SOP #BR-0021

BRL Procedure for the Analysis of Water, Sediment, and Tissue by EPA Method 1632, Revision A (1/01): Chemical Speciation of Arsenic in Water and Tissue by Hydride Generation Quartz Furnace Atomic Absorption Spectrometry

Brooks Rand Labs

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BRL Procedure for the Analysis of Water, Sediment, and Tissue by EPA Method 1632, Revision A (1/01): Chemical Speciation of Arsenic in Water and Tissue by Hydride Generation Quartz Furnace Atomic Absorption Spectrometry

1.0 SCOPE AND APPLICATION

1.1 This procedure describes the necessary steps required for determination of the concentrations of arsenic (As) species present in solid or water samples. The specific inorganic species determined are total inorganic As (As(In)), trivalent As (As(III)), and pentavalent As (As(V)) (calculated by difference between total As(In) and As(III)). The organic species determined are monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA).

1.2 This procedure is necessary to provide additional information regarding the type of arsenic species present relative to total arsenic concentration present in a sample.

1.3 The procedure provides a list of the required reagents, standards, and instrumentation used to perform preparation and analysis for the aforementioned arsenic species. Where it is deemed necessary, detailed information is given regarding acquisition of these components.

1.4 The procedures employed by Brooks Rand LLC (BRL) are based on EPA Method 1632 Revision A Draft (EPA, 2001) and a document prepared by Battelle Laboratories (Crecelius et al., 1986) as well as internally developed improvements.

2.0 SUMMARY OF METHOD

2.1 Aqueous samples, preserved with 3 mL 6 M HCl per liter of sample, do not require preparation.

2.2 Tissue samples being analyzed for all As species are prepared by digestion with 2 M hydrochloric acid (HCl) (for As(In) and As(III)) or 2 M sodium hydroxide (NaOH) (for MMA and DMA) and heat, as per EPA Method 1632 Revision A Draft (EPA, 2001).

2.3 Sediment samples being analyzed for total inorganic arsenic content are prepared with 2 M HCl, as per EPA Method 1632 Revision A Draft (EPA, 2001). Sediments being analyzed for As(III) are prepared with 0.1 M phosphoric acid (H_3PO_4) (Crecelius et al, 1986).

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2.4 Refer to Section 2.0 of EPA Method 1632 Revision A Draft (EPA, 2001) for a summary of sample analysis. Specific changes to the analytical procedure adopted by BRL will be detailed in subsequent sections of this document.

3.0 DEFINITIONS

3.1 Refer to Section 3.0 of EPA Method 1632 Revision A Draft (EPA, 2001) for definitions important to the application of this method. Additional terms are defined in the glossary in EPA Method 1632 Section 17.0.

4.0 CONTAMINATION AND INTERFERENCES

4.1 Refer to Section 4.0 of EPA Method 1632 Revision A Draft (EPA, 2001) for requirements and suggestions for maintaining a laboratory that is free from contamination.

4.2 The condensation of water vapor in the transfer line between the sample trap and the atomizer may interfere with arsenic determination, and particularly with DMA recovery. To minimize this effect, BRL places a water condensation trap (of similar shape to sample traps) in an ice bath between the bubbler and sample trap during sample purging.

5.0 SAFETY

5.1 Refer to Section 5.0 of EPA Method 1632 Revision A Draft (EPA, 2001) for information pertaining to maintaining a safe working environment.

6.0 APPARATUS AND MATERIALS

6.1 Refer to Section 6.0 of EPA Method 1632 Revision A Draft (EPA, 2001) for information pertaining to Sampling Equipment (Section 6.1), Equipment for bottle and glassware cleaning (Section 6.2), and pipettors (Section 6.7).

6.2 Routine preventative maintenance for the equipment used in this procedure is described in detail in the standard operating procedure BR-1205 (Preventative Maintenance).

6.3 Detector: A Buck Scientific AAS 205 with an Arsenic hollow cathode lamp. The AAS is fitted with a quartz tube furnace (1.0 cm i.d. and 7.0 cm long) in which an air-hydrogen flame burns.

6.4 Recorder: BRL Guru® integrating software is used to integrate the analytical signal. Refer to the "Brooks Rand, LLC Model III Operation Manual" for Guru® software/integrator operating instructions. Guru® software requires an

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IBM compatible computer (Pentium® II, 400MHz, 128MB RAM minimum) and running MS Windows® 98SE/ME/NT4/NT2000/XP.

6.5 Hydride generation system: The hydride generation system, cryogenic trap precollection and AA detection systems are shown in Figure 1. A 30 mL midget impinger with sparging-tube cap assembly and a silicone bubbler septum injection port serves as a reaction vessel and stripper. A peristaltic pump capable of

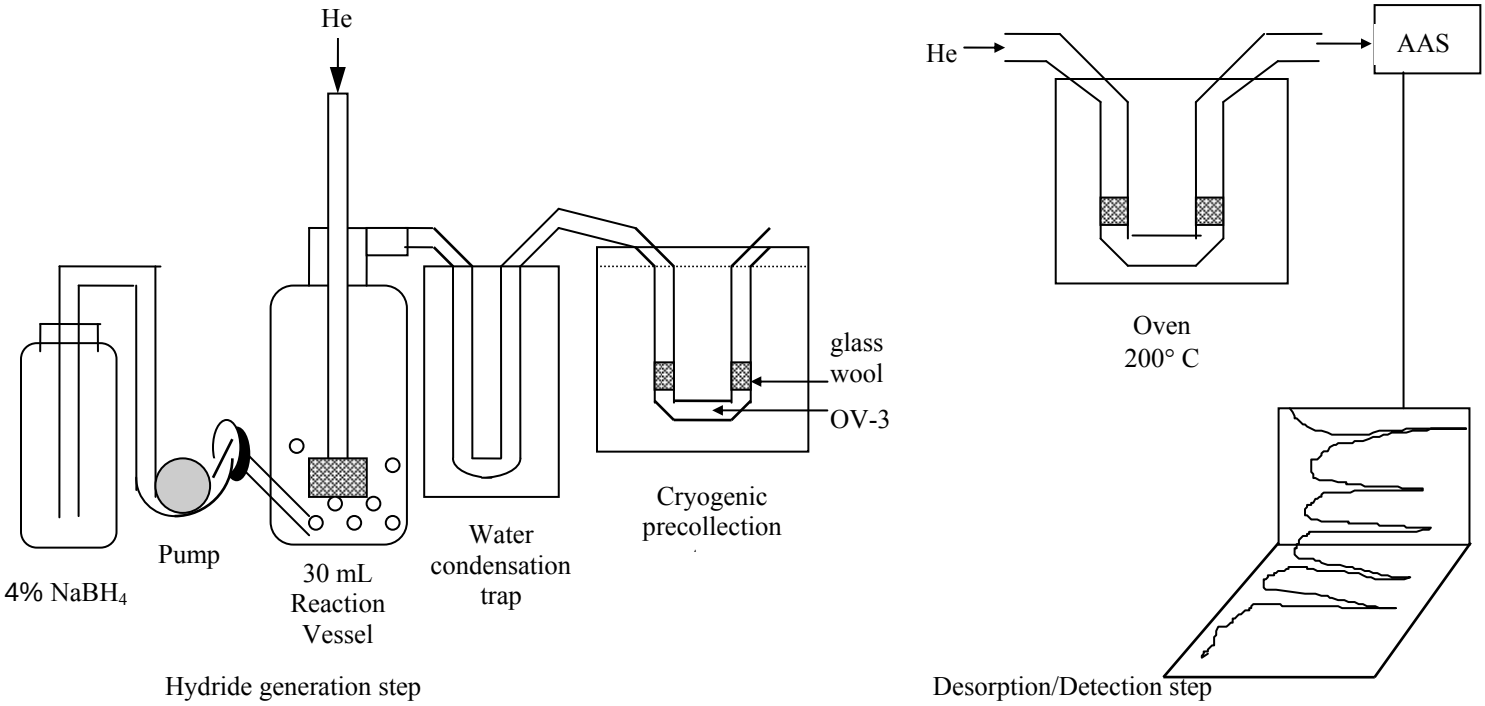

Figure 1. Sample Flow Train

accurately delivering 0.5-5.0 mL/min is used for pumping the $NaBH_4$ solution. A water condensation trap is constructed from a 30 cm length of 6.2 mm o.d x 4.0 mm i.d. borosilicate glass tube bent into a "U" shape. This trap is immersed in an ice water bath during use. A cryogenic trap for the collection of hydrides is constructed from a 30 cm length of 6.2 mm o.d. x 4.0 mm i.d. borosilicate glass tube packed with 5 cm of 15 % OV-3 on Chromasorb W-AW-DMCS, 45-60 mesh, or 5 cm of 3% OV-3 on Chromasorb W-AW-DMCS, 60-80 mesh. The OV-3 column is held in place with silane treated glass wool plugs. If necessary, the collection traps are conditioned by connecting the input side of the trap with silicone rubber tubing to He at a flow rate of 40 mL/min and placing the trap in an oven at 175 ºC for 2 hours. Two 25 µL aliquots of GC column conditioner (Silyl-8®, Supelco, Inc., or equivalent) are injected through the silicone tubing into the

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trap. The trap is reconnected to the helium (He) flow and returned to the oven for 24 hours. A cylindrical oven capable of maintaining a temperature of 100 ºC with a fitted insulated cap is used for rapidly heating the trap to quickly release hydrides. All connections are made with Teflon® connectors and Teflon® tubing.

6.6 Analytical Balance: A standard laboratory balance suitable for sample preparation (0-300 g; ± 0.01 g).

6.7 Centrifuge: Suitable for 50 mL tubes or 40 mL vials and capable of 3000 rpm.

6.8 Sample containers: 50 mL environmental express tubes, rinsed three times with deionized water before using, are used for the preparation of solid samples. 50 mL polypropylene centrifuge tubes with tight fitting lids are also used for the preparation of sediments for As (III) analysis.

6.9 Compressed gases: Hydrogen, Grade 5.0 or Ultra High Purity; Helium, Grade 5.0 or UHP; Air, two-stage compressed air filtration (Balston® 73-099 AA gas purifier); Nitrogen, Grade 4.8 or pre-purified.

6.10 Liquid Nitrogen (LN_2): A 10 L dewar of LN_2 is used to replenish the 1 L flask used to cool the precollection traps.

7.0 REAGENTS AND STANDARDS

7.1 Deionized water (DIW): ASTM Type I DIW is used throughout the sample preparation and analysis procedures.

7.2 HCl: Analytical grade or trace metal grade with a suitably low blank value for the respective analyte. Prepare 2 M HCl (used in sample preparation) by combining concentrated HCl and DIW in a 1:6 ratio. Prepare 6 M HCl (used in analysis) by combining equal volumes of concentrated HCl and DIW.

7.3 NaOH: Baker NaOH pellets are used to prepare a 2 M solution by weighing out 80.0 g and dissolving in DIW to 1 L.

> *Caution: This solution will generate a large amount of heat. Add the pellets slowly and do not cap the container tightly until the solution has cooled to room temperate.*

This solution has been shown to be high in inorganic arsenic. For this reason, this solution can only be used in the analysis for MMA and DMA.

7.4 Hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$): Dissolve 300 g of reagent grade $NH_2OH{\cdot}HCl$ in DIW to 1 L. The solution is purged overnight after the

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addition of 1.0 mL 20% stannous chloride solution with mercury-free N_2 at 400-500 mL·min^{-1} to remove all traces of mercury.

7.5 H_3PO_4: Analytical grade with a suitably low blank value for the respective analyte. Prepare 0.1 M H_3PO_4 by adding 6.8 mL 85% H_3PO_4 to ~900 mL DIW and bringing to a final volume of 1 L.

7.6 Reducing agent: A 4% sodium tetrahydroborate ($NaBH_4$) (w/v) in 0.1% potassium hydroxide (KOH) (w/v) solution is prepared using 98% purity $NaBH_4$. This solution is stable for approximately 8 hours and is replaced daily.

7.7 Standards: Stock solutions of 1000 µg/mL are either purchased or prepared for As(III) in 2% HCl (v/v), High Purity Standards, catalog #10003-6, As(V) in 0.5% sodium hydroxide (NaOH) (w/v), High Purity Standards, catalog #10003-7, Methylarsine dibromide in 0.1M NaOH, Alfa Aesar, catalog #89766, and Dimethylarsinic acid (Cacodylic acid) in 1% HCl (v/v), Sigma, catalog #C0250. Intermediate standards of 10 µg/mL are stable for 30 days. A standard from an independent source for calibration verification is made monthly at 100 ng/mL and preserved with 0.30 mL of 6M HCl.. A working standard of 10 ng/mL (calibration standard) is made daily.

7.8 Tris·HCl buffer: 2.5M TRIS and 2.475M HCl solution prepared from TRIS Hydrochloride, J.T. Baker Ultrapure bioreagent, catalog #4103-01, buffers at pH 6.2 when diluted 50 fold in the reaction vessel.

7.9 10% 1,10-phenanthroline in 50% methanol: Used as needed for minimizing transition metal interference. Methanol is used to dissolve the reagent prior to addition of DIW.

7.10 20% KOH: Used for pH adjustment as needed.

7.11 pH indicator: Methyl Red pH range 5-7.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Refer to Section 8.0 of EPA Method 1632 Revision A Draft (EPA, 2001) for information regarding these topics.

8.2 Pre-preserved bottles are shipped such that collected samples will be preserved at 0.4% 6M HCl. The samples must be stored cold (0-4 ºC) and the pH must be checked upon receipt. If pH>2, then additional 6M HCl must be added to the sample until pH≤2. The original pH of the samples upon receipt must be noted in the sample receiving log.

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8.3 Sediment and biota samples are stored frozen at less than -18 ºC or freeze-dried at room temperature. The holding time for properly stored solid samples is up to 2 years long.

9.0 QUALITY CONTROL / QUALITY ASSURANCE

9.1 Refer to Section 9.0 of EPA Method 1632 Revision A Draft (EPA, 2001) for information pertaining to Initial Demonstration of Capabilities (Section 9.2).

9.2 Quality Assurance Samples:

9.2.1 Refer to Tables 3 and 4 for summaries of QC criteria (frequency and acceptance limits) used at Brooks Rand. Table 5 shows a typical run order for the analysis of an analytical batch. Both tables are located in Section 18.0 of this document.

9.2.2 Standard and Certified Reference Materials: Brooks Rand does not currently have reference materials certified for arsenic speciation. However MESS-3 (marine sediment) show that the arsenic contained in these reference materials is primarily in the form of inorganic arsenic. On this basis, these reference materials are routinely analyzed with all inorganic arsenic analyses. The value for MESS-3 is periodically updated using the 30 most recent data points. Historically, the value determined at Brooks Rand for inorganic arsenic in MESS-3 ranges from **18 mg/kg** to **19 mg/kg** with a typical standard deviation around **2 mg/kg**. The recovery limits based on this value have been set at 65-135%.

Additionally, Brooks Rand has collected historical recovery data for trivalent arsenic in PACS-2 (marine sediment) and routinely prepares and analyzes this reference material with each trivalent arsenic in sediments analysis. Similar to MESS-3 for inorganic arsenic analysis, the trivalent arsenic value for PACS-2 is periodically updated using the 30 most recent data points. Historically, the value determined at Brooks Rand for trivalent arsenic in PACS-2 ranges from **4.5 mg/kg** to **5.5 mg/kg** with a typical standard deviation around **0.9 mg/kg**. The recovery limits based on this value have been set at 50-150%.

9.2.3 Performance Evaluation Samples: As an external check performance evaluation samples are usually analyzed for all conventional analytical parameters at BRL. No performance evaluation samples exist for arsenic speciation.

9.2.4 Method Blanks: A method blank (BLK) is an aliquot of reagent water (DIW) that is treated as a sample in that it undergoes the same analytical process as the corresponding field samples. Method blanks are

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used to monitor laboratory performance, contamination introduced during the analytical procedure, and for determining method detection limits. Three method blanks (BRL usually prepares four) are required per analytical batch.

9.2.5 Method Duplicates (DUP): Method duplicates are not routinely analyzed as part of this method for waters and are only analyzed at the specific request of clients or with samples suspected of being inhomogeneous. For solid samples a DUP is prepared and analyzed with every MS/MSD pair.

9.2.6 Matrix Spikes (MS) and Matrix Spike Duplicates (MSD): Both samples and method blanks can be spiked with a known amount of analyte. Both samples and spiked samples are then analyzed. Bias is then estimated by calculating the percent recovery of the known amount.

$$\% \text{ Recovery} = 100 * \frac{\text{(spiked sample result - sample result)}}{\text{(known amount spiked)}}$$

Spiked samples are used to monitor the analytical method and the effect of the sample matrix on analytical technique. A MS/MSD pair should be analyzed at a frequency of one per ten native client samples.

Liquid spikes are performed for all analysis except for trivalent arsenic in solids. Instead, the solid reference material PACS-2 is typically used to spike solid trivalent arsenic samples.

9.2.7 Preventative Maintenance: Instrument logbooks are maintained for all equipment. These logbooks contain a complete history of past performance and maintenance. When QC criteria are not met from an analysis, all instrumentation is thoroughly checked and appropriate maintenance is taken.

9.2.8 Supplies and Reagents: All supplies (i.e., glassware, chemicals, reagents) are of the best possible quality to ensure proper instrument calibration and to avoid contamination. All reagents used are prepared from Analytical Reagent Grade (AR) chemicals or higher purity grades, unless such purity is not available. Each reagent is clearly labeled with the composition, concentration, date prepared, initials of preparer, expiration date, and special storage requirements, if any. Reagent solutions are stored in appropriate glass or plastic containers, under conditions designed to maintain their integrity (refrigerated, dark, etc.). Shelf life is listed on the label, and the reagent is discarded after it has expired. Water used in the laboratory is deionized and periodically checked for purity by analyzing instrument water blanks by ICP-MS. All acids used are either

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glass-distilled or special grade for trace metal analysis and are tested for purity prior to use. Reagent solutions are checked for contamination by employing reagent blanks before use in any analysis.

9.2.9 Method Detection Limits and Quantitation Limits: The method detection limit (MDL) is the minimum concentration of an analyte of interest that can be measured and reported with 95 percent confidence that the value is above zero. MDLs are determined by following 40 CFR 136, Appendix B.

9.2.10 Method detection limits for the various species and matrices are listed in Table 2 found in Section 18.0 of this document.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Refer to Section 10.0 of EPA Method 1632 Revision A Draft (EPA, 2001) for information regarding calibration.

10.2 Calibration: All standards used to prepare the calibration standard solutions are obtained from chemical suppliers, are of high purity and concentration, and are traceable to National Research Council of Canada (NRCC) or National Institute of Standards and Technology (NIST) standard reference materials. These commercial standards are used as stock standards. Working standards are made from the stock standards at appropriate concentrations to cover the linear range of the instrument as outlined in the individual procedures. The working standards used for initial calibration curves are independent of calibration check standards and spiking solutions. Preparation of all standards is recorded in LIMS.. See Table 2 for a summary of all QA criteria and frequency requirements. All solutions are labeled as follows: name of solution, concentration of solution, date prepared, and analyst's initials.

10.3 Calibration Verification: Calibration verification standards are used to check the standard calibration curve and assess instrument drift. A calibration check consists of a mid-level standard and a calibration blank. Calibration checks are performed immediately after the calibration curve, after every ten samples and at the end of the analytical batch. Calibration check standards are prepared in the laboratory from standards traceable to the NRCC or NIST. The experimental results of the check sample are compared with the true values, and the percent recovery of the check standard is calculated. If the percent recovery falls within the acceptance ranges listed in Table 2, then sample analysis proceeds. However, if the percent recovery does not fall within the acceptance range, the problem is identified and corrected, and the instrument calibration sequence begins again.

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11.0 SAMPLE PREPARATION AND ANALYSIS

11.1 Aqueous samples do not require preparation.

11.2 The following procedure (EPA Method 1632 Revision A Draft, 2001) is used for the extraction of sediment samples for As(In), MMA, and DMA, as well as for extraction of biota samples for As (In) and As(III).

11.2.1 Transfer 500 mg of wet, homogenized sample to a 50 mL environmental express tube rinsed three times with DIW.

11.2.2 Add 10 mL 2 M HCl.

11.2.3 Spike sample preparations with appropriate standard(s). As(In) preparations should be spiked with both As(III) and As(V). If approximate background levels are unknown, spike at a standard level of ~10µg/g.

11.2.4 Digest sample preparations in oven at 75-85 ºC for 16 hours. Preparations are good for a week. Keep refrigerated.

11.3 The following procedure (EPA Method 1632 Revision A Draft, 2001) is used for the extraction of biota samples for MMA and DMA.

11.3.1 Transfer 500 mg of wet, homogenized sample to a 50 mL environmental express tube rinsed three times with DIW.

11.3.2 Add 10 mL 2 M NaOH.

11.3.3 Spike sample preparations with appropriate standard(s). If approximate background levels are unknown, spike at a standard level of ~10µg/g.

11.3.4 Digest sample preparations in oven at 75-85 ºC for 16 hours. No holding time has been established yet but 24 hours later, recoveries come out acceptable.

11.4 The following procedure is used for the extraction of sediment samples for As(III) (Crecelius et al, 1986).

11.4.1 Transfer 500 mg of wet, homogenized sample to a 50 mL centrifuge tube that has been rinsed three times with DIW.

11.4.2 Add 40 mL of 0.5M H_3PO_4 / 0.1 M $NH_2OH{\cdot}HCl$.

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11.4.3 Spike sample preparations as appropriate with the CRM PACS-2.

11.4.4 Cap tubes tightly and placed in the tumbler for an hour.

11.4.5 Centrifuge for 20-30 minutes at 2500 RPM.

11.4.6 Pipette out 2 mL of the supernatant into pre-rinsed centrifuge vials and dilute up to 50 mL with 0.5% HCl.

11.4.7 Refrigerate preparations until analysis, which should be carried out within 48 hours of preparation.

11.5 Hydride Generation-Atomic Absorption Spectrometry:

11.5.1 Turn on the AA instrument Sufficient lamp warm-up is crucial to instrument set-up; generally allow 15 to 30 for the lamp to stabilize. The operation parameters, including lamp power (Refer to Section 18.0). *Note: For the rotometers presently used the gas flow rates listed correspond as follows, H_2/He/Air = 100mm/52mm/155mm.*

11.5.2 Make sure the fume vent is and all gases are on, and light the flame by exposing one or both ends of the quartz cuvette to an electro-spark. A flame should be burning out the ends of the cell. After allowing to heat for five minutes, place a flat metal spatula over each end of the cell in sequence in order to confine the flame to the center of the cuvette. Check for the flame by placing the spatula near each end of the cell and observing water condensate on the spatula.

11.5.3 The sample flow train (see Figure 1) is established with the following components:

4% $NaBH_4$
Peristaltic pump
Reaction vessel with side port and septum
Water trap in an ice bath
1 L Dewar flask with LN_2
Cylindrical oven
Heated transfer line from the collection trap to the atomizer
Helium carrier gas

11.6 As(III) determination: To analyze a water sample, add an aliquot to the reaction vessel with DIW such that the total volume is 20 mL. To analyze biota and sediment preparations, add an aliquot (0.01-2.0 mL) of extract to DIW in the reaction vessel and bring to a total volume of 20 mL. Add 0.025 mL methyl red indicator, swirl the sample, and adjust the pH using 20% KOH such that the pH changes from 4.2 (pink) to 6.2 (yellow). Add 0.50 mL of the Tris·HCl buffer.

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Close the reaction vessel and, in order to prevent loss of sample, connect the side port to the pump tubing that will deliver 4% $NaBH_4$. Next, connect the vessel in-line with the water trap, the hydride collection trap (already immersed in LN_2 for approximately three minutes minimum), and the heated (to 80 ºC.) transfer line. 4% $NaBH_4$ is pumped into the reaction vessel at 1.5 mL/min for 2.5 minutes while purging with He. Disconnect the vessel from the flow train, and connect the carrier gas directly to the collection trap while still immersed in LN_2. Once the signal stabilizes, in quick succession engage the integration program, remove the trap from the LN_2, and place it in the cylindrical oven (set at 100 ºC). Run time for the integration program is one minute. After one minute, remove the trap from the oven, reconnect the gas flow so it bypasses the trap and reaction vessel, and prepare another sample. The water trap and the collection trap are connected separately to N_2 or airflow (approx. 5 psi) while not in use to ensure dryness (by visual inspection) before being used again.

11.7 As(In), MMA, and DMA determinations: Add an aliquot of water or solid sample preparation (0.010 to 2.0 mL) to DIW in the reaction vessel and bring to a final volume of 20 mL. For MMA and DMA biota preparations, add 6 M HCl until the pH in the reaction vessel is less than 1.5 before connecting the reaction vessel. For all other MMA and DMA preparations and all As(In) preparations, add 0.5 mL 6 M HCl and connect the reaction vessel as described previously.

Note: The level of the water trap in the ice bath can have a significant affect on the consistency of the recovery of analyte. This is especially pronounced with the recovery of DMA. It is important to have a method of holding the water trap at a specific level to prevent it from sinking into the ice bath as the ice melts.

If the only analyte of interest is As(In), then purge the sample with He while adding 4% $NaBH_4$ at a rate of 1.5 mL/min for 2 minutes and desorb in the oven (set at 100 ºC) for an integration period of 1 minute. To conduct analysis for As(In), MMA, and DMA, purge the sample with He while adding 4% $NaBH_4$ at a rate of 1.5 mL/min for 2.5 minutes and desorb above a halogen lamp with a surface cool to the touch. The trap should be held parallel to the bench top and be slowly lowered onto the lamp. The trap curve should be as close as possible to the center of the lamp without actually coming in contact with the lamp.. The arsines are eluted according to increasing boiling points with As(In) first, followed by MMA, and, lastly, DMA. To improve separation between MMA and DMA it can be helpful to touch the trap to the lamp just as the MMA peak is finishing. After the elapsed time, remove the trap from the lamp and turn the lamp off to re-cool the surface, reconnect the gas flow so it bypasses the trap and reaction vessel, and prepare another sample. The water trap and the collection trap are separately connected to N_2 or air flow (approx. 5 psi) to ensure dryness before being used again.

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Analytical Notes: As a consequence of sequential peak elution, interference may occur between one or more of the eluted peaks. This effectively limits the volume of sample able to be analyzed and, subsequently, the detection limit of one or more of the desired determinations. Interferences due to transition metals present in the matrix may be lessened if the sample is prepared as above in 1% 1,10-Phenanthroline. This is best achieved using a fraction of the prepared extraction to minimize the amount of this reagent needed. Beware of adding large aliquots of sample until data for all species has been gathered. It is possible to contaminate the reaction vessel with an undetermined species while attempting to obtain a response for the specie to be determined.

Due to the high As(In) contamination present in the NaOH reagent, preparation and analysis of biota samples must be performed separately for As(In) verses MMA and DMA.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Calculations are performed as follows:

12.1.1 $$\text{Coefficient } C_X = \frac{D_X}{(P_X - CB_{avg})}$$

Where D_X is the amount of standard in ng and P_X is the peak area signal of the standard and $x = 1 \rightarrow n$. CB_{avg} is the average peak area signal of the calibration blanks. The Calibration Coefficient (C) is then calculated as the average of $C_1, C_2, C_3, \rightarrow C_n$.

12.1.2 As species ng present in sample aliquot,

$$N = C * (S_1 - CB_{avg})$$

Where S_1 is the peak area signal of the sample measured.

12.1.3 As species concentration of water sample,

$$\mu g / L = N / (V_A * V_D) / V_S$$

Where V_A is the total analyzed volume (mL of DIW plus sample aliquot), V_D is the dilution volume (mL), and V_S is the sample volume (mL).

12.1.4 As species concentration of solid sample,

$$\mu g/g = \{[N / (V_A * V_D)] - BLK_{Tng}\} / M_S$$

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Where BLK_{Tng} is the average total ng of analyte present in the method blanks (See Section 12.1.6 below) and M_S is the sample mass (mg).

12.1.5 For a batch of water samples, calibration blanks serve as method blanks (BLK) and are calculated as:

$$BLK\ \mu g / L = N / 20$$

Where the number 20 represents 20 mL of DIW analyzed. Since the method blanks are equivalent to calibration blanks for water analyses, N is not CB_{avg} when determining the BLK concentration.

12.1.6 For a batch of solid samples, prepared method blanks are analyzed at maximum volume. The total ng of analyte present in the preparation is calculated as follows:

$$BLK_{Tng} = N / (VA * VD)$$

N is CB_{avg} corrected when calculating results for solid method blanks.

$$BLK\ \mu g/g = BLK_{Tng} / M_R$$

Where M_R is a mass of 500 mg or other mass representative of the sample masses prepared.

12.2 BRL routinely calibration blank corrects all data, including calibration standard results, and routinely method bank corrects all solid sample results unless specifically requested not to do so by the client. Since calibration blanks are equivalent to method blanks for water analyses, all water sample results are essentially already method blank corrected.

BRL data spreadsheets are set up to calculate both blank corrected and uncorrected data. For those clients that do not allow blank correction, all of the calculations used to determine results, including the calculation of the calibration coefficient, do not include any subtraction of calibration or method blank data.

13.0 METHOD PERFORMANCE

13.1 Refer to Section 13.0 of EPA Method 1632 Revision A Draft (EPA, 2001) for general method performance.

13.2 Refer to Brooks Rand Control Charts for internal method performance.

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14.0 POLLUTION PREVENTION

14.1 Refer to Section 14.0 of EPA Method 1632 Revision A Draft (EPA, 2001) for pollution prevention

15.0 WASTE MANAGEMENT

15.1 Refer to Section 15.0 of EPA Method 1632 Revision A Draft (EPA, 2001) for waste management

16.0 REFERENCES

16.1 Refer to Section 16.0 of EPA Method 1632 Revision A Draft (EPA, 2001) for additional references.

16.2 Andreae, M.O. "Determination of Arsenic Species in Natural Waters" *Analytical Chemistry*, 49:820-823 (1977).

16.3 Braman R.S. et.al. "Separation and Determination of Nanogram Amounts of Inorganic Arsenic and Methylarsenic Compounds" *Analytical Chemistry*, 49:621-625 (1977).

16.4 Crecelius, E.A. et.al. "Speciation of Selenium and Arsenic in Natural Waters and Sediments, Volume 2: Arsenic Speciation" Electric Power Research Institute (1986).

16.5 EPA. "Inorganic Arsenic in Water by Hydride Generation Quartz Furnace AA", EPA Method 1632 (1996).

16.6 EPA. "Chemical Speciation of Arsenic in Water and Tissue by Hydride Generation Quartz Furnace Atomic Absorption Spectrometry," EPA Method 1632 Revision A (January 2001).

17.0 GLOSSARY

17.1 Refer to Section 18.0 of EPA Method 1632 Revision A Draft (EPA, 2001) for glossary of terms.

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18.0 TABLES

Table 1: AAS Conditions for Determination of Arsenic Species

Lamp Power:	400 mA
Wavelength:	193.7 nm
Slit Width:	2.0 nm
Time Constant (t):	0.5 seconds
Gas Flow Rates:	H_2/He/Air = 100/52/150 mL/min

Table 2: MDL and ML

Analyte	WATER		SOLIDS (Biota & Sediment)	
	MDL	MRL (PQL)	MDL	MRL (PQL)
Inorganic Arsenic	0.008 µg/L	0.025 µg/L	0.003 mg/kg	0.010 mg/kg
Arsenic (III)	0.011 µg/L	0.030 µg/L	0.03 mg/kg (Sediment) 0.003 mg/kg (Biota)	0.10 mg/kg (Sediment) 0.010 mg/kg (Biota)
MMA	0.008 µg/L	0.025 µg/L	0.003 mg/kg	0.010 mg/kg
DMA	0.008 µg/L	0.025 µg/L	0.003 mg/kg	0.010 mg/kg

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Table 3. Quality control criteria for the analysis of arsenic species in aqueous samples.

QC Sample	Measure	Minimum Frequency	Criteria	Corrective Action
Calibration Blanks (method blanks are equivalent to calibration blanks for aqueous samples)	Contamination from bubbler/reagents	Before & after calibration, each CCV, and any unusually high result. Currently ≥ 1.5x the high standard	Avg ≤2x MDL and StDev ≤2/3x MDL or Avg < 1/10th of associated samples*	Clean and test bubblers until criteria met prior to any further analysis. Reanalyze all affected samples
Calibration Standards	Acceptability of the Calibration Curve	Each day prior to analysis and whenever ICV fails	RSD ≤ 20% $R^2 \geq 0.995$ Recovery between 80%-120%	Reanalyze suspect calibration standard. If criteria still not met, then remake standards and recalibrate the instrument
Initial Calibration Verification (ICV)	Independent check of system performance	Immediately following the calibration of the instrument	IA, As^{3+} & MMA Rec = 80–120% DMA Rec = 70–130%	Correct problem prior to continuing analysis, recalibrate if necessary
Continuing Calibration Verification (CCV)	Accuracy	At beginning and end and 1 per 10 sample preparations	IA, As^{3+} & MMA Rec = 80–120% DMA Rec = 70–130%	Halt analysis, correct problem, recalibrate, and reanalyze affected samples
Aqueous Certified Reference Material (CRM)	Accuracy	1 per batch	IA Rec = 75–125%	Correct problem prior to continuing analysis
Matrix Spike/Spike Duplicate (MS/MSD)	Accuracy and Precision within a given matrix	1 per 10 client samples	IA Rec = 65–135% RPD≤35% As^{3+} Rec = 65–135% RPD≤35% MMA Rec = 65–135% RPD≤25% DMA Rec = 60–140% RPD≤35%	If **recoveries** similar but fail recovery criteria, an interference is present in the sample and the result must be qualified. If **RPD** criteria not met, then the system is not in control. Correct problem and reanalyze all associated samples.

* If the client does not allow blank correction, then each calibration blank must be less than the MDL or less than 1/10th the lowest sample result.

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Table 4. Quality control criteria for the analysis of arsenic species in solid samples.

QC Sample	Measure	Minimum Frequency	Criteria	Corrective Action
Calibration Blanks	Contamination from bubblers/reagents	Following calibration, each CV, and any unusually high result. Currently ≥ 2x the high standard	each ≤ MDL or < 1/10th of associated samples (measured PA units)	Clean and test bubblers until criteria met prior to any further analysis. Reanalyze all affected samples
Calibration Standards	Acceptability of the Calibration Curve	Each day prior to analysis and whenever ICV fails	RSD ≤ 20% $R^2 \geq 0.995$ Recovery between 80%-120%	Reanalyze suspect calibration standard. If criteria still not met, then remake standards and recalibrate the instrument
Initial Calibration Verification (ICV)	Independent check of system performance	Immediately following the calibration of the instrument	IA, As^{3+} & MMA Rec = 80–120% DMA Rec = 70–130%	Correct problem prior to continuing analysis, recalibrate if necessary
Continuing Calibration Verification (CCV)	Accuracy	At beginning and end and 1 per 10 sample preparations	IA, As^{3+} & MMA Rec = 80–120% DMA Rec = 70–130%	Halt analysis, correct problem, recalibrate, and reanalyze affected samples
Method Blank (BLK)	Contamination from reagents, lab ware, etc.	3 per batch	Avg ≤2x MDL and StDev ≤2/3x MDL or Avg < 1/10th of associated samples*	Halt analysis, correct problem. Reanalyze affected samples. Qualify data as necessary.
Solid Certified Reference Material MESS-3 (IA) PACS-2 (As^{3+})	Accuracy	1 per batch	IA Rec = 65–135% As^{3+} Rec = 50–150%	Correct problem prior to continuing analysis
Laboratory Fortified Blank (BS) equiv. to an OPR as defined by EPA 1632	Accuracy	1 per batch	IA Rec = 65–135% As^{3+} Rec = 50–150% MMA Rec = 60–140% DMA Rec = 60–140%	Correct problem prior to continuing analysis
Matrix Spike/Spike Duplicate	Accuracy and Precision within a given matrix	1 per 10 client samples	IA Rec = 65–135% RPD≤35% As^{3+} Rec = 50–150% RPD≤35% MMA Rec = 60–140% RPD≤25% DMA Rec = 60–140% RPD≤35%	If **recoveries** similar but fail recovery criteria, an interference is present in the sample and the result must be qualified. If **RPD** criteria not met, then the system is not in control. Correct problem and reanalyze all associated samples.
Post Preparation Spikes (PS)	A spike prepared at the instrument directly onto an aliquot of the prepared sample.	If no appropriate matrix spike	Equivalent to the MS recovery criterion in waters for each As species	Attempt to overcome any matrix effects and reanalyze the sample. Qualify data as necessary.

* If the client does not allow blank correction, then each method blank must be less than the MDL or less than 1/10th the lowest sample result.

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Table 5. Typical run sequence for the analysis of samples.

Run	Run Name	Section Name	Analyze	Requirements
01 02 03	Calib. Blank Calib. Blank Calib. Blank	Calibration Blanks	CB CB CB	
04 05 06 07	0.5 ng std 2.0 ng std 5.0 ng std 30 ng std	Calibration	0.5 ng std 2.0 ng std 5.0 ng std 30 ng std	RSD≤20% $R^2 \geq 0.995$ Recovery between 80%-120%
08 09 09	Calibration Check Verification Standard Independent Source Standard Calib. Blank Check	Calibration Verification	5.0 ng std (CCV) 5.0 ng std (ICV) CB	Vary by species
10 11 12	Method Blank #1 Method Blank #2 Method Blank #3	Method Blanks	BLK BLK BLK	Depends on whether blank corrected data or no blank correction
13 14	CRM-1 CRM-2	Certified Reference Materials	CRM (if appropriate)	Vary by species
15 16 17	Sample 01 Sample 01-MS Sample 01-MSD	Sample Analysis Matrix Spike/Matrix Spike Duplicate Analysis	Sample 01 Spike of 01 Spike Dup. of 01	Vary by species
18 19 20	Sample 02 Sample 02-MS Sample 02-MSD	Sample Analysis Matrix Spike/Matrix Spike Duplicate Analysis	Sample 02 Spike of 02 Spike Dup. of 02	Vary by species
21 22	Calib. Check Sample Calib. Blank Check	Calibration Verification	5.0 ng std (CCV) CB	Vary by species
23 24 25 26 27 28 29 30	Sample 03 Sample 04 Sample 05 Sample 06 Sample 07 Sample 08 Sample 09 Sample 10	Sample Analysis	Sample 03 Sample 04 Sample 05 Sample 06 Sample 07 Sample 08 Sample 09 Sample 10	
31 32	Calib. Check Sample Calib. Blank Check	Calibration Verification	5.0 ng std (CCV) CB	Vary by species
33 34 35 36 37 38 39 40 41 42	Sample 11 Sample 12 Sample 13 Sample 14 Sample 15 Sample 16 Sample 17 Sample 18 Sample 19 Sample 20	Sample Analysis	Sample 11 Sample 12 Sample 13 Sample 14 Sample 15 Sample 16 Sample 17 Sample 18 Sample 19 Sample 20	
43 44	Calib. Check Sample Calib. Blank Check	Calibration Verification	5.0 ng std (CCV) CB	Vary by species

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EXAMPLE LOG

Total As / Total Se / As Species _____
Methods BR-0020 (HGAAS) & BR-0021 Mod. of EPA 1632 (HGAAS)

Matrix: Water / Sediment / Biota / Other _____

Batch #: _____ Prep Date/Time: _____

Prepared By: _____

Project #(s): _____ Page _____ of _____

Flask #	Sample I.D.	Sample Wt. / Vol. (mg) / (mL)	Flask #	Sample I.D.	Sample Wt. / Vol. (mg) / (mL)

Matrix Spike/Matrix Spike Duplicate

Sample I.D.	Spike I.D.	Spike std. Conc. ng/mL / µg/mL	Spike Vol. (mL)	Spike Conc. (µg/g) / (µg/L)

.

Spike Witnessed by:

_____

Initials / Date

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EXAMPLE LOG

Total As / Total Se / As Species _____
Methods BR-0020 (HGAAS) & BR-0021 Mod. of EPA 1632 (HGAAS)

Matrix: Water / Sediment / Biota / Other _____

Batch #: _____ Prep Date/Time: _____

Prepared By: _____ Page _____ of _____

Ongoing Precision and Recovery Sample (OPR) or Certified reference Material (CRM)

OPR / CRM I.D.	Certified Conc. (µg/g) / (µg/L)	Source I.D. (for OPR)

REAGENTS: *Volume & ID #*

HNO_3: _____

H_2SO_4: _____

HCl: _____

$H_3PO_4/NH_2OH{\bullet}HCl$: _____

DIGESTION:

Target Temperature	Measured Temperature	Time

DILUTION INFORMATION:

Final Volume of Preparation: _____

Volume of Prep Subsampled: _____

Dilution Media: _____

Final Dilution Volume: _____

ADDITIONAL COMMENTS: _____

_____

_____

_____

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EXAMPLE BENCH SHEET

As(In) □ As(III) □ MMA □ DMA □ Analysis Sheet

Page 1 of____

Sequence:_____ Matrix:_____

Analyst:_____ Date:_____ 30% NH_2OHHCl:_____

Standards: Cal Std 10 ng /mL:_____ ICV Std:_____ 4% $NaBH_4$:_____

4% $NaBH_4$ add. & purge time (m:s):_____ HCl: _____

Run #	Sample ID	Analyzed volume (mL)	Dilution Factor	Analysis comments / For spiked QC: Source sample, standard ID, and spiked amount

Comments:_____

_____

Method SRM:_____

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As(In) ☐ As(III) ☐ MMA ☐ DMA ☐ Analysis Sheet

Page 1 of____

Batch:____________________ Matrix:____________________________

Analyst:__________________ Date:_____________________________

Run #	Sample ID	Analyzed Volume (mL)	Dilution Factor	Analysis comments / For spiked QC: Source sample, standard ID, and spiked amount

Comments:____

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Appendix to Brooks Rand Procedure for EPA Draft Method 1632, Revision A (01/01)

Differences between EPA Draft Method 1632, Revision A, and Brooks Rand SOP #BR-0021

The following differences exist between EPA Draft Method 1632a (with relevant sections marked in boldface), and the practices employed by Brooks Rand (SOP #BR-0021):

- EPA Draft Method 1632, Revision A, (hitherto referred to as simply 1632a) states that grade 4.5 (standard grade) helium gas is adequate for use in the analysis of samples (**Section 7.7**). Brooks Rand uses grade 4.8 (high purity) helium gas for all arsenic analysis.

- 1632a states that grade 4.5 (standard grade) hydrogen gas is adequate for use in the analysis of samples (**Section 7.8**). Brooks Rand uses grade 4.8 (high purity) hydrogen gas for all arsenic analysis.

- 1632a calls for samples to be preserved in the field with sufficient 6M HCl to bring the pH to < 2 (**Section 8.3**). Since most of Brooks Rand's clients do not have adequate training for acidifying samples in the field, Brooks Rand routinely sends bottles prepreserved with acid such that samples will contain 0.4% 6M HCl. This concentration corresponds to the concentration suggested in the method. The pH of each sample is checked upon receipt. Any sample with a pH > 2 is qualified, additional acid is added, and the client is contacted.

- The method blank criteria for 1632a requires that the result from a single method blank must be less than the MDL or if a minimum of three method blanks are analyzed, then the average result plus two standard deviations must be less than the regulatory compliance level (**Section 9.6.2.3**). Brooks Rand routinely performs method blank correction of its results and, therefore, finds that reproducibility in the method blanks is more relevant than the absolute value of the method blanks. Brooks Rand runs a minimum of three method blanks with every batch. The average of the method blanks must be ≤ two times the MDL with a standard deviation ≤ $2/3^{rd}$ the MDL or the highest method blank must be < $1/10^{th}$ the sample results. Brooks Rand defers to the 1632a criteria for those clients that absolutely do not allow for method blank correction.

- 1632a does not call for calibration blank correction of the calibration coefficients (**Section 10.1.3**). Brooks Rand uses the average result of the first three calibration blanks analyzed at the beginning of the batch to correct the calibration coefficients unless the client absolutely does not allow for calibration blank correction.

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- **Sections 11.4.1.1 and 11.5.1.1** in 1632a calls for analyzing up to 70 mL of sample in a 125 mL reaction vessel (bubbler). Brooks Rand utilizes smaller bubblers and analyzes a maximum of 20 mL of sample.

- For inorganic arsenic, MMA, and DMA analyses, 1632a states that 5.0 mL of 6M HCl should be added to water samples and 1.0 mL of 6M HCl should be added to biota samples in the bubbler (**Sections 11.4.1.1 and 11.4.1.2**). Since Brooks Rand analyzes similar, smaller volumes for all matrices (aqueous, biota, and sediment), Brooks Rand adds 0.5 mL of 6M HCl to all samples in the bubbler. Note that the volume of 6 M HCl added to MMA and DMA biota preparations is not specified beyond enough to bring the pH below 1.5.

- For inorganic arsenic, MMA, and DMA analyses, 1632a states that 10 mL of $NaBH_4$ is manually added slowly to the bubbler. Brooks Rand pumps $NaBH_4$ into the bubbler at a rate of 1.5 mL/minute to compensate for the smaller sample volume used in the analysis.

- 1632a states that traps should be heated to a temperature of 75-85º C for several minutes (**Section 11.4.2.1**); whereas, Brooks Rand has found that heating the trap at 100º C for one minute is sufficient.

- For pH adjustment of trivalent arsenic samples, 1632a calls for the bubbler addition of 3.0 mL of Tris Buffer for aqueous samples and 1.0 mL of Tris Buffer for biota samples (**Section 11.5.1**). Due to the smaller bubbler size used, Brooks Rand has determined that the addition of 0.5 mL of Tris Buffer to the bubbler is sufficient to buffer the sample preparation regardless of matrix type.

- 1632a allows for method blank correction of results if required or requested (**Section 12.1**); whereas, Brooks Rand routinely method blank corrects all sample results unless specifically requested not to do so.

- 1632a does not include calibration blank correction of samples results in its calculations section (**Section 12.1**). Brooks Rand routinely subtracts the average measured peak area of the first 3 calibration blanks from the measured peak area of each sample unless the client absolutely does not allow for calibration blank correction.

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