Simultaneous Optimization of Sulfur and Arsenic Analysis for Organo-Thioarsenic Species Determination using HPLC Coupled to ICP-Triple Quadrupole MS

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Outline

- The study of sulfur and arsenic in biological systems, why?
- Challenges of simultaneously measuring sulfur and arsenic by conventional ICP-MS
- Introduction to ICP-MS/MS for elimination of common interferences on sulfur and other elements
- Application of MS/MS technology using ICP triple-quad MS to the simultaneous measurement of As and S in HPLC
- Preliminary determination of sulfur and arsenic species in *Zea mays* exposed to arsenic as As(V)
- Future work

Background

- All studied living organisms have mechanisms for arsenic detoxification
- Arsenic, having highly toxic species, has a strong affinity for sulfur
- Sulfhydryl groups are known to play a key role in bio-accumulation/sequestration and bio-transformation of Arsenic
- Arsenite binds to a variety of sulfur-rich peptides and proteins, such as glutathione, metallothionein, actin and tubulin, galectin I thioredoxin peroxidase II, and other macromolecular constituents of tissues (Hansen et al, 2004)
- ICP-MS coupled to HPLC has been the traditional method for determination of Thioarsenical species
- However, S-As compounds have been difficult to detect and identify in biological matrices – most likely due to poor stability of many S-As species and difficulty of measuring sulfur and arsenic together at sufficiently low detection limits (Raab et al, 2007).

Challenges using conventional ICP-MS

Both sulfur and arsenic suffer from common, intense polyatomic interferences.

Element	Isotopes	Interferences
arsenic	75	ArCl, CaCl, Nd^{++}, Sm^{++}
sulfur	32,33,34	$^{16}O_2$,$^{16}O^{17}O$,$^{17}O_2$,$^{16}O^{18}O$,$^{18}O^{14}N$, $^{17}O^{15}N$,$^{18}O^{15}N$

Some of the interferences can be removed effectively using He collision mode i.e. ArCl and CaCl on As.

Doubly-charged interferences (Nd^{++}, Sm^{++}) on As cannot be removed with traditional ICP-MS techniques

Some can be removed using Xe reaction mode, i.e. O_2 interferences on S.

But, the interferences on sulfur aren't removed well by He collision mode and As doesn't recover well in Xe mode – therefore simultaneous determination in narrow LC peaks is highly compromised.

What about mass-shift mode using O_2 in the cell with conventional (SQ) reaction cell ICP-MS?

Both As and S can be measured at their respective oxides using mass shift mode with oxygen to form AsO (mass 91) and SO (mass 48,49, 50)…

But…

What about other (new) interferences at those masses (91, 48, 49, 50)?

Analyte	mass	interferences
AsO	91	^{91}Zr and multiple possible polyatomic ions depending on matrix
SO	48, 49, 50	Ti (48, 49, 50) interferes with all 3 sulfur isotopes. ^{50}V and ^{50}Cr interfere with $^{34}S^{16}O$ and ^{48}Ca interferes with $^{32}S^{16}O$ etc.

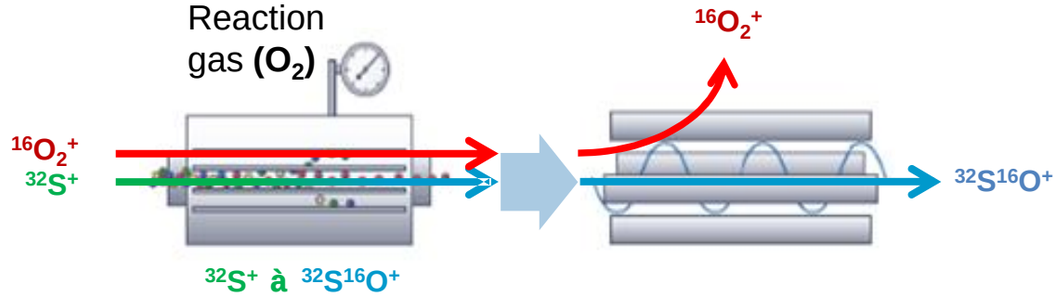
Mass shift mode using oxygen isn't reliable in complex, unknown or variable matrices using conventional (SQ) reaction cell ICP-MS

ICP-QMS With CRC: O_2 Reaction Mode to Avoid $^{16}O_2^+$ Interference on $^{32}S^+$

Reaction using O_2 cell gas to avoid $^{16}O_2^+$ overlap on $^{32}S^+$

$^{32}S^+ + O_2$ <cell gas> à $^{32}S^{16}O^+$ (mass 48)

$^{16}O_2^+ + O_2$ à no (or very slow) reaction



No way to control ions that enter the cell

Quad (m/z 48) – allows through only ions at m/z 48

$^{32}S^+$ reacts with O_2 cell gas to form $^{32}S^{16}O^+$ product ion at m/z 48. Original O_2^+ interference doesn't react with O_2 cell gas and remains at m/z 32.

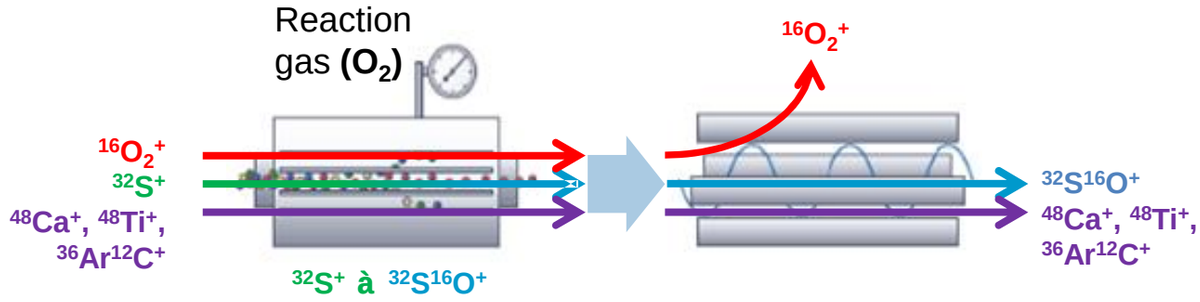
Problem Solved? Not quite! With ICP-QMS, Other Overlaps May Occur on $^{32}S^{16}O^{+}$ Product Ion Mass

Avoiding $^{16}O_2^{+}$ overlap on $^{32}S^{+}$ with O_2 cell gas

$^{32}S^{+}$ + O_2 <cell gas> à $^{32}S^{16}O^{+}$ (mass 48)

$^{16}O_2^{+}$ + O_2 à no (or very slow) reaction

$^{48}Ca^{+}$, $^{48}Ti^{+}$, $^{36}Ar^{12}C^{+}$ + O_2 à no (or incomplete) reaction



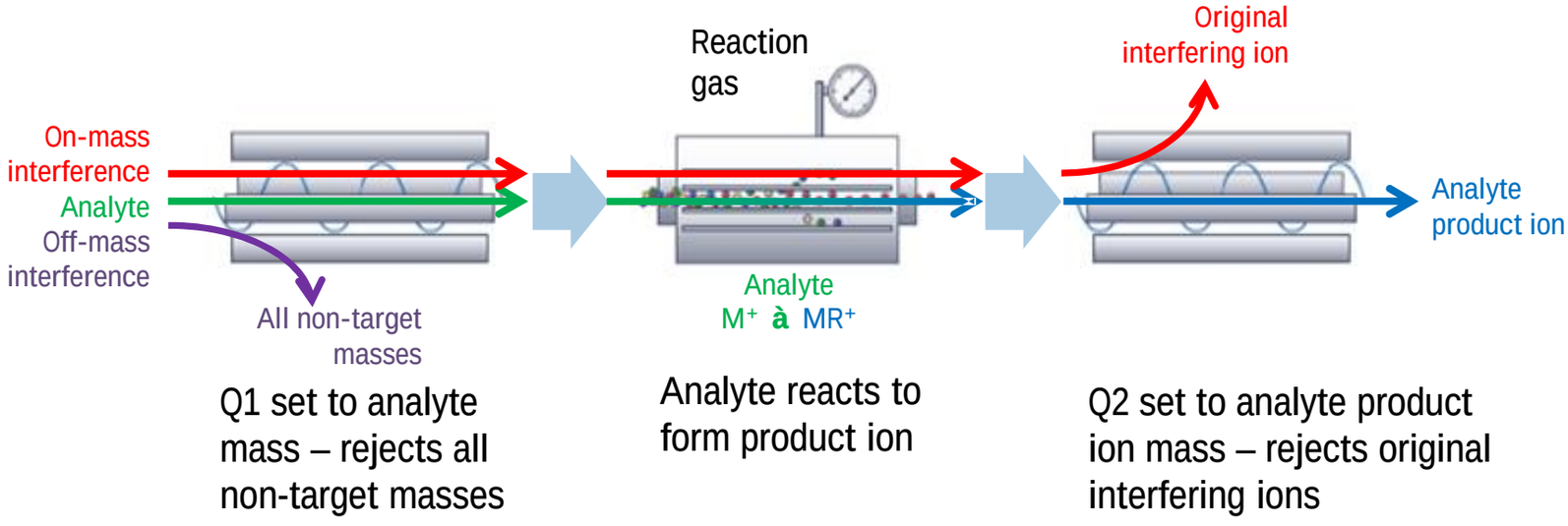
No way to control ions that enter the cell

Quad (m/z 48) – allows through **all** ions at m/z 48

O_2^{+} polyatomic can be removed, but other co-existing ions may occur at SO^{+} product ion mass (m/z 48). These cannot be removed/rejected in ICP-QMS.

Mass-shift mode using ICP-MS/MS (reactive analyte such as S or As)

2. Mass-Shift Measurement: <u>Reactive analyte</u> reacts with chosen cell gas, is moved to a new product ion mass and can be separated from <u>unreactive interferences</u>. No existing ions can overlap new analyte product ion, as all non-target masses are rejected by Q1



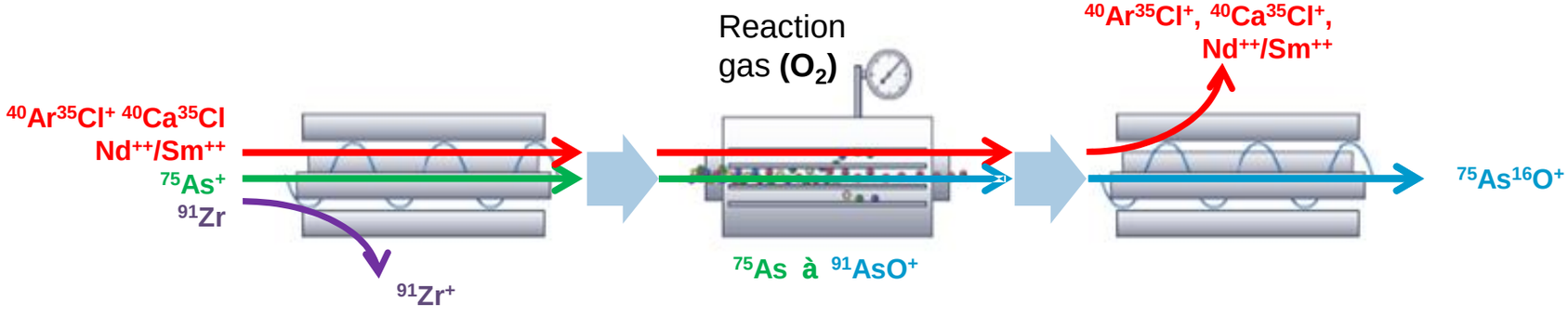
With ICP-MS/MS, Q1 rejects all non-target masses, ensuring no existing ions (analyte, matrix, or polyatomic) can overlap new analyte product ion

ICP-MS/MS Mass-Shift mode for As with O_2 Cell Gas

$^{75}As^+ + O_2$ <cell gas> à $^{91}(AsO)^+$

$^{40}Ar^{35}Cl^+$, $^{40}Ca^{35}Cl^+$, Sm^{++}, $Nd^{++} + O_2$ à no reaction

Q1 rejects $^{91}Zr^+$ ions that would overlap AsO^+ at mass 91



Q1 set to m/z 75, so rejects all ions except m/z 75. ^{91}Zr at mass 91 is rejected

As^+ reacts with O_2 cell gas to form AsO^+ product ion. $^{40}Ar^{35}Cl^+$, $^{40}Ca^{35}Cl^+$, Nd^{++}/Sm^{++} don't react and stay at m/z 75

Q2 set to m/z 91, AsO^+ product ion mass – rejects original on-mass interferences

Allows measurement of AsO^+ at product ion mass, after removal of original $ArCl^+/CaCl^+/REE^{++}$ interference, and $^{91}Zr^+$ overlap on AsO^+ product ion mass

Confirmation of interference removal for As in various reference materials by ICP-MS/MS

As measured as $^{91}AsO^{+}$

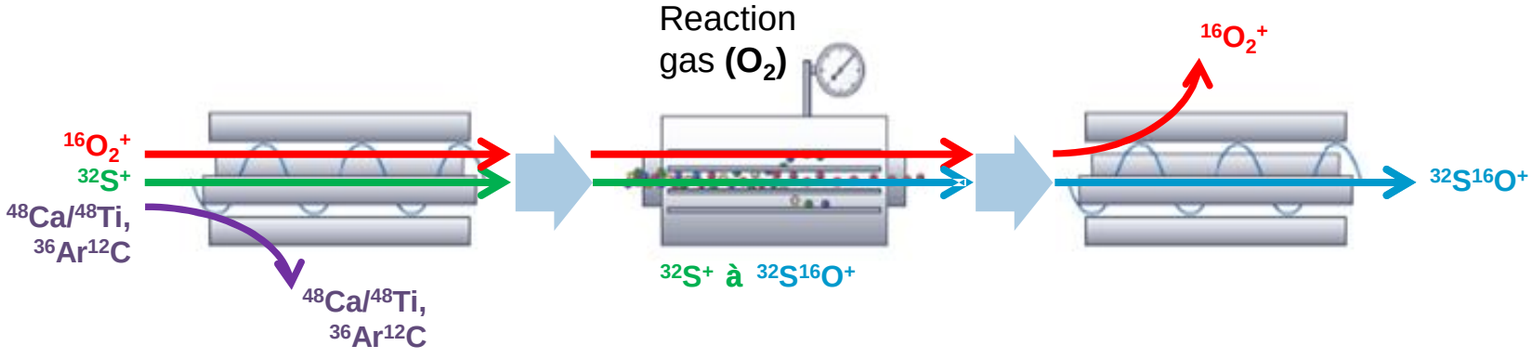
	Certified (ng/mL in Solution)	Measured As 75 -> 91	
Sample Name	As	Conc.	recovery
NIST 1573a Tomato	0.47	0.50	1.07
NIST 1573a Tomato	0.43	0.47	1.10
NIST 1575a Pine needles	0.16	0.16	1.00
NIST 1575a Pine needles	0.16	0.16	1.01
NIST 1515 apple	0.16	0.16	1.01
NIST 1515 apple	0.16	0.16	1.01
NIST 1643 e water	5.9	5.97	1.01
NIST 1643 e water	5.9	5.92	1.00
JSAC 0302-3 river water	0.52	0.49	0.95
JSAC 0302-3 river water	0.52	0.50	0.96
JSI sedimentary rock	14.6	13.59	0.93
JSI sedimentary rock	14.6	13.50	0.92
NIST 1566a oyster	32.97	31.66	0.96
NIST 1566a oyster	36.25	34.69	0.96
NCS zc 81002 hair	1.43	1.38	0.97
NCS zc 81002 hair	1.43	1.37	0.96
NIST 2976 mussel	13.43	14.87	1.11
NIST 2976 mussel	13.43	14.05	1.05
NIST 1646a sediment	17.13	14.51	0.85
NIST 1646a sediment	17.13	14.45	0.84

Sulfur isotopes measured under the same conditions

$^{32}S^{+}$ + $^{16}O_2$ <cell gas> à $^{32}S^{16}O^{+}$ (mass 48)

$^{16}O_2^{+}$ + O_2 à no reaction

Q1 rejects other ions that would overlap SO^{+} at mass 48



Q1 (m/z 32) – rejects all ions apart from m/z 32

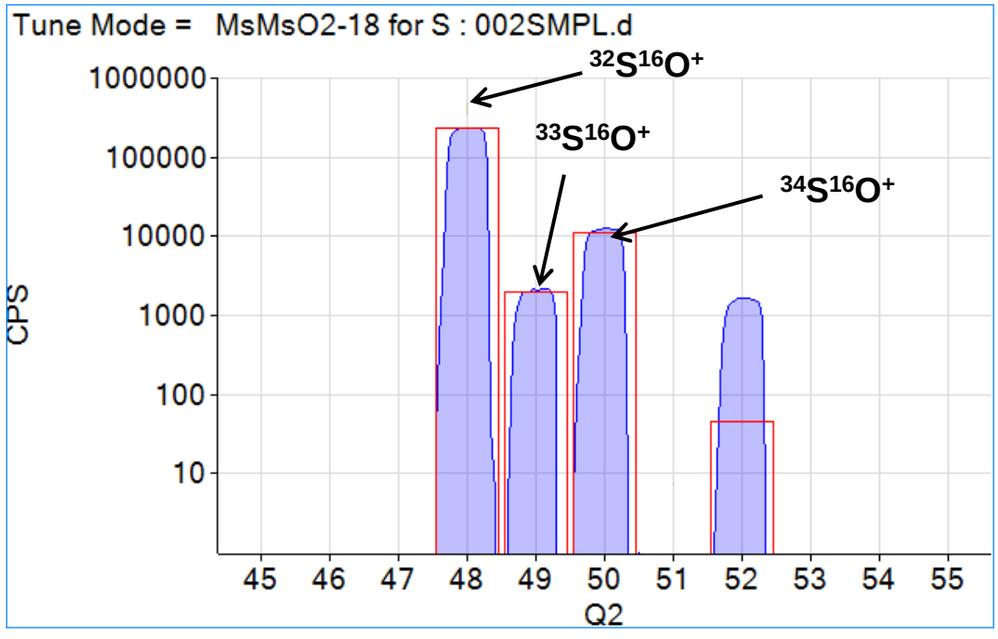
Q2 (m/z 48) – rejects all ions apart from m/z 48

If Q1 and Q2 are synchronously scanned (32/48, 33/49, 34/50), then sulfur isotope accuracy is maintained as well.

Sulfur – Measured as SO^+ in O_2 Mode

Isotopic template match for ^{32}S, ^{33}S, ^{34}S (~ 30ppb S)

High background at 52 is due to $^{36}Ar^{16}O$. S isotope at m/z 36 (SO at m/z 52) is too low to be analytically useful (0.02% relative abundance)



Q1 – Q2 mass difference is 16, so only the + ^{16}O transition is measured

Ensures S isotope abundance is maintained – no overlap from $^{32}S^{18}O^+$ on $^{34}S^{16}O^+$, for example

Applying ICP-MS/MS to real samples for simultaneous determination of S and As

HPLC Conditions	
HPLC	Agilent 1260
solvent A	0.1 % (v/v) formic acid in water
solvent B	0.1 % (v/v) formic acid in MeOH
gradient	linear from 0-20 min, 0-20 % solvent B, 10 min at 20 % solvent B
column temperature	ambient
sample volume	0.1 mL
ICP- MS/MS Conditions	
ICP-MS	Agilent 8800 triple quadrupole ICP-MS/MS
Plasma Power	1550 W
Carrier Gas	0.9 L/min
Sampling Depth	8 mm
O_2 Cell Gas	0.4 ml/min

Comparative calibrations in mobile phase

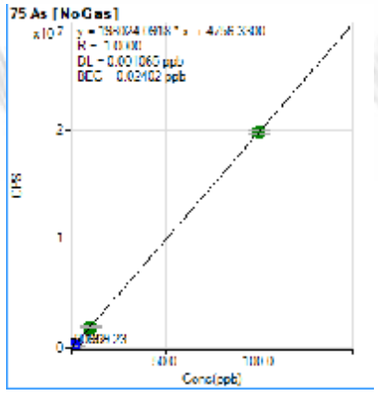
S and As single quad mode (no gas and He) vs MS/MS mode with O_2 mass shift

As BEC and DL is almost the same in all 3 modes (DL ~1-2 ppt) because there are no interferences in the calibration mobile phase.

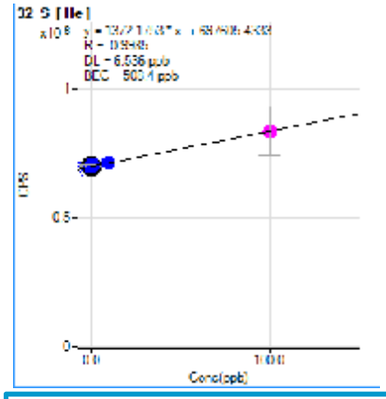
Sulfur improves from not measurable in no gas mode, to 6.5ppb in He mode to <40ppt in MS/MS mode

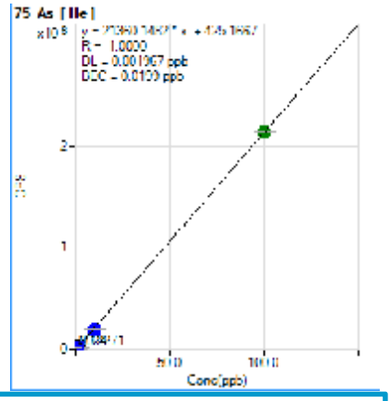
No gas mode

^{32}S
Not possible



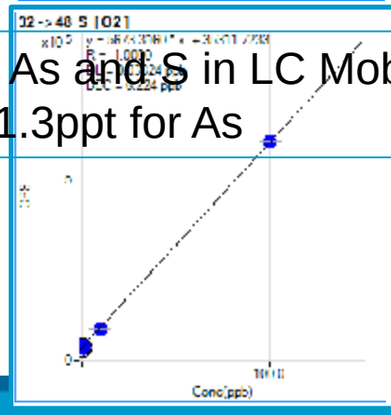
He mode

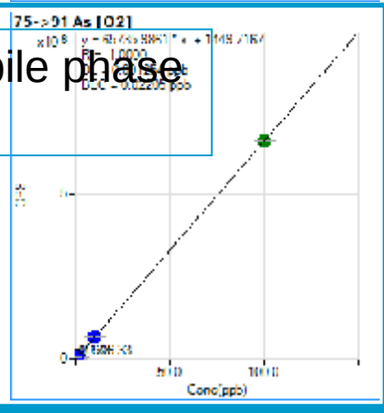




Simultaneous DL for As and S in LC Mobile phase 38ppt for sulfur and 1.3ppt for As

MS/MS mode





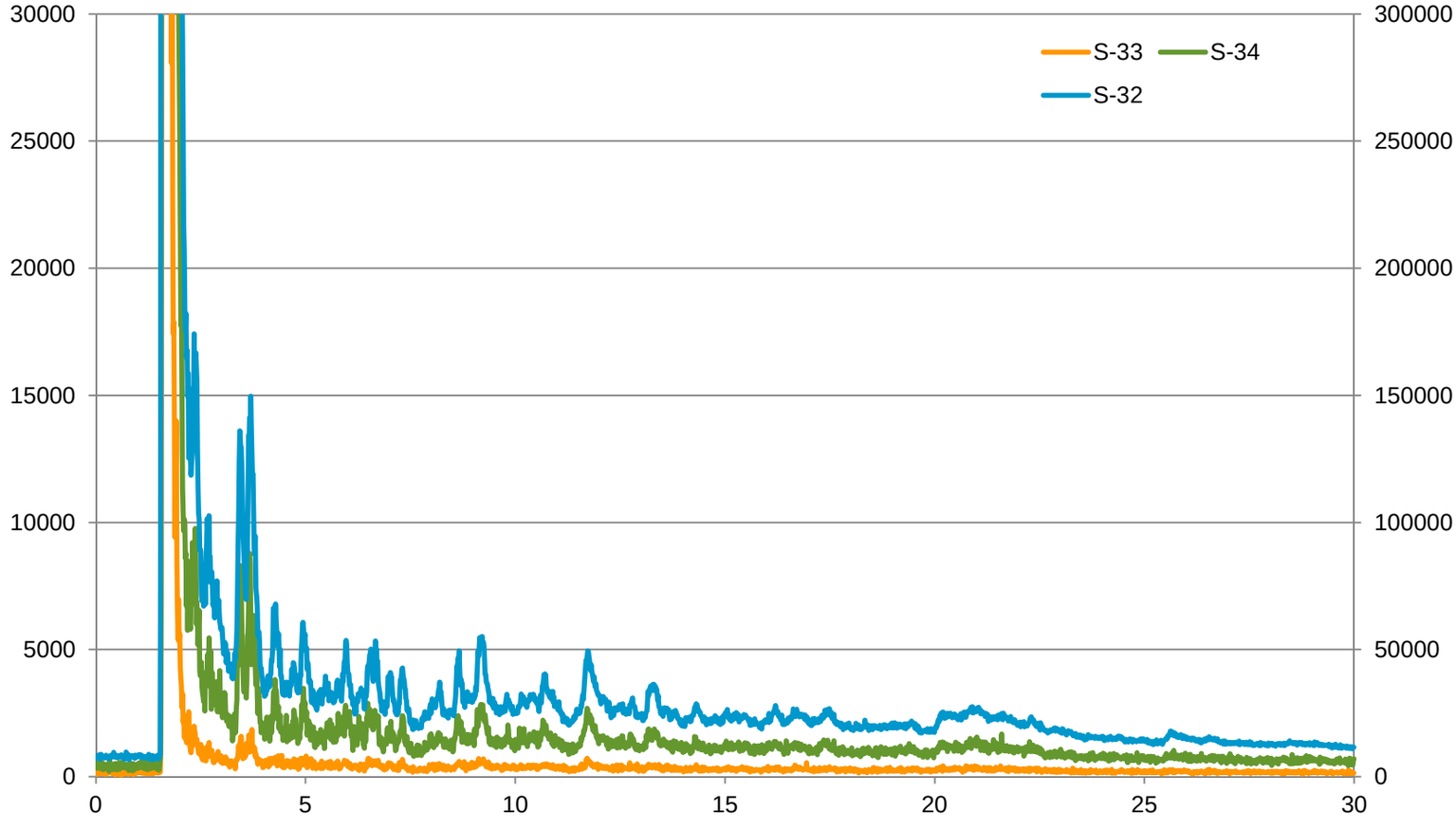
Cultivation of Arsenic containing Zea mays

Maize (Zea mays) plants were grown from seed for 12 weeks in Vermiculite and fertilized once a week except in the last week, when no fertilizer was used.

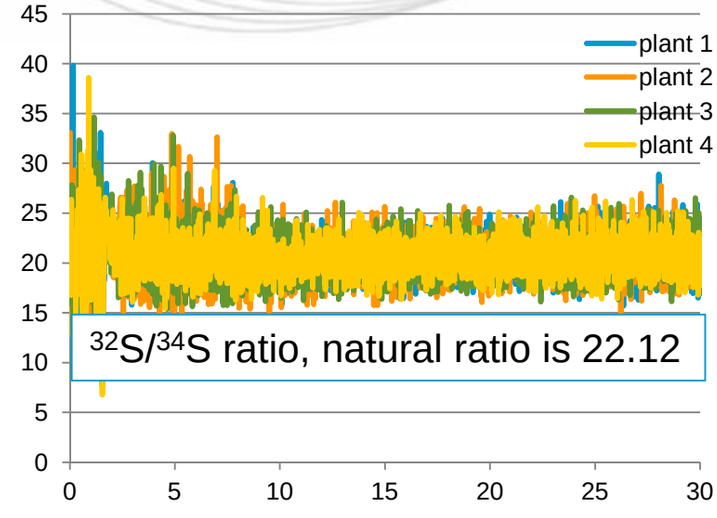
The roots were freed of Vermiculite before the plants were exposed to arsenic in the form of As(V) for 48 h.

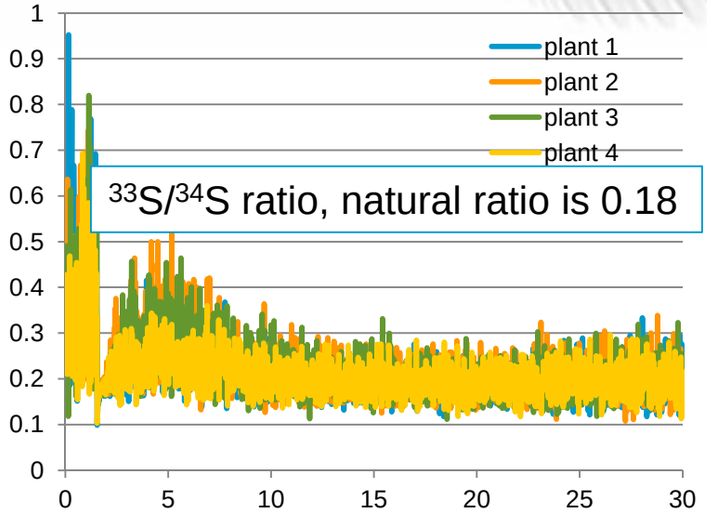
After the incubation period, the plants were separated into root and shoot. Each part of the plant was ground separately under liquid nitrogen and extracted with 1% formic acid (solid/liquid1:3) for 30 min at 0C. After that, the extract was filtered (0.45 mm) and injected onto the HPLC column.

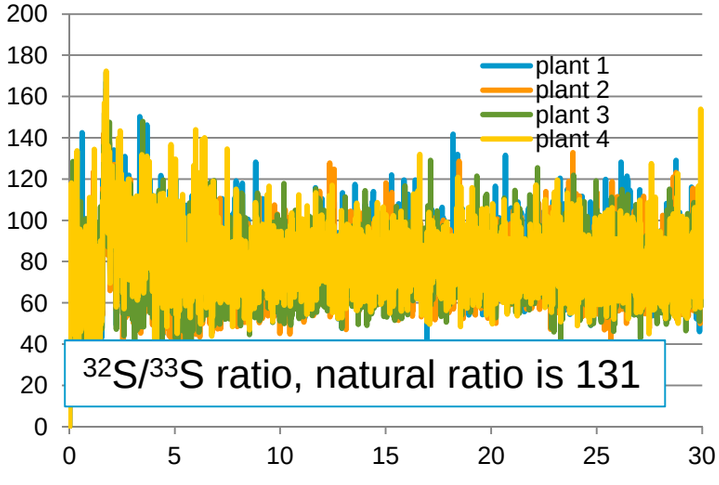
Plant 1 – sulfur isotope chromatograms



Measurement of sulfur isotope ratios in Maize extracts



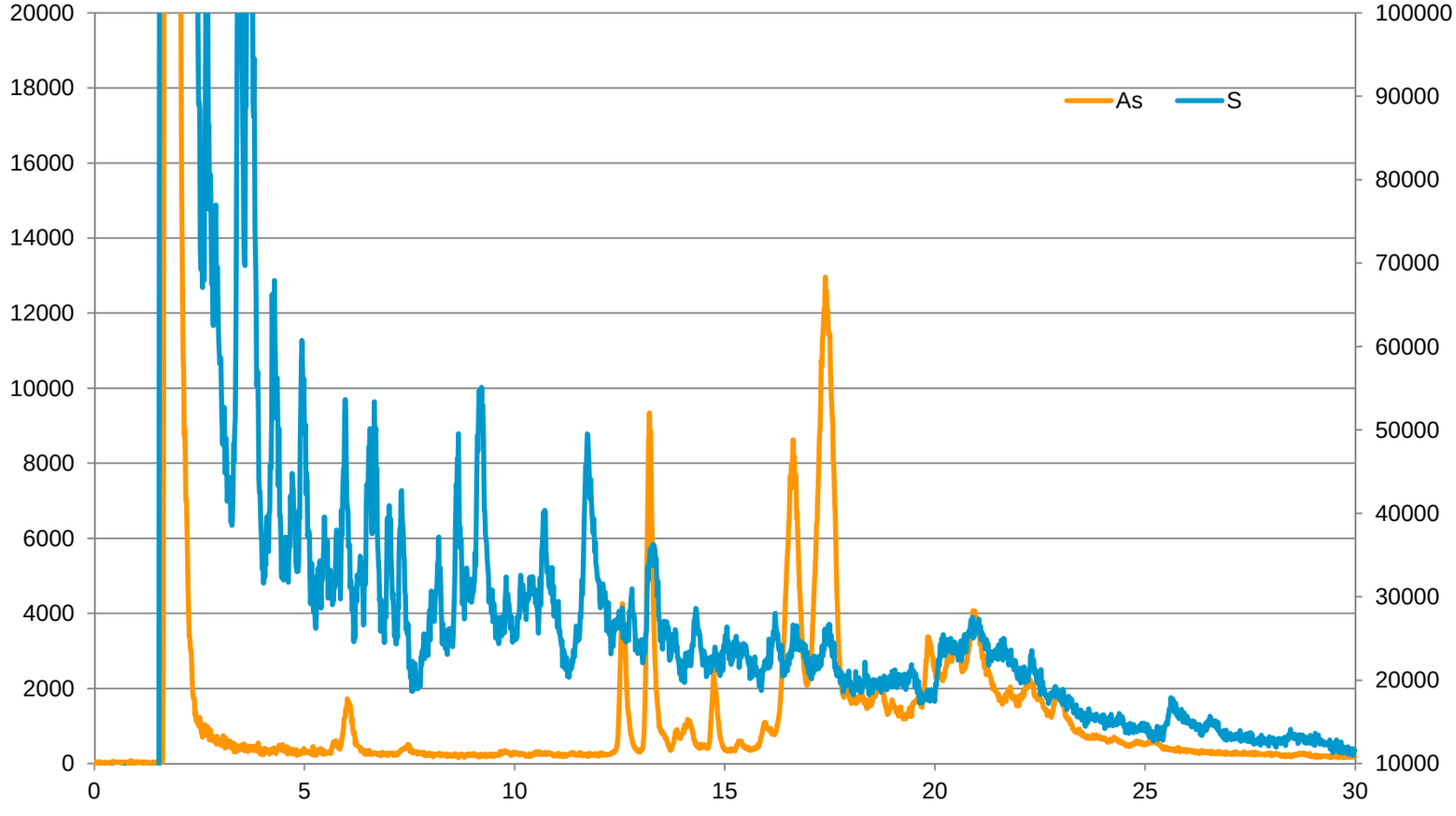




32/34 and 33/34 ratios are close to expected.

32/33 is lower than expected due to low abundance for ^{33}S (0.76% relative abundance and noise at *m/z* 33

Plant 1 – simultaneous determination of ^{32}Sulfur and arsenic as SO and AsO



Conclusions

- Reliable and simultaneous analysis/measure of As and S at low ppt in **complex sample matrix**
- Sulfur IR (^{32}S and ^{34}S) can accurately be measured, validating sulfur determination.
- ID of various known sulfur species?
- Compound Independent Calibration (CIC)
- By eliminating possible interferences on AsO and SO via the first quadrupole of the ICP-QQQ, oxygen reaction mode can be reliably used to simultaneously measure arsenic and sulfur at ppt concentrations in complex mixtures of plant extracts and LC mobile phase.

Future work

- Current procedure for preparing plant extracts results in very “peak rich”, complex chromatograms, especially for sulfur species. Additional clean up, or improved chromatographic resolution will be required to positively identify the compounds using alternative MS techniques such as electrospray MS.
- Combining parallel ESI-MS with ICP-QQQ can be used to simultaneously locate, identify and quantify previously unreported sulfur-arsenic species in sufficiently resolved chromatograms of biological extracts.

THANK YOU