Accurate determination of sulfur in biodiesel using Isotope Dilution-Triple Quadrupole ICP-MS (ID-ICP-QQQ)

Application note

Petrochemical

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Introduction

The development of accurate, sensitive and fast analytical methods for the determination of low levels of sulfur (S) in organic matrices is important in several applications. In this note we concentrate on the determination of S in biodiesel, but the methodology is also applicable to the determination of S (plus other elements) in other matrices such as biological materials and S-containing drugs.

Biodiesel is a renewable fuel derived from natural oils, which can be used in diesel-engine vehicles with little or no modification. Biodiesel refers to a collection of alkyl esters derived from renewable or "bio" sources such as cooking oils, animal fats, or plant oils. In its pure form, it is termed fatty acid methyl ester (FAME). Sulfur content of biofuels is of interest due to emission legislation specified by governmental agencies (such as ASTM and ISO) concerning the maximum sulfur content in fuels, and the low sulfur requirements of modern diesel engines.

Traditional analytical techniques for analysis of biofuels are Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) and Atomic Absorption Spectrometry (AAS/FAAS). However, these techniques suffer from intense interferences due to an increased background continuum from the carbon matrix, so they are limited to a few elements or have insufficient detection limits. Sulfur determination in organic matrices is also challenging for ICP-Mass Spectrometry (ICP-MS) [1,2] even when a collision/reaction cell (CRC) is employed, due to the occurrence of spectral overlaps from multiple polyatomic ions for all isotopes of S (Table 1). Sector field high resolution ICP-MS (HR-ICP-MS) has also been applied, but this type of instrument comes at a higher cost than quadrupole-based instruments, and is therefore less widely used. Another disadvantage of HR-ICP-MS is a 10-fold reduction in ion transmission efficiency (and therefore sensitivity) at the mass resolution required to separate the sulfur isotopes from their overlaps.

In this study, we assess the suitability of a new type of ICP-MS instrument, Triple Quadrupole ICP-MS or ICP-QQQ, operating in MS/MS mode for the determination of S in biodiesel using isotope dilution (ID)-MS.

Table 1. S-isotopes and SO^+ product ions with their natural isotopic abundance and the most important potentially interfering ions that hamper the accurate determination of S

Isotope	Abundance (%)	Ions causing spectral interference
$^{32}S^+$	95.04	$^{16}O^{16}O^+$, $^{14}N^{18}O^+$, $^{15}N^{16}O^1H^+$
$^{33}S^+$	0.75	$^{32}S^1H^+$, $^{16}O^{16}O^1H^+$, $^{16}O^{17}O^+$, $^{15}N^{18}O^+$, $^{14}N^{18}O^1H^+$
$^{34}S^+$	4.20	$^{33}S^1H^+$, $^{16}O^{18}O^+$
$^{32}S^{16}O^+$	95.04	$^{48}Ti^+$, $^{48}Ca^+$, $^{36}Ar^{12}C^+$
$^{33}S^{16}O^+$	0.75	$^{49}Ti^+$, $^{32}S^{17}O^+$, $^{31}P^{18}O^+$
$^{34}S^{16}O^+$	4.20	$^{50}Ti^+$, $^{50}Cr^+$, $^{50}V^+$, $^{38}Ar^{12}C^+$, $^{36}Ar^{14}N^+$, $^{32}S^{18}O^+$, $^{33}S^{17}O^+$

Experimental

Instrumentation

All measurements were carried out with an Agilent 8800 Triple Quadrupole ICP-MS.

In the 8800, an octopole-based CRC is located in between two quadrupole analyzers (Figure 1). The mass "window" passed by the first quadrupole (Q1) analyzer can be varied from "fully open" in "Single-Quad" mode, down to unit mass width in MS/MS mode. The octopole CRC can be used in vented mode or can be pressurized with either a collision gas (to remove polyatomic ions by kinetic energy discrimination (KED), or to induce collision-induced dissociation), a reactive gas (to selectively react with either the interfering or the target ion to attain interference-free measurement) or a combination of both.

In this study, the ICP-QQQ instrument was operated in three different modes:

1. Single-quad (SQ) mode with Q1 operating as an ion guide (further described as the "SQ ion guide" mode)
2. Single-quad (SQ) mode with Q1 acting as a bandpass filter, with the bandpass sufficiently wide to allow both S^+ and SO^+ ions to pass through the system (further described as the "SQ bandpass" mode)
3. MS/MS mass-shift mode with Q1 set to the mass of the target precursor ion, the S^+ isotopes in this case, and Q2 set to the mass of the appropriate reaction product ion, SO^+ in this case (further described as "MS/MS" mode).

The fact that the instrument can be used in these three different modes makes it very well suited for

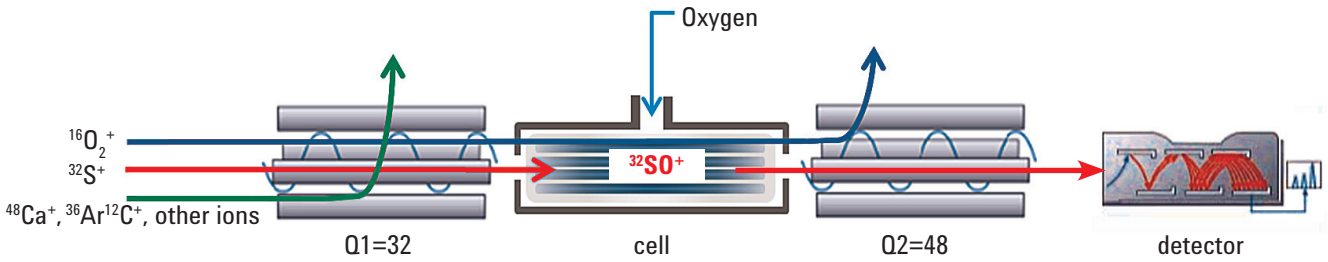

Figure 1. Schematic representation of the operating principle of the ICP-QQQ system, functioning in MS/MS mode. Non-target ions (in this case $^{48}Ca^+$, $^{36}Ar^{12}C^+$ and other ions) are rejected by Q1 before the ions enter the collision/reaction cell, leading to an interference-free determination of ^{32}S as $^{32}S^{16}O^+$ at m/z=48

a large variety of applications, as well as for clearly demonstrating the strength of using MS/MS mode, which is unique to ICP-QQQ. MS/MS mode was utilized to provide interference-free conditions for monitoring the signals of both ^{32}S and ^{34}S for isotope dilution purposes in a demanding matrix.

The ICP-QQQ was configured for the analysis of organic solvents, which involves reducing the amount of organic vapor entering the plasma. The Peltier-cooled spray chamber was set to -5 °C and the standard torch (which has an injector tube of 2.5 mm internal diameter) was replaced by an organics torch with a 1 mm i.d. injector. To prevent carbon-build up on the interface, O_2 was added (as a 20% mixture in Ar) to the spray chamber. For maximum sensitivity, the ion lenses were optimized for the different plasma conditions when running organics. Table 2 summarizes the instrument operating conditions used.

Table 2. Instrumental settings for the ICP-QQQ

Parameter	Value
RF power	1450 W
Carrier gas flow rate	0.98 L/min
O_2 option gas flow rate	75 mL/min
Spray chamber temperature	-5 °C
ORS^3 reaction gas flow rate (O_2)	0.4 mL/min
Q1 bias	-2 V
Octopole bias	-9 V
Q2 bias	-18 V

Samples and reagents

Reference biodiesel material NIST SRM 2773 (National Institute of Standards and Technology, USA) was used to check the accuracy of the method. The 98.8% ^{34}S spike (sodium sulfate) used for isotope dilution was obtained from Isoflex (USA) and the single element 10 g/kg inorganic S standard solution (used for preparing calibration standards and for reverse ID-MS) from SPEX CertiPrep (USA). Only high-purity reagents were used for sample preparation. Water was purified by means of a Direct Q-3 Milli-Q system (Millipore, USA), while HNO_3 (pro analysis, ChemLab, Belgium) was further purified by sub boiling distillation. All samples and standards were diluted in absolute ethanol (USP grade for analysis, Fisher Scientific, UK), containing 0.14 M HNO_3.

Sample preparation

To check the linearity of the calibration curves, sulfur standards with concentrations ranging between 0 and 850 µg/kg were prepared, by diluting a 10 g/kg S single element standard solution with ethanol.

For the isotope dilution experiment, a solution of the ^{34}S spike material was prepared by dissolving 0.1 g powder into 10 mL of 0.14 M HNO_3, to give a concentration of approximately 2.25 mg/g S (stock solution A). Subsequently, 250 mL of spike solution A was mixed with 65 mL of a S standard solution of natural isotopic composition (1 g/kg) and further diluted to a volume of 25 mL with 0.14 M HNO_3. In this way, the ^{34}S enrichment of the spike was reduced to ~90%, which allows a more accurate and precise characterization of the spike. The concentration of this 'diluted' ^{34}S spike solution (solution B) was determined to be 0.711 mmol/g ^{34}S by reverse ID-MS using a S standard solution of natural isotopic composition.

Approximately 1 g of the biodiesel sample (NIST SRM 2773) was directly weighed into a 15 mL polypropylene tube and an accurately weighed amount of spike solution B (~0.2 g) was added. The solution was further diluted to 25 mL with ethanol. Three different mixtures of sample and spike were prepared to allow evaluation of the reproducibility of the method. Blanks were obtained using exactly the same procedure, but without addition of the biodiesel sample.

Results and discussion

The linearity of the calibration curves obtained for a set of S-standards (with concentrations ranging between 0 and 850 µg/kg), diluted with pure ethanol, was evaluated for each of the three different modes of operation.

In single quad (SQ) ion guide mode, S-intensities were measured directly as the elemental ions at Q2 *m/z* of 32, 33 and 34. In the SQ bandpass and MS/MS modes, oxygen reaction gas was added into the octopole cell to convert the S^+ ions into SO^+ ions, which were measured at Q2 *m/z* of 48, 49 and 50, corresponding to the SO^+ product ion mass from $^{32}S^+$, $^{33}S^+$ and $^{34}S^+$ respectively. In MS/MS mode, where only a single precursor ion mass is permitted to enter the cell for each product ion being measured, the corresponding values for Q1 were 32, 33 and 34, respectively (S^+ precursor ions).

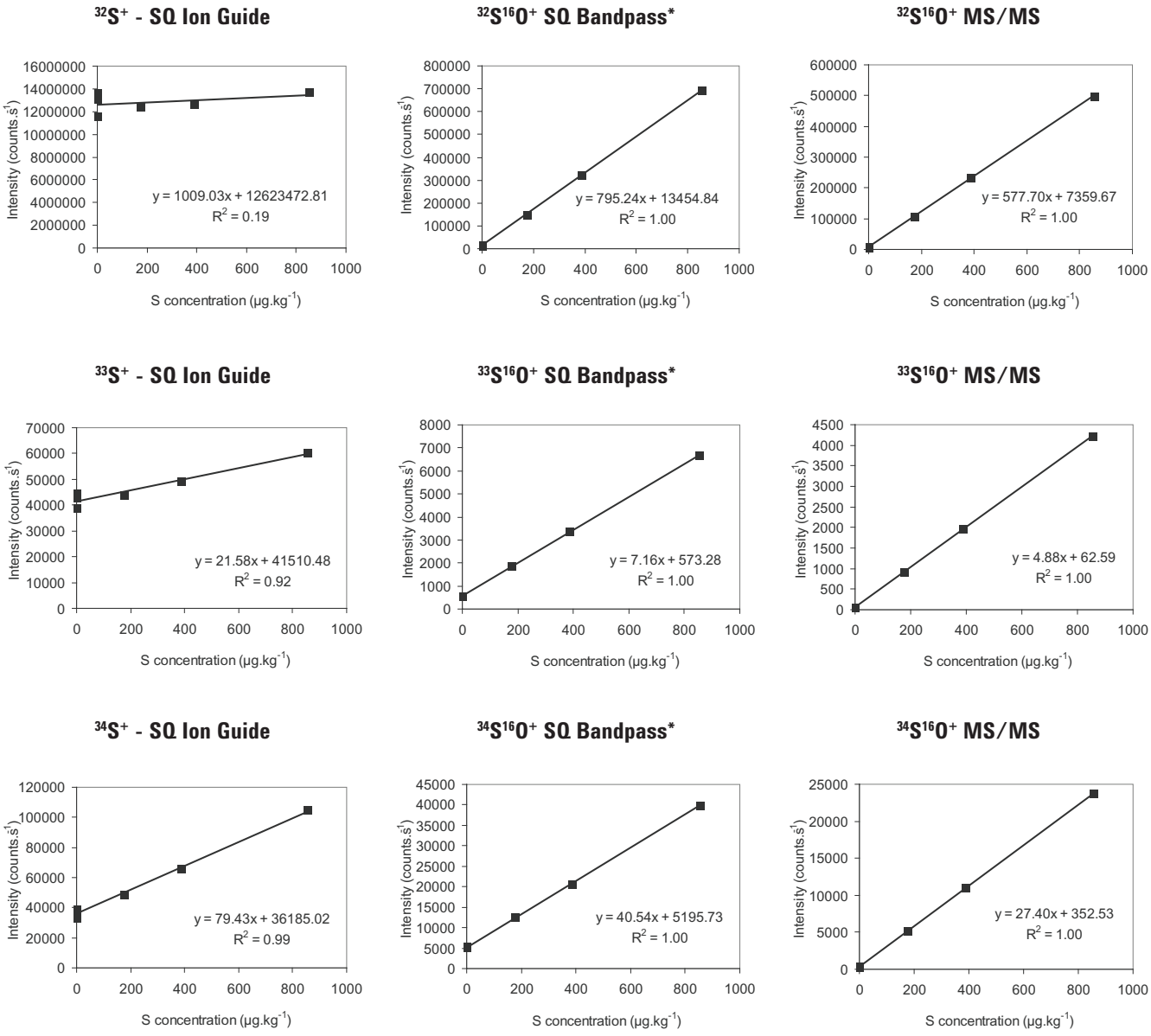

Figure 2. Calibration curves obtained for $^{32}S^+$, $^{33}S^+$ and $^{34}S^+$ (SQ ion guide mode) and for $^{32}S^{16}O^+$, $^{33}S^{16}O^+$ and $^{34}S^{16}O^+$ (SQ bandpass mode and MS/MS mode) for a series of standards with concentrations ranging between 0 and 850 µg/kg. *Reproduced by permission of The Royal Society of Chemistry (RSC)*

* Note: The SQ Bandpass product ions measured are labelled as $^{xx}S^{16}O^+$, but since SQ Bandpass mode (in common with bandpass filtering on conventional ICP-QMS) cannot precisely control the ions that enter the cell, the signals at these masses are actually due to a combination of different product ions. For example at *m/z* 50, as well as the target product ion $^{34}S^{16}O^+$, a second product ion $^{32}S^{18}O^+$ will also be present.

It can be seen from the calibration curves presented in Figure 2 that an accurate determination of S in SQ ion guide mode is almost impossible, as a consequence of the (oxygen-based) spectral overlaps which are always present (see Table 1). The situation clearly improves when the instrument is operated in "reaction mode" and O_2 is added into the cell. While the linearity of the calibration curves is good for both the SQ bandpass and MS/MS modes, an offset of the origin of the calibration curves is clearly visible for SQ bandpass (especially for *m/z* 49 and *m/z* 50). This shows that there is still a considerable spectral overlap at these mass-to-charge ratios when the instrument is operated in SQ bandpass mode, a finding that corresponds to the data obtained by De Wolf et al. [3].

However, in MS/MS mode, the blank values are drastically reduced and the signal intensities obtained at *m/z* ratios of 48, 49 and 50 follow the natural isotopic pattern of S. This indicates that, by using MS/MS mode, S can be determined (practically) interference-free in organic matrices via, at least, its two main isotopes. A further benefit of MS/MS mode on the ICP-QQQ is that it allows selective ion transitions to be measured. For a mass-shift of +16 (Q2 set to Q1 +16), only the addition of a ^{16}O atom is measured. No inter-isotopic interferences can occur, such as the overlap of $^{32}S^{18}O^+$ on $^{34}S^{16}O^+$ at *m/z* 50, that happens in O_2 reaction mode in "SQ Bandpass" and on conventional quadrupole ICP-MS.

The limits of detection (LOD) obtained for this method were calculated based on the standard deviation of the response (s) and the slope of the calibration curve (m), according to the formula: LOD = 3 (s m^{-1}). The standard deviation of the response was determined based on the standard deviation of the y-intercept of the regression lines.

LOD values of 5, 4 and 7 µg/kg (or 4, 3 and 6 µg/L) sulfur were obtained based on $^{32}S^{16}O^+$, $^{33}S^{16}O^+$ and $^{34}S^{16}O^+$, respectively. The fact that the LODs for all three S isotopes were very similar, despite the large variation in their natural isotopic abundance, illustrates that the LOD is limited by the S concentration present as contamination in the ethanol used. When LOD calculations are only based on three times the internal standard deviation (i.e. the standard deviation for repeated measurements of one calibration blank), much lower sulfur LOD values are obtained using $^{32}S^{16}O^+$ (0.5 µg/kg or 0.4 µg/L) and $^{34}S^{16}O^+$ (2 µg/kg or 1.6 µg/L), but not using $^{33}S^{16}O^+$ (6 µg/kg or 5 µg/L). The instrumental LODs calculated in this way reflect the differences in natural isotopic abundance among the S isotopes. Although there are few LOD-values for S in organic matrices reported in the literature, some values are given for comparison. Smith et al. [4], Sulyok et al. [5] and De Wolf et al. [3] published the values of 16 µg/L, 100 µg/L, and 10 µg/L, respectively, via $^{32}S^{16}O^+$. However, a much higher value was obtained using $^{34}S^{16}O^+$ (300 µg/L) [3]. These LOD values were reduced to 1 µg/L and 2 µg/L based on $^{32}S^+$ and $^{34}S^+$, respectively, using HR-ICP-MS.

The strength of the MS/MS technique becomes even more apparent when S is determined in a matrix containing a large amount of Ca or Ti (Table 1). When the instrument is operated in SQ bandpass mode (which is equivalent to a conventional quadrupole ICP-MS with a bandpass cell) it must be setup so the mass range of ions passed to the cell includes both the S^+ precursor ions and the SO^+ product ions. Under these conditions the Ca and Ti ions at *m/z* 48 will also enter the cell and will then be transmitted by Q2 (set to *m/z* 48 for the measurement of $^{32}S^{16}O^+$), such that they are detected at the same nominal mass-to-charge ratio as the $^{32}SO^+$ ions. However, in MS/MS mode, where the Q1 is set to pass only the specific S precursor ion mass (in this case *m/z* 32) with unit mass resolution, the Ca and Ti ions are rejected from the ion beam and therefore do not enter the cell and Q2 (Figure 1). This is demonstrated in Figure 3, where spectral peaks in the mass range of 47.55 to 50.55 amu are shown for (i) an ethanol blank and (ii) an ethanol blank containing 50 µg/L Ca and Ti. For each solution, spectral peaks are shown for both SQ bandpass mode (A) and MS/MS mode (B). It can be seen that in SQ bandpass mode, the signal intensities obtained at *m/z* 48, 49 and 50 clearly increase when Ca and Ti are added to the sample, while in MS/MS mode there are no significant differences between the two spectra.

Furthermore, when comparing the signal intensities obtained for the ethanol blank in SQ bandpass and MS/MS modes (C), the peaks are lower in MS/MS mode and, in contrast to the SQ bandpass spectrum, the MS/MS mode spectrum matches the natural isotopic

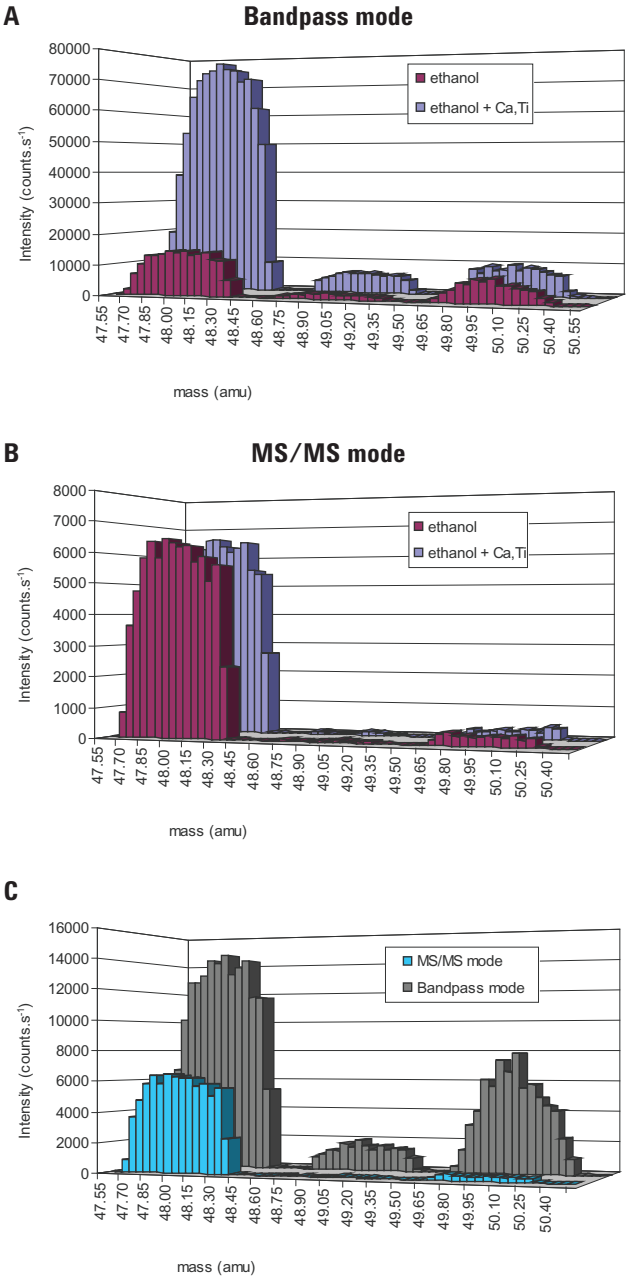

Figure 3. Comparison of the spectra obtained for the mass range from 47.55 to 50.55 amu for (i) an ethanol blank and (ii) an ethanol blank containing Ca and Ti, using the 8800 operated in SQ bandpass mode (A) and MS/MS mode (B). (C) shows the comparison for the ethanol blank solution in both SQ bandpass and MS/MS modes. *Reproduced by permission of The Royal Society of Chemistry (RSC)*

pattern of S. This again proves that the blank values obtained in SQ bandpass mode are derived partly from the S present in the blanks, but also partially arise from interfering species.

Determination of S in NIST SRM 2773 (biodiesel) by isotope dilution ICP-QQQ

An ICP-MS/MS method was developed for the determination of S in the biodiesel reference material NIST SRM 2773. This material is a commercial 100% biodiesel produced from animal-tallow based feedstocks and comes with a certified mass fraction value for S of 7.39±0.39 µg/kg. The only sample pretreatment step that is required for the analysis of biodiesel samples by ICP-MS is simple dilution in ethanol.

As the spike material was enriched in ^{34}S, the $^{32}S/^{34}S$ ratio was used for the ID approach (with ^{32}S and ^{34}S measured at *m/z* 48 ($^{32}S^{16}O^{+}$) and *m/z* 50 ($^{34}S^{16}O^{+}$), respectively). All raw signal intensities were blank-corrected with those obtained for an ethanol blank. No mass bias correction was performed because all isotope ratios were determined experimentally and mass discrimination can be assumed to be constant. Three different blends of SRM sample + spike and two blends of ethanol blank + spike were analyzed by means of ID-ICP-MS/MS. The average blank value was calculated to be 0.011 µg/g and was subtracted from the results obtained. The results from the S determination by ID-ICP-MS/MS are summarized in Table 3. The results obtained for each of the three SRM samples agree well with the certified value within the experimental uncertainty. Furthermore, an evaluation of the 95% confidence interval shows that the method delivers results that are not only accurate, but also reproducible.

Table 3. Results of S determination in NIST SRM 2773 (biodiesel) by isotope dilution (ID-)ICP-MS/MS

Sample	Concentration (µg/g)	Certified value (µg/g)
SRM 2773 – 1	7.234	7.39 ± 0.39
SRM 2773 – 2	7.227	7.39 ± 0.39
SRM 2773 – 3	7.231	7.39 ± 0.39
Average	7.231	7.39 ± 0.39
Standard deviation	0.003	
95% Confidence interval	7.231±0.015	

Conclusions

A new method has been developed for the determination of S in organic matrices using an Agilent 8800 ICP-QQQ operating in MS/MS mode. Interferences on S were eliminated by reacting the analyte ions with O_2 and measuring the isotope-specific SO^+ product ions at M + 16 amu. Several authors have reported on the determination of S using conventional quadrupole ICP-MS after converting the S^+ ions into SO^+ ions by means of O_2 as a reaction gas. However, in organic matrices and/or matrices containing Ca, Ti or Cr (that overlap with SO^+), these methods did not provide sufficient accuracy for isotope dilution or isotope ratio analysis.

The 8800 ICP-QQQ is able to provide enhanced interference reduction for both ^{32}S and ^{34}S for isotope dilution purposes by utilizing MS/MS mode. The first quadruple operates as a unit mass filter, selecting the mass of the ions that are passed to the CRC, and rejecting all non-target masses e.g., Ca, Ti or ArC, before they can enter the cell. Controlling the ions that enter the cell means that reactions are consistent and predictable, regardless of the other matrix and analyte elements present in the sample. By mass shifting S^+ + 16 amu by reaction with O_2, and performing indirect measurement of S as the SO^+ product ion, all potential interferences on S^+ are removed or avoided, including O_2, NO, NOH and NOH_2.

The new MS/MS method was successfully applied to the S determination in a biodiesel reference material. Although only biodiesel has been analyzed in this initial study, it may be assumed that a very large variety of samples can be analyzed for their S content in the same way. We plan to develop this method to the use of on-line species-unspecific isotope dilution for quantification of S-containing materials via reverse phase HPLC-ICP-MS.

More information

For a full account of this application see publication: Accurate determination of S in organic matrices using isotope dilution ICP-MS/MS, Lieve Balcaen, Glenn Woods, Martín Resano and Frank Vanhaecke, *J. Anal. At. Spectrom.*, 2013, 28, 33-39

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