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Compound-Specific $\delta^{34}S$ Analysis of Volatile Organics by Coupled GC/Multicollector-ICPMS

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We have developed a highly sensitive and robust method for the analysis of $\delta^{34}S$ in individual organic compounds by coupled gas chromatography (GC) and multicollector inductively coupled plasma mass spectrometry (MC-ICPMS). The system requires minimal alteration of commercial hardware and is amenable to virtually all sample introduction methods. Isobaric interference from O_2^+ is minimized by employing dry plasma conditions and is cleanly resolved at all masses using medium resolution on the Thermo Neptune MC-ICPMS. Correction for mass bias is accomplished using standard−sample bracketing with peaks of SF_6 reference gas. The precision of measured $\delta^{34}S$ values approaches 0.1‰ for analytes containing >40 pmol S and is better than 0.5‰ for those containing as little as 6 pmol S. This is within a factor of 2 of theoretical shot-noise limits. External accuracy is better than 0.3‰. Integrating only the center of chromatographic peaks, rather than the entire peak, offers significant gain in precision and chromatographic resolution with minimal effect on accuracy but requires further study for verification as a routine method. Coelution of organic compounds that do not contain S can cause degraded analytical precision. Analyses of crude oil samples show wide variability in $\delta^{34}S$ and demonstrate the robustness and precision of the method in complex environmental samples.

Sulfur is an important constituent of many natural and anthropogenic organic compounds. Its reactivity and facile redox chemistry make it an important intermediary in a variety of natural biogeochemical processes,[1,2] as well as a key component of some environmental and atmospheric contaminants.[3] Specific redox transitions of sulfur are linked to large isotopic fractionations, and the record of those fractionations is potentially well-preserved by individual organosulfur compounds. Thus, measurements of the sulfur-isotopic composition of specific organic molecules are potentially very useful for tracing both the origins of those compounds and the processes that have affected them. Potential research applications for such measurements include tracking sources of atmospheric trace gases,[3,4] studying the sulfurization of natural organic matter,[5] producing paleoenvironmental and paleoatmospheric proxy records,[2,6] identifying the provenance of industrial products and contaminants,[7,8] and investigation of both biogenic and thermogenic alterations and sources in crude oils.[9]

The conventional approach to sulfur-isotopic analysis of organic materials is based on combustion to SO_2 in an elemental analyzer (EA), followed by measurement of $^{34}S/^{32}S$ ratios in a gas-source isotope ratio mass spectrometer (IRMS).[10] Conversion to SF_6 with analysis by IRMS has also been used to examine ^{33}S and ^{36}S isotopes.[11] Both types of analysis are necessarily restricted to bulk materials, or to compounds or fractions that can be purified in milligram quantities. There is thus no simple and robust analytical route to sulfur-isotopic analysis of *individual* molecular species. Efforts to develop compound-specific ^{34}S analysis by coupling gas chromatography (GC) and IRMS via a combustion reactor have been ongoing for over a decade, but remain largely unsuccessful. The main difficulty in this approach is the need for continuous oxidation/reduction and separation of the combustion products (e.g., H_2O, CO_2) from SO_2.

A potentially more tractable approach involves the coupling of GC separation to a multicollector inductively coupled plasma mass spectrometer (MC-ICPMS). This avoids most of the problems associated with using a combustion interface to couple GC and IRMS, because organic species are atomized and ionized in the plasma source. The measurement of monatomic ions ($^{32}S^+$) rather than molecular ions (SO_2^+) has another advantage, namely, that it avoids the need for ^{18}O or ^{17}O corrections in the isotopic ratio of SO_2. The main hurdle to measuring S^+ isotopes by ICPMS is the existence of significant isobaric interferences between S and O_2 ions (i.e., $^{32}S^+$ and $^{16}O^{16}O^+$, $^{34}S^+$, and $^{18}O^{16}O^+$). Previous studies have shown that a mass resolution ($M/\Delta M$) of 3000−6000 is sufficient to cleanly resolve this interference,[12−14] and most modern MC-ICPMS instruments are capable of such resolution.

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There has also been a substantial amount of antecedent work on the coupling of GC and ICPMS instrumentation and on the use of ICPMS for measuring S isotopes or quantities. For example, GC/ICPMS methods have been demonstrated for analysis of the isotopic composition of Br, Cl, Se, and Hg in volatile organic species.[15−18]

Prohaska et al.[12] reported δ^{34}S measurements of aqueous solutions of ZnS (sphalerite) by MC-ICPMS down to concentrations of 1 ng S/mL, with a reported internal precision of 1‰. They employed a desolvator to dramatically decrease isobaric interferences from water, allowing them to work in low resolution (nominally ~400) on their ICPMS. Clough et al.[13] measured aqueous solutions of both organic and inorganic S compounds by ICPMS and reported better than 1‰ total uncertainties for analyte concentrations of 10 μg S/mL. Craddock et al.[14] reported δ^{34}S values for S minerals in both aqueous solution and solid matrixes analyzed by laser ablation (LA-ICPMS) with precision and accuracy of 0.1−0.3‰. Krupp et al.[19] described δ^{34}S measurements of gaseous SF_6 standards using GC/ICPMS and reported 0.3‰ precision for peaks just 5 s wide and containing <1 nmol S injected. In addition to the analysis of isotopic compositions, GC/ICPMS has also proven to be a very sensitive and robust method for the quantitative analysis of organosulfur in complex environmental samples and oils.[20]

These studies notwithstanding, none have yet described a system capable of routine isotopic analysis of individual organosulfur compounds in complex environmental samples. Here we present a precise, accurate, and sensitive method for the measurement of picomole-level organosulfur compounds by directly coupling GC and ICPMS. The method is simple and robust and can accommodate virtually all modern GC methods (including sample introduction, columns, temperature programs, flow rates, and auxiliary detectors) with little modification.

MATERIALS AND METHODS

Reagents and Standard Compounds. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) and are analytical grade (>97% purity) with no further purification. Solutions of different concentrations were prepared in appropriate solvents (GC-grade hexane and dichloromethane) for testing the system. Reference gas SF_6 was purchased from Scott Specialty Gases (Pennsylvania) as a 2% mixture in He. The sulfur-isotopic composition of eight organosulfur standards was analyzed at both the University of California, Riverside and the Hebrew University of Jerusalem using standard EA-IRMS methods.[10,21] The sulfur isotope reference materials NBS-123 ($BaSO_4$; $\delta^{34}S$ = 21.3‰), IAEA-S-1 (Ag_2S; −0.3‰), IAEA-S-2 (Ag_2S; 22.65‰), and IAEA-S-3 (Ag_2S −32.5‰) were purchased from NIST and used for calibration of those analyses.[22−24]

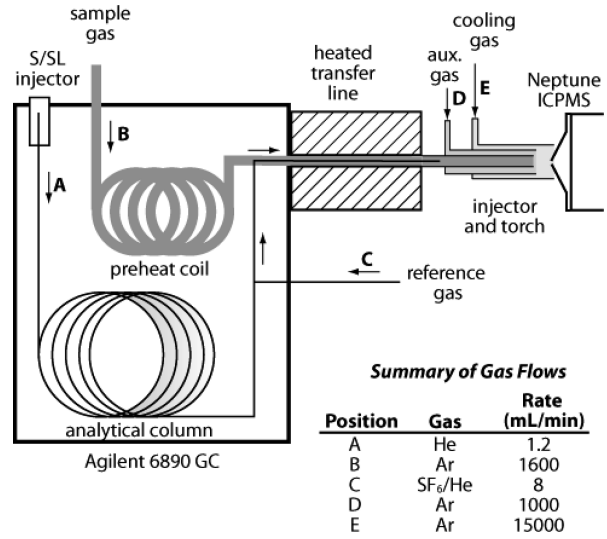

Figure 1. Schematic layout of instrumentation components (not to scale). Helium flows are shown in solid black; argon flows are shaded gray. Flow rates are indicated by letters A−E and correspond to the inset table. GC carrier gas and Ar sample gas flow coaxially through the transfer line and into the injector, where they mix. SF_6 reference gas (50 ppm S) is supplied via the apparatus described in Figure 2. The transfer line shown here combines aspects of both interfaces that were used, with salient differences noted in the text.

Instrumentation. The GC/ICPMS system employed here consists of a stock Agilent 6890 GC equipped with a split/splitless injector that is coupled to a Thermo Neptune MC-ICPMS via a heated transfer line (Figure 1). The Neptune is a double-focusing magnetic-sector instrument equipped with eight moveable Faraday detectors and one fixed detector for simultaneous detection of different masses. The Faraday detectors were positioned to simultaneously collect $^{32}S^+$, $^{33}S^+$, and $^{34}S^+$. The heaviest S isotope (^{36}S) cannot be measured because of severe interference from ^{36}Ar. When the Neptune is operated with low resolution, S^+ and O_2^+ peaks fully overlap and must be measured together. When medium or high resolution is used, the wide exit slits employed by the Neptune result in two flat-topped but partially overlapping peaks. In this case, S^+ is measured as a shoulder on the low-mass side of the larger (overlapping) peak. In all cases the system was operated with "dry" plasma conditions, i.e., with no aqueous vapor added to the gas streams. Relevant GC and ICPMS parameters are listed in Table 1.

We employed two interface systems for this study. The first is a home-built system used for initial testing and troubleshooting of the GC/ICPMS system. Analytes are carried in He carrier gas from the GC to the Neptune ion source via a fused-silica capillary (0.32 mm i.d., methyl deactivated). That capillary is contained

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Table 1. Operating Parameters for the GC/ICPMS System Employed in This Study

gas chromatograph	Agilent 6890
capillary column	HP DB-5, 30 m × 0.25 mm × 0.25 μm
injector	S/SL, 300 °C
carrier gas	He, 1.2 mL/min
oven temperature program	80 °C (5 min), 10 °C/min, 300 °C (10 min)
transfer line temperature	280−330 °C
reference gas carrier flow	7.7−23 mL/min
mass spectrometer	Thermo Neptune
cooling flow (Ar)	15 000 mL/min
auxiliary flow (Ar)	1000 mL/min
sample gas flow (Ar)	1550−1650 mL/min
extraction voltage	2000 V
resolution	medium resolution, 5000 resolving power ($m/\Delta m$, 5−95%)
RF power	1200 W
sample cone	nickel 1.1 mm aperture
skimmer cone	nickel 0.8 mm aperture
ion lenses	optimized for sensitivity and peak shape
detection system	Faraday cups
integration time	0.189 s

within a 3.2 mm o.d. copper tube that conducts Ar sample gas to the ICP torch. The capillary extends to near the end of the torch, such that He and Ar gas streams flow coaxially through the transfer line and mix only as they enter the plasma. The transfer line is heated via flexible heating tape to ~280 °C and insulated with glass wool to prevent condensation of analytes. A standard ICP torch (Thermo Fisher Scientific, Germany) is modified by removing the ground-glass ball joint, and connection to the transfer line is made via a Swagelok union wrapped in heating tape. Argon makeup gas ("sample gas", 1600 mL/min) is preheated in the GC oven in a coiled 5 m × 3.2 mm o.d. copper tube before entering the transfer line about 10 cm before the torch (not shown in Figure 1).

The second interface is a commercial system (Thermo Fisher Scientific) that was used to collect most of the data reported here, except where noted. It was designed to connect with the Element 2 ICPMS and work under wet plasma conditions but also fits the ion source of the Neptune ICPMS. It differs from our home-built system in several ways. First, the transfer line is longer (~1 m) and more flexible, to permit easy alignment and adjustment of the torch for system optimization. It is uniformly heated up to 350 °C to accommodate high-boiling analytes. Second, preheated Ar enters the transfer line within the GC oven (as shown in Figure 1) to minimize the risk of cold spots and flows through the transfer line within a 1.6 mm o.d. stainless steel tube. Third, a specially shortened torch is used to reduce the length of unheated glass where analytes can condense. Connection of the transfer line to the torch is via a ground-glass ball joint. The GC transfer capillary extends ~1 cm past this ball joint (as shown in Figure 1), such that He and Ar streams mix within the torch injector tube. The custom ICP torch also contains a second sample inlet tube for introduction of liquid samples (not shown in Figure 1) or the addition of water vapor for work in "wet plasma" conditions. This second inlet was blanked off for our studies. No adverse effects were observed operating the commercial system under dry plasma conditions. Both the home-built and commercial interfaces yield similar sensitivity, accuracy, and precision for $\delta^{34}S$ analyses. However, the commercial interface is more capable of transmitting high-boiling analytes to the plasma ion source without peak broadening.

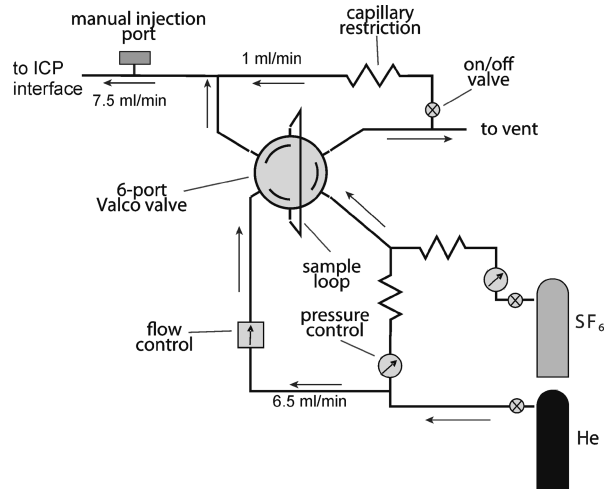

Figure 2. Schematic layout of the apparatus used to introduce a continuous flow or discrete peaks of SF_6 reference gas at variable concentrations. Reference gas is 2% SF_6 in He and is further diluted to ~250 ppm SF_6 in He (~50 ppm S). This gas is used for instrument tuning, testing, and calibration of analyte isotope ratios.

Tuning and Calibration. To provide a reference gas for instrument tuning and calibration, we constructed a gas inlet system that allows addition of either a continuous or time-varying stream of SF_6 in He to the GC effluent (Figure 2). SF_6 was employed as a calibration gas because it is nontoxic, chemically inert, odorless, and relatively inexpensive. The reference gas (2% SF_6 in He) is first diluted in a variable stream of He allowing its concentration to be adjusted over an order of magnitude. The diluted reference gas can then either flow continuously to the GC (for instrument tuning) or is sampled via a six-port two-position valve (Valco Instrument Co., Texas) equipped with a 10 μL loop to produce discrete peaks for calibration of GC analytes. In the latter mode, peaks of SF_6 (~250 ppmv) are carried to the GC in a He stream that is controlled via a mechanical flow controller (Porter Instruments, Pennsylvania). Adjusting the flow rate of this carrier gas stream allows us to vary the width of SF_6 calibration peaks, which can be <10 s wide. The entire reference gas system is operated at room temperature, and both flows join the GC carrier gas at a point just before the transfer line (shown in Figure 1). An advantage of this configuration is that the increased He flow rate helps to maintain peak shapes as GC analytes traverse the transfer line.

Data Processing. The GC/ICPMS produces transient signals that the latest Neptune software can collect but not process to yield $\delta^{34}S$ values. In the present study, we employed algorithms originally developed for GC−combustion−IRMS by Ricci et al.[25] that are implemented as Visual Basic code within Microsoft Excel.[26] Ion currents are integrated by the Neptune software in 189 ms increments, the smallest integration unit available in our software version (3.1.0.27), then exported to Excel in

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ASCII format. All subsequent processing uses the custom Visual Basic code. Chromatographic peaks are defined using the m/z 32 data stream with a starting slope of 0.2 mV s^{-1} and ending slope of 0.4 mV s^{-1}, and this peak definition is transferred to all three data channels (i.e., m/z 32, 33, and 34) without adjusting for time shifts caused by isotope chromatography (see the Results section). Background signals are estimated independently for each data channel by averaging 30−100 points preceding each peak, depending on the complexity of the chromatogram. Peak areas (i.e., integrated ion currents) are then calculated with background subtraction, raw ion-current ratios ($^{34}S/^{32}S$) are calculated from peak areas, and calibrated isotope ratios are obtained by comparison to SF_6 reference gas peaks in the same chromatogram. The results are expressed in conventional $\delta^{34}S$ notation as a permil (‰) deviation from the CDT standard:

$$\delta^{34}S = ({}^{34}R_{sample}/{}^{34}R_{std}) - 1 \quad (1)$$

where ^{34}R is the integrated $^{34}S/^{32}S$ ion-current ratio of the sample and standard peaks. Details of these calculations are provided by Ricci et al.[25]

RESULTS

Isobaric Interferences. Significant isobaric interferences can confound the measurement of ^{32}S, ^{33}S, and ^{34}S ions by ICPMS. These include $^{16}O^{16}O$ and $^{14}N^{18}O$ (at m/z 32), $^{16}O^{17}O$ and ^{32}SH (at m/z 33), and $^{16}O^{18}O$ and $^{32}SH_2$ (at m/z 34). O_2 interferences are by far the most abundant. A mass resolution of 1800 is theoretically sufficient to separate ^{32}S at m/z 31.97207 from its major interfering polyatomic ion $^{16}O^{16}O$ at m/z 31.98982 (ref 6). However, in practice a higher resolution is required because the interfering species can be much more abundant than ^{32}S and there is a distinct low-mass tail (i.e., the problem of abundance sensitivity). Craddock et al.[14] reported that a resolving power $M/\Delta M$ of ∼5000−6000 is sufficient to separate major oxygen interferences, including contributions from O_2 tailing and scattered ions, from sulfur isotopes of interest. Those authors suggested working in high-resolution mode of the Neptune ($M/\Delta M$ ∼10 000) for best results in "wet" plasma conditions, wherein an aqueous nitric acid solution is continuously nebulized to the plasma source.

When measuring sulfur under "dry" plasma conditions, i.e., in a He carrier gas with no spray chamber connected, isobaric interferences are much lower (Figure 3), presumably because most interferences arise from the aqueous vapor. Under these conditions, Ar sample gas (flow B in Figure 1) is the most important parameter affecting both sensitivity and the relative abundance of $^{16}O^{16}O^+$. Increasing Ar sample gas flow increases sensitivity for all species up to ∼1600 mL/min (Figure 4). However, the abundance of $^{16}O^{16}O^+$ increases disproportionately, rising from ∼1% of ^{32}S at 1200 mL/min to ∼33% of ^{32}S at 1800 mL/min. Thus, a flow rate of 1500−1700 mL/min provides the optimal compromise between best sensitivity and low $^{16}O^{16}O^+$ abundance.

To compare the performance of our system operating at different mass resolutions and with different levels of isobaric interference, we measured SF_6 reference gas in low-, medium-, and high-resolution modes with Ar sample gas set to 1200, 1400, or 1600 mL/min (Table 2). Because sensitivity varies significantly with flow rate, SF_6 concentration was adjusted to achieve roughly constant peak heights in all tests in order to minimize the influence of changing signal/noise ratios on this comparison. There is no significant difference in accuracy or precision when operating in medium or high resolution, though measured $^{34}S/^{32}S$ ratios did change as a result of changing instrumental mass bias. Because medium resolution offers higher ion transmission, and thus better sensitivity, it is the preferred mode of operation for these analyses. The difference in this conclusion from that of Craddock et al.[14] arises because isobaric interferences are at least an order of magnitude smaller under dry plasma conditions than for wet plasma.

Operation of the Neptune in low resolution yielded much poorer precision when Ar sample gas flow is adjusted for maximal sensitivity, resulting in large isobaric interferences. This degradation in performance can be mostly, but not entirely, remediated

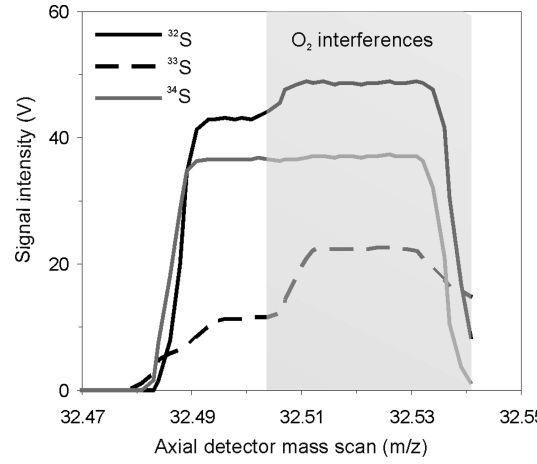

Figure 3. Scan over the center peak (m/z = 32.5) while introducing SF_6 in He (50 ppm S). Ar sample gas flow rate is 1.6 L/min at medium resolution. Signals for ^{33}S (broken line) and ^{34}S (gray line) are multiplied by 18 to match the ^{32}S scale (black line). The gray shaded area highlights the overlap between S^+ and O_2^+. Measurements of S isotopes must be made on the interference-free plateau at lower mass.

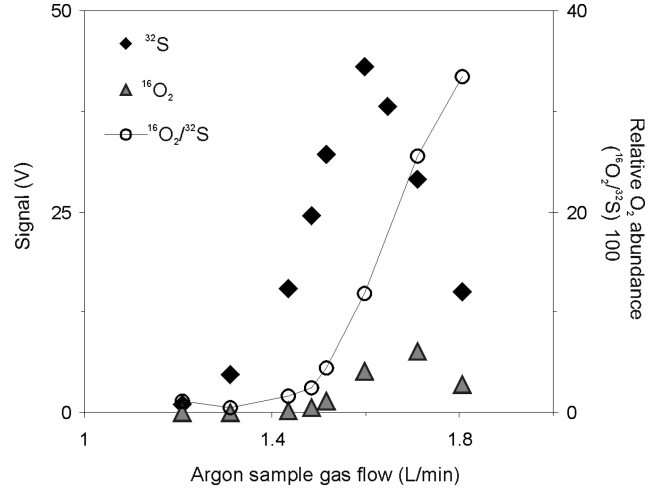

Figure 4. Effect of Ar sample gas flow rate on sensitivity and O_2^+ formation for SF_6 (50 ppm S in He). Diamonds are ^{32}S intensity in volts, triangles are $^{16}O_2$, and circles represent the $^{16}O_2/^{32}S$ ratio (right axis).

Table 2. Effect of Ar Sample Gas Flow Rate on $^{34}S/^{32}S$ Ratio at Varying Mass Resolution

flow (mL/min)	$^{32}S/^{16}O_2$	low resolution (400) $^{34}S/^{32}S$ (RSD × 1000)	medium resolution (5000) $^{34}S/^{32}S$ (RSD × 1000)	high resolution (10 000) $^{34}S/^{32}S$ (RSD × 1000)
1600	8.5	0.04756 (6.49)	0.04734 (0.11)	0.04732 (0.22)
1400	58.8	0.04806 (1.11)	0.04807 (0.22)	0.04807 (0.29)
1200	87.7	0.04848 (0.49)		

by lowering sample gas flow to 1200 mL/min to reduce $^{16}O^{16}O^+$ formation. In this case the ~5-fold drop in ion yield is roughly canceled by the ~5-fold greater ion transmission of low versus medium resolution, such that both modes of operation yield a similar sensitivity. Although the attainable precision using low resolution (~0.5‰) is still worse than can be achieved with medium resolution (~0.2‰), this does suggest that useful compound-specific S isotope measurements could still be made on ICPMS instruments that are not capable of high mass resolution.

Analysis of ^{33}S is possible, but more difficult for several reasons. The lower abundance of ^{33}S (0.75%) relative to ^{34}S (4.2%) leads to a theoretical drop in performance (i.e., sensitivity at a given sample size) of 2.4-fold. In practice, we find that $\delta^{33}S$ precision is lower by 4−10-fold compared with $\delta^{34}S$. This is mainly due to isobaric interference from $^{32}S^1H$, which requires a resolution of >12 000 to be fully separated.[14] Lowering the Ar sample gas flow rate reduces this interference significantly and improves precision to within 4−5-fold that of $\delta^{34}S$ but also lowers sensitivity dramatically and thus requires much larger samples. Moreover, significant systematic errors are likely to arise in samples where the abundance of H varies throughout a chromatogram, due to changing levels of SH formation. Because of these complications, in the present study we focused solely on the measurement of $\delta^{34}S$.

Calibration. Several approaches have been previously employed to correct for instrumental mass bias in S-isotopic analyses, including calibration by sample−standard bracketing[27] or by using $^{37}Cl/^{35}Cl$ or $^{30}Si/^{29}Si$ internal isotope spikes.[13,28] The latter approach is not feasible for our application, because the Neptune is unable to measure m/z 29 and 34 simultaneously. This would necessitate changes in the MS magnetic field to jump between sample and standard, preventing continuous measurement of the GC chromatogram. A second disadvantage is that employing internal standards would require either adding a constant amount of reference gas to the sample stream or else spraying a liquid standard into the ICPMS.

The approach we adopt here is to introduce peaks of SF_6 reference gas at frequent time intervals between analytes (i.e., sample−standard bracketing), the approach used by virtually all IRMS methods[29] and recently used for S-isotope measurements in LA-ICPMS.[14] Peaks of SF_6 in He are produced by a six-port sampling valve (Figure 2) and are added to the GC effluent immediately downstream from the analytical column (Figure 1) at times bracketing the analytes of interest. The main difficulty in this approach is that calibration peaks can only be added when there is open space in the chromatogram. For complex natural samples, these windows can often be tens of minutes apart. Thus, the instrumental mass bias must be very stable to tolerate only sporadic calibration. We examined this requirement in two ways. First, a continuous flow of SF_6 was introduced and monitored over a period of 35 min, resulting in a $^{34}S/^{32}S$ ratio that drifted by only 0.16‰ (maximum peak-to-peak variation). Repeated tests yielded equivalent results. Second, SF_6 peaks were introduced at the beginning and end of a 20 min GC program in which the oven temperature ramped from 60 to 250 °C, resulting in a rising background. The $^{34}S/^{32}S$ ratios between the peaks differed by only 0.12‰ ($n = 10$) on average. We conclude that instrumental mass bias in this GC/ICPMS system is sufficiently stable to allow sample−standard bracketing with minimal loss of accuracy, even when standards are spaced >20 min apart.

Precision and Sensitivity. The attainable precision of compound-specific $\delta^{34}S$ analyses by GC/ICPMS was assessed using both SF_6 reference gas, which does not enter the GC analytical column, and hexylthiophene that was injected into the split/splitless injector of the GC at a 1:10 split ratio (Figure 5). Results for both compounds are similar with SF_6 precision only slightly better, indicating that the GC does not contribute significantly to analytical uncertainty. Data for hexylthiophene are slightly noisier because fewer ($n = 3$) replicates were measured than for SF_6 ($n = 5$). Both estimates of precision closely approach the "shot-noise limit" based on ion-counting statistics for peaks containing >40 pmol S (>2 V·s) and average 0.10−0.15‰ (1σ). The shot-noise limit for $\delta^{34}S$ is calculated as

$$\sigma_{SNL} = \sqrt{\frac{1}{N_{sample}} + \frac{1}{N_{ref}}} \times 1000 \qquad (2)$$

where N is the total number of ^{34}S ions counted in the sample and reference peaks, and the factor 1000 converts σ_{SNL} to units of permil.[30] At lower sample sizes, attainable precision for $\delta^{34}S$ approaches the shot-noise limit within a factor of about 2, and is still better than 0.5‰ for peaks containing only 6 pmol S. The specific reasons for degradation of performance above shot-noise limits are not known. A statistical model for uncertainties introduced by background subtraction when those signals can only be estimated over a short time period (as is the case here) was developed by Merritt and Hayes.[30] Analogous calculations using our data suggest that background correction is insignificant in our case and cannot explain the offset between practice and theory. Regardless, the 2-fold difference is already as good as that achieved by other forms

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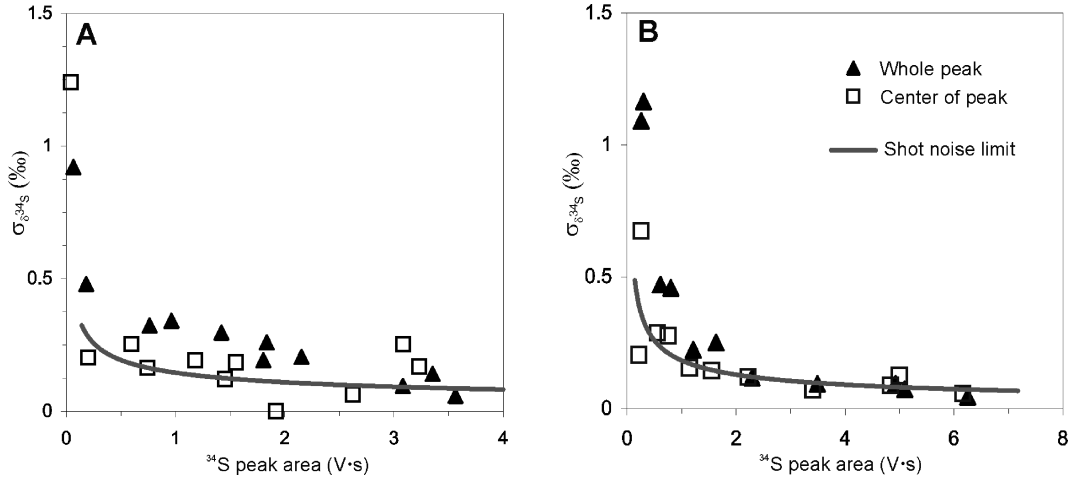

Figure 5. Attainable precision for δ^{34}S analyses of hexylthiophene (A) and SF_6 (B) peaks. Each triangle represents the standard deviation of δ^{34}S values for three separate injections of hexylthiophene (calibrated against SF_6 reference gas) or five injections of SF_6 through the reference gas apparatus. Peaks were integrated using the normal method of whole peak integration. Squares indicate results obtained from integration of only the center of the peak where there is a relatively stable $^{34}S/^{32}S$ ratio plateau. The gray line is the calculated theoretical precision limit based on counting statistics (shot noise). One picomole of S injected is equivalent to approximately 0.05 V·s peak area.

Table 3. Comparison of δ^{34}S Values for Eight Organosulfur Standards Measured by EA-IRMS and GC/ICPMS

compound	EA-IRMS (‰)	standard error (‰)	repetitions	GC-ICPMS (‰)	standard error (‰)	peak area ^{34}S (V·s)	repetitions
dihexylsulfide	5.31	0.08	5	5.33	0.09	1.2	6
dibenzothiophene	3.31	0.07	7	3.31	0.09	2.3	6
1-octadecanethiol	30.55	0.24	6	30.71	0.27	1.7	6
3-octylthiophene	−2.22	0.04	8	−2.10	0.12	2.3	6
1-dodecanethiol	−6.84	0.05	7	−7.00	0.16	0.5	7
4,6-diethyldibenzothiophene	−3.54	0.07	5	−3.58	0.13	2.1	6
3-hexylthiophene	6.1	0.08	6	5.9	0.04	1.6	3
benzothiophene	14.28	0.05	5	13.64	0.23	2.3	6
linear regression: slope = 0.999, intercept = 0.095, R^2 = 0.999							

of isotope ratio monitoring GC/IRMS, such as for compound-specific ^{13}C or ^{15}N analyses.[31]

Krupp et al.[19] reported a significant improvement in the precision of δ^{34}S data by integrating only a small portion in the center of each peak. Although offering the potential advantage of increased signal/noise ratios, such an approach to measuring isotope ratios can fail for two reasons. First, if peak shapes for m/z 32 and 34 are not identical because of mismatched time constants in the electrometer circuits, the isotope ratio can be strongly biased in a way that is dependent on peak height.[26] Second, for lighter isotopes (e.g., ^{1}H and ^{13}C) the phenomenon of isotope chromatography leads to peaks which are strongly inhomogeneous in isotopic composition. In both cases, integration of the entire peak would be required for accurate measurements. Because Krupp et al.[19] did not test their approach on organosulfur peaks that were separated by GC, its feasibility remains uncertain.

Nevertheless, the approach appears promising for S isotopic analyses. Plots of the $^{34}S/^{32}S$ ratio across any given peak are invariant and show no evidence for isotope chromatography (data not shown). Time constants for the m/z 32 and 34 data channels, which utilize 10^{11} Ω feedback resistors, are <0.1 s. To examine this approach quantitatively, we reprocessed all data for hexylthiophene and SF_6 integrating only the center, tallest portion of each peak where the $^{34}S/^{32}S$ ratio is stable and flat. We used an algorithm that finds these plateaus in $^{34}S/^{32}S$ ratios by comparing the standard deviation of a window at least seven points wide (>1.32 s) to a user-controlled value of. $1-5 \times 10^{-4}$ (RSD 0.2−1%). The exact value used depends on the shape and size of the peak. This approach increased δ^{34}S precision by a factor of 1.5−2 for most peaks and approaches the shot-noise limit (see Figure 5), while changing the measured δ^{34}S values by only ~0.2‰ on average. Isotope chromatography apparently does not lead to significant separation of ^{32}S and ^{34}S isotopologues under the conditions employed here, and integration of peak centers could offer significant advantages in both precision and the resolution of complex chromatograms. However, we are hesitant to universally recommend this procedure until the lack of isotope chromatography can be demonstrated more systematically, using a wider range of analyte chemistries and analytical columns.

External Accuracy. Eight organosulfur standards were measured both by GC/ICPMS and by EA-IRMS, with the latter calibrated against several NIST standards to assess the external accuracy of δ^{34}S analyses (Table 3). The δ^{34}S values are generally in agreement to better than 0.3‰, which are identical within 1σ uncertainties. The one significant exception is benzothiophene with a δ^{34}S difference of 0.64‰. These organic sulfur standards represent a wide range of δ^{34}S values, chemical

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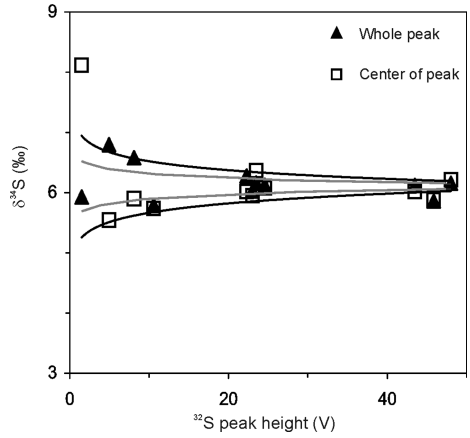

Figure 6. Values of $\delta^{34}S$ for hexylthiophene as a function of peak height. Triangles are the integration of the whole peak, whereas squares are integration of the center of the peak. The gray (1σ) and black (2σ) lines represent limiting precision based on ion-counting statistics.

structures, molecular weights, and boiling points. The results also highlight the fact that industrially produced organosulfur compounds possess a wide range of S-isotopic compositions, a property that should facilitate the use of isotopic measurements to trace their provenance.

Linearity, defined as the ability to produce constant $\delta^{34}S$ values across widely varying peak sizes, is a second important component of accuracy. Peaks of hexylthiophene that vary in height from 1.5 to 48 V (for m/z 32) are very uniform in their $^{34}S/^{32}S$ ratio, with peaks larger than ~10 V exhibiting a standard deviation of 0.16‰ (Figure 6). Below this size the standard deviation rises to 0.45‰, consistent with limitations imposed by shot noise. The data clearly demonstrate the linearity of $^{34}S/^{32}S$ measurements over a wide range of ^{32}S intensities. Integration of the center peak as described above shows a very similar trend.

Matrix Effects. Changes in instrumental mass bias due to sample matrix are well-known in many ICPMS applications and require matrix-matching of sample and standard.[32] In contrast, most GC/IRMS applications are relatively free from matrix effects, because each sample component is chromatographically separated from the solvent and other analytes. Although this must also be true in GC/ICPMS analyses of organosulfur compounds, the coelution of different analytes with varying chemistry could produce a type of matrix effect. As a concrete example of this phenomenon, elution of the solvent peak (generally hexane) has a substantial impact on the ion currents, even though it contains no sulfur, and yields poor precision when analytes elute close to the solvent peak (data not shown).

To test for matrix effects arising from coelutions, we analyzed solutions containing dihexylsulfide at a constant concentration (25 pmol per injection) together with varying amounts of *n*-pentadecane, such that the molar ratio of pentadecane/dihexylsulfide varied between 0 and 200. These two peaks partially coelute under the GC conditions employed (Figure 7a). The results show that up to a ratio of 10:1 pentadecane/dihexylsulfide there is only minor effect on precision and accuracy. When the ratio increases to 200:1 it substantially degrades precision (Figure 7c). The effect on accuracy is uncertain given the relatively small number of

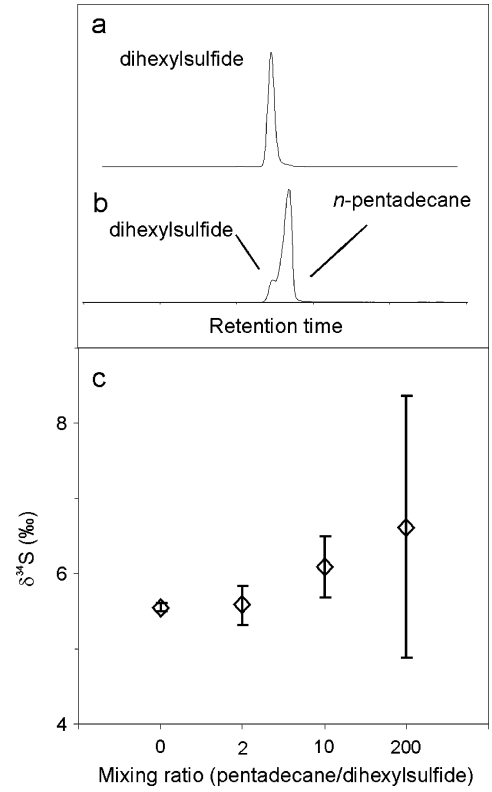

Figure 7. Effect of a hydrocarbon coeluting with the organosulfur analyte: (a) ^{32}S trace measured by GC/ICPMS; (b) TIC (total ion current) trace measured by GC/MS; (c) $\delta^{34}S$ values (1σ, $n = 3$) of dihexylsulfide measured by GC/ICPMS. $\delta^{34}S$ values were calibrated against an internal standard (hexylthiophene) that elutes at a different time.

standards analyzed. The mean $\delta^{34}S$ values for pentadecane/dihexylsulfide mixtures across this range differ by more than 1‰, though this difference is not statistically significant ($P = 0.40$). The mechanistic basis for this effect is not understood but could relate to transient formation of hydrides such as $^{32}SH_2$ which cannot be mass-resolved. We have not yet tested other analyte chemistries, so the matrix effect should be regarded as probable for all coeluting organic compounds. This effect may impose limitations on the precision that can be achieved for some complex samples with low organosulfur contents and significant coelutions. Preparative concentration of S-containing fractions, by column chromatography or high-performance liquid chromatography (HPLC), should prove beneficial in those cases.

Demonstration in Natural Samples. To demonstrate the utility of our new method in environmental samples, we analyzed a low-sulfur crude oil from the Caspian Sea area. This is a light crude oil that contains 0.5 wt % sulfur with no asphaltene fraction and very low amounts of resins (4%), as described by Zhang et al.[33] In many ways, crude oil represents the ultimate test for our analytical system. It is highly complex, yielding a very dense and noisy chromatogram containing hundreds of compounds of widely varying chemistry and relatively small amounts of S, together with numerous coeluting hydrocarbon compounds that do not appear in the chromatogram. Most relevant environmental samples will be less complex than crude oil. The oil was diluted in hexane and injected into the GC (split ratio 1:10) with no additional treatment. The results show widely

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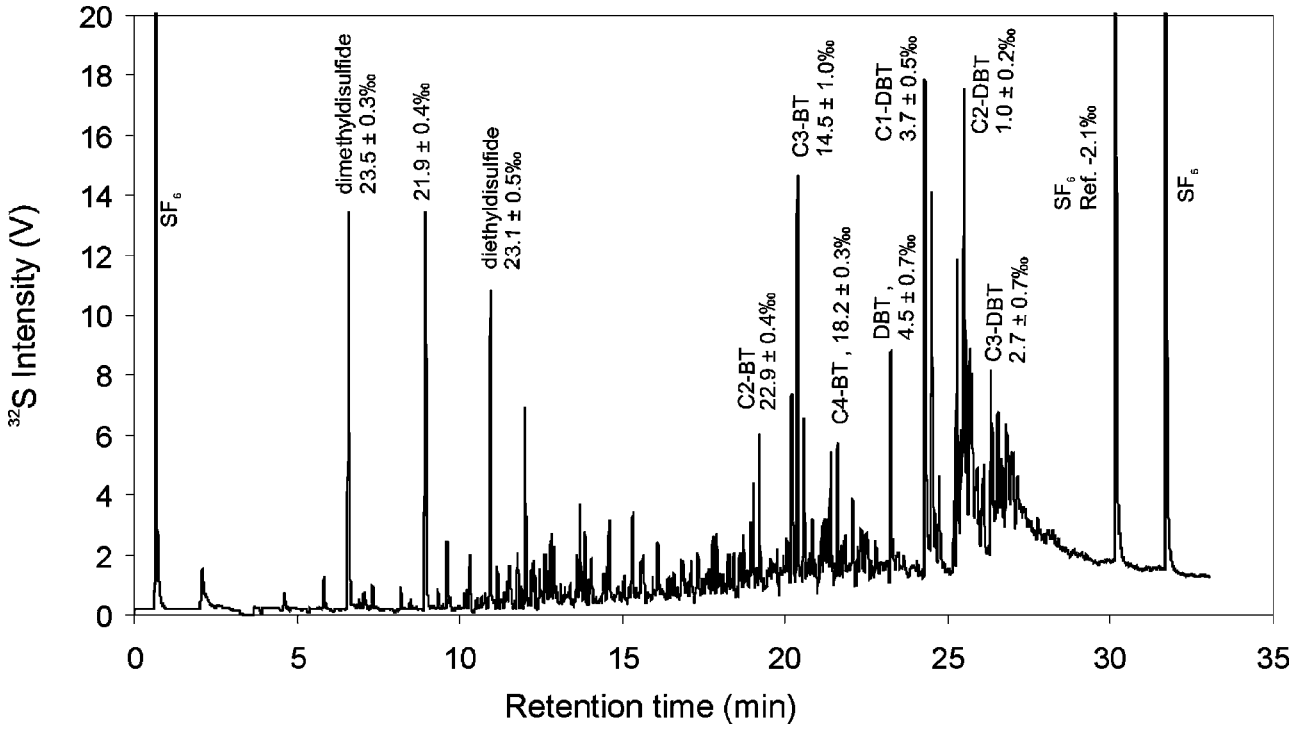

Figure 8. GC/ICPMS chromatogram of a low-sulfur Caspian Sea crude oil. Labels on the chromatogram denote compounds (DBT = dibenzothiophene and BT = benzothiophene) and their measured δ^{34}S values ($\pm 1\sigma$). Values are the averages of three separate injections. The average SD for all compounds is 0.5‰. SF_6 ($\delta^{34}S = -2.1$‰) was used as an internal standard for isotopic calibration. The extra SF_6 peak in the end of the chromatogram used for verification of the internal standard precision (≤0.2‰). Identification of the compounds is based on comparison of retention time and intensity to authentic standards measured by GC-SCD (sulfur chemiluminescence detection).

varying δ^{34}S values ranging from 1‰ to 23.5‰. Precision for the individual δ^{34}S values is in the range of 0.2−1.0‰ (1σ, $n = 3$) for peaks containing 2−15 pmol S (Figure 8). This is surprisingly close to the estimates of limiting precision for pure standards (Figure 5) and is clearly adequate to discern important differences among analytes. The average precision for all peaks is about 0.5‰.

These data provide the first indication that individual organosulfur compounds within a single crude oil will not always have homogeneous δ^{34}S values. The pattern of ^{34}S enrichment in sulfides and benzothiophenes, together with ^{34}S depletion in dibenzothiophenes, suggests the incorporation of multiple sulfur sources into the oil. Lower δ^{34}S values presumably reflect those inherited from the source kerogen, whereas higher δ^{34}S values indicate reaction of the oil with inorganic sulfur species formed by the thermochemical reduction of sulfate.[9,34] This pattern thus demonstrates the great potential of compound-specific ^{34}S analysis to trace mechanisms and pathways of sulfur incorporation in oils specifically and in organic matrixes more generally.

CONCLUSIONS

We have demonstrated the coupling of GC and MC-ICPMS via a heated gas transfer line as a simple and robust method for measuring the δ^{34}S values of individual organosulfur compounds within complex mixtures. The system requires minimal alteration to commercially available hardware and is amenable to virtually all sample introduction methods. Isobaric interference from O_2 is minimized by employing dry plasma conditions and is cleanly resolved at all masses by using medium resolution on the Thermo Neptune ICPMS. Correction for mass bias is accomplished using standard−sample bracketing with peaks of SF_6 reference gas. The precision of measured δ^{34}S values approaches 0.1‰ for analytes containing >40 pmol S and is better than 0.5‰ for those containing as little as 6 pmol S. This is within a factor of 2 of theoretical shot-noise limits. External accuracy is better than 0.3‰. Integrating only the center of chromatographic peaks, rather than the entire peak, offers a significant gain in precision and chromatographic resolution with minimal effect on the accuracy. However, the full effects of this procedure on accuracy have not yet been explored and further study is required. Coelution of organic compounds that do not contain S can cause degraded analytical precision.

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