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Critical Considerations for Data Quality in Elemental Speciation

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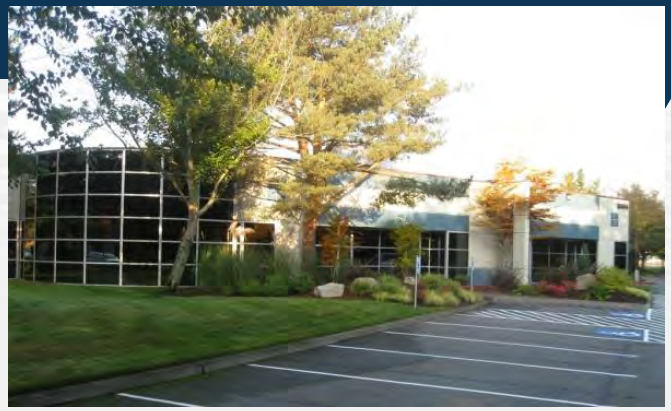
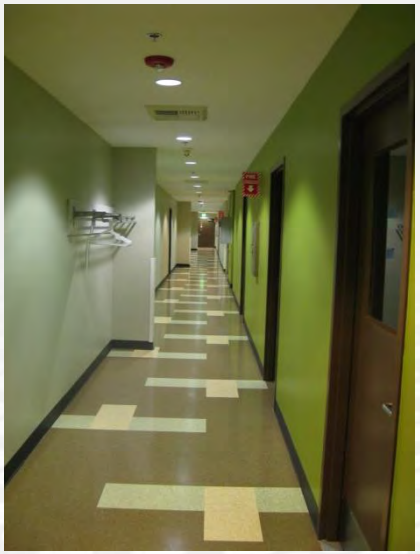
Who is Brooks Applied Labs?



- Brooks Rand Labs and Applied Speciation and Consulting merged on April 1, 2015 creating Brooks Applied Labs
- Provide routine analyses for compliance purposes (NELAC, CLIA Certified, GMP, FDA Compliant)
- Routinely perform research to understand limitations of compliance methods and rectify them
- Routinely perform internal and contract research to better understand the chemistry in the presented sample, processes, and risk assessment
- Routine speciation analyses of: Cr, As, Se, Hg, Mn, Tl, Sb, V, Cu, Co, Pb, Fe, Cl, Br, I, Zn
- Applied scientific principles for generation of robust quality systems



Interest in Elemental Speciation Analysis

- Speciation analysis is the analytical activity of identifying and/or measuring the quantities of one or more individual chemical species in a sample.
  - The chemical species are specific forms of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure.
- Different forms of an element can have totally different properties.
  - Essential for predicting and modeling fate, risk, and effects, Critical for toxicology, bioavailability, and bioaccumulation.
  - In fact, speciation of an element can even impact total elemental analysis.
- Speciation Analysis Can Answer Tough Questions
    - Do I have hexavalent chromium in my drinking water?
    - Why doesn't my treatment work?
    - Is there inorganic arsenic or methylmercury in my diet (fish, milk, supplements, etc)?

Interest in Elemental Speciation Analysis

- More scientists are interested in speciation analysis
  - Over 400 papers* between 2000-2003 on arsenic speciation only! Information overload?
- There are only a few commercial laboratories performing routine speciation analysis
- Promulgated methods for speciation analyses are limited resulting in a haphazard approach to quality
- Many commercial laboratories will blindly apply methods without a comprehensive understanding of the chemistry. A major problem is surfacing as speciation analyses are more widely requested. Which laboratory results can you believe?

Experience in Speciation Analysis

gained knowledge through direct observation or participation

- Many types of samples processed for speciation analysis in our lab

- Algae, kelp, etc
- Cosmetics
- Milk (cow, soymilk, rice milk etc)
- Human organs (brain, kidney, stomach contents, etc), semen
- Blood, urine (human, rats, etc)
- Wastes (landfill, sludges, etc)
- Soils, sediments
- Various types of fish
- Fish eggs, fish meal
- Mussels, shellfish, clams
- Nutraceuticals
- Pharmaceuticals (APIs, excipients)
- Water (almost all water sources)
- Rice and rice products
- Wine, wine cooler, beer, juices, etc
- Cheese, cheese brines
- Yeast

"Current" EPA Methods for Speciation Analysis

- Method 1632: Arsenic Speciation by Hydride Generation Quartz Furnace Atomic Absorption Spectrometry (Based on a paper by M.O. Andreae (1977))
- Method 1630: Methyl Mercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and CVAFS (Based on Nicolas Bloom et al (1988))
- Method 7199: Determination of hexavalent chromium in drinking water, groundwater, and industrial wastewater effluents by ion chromatography. (Based on Arar et al. (1991))
- Excellent methods but they utilize reaction-based analytical techniques
  - Reaction based methods are more prone to interferences
- Data Quality issues due to QA/QC holes
- Almost all new methods in the literature use better instrumentation such as ICP-MS
  - More sensitive and selective (no need for preconcentration and or reaction chemistry)
  - Allows for species specific isotope dilution analysis (SIDMS)
  - Allows for monitoring non-target species and species transformations

Quality Control Criteria for 1632a

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

	IPR (Section 9.2)		OPR	Calibration Verification	MS/MSD (Section 9.3)	
Analyte[2]	s	X	(Section 9.7)	(Section 9.5)	%R	RPD
IA	< 25%	60-140%	50-150%	80-120%	50-150%	< 35%
As^{+3}	< 25%	40-160%	30-170%	70-130%	30-170%	< 35%
MMA	< 20%	70-130%	60-140%	80-120%	60-140%	< 25%
DMA	< 30%	50-150%	40-160%	70-130%	40-160%	< 40%

[1] Acceptance criteria based on quality control data generated during As speciation analysis for the Cook Inlet Study (1998). Details can be found in Reference 16.16.

2 IA - Inorganic arsenic (As^{+3} + As^{+5}); MMA - monomethylarsonic acid; DMA - dimethylarsinic acid.

As Speciation in Tissues Extraction Methods from Literature

- 0.83% TMAOH
- 2M HCl (EPA Method 1632)
- Water
- Water:Methanol
- TFA
- Phosphoric acid
- Enzymes
- Shaking/mixing
- Sonication
- MW-assisted
- Heating
- Sub/supercritical fluid
- ASE
- Soxhlet

What is expected?

- 100% recovery of all arsenic species in ANY matrix without ANY species interconversion
- Recovery is relative to the “total” arsenic concentration
- In our experience, there are no methods that work on every sample matrix for every possible molecular form.
- Our goal is to extract as much As species as possible without any species interconversion and to support as many species as possible

Best Extraction Method?

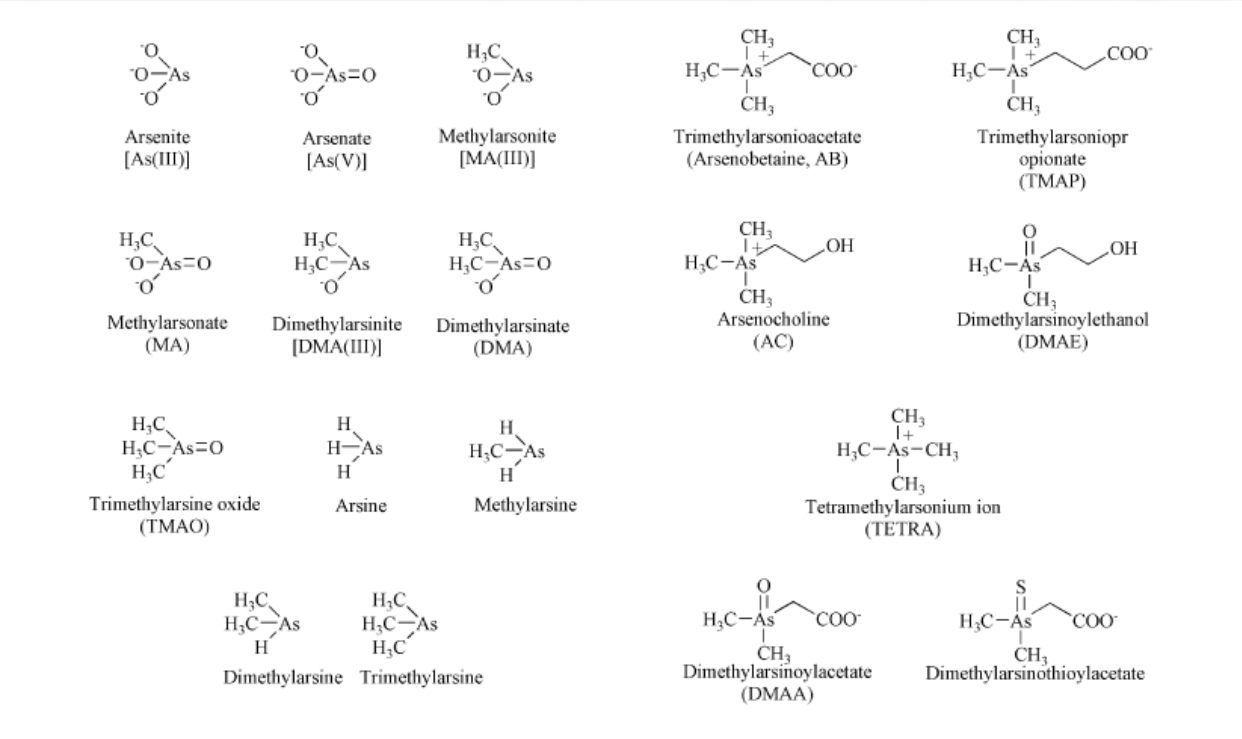
- Depends on the matrix but there are no guarantees
- Even for similar matrices different extraction methods can work significantly better
- Can we come up with a single extraction method for regulatory purposes?
    - Good luck…
- Sequential extraction?
    - Could be expensive!

Best Extraction Method?

- Depends on the matrix and target species
  - Small ionic molecules can be extracted using specific pH and ionic extract solutions
  - Small non-ionic molecules require organic solvent extraction
  - Large molecules require a more delicate extraction to mitigate molecular degradation
  - Derivatization during extraction? Depends on species and whether process induces conversion. Certain chelating agents, such as EDTA, are also reducing agents.
  - Each group of species (e.g. ionic, non-ionic, large molecule) often requires different analytical approaches

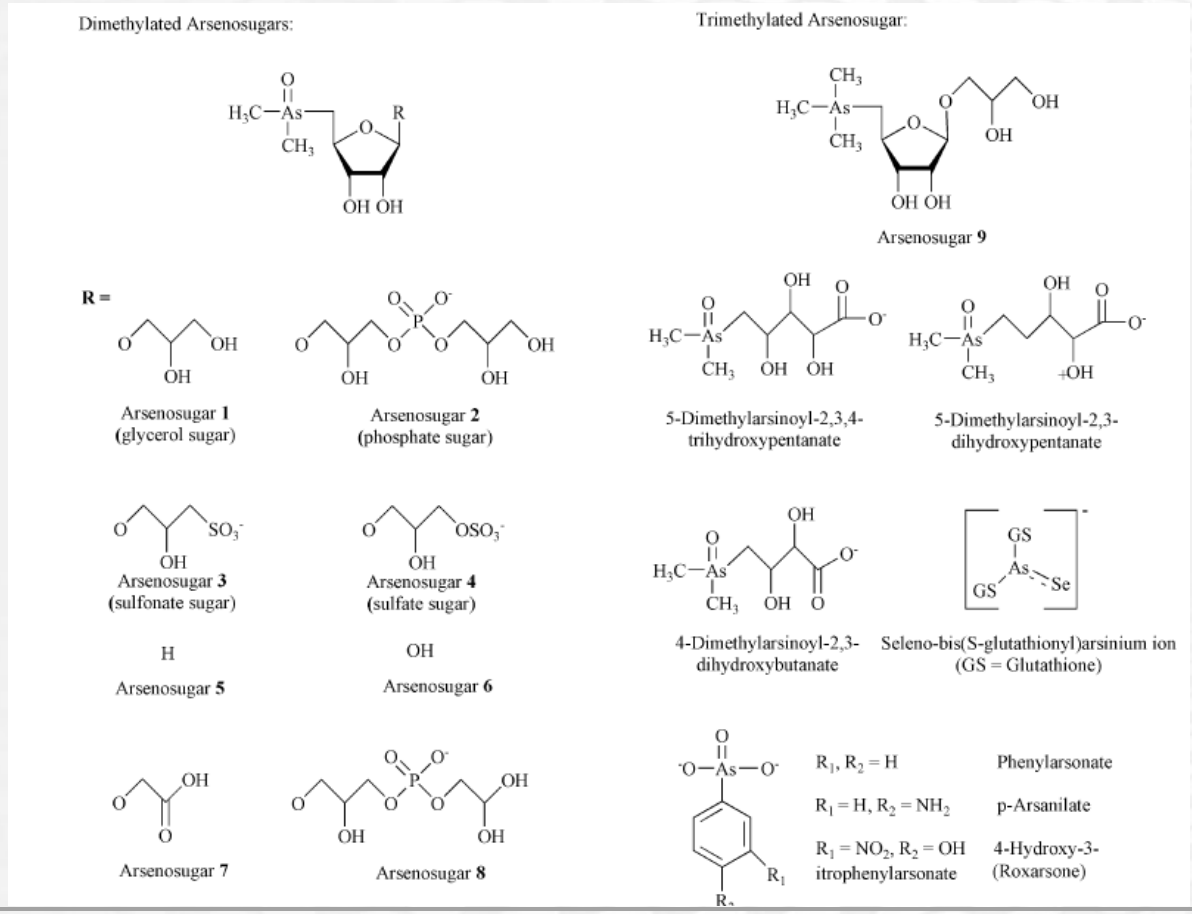
Inorganic vs. Organic Arsenic

- Organoarsenic species are defined as As bound to at least one C atom



Inorganic vs. Organic Arsenic

Readily degrades to DMAs



Species Interconversion

- EPA Method 1632 uses 2M HCl in a closed vessel extraction @ 80oC for 16hrs
  - Method 1632 has been commonly used to determine "inorganic" arsenic in fish tissue
- In our experience, we extract more Inorganic Arsenic with this method than any other method
- Question: Are we extracting more or are we breaking up proteins and possibly As-C bonds ?
- We need to incorporate QA/QC protocols to identify if this happens or not…

CRMs

- A CRM should be run with every batch of samples but we need better CRMs.
    - NRC and IRMM has various RMs for speciation
    - NIST is working on it
- 100% extraction efficiency for CRMs does not mean 100% recovery of all As species in real samples.
- CRM's are usually highly processed (freeze dried, well homogenized)
- Looking for collaborations to see the effects of freeze-drying process (contamination, oxidation and extraction efficiency)
    - Couldn't find any literature data.

QA/QC

- LCS using every standard available
  - Effect of extraction on species stability (A good method should not cause oxidation/reduction/degredation, etc)
- MS/MSD using every standard available
  - Effect of extraction + matrix on species stability (reduction of species, creation of new species)
- AS/ASD using every standard available
  - Effect of extraction + matrix on chromatography (co-elution, misidentified peaks)
- Compound independent calibration is possible.
  - Allows accurate quantification of unknown species without any standards
  - The RPD between the slopes of each species should be less than 5% (If not, suspect impurities, signal depression/enhancement)

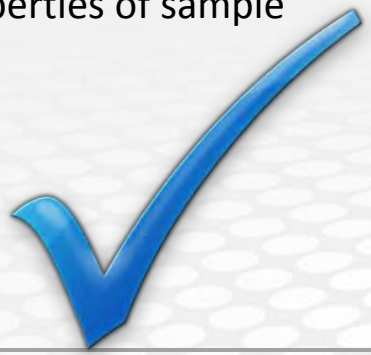
Extraction of As Species from Tissues using Proposed EPA 6870 Method

TMAH Extraction (mg/Kg)	TV	As(V) Found	% Rec
STD 01-18-06 As in Oil	100	69.340	69.3
Triphenylarsine (01-22-08)	12.3	0.021	0.17
Triphenylarsine Oxide (01-22-07)	6.15	0.017	0.28

After HAc Neutralization (mg/Kg)	TV	As(V) Found	% Rec
STD 01-18-06 As in Oil	100	118.672	118.7
Triphenylarsine (01-22-08)	11.6	0.511	4.41
Triphenylarsine Oxide (01-22-07)	5.80	0.201	3.47

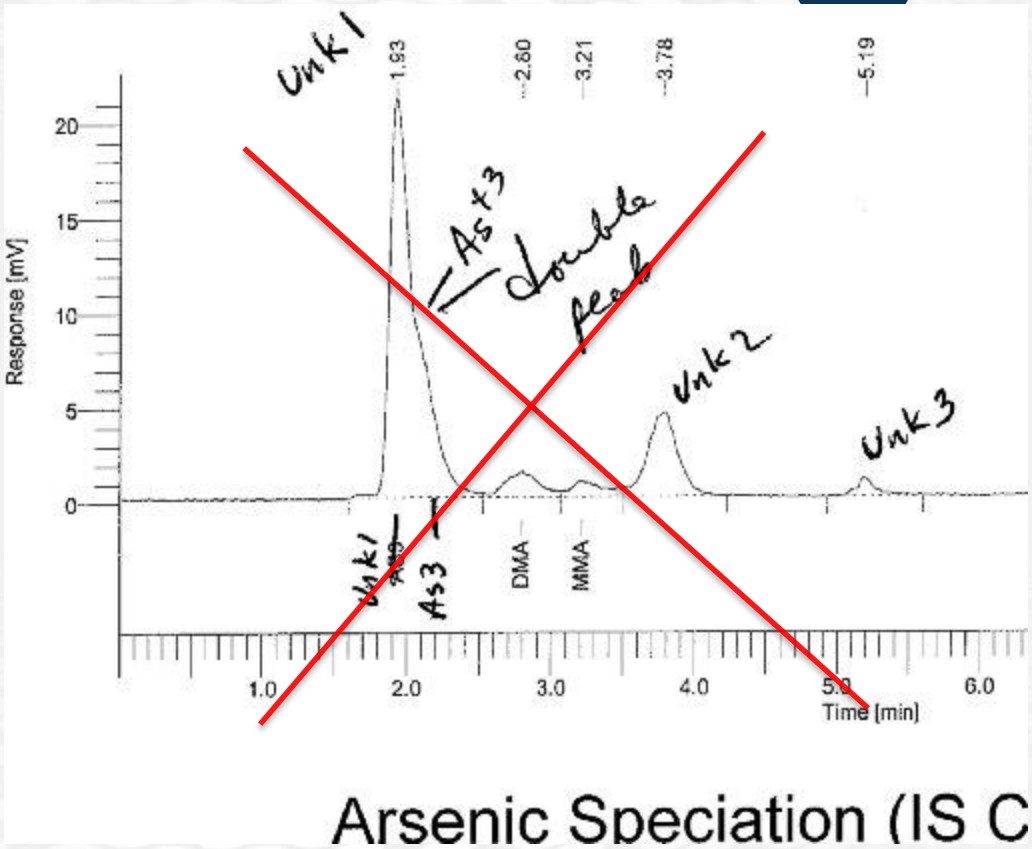
QA/QC

- Correlation of sum of species with total analyte in the sample and in the extract is very valuable.
    - Extraction efficiency and chromatography efficiency
- Failed Spikes/low recoveries can tell us something
    - Low As(III) recoveries due to lipid content
    - Low As(V) recoveries due to Fe content
    - Oxidation/reduction can usually be monitored by As(III)/As(V) of the spikes
    - Intrinsic chemical properties of sample

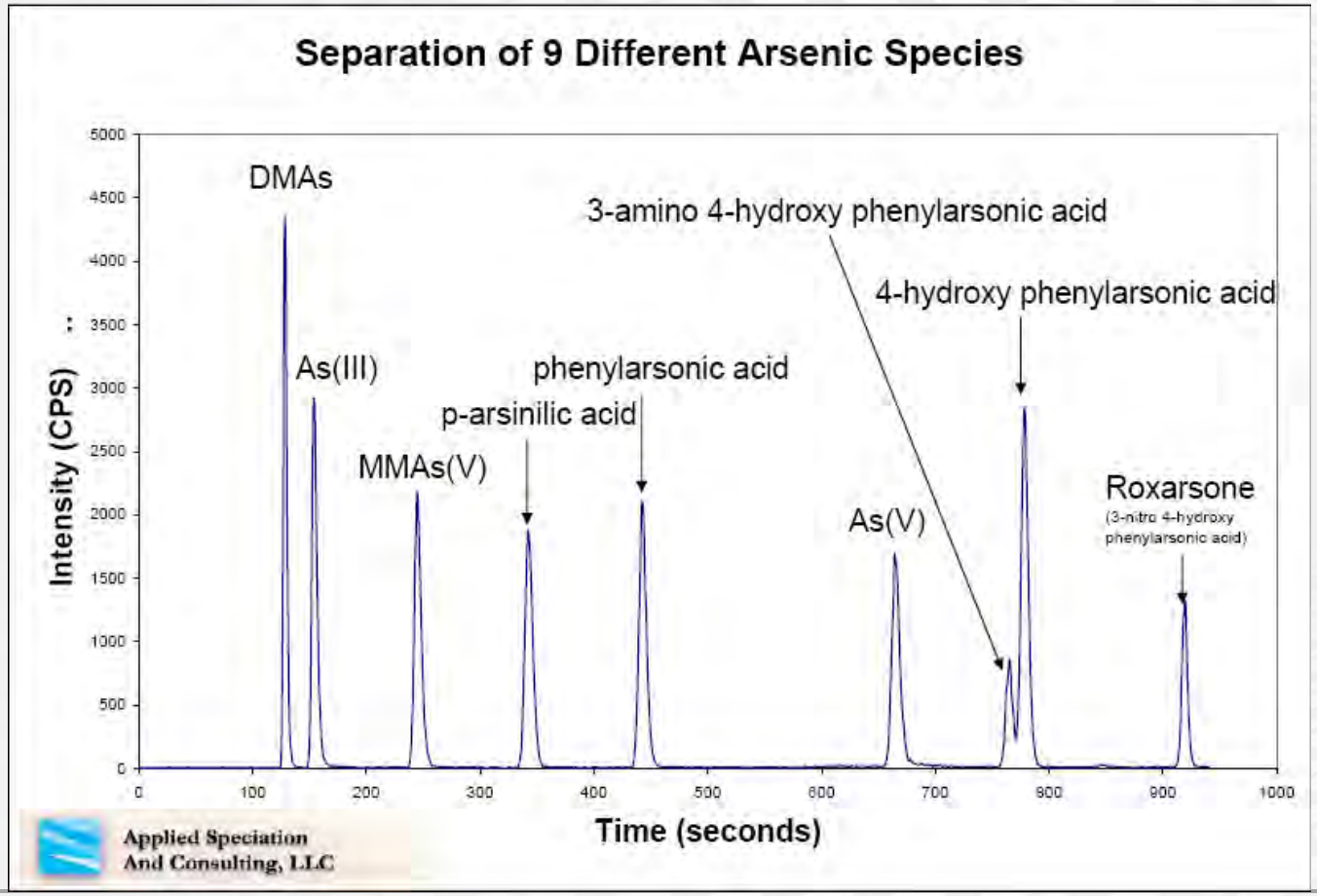


Speciation Methods Gone Wrong!

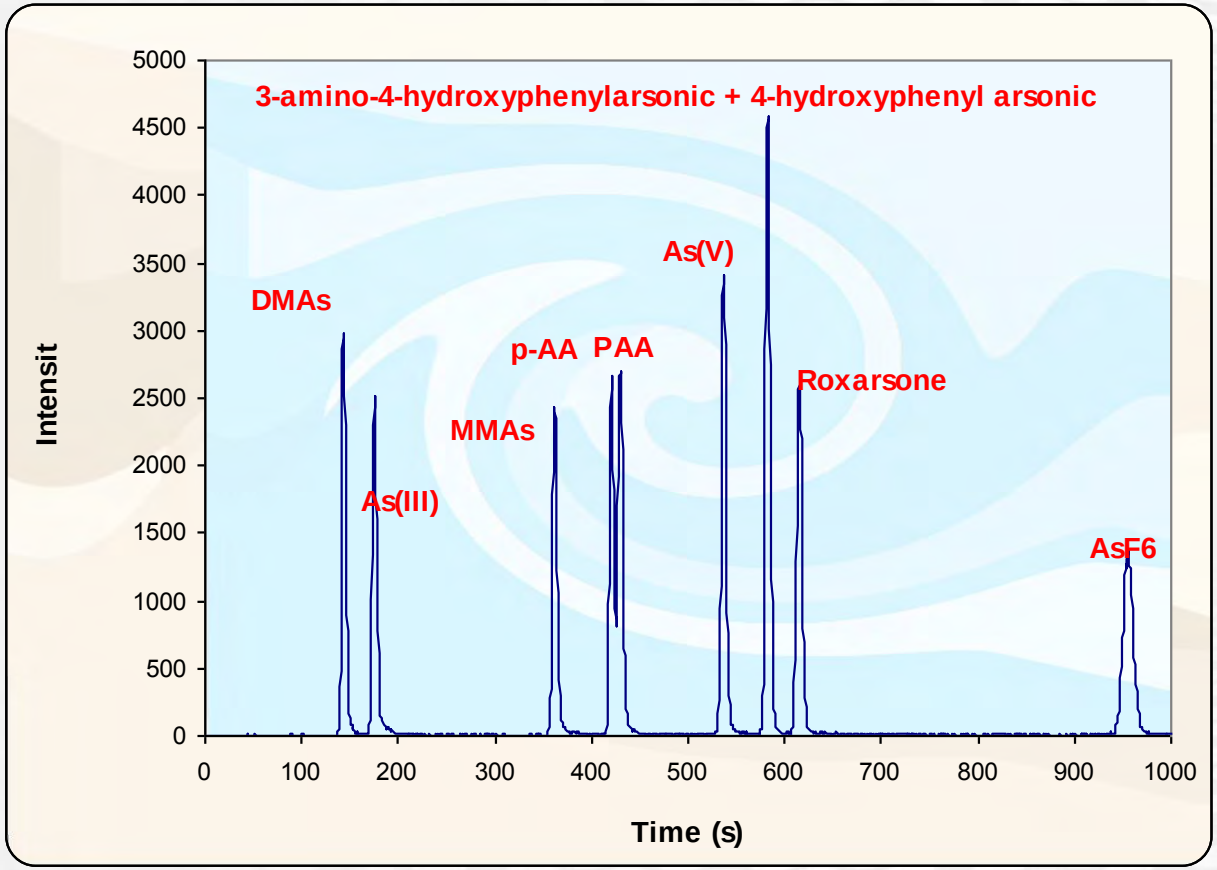
- Target Analyte species should be retained on the column.
- Species that elute in the dead volume can cause false identification/quantification
- Extra attention to tailing/shouldering peaks (especially on Inorganic As species).



Separation of Different As Species

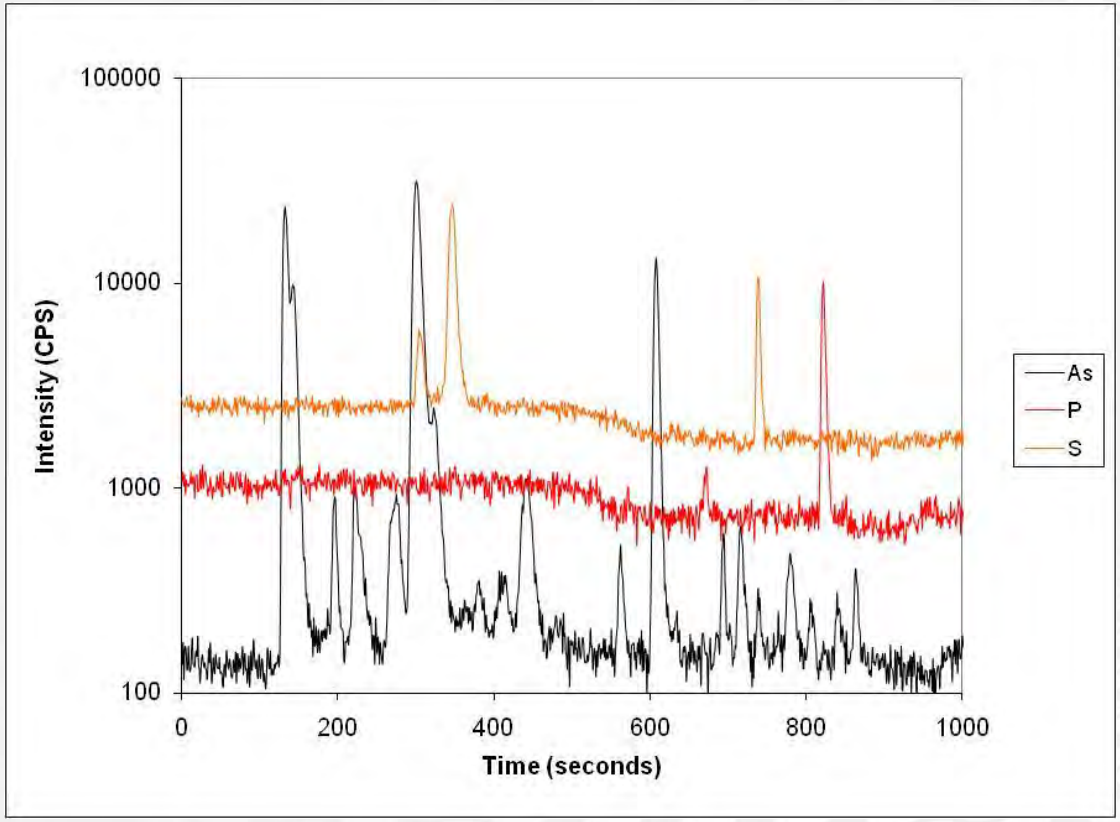


Separation of Different As Species

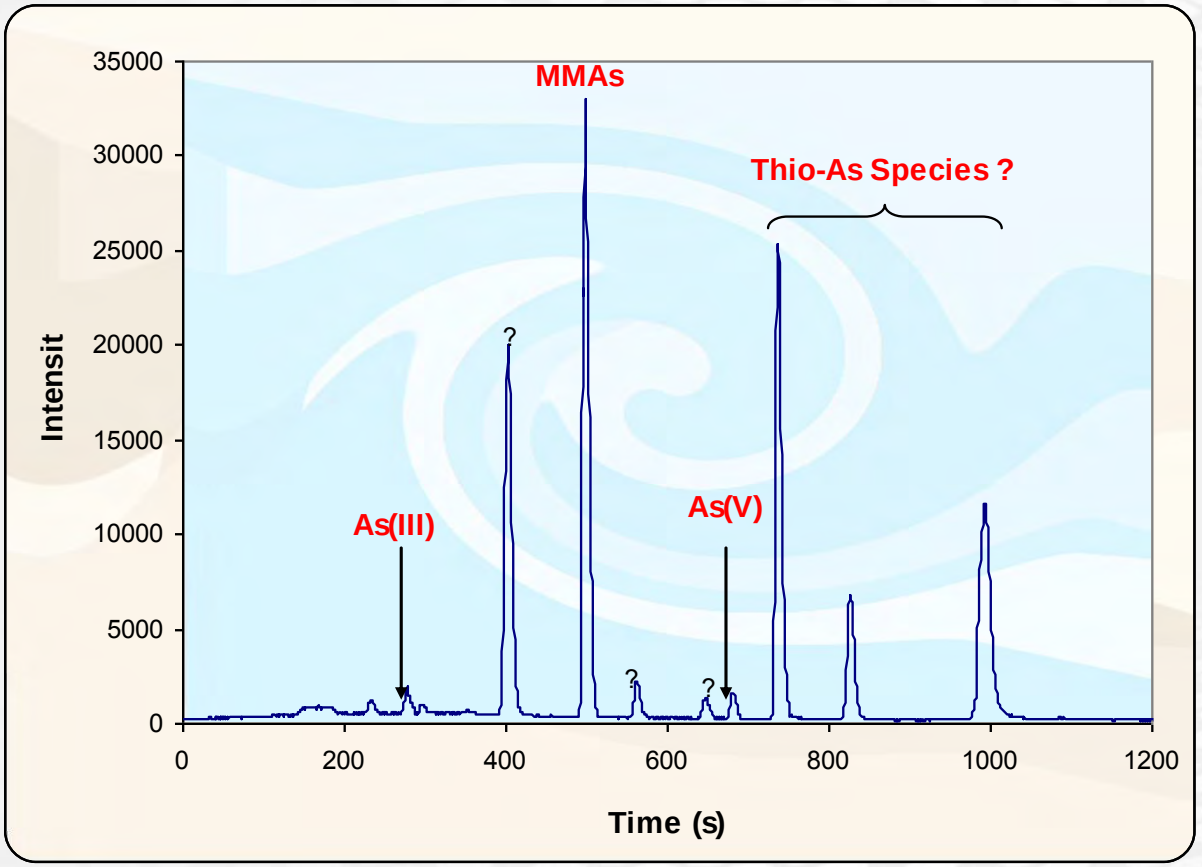


Why do we need this separation power?

Algae Extract



Wastewater Sample (500X dilution)

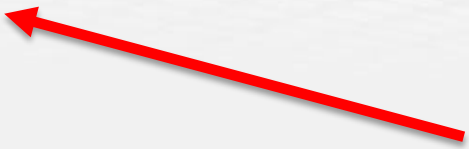


Preservation of Arsenic Speciation

- Even the most sophisticated analytical methods for speciation are useless if it cannot be assured that the species distribution in the sample remains unchanged between collection and analysis.
- Speciation in the field = No need for preservatives
- Temperature, pH, light, dissolved oxygen, container material, microbiological activity, or other water constituents, have previously been identified as potentially-detrimental to the stability of As species in waters.
- Is there a universal preservative ?

Preservation of Arsenic Speciation

- Hydrochloric acid
- Complexation
- Flash freezing (cryo freezing)
- Anoxic sample containers



Stability and Preservation of Se Species

- Apart from filtration, no preservatives should be used.
  - The stability of different Se species is not well understood and changes in pH may cause species interconversion
- Cryofreezing in the field may work well for some samples
- Selenite, selenate, SeCN in filtered samples sent over blue ice and kept in the refrigerator were found to be stable 21 days
  - MSe(IV) and SeMet were not stable

<u>HNO_3 should NEVER be used!!</u>

Field Spikes

- BAL also utilizes field spikes to confirm preservation of species information.
- A stock solution of target analyte is added to specific samples
- Field spike samples are analyzed to determine if any oxidation or co-precipitation reactions occur during sampling and shipping. Interpretation can be tricky!

Check the purities of your standards

- Almost all standards gravimetrically
- The total concentration of the analyte may be correct but speciation may not…
- A lot more often than people think…
  - Received a 1000ppm MeHg std that contained 300ppm Hg(II)
  - Selenite std that contained selenate

Certification Process for Speciation Standards

APPLIED SPECIATION AND CONSULTING, LLC

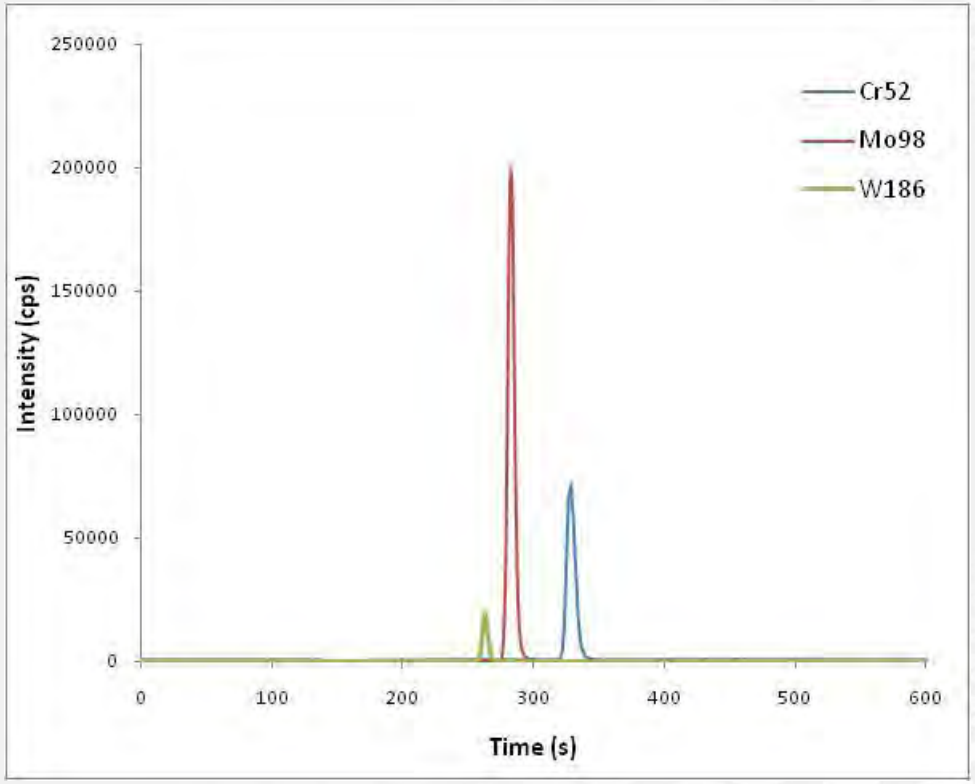
Certificate of Analysis

Standard Name:	**Dimethylselenide (DMSe) Calibration**
Date Prepared:	5/14/2009
Standard ID:	STD-01-16-12
Analyte (Unit):	DMSe (ug/L as Se)
Analytical SOP:	N/A
Analytical Description:	N/A
Preparation SOP:	N/A
Preparation Description:	N/A
Matrix:	Acetonitrile (CH_3CN)
Date:	08/11/09
Dataset ID::	See below

Analysis	Date Digested	Dataset ID	Dataset Sample ID	Concentration*
1)	5/21/2009	ICPMS-090514-TM-Se	1100 DmSe	327.4
2)	5/23/2009	ICPMS-090525-TM-DMSe	DMSe cal	334.0
3)	5/23/2009	ICPMS-090525-TM-DMSe	DMSe cal D	318.2
4)	5/23/2009	ICPMS-090525-TM-DMSe	DMSe cal T	325.5
5)	5/5/2009	ICPMS-090610-TM-SeOx	DMSe Cal	326.0
6)	5/5/2009	ICPMS-090610-TM-SeOx	DMSe Cal MD	324.9
7)	5/5/2009	ICPMS-090610-TM-SeOx	DMSe Cal MT	329.1
			Mean =	327.1
			Standard Deviation =	7.1
			Relative Standard Deviation (%) =	2.2

Certified Value: 327.1 mg/L as Se

Internal Standards?



Molybdate and Tungstate as Internal Standards for Cr(VI)

Isotopically enriched Se speciation standards used for As

Best Internal Standard is the enriched $^{53}Cr(VI)$ but they have $^{52}Cr(VI)$ impurities

Conclusions

- IC-ICP-MS has been applied for As, Se, Co, and Hg speciation in many different matrices successfully at BAL
  - BAL does not have a universal method for any speciation analysis
  - Different methods for different matrices is necessary
  - ASC-SOP 015.1 “Method Development and Validation”
- Conventional ICP-MS instruments can produce false positives
  - Use of reaction cell instruments are highly recommended!

Primary QC Concerns for ALL Elemental Speciation Methods

- From start to finish…
  - Sample collection
  - Sample Storage
  - Sample preparation (extraction, derivatization)
  - Sample Analysis
  - Quality review of data
  - Does the data make sense?
    - Total Se = 50ug/L ; Sum of species during speciation analysis = 2,500ug/L

Primary QC Concerns for ALL Elemental Speciation Methods

- From start to finish…
  - **Sample collection**:
    - Sample container must be compatible with target and non-target species
    - Preservative must not alter chemical equilibrium of sample
    - Temperature must be taken into consideration
    - Holding time studies are a must during validation process
    - Acids and bases should be taken very critically. Non-target species may precipitate resulting in loss of target species
    - Chemical system MUST be taken into consideration when selecting preservative. Sulfur driven environments interact completely different than standard Pourbaix diagrams
    - Application of field spikes

Primary QC Concerns for ALL Elemental Speciation Methods

- From start to finish…

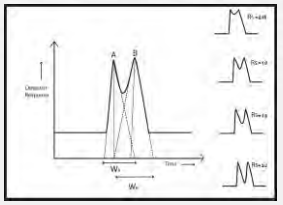
  - **Sample storage**:
    - Short and long term storage must be studied with different preservatives to ascertain optimal conditions
    - Cryofreezing is a great option but is not always appropriate
    - Stabilize non-target species? May be necessary…Fe and Mn

Primary QC Concerns for ALL Elemental Speciation Methods

- From start to finish…
  - **Sample preparation**:
    - Multiple dilutions should be applied to identify if change in ionic strength or DO induces molecular conversion
    - 1st rule of extractions: Thou shall not induce molecular conversion
    - 2nd rule of extractions: See #1
    - Extraction method should be selected in accordance with properties of target molecules (ionic, non-ionic, macro)
    - Holding time for the extracts must be understood
    - Optimal storage conditions of extracts must be studied
    - QC must be driven by chemistry and not writ compliance requirements (sample equilibrium must be taken into consideration)
    - Extract solution concentrations should also be studied to identify prevalence of catalytic reactions

Primary QC Concerns for ALL Elemental Speciation Methods

- From start to finish…



  - **Sample analysis:**
    - Resolution MUST support target and non-target species
    - Dual column validation necessary to confirm co-elution is minimized
    - Internal standards are a must to identify issues with analytical system or bench top preparation
    - Reaction based methods should be minimized if not completely eliminated (failure is not if but when)
    - Volatility of species (partial pressures) must be taken into consideration. Not all molecular forms of elements have the same response factors for ICP-MS!
    - Groups of species with similar partial pressures must yield same response factors in calibration (impurities, impurities, impurities)

Primary QC Concerns for ALL Elemental Speciation Methods

- From start to finish…
  - **QC Review**:
    - Integration for each peak must be reviewed (no shoulders or peak splitting allowed)
    - Do any QC failures follow equilibrium of sample?
    - Is there mass balance?
    - Second source and tertiary source verification is of utmost importance without the availability of CRMs
    - Continuing calibration blanks (CCB) must be focused on
      - Indicator of column health
      - Indicator of precipitate or particle accumulation
      - Indicator of greater bias at low concentrations

Does the data make sense?

Future of Speciation Analysis

- More work is needed for wide spread adaptation of the technique
  - New CRMs (NIST, NRC, IRMM)
  - Better Standards (NIST and commercial)
  - Guidance on acceptable methodology (EPA, FDA, etc)
  - Establishing (better) QA/QC requirements (EPA, FDA, etc)
- More interdisciplinary collaborations are needed
- Experience is very important
  - While setting up an LC-ICP-MS system is very easy, the most important things to consider are:
    - Knowledgeable project managers
    - Experienced analysts who are familiar with both IC/LC and ICP-MS systems
    - Analysts that can interpret and report the data

Concerns

Instrument manufacturers are providing “kits” for speciation to sell more instruments. Methods are rarely validated and the scope of applicability is not defined.

If methods are not available the next best thing from a regulatory standpoint is guidance documents.

Demand for elemental speciation is increasing. The financial aspect of the market will attract more laboratories to offer speciation services.

Haphazard application of science will result in legal issues, reduced desire to use new technologies, and regulatory headaches

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