Orbitrap Exploris™ Isotope Solutions: Isotope Fingerprints

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The world leader in serving science

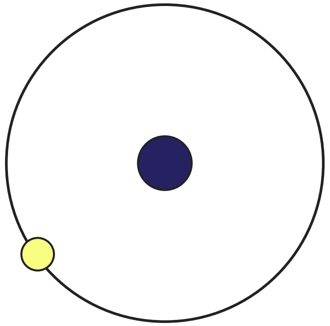
Isotope Ratios as Fingerprints

- Materials have a unique chemical signature that allows them to be identified using untargeted and targeted analysis.

- Isotope Ratio Mass Spectrometry (IRMS) is used to measure the stable isotope ratios of specific elements and compounds: “super-targeted” analysis.

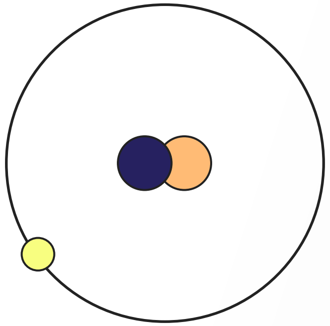
Isotope Ratios as Fingerprints

- Atoms consist of protons (+), neutrons (N), and electrons (−)
- Atoms of the same element always have the same number of protons, but may have different numbers of neutrons
- Atoms of an element with different numbers of neutrons are called **isotopes**
- Isotopes can be radioactive (they transform with time to other isotopes) or stable -> **we focus on stable isotopes**



$^{1}_{1}H$

Protium



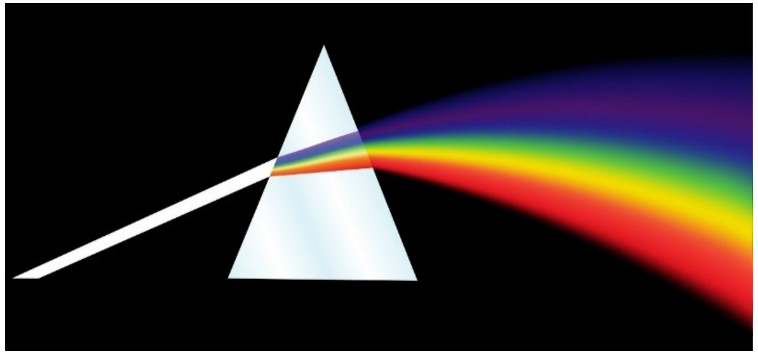
$^{2}_{1}H$

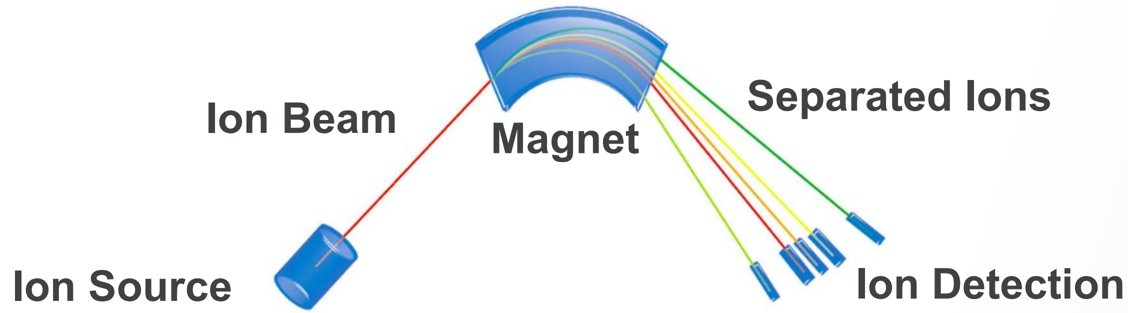
Deuterium

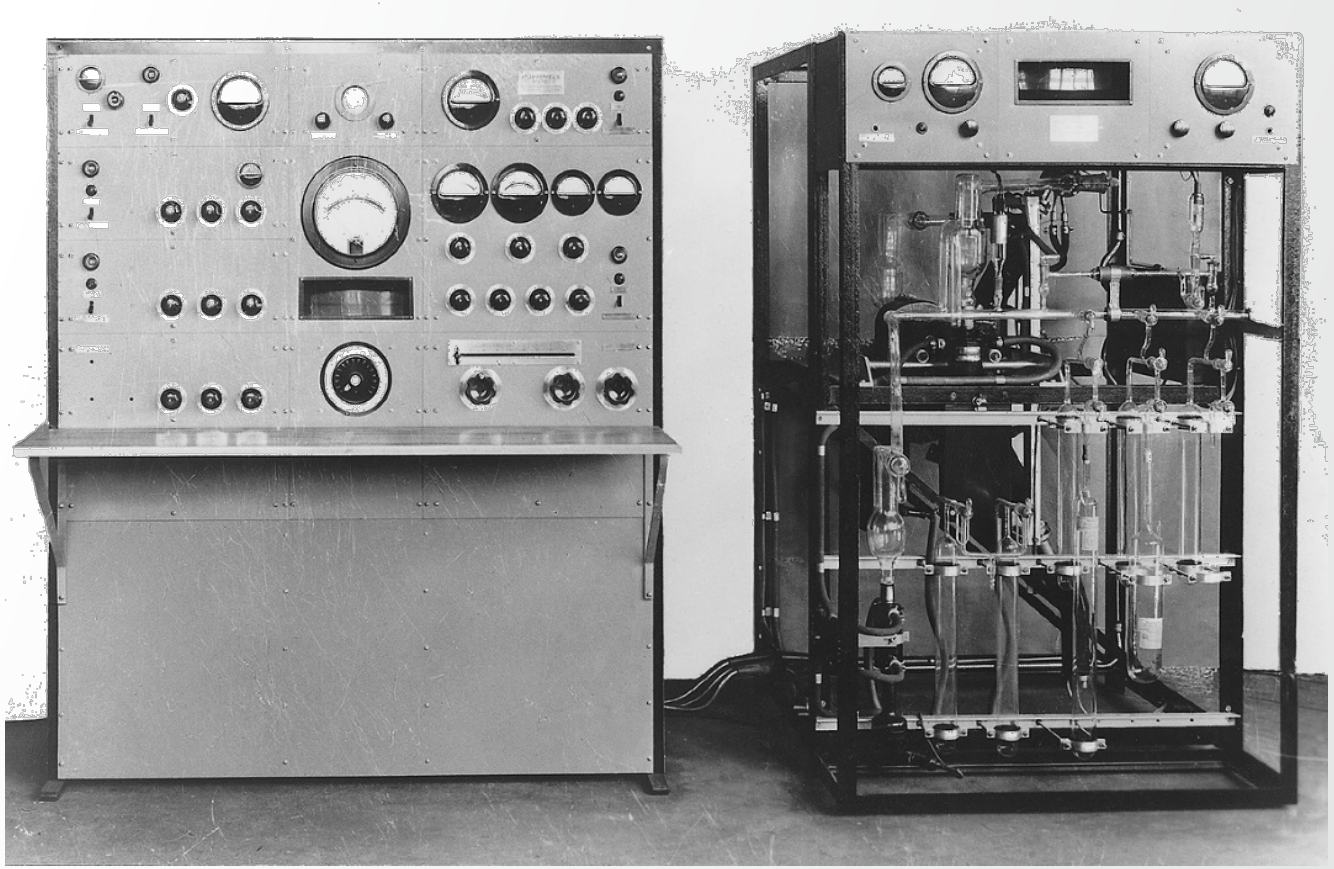
$^{2}H/^{1}H$ ratio →

Isotope Fingerprints

Isotope Ratio Mass Spectrometry

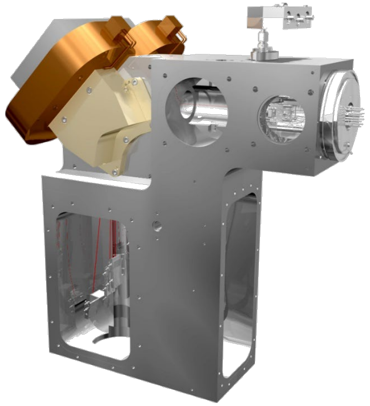






CH3 IRMS (1950)

The current Thermo Scientific™ gas IRMS portfolio



- **Thermo Scientific™ DELTA Q™ IRMS**
- Sensitivity up to 800 M/I
- Mass range up to *m*/*z* 96
- Accommodates up to 10 collectors

- **Thermo Scientific™ 253 Plus™ 10 kV IRMS**
- High-precision isotope analysis
- Long term stability and robustness
- Complete automation for ease-of-use

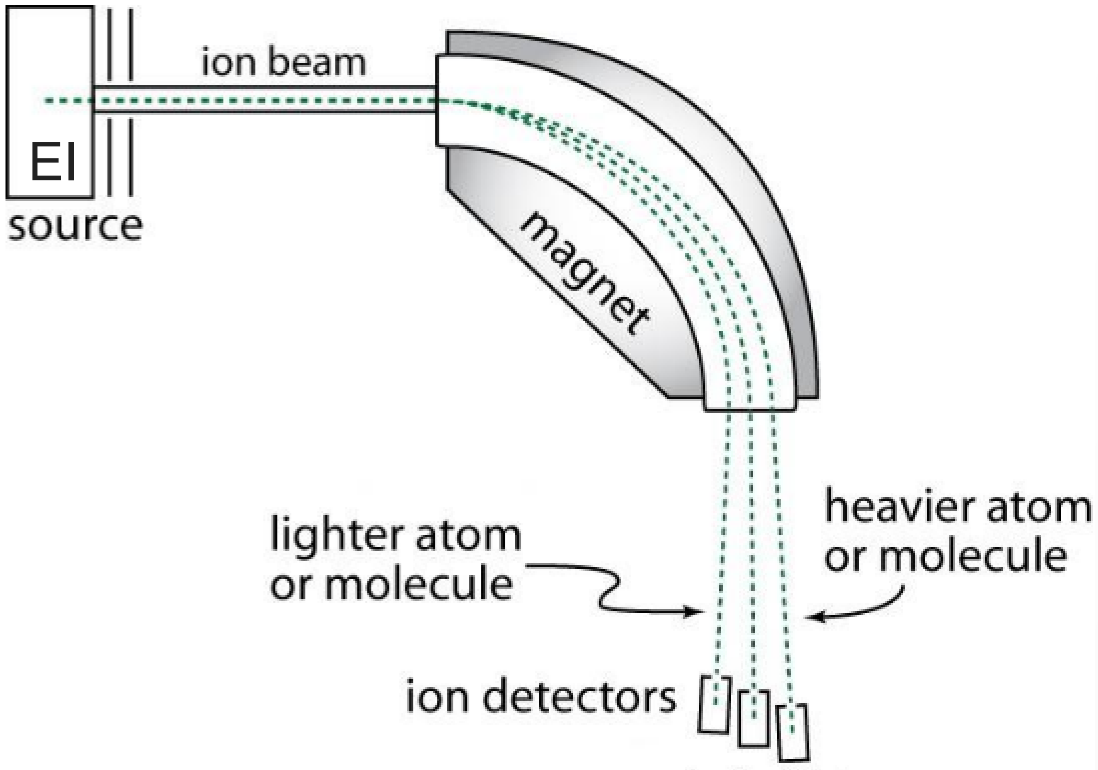
- **Thermo Scientific™ Ultra™ HR-IRMS**
- Double-focusing magnetic-sector mass analyzer
- Variable collector array
- Dual viscous flow inlet systems
- Optional (Multi) Ion Counting and RPQ

From sample to Isotope Fingerprints

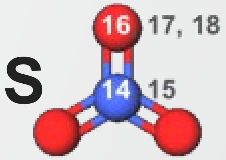
Mass spectrometers for isotope ratio analysis

Classic isotope-ratio MS N_2, O_2, N_2O

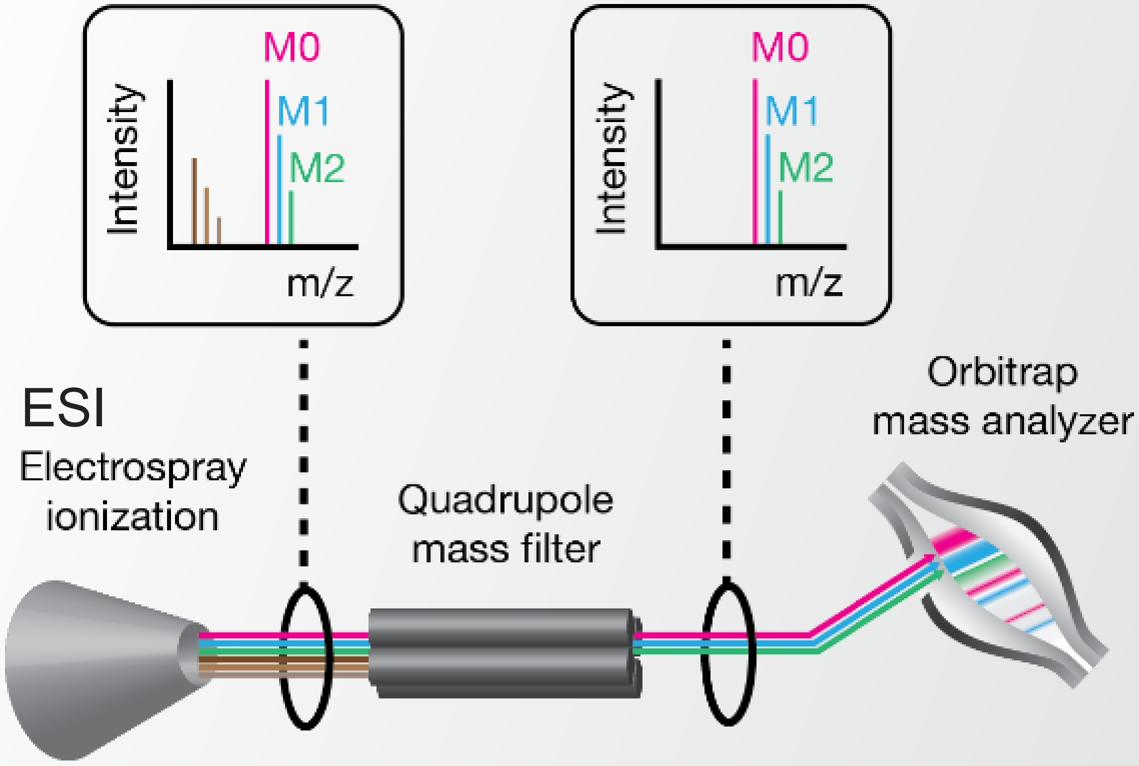
- **Indirect**: Conversion into **simple gases**
- Combining isotopologues on **few signals**



Thermo Scientific Orbitrap™ MS



- **Direct**: Intact isotopologues
- Separation of **all species** by HRMS



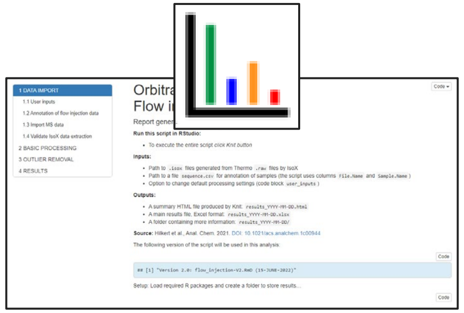
Orbitrap Exploris Isotope Solutions

Thermo Scientific™ Orbitrap Exploris™ Isotope Solutions includes:

Thermo Scientific™
Orbitrap Exploris™
120/240/480 MS

Dual Syringe Inlet

Data evaluation Package
for Isotope Ratio MS



Optional Thermo
Scientific™ Vanquish™
Neo UHPLC System

Oxyanions: refine understanding of element cycling

Atmosphere

Soil

Water

Oxyanions are important chemical constituents of virtually every environment on Earth.

What's inside the Orbitrap Exploris MS

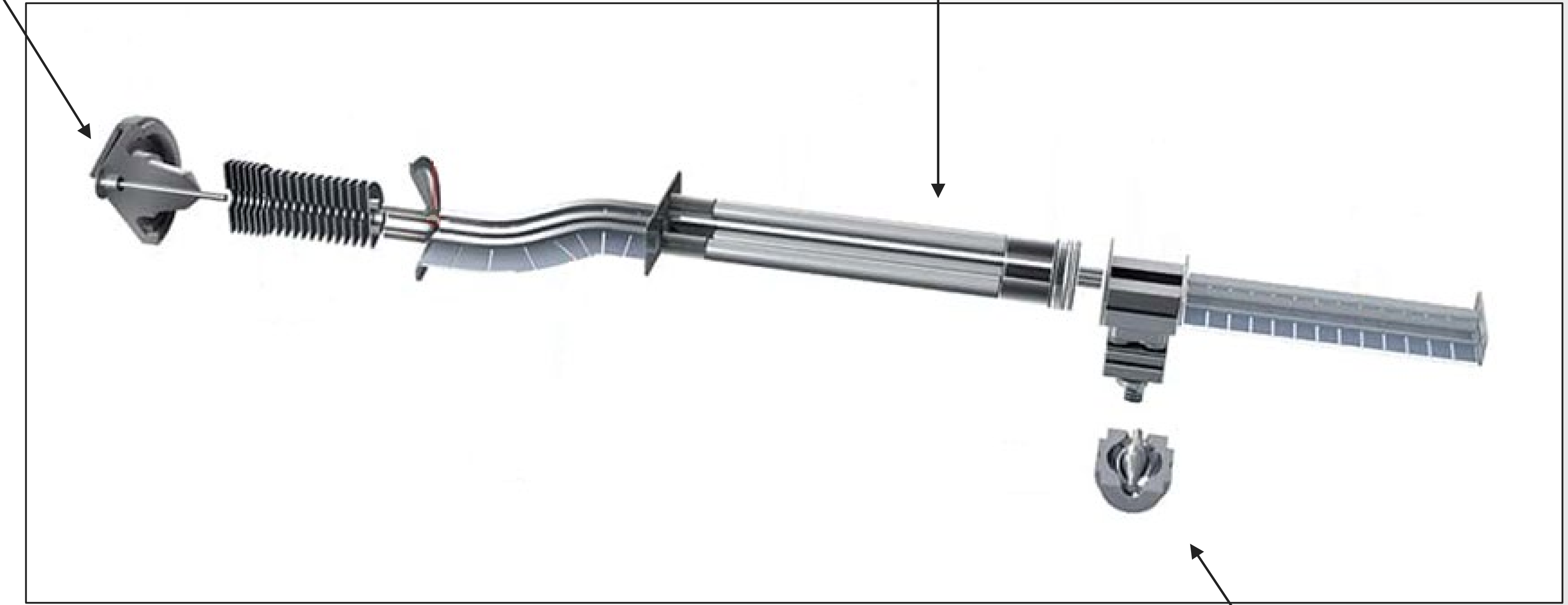
Essential components for isotope ratio analysis

1. ESI Source

Electrospray Ionization

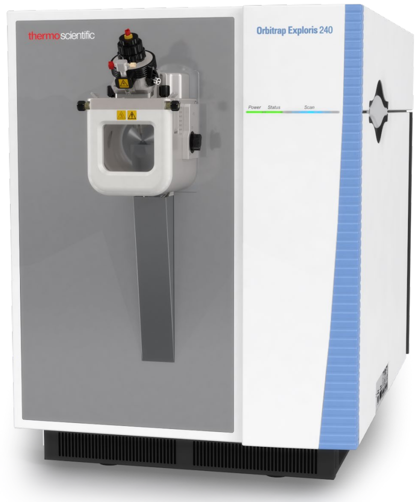
2. Advanced Quadrupole

m/*z* Range Selection

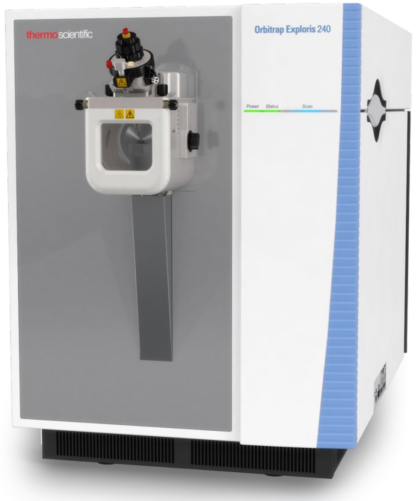


3. Orbitrap Mass Analyzer

Image Current Detection and FT Analysis

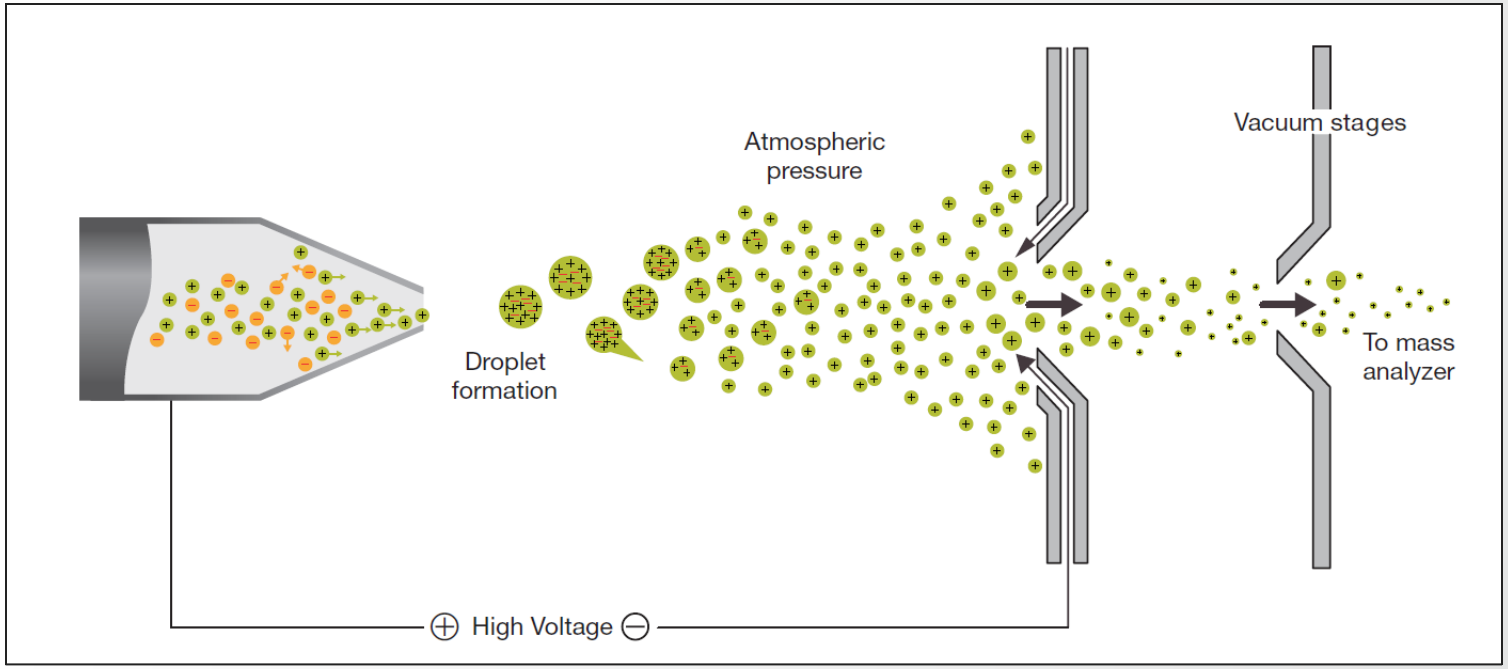


Electro Spray Ionization (ESI)



Ionization source:

1. Ionization of target molecules in liquid phase
2. Transfer of intact molecular ions to the gas phase
3. Transfer into the MS



Important parameters for Orbital analyzer

Isotope ratio analysis

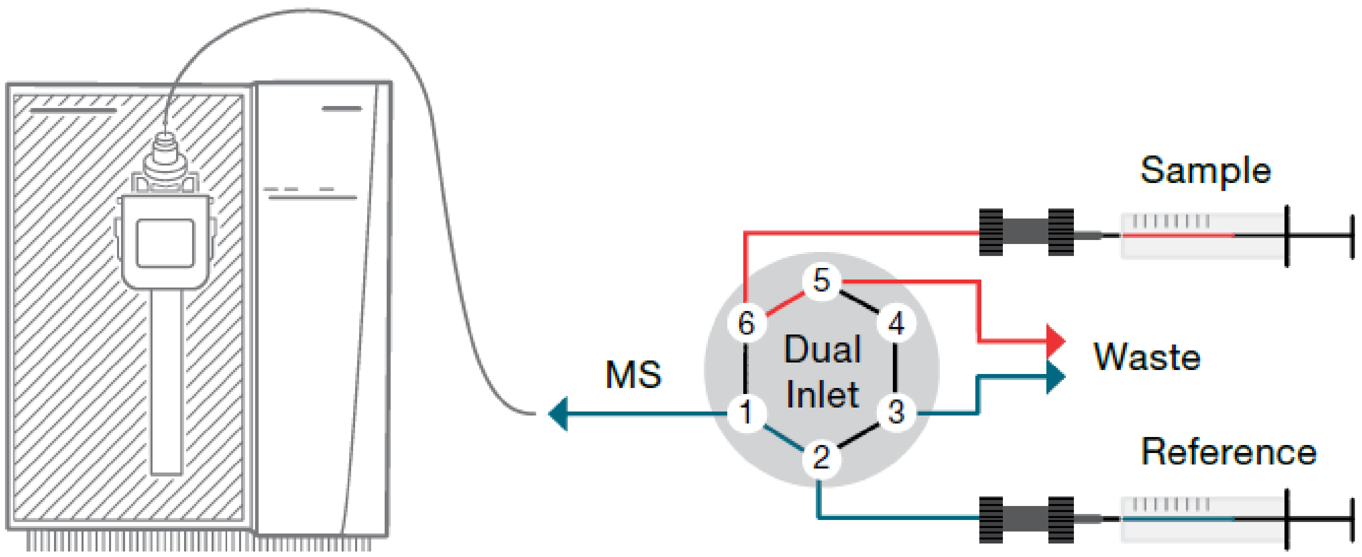
- Resolution:
  - Orbitrap analyzes a specific number of ions (ion package) per scan
  - Every scan results in a mass spectrum
  - Higher resolution requires longer scan time:

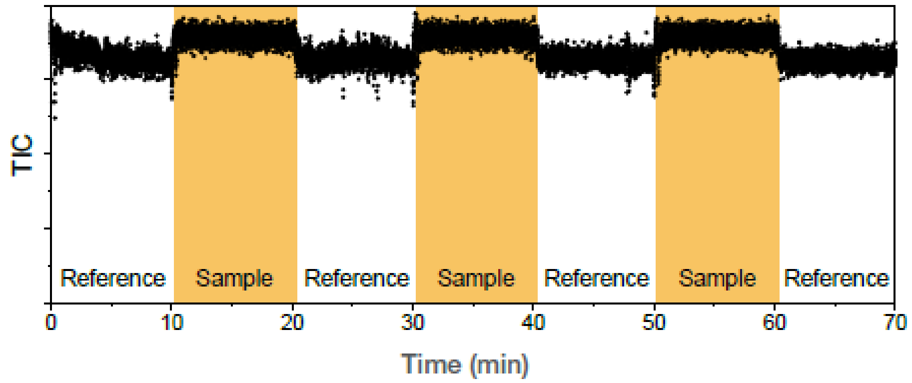
	Resolution at *m*/*z* 200	Scans per second
NO_3^-	15,000	22
	30,000	12
HSO_4^-	45,000	10
	60,000	7
Organics	120,000	3
	240,000	1.5

Dual Syringe Inlet

Sample introduction technique

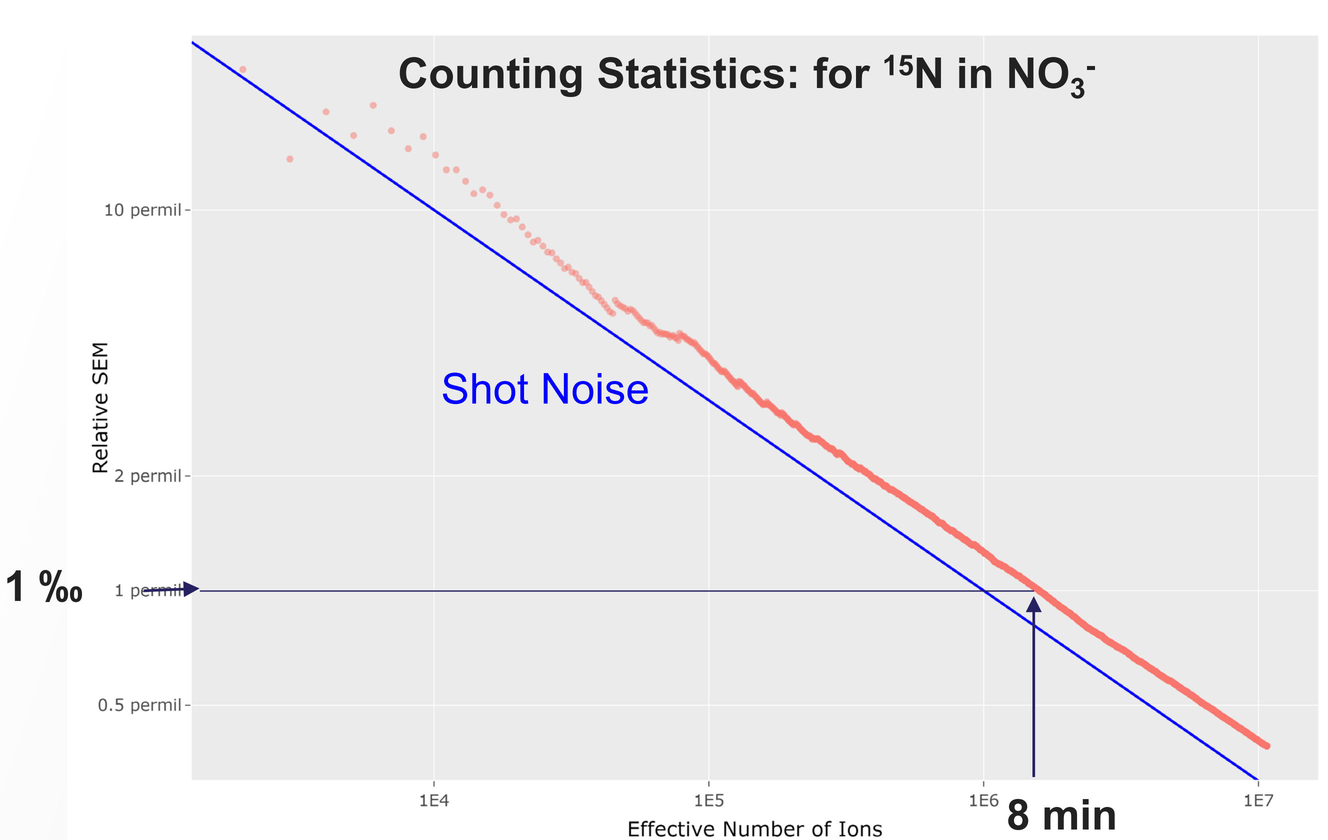
- **Dual Syringe Inlet System** utilizing the diverter valve option of the Orbitrap Exploris MS
- Direct infusion of sample (**50 µM** in MeOH) with a flow rate of **4 µl/min** via a syringe pump; sample reference comparison by switching of a diverter valve.





Isotope ratio methodology for Orbitrap MS

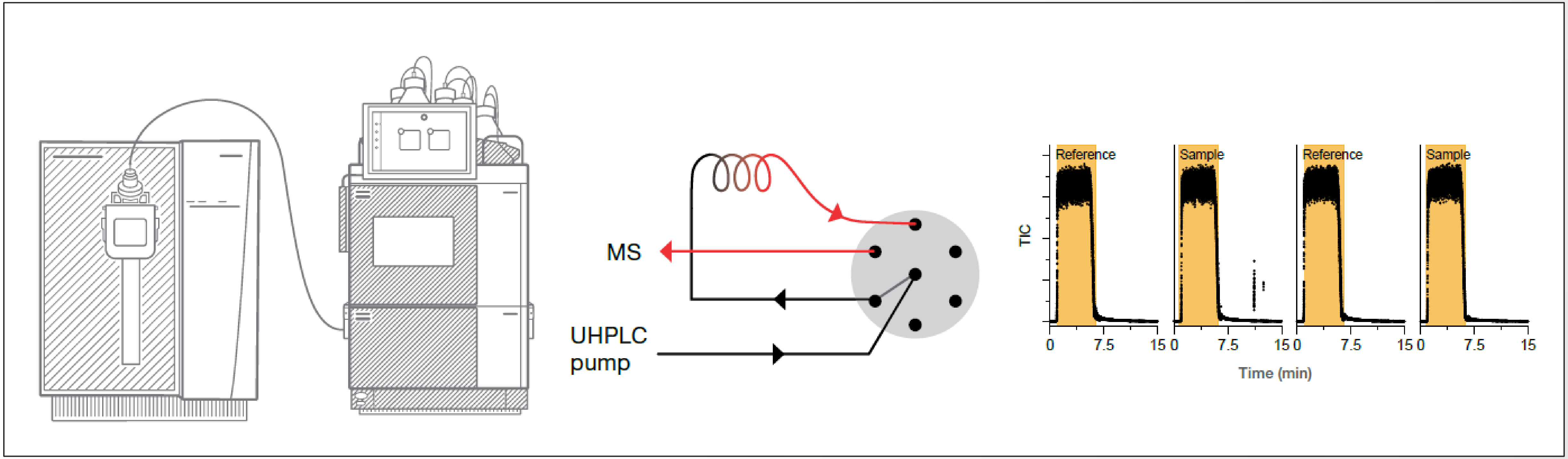
- **Precision: maximizing ion counts**
  - Longer analysis times achieve greater precision.
  - Lower resolution setting requires less time for scanning.
  - Optimize mass range maximize the number of target ions.
- **Accuracy: Sample/Standard comparison**
  - NO_3^- Analysis of signals (reference/sample) for 6–9 mins



Automated In-Flow Injection

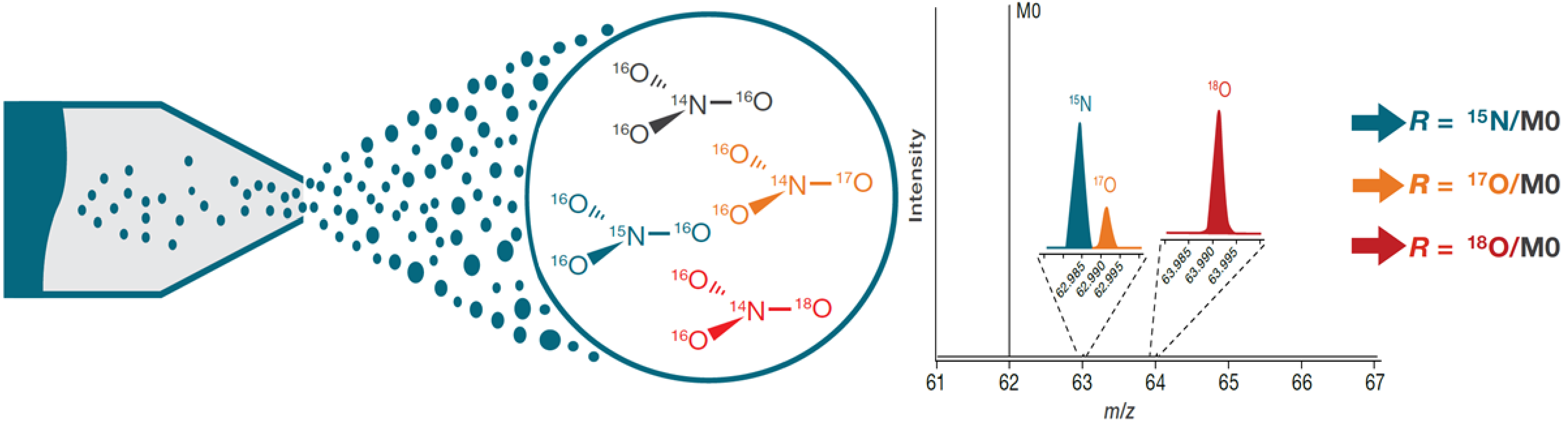
Sample introduction technique

- **In-flow Injection** - Vanquish Neo UHPLC System
- Loop-injection of **20-30 µL** sample (**50 µM** in MeOH) by the Autosampler into a flow of **4 µL/min** of MeOH.



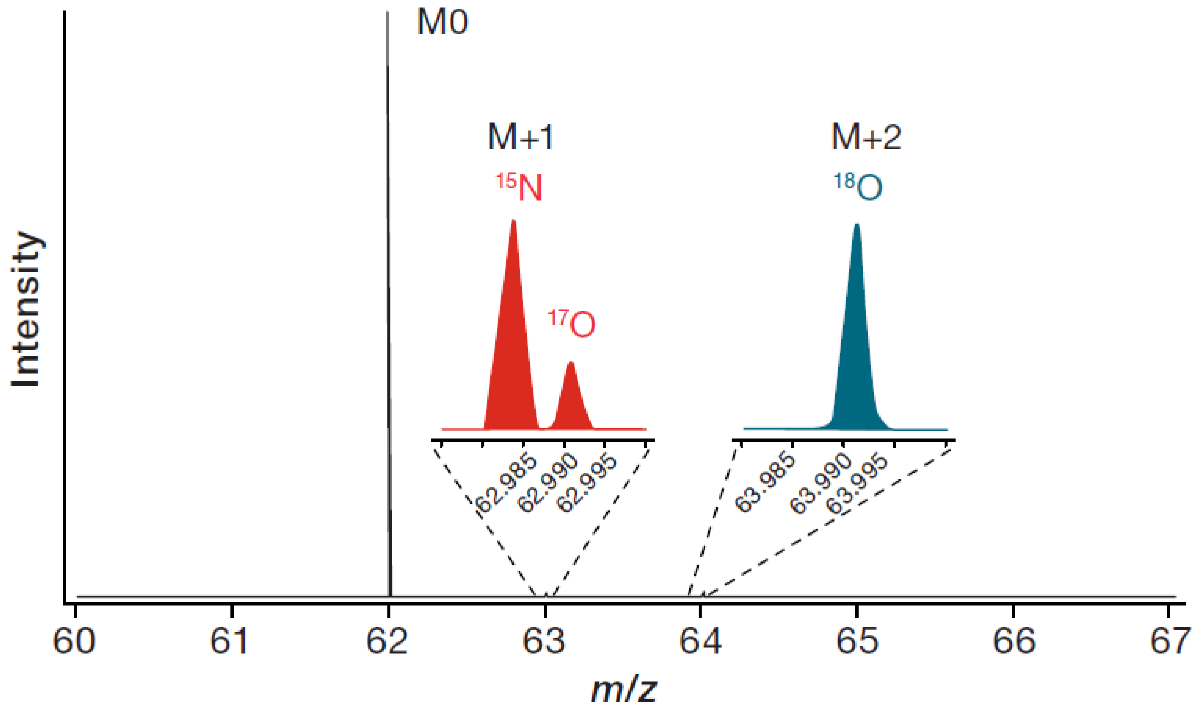
Orbitrap for isotopes: workflow for nitrate

Nitrate simplest model (4 atoms with isotope species: ^{14}N, ^{15}N, ^{16}O, ^{17}O, ^{18}O)



ESI-Orbitrap for isotopes – methodology

‘M0’ experiment

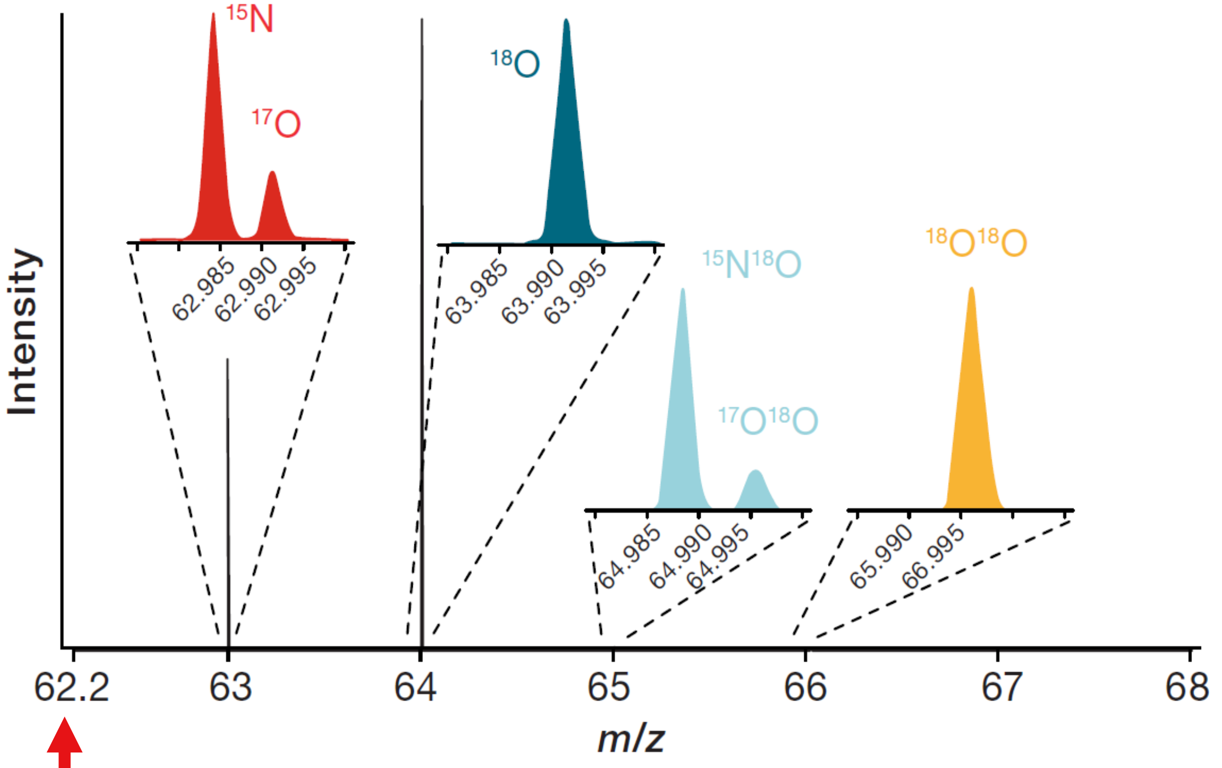


	m/z	Isotopologue	Abundance	Percentage
M0	**61.9884**	$^{14}N^{16}O_3$	**989242**	**98.9**
M+1	**62.9854**	$^{15}N^{16}O_3$	**3637**	**0.36**
	62.9926	$^{14}N^{17}O^{16}O_2$	**1127**	**0.11**
M+2	63.9896	$^{15}N^{17}O^{16}O_2$	4.1	
	63.9926	$^{14}N^{18}O^{16}O_2$	**5951**	**0.60**
	63.9968	$^{14}N^{17}O_2{}^{16}O$	0.4	
M+3	64.9897	$^{14}N^{18}O^{16}O_2$	21.9	
	64.9938	$^{15}N^{17}O_2{}^{16}O$	< 0.1	
	64.9968	$^{15}N^{17}O^{18}O^{16}O$	4.5	
	65.0010	$^{14}N^{17}O_3$	< 0.1	
M+4	65.9939	$^{15}N^{18}O^{16}O_2$	< 0.1	
	65.9969	$^{15}N^{18}O_2{}^{16}O$	11.9	
	65.9981	$^{15}N^{17}O_3$	< 0.1	
	66.0011	$^{15}N^{17}O_2{}^{18}O$	< 0.1	

- M0 peak as basepeak

ESI-Orbitrap for isotopes – methodology

‘noM0’ experiment



Mass Range!

	m/z	Isotopologue	Abundance	Percentage
~~**M0**~~	~~61.9884~~	~~$^{14}N^{16}O_3$~~	~~989242~~	~~**98.9**~~
M+1	**62.9854**	$^{15}N^{16}O_3$	**3637**	**33.81**
	62.9926	$^{14}N^{17}O^{16}O_2$	**1127**	**10.48**
M+2	63.9896	$^{15}N^{17}O^{16}O_2$	4.1	
	63.9926	$^{14}N^{18}O^{16}O_2$	**5951**	**55.32**
	63.9968	$^{14}N^{17}O_2{}^{16}O$	0.4	
M+3	**64.9897**	$^{15}N^{18}O^{16}O_2$	**21.9**	**0.20**
	64.9938	$^{15}N^{17}O_2{}^{16}O$	< 0.1	
	64.9968	$^{14}N^{17}O^{18}O^{16}O$	**4.5**	**0.04**
	65.0010	$^{14}N^{17}O_3$	< 0.1	
M+4	65.9939	$^{15}N^{18}O^{16}O_2$	< 0.1	
	65.9969	$^{14}N^{18}O_2{}^{16}O$	**11.9**	**0.11**
	65.9981	$^{15}N^{17}O_3$	< 0.1	
	66.0011	$^{14}N^{17}O_2{}^{18}O$	< 0.1	

- ^{15}N or ^{18}O peak as basepeak

Dual Inlet measurements of nitrate

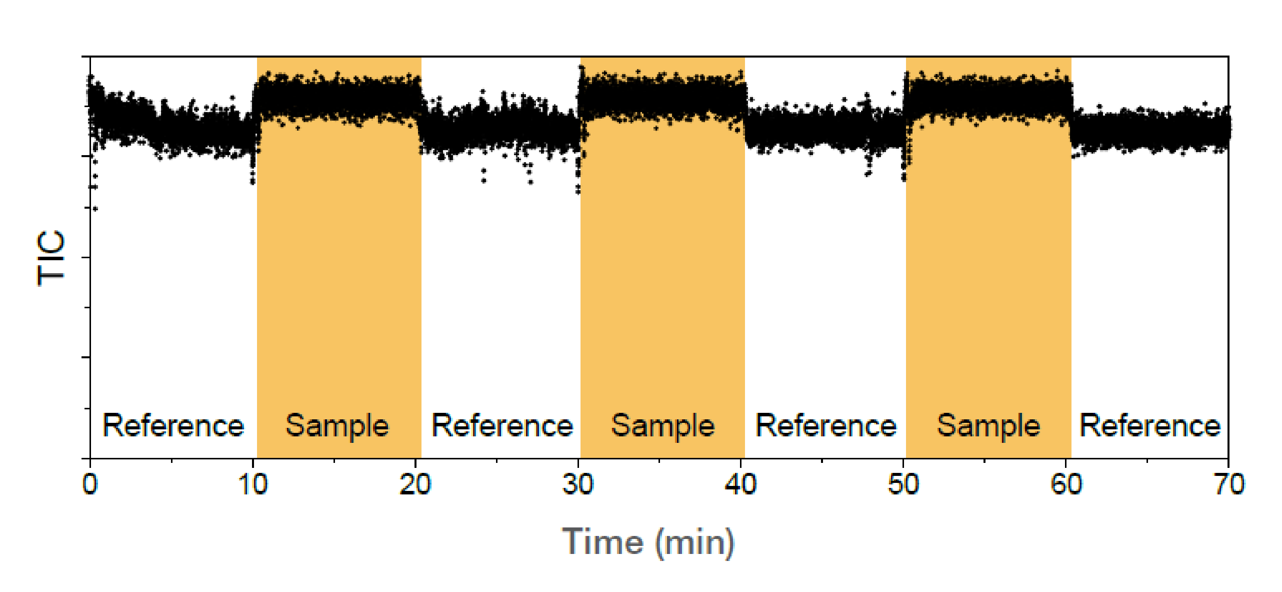
‘M0’ experiment:

- Three nitrate reference materials available from USGS:[1]

	$\delta^{15}N_{AIR}$	$\delta^{18}O_{VSMOW}$	$^{17}O_{VSMOW}$
USGS32	+ 180 ‰	+ 25.7 ‰	-
USGS34	- 1.8 ‰	- 27.9 ‰	- 14.8 ‰
USGS35	+2.7 ‰	+ 57.5 ‰	+ 51.5 ‰

Quality control: $^{15}N/^{14}N$ isotope ratio analysis

Block	Description	Ratio ($^{15}N/^{14}N$)	$\delta^{15}N_{USGS32/Air}$ [‰]	$\delta^{15}N_{USGS32/Air}$ [‰]	Std. Dev.
1	Reference (USGS35)	0.00430			
2	Sample (USGS32)	0.00506	179.5		
3	Reference (USGS35)	0.00430			
4	Sample (USGS32)	0.00506	179.3	179.6	0.4
5	Reference (USGS35)	0.00430			
6	Sample (USGS32)	0.00506	180.1		
7	Reference (USGS35)	0.00430			



[1] Böhlke, J.K .; et al. Rapid Commun. Mass Spectrom., 2003, 17, p. 1835−1846.

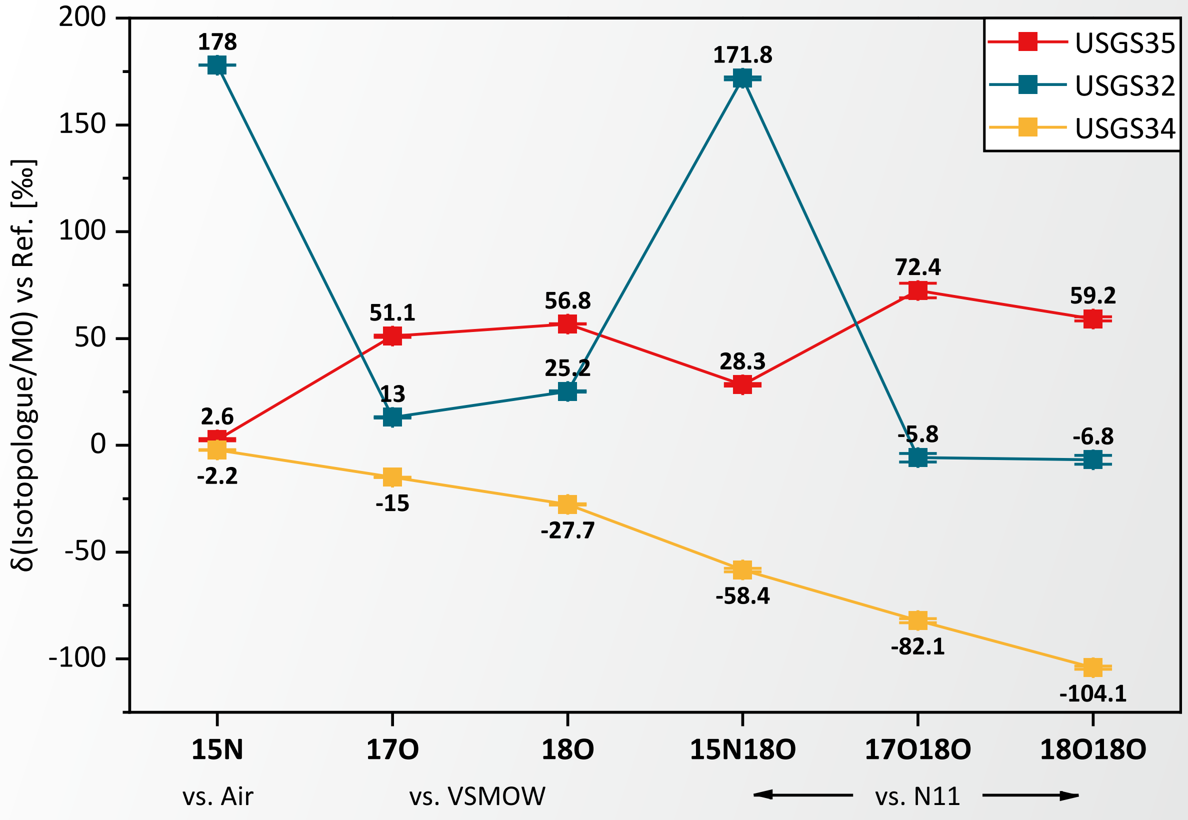
Referencing scheme for nitrate

Analysis of international standards for nitrate

- Isotope ratio data of ‘M0’ and ‘noM0’ experiments using N11 as a working standard:[1]

	$\delta^{15}N_{AIR}$	$\delta^{18}O_{VSMOW}$	$^{17}O_{VSMOW}$
USGS32	+ 180 ‰	+ 25.7 ‰	-
USGS34	- 1.8 ‰	- 27.9 ‰	- 14.8 ‰
USGS35	+2.7 ‰	+ 57.5 ‰	+ 51.5 ‰

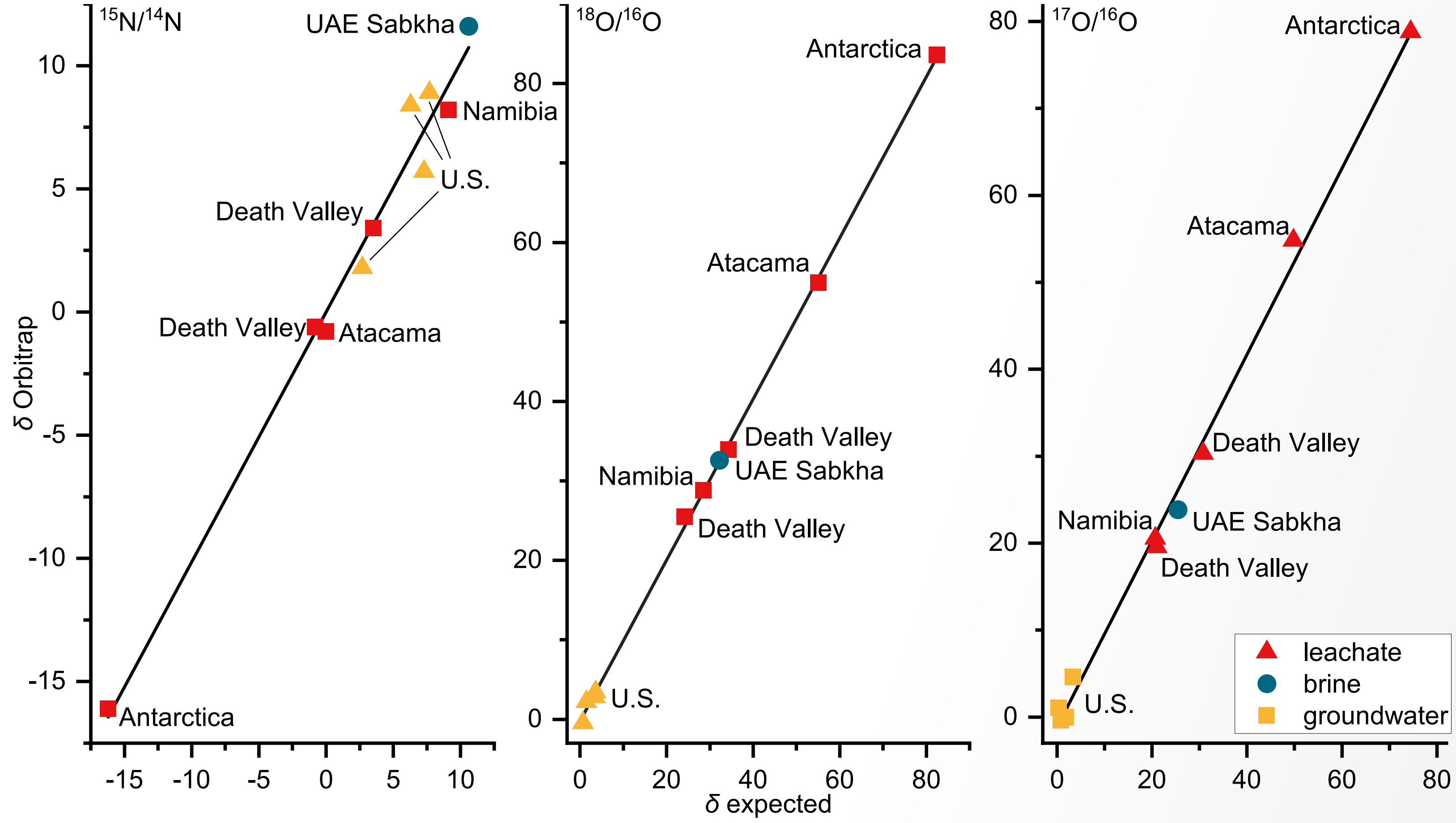
Isotope ratio data of six of nitrate`s most abundant isotopologues using the In-Flow Injection setup.



[1] Böhlke, J.K .; et al. Rapid Commun. Mass Spectrom., 2003, 17, p. 1835−1846.

Nitrate analysis of environmental samples

'M0' experiment



Dual Syringe Inlet methodology

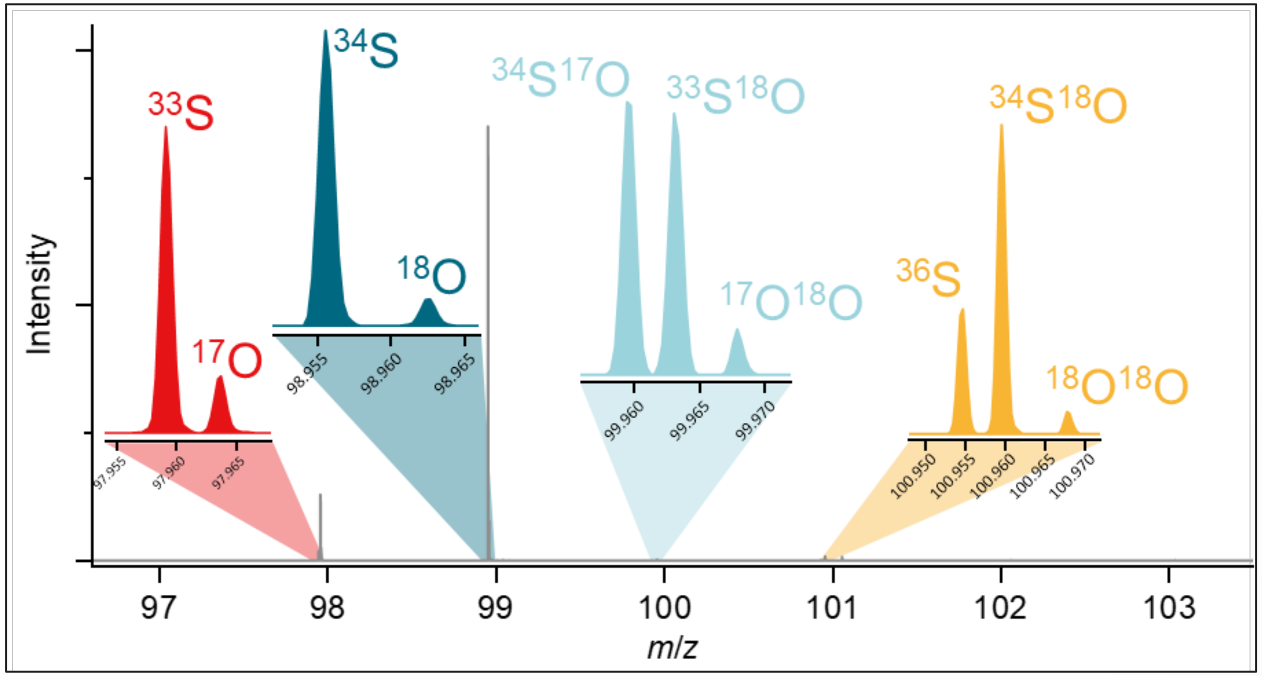
- Sample prep: "Dilute and Shoot"
  - Dilution to **1µM** (**62 pg** NO_3^-/µL) for high salinity samples
- Results $\delta^{15}N$ and $\delta^{18}O$
  - Precision: < 1‰
  - Difference Orbitrap – Expected: < 1‰
- Takeaway
  - ESI tolerates up to **100-fold Cl^-** load
  - Ground waters diluted up to **1/500**

[2] Hilkert, A.; et al. Anal. Chem. 2021, 93, 9139−9148.

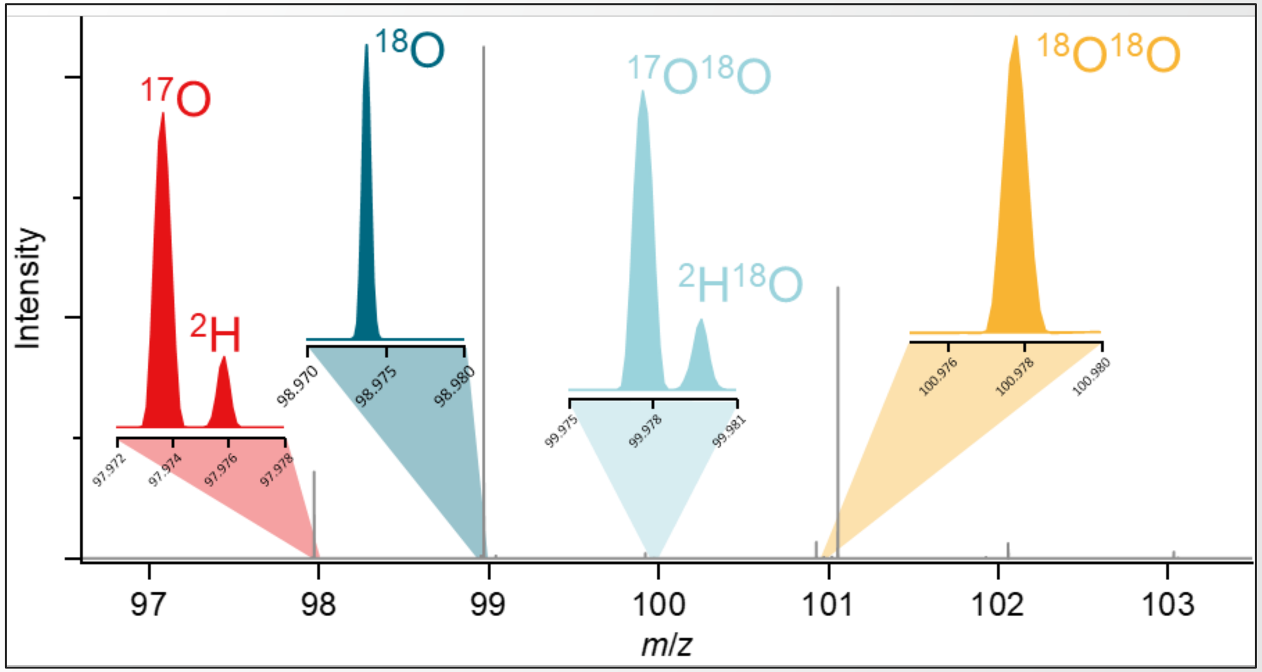
Other oxyanions – sulfate and phosphate

Thermo Fisher SCIENTIFIC

Sulfate ionized as HSO_4^-



Phosphate ionized as $H_2PO_4^-$



Stable Isotope Analysis of Intact Oxyanions Using Electrospray Quadrupole-Orbitrap Mass Spectrometry

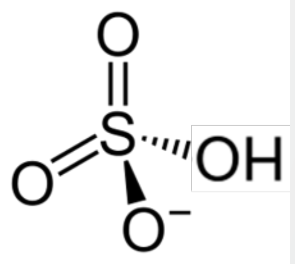
Cajetan Neubauer,* Antoine Crémière, Xingchen T. Wang, Nivedita Thiagarajan, Alex L. Sessions, Jess F. Adkins, Nathan F. Dalleska, Alexandra V. Turchyn, Josephine A. Clegg, Annie Moradian, Michael J. Sweredoski, Spiros D. Garbis, and John M. Eiler

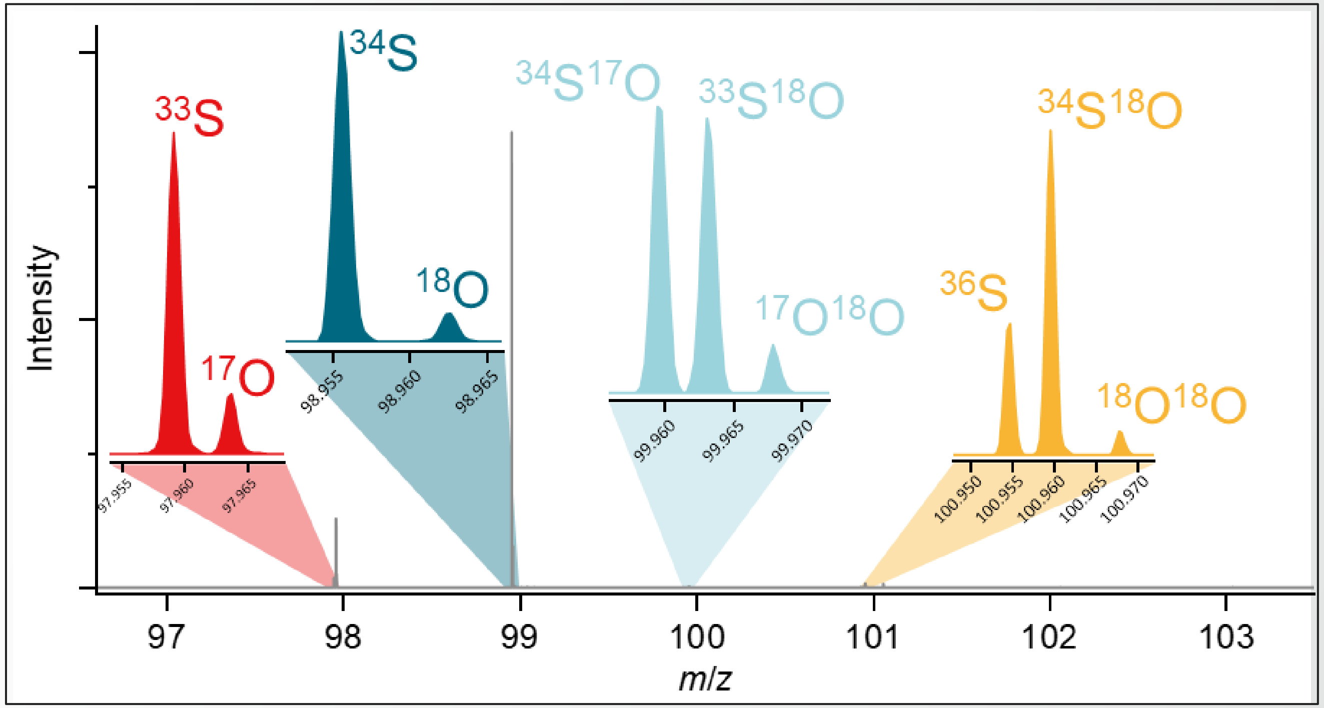
Cite This: *Anal. Chem.* 2020, 92, 3077–3085 Read Online

Isotope mapping of sulfates

	m/z	Isotopologue	Abundance
M0	**96.9596**	$^{1}H^{32}S^{16}O_4$	9408592
M+1	**97.9590**	$^{1}H^{33}S^{16}O_4$	7427
	97.9638	$^{1}H^{32}S^{17}O^{16}O_3$	1433
M+2	**98.9554**	$^{1}H^{34}S^{16}O_4$	42084
	98.9638	$^{1}H^{32}S^{18}O^{16}O_3$	7632
M+3	**99.9596**	$^{1}H^{34}S^{17}O^{16}O_3$	63
	99.9632	$^{1}H^{33}S^{18}O^{16}O_3$	59
	99.9680	$^{1}H^{32}S^{17}O^{18}O^{16}O_2$	9
M+4	**100.9546**	$^{1}H^{36}S^{16}O_4$	145
	100.9596	$^{1}H^{34}S^{18}O^{16}O_3$	333
	100.9680	$^{1}H^{32}S^{18}O_2{}^{16}O_2$	44

$$\begin{pmatrix} ^{32}S \\ ^{33}S \\ ^{34}S \\ ^{36}S \end{pmatrix} \times \begin{pmatrix} ^{16}O \\ ^{17}O \\ ^{18}O \end{pmatrix}$$





Comprehensive IRMS of sulfates

- Dual Inlet experiment:

Reference: S-3477 (Working Standard)

Sample: S-MIF-1, S-MIF-2

- Reported isotope data:[3]

S-3477 δ^{XX} [‰]	
33S	(3.33)
34S	**6.48**
36S	(12.35)
17O	(6.77)
18O	**13.02**
Δ33	na (0)
Δ36	na (0)
Δ17	na (0)

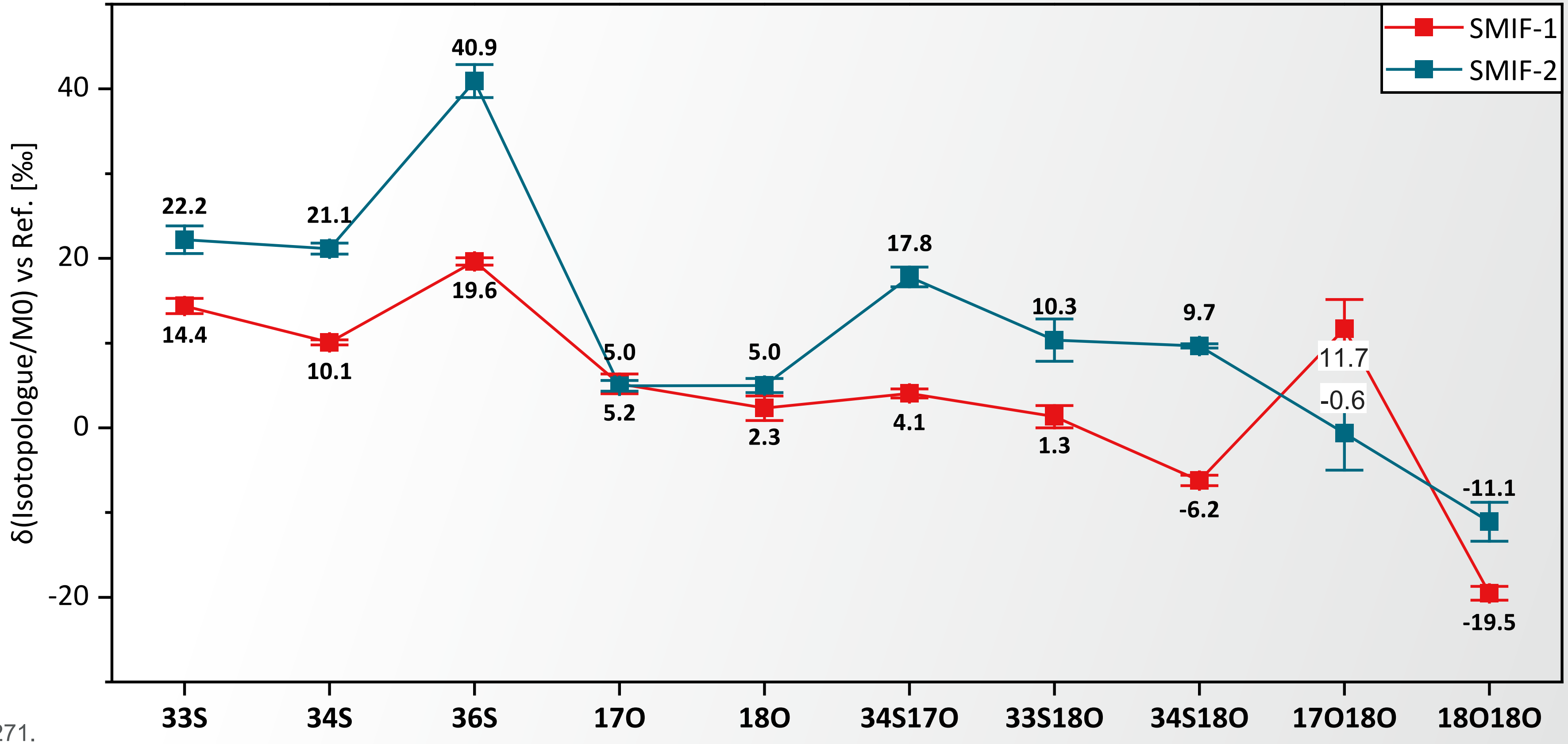
Böhlke, USGS

S-MIF1 δ^{XX} [‰]	
33S	**14.81**
34S	**10.26**
36S	**19.47**
17O	na
18O	na
Δ33	**9.54**
Δ36	**-0.14**
Δ17	**3.3**

S-MIF2 δ^{XX} [‰]	
33S	**22.42**
34S	**21.53**
36S	**40.73**
17O	na
18O	na
Δ33	**11.39**
Δ36	**--0.33**
Δ17	**3.3**

Samples provided by Joel Savarino
Institut des Géosciences de l'Environnement/CNRS

- Measured Isotope Data:

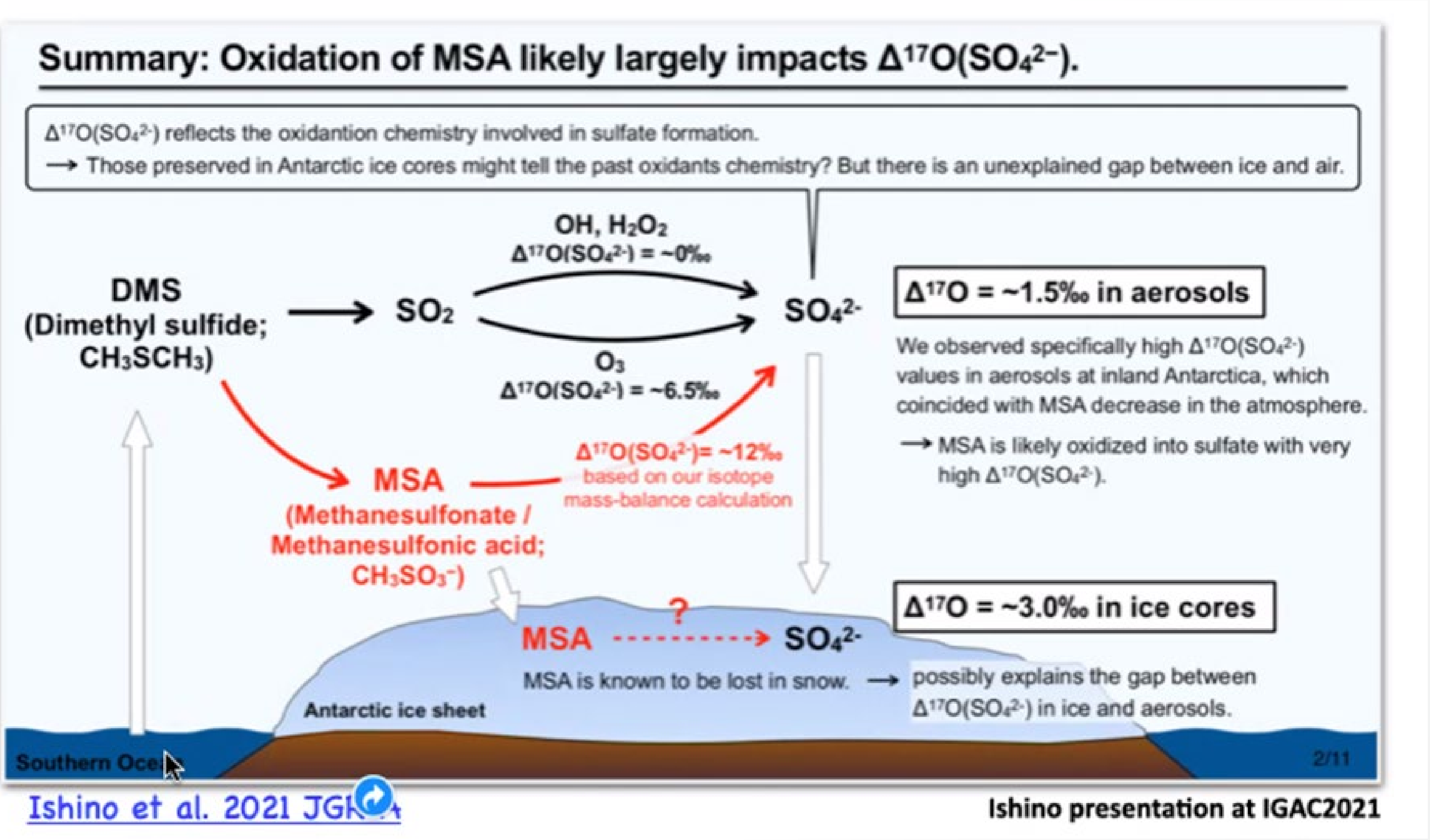


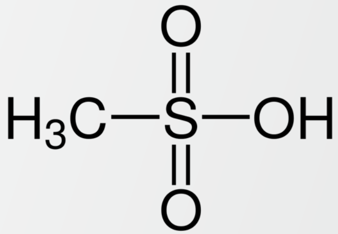
Preliminary results - unpublished data

[3] Geng L.; et al. J. Anal. At. Spectrom., 2019, 34, 1263–1271.

Comprehensive IRMS of methanesulfonic acid (MSA)

- Easy access to test new hypotheses

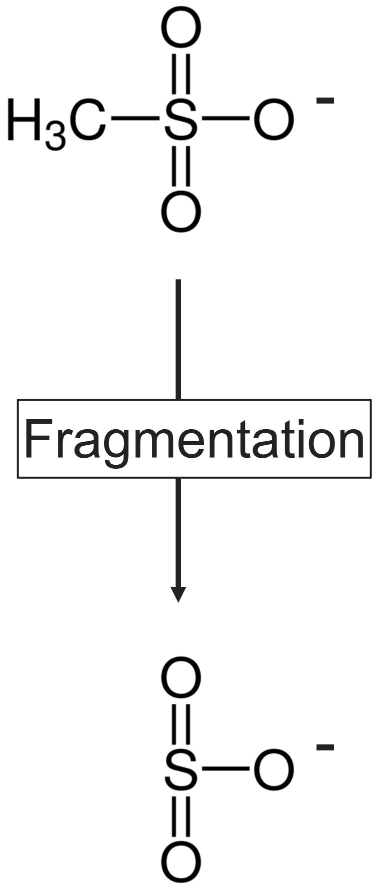


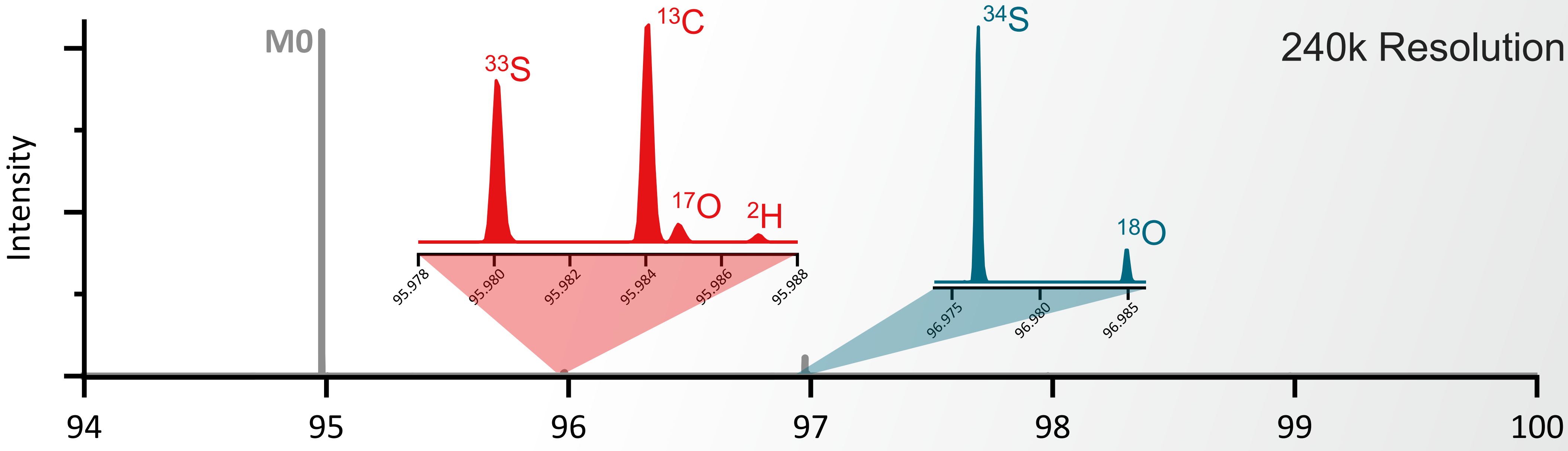


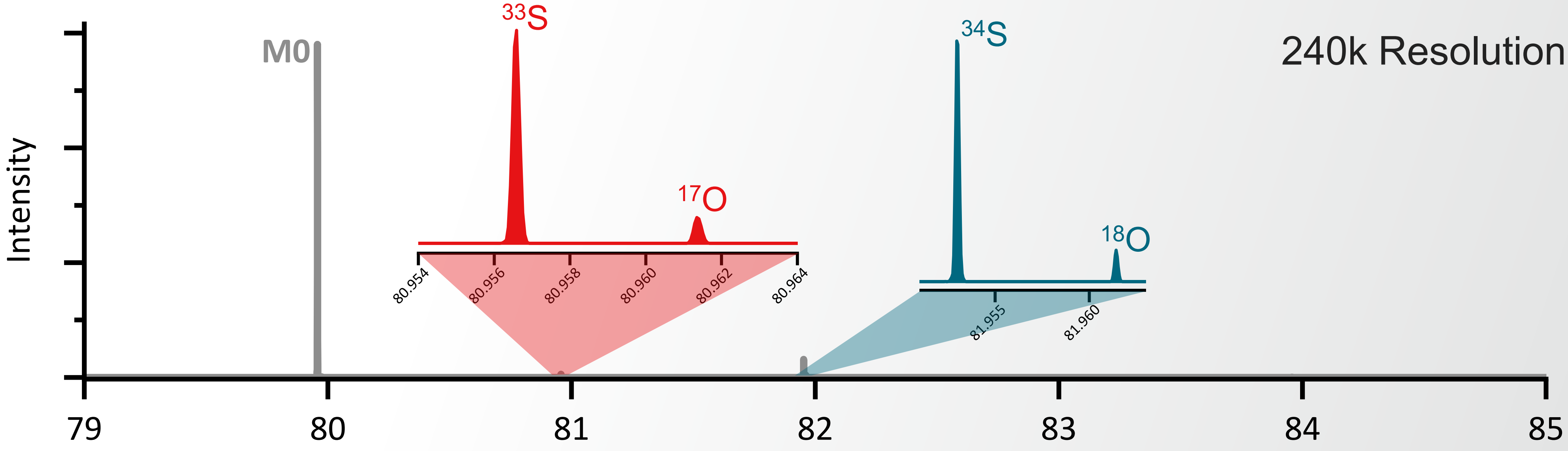
[4] Ishino S.; et al. J. Geophys. Res. Atmos., 2021, 126 (6).

[5] Wang K.; et al. Atmos. Chem. Phys., 2021, 21, 8357–8376.

First results of methanesulfonic acid (MSA)



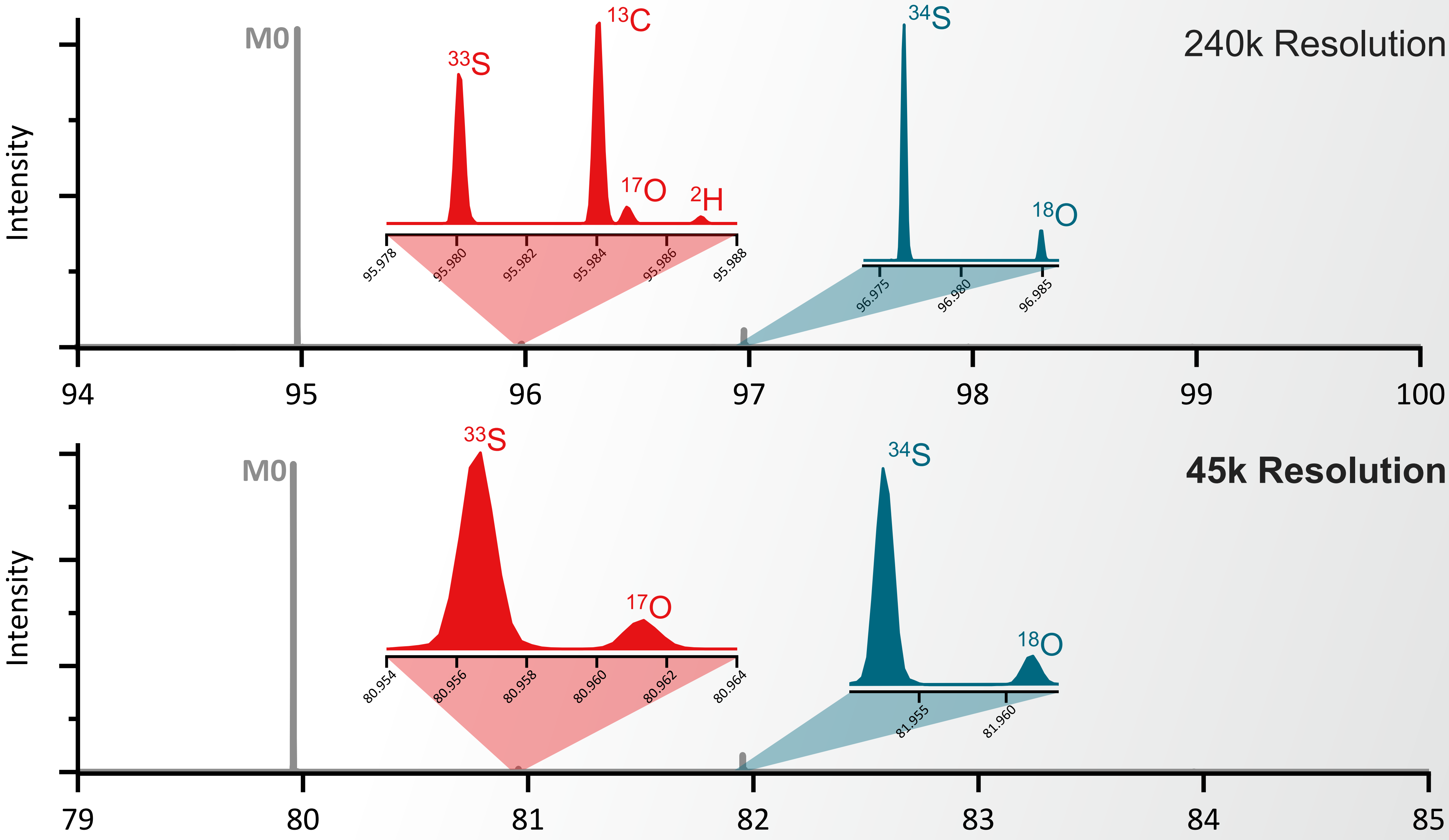




Fragmentation of methanesulfonic acid (MSA)

~ 1 scan/s

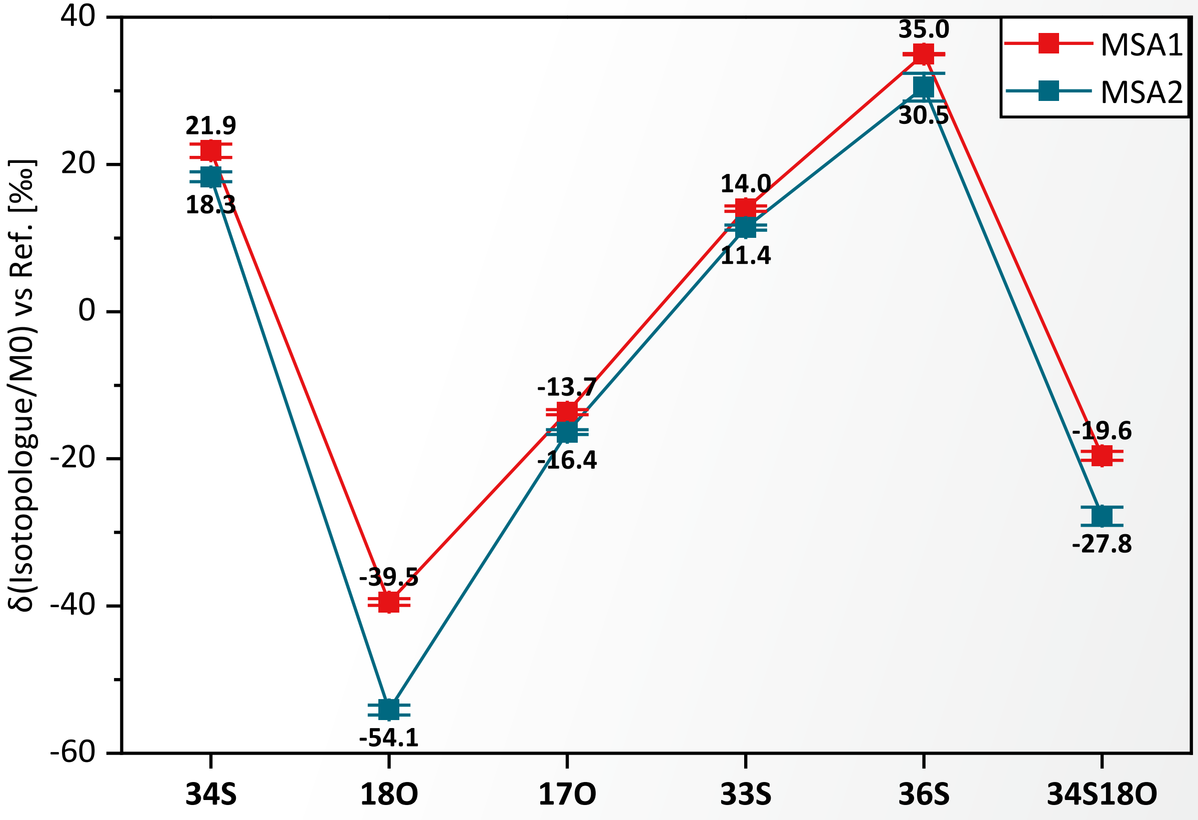
~ 10 scan/s



Analysis of two MSA samples

'noM0' experiments:

- **Dual Inlet analysis** of two MSA samples vs a working standard (Sigma-Aldrich)
  - 3x70 min runs → error bars - st. dev. of the 3 runs



Samples provided by Shohei Hattori
Tokyo Tech & Nanjing

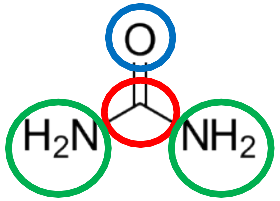
Preliminary results - unpublished data

Outlook: isotopic anatomy of organic compounds

Thermo Fisher SCIENTIFIC

- Examples: amino acids, urea
- Position specific isotope analysis by fragmentation of organic molecules

Urea



^{13}C **± 0.5 ‰**

^{15}N **± 0.5 ‰** (no M0)

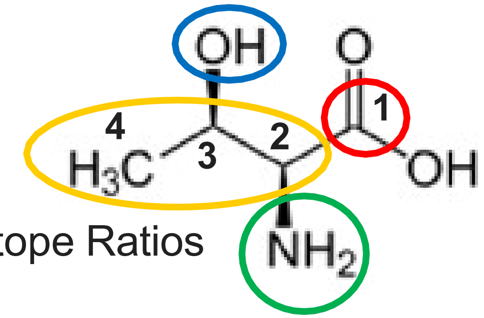
^{2}H ± 1.5 ‰ (no M0)

^{18}O ± 0.8 ‰ (no M0)

Dual Syringe Inlet Experiment

- Concentration: 50 pmol/µL
- Resolution:15000

Threonine



- Compound Specific Isotope Ratios

$^{13}C_{bulk}$ **± 0.6 ‰**

^{15}N ± 2 ‰

$^{2}H_{bulk}$ ± 3 ‰

$^{18}O_{bulk}$ ± 1 ‰

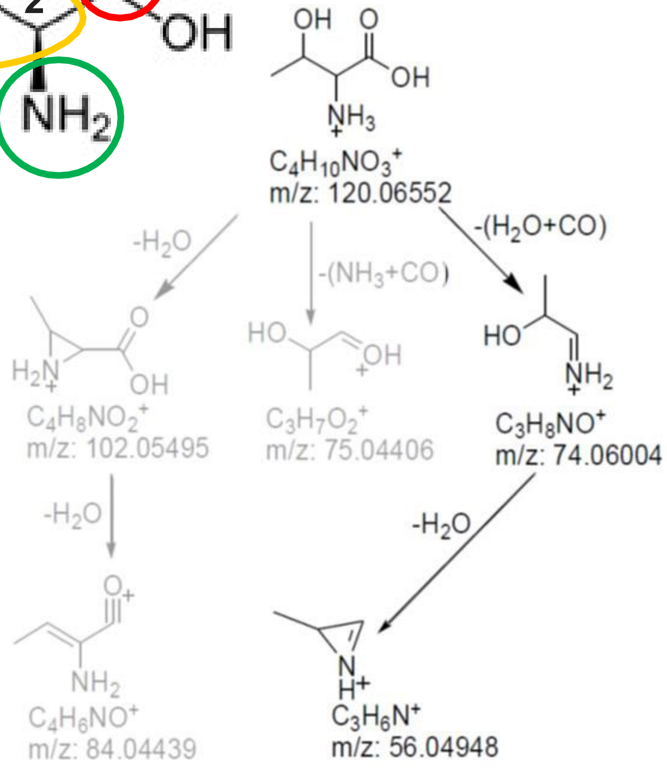
- Position Specific Isotope Ratios

$^{13}C_{C-1}$ **± 0.9 ‰**

$^{13}C_{C-2,3,4}$ **± 0.6 ‰**

$^{18}O_{\beta-OH}$ **± 2.5‰**

$^{2}H_{C3H6N}$ **± 3 ‰**

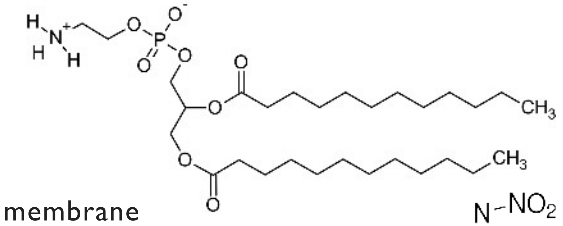


Fragmentation according Zhang et al, 2019

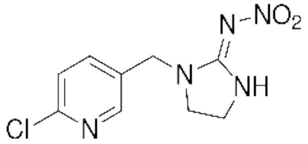
Molecular targets for isotope ratio analysis

Thermo Fisher SCIENTIFIC

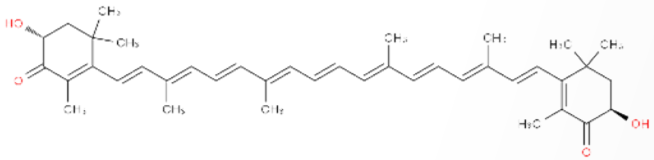
Application areas: ecology, environmental and paleoclimate research and similar disciplines



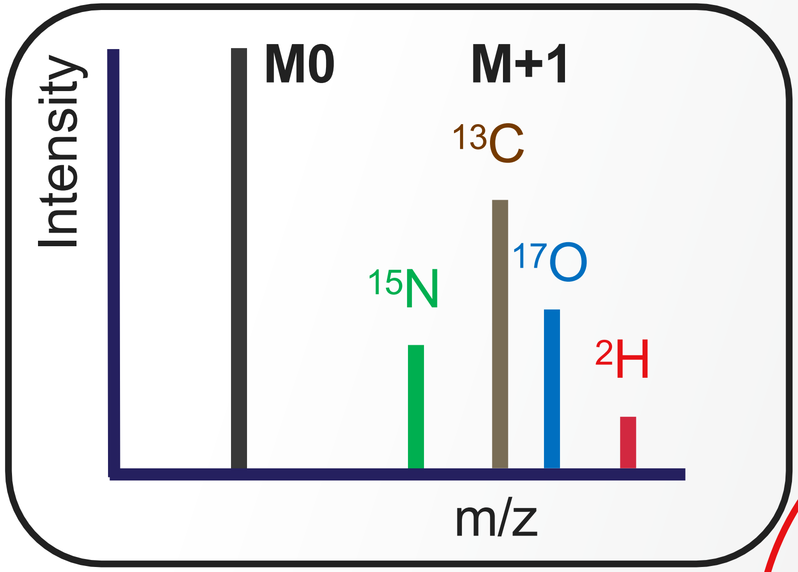
membrane lipids

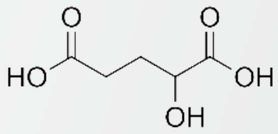


pesticide

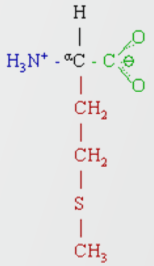


pigments

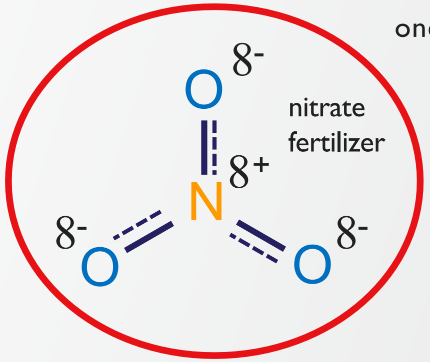


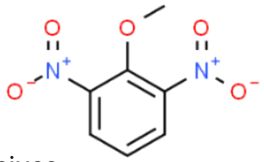


oncometabolites

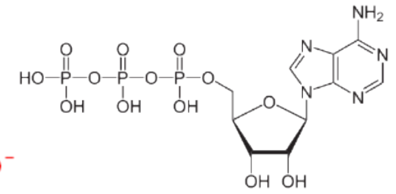


amino acids

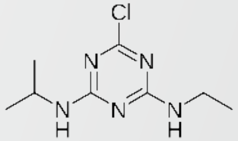




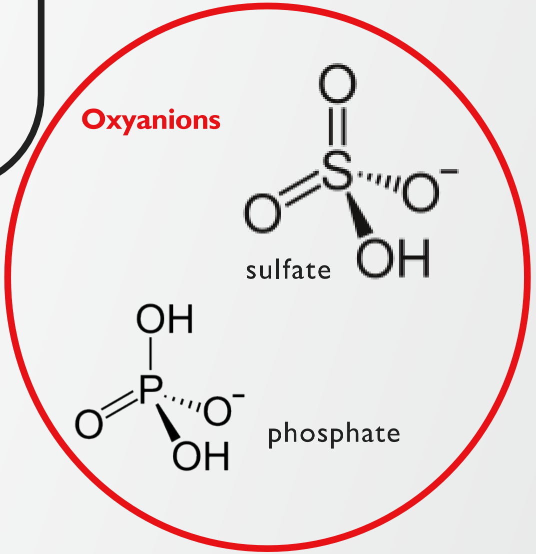
explosives

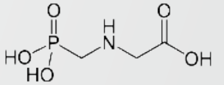


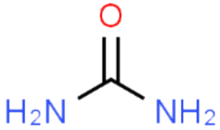
ATP



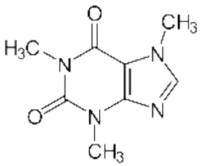
herbicides



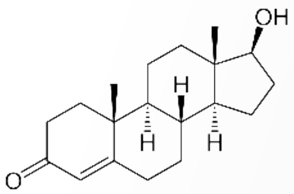




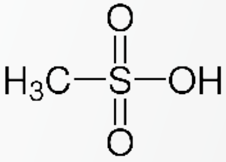
urea



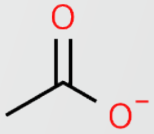
caffeine



steroid hormones



aerosols



primary metabolites

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Charles Cartwright

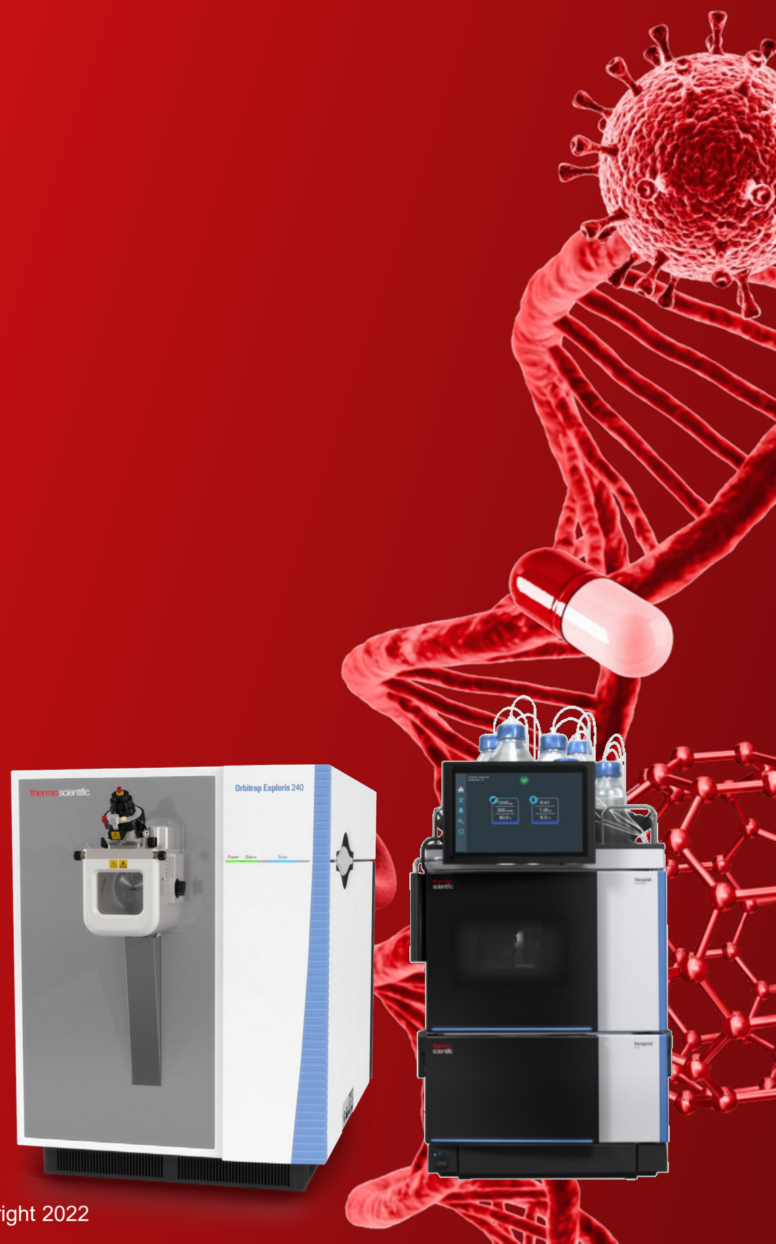
John K. Böhlke
Stan Mroczkowski

John Eiler
Nathan Dalleska

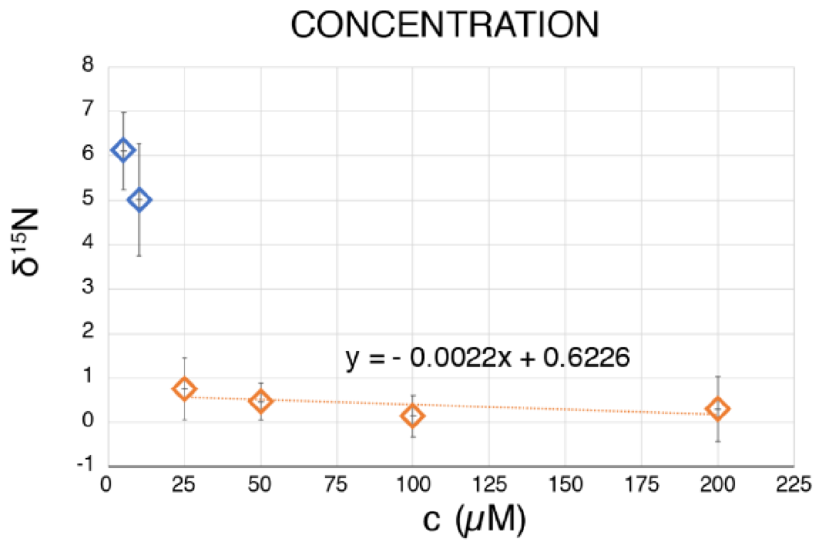
Sebastian Kopf

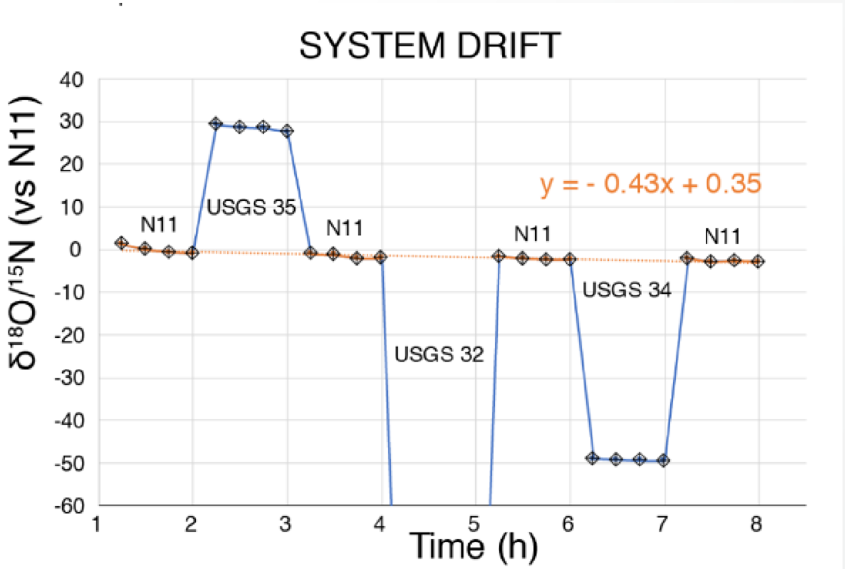
Xingchen Wang

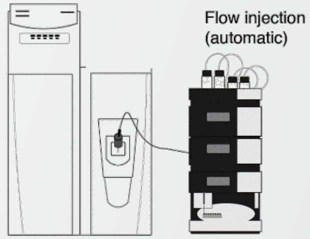
Thank you

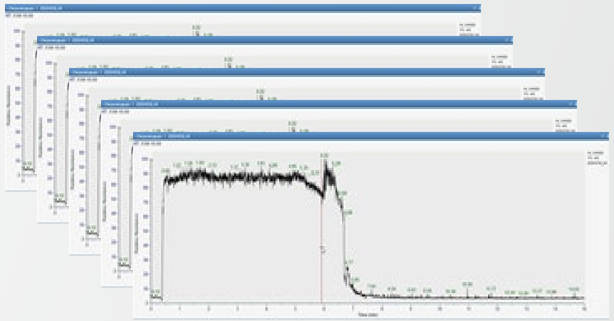


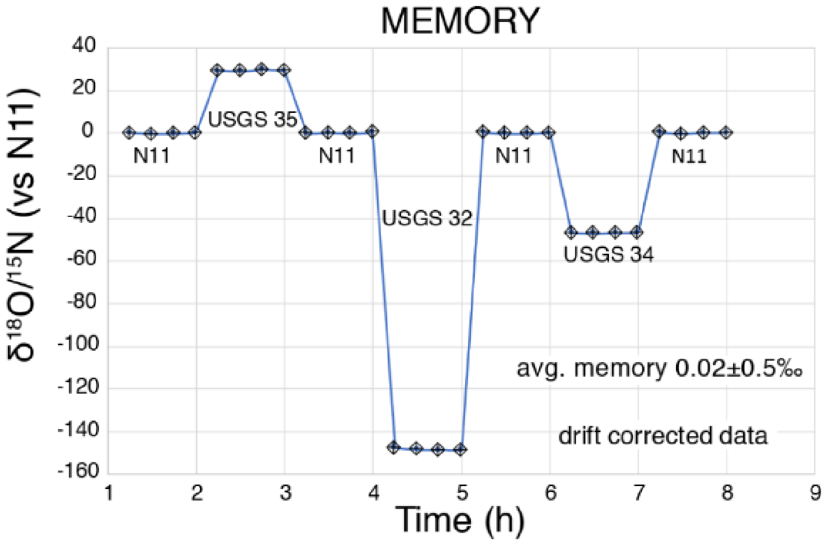
Basic characteristics for Orbitrap IRMS

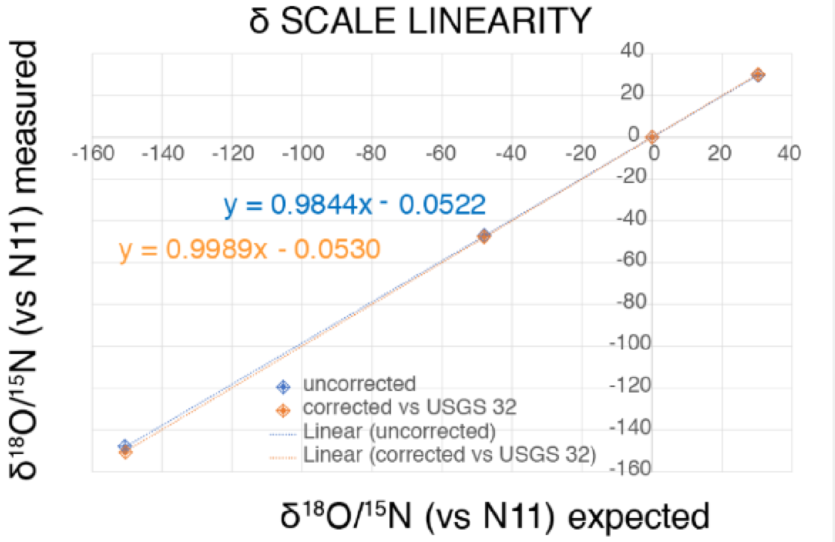








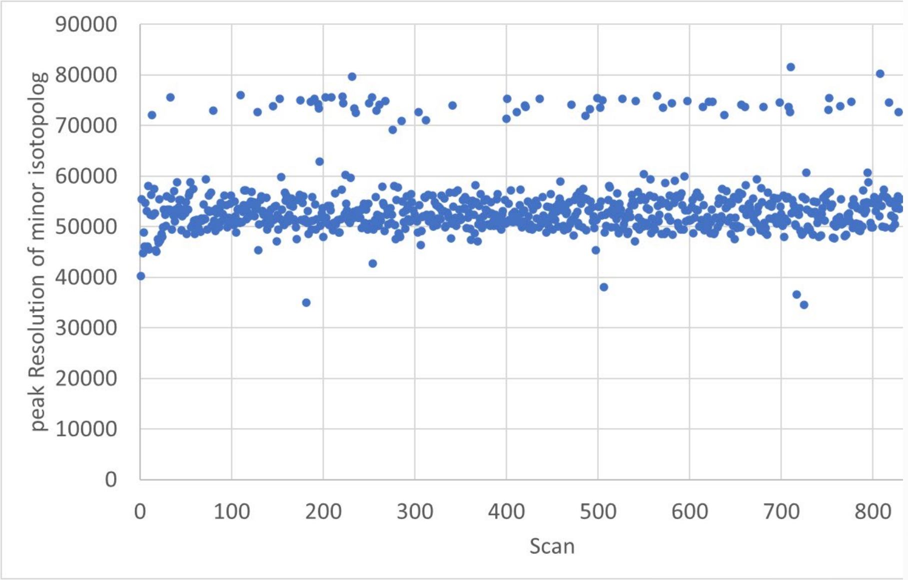




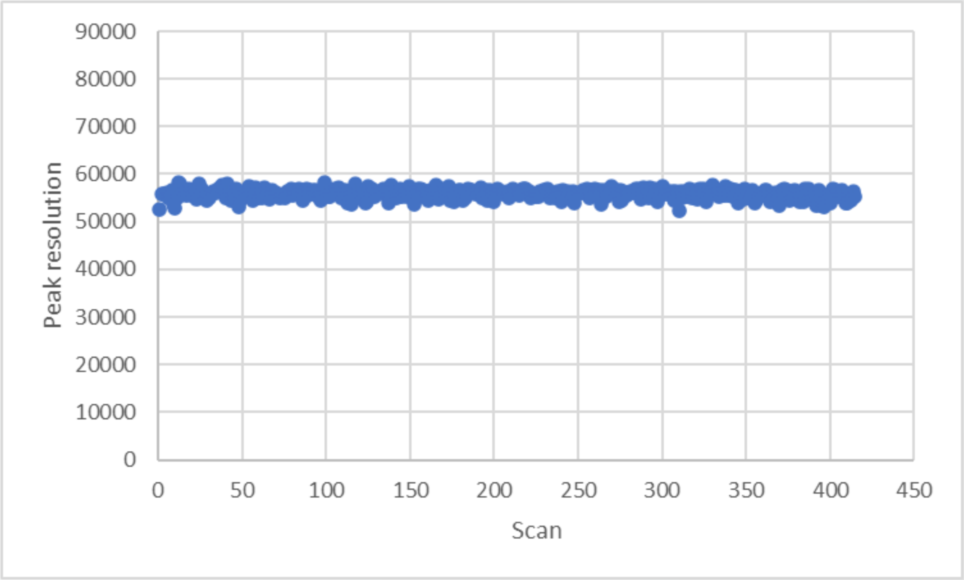
eFT

- Intensity of very low abundant isotope species being affected by eFT not being triggered consistently (absorption and magnitude mode)
  - Increase of µScans
  - Use of magnitude mode only (turn off eFT)
  - PhiSDM?

34S18O clumped isotopolog at 5µscans

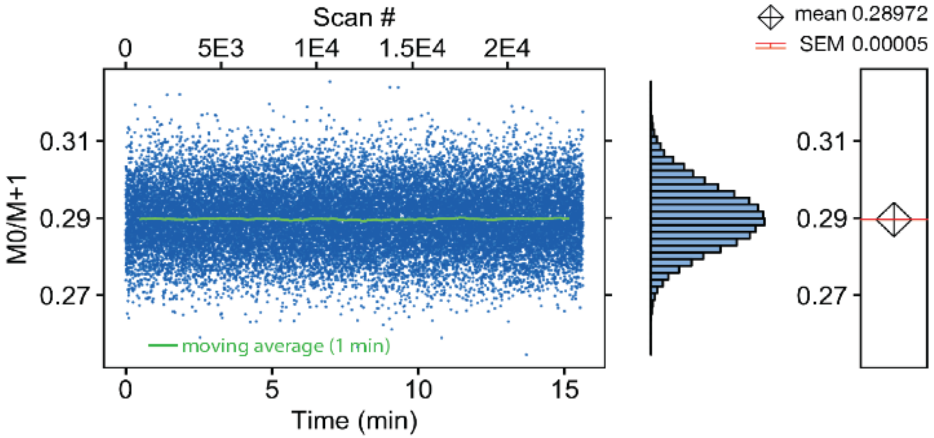


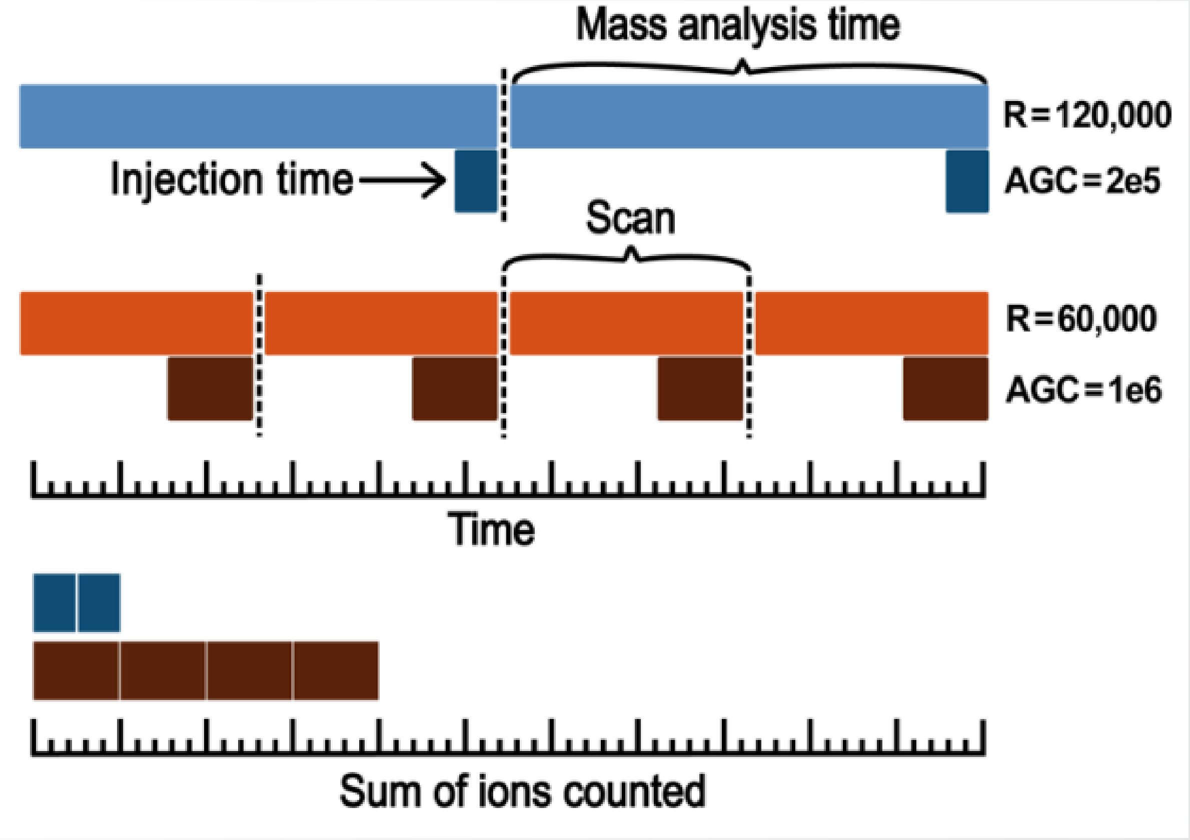
34S18O clumped isotopolog at 10µscans



How to increase precision for Orbitrap IRMS

- Ion statistics:





- **15,000 Resolution requires 40 ms / scan**
- **24 Scans per second**

[1] https://doi.org/10.1021/acs.analchem.1c04141 Anal. Chem. 2022, 94, 1092−1100

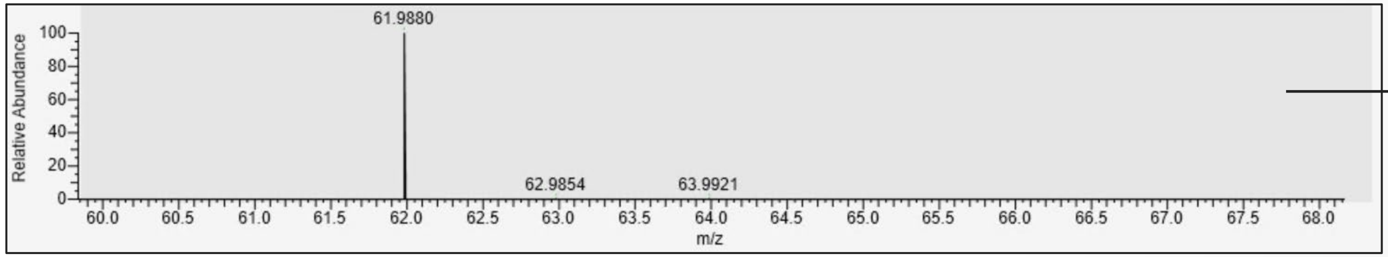
[2] https://doi.org/10.1016/j.jasms.2009.03.024 J Am Soc Mass Spectrom 2009, 20, 8, 1486–1495

Determining ion counts from Orbitrap measurements

ThermoFisher SCIENTIFIC

(1) $$R(^{15}\mathrm{N}) = \frac{N(15\mathrm{N}^{16}\mathrm{O}_4)}{N(14\mathrm{N}^{16}\mathrm{O}_4)}$$

- Calculating the ion count (N) for every isotopolog in each scan based on its Orbitrap signal



→ Ion counts

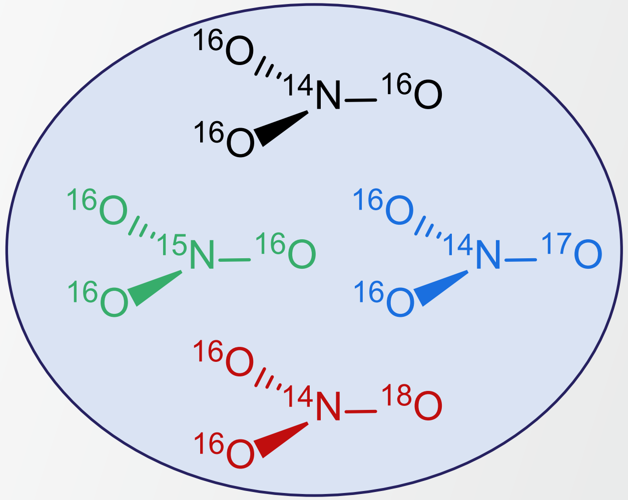
(4) $$N = \left(\frac{S}{N_P}\right)\left(\frac{C_N}{z}\right)\sqrt{\frac{R_N}{R}}$$

N = Ion counts
C_N = Empirical factor
S/N_P = Signal-to-noise ratio
z = charge of the ion
R = Orbitrap resolution
R_N = Reference resolution (240,000)

[1] https://doi.org/10.1021/acs.analchem.1c04141 Anal. Chem. 2022, 94, 1092−1100
[2] https://doi.org/10.1016/j.jasms.2009.03.024 J Am Soc Mass Spectrom 2009, 20, 8, 1486–1495

Isotope ratios

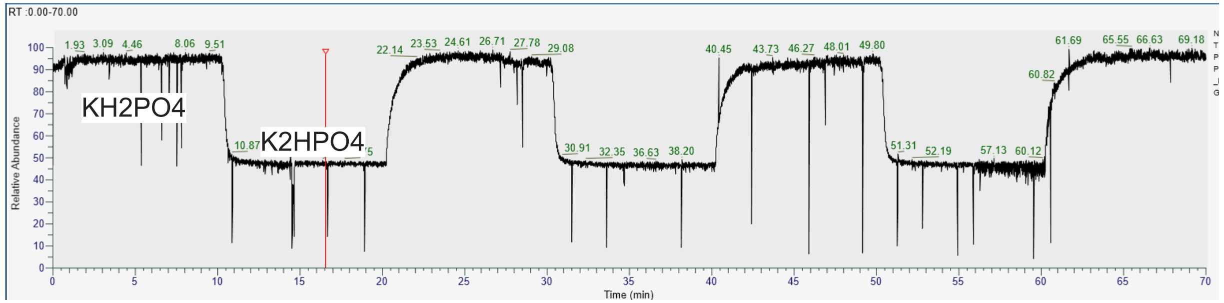
- Ratio (R) calculated by dividing the ion count (N) of the heavier isotope by the ion count of the lighter isotope
- For ^{15}N in Nitrate:



(1) $$R(^{15}\mathrm{N}) = \frac{N(15\mathrm{N}^{16}\mathrm{O}_3{}^{-})}{N(14\mathrm{N}^{16}\mathrm{O}_3{}^{-})}$$

- For the comparison of isotope ratios of natural abundances in between samples a working standards is sued
- The difference in the ratios expressed as δ-values

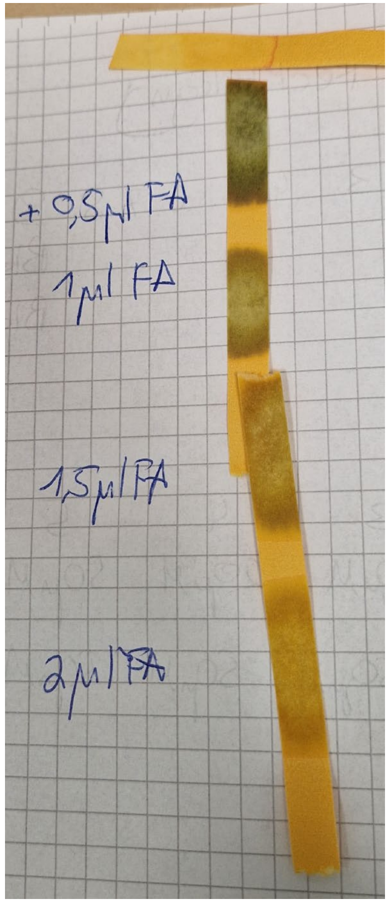
(2) $$\delta(^{15}\mathrm{N}) = \left(\frac{R_{sample}(^{15}\mathrm{N})}{R_{standard}(^{15}\mathrm{N})} - 1\right) \cdot 1000\ [‰]$$

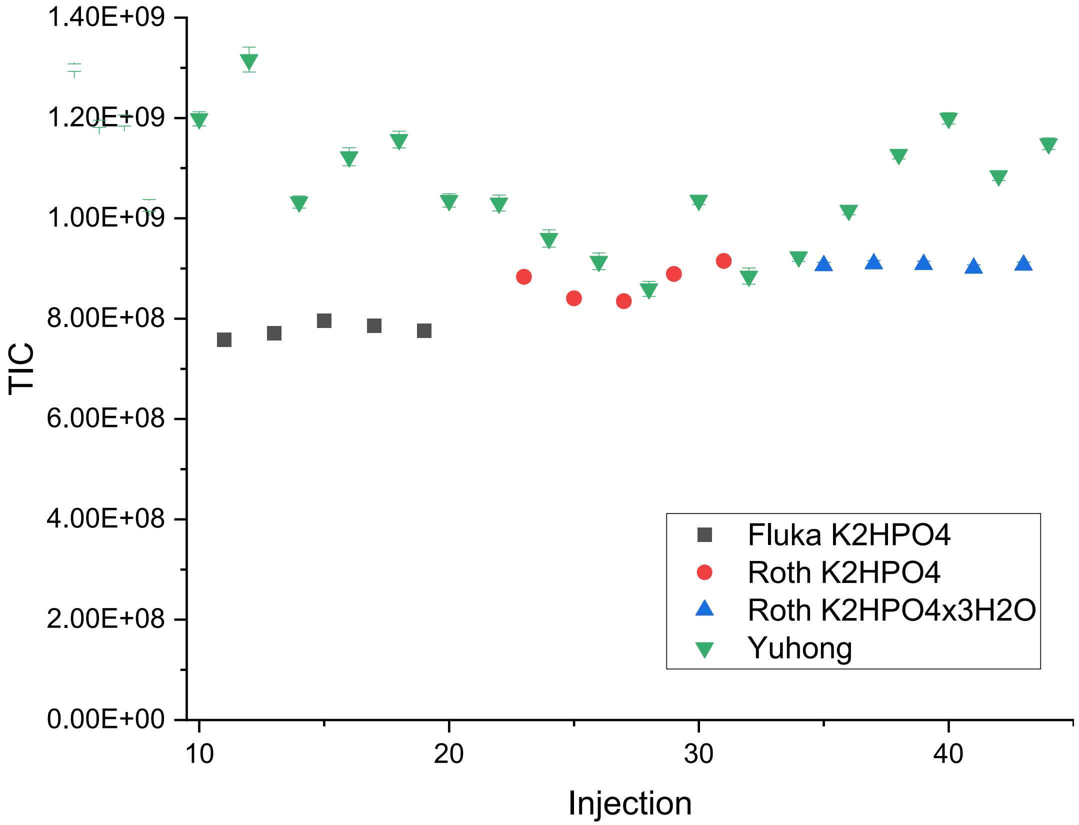


Without pH adjustment:

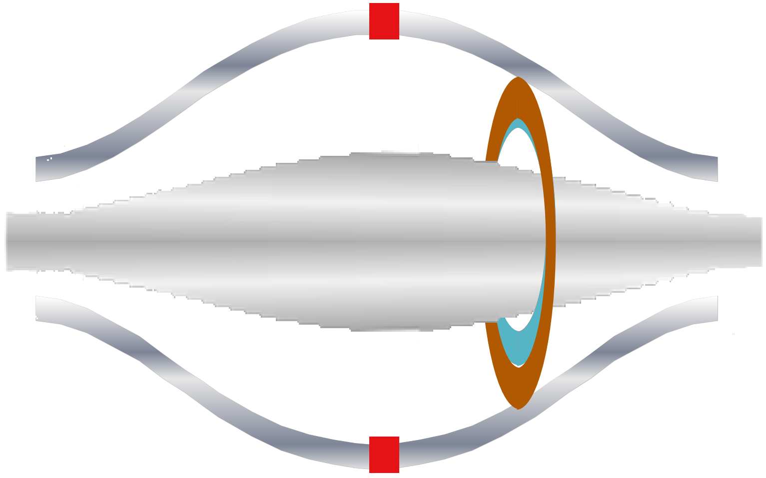
- KH_2PO_4: 2.44E9 Intensity
- K_2HPO_4: 1.16E9 Inensity

K_2HPO_4 gave <50% the intensity of KH_2PO_4

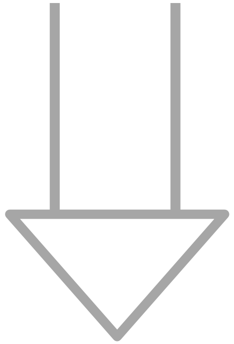


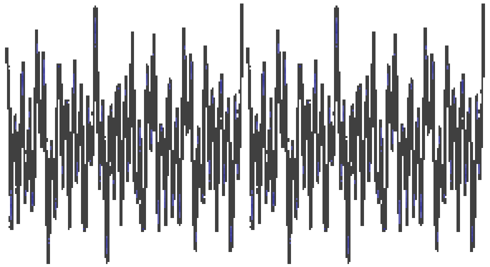


Orbitrap Mass Analyzer



$$\omega = \sqrt{\frac{k}{m/z}}$$





- Ions injected into the Orbitrap are trapped in an electrostatic field
- Each ion oscillates axially with a frequency that is proportional to its mass
- An image current of these oscillations is measured using a split outer electrode
- This image is then converted to a frequency spectrum using Fourier transform
- Frequency spectrum is converted to Mass Spectrum
- The longer a signal (transient) is measured, the higher the resolution