Development of a Direct Procedure for the Measurement of Sulfur Isotope Variability in Beers by MC-ICP-MS

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In this work, a multi-collector inductively coupled plasma mass spectrometer (MC-ICP-MS) was evaluated for the direct measurement of sulfur stable isotope ratios in beers as a first step toward a general study of the natural isotope variability of sulfur in foods and beverages. Sample preparation consisted of a simple dilution of the beers with 1% (v/v) HNO_3. It was observed that different sulfur isotope ratios were obtained for different dilutions of the same sample indicating that matrix effects affected differently the transmission of the sulfur ions at masses 32, 33, and 34 in the mass spectrometer. Correction for mass bias related matrix effects was evaluated using silicon internal standardization. For that purpose, silicon isotopes at masses 29 and 30 were included in the sulfur cup configuration and the natural silicon content in beers used for internal mass bias correction. It was observed that matrix effects on differential ion transmission could be corrected adequately using silicon internal standardization. The natural isotope variability of sulfur has been evaluated by measuring 26 different beer brands. Measured $\delta^{34}S$ values ranged from -0.2 to 13.8 ‰. Typical combined standard uncertainties of the measured $\delta^{34}S$ values were ≤ 2 ‰. The method has therefore great potential to study sulfur isotope variability in foods and beverages.

KEYWORDS: Sulfur isotope ratios; natural variability; beer; MC-ICP-MS; matrix effects

INTRODUCTION

The natural variability of the isotopic composition of an element needs to be evaluated for different purposes. First, the isotopic composition of a given element in a certain product is acquired during its manufacture, and it is unique to its history and origin. When two separate samples of the same product are chemically the same and have identical isotopic profiles, then there is a high degree of certainty that both samples originate from the same source. Manufacturing and/or biological processes can result in the formation of products whose stable isotope composition is characteristic of that process. Therefore, the isotopic composition of different elements (C, S, Sr, Pb, etc.) can be used for traceability purposes.

A second purpose of these natural variability studies is to evaluate the minimum amount of an enriched isotope which would be needed for tracer work. For example, if we want to follow the metabolic pathway of a certain element in the human body by using an isotopically enriched tracer, the amount of tracer to be used should be enough to change the isotopic composition of the element above the natural variability limits. The isotopic composition of most elements in the human body depends mainly on food and drink intake. Therefore, the natural isotope variability of the elements in foods and beverages could be used to estimate the natural variability in the human body.

Differences in sulfur isotopic composition, observed in sulfur-containing compounds from different sources (*1*), have been widely reported (*2−14*). The relative difference between measured isotope ratios of an element is usually expressed using the per mil notation (‰). Sulfur $^{34}S/^{32}S$ isotope ratios, expressed as $\delta^{34}S$ ‰ values, have been reported to discriminate geographical origin in meat (*15*), butter produced from cow's milk (*16*), and cheese (*17*). Natural variations of $^{34}S/^{32}S$ isotope amount ratios often occur around the third or fourth significant figure and are commonly measured by gas source isotope ratio mass spectrometry (GS-IRMS) after a tedious and laborious sample preparation procedure. Quadrupole collision/reaction cell and sector field ICP-MS instruments have been used for the measurement of sulfur isotope ratios (*18−22*). Because of plasma instabilities and because data acquisition is performed in a sequential mode, the precision obtained in these studies allow only relatively large variations in sulfur isotopic composition to be detected. Continuous flow methods involving the use of MC-ICP-MS have recently been reported (*23−27*). The use of MC-ICP-MS offers the capability of detecting and measuring multiple isotopes simultaneously with minimal sample preparation (e.g., in comparison with GS-IRMS) and typical instrumental precision from 0.1% to < 0.005% (*12*, *13*, *28*) depending on sulfur concentration.

The main problem affecting isotope ratio measurements by MC-ICP-MS is the existence of matrix effects on the mass bias correction factor. The transmission of the ions in the mass spectrometer will change slightly in the presence of high concentrations of the matrix, and this change will not be the same for different isotopes of the same element. Therefore, apparent changes in isotope ratios could be due to matrix effects and not

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to real changes in the sample. There are different alternatives to eliminate or correct for these matrix effects. The traditional solution is the separation of the element from the matrix and the use of a certified isotopic standard of the element for external mass bias correction. As an alternative, internal mass bias correction, using another element close in mass, can be applied. For sulfur measurements, silicon internal standardization has been used to correct for mass bias effects (25). It was observed that silicon could correct for matrix effects from sodium, chlorine and calcium in waters (25). In this article, we explore the capabilities of silicon for the correction of mass bias related matrix effects in the determination of sulfur natural isotope variations by MC-ICP-MS in samples containing large amounts of dissolved organic matter. Then, a simple dilution of the sample would be enough, opening the way for large studies on sulfur isotope variations in foods and beverages with a very simple sample preparation. To demonstrate the potential of the method, 26 beer samples representative of those currently on sale in the U.K. were analyzed. $\delta^{34}S$ values, reported relative to the Vienna Canyon Diablo Troilite (VCDT) scale (29), were obtained. NIST RM8553 was used as the working standard to anchor our results to the VCDT scale.

EXPERIMENTAL SECTION

Reagents and Standards. High purity reagents were used throughout. Water was taken directly from an 18.2 MΩcm supply (Elga Ltd., High Wycombe, U.K.). Three sulfur reference materials (RM) were employed in this study: (a) National Institute of Science and Technology (NIST) RM8553, Soufre de Lacq-Elemental sulfur, $\delta^{34}S = 16 \pm 0.3$ (‰); (b) NIST RM8554, IAEA-S1 Silver sulfide (AgS), $\delta^{34}S = -0.3 \pm 0.3$ (‰); and (c) NIST RM8556, NBS 123 Sphalerite, $\delta^{34}S = 17.44 \pm 0.3$ (‰).

Each reference material was separately prepared by digesting an aliquot in a closed vessel microwave digester (Multiwave 3000, Perkin-Elmer, Beaconsfield, U.K.) with high purity HNO_3 acid (ROMIL, Cambridge, U.K.) and subsequent dilution with high purity water. Two reference values are given for RM8553. This work adopts the $\delta^{34}S$ value of $+16 \pm 0.3$ ‰, and RM8553 was selected as the main working standard because it required the least sample preparation. Both RM8554 and RM8556 were used to evaluate instrumental performance. The Si internal standard source was an Assurance Spex CertiPrep ICP (1,000 $\mu g\ g^{-1}$) standard solution (Spex Certiprep Ltd., Stanmore, Middlesex, U.K.). The isotopic composition of the Spex Si standard was measured in-house using the certified IRMM017 Si material for bracketing during the MC-ICP-MS measurements. The Spex silicon standard was added to RM8553, RM8554, and RM8556 to correct for mass bias in the sulfur measurements. The direct addition of IRMM017 to the sulfur isotopic standards RM8553, RM8554, and RM8556 was not performed in order to save the expensive IRMM017 standard.

Instrumentation. The MC-ICP-MS instrument used in this study was Neptune (ThermoFisherScientific, Bremen, Germany). Neptune has an argon plasma ion source, forward Nier-Johnson geometry, and nine Faraday cup detectors. Typical operating conditions have been published elsewhere (30). The instrument, located at LGC, can be used to measure simultaneous ion signals in three resolution modes, low, medium, and high. The spectral interferences affecting the accuracy of the sulfur and silicon isotope ratio measurements (mainly $^{16}O_2^+$, $^{16}O^{18}O^+$, $^{14}N^{14}N^1H^+$, and $^{14}N^{16}O^+$, respectively) were resolved using the Neptune instrument working at medium resolution. The potential hydride interference of $^{28}Si^1H^+$ previously reported (31) was not detected. A typical mass scan obtained for sulfur and silicon isotope ratio measurements working at medium resolution in the Neptune MC-ICP-MS is shown in **Figure 1**. The axial faraday cup (C) was fixed at mass 31.26 (amu) for multiple interference-free simultaneous measurements of ^{29}Si (L4), ^{30}Si (L1), ^{32}S (H1), and ^{34}S (H4) isotopes. In this scan, the signal intensities of Si isotopes 29 and 30 and ^{34}S have been normalized to that of ^{32}S.

The instrumental mass discrimination of the measured $^{34}S/^{32}S$ isotope amount ratio was corrected by interpolation of the mass discrimination observed for $^{30}Si/^{29}Si$ isotope amount ratio using Russell's equation (32).
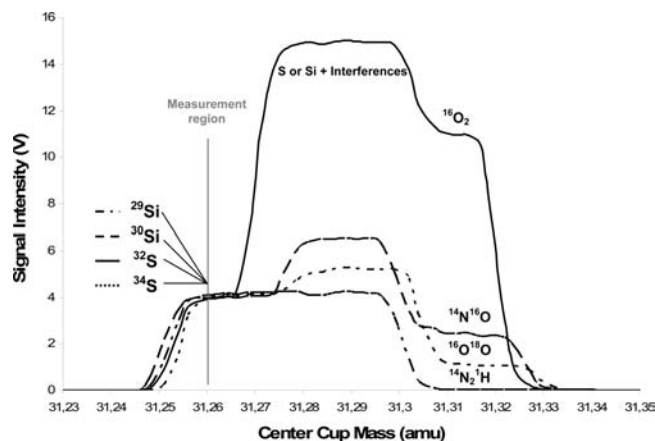
Figure 1. Typical mass scan obtained for sulfur and silicon isotope ratio measurements working at medium resolution in the Neptune MC-ICP-MS instrument. The center Faraday cup has been fixed at mass 31.26 (amu) for multiple interference free simultaneous measurements of S and Si isotopes. In this scan, the signal intensities of Si isotopes and ^{34}S have been normalized to that of ^{32}S.

The corrected ratio is a function of the two measured isotope ratios and the relative atomic mass difference between the isotope pairs. For the beer samples, the $^{30}Si/^{29}Si$ reference isotope amount ratio used was taken from the last compilation of the IUPAC values (1) including the natural variability in the silicon isotope abundances as uncertainty. For the sulfur isotopic reference materials, the $^{30}Si/^{29}Si$ reference isotope amount ratio used was that measured for the Spex silicon standard. Mass bias was typically 3.3% per atomic mass unit.

It is important to highlight that the mass difference between ^{29}Si and ^{34}S is close to the limit of the mass range of the Neptune's Faraday detector array ($\pm 8.5\%$ relative to the mass focused into the fixed center channel). However, by adjusting the dispersion and focus lenses to -5 V and 15 V, respectively, and fixing the center cup at mass 31.26 amu, all of the isotopes of interest can be measured within the same cup configuration, as previously reported by Mason et al (18). As a result, this cup configuration drastically reduces the time needed for the analysis of liquid solutions when compared to a similar analysis reported before where S and Si isotope ratios ($^{30}Si/^{28}Si$) had to be monitored using two separate cup configurations (25). In addition, the new method can be applied to the measurement of transient signals where a mass bias correction can be performed simultaneously. For the method used in this study, each complete acquisition comprised 5 cycles divided into 5 blocks, and the total acquisition time for each sample measurement was less than 4 min. A microconcentric glass nebulizer (Micromist) and a quartz sample introduction system (SIS) spray chamber were used in this study. Operating conditions and data acquisition parameters are summarized in **Table 1**.

Measurement of $\delta^{34}S$ in Beers by MC-ICP-MS. A suite of 26 beers representative of those currently on sale at specialized stores and supermarkets in England were measured. Sample preparation was kept to a minimum, and beer samples were simply diluted with 1% (v/v) HNO_3 to approximately 1:3 for direct measurement in the MC-ICP-MS. No problems with signal losses, blocked cones, or nebulizers were observed. NIST reference materials were doped with the Spex silicon standard at approximately 5 $\mu g\ g^{-1}$ to correct for mass bias effects using the equation:

$$\left(\frac{^{34}S}{^{32}S}\right)_{cor} = \frac{\left(\frac{^{34}S}{^{32}S}\right)_{meas}}{\left(\frac{\left(\frac{^{30}Si}{^{29}Si}\right)_{meas}}{\left(\frac{^{30}Si}{^{29}Si}\right)_{ref}}\right)^{\left(\frac{\ln\left(\frac{Mass^{34}S}{Mass^{32}S}\right)}{\ln\left(\frac{Mass^{30}Si}{Mass^{29}Si}\right)}\right)}} \quad (1)$$

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Table 1. Operating Conditions and Data Acquisition Parameters for the Measurement of Sulfur and Silicon Isotope Ratios in Beer Samples

MC-ICP-MS Settings		
Rf power	1350 W	
cool gas flow	14.0 L min^{-1} Ar	
auxiliary gas flow	0.7 L min^{-1} Ar	
sample gas flow	1.0 L min^{-1} Ar	
sampler and skimmer cones	Ni	
extraction voltage	−2000 V	
focus lens	15 V	
dispersion Lens	−5 V	

Data Acquisition Parameters		
resolution mode	medium	
	L4	^{29}Si
	L1	^{30}Si
cup configuration	C	31.26
	H1	^{32}S
	H4	^{34}S
acquisition method	5 blocks, 5 cycles, 4.194 s integration	

where the abbreviations cor, meas, and ref indicate corrected, measured, and reference, respectively. Silicon was present in beer samples at relatively high levels; thus, the external addition of silicon to beer samples was not required. For the beer samples, the reference isotope ratio for silicon was taken from the IUPAC tables (*1*), and then eq **1** was applied. NIST RM8553 was used as the working standard for δ^{34}S measurements. A solution containing NIST RM8553 and silicon was analyzed every 5 samples and corrected for mass bias also using eq **1**. In this case, the reference silicon isotope ratio was that measured for the Spex silicon standard. Then, the δ^{34}S value relative to the VCDT was calculated using:

$$\partial^{34}S_{VCDT} = \left(\left(\frac{\left(\frac{^{34}S}{^{32}S}\right)_{beer\_cor}}{\left(\frac{^{34}S}{^{32}S}\right)_{RM8553\_cor}}\right) \times \left(\frac{\partial^{34}S_{RM8553}}{1000}+1\right) - 1\right) \times 1000 \quad (2)$$

Finally, NIST RM8554 and NIST RM8556 were also analyzed to monitor the performance of the instrument at both ends of the range of variation in δ^{34}S. In total, 26 beers and 3 sulfur reference materials, as summarized in **Table 2**, were analyzed.

Evaluation of Uncertainty Sources. Combined standard uncertainties were calculated following the Kragten spreadsheet method (*33*) taking into account instrumental variability, the uncertainties associated with the reference materials used in the calculation, and the mass bias correction applied to the observed isotope ratios. In total, seven uncertainty sources were identified, and they are shown in **Table 3**. The first six uncertainty sources arise from the application of eq **1** both to the beer sample and to the RM8553. The final uncertainty source is the uncertainty in the δ^{34}S value of RM8553 relative to the VCDT. Typical values for one of the samples are shown in **Table 3**. For the four measured isotope ratios, their uncertainties were taken as the standard error of the mean. For the reference silicon isotope ratio values, the uncertainties were calculated as described in the Results and Discussion section.

Finally, the uncertainty of each δ^{34}S value was calculated in accordance with error propagation laws as described in EURACHEM Guide (*34*) using the Kragten procedure. Combined standard uncertainties for the measured samples are given in **Table 2**.

RESULTS AND DISCUSSION

Uncertainties of the Reference Silicon Isotope Values. (*a*). *Uncertainty of the Reference IUPAC Isotope Ratio.* The last representative isotope composition of silicon as tabulated by the IUPAC (*1*) indicates that the isotope abundances of ^{28}Si, ^{29}Si, and ^{30}Si were 0.92223(19), 0.04685(8), and 0.03092(11), respectively, where the values in parentheses indicate expanded uncertainties

due to natural variability. From these data, the reference isotope ratio ^{30}Si/^{29}Si would be 0.65998. For the calculation of the uncertainty of this value, we have taken into account the recent paper by Meija and Mester (*35*) in which they demonstrate that isotope abundances are correlated variables of constant sum and that the correlation coefficients between the variables will need to be taken into account for the calculation of the uncertainties in derived variables, such as atomic weights (*35*) or, in this case, isotope ratios.

For this purpose, we have taken the general equation for error propagation (eq **3** where $u(x_i)$ are the standard uncertainties for each parameter and $u(x_i,x_k)$ the covariances):

$$u(y(x_1, x_2 ... x_n)) = \sqrt{\sum_{i=1,n}\left(\frac{\partial y}{\partial x_i}\cdot u(x_i)\right)^2 + \sum_{i,k=1,n}\left(\frac{\partial y}{\partial x_i}\cdot\frac{\partial y}{\partial x_k}\cdot u(x_i, x_k)\right)}$$

$$(3)$$

Then, we can calculate the covariances $u(x_i,x_k)$ if we know the correlation coefficients between the variables using:

$$u(x_i, x_k) = r_{ik}\cdot u(x_i)\cdot u(x_k) \quad (4)$$

For the particular case of the uncertainty of a ratio R, u_R, of two correlated variables, x and y, the application of eqs **3** and **4** was derived in eq **5** as follows:

$$u_R = R \times \sqrt{\left(\frac{u_x}{x}\right)^2 + \left(\frac{u_y}{y}\right)^2 - 2 \times r_{xy} \times \left(\frac{u_x}{x}\right) \times \left(\frac{u_y}{y}\right)} \quad (5)$$

where r_{xy} is the correlation coefficient between the variables x and y, and u_x and u_y their standard uncertainties. For bi-isotopic elements, the correlation coefficients are always −1 (*35*); therefore, the uncertainty in the isotope ratio would be larger than that calculated assuming no correlation between the variables (the correlation always adds uncertainty to the isotope ratio of bi-isotopic elements as shown in eq **5**). For tri-isotopic elements, such as silicon, the correlation coefficients will need to be evaluated. In a previous paper, we have calculated the correlation coefficients between the isotopes of silicon by applying the mass dependent fractionation law (*36*). We observed a perfectly negative correlation ($r = -1$) for the ^{29}Si/^{28}Si and ^{30}Si/^{28}Si ratios and a positive correlation ($r = 1$) for the ratio ^{30}Si/^{29}Si. Therefore, using eq **5**, we have calculated the standard uncertainty for the ^{30}Si/^{29}Si ratio to be 0.00061, which is about half of that calculated assuming no correlation between the isotope abundances (0.00130). However, the uncertainty of the natural ^{30}Si/^{28}Si ratio increased only from 0.000060 to 0.000063 when the correlation was taken into account.

(*b*). *Isotope Ratios and Uncertainties of the Spex Silicon Standard.* A series of 30 measurements of the Spex silicon standard together with 11 measurements of the IRMM 017 silicon isotopic reference material were performed by bracketing the reference material between every three samples of the Spex standard. The final value found for the ratio ^{30}Si/^{29}Si in the Spex standard was 0.659477 with a standard error of the mean ($n = 30$) of 0.000027. This is only slightly larger than the standard uncertainty of the ratio ^{30}Si/^{29}Si in the IRMM 017 reference material, which was 0.000018 for a certified ratio of 0.659600.

Matrix Effects on Mass Bias. Matrix effects can have a major impact on the precision and accuracy of isotope ratio measurements by MC-ICP-MS (*37*); therefore, understanding their impact for any given isotopic system is an important aspect of method development. Beer constituents comprise more than 800 compounds (*38*), and therefore, matrix effects could affect isotope

Table 2. $\delta^{34}S_{VCDT}$ Values, Measurement Standard Deviations, and Combined Standard Uncertainties for Beer Samples, Using NIST RM8553 As the Working Standard[a]

| beer	$\delta^{34}S_{VCDT}$ (‰) average ($n = 3$)	standard deviation (‰) ($n = 3$)	combined standard Uc (‰)	% Uc (1)	% Uc (2)	% Uc (3)	% Uc (4)	% Uc (5)	% Uc (6)	% Uc (7)
Asahi	7.9	0.1	1.8	1.0	8.5	87.9	1.3	0.5	0.2	0.7
Baltika	4.7	0.2	2.0	2.2	21.2	74.7	0.8	0.3	0.1	0.6
Becks	3.4	0.8	1.9	1.6	3.6	91.9	1.6	0.4	0.2	0.7
Bock	1.9	0.4	1.8	0.9	2.1	94.6	0.9	0.5	0.2	0.8
Brahma	4.2	0.5	1.8	3.4	2.1	92.5	0.6	0.5	0.2	0.7
Brooklyn	−0.2	0.3	1.8	0.9	1.0	95.1	1.7	0.4	0.2	0.8
Budweiser	4.8	0.2	1.8	4.6	0.5	92.2	1.1	0.7	0.2	0.7
Carling	10.3	0.4	1.9	0.6	14.6	82.8	0.8	0.5	0.2	0.7
Cobra	7.9	0.5	1.9	3.3	8.5	85.8	1.3	0.4	0.2	0.7
Corona	10.7	0.2	1.7	0.4	1.4	95.8	0.9	0.5	0.2	0.8
Cusqueña	6.8	0.8	1.9	0.7	0.8	95.8	1.5	0.3	0.2	0.8
Desperado	9.1	0.3	1.8	4.4	4.7	88.7	0.8	0.5	0.2	0.7
Duvel	10.0	0.5	1.8	3.1	3.4	91.2	0.9	0.5	0.2	0.7
Erdinger	5.9	0.2	1.8	2.4	10.6	84.8	0.8	0.5	0.2	0.7
Estrella Damm	4.6	0.3	1.8	3.0	2.4	93.0	0.6	0.1	0.2	0.7
Guinness	9.8	0.6	1.8	1.3	4.1	92.0	1.5	0.3	0.2	0.7
Heineken	5.6	0.6	1.6	1.5	6.1	89.7	1.2	0.6	0.2	0.7
Kronenbourg	6.8	0.9	2.0	0.7	12.3	84.5	1.1	0.6	0.2	0.7
Leffe	2.0	0.8	1.9	1.1	3.1	93.2	1.5	0.3	0.2	0.7
Peeterman	7.5	0.4	1.9	0.9	13.9	83.1	0.8	0.5	0.2	0.7
Peroni	7.0	0.7	1.9	1.9	2.2	93.2	1.4	0.4	0.2	0.7
Pilsner	4.4	0.5	1.8	0.3	0.7	96.8	0.7	0.5	0.2	0.8
San Miguel	13.8	0.4	1.8	0.5	2.3	95.0	0.6	0.5	0.2	0.8
Shinga	9.6	0.4	1.8	1.4	6.9	89.0	1.5	0.3	0.2	0.7
Stella	7.8	0.8	1.9	1.4	0.5	95.1	1.7	0.4	0.2	0.8
Tiger	7.5	0.6	1.9	1.9	6.9	88.8	1.4	0.2	0.2	0.7
NIST RM 8554	−1.4	0.2	0.5	41.4	6.4	0.0	30.7	7.5	0.0	14.0
NIST RM 8556	17.3	0.3	0.4	15.0	4.4	0.0	43.4	13.0	0.0	24.2
			average of beer samples	1.7	5.6	90.3	1.1	0.4	0.2	0.7

[a] Full uncertainty budgets have been calculated for each sample and are summarized below. Seven uncertainty sources have been identified: % Uc (1) is the uncertainty of the measured $^{34}S/^{32}S$ isotope ratio in the beer sample, % Uc (2) is the uncertainty of the measured $^{30}Si/^{29}Si$ isotope ratio in the beer sample, % Uc (3) is the uncertainty of the IUPAC $^{30}Si/^{29}Si$ natural abundance isotope ratio, % Uc (4) is the uncertainty of the measured $^{34}S/^{32}S$ isotope ratio for the working standard, % Uc (5) is the uncertainty of the measured $^{30}Si/^{29}Si$ isotope ratio for the working standard, % Uc (6) is the uncertainty of the SPEX certified in house $^{30}Si/^{29}Si$ isotope ratio, and % Uc (7) is the uncertainty of the $\delta^{34}S_{VCDT}$ value of the working standard.

**Table 3.** Uncertainty Sources for the Measurement of $\delta^{34}S$ in Beer Samples

uncertainty source	typical value	standard uncertainty	comment
$^{34}S/^{32}S$ measured isotope ratio in beers	0.049530	0.000007	standard deviation of the mean ($n = 5$)
$^{30}Si/^{29}Si$ measured isotope ratio in beers	0.70397	0.00016	standard deviation of the mean ($n = 5$)
$^{30}Si/^{29}Si$ reference value IUPAC for beer samples	0.65998	0.00061	propagated uncertainty taking into account the correlation coefficients
$^{34}S/^{32}S$ measured isotope ratio in RM8553	0.049994	0.000008	standard deviation of the mean ($n = 5$)
$^{30}Si/^{29}Si$ measured isotope ratio in RM8553	0.703976	0.000050	standard deviation of the mean ($n = 5$)
$^{30}Si/^{29}Si$ corrected value Spex standard for RM8553	0.659477	0.000027	propagated uncertainty using IRMM 017 silicon as reference
$\delta^{34}S$ value for RM8553	16	0.15	certified value and uncertainty ($k = 2$)

ratio measurements. Although beer is usually made up of four main ingredients, water, hops, malted barley, and yeast (*39*), it is the precise recipe and timing of the brewing process that gives one beer a different taste from another. In general, the composition of beer varies from one brewery to another, but the following ingredients are present in all recipes: carbohydrates, ethanol (being the most important alcohol present), protein derived material, amines, nucleic acid derivatives, hop bitter acids, small amounts of vitamins of the B-group, phenolic compounds, and a range of inorganic compounds, which include major cations, trace metals, and anions. A wide variety of volatile sulfur compounds have also been reported in beer formulations (*40*). Given the complexity of beer matrices, it is expected that many of these components (*41*) may contribute as a potential source of matrix depending mass bias. Typical concentration of S in beer varies from 10 to 50 μg g^{-1}. Silicon content in beer also varies from 10 to 50 μg g^{-1} and would show small isotopic variations typical of those tabulated by the IUPAC (*1*). **Figure 2** shows the

measured $^{30}Si/^{29}Si$ isotope ratios in a group of beer samples and in the NIST RM8553, 8554, and 8556 sulfur isotopic standards as a function of the order in which the samples were measured. Beer samples were simply diluted 1:3 with 1% (v/v) HNO$_3$. As can be observed, the measured silicon isotope ratios in the NIST reference materials, corresponding to the Spex silicon standard added, are fairly stable with time with a small decrease at the end of the measurement series. The deviations from the expected ratio in the silicon standard ($^{30}Si/^{29}Si = 0.659477 \pm 0.000027$) are due to mass bias effects. However, the measured silicon isotope ratios in the beer samples are clearly different from those measured for the reference materials and changed depending on the nature of the measured sample. The maximum variation expected in the $^{30}Si/^{29}Si$ isotope ratios from natural variations would be in the range of 0.65998 ± 0.00061, as calculated before. Therefore, the observed variations in the measured silicon isotope ratios cannot be explained only by natural variability and should indicate the presence of matrix effects on the instrumental mass bias.

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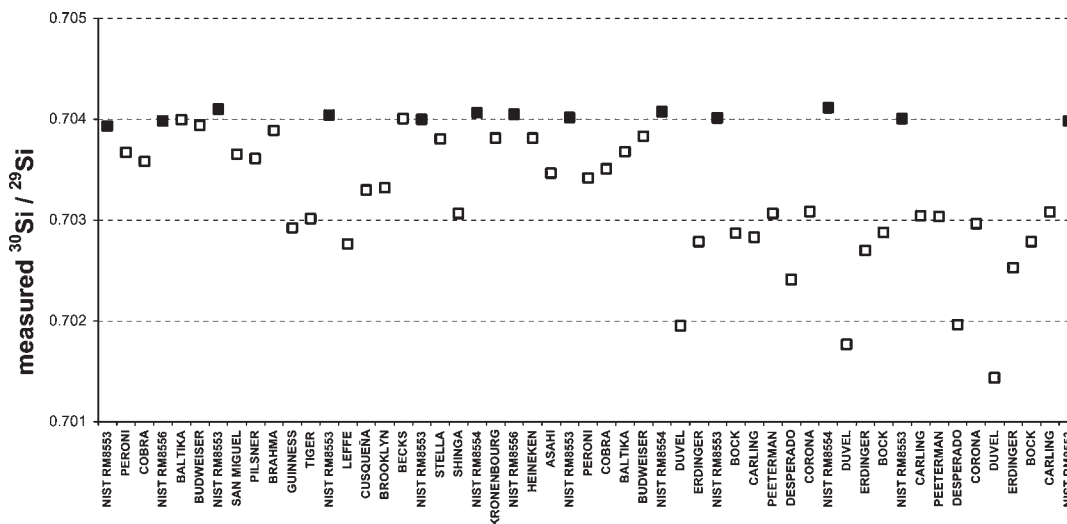
Figure 2. Measured $^{30}Si/^{29}Si$ isotope ratio plotted as a function of the order in which the NIST reference materials (■) and beer samples (□) were analyzed.
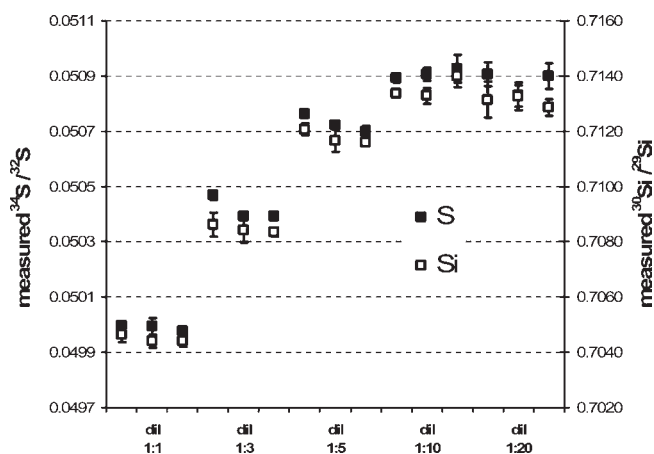
Figure 3. Measured $^{34}S/^{32}S$ and $^{30}Si/^{29}Si$ isotope amount ratios in a beer sample with increasing dilution factor. Error bars represent the instrumental uncertainty ($n = 3$).

The question is if similar changes are obtained for sulfur and if mass bias correction can be applied safely using silicon. For a complex system like beer, it is not feasible to study the matrix effects of each component by its addition to a certified sulfur standard as performed by Clough et al. for water (*25*). Therefore, in order to demonstrate the presence of matrix effects on the measured silicon isotope ratios, the effect of sample dilution was evaluated. If matrix effects were not present, the dilution of the sample would not change its isotopic composition.

Five dilution factors were tested, namely, 1:1, 1:3, 1:5, 1:10, and 1:20. In a first experiment, the Spex silicon standard was added to all dilutions of the same sample to a final concentration of 15 μg g^{-1} to ensure good counting statistics for silicon. In a separate experiment, the same solutions were prepared without the addition of exogenous silicon. Similar results were obtained in both experiments. **Figure 3** shows the measured $^{34}S/^{32}S$ and $^{30}Si/^{29}Si$ isotope ratios (in triplicate dilutions) for the different dilutions of the same beer sample without silicon addition. As can be observed, isotope ratios increased with sample dilution up to a dilution factor of 10. After that, no further changes in the isotope ratios were observed. The increase in the isotope ratios with sample dilution indicates that matrix effects decrease the mass bias factor as the relative transmission of the heavier isotope is decreased against the
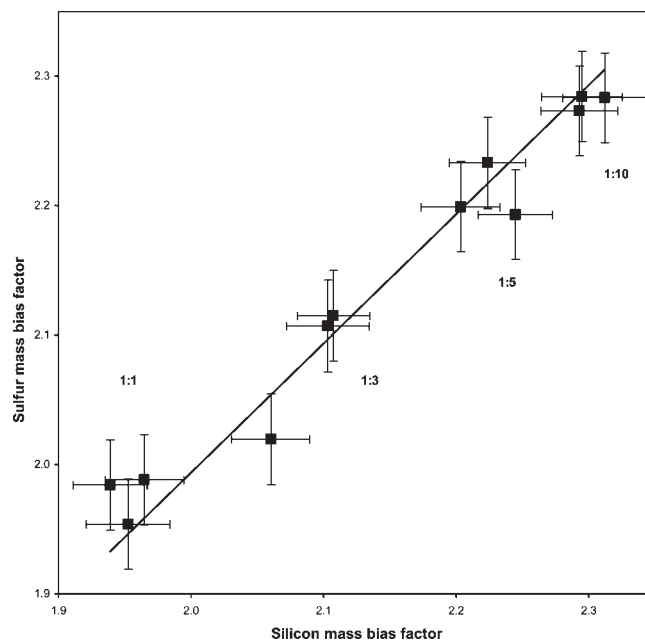
Figure 4. Correlation between the mass bias factors for sulfur and silicon for the different dilutions of the beer shown in **Figure 3**. Error bars indicate combined standard uncertainties.

lighter isotope. Similar observations, opposite to the expected behavior, have been reported previously (*32*).

In order to verify if silicon can be used for internal mass bias correction and to correct for matrix effects simultaneously the experimental mass bias factors (β in the Russell equation) were calculated for both elements in the following manner: for silicon, the natural IUPAC $^{30}Si/^{29}Si$ isotope ratio (0.65998 ± 0.00061) was used to calculate the mass bias factors and their uncertainties for all dilution factors of the same beer sample using the Russell equation and applying the Kragten procedure for the uncertainty propagation. Then, for sulfur, the isotope ratios measured at the dilution factor of 1:20 (where no matrix effects seemed to be present) were corrected with the β value calculated for silicon at this dilution factor ($\beta = 2.25 \pm 0.03$). Then, the β values for sulfur at the other dilution factors were calculated using the corrected isotope ratio value at the dilution of 1:20 as the reference. The Kragten procedure of uncertainty propagation was also applied

here. All β values calculated for silicon and sulfur at the dilution factors of 1:1, 1:3, 1:5, and 1:10 measured in triplicate are plotted in **Figure 4** including the uncertainty estimates. The data was adjusted by least-squares to a function of $y = ax$ with a given intercept of 0. The observed slope was $a = 0.997 \pm 0.004$ indicating that the mass bias factors measured for silicon could be applied to sulfur regardless of the dilution factor of the beer sample.

Finally, the different dilution factors of the same beer, both with and without the addition of silicon, were compared to the NIST RM8553 for the calculation of the $\delta^{34}S_{VCDT}$ values. The corrected $^{34}S/^{32}S$ isotope ratios for both sets of dilutions using silicon $^{30}Si/^{29}Si$ internal standardization are shown in **Figure 5**, expressed as $\delta^{34}S_{VCDT}$ (‰) using NIST RM8553 as the working standard. As can be observed, no significant differences were obtained with and without the addition of silicon to the samples. Also, no significant changes in the $\delta^{34}S$ values were detected for different dilution factors of the same sample except, perhaps, for the 1:1 dilution. A dilution factor of 1:3 was selected for further analyses in order to keep the maximum sensitivity possible, and no addition of exogenous Si was finally performed.
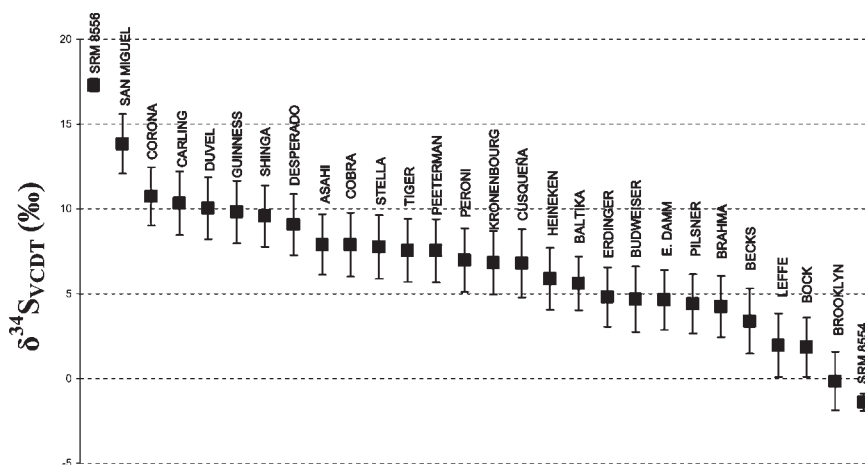
Analysis of Beers. The natural variability of sulfur isotope ratios in beers was measured by obtaining $\delta^{34}S_{VCDT}$ values for a suite of bottled beers representative of those currently sold in the U.K. NIST RM8553 was used as the working standard to anchor the results to the VCDT scale. NIST RM8554 and RM8556 were used to test instrumental performance. **Figure 6** illustrates the

range of $\delta^{34}S_{VCDT}$ values in the beer samples investigated in this study, while the mean $\delta^{34}S_{VCDT}$ values ($n = 3$), measurement standard deviations, combined standard uncertainties, and each individual contribution (in %) are summarized in **Table 2**. Typical combined standard uncertainties of measured $\delta^{34}S_{VCDT}$ values ranged from 1.5 to 2.0 ‰. The major contributor to the standard uncertainty of the $\delta^{34}S_{VCDT}$ values in the beer samples was the uncertainty of the natural isotope composition of silicon used for mass bias assessment (ca. 90%). The experimental uncertainty in the measured silicon isotope ratio in the beer samples (ca. 5%) was the second important source of uncertainty. For the NIST RM8554 and RM8556 standards, the main sources of uncertainty were the uncertainty of the $^{34}S/^{32}S$ measured isotope ratio both in the sample and in the working standard RM8553 and the uncertainty of the $\delta^{34}S_{VCDT}$ value for NIST RM8553, typically contributing 20% relative to the combined standard uncertainty. In this case, the Spex silicon standard was used for mass bias assessment, and its uncertainty was much lower than the natural silicon variation used for the beer samples. **Table 2** shows also how important it is to calculate a full uncertainty budget because, if the measurement precision is reported as the standard deviation of replicate measurements, the actual measurement uncertainty will be underestimated.

Sulfur Isotope Ratios in Beers: Applications to Authenticity Testing and/or Tracer Studies. One of the objectives of this work was the determination of $\delta^{34}S$ values in beer samples with a view to the discrimination of beers of different brands. Manufacturing and/or biological processes can result in the formation of products whose stable isotope composition is characteristic of that process. Variations in isotopic ratios result from a complex interplay of historic, biological, and geological processes (*1*); so in this case, it is difficult to unambiguously identify a single source. However, the isotope ratio measurements can still be used to profile sources, and the combination with other analysis could be used to fingerprint beer brands. Brooklyn (North American beer) presented the lowest $\delta^{34}S$, whereas San Miguel (Spanish beer) showed the highest $\delta^{34}S$ value. We do not know yet if the isotopic composition of beer from the same manufacturer will be constant or whether it will vary depending on the manufacturing time of the year or other sources of variation. We must say that the analytical method presented in this work will not be able to distinguish between different beers of similar origin. In that case, the technique will need to be used in combination with other existing techniques (complementary information such as multi-element and multi-isotope analysis (*42*)).

Figure 5. Corrected $^{34}S/^{32}S$ isotope ratios using $^{30}Si/^{29}Si$ internal standardization of (a) diluted samples with no added Silicon (■) and (b) diluted samples with added silicon (□). The results are expressed as $\delta^{34}S_{VCDT}$ (‰) using NIST RM8553 as the working standard. Error bars represent the combined standard uncertainty for each value.

Figure 6. Neptune MC-ICP-MS $\delta^{34}S_{VCDT}$ values for the suite of beer samples. Values obtained for NIST RM8556 and NIST RM8554 are also included. Error bars represent the combined standard uncertainty for each value.

For tracer studies, which are going to be performed in our laboratory using ^{34}S labeled yeast (*30*), the results obtained were very informative. The natural variability of sulfur isotope composition was previously measured for bottled water samples to be between −12 and +22 ‰ (*25*). Our results showed a narrower range of variation, ca. between −0.2 and +13.8 ‰. On the basis of these data, we could calculate the minimum amount of enriched ^{34}S which would be needed for a tracer experiment assuming that measurement uncertainty will be around 2 ‰. For example, a small laboratory animal (e.g., a Wistar rat weighting ca. 100 g) will contain ca. 0.175 g of protein sulfur. The administration of a single dose of 1 mg of enriched ^{34}S as isotopically labeled yeast will change the ^{34}S/^{32}S isotope ratio of sulfur in proteins, if completely equilibrated, from 0.045 to 0.051 which corresponds to a δ^{34}S$_{VCDT}$ value of about +100. This change is now much larger than the expected natural variation of sulfur isotope ratios, and it is calculated assuming total isotope equilibration between sulfur isotopes in the body. As this is very unlikely to occur, sulfur isotope enrichment in certain serum proteins should be easily detected using this tracer amount.

To conclude, silicon internal standardization has been shown to be an effective method to correct for mass bias matrix effects in samples containing high amounts of dissolved organic matter. Our results are in agreement with those reported by Clough et al. (*25*) for the study of sulfur isotope variations in bottled mineral waters. The potential of the method has been tested with 26 beer samples from different brands. The δ^{34}S values have been calculated, and typical combined standard uncertainties of measured δ^{34}S values were ≤2 ‰. Although the discrimination power of sulfur isotope ratios could not be used to distinguish every single beer, results show the potential of the method to distinguish brands and to estimate the natural variability of sulfur in foods and beverages for future tracer experiments.

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