The MAK Collection for Occupational Health and Safety

Mercury and mercury compounds – Determination of mercury in blood and in urine by Cold Vapour AAS

Biomonitoring Method – Translation of the German version from 2019

P. Heitland[1], T. Göen[2,*], A. Hartwig[3,*], MAK Commission[4,*]

1 *Method development, Medical Laboratory Bremen, Haferwende 12, 28357 Bremen, Germany*

2 *Head of 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, Deutsche Forschungsgemeinschaft, Friedrich-Alexander-Universität Erlangen-Nürnberg, Institute and Outpatient Clinic of Occupational, Social, and Environmental Medicine, Henkestraße 9–11, 91054 Erlangen, Germany*

3 *Chair of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Institute of Applied Biosciences, Department of Food Chemistry and Toxicology, Karlsruhe Institute of Technology (KIT), Adenauerring 20a, Building 50.41, 76131 Karlsruhe, Germany*

4 *Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Kennedyallee 40, 53175 Bonn, Germany*

***** *email: T. Göen (thomas.goeen@fau.de), A. Hartwig (andrea.hartwig@kit.edu), MAK Commission (arbeitsstoffkommission@dfg.de)*

Keywords: mercury; methylmercury; biomonitoring; blood; urine; CV-AAS

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Biomonitoring Methods

P. Heitland[1], T. Göen[2, *], A. Hartwig[3, *], MAK Commission[4, *]

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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 validated the presented biomonitoring method.

Mercury is determined by flow injection cold vapour atomic absorption spectrometry (CV-AAS). The digested blood or urine samples are stabilised with potassium permanganate, introduced into the acid carrier flow (hydrochloric acid) and mixed with the reducing agent sodium borohydride. Mercury vapour formed by reduction is transported with an argon flow into the atomisation cell of the AA spectrometer.

Calibration is performed using matrix matched calibration solutions. The mercury concentrations in real samples are calculated from the linear relationship between the measured absorbance and the mass concentration of mercury.

Keywords

mercury; methylmercury; urine; blood; biomonitoring; Analyses in Biological Materials; flow injection system; cold vapour atomic absorption spectrometry; CV-AAS

Author Information

[1] Method development, Medical Laboratory Bremen, Haferwende 12, 28357 Bremen, Germany

[2] Chair of the working group "Analyses in Biological Materials", Deutsche Forschungsgemeinschaft, Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine, Friedrich-Alexander University Erlangen-Nürnberg (FAU), Henkestr. 9–11, 91054 Erlangen, Germany

[3] Chair of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Department of Food Chemistry and Toxicology, Institute of Applied Biosciences, Karlsruhe Institute of Technology (KIT), Adenauerring 20a, Building 50.41, 76131 Karlsruhe, Germany

[4] Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Kennedyallee 40, 53175 Bonn, Germany

[*] Email: T. Göen (thomas.goeen@fau.de), A. Hartwig (andrea.hartwig@kit.edu), MAK Commission (arbeitsstoffkommission@dfg.de)

Mercury and mercury compounds – Determination of mercury in blood and in urine by Cold Vapour AAS

Matrix:	Blood and urine
Hazardous substances:	Mercury and mercury compounds
Analytical principle:	Flow injection-cold vapour atomic absorption spectrometry (FI-CV-AAS)
Completed in:	April 2008

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

Hazardous substance	CAS	Parameter	CAS
Mercury and mercury compounds	7439-97-6 (Mercury)	Mercury	7439-97-6

Summary

Mercury is determined by flow injection cold vapour atomic absorption spectrometry (CV-AAS).

The digested blood or urine samples are stabilised with potassium permanganate, introduced into the acid carrier flow (hydrochloric acid) and mixed with the reducing agent sodium borohydride. Mercury vapour formed by reduction is transported with an argon flow into the atomisation cell of the AA spectrometer.

Calibration is performed using matrix matched calibration solutions. The mercury concentrations in real samples are calculated from the linear relationship between the measured absorbance and the mass concentration of mercury.

Reliability data of the method

Mercury (Hg) in blood

Within-day precision: Standard deviation (rel.) s_w = 2.0% or 1.6%
Prognostic range u = 4.5% or 3.6%
at a spiked concentration of 3.4 µg or 15 µg Hg per litre blood and where n = 10 determinations

Day-to-day precision:	Standard deviation (rel.)	s_w = 4.8% or 4.7%
	Prognostic range	u = 10.0% or 9.8%
	at a spiked concentration of 3.4 µg or 15 µg Hg per litre blood and where n = 20 determinations	
Accuracy:	Recovery rate	r = 102% or 98%
	in a blood sample with an initial value of 2 µg Hg per litre and a spiked concentration of 10 µg Hg^{2+} or methylmercury per litre and where n = 3 determinations	
Detection limit:	0.04 µg Hg per litre blood	
Quantitation limit:	0.12 µg Hg per litre blood	

Mercury (Hg) in urine

Within-day precision:	Standard deviation (rel.)	s_w = 1.2% or 1.1%
	Prognostic range	u = 2.7% or 2.5%
	at a spiked concentration of 41.0 µg or 127 µg Hg per litre urine and where n = 10 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 7.9% or 6.2%
	Prognostic range	u = 16.5% or 13.0%
	at a spiked concentration of 41.0 µg or 127 µg Hg per litre urine and where n = 20 determinations	
Accuracy:	Recovery rate	r = 98%
	at a spiked concentration of 10 µg Hg per litre urine and where n = 3 determinations	
Detection limit:	0.04 µg Hg per litre urine	
Quantitation limit:	0.12 µg Hg per litre urine	

General information on the hazardous substance

Mercury

Mercury (Hg, atomic number 80, relative atomic mass 200.59) takes the 62nd place in the elemental abundance and is thus one of the rare elements. Mercury is the only metallic element that is liquid at room temperature and as such has a vapour pressure leading to toxicologically relevant air concentrations. In inorganic form, mercury exists in two oxidation states: Hg^{1+} and Hg^{2+}, with Hg^{2+} occurring more frequently. There are also numerous organic mercury compounds, of which methylmercury is one of the most stable forms. In nature, mercury is released by volcanoes and from the sea. The combustion of fossil fuels (coal, oil) and the incineration of domestic waste are anthropogenic sources of mercury emission into the atmosphere.

Metallic mercury is used in thermometers, manometers, mercury vapour lamps, energy-saving light bulbs and special batteries as well as in mining of metals. Amalgams in tooth filling materials also contain elemental mercury. Organic and inorganic mercury compounds are used as fungicides and insecticides and as seed dressings, wood preservatives and for pelt tanning [Hartwig 2011, translated].

In environmental medicine, for example, mercury vapour or dissolved ionic mercury from amalgam fillings play a major role [Berglund 1993; Ferracane et al. 1995; Jokstad et al. 1992]. Besides, the consumption of foodstuffs, in particular of fish and crustaceans, contributes to the uptake of mercury [Björnberg et al. 2003; Mahaffey et al. 2004; Sanzo et al. 2001; Svensson et al. 1992]. In addition, exposure may result from indoor contamination, for example from broken mercury thermometers, from batteries or from paints containing mercury. Residential proximity to mercury-emitting industry is also a source of exposure [ATSDR 1999].

At work, dental personnel may be occupationally exposed to mercury [Aydin et al. 2003; Bittner et al. 1998; Ritchie et al. 2002], while industrial workers can be exposed, e.g. at a fluorescent lamp manufacturing factory [El-Safty et al. 2003; Soleo et al. 1997] or at a thermometer-manufacturing facility [Ehrenberg 1991], in the production [Dantas and Queiroz 1997] and extraction [Boffetta et al. 2001; Queiroz et al. 1999] of mercury, in the chloralkali process using the mercury cell method [Camerino et al. 2002; Cárdenas et al. 1993; Ellingsen et al. 2001] and in further tasks [Abdennour et al. 2002].

The German Environment Agency (Umweltbundesamt) published reference values for the non-occupationally exposed general population: 1 µg mercury per litre urine (adults aged 18 to 69 years without amalgam fillings; first morning void) and 2.0 µg mercury per litre blood (adults aged 18 to 69 years; up to three servings of fish per month) [Schulz et al. 2011] (see also Table 1). Much higher concentrations can be found in the blood and urine of occupationally exposed individuals. Mercury concentrations ranging from 4–169 µg/L urine were measured in 44 workers at a chloralkali plant [Cárdenas et al. 1993] and concentrations in the range of 2–55 µg mercury/L urine were found in industrial workers in mercury production [Dantas and Queiroz 1997]. Data on mercury levels of other occupationally exposed groups have been published [Abdennour et al. 2002; Aydin et al. 2002; Boffetta et al. 2001; Ellingsen et al. 2001; Queiroz et al. 1999].

At the workplace, most exposure is to mercury vapour. Exposure to dusts containing inorganic mercury compounds, on the other hand, is very rare. Approximately 80% of mercury vapour is absorbed by the lungs; elemental mercury is practically not absorbed by the gastrointestinal tract. All biological and toxicological effects of exposure to elemental mercury are put down to the action of mercury ions. The central nervous system is the target organ after long-term exposure of humans to mercury vapours [Hartwig 2011, translated].

7–15% of an ingested dose of mercury (II) compounds are absorbed from the gastrointestinal tract, where the amount absorbed correlates with the solubility of the compound in water [Greim 1999, translated]. In the blood, the divalent mercury ion binds to sulfhydryl groups of plasma constituents and erythrocyte proteins. The ion accumulates in the liver, but mainly in the kidney, which is also the target organ of long-term exposure to mercury [Hartwig 2011, translated].

Mercury in the body has an estimated elimination half-life of about 58 days. For individual compartments, major deviations from this value were found. For example, the biological half-life of mercury in the brain can be several years [Greim 1999, translated].

Mercury and its inorganic compounds are also absorbed through the skin and have a sensitising effect on the skin. For this reason, mercury and its inorganic compounds

are designated with an "H" and "Sh" by the Commission [DFG 2018]. Moreover, mercury and its compounds are classified by the Commission as category 3B carcinogens [DFG 2018].

For more detailed information on the toxicological evaluation of mercury and its compounds, please refer to the respective MAK Value Documentations of the Commission [Greim 1999, translated; Hartwig 2011, translated].

Both blood and urine are suitable sample matrices to quantify mercury exposure. The determination of mercury in whole blood is used to assess exposure to inorganic and organic mercury in the case of long-term exposure. As organic mercury accumulates in the erythrocytes, an additional analysis of the erythrocytes and plasma of a blood sample may indicate the binding form of mercury. The determination of mercury in urine is performed to detect exposure to inorganic mercury. For mercury and its inorganic compounds in urine, the Commission established a biological tolerance value (Biologischer Arbeitsstoff-Toleranzwert; BAT Value) of 25 µg/g creatinine [DFG 2018].

Table 1 and Table 2 show selected study data on mercury concentrations in the blood and urine of the general population and of occupationally exposed workers.

Table 1: Reference values for mercury in blood and in urine [Schulz et al. 2011].

Sample material	Groups of persons/ age	Reference year	Reference value	HBM I value	HBM II value
First morning void	Children (3–14 years), without amalgam fillings	2003/2006	0.4 µg/L		
	Adults (18–69 years), without amalgam fillings	1997/1999	1 µg/L		
Whole blood	Children (3–14 years), up to three servings of fish per month	2003/2006	0.8 µg/L		
	Adults (18–69 years), up to three servings of fish per month	1997/1999	2 µg/L		
First morning void	General population			5 µg/g creatinine or 7 µg/L	20 µg/g creatinine or 25 µg/L
Whole blood	General population			5 µg/L	15 µg/L

Table 2: Selected study data on mercury concentrations in blood and urine of occupationally exposed workers.

Study	Collective	Number of samples n	Mercury concentration median	
			Blood [µg/L]	Urine [µg/g Crea]
Schecter et al. 2018	Electronic scrap recycling	40	2.49	0.52
Goodrich et al. 2016	Dentists	434 (blood) 606 (urine)	3.67	1.07[a]
Zeneli et al. 2016	Power plant	70	1.53	–
Dantas and Queiroz 1997	Mercury production	36	–	19.4
Cárdenas et al. 1993	Chloralkali plant	44	7.2	21.9

[a]converted assuming 1.2 g creatinine/L

Contents

1 General principles

Mercury is determined by flow injection cold vapour atomic absorption spectrometry (CV-AAS).

The digested blood or urine samples are stabilised with potassium permanganate, introduced into the acid carrier flow (hydrochloric acid) and mixed with the reducing agent sodium borohydride. Mercury vapour formed by reduction is transported with an argon flow into the atomisation cell of the AA spectrometer.

Calibration is performed using matrix matched calibration solutions. The mercury concentrations in real samples are calculated from the linear relationship between the measured absorbance and the mass concentration of mercury.

2 Equipment, chemicals and solutions

2.1 Equipment

- Cold vapour atomic absorption spectrometer with integrated flow injection system and autosampler (e.g. FIMS 400 by Perkin-Elmer)
- Analytical balance (e.g. Sartorius)
- 1000 mL PE bottle with Dispensette (variably adjustable between 0.5 and 5 mL) (e.g. Brand)
- Various volumetric flasks made of glass (e.g. Schott)
- 100 mL measuring cylinder (e.g. Brand)
- Microlitre pipettes with adjustable volume between 10–100 µL or 100–1000 µL and suitable pipette tips (e.g. Eppendorf)
- 10 mL sample vessels made of plastic (polystyrene) for the autosampler (e.g. Sarstedt)
- Filter paper, 55 mm in diameter (e.g. Schleicher & Schuell No. 595)
- Vortex-mixer (e.g. REAX 2000 by Heidolph)
- 250 mL screw cap containers for urine collection (e.g. Sarstedt)
- 10 mL blood collection tubes (e.g. Sarstedt S-Monovette®)
- Digestion block heater (e.g. Liebisch, Bielefeld)
- Glass vials for digestion (e.g. Macherey-Nagel, Düren)

2.2 Chemicals

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

- Mercury standard solution, 1 g/L (e.g. SpexCertiPrep No. PLHG4-2M)
- Potassium permanganate (e.g. Merck No. 105084)
- Potassium peroxodisulfate (e.g. Merck No. 105091)
- Sodium borohydride (e.g. Merck No. 106371)
- Sodium hydroxide pellets, EMSURE® (e.g. Merck No. 106469)
- Nitric acid 65%, Suprapur® (e.g. Merck No. 100441)
- Hydrochloric acid 30%, Suprapur® (e.g. Merck No. 100318)
- Sulfuric acid 96%, Suprapur® (e.g. Merck Nr. 100714)

- Antifoam Dow Corning DB 110 A (e.g. Perkin-Elmer No. B0507226)
- Deionised water
- Argon 5.0 (e.g. Linde)

2.3 Solutions

- Potassium permanganate solution (5%)
  Exactly 20.0 g potassium permanganate are weighed into a 200 mL volumetric flask and dissolved in deionised water. The flask is then filled with deionised water.
- Hydrochloric acid solution (2.4%)
  200 mL deionised water are placed into a 1000 mL volumetric flask, to which 80 mL of 30% hydrochloric acid are added. The flask is then filled to the mark with deionised water.
- Sodium borohydride solution (0.2%)
  Exactly 2 g sodium borohydride and 0.7 g sodium hydroxide pellets are weighed into a 1000 mL volumetric flask, to which 2 mL of the antifoam are added. The chemicals are dissolved in deionised water and the flask is then filled to the mark with deionised water.
- Digestion solution
  Exactly 10 g potassium peroxodisulfate are weighed into a 500 mL volumetric flask and dissolved in 25 mL concentrated sulfuric acid and 200 mL nitric acid. The solution should be left to stand for a while as initially increased foaming may occur.

The prepared potassium permanganate solution and the hydrochloric acid solution are stable for one month if stored at room temperature. The sodium borohydride solution and the digestion solution are stable for two or three days, respectively, if stored at room temperature.

2.4 Calibration standards

- Stock solution (1000 µg/L)
  Approximately 50 mL deionised water and 5 mL concentrated nitric acid are placed into a 100 mL volumetric flask, to which 100 µL of the 1000 mg/L mercury standard solution are pipetted. The volumetric flask is filled up to the mark with deionised water and the solution is homogenised by shaking.
- Spiking solution 1 (100 µg/L)
  1 mL of the stock solution is pipetted into a 10 mL volumetric flask, which is then filled up to the mark with deionised water.
- Spiking solution 2 (10 µg/L)
  100 µL of the stock solution are pipetted into a 10 mL volumetric flask, which is then filled up to the mark with deionised water.

All solutions are freshly prepared in quartz volumetric flasks every working day and used within half an hour to prepare the calibration standards. If the stock solution and the spiking solutions do not prove stable even in this short time, 100 µL each of the potassium permanganate solution can be added for stabilisation.

Calibration standards in the concentration range of up to 2 µg mercury per litre blood or 10 µg mercury per litre urine are prepared by diluting the spiking solutions according to the schemes given in Tables 3 and 4.

For matrix adapted calibration, each calibration standard is prepared using 1.5 mL of a digested sample (0.5 mL blood or urine + 1 mL digestion solution). Larger volumes of this digested material can be prepared. However, it must be ensured that the mercury concentration is below the limit of quantitation. 100 µL each of the potassium permanganate solution and the appropriate amount of spiking solution are added to the cooled digested material which is then made up to 5 mL with deionised water.

Table 3: Pipetting scheme for the preparation of calibration standards to determine mercury in blood.

Digested blood sample	$KMnO_4$ solution	Spiking solution 2	Deionised water	Conc. calibration standards
[µL]	[µL]	[µL]	[µL]	[µg/L]
1500	100	0	3400	0
1500	100	50	3350	0.1
1500	100	100	3300	0.2
1500	100	250	3150	0.5
1500	100	500	2900	1.0
1500	100	1000	2400	2.0

Table 4: Pipetting scheme for the preparation of calibration standards to determine mercury in urine.

Digested urine sample	$KMnO_4$ solution	Spiking solution 1	Deionised water	Conc. calibration standards
[µL]	[µL]	[µL]	[µL]	[µg/L]
1500	100	0	3400	0
1500	100	50	3350	1
1500	100	100	3300	2
1500	100	250	3150	5
1500	100	500	2900	10

3 Specimen collection and sample preparation

3.1 Specimen collection

As with all trace element analyses, the reagents and materials used must be of the highest purity. Any contamination must also be avoided during sampling.

Both lithium heparin blood and potassium EDTA blood are suitable for the determination of mercury in blood. It must be ensured, however, that the blood collection devices are free of mercury. If analysis cannot be performed immediately, the blood can be stored in the refrigerator at +4 °C for about one week. For long-term storage (weeks or months), storage at −20 °C is recommended.

For the determination of mercury in urine, the polyethylene containers used for specimen collection must be cleaned with 1% nitric acid prior to use. For acid cleaning, 1% nitric acid is filled into the containers and left to stand for at least two hours. The containers are then thoroughly rinsed with ultrapure water and dried. For the determination of environmental exposure, 24-hour urine is best suited for analysis, but spot urine or first morning void can also be used. In the case of occupational exposure, spot urine is collected, there being no restrictions with regard to the sampling time. If the preparation and analysis of the urine samples is not possible within 1–2 days after sampling, the urine should be acidified with 1 mL concentrated nitric acid per 100 mL urine and can then be stored in the refrigerator at +4 °C. For long-term storage (weeks or months), storage at −20 °C is recommended.

3.2 Sample preparation

The blood or urine samples are brought to room temperature and thoroughly mixed. A 0.5 mL aliquot of the blood or urine sample is pipetted into a screw top glass vial. 1 mL of the digestion solution is added and the sample digested at 90 °C for 45 min. After cooling, 100 µL of the potassium permanganate solution are added to the digested sample. The sample is then made up to 5 mL with deionised water and homogenised on a vortex-mixer.

If the sample still has to be transferred into a plastic autosampler vial, polystyrene is a better material than polyethylene or polypropylene with regard to the stability of mercury.

With the blood samples, it may happen that there are still particles in the digested sample. In such cases, the volume of the digestion solution should be increased from 1 mL to 1.5 mL and the amount of added water should be reduced accordingly. The digested solution can also be filtered prior to final dilution. Blood samples that were prepared in this way yielded no false low mercury levels.

4 Operational parameters

Analysis was performed using a cold vapour atomic absorption spectrometer coupled to a flow injection system with autosampler.

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

4.1 Flow injection

The system is equipped with two peristaltic pumps and a multiport valve. When the multiport valve is in the FILL position, the sample loop is filled with an exact sample volume. When the valve is switched to the INJECT position, the sample is introduced into the carrier stream and transported to the mixing section where the sample is mixed for reduction with sodium borohydride. The resulting reaction mixture is then transported to a gas/ liquid separator where the elemental mercury is released by the argon carrier gas and swept into the quartz cell of the spectrometer.

To stabilise the sample, it may be useful to add additional potassium permanganate solution "online" to the acidified sample solution. Within this method a t-piece was used to add 1% potassium permanganate solution before the acidified sample was mixed with the sodium borohydride solution. 1% potassium permanganate solution is prepared by diluting the respective 5% solution with deionised water.

The flow injection flow chart is shown in Figure 1.

The flow injection parameters are given in Table 5 and 6.

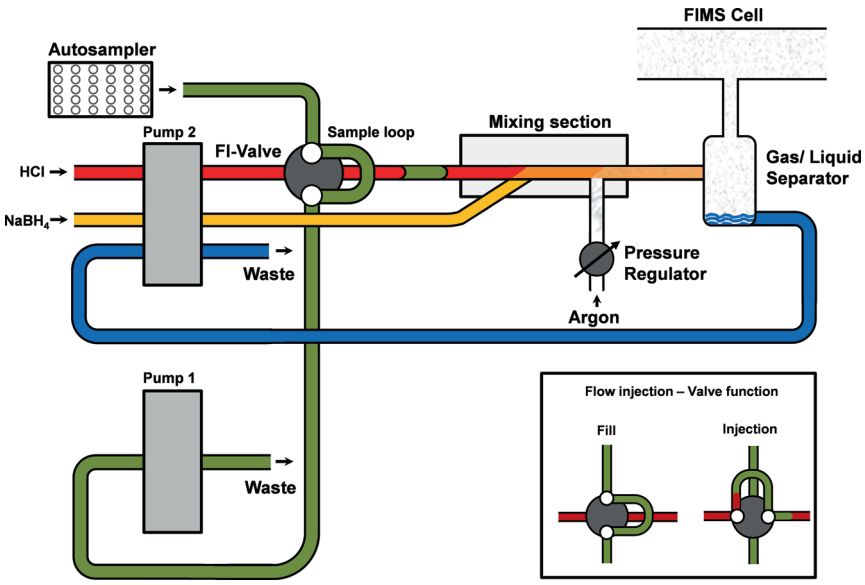

Figure 1: Flow chart for the cold vapour AAS.

Table 5: Flow injection parameters for the determination of mercury in blood and in urine.

Step	Duration [s]	Pump 1	Pump 2	Valve position	Measurement
Prefill	15	100	120	Fill	
1	10	100	120	Fill	
2	30	0	120	Inject	x

Table 6: Flow injection system for the determination of mercury in blood and in urine: information on the peristaltic pump tubings.

Solution	Concentration	Pump tubing
Hydrochloride acid solution	2.4% (V/V)	yellow/blue
Sodium borohydride solution	0.2% (m/V)	red/red
Potassium permanganate solution	1% (m/V)	white/orange
Sample solution	–	red/red

4.2 Cold vapour atomic absorption spectrometer

Wavelength	253.7 nm
Slit width	0.7 nm
Read time	30 s
Read delay	4 s
BOC time	2 s
Argon gas flow	50 mL/min
Cell temperature	80 °C
Evaluation	Peak height

5 Analytical determination

The samples prepared and diluted 1:10 according to Section 3 are mixed with the acid carrier in the flow injection system. Immediately afterwards, the 1% potassium permanganate solution is added. The mercury in the sample is reduced to elemental mercury by the sodium borohydride solution and then swept with the carrier gas into the quartz cell of the spectrometer. Absorbance measurements are performed in triplicate, using the mean value for data output.

Reagent blank values are included at the beginning of each analytical run, following the calibration samples and after the quality control samples.

6 Calibration

The calibration solutions described in Section 2.4 are analysed in the same way as the samples using FI-CV-AAS (see Section 4). A calibration graph is obtained by plotting the measured absorbance of the individual calibration standards against the respective mercury concentration. Under the conditions described, the calibration graph is linear in the range from the detection limit to 2 µg mercury/L (blood) or up to 10 µg mercury/L (urine). Recalibration should be performed if the quality control results suggest systematic errors. Figure 2 (in the Appendix) shows an example of a calibration graph for the determination of mercury in blood and in urine.

7 Calculation of the analytical results

The analyte concentration in µg mercury/L is calculated by entering the measured absorbance (peak height) into the corresponding calibration graph taking into account the 1:10 dilution of the samples. Any reagent blank values are accounted for by subtraction. This calculation is usually performed by the spectrometer software.

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, at least three quality control samples with known and constant analyte concentrations are analysed within each analytical run. Control materials from various manufacturers and certified reference materials are commercially available for mercury in blood and in urine. Therefore, two or more control materials with different concentrations should be used for quality control in order to cover a wide concentration range. The control materials should be analysed after calibration, after every twentieth sample and at the end of the analytical run.

The measured values of the control samples analysed within each analytical run should be within the specified tolerance ranges.

9 Evaluation of the method

The reliability of the method was verified by comprehensive validation and by successful participation in round robin tests.

9.1 Precision

To determine within-day precision, blood with 3.4 µg or 15 µg mercury per litre and urine with 41 µg or 127 µg mercury per litre was used. These samples were processed ten times in parallel and then analysed. The obtained within-day precision data are given in Table 7.

Table 7: Within-day precision for the determination of mercury in blood and in urine (n = 10).

Matrix	Concentration [µg/L]	Standard deviation (rel.) s_w [%]	Prognostic range u [%]
Blood	3.4	2.0	4.5
	15.0	1.6	3.6
Urine	41.0	1.2	2.7
	127	1.1	2.5

To determine day-to-day precision, the same control materials were processed on twenty different days and the mercury concentration was determined. The results are summarised in Table 8.

Table 8: Day-to-day precision for the determination of mercury in blood and in urine (n = 20).

Matrix	Concentration [µg/L]	Standard deviation (rel.) s_w [%]	Prognostic range u [%]
Blood	3.4	4.8	10.0
	15.0	4.7	9.8
Urine	41.0	7.9	16.5
	127	6.2	13.0

9.2 Accuracy

The accuracy of the method was validated by internal and external quality control. For internal quality control, the control materials ClinChek Blood 1 and 2 (Recipe) as well as Lyphochek Urine 1 and 2 (Biorad) were analysed on twenty days. The results are presented in Table 9.

To determine the recovery rate, blood and urine samples were spiked with 10 µg Hg^{2+}/L, processed three times and analysed. The recovery was calculated on the basis of the determined concentrations in the spiked material by subtracting any background levels in the unspiked material. The recovery rates in blood and urine were 102 and 98%, respectively. In addition, blood was spiked with 10 µg methylmercury/L, processed three times and analysed. The recovery rate was 98%. This recovery rate of nearly 100% ensures that even organic methylmercury is completely reduced to elemental mercury and detected by this method.

External quality control was ensured by successful participation in the German external quality assessment scheme (G-EQUAS) No. 38. The results obtained with this method are summarised in Table 10.

Table 9: Recovery rates for the determination of mercury in blood and in urine (n = 20).

Material	Matrix	Nominal value [µg/L]	Measured value [µg/L]	Mean rel. recovery [%]
ClinChek 1	Blood	3.5	3.6	103
ClinChek 2		15.0	14.8	98.7
Lyphochek 1	Urine	41.0	42.0	102
Lyphochek 2		127	128	101

Table 10: Results of participation in the German external quality assessment scheme (GEQUAS) to determine the accuracy of the method.

G-EQUAS	Matrix	Nominal value	Measured value	Accuracy
		[µg/L]	[µg/L]	[%]
No. 38	Blood	1.1	1.2	109
		2.3	2.7	117
		12.0	12.7	106
		15.7	14.7	93.6
No. 38	Urine	1.5	1.5	100
		3.0	3.4	113
		16.7	18.6	111
		46.3	50.9	110

9.3 Limits of detection and limits of quantitation

Under the analytical conditions specified, the detection limits for the undiluted blood and urine samples were determined to be 0.04 µg mercury per litre. The detection limits were determined using three times the standard deviation of the absorbance of blank values (n = 10) (Table 11).

Table 11: Detection limits and quantitation limits for the determination of mercury in blood and in urine (n = 10).

Matrix	Detection limit [µg/L]	Quantitation limit [µg/L]
Blood	0.04	0.12
Urine	0.04	0.12

9.4 Sources of error

The analytical method presented herein permits the specific and sensitive determination of both occupational and environmental exposure to mercury and mercury compounds.

In order to determine mercury concentrations in blood and in urine correctly even at lower levels, the user of the method should always be aware of the risk of mercury contamination from reagents, vessels or ambient air. All chemicals used should be checked for blank values at regular intervals and the digestion vials, volumetric flasks, tubes and pipettes used should also meet the highest purity standards. Clean-room conditions are not mandatory, but beneficial.

However, it must also be taken into account that various mechanisms can lead to analyte loss. For example, evaporation, adsorption on vessel walls, suspended matter or colloids, complexation or amalgam formation may lead to false low results. With regard to the stability of mercury, polystyrene is a better material for autosampler vials than polyethylene or polypropylene and should be preferred.

For digestion and stabilisation of mercury in the solution, the use of oxidising acids (nitric acid, sulfuric acid, perchloric acid) in combination with strong oxidising agents (potassium dichromate, potassium permanganate, potassium peroxodisulfate) is suggested in the literature. Due to special safety precautions required for perchlorate and the toxicity of chromium(VI) compounds, a combination of nitric acid, sulfuric acid, potassium permanganate and potassium peroxodisulfate was chosen for this method. A disadvantage is the possible precipitation of manganese dioxide, which may occur in urine samples. The urine samples should therefore be analysed as soon as possible after the addition of potassium permanganate.

Any matrix effects that may occur can be well compensated for by using matrix-matched calibration standards. Besides, the organic matrix of the biological material is destroyed by the strong oxidative digestion.

As there is a risk of carry-over in the flow injection tubing, 1% potassium permanganate solution is added "online" to the sample solution mixed with the hydrochloric acid carrier solution. This avoids false low results that occasionally occur in real urine samples and also increases the stability of the measuring signal. In order to prevent the formation of foam when adding the reducing agent (sodium borohydride) to the sample, an antifoam is used, which is provided by the manufacturer of the instrument.

10 Discussion of the method

This method, which is a combination of flow injection and cold vapour AAS, enables the reliable determination of mercury in both blood and urine. Due to the high detection sensitivity of the cold vapour technique for mercury, it can be used for the determination of both occupational and environmental exposure.

Blood and urine samples are processed in the same way. The strong oxidative digestion with potassium permanganate, potassium peroxodisulfate, sulfuric acid and nitric acid takes only 45 min and usually produces clear solutions. Even mercury, present as methylmercury, is determined quantitatively in real samples using this digestion method. While other digestion methods often lead to false low results for organic

mercury, the use of potassium peroxodisulfate in the digestion procedure described leads to recovery rates of almost 100%.

Instruments used: FIMS 400 AAS spectrometer with AS 90 autosampler manufactured by Perkin-Elmer.

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12 Appendix

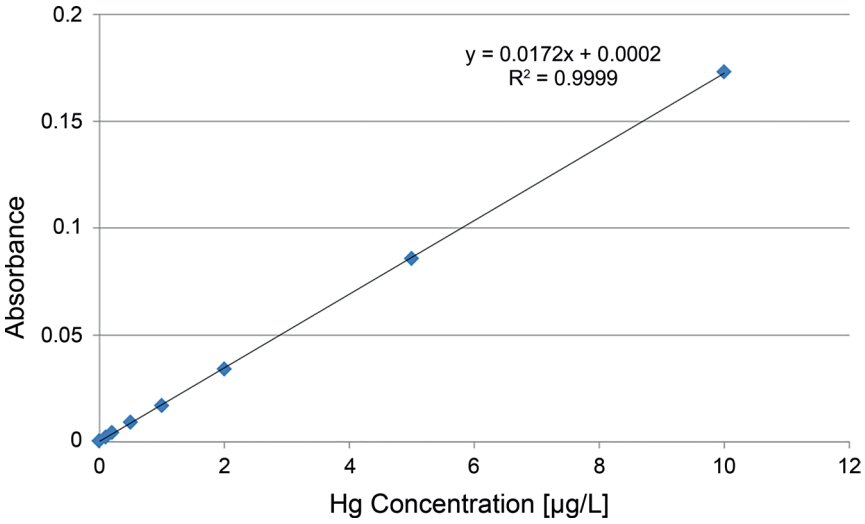

Figure 2: Calibration graph and linear dynamic range for the determination of mercury in blood and urine.