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1-Bromopropane and 2-bromopropane – Determination of 1-bromopropane and 2-bromopropane in urine by dynamic headspace GC/MS

Biomonitoring Method – Translation of the German version from 2019

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Biomonitoring Methods

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Abstract

The working group "Analyses in Biological Materials" of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area verified the presented biomonitoring method. The analytical method described herein permits the sensitive determination of both unmetabolised 1-bromopropane and 2-bromopropane in urine. Prior to the determination using GC-MS in the Single-Ion-Mode (SIM), the analytes are extracted and enriched using Headspace Solid Phase Dynamic Extraction (SPDE). To this end, the urine samples are incubated at 50 °C and the analytes are extracted from the gas phase using SPDE. The headspace extract is then injected into the gas chomatograph for separation and mass spectrometric analysis. Calibration standards are prepared in urine and processed in the same way as the samples to be analysed. Deuterated benzene is used as the internal standard (IS).

Keywords

1-bromopropane; 2-bromopropane; urine; biomonitoring; Analyses in Biological Materials; headspace-solid phase dynamic extraction; HS-SPDE; gas chromatography mass spectrometry; GC-MS

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Matrix:	Urine
Hazardous substances:	1-Bromopropane, 2-bromopropane
Analytical principle:	Headspace-gas chromatography with mass spectrometric detection (HS-GC/MS)
Completed in:	October 2013

Overview of the parameters that can be determined with this method and the corresponding hazardous substances:

Hazardous substance	CAS	Parameter	CAS
1-Bromopropane	106-94-5	1-Bromopropane	106-94-5
2-Bromopropane	75-26-3	2-Bromopropane	75-26-3

Summary

The analytical method described herein permits the sensitive determination of both unmetabolised 1-bromopropane and 2-bromopropane in urine. Prior to the determination using GC-MS in the Single-Ion-Mode (SIM), the analytes are extracted and enriched using Headspace-Solid Phase Dynamic Extraction (SPDE). To this end, the urine samples are incubated at 50 °C and the analytes are extracted from the gas phase using SPDE. The headspace extract is then injected into the gas chromatograph for separation and mass spectrometric analysis. Calibration standards are prepared in urine and processed in the same way as the samples to be analysed. Deuterated benzene is used as the internal standard (IS).

Reliability data of the method

1-Bromopropane

Within-day precision:	Standard deviation (rel.)	s_w = 3.83%, 2.34% or 2.41%
	Prognostic range	u = 9.37%, 5.73% or 5.90%
	at spiked concentrations of 0.22 µg, 2.17 µg or 16.3 µg 1-bromopropane per litre urine and where n = 7 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 6.76%, 2.05% or 2.91%
	Prognostic range	u = 16.5%, 5.02% or 7.12%
	at spiked concentrations of 0.22 µg, 2.17 µg or 16.3 µg 1-bromopropane per litre urine and where n = 7 determinations	
Accuracy:	Recovery rate (rel.)	r = 95.3%, 106% or 107%
	at spiked concentrations of 0.22 µg, 2.17 µg or 16.3 µg 1-bromopropane per litre urine and where n = 7 determinations	
Detection limit:	0.01 µg 1-bromopropane per litre urine	
Quantitation limit:	0.03 µg 1-bromopropane per litre urine	

2-Bromopropane

Within-day precision:	Standard deviation (rel.)	s_w = 6.89%, 3.47% or 6.08%
	Prognostic range	u = 16.9%, 8.49% or 14.9%
	at spiked concentrations of 0.21 µg, 2.10 µg or 15.7 µg 2-bromopropane per litre urine and where n = 7 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 7.00%, 5.50% or 6.77%
	Prognostic range	u = 17.1%, 13.5% or 16.6%
	at spiked concentrations of 0.21 µg, 2.10 µg or 15.7 µg 2-brompropane per litre urine and where n = 7 determinations	
Accuracy:	Recovery rate (rel.)	r = 88.0%, 92.3% or 108%
	at spiked concentrations of 0.21 µg, 2.10 µg or 15,7 µg 2-bromopropane per litre urine and where n = 7 determinations	
Detection limit:	0.01 µg 2-bromopropane per litre urine	
Quantitation limit:	0.04 µg 2-bromopropane per litre urine	

General information on 1-bromopropane and 2-bromopropane

1-Bromopropane is primarily used as a solvent for fats, waxes or resins. One area of application is, for example, the degreasing of metal parts, optical instruments or electronic components. It is also used as a solvent in spray adhesives or as an intermediate in the synthesis of other compounds including pharmaceuticals, insecticides, quaternary ammonium compounds, flavours or fragrances. Besides, 1-bromopro-

pane is used as a substitute for ozone-depleting substances. 1-Bromopropane is known to be contaminated by small amounts of 2-bromopropane, which is formed as a byproduct during production. By using improved production procedures, it was possible to reduce the amount of 2-bromopropane from 0.1 to 0.2% to now 0.05% and less [Hartwig 2011; NTP 2013; Valentine et al. 2007].

Critical properties associated with 1-bromopropane are its irritating effects, neurotoxicity, carcinogenicity and its adverse effect on fertility. Based on evidence from long-term studies in rats and mice, the compound is to be considered carcinogenic to humans and has been classified by the Commission as carcinogen of category 2. 2-Bromopropane likewise proved to be neurotoxic. In animal studies, systemically available 1-bromopropane was detected after inhalative exposure. Besides, this compound has been designated with an "H" in the List of MAK and BAT Values, indicating that it can be absorbed through the skin in toxicologically relevant amounts in case of dermal exposure [DFG 2018; Hartwig 2011].

Experiments on rodents have shown that absorbed 1-bromopropane is predominantly eliminated by exhalation either of the unchanged substance or of volatile metabolites such as carbon dioxide. Another major route of elimination is the excretion of metabolites in urine, either by direct glutathione conjugation and subsequent degradation to *S-n*-propyl mercapturic acid or by CYP450-catalysed oxidation via 1-bromo-2-hydroxypropane, from which numerous derivatives may result. A detailed description of the metabolism of 1-bromopropane can be found, inter alia, in the corresponding MAK Value Documentation of the Commission [Hartwig 2011]. The concentration of *S-n*-propyl mercapturic acid in urine can be used to quantify internal exposure to 1-bromopropane. There is an EKA correlation available that describes the relation between the air concentration of 1-bromopropane at the workplace and the urinary concentration of this biomarker [DFG 2018; Drexler and Hartwig 2011]. An analytical method which permits the quantification of *S-n*-propyl mercapturic acid and of the isomeric *S-iso*-propyl mercapturic acid, the metabolite of 2-bromopropane, has been published by the Commission [Eckert et al. 2016].

In the past, bromide in urine and unchanged bromopropane in urine were also used as biomarkers of exposure to 1-bromopropane. In particular post-shift unmetabolised 1-bromopropane in urine correlated with 1-bromopropane in air. However, estimates suggest that only 0.002% of inhaled 1-bromopropane is excreted unchanged in urine [Ichihara et al. 2004; Kawai et al. 2001]. According to Kawai et al. [2001], 1-bromopropane excretion levels of approximately 0.6 µg/L urine and lower can be expected at air concentrations at the lower end of the EKA correlation (≤1 ppm). The method described hereinafter should therefore allow reliable detection of 1-bromopropane and 2-bromopropane even at these low concentrations.

Contents

1 General principles

The analytical method described herein permits the sensitive determination of both unmetabolised 1-bromopropane and 2-bromopropane in urine. Prior to the determination using GC-MS in the Single-Ion-Mode (SIM), the analytes are extracted and enriched using Headspace-Solid Phase Dynamic Extraction (SPDE). To this end, the urine samples are incubated at 50 °C and the analytes are extracted from the gas phase using SPDE. The headspace extract is then injected into the gas chromatograph for separation and mass spectrometric analysis. Calibration standards are prepared in urine and processed in the same way as the samples to be analysed. Deuterated benzene is used as the internal standard (IS).

2 Equipment, chemicals and solutions

2.1 Equipment

- Gas chromatograph with split/splitless injector, mass selective detector (MSD) and data processing system
- Autosampler with SPDE option (e. g. Chromtech GmbH), consisting of:
  - Combi PAL autosampler with control software ("Cycle Composer")
  - Gas tight 2.5 mL headspace syringe
  - 74 × 0.8 mm conical tip needle with side port, coated on its inner side with the extraction phase (polydimethylsiloxane with 10% activated carbon; 50 µm film thickness)
  - Syringe temperature controller
  - Temperature controlled sample tray ("Single Magnet Mixer")
  - Extraction cooler for thermostatting the SPDE needle during extraction
  - Heated flush station for cleaning and conditioning of the extraction needle
  - Gas station for the aspiration of the desorption gas (nitrogen)
- Gas chromatographic column: VF-WAXms 60 m × 0.25 mm × 0.5 µm (e. g. Agilent Technologies)
- 20 mL headspace screw cap vials (e. g. CS-Chromatographie Service GmbH)
- Magnetic headspace screw caps with PTFE septa (e. g. CS-Chromatographie Service GmbH)
- 8 mL screw cap vials with PTFE septa (e. g. CS-Chromatographie Service GmbH)
- Microlitre syringes 1–10 µL and 10–100 µL (e. g. VWR)
- Microlitre pipettes with adjustable volumes between 10–100 µL and 100–1000 µL, and matching pipette tips (e. g. Eppendorf)
- Manual dispenser for dispensing 2 µL aliquots (e. g. Eppendorf Multipette)
- 10 mL and 100 mL volumetric flasks (e. g. Brand)
- Analytical balance (e. g. Mettler-Toledo)
- Glas container with PTFE-coated screw caps for urine collection (e. g. VWR)

2.2 Chemicals

Unless otherwise specified, all chemicals must be at least p. a. grade.

- Acetone (e. g. Sigma-Aldrich No. 1.00012)
- Benzene-d_6, 99.5% (e. g. Dr. Ehrenstorfer No. C10535200)
- 1-Bromopropane, 99% (e. g. Sigma-Aldrich No. B78106)
- 2-Bromopropane, 99% (e. g. Sigma-Aldrich No. B78114)
- Methanol (e. g. Merck No. 1.06007)
- Tetraethylene glycol dimethyl ether, 99% (e. g. Sigma-Aldrich No. 172405)
- Helium 5.0 (e. g. Linde)
- Nitrogen 5.0 (e. g. Linde)
- double distilled water

2.3 Internal standard

- IS stock solution
  Approx. 9 mL tetraethylene glycol dimethyl ether are placed into a 10 mL volumetric flask pre-flushed with nitrogen. Then, 3 µL benzene-d_6 (density 0.950 mg/µL), equivalent to a mass of 2.85 mg, are added using a microlitre syringe and the flask is filled to the mark with tetraethylene glycol dimethyl ether. The concentration of the resulting stock solution is 285 mg/L.

- IS spiking solution
  Approx. 8 mL tetraethylene glycol dimethyl ether are placed into a 10 mL volumetric flask pre-flushed with nitrogen. Then, 400 µL of the IS stock solution are added and the flask is filled to the mark with tetraethylene glycol dimethyl ether. The concentration of the resulting spiking solution is 11.4 mg/L.

After being transferred into 8 mL screw cap vials, the solutions are stable for at least six months when stored at +4 °C.

2.4 Calibration standards

- Stock solution
  Approx. 9 mL tetraethylene glycol dimethyl ether are placed into a 10 mL volumetric flask pre-flushed with nitrogen. Then, 2 µL each of 1-bromopropane (density 1.354 mg/µL) and 2-bromopropane (density 1.310 mg/µL), equivalent to a mass of 2.708 mg and 2.620 mg, respectively, are added using a microlitre syringe and the flask is filled to the mark with tetraethylene glycol dimethyl ether. The concentrations of this stock solution are 270.8 mg 1-bromopropane and 262.0 mg 2-bromopropane per litre.

- Spiking solution I
  Approx. 5 mL tetraethylene glycol dimethyl ether are placed into a 10 mL volumetric flask pre-flushed with nitrogen. The flask is then closed and weighed. With a suitable pipette about 2.0 mL of the stock solution are added and the flask is then closed and weighed anew. Afterwards, the flask is filled to the mark with tetraethylene glycol dimethyl ether. The mass of the added stock solution is determined by calculating the mass difference and is then converted to the corresponding volume using the density of tetraethylene glycol dimethyl ether (1.009 g/mL). If exactly 2.0 mL of the stock solution are added, the concentrations of the resulting solution are 54.2 mg 1-bromopropane and 52.4 mg 2-bromopropane per litre.

- Spiking solution II
  Approx. 9 mL tetraethylene glycol dimethyl ether are placed into a 10 mL volumetric flask pre-flushed with nitrogen. Then, 100 µL of the stock solution are added using a microlitre syringe and the flask is filled to the mark with tetraethylene glycol dimethyl ether. The concentrations of this solution are 2.71 mg 1-bromopropane and 2.62 mg 2-bromopropane per litre.

- Spiking solution III
  Approx. 9 mL tetraethylene glycol dimethyl ether are placed into a 10 mL volumetric flask pre-flushed with nitrogen. Then, 4 µL of the stock solution are added using a microlitre syringe and the flask is filled to the mark with tetraethylene glycol dimethyl ether. The concentrations of the resulting solution are 0.108 mg 1-bromopropane and 0.105 mg 2-bromopropane per litre.

After being transferred into 8 mL screw cap vials, the stock solution and the spiking solutions are stable for at least six months when stored at +4 °C.

Calibration standards can be prepared in a concentration range of approx. 0.02 to 100 µg/L by diluting the spiking solutions with pooled urine. Depending on the expected analyte concentrations, a five-point calibration in the concentration range between 0.2 and 20 µg/L is sufficient in most cases. A corresponding five-point calibration consisting of an unspiked urine sample and four calibration standards was also used to determine precision and recovery data (see Section 9).

The calibration standards are directly prepared in the 20 mL headspace vials used for analysis. To this end, the vials are initially pre-flushed with nitrogen for about 15 s and covered loosely with the screw caps. Then 5 mL pooled urine are pipetted into each vial. Using microlitre syringes, the spiking solutions are added into the prepared vials according to the scheme shown in Table 1.

The calibration standards should be freshly prepared for each analytical run and be analysed immediately. It is not advisable to store the prepared calibration standards (cf. Section 9.5).

Table 1: Pipetting scheme for the preparation of calibration standards in pooled urine.

Calibration standard	Spiking solution	Volume of pooled urine	Volume of spiking solution	concentration 1-bromopropane	concentration 2-bromopropane
		[µL]	[µL]	[µg/L]	[µg/L]
1	–	5000	0	0	0
2	III	5000	10	0.22	0.21
3	II	5000	2	1.08	1.05
4	II	5000	10	5.42	5.24
5	I	5000	2	21.7	21.0

3 Specimen collection and sample preparation

3.1 Specimen collection

Glass container with PTFE-coated screw caps are used for urine collection. A contaminant-free environment must be ensured. Immediately following the urine void, a 5 mL aliquot of the sample is to be transferred to a 20 mL headspace screw cap vial pre-flushed with nitrogen.

The sample can be stored in the headspace vial only for a few days in the refrigerator. Longer-term storage (over one week) even of cooled samples entails analyte loss.

3.2 Sample preparation

5 mL of the urine sample are spiked with 2 µL of the IS spiking solution. To this end, the vial is decapped and 2 µL of the IS spiking solution are added using a manual dispenser. The vial is recapped immediatly and gently swirled to mix the contents thoroughly. The resulting concentration of the internal standard in the sample is 4.56 µg/L.

4 Operational parameters

4.1 Headspace-SPDE

Incubation:	18 min at 50 °C
Sorbent:	polydimethylsiloxane with 10% activated carbon
Syringe temperature:	60 °C
Needle temperature:	–5 °C
Extraction volume:	1250 µL
Number of strokes:	40
Flow rate during extraction:	200 µL/s
Desorption gas:	nitrogen 5.0
Desorption volume:	250 µL
Desorption temperature:	250 °C (GC injector)
Desorption flow rate:	50 µL/s
Needle reconditioning:	18 min at 250 °C (nitrogen flushing)

4.2 Gas chromatography

Capillary column:	Material:	Fused silica
	Stationary phase:	VF-WAXms
	Length:	60 m
	Inner diameter:	0.25 mm
	Film thickness:	0.5 µm
Detector:	MSD	
Temperatures:	Column:	Initial temperature 38 °C, 2 min isothermal; increase at a rate of 5 °C/min to 90 °C, then increase at a rate of 30 °C/min to 220 °C, 5 min isothermal
	Injector:	250 °C
	Transfer line:	280 °C
Carrier gas:	Helium 5.0	
	Flow rate:	1.5 mL/min, constant
Injection volume:	250 µL	
Split:	1:10	

4.3 Mass spectrometry

Ionisation type:	Electron ionisation (EI)
Ionisation energy:	70 eV
Source temperature:	230 °C
Quadrupole temperature:	150 °C
Dwell time:	100 ms
Detection mode:	Single Ion Monitoring

All parameters are guide values only and may have to be optimised in accordance with the manufacturer's specifications.

5 Analytical determination

The samples prepared in accordance with Section 3.2 are extracted using the SPDE system described. The enriched analytes are thermally desorbed in the GC injector for analysis and transferred to the GC/MS column in a stream of desorption gas. The analytes are identified on the basis of their retention times and characteristic ion traces. The temporal profiles of the ion traces shown in Table 2 are recorded in the SI-mode to identify and quantify the analytes. Two quality control samples (see Section 8) as well as one reagent blank consisting of double distilled water are included in each analytical run.

Table 2: Retention times and detected ion traces for the determination of 1-bromopropane and 2-bromopropane in urine.

Analyte	Retention time	Ion trace [*m/z*]	
	[min]	Quantifier	Qualifier
2-Bromopropane	6.17	122	124
1-Bromopropane	7.32	122	124
Benzene-d_6 (IS)	9.52	84	82

The retention times given in Table 2 are intended to be a rough guide only. Users of the method must ensure proper separation performance of the capillary column used, influencing the resulting retention behaviour of the analytes. Figure 1 (in the Appendix) shows as an example a chromatogram of a spiked pooled urine sample.

6 Calibration

The calibration standards (Section 2.4) are processed in the same way as the samples to be analysed (Section 3) and analysed according to Sections 4 and 5. Calibration curves are obtained by plotting the peak area ratios of the respective analyte and the IS against the spiked concentrations. The calibration curves for both analytes are linear between the detection limit and 100 µg/L. In most cases, however, calibration in a concentration range up to 20 µg/L has proved to be sufficient. Figure 2 (in the Appendix) shows an example of the calibration curves for both analytes.

7 Calculation of the analytical results

The analyte concentrations in the urine samples are calculated using the calibration function of the respective analytical run (Section 6). The analyte concentration in a urine sample is determined by calculation of the peak area ratio of the analyte and the IS. The ratio thus obtained is entered in the calibration function (Section 6) to give the analyte concentration in µg/L.

Any reagent blank values have to be subtracted from the analytical results. However, no reagent blank values were observed during method development.

8 Standardisation and quality control

Quality control of the analytical results is carried out as stipulated in the guidelines of the Bundesärztekammer (German Medical Association) and in a general chapter of the MAK Collection for Occupational Health and Safety [Bader et al. 2010; Bundesärztekammer 2014].

To check precision, two quality control samples with known concentrations of 1-bromopropane and 2-bromopropane (Q_{low} and Q_{high}) are analysed within each

analytical run. As material for quality control is not commercially available, it must be prepared in the laboratory. To this end, 5 mL aliquots of pooled urine were transfered in nitrogen-flushed headspace vials and spiked with a standard solution of the analytes. The concentration of the control material should lie within the relevant concentration range (e. g. Q_{low} c = 0.2 µg/L and Q_{high} c = 2.0 µg/L). After thorough mixing, the quality control material thus prepared is stored in the refrigerator. Due to the limited shelf life of the samples, it is advisable to prepare only a week's supply of control material at once.

The nominal values and the tolerance ranges of the quality control material are determined in the course of a pre-analytical period [Bader et al. 2010]. The measured values of the control samples analysed within each analytical run should lie within the determined tolerance ranges.

9 Evaluation of the method

The reliability of the method was confirmed by comprehensive validation and by implementation and validation of the method in an independent laboratory.

9.1 Precision

To determine within-day precision, pooled urine samples from individuals not occupationally exposed to bromopropane were spiked with the analytes at three different concentrations. Each sample was then processed seven times in parallel and analysed. Spiking of the samples was done as described in Section 2.4. The obtained within-day precision data are presented in Table 3.

Table 3: Within-day precision for the determination of 1-bromopropane and 2-bromopropane in urine (n = 7).

Analyte	Spiked concentration	Standard deviation (rel.) s_w	Prognostic range u
	[µg/L]	[%]	[%]
1-Bromopropane	0.22	3.83	9.37
	2.17	2.34	5.73
	16.3	2.41	5.90
2-Bromopropane	0.21	6.89	16.9
	2.10	3.47	8.49
	15.7	6.08	14.9

To determine day-to-day precision, the spiked pooled urine samples were prepared, processed and analysed on seven consecutive days. The samples prepared in headspace vials were stored upside down in the refrigerator (+4 °C) until analysis. This prevented analyte losses caused by contact of the gas phase with the headspace vial septum. The obtained day-to-day precision data are presented in Table 4.

9.2 Accuracy

Recovery experiments were performed to determine the accuracy of the method. To this end, pooled urine from individuals not occupationally exposed to bromopropane was spiked with 1-bromopropane and 2-bromopropane at three different concentrations and subsequently analysed. Seven replicate determinations of the samples yielded the relative recovery rates shown in Table 5.

Table 4: Day-to-day precision for the determination of 1-bromopropane and 2-bromopropane in urine (n = 7).

Analyte	Spiked concentration	Standard deviation (rel.) s_w	Prognostic range u
	[µg/L]	[%]	[%]
1-Bromopropane	0.22	6.76	16.5
	2.17	2.05	5.02
	16.3	2.91	7.12
2-Bromopropane	0.21	7.00	17.1
	2.10	5.50	13.5
	15.7	6.77	16.6

Table 5: Relative recovery rates for the determination of 1-bromopropane and 2-bromopropane in urine (n = 7).

Analyte	Spiked concentration	Mean relative recovery r	Range
	[µg/L]	[%]	[%]
1-Bromopropane	0.22	95.3	90–101
	2.17	106	102–110
	16.3	107	104–112
2-Bromopropane	0.21	88.0	81–97
	2.10	92.3	88–97
	15.7	108	92–113

9.3 Matrix effects

In addition, the precision and accuracy of the method were determined in different urine samples. To this end, six individual urine samples (creatinine level of 0.29 to 2.91 g/L) were processed and analysed, both unspiked and spiked with the analytes. The obtained precision data and relative recovery rates are presented in Tables 6 and 7.

To further investigate matrix effects, calibration standards were prepared in water and in pooled urine, processed and analysed.

Table 8 shows the slopes of the calibration curves in water and in urine for the respective analytes ($R^2 > 0.999$). A comparison of the slopes of the calibration curves indicates a negligible influence of the sample matrix on the analytical results.

9.4 Limits of detection and limits of quantitation

The limits of detection and of quantitation were calculated based on DIN 32645 [DIN 2008] using ten calibration standards in urine in a concentration range between 0.02 and 0.20 µg/L and an unspiked urine sample. The obtained limits of detection and quantitation are shown in Table 9.

9.5 Sources of error

When determining 1-bromopropane and 2-bromopropane in urine, sample contamination and analyte losses are potential sources of error. Urine samples should therefore be collected in a contaminant-free environment, using glass containers with PTFE-lined screw caps for urine collection. An aliquot of the collected urine samples should be transferred to a nitrogen-flushed headspace vial immediately after sampling, as is usual for headspace analyses.

In addition to the relatively high volatility of the analytes, their partly limited stability in aqueous solutions must be taken into account. Considerable analyte loss occurred in particular for 2-bromopropane during long-term storage of the standards prepared in water or in urine. Such loss is due to the hydrolysis of 2-bromopropane to isopropanol. By contrast, 1-bromopropane decomposes much more slowly to 1-propanol [Vollhardt and Schore 2011] so that hardly any losses occur. Due to the limited stability of 2-bromopropane in aqueous media, the samples stored in the refrigerator at +4 °C should be analysed within one week. Freezing the samples at –20 °C provides no benefits in terms of analyte loss. Considerable losses are also to be expected if the urine samples are stored in plastic containers at –80 °C [B'Hymer and Cheever 2005].

The calibration standards and quality control samples prepared in water or urine should also be freshly prepared due to the limited analyte stability. By contrast, the standard solutions prepared in tetraethylene glycol dimethyl ether that are used for the preparation of standards and control material are stable for at least six months even when stored at +4 °C.

To avoid carry-over of the analytes, the conditioning phase of the SPDE system must be long enough. Conditioning of the system throughout sample incubation time has proved successful in practice.

Table 6: Within-day precision for the determination of 1-bromopropane and 2-bromopropane in individual urine samples (n = 6).

Analyte	Spiked concentration	Standard deviation (rel.) s_w	Prognostic range u
	[µg/L]	[%]	[%]
1-Bromopropane	1.08	6.30	16.2
2-Bromopropane	1.05	6.50	16.7

Table 7: Relative recovery rates for the determination of 1-bromopropane and 2-bromopropane in individual urine samples (n = 6).

Analyte	Spiked concentration	Mean relative recovery	Range
	[µg/L]	[%]	[%]
1-Bromopropane	1.08	101	88–108
2-Bromopropane	1.05	96.6	86–105

Table 8: Comparison of the calibration curves in water and in pooled urine.

Analyte	Calibration function in water	Calibration function in pooled urine	Difference of the slopes of the calibration curves [%]
1-Bromopropane	y = 0.01741*x	y = 0.01729*x	–0.7
2-Bromopropane	y = 0.00780*x	y = 0.00758*x	–2.8

Table 9: Limits of detection and quantitation for the determination of 1-bromopropane and 2-bromopropane in urine.

Analyte	Detection limit [µg/L]	Quantitation limit [µg/L]
1-Bromopropane	0.01	0.03
2-Bromopropane	0.01	0.04

10 Discussion of the method

The method described herein enables the sensitive detection of 1-bromopropane and 2-bromopropane in urine after enrichment by Headspace-Solid Phase Dynamic Extraction (HS-SPDE). Compared to the methods previously described in the literature, for which detection limits of up to 0.5 µg/L are given [Kawai et al. 2001; Ichihara et al. 2004; B'Hymer and Cheever 2005], sensitivity could be considerably improved to a LOD of 10 ng/L by using the SPDE technique. In general, the method's sensitivity should permit reliable detection of exposure also in the lower range of the EKA correlation evaluated for 1-bromopropane in air and *S*-*n*-propyl mercapturic acid in urine (air concentrations ≤ 1 mL/m^3) [DFG 2018].

The use of the headspace technique provides chromatograms with minimum to none matrix interference. The analytes are well separated from potential other sample components, such as acetone, to ensure that interference-free ion chromatograms are obtained.

With precision data ≤ 7%, the method is characterised by excellent reproducibility. Mean relative recovery rates between 88 and 108% confirm the method's reliability also in terms of accuracy. Validation also showed that the analytical results are practically unaffected by matrix effects. This enables the use of calibration standards that are prepared in water instead of pooled urine. The calibration curves are linear up to a concentration range of at least 100 µg/L.

A critical aspect of the method is probably the limited stability of aqueous analyte solutions, which prevents, among other things, long-term storage of prepared calibration standards and quality control materials. As calibration standards and control materials are each freshly prepared from the same spiking solutions, changes in the concentration of the spiking solutions are not directly apparent during analysis. These, however, manifest themselves in a change in the calibration curve slope. For the purposes of quality control, not only the course of the analyte concentrations in the quality control materials should be observed but also the changes in the calibration curve slope.

Using SPDE, the gas phase of the sample is brought into contact with a sorbent material. The sorbent (e. g. polydimethylsiloxane with 10% activated carbon) is coated at the inner side of a stainless steel needle of a temperature-controlled syringe. The analytes are enriched by repeatedly aspirating and ejecting aliquots of the sample headspace using the syringe. Optionally, the syringe needle can be cooled to further increase the enrichment of volatile substances. The analytes are thermally desorbed after insertion of the syringe needle into the hot GC injector by injecting nitrogen as desorption gas.

The development of such a SPDE method requires the optimisation of numerous parameters concerning the enrichment and desorption of the analytes. The selection of the enrichment conditions was based on the literature [Sieg et al. 2008]. The extraction yield could be further increased by using an extraction cooler for thermostatting the enrichment phase to –5 °C.

The selection of the desorption conditions (desorption flow rate, desorption gas volume) affects the size and shape of the analyte signals. The conditions described were optimised to obtain the highest possible signal intensities and a symmetrical peak shape at a given initial temperature of the oven program of 38 °C. If necessary,

chromatography could be further improved by active oven cooling or a cold trap at the beginning of the column, which enables better focussing of the analytes prior to chromatographic separation.

The molecular ions at *m/z* 122 and *m/z* 124 are used for mass spectrometric detection of the analytes. Considerable less interference peaks were observed in the chromatograms of these ions than in the chromatograms of the fragment at *m/z* 43 that shows a higher intensity (see also the mass spectrum of 1-bromopropane in Figure 3 as an example). However, the fragment ion at *m/z* 43 could be used as an additional qualifier.

During method development and validation, the long-term stability of the instruments used proved to be very good. Even after several hundred measurements, performance was impaired neither with regard to the SPDE system nor with regard to the GC/MS system.

Although the method includes sample enrichment using SPDE, it is also possible to work with another enrichment system, such as ITEX (In Tube Extraction). Thus, the external method verification was performed using an ITEX system and produced comparably good results in method validation. Basically,1-bromopropane and 2-bromopropane in urine can also be determined with static headspace-GC/MS without prior analyte enrichment, which was also tested during external method verification. Using this approach, the detection limit was found to be 0.33 µg 1-bromopropane and 0.44 µg 2-bromopropane per litre urine with a given good precision (s_w < 8%) and accuracy (mean rel. recovery 87.6–105%).

Instruments used: Gas chromatograph 6890 A with split/splitless injector, mass spectrometric detector MSD 5973N (Agilent Technologies) and CombiPal autosampler with option of dynamic headspace enrichment by Solid Phase Dynamic Extraction (SPDE) (CTC Analytics AG, Chromtech GmbH).

11 References

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12 Appendix

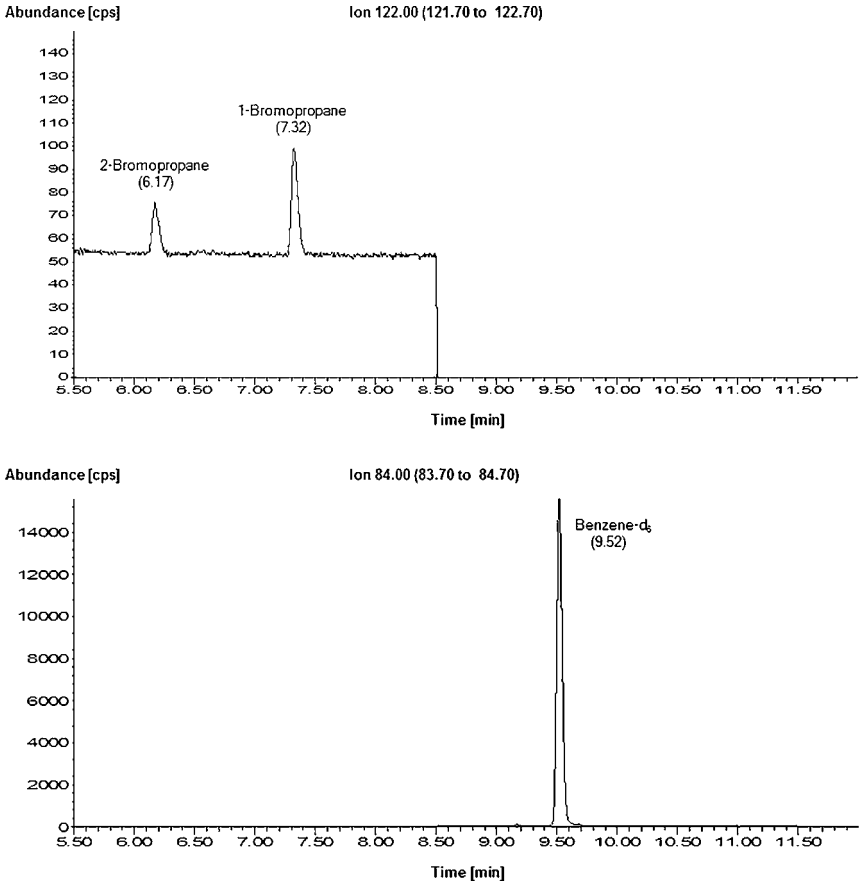

Figure 1: SIM chromatograms of a pooled urine sample spiked with 0.22 µg 1-bromopropane and 0.21 µg 2-bromopropane per litre (benzene-d_6 (IS): 4.56 µg/L).

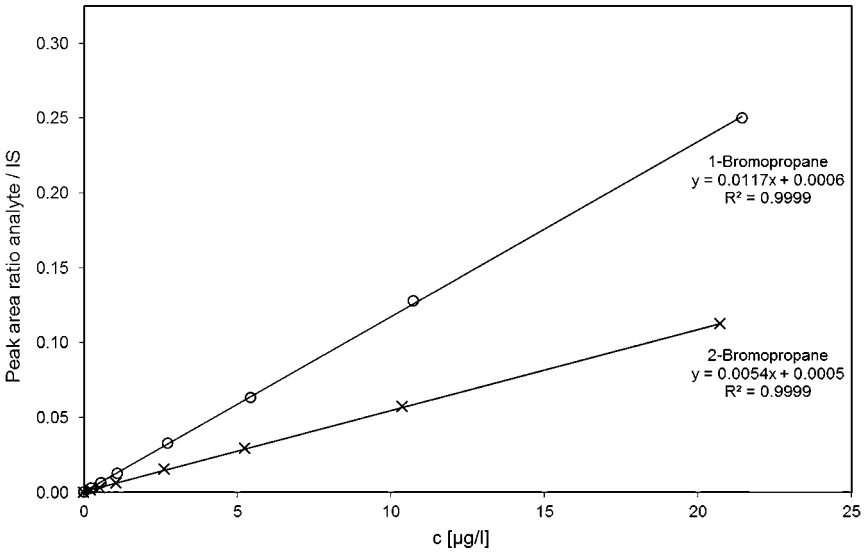

Figure 2: Calibration curves in urine for 1-bromopropane and 2-bromopropane.

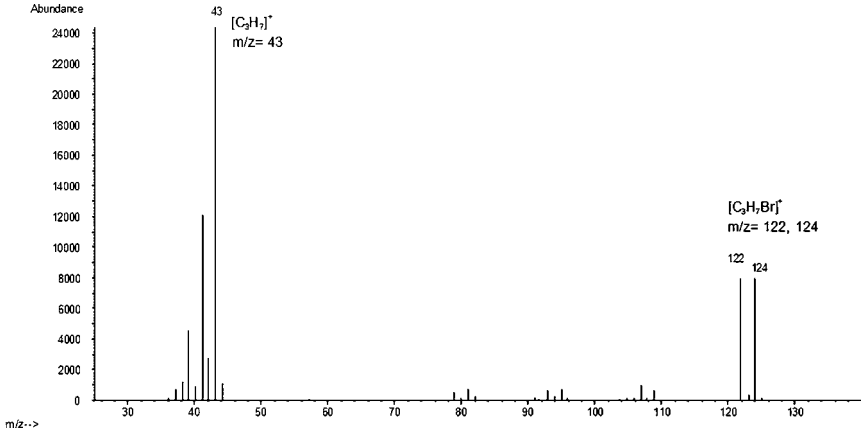

Figure 3: Mass spectrum of 1-bromopropane.