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Antimony and its compounds – Determination of antimony species in urine by HPLC-ICP-MS

Biomonitoring Method – Translation of the German version from 2020

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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 developed the presented biomonitoring method.

The analytical method described hereinafter consists of two sub-procedures and permits the determination of antimonite (Sb(III)) and antimonate (Sb(V)) as well as of trimethylantimony ion (Sb(V)) in urine. The limits of quantitation allow the quantification of the aforementioned antimony species both at occupational and environmental exposure levels.

The antimony species are separated by anion exchange chromatography, using two sub-procedures with different columns and eluents to quantify antimonite and antimonate or the trimethylantimony ion and antimonate. The isotope ^{121}Sb is used for element-specific detection of the antimony species by inductively coupled plasma-mass spectrometry (ICP-MS).

Sample preparation is carried out by dilution of urine aliquots with the respective eluents and subsequent filtration. Calibration is performed using either mixed standards of the three antimony species or the standard addition method.

1 Characteristics of the method

Matrix Urine

Analytical principle Anion exchange chromatography – HPLC-ICP-MS

Parameters and corresponding hazardous substances

Hazardous substances	CAS No.	Parameter	CAS No.
Antimony and its compounds	7440-36-0 (Antimony)	Antimonite (Sb(III)), e.g. $Sb(OH)_4^-$ or SbO_2^-	–
		Antimonate (Sb(V)), e.g. $Sb(OH)_6^-$ or SbO_3^-	–
		Trimethylantimony ion (Sb(V)) e.g. $(CH_3)_3Sb(OH)^+$	–

Reliability data

Antimonite (Sb(III)) [sub-procedure 1]

Within-day precision:	Standard deviation (rel.)	s_w = 4.46%
	Prognostic range	u = 12.4%
	at a determined concentration level of 1.4 µg antimony per litre urine and where n = 5 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 6.66%
	Prognostic range	u = 17.1%
	at a determined concentration level of 3.3 µg antimony per litre urine and where n = 6 determinations	
Accuracy:	Recovery rate (rel.)	r = 72.0%
	at a nominal concentration of 2.0 µg antimony per litre urine and where n = 5 determinations	
Detection limit:	0.5 µg antimony per litre urine	
Quantitation limit:	1.7 µg antimony per litre urine	

Antimonate (Sb(V)) [sub-procedure 1]

Within-day precision:	Standard deviation (rel.)	s_w = 5.01%
	Prognostic range	u = 13.9%
	at a determined concentration level of 0.72 µg antimony per litre urine and where n = 5 determinations	

Day-to-day precision:	Standard deviation (rel.)	s_w = 7.17%
	Prognostic range	u = 18.4%
	at a determined concentration level of 1.2 µg antimony per litre urine and where n = 6 determinations	
Accuracy:	Recovery rate (rel.)	r = 91.1%
	at a nominal concentration of 0.79 µg antimony per litre urine and where n = 5 determinations	
Detection limit:	0.4 µg antimony per litre urine	
Quantitation limit:	1.3 µg antimony per litre urine	

Trimethylantimony ion (Sb(V)) [sub-procedure 2]

Within-day precision:	Standard deviation (rel.)	s_w = 5.44%
	Prognostic range	u = 15.1%
	at a determined concentration level of 3.1 µg antimony per litre urine and where n = 5 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 7.90%
	Prognostic range	u = 21.9%
	at a determined concentration level of 3.1 µg antimony per litre urine and where n = 5 determinations	
Accuracy:	Recovery rate (rel.)	r = 96.9%
	at a nominal concentration of 3.2 µg antimony per litre urine and where n = 5 determinations	
Detection limit:	0.04 µg antimony per litre urine	
Quantitation limit:	0.14 µg antimony per litre urine	

Antimonate (Sb(V)) [sub-procedure 2]

Within-day precision:	Standard deviation (rel.)	s_w = 4.39%
	Prognostic range	u = 12.2%
	at a measured concentration of 3.5 µg antimony per litre urine and where n = 5 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 6.37%
	Prognostic range	u = 17.7%
	at a measured concentration of 3.5 µg antimony per litre urine and where n = 5 determinations	

Accuracy:	Recovery rate (rel.)	r = 113%
	at a nominal concentration of 3.1 µg antimony per litre urine and where n = 5 determinations	
Detection limit:	0.04 µg antimony per litre urine	
Quantitation limit:	0.13 µg antimony per litre urine	

2 General information on antimony and its compounds

Antimony (Sb) is a shiny, brittle metalloid, which is a member of Group 15 of the periodic table closely related to arsenic, to which it is chemically, physically and toxicologically very similar. It is found in the earth's crust at about 0.3 mg/kg and is thus relatively rare. Global antimony consumption in 2008 was around 160,000–190,000 t (Erdmann et al. 2011). Antimony is predominantly used (60–70%) in form of antimony trioxide as flame retardant, especially in plastics, rubber and textiles as well as in the manufacture of electrical and electronic devices. Furthermore, antimony alloy is used to harden the grid of car batteries improving the cycle life. Moreover, polyethylene terephthalate (PET) manufacture involves the use of antimony trioxide as a catalyst. The glass and ceramic industry uses various antimony compounds as pigments, glazes and decolourising agents. Further uses include the production of plastic stabilisers, pigments, fireworks, ammunition, slide bearings, medicines, pesticides, fluorescent lamps and semiconductors. On the whole, the latter uses are of minor importance in quantitative terms (Erdmann et al. 2011).

Antimony is mainly released into the environment from mining and ore smelting as well as from metal processing industries, combustion processes and ammunition. It is detectable at low concentration levels in almost all environmentally relevant media.

The daily dietary intake of antimony is estimated at about 5–38 µg (Domingo et al. 2012; Iyengar et al. 1987) and is thus below the tolerable daily intake (TDI) level of 6 µg/kg body weight/day (WHO 2003). These figures show that the non-occupationally exposed population is only slightly exposed to antimony, which is also reflected in the low urinary antimony levels of the non-occupationally exposed population. The Commission derived a BAR value of 0.2 µg antimony per litre urine (Göen et al. 2020).

At the workplace, the inhalation of dusts containing antimony or of gaseous antimony hydride are the most important sources of exposure. Ingestion or dermal absorption play a minor role in occupational exposure (Schaller 2005). In humans, antimony is excreted independent of its valence mainly via the kidneys (Gebel 1997). For renal excretion of antimony (measured as total antimony), a short half-life of about 34 hours and a longer half-life of about 90 hours were determined in workers (Kentner et al. 1995).

As the toxicity of antimony compounds depends both on the solubility of the respective compound and on the oxidation state of antimony (Greim 2007), it is important to analyse the species in order to assess exposure to antimony correctly. The pentavalent and trivalent antimony species can be transformed into each other under physiological conditions (Godfrey et al. 1998). Furthermore, methylated Sb(V) species have been detected in human urine (Krachler and Emons 2001; Quiroz et al. 2011; Ye et al. 2018). Only few data on antimony species in human urine have been published in the literature so far. These data are summarised in Table 1.

Tab. 1 Data on antimony species in human urine

Group	Number of samples	Antimony level [µg/l]				References
		Antimony (total)	Sb(V)	Sb(III)	$(CH_3)_3Sb(OH)^+$	
General population	2	< 0.12	< 0.060	< 0.025	< 0.036–0.09	Krachler and Emons 2001
Workers	2	5.1–8.3	2.0–5.9	< 0.025–0.15	0.40–0.57	
General population	1	not analysed	0.94	< 0.27	not analysed	Li et al. 2006
Exposed general population [b)]	8	< LOQ–6.3	< 0.60–6.2	< 0.62	< 0.59	Quiroz et al. 2011
Exposed general population [c)]	63	39 [a)]	8.1	0.1	31	Ye et al. 2018

[a)] Value read off from diagram.
[b)] Industrial area in Chile, exposure pathway not specified.
[c)] Antimony mining in China, dietary exposure. The specified values are mean values.

It has not been conclusively clarified in which configuration the antimony species are actually present in urine. Lintschinger et al. (1997, 1998) discussed that Sb(III) is present as chelate complexes in urine. According to Lintschinger et al. (1997, 1998), these chelate complexes are completely transformed into EDTA complexes in the EDTA-containing eluent also used in this method. Inorganic Sb(V) should be present in urine as $Sb(OH)_6^-$ and trimethylated Sb(V) as $(CH_3)_3SbOH^+$ (Hansen and Pegantis 2008).

For a detailed toxicological evaluation of antimony, please refer to the respective MAK Value Documentation (Greim 2007). Antimony and its inorganic compounds have been classified by the Commission in Carcinogen Category 2 and as Category 3B germ cell mutagens (DFG 2019). Due to the carcinogenicity classification, no BAT value can be derived for antimony and its compounds, however, a BAT documentation on antimony is published in the MAK Collection Part II: BAT value documentations (Schaller 2005). Moreover, the Commission derived a BAR value of 0.2 µg antimony/l urine (Göen et al. 2020). Due to the long elimination half-life of antimony, in the case of long-term exposure at the workplace, sampling should be done at the end of shift after several shifts (Göen et al. 2020).

3 General principles

The analytical method described hereinafter consists of two sub-procedures and permits the determination of antimonite (Sb(III)) and antimonate (Sb(V)) as well of trimethylantimony ion (Sb(V)) in urine. The limits of quantitation allow the quantification of the aforementioned antimony species both at occupational and environmental exposure levels.

The antimony species are separated by anion exchange chromatography, using two sub-procedures with different columns and eluents to detect antimonite and antimonate or the trimethylantimony ion and antimonate. The isotope ^{121}Sb is used for element-specific detection of the antimony species by ICP-MS.

Sample preparation is carried out by dilution of urine aliquots with the respective eluents and subsequent filtration. Calibration is performed using either mixed standards of the three antimony species or the standard addition method.

4 Equipment, chemicals and solutions

4.1 Equipment

- HPLC system with a gradient pump, 6-port injection valve (e.g. Rheodyne®, IDEX Health & Science LLC, CA, USA) and 100 µl PEEK (polyetheretherketone) sample loop; PEEK material for all wettable surfaces and capillary tubing (e.g. Merck KGaA, Darmstadt, Germany)
- Plastic syringe with steel injection needle for HPLC (1 ml fine dosage syringe (e.g. B. Braun Melsungen AG, Melsungen, Germany)) with Kel-F hub side port needle (Luer Lock (e.g. Hamilton Bonaduz AG, Bonaduz, Switzerland, No. 90516))
- Inductively coupled plasma mass spectrometer (e.g. Elan® 5000, PerkinElmer Inc., Rodgau, Germany) with pneumatic nebuliser (e.g. MEINHARD® nebuliser, PerkinElmer Inc., Rodgau, Germany)
- Analytical column (sub-procedure 1): PRP-X100 250 mm × 4.6 mm × 5 µm (PEEK) (e.g. Hamilton Bonaduz AG, Bonaduz, Switzerland, No. 79181) with PRP-X pre-column 8 mm × 3 mm × 10 µm (PEEK) (e.g. Hamilton Bonaduz AG, Bonaduz, Switzerland, No. 79354)
- Analytical column (sub-procedure 2): IonPac™ AS4 250 mm × 4 mm × 13 µm (e.g. Dionex™, Thermo Fisher Scientific GmbH, Dreieich, Germany, No. 35311) with IonPac™ AG4 pre-column 50 mm × 4 mm × 13 µm (e.g. Dionex™, Thermo Fisher Scientific GmbH, Dreieich, Germany, No. 35310)
- 0.2 µm cellulose nitrate membrane filters (e.g. Minisart RC 25, Sartorius AG, Göttingen, Germany)
- Analytical balance (e.g. Sartorius AG, Göttingen, Germany)
- pH meter (e.g. Knick Elektronische Messgeräte GmbH & Co. KG, Berlin, Germany)
- pH electrode (e.g. Mettler-Toledo GmbH, Gießen, Germany)
- 15 ml polyethylene tubes (e.g. Becton Dickinson GmbH, Heidelberg, Germany)
- Various pipettes (e.g. Eppendorf AG, Hamburg, Germany)
- Various volumetric flasks and beakers made of polyethylene (e.g. VWR International GmbH, Darmstadt, Germany)

4.2 Chemicals

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

- Nitric acid 65%, Suprapur (e.g. Merck KGaA, Darmstadt, Germany, No. 100441)
- Trimethylantimony dichloride (synthesis according to Dood et al. (1992))
- Potassium hexahydroxoantimonate(V), 99% (e.g. Fluka™, Honeywell Deutschland Holding GmbH, Offenbach, Germany, No. 60500)
- Potassium antimony(III)tartrate 3 × H_2O, 99% (e.g. Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany, No. 230057)
- Ethylenediaminetetraacetic acid (EDTA) (e.g. Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany, No. 431788)

- Sodium chloride, Suprapur (e.g. Merck KGaA, Darmstadt, Germany, No. 1.06406)
- Tetramethylammonium hydroxide (TMAH), 25% (e.g. Tama Chemicals Co. Ltd., Japan, No. AA-TMAH)
- Ammonia solution 25%, Suprapur (e.g. Merck KGaA, Darmstadt, Germany, No. 1.05428)
- Ultrapure water (e.g. Milli-Q®, Merck KGaA, Darmstadt, Germany)
- Argon 4.6 (e.g. Linde AG, Pullach, Germany)
- Urine control material (e.g. ClinChek®, RECIPE Chemicals + Instruments GmbH, München, Germany)

4.3 Solutions

Mobile phases for HPLC

- Sub-procedure 1: 20 mM EDTA solution (pH 4.7)

  Exactly 5.845 g EDTA are weighed into a 1000 ml volumetric flask. After adding approximately 980 ml ultrapure water, the pH is adjusted to 4.7 by adding 25% ammonia solution (approximately 4 ml). As EDTA goes slowly into solution and the pH adjustment might be delayed, at first only 2.5–3 ml of the ammonia solution should be added. Further additions should then be made slowly and dropwise. Afterwards, the flask is made up to the mark with ultrapure water.

- Sub-procedure 2: 3 mM TMAH solution with 17 mM sodium chloride

  Exactly 1.07 ml of the 25% TMAH solution are pipetted into a 1000 ml volumetric flask and diluted with approximately 500 ml ultrapure water. After adding exactly 993.5 mg sodium chloride, the flask is made up to the mark with ultrapure water.

The respective mobile phase is freshly prepared for each analytical run, filtered before use (0.45 µm cellulose filter) and degassed for five minutes in an ultrasonic bath.

4.4 Calibration standards

- Antimony(III) stock solution (200 mg Sb/l)

  Exactly 5.49 mg potassium antimony(III) tartrate are weighed into a 10 ml volumetric flask and dissolved in ultrapure water. The flask is then made up to the mark with ultrapure water.

- Antimony(V) stock solution (200 mg Sb/l)

  Exactly 4.32 mg potassium hexahydroxoantimonate(V) are weighed into a 10 ml volumetric flask and dissolved in ultrapure water. The flask is then made up to the mark with ultrapure water.

- Stock solution of the methylated Sb(V) (200 mg Sb/l)

  Exactly 3.91 mg trimethylantimony dichloride are weighed into a 10 ml volumetric flask and dissolved in ultrapure water. The flask is then made up to the mark with ultrapure water.

All stock solutions are prepared in PE volumetric flasks and stored in the refrigerator at 4 °C. Under these conditions, the stock solutions are stable for at least 12 months.

- Working solution 1 (20 mg Sb/l)

  Dilute 100 µl each of the stock solutions of Sb(III), Sb(V) and the methylated Sb(V) with 900 µl each of the respective eluent for HPLC (see Section 4.3) and mix thoroughly.

- Working solution 2 (1 mg Sb/l)

  Dilute 50 µl each of working solution 1 of Sb(III), Sb(V) and the methylated Sb(V) with 950 µl each of the respective eluent for HPLC (see Section 4.3) and mix thoroughly.

- Spiking solution 1 (10 µg Sb/l)

  Dilute 50 µl each of working solution 2 of Sb(III), Sb(V) and the methylated Sb(V) with 4950 µl each of the respective eluent for HPLC (see Section 4.3) and mix thoroughly.

- Spiking solution 2 (0.2 µg Sb/l)

  4900 µl each of the respective eluent for HPLC (see Section 4.3) are added to 100 µl each of spiking solution 1 of Sb(III), Sb(V) and the methylated Sb(V) and mixed thoroughly.

All working solutions and spiking solutions are freshly prepared every day in PE flasks and stored in the refrigerator at 4 °C until use.

Calibration standards in the concentration range between 0.025 and 2 µg Sb/l are prepared in PE vials in the respective eluent for HPLC. To this end, the spiking solutions are mixed with the respective eluent according to the pipetting scheme given in Table 2 to a final volume of 1 ml.

The calibration standards are freshly prepared every working day immediately before use, since the species ratio can shift at the very low concentration levels of the standard solutions.

Tab. 2 Pipetting scheme for the preparation of calibration standards used to determine antimony species in urine

Calibration standard	Spiking solution 1 [µl]	Spiking solution 2 [µl]	Mobile phase [µl]	Concentration of the calibration standard [µg/l]
0	–	–	1000	0
1	–	125	875	0.025
2	–	250	750	0.05
3	–	500	500	0.1
4	–	1000	–	0.2
5	50	–	950	0.5
6	100	–	900	1.0
7	200	–	800	2.0

5 Specimen collection and sample preparation

5.1 Specimen collection

The polyethylene containers used for specimen collection are rinsed three times each with 10% nitric acid and then with ultrapure water. Afterwards, they are allowed to dry. If not immediately analysed, the urine samples obtained may be stored in the refrigerator at 4 °C or deep-frozen at −18 °C. The urine sediment precipitating during storage in the refrigerator or freezer does not contain any antimony (Domingo et al. 2012). However, the exclusive analysis of the supernatant means that the sample is concentrated, even if only slightly. For this reason, and as currently only little information is available on the stability of antimony species, the urine samples should be processed and analysed as soon as possible after sampling.

5.2 Sample preparation

The urine samples are each divided into two aliquots, which are then processed according to sub-procedure 1 or sub-procedure 2 as the simultaneous quantitation of all antimony species to be determined is not possible.

Sample preparation for antimony species determination consists of diluting the urine with subsequent filtration. Depending on the concentration of the antimony species in the sample, the urine is diluted in a ratio of 1 : 3 to 1 : 10 with the mobile phase used for the respective chromatographic separation. The diluted urine is then filtered through a 0.2 µm syringe filter to remove precipitated particles.

6 Operational parameters

Analysis was performed using an HPLC system coupled with an ICP-MS.

6.1 High-performance liquid chromatography

Pump:	Beckman 127 HPLC gradient pump (Beckman Coulter GmbH, Krefeld, Germany)
Injector:	Rheodyne® (IDEX Health & Science LLC, CA, USA)
Injection volume:	100 µl PEEK sample loop

Sub-procedure 1

Pre-column:	Material:	PSDVB/trimethylammonium
	Column packing:	PRP-X
	Length:	8 mm
	Inner diameter:	3 mm
	Particle size:	10 µm
Analytical column:	Material:	PSDVB/trimethylammonium
	Column packing:	PRP-X100
	Length:	250 mm
	Inner diameter:	4.6 mm
	Particle size:	5 µm
Analytical principle:		Anion exchange
Mobile phase:		20 mM EDTA; pH 4.7
Flow rate:		1.5 ml/min

Sub-procedure 2

Pre-column:	Material:	Quaternary ammonium salt
	Column packing:	IonPac™ AG4
	Length:	50 mm
	Inner diameter:	4 mm
	Particle size:	13 µm
Analytical column:	Material:	Quaternary ammonium salt
	Column packing:	IonPac™ AS4
	Length:	250 mm
	Inner diameter:	4 mm
	Particle size:	13 µm
Analytical principle:		Anion exchange
Mobile phase:		3 mM TMAH; 17 mM sodium chloride
Flow rate:		1.5 ml/min

6.2 Inductively coupled plasma mass spectrometry

The settings described below are intended as a rough guide only. These parameters must be optimised individually for each device. Additional settings and parameter optimisation may be required when using instruments from other manufacturers.

HPLC-ICP-MS coupling:	directly from the HPLC column to the nebuliser using a PEEK capillary tubing
Nebuliser system:	Meinhard® nebuliser with cyclonic spray chamber
ICP-MS:	ELAN® 5000, Perkin Elmer Inc., Rodgau, Germany
Power output:	1200 W
Cones:	Nickel
Plasma gas:	15.0 l/min
Auxiliary gas:	1 l/min
Nebuliser gas:	ca. 780 ml/min, optimised daily to the signal intensity of ^{121}Sb(V)
Data acquisition:	Isotope ^{121}Sb, dwell time 500 ms, usually 700 repetitions (350 s data recording with two data points per second)
Data evaluation:	ADL Chrommanager, Advanced Chemistry Development Inc., Toronto, Canada

7 Analytical determination

100 µl each of the samples prepared according to Section 5 are injected into the HPLC-ICP-MS system. Reagent blank values (ultrapure water or eluent instead of the urine sample) are included in each analytical run.

Especially at low antimony levels, it is advisable to measure the urine samples in triplicate. In this case, the result of the analysis is the mean value of the three measurements.

Figures 1 and 2 (Appendix) show examples of chromatograms for the determination of antimony species using sub-procedure 1 or 2.

8 Calibration

The calibration standards described in Section 4.4 are prepared and processed in the same way as the samples (see Section 5) and analysed using HPLC-ICP-MS (see Section 6). Calibration graphs are obtained by plotting the peak areas of the analytes against the respective concentration of the calibration standards. Under the described conditions, the calibration graphs are linear for all analytes in the range from the detection limit to 1 µg Sb/l. The linearity of the working range has been verified and confirmed up to 2 µg Sb/l. As an example, Figures 3 and 4 (Appendix) show the calibration graphs of the analytes in the respective eluent.

The slopes of the calibration graphs for standards prepared in mobile phase are different from the calibration graphs made up in urine. To compensate this matrix effect, standard addition must be used whenever possible. For this, a series of increasing volumes of spiking solution is added to aliquots of each urine sample. The concentration and volume of the spiking solution added should be chosen to increase the concentration of the unknown by about 30% in each succeeding vial.

9 Calculation of the analytical results

In the case of external calibration, the analyte concentrations in a sample are calculated by entering the peak areas of the ^{121}Sb mass peak in the calibration functions of the respective analytical run.

If the standard addition method is used, the peak areas of the analyte peaks yield a straight line, which is extended down to the x-axis (y = 0). The point of intersection with the abscissa is the antimony concentration of the sample in µg/l.

Any reagent blank values have to be subtracted from the analytical results. If a measured value is out of the calibration range, the urine sample is diluted with the respective mobile phase in a ratio of 1 : 10 and processed and analysed anew.

10 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, at least one quality control sample with known and constant analyte concentrations is analysed within each analytical run. The analyte levels of the quality control material should be within the relevant concentration range.

The developer of this method used lyophilised ClinChek® urine from RECIPE Chemicals + Instruments GmbH as quality control material. The freeze-dried urine was reconstituted according to the manufacturer's instructions by adding ultrapure water. The analyte level in the reconstituted urine was 12 µg antimony (total) per litre. The individually quantified antimony species were summed up and compared with the total antimony level stated by

the manufacturer (information on the individual species in the control material was not available). Aliquots of this control material were processed according to Section 5 and subsequently analysed.

The nominal value and the tolerance ranges of the quality control material are determined in 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.

11 Evaluation of the method

The reliability of the method was verified by comparative analysis of urine samples in two independent laboratories.

11.1 Within-day precision

On the one hand, within-day precision was determined using the calibration standards prepared according to Section 4.4. The material was processed several times in parallel and analysed. The results are presented in Table 3.

Tab. 3 Within-day precision for the determination of antimonite and antimonate as well as the trimethylantimony ion and antimonate using calibration standards

Analyte	Concentration of calibration standard [µg/l]	Standard deviation (rel.) s_w [%]	Prognostic range u [%]
Sub-procedure 1 (n = 10)			
Antimonite (Sb(III))	0.5	3.68	8.32
Antimonate (Sb(V))	0.5	4.12	9.32
Sub-procedure 2 (n = 6)			
Trimethylantimony ion (Sb(V))	1.0	5.68	14.6
Antimonate (Sb(V))	1.0	3.28	8.43

On the other hand, within-day precision was also determined in urine. For sub-procedure 1, urine with a background level of 0.29 Sb(V) per litre was used. There was no background level of Sb(III). The urine was spiked with 2.0 µg Sb(III) and 0.5 µg Sb(V) per litre and analysed five times in a 1 : 3 dilution. Within-day precision in urine was also determined for sub-procedure 2. The urine was spiked with 1 µg antimony per litre urine in the form of trimethyl antimony dichloride and Sb(V), respectively. This urine was diluted in a ratio of 1 : 10 with the eluent and analysed five times. The obtained precision data are given in Table 4.

Tab. 4 Within-day precision for the determination of antimonite and antimonate as well as the trimethylantimony ion and antimonate in urine

Analyte	Spiked concentration [µg/l urine]	Determined concentration [µg/l urine]	Standard deviation (rel.) s_w [%]	Prognostic range u [%]
Sub-procedure 1 (n = 5)				
Antimonite (Sb(III))	0	0	–	–
	2.0	1.44	4.46	12.4
Antimonate (Sb(V))	0	0.29	4.99	13.9
	0.5	0.72	5.01	13.9

Tab. 4 *(continued)*

Analyte	Spiked concentration [µg/l urine]	Determined concentration [µg/l urine]	Standard deviation (rel.) s_w [%]	Prognostic range u [%]
Sub-procedure 2 (n = 5)				
Trimethyl-antimony ion (Sb(V))	0	2.2	7.90	21.9
	1.0	3.1	5.44	15.1
Antimonate (Sb(V))	0	2.1	5.74	15.9
	1.0	3.5	4.39	12.2

11.2 Day-to-day precision

Day-to-day precision was determined by processing and analysing calibration standards and spiked urine samples. The obtained precision data for the two sub-procedures are presented in Tables 5 and 6.

Tab. 5 Day-to-day precision for the determination of antimonite and antimonate as well as the trimethylantimony ion and antimonate using calibration standards

Analyte	Concentration of calibration standard [µg/l]	Standard deviation (rel.) s_w [%]	Prognostic range u [%]
Sub-procedure 1 (n = 4)			
Antimonite (Sb(III))	0.5	4.23	13.5
Antimonate (Sb(V))	0.5	4.96	15.8
Sub-procedure 2 (n = 4)			
Trimethylantimony ion (Sb(V))	1.0	5.81	18.5
Antimonate (Sb(V))	1.0	3.37	10.7

Tab. 6 Day-to-day precision for the determination of antimonite and antimonate as well as the trimethylantimony ion and antimonate in spiked urine

Analyte	Spiked concentration [µg/l urine]	Determined concentration [µg/l urine]	Standard deviation (rel.) s_w [%]	Prognostic range u [%]
Sub-procedure 1 (n = 6)				
Antimonite (Sb(III))	3.3	2.35	6.66	17.1
Antimonate (Sb(V))	1.2	1.75	7.17	18.4
Sub-procedure 2 (n = 5)				
Trimethylantimony ion (Sb(V))	1.0	3.1	7.90	21.9
Antimonate (Sb(V))	1.0	3.5	6.37	17.7

11.3 Accuracy

The relative recovery rates were determined on the basis of the within-day precision data in urine. The results are presented in Table 7.

Tab. 7 Relative recovery rates for the determination of antimonite and antimonate as well as the trimethylantimony ion and antimonate in urine

Analyte	Nominal value [µg/l urine]	Measured value [µg/l urine]	Recovery (rel.) [%]
Sub-procedure 1 (n = 5)			
Antimonite (Sb(III))	2.0	1.44	72.0
Antimonate (Sb(V))	0.79	0.72	91.1
Sub-procedure 2 (n = 5)			
Trimethylantimony ion (Sb(V))	3.2	3.1	96.9
Antimonate (Sb(V))	3.1	3.5	113

11.4 Matrix effects

To determine the absolute recovery for sub-procedure 1, the peak areas in urine were quantified against the standard calibration curve. With absolute recovery rates of 34% for Sb(III) and 25% for Sb(V), a clear matrix effect could be stated. No matrix effect was found for sub-procedure 2, which is probably due to the higher dilution of the urine compared to sub-procedure 1.

11.5 Limits of detection and limits of quantitation

The limits of detection and quantitation of the analytes were set as 3 and 10 times the signal to noise ratio using a 0.5 µg/l (sub-procedure 1) or 0.2 µg/l (sub-procedure 2) calibration standard, respectively. The results are presented in Table 8.

Tab. 8 Detection limits and quantitation limits of the antimony species analysed, based on a 0.5 µg/l (sub-procedure 1) or 0.2 µg/l (sub-procedure 2) calibration standard

Sub-procedure	Analyte	Detection limit [µg/l]	Quantitation limit [µg/l]
1	Antimonite (Sb(III))	0.047	0.150
	Antimonate (Sb(V))	0.022	0.077
2	Trimethylantimony ion (Sb(V))	0.014	0.046
	Antimonate (Sb(V))	0.006	0.021

In urine the detection limits and quantitation limits were somewhat higher than in pure standard solutions and yielded the results given in Table 9.

Tab. 9 Detection limits and quantitation limits of the antimony species in urine

Sub-procedure	Analyte	Detection limit [µg/l]	Quantitation limit [µg/l]
1	Antimonite (Sb(III))	0.5	1.67
	Antimonate (Sb(V))	0.4	1.33
2	Trimethylantimony ion (Sb(V))	0.04	0.14
	Antimonate (Sb(V))	0.04	0.13

11.6 Sources of error

It is absolutely necessary to filter the urine samples prior to analysis to prevent urine sediment from entering the chromatographic system, where it would significantly reduce the separation performance and lifetime of the analytical column.

Although the species separation is completed within a few minutes with both sub-procedures, with sub-procedure 1 samples should be injected only every 10 minutes to avoid a shift of the retention times (especially those of the Sb(III) signal) and to allow the analytical column to regenerate.

Since antimony is present as a trace compound in glass, no glass ware or HPLC syringes made of glass should be used. This would lead to increased blank values and non-reproducible Sb(V) measurements, especially when alkaline eluents are used (Lintschinger et al. 1998).

Sb(III) solutions with concentrations in the lower ng per litre range are oxidised to Sb(V) within a few hours. To avoid this, the calibration standards are freshly prepared prior to use. With sub-procedure 1, diluting the samples and the standard solutions with the EDTA containing mobile phase leads to complexation and thus stabilisation of the Sb(III) (Lintschinger et al. 1998).

The matrix effect observed for sub-procedure 1 during method validation shows how important it is to use the standard addition method for calibration. Therefore, the calibration standards prepared according to Section 4.4 were mainly used to ensure the linearity of the working range.

12 Discussion of the method

This method is based on the method developed by Lintschinger et al. (1997, 1998) and permits the determination of antimonite (Sb(III)), antimonate (Sb(V)) and of the trimethylantimony ion (Sb(V)) in urine by coupling anion exchange chromatography with plasma-mass spectrometry (ICP-MS). This coupling allows the sensitive determination of the antimony species in the urine of both non-exposed and occupationally exposed individuals. Before injecting a urine sample on the analytical column, the urine is diluted with the respective eluent and filtered. The HPLC system is connected directly to the nebuliser of the ICP-MS with a short piece of PEEK tubing. The ICP-MS serves as an extremely sensitive, element-specific detector for the antimony species analysed.

Species separation, precision and detection limits in both standard solutions and urine are satisfactory. However, the three antimony species cannot be analysed in a single analysis, since the separation of $(CH_3)_3Sb(OH)^+$ and antimonate requires other conditions than the separation of antimonite and antimonate. This method therefore consists of two different procedures, using different chromatographic columns and eluents, which were optimised separately. It is therefore necessary to divide the urine samples into two aliquots prior to analysis, each of which is analysed using one of the two sub-procedures.

Antimonate is determined using the Sb(III)/Sb(V) method (sub-procedure 1), since antimonate can be detected by sub-procedure 1 with less interferences, whereas the trimethylantimony ion is determined using sub-procedure 2.

For sub-procedure 2 an IonPac™ AS4 column was used. This is a column based on a quaternary ammonium exchange function, which separates the antimony species very well. The mobile phase tested and proven by Lintschinger et al. (1998) was used as eluent. In order to minimise interferences when analysing the urine samples, sodium chloride was added to the eluent. 17 mM sodium chloride proved to be the optimal concentration to minimise the interference on the $(CH_3)_3Sb(OH)^+$ signal (false positive) and to minimise the signal suppression on the antimonate signal (false negative).

The antimonate concentration determined using sub-procedure 2 is slightly lower than that determined using sub-procedure 1. This is possibly due to the fact that the suppression on the antimonate signal observed for sub-procedure 2 without sodium chloride in the eluent cannot be excluded quantitatively even with 17 mM sodium chlo-

ride. Antimonate should therefore be quantified using sub-procedure 1, whereas the result from sub-procedure 2 can at least be used for comparison. In the case of extended long-term measurements using sub-procedure 2, it must be taken into account that a sodium chloride concentration of 17 mM per litre can cause faster wear out of the ICP-MS cones.

The analysed species seem to correspond to the total antimony in the urine, since no other compounds were found during method development. Even prolonged flushing with sodium chloride solution did not result in further peaks or a prolonged baseline rise. However, it is possible that any monomethyl and dimethylantimony(V) compounds present, elute with the trimethylantimony ion. As the methylated compounds elute shortly after the dead time with the anion exchange HPLC method presented herein, it was not possible to differentiate between the individual methyl compounds any further.

Instruments used HPLC system with a gradient pump (e.g. Beckman 127, Beckman Coulter GmbH, Krefeld, Germany), 6-port injection valve (e.g. Rheodyne®, IDEX Health & Science LLC, CA, USA) and 100 µl PEEK sample loop; Inductively coupled plasma mass spectrometer (e.g. ELAN® 5000, Perkin Elmer Inc., Rodgau, Germany) with pneumatic nebuliser with cyclonic spray chamber (e.g. Meinhard® nebuliser, Perkin Elmer Inc., Rodgau, Germany); Software for chromatogram evaluation: (e.g. ADL Chrommanager, Advanced Chemistry Development Inc., Toronto, Canada).

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Appendix

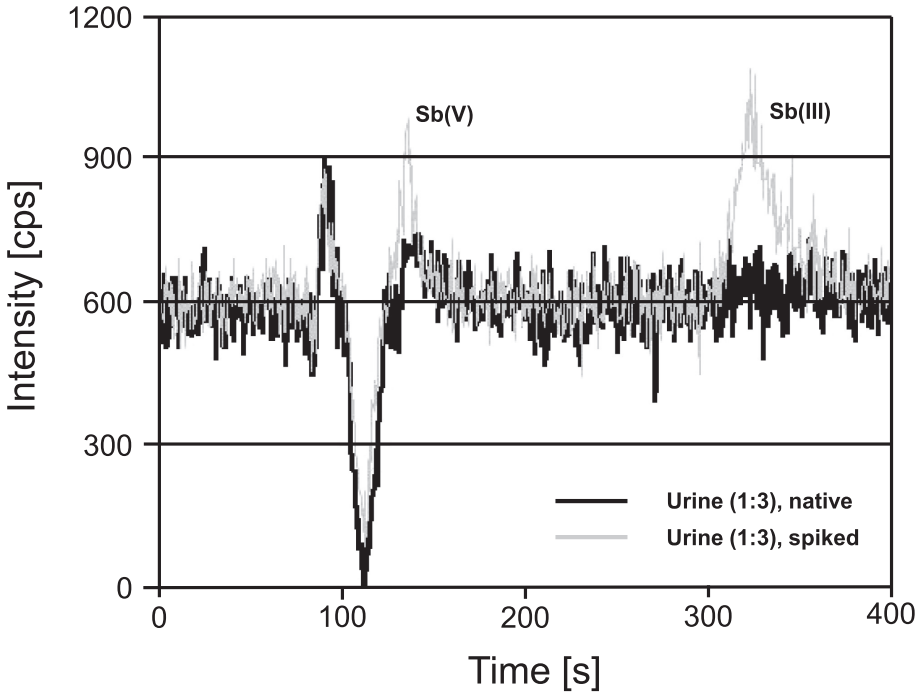

Fig. 1 Chromatogram of a urine sample spiked with 0.4 µg/l antimonate Sb(V) and 1.1 µg/l antimonite Sb(III) (sub-procedure 1)

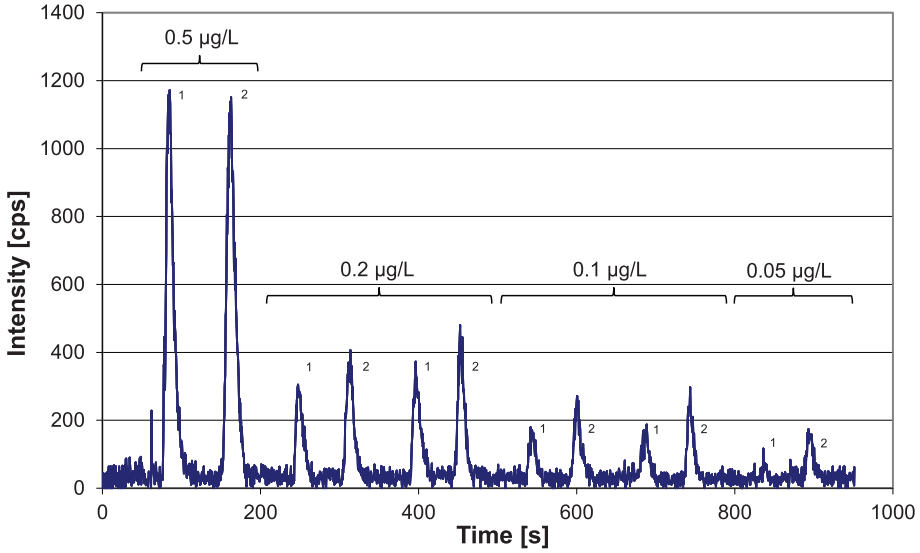

Fig. 2 Chromatogram of spiked urine samples in the concentration range of 0.05–0.5 µg/l (sub-procedure 2), multiple injections (1: trimethylantimony ion; 2: antimonate)

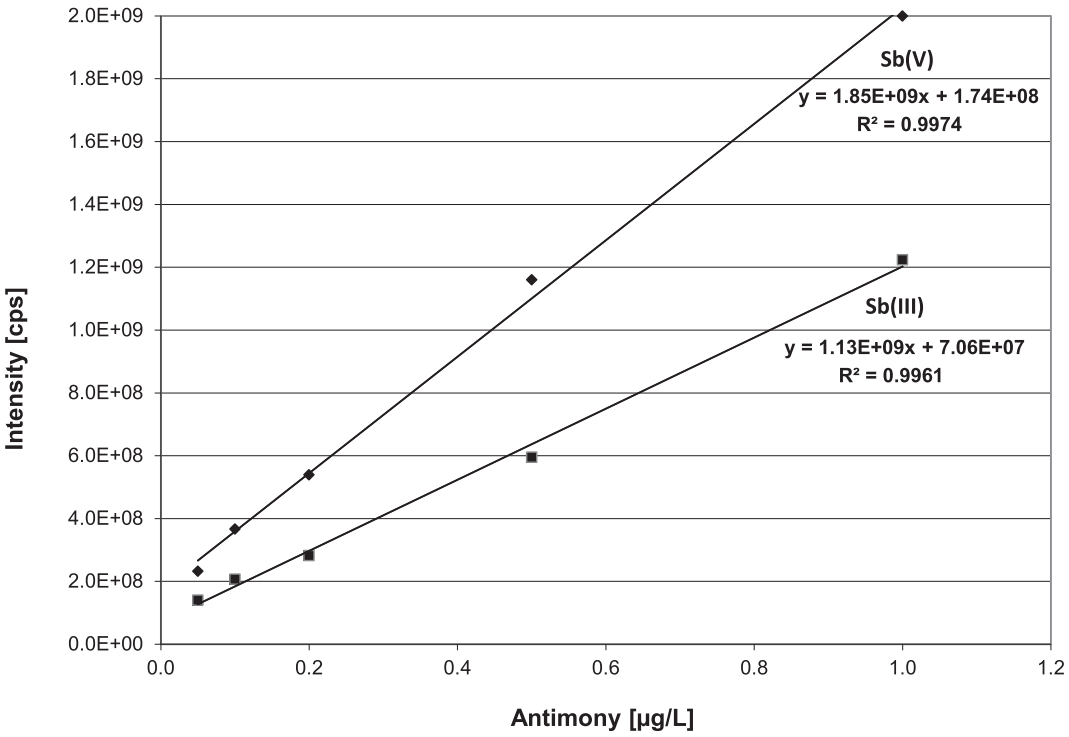

Fig. 3 Calibration curve for antimonite Sb(III) and antimonate Sb(V) in the eluent (sub-procedure 1)

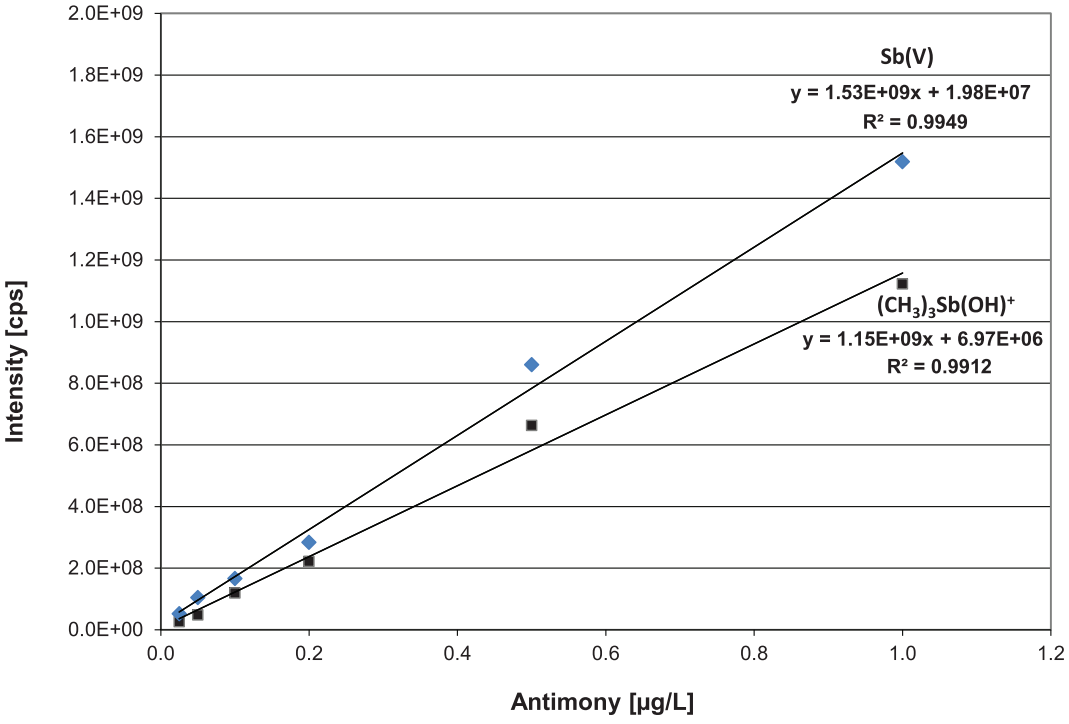

Fig. 4 Calibration curve for $(CH_3)_3Sb(OH)^+$ and antimonate Sb(V) in the eluent (sub-procedure 2)