

---

# Laboratory Procedure Manual

*Analyte:* arsenobetaine, arsenocholine,  
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:* 3000.15 (Formerly 0161A/01-OD)

*Revised:* **February 29, 2016**

*As performed by:*

Inorganic and Radiation Analytical Toxicology Branch  
Division of Laboratory Sciences  
National Center for Environmental Health

*Contact:*

Robert L. Jones, PhD  
Phone: 770-488-7991  
Fax: 770-488-0509  
Email: [RLJones@cdc.gov](mailto:RLJones@cdc.gov)

James L. Pirkle, MD, PhD  
Director, Division of Laboratory Sciences

***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\_I and UASS\_I. One method was used to measure both the urinary arsenics and urinary arsenics for smokers. However, these results are released as 2 separate data files.

<b>File Name</b>	<b>Variable Name</b>	<b>SAS Label (and SI units)</b>
UAS_I UASS_I	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)
	URXUTM	Urinary Trimethylarsine Oxide (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 high performance liquid chromatography (HPLC) 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<sup>-5</sup> 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 <sub>a</sub>
Arsenobetaine	AB	$\begin{array}{c} \text{CH}_3 \\   \\ \text{H}_3\text{C}-\text{As}^+-\text{CH}_2\text{COOH} \\   \\ \text{CH}_3 \end{array}$	-
Arsenocholine	AC	$\begin{array}{c} \text{CH}_3 \\   \\ \text{H}_3\text{C}-\text{As}^+-\text{CH}_2\text{CH}_2\text{OH} \\   \\ \text{CH}_3 \end{array}$	-
Monomethyl arsonic acid	MMA	$\begin{array}{c} \text{OH} \\   \\ \text{H}_3\text{C}-\text{As}^{(\text{V})} \\ // \quad \backslash \\ \text{O} \quad \text{OH} \end{array}$	4.1, 8.7
Dimethylarsinic acid	DMA	$\begin{array}{c} \text{CH}_3 \\   \\ \text{H}_3\text{C}-\text{As}^{(\text{V})} \\ // \quad \backslash \\ \text{O} \quad \text{OH} \end{array}$	6.2
Trimethylarsine oxide	TMAO	$\begin{array}{c} \text{CH}_3 \\   \\ \text{H}_3\text{C}-\text{As}^{(\text{V})} \\ // \quad \backslash \\ \text{O} \quad \text{CH}_3 \end{array}$	-
Arsenic (V) acid (arsenate)	As(V)	$\begin{array}{c} \text{OH} \\   \\ \text{O}=\text{As}^{(\text{V})} \\ / \quad \backslash \\ \text{HO} \quad \text{OH} \end{array}$	2.2, 7.0, 11.5
Arsenous (III) acid (arsenite)	As(III)	$\begin{array}{c} \text{OH} \\   \\ \text{HO}-\text{As}^{(\text{III})} \\   \\ \text{OH} \end{array}$	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 should take the Hazardous Chemical Waste Management for CDC Workers course upon initial hire and yearly refreshers thereafter. Place disposable plastic, glass, and paper (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, wipe down all work surfaces where human biological fluids were handled with a 10% (v/v) sodium hypochlorite solution. 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 should be read before operating the 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.

### **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. 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.**

## 3. DATA SYSTEM MANAGEMENT

---

To maintain the integrity of specimen and analytical data generated by this method, eliminate hand entry of specimen identifiers or analytical results whenever possible, proofread all transcribed data, and 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:\NexIONdata (include all subdirectories)
- ✓ C:\hplc (include subdirectories “data” and “methods as well as other relevant directories)
- ✓ C:\Event Log Backup
- ✓ Any other relevant files or folders.

**Daily.** Full data backups onto an encrypted, external hard drive (if equipped to do so).

**Weekly.** Full data backups onto one or more recordable compact discs (CD-R) or digital video discs (DVD). These backups are made in duplicate.

### (2) Backup Procedures

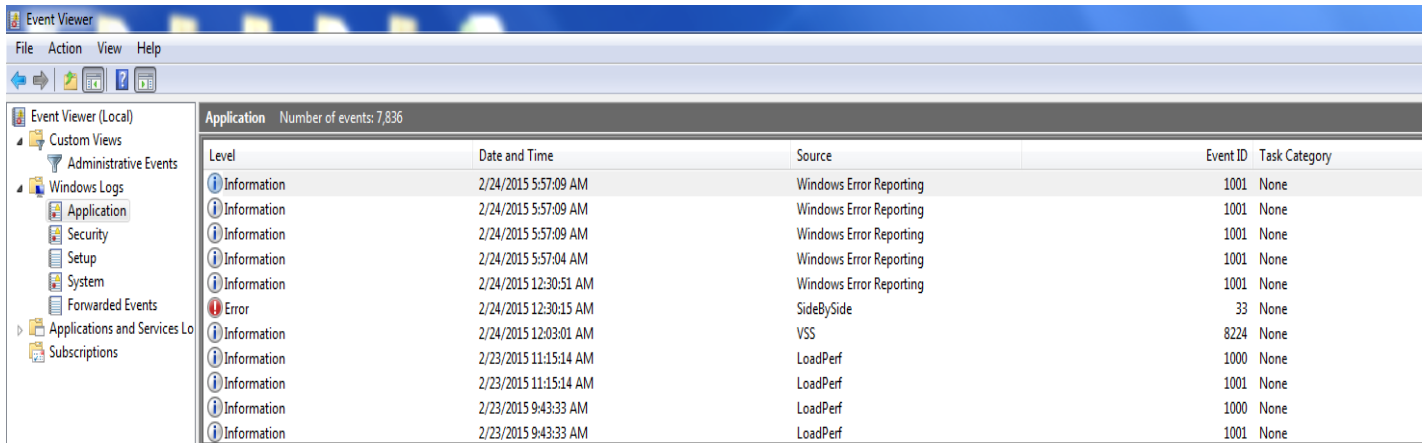
Whenever making a backup (daily or weekly) include the directories and subdirectories:

- ✓ C:\NexIONdata (include all subdirectories)
- ✓ C:\hplc (include subdirectories “data” and “methods as well as other relevant directories)
- ✓ Other relevant folders.

Before making weekly backups, saving a copy of the Windows™ event log in the active “NexIONdata” directory 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).

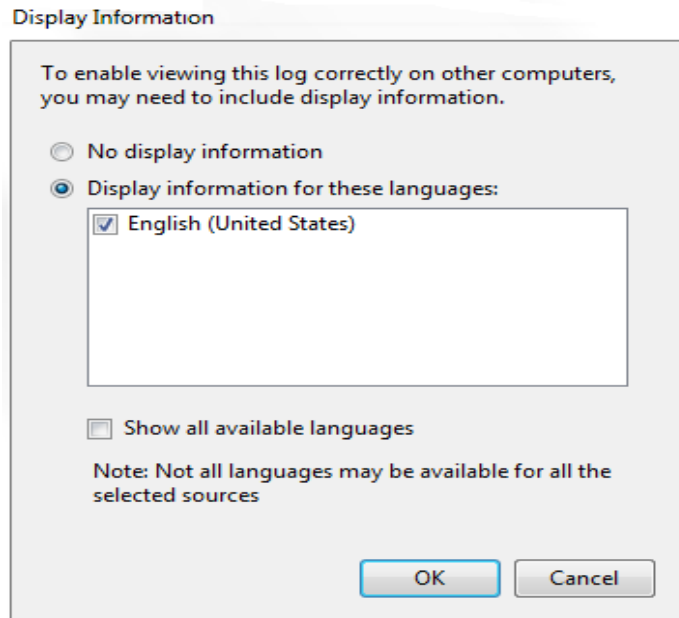
#### (a) Secondary Hard Drive Backups

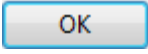
Before making a CD or DVD backup, saving the information from the Windows Event Viewer in C:\Event Log Backup will ensure archiving of all recent software system events including communications between ICP-DRC-MS and NexION® software, as well as times of hard drive defragmentation, and other Windows™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

**(b) Compact Disc Backups**

- Use CD-R disks only (recordable compact disks), *not* CD-RW disks (rewritable compact disks) so that after creation the recordable compact disk cannot be over-written. DVD discs are also acceptable when transferring large amounts of data.
- Use Roxio Creator or an equivalent software program to make CD-R/DVD backups .
- Back up the oldest data on the hard drive in duplicate onto two CD-R disks. Manually select each dataset folder (subdirectories under “C:\NexIONdata\dataset” and “C:\hplc\data”) and other relevant files (i.e., optimization, tuning, and sample files) that are to be included on these backups.
- Back up the most recent data on the hard drive in duplicate onto two CD-R/DVD disks. Manually select each dataset folder (subdirectories under “C:\NexIONdata\dataset” and “C:\hplc\data”) and other relevant files) that are to be included on these backups.
- Keep one copy of the disk in a building other than the laboratory (in case of fire). Keep the other copy in or near the ICP-MS laboratory for easy retrieval, if necessary.

d. Documentation of System Maintenance

**Computer Maintenance:** Record any maintenance of computer hardware or HPLC or ICP-DR-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 relating to instrument conditions (\*dac) and mass cal (default.tun). Whenever a service call is placed, store copies of any documents related to the service call in the instrument logbook.

#### 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 5.0-mL polypropylene cryogenic vials (e.g., Nalgene, Item # 5000-0050), 2 mL polypropylene cryogenic vials (e.g. Nalgene, item # 500-0020), 2 mL fluidX 2D coded cryo tubes (fluidX part 65-



7532), or equivalent storage containers. Screen lots of specimen collection cups, containers, and sample 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 sample as it was 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 inter-conversion of arsenic species, immediately store or transport urine specimens at approximately  $\leq -70^{\circ}\text{C}$ . Upon receipt, they must remain frozen at approximately  $\leq -70^{\circ}\text{C}$  until time for analysis. Refreeze remaining portions at approximately  $\leq -70^{\circ}\text{C}$ . Samples thawed and refrozen several times may be compromised.

c. Criteria for an Unacceptable Specimen

The criteria for an unacceptable specimen 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, AND QUALITY CONTROL MATERIAL

---

a. Chemicals

1. Deionized (DI) water, high purity (approximately 18 M $\Omega$ -cm resistivity), Aqua Solutions, or equivalent
2. Ammonium carbonate, (CAS# 506-87-6), MW 96.09, GFS Chemicals, Item #839-12471, 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 Inc., 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,  $\geq 99.999\%$  purity, Airgas South, Inc., Item # X02AR90C350976, or equivalent.
10. Double-distilled nitric acid, 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, GFS Chemicals, Item # 2482, or equivalent.
15. Bleach (10% sodium hypochlorite solution), Fisher Scientific, Item # 23-640-501, or equivalent.
16. Base urine pooled from anonymous donors or purchased from a vendor.
17. Potassium Persulfate, purified GFS Chemicals, Item #61712, or equivalent.

#### b. Standards

The following is a list of possible sources of material for the seven arsenic species. A mixture of the sources is used at any given time to provide calibration material that is from different sources. Other sources can be used, and please note that the availability of the sources listed is subject to change without notice.

1. Arsenic (III) oxide,  $\text{As}_2\text{O}_3$ , CAS 1327-53-3, MW 197.84, Sigma-Aldrich, Item # 202673, or equivalent.
2. Arsenic (III) speciation standard in 2% $\text{HCl}$ , Spex CertiPrep, Item # SPEC-AS3, or equivalent.
3. Arsenic (III) in 2% $\text{HCl}$ , Inorganic Ventures, Item # CGAS(3)1-1, or equivalent.
4. Arsenic (III) in 2% $\text{HCl}$ , High Purity Standards, Item # 10003-6, or equivalent.
5. Arsenic (V) oxide hydrate,  $\text{As}_2\text{O}_5 \cdot x\text{H}_2\text{O}$ , CAS 12044-50-7, MW 229.84, Sigma-Aldrich, Item # 363456, or equivalent source or vendor.
6. Arsenic (V) in water, Spex CertiPrep, Item # SPEC-AS5, or equivalent.
7. Arsenic (V) in water, Inorganic Ventures, Item # CGAS(5)1-1, or equivalent.
8. Arsenic (V) in water, High Purity Standards, Item # 10003-7, or equivalent.
9. Arsenobetaine (AB),  $(\text{CH}_3)_3\text{AsCH}_2\text{COOH}$ , CAS 64436-13-1, MW 178.06, Sigma-Aldrich, Item # 11093, or equivalent.
10. Arsenobetaine (AB),  $(\text{CH}_3)_3\text{AsCH}_2\text{COOH}$ , CAS 64436-13-1, MW 178.06, Wako USA, Item # 321-34911, or equivalent.
11. Arsenobetaine (AB),  $(\text{CH}_3)_3\text{AsCH}_2\text{COOH}$ , CAS 64436-13-1, MW 178.06, Argus, Vernio, Italy, Item # AR60008, or equivalent.
12. Arsenocholine bromide (AC),  $(\text{CH}_3)_3\text{AsCH}_2\text{CH}_2\text{OH} \cdot \text{Br}$ , MW 244.99, Argus, Vernio, Italy, Item # AR60010, or equivalent.
13. Arsenocholine bromide (AC),  $(\text{CH}_3)_3\text{AsCH}_2\text{CH}_2\text{OH} \cdot \text{Br}$ , MW 244.99, Wako USA, Item # 328-34921, or equivalent.
14. Dimethylarsinic acid (DMA),  $(\text{CH}_3)_2\text{As}(\text{OH})_2$ , MW 138.01, Sigma-Aldrich, Item # PS-51, or equivalent.
15. Cacodylic Acid (DMA),  $(\text{CH}_3)_2\text{As}(\text{O})\text{OH}$ , CAS 75-60-5, MW 138.00, Sigma-Aldrich, Item # 20835-10G-F, or equivalent.
16. Monomethylarsonic acid disodium salt (MMA),  $\text{CH}_3\text{AsO}_3\text{Na}$ , CAS 144-21-8, MW 183.93, Argus Chemicals, Vernio, Italy, Item # AR60009, or equivalent
17. Disodium methyl arsenate (MMA),  $\text{CH}_3\text{AsO}_3 \cdot 6\text{H}_2\text{O}$ , CAS 144-21-8, MW 291.9, Sigma-Aldrich, Item # PS-281, or equivalent.

18. Monosodium acid methane arsonate sesquihydrate (MMA), CAS 2163-80-6, Sigma-Aldrich, Item #, PS-429, or equivalent.
19. Trimethylarsine oxide (TMAO), (CH<sub>3</sub>)<sub>3</sub>AsO, CAS 4964-14-1, MW 136.03, Argus Chemicals, Vernio, Italy, Item # AR60011, or equivalent.

### c. Quality Control Material

Quality control (QC) materials are prepared from pools of human urine collected from several anonymous donors. See the “Preparation of Quality Control Material” section of this method for details on preparation. The two 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 *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 same 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 extended calibration curve are designated as S0, S4, S5, S6, and S7.

## 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®.
3. Chiller 1 HP 230V/60HZ TP Quiet, PolyScience, 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 *2012/2013 Consumables Catalog and 2012 NexION® 300 ICP-MS Consumables and Supplies Reference Guide* 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.), Item # N8145202, and for waste (3.16 mm i.d.), 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. 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 pumps oil for the roughing pump, Item # N1845003, 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 # F1-C16, 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 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. Dionex 2L HPLC reservoir bottles with cap, Dionex, Item # 44129, or equivalent

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  $\geq 18$  M $\Omega$ -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  $\geq 18,000$  rcf for centrifugation of 1.5 mL capacity microcentrifuge tubes.
4. 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.
5. 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.
6. pH meter with one hundredths of a pH unit readout or better, fitted with glass electrode (pH probe), Accumet XL-150, or equivalent
7. Hamilton Microlab 625 Diluter, Hamilton Company, Item # ML625-DIL, or equivalent
8. Calibrated hand-held adjustable pipettes that cover the range of accurate

liquid delivery from 50  $\mu\text{L}$  to 5000  $\mu\text{L}$ . Eppendorf™ Xplorer™, Fisher Scientific, or Sartorius Picus NxT, Sartorius Corporation, single channel electronic programmable pipettes, or equivalent

9. Honeywell Xenon 1902 USB 2D barcode reader, Computech International, Item # 1902HHD-0USB-5F, or equivalent
10. "Repeater Plus" Pipetter, Fisher Scientific, Item # 2226020-1, or equivalent pipetting device(s) capable of accurately dispensing multiple microliter aliquots of liquid.
11. Vortex mixer, VWR, Item # 945303, or equivalent.
12. Gas regulator for 10% DRC™ gas, Matheson Tri-Gas Products, or equivalent.
13. Gas regulator for argon gas, Matheson Tri-Gas Products, or equivalent.
14. CEM Discover SP-D closed vessel microwave, CEM Corporation, or equivalent
15. Stormat Sealer, PerkinElmer®, Item# CUSG01273, or equivalent.

c. Computer Software

1. PdfFactory Pro 5 or later version, FinePrint Software, LLC, [www.fineprint.com](http://www.fineprint.com) or equivalent. This product is used for creating electronic Portable Document Files (pdf) directly from Microsoft® Windows print dialog box.
2. A custom Microsoft Excel® macro procedure named "Extract TC Data". See *Appendix a* section in the *Appendix* for description and macro code.
3. NexION® instrument control and data handling software, version 1.5 or greater, PerkinElmer®.
4. Chromatography data handling software, specifically, TotalChrom™ Workstation, version 6.0.2 or later (PerkinElmer®) with ChromLink 2.0 or greater, or equivalent

d. Supplies

1. 2-200  $\mu\text{L}$  pipette tips, 960 tips per case, Fisher Scientific, Item # 05-403-66, or equivalent.
2. 20-300  $\mu\text{L}$  pipette tips, 960 tips per case, Fisher Scientific, Item # 05-403-67, or equivalent.
3. 50-1000  $\mu\text{L}$  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 # 05-403-117, or equivalent.
5. 0.5-200  $\mu\text{L}$  LowRet pipette tips, 960 tips per case, Sartorius Corporation, Item # LH-L790200, or equivalent
6. 5-350  $\mu\text{L}$  LowRet pipette tips, 960 tips per case, Sartorius Corporation, Item # LH-L790350, or equivalent
7. 10-1000  $\mu\text{L}$  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. 0.75 mL 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, 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, fluidX, UK, Item # 170-01-110.
16. fluidX racks for fluidX 2D cryotubes, fluidX, UK, Item # 65-9451.
17. 2D coded jacket cryotubes external threaded with yellow caps 480 per case, fluidX UK, Item # SP-2003.
18. 2D coded jacket cryotubes external threaded with blue caps 480 per case, fluidX UK, Item # SP-2004.
19. 2D coded jacket cryotubes external threaded with orange caps 480 per case, fluidX UK, 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 pipe tip – 200µL 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, Kimberly-Clark Corp., Item # 06-666-11, or equivalent.
27. Teflon™-coated magnetic stir bars (2), VWR, Item #58948-974, or equivalent.
28. Nitrile, powder-free examination gloves (N-Dex®, Best Manufacturing Co., Menlo, GA), or equivalent.
29. Biohazard autoclave bags (Fisher Scientific), or equivalent.
30. 35 mL Pyrex vials, CEM Corporation, Item # 909036, or equivalent.
31. 35 mL 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 tube “Base Urine – Speciated Arsenic” or a similar designation.

c. Preparation of Working Solutions

1. **25% (%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 1 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  $8.60 \pm 0.05$ . 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.** 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  $8.00 \pm 0.1$ . 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 (Rinse Solution) Preparation. 5% (v/v) acetonitrile** (HPLC grade). Mix 50 mL of acetonitrile with 950 mL of 18 M $\Omega$ -cm water. Prepare as needed. Expiration date is 1 year from the date made.
4. **HPLC Buffer D (Autosampler Wash Solution) Preparation. 5% (v/v) acetonitrile** (HPLC grade). Mix 50 mL of acetonitrile with 950 mL of 18 M $\Omega$ -cm water. Prepare as needed. Expiration date is 1 year from the date made.
5. **Internal Standard (2.5  $\mu$ g/L) Preparation.** May be prepared ahead of time before the day of analysis. To an empty 1 liter PP bottle labeled "Internal Standard", add 5.0 mL of methanol. Next, add 50  $\mu$ L of 50 mg/L trimethylarsine oxide (TMAO Internal Standard Stock Solution). Fill with 18 M $\Omega$ -cm water to the 1000 mL mark. Mix thoroughly. Prepare as needed. Expiration date is 1 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. **Wear appropriate personal protective gear. At a minimum, wear a laboratory coat, safety glasses, and latex or nitrile gloves.** Clean up any spill that occurs according to applicable hazardous material spill procedures.

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

***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  $\mu$ g/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, prepare the arsenic Stock Standards described in TABLE 7-2 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	Water ( $\geq 18$ M $\Omega$ -cm)	10.00
Arsenocholine bromide (or other salt equivalent)	0.20–0.25	Water ( $\geq 18$ M $\Omega$ -cm)	50.00
Disodium methyl arsenate	0.20–0.25	Water ( $\geq 18$ M $\Omega$ -cm)	50.00
Trimethylarsine oxide	0.10–0.125	Water ( $\geq 18$ M $\Omega$ -cm)	50.00
Dimethylarsinic acid	0.10–0.125	Water ( $\geq 18$ M $\Omega$ -cm)	50.00
Arsenic (V) oxide hydrate	0.10–0.125	Water ( $\geq 18$ M $\Omega$ -cm)	50.00
Arsenic (III) oxide	0.10–0.125	Dissolve in 1.5 ml of 6N HCl with mild heating. Add water ( $\geq 18$ M $\Omega$ -cm) to complete volume	50.00

2. Tightly cap the storage vessels for future use. Expiration date is 1 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 (F.W.) and number of arsenic atoms per molecule for each species. A list of commonly used formula weights 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 and should be used instead.**

TABLE 7-2: FORMULA WEIGHTS OF ARSENIC SPECIES

Arsenic Species	Formula Weight	Number of Arsenic Atoms per Molecule	Formula
Arsenobetaine	178.06	1	$(\text{CH}_3)_3\text{AsCH}_2\text{COOH}$
Arsenocholine bromide	244.99	1	$(\text{CH}_3)_3\text{AsCH}_2\text{CH}_2\text{OH}\cdot\text{Br}$
Disodium methyl arsenate	291.9	1	$\text{CH}_3\text{AsO}_3\cdot 6\text{H}_2\text{O}$
Trimethylarsine oxide	136.03	1	$(\text{CH}_3)_3\text{AsO}$
Dimethylarsinic acid	138.01	1	$(\text{CH}_3)_2\text{As}(\text{OH})_2$
Arsenic (V) oxide hydrate	229.84	2	$\text{As}_2\text{O}_5\cdot x\text{H}_2\text{O}$
Arsenic (III) oxide	197.84	2	$\text{As}_2\text{O}_3$

*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, and 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 100 mL of 3% potassium persulfate ( $\text{K}_2\text{S}_2\text{O}_8$ ) in  $\geq 18\text{-M}\Omega$  deionized water. Expiration date is 1 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%  $\text{K}_2\text{S}_2\text{O}_8$  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  $\text{K}_2\text{S}_2\text{O}_8$ ) and triplicate certified AsV solution 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. Using an analytical balance, once digestion vessels are at room temperature, quantitatively transfer (with  $\geq 18\text{-M}\Omega$  de-ionized water) each digested solution into separate labeled 50 mL centrifuge tubes. Bring the final weight to 50.0 g using  $\geq 18\text{-M}\Omega$  deionized water, and record the total weight to three significant digits. The expected arsenic concentration of each solution should be approximately 100  $\mu\text{g/L}$  as arsenate (AsV).
5. Dispose 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.
6. Using an analytical balance, once digestion vessels are at room temperature, quantitatively transfer (with  $\geq 18\text{-M}\Omega$  de-ionized water) each digested solution into separate labeled 50 mL centrifuge tubes. Bring the final weight to 50.0 g using  $\geq 18\text{-M}\Omega$  deionized water, and record the total weight to three significant digits. The expected arsenic concentration of each solution should be approximately 100  $\mu\text{g/L}$  as arsenate (AsV).

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  $\mu\text{g/L}$  gallium in deionized water by diluting 2 mL of 1000  $\mu\text{g/L}$  stock Ga in 1000 mL of deionized 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 ppm AsV “spiking solution” in deionized water. Weigh 0.25 g of 1,000 mg/L certified arsenate solution and bring to a final weight of 50.0 g using deionized 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  $\mu\text{g/L}$  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  $\mu\text{g/L}$  Ga solution). Record all weights. Final concentrations should be approximately 50  $\mu\text{g/L}$ .
4. For each new 50  $\mu\text{g/L}$  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 1

Tube #	Labels		
	Analyte	Replicate	Spike in $\mu\text{g/L}$
1	AB	1	0
2	AB	1	25
3	AB	1	50
4	AB	1	100

*Note: A spike of 0  $\mu\text{g/L}$  corresponds to an unspiked sample for which a value will be determined in subsequent steps via total arsenic analysis.*

- 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  $\mu\text{g/L}$  microwave-digested solution:

- Into the tube labeled 0  $\mu\text{g/L}$ , transfer by weight approximately 10.0 g of the approximately 50  $\mu\text{g/L}$  diluted microwave-digested solution prepared in Step 8.g.3. Record the weight.
- Into the tube labeled 25  $\mu\text{g/L}$ , transfer by weight approximately 0.05 g of the approximately 5 ppm spiking solution prepared in step 8.g.2. Additionally, transfer by weight 10.0 g of the approximately 50  $\mu\text{g/L}$  diluted microwave-digested solution prepared in Step 8.g.3. Record all weights.
- Into the tube labeled 50  $\mu\text{g/L}$ , transfer by weight approximately 0.10 g of the approximately 5 ppm spiking solution prepared in step 8.g.2. Additionally, transfer by weight 10.0 g of the approximately 50  $\mu\text{g/L}$  diluted microwave-digested solution prepared in Step 8.g.3. Record all weights.
- Into the tube labeled 100  $\mu\text{g/L}$ , transfer by weight approximately 0.20 g of the approximately 5 ppm spiking solution prepared in step 8.g.2. Additionally, transfer by weight 10.0 g of the approximately 50  $\mu\text{g/L}$  diluted microwave-digested solution prepared in Step 8.g.3. Record all weights. TABLE 7-4: MSA TABLE 2 shows an example of the information that needs to be recorded for each analyte and corresponding tube.

TABLE 7-4: METHOD OF STANDARD ADDITIONS TABLE 2

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

#### h. Determining the Total Arsenic Concentration

- 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	$M1MSA = \frac{V2MSA * M2MSA}{V1MSA}$	$M1MW = \frac{V2MW * M2MW}{V1MW}$
-------------	---------------------------------------	-----------------------------------

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. From each 10,000 µg/L solution prepare a 100 µg/L solution.
2. Using the CLIA "Urine arsenic species HPLC-ICP-DRC-MS" method, analyze each arsenic species for the presence of significant levels (>2%) 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 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 should 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. Expiration is 1 year from date of preparation.

1. Prepare concentrated Stock Internal Standard, 50 mg/L trimethylarsine oxide (TMAO). Dilute a calculated volume ( $\mu\text{L}$ ) of trimethylarsine oxide (TMAO) stock standard in 18 M $\Omega$ .cm water to make a total volume of 20 mL. Calculate the number of  $\mu\text{L}$  of stock TMAO standard to add using Equation 2:

Equation 2

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

k. Preparation of Working Calibrators

At any given time, it is necessary to have at least two sets of Working Calibrators in storage from two independent preparations of Stock Standards. The original material should be from different lots, preferably from alternate vendors. Alternate usage of the different sets of working calibrators throughout the analytical runs.

**(1) Preparation of Intermediate “Mixed Species” Calibration Solutions**

Prepare the intermediate mixed species calibration solutions on the day of analysis.

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  $\mu\text{g/L}$ ” (or similar designation). Likewise, add 7.5 mL of ammonium acetate pH 5 to another dedicated 50 mL volumetric flask labeled “TMAO 250  $\mu\text{g/L}$ ” 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 $\mu\text{g/L}$  mixed arsenic species solution. To the flask labeled “Mixed As Species 250  $\mu\text{g/L}$ ”, add each of the following Intermediate Standards: AC, AB, DMA, MMA, As(III) and As(V) - six solutions in all so that each analyte’s final concentration will be 250  $\mu\text{g/L}$  at a volume of 50.0 mL. Use of the dilution equation  $C_1 \cdot V_1 = C_2 \cdot V_2$  is helpful, where  $C_1$  is the pure measured value of

an analyte + 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 18 MΩ·cm 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, $\mu\text{L}$		Sample Syringe Size, $\mu\text{L}$	Target Arsenic Concentration (Nominal Conc. entered into HPLC software)	
	Diluent Syringe	Sample Syringe			
<b>S0 / T0</b>	5000	0	<b>100</b>	<b>0</b>	<b>(0)</b>
	5000	0			
<b>S0 DUP / T0 DUP</b>	5000	0	<b>100</b>	<b>0</b>	<b>(0)</b>
	5000	0			
<b>S1 / T1</b>	5000	0	<b>100</b>	<b>0.50</b>	<b>(2)</b>
	5000	0			
	5000	0			
	5000	0			
	4950	50			
<b>S2 / T2</b>	4950	50	<b>100</b>	<b>2.50</b>	<b>(10)</b>
	4950	50			
<b>S3 / T3</b>	4750	250	<b>500</b>	<b>12.5</b>	<b>(50)</b>
	4750	250			
<b>S4 / T4</b>	4250	750	<b>1000</b>	<b>37.5</b>	<b>(150)</b>
	4250	750			
<b>S4 DUP / T4 DUP</b>	4250	750	<b>1000</b>	<b>37.5</b>	<b>(150)</b>
	4250	750			
<b>S5 / T5</b>	2500	800	<b>1000</b>	<b>62.5</b>	<b>(250)</b>
	2500	850			
	2500	850			
<b>S6 / T6</b>	1000	1000	<b>1000</b>	<b>125.0</b>	<b>(500)</b>
	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.

a. 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 90 minutes at 5,250 x g in a preparative table-top centrifuge cooled to 4°C 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 2.5 years from the date made.

#### b. Processing of Urine Samples and QC Material

Process a chosen number of urine samples and QC material on the day of analysis. One run is defined as the analysis of a contiguous set of samples (typically 20) 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  $\mu$ L of diluent (for flushing).

(f) Repeat steps (a) – (e) until the program is stopped.

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 20,800 x g in a refrigerated

centrifuge pre-cooled to approximately 4°C.

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 150µg/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"), add 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.
  - (d) Pump with autosampler. Set the autosampler on top of the pump.
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

The NexION® Instrument Control software version 1.5 or greater should be installed on the computer controlling the NexION®. If it is not, contact a PerkinElmer® service representative to get the latest version of the software installed.

It is preferable to install PerkinElmer®'s TotalChrom™ Workstation package on the same computer that contains the NexION® Instrument Control software\*. It is advised that a PerkinElmer® representative install and initially configure TotalChrom™. Alternatively, laboratory personnel can install and configure TotalChrom themselves by following instructions contained in *TotalChrom Workstation User's Guide*, or by getting help from PerkinElmer® Technical Services. As part of installing TotalChrom™, create a folder named “hplc” on the root level of the C drive. Create the following subfolders inside the “hplc” folder: “methods”, “data”, “optimization”, and “reports”. Feel free to create additional subfolders as the need arises. This method assumes that version 6.2.0 of TotalChrom™ Workstation package is installed.

ChromLink™ 2.0 or a greater version (PerkinElmer®) is installed into its own program folder. The installation of ChromLink™ is quick and straightforward when using the supplied installation utility.

PDFFactory Pro 1.52 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 solving chromatographic integration challenges, 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

---

\* Raw signal versus time data is collected by the NexION® software and stored on NexION® controller computer's hard drive as a “NetCDF” file before it is read by the TotalChrom Workstation software. For the purposes of this arsenic speciation method, TotalChrom Workstation is used only for post-run data processing and is not used to operate the HPLC or the NexION®.



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 unless you are able to get one pump to work for both purposes.

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 interfacing of the switching valves to the HPLC system has to be made by a PerkinElmer service engineer.

#### e. Six-Port Switching Valves

Each 6-port switching valve’s DIP switches need to be configured upon initial installation. If the switching valves are not put into “pulse mode”, the units will not respond to the ~1 second contact closure from the HPLC autosampler. 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.

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.
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 7.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. Plug-in the A/C power adapter to the back of each unit and into a regular 110V outlet. The switching valve will automatically come on, and one of the diode indicators will light up. Press the "Local/Remote" button on the front of the unit so that the top yellow indicator light is on.
  - (a) For Switching Valve #1: Press the left arrow button until the LCD display indicates "1". Press the "Local/Remote" button again so that the bottom green indicator light is now on, representing "remote."
  - (b) For Switching Valve #2: Press the left arrow button until the LCD display indicates "2." Ensure that the top yellow indicator light is on, representing "local."

*Note: These LCD positions are for a typical HPLC-ICP-DRG-MS analysis run. Users may toggle between "local" and "remote" and positions "1" and "2", for instrument optimization or other tasks.*

The switching valves are now ready for operation.

## 9. INSTRUMENT SETUP AND CONFIGURATION

---

### a. Programming the Agilent Infinity 1260 System

To improve work flow, instrument setup described in this section may be completed after installation of the HPLC system.

1. On the Agilent Instant Pilot welcome screen click "Method".
2. In the "System" section tab to the row "Stoptime". Click "Edit" and enter "9.5" to set the run time in minutes. Click "Done". This will automatically update the stoptime in the Quat Pump section.
3. In the "Quat Pump" section tab down to the row "Flow". Click "Edit", enter "1", and click "Done".
4. Click the "Timetable" tab along the bottom to set up the buffer gradient. Click "Insert" and select option "1 Quat Pump". In the "Time" column enter "0.10" then press "enter". In the "Setting" column tab over to the "%B, %C, %D" option and press "Enter". In the "Value" column, enter "0" for B, but leave the C and D areas at "OFF". Once entered click "Done". Repeat these steps until all the rows of TABLE 9-1 below have been entered for the Quat Pump. Click "Exit".

TABLE 9-1: HPLC PUMP TIMETABLE

TIME	SETTING	VALUE
0.10	%B, %C, %D	0, OFF, OFF
5.00	%B, %C, %D	100, OFF, OFF
9.00	%B, %C, %D	100, OFF, OFF

5. The pump is now programmed.
6. In the "HiP ALS" section tab down to the "Injection Volume" row. Click "Edit" and enter "20" if this value is not already set at "20". Click "Done".
7. Tab to the row "Injection Mode" and click "Edit". Arrow to the option "Injector Program" and then click "Done".
8. Arrow to the "Injector Program" row and click "Edit". Click "Insert" and select option "8 More". Tab over to the new window that appears and select option "3 Wait". In the new window that appears select option "1 Wait". Enter "6.5" and then click "Done". This is the equilibration time for the column.
 

**NOTE:** This will appear as a "Prerun" once you begin a sequence and does not go into the total sample analysis time.
9. At the "Injector Program" home screen click "Insert" and select option "1 Draw". Tab over to the new window and select "Draw def. from sample" then click "Done". Once back in the home screen click "Insert" and select option "4 Inject" then "Done". The injector program is now complete. Click "Exit" to return to the "Method" screen.
10. Set the wash position by tabbing over to the row and click "Edit". Tab over to sample position and select "V" then press "Enter". Tab to the next column and

key in "1". Press "Done". The wash position has been set as "V1".

11. Click the "Timetable" button at the bottom of the Instant Pilot. Click "Insert" and select option "2 HiP ALS". In the "Time" column key in "0.00" press "Enter". In the "Setting" column, tab over to the "External Contact 2" option and press "Enter". In the "Value" column, tab over to "Open". Once entered, click "Done". Repeat these steps until all the rows of TABLE 9-2 below have been entered for the HiP ALS. Click "Exit" when complete.

TABLE 9-2: HPLC Autosampler Timetable

TIME	SETTING	VALUE
0.00	External Contact 2	Open
0.10	External Contact 1	Closed
0.40	External Contact 2	Closed
0.50	External Contact 1	Open
0.50	External Contact 2	Open
0.90	External Contact 1	Closed
1.00	External Contact 1	Open
1.10	External Contact 2	Closed

12. The autosampler is now programmed.
13. Set the thermostatted column compartment (TCC) by arrowing down to the "TCC" section to the row "Temp (Left)" and clicking "Edit".
14. Arrow to the "Value" column and select the "Set Temp. to" option and press "enter". In the next column key in "35" and click "Done".
15. Arrow to the next row "Temp (Right)" and click "Edit". In the "Value" column arrow to the option "same as left" and click "Done".
16. The TCC is now programmed.
17. Click "Exit" and when prompted click "Yes" to save and name the method "As Speciation".
18. To program the "WASH" method click "Method" from the welcome screen. Arrow down to the "Stoptime" row and click "Edit". Enter "20" and click "Done". Arrow to the row "%B, %C, %D" and click "Edit". Tab over and key in "0", press "Enter", key in "0", press "Enter", and key in "100", press "Enter". The row will read "0,0,100" when done. Click "Timetable" at the bottom of the Instant Pilot. If anything appears in the timetable click "Delete" on the right hand side and select option "2 Delete All". Click "Exit" and when prompted click "Yes" to save and name the method "WASH".
19. This completes the programming the methods for the Agilent 1260 system.

#### b. ICP-DRC-MS Instrument Setup

To improve workflow, complete the programming steps described in this section before the day of analysis.

## (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. 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 “1”, change the value in the field named “Flow Change” to “1”. 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” 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 7- into this window :

---

TABLE 9-3: NEXION® TIMING PARAMETERS

---

Parameter	Setting
Sweeps/Reading:	1
Readings/Replicate:	1403
Number of Replicates:	1
Mass Cal File:	C:\NexIONdata\masscal\default.tun
Optimization File:	C:\NexIONdata\conditions\As_HPLC.dac

---

- (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 enter key. 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 9-4-.

---

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

TABLE 9-4: 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 following information:

TABLE 9-5: 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 9-6.

TABLE 9-6: 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 from TABLE 9-7.

TABLE 9-7: NEXION® REPORT PARAMETERS

Parameter	Setting
Report View   Send to Printer:	Unchecked
Report Options Template:	<leave empty> *
Automatically Generate NetCDF File:	C:\NexIONdata\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 3:
  - (a) Make the active method file the active window (do this by clicking on the tool bar icon that looks like a peak with a red arrow on crest of it). 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 8. *Note: Values in TABLE 9-8 are suggested starting values. Instruments vary in their optimal parameter values, and analysts should use their discretion.*

TABLE 9-8: NEXION® OPTIMIZATION PARAMETERS

Parameter	Setting	Parameter	Setting
Nebulizer Gas Flow (NEB):	0.9*	Cell Rod Offset [CRO]	-12*
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*	DRC™ Mode CRO	-2*
Discriminator Threshold:	12	DRC™ Mode AFT	250*
Deflector Voltage:	-10*	DRC™ Mode RPa	0
Quadrupole Rod Offset [QRO]	0*	DRC™ Mode RPq	0.50*
Cell Entrance Voltage	-3*	DRC™ Mode Cell Gas A	0.55*
Cell Exit Voltage	-3*	DRC™ Mode Cell Gas B	0
Cell Rod Offset {CRO}	-12*		
DRC™ Mode NEB	0.9*		

\*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.

3. The NexION® method “as\_hplc.dac” is now loaded into memory.

#### (4) Creating the NexION® Sample Table “.sam” 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 “New” button for “Dataset”, the second item on the list. Navigate to the folder “C:\hplc\data” and enter the file name “As<yymmdd>” (where yy = last 2 digits of current year, mm = month, and dd = date of run, for example, As110201 denotes an Arsenic Speciation run on Feb 1, 2011) and click the “Create” button. The new dataset folder has been created and is now active. Click “OK” in the Review Files Window.
2. Click on the tool bar icon that looks like three Erlenmeyer flasks. Choose File > New on the menu bar. A new window will appear titled “Samples – [Untitled]”. Click the “Batch” tab. Click on the box for the “Use Manual Sampling: to place a check mark to indicate usage of manual sampling.
3. Click on the “Sample Template...” button. A dialog box titled “Sample Template Data” will appear. Enter the information for TABLE 9-9.



---

TABLE 9-9: SAMPLE TEMPLATE DATA

---

Parameter	Setting
Sample ID Prefix:	<leave empty>
Sample ID Number:	101
Sample ID Suffix:	_ <a single underscore character>
Increment:	1
Autosampler Position – Number:	100
Autosampler Position – Increment:	0
Range – Start Row:	1
Range – End Row:	5

---

Click the “Generate” button.

4. Again, click on the “Sample Template...” button. The same dialog box titled “Sample Template Data” will appear. Enter this information:

---

TABLE 9-10: SAMPLE TEMPLATE DATA

---

Parameter	Setting
Sample ID Prefix:	<empty>
Sample ID Number:	001
Sample ID Suffix:	_ <a single underscore character>
Increment:	1
Autosampler Position – Number:	1
Autosampler Position – Increment:	1
Range – Start Row:	6
Range – End Row:	105

---

Click the “Generate” button.

5. From the menu bar, choose File > Save As and save the file in the directory “C:\hplc\data\” using the name “As<yymmdd>.sam” (where yy = last 2 digits of the current year, mm = month, and dd = date of run).
6. It is a good idea to save a copy of this file as a template, thereby avoiding the need to re-create it every time.

## 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.

#### **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" and "E" are placed into bottles containing 5% acetonitrile in water. Press the "rinse" key on the autosampler to prime and rinse the autosampler fluid path.
2. Open the purge port by turning its knob counterclockwise  $\sim\frac{1}{4}$  to  $\frac{1}{2}$  turn.
3. From the welcome screen on the Instant Pilot control, click the tab "Control" and select "1 System: On/Off".
4. On the row labeled "Quat Pump" click the corresponding "On". The pump will first initialize and then go to "On" mode.
5. Click "exit" to return to the welcome screen.
6. From the welcome screen select "Status". In the status screen, the rectangular box in the upper right hand corner will have buffer percentages. Allow the pump to purge at 100% A until all bubbles are flushed out of the tubing.
7. Tab over to the box corresponding to "%B" and change the value by entering "100" followed by clicking "Enter". Allow buffer B to purge until all bubbles are flushed out of the tubing.
8. Once buffer B has purged enter a "0" in the box "%B" to return the buffer to 100% A. Click "Exit" to return to the welcome screen.
9. Place the pump in standby mode until ready to begin analysis. Click "Control" and select "1 System: On/Off". At the bottom of the screen click "Quat Pump" and select option "3 Pump to Stand By". Click "Exit" to return to the welcome screen.
10. Close the valve (turn the knob clockwise), and replace the cover.

### (3) Adjusting the Peristaltic Pumps

1. Check the external peristaltic pump's tubing for signs of wear, which will be evident by flattening of the tubing and pinch-roller marks. Excessively worn tubing should 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. Close the remaining clamps of the other channels.
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. Ensure that the digit "1" is displayed on switching valve #1. If it is not, press the "local/remote" button until a yellow light indicates local mode. Then, toggle the arrows to display a value of "1" in the digital window. Additionally, ensure that a value of "1" is in the display on switching valve #2.
3. Launch the NexION® 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.
4. 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  $8 \times 10^{-7}$  and  $1.8 \times 10^{-6}$  torr). Keep a record of any maintenance procedures along with the base vacuum pressure in the *Daily Maintenance Checklist* logbook.
5. 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 carboy jug.

6. In the INSTRUMENT window of the NexION® software, click the “Main” tab and click the plasma “Start” button to ignite the plasma. In the same 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.

7. 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*).
8. 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 24. 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 should be correct. Re-insert the sample probe into the rinse solution.
9. 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.
10. Let the ICP-DRC-MS warm up for 30-45 minutes.
11. 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 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 “as\_hplc.dac” file then click the “Open” button. Return to File > Review Files and click the “Load” button for “Method”, the first item on the list. Navigate to the folder “C:\NexIONdata\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.

12. 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 should give counts >2000 cps (in Standard Mode). 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”.
13. 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*.
14. 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 approximately 7 psi.
3. Click on the “Manual Adjust” tab of the “Conditions” window and enter a value of “0” in the appropriate cell-gas field (cell-gas A or B, depending on how the instrument is set up). Then enter a value of 0.6\* in the same field. A clicking should be heard from the ICP-DRC-MS cell-gas solenoid as the flow turns on.
4. Monitor the flow on the mass-flow controller by clicking on the “Diagnostics” tab of the INSTRUMENT window of the NexION® program and look for a field labeled “Cell Gas A”. The flow should 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. After the analysis of the DRC™ mode Daily performance check is complete and deemed satisfactory, change the selections on the 6-port switching valves #1 and #2 to match the values described below:
  - (a) Switching Valve #1: Remote, "1"
  - (b) Switching Valve #2: Local, "2"
8. 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 NexION® Sample Table

1. Click on the tool bar icon that looks like three Erlenmeyer flasks. If the current Samples window is not this run's sample file, then choose File > Open on the menu bar and navigate to and open this run's current data folder in "C:\hplc\data". Click on the file named "As-<yymmdd>.sam" (yy = year, mm = digit month, dd = date) and open it. The Samples window will be the one created in the *Creating the NexION® Sample Table ".sam" file* section.
2. Fill in the name of each sample by double-clicking after the "\_" (underscore) in the cell matching its "A/S Loc". 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, an Excel workbook macro 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.
  - Autosampler tray position 100 will contain the vial containing an excess low
  - Autosampler tray position 100 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.
  - Autosampler tray position 99 needs to contain a blank vial (even an empty vial will do).
  - Insert a "Bk" between the equilibrators. Also insert blank checks throughout the run as needed to show that carryover is not occurring.
  - 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.

TABLE 10-1: NEXION® SAMPLES TABLE

A/S Loc.	Batch ID	Sample ID	Measurement Action	Method	...	Sample Type
100		101_EQ	Run Sample			Sample
100		102_EQ	Run Sample			Sample
100		103_EQ	Run Sample			Sample
100		104_EQ	Run Sample			Sample
100		105_EQ	Run Sample			Sample
1		001_Bk	Run Sample			Sample
2		002_S0	Run Sample			Sample
3		003_S1	Run Sample			Sample
4		004_S2	Run Sample			Sample
5		005_S3	Run Sample			Sample
6		006_S4	Run Sample			Sample
7		007_T0	Run Sample			Sample
8		008_T1	Run Sample			Sample
9		009_T2	Run Sample			Sample
10		010_T3	Run Sample			Sample
11		011_T4	Run Sample			Sample
12		012_Bk check	Run Sample			Sample
13		013_LU-XXXX	Run Sample			Sample
14		014_HU-XXXX	Run Sample			Sample
15		015_Bk check	Run Sample			Sample
...	...	...rows for 20 samples omitted for brevity		...		...
36		036_Bk check	Run Sample			Sample
37		037_LU-XXXX	Run Sample			Sample
38		038_HU-XXXX	Run Sample			Sample

- The sample names will resemble those typed in bold in the example table above. In the example table above, a run of 20 samples is shown so the last vial ends up being placed in A/S Location #38. Of course, the actual position of the last sample depends on the total number of vials in the autosampler tray. Note that, 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 ELAN Samples window, i.e. clear all rows after the last row by selecting them and press Ctrl-Delete.
- The numbers preceding the underscore character (with the exception of sample numbers "101" through "105") correspond to the order of injection. These numbers will later help the analyst find individual chromatograms based on injection number rather than being forced to scroll long lists of alphabetically-sorted file names in Windows Open File dialog boxes and looking for specific sample names during post-run data processing in TotalChrom™.
- When satisfied that the Sample table entries are correct, choose File > Save.  
Analysts may print the NexION® Sample table by choosing the File > Print Setup > Reports command. In the ensuing dialog box, select the preferred

printer and click OK. Next, choose File > Print and then click the Print button. Printouts may be helpful for the correct vial positions when loading samples into the HPLC autosampler tray.

e. Instrument Shut Down

1. Shut off 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 controller computer, visit the NexION® Instrument Control Session application and open the “Dataset” window. 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.
5. If you set up the autostop, make sure wash time is set at 20 minutes.

## 11. POST-RUN DATA ANALYSIS

---

a. Configuration of TotalChrom™ Integration 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 TotalChrom™ to properly integrate peaks and identify components with minimum operator intervention. Therefore, the analyst should pay particular attention to the chromatograms produced in every run and make necessary adjustments as warranted. The analyst should be familiar with the frequently used integration functions of TotalChrom™ which are described in Chapter 18 of *TotalChrom Workstation User’s Guide: Volume II*.

1. The creation of a new method file in TotalChrom™ is done the first time TotalChrom™ is setup, or it will need to be recreated if the file “Arsenic1.mth” cannot be found or has been corrupted. In the TotalChrom™ Navigator window, choose the menu item Build > Method. In the next dialog box, click the “Create a new method” radio button and click OK. The default method will load into the method editor.
2. Choose the menu item Process > Integration. Click on the “Integration” tab in the “Process” window. Enter the information shown in TABLE 11-1.



TABLE 11-1: INTEGRATION

Basic Parameters		Advanced Parameters	
Bunching Factor :	1	<i>Peak Separation Criteria</i>	
Noise Threshold :	5	Width ratio :	0.2
Area Threshold :	25	Valley to peak ratio :	0.01
		<i>Exponential Skim Criteria</i>	
		Peak height ratio :	5
		Adjusted height ratio :	4
		Valley height ratio :	2

The analyst may make appropriate changes to one or more of the Integration parameters in TABLE 11-1 if necessary.

Click on the “Baseline Timed Events” tab. As a guideline, enter the information shown in TABLE 11-2 or other parameters as determined to be appropriate.

TABLE 11-2: BASELINE TIMED EVENTS

*Defined Events*

Time	Event	Value	Code	Level
0.000	Smooth Peak Ends On	5	+SM	
0.000	Locate Maximum On		LM	
0.000	Set Bunching Factor	3	BF	
0.000	Disable Peak Detection		- P	
0.350	Enable Peak Detection		+P	
0.500	End Peak Detection Inhibit		+I	
1.000	End Peak Detection Enable		- I	
1.000	Locate Maximum Off		-LM	
1.000	Set Bunching Factor	1	BF	
1.200	Common Baseline On		+CB	
2.200	Set Bunching Factor	2	BF	
2.250	Common Baseline Off		- CB	
6.250	Set Noise Threshold	3	NT	
6.250	Set Area Threshold	15	AT	
8.750	Peak End Detection Inhibit		+I	
10.80	Peak End Detection Enable		- I	

Be sure there is no checkmark in the box for “Correct actual times of all baseline events based on actual RT of nearest reference peak”. The parameters in TABLE 11-2 are starting points. The analyst may make appropriate changes to one or more of the Baseline Timed Events if necessary.

3. Click on the “Optional Reports” tab. Uncheck the box for “Keep temporary files”.

- Click on the “Replot” tab. Enter the information shown in TABLE 11-3.

TABLE 11-3: REPLOT

Plots	Miscellaneous
Generate a separate replot : not checked	Start plot at end of delay : Checked
Retention Labels : Peak crests*	Gradient overlay : not checked*
Component Labels : Actual time	Draw baselines : checked*
Scaling Type : Autozero offset	Timed Events : checked*
<i>Scaling Parameters</i>	X axis label : Time [min]
Full scale (mV) : 2.000*	Y axis label : Intensity [cps]

\*These parameters maybe altered to suit the analyst.

It is unnecessary to click on the “User Programs” tab because it is not used. Close the Process window by clicking on the “OK” button. The parameters in TABLE 11-3 are starting points. The analyst may make appropriate changes to one or more of the Replot parameters if necessary. In the Method Editor window, choose the menu item Components > Global Information. Click on the “Global Information” tab in the “Global Information” window. Enter the information shown in TABLE 11-4:

TABLE 11-4: Global Information

Volume units : $\mu\text{L}$	<i>Unidentified Peak Quant.</i>
Quantitation units : $\mu\text{g/L}$	Calibration factor : 1.000e+99
Sample Volume : 1.000	Always use calib. Factor : selected
Void time (min) : 0.000	
<i>Calibration</i>	
Internal Standard : selected	<i>RRT Calculation</i>
Reject outliers during calibration : Not checked	Use first peak in run as RRT reference: selected
<i>Sample Amount Options</i>	
Correct amounts for calibration standards : Not checked	
Convert unknown samples to concentration units: Not checked	

The “LIMS Results” tab is not used. Click the “OK” button to close the window. The parameters in TABLE 11-4 are starting points. The analyst may make appropriate changes to one or more of the Global Information parameters if necessary.

- In the Method Editor window, choose the menu item Components > New Component. The white list box in the left portion of the window will be empty. Click in the empty field labeled “Name” and type “IS”. Press the tab key and enter “0.6” in the field labeled “Retention time”. Put a checkmark in the box labeled “This component is an internal standard”. Select the radio button labeled “Peak” if it is not already selected. Leave the other fields and check

boxes unaltered. Click the “New Component” button. Enter each of the component names and parameters listed in 11-5.

TABLE 11-5: METHOD EDITOR – COMPONENTS SETTINGS

Name	Retention Time	Absolute window	Relative window	Find tallest peak	Is a retention reference?	Internal Standard	This Component is an Int. Std?	Use as a RRT reference?
IS	0.6	5	3	No	No		Yes	No
AC	1.70	3	3	No	No	IS	No	No
AB	2.00	0	3	No	No	IS	No	No
TMAO	2.10	3	3	No	No	IS	No	No
AsIII	2.50	5	3	No	No	IS	No	No
DMA	4.00	5	3	No	No	IS	No	No
MMA	9.70	50	3	No	No	IS	No	No
AsV	11.90	0	3	No	No	IS	No	No

Click the “New Component” button before starting a new component. After entering the last component, click the “OK” button. The values for Retention Time, Absolute Window and Relative Window serve as starting points. The analyst may alter these values as actual chromatographic results may dictate.

- In the Method Editor window, choose the menu item Components > Defaults. Click on the “Identification” tab. Enter the information shown in TABLE 11-6.
- Click on the “Calibration” tab in Components Defaults Window. Enter the information shown in TABLE 11-7. To set up the method for the extended calibration, enter 150 for S1, 250 for S2, 500 for S3, and 1000 for S4. Save this method using a different name such as “Extended Calibration (S4-S7)” to let the analyst know which calibration it covers. The same has to be done for the “T” series calibrators.

TABLE 11-6: COMPONENTS DEFAULTS — IDENTIFICATION

Component Type :	Peak	Reference :	Blank
Absolute window :	5	Internal Standard :	IS
Relative window :	5	Find tallest peak :	Not checked

TABLE 11-7: COMPONENTS DEFAULTS — CALIBRATION

		<u>Level</u>	<u>Amount</u>
Calibration Type :	Use Curve	S0	1.0000E-6
Curve Type :	1 <sup>st</sup> Order	S1	2
Scaling :	None	S2	10
Weighing :	None	S3	50
Purity (%) :	100	S4	150
Response :	Area		
Origin Treatment – Include :	Not checked		
Origin Treatment – Force :	Not checked		

8. The “User Values/LIMS” tab is not used. Close the “Components Defaults” window by clicking the “OK” button.
9. In the Method Editor window, click on “Components” in the menu bar. If the menu item “Delete All Components” is not grayed out, select it and click the “OK” button when prompted to “Delete all components, calibration levels, and calibration replicates”. Click the “OK” button.
10. In the Method Editor window, choose the menu item Components > New Component. The white list box in the left portion of the window will be empty. Click in the empty field labeled “Name” and type “IS”. Press the tab key and enter “0.6” in the field labeled “Retention time”. Put a checkmark in the box labeled “This component is an internal standard”. Select the radio button labeled “Peak” if it is not already selected. Leave the other fields and check boxes unaltered. Click the “New Component” button. Enter each of the component names and parameters listed in 11-5.
11. In the Method Editor window, choose the menu item Components > Edit Component then click the “Calibration” tab. Click to highlight “TMAO” in the component list box. Click on the cell containing “S0” and type “T0” (capital “T”) on the keyboard followed by the Enter key. In this manner, replace “S0” through “S4” with “T0” through “T4”, respectively. Click the “OK” button when finished.
12. In the Method Editor window, choose the menu item File > Description, and type in your name and the date this method was created. Add any other pertinent information at this time. Click the OK button to close window.
13. In the Method Editor window, Choose File > Save As. A window appears inviting you to enter any information pertinent to this method, which will be saved with the method. Enter your name and the date this method was created. Click “OK” and a “TotalChrom™ File-Save-As” dialog box will open. Navigate the directory tree to get to the folder C:\HPLC\Methods. Double-click on this folder. In the “File name:” field, enter “Arsenic1.mth”. If there is already a file in that folder with the same name, highlight that file and right-click the mouse. Choose “Rename” and give the file a new name (e.g. add “backup” to the name). Click “Save’ and close the Method Editor window.

b. Configuration of ELAN ChromLink™

ELAN ChromLink™ should be configured after initial installation of the program. At least one recent ELAN NetCDF file (with the “.nc” extension) containing data for the mass of interest that was collected since the last update of the “default.tun” file will need to be available in order to complete this step.

1. Launch TotalChrom™ Navigator. In the TotalChrom™ Navigator window that appears, choose the menu item Apps > ChromLink (alternatively, you may launch ChromLink™ from the operating system Start > Programs menu).
  - (a) Inside the ELAN ChromLink window, click on Configuration > Default TotalChrom Method. Click on the “Browse...” button and navigate to the directory C:\hplc\methods\. Select “Arsenic 150ppb LDR.mth” and click the “Open” button. “C:\hplc\methods\Arsenic 150ppb LDR.mth” will now be the ChromLink™ default method. Click “OK” to close the “Default TotalChrom Method” window.
  - (b) Inside the ELAN ChromLink window, click on “Set”. The “Operating Mode” window will open. Click on the “Manual – process single ELAN NetCDF file” radio button then click the “Review ELAN NetCDF mass file components before processing” radio button. Click “OK” to close the window. Click on the “Browse...” button for “ELAN NetCDF file – location/file to be converted” field. An open file dialog box will open, prompting you to choose a file. Navigate to the latest working HPLC Data folder and choose any file with the “.nc” extension (perhaps one of the calibrators). Click “Open”. The dialog box will disappear and you will be returned to the “ELAN ChromLink” window. The path and file to be converted will now be shown in the field called “ELAN ChromLink file – location/file to be converted”.
  - (c) Click on the “Start Processing ELAN Data Files” button. The “Processing ELAN Data” window will briefly open, followed by a window called “Mass Components in ELAN Data File”. The file name being processed will appear in the ELAN File Contents panel along with its “Mass” and “Nominal Name”. Write down the mass value. The next panel called Configured Mass Components will show the mass and nominal name of the mass components that will be identified from the configured list and be processed as separate TotalChrom RAW files. If the fields for “Mass” and “Nominal Name” are empty, or are different compared to the corresponding fields for Configured Mass Components, then click the “Edit Configured Mass Components” button. A new window called “List of Mass Components” will open. Click on the cell in the table at the top of the window containing the mass that you wrote down earlier. Next, Click on the field named “Nominal Names” in the “Names” panel and enter “As”. Be sure the field named “ELAN Name (mass)” contains the mass value that you wrote down earlier. Leave the field “Expression” unchanged. Next, click on the “Browse...” button and navigate to “C:\hplc\methods\arsenic1.mth” then click “OK”. Put a checkmark in the box for “Process this mass component to produce its own TotalChrom RAW file”. Make sure that the box for “Process this mass component as part of an expression” is unchecked. Now, click the “Update Selected Mass Component” button. One line should now show the following information:

Nom. Name	Mass	Expression	raw?	In expr?	TotalChrom Method File
As	74.92 <sup>†</sup>		X		C:\HPLC\Methods\Arsenic 150ppb LDR.mth

<sup>†</sup> Or some value close to the atomic weight of arsenic.

If not, repeat the above steps, except this time click the “Add as a new mass component” button. Delete unnecessary lines by clicking on the line then clicking the “Delete selected mass component” button. When satisfied that the List of Mass Components window is properly configured, click the “Close” button. Next, click the “Close” button to close the “Mass Components in ELAN Data File” window. Close the “Processing ELAN Data” window by clicking its “Close” button.

- (d) Inside the ELAN ChromLink window, click on the “Set” button. A window titled “Operating Mode” will open. Click on the “Automatic – process all ELAN NetCDF files in specified location” radio button. The lower radio buttons will gray out. Click “OK” to close the window.
2. At this time, ChromLink™ may be closed by selecting File > Exit. Click “OK” at the dialog box asking if you want to quit ChromLink™.
3. In addition to configuring ChromLink™ itself, it is necessary to alter one value in the “seed” method file that ChromLink™ uses to set a select number of parameters to certain default values. This step only needs to be done once following the installation of ChromLink™.
  - (a) In the TotalChrom™ Navigator window, choose the menu item Build > Sequence and a dialog box called “Startup” will appear. Click on the radio button labeled “Load sequence stored on disk” then click the OK button. Navigate to the folder on the C drive that contains the ChromLink™ program file (usually in C:\PenExe\ChromLink but if it is not there, check under the C:\Program Files directory). Click on the sequence file “seed.seq” to highlight it. If this file is missing, reinstall ChromLink™. Click “Open”. A spreadsheet style sequence table will present itself in a window called “Sequence Information – Channel A”. There will be a minimized window for channel B data, ignore this window. Scroll across to the “Int Std Amt” column and click on the first cell in row 1 of this column. Replace the existing value with the concentration of working Internal Standard which is 2.5 µg/L.
  - (b) Choose menu item File > Save. Close the Sequence Editor window by choosing File > Exit from the menu bar.

### c. Data Processing and Analysis

1. Open Microsoft Windows® File Explorer and open the current working HPLC data directory (e.g., C:\HPLC\Data\). Select all files ending with the .rst and .idx and “delete” them.
2. If it is not already open, launch TotalChrom™.
3. If ChromLink was not run in real-time data collection mode during the run as described in step 16 under *Starting the Run* (see page **Error! Bookmark not defined.**), do the following:
  - (a) In the TotalChrom™ Navigator window, choose menu item Apps >

ChromLink. Choose the menu item Configuration > Mass Details and check the Nominal Name and Mass for arsenic. If it is missing or altered then ChromLink™ needs to be configured (see *Configuration of ELAN ChromLink™* on page 55 for details).

- (b) Check that the Mode field indicates “Automatic – Process all NetCDF files in specified location”. If it does not, click the “Set” button to the right of this field and in the resulting “Operating Mode” dialog box click the “Automatic – process all ELAN NetCDF files in specified location” radio button. Click “OK”. Next, check that the Field labeled “ELAN NetCDF file – location/file to be converted” indicates the correct data folder. This should be “C:\NexIONdata\Reportoutput\\*.nc”. If it is not, click the Browse button to the right of it, and in resulting dialog box, navigate to that folder. Double-click on that folder then click “OK” to close the front most dialog box. Click the Browse button to the right of the field labeled “ELAN ChromLink file location...”. In the dialog box “Select TotalChrom™ Data Location”. Navigate to the folder containing the run data and double-click on it. Click “OK” to close that dialog box. In the ELAN ChromLink window, click the button “Start Processing ELAN Data Files” to start processing of the run data. A new dialog box will open and provide current information on the status of the data conversion.
  - (c) When data conversion by ChromLink is completed within a minute or two, a message in the Step field will indicate “Successfully Finished”. Click “Close”. At this point, you may close the ELAN ChromLink application by choosing File > Exit or clicking on the window “x” box. In the resulting “OK to quit?” confirmation dialog box, click “OK”.
4. In the TotalChrom™ Navigator window, choose the menu item Build > Method. Click the “Load method stored on disk” radio button and click “OK”. In the TotalChrom™ File-Open” dialog box, find C:\HPLC\Methods folder and open “Arsenic 150ppb LDR.mth” file. The template method file should now be loaded. If instead of loading the method file an error message says the file is unavailable because it is in use and asks if you would like to open it in Read-Only mode, click “No”. Cancel the Open-File dialog box, and exit the Graphic Method Editor. In the Navigator window, choose menu item Admin > CAM Administrator. A window will appear with two panes. In the left pane, click on the “+” sign in front of “TotalChrom Servers” to expand it. Click on the computer icon on the next line that just appeared to highlight it. In the right pane, under the heading “Resource/Instrument”, select the first item. If there is more than one item, select every item by shift-clicking on each item. Every item should now be highlighted. Choose Edit > Remove Locks (or press the Delete key on the keyboard). Next, click on the “+” sign in front of “Users” to expand it. Click to highlight your TotalChrom™ user name that appeared. In the right pane, under the heading “Resource/Instrument”, select every item and Choose Edit > Remove Locks. This action serves to unlock files and make them available for editing. If in the future, TotalChrom™ complains that files cannot be edited because they are locked, use CAM Administrator to unlock them. Choose File > Exit to quit CAM Administrator. Start again at the beginning of this step to open the Method Editor.
5. Choose File > Save As. At the next window you will be given the option to enter information about the method which can be done at your discretion. Click “OK” and a TotalChrom™ File-Save-As” dialog box opens. Navigate the directory tree to get to the folder that contains the NexION data files for this run (typically in the folder C:\HPLC\Data\). Double-click on this folder. In the “File name:” field, enter the same name as it exactly appears for the folder

that will contain it (i.e. As<yymmdd> convention where yy = last two digits of the year, mm = two digit month, dd = two digit date). Click “Save” then close the “Method Editor” window.

6. In the TotalChrom™ Navigator window, choose the menu item Build > Graphic Edit. Choose File > Open from the menu bar and navigate the file-open dialog box to the folder containing the method file created in the preceding step. Click on that file and then click “Open”. Return to Graphic Method Editor’s menu bar and choose File > New Data File. Navigate to C:\HPLC\Data\ and double-click on the folder containing the run data. Find and click on a data file (indicated by the “.raw” extension) that corresponds to the “S4” calibrator run. When this file appears in the File Name field, click the Open button. In the File-Open dialog box that appears, click “Cancel”. If a message box appears with the warning “Unable to open this file: default.mth”, click OK to clear the message (you do not have to go to CAM Administration to unlock it). Do the same if another message warning box appears (i.e. click OK again to clear it). You should be in the “Graphic Method Editor - <path to method file>” window and see a chromatogram.
7. Choose menu item Calibration > Show Windows and retention window bars (looks like “H” style error bars) will appear. Each retention time window bar should be located above the chromatographic baseline and contain an identified peak within its bounds. If there are any bars at the bottom of the chromatogram located below the baseline, choose menu item Calibration > Edit Components. Click on the first arsenic species peak that falls outside its retention time window to select it. In the group of data fields located on the right side of the window, click on the “Name” dropdown arrow (located on the right side of the data entry field) and choose the appropriate species by name. Next, click on the “ISTD” field’s dropdown arrow and choose “IS”. Be sure the “ISTD” checkbox is unchecked unless you are editing the “IS” peak; in this case put a checkmark in the “ISTD” checkbox. It is usually not necessary to alter the retention time window’s “Absolute” and “Relative” window parameters, but you may do so if experience dictates that a change will be beneficial. Click the Next or Prev button. Repeat these steps for each arsenic species peak that was not properly identified because it was outside its retention time window. Since TMAO is not present in any of the “S” calibrators, choose File > New Data and open one of the “T” series calibrators that contain TMAO (e.g., T4) and confirm its identity and retention time window using the same process as was used for S4. When the editing of peak retention time windows is completed, click on the menu bar item “Return”. Choose File > Save followed by File > Exit.
8. Launch Microsoft® Excel and choose menu item HPLC > Create TC Sequence File (the Excel macro “Extract TC Data.xls” must be installed in Excel’s Startup folder). In the open file dialog box, navigate to the current working HPLC data folder. Click on a RAW file then click the “Open” button. Wait about 30 seconds until a “Done” message box appears. Excel will create two sequence files, one containing just the calibrators (name ending with “calib.seq”) and the other file containing all samples and calibrators (named As<yymmdd>.seq). You may leave Excel open.

Skip the following steps (a) through (c) unless, for some reason, the Excel menu item HPLC > Create TC Sequence File cannot be run:

- (a) In the TotalChrom™ Navigator window, choose the menu item Build > Sequence and a dialog box called “Startup” will appear. Click on the radio button labeled “Load sequence stored on disk” then click the OK button. Navigate to the folder containing the run data and click on the



sequence file (ends with “.seq”) corresponding to the run (typically named in the As\_yymmdd.seq format). Click “Open”. A spreadsheet styled sequence table will present itself in a window called “Sequence Information – Channel A”. There will be a minimized window for channel B data. Ignore this window. Look for the “Method” column and click on the first cell in row 1 in this column. Right click the mouse and a contextual menu will appear, choose “Browse”. In the resulting File-Select dialog box, navigate and choose the method file (ending in “.mth”) created earlier. Click “Select”. The path and name of the new method file will replace the default information in this cell. Right click this cell again and choose Fill Down. The new file name information will fill down to every cell in the “Method” column. Look for the “Study Name” column and click on the first cell in row 1 in this column. Note that this cell contains redundant information that is already in the Name column. Press the delete key to clear this cell. Right click the mouse and a contextual menu will appear, choose “Fill Down”. Right click this cell again and choose Fill Down. This will clear every cell in the “Study Name” column. Choose menu item File > Save. Do not close this window yet.

- (b) Position the mouse cursor over the first row number (in the Row column on the far left side of the window) that is NOT a calibrator. The cursor should be in the form of a fat plus sign. If it looks like small vertical double-ended arrow, move the mouse slightly up or down until it changes to a fat plus sign. Press and hold down the left mouse button and drag down across all the row numbers that are not calibrators. Check that you have not accidentally included calibrators, otherwise, deselect all the rows and try selecting again. Once you are sure that none of the calibrators are selected, choose menu item Edit > Delete. Repeat this process until only rows corresponding to calibrators are present in the sequence table. Click the cell in the first row in the “Type” column and a dropdown menu should appear. Choose “Cal:Replace”. Right-click on the same cell and choose Fill Down. At this time, “Cal:Replace” should appear in every cell in the “Type” column.
- (c) Inspect the Sequence Editor window for Excel-styled workbook tabs at the bottom. Locate the tab labeled “Calibration” (if you do not see it on first look, click on the small right arrow just left of the first tab, this will cause the tabs to “scroll” left and reveal additional tabs). Click on the top cell in the “Cal Level” column. A dropdown menu will reveal “S0”, “S1”, “S2” through to “T4” menu choices. Moving down the column, for each calibrator you will need to assign its level by choosing the correct menu item from the dropdown menu. When all calibrators are assigned their appropriate level, click on the cell in the “Calib Rpt” column corresponding to “S4” calibrator. A pop-down menu should appear; choose “Short”. Likewise, change the “Calib Rpt” for “T4” from “None” to “Short”. Choose menu item File > Save As. A window showing a Description field appears, just click OK and Save As dialog box will appear. Name the new sequence file the same name as the original sequence file except add the word “calib” to the end of the file name (be sure to separate the words by a space). Click the Save button. Then close the Sequence Editor window by choosing File > Exit from the menu bar.

9. In the TotalChrom™ Navigator window, again choose menu item Sequence.

Open the sequence file created in the previous step. It will look like “AsYYMMDD.seq”). A sequence table containing all of the items represented in the run’s NexION® sample file will open. Various field changes will have to be made. This step is important for proper database importing. In the “Type” field, use the drop-down menu to select the proper representation for each item in the entire list. In this field, Calibrators should be marked “Cal:Replace,” the first blank should be marked “Blank,” quality control materials and other control materials should be marked “Ctrl Sample,” and all other items, including additional blanks and those assessed for quantitative measure and equilibrators, should be marked “Sample,” unless another title in the drop-down menu is appropriate. Next, ensure that the “Cal level” field is populated for calibrator sample rows only. If this field is not populated, select calibrator levels from a drop down menu by clicking in the field. Make sure these selected calibrator levels correspond to names in the “Type” field for each row. (For instance, a name of 002\_S0 in row 2 should correspond to a Cal level of “S0” in row 2.) Next, ensure that the proper method is listed in the “Method” field of each row. (This method should be the same that was “saved as” in step 5 of this section). Lastly, at the bottom of the sequence table, click on the “calibration” tab. A new sequence table will display; ensure that each row in the field “Cal levels” is accurate. Save this sequence file (File > Save) and close.

10. In the TotalChrom™ Navigator window, choose the menu item Reprocess > Batch. A new window appears titled “Batch Reprocessing”. Choose menu item File > Sequence and the “From Sequence” window appears. Locate the top field labeled “Sequence file” and look for a button with an open folder icon immediately to the right of the field. Click this button and navigate, if necessary, to the folder containing the run’s sequence files. Click on the sequence file whose name ends with “calib.seq” and click the Open button. You will be returned to the previous window. Set each parameter in this window to the values shown in TABLE 11-8.

TABLE 11-8: TOTALCHROM™ NAVIGATOR – REPROCESS BATCH

Parameter Name	Parameter Setting
Starting Row	: 1
Ending Row	: <number of rows in sequence file>
Channel A	: Checked
Channel B	: Not Checked
Start Analysis	: Peak Detection
End Analysis	: Calibration
Batch Execution	: Interactive
Batch Printer	: None
Batch Plotter	: None
Enable Optional Reports in Method	: Not Checked
Use Method as Result File	: Grayed Out
Overwrite Existing Result Files	: Checked
Raw File Treatment	: Update existing raw file header with new sequence

11. Click “OK”.

Choose menu item Reprocess > Start. The middle panel will contain a list of raw file waiting to be processed. Reprocessing of the chromatographic raw data will commence. The bottom panel in the window will update with each file's name as it is processed. When processing is done, this panel will be clear of files. Close this window.

12. In the TotalChrom™ Navigator window, choose the menu item Build > Method and open the method file for this run. If you get an error message telling you that you can only open this in read-only mode, then unlock the files by doing these steps:
  - (a) Click "No" to cancel the error message. Select File > Exit to close the Method Editor.
  - (b) In TotalChrom Navigator, select Admin > CAM Administration.
  - (c) In the CAM Admin Tool window, click in the file explorer-like window on top of the "+" sign in front of "TotalChrom Servers" to expand it. Click on the server name. A list of files will reveal themselves in the right-hand window panel.
  - (d) Click on the first file, hold down the shift key and click on the last file in the list. This will highlight all the files in the list. Press the keyboard's "delete" key. This does not delete the actual files but only unlocks them.
  - (e) Select File > Exit in the CAM Admin Tool window to close it.
  - (f) Return to Method Editor window by selecting Build > Method in the TotalChrom™ Navigator window. Begin this step again.
13. Return to the menu bar and choose Window > Component List. Position the mouse on any of the four corners or edges of the Component List window until the mouse cursor turns to a double-headed arrow. Expand the window until it fills its parent window.
14. The Component List window is divided into three panels. The left panel lists each component by peak number, retention time and component name. Clicking on any of the components will reveal that component's calibration data and calibration curve in the middle and right panels, respectively. Consecutively click on each component one at a time and individually inspect each component's calibration curve. It is important to note whether the calibration curve meets requirements for linearity, slope and intercept.
  - (a) Watch for calibration points that obviously fail to display their expected Response Ratio (i.e. fall away from the regression line compared to their neighboring points). Typically, a point with a Response Ratio equal to zero indicates that a component peak was missed during peak identification phase of calibration processing. Likewise, a point that falls far from the calibration curve might be because that component's peak was misidentified (perhaps confused for another component). Inspection of nearby eluting component's calibration curve might reveal an oppositely misaligned point for the same calibration level. Return to Graphic Editor and alter the retention time windows as necessary, so all peaks will be correctly identified upon reprocessing.
  - (b) While the regression line does not have to intersect every point, be especially mindful of "R-squared" value at the top left of the right panel. R-squared must exceed 0.990 ("two nines") for each component. R-squared values >0.999 is common for this procedure.

- (c) Corrective action will need to be taken in any case of failure in the above rules. Possible steps include:
- (i) Checking chromatograms to see if an autosampler injection was missed. If so, all samples will have to be re-analyzed.
  - (ii) Inspecting previous runs in database for deviating trends among calibration curves from separate runs.
  - (iii) Inspecting chromatograms from previous runs to assess chromatographic shifts in retention time, peak height, or peak broadness. These shifts could be a result of errors in buffer preparation, contaminated buffer solutions, an alteration of pH of buffer solutions, or a poor column.
  - (iv) Deleting one extreme outlier point from the calibration curve if, in doing so, adequate linearity is achieved. This step should not be common practice (for no more than 2 runs in a row should an analyst have to perform this corrective measure). Analysts must include record of this action in a run summary email to supervisor(s).
15. If the calibration curves pass inspection, close the Method Editor.
16. In the TotalChrom™ Navigator window, choose the menu item Reprocess > Batch to reopen the “Batch Reprocessing” window. Choose File > Sequence and click the button with the open folder icon located right of the field labeled “Sequence file”. Navigate, if necessary, to the folder containing the run’s sequence files and click on the other sequence file whose name does not contain “calib”. Click “Open”. Upon return to the previous window, set End Analysis to “Quantitation” and Batch Printer to “None”. All other parameters should remain unchanged.
17. Click “OK” to close the front window. Next, click the green “start arrow” button. Reprocessing of the chromatographic raw data will commence.
18. In the TotalChrom™ Navigator window, choose the menu item Reprocess > Results. A new window should open called “Reprocess Results”. If you get an error message telling you that you can only open this in read-only mode, then unlock the files (follow the procedure described in step 4 of this section). Select from the menu File > Open. In the open file dialog box, click on the “Files of type:” dropdown menu and select “IDX files (\*.idx)”. Navigate to the folder containing this run’s data and click on the newest file (in the format of “As<yymmdd>-<today’s date>-<time of reprocessing>”). Click “Open”. A chromatogram will be presented for the first sample in the sequence in the Reprocess Results window. Carefully inspect the chromatogram one peak at a time for correct peak identification and accurate baseline. If you are satisfied that there are no integration problems, proceed to the next sample’s chromatogram by selecting File > Next File from the menu bar. Examine all chromatograms in this manner and make corrections in peak identity and integration as necessary. Make notes concerning issues encountered with individual chromatograms and changes that were made. If a chromatogram is changed or edited in any way, be sure to select File > Save to save your changes. See the chapter titled “Developing Processing Parameters in the Method” in the PerkinElmer® *TotalChrom™ Workstation Users Guide* for a detailed explanation on how to use integration events to optimize the integration of a chromatogram. After review of each and every chromatogram, select File > Exit from the Reprocess Results menu bar.
19. Repeat step 16 except set both Start and End Analysis to “Report Generation”. Set Batch Printer to “Find Print Factory Pro”. A new window will open titled “PdfFactory Pro: <##> Jobs (## pages, ### Kb)”. When reprocessing has completed, click the Save button on the PdfFactory Pro

window. In the Save As dialog box that appears, navigate to the run's data folder and create a new pdf file named "As<yymmdd> report". Be sure to include a space between "report" and the first word of the new file. Click the "Save" button. This pdf file is to be kept and backed up, for archival purposes, in the same folder with all the other chromatographic data files for this run. Click the "Close" button to close PdfFactory Pro window.

20. Open Microsoft Excel and choose HPLC > Extract TC Data. In the dialog box which follows, choose the sequence file created in step 10 of this section and click Open. Immediately a macro will run that will transform the data into a format that is easily exported into the database. Just before the macro finishes, a Save As dialog box will open giving you the opportunity to save the file as an Excel workbook. Save the file type as a Word 97-2003 document and give the file a name as follows: "As<yymmdd> results". *Note: For runs containing multiple groups of samples (each group being bracketed by its own quality control material), separate filenames will be necessary for each group. The newly-created "As<yymmdd> results" Excel file should be broken into multiple smaller files, labeled as "As<yymmdd> results Run 1", "As<yymmdd> results Run 2", and so forth, where Run 1 corresponds to the first unique group in the spreadsheet, Run 2 corresponds to the second unique group in the spreadsheet.*

Each multi-tabbed Excel workbook contains a worksheet suitable for data exportation to the MS SQL Server 7™ database. Clicking on additional tabs will show worksheets for (a) summary table for easy visual review of the data, (b) quality control results, (c) calibration data with regression statistics and plotted calibration curves for each arsenic species, (d) instrument stability chart showing degree of consistency of internal standard peak areas plotted as a function of injection #, and (e) raw data (two tabs).

21. The data processing portion on the instrument controller computer is now complete. At this point you may close Microsoft Excel® and TotalChrom™ Navigator.

## 12. RECORDING OF SAMPLE AND QC DATA

---

### a. Transferring the Data to the LIMS

1. Transfer the "As<yymmdd> results".xls file (or the files representing each run) 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\ and study name or ID, for example,  
".....\Nutritional\Instruments\NexION\NexION - G\2013\As130110"
2. 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).
3. 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 allow 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 report.

## 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 two times in 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 assesses all levels of the analyte concentrations. The data from these materials are 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 should 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 or problem vials can be

identified and eliminated, the characterization of the QC is the next step. If problems do exist, the source(s) of the problem has to be identified and the pool has to be re-made and dispensed again.

To complete the characterization that will allow you to assess limits for each pool, analyze a minimum of twenty samples of each pool (low and high) on 20 different days, preferably among all of the instruments that will be used to analyze this method. During the 20 characterization runs, previously characterized QCs or pools with target values assigned by outside laboratories are also analyzed to evaluate each run's QC. Once analysis is complete, calculate the mean and standard deviation for each pool from the concentration results. These values will be used to establish the limits for each pool.

#### b. Precision and Accuracy

**QC Results Evaluation.** After completing a run, consult the QC limits 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, accept the run.
2. If one of two QC results is outside the 2s limits, apply the rules below and reject the run if any condition is met.
  - 1<sub>3s</sub>** – Average of both low QCs OR average of both high QCs is outside of a 3s limit.
  - 2<sub>2s</sub>** – Average of both low QCs AND average of both high QCs is outside of 2s limit *on the same side of the mean.*
  - R<sub>4s</sub> sequential** – Average of both low QCs AND average of both high QCs is outside of 2s limit *on opposite sides of the mean.*
  - 10<sub>x</sub> sequential** – The 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.

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 should 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 variability and drift in internal standard raw peak areas over the course of the run. Irreproducibility that exceeds 15% and drift >20% or sudden large changes in internal standard peak area likely indicates that there was a problem in plasma stability.

Setup a new run for the reanalysis of the patient samples affected by the previous failed run. Be sure to use freshly thawed calibrators and QC material.

If these three 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 should be reported for runs that are not in statistical control.

## 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:  $con_{LOD} = [\text{mean}_b + 1.645 \cdot (s_b + B)] / (1 - 1.645 \cdot A)$  where  $\text{mean}_b$  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:  $con_{LOD} = [-b \pm \sqrt{b^2 - 4ac}] / 2a$  where  $a = 1.645 \cdot A$ ,  $b = (1.645 \cdot B) - 1$ , and  $c = \text{mean}_b + 1.645 \cdot (s_b + C)$ . 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
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 above 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 (<sup>75</sup>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 <sup>1</sup> , µg/L
Arsenobetaine	0.940 – 50.4
Arsenocholine	<LOD
Monomethylarsonic acid	<LOD – 2.01
Dimethylarsinic acid	3.5 – 17.4
Arsenous (III) acid	<LOD
Arsenic (V) acid	<LOD

---

<sup>1</sup> Above ranges are taken from NHANES population data for the 2009 to 2010 survey years as provided in the *Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, September 2013*. Sample sizes were n=2870 for arsenobetaine, n=2852 for arsenic (V) acid, and n=2871 for all other species.

## 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 the laboratory database as the “1UB”) should 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  $\leq -70^{\circ}\text{C}$  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  $\leq -70^{\circ}\text{C}$ ) 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 should notify the supervising physician or principal investigator as soon as possible. The most expeditious means should 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 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 (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, twice per year, if an analytical method is performed on more than one instrument, then an instrument-to-instrument comparison has to be performed. This is usually done in conjunction with the extended linear range verification study, if applicable. 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.

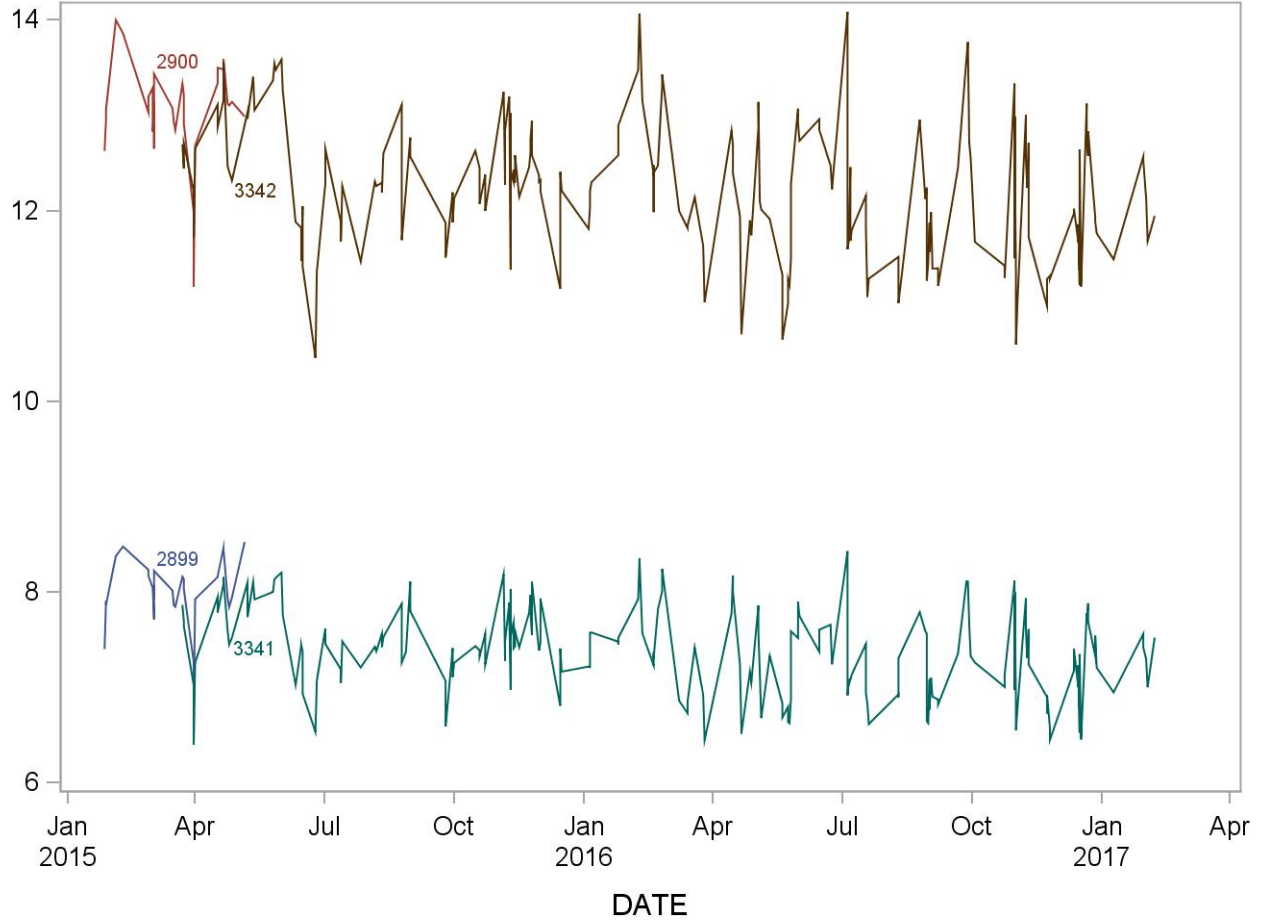
## 26. SUMMARY STATISTICS AND QC GRAPHS

---

See following pages

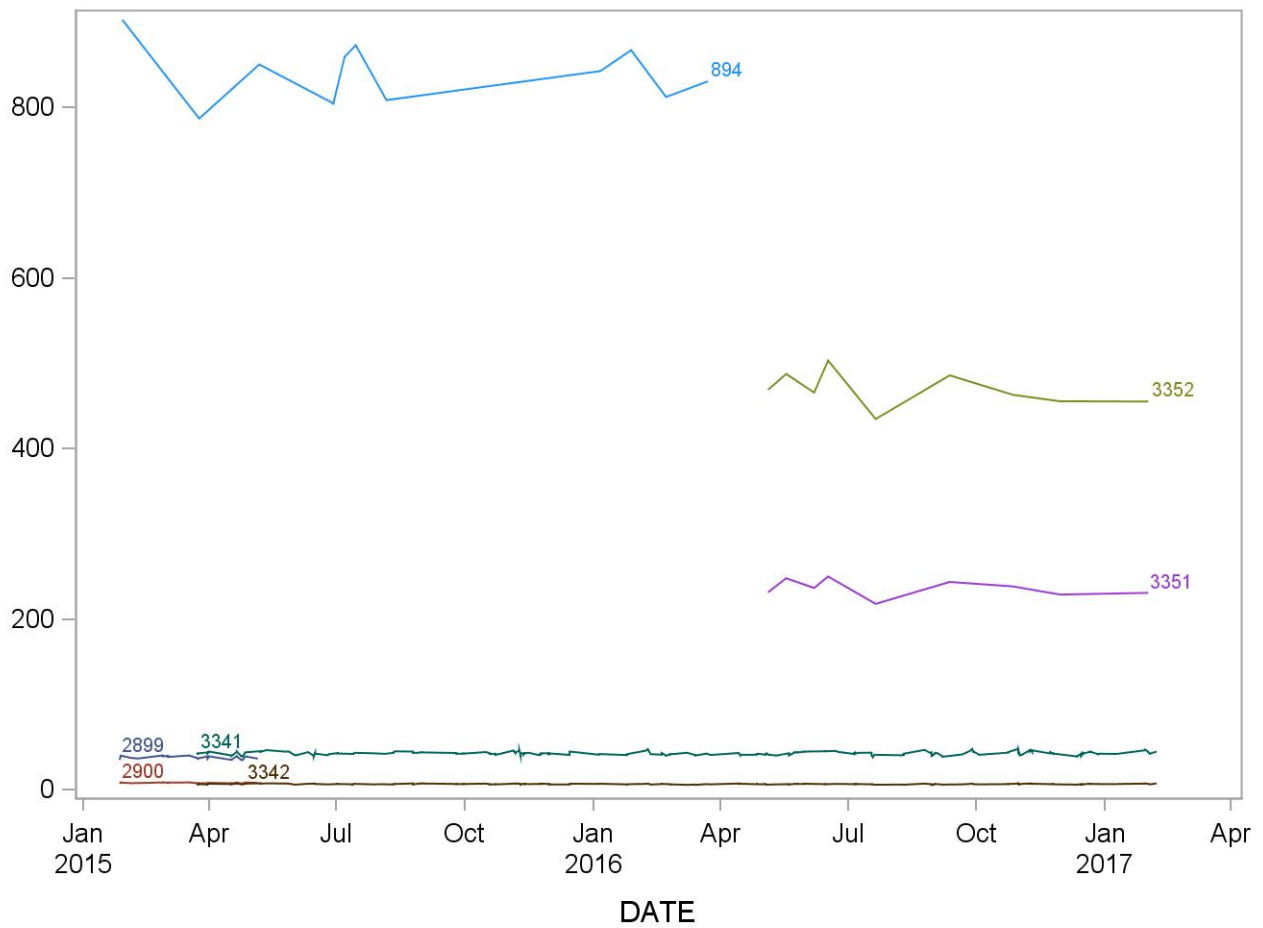
### Summary Statistics and QC Chart for Urinary Arsenic acid (µg/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
2899	32	27JAN15	06MAY15	7.995	0.355	4.4
2900	32	27JAN15	06MAY15	13.039	0.504	3.9
3341	203	23MAR15	07FEB17	7.371	0.450	6.1
3342	203	23MAR15	07FEB17	12.212	0.707	5.8



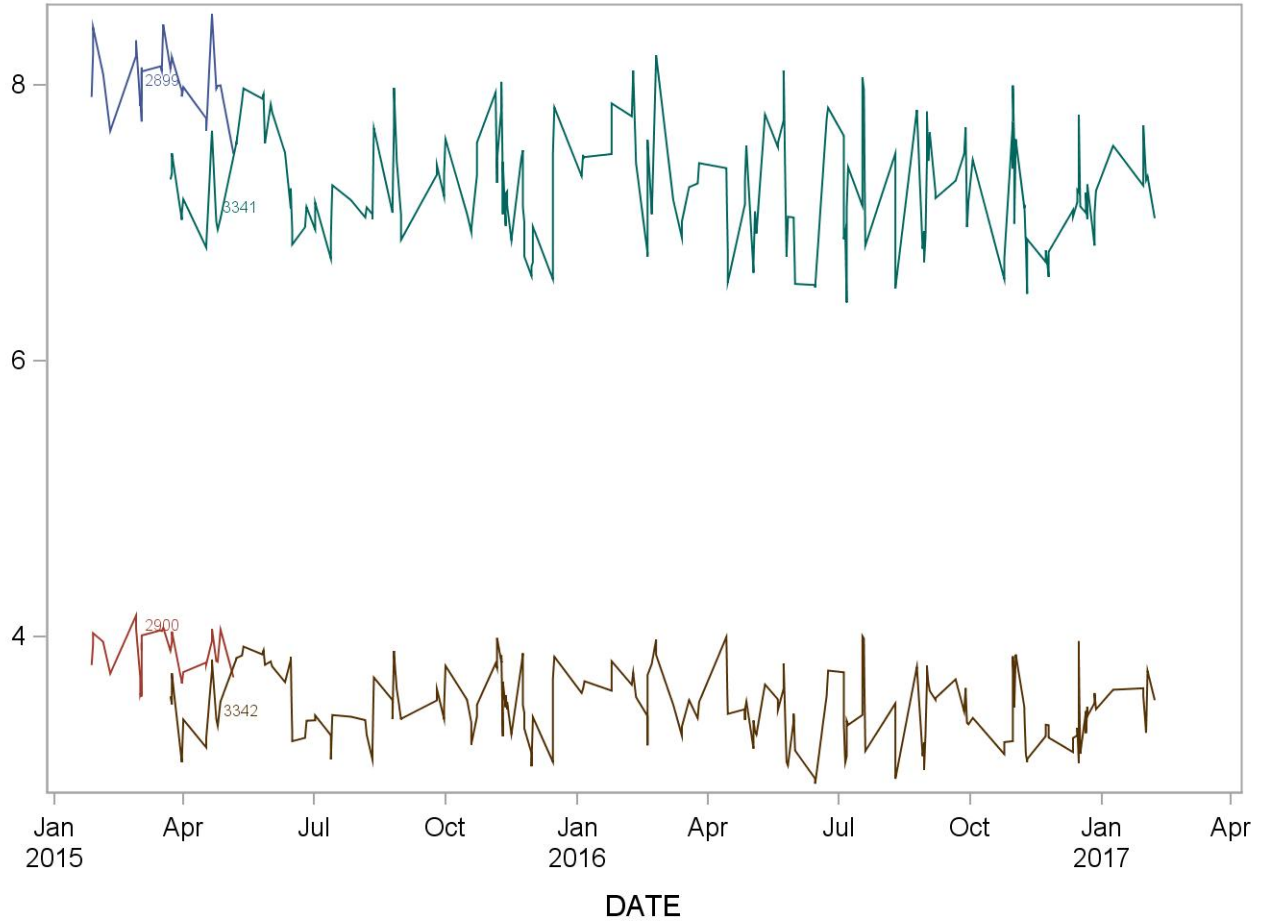
### Summary Statistics and QC Chart for Urinary Arsenobetaine (µg/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
2899	32	27JAN15	06MAY15	37.991	1.836	4.8
2900	32	27JAN15	06MAY15	7.959	0.407	5.1
894	12	29JAN15	23MAR16	837.317	34.663	4.1
3341	203	23MAR15	07FEB17	42.669	1.951	4.6
3342	203	23MAR15	07FEB17	6.577	0.394	6.0
3351	9	05MAY16	01FEB17	236.193	10.198	4.3
3352	9	05MAY16	01FEB17	468.965	20.618	4.4



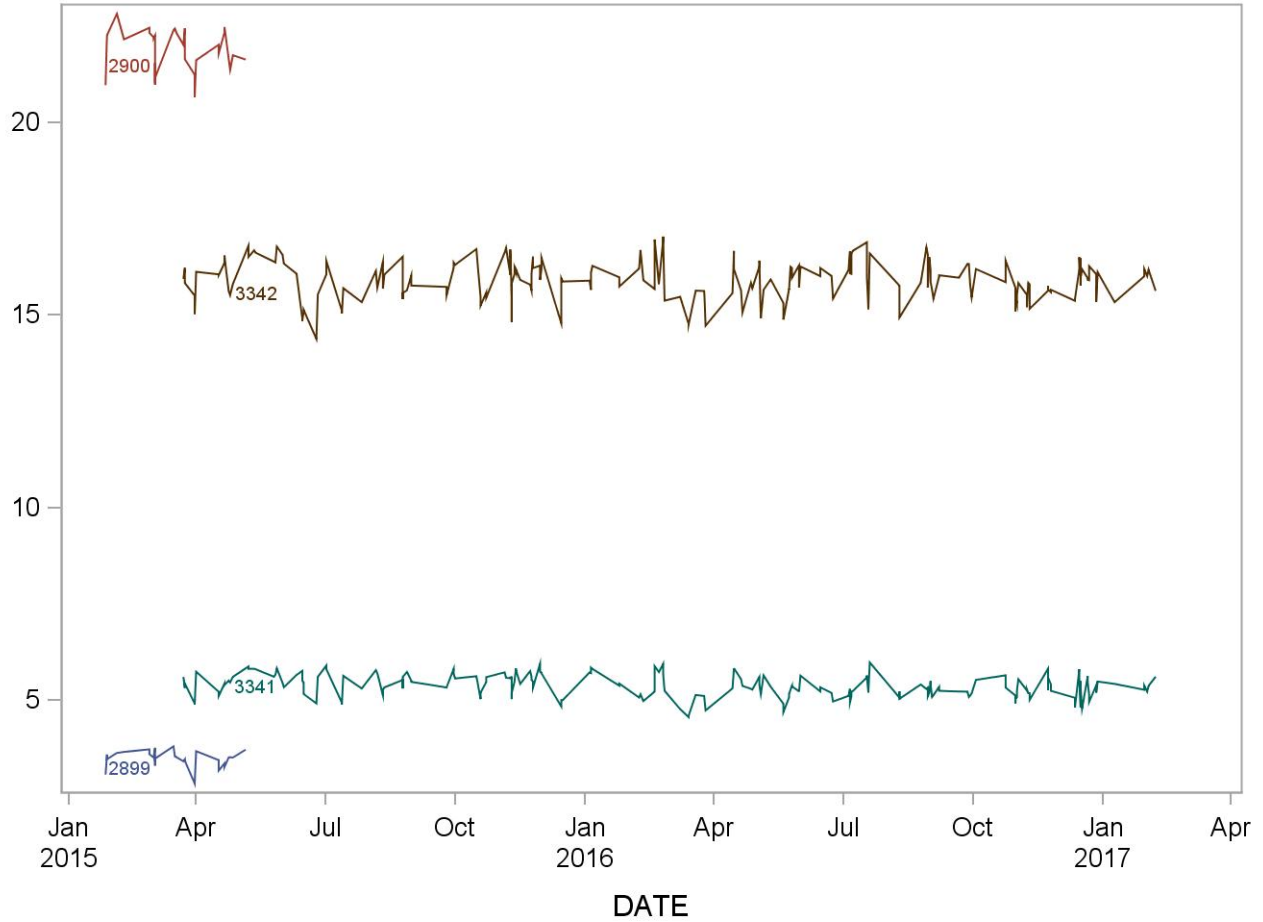
### Summary Statistics and QC Chart for Urinary Arsenocholine ( $\mu\text{g/L}$ )

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
2899	32	27JAN15	06MAY15	8.039	0.248	3.1
2900	32	27JAN15	06MAY15	3.876	0.163	4.2
3341	202	23MAR15	07FEB17	7.252	0.397	5.5
3342	202	23MAR15	07FEB17	3.492	0.248	7.1



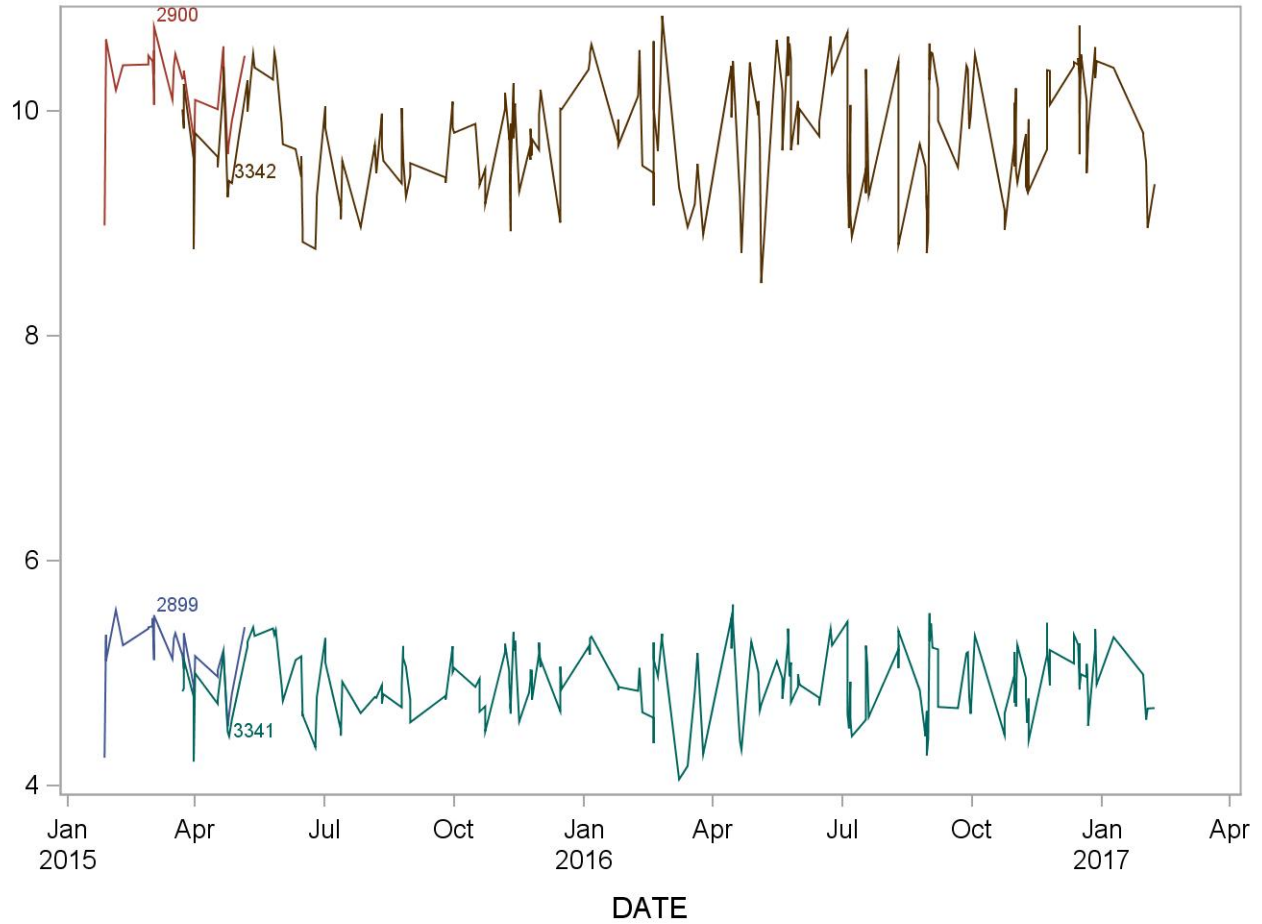
### Summary Statistics and QC Chart for Urinary Dimethylarsinic acid ( $\mu\text{g/L}$ )

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
2899	32	27JAN15	06MAY15	3.4680	0.2294	6.6
2900	32	27JAN15	06MAY15	21.8947	0.5364	2.4
3341	204	23MAR15	07FEB17	5.3772	0.2938	5.5
3342	204	23MAR15	07FEB17	15.8964	0.5121	3.2



### Summary Statistics and QC Chart for Urinary Monomethylarsonic acid ( $\mu\text{g/L}$ )

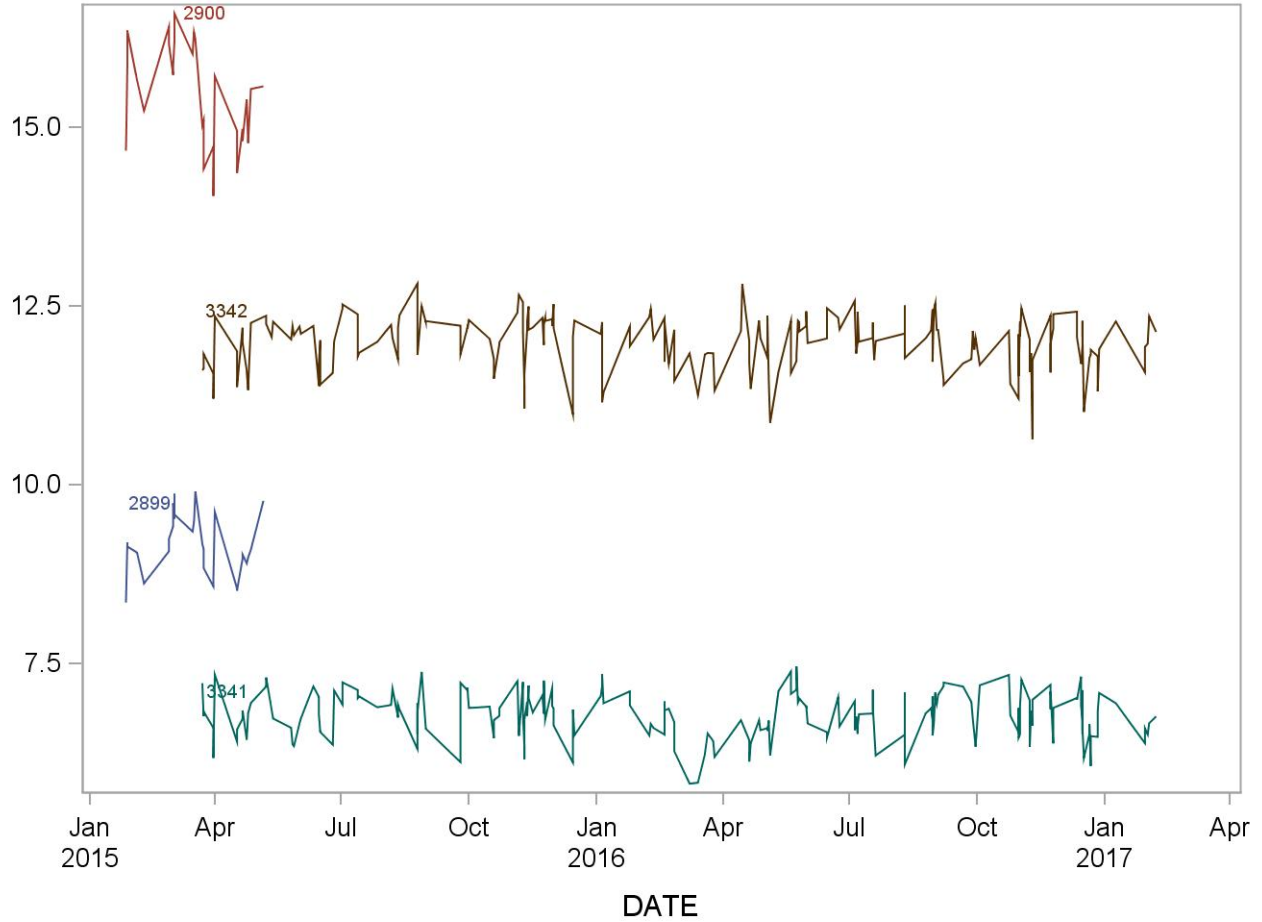
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
2899	32	27JAN15	06MAY15	5.1658	0.3210	6.2
2900	32	27JAN15	06MAY15	10.2085	0.3839	3.8
3341	203	23MAR15	07FEB17	4.9344	0.3129	6.3
3342	203	23MAR15	07FEB17	9.8089	0.5169	5.3





### Summary Statistics and QC Chart for Urinary arsenous acid ( $\mu\text{g/L}$ )

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
2899	32	27JAN15	06MAY15	9.198	0.437	4.8
2900	32	27JAN15	06MAY15	15.558	0.722	4.6
3341	203	23MAR15	07FEB17	6.770	0.324	4.8
3342	203	23MAR15	07FEB17	11.990	0.387	3.2



## REFERENCES

---

1. Styblo M, Drobna Z, Jaspers I, Lin S, Thomas DJ. The role of biomethylation in toxicity and carcinogenicity of arsenic: a research update. *Environmental Health Perspectives*. 2002;110:767-71.
2. Sakurai T. Biological effects of organic arsenic compounds in seafood. *Applied Organometallic Chemistry* 2002;16:401-405.
3. Francesconi KA, Edmonds JS. Arsenic and marine organisms. *Advances in Inorganic Chemistry*, Vol. 44, 1997:147-189.
4. Kojima C, Sakurai T, Ochiai M, Kumata H, Qu W, Waalkes MP, et al. Cytotoxicological aspects of the organic arsenic compound arsenobetaine in marine animals. *Applied Organometallic Chemistry* 2002;16:421-426.
5. Sakurai T, Kaise T, Saitoh T, Matsubara C. Evaluation of in vitro cytotoxicity of tetramethylarsonium hydroxide in marine animals. *Applied Organometallic Chemistry* 1999;13:101-106.
6. Agency for Toxic substances and Disease Registry (ATSDR). Toxicological profile for arsenic. Atlanta, GA: Public Health service, 2000.
7. Hall AH. Chronic arsenic poisoning. *Toxicol Lett* 2002;128:69-72.
8. Ahsan H, Perrin M, Rahman A, Parvez F, Stute M, Zheng Y, et al. Associations between drinking water and urinary arsenic levels and skin lesions in Bangladesh. *J.Occup.Environ.Med.* 2000;42:1195-1201.
9. Anawar HM, Akai J, Mostofa KM, Safiullah S, Tareq SM. Arsenic poisoning in groundwater: health risk and geochemical sources in Bangladesh. *Environ Int* 2002;27:597-604.
10. Das D, Chatterjee A, Mandal BK, Samanta G, Chakraborti D, Chanda B. Arsenic in ground water in six districts of West bengal, India: the biggest arsenic calamity in the world. Part 2. Arsenic concentration in drinking water, hair, nails, urine, skin-scale and liver tissue (biopsy) of the affected people. *Analyst* 1995;120:917-924.
11. Hsueh YM, Huang YL, Huang CC, Wu WL, Chen HM, Yang MH, et al. Urinary levels of inorganic and organic arsenic metabolites among residents in an arseniasis-hyperendemic area in Taiwan. *J.Toxicol.Environ.Health A* 1998;54:431-444.
12. Kreiss K, Zack MM, Landrigan PJ, Feldman RG, Niles CA, Chirico-Post J, et al. Neurologic evaluation of a population exposed to arsenic in Alaskan well water. *Arch.Environ.Health* 1983;38:116-121.
13. Valentine JL, Kang HK, Spivey G. Arsenic levels in human blood, urine, and hair in response to exposure via drinking water. *Environ.Res.* 1979;20:24-32.
14. Lewis DR. Dopant materials used in the microelectronics industry. *Occup Med* 1986;1:35-47.

15. Horng CJ, Tsai JL, Lin SR. Determination of urinary arsenic, mercury, and selenium in steel production workers. *Biol.Trace Elem.Res.* 1999;70:29-40.
16. Vahter M. Environmental and occupational exposure to inorganic arsenic. *Acta Pharmacol.Toxicol.(Copenh)* 1986;59 Suppl 7:31-34.
17. Hwang YH, Chen SC. Monitoring of low level arsenic exposure during maintenance of ion implanters. *Arch.Environ.Health* 2000;55:347-354.
18. Apostoli P, Alessio L, Romeo L, Buchet JP, Leone R. Metabolism of arsenic after acute occupational arsine intoxication. *J.Toxicol.Environ.Health* 1997;52:331-342.
19. Battista G, Bartoli D, Iaia TE, Dini F, Fiumalbi C, Giglioli S, et al. Art glassware and sinonasal cancer: report of three cases. *Am.J.Ind.Med.* 1996;30:31-35.
20. Aitken I. Arsenic poisoning associated with the burning of arsenic-treated timber. *P.N.G.Med.J.* 1976;19:103-104.
21. Gollop BR, Glass WI. Urinary arsenic levels in timber treatment operators. *N.Z.Med.J.* 1979;89:10-11.
22. Nygren O, Nilsson CA, Lindahl R. Occupational exposure to chromium, copper and arsenic during work with impregnated wood in joinery shops. *Ann.Occup.Hyg.* 1992;36:509-517.
23. Rosenberg MJ, Landrigan PJ, Crowley S. Low-level arsenic exposure in wood processing plants. *Am.J.Ind.Med.* 1980;1:99-107.
24. Takahashi W, Pfenninger K, Wong L. Urinary arsenic, chromium, and copper levels in workers exposed to arsenic-based wood preservatives. *Arch.Environ.Health* 1983;38:209-214.
25. Peters HA, Croft WA, Woolson EA, Darcey B, Olson M. Hematological, dermal and neuropsychological disease from burning and power sawing chromium-copper-arsenic (CCA)-treated wood. *Acta Pharmacol Toxicol (Copenh)* 1986;59 Suppl 7:39-43.
26. Brown RM, Newton D, Pickford CJ, Sherlock JC. Human metabolism of arsenobetaine ingested with fish. *Hum Exp Toxicol* 1990;9:41-6.
27. Kaise T, Horiguchi Y, Fukui S, Shiomi K, Chino M, Kikuchi T. Acute Toxicity and Metabolism of Arsenocholine in Mice. *Applied Organometallic Chemistry* 1992;6:369-373.
28. Kaise T, Ochi T, Oya-Ohta Y, Hanaoka K, Sakurai T, Saitoh T, et al. Cytotoxicological aspects of organic arsenic compounds contained in marine products using the mammalian cell culture technique. *Applied Organometallic Chemistry* 1998;12:137-143.
29. Kaise T, Watanabe S, Itoh K. The Acute Toxicity of Arsenobetaine. *Chemosphere* 1985;14:1327-1332.
30. Marafante E, Vahter M, Dencker L. Metabolism of arsenocholine in mice, rats and rabbits. *Sci.Total Environ.* 1984;34:223-240.
31. Yamauchi H, Kaise T, Yamamura Y. Metabolism and excretion of orally administered arsenobetaine in the hamster. *Bull Environ Contam Toxicol* 1986;36:350-5.

32. Tam GK, Charbonneau SM, Bryce F, Pomroy C, Sandi E. Metabolism of inorganic arsenic (74As) in humans following oral ingestion. *Toxicol Appl Pharmacol* 1979;50:319-22.
  33. Pomroy C, Charbonneau SM, McCullough RS, Tam GK. Human retention studies with 74As. *Toxicol.Appl.Pharmacol.* 1980;53:550-556.
  34. Buchet JP, Lauwerys R, Roels H. Urinary excretion of inorganic arsenic and its metabolites after repeated ingestion of sodium metaarsenite by volunteers. *Int.Arch.Occup.Environ.Health* 1981;48:111-118.
  35. Baranov VI, Tanner SD. A dynamic reaction cell for inductively coupled plasma mass spectrometry (ICP-DRC-MS). Part 1. The rf-field energy contribution in thermodynamics of ion-molecule reactions. *J. Anal. At. Spectrom.* 1999;14:1133-1142.
  36. Tanner S, Baranov VI, Vollkopf U. A dynamic reaction cell for inductively coupled plasma mass spectroscopy (ICP-DRC-MS). Part III. Optimization and analytical performance. *J. Anal. At. Spectrom.* 2000;15:1261-1269.
  37. Centers for Disease Control and Prevention. Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, September 2013.
-

## APPENDIX

### d. Appendix A: 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.

<b>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).</b>				
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 33.95 – 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.

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

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

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

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