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Water quality — Determination of arsenic(III) and arsenic(V) species — Method using high performance liquid chromatography (HPLC) with detection by inductively coupled plasma mass spectrometry (ICP-MS) or hydride generation atomic fluorescence spectrometry (HG-AFS)

Qualité de l'eau — Détermination des formes chimiques (III) et (V) d'arsenic — Méthode par chromatographie en phase liquide à haute performance (HPLC) avec détection par spectrométrie de masse par torche à plasma (ICP-MS) ou génération d'hydrure fluorescence atomique (HG-AFS)

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

Introduction

In the environment, metals and metalloids are found in the form of various chemical species. Chemical speciation makes it possible to identify and quantify these different species. For the same metal or metalloid, given that the toxicity of each compound may vary significantly, it can be useful to quantify each of the species present in a given sample. For arsenic, the toxicity of the various species varies considerably; inorganic species are recognized as being more toxic than organic species and, for example, the toxicity of As(III) is greater than that of As(V). This method is primarily applicable to the determination of arsenite (As(III)) and arsenate (As(V)) as these are the main species of interest and are the predominant species found in potable water samples from the underlying geological strata in many parts of the world. Arsenic speciation measurements are important to establish and select the best water treatment technology for arsenic removal from raw waters containing significant levels of arsenic.

Water quality — Determination of arsenic(III) and arsenic(V) species — Method using high performance liquid chromatography (HPLC) with detection by inductively coupled plasma mass spectrometry (ICP-MS) or hydride generation atomic fluorescence spectrometry (HG-AFS)

WARNING — Persons using this document should be familiar with normal laboratory practice. This document is not intended to cover any safety problems associated with its use, if applicable. It is the responsibility of the user to establish appropriate safety and health practices.

IMPORTANT — It is absolutely essential that tests conducted in accordance with this document be carried out by suitably qualified staff.

1 Scope

This document specifies a method primarily developed for the determination of inorganic arsenic species (arsenite (As(III)) and arsenate (As(V)) dissolved in a sample after a preservation process in waters with a low total organic carbon content such as potable water, tap water, surface waters, ground waters and rain waters. Information is provided on the determination of potentially relevant organo-arsenic species such as methylarsonic acid (MMA) and dimethylarsinic acid (DMA) which may be encountered at very low levels in natural surface waters.

The linear working dynamic range depends on the operating conditions and the method of detection used; under standard conditions, it typically ranges from 0,5 µg/l to 50 µg/l for each species. Samples containing arsenic at concentrations higher than the linear dynamic range can be analysed after suitable dilution.

This method is based on high performance liquid chromatography separation of arsenic species with either inductively coupled mass spectrometry (ICP-MS) or hydride generation atomic fluorescence spectrometry (HG-AFS) as a method of detection.

The sensitivity of this method depends on the method of detection and the instrumental operating conditions selected. The limit of quantification (LOQ) of the method also depends on the operating conditions of the analytical system used and the extent of the calibration range used. LOQ examples for As(III) and As(V) are provided; LOQs are generally less than 1 µg/l.

This document does not apply to arsenobetaine and other organic arsenic species which are not present in natural water samples.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 5667-1, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes and sampling techniques*

ISO 5667-3, *Water quality — Sampling — Part 3: Preservation and handling of water samples*

ISO 17294-1, *Water quality — Application of inductively coupled plasma mass spectrometry (ICP-MS) — Part 1: General guidelines*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org./obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
analyte
substance to be determined

[SOURCE: ISO/TS 28581:2012, 3.1]

3.2
blank calibration solution
solution prepared in the same way as the calibration solution but leaving out the analyte

[SOURCE: ISO 17294-1:2004, 3.3]

3.3
calibration solution
solution used to calibrate the instrument, prepared from (a) stock solution(s) or from a certified standard

[SOURCE: ISO 17294-1:2004, 3.4]

3.4
stock solution
solution with accurately known analyte concentration(s), prepared from suitably pure chemicals

[SOURCE: ISO 17294-1:2004, 3.30]

3.5
determination
entire process from preparing the test sample solution up to and including measurement and calculation of the final result

[SOURCE: ISO 17294-1:2004, 3.6]

3.6
limit of quantification
LOQ
lowest concentration of an analyte that can be determined with a specified degree of accuracy and precision

3.7
limit of detection
LOD
lowest concentration of an analyte that can be detected with a specified degree of accuracy and precision

4 Principle

The different arsenic species are separated using a specific column in a high performance liquid chromatograph (HPLC). The separation of arsenic species in natural water is typically achieved using strong anion exchange ion chromatography. The species can be separated using isocratic conditions however faster more efficient separations can be achieved using gradient elution.

This method permits the use of ICP-MS (see Annex A) or HG-AFS (see Annex B) for individual detection of the various arsenic species.

Annex C provides information on the determination of organic arsenic species.

Annex D provides information on the stability of arsenic species using different storage and preservations.

5 Interferences

Retention time shift may occur for some water samples, especially when they are enriched with significant levels of various water matrix ions. These ions compete with the active sites on the column. Sample spikes using each of the arsenic species should therefore be used to confirm species identity if a retention time shift is observed. The sample may also be diluted to overcome this effect or a smaller injection volume may be used with an inferior LOQ. The co-elution of organic arsenic species with arsenite (As(III)) and arsenate (As(V)) may cause a positive interference; therefore, the separation conditions should be well proven and established.

To detect ICP-MS interferences, follow the procedure specified in Annex A.

To detect HG-AFS interferences, follow the procedure specified in Annex B.

6 Apparatus

Due to significant differences between the various instrument models and brands available, it is not possible to give detailed instructions on their operation. The operator shall thus refer to the instructions provided by the manufacturer of each instrument.

Usual laboratory glassware and equipment and, in particular, the following:

6.1 High performance liquid chromatograph (HPLC), including a column for analyte separation and optionally a chromatographic guard column.

The HPLC system may be equipped with an autosampler, in-line degassing system and auto injection system for introducing the sample. In most cases, an isocratic pump can be used; although, the use of gradient pumps to provide optimal separation times is permitted.

NOTE Various column/eluent pairings can be used for separating arsenic species. A strong anion exchange column is typically used. Some examples are provided in Annexes A and B.

6.2 Vacuum filtration system, for filtering the eluent and reagents prepared.

6.3 pH meter, for adjusting pH of eluent to one decimal place between 1,0 and 14,0.

6.4 Inductively coupled plasma mass spectrometer (ICP-MS).

See Annex A.

6.5 Hydride generation atomic fluorescence spectrometer (HG-AFS).

See Annex B.

7 Reagents and standards

7.1 General requirements

All reagents shall be of known analytical grade. The concentration of the analyte or interfering substances in the reagents and water should be negligible compared to the lowest concentration to be determined.

NOTE Standard stock solutions are commercially available or can be prepared using chemicals of known analytical purity.

Any reagents used in the preparation of the mobile phases of the HPLC procedure are column and instrument specific and are not included here.

7.2 Water, grade 1, as defined in ISO 3696.

7.3 HPLC grade water.

HPLC grade water is used to prepare the mobile phase and the calibration solutions and to produce the sample dilutions. It can be prepared by suitably purifying water (7.2).

7.4 Sodium hydroxide.

7.5 Sodium hydroxide solution, 1 mol/l.

Weigh 4 g of sodium hydroxide pellets (7.4) and add them to a 100 ml beaker. Then add approximately 50 ml de-ionized water and stir until the pellets have dissolved. Transfer to a 100 ml volumetric flask and make up to the mark with de-ionized water (7.2)

7.6 Hydrochloric acid, mass fraction 35 % to 37 %.

7.7 Hydrochloric acid preservation solution for HG-AFS, approximately 6 mol/l.

Carefully add 500 ml of hydrochloric acid (7.6) to 250 ml of water (7.2). Make up to a final volume of 1 000 ml with water (7.2).

7.8 Nitric acid, mass fraction, 68 % to 72 %.

7.9 Nitric acid preservation solution for ICP-MS, approximately 6 mol/l.

Carefully add 365 ml of nitric acid (7.8) to 250 ml of water (7.2). Make up to a final volume of 1 000 ml with water (7.2).

7.10 Standard substances

As(III): Arsenious oxide As_2O_3 (CAS No. 1327-53-3);

As(V): Di-sodium hydrogen arsenate $Na_2HAsO_4 \cdot 7H_2O$ (CAS No. 10048-95-0);

DMA: Dimethylarsinic acid, refer to Annex C;

MMA: Methylarsonic acid acid, refer to Annex C.

7.11 Stock solutions 1 000 mg/l

For each of the species, As(III) and As(V), prepare a stock solution with a concentration of 1 000 mg/l expressed as As.

These solutions are prepared from the standard substances (7.10).

In 100 ml volumetric flasks, dissolve the appropriate mass of each substance (7.10) as shown in Table 1.

Commercially available As(III) and As(V) stock solutions of the required concentration can also be used.

Table 1 — Preparation guidelines for 1 000 mg/l arsenic standards

Species	Mass g	Final volume ml
As(III)	0,132	4 ml of 1 mol/l NaOH (7.5) made up to 100 ml with water (7.2)
As(V)	0,416	100 ml in water (7.2)

These solutions, when stored protected from light and at 4 °C, are considered to be stable for one year.

7.12 Single component stock solutions 10 mg/l

Pipette 1 ml of 1 000 mg/l stock solution (7.11) and dilute to 100 ml in a volumetric flask.

The 10 mg/l single-component working solutions are also stable for one year if they are stored protected from light and at 4 °C and stabilized in 0,04 mol/l NaOH (7.5) for As(III).

7.13 Calibration solutions

The calibration solutions are prepared from the 1 000 mg/l stock solutions (7.12). Tables 2 and 3 are given as examples. The method is primarily for As(III) and As(V). It may be necessary to include DMA and MMA standards to prove that the chromatographic separation is acceptable. Additional guidance is provided in Annex C. Working standards should be prepared daily.

Table 2 — Preparation guidelines for working arsenic standards

Concentration µg/l	Volume of each stock solution µl	Solution for dilution	HPLC grade water (7.3)
1 000	100 µl of each stock solution	1 000 mg/l (7.11) As(III), As(V)	100 ml
5	500 µl	1 000 µg/l	100 ml
10	1 000 µl	1 000 µg/l	100 ml
25	2 500 µl	1 000 µg/l	100 ml
50	5 000 µl	1 000 µg/l	100 ml

To prepare standard solutions below 5 µg/l use the 10 mg/l intermediate stock to produce a mixed standard of 100 µg/l. Table 3 is given as an example.

Table 3 — Preparation guidelines for working arsenic standards

Concentration µg/l	Volume of each working solution µl	Solution for dilution	HPLC grade water (7.3)
100	1 000 µl of each solution	10 mg/l (7.12) As(III), As(V)	100 ml
0,2	200 µl	100 µg/l	100 ml
0,5	500 µl	100 µg/l	100 ml
1,0	1 000 µl	100 µg/l	100 ml
2,0	2 000 µl	100 µg/l	100 ml

7.14 Eluents

Various eluents can be used and the choice depends on the type of separation column chosen. The eluent compositions described in Annex A and Annex B and in the associated tables are given as examples. Given the number of possible eluents that can be used, this document does not provide guidance on how to prepare these eluents.

7.15 HPLC trace analysis grade methanol.

8 Sampling, preservation and storage of samples

During the elaboration of this document, the stability of dissolved arsenic species was studied using various methods of storage and preservation. A summary of the findings of this work is provided in Annex D. Given the possible transformation of arsenate (As(V)) and arsenite (As(III)) species, it is imperative that all laboratories using this document conduct their own investigations to demonstrate that their selected storage and preservation approach is suitable for the water samples that their laboratory may analyse.

This document is based on the preservation of dissolved arsenic species contained in the sample. Depending on the type of sample, filtering at 0,45 µm, preferably on-site, is recommended followed by acid stabilization, particularly in the case of ground waters and surface waters.

In the case of samples of water intended for human consumption with turbidity levels < 2 NFU, filtering at 0,45 µm is not required.

If filtration and subsequent stabilization with acid are not feasible on-site, to minimize variations in terms of speciation, these procedures shall be carried out on receipt in the laboratory and within not more than 48 h of sampling. Sample preservation with acid shall always be carried out after filtration.

For HG-AFS, samples should be preserved by the addition of 300 µl of 6 mol/l HCl (7.7) per 100 ml of sample. This equates to a final concentration of 0,018 mol/l HCl.

For ICP-MS, nitric acid stabilization can be used to avoid polyatomic $^{40}Ar^{35}Cl$ interference for ^{75}As. This stabilization approach has not been fully tested; therefore, additional verification is strongly advised.

In this case, samples should be preserved by the addition of 300 µl of 6 mol/l HNO_3 per 100 ml of sample. This equates to a final concentration of 0,018 mol/l HNO_3.

Samples may be shipped at room temperature but should be stored in the laboratory at 4 °C until they are analysed.

Additional guidance on stability, preservation and storage is provided in Annex D and in the Bibliography.

In general terms, sampling shall be performed in accordance with ISO 5667-1 and ISO 5667-3, using suitable sampling containers that have been validated for the stability and storage of arsenic species.

9 Procedure

9.1 HPLC instrument optimization

Use the instrument under the manufacturer's standard conditions.

Start up the HPLC system, set the pump flow rate and couple the column outlet to the detection system. Ensure that the baseline is stable and the eluent has sufficiently flushed the column.

Before running the calibration, check that the chromatographic conditions selected enable satisfactory separation with minimum resolution between each peak for the highest concentration of standard solutions.

Independent from the detection system, identify the analyte by comparing the retention times for the samples and reference standards. The minimum requirements for identification are retention times within ±0,1 min and relative retention times within ±0,5 % over the total run of a chromatogram.

A retention time shift may occur for some samples, especially when they are enriched with significant levels of various water matrix ions. Sample spikes of each arsenic species should therefore be used to confirm the species identity if the retention shift observed is greater than ±0,2 min. The sample may be diluted to overcome this effect or a smaller injection volume may be used with an inferior LOQ.

Annex A provides more detailed information on HPLC-ICP-MS using different separations.

Annex B provides more detailed information on HPLC-HG-AFS using different separations.

Annex C provides information on the separation of organic arsenic species.

Annex D provides information on sample storage, preservation and stability of arsenic species.

9.2 Calibration

As a general rule, proceed as follows.

— Prepare and measure the blank calibration solution and the calibration solutions prepared in 7.13.

— Prepare a calibration graph in accordance with the manufacturer's instructions according to the processing software used for signal acquisition in coupling mode.

9.3 Sample measurement

The water samples can be injected with or without dilution depending on the arsenic concentrations measured previously.

The preliminary arsenic analysis of the filtered sample provides information on any dilution required prior to injecting the sample and serves to check the consistency of the results. It is important to ensure that the sum of the species measured remains less than or equal to the total arsenic value measured. Regulatory total arsenic measurements are based on unfiltered samples and, therefore, may provide a significantly higher result than total dissolved arsenic.

10 Expression of results

The results obtained are expressed as µg/l As, applying the dilution factors used for each sample. Give the results to a maximum of two significant digits.