

**Determination of Arsenic and Selenium in
Environmental Samples by ICP-MS after
Hydride Formation**

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1 German Environmental Specimen Bank

The German Environmental Specimen Bank (ESB) is an instrument for the monitoring of the environment. It is in the responsibility of the Federal Ministry for the Environment, Nature Protection and Reactor Safety (BMU) and technically and administratively coordinated by the Federal Environment Agency (Umweltbundesamt). The ESB collects ecologically representative environmental specimens as well as human samples, stores them and examines the archived material for environmental relevant substances.

The long-term storage is performed under conditions that exclude a change of state or a loss of chemical characteristics as far as possible during a period of several decades. By this means the archive provides specimens for a retrospective monitoring of such substances, whose hazard potential for the environment or human health are not yet known.

Comprehensive information on the German ESB is available at www.umweltprobenbank.de (English language pages available).

2 General information

This guideline for determining the content of hydride-forming elements in environmental samples by means of mass spectrometry following ionisation in inductively coupled plasma (ICP-MS: inductively coupled plasma mass spectrometry), describes a process for determining the dissolved elements arsenic and selenium. In compiling this guideline, parts of the draft standard DIN ISO 20280 'Soil Quality - Determination of Arsenic, Antimony and Selenium in *aqua regia* Soil Extracts by Means of Electrothermal or Hydride Atomic-Absorption Spectrometry' (April 2003) were also taken into account.

This guideline is a continuation of the process guidelines developed by the German Environmental Specimen Bank for analysing digests of environmental samples (UMWELTBUNDESAMT 1996). However, the method described there is also hydride atomic-absorption

spectrometry. Due to its higher specificity and sensitivity, the coupling with ICP-MS is now preferred for the purposes of the Environmental Specimen Bank.

This method is used for the As and Se analysis of spruce and pine shoots as well as for beech and poplar leaves. In principle, the method described here can also be used for other types of biological sample. Where samples are used for which no empirical data are available, a suitable process for validation should be carried out (see section 8).

The lower range of application of the method described depends on the matrix in question and the types of interference connected therewith. For arsenic and selenium it is approximately 6 ng/g or 3 ng/g, respectively, with reference to the dry mass.

3 Description of method

For determining low element contents in environmental samples, it is often possible to use ICP-MS because this is generally a highly sensitive method of analysis which is free of interferences (see guideline 'Determination of the Elemental Content of Environmental Samples Using Inductively Coupled Plasma Mass Spectrometry (ICP-MS)' (RÜDEL ET AL., 2011b)). For arsenic (As) and selenium (Se) matrix effects may occur in environmental samples which result in unacceptably high limits of detection. These are partly due to the fact that the ionisation energy of As (9.81 eV) and Se (9.75 eV) is relatively high. This means that where high concentrations of elements with low ionisation energies are present (e.g. Na 5.14 eV) ionisation suppression may take place in the plasma. A further problem may be the spectral interference of polyatomic species such as ArCl or ArAr. $^{40}\text{Ar}^{35}\text{Cl}$ interferes with the only arsenic isotope ^{75}As . Ar/Cl- and Ar/Br polyatomic species interfere with all the relevant Se isotopes. Polyatomic interferences can be reduced by using special plasma conditions (cold plasma conditions; implemented as so called Shield Torch System for ICP-MS units manufactured by Agilent).

In order to achieve optimum sensitivity, the analyte must be separated from the matrix and the argon-based interference reduced. Similar to a number of other elements (Sb, Te, Bi, Ge, Sn, Pb) As and Se form gaseous hydrides. Matrix interference can be substantially eliminated by means of a constant flow of Ar. The present guideline describes the standard procedure for determining arsenic and selenium contents.

Before testing with the ICP-MS, the sample material has to be dissolved. Routinely, this is done by means of digestion with acids e.g. in a pressure digestion system with microwave heating (see 'Procedure for Digestion of Environmental Samples', RÜDEL ET AL., 2011a). If the elements being detected are not present in the optimum oxidation stage before hydride formation, a preliminary reduction process is necessary (see 6.3 and 6.4).

If necessary other acidic aqueous solutions can also be examined (e.g. *aqua regia* extracts of soils and sediments). Suitable measures should be taken to ensure the transferability to the other matrix (e.g. by applying standard addition tests, testing of suitable certified reference materials) (see section 8).

4 Apparatus

4.1 Vessels for element solutions

The stability of diluted element solutions (both sample and standard solutions) is determined substantially by the material of the vessels used. The suitability of the material for the intended purpose must always be ensured beforehand. For determining elements in the trace range, vessels of glass or polyvinyl chloride (PVC) should not be used. Vessels made of perfluoralkoxy plastics (PFA), hexafluoro-ethylene-propylene (FEP) or quartz are more suitable. In many cases, high-density polyethylene (e.g. HDPE vessels which are used for scintillation measurements) and polypropylene may also be used. When they are being re-used, the vessels must be rinsed with nitric acid or 'steam cleaned' with boiling concentrated nitric acid in a closed system.

4.2 Mass spectrometer

Mass spectrometers with inductively coupled plasma (ICP-MS) are suitable for the range 70 to 240 m/z . The resolution within this range must be at least 1 m_r/z (m_r = relative mass of an isotope; z = charge number).

The following components are important for reproducible operation:

- mass flow controller for the nebuliser gas and (if possible) also for the auxiliary and cooling gas
- nebulising system with controlled low-impulse pump.
- Argon, minimum purity 99.99 %.

4.3 Hydride system

A hydride unit is also required as an accessory to the mass spectrometer. This should consist of a pump for infeeding the reduction agent, cross piece and mixing coil for mixing sample and reduction agent, and a phase separator for separating the hydride gas from the sample fluid.

5 Reagents

5.1 General notes

All chemicals used must be of the highest commercially available degree of purity. The reagents should be selected to ensure that the content of the element to be determined or interfering elements should be negligible in relation to the lowest element concentration to be determined.

If possible, nitric acid should always be used for preservation as well as for digestion since this minimises interferences by polyatomic ions (see section 8.2.2).

NOTE: For element analysis carried out by the Environmental Specimen Bank, reagents (e.g. nitric acid) of 'suprapur' and 'ultrapur' qualities are used (supplier: Merck, Darmstadt). As an alternative, acid produced in-house by sub-boiling may also be used. Every charge is analysed to

determine the content of the elements being analysed.

5.2 List of reagents

- 5.2.1 Water from a high-purity water system, Quality: specific resistance > 18.2 MΩ cm.
- 5.2.2 Nitric acid, $\rho(\text{HNO}_3) > 1.39 \text{ g/mL}$ ($\geq 65 \%$).
- 5.2.3 Hydrochloric acid, $\rho(\text{HCl}) > 1.15 \text{ g/mL}$ ($\geq 30 \%$).
- 5.2.4 Hydrochloric acid, dilute (15 %), from 5.2.3 with high-purity water (5.2.1).
- 5.2.5 Hydrochloric acid, dilute (5 %), from 5.2.3 with high-purity water (5.2.1).
- 5.2.6 KJ/ascorbic-acid mixture made by mixing 5 g suprapur potassium iodide and 5 g high-purity ascorbic acid in 100 mL of high-purity water (5.2.1).
- 5.2.7 Pre-reduction solution consisting of 1 part KJ/ascorbic acid mixture (5.2.6) and 1 part hydrochloric acid (5.2.3); mixed afresh daily.
- 5.2.8 NaBH_4 -reduction solution consisting of: 5 g NaBH_4 plus 1 g NaOH filled up to 1000 mL with high-purity water.

5.3 Element stock solutions

Commercially available solutions of the elements As and Se are used as single- or multi-element standard solutions (1000 mg/L each). The solutions are usable for several years. The shelf life stated by the manufacturer should always be observed. Only standard solutions with certificate should be used.

5.4 ICP-MS tune solution

To optimise the mass-spectrometer function, an ICP-MS tuning solution consisting of an As or Se solution (5 µg/L) is used. This is made by diluting an As or Se standard solution (see 5.3 above).

5.5 Instrument blank solution

5% hydrochloric acid (see 5.2.25) is used as instrument blank solution.

6 Test procedure

6.1 Calibration solution

Commercial standards are used as calibration solutions (e.g. single or multi-element standard solutions containing 1000 mg/L per element) which are diluted as required. The calibration procedure is carried out by means of the respective instrument software options. As a rule, five calibration solutions and one blank solution are used. The quality of the calibration function within the working range should be verified.

The standards should be treated in exactly the same way as the samples. The acid content should also be adjusted to that of the samples.

6.2 Quality-assurance samples

To verify the perfect function of all components, various quality-assurance samples should be analysed at each test. These are (at least) zero samples, blank samples (reagent blank values) and (if available) certified reference materials and/or laboratory-internal reference samples.

NOTE: For the analysis of ESB samples, the ratio of specimens digests to quality-assurance samples is always 2:1 at the most. Table 1 shows a selection of the reference materials used for element analysis of ESB samples

6.3 Preparation of samples for As analysis

All samples are digested with HNO_3 (RÜDEL ET AL., 2011a) and high-purity water (5.2.1) replenished to 20.0 mL. From this solution, 2.5 mL are removed and placed in a 15 mL test tube, to which 5 mL of pre-reduction solution are added (5.2.7).

Following a reaction time of 2 h, 2.5 mL of high-purity water (5.2.1) are added. After being shaken briefly, 10 mL of sample solution are available for

Table 1: Selection of available reference materials.

Designation	Code	Certified by	Certified for (e.g.)
Beech leaves	CRM 100	BCR	As, Se (information values)
Spruce needles	CRM 101	BCR	As (information value)
Poplar leaves	GBE 07604	Institute of Geophysical and Geo-chemical Exploration (Langfang, China)	As
Pine needles	NIST 1575a	NIST	As, Se
Sea lettuce (algae)	CRM 279	BCR	As, Se
Water, certified	TMDA-54.4	National Water Research Institute, Canada	As, Se

testing and the samples are diluted by 1:4. Samples, reference samples, stock solution for standards and ICP-MS tune solution (As solution 5 µg/L) are treated according to the above instructions.

5% suprapur HCl (5.2.5) is used as blank solution for analysis, for dilution of standards and as rinsing solution.

6.4 Preparation of samples for Se analysis

For the Se analysis, 4 mL of the digestion solution are pipetted into a 15 mL test tube and filled up with 4 mL HCl conc. (5.2.3). Thus the samples are diluted 1:2. The test tubes are closed with a cap and carefully shaken. Standard solutions, ICP-MS tune solution (Se solution 5 µg/L) and certified water (e.g. TMDA 54.4) are mixed in HCl 15% suprapur (5.2.4) or diluted with HCl 30% in order to obtain a 15% HCl solution. Afterwards, these solutions are heated to 90°C for approx. 20 min in a water bath.

After cooling to room temperature, the samples are ready for analysis. Further dilutions of the parent solution, tune solution and certified water are carried out with HCl 15% (5.2.4).

HCl 15% suprapur is used as blank for the analysis and HCl 5% is used as the rinsing solution.

6.5 Hydride formation

An NaBH₄ solution (5.2.8) is used for hydride formation. This solution is prepared freshly every day when tests are to be carried out.

The NaBH₄ solution is mixed directly with the sample using a separate pump, a cross piece and a mixing coil and is then fed into the phase separator. Using Ar as carrier or makeup gas, the hydride gas is fed into the plasma. The remaining sample solution is then pumped away as waste.

7 ICP-MS measurement procedure

7.1 General notes

Mass spectrometers with inductively coupled plasma (ICP-MS) have a linear relationship over several times the power of ten between the impulse rates measured and the concentration of an element. This means that quantification by means of external calibration with a linear regression line is possible. The calibration should be adjusted to the working range in question. Even for the lowest concentration values, sufficient measuring sensitivity and reproducibility must be assured.

7.2 Preparation of the unit

The ICP-MS should be started and set in accordance with the instructions of the manufacturer. The masses of the elements being

detected are entered. To ensure sufficient stability, it should be started up at least half an hour before the measuring operation. A tune solution (5.4) is used to test the unit with regard to resolution, mass calibration, sensitivity and stability of signal. In case of interference (high rates of oxide formation, formation of double-charged ions) the settings are varied to reduce the interference as far as possible.

Should the concentrations of the measurement solutions vary strongly, the rinsing times may be increased.

7.3 Calibration of the ICP-MS system

Calibration is carried out on a daily basis using the appropriate program options of the apparatus. For each element being analysed, one straight calibration line with at least four concentrations should be determined. One blank-value solution is also measured in each case. For each measurement, at least three measurements are carried out automatically by the unit and then averaged.

The quality of the calibration function within the working range should be verified. The coefficient of correlation r should be > 0.995 . Should $r < 0.995$ and the calibration still be used, the reason must be explicitly stated (e.g. calibration in the lowest application range of the method and resulting higher measurement uncertainty).

The correctness of the calibration is verified with an aqueous certified reference sample.

7.4 Measurement of the sample solutions

Following calibration of the mass spectrometer, the sample solutions can now be analysed. The correctness of the calibration is verified regularly by analysing an aqueous certified reference sample after about 20 samples. If the deviations from the target value are $> 20\%$ in the range up to five times the limit of quantification, the unit should be recalibrated. Blank value solutions are also measured at regular intervals to detect any possible carry over.

8 Validation of method

For method validation, standard addition tests can be carried out. Different concentrations of the element being determined (e.g. approx. 50% and 100% of the concentration anticipated in the sample) are added to two parts of that sample. These part-samples along with a further part-sample (without addition of the element) are then analysed. By extrapolating the straight lines (measurement signal as linear function of the mass concentration of the analysis element) it is then possible to determine the original concentration of the sample. This procedure is described in detail in DIN 32633 / DEV E7.

With concentrations substantially above the limit of quantification, it is also possible to use the dilution method for verification. To do this, a sample is first analysed in the undiluted state and then after dilution by a factor of between 5 and 10. If no interference occurs, the results converted for the original solution should be identical with due consideration of measurement uncertainty. The criterion of quality is a correspondence of $100 \pm 10 \%$.

Another means of method validation is the analysis of a certified reference material. A correspondence of $100 \pm 20 \%$ is the criterion of good quality.

For method validation, the following process parameters should also be determined:

Selectivity / specificity: these are met if the figure measured for the chemical blank value is less than the lowest validated concentration.

Reproducibility: the reproducibility is calculated from the correspondence data of the reference materials via the relative standard deviation (S_{rel}). This condition is fulfilled if the following applies: $S_{rel} < 10 \%$.

Lowest limit of measuring range: the lowest limit of the method is the calibration point for which the signal-noise ratio is no less than 6:1.

Limit of detection / limit of quantification: The limit of detection is calculated from blank value analyses (DIN 32645: blank test method, quick

estimate). The limit of quantification is obtained by multiplying the limit of detection by a factor of 3.

9 Documentation

For the raw data, at least the following information should be documented:

- date of processing;
- name of user;
- unit settings;
- preparation of samples;
- designation of samples (e.g. ESB code).

10 Interferences

Due to the selective preparation of samples (hydrogenation) no interference is anticipated as a rule.

11 Evaluation

The analysis data are evaluated by the software in the mass spectrometer. The automatic evaluation must be verified for plausibility. During evaluation, the analysis data are calculated in accordance with the mathematical corrections selected and then related to the internal standard. For the purposes of calibration, linear regression is carried out in order to calculate the slope, ordinate intercepts and the coefficients of correlation (r). The concentrations of all the analysis solutions (blank values, aqueous reference materials, digestion solutions) are determined on the basis of the linear calibration lines.

Should the concentration of an element in the blank-value sample be higher than 10% with reference to the concentration in the sample, the values are adjusted by the blank value (i.e. this concentration is subtracted). Any dilution operations must also be taken into account in all calculations. It must be possible to reconstruct all the calculations carried out by means of the

electronically stored data or by means of archived printouts.

When examining digested samples of solids, the results must refer to the solid (i.e. to dry mass or fresh mass). The concentrations of the elements in the digestion solutions are generally determined by the ICP-MS software. The further calculation of the element contents in the solid matter is done by means of the following equation:

$$\omega_E = V / M * \rho_E * F_V * F_U$$

Where:

ω_E proportion by mass of the element in the solid matter, stated, for example as $\mu\text{g/g}$;

M mass of the sample used, stated in mg (e.g. 200 mg);

V volume to which replenished, stated in mL (e.g. 20.0 mL);

ρ_E concentration of the element under consideration in the digestion solution, stated for example in $\mu\text{g/L}$;

F_V dilution factor for hydride ICP-MS analysis (e.g. 4 for As, 2 for Se);

F_U conversion factor (1 L/mL * mg/g).

12 Reporting

The results refer to the amount of solid material used (dry mass or fresh mass).

All results should be stated to three significant places.

EXAMPLES: selenium (Se) 123 ng/g; arsenic (As) 12,3 $\mu\text{g/g}$; selenium (Se) 1,23 $\mu\text{g/g}$; arsenic (As) 0,123 $\mu\text{g/g}$.

Measurement results are subject to a degree of uncertainty. In the working range of a process, the measurement uncertainty increases as the concentration in the sample decreases. The degree of uncertainty of a measured figure can be

determined in a number of ways which are described in 'ISO Guide to the Expression of Uncertainty in Measurement (GUM)' (ISO, 1995) and guideline 'Quantifying Uncertainty in Analytical Measurement' (EURACHEM/CITAC, 2000). A practical means of determining uncertainty is the so-called Nordtest method (MAGNUSSON ET AL., 2003; calculation of the uncertainty from duplicate measurements of certified reference materials and interlaboratory comparison results).

NOTE: For the analysis of Environmental Specimen Bank samples, generally six subsamples from one homogenate are used. The standard deviation of the average value is regarded as the measurement uncertainty of the result. The correctness of the results is verified with the help of certified reference materials. Representative data are given in the appendix.

13 Analysis report

The following data should be documented in the analysis report:

- reference to this guideline,
- sample identity,
- concentration of the elements in question with reference to the percentage of solid matter (depending on material used, with reference to fresh or dry mass),
- statement of measurement uncertainty if applicable,
- data on preliminary treatment of the sample and digestion,
- any deviations from this guideline.

14 Representative analysis results

Representative results of analyses are given in the appendix:

a) Results of the analysis of certified reference materials,

b) Results of the analysis of reference materials from the Environmental Specimen Bank,

c) Results of the analysis of representative samples from the Environmental Specimen Bank.

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Appendix: Representative Analysis Results

a) Results of the analysis of certified reference materials.

Reference material	Element	Certified Content	Correspondence	Comment
Spruce needles BCR CRM 101	As	0.22 µg/g	95.5 ± 4.1 (n = 6)	Information value
	Se	0.048 µg/g	91.7 ± 6.3 (n = 4)	Reference value
Beech Leaves BCR CRM 100	As	0.51 µg/g	104 ± 7 (n = 6)	Information value
	Se	0.15 µg/g	102 ± 23 (n = 4)	Information value
Poplar Leaves GBW07604	As	0.37 µg/g	90.3 ± 1.6 (n = 3)	Certified value
	Se	0.14 µg/g	88.6 ± 6.4 (n = 3)	Certified value

b) Results of the analysis of Environmental Specimen Bank reference materials;

IS UPB - information system of the German ESB (Informationssystem Umweltprobenbank).

ESB reference material	Element	Content (from IS UPB)	Correspondence
Spruce shoots, Berchtesgaden 0110/0/0387/01101/0/584	As	0.05 µg/g	110 ± 8 (n = 4)
	Se	0.01 µg/g	130 ± 20 (n = 4)
Beech leaves, Bornhöved 0410/0/0893/03103/0/266	As	0.05 µg/g	98.0 ± 22.0 (n = 6)
	Se	0.03 µg/g	110 ± 30 (n = 4)
Poplar, Halle 0310/0/0891/11110/0/524	As	0.252 µg/g	87.3 ± 5.2 (n = 3)
	Se	0.390 µg/g	82.1 ± 3.3 (n = 4)

c) Examples of results of the analysis of representative Environmental Specimen Bank samples.

Sample	Element	Content	Comments
Spruce shoots, Hochharz 2005 0110/0/0405/12101/0/xxx	As	0.053 ± 0.007 µg/g	(n = 6)
	Se	0.032 ± < 0.001 µg/g	(n = 6)
Pine shoots, Dübener Heide 2005 0210/0/0305/11200/0/xxx	As	0.038 ± 0.002 µg/g	(n = 6)
	Se	0.032 ± 0.002 µg/g	(n = 6)
Beech leaves, Hochharz 2006 0410/0/0806/12102/0/xxx	As	0.029 ± 0.001 µg/g	(n = 5)
	Se	0.027 ± 0.001 µg/g	(n = 6)
Poplar leaves, Leipzig 2006 0310/0/0806/11111/0/xxx	As	0.135 ± 0.003 µg/g	(n = 6)
	Se	0.246 ± 0.008 µg/g	(n = 6)