A Comparison of Three Methods for Arsenic Speciation in Biological Tissues

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Select Arsenic Species

Inorganic Species

- arsenite [As(III)]
- arsenate [As(V)]

Organic Species

- arsenobetaine [AsB]
- arsenocholine [AsC]

Methylated Species

- monomethylarsine [MMA]
- dimethylarsine [DMA]
- trimethylarsine [TMA]
- trimethylarsine oxide [TMAO]

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Relative Toxicity

Species	Charge	Toxicity
AsB	cation	non-toxic
AsC	cation	non-toxic
MMA	anion	moderately toxic
DMA	anion	moderately toxic
TMA	cation	moderately toxic
TMAO	cation	moderately toxic
As(V)	anion	toxic
As(III)	anion	very toxic

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EPA Method 1632

- hydride generation reaction with volatile species
- cryogenic trap
- heating element – different boiling points for different species
- atomic absorbance spectrophotometer

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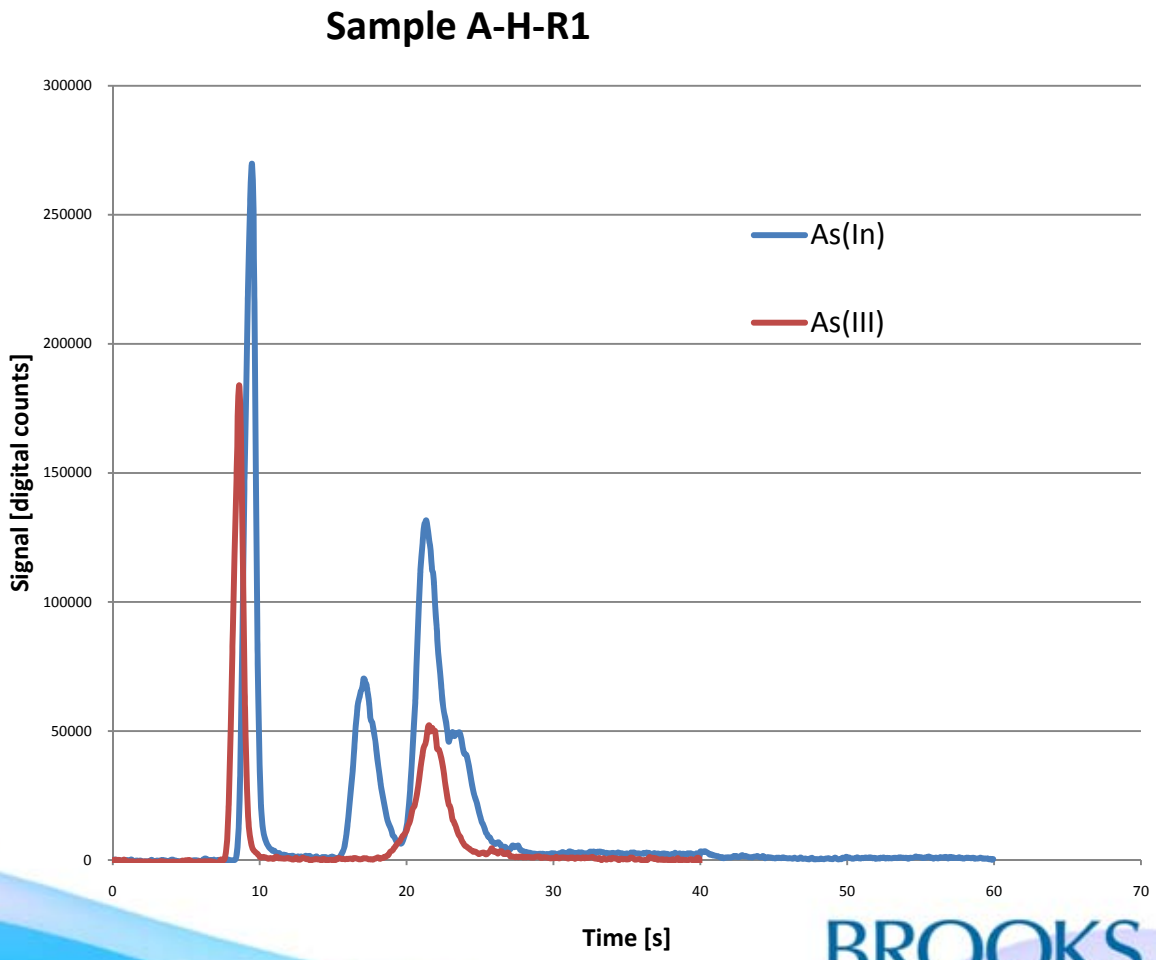
As(III) & As(V) Analysis

- As(III) and As(V) have the same boiling point
- As(In) = As(III) + As(V)
- For biota, As(III) and As(In) prepared by the same digestion method.
- As(III) directly quantifiable – analysis within very specific pH range
    - requires separate prep
- As(In) – As(III) = As(V)
    - two complete digestions and analyses



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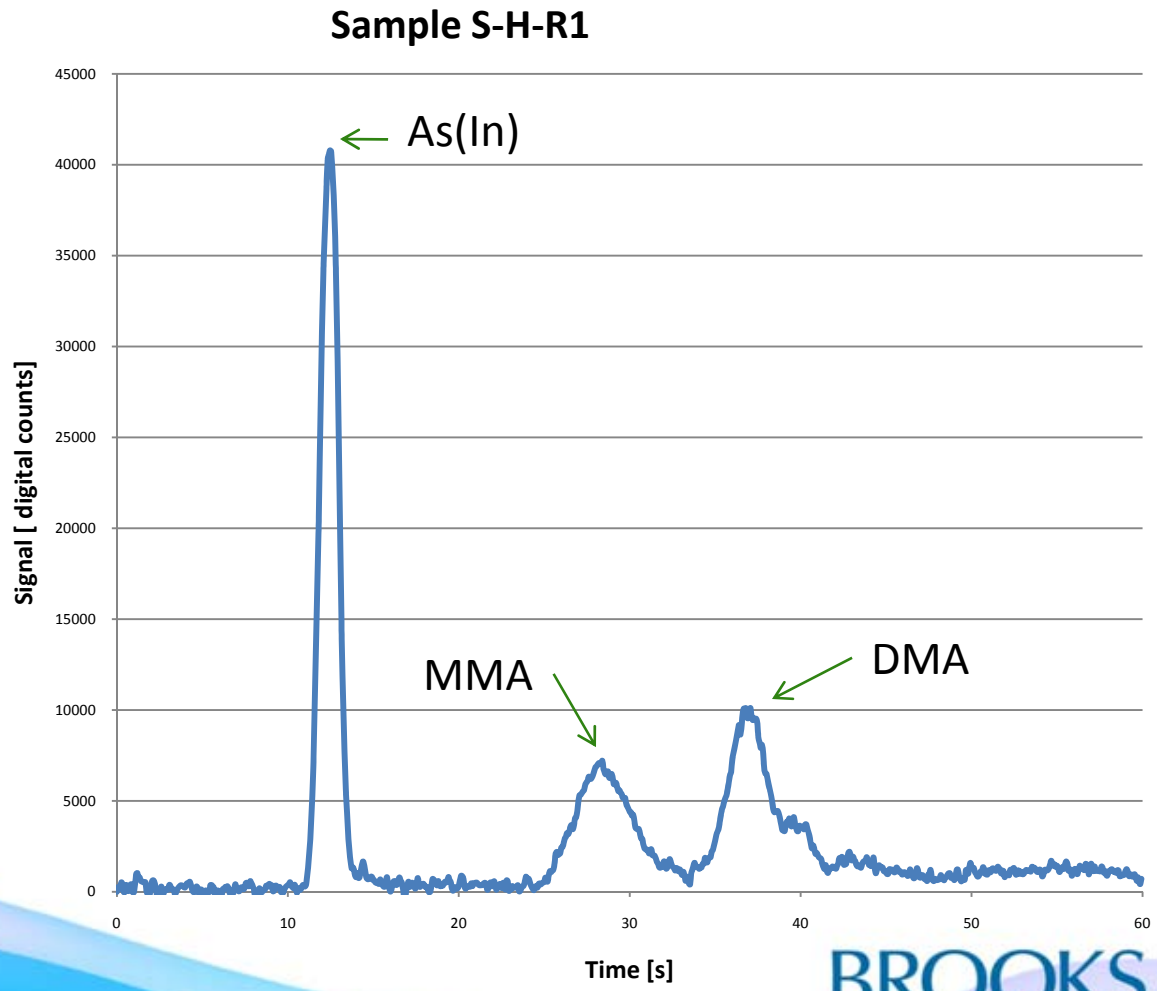
MMA & DMA Analysis

- For biota, separate digestion method – NaOH.
- MMA and DMA have very different boiling points
  - able to analyze for both in the same run
- not easy to achieve baseline separation



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EPA Method 1632 – Summary

Pros

- very low detection limits: 0.5 ng or 0.025 µg/L in reaction vessel
- demonstrated method for As speciation – first drafted 1998
- As(III) – the most toxic species – is directly quantifiable
- MMA and DMA analysis is pretty good

Cons

- very narrow calibration range: 0.5 to 30 ng
  - in other words, 0.025 to 1.5 µg/L in reaction vessel
  - necessitates dilutions
- multiple digestions for multiple analytes
- As(V) is not directly quantified
- As(III) analysis requires titration
- no analysis for arsenic cation species

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Initial Demonstration of Proficiency for the Multilaboratory Validation of Arsenic Speciation Methods 3110 and 6870

EPA INTERCOMPARISON STUDY

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Extraction by EPA 3110

- heated digestion
- centrifugation of sample material
- neutralization and heating of digestion extract
  - Per EPA Sec. 11.2.3, it is noted that some arsenicals are lost in the neutralization process.
- centrifugation and further heating of neutralized extract

Certified Reference Material	Certified Value (mg/kg)	Average Recovery (%)
DOLT-3 Dogfish Liver	10.2	84
DOLT-4 Dogfish Liver	9.66	88
DORM-2 Dogfish Muscle	18	85
DORM-3 Fish Protein	6.88	93
GBW 08571 Mussel	6.1	99
IAEA-407 Fish Tissue	12.6	97
TORT-2 Lobster	21.6	82

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Total Arsenic Recoveries

Sample	Sample	Total Arsenic in Sample (ng/g)	Total Arsenic in Extract (ng/g)	Extraction Efficiency (%)
A-L-R1	Rep 1	34900	31700	91
A-H-R1	Rep 1	164000	151000	92
A-H-R2	Rep 2	161400	155000	96
S-L-R1	Rep 1	8490	6980	82
S-H-R1	Rep 1	63600	60400	95
S-H-MS	Matrix Spike	82130	75330	92
LCS BCR-627	LCS	4940	4020	81

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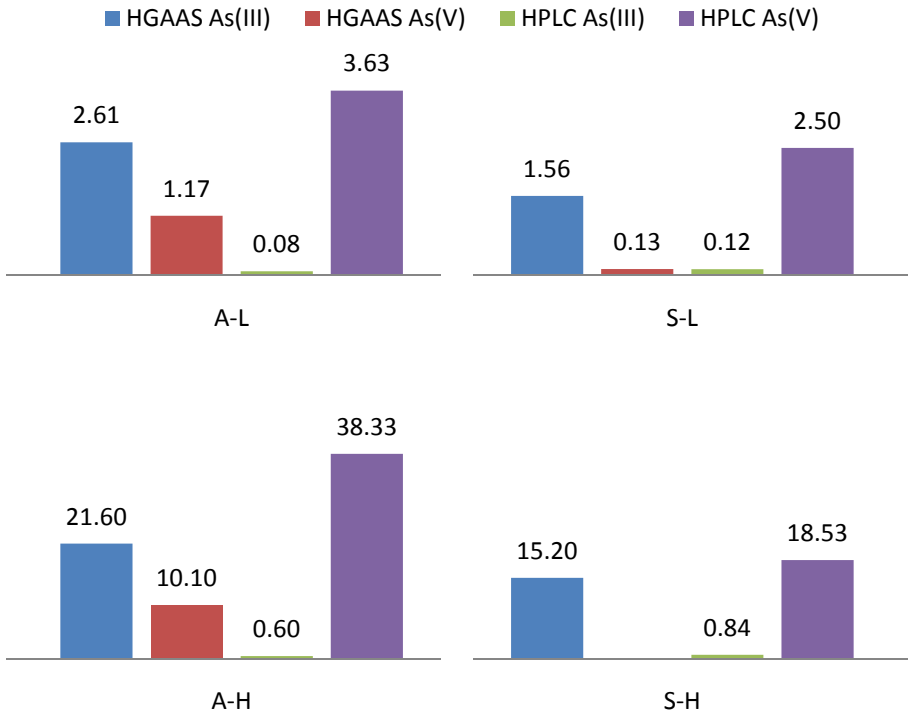
Extraction by EPA 3110

- Per EPA Sec. 1.2, digestion extract (TMAOH) favors As(V) stability at higher pH.
- TMAOH can act as an oxidizing agent and push conversion of As(III) to As(V).



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Extraction by EPA 3110

Pros

- single digestion for cation and anion analysis
- Bigger mention for cation analysis!

Cons

- unknown stability of species over time
- conversion of As(III) to As(V)

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EPA Method 6870

- HPLC-ICP-MS
- encompasses 3 analyses: total arsenic in extract (via ICP), cations, and anions
- separate ion-exchange columns for anionic and cationic analysis
- isocratic separation of the mobile phase

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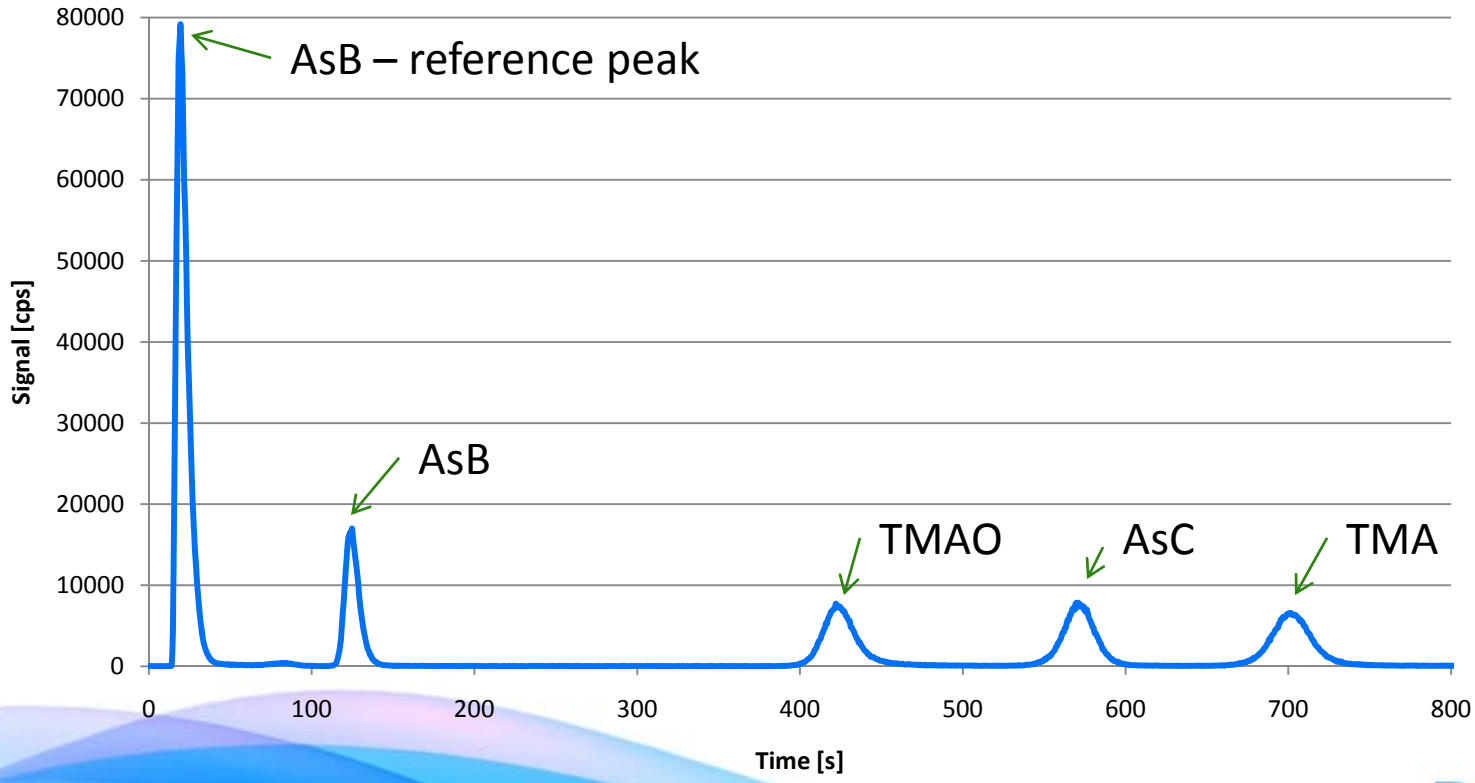
Cations – Calibration 5 µg/L



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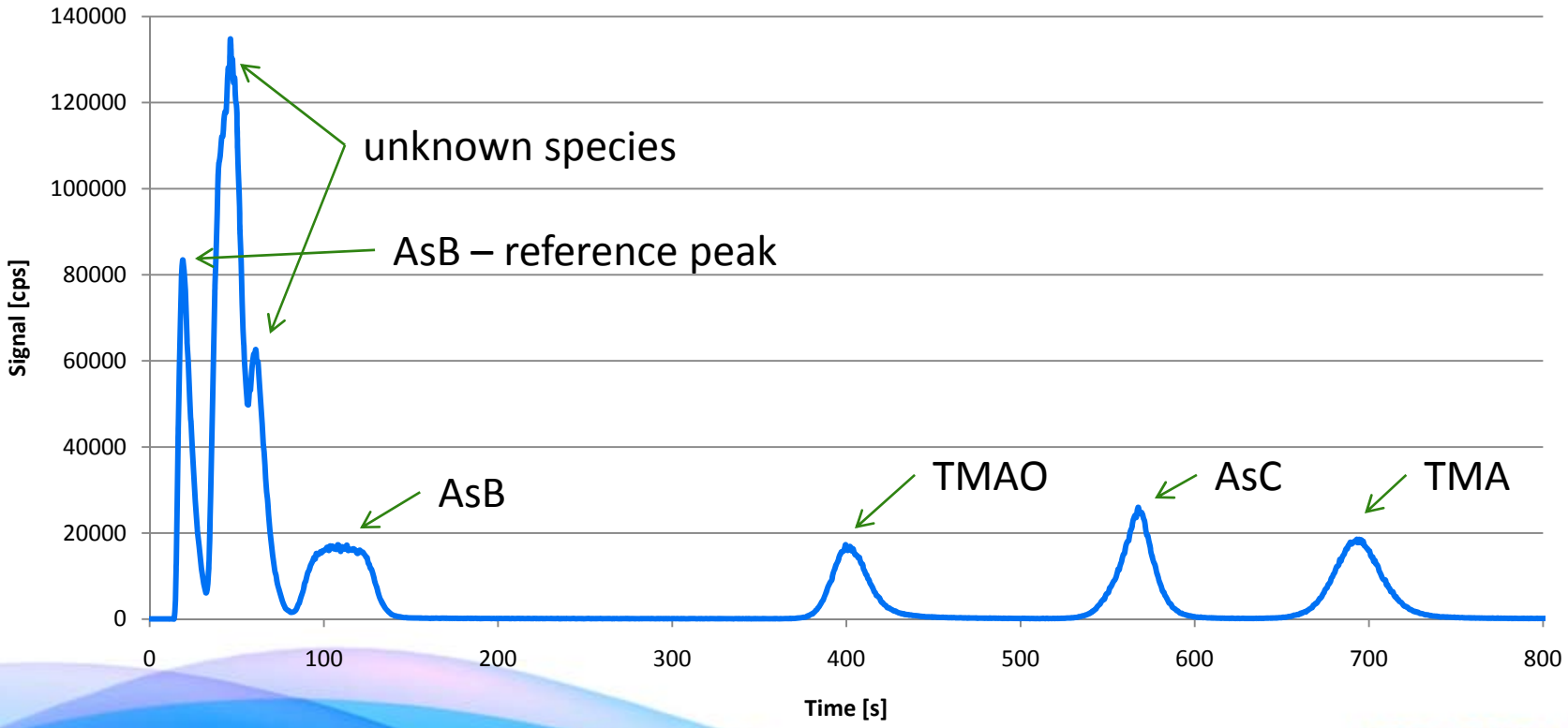
Cations – Sample S-H-R1



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Cations – QC Results

Sample	Description	AsB	TMAO	AsC	TMA
A-L-MS	Matrix Spike	89%	94%	92%	94%
A-L-MSD	MS Duplicate	88%	88%	80%	89%
A-H-MS	Matrix Spike	67%	67%	63%	66%
A-H-MSD	MS Duplicate	77%	69%	66%	59%
S-L-MS	Matrix Spike	105%	69%	95%	95%
S-L-MSD	MS Duplicate	100%	70%	92%	93%
S-H-MS	Matrix Spike	77%	64%	84%	86%
S-H-MSD	MS Duplicate	74%	57%	64%	69%
LCS BCR-627-MS	LCS Spike	223%	119%	136%	137%
BLANK SPIKE-R1	Rep 1	116%	99%	97%	98%
BLANK SPIKE-R2	Rep 2	115%	99%	98%	99%
BLANK SPIKE-R3	Rep 3	113%	99%	98%	98%

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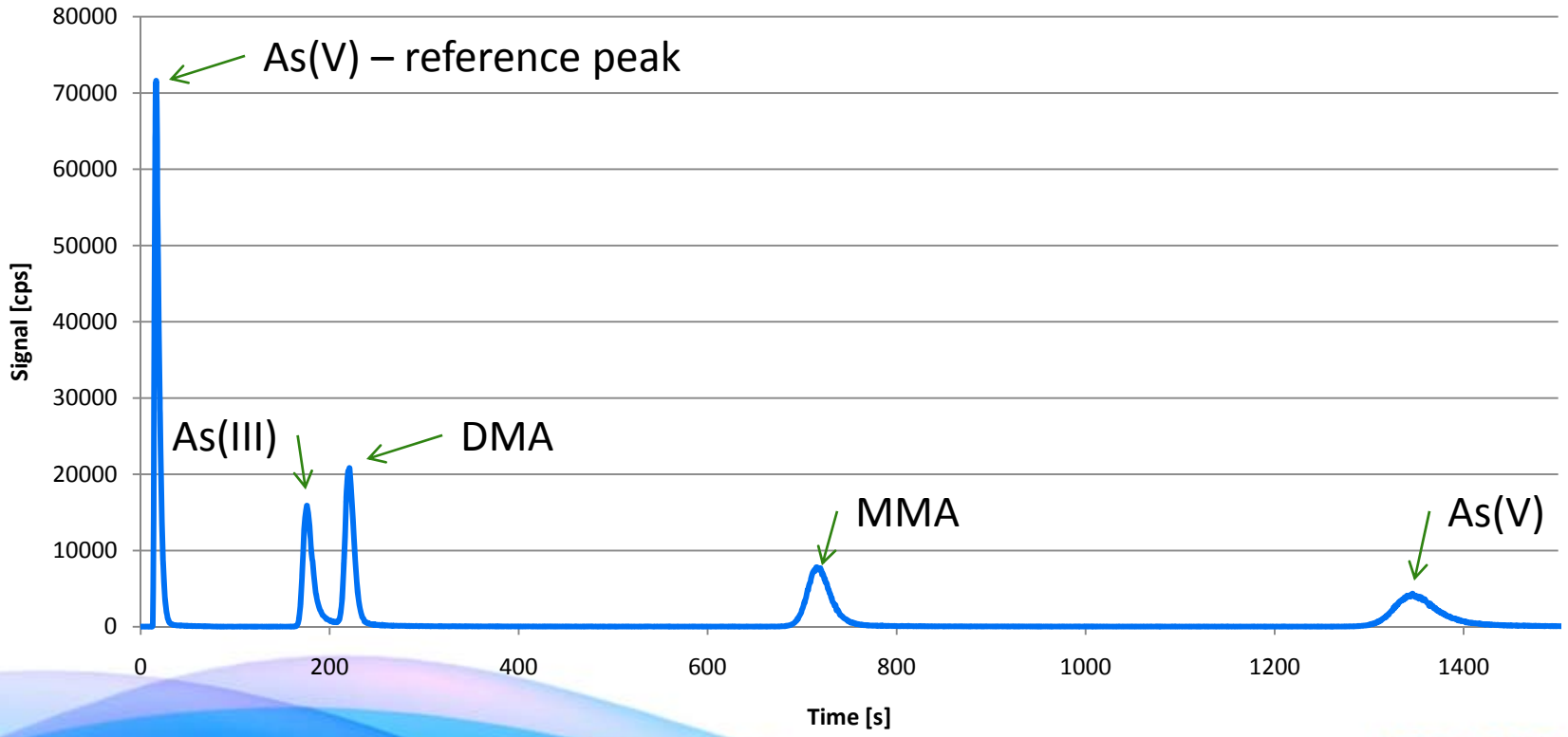
Anions – Calibration 10 µg/L



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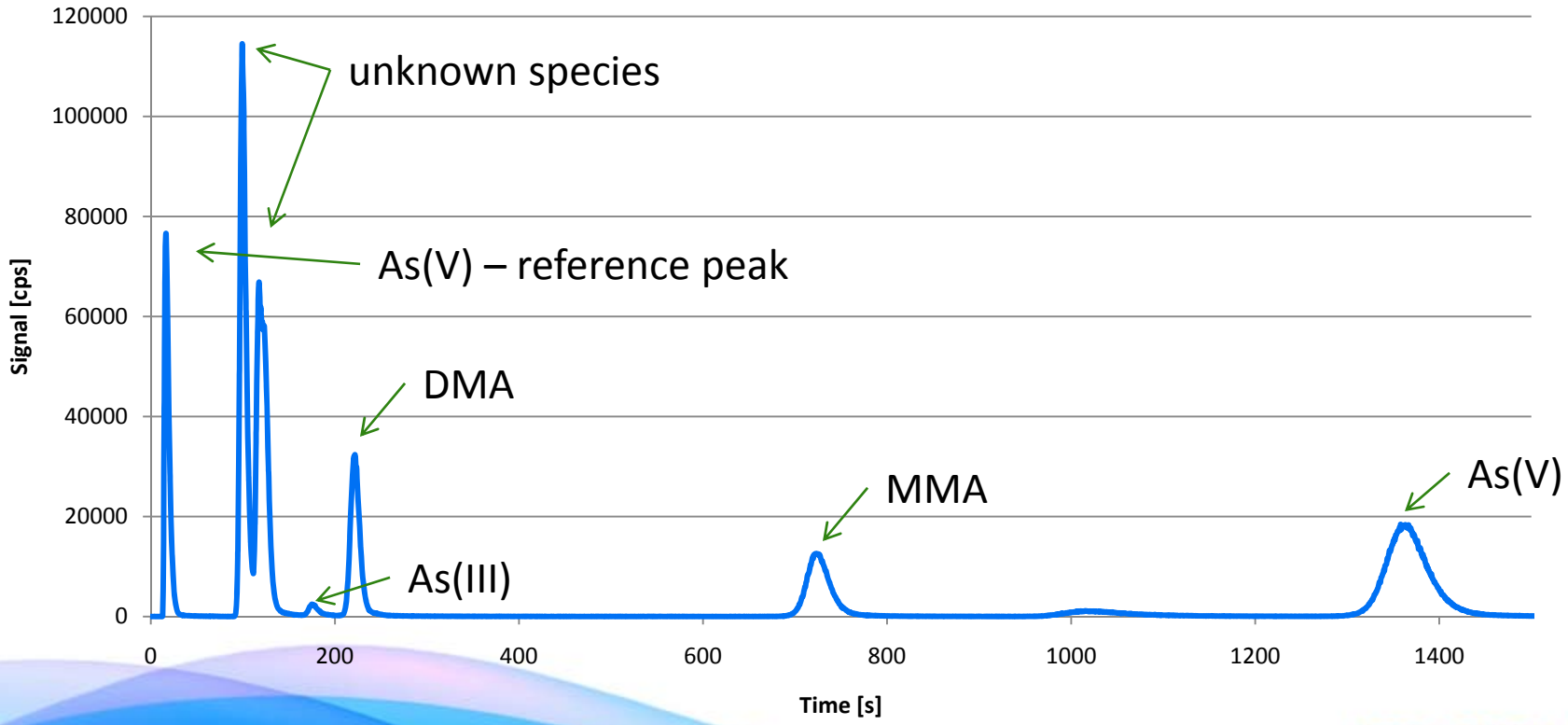
Anions – Sample S-H-R1



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Anions – QC Results

Sample	Description	As(III)	DMA	MMA	As(V)
A-L-MS	Matrix Spike	4%	101%	108%	204%
A-L-MSD	MS Duplicate	3%	105%	108%	212%
A-H-MS	Matrix Spike	1%	73%	85%	151%
A-H-MSD	MS Duplicate	0%	90%	124%	207%
S-L-MS	Matrix Spike	8%	66%	69%	197%
S-L-MSD	MS Duplicate	8%	68%	68%	189%
S-H-MS	Matrix Spike	15%	81%	85%	215%
S-H-MSD	MS Duplicate	9%	64%	71%	175%
LCS BCR-627-MS	LCS Spike	12%	201%	199%	439%
BLANK SPIKE-R1	Rep 1	7%	150%	153%	308%
BLANK SPIKE-R2	Rep 2	9%	155%	154%	309%
BLANK SPIKE-R3	Rep 3	7%	150%	151%	303%

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EPA Method 6870 – Summary

Pros

- anion and cation analyses potentially covers 8 species
- HPLC-ICP-MS has a wider calibration range: 0.25-10 µg/L
- direct quantification of all species
- ease and simplicity of use:
  - standard mode for ICP-MS
  - isocratic separation for HPLC

Cons

- reference peak does not monitor for within run matrix effects
- close peaks for As(III) and DMA – no baseline separation
- ICP-MS standard mode is susceptible to polyatomic interferences leading to biased results

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A Comparison: EPA 1632 vs EPA 6870

	EPA 1632	EPA 6870
species	4	8
digestions	3	1
analyses	3	2

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Our Recommendations

EPA 3110

- Different digestion solution?
  - HNO_3
  - HCl
  - NAOH
- test for preservation properties as well

EPA 6870

- continuous internal standard introduction to monitor matrix effects
- gradient-step separation to get baseline separation of As(III) and DMA
- DRC mode to alleviate polyatomic interferences

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Interference Reduction Technology

Certified Reference Material	Certified Value (mg/kg)	Average Recovery in Standard Mode (%)	Average Recovery in DRC Mode (%)
DOLT-4 Dogfish Liver	9.66	88	91
DORM-2 Dogfish Muscle	18	85	92
DORM-3 Fish Protein	6.88	93	97
IAEA-407 Fish Tissue	12.6	97	107
TORT-2 Lobster	21.6	82	97

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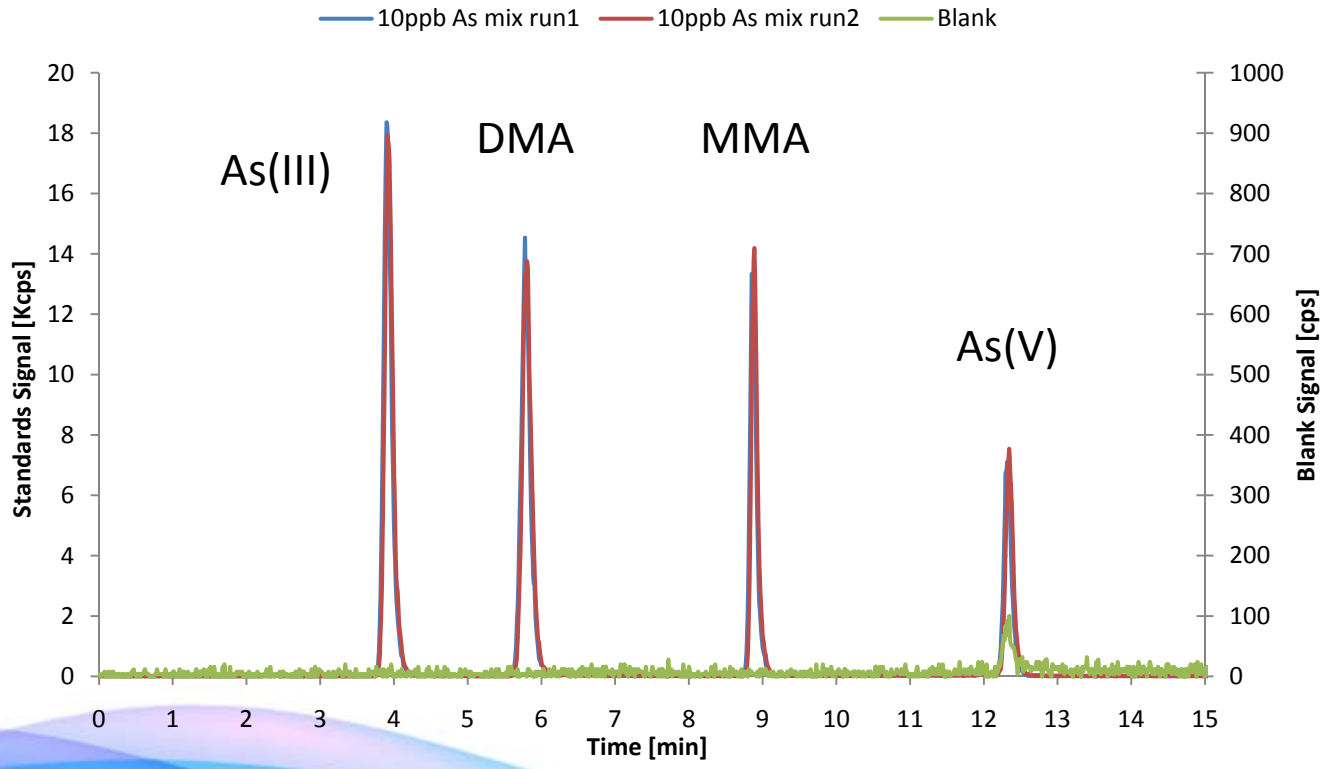
Gradient-Step Separation



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Questions?

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