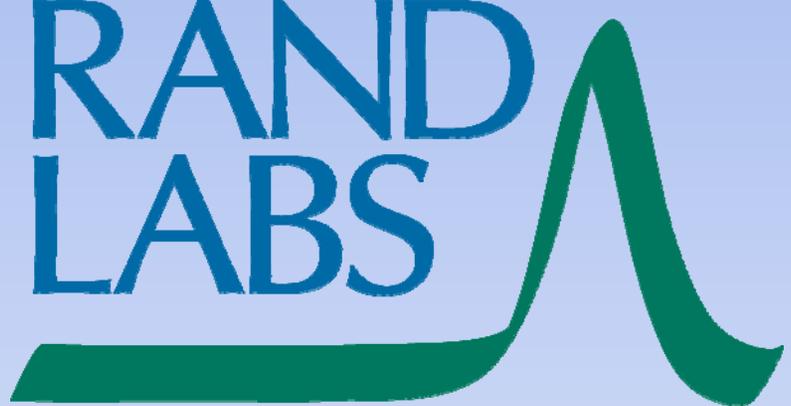


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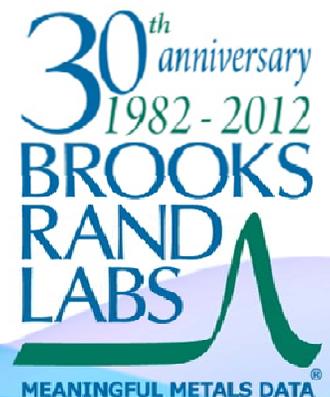
MEANINGFUL METALS DATA®

A Comparison of Arsenic Speciation Methods for Biological Tissues

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Various Relevant Arsenic Species

Species	Abbreviation
arsenous acid (arsenite)	As ^{III}
arsenic acid (arsenate)	As ^V
monomethylarsonic acid	MMA
dimethylarsinic acid	DMA
trimethylarsine oxide	TMAO
tetramethylarsonium ion	TMA
arsenobetaine	AsB
arsenocholine	AsC

Toxicity of Arsenic

Elevated arsenic concentrations in seafood are not necessarily indicative of toxicity:

- less toxic: AsB, AsC
- moderately toxic: MMA, DMA, TMAO, TMA
- highly toxic: As^{III}, As^V (inorganic forms)

Need for a Standardized Method

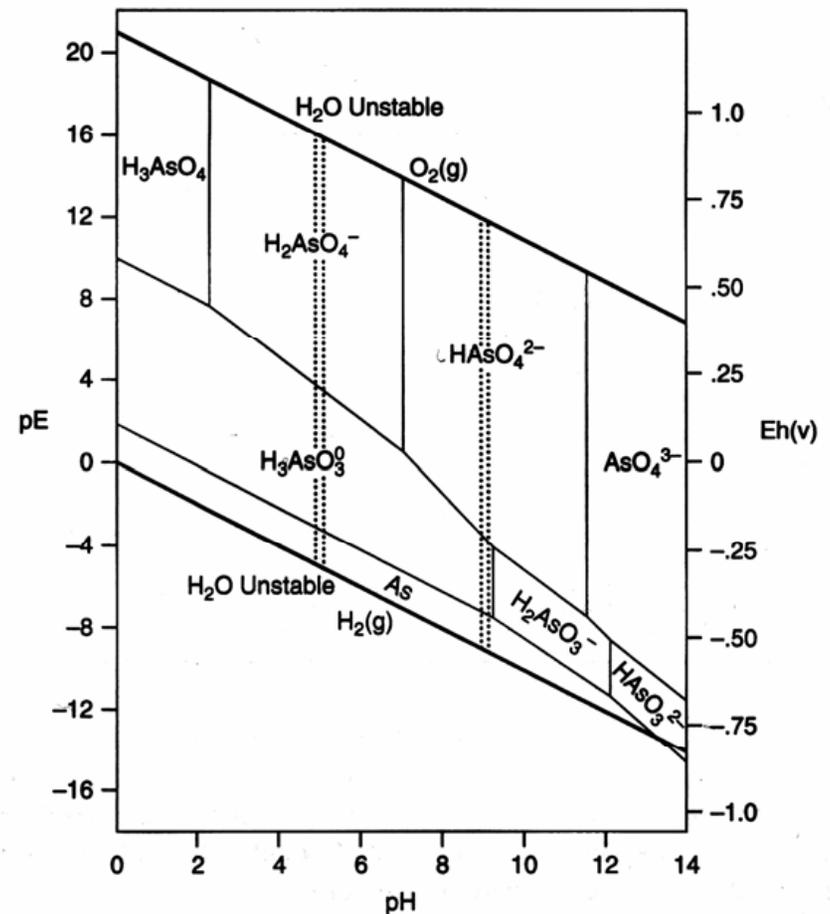
- ~1000 published papers on arsenic speciation techniques
- unique/proprietary methods = poor data intercomparability between laboratories
- proposed solution:
 - EPA Draft Method 3110 (sample preparation)
 - EPA Draft Method 6870 (sample analysis)

EPA Method 3110 (Draft)

- highly complex process
- labor intensive
- species extraction with tetramethylammonium hydroxide (TMAOH) and acetic acid

Known Issues with EPA Method 3110

Section 1.2 *It is difficult to preserve the oxidation state of inorganic arsenic, as As^{III} converts to As^V , and vice versa...*



Species Conversion during Extraction Process

- conversion from As^{III} to As^{V} is acknowledged
- the relative differences in the toxicity of As^{III} and As^{V} are not well understood
- it is important to be able to quantify the concentrations of both species in real samples

Experimental Modifications

- less complex and faster digestion procedure
- avoid multiple pH adjustments
- less-oxidizing extractant, such as trifluoroacetic acid (TFA), hydrochloric acid (HCl), or methanol (MetOH)

Extraction Efficiency

SRM	EPA 3110	TFA	HCl	MetOH
DORM-3	99%	91%	92%	86%
TORT-2	97%	97%	95%	100%
NIST-2976	82%	96%	107%	101%
BCR-627	103%	n/a	n/a	90%

Conversion of $\text{As}^{\text{III}} \rightleftharpoons \text{As}^{\text{V}}$

EPA 3110	TFA	HCl	MetOH
97% ± 3	47% ± 41	8% ± 4	> 5%

- TFA causes inconsistent species conversion
- HCl and MetOH minimize species conversion
- However, the procedure suggests avoiding the use of chloride due to polyatomic interferences during analysis.

Simplified Digestion Procedure

Hot Block Digestion with MetOH

- 2 extractions over 2 days
- 6 hours labor

Microwave Digestion using MetOH

- 30 minute extraction
- 2 hours labor

SRM	First Extraction	Second Extraction	Combined Extractions	Microwave Digestion
DORM-3	81%	16%	96%	86%
TORT-2	81%	27%	107%	100%
NIST-2976	90%	23%	113%	101%

% recoveries of Total As

EPA Method 6870 (Draft)

- developed for seaweed, finfish, and shellfish
- ion chromatography (IC) to separate species
- detection by inductively coupled plasma – mass spectrometry (ICP-MS)

EPA Method 6870 (Draft)

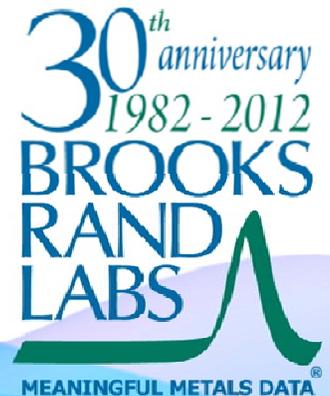
Anion Species (As^{III} , As^{V} , DMA, MMA)

- column compatible with a pH 9 ammonium carbonate eluent
- recommended PRP X-100 (Hamilton)
- 30 minutes per injection with isocratic elution

Cation Species (AsB, AsC, TMAO, TMA)

- column compatible with pH 2.7 pyridinium eluent
- recommended Chrompack Ionospher (Varian)
- 20 minutes per injection with isocratic elution

Total Arsenic by EPA Method 6020 recommended



Known Issues with EPA Method 6870

4.1 Isobaric polyatomic interferences result when ions containing more than one atom have the same nominal mass-to-charge (m/z) ratio as the analyte of interest, and therefore cannot be resolved by the mass spectrometer.

Isobaric Polyatomic Interferences

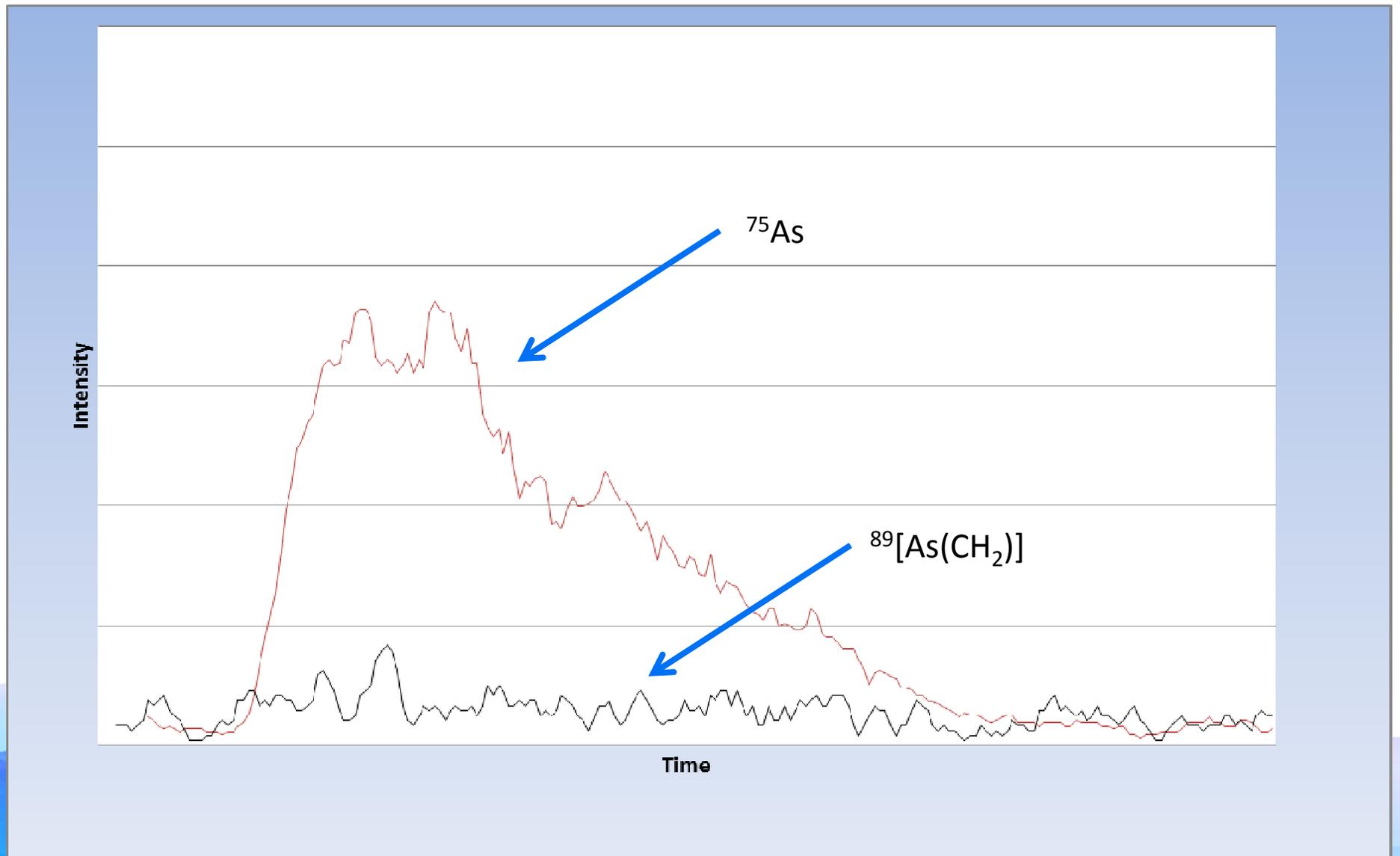
Arsenic is monoisotopic with an m/z of 75:

- anion interference Cl^- as $^{40}\text{Ar}^{35}\text{Cl}$
- cation interference K^+ as $^{36}\text{K}^{39}\text{Ar}$

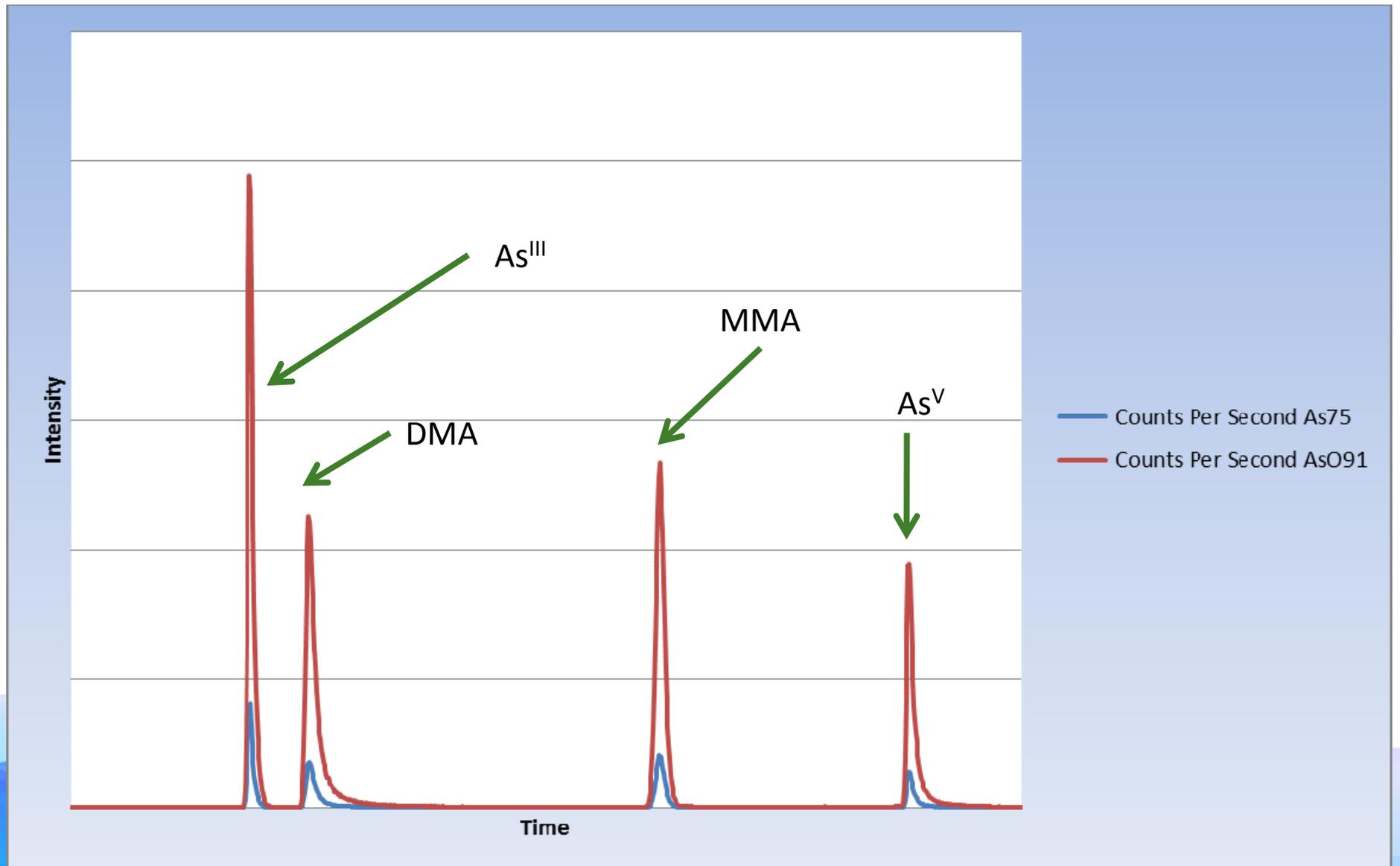
Interferences can be resolved by utilizing a reaction gas (DRC) to create a second isotope:

- O_2 to create AsO at m/z 91
- CH_4 to create $\text{As}(\text{CH}_2)$ at m/z 89

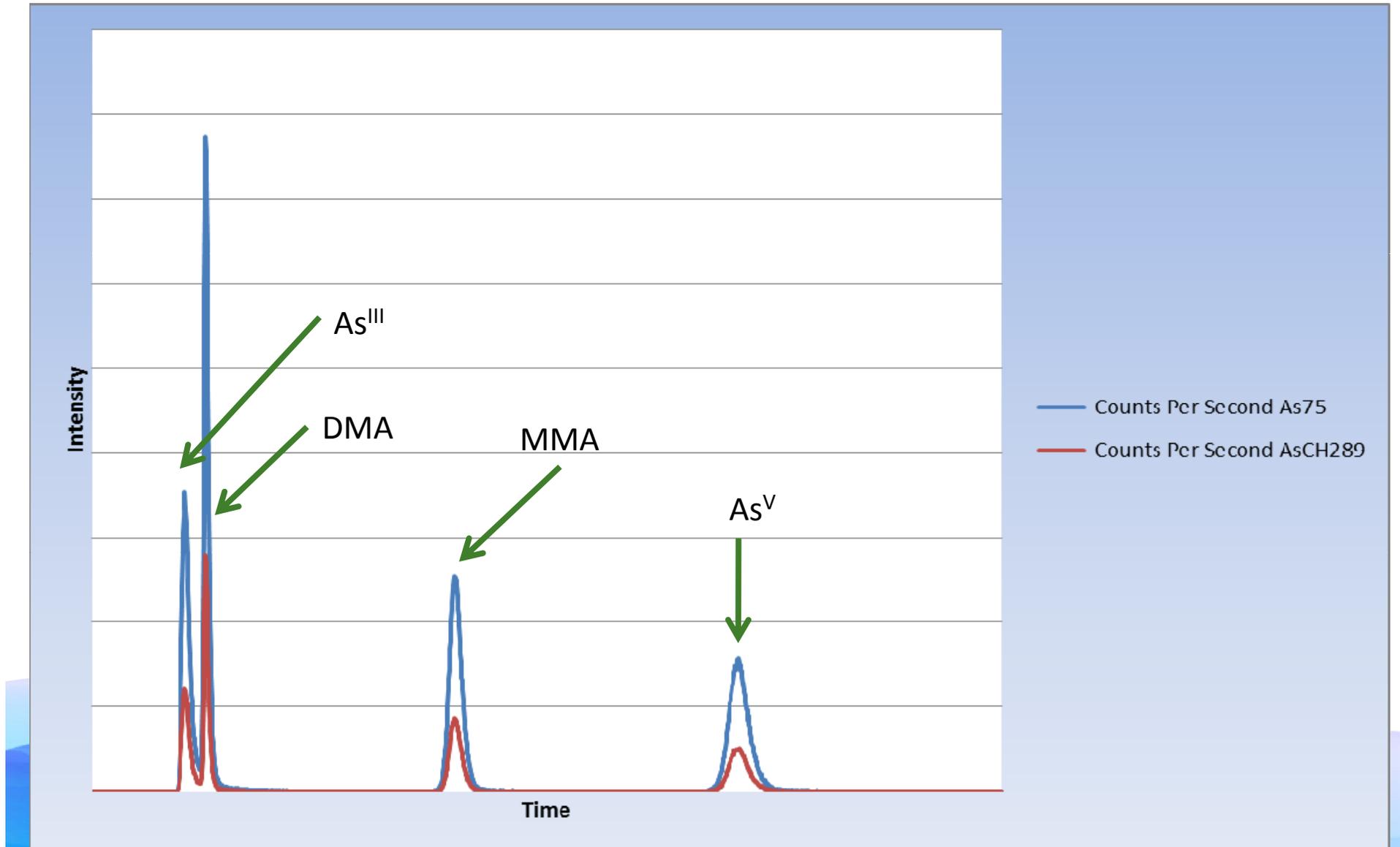
Example Chloride Interference



Oxygen as a Reactive Gas



Methane as a Reactive Gas



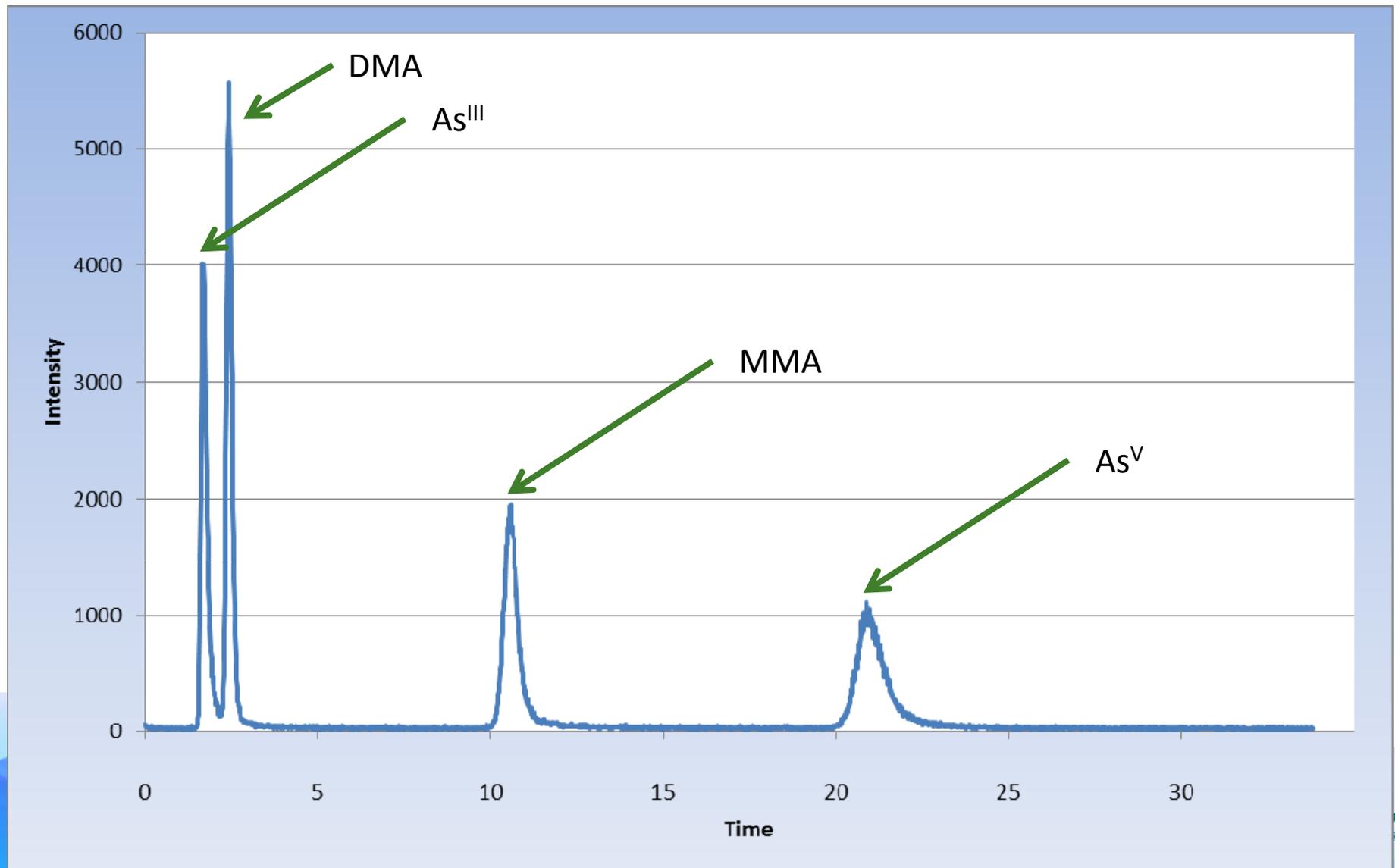
Known Issues with EPA Method 6870

4.6 Peak broadening effects may limit an analyst's ability to obtain adequate chromatographic resolution and hence, accurate peak integrations.

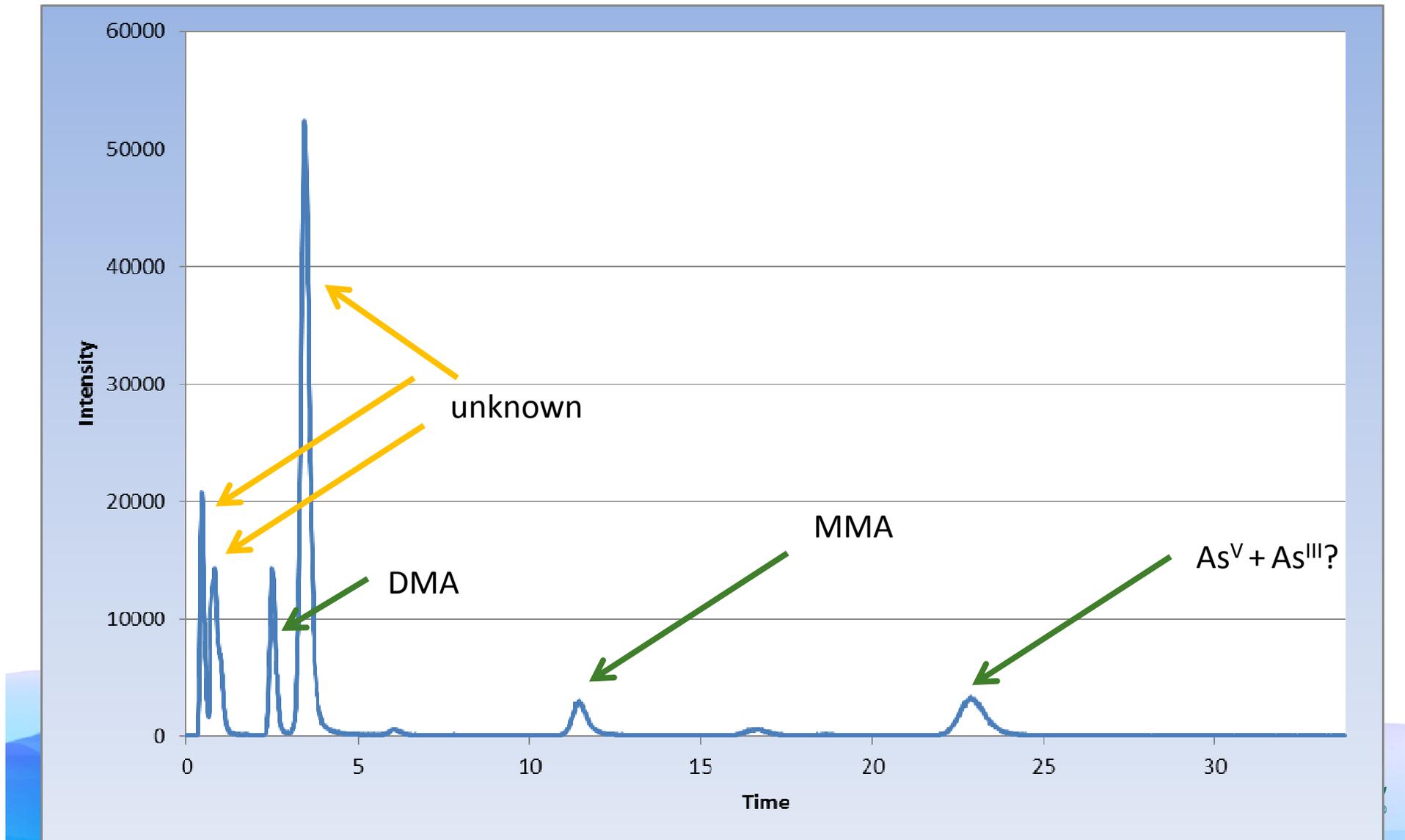
Known Issues with EPA Method 6870

6.8.9 and 6.8.10 are very specific in the eluents therefore the columns that can be used.

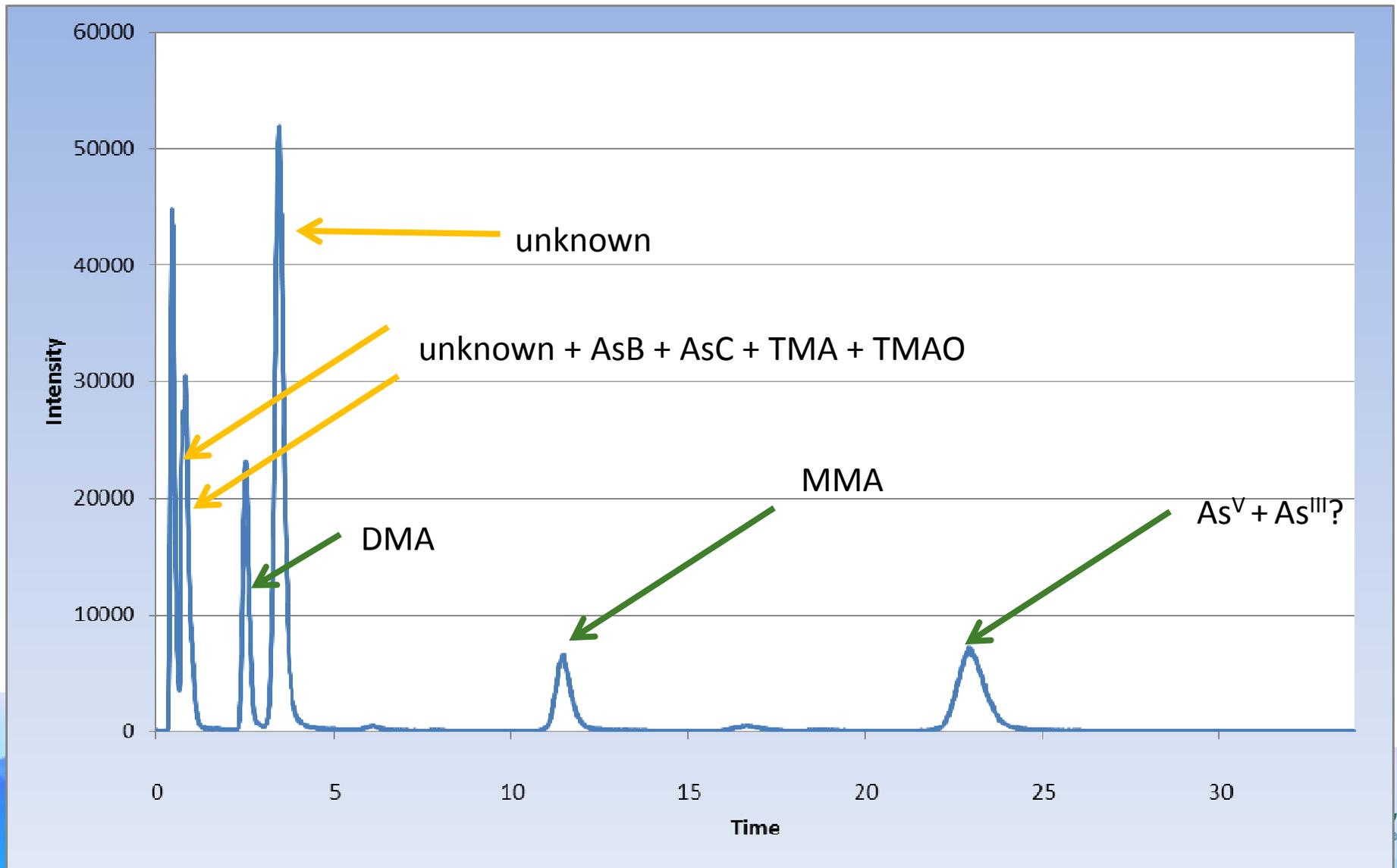
Calibration of Anions



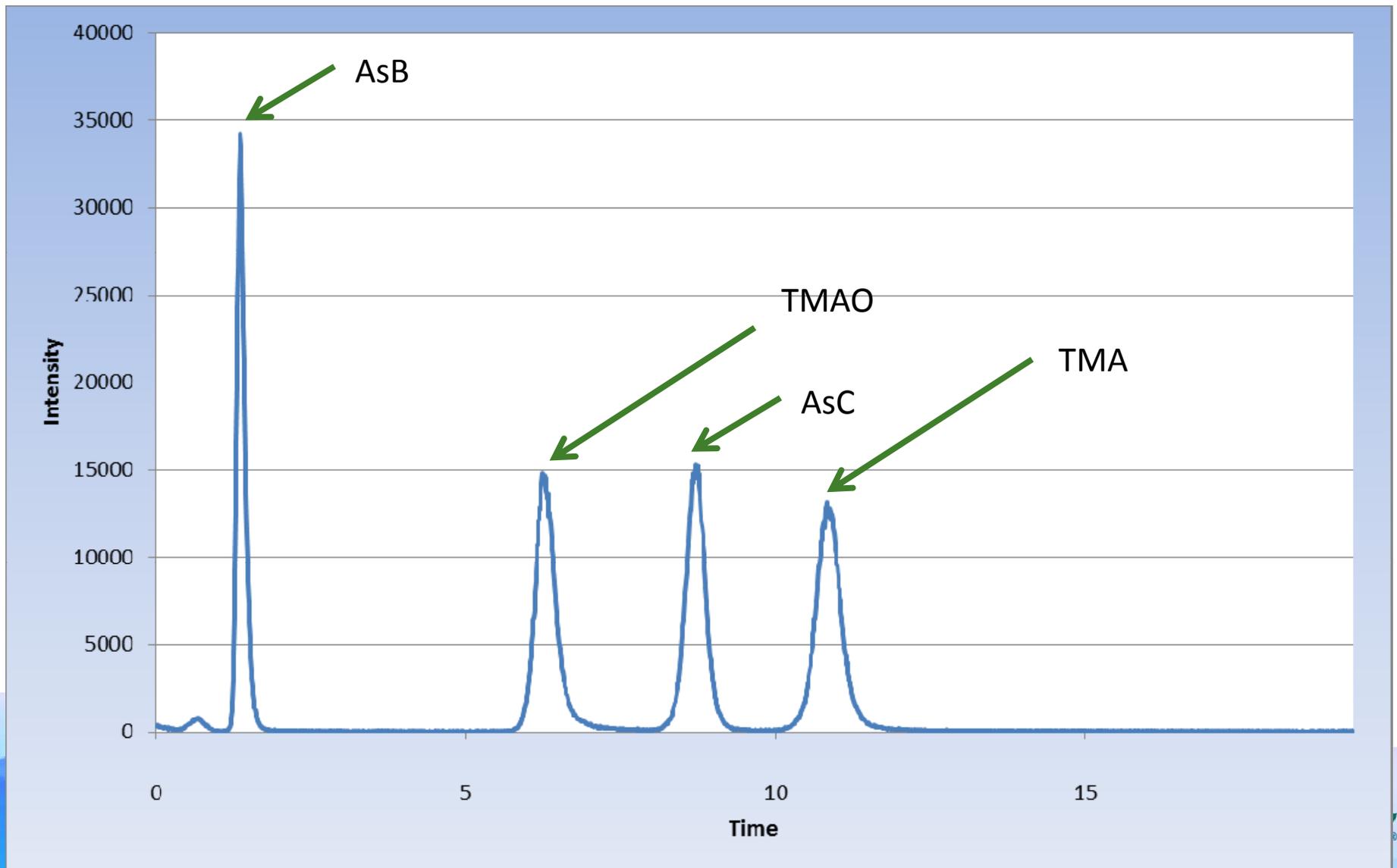
Sample Anion Results



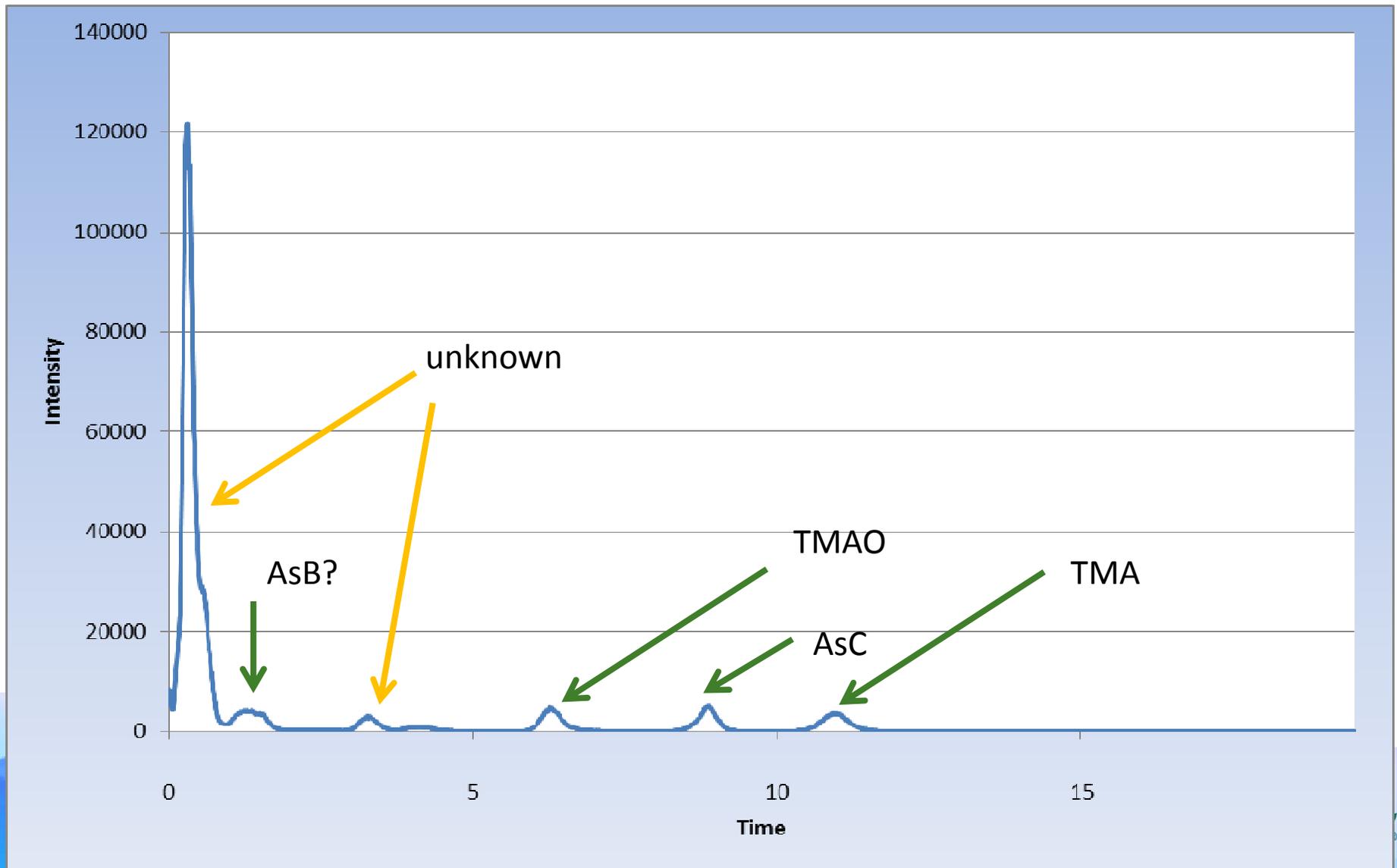
Matrix Spike Results



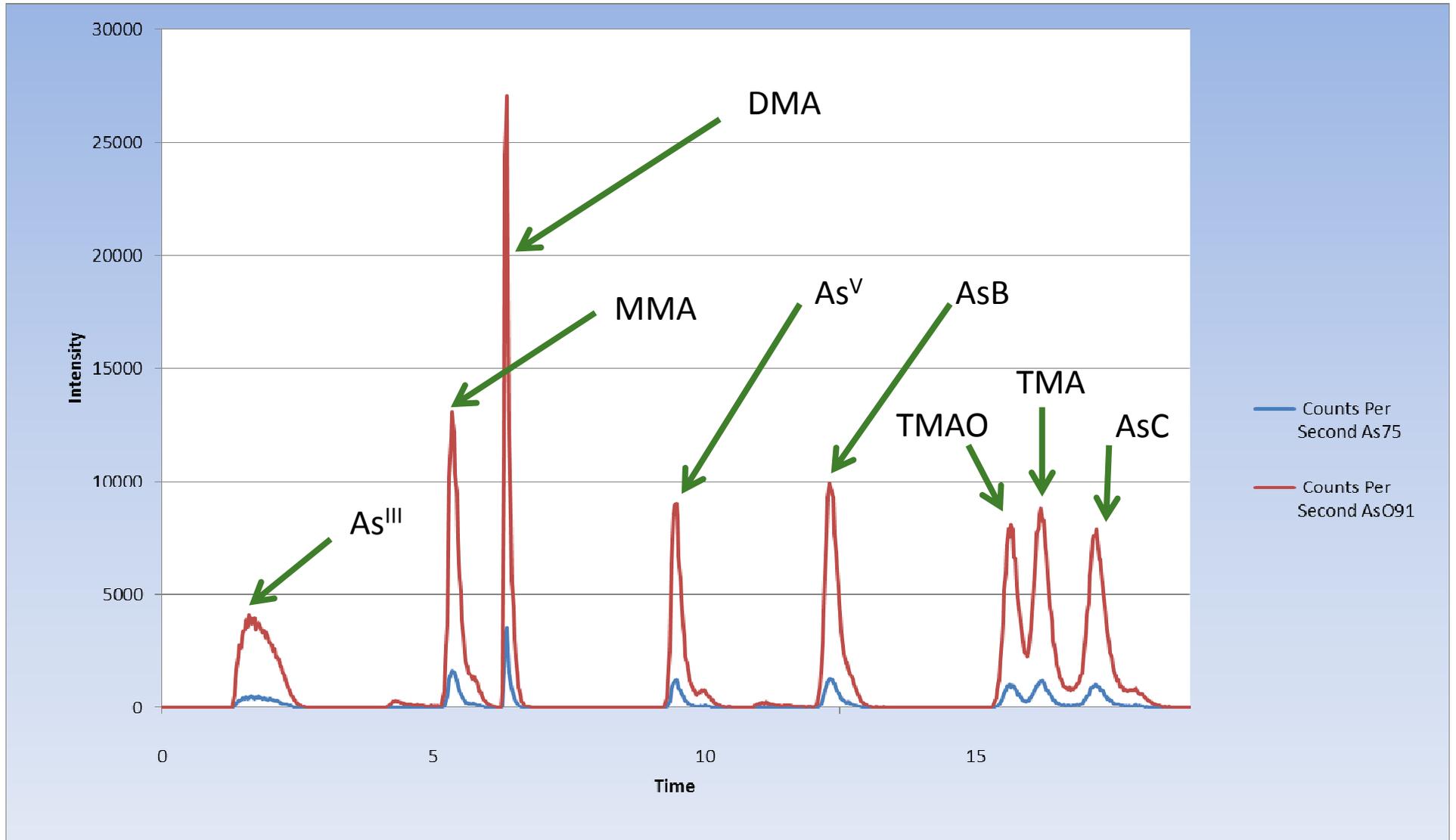
Calibration of Cations



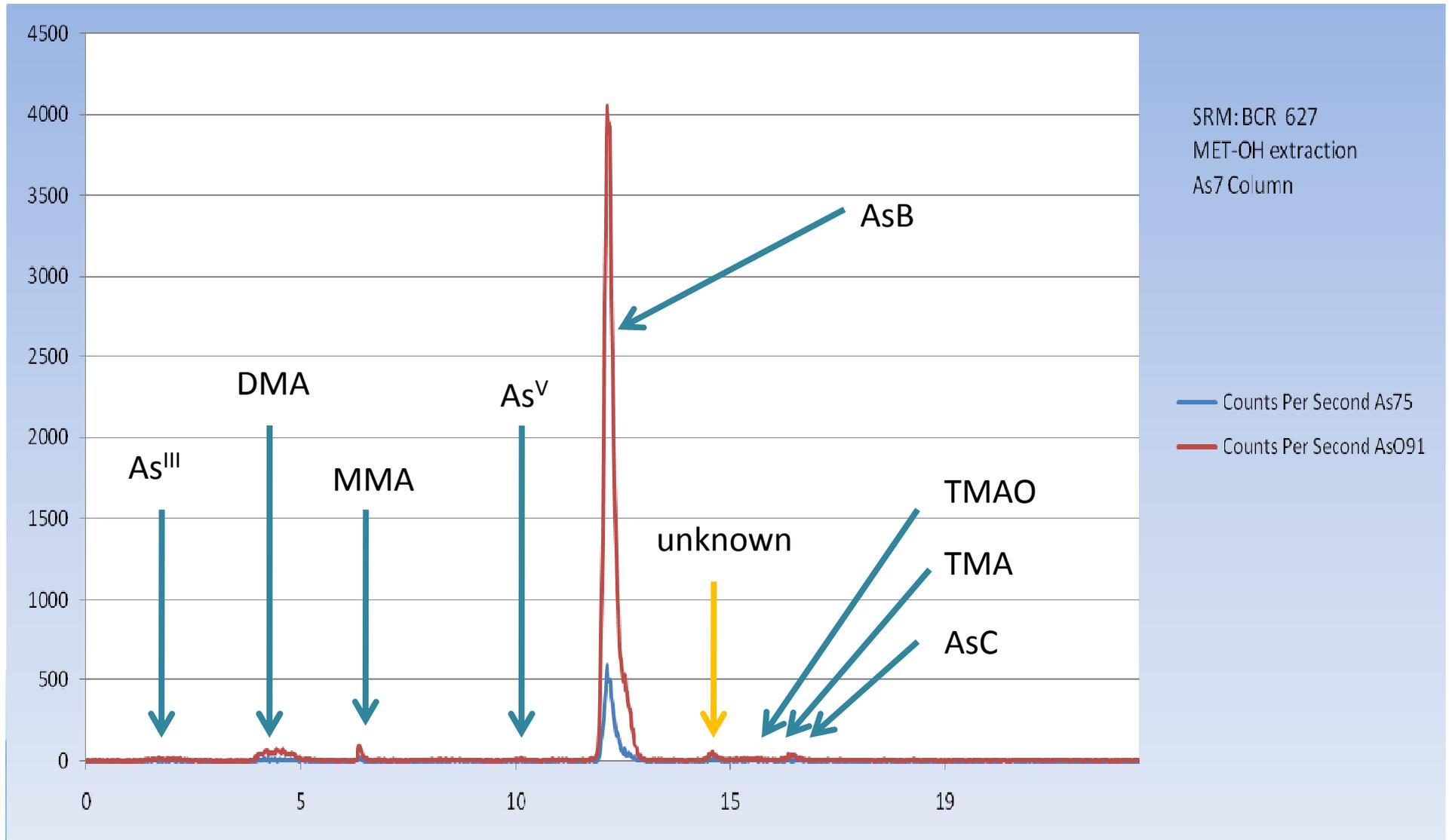
Sample Cation Results



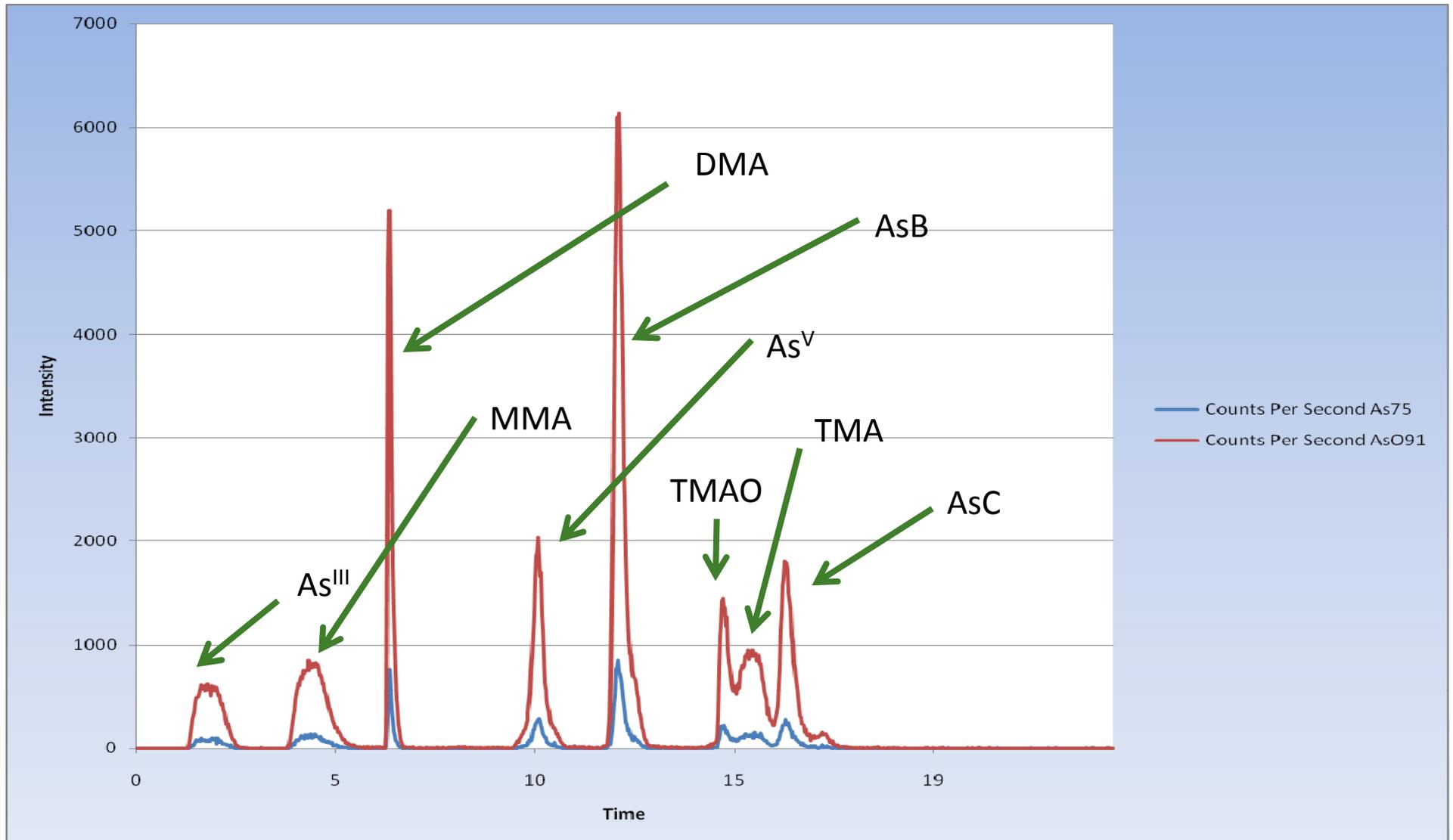
As7 Column Calibration with a Gradient Elution Curve



As7 Column Sample results



As7 Column Spiked Sample results



Results of SRMs (LOQ 0.02 mg/kg)

SRM	AsIII	DMA	MMA	AsV	AsB	TMAO	AsC	TMA
DORM-3	0.07	0.60	0.06	0.26	4.69	0.06	0.07	0.03
TORT-2	0.26	1.35	0.50	0.58	13.85	0.05	0.07	0.02
NIST-2976	0.17	0.56	0.13	0.05	9.39	0.08	0.05	0.04
BCR-627	0.04	0.16	0.04	> 0.02	4.17	0.05	> 0.02	0.03

SRM	Sum of 8 Known Species	Certified Value for Arsenic	Percent Recovery
DORM-3	5.8	6.8	86%
TORT-2	16.7	21.6	77%
NIST-2976	10.5	13.3	79%
BCR-627	4.5	4.8	94%

Spike Recoveries

SRM	AsIII	AsV	MMA	DMA	TMAO	TMA	AsB	AsC
DORM-3	103%	104%	86%	105%	*49%	98%	99%	93%
TORT-2	98%	105%	94%	101%	*55%	100%	89%	86%
NIST-2976	77%	100%	56%	103%	93%	105%	100%	92%
BCR-627	73%	109%	101%	106%	*67%	112%	104%	108%

* Peak overlap from potential Arsine-phosphate compound

Certified Reference Material

BCR-627	Certified Value	Recovery
DMA	0.15 mg/kg	106%
AsB	3.9 mg/kg	107%

Proposed Changes Summary

3110/6870

- 2 day prep (20 samples)
- Complex 10 step
- Most toxic forms (As^{III} , As^{V}) not differentiated
- Many forms not quantified in actual matrix (including unknowns)
- 3 analyses per sample (including total)

BRL Method

- 2 h prep (20 samples)
- Simple 3 step
- Most forms of As differentiated
- Most forms of As quantified
- 2 analyses per sample (including total)

Questions?



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