UMWELTPROBENBANK DES BUNDES

Guidelines for Chemical Analysis

Digestion of Environmental Samples

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Für Mensch und Umwelt

Contents

Guidelines for sampling, transport, storage and chemical characterisation of environmental and human samples
March 2011, V 2.0.2

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

The subject of the present guideline for the 'Digestion of Environmental Samples' is the mineralisation of animal and plant specimen material after addition of acid at high temperature (≥ 180°C) and under high pressure (≥ 30 bar) in a microwave unit. The digest solutions can be used for elemental analysis by means of atomic-emission and mass spectrometry. This guideline is a continuation of the standard work procedures used by German Environmental Specimen Bank (UMWELTBUNDESAMT 1996). In addition, instructions have also been included from the standard method for determining elemental traces in food samples L 00.00 19/1 'Pressurised Digestion' (DIN EN 13805: 06.2002).

3 Field of application

This guideline is regularly used by the ESB for digesting samples of the following types: bladder wrack, blue mussels, eel pout (muscle tissue and liver), herring gull egg content, zebra mussels, bream (muscle tissue and liver), spruce shoots, pine shoots, poplar leaves, beech leaves, earthworm, roe-deer liver, feral pigeon egg content. The method described below can also be applied to other biological sample types. Where samples are used for which no empirical data are yet available, it is particularly important to verify the completeness of the digestion process.

On the basis of experience gained, digest solutions prepared in accordance with this guideline are suitable for determining contents of the following elements: As, Cd, Co, Cr, Cu, Ni, Pb, Se, Tl (using ICP-MS; Se in bladder wrack and low contents of As and Se in plant samples by hydride-ICP-MS); Ba, Cu, Ca, Fe, K, Mg, Mn, P, S, Zn (using ICP-OES); Cd, Co, Cr, Cu, Ni, Pb (using ET-AAS); Hg (using a direct mercury analyzer, DMA, or CV-AAS).

4 Terminology

For the purposes of this guideline, the following terms are defined:

4.1 Digest

Dissolving of a sample in acid or acid mixtures or other reagents, normally carried out at high temperature (≥ 180°C) and under pressure (≥ 30 bar). This causes mineralisation of the organic constituents of the sample.

4.2 Microwave unit

A microwave unit consists of the actual microwave chamber, the control system including software, and special closable digestion vessels of acid-resistant, heat and pressure-resistant material which are suitable for the microwave chamber.

4.3 Water content

The water content of a sample is the difference between the fresh mass and the dry mass and is expressed in %. According to this guideline, the dry-mass content is determined in % from the dry

residue following several days of freeze-drying (until constant mass is achieved).

5 Safety notes

All operations must be carried out by qualified personnel.

The acids prescribed by these guidelines are highly corrosive and some are toxic. Since they are also used at high temperatures and under high pressure, special safety precautions are necessary. All digestion vessels must be filled and emptied in a fume hood. Always wait until the vessel has cooled before opening it. The microwave unit must be connected with a suitable ventilation device or be located inside a fume hood.

6 Description of method

Unless special solid-analysis systems are being used, solid samples must be dissolved before analysis using atomic-emission and mass-spectrometric methods. In most cases, acid digestion is used for processing biological samples. To minimise the time required and ensure complete digestion, this process is generally performed at high temperatures and under high pressure. To heat the samples, microwave radiation is being used increasingly as the source of energy. For this purpose, special devices are used which can be programmed and have temperature and pressure-monitoring systems and which are capable of processing large quantities of sample material in vessels of suitable material. Following digestion, the samples for analysis are generally clear solutions.

7 Apparatus

7.1 Vessels for elemental solutions

The stability of digest solutions up to analysis 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 perfluoroalkoxy plastics (PFA), hexafluoroethylene-propylene (FEP) or quartz glass 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 ‘acid vapour cleaned’ with boiling concentrated nitric acid in closed systems.

7.2 Freeze-drying unit

Complete system consisting of a freeze-drying chamber, cooling unit and vacuum pump.

7.3 Microwave digestion unit

A closed microwave unit with programmable power consumption as well as a temperature and pressure-monitoring system must be used. Always use the digestion vessels of PFA or quartz recommended by the manufacturer and which are resistant to the reagents used.

NOTE 1: Always observe the manufacturer's safety instructions when operating the microwave unit.

NOTE 2: An UltraClave II (manufactured by MLS GmbH, Leutkirch) is used for ESB samples.

7.4 Analysis balance

Analysis balance with precision of $\pm$ 0.1 mg.

8 Reagents

8.1 General

The digestion reagents used must be suitable for trace analysis. The digestion reagents must be of at least high-purity quality. Even better qualities are available for ultra-trace analysis.

NOTE: For elemental analyses 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 by sub-boiling may also be used which has been purified and analyzed for possible impurities.

8.2 List of reagents

8.2.1 Water from a high-purity water system, Quality: specific resistance > 18.2 MΩ cm.

8.2.2 Nitric acid, ρ (HNO_3) > 1.39 g/mL (≥ 65 %).

8.2.3 Nitric acid, dilute (20 %), c (HNO_3) = 3.5 mol/L.

8.2.4 Nitric acid, dilute (13 %), c (HNO_3) = 2.2 mol/L.

8.2.5 Hydrofluoric acid, ρ (HF) = 1.13 g/mL.

8.2.6 Boric acid solution, prepared by dissolving 4 g $B(OH)_3$ in 100 mL water (see 8.2.1).

9 Digestion procedure

9.1 General notes

The microwave unit should be used according to the operating instructions and any additional internal laboratory procedural instructions.

9.2 Safety note

During digestion of organic samples containing carbon, large quantities of carbon dioxide are produced under digestion conditions. This leads to a rapid increase in pressure in the enclosed digestion vessels. The pressurised vessels should therefore be vented and opened only in a fume hood or with suitable ventilation. Always comply strictly with the safety instructions of the digestion apparatus being used.

9.3 Cleaning the apparatus

Since many of the elements being determined in digests are present in traces only (concentration range in digest solution: ng/L to µg/L), contamination of the samples should be carefully avoided.

Always clean the apparatus before use (e.g. by 'acid vapour cleaning' with nitric acid (see 8.2.2) or first rinsing with dilute nitric acid (see 8.2.4) and then with high-purity water (see 8.2.1).

9.4 Freeze-drying of sample material

In many cases it is advisable to freeze-dry biological sample material for element analysis before digestion. In addition, the percentage of dry mass (or the water content in the fresh sample) can also be determined. For this purpose, the sample material must be frozen to a temperature of less than -20°C. Should it be necessary to freeze-dry solutions, this should be done in cylinders which are revolved in a coolant (distribution of the solution over as large a surface as possible, prevention of glass breakage through volume expansion). Deep-frozen samples and material which is stored in liquid nitrogen can be freeze-dried directly.

NOTE: The freeze-drying apparatus is operated with a vacuum, which means that there is a risk of implosion. The evacuation of the apparatus is a particularly critical operation and must be carried out with great care.

The freeze-drying process is not complete as long as ice caused by condensation continues to form on the vessels. Depending on the water content, and the thickness of the layer of material being dried, the freeze-drying process may take between several hours and several days. It should also be verified whether a further reduction in weight is detected by prolonging the duration of freeze-drying, i.e. interruption of the freeze-drying process, weighing of the sample, continuation of the process, second weighing of sample. If the change in net weight of the sample is less than 0.5% between two weighing operations on different days, the sample may be regarded as dry.

NOTE: As a general rule, ESB samples are freeze-dried directly in the glass vessels in which they are usually stored (20 mL bottles of high-quality glass with plastic caps, of the type normally used for scintillation measurements). Before freezing, the caps are loosened by a quarter turn but are not removed. This avoids cross-contamination of samples being freeze-dried at the same time. The weight of the empty glass bottles is documented before being filled with sample material.

9.5 Digestion of freeze-dried samples

If the samples are being processed in freeze-dried form, between 100 and 500 mg of the material is weighed precisely using a laboratory balance (see 7.4). The sample is weighed directly in the digestion vessel.

NOTE 1: It may be advisable to treat the digestion vessel with an anti-static blower beforehand. Suitable digestion reagents are then added to the weighed quantity of sample material. For organic material, nitric acid (see 8.2.2) is sufficient in many cases. Any spontaneous reaction is allowed to subside. When the digestion vessel has been closed and placed in the digestion system, the unit is either programmed or set to one of the resident programs according to the manufacturer's instructions. The digestion parameters are adjusted to the specific sample. The selected conditions (microwave energy, duration of digestion, temperature, pressure) are documented in the raw data.

NOTE 2: For the analysis of ESB samples, the following weights and settings are used for the UltraClave II (MLS GmbH): approx. 200 mg freeze-dried material, 5 mL nitric acid to 8.2.2 in quartz vessels. Program: heat for 25 min. to maximum temperature of 220°C, hold at 220 °C for 30 min., allow to cool for approx. 60 min. Initial pressure 40 bar, increase during digestion to 60 - 100 bar depending on matrix. Maximum pressure possible 200 bar, total duration approx. 2 h.

9.6 Digestion of fresh sample material

For some tasks it may be advisable to digest fresh biological material directly. In this case, the weight of material used depends on the dry-mass percentage of the sample. However, the dry-mass equivalent of the sample should not exceed 500 mg. The subsequent stages in the process are identical to those used for freeze-dried material.

9.7 Venting of digestion vessels

After cooling down, the digestion vessels are carefully vented. It should be ensured that the safety valve has not opened during the digestion process thereby leading to a loss of volatile elements. Any error signals from the microwave digestion unit should be documented in the report of results.

9.8 Procedure in case of incomplete digestion

If the digestion solution is not clear, a few mL of reagent are added and the digestion process repeated. It may also be advisable to repeat the digestion with a smaller quantity of material. If the undissolved residues are inorganic material, (e.g. sand grains when digesting bladder wrack), it may be useful to add 1 mL of hydrofluoric acid (see 8.2.5).

NOTE 1: Caution! Danger of severe burns when working with hydrofluoric acid.

After digestion, the excess hydrofluoric acid should be complexed with tetrafluoroborate adding 10 mL boric acid solution (see 8.2.6).

NOTE 2: After complexing with boric acid, it is no longer possible to determine the boron content in samples.

9.9 Storage of samples

Following digestion, the solutions are transferred to volumetric flasks to be replenished to a defined volume depending on elemental content and measuring method (e.g. 10 mL, 20 mL or 25 mL). The digestion vessel is rinsed with high-purity water (see 8.2.1) and the rinsing solution is added to the digest solution. The volumetric cylinder is then replenished to the nominal volume with high-purity water. The solutions are stored at ambient temperature.

9.10 Shelf life

The elemental content in question should be determined as soon as possible after the digestion process. Although the digestions solutions probably remain stable for a fairly long period of time (up to two months at least, based on past experience) longer periods of storage may lead to distortions of elemental contents caused by adsorption at the walls of the vessel as well as precipitation. If samples stored for longer periods of time are to be used, their suitability should be verified.

9.11 Quality assurance

The following quality-assurance samples are also digested along with the samples under analysis:

- blank samples (reagents only);
- certified reference material which is as similar as possible to the material under analysis with regard to matrix composition, and content of main target and interfering elements;
- laboratory-internal reference material characterised by the user.

NOTE: In the analysis of ESB material, the proportion of samples to quality assurance samples is always 2:1 at the most. The laboratory internal reference material used is sample material from previous years for which the required elemental-analysis data are known.

10 Calculation of water content

The water content in the fresh mass of a sample is calculated using the following formula:

$WC = (M_F – M_D) / M_F * 100$

where

WC water content,

M_F fresh mass of sample,

M_D dry residue after freeze-drying.

If the fresh sample is being weighed in a vessel of known weight, the following equation may also be used:

$WC = (V_F – V_D) / (V_F – V_E) * 100$

where

V_F mass of empty vessel plus fresh mass of sample,

V_D mass of empty vessel plus dry residue after freeze-drying,

V_E weight of empty vessel.

11 Report of results

The following digest data must be recorded:

- Reference to this guideline;
- Sample identity;
- Description of any prior treatment of sample (e.g. freeze-drying);
- Quantity of sample used for digestion;
- Water content in %, if determined in accordance with these guidelines;
- Volume of solution to which the digestion solution was replenished;
- Approximate acid content of digestion solution;
- Reference to any discolouration or cloudiness in the solution and to any particles or residues of undigested material;
- Data (degree of purity, concentration) on acid used and of any other reagents used for digestion.
- Data on accompanying quality-assurance samples.

12 Bibliography

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