The MAK Collection for Occupational Health and Safety

Boric acid and tetraborates – Determination of boron in urine by ICP-OES

Biomonitoring Method – Translation of the German version from 2019

B. Michalke[1], P. Grill[1], J. Morton[2], T. Göen[3,*], A. Hartwig[4,*], MAK Commission[5,*]

1 *Method development, Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Research Unit Analytical BioGeoChemistry, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany*

2 *External verification, Health & Safety Laboratory, Harpur Hill, Buxton SK17 9JN, Vereinigtes Königreich*

3 *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*

4 *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*

5 *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: boron; boric acid; borax; disodium tetraborate; biomonitoring; urine; ICP-OES

Citation Note: Michalke B, Grill P, Morton J, Göen T, Hartwig A, MAK Commission. Boric acid and tetraborates – Determination of boron in urine by ICP-OES. Biomonitoring Method – Translation of the German version from 2019. MAK Collect Occup Health Saf [Original edition. Weinheim: Wiley-VCH; 2019 Jul;4(3):1713-1726]. Corrected republication without content-related editing. Düsseldorf: German Medical Science; 2025. https://doi.org/10.34865/bi1004335kske2219_w

Republished (online): 30 Apr 2025

Originally published by Wiley-VCH Verlag GmbH & Co. KGaA; https://doi.org/10.1002/3527600418.bi1004335kske2219

Manuscript completed: 19 Nov 2015

Published (online): 25 Jul 2019

The commission established rules and measures to avoid conflicts of interest.

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Biomonitoring Methods

B. Michalke[1], P. Grill[1], J. Morton[2], T. Göen[3,*], A. Hartwig[4,*], MAK Commission[5,*]

DOI: 10.1002/3527600418.bi1004335kske2219

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 and validated the presented biomonitoring method.
The analytical method described hereinafter is used to determine the boron concentration in urine using inductively coupled plasma optical emission spectrometry (ICP-OES). The method is rapid, simple, reliable, adequately sensitive and also suitable for routine use in laboratories with high sample throughput. It is possible to determine boron at both occupational and environmental concentrations. Sample preparation is performed by 1/20 (V/V) dilution of urine with 5% nitric acid, which largely reduces matrix interferences.

Keywords

boron; borax; disodium tetraborate; urine; biomonitoring; Analyses in Biological Materials; inductively coupled plasma optical emission spectrometry; ICP-OES

Author Information

[1] Method development, Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Research Unit Analytical BioGeoChemistry, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany
[2] External verification, Health & Safety Laboratory, Harpur Hill, Buxton SK17 9JN, United Kingdom
[3] 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
[4] 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
[5] 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)

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Boric acid and tetraborates – Determination of boron in urine by ICP-OES

Matrix:	Urine
Hazardous substances:	Boric acid and tetraborates
Analytical principle:	Inductively coupled plasma optical emission spectrometry (ICP-OES)
Completed in:	November 2015

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

Hazardous substance	CAS	Parameter	CAS
Boric acid	10043-35-3	Boron	7440-42-8
Disodium tetraborate	1330-43-4		
Disodium tetraborate-tetrahydrate (kernite)	12045-87-3		
Disodium tetraborate-pentahydrate	12179-04-3		
Disodium tetraborate-decahydrate (borax)	1303-96-4		

Summary

The analytical method described hereinafter is used to determine the boron concentration in urine using inductively coupled plasma optical emission spectrometry (ICP-OES). The method is rapid, simple, reliable, adequately sensitive and also suitable for routine use in laboratories with high sample throughput. It is possible to determine boron at both occupational and environmental concentrations. Sample preparation is performed by 1/20 (V/V) dilution of urine with 5% nitric acid, which largely reduces matrix interferences.

Reliability data of the method

Boron

Within-day precision:	Standard deviation (rel.)	s_w = 1.4%, 1.0% or 1.2%
	Prognostic range	u = 3.1%, 2.2% or 2.7%
	at a concentration of 2500 µg, 3500 µg or 5500 µg boron per litre urine and where n = 10 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 1.6%, 1.8% or 1.7%
	Prognostic range	u = 3.7%, 4.2% or 3.9%
	at a concentration of 2500 µg, 3500 µg or 5500 µg boron per litre urine and where n = 10 determinations	
Accuracy:	Recovery rate (rel.)	r = 103% or 102%
	at a spiked concentration of 1000 µg or 3000 µg boron per litre urine and where n = 10 determinations	
Detection limit:	50 µg boron per litre urine	
Quantitation limit:	180 µg boron per litre urine	

General information on the hazardous substance

Boron (B, relative atomic mass 10.8, atomic number 5) is widely distributed in nature in the form of inorganic borates at low concentrations. The economically most attractive boron-bearing minerals are borax (tincal), colemanite, ulexite and kernite with Turkey, the USA, Russia and China owning the largest deposits [Moore 1997].

Boron compounds are primarily used in the production of borosilicate glass and glass fibres, as well as ceramic glazes and enamels. Besides, they are also used in the production of detergents, fertilisers and biocides [ECHA 2010; Moore 1997].

The mining of minerals and processing of refined sodium borate compounds may lead to occupational exposure of workers. However, occupational exposure to boron may occur in the above-mentioned industrial sectors, as well. There is also a dietary boron intake of the general population. Boron concentrations in drinking water are regulated in Germany with a limit value of 1 mg/L [TrinkwV 2001], while the level of 0.3 mg boron per litre drinking water is rarely exceeded. Bottled water, in contrast, contains significantly more boron with an average level of 0.75 mg/L [Moore 1997].

The dietary intake provides boron from various foods. Nuts, dried fruit, fruit, vegetables, wine and beer are particularly rich in boron. According to the EFSA Scientific Panel on Dietetic Products, Nutrition and Allergies, the average daily intake of boron from food is 1.5 mg/d (97.5th percentile: 2.6 mg/d) and 0.2–0.6 mg/d from drinking water [EFSA 2004; Moore 1997]. In general, the background boron contents in soils are extremely variable. The background levels measured in human individuals are usually < 3000 µg boron/L urine. A study carried out on 132 adults in Great Britain yielded a median background level of 830 µg boron per litre urine (95th percentile: 2340 µg boron per litre urine) [Morton et al. 2014].

The toxicokinetics of boric acid have been investigated in animal studies and in humans. At the workplace boric acid and tetraborates occur in the form of dust/aerosol. There is practically no absorption through the intact skin. After ingestion, boric acid is rapidly and completely absorbed. The exact amount of uptake via the respiratory tract is not clear; a systemic uptake of dust deposited in the respiratory tract via swallowing is thereby possible [Hartwig 2014].

After the intake of boric acid or salts of boric acid, 98.4% of boric acid is present in the organism as undissociated boric acid [Woods 1994] and is almost entirely eliminated unchanged with the urine [Murray 1995]. In humans, the plasma half-life of boric acid is about 21 h [Jansen et al. 1984]. Therefore, sampling to determine the boron concentration in urine after workplace exposure should be performed at the end of any shift [Bolt et al. 2017]. The boric acid and borate levels in biological materials are generally related to elemental boron (B) [Bolt et al. 2017].

Boric acid and tetraborates occur in workplaces in the form of dust/aerosol and causes acute respiratory irritation. The MAK values established on the basis of this irritation by means of studies involving human volunteers are 10 mg boric acid/m^3 E (1.8 mg boron/m^3 E) or 5 mg disodium tetraborate pentahydrate/m^3 E (0.75 mg boron/m^3 E). The MAK value of 0.75 mg boron/m^3 E also applies to other tetraborates and their hydrates [Hartwig 2014]. Besides, the Commission has classified boric acid in Pregnancy Risk Group B and tetraborates in Pregnancy Risk Group C [DFG 2018]. For a detailed toxicological evaluation of boric acid and tetraborates, please refer to the respective MAK Value Documentations [Hartwig 2014; Henschler 1993].

Assessment values in biological material are not available for boron owing to the fact that local irritation is the main effect and that there is no systemic toxicity at these concentrations [Bolt et al. 2017]. Some studies, however, have investigated boron concentrations in the urine of occupationally exposed individuals. For example, Robbins et al. [2010] found mean levels of 16.7 ± 31.4 mg boron per litre in the urine of 66 workers exposed to boron. Another study investigated the levels in 102 boron exposed workers in Turkey and found mean concentrations of 6.6 ± 4.2 mg boron per litre urine [Duydu et al. 2011].

Contents

1 General principles

The analytical method described hereinafter is used to determine the boron concentration in urine using inductively coupled plasma optical emission spectrometry (ICP-OES). The method is rapid, simple, reliable, adequately sensitive and also suitable for routine use in laboratories with high sample throughput. It is possible to determine boron at both occupational and environmental concentrations. Sample preparation is performed by 1/20 (V/V) dilution of urine with 5% nitric acid, which largely reduces matrix interferences.

2 Equipment, chemicals and solutions

2.1 Equipment

- ICP-OES with autosampler (e.g. Ciros Vision by Spectro Analytical Instruments GmbH or Optima 7300 DV by Perkin Elmer Inc., USA)

- Distillacid BSB-939-IR sub-boiling distillation system (e.g. Berghof Products + Instruments GmbH)
- Piston stroke pipettes, with adjustable volumes between 1–10 µL, 10–100 µL or 100–1000 µL with matching pipette tips (e.g. Eppendorf)
- 10 mL quartz vials (e.g. Schott)
- 250 mL urine sample containers with screw caps (e.g. Sarstedt No. 77.577)

2.2 Chemicals

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

- Concentrated high-purity nitric acid, purified by acid distillation
- Boron standard solution, 1000 mg/L (e.g. Perkin Elmer No. N9303760)
- Ultrapure water, fully deionised (> 18 MΩ×cm) (e.g. Milli-Q® plus VE System)
- Pooled human urine with a boron concentration as low as possible
- Argon 5.0 (e.g. Linde)

2.3 Solutions

All solutions are prepared in quartz vials to avoid contamination.

- Nitric acid (5%)
  500 µL of high-purity nitric acid are added to 9500 µL ultrapure water in a quartz vial. The solution is thoroughly mixed and is stable for at least 14 days when stored in the refrigerator at +4 °C.

2.4 Calibration standards

- Stock solution (10 mg/L)
  100 µL of the 1000 mg/L boron standard solution are pipetted into a 10 mL quartz vial. Then 9900 µL ultrapure water are added and the solution is mixed thoroughly. The boron stock solution is stored in the refrigerator at +4 °C and is freshly prepared every 14 days.
- Spiking solution (1 mg/L)
  1 mL of the stock solution and 9000 µL 5% nitric acid are pipetted into a 10 mL quartz vial. The solution is mixed thoroughly. The spiking solution is freshly prepared every working day.

The calibration standards are prepared in 5% nitric acid according to the pipetting scheme shown in Table 1. These calibration standards, prepared according to the pipetting scheme in Table 1, are ready to be analysed. The given concentration levels represent the boron levels in the 1/20 (V/V) diluted urine samples (see Section 3.2).

Table 1 Pipetting scheme for the preparation of calibration standards used to determine boron in urine.

Concentration of calibration standard	Volume of spiking solution	Volume of stock solution	Volume of 5% nitric acid	Final volume
[µg/L]	[µL]	[µL]	[µL]	[mL]
0	–	–	10 000	10
10	100	–	9900	10
25	250	–	9750	10
50	500	–	9500	10
100	–	100	9900	10
250	–	250	9750	10
500	–	500	9500	10
1000	–	1000	9000	10
2500	–	2500	7500	10

In order to check whether the 1/20 dilution of urine sufficiently eliminates matrix effects, matrix-adapted calibration can additionally be performed by standard addition of the analyte to a pooled human urine sample low in boron. To prepare three calibration standards, defined volumes of boron stock solution and 5% nitric acid are added to this urine so that the urine is diluted at a ratio of 1:20 (V/V). In 1/20 diluted real samples, boron is quantified at levels between 100 µg and 150 µg per litre urine. The concentration for the standard addition should be chosen adequately.

3 Specimen collection and sample preparation

3.1 Specimen collection

Reagents and vials of the highest purity are used. Any contamination must also be avoided during sampling. The urine should be collected in polyethylene containers pre-cleaned with 1% nitric acid.

24-h urine collection is ideal for determining the background exposure to boron. However, spot urine or first morning voids can also be used. For occupational exposure scenarios, post-shift sampling of spot urine is advisable [Bolt et al. 2017].

3.2 Sample preparation

If it is not possible to determine boron within 1–2 days after sampling, the urine sample should be acidified with 1 mL of concentrated high-purity nitric acid per 100 mL urine and can thus be stored in the refrigerator at +4 °C. For long-term storage over weeks or months, storage at –20 °C is recommended.

The urine sample is brought to room temperature and mixed thoroughly. 500 µL of the homogenised sample are added to 9500 µL 5% nitric acid in a 10 mL quartz vial.

4 Operational parameters

Analysis is performed using an inductively coupled plasma optical emission spectrometer.

4.1 Sample feeding and plasma settings

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

Sample delivery:	Peristaltic pump, flow rate: 1.0 mL/min
Spray chamber:	Cyclon type
Nebuliser:	Seaspray
Nebuliser gas:	0.7 L/min argon
Injector tube (torch):	1.8 mm inner diameter
Plasma power:	1200 W
Plasma gas:	14 L/min argon
Auxiliary gas:	0.6 L/min argon

In principle, other nebulisers can also be used for sample introduction.

4.2 Optical emission spectrometry

Due to the different spectrometer types, the OES settings also depend on the respective instrumentation and always have to be optimised individually. Here, too, additional settings and parameter optimisation may be required when using spectrometers from other manufacturers. The following settings are therefore intended as a rough guide only.

Wavelength	249.773 nm
Evaluation	Peak area
Background correction	on both sides, 1st degree polynominal

5 Analytical determination

The urine samples diluted according to Section 3 are introduced directly into the ICP and analysed by optical emission spectrometry.

The mean value of three emission measurements is used for data output.

6 Calibration

The calibration standards are prepared according to Section 2.4 and analysed by ICP-OES (see Section 4). A calibration graph is obtained by plotting the measured intensities of the emission lines against the respective boron concentration. Under the described conditions, the calibration graph is linear in the range between the detection limit and at least 2500 µg boron per litre, corresponding to 50.000 µg boron per litre undiluted urine. Recalibration is recommended if the quality assurance results indicate systematic deviations. Figure 1 (in the Appendix) shows an example of a calibration graph for the determination of boron in urine.

7 Calculation of the analytical results

Taking into account the 1/20 dilution of the urine samples, the boron concentrations in µg per litre urine are calculated by inserting the intensities of the spectral lines (peak area) determined for the analysed sample into the calibration function. Any reagent blank values have to be subtracted from the analytical results. This data analysis 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, quality control samples with known and constant analyte concentrations are analysed within each analytical run. As neither control materials nor certified reference materials are commercially available for boron in urine, the control material must be prepared in the laboratory by spiking pooled urine. The analyte concentration in the quality control material should be within the relevant range (e.g. 250 µg/L corresponding to 5.000 µg boron per litre undiluted urine). Aliquots of these samples are stored at –20 °C and are included in each analytical run as quality control samples. The nominal value and the tolerance ranges of the quality control material are determined during a pre-analytical period (one preparation and analysis of the control material each on 10 different days). The measured values of the control samples assayed within each analytical run should be within the specified tolerance ranges [Bader et al. 2010].

9 Evaluation of the method

The reliability of the method was verified by comprehensive validation as well as by implementation and validation of the method in a second, independent laboratory.

9.1 Precision

To determine within-day precision, urine was spiked with 1000 µg or 3000 µg boron per litre, corresponding to a concentration of 50 µg/L and 150 µg/L in the 1/20 diluted urine, respectively. The unspiked urine samples and the spiked samples were processed ten times in parallel and then analysed. The obtained within-day precision data are presented in Table 2.

To determine day-to-day precision, the same control materials were processed on ten different days and the boron concentration was determined. The obtained day-to-day precision data are presented in Table 3.

Table 2 Within-day precision for the determination of boron in urine (n = 10).

Spiked concentration [µg/L urine]	Measured concentration [µg/L urine]	Standard deviation (rel.) s_w [%]	Prognostic range u [%]
0	2480	1.38	3.12
1000	3580	0.96	2.17
3000	5640	1.17	2.65

Table 3 Day-to-day precision for the determination of boron in urine (n = 10).

Spiked concentration [µg/L urine]	Measured concentration [µg/L urine]	Standard deviation (rel.) s_w [%]	Prognostic range u [%]
0	2500	1.64	3.71
1000	3520	1.84	4.16
3000	5560	1.73	3.91

9.2 Accuracy

The accuracy of the method was determined on the basis of the day-to-day precision data. It was determined to be 103% and 102% after spiking with 1000 µg and 3000 µg boron per litre urine, corresponding to a concentration of 50 µg/L and 150 µg/L in the 1/20 diluted urine, respectively (n = 10).

The comparability of the method presented herein was validated by an interlaboratory comparison. To this end, pooled urine was spiked with 200 µg or 2400 µg boron per litre urine (corresponding to a spiked concentration of 10 µg/L and 120 µg/L in the 1/20 diluted urine, respectively) and aliquots of both native and spiked urine were sent to three laboratories. The boron concentrations of the respective samples were determined in the three laboratories using different methods. In addition to the described optical emission spectrometry, mass spectrometry and high-resolution mass spectrometry were used. The results of this interlaboratory comparison are shown in Table 4.

Table 4 Determination of boron in urine: comparison of laboratories and methods.

Laboratory	Method	Measured mean concentration [µg/L urine]		
		Native sample	Spiked concentration 200 µg/L urine	Spiked concentration 2400 µg/L urine
1	ICP-OES [a]	1220	1460	3740
	ICP-MS	1360	1710	3920
	ICP-HRMS	1240	1350	3610
2	ICP-MS [b]	1274	1508	3894
3	ICP-OES	1355	1588	4140
	ICP-MS	1362	1609	4089

[a] method described herein; [b] use of beryllium as internal standard

If the results are related to the method described herein (ICP-OES, laboratory 1), very similar boron concentrations are found in the other laboratories or with the other methods. The mean recovery for the unspiked urine and the urine spiked with 200 µg/L and 2400 µg/L, was 106%, 104% and 104%, respectively.

9.3 Limit of detection and limit of quantitation

The detection limit was calculated from the signal-to-noise ratio and the standard deviation of the spectral background intensity according to the 3 s criterion (n = 10). Under the specified conditions, it was determined to be 50 µg boron per litre urine for the undiluted sample. The calculated quantitation limit (10 s criterion) was 180 µg boron per litre urine, likewise for the undiluted urine sample.

9.4 Sources of error

The analytical method presented herein permits the specific and sensitive determination of both occupational and environmental exposure to boric acid and tetraborates.

In order to determine the correct boron concentration in urine, the user of the method should always be aware of the risk of boron contamination caused by reagents and glassware. All chemicals used should therefore be checked for blank values at regular intervals and the vessels, tubes and pipettes used should also meet the highest purity standards. Basically, no glassware should be used, but only laboratory equipment made of plastic (with a smooth surface, e.g. Nalgene) or quartz.

Due to the use of relatively highly diluted urine, no matrix effects or interferences are seen due to nebulisation or emission. Both boron lines are interference-free. The more sensitive spectral line at 249.773 nm is used for analysis (Figure 2 in the Appendix).

10 Discussion of the method

Various analytical methods for the determination of boron in biological materials, such as atomic absorption spectrometry, ICP mass spectrometry or optical emission spectrometry (ICP-OES) are described in the literature. Boron is most commonly analysed in biological materials by ICP-MS or ICP-OES, with ICP-MS being more suitable for low-concentration samples. Urine samples with background boron levels of 2500 to 3000 µg per litre have to be diluted several times in order to fit the measuring range of the mass spectrometer and to avoid unnecessary contamination of the system with high amounts of various elements. Therefore, the more cost-effective ICP-OES is best suited for the determination of boron in urine, since only a single dilution step is needed to fit the boron concentration to the measuring range of the instrument. Therefore, a 1/20 dilution of urine proved to be adequate. A better sensitivity could be achieved using a lower dilution ratio, although in this case precision data may be adversely influenced due to possible matrix effects. However, since the background levels of about 2500 to 3000 µg boron per litre urine are far above the quantitation limit (180 µg/L), higher precision is preferred over a lower detection limit.

The presented method enables the reliable determination of boron concentrations in urine. It permits a sensitive determination of both the background exposure of the general population and the exposure of occupational exposed individuals. The recovery rates of almost 100% (see Section 9.2) determined during method validation show that the use of the standard addition method is not essential.

Among the critical aspects of boron determination are the risk of contamination, especially from borosilicate glassware, as well as the risk of analyte loss. Analyte loss may occur in particular if acidified samples are heated. For this reason, and in order to keep the procedure as simple as possible, the sample is merely diluted and the urine analysed without further digestion. All work steps during sampling and sample preparation should be performed in pre-cleaned plastic or quartz vessels.

In conclusion, the method enables a rapid, simple, reliable and adequately sensitive boron determination and is also suitable for routine use in laboratories with a high sample throughput.

Instruments used: Inductively coupled argon plasma atomic emission spectrometer (ICP-OES): Ciros Vision (SPECTRO Analytical Instruments GmbH) or Optima 7300 DV (Perkin Elmer Inc., USA).

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Method development: B. Michalke, P. Grill
External verification: J. Morton
Chair of the working group "Analyses in Biological Materials", Deutsche Forschungsgemeinschaft: Th. Göen
Chair of the "Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area", Deutsche Forschungsgemeinschaft: A. Hartwig
Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft: MAK Commission

12 Appendix

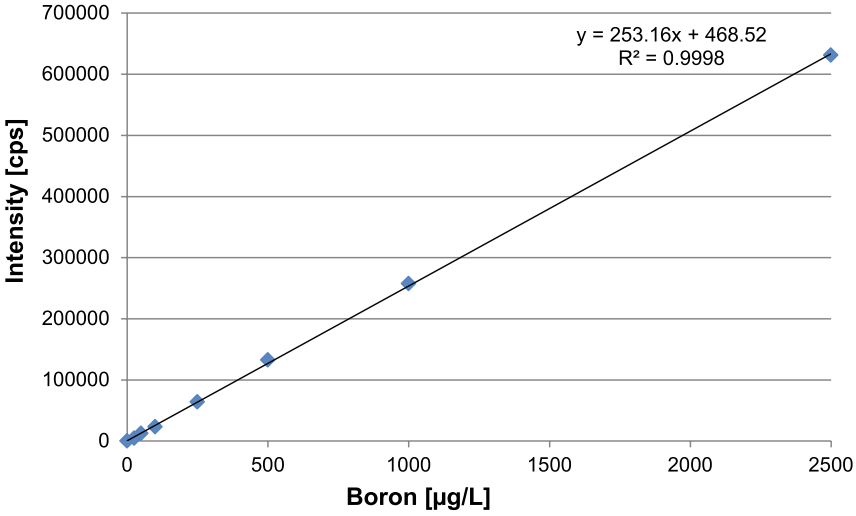

Figure 1 Calibration graph for the determination of boron in urine.

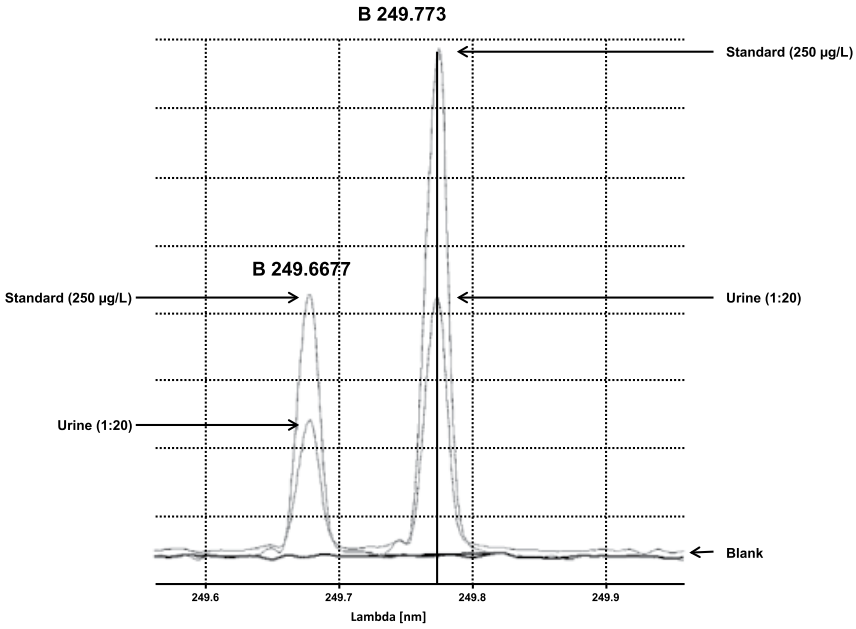

Figure 2 Emission lines of boron.