Laboratory Procedure Manual

Analyte: Arsenobetaine, Arsenocholine, Trimethylarsine Oxide, Monomethylarsonic Acid, Dimethylarsinic Acid, Arsenous (III) Acid, Arsenic (V) Acid

Matrix: Urine

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

Method No: 3000.14 (Formerly 0161A/01-OD)

Revised: April 08, 2014

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

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Important Information for Users

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

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

This method file describes measurements of UAS_H and UASS_H. 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.

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

1. CLINICAL RELEVANCE AND TEST PRINCIPLE

a. Clinical Relevance

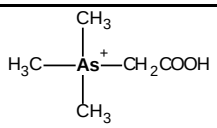
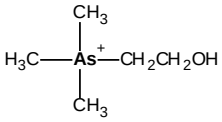
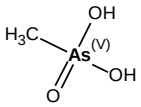
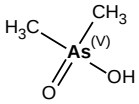
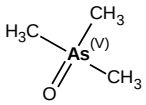
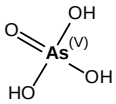
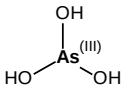
People encounter arsenic in many chemical forms that vary in toxicity. The most toxic of the naturally-occurring arsenic compounds are inorganic forms of arsenic and their monomethylated metabolites (1). Less toxic are the organic arsenic compounds (2-5). Exposure to inorganic arsenic can result in a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

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

b. Test Principle

The concentrations of arsenate [As (V)], arsenite [As (III)], MMA, DMA, TMAO, AC, and AB are determined by using 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^{-5} torr. The mass spectrometer permits detection of ions at each mass-to-charge ratio in rapid sequence, which allows the determination of individual isotopes of an element. Once inside the mass spectrometer, the ions pass through the ion optics, then through the DRC™ and finally through the mass-analyzing quadrupole before being detected as they strike the surface of the detector. The ion optics uses an electrical field to focus the ion beam into the DRC™. The DRC™ component is pressurized with an appropriate reaction gas and contains a quadrupole. In the DRC™, elimination or reduction of argon-based polyatomic interferences takes place through the interaction of the reaction gas with the interfering polyatomic species in the incoming ion beam. The quadrupole in the DRC™ allows elimination of unwanted reaction by-products that would otherwise react to form new interferences.

TABLE 1-1: SPECIES OF ARSENIC

Name	Abbreviation	Structural Formula	pK_a
Arsenobetaine	AB		-
Arsenocholine	AC		-
Monomethyl arsonic acid	MMA		4.1, 8.7
Dimethylarsinic acid	DMA		6.2
Trimethylarsine oxide	TMAO		-
Arsenic (V) acid (arsenate)	As(V)		2.2, 7.0, 11.5
Arsenous (III) acid (arsenite)	As(III)		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

Whenever possible, use bar code scanners to enter sample identifiers into the ICP-DRC-MS computer software to avoid transcription errors and to speed up sample processing. When bar code scanners cannot be used, proofread transcribed data after entry. Handle or transfer data electronically when reporting or moving data to other computerized data-handling software. In the Inorganic and Radiation Analytical Toxicology Branch (IRAT), sample analysis results generated by this method are stored long-term on compact discs and on the CDC network shared drive. The results should include at least the analysis date, quality-control (QC) results for the run, results of specimen analysis by specimen identification (ID), and method identifier.

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

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

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

(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

- If the computer is equipped with a secondary hard drive, configure Microsoft Windows® Backup™ (located in Start > Programs > Accessories > System Tools) or other applicable program to do a daily backup of the NexION® data system computer's data directories (see *Backup Procedures*).

(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 equivalent software to make CD 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.
- Verify that backup CD-R disks operate correctly before deleting any data from the hard drive. To verify the operation of a CD-R disk, open any file on the disk by using the appropriate computer software (ICP-DRC-MS software).
- After verifying that all backups are operational, delete the original data from the hard drive.

Keep one copy of the CD-R disk in a building other than the laboratory (in case of fire). Keep the other near the ICP-MS laboratory.

d. Documentation of System Maintenance

Computer Maintenance: Record any maintenance of computer hardware or HPLC or ICP-DRC-MS software in the instrument logbook. Place other electronic records related to integrity of the data and hard drive in the Windows™ event log. Back up the event log on a regular basis by saving a copy in the active "NexIONdata" directory. The event log will then be backed up along with the NexION® data when backup CD-R disks are made.

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).

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. To prevent inter-conversion of arsenic species, immediately store or transport urine specimens at approximately ≤ -70°C. Upon receipt, they must remain frozen at approximately ≤ -70°C until time for analysis. Refreeze remaining portions at approximately ≤ -70°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. Water, high purity (≥18 MΩ·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, ≥ 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, As_2O_3, CAS 1327-53-3, MW 197.84, Sigma-Aldrich, Item # 202673, or equivalent.
2. Arsenic (III) speciation standard in 2%HCl, Spex CertiPrep, Item # SPEC-AS3, or equivalent.
3. Arsenic (V) oxide hydrate, $As_2O_5 \cdot xH_2O$, CAS 12044-50-7, MW 229.84, Sigma-Aldrich,

Item # 363456, or equivalent source or vendor.

4. Arsenic (V) speciation standard in water, Spex CertiPrep, Item # SPEC-AS5, or equivalent.
5. Arsenobetaine, $(CH_3)_3AsCH_2COOH$, CAS 64436-13-1, MW 178.06, Sigma-Aldrich, Item # 11093, or equivalent.
6. Arsenobetaine, $(CH_3)_3AsCH_2COOH$, CAS 64436-13-1, MW 178.06, Wako USA, Item # 321-34911, or equivalent.
7. Arsenobetaine, $(CH_3)_3AsCH_2COOH$, CAS 64436-13-1, MW 178.06, Argus, Vernio, Italy, Item # AR60008, or equivalent.
8. Arsenocholine bromide, $(CH_3)_3AsCH_2CH_2OH{\cdot}Br$, MW 244.99, Argus, Vernio, Italy, Item # AR60010, or equivalent.
9. Arsenocholine bromide, $(CH_3)_3AsCH_2CH_2OH{\cdot}Br$, MW 244.99, Wako USA, Item # 328-34921, or equivalent.
10. Dimethylarsinic acid, $(CH_3)_2As(OH)_2$, MW 138.01, Sigma-Aldrich, Item # PS-51, or equivalent.
11. Cacodylic Acid, $(CH_3)_2As(O)OH$, CAS 75-60-5, MW 138.00, Sigma-Aldrich, Item # 20835-10G-F, or equivalent.
12. Disodium methyl arsenate, $CH_3AsO_3{\cdot}6H_2O$, CAS 144-21-8, MW 291.9, Sigma-Aldrich, Item # PS-281, or equivalent.
13. Monosodium acid methane arsonate sesquihydrate, CAS 2163-80-6, Sigma-Aldrich, Item #, PS-429, or equivalent.
14. Trimethylarsine oxide, $(CH_3)_3AsO$, CAS 4964-14-1, MW 136.03, Argus Chemicals, Vernio, Italy, Item # AR60011, or equivalent.
15. Trimethylarsine oxide, $(CH_3)_3AsO$, CAS 4964-14-1, MW 136.02, Wako USA, Item # 321-34891, 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
low pool	LU-yy###
high pool	HU-yy###
elevated pool	EU-yy###

Where yy is the last two digits of production year and ### is the assigned pool identification number.

QC material that is to be used for bench quality control purposes will need to be “characterized” as described in the section *Establish QC limits for each QC pool.*

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

1. HPLC pump, specifically, PerkinElmer® Series 200™ Pump, made with biocompatible materials consisting of polyethylethylketone (PEEK) and other polymers in the fluid path (PerkinElmer®), or equivalent.
2. HPLC autosampler, specifically, PerkinElmer® Series 200™ Autosampler, made with biocompatible materials consisting of PEEK and other polymers in the fluid path (PerkinElmer® LAS), Item # N2930035, or equivalent.
3. Autosampler temperature cooling tray for 100 samples, specifically, PerkinElmer® Series 200™ Peltier Cooling Tray and Assembly, or equivalent.
4. Autosampler tray, double microtiter peltier tray, (PerkinElmer®), Item # N2930037, or equivalent.
5. Column oven, specifically, PerkinElmer® Series 200™ Column Oven, or equivalent.
6. Vacuum degasser, PerkinElmer®, or equivalent.
7. Chromatography data handling software, specifically, TotalChrom™ Workstation, version 6.0.2 or later (PerkinElmer®), or equivalent.
8. Anion-exchange HPLC column, specifically, PRP-X100™, 4.6 X 150 mm dimensions, 5 µm particle size in PEEK hardware, Hamilton Company, Item # 79174, or equivalent.
9. Autosampler injection needle, stainless steel, PerkinElmer®, Item # N2930023, or equivalent.
10. HPLC sampling syringe 250µL, PerkinElmer®, Item # 0992-3270, or equivalent.
11. HPLC syringe 2500µL, PerkinElmer®, Item # 0992-3219, or equivalent.
12. Autosampler injector loop, 20 µL, ChromTech or IDEX Health & Science, Item # 9055-022, or equivalent.
13. Autosampler injector loop, 200 µL, ChromTech or IDEX Health & Science, Item # 9055-025, or equivalent.
14. Replacement precolumn blue microfilter 0.5 µm 10/pk, ChromTech or IDEX Health & Science, Item # A-735X, or equivalent.
15. Precolumn microfilter assembly PEEK, ChromTech or IDEX Health & Science, Item # M-560, or equivalent.
16. HPLC tubing, 0.007” I.D. X 1/16” O.D., polyethylethylketone (PEEK), 5 feet length, IDEX Health & Science, Item # 1536, or equivalent.
17. Peek sample uptake fitting, Analytical West, Item #500-QD-PEEK, or equivalent
18. Four “end-of-tubing” prefilters (10 µm) for each HPLC reservoir bottle, (ChromTech or IDEX Health and Science, Item # A-438, or equivalent.
19. In-line HPLC post-pump filter (2 µm), ChromTech or IDEX Health and Science, Item # A-430, or equivalent.
20. Luer fitting plastic syringe, 10 mL or larger.
21. RheBuild Kit for autosampler valve, ChromTech or IDEX Health & Science, Item # 9725-999, or equivalent.

22. Rheodyne PEEK dual mode injector valve for autosampler, ChromTech or IDEX Health & Science, Item # 9725, or equivalent.
23. Vespel rotor seal for 6 port switching valves, ChromTech or IDEX Health & Science, Item # 7750-016, or equivalent.
24. Stator Rheodyne PEEK for 6-port switching valve, ChromTech or IDEX Health & Science, Item # 9750-021, or equivalent.
25. Dionex 2L HPLC reservoir bottles with cap, Dionex, Item # 44129, or equivalent
26. 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. Equipment

1. Water purification system for providing ultrapure water with a resistivity ≥18 MΩ·cm, Aqua Solutions, or equivalent.
2. Caliper Automated Staccato Sample Preparation System, Caliper Life Science Technologies, or equivalent.
3. Eppendorf® Model 5417R refrigerated centrifuge fitted with FA45-24-11 fixed angle rotor or equivalent refrigerated centrifuge capable of ≥18,000 rcf for centrifugation of 1.5 mL capacity microcentrifuge tubes.
4. 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. Gilson 402™ Programmable Diluter-Dispenser (or equivalent) equipped with 10.0-mL dispensing syringe and a 2-mL sampling syringe.
8. Calibrated hand-held adjustable pipetters that cover the range of accurate liquid delivery from 50 µL to 5000 µL. Eppendorf™ Xplorer™ electronic programmable pipetters (Fisher Scientific), 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. Ethos EZ microwave Digestion Labstation with PRO-24 High Throughput Rotor, Milestone, Inc., or equivalent.
15. Stormat Sealer, PerkinElmer®, Item# CUSG01273, or equivalent.

c. Computer Software

1. PdfFactory Pro 1.52 or later version, FinePrint Software, LLC, 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. TotalChrom™ Workstation with ChromLink 2.0 or greater

d. Supplies

1. 2-200 μL pipette tips, 960 tips per case, Fisher Scientific, Item # 05-403-66, or equivalent.
2. 20-300 μL pipette tips, 960 tips per case, Fisher Scientific, Item # 05-403-67, or equivalent.
3. 50-1000 μL pipette tips, 960 tips per case, Fisher Scientific, Item # 05-403-68, or equivalent.
4. 5 mL pipette tips, 500 tips per case, Fisher Scientific, Item # 05-403-117, or equivalent.
5. Acid-cleaned 2 liter polyethylene (PE) bottles. To acid-wash containers, rinse with 10% (v/v) reagent-grade nitric acid, followed by rigorous rinsing with ≥18 MΩ·cm water. Repeat this process several times depending on prior use of the containers.
6. Acid-cleaned 1 liter PE bottles. To acid-wash containers, rinse with 10% (v/v) reagent-grade nitric acid followed by rigorous rinsing with ≥18 MΩ·cm water. Repeat this process several times depending on prior use of the containers.
7. Acid-cleaned 500 mL PE bottles. To acid-wash containers, rinse with 10% (v/v) reagent-grade nitric acid followed by rigorous rinsing with ≥18 MΩ·cm water. Repeat this process several times depending on prior use of the containers.
8. Acid-cleaned 100 mL PE bottles. To acid-wash containers, rinse with 10% (v/v) reagent-grade nitric acid followed by rigorous rinsing with ≥18 MΩ·cm water. Repeat this process several times depending on prior use of the containers.
9. 1.5 mL polypropylene (PP) microcentrifuge tubes, Fisher Scientific, Catalogue # 05-402, or equivalent.
10. 0.75mL virgin polypropylene HPLC vials, National Scientific, Item # C4011-15, or equivalent.
11. Red Snap-it seal T/S SLT septa, case of 1000, National Scientific, Item # C4011-55R, or equivalent.
12. Tube racks for 1.5 mL microcentrifuge tubes (approximately 6), Fisher Scientific, 05-405-3, or equivalent.
13. Vial rack for HPLC autosampler vials, 50-position, Fisher Scientific, Item # 03-375-9, or equivalent.
14. fluidX racks for Nalgene cryovials, fluidX, UK, Item # 170-01-110.

15. fluidX racks for fluidX 2D cryotubes, fluidX, UK, Item # 65-9451.
16. 2D coded jacket cryotubes external threaded with yellow caps 480 per case, fluidX UK, Item # SP-2003.
17. 2D coded jacket cryotubes external threaded with blue caps 480 per case, fluidX UK, Item # SP-2004.
18. 2D coded jacket cryotubes external threaded with orange caps 480 per case, fluidX UK, Item # SP-1007.
19. Corning 2mL V-bottom non-sterile 96 well plate, case of 100, Fisher Scientific, Item # 07-200-701, or equivalent.
20. Non-sterile deep well plates 1 ml capacity 50/case. Fisher Scientific, Item # 12-566-120, or equivalent.
21. Easy Pierce Foil sealing tape, Thermo Scientific, Item # AB-3738, or equivalent.
22. Nested pipette tips 776 pipe tip – 200µL SLV 30-96 trays, PerkinElmer®, Item # 105649, or equivalent.
23. Silicone STORMAT-96, 50/case, PerkinElmer®, Item # 6008096, or equivalent.
24. Eppendorf Repeater pipetter tips, “Combitips Plus” 5 mL, Fisher Scientific, Item # 21-381-330, or equivalent.
25. Kay-Dry™ paper towels and Kim-Wipe™ tissues, Kimberly-Clark Corp.,, Item # 06-666-11, or equivalent.
26. Teflon™-coated magnetic stirs bars (2), VWR, Item #58948-974, or equivalent.
27. Cotton swabs (Hardwood Products Co. ME), 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. 35mL Pyrex vials, CEM Corporation, Item # 909036, or equivalent.
31. 35mL SP-D Plus Pressure Caps, CEM Corporation, Item # 909350, or equivalent,
32. Explorer Vessel Racks, CEM Corporation, Item # 171825, or equivalent.
33. 35 mL Teflon PFA Liner for 35 mL Pyrex vessels, CEM Corporation, Item # 171968, or equivalent.
34. Thermowell for long type sensor for Ethos microwave, Milestone Inc., Item # APC2018, or equivalent.
35. 15 mL conical polypropylene centrifuge tube, VWR, Item # 82050-276, or equivalent.
36. 50 mL polypropylene centrifuge tubes, Fisher Scientific, Item # 14-432-22 or equivalent.

7. INSTRUMENT SETUP AND CONFIGURATION

a. HPLC Hardware Setup

A PerkinElmer® representative should perform the initial installation of the Series 200 HPLC system. If the instrument has been moved, laboratory personnel can assemble and configure the system according to the PerkinElmer® supplied user manuals for the Series 200 LC Pump and the Series 200 Autosampler and the SOP for this analytical method. The following should be considered 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, from left to right, 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
   (d) Pump with autosampler. Set the autosampler on top of the pump.
4. Replace the existing injection sample loop with a 20 µL loop.
5. If the autosampler has a titanium needle instead of a stainless steel needle, replace it with a stainless steel needle because titanium is potentially contaminated with arsenic. Refer to the PerkinElmer® Series 200 LC Autosampler user manual for instructions on needle replacement.
6. Replace all stainless steel HPLC tubing beyond the HPLC pump's purge port with "yellow" PEEK tubing, 0.007" I.D. X 1/16" O.D. Use "yellow" PEEK tubing for the remainder of the fluid path. Keep tubing lengths short but long enough to allow the connected components to be moved if necessary.
7. Install a PEEK construction in-line filter (10 µm) between the HPLC pump and the autosampler.
8. Do not use the column oven's stainless steel tubing to preheat the mobile phase before it enters the column. The preheating is unnecessary and the extra tubing length will not help the quality of the chromatography.
9. 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[1]. 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

[1] 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®.

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 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

Make the necessary electrical connections between specific I/O terminals of the HPLC autosampler, the pump, the ICP-DRC-MS, and the 6-port switching valves. The event I/O terminals are prominently labeled and are located on the right side of each instrument. For the 6-port switching valves, the terminals are located on the back of each unit.

1. Between the HPLC pump and the autosampler, use two 12-inch long common telephone wires with RJ-11 connectors to connect:
   (a) the HPLC pump's terminal labeled "RDY" to the HPLC autosampler's terminal labeled "RDY IN" and
   (b) the HPLC pump's terminal labeled "EXT RUN" to the HPLC autosampler's terminal labeled "INJ 1".
2. Use the supplied cable (PerkinElmer® Item # B3001203) to connect the Pump's "RUN OUT" terminal to the ICP-DRC-MS's "AUXILLARY I/O".
3. Use the supplied cable (PerkinElmer® Item # N2600418) to connect the pump's "TE 1" terminal to the electrically-activated terminal block I/O on the back of 6-port switching valve #1.
4. Use a standard serial cable to connect an external desktop computer to the back of 6-port switching valve #2.

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 PerkinElmer® Series 200 HPLC autosampler. This only needs to be done once for each new unit. (Note that settings of the front panel buttons should still 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. Screw the cover back on the switching valve unit. For switching valve #1, connect the timed event wire leads that come from the "TE1" timed event output on the Series 200 HPLC pump to I/O junctions #4 and GND on the back of the EV750-100 (order of connection of colored wires does not matter).

3. For switching valve #2, connect a standard serial cable from a desktop computer to the serial port on the back of the EV750-100-S2.
4. Position both 6-port switching valves close to and on the left side of the HPLC Series 200 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 7-1-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 7-1-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.,

5. 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-DRC-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.

8. 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 18 MΩ·cm water. Adjust pH to 5.0 by adding concentrated glacial acetic acid drop-wise. Bring volume to 1000 mL with ≥ 18 MΩ·cm water, and mix thoroughly. Expires in 1 year. 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 ≥ 18 MΩ·cm 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 ≥ 18 MΩ·cm water, and mix thoroughly. Expires in 1 year. Prepare ahead of time or as needed. *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 ≥ 18 MΩ·cm water. Bring volume to 1000 mL with ≥ 18 MΩ·cm water, and mix thoroughly. This solution needs to be filtered before use. Expires in 1 year. 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 ≥ 18 MΩ·cm water. Bring volume to 1000 mL with ≥ 18 MΩ·cm water, and mix thoroughly. Expires in 1 year. 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 ≥18 MΩ·cm water. Bring volume to 1000 mL with ≥ 18 MΩ·cm water, and mix thoroughly. Expires in 1 year. Prepare ahead of time or as needed.
6. **5% Acetonitrile.** To make autosampler rinse solution, add 50 mL of acetonitrile to 950 mL of ≥ 18 MΩ·cm water, and mix thoroughly. Expires in 1 year. Prepare ahead of time or as needed.

b. Preparation of Matrix-Matched Material (“Base Urine”)

Collect human urine from anonymous donors following the same collection procedure used for the preparation of QC material. Assay each donation for, if possible, speciated arsenic or, alternatively, for total arsenic. Exclude urine donations that exceed 10 µg/L for each arsenic species or 25 µg/L total arsenic. Pool a sufficient number of urine donations to make a volume greater than 2 liters. Divide the pool into 40 mL aliquots and store at approximately ≤ -70°C in 50 mL centrifuge tubes labeled “Base Urine – Speciated Arsenic” or a similar designation.

c. Preparation of Working Solutions

1. **25% ($^{v}/_{v}$) Base Urine Pool in 0.075 M Ammonium Acetate, pH 5.** May be prepared ahead of time before the day of analysis. To a clean 250 mL polypropylene (PP) bottle, add 30 mL of 0.5 M ammonium acetate pH 5 to a clean 250 mL PP bottle, 50 mL of “Base Urine – Speciated Arsenic”, and 120 mL of 18 MΩ·cm water. Mix thoroughly. Store refrigerated at approximately 4°C. Prepare as needed. Expiration

date is 1 day from the date made.

2. **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 liter or greater capacity beaker containing a clean magnetic stir bar add approximately 1.9 L of 18 MΩ·cm water (≥ 18 MΩ·cm). 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.

   Be sure solution is being mixed on a magnetic stir plate. Using a glass electrode (pH probe), monitor pH while slowly adding either glacial acetic acid (to lower pH) or 10% ammonium hydroxide (to increase pH) drop wise to bring the pH to 8.60 ± 0.05. In this case, an Eppendorf® Repeater Pipetter fitted with a 2.5 mL combitip works well for adjusting pH. After complete mixing, transfer the beaker's contents to a 2 L graduated cylinder. Bring volume to 2000 mL with 18 MΩ·cm water. Transfer entire 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. Expiration date is 2 weeks from the date made.

3. **HPLC Buffer B Preparation.** May be prepared ahead of time before the day of analysis. To a clean 2 liter or greater capacity beaker containing a clean magnetic stir bar, add approximately 1.85 L of 18 MΩ·cm water (≥ 18 MΩ·cm). 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

   Ensure that the temperature-adjust mode is enabled on the pH meter to be used. Be sure solution is being mixed on a magnetic stir plate. Using a glass electrode (pH probe), monitor pH while slowly adding either glacial acetic acid (to lower pH) or 10% ammonium hydroxide (to increase pH) drop wise to bring the pH to 8.0 ± 0.1. After complete mixing, transfer the beaker's contents to a 2 L graduated cylinder and add 18 MΩ·cm water to bring the volume to 2000 mL. Transfer entire 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. Expiration date is 2 weeks from the date made.

4. **HPLC Buffer C (Rinse Solution) Preparation. 5% (v/v) acetonitrile** (HPLC grade). Mix 50 mL of acetonitrile with 950 mL of 18 MΩ·cm water. Prepare as needed. Expiration date is 1 year from the date made.

5. **HPLC Buffer D (Autosampler Wash Solution) Preparation. 5% (v/v) acetonitrile** (HPLC grade). Mix 50 mL of acetonitrile with 950 mL of 18 MΩ·cm water. Prepare as needed. Expiration date is 1 year from the date made.

6. **Internal Standard (2.5 µg/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 µL of 50 mg/L trimethylarsine oxide (TMAO Internal Standard Stock Solution). Fill with 18 MΩ·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^3.*

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

Definitions:

- **Stock Standard:** Initial solution of one of seven arsenic species prepared by dissolving solid or liquid standard material into aqueous or acidic solution.
- **Intermediate Standard**: A 10,000 ppb 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 8-2: 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 (≥18 MΩ·cm)	10.00
Arsenocholine bromide (or other salt equivalent)	0.20–0.25	Water (≥18 MΩ·cm)	50.00
Disodium methyl arsenate	0.20–0.25	Water (≥18 MΩ·cm)	50.00
Trimethylarsine oxide	0.10–0.125	Water (≥18 MΩ·cm)	50.00
Dimethylarsinic acid	0.10–0.125	Water (≥18 MΩ·cm)	50.00
Arsenic (V) oxide hydrate	0.10–0.125	Water (≥18 MΩ·cm)	50.00
Arsenic (III) oxide	0.10–0.125	Dissolve in 1.5 ml of 6N HCl with mild heating. Add water (≥18 MΩ·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 7-3; however, formula weights provided by the chemical manufacturers, if different, supersede the values presented in the table and should be used instead.

TABLE 8-3: FORMULA WEIGHTS OF ARSENIC SPECIES

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

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

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

e. Preparation of Intermediate Standards

Into separate 50 mL centrifuge tubes, gravimetrically prepare 10,000 ppb 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 ppb. 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 ($K_2S_2O_8$) in ≥18-MΩ 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% $K_2S_2O_8$ to each vessel. (A volumetric measure is sufficient here, as the final solution, post-microwave assisted digestion, will be gravimetrically brought to 50.0 g total weight). Additionally, triplicate blanks (0.5 g water + 10.0 mL $K_2S_2O_8$) and triplicate certified AsV 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 ≥18-MΩ de-ionized water) each digested solution into separate labeled 50 mL centrifuge tubes. Bring the final weight to 50.0 g using ≥18-MΩ deionized water, and record the total weight to three significant digits. The expected arsenic concentration of each solution should be approximately 100 ppb 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 ≥18-MΩ de-ionized water) each digested solution into separate labeled 50 mL centrifuge tubes. Bring the final weight to 50.0 g using ≥18-MΩ deionized water, and record the total weight to three significant digits. The expected arsenic concentration of each solution should be approximately 100 ppb 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 ppb gallium in deionized water by diluting 2 mL of 1000 µg/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 ppb 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 ppb Ga solution). Record all weights. Final concentrations should be approximately 50 ppb.
4. For each new 50 ppb 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 7-4. Each tube will be used to prepare new solutions for MSA in subsequent steps.

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

TABLE 8-4: METHOD OF STANDARD ADDITIONS TABLE 1

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

5. The solutions mentioned in Step 4 and outlined in TABLE 8-4 must be prepared. For accuracy, it is important to prepare each solution in the following order:

   For each 50 ppb microwave-digested solution:

   (i) Into the tube labeled 0 ppb, transfer by weight approximately 10.0 g of the approximately 50 ppb diluted microwave-digested solution prepared in Step 8.g.3. Record the weight.
   (ii) Into the tube labeled 25 ppb, 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 ppb diluted microwave-digested solution prepared in Step 8.g.3. Record all weights.
   (iii) Into the tube labeled 50 ppb, 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 ppb diluted microwave-digested solution prepared in Step 8.g.3. Record all weights.
   (iv) Into the tube labeled 100 ppb, 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 ppb diluted microwave-digested solution prepared in Step 8.g.3. Record all weights. TABLE 7-5: MSA TABLE 2 shows an example of the information that needs to be recorded for each analyte and corresponding tube.

TABLE 8-5: 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, 0ppb	n/a	n/a	10.0g	*9.8973g*
AB, Replicate 1, 25ppb	0.05g	0.0517g	10.0g	9.8269g
AB, Replicate 1, 50ppb	0.10g	0.0997g	10.0g	9.8738g
AB, Replicate 1, 100ppb	0.20g	0.2051g	10.0g	9.8571g

h. Determining the Total Arsenic Concentration

1. Analyze each MSA solution for total arsenic content using a validated ICP-MS method. Ensure that the method uses Ga as an internal standard.
2. After the analysis is complete, prepare an MSA calibration curve for each solution, and calculate the concentration of the unknown sample by dividing the y-intercept by the slope.
3. Using the recorded weights for each MSA solution, calculate the exact concentrations of arsenic in each tube.

4. Once the arsenic concentration of each MSA solution has been determined, perform blank subtraction, and then use all recorded weights for each replicate to back-calculate the exact 10,000 ppb concentration of the Intermediate Standard solutions prepared in section 8.e. An example of the necessary calculation is given below:

Intermediate Standard Concentration Determination

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

Key:

V2MSA	Final Wt (g) of solution prepared in Step 8.g.3
M2MSA	Measured concentration (ppb) as determined from MSA
V1MSA	Initial Wt (g) of solution prepared in Step 8.g.3
M1MSA	Exact concentration (ppb) of microwave-digested solution (Step 8.f.2)
V2MW	Final wt (g) of solution prepared in Step 8.f.5
M2MW	The M1MSA concentration (ppb)
V1MW	Added amount (g) of Intermediate Standard from step 8.f.2
M1MW	Exact concentration (ppb) 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 ppb solution, prepare a 100 ppb 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 (µL) of trimethylarsine oxide (TMAO) stock standard in 18 MΩ·cm water to make a total volume of 20 mL. Calculate the number of µL of stock TMAO standard to add using Equation 2:

Equation 2

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

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. Arsenic speciation analysts should alternate between both sets of working calibrators from run to run.

(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 µg/L" (or similar designation). Likewise, add 7.5 mL of ammonium acetate pH 5 to another dedicated 50 mL volumetric flask labeled "TMAO 250 µg/L" or something similar.
2. To each flask, add 12.5 mL of base urine.
3. Based on the pure measured value of each Intermediate Standard (which takes into consideration the addition of any impurities per analyte) prepare the 250µg/L mixed arsenic species solution. To the flask labeled "Mixed As Species 250 µg/L", add each of the following Intermediate Standards: AC, AB, DMA, MMA, As(III) and As(V) - six solutions in all so that each analyte's final concentration will be 250 µg/L at a volume of 50.0 mL. Use of the dilution equation C1*V1 = C2*V2 is helpful, where C1 is the pure measured value of an analyte + 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 ten clean 15 mL PP screw-top Falcon® (or equivalent) tubes with caps as follows: “Mix S0”, “Mix S0 DUP”, “Mix S1”, “Mix S2”, “Mix S3”, “Mix S4”, “Mix S4 Dup”, “Mix S5”, “Mix S6”, and “Mix S7”. Label an additional ten 15 mL PP screw-top tubes as follows: “TMAO T0”, “TMAO T0 DUP”, “TMAO T1”, “TMAO T2”, “TMAO T3”, and “TMAO T4”, “TMAO T4 DUP”, “TMAO T5”, “TMAO T6”, and “TMAO T7”. Arrange these tubes in order and place in a test tube rack.
2. Inspect a Gilson 402™ (or equivalent) Dilutor/Dispenser to ensure it has a clean 10 mL dispensing syringe and a clean 1 mL sample syringe. It is helpful to examine the rubber gasket for precipitate or dirt. If precipitates or dirt are found on either syringe, replace that syringe with a new one. If possible, it helps to have syringes dedicated to calibrator preparation.
3. Place the tubing that will draw diluent into the dilution syringe into a buffered solution consisting of 25% base urine in 0.075 M ammonium acetate (pH 5). Place a waste beaker in position to collect the effluent liquid from the tip of the sample/dispense tubing. Prime the liquid path by pressing the “Prime” button on the Gilson and allow the syringes to cycle 3 to 4 times before stopping the prime function with a press of the “Start/Stop” button.
4. To make the “S0” calibrator, set the diluent syringe volume to “10000” and the sample syringe volume to “0”. Press the “Start/Stop” button once to draw 10 mL of diluent solution (25% urine in 0.075 M ammonium acetate pH 5) into the diluent syringe then press again to dispense 10 mL to the “Mix S0” tube. Repeat this for the “Mix S0 DUP” tube.
5. To make the calibrator levels “S1” to “S7”, set the diluent syringe volume to the appropriate values indicated in TABLE 8- for the diluent and sample syringe volumes. Submerge the tip of the Gilson 402™ sample/dispense tubing into the “Mixed As Species 250 µg/L” intermediate solution and press the “Start/Stop” button on the Gilson. A volume in µL (indicated in the appropriate row in the third column of TABLE 7-6) will be aspirated. Place the appropriately labeled receiving tube (“S1…”S7”) under the sample/dispense tubing and press the “Start/Stop” button. The indicated volume of diluent and sample will be dispensed to make a total volume of 10 mL of diluted calibrator in each tube. If necessary, repeat the dispense step into the same tube to make a total dispensed volume equal to 10 mL.

TABLE 8-6: GILSON 402™ SETTINGS FOR MAKING DILUTED SERIES

Calibrator Level	Gilson Volume Setting, µL		Target Arsenic Concentration (Nominal Conc. entered into HPLC software)	
	Diluent Syringe	Sample Syringe		
S0 / T0	10000	0	0	(0)
S1 / T1	9980	20	0.50	(2)
S2 / T2	9900	100	2.50	(10)
S3 / T3	9500	500	12.5	(50)
S4 / T4	8500	1500	37.5	(150)
S5 / T5	7500	2500	62.5	(250)
S6 / S7	5000	5000	125.0	(500)
S7	0	10000	250.0	(1000)

6. Repeat the preceding step for each calibrator level. Between calibrators, rinse the tip of the sample/dispense tubing with ≥ 18 MΩ·cm water from a polypropylene wash bottle.
7. Repeat steps four and five, this time using “TMAO 250 µg/L” to make “T0” through “T7”.
8. Cap all tubes. Mix them thoroughly by vortexing and/or inverting repeatedly.
9. 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. Expires in 1 year. 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.

I. Preparation of Quality Control Material

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 2 weeks or less. For longer periods, freeze the urine donations until needed. Assay each donation for, if possible, speciated arsenic or, alternatively, for total arsenic. Assign urine donations to a “low” pool or to a “high” pool according to whether its arsenic concentration exceeds a predetermined threshold value (i.e. 25 µg/L). Do not pool urine donations with an arsenic concentration that exceeds the threshold by more than a factor of 10. After pooling urine donations into their respective pools, clarify each pool by centrifugation in acid-washed 1 liter centrifuge bottles (90 minutes at 5,250 *x g* in a preparative table-top centrifuge cooled to 4ºC is typically sufficient, but additional centrifugation may be needed). Pour off the supernatant and dispose of the pellets. 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. 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. Arsenic speciation pools have to be prepared under nitrogen, in an oxygen free environment.

m. 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. Two runs are typically analyzed on one instrument in a day. 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 does not exceed 24 hours. 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 step 1 below.

1. Identify, gather, and thaw the necessary specimen tubes containing the urine samples for the batch ("run") to be analyzed.
2. 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).
3. Label the required number of Eppendorf® (or equivalent) 1.5 mL microcentrifuge tubes corresponding to the samples and bench QC samples to be run. Label a pair of microcentrifuge tubes for each bench QC, 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. It is helpful to use preprinted barcode labels to improve efficiency and reduce the chance of labeling errors.
4. Attach a bottle of 0.1M ammonium acetate solution to a Gilson 402™ Dilutor/Dispenser diluent draw-line. In order to minimize evaporation, it is helpful to use a capped bottle with a small hole in the cap that is slightly larger than the outer diameter of the draw-line. Insert the draw-line through the hole and assure that the end of the draw line is completely submersed in ammonium acetate solution. *It is important that the end of the line remain submersed throughout sample preparation in order to prevent air bubbles.*
5. Using the "Prime" function on the Gilson 402™ Dilutor/Dispenser, flush lines with 0.1M ammonium acetate solution and empty into a small waste container.
6. If not done so at a previous date, create a sample preparation method on the Gilson 402™ Dilutor/Dispenser that will in a step-wise, sequential, and user-controlled fashion:
   (a) Uptake 10 µL of air into sample draw-line.
   (b) Uptake 200 µL of sample (urine) into sample draw-line.
   (c) Uptake 600 µL of 0.1M ammonium acetate solution into the diluent draw-line.
   (d) Dispense 800 µL of sample + diluent mix (200 µL sample + 600 µL 0.1M ammonium acetate solution).
   (e) Dispense 1000 µL of diluent (for flushing).
   (f) Repeat steps (a) – (e) until the program is stopped.

   The method should be set up such that no step shall execute until the user has

pressed the black button on the black dispenser control device.

Name and save this method into memory.

For detailed instructions on programming, please consult the Gilson 402™ Dilutor/Dispenser *instrument 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).

7. Recall the sample preparation method created in step 6. Using this method, follow its steps (including the 10 µL air uptake) to mix 200 µL of sample from each specimen tube with 600 µL of 0.1M ammonium acetate solution and to transfer the mix to each sample's respective 1.5 mL microcentrifuge tube. *Note that calibrators, which have been pre-made, do not undergo this type of sample preparation.*

8. After each sample/diluent mix is transferred, dispense 1000 µL of 0.1M ammonium acetate solution (pH 5) into a waste container and repeat the process for each remaining sample. Wipe the dispensing tip with a clean KimWipe or rinse with DI water to avoid sample to sample contamination.
9. After capping all 1.5 mL microcentrifuge tubes containing sample/diluent mix, vortex each for 3-5 seconds. Next, centrifuge tubes for 5 minutes at 14,000 rpm in a refrigerated centrifuge pre-cooled to ≤ 4°C.
10. 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.
11. Cap all autosampler vials with the proper fitting "snap-cap" septum caps.
12. Thaw one "set" of calibrators, including S0 – S4 and T0 – T4, 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 200µl of DI water and 600 µL of 0.1M ammonium acetate solution.
14. For each run, one extra sample of 200 µL LU-xxxx + 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 according to step 7 of this section.
15. If barcode labels have been affixed to the vials, at the appropriate time, use the barcode scanner attached to the instrument computer to scan the sample ID from the barcode label on each sample and QC vial before placing it into position in the HPLC autosampler tray.

n. HPLC Instrument Setup

To improve work flow, instrument setup described in this section may be completed before the day of analysis.

(1) Programming the HPLC Pump Methods

1. On the PerkinElmer® Series 200 Pump control panel, press the Quit button to bring the pump controller to the starting screen.

2. If the method number for the correct stored pump program is known, that method can be called up into active memory. Press the function key F6 (labeled as “DIR” on screen). The screen changes and presents a table with column headers “Method”, “Name” and “Last Modified”. Press function key F4 (“RCL”) and you will be prompted to enter a 2-digit number. Press the number for the correct method (e.g., “1”) followed by the “enter” key. The stored method will be loaded into memory and becomes the active pump program. The method can be inspected by pressing the function key F2 (“PUMP”) and using the up or down arrow keys to scroll the program steps.

3. If the correct pump program cannot be found, then the pump program will have to be reentered. To do this, execute the following steps:

   (a) Press the Quit button, then the function key F6 (labeled as “DIR” on screen). The screen changes and presents a table with column headers “Method”, “Name” and “Last Modified”. Press function key F4 (“RCL”) and you will be prompted to enter a 2-digit number. Press “0” followed by the “enter” key. The “DEFAULT” method will now be loaded.

   (b) Press the function key F2 (“PUMP”). A new screen presents a table with column headers “Step”, “Time”, “Flow”, “%A”, “%B”, “%C”, “%D” and “Curve”. The default method has only one line, Step “0”, with the “>” marker just left of it. The highlighted data field is “Time” and contains “10.0”. Press “6.5” on the keypad followed by the right arrow key to replace the previous number. The highlight will advance right to “Flow”. Press 1 then the right arrow key. The next field is “%A”; enter 100 then press the right arrow key. Repeat this for fields “%B”, “%C” and “%D”, entering 0 for each. Press the right arrow key again to return to “Time”.

   (c) Press the “insert” key on the pump controller keypad. A new step, Step “1”, is added and is a replicate of the step before it. Input the data indicated in the table below. Notice that the last column “CURV” in step 1 calls for the input of a number. Likewise, create Step “2”, using the data from the table below. Note that you can move back and forth between fields, and up and down from one step to another, by using the left, right, up and down arrow keys on the keypad.

TABLE 8-7: HPLC PUMP PROGRAM SETTINGS

STEP	TIME	FLOW	%A	%B	%C	%D	CURV
0	6.5	1.00	100	0.0	0.0	0.0	
1	5.0	1.00	0.0	100	0.0	0.0	1
2	4.5	1.00	0.0	100	0.0	0.0	

   (i) Press the function key F6 (“STOR”). You will be prompted to enter a method number, press “1” then the “enter” key. If a message comes up asking if you want to overwrite the existing method stored at that location, press the “1” key for “Yes”. Next you will be prompted to name the method, press the “0” key for “No”. (You may enter “Yes” and create a name for the method but this is optional).

   (ii) Press the function key F3 (“T.E.”). This is for entering timed events. Since an electrical-activated external switching valve is going to be used, the timing of a brief ~1 second contact closure needs to be programmed.

EVENT	TIME	T.E.1	T.E.2
1	0.5	YES	-
2	1.0	YES	-

(iii) Enter the parameters from the above table using the same technique as was used to create the pump method. Note that to successfully input the minutes for each event, you need to press “enter” after inputting the number, instead of using the right arrow key. Timed Event #1 (“T.E.1”) is turned on by pressing “1” (“Yes”) on the keypad. Store a “ready” time (the number of minutes the pump waits during re-equilibration before allowing the next injection) by pressing function key F8 (“RDY”) and input “30” followed by the “enter” key. Press function key F6 (“STOR”) and respond to the prompt for a method number by inputting the same method number (e.g., “1”) used to store the pump program. Respond to the next two prompts with a “yes” then a “no”.

4. Press the function key F4 (“PRESS”). Press function key F4 (“MAX”) then input “3000” followed by the “enter” key. Press function key F3 (“MIN”) then input “100” followed by the “enter” key. Press function key F6 (“STOR”) and respond to the prompt for a method number by inputting the same method number used to store the pump program. Respond to the next two prompts with a “yes” then a “no”.

5. Create a separate HPLC pump method (e.g. method “19”) which will serve as a column wash method after the completion of a batch run:

   (a) Follow step 3 above to input the following in TABLE 7-:

TABLE 7-8: HPLC PUMP COLUMN WASH PROGRAM

STEP	TIME	FLOW	%A	%B	%C	%D	CURV
0	0	1.00	100	0.0	0.0	0.0	
1	20	1.00	0.0	0.0	0.0	100	0
2	HALT	0	-	-	-	-	

   (b) No Timed Events need to be programmed.

6. Store this method using a number of your choosing (e.g. “19”).

7. To link the pump methods into a sequence, do the following:

   (a) Press soft key F5 (“SEQ”) on the HPLC pump. Press F8 (“DELS”) to clear any preexisting sequence. The following fields will be shown: “SET”, “METHOD”, “FIRST”, “LAST” and “INJ”. The “METHOD” field will be active highlighted field.

   (b) Input “1” then press the “enter” key. The next highlighted field will be “FIRST”; input “100” then press the “enter” key. The “LAST” field will automatically change to 100. Press the “enter” key again. The last field “INJ” will now be highlighted; input “5” then press the “enter” key.

   (c) A second line for set 2 will automatically be created. Press the “enter” key to advance to the “FIRST” field and input “1” followed by the “enter” key. In the “LAST” field, enter the number of samples to be analyzed. If you do not yet know this number, leave the default number alone (it can be changed later). Advance to the “INJ” field by pressing the “enter” key and input “1” followed by the “enter” key.

(d) A third line for set 3 will automatically be created. Change the “METHOD” field to a value corresponding to the method number for the “column wash” pump method (e.g., 19), then change the “FIRST” and “LAST” fields to 99. Leave the “INJ” field set to “1”.

(e) Do not press the soft key F6 (“LINK”). This will be done later before the start of a batch run.

8. The HPLC pump is now programmed.

(2) Programming the HPLC Autosampler

1. On the Series 200 Autosampler, press the “quit” key. This brings up the “READY” screen. Next, press function key F6 (“DIR”) to show the method names directory. Press function key F4 (“RCL”) and input “0” followed by the “enter” key. Answer the next prompt with “yes” and you will be returned to the starting screen.

2. Press function key F2 (“METH”). A new screen presents a table with column headers “First”, “Last”, “Volume”, “Replicates” and “Time”. The highlighted data field is “First” and contains a value of “1”. Key in “100”. Complete the entry by pressing the “enter” key. It will automatically advance the highlight to the data field “Last” which already contains a value of “100”. Since this method will only make injections from position “100”, press “enter”. The next highlighted field is “VOLUME”. Input a value that is 2.5X the size of the autosampler’s injection loop. For instance, if the autosampler’s injection valve has a 20 µL loop installed, input 50 for “VOLUME”. Enter 5 as the number of “REPLICATES” so that five replicate injections will be made from the vial in position 100. Leave the default value for “TIME” unchanged. Press the function key F6 (“STOR”), key in “1” then “enter”. Respond to the next two prompts with a “yes” then a “no”.

3. To program the second method, it is not necessary to exit the existing one. Since the method just programmed was stored, you can edit the existing method in memory and save it to a different location. Using the arrow keys on the autosampler panel, highlight data field “FIRST” which will contain a value of “100”. Input the starting vial position from which the autosampler is to make its first injection, which is usually “1”. Press the “enter” key. Enter the number for the last vial position for this sample set, and then press the “enter” key. The highlighted field is “VOLUME” which is still the volume programmed for Method 1. Press the “enter” key to advance to “REPLICATES”. Replace the existing value by keying in 1. Press “enter”. Leave the default value for “TIME” unchanged. Press the function key F6 (“STOR”). Key in “2” then “enter”. Respond to the next two prompts with a “yes” then a “no”.

4. Program a third method that will be used in conjunction with the column wash pump program. This method will do an injection for a blank or empty vial in autosampler tray position 99. Set “First” and “Last” vial position fields to “99”. Set the “Replicate” field to “1”. While the “Volume” field can be set to any value, a value of “1” is preferred. Press the function key F6 (“STOR”), key in a number of your choosing (e.g., “19”) then press “enter”. Respond to the next two prompts with a “yes”. Input a name for this method (“i.e., “WASH”) then press “enter”.

   If necessary, additional methods may be created following the steps in this section.

5. At the “READY” screen, press function key F5 (“SEQ”). Press F8 (“DELS”) to delete previous sequences, if present. Three columns will be shown: “SET”, “METHOD”, and “INJECTIONS”. The “METHOD” column will be highlighted; key in “1” followed by the “enter” key. The table will automatically create and jump to a line for set 2. Enter “2” then press “enter”. You will again be prompted to enter a method number for set 3. Enter “3” then press “enter”. You will be prompted to enter a fourth set, but that will not

be necessary unless more than three methods need to be linked together. You can scroll the sets by using the up and down arrow keys. If you inadvertently created too many sets, position the “>” symbol to point at the unwanted set and press the “delete” key. The “INJECTIONS” column, which shows the number of injections programmed for each method, cannot be edited.

6. Link the methods together by pressing the F6 function key (“LINK”).
7. The HPLC autosampler is now programmed.

o. 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[2] 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 TABLE8-9 into this window :

[2]Actual file names may differ from those presented throughout this document.

TABLE 8-9: 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 8-10.

TABLE 8-10: 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 8-11: 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 following information:

TABLE 8-12: 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 following information:

TABLE 8-13: 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 TABLE 7-1. *Note: Values in* TABLE 8-13 *are suggested starting values. Instruments vary in their optimal parameter values, and analysts should use their discretion.*

TABLE 8-14: 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*		
Discriminator Threshold:	12	DRC™ Mode CRO	-2*
Deflector Voltage:	-10*		
Quadrupole Rod Offset [QRO]	0*	DRC™ Mode AFT	250*
Cell Entrance Voltage	- 3*	DRC™ Mode RPa	0
Cell Exit Voltage	-3*	DRC™ Mode RPq	0.50*
Cell Rod Offset {CRO}	-12*	DRC™ Mode Cell Gas A	0.55*
DRC™ Mode NEB	0.9*	DRC™ Mode Cell Gas B	0

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

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

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 following information:

TABLE 8-15: 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 8-16: 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.

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

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

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

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

Important!

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

(2) Priming the HPLC Pump

1. Turn on the HPLC Series 200 Vacuum Degasser (switch is on back of unit).
2. 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.
3. On the HPLC pump, open the door that accesses the pump head. Attach a 30 mL or larger plastic syringe to the purge port. Open purge port by turning its knob counterclockwise ~¼ to ½ turn.

Important!

Be sure the HPLC pump's purge port is open and that a syringe is attached before completing the next step. While there is no specific danger to the analyst, an over-pressure situation can occur that, under certain circumstances, could damage the HPLC pump, column or other components.

4. At the HPLC pump control panel, press the "Purge" button. Press F4 ("%A") followed by F3 ("FLOW"). Key in 2 then press the "enter" key. The pump will immediately start and quickly ramp up to a flow rate of 2 mL/min.
5. Allow the pump to fill the syringe with about 15 mL of buffer A. Watch the reservoir tubing for air bubbles, which should be flushed out. If there are air bubbles inside the tubing, tap the tubing to free the air bubble(s). The tubing should be bubble-free after ~10 mL of buffer has been pumped through.
6. Press F5 ("%B"). The pump will now switch to reservoir B. Again, watch for bubbles and make sure they are flushed out of the tubing. Allow another 15 mL of buffer to be pumped or until the syringe is almost full, then press the "stop" key to stop flow.
7. Close the purge port (turn knob clockwise), remove syringe, and close door. Dispose of syringe contents to waste or in a sink.

(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).

q. 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 8×10^{-7} and 1.8×10^{-6} torr). Keep a record of any maintenance procedures along with the base vacuum pressure in the *Daily Maintenance Checklist* logbook.

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 should establish a more or less steady intensity after 5–10 seconds.

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”.

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.*

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.

r. 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.

s. 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". Type in 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. If barcodes are used on the sample labels, use the barcode scanner attached to the ICP-DRC-MS computer to scan the sample ID from the barcode on each sample before placing it into position in HPLC autosampler tray. 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 QC sample, called "EQ", which will be injected with five replicates during the initial system equilibration period that occurs 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 should lapse between the time that the actual analytical run starts (the analysis of the "Bk" in autosampler location 1) and the analysis of the last vial is complete. Keep this in mind when determining how many samples will be analyzed.

TABLE 8-17: 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™.

4. When satisfied that the Sample table entries are correct, choose File > Save.
5. 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.

t. Starting the Run

1. Restart the NexION® controller computer by going to the Windows Start button and choosing “Restart”. This serves to purge the computer of possible memory/register conflicts and will give the system and NexION® software a fresh start. It is unnecessary to shutoff the plasma as the NexION® instrument will sustain it while the computer does a restart.
2. Check the waste carboy. If more than two-thirds full, discard of the waste using the protocol outlined in SOP DLS 3500 *Handling Corrosive Liquid Laboratory Waste*.
3. Check that the tubing that draws internal standard is inserted into the bottle containing Internal Standard and that there is sufficient quantity of Internal Standard.
4. Check that there is sufficient mobile phase to last the entire run. In addition, be sure that Bottle D and the HPLC Autosampler’s wash bottle contains sufficient amount of 5% (v/v) acetonitrile. It is very important that line D does not become filled with air bubbles at any point during analysis.
5. Set the HPLC Series 200 Column Oven to 35°C if it is not already at that temperature.
6. Check for stray “.nc” files by using Microsoft Windows® File Explorer to look inside the C:\NexIONdata\ReportOutput folder. Move any existing files that end with “.nc” extension to another folder so that the C:\NexIONdata\ReportOutput folder is empty of “.nc” files. Close Windows File Explorer.
7. Launch NexION® Instrument Control program if it is not already. Do not launch or start any other programs at this time.
8. Check that the correct Sample file in the window “Instrument Control Session” is active. If it is not correct, load the correct Sample file. In this window, note the injection number of the last vial as indicated by the numbered prefix leading the first underscore (“_”) character (e.g., 42 from “042_samplename”).
9. Check that the HPLC pump methods are correctly programmed according to *Programming the HPLC Pump Methods*. On the HPLC pump, press the soft key F5 (“SEQ”). Confirm that there are just three lines indicating sets 1, 2 and 3. Check that sets 1 and 3 are configured properly. Press soft key “SET”, input 2 then press the “enter” key. Press soft key F4 (“LAST”) and input the injection number of the last vial noted in the preceding step. Press the “enter” key. Press soft key F6 (“LINK”) then press the “return” key. The display will return to the top level. Confirm that the top line in the pump control panel display displays “METHOD01 STORD SHTDN Q01.100.00” indicating that the pump methods are now linked. Do <u>not</u> press the soft key F8 (“STRT”) at this time.
10. Check that the HPLC autosampler methods are correctly programmed according to *Programming the HPLC Autosampler*. On the HPLC autosampler, press the soft key F6 (“DIR”), followed by soft key F4 (“RCL”). Input 2 then press “enter” followed by “yes”. Next, press F2 (“METH”). A new screen presents a table with column headers “First”, “Last”, “Volume”, “Replicates” and “Time”. Using the arrow keys on the autosampler panel, highlight data field “LAST” and input the injection number of the last vial noted in the preceding two steps. Press the “enter” key. Next, press F6 (“STOR”), input 2 then press the “enter” key. Respond to the next two prompts with a “yes” then a “no”. Press the “return” key. If the word “LINKED” does not appear on the autosampler display, then link the methods together by pressing the F6 function key (“LINK”).
11. Check that the correct NexION® method is loaded and active in the window “Instrument Control Session”. If it is not correct, load the correct Method file. Check under the Sampling tab that “Peristaltic pump under computer control” is checked, and

the pull-down menu “Sampling” indicates “External”.

12. Check that the DRC™ gas is indeed flowing by making the NexION®'s Instrument window active and clicking on the Diagnostics tab. Inspect the Cell Gas A, its value should be fluctuating at 0.6 (or other optimal value) ± 0.01 mL/min. If it is not, see section *Turning on the Reaction Cell Gas* for details to turn on the DRC™ gas flow. Make the conditions window active and Choose File > Save to save the method file.

13. Choose File > Review Files in the “Instrument Control Session” menu bar. In the next window, click the “Load” button for “Dataset” (second item) and navigate to this run's data folder, double-click on it and click on the “OK” button. The correct Dataset path should now be indicated. Click the “Done” button.

14. Check that all blanks, calibrators, QC and sample vials are loaded into their correct positions in the HPLC autosampler tray, as designated by the NexION® Sample window (or its printout).

15. Press function key F5 (“SEQ”) on the Autosampler and check that the total number of injections (i.e., the sum of all injections for each listed method) agrees with the number of vials + 4 (accounting for the extra 4 injections of the equilibrator vial in position #100) in the autosampler tray. Press the “return” key to get back to the main screen. See section *Programming the HPLC Autosampler* for details on how to program the autosampler. Check that the HPLC autosampler's methods are linked and that the word “LINKED” appears in the autosampler's information screen.

16. This step is optional but offers the advantage that the NexION® data files will be converted in real time to TotalChom™ “.raw” files that have names containing a date-time stamp corresponding to actual time of injection.

    (a) Launch TotalChrom™ Navigator. In the resulting TotalChrom™ Navigator window, choose menu item Apps > ChromLink (alternatively, you may launch ChromLink™ from the operating system Start > Programs menu).

    (b) In the ChromLink™ program window, choose the menu item Configuration > Mass Details and check the Nominal Name and Mass for arsenic. If it is missing or the NexION® tune (“default.tun”) file was re-optimized earlier then ChromLink™ needs to be configured (see *Configuration of ELAN ChromLink*™ on page 54 for details). To save time, the analyst may choose to close the TotalChom™ Navigator and ChromLink™ windows and skip step 16 in its entirety. Data file conversion via ChromLink™ can easily be done during post-run data reprocessing.

    (c) In the ChromLink program window, click on the “Browse” button to the right of the “ELAN ChromLink file location” field. Navigate to the current working folder, double-click on it then click the “OK” button so that ChromLink knows where to save its processed files.

    (d) Otherwise, refer to step (b) of *Data Processing and Analysis* for details on proper setting of the ELAN ChromLink™ window's parameter fields. In the ELAN ChromLink window, click the button “Start Processing ELAN Data Files” to put ChromLink in watch mode so it will process each data for each injection in real time. A new dialog box will open and indicate it is ready to convert data and waiting for the first file.

17. Click on the NexION® “Instrument Control Session” window to make it active, and then click the mouse in the Samples window on the corner rectangle of the sample table (where row headers intersect column names). The entire sample table will become highlighted (dark background). Click the “Build Run List” button. A “Run List” Box will appear. This is where you can review and append the sample list and indicate “Autostop” when the batch is completed. Click the “Analyze Batch” button. A Run

Progress box will appear indicating that the NexION® software is now waiting for a signal from the HPLC that indicates the occurrence of an injection.

18. If the HPLC pump is not already pumping, press function key F8 ("STRT"). This will start the flow of Buffer A and put the pump into a "wait for injection" mode.
19. On the HPLC autosampler, press the "start" key. If the equilibration wait time has been reached, the autosampler will immediately begin its injection sequence. Otherwise, it will respond with the message 'WAITING FOR EXTERNAL READY" and wait for the equilibration wait time to complete.
20. Open the NexION® "Instrument Control Session" Real-Time window by clicking the tool bar button that looks like a Gaussian distribution (or a burgundy chromatographic peak, if you prefer). After the Real-Time window opens, click on the drop-down menu and select "Signal". Real-time data will now be displayed.

When the HPLC pump's equilibration time has been reached, the autosampler will seek the first vial and make an injection. A blue bar in the NexION®'s "Measurement Status" box will now indicate that data is being collected. The system can now run unattended.

Check the progress of the run after 2 or 3 injections. Note the chromatograms appearing in the NexION®'s Real Time window. Adjust the signal scale in the Real Time window, as necessary. Compare the positions and peak heights of each arsenic species relative to the internal standard. It helps to visually compare it to a printed reference chromatogram. If abnormalities in retention time, peak height or shape are readily apparent, the analyst may need to stop the autosampler and pump and abort the run in the NexION® program. The HPLC pump and autosampler are stopped by pressing the "stop" buttons on their respective control panels. Correct the problem(s) and restart the run.

u. 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, for example, if a sample vial is 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.

9. 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 TotalChom™ 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 8-1.

TABLE 9-1: INTEGRATION

Basic Parameters		Advanced Parameters	
Bunching Factor :	1	*Peak Separation Criteria*	
Noise Threshold :	5	Width ratio :	0.2
Area Threshold :	25	Valley to peak ratio :	0.01
		Exponential Skim Criteria	
		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 9-1 if necessary.

Click on the "Baseline Timed Events" tab. As a guideline, enter the information shown in TABLE 8-2 or other parameters as determined to be appropriate.

TABLE 9-2: 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 9-2 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”.
4. Click on the “Replot” tab. Enter the information shown in TABLE 8-3.

TABLE 9-3: 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*
Scaling Parameters		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 9-3 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 8-4:

TABLE 9-4: Global Information

Volume units :	µL	*Unidentified Peak Quant.*	
Quantitation units :	µg/L	Calibration factor :	1.000e+99
Sample Volume :	1.000	Always use calib. Factor :	selected
Void time (min) :	0.000		
Calibration			
Internal Standard	selected		
		RRT Calculation	
Reject outliers during calibration :	Not checked	Use first peak in run as RRT reference:	selected
Sample Amount Options			
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 9-4 are starting points. The analyst may make appropriate changes to one or more of the Global Information parameters if necessary.

5. 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 8-5.

TABLE 9-5: 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.

6. In the Method Editor window, choose the menu item Components > Defaults. Click on the “Identification” tab. Enter the information shown in TABLE 8-6.
7. Click on the “Calibration” tab in Components Defaults Window. Enter the information shown in TABLE 8-7. 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 9-6: COMPONENTS DEFAULTS — IDENTIFICATION

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

TABLE 9-7: COMPONENTS DEFAULTS — CALIBRATION

		Level	Amount
Calibration Type :	Use Curve	S0	1.0000E-6
Curve Type :	1st 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 9-5.

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†		X		C:\HPLC\Methods\Arsenic 150ppb LDR.mth

† 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\<current working folder>). 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 47), 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:\NexION data\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 8-8.

TABLE 9-8: 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 is 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.

10. RECORDING OF SAMPLE AND QC DATA

a. Transferring the Data to the Central Database

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. From a computer that has access to the Microsoft Access™ or MS SQL Server 7™ database used for tracking data start the program. A “GoTo2 : Form” window should automatically open. If it does not, you may have to open it manually.
3. Click the “Add Sample Results to Database” button. New buttons will appear. Click the “Import Instrument Data File” button. For “Instrument”, choose “NexION-X” (or the appropriate instrument). For “Assay”, choose “As Speciation 2”. Choose the correct analyst from the drop-down list and the appropriate study. It is not necessary to fill-in the “IS Lot Number” Field. Click “Import”. Select the location of the data file on the network drive and press the “Open” button.
4. In the “Imported Results” table, pressing the “Find X’s” button will show only those samples whose sample ID is not recognized as a valid QC pool ID or sample ID for this study. (Sample IDs are set up when the study is logged into the database). If necessary, corrections to sample IDs and dilution factors can be made in this table (e.g., correction of transcription errors and adjustment for level of dilution). If samples were diluted for analysis, both the sample ID and the dilution factor need to be edited in this table before the values are transferred to the database. First, change the dilution factor to reflect the way that the sample was analyzed then edit the sample ID to remove any comments about the level of dilution at which the sample was analyzed. (The replace command is useful here.) Delete X, select Filtered
5. When corrections to sample IDs are made, press the “Recheck” button to evaluate the sample IDs. Any sample or analyte row marked “Not Recognized” will not be transferred to the database when the “Transfer” button is pressed.
6. Press the “Transfer” button to import data into the database.

b. QC Data

Once data is transferred to the Microsoft Access™ (or MS SQL Server 7™) database, quality control (QC) samples must be assessed for pass or failure through the generation of QC reports. The database allows for the printing of several types of QC reports. If necessary, keep a copy of the report with the analysis printouts from the run (if the data needs to be printed). The QC reports can be stored electronically.

11. FINAL REVIEW OF THE DATA

c. Analysis Printouts and Analyst Run Report

Per the guidelines of each study, bind the analysis printouts with a printout of the calibration curve and curve statistics and place them in the study folder(s). For some studies, this step is not necessary.

d. Plotting QC Results

When the Microsoft Access™ or MS SQL Server 7™ database is used, QC plots are updated automatically when the data are imported into the database. Monitor these plots regularly for any trends in the bench QC results. If trends are observed, contact the laboratory supervisor.

e. Supervisor Review

The Microsoft Access™ or MS SQL Server 7™ database allows the supervisor to review the QC and sample results directly in the database. After the supervisor reviews the data, he or she may mark results as "Ready to Report."

12. LIMIT OF DETECTION AND LINEAR RANGE TESTED

The limits of detection (LOD) for arsenic species in urine specimens are based on data taken from a minimum of 60 analytical runs. At least four levels are used in each run with one level being below the LOD. The matrix blank can be used to satisfy the criterion of having a level below the LOD. Using the data from at least 60 runs, regression can be used to validly predict the standard deviation at the LOD concentration. The LOD will be three times this calculated standard deviation, and this will represent the method detection limit. Report results below the detection limit as "< LOD" (where "LOD" is the calculated lowest detection limit). The LOD calculation is reevaluated once every two years.

TABLE 12-1: LIMITS OF DETECTION (LOD) AND LINEAR RANGE TESTED (LRT) FOR AS SPECIES

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

13. REPORTABLE RANGE OF RESULTS

Urine arsenic results are reportable in the range of greater than the LOD, where LOD is the calculated limit of detection. 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. An elevated QC needs to be analyzed within the extended calibration run along with the patient sample that falls within that range. The elevated QC sample only needs to be analyzed once within that run, and the sample result should be reported from that run. The maximum dilution allowed for each analyte is a 1:50 dilution making the reportable range 50,000 µg/L for this analytical method.

Results Greater Than Range of Linearity Tested: Perform an extra dilution on any urine sample whose concentration is greater than 110% of the Linear Range Tested ("LRT") concentration in TABLE 12-1 (the highest concentration for linear range tested).

14. SPECIAL PROCEDURE NOTES – CDC MODIFICATIONS

None applicable for this method.

15. 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_{3s} – Average of both low QCs <u>OR</u> average of both high QCs is outside of a 3s limit.

   2_{2s} – Average of both low QCs <u>AND</u> average of both high QCs is outside of 2s limit *on the same side of the mean*.

   R_{4s} sequential – Average of both low QCs <u>AND</u> average of both high QCs is outside of 2s limit *on opposite sides of the mean*.

   10_x sequential – The previous nine average QCs results (for the previous nine runs) were *on the same side of the mean* for either the low <u>OR</u> 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.

16. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

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

17. REFERENCE RANGES

The reference range for each arsenic species (see TABLE 17-1) 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 17-1: REFERENCE RANGES FOR ARSENIC SPECIES

Species Chemical Name	Reference Range[1], µg/L
Arsenobetaine	0.940 – 50.4
Arsenocholine	<LOD
Trimethylarsine oxide	<LOD
Monomethylarsonic acid	<LOD – 2.01
Dimethylarsinic acid	3.5 – 17.4
Arsenous (III) acid	<LOD
Arsenic (V) acid	<LOD

[1] 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.

18. 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.

19. SPECIMEN STORAGE AND HANDLING DURING TESTING

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

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

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

21. 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).

22. 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 means (e.g., Microsoft Access™, encrypted USB devices, or CD-R backups) to track specimens. Maintain records, including related quality assurance (QA) and QC data, for 3 years or longer. Keep duplicate records (off site, if sensitive or critical) 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.

23. BI-ANNUAL INSTRUMENT-TO-INSTRUMENT COMPARISON

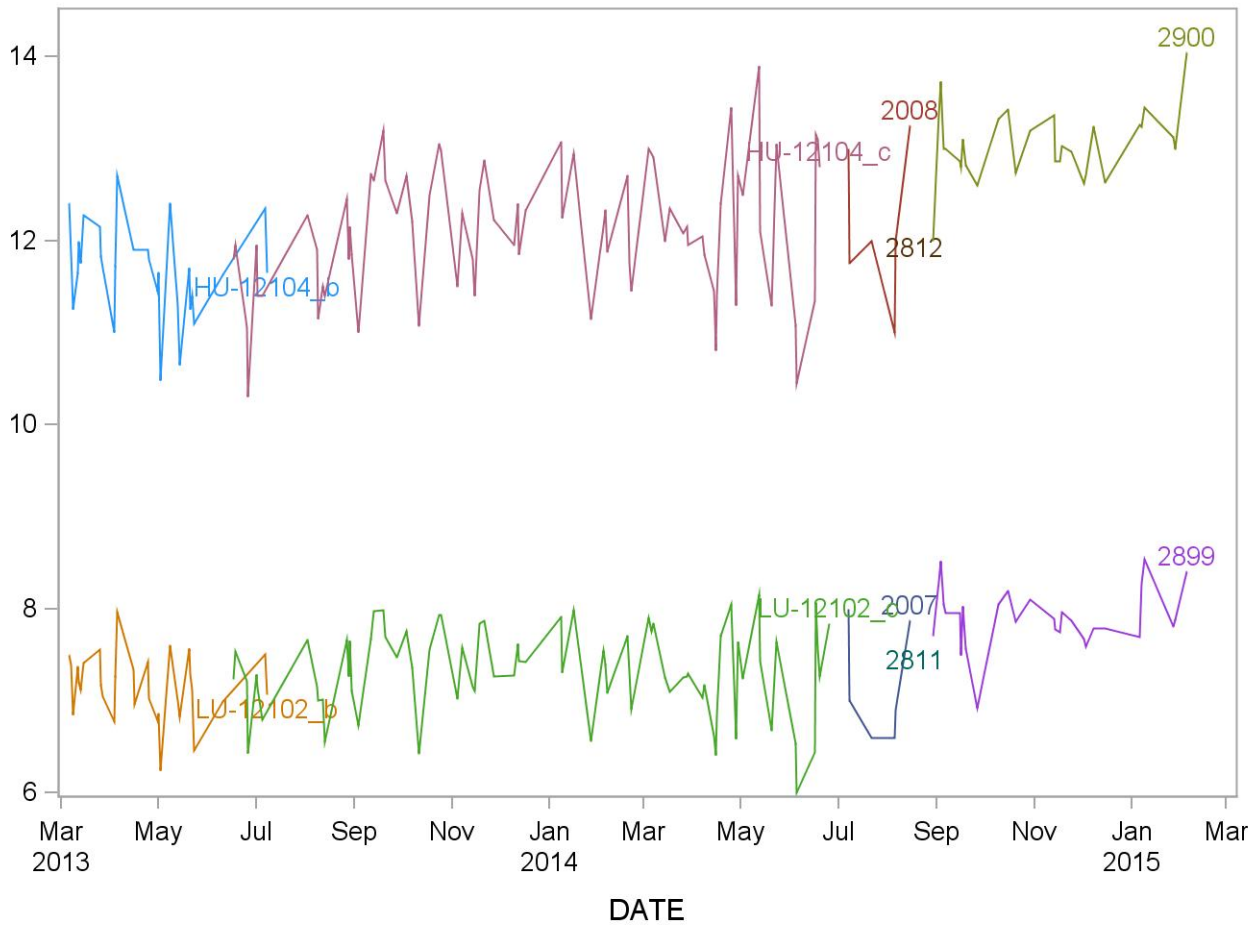
Per CLIA requirements, twice per year, if an analytical method is performed on more than one instrument, then an instrument-to-instrument comparison has to be performed. This is usually done in conjunction with the extended linear range verification study. The same samples have to be analyzed on each 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.

24. SUMMARY STATISTICS AND QC GRAPHS

See following pages

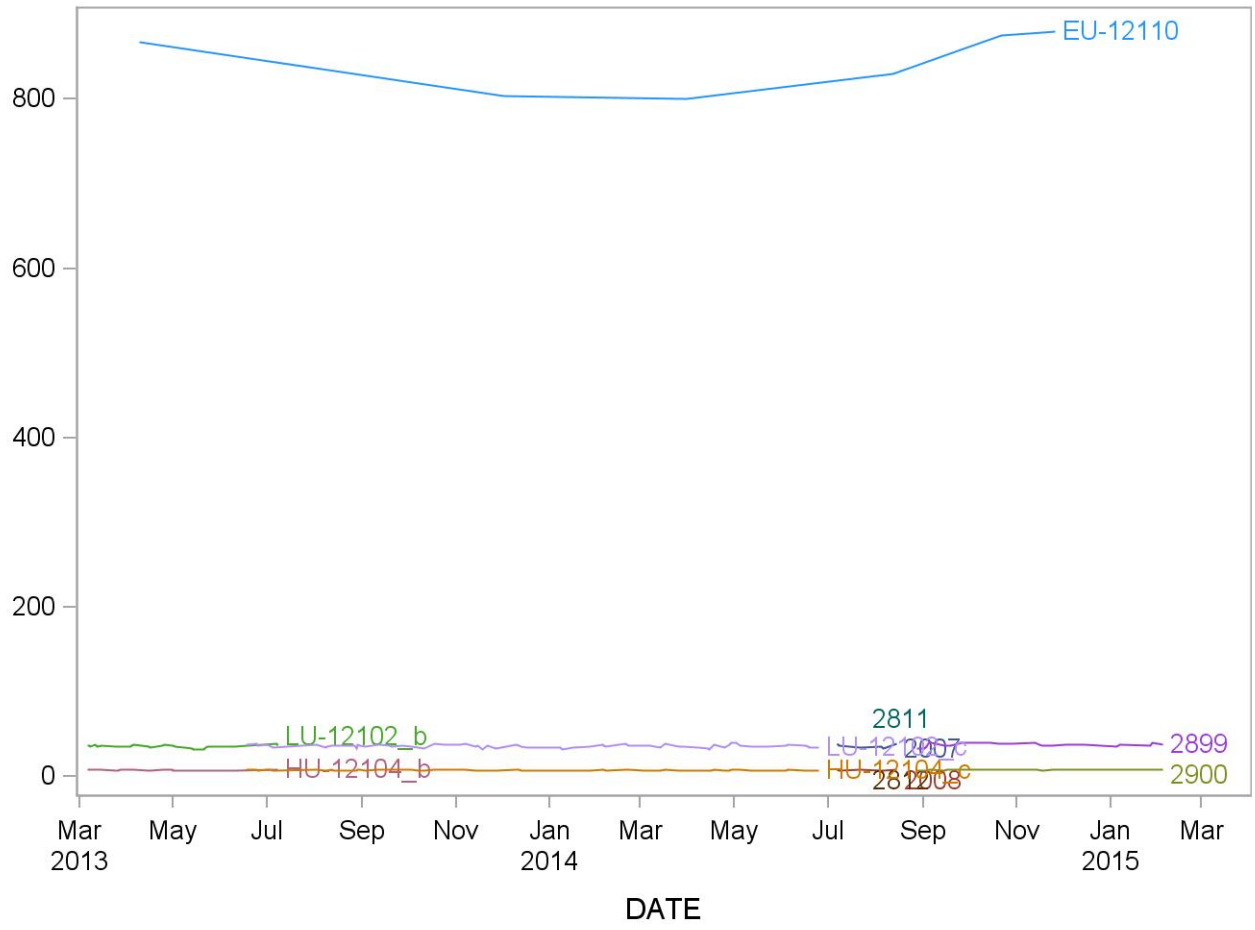
2013-2014 Summary Statistics and QC Chart for Urinary Arsenic acid (µg/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU-12104_b	40	06MAR13	08JUL13	11.757	0.560	4.8
LU-12102_b	40	06MAR13	08JUL13	7.180	0.380	5.3
HU-12104_c	107	17JUN13	19JUN14	12.117	0.711	5.9
LU-12102_c	108	17JUN13	25JUN14	7.327	0.475	6.5
2008	8	07JUL14	15AUG14	12.250	0.845	6.9
2007	8	07JUL14	15AUG14	7.231	0.608	8.4
2812	2	18AUG14	18AUG14	11.750	0.354	3.0
2811	2	18AUG14	18AUG14	7.275	0.035	0.5
2900	41	29AUG14	04FEB15	13.071	0.415	3.2
2899	41	29AUG14	04FEB15	7.917	0.323	4.1



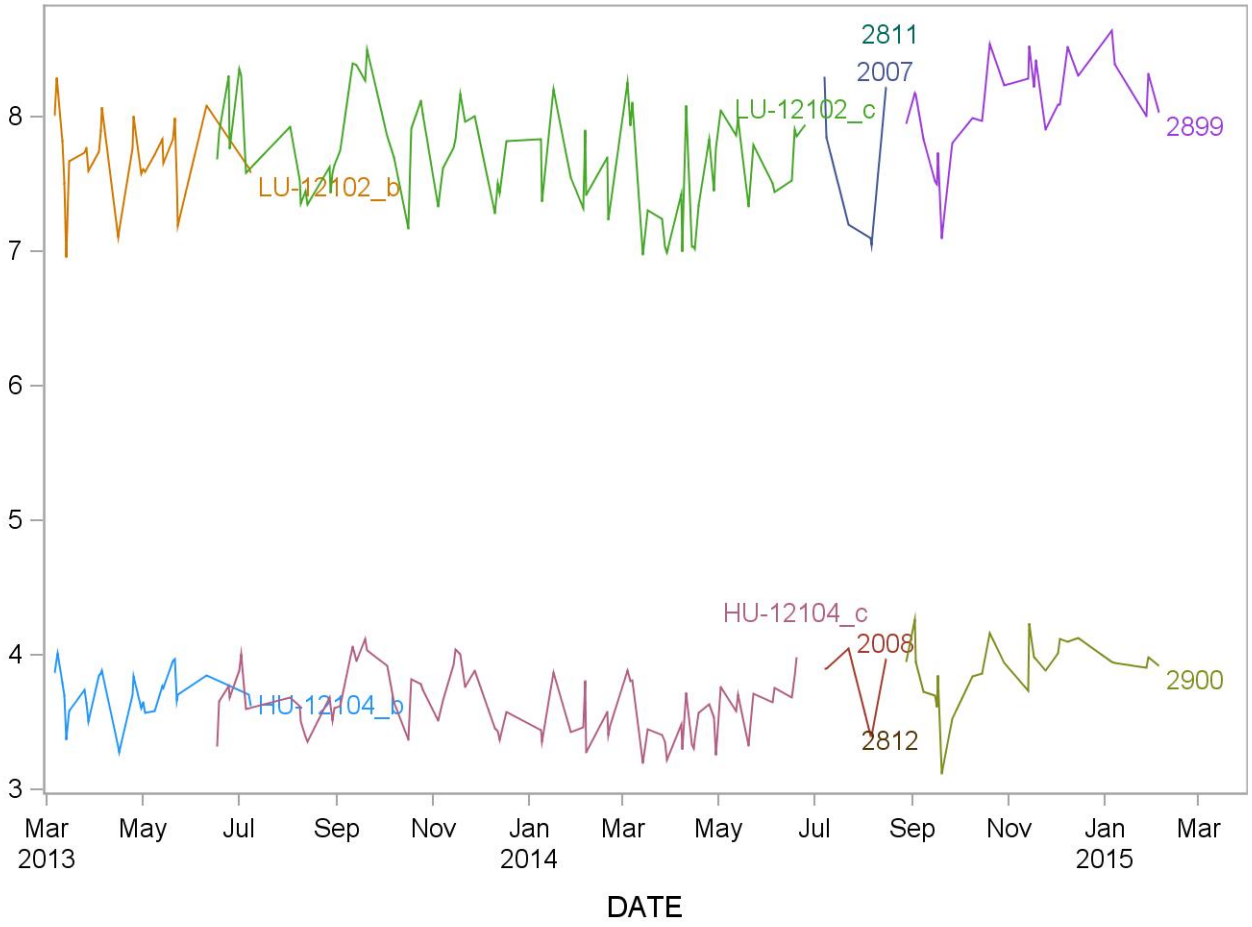
2013-2014 Summary Statistics and QC Chart for Urinary Arsenobetaine (µg/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU-12104_b	40	06MAR13	08JUL13	7.5385	0.3850	5.1
LU-12102_b	40	06MAR13	08JUL13	35.6500	1.4998	4.2
EU-12110	6	09APR13	26NOV14	842.8333	36.2128	4.3
HU-12104_c	108	17JUN13	25JUN14	7.4461	0.4026	5.4
LU-12102_c	108	17JUN13	25JUN14	36.0847	1.7039	4.7
2008	7	07JUL14	15AUG14	7.5714	0.4803	6.3
2007	7	07JUL14	15AUG14	35.9286	2.2254	6.2
2812	2	18AUG14	18AUG14	8.8500	0.0000	0.0
2811	2	18AUG14	18AUG14	50.0000	0.0000	0.0
2900	41	28AUG14	04FEB15	7.8110	0.3592	4.6
2899	41	28AUG14	04FEB15	37.5397	1.8222	4.9



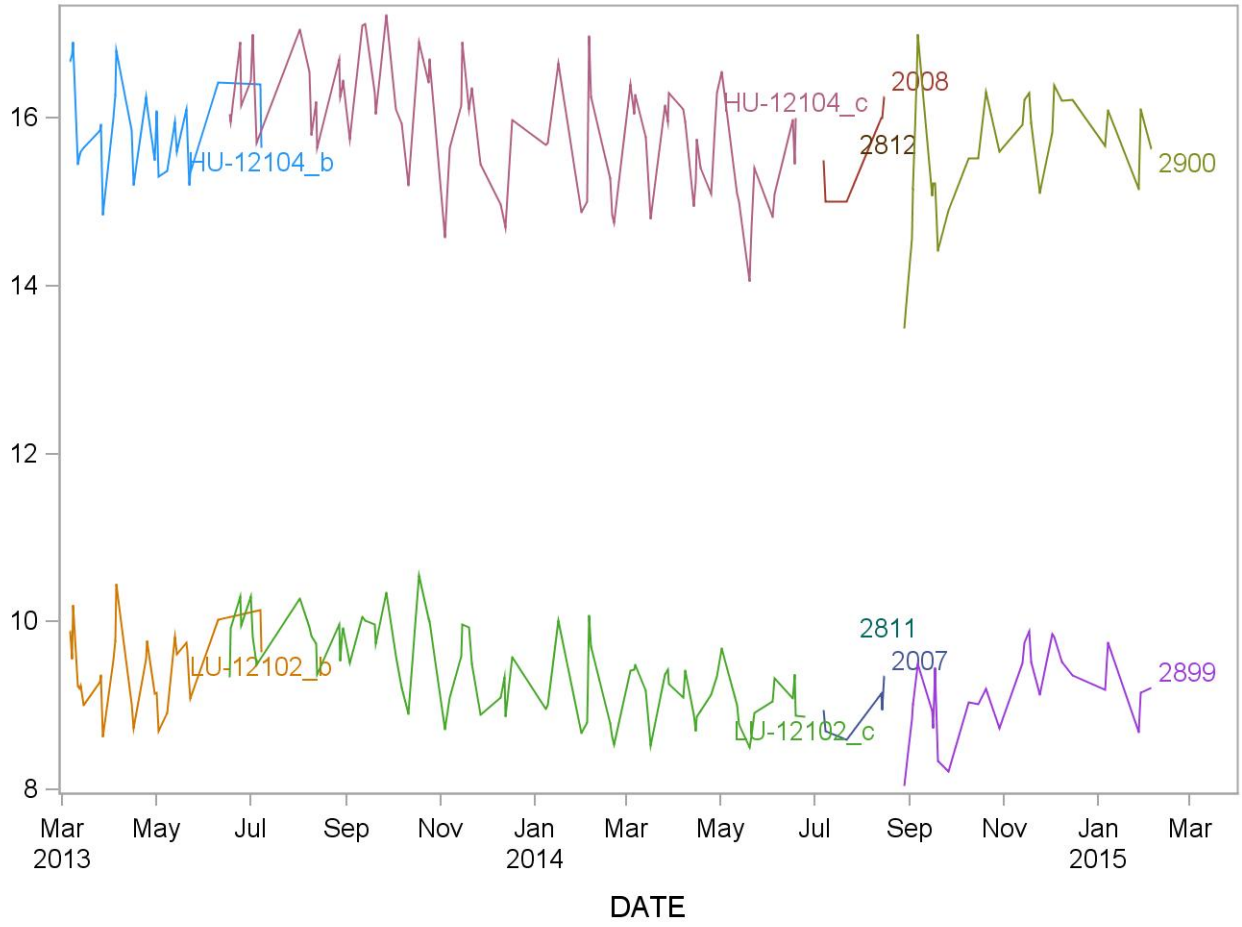
2013-2014 Summary Statistics and QC Chart for Urinary Arsenocholine (µg/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU-12104_b	40	06MAR13	08JUL13	3.7166	0.1981	5.3
LU-12102_b	40	06MAR13	08JUL13	7.7215	0.3258	4.2
HU-12104_c	105	17JUN13	19JUN14	3.6227	0.2251	6.2
LU-12102_c	106	17JUN13	25JUN14	7.6858	0.3852	5.0
2008	8	07JUL14	15AUG14	3.8125	0.2615	6.9
2007	8	07JUL14	15AUG14	7.7250	0.5372	7.0
2812	2	18AUG14	18AUG14	3.2500	0.0707	2.2
2811	2	18AUG14	18AUG14	8.5000	0.1414	1.7
2900	41	28AUG14	04FEB15	3.8945	0.2409	6.2
2899	41	28AUG14	04FEB15	8.0786	0.3652	4.5



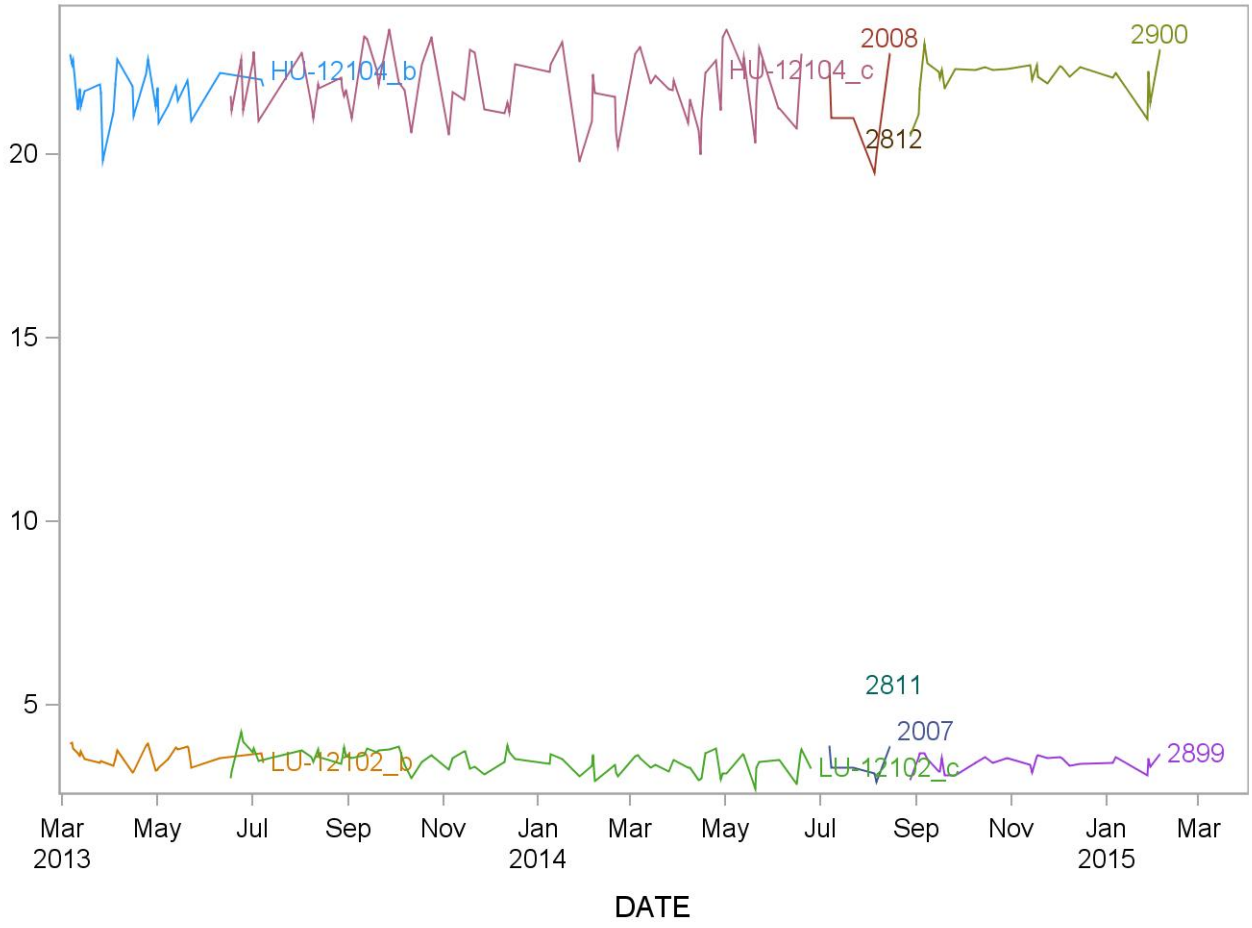
2013-2014 Summary Statistics and QC Chart for Urinary Arsenous acid (µg/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU-12104_b	40	06MAR13	08JUL13	15.886	0.553	3.5
LU-12102_b	40	06MAR13	08JUL13	9.428	0.466	4.9
HU-12104_c	106	17JUN13	19JUN14	15.903	0.756	4.8
LU-12102_c	107	17JUN13	25JUN14	9.410	0.523	5.6
2008	8	07JUL14	15AUG14	15.625	0.582	3.7
2007	8	07JUL14	15AUG14	8.969	0.309	3.4
2812	2	18AUG14	18AUG14	15.500	0.000	0.0
2811	2	18AUG14	18AUG14	9.400	0.141	1.5
2900	41	28AUG14	04FEB15	15.598	0.720	4.6
2899	41	28AUG14	04FEB15	9.167	0.464	5.1



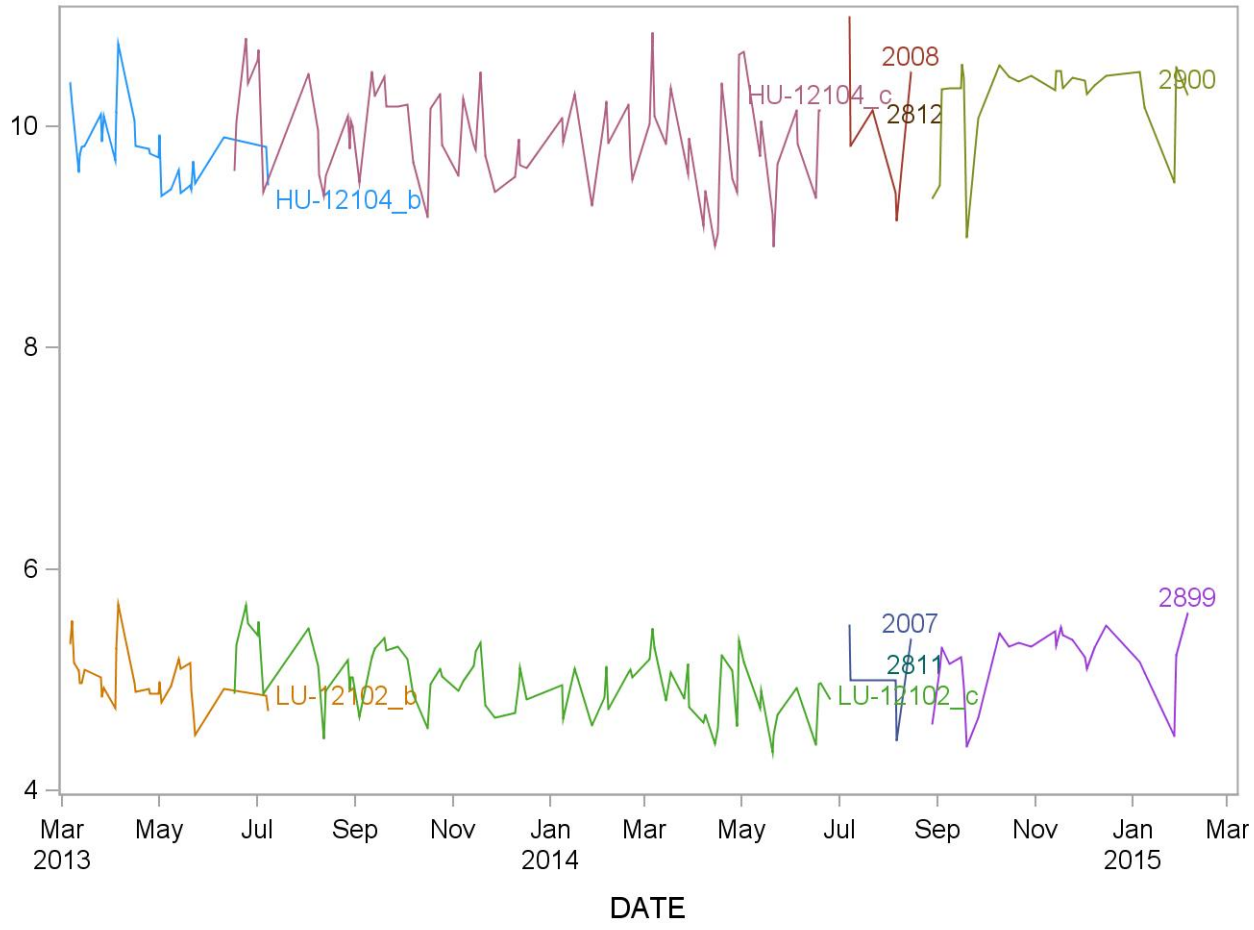
2013-2014 Summary Statistics and QC Chart for Urinary Dimethylarsonic acid (µg/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU-12104_b	40	06MAR13	08JUL13	21.731	0.688	3.2
LU-12102_b	40	06MAR13	08JUL13	3.583	0.243	6.8
HU-12104_c	107	17JUN13	19JUN14	21.875	0.907	4.1
LU-12102_c	108	17JUN13	25JUN14	3.445	0.298	8.7
2008	8	07JUL14	15AUG14	21.313	1.308	6.1
2007	8	07JUL14	15AUG14	3.450	0.394	11.4
2812	2	18AUG14	18AUG14	20.000	0.000	0.0
2811	2	18AUG14	18AUG14	5.125	0.177	3.4
2900	41	28AUG14	04FEB15	22.121	0.480	2.2
2899	41	28AUG14	04FEB15	3.448	0.208	6.0



2013-2014 Summary Statistics and QC Chart for Urinary Monomethylacrsonic acid (µg/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HU-12104_b	40	06MAR13	08JUL13	9.826	0.343	3.5
LU-12102_b	40	06MAR13	08JUL13	5.013	0.262	5.2
HU-12104_c	106	17JUN13	19JUN14	9.901	0.461	4.7
LU-12102_c	107	17JUN13	25JUN14	4.972	0.290	5.8
2008	8	07JUL14	15AUG14	10.044	0.677	6.7
2007	8	07JUL14	15AUG14	5.088	0.376	7.4
2812	2	18AUG14	18AUG14	9.975	0.035	0.4
2811	2	18AUG14	18AUG14	5.000	0.071	1.4
2900	41	28AUG14	04FEB15	10.238	0.438	4.3
2899	41	28AUG14	04FEB15	5.181	0.328	6.3



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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.

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

Analyte	Target Mean and 2SD Range	25°C (decreased)	35°C (per method)	45°C (increased)
AC	7.59 6.83 – 8.35	7.06 6.83 – 7.30	7.44 7.06 - 7.81	6.87 6.42 – 7.33
AB	24.07 21.79 – 26.35	24.7 23.68 – 25.72	24.01 23.24 – 24.78	25.2 23.43 - 26.97
TMAO	32.25 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

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

Test Details:

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

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

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

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

Test Details:

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

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

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

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

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

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

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

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

Test Details:

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

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

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