tr>
</tbody>
</table>

Water or urine with DMF, MMF, DMA or MMA was analyzed in triplicate. a Final concentration of DMF, MMF, DMA or MMA in µmoles/l. b Coefficients of variation (i.e., standard deviation divided by mean; in %). c The mean for the spiked urine samples divided by the mean for the spiked water samples (in %). d Urine samples from three different nonexposed persons were utilized. e Determined in reference to an internal standard.

### Table 2. Amounts of DMF, DMA, MMF and MMA in the urine of rats given DMF and DMA either separately or in combination

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose a</th>
<th>BMF, DMA, MMF or MMA in urine b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DMF</td>
</tr>
<tr>
<td>DMF</td>
<td>1.37 µmoles/kg b.w.</td>
<td>81.7 ± 12.4</td>
</tr>
<tr>
<td>DMA</td>
<td>1.15 µmoles/kg b.w.</td>
<td>88.1 ± 28.9</td>
</tr>
<tr>
<td>DMF+DMA</td>
<td>1.37 µmoles/kg b.w.</td>
<td>75.8 ± 14.6</td>
</tr>
</tbody>
</table>

Urine excreted in 24 h after the injection was collected. Values in the table are mean ± SD (3 determinations) in µmoles/kg b.w. The asterisk (*) indicates that the difference from MMA in the urine of the DMA group is statistically significant (p<0.05). a Diluted with corn oil (to a volume of 1 ml/kg b.w.) and given i.p. b In the urine collected over a 24-h period.

**Fig. 3.** Correlation of DMF with MMF. The calculated regression line is $Y=10.1 + 0.020X$ ($r=0.647$, $n=27$, $p<0.01$), where $X$ is MMF and $Y$ is DMF.

**Fig. 4.** Correlation of DMA with MMA. The calculated regression line is $Y=3.7 + 0.017X$ ($r=0.777$, $n=27$, $p<0.01$), where $X$ is MMA and $Y$ is DMA.
of MMF correlated significantly (p<0.01) with that of DMF, and that the MMA level also showed significant correlation (p<0.01) with that of DMA, although the variation around each regression line was wide in either case. From a quantitative viewpoint, the ratio of DMF to MMF and that of DMA to MMA were essentially the same, i.e., 0.02 to 0.03, suggesting that much less parent compounds are excreted in urine than the corresponding metabolites under occupational exposure conditions.

Discussion

GC conditions are established in the present study so that two homologous solvents of MMF and DMA, and also their respective metabolites of MMF and MMA in urine can be measured simultaneously. It is now possible to make separate determination of urine for MMF and MMA even when the workers are exposed to a combination of DMF and DMA. It was further made clear that the same analytical system allows the detection of the two parent compounds, DMF and DMA, both of which are also excreted in urine at measurable levels. Thus, DMF and DMA are among the solvents, for which urinalysis for the solvent per se is a promising tool for biological exposure monitoring. Data on the intensity of exposure of the workers to DMF and DMA were unfortunately not available in the present study. The urinary MMF concentrations (up to about 900 µmoles/l or 66 mg MMF/l; Fig. 3) indicate that the best estimate for DMF in breathing zone air (if no skin absorption is considered) will be about 40 ppm as the maximum, according to the exposure - excretion relation previously established. A similar estimation from urinary MMA (up to 1,000 µmoles/l or 78 mg MMA/l) suggests that DMA in air may be equal to or slightly higher than that of DMF, although this estimation might not be precise due to formation of metabolite(s) other than monohydroxymethyl derivatives.

The animal study with i.p. injection of DMF and DMA into rats (Table 2), although small in scale, suggested that the metabolism of DMF and DMA will not interact with each other at least at low doses. Independent metabolism between homologous solvents of toluene and xylenes as well as between heterogenous combination of toluene and isopropyl alcohol, or toluene and methyl ethyl ketone has been previously described.

It is desirable to examine quantitative relationship of DMF, DMA, MMF and MMA in urine with DMF and DMA in breathing zone air. In this connection, it was also unfortunate that no occupational hygiene information was available to evaluate the possibility that the workers had skin contact with DMF or DMA which are known to penetrate through intact skin. Nevertheless, the observation that, in urine, DMF and DMA correlate with MMF and MMA, respectively (Figs. 3 and 4), suggests that DMF and DMA, MMF and MMA are all promising candidates for biological exposure monitoring. In fact, it is known that DMF and MMF in urine correlate significantly (p<0.01) with time-weighted average intensity of exposure to DMF vapor.

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