Laboratory Procedure Manual

Analyte: arsenobetaine, arsenocholine, trimethylarsine oxide, monomethylarsonic acid, dimethylarsinic acid, arsenous (III) acid, arsenic (V) acid

Matrix: Urine

Method: Urine arsenic speciation HPLCICPDRCMS
(Renamed from High Performance Liquid Chromatography Inductively Coupled Plasma Dynamic Reaction Cell Mass Spectrometry (HPLC-ICP-DRC-MS))

Method No: **DLS-3000.15-03**

Adopted:

Revised: **October 15, 2018**

As performed by: Inorganic and Radiation Analytical Toxicology Branch
Division of Laboratory Sciences
National Center for Environmental Health

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Important Information for Users

CDC periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

This document details the Lab Protocol for testing the items listed in the following table.

This method file describes measurements of UAS_J.

File Name	Variable Name	SAS Label (and SI units)
UAS_J	URXUAS3	Urinary Arsenous Acid (µg/L)
	URXUAS5	Urinary Arsenic Acid (µg/L)
	URXUAB	Urinary Arsenobetaine (µg/L)
	URXUAC	Urinary Arsenocholine (µg/L)
	URXUDMA	Urinary Dimethylarsinic Acid (µg/L)
	URXUMMA	Urinary Monomethylarsonic Acid (µg/L)

1. CLINICAL RELEVANCE AND TEST PRINCIPLE

a. Clinical Relevance

People encounter arsenic in many chemical forms that vary in toxicity. The most toxic of the naturally-occurring arsenic compounds are inorganic forms of arsenic and their monomethylated metabolites (1). Less toxic are the organic arsenic compounds (2-5). Exposure to inorganic arsenic can result in a variety of adverse health effects, such as skin disorders, nerve impairment, cancer of the liver, bladder, kidneys, prostate, and lungs, and even death from large doses (6, 7). People may be exposed to inorganic arsenic through activities such as drinking water contaminated from geological sources (8-14) or occupational exposure (15-19), especially breathing air contaminated with sawdust or smoke from wood treated with chromated copper arsenic preservatives (20-25). Organic arsenic compounds are generally less toxic and may be encountered by ingesting various types of fish, shellfish, or seaweed (26-31).

The method described in this manual assesses arsenic exposure, as defined by exposure to individual arsenic species by analyzing urine through the use of high performance liquid chromatography (HPLC) coupled to inductively-coupled plasma dynamic reaction cell-mass spectrometry (ICP-DRC-MS). Urine is analyzed because urinary excretion is the major pathway for eliminating arsenic from the mammalian body (32-34). This hyphenated method will provide accurate quantification of seven urinary arsenic species: inorganic species - arsenite (valence III) and arsenate (valence V), their metabolites - monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), and organic forms of arsenic which include trimethylarsine oxide (TMAO), arsenocholine (AC), and arsenobetaine (AB) (see TABLE 1-1).

b. Test Principle

The concentrations of arsenate [As(V)], arsenite [As(III)], MMA, DMA, TMAO, AC, and AB are determined by using a high performance liquid chromatography (HPLC) system to separate the species coupled to an ICP-DRC-MS to detect the arsenic species. This analytical technique is based on separation by anion-exchange chromatography (IC) followed by detection using quadrupole ICP-MS technology and includes DRC™ technology (35) which minimizes or eliminates many argon-based polyatomic interferences (36). Column separation is largely achieved due to differences in charge-charge interactions of each negatively-charged arsenic component in the mobile phase with the positively-charged quaternary ammonium groups bound at the column's solid-liquid interface. Upon exit from the column, the chromatographic eluent goes through a nebulizer where it is converted into an aerosol upon entering the spray chamber. Carried by a stream of argon gas, a portion of the aerosol is transported through the spray chamber and then through the central channel of the plasma, where it is heated to temperatures of 6000-8000°K. This thermal energy atomizes and ionizes the sample. The ions and the argon enter the mass spectrometer through an interface that separates the ICP, which is operating at atmospheric pressure (approximately 760 torr), from the mass spectrometer, which is operating at approximately 10^{-5} torr. The mass spectrometer permits detection of ions at each mass-to-charge ratio in rapid sequence, which allows the determination of individual isotopes of an element. Once inside the mass spectrometer, the ions pass through the ion optics, then through the DRC™ and finally through the mass-analyzing quadrupole before being detected as they strike the surface of the detector. The ion optics uses an electrical field to focus the ion beam into the DRC™. The DRC™ component is pressurized with an appropriate reaction gas and contains a quadrupole. In the DRC™, elimination or reduction of argon-based polyatomic interferences takes place through the interaction of the reaction gas with the interfering polyatomic species in

the incoming ion beam. The quadrupole in the DRC™ allows elimination of unwanted reaction by-products that would otherwise react to form new interferences.

TABLE 1-1: SPECIES OF ARSENIC

Name	Abbreviation	Structural Formula	pK_a
Arsenobetaine	AB	H_3C—$As^+(CH_3)_2$—CH_2COOH	-
Arsenocholine	AC	H_3C—$As^+(CH_3)_2$—CH_2CH_2OH	-
Monomethyl arsonic acid	MMA	H_3C—$As^{(V)}(=O)(OH)(OH)$	4.1, 8.7
Dimethylarsinic acid	DMA	H_3C—$As^{(V)}(=O)(CH_3)(OH)$	6.2
Trimethylarsine oxide	TMAO	H_3C—$As^{(V)}(=O)(CH_3)(CH_3)$	-
Arsenic (V) acid (arsenate)	As(V)	$O=As^{(V)}(OH)(OH)(HO)$	2.2, 7.0, 11.5
Arsenous (III) acid (arsenite)	As(III)	$As^{(III)}(OH)(HO)(OH)$	9.2, 12.1, 13.4

2. SAFETY PRECAUTIONS

Precautionary information that is important to protecting personnel and safeguarding equipment will be presented inside a box, such as this one, throughout the procedure where appropriate.

Follow universal precautions. Wear gloves, a lab coat, and safety glasses while handling human blood, plasma, serum, urine or other bodily fluid or tissue. Operators of this method must take the Hazardous Chemical Waste Management for CDC Workers course upon initial hire and yearly refreshers thereafter. Place disposable items (e.g., pipette tips, autosampler tubes, and gloves) that come in contact with human biological fluids, such as urine, in a biohazard autoclave bag. Keep these bags in appropriate containers until they are sealed and autoclaved. When work is finished, spray all work surfaces where human biological fluids were handled with a broad spectrum disinfectant or other appropriate disinfectant. Dispose of all diluted biological specimens in a biohazard autoclave bag at the end of the analysis according to CDC/DLS guidelines for disposal of hazardous waste.

PerkinElmer® provides safety information that must be read before operating the ICP-MS instrument. This information is found in the *PerkinElmer® NexION® 300D ICP-DRC-MS System Safety Manual*. Possible hazards include ultraviolet radiation, high voltages, radio-frequency radiation, and high temperatures.

Handle acids and bases with extreme care; they are caustic and toxic. Reagents used in this study include those listed in Section 5. Safety Data Sheet documents (SDS) for these chemicals are readily accessible as hard copies in the lab. If needed, SDS for other chemicals can be viewed at http://www.ilpi.com/msds/index.html or through CDC networked computers at http://intranet.cdc.gov/ossam.

Caution!

Exercise caution when handling and dispensing concentrated nitric acid. Always remember to add acid to water. Nitric acid is a corrosive chemical that is capable of severe eye and skin damage. At a minimum, wear powder-free gloves, a lab coat, and safety glasses. If nitric acid comes in contact with any part of the body, quickly wash the exposed area with copious quantities of water for at least 15 minutes and refer to the SDS for full information.

3. DATA SYSTEM MANAGEMENT

To maintain the integrity of specimen and analytical data generated by this method, eliminate hand entry of specimen identifiers (IDs) or analytical results whenever possible. When this is not possible, proofread all transcribed data. Regularly back up the ICP-MS computer's hard drive. It is recommended that a defragmentation program be run on the computer's hard drive on a periodic basis.

a. Data Entry and Transfer

Enter sample identifiers into the ICP-DRC-MS computer software either by using barcode scanners to scan the ID directly from the vials or queue sheets or by copying the IDs from an Excel file with sample IDs exported directly from the Laboratory Information Management System (LIMS). This will eliminate transcription errors and speed up sample processing. If hand entry of a sample ID is necessary, proofread transcribed data after entry. Transfer data electronically when reporting or moving data to other computerized data-handling software programs or storage locations.

b. Routine Computer Hard Drive Maintenance

Defragment the computer hard drive by using software such as Microsoft Windows® Disk Defragmenter (located in Start > Programs > Accessories > System Tools) or an equivalent program to maximize computer performance and maintain data integrity for files on the hard drive. An entry will automatically be logged in the Windows™ system event log when this process is performed providing documentation of this step.

c. Data Backup

(1) Schedule of Data Backups

Sample results generated by this analytical method are stored long-term on compact discs and on the CDC network shared drive. The stored results must include at least the analysis date, quality-control (QC) results for the run, and the results of specimen analysis by specimen identifier (ID).

Whenever making a backup (daily or weekly) include the directories and subdirectories:

- ✓ C:\Users\Public\Documents\PerkinElmer Syngistix\ICPMS (include all subdirectories)
- ✓ C:\hplc (include subdirectories “data” C:\Users\Public\Documents\PerkinElmer Syngistix\ICPMS include the Application, Security, and System logs
- ✓ Any other relevant files or folders.

Daily. Full data backups onto the laboratory’s (Isolated Secure Network Environment (ISLE).

Weekly. Full data backups onto one or more recordable compact discs (CD-R) or digital video discs (DVD).

(2) Backup Procedures

Before making a CD or DVD backup, saving the information from the Windows Event Viewer in C:\Event Log Backup will ensure archiving of all recent software system events including communications between ICP-DRC-MS and NexION® software, as well as times of hard drive defragmentation, and other Windows™ system events.

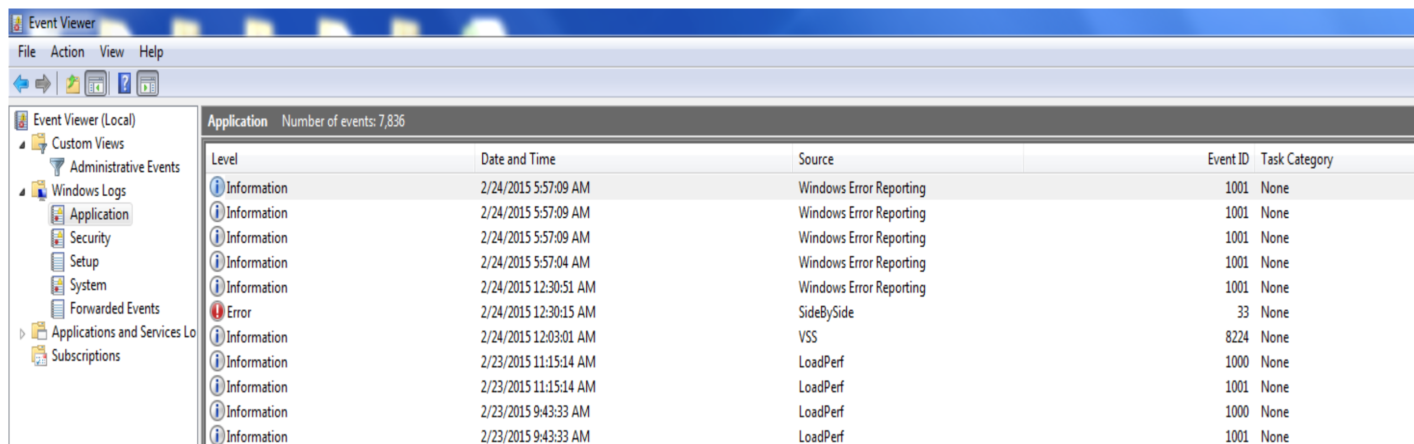
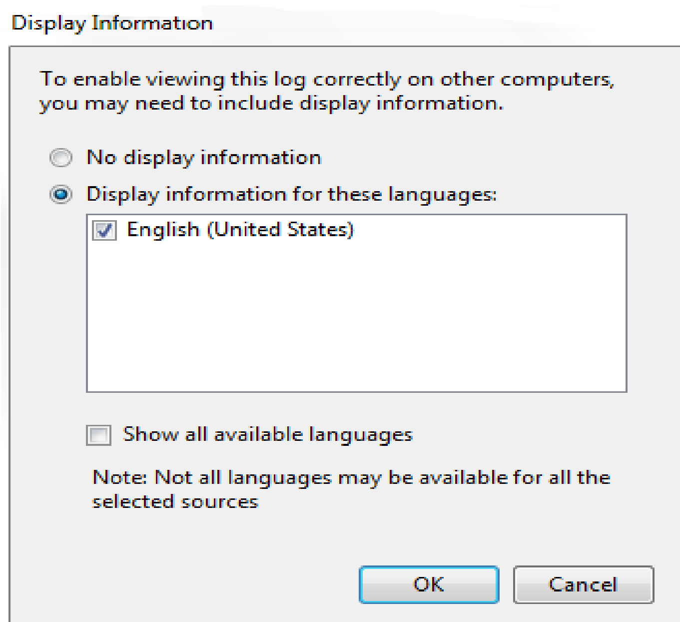


Figure 2-1: Event Viewer

If the computer has Windows 7 operating system, go to the Event Viewer and expand the "Windows Logs" folder. Right click on Application. Choose "Save All Events As…" Save the file in the folder "C:/Event Log Backup". Name the file "Application NexION-I MM-DD-YY" where I is the instrument name (E, G, H, L, etc.) and "MM" is the month, "DD" is the day, and "YY" is the last two digits of the year. The following window will pop up.



Select English as the language and click .

Repeat this procedure for the Security and System folders.

If the computer system is running on Windows XP, go to the Event Viewer and right click on Application. Choose "Save Log File As" and save the file as "Event Log Backup (application).evt". Repeat this procedure for the Security and System Logs.

(a) Secondary Hard Drive Backups

- Each instrument computer is configured to backup the relevant data and files at a specified time each day. The backup files are stored on the laboratory's ISLE.

(b) Compact Disc Backups

- Use CD-R disks only (recordable compact disks) *not* CD-RW disks (rewritable compact disks) so that the compact disk cannot be over-written after creation. DVD discs are also acceptable when transferring large amounts of data (or working with large databases).
- Back up the most recent project data on the hard drive onto a CD-R/DVD disk. In Empower, select the "Configure the System" option. On the left of the screen select the Project folder. On the right select the current project folder to backup Go to "File" → "Backup Project". Browse to the CD or DVD drive to select the CD to burn the backup files. Once done go to the window and eject the disk.
- Backup the Empower database data on the hard drive monthly onto a DVD disk. In Empower, select the "Configure the System" option. Go to "File" → "Backup Database". Browse to DVD drive to select the DVD to burn the backup files. Once done go to the window and eject the disk
- Keep the disk in a building other than the laboratory (in case of fire).

(c) Deleting Data from the Hard Drive

- If data needs to be deleted from the hard drive (i.e. for computer replacement or upgrade, to free up hard drive space, etc.) backup all of the data on the hard drive in duplicate using one or more disks.
- Verify that backup disks operate correctly before deleting any data from the hard drive. To verify the operation of a disk, open any file on the disk by using the appropriate computer software (ICP-DRC-MS software).
- After verifying that all backups are operational, delete the original data from the hard drive.
- Keep one copy of the disk in a building other than the laboratory (in case of fire). Keep the other copy in the ICP-MS laboratory.

d. Documentation of System Maintenance

Computer Maintenance:

Record any maintenance of computer hardware or HPLC or ICP-DRC-MS software in the instrument logbook. This includes disk defragmentation, software upgrades, software patches, etc.

Instrument Maintenance: Document system maintenance in hard copies of data records (i.e., daily maintenance checklists, PerkinElmer® service records, and instrument log book) as well as in electronic records (e.g. hard copy of the daily performance check report). Whenever a service call is placed, store copies of any documents related to the service call in the instrument logbook and record the service in STARLIMS.

4. COLLECTING, STORING, AND HANDLING SPECIMENS; CRITERIA FOR REJECTING SPECIMENS

a. Specimen Type

Specimen type is human urine. No special instructions for fasting or special diets are required of patient or study subjects.

b. Specimen Collection, Handling, and Storage

Optimal amount of specimen is 1.0 mL; the minimum is 0.25 mL. Use sterile specimen containers for specimen acquisition. Acceptable containers for allotment of urine for this method include 5 mL polypropylene cryogenic vials (e.g., Nalgene, Item # 5000-0050), 2 mL polypropylene cryogenic vials (e.g. Nalgene, item # 5000-0020), 2 mL fluidX 2D coded cryo tubes (fluidX part 65-7532), or equivalent storage containers. Screen lots of specimen collection cups, containers, and sample vials and tubes for total arsenic contamination prior to use.

Specimen handling conditions are outlined in the Division of Laboratory Sciences' Policies and Procedures Manual. Every attempt is made to ensure that specimens derived from outside direct CDC control are collected, stored, and transported in a manner that maintains the integrity of the samples as they were collected. This includes, but is not limited to, recognized proper sampling technique, storage in pre-screened containers, packaging and transport in suitably labeled containers that will maintain a temperature of approximately 4°C or lower throughout transport. To prevent possible inter-conversion of arsenic species, immediately store or transport urine specimens at approximately -70°C or a lower temperature. Upon receipt, they must remain frozen at approximately -70°C or a lower temperature until time for analysis. Refreeze remaining portions at the same temperature. Samples thawed and refrozen several times may be compromised.

c. Criteria for an Unacceptable Specimen

The criteria for deeming a specimen unacceptable are low volume sample volumes (< 0.25 mL), suspected contamination due to improper collection procedures or collection devices, and/or contamination during sample preparation/analysis. Specimen contact with dust or dirt may compromise test results. In all cases, request a second urine specimen, if possible.

5. CHEMICALS, STANDARDS, QUALITY CONTROL MATERIALS, AND WORKING CALIBRATORS

a. Chemicals

1. Deionized (DI) water, high purity (approximately 18 MΩ·cm resistivity), Aqua Solutions, or equivalent
2. Ammonium carbonate, (CAS# 506-87-6), MW 96.09, GFS Chemicals, Item # 839, or equivalent
3. Tris(hydroxymethyl)aminomethane, (CAS# 77-86-1), MW 121.14, Bio-refined, GFS Chemicals, Item # 1948, or equivalent
4. Ammonium Sulfate, (CAS# 7783-20-2), MW 132.13, GFS Chemicals, Item # 1906, or equivalent
5. Ammonium Acetate, (CAS# 631-61-8), MW 77.08, GFS Chemicals, Item # 547, or equivalent
6. Acetic Acid, Glacial (CAS# 64-19-7) M.W. 60.05, GFS Chemicals, Item # 624, or equivalent
7. Ammonium Hydroxide, (CAS# 1336-21-6) M.W. 35.05, Fisher Scientific, Item # A470500, or equivalent
8. Methanol (CAS# 67-56-1) M.W. 32.04, GFS Chemicals, Item # 2483, or equivalent
9. 10% hydrogen in argon gas mixture, ≥ 99.999% purity, Airgas South, Inc., Item # X02AR90C350976, or equivalent
10. Double-distilled nitric acid (CAS# 7697-37-2), GFS Chemicals, Item # 621, or equivalent
11. 1,000 mg/L Gallium, SPEX CertiPrep, Item # PLGA2-2Y, or any equivalent traceable to the National Institute for Standards and Technology
12. Certified pH 7 and pH 10 buffer solutions, Fisher Scientific, Item # SB107-500 and SB115-500, or equivalents
13. Liquid argon, Airgas South, Inc., or equivalent
14. Acetonitrile, HPLC grade (CAS# 75-05-8), GFS Chemicals, Item # 2482, or equivalent
15. Accel TB™ Ready-to-use disinfectant, Fisher Scientific, Item # 19-130-5975, or equivalent broad spectrum disinfecting agent
16. Base urine pooled from anonymous donors or purchased from a vendor
17. Potassium Persulfate, purified (CAS# 7727-21-1), GFS Chemicals, Item #557, or equivalent

b. Standards

A mixture of the sources is used to make each set of calibration materials. Alternate sources can be used, and the availability of the sources listed is subject to change without notice.

1. Arsenic (III) oxide, As_2O_3, CAS 1327-53-3, MW 197.84, Sigma-Aldrich (Item # 202673), Santa Cruz Biotechnology (Item # sc-210837), or equivalent
2. Arsenic (III) in 2%HCl, Spex CertiPrep (Item # SPEC-AS3), Inorganic Ventures (Item # CGAS(3)1-1), High Purity Standards (Item # 10003-6), ChemService (Item # X10003-6-100ML), or equivalent
3. Arsenic (V) oxide hydrate, $As_2O_5 \cdot xH_2O$, CAS 12044-50-7, MW 229.84, Sigma-Aldrich (Item # 363456) or equivalent source
4. Arsenic (V) in water, Spex CertiPrep (Item # SPEC-AS5), Inorganic Ventures, (Item # CGAS(5)1-1), High Purity Standards (Item # 10003-7), ChemService (Item # X10003-7-100ML), or equivalent
5. Arsenobetaine (AB), $(CH_3)_3AsCH_2COOH$, CAS 64436-13-1, MW 178.06, Sigma-Aldrich (Item # 11093-50MG), Wako USA (Item # 321-34911), Argus, Vernio, Italy (Item # AR60008), Santa Cruz Biotechnology (Item # sc-227279), or equivalent
6. Arsenocholine bromide (AC), $(CH_3)_3AsCH_2CH_2OH \cdot Br$, CAS 71802-31-8, MW 244.99, Argus, Vernio, Italy (Item # AR60010), Wako USA (Item # 328-34921 or Fisher Scientific Catalog # 50-994-368), or equivalent
7. Dimethylarsinic acid (DMA), $(CH_3)_2As(O)OH$, CAS 75-60-5, MW 138.00, Sigma-Aldrich or ChemService (Item # N11779-500MG) or equivalent
8. Cacodylic Acid (DMA), $(CH_3)_2As(O)OH$, CAS 75-60-5, CAS 75-60-5, MW 138.00, Sigma-Aldrich (Item # 20835-10G-F) or equivalent
9. Monomethylarsonic acid disodium salt (MMA), $CH_3AsO_3Na_2$, CAS 144-21-8, MW 183.93, Argus Chemicals, Vernio, Italy (Item # AR60009) or equivalent
10. Disodium methyl arsonate hexahydrate (MMA), $CH_3AsO(ONa)_2 \cdot 6H_2O$, CAS 144-21-8, MW 291.9, ChemService (Item # N-11817-500MG) or equivalent
11. Monosodium acid methane arsonate sesquihydrate (MMA), CAS 2163-80-6, MW 189, ChemService (Item # N-12495-100MG) or equivalent
12. Trimethylarsine oxide (TMAO), $(CH_3)_3AsO$, CAS 4964-14-1, MW 136.03, Argus Chemicals, Vernio, Italy (Item # AR60011) or equivalent

c. Quality Control Materials

Quality control (QC) materials are prepared from pooled human urine collected from several anonymous donors. See the *Preparation of Quality Control Material* section of this method for details on preparation. The five urine QC pools made for arsenic speciation are designated as:

QC level	QC Designation ID
Base pool	Base Urine – Speciated Arsenic
Low pool	LU-YY102
High pool	HU-YY104
Elevated pool A	EU-YY110
Elevated pool B	EU-YY112

where "YY" is the last two digits of the production year and the last three numbers in the name represent the assigned pool identification number for the arsenic speciation pools.

QC material that is to be used for bench quality control purposes (i.e. LU, HU, and EU pools) will need to be "characterized" as described in the section 14 a. (Establish QC limits for each QC Pool).

d. Working Calibrators

Working calibrators are prepared from pooled human urine collected from several anonymous donors. See the *Preparation of Quality Control Material* section of this method for details on preparation. The calibrators are diluted with the same diluent and in the same ratio as patient samples; therefore, frozen calibrators do not have to be prepared before usage. They are simply thawed and placed on the HPLC autosampler for analysis.

Two types of calibrators are used for this method – the normal calibration range and the extended (or elevated) calibration range. The normal calibration curve is used routinely, and it covers concentrations from the LOD up to 150 µg/L. The extended calibration curve range is analyzed with samples with analyte concentrations between 150 µg/L up to 1000 µg/L. The extended calibration curve is only used after a sample has been analyzed with the normal calibration curve. If an extended calibration curve is needed, the sample is prepared again and reanalyzed with the extended calibration curve and appropriate QC.

The calibration points in the normal calibration curve are designated as S0, S1, S2, S3, and S4. The calibration points in the normal calibration curve for the T series are designated as T0, T1, T2, T3, and T4.

The calibration points in the extended calibration curve are designated as S0, S4, S5, S6, and S7. The calibration points in the extended calibration curve for the T series are designated as T0, T4, T5, T6, and T7.

6. INSTRUMENTATION, EQUIPMENT, SOFTWARE, AND SUPPLIES

a. Instrumentation

(1) ICP-DRC-MS System

1. Inductively-coupled plasma mass spectrometer, specifically the NexION® 300D ICP-Mass Spectrometer with Universal Cell Technology (UCT™) and Dynamic Reaction Cell (DRC™) capability, PerkinElmer® LAS, or equivalent

2. NexION® instrument control and data handling software, version 1.5 or greater, PerkinElmer® or Syngistix for ICP-MS software version 1.1 or greater
3. Chiller 1 HP 230V/60HZ TP Quiet, PolyScience through PerkinElmer® (Item # N0772046) or equivalent
4. Two external peristaltic 2-, 3-, or 4-channel peristaltic pumps, “Minipuls 3”, Gilson Inc., or equivalent
5. NexION® 300 ICP-MS Detector, PerkinElmer® (Item # N8145000) or equivalent

(2) Replacement and Periodic Maintenance of Key Components

Part numbers listed below are PerkinElmer® part numbers from their *2015-2016 Analytical Consumables and Supplies Catalog* unless indicated. Equivalent high quality parts from other suppliers may be used as noted.

1. Peristaltic pump tubing for sample and internal standard (0.76 mm i.d.), Analytical West (Item # PT-2130P), and for waste (3.18 mm i.d.), Analytical West (Item # N8122012) or equivalent
2. Autosampler probe assembly (Item # B3000161) or equivalent
3. Torch O-Ring kit, package of four (Item # N8120100) or equivalent
4. Platinum sampler cone (Item # W1033614) or equivalent
5. Platinum skimmer cone (Item # W1026907) or equivalent
6. Aluminum hyper skimmer cone (Item # W1033995) or equivalent
7. Sampler gasket (Item # W1040148) or equivalent
8. Hyper skimmer o-ring (Item # 09902123) or equivalent
9. Hyper skimmer screw (Item # WE027484) or equivalent
10. Cone removal tool (Item # W1034694) or equivalent
11. Torch alignment tool (Item # WE015554) or equivalent
12. Quartz cyclonic spray chamber (Item # N8145013) or equivalent
13. Quartz ball injector 2.0 mm i.d. (Item # WE023948) or equivalent
14. Demountable quartz torch (Item # N8122006) or equivalent
15. Load coil (Item # WE021816) or equivalent
16. Fombulin® GV80 pump oil for the roughing pump (Item # N8145003) or equivalent
17. PE Sciex coolant (Item # WE016558A) or equivalent
18. NexION® trap coaxial foreline (Item # W1036511) or equivalent
19. NexION® exhaust filter kit SV40BI (Item # N8145005) or equivalent
20. TQ+ Quartz nebulizer, Meinhard (Item # TQP-50-A0.5) or equivalent
21. Nebulizer connection kit, Meinhard (Item # F2-50) or equivalent

(3) HPLC System – Agilent Infinity 1260 Bio-Inert System - Equipment and Consumables

Part numbers listed below are all Agilent part numbers unless otherwise specified.

1. HPLC pump, specifically Agilent 1260 Infinity Bio-Inert Quaternary Pump (Item # G5611A) or equivalent

2. HPLC autosampler, specifically Agilent 1260 Bio-Inert High Performance Autosampler (Item # G5667A) or equivalent
3. Thermostat, specifically Thermostat for 1200 ALS/Fraction Collector (Item # G1330B) or equivalent
4. Column compartment, specifically Agilent 1200 Series Thermostatted Column Compartment (Item # G1316B) or equivalent
5. Control module, specifically Agilent Instant Pilot (Item # G4208-67001) or equivalent
6. Stator face assembly for p/n 0101-0921 valve (Item # 0100-1851) or equivalent
7. Frit, PTFE, 5/pk (Item # 01018-22707) or equivalent
8. Peristaltic pump, silicone tubing (Item # 5042-8507) or equivalent
9. PTFE solvent tubing 5 m, 1.5 mm ID, 3 mm od (Item # 5062-2483) or equivalent
10. Frit adapter, 3 mm 4/pk (Item # 5062-8517) or equivalent
11. Bio-inert 2 position/6 port injection valve for 1260 Bio-inert high performance autosampler (Item # 5067-4131) or equivalent
12. Tubing Kit-degasser to pump (Item # G1322-67300) or equivalent
13. Tray for well plate autosampler which holds 2 well plates (max 50mm high) and 10 X 2 vials, Agilent G1367A, G1377A, G2258A well plate samplers (Item # G2258-60011) or equivalent
14. 100 position tray for 2 mL vials with inserts (Item # G4226-60021) or equivalent
15. Capillary starter kit, bio-inert (Item # G5611-68711) or equivalent
16. Accessory kit, for 1260 Bio-inert Quaternary LC (Item # G5611-68755) or equivalent
17. Tool for needle adjustment (Item # G5667-40500) or equivalent
18. Bio-inert needle assembly for 1260 Bio-inert high performance autosampler (Item # G5667-87200) or equivalent
19. Bio-inert stator for Bio-inert 2 position / 6 port injection valve (Item # 5068-0060) or equivalent
20. Rotor, 12-position/13-port bio valve, 200 bar (Item # 5068-0099) or equivalent
21. Bio-inert sample loop, 100 µL (Item # G5667-81006) or equivalent
22. Rotor seal, 3 grooves, max 600 bar, for thermostatted column compartment, switching valve p/n 0101-0920 or G1158A switching valve (Item # 0101-1409) or equivalent

(4) HPLC Supplies

1. Anion-exchange HPLC column, specifically, PRP-X100™, 4.6 X 150 mm dimensions, 7 µm particle size in Polyether ether ketone (PEEK) hardware, Hamilton Company (Item # 79665) or equivalent
2. Autosampler injector loop, 200 µL, ChromTech or IDEX Health & Science (Item # 9055-025) or equivalent
3. Replacement precolumn blue microfilter 0.5 µm 10/pk, ChromTech or IDEX Health & Science (Item # A-735X) or equivalent
4. Precolumn microfilter assembly PEEK, ChromTech or IDEX Health & Science

(Item # M-560) or equivalent

5. HPLC tubing, 0.007” I.D. X 1/16” O.D., polyethylethylketone (PEEK), 5 feet length, ChromTech or IDEX Health & Science (Item # 1536) or equivalent
6. Vespel rotor seal for 6 port switching valves, ChromTech or IDEX Health & Science (Item # 7750-016) or equivalent
7. Stator Rheodyne PEEK for 6-port switching valve, ChromTech or IDEX Health & Science (Item # 9750-021) or equivalent
8. RheBuild Kits for 6-port switching valve, ChromTech or IDEX Health & Science (Item # 7501-999) or equivalent
9. Dionex 2L HPLC reservoir bottles with cap, Dionex (Item # 44129) or equivalent
10. Electrically-activated 6-port switching valve, IDEX Health & Science (Item # EV750-100-S2) or equivalent. An additional switching valve may also be used as demonstrated in Section *8 Instrument Setup and Configuration*.

b. Laboratory Equipment

1. Water purification system for providing ultrapure water with a resistivity of approximately 18 MΩ·cm, Aqua Solutions or equivalent
2. Caliper Automated Staccato Sample Preparation System, Caliper Life Science Technologies or equivalent
3. Eppendorf® Model 5417R refrigerated centrifuge fitted with FA45-24-11 fixed angle rotor or equivalent refrigerated centrifuge capable of ≥18,000 rcf for centrifugation of 1.5 mL capacity microcentrifuge tubes
4. Eppendorf® Model 5427R refrigerated centrifuge fitted with FA-45-48-11 fixed angle rotor or equivalent refrigerated centrifuge capable of ≥16 000 g for centrifugation of 1.5 mL capacity microcentrifuge tubes
5. High-precision analytical balance capable of accurately weighing milligram amounts of material to the tenth of a milligram or better, Sartorius™ Cubis™ MSA Precision balance, Item # MSA1203S100, Fisher Scientific or equivalent
6. Analytical balance for routine weighing of material to the nearest hundredth of a gram and with a loading capacity of at least 200 g, Sartorius™ Cubis™ MSA Analytical balance, Item # MSA324S100DI, Fisher Scientific or equivalent
7. pH meter with one hundredths of a pH unit readout or better, fitted with glass electrode (pH probe), Accumet XL-150 or equivalent
8. Hamilton Microlab 625 Diluter, Hamilton Company (Item # ML625-DIL) or equivalent
9. Calibrated hand-held adjustable pipettes that cover the range of accurate liquid delivery from 50 µL to 5000 µL. Eppendorf™ Xplorer™, Fisher Scientific, or Sartorius Picus NxT, Sartorius Corporation, single channel electronic programmable pipettes, or equivalent
10. Honeywell Xenon 1902 USB 2D barcode reader, Computech International (Item # 1902HHD-0USB-5F) or equivalent
11. “Repeater Plus” Pipette, Fisher Scientific (Item # 2226020-1) or equivalent pipetting device(s) capable of accurately dispensing multiple microliter aliquots of liquid

12. Vortex mixer, VWR (Item # 945303) or equivalent
13. Gas regulator for 10% DRC™ gas, Matheson Tri-Gas Products or equivalent
14. Gas regulator for argon gas, Matheson Tri-Gas Products or equivalent
15. CEM Discover SP-D closed vessel microwave, CEM Corporation or equivalent
16. Stormat Sealer, PerkinElmer® (Item # CUSG01273) or equivalent

c. Computer Software

1. PdfFactory Pro 5 or later version, FinePrint Software, LLC, www.fineprint.com or equivalent. This product is used for creating electronic Portable Document Files (pdf) directly from Microsoft® Windows print dialog box
2. Syngistix for ICP-MS software version, 1.1 or greater.
3. Chromatography data handling software, specifically, Waters Empower 3 Chromatography Data Software

d. Supplies

1. 2-200 μL pipette tips, 960 tips per case, Fisher Scientific (Item # 05-403-66) or equivalent
2. 20-300 μL pipette tips, 960 tips per case, Fisher Scientific (Item # 05-403-67) or equivalent
3. 50-1000 μL pipette tips, 960 tips per case, Fisher Scientific (Item # 05-403-68) or equivalent
4. 5 mL pipette tips, 500 tips per case, Fisher Scientific (Item # 05-403-71) or equivalent
5. 0.5-200 μL LowRet pipette tips, 960 tips per case, Sartorius Corporation (Item # LH-L790200) or equivalent
6. 5-350 μL LowRet pipette tips, 960 tips per case, Sartorius Corporation (Item # LH-L790350) or equivalent
7. 10-1000 μL LowRet pipette tips, 960 tips per case, Sartorius Corporation (Item # LH-L791000) or equivalent
8. 5 mL pipette tips, 1000 tips per case, Sartorius Corporation (Item # 780308) or equivalent
9. Acid-washed 2 liter, 1 liter, 500 mL, 100 mL, and 50 mL polyethylene (PE) bottles. To acid-wash containers, rinse with 10% (v/v) reagent-grade nitric acid, followed by rigorous rinsing with DI water to remove all traces of the acid. Repeat this process several times depending on prior use of the containers.
10. 1.5 mL polypropylene (PP) microcentrifuge tubes, Fisher Scientific (Item # 05-402-25) or equivalent
11. 0.75mL virgin polypropylene HPLC vials, National Scientific (Item # C4011-15) or equivalent
12. Red Snap-it seal T/S SLT septa, case of 1000, National Scientific (Item # C4011-55R) or equivalent
13. Tube racks for 1.5 mL microcentrifuge tubes (approximately 6), Fisher Scientific (Item # 05-405-3) or equivalent

14. Vial rack for HPLC autosampler vials, 50-position, Fisher Scientific (Item # 03-375-9) or equivalent
15. fluidX racks for Nalgene cryovials, Brooks Automation (Item # 170-01-110)
16. fluidX racks for fluidX 2D cryotubes, Brooks Automation (Item # 65-9451)
17. 2D coded jacket cryotubes external threaded with yellow caps 480 per case, Brooks Automation (Item # SP-2003)
18. 2D coded jacket cryotubes external threaded with blue caps 480 per case, Brooks Automation (Item # SP-2004)
19. 2D coded jacket cryotubes external threaded with orange caps 480 per case, Brooks Automation (Item # SP-1007)
20. Corning 2mL V-bottom non-sterile 96 well plate, case of 100, Fisher Scientific (Item # 07-200-701) or equivalent
21. Non-sterile deep well plates 1 ml capacity 50/case. Fisher Scientific (Item # 12-566-120) or equivalent
22. Easy Pierce Foil sealing tape, Thermo Scientific (Item # AB-3738) or equivalent
23. Nested pipette tips 776 pipette tip – 200 µL SLV 30-96 trays, PerkinElmer® (Item # 105649) or equivalent
24. Silicone STORMAT-96, 50/case, PerkinElmer® (Item # 6008096) or equivalent
25. Eppendorf Repeater pipette tips Combitips Plus 5 mL, Fisher Scientific (Item # 21-381-330) or equivalent
26. Kay-Dry™ paper towels and Kim-Wipe™ tissues, Fisher Scientific (Item # 06-666-11) or equivalent
27. Teflon™-coated magnetic stirs bars, VWR (Item # 58948-974) or equivalent
28. Nitrile, powder-free examination gloves, any vendor
29. Biohazard autoclave bags, Fisher Scientific or equivalent
30. 35mL Pyrex vials, CEM Corporation (Item # 909036) or equivalent
31. 35mL SP-D Plus Pressure Caps, CEM Corporation (Item # 909350) or equivalent
32. Explorer Vessel Racks, CEM Corporation (Item # 171825) or equivalent
33. 35 mL Teflon PFA Liner for 35 mL Pyrex vessels, CEM Corporation (Item # 171968) or equivalent
34. 15 mL conical polypropylene centrifuge tube, VWR (Item # 82050-276) or equivalent
35. 50 mL polypropylene centrifuge tubes, Fisher Scientific (Item # 14-432-22) or equivalent

7. STANDARD PROCEDURE

a. Preparation of Stock Solutions

1. **0.5 M Ammonium Acetate, pH 5.** Dissolve 27.2 g of ammonium acetate and 8.0 mL of glacial acetic acid into approximately 950 mL of DI water. Adjust pH to 5.0 by adding concentrated glacial acetic acid drop-wise. Bring volume to 1000 mL with DI water, and mix thoroughly. This solution expires one year from the date made. Prepare ahead of time or as needed.
2. **0.1 M Ammonium Acetate, pH 5.** Dissolve 5.44 g of ammonium acetate and 1.68 mL of concentrated glacial acetic acid into approximately 950 mL of DI water. Adjust pH to 5.0 using drop wise additions of either 10% ammonium hydroxide or glacial acetic acid. Bring volume to 1000 mL with DI water, and mix thoroughly. This solution expires one year from the date made. *Note: This solution is not prepared as a dilution of 0.5 ammonium acetate, pH 5.*
3. **0.5 M Ammonium Carbonate.** Dissolve 48.05 g of ammonium carbonate into approximately 900 mL of DI water. Bring volume to 1000 mL with DI water, and mix thoroughly. This solution needs to be filtered before use. This solution expires one year from the date made. Prepare ahead of time or as needed.
4. **0.5 M TRIS Buffer.** Dissolve 60.57 g of tris(hydroxymethyl)aminomethane in approximately 900 mL of DI water. Bring volume to 1000 mL with DI water, and mix thoroughly. This solution expires one year from the date made. Prepare ahead of time or as needed.
5. **0.5 M Ammonium Sulfate.** Dissolve 66.07 g of ammonium sulfate in approximately 900 mL of DI water. Bring volume to 1000 mL with DI water, and mix thoroughly. This solution expires one year from the date made. Prepare ahead of time or as needed.
6. **5% Acetonitrile.** To make autosampler rinse solution, add 50 mL of acetonitrile to 950 mL of DI water, and mix thoroughly. This solution expires one year from the date made. Prepare ahead of time or as needed.

b. Preparation of Base Urine Pool

Collect human urine from anonymous donors in clean, trace metals-free urine cups. Refrigerate urine donations at approximately 4°C as soon as possible for periods of two weeks or less. For longer periods, freeze the urine donations until needed. If possible, analyze each donation for speciated arsenic. If speciated arsenic analysis is not possible, the specimens have to be analyzed for total arsenic. If total arsenic analysis is used, exclude specimens that contain more than 5 µg/L of arsenic. If speciated arsenic analysis of each cup is used, exclude donated specimens that contain detectable amounts of AC, TMAO, AsIII, and MMA. Exclude specimens that have more than 2.5 µg/L total of AB and DMA combined. Reserve approximately two liters of urine that fit this criteria. Clarify the urine (through centrifugation), and mix it thoroughly. Dispense approximately 40 mL of this pooled urine into 50 mL centrifuge tubes and store at approximately -70°C or a lower temperature. Label the tubes “Base Urine – Speciated Arsenic” or a similar designation.

c. Preparation of Working Solutions

1. **25% ($^{v}/_{v}$) Base Urine Pool in 0.075 M Ammonium Acetate, pH 5.** May be prepared ahead of time before the day of analysis. To a clean 500 mL polypropylene (PP) bottle, add 75 mL of 0.5 M ammonium acetate pH 5, 125 mL of “Base Urine – Speciated Arsenic”, and 300 mL of DI water for a total volume of 500 mL. Mix thoroughly. Store refrigerated at approximately 4°C. Prepare as needed. The expiration date is one day from the date made.

1. **HPLC Buffer A Preparation (10mM Ammonium Carbonate/10 mM TRIS/0.5% MeOH pH 8.6).** May be prepared ahead of time before the day of analysis. To a clean 2 L or greater capacity beaker containing a clean magnetic stir bar add 1.91L (2 liters with 90 mL removed) of DI water. Add the following:

   (a) 40.0 mL of 0.50 M ammonium carbonate

   (b) 40.0 mL of 0.50 M TRIS buffer

   (c) 10 mL of methanol.

   While the solution is being mixed on a magnetic stir plate, use a pH meter fitted with a glass electrode to monitor pH. Using a repeater pipette, slowly add either glacial acetic acid (to lower pH) or 10% ammonium hydroxide (to increase pH) drop-wise to bring the pH to 8.60 ± 0.05. After complete mixing, transfer the beaker’s contents to HPLC “Bottle A” (or appropriately labeled container). Cap and mix thoroughly. Label bottle “10 mM Amm. Carbonate / 10 mM TRIS / 0.5% MeOH / pH 8.6” (or other appropriate notation to indicate contents). Prepare as needed. The expiration date is two weeks from the date made.

2. **HPLC Buffer B Preparation (15mM Ammonium Sulfate/10mM Ammonium Carbonate/10 mM TRIS/0.5% MeOH pH 8.0).** May be prepared ahead of time before the day of analysis. To a clean 2 L or greater capacity beaker containing a clean magnetic stir bar add 1.85 L (2 liters with 150 mL removed) of DI water. Add the following:

   (a) 40.0 mL of 0.50 M ammonium carbonate

   (b) 40.0 mL of 0.50 M TRIS buffer

   (c) 10 mL of methanol.

   (d) 60.0 mL of 0.50 M ammonium sulfate

   While the solution is being mixed on a magnetic stir plate, use a pH meter fitted with a glass electrode to monitor pH. Using a repeater pipette, slowly add either glacial acetic acid (to lower pH) or 10% ammonium hydroxide (to increase pH) drop-wise to bring the pH to 8.00 ± 0.1. After complete mixing, transfer the beaker’s contents to HPLC “Bottle B” (or appropriately labeled container). Cap and mix thoroughly. Label bottle “15 mM Amm. Sulfate / 10 mM Amm. Carbonate / 10 mM TRIS / 0.5% MeOH / pH 8.0” (or other appropriate notation to indicate contents). Prepare as needed. The expiration date is two weeks from the date made.

3. **HPLC Buffer C (Autosampler Wash Solution) Preparation. 5% (v/v) acetonitrile** (HPLC grade). Mix 50 mL of acetonitrile with 950 mL of DI water. Prepare as needed. The expiration date is one year from the date made.

4. **Internal Standard (2.5 µg/L) Preparation.** May be prepared ahead of time before the day of analysis. Add approximately 950 mL DI water followed by 5.0 mL of methanol to an empty 1 liter PP volumetric flask. Add 50 µL of 50 mg/L

trimethylarsine oxide (TMAO Internal Standard Stock Solution) to the container and fill with DI water to the 1000 mL mark. Mix thoroughly. Prepare as needed. The expiration date is one year from the date made.

d. Preparation of Stock Standards (Concentrated)

CAUTION!
Arsenic compounds are toxic! Take extra care to avoid accidental ingestion or inhalation of these materials. **Utilize appropriate personal protective equipment. At a minimum, wear a laboratory coat, safety glasses, and latex or nitrile gloves.** Clean up any spills that occur according to applicable hazardous material spill procedures.

Note 1*: All preparations will be performed gravimetrically (wt/wt), unless otherwise noted. All gravimetric measurements assume the density of water equal to 1g/cm^3.*

Note 2*: The steps outlined in Sections 8.b, 8.d. – 8.h may be optionally outsourced under contract to a partner facility.*

Definitions:

- **Stock Standard:** Initial solution of one of seven arsenic species prepared by dissolving solid or liquid standard material into aqueous or acidic solution.
- **Intermediate Standard** A 10,000 µg/L solution prepared from dilution of a Stock Standard.
- **Working Calibrator**: A dilution of the Intermediate Standards prepared in urine and ammonium acetate buffer. A Working Calibrator is used in the urine arsenic species HPLC-ICP-DRC-MS analysis to build a calibration curve.

Use a high precision analytical balance capable of accurately weighing material to the tenth of a milligram or better. It is important to use the balance in a vibration-free environment that is free of air drafts and away from direct sun light, to the fullest extent possible.

1. Using a clean Teflon-coated spatula, or equivalent apparatus, prepare the arsenic Stock Standards described in TABLE 7-1 in 50.0 mL centrifuge tubes or other suitable storage vessels. Record the weights of the initial solid arsenic material for each species and all final weights of the corresponding arsenic solutions after dissolution.

TABLE 7-1: PREPARATION OF STOCK STANDARDS

Arsenic Species	Range to Weigh (g)	Solvent Used to Dissolve	Final Weight (g) After Dissolution
Arsenobetaine	0.02–0.03	DI Water	10.00
Arsenocholine bromide (or other salt equivalent)	0.20–0.25	DI Water	50.00
Disodium methyl arsenate	0.20–0.25	DI Water	50.00
Trimethylarsine oxide	0.10–0.125	DI Water	50.00
Dimethylarsinic acid	0.10–0.125	DI Water	50.00
Arsenic (V) oxide hydrate	0.10–0.125	DI Water	50.00
Arsenic (III) oxide	0.10–0.125	Dissolve in 1.5 ml of 6N HCl with mild heating. Add DI water to complete volume	50.00

2. Tightly cap the storage vessels for future use. The expiration date is one year from the date weighed.
3. Calculate the concentration of each Stock Standard using the recorded weights for each species. The resulting units of concentration are milligrams per liter (mg/L). Record these values in a laboratory notebook.
4. Calculate the "Arsenic (As) atomic equivalent" concentration of each arsenic species concentrated standard using Equation 1.

Equation 1

$$\text{"As atom equivalent" conc. in mg/L} = \frac{\text{concentration of concentrated stock standard in g/L} \times \text{\# As atoms per species molecule} \times 74.92 \text{ atomic wt. As}}{\text{F.W. of As species} \times 10^{-3}\ \text{g/mg}}$$

Insert into the equation the appropriate values for the formula weights (also referred to as molecular weights) and number of arsenic atoms per molecule for each species. A list of commonly used formula weights (F.W.) for each arsenic species is shown in TABLE 7-2. ***However, formula weights provided by the chemical manufacturers, if different, supersede the values presented in the table.***

TABLE 7-2: FORMULA WEIGHTS OF ARSENIC SPECIES

Arsenic Species	Formula Weight	Number of Arsenic Atoms per Molecule	Formula
Arsenobetaine	178.06	1	$(CH_3)_3AsCH_2COOH$
Arsenocholine bromide	244.99	1	$(CH_3)_3AsCH_2CH_2OH \cdot Br$
Disodium methyl arsenate	291.9	1	$CH_3AsO_3 \cdot 6H_2O$
Trimethylarsine oxide	136.03	1	$(CH_3)_3AsO$
Dimethylarsinic acid	138.01	1	$(CH_3)_2As(OH)_2$
Arsenic (V) oxide hydrate	229.84	2	$As_2O_5 \cdot xH_2O$
Arsenic (III) oxide	197.84	2	As_2O_3

Note: All arsenic solutions from this point forward are referenced in terms of arsenic concentration.

5. Record the “arsenic (As) atomic equivalent” concentration value on each arsenic stock standard storage vessel.

e. Preparation of Intermediate Standards

Into separate 50 mL centrifuge tubes, gravimetrically prepare 10,000 µg/L solutions of each stock standard. Use the arsenic atomic equivalent for each species to determine the appropriate dilution needed to obtain the expected concentration of approximately 10,000 µg/L. Record all weights. Calculate the expected concentration of each solution marking these values in a laboratory notebook. *Note: Exact arsenic concentrations will be determined by DRC-ICP-MS in subsequent steps.*

f. Microwave Digestion for Conversion to Arsenate (AsV)

Even at the same arsenic concentration, different arsenic species can produce different instrument responses during analysis; therefore, it is important to convert each arsenic species to one common chemical form prior to analysis for total arsenic. Digestion of the arsenic species by microwave-assisted oxidation to arsenate (AsV) allows for analysis with an instrument calibrated using aqueous arsenate calibrators. This prevents introduction of systematic errors that otherwise might be caused by the determination of undigested arsenic species concentrations calculated using an inorganic arsenate calibration curve.

1. Prepare 1000 mL of 3% potassium persulfate ($K_2S_2O_8$) in deionized water. The expiration date is one day from the date made. This solution may be prepared by weight/volume.
2. Perform the following steps in triplicate: For each of the Intermediate Standard solutions, gravimetrically transfer 0.5 g into a microwave Teflon vessel and record the weight to three significant digits. Add 10 mL of 3% $K_2S_2O_8$ to each vessel. (A volumetric measure is sufficient here, as the final solution, post-microwave assisted digestion, will be gravimetrically brought to 50.0 g total weight). Additionally, triplicate blanks (0.5 g water + 10.0 mL $K_2S_2O_8$) and triplicate certified AsV solutions are desired for quality control if space permits in the microwave.
3. Perform microwave-assisted digestion of these solutions using the CEM Discover SP-D microwave program “As Calib Digest” or a suitable program. For detailed instructions on microwave digestion, refer to DLS 3515 (*Operation of the CEM Corporation Discover SP-D microwave for the Arsenic Speciation Analytical Method*).
4. Once digestion vessels are at room temperature, quantitatively transfer each digested solution into separate labeled 50 mL centrifuge tubes using DI water. Use an analytical balance to make the transfer. Bring the final weight to 50.0 g using DI water, and record the total weight to three significant digits. The expected arsenic concentration of each solution is approximately 100 µg/L as arsenate (AsV).
5. To determine if all arsenic species have been converted to AsV, measure an aliquot of each species by the current CLIA urine arsenic species HPLC-DRC-

ICP-MS method. Since this step is performed solely to confirm that the species are no longer present in their original forms, this may be performed in a qualitative manner.

g. Method of Standard Additions (MSA)

Note: It is imperative that the total arsenic concentration of each solution be determined by the Method of Standard Additions (MSA), and that all solutions are prepared gravimetrically (wt/wt) unless otherwise noted.

1. Prepare 1000 mL of 2 µg/L gallium in DI water by diluting 2 mL of 1000 µg/L stock Ga in 1000 mL of DI water. This solution will be used to dilute post-microwave digested solutions. This solution does not need to be prepared gravimetrically.
2. Prepare a 5 µg/mL AsV "spiking solution" in DI water. Weigh 0.25 g of 1,000 mg/L certified arsenate solution and bring to a final weight of 50.0 g using DI water Record all weights, and calculate the exact concentration of this solution. This concentration will be used in steps 8. g.5.ii, iii, and iv.
3. Using the 2 µg/L Ga solution prepared in Step 1, dilute each of the microwave-digested solutions 1:2 into new 50 mL centrifuge tubes by weight, with a final weight of 50.0 g. (For example, dilute 25.0 g of each microwave digested solution to 50.0 g total weight using the 2 µg/L Ga solution). Record all weights. Final concentrations are approximately 50 µg/L.
4. For each new 50 µg/L microwave-digested solution, label four new 15 mL centrifuge tubes, incorporating the analyte name, replicate, and MSA spike concentration. An example (demonstrating only one replicate for one microwave-digested solution) is shown below in TABLE 7-3. Each tube will be used to prepare new solutions for MSA in subsequent steps.

TABLE 7-3: METHOD OF STANDARD ADDITIONS TABLE A

	Labels		
Tube #	**Analyte**	**Replicate**	**Spike in µg/L**
1	AB	1	0
2	AB	1	25
3	AB	1	50
4	AB	1	100

Note: A spike of 0 µg/L corresponds to an unspiked sample for which a value will be determined in subsequent steps via total arsenic analysis.

5. The solutions mentioned in Step 4 and outlined in TABLE 7-3 must be prepared. For accuracy, it is important to prepare each solution in the following order:

   For each 50 µg/L microwave-digested solution:

   (i) Into the tube labeled 0 µg/L, transfer by weight approximately 10.0 g of the approximately 50 µg/L diluted microwave-digested solution prepared in Step 8.g.3. Record the weight.
   (ii) Into the tube labeled 25 µg/L, transfer by weight approximately 0.05 g of the approximately 5 µg/mL spiking solution prepared in step 8.g.2. Additionally, transfer by weight 10.0 g of the approximately 50 µg/L diluted microwave-digested solution prepared in Step 8.g.3. Record all weights.

(iii) Into the tube labeled 50 µg/L, transfer by weight approximately 0.10 g of the approximately 5 µg/mL spiking solution prepared in step 8.g.2. Additionally, transfer by weight 10.0 g of the approximately 50 µg/L diluted microwave-digested solution prepared in Step 8.g.3. Record all weights.

(iv) Into the tube labeled 100 µg/L, transfer by weight approximately 0.20 g of the approximately 5 µg/mL spiking solution prepared in step 8.g.2. Additionally, transfer by weight 10.0 g of the approximately 50 µg/L diluted microwave-digested solution prepared in Step 8.g.3. Record all weights. TABLE 7-4: MSA TABLE B is an example of the information that needs to be recorded for each analyte and corresponding centrifuge tube.

TABLE 7-4: EXAMPLE METHOD OF STANDARD ADDITIONS TABLE B

Tube	Target Weight, Spiking Solution	Measured Weight, Spiking Solution	Target Weight, Final Solution	Measured Weight, Final Solution
AB, Replicate 1, 0µg/L	n/a	n/a	10.0g	9.8973g
AB, Replicate 1, 25µg/L	0.05g	0.0517g	10.0g	9.8269g
AB, Replicate 1, 50µg/L	0.10g	0.0997g	10.0g	9.8738g
AB, Replicate 1, 100µg/L	0.20g	0.2051g	10.0g	9.8571g

h. Determining the Total Arsenic Concentration

1. Analyze each MSA solution for total arsenic content using a validated ICP-MS method. Ensure that the method uses Ga as an internal standard.
2. After the analysis is complete, prepare an MSA calibration curve for each solution, and calculate the concentration of the unknown sample by dividing the y-intercept by the slope.
3. Using the recorded weights for each MSA solution, calculate the exact concentrations of arsenic in each tube.
4. Once the arsenic concentration of each MSA solution has been determined, perform blank subtraction, and then use all recorded weights for each replicate to back-calculate the exact 10,000 µg/L concentration of the Intermediate Standard solutions prepared in section 8.e. An example of the necessary calculation is given below:

Intermediate Standard Concentration Determination

calculation	**M1MSA**= V2MSA*M2MSA/V1MSA	**M1MW**= V2MW*M2MW/V1MW

Key:

V2MSA Final Wt (g) of solution prepared in Step 8.g.3

M2MSA Measured concentration (µg/L) as determined from MSA

V1MSA	Initial Wt (g) of solution prepared in Step 8.g.3
M1MSA	Exact concentration (µg/L) of microwave-digested solution (Step 8.f.2)
V2MW	Final wt (g) of solution prepared in Step 8.f.5
M2MW	The M1MSA concentration (µg/L)
V1MW	Added amount (g) of Intermediate Standard from step 8.f.2
M1MW	Exact concentration (µg/L) of Intermediate standard

5. After the concentration for each replicate of the Intermediate Standards has been determined, calculate the average concentration of all Intermediate Standard replicates per analyte.
6. Additionally, calculate the average concentration of all microwave blanks prepared in Step 8.f.2. Subtract the average blank value from all Intermediate Standard concentrations.

i. Assessment of Purity by HPLC-DRC-ICP-MS

1. Prepare a 100 µg/L solution by making a dilution of each 10,000 µg/L solution.
2. Using the CLIA “Urine arsenic species HPLC-ICP-DRC-MS” method, analyze each solution for the presence of arsenic species impurities which are defined as the presence of any other arsenic species that are included in Table 1-1 of this method.
3. Tabulate all impurities for each solution. If significant levels of impurities (>10%) are found, consider purchasing additional standard solid material from an alternative source and remake the stock standard. If the total amount of impurities is small (<5%), it is permissible to calculate a correction factor which will be used to adjust the volumes in the next dilution step (i.e., preparation of Working Calibrators) so that the final concentration reaches the intended value.
4. Correct the measured Intermediate Standard concentrations based on their purities. For example, if the Intermediate Standard solution of arsenobetaine (*AB*) was found to have 2% total impurities, the measured value will be multiplied by 98% to obtain the pure value of this Intermediate Standard.
5. For AB, AC, DMA, MMA, AsIII, or AsV solutions that are found to have impurities, be sure to add the concentrations of the impurities to the value of the species because once combined in the mixed calibrators the impurities will contribute to the final value for that species. Impurities in the TMAO solution will not be used in the calculation because TMAO will not be combined with the other species.

j. Preparation of Concentrated Stock Internal Standard

Prepare ahead of time before the day of analysis. The expiration date is one year from the date of preparation.

1. Prepare concentrated Stock Internal Standard, 50 mg/L trimethylarsine oxide (TMAO). Dilute a calculated volume (µL) of trimethylarsine oxide (TMAO) stock standard in DI water to make a total volume of 20 mL.Calculate the number of µL of stock TMAO standard to add using Equation 2:

Equation 2

$$\mu L \text{ of stock TMAO standard to add} = \frac{50 \text{ ppm} \times 20 \text{ mL} \times 1000\ \mu L/mL}{\text{actual ppm of stock TMAO standard}}$$

k. Preparation of Working Calibrators

At any given time, it is necessary to have at least three sets of Working Calibrators in storage from two independent preparations of Stock Standards. When possible, the original material should be from different lots, preferably from alternate vendors. This is not always feasible due to the limited availability/sources of some of the arsenic compounds. Alternate usage of the different sets of working calibrators throughout the analytical runs.

(1) Preparation of Intermediate "Mixed Species" Calibration Solutions

To prepare the intermediate mixed species calibration solutions:

1. Add 7.5 mL of 0.5 M ammonium acetate (pH 5) to a clean 50 mL volumetric flask labeled "Mixed As Species 250 µg/L" (or similar designation). Likewise, add 7.5 mL of ammonium acetate pH 5 to another dedicated 50 mL volumetric flask labeled "TMAO 250 µg/L" or something similar.
2. To each flask, add 12.5 mL of base urine.
3. Based on the pure measured value of each Intermediate Standard (which takes into consideration the addition of any impurities per analyte) prepare the 250 µg/L mixed arsenic species solution. To the flask labeled "Mixed As Species 250 µg/L", add each of the following Intermediate Standards: AC, AB, DMA, MMA, As(III) and As(V) - six solutions total - so that each analyte's final concentration will be 250 µg/L at a volume of 50.0 mL. Use of the dilution equation $C1*V1 = C2*V2$ is helpful, where C1 is the pure measured value of an analyte plus the sum of any impurities in the form of that analyte from the other Intermediate Standards and V1 is the weight of each Intermediate Standard for which to solve. Do not account for any impurities from TMAO in this step, as TMAO will be diluted in a separate flask and not mixed with the other arsenic species.
4. Repeat Step 3 for the TMAO Intermediate Standard. Using the flask labeled "TMAO 250 µg/L", based on the pure measured value weigh the appropriate amount of TMAO Intermediate Standard to obtain a 250 µg/L solution based on a final volume of 50.0 mL.
5. Bring both flasks to a final volume of 50.0 mL using DI water. Mix the contents of each flask thoroughly. Transfer each solution to an appropriately labeled 50 mL centrifuge tube or equivalent container. This solution expires in 8 hours unless it is frozen. If the solution is frozen, the expiration date is 1 year from the date made.

(2) Preparation of Working Calibrator Series

1. Label nine 15 mL PP screw-top conical centrifuge tubes with caps as follows: "S0", "S0 DUP", "S2", "S3", "S4", "S4 Dup", "S5", "S6", and "S7". Label an additional nine 15 mL PP centrifuge tubes as follows: "T0", "T0 DUP", "T2", "T3", and "T4", "T4 DUP", "T5", "T6", and "T7". Arrange these tubes in order and place in a test tube rack. Label two 50 mL PP screw-top conical centrifuge tubes with caps as follows: "S1" and "T1".

2. Inspect a Hamilton Microlab 625 diluter (or equivalent) to ensure that it has a clean 5 mL syringe on the left valve and a clean sample syringe (sizes vary according to volume dispensed see TABLE 7-5) on the right valve. If possible, it helps to have syringes dedicated to calibrator preparation.
3. Program a method into the Hamilton Microlab 625 diluter using the values in TABLE 7-5 prior to the first time that the calibrators are made. The method can be saved and recalled each time calibrator preparation is needed. Please refer to the Hamilton, *Microlab® 600 Advanced Manual Wizards and Custom Method Operation* manual for assistance with programming.
4. Thoroughly rinse the system out with DI water by priming. Place the fill line tubing into a container of DI water. Place an empty waste beaker in position to collect the effluent liquid from the tip of the dispensing probe. Press “Prime” to start the process and “Prime” to stop the process. Remove the fill line tubing from the water and use the prime function to clear all of the residual water from the system.
5. Place the fill line tubing into the buffered solution consisting of 25% base urine in 0.075 M ammonium acetate (pH 5). Place an empty waste beaker in position to collect the effluent liquid from the tip of the dispensing probe. Don the appropriate PPE (face shield). Prime the system. Allow the syringes to cycle the diluent through the syringes three to four times before pressing stop.
6. Recall the method for calibrator preparation.
7. To make the “S0” and “S0 DUP” calibrators, the unit will simply draw diluent from the 500 mL PP bottle to be dispensed into the labeled 15 mL centrifuge tubes. Align the labeled 15 mL conical vial under the tip of the probe, and press the button (“trigger”) on the probe to dispense the solution into the centrifuge tube.
8. Cap each centrifuge tube when the dispensing for that tube is complete.
9. To make the calibrator levels “S1” through “S6”, the tip of Microlab 625 sampling probe needs to be submerged in the “Mixed As Species 250 µg/L” intermediate solution prior to triggering the unit to start the dispensing steps. When the tip of the probe is submerged in the solution, press the trigger to draw up the solution. Once the appropriate amount of 250 µg/L mixed species is drawn into the probe, align the appropriate 15 mL centrifuge tube (or 50 mL centrifuge tube for S1) under the tip of the probe. When the trigger is pressed again, the indicated volume of diluent and sample will be dispensed to make a total volume of 10 mL of diluted calibrator in each tube (25 mL for S1 and T1).
10. Between calibrators, rinse the tip of the sample/dispense tubing with DI water from a polypropylene wash bottle (or use disposable tips if the unit is so equipped). Flushes are programmed into the method between all of the calibrators except after S0. Direct the probe to the waste container to collect the diluent that is flushed out.
11. For “S7”, the Hamilton Microlab 625 will not be used. Add 10 mL of the undiluted “Mixed As Species 250 µg/L” to the labeled tube for “S7”.
12. After all of the S series calibrators have been made, repeat this process for the T series calibrators using “TMAO 250 µg/L”.
13. Mix all of the tubes thoroughly by vortexing and inverting repeatedly.
14. Divide each calibrator into twenty (or less) 0.5 mL aliquots contained in correspondingly labeled clean HPLC autosampler vials. Cap each with a “snap-cap” septum cap. Store at approximately -70°C or lower. The frozen calibrators expire one year from the date made. Store sets of S0 through S4 together for

the normal calibration range and S0, S4, S5, S6, and S7 together for the extended calibration range. Do the same for the T series calibrators.

TABLE 7-5: HAMILTON MICROLAB 625 SETTINGS FOR MAKING DILUTED SERIES CALIBRATORS

Calibrator Level	Microlab 625 Volume Setting, µL		Sample Syringe Size, µL	Target Arsenic Concentration (Nominal Conc. entered into HPLC software)	
	Diluent Syringe	Sample Syringe			
S0 / T0	5000	0	**100**	**0**	**(0)**
	5000	0			
S0 DUP/ T0 DUP	5000	0	**100**	**0**	**(0)**
	5000	0			
S1 / T1	5000	0		**0.50**	**(2)**
	5000	0	**100**		
	5000	0			
	5000	0			
	4950	50			
S2 / T2	4950	50	**100**	**2.50**	**(10)**
	4950	50			
S3 / T3	4750	250	**500**	**12.5**	**(50)**
	4750	250			
S4 / T4	4250	750	**1000**	**37.5**	**(150)**
	4250	750			
S4 DUP / T4 DUP	4250	750	**1000**	**37.5**	**(150)**
	4250	750			
S5 / T5	2500	800	**1000**	**62.5**	**(250)**
	2500	850			
	2500	850			
S6 / T6	1000	1000	**1000**	**125.0**	**(500)**
	1000	1000			
	1000	1000			
	1000	1000			
	1000	1000			

Note: S7/T7 do not have to be diluted with the Hamilton diluter. See Section 7.k.(2).11.

l. Preparation of Quality Control Material

Collect human urine from anonymous donors following the same collection procedure used for the preparation of the base urine pool. Assay each donation for speciated arsenic. Total arsenic analysis is insufficient as a means to determine the baseline levels of arsenic in the individual cups. Assign each urine donation to the “low”, “high”, or “elevated” pools pool according to whether its falls below the spike levels for each of the individual analytes in each pool. Assign specimens to the low and high pools first to ensure that you have a sufficient quantity of specimens with values within the targeted spike levels that are desired.

After pooling urine donations into their respective pools, clarify each pool by centrifugation in acid-washed 1 liter centrifuge bottles. A centrifugation time of 60 minutes at 5,250 *x g* in a preparative table-top centrifuge cooled to 4ºC is typically sufficient but additional centrifugation may be needed. Pour off the supernatant into a larger container and dispose of the residual solution/sediment in each bottle. Thoroughly mix the pooled urine, and analyze an aliquot using this analytical method (DLS 3000) to determine the levels of the endogenous urine species. To each pool, add a calculated volume of the chosen arsenic species standard solution to raise the concentration of that arsenic species to the desired value. Arsenic speciation pools have to be prepared under nitrogen in an oxygen free environment. While maintaining constant stirring of each pool, aliquot 1.5 mL (or more) of urine into a sufficient number of pre-labeled 2 mL vials to provide QC material for 1000 or more runs. Store aliquotted QC material at approximately -70°C or lower. The expiration date of the pools is approximately 2.5 years from the date made.

m. Processing of Urine Samples and QC Material

Process a chosen number of urine samples and QC materials on the day of analysis. One run is defined as the analysis of a contiguous set of samples (typically 20 but this number can very) bracketed by bench QC material at the beginning and end of the set. Each bench QC level needs to be analyzed at the beginning and end of a run in separate tubes/vials. *Sharing of even a single QC tube or vial for more than one QC determination is disallowed*. It is permissible to “piggyback” two runs in succession following a single calibration done during a single autosampler load (such as for an overnight analysis), as long as each run of samples is bracketed by its own uniquely co-prepared bench QC material. The number of samples per run can exceed 20 as long as the total analysis time for all vials under a single calibration does not exceed 24 hours. The 24 hour time limit includes the analysis of the calibrators as well as all QC, patient samples, and blanks. The sample preparation process can be completely automated using the Caliper sample preparation station. For detailed instructions on sample preparation using this method, refer to DLS-3503 (*Operation of the Caliper Staccato Automated Sample Preparation Station for the Arsenic Speciation Analytical Method*). For manual sample preparation, proceed with the steps below.

1. Prior to initial use of the Hamilton Microlab 625, program a method in the unit that will be used for arsenic speciation sample preparation. The method can be saved and recalled each time calibrator preparation is needed. Use a name such as “DLS 3000 Sample Prep” or some equivalent. Please refer to the Hamilton, *Microlab® 600 Advanced Manual Wizards and Custom Method Operation* manual for assistance with programming.
2. In a step-wise, sequential, and user-controlled fashion the method will:

(a) Uptake 600 µL of 0.1M ammonium acetate solution into the diluent draw-line.

(b) Uptake 10- 20 µL of air into the sample draw-line.

(c) Uptake 200 µL of sample (urine) into the sample draw-line.

(d) Dispense 800 µL of sample/diluent mix (200 µL sample and 600 µL 0.1M ammonium acetate solution).

(e) Dispense 1000 µL of diluent (for flushing).

(f) Repeat steps (a) – (e) until all samples, QC, and blanks are prepared.

The method must be set up such that no step shall execute until the user has pressed the trigger button on the probe.

Name and save this method into memory.

For detailed instructions on programming, please consult the Microlab® 600 Advanced Manual Wizards and Custom Method Operation manual.

CAUTION!
Work with open vials or tubes containing biological samples in a biological safety cabinet (BSC). Wear appropriate personal protective equipment (gloves, lab coat and safety glasses).

3. Insert the Hamilton Microlab 625 sample fill line into the cap of a bottle of 0.1M ammonium acetate solution. To minimize evaporation, use a capped bottle with a small hole in the cap that is slightly larger than the outer diameter of the fill line tubing. After inserting the fill line through the hole of the cap, verify that the end of the fill line is completely submersed in the ammonium acetate solution. *It is important that the end of the line remain submersed throughout sample preparation in order to prevent air bubbles and the possibility that the full volume of diluent will not be dispensed.*
4. Put on the appropriate PPE (face shield) and use the prime function to flush the 0.1M ammonium acetate solution through the dispenser. Three to four priming cycles is sufficient. Make sure that an empty waste container is placed at the end of the sample probe prior to starting the priming process. Do not leave the unit unattended while priming.
5. Identify, gather, and thaw the necessary specimen tubes containing the urine samples for the each batch (“run”) to be analyzed.
6. Likewise, for each batch run, thaw one tube each of low and high bench QC samples “LU-yyxxx” and “HU-yyxxx” (for explanation of nomenclature see the *Quality Control Material* section).
7. Label the required number of 1.5 mL microcentrifuge tubes that will be used for patient samples, blanks, and bench QC that will be analyzed. Label a pair of microcentrifuge tubes for each bench QC level (LU and HU or EU and EU) since each bench QC will be injected at the beginning and end of each batch run and need to be contained in separate tubes. Likewise label an equal number of HPLC autosampler vials and set these aside for later use. If two runs will be done under a single calibration, label four microcentrifuge tubes and HPLC vials for each QC level. Use preprinted barcode labels to improve efficiency and reduce the chance of labeling errors.
8. Recall the sample preparation method created in steps 1 and 2. Using this method, follow its steps to deposit the diluted sample into its respective, pre-labeled 1.5 mL microcentrifuge tube. Cap the microcentrifuge tube. *Note that*

calibrators, which have been pre-made, do not undergo this type of sample preparation.

9. Between samples, wipe the dispensing tip with a clean KimWipe or rinse with DI water to avoid sample to sample contamination.
10. When this process is complete, vortex each capped microcentrifuge tube for 3-5 seconds. Next, centrifuge tubes for 5 minutes at a minimum of approximately 16 049 x *g* to 20 800 x *g* in a refrigerated centrifuge pre-cooled to approximately 4°C. The *g* force will depend on the rotor used and the level the vials are at on the rotor (for the FA-45-48-11 rotor with two levels).
11. Following centrifugation, transfer approximately 0.6 mL of the supernatant to the appropriately pre-labeled HPLC autosampler vials. Be careful not to disturb any pellet that might be present at the bottom of the microcentrifuge tube during transfer. Cap all autosampler vials with the proper fitting “snap-cap” septum caps.
12. Thaw one “set” of calibrators, including S0 – S4 and T0 – T4, if needed, at room temperature unless you are analyzing samples that have been determined to have species concentrations greater than 150 µg/L. In that case, thaw a set of calibrators that cover the extended calibration range (i.e. S0, S4, S5, S6, and S7 and/or T0, T4, T5, T6 and T7). Vortex each thawed calibrator for 3-5 seconds.
13. To autosampler vials labeled “Bk” (which stands for “Blank”), 0.6 mL of the samples prepared by adding 200 µL of DI water and 600 µL of 0.1M ammonium acetate solution.
14. For each run, one extra sample of 200 µL LU-xxxx and 600 µL 0.1M ammonium acetate solution is needed. This sample is used for instrument equilibration and conditioning only, so an LU-xxxx vial from a previous day’s run containing leftover sample will suffice. If there are no leftover samples, one may be made using this sample preparation procedure.
15. After sample preparation is complete, use a broad spectrum disinfectant, or other appropriate cleaner, to clean up the laboratory prep areas used as well as any spills that may have occurred.

8. INSTRUMENT SETUP AND CONFIGURATION

a. HPLC Hardware Setup

The initial installation of the HPLC system will be performed by a representative from the vendor. If the instrument has been moved, laboratory personnel can assemble and configure the system according to the Agilent supplied user manuals and the SOP for this analytical method. The following considerations are necessary in planning a new location:

1. Two 6-outlet A/C power strips rated for 15-amp duty will be required to plug-in all HPLC equipment and its associated components.
2. The HPLC equipment needs to be placed adjacent to the right side of the ICP-DRC-MS instrument. Allow for approximately 4 to 7 feet of bench space for the HPLC equipment and data processing computer.
3. HPLC components need to be arranged in the following order:
   (a) Six-port switching valve “#1” (EV750-100)

(b) Six-port switching valve “#2” (EV750-100-S2). This switching valve must have a serial port for connection to an external desktop computer.

(c) Column oven. The column oven can be placed under the pump and autosampler.

(d) Pump with autosampler. Set the pump on top of the autosampler.

4. The Infinity system comes with the appropriate loop installed.
5. Use “yellow” PEEK tubing (0.007” I.D. X 1/16” O.D.) throughout the fluid path. Keep tubing lengths short but long enough to allow the connected components to be moved if necessary.
6. Attach via a plastic tie clamp (or equivalent) one end of a given length of ¼” diameter Tygon™ tubing to the end of the bench holding the HPLC equipment. Position the other end of this Tygon™ tubing to empty into a large waste container. Position this tubing close enough to the 6-port switching valves so that HPLC drain tubing from ports 5 and 6 (dedicated for effluent waste) on switching valves #1 and #2, respectively, will easily fit into the Tygon tubing.

b. Software Installation

Syngistix for ICP-MS software version 1.1 or greater must be installed. If it is not, contact a PerkinElmer® service representative to get the latest version of the software installed.

Empower Quickstart software version 3 must be installed. It is advised that a PerkinElmer® representative install and initially configure Empower software to work with instrumentation. This includes the setup of the method file for DLS method 3000.

PdfFactory Pro 5 or a greater version (FinePrint Software, LLC) is software used for creating electronic Portable Document Format (PDF). Documents are “printed” from the Microsoft® Windows print dialog box, but instead of printing on paper, the document appears on the screen in preview form. The document can then be either printed in hardcopy or saved to a hard drive or disk as a PDF file. Because of the “trial and error” nature of chromatographic integration, frequent reprocessing and reprinting may be required. In these instances, generating PDF documents with the option to print a hardcopy becomes an indispensable tool.

c. Peristaltic Pump Setup

An external peristaltic pump offers a number of advantages over the peristaltic pump built into the ICP-DRC-MS. It can be started, stopped and its speed set independent of the ICP-DRC-MS control software. This “feature” is important when one considers that the HPLC pump “knows nothing” about the state of affairs beyond the HPLC system, and it will continue to pump mobile phase regardless of whether the spray chamber is being drained or not. Likewise, the built-in peristaltic pump is under NexION® software control only and cannot be operated in manual mode. While it is feasible to configure the NexION® software to force the built-in peristaltic pump to keep emptying the spray chamber after completing an analysis, it is nonetheless easy to make a mistake during the NexION® program setup. The built-in pump’s timing and speed is set in the NexION®’s sample file and not the method file. The sample file is created each time before a batch run posing the risk that the built-in peristaltic pump’s timing and speed could be set up incorrectly or forgotten by the analyst. If this happens, the software will stop the built-in peristaltic pump well before the HPLC pump stops, causing the spray chamber to flood and the plasma to be extinguished. Since the HPLC pump can only be stopped manually, it is quite possible for the HPLC to pump mobile phase for an extended time before it auto-stops causing extensive flooding of the torch box and the ICP-DRC-MS, which can result in damage to the instrument electronics.

Set up one multi-channel peristaltic pump (Gilson “Minipuls 3” or equivalent) near the ICP- MS spray chamber for the purpose of draining liquid from the spray chamber and a separate multi-channel pump next to that one for the purpose of pumping the internal standard.

1. Do not connect the peristaltic pumps to the control computer; connect the pump to A/C power only. Run the pump in manual mode only.
2. Designate one peristaltic pump for nebulizer waste tubing. Press the + or – (dependent upon direction) to set to control to approximately 6.25 or greater.
3. Designate the other peristaltic pump for post-column internal standard tubing. Press the + or – (dependent upon direction) to set to control to approximately 5.75 or greater.

d. Electrical Connections

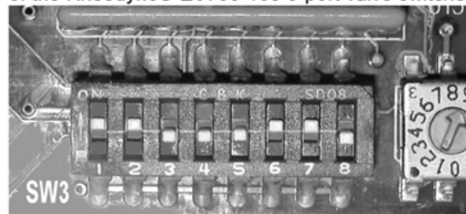
For the Agilent HPLC system, the initial connection of the switching valves to the HPLC system, and configuration to work with Empower software has to be completed by an authorized PerkinElmer service engineer.

e. Six-Port Switching Valves

Each 6-port switching valve’s set of DIP switches need to be configured upon initial installation. The valves will not respond to the ~1 second contact closure from the HPLC autosampler if they are not put into “pulse mode”. This only needs to be done once for each new unit. (Settings of the front panel buttons must be checked before the start of each chromatographic run). For each 6-port switching valve:

1. Turn the switching valve unit upside down and remove the 4 screws. Remove the metal cover and look for DIP switch “SW3” on the printed circuit board. Use a small flat-head screwdriver to adjust the position of each switch so that it matches the following illustration. This illustration applies to both EV750-100 and EV750-100-S2.

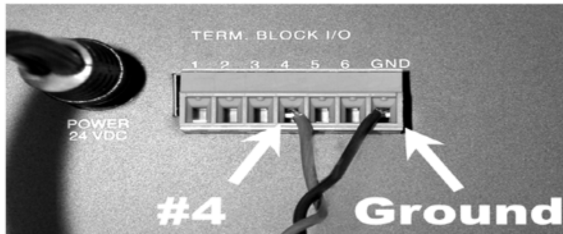
Detail close-up of DIP switch “SW3” on circuit board of the Rheodyne® EV750-100 6-port valve switcher.



Rheodyne® EV750-100 DIP Switch “SW3”								
DIP switch #	1	2	3	4	5	6	7	8
Position	up	up	down	down	down	up	up	down

2. A PerkinElmer service engineer will configure a remote cable to connect the Agilent HPLC to the left and right switching valves.

Detail of Terminal Block I/O located on back of the Rheodyne® EV750-100 6-port valve.



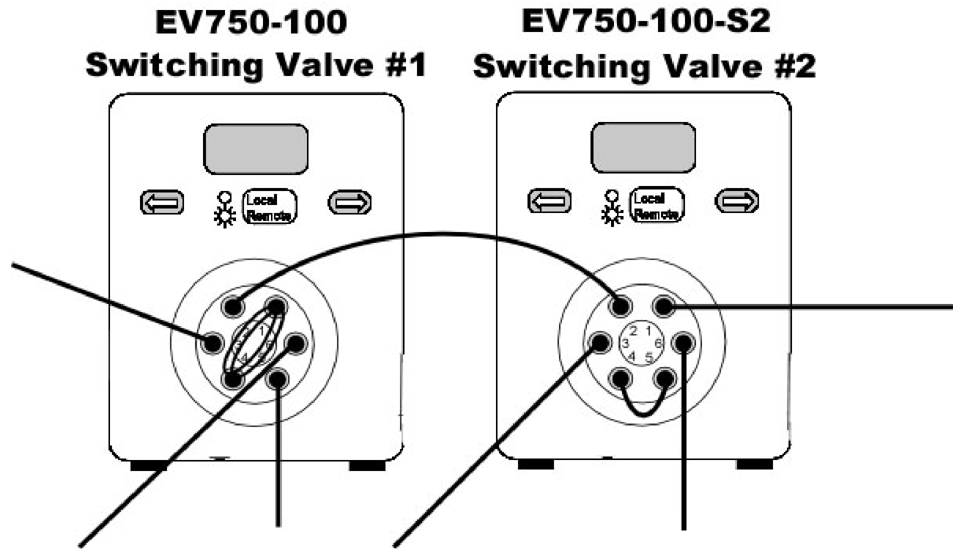
3. Position both 6-port switching valves close to and on the left side of the HPLC Column Oven, adhering to the order listed in Section 8.a.3. Using supplied HPLC pressure fittings, make HPLC tubing connections to the appropriate ports as described in TABLE 8-1-A and TABLE 8-1-B.

TABLE 8-1-A: TUBING CONNECTIONS ON EV750-100

Valve Port	Flow Direction		Tubing Description
	From	To	
#1	Valve Port #1	Sample Loop	PEEK (200 µL loop)
#2	Valve Port #2 on EV750-100-S2	Valve Port #2	HPLC tubing, 0.007″ i.d., 1/16″ O.D., “yellow” PEEK
#3	Valve Port #3	Nebulizer	PEEK Sample Uptake Fitting
#4	Sample Loop	Valve Port #4	PEEK (200 µL loop)
#5	Valve Port #5	Waste	PP or PFA Tubing, 0.03″ i.d., 1/16″ o.d.,
#6	Internal Standard (via external peristaltic pump)	Valve Port #6	PP or PFA Tubing, 0.03″ i.d., 1/16″ o.d.,

TABLE 8-1-B: TUBING CONNECTIONS ON EV750-100-S2

Valve Port	Flow Direction		Tubing Description
	From	To	
#1	HPLC column	Valve Port #1	HPLC tubing, 0.007″ i.d., 1/16″ O.D., “yellow” PEEK
#2	Valve Port #2	Valve Port #2 on EV750-100	HPLC tubing, 0.007″ i.d., 1/16″ O.D., “yellow” PEEK
#3	Line-In for Various Solutions (via ICP-MS peristaltic pump)	Valve Port #3	PP or PFA Tubing, 0.03″ i.d., 1/16″ o.d.,
#4	Valve Port #4	bridge	HPLC tubing, 0.007″ i.d., 1/16″ O.D., “yellow” PEEK
#5	bridge	Valve Port #5	HPLC tubing, 0.007″ i.d., 1/16″ O.D., “yellow” PEEK
#6	Valve Port #6	Tygon™ Waste Line	PP or PFA Tubing, 0.03″ i.d., 1/16″ o.d.,



4. The switching valves are now ready for operation.

9. INSTRUMENT SETUP AND CONFIGURATION

a. Method Set Up in Empower Quickstart

The setup of the method in Empower Quickstart should be completed by a PerkinElmer engineer.

1. In Empower Quickstart, select "View Method" from the left hand side of the window. Click File → Open → Select method "As_HPLC1_DRC". On the "Instrument Method" tab verify all of the below.
2. In the "NexION 300D" window, verify all of the information in TABLE 9-1 is correct.

TABLE 9-1: NEXION® EMPOWER PARAMETERS

Settings	
Run Time (min):	9.5
Mass Calibration:	C:\Users\Public\Documents\PerkinElmerSyngistix\ICPMS\MassCal\default.tun
Conditions:	C:\Users\Public\Documents\PerkinElmerSyngistix\ICPMS\Conditions\As_HPLC.dac
Mode:	DRC
Cell Gas A Flow (mL/min):	0.6
Cell Gas B Flow (mL/min):	0
Advanced	
QID:	On
Detector:	Pulse
Sampling (pts/s):	3.9
Readings:	2244
Plasma Off at End of Method:	Unchecked
Diverter Valve	
Delay after Start (min):	0
Switch at (min):	0.0
Post- Injection Events	
Time (min):	0.0
Valve Position:	LC to ICP-MS

Add Analytes

Name	Mass	Dwell Time (ms)	RPa	RPq	MSIS	Corr	Interference Corrections
As75	74.9216	250.0	0.0	0.50	…	…	

3. In the “Quat. Pump.” Tab of the “AgilentLC” window, verify the information in TABLE 9-2 is correct.

TABLE 9-2: QUAT. PUMP (AGILENT 1260 INFINITY) PARAMETERS

Flow						
1.000:	mL/min					
Solvents						
A:						
B:	Check box; 0.0%					
C:	0.6					
D:	0					
Pressure Limits						
Min:	0.00 bar					
Max:	600.00 bar					
Stoptime	3.9					
Deselect radio button:	As Injector/No Limit					
Select radio button:	9.50 min					
Posttime						
Select radio button:	Off					
Deselect radio button:	0.0 (greyed out)					
Timetable						
Time (min)	A(%)	B(%)	C(%)	D(%)	Flow (mL/min)	Max Pressure Limit (bar)
0.00	100.00	0.0	0.0	0.0	1.0.00	600.00
5.00	0.0	100.0	0.0	0.0	---	---
9.50	0.0	100.0	0.0	0.0	---	---

4. In the "Sampler" tab on the "AgilentLC" window, verify all the information in TABLE 9-3 is correct.

TABLE 9-3: HIP SAMPLER (AGILENT 1260 INFINITY) PARAMETERS

Injection	
Injection volume:	20.00 µl
Needle Wash	
Checked	Enable Needle Wash
Mode:	Flush Port
Time:	1.0 s
Location:	
Repeat:	
Auxiliary	
Draw Speed:	200.0 µL/min
Eject Speed:	200.0 µL/min
Draw position:	0.0 mm
Equilibration time:	2.0 sec
Sample flush out factor:	5.0 times injection volume
Unchecked:	Vial/Well bottom sensing
Stoptime	3.9
Select radio button:	As Pump/No Limit
Deselect radio button:	1.00 (greyed out)
Posttime	
Select radio button:	Off
Deselect radio button:	1.00 (greyed out)
High Throughput	
Unchecked	Automatic delay volume reduction
Unchecked	Enable overlapped injection
Greyed out	When Sample is Flushed Out
Greyed out	After Period of Time

5. In the Column Comp." tab of the "AgilentLC" window, verify all of the information in TABLE 9-4 is correct.

TABLE 9-4: COLUMN COMP. (AGILENT 1260 INFINITY) PARAMETERS

Temperature (for both left and right)	
Select radio button for specific temp:	35.0 °C
Valve	
Port 1 -> 2	
Enable Analysis (for both right and left)	
Checked	When front door open
Radio button for:	When temperature is within +/- 0.8 °C
Stoptime	3.9
Select radio button:	As Pump/Injector
Deselect radio button:	1.00 (greyed out)
Posttime	
Select radio button:	Off
Deselect radio button:	1.00 (greyed out)

6. Select the the "PCI Valve" tab, verify that that the table reads Time (min): 0.5 and Pulse (Min): 0.5.
7. On the "Pretreatment Method" tab select the "AglientLC" icon and verify the following information in TABLE 9-5.

TABLE 9-5: PRETREATMENT METHOD (AGILENT 1260 INFINITY) PARAMETERS

Function	Parameter
Wait:	Wait 6.5 min.
Draw:	Draw default volume from sample with default speed using default offset
Inject:	Inject

8. Leave all other tabs in the Instrument Method as is. The method "As_HPLC1_DRC" is ready for use.
9. Open the method "As_HPLC1_DRC_WASH" and verify that the run time for the "NexION 300D" is set to 20 min. On the "AgilentLC" tab for the "Quat. Pump" verify that Solvents D: is checked and set to 100.0% and the stoptime is set for 20.00 min. The "Column Comp." should have not controlled selected for temperature. Once those have been verified "As_HPLC1_DRC_WASH" is ready for use.
10. Open the method "SHUTDOWN_CDC" in the "Instrument" tab verify the following: for the "NexION 300D" that the box is checked for "Plasma Off at End of Method". On the "AgilentLC" tab, for the "Quat. Pump" the flow is 0.000 mL/min and the stoptime is 1.00 min. Once those have been verified "SHUTDOWN_CDC" is ready for use.

b. ICP-DRC-MS Instrument Setup

To improve workflow, complete the programming steps described in this section before the instrument is used for its first analytical run.

(1) Programming the DRC™ Gas Flow Delay Parameter

A special NexION® DRC™ setting, called "Flow Delay", needs to be changed from its default setting to avoid the problem of the NexION® software forcing a time delay of several seconds before collecting data at the start of a chromatographic run in DRC™ mode. This change only needs to be done once per software installation or upgrade, or if the setting was deliberately changed by a field service engineer. It is a good idea to inform the service engineer who intends to perform work on the instrument of the importance of returning the "Flow Delay" to the non-default value of 1. This must be done in Service mode.

1. From within the NexION® software in the "Instrument Control Session" window, choose menu item Options > Configuration.
2. Click on the "set" button under the "Cell Gas Delays" section. If the "Flow Delay" (Gas changes while in DRC™ Mode) setting is a value other than "60", change the value in the field named "Flow Change" to "60". Click "Apply" then click "Close."

(2) Programming the NexION® ".mth" file

1. If it is not already open, launch the NexION® program and in the "Instrument Control Session" window, choose menu item File > Review Files. Click the "Load" button for "Method", the first item on the list. Navigate to the folder "C:\NexIONdata\Method" or alternatively "C:\NexIONdata\Method\As Spec Methods" and click on "As_HPLC-1_drc.mth" file* then click the "Open" button.
2. Proceed to step three unless, the "As_HPLC-1_drc.mth" file cannot be found, or it has been changed or corrupted in a manner that makes its use questionable. If this is the case, cancel the open file dialog box and close the Review Files window by clicking "Done". Perform the following steps:
   (a) Make the active method file the active window. Do this by clicking on the tool bar icon that looks like a notepad with a "Cu" on it. Click File > New on the menu bar and then choose "Data Only" in the New Method window that appears. Click "OK "then maximize the window. Enter the information in TABLE 9-6 into this window :

TABLE 9-6: NEXION® TIMING PARAMETERS

Parameter	Setting
Sweeps/Reading:	1
Readings/Replicate:	1403
Number of Replicates:	1
Mass Cal File:	C:\NexIONdata\masscal\default.tun
Mass Cal File (Syngistix):	C:\Users\Public\Documents\PerkinElmerSyngistix\ICPMS\MassCal\default.tun
Optimization File:	C:\NexIONdata\conditions\As_HPLC.dac
Optimization File (Syngistix):	C:\Users\Public\Documents\PerkinElmerSyngistix\ICPMS\Conditions\As_HPLC.dac

*Actual file names may differ from those presented throughout this document.

(b) On the first line of the worksheet-like table, click in the cell of row 1 of the "Analyte (*)" column. Type "As" then the press "Enter". The row will suddenly be filled-in with arsenic's "Begin Mass (amu)" of 74.92 (or something close) and several default parameters. Tab from cell to cell to fill in the information shown in TABLE 9-7.

TABLE 9-7: NEXION® ANALYTE PARAMETERS

Parameter	Setting
Analyte:	As
Begin Mass (amu):	74.9216 (or something close will be automatically entered by software)
End Mass:	<leave empty>
Scan Mode:	Peak Hopping
MCA Channels:	1
Dwell Time:	488
Integration Time:	(automatically determined by software)
Corrections:	<leave empty>
Mode:	<right click to choose> DRC
Cell Gas A:	<instrument specific> 0.55
Cell Gas B:	<leave empty>
RPa:	<leave empty>
RPq:	<instrument specific> 0.50

(c) Click on the "Processing" tab and enter the information from TABLE 9-8.

TABLE 9-8: NEXION® PROCESSING PARAMETERS

Parameter	Setting
Detector:	Pulse
Measurement Unit:	Cps
Process Spectral Peak:	Average
Process Signal Profile:	Average
Baseline Readings:	0
Apply Smoothing:	Checked
Factor:	5
Auto Lens:	On
Isotope Ratio Mode:	Off

(d) Skip the "Equation" tab. Click on the "Sampling" tab and enter the information from TABLE 9-9.

TABLE 9-9: NEXION® SAMPLING PARAMETERS

Parameter	Setting
Peristaltic Pump Under Computer Control:	Checked
Peristaltic Pump:	
Sample Flush:	Time: 0 Speed: 0.0
Read Delay:	Time: 0 Speed: 0.0
Analysis:	Time: 0 Speed: 0.0
Wash:	Time: 0 Speed: 0.0
Sampling:	External

(e) Click on the “Report” tab and enter the information TABLE 9-10.

TABLE 9-10: NEXION® REPORT PARAMETERS

Parameter	Setting
Report View | Send to Printer:	Unchecked
Report Options Template:	<leave empty> *
Automatically Generate NetCDF File:	C:\NexIONdata\reportoutput\
Automatically Generate NetCDF File (Syngistix):	C:\Users\Public\Documents\PerkinElmerSyngistix\ICPMS\ReportOutput\
Report to File | Send to File:	Unchecked
Report Options Template:	<leave empty> *
Report File Name:	<leave empty> *
Report Format:	Use separator
File Write Option	Append

* Content of these fields is not important since Send To Printer/File is unchecked.

(f) Choose menu item File > Save As and navigate to “C:\NexIONdata\Methods\” folder. Enter “As_HPLC-1_drc.mth” as the name of the method file and click the “Save” button.

3. The NexION® method “As_HPLC-1_drc.mth” is now loaded into memory.

(3) Programming the NexION® “.dac” file

1. If it is not already open, launch the NexION® program and in the “Instrument Control Session” window, choose menu item File > Review Files. Click the “Load” button for “Conditions”, the sixth item on the list. Navigate to the folder “C:\NexIONdata\Conditions” and click on the “as_hplc.dac” file then “Open”.
2. If the “as_hplc.dac” file cannot be found, or it has been changed or corrupted in a manner that makes its use questionable, cancel the open file dialog box and close the Review Files window by clicking the “Done” button. Do the following steps; otherwise, proceed to step 4:

   (a) Make the active method file the active window (do this by clicking on the tool bar icon that looks like two gears). Then click File > Open on the menu bar, navigate to the folder “C:\NexIONdata\conditions\”. Click on the most current “default.dac” file then click the OK button. Complete the “Current Value” column with the information in TABLE 9-11. *Note:*

Values in TABLE 9-8 *are suggested starting values. Instruments vary in their optimal parameter values, and analysts will use their discretion and expertise in establishing final parameters.*

TABLE 9-11: NEXION® OPTIMIZATION PARAMETERS

Parameter	Setting	Parameter	Setting
Nebulizer Gas Flow (NEB):	0.9*		
Auxiliary Gas Flow:	1.2	DRC™ Mode NEB	0.9*
Plasma Gas Flow	18	DRC™ Mode QRO	-7.5*
ICP RF Power:	1600	DRC™ Mode Cell Entrance Voltage	-4*
Analog Stage Voltage:	- 1600*	DRC™ Mode Cell Exit Voltage	-4*
Pulse Stage Voltage:	1000*		
Discriminator Threshold:	12	DRC™ Mode CRO	-2*
Deflector Voltage:	-10*		
Quadrupole Rod Offset [QRO]	0*	DRC™ Mode AFT	250*
Cell Entrance Voltage	- 3*	DRC™ Mode RPa	0
Cell Exit Voltage	-3*	DRC™ Mode RPq	0.50*
Cell Rod Offset {CRO}	-12*	DRC™ Mode Cell Gas A	0.55*
		DRC™ Mode Cell Gas B	0

*Suggested starting values only. Optimum parameters will depend on outcome of the optimization procedure.

(b) Choose menu item File > Save As in the NexION® "Instrument Control Session" window menu bar and navigate to "C:\NexIONdata\Conditions\" folder. Enter "as_hplc.dac" as the name of the optimization file and click the "Save" button.

The NexION® method "as_hplc.dac" is now loaded into memory.

(4) Creating the Empower Sample Set Method Set

NOTE: T*his will take you through the initial process of setting up a sample set method. This only needs to be done once upon initial set up. It will be saved on each instrument with the title XXX. The daily sample set method can be made by opening the generic sample set method and editing as needed using steps C. xiv. 5.*

1. Click "File" → "New Sample Set" → "Using Wizard…"
2. Select the radio button for "Create a sample set method using this wizard" → "Next".
3. Ensure that the radio button for "LC or PDA/MS" is selected.
4. Click on the "Define Plate" button.
   (a) Click in the row beneath the heading "Plate Type Name" and select the desired plate format depending on the type of sample geometry to be used. The plate layout position will automatically fill in the number 1. For analysis using HPLC vials the configuration is "54VialPlate". Click in row 2 and it will automatically fill in the plate type used in row 1 and set the plate layout position as 2. Click "Ok" → "Next".
5. In the "Set Location of Standards" window

(a) Select the radio button for “At start of sample set”. In the “Start loading vials in tray position:” set to “1:A,1”. Click “Next”.

6. In “Specify Calibration Options” window, ensure the radio button for “Each standard vial contains a different level standard” is selected → “Next”.

7. In the “Describe Standard Bracket” window

   (a) Fill in the number of samples set for the number of standards to be used. This should be your EQ + the samples of your calibration curve. NOTE: For EQ plus S and T series, the number is 11 and for EQ plus only S series the number is 6.

   (b) Number of injections = 1.

   (c) Injection volume = 20.0.

   (d) Run time = 9.5.

   (e) In the “Method Set” select the one titled “As_HPLC1_DRC”.

   (f) Click “Next”.

8. In the “Describe Samples:” window

   (a) Fill in the number of samples for the number of QC, blanks, and samples to be used.

   (b) Number of injections = 1.

   (c) Injection volume = 20.0.

   (d) Run time =9.5.

   (e) In the “Method Set” select, the one titled “As_HPLC1_DRC”.

   (f) Click “Next”.

9. In the “Identification” Window, all options for “How should your standard be identified?” will be grayed out.

   (a) Leave “SampleName” as Std.

10. In the “How should your samples be identified?” Options are as follows.

    (a) Leave the “Area_Correction” as “None” → “Next”.

    (b) Leave the “Blank_Correction” as “None” → “Next”.

    (c) Leave “Column Name” empty → “Next”.

    (d) Leave “Column Serial Number” empty → “Next”.

    (e) Leave “Sample Matrix” empty → “Next”.

    (f) Leave “SampleName” as Unk. Leave incrementing prefix and suffix empty. → “Next”.

11. In the “Describe Runtime Options”

    (a) Select the radio button for “Run and process”.

    (b) Leave “Interactive system suitability:” as “Continue on fault”.

    (c) Click “Next”.

12. Review the information in the “Summary” window. If correct, select “Finish”.

13. The “Component Editor” window pops up.

(a) At the top of the window, select “Copy from the Process Method”. This will populate the “Component” column.

(b) The “SampleSet Type” drop down should have “STANDARDS & UNKNOWNS” selected.

(c) In the row for “ISTD” put the value of 1.0 for all columns title “Value(Standard) or Value(Unknown).

(d) For AC, AB, AsIII, DMA, MMA, and AsV fill in the component editor table as below in TABLE 9-12. NOTE: For this part, all fields for TMAO are blank.

(e) For TMAO fill in the next 5 columns. See TABLE 9-13.

(f) The “Value (Unknown)” columns should only have “1.000000” in the ISTD row.

(g) Click “OK”.

(h) Your wizard input will now populate the “Sample Set Method” Table at the top of the “Quickstart” screen.

TABLE 9-12. S Series Calibrators

Component	Value (Standard)	Value (Standard)	Value (Standard)	Value (Standard)	Value (Standard)
ISTD	1.000000	1.000000	1.000000	1.000000	1.000000
AC	0.000000	2.000000	10.000000	50.000000	150.000000
AB	0.000000	2.000000	10.000000	50.000000	150.000000
TMAO					
AsIII	0.000000	2.000000	10.000000	50.000000	150.000000
DMA	0.000000	2.000000	10.000000	50.000000	150.000000
MMA	0.000000	2.000000	10.000000	50.000000	150.000000
AsV	0.000000	2.000000	10.000000	50.000000	150.000000

TABLE 9-13. S and T Series Calibrators

Component		Value (Standard)	Value (Standard)	Value (Standard)	Value (Standard)	Value (Standard)	Value (Standard)
ISTD	Note: There are	1.000000					
AC	4 preceding	150.000000					
AB	columns for	150.000000					
TMAO	“Value (Standard)”		0.000000	2.000000	10.000000	50.000000	150.000000
AsIII	that have been	150.000000					
DMA	excluded from this	150.000000					
MMA	table due to space	150.000000					
AsV	limitations.	150.000000					

14. In the "Sample Set Method" make the following edits,
    (a) Change the "# of Injs" to 5 and the "SampleName" to EQ for the sample in row 2.
    (b) Change the "Label" to STBlank and the "SampleName" to S0 for the sample in row 3. NOTE: Correct spelling and capitalization is critical to ensure that this sample is used as the standard blank.
    (c) For all subsequent standards, change the "SampleName" to S1, S2, etc.
    (d) For the first unknown sample, ensure the "Label" and "SampleName" column read BLANK. NOTE: Correct spelling and capitalization is critical to ensure that this sample is used as the sample blank.
    (e) The sample ID list including all subsequent blanks and QC can be made using the "ELAN Sample Naming Wrksht".
        (i) Once the naming worksheet is done copy the column "Sample ID".
        (ii) Paste in information in by clicking on the first "Unk" cell after the "BLANK".
    (f) Add two rows by clicking on the row after your last Bk sample.
        (i) In the first, change "SampleName" to "WASH", The "Method Set/Report Method" to "As_HPLC1_DRC_WASH), "Run Time (Minutes)" to 20.00, ensure there is an empty vial in the tray position of the WASH sample.
        (ii) In the second row, change the "Method Set/Report Method" to "SHUTDOWN_CDC)". NOTE: The Plate/Well position for the shutdown method should be the same as the wash sample.
    (g) Save the Sample Set Method by clicking "File" → "Save Sample Set Method As…" → Filename should be "Vials S&T" or "Vials S only". NOTE: If opening one of the templates go through the "Save as" procedure again using Filename "AsYYMMDD".

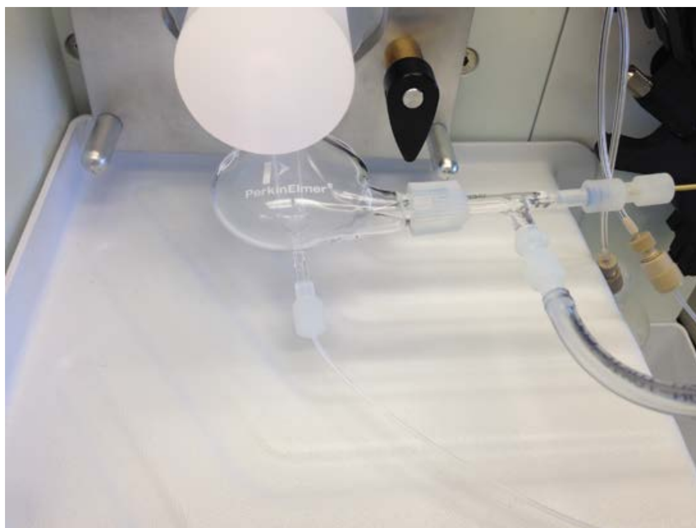
10. INSTRUMENT START UP, ANALYSIS, AND SHUT DOWN

a. HPLC–ICP-DRC-MS System Connection and Startup

(1) Interfacing the HPLC Column to the ICP-DRC-MS Nebulizer

1. Turn off the ICP-MS plasma if it is on.
2. Remove any non-HPLC tubing that may have been installed in the nebulizer.

   Connect the HPLC column effluent tubing (coming from port #3 of switching valve #1) to the ICP-DRC-MS nebulizer/spray chamber assembly as shown below.



> **Important!**
>
> Inspect the tubing-nebulizer interface. It is important that there is no gap between the end of the HPLC tubing and the portion of the nebulizer where it abruptly narrows to a capillary tube. Small gaps can contribute significantly to chromatographic peak broadening and tailing.

(2) Priming the HPLC Pump on the Agilent System

1. If it has not already been done, place each mobile phase reservoir tubing into the correct reservoir bottles (i.e. place end of tubing “A” into the bottle containing HPLC Buffer A, and end of tubing “B” into the bottle containing HPLC Buffer B). Reservoir tubing “D” is placed into a bottle containing 5% acetonitrile in water.
2. Open the purge port by turning the black knob on the pump counterclockwise ~¼ to ½ turn.
3. From the run samples screen in Empower Quickstart, right-click in the “Quat. Pump” HPLC control window and select “Method”.
4. In the section labeled “Flow change 0.000 mL/min to 1.000 mL/min. The pump will first initialize and then go to “On” mode.
5. Click “ok” to return to the run samples screen.
6. Allow the pump to purge at 100% A until all bubbles are flushed out of the tubing.
7. Once A has purged, right-click in the “Quat. Pump” HPLC control window and select “Method”. In the “Solvents” section change B: 0.0% to B: 100.0%.
8. Click “ok” to return to the run samples screen.
9. Once buffer B has purged right-click in the “Quat. Pump” HPLC control window and select “Method”. Change B: 100.0% to 0.0%. Change the “Flow” from 1.000 mL/min to 0.000 mL/min. Click “Ok” to return to the run samples screen.
10. Close the valve (turn the knob clockwise).

(3) Adjusting the Peristaltic Pumps

1. Check the external peristaltic pump’s tubing for signs of wear. Flattening of the tubing is indicative of excessive wear. Excessively worn tubing must be

replaced.

2. If necessary, install new large diameter white-black two-stop peristaltic pump tubing on the bottom channel of the external peristaltic pump that controls the draining of the spray chamber. If the pump is rotating clockwise, connect the left end of the white-black tubing to the tubing that empties the ICP-MS' spray chamber. Connect the right end of the "white-black" tubing to the Tygon waste line that leads to the large liquid waste carboy. Close the channel clamp. Do a preliminary tightening of the peristaltic pump channel's tension clamps on the "white-black" pump tubing. Later, when you are able to observe liquid actively draining from the spray chamber, you will make further adjustments to the tension clamps so that the spray chamber will properly drain without applying excessive pressure on the tubing.
3. On the peristaltic pump that controls the internal standard flow, install new black-black two-stop peristaltic pump tubing on the top channel and close its clamp. Note that the peristaltic pump will rotate counterclockwise. Into the right end of the black-black peristaltic tubing, insert the free end of the tubing that will draw Internal Standard solution (i.e. the one that will come from the Internal Standard bottle). Into the left end of the black-black peristaltic tubing, insert the tubing that will carry Internal Standard to Port #6 of switching valve #1 (see TABLE 8-1-A).
4. On the instrument peristaltic pump, install new black-black pump tubing and close the clamp. Note that the peristaltic pump will rotate counterclockwise. Into the bottom end of the black-black tubing, insert the end of the sampling probe that will draw daily solution. Into the top end of the tubing, insert the tubing that will carry daily solution to port #3 of switching valve #2 (see TABLE 8-1-B).

b. ICP-DRC-MS Warm Up and Performance Check

1. Perform a pre-ignition check of the ICP-DRC-MS according to PE recommendations specified in the manual.
2. Launch the NexION® Syngistix for ICP-MS program and note whether all graphical indicators of instrument readiness are green. If not, take the appropriate actions described in the instrument's software and hardware manual.
3. Perform necessary daily maintenance checks as described in Chapter 2 of the *NexION® 300 ICP/MS System Maintenance Guide* (e.g., argon supply, interface components, cleanliness, positioning, and interface pump oil condition). Note the base vacuum pressure in the INSTRUMENT window of the software. (Before igniting the plasma, the vacuum is typically between 8×10^{-7} and 1.8×10^{-6} torr). Keep a record of any maintenance procedures along with the base vacuum pressure in the *Daily Maintenance Checklist* logbook.
4. Start the external peristaltic pump that controls the waste coming from the spray chamber by pressing the appropriate arrow on the peristaltic pump control panel. Press either the up or down arrow keys to adjust the peristaltic pump speed to approximately "6.25 or greater". Ensure that the direction of rotation is correct so that the spray chamber is being drained and that waste liquid will go to the waste container.
5. In the INSTRUMENT window of the NexION® software, click the "Main" tab and click the plasma "Start" button to ignite the plasma, or alternatively in Empower Quickstart in the RUN SAMPLES window click on "Turn Plasma On" in the NexION ICP-MS section. In the Syngistix INSTRUMENT window, the ignition

sequence bar (blue progress bar) will start to expand to the right, indicating the approximate time before plasma ignition. Before the bar reaches its end, look at the spray chamber on the ICP-MS and watch for plasma ignition. Proper ignition will occur suddenly and with a single audible “pop”. A bright white light will emanate from the injector assembly that connects to the spray chamber. The light may at first flicker, but it will establish a more or less steady intensity after 5–10 seconds unless a problem exists with the system.

On a rare occasion, the plasma may ignite emitting an orange, violently flickering light, and electrical discharge noises will be heard. In this case, immediately **shut off the plasma by pressing the button next to the photo depicting a plasma** on the ICP-DRC-MS instrument’s front control panel. Wait 30 seconds then investigate the cause of the plasma misfire.

6. On occasion, the plasma may extinguish a few seconds after ignition. Promptly reignite by pressing the “Start” button on the ICP-DRC-MS instrument’s front control panel. Usually, the plasma will stay lit after the second try, but if not, investigate the cause of the plasma instability (refer to the *NexION® 300 ICP/MS System Maintenance Guide*).
7. Soon after the plasma ignites, place the sample probe (the one connected to the instrument peristaltic pump’s “black-black” tubing, PerkinElmer® P/N B300-0161) into the daily performance check solution. In The NexION® software, click the “Devices” tab and set the speed on the peristaltic pump to approximately 20 if the pump fails to start when the plasma is lit. Watch the tubing that drains the spray chamber for a half minute or so. If the tubing is filling with liquid and you do not see bubbles being carried away from the spray chamber drain (and especially if you see liquid starting to rise within the spray chamber) immediately remove the sample probe from the rinse solution. Check that the peristaltic pump is rotating in the proper direction so that the spray chamber is draining. If not, immediately correct the direction of rotation on the peristaltic pump. Next, tighten the thumb screw on the tension clamp of the peristaltic pump about ¼ turn. Examine smoothness of flow of liquid draining from the spray chamber. If there is no liquid flow or if it continually “starts and stops”, tighten the thumb screw again. Keep tightening the thumb screw until large bubbles flow through the drain line at a consistent pace. Now, slowly loosen the thumb screw until the flow stops or becomes hesitant. Make one final adjustment by tightening the thumb screw ½ turn. At this point, the tension on the peristaltic pump tubing will be correct, unless a problem exists. Re-insert the sample probe into the rinse solution.
8. Repeat the preceding steps for adjusting the tension clamp for the “black-black” tubing on the channel if the flow is not steady and consistent.
9. Let the ICP-DRC-MS warm up for 30-45 minutes.
10. The following step is for the initial method setup only:
    (a) While the instrument is warming up, in the NexION® program window titled “Instrument Control Session”, choose menu item Review. Click the “Load” button for “Conditions”, the sixth item on the list. Navigate to the folder “C:\Users\Public\Public Documents\PerkinElmer Syngistix\ICPMS\Conditions” and click on “as_hplc.dac” file then click the “Open” button. Return to Review and click the “Load” button for “Method”, the first item on the list. Navigate to the folder “C:\Users\Public\Public Documents\PerkinElmer Syngistix\ICPMS\\Method” and click on “Daily Performance.mth” file

then click the “Open” button. Add a new line for arsenic “As” in the Quantitative Analysis Method window. Set the Dwell Time to 50. Do a File > Save and save the edited method as “As_HPLC_daily.mth”. Click on the Sampling tab and uncheck “Peristaltic Pump under Computer Control”. Return to the Timing tab.

11. After warm-up, complete the appropriate daily optimization procedures as described in Chapter 4 of the *NexION® Software Guide.* Include beryllium (m/z 9) in the mass calibration, and be sure to use mass calibration solution containing 10 μg/L beryllium if a mass calibration is needed. Do the autolens optimization and daily performance check by using a 1 μg/L multi-element solution that includes 1 μg/L of arsenic. Instrument response for 1 μg/L arsenic will give counts >2000 cps (in Standard Mode) when the instrument is properly optimized. Fill in the *Daily Maintenance Checklist* in the instrument logbook according to the completed optimization procedures. If a tuning (mass-calibration) procedure was done, save it to the file “default.tun,” and also in a separate file containing the analysis date “default_MMDDYY.tun” (where MM=month, DD=day, and YY=year). Save the new optimization parameters (i.e., torch alignment, autolens values, nebulizer gas flow rate, and deflector voltages) to the file “As_HPLC.dac”.
12. Also perform a daily performance check in DRC™ mode to record the arsenic counts that you receive. Record the DRC™ arsenic counts on the *Daily Maintenance Checklist.* Refer to section 10 c.
13. If an HPLC analysis is to be run the same day, you may leave the plasma on until it is time to convert the nebulizer to interface with the HPLC. If not, press “Stop” on the NexION® control panel to turn off the plasma.

c. Turning on the Reaction Cell Gas

1. Flush the cell gas for 20 seconds by lifting the lever on the right side of the instrument.
2. Start the flow of the reaction-cell gas (10% hydrogen, 90% argon) and allow the cell conditions to equilibrate. Make sure the regulator on the reaction-cell gas cylinder is set to 5-7 psi.
3. Click on the “Manual Adjust” tab of the “Conditions” window and choose “DRC” from the drop menu under Mode.
4. Monitor the flow on the mass-flow controller by clicking on the “Diagnostics” tab of the INSTRUMENT window of the NexION® program and in the filter drop down menu, select “Gas Flow Control”. Look for a field labeled “Cell Gas A”. The flow will reach approximately “0.6*” within 10–15 seconds. If possible, allow 30 minutes for the cell to equilibrate before beginning analysis, with the cell gas flowing at 0.6 mL/min*. Note: The cell gas will automatically turn off after 45 minutes if the analysis has not begun.

   *Or the DRC™ gas value that is found to be optimal.

5. Once the cell gas has warmed up, perform a DRC™ neb gas optimization and a deflector voltage optimization. Update the values if needed. Perform a DRC™ Mode Daily Optimization Check and record the results in the *Daily Maintenance Checklist*.
6. Save the new optimization parameters to the file “As_HPLC.dac.” If needed, save it again to a new file named “default <yymmdd.dac>” (where yy=year, mm=month, and dd=day; do not include the brackets in the file name).

7. Place the free end of the tubing that will carry the internal standard into a bottle containing 1 liter of the Internal Standard solution.

d. Entering Sample Names into the Empower Quickstart Sample Set Method

1. Click on the Run Samples tab on the left hand side of Empower Quickstart. If the current Sample Set Method is not this run's sample set, then choose File > New Sample Set > Using Sample Set Method… on the menu bar and navigate to and open this run's current data folder in the Sample Set Method window. Click on the file named "As<yymmdd> (yy = year, mm = digit month, dd = date) and open it. The Sample Set Method window will be the one created in the *Creating the* section.
2. Fill in the name of each sample by double-clicking in the "SampleName" cell matching its "Plate/Well" location. Use a barcode scanner to input the sample name and press "Enter" on the keyboard. In this manner, enter the name of every blank, calibrator, quality control, and sample that will analyzed in the run. Alternatively, a Sample Set Method template saved in Empower can be used to create a sample list, which puts each sample into the correct autosampler formatting to help reduce error.
3. Keep the following in mind while filling out the Samples table.
   (a) Autosampler tray position 1:A,1 will contain the vial containing a low bench QC sample called "EQ", which will be injected with five replicates during the initial system equilibration period before the start of calibration. "EQ" is not used for QC but is strictly for equilibrating the HPLC and conditioning the ICP-DRC-MS.
   (b) Autosampler tray position at the end of the sample run needs to contain a blank vial (even an empty vial will do). **NOTE:** This will change from run to run if the number of samples analyzed is different from previous days.
   (c) Insert a "Bk" between the equilibrators and S0. Also insert blank checks throughout the run as needed to show that carryover is not occurring.
   (d) No more than 24 hours can elapse between the time that the actual analytical run starts (the analysis of S0) and the analysis of the last vial is complete. Keep this in mind when determining how many samples will be analyzed.

4. For example, in a run of 15 samples, the last vial ends up being placed in Plate/Well position 1:D,8. Of course, the actual position of the last sample depends on the total number of vials in the autosampler tray. Note: if more than one group of samples is to be analyzed, each group needs to be bracketed by its own QC. In some instances, on the sample table this rule will result in four QC samples being run in succession (for instance, LU-xxxx, HU-xxxx, LU-xxxx, HU-xxxx). Be sure to delete all unused rows after the last vial in the Sample Set Method window, i.e. clear all rows after the last row by selecting them, right-clicking and select "Delete". **NOTE:** As stated in the *Creating the Empower Sample Set Method* section be sure that the last two rows have the same Plate/Well designation and an empty vial is in the autosampler tray.
5. When satisfied that the Sample Set Method table entries are correct, choose File > Save.

e. Starting the Run

1. Check the waste container. If more than two-thirds full, discard of the waste

using the protocol outlined in SOP DLS 3500 *Handling Corrosive Liquid Laboratory Waste*.

2. Ensure that the tubing that draws internal standard is inserted into the bottle containing internal standard and that there is a sufficient amount of solution to complete the run.
3. Ensure that there is sufficient mobile phase to last the entire run. In addition, be sure that Bottle D and the HPLC Autosampler's wash bottle contains sufficient amount of 5% (v/v) acetonitrile. It is very important that line D does not become filled with air bubbles at any point during analysis.
4. Set the 1260 Column Oven to 35°C if it is not already at that temperature. Verify that the HiP ALS temperature is set at 4°C. Both can be checked in Empower Quickstart in the HPLC control windows of "Column Comp." and "HiPSampler", respectively.
5. Launch Syngistix for ICP-MS® Instrument Control program if it is not already. Do not launch or start any other programs at this time.
6. Check that the correct Sample Set Method file in the window Run Samples Sample Queue" is active. If it is not correct, load the correct Sample file. Check that the correct Empower method is loaded and active in the window "Run Samples > Sample Queue". If it is not correct, load the correct Method file.
7. Check that the DRC™ gas is indeed flowing by making the NexION®'s Instrument window active and clicking on the Diagnostics tab. Inspect the Cell Gas A. Its value will be fluctuating around 0.6 (or other optimal value) ± 0.01 mL/min. If it is not, see section *Turning on the Reaction Cell Gas* for details to turn on the DRC™ gas flow. Make the conditions window active and Choose File > Save to save the method file.
8. Check that all blanks, calibrators, QC and sample vials are loaded into their correct positions in the HPLC autosampler tray, as designated by the run's Sample Set Method.
9. Open the "View Acquisition" tab on the left hand side of Empower Quickstart, click the icon of three test tubes with a green play button to start the run.
10. If the HPLC pump is not already pumping, Empower Quickstart will start the flow of buffer A. This will start the prerun on the HPLC control window. **NOTE:** There is no run time displayed during the pre-run.
11. Open the NexION® "Instrument Control Session" Real-Time window by clicking the tool bar button that looks like a Gaussian distribution (burgundy chromatographic peak). After the Real-Time window opens, click on the drop-down menu and select "Signal". Real-time data will now be displayed. Real time data is also displayed in the View Acquisition window of Empower Quickstart.
12. When the HPLC pump's equilibration time has been reached, the autosampler will seek the first vial and make an injection. The HPLC controls will switch to all green status bars and show the current run time for the sample in Empower Quickstart. The system can now run unattended.
13. Check the progress of the run after 2 or 3 injections. Note the chromatograms appearing in the NexION®'s Real Time window. Adjust the signal scale in the Real Time window, as necessary. Compare the positions and peak heights of each arsenic species relative to the internal standard. It helps to visually compare it to a printed reference chromatogram. If abnormalities in retention time, peak height or shape are readily apparent, the analyst may need to stop the autosampler and pump and abort the run in the Empower Quickstart

program. To do this click the icon of three test tubes with a red stop button. The HPLC pump is stopped by right-clicking in the “Quat. Pump” window of the HPLC control and selecting “Method”. Change the “Flow” from 1.000 mL/min to 0.000 mL/min. Click “Ok”. Correct the problems(s) and restart the run.

f. Instrument Shut Down

1. Shut off the ICP-DRC-MS plasma if it has not already been done. Stop all peristaltic pumps and loosen tensioning bars and tubing.
2. Check that the HPLC autosampler controller readout indicates that the sequence was successfully completed. If not, note the message and investigate the reason for the message (i.e. sample vial was missing).
3. At the instrument computer, check the Sample Set Method and confirm that all samples were analyzed.
4. Remove the calibrator, QC and sample vials from the HPLC tray. Discard them according to CDC biohazard waste disposal guidelines.

11. POST-RUN DATA ANALYSIS

a. Configuration of Empower Quickstart Processing Method

The following information is presented as a starting point to help the analyst develop robust integration method parameters that will work best for most chromatography data. Many of these parameters will work just fine as presented below. However, the separation chemistry of HPLC columns can vary due to frequency of use, column replacement, or because of individual sample “oddities”. Some parameters may need to be adjusted from time to time to maximize the ability of Empower to properly integrate peaks and identify components with minimum operator intervention. Therefore, the analyst must pay particular attention to the chromatograms produced in every run and make necessary adjustments as warranted. The creation of a new processing method in Empower is done the first time by a PerkinElmer technician.

1. Choose the left hand menu item View Method > Processing. Click on the “Integration” tab in the “Processing” window. Enter the information shown in TABLE 11-1. The analyst may make appropriate changes to one or more of the Integration parameters if necessary.

TABLE 11-1: INTEGRATION

Integration Algorithm	ApexTrack		*Peak Integration*	
			Liftoff %:	0.000
Apex Detection			Touchdown %:	0.500
Start (min):	0.227		Minimum Area:	200
End (min):	10.153		Minimum Height:	50
Peak Width (sec):	8.98			
Detection Threshold:	6.400e+000	Time (min)	Type	Value
		0.000	Set Peak Width (sec)	60.000
		1.000	Set Peak Width (sec)	18.770

2. Click on the “Smoothing/Offset” tab. As a guideline leave the information in the tab on the default.
3. Click on the “Components” tab. As a guideline, enter the information shown in TABLE 11-2. Be sure there is a checkmark in the box for “Include Internal Std Amounts in % Amount Calculation”. The parameters in TABLE 11-2 are starting points. The analyst may make appropriate changes to one or more of the Components parameters if necessary.

TABLE 11-2: Components

Average By:	None
RT Window (%):	6.00
Sample Value Type:	Concentration
Update RT:	Never
CCalRef1:	ISTD
RT Reference Used to Name Unnamed Peaks by RRT:	Empty

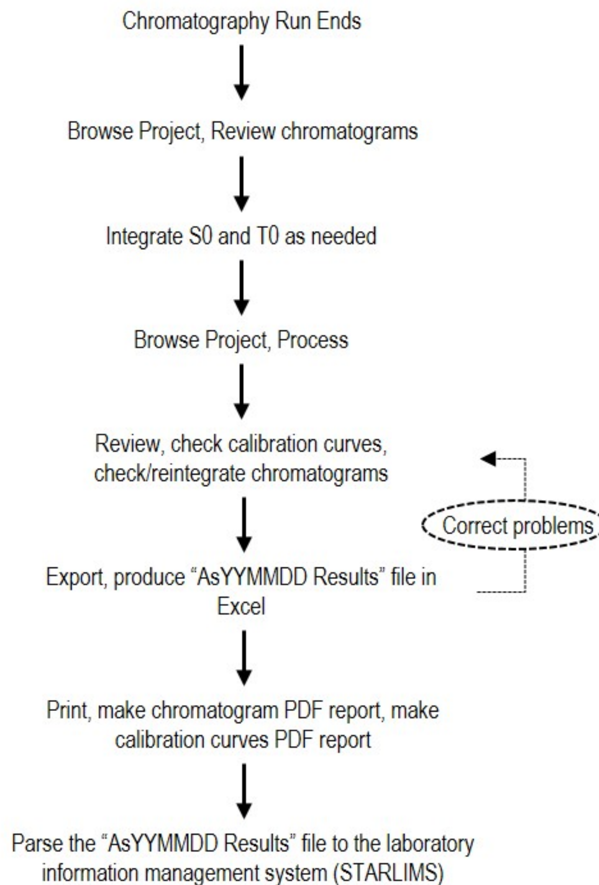
Name	*Retention Time (min)*	*RT Window (min)*	*Peak Match*	*Channel*	*Y Value*	*X Value*	*Fit*	*Weighting*	*Internal Std*	*Impurity RRF*
ISTD	0.635	0.353	Closet	As75	Corrected_Area	Corrected_Concentration	Linear	None		
AC	1.327	0.077	Closet	As75	Corrected_Area	Corrected_Concentration	Linear	None	ISTD	1.000
AB	1.665	0.042	Closet	As75	Corrected_Area	Corrected_Concentration	Linear	None	ISTD	1.000
TMAO	1.782	0.035	Closet	As75	Corrected_Area	Corrected_Concentration	Linear	None	ISTD	1.000
AsIII	2.195	1.000	Closet	As75	Corrected_Area	Corrected_Concentration	Linear	None	ISTD	1.000
DMA	3.392	1.000	Closet	As75	Corrected_Area	Corrected_Concentration	Linear	None	ISTD	1.000
MMA	6.735	2.000	Closet	As75	Corrected_Area	Corrected_Concentration	Linear	None	ISTD	1.000
AsV	8.443	0.750	Closet	As75	Corrected_Area	Corrected_Concentration	Linear	None	ISTD	1.000

4. Leave all default information for the tabs “Impurity”, “Peak Ratios (MS Ion Ratios)”, “Default Amounts/Purity”, “Named Groups”, “Timed Groups”, and “Noise and Drift”.

b. Data Processing and Analysis

Refer to Figure 1 below “Post-Run Data Processing Work Flow Diagram” for a summary representation of the important aspects of post-run data processing.

Figure 1: Post-Run Data Processing Work Flow Diagram



1. Open “Quickstart” and click on “Browse Project”. Click on “Result Sets” tab if not already selected then “Update” in the top tool bar. This will refresh the list of result sets and include the last set analyzed on the system.
2. Highlight the “Result Set Name” for reprocessing →right – click “Review”. In the resulting “Replace” window click “Yes”. Click on “Injection 1” of the EQ sample drop down on the left side of the screen. In the top toolbar, click “Next injection” to go through all samples to check for peak identification.
3. For S0 and T0 (when applicable) analyst must manually integrate for all applicable peaks not detected by the software. This will ensure each calibration curve has the required five points. Zoom in as needed on the chromatogram and manually integrate on the baseline for AC, AB, AsIII, DMA, MMA, and AsV on S0 as needed. Repeat on the T0 chromatogram for TMAO only. To zoom out right click →“FullView”. Click the icon for “Show components” to remove the indicators. NOTE: When changes are made to any chromatogram once done, immediately go to File → Save → “Result”. Do not move to the next chromatogram without doing this and do not select any option enabled except “Result”.

4. Click on “Browse Project” on the left side. Highlight the “Result Set Name” being reprocessed → Right-click →Click “Process”. In the window “Background Processing and Reporting” in the “Processing” section. Check “Clear Calibration” and “Use Existing Integration” if not already done. For “How” select “Calibrate and Quantitate” from the drop down if not already selected. Then click “Ok”. Wait 30 seconds and then click “Update” at the top. A new Result Set Name line will appear above the previous one that was being used for processing.
5. Highlight the new “Result Set Name” → right-click → select “Review”. The result set with S0 and T0 integration is now loaded.
6. Check the calibration curves for each species. In the top toolbar click on the icon that looks like a liquid drop with a line through it for “Calibration Curve”. In the new window, select each species from the “Component” drop down. Verify the following:
    (a) There are five points per curve.
    (b) The R^2 value is 0.9990 or higher.
    (c) Slopes range from 9.0E-3 to 1.3E-2.
7. Check chromatograms for QC and samples. Make adjustments as needed. **NOTE:** When changes are made to any chromatogram once done, immediately go to File → Save → “Result”. Do not move to the next chromatogram without doing this and do not select any option enabled except “Result”.
8. To export the results go back to “Browse Project” → “Results Sets” tab → right-click on the “Result Set Name” desired →“Export”. In the “Exporting” section of the “Background Processing and Reporting” →check “Export” → select the radio button for “Use specified export method” if not already selected. In the drop down, select “Export basic_Final conc” → Click “Ok”.
9. Open Excel. In Excel, select “File” → “Open”. Navigate to the C: drive. Change “All Excel Files” to “All files” in the drop down menu. Sort by date modified and select the export file with a filename exportXXXXX (this file is a text file). NOTE: The XXXXX will change based on the file selected for export in Empower but will always be five numbers. Click “Ok”.
10. In the “Text Import Wizard” window. Select the radio button for “Delimited” → “Next”. Uncheck the box for “Tab” and check the box for “Comma” → next. Click “Finish” on the last window. The file for your Empower results opens.
11. Save this file with the filename format of AsYYMMDD Results to the corresponding run folder on the C: drive. Cut and paste export file from the C: into proper run folder for the day.
12. To print one PDF file of all sample chromatograms go back to “Browse Project” →”Results Sets” tab → right-click on the “Result Set Name” desired →“Print”.
13. In the “Reporting” section of the “Background Processing and Reporting” →check “Print” → select “pdfFactory Pro” from the drop down menu → select the radio button for “Use specified report method” if not already selected. In the drop down, select “Arsenic Speciation” → Click “Ok”.
14. pdfFactory Pro will open and compile the file. Save the file, with the format AsYYMMDD Report, to the corresponding run folder for the day on the C: drive.
15. To print one PDF file of all calibration curves go back to “Browse Project” →the

"Curves" tab → highlight all of the analyte calibration curves → right-click →"Print".

16. In the "Reporting" section of the "Background Processing and Reporting" →check "Print" → select "pdfFactory Pro" from the drop down menu →, click "Ok". In the drop down, select "Arsenic Speciation" → Click "Ok".
17. pdfFactory Pro will open and compile the file. Save the file, with the format AsYYMMDD Calibration Report, to the corresponding run folder for the day on the C: drive.
18. The data processing portion on the instrument controller computer is now complete.

12. RECORDING OF SAMPLE AND QC DATA

a. Transferring the Data to the LIMS

1. Transfer the entire folder labeled "As<yymmdd>" via encrypted USB drive or other data media to the appropriate subdirectory on the network drive where exported data are stored. (Note that directories are named according to instrument\year\month\ for example,

   "\.........\Nutritional\Instruments\NexION\NexION - G\2013\As130110"
2. Use the current "Speciation Lab Parsing Application" file to convert the file saved in section 11 b.11 to a multi-tabbed Excel workbook suitable for data exportation to the LIMS.
3. Import the instrument file into the laboratory information management system with appropriate documentation (e.g. instrument ID, analyst, calibration standards lot number, and run or sample specific comments).
4. Save all instrument data for that day on the shared network drive.

b. QC Data

Once data is transferred to DLS STARLIMS, quality control (QC) samples must be assessed for pass or failure through the generation of QC reports. The QC reports are stored electronically.

13. FINAL REVIEW OF THE DATA

a. Run Data

Instead of printing the data onto paper, it should be stored as a PDF that can be reviewed. If the samples analyzed are part of a study that has an associated study folder, indicate the shared drive location of the electronically stored data.

b. Plotting QC Results

Importing data into DLS STARLIMS allows for the capture of quality control data that can be monitored through the generation of statistical quality control plots using SAS. The analyst must monitor these plots regularly for any trends in the bench QC results. If trends are observed, contact the laboratory supervisor, and investigate the cause of

the trend(s). The QC results for a particular method must also be reviewed periodically by the team leader or lab chief to verify that trends in the data do not exist.

c. Supervisor Review

DLS STARLIMS allows a person with supervisory authority to review the QC and sample results in the system (electronically). After the supervisor reviews the data, he or she may release the data for reporting.

14. QUALITY CONTROL PROCEDURES

The Inorganic and Radiation Analytical Toxicology Branch uses the method described in this protocol for environmental and occupational health screening studies.

The analyst inserts bench QC specimens at the beginning and end of each analytical run (a set of consecutive assays performed without interruption) so that judgments may be made on the day of analysis. Taking these samples through the complete analytical process exposes them to the same treatment and conditions as the patient samples. The data from these QC materials is then used to estimate methodological imprecision and to assess the magnitude of any time-associated trends.

The bench QC pools used in this method comprise two levels of concentrations spanning the “low-normal” and “high-normal” ranges for each arsenic species. Both of these pools are analyzed after the calibration standards are analyzed but before any patient samples are analyzed. These bench QCs will be analyzed again at the end of the run. If a second run of samples are analyzed using the same calibration curve as the first run, the QC results obtained from the second run’s own bench QC samples need to be analyzed and treated independent of the first run.

a. Establish QC limits for each QC pool.

A run to assess the homogeneity of the pools is performed after the pools are aliquotted into individual vials. Vials are randomly chosen and randomly analyzed, and the first and last vials dispensed are always included in the homogeneity study. Unlike the characterization of the QC, the homogeneity study can be completed in a single run. Once analysis is complete, the data is evaluated in terms of QC recovery to determine whether or not trends exist in QC during the dispensing of the pool. If the pool does not vary from beginning to end, characterization of the QC pool is the next step. If problems exist, identify the source(s). The pool has to be re-made and dispensed again unless problem vials can be identified and eliminated.

To complete the characterization that will allow you to assess limits for each pool, analyze a minimum of forty samples of each pool (one at the beginning of the run and one at the end of the run) on 20 different runs, preferably among all of the instruments that will be used to analyze this method. During the 20 characterization runs, QCs from previously characterized pools are also analyzed to evaluate the analytical performance of each run. Once analysis is complete, SAS within DLS STARLIMS is used to calculate the mean and standard deviation for each pool from the concentration results. The statistical limits for each pool are set using this process.

b. Precision and Accuracy

QC Results Evaluation. After completing a run and reprocessing the data, check the results of the QC to determine whether the run is “in control” for each of the seven analytes. *The QC rules*

apply to the average of the beginning and ending analyses of each of the bench QC pools. The QC rules are as follows:

1. If both the low-and the high-QC results are within the 2s limits, the run will be accepted.
2. If one of two QC run means is outside the $2S_m$ limits, the run will be evaluated to determine if any of the following are true. The run will be rejected if any of these conditions are met.
    - **Extreme Outlier** – run mean beyond characterization mean +/- $4S_m$.
    - **1_{3s}** – Average of low QCs OR average of high QCs is outside of a 3s limit.
    - **2_{2s}** – Average of low QCs AND average of high QCs is outside of 2s limit *on the same side of the mean*.
    - **10_x sequential** – The current and previous nine average QCs results (for the previous nine runs) were *on the same side of the mean* for either the low OR high QC.
3. If one of the four individual QC results is outside a 2Si limit, the run will be evaluated to determine if any of the following are true. The run will be rejected if any of these conditions are met.
    (a) **Extreme Outlier** – One individual results is beyond the characterization mean +/- $4S_i$.
    (b) R_{4S}– Within run ranges for all pools in the same run exceed $4S_w$. This rule is applied within runs only. If the run is declared "out of control," the analysis results for all patient samples analyzed during that run are invalid for reporting for the affected analytes.

c. Remedial Action If Calibration or QC Systems Fail to Meet Acceptable Criteria

If an analyte fails to pass QC based upon the QC report, the following steps must be taken, if possible:

- Check the chromatograms for each blank, calibrator, QC, and sample for proper peak integration and identification. Check that the internal standard peak was properly integrated and identified. Change integration parameters or manually reintegrate peaks, if necessary, and reprocess the run in TotalChom™.
- Check the ICP-DRC-MS stability during the run by examining the degree of drift in internal standard raw peak areas over the course of the run. Drift >20% or sudden large changes in internal standard peak area can indicate a problem with plasma instability. Monitor QC recovery to determine if that is the case.
- If the QC failure is likely attributed solely to the calibrators, the run can be repeated with a new set of calibrators using the prepared samples and QC from the original run. This can be done only if the two runs (original and repeat) are performed on two consecutive days. If more than one day has lapsed since the QC failure was noticed, both the samples and QC must be re-prepared.
- If using new calibrators does not resolve the issue, further troubleshooting will be needed. All samples and QC will need to be re-prepared for a new run if the source of the problem is suspected to be the QC or cannot be confirmed.

- If these steps do not result in correction of the out-of-control values for QC materials, consult the supervisor for other appropriate corrective actions. No analytical results will be reported for runs that are not within acceptable statistical control limits.

15. LIMIT OF DETECTION AND LINEAR RANGE TESTED

The limits of detection (LOD) for arsenic species in urine specimens were calculated in accordance to the guidelines established by the Division of Laboratory Sciences. The method used to calculate the LODs is based on the recommendations of the Clinical and Laboratory Standards Institute (CLSI) and take both Type I and Type II errors into consideration. According to the guidelines established by our division, at least four concentration levels are to be analyzed in each run with one level having analyte concentrations below the projected LOD. For this method, the matrix-matched calibration blank was used to satisfy the criterion of having a level below the LOD while the first three calibrators of the standard calibration curve were used for the other three levels. All samples used for LOD calculations were human urine specimens. Multiple analysts were used for the measurements which covered over 60 runs over at least 60 days.

According to division protocol, for linear relationships the following equation is used to calculate the LOD: $conc_{LOD} = [mean_b + 1.645*(s_b + B)] / (1-1.645*A)$ where $mean_b$ is the mean of the blank, s_b is the standard deviation of the blank, B is the y-intercept, and A is the slope. For quadratic relationships ($y=Ax^2 + Bx+C$), the following equation is used to calculate the LOD: $conc_{LOD} = [-b \pm sqrt(b^2-4ac)] / 2a$ where a=1.645*A, b= (1.645*B)-1, and c= $mean_b + 1.645*(s_b+C)$. With both linear and quadratic relationships, the resulting calculated LOD has a 5% false negative rate. TABLE 15-1 lists the LODs calculated for this method.

As stated in the DLS Policies and Procedures Manual “uncertainty increases as values approach the limit of detection.” Division policy prohibits reporting results lower than the lowest calibration curve point. Report results below the detection limit as “< LOD” (where “LOD” is the calculated lowest detection limit). The LOD calculation is reevaluated whenever major changes to the method occur.

During method development, it was established that the arsenic species were linear up to 1000 µg/L.

TABLE 15-1: LIMITS OF DETECTION (LOD) AND LINEAR RANGE TESTED (LRT) FOR AS SPECIES

Species Chemical Name	Abbreviated Name	Limit of Detection, µg/L	Highest Concentration for Linear Range Tested, µg/L
Arsenobetaine	AB	1.16	1000
Arsenocholine	AC	0.11	1000
Trimethylarsine oxide	TMAO	0.17	1000
Monomethylarsonic acid	MMA	0.20	1000
Dimethylarsinic acid	DMA	1.91	1000
Arsenous (III) acid	As(III)	0.12	1000
Arsenic (V) acid	As(V)	0.79	1000

16. REPORTABLE RANGE OF RESULTS

When a sample result for any analyte is greater than the highest calibrator for the same analyte within the run (150 µg/L but less than 1000 µg/L), the sample needs to be reanalyzed using the extended calibration curve. Bracketing elevated QC needs to be analyzed for the extended calibration run along with the patient sample that falls within that range. The sample result will be reported from that run. The maximum dilution allowed for each analyte is a 1:50 dilution; therefore, urine arsenic results are reportable in the range of greater than the LOD up to 50,000 µg/L.

If a patient result for a particular analyte is slightly above 150 µg/L when measured with the normal calibration curve but falls slightly below 150 µg/L when measured with the extended calibration curve, the following measure should be taken. Dilute the sample using the smallest dilution factor possible (i.e. dilution factor of 2) and analyze with the normal calibration curve and bench QC. If the sample volume is limited, a larger dilution factor can be used, but it cannot exceed 50.

Results Greater Than Range of Linearity Tested: Perform an extra dilution on any urine sample whose concentration is greater than the Linear Range Tested (“LRT”) concentration in TABLE 15-1 (the highest concentration for linear range tested). A 1:50 dilution is the maximum dilution that may be performed.

17. SPECIAL PROCEDURE NOTES – CDC MODIFICATIONS

None applicable for this method.

18. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

The argon chloride (ArCl) interferences on arsenic (^{75}As) are eliminated by the operation of the DRC™ under the parameters noted in the sections above during the speciated arsenic analysis.

19. REFERENCE RANGES

The reference range for each arsenic species (see TABLE 19-1) is based on literature reports and from periodic review of accumulated data collected during the analysis of urine samples representing a normal, healthy population believed to be free of unusual exposure to arsenic.

TABLE 19-1: REFERENCE RANGES FOR ARSENIC SPECIES

Species Chemical Name	Reference Range[1], µg/L
Arsenobetaine	<LOD – 30.5
Arsenocholine	<LOD – 0.320
Trimethylarsine oxide	<LOD
Monomethylarsonic acid	0.460 – 1.53
Dimethylarsinic acid	3.08 – 11.8
Arsenous (III) acid	0.470 – 1.19
Arsenic (V) acid	<LOD

[1] Above ranges (50th percentile to 95th percentile) are taken from NHANES population data for the 2013 to 2014 survey years (2011 to 2012 for TMAO) as provided in the *Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, March 2018.* Sample sizes were n=2517 for trimethylarsine oxide and n=2654 for all other species. (http://www.cdc.gov/exposurereport/)

20. ACTION-LEVEL RESULTS

Concentrations for the non-dietary arsenic species - DMA, MMA, As (III), and As (V) - that are observed to be greater than the “first upper boundary” (defined in DLS STARLIMS as the “1UB”) must be confirmed by repeat analysis of a new sample preparation. The 1UB for these four species is 50 µg/L. Report the original result, as long as the confirmation is within 10% of the original. Continue repeat analysis until a concentration can be confirmed.

If a patient sample has a non-dietary arsenic concentration greater than 50 µg/L, the levels will have to be reported by fax, telephone, or E-mail to the supervising physician or principal investigator. This is done by the reviewer(s) of the data and not the analyst.

21. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens may reach and maintain ambient temperature during analysis. Take stringent precautions to avoid external contamination. After the samples are analyzed, return them to approximately -70°C freezer storage as soon as possible.

22. ALTERNATE METHODS FOR PERFORMING TEST AND STORING SPECIMENS IF TEST SYSTEM FAILS

If the analytical system fails, freezer storage (approximately -70°C or lower) is recommended until the analytical system is restored to full functionality.

23. TEST-RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

Report test results as outlined in the *DLS Policies and Procedures Manual*. For critical calls, the supervisor must notify the supervising physician or principal investigator as soon as possible. The most expeditious means must be used (e.g., telephone, FAX, or E-mail).

24. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

For select studies, the person that receives specimens or samples delivered to the Inorganic and Radiation Analytical Toxicology Branch sets up a “Specimen Folder.” He or she is to fill out a tracking form and place it in the folder to be given to the analyst performing the analysis. The form tracks location, status, and final disposition of the specimens. When sample analysis is completed, update and place the tracking form in the Specimen folder.

Use standard electronic record keeping means (e.g., usage of shared network drives, encrypted USB devices, or CD-R backups) to track specimens. Keep duplicate records in electronic or hard-copy format. Use only numerical identifiers (e.g., case ID numbers); all personal identifiers are available only to the medical supervisor or project coordinator to safeguard confidentiality. Refer to the DLS Policies and Procedures Manual for up-to-date record retention guidelines.

25. BI-ANNUAL INSTRUMENT-TO-INSTRUMENT COMPARISON

Per CLIA requirements, if an analytical method is performed on more than one instrument, then an instrument-to-instrument comparison has to be performed twice per year. The same samples have to be analyzed on each instrument, and the Pearson Product Moment Correlation Coefficient for the results for each species has to be greater than 0.95. If not, remedial action has to be taken.

26. METHOD PERFORMANCE DOCUMENTATION

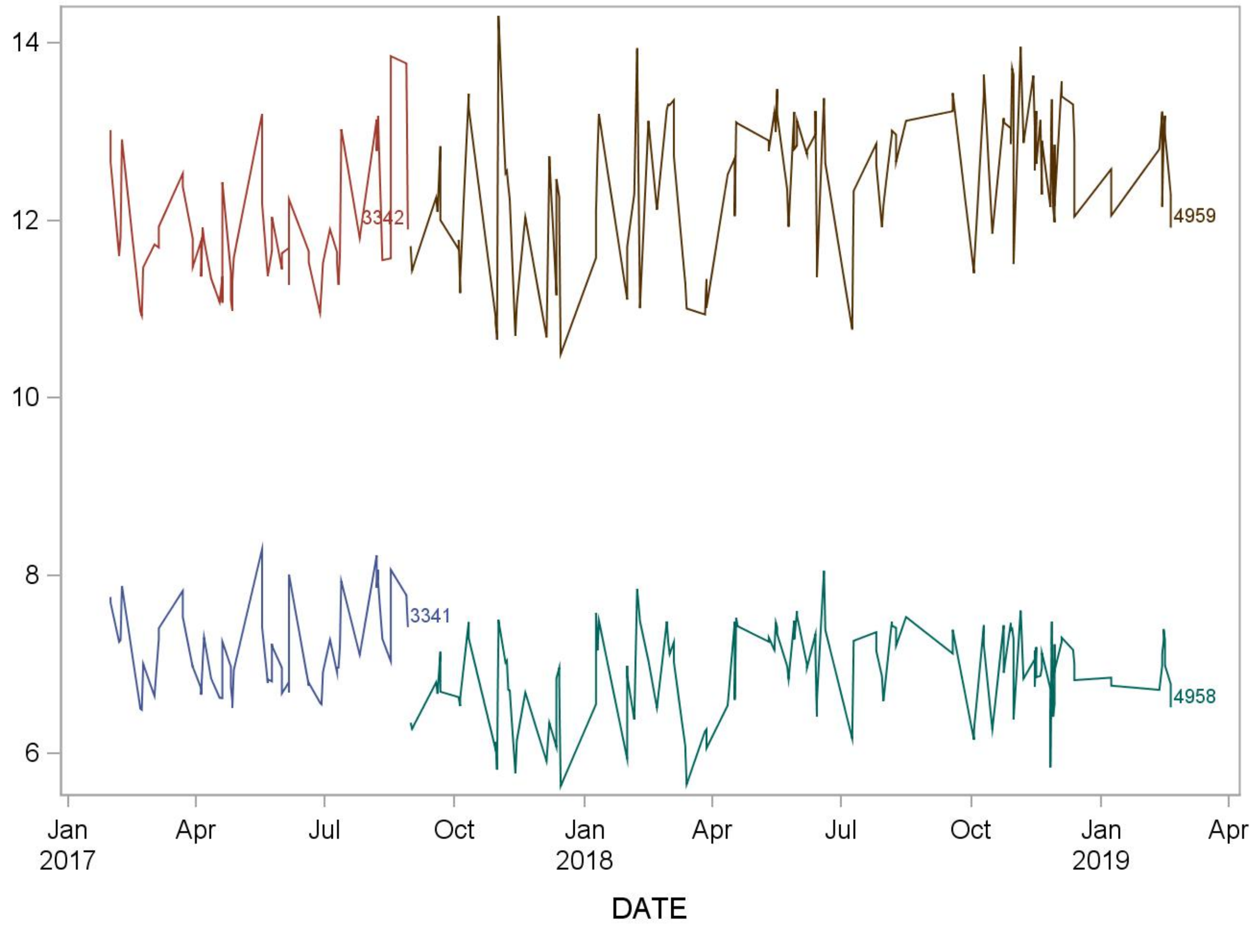
Method performance documentation for this method including accuracy, precision, sensitivity, specificity and stability is provided in Appendix A of this method documentation. T**he signatures of the Branch Chief and Director of the Division of Laboratory Sciences on the first page of this procedure denote that the method performance is fit for the intended use of the method.**

27. SUMMARY STATISTICS AND QC CHARTS

Please see following pages.

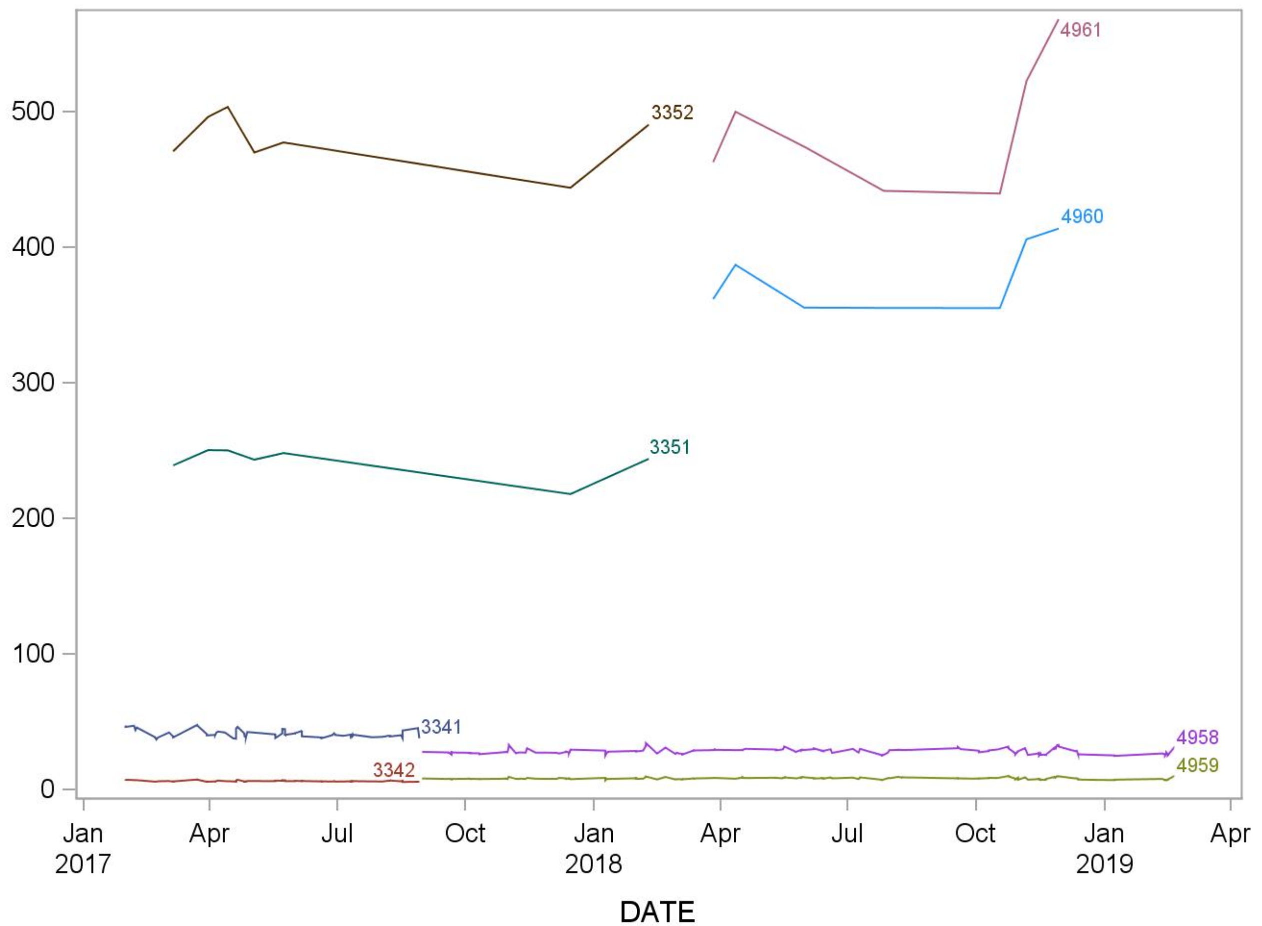
2017-2018 Summary Statistics and QC Chart for Urinary Arsenic acid (µg/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
3341	62	31JAN17	29AUG17	7.1491	0.4911	6.9
3342	62	31JAN17	29AUG17	11.8694	0.7055	5.9
4958	141	31AUG17	19FEB19	6.9156	0.5048	7.3
4959	141	31AUG17	19FEB19	12.4665	0.8429	6.8



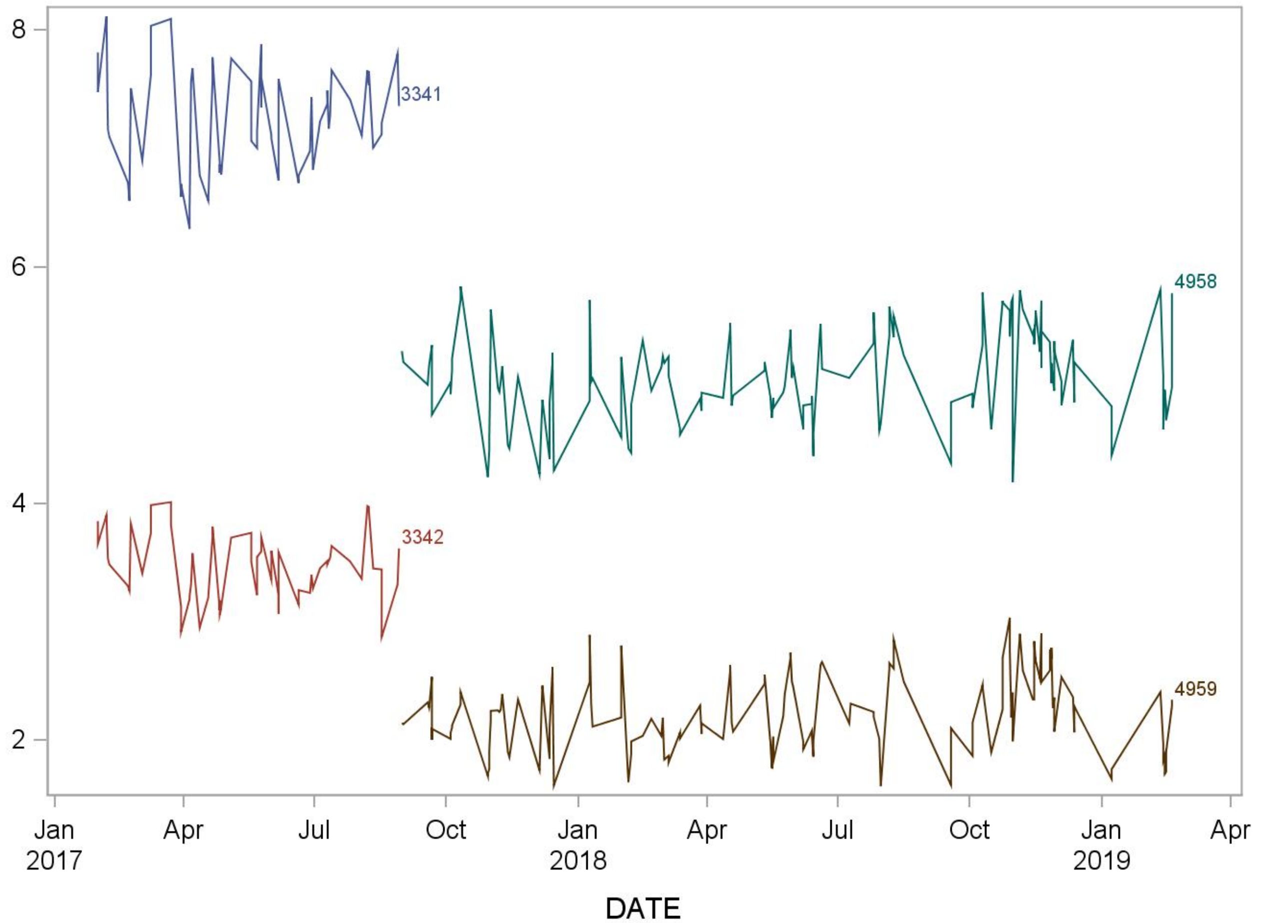
2017-2018 Summary Statistics and QC Chart for Urinary Arsenobetaine (µg/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
3341	63	31JAN17	29AUG17	41.172	2.833	6.9
3342	63	31JAN17	29AUG17	6.298	0.445	7.1
3351	7	06MAR17	09FEB18	241.705	11.303	4.7
3352	7	06MAR17	09FEB18	478.508	20.047	4.2
4958	142	31AUG17	19FEB19	28.258	1.963	6.9
4959	142	31AUG17	19FEB19	8.255	0.726	8.8
4960	7	27MAR18	29NOV18	376.074	25.566	6.8
4961	7	27MAR18	29NOV18	486.623	46.638	9.6



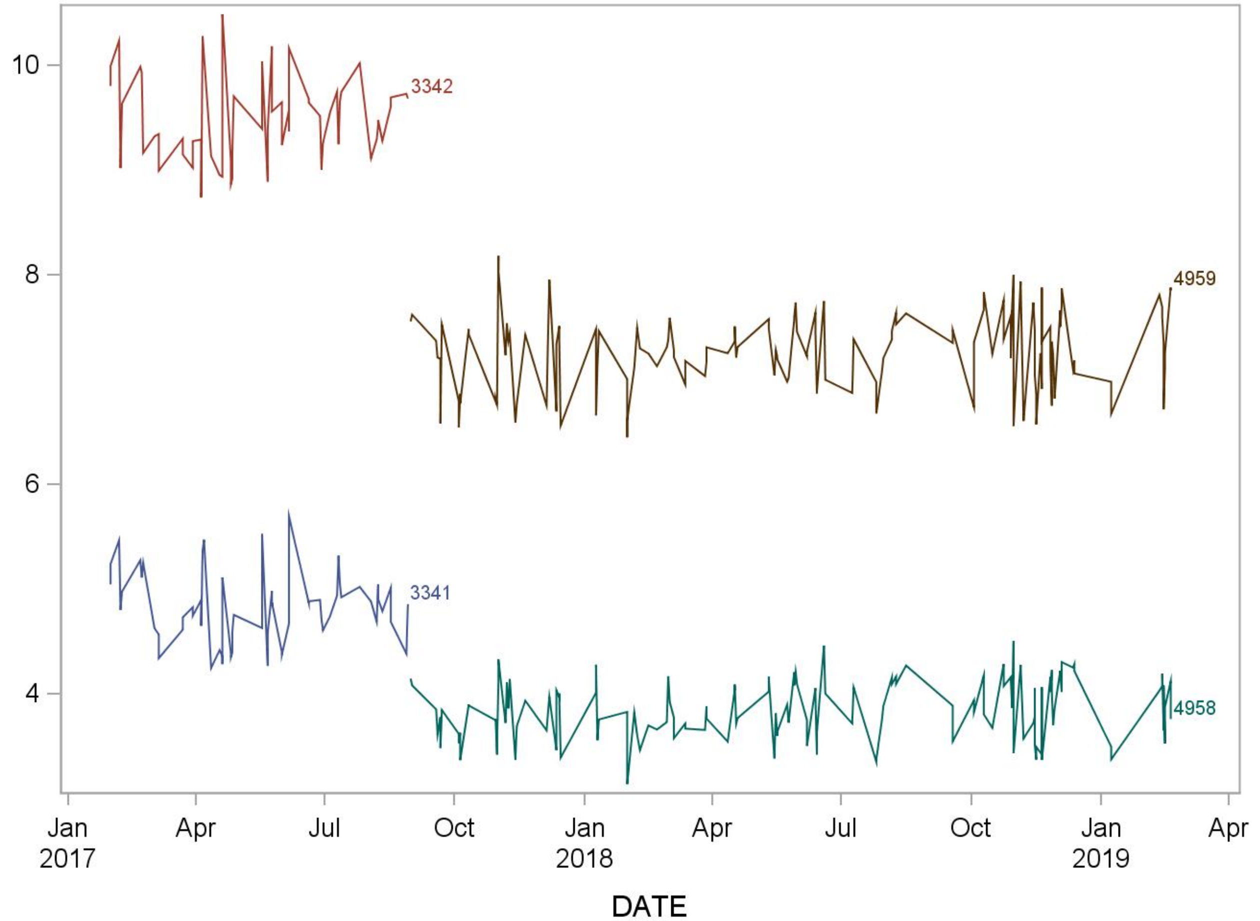
2017-2018 Summary Statistics and QC Chart for Urinary Arsenocholine (µg/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
3341	62	31JAN17	29AUG17	7.240	0.445	6.1
3342	62	31JAN17	29AUG17	3.465	0.289	8.3
4958	141	31AUG17	19FEB19	5.077	0.399	7.8
4959	141	31AUG17	19FEB19	2.239	0.333	14.9



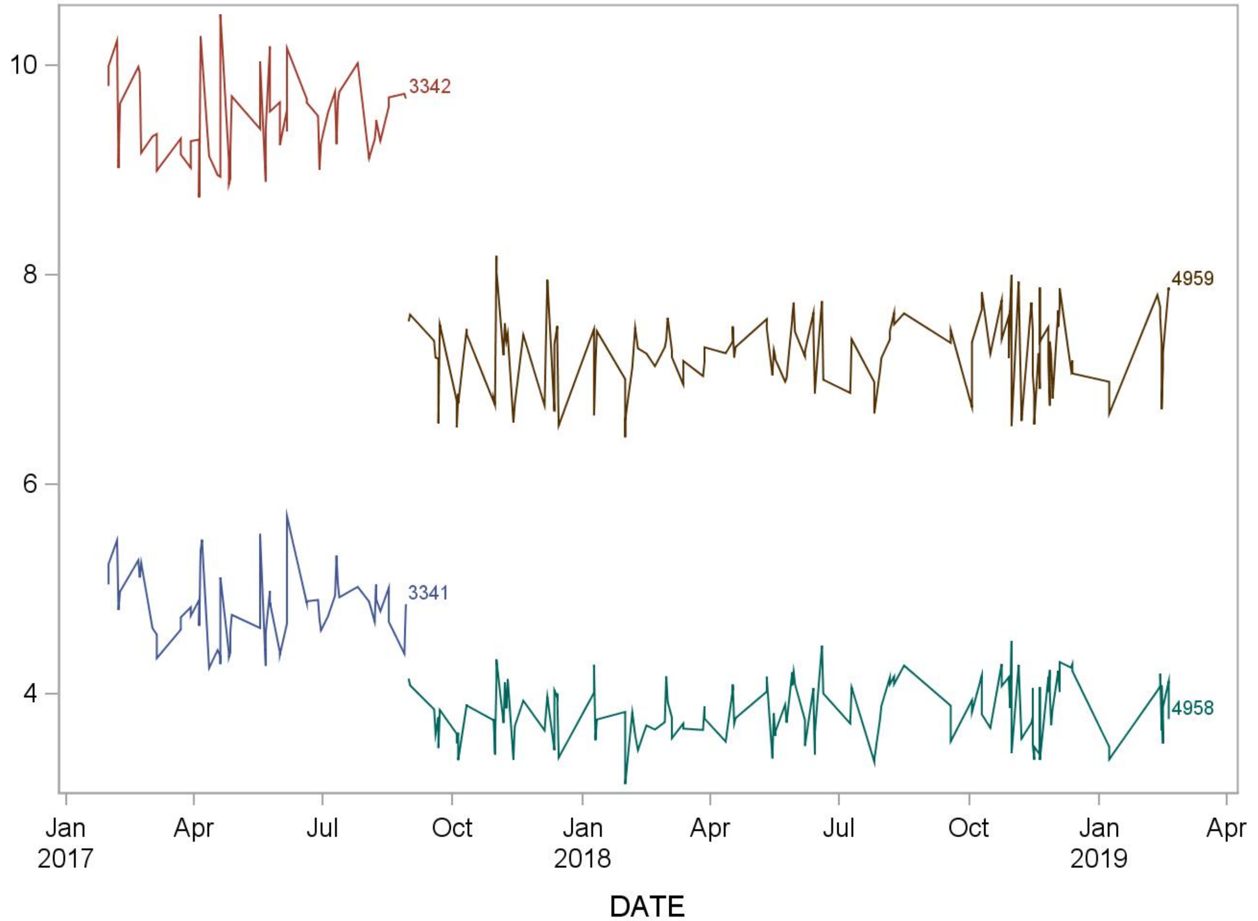
2017-2018 Summary Statistics and QC Chart for Urinary Dimethylarsinic acid (µg/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
3341	62	31JAN17	29AUG17	5.4398	0.2997	5.5
3342	62	31JAN17	29AUG17	16.0494	0.4821	3.0
4958	141	31AUG17	19FEB19	6.0508	0.3393	5.6
4959	141	31AUG17	19FEB19	11.3460	0.4532	4.0



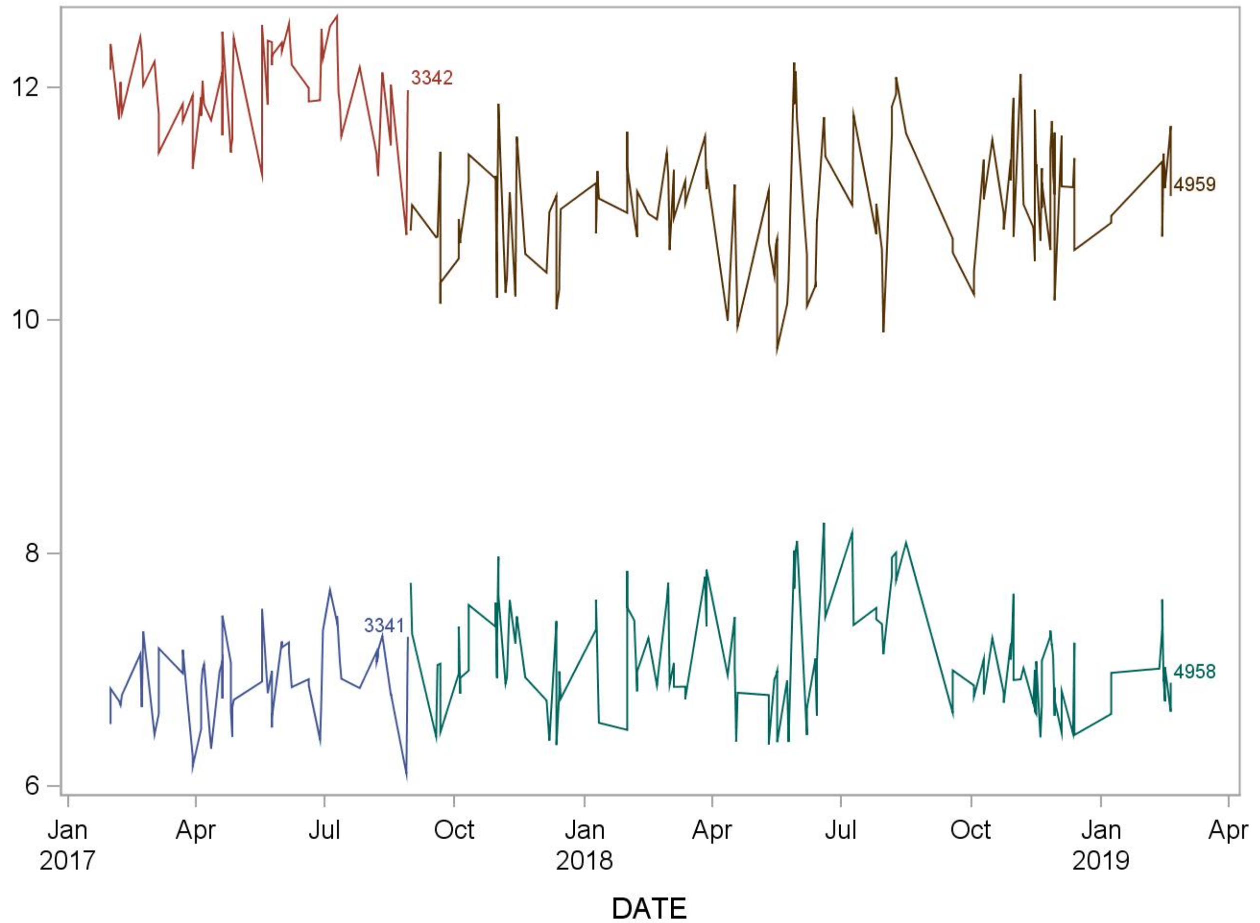
2017-2018 Summary Statistics and QC Chart for Urinary Monomethylarsonic acid (µg/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
3341	62	31JAN17	29AUG17	4.8113	0.3368	7.0
3342	62	31JAN17	29AUG17	9.4841	0.4044	4.3
4958	141	31AUG17	19FEB19	3.8379	0.2866	7.5
4959	141	31AUG17	19FEB19	7.2562	0.3762	5.2



2017-2018 Summary Statistics and QC Chart for Urinary arsenous acid (µg/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
3341	62	31JAN17	29AUG17	6.9126	0.3436	5.0
3342	62	31JAN17	29AUG17	11.9629	0.4122	3.4
4958	141	31AUG17	19FEB19	7.0951	0.4476	6.3
4959	141	31AUG17	19FEB19	11.0128	0.5279	4.8



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29. APPENDIX

a. Appendix A: Method Performance Documentation

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration

Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name:	Arsenic Speciation in Urine by ICP-DRC-MS-HPLC
Method #:	3000
Matrix:	Urine
Units:	µg/L
Analyte:	**Arsenocholine (UASC)**

	Replicate	Sample 1 Spike concentration	Sample 1 Measured concentration Day 1	Sample 1 Measured concentration Day 2	Sample 1 Mean	Sample 1 Recovery (%)	Sample 2 Spike concentration	Sample 2 Measured concentration Day 1	Sample 2 Measured concentration Day 2	Sample 2 Mean	Sample 2 Recovery (%)
Sample	1	0	0	0	0.1		0	0	0	0.0	
	2		0	0				0	0		
	3		0.56	0.0				0	0.0		
Sample + Spike 1	1	10	9.7	9.1	9.4	92.6	15	14	14	13.8	92.2
	2		9.5	9.1				14	14		
	3		9.5	9.2				14	13		
Sample + Spike 2	1	20	19	18	18.5	92.0	25	23	23	23.0	92.0
	2		19	18				23	23		
	3		19	18				23	23		
Sample + Spike 3	1	30	28	27	27	90.2	35	32	32	32	91.4
	2		27	27				32	32		
	3		27	27				32	32		

Mean recovery (%)	SD (%)
91.7	0.8

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: Arsenic Speciation in Urine by ICP-DRC-MS-HPLC
Method #: 3000
Matrix: Urine
Units: µg/L
Analyte: **Arsenobetaine (UASB)**

	Replicate	Sample 1 Spike concentration	Sample 1 Measured concentration Day 1	Sample 1 Measured concentration Day 2	Sample 1 Measured concentration Mean	Sample 1 Recovery (%)	Sample 2 Spike concentration	Sample 2 Measured concentration Day 1	Sample 2 Measured concentration Day 2	Sample 2 Measured concentration Mean	Sample 2 Recovery (%)
Sample	1	0	0	0	0.0		0	0	0	0.0	
	2		0	0				0	0		
	3		0	0.0				0	0.0		
Sample + Spike 1	1	10	10	9.4	9.6	96.3	15	15	14	14.7	97.8
	2		9.7	9.4				15	15		
	3		9.9	9.4				15	14		
Sample + Spike 2	1	20	21	20	20.3	101.6	25	25	22	23.2	92.7
	2		20	20				24	22		
	3		21	20				24	22		
Sample + Spike 3	1	30	30	27	28	93.9	35	34	32	33	93.3
	2		29	27				33	31		
	3		29	27				34	32		

Mean recovery (%)	SD (%)
95.9	3.4

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: Arsenic Speciation in Urine by ICP-DRC-MS-HPLC
Method #: 3000
Matrix: Urine
Units: µg/L
Analyte: **Trimethylarsine oxide (UTMO)**

		Sample 1					Sample 2				
	Replicate	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)
Sample	1	0	0	0	0.0		0	0	0	0.0	
	2		0	0				0	0		
	3		0	0.0				0.24	0.0		
Sample + Spike 1	1	10	9	9.8	9.5	94.7	15	13	15	14.2	94.2
	2		9.2	9.9				14	15		
	3		8.9	10				13	15		
Sample + Spike 2	1	20	18	20	19.0	95.0	25	22	25	23.5	93.8
	2		18	20				22	25		
	3		18	20				22	25		
Sample + Spike 3	1	30	27	30	29	95.0	35	31	36	33	95.1
	2		27	30				31	36		
	3		27	30				31	35		

Mean recovery (%)	SD (%)
94.6	0.5

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration

Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name:	Arsenic Speciation in Urine by ICP-DRC-MS-HPLC
Method #:	3000
Matrix:	Urine
Units:	µg/L
Analyte:	**Arsenous (III) Acid (UAS3)**

	Replicate	Sample 1					Sample 2				
		Spike concentration	Measured concentration			Recovery (%)	Spike concentration	Measured concentration			Recovery (%)
			Day 1	Day 2	Mean			Day 1	Day 2	Mean	
Sample	1	0	0	0	0.0		0	0	0	0.0	
	2		0.24	0				0	0		
	3		0	0.0				0.16	0.0		
Sample + Spike 1	1	10	9.5	9.7	9.5	94.3	15	14	15	14.0	93.2
	2		9.6	9.2				14	14		
	3		9.2	9.6				14	13		
Sample + Spike 2	1	20	18	19	18.8	94.0	25	23	24	23.5	93.9
	2		18	19				23	24		
	3		19	20				23	24		
Sample + Spike 3	1	30	28	28	28	94.3	30	32	34	33	109.9
	2		28	29				32	34		
	3		28	29				32	34		

Mean recovery (%)	SD (%)
96.6	6.5

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: Arsenic Speciation in Urine by ICP-DRC-MS-HPLC
Method #: 3000
Matrix: Urine
Units: µg/L
Analyte: **Dimethylarsinic Acid (UDMA)**

		Sample 1					Sample 2				
			Measured concentration					Measured concentration			
	Replicate	Spike concentration	Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Day 1	Day 2	Mean	Recovery (%)
Sample	1	0	0	0.84	0.4		0	0	0.81	0.4	
	2		0	0.80				0	0.77		
	3		0	0.84				0	0.84		
Sample + Spike 1	1	10	12	11	11.2	107.5	15	17	16	16.3	106.2
	2		11	11				16	16		
	3		11	11				17	16		
Sample + Spike 2	1	20	22	21	21.5	105.4	25	27	25	26.3	103.7
	2		22	21				27	25		
	3		22	21				28	26		
Sample + Spike 3	1	30	32	30	31	101.4	35	37	36	36	102.7
	2		31	30				38	35		
	3		32	30				37	35		

Mean recovery (%)	SD (%)
104.5	2.3

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration

Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name:	Arsenic Speciation in Urine by ICP-DRC-MS-HPLC
Method #:	3000
Matrix:	Urine
Units:	µg/L
Analyte:	**Monomethylarsonic Acid (UMMA)**

	Replicate	Sample 1					Sample 2				
		Spike concentration	Measured concentration			Recovery (%)	Spike concentration	Measured concentration			Recovery (%)
			Day 1	Day 2	Mean			Day 1	Day 2	Mean	
Sample	1	0	0	0	0.0		0	0	0	0.0	
	2		0	0				0	0		
	3		0	0.0				0	0.0		
Sample + Spike 1	1	10	9.3	9.1	9.2	91.8	15	14	14	13.7	91.1
	2		9.3	9				14	13		
	3		9.4	9				14	13		
Sample + Spike 2	1	20	18	18	18.0	90.0	25	23	23	22.8	91.3
	2		18	18				23	23		
	3		18	18				23	22		
Sample + Spike 3	1	30	27	27	27	90.0	35	32	31	32	90.5
	2		27	27				32	31		
	3		27	27				32	32		

Mean recovery (%)	SD (%)
90.8	0.8

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name:	Arsenic Speciation in Urine by ICP-DRC-MS-HPLC
Method #:	3000
Matrix:	Urine
Units:	µg/L
Analyte:	**Arsenic (V) Acid (UAS5)**

		Sample 1					Sample 2				
		Spike concentration	Measured concentration			Recovery (%)	Spike concentration	Measured concentration			Recovery (%)
	Replicate		Day 1	Day 2	Mean			Day 1	Day 2	Mean	
Sample	1	0	0	0.13	0.1		0	0	0.16	0.1	
	2		0	0.20				0	0.14		
	3		0	0.17				0	0.17		
Sample + Spike 1	1	10	9.8	9.1	9.5	94.0	15	15	14	14.2	93.9
	2		10	9.0				14	14		
	3		10	9.0				15	13		
Sample + Spike 2	1	20	19	18	18.7	92.9	25	25	23	23.8	95.0
	2		19	18				25	23		
	3		20	18				24	23		
Sample + Spike 3	1	30	28	27	28	92.5	35	34	32	33	93.6
	2		29	27				34	32		
	3		29	27				33	32		

Mean recovery (%)	SD (%)
93.7	0.9

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	Arsenic Speciation in Urine by ICP-DRC-MS-HPLC
Method #:	3000
Matrix:	Urine
Units:	µg/L
Analyte:	**Arsenocholine (UASC)**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	7.4	7.3	7.32	0.004244523	0.004244523	107.0550281
2	7.3	7.4	7.36	0.00662596	0.00662596	108.2862145
3	7.8	8.1	7.94	0.030572523	0.030572522	125.9586048
4	8.1	8.1	8.10	0.001524902	0.001524902	131.1665454
5	6.7	6.7	6.73	0.000254403	0.000254403	90.65176601
6	7.0	7.0	7.02	0.000167703	0.000167703	98.68579561
7	7.1	7.2	7.17	0.003642122	0.003642123	102.9339868
8	7.2	7.6	7.39	0.034614602	0.034614602	109.2788928
9	7.3	7.5	7.42	0.00599076	0.00599076	110.2167045
10	7.2	7.1	7.17	0.004699103	0.004699103	102.8020266

Grand sum	147.249	**Grand mean**	7.36245

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.1846732	0.01846732	0.135894518	1.85
Between Run	2.92216517	0.324685019	0.391291259	5.31
Total	3.10683837		0.414217539	**5.63**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	3.5	3.6	3.57	0.00046656	0.00046656	25.475522
2	3.6	3.4	3.51	0.008789063	0.008789063	24.65353981
3	3.9	4.0	3.93	0.003300502	0.003300503	30.89058601
4	4.0	4.1	4.05	0.00229441	0.00229441	32.75965568
5	3.4	3.1	3.22	0.019866902	0.019866903	20.68080985
6	3.2	3.0	3.09	0.00570025	0.00570025	19.0962
7	3.4	3.4	3.40	6.80625E-05	6.80625E-05	23.12748061
8	3.6	3.5	3.54	0.00552049	0.00552049	25.119872
9	3.6	3.7	3.64	0.00062001	0.00062001	26.4919205
10	3.6	3.5	3.53	0.000838103	0.000838103	24.94227821

Grand sum	70.9567	**Grand mean**	3.547835

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	0.094928705	0.009492871	0.097431363	2.75
Between Run	1.4952009	0.166133433	0.279857609	7.89
Total	1.590129605		0.29633284	**8.35**

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	Arsenic Speciation in Urine by ICP-DRC-MS-HPLC
Method #:	3000
Matrix:	Urine
Units:	µg/L
Analyte:	**Arsenobetaine (UASB)**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	43	42	42.32	0.321999503	0.321999503	3581.939408
2	42	42	42.09	0.00423801	0.00423801	3543.674972
3	43	44	43.24	0.23668225	0.23668225	3739.516273
4	45	44	44.41	0.075103403	0.075103403	3943.901128
5	41	41	41.23	0.00096721	0.00096721	3399.858784
6	45	43	43.78	0.522078502	0.522078503	3833.683266
7	45	45	44.67	0.01500625	0.01500625	3990.549785
8	39	41	40.22	1.36819809	1.36819809	3236.08516
9	44	44	44.37	0.009672723	0.009672722	3938.272375
10	45	43	43.81	1.204616003	1.204616003	3838.062691

Grand sum 860.2949 **Grand mean** 43.014745

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	7.517123885	0.751712389	0.867013488	2.02
Between Run	40.1780945	4.464232723	1.362446391	3.17
Total	47.69521839		1.614921842	**3.75**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	6.6	6.5	6.55	0.006947223	0.006947223	85.69368613
2	6.3	6.3	6.29	1.369E-05	1.369E-05	79.22383688
3	6.7	6.7	6.69	0.000225	0.000225	89.38112402
4	6.7	6.9	6.76	0.008602562	0.008602563	91.35329281
5	6.7	6.2	6.44	0.057384203	0.057384202	82.90727681
6	6.2	6.3	6.21	0.002185563	0.002185562	77.01770161
7	7.0	7.0	6.96	4.624E-05	4.624E-05	96.827528
8	6.1	5.9	6.00	0.017121723	0.017121722	71.91242665
9	7.0	7.1	7.03	0.000540563	0.000540563	98.76448513
10	7.1	7.2	7.15	0.001564203	0.001564203	102.2178318

Grand sum 132.1155 **Grand mean** 6.605775

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	0.189261935	0.018926194	0.137572503	2.08
Between Run	2.573922803	0.285991423	0.365421147	5.53
Total	2.763184738		0.390459739	**5.91**

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	Arsenic Speciation in Urine by ICP-DRC-MS-HPLC
Method #:	3000
Matrix:	Urine
Units:	µg/L
Analyte:	**Trimethylarsine oxide (UTMO)**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	44	45	44.09	0.217575602	0.217575603	3888.729231
2	45	45	44.89	0.045646323	0.045646323	4030.915536
3	46	47	46.64	0.17073424	0.17073424	4350.859045
4	46	48	47.02	0.40755456	0.40755456	4422.061733
5	44	43	43.14	0.373137722	0.373137722	3721.644675
6	49	46	47.64	1.876763003	1.876763003	4539.301177
7	47	48	47.36	0.295990403	0.295990402	4485.153058
8	43	46	44.15	1.97993041	1.97993041	3899.13377
9	47	47	47.22	0.000241802	0.000241802	4459.50402
10	47	48	47.28	0.38489616	0.38489616	4471.685708

Grand sum 918.8894 **Grand mean** 45.94447

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	11.50494045	1.150494045	1.072610854	2.33
Between Run	51.10148239	5.677942488	1.504567786	3.27
Total	62.60642284		1.847760338	**4.02**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	6.8	6.7	6.72	0.001751423	0.001751422	90.18647905
2	6.9	6.7	6.78	0.00820836	0.00820836	91.8961245
3	7.7	7.3	7.49	0.02782224	0.02782224	112.2661217
4	7.4	7.8	7.60	0.02405601	0.02405601	115.51696
5	6.4	6.0	6.19	0.032815322	0.032815323	76.56660005
6	7.3	6.8	7.05	0.05326864	0.05326864	99.489618
7	7.3	7.2	7.22	0.00344569	0.00344569	104.3665729
8	6.4	6.4	6.39	0.000764523	0.000764522	81.58115113
9	7.0	6.9	6.98	0.003209223	0.003209222	97.49245885
10	7.6	7.8	7.69	0.011674802	0.011674803	118.3691138

Grand sum 140.2233 **Grand mean** 7.011165

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	0.334032465	0.033403247	0.182765551	2.61
Between Run	4.602506841	0.511389649	0.488869309	6.97
Total	4.936539306		0.521916131	**7.44**

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	Arsenic Speciation in Urine by ICP-DRC-MS-HPLC
Method #:	3000
Matrix:	Urine
Units:	µg/L
Analyte:	**Arsenous (III) Acid (UAS3)**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	7.2	7.3	7.22	0.00443556	0.00443556	104.3579045
2	6.8	6.8	6.76	0.00011025	0.00011025	91.43035538
3	6.7	7.3	6.97	0.094156922	0.094156922	97.07678461
4	6.7	7.3	6.96	0.087025	0.087025	96.8971205
5	6.5	6.4	6.43	0.000729	0.000729	82.66151042
6	6.7	6.5	6.60	0.01110916	0.01110916	87.04609568
7	7.2	7.5	7.34	0.013924	0.013924	107.8539845
8	6.3	6.9	6.60	0.074610923	0.074610923	87.05533201
9	6.6	6.6	6.62	0.000183603	0.000183603	87.76137613
10	7.1	7.1	7.09	9.604E-05	9.604E-05	100.5617256

Grand sum	137.1891	**Grand mean**	6.859455

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.572760915	0.057276092	0.239324239	3.49
Between Run	1.659731395	0.184414599	0.252129439	3.68
Total	2.23249231		0.347628171	**5.07**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	12	11	11.60	0.06105841	0.06105841	268.9761792
2	12	11	11.62	0.12131289	0.12131289	270.0395041
3	12	12	12.06	0.07941124	0.07941124	291.0705409
4	12	13	12.14	0.16459249	0.16459249	294.8174749
5	12	11	11.29	0.217156	0.217156	255.0004611
6	12	11	11.55	0.01679616	0.01679616	266.80962
7	12	12	12.35	0.00292681	0.00292681	305.1882768
8	12	11	11.34	0.02829124	0.02829124	257.1095585
9	11	12	11.44	0.060294802	0.060294802	261.8776322
10	13	13	12.95	0.085702562	0.085702562	335.283281

Grand sum	236.69	**Grand mean**	11.8345

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	1.67508521	0.167508521	0.409278049	3.46
Between Run	5.06472379	0.562747088	0.444543905	3.76
Total	6.739809		0.604258061	**5.11**

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	Arsenic Speciation in Urine by ICP-DRC-MS-HPLC
Method #:	3000
Matrix:	Urine
Units:	µg/L
Analyte:	**Dimethylarsinic Acid (UDMA)**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	5.6	5.6	5.61	0.00031684	0.00031684	62.91727488
2	5.3	5.3	5.32	0.000499523	0.000499523	56.68888721
3	5.8	6.0	5.92	0.00900601	0.00900601	70.021778
4	5.6	6.1	5.85	0.06390784	0.06390784	68.48712648
5	5.2	5.1	5.16	0.004323063	0.004323062	53.18001581
6	5.0	4.9	4.91	0.002025	0.002025	48.30658632
7	5.8	5.7	5.74	0.00574564	0.00574564	65.97098978
8	5.1	5.6	5.35	0.047067302	0.047067302	57.30600625
9	5.6	5.6	5.63	4.2025E-06	4.2025E-06	63.48278521
10	5.6	5.6	5.60	0.000200222	0.000200223	62.80626965

Grand sum	110.2133	**Grand mean**	5.510665

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.266191285	0.026619128	0.163153696	2.96
Between Run	1.81914472	0.202127191	0.296233069	5.38
Total	2.085336005		0.338191011	**6.14**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	16	16	15.94	0.002688423	0.002688423	507.9281281
2	16	16	16.22	0.03591025	0.03591025	525.9367714
3	17	16	16.40	0.062675123	0.062675123	538.1135374
4	17	17	16.93	0.05909761	0.05909761	573.4665245
5	15	15	15.10	0.016448062	0.016448062	456.0049001
6	16	15	15.50	0.04414201	0.04414201	480.2582304
7	16	16	16.12	0.00446224	0.00446224	519.4638049
8	15	15	14.94	0.067210562	0.067210563	446.4579974
9	16	16	16.13	0.00804609	0.00804609	520.1409058
10	16	16	16.29	0.025520062	0.025520063	531.0051661

Grand sum	319.1251	**Grand mean**	15.956255

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	0.652400865	0.065240087	0.25542139	1.60
Between Run	6.734493665	0.748277074	0.584395836	3.66
Total	7.38689453		0.637776278	**4.00**

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	Arsenic Speciation in Urine by ICP-DRC-MS-HPLC
Method #:	3000
Matrix:	Urine
Units:	µg/L
Analyte:	**Monomethylarsonic Acid (UMMA)**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	4.7	5.0	4.84	0.013398063	0.013398062	46.83280981
2	4.9	4.8	4.86	0.00398161	0.00398161	47.31504642
3	5.3	5.3	5.33	0.00029584	0.00029584	56.81140418
4	5.1	5.3	5.21	0.012667502	0.012667502	54.22465661
5	4.8	4.9	4.87	0.002308802	0.002308802	47.51857585
6	5.0	4.6	4.79	0.03976036	0.03976036	45.86521088
7	4.9	5.1	5.00	0.018782703	0.018782702	49.96300685
8	4.9	5.2	5.07	0.024009502	0.024009503	51.44529613
9	4.9	5.0	4.94	0.00801025	0.00801025	48.85463552
10	5.0	5.2	5.09	0.013653922	0.013653923	51.73987813

Grand sum	100.0026	**Grand mean**	5.00013

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.27373711	0.027373711	0.165450026	3.31
Between Run	0.544520012	0.060502224	0.1287022	2.57
Total	0.818257122		0.209613853	**4.19**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	10	10	10.01	0.022365203	0.022365202	200.5904351
2	10	9.5	9.85	0.12866569	0.12866569	193.9071245
3	11	10	10.41	0.08856576	0.08856576	216.5655096
4	10	11	10.39	0.01898884	0.01898884	215.7172205
5	9.5	9.0	9.25	0.08614225	0.08614225	171.236018
6	9.7	9.5	9.57	0.014125323	0.014125323	183.2559401
7	9.8	9.9	9.80	0.002204302	0.002204303	192.2466361
8	9.3	9.1	9.18	0.008454803	0.008454802	168.363085
9	9.7	9.5	9.60	0.01159929	0.01159929	184.1856245
10	9.7	11	10.20	0.25847056	0.25847056	208.2064992

Grand sum	196.5136	**Grand mean**	9.82568

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	1.27916404	0.127916404	0.357654028	3.64
Between Run	3.394343472	0.377149275	0.353010531	3.59
Total	4.673507512		0.502526456	**5.11**

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	Arsenic Speciation in Urine by ICP-DRC-MS-HPLC
Method #:	3000
Matrix:	Urine
Units:	µg/L
Analyte:	**Arsenic (V) Acid (UAS5)**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	7.8	7.9	7.86	0.00314721	0.00314721	123.6315226
2	7.7	7.7	7.69	0.00128881	0.00128881	118.3706525
3	7.9	8.0	7.96	0.00380689	0.00380689	126.7518576
4	7.7	7.8	7.75	0.003335062	0.003335063	120.0800542
5	7.6	7.2	7.41	0.047982903	0.047982902	109.8680761
6	7.1	7.0	7.02	0.003969	0.003969	98.6029245
7	7.2	7.4	7.26	0.009321902	0.009321903	105.419556
8	7.5	8.0	7.76	0.04562496	0.04562496	120.5438645
9	7.6	7.6	7.59	5.29E-06	5.29E-06	115.0887233
10	7.7	7.9	7.80	0.006217323	0.006217323	121.68156

Grand sum	152.2154	**Grand mean**	7.61077

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.2493987	0.02493987	0.157923621	2.08
Between Run	1.562391482	0.173599054	0.272634539	3.58
Total	1.811790182		0.315070566	**4.14**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	13	13	12.69	0.00355216	0.00355216	322.1381914
2	12	12	12.44	0.00275625	0.00275625	309.6266355
3	13	13	12.93	0.01361889	0.01361889	334.5715384
4	12	13	12.83	0.162933322	0.162933323	329.0766851
5	12	12	11.93	0.00367236	0.00367236	284.8263914
6	12	12	11.88	0.10252804	0.10252804	282.4256378
7	13	13	12.65	0.008639703	0.008639703	319.9362192
8	12	12	12.19	0.036005063	0.036005063	297.2238948
9	13	12	12.40	0.05841889	0.05841889	307.6638568
10	13	13	12.75	0.01996569	0.01996569	325.0485045

Grand sum	249.4035	**Grand mean**	12.470175

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	0.824180735	0.082418074	0.287085481	2.30
Between Run	2.432264403	0.2702516	0.30645842	2.46
Total	3.256445138		0.419922418	**3.37**

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: 2015 LU and HU samples three times frozen at approximately -80°C and then thawed (3 freeze-thaw cycles)
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: 2015 LU and HU QC original samples (not yet prepared for instrument analysis) stored at room temperature for 1 day
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: 2015 LU and HU QC samples processed samples (ready for instrument analysis) stored at room temperature for 1 day
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: 2015 LU and HU bench QC samples stored at approximately -80°C for 2 years.

All stability sample results should be within ±15% of nominal concentration

Method name: Arsenic Speciation in Urine by ICP-DRC-MS-HPLC
Method #: 3000
Matrix: Urine
Units: µg/L
Analyte: **Arsenocholine (UASC)**

Quality material 1	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	7.4	7.2	7.4	6.8	7.4	7.5	7.4	7.4
Replicate 2	7.6	6.7	7.6	6.8	7.6	7.7	7.3	7.9
Replicate 3	7.4	7.0	7.4	6.9	7.4	7.4	7.5	8.0
Mean	7.466666667	6.966666667	7.466666667	6.8	7.466666667	7.533333333	7.431666667	7.8
% difference from initial measurement	--	**-6.7**	--	**-8.5**	--	**0.9**	--	**4.4**

Quality material 2	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	3.3	3.7	3.3	3.4	3.3	3.5	3.5	3.5
Replicate 2	3.3	3.4	3.3	3.4	3.3	3.7	3.6	3.8
Replicate 3	3.4	3.4	3.4	3.4	3.4	3.7	3.6	3.9
Mean	3.333333333	3.5	3.333333333	3.4	3.333333333	3.633333333	3.571333333	3.7
% difference from initial measurement	--	**5.0**	--	**2.0**	--	**9.0**	--	**4.1**

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: 2015 LU and HU samples three times frozen at approximately -80°C and then thawed (3 freeze-thaw cycles)
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: 2015 LU and HU QC original samples (not yet prepared for instrument analysis) stored at room temperature for 1 day
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: 2015 LU and HU QC samples processed samples (ready for instrument analysis) stored at room temperature for 1 day
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: 2015 LU and HU bench QC samples stored at approximately -80°C for 2 years.

All stability sample results should be within ±15% of nominal concentration

Method name: Arsenic Speciation in Urine by ICP-DRC-MS-HPLC
Method #: 3000
Matrix: Urine
Units: µg/L
Analyte: **Arsenobetaine (UASB)**

Quality material 1	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	43	40	43	39	43	41	43	42
Replicate 2	42	37	42	38	42	43	43	43
Replicate 3	43	38	43	39	43	41	45	43
Mean	42.66666667	38.33333333	42.66666667	38.7	42.66666667	41.66666667	43.55666378	42.8
% difference from initial measurement	--	**-10.2**	--	**-9.4**	--	**-2.3**	--	**-1.7**

Quality material 2	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	6.3	6.3	6.3	6.6	6.3	6.3	6.6	6.5
Replicate 2	6.5	6.4	6.5	6.7	6.5	6.4	6.7	6.7
Replicate 3	6.6	6.2	6.6	6.4	6.6	6.2	6.9	6.8
Mean	6.466666667	6.3	6.466666667	6.6	6.466666667	6.3	6.711298496	6.7
% difference from initial measurement	--	**-2.6**	--	**1.5**	--	**-2.6**	--	**-0.3**

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: 2015 LU and HU samples three times frozen at approximately -80°C and then thawed (3 freeze-thaw cycles)
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: 2015 LU and HU QC original samples (not yet prepared for instrument analysis) stored at room temperature for 1 day
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: 2015 LU and HU QC samples processed samples (ready for instrument analysis) stored at room temperature for 1 day
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: 2015 LU and HU bench QC samples stored at approximately -80°C for 2 years.

All stability sample results should be within ±15% of nominal concentration

Method name:	Arsenic Speciation in Urine by ICP-DRC-MS-HPLC
Method #:	3000
Matrix:	Urine
Units:	µg/L
Analyte:	**Trimethylarsine oxide (UTMO)**

Quality material 1	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	46	47	46	45	46	44	46	45
Replicate 2	45	41	45	42	45	46	46	45
Replicate 3	46	46	46	44	46	45	46	46
Mean	45.66666667	44.66666667	45.66666667	43.7	45.66666667	45	46.16774035	45.4
% difference from initial measurement	--	**-2.2**	--	**-4.4**	--	**-1.5**	--	**-1.6**

Quality material 2	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	6.6	7.0	6.6	6.7	6.6	6.8	7.1	6.6
Replicate 2	6.5	6.7	6.5	6.7	6.5	7.0	7.2	7.1
Replicate 3	6.7	7.3	6.7	6.8	6.7	6.9	7.2	6.9
Mean	6.6	7.0	6.6	6.7	6.6	6.9	7.152642946	6.9
% difference from initial measurement	--	**6.1**	--	**2.0**	--	**4.5**	--	**-4.1**

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: 2015 LU and HU samples three times frozen at approximately -80°C and then thawed (3 freeze-thaw cycles)
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: 2015 LU and HU QC original samples (not yet prepared for instrument analysis) stored at room temperature for 1 day
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: 2015 LU and HU QC samples processed samples (ready for instrument analysis) stored at room temperature for 1 day
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: 2015 LU and HU bench QC samples stored at approximately -80°C for 2 years.

All stability sample results should be within ±15% of nominal concentration

Method name: Arsenic Speciation in Urine by ICP-DRC-MS-HPLC
Method #: 3000
Matrix: Urine
Units: µg/L
Analyte: **Arsenous (III) Acid (UAS3)**

Quality material 1	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	7.3	7.9	7.3	7.2	7.3	7.2	7.0	6.9
Replicate 2	7.6	6.5	7.6	7.1	7.6	7.8	7.2	7.0
Replicate 3	7.6	7.6	7.6	6.9	7.6	7.1	7.2	7.3
Mean	7.5	7.333333333	7.5	7.1	7.5	7.366666667	7.137330742	7.1
% difference from initial measurement	--	**-2.2**	--	**-5.8**	--	**-1.8**	--	**-1.1**

Quality material 2	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	12	13	12	11	12	13	12	12
Replicate 2	13	13	13	11	13	12	12	12
Replicate 3	12	13	12	12	12	13	12	12
Mean	12.33333333	13.0	12.33333333	11.3	12.33333333	12.66666667	11.95737205	12.3
% difference from initial measurement	--	**5.4**	--	**-8.1**	--	**2.7**	--	**2.7**

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: 2015 LU and HU samples three times frozen at approximately -80°C and then thawed (3 freeze-thaw cycles)
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: 2015 LU and HU QC original samples (not yet prepared for instrument analysis) stored at room temperature for 1 day
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: 2015 LU and HU QC samples processed samples (ready for instrument analysis) stored at room temperature for 1 day
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: 2015 LU and HU bench QC samples stored at approximately -80°C for 2 years.

All stability sample results should be within ±15% of nominal concentration

Method name: Arsenic Speciation in Urine by ICP-DRC-MS-HPLC
Method #: 3000
Matrix: Urine
Units: µg/L
Analyte: **Dimethylarsinic Acid (UDMA)**

Quality material 1	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	5.6	6.0	5.6	5.3	5.6	5.3	5.8	5.4
Replicate 2	5.6	5.4	5.6	5.2	5.6	5.8	5.6	5.4
Replicate 3	5.5	5.6	5.5	5.4	5.5	5.5	5.6	5.7
Mean	5.566666667	5.666666667	5.566666667	5.3	5.566666667	5.533333333	5.657992135	5.5
% difference from initial measurement	--	**1.8**	--	**-4.8**	--	**-0.6**	--	**-2.7**

Quality material 2	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	17	17	17	16	17	17	16	16
Replicate 2	17	17	17	16	17	17	16	16
Replicate 3	16	17	16	16	16	17	16	16
Mean	16.66666667	17.0	16.66666667	16.0	16.66666667	17	16.24349934	16.0
% difference from initial measurement	--	**2.0**	--	**-4.0**	--	**2.0**	--	**-1.5**

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: 2015 LU and HU samples three times frozen at approximately -80°C and then thawed (3 freeze-thaw cycles)
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: 2015 LU and HU QC original samples (not yet prepared for instrument analysis) stored at room temperature for 1 day
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: 2015 LU and HU QC samples processed samples (ready for instrument analysis) stored at room temperature for 1 day
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: 2015 LU and HU bench QC samples stored at approximately -80°C for 2 years.

All stability sample results should be within ±15% of nominal concentration

Method name: Arsenic Speciation in Urine by ICP-DRC-MS-HPLC
Method #: 3000
Matrix: Urine
Units: µg/L
Analyte: **Monomethylarsonic Acid (UMMA)**

Quality material 1	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	4.6	4.9	4.6	5.3	4.6	4.7	5.1	5.4
Replicate 2	4.4	4.5	4.4	5	4.4	4.6	4.9	5.4
Replicate 3	4.3	4.9	4.3	4.7	4.3	4.6	5.3	5.4
Mean	4.433333333	4.766666667	4.433333333	5.0	4.433333333	4.633333333	5.090569281	5.4
% difference from initial measurement	--	**7.5**	--	**12.8**	--	**4.5**	--	**5.6**

Quality material 2	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	9.1	10	9.1	9.6	9.1	9.8	10	11
Replicate 2	9.2	10	9.2	9.3	9.2	10	10	11
Replicate 3	9.4	10	9.4	9.5	9.4	9.8	10	11
							9.683117293	
Mean	9.233333333	10.0	9.233333333	9.5	9.233333333	9.866666667	10.05677324	10.7
% difference from initial measurement	--	**8.3**	--	**2.5**	--	**6.9**	--	**6.1**

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: 2015 LU and HU samples three times frozen at approximately -80°C and then thawed (3 freeze-thaw cycles)
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: 2015 LU and HU QC original samples (not yet prepared for instrument analysis) stored at room temperature for 1 day
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: 2015 LU and HU QC samples processed samples (ready for instrument analysis) stored at room temperature for 1 day
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: 2015 LU and HU bench QC samples stored at approximately -80°C for 2 years.

All stability sample results should be within ±15% of nominal concentration

Method name: Arsenic Speciation in Urine by ICP-DRC-MS-HPLC
Method #: 3000
Matrix: Urine
Units: µg/L
Analyte: **Arsenic (V) Acid (UAS5)**

Quality material 1	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	7.4	7.2	7.4	7.1	7.4	7.1	7.8	7.2
Replicate 2	7.5	7.0	7.5	6.9	7.5	7.4	7.6	7.6
Replicate 3	7.3	6.9	7.3	7.6	7.3	7.1	7.9	7.7
Mean	7.4	7.033333333	7.4	7.2	7.4	7.2	7.789574431	7.5
% difference from initial measurement	--	**-5.0**	--	**-2.7**	--	**-2.7**	--	**-3.7**

Quality material 2	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	12	12	12	12	12	12	13	12
Replicate 2	13	12	13	12	13	13	13	13
Replicate 3	12	12	12	11	12	12	13	13
Mean	12.33333333	12.0	12.33333333	11.7	12.33333333	12.33333333	13.4042847	12.4
% difference from initial measurement	--	**-2.7**	--	**-5.4**	--	**0.0**	--	**-7.3**

LOD, specificity and fit for intended use

Method name:	Arsenic Speciation in Urine by ICP-DRC-MS-HPLC
Method #:	3000
Matrix:	Urine
Units:	µg/L

Analytes	Limit of Detection (LOD)	Interferences successfully checked in at least 50 human samples	Accuracy, precision, LOD, specificity and stability meet performance specifications for intended use
Arsenocholine	0.11	yes	yes
Arsenobetaine	1.16	yes	yes
Trimethylarsine oxide	0.17	yes	yes
Arsenous (III) Acid	0.12	yes	yes
Dimethylarsinic Acid	1.91	yes	yes
Monomethylarsonic Acid	0.2	yes	yes
Arsenic (V) Acid	0.79	yes	yes

b. Appendix B: Ruggedness Testing

Critical Parameter Test 1: This test evaluated the significance of the column oven temperature while analyzing urine samples for seven arsenic species (AC, AB, TMAO, AsIII, DMA, MMA, and AsV). The column oven temperature was increased and decreased by 10°C increments (roughly 28.5%). The results are presented in the table below.

Test Details:
1. Prepare a set of samples for analysis in triplicate (seven separate vials of low bench QC, each prepared in triplicate).
2. Analyze the samples along with calibrators and QC as normal.
3. Analyze the same samples again at 25°C on day two and at 35°C on day three.

Critical Test Parameter 1. Ruggedness Testing Results: Evaluating the significance of column oven temperature on sample stability. Tests performed on March 21, 2013, March 22, 2013, and March 25, 2013 by Joshua Godshaw. Results below are the average of the 21 replicates of the low bench QC analyzed as samples in each run (in µg/L).

Analyte	Target Mean and 2SD Range	25°C (decreased)	35°C (per method)	45°C (increased)
AC	7.59 6.83 – 8.35	7.06 6.83 – 7.30	7.44 7.06 - 7.81	6.87 6.42 – 7.33
AB	24.07 21.79 – 26.35	24.7 23.68 – 25.72	24.01 23.24 – 24.78	25.2 23.43 - 26.97
TMAO	32.25 30.55 – 33.95	32.15 31.50 – 33.25	31.95 31.01 – 32.90	31.87 29.93 – 33.83
AsIII	7.10 6.32 – 7.88	7.13 6.25 – 8.00	7.18 6.48 – 7.87	7.49 6.60 – 8.39
DMA	3.97 3.34 – 4.39	3.88 3.65 – 4.12	3.80 3.58 - 4.01	3.71 3.37 – 4.05
MMA	4.46 3.94 – 4.97	4.24 4.10 – 4.38	4.52 4.33 – 4.71	4.23 3.98 – 4.49
AsV	7.98 6.92 – 9.04	7.42 6.85 – 7.99	7.20 6.54 - 7.86	7.24 6.54 – 7.95

Conclusion: All of the analytes fell within 2SD of the mean at all temperatures; however, it was confirmed that the column oven temperature of 35°C was the optimal choice due to the sharpness of the peaks (no tailing, etc.) obtained at that temperature.

Critical Parameter Test 2: This test evaluated the significance of the concentration of ammonium acetate in the diluent on quality control sample recovery. The amount of ammonium acetate in the diluent was increased and decreased by 0.025M increments (roughly 25%). The results are presented in the table below.

Test Details:
1. Prepare three sets of samples for analysis in triplicate (seven separate vials of low bench QC, each prepared in triplicate) for each of the three concentrations.
2. Analyze the samples along with calibrators and QC as normal.

Critical Parameter Test 2. Ruggedness Testing Results: Evaluating the significance of the concentration of ammonium acetate in the diluent on quality control sample recovery. Tests performed on April 03, 2013, April 04, 2013, and April 08, 2013 by Joshua Godshaw. Results below are the average of the 21 replicates of the low bench QC analyzed as samples in each run (in µg/L).

Analyte	Target Mean and 2SD Range	0.075M (decreased)	0.1M (per method)	0.125M (increased)
AC	7.59 6.83 – 8.35	6.76 6.42 – 7.09	7.30 6.82 – 7.79	7.40 7.15 – 7.66
AB	24.07 21.79 – 26.35	24.55 23.74 – 25.35	24.92 23.89 – 25.95	25.43 24.77 – 26.09
TMAO	32.25 30.55 – 33.95	31.19 30.00 – 32.39	32.34 31.32 – 33.36	32.51 31.88 – 33.14
AsIII	7.10 6.32 – 7.88	6.91 6.33 – 7.48	6.91 6.25 – 7.57	6.90 6.35 – 7.45
DMA	3.97 3.34 – 4.39	3.95 3.66 – 4.24	3.81 3.52 – 4.10	3.82 3.56– 4.08
MMA	4.46 3.94 – 4.97	4.28 4.01 – 4.54	4.50 4.22 – 4.79	4.72 4.60 – 4.85
AsV	7.98 6.92 – 9.04	7.33 6.55 – 8.11	7.96 7.41 – 8.51	7.91 7.35 – 8.46

Conclusion: Results using 0.1M ammonium acetate in the diluent and results using 0.125M ammonium acetate in the diluent all fell within 2 SD of the mean. A concentration of 0.075M ammonium acetate produced QC results outside of the mean for arsenocholine. 0.1M was confirmed to be the optimum ammonium acetate diluent concentration.

Critical Parameter Test 3: This test evaluated the significance of the dynamic reaction cell (DRC™) gas flow rate on quality control sample recovery while analyzing urine samples for arsenic species. On the instrument used, the normal cell gas flow rate is 0.6 mL/min. The DRC™ cell gas flow rate was increased and decreased by 0.12 increments (20%) giving reduced and elevated settings of 0.48 mL/min and 0.72 mL/min, respectively. The results are presented in the table below.

Test Details:
1. Prepare a set of samples for analysis in triplicate (three separate vials of low bench QC, each prepared in triplicate).
2. Analyze the samples along with calibrators and QC with a cell gas flow of 0.55 mL/min (per method) on day one.
3. Analyze the same samples again with a cell gas flow of 0.44 mL/min on day two and 0.66 mL/min on day three.

Critical Parameter Test 3. Ruggedness Testing Results: Evaluating the significance of dynamic reaction cell gas flow rate on quality control sample stability. Tests performed on March 26, 2013, March 27, 2013, and March 29, 2013 by Joshua Godshaw. Results below are the average of the 9 replicates of the low bench QC analyzed as samples in each run (in µg/L).

Analyte	Target Mean and 2SD Range	0.44 mL/min (decreased)	0.55 mL/min (per method)	0.66 mL/min (increased)
AC	7.59 6.83 – 8.35	7.82 7.32 – 8.32	7.68 7.05 – 8.31	7.83 7.26 – 8.39
AB	24.07 21.79 – 26.35	24.03 23.15 – 24.91	25.85 25.23 - 26.46	24.61 23.64 – 25.58
TMAO	32.25 30.55 – 33.95	30.90 29.94 – 31.85	33.14 32.18 – 34. 10	32.29 31.63 – 32.95
AsIII	7.10 6.32 – 7.88	7.12 6.52 – 7.88	7.38 6.63 – 8.13	7.67 7.05 – 8.29
DMA	3.97 3.34 – 4.39	3.93 3.63 – 4.24	4.02 3.71 – 4.33	4.42 4.13 – 4.71
MMA	4.46 3.94 – 4.97	4.29 4.08 – 4.49	4.40 4.25 – 4.56	4.67 4.51 – 4.82
AsV	7.98 6.92 – 9.04	7.45 6.75 - 8.14	7.83 7.22 – 8.44	7.92 7.32 – 8.51

Conclusion: A DRC gas flow of 0.44 mL/min produced some results that were close to being outside of 2SD, and 0.66 mL/min produced DMA results that were outside of 2 SD. A DRC gas flow of 0.55 mL/min was confirmed to be the optimum choice.

Critical Parameter Test 4: This test evaluated the significance of the pH of buffer B on while analyzing urine quality control samples for seven arsenic species. The pH of the buffer was increased and decreased by 1.6 increments (20%). The results are presented in the table below.

Test Details:
1. Prepare a set of samples for analysis in triplicate (seven separate vials of low bench QC, each prepared in triplicate).
2. Analyze the samples along with calibrators and QC as normal.
3. Analyze the same samples again at a buffer B pH of 6.4 on day two and at a buffer B pH of 9.6 on day three.

Critical Parameter Test 4. Ruggedness Testing Results: Evaluating the significance of the pH of buffer B on quality control sample stability. Tests performed on April 10, 2013, April 11, 2013, and April 12, 2013 by Joshua Godshaw. Results below are the average of the 21 replicates of the low bench QC analyzed as samples in each run (in µg/L).

Analyte	Target Mean and 2SD Range	6.4 (decreased)	8.0 (per method)	9.6 (increased)
AC	7.59 6.83 – 8.35	7.30 7.03 – 7.57	6.94 6.23 – 7.64	6.99 6.68 – 7.30
AB	24.07 21.79 – 26.35	24.60 23.67 – 25.52	25.32 24.71 – 25.93	22.40 21.11 – 23.69
TMAO	32.25 30.55 – 33.95	31.27 30.38 – 32.16	31.57 30.88 – 32.25	29.08 27.60 – 30.56
AsIII	7.10 6.32 – 7.88	6.92 6.38 – 7.46	7.12 6.49 – 7.75	6.28 5.78 – 6.78
DMA	3.97 3.34 – 4.39	3.69 3.39 – 3.98	3.83 3.49 – 4.16	3.36 3.11 – 3.61
MMA	4.46 3.94 – 4.97	4.24 3.99 – 4.49	4.36 4.14 – 4.58	3.32 2.99 – 3.64
AsV	7.98 6.92 – 9.04	7.26 6.78 – 7.73	7.92 7.46 – 8.39	5.56 4.64 – 6.49

Conclusion: A buffer B pH of 9.6 produced some values outside of 2 SD for all of the analytes that elute while buffer B is flowing through the system. A pH of 8.0 for buffer B was confirmed to be optimal for this method.

Critical Parameter Test 5: This test evaluated the significance of the RPq on quality control sample recovery while analyzing urine samples for arsenic species. On the instrument used, the normal RPq is 0.5. The RPq was increased and decreased by 0.1 increments (20%) giving reduced and elevated settings of 0.4 and 0.6, respectively. The results are presented in the table below.

Test Details:
1. Prepare a set of samples for analysis in triplicate (seven separate vials of low bench QC, each prepared in triplicate).
2. Analyze the samples along with calibrators and QC with a RPq of 0.5 (per method) on day one.
3. Analyze the same samples again with a RPq of 0.4 on day two and 0.6 on day three.

Critical Parameter Test 5. Ruggedness Testing Results: Evaluating the significance of RPq on quality control sample stability. Tests performed on June 03, 2013, June 04, 2013, and June 05, 2013 by Jennifer Ysseldyke. Results below are the average of the 21 replicates of the low bench QC analyzed as samples in each run (in µg/L).

Analyte	Target Mean and 2SD Range	0.4 (decreased)	0.5 (per method)	0.6 (increased)
AC	7.59 6.83 – 8.35	7.72 6.91 – 8.53	6.53 5.79 – 7.27	6.91 6.36 – 7.45
AB	24.07 21.79 – 26.35	27.49 25.49 – 29.48	24.65 22.57 – 26.73	25.31 21.96 -28.66
TMAO	32.25 30.55 – 33.95	33.84 31.58 – 36.10	32.25 29.81 – 34.69	33.90 31.19 – 36.61
AsIII	7.10 6.32 – 7.88	8.21 7.28 - 9.14	6.91 6.10 - 7.72	7.38 6.75 - 8.01
DMA	3.97 3.34 – 4.39	4.55 4.08 – 5.03	3.49 3.03 – 3.94	3.63 3.38 - 3.89
MMA	4.46 3.94 – 4.97	4.99 4.31 – 5.66	4.01 3.65 – 4.36	4.20 3.85 – 4.56
AsV	7.98 6.92 – 9.04	8.16 6.87 – 9.45	7.27 6.19 – 8.34	7.25 6.34 – 8.16

Conclusion: A RPq of 0.5 mL was confirmed to be the optimal RPq for this method. The failure for AC was an anomaly.

c. Appendix C: Sample Chromatographic Report

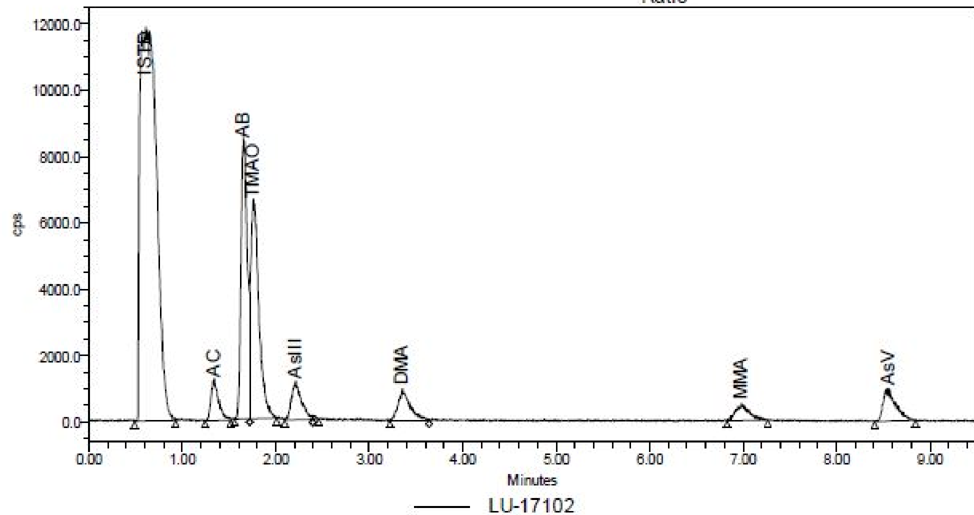
Example chromatogram of a bench QC sample below:

Arsenic Speciation

SAMPLE INFORMATION

Sample Name:	LU-17102	Date Acquired:	3/15/2018 3:22:40 PM EDT
Sample Type:	Unknown	Acq. Method Set:	As_HPLC1_DRC
Vial:	1:B,4	Date Processed:	3/16/2018 7:08:32 AM EDT
Injection #:	1	Processed By:	System/Administrator
Injection Volume:	20.00 ul	Processing Method:	As_HPLC1_DRC
Run Time:	9.5 Minutes	Channel Name:	As75
Sample Set Name:	As180315	Proc. Chnl. Descr.:	As75
Acquired By:	System	Calibration ID:	34511

Area Correction Applied:	None	Sample Weight:	1.00000
Blank Subtraction Applied:	None	Dilution:	1.00
Label:	U0102	Calibrated By:	Corrected_Area, Corrected_Area Ratio



	Peak Name	RT	Height	Area	Corrected Area	Final_Concentration	Units
1	ISTD	0.610	11742	146609	146609		
2	AC	1.341	1221	6955	6955	5.617	
3	AB	1.657	8323	39607	39607	27.618	
4	TMAO	1.762	6620	39570	39570	29.864	
5	AsIII	2.217	1130	8279	8279	7.049	

Reported by User: System
Report Method: Arsenic Speciation
Report Method ID: 32724
Page: 1 of 2

Project Name: CDC_Speciation Default
Date Printed:
3/16/2018
12:56:49 PM US/Eastern

	Peak Name	RT	Height	Area	Corrected Area	Final_Concentration	Units
6		2.402	109	224	224		
7	DMA	3.357	921	8296	8296	5.549	
8	MMA	6.981	487	4915	4915	3.577	
9	AsV	8.557	943	8951	8951	7.557	

Reported by User: System
Report Method: Arsenic Speciation
Report Method ID: 32724
Page: 2 of 2

Project Name: CDC_Speciation Default
Date Printed:
3/16/2018
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d. Appendix D: HPLC Batch Run "Results" File in Microsoft Excel®

A screen shot of a "results" file in Microsoft Excel® after exporting data with Empower Quickstart.

As180315 Results [Compatibility Mode] - Excel

	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	Label	Sample ID	Sample Ty	Date Acqu	Dilution	Analyte ID	RT	Height	Area	Norm Inte	R^2	Final_Concentration	Column S	Calibratio	Comments
2	STBlank	S0	Standard	3/15/2018	1	ISTD	0.606	14132	174766	0	1		695	As28-yie6170917	
3	STBlank	S0	Standard	3/15/2018	1	AC	1.32	43	75	0.003873	0.99995		695	As28-yie6170917	
4	STBlank	S0	Standard	3/15/2018	1	AB	1.654	106	473	0.009452	0.999948		695	As28-yie6170917	
5	STBlank	S0	Standard	3/15/2018	1	TMAO	1.782						695	As28-yie6170917	
6	STBlank	S0	Standard	3/15/2018	1	AsIII	2.225	49	106	-0.001	0.999802		695	As28-yie6170917	
7	STBlank	S0	Standard	3/15/2018	1	DMA	3.352	111	630	0.016115	0.999824		695	As28-yie6170917	
8	STBlank	S0	Standard	3/15/2018	1	MMA	6.758	7	60	0.00646	0.999814		695	As28-yie6170917	
9	STBlank	S0	Standard	3/15/2018	1	AsV	8.542	106	493	0.009153	0.999953		695	As28-yie6170917	
10	S0103	S1	Standard	3/15/2018	1	ISTD	0.649	13876	173828	0	1		695	As28-yie6170917	
11	S0103	S1	Standard	3/15/2018	1	AC	1.346	596	2785	0.003873	0.99995		695	As28-yie6170917	
12	S0103	S1	Standard	3/15/2018	1	AB	1.66	718	3936	0.009452	0.999948		695	As28-yie6170917	
13	S0103	S1	Standard	3/15/2018	1	TMAO	1.782						695	As28-yie6170917	
14	S0103	S1	Standard	3/15/2018	1	AsIII	2.19	387	2989	-0.001	0.999802		695	As28-yie6170917	
15	S0103	S1	Standard	3/15/2018	1	DMA	3.365	462	4213	0.016115	0.999824		695	As28-yie6170917	
16	S0103	S1	Standard	3/15/2018	1	MMA	6.876	270	2600	0.00646	0.999814		695	As28-yie6170917	
17	S0103	S1	Standard	3/15/2018	1	AsV	8.525	411	3699	0.009153	0.999953		695	As28-yie6170917	
18	S0104	S2	Standard	3/15/2018	1	ISTD	0.629	13927	171255	0	1		695	As28-yie6170917	
19	S0104	S2	Standard	3/15/2018	1	AC	1.344	2775	15338	0.003873	0.99995		695	As28-yie6170917	
20	S0104	S2	Standard	3/15/2018	1	AB	1.657	3455	18429	0.009452	0.999948		695	As28-yie6170917	
21	S0104	S2	Standard	3/15/2018	1	TMAO	1.782						695	As28-yie6170917	
22	S0104	S2	Standard	3/15/2018	1	AsIII	2.215	1868	14964	-0.001	0.999802		695	As28-yie6170917	
23	S0104	S2	Standard	3/15/2018	1	DMA	3.375	1750	17860	0.016115	0.999824		695	As28-yie6170917	
24	S0104	S2	Standard	3/15/2018	1		6.03	56	220				695	As28-yie6170917	
25	S0104	S2	Standard	3/15/2018	1	MMA	6.892	1336	15225	0.00646	0.999814		695	As28-yie6170917	
26	S0104	S2	Standard	3/15/2018	1	AsV	8.556	1704	15014	0.009153	0.999953		695	As28-yie6170917	
27	S0105	S3	Standard	3/15/2018	1	ISTD	0.651	13545	169137	0	1		695	As28-yie6170917	
28	S0105	S3	Standard	3/15/2018	1	AC	1.348	12250	68584	0.003873	0.99995		695	As28-yie6170917	
29	S0105	S3	Standard	3/15/2018	1	AB	1.659	15955	83468	0.009452	0.999948		695	As28-yie6170917	
30	S0105	S3	Standard	3/15/2018	1	TMAO	1.782						695	As28-yie6170917	
31	S0105	S3	Standard	3/15/2018	1	AsIII	2.212	8543	66972	-0.001	0.999802		695	As28-yie6170917	
32	S0105	S3	Standard	3/15/2018	1		2.931	95	293				695	As28-yie6170917	
33	S0105	S3	Standard	3/15/2018	1	DMA	3.355	7781	79003	0.016115	0.999824		695	As28-yie6170917	
34	S0105	S3	Standard	3/15/2018	1	MMA	6.9	6050	73221	0.00646	0.999814		695	As28-yie6170917	
35	S0105	S3	Standard	3/15/2018	1		7.329	119	468				695	As28-yie6170917	
36	S0105	S3	Standard	3/15/2018	1	AsV	8.532	6977	65785	0.009153	0.999953		695	As28-yie6170917	
37	S0106	S4	Standard	3/15/2018	1	ISTD	0.614	13488	166733	0	1		695	As28-yie6170917	
38	S0106	S4	Standard	3/15/2018	1	AC	1.345	35611	203095	0.003873	0.99995		695	As28-yie6170917	
39	S0106	S4	Standard	3/15/2018	1	AB	1.658	46722	241724	0.009452	0.999948		695	As28-yie6170917	
40	S0106	S4	Standard	3/15/2018	1	TMAO	1.782						695	As28-yie6170917	
41	S0106	S4	Standard	3/15/2018	1	AsIII	2.208	25241	205186	-0.001	0.999802		695	As28-yie6170917	
42	S0106	S4	Standard	3/15/2018	1		2.519	862	3832				695	As28-yie6170917	
43	S0106	S4	Standard	3/15/2018	1		2.642	428	3031				695	As28-yie6170917	
44	S0106	S4	Standard	3/15/2018	1		2.837	219	709				695	As28-yie6170917	
45	S0106	S4	Standard	3/15/2018	1		2.908	143	1158				695	As28-yie6170917	

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