Technical note | 002527

Isotope Ratio MS

Isotope ratio analysis of sulfate using Orbitrap Exploris Isotope Solutions

Author

Nils Kuhlbusch[1,2], Dieter Juchelka[1], Issaku Kohl[1], Andreas Hilkert[1], Alexandra V. Turchyn[3], Joel Savarino[4]

[1]Thermo Fisher Scientific, Bremen, Germany
[2]University of Muenster, Department of inorganic and analytical chemistry, Muenster, Germany
[3]University of Cambridge, Department of Earth Sciences, Cambridge, UK
[4] Univ. Grenoble Alpes, CNRS, IRD, Grenoble, France

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Introduction

The measurement of the isotopic composition of sulfate in water samples is crucial for identifying how the sulfate was made and where it was derived from. Different sources of sulfate, such as atmospheric deposition, weathering of rocks, volcanic activity or industrial pollution have different isotopic signatures. By measuring the isotopic composition, we can determine the origin of the sulfate, from natural to anthropogenic sources, thus helping to assess the impact of human activities on water quality.

Moreover, the isotopic composition can provide insights into the processes involved in the global biogeochemical sulfur cycle, such as microbial redox transformations, precipitation or dissolution of minerals, or atmospheric reactions. Similarly, the isotopic composition of sulfate can be used to trace water sources and movement in hydrological systems. However, while the sulfur isotopic composition of sulfate mostly reflects the source, the oxygen isotopic composition in general traces the pathway of sulfur oxidation to sulfate, (Figure 1). As a result, increasing numbers of researchers find it crucial to utilize both oxygen and sulfur isotope ratios and their relationship for deeper insight into the sources and processes within natural environments involving sulfur. Up to now, there has been no analytical solution providing both sulfur and oxygen isotope ratios of sulfate simultaneously from the same sample aliquot.[1-3]

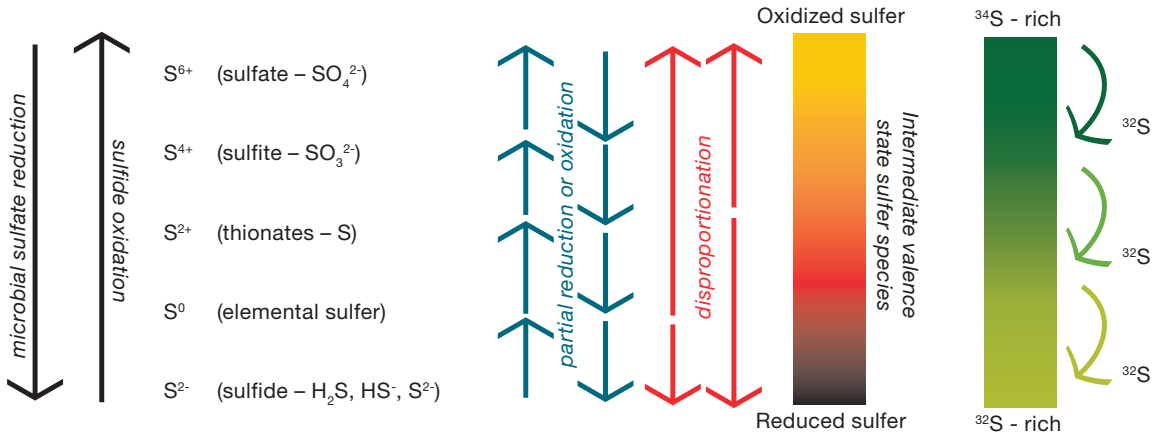

Figure 1. Schematic of the two prevalent sulfur species, oxidized sulfate and reduced sulfide, reactive intermediates, and common redox cycling pathways. Adapted from Turchyn *et al.* 2022.[3]

In this technical note we demonstrate how Thermo Scientific™ Orbitrap Exploris™ Isotope Solutions allow for comprehensive isotope ratio analysis of sulfur and oxygen within intact molecular sulfate ions, including less abundant variants such as $\delta^{17}O$, $\delta^{33}S$, and $\delta^{36}S$, and the ^{34}S–^{18}O "clumped" sulfate.

Isotope ratios by Orbitrap MS technology

Orbitrap Exploris Isotope Solutions enable measurement and calculation of isotope ratios directly from the relative abundances of a compound's isotopocules in solution. Intact molecular ions are produced by electrospray ionization (ESI) and delivered to the Orbitrap analyzer. In addition, controlled fragmentation of the molecular ions can be used to deduce site-specific isotope compositions of organic compounds. Isotope ratios of unknown samples are analyzed relative to a reference with known isotope ratios, which allows reporting of results relative to international standards.

Equipment and methodology

To fully characterize the isotopic structure of sulfate material the corresponding salt is dissolved in water at 100 mM concentration. These stock solutions are diluted with methanol to 50 µM working solutions for Orbitrap measurement.

The Orbitrap Exploris Isotope Solutions for sulfate analyses presented here includes the Thermo Scientific™ Orbitrap Exploris™ 120/240/480 MS and data evaluation package for Isotope Ratio MS. Two sample introduction methods developed for sample/reference comparison are available:

1. Dual Syringe Inlet system based on a syringe pump and a diverter valve
2. An automated In-flow Injection approach utilizing the Thermo Scientific™ Vanquish™ Neo UHPLC System

For In-flow Injection measurements, the Vanquish Neo UHPLC System is coupled to the Orbitrap Exploris MS. The UHPLC pump module delivers a constant flow of LC/MS-grade methanol at a flow rate of 4 µL/min. The autosampler is equipped with a 25 µL sample loop for In-flow Injections, resulting in 6–8 min wide plateau peaks. Each injection results in a total run time of 15 min per sample, including a 7–9 min wash out time to ensure that no sample is left in the loop. The presented workflow setup comprises of two alternative injection patterns to set up a sequence of In-flow injections.

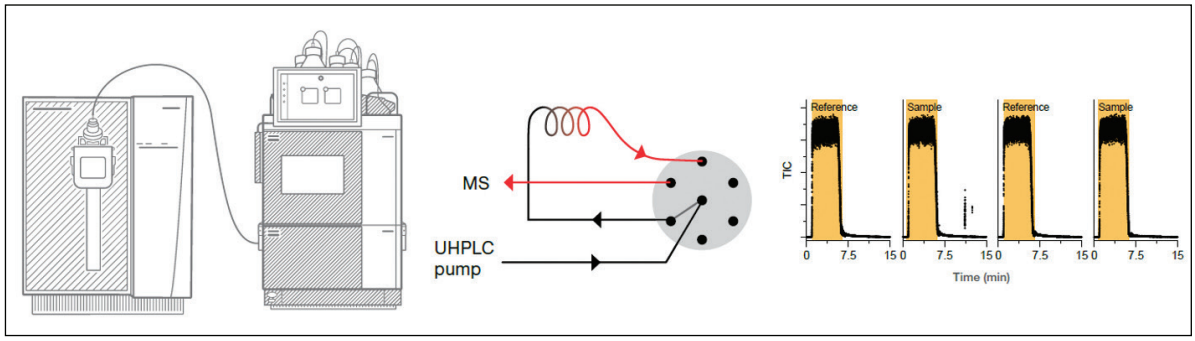

Figure 2. In-flow Injection measurement showing alternating injections of sample and reference, and system flushing with methanol

Orbitrap Exploris MS settings

Source parameters and settings used for the isotope ratio analysis are listed in Table 1.

Table 1. Ion source settings for Orbitrap Exploris 480 MS. (According settings for Orbitrap Exploris 120/240 MS).

Sheath gas (Arb)*	0
Auxiliary gas (Arb)	7 (2)
Sweep gas (Arb)	0
Neg ion spray Voltage (V)	~ 2400
Spray current (observed)	<0.2 µA
Ion transfer tube temp (˚C)	280

*Arb = Arbitrary units

Different to conventionally used sector field MS, the Orbitrap Exploris MS does not analyze a continuous stream of ions but ion packages that are collected after the quadrupole mass filter and injected into the Orbitrap one by one. The size of these packages can be controlled by adjusting the target of the active gain control (AGC). The quadrupole mass filter can be used to control the ion composition of each injected ion package based on the selected range of *m/z*. This way potentially interfering ions can be removed from the ion package selecting all or only some of sulfate isotopocule ions.

Two different methodologies were applied for isotope ratio analysis utilizing different scan ranges of the quadrupole mass filter:

1. 'M0' experiment - includes the predominant monoisotopic ion (M0) consisting of the light isotopes ^{1}H, ^{32}S and ^{16}O as base peak
2. 'noM0' experiment - excludes the M0 amplifying the minor peaks, including doubly substituted isotopocules

The scan parameters used for the experiments are listed in Table 2.

Table 2. Scan parameters used for isotope ratio analysis with the Orbitrap Exploris 480 MS. (According settings for Orbitrap Exploris 120/240 MS).

Scan type	Full scan
Scan ranges (*m/z*)	93–105 ('M0') 97.4–105 ('noM0')
Orbitrap Resolution	45,000 ('M0') or 60,000 ('noM0')
Polarity	Negative
Microscans	10
AGC Target	Custom
Normalized AGC Target (%)	100 ('M0') 10 ('noM0')
Maximum injection time (ms)	1,000
RF Lens (%)	100 (70)

Data acquisition and evaluation

Thermo Scientific™ Xcalibur™ Software is used for instrument setup and data acquisition. Every In-Flow Injection results in a RAW file that includes one mass spectrum for every Orbitrap scan. The full process used to ultimately create a single mass spectrum is referred to as "scan" (read more in Technical note **TN002087**).

The resulting RAW files are processed by the Thermo Scientific™ IsoX™ Software to extract all relevant parameters for the calculation of isotope ratios. To determine the number of ions *(N)* entering the analyzer, the signal-to-noise ratio (S/N_p) of a given spectral peak is determined for every single Orbitrap scan (read more in Technical note **TN001482**).

The resulting IsoX Software output files, including all the data and parameters needed for the further evaluation steps, are simple tab-delimited files and can be opened as spreadsheets. For processing of multiple RAW files, a combined IsoX Software output file can be created.

Further processing of the IsoX output files can be performed using commonly used data science statistical computing programs. R scripts were used for the evaluation of the presented data. Isotope ratios *(R)* calculated by the R scripts are saved in different data formats to enable flexible data evaluation.

Calculation of δ-values was performed using Microsoft™ Excel™. Presented sample (Sam) and characterized material (SMIF-1, SMIF-2, S-hot, S-^{18}O-depleted) data were measured against a solution of our in-house working standard (S3744, Std). Formula (1) shows the calculation of δ-values against a standard for isotope ratios of $^{34}S/^{32}S$ as an example:

$$\delta^{34}S_{Sam/Std} = \frac{R(^{1}H^{34}S^{16}O_4/^{1}H^{32}S^{16}O_4)_{Sam}}{R(^{1}H^{34}S^{16}O_4/^{1}H^{32}S^{16}O_4)_{Std}} - 1 \quad (1)$$

To calculate the δ-values against international primary reference materials, known values of the working standard ($\delta^{34}S_{Std/CDT}$) and the measured sample values ($\delta^{34}S_{Sam/Std}$) were evaluated using equation (2).

$$\delta^{34}S_{Sam/VCDT} = \delta^{34}S_{Sam/Std} + \delta^{34}S_{Std/VCDT} + \delta^{34}S_{Sam/Std} \cdot \delta^{34}S_{Std/VCDT} \quad (2)$$

Results

Sulfate is measured as HSO_4^- in a negative ESI mode. The sulfate molecular ion consists of four oxygen, one sulfur and one hydrogen atom. Different combinations of the elements` stable isotopes result in 10 most abundant isotopocules, which are listed in Table 3. M0, the most abundant isotopocule, refers to the monoisotopic molecule consisting of only the light isotopes ^{32}S,^{16}O and ^{1}H. All other isotopocules show single or multiple substitutions by the heavy isotopes ^{33}S, ^{34}S, ^{36}S, ^{17}O and ^{18}O. Throughout this and the following paragraphs the heavy isotope substitutions will be used as shortcuts for their corresponding sulfate isotopocules.

Based on their cardinal masses, the isotopocules can be grouped as M+X (Table 3). M referring to the cardinal *m/z* of the unsubstituted molecular ion and X being the added *m/z* due to heavy isotope substitution. Using Orbitrap MS technology the ten most abundant isotopocules can be detected in a single run (Figure 3). Typically, a scan range of 93–105 is selected to apply the so called 'M0' methodology. The peaks are labeled according to the heavy isotope substitutions present in the corresponding sulfate ion.

Table 3. Abundance out of 1 million isotopocules and *m/z* of sulfate`s isotopocules in the range of 96–105 *m/z*

Cardinal Mass	Accurate Mass	Isotopocule	Heavy Isotope substitution(s)	Abundance
M0	96.9601	$^{1}H^{32}S^{16}O_4$	-	940592
M+1	97.9595	$^{1}H^{33}S^{16}O_4$	^{33}S	7427
M+1	97.9643	$^{1}H^{32}S^{17}O^{16}O_3$	^{17}O	1433
M+2	98.9559	$^{1}H^{34}S^{16}O_4$	^{34}S	42084
M+2	98.9644	$^{1}H^{32}S^{18}O^{16}O_3$	^{18}O	7632
M+3	99.9601	$^{1}H^{34}S^{17}O^{16}O_3$	$^{34}S^{17}O$	63
M+3	99.9633	$^{1}H^{33}S^{18}O^{16}O_3$	$^{33}S^{18}O$	59
M+3	99.9681	$^{1}H^{32}S^{17}O^{18}O^{16}O_2$	$^{17}O^{18}O$	9
M+4	100.9551	$^{1}H^{36}S^{16}O_4$	^{36}S	145
M+4	100.9601	$^{1}H^{34}S^{18}O^{16}O_3$	$^{34}S^{18}O$	333
M+4	100.9686	$^{1}H^{32}S^{18}O_2{}^{16}O_2$	$^{18}O^{18}O$	44

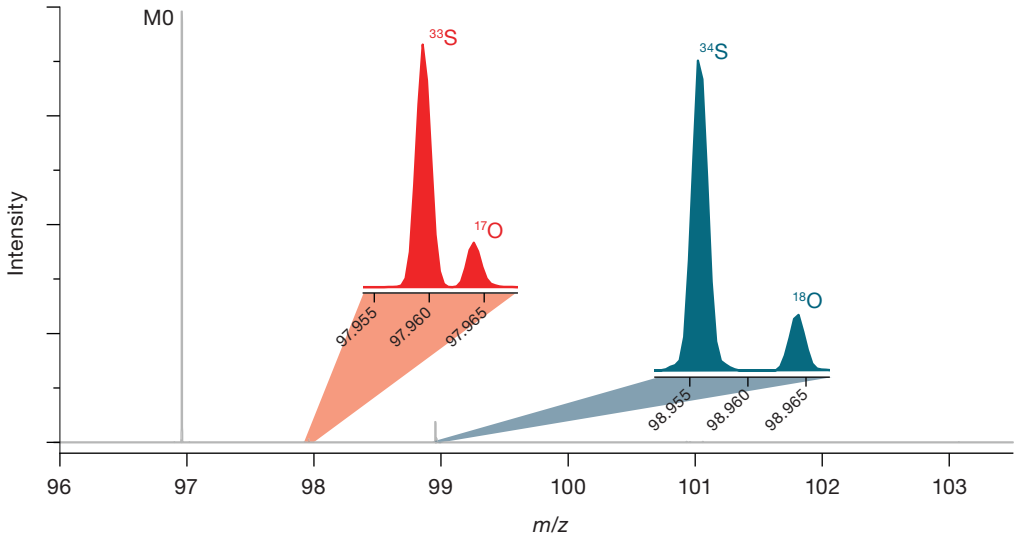

Figure 3. Mass spectrum of sulfate with 'M0' methodology (scan range 93–105 *m/z*). The peaks are labeled according to the heavy isotope substitutions in the corresponding sulfate ion.

Raw data acquired by 'M0' methodology can be used to calculate the isotope ratios of all singly substituted species over the unsubstituted (M0) from a single run.

In case of 'M0' experiments ~94 % of the ions within a single ion package are M0 ions (see Table 3). All other isotopocules make the last 6 %, not letting enough ions of the minor isotopocules enter the Orbitrap to be detected.

Shifting the scan range of the quadrupole mass filter to higher *m/z* (97.4–105) excludes the M0-ion from the ion package entering the Orbitrap-analyzer. This so-called 'noM0' methodology enables the detection and the calculation of isotope ratios for the minor clumped isotopocules. Figure 4 shows the mass spectrum of a 'noM0' measurement. The peaks are labeled according to the heavy isotope substitutions in the corresponding sulfate ion.

The M0 ion being excluded from entering the Orbitrap allows the analysis of all other isotopocules at higher intensities. Being the most abundant species in these 'noM0' experiments, the peaks of the isotopocules with single substitutions of either ^{34}S or ^{18}O are typically used as base peak for isotope ratio calculation. The 'M0' methodology provides the link to the major light isotopes (^{32}S and ^{16}O) by calculating the isotope ratios $^{34}S/^{32}S$ and $^{18}O/^{16}O$. Ratios of the clumped isotopocules over the major isotopes can be calculated using the base peak as the mediator.

The isotope ratios of sulfate isotopocules can be determined with high precision and accuracy by combining the data acquired in both experiments. Additional automation of the isotope ratio analysis is achieved utilizing the Vanquish Neo UHPLC System.

Full characterization of sulfate standard materials

During In-Flow injection measurements, the Vanquish Neo Autosampler performs alternating loop injections of working solutions of a known working standard and a sulfate sample. S3744 was used as reference material for sample/reference-comparison.[4] For verification purposes this procedure was carried out with isotopically well-characterized sulfates SMIF-1 and SMIF-2 as samples.[5] In addition to that, two samples (S-hot and S-^{18}O-depleted) were analyzed with the described methodology. S-hot is made of sulfuric acid placed in an evacuated quartz ampoule and heated to 1,000 ˚C for 10 hours in an oven. After fast cooling, the sulfuric acid is recovered in water and converted to sodium sulfate with the mean of ion exchange resin and finally dried. S-^{18}O-depleted is made of sodium sulfide (Na_2SO_3) equilibrated in depleted ^{18}O water (purified Antarctic water, $\delta^{18}O \approx -50$ ‰) followed by hydrogen peroxide oxidation to sulfate and finally dried. All results are presented in Table 4 and Figure 5. Every value is calculated as the average of six sample injections, the error bars indicating the calculated standard error by the mean.

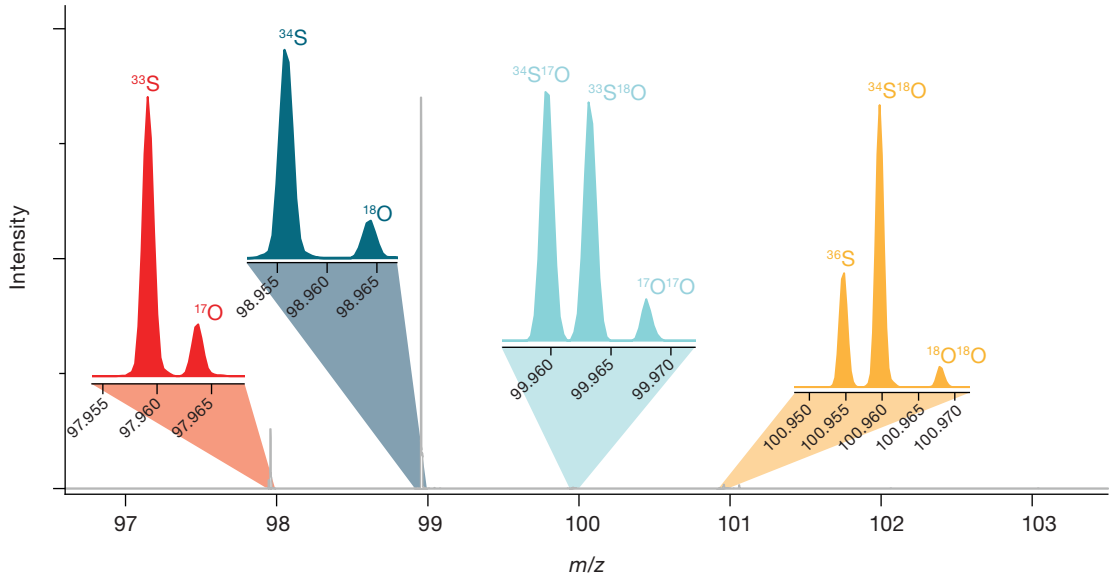

Figure 4. Mass spectrum of sulfate with 'noM0' methodology (scan range 97.4–105 *m/z*). The peaks are labeled according to the heavy isotope substitutions of their corresponding sulfate isotopocule ion.

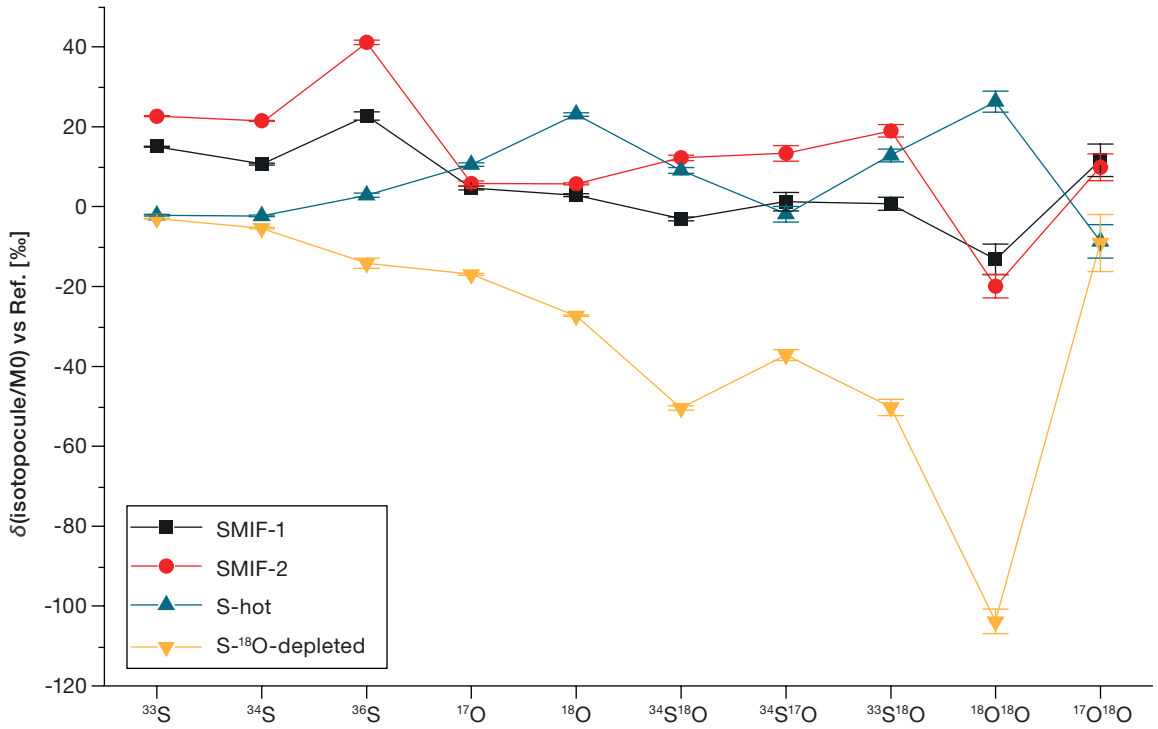

Figure 5. Ratios of sulfate`s most abundant isotopocules measured for the analyzed materials using the In-Flow Injection workflow. δ values were calculated versus Vienna-Canyon Diablo Troilite (VCDT) for sulfate isotopes, versus Vienna Standard Mean Ocean Water oxygen (VSMOW) for oxygen isotopes and versus the working standard S3744 for all clumped isotopocules.

Table 4. Exemplary data of Isotope ratios of sulfate`s most abundant isotopocules measured for the analyzed materials using the In-Flow Injection workflow. All ratios were calculated over M0 and against the shown standard.

Isotopocule	$\delta^{33}S$*	$\delta^{34}S$	$\delta^{36}S$*	$\delta^{17}O$*	$\delta^{18}O$	$\delta^{34}S^{18}O$	$\delta^{34}S^{17}O$	$\delta^{33}S^{18}O$	$\delta^{18}O^{18}O$	$\delta^{17}O^{18}O$
Standard	VCDT			VSMOW		S3744				
SMIF-1	15.0±0.1	10.7±0.2	22.7±1.0	4.6±0.5	2.9±0.4	-3.0±0.5	1.2±2.3	0.7±1.7	-13.2±3.9	11.6±4.1
SMIF-2	22.7±0.1	21.5±0.1	41.1±0.6	5.8±0.6	5.7±0.2	12.2±0.6	13.4±2.0	19.0±1.5	-19.9±2.9	9.8±3.4
S-hot	-2.2±0.2	-2.3±0.2	2.9±0.5	10.5±0.5	23.1±0.4	9.0±0.7	-1.9±1.9	12.8±1.6	26.3±2.6	-8.7±4.2
S-^{18}O-depleted	-3.0±0.1	-5.4±0.2	-14.1±1.3	-17.0±0.2	-27.3±0.2	-50.4±0.6	-37.1±1.4	-50.3±2.0	-103.8±3.0	-9.1±7.2

*A stochastic stable isotope distribution is assumed for S3744 which forms the basis for the calculation of $\delta^{33}S$, $\delta^{36}S$ and $\delta^{17}O$

Optimization of methodology for real environmental samples

The sulfur and oxygen isotopic composition of sulfate is commonly analyzed in different environmental samples such as water, sedimentary rocks, ice/marine cores or aerosols. While the analysis of pure sulfate reference materials bears little room for variations, real samples can differ fundamentally. Sulfate as well as other matrix ions are present in real samples in various concentrations. While low sulfate concentrations can cause instability of the electro spray and low ionization yields, the presence of sodium leads to the formation of a monosodium sulfate adduct as by-product during ionization (Figure 6, top). Both of these processes can affect the quality of the results and lead to a lack of isotope ratio linearity, i.e. $\delta^{34}S$ and $\delta^{18}O$ of standards and sample; isotope ratios vary with the sulfate and matrix concentration.

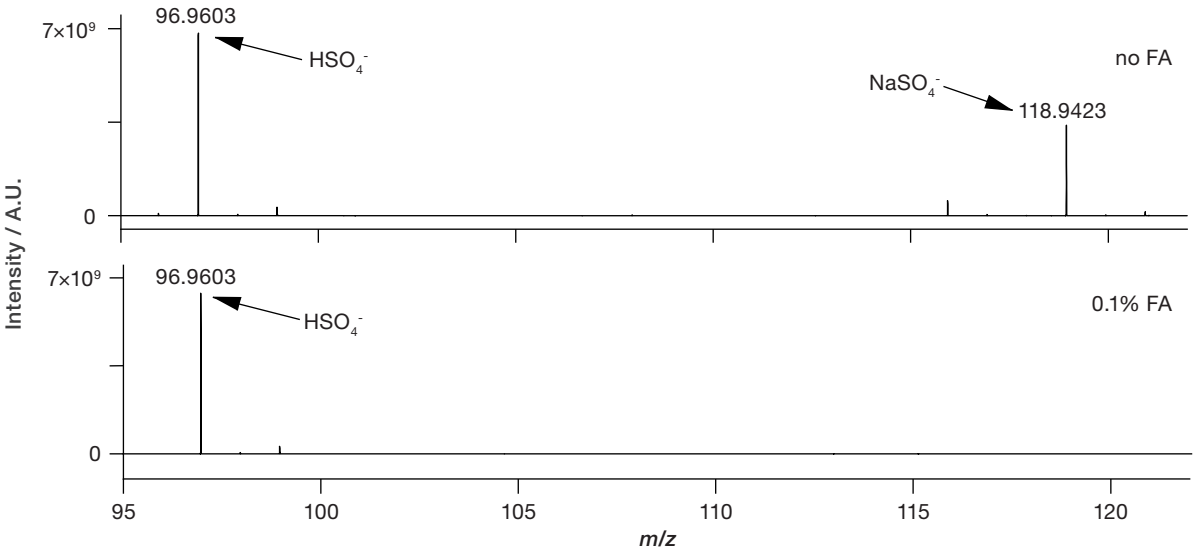

Figure 6. Mass spectrum (95–125 *m/z*) of sulfate S-hot with (top) and without (bottom) formic acid added to the solution

In ESI-MS applications, adduct formation is commonly observed and needs to be managed for optimal sensitivity and linearity. To control adduct formation and increase linearity, ESI ionization additives (typically acids, bases or buffers) can be added to the liquid.[6]

It was found for filtered but otherwise untreated river water samples, that adding formic acid to the sample and standard can suppress the formation of the monosodium sulfate adduct ($NaSO_4^-$, *m/z*: 118.9423) and improve the electrospray stability. Using this improved methodology decreases possible matrix effects caused by differing concentrations of counter cations within or in between samples and sample batches.

For method evaluation, a working range between 1 and 100 µM of sulfate was tested. The isotopic composition ($\delta^{18}O$ and $\delta^{34}S$) of the standard material S3744 was measured in different concentrations relative to a reference solution concentration of 10 µM. Formic acid was added to the samples and standard with the concentration of 0.1% (*v/v*).

Application of optimized methodology on real environmental samples

Various samples of river water were taken from a region in the northern part of India at different points along the river. The sulfate concentration in river water can vary depending on the geographical location and the strength of the hydrological cycle. The sulfate concentration of the analyzed river water samples ranged from 37 to 139 milligrams per liter (approx. 0.39–1.45 mM). For sample preparation, water samples were diluted in methanol 1:100 to achieve a final concentration range of 3.9–14.5 µM. To quantify the effect of formic acid additive on the sulfate isotope ratios of real samples, one replicate of every sample was analyzed with and one without the addition of 0.1 % formic acid. Figure 8 shows the resulting $\delta^{34}S$ and $\delta^{18}O$ values determined with formic acid plotted versus the values determined without formic acid for each of the samples.

Formic acid having no effect on the isotope ratio analysis of a sample would lead to its data point lying on the 1:1 line shown in the graphs. While the 1:1 line is within 2 times standard deviation for a majority of the samples, some samples show a significant effect due to the additive. This could potentially be caused by artifacts due to sulfate concentrations that are in the very high or very low concentration range of the sample batch or due to higher/lower concentration of matrix ions that are not corrected for, without the use of additive. The wider spread of data points for the oxygen isotope analysis compared to the ones analyzed for sulfur could be explained by the molecular structure of sulfate. Since all bonds that are built or broken during the process of ESI are connected to the oxygen atoms, the isotopic composition measured for them will be most affected by the ionization process.

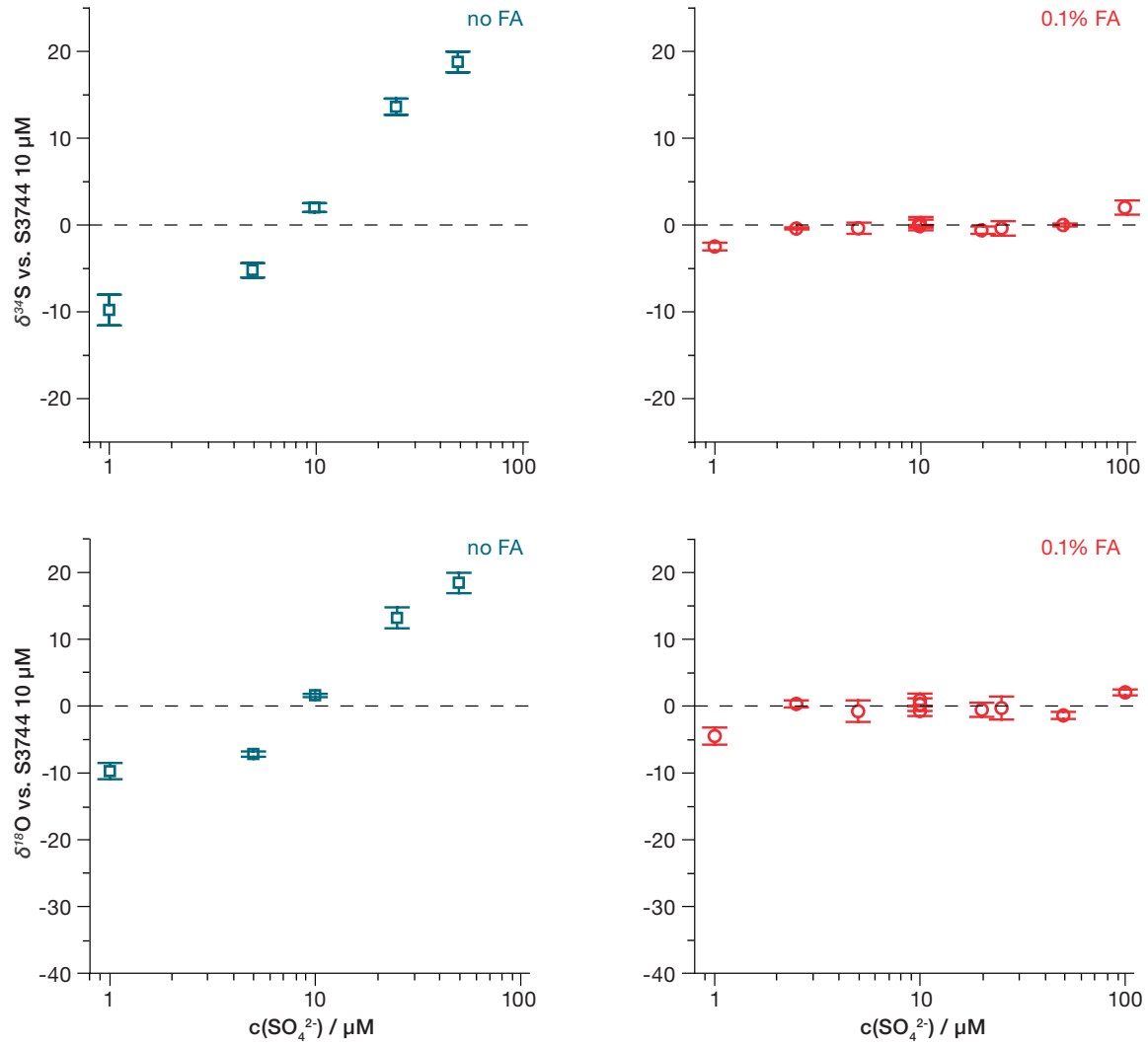

Figure 7. Isotopocule ratio linearity for different sulfate concentrations with and without formic acid (FA). Error bars indicate the standard deviation of three injections and x-axis is scaled logarithmic.

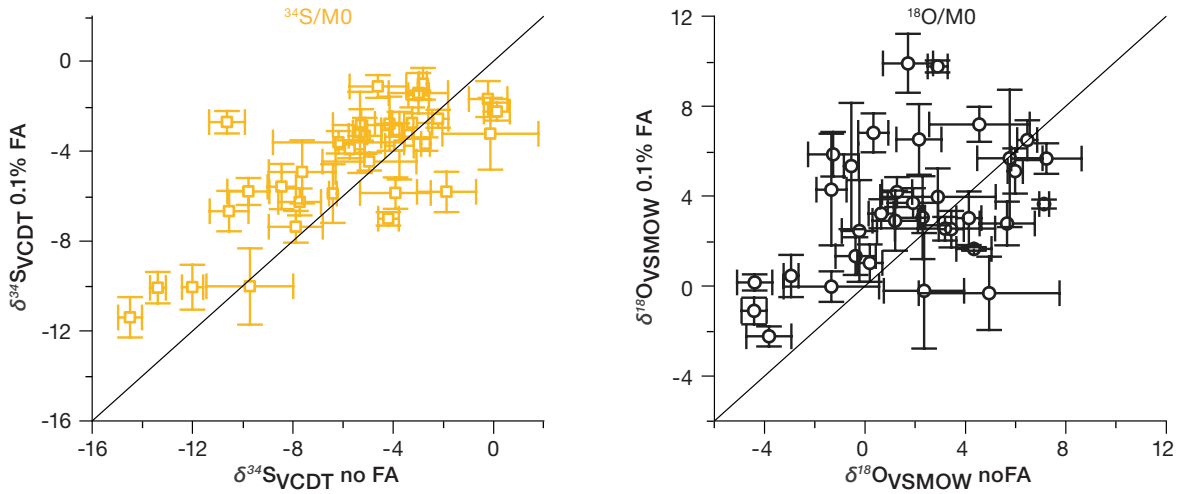

Figure 8. Isotopic composition determined for sulfates ^{34}S (left) and ^{18}O (right) isotopocules over M0 in river waters samples. Samples were analyzed versus a 10 µM solution of working standard S3744. δ values were calculated versus international primary reference VCDT/VSMOW. Data achieved with the addition of formic acid (0.1 % FA) to sample and standard solutions was plotted against data achieved without the addition of formic acid (no FA).

Isotopic compositions of Riverine Sulfate

Figure 9 shows the $\delta^{18}O$ and $\delta^{34}S$ of the river water from a region in the northern part of India analyzed with formic acid added to the solution used for analysis. Data follows a trend defined by mixing between sulfate produced from dissolution of ancient evaporite minerals, with more positive $\delta^{18}O$ and $\delta^{34}S$ values, and sulfate produced from oxidative weathering of reduced sulfur minerals, most notably pyrite. The pyrite derived sulfate end member is characterized by more negative $\delta^{34}S$ and $\delta^{18}O$, the low $\delta^{34}S$ is due to pyrite having low $\delta^{34}S$, while the low $\delta^{18}O$ is due to the incorporation of water-oxygen atoms into the sulfate during oxidative weathering of pyrite (Figure 9). Deeper discussion of the implications and interpretations of this data set will be considered in the context of a wider geochemical environmental characterization in a future publication.

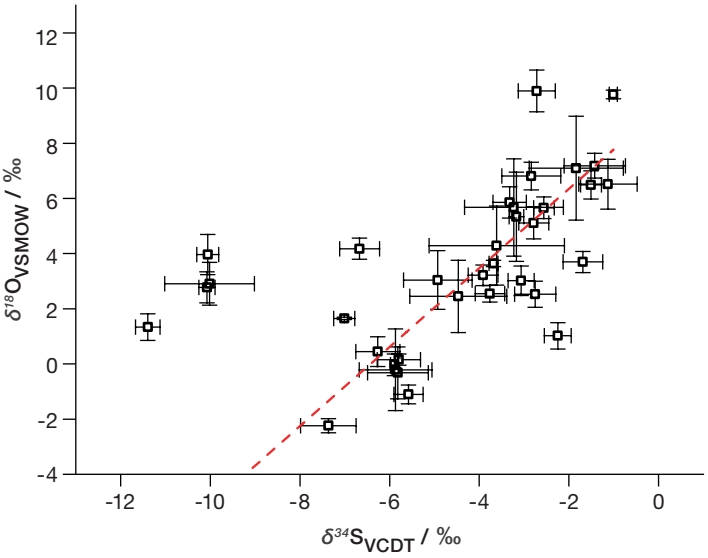

Figure 9. $\delta^{18}O_{VSMOW}$ plotted versus $\delta^{34}S_{VCDT}$ for river water sample determined by performing 'M0' experiments on Orbitrap Exploris 240 MS

Conclusions

By utilizing the UHPLC in-Flow Injection, Orbitrap Exploris Isotope Solutions allow the isotope ratio analyses of sulfate with faster analysis time and reduced sample size compared to conventional IRMS approaches. Soft ionization by ESI-technology enables the analysis of intact sulfate ions. This allows for simultaneous measurement of $\delta^{18}O$ and $\delta^{34}S$ for a single sulfate aliquot for the first time. Measurement of molecular ion isotopocules also enables the determination of non-stochastic isotopic distributions for the clumping of heavy isotopes (e.g. $\Delta^{34}S^{18}O$). This isotopic information, which would be lost during combustion or fluorination in conventional sulfate-IRMS methodology, can open new dimensions in understanding the individual pathways of biogeochemical cycling.

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