Method 1632

Chemical Speciation of Arsenic in Water and Tissue by Hydride Generation Quartz Furnace Atomic Absorption Spectrometry

Revision A

August, 1998

U.S. Environmental Protection Agency
Office of Water
Engineering and Analysis Division (4303)
401 M Street S.W.
Washington, D.C. 20460

Acknowledgments

Method 1632 was prepared under the direction of William A. Telliard of the U.S. Environmental Protection Agency's (EPA's) Office of Water (OW), Engineering and Analysis Division (EAD). The method was prepared under EPA Contract 68-C3-0337 by the DynCorp Environmental Programs Division with assistance from Quality Works, Inc. and Interface, Inc. The method is based on procedures developed by Eric Crecelius of the Battelle Marine Sciences Laboratory in Sequim, Washington.

Disclaimer

This draft method has been reviewed and approved for publication by the Analytical Methods Staff within the Engineering and Analysis Division of the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use. EPA plans further validation of this draft method. The method may be revised following validation to reflect results of the study.

EPA welcomes suggestions for improvement of this method. Suggestions and questions concerning this method or its application should be addressed to:

W.A. Telliard
Engineering and Analysis Division (4303)
U.S. Environmental Protection Agency
401 M Street SW
Washington, D.C. 20460
Phone: 202/260-7134
Fax: 202/260-7185

Introduction

This analytical method supports water quality monitoring programs authorized under the Clean Water Act (CWA, the "Act"). CWA Section 304(a) requires EPA to publish water quality criteria that reflect the latest scientific knowledge concerning the physical fate (e.g., concentration and dispersal) of pollutants, the effects of pollutants on ecological and human health, and the effect of pollutants on biological community diversity, productivity, and stability.

CWA Section 303 requires each state to set a water quality standard for each body of water within its boundaries. A state water quality standard consists of a designated use or uses of a waterbody or a segment of a waterbody, the water quality criteria that are necessary to protect the designated use or uses, and an antidegradation policy. These water quality standards serve two purposes: (1) they establish the water quality goals for a specific waterbody, and (2) they are the basis for establishing water quality-based treatment controls and strategies beyond the technology-based controls required by CWA Sections 301(b) and 306.

In defining water quality standards, the state may use narrative criteria, numeric criteria, or both. However, the 1987 amendments to CWA required states to adopt numeric criteria for toxic pollutants (designated in Section 307(a) of the Act) based on EPA Section 304(a) criteria or other scientific data, when the discharge or presence of those toxic pollutants could reasonably be expected to interfere with designated uses.

In some cases, these water quality criteria (WQC) are as much as 280 times lower than the MDL achievable using existing EPA methods and required to support technology-based permits. Therefore, EPA developed new sampling and analysis methods to specifically address state needs for measuring toxic metals at water quality criteria levels, when such measurements are necessary to protect designated uses in state water quality standards. The latest criteria published by EPA are those listed in the National Toxics Rule (58 FR 60848) and the Stay of Federal Water Quality Criteria for Metals (60 FR 22228). These rules include WQC for 13 metals, and it is these criteria on which the new sampling and analysis methods are based. Method 1632 was specifically developed to provide reliable measurements of arsenic at EPA WQC levels using hydride generation quartz furnace atomic absorption techniques. It has since been modified to include analysis of arsenic species due to interest expressed during the development of Method 1632, Revision 1.0.

In developing methods for determination of trace metals, EPA found that one of the greatest difficulties was precluding sample contamination during collection, transport, and analysis. The degree of difficulty, however, is highly dependent on the metal and site-specific conditions. This method is designed to preclude contamination in nearly all situations. It also contains procedures necessary to produce reliable results at the lowest ambient water quality criteria published by EPA. In recognition of the variety of situations to which this method may be applied, and in recognition of continuing technological advances, Method 1632 is performance based. Alternative procedures may be used so long as those procedures are demonstrated to yield reliable results.

Requests for additional copies of this publication should be directed to:

U.S. EPA NCEPI
11209 Kenwood Road
Cincinnati, OH 45242
(513) 489-8190

Note: This method is performance based. The laboratory is permitted to omit any step or modify any procedure provided that all performance requirements in this method are met. The laboratory may not omit any quality control analyses. The terms “shall,” “must,” and “may not” define procedures required for producing reliable data at water quality criteria levels. The terms “should” and “may” indicate optional steps that may be modified or omitted if the laboratory can demonstrate that the modified method produces results equivalent or superior to results produced by this method.

Method 1632

Chemical Speciation of Arsenic in Water and Tissue by Hydride Generation Quartz Furnace Atomic Absorption Spectrometry

1.0 Scope and Application

1.1 This method is for determination of total inorganic arsenic (As), arsenite (As^{+3}), arsenate (As^{+5}), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) in filtered and unfiltered water and in tissue by hydride generation and quartz furnace atomic absorption detection. The method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act. The method is based on a contractor-developed method (Reference 16.1) and on peer-reviewed, published procedures for the determination of As in aqueous samples (Reference 16.2).

1.2 This method is accompanied by Method 1669: *Sampling Ambient Water for Determination of Trace Metals at EPA Water Quality Criteria Levels* (the Sampling Method). The Sampling Method is necessary to preclude contamination during the sampling process.

1.3 This method is designed for measurement of dissolved and total As and As species in water (range 0.01-50 μg/L for As and As species) and in tissue (range 0.01-500 μg/g dry weight for As and As species). This method is not intended for determination of As or As species at concentrations normally found in treated and untreated discharges from industrial facilities. Existing regulations (40 CFR Parts 400-500) typically limit concentrations in industrial discharges to the part-per-billion (ppb) range, whereas ambient As concentrations are normally in the low part-per-trillion (ppt) to low part-per-billion range. No regulations currently exist for As species.

1.4 The detection limits and quantitation levels in this method are usually dependent on the level of background elements rather than instrumental limitations. The method detection limits (MDL; 40 CFR 136, Appendix B) for total inorganic As, As^{+3}, and As^{+5} have been determined to be 3 ng/L when no background elements or interferences are present (Table 1). The minimum level (ML) has been established at 10 ng/L. The MDLs for MMA and DMA are about 15 ng/L, and the MLs are about 45 ng/L (Table 1).

1.5 The ease of contaminating water samples with As and interfering substances cannot be overemphasized. This method includes suggestions for improvements in facilities and analytical techniques that should maximize the ability of the laboratory to make reliable trace metals determinations and minimize contamination (Section 4.0). Additional suggestions for improvement of existing facilities may be found in EPA's *Guidance for Establishing Trace Metals Clean Rooms in Existing Facilities*, which is available from the National Center for Environmental Publications and Information (NCEPI) at the address listed in the introduction to this document.

1.6 Clean and ultraclean—The terms "clean" and "ultraclean" have been applied to the techniques needed to reduce or eliminate contamination in trace metals determinations. These terms are not used in this method because they lack an exact definition. However, the information provided in this method is consistent with EPA's summary guidance on clean and ultraclean techniques.

1.7 This method follows the EPA Environmental Methods Management Council's "Format for Method Documentation."

1.8 This method is "performance based." The analyst is permitted to modify the method to overcome interferences or lower the cost of measurements if all performance criteria are met. Section 9.1.2 gives the requirements for establishing method equivalency.

1.9 Any modification of this method, beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures at 40 CFR 136.4 and 136.5.

1.10 Each analyst who uses this method must demonstrate the ability to generate acceptable results (Section 9.2).

1.11 This method is accompanied by a data verification and validation guidance document, *Guidance on the Documentation and Evaluation of Trace Metals Data Collected for CWA Compliance Monitoring*. Before this method is used, data users should state data quality objectives (DQOs) required for a project.

2.0 Summary of Method

2.1 Water samples (100 to 1000 mL) are collected directly into cleaned fluoropolymer, conventional or linear polyethylene, polycarbonate, or polypropylene bottles using sample handling techniques specially designed for collection of metals at trace levels (Reference 16.3). Water samples are preserved in the field by the addition of 3 mL of pretested 6M HCl per liter of sample. The recommended holding time is 28 days.

2.2 Tissue samples (10 to 50 g wet weight) are collected into glass or plastic jars. Tissue samples are either freeze-dried and stored at room temperature or stored frozen at -18°C. Prior to analysis, tissue samples are digested in HCl or NaOH at 80°C for 16 hours. The digestate is analyzed with the same procedure as a water sample. Matrix spike recoveries indicate that As^{+3} is more stable in HCl than NaOH.

2.3 An aliquot of sample is placed in a specially designed reaction vessel, and 6M HCl is added.

2.4 Four percent $NaBH_4$ solution is added to convert inorganic As, MMA, and DMA to volatile arsines.

2.5 Arsines are purged from the sample onto a cooled glass trap packed with 15% OV-3 on Chromasorb® WAW-DMCS0, or equivalent.

2.6 The trapped arsines are thermally desorbed, in order of increasing boiling points, into an inert gas stream that carries them into the quartz furnace of an atomic absorption spectrophotometer for detection. The first arsine to be desorbed is AsH_3, which represents total inorganic As in the sample. The MMA and DMA are desorbed and detected several minutes after the arsine.

2.7 Quality is ensured through calibration and testing of the hydride generation, purging, and detection systems.

2.8 To determine the concentration of As^{+3}, another aliquot of sample is placed in the reaction vessel and Tris-buffer is added. The procedure in Sections 2.4 through 2.7 is repeated to quantify only the arsine produced from As^{+3}.

2.9 The concentration of As^{+5} is the concentration of As^{+3} subtracted from the concentration of inorganic As.

3.0 Definitions

3.1 Apparatus—Throughout this method, the sample containers, sampling devices, instrumentation, and all other materials and devices used in sample collection, sample processing, and sample analysis that come in contact with the sample and therefore require careful cleaning will be referred to collectively as the Apparatus.

3.2 Dissolved Inorganic Arsenic—All $NaBH_4$-reducible As^{+3} and As^{+5} found in aqueous solution filtrate after passing the sample through a 0.45 µM capsule filter.

3.3 Total Arsenic—All inorganic and organic arsenic found in aqueous solution (the sum of As^{+3}, As^{+5}, MMA, and DMA).

3.4 Total Inorganic Arsenic—All $NaBH_4$-reducible As^{+3} and As^{+5} found in aqueous solution. In this method, total inorganic arsenic and total recoverable inorganic arsenic are synonymous.

3.5 Definitions of other terms used in this method are given in the glossary at the end of the method.

4.0 Contamination and Interferences

4.1 Preventing ambient water samples from becoming contaminated during the sampling and analytical processes constitutes one of the greatest difficulties encountered in trace metal determinations. Over the last two decades, marine chemists have come to recognize that much of the historical data on the concentrations of dissolved trace metals in seawater are erroneously high because the concentrations reflect contamination from sampling and analysis rather than ambient levels. Therefore, it is imperative that extreme care be taken to avoid contamination when collecting and analyzing ambient water samples for As and/or As species at trace levels.

4.2 Samples may become contaminated by numerous routes. Potential sources of trace metal contamination during sampling include: metallic or metal-containing labware, containers, sampling equipment, reagents, and reagent water; improperly cleaned and stored equipment, labware, and reagents; and atmospheric inputs such as dirt and dust. Even human contact can be a source of trace metal contamination.

4.3 Contamination Control

4.3.1 Philosophy—The philosophy behind contamination control is to ensure that any object or substance that contacts the sample is arsenic-free and free from any material that may contain As, As species, or material that might interfere with the analysis of samples.

4.3.1.1 The integrity of the results produced must not be compromised by contamination of samples. This method and the Sampling Method give requirements and suggestions for control of sample contamination.

4.3.1.2 Substances in a sample cannot be allowed to contaminate the laboratory work area or instrumentation used for trace metal measurements. This method gives requirements and suggestions for protecting the laboratory.

4.3.1.3 Although contamination control is essential, personnel health and safety remain the highest priority. The Sampling Method and Section 5.0 of this method give requirements and suggestions for personnel safety.

4.3.2 Avoiding contamination—The best way to control contamination is to completely avoid exposure of the sample to contamination in the first place. Avoiding exposure means performing operations in an area known to be free from contamination. Two of the most important factors in avoiding/reducing sample contamination are (1) an awareness of potential sources of contamination and (2) strict attention to the work being done. Therefore, it is imperative that the procedures described in this method be carried out by well-trained, experienced personnel.

4.3.3 Use a clean environment—The ideal environment for processing samples is a class 100 clean room (Section 1.5). If a clean room is not available, all sample preparation should be performed in a class 100 clean bench or a nonmetal glove box fed by arsenic- and particle-free air or nitrogen. Digestions should be performed in a nonmetal fume hood situated, ideally, in the clean room.

4.3.4 Minimize exposure—Any apparatus that will contact samples, blanks, or standard solutions should be opened or exposed only in a clean room, clean bench, or glove box so that exposure to an uncontrolled atmosphere is minimized. When not in use, the apparatus should be covered with clean plastic wrap and stored in the clean bench, in a plastic box, or in a glove box, or bagged in clean zip-type bags. Minimizing the time between cleaning and use will also minimize contamination.

4.3.5 Clean work surfaces—Before a given batch of samples is processed, all work surfaces in the hood, clean bench, or glove box in which the samples will be processed should be cleaned by wiping with a lint-free cloth or wipe soaked with reagent water.

4.3.6 Wear gloves—Sampling personnel must wear clean, nontalc gloves during all operations involving handling of the apparatus, samples, and blanks. Only clean gloves may touch the apparatus. If another object or substance is touched, the glove(s) must be changed before again handling the apparatus. If it is even suspected that gloves have become contaminated, work must be halted, the contaminated gloves removed, and a new pair of clean gloves put on. Wearing multiple layers of clean gloves will allow the old pair to be quickly stripped with minimal disruption to the work activity.

4.3.7 Use metal-free apparatus—All apparatus used for determination of As and/or As species at ambient water quality criteria levels must be nonmetallic and free of material that may contain metals.

4.3.7.1 Construction materials—Only fluoropolymer (FEP, PTFE), conventional or linear polyethylene, polycarbonate, or polypropylene containers should be used for samples that will be analyzed for As. PTFE is less desirable than FEP because the sintered material in PTFE may contain

contaminants and is susceptible to serious memory effects (Reference 16.4). All materials, regardless of construction, that will directly or indirectly contact the sample must be cleaned using the procedures given (Section 6.1.2) and must be known to be clean and arsenic-free before proceeding.

4.3.7.2 Serialization—It is recommended that serial numbers be indelibly marked or etched on each piece of apparatus so that contamination can be traced. Logbooks should be maintained to track the sample from the container through the labware to injection into the instrument. It may be useful to dedicate separate sets of labware to different sample types; e.g., receiving waters and effluents. However, the apparatus used for processing blanks and standards must be mixed with the apparatus used to process samples so that contamination of all labware can be detected.

4.3.7.3 The laboratory or cleaning facility is responsible for cleaning the apparatus used by the sampling team. If there are any indications that the apparatus is not clean when received by the sampling team (e.g., ripped storage bags), an assessment of the likelihood of contamination must be made. Sampling must not proceed if it is possible that the apparatus is contaminated. If the apparatus is contaminated, it must be returned to the laboratory or cleaning facility for proper cleaning before it is used in any sampling activity.

4.3.8 Avoid sources of contamination—Avoid contamination by being aware of potential sources and routes of contamination.

4.3.8.1 Contamination by carryover—Contamination may occur when a sample containing low concentrations of As is processed immediately after a sample containing relatively high concentrations of As. To reduce carryover, the sample introduction system may be rinsed between samples with dilute acid and reagent water. When an unusually concentrated sample is encountered, it should be followed by analysis of a method blank to check for carryover. Samples known or suspected to contain the lowest concentration of As should be analyzed first followed by samples containing higher levels.

4.3.8.2 Contamination by samples—Significant laboratory or instrument contamination may result when untreated effluents, in-process waters, landfill leachates, and other samples containing high concentrations of As are processed and analyzed. This method is not intended for application to these samples, and samples containing high concentrations should not be permitted into the clean room and laboratory dedicated for processing trace metal samples.

4.3.8.3 Contamination by indirect contact—apparatus that does not directly come in contact with the samples may still be a source of contamination. For example, clean tubing placed in a dirty plastic bag may pick up contamination from the bag and subsequently transfer the contamination to the sample. Therefore, it is imperative that every piece of the apparatus

that is directly or indirectly used in the collection, processing, and analysis of ambient water samples be thoroughly cleaned (see Section 6.1.2).

4.3.8.4 Contamination by airborne particulate matter—Less obvious substances capable of contaminating samples include airborne particles. Samples may be contaminated by airborne dust, dirt, particles, or vapors from unfiltered air supplies; nearby corroded or rusted pipes, wires, or other fixtures; or metal-containing paint. Whenever possible, sample processing and analysis should occur as far as possible from sources of airborne contamination.

4.4 Interferences—Water vapor may condense in the transfer line between the cold trap and the atomizer if it is not well heated. Such condensation can interfere with the determination of DMA.

5.0 Safety

5.1 The toxicity or carcinogenicity of each chemical used in this method has not been precisely determined; however, each compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. It is recommended that the laboratory purchase a dilute standard solution of the As and/or As species in this method. If solutions are prepared from pure solids, they shall be prepared in a hood, and a NIOSH/MESA-approved toxic gas respirator shall be worn when high concentrations are handled.

5.2 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a current awareness file of OSHA regulations for the safe handling of the chemicals specified in this method. A reference file of material safety data sheets (MSDSs) should also be made available to all personnel involved in these analyses. It is also suggested that the laboratory perform personal hygiene monitoring of each analyst who uses this method and that the results of this monitoring be made available to the analyst. Additional information on laboratory safety can be found in References 16.5-16.8. The references and bibliography given in Reference 16.8 are particularly comprehensive in dealing with the general subject of laboratory safety.

5.3 Samples suspected to contain high concentrations of As and/or As species are handled using essentially the same techniques used in handling radioactive or infectious materials. Well-ventilated, controlled access laboratories are required. Assistance in evaluating the health hazards of particular laboratory conditions may be obtained from certain consulting laboratories and from State Departments of Health or Labor, many of which have an industrial health service. Each laboratory must develop a strict safety program for handling As and/or As species.

5.3.1 Facility—When samples known or suspected of containing high concentrations (> 50 µg/L) of total As are handled, all operations (including removal of samples from sample containers, weighing, transferring, and mixing) should be performed in a glove box demonstrated to be leaktight or in a fume hood demonstrated to have adequate air flow. Gross losses to the laboratory ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical and animal work presents no inhalation hazards except in an accident.

5.3.2 Protective equipment—Disposable plastic gloves, apron or laboratory coat, safety glasses or mask, and a glove box or fume hood adequate for radioactive work should be used when handling arsenic powders. During analytical operations that may give rise to aerosols or dusts, personnel should wear respirators equipped with activated carbon filters.

5.3.3 Training—Workers must be trained in the proper method of removing contaminated gloves and clothing without contacting the exterior surfaces.

5.3.4 Personal hygiene—Hands and forearms should be washed thoroughly after each manipulation and before breaks (including coffee, lunch, and shift).

5.3.5 Confinement—Isolated work areas posted with signs, with their own segregated glassware and tools, and with plastic absorbent paper on bench tops will aid in confining contamination.

5.3.6 Effluent vapors—The effluent vapors from the AAS should pass through either a column of activated charcoal or a trap designed to remove As and/or As species.

5.3.7 Waste handling—Good waste handling technique includes minimizing contaminated waste. Plastic bag liners should be used in waste cans. Janitors and other personnel must be trained in the safe handling of waste.

5.3.8 Decontamination

5.3.8.1 Decontamination of personnel—Use any mild soap with plenty of scrubbing action.

5.3.8.2 Glassware, tools, and surfaces—Satisfactory cleaning may be accomplished by washing with any detergent and water.

5.3.9 Laundry—Clothing known to be contaminated should be collected in plastic bags. Persons who convey the bags and launder the clothing should be advised of the hazard and trained in proper handling. If the launderer knows of the potential problem, the clothing may be put into a washing machine without contact. The washing machine should be run through a full cycle before being used for other clothing.

6.0 Apparatus and Materials

NOTE: *The mention of trade names or commercial products in this method is for illustrative purposes only and does not constitute endorsement or recommendation for use by the Environmental Protection Agency. Equivalent performance may be achievable using apparatus, materials, or cleaning procedures other than those suggested here. The laboratory is responsible for demonstrating equivalent performance.*

6.1 Sampling Equipment

6.1.1 Sample collection bottles—Fluoropolymer, conventional or linear polyethylene, polycarbonate, or polypropylene, 500-1000 mL.

6.1.2 Cleaning—Sample collection bottles, glass jars, and glass vials are cleaned with liquid detergent and thoroughly rinsed with reagent water. The bottles are then immersed in 1N trace metal grade HCl for at least 48 hours. The bottles are thoroughly rinsed with reagent water, air dried in a class 100 area, and double-bagged in new polyethylene zip-type bags until needed.

NOTE: *Plastic sample bottles should never be cleaned with HNO_3 as it oxidizes chemicals that may remain in the plastic.*

6.1.3 Tissue digestion vials— Glass scintillation vials (25-mL) and fluoropolymer lids are used for the digestion of tissue samples.

6.2 Equipment for Bottle and Glassware Cleaning.

6.2.1 Vats—Up to 200-L capacity, constructed of high-density polyethylene (HDPE) or other nonmetallic, noncontaminating material suitable for holding dilute HCl.

6.2.2 Laboratory sink—In class 100 clean area, with high-flow reagent water for rinsing.

6.2.3 Clean bench—Class 100, for drying rinsed bottles.

6.3 Atomic Absorption Spectrophotometer (AAS)—Any atomic absorption spectrophotometer may serve as a detector. A bracket is required to hold the quartz atomizer in the optical path of the instrument. Table 3 gives typical conditions for the spectrophotometer.

6.3.1 Electrodeless discharge lamp—For measuring As at 193.7 nm.

6.3.2 Quartz cuvette burner tube (Reference 16.2)—70 mm long and 9 mm in diameter with two 6 mm o.d. side tubes, each 25 mm long. Figure 1a shows a schematic diagram of the tube and bracket.

6.4 Reaction vessel—Figure 1b shows the schematic diagram for the vessel used for the reaction of the sample with sodium borohydride. The system consists of the following:

6.4.1 125-mL gas wash bottle—Corning # 1760-125, or equivalent, onto which an 8 mm o.d. sidearm inlet tube 2 cm long has been grafted. A smaller reaction vessel (30-mL size) can be used for up to 5 mL aqueous samples and tissue digestates.

6.4.2 Silicone rubber stopper septum—Ace Glass #9096-32, or equivalent.

6.4.3 Four-way fluoropolymer stopcock valve—Capable of switching the helium from the purge to the analysis mode of operation.

6.4.4 Flow meter/needle valve—Capable of controlling and measuring gas flow rate to the reaction vessel at 150 (±30) mL/min.

6.4.5 Silicone tubing—All glass-to-glass connections are made with silicone rubber sleeves.

6.5 Cryogenic trap—Figure 1c shows the schematic diagram for the trap. It consists of the following:

6.5.1 Nichrome wire (22-gauge).

6.5.2 Variacs for controlling Nichrome wire.

6.5.3 A 6 mm o.d. borosilicate glass U-tube about 30 cm long with a 2 cm radius of bend (or similar dimensions to fit into a tall wide mouth Dewar flask), which has been silanized and packed halfway with 15% OV-3 on Chromasorb® WAW-DMCS (45-60 mesh), or equivalent. The ends of the tube are packed with silanized glass wool.

6.5.3.1 Conditioning the trap—The input side of the trap (the side that is not packed) is connected with silicone rubber tubing to He at a flow rate of 40 mL/min, and the trap is placed in an oven at 175°C for two hours. At the end of this time, two 25 µL aliquots of GC column conditioner (Silyl-8®, Supelco, Inc., or equivalent) are injected through the silicone tubing into the glass trap. The trap is returned to the oven, with the He still flowing, for 24 hours.

6.5.3.2 After conditioning, the trap is wrapped with approximately 1.8 m of 22-gauge Nichrome wire, the ends of which are affixed to crimp-on electrical contacts.

6.5.3.3 The trap is connected by silicone rubber tubing to the output of the reaction vessel. The output side of the trap is connected by 6 mm o.d. borosilicate tubing that has been wrapped by Nichrome wire to the input of the flame atomizer.

6.5.4 Dewar flask—Capable of containing the trap described in Section 6.5.3.

6.6 Recorder/Integrator—Any integrator with a range compatible with the AAS is acceptable.

6.7 Pipettors—All-plastic pneumatic fixed volume and variable pipettors in the range of 10 µL to 5.0 mL.

6.8 Analytical balance—Capable of weighing to the nearest 0.01 g.

7.0 Reagents and Standards

7.1 River/Reagent Water—Water demonstrated to be free from As and/or As species at the MDL as well as potentially interfering substances. Prepared by distillation or collected from the field. It has been observed that deionized water can have an oxidizing potential that diminishes As^{+3} response (References 16.1 and 16.2).

7.2 Hydrochloric Acid—Trace-metal grade, purified, concentrated, reagent-grade HCl.

7.2.1 6M Hydrochloric Acid—Equal volumes of trace metal grade concentrated HCl and river/reagent water (Section 7.1) are combined to give a solution approximately 6M in HCl.

7.2.2 2M Hydrochloric acid—Trace metal grade concentrated HCL (Section 7.2) and river/reagent water (Section 7.1) are combined in a 1:6 ratio to give a solution approximately 2M in HCl.

7.3 Tris Buffer—394 g of Tris-HCl (tris hydroxymethyl aminomethane hydrochloride) and 2.5 g of reagent grade NaOH are dissolved in river/reagent water (Section 7.1) to make 1.0 L of a solution that is 2.5 M tris and 2.475 M HCl. The pH is about 6.2 when diluted 50-fold with river/reagent water.

7.4 Sodium Hydroxide (NaOH)— Reagent grade NaOH.

7.4.1 2M NaOH—Add 80 g of reagent grade NaOH to a 1-L flask. Add about 700 mL of river/reagent water. After the solid dissolves, dilute to 1 L to give a 2M NaOH solution.

7.4.2 0.02M NaOH—Add 10.0 mL of 2M NaOH (Section 7.4.1) to a 1-L flask. Dilute to 1 L to give a 0.02M NaOH solution.

7.5 Sodium Borohydride Solution—Four grams of > 98% $NaBH_4$ (previously analyzed and shown to be free of measurable As) are dissolved in 100 mL of 0.02 M NaOH solution. This solution is stable for only 8-10 hours, and must be made daily.

7.6 Liquid Nitrogen (LN2)—For cooling the cryogenic trap.

7.7 Helium—Grade 4.5 (standard laboratory grade) helium.

7.8 Hydrogen—Grade 4.5 (standard laboratory grade) hydrogen.

7.9 Air—Grade 4.5 (standard laboratory grade) air.

7.10 Quality Control Sample (QCS)—River water and marine water that contain certified concentrations of total As may be purchased. Certified reference materials for As species are not currently available. If the QCS is made in the laboratory, it should be made from a source different than the one used to prepare calibration standards. Spike river/reagent water with the analyte(s) of interest at a concentration one to five times the MDL. Preserve the QCS with sufficient HCl to bring the pH to < 2.

7.11 Precision and Recovery Sample (PAR)—The PAR should be prepared in the same manner as the calibration standards (Section 7.13) by combining method analytes at appropriate concentrations. The PAR must be carried through the same entire preparation scheme as the samples (Sections 9.2.2 and 9.6).

7.12 Ascorbic acid

7.12.1 10% Ascorbic acid—Add 10 g reagent ascorbic acid to about 70 mL of river/reagent water (Section 7.1) and swirl to dissolve. After the powder dissolves, dilute to 100 mL, producing a solution which is stable for one year when stored at 4°C.

7.12.2 0.1% Ascorbic acid—Dilute 100 mL of 10% ascorbic acid solution to 1 L with river/reagent water. This solution should be made as needed.

7.13 Arsenic Standards—It is recommended that laboratories purchase standard solutions of 1000 mg/L and dilute them to make calibration solutions (Section 7.13.5). Sections 7.13.1 through 7.13.4 give directions for making standard solutions if a source is not readily available.

7.13.1 Arsenite (As^{+3}) Standards—A 1000 mg/L stock solution is made up by the dissolution of 1.73 g of reagent grade $NaAsO_2$ in 1.0 L river/reagent water (Section 7.1) containing 0.1% ascorbic acid (Section 7.12.2). This solution is kept refrigerated in an amber bottle. A 1.0 mg/L working stock solution is made by dilution with 0.1% ascorbic acid solution (Section 7.12.2) and refrigerated in an amber bottle. Under these conditions this working stock solution has been found stable for at least one year.

Further dilutions of As^{+3} for analysis, or of samples to be analyzed for As^{+3}, are made in filtered river/reagent water (Section 7.1). It has been observed that deionized water can have an oxidizing potential that causes a diminished As^{+3} response at low levels (1 μg/L and less; Reference 16.9). Dilute As^{+3} standards are prepared daily.

7.13.2 Arsenate (As^{+5}) Standards—To prepare a 1000 mg/L stock solution, 4.16 g of reagent grade Na_2HASO_4 -$7H_2O$ are dissolved in 1.0 L of river/reagent water (Section 7.1). This stock solution has been found to be stable for at least 10 years. Working standards are prepared by serial dilution with river/reagent water (Section 7.1) and prepared monthly. Reagent water is acceptable for As^{+5} standards since this standard is in an oxidized form.

7.13.3 Monomethylarsonate (MMA) Standards—To prepare a stock solution of 1000 mg/L, 3.90 of $CH_3AsO(ONa)_2$ -$6H_2O$ is dissolved in 1.0 L of river/reagent water (Section 7.1). This stock solution has been found to be stable for at least 10 years. Working standards are prepared by serial dilution with river/reagent water (Section 7.1). Dilute standards are prepared weekly.

7.13.4 Dimethylarsinate (DMA) Standards—To prepare a stock solution of 1000 mg/L, 2.86 g of reagent grade $(CH_3)_2AsO_2Na$-$3H_2O$ (cacodylic acid, sodium salt) is dissolved in 1.0 L river/reagent water (Section 7.1). This stock solution has been found to be stable for at least 10 years. Dilute standards are prepared weekly.

7.13.5 Calibration solutions—Using the standard As and As species solutions, (Sections 7.13.1 through 7.13.4), prepare two sets of calibration solutions in river/reagent water. One set of calibration solutions should contain total inorganic As (As^{+3} + As^{+5}), MMA, and DMA. The other set of calibration solutions should contain As^{+3}. Each calibration solution set should consist of three standards at concentration levels that encompass the range of the method. The low concentration for As and each As species must be at the minimum level of the method.

8.0 Sample Collection, Preservation, and Storage

8.1 Sample Collection—Aqueous samples are collected as described in the Sampling Method (Reference 16.3). Tissue samples are collected as described in Reference 16.10.

8.2 Sample Filtration—This step is not required if total As and/or As species are the target analyte(s). For dissolved As and/or As species, samples and field blanks are filtered through a 0.45 µM capsule filter at the field site as described in the Sampling Method. If the dissolved As species are required analytes, the water sample must be field filtered without contact to air. This can be accomplished by using the capsule filter and exercising care during the filtration process. The extra care is necessary because anoxic water may contain high concentrations of soluble iron and manganese that rapidly precipitate when exposed to air. Iron and manganese hydroxy/oxides precipitates remove dissolved As from water. After the sample is filtered, however, the concern is not as great. The samples are preserved through acidification, and when the water is acidified these precipitates will dissolve.

8.3 Water Sample Preservation—Preservation of the samples must be performed in the field to reduce changes in As speciation that may occur during transport and storage. Water samples should be acidified to pH of < 2 with hydrochloric acid (3 mL 6M HCl/L sample) and stored for less than 28 days at 0-4°C. Other preservation techniques for water and a variety of matrices have been explored (References 16.1 and 16.11 through 16.13). If As species are not target analytes, the samples may be preserved upon receipt by the laboratory.

8.3.1 Wearing clean gloves, remove the cap from the sample bottle, add the volume of reagent grade acid that will bring the pH to < 2, and recap the bottle immediately. If the bottle is full, withdraw the necessary volume using a precleaned pipette and then add the acid.

NOTE: *When testing a sample's pH, do not dip pH paper or a pH meter into the sample; remove a small aliquot with a clean pipette and test the pH of the aliquot.*

8.3.2 Store the preserved sample for a minimum of 48 hours at 0-4°C to allow the As adsorbed on the container walls to completely dissolve in the acidified sample.

8.3.3 Sample bottles should be stored in polyethylene bags at 0-4°C until analysis.

8.4 Tissue Sample Preservation—Tissue samples can be frozen in the sampling containers at -18°C or freeze-dried and stored at room temperature. Holding time has not been evaluated but limited data indicates dry samples are stable for several years without detectable change.

9.0 Quality Control/Quality Assurance

9.1 Each laboratory that uses this method is required to operate a formal quality assurance program (Reference 16.3). The minimum requirements of this program consist of an initial demonstration of laboratory capability, analysis of samples spiked with As and/or As species to evaluate and document data quality, and analysis of standards and blanks as tests of continued performance. To determine if the results of analyses meet the performance characteristics of the method, laboratory performance is compared to established performance criteria.

9.1.1 The analyst shall make an initial demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 9.2.

9.1.2 In recognition of advances that are occurring in analytical technology, the analyst is permitted to exercise certain options to eliminate interferences or lower the costs of measurements. These options include alternate digestion, concentration, and cleanup procedures, and changes in instrumentation. Alternate determinative techniques such as the substitution of a colorimetric technique or changes that degrade method performance are not allowed. If an analytical technique other than the techniques specified in this method is used, that technique must have a specificity equal to or better than the specificity of the techniques in the referenced method for the analytes of interest.

9.1.2.1 Each time this method is modified, the analyst is required to repeat the procedures in Section 9.2. If the change will affect the detection limit of the method, the laboratory is required to demonstrate that the MDL (40 CFR Part 136, Appendix B) is lower than the MDL for this method or one-third of the regulatory compliance level, whichever is higher. If the change will affect calibration, the analyst must recalibrate the instrument according to Section 10.0 of this method.

9.1.2.2 The laboratory is required to maintain records of modifications made to this method. These records include the following, at a minimum:

9.1.2.2.1 The names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and of the quality control officer who witnessed and will verify the analyses and modification.

9.1.2.2.2 A listing of metal measured (As and/or As species), by name and CAS Registry number.

9.1.2.2.3 A narrative stating reason(s) for the modification(s).

9.1.2.2.4 Results from all quality control (QC) tests comparing the modified method to this method, including:

(a) Calibration (Section 10.0)
(b) Calibration verification (Section 10.2)
(c) Initial precision and recovery (Section 9.2)
(d) Analysis of blanks (Section 9.5)
(e) Matrix spike/matrix spike duplicate analysis (Section 9.3)
(f) Ongoing precision and recovery (Section 9.6)

9.1.2.2.5 Data that will allow an independent reviewer to validate each determination by tracing the instrument output (peak height, area, or other signal) to the final result. These data are to include, where possible:

(a) Sample numbers and other identifiers
(b) Preparation dates
(c) Analysis dates and times
(d) Analysis sequence/run chronology
(e) Sample volume
(f) Volume before each preparation step
(g) Volume after each preparation step
(h) Final volume before analysis
(i) Dilution data
(j) Instrument and operating conditions (make, model, revision, modifications)
(k) Sample introduction system (ultrasonic nebulizer, hydride generator, flow injection system, etc.)
(l) Operating conditions (ashing temperature, temperature program, flow rates, etc.)
(m) Detector (type, operating conditions, etc.)
(n) Printer tapes and other recordings of raw data
(o) Quantitation reports, data system outputs, and other data to link the raw data to the results reported

9.1.3 Analyses of blanks are required to demonstrate freedom from contamination. Section 9.5 describes the required blank types and the procedures and criteria for analysis of blanks.

9.1.4 The laboratory shall spike at least 10% of the samples with As and/or As species to monitor method performance. Section 9.3 describes this test. When results of these spikes indicate atypical method performance for samples, an alternative extraction or cleanup technique must be used to bring method performance within acceptable limits. If method performance for spikes cannot be brought within the limits given in this method, the result may not be reported for regulatory compliance purposes.

9.1.5 The laboratory shall, on an ongoing basis, demonstrate through calibration verification and through analysis of the ongoing precision and recovery aliquot that the analytical system is within specified limits. Sections 10.2 and 9.6 describe these required procedures.

9.1.6 The laboratory shall maintain records to define the quality of data that are generated. Section 9.3.4 describes the development of accuracy statements.

9.2 Initial Demonstration of Laboratory Capability.

9.2.1 Method detection limit—To establish the ability to detect each analyte (As and/or As species), the analyst must determine the MDL for each analyte per the procedure in 40 CFR 136, Appendix B using the apparatus, reagents, and standards that will be used in the practice of this method. The laboratory must produce an MDL for each analyte that is no more than one-tenth the regulatory compliance level or that is less than the MDL listed in Table 1, whichever is greater.

9.2.2 Initial precision and recovery (IPR)—To establish the ability to generate acceptable precision and recovery, the analyst shall perform the following operations.

9.2.2.1 Analyze four aliquots of river/reagent water (Section 7.1) spiked with the analyte(s) of interest at two to three times the ML (Table 1) according to the procedures in this method. All digestion, extraction, and concentration steps, and the containers, labware, and reagents that will be used with samples must be used in this test.

9.2.2.2 Using results of the set of four analyses, compute the average percent recovery (X) of each analyte in each aliquot and the standard deviation (s) of the recovery of the analyte.

9.2.2.3 Compare s and X for each analyte with the corresponding limits for initial precision and recovery in Table 2. If s and X meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, s exceeds the precision limit or X falls outside the range for accuracy, system performance is unacceptable. The analyst should correct the problem and repeat the test (Section 9.2.2.1).

9.2.3 Quality Control Sample (QCS)—When beginning the use of this method, on a quarterly basis, or as required to meet data quality needs, the calibration standards and acceptable instrument performance must be verified with the preparation and analyses of a QCS (Section 7.10). To verify the calibration standards, the determined mean concentration from three analyses of the QCS must be within ± 10% of the stated QCS value. If the QCS is not within the required limits, an immediate second analysis of the QCS is recommended to confirm unacceptable performance. If the calibration standards and/or acceptable instrument performance cannot be verified, the source of the problem must be identified and corrected before proceeding with further analyses.

9.3 Method Accuracy—To assess the performance of the method on a given sample matrix, the laboratory must perform matrix spike (MS) and matrix spike duplicate (MSD) sample analyses on 10% of the samples from each site being monitored, or at least one MS sample analysis and one MSD sample analysis must be performed for each sample set (samples collected from the same site at the same time, to a maximum of 10 samples), whichever is more frequent.

9.3.1 The concentration of the MS and MSD is determined as follows:

9.3.1.1 If, as in compliance monitoring, the concentration of analyte(s) in the sample is being checked against a regulatory concentration limit, the spike must contain the analyte(s) at that limit or at one to five times the background concentration, whichever is greater.

9.3.1.2 If the concentration(s) is not being checked against a regulatory limit, the concentration(s) must be at one to five times the background concentration or at one to five times the ML(s) in Table 1, whichever is greater.

9.3.2 Assessing spike recovery

9.3.2.1 Determine the background concentration (B) of As and/or As species by analyzing one sample aliquot according to the procedures in Section 11.0.

9.3.2.2 Prepare a matrix spiking concentrate that will produce the appropriate level(Section 9.3.1) of analyte(s) of interest in the sample when the concentrate is added.

9.3.2.3 Spike two additional aliquots with the matrix spiking concentrate and analyze these aliquots to determine the concentration after spiking (A).

9.3.2.4 Calculate each percent recovery (P) as 100(A-B)/T, where T is the known true value of the spike.

9.3.3 Compare the percent recovery (P) with the corresponding QC acceptance criteria in Table 2. If P falls outside the designated range for recovery, the result has failed the acceptance criteria.

9.3.3.1 If the system performance is unacceptable, analyze the ongoing precision and recovery standard (Section 9.6). If the OPR is within acceptance criteria (Table 2), the analytical system is within specified limits and the problem can be attributed to the sample matrix.

9.3.3.2 For samples that exhibit matrix problems, further isolate As and/or As species from the sample matrix using chelation, extraction, concentration, hydride generation, or other means, and repeat the accuracy test (Section 9.3.2).

NOTE: *The use of these techniques to reduce matrix problems may affect the speciation of the As in solution.*

9.3.3.3 If the matrix problems can not be corrected and the recovery for As and/or As species remains outside the acceptance criteria, the analytical result in the unspiked sample is suspect and may not be reported for regulatory compliance purposes.

9.3.4 Recovery for samples should be assessed and records maintained.

9.3.4.1 After the analysis of five samples of a given matrix type (river water, lake water, etc.) for which As and/or As species passes the tests in Section 9.3.3, compute the average percent recovery (R) and the standard deviation of the percent recovery (SR). Express the accuracy assessment as a percent recovery interval from R-2SR to R+2SR for each matrix. For example, if R = 90% and SR = 10% for five analyses of river water, the accuracy interval is expressed as 70-110%.

9.3.4.2 Update the accuracy assessment in each matrix regularly (e.g., after each 5-10 new measurements).

9.4 Precision of Matrix Spike Duplicates

9.4.1 Calculate the relative percent difference (RPD) between the MS and MSD using the concentrations found in the MS and MSD (Equation 1). Do not use the recoveries

calculated in Section 9.3.2.4 for this calculation because the RPD of recoveries is inflated when the background concentration is near the spike concentration.

Equation 1

$$RPD = 100 * \frac{\left(\left|D_1 - D_2\right|\right)}{\frac{1}{2}\left(D_1 - D_2\right)}$$

where:

RPD = Relative percent difference
D_1 = Concentration of the analyte in the MS sample.
D_2 = Concentration of the analyte in the MSD sample.

9.4.2 Compare the RPD with the limits in Table 2. If the criteria are not met, the analytical system performance is judged to be unacceptable. Correct the problem and reanalyze all samples in the sample set associated with the MS/MSD that failed the RPD test.

9.5 Blanks—Blanks are analyzed to demonstrate freedom from contamination.

9.5.1 Method blanks—The method blank is used to determine if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus. It is an aliquot of river/reagent water (Section 7.1) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with samples.

9.5.1.1 Prepare a method blank with each sample batch (samples of the same matrix started through the preparation process on the same 12-hour shift, to a maximum of 10 samples). Analyze the blank immediately after analysis of the OPR (Section 9.6) to demonstrate freedom from contamination.

9.5.1.2 If As and/or As species or any potentially interfering substance is found in the blank at a concentration equal to or greater than the MDL (Table 1), sample analysis must be halted, the source of the contamination determined, the problem corrected, and the sample batch and a fresh method blank reanalyzed.

9.5.1.3 Alternatively, if a sufficient number of blanks (three minimum) are analyzed to characterize the nature of a blank, the average concentration plus two standard deviations must be less than the regulatory compliance level.

9.5.1.4 If the result for a single blank remains above the MDL or if the result for the average concentration plus two standard deviations of three or more blanks exceeds the regulatory compliance level, results for samples associated with those blanks may not be reported for regulatory compliance purposes. Stated another way, results for all initial precision and recovery tests (Section 9.2) and all samples must be associated with

an uncontaminated method blank before these results may be reported for regulatory compliance purposes.

9.5.2 Field blank

9.5.2.1 Analyze the field blank(s) shipped with each set of samples (samples collected from the same site at the same time, to a maximum of 10 samples). If the samples are filtered for the determination of dissolved As and/or As species, the field blank shall be filtered as well. Analyze the blank immediately before analyzing the samples in the batch.

9.5.2.2 If As and/or As species or any potentially interfering substance is found in the field blank at a concentration equal to or greater than the MDL (Table 1), or greater than one-fifth the level in the associated sample, whichever is greater, results for associated samples may be the result of contamination and may not be reported for regulatory compliance purposes.

9.5.2.3 Alternatively, if a sufficient number of field blanks (three minimum) are analyzed to characterize the nature of the field blank, the average concentration plus two standard deviations must be less than the regulatory compliance level or less than one-half the level in the associated sample, whichever is greater.

9.5.2.4 If contamination of the field blanks and associated samples is known or suspected, the laboratory should communicate this to the sampling team so that the source of contamination can be identified and corrective measures taken before the next sampling event.

9.5.3 Equipment blanks—Before any sampling equipment is used at a given site, the laboratory or cleaning facility is required to generate equipment blanks to demonstrate that the sampling equipment is free from contamination. Two types of equipment blanks are required: bottle blanks and sampler check blanks.

9.5.3.1 Bottle blanks—After undergoing appropriate cleaning procedures (Section 6.1.2), bottles should be subjected to conditions of use to verify the effectiveness of the cleaning procedures. A representative set of sample bottles should be filled with river/reagent water (Section 7.1) acidified to pH < 2 and allowed to stand for a minimum of 24 hours. Ideally, the time that the bottles are allowed to stand should be as close as possible to the actual time that sample will be in contact with the bottle. After standing, the water should be analyzed for any signs of contamination. If any bottle shows signs of contamination, the problem must be identified, the cleaning procedures corrected or cleaning solutions changed, and all affected bottles cleaned again.

9.5.3.2 Sampler check blanks—Sampler check blanks are generated in the laboratory or at the equipment cleaning contractor's facility by processing river/reagent water (Section 7.1) through the sampling devices using the same procedures that are used in the field (see Sampling Method). Therefore, the "clean hands/dirty hands" technique used during field sampling should be followed when preparing sampler check blanks at the laboratory or cleaning facility.

9.5.3.2.1 Sampler check blanks are generated by filling a large carboy or other container with river/reagent water (Section 7.1) and processing the river/reagent water (Section 7.1) through the equipment using the same procedures that are used in the field (see Sampling Method). For example, manual grab sampler check blanks are collected by directly submerging a sample bottle into the water, filling the bottle, and capping. Subsurface sampler check blanks are collected by immersing the sampler into the water and pumping water into a sample container. "Clean hands/dirty hands" techniques must be used.

9.5.3.2.2 The sampler check blank must be analyzed using the procedures in this method. If As and/or As species or any potentially interfering substance is detected in the blank, the source of contamination or interference must be identified and the problem corrected. The equipment must be demonstrated to be free from As and/or As species before the equipment may be used in the field.

9.5.3.2.3 Sampler check blanks must be run on *all* equipment that will be used in the field. If, for example, samples are to be collected using both a grab sampling device and a subsurface sampling device, a sampler check blank must be run on both pieces of equipment.

9.6 Ongoing Precision and Recovery

9.6.1 Prepare an OPR (laboratory-fortified method blank) in the same manner as IPR aliquots were prepared (Section 9.2) with each sample batch (samples of the same matrix started through the extraction process on the same 12-hour shift, to a maximum of 10 samples) by spiking an aliquot or river/reagent water (Section 7.1) with the analyte(s) of interest .

9.6.2 Analyze the OPR aliquot before analyzing the method blank and samples from the same batch.

9.6.3 Compute the percent recovery of As and/or As species in the OPR aliquot using the procedure given in Section 9.3.2.

9.6.4 Compare the concentration to the limits for ongoing recovery in Table 2. If the acceptance criteria are met, system performance is acceptable and analysis of blanks and samples may

proceed. If, however, recovery falls outside of the range given, the analytical processes are not being performed properly. Correct the problem, prepare the sample batch again, and repeat the OPR test (Section 9.6).

9.6.5 Add results that pass the specifications in Section 9.6.4 to IPR and previous OPR data for As and/or As species in each matrix. Update QC charts to form a graphic representation of continued laboratory performance. Develop a statement of laboratory accuracy in each matrix type by calculating the average percent recovery (R) and the standard deviation of percent recovery (SR). Express the accuracy as a recovery interval from R-2SR to R+2SR. For example, if R = 95% and SR = 5%, the accuracy is 85-105%.

9.7 The specifications in this method can be met if the instrument used is calibrated properly and then maintained in a calibrated state. A given instrument will provide the most reproducible results if dedicated to the settings and conditions required for the analyses of As and/or As species by this method.

9.8 Depending on specific program requirements, field duplicates may be collected to determine the precision of the sampling technique. The relative percent difference (RPD) between field duplicates should be less than 20%.

10.0 Calibration and Standardization

10.1 Calibration—Calibrate with a minimum of three points, one of which must be the ML (Table 1), and another that must be near the upper end of the linear dynamic range. Calibration is required before any samples or blanks are analyzed.

10.1.1 External standard calibration

10.1.1.1 Calculate the calibration factor (CF) for As and/or As species in each CAL solution using the following equation and the height or area produced.

Equation 2

$$CF = \frac{R_x}{C_x}$$

where,

CF = Calibration factor
R_x = Height or area of the signal for As or As species
C_x = Concentration of As or As species standard injected (ng/L)

10.1.1.2 For each analyte of interest, calculate the mean calibration factor (CF_m), the standard deviation of the CF_m (SD), and the relative standard deviation (RSD) of the mean, where RSD = 100 x SD/CF_m.

10.1.1.3 Linearity—If the RSD of the CF_m is less than 25% over the calibration range, the CF_m may be used to calculate sample concentrations.

10.2 Calibration Verification—Immediately after calibration, an initial calibration verification should be performed. Adjustment of the instrument is performed until verification criteria are met. Blanks and samples may not be analyzed until these criteria are met.

10.2.1 Verify the specificity of the instrument for As and/or As species and adjust the wavelength or tuning until the resolving power specified in this method is met.

10.2.2 Inject the mid-point calibration standard (Section 10.1) or a dilution of the QCS (Section 7.10) with a concentration near the midpoint of the calibration range.

10.2.3 Compute the percent recovery of As and/or As species using the mean calibration factor obtained in Section 10.1.1.2.

10.2.4 Compare the recovery with the corresponding limit for calibration verification in Table 2. If acceptance criteria are met, system performance is acceptable and analysis of blanks and samples may continue using the response from the initial calibration. If acceptance criteria are not met, system performance is unacceptable. Locate and correct the problem and/or prepare a new calibration check standard and repeat the test (Sections 10.2.2 through 10.2.4), or recalibrate the system (Sections 10.1 and 10.2).

10.2.5 Calibration should be verified following every ten samples by analyzing the mid-point calibration standard. If the recovery does not meet the acceptance criteria specified in Table 2, analysis must be halted, the problem corrected, and the instrument recalibrated. All samples after the last acceptable calibration verification must be reanalyzed.

10.3 Analyze a calibration blank following every calibration verification to demonstrate that there is no carryover of the analytes of interest and that the analytical system is free from contamination. If the concentration of an analyte in the blank result exceeds the MDL, correct the problem, verify the calibration (Section 10.2), and repeat the analysis of the calibration blank.

11.0 Procedure

11.1 Set up the AAS system according to manufacturer's instructions. The settings in Tables 3 and 4 can be used as a guide. Calibrate the instrument according to Section 10.0.

NOTE: *Precision and sensitivity are affected by gas flow rates and these must be individually optimized for each system, using the figures in Table 4 as an initial guide.*

11.2 To light the flame, turn on the air and H_2, and expose the end of the quartz cuvette to a flame. At this point, a flame will be burning out the ends of the tube. Allow the tube to heat for approximately five minutes, then place a flat metal spatula over each end of the tube in sequence. An invisible air/hydrogen flame should now be burning in the center of the cuvette. To check for the flame, place a mirror near the end of the tube and observe condensation of water vapor or turn-off the room light to observe the flame.

11.3 Digest tissue samples by adding 10 mL of 2M HCl to 0.5 g of either wet or dry tissue in a 25-mL glass scintillation vial. Cap the vial with a fluoropolymer lid and heat overnight (16 hours) in an

oven at 80°C. Cool and analyze the overlying liquid. Tissue may also be digested in 2M NaOH overnight at 80°C; however, As^{+3} and As^{+5} are more stable in HCl than NaOH. If only total inorganic As, MMA, and DMA are required, then the advantage of the NaOH digestion is that, if it is available, ICP-MS can be used to quantify total As (Reference 16.14).

11.4 Total inorganic As (As^{+3} plus As^{+5}), MMA, and DMA determination.

11.4.1 Purging of Samples

11.4.1.1 Analysis of water samples for detection limits < 0.01 μg/L of As or As species—Place a known volume of aqueous sample (up to 70 mL) into the large (125-mL) reaction vessel. If less than 70 mL of sample is used, add sufficient river/reagent water (Section 7.1) to result in a total volume of 70 mL. Add 5.0 mL of 6M HCl. Set the four-way valve on the reaction vessel to pass the flow of He through the sample and onto the trap and begin purging the vessel with He.

11.4.1.2 Analysis of tissue digestates and of water samples for detection limits between > 0.1 μg/L of As or As species—Place a known volume of aqueous sample (up to 5 mL) or 0.5 mL of tissue digestate into the small (30 mL) reaction vessel. Add 1.0 mL of 6M HCl. Set the four-way valve on the reaction vessel to pass the flow of He through the sample and onto the trap and begin purging the vessel with He.

11.4.1.3 Lower the trap into a Dewar flask containing LN_2 and top the flask off with LN_2 to a constant level.

11.4.1.4 For a large reaction vessel, add 10 mL of $NaBH_4$ solution slowly (over a period of approximately two minutes) through the rubber septum with a disposable hypodermic syringe and begin timing the reaction. For a small reaction vessel, add 2.0 mL of $NaBH_4$ slowly over a 1-minute period. After seven minutes, turn the stopcock on the four-way valve to bypass the reaction vessel and pass helium directly to the trap. Arsines are purged from the sample onto the cooled glass trap packed with 15% OV-3 on Chromasorb® WAW-DMCS0, or equivalent.

11.4.2 Sample Analysis

11.4.2.1 Quickly remove the trap from the LN_2, activate the heating coils to heat the trap and begin recording output from the AAS system. The transfer line is maintained at 80°C. The trapped arsines are thermally desorbed, in order of increasing boiling points, into an inert gas stream that carries them into the quartz furnace of an atomic absorption spectrophotometer for detection. The first arsine to be desorbed is AsH_3, which represents total inorganic As in the sample. The MMA and DMA are desorbed and detected several minutes after the arsine.

11.4.2.2 To ensure that all organic reduction products have been desorbed from the trap, maintain the trap temperature at 80°C and keep He flowing through the trap for at least three minutes between samples.

11.4.3 The trap should be cooled for one minute before re-using for another analysis to reduce the possibility of it cracking in the LN2.

11.5 Arsenite (As^{+3}) Determination

11.5.1 pH Adjustment

11.5.1.1 Analysis of water samples for detection limits < 0.01 µg/L—Place a known volume (up to 70 mL) in the large (125-mL) reaction vessel. If less than 70 mL of sample is used, add sufficient river/reagent water (Section 7.1) to result in a total volume of 70 mL. Add 3.0 mL of Tris buffer to bring the sample's pH to 5 to 7. If the sample is strongly acidic or basic, it must be either neutralized or have more buffer added to obtain a pH of 5 to 7.

11.5.1.2 Analysis of tissue digestates and or water samples for detection limits > 0.01 µg/L—Place a known volume of aqueous sample (up to 5-mL) or 0.5 mL of tissue digestate into a small reaction vessel. Add 1.0 mL of Tris buffer. If the sample is strongly acidic or basic, it must be either neutralized or have more buffer added to obtain a pH of 5 to 7.

11.5.2 Purging of Samples—For a large reaction vessel, add 3.0 mL of $NaBH_4$ solution quickly (about 10 seconds) through the rubber septum with a disposable hypodermic syringe and begin timing the reaction. For a small reaction vessel, add 1.0 mL of $NaBH_4$ in a short injection (about 10 seconds). The injections are quicker in this section than in Section 11.4.1 because rapid evolution of H_2 does not occur at a neutral pH. After seven minutes, turn the stopcock on the four-way valve to bypass the reaction vessel and pass helium directly to the trap. Arsines are purged from the sample onto the cooled glass trap packed with 15% OV-3 on Chromasorb® WAW-DMCS0, or equivalent.

11.5.3 Sample Analysis—Follow the procedure in Sections 11.7 through 11.9 to complete the determination of As^{+3} concentration. During this procedure, small, irreproducible quantities of organic arsines may be released at this pH and should be ignored. This separation of arsenite is reproducible and essentially 100% complete.

11.6 Arsenate (As^{+5}) Determination—The concentration of As^{+5} is calculated by subtracting the As^{+3} determined in Section 11.5 from the total inorganic As determined on an aliquot of the same sample in Section 11.4.

12.0 Data Analysis and Calculations

12.1 Compute the concentration of As and/or As species in ng/L (parts-per-trillion; ppt, Equation 3) using the calibration data (Section 10.1).

Equation 3

$$[\mathrm{As}](\mathrm{ng/L}) = \frac{R_x}{CF_m}$$

where,

R_x = Height or area of signal for As or the As species in the sample
CF_m = Mean calibration factor for As or the As species

12.2 If the concentration exceeds the linear dynamic range of the instrument, dilute the sample by successive factors of 10 until the concentration is within the linear dynamic range.

12.3 Report results at or above the ML for As and/or As species found in samples and determined in standards. Report all results for As and/or As species found in blanks, regardless of level.

12.4 Report results to one significant figure at or below the MDL, two significant figures between the MDL and ML, and three significant figures at or above the ML.

12.5 Do not perform blank subtraction on the sample results.

13.0 Method Performance

13.1 The method detection limit (MDL) listed in Table 1 for As and the quality control acceptance criteria for As listed in Table 2 were validated in two laboratories (Reference 16.15). The MDLs listed for As species are based on data collected during the development of the method. The quality control acceptance criteria for As species will be developed in a single laboratory validation of this method.

14.0 Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Many opportunities for pollution prevention exist in laboratory operation. EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option. The acids used in this method should be reused as practicable by purifying with electrochemical techniques. The only other chemicals used in this method are the neat materials used in preparing standards. These standards are used in extremely small amounts and pose little threat to the environment when managed properly. Standards should be prepared in volumes consistent with laboratory use to minimize the disposal of excess volumes of expired standards.

14.2 For information about pollution prevention that may be applied to laboratories and research institutions, consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's Department of Governmental Relations and Science Policy, 1155 16th Street NW, Washington DC 20036, 202/872–4477.

15.0 Waste Management

15.1 The laboratory is responsible for complying with all federal, state, and local regulations governing waste management, particularly hazardous waste identification rules and land disposal restrictions, and for protecting the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

15.2 Acids and samples at pH < 2 must be either neutralized before being disposed or handled as hazardous waste.

15.3 For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* and *Less is Better: Laboratory Chemical Management for Waste Reduction,* both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington, DC 20036.

16.0 References

16.1 Crecelius, E.A., Bloom, N.S., Cowan, C.E., and Jenne, E.A., *Speciation of Selenium and Arsenic in Natural Waters and Sediments, Volume 2: Arsenic Speciation,*. Final Report, Prepared for Electric Power Research Institute, Palo Alto, CA by Battelle, Pacific Northwest Laboratories, Richland, WA, 1986.

16.2 Andreae, M.O. "Determination of Arsenic Species in Natural Waters," *Anal. Chem.* 1977, *49,* 820.

16.3 Method 1669, "Method for Sampling Ambient Water for Determination of Metals at EPA Ambient Criteria Levels," U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Engineering and Analysis Division (4303), 401 M St SW, Washington, DC 20460 (January 1996).

16.4 Patterson, C.C. and Settle, D.M. "Accuracy in Trace Analysis"; In *National Bureau of Standards Special Publication 422*; LaFleur, P.D., Ed., U.S. Government Printing Office, Washington, DC, 1976.

16.5 "Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Centers for Disease Control, NIOSH, Publication 77-206, August 1977, NTIS PB-277256.

16.6 "OSHA Safety and Health Standards, General Industry," OSHA 2206, 29 CFR 1910.

16.7 "Safety in Academic Chemistry Laboratories," ACS Committee on Chemical Safety, 1979.

16.8 "Standard Methods for the Examination of Water and Wastewater," 18th ed. and later revisions, American Public Health Association, 1015 15th Street NW, Washington DC 20005, 1-35: Section 1090 (Safety), 1992.

16.9 Andrae, M.O., 1983. "Biotransformation of arsenic in the marine environment." In W.H. Lederer and R.J. Fensterheim (Eds.), Arsenic: Industrial, Biomedical, Environmental Perspectives. Van Nostrand-Reinhold, New York, pp. 378-392.

16.10 Lauenstein, G.G. and A.Y. Cantillo (Eds.). July, 1983. Silver Spring, MD. NOAA Technical Memorandum NOS ORCA 71. Sampling and Analytical Methods of the National Status and Trends Program National Benthic Surveillance and Mussel Watch Projects 1984-1992, Volume 1: Overview and Summary of Methods.

16.11 Aggett, J. and Kriegman, M.R. "Preservation of Arsenic(III) and Arsenic(V) in Samples of Sediment Interstitial Water," *Analyst* 1987, 112, 153.

16.12 Wing, R., D. K. Nordstrom, and G.A. Parks. "Treatment of Groundwater Samples to Prevent Loss or Oxidation of Inorganic Arsenic Species."; In *Analytical Characterization of Arsenic in Natural Waters*. R. Wing's Master's Thesis, 1987, Stanford University.

16.13 Crecelius, E. and J. Yager. "Intercomparison of Analytical Methods for Arsenic Speciation in Human Urine." *Environmental Health Perspectives* 1997, 105, 650.

16.14 Method 1640, "Determination of Trace Elements in Water by Preconcentration and Inductively Coupled Plasma-Mass Spectrometry," U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Engineering and Analysis Division (4303), 401 M St SW, Washington, DC 20460 (April, 1997). Draft.

16.15 "Results of the EPA Method 1632 Validation Study," July 1996. Available from the EPA Sample Control Center, 300 N. Lee St., Alexandria, VA 22314; 703/519-1140.

17.0 Glossary

The definitions and purposes below are specific to this method, but have been conformed to common usage as much as possible.

17.1 Ambient Water—Waters in the natural environment (e.g., rivers, lakes, streams, and other receiving waters), as opposed to effluent discharges.

17.2 Calibration Standard (CAL)—A solution prepared from a dilute mixed standard and/or stock solutions and used to calibrate the response of the instrument with respect to analyte concentration.

17.3 Equipment Blank—An aliquot of river/reagent water (Section 7.1) that is subjected in the laboratory to all aspects of sample collection and analysis, including contact with all sampling devices and apparatus. The purpose of the equipment blank is to determine if the sampling devices and apparatus for sample collection have been adequately cleaned before shipment to the field site. An acceptable equipment blank must be achieved before the sampling devices and apparatus are used for sample collection. In addition, equipment blanks should be run on random, representative sets of gloves, storage bags, and plastic wrap for each lot to determine if these materials are free from contamination before use.

17.4 Field Blank—An aliquot of river/reagent water (Section 7.1) that is placed in a sample container in the laboratory, shipped to the field, and treated as a sample in all respects, including contact with

the sampling devices and exposure to sampling site conditions, storage, preservation, and all analytical procedures, which may include filtration. The purpose of the field blank is to determine if the field or sample transporting procedures and environments have contaminated the sample.

17.5 Field Duplicates (FD1 and FD2)—Two separate samples collected in separate sample bottles at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.

17.6 Initial Precision and Recovery (IPR)—Four aliquots of the ongoing precision and recovery standard analyzed to establish the ability to generate acceptable precision and accuracy. IPR tests are performed before a method is used for the first time and any time the method or instrumentation is modified.

17.7 Intercomparison Study—An exercise in which samples are prepared and split by a reference laboratory, then analyzed by one or more testing laboratories and the reference laboratory. The intercomparison, with a reputable laboratory as the reference laboratory, serves as the best test of the precision and accuracy of the analyses at natural environmental levels.

17.8 Matrix Spike (MS) and Matrix Spike Duplicate (MSD)—Aliquots of an environmental sample to which known quantities of the analytes are added in the laboratory. The MS and MSD are analyzed exactly like samples. Their purpose is to quantify the bias and precision caused by the sample matrix. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the MS and MSD corrected for background concentrations.

17.9 May—This action, activity, or procedural step is optional.

17.10 May Not—This action, activity, or procedural step is prohibited.

17.11 Method Blank—An aliquot of river/reagent water (Section 7.1) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with samples. The method blank is used to determine if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

17.12 Minimum Level (ML)—The lowest level at which the entire analytical system must give a recognizable signal and acceptable calibration point for the analyte. It is equivalent to the concentration of the lowest calibration standard, assuming that all method-specified sample weights, volumes, and cleanup procedures have been employed. The ML is calculated by multiplying the MDL by 3.18 and rounding the result to the number nearest to (1, 2, or 5) x 10^n, where n is an integer.

17.13 Must—This action, activity, or procedural step is required.

17.14 Ongoing Precision and Recovery (OPR) Standard—A method blank spiked with known quantities of analytes. The OPR is analyzed exactly like a sample. Its purpose is to assure that the results produced by the laboratory remain within the limits specified in the referenced methods for precision and accuracy.

17.15 Quality Control Sample (QCS)—A sample containing all or a subset of the analytes at known concentrations. The QCS is obtained from a source external to the laboratory or is prepared from a source of standards different from the source of calibration standards. It is used to check laboratory performance with test materials prepared external to the normal preparation process.

17.16 Reagent Water—Water demonstrated to be free of As, As species, and potentially interfering substances at the MDLs for As and/or As species.

17.17 River Water—Ambient freshwater containing arsenic species at concentrations below the MDLs.

17.18 Should—This action, activity, or procedural step is suggested but not required.

17.19 Stock Solution—A solution containing an analyte that is prepared using a reference material traceable to EPA, the National Institute of Science and Technology (NIST), or a source that will attest to the purity and authenticity of the reference material.

Section 18.0 Tables and Figures

TABLE 1. ARSENIC ANALYSIS USING METHOD 1632: LOWEST WATER QUALITY CRITERION, METHOD DETECTION LIMIT, AND MINIMUM LEVEL

Metal	Lowest Ambient Water Quality Criterion [1]	Method Detection Limit (MDL) and Minimum Level (ML)	
		MDL [2]	ML [3]
Arsenic (As)	18 ng/L	3 ng/L	10 ng/L
Arsenite (As^{+3})		3 ng/L	10 ng/L
Arsenate (As^{+5})		3 ng/L	10 ng/L
Monomethylarsonic acid (MMA)		~ 15 ng/L	~ 45 ng/L
Dimethylarsinic acid (DMA)		~ 15 ng/L	~ 45 ng/L

[1] Lowest of the freshwater, marine, and human health ambient water quality criteria promulgated by EPA for nine states and the District of Columbia at 40 CFR Part 131 on May 4, 1995 (60 FR 22229).

[2] Method Detection Limit as determined by the procedure in 40 CFR Part 136, Appendix B.

[3] Minimum Level (ML).

TABLE 2. QUALITY CONTROL ACCEPTANCE CRITERIA FOR PERFORMANCE TESTS IN EPA METHOD 1632[1]

	IPR (Section 9.2)		OPR	Calibration Verification	MS/MSD (Section 9.3)	
Metal	s	X	(Section 9.6)	(Section 10.2)	%R	RPD
As	< 42%	59-143%	55-146%	76-116%	55-146%	< 20%
As^{+3}						
As^{+5}						
MMA						
DMA						

[1]Missing values will be determined during laboratory validation of this method

TABLE 3: TYPICAL SPECTROPHOTOMETER SETTINGS

Parameter	Typical Setting
EDL energy	59
EDL power	8 W
Wavelength	193.7 nm
Slit width	0.7 nm

TABLE 4: TYPICAL FLOWS AND PRESSURES FOR GASES IN THE HYDRIDE GENERATION SYSTEM

Gas	Flow Rate (mL/min)	Pressure (lb/in^2)
He	150	10
H_2	350	20
Air	180	20

Figure 1. Arsenic Speciation Apparatus: (a) Quartz Cuvette Burner Tube, (b) Reaction Vessel, and (c) Schematic Diagram