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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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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.

EXAMPLE

Arsenic(III)	1,2 µg/l
Arsenic(V)	0,5 µg/l

11 Test report

The test report shall contain at least the following information:

a) the test method used, together with a reference to this document, i.e. ISO/TS 19620;

b) the instrumental and separation conditions used;

c) complete identification of the sample;

d) expression of the results according to Clause 10;

e) any details not specified in this document or considered to be optional, and any particular factor liable to have affected the results.

Annex A
(normative)

Detection using inductively coupled plasma mass spectrometry (ICP-MS)

A.1 Principle

For ICP-MS, the eluted species are entrained in an argon plasma by means of a nebulizer and a nebulization chamber. The arsenic compounds contained in the sample are atomized and ionized in the plasma. The ions produced are introduced into the mass spectrometer chamber where they are routed via a series of charged lenses and separated by a quadrupole, to be ultimately captured by a detector. The concentration of an element with a specific mass is determined by comparing the quantities of ions detected in the sample and in the calibration solutions.

A.2 Interferences

For ICP-MS, the interferences encountered for the arsenic assay essentially consist of interferences caused by polyatomic species formation. Non-specific interferences (or the matrix effects) are described in ISO 17294-1. Polyatomic ions are formed by concordance of gaseous compounds in the plasma, reagents and the sample matrix (for example, interference with the relative mass of 75As by 40Ar35Cl and 40Ca35Cl). This interference is particularly important for the element arsenic. The mass corresponding to the polyatomic ion ClO having a mass 51 should be monitored if the matrix under analysis contains a high concentration of chloride ions. The chromatographic separation system frequently provides for the separation of chloride ions from the species under analysis.

The impact of this effect on the measurement is dependent on the system used; this impact shall be known. If mathematical corrections are used, the fact that the extent of the interference depends on the plasma setting (oxide formation rate, for example) and the mass concentration of the interfering element, which is, in general, a variable component of the sample solution, shall be taken into account.

Using a reaction/collision chamber after the skimmer cone makes it possible to reduce the polyatomic interference caused by the molecular species 40Ar35Cl formed. Regardless of the interference reduction system used, chloride ion related interference shall be controlled in accordance with ISO 17294-1.

NOTE An internal standard can be used to check instrumental drift. For example, a solution containing 10 µg/l of germanium can be injected.

A.3 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.

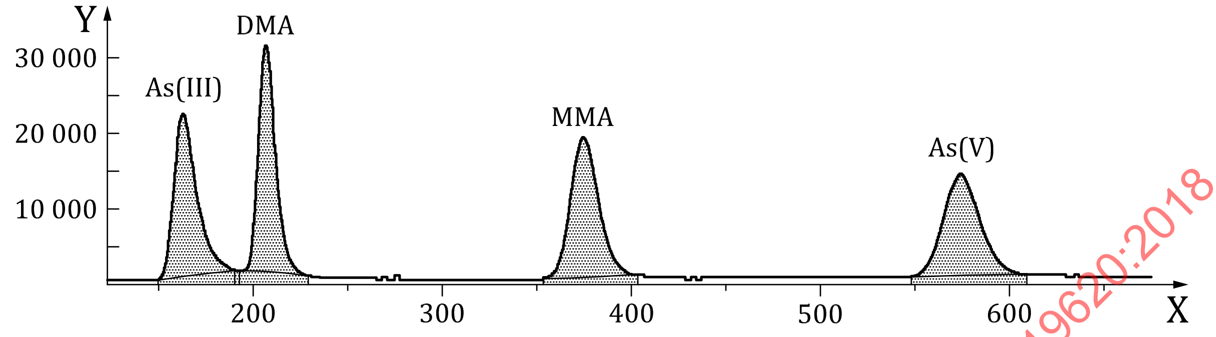
A.3.1 Mass spectrometer, with argon plasma as an ion source (ICP-MS).

Depending on the instruments used, the mass spectrometers may be equipped with an interference reduction device positioned upstream from the mass analyser.

NOTE Further information on the instrumentation is described in ISO 17294-1:2004, Clause 5.

A.4 Procedure

Use the instrument under the manufacturer's standard conditions. The following examples are provided as guidance only and do not endorse specific vendor instrumentation. (See Figures A.1 to A.6 and Tables A.1 to A.18.)



Key

X retention time in seconds, s

Y counts per second, cps

Figure A.1 — Typical chromatogram representing four arsenic species containing 10 µg As/l in 1 % 10 mM EDTA (injection volume 50 µl)

Table A.1 — ICP-MS conditions

Instrument	ICP-MS Thermo Elemental®[a]
Power	1,25 KW
Plasma-forming gas	14,0 l/min
Auxiliary gas	0,7 l/min
Nebulizer	Conikal 1 ml
Interface	Xs nickel
Acquisition	Peak jumping

[a] CP-MS Thermo Elemental® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

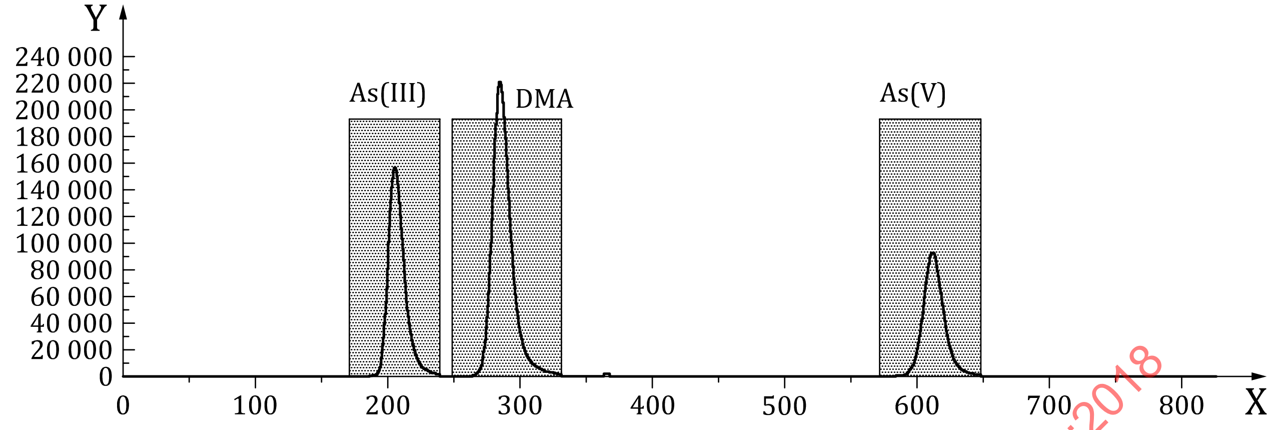
Table A.2 — Chromatographic conditions

Pump	Agilent 1100® series[a]
Flow rate	Isocratic 1 ml/min
Column	Hamilton PRP®[a]-1 (l = 250 mm, ID = 4,1 mm, particle size 10 µm)
Eluent	0,5 mM ammonium monohydrogen phosphate 0,5 mM tetrabutylammonium phosphate, 3 % volume fraction methanol at pH = 9
Injection loop	50 µl

[a] Agilent 1100 series® and Hamilton PRP® are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

Table A.3 — Calibration conditions

Number of standards	4
Calibration dynamic range	5 µg/l to 50 µg/l
Limit of detection	0,2 µg/l
Limit of quantification	1 µg/l



Key

X retention time in seconds, s

Y counts per second, cps

Figure A.2 — Typical chromatogram representing three arsenic species containing 25 µg/l (injection volume 20 µl)

Table A.4 — ICP-MS conditions

Instrument	ICP-MS Series X II Thermo Elemental®[a]
Power	1,25 KW
Plasma-forming gas	14,0 l/min
Auxiliary gas	0,7 l/min
Nebulizer	Concentric nebulizer 1 ml
Interface	Xt nickel + CCT
Acquisition	Scan

[a] ICP-MS Series X II Thermo Elemental® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

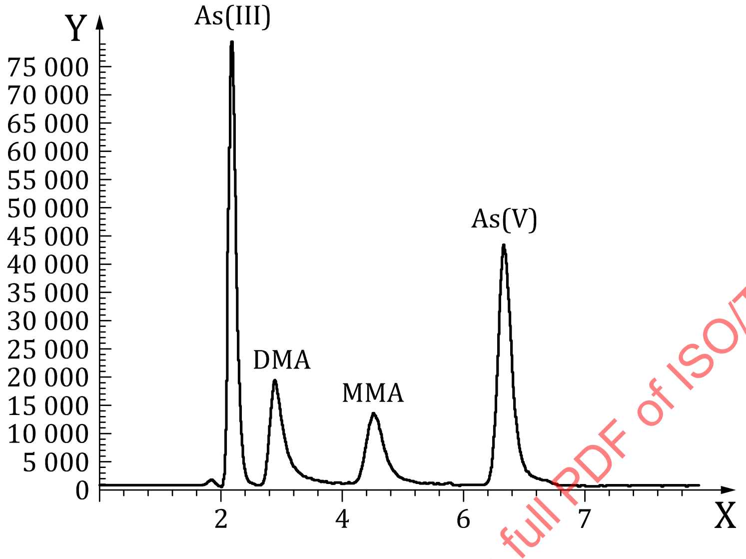
Table A.5 — Chromatographic conditions

Pump	Thermo P 1000®[a]
Flow rate	Elution gradient: fixed flow rate at 1 ml/min
Column	Hamilton PRP-X 100®[a] (l = 250 mm, ID = 4,1 mm, particle size 10 µm)
Eluent	A: $(NH_4)_2HPO_4$ / $(NH_4)H_2PO_4$: 10 mmol/l, 3 % volume fraction methanol at pH = 7
	B: $(NH_4)HPO_4$: 100 mmol/l, 3 % volume fraction methanol at pH = 8,5
Gradient	A-B (45 %-55 %) 11 min
	A-B (0 %-100 %): 2 min
	A-B (45 %-55 %): 5 min
Injection loop	20 µl

[a] Thermo P 1000® and Hamilton PRP-X 100® are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

Table A.6 — Calibration conditions

	As(III)	As(V)
Number of standards	4 points	4 points
Calibration dynamic range	2,5 µg/l to 25 µg/l	2,5 µg/l to 25 µg/l
Limit of detection (theoretical)	0,15 µg/l	0,15 µg/l
Limit of quantification (theoretical)	0,5 µg/l	0,5 µg/l



Key

X retention time in milliseconds, ms

Y counts per second, cps

Figure A.3 — 10 µg/l As + 1 % 10 mM EDTA; 100 µl injected; order of elution, As(III), DMA, MMA and As(V), respectively (injection volume 100 µl)

Table A.7 — ICP-MS condition

Instrument	ICP-MS 7500ce AGILENT®[a]
Power	1,55 KW
Plasma-forming gas	15 l/min
Auxiliary gas	
Nebulizer	Micromist 1 ml
Interface	Skimmer and sampler made of nickel
Acquisition	Time resolved analysis

[a] ICP-MS 7500ce AGILENT® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

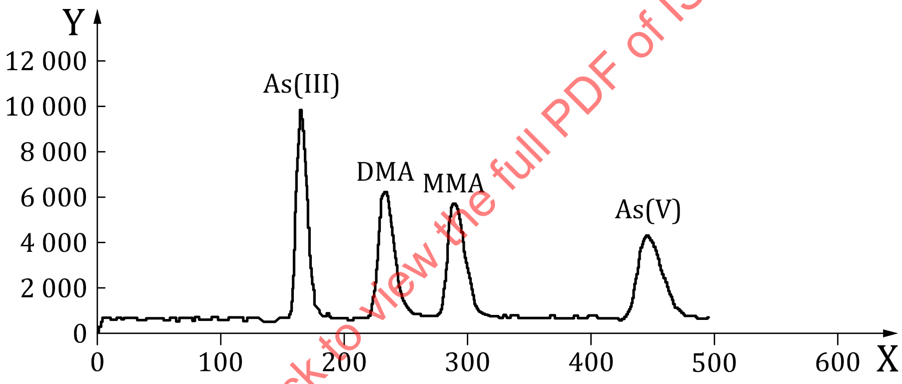
Table A.8 — Chromatographic conditions

Pump	Dionex ICS-3000®[a]
Flow rate	Isocratic 1 ml/min
Column	Hamilton PRP-X100®[a] (l = 250 mm, ID = 4,1 mm, particle size 10 µm)
Eluent	2,5 mM Na dihydrogen phosphate + 0,5 mM EDTA-2Na + 2,5 % MeOH by volume fraction at pH = 6
Injection loop	100 µl

[a] Dionex ICS-3000® and Hamilton PRP-X100® are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

Table A.9 — Calibration conditions

Number of standards	5
Calibration dynamic range	0,5 µg/l to 30 µg/l
Limit of detection (calculated)	0,2 µg/l
Limit of quantification	0,5 µg/l for As(III) and As(V)



Key

X retention time in seconds, s

Y counts per second, cps

Figure A.4 — Typical chromatogram representing four arsenic species containing 5 µg/l (injection volume 50 µl)

Table A.10 — ICP-MS conditions

Instrument	PQ3®[a]
Power	1 380 W
Plasma-forming gas	14,0 and 0,85 l/min
Auxiliary gas	
Nebulizer	Concentric nebulizer
Interface	Xs
Acquisition	Dwell time 700 ms, acquisition 400 s, Transient TRA

[a] PQ3® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

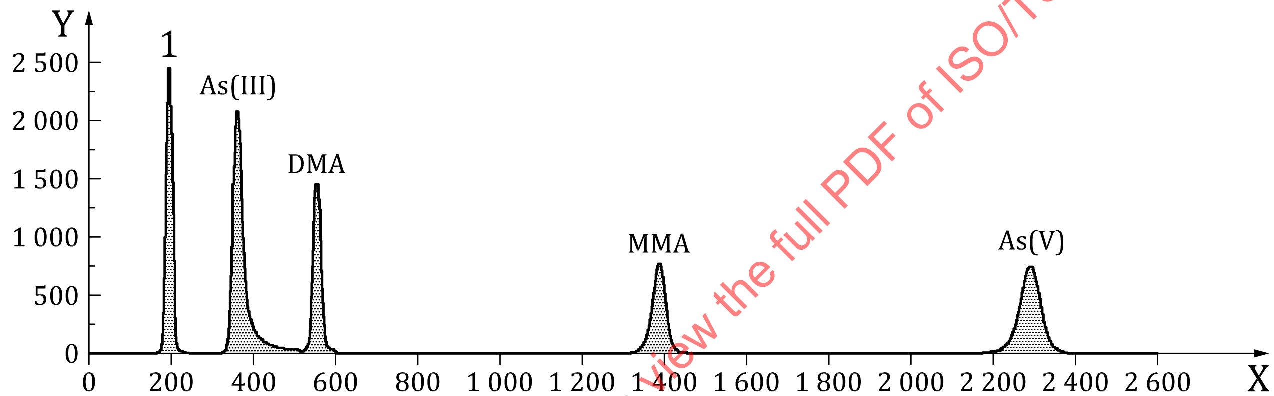
Table A.11 — Chromatographic conditions

Pump	Varian 9010®[a] manual injection
Flow rate	Isocratic: 1,2 ml/min
Column	Hamilton PRP-X 110®[a] (l = 250 mm, ID = 4,1 mm, particle size 10 µm)
Eluent	(0,02 M $NH_4)_2HPO_4$ — pH 6
Injection loop	50 µl

[a] Varian 9010® and Hamilton PRP-X 110® are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

Table A.12 — Calibration conditions

Number of standards	4
Calibration dynamic range	1 µg/l-5 µg/l-10 µg/l-25 µg/l
Limit of quantification	0,5 µg/l (1 µg/l As(V))



Key

X retention time in seconds, s

Y counts per second, cps

Figure A.5 — Typical chromatogram of four arsenic species + 20 µg/l arsenobetaine (internal standard)

Table A.13 — ICP-MS conditions

Instrument	Thermo X Series II™[a]
Power	1 400 W
Plasma-forming gas	13,0 l/min
Auxiliary gas	0,7 l/min
Nebulizer	Concentric nebulizer
Interface	Sampler and skimmer made of Ni Xt + CCT
Acquisition	Time resolved analysis

[a] Thermo X Series II™ is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

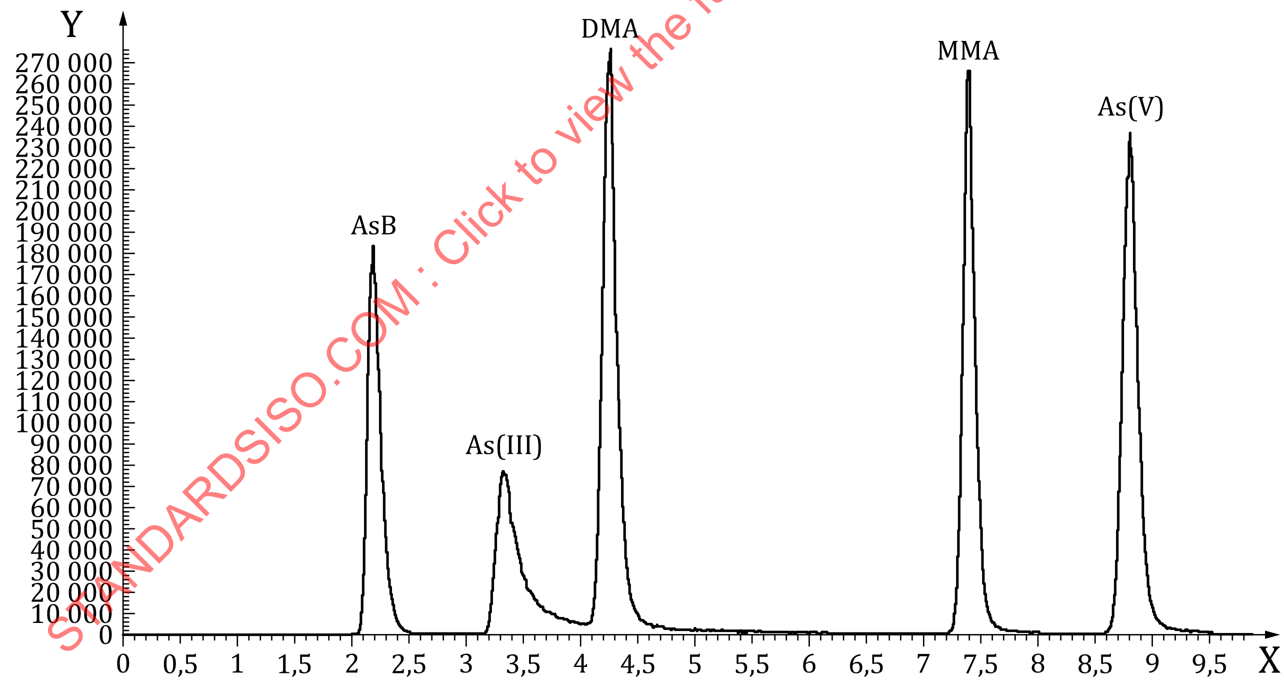
Table A.14 — Chromatographic conditions

Pump	SPECTRA system P1000XR®[a] with AS3000 sample changer (PEEK pumps and injection system)
Flow rate	1,0 ml/min
Column	Hamilton PRP-X 100®[a] PEEK (l = 250 mm, ID = 4,1 mm, particle size 10 µm)
Eluent	A = 10 mM $(NH_4)_2CO_3$ B = 20 mM $(NH_4)_2CO_3$ C = Methanol
Gradient	A-B-C (97 %-0 %-3 %) 1 min to 8 min A-B-C (0 %-97 %-3 %) 9 min to 40 min A-B-C (97 %-0 %-3 %) 40 min to 50 min
Injection loop	50 µl

[a] SPECTRA system P1000XR® and Hamilton PRP-X 100® are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

Table A.15 — Calibration conditions

Number of standards	Blank + 5 points
Calibration dynamic range	1 µg/l to 20 µg/l
Limit of quantification	Verified at 1 µg/l for each element in a real matrix



Key

X retention time in minutes, min

Y counts per second, cps

Figure A.6 — Typical chromatogram of four arsenic species + 10 µg/l arsenobetaine (AsB)

Table A.16 — ICP-MS conditions

Instrument	Bruker Aurora M90®[a]
Mode	High-Sensitivity
Power	1,4 kW
Plasma-forming gas	15 l/min
Auxiliary gas	1,5 l/min
Nebulizer	Concentric pneumatic
Acquisition mode	Time resolved scan
Dwell time	500 ms

[a] Bruker Aurora M90® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

Table A.17 — Example chromatographic conditions

Pumps	Varian 210®[a]
Sample changer	Varian 410®[a]
Column	Hamilton PRP-X 100®[a] (l = 250 mm, ID = 4,1 mm, particle size 10 μm)
Injection loop	100 μl
Flow rate	1 ml/min
Eluent	12,5 mM ammonium carbonate at pH 9 (A) 60 mM ammonium carbonate at pH 9 (B)
Gradient	0 min 100 % A 2,0 min 80 % A 20 % B 2,9 min 60 % A 40 % B 3,.0 min 30 % A 70 % B 6,0 min 0 % A 100 % B 10,0 min 0 % A 10 0 % B

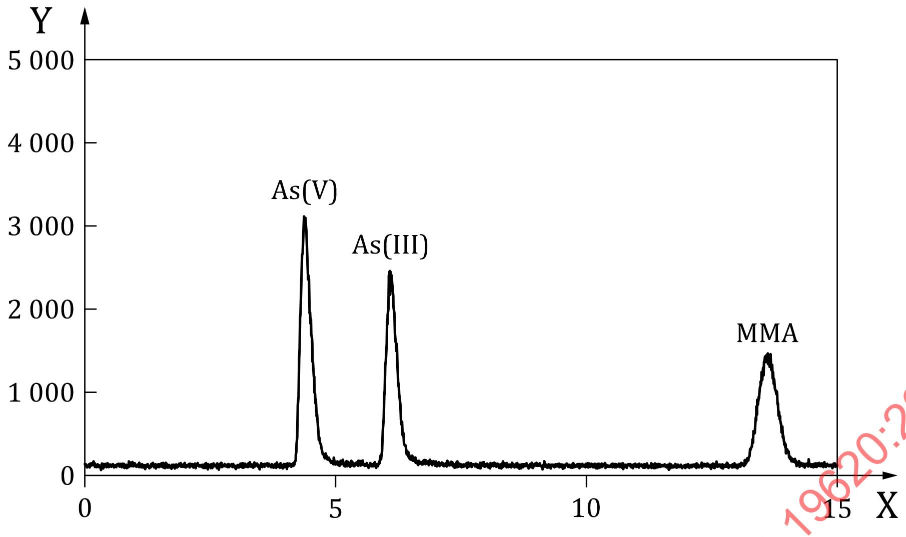
[a] Varian 210®, Varian 410® and Hamilton PRP-X 100® are examples of a suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

Table A.18 — Calibration conditions

Number of standards	Blank + 5 points
Calibration range	0,1 μg/l–0,5 μg/l–1,0 μg/l–5,0 μg/l–10,0 μg/l
Instrument limit of detection	5 ng/l
Limit of quantification	Verified at 0,2 μg/l for each species in a real matrix

A.5 Examples of experimental conditions (References [10] and [11])

See Figures A.7 to A.8 and Tables A.19 to A.24.



Key

X retention time in minutes, min

Y As75, counts per second, cps

NOTE Order of elution: As(V), As(III) and methylarsonic acid acid (MMA), respectively.

Figure A.7 — Chromatogram representing three arsenic species containing 1 µg/l As (injection volume 200 µl)

Table A.19 — ICP-MS conditions

Instrument	Agilent 7500c®[a]
Plasma RF power	1,5 kw
Plasma gas	15 l/min
Nebulizer	Quartz concentric nebulizer
Reaction/collision cell function	Not used (no gas mode)
Skimmer and sampling cone	Made of nickel
Data acquisition	Time resolved analysis

[a] Agilent 7500c® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

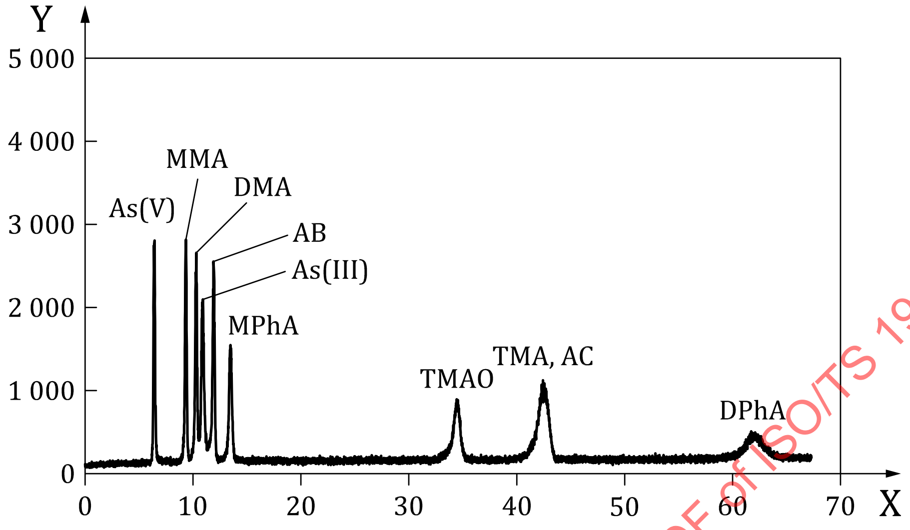
Table A.20 — Chromatographic conditions

Pump	Yokogawa IC7000®[a]
Flow rate	1,5 ml /min
Column	Shimadzu SCR-102H®[a] (l = 250 mm, ID = 8,0 mm, particle size 7 µm)
Eluent	HNO_3, pH 2,0
Injection loop	200 µl

[a] Yokogawa IC7000® and Shimadzu SCR-102H® are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

Table A.21 — Calibration conditions

Number of standards	Blank + 4 points
Calibration dynamic range	0,04 µg/l to 4 µg/l As
Limit of quantification	0,04 µg/l As for As(III) and As(V)



Key

X retention time in minutes, min

Y As75, counts per second, cps

NOTE Order of elution: As(V), methylarsonic acid acid (MMA), dimethylarsenic acid (DMA), As(III), arsenobetaine (AsB), monophenylarsonic acid (MPhA), trimethylarsenine oxide (TMAO), tetramethyarsonium (TMAs), arsenocholine (AC) and diphenylarsinic acid (DPhA), respectively.

Figure A.8 — Chromatogram representing 10 arsenic species containing 10 µg /l As (injection volume 20 µl)

Table A.22 — ICP-MS conditions

Instrument	Agilent 7500c®[a]
Plasma RF power	1,5 kw
Plasma gas	15 l/min
Nebulizer	Quartz concentric nebulizer
Reaction/collision cell function	Not used (no gas mode)
Skimmer and sampling cone	Made of nickel
Data acquisition	Time resolved analysis

[a] Agilent 7500c® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

Table A.23 — Chromatographic conditions

Pump	Yokogawa IC7000®[a]
Flow rate	1 ml/min
Column	Tosoh TSKgel Oapak-A®[a] (l = 300 mm, ID = 7,8 mm, particle size 5 µm)
Eluent	0,35mM Na_2SO_4 at pH 3,8 adjusted with H_2SO_4
Injection loop	20 µl

[a] Yokogawa IC7000® and Tosoh TSKgel Oapak-A® are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

Table A.24 — Calibration conditions

Number of standards	Blank + 4 points
Calibration dynamic range	0,2 µg/l to 10 µg/l As
Limit of quantification	0,2 µg/l As for As(III) and As(V)

Annex B
(normative)

Detection using hydride generation atomic fluorescence (HG-AFS)

B.1 Principle

For hydride generation atomic fluorescence, the eluted arsenic species are acidified and subsequently reduced using a sodium tetrahydroborate reductant to form the species specific covalent gaseous hydride. After gas liquid separation, the arsenic hydride species are delivered to an atomizer such as hydrogen diffusion flame using an argon carrier gas. Moisture is removed from this stream using a hygroscopic membrane dryer (e.g. NafionTM). After atomisation, the arsenic is determined by atomic fluorescence spectrometry (AFS) equipped with an arsenic boosted discharge hollow cathode lamp.

The concentration of arsenic is determined by comparing the quantity of arsenic detected in the sample in relation to the calibration solutions of each species.

B.2 Interferences

The hydride generation technique is prone to interferences by transition and easily reducible metals. For the majority of natural water samples, this type of interference should not be significant. The user should carry out recovery tests on typical waters and also determine the maximum concentrations of potentially interfering elements, using appropriate methods. If such interferences are indicated, the level of interferences should be assessed by performing spike recoveries. It is important that the excitation source does not contain any significant amount of other hydride-forming elements (e.g. antimony when analysing for arsenic) that emit fluorescent radiation over the band pass of the interference filter used in the detector.

B.3 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.

B.3.1 Hydride generation apparatus with atomic fluorescence spectrometer, including a peristaltic pump unit to deliver reagents and load sample injection loop, a mixing manifold to produce covalent hydride species, a gas-liquid separator to separate hydride species from liquid phase reagents and a moisture removal device.

B.3.2 Atomic fluorescence spectrometer equipped with an arsenic excitation source to promote atomic fluorescence of the arsenic hydride species after atomization.

The concentration and the flow of reagents are specifically related to the hydride generation apparatus. The user should use optimal conditions to ensure that the best analytical performance is obtained.

B.4 Reagents

Prepare an appropriate quantity of reagent for the number of samples to be analysed. Guidance notes are based on a 1 l volume.

B.4.1 Hydrochloric acid, mass fraction 35 % to 37 %.

B.4.2 Hydrochloric acid hydride generation solution, volume fraction 25 %.

Carefully add 250 ml of high purity hydrochloric acid (B.4.1) to 750 ml of water (7.2).

B.4.3 Sodium tetrahydroborate, $NaBH_4$, analytical reagent grade pellets.

B.4.4 Sodium tetrahydroborate, 0,7 % m/v, / **sodium hydroxide hydride generation solution**, 0,4 % m/v.

Weigh 7 g of $NaBH_4$ pellets (B.4.3) and transfer to a 1 l flask. Add 4 g of NaOH pellets (7.4) and make up the mark with water (7.2). Filter if necessary and prepare daily.

B.5 Procedure

Use the instrument under the manufacturer's standard conditions. The following examples are provided as guidance only and do not endorse specific vendor instrumentation. (See Figures B.1 to B.6 and Tables B.1 to B.16.)

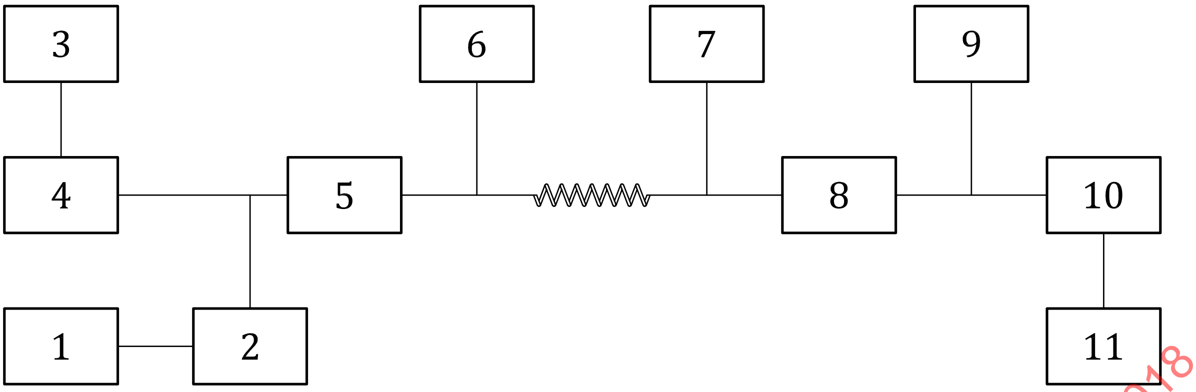
The efficiency of hydride generation is highly dependent on the design and operating conditions of the apparatus and concentration of reagents. The hydride generation and measurement should be optimized to ensure that that optimal performance is achieved. Example conditions are provided in Table B.1.

A block diagram showing the instrumental layout is shown in Figure B.1.

Table B.1 — Typical HG-AFS conditions

HCl concentration	25 % volume fraction
HCl flow rate	1,7 ml/min
NaBH4 concentration	0,7 % m/v in 0,4 % m/v NaOH
NaBH4 flow rate	5 ml/min
Argon carrier gas flow rate	250 ml/min
Air dryer gas flow rate	2,5 l/min
Aux. hydrogen flow rate	Optional (not used in example shown)
Primary current	27,5 mA
Boost current	35,0 mA
Acquisition	Peak area
HG-AFS	PSA Millennium ExcaliburTM 10.055[a]

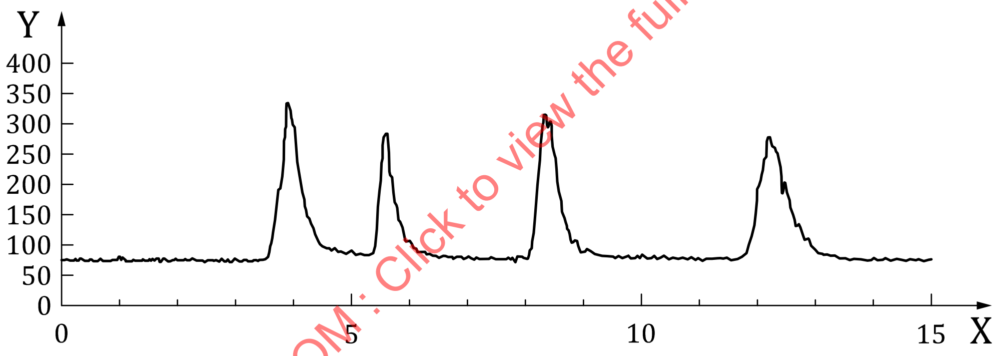
[a] PSA Millennium Excalibur 10.055 is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.



Key

1	autosampler	7	NaBH4
2	injection value	8	GLS
3	mobile phase	9	AUX H2
4	LC-pump	10	Nafion Dryer
5	column	11	AFS
6	HCL		

Figure B.1 — Example layout for HPLC-HG-AFS



Key

X time in minutes, min

Y AFS signal

Figure B.2 — Typical chromatogram representing four arsenic species containing 5 µg/l; As(III), DMA, MMA, and As(V) (in order of elution)

Table B.2 — Typical HG-AFS conditions

Same conditions as in Table B.1.

Table B.3 — Example chromatographic conditions

Flow rate	Elution gradient: fixed flow rate at 1 ml/min
Column	Hamilton PRP-X 100®[a] (l = 250 mm, ID = 4,1 mm, particle size 10 µm)
Eluent	A: 12,5 mM ammonium carbonate at pH 9
	B: 60 mM ammonium carbonate at pH 9

[a] Hamilton PRP-X 100® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

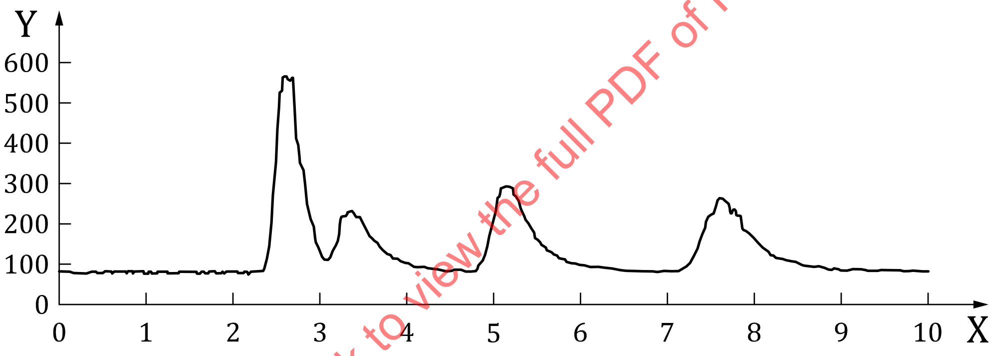
Table B.3 *(continued)*

Flow rate	Elution gradient: fixed flow rate at 1 ml/min
Gradient	0 min: 100 % A, 0 % B
	2 min: 0 % A, 100 % B
	10 min: 100 % A, 0 % B
Injection loop	50 µl to 250 µl

[a] Hamilton PRP-X 100® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

Table B.4 — Example calibration conditions

Number of standards	Blank + 5 points
Calibration dynamic range	0,5 µg/l to 50 µg/l
	0,2 µg/l to 2 µg/l
Limit of detection (3σ_{n-1} calculated)	0,05 µg/l for As(III), As(V), DMA and MMA
Limit of quantification	0,16 µg/l for As(III), As(V), DMA and MMA



Key

X time in minutes, min

Y AFS signal

Figure B.3 — Example chromatogram representing four arsenic species containing 5 µg/l; As(III), DMA, MMA, and As(V) (order of elution)

Table B.5 — Typical HG-AFS conditions

Same conditions as in Table B.1.

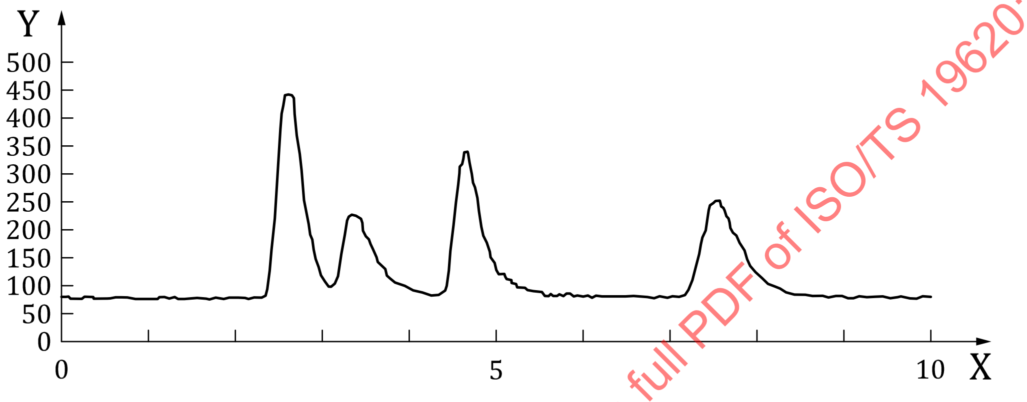
Table B.6 — Example chromatographic conditions

Pump	Isocratic®[a]
Flow rate	Isocratic conditions: fixed flow rate at 1 ml/min
Column	Hamilton PRP-X 100®[a] (l = 250 mm, ID = 4,1 mm, particle size 10 µm)
Eluent	2,5 mM NaH_2PO_4 + 0,5 mM 2Na EDTA + 2,5 % volume fraction methanol at pH = 6
Injection loop	5 µl to 250 µl

[a] Isocratic® and Hamilton PRP-X100® are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

Table B.7 — Example calibration conditions

Number of standards	Blank + 5 points
Calibration dynamic range	0,5 µg/l to 50 µg/l
	0,2 µg/l to 2 µg/l
Limit of detection (calculated)	0,05 µg/l for As(III)
	0,1 µg/l for As(V) and MMA
	0,2 µg/l for DMA
Limit of quantification	0,16 µg/l for As(III)
	0,33 µg/l for As(V) and MMA
	0,66 µg/l for DMA



Key

X time in minutes, min

Y AFS signal

Figure B.4 — Typical chromatogram representing four arsenic species containing 5 µg/l; As(III), DMA, MMA, and As(V) (order of elution)

Table B.8 — Typical HG-AFS conditions

Same conditions as in Table B.1.

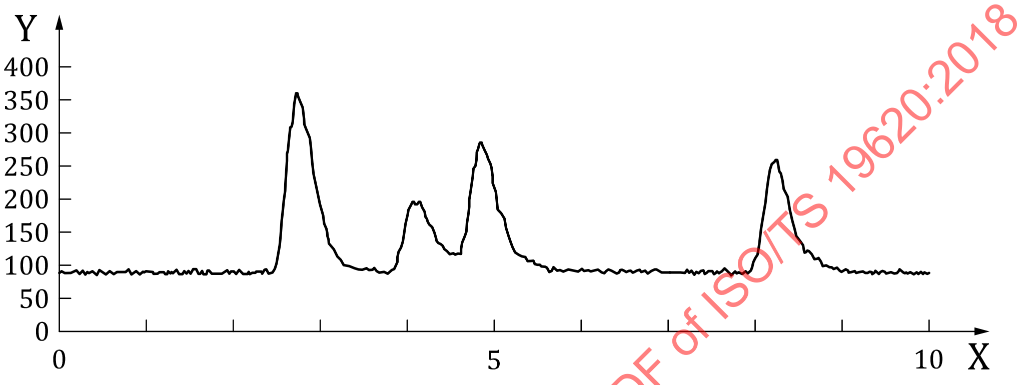
Table B.9 — Chromatographic conditions

Pump	Isocratic®[a]
Flow rate	Isocratic conditions: fixed flow rate at 1 ml/min
Column	Hamilton PRP-X 100®[a] (l = 250 mm, ID = 4,1 mm, particle size 10 µm)
Eluent	20 mM $(NH_4)_2HPO_4$ at pH = 6
Injection loop	50 µl to 250 µl

[a] Isocratic® and Hamilton PRP-X 100® are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

Table B.10 — Calibration conditions

Number of standards	Blank + 5 points
Calibration dynamic range	0,5 µg/l to 50 µg/l
	0,2 µg/l to 2 µg/l
Limit of detection (calculated)	0,05 µg/l for As(III) and MMA
	0,1 µg/l for As(V) and DMA
Limit of quantification	0,16 µg/l for As(III) and MMA
	0,33 µg/l for As(V) and DMA



Key

X time in minutes, min

Y AFS signal

Figure B.5 — Typical chromatogram representing four arsenic species containing 5 µg/l; As(III), DMA, MMA, and As(V) (order of elution)

Table B.11 — Typical HG-AFS conditions

Same conditions as in Table B.1.

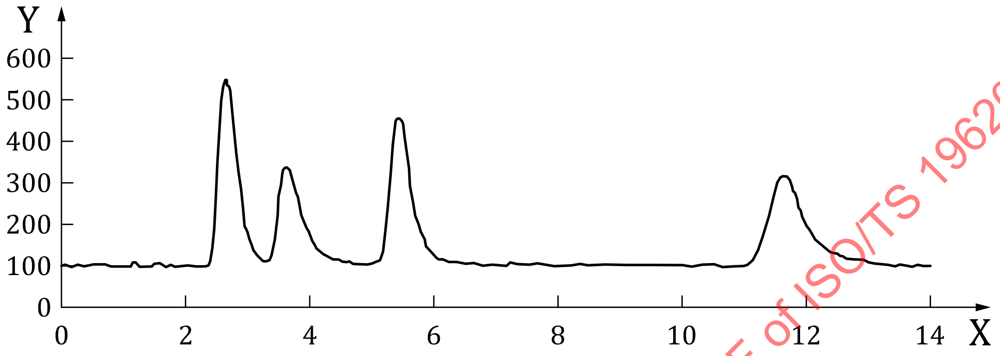
Table B.12 — Chromatographic conditions

Pump	Binary®[a]
Flow rate	Eluent gradient: fixed flow rate at 1 ml/min
Column	Hamilton PRP-X 100®[a] (l = 250 mm, ID = 4,1 mm, particle size 10 µm)
Eluent	A: 10 mM $(NH_4)_2HPO_4/(NH_4)H_2PO_4$ in 3 % methanol at pH = 7
	B: 100 mM $(NH_4)_2HPO_4$ in 3 % methanol at pH = 8,5
Gradient	0 min: 100 % A, 0 % B
	3 min: 0 % A, 100 % B
	10 min: 100 % A, 0 % B
Injection loop	50 µl to 250 µl

[a] Binary® and Hamilton PRP-X100® are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

Table B.13 — Example calibration conditions

Number of standards	Blank + 5 points
Calibration dynamic range	0,5 µg/l to 50 µg/l
	0,2 µg/l to 2 µg/l
Limit of detection (calculated)	0,05 µg/l for As(III), MMA and As(V)
	0,1 µg/l for DMA
Limit of quantification	0,16 µg/l for As(III), MMA and As(V)
	0,33 µg/l for DMA



Key

X time in minutes, min

Y AFS signal

Figure B.6 — Example chromatogram representing four arsenic species containing 5 µg/l; As(III), DMA, MMA, and As(V) (order of elution)

Table B.14 — Typical HG-AFS conditions

Same conditions as in Table B.1.

Table B.15 — Chromatographic conditions

Pump	Isocratic®[a]
Flow rate	Eluent gradient: fixed flow rate at 1 ml/min
Column	Hamilton PRP-X 100®[a] (l = 250 mm, ID = 4,1 mm, particle size 10 µm)
Eluent	20 mM NaH_2PO_4/Na_2HPO_4 at pH = 6,2
Injection loop	50 µl to 250 µl

[a] Isocratic® and Hamilton PRP-X 100® are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

Table B.16 — Calibration conditions

Number of standards	Blank + 5 points
Calibration dynamic range	0,5 µg/l to 50 µg/l
	0,2 µg/l to 2 µg/l
Limit of detection (calculated)	0,05 µg/l for As(III) and MMA
	0,1 µg/l for DMA and As(V)
Limit of quantification	0,16 µg/l for As(III) and MMA
	0,33 µg/l for DMA and As(V)

Annex C
(informative)

Determination of organic arsenic species

C.1 General

This method can be used to determine organic arsenic species such as methylarsonic acid (MMA) and dimethylarsinic acid (DMA). These have lower toxicity than inorganic arsenic species and are normally present in lower concentrations in water samples.

C.2 Standard substances

DMA: Dimethylarsinic acid $(CH_3)_2$ $AsO_2{\cdot}Na\ 3H_2O$ (CAS No. 124-65-2)

MMA: Methylarsonic acid acid $CH_3AsO(ONa)_2{\cdot}6H_2O$ (CAS No. 144-21-8)

C.2.1 Stock solutions

For each of the species, DMA and MMA, prepare a stock solution with a concentration of 1 000 mg/l expressed as As.

These solutions are prepared from the standard substances (C.2).

In 100 ml flasks, dissolve a suitable mass of each substance (C.2) as shown in Table C.1.

Commercially available stock solutions of the required concentration can also be used if available and the species is known.

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

Species	Mass g	Final volume ml
DMA	0,286	100 ml in water (7.3)
MMA	0,390	100 ml in water (7.3)

These solutions, when stored protected from light and at 4 °C, are considered to be stable for one year. The intermediate stock solution at 10 mg/l is considered to be stable for six months.

C.2.2 Calibration solutions

The calibration solutions are prepared from the 1 000 mgl stock solutions (C.2.1). Tables C.2 and C.3 are given as examples.

Table C.2 — Preparation guidelines for working arsenic standards

Concentration µg l^{-1}	Sample volume µl	Solution for dilution	HPLC grade water (7.3)
1 000	100 µl of each solution	1 000 mg/l DMA, MMA (C.2)	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 C.3 is given as an example.

Working standards should be prepared daily.

Table C.3 — Preparation guidelines for working arsenic standards

Concentration µg l^{-1}	Sample volume µl	Solution for dilution	HPLC grade water (7.3)
100	1 000 µl of each solution	10 mg l-1 DMA, MMA (C.2)	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

Annex D
(informative)

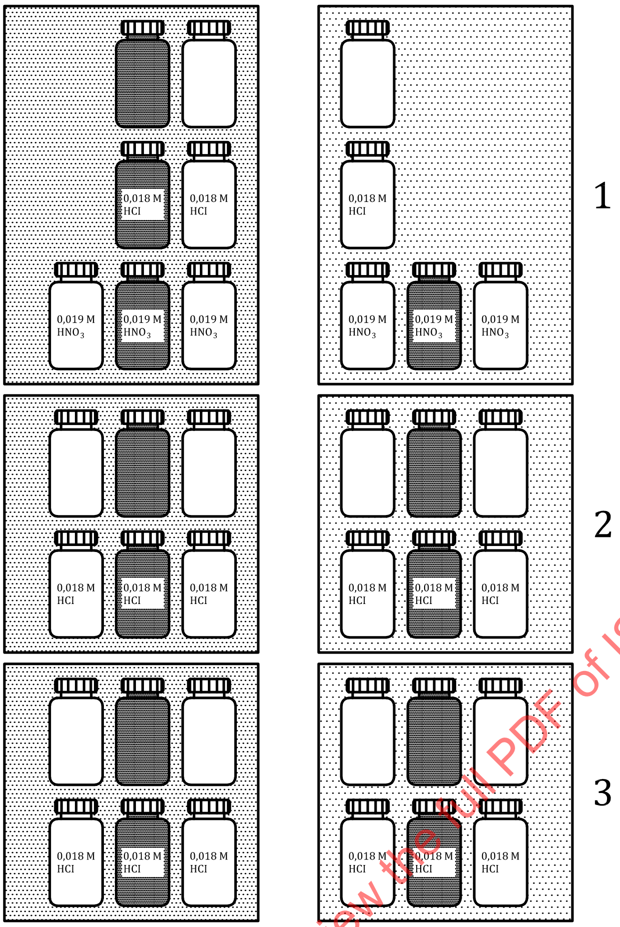
Storage and preservation of arsenic species in water samples

D.1 General

De-ionized water, bottled drinking water and river water were chosen for the arsenic stability test. The samples were spiked with 10 µg/l As(III), DMA, MMA and As(V) and stored in three different plastic bottles: polyethylene bottle, fluorinated polyethylene, and amber polyethylene. One batch of samples was acidified with 750 µl of 6 mol/l HCl per 250 ml samples (final concentration: 0,018 mol/l HCl). This acidification is proposed in US EPA method 1632 (Reference [17]), a chemical speciation method for arsenic in water and tissue. Hall et al. (Reference [9]) proposed the storage of the samples at 5 °C which avoids any oxidation state changes between As(III) and As(V).

A separate batch of de-ionized water samples spiked with a 10 µg/l mix of As(III), DMA, MMA and As(V) was acidified with 750 µl of 6 mol/l HNO_3 to a volume of 250 ml (final concentration of 0,018 mol/l HNO_3).

The samples were stored either in a refrigerator or at room temperature. The average temperature of the refrigerator was 4 °C and the room temperature varied from 18 °C up to 30 °C (variations of temperature in summer). Figure D.1 shows a scheme of the experimental set-up.



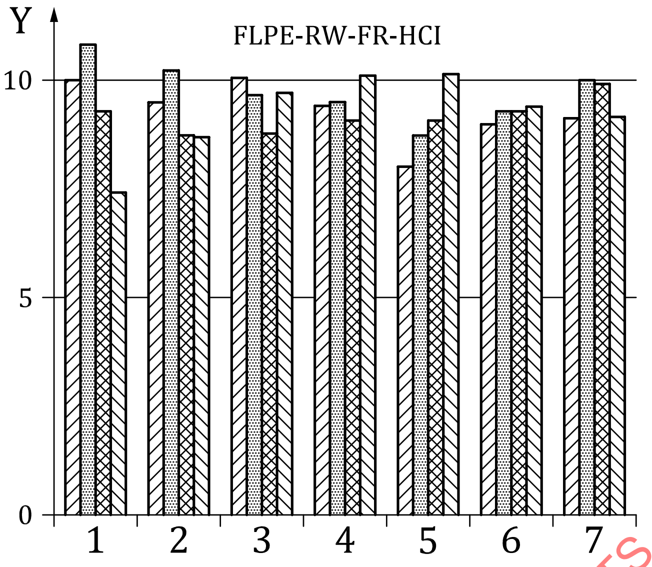
Key

1 de-ionized water

2 bottled drinking water

3 river water

Figure D.1 — Experimental set-up



Key

▨	*c*[As(III)]	1	1 day	5	6 weeks
░	*c*(DMA)	2	1 week	6	8 weeks
▦	*c*(MMA)	3	2 weeks	7	12 weeks
▧	*c*(AsV)	4	3 weeks	Y	ppb

Figure D.2 — Example graph for stability test

The coding of the header consists of four different abbreviations:

— The first code refers to the storage bottle (PE is polyethylene, BPE is amber polyethylene and FLPE is fluorinated polyethylene).

— The second code is the sample matrix (DI is de-ionized water, DW is drinking water and RW is river water).

— The third code is the temperature (FR is refrigerator, RT is room temperature).

— The final code refers to the addition of acid (HCl for hydrochloric acid, HNO_3 for nitric acid).

An example graph is shown in Figure D.2. The sample here is an acidified river water sample (with HCl) stored in fluorinated polyethylene bottle in the refrigerator.

D.2 Results

One batch of samples (de-ionized water, drinking water and river water) was spiked with 10 µg/l As(III), DMA, MMA and As(V), then monitored over a period of 12 weeks via speciation. The DMA and MMA were stable in all the different samples as were As(III) and As(V) in de-ionized water. As(III) and As(V) were found to interchange in the drinking and river water, As(V) is the favoured oxidation state [see Figure D.3 a) to h)].

When the samples were acidified with hydrochloric acid and stored in the refrigerator, all species showed good stability in all of the water matrices. There were slight variations and losses found but overall, the species were relatively stable [see Figure D.4 a) to h)].

A second batch of water samples was spiked with 10 µg/l As(III), DMA, MMA and As(V) and then stored at room temperature. DMA was the only species with good stability throughout the 12 week period in all three sample containers (polyethylene, amber polyethylene, fluorinated polyethylene).

MMA decreased significantly (down to 3,9 µg/l for the de-ionized water sample and down to 6 µg/ l for the other types of water samples) during the same period. This drop only occured in the polyethylene and amber polyethylene bottle but interestingly not in fluorinated polyethylene. As(III) and As(V) interconvert over the test period. The storage bottle, the sample matrix and the temperature seem to have had an influence on the oxidation state of the inorganic arsenic. For example, in a polyethylene bottle the preferred oxidation state was As(V) for the drinking water, while the preferred oxidation state in an amber bottle changed with time [see Figure D.5 a) to g)].

A third batch of samples was stored at room temperature but was additionally stabilized with HCl. All four species showed an improved stability. MMA and DMA were very stable but As(III) and As(V) were found to have lower recoveries of around 70 % to 80 % in some cases. This suggests that inorganic arsenic species may have a tendency to absorb or adsorb during storage on bottle walls [see Figure D.6 a) to g)].

Hydrochloric acid is not a suitable stabilizer when the sample is analysed with LC-ICP-MS. The argon chloride cluster interferes at a mass of 75. When using LC-ICP-MS, stabilization of the samples with nitric acid is preferred. The samples were stabilized in 0,018 mol/l nitric acid and no significant difference between storage in the refrigerator or at room temperature was observed [see Figure D.7 a) to f)].

When the samples were stored at room temperature without acid stabilization, MMA was found to decrease. In order to study this further to monitor for interconversion of species, an additional test was performed using a different batch of de-ionized water spiked with the single species only (10 µg/l) and stored at room temperature. The species showed a good stability over the 12 weeks and no conclusion about adsorption or species interconversion could be drawn [see Figure D.8 a) to d)]