

Acrylonitrile and *N,N*-dimethylformamide – Determination of *S*-(2-cyanoethyl)mercapturic acid and *S*-(*N*-methylcarbamoyl)mercapturic acid in urine by LC-MS/MS

Biomonitoring Method – Translation of the German version from 2026

Keywords

acrylonitrile; *N,N*-dimethylformamide; CEMA; AMCC; biomonitoring; urine; LC-MS/MS

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Abstract

The working group “Analyses in Biological Materials” of the German Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission) developed and verified this biomonitoring method for the simultaneous determination of the mercapturic acid of acrylonitrile (*S*-(2-cyanoethyl)mercapturic acid, CEMA) and that of *N,N*-dimethylformamide (*N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine, AMCC) in urine by LC-MS/MS. For determination, the urine samples are buffered to a pH value of 2.5 and online enrichment is performed. Using integrated, automatic column-switching, the analytes are transferred in back-flush mode onto the analytical column and quantified by tandem mass spectrometry. Calibration is carried out using calibration standards prepared in pooled urine and processed analogously to the samples. The isotope-labelled mercapturic acids, CEMA-D₃ and AMCC-D₃, are used as internal standards. The method provides reliable and accurate analytical results, as shown by the good precision data with standard deviations no greater than 10%. Good accuracy data were obtained with mean relative recoveries in the range of 102–105%. Limits of quantitation of 6.0 µg CEMA/l and 7.5 µg AMCC/l were calculated. The method thus enables the biomonitoring of acrylonitrile and DMF in occupational and environmental exposure ranges.

1 Characteristics of the method

Matrix and analytical principle

Matrix	Urine
Analytical principle	Liquid chromatography with tandem mass spectrometry (LC-MS/MS)

Parameters and corresponding hazardous substances

Hazardous substance	CAS No.	Parameter	CAS No.
Acrylonitrile	107-13-1	<i>S</i> -(2-Cyanoethyl)mercapturic acid (CEMA)	74514-75-3
<i>N,N</i> -Dimethylformamide (DMF)	68-12-2	<i>N</i> -Acetyl- <i>S</i> -(<i>N</i> -methylcarbamoyl)cysteine (AMCC)	103974-29-4

Reliability criteria

CEMA

Within-day precision:	Standard deviation (rel.) Prognostic range at a concentration of 35 µg or 425 µg CEMA per litre of urine and n = 8 determinations	$s_w = 4.8\%$ or 3.3% $u = 11.4\%$ or 7.8%
Day-to-day precision:	Standard deviation (rel.) Prognostic range at a concentration of 36 µg or 454 µg CEMA per litre of urine and n = 10 determinations	$s_w = 6.4\%$ or 3.2% $u = 14.5\%$ or 7.2%
Accuracy:	Recovery (rel.) at a spiked concentration of 31 µg or 412 µg CEMA per litre of urine and n = 8 determinations	$r = 105\%$ or 103%
Limit of detection:	2.0 µg CEMA per litre of urine	
Limit of quantitation:	6.0 µg CEMA per litre of urine	

AMCC

Within-day precision:	Standard deviation (rel.) Prognostic range at a concentration of 46 µg or 454 µg AMCC per litre of urine and n = 8 determinations	$s_w = 5.9\%$ or 3.4% $u = 14.0\%$ or 8.0%
Day-to-day precision:	Standard deviation (rel.) Prognostic range at a concentration of 44 µg or 442 µg AMCC per litre of urine and n = 10 determinations	$s_w = 8.6\%$ or 9.6% $u = 19.5\%$ or 21.7%

Accuracy:	Recovery (rel.) at a spiked concentration of 34 µg or 428 µg AMCC per litre of urine and n = 8 determinations	$r = 102\%$ or 104%
Limit of detection:	2.5 µg AMCC per litre of urine	
Limit of quantitation:	7.5 µg AMCC per litre of urine	

2 General information on acrylonitrile and dimethylformamide

Acrylonitrile

At room temperature, acrylonitrile presents as a colourless, volatile liquid which exhibits a pungent odour and tends towards spontaneous polymerisation (IFA 2026). It is largely used as a starting material for the production of polyacrylonitrile and its copolymers, such as styrene-acrylonitrile, acrylonitrile butadiene styrene, or acrylonitrile butadiene rubber (Stayner et al. 2024). Acrylonitrile is further used for the industrial production of acrylic acid, acrylic esters, and acrylamide (IARC 1999, 2025) and is also applied as an insecticide (Brazdil 2026). Moreover, acrylonitrile is a component of tobacco smoke (IARC 1999; Kraus et al. 2021). The Commission has categorised acrylonitrile as a Category 2 carcinogen and has given the substance both an H designation (danger from percutaneous absorption) as well as an Sh designation (danger of sensitisation of the skin) (DFG 2025). The IARC has categorised acrylonitrile as carcinogenic to humans (Group 1), as there is sufficient evidence that exposure to acrylonitrile causes lung cancer (IARC 2025; Stayner et al. 2024). A summary of the toxicological properties of acrylonitrile can be found in the corresponding documentations (Greim 2007; IARC 2025).

In addition to occupational exposure in chemical industries, the general population can be exposed to acrylonitrile via tobacco smoke and, to a lesser extent, via the use of e-cigarettes (Chen et al. 2023; Stayner et al. 2024). Acrylonitrile is rapidly absorbed and distributed throughout the body after uptake via oral, dermal, or inhalation routes (Greim 2007). Animal studies showed that 40–100% of an orally administered dose of acrylonitrile is excreted with the urine within 24 hours. Excretion via the lung or faeces was found to be much less relevant (Kraus et al. 2021). With respect to elimination via the kidneys, the conjugation of acrylonitrile and its metabolites with glutathione, and the resulting formation of mercapturic acids, is very important. Figure 1 shows a simplified metabolism scheme for acrylonitrile in humans (according to Fennell et al. (1991) and Sumner et al. (1999)). *S*-(2-cyanoethyl)mercapturic acid (CEMA) is formed via conjugation with glutathione and further metabolic conversion of these conjugates. Other possible mercapturic acids, the formation of which have been described in animal studies following acrylonitrile exposure, include *N*-acetyl-*S*-(1-cyano-2-hydroxyethyl)cysteine and *N*-Acetyl-*S*-(2-hydroxyethyl)cysteine (Kraus et al. 2021). The elimination half-life of CEMA has been reported to be about 8 hours (Jakubowski et al. 1987). For this reason, CEMA constitutes a classic short-term biomarker for acrylonitrile exposure. As a directly alkylating agent, absorbed acrylonitrile easily binds to macromolecules, such as DNA, proteins, or haemoglobin (Greim 2007; IARC 1999; Kraus et al. 2021). As a result, the haemoglobin adduct *N*-(2-cyanoethyl)valine can likewise be determined as a long-term biomarker for acrylonitrile exposure. Methods on the determination of *N*-(2-cyanoethyl)valine in blood have already been published by the Commission (Christ et al. 2023; Schettgen et al. 2016; van Sittert et al. 1997).

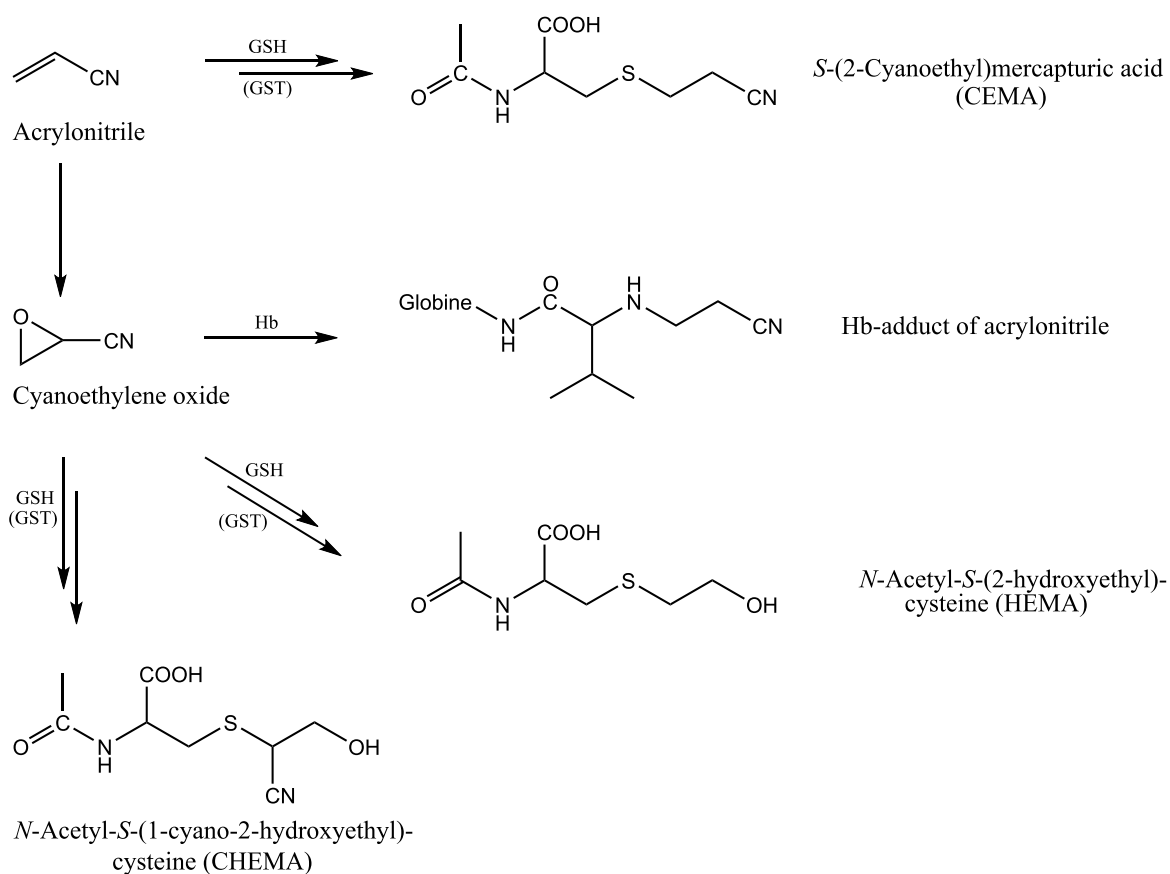


Fig. 1 Simplified metabolism scheme of acrylonitrile (according to Fennell et al. (1991) and Sumner et al. (1999))

With regard to assessment values in biological materials, the Commission has established two biological reference values (*Biologischer Arbeitsstoff-Referenzwert*, BAR) for acrylonitrile: 15 µg/g creatinine for the mercapturic acid CEMA in urine, whereby sample collection should take place at the end of a shift; and 12 pmol/g globin for the haemoglobin adduct *N*-(2-cyanoethyl)valine, whereby sample collection should take place after at least three months of exposure. In addition, exposure equivalents for carcinogenic substances (*Expositionsäquivalente für krebserzeugende Arbeitsstoffe*, EKA) have been established for *N*-(2-cyanoethyl)valine (DFG 2025). As both smokers and non-smokers generally exhibit higher values, both BARs were derived for non-smokers (Schettgen et al. 2009). Information on background exposure to CEMA in both smokers and non-smokers from the general population can be found in the 2021 BAR/EKA documentation (Jäger et al. 2021). More recent data on CEMA concentrations in urine of the non-occupationally exposed general population are presented in Table 1. Table 2 lists studies on CEMA concentrations in urine from workers occupationally exposed to acrylonitrile.

Tab. 1 CEMA in urine from the non-occupationally exposed general population

Study collective (country; n; sample)	QF [%]	LOD [µg/l]	LOQ [µg/l]	Mean (SD)	GM	Median	P95	Min–Max	References
Cigarette smokers (China; 12; 24h urine)	–	–	–	316 (203) µg/24 h	–	–	–	–	Li et al. 2024
E-cigarette smokers (China; 11; 24h urine)	–	–	–	306 (230) µg/24 h	–	–	–	–	
Non-smokers (USA; 63; spot urine)	–	–	–	–	0.376 µg/l	–	–	< LOD–8.85 µg/l	Chen et al. 2023
Cigarette smokers (USA; 22; spot urine)	–	–	0.015	–	0.588 µg/l	–	–	4.11–2056 µg/l	
E-cigarette smokers (USA; 33; spot urine)	–	–	–	–	115 µg/l	–	–	< LOD–128 µg/l	
Non-smokers (Spain; 25; spot urine)	–	–	–	< LOD	–	< LOD	< LOD	< LOD	Gallart-Mateu et al. 2023
Cigarette smokers (USA; 14; spot urine)	–	3.3	11.0	180 (80) µg/g creatinine	–	169 µg/g creatinine	52 µg/g creatinine	41–370 µg/g creatinine	
E-cigarette smokers (USA; 63; spot urine)	–	–	–	< LOD	–	< LOD	< LOD	< LOD	
Non-smokers (Italy; 38; spot urine)	63	–	–	–	–	0.9 µg/g creatinine	2.1 µg/g creatinine	–	Frigerio et al. 2020 b
Cigarette smokers (Italy; 22; spot urine)	100	–	0.9	–	–	163 µg/g creatinine	358 µg/g creatinine	–	
E-cigarette smokers (Italy; 7; spot urine)	86	–	–	–	–	2.7 µg/g creatinine	36.5 µg/g creatinine	–	
Non-smokers (Taiwan; 381; spot urine)	–	–	–	2.98 (6.91) µg/l; 2.09 (6.65) µg/g creatinine	–	–	–	–	Lin et al. 2018
Second-hand smokers (Taiwan; 326; spot urine)	–	0.077	–	2.98 (6.86) µg/l; 1.99 (5.99) µg/g creatinine	–	–	–	–	
Cigarette smokers (Taiwan; 36; spot urine)	–	–	–	98.8 (2.52) µg/l; 76.8 (2.35) µg/g creatinine	–	–	–	–	

GM: geometric mean; LOD: limit of detection; LOQ: limit of quantitation; n: sample size; P95: 95th percentile; QF: quantitation frequency (measured values above the LOQ)

Tab. 2 CEMA in urine from occupationally exposed workers

Study collective (country; n; sample)	QF [%]	LOD [$\mu\text{g/l}$]	LOQ [$\mu\text{g/l}$]	Mean (SD)	GM	Median	P95	Min–Max	References
Coke-oven workers, non-smokers (Poland; 49; spot urine, post-shift)	88	–	0.9	3.7 $\mu\text{g/l}$	–	–	9.6 $\mu\text{g/l}$	–	Frigerio et al. 2020 a
Controls, non-smokers (Poland; 49; spot urine)	98	–		1.4 $\mu\text{g/l}$	–	–	16.6 $\mu\text{g/l}$	–	
Highly exposed workers (Taiwan; 26; spot urine, post-shift)	–	–	0.1	–	230 $\mu\text{g/g}$ creatinine	–	–	3.43–2460 $\mu\text{g/g}$ creatinine	Wu et al. 2023
Slightly exposed workers (Taiwan; 53, of which about 1/3 were smokers; spot urine, post-shift)	–	0.05		–	14.3 $\mu\text{g/g}$ creatinine	–	–	0.10–289 $\mu\text{g/g}$ creatinine	
Controls (Taiwan; 18, of which 2 were smokers; spot urine)	–	–		–	1.26 $\mu\text{g/g}$ creatinine	–	–	<LOD–61.3 $\mu\text{g/g}$ creatinine	

GM: geometric mean; LOD: limit of detection; LOQ: limit of quantitation; n: sample size; P95: 95th percentile; QF: quantitation frequency (measured values above the LOQ)

Dimethylformamide (DMF)

At room temperature, DMF presents as a colourless, low-volatile liquid which is miscible with water as well as many organic solvents. DMF is primarily used as a solvent. The most important industrial application of DMF as a solvent is in the production of polyacrylonitrile (IARC 2018). At these workplaces, combined exposure to DMF and acrylonitrile is to be expected in workers. The IARC has categorised DMF as probably carcinogenic to humans (Group 2A) (IARC 2018). The Commission has categorised DMF as a Category 4 carcinogen and has given the substance an H designation (danger from percutaneous absorption). A MAK value of 5 ml/m^3 (15 mg/m^3) has been established by the Commission (DFG 2025). DMF has been further assigned to Pregnancy Risk Group B. Notes regarding the prerequisites for classification into Pregnancy Risk Group C can be found in the corresponding documentation published by the Commission (Hartwig and MAK Commission 2017 b). A summary of the toxic properties of DMF can be found in the corresponding documentations (Greim 1997; Hartwig 2010; Hartwig and MAK Commission 2017 a, 2020; IARC 2018).

After exposure via oral, inhalation, or dermal routes, DMF is easily absorbed and distributed throughout the body (Greim 1997). In humans, about half of an inhaled dose of DMF is excreted with the urine (IARC 2018). Important urinary DMF metabolites include *N*-methylformamide (NMF), *N*-hydroxymethyl-*N*-methylformamide (HMMF), as well as *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine (AMCC). The half-lives of NMF and HMMF are each reported as 2–7 hours. At 23–36 hours, the half-life of AMCC is considerably longer (IARC 2018; Kafferlein et al. 2005); as a result, an accumulation of AMCC over the course of a workweek has been observed in cases of regular occupational exposure to DMF (IARC 2018; Seitz et al. 2018). The half-life of DMF in blood has been reported to be 1–2 hours (Hartwig 2010). Figure 2 shows a simplified scheme of DMF metabolism in humans (according to IARC (2018) and Kafferlein et al. (2005)). Human studies and results from animal experiments verify the formation of HMMF as the first product of enzymatic oxidation. NMF is formed under formaldehyde release and can be further oxidised to *N*-hydroxymethylformamide, which forms formamide and releases formaldehyde. The mercapturic acid AMCC is formed by the glutathione conjugation of a reactive intermediate (methylisocyanate), which is formed by the oxidation of NMF. The glutathione conjugate *S*-(*N*-methylcarbamoyl)glutathione undergoes the mercapturic-acid metabolic pathway and is then excreted as AMCC with the urine. Methylisocyanate can also react with macromolecules, forming, for example, haemoglobin adducts.

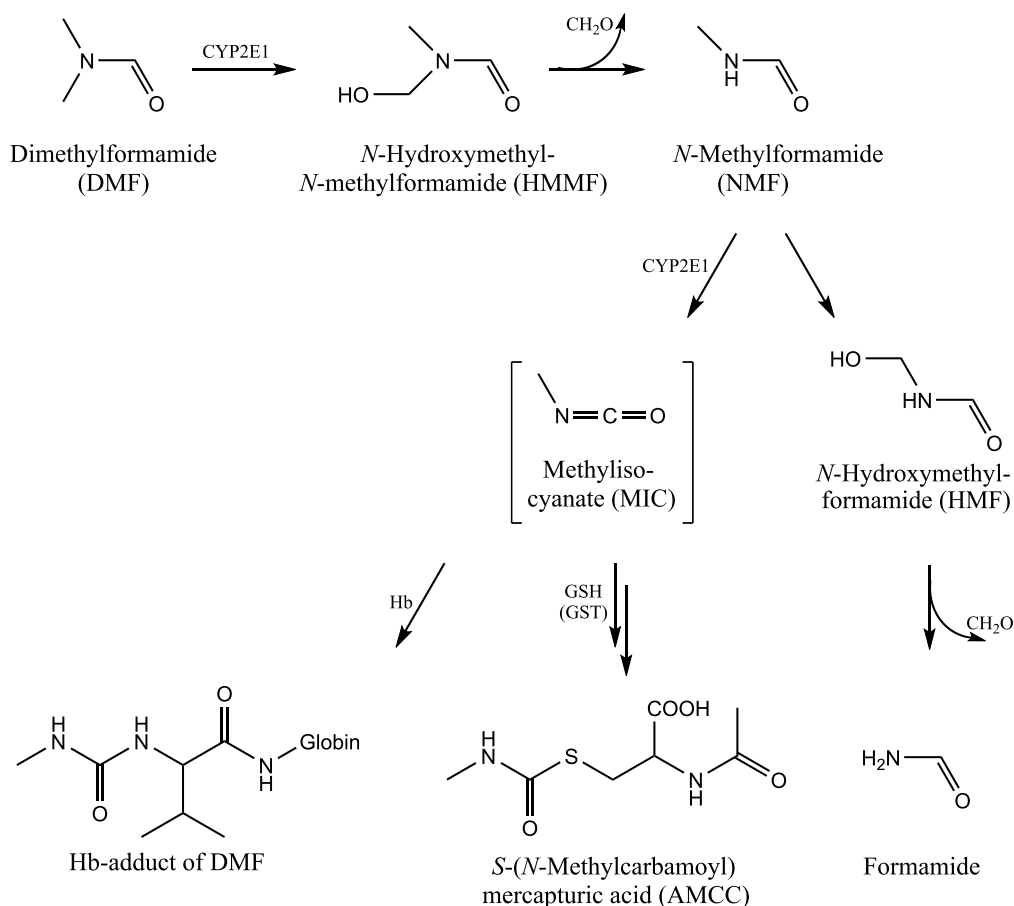


Fig. 2 Simplified metabolism scheme of DMF (according to IARC (2018) and Kafferlein et al. (2005))

NMF and HMMF, metabolites which are excreted with the urine, may also serve as suitable biomarkers for the measurement of DMF exposure. The Commission has already published valid analytical methods for the determination of these parameters in urine (Kafferlein et al. 2013; Scherer et al. 2018; Will et al. 2016). HMMF, NMF, and AMCC are short-term biomarkers which serve as indicators for DMF exposure which has taken place only a few hours or days prior to sample collection (Kafferlein et al. 2016).

Additionally, the formation of the haemoglobin adduct *N*-methylcarbamoylvaline (NMVal) is observed after exposure to DMF; this substance is similarly considered a reaction product of the intermediately formed methylisocyanate (Angerer et al. 1998; Seitz et al. 2018). NMVal is used as a biomarker for cumulative DMF exposure over the three months preceding sample collection. A method on the determination of NMVal after transformation into the corresponding hydantoin derivative, 3-methyl-5-isopropylhydantoin (MIH), has already been published by the Commission (Kafferlein et al. 2016).

The Commission has established two biological tolerance values (*Biologischer Arbeitsstoff-Toleranzwert*, BAT) for DMF as assessment values in biological materials: 20 mg/l urine for the sum of NMF and HMMF as well as 25 mg/g creatinine for the mercapturic acid AMCC; in each case, sample collection should take place at the end of a shift or after several previous shifts (DFG 2025). Notes regarding the prerequisites for classification into Pregnancy Risk Group C as well as information on background levels of common DMF biomarkers can be found in the corresponding BAT documentation published by the Commission (Goen et al. 2020).

Data on AMCC concentrations in urine from the occupationally non-exposed general population are presented in Table 3. Table 4 provides AMCC concentrations from workers occupationally exposed to DMF.

Tab. 3 AMCC in urine from the non-occupationally exposed general population

Smoking status (country; n; sample)	QF [%]	LOD [$\mu\text{g/l}$]	LOQ [$\mu\text{g/l}$]	Mean (SD)	GM	Median	P95	Min–Max	References
Non-smokers (USA; 1377; spot urine)	–	–	6.26	–	0.11 mg/l	–	0.47 mg/l	–	NCEH 2021
Smokers (USA; 779; spot urine)	–	–		–	0.39 mg/l	–	1.58 mg/l	–	
Non-smokers (Italy; 38; spot urine)	100	–	2	–	–	0.142 mg/g creatinine	0.434 mg/g creatinine	–	Frigerio et al. 2020 b
Cigarette smokers (Italy; 22; spot urine)	100	–		–	–	0.405 mg/g creatinine	0.844 mg/g creatinine	–	
E-cigarette smokers (Italy; 7; spot urine)	100	–		–	–	0.243 mg/g creatinine	0.519 mg/g creatinine	–	
Non-smokers (Germany; 14; spot urine)	–	–	5.0	–	–	0.11 mg/l (0.10 mg/g creatinine)	–	<LOQ–0.50 mg/l (0.047–0.45 mg/g creatinine)	Schettgen et al. 2008
Smokers (Germany; 14; spot urine)	–	–		–	–	0.82 mg/l (0.59 mg/g creatinine)	–	0.12–1.45 mg/l (0.20–1.15 mg/g creatinine)	
Non-smokers (Italy; 21; spot urine)	–	–	1800	5 (2.5) mg/l	4.5 mg/l	3.8 mg/l	–	1.9–10.5 mg/l	Perbellini et al. 2001
Smokers (Italy; 19; spot urine)	–	–		4.5 (1.6) mg/l	4.2 mg/l	4.4 mg/l	–	1.8–7.3 mg/l	

GM: geometric mean; LOD: limit of detection; LOQ: limit of quantitation; n: sample size; P95: 95th percentile; QF: quantitation frequency (measured values above the LOQ)

Tab. 4 AMCC in urine from occupationally exposed workers

Study collective (country; n; sample)	QF [%]	LOD [$\mu\text{g/l}$]	LOQ [$\mu\text{g/l}$]	Mean (SD)	GM	Median	P95	Min–Max	References
Workers of a synthetic-leather factory (China; 263; spot urine)	–	–	200	–	–	84.8 mg/l	278 mg/l	–	Wu et al. 2017
Workers in the production of synthetic fibres (Germany; 208; spot urine)	–	5.0	15	9.42 (10.4) mg/g creatinine	–	4.84 mg/g creatinine	–	<LOQ–49.6 mg/g creatinine	Kilo et al. 2016
Controls in the production of synthetic fibres (Germany; 174; spot urine)	–			0.28 (0.21) mg/g creatinine	–	0.21 mg/g creatinine	–	<LOQ –1.16 mg/g creatinine	
Highly exposed workers in an organic-chemical factory (China; 22; spot urine)	–	–	400	–	45.5 mg/l (19.6 mg/g creatinine)	–	–	7.4–145.7 mg/l	He et al. 2010
Workers in the polyacrylic-fibre industry (Germany; 34; spot urine)	–	–	1000	22.5 mg/l	–	12.4 mg/l	73.9 mg/l	1.0–134 mg/l	Käfferlein et al. 2004
	–	–	30	20.2 mg/l	–	11.4 mg/l	64.4 mg/l	0.6–117 mg/l	
Workers of a synthetic-leather factory (Italy; 20; spot urine)	–	–	1.8 mg/l	63.5 mg/l	55.2 mg/l	59.1 mg/l	–	16–170 mg/l	Perbellini et al. 2001
Workers in the polyacrylic-fibre industry (Germany; 92; spot urine)	–	–	0.5 mg/l	14.2 (15.6) mg/g creatinine	–	8.78 mg/g creatinine	46.8 mg/g creatinine	<LOQ–82.1 mg/g creatinine	Käfferlein et al. 2000

Tab. 4 (continued)

Study collective (country; n; sample)	QF [%]	LOD [$\mu\text{g/l}$]	LOQ [$\mu\text{g/l}$]	Mean (SD)	GM	Median	P95	Min-Max	References
Workers in the production of polyurethane (Japan; 10; spot – urine)	–	–	–	22.0 (4.6) mg/g creatinine	–	–	–	–	Sakai et al. 1995

GM: geometric mean; LOD: limit of detection; LOQ: limit of quantitation; n: sample size; P95: 95th percentile; QF: quantitation frequency (measured values above the LOQ)

3 General principles

The method described herein serves the simultaneous measurement of the mercapturic acids of acrylonitrile (*S*-(2-cyanoethyl)mercapturic acid, CEMA) and dimethylformamide (*N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine, AMCC) in urine. For determination, the urine samples are buffered to a pH value of 2.5 and online enrichment is performed. Using integrated, automatic column-switching, the analytes are transferred onto the analytical column in back-flush mode, where they are chromatographically separated. Quantification was performed by tandem-mass spectrometry. Calibration is carried out using calibration standards prepared in pooled urine and processed analogously to the samples. The isotope-labelled mercapturic acids, CEMA- D_3 and AMCC- D_3 , are added to the urine samples as internal standards.

4 Equipment, chemicals, and solutions

4.1 Equipment

- HPLC system with a binary and a quaternary pump, a vacuum degasser, and an autosampler (e.g. Series 1100 with G 1311 A, G 1322 A, and G 1313 A, Agilent Technologies Deutschland GmbH, Waldbronn, Germany)
- Triple-quadrupole mass spectrometer (e.g. API 2000, AB SCIEX Germany GmbH, Darmstadt, Germany)
- Analytical HPLC column (e.g. No. 00F-4337-Y0, SynergiTM MaxRP C12 (3.0 mm $\times$ 150 mm, 4 μm), with a C12 pre-column, Phenomenex Ltd. Deutschland, Aschaffenburg, Germany)
- Precolumn filter (4.6 mm $\times$ 3 mm, pore size of 0.5 μm) with cartridge (e.g. No. 57677 and No. 57675U, Restek Corporation, Bellefonte, USA)
- Cartridge holder (e.g. No. 150017, manu-CART[®] for LiChroCart[®] 25-4 HPLC, Merck KGaA, Darmstadt, Germany)
- Preconcentration column used during method development, no longer commercially available (No. 150947, restricted access material (RAM phase), LiChrospher RP-18 ADS LiChroCart[®] 25-4 (25 mm $\times$ 4 mm, 25 μm), Merck KGaA, Darmstadt, Germany)
- Preconcentration column used during external method verification (No. 00M-S036-B0-CB, StrataTM-X 25 μm On-Line Extraction (20 $\times$ 2.0 mm), restricted access material (RAM phase), Phenomenex Ltd. Deutschland, Aschaffenburg, Germany)
- Laboratory centrifuge (e.g. MegafugeTM, Heraeus Holding GmbH, Hanau, Germany)
- Vortex shaker (e.g. Vortex 2, IKA-Werke GmbH & Co. KG, Staufen, Germany)
- 1.8-ml threaded vials, clear, with 8-mm screw caps with PTFE-lined septa (e.g. No. 548-0018 and No. 548-0024, VWR International GmbH, Darmstadt, Germany)
- 13-ml plastic tubes with caps (e.g. No. HERE1130708, VWR International GmbH, Darmstadt, Germany)
- Microlitre pipettes, variably adjustable (e.g. Eppendorf AG, Hamburg, Germany)
- Multipettes[®] (e.g. M4, Eppendorf AG, Hamburg, Germany)

- Various volumetric flasks and glass beakers (e.g. DURAN®, Schott AG, Mainz, Germany)
- 1000-ml HPLC bottles (e.g. DURAN®, Schott AG, Mainz, Germany)
- Magnetic stirrer (e.g. RSM-10HS, Phoenix Instrument GmbH, Garbsen, Germany)
- pH meter (e.g. Mettler-Toledo GmbH, Gießen, Germany)
- Urine-collection cups (e.g. polypropylene) (e.g. Sarstedt AG & Co. KG, Nümbrecht, Germany)

4.2 Chemicals

Unless otherwise specified, all chemicals must be a minimum of *pro analysi* grade.

- AMCC (*N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine) (e.g. No. 90914, Merck KGaA, Darmstadt, Germany)
- CEMA (*N*-acetyl-*S*-(cyanoethyl)cysteine) (e.g. custom synthesis, Max Planck Institute for Multidisciplinary Sciences, Facility for Synthetic Chemistry, Göttingen, Germany)
- AMCC-D₃ (e.g. No. A186 627, Toronto Research Chemicals Inc., Toronto, Canada)
- CEMA-D₃ (e.g. No. A172087, Toronto Research Chemicals Inc., Toronto, Canada)
- Acetic acid (glacial) 100%, anhydrous for analysis, EMSURE® ACS (e.g. No. 1.00063, Merck KGaA, Darmstadt, Germany)
- Ammonium formate ≥ 99.0%, LiChropur™ (e.g. No. 70221, Merck KGaA, Darmstadt, Germany)
- Formic acid 98–100%, for analysis, EMSURE® ACS (e.g. No. 1.00264, Merck KGaA, Darmstadt, Germany)
- Methanol ≥ 99.9%, LiChrosolv™ (e.g. No. 1.06035, Merck KGaA, Darmstadt, Germany)
- Ultra-pure water (e.g. No. 83645-320, VWR International GmbH, Darmstadt, Germany)

4.3 Solutions

- Ammonium formate buffer (50 mmol/l, pH 2.5)
Exactly 1.58 g ammonium formate are weighed into a glass beaker and dissolved in about 400 ml of ultra-pure water. Using a pH meter and formic acid, the pH value is subsequently adjusted to pH 2.5. The solution is quantitatively transferred into a 500-ml volumetric flask, which is then made up to the mark with ultra-pure water.
- Aqueous acetic acid (1%)
About 800 ml of ultra-pure water are placed in a 1000-ml volumetric flask and 10 ml of glacial acetic acid are added. The volumetric flask is then made up to the mark with ultra-pure water.

The solutions are stable for at least three months when stored at room temperature.

4.4 Internal standards (ISTDs)

- ISTD stock solutions (1000 mg/l)
10 mg each of AMCC-D₃ and CEMA-D₃ are weighed exactly into separate 10-ml volumetric flasks and dissolved in methanol. The flasks are then made up to the mark with methanol.
- ISTD spiking solution (30 mg/l AMCC-D₃ and 10 mg/l CEMA-D₃)
300 µl of the AMCC-D₃ stock solution as well as 100 µl of the CEMA-D₃ stock solution are pipetted into a 10-ml volumetric flask, which is then made up to the mark with methanol.

The ISTD stock and spiking solutions are stored at –20 °C and are stable for at least six months under these conditions.

4.5 Calibration standards

- Stock solutions (1000 mg/l)
10 mg each of CEMA or AMCC are weighed exactly into separate 10-ml volumetric flasks and dissolved in methanol. The flasks are then made up to the mark with methanol.
- Spiking solution I (10 mg/l)
100 µl of each stock solution are pipetted into a 10-ml volumetric flask. The flask is then made up to the mark with methanol.
- Spiking solution II (1 mg/l)
1 ml of Spiking solution I is pipetted into a 10-ml volumetric flask. The flask is then made up to the mark with methanol.

The stock and spiking solutions are stored at -20°C and are stable for at least six months under these conditions.

For calibration, pooled urine from persons with no occupational exposure to the hazardous substances in question is used. To prepare the calibration standards, the urine is mixed with the analyte spiking solutions according to the pipetting scheme given in Table 5. Unspiked pooled urine is additionally included to ascertain the blank value. The background concentrations of the individual analytes in urine are accounted for by subtraction.

Tab. 5 Pipetting scheme for the preparation of calibration standards for the determination of CEMA and AMCC in urine

Calibration standard	Spiking solution II [µl]	Spiking solution I [µl]	Pooled urine [µl]	Analyte concentration [µg/l]
0	0	–	1000	0
1	10	–	990	10
2	50	–	950	50
3	–	10	990	100
4	–	25	975	250
5	–	50	950	500
6	–	100	900	1000

If necessary, the calibration standards can be prepared in a larger batch. In this case, the prepared calibration standards are divided into 1-ml aliquots and stored in 13-ml plastic tubes at -20°C . Under these conditions, the calibration standards are stable for at least six months.

5 Specimen collection and sample preparation

5.1 Specimen collection

The urine samples are collected in sealable urine-collection cups and are stored at -20°C until sample preparation. When stored in this manner, the analytes in urine are stable for at least six months.

5.2 Sample preparation

Prior to analysis, the samples are thawed at room temperature and thoroughly mixed. A 1-ml aliquot of the urine sample is transferred into a 13-ml plastic tube and mixed with 1 ml of the ammonium formate buffer, 20 µl of formic acid, and 20 µl of the ISTD spiking solution. The sample is thoroughly mixed on a vortex shaker and then centrifuged at $1500 \times g$.

for 10 min. Subsequently, 1 ml of the supernatant is transferred into a 1.8-ml vial. For analysis, 100 µl of each sample are injected into the LC-MS/MS device.

6 Operational parameters

Analytical determination was carried out on a device configuration comprised of an HPLC system and a tandem mass spectrometer. For online sample workup and separation by HPLC, two HPLC pumps are required (here: isocratic Pump P1 and quaternary Pump P2). The preconcentration column and the analytical column are coupled via a six-port valve ([Figure 3](#)).

6.1 Liquid chromatography

Preconcentration column:	LiChrospher RP-18 ADS (25 mm × 4 mm, 25 µm)
Analytical column:	Synergi™ MaxRP C12 (3.0 × 150 mm, 4 µm)
Separation principle:	Reversed phase
Mobile phase of Pump P1:	Eluent A: aqueous acetic acid (1%)
Mobile phases of Pump P2:	Eluent B: ultra-pure water Eluent C: methanol Eluent D: aqueous acetic acid (1%)
Flow rate of Pump P1:	0.5 ml/min
Flow rate of Pump P2:	0.4 ml/min
Injection volume:	100 µl
Gradient programme for Pump P2:	see Table 6
Valve-switching programme:	see Table 7

Tab. 6 Gradient programme of quaternary Pump P2 for the chromatographic separation of the analytes

Time [min]	Eluent B [%]	Eluent C [%]	Eluent D [%]
0	75	5	20
1.5	75	5	20
6.5	30	50	20
9.0	30	50	20
11.0	10	70	20
13.0	10	70	20
16.0	75	5	20
23.0	75	5	20

Tab. 7 Switching programme of the six-port valve (see also [Figure 3](#))

Time [min]	Switch position	Description
0.0–1.5	A	Enrichment of analytes on RAM phase via Pump P1, equilibration of analytical column via Pump P2
1.5–13.0	B	Transfer of analytes from RAM phase onto analytical column via Pump P2, chromatographic separation of analytes
13.0–23.0	A	Reconditioning of RAM phase via Pump P1, equilibration of both columns

It is recommended to add a particle filter before the RAM phase to avoid clogging. All other parameters must be optimised according to manufacturer specifications. Figure 3 depicts a schematic diagram of the switch positions.

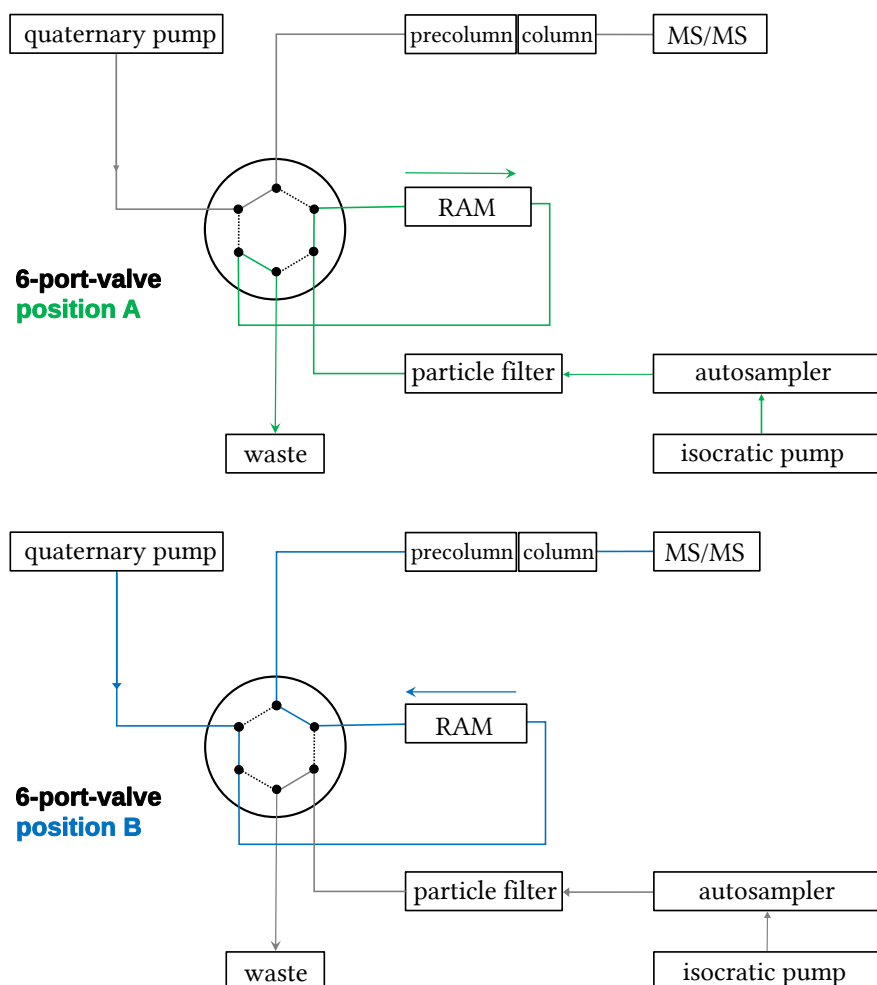


Fig. 3 Schematic diagram of the LC-MS/MS system including an illustration of switch options A (green) and B (blue) of the six-port valve

6.2 Tandem mass spectrometry

Ionisation:	Electrospray, negative (ESI ⁻)
Ion-spray voltage:	−4500 V
Source temperature:	475 °C
Dwell time:	100 ms
Curtain gas:	Nitrogen, 241 kPa
Nebulising gas:	Nitrogen, 241 kPa
Turbo-heater gas:	Nitrogen, 483 kPa
Collision gas:	Nitrogen, 3 instrument units
Detection mode:	Multiple Reaction Monitoring (MRM)
Parameter-specific settings:	see Table 8

The instrument-specific settings must be ascertained and adjusted individually by the user for the MS/MS system used. The device-specific parameters indicated above were determined and optimised for the system used during method development.

Tab. 8 Parameter-specific settings and retention times of the analytes

Analyte or ISTD	Precursor ion (m/z)	Product ion (m/z)	Collision energy [V]	Retention time [min]
AMCC	219.0	84.0	–24	10.03
		161.9	–12	
CEMA	215.0	85.9	–18	10.48
		162.0	–13	
AMCC-D ₃	222.0	165.2	–10	10.01
CEMA-D ₃	218.0	85.9	–20	10.43

The retention times given in Table 8 can only serve as a point of reference. The user of the method must ensure the separation performance of the column used and the resulting retention behaviour of the analytes.

Figures 4 and 5 show representative chromatograms of processed native urine samples.

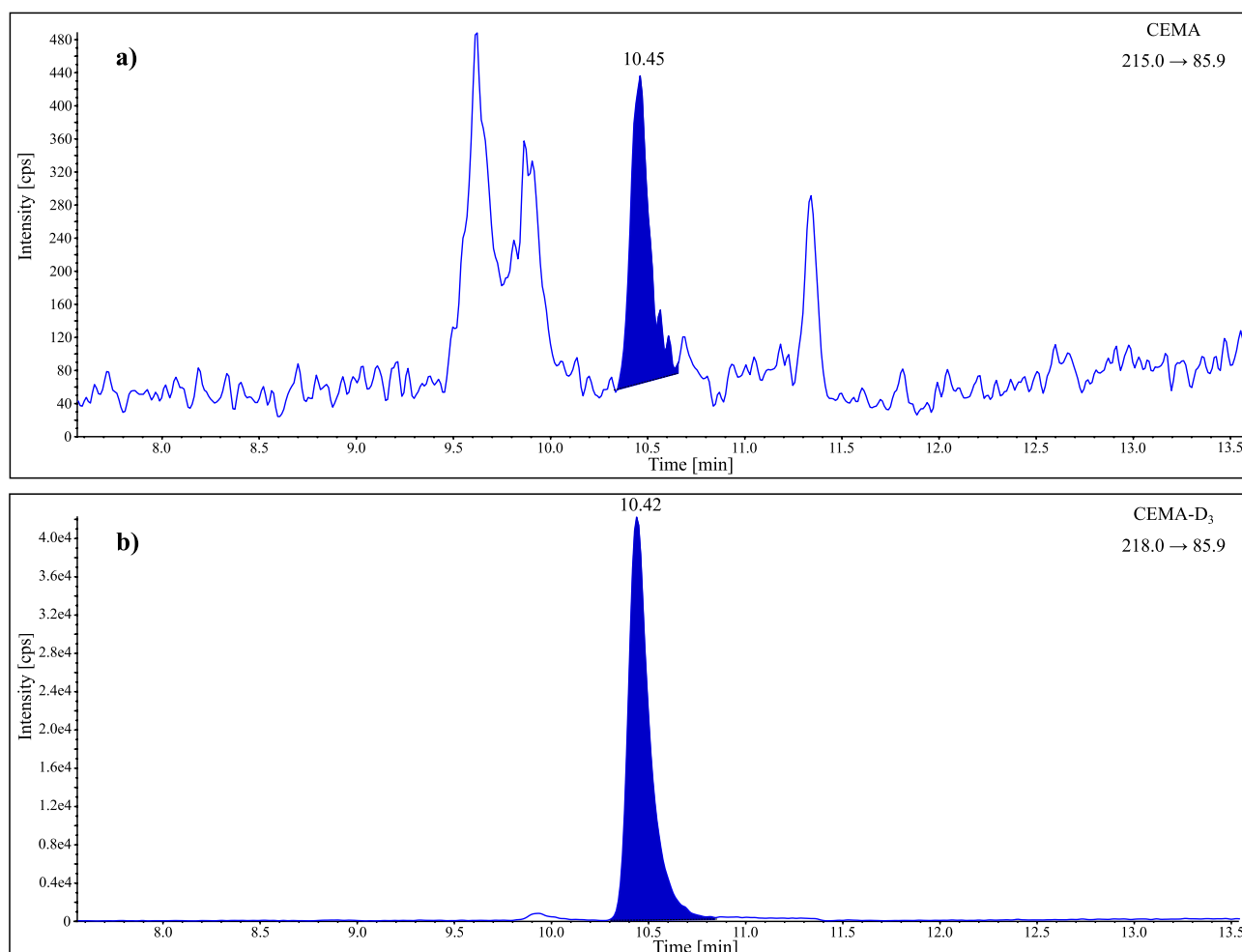


Fig. 4 Chromatogram of a processed native urine sample (measured concentrations: CEMA 4.2 µg/l; AMCC < LOD)

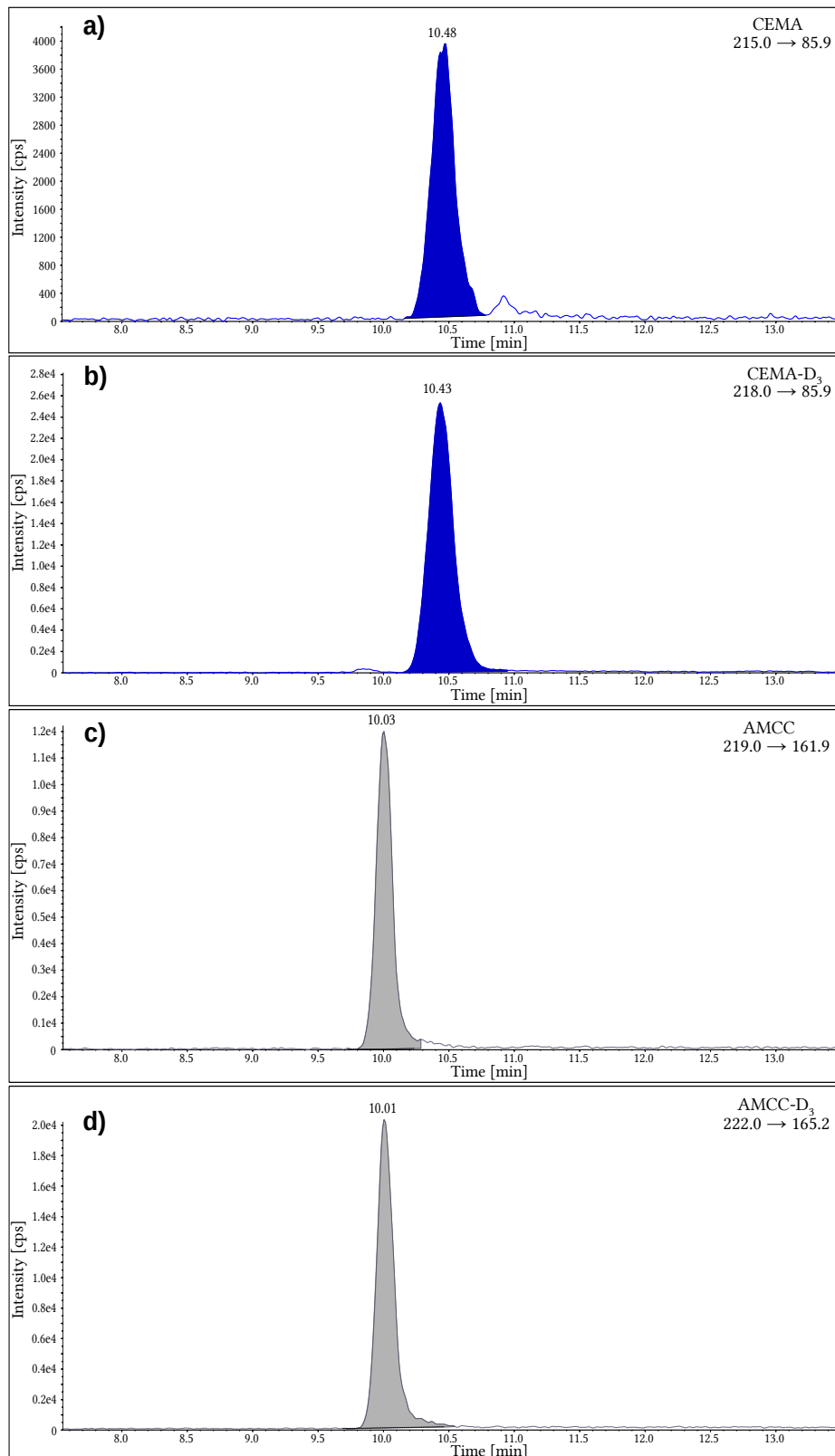


Fig. 5 Chromatogram of a processed native urine sample (measured concentrations: CEMA 74.4 µg/l; AMCC 249.1 µg/l)

7 Analytical determination

For the analytical determination of the urine samples processed according to [Section 5.2](#), an aliquot of 100 µl is injected into the LC-MS/MS system by the autosampler. After online enrichment and chromatographic separation, the analytes are identified by their retention times and characteristic ion traces (see [Table 8](#)). Pooled urine, serving as a blank value, and at least one quality-control sample are included as part of each analytical series.

8 Calibration

The calibration standards (see [Section 4.5](#)) are processed analogously to the urine samples as described in [Section 5](#) and analysed according to [Sections 6](#) and [7](#). The calibration curves are generated by plotting the quotients of the peak areas of the analytes and those of the corresponding ISTDs against the spiked concentrations of the calibration standards. The calibration curves are linear in the range from the detection limit up to 1000 µg/l. Representative calibration curves are depicted in [Figure 6](#).

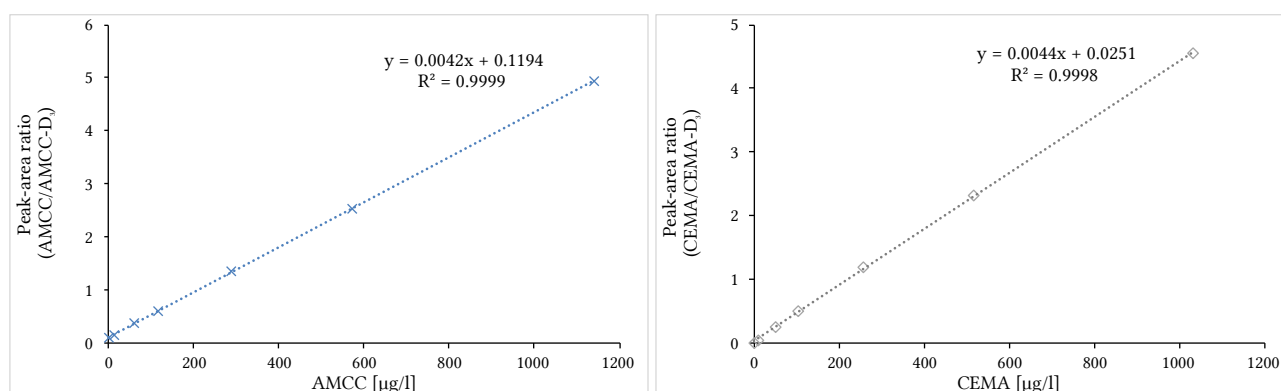


Fig. 6 Representative calibration curves for the determination of CEMA and AMCC in urine

9 Calculation of analytical results

The analyte concentration of a sample is determined by dividing the peak area of the analyte by the peak area of the corresponding ISTD and inserting the quotient thus calculated into the calibration function of the corresponding analytical run, yielding the analyte concentration in µg/l. Any reagent blank values are accounted for by subtraction.

10 Standardisation and quality control

Quality assurance of the analytical results is carried out as stipulated in the guidelines of the *Bundesärztekammer* (German Medical Association) and in a general chapter published by the Commission (Bader et al. 2010; Bundesärztekammer 2023).

For precision control, at least two urine samples with known and constant analyte concentrations are included as quality controls in each analytical run. Since such quality-control material is not commercially available, it must be prepared in the in-house laboratory. To this end, pooled urine is spiked with a defined amount of the analytes, aliquoted, and stored at -20°C. Of this control material, two concentration levels per analyte are prepared such that the concentrations of the control materials lie within the relevant range (e.g. 40 µg/l and 400 µg/l). The nominal values and tolerance ranges

of the quality-control materials are ascertained as part of a pre-analytical period (one analysis of the control materials per day on ten different days) (Bader et al. 2010).

In addition, a reagent blank comprising 1000 µl of ultra-pure water is included in each analytical run. The reagent blank is processed and analysed analogously to the urine samples.

11 Evaluation of the method

The reliability of this method was confirmed by comprehensive validation as well as by replication and verification in a second, independent laboratory.

11.1 Precision

To determine within-day precision, the quality-control material prepared as described in Section 10 was processed and analysed in parallel. Eightfold determination of these urine samples yielded the precision data given in Table 9.

Tab. 9 Within-day precision for the determination of CEMA and AMCC in urine (n = 8)

Analyte	Measured concentration [µg/l]	Standard deviation (rel.) s_w [%]	Prognostic range u [%]
CEMA	35.4	4.8	11.4
	425	3.3	7.8
AMCC	45.9	5.9	14.0
	454	3.4	8.0

To determine day-to-day precision, the quality-control samples were processed and analysed on ten different days. The precision data thus obtained are documented in Table 10.

Tab. 10 Day-to-day precision for the determination of CEMA and AMCC in urine (n = 10)

Analyte	Measured concentration [µg/l]	Standard deviation (rel.) s_w [%]	Prognostic range u [%]
CEMA	35.9	6.4	14.5
	454	3.2	7.2
AMCC	43.8	8.6	19.5
	442	9.6	21.7

11.2 Accuracy

Accuracy was determined using the within-day precision data while accounting for the analyte background levels measured in the pooled urine. The relative recoveries thus obtained are presented in Table 11.

Tab. 11 Mean relative recoveries for the determination of CEMA and AMCC in urine (n = 8)

Analyte	Spiked concentration [µg/l]	Mean recovery (rel.) r [%]	Range [%]
CEMA	30.9	105	98.7–114
	412	103	99.0–110
AMCC	34.2	102	91.6–112
	428	104	98.2–110

11.3 Limits of detection and quantitation

The detection limit was estimated from the threefold signal-to-noise ratio. The quantitation limit was analogously calculated from the ninefold signal-to-noise ratio. Table 12 provides the limits of detection and quantitation thus calculated for the analytes.

Tab. 12 Limits of detection and quantitation for the determination of CEMA and AMCC in urine

Analyte	Detection limit [µg/l]	Quantitation limit [µg/l]
CEMA	2.0	6.0
AMCC	2.5	7.5

11.4 Sources of error

For urine samples with high matrix burdens, a quenching effect may arise which suppresses the ionisation of the analytes, which may – in some cases – lead to higher detection limits than those given above. Any potential matrix effects can be efficiently compensated by the use of the isotope-labelled internal standards.

In order to maintain proper chromatographic resolution, both the precolumn and the particle filter should be renewed after approximately 100 injections. During external method verification, the decision was made to forgo the use of a precolumn filter.

Since the preconcentration column used during method development (LiChrospher RP-18 ADS LiChroCart® 25-4 (25 mm × 4 mm, 25 µm), Merck KGaA, Darmstadt, Germany) was no longer commercially available at the time of external verification, a different column (Strata™-X 25 µm On-Line Extraction (20 × 2.0 mm), Phenomenex Ltd. Deutschland, Aschaffenburg, Germany) was used. Furthermore, only 20 µl of each sample was injected into the LC-MS/MS system, rather than 100 µl, during external verification; this approach allowed for an improved peak form. Even with these changes, the method was easily replicable. An injection volume of 100 µl is highly recommended for the determination of low analyte levels.

12 Discussion of the method

The analytical method hereby presented enables the simultaneous determination of the mercapturic acids CEMA and AMCC as specific biomarkers of the hazardous substances acrylonitrile and dimethylformamide in human urine samples. This procedure is especially important for the biomonitoring of workers in the production of polyacrylonitrile fibres, where simultaneous exposure to both substances is to be expected (IARC 2018).

In general, this method is characterised by simple sample workup, as the samples are enriched and purified by online SPE with automatic column-switching after dilution with a buffer; this approach enables a high sample throughput. The collected validation data prove the good reproducibility and accuracy of the method, with relative standard deviations of less than 6% for within-day measurements and of less than 10% for day-to-day measurements. With mean relative recoveries between 102% and 105% the accuracy of the method can likewise be described as excellent.

With quantitation limits of 7.5 µg AMCC and 6.0 µg CEMA per litre of urine, the method allows for the sensitive measurement of AMCC background levels as well as, to a lesser extent, CEMA background levels in the non-smoking, non-occupationally exposed general population. The consistently excellent validation data were confirmed by the external verification of the method in a second, independent laboratory.

In older methods for the determination of AMCC in urine, the reported sensitivity is considerably lower than has been achieved with this method. Perbellini et al. (2001) ascertained a quantitation limit for AMCC of 1800 µg/l with their

HPLC-UV method. He et al. (2010) reported a quantitation limit for AMCC in a similar range, whereby the urine sample was analysed, after SPE, by HPLC and photodiode-array detector. The determination of AMCC by UPLC-MS/MS led to a detection limit of 200 µg/l (Sohn et al. 2005; Wu et al. 2017). Using HPLC-MS/MS, Schettgen et al. (2013) ascertained a quantitation limit of 15 µg/l when determining AMCC in urine. In contrast, an even higher sensitivity was reported by Frigerio et al. (2019), whereby, alongside AMCC and CEMA, further mercapturic acids were simultaneously measured in urine. Using LC-MS/MS, quantitation limits of 2 µg/l and 0.9 µg/l were achieved for AMCC and CEMA, respectively.

In summary, the method herein described is very suitable for the measurement of simultaneous occupational exposure to acrylonitrile and DMF via the determination of the specific urinary metabolites as well as for the verification of compliance with the current biological assessment values for both hazardous substances. With respect to urinary CEMA concentrations, the method is further able to distinguish between smokers and non-smokers.

Instruments used HPLC system (Series 1100) with a binary pump and a quaternary pump (G 1311 A), a vacuum degasser, (G 1322 A), and an autosampler (G 1313 A) (Agilent Technologies Deutschland GmbH, Waldbronn, Germany); triple-quadrupole mass spectrometer (API 2000, AB SCIEX Germany GmbH, Darmstadt, Germany)

Notes

Competing interests

The established rules and measures of the Commission to avoid conflicts of interest (www.dfg.de/mak/conflicts_interest) ensure that the content and conclusions of the publication are strictly science-based.

The views expressed in these publications are those of the individual authors acting in their personal capacity as experts and do not represent the positions of their respective institutions or employers.

References

- Angerer J, Göen T, Krämer A, Kählerlein HU (1998) N-methylcarbamoyl adducts at the N-terminal valine of globin in workers exposed to N,N-dimethylformamide. *Arch Toxicol* 72(5): 309–313. <https://doi.org/10.1007/s002040050507>
- Bader M, Barr D, Göen T, Schaller KH, Scherer G, Angerer J (2010) Reliability criteria for analytical methods. *Biomonitoring Method*, 2010. In: Angerer J, Hartwig A, editors. *The MAK-Collection for Occupational Health and Safety. Part IV: Biomonitoring Methods. Volume 12*. Weinheim: Wiley-VCH. p. 55–101. Also available from <https://doi.org/10.1002/3527600418.bireliabe0012>
- Brazdil JF (2026) Acrylonitrile. In: *Ullmann's Encyclopedia of Industrial Chemistry*, 7th ed. Weinheim: Wiley-VCH. p. 1–10. https://doi.org/10.1002/14356007.a01_177.pub3
- Bundesärztekammer (2023) Richtlinie der Bundesärztekammer zur Qualitätssicherung laboratoriumsmedizinischer Untersuchungen. *Dtsch Arztebl* 120(21–22): 1–37. https://doi.org/10.3238/arztebl.2023.rili_baek_QS_Labor
- Chen M, Carmella SG, Lindgren BR, Luo X, Ikuemonisan J, Niesen B, Thomson NM, Murphy SE, Hatsukami DK, Hecht SS (2023) Increased levels of the acrolein metabolite 3-hydroxypropyl mercapturic acid in the urine of e-cigarette users. *Chem Res Toxicol* 36(4): 583–588. <https://doi.org/10.1021/acs.chemrestox.2c00145>
- Christ T, Ellrich D, Leng G, Schmidtkunz C, Gries W, Pilz F, Scherer G, Göen T, Hartwig A, MAK Commission (2023) Alkylating substances – Determination of haemoglobin adducts as N-methylvaline, N-ethylvaline, N-propylvaline, N-butylvaline, N-(2-hydroxypropyl)valine, N-(2-hydroxyethyl)valine, N-(2-cyanoethyl)valine, N-benzylvaline, and N-(2-carbamoyl)valine in the erythrocyte fraction of whole blood by GC-MS/MS. *Biomonitoring Method – Translation of the German version from 2023. MAK Collect Occup Health Saf* 8(3): Doc074. https://doi.org/10.34865/bi33488e8_3or
- DFG (Deutsche Forschungsgemeinschaft), editor (2025) List of MAK and BAT Values 2025. Maximum Concentrations at the Workplace and Assessment Values in Biological Material. Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, report 61. Düsseldorf: German Medical Science. https://doi.org/10.34865/mbwl_2025_eng
- Fennell TR, Kedderis GL, Sumner SCJ (1991) Urinary metabolites of [1,2,3-¹³C]acrylonitrile in rats and mice detected by ¹³C nuclear magnetic resonance spectroscopy. *Chem Res Toxicol* 4(6): 678–687. <https://doi.org/10.1021/tx00024a013>
- Frigerio G, Mercadante R, Polledri E, Missineo P, Campo L, Fustinoni S (2019) An LC-MS/MS method to profile urinary mercapturic acids, metabolites of electrophilic intermediates of occupational and environmental toxicants. *J Chromatogr B Analyt Technol Biomed Life Sci* 1117: 66–76. <https://doi.org/10.1016/j.jchromb.2019.04.015>

- Frigerio G, Campo L, Mercadante R, Mielżyńska-Švach D, Pavanello S, Fustinoni S (2020 a) Urinary mercapturic acids to assess exposure to benzene and other volatile organic compounds in coke oven workers. *Int J Environ Res Public Health* 17(5): 1801. <https://doi.org/10.3390/ijerph17051801>
- Frigerio G, Mercadante R, Campo L, Polledri E, Boniardi L, Olgiati L, Missineo P, Fustinoni S (2020 b) Urinary biomonitoring of subjects with different smoking habits. Part I: profiling mercapturic acids. *Toxicol Lett* 327: 48–57. <https://doi.org/10.1016/j.toxlet.2020.03.010>
- Gallart-Mateu D, Dualde P, Coscollà C, Soriano JM, Garrigues S, de la Guardia M (2023) Biomarkers of exposure in urine of active smokers, non-smokers, and vapers. *Anal Bioanal Chem* 415(27): 6677–6688. <https://doi.org/10.1007/s00216-023-04943-w>
- Göen T, Drexler, Hans J, Hartwig A, MAK Commission (2020) N,N-Dimethylformamide – Addendum for re-evaluation of the BAT value. Assessment Values in Biological Material – Translation of the German version from 2019. *MAK Collect Occup Health Saf* 5(3): Doc059. https://doi.org/10.34865/bb6812e5_3ad
- Greim H, editor (1997) Dimethylformamide. MAK Value Documentation, 1992. In: Occupational Toxicants. Volume 8. Weinheim: VCH. p. 1–44. Also available from <https://doi.org/10.1002/3527600418.mb6812e0008>
- Greim H, editor (2007) Acrylonitrile. MAK Value Documentation, 1978, 1999. In: The MAK-Collection for Occupational Health and Safety. Part I: MAK Value Documentations. Volume 24. Weinheim: Wiley-VCH. Also available from <https://doi.org/10.1002/3527600418.mb10713e0024>
- Hartwig A, editor (2010) Dimethylformamide. MAK Value Documentation, 2006. In: The MAK-Collection for Occupational Health and Safety. Part I: MAK Value Documentations. Volume 26. Weinheim: Wiley-VCH. p. 153–182. Also available from <https://doi.org/10.1002/3527600418.mb6812e0026b>
- Hartwig A, MAK Commission (2017 a) Dimethylformamide. MAK Value Documentation, addendum – Translation of the German version from 2016. *MAK Collect Occup Health Saf* 2(1): 7–20. [Corrected republication without content-related editing. Düsseldorf: German Medical Science; 2026. Available from https://doi.org/10.34865/mb6812e6017_w]
- Hartwig A, MAK Commission (2017 b) Dimethylformamide. MAK Value Documentation, addendum – Translation of the German version from 2017. *MAK Collect Occup Health Saf* 2(4): 1545–1547. [Corrected republication without content-related editing. Düsseldorf: German Medical Science; 2026. Available from https://doi.org/10.34865/mb6812e6217_w]
- Hartwig A, MAK Commission (2020) N,N-Dimethylformamide. MAK Value Documentation, supplement – Translation of the German version from 2019. *MAK Collect Occup Health Saf* 5(1): Doc007. https://doi.org/10.34865/mb6812e5_1
- He J, Wang P, Zhu J, Wu G, Ji J, Xue Y (2010) Role of urinary biomarkers of N,N-dimethylformamide in the early detection of hepatic injury among occupational exposed workers. *Int Arch Occup Environ Health* 83(4): 399–406. <https://doi.org/10.1007/s00420-010-0520-8>
- IARC (International Agency for Research on Cancer) (1999) Acrylonitrile. In: Re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide. IARC monographs on the evaluation of carcinogenic risks to humans. Volume 71. Lyon: IARC Press. p. 43–108. <https://publications.iarc.who.int/download/mono71.pdf>, accessed 12 May 2026
- IARC (International Agency for Research on Cancer) (2018) N,N-Dimethylformamide. In: Some industrial chemicals. IARC monographs on the evaluation of carcinogenic risks to humans. Volume 115. Lyon: IARC Press. p. 119–170. <https://publications.iarc.who.int/download/Vol115-BOOK.pdf>, accessed 12 May 2026
- IARC (International Agency for Research on Cancer) (2025) Acrylonitrile. In: Talc and acrylonitrile. IARC monographs on the identification of carcinogenic hazards to humans. Volume 136. Lyon: IARC Press. p. 471–778. https://publications.iarc.who.int/download/Volume136_Nov2025.pdf, accessed 28 May 2026
- IFA (Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung) (2026) Acrylonitrile. GESTIS Substance Database. <https://gestis.dguv.de/data?name=011410&lang=en>, accessed 12 May 2026
- Jäger T, Drexler H, Hartwig A, MAK Commission (2021) Acrylonitrile – Addendum for re-evaluation of EKA and BAR. Assessment Values in Biological Material – Translation of the German version from 2021. *MAK Collect Occup Health Saf* 6(1): Doc014. https://doi.org/10.34865/bb10713e6_1ad
- Jakubowski M, Linhart I, Pielas G, Kopecký J (1987) 2-Cyanoethylmercapturic acid (CEMA) in the urine as a possible indicator of exposure to acrylonitrile. *Br J Ind Med* 44(12): 834–840. <https://doi.org/10.1136/oem.44.12.834>
- Käfferlein HU, Göen T, Müller J, Wrbitzky R, Angerer J (2000) Biological monitoring of workers exposed to N,N-dimethylformamide in the synthetic fibre industry. *Int Arch Occup Environ Health* 73(2): 113–120. <https://doi.org/10.1007/s004200050016>
- Käfferlein HU, Mráz J, Ferstl C, Angerer J (2004) Analysis of metabolites of N,N-dimethylformamide in urine samples. *Int Arch Occup Environ Health* 77(6): 427–432. <https://doi.org/10.1007/s00420-004-0538-x>
- Käfferlein HU, Ferstl C, Burkhart-Reichl A, Hennebrüder K, Drexler H, Brüning T, Angerer J (2005) The use of biomarkers of exposure of N,N-dimethylformamide in health risk assessment and occupational hygiene in the polyacrylic fibre industry. *Occup Environ Med* 62(5): 330–336. <https://doi.org/10.1136/oem.2004.017129>
- Käfferlein HU, Angerer J, Leng G (2013) N-Acetyl-S-(N-methylcarbamoyl)-cysteine (AMCC), N-hydroxymethyl-N-methylformamide (HMMF) and N-methylformamide (NMF) in urine. Biomonitoring Method, 2012. In: Göen T, Hartwig A, editors. The MAK-Collection for Occupational Health and Safety. Part IV: Biomonitoring Methods. Volume 13. Weinheim: Wiley-VCH. p. 3–19. Also available from <https://doi.org/10.1002/3527600418.bi6812e0013>
- Käfferlein HU, Angerer J, Leng G, Gries W, Eckert E, Ferstl C, Göen T, Hartwig A, MAK Commission (2016) 3-Methyl-5-isopropylhydantoin as hemoglobin adduct of N,N-dimethylformamide and methylisocyanate. Biomonitoring Method, 2015. *MAK Collect Occup Health Saf* 1(1): 554–577. <https://doi.org/10.1002/3527600418.bi6812e2115a>

- Kilo S, Göen T, Drexler H (2016) Cross-sectional study on N,N-dimethylformamide (DMF); effects on liver and alcohol intolerance. *Int Arch Occup Environ Health* 89(8): 1309–1320. <https://doi.org/10.1007/s00420-016-1164-0>
- Kraus T, Angerer J, Bader M, Schettgen T (2021) Acrylonitrile – Addendum for evaluation of a BAR. Assessment Values in Biological Material – Translation of the German version from 2010. MAK Collect Occup Health Saf: Doc918. https://doi.org/10.34865/bb10713eoj21_1ad
- Li C, Guo Y, Duan K, Wang Z, Wu Z, Jiang X, Yang L, Hu S, Li S, Huang M, Zhong G (2024) Changes in biomarkers of exposure and withdrawal symptom among Chinese adult smokers after completely or partially switching from combustible cigarettes to an electronic nicotine delivery system. *Intern Emerg Med* 19(3): 669–679. <https://doi.org/10.1007/s11739-023-03518-y>
- Lin C-Y, Lee H-L, Sung F-C, Su T-C (2018) Investigating the association between urinary levels of acrylonitrile metabolite N-acetyl-S-(2-cyanoethyl)-L-cysteine and the oxidative stress product 8-hydroxydeoxyguanosine in adolescents and young adults. *Environ Pollut* 239: 493–498. <https://doi.org/10.1016/j.envpol.2018.04.010>
- NCEH (National Center for Environmental Health (U.S.). Division of Laboratory Sciences) (2021) Fourth national report on human exposure to environmental chemicals. Updated tables, March 2021, Volume four: analysis of chemicals found in cigarette smoke in a special sample of U.S. adults, NHANES 2011–2016. Atlanta, GA: CDC. https://stacks.cdc.gov/view/cdc/105343/cdc_105343_DS1.pdf, accessed 28 May 2026
- Perbellini L, Maestri L, Veronese N, Romani S, Brugnone F (2001) Analysis of urinary N-acetyl-S-(N-methylcarbamoyl)cysteine, the mercapturic acid derived from N,N-dimethylformamide. *J Chromatogr B Biomed Sci Appl* 759(2): 349–354. [https://doi.org/10.1016/s0378-4347\(01\)00242-0](https://doi.org/10.1016/s0378-4347(01)00242-0)
- Sakai T, Kageyama H, Araki T, Yosida T, Kuribayashi T, Masuyama Y (1995) Biological monitoring of workers exposed to N,N-dimethylformamide by determination of the urinary metabolites, N-methylformamide and N-acetyl-S-(N-methylcarbamoyl) cysteine. *Int Arch Occup Environ Health* 67(2): 125–129. <https://doi.org/10.1007/BF00572236>
- Scherer G, Gilch G, Aust B, Blaszkewicz M, Göen T, Hartwig A, MAK Commission (2018) Formamide, dimethylformamide – Determination of formamide in urine by gas chromatography mass spectrometry. *Biomonitoring Method*, 2018. MAK Collect Occup Health Saf 3(1): 382–397. [Corrected republication without content-related editing, Düsseldorf: German Medical Science; 2025. Available from https://doi.org/10.34865/bi7512e2218_wj]
- Schettgen T, Musiol A, Kraus T (2008) Simultaneous determination of mercapturic acids derived from ethylene oxide (HEMA), propylene oxide (2-HPMA), acrolein (3-HPMA), acrylamide (AAMA) and N,N-dimethylformamide (AMCC) in human urine using liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom* 22(17): 2629–2638. <https://doi.org/10.1002/rcm.3659>
- Schettgen T, Musiol A, Alt A, Ochsmann E, Kraus T (2009) A method for the quantification of biomarkers of exposure to acrylonitrile and 1,3-butadiene in human urine by column-switching liquid chromatography-tandem mass spectrometry. *Anal Bioanal Chem* 393(3): 969–981. <https://doi.org/10.1007/s00216-008-2510-1>
- Schettgen T, Scherer G, Sterz K (2013) Mercapturic acids (N-acetyl-S-2-carbamoyl-L-cysteine, N-acetyl-S-2-hydroxyethyl-L-cysteine, N-acetyl-S-3-hydroxypropyl-L-cysteine, N-acetyl-S-2-hydroxypropyl-L-cysteine, N-acetyl-S-(N-methylcarbamoyl)-L-cysteine) in urine. *Biomonitoring Method*, 2012. In: Göen T, Hartwig A, editors. *The MAK-Collection for Occupational Health and Safety. Part IV: Biomonitoring Methods. Volume 13*. Weinheim: Wiley-VCH. p. 123–162. Also available from <https://doi.org/10.1002/3527600418.bi0mercapace0013>
- Schettgen T, Müller J, Ferstl C, Angerer J, Weiss T, Leng G, Göen T, Hartwig A, MAK Commission (2016) Haemoglobin adducts of ethylene oxide (N-(2-hydroxyethyl)valine), propylene oxide (N-(2-hydroxypropyl)valine), acrylonitrile (N-(2-cyanoethyl)valine), acrylamide (N-(2-carbamoyl)valine) and glycidamide (N-(2-hydroxy-2-carbamoyl)valine). *Biomonitoring Method*, 2015. MAK Collect Occup Health Saf 1(1): 473–506. <https://doi.org/10.1002/3527600418.bi7521e2115>
- Seitz M, Kilo S, Eckert E, Müller J, Drexler H, Göen T (2018) Validity of different biomonitoring parameters for the assessment of occupational exposure to N,N-dimethylformamide (DMF). *Arch Toxicol* 92(7): 2183–2193. <https://doi.org/10.1007/s00204-018-2219-7>
- van Sittert NJ, Angerer J, Bader M, Blaszkewicz M, Ellrich D, Krämer A, Lewalter J (1997) N-2-Cyanoethylvaline, N-2-Hydroxyethylvaline, N-Methylvaline (as evidence of exposure to acrylonitrile, ethylene oxide as well as methylating agents). *Biomonitoring Method*, 1996. In: Angerer J, Schaller KH, Greim H, editors. *Analyses of Hazardous Substances in Biological Materials. Volume 5*. Weinheim: VCH. p. 181–210. Also available from <https://doi.org/10.1002/3527600418.bi2176851e0005>
- Sohn JH, Han MJ, Lee MY, Kang S-K, Yang JS (2005) Simultaneous determination of N-hydroxymethyl-N-methylformamide, N-methylformamide and N-acetyl-S-(N-methylcarbamoyl)cysteine in urine from workers exposed to N,N-dimethylformamide by liquid chromatography-tandem mass spectrometry. *J Pharm Biomed Anal* 37(1): 165–170. <https://doi.org/10.1016/j.jpba.2004.10.001>
- Stayner LT, Carreón-Valencia T, Demers PA, Fritz JM, Sim MR, Stewart P, Tsuda H, Cardenas A, Consonni D, Davies L, De Matteis S, Felley-Bosco E, Ghio AJ, Göen T, Grosse Y, Gualtieri AF, Josephy PD, Koutros S, Linhart I, Louro H, O'Brien KM, Panzacchi S, Peña L, Rössner P, Schildkraut JM, Stefaniak AB, Wentzensen N, Wild P, Xu Y, de Conti A, Facchin C, Wedekind R, Ahmadi A, Blanco J, Chittiboyina S, Kulasingam S, MacLehose R, Motlhale M, Shah S, Suonio E, Mattock H, Kunzmann A, Madia F, Pasqual E, Benbrahim-Tallaa L, Schubauer-Berigan MK (2024) Carcinogenicity of talc and acrylonitrile. *Lancet Oncol* 25(8): 962–963. [https://doi.org/10.1016/s1470-2045\(24\)00384-x](https://doi.org/10.1016/s1470-2045(24)00384-x)
- Sumner SC, Fennell TR, Moore TA, Chanas B, Gonzalez F, Ghanayem BI (1999) Role of cytochrome P450 2E1 in the metabolism of acrylamide and acrylonitrile in mice. *Chem Res Toxicol* 12(11): 1110–1116. <https://doi.org/10.1021/tx990040k>
- Will W, Bader M, Göen T, Hartwig A, MAK Commission (2016) N-Methylformamide and N-methylacetamide in urine. *Biomonitoring Method*, 2015. MAK Collect Occup Health Saf 1(1): 536–553. <https://doi.org/10.1002/3527600418.bi6812e2115b>
- Wu Z, Liu Q, Wang C, Xu B, Guan M, Ye M, Jiang H, Zheng M, Zhang M, Zhao W, Jiang X, Leng S, Cheng J (2017) A comparative benchmark dose study for N,N-dimethylformamide induced liver injury in a Chinese occupational cohort. *Toxicol Sci* 158(1): 140–150. <https://doi.org/10.1093/toxsci/kfx076>

Wu K-Y, Wu C-F, Luo Y-S, Huang Y-F, Uang S-N, Lee Y-Y, Chiang S-Y (2023) Study of urinary mercapturic acids as biomarkers of human acrylonitrile exposure. *Toxicol Lett* 373: 141–147. <https://doi.org/10.1016/j.toxlet.2022.11.006>