Isotopic "Fingerprinting" of PFAS – via the Thermo Scientific™ Orbitrap Exploris™ Isotope Solutions

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The world leader in serving science

Outline

- Thermo Fisher's magnetic sector portfolio
- Why use an orbitrap for natural abundance isotope measurements?
- Proof of concept: natural abundance ratios on an orbitrap
- Measurement technique
- PFAS data
- Questions

The current Thermo Scientific™ gas IRMS portfolio

We currently offer a scaled portfolio of MS and sample introduction solutions

- **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

Oxyanions: refine understanding of element cycling

Atmosphere ⟷ Soil ⟷ Water

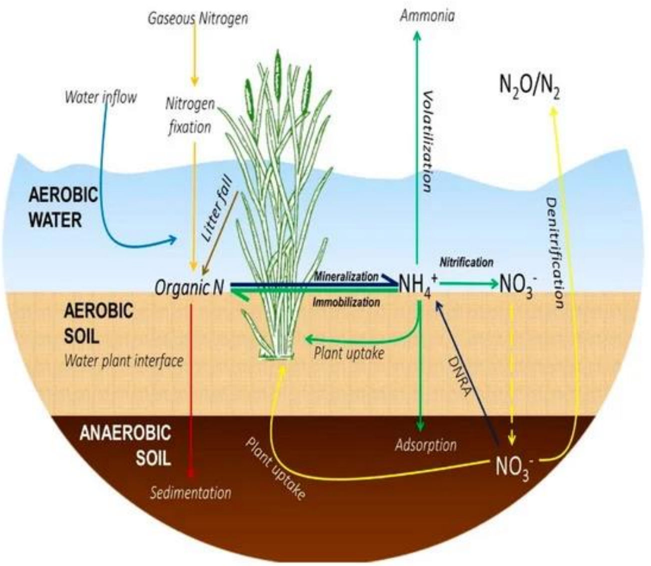
NO_3^- SO_4^{-2} PO_4^{-3}

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

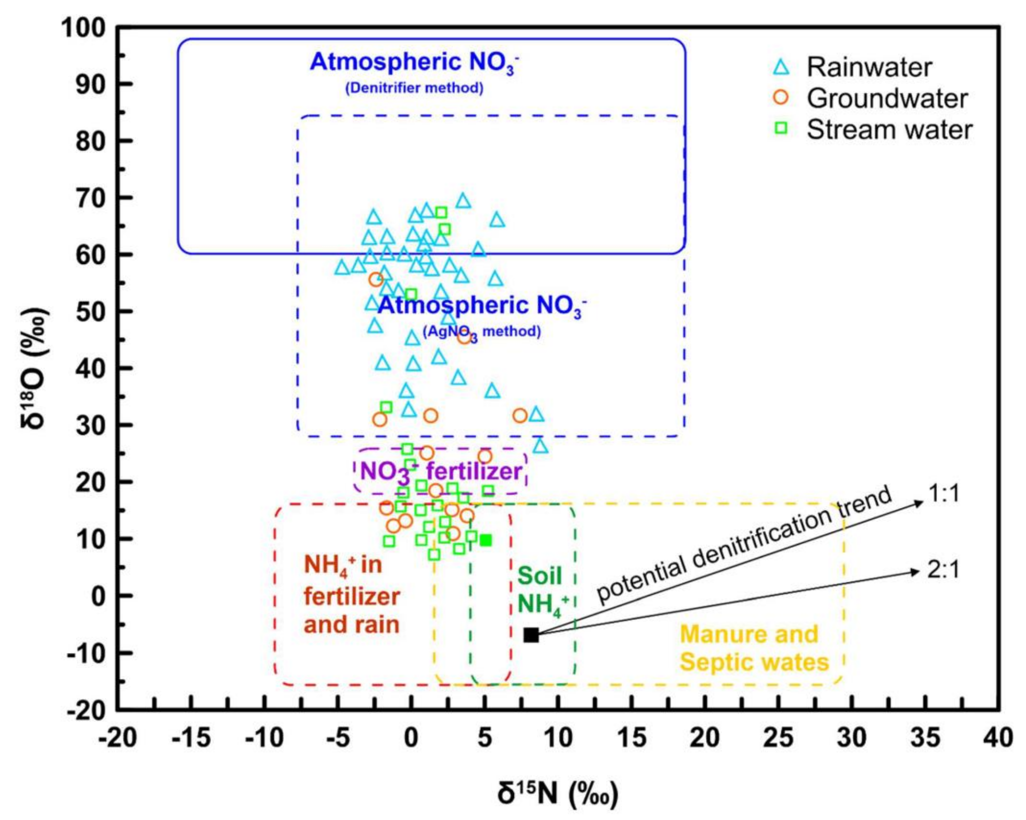
What are current isotope ratio techniques missing?

A wish list

- Direct measurement of intact molecules
- Access to liquid, polar samples
- Higher sensitivity
- Less sample preparation, eliminate solid → gas process
- Position specific isotope analysis



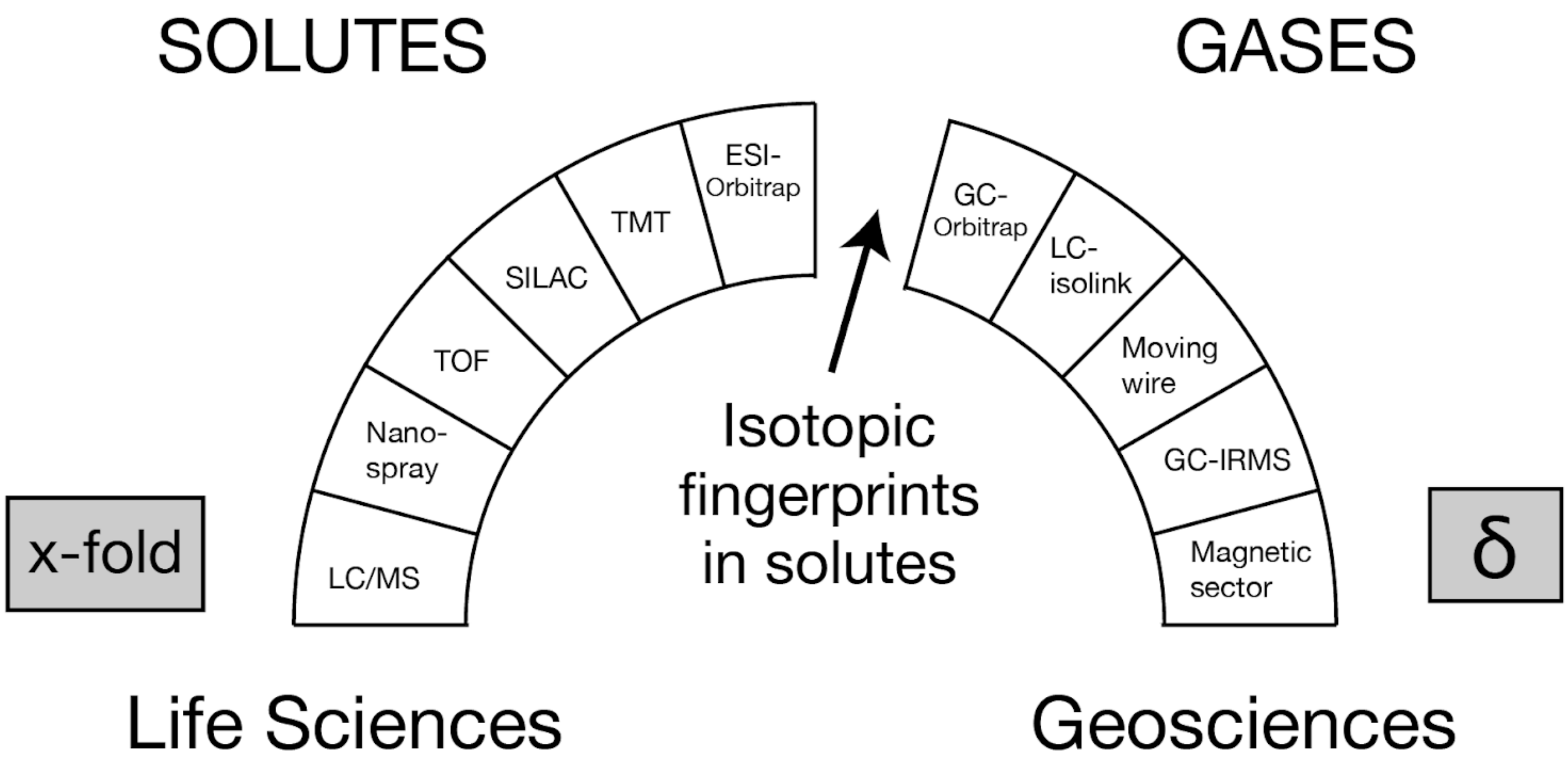
Nitrogen **2021**, 2, 196–217. **https://doi.org/10.3390/nitrogen2020013**



Water **2020**, *12*(1), 138; **https://doi.org/10.3390/w12010138**

An opportunity to bridge a gap exists

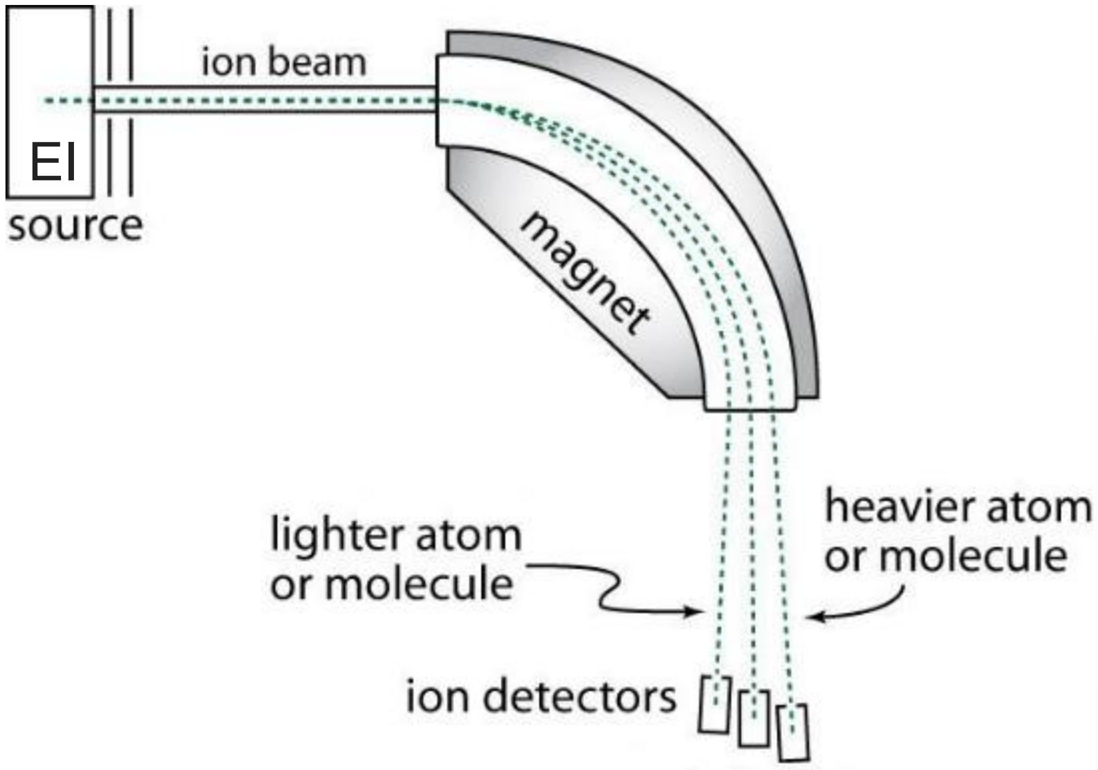
Combine technology developed for Life Science with approaches from stable isotope geochemistry



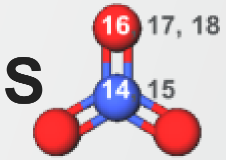
Mass spectrometers for isotope ratio analysis

Classic isotope-ratio MS N_2, O_2, N_2O

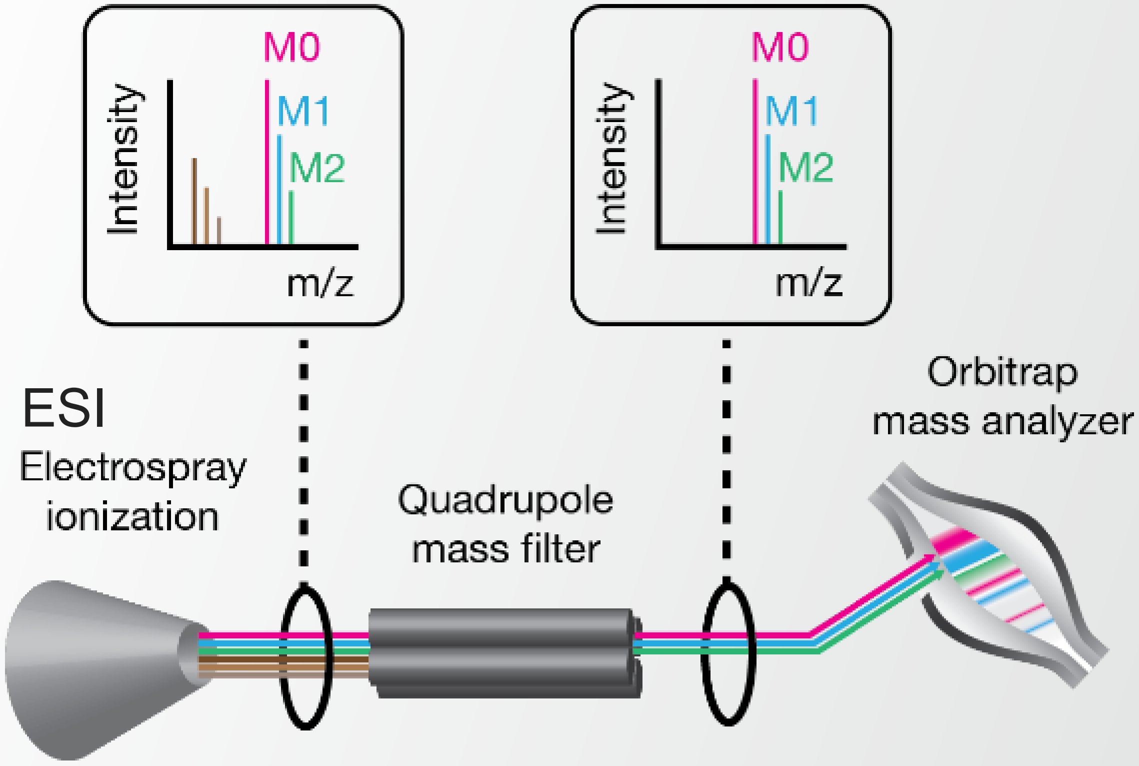
- **Indirect**: Conversion into **simple gases**
- Combining isotopologues on **few signals**



Thermo Scientific Orbitrap™ MS



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



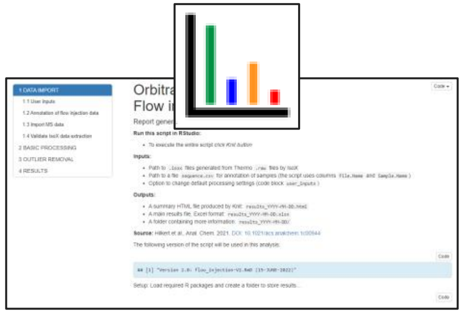
Orbitrap Exploris Isotope Solutions

Thermo Scientific™ Orbitrap Exploris™ Isotope Solutions includes:

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

Dual Syringe Inlet

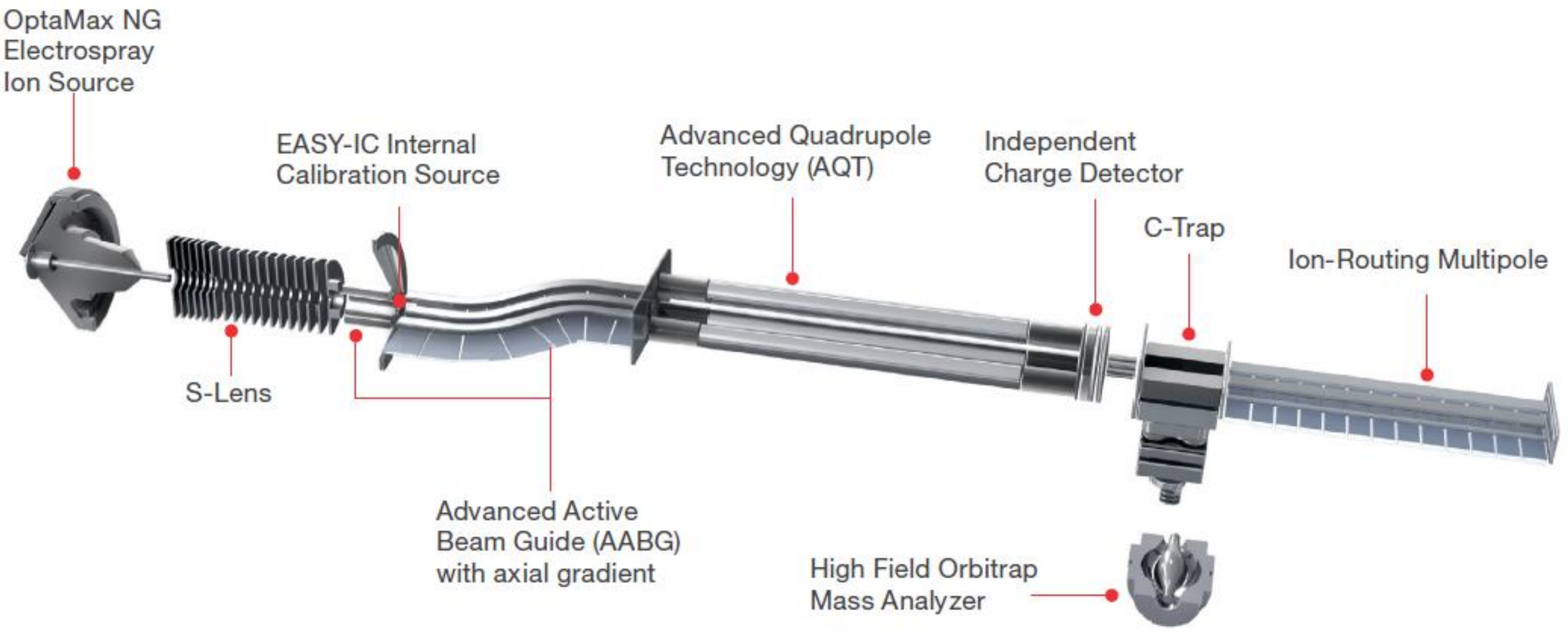
Data evaluation Package for Isotope Ratio MS



Optional Thermo Scientific™ Vanquish™ Neo UHPLC System

What's inside the Orbitrap Exploris MS

Essential components for isotope ratio analysis

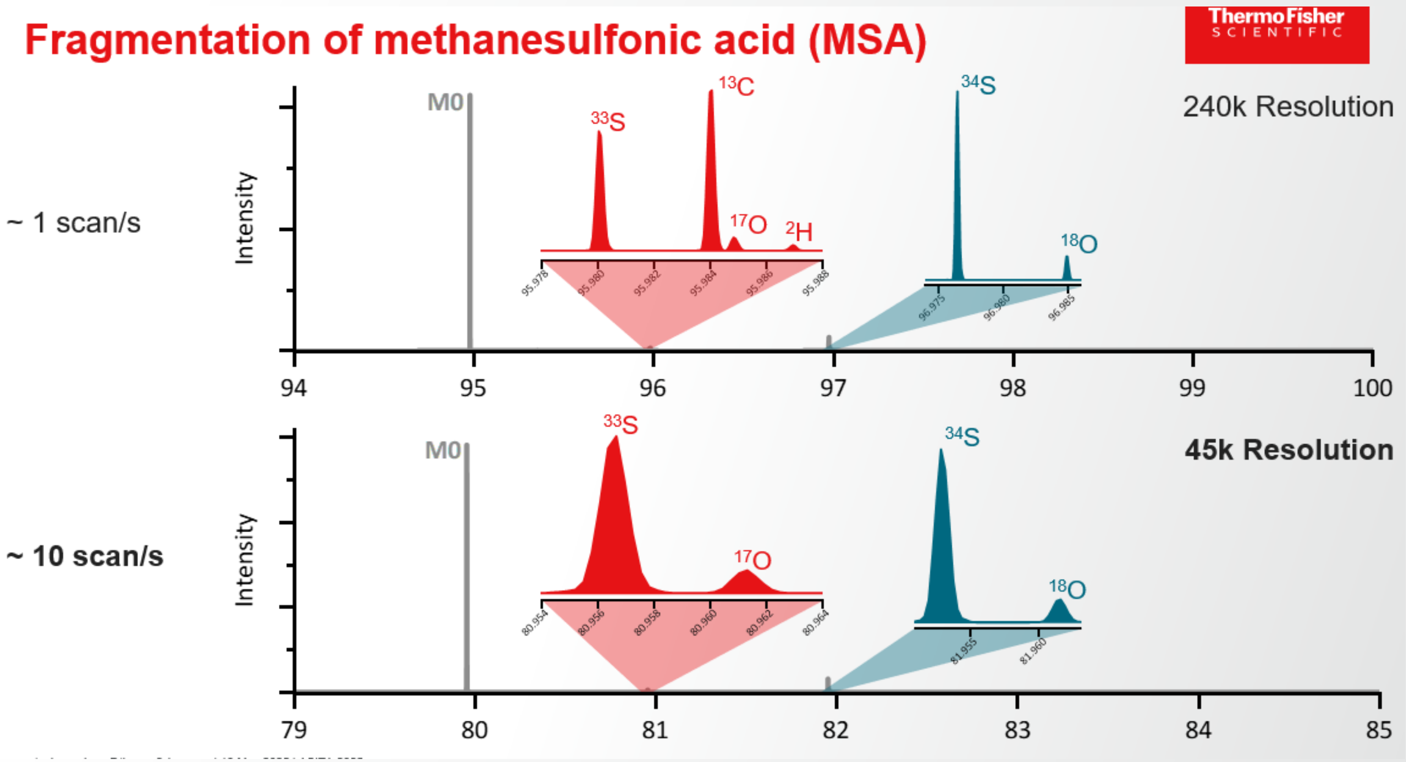


Important parameters for Orbital analyzer

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:

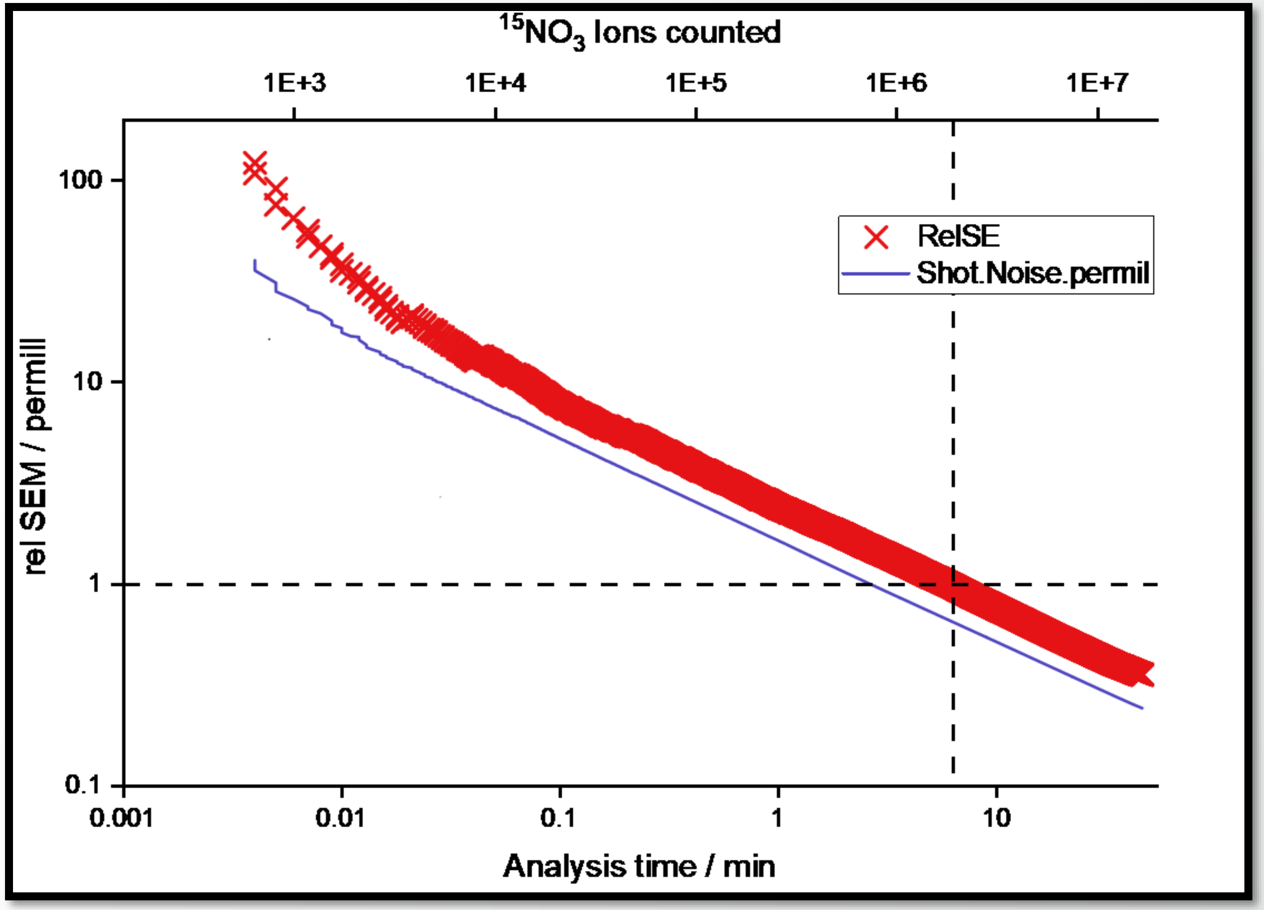
	Resolution at *m/z* 200	Scans per second
NO_3^-	15,000	22
	30,000	12
HSO_4^-	45,000	10
	60,000	7
Organics	120,000	3
	240,000	1.5



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.

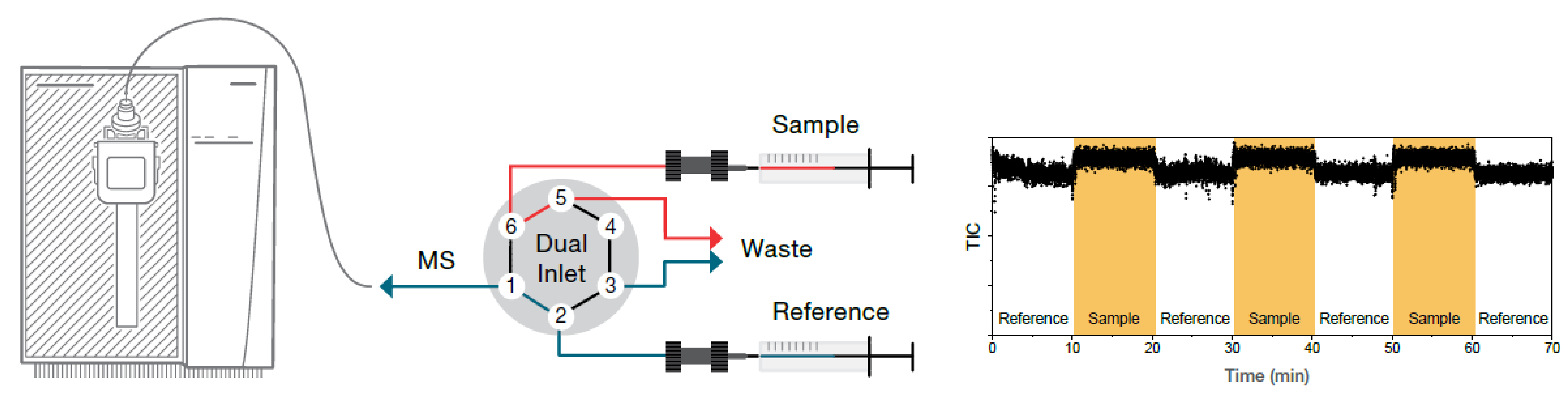
- **Accuracy: Sample/Standard comparison**
  - NO_3^- Analysis of signals (reference/sample) for 6–9 mins



Dual Syringe Inlet

Sample introduction technique

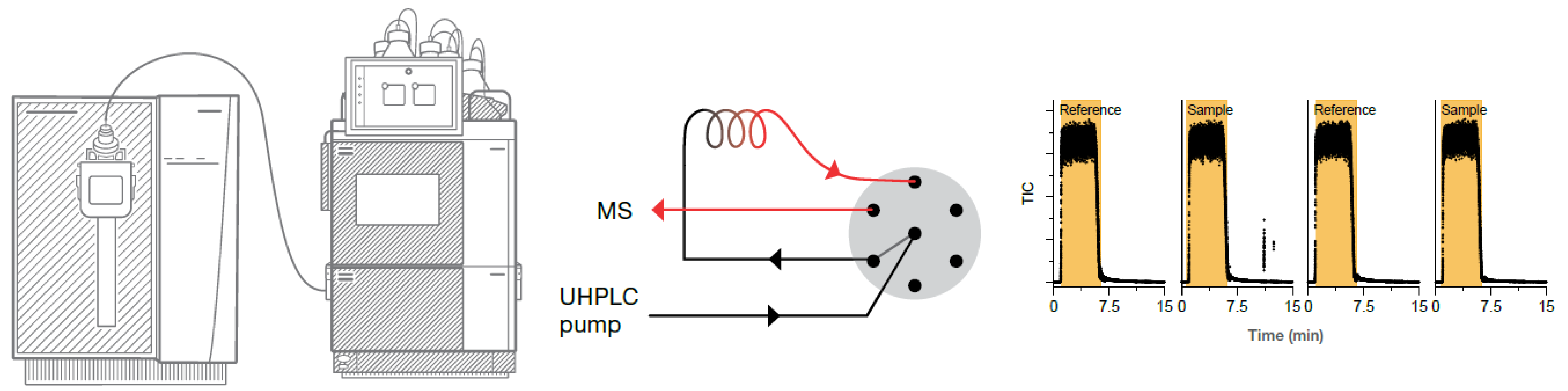
- **Dual Syringe Inlet System** utilizing the diverter valve option of the Orbitrap Exploris MS
- Direct infusion of sample (**50 µM** in MeOH) with a flow rate of **4 µl/min** via a syringe pump; sample reference comparison by switching of a diverter valve.



Automated In-Flow Injection

Sample introduction technique

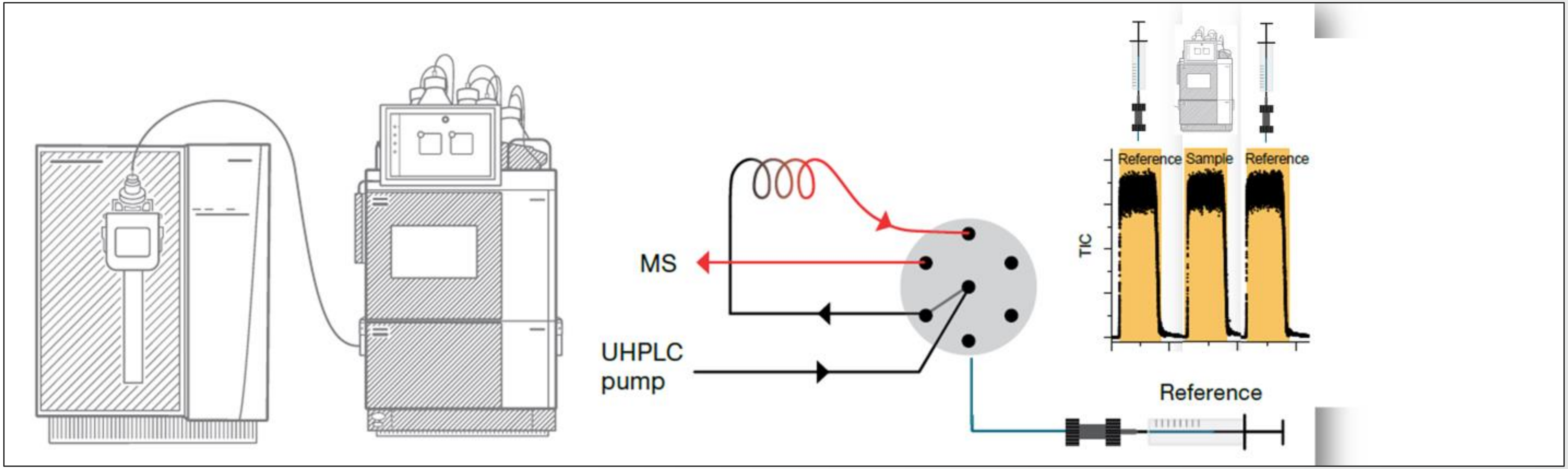
- **In-flow Injection** - Vanquish Neo UHPLC System
- Loop-injection of **20-30 µL** sample (**50 µM** in MeOH) by the Autosampler into a flow of **4 µL/min** of MeOH.



Automated In-Flow Injection with Syringe Reference

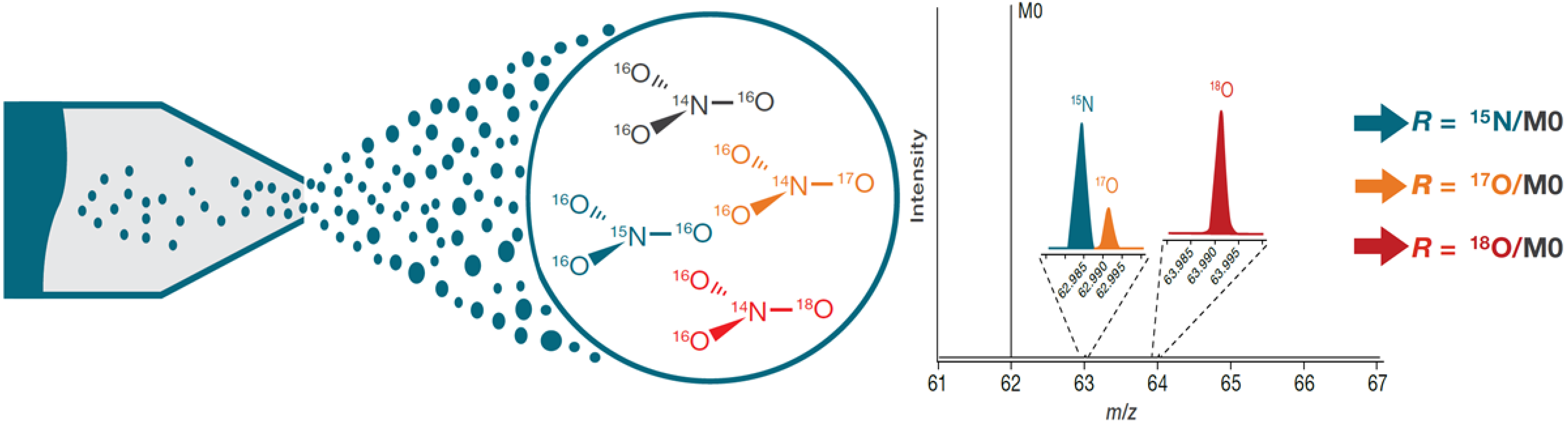
Sample introduction technique

- **In-flow Injection** - Vanquish Neo UHPLC System
- Syringe injected reference material to control for ESI instrument drift. Increases orbitrap uptime while loop is washed out.



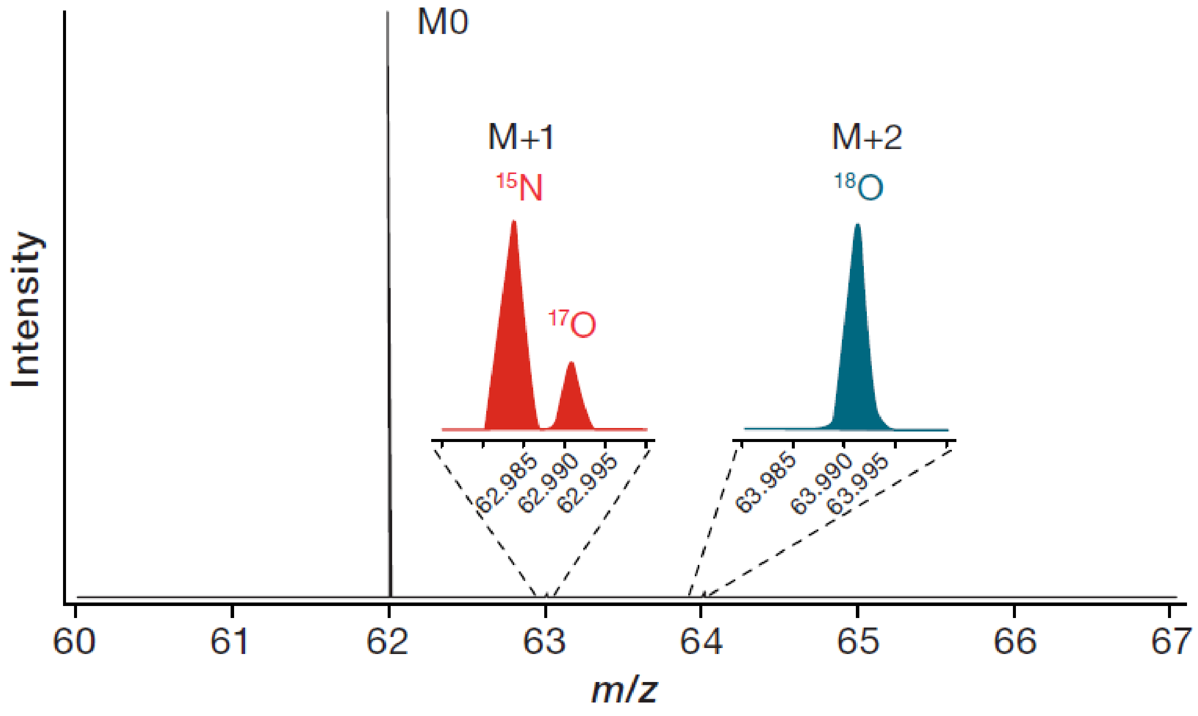
Orbitrap for isotopes: workflow for nitrate

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

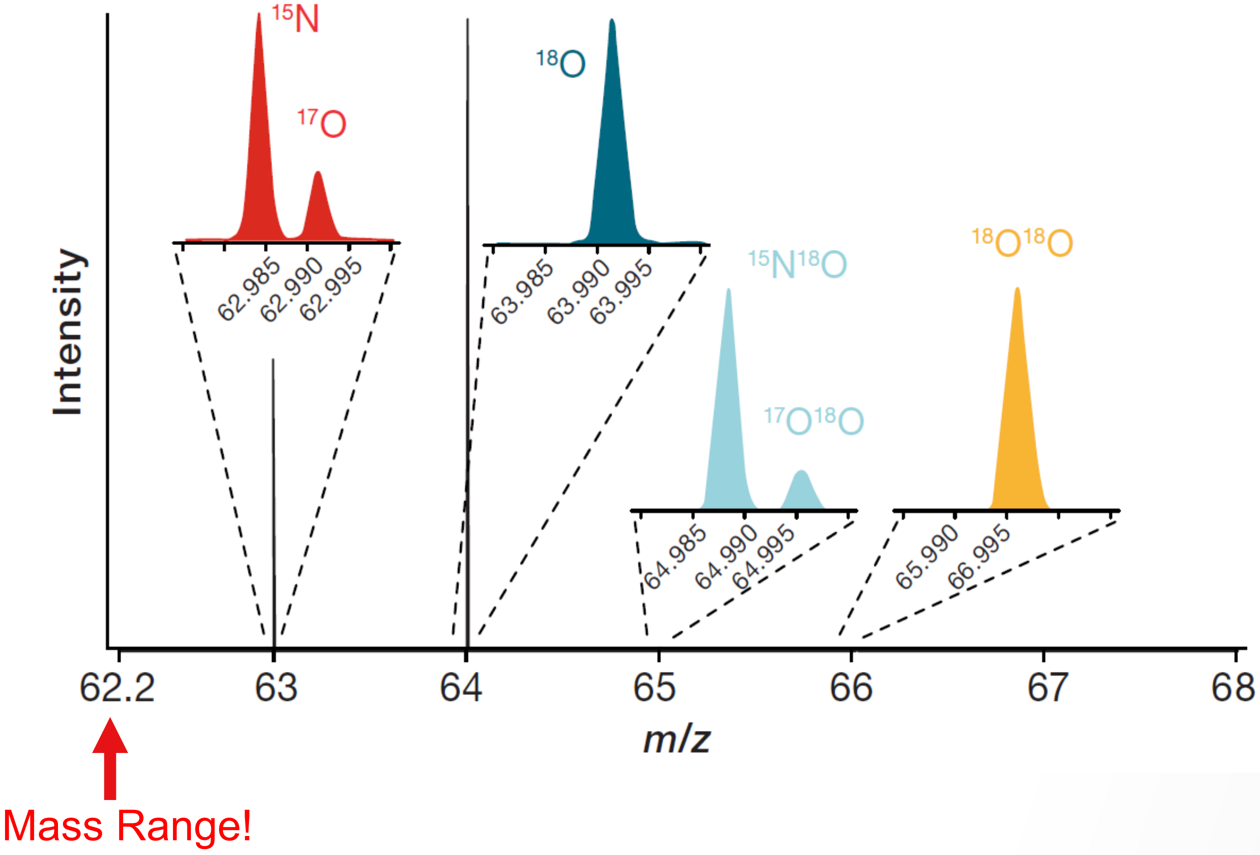


- M0 peak as basepeak

	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	

ESI-Orbitrap for isotopes – methodology

'noM0' experiment



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

	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	

Dual Inlet measurements of nitrate

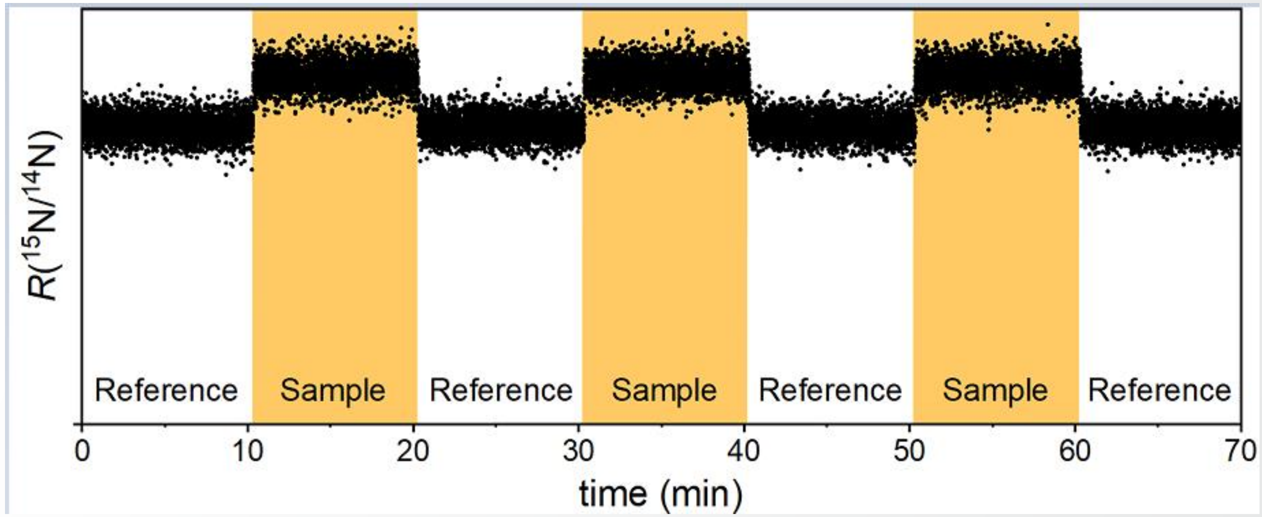
'M0' experiment:

- 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.

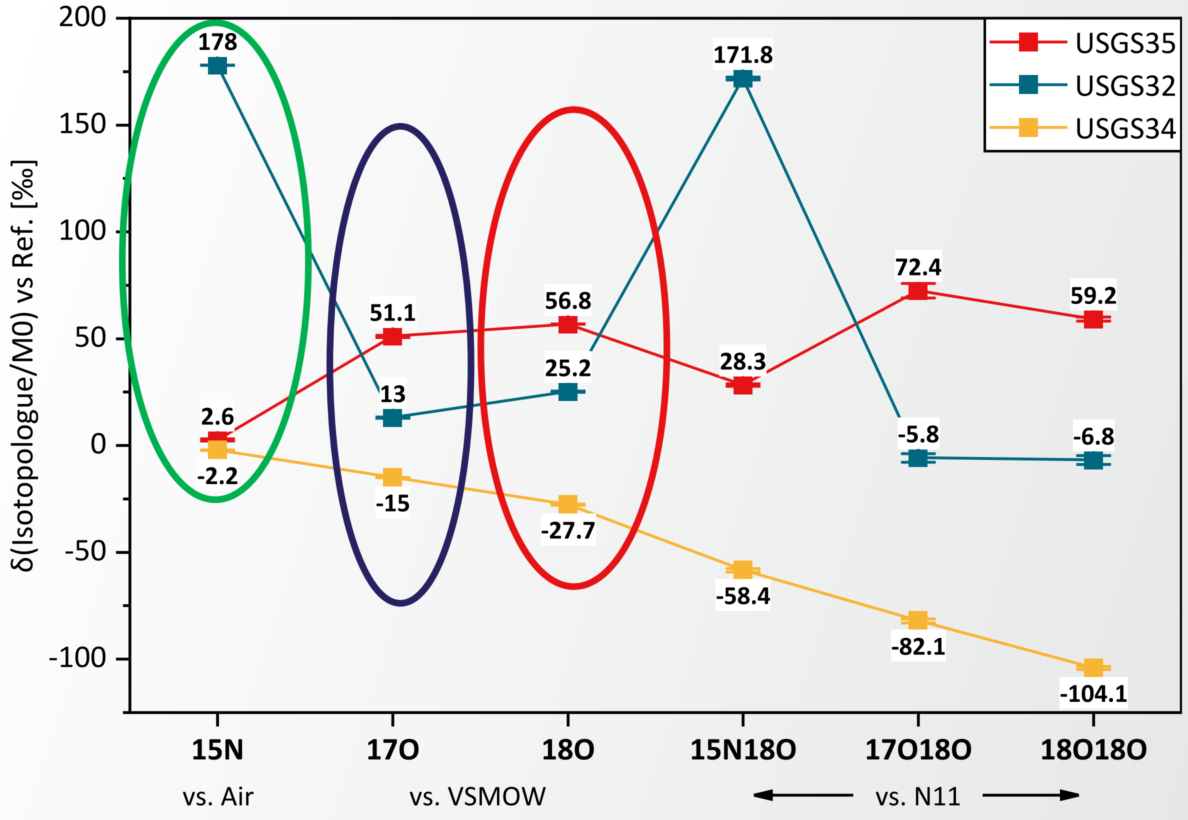
Referencing scheme for nitrate

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 ‰

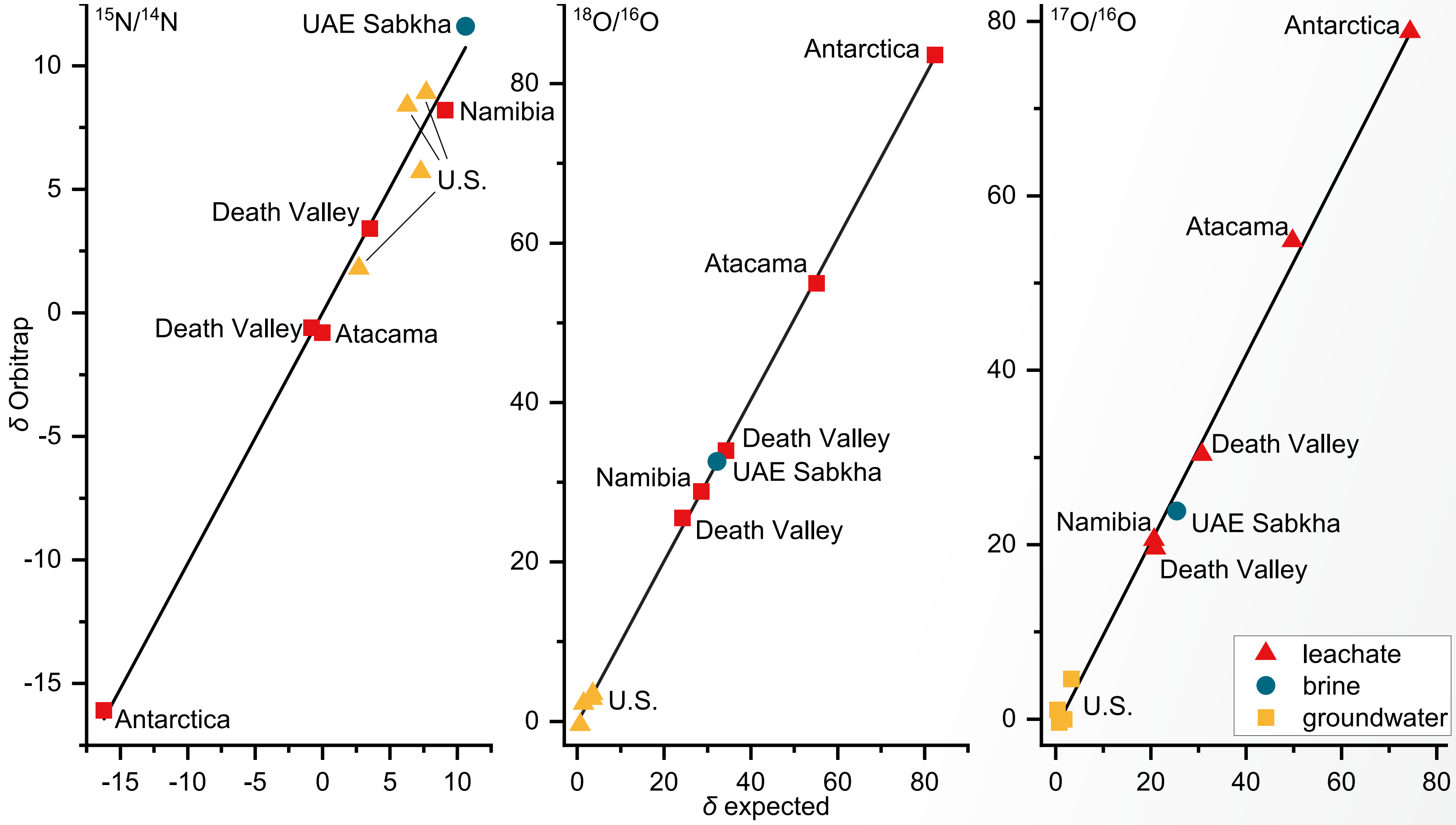
Isotope ratio data of six of nitrate`s most abundant isotopologues using the In-Flow Injection setup.



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

Nitrate analysis of environmental samples

‘M0’ experiment



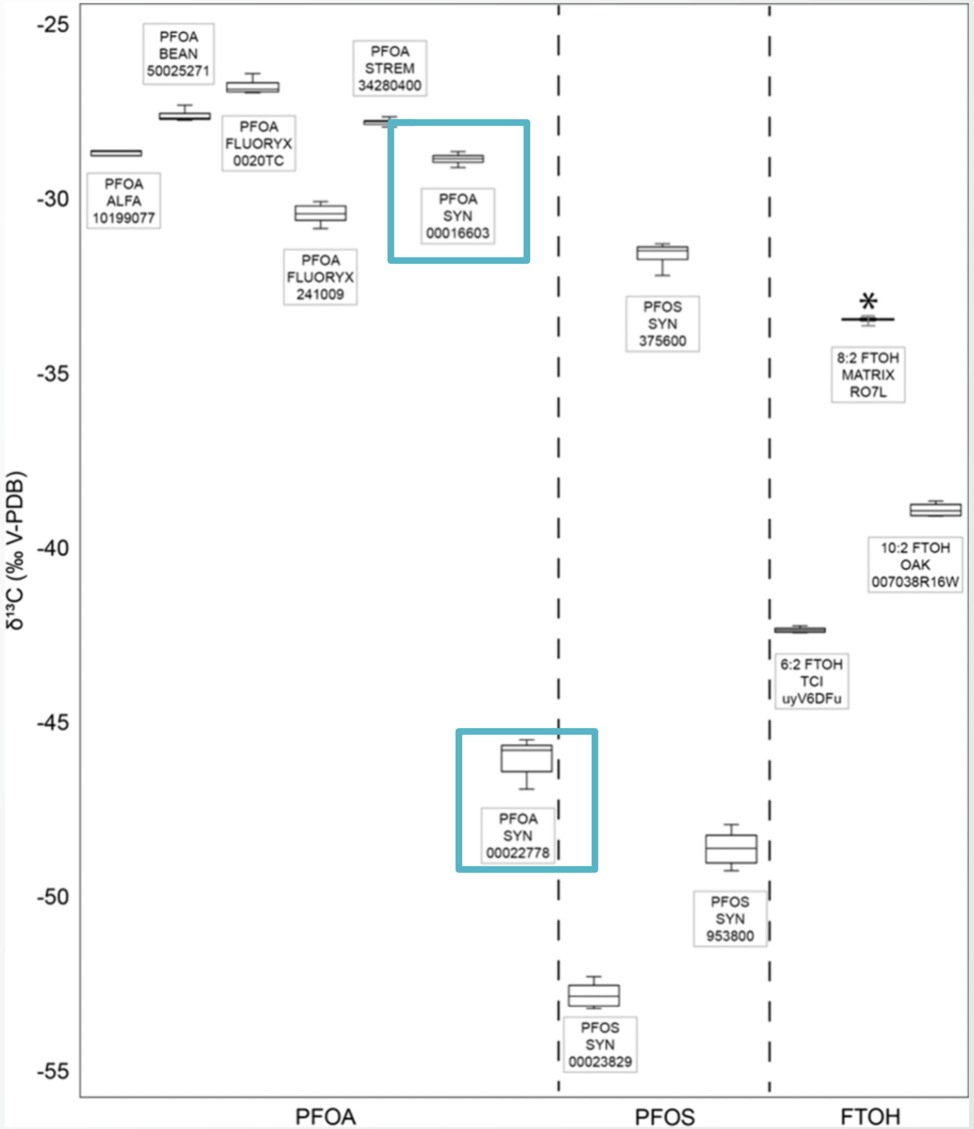
Dual Syringe Inlet methodology

- 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.

Carbon stable isotope analysis of PFAS sources

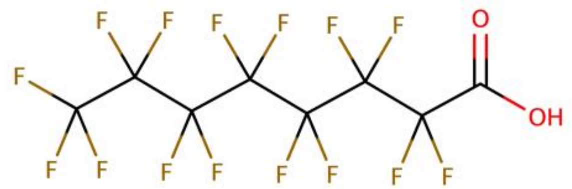
- Carbon isotope analysis of PFAS, PFOA and FTOH were acquired from multiple vendors and where possible, from multiple lots.
- There was variation in the carbon isotope values among different compounds, manufacturers, and batches.
  - Ex. PFOA Syn $\delta^{13}C$ lots differed (−28.9 ± 0.3‰, and −46.1 ± 0.7‰).
- Variability among mean carbon isotope values makes EA-IRMS a potentially powerful tracking tool for PFAS contaminants.

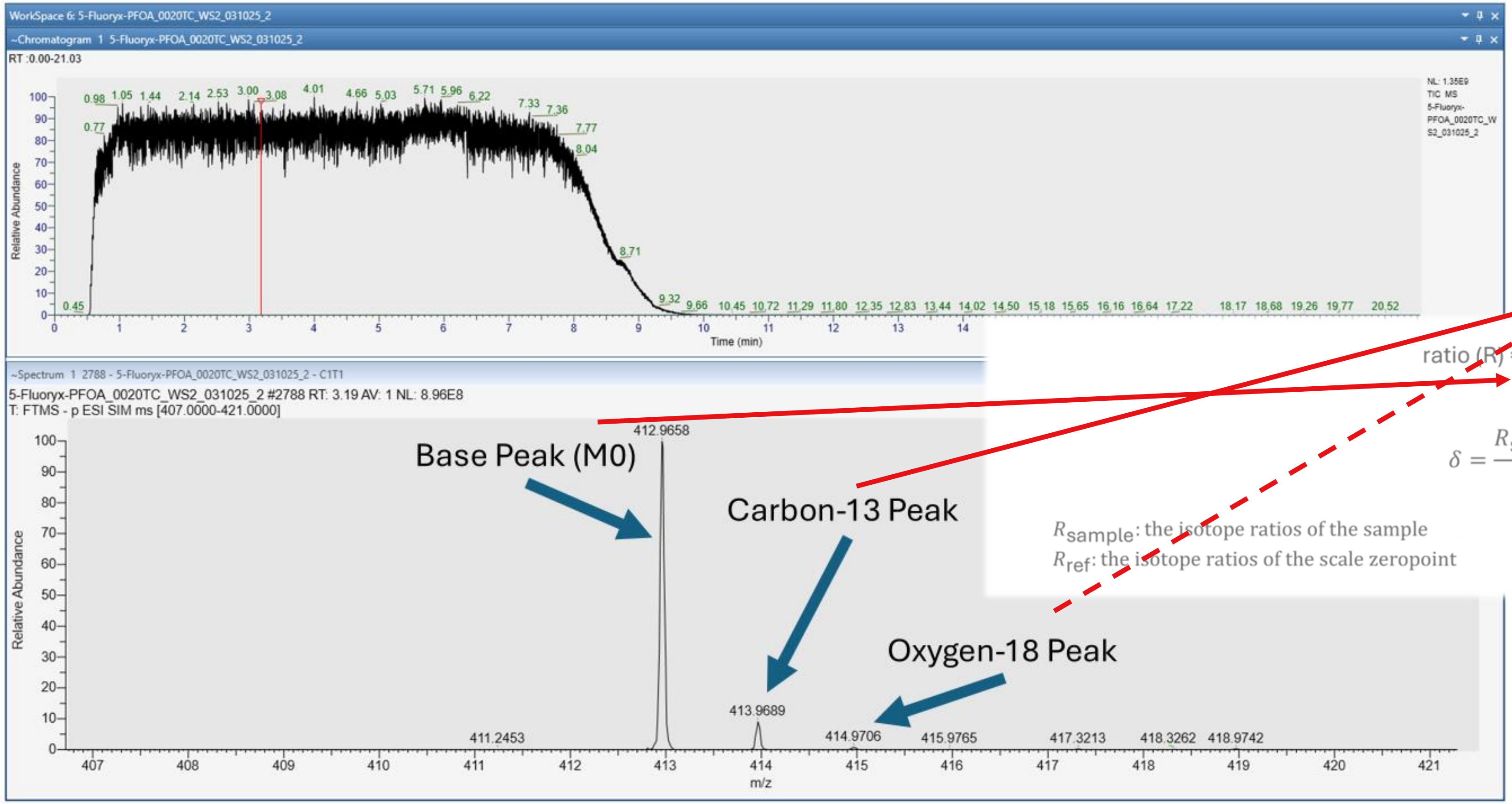


- Dombrowski et al (2025). *Environmental Science & Technology Letters* https://doi.org/10.1021/acs.estlett.5c00021

8 μM solution of PFOA run on Orbitrap

PFOA Analysis





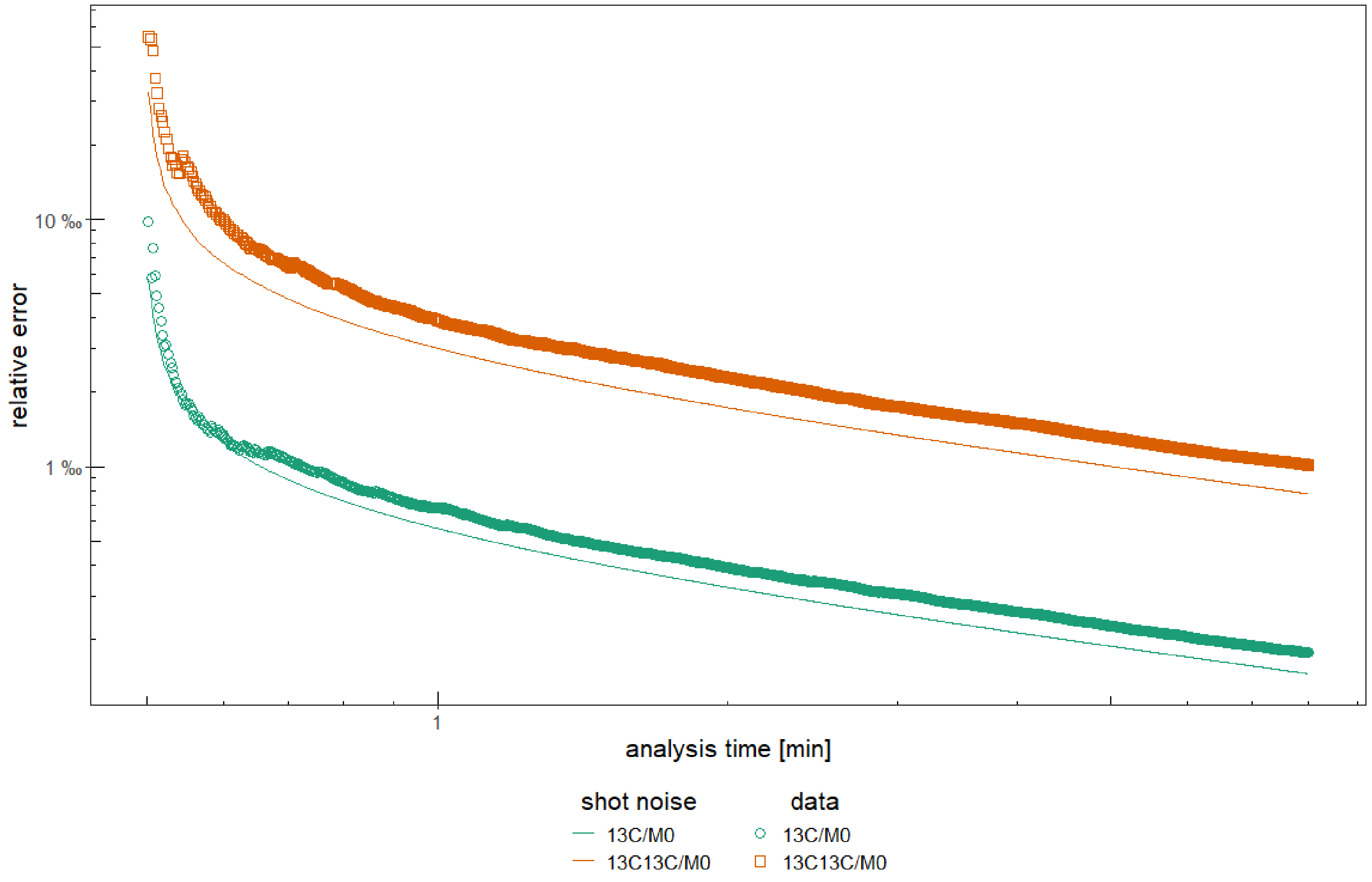
$$\text{ratio }(R) = \frac{\text{heavier isotope}}{\text{lighter isotope}}$$

$$\delta = \frac{R_{\text{sample}}}{R_{\text{ref}}} - 1$$

R_{sample}: the isotope ratios of the sample

R_{ref}: the isotope ratios of the scale zeropoint

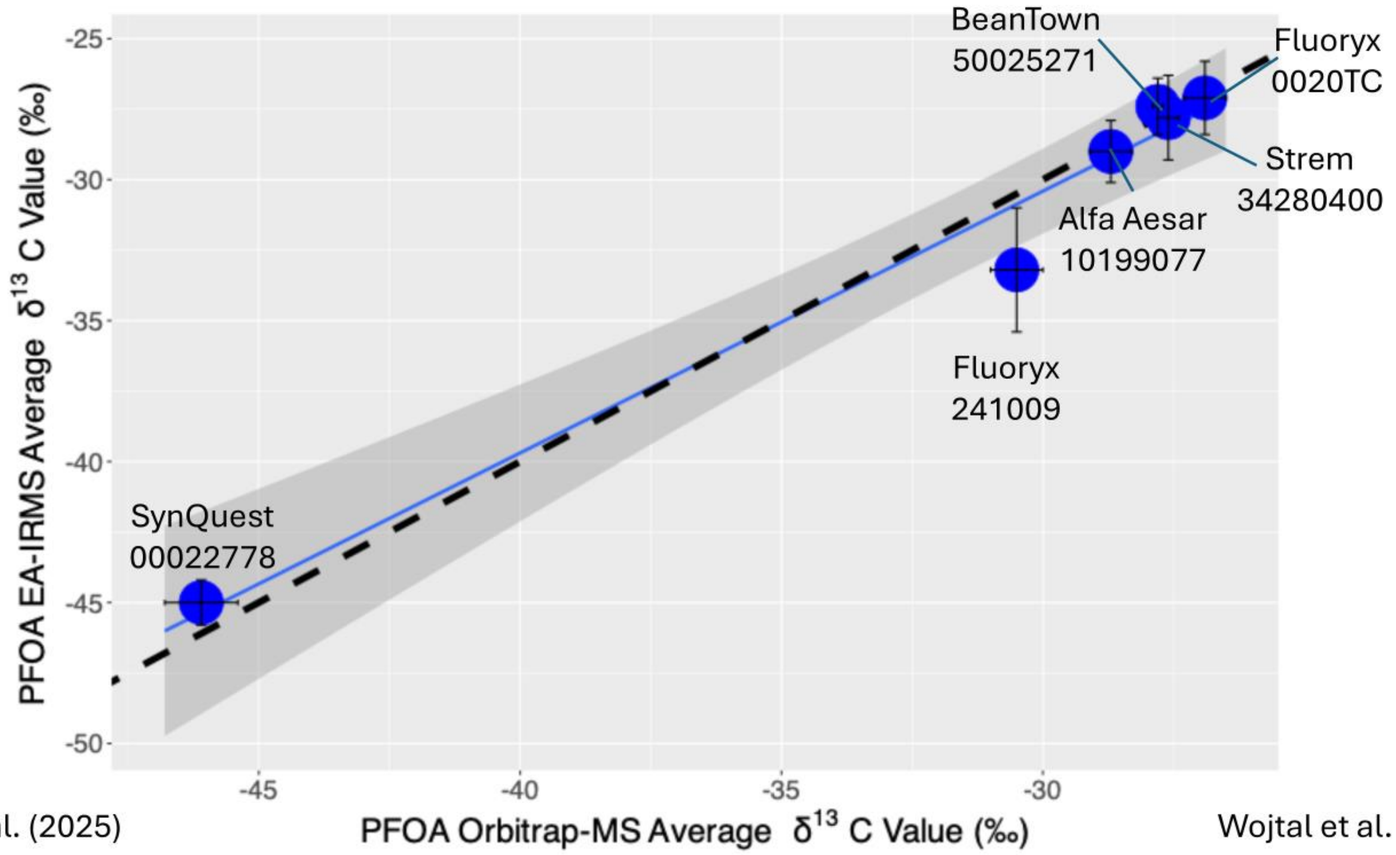
8 µM solution of PFOA run on Orbitrap



EA-IRMS vs Orbitrap IRMS Data

Thermo Fisher SCIENTIFIC

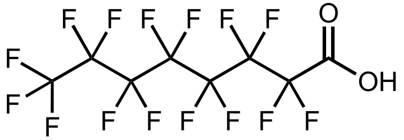
Orbitrap Method Validation



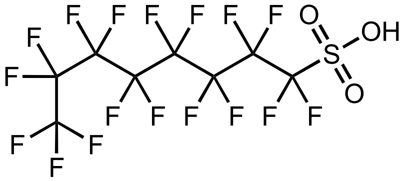
Dombrowski et al. (2025)

Wojtal et al. (*In Prep*)

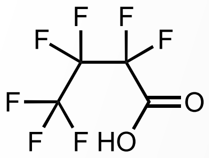
PFAS



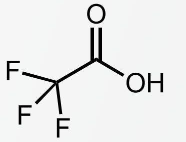
PFOA
Chemical Formula: $C_8HF_{15}O_2$
Exact Mass: 413.97370



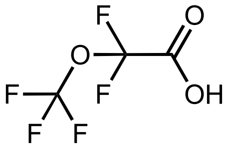
PFOS
1763-23-1
Chemical Formula: $C_8HF_{17}O_3S$
Exact Mass: 499.93749



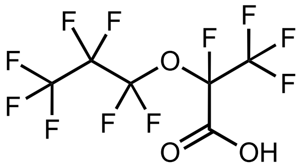
PFBA
Chemical Formula: $C_4HF_7O_2$
Exact Mass: 213.98648



TFA
Chemical Formula: $C_2HF_3O_2$
Exact Mass: 113.99286

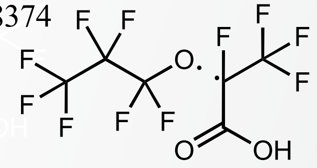


PFMOAA 674-13-5
Chemical Formula:
$C_3HF_5O_3$
Exact Mass: 179.98458



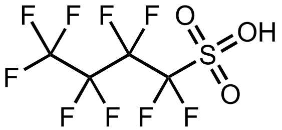
HFPO-DA
13252-13-6

Chemical Formula: $C_3F_7O^{\bullet}$
Exact Mass: 184.98374



Chemical Formula: $C_3HF_4O_2^{\bullet}$
Exact Mass: 144.99127

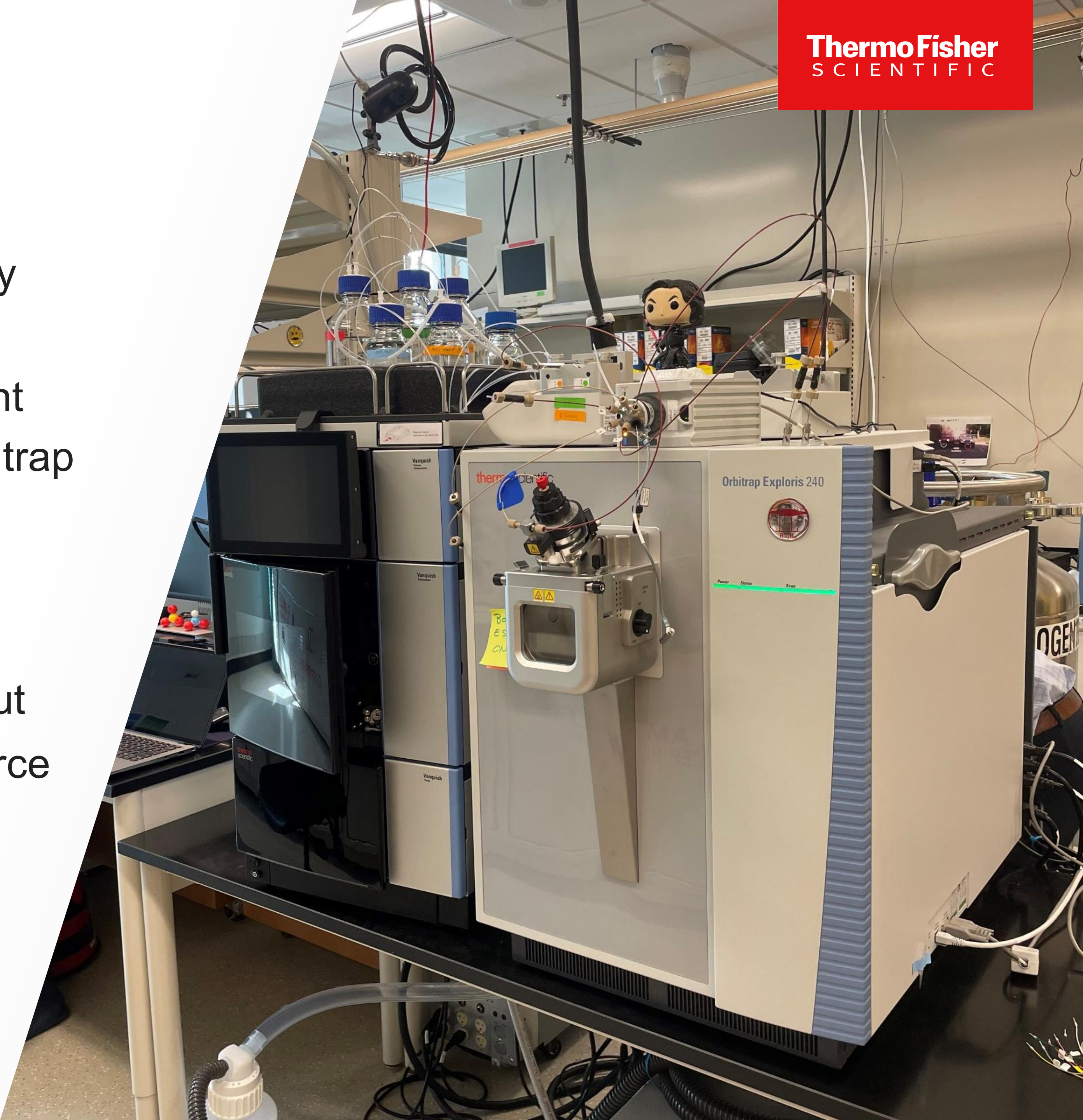
HFPO-DA in-source fragmentation very common



PFBS
375-73-5
Chemical Formula: $C_4HF_9O_3S$
Exact Mass: 299.95027

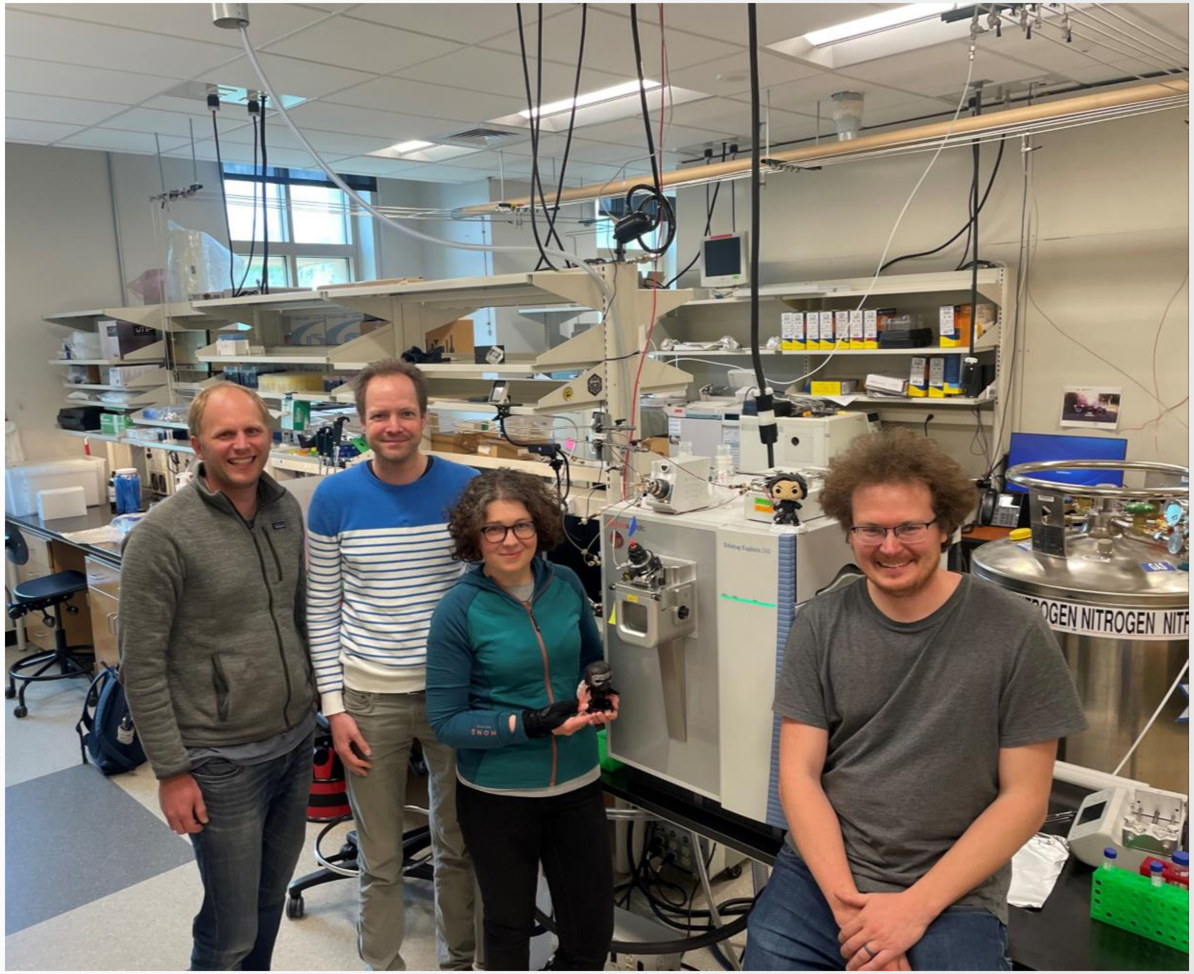
Conclusions

- PFAS compounds are possible to isotopically fingerprint using Orbitrap IRMS
- Preliminary results show excellent agreement between traditional EA-IRMS and novel Orbitrap IRMS measurements
- Workflows on Orbitrap IRMS with PFAS compounds in solution are less damaging to instrumentation and provide faster throughput
- Orbitrap IRMS can be a powerful tool in source identification of PFAS contaminated sites.



Demo Lab at CU Boulder

- Orbitrap 240 with Vanquish Neo UHPLC
- Thermo Fisher: Brett Davidheiser
- PIs: Sebastian Kopf and Cajetan Neubauer
- Post Doc: Kristyna Kantnerova (now Assistant Prof at UCT Prague)



Thank you to our collaborators

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Uwe Rickens
Tabiwang Arrey
Colin Wirth
Christian Klaas
Charles Cartwright

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Stan Mroczkowski

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Nathan Dalleska

Caltech

Sebastian Kopf

University of Colorado Boulder

Xingchen Wang

BOSTON COLLEGE

Ralph Mead
Chad Lane
Paul Wojtal

UNCW UNIVERSITY of NORTH CAROLINA WILMINGTON

Thank you