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A comparison of several published and in-house methods for quantitation of selenomethionine (SeMet) in yeast was undertaken using species specific isotope dilution (ID) with a 78Se enriched SeMet. An in-house method was based on digestion of samples by refluxing for 16 h with 4 M methanesulfonic acid, derivatization of SeMet with cyanogen bromide and extraction into chloroform for determination by GC ICP-MS. Concentrations of SeMet were obtained in yeast based on measurement of 78Se/74Se and 82Se/74Se ratios, respectively, in agreement with a value of 3417 ± 27 µg g⁻¹ (one standard deviation, n = 6) obtained using ID GC-MS detection based on the ratio of 106/100 of SeCN⁻ ion. The SeMet accounts for 67% of the total Se in the sample. A method detection limit (three standard deviations) of 0.9 µg g⁻¹ was estimated for SeMet based on a 0.25 g subsample. Significantly lower concentrations of 2220 ± 7 and 2215 ± 9 µg g⁻¹ (one standard deviation, n = 4) with RSDs of 0.30 and 0.41% were obtained by ID GC ICP-MS using 78Se/74Se and 82Se/74Se ratios, respectively, following digestion with 2% SnCl₂ in 0.1 M HCl and reaction with CNBr to form volatile CH₃SeCN. Similar SeMet concentrations of 3415 ± 200 and 3447 ± 198 µg g⁻¹ (one standard deviation, n = 6) and significantly degraded precisions of 5.86 and 5.74%, respectively, were obtained in yeast using 78Se/74Se and 82Se/74Se ratios, respectively, following digestion with 4 M methanesulfonic acid and derivatization with methyl chloroformate.

Introduction

Interest in the speciation of Se in foods and supplements has increased dramatically as a result of the numerous health benefits bestowed by Se, including protection of cells against the effects of free radicals, the normal functioning of the immune system and thyroid gland1–3 as well as protection against various forms of cancers, including those of the lung, colorectal and prostate.4–7 Since common foods in some regions of the world have very low selenium content, consumption of Se enriched yeast supplements is popular. Selenomethionine (SeMet) is generally the dominant Se species in such supplements and is less toxic than inorganic selenium, and possesses higher bioavailability.8–11

Although the most frequently used procedures for the extraction of SeMet from yeast are based on enzymatic hydrolysis8–11 with protease, proteinase K or a mixture of proteolytic enzymes, methanesulfonic acid has also been found efficient for this purpose.12,13 As has cyanogen bromide in combination with use of SnCl₂ in 0.1 M HCl pretreatment.14 However, incomplete degradation of proteins with cyanogen bromide has also been reported,15 which may bias analytical results.

Gas chromatography (GC) and high performance liquid chromatography (HPLC) are currently the most commonly used separation techniques for determination of SeMet in combination with detection by flame photometry,16 atomic emission,17 mass spectrometry12,14,17–19 and inductively coupled plasma mass spectrometry (ICP-MS).8,11,20–24 In particular, ICP-MS has been used as a sensitive and selective detector for speciation analysis for the past decade. In addition to its high sensitivity, large dynamic range and multi-element capability, isotope dilution (ID) calibration can also be implemented.

Isotope dilution mass spectrometry (ID-MS) has been widely employed for trace element analysis in a variety of sample matrices to yield highest accuracy and precision.25 Applications of ID-MS to species specific determinations have generally been limited due to a lack of commercially available species specific spikes.26 Despite the advantages offered by species specific isotope dilution methodology, very few applications for the determination of SeMet have been published.8,12,14 Moreover, the accuracy of the data cannot be directly verified due to an absence of reference materials certified for SeMet content. The National Research Council Canada has recently embarked on a project to address the need for a yeast Certified Reference Material (CRM) for validation of measurements of methionine (Met), SeMet and total Se. As certification requires good agreement of results generated by at least two independent methods of analysis, development of independent methodologies possessing the highest possible accuracy and precision is required. The objective of this study was to develop alternative GC ICP-MS detection methods to complement a recently reported approach based on isotope dilution GC-MS detection of Met and SeMet using 13C enriched spikes as well as a 78Se enriched SeMet spike.12

Experimental

Instrumentation

A ThermoFinnigan Element2 (Bremen, Germany) sector field inductively coupled plasma mass spectrometer (ICP-MS) was used, equipped with a Scott-type double pass glass spray chamber, and a PFA self aspirating nebulizer (Elemental Scientific, Omaha, NE, USA). A plug-in quartz torch with a sapphire injector and a Ag guard electrode were used. Optimization of
the Element2 was performed as recommended by the manufacturer. Detector dead time was determined following the procedure of Nelms et al.\textsuperscript{27} (method 2); a value of 18 ns was derived from a plot of the measured \(^{238}\text{U}/^{234}\text{U}\) ratios vs. U standard solutions of 0.5, 1.0 and 2.5 ng ml\(^{-1}\) at slope of zero.

A Varian 3400 gas chromatograph (Varian Canada Inc., Georgetown, Ontario, Canada) equipped with a MXT-5 metal column (5% diphenyl, 95% poly(dimethylsiloxane), 30 m x 0.28 mm i.d. with a 0.5 \(\mu\)m film thickness) was used for separation of Se species. The GC was coupled to the ICP-MS instrument using a home-made interface and a transfer line similar to that described previously\textsuperscript{29–32} The latter was maintained at 180 °C.

For comparison purposes, a Hewlett Packard HP 6890, 5973 GC-MS (Agilent Technologies Canada Inc., Mississauga, Ontario, Canada), fitted with a DB-5MS column (Iso-Mass Scientific Inc., Calgary Alberta, Canada) was also used for the determination of SeMet in the yeast extract.

A 10 \(\mu\)l liquid sampling syringe (Hamilton Company, Nevada, USA) was used for the injection of chloroform extracts of samples.

Reagents and solutions

Hydrochloric acid was purified in-house prior to use by sub-boiling distillation of reagent grade feedstock in a quartz still. Environmental grade aqueous ammonia was purchased from Anachemia Science (Montreal, Quebec, Canada). OmniSolv \(\circ\) methanol (glass-distilled) and chloroform were purchased from EM Science (Gibbstown, NJ, USA). High purity de-ionized water (DIW) was obtained from NuoPure mixed bed ion exchange systems fed with reverse osmosis domestic feed water (Barnstead/Thermolyne Corp., Iowa, USA). Methanesulfonic acid (98% purity), methyl chloroformate (99% purity) and 3 M (Barnstead/Thermolyne Corp., Iowa, USA) was obtained from Sigma Aldrich Canada (Oakville, Ontario, Canada).

Natural abundance high purity SeMet was purchased from Sigma Aldrich Canada (Oakville, Ontario, Canada). Individual stock solutions of 1990.3 and 2185.6 \(\mu\)g ml\(^{-1}\) were gravimetrically prepared in 1% HCl and kept refrigerated until used.

A \(^{74}\text{Se}\) SeMet (\(^{74}\text{SeMet}\)) was provided by the Food Composition Laboratory (USDA, Beltsville, MD, USA) and used to prepare a stock solution of approximately 450 \(\mu\)g ml\(^{-1}\) in 1% HCl. The concentration of the \(^{74}\text{SeMet}\) spike was verified by reverse spike isotope dilution using the natural abundance SeMet standards.

Lalmin Se yeast was obtained from Rosell-Lallemand (Montreal, Canada) and used as a test sample for method development.

Safety considerations

Methyl chloroformate is a highly toxic and flammable substance. Cyanogen bromide is a highly toxic substance. Material Safety Data Sheets must be consulted and essential safety precautions employed for all manipulations including waste disposal and decontamination.

Sample preparation for final quantitation of SeMet in yeast using 4 M methanesulfonic acid digestion and CNBr derivatization

The extraction procedure used in this study followed that described previously\textsuperscript{12} and is similar to that reported by Wrobel et al.\textsuperscript{13} Analyte in the extract was subsequently derivatized to CH\(_3\)SeCN using CNBr, as reported by Wolf et al.\textsuperscript{14} Three sample blanks and six sub-samples of yeast were prepared at the same time. In brief, 0.25 g of yeast was spiked with 0.600 ml of 450 \(\mu\)g ml\(^{-1}\) \(^{74}\text{Se}\) enriched SeMet. After addition of 17.4 ml of DIW and 6 ml of methanesulfonic acid (resulting in a concentration of 4 M for methanesulfonic acid and 24 ml volume in total), the contents were refluxed on a hot plate for 16 h. When cool, the solutions were centrifuged at 2000 rpm for 10 min and a 1 ml volume of the supernatant was transferred to a 10 ml glass vial. After 0.475 ml aqueous ammonia, 1.48 ml of 4% SnCl\(_2\) in 0.2 M HCl (resulting a 2% SnCl\(_2\) in 0.1 M HCl solution) and 0.50 ml of 3 M CNBr in CHCl\(_3\) were added, the vials were capped and shaken on a vortex for 5 min and maintained at 37 °C for 24 h. Derivatized analyte was extracted into 1 ml of chloroform for GC ICP-MS analysis.

Six reverse spike isotope dilution calibration samples were prepared to quantify the concentration of the \(^{74}\text{Se}\) enriched SeMet spike. A 0.200 ml volume of \(^{74}\text{Se}\) Se enriched SeMet spike solution and 0.100 ml of 1990.3 \(\mu\)g ml\(^{-1}\) (or 2185.6 \(\mu\)g ml\(^{-1}\)) natural abundance SeMet solution were accurately pipetted into a vial and diluted with 4 M methanesulfonic to 10 ml. Subsequent derivatization of the analyte was described as above using 1 ml of solution.

The digested yeast samples and the six reverse spike ID calibration samples were analyzed by GC ICP-MS on the same day. Following injection of the sample onto the GC column, data acquisition on the Element2 was manually triggered. Isotopes of \(^{74}\text{Se}, ^{78}\text{Se}\) and \(^{82}\text{Se}\) were monitored during every run. Mass bias correction was implemented based on the theoretical natural abundance ratio of an isotope pair divided by the mean value of the isotope pair measured in a natural abundance SeMet standard. At the end of the chromatographic run, the acquired raw data were transferred to an off-line computer for further processing using a in-house software to yield both background corrected peak height and peak area information. In this work, only peak areas were used to generate \(^{78}\text{Se}/^{74}\text{Se}\) or \(^{82}\text{Se}/^{74}\text{Se}\) ratios, from which the analyte concentrations in the yeast samples were calculated. The GC ICP-MS operating conditions are summarized in Table 1. For comparison purpose, these samples were also analyzed by GC-MS (Table 2).

Sample preparation using 4 M methanesulfonic acid digestion and methyl chloroformate derivatization

Sample preparation has been described previously.\textsuperscript{12} Three sample blanks and six sub-samples of yeast were prepared. Methanesulfonic acid digestion was as described above. After...
Sample preparation using CNBr with 2% SnCl₂ in 0.1 M HCl pretreatment

The sample preparation procedure followed that reported by Woll et al. Three sample blanks and four sub-samples of yeast were prepared. Sub-samples of 0.10 g yeast were spiked with 0.301 ml of 450 µg ml⁻¹ ⁷⁶Se enriched SeMet. After addition of 2 ml of 2% SnCl₂ in 0.1 M HCl, the vials were vortex mixed for 5 min and then heated in a water bath at 37 °C for 24 h. After addition of 0.50 ml of 3 M CNBr in CHCl₃, the vials were then maintained at 37 °C for a further 24 h to cleave the CH₃Se group and form volatile CH₃SeCN, which was then extracted into 1 ml of chloroform and diluted 10-fold in chloroform prior to analysis by GC ICP-MS.

Results and discussion

Optimization of the GC-ICP-MS system

Optimization of the ICP-MS system was undertaken as recommended by the manufacturer using liquid sample introduction of 1 ng ml⁻¹ multi-element standard to achieve stable and high sensitivities for Li, In and U. Mass calibration was only performed once per week because of the good stability of the Element2. The plasma was then extinguished and the spray chamber and nebulizer assembly replaced with the heated GC transfer line and its ball joint adapter. Optimization of GC ICP-MS system was similar to that described previously and is briefly summarized here.

The distance between the injector tip and the end of the transfer line had no significant effect on the resulting sensitivity over the range 0 to 12 mm; consequently, a 5 mm distance was used. Similarly, the length of the transfer line had little effect on the analyte peak shape, due to a short residence time. Therefore, for flexibility and ease of handling of the GC ICP-MS, a 150 cm long heated PTFE line was used in this study.

The optimum Ar carrier gas flow rate is related to that of the He effluent from the GC column as it was introduced through a side arm of the interface and was optimized for a He pressure of 40 psi. The effect of Ar carrier gas flow in the range of 0.2 to 0.45 l min⁻¹ was studied by examining response following injections of 1 µl of 100 µg ml⁻¹ SeMet standard solution in chloroform. An optimum Ar carrier gas flow rate of 0.275 l min⁻¹ was found and used for all subsequent studies.

Results for 4 M methanesulfonic acid digestion and methyl chloroformate derivatization

An isotope dilution GC-MS method using either the ¹³C enriched or ⁷⁶Se enriched SeMet spike for the determination of SeMet in yeast based on a 4 M methanesulfonic acid reflux digestion and methyl chloroformate derivatization was previously reported. Yeast extracts prepared using this sample preparation approach were tested for the determination of SeMet using GC ICP-MS. Optimization of GC ICP-MS for methyl chloroformate derivatized SeMet [CH₃SeCH₂CH₂CH (COOCH₃)NHCOOCH₃] was performed in a similar manner to that described earlier for CH₃SeCN. Optimum conditions were: injector and interface temperature both at 280 °C, transfer line temperature at 260 °C and column temperature rising from 120 °C (1 min) to 260 °C (2 min) at 20 °C min⁻¹. As shown in Fig. 2, some decomposition of the derivatized SeMet was observed, as indicated by the peak at 460 seconds (⁷⁶Se from spiked sample), similar to that reported by Haberhauer-Troyer et al. Additionally, significant tailing of the derivatized SeMet peak and much lower sensitivity were also evident despite optimized conditions. This may arise as a result of inefficient transport of CH₃SeCH₂CH₂CH (COOCH₃)NHCOOCH₃ to the ICP-MS because of its adsorption on the transfer line.

Isotope dilution and reverse isotope dilution were used for quantitation of SeMet content in the yeast sample following 4 M methanesulfonic acid digestion and methyl chloroformate derivatization using GC ICP-MS. The following equation was
HCl. Since CH normalized to a sample weight of 0.25 g.

\[ C = C_s - m_x \frac{v_y B_x}{B_z} \left( n A_{xz} \right) \]

where \( C \) is the blank corrected SeMet concentration (\( \mu g \) g\(^{-1} \)) in the yeast; \( C_s \) is the concentration of primary assay SeMet standard (\( \mu g \) ml\(^{-1} \)); \( v_x \) is the volume (ml) of spike used to prepare the blend solution of sample and spike; \( v_y \) is the volume (ml) of primary assay SeMet standard solution; \( A_z \) is the abundance of the reference isotope in the spike; \( B_z \) is the abundance of the reference isotope in the yeast; \( A_x \) is the abundance of the reference isotope in the sample or primary assay standard; \( m_x \) is the mass (g) of yeast sample used; \( m_y \) is the mass (g) of spike used to prepare the blend solution of spike and primary assay SeMet standard solution; \( R_m \) is the measured reference/spike isotope ratio (mass bias corrected) in the blend solution of sample and spike; \( R_n \) is the measured reference/spike isotope ratio (mass bias corrected) in the blend solution of spike and natural abundance SeMet standard; \( C_0 \) is the analyte concentration in the blank (\( \mu g \) g\(^{-1} \)) normalized to a sample weight of 0.25 g.

SeMet concentrations of 3415 ± 200 and 3447 ± 198 \( \mu g \) g\(^{-1} \) (one standard deviation, \( n = 6 \)) with RSDs of 5.86% and 5.74%, respectively, were obtained based on \(^7\)Se/\(^3\)Se and \(^8\)Se/\(^3\)Se ratios, respectively. Although these results are not significantly different from the 3418 ± 8 \( \mu g \) g\(^{-1} \) (one standard deviation, \( n = 6 \), RSD of 0.24%) reported previously using GC-MS inspection,\(^{12} \) the precision of determination obtained using GC ICP-MS is 24-fold inferior than that obtained using GC-MS. This is due to poor signal-to-noise ratio and poor peak shape obtained with GC ICP-MS using this home-made interface and transfer line, as evident in Fig. 2.

Results for digestion with 2% SnCl\(_2\) in 0.1 M HCl and CNBr derivatization

Wolf et al.\(^{14} \) recently reported on an ID GC-MS method based on cleavage of the CH\(_3\)Se group with CNBr and formation of volatile CHSeCN following digestion with 2% SnCl\(_2\) in 0.1 M HCl. Since CHSeCN is much more volatile than the methyl chloroformate derivatized SeMet, higher sensitivity and symmetric peaks are expected as a result of its more efficient transport to the ICP.

This sample preparation procedure\(^{2,4} \) was thus adopted for determination of SeMet in yeast using ID GC ICP-MS detection. Indeed, symmetric peaks and high sensitivity were obtained in a yeast extract, similar to results shown in Fig. 1. SeMet concentrations of 2220 ± 7 and 2215 ± 9 \( \mu g \) g\(^{-1} \) (one standard deviation, \( n = 4 \)) with RSDs of 0.30 and 0.41%, respectively, were obtained based on \(^7\)Se/\(^3\)Se and \(^8\)Se/\(^3\)Se ratios, respectively. A concentration of 2209 ± 46 \( \mu g \) g\(^{-1} \) (one standard deviation, \( n = 3 \)) was obtained by an independent laboratory of Food Composition Laboratory (FCL) using the same sample preparation protocol but with quantitation by ID GC-MS, confirming the above results.

These results are significantly lower (35%) than those obtained using a sample preparation procedure based on 4 M methanesulfonic acid digestion and methyl chloroformate derivatization, irrespective of quantitation by GC-MS\(^{5,7} \) or GC ICP-MS. Although incomplete peptide cleavage at Met residues with CNBr has been reported,\(^{31-33} \) it should be noted that the cleavage of CH\(_3\)S from Met by CNBr to form CH\(_3\)SeCN (or, similarly, of CH\(_3\)Se to form CH\(_3\)SeCN in the case of SeMet), which occurs during a preliminary step in the cleavage reaction, occurs even when peptide bond cleavage does not go to completion.\(^{31-33} \) Under the relatively mild digestion conditions used (0.1 M HCl, 37 °C), there may be some portion of the total endogenous SeMet that is inaccessible to CNBr, e.g., due to incomplete breakdown of cell structures, which may bias analytical results.

Results for final quantitation of SeMet in yeast using GC ICP-MS

Efficient release of SeMet from yeast can be achieved without degradation of analyte\(^{12} \) using a 16 h 4 M methanesulfonic acid reflux digestion. In combination with CNBr to derivatize the analyte to CH\(_3\)SeCN, drawbacks associated with poor transport efficiency of CH\(_3\)SeCH\(_2\)CH\(_2\)CH(COOCH\(_3\))NHCOOCH\(_3\) leading to poor sensitivity and peak tailing are overcome and the low extraction efficiency encountered with use of CNBr with 2% SnCl\(_2\) in 0.1 M HCl pretreatment avoided. As shown in Fig. 1, good sensitivity and peak profile for SeMet was obtained using this approach by GC ICP-MS and the entire chromatographic run was accomplished in 7 min.

ID-MS is capable of compensating for any loss of analyte during subsequent sample preparation (e.g., during derivatization), suppression of ion intensities by concomitant elements present in the sample matrix and for instrument drift, providing isotopic equilibration is achieved between the added spike and the endogenous analyte in the sample. In addition, an interference free pair of isotopes must be available for ratio measurements, care must be taken to avoid any contamination during the process, and an optimum measurement procedure must be used to achieve accurate ratio measurements.

In practice, validation of the achievement of equilibration of the enriched spike and the endogenous analyte in the sample is not easy. In an effort to ensure equilibration, a series of experiments was conducted. No significant difference in SeMet concentrations in yeast was obtained using either 8 or 16 h of digestion in 4 M methanesulfonic acid. A previous study\(^{12} \) also concluded that no degradation of analyte occurs during 16 h of digestion. Furthermore, \(^7\)Se/\(^3\)Se and \(^8\)Se/\(^3\)Se ratios in a spiked yeast extract, which had been stored in the dark at 4 °C following the initial measurement, were re-measured four weeks later. No significant difference was found between the two sets of results, suggesting that equilibration between the added spike and the endogenous SeMet in the sample is most likely achieved prior to the derivatization reaction.

Mass bias corrected ratios of 26.62 ± 0.38 and 9.77 ± 0.12 (one standard deviation, \( n = 3 \)) obtained in a spiked yeast...
extract are not significantly different from the expected natural abundance ratios of 26.74 and 9.821 for $^{75}\text{Se}^{75}\text{Se}$ and $^{82}\text{Se}^{82}\text{Se}$, respectively, confirming that no significant spectroscopic interference on any of the three isotopes arises from the sample matrix, permitting accurate results to be obtained using either isotope pair.

Isotope dilution and reverse isotope dilution were used for the final quantitation of SeMet content in the yeast sample using eqn. (1). SeMet concentrations of 3434 ± 19 and 3419 ± 15 μg g⁻¹ (one standard deviation, n = 6) with RSDs of 0.55 and 0.42%, respectively, were obtained in yeast using $^{75}\text{Se}^{75}\text{Se}$ and $^{82}\text{Se}^{82}\text{Se}$ ratios, respectively, in agreement with those generated with GC ICP-MS using 4 M methanesulfonic acid digestion and methyl chloroformate derivatization, shown in Table 3. More than a 10-fold improvement in precision of measurement of SeMet was obtained as a result of the more efficient transport of CH₃SeCN to the detector. Based on a total Se concentration of 2063 ± 13 μg g⁻¹ (one standard deviation, n = 4) measured previously in this yeast, SeMet concentration as Se accounts for about 67% of the total Se.

A subsequent, comparative analysis of these samples was performed using GC-MS. A software program (Isotope Pattern Calculator v 3.0) developed by Yan was used to calculate the relative abundance of the derivatized SeMet ion and its fragment ions for different C or Se isotopes. As shown in Fig. 3(b), skewed isotope patterns for CH₃SeCN⁺ ion (m/z 115–123) and its CH₃Se⁺ fragment ion (m/z 88–97) were observed, compared to their calculated relative abundances. This may arise because of interferences on these masses or as a result of deprotonation of some of these ions. Good isotope patterns for the fragment ion SeCN⁻ (m/z 100–108) was obtained. Thus, ions at m/z 106 and 100 were selected as reference and spike ions for ID analysis using a $^{75}\text{Se}$ enriched SeMet to generate the final concentration of SeMet in the yeast. A measured ratio of 55.75 ± 0.14 (one standard deviation, n = 4) at m/z 106/100 in an unspiked yeast extract was not significantly different from the expected natural abundance ratio of 55.7426 (48.899%/0.8772%), confirming the absence of any significant spectroscopic interference on the selected ions arising from the sample matrix. A concentration of 3417 ± 27 μg g⁻¹ (one standard deviation, n = 6) with RSD of 0.78% was obtained, in agreement with that measured by GC ICP-MS. The results obtained for SeMet with either ID GC ICP-MS or ID GC-MS using 4 M methanesulfonic acid digestion and CNBr derivatization were also confirmed by FCL using identical sample preparation to yield 3296 ± 37 μg g⁻¹ (one standard deviation, n = 3), a value within 3.5% of values produced in our laboratory.

### Results for final quantitation of SeMet in yeast using GC-MS

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### Conclusions

A precise method has been developed for the determination of SeMet in yeast using ID GC ICP-MS using 4 M methanesulfonic acid digestion with 4 M methanesulfonic acid and derivatization with CNBr. A species specific a $^{75}\text{Se}$ enriched SeMet spike used for this purpose provided values in a good agreement with those obtained using GC-MS. Good precision (better than 0.6% RSD for SeMet) was obtained using the present method, clearly demonstrating the superior capability of ID. The SeMet content is equivalent to 67% of the total selenium in this yeast sample. The proposed method has sufficiently low detection power (0.9 μg g⁻¹) for SeMet to make it well suited for the certification of SeMet in a proposed CRM yeast material.

The method was based on use of CNBr to cleave the CH₃Se group following pretreatment with 2% SnCl₂ in 0.1 M HCl resulted in a 35% lower concentration of SeMet than that obtained using the method proposed in this study. Use of 4 M methanesulfonic acid digestion and methyl chloroformate derivatization produced similar results for SeMet but the precision of determination is 10-fold inferior than that obtained using the method proposed herein.

### Acknowledgements

The authors are grateful to the Institute Rosell-Lallemand for financial supporting this research and providing the yeast sample used in this study.

### Results for final quantitation of SeMet in yeast using GC-MS

Table 3 Results for SeMet, μg g⁻¹, in yeast

<table>
<thead>
<tr>
<th>Method used</th>
<th>SeMet</th>
</tr>
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<tr>
<td>GC-MS² using 4 M methanesulfonic acid digestion and methyl chloroformate derivatization (n = 6)</td>
<td>3417 ± 8</td>
</tr>
<tr>
<td>GC-MS this study using 4 M methanesulfonic acid digestion and CNBr derivatization (n = 6)</td>
<td>3417 ± 27</td>
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<table>
<thead>
<tr>
<th>Method used</th>
<th>SeMet ($^{75}\text{Se}^{75}\text{Se}$)</th>
<th>SeMet ($^{82}\text{Se}^{82}\text{Se}$)</th>
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<tbody>
<tr>
<td>GC ICP-MS this study using 4 M methanesulfonic acid digestion and CNBr derivatization (n = 6)</td>
<td>3434 ± 19</td>
<td>3419 ± 15</td>
</tr>
<tr>
<td>GC ICP-MS this study using CNBr with 2% SnCl₂ in 0.1 M HCl pretreatment (n = 4)</td>
<td>2220 ± 7</td>
<td>2215 ± 9</td>
</tr>
<tr>
<td>GC ICP-MS this study using 4 M methanesulfonic acid digestion and methyl chloroformate derivatization (n = 6)</td>
<td>3415 ± 200</td>
<td>3447 ± 198</td>
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References