Critical Considerations for Data Quality in Elemental Speciation Analysis

Hakan Gürleyük, Ph.D.

hakan@appliedspeciation.com

Who is Applied Speciation?

- Started in 2005, doubling in size and revenue almost every year after that
  - 17,000 sq ft state-of-the-art laboratory
  - **4** Elan DRCII, **3** Elan 6000
  - PE Series 200 HPLC systems, Various IC Systems,
- Provide routine analyses for compliance purposes (NELAC, CLIA Certified, 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
- Internal Seminars, Internal Poster competitions
- Strong dedication to client satisfaction and data quality

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)?
- 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

*Analyst, 2004, **129**, 373–395

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)
  - Dyes and paints
  - 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)

Quality Control Criteria for 1632a

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

Analyte[2]	IPR (Section 9.2) s	IPR (Section 9.2) X	OPR (Section 9.7)	Calibration Verification (Section 9.5)	MS/MSD (Section 9.3) %R	MS/MSD (Section 9.3) 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?

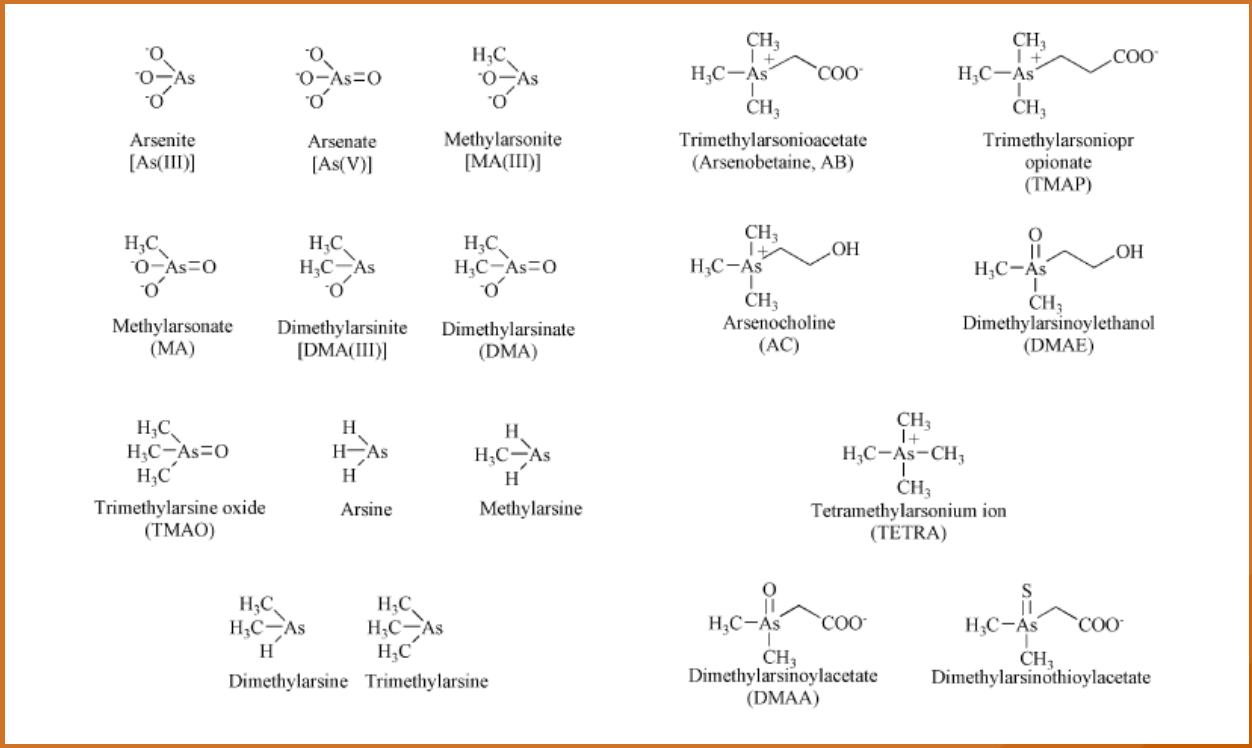
- 100% recovery of all arsenic species in ANY matrix without ANY species interconversion
- In our experience, there are no methods that work on every sample matrix.
- Our goal is to extract as much As species as possible without any species interconversion

Best Extraction Method?

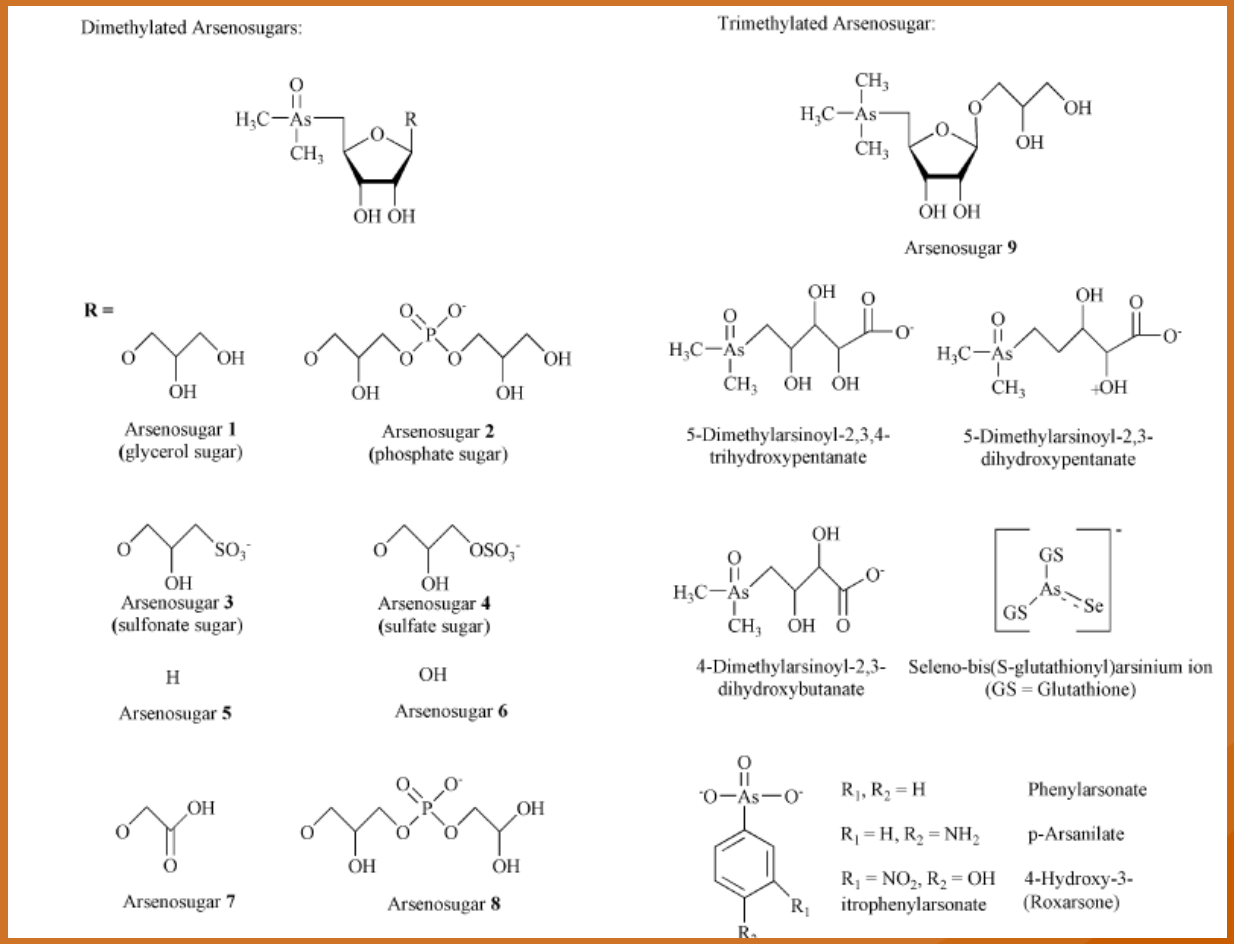
- Depends on the matrix but there are no guarantees
- Even for similar matrices different extraction methods can work significantly better
- Can we come up with a single extraction method for regulatory purposes?
  - Good luck…
- Sequential extraction?
  - Could be expensive!

Inorganic vs. Organic Arsenic

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



Inorganic vs. Organic Arsenic



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

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

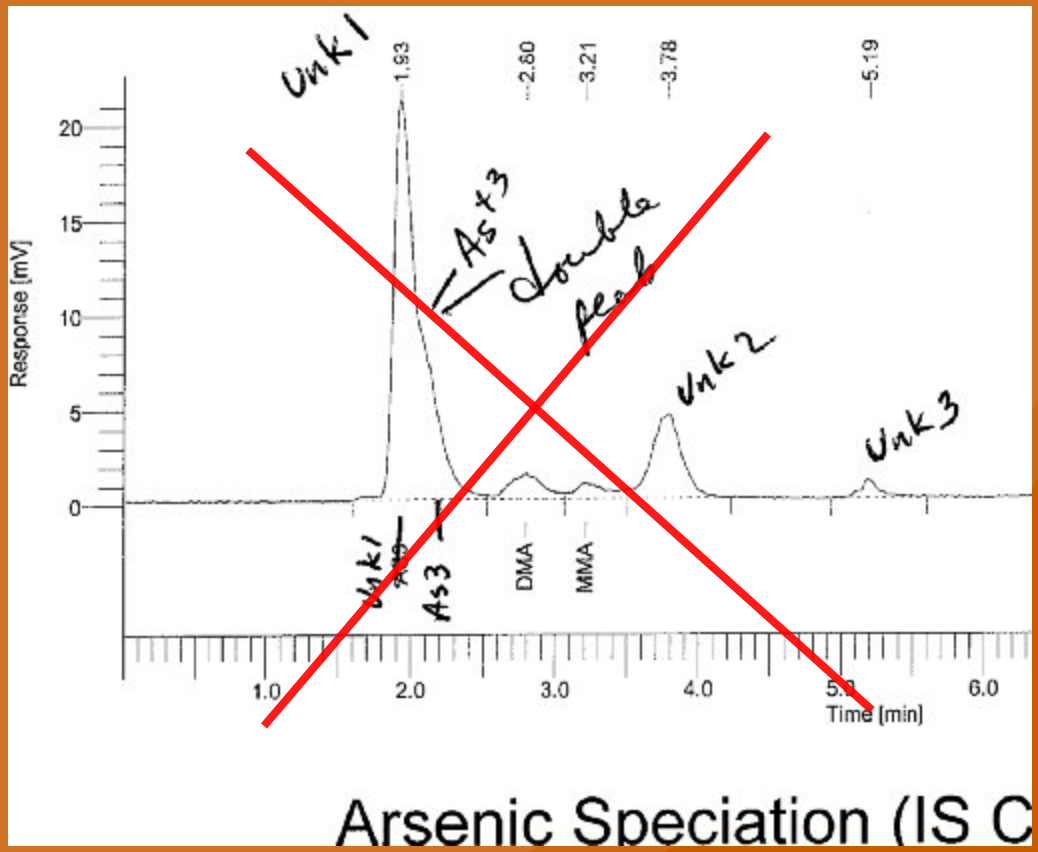
After HAc Neutralization (mg/Kg)	TV	As(V) Found	% Rec
STD 01-18-06 As in Oil	100	118.672	118.7
Triphenylarsine (01-22-08)	11.6	0.511	4.41
Triphenylarsine Oxide (01-22-07)	5.80	0.201	3.47

QA/QC

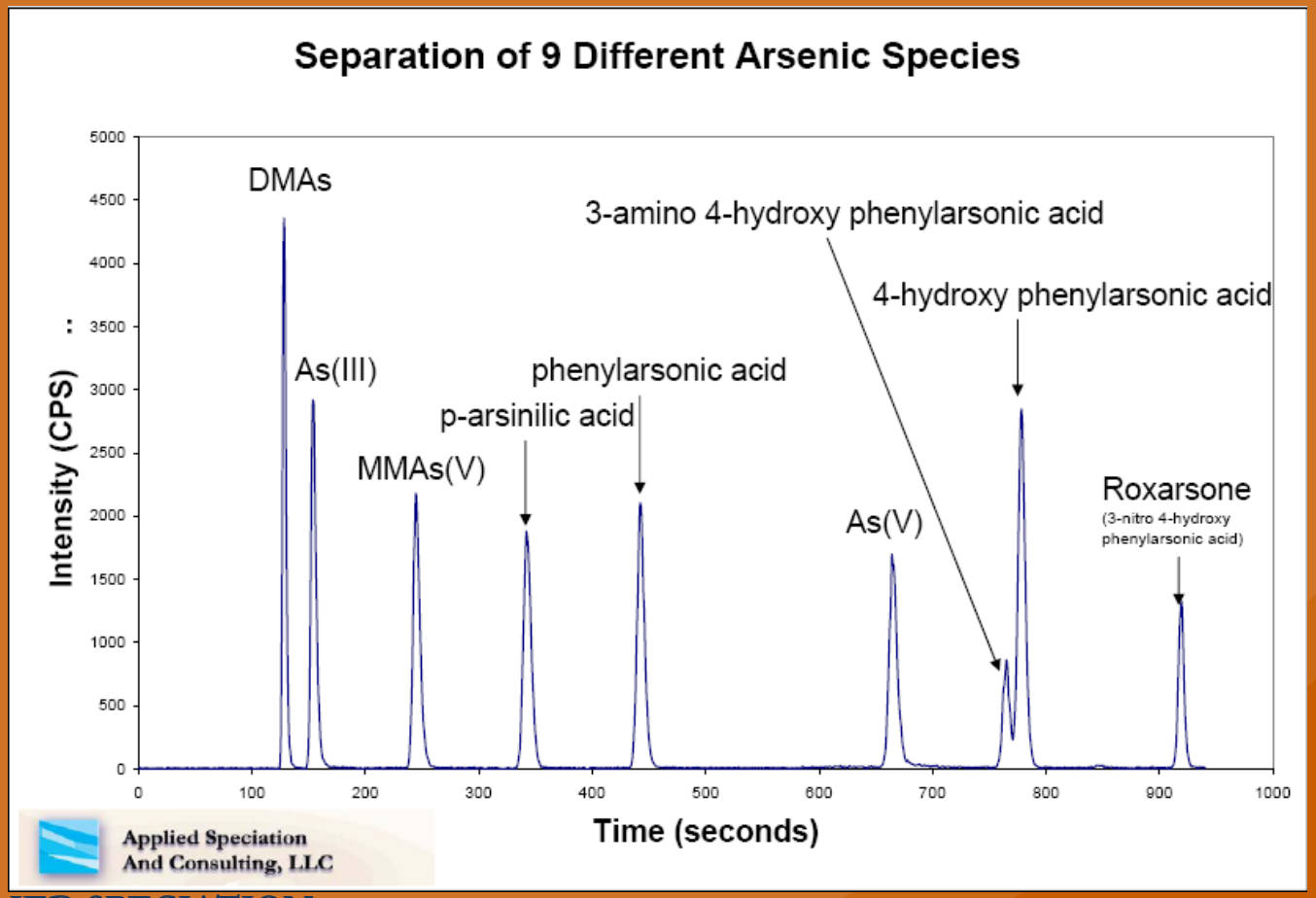
- Correlation of sum of species with total analyte in the sample and in the extract is very valuable.
  - Extraction efficiency and chromatography efficiency
- Failed Spikes/low recoveries can tell us something
  - Low As(III) recoveries due to lipid content
  - Low As(V) recoveries due to Fe content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

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



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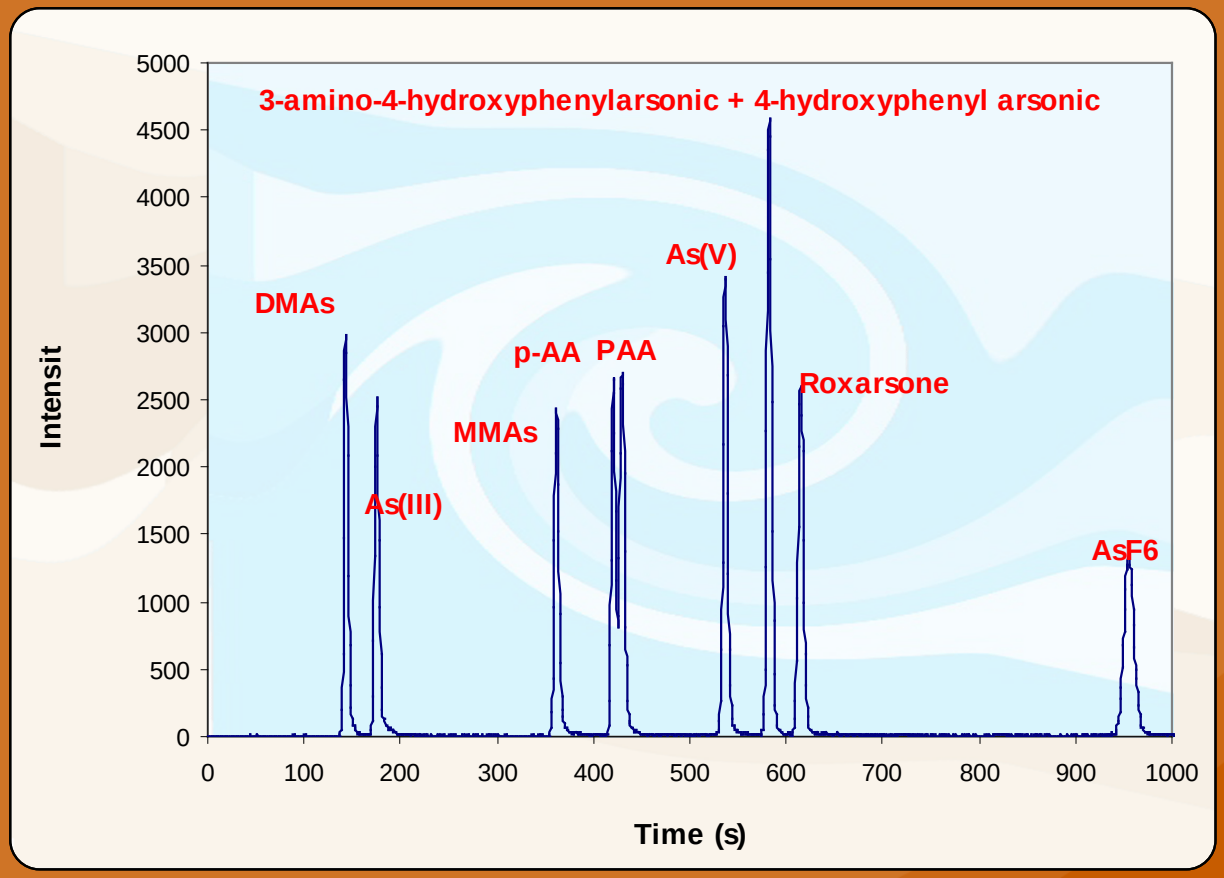
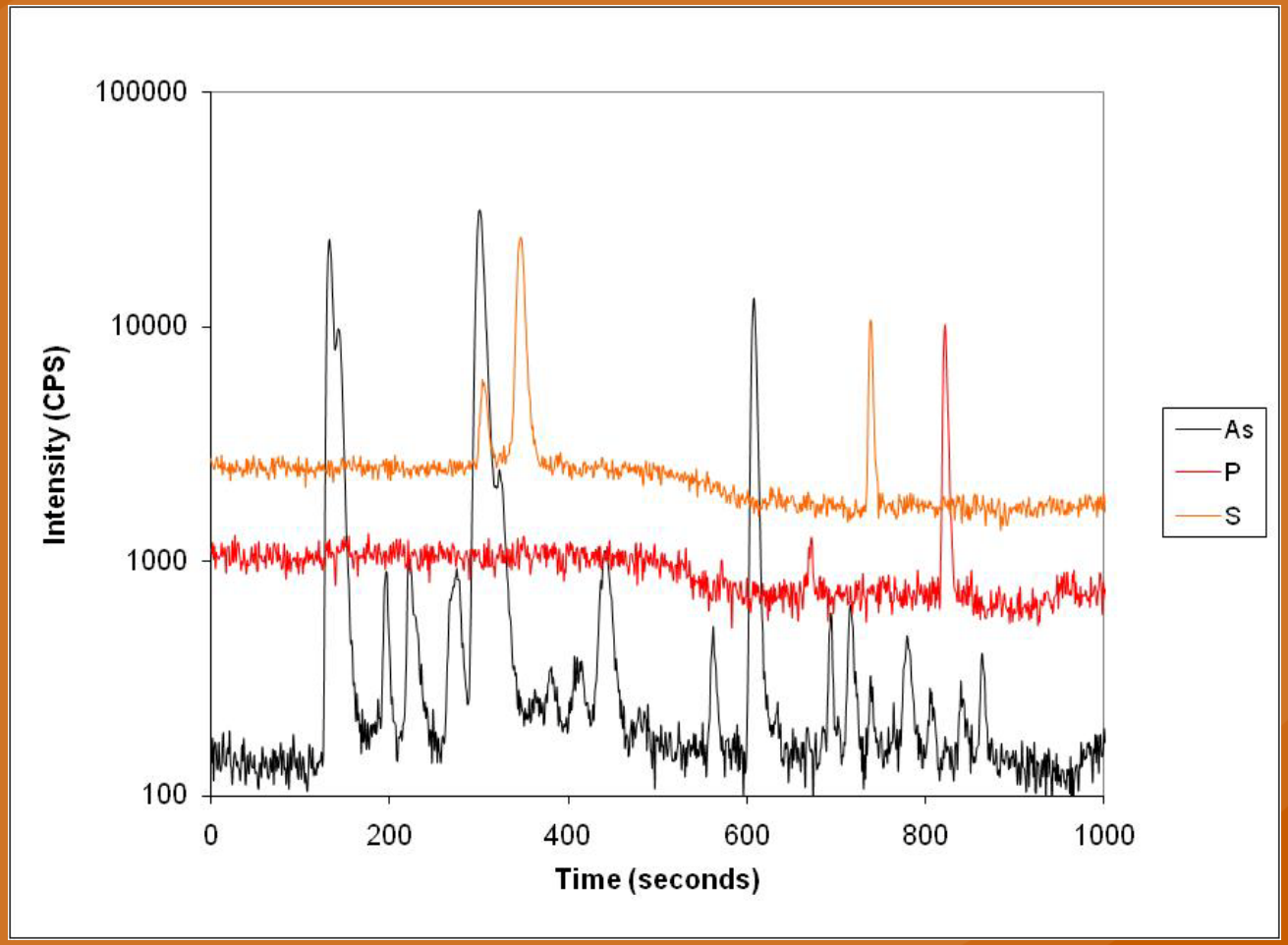
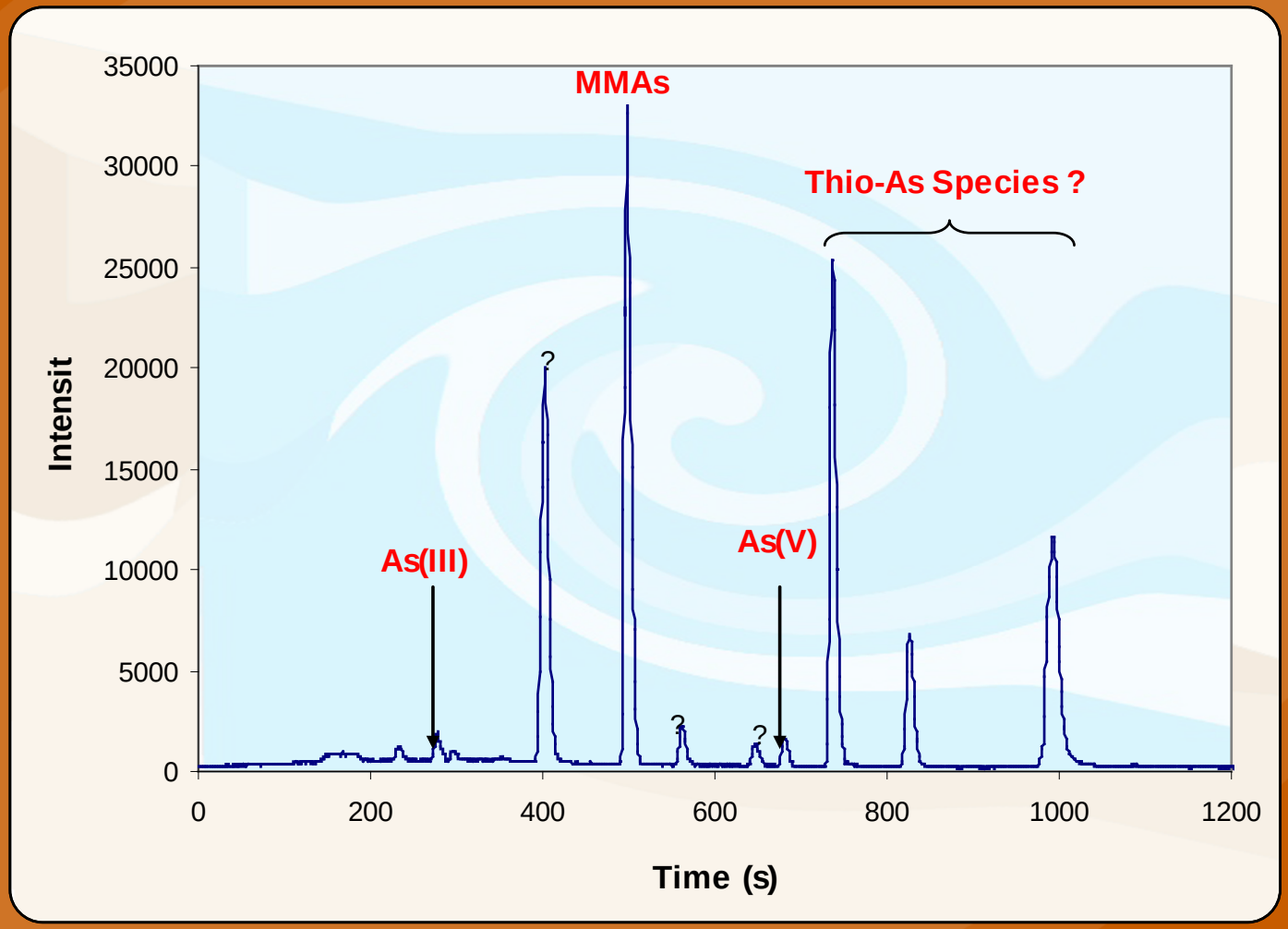


Why do we need this separation power?

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)

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

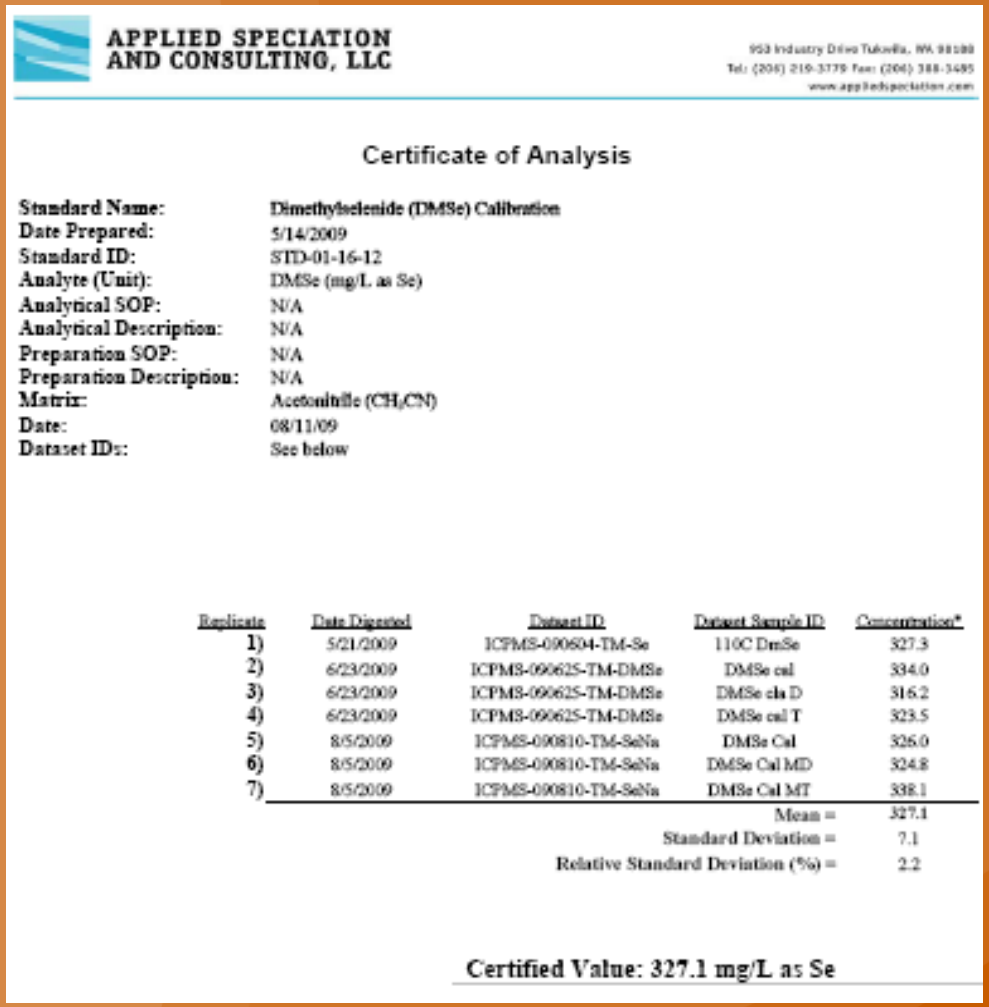
Field Spikes

- Applied Speciation also utilizes field spikes to confirm preservation of species information.
- A stock solution of target analyte is added to specific samples
- These samples are analyzed to determine if any oxidation or co-precipitation reactions occur during sampling and shipping

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

953 Industry Drive Tukwila, WA 98188
Tel: (206) 219-3778 Fax: (206) 388-3485
www.appliedspeciation.com

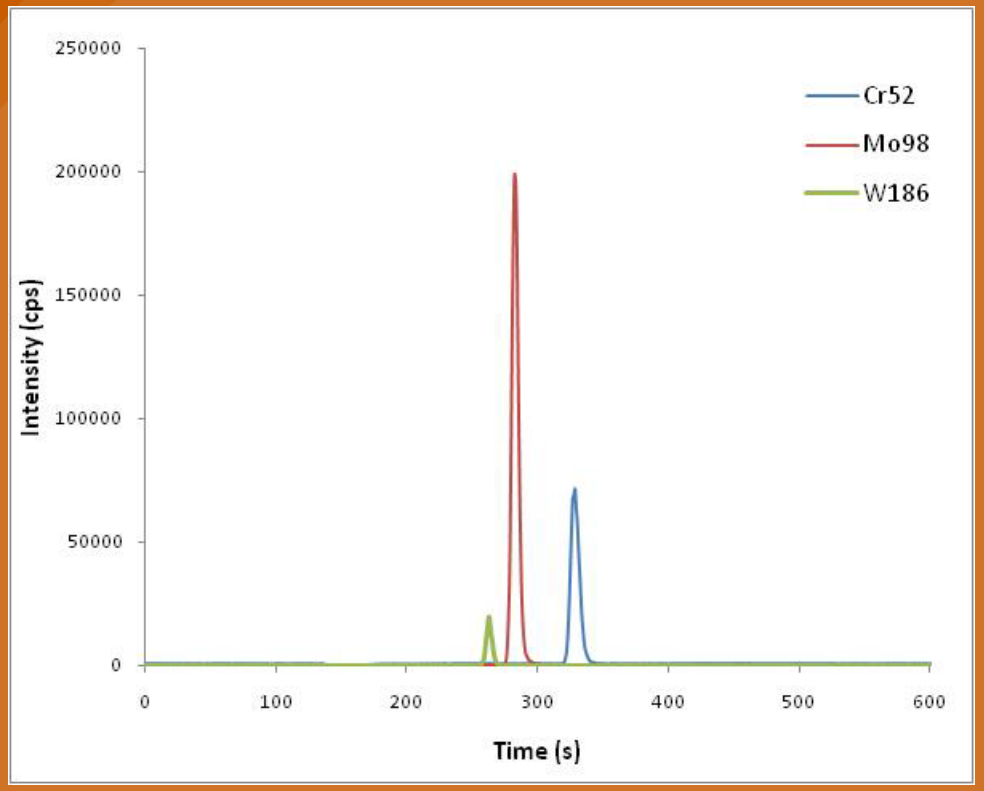
Certificate of Analysis

Standard Name:	**Dimethylselenide (DMSe) Calibration**
Date Prepared:	5/14/2009
Standard ID:	STD-01-16-12
Analyte (Unit):	DMSe (mg/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 IDs:	See below

Replicate	Date Digested	Dataset ID	Dataset Sample ID	Concentration*
1)	5/21/2009	ICPMS-090604-TM-Se	110C DmSe	327.3
2)	6/23/2009	ICPMS-09062S-TM-DMSe	DMSe cal	334.0
3)	6/23/2009	ICPMS-09062S-TM-DMSe	DMSe cla D	316.2
4)	6/23/2009	ICPMS-09062S-TM-DMSe	DMSe cal T	323.5
5)	8/5/2009	ICPMS-090810-TM-SeNa	DMSe Cal	326.0
6)	8/5/2009	ICPMS-090810-TM-SeNa	DMSe Cal MD	324.8
7)	8/5/2009	ICPMS-090810-TM-SeNa	DMSe Cal MT	338.1
			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)

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 and Hg speciation in many different matrices successfully at Applied Speciation
  - ASC 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!

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

Acknowledgements

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  - Russ Gerads, Ben Wozniak, Tyler Kennedy, Jacob Meyer
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