The MAK-Collection for Occupational Health and Safety

GMS PUBLISSO ZB MED-Publikationsportal Lebenswissenschaften

Iodine and inorganic iodides – Determination of iodide in serum/plasma or urine by ion chromatography-ICP-MS

Biomonitoring Method – Translation of the German version from 2021

B. Michalke[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 Executive (HSE), 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 (FAU) Erlangen-Nürnberg, 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

iodine; inorganic iodides; biomonitoring; serum; plasma; urine; ion chromatography; ICP-MS

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 verified the presented biomonitoring method.

This procedure enables the determination of iodide concentration in serum/plasma or urine by ion chromatography coupled with inductively coupled plasma-mass spectrometry (IC-ICP-MS). The method is reliable, sensitive, and – for a coupling method – relatively fast, and therefore suitable for routine testing in laboratories with high sample throughput. Using this method, iodide concentrations can be determined which are relevant for both occupational health and environmental medicine.

Sample preparation is carried out using ultra-pure water to dilute serum/plasma by a factor of 1 : 3 (v/v) and urine by a factor of 1 : 10 (v/v). Matrix interferences are significantly reduced by diluting the samples. Calibration is performed using matrix-matched calibration standards which are prepared in either serum/plasma or pooled urine.

Citation Note:
Michalke B, Morton J, Göen T, Hartwig A, MAK Commission. Iodine and inorganic iodides – Determination of iodide in serum/plasma or urine by ion chromatography-ICP-MS. Biomonitoring Method – Translation of the German version from 2021. MAK Collect Occup Health Saf. 2021 Mar;6(1):Doc023. DOI: https://doi.org/10.34865/bi755356e6_1or

Manuscript completed:
09 May 2018

Publication date:
31 Mar 2021

1 Characteristics of the method

Matrix Serum/plasma or urine

Analytical principle Ion chromatography coupled with inductively coupled plasma-mass spectrometry (IC-ICP-MS)

Parameter and corresponding hazardous substances

Hazardous substance	CAS No.	Parameter	CAS No.
Iodine	7553-56-2		
Ammonium iodide	12027-06-04		
Calcium iodide	10102-68-8		
Magnesium iodide	10377-58-9	Iodide	–
Potassium iodide	7681-11-0		
Sodium iodide	7681-82-5		
Methyl iodide	74-88-4		

Reliability data

Iodide in serum/plasma

Within-day precision:	Standard deviation (rel.) Prognostic range at a concentration of 1.63 µg iodide per litre of serum and n = 6 determinations	s_w = 4.9% u = 12.5%
Day-to-day precision:	Standard deviation (rel.) Prognostic range at a concentration of 1.64 µg iodide per litre of serum and n = 6 determinations	s_w = 5.3% u = 13.7%
Accuracy:	Recovery rate (rel.) at a spiked concentration of 5 µg iodide per litre of serum and n = 6 determinations	r = 99%
Detection limit:	0.35 µg iodide per litre of serum/plasma	
Quantitation limit:	1.2 µg iodide per litre of serum/plasma	

Iodide in urine

Within-day precision:	Standard deviation (rel.) Prognostic range at a concentration of 5.75 µg iodide per litre of urine and n = 6 determinations	s_w = 1.4% u = 3.6%
Day-to-day precision:	Standard deviation (rel.) Prognostic range at a concentration of 5.82 µg iodide per litre of urine and n = 6 determinations	s_w = 2.3% u = 5.9%
Accuracy:	Recovery rate (rel.) at a spiked concentration of 10 µg or 20 µg iodide per litre of urine and n = 6 determinations	r = 107% or 99%
Detection limit:	0.5 µg iodide per litre of urine	
Quantitation limit:	1.7 µg iodide per litre of urine	

2 General information on iodine and iodide

Iodine is a greyish-black, metallic, solid substance which is volatile and even sublimates at room temperature. Iodine is poorly soluble in water; however, it does dissolve well in aqueous potassium iodide or hydrogen iodide solutions under the formation of triiodine (I_3). Moreover, iodine is readily soluble in ethanol, carbon tetrachloride, chloroform, and other organic solvents. Iodine is widely distributed in the environment, but only in small concentrations. Higher concentrations of iodine or its compounds (up to 1%) are found in Chile saltpetre, in the form of lautarite ($Ca(IO_3)_2$), as well as in the form of iodide in the ashes of burnt seaweed. Organic iodine compounds can be isolated from marine algae, seaweed, and sea sponges. In the form of hydrogen iodide, iodine can be found in small amounts in volcanic gases. The inorganic salts of this hydroiodic acid are known as iodides and are very soluble in water.

The most important fields of application for iodine are those of hygiene (e.g. as disinfectants, antiseptics, and fungicides), medicine (e.g. for radioiodine diagnostics and therapy), and as a catalyst in synthesis chemistry (e.g. for the synthesis of acetic acid from ethylene). Iodine is also present in halogen lamps and is further used in the production of very pure metals (e.g. titanium, zirconium, and hafnium).

Iodine is an essential micronutrient. The main source for iodine intake is nutrition. The daily requirement of iodine for adults is specified to be about 150–200 µg (ATSDR 2004). In Germany, it is recommended not to exceed a dietary uptake of 500 µg iodine/day (D-A-CH 2015; Domke et al. 2004). In Germany, however, the soil has a very low iodine content, which means that all domestic plant- and animal-based food products are largely categorised as iodine-deficient. The only food products in which iodine can be found in any considerable amounts are saltwater fish and seafood. In 2000, Germany was still considered to be an iodine-deficient country, even though iodine supply has significantly improved due to the usage of iodised table salt (20 mg of iodine/kg) since the beginning of the 1980s (D-A-CH 2015). Referring to the iodine concentration in the soil, the *Bundesinstitut für Risikobewertung* (Federal Institute for Risk Assessment, BfR) still designates Germany as an iodine-deficient region in its most recent publication on iodine (BfR 2020).

Following oral consumption of water-soluble iodides, gastrointestinal absorption is almost complete. Inhaled iodine is also easily absorbed and retained at a rate of nearly 100% (ATSDR 2004). As a component of thyroid hormones, iodine is essential for the human organism. The absolute iodine content in the body of an adult in good health is estimated at around 10–20 mg, wherein the thyroid gland functions as a storage organ, holding about 70–80% of the total amount of iodine. Since iodine is present not merely in the thyroid gland but in many other parts of the body, where it engages in the process of metabolism, thyroid hormones are only suitable to a limited extent for obtaining information regarding the balance of iodine in the body or excessive iodine exposure (Michalke et al. 1996). A heightened oral intake of iodine can lead to thyroid dysfunction. Observed illnesses include hyperthyroidism, autoimmune diseases (e.g. Graves' disease and Hashimoto's thyroiditis), as well as hypothyroidism and goitre (Hartwig and MAK Commission 2017 a; WHO 2009).

Due to regional differences in iodine uptake, iodine excretion varies widely both throughout the day and over the course of several days, allowing no conclusive derivation of biological values. About 11% of the absorbed amount per day is excreted with the faeces, whereas the majority of absorbed iodine (89%) is excreted with the urine (Domke et al. 2004). In Germany, the concentration of iodine in urine – depending on regional circumstances – lies between 90 and 190 µg of iodine per litre. Normal serum levels lie between 50 and 100 µg of iodine per litre (Michalke et al. 2000 a). It should be noted that iodide represents only about 2–7%, in some cases up to about 13%, of total iodine in serum, whilst it is the main metabolite in urine (Michalke et al. 1996). According to Michalke et al. (2000 b), about 86% of the iodine in urine is present as iodide, about 3% as triiodothyronine, and 7% as thyroxine. In addition, two unknown species were detected in urine in very small amounts. These were possibly tetra- and triiodothyroacetic acid, two degradation products of triiodothyronine and thyroxine.

The MAK value for iodine and inorganic iodides was withdrawn in 2006, and the substance was categorised into Section II b of the List of MAK and BAT Values, as currently, no MAK value or assessment value in biological materials can be derived. Further information regarding the toxicological evaluation of iodine and inorganic iodides is available in the corresponding documentations issued by the Commission (Hartwig and MAK Commission 2017 a, b; Nasterlack et al. 2018).

3 General principles

The method described herein enables the determination of iodide concentration in serum/plasma or urine by ion chromatography (IC) coupled with inductively coupled plasma-mass spectrometry (ICP-MS). The method is reliable, sensitive, and – for a coupling method – relatively fast, and therefore suitable for routine testing in laboratories with high sample throughput. Using this method, iodide concentrations can be determined which are relevant for both occupational health and environmental medicine.

Sample preparation is carried out using ultra-pure water to dilute serum/plasma by a factor of 1 : 3 (v/v) and urine by a factor of 1 : 10 (v/v). Matrix interferences are significantly reduced by diluting the samples. Calibration is performed using matrix-matched calibration standards which are prepared in either serum/plasma or pooled urine.

4 Equipment, chemicals, and solutions

4.1 Equipment

- HPLC system with autosampler (e.g. Knauer 1100 Smartline inert Series Gradient, KNAUER Wissenschaftliche Geräte GmbH, Berlin, Germany)
- Anion-exchange column (e.g. Thermo-Dionex AG11 40 × 4 mm with a sample volume of 20 µl, Thermo Fisher Scientific GmbH, Dreieich, Germany)
- ICP-MS (e.g. NexION by PerkinElmer Inc., Rodgau, Germany)
- Sub-boiling apparatus BSB-939-IR (e.g. Berghof Products + Instruments GmbH, Eningen, Germany)
- Analytical balance (e.g. Mettler-Toledo GmbH, Gießen, Germany)
- Various beakers, volumetric flasks, and threaded glass vials (e.g. VWR International GmbH, Darmstadt, Germany)
- Variably adjustable microlitre pipettes with matching tips (1–10 µl, 10–100 µl, and 100–1000 µl) (e.g. Eppendorf AG, Hamburg, Germany)
- 1.8-ml screw-neck vials with matching caps (e.g. MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany)
- Blood-collection tubes for serum extraction (e.g. S-Monovette®, Sarstedt AG & Co. KG, Nümbrecht, Germany)
- Blood-collection tubes for plasma extraction (with additional anticoagulant) (e.g. EDTA-Vacutainer®, Becton Dickinson GmbH, Heidelberg, Germany)
- Urine-collection containers (e.g. Sarstedt AG & Co. KG, Nümbrecht, Germany)

4.2 Chemicals

Unless otherwise specified, all chemicals must be at least *pro analysi* grade.

- Certified iodide standard, 1000 mg/l (e.g. No. AS-19-2Y, SPEX CertiPrep, Rickmansworth, United Kingdom)
- Seronorm™ Pharmaca control serum L-1 (No. 101405, invicon diagnostic concepts GmbH, Munich, Germany)
- ClinChek® Urine Control L-1 (No. 17080, RECIPE Chemicals + Instruments GmbH, Munich, Germany)
- Ammonium acetate (e.g. No. 101116, Merck KGaA, Darmstadt, Germany)

- Acetic acid, 30% (e.g. No. 159166, Merck KGaA, Darmstadt, Germany)
- Methanol for liquid chromatography (e.g. No. 106018, Merck KGaA, Darmstadt, Germany)
- Tris(hydroxymethyl)aminomethane (Tris) (e.g. No. 108382, Merck KGaA, Darmstadt, Germany)
- Ultra-pure water (e.g. Milli-Q plus VE system (> 18 MΩ), Merck KGaA, Darmstadt, Germany)
- Argon 4.6 (Linde GmbH, Pullach, Germany)

4.3 Solutions

- HPLC eluent A: 10 mmol/l Tris-acetic acid-buffer (pH 8.0)

  Exactly 1.21 g of Tris are weighed into a 1000-ml beaker and dissolved in 900 ml of ultra-pure water. The pH value is adjusted to pH 8.0 with 30% acetic acid. The solution is transferred into a 1000-ml volumetric flask and made up to the mark with ultra-pure water. The buffer is filtrated and stored in the refrigerator at 4 °C.

- HPLC eluent B: 10 mmol/l Tris-acetic acid-buffer with 500 mmol/l ammonium acetate and 5% methanol (pH 8.0)

  Exactly 1.21 g of Tris and exactly 38.54 g of ammonium acetate are weighed into a 1000-ml beaker and dissolved in 900 ml of ultra-pure water. The pH value is adjusted to pH 8.0 with 30% acetic acid; 50 ml of methanol are then added. The solution is transferred into a 1000-ml volumetric flask and made up to the mark with ultra-pure water. The buffer is filtered and stored in the refrigerator at 4 °C.

The solutions are stable for at least two weeks when stored in the refrigerator.

4.4 Calibration standards

- Iodide stock solution (10 mg/l)

  1 ml of the certified iodide standard (1000 mg/l) is pipetted into a 100-ml volumetric flask. The flask is made up to the mark with ultra-pure water.

- Iodide spiking solution I (100 µg/l)

  1 ml of the stock solution is pipetted into a 100-ml volumetric flask. The flask is made up to the mark with ultra-pure water.

- Iodide spiking solution II (20 µg/l)

  2 ml of the spiking solution I are pipetted into a 10-ml volumetric flask. The flask is made up to the mark with ultra-pure water.

The iodide stock solution and the iodide spiking solutions are stable for at least six months when stored at –20 °C.

For matrix-adapted calibration, the calibration standards are freshly prepared in serum/plasma or pooled urine. For this purpose, the iodide spiking solutions are diluted with serum/plasma or pooled urine and ultra-pure water in 4-ml threaded glass vials, according to the pipetting schemes presented in Tables 1 and 2. The calibration-standard solutions are already diluted to factors of 1 : 3 (serum/plasma) or 1 : 10 (urine) and are analysed without further preparation.

Tab. 1 Pipetting scheme for the preparation of calibration standards for the determination of iodide in serum/plasma

Calibration standard	Serum/plasma [µl]	Spiking solution I [µl]	Spiking solution II [µl]	Ultra-pure water [µl]	Final volume [µl]	Iodide conc. in 1 : 3 diluted serum/plasma [µg/l]	Iodide conc. in undiluted serum/plasma [µg/l]
0	300	0	0	600	900	0	0
1	300	0	45	555	900	1	3
2	300	0	90	510	900	2	6
3	300	45	0	555	900	5	15
4	300	90	0	510	900	10	30
5	300	180	0	420	900	20	60

Tab. 2 Pipetting scheme for the preparation of calibration standards for the determination of iodide in urine

Calibration standard	Pooled urine [µl]	Spiking solution I [µl]	Ultra-pure water [µl]	Final volume [µl]	Iodide conc. in 1 : 10 diluted urine [µg/l]	Iodide conc. in undiluted urine [µg/l]
0	100	0	900	1000	0	0
1	100	50	850	1000	5	50
2	100	100	800	1000	10	100
3	100	200	700	1000	20	200
4	100	300	600	1000	30	300

5 Specimen collection and sample preparation

5.1 Specimen collection

Serum/plasma

For the collection of serum, disposable and sterile blood-collection tubes are used. For example, neutral Monovettes® without additional coagulation activators would be suitable. In order to extract the serum, the blood sample should thoroughly coagulate for a minimum of 20 minutes (no more than one hour). After centrifugation for 15 minutes at 2000 × *g* (15 °C), the supernatant is transferred into neutral sample vials.

For the collection of plasma, blood is drawn using disposable blood-collection tubes with additional anticoagulant (e.g. EDTA-Vacutainer®). The tube is immediately swivelled slightly to ensure homogenous distribution. The sample is then centrifuged at 2000 × *g* for 10 minutes. Afterwards, the plasma is pipetted into a new sample vial.

Iodide concentrations in serum/plasma must be determined shortly after specimen collection in order to avoid analyte losses. The storage of serum/plasma seems to be possible at 4 °C for a maximum of one week without analyte loss.

Urine

Urine samples are collected in urine-collection containers. 24-hour urine collection is generally considered more suitable for the verification of iodine and iodide levels compared with spontaneous urine, which only reflects recent exposure. When monitoring iodine exposure at the workplace, it is advisable to collect spot urine samples after several shifts or at the end of a shift. Urine samples can be stored in the refrigerator at 4 °C for a few days. For longer term storage, over a period of weeks or months, the urine should be frozen at –20 °C.

5.2 Sample preparation

The serum/plasma or urine samples are brought to room temperature and thoroughly mixed. For sample preparation, ultra-pure water is used to dilute the serum/plasma to 1 : 3 (v/v) and the urine to 1 : 10 (v/v).

The calibration standards, prepared in the respective matrix, are already accurately diluted and can be analysed without further work-up.

6 Operational parameters

Analytical determination is carried out using a combination of an HPLC system with an anion-exchange column and an ICP-MS.

6.1 High-performance liquid chromatography

Analytical column:	Material:	Ethyl vinyl benzene cross-linked with 55% divinyl benzene
	Functional group:	Alkanol quaternary ammonium ion
	Length:	40 mm
	Inner diameter:	4.0 mm
	Particle size:	9 µm
Separation principle:	Anion-exchange	
Eluent:	Eluent A	Tris-acetic acid-buffer (10 mmol/l; pH 8.0)
	Eluent B	Tris-acetic acid-buffer (10 mmol/l) with 500 mmol/l ammonium acetate and 5% methanol (pH 8.0)
Flow rate:	0.9 ml/min	

The gradient program is given in Table 3 and represented graphically in Figure 1. The chromatographic column is cleaned by flushing with a high ionic-strength eluent in the time interval from 6 to 8 minutes. Re-equilibration is carried out in the time interval from 8 to 10 minutes using eluent A.

Tab. 3 Gradient program for the determination of iodide in serum/plasma or urine

Time [min]	Eluent A [Vol. %]	Eluent B [Vol. %]
0	100	0
1.5	100	0
4	60	40
5	30	70
6	30	70
7	0	100
8	0	100
8.5	100	0
10	100	0

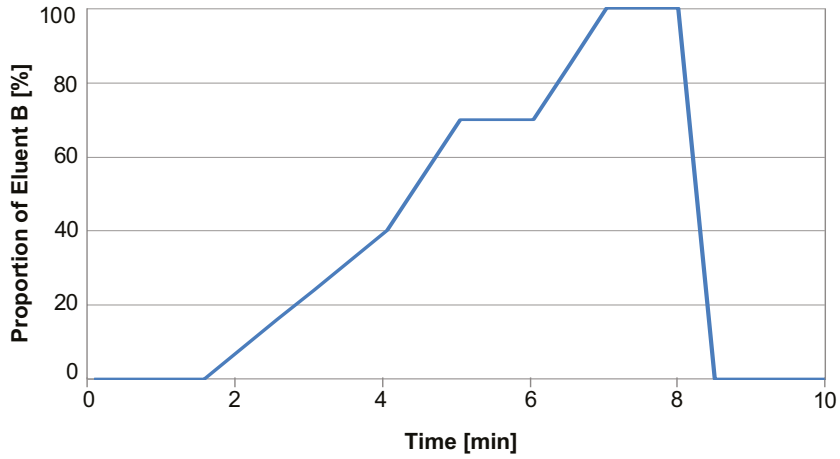

Fig. 1 Graphical representation of the gradient program for the determination of iodide in serum/plasma or urine

6.2 Sample introduction and plasma adjustment

The following ICP-parameters can only serve as a point of reference and must be appropriately optimised for each instrument. It may be necessary to make additional adjustments for instruments from other manufacturers. Generally, other kinds of nebulisers may be used for sample introduction.

Plasma performance:	1.2 kW	
Gas flow rates:	Nebuliser gas	0.96 l Ar/min, optimised daily
	Intermediate gas	0.6 l Ar/min
	Outer gas	15 l Ar/min
Torch:	Inner diameter	1.8 mm
Sample introduction:	HPLC effluent, 0.9 ml/min	
Nebuliser:	Seaspray	
Nebuliser chamber:	Cyclone	

6.3 Mass spectrometry

The mass-spectrometric adjustments also depend on the individual design of the instrument and must be optimised accordingly. The measurements for the determination of iodide are run in standard mode; as such, neither the collision cell nor the dynamic reaction cell mode are necessary and, in turn, no specific cell gases or device adjustments are required. As a general rule, the ICP-MS should complete the daily optimisation routine and reach the individual specification values as prescribed by the manufacturer. A dwelltime of 100–150 ms should be chosen in order to achieve a sufficient chromatographic resolution.

7 Analytical determination

The coupling of ion chromatography with ICP-MS enables the determination of the various iodine species that are present in both serum/plasma and urine.

The samples, diluted according to Section 5.2, are directly measured. The iodine species in the samples are separated on the anion-exchange chromatographic column, wherein special care must be taken to ensure a clean separation of iodide from the remaining iodine species. Quantification is carried out by ICP-MS. The iodine is measured selectively online at m/z = 127. Representative chromatograms of a serum and a urine sample are given in Figures 2 and 3.

The samples are analysed three times, and the mean value is used for data output.

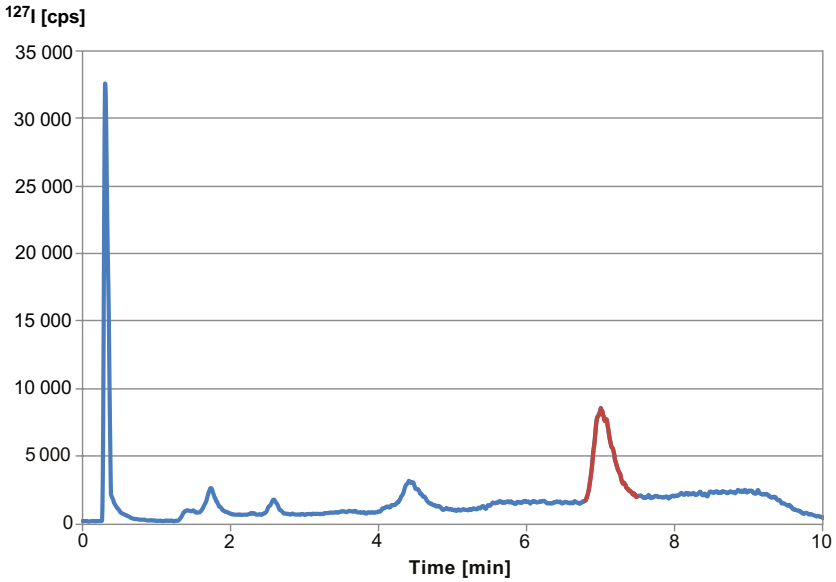

Fig. 2 Chromatogram of a 1 : 3 (v/v) diluted serum sample

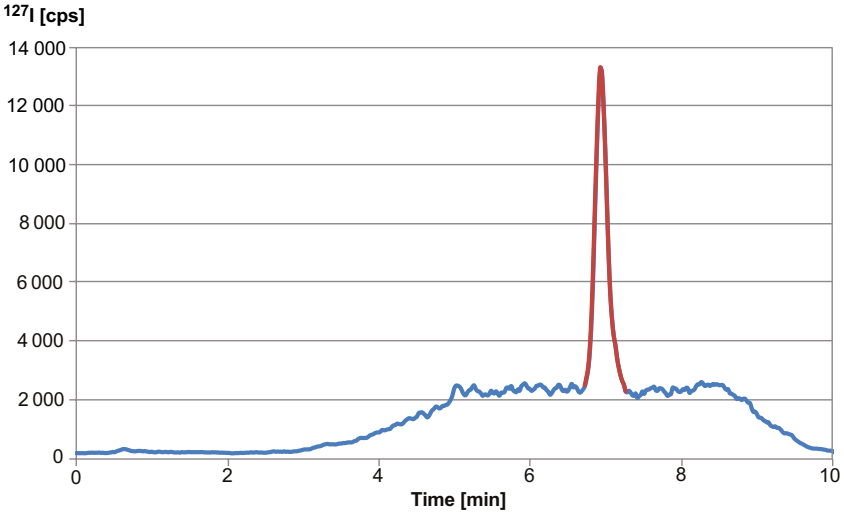

Fig. 3 Chromatogram of a 1 : 10 (v/v) diluted urine sample

8 Calibration

Quantification using aqueous standard solutions results in an overestimation of the iodide concentration by about 20%. It is therefore recommended to use matrix-matched calibration curves for quantification. The calibration standards, which are prepared in the corresponding sample matrices as indicated in Section 4.4, are analysed directly by IC-ICP-MS.

Plotting the peak areas of the iodide peak against the spiked iodide concentration results in a linear calibration curve in the range from the quantitation limit up to at least 20 µg/l of iodide in serum/plasma or 30 µg/l of iodide in urine. Figures 4 and 5 show representative calibration curves prepared in serum and urine.

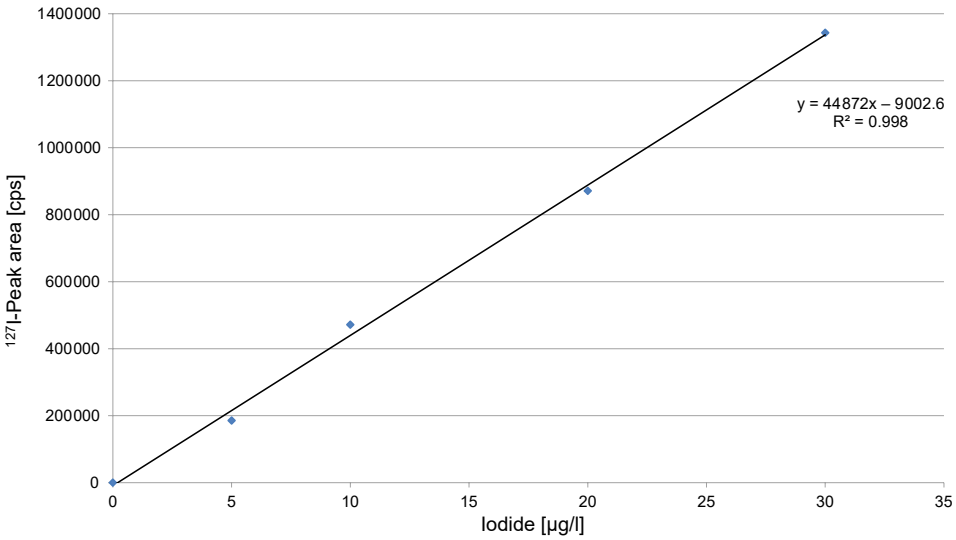

Fig. 4 Matrix-matched calibration curve for the determination of iodide in serum/plasma; the given concentrations correspond to the iodide levels in the 1 : 3 (v/v) diluted sample

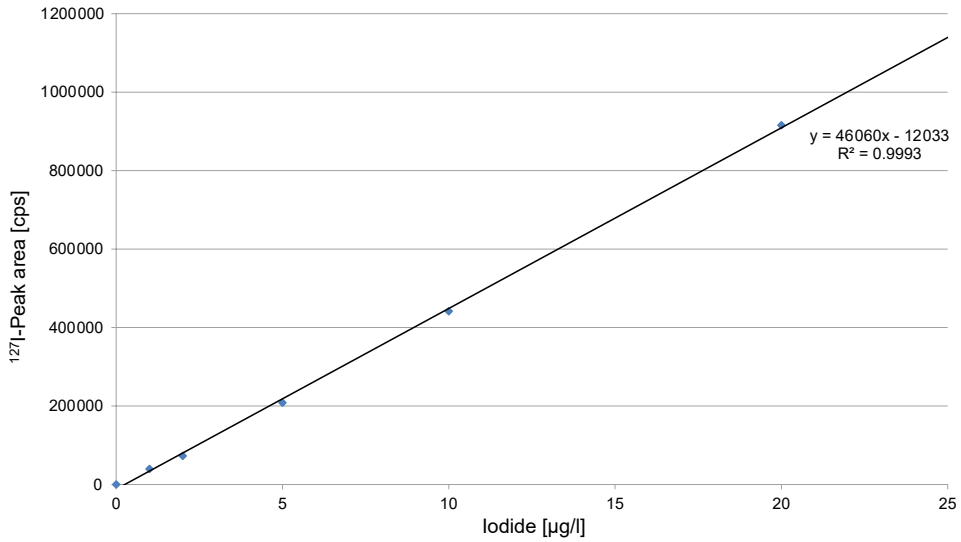

Fig. 5 Matrix-matched calibration curve for the determination of iodide in urine; the given concentrations correspond to the iodide levels in the 1 : 10 (v/v) diluted sample

9 Calculation of the analytical results

To calculate the analyte concentration in a serum/plasma or urine sample, the peak area of the iodide peak is determined. With the aid of the corresponding calibration function, the respective iodide concentration is calculated in µg/l of either serum/plasma or urine, taking into account the dilution of the sample. If the measured concentration lies above the calibration range, the corresponding sample is diluted with ultra-pure water, reprocessed, and newly analysed.

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 published by the Commission (Bader et al. 2010; Bundesärztekammer 2014).

Control material for the determination of iodide in serum/plasma or urine is commercially available from various manufacturers (e.g. RECIPE Chemicals + Instruments GmbH, Munich, Germany or invicon diagnostics concepts GmbH, Munich, Germany). The control materials should be analysed after calibration, after every twentieth sample, and at the end of each analytical run.

11 Evaluation of the method

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

11.1 Precision

To ascertain within-day precision, iodide in native serum or urine was repeatedly analysed. For within-day precision, sixfold determination of the samples resulted in the relative standard deviations and corresponding prognostic ranges shown in Table 4.

Tab. 4 Within-day precision for the determination of iodide in serum or urine (n = 6)

Matrix	Determined concentration [µg/l]	Standard deviation (rel.) s_w [%]	Prognostic range u [%]
Serum	1.63	4.9	12.5
Urine	5.75	1.4	3.6

The day-to-day precision was also determined. For this purpose, the same materials were processed on six different days and analysed as specified above. The precision data determined are given in Table 5.

Tab. 5 Day-to-day precision for the determination of iodide in serum or urine (n = 6)

Matrix	Determined concentration [µg/l]	Standard deviation (rel.) s_w [%]	Prognostic range u [%]
Serum	1.64	5.3	13.7
Urine	5.82	2.3	5.9

11.2 Accuracy

In order to determine the accuracy of the method, a serum sample was spiked with 5 µg iodide per litre and measured six times. Accordingly, a urine sample was spiked with 10 µg or 20 µg iodide per litre of urine, respectively, and the recovery rates were determined (n = 6). The relative recovery rates determined in these samples are shown in Table 6.

Tab. 6 Mean relative recovery rates for the determination of iodide in serum or urine (n = 6)

Matrix	Nominal concentration [µg/l]	Measured concentration [µg/l]	Relative recovery rate *r* [%]
Serum	5	5.1[a)]	99
Urine	10	10.7[a)]	107
	20	19.8[a)]	99

[a)] after subtraction of background levels

In addition, control material for both matrices was analysed for the purpose of internal quality assurance. The reference value indicated for total iodine in urine was 115 µg/l for the ClinChek® control urine, whereby iodide was expected to be the main iodine species in urine. An estimation of the peak areas in the analysed chromatogram revealed that about 90% of iodine was present as iodide and the remaining 10% was present in the form of other iodine species. According to Michalke et al. (2000 b), 86% of the iodine in urine is present as iodide. As a result, about 104 µg/l (90%) or 99 µg/l (86%) iodide were expected in the control urine.

For additional verification, a control serum by Seronorm™ was also analysed. The reference value for total iodine in this serum was indicated to be 54 ± 1.2 µg/l, whereas no value was specified for iodide. The quantitation yielded 54.6 µg/l for total iodine and 1.6 µg/l for iodide. The results are summarised in Table 7.

Tab. 7 Determination of total iodine and iodide in certified control material

Control material	Nominal concentration [µg/l]		Measured concentration [µg/l]		Recovery rate *r* [%]	
	Total iodine	Iodide	Total iodine	Iodide	Total iodine	Iodide
Serum, Seronorm™	54 ± 1.2	–	54.6	1.6	101	–
Urine, ClinChek®	115	99[a)]	115	98.4	100	99.4

[a)] expected concentration

11.3 Limits of detection and quantitation

The limit of detection was estimated from the standard deviation of the spectral underground intensity on the basis of a signal-to-noise ratio of 3 : 1. The limit of quantitation was similarly ascertained from the tenfold signal-to-noise ratio. The determined values, as relevant to the determination of iodide in serum/plasma or urine, are presented in Table 8.

Tab. 8 Limits of detection and quantitation for the determination of iodide in serum/plasma or urine

Matrix	Detection limit [µg/l]	Quantitation limit [µg/l]
Serum/plasma	0.35	1.2
Urine	0.5	1.7

11.4 Sources of error

The chromatographic-separation performance should be monitored on a regular basis, especially when analysing high-matrix samples, such as serum or plasma. Within the course of method development, column lifetime has spanned several hundred serum analyses, although, as expected, the retention times decreased consistent with the wearout of the chromatographic column. The iodide peak was, nevertheless, completely separated from the other iodine compounds and could be determined with consistent precision and accuracy.

Sample preparation is carried out by simply diluting the samples with ultra-pure water. As such, only freshly prepared ultra-pure water should be used in order to avoid potential iodine contaminations. Eluents should also be prepared

using fresh ultra-pure water. In general, there is a low blank value which originates from the chemicals used in the preparation of the eluents. This blank value is, however, stable and does not interfere with iodide determination.

During method development, protein precipitation in serum samples was tested using 30% ethanol. The precipitation led to a reduced protein matrix and improved reproducibility with relative standard deviations of 2% instead of 5%, but also to an overestimation of the iodide concentration by about 10%. The usage of tellurium as an internal standard was also tested, but it did not improve the method compared to determination without an internal standard.

Special attention must be given to the storage of serum and plasma samples for correct iodide determination. By repeated measurements of identical samples, it was shown that, with short-term storage of about one week, correct analyte concentrations in serum/plasma could still be quantified. In case of longer term storage of the samples, a conversion into other iodine compounds occurred which could not be detected with this method. As a result, a lower iodide concentration was determined with an unchanged total iodine concentration.

12 Discussion of the method

The analytical method described herein allows a simple, reliable, and sensitive determination of iodide in serum/plasma or urine. With good precision data and good recovery rates, the IC-ICP-MS method meets the requirements for analytical methods in terms of both precision and accuracy.

With quantitation limits of 1.2 µg iodide per litre of serum/plasma and 1.7 µg iodide per litre of urine, iodide determination is possible in the context of both occupational health and environmental medicine. However, as iodine excretion is generally characterised by a wide range of variation, individual assessment of measured values becomes more difficult (Hartwig and MAK Commission 2017 b).

A short anion-exchange ion-chromatography column was chosen for the separation of the iodine species in order to guarantee a short runtime. With this column, a total runtime of 10 minutes per sample (including the re-equilibration of the column) was achieved, meaning that this method is also suitable for laboratories with high sample throughput. In principle, the method can also be used to analyse further iodine compounds, although the analysis runtime may have to be extended as necessary.

Instruments used HPLC system with autosampler (Knauer 1100 Smartline inert Series Gradient, KNAUER Wissenschaftliche Geräte GmbH, Berlin, Germany); anion-exchange column (Thermo-Dionex AG11 (40 × 4 mm) with a sample volume of 20 µl, Thermo Fisher Scientific GmbH, Dreieich, Germany); ICP-MS (NexlON, PerkinElmer Inc., Rodgau, Germany)

References

ATSDR (Agency for Toxic Substances and Disease Registry) (2004) Toxicological profile for iodine. ATSDR, Atlanta, GA. https://www.atsdr.cdc.gov/toxprofiles/tp158.pdf, accessed 12 Oct 2020

Bader M, Barr D, Göen T, Schaller KH, Scherer G, Angerer J (2010) Reliability criteria for analytical methods. Biomonitoring Method, 2010. In: Angerer J, Hartwig A (eds) The MAK-Collection for Occupational Health and Safety. Part IV: Biomonitoring Methods, vol 12. Wiley-VCH, Weinheim, 55–101. Also available from DOI: https://doi.org/10.1002/3527600418.bireliabe0012

BfR (German Federal Institute for Risk Assessment) (2020) Iodine intake in Germany on the decline again – tips for a good iodine supply. BfR, Berlin. https://www.bfr.bund.de/cm/349/iodine-intake-in-germany-on-the-decline-again-tips-for-a-good-iodine-supply.pdf, accessed 14 Jan 2021

Bundesärztekammer (2014) Richtlinie der Bundesärztekammer zur Qualitätssicherung laboratoriumsmedizinischer Untersuchungen. Dtsch Ärztebl 111(38): A1583–A1618

D-A-CH (Deutsche Gesellschaft für Ernährung; Österreichische Gesellschaft für Ernährung; Schweizerische Gesellschaft für Ernährungsforschung; Schweizerische Vereinigung für Ernährung) (ed) (2015) Iod. In: Referenzwerte für die Nährstoffzufuhr, 2nd ed. Umschau Braus GmbH, Verlagsgesellschaft, Frankfurt a M, 179–194

Domke A, Großklaus R, Niemann B, Przyrembel H, Richter K, Schmidt E, Weißenborn A, Wörner B, Ziegenhagen R (eds) (2004) Risk assessment of iodine. In: Use of minerals in foods. Toxicological and nutritional-physiological aspects, Part II. Federal Institute for Risk Assessment, Berlin, 189–226. https://www.bfr.bund.de/cm/350/use_of_minerals_in_foods.pdf, accessed 14 Jan 2021

Hartwig A, MAK Commission (2017 a) Iodine and inorganic iodides. MAK Value Documentation, 2014. MAK Collect Occup Health Saf 2(2): 375–415. DOI: https://doi.org/10.1002/3527600418.mb755356e5717

Hartwig A, MAK Commission (2017 b) Iodine. MAK Value Documentation, 2006. MAK Collect Occup Health Saf. DOI: https://doi.org/10.1002/3527600418.mb755356e4017

Michalke B, Schramel P, Hasse S (1996) Determination of free iodide in human serum: separation from other I-species and quantification in serum pools and individual samples. Mikrochim Acta 122(1–2): 67–76. DOI: https://doi.org/10.1007/BF01252407

Michalke B, Schramel P, Witte H (2000 a) Iodine speciation in human serum by reversed-phase liquid chromatography-ICP-mass spectrometry. Biol Trace Elem Res 78(1–3): 81–91. DOI: https://doi.org/10.1385/BTER:78:1-3:81

Michalke B, Schramel P, Witte H (2000 b) Method developments for iodine speciation by reversed-phase liquid chromatography-ICP-mass spectrometry. Biol Trace Elem Res 78(1–3): 67–79. DOI: https://doi.org/10.1385/BTER:78:1-3:67

Nasterlack M, Drexler H, Hartwig A, MAK Commission (2018) Iodine and inorganic iodides. BAT Value Documentation, 2016. MAK Collect Occup Health Saf 3(3): 1535–1545. DOI: https://doi.org/10.1002/3527600418.bb755356e2218

WHO (World Health Organisation) (2009) Iodine and inorganic iodides: human health aspects. Concise international chemical assessment document, No. 72. WHO, Geneva. https://apps.who.int/iris/bitstream/handle/10665/43579/9789241530729_eng.pdf?sequence=1&isAllowed=y, accessed 12 Oct 2020