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# Simultaneous determination of two human urinary metabolites of *N*,*N*-dimethylformamide using gas chromatography–thermionic sensitive detection with mass spectrometric confirmation

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**Abstract**

Two human urinary metabolites of the industrial solvent *N*,*N*-dimethylformamide (DMF), *N*-hydroxymethyl-*N*-methylformamide (HMMF) and *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine (AMCC), were assayed using a new analytical method (gas chromatography and thermionic sensitive detection). Clean-up of urine samples includes a liquid–liquid extraction step followed by a solid-phase extraction step to separate HMMF and AMCC from other urine components. During clean-up, AMCC is converted into ethyl-*N*-methylcarbamate (EMC), and during gas chromatography, HMMF is degraded in the injector to *N*-methylformamide (NMF). All the validation data necessary for a quantitative procedure are given. The method was applied to urine samples from workers exposed to DMF and from the general population. The results were confirmed by mass spectrometric determination. For this purpose a further liquid–liquid extraction step was introduced in the clean-up procedure. Background levels of AMCC in the general population were identified. © 1999 Elsevier Science B.V. All rights reserved.

*Keywords:* *N*,*N*-Dimethylformamide; *N*-Hydroxymethyl-*N*-methylformamide; *N*-Acetyl-*S*-(*N*-methylcarbamoyl)cysteine

## 1. Introduction

*N*,*N*-Dimethylformamide (DMF) is an important industrial chemical world-wide. In 1989, about 250,000 tons were produced [1,2]. It is commonly used for the manufacture of films, fibres and coatings and for making polyurethane laquers for clothing and accessories made of synthetic leather. The main primary metabolite of DMF is *N*-(hydroxymethyl)-*N*-methylformamide (HMMF) after hydroxylation of one of its methyl moieties by cytochrome P450 CYP2E1 [3,4]. Only small urinary amounts of *N*-methylformamide (NMF) are found [5]. However, during analysis using gas chromatography (GC) HMMF undergoes thermolytic degradation to formaldehyde and NMF [6,7]. As a consequence the total amount of HMMF and NMF is determined by GC analysis in the form of NMF [8]. However, the most important secondary metabolite of DMF in humans is *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine (AMCC)

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[9,10]. The half-life of AMCC in humans is assumed to be about 23 h, while the half-life of HMMF is approx. 2 h. The characterisation of AMCC as a major metabolite of DMF in humans was a great step forward in understanding the mechanism by which DMF exhibits its toxicity. However, AMCC and other *N*-methylcarbamoylated species were also identified in animals and humans as metabolites of methyl isocyanate (MIC) [11,12], the chemical which caused the Bhopal disaster [13]. Therefore, MIC is assumed to be the reactive intermediate in the metabolism of DMF [14,15] (Fig. 1) and MIC may also be responsible for the hepatotoxicity of DMF [16]. Consequently, the American Conference of Governmental Industrial Hygienists (ACGIH) established a new biological exposure index (BEI) for AMCC several months ago for the first time (40 mg/l) [17], although AMCC is not yet commercially available. For biological monitoring of exposure to DMF, only the determination of urinary levels of NMF is commonly used. This parameter is recommended by the Deutsche Forschungsgemeinschaft (DFG) in Germany and also by the ACGIH in the United States for the surveillance of workers exposed to DMF in industry [18]. The biological tolerance value (BAT) for NMF in Germany and also the BEI in the United States is 15 mg/l.[1] The analytical methods used for the determination of NMF, however, have several disadvantages with regard to sample preparation and clean-up procedures [19]. Moreover, none of these methods are able to determine NMF and AMCC in one analytical run. The above facts have created a substantial need for a valuable, sensitive, and specific method for the simultaneous analysis of NMF and AMCC in urine samples of persons exposed to DMF. Therefore, we developed such a method for the simultaneous determination of NMF and ethyl-*N*-methylcarbamate (EMC). EMC is formed as a degradation product of AMCC during our clean-up procedure.[2] Dimethylpropionic acid amide (DMPA) is used for internal standardisation. Separation and detection is accomplished by GC–TSD and GC–MS.

[1]The BEI in the United States was changed in 1999 from 40 mg NMF/g creatinine in urine samples to 15 mg/l.

[2]Whenever NMF and AMCC are mentioned in this article, please keep in mind that HMMF and AMCC are measured. However, NMF and EMC are detected.

## 2. Experimental

### 2.1. Instrumentation

GC–TSD analysis was carried out on a Varian 3400 gas chromatograph fitted with a temperature-programmable 1075 split/splitless capillary injector, a thermionic sensitive detector (TSD), and a programmable 8200 CX autosampler. Split injection with a split rate of 20 ml/min was used. The carrier gas was helium at 20 p.s.i. (1 p.s.i.=6890 Pa). Nitrogen was used as makeup gas. The injector and the TSD were maintained at 280°C. For separation of the analytes a DX-4 column (15% dimethylpolysiloxane, 85% polyethylene glycol), 60 m in length with an inner diameter of 0.25 mm and a phase film thickness of 0.25 μm, was used (J&W Scientific, Folsom, USA). One microlitre of the sample was injected. The column temperature was 100°C for 10 min, then raised to 140°C at 3°C/min and then to 240°C at 25°C/min. This temperature was held for 15 min. Retention times ($t_R$): DMPA, 15.1±0.2 min; EMC, 16.2±0.3 min; NMF, 22.3±0.3 min.

GC–MS was performed on a gas chromatograph HP 5890 Series II fitted with a mass selective detector HP 5972 and a split/splitless injector system HP 7673 operating in the splitless mode (Hewlett-Packard, Waldbronn, Germany). The inlet purge off time was set to 1.0 min. The electron energy was 70 eV and the electron multiplier voltage 2400 V. The detector and injector temperature was 280°C. Samples analysed by GC–MS were injected (1 μl) on a capillary column with the stationary phase HP-Innowax (100% polyethylene glycol, Hewlett-Packard). The column was 60 m in length with an inner diameter of 0.32 mm and a film thickness of 0.25 μm. Helium was used as carrier gas with a constant flow of 1.2 ml/min. The initial column temperature of 80°C was held for 15 min and then raised to 100°C at 20°C/min. This temperature was held for 11 min and then raised to 120°C at 20°C/min. After 5 min, the temperature was raised again to 240°C at 25°C/min. The final temperature was held for 15 min. For identifying EMC and NMF in human urine samples, multiple-ion detection (MID) of two masses was carried out. Using GC–MS–EI–MID it was possible to confirm the GC–TSD results and to identify EMC and NMF with certainty. Retention

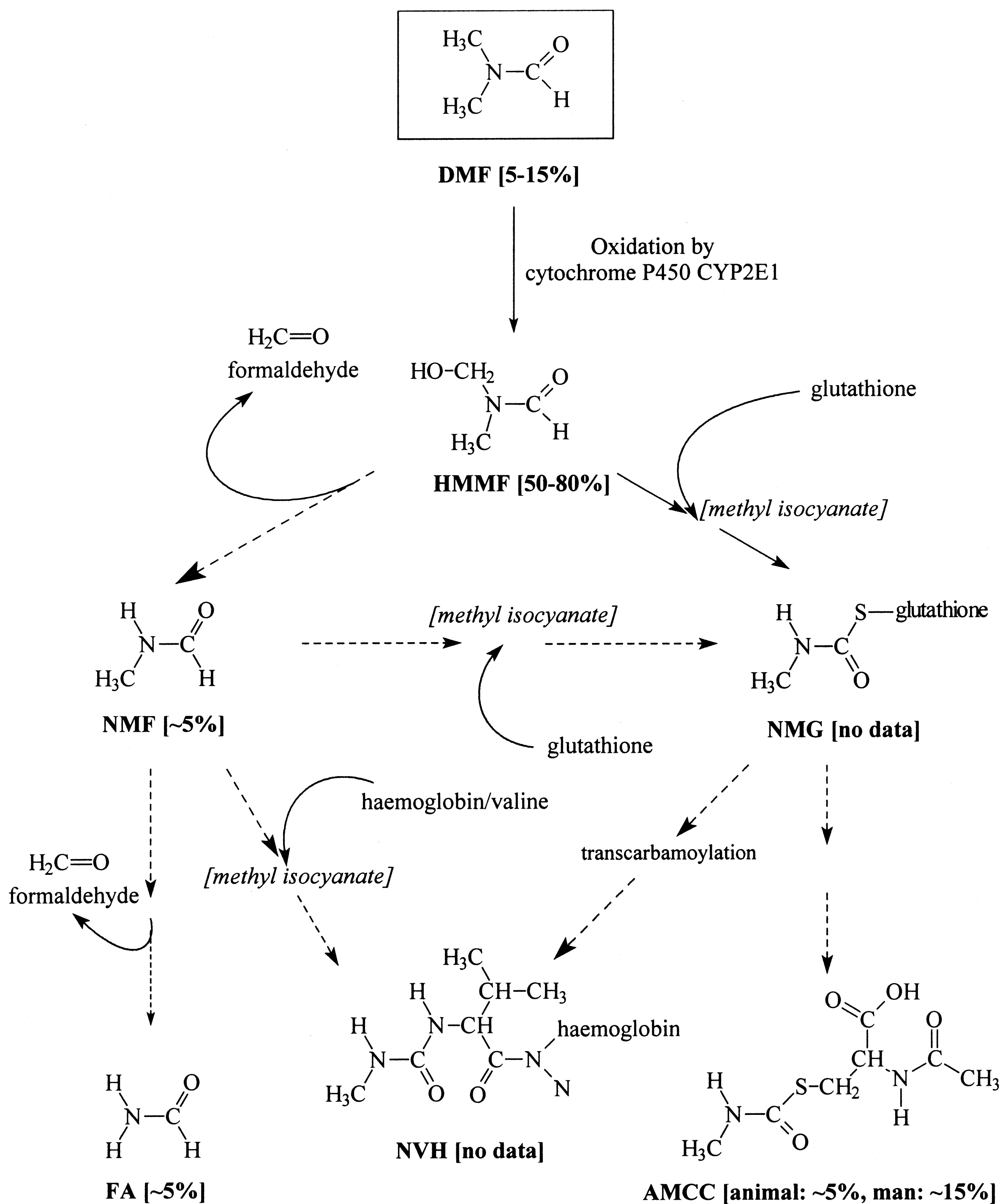


Fig. 1. Metabolism of DMF. The amounts of metabolites are also shown. Abbreviations: DMF, *N*,*N*-dimethylformamide; HMMF, *N*-hydroxymethyl-*N*-methylformamide; NMF, *N*-methylformamide; NMG, *N*-methylcarbamoylglutathione; FA, formamide; NVH, *N*-methylcarbamoyl valine haemoglobin; AMCC, *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine.

times ($t_R$) and monitored ions are as follows: DMPA, 19.8±0.2 min, $m/z$ 101, 72 [ion ratio, 53:47 (±2%)]; EMC, 22.3±0.2 min, $m/z$ 103, 75 [ion ratio, 37:63 (±2%)]; NMF, 31.6±0.3 min, $m/z$ 59, 30 [ion ratio, 61:39 (±3%)].

### 2.2. Reagents

*N*,*N*-Dimethylpropionic acid amide (DMPA) was provided by Fluka (Deisenhofen, Germany). *N*-Methylformamide (NMF) was obtained from Sigma (St. Louis, MO, USA). Ethyl-*N*-methylcarbamate (EMC) was obtained from ICT Tokyo Kasei Chemicals (Tokyo, Japan), while the cation-exchange resin AG® 50W-X8 (100–200 mesh hydrogen form) was provided by BIO-RAD laboratories (Richmond, VA, USA). *N*-Acetyl-*S*-(*N*-methylcarbamoyl)cysteine (AMCC) was synthesised at our institute. All other chemicals used for clean-up of the samples were of analytical grade, normally found in a laboratory, and were obtained from Merck (Darmstadt, Germany).

### 2.3. Synthesis of N-acetyl-S-(N-methylcarbamoyl)cysteine

AMCC was synthesised by the reaction of *N*-acetylcysteine (ACC) and methyl isocyanate (MIC). For this, a solution of ACC (1.2 mmol) in water (5 ml) is prepared and the pH is adjusted to about 7 using 0.1 *M* sodium peroxide solution. MIC (2.4 mmol) in acetone (4.0 ml) is added to this solution. The mixture was stirred at room temperature for about 2 h and the AMCC extracted with 2×20 ml ethyl acetate. The reaction solution was concentrated using a vacuum evaporator and purified by repeated recrystallisation in EtOH. This gave the desired product in 78% yield and >95% purity. MIC itself was synthesised by the reaction of sodium azide and acetyl chloride in the presence of a phase-transfer catalyst, as presented elsewhere in detail [20]. Several precautions have to be taken if MIC is handled in the laboratory. These can be found in detail in the Hazardous Substance Data Base of the EPA.[3] Spectroscopic data: $^{1}$H-NMR (400 MHz, $D_2O$): δ 2.01 (s, 3 H, COC***H₃***), δ 2.76 (s, 3 H, NHC***H₃***), δ 3.22 (dd, 1 H, Cys-β-C***H₂***), δ 3.45 (dd, 1 H, Cys-β-C***H₂***) and δ 4.59 (dd, 1 H, Cys-α-C***H***). $^{13}$C-NMR (100 MHz, $D_2O$): δ 21.9 (CO***C***$H_3$), δ 27.6 (Cys-***C***$H_2$), δ 30.7 (NH***C***$H_3$), δ 53.4 (Cys-***C***H), δ 169.3 (S***C***O), δ 173.8 (***C***$O_2$H), and δ 174.2 (***C***O$CH_3$). FT-IR (KBr, $cm^{-1}$): 3309.8, 2940.9, 2447.8, 1712.2, 1668.5, and 1555.3. MS–EI (70 eV): $m/z$ 43, 60, 76, 86, and 163. FAB–MS ($Cs^+$, 16 keV): $m/z$ 221 ($M+H^+$), 353 ($M+Cs^+$).

[3]Available free on the Internet (National Library of Medicine, National Institute of Health: http://toxnet.nlm.nih.gov/).

### 2.4. Preparation of standard solutions

Calibration standard solutions of AMCC and NMF were prepared in pooled urine of non-exposed persons with concentrations ranging from 1.0 to 100.0 mg/l. A solution of the internal standard DMPA was obtained by diluting 10 mg DMPA (10.9 μl) with bidistilled water in a 100 ml glass volumetric flask ($c = 100$ mg/l). The solutions described above were only used for standardisation and calibration of the method. A 25% hydrochloric acid (HCl) solution (128 ml) is diluted to the mark in a 1 l volumetric flask for preparing 1 *M* HCl. All solutions described above are stable at −18°C for more than 6 months.

### 2.5. Sample preparation and analysis

Urine samples ($N = 184$) from 23 workers exposed to DMF were collected pre- and post-shift on four consecutive days. Twenty-four-hour urine samples of 42 non-exposed persons were also collected. The urine was stored in sealable plastic bottles and kept in a deep freezer until processing. In this way the urine can be stored for several years. For NMF and AMCC analysis a 20 ml vial is prepared with 2.5 g solid NaCl. Then, 5 ml of the urine sample, 500 μl 1 *M* HCl, and 1 ml internal standard are added. The sample is sealed and shaken for about 30 s (there must be a residue of NaCl on the bottom). The urine is then extracted twice using 5 ml tetrahydrofuran (THF). For this purpose, the sample is shaken for 10 min and centrifuged for 5 min at 2600 *g*. The organic phase is transferred to a new vial containing 1.0 g cation-exchange resin and is shaken gently for about 1 h at room temperature. After decanting the solvent

the resin is washed using a further 3 ml THF. The combined organic solvents are transferred to a new vial and evaporated carefully to about 500 μl under a stream of nitrogen. It is noteworthy that the sample is not allowed to dry. Heating is not allowed during evaporation. Two millilitres of EtOH are added. A new 5 ml vial is filled with 1.5 g anhydrous potassium carbonate ($K_2CO_3$). The ethanolic solution is now added to the $K_2CO_3$ in a single step. The sample must be shaken immediately for about 15 s. During this workup step AMCC is converted into EMC. After centrifugation at 2600 *g*, 1 μl of the ethanolic supernatant is injected into the GC–TSD system.

For the GC–MS–EI–MID procedure, 1 ml of the ethanolic supernatant is mixed with 3 ml bidistilled water and extracted twice using 5 ml methylenechloride ($CH_2Cl_2$). For this procedure, the samples are shaken for 10 min and centrifuged for 5 min. Combined organic phases are added to sufficient anhydrous sodium sulphate ($Na_2SO_4$) and the samples are dried for 15 min. The $CH_2Cl_2$ is transferred to a new vial and the $Na_2SO_4$ is washed with a further 3 ml $CH_2Cl_2$. The combined organic phases are evaporated under nitrogen to about 100 μl. After transferring to a micro insert and addition of 50 μl EtOH the samples are evaporated to a final volume of 50 μl. One microlitre is injected and analysed by GC–MS–EI–MID. The complete sample preparation scheme is shown in Fig. 2.

### *2.6. Optimising clean-up conditions*

In order to optimise the clean-up conditions and to assess the quality of several single steps during clean-up, various solutions in pooled urine were prepared. These solutions were not used for standardisation or calibration of our analytical method.

1. A standard solution was prepared in pooled urine to determine the extraction rates for AMCC, NMF, and DMPA using diethylether. The concentration of each analyte was 50 mg/l. Different conditions were used (saturation with NaCl, addition of HCl, a combination of both).
2. After examining the best starting conditions for clean-up several polar and non-polar solvents were also used to optimise the extraction step (aliphatic and aromatic and also chlorinated hydrocarbons, different ethers and ketones, ethyl acetate, acetonitrile, etc.). Double experiments were carried out.
3. Several batch methods using different solid-phase extractions (SPE) were performed for further clean-up of the samples. For this purpose, a cation-exchange resin, an anion-exchange resin, a 20%-deactivated silica gel, a $C_{18}$ reversed phase, and a combination of a cation and an anion resin in series were examined for their sample cleaning properties.
4. To determine the yield of EMC by the reaction of AMCC with EtOH, AMCC in EtOH was converted to EMC in the presence of THF and $K_2CO_3$ in one single step. The samples were shaken immediately. The reaction was observed 10 times at two different concentrations (250 and 25 mg/l).
5. The rate of conversion of AMCC to EMC was also examined as a function of different amounts of $K_2CO_3$ (0.25–2.5 g). Experiments were carried out five times for each amount of $K_2CO_3$.

### *2.7. Calibration process, calculation of the analytical result and quality control*

For calibration, standard solutions of AMCC and NMF (1.0–100.0 mg/l) in pooled urine from persons non-exposed to DMF were analysed as described. The quotient of the areas under the curve (AUC) of EMC and NMF versus the AUC of the internal standard was plotted as a function of concentration. These calibration curves were used for the calculation of the AMCC and NMF content of each urine sample. The concentrations are given in mg/l urine. As our calibration standards contained AMCC and not EMC, it is not necessary to calculate the AMCC content on the basis of EMC measures.

At present, no quality control material is commercially available. Therefore, one spiked pooled urine sample with AMCC and NMF was used as quality control material. The concentrations were 40 and 30 mg/l urine, respectively. The sample was divided into aliquots and stored at −18°C.

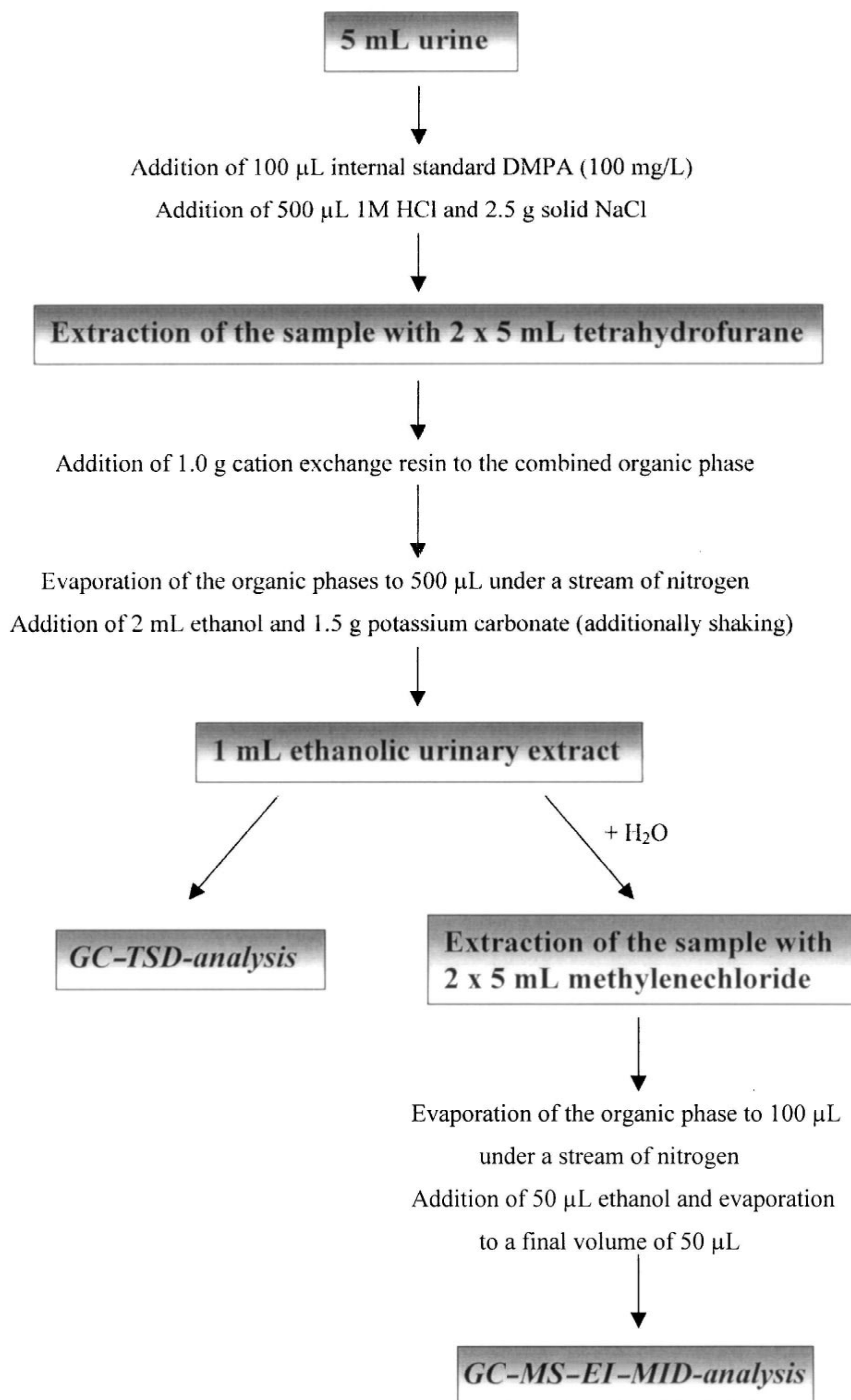


Fig. 2. Sample preparation for the simultaneous determination of HMMF and AMCC in urine samples by GC–TSD and GC–MS–EI–MID.

### 2.8. Reliability of the developed method

The reliability of the method was demonstrated in detail as it is recommended by the DFG in Germany. In order to determine the imprecision from day to day (BDI) the above-described quality control urine samples were used. Five samples were analysed on five different days together with a complete calibration curve and the BDI was calculated. Recovery experiments were carried out in order to test the accuracy of the method. For this purpose, pooled urine samples spiked with 10.0, 25.0 and 50.0 mg AMCC or NMF per litre urine were prepared and processed as described. The results were calculated using a calibration curve which was prepared from a different pooled urine and which was also analysed as described above. Please note that, using this procedure, all the losses during sample preparation are compensated for ('relative recovery'). However, the losses during sample preparation were also determined by processing the same urine samples eight times as described. The results were compared with standard solutions which were analysed after addition of the internal standard, but without further sample processing. Therefore, the 'absolute recovery' can be calculated (100% minus losses during sample preparation).[4] The limits of detection (LOD) for AMCC and NMF were calculated from a signal-to-noise ratio of 3:1. In each analytical series, one calibration standard, one blank water sample (reagent blank), and one quality control sample were included.

## 3. Results and discussion

For the quantitative analysis of NMF in persons exposed to DMF several clean-up procedures and determination methods have been described [18,21–30]. However, in some of these reports urinary NMF concentrations were underestimated because of the analytical conditions used [21,29,30]. The injection temperatures for GC analysis were maintained lower than 250°C. These temperatures were not sufficient to convert HMMF to NMF completely. However, none of the methods given above allows the determination of AMCC and NMF in one analytical run. For the simultaneous determination of NMF and AMCC, only one method was traced in the literature [31]. One millilitre of urine is diluted with a threefold amount of water and treated with 2 ml EtOH and 1.5 g $K_2CO_3$ to produce EMC, which is extracted in EtOH and measured by GC and nitrogen–phosphorus detection (GC–NPD). No further clean-up of the samples was performed to separate interfering urine components. The LOD for both analytes is given as 1.0 mg/l. The method was used successfully to identify AMCC and NMF in urine samples of persons exposed to DMF and was a step forward in investigating DMF exposure. It was also used by Sakai et al. to determine AMCC in exposed workers [32], while Casal-Lareo et al. used a different clean-up procedure [33]. The latter method was only capable of detecting AMCC and not NMF, however. There, 1 ml urine is extracted with diethylether after addition of HCl and solid NaCl. The extracts are dehydrated and evaporated under nitrogen. AMCC is silylated by adding 100 μl of a derivatising agent [*N*,*O*-bis(trimethylsilyl)trifluoroacetamide + 1% trimethyl-chlorosilane] and analysed by GC–MS. The samples are injected into the GC without further clean-up. The LOD for this method is also given as 1 mg/l. The methods described above do not include every aspect of relevance for a method used for occupational health surveillance, e.g. their accuracy was not demonstrated in detail, and no standard operating procedures for the analysis and no purity data for the standards (especially AMCC) were described.

### 3.1. Synthesis of AMCC

We synthesised AMCC in our laboratory and checked its purity and identity using several analytical methods (see Experimental section). The synthesis of AMCC has already been reported by Kestell et al. [34], Mráz [31] and Slatter et al. [35]. However, the first two methods are very time-con-

[4] It is important to know that, in Germany, there is a distinction between 'relative recovery' and 'absolute recovery' by definition, while this is not the case in the US. If the 'recovery' is determined in the US to be 80%, consequently the losses must be 20%. This is not necessarily the case in Germany. However, it is beyond the scope of this article to discuss the pros and cons of the different definitions.

suming, while the latter is simple, fast, and results in good yields. The synthesis presented in this article is similar to the method of Slatter et al. and the synthesis for the corresponding methyl isothiocyanate derivative described by Valentine et al. [36].

### 3.2. Extraction of AMCC and NMF

The extraction properties of AMCC, NMF and DMPA were examined as described in the Experimental section. First, diethylether was used under different conditions. This solvent has already been applied in the extraction of AMCC [22]. AMCC and NMF are extracted only if the urine sample is saturated with NaCl and if 1 *M* HCl is added. Nevertheless, AMCC was very badly extracted using diethylether. This may be explained by the low polarity of the solvent. For the analysis of other mercapturic acids, such as *S*-phenylmercapturic acid, ethyl acetate was shown to be more appropriate [18,37,38]. Nevertheless, ethyl acetate was also shown not to be sufficiently effective for AMCC. NaCl saturation permits us to use organic solvents for extraction, which would be soluble in urine under regular conditions. THF turned out to be the only solvent which extracts AMCC, NMF, and DMPA in high amounts from the biological matrix, e.g. it extracts AMCC 20 times better than diethylether.

### 3.3. Batch SPE clean-up

THF is a very polar solvent. Consequently, the use of this solvent results in a higher analytical background, which must be reduced using further clean-up steps. Several SPE materials were investigated using batch methods. Only a cation-exchange resin resulted in further clean-up and reproducible AMCC and NMF values. Therefore, increasing sensitivity is achieved by increasing the analyte/background peak ratio. After SPE and transfer of the solvent to a new vial, THF is evaporated under a stream of nitrogen. Heating should be avoided and the samples should not be allowed to dry out in order to avoid losses of the volatile DMPA and NMF.

### 3.4. Conversion of AMCC to EMC

As AMCC is a mercapturic acid it cannot be injected underivatised into the GC system. Silylation used by Casal-Lareo et al. is a matter of some controversy if the method is used in routine analysis. Therefore, we wanted to avoid such a derivatization procedure, although modern silylation reagents [e.g., *N*-methyl-*N*-(trimethylsilyl)-2,2,2-trifluoroacetamide] are not reported to influence MS.[5] Preliminary experiments for methylation using diazomethane and boron trifluoride methanol solution did not result in high yields (about 60 or 45% yield in diethylether, respectively). As urine samples are used for the investigation of exposed persons, this yield is assumed to decrease under regular work-up conditions. However, diazomethane is carcinogenic and should also be avoided as far as possible. Therefore, we decided to use the conversion of AMCC to EMC in the presence of EtOH and $K_2CO_3$, as already described by Mráz [31]. The reliability and accuracy of this conversion have been demonstrated in detail. The yield of EMC seems to underlie great imprecision, because no EMC will be found if the samples are not shaken immediately after addition of the ethanolic solution to $K_2CO_3$. It was found that the conversion yield decreases with decreasing $K_2CO_3$ and was not observed at the lowest amount of $K_2CO_3$ used in our experiments (0.25 g). The maximum rate of conversion was observed at concentrations of $\geq$2.0 g $K_2CO_3$. Because of the practicability of the method, 1.5 g $K_2CO_3$ turned out to be the maximum amount. The yield of the conversion of AMCC to EMC under the conditions described above ranged between 55.1 and 66.8% (mean 58.7%) using a concentration of 250 mg/l AMCC, while it was between 50.4 and 63.8% (mean 56.9%) at 25 mg/l. The standard deviation of the conversion was 9.8%, thus contributing a great part to the imprecision of the method. Mráz reported a mean conversion of about 64% [31].

### 3.5. Reliability of the method

The linearity of the calibration curves and possible differences between the calibration curves using

[5] Such reagents were not tested in our preliminary experiments because we wanted to confirm our results by GC–MS. Over the last few years our experience has shown that silylation greatly influences the detection of mass fragments in the low-mass range (up to $m/z$ 100, however for NMF it was necessary to detect $m/z$ 59 and $m/z$ 30!).

pooled urine or water were investigated. Both calibration curves were linear in the range 1.0 to 100.0 mg/l for the analytes AMCC and NMF. No differences in the slopes of calibration curves could be observed after studying five calibration curves for pooled urine and also for water. This shows that, due to our clean-up procedure, there are no observable matrix effects which may influence the accuracy of the results. Although there would be no necessity to use urinary calibration standards, it was observed that evaporation of THF extracts under nitrogen is easier using urine standard material (probably due to the lower water content of THF).

We determined a BDI of 10.8% for AMCC at a concentration of 40 mg/l (39.74±4.31 mg/l) analysing five urine samples spiked with AMCC. During the analysis of the urine samples of persons exposed to DMF one calibration standard and one quality control were included in each analytical series. The BDI during the analysis of 184 urine samples of DMF-exposed workers and 42 control individuals using the GC–TSD method is shown in Fig. 3 for AMCC. Please note that the quality control samples never exceeded twice the standard deviation over a period of about 2 months. The BDI over the same period for NMF at a concentration of 30 mg/l was found to be 6.9% (29.06±2.01 mg/l, not shown). This indicates the robustness of the developed method and therefore the method should be suitable for the analysis of AMCC and NMF in one chromatographic run achieving reproducible values. However, while the imprecision is low for NMF, it is higher for AMCC. This is mainly caused by the conversion of AMCC to EMC described above, where optimum conditions cannot be achieved.

The mean losses during sample treatment at three different concentrations were between 59.7 and 64.8% for AMCC and between 21.5 and 29.2% for NMF, dependent on the concentration. The losses during sample preparation are compensated for using our calibration procedure. This is evidenced by the determination of the relative recovery of the analytes. The mean values were in the range between 81.3 and 88.7% for AMCC and between 94.6 and 99.3% for NMF. Detailed values are presented in Table 1. Under the analytical conditions described, the LOD is 0.5 mg/l urine for AMCC and 1.0 mg/l for NMF. These LODs are sufficient for the bio-

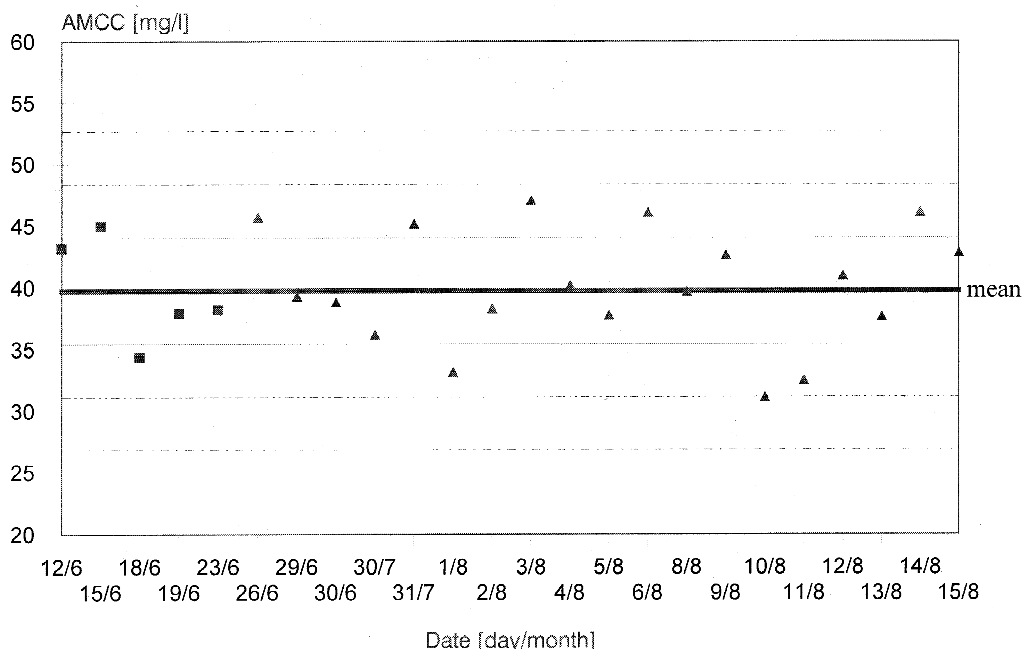


Fig. 3. Internal quality control using pooled urine spiked with 40 mg/L AMCC. (■) Pre-series sample; (▲) quality samples analysed within a series of population samples; the first, second, and third standard deviations are given.

Table 1
Losses during sample preparation and (relative) recovery of AMCC and NMF during clean-up of urinary samples using GC–TSD analysis

| Conc. (mg/L) | Losses (%) ($N=8$) | | Recovery (%) ($N=8$) | |
|---|---|---|---|---|
| | AMCC | Total NMF | AMCC | Total NMF |
| 10 | 63.3 | 29.2 | 81.3 | 99.3 |
| 25 | 64.8 | 21.5 | 81.4 | 94.6 |
| 50 | 59.7 | 21.5 | 88.7 | 96.7 |

logical monitoring of DMF-exposed persons. No AMCC or NMF could be traced in control individuals using the GC–TSD method.

### 3.6. GC–TSD analysis

A GC–TSD chromatogram of a urine sample of one worker exposed to DMF in the polyacrylic fibre industry is shown in Fig. 4. As can be seen, there is an interfering peak of an unknown substance next to NMF, which is not completely separated. However, using a temperature program with slow ramp rates, a suitable column, and helium as carrier gas we were able to determine that this peak is not coeluting with NMF. The still unidentified substance appears in about 70% of urine samples analysed from exposed and control individuals. It was not possible to identify the substance by MS, because the concentration for a full-scan mode was too low. We found higher NMF values compared with our results, especially in the lower concentration range, if we used other methods recommended for the determination of NMF. Possibly, these methods are not able to separate this interfering peak. About 20 urine samples can be analysed during 1 day using our sample preparation and analytical procedure. The efficiency of the developed method is due to a simple liquid–liquid extraction step followed by a SPE batch method to separate AMCC and HMMF from the biological matrix. Moreover, the analytical background is reduced, because in most cases for biological monitoring of NMF urine sample mixtures were injected into the GC system using no extraction. Furthermore, the whole GC system is treated with care using our procedure. The use of

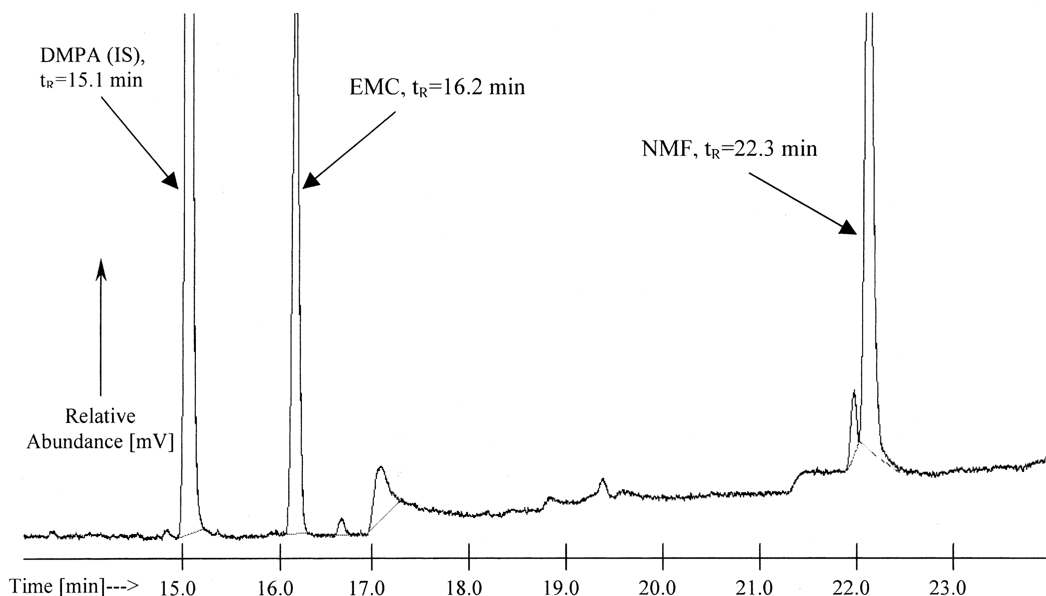


Fig. 4. GC–TSD chromatogram of a urine sample from a person exposed to DMF in the polyacrylic fibre industry. 34.4 mg/L AMCC (EMC, $t_R = 16.2$ min) and 25.1 mg/L NMF ($t_R = 22.3$ min) were found. In order to present a worst-case chromatogram concerning NMF analysis, a chromatogram with a peak of a still unknown substance is shown (see discussion in the text); internal standard DMPA: $t_R = 15.1$ min.

helium as carrier gas increases the separation efficiency of the method in contrast to nitrogen. Split injection and higher column temperatures result in better peak shape and higher signal-to-noise ratio than achieved using splitless injection. TSD detection was shown to be very specific and sensitive for nitrogen-containing substances.

### 3.7. GC–MS–EI–MID analysis

We wanted to confirm our results using GC–MS, because this analytical approach shows higher specificity. Therefore, we injected the same samples analysed by GC–TSD into a GC–MS system with electron ionisation using a different column and a different temperature program. EMC and NMF in urine samples of exposed persons were identified by investigating two masses for each analyte (and their ratio) and comparing them to peaks and mass ratios obtained from EMC and NMF standard solutions in EtOH. As the adjustment of the GC–MS did not change, the retention times and percentages of the observed mass fragments also did not vary greatly (not even near the LOD; the deviations are presented in the Experimental section). However, we observed that the analytical background near the peaks of both analytes is higher using GC–MS than GC–TSD. This is mainly due to the low masses observed. Therefore, we included an additional extraction step using $CH_2Cl_2$ as described in the Experimental section. The performed liquid–liquid extraction is a proven procedure for the clean-up and determination of carbamates in ethanolic solutions [39,40]. It has already been applied to EMC, e.g. in wines [41,42]. Extraction of the samples is followed by evaporation of the organic solvent under a stream of nitrogen. This procedure simply yields a preconcentration of the sample. EtOH serves as a keeper which is added at the end during the evaporation. The LODs were determined to be 0.03 mg/l for EMC and 0.10 mg/l for NMF for two fragment ions each. If urine samples from exposed workers are analysed, a third diagnostic ion for EMC (*m/z* 103, 75, 58) can be included, while this is not possible for NMF. The masses observed for NMF are very low anyway (*m/z* 59, 30). Using this procedure, 46 out of 184 urine samples from workers exposed to DMF were also analysed by GC–MS–EI–MID. The obtained AMCC

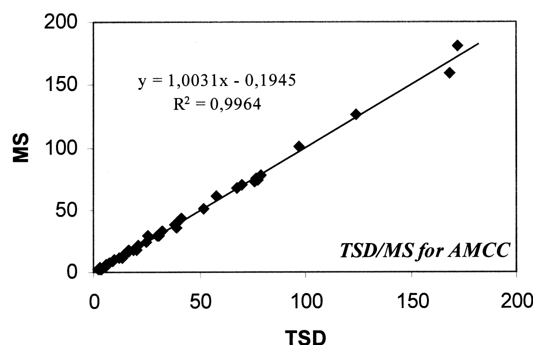


Fig. 5. Linear regression analysis between GC–TSD and GC–MS–EI–MID for the determination of AMCC in 46 urinary samples from workers exposed to DMF. The only differences between the two methods are the additional extraction step for GC–MS using $CH_2Cl_2$ and the use of different detectors; values are mg/L.

and NMF concentrations were compared with the corresponding AMCC and NMF levels obtained by GC–TSD by linear regression analysis. The levels of both analytes correlate well ($R^2 = 0.9964$ for AMCC and $R^2 = 0.9973$ for NMF). The result for AMCC is presented in Fig. 5. A GC–MS–EI–MID chromatogram obtained using this clean-up procedure from an exposed worker is shown in Fig. 6. Using this method and the additional clean-up step we were also able to determine AMCC in the general population for the first time (Fig. 7). However, no NMF could be detected. As the peak in the chromatogram is caused by EMC and not AMCC, it may be concluded that EMC and not AMCC occurs in urine samples of non-exposed persons. However, no EMC could be traced in human urine samples if the conversion step is not carried out. Precursors other than AMCC are not known for EMC.[6] Therefore, to our knowledge, the EMC peak results from AMCC in urine. A short summary of the results of the biological monitoring of DMF-exposed persons in the polyacrylic fibre industry and control individuals is given in Table 2.

[6] *N*-Methylurea is possibly present in urine samples. The theoretical formation of EMC by the conversion of *N*-methylurea in the presence of EtOH and $K_2CO_3$ was shown not to occur during our clean-up procedure.

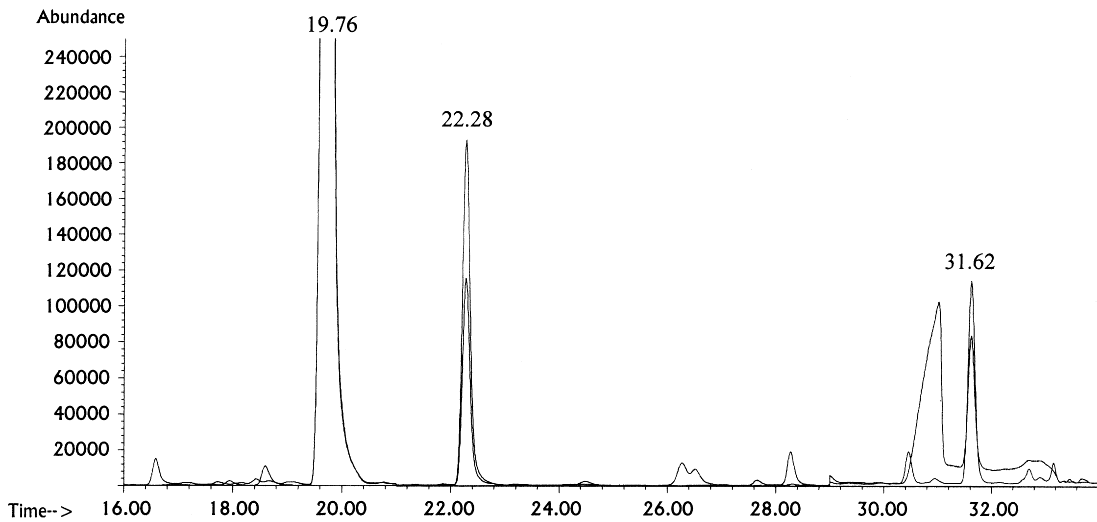


Fig. 6. GC–MS–EI–MID chromatogram of a urine sample from an exposed individual working in the polyacrylic fibre industry, containing 11.90 mg/L AMCC ($t_R$ = 22.28 min, *m/z* 103, 75) and 14.30 mg/L total NMF ($t_R$ = 31.62 min, *m/z* 59, 30). The sample was worked up using the additional extraction step with $CH_2Cl_2$; internal standard DMPA: $t_R$ = 19.76 min, *m/z* 101, 72.

## 4. Summary and prospects

As a whole the fully validated GC–TSD method for the determination of two important metabolites of DMF, HMMF and AMCC, fulfils the requirements for the surveillance of DMF-exposed individuals in occupational medicine. The results were confirmed by GC–MS.

In future, the use of HPLC–$MS^{(n)}$ will greatly influence the analysis of polar compounds such as

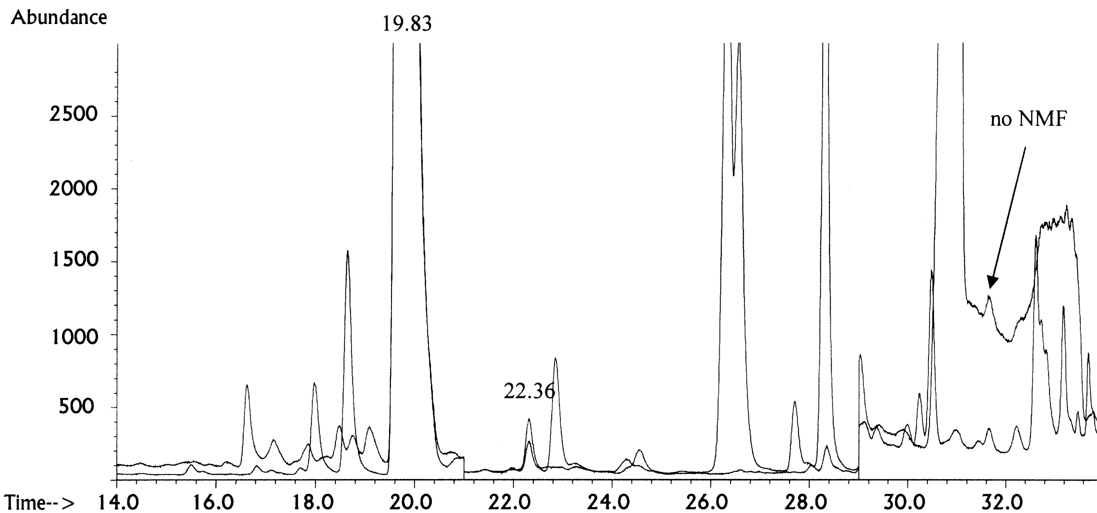


Fig. 7. GC–MS–EI–MID chromatogram of a urine sample from a non-exposed person, containing 67 μg/L AMCC ($t_R$ = 22.36 min, *m/z* 103, 75). No NMF ($t_R$ = 31.60 min, *m/z* 59, 30) could be detected, because the peak at this retention time does not have the correct ion ratios; internal standard DMPA: $t_R$ = 19.83 min, *m/z* 101, 72.

Table 2
Results of biological monitoring for NMF and AMCC in 92 pre-shift and 92 post-shift urine samples and 42 urine samples from control individuals. Mean and median concentrations are given in mg/L together with the ranges. Urine samples of exposed persons were analysed by GC–TSD, while urine samples of controls were analysed by GC–MS–EI–MID using an additional clean-up step

| Subject | Mean | Median | Range |
|---|---|---|---|
| NMF pre-shift urine (mg/L) | 2.05 | <LOD | <LOD–38.80 |
| NMF post-shift urine (mg/L) | 13.08 | 6.44 | <LOD–108.70 |
| AMCC pre-shift urine (mg/L) | 21.44 | 8.73 | <LOD–168.79 |
| AMCC post-shift urine (mg/L) | 30.31 | 12.39 | <LOD–204.90 |
| NMF control persons (mg/L) | <LOD | <LOD | <LOD |
| AMCC control persons (mg/L) | 0.040 | 0.039 | <LOD–0.078 |

AMCC. Possibly, a HPLC–MS method can greatly simplify the above-described method because no derivatization should be necessary.

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