

# Fast and accurate absolute-quantification of proteins and antibodies using Isotope Dilution-Triple Quadrupole ICP-MS

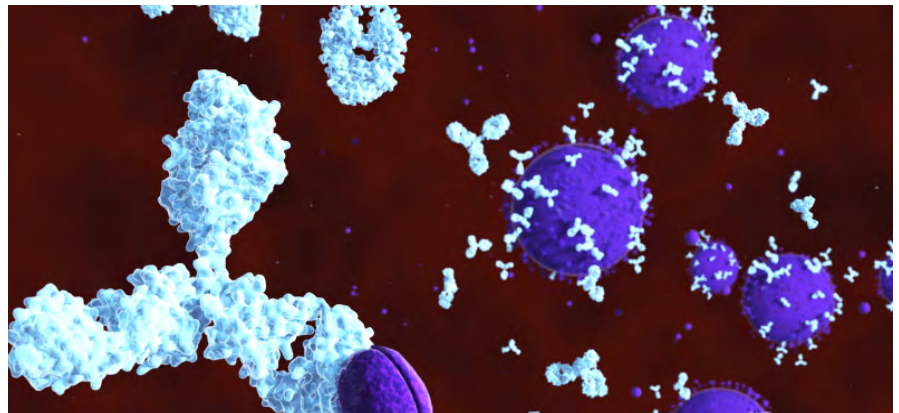
## Application note

### Proteomics

#### Authors

Philippe De Raeve, Juliusz Bianga

Quality Assistance S.A.  
Contract Research Organisation  
Belgium



#### Introduction

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is used increasingly in metallomic studies to analyze metals and metal species and their interactions within biological and ecological systems. It is an elemental detection technique that uses a very high temperature argon plasma as the ion source. As a result, a given concentration of an element provides a signal that is broadly consistent, irrespective of the compound structure or sample matrix. Key to achieving accurate and reliable measurements is the ability to reduce and eliminate polyatomic interferences that otherwise would bias the analytical results. Recent development of a triple quadrupole ICP-MS (ICP-QQQ) dramatically improves the efficiency and reliability of removal of spectral interferences, making it easier to analyze elements with spectral overlaps such as iron, sulfur and selenium. Furthermore, the more effective removal of spectral overlaps allows access to multiple isotopes of many elements, enabling quantification of metalloproteins and peptides through the use of isotope dilution mass spectrometry (IDMS) analysis, which eliminates the requirement for compound specific calibration standards [1]. This absolute quantification technique allows accurate



**Agilent Technologies**

quantification without the need for a reference standard, which is a major benefit of ID-ICP-QQQ for life science research, where many compounds are unknown.

More traditional “absolute” methods of analysis for protein quantification include colorimetry, amino acid (AA) analysis and UV-Vis spectroscopy but each of these techniques has its limitations. Colorimetry, which relies on a color reagent to determine the concentration of a chemical element or chemical, and often requires a Bovine Serum Albumin (BSA) standard, provides poor precision and repeatability. The accuracy and precision achieved with AA analysis is better, although sample preparation involving protein hydrolysis and AA derivatization is time consuming. UV-Vis spectroscopy requires knowledge of the molar extinction coefficient, i.e. prior availability of the reference protein, and spectral interferences often lead to positive bias. In addition, classical relative methods such as enzyme-linked immunosorbent assay (ELISA), LC-MS, LC-UV all require calibration with a standard solution of the protein to be assayed, and such standards are not readily available in most cases. Clearly there is a requirement for a fast, generic method that provides accurate protein quantification without the need for a reference material, in this case a protein standard.

ICP-MS is a trace metals technique, but relatively few proteins contain a significant quantity of a metal as part of their normal composition; examples include hemoglobin (Fe) and superoxide dismutase (Cu/Zn, Mn). However, most proteins (> 95%) do contain sulfur from methionine and cysteine residues [2, 3]. For example, BSA has the formula  $C_{2932}H_{4614}N_{780}O_{898}S_{39}$  and is therefore 1.833% S by weight. Sulfur determination is challenging for conventional quadrupole ICP-MS due to the element’s high ionization potential (10.4 eV) that leads to low sensitivity, and to the occurrence of spectral overlaps from multiple polyatomic ions for all isotopes of S (Table 1), especially in an organic matrix. High resolution ICP-MS (HR-ICP-MS) can be used for the analysis of S, but, apart from the expense of the technique, it suffers a 10-fold reduction in ion transmission efficiency (sensitivity) at the mass resolution required to separate the S isotopes from the spectral interferences.

In this study, we evaluated an Agilent 8800 ICP-QQQ and isotope dilution analysis (ID-ICP-QQQ) of sulfur, for the quantification of NIST BSA 927e standard reference material (SRM), a human immunoglobulin mixture, and a trastuzumab solution. The 8800 ICP-QQQ is ideally suited for IDMS analysis of S as it removes multiple matrix interferences using MS/MS, freeing up the three most abundant S isotopes for accurate measurement.

**Table 1.** S-isotopes and SO<sup>+</sup> product ions with their natural isotopic abundance and their relevant potentially interfering ions

| Isotope                                      | Abundance (%) | Ions causing spectral interference   |
|--|---------------|--|
| <sup>32</sup> S <sup>+</sup>                 | 95.04         | <sup>16</sup> O <sup>16</sup> O <sup>+</sup> , <sup>14</sup> N <sup>18</sup> O <sup>+</sup> , <sup>15</sup> N <sup>16</sup> O <sup>1</sup> H <sup>+</sup>  |
| <sup>33</sup> S <sup>+</sup>                 | 0.75          | <sup>32</sup> S <sup>1</sup> H <sup>+</sup> , <sup>16</sup> O <sup>18</sup> O <sup>1</sup> H <sup>+</sup> , <sup>16</sup> O <sup>17</sup> O <sup>+</sup> , <sup>15</sup> N <sup>18</sup> O <sup>+</sup> , <sup>14</sup> N <sup>18</sup> O <sup>1</sup> H <sup>+</sup>                      |
| <sup>34</sup> S <sup>+</sup>                 | 4.20          | <sup>33</sup> S <sup>1</sup> H <sup>+</sup> , <sup>16</sup> O <sup>18</sup> O <sup>+</sup>   |
| <sup>32</sup> S <sup>16</sup> O <sup>+</sup> | 95.04         | <sup>48</sup> Ti <sup>+</sup> , <sup>48</sup> Ca <sup>+</sup> , <sup>36</sup> Ar <sup>12</sup> C <sup>+</sup>  |
| <sup>33</sup> S <sup>16</sup> O <sup>+</sup> | 0.75          | <sup>48</sup> Ti <sup>+</sup> , <sup>32</sup> S <sup>16</sup> O <sup>1</sup> H <sup>+</sup> , <sup>32</sup> S <sup>17</sup> O <sup>+</sup>   |
| <sup>34</sup> S <sup>16</sup> O <sup>+</sup> | 4.20          | <sup>50</sup> Ti <sup>+</sup> , <sup>50</sup> Cr <sup>+</sup> , <sup>50</sup> V <sup>+</sup> , <sup>38</sup> Ar <sup>12</sup> C <sup>+</sup> , <sup>36</sup> Ar <sup>14</sup> N <sup>+</sup> , <sup>32</sup> S <sup>18</sup> O <sup>+</sup> , <sup>33</sup> S <sup>17</sup> O <sup>+</sup> |

## Experimental

### Instrumentation

All measurements were carried out with an Agilent 8800 Triple Quadrupole ICP-MS fitted with the standard glass concentric nebulizer, x-lens, Peltier-cooled double-pass Scott-type spray chamber, and standard nickel interface cones. A standard one-piece quartz torch with 2.5 mm internal diameter (ID) injector was used throughout. The operating parameters, given in Table 2, are typical for S analysis, except that a relatively low nebulizer gas flow was used in order to attenuate the S sensitivity and ensure that all measurements were carried out in the pulse counting range of the detector.

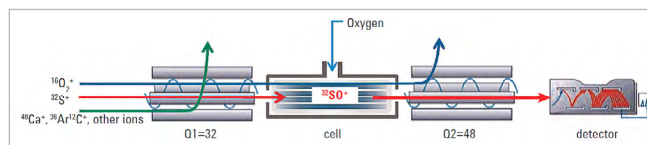
**Table 2.** Operating conditions for the 8800 ICP-QQQ

| Parameter  | Setting                              |
|--|--------------------------------------|
| RF power (W)                                       | 1550                                 |
| Nebulizer type                                     | MicroMist                            |
| Nebulizer gas flow (L/min)                         | 0.25                                 |
| Dilution gas flow (L/min)                          | 0.85                                 |
| O <sub>2</sub> cell gas flow rate (mL/min)         | 0.35                                 |
| <b>Data acquisition</b><br><i>m/z</i> of ion pairs | 32 shifted to 48<br>34 shifted to 50 |

The 8800 ICP-QQQ has a tandem mass spectrometer configuration, with two quadrupole mass filters (Q1 and Q2) situated either side of the Octopole Reaction System (ORS<sup>3</sup>) collision/reaction cell. In MS/MS mode, Q1 operates as a mass filter, allowing only the target analyte mass to enter the cell, and rejecting all other masses. Because plasma, matrix and all non-target analyte ions are excluded by Q1, the reaction processes in the ORS<sup>3</sup> are precisely controlled and consistent, so accurate measurements can be performed even in complex, high matrix samples, while sensitivity is significantly increased. The ORS<sup>3</sup> cell can be pressurized with either a collision gas (to remove polyatomic ions by kinetic energy discrimination (KED), or by collision-induced dissociation (CID)), 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 8800 ICP-QQQ was operated in MS/MS mass-shift mode using oxygen as the cell gas. Q1 was set to  $m/z$  32 ( $^{32}\text{S}^+$ ) and 34 ( $^{34}\text{S}^+$ ) to allow the target precursor ions to pass into the cell and react with  $\text{O}_2$  to form  $\text{SO}^+$  product ions at  $M+16$ . Q2 was set to  $m/z$  48 and 50 to measure the product ions  $^{32}\text{S}^{16}\text{O}^+$  and  $^{34}\text{S}^{16}\text{O}^+$  respectively. The principles of MS/MS mass-shift mode to avoid both on-mass and product ion overlaps are explained in Figure 1, using  $^{32}\text{S}$  as an example.

Operation in MS/MS mode ensures that no isotopic overlaps can occur on the target product ions, since a) only one S isotope mass is permitted to enter the cell at any time, so no other S isotope can contribute to the product ion signal (e.g.  $^{32}\text{S}^{18}\text{O}^+$  cannot overlap on the  $^{34}\text{S}^{16}\text{O}^+$  product ion), and b) only the +  $^{16}\text{O}$  atom addition reaction is being monitored, as a result of the fixed +16 amu mass difference between Q1 and Q2. Again, this means that there is no contribution from reactions with other oxygen isotopes. Both of these unwanted reaction pathways can and do contribute to the signals when  $\text{O}_2$  mass shift mode is used on an ICP-MS system without MS/MS capabilities.



**Figure 1.** Schematic showing the principles of MS/MS mode operation on the 8800 ICP-QQQ. Off-mass ions (those at masses other than  $m/z$  32, i.e.  $^{40}\text{Ca}^+$ ,  $^{36}\text{Ar}^{12}\text{C}^+$  and all other ions) are rejected by Q1 and are prevented from entering the collision/reaction cell. Only  $^{32}\text{S}^+$  and any on-mass interferences (i.e.  $^{16}\text{O}_2^+$ ) enter the cell, and only  $^{32}\text{S}^+$  reacts with the cell gas ( $\text{O}_2$  in this case) to form the product ion  $\text{SO}^+$ . The diatomic oxygen ion ( $^{16}\text{O}_2^+$ ), which enters the cell alongside  $^{32}\text{S}^+$ , does not react with  $\text{O}_2$  at an appreciable rate (kinetically very slow) if at all. Q2 is set to mass 48, so the unreacted  $^{16}\text{O}_2^+$ , which remains at  $m/z$  32, is rejected, leading to an interference-free determination of  $^{32}\text{S}^+$  as  $^{32}\text{S}^{16}\text{O}^+$  at  $m/z$  48.

### Isotope dilution

In a simple isotope dilution method, a sample containing an unknown amount of S, which is primarily composed of the major  $^{32}\text{S}$  isotope (94.93% abundance), is spiked with a known amount of a certified enriched isotopic standard solution containing  $^{34}\text{S}$ . An aliquot of the resulting solution is analyzed and the ratio of  $^{34}\text{S}$  to  $^{32}\text{S}$  is measured. From the measured ratio and the known amount of  $^{34}\text{S}$ , it is possible to calculate the amount of  $^{32}\text{S}$  and therefore the total S concentration (based on natural isotopic abundances) in the original sample. However, as the  $^{34}\text{SO}_4^{2-}$  spiking solution used in this study was prepared by oxidation of a powder of  $^{34}\text{S}$  sulfur, it is impossible to know the exact concentration of  $^{34}\text{S}$  present in the spike. Therefore, a high accuracy technique known as double IDMS was required in this study per Equation 1. The concentration of  $^{34}\text{S}$  in the  $\text{H}_2^{34}\text{SO}_4$  solution is determined by reverse IDMS using a National Institute of Standards and Technology (NIST) certified solution of  $\text{SO}_4$  with natural sulfur isotopic abundance as the reference standard. Double IDMS also compensates for any sensitivity differences observed in ICP-MS between  $^{32}\text{S}$  and  $^{34}\text{S}$  i.e. mass bias correction is automatically applied. Other studies using IDMS with the 8800 ICP-QQQ can be found in references 4 and 5.

**Equation 1.** The double IDMS equation used in this study

$$w_x = w_z \cdot \frac{m_y \cdot m_z}{m_x \cdot m_{y'}} \cdot \frac{R_y - R_{xy}}{R_{xy} - R_x} \cdot \frac{R_{zy} - R_z}{R_y - R_{zy}}$$

- x refers to the sample
- y and y' refer to the  $^{34}\text{SO}_4$  spiking solution
- z refers to the NIST  $\text{SO}_4$  standard solution
- $w_x$  is the sulfur mass fraction ( $\mu\text{g/g}$ ) in the sample
- $w_z$  is the sulfur mass fraction ( $\mu\text{g/g}$ ) in the NIST  $\text{SO}_4$  standard solution
- $m_i$  is the mass of sample, standard, or spiking solution
- $R_i$  is the  $^{34}\text{S}/^{32}\text{S}$  ratio measured by ICP-QQQ in the unspiked and spiked solutions
- $R_x$  is the  $^{34}\text{S}/^{32}\text{S}$  ratio measured in the sample solution
- $R_y$  is the  $^{34}\text{S}/^{32}\text{S}$  ratio measured in the spiking solution
- $R_z$  is the  $^{34}\text{S}/^{32}\text{S}$  ratio measured in the  $\text{SO}_4$  standard solution
- $m_x$  is spiked with  $m_y$  and ratio  $R_{xy}$  is measured
- $m_z$  is spiked with  $m_{y'}$  and ratio  $R_{zy}$  is measured

### Sample preparation

Three solutions were prepared for microwave digestion. First, an amount of sample (BSA, trastuzumab solution or human immunoglobulin mixture) estimated to contain approximately 50  $\mu\text{g}$  sulfur was weighed into a disposable glass tube. 50  $\mu\text{g}$  of  $^{34}\text{S}$  (as  $\text{H}_2^{34}\text{SO}_4$ ) was added, followed by 2 mL of 69%  $\text{HNO}_3$ , 0.5 mL of 37%  $\text{HCl}$ , and 1 mL of 30 %  $\text{H}_2\text{O}_2$ . Once the microwave digestion program had finished, the digest was transferred and diluted to 50 mL with  $\text{H}_2\text{O}$ . The concentration of S in solution was about 1 ppm.

Secondly, a standard was prepared for the double IDMS measurements. 50  $\mu\text{g}$  of 1000 mg/L sulfur ICP-MS standard (natural isotopic abundance) was weighed into another glass tube, and then the same procedure set out above was followed.

**Table 3.** Analysis of different sample digests of NIST 927e BSA SRM solution

| Actual weight of NIST 927e BSA solution (mg) | Nominal mass of BSA (mg, approx.) | Sulfur ( $\mu\text{g}$ , approx.) | Sulfur ( $\mu\text{g/g}$ , approx.) | Average recovery (%) | RSD(%) | n |
|--|-----------------------------------|-----------------------------------|-------------------------------------|----------------------|--------|---|
| 26.05; 24.67; 23.37                          | 1.6                               | 30                                | 0.6                                 | 101.29               | 0.34   | 3 |
| 30.93; 32.09; 31.34                          | 2.1                               | 40                                | 0.8                                 | 101.30               | 0.21   | 3 |
| 40.33; 40.19; 39.72; 39.60; 40.01; 40.30     | 2.6                               | 50                                | 1.0                                 | 101.26               | 0.22   | 6 |
| 47.71; 48.30; 48.12                          | 3.2                               | 60                                | 1.2                                 | 101.17               | 0.13   | 3 |
| 55.56; 55.72; 56.03                          | 3.7                               | 70                                | 1.4                                 | 101.17               | 0.12   | 3 |

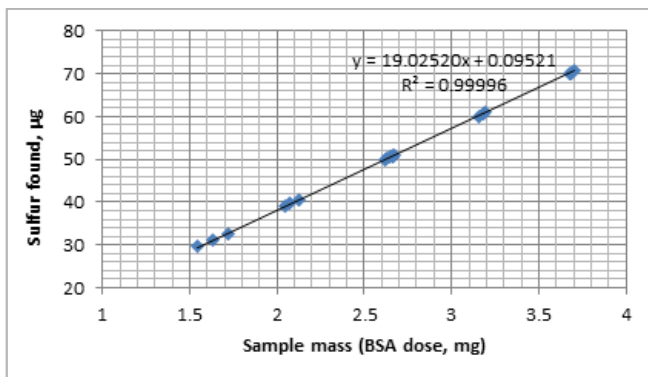
Finally, a sample blank was prepared to check whether the drug product was contaminated by non-proteinaceous sulfur. An aliquot of the sample solution was filtered through a 3 KDa molecular weight cut-off membrane. Then an amount of the filtrate equivalent to the sample weight was weighed into a disposable glass tube and the same sample prep procedure as outlined for the sample was followed.

## Results and discussion

### Method verification

BSA was selected as a model compound to test the method. The standard reference material (SRM) used was NIST 927e Bovine Serum Albumin (7% solution) that contains  $67.38 \pm 1.38 \text{ g/L}$  ( $\pm 2.0 \%$ ) of S.

As part of the verification, several separate weighings (per the levels shown in Table 3) of the BSA SRM were digested and measured. Each solution was analyzed using the ICP-QQQ and the sulfur concentration was determined using the IDMS method described. The results shown in Table 3 show excellent accuracy and precision, with recoveries of nearly 100% and RSD less than 0.35%. A plot of the data (Figure 2) shows the excellent linearity ( $R = 0.99998$ ) of the analytical method.



**Figure 2.** Data plot showing linearity of the method. The individual points represent the different sample preparations at each level, containing slightly different doses of BSA

### Sample dilution

In order to test the effect of sample dilution on the digestion efficiency, variable volumes of water (0.9 to 5 mL) were added to constant amounts of BSA solution (40 mg) and of  $^{34}\text{SO}_4$ , and constant volumes of digestion reagents (2 mL  $\text{HNO}_3$ , 0.5 mL  $\text{HCl}$ , 1 mL  $\text{H}_2\text{O}_2$ ), before digestion. The sulfur concentration recovery results given in Table 4 also show excellent accuracy and precision, with average recoveries at 100% and RSD less than 0.35%.

**Table 4.** Effects of sample dilution on the method

| BSA solution (mg) | $\text{H}_2\text{O}$ (mL) | Mean recovery (%) | %RSD (n = 3) |
|-------------------|---------------------------|-------------------|--------------|
| 40                | 0.9                       | 100.51            | 0.32         |
| 40                | 1.4                       | 100.92            | 0.34         |
| 40                | 2.0                       | 100.59            | 0.18         |
| 40                | 2.5                       | 100.64            | 0.07         |
| 40                | 3                         | 100.32            | 0.14         |
| 40                | 4                         | 100.89            | 0.15         |
| 40                | 5                         | 100.77            | 0.18         |

### Matrix effects

Matrix effects were investigated as drug products containing proteins or antibodies are usually presented in a formulation buffer which may contain parts or a combination of the following: 0.9% NaCl, Phosphate-Buffered Saline (PBS), 10% sucrose, 0.1% polysorbate80, 15 mg/mL glycine, 15 mg/mL histidine, and in the worst case scenario, a mixture of all of the above. Aligning with the previous tests, the same amount of BSA was spiked with the different

formulation ingredients as shown in Table 5, then digested and analyzed using the 8800 ICP-QQQ. The results (Table 5) show that there were no apparent matrix effects, as indicated by the mean sulfur recoveries of 100% and RSD less than 0.65%.

**Table 5.** Matrix effect test

| Matrix             | Mean recovery (%) | % RSD (n = 3) |
|--------------------|-------------------|---------------|
| 0.9% NaCl          | 100.27            | 0.05          |
| PBS                | 100.24            | 0.36          |
| 10% sucrose        | 100.16            | 0.61          |
| 0.1% PS-80         | 100.26            | 0.57          |
| 15 mg/mL glycine   | 99.61             | 0.37          |
| 15 mg/mL histidine | 100.76            | 0.23          |
| All of above       | 100.33            | 0.21          |

### Non-protein sulfur: Sulfate

A NIST BSA 927e solution was spiked with  $\text{SO}_4$  (~ 5% of BSA sulfur). Part of this solution was filtered through a 3 kDa molecular weight cut-off membrane to separate any possible low molecular weight sulfur-containing contaminants. The corrected BSA recovery in the spiked solution was 99.6 % (Table 6).

**Table 6.** Recovery of sulfate

| Sample                   | Sulfur measured ( $\mu\text{g/g}$ ) |
|--------------------------|-------------------------------------|
| A: unspiked BSA solution | 36.50                               |
| B: spiked BSA solution   | 38.81                               |
| C: filtered solution     | 2.44                                |
| B-C (calculated)         | 36.37                               |

### Non-protein sulfur: Methionine

Methionine was added to solutions of NIST BSA 927e and human immunoglobulin (IgG). The solutions were then filtered and methionine was measured in the original solutions and in the filtrate. The results given in Table 7 show excellent recovery for methionine in the filtrate at 99.1 and 99.5% respectively. This clearly indicates that any possible contamination of low molecular weight sulfur-containing molecules can be selectively removed through filtration.

**Table 7.** Recovery of methionine

|                             | NIST BSA solution | IgG solution |
|-----------------------------|-------------------|--------------|
| Methionine added (mM)       | 2.05              | 2.05         |
| Methionine in filtrate (mM) | 2.03              | 2.04         |
| % Recovery                  | 99.1              | 99.5         |

### Stability of solutions

Standard, sample and blank solutions, after digestion and dilution, were stored in clean polypropylene tubes and were tested for stability on 7 consecutive days. The  $^{34}\text{S}/^{32}\text{S}$  ratios measured on the different days showed minimal change (1% in 7 days), which means that all solutions are stable for at least 7 days.

### Intermediate precision

To test the ruggedness of the method an external precision test was performed over separate days by separate analysts. On day one, an analyst (Analyst 1) carried out 6 independent determinations (different weighings) of the same solutions of NIST BSA 927e (67.38 mg/mL) and of human IgG. On a separate day, a second analyst (Analyst 2) also carried out the same 6 separate preparations and determinations. The results shown in Table 8 demonstrate excellent external precision of 0.6% and 0.7% RSD for the 12 preparations of BSA and IgG respectively.

**Table 8.** Precision of method

| Sample           | Analyst 1 |               | Analyst 2 |               | Total RSD (%)<br>n = 12 |
|------------------|-----------|---------------|-----------|---------------|-------------------------|
|                  | Average   | % RSD (n = 6) | Average   | % RSD (n = 6) |                         |
| NIST BSA (mg/mL) | 67.28     | 0.41          | 67.50     | 0.52          | 0.6                     |
| IgG (mgS/g)      | 8.39      | 0.13          | 8.33      | 0.19          | 0.7                     |

### Trastuzumab solution

Trastuzumab is a monoclonal antibody that interferes with the human epidermal growth factor receptor 2 (HER2/neu receptor). It is widely used to control the growth of cancer cells that produce too much of the HER2 protein seen in some forms of breast cancers and stomach cancers. The measured concentration for sulfur in the trastuzumab solution was in good agreement with the expected value as shown in Table 9.

**Table 9.** Trastuzumab solution

|        | Expected concentration | Measured concentration | %RSD, (n=3) | %Recovery |
|--------|------------------------|------------------------|-------------|-----------|
| Sulfur | 21 mg/mL               | 20.53 mg/g             | 0.02        | 97.8      |

## Conclusions

The Agilent 8800 ICP-QQQ with MS/MS mode provides high sensitivity and effective interference reduction for multiple sulfur isotopes, allowing accurate determination of sulfur in biological molecules such as antibodies using IDMS. The high-accuracy IDMS method based on the determination of  $^{34}\text{S}/^{32}\text{S}$  ratios was achieved by reacting the analyte ions with  $\text{O}_2$  and measuring the isotope-specific  $\text{SO}^+$  product ions at  $M + 16$  amu. The method, which requires only very common reagents and standards, was found to provide:

- High sensitivity, so only requires a low concentration of the analyte protein
- Good reproducibility: RSD < 1 %
- Excellent accuracy: < 2 % bias
- Matrix tolerance: results were consistent in the presence of various types and combination of formulation buffer
- Wide applicability: the same method can be used for the analysis of any sulfur-containing protein or peptide and possibly any other small sulfur-containing molecules
- Fast: all results were obtained within half a day of lab work in routine use

The ID-ICP-QQQ method clearly demonstrates the capability of accurate and precise quantitative analysis of biological molecules such as pure proteins and antibodies, without the need for compound-specific calibration standards.

## Acknowledgement

Quality Assistance S.A. is a leading European contract research organization providing the pharmaceutical industry with all the analytical services required by EMA and FDA regulations for the development and marketing of innovative human medicinal products. Find out more about the company at [www.quality-assistance.com](http://www.quality-assistance.com) or contact Philippe De Raeve at [philippe.deraeve@quality-assistance.be](mailto:philippe.deraeve@quality-assistance.be)



## References

1. Yasumi Anan, Yoshiko Hatakeyama, Maki Tokumoto and Yasumitsu Ogra, Analysis of selenoproteins in rat serum by Triple Quadrupole ICP-MS, Agilent publication 5991-2750EN (2013)
2. Wind M, Wegener A, Eisenmenger A, Keller R, Lehmann WD, (2003) *Angewandte Chemie International* 42:3425-3427
3. Zhang H, Yan W, Aebersold R, (2004) *Current Opinions Chem. Biol.* 8 (1) 66-75
4. Naoki Sugiyama, The accurate measurement of selenium in twelve diverse reference materials using on-line isotope dilution with the 8800 Triple Quadrupole ICP-MS in MS/MS mode. Agilent publication 5991-0259EN (2012)
5. Lieve Balcaen, Frank Vanhaecke, Glenn Woods, Martín Resano, Accurate determination of sulfur in biodiesel using Isotope Dilution-Triple Quadrupole ICP-MS (ID-ICP-QQQ), Agilent publication 5991-2337EN (2013)

**[www.agilent.com/chem](http://www.agilent.com/chem)**

For research use only. Not for use in diagnostic procedures.

Information subject to change without notice.

© Agilent Technologies, Inc. 2015

Published September 7 2015

Publication number: 5991-6118EN

