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Inorganic & Organic Certified Reference Materials

Your Science is Our Passion.™

Transitioning Arsenic Speciation Analysis from the Inorganic to the Organic Laboratory: Novel Methods for Separation of Inorganic and Organic Arsenic Species by LCMS

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What is Speciation? & Why is it Important?

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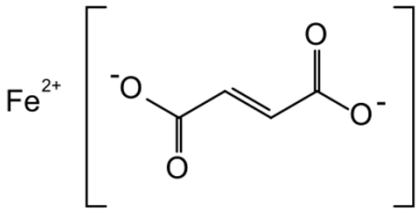
- IUPAC Definition of Speciation:

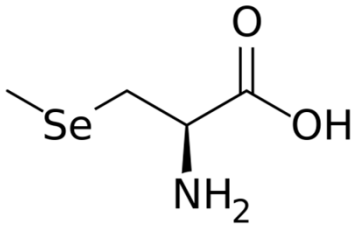
Distribution of an element amongst defined chemical species in a system.

  - *Isotopic composition*
  - *Electronic or oxidation state*
  - *Molecular structure*
- Biological Activity
  - *Nutrition:*
    - Some Fe forms higher bioavailability as nutrient
  - *Disease treatment:*
    - Organoselenium compounds scavenge free radicals & prohibit tumor growth
  - *Toxicity:*
    - Generally, Organometallics > toxic than Inorganic or Elemental forms (except As)

Z	Element	Negative Oxidation States					Positive Oxidation States					
24	Cr	−4		−2	−1	0	1	2	3	4	5	6
33	As		−3	−2	−1	0	1	2	3	4	5	
34	Se			−2	−1	0	1	2	3	4	5	6
48	Cd			−2		0	1	2				
80	Hg			−2		0	1	2		4		
82	Pb	−4		−2	−1	0	1	2	3	4		



(E)-But-2-enedioate; iron(2+)



3-(Methylselanyl)-L-alanine

Metal Toxicity

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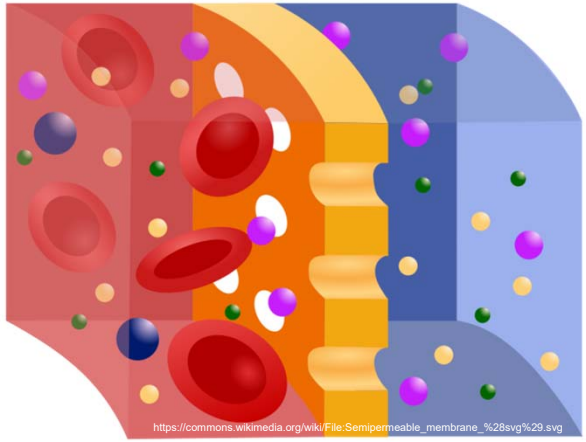
- Species Toxicity effected by:
  - *Absorption:*
    - Binding to an to unabsorbable constituent reduces bioavailability & toxicity
  - *Distribution:*
    - Inability to cross biological barriers (i.e. blood/brain, intestines) reduces bioavailability & toxicity
  - *Biotransformation:*
    - accumulation, modification and excretion from tissues
- Traditional metal species of concern:
  - *Pb, Cd, Hg, As, Cr*
  - *Usually, Organic forms > toxic than Inorganic*
- Arsenic (As) - exception
  - *Inorganic Species = higher toxicity*



Arsenic Species		LD_{50} (mg/kg)
Arsenite	As (III)	14
Arsenate	As (V)	20
Arsine	AsH3	3
Monomethylarsonic Acid	MMA	700 - 1800
Dimethylarsinic Acid	DMA	700 - 2600
Arsenocholine		> 10000
Arsenobetaine		> 10000

Why are we slow to adapt to Speciation?

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- Difficult Sampling Collection & Preservation
  - *Distribution must be preserved or in-situ analysis*
    - Effects of Collection & Preservation
      - Acidification or additions (Δ pH)
      - Microbial activity
      - Time: redox reactions due Δ O_2 saturation
      - Temperature
      - Volatility
      - Absorption into/through container
      - Contamination from container
- Difficult Sample Preparation
  - *Extraction: (methylation of Hg from sediments)*
    - *Heat*
    - *Oxidizers*
  - *Derivatization:*
    - *Methyl-Hg & Ethyl-Hg formed in derivatization rxn between Inorganic Hg & Na tetra(n-propyl)borate*

Material	Total # of Elements	Total ppm	Major Impurities
PS	8	4	Na, Ti, Al
Teflon-TFE	24	19	Ca, Pb, Fe, Cu
LDPE	18	23	Ca, Ti, Zn
PC	10	85	Cl, Br, Al
PMP	14	178	Ca, Mg, Zn
Teflon-FEP	25	241	K, Ca, Mg
Borosilicate Glass	14	497	Si, B, Na
PP	21	519	Cl, Mg, Ca
HDPE	22	654	Ca, Zn, Si

Why are we slow to adapt to Speciation?

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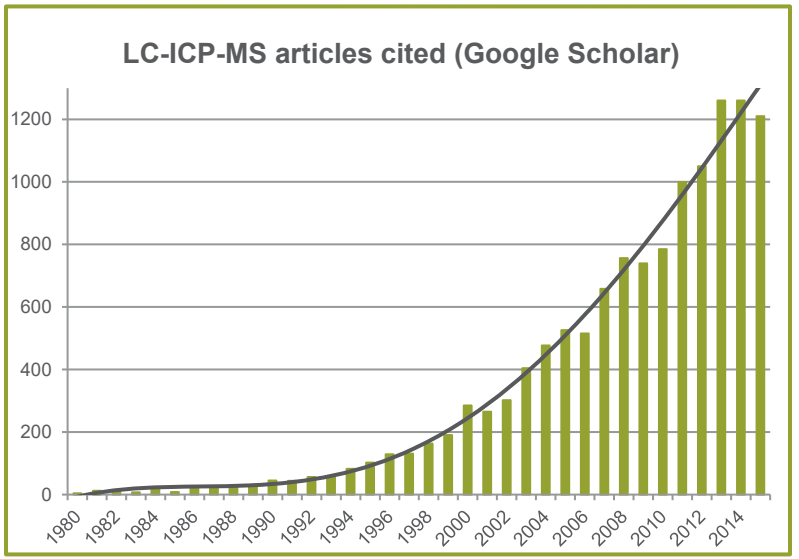
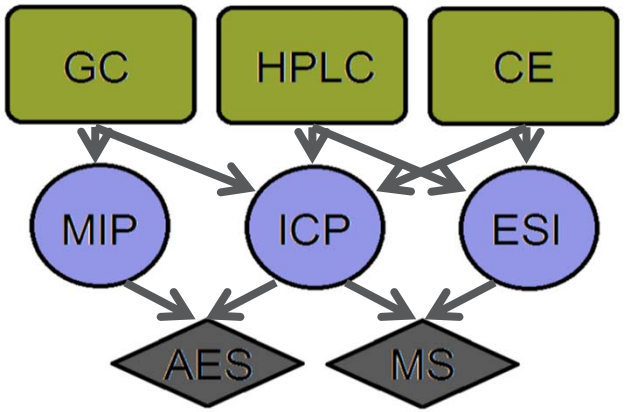
- Specialized analysis: Hyphenated technologies
  - *LC-ICP-MS most common analysis*
  - *Other analysis: CE, GC etc.*
- Analysis can change or inaccurately quantitate distribution
  - *Chromatographic Separation*
    - Co-elution or loss of species to void volume
    - Column yield should be checked by mass balance with total element concentration
  - Techniques causes distribution changes
    - HPLC: Sb & As species transform via oxygen in the mobile phase & interaction of species with the mobile phase
    - GC: Phenyl mercury salts to Diphenyl mercury
    - ESI-MS: redox reactions at ESI source





Why are we slow to adapt to Speciation?

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- Limited number of clear directives, regulations and procedures
  - *Most regulations concern organotin & Cr (VI)*
  - *New regulations for pharmaceuticals*
  - *Some regulations drinking water regulations*
- Lack or limited reference materials
  - *Chemical species distribution must be*
    - Stable: validated stability
    - Homogeneous
    - Short-term & Long-term must be validated
  - *Changes in temperature, time, pH, other elements can effect distribution*
  - *Specialized equipment for analysis*
  - *New methods need development & validation*

Inorganics Instrumentation: Start

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- HPLC: Agilent 1260 Series HPLC
  - *Proprietary Method*
- ICP-MS: Agilent 7500
  - *RF power: 1550*
  - *Carrier gas flow rate: 1.05 ml/min*
  - *Plasma gas flow rate: 15 ml/min*
  - *Auxiliary gas flow rate: 0 ml/min*
  - *Nebulizer: Micro Mist*
  - *Spray Chamber: 2 C*
  - *Interface cones: Nickle Cones*
  - *Integration Time: 0.5 seconds*

As Calibration

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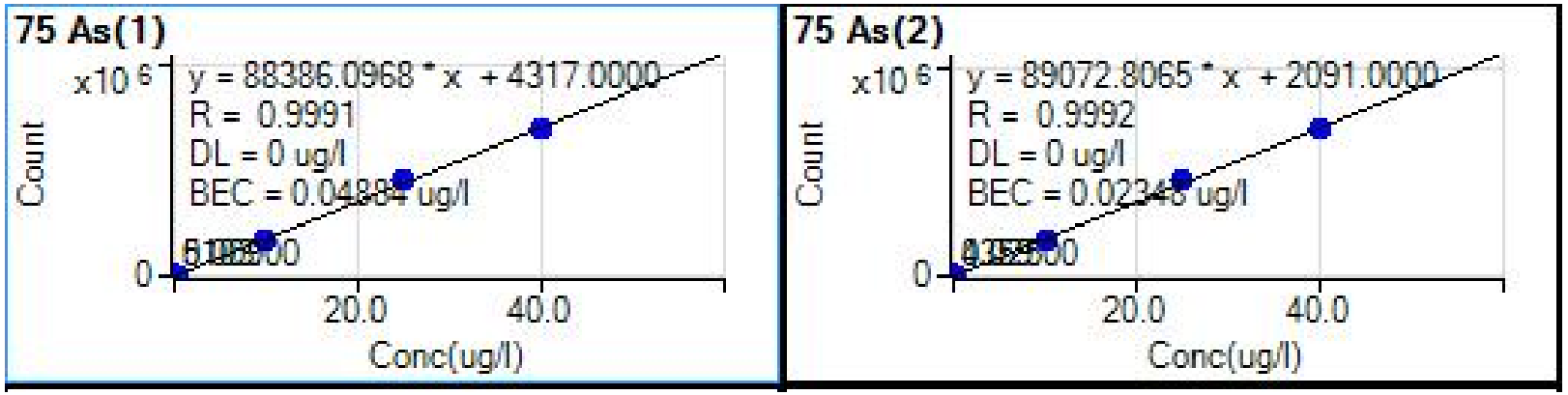

Inorganic Speciation of Arsenic

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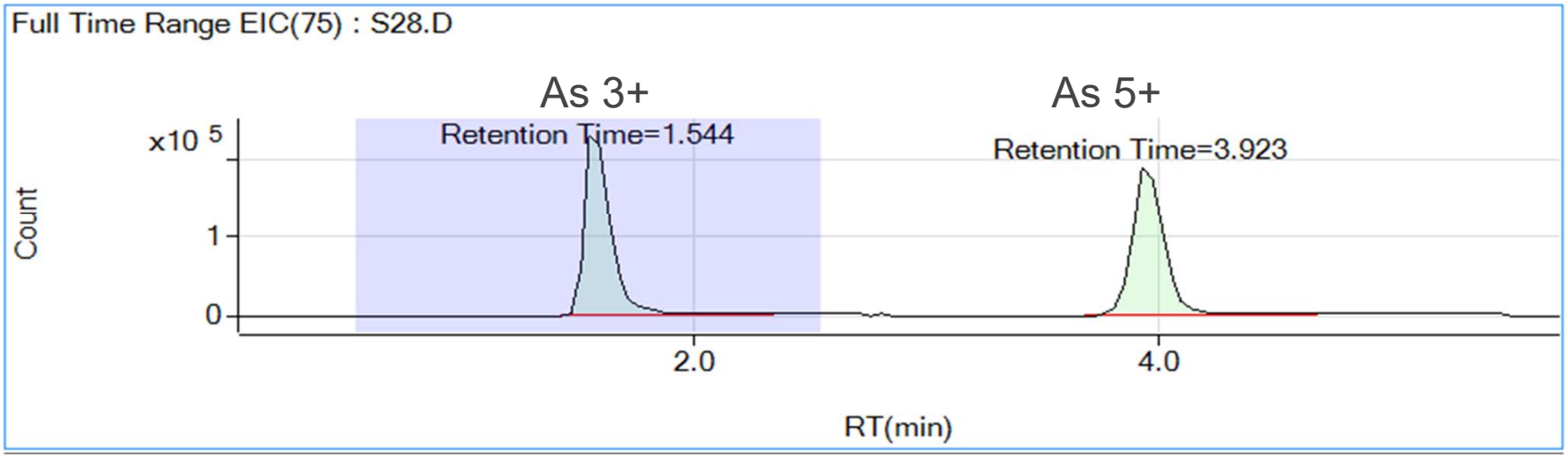
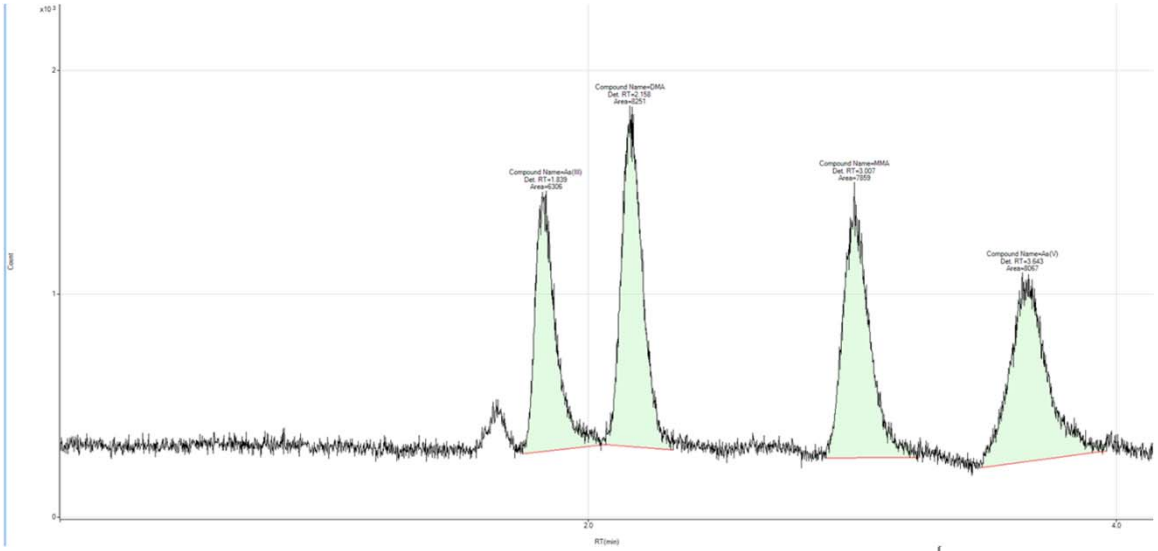
Arsenic





MMA/DMA

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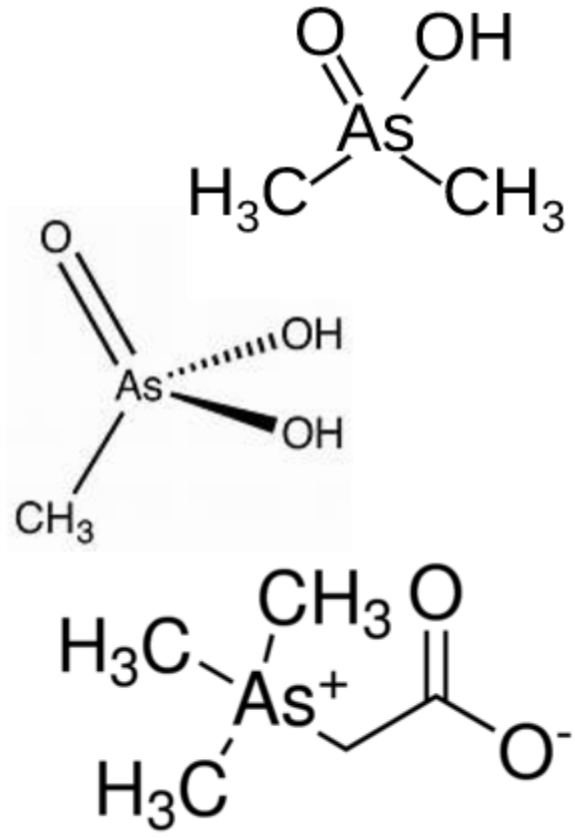
- Organic arsenic species; not as clearly separated
  - Dimethyl arsenic acid – (DMA)
  - Monomethyl arsenic acid – (MMA)
  - Arsenobetaine
  - Arsenocholine
  - *Many other As species*
- Not well characterized with LC-ICP-MS



Change to LCMS system

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- Project and product changes forced decommission of LC-ICP-MS
  - *Arsenic species standards discontinued*
- Continued need for organic arsenic species standards with arsenic impurities
  - *Food Industry*
    - SPEX Certiprep study: As in Cider
  - *Water*
- Project started to convert to LCMS

Instrumentation

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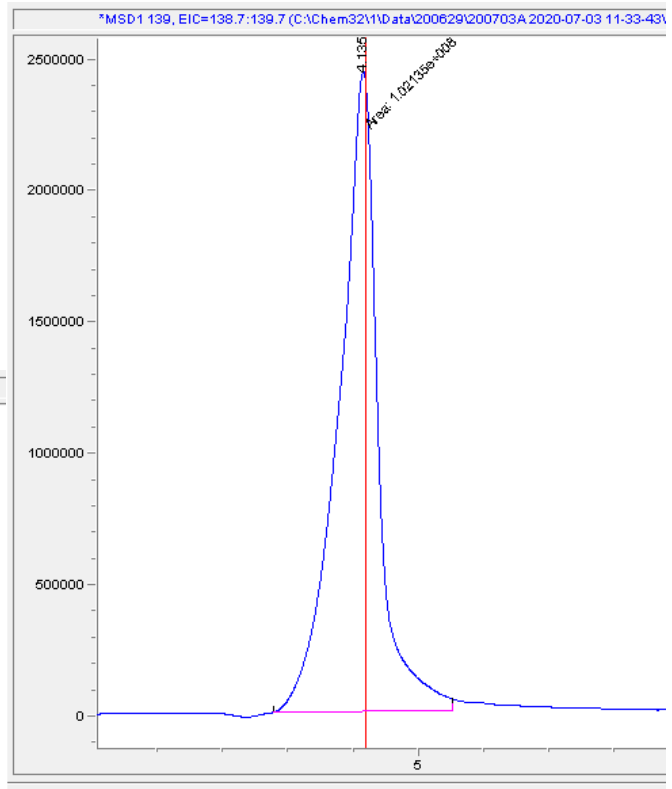
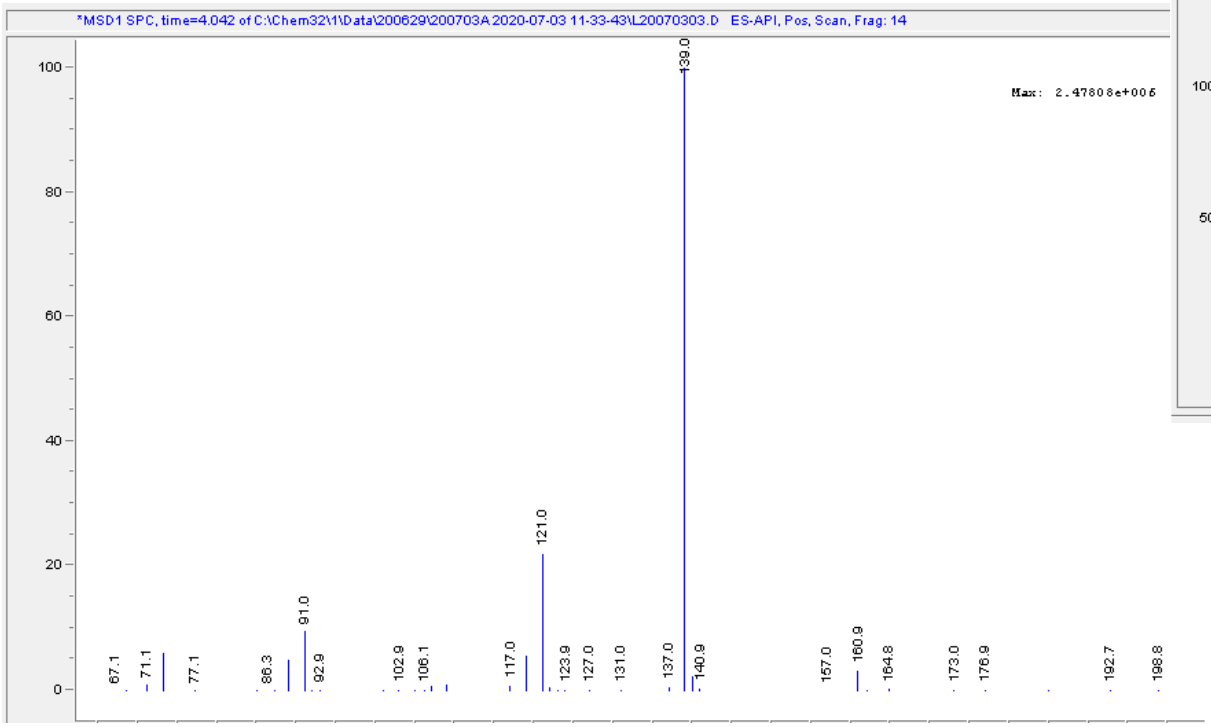
- HPLC: Agilent 1260 Series HPLC
  - *Method develop in progress*
    - Columns being tested
      - **Speciation column**
        - » **80% WATER/20% ACN + 0.05% FA ISOCRATIC**
      - C18
      - HILIC
- HPLC-MS: Agilent LCMS
  - *MS Detector Settings:*
    - Fragmentor – 140
    - Drying Gas Flow – 12 L/min
    - Capillary Voltage – 1500
- Flow injection analysis to optimize ionization for LCMS ESI

DMA

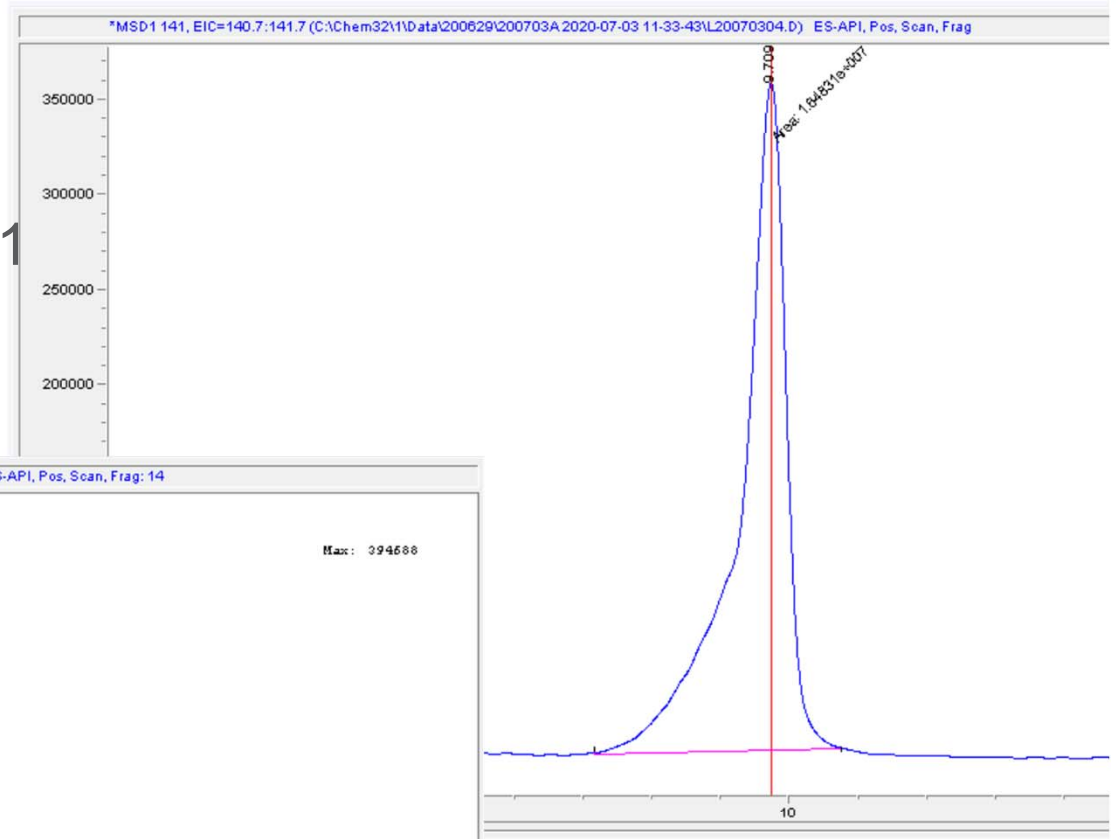
- Molecular weight 138; not just 75 for Arsenic
- Good response
- Unique spectrum: Base 139 m+1; no 75

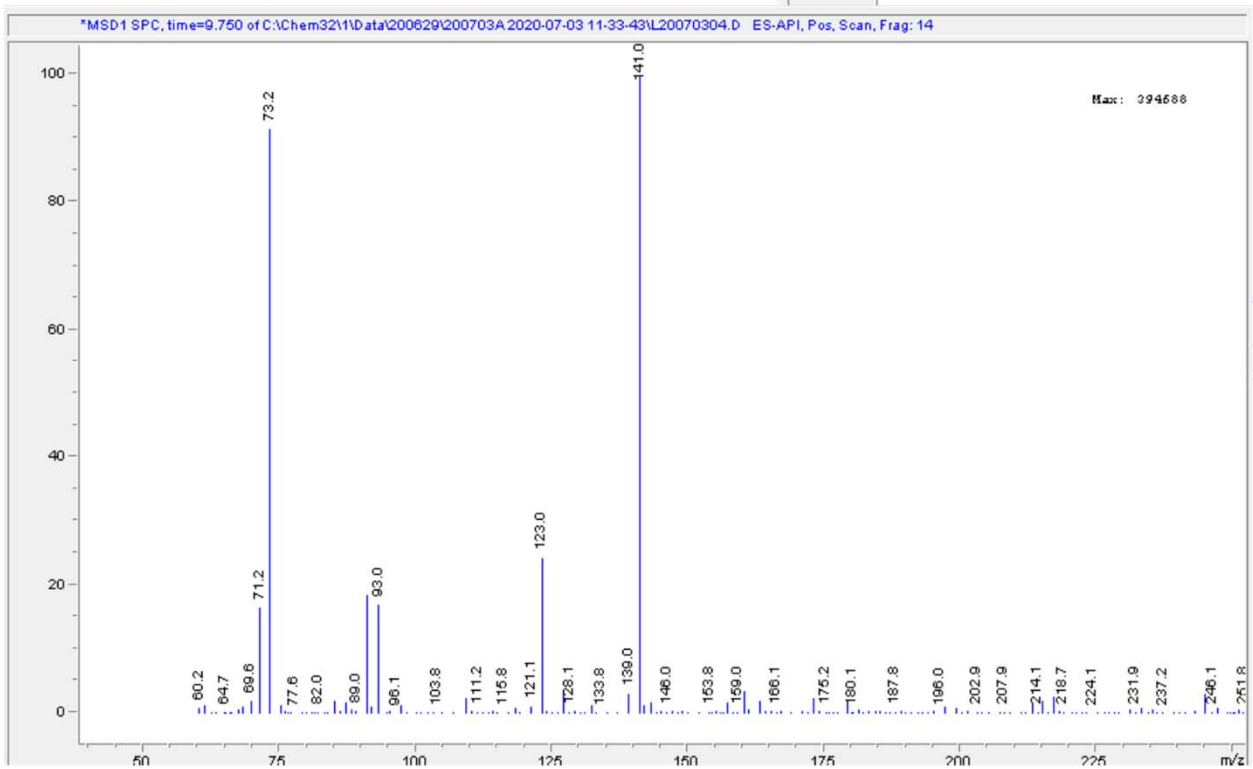




MMA

- Molecular weight 140;
- Good response
- Unique spectrum: Base 141 m+1; no 75; but 73





DMA vs. MMA

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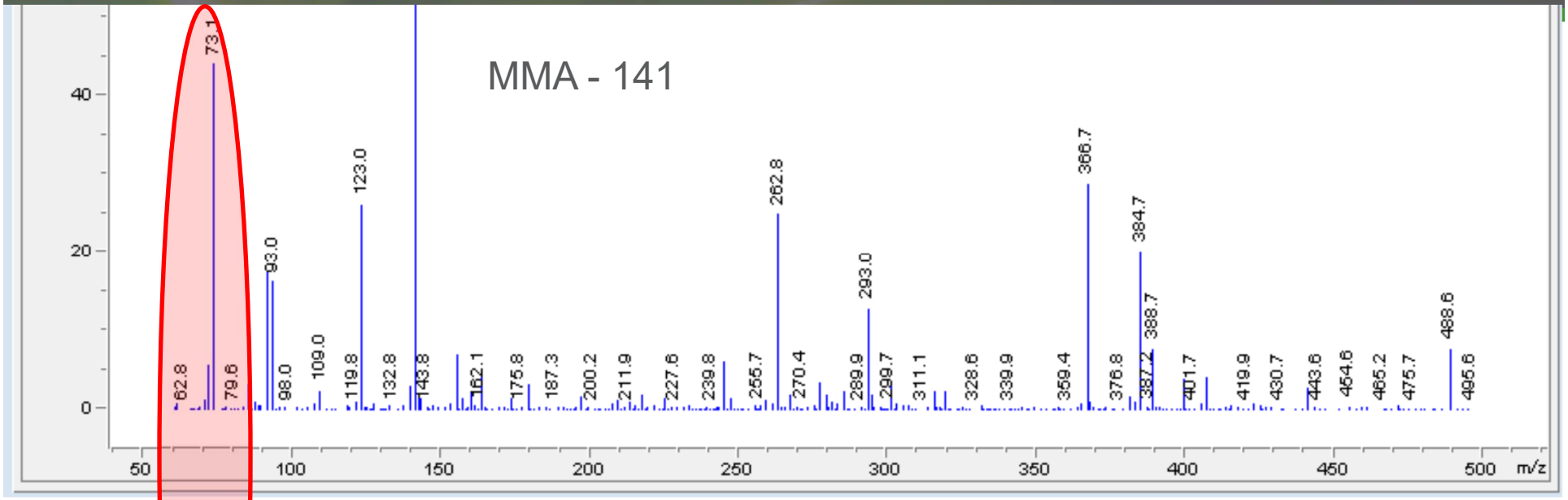

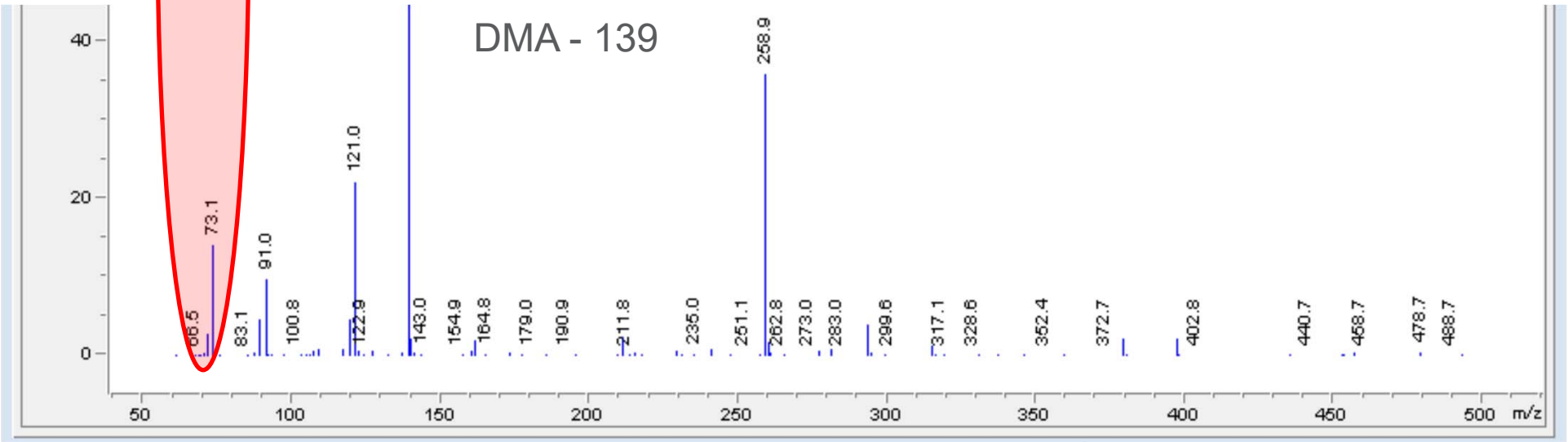

As (III)

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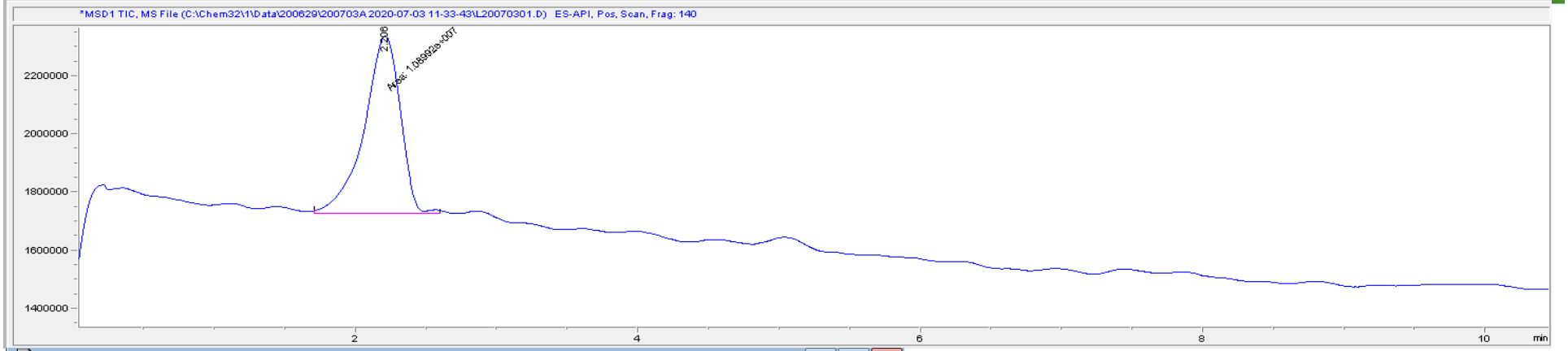

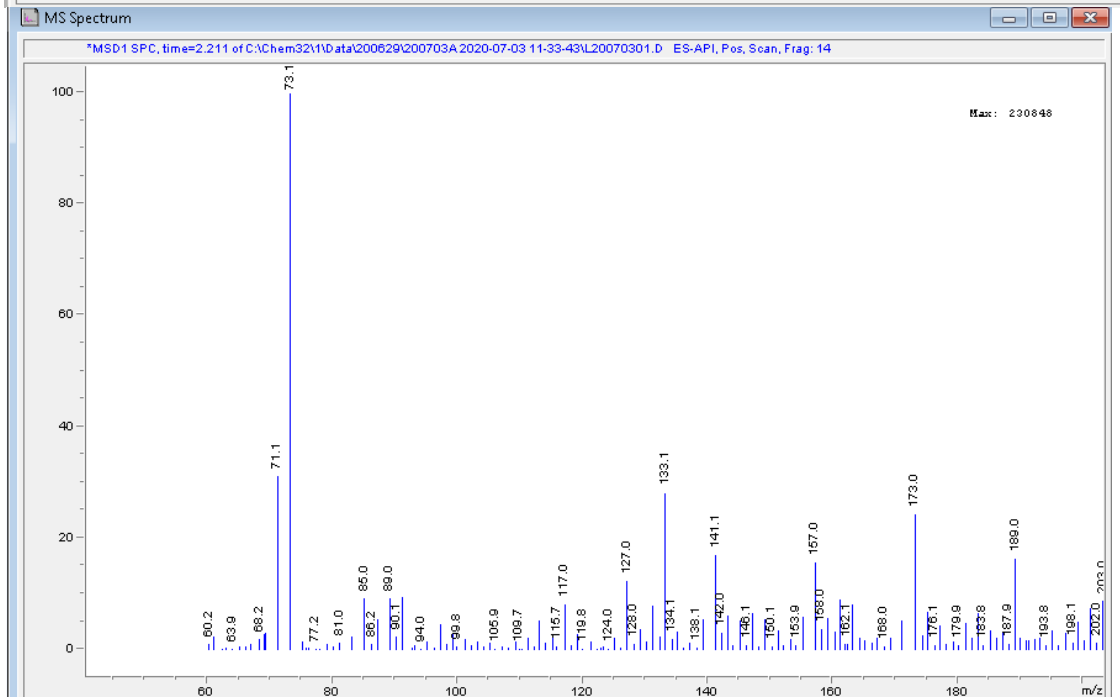

- Molecular wt: 75
- Good response; but unretained
- Unique spectrum: 73

As (V)

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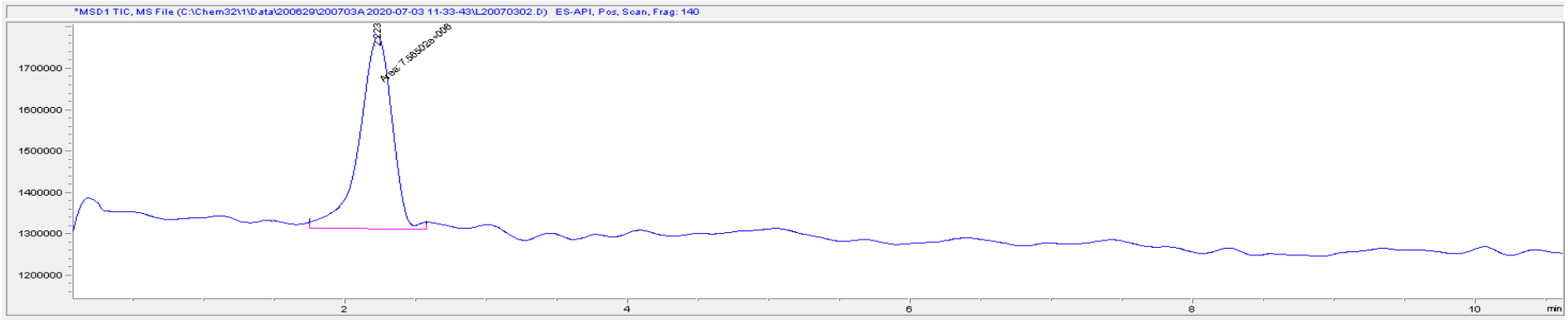

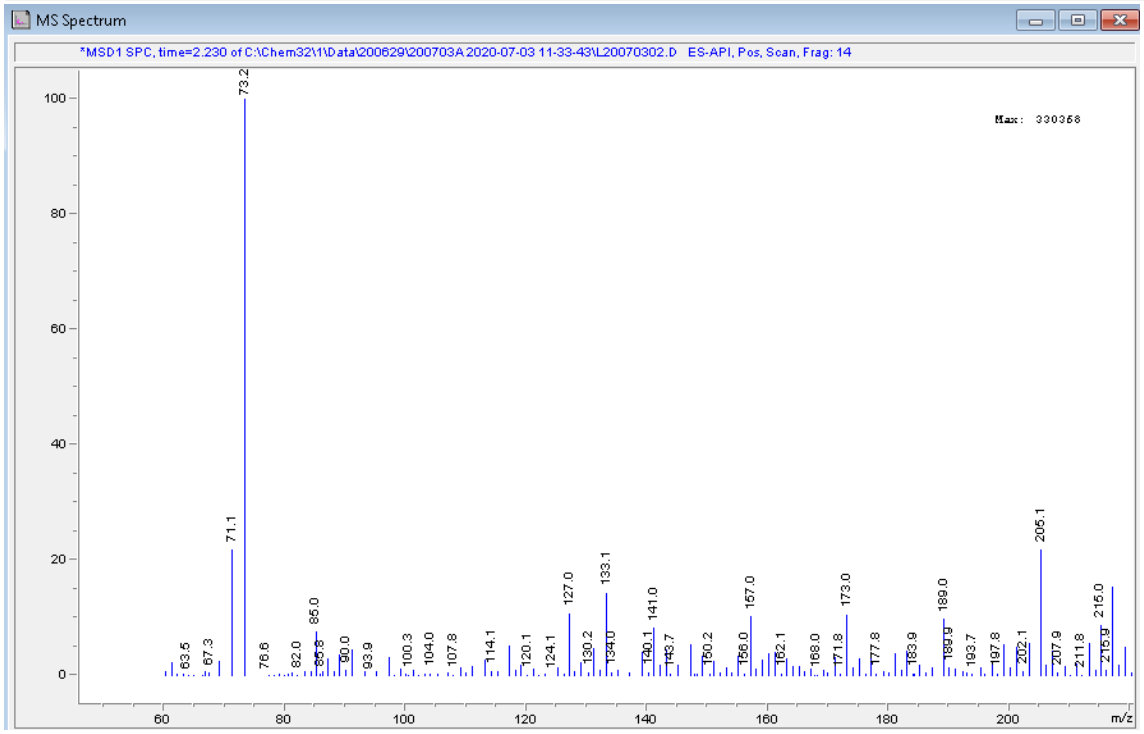

- Molecular wt: 75
- Good response; but unretained
- Unique spectrum: 73

As (III) & As (V) spectrums

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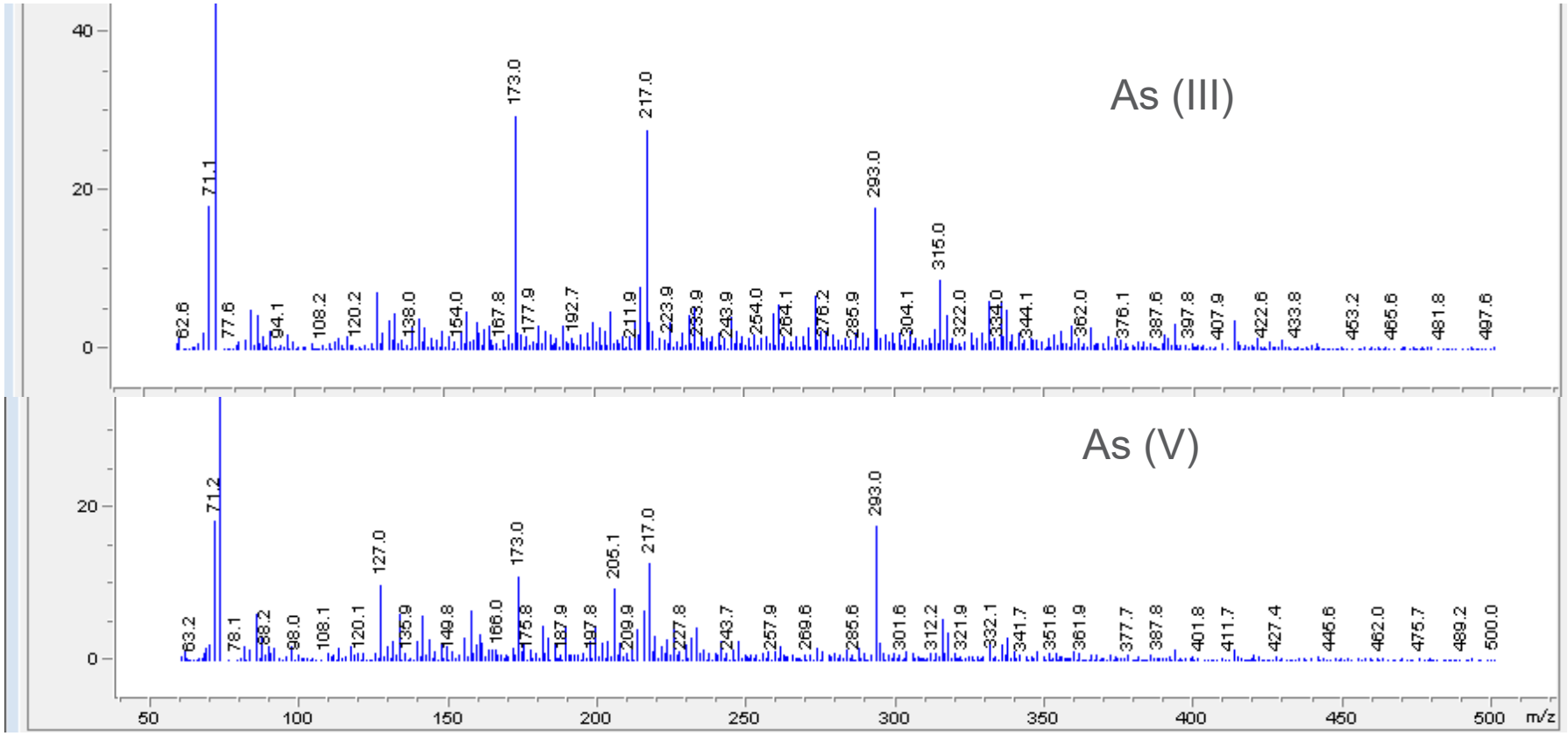

Impurities

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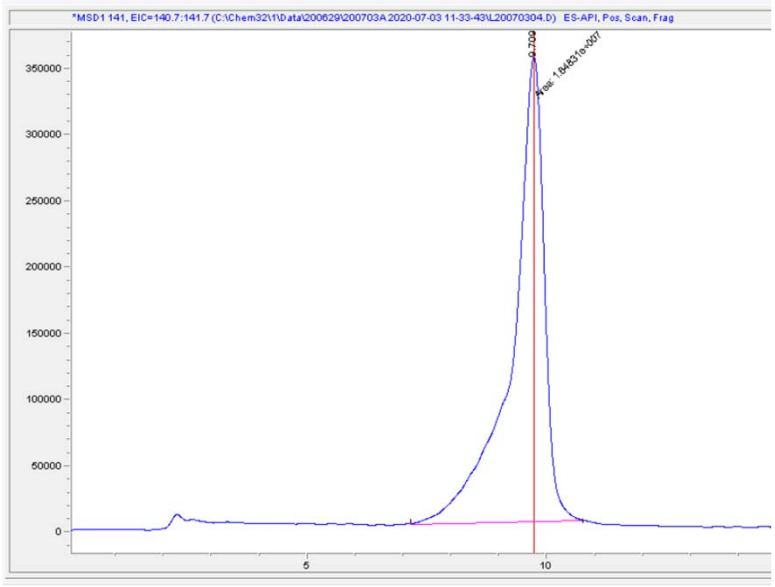
- One question was to find if MMA had DMA, As III and As V impurities and vice versa.
- Current work shows little to no impurities interference
  - *Some small spectral overlaps to investigate but no significant EIC at current retention times.*



Future Work

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- Column and mobile phase changes to push for inorganic retentions
- Clean up spectrum to use for quantitation and ID
- Reintroduction of DMA and MMA standards with certified impurities

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Questions?

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