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Technical Notes

Determination of Methionine and Selenomethionine in Selenium-Enriched Yeast by Species-Specific Isotope Dilution with Liquid Chromatography−Mass Spectrometry and Inductively Coupled Plasma Mass Spectrometry Detection

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Selenomethionine (SeMet) and methionine (Met), liberated by acid hydrolysis of selenium-enriched yeast, were quantified by liquid chromatography−mass spectrometry (LC/MS) using standard additions calibrations as well as isotope dilution (ID) based on species-specific ^{13}C-enriched spikes. LC inductively coupled plasma mass spectrometry (ICPMS) was also employed for the quantification of SeMet, and ^{74}Se-enriched SeMet was used for ID calibration. The results were evaluated to ascertain the feasibility of using these methods in a campaign to certify selenized yeast. Good agreement was found between the methods, which, when averaged, gave concentrations of 5482.2 ± 101 and 3256.9 ± 217.4 μg/g for Met and SeMet, respectively. This corresponds to a 1.68:1 Met-to-SeMet ratio in the yeast. Quantification by ID LC/MS and LC ICPMS yields the most precise sets of results with relative standard deviations in the range 0.5−1.3% (n = 6). A total selenium concentration of 2064.6 ± 45.4 μg/g was obtained for this yeast material. The extraction efficiency and a mass balance budget were determined. Acid hydrolysis liberated 81.0% of the total selenium present. SeMet comprised 79.0% of the extracted selenium and 63.9% of the total selenium present in the yeast.

Since the discovery in 1957 that selenium is an essential trace element for mammals,[1] it has been increasingly implicated in a beneficial role for human health. Selenium is an essential trace element, present in several proteins as selenocysteine, and selenium availability is directly linked to the regulation of selenoprotein expression.[2] Due to low levels of selenium available in the average human diet in certain geographic areas,[3] it has become increasingly popular to use supplements. As well as maintaining adequate levels of selenium in the body, the use of supplements has been shown to prevent cancer in humans,[4] act as an antiviral agent, and play a preventative role in several syndromes associated with deficiencies.[5] Selenium is most often supplemented in the form of selenomethionine (SeMet): the major form found naturally occurring in foods and generally thought of as one of the most bioavailable. Selenium-enriched yeast has become the most popular matrix for food supplements as it contains SeMet as the major species and is economically viable. Inorganic selenium in yeast growth media intrudes on the sulfur assimilation pathway, ultimately forming SeMet. The SeMet is then believed to be nonspecifically incorporated into the protein in the place of Met.[5]

Due to limited regulations for food supplements, there is a need for robust methodology characterizing Se-enriched supplements to ensure that they contain the appropriate dosage and the particular form of selenium that is most beneficial. Most studies have focused on identification and quantification of SeMet in supplements. For the quantification of SeMet, degradation of all proteins to their constituent amino acids, either enzymatically or via acid hydrolysis, is required. Many protocols employ an element specific mode of detection, such as inductively coupled plasma mass spectrometry (ICPMS) coupled with liquid chromatography (LC).[6−11] Increasingly, electrospray mass spectrometry (ES MS)

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has been utilized,[12−14] where the distinguishing isotopic profile of selenium can be exploited for identification. However, ES MS has not been the method of choice for quantification of SeMet. Similarly, in gas chromatography/mass spectrometry (GC/MS), the characteristic selenium isotopic profile can be observed,[10,15−17] but derivatization of the selenium species is needed to render a volatile product for separation on the GC column.

In regulated environments, quantitative measurements are validated using certified reference materials (CRMs), against which an analyst may compare results. Unfortunately, production of CRMs is laborious and costly and so a limited range of materials is commercially available. With selenium becoming increasing relevant to the scientific community, it is the objective of the certified reference materials program of the National Research Council Canada to produce a CRM for selenized yeast. Optimization of the extraction procedure and the development of an in-house reference method based on isotope dilution (ID) GC/MS have already been completed and described elsewhere.[18−20] Good agreement among quantitative measurements derived from use of several different analytical techniques is needed for certification. This study was undertaken to provide accurate and precise data for SeMet and Met concentrations in a selenium-enriched yeast using LC-based techniques. Results are compared to those generated using in-house reference method.

Several calibration strategies for quantification of analyte compounds in a given matrix exist, including external calibration, standard additions (prior to and after the sample treatment), and isotope dilution. The latter, a more accurate and precise method than the method of standard additions, has been increasingly applied to bioinorganic speciation, especially for organotin[21] and organomercury[22] species-specific measurements. To date though, there are limited applications of species-specific isotope dilution for the speciation of selenium. Selenite (^{78}Se-selenite) and selenate (^{78}Se-selenate) were determined offline in natural water samples with microwave induced plasma MS.[23] Species-specific ID of SeMet in enriched yeast supplements was determined with ID (^{74}Se-SeMet) GC/MS [16] and LC/ICPMS (^{77}Se-SeMet).[24] Speciation of selenium has been attempted with postcolumn ID ICPMS.[25,26]

Table 1. Experimental Conditions

ICPMS Operating Conditions	
nebulizer gas flow	0.7 L/min
ICP rf power	1100 W
lens voltage	10 V
ES MS Operating Conditions	
spray voltage	3000 V
capilliary temp	350 °C
sheath gas flow	40
auxiliary gas flow	10
tube lens	100
Anion-Exchange Chromatography Gradient Conditions	
eluent A	20 mM ammonium acetate/ acetic acid, pH 4.7
eluent B	200 mM ammonium acetate/ acetic acid, pH 4.7
0−5 min	100% eluent A
5−30 min	100−0% eluent A, 0−100% eluent B
30−40 min	100% eluent B
flow rate	1.5 mL/min
Reversed-Phase Chromatography Gradient Conditions	
eluent A	0.02% formic acid, 99.8% water
eluent B	0.02% formic acid, 99.8% acetonitrile
0−5 min	100% eluent A
5−30 min	100−50% eluent A, 0−50% eluent B
30−40 min	50% eluent B
flow rate	0.2 mL/min

The majority of ID applications to selenium, however, concern total selenium concentrations.[15,26−28] Species-specific ID of bioinorganic compounds with ^{13}C-enriched compounds has not as yet been exploited despite its customary use in LC/MS applications for a range of small molecules in biological matrixes, including amino acid analysis.[29,30] Thus, it was proposed to quantify SeMet in the selenized yeast by species-specific ID using ^{13}C-SeMet (LC/MS) in addition to ID quantification with ^{74}Se-SeMet (LC/ICPMS). Moreover, with LC/MS, the simultaneous quantification of Met by species-specific ID using ^{13}C-Met can be achieved.

EXPERIMENTAL SECTION

Instrumentation. A Thermo Finnigan TSQ quantum AM triple quadrupole instrument (San Jose, CA) was used for ES MS analysis. ES MS conditions (e.g., capillary voltage, lens voltage, multipole offset, and entrance voltage) were optimized for selenomethionine using the standard tune procedure, and typical conditions are outlined in Table 1. An ELAN 6000 (PE-Sciex, Thornhill, ON, Canada) ICPMS equipped with a Ryton spray chamber and cross-flow nebulizer was used for the detection of selenium species. ICPMS parameters (nebulizer gas flow, rf power, and lens voltage) were optimized daily using a standard procedure recommended by the manufacturer, and typical conditions are outlined in Table 1.

Anion-exchange HPLC separations were achieved using a Hamilton PRPx-100 (250 × 4.6 mm × 5 μm) column (Hamilton,

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Reno, NV) with a PRPx-100 guard column (Hamilton). A Dionex BioLC, model LCM (Dionex Corp., Sunnyvale, CA) fitted with a 100-μL injection loop was employed for the anion-exchange HPLC separations. Reversed-phase separations were undertaken using a Prevail C18 (150 × 2.1 mm × 5 μm) column (Alltech, Deerfield, IL). A Hewlett-Packard HP 1100 pump with autosampler was used for the reversed-phase separations.

A CEM MDS-2100 closed-vessel microwave digestion system (Matthews) was employed for complete digestion of the yeast tissue for determination of total Se.

Reagents and Solutions. Analytical reagent grade chemicals were used throughout. Water was purified to 18.2 MΩ·cm resistivity using a NANOpure mixed-bed ion-exchange system fed with reverse osmosis domestic feedwater (Barnstead/Thermolyne, Dubuque, IA). Acetic acid was purified in-house by sub-boiling distillation of reagent grade feedstock. Methanesulfonic acid (98% purity) was purchased from Fluka (Oakville, ON, Canada). OmniSolv acetonitrile and formic acid (98% purity) were purchased from EM Science (Gibbstown, NJ). Ammonium acetate was obtained from Sigma Aldrich (Oakville, ON, Canada).

Eluent B for anion-exchange chromatography was prepared from a mixture of 200 mL of acetic acid (1 M) and 200 mL of ammonium acetate (1 M) diluted to 1 L with water. Eluent A was prepared by diluting eluent A 10-fold. Eluents A and B for reversed-phase chromatography were prepared by adding 200 μL of formic acid to 1 L of water and 1 L of acetonitrile, respectively. The gradient elution programs employed for anion-exchange and reversed-phase chromatography are outlined in Table 1.

Standard stock solutions (1000 μg/mL) of natural abundance L-methionine, seleno-DL-methionine and seleno-DL-cystine (Sigma Aldrich) were prepared by dissolving the target compound in water. Selenite and selenate were prepared from Na_2SeO_3 and Na_2SeO_4, respectively, purchased from Sigma Aldrich, and dissolved in 0.8 M HCl. Selenoadenosylhomocysteine was isolated from an aqueous extract of selenized yeast by 2D LC. The chromatographic purity was verified by LC/ICPMS. ES MS^n was employed to confirm the identity of the species. A 1000 μg/mL elemental solution for determination of total selenium was purchased from SCP Science (Baie d'Urfe, PQ, Canada). For isotope dilution analysis, high-purity ^{13}C-enriched L-methionine and ^{13}C-enriched DL-selenomethionine, purchased from Sigma Aldrich, were gravimetrically prepared in 1% HCl. ^{74}Se-enriched L-selenomethionine (^{74}SeMet) was donated by Dr. W. Wolf (Food Composition Laboratory, USDA, Beltsville, MD), and a stock solution of 450 μg/mL was prepared in 1% HCl. The solution concentration was verified using reverse isotope dilution GC/MS. Working solutions were prepared on the day of analysis by appropriate dilution of the stock solutions with water. The stock solutions were kept at 4 °C in the dark.

Selenium-Enriched Yeast. The Se-enriched yeast tissues were provided by Lallemand-Institut Rosell (Montreal, PQ, Canada). Yeast preparation essentially consisted of growth in a Se-rich medium, followed by a heat treatment process intended to stop yeast growth and inhibit enzymatic activity.

Procedures and Sample Preparation. *Microwave Digestion for Total Selenium Content Determination.* For total analysis, a subsample (100 mg) of selenium-enriched yeast was weighed into a clean, dry Teflon microwave bomb. Nitric acid (7 mL) and hydrogen peroxide (200 μL) were added, and the sample was digested by closed-vessel microwave digestion. The digestion conditions were as follows: 10 min at 20 psi and 40% power, 10 min at 40 psi and 40% power, 15 min at 85 psi and 60% power, and 30 min at 120 psi and 70% power. After cooling, the contents of the bomb were transferred to a volumetric flask and diluted to 50 mL with water. The selenium content was then determined by the method of standard additions with off-line ICPMS.

Acid Hydrolysis: Postsample Treatment Standard Addition for Determination of SeMet and Met by LC/MS and Determination of SeMet Content by LC/ICPMS. The extraction procedure employed for the yeast was based on a method developed by Wrobel et al.[8] For each replicate, one subsample (250 mg) of selenium-enriched yeast was weighed into a clean, dry conical flask. The appropriate volumes of water and methanesulfonic acid were added to give a concentration of 4 M methanesulfonic acid (24 mL). The sample was subjected to reflux on a hot plate for 16 h with glass beads acting as antibumping granules. After cooling, the digest was transferred to a volumetric flask and diluted to 100 mL with water. Known masses of SeMet and Met were added to aliquots of the prepared sample, to make solutions that contained close to two and three times the concentration of the target species. The solutions were filtered (0.45 μm) prior to injection.

Acid Hydrolysis: Presample Treatment Standard Addition Spiking for Determination of SeMet and Met Content by LC/MS and Determination of SeMet Content by LC/ICPMS. For each replicate, three subsamples (250 mg) of selenium-enriched yeast were weighed into clean, dry conical flasks. Known masses of SeMet and Met were added to the yeast to make final solutions that contained close to two and three times the concentration of the target species. The above acid reflux procedure was followed, and the digests, diluted to 100 mL with water, were filtered (0.45 μm) prior to injection.

Acid Hydrolysis: Isotope Dilution Spiking for Determination of SeMet and Met Content by LC/MS. For each replicate, one subsample (250 mg) of selenium-enriched yeast was weighed into a clean, dry conical flask. A suitable mass of ^{13}C-SeMet and ^{13}C-Met spikes was added to the yeast, and the above acid reflux procedure was followed. The digests were diluted to 100 mL with water and filtered (0.45 μm) prior to injection.

Acid Hydrolysis: Isotope Dilution Spiking for Determination of SeMet Content by LC/ICPMS. For each replicate, one subsample (250 mg) of selenium-enriched yeast was weighed into a clean, dry conical flask. A known mass of ^{74}Se-SeMet was added to the yeast, and the above acid reflux procedure was followed. The digests were diluted to 100 mL with water and filtered (0.45 μm) prior to injection.

RESULTS AND DISCUSSION

Determination of Total Selenium Content. For this study, closed-vessel microwave digestion with nitric acid and hydrogen peroxide was employed. The yeast was completely solubilized and Se quantified by the method of standard additions using ICPMS. A total selenium concentration of 2064.6 ± 45.4 μg/g (n = 6, RSD = 2.2%) was obtained.

Extraction of SeMet and Met. A complete hydrolysis of yeast protein into its constituent amino acids is necessary for the quantitative extraction of SeMet and Met. Aqueous extractions

Table 2. Isotopic Distributions of Natural and Enriched Forms of Met and SeMet Used in Eq 1 for LC Species-Specific Isotope Dilution MS

m/z	natural abundance $C_5H_{12}O_2NS^+$	enriched $^{13}CC_4H_{12}O_2NS^+$ (99.3%)
149	0.000 00	0.000 00
150	0.890 69	0.006 30
151	0.060 50	0.894 39
152	0.045 67	0.051 37
153	0.002 69	0.045 31
154	0.000 42	0.002 21
155	0.000 02	0.000 40
156	0.000 00	0.000 02

m/z	natural abundance $C_5H_{12}O_2NSe^+$	enriched $^{13}CC_4H_{12}O_2NSe^+$ (99.1%)
191	0.000 00	0.000 00
192	0.008 35	0.000 08
193	0.000 50	0.008 37
194	0.087 96	0.001 21
195	0.076 86	0.088 80
196	0.227 81	0.078 10
197	0.013 79	0.227 48
198	0.466 74	0.015 59
199	0.027 95	0.467 63
200	0.084 52	0.023 71
201	0.005 03	0.084 46
202	0.000 46	0.004 13
203	0.000 02	0.000 42
204	0.000 00	0.000 02

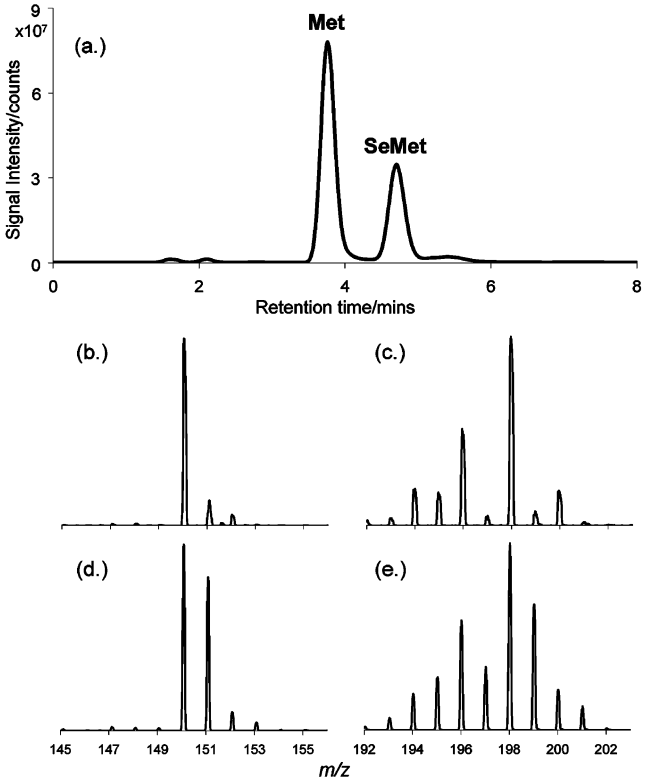

Figure 1. (a) Reversed-phase SIM MS chromatogram of selenized yeast digest; (b) mass spectrum of natural abundance Met; (c) mass spectrum of natural abundance SeMet; (d) mass spectrum of Met in the selenized yeast digest with ^{13}C-enriched spike; (e) mass spectra of SeMet in the selenized yeast digest with ^{13}C-enriched spike.

are incapable of digesting proteins and liberating the bulk of SeMet from an enriched yeast; generally only 10−25% of the total selenium is extracted.[7,31] Recent studies employing enzymatic digestion have shown that SeMet accounted for 50%[6] and 46%[8] of the total selenium in a supplement and a selenized yeast, respectively. Acid hydrolysis of the same samples yielded SeMet extraction efficiencies of 63%[6] and 65%[8] in the supplement and selenized yeast, respectively. Although enzymatic digestion is a popular extraction method for samples of high protein content, it has been suggested that incomplete digestion of the proteins occurs.[8] An in-house study of several extraction protocols[20] concluded that using methanesulfonic acid for acid hydrolysis, based on a slightly modified method developed by Wrobel et al.,[8] was the most effective, reproducible, and cost-efficient approach for yeast.

Isotope Dilution Calculations. The following equation was used in isotope dilution analysis for the quantitation of analytes in the yeast digest:

$$C_x = C_y \frac{v_y}{m_x} \frac{A_y - B_y R_n}{B_x R_n - A_x} \frac{AW_x}{AW_y} \quad (1)$$

where C_x is the analyte concentration (μg/g), C_y is the spike concentration (μg/g), v_y is the volume (mL) of spike used to prepare the blend solution of sample and spike, m_x is the mass (g) of sample used, A_y is the abundance of the reference ion in the spike, B_y is the abundance of spike ion in the spike, A_x is the abundance of reference ion in the sample, B_x is the abundance of spike ion in the sample, R_n is the measured reference/spike ion ratio (mass bias corrected) in the blend solution of sample and spike, AW_x is the atomic weight of analyte in the sample, and AW_y is the atomic weight of analyte in the spike.

The relative abundances of natural and enriched species for isotope dilution analysis with LC/MS were calculated by a software program (Isotope Pattern Calculator v 3.0) developed by Yan[32] and are summarized in Table 2. These data were used to calculate the theoretical reference-to-spike ion ratios needed for eq 1. For isotope dilution analysis using ICPMS, the isotopic compositions of Se isotopes recommended by IUPAC[33] were used for the calculations. The mass bias correction factor (the ratio of "theoretical reference-to-spike ion ratio" to "measured reference-to-spike ion ratio") was based on a set of five replicate measurements of natural abundance standards, matrix matched with the same concentration of methanesulfonic acid as in the samples.

LC/MS Analysis of Yeast Digest. The most commonly used separation mechanism for the on-line analysis of amino acids by LC/MS is reversed-phase ion pairing.[29,30] As only two amino acids were the target of this study, reversed phase without an ion-pairing agent (which can sometimes compete with the target analyte for ionization in the ES MS source) was employed. Figure 1a shows a typical selected ion monitoring (SIM) LC/MS chromatogram of the yeast digest. A low v/v percentage of formic acid (0.02%) in the eluent was found to favor the separation of Met and SeMet,

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Table 3. Methionine and Selenomethionine Concentrations Determined in Selenium-Enriched Yeast A[a]

	Met μg/g by GC/MS[b]	Met, μg/g by LC/MS		
	^{13}C ID	presample prep std addn	postsample prep std addn	^{13}C-enriched ID
mean	5955	5368	5606	5522
SD	33	316	189	26
RSD (%)	0.55	5.9	3.4	0.5
LOD	3.4	1.56	1.00	0.87

	SeMet μg/g by GC/MS[b]	SeMet, μg/g by LC/MS		
	^{13}C ID	presample prep std addn	postsample prep std addn	^{13}C-enriched ID
mean	3404	3210	3462	3395
SD	12	252	147	45
RSD (%)	0.35	7.9	4.2	1.3
LOD	1.0	0.59	0.95	1.79

	SeMet μg/g by GC/MS[b]	SeMet, μg/g by LC−ICPMS		
	^{74}Se ID	presample prep std addn	postsample prep std addn	^{74}Se-enriched ID
mean	3417	2923	3102	3450
SD	8	211	96	22
RSD (%)	0.23	7.2	3.1	0.6
LOD		1.68	0.96	1.41

[a] LOD are listed for each technique and method of quantification employed. [b] Data from ref 18.

and baseline separation is achieved despite the similarity of the compounds. The insets show the mass spectra of Met (Figure 1b) and SeMet (Figure 1c), distinguished by the natural isotopic profiles of S and Se, respectively. The theoretical isotopic compositions of these species are given in Table 2. For standard additions, the molecular ions of protonated Met at m/z 150 and protonated SeMet at m/z 198 were monitored in SIM mode for integration. The results for the quantitation of Met and SeMet by pre- and postsample preparation standard additions are presented in Table 3.

Panels d and e of Figure 1 illustrate how the natural isotopic profiles of Met and SeMet are distorted due to the addition of ^{13}C-enriched spikes. Reference and spike ions at m/z 150 and 151 for Met and m/z 198 and 199 for SeMet, respectively, were monitored in SIM mode for integration. Peak areas were used to calculate the reference-to-spike ion ratios of Met (m/z 150/151) and SeMet (m/z 198/199) for use in eq 1. Concentrations of 5521.7 ± 26.2 and 3395.0 ± 44.5 μg/g for Met and SeMet, respectively, were obtained.

Analysis of Yeast Digest by LC/ICPMS. LC coupled to ICPMS for the analysis of organoselenium compounds is a popular technique in bioinorganic speciation studies. The ICP source is not compatible with the high carbon content of organic solvents unless a stream of oxygen (necessary to oxidize the carbon) is introduced into the plasma with the sample. To eliminate the need for oxygen, reversed-phase chromatography is often used with a small volume of methanol and an ion-pairing agent,[6−8,10] or ion-exchange chromatography with aqueous eluents is employed.[12,24] In this study, anion-exchange chromatography with a gradient previously developed for the separation of water-soluble compounds from selenized yeast was used.[34] Figure 2a shows the elution profile for several calibration standards to which that for the acid digest of the selenized yeast was compared. The major peak can be seen to agree with the retention time of SeMet (Figure 2b). A proportional increase in peak size due to addition of the internal standard of SeMet to the yeast sample is also shown in Figure 2b. The concentration of SeMet determined in the yeast by pre- and postsample preparation standard additions with LC/ICPMS is summarized in Table 3.

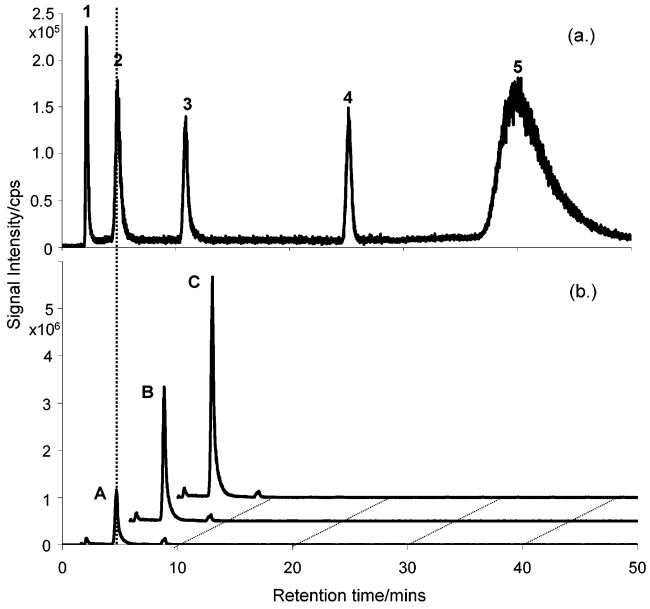

Figure 2. Anion-exchange ICPMS chromatograms of (a) Se standards. Peaks: 1, seleno-DL-cystine; 2, seleno-DL-methionine; 3, selenite; 4, selenate; 5, selenoadenosylhomocysteine, (b) Selenized yeast digest on AE ICPMS with the internal standard addition of SeMet on two levels (the chromatograms are shifted on the x and y axis to improve clarity). Peaks: A, yeast digest; B, yeast digest + 7.5 μg/g SeMet; C, yeast digest + 15 μg/g SeMet.

The same principles of ID based on eq 1 used for LC/MS apply to the analysis of the selenized yeast digest by LC/ICPMS, except that elemental isotopes replace the molecular ions monitored in LC/MS. In Figure 3, chromatograms a and b illustrate the elution profiles obtained for a natural abundance standard and a spiked yeast digest, respectively. In each chromatogram, the isotopes of selenium at 74, 78, and 82 are shown. The increase in the peak area of isotope 74 due to the addition of the ^{74}Se-enriched SeMet spike is clearly evident (Figure 3b). The extent of enhancement is confirmed by the theoretical distribution of isotopes calculated and displayed in insets A and B. The selenium isotopes at 74, 77, 78, and 82 were monitored and used to calculate the 77/74, 78/74, and 82/74 isotope ratios necessary for the quantification of SeMet. The concentration of SeMet determined by ID LC/ICPMS in the yeast using the 82/74 reference-to-spike isotope ratio, was 3449.8 ± 22.3 μg/g.

Evaluation of SeMet and Met Quantification by LC Techniques. As noted earlier, several calibration strategies for

(34) McSheehy, S.; Szpunar, J.; Haldys, V.; Tortajada, J. *J. Anal. At. Spectrom.* **2002**, *17*, 507−514.

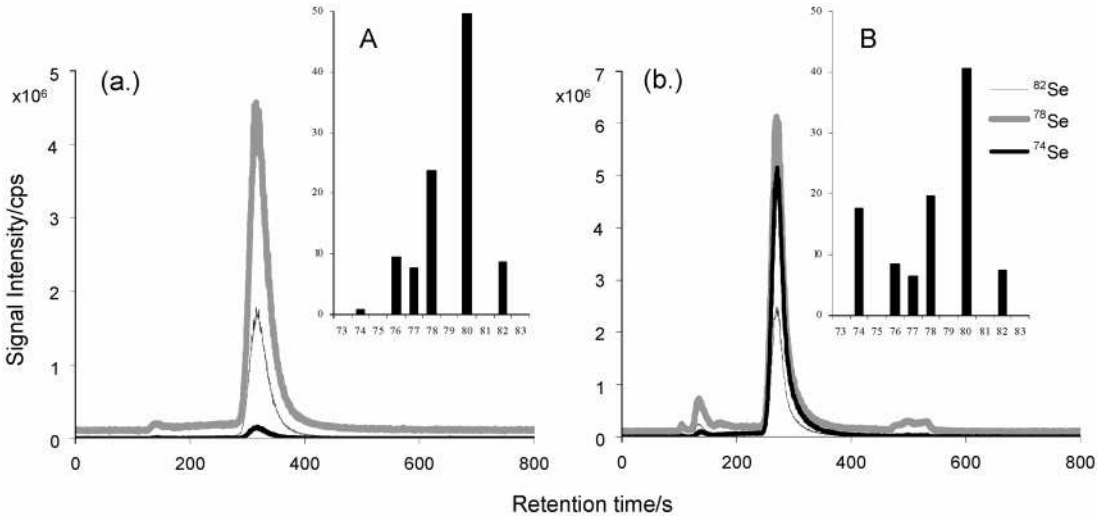

Figure 3. AE ICPMS chromatograms of (a) natural abundance standard and (b) selenized yeast digest spiked with ^{74}Se-enriched SeMet. Insets A and B show the respective theoretical distributions of Se isotopes.

quantification of bioinorganic species exist. In this study, six methods of quantifying SeMet, of which three were also used for quantifying Met in the acid hydrolysis digests, were compared (cf. Table 3). External calibration was not considered an appropriate technique for use in this study, as it does not compensate for interferences ascribed to matrix effects. There is generally good agreement between the sets of results, with the major discrepancy being a low result obtained for SeMet based on standard additions with LC/ICPMS (an average of 13% lower for standard addition) compared to the ID LC/ICPMS technique. The ID analyses show a great improvement in precision (expressed as relative standard deviation, RSD) over the standard additions technique and are more fit for the accurate and precise measurements needed for these analytes. Species-specific isotope dilution also provides a quantitation technique that accounts for matrix effects.

The SeMet ID data obtained in this study are in good agreement with reference data generated by ID using ^{74}Se-enriched and ^{13}C-enriched spikes combined with GC/MS[18] (Table. 3). However, the Met data obtained by LC techniques in this study are, on average, ~8% lower than the data obtained by the GC/MS reference method. This discrepancy could have arisen due to the difference in mass bias obtained with the techniques GC/MS and LC/MS. It has been observed that the mass bias for LC/MS suffers greater variation from day to day and is less precise than the mass bias obtained for GC/MS. Since this set of results was obtained, efforts have been made, with success to improve the mass bias of the LC/MS technique for the certification campaign, by manipulation of the ES MS parameters.

The LODs for ID LC/MS and ID LC/ICPMS methodologies are based on three standard deviations of the average response from spiked blank samples (n = 3) with concentrations normalized to 0.25 g of yeast test sample and are comparable to the ID GC/MS reference method. The LODs for LC/MS and LC/ICPMS methodologies were based on three standard deviations of the average response from blanks (n = 3) with concentrations normalized to 0.25 g of yeast test sample and are also found to have values comparable to those obtained by other calibration methods.

CONCLUSIONS

Two analytical techniques, LC/MS and LC/ICPMS, were developed based on species-specific isotope dilution for the determination of Met and SeMet in selenized yeast. Results obtained are in good agreement with a GC/MS reference method developed in-house and will likely prove to provide satisfactory parallel techniques for the certification of this candidate yeast CRM. LC provides a relatively straightforward alternative technique for quantitation of Met and SeMet wherein no derivatization procedures are required as in GC. However, the sample analysis time for LC is considerably greater compared to GC.

Mean values of the set of results for Met and SeMet give a Met-to-SeMet ratio of 1.68:1. This information is useful for subsequent investigations into the mechanism of incorporation of Se into yeast and for supplementary data characterizing the proposed reference material.

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