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Yang, L.; Sturgeon, R.; Mester, Z.

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Dried-droplet laser ablation ICP-MS of HPLC fractions for the determination of selenomethionine in yeast

Lu Yang,* Ralph E. Sturgeon and Zoltán Mester

Institute for National Measurement Standards, National Research Council Canada, Ottawa, Ontario, Canada K1A 0R6. E-mail: Lu.Yang@nrc.ca; Fax: 613-993-2451

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Offline coupling of high performance liquid chromatography (HPLC) to dried droplet laser ablation (LA) ICP-MS detection is described. The method is based on the offline spotting of HPLC fractions onto a substrate followed by LA introduction of the dried sample residues to an ICP-MS for elemental analysis. Quantitation of selenomethionine (SeMet) in yeast using species specific isotope dilution (ID) was achieved following digestion of samples in 4 M methanesulfonic acid and HPLC separation of species. Chromatographic fractions having retention times of 180–210 seconds were collected for each sample. Dried micro-droplets from each fraction were ablated from a polystyrene substrate and quantitated for SeMet. Concentrations of 3301 ± 18 and $3309 \pm 24 \mu\text{g g}^{-1}$ (one standard deviation, $n = 4$) with RSDs of 0.55% and 0.73% were obtained based on measured $^{78}\text{Se}/^{74}\text{Se}$ and $^{82}\text{Se}/^{74}\text{Se}$ ratios, in good agreement with the values of 3309 ± 19 and $3305 \pm 26 \mu\text{g g}^{-1}$ (one standard deviation, $n = 4$, RSDs of 0.58% and 0.79%), respectively, obtained by direct HPLC-ICP-MS analysis. The proposed method provides a satisfactory alternative technique for the quantitation of SeMet in yeast.

Introduction

Laser ablation ICP-MS has become one of the more powerful techniques for direct analysis of solids because of its high sensitivity, rapid throughput, microanalysis and depth profiling capabilities.^{1–14} However, elemental fractionation,¹⁵ leading to non-stoichiometric response, frequently occurs, although the sources of this problem are still debated.^{2,3,16,17} This makes it difficult to achieve quantitative analytical results using this technique, especially if no suitable standard reference materials of similar matrix composition are available for calibration. Isotope dilution approaches have been applied by several research groups^{10–14} for the direct determination of trace elements in various solid samples using LA-ICP-MS in attempts to achieve enhanced accuracy and precision of results. However, because of inhomogeneous distributions of the analyte or spike in the solid samples, relatively poor precision persists, depending on the analyte measured.

The application of LA to the analysis of aqueous samples has been limited.^{18–22} Recently, a quantitative and fast LA-ICP-MS method based on complete ablation of dried-droplets for the determination of trace metals in water and selenium in yeast digest was reported by Yang *et al.*¹⁹ Good precision of 0.50% RSD, comparable to the 0.40% RSD obtained with liquid sample introduction using ICP-MS, was reported for Se in the yeast digest using ID with LA. This was achieved because complete sample ablation eliminated elemental fractionation during the sampling stage and the homogeneous distribution of analyte and spike in solution ensured isotopic equilibration.

The rate limiting step for on-line LC-ICP-MS is the slow LC separation step. In addition, organic solvents used in LC separations potentially make for more difficult ICP-MS measurements (for example, no normal phase separations can be simply performed). Utilizing offline chromatographic separation and spotting the chromatographic fractions on a substrate offers the possibility of using LA for sample introduction to ICP-MS. Laser ablating dried residues corresponding to LC fractions can be rapidly accomplished and the major matrix components of the LC solvent are completely eliminated by the drying process.

The offline coupling of LC with LA-ICP-MS can be applied to the determination of SeMet in yeast using fraction collected eluent from a HPLC chromatographic separation of the species in a yeast extract. Faster analysis and lower cost per sample can thus be achieved. The objective of this study was to evaluate the possibility of undertaking quantitative ICP-MS analysis of SeMet in HPLC eluent based on LA of dried droplets representative of specific chromatographic fractions. To the best of our knowledge, this is the first report of such application for speciation of Se in biological samples.

Experimental

Instrumentation

A PerkinElmer SCIEX ELAN 6000 ICP-MS (Concord, Ontario, Canada) equipped with a Gem-tipped cross-flow nebulizer mated to a quartz torch and alumina sample injector tube was used. A double pass Rytan[®] spray chamber was mounted outside the torch box and maintained at room temperature. Optimization of the ELAN 6000 and dead time correction were performed as recommended by the manufacturer. Typical operating conditions for the HPLC-ICP-MS systems are summarized in Table 1.

Anion-exchange HPLC separations were achieved using a Hamilton PRPx-100 (250 × 4.6 mm × 5 μm) column (Hamilton, Reno, NV, USA) with a PRPx-100 guard column (Hamilton) and 0.45 pore size filter. A Dionex Model LCM (Dionex Corp., Sunnyvale, CA, USA) fitted with a 100 μl injection loop was employed for the anion exchange HPLC separations. The coupling of HPLC to ICP-MS was accomplished by directing the eluent from the column to the nebulizer through a 0.3 m length of PEEK tubing (0.0762 mm id, 1.59 mm od).

A frequency quadrupled ND-YAG laser system operating at 266 nm (LUV266, New Wave Research, Inc., Fremont, USA) was used for sample ablation. Argon served to transport sample aerosol to the ICP-MS. Laser ablation was performed at 100% applied laser energy (3.4 mJ).

Table 1 HPLC, LA and ICP-MS operating conditions

ICP-MS	
Rf power	1200 W
Plasma Ar gas flow rate	15.0 l min ⁻¹
Auxiliary Ar gas flow rate	1.0 l min ⁻¹
Nebulizer Ar gas flow rate	0.85 l min ⁻¹ for liquid introduction
Sampler cone (nickel)	1.00 mm aperture
Skimmer cone (nickel)	0.88 mm aperture
Lens voltage	7.50 V
Scanning mode	Peak hopping
Points per peak	1
Dwell time	20 ms
Sweeps per reading	1
Readings per replicate	180
Number of replicates	1
HPLC	
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
5–10 min	100–0% eluent A, 0–100% eluent B
10–15 min	100% eluent B
Flow rate	1.5 ml min ⁻¹
LUV266 laser system	
Energy	100% (3.4 mJ)
Size of laser beam	400 µm
Repetition rate	20 Hz
LA mode	Raster, scan speed of 400 µm s ⁻¹
Ar carrier gas flow rate	0.700 l min ⁻¹

Polystyrene weighing boats (VWR International, Mississauga, Ontario, Canada) were used as substrates on which all samples were deposited.

Reagents and solutions

Hydrochloric acid and acetic acid were purified in-house prior to use by sub-boiling distillation of reagent grade feedstock in a quartz still. High purity de-ionized water (DIW) was obtained from a NanoPure mixed bed ion exchange system fed with reverse osmosis domestic feed water (Barnstead/Thermolyne Corp, Iowa, USA). Methanesulfonic acid (98% purity) and ammonium acetate were obtained from Sigma-Aldrich Canada (Oakville, Ontario, 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 DIW. Eluent A was prepared by diluting eluent B 10-fold. The gradient elution program employed for anion exchange chromatography is outlined in Table 1.

Natural abundance high purity SeMet, seleno-DL-cystine (SeCys) and seleno-DL-ethionine (SeEt) compounds were purchased from Sigma-Aldrich Canada. Individual stock solutions of 1000–2500 µg ml⁻¹ were gravimetrically prepared in 1% HCl solution and kept refrigerated until used.

A ⁷⁴Se enriched SeMet (⁷⁴SeMet) compound was obtained from W. Wolf (Food Composition Laboratory, USDA, Beltsville, MD, USA) and used to prepare a stock solution of approximately 450 µg mL⁻¹ in 1% HCl. The concentration of ⁷⁴SeMet spike was verified by reverse spike isotope dilution using the natural abundance SeMet standards.

Lalmin Se yeast was obtained from Lallemand-Institut Rosell (Montreal, QC, Canada) and used as the test sample for this study.

Sample preparation and procedure for direct HPLC-ICP-MS analysis

The extraction procedure used in this study followed that described previously^{23,24} and is similar to that reported by Wrobel, *et al.*²⁵ Three sample blanks and four 0.20 g subsamples of yeast were prepared at the same time. Samples were diluted 5-fold prior to HPLC-ICP-MS analysis. Four reverse spike isotope dilution calibration samples were prepared to quantify the concentration of the ⁷⁴Se enriched SeMet spike.^{23,24} These samples were diluted 10-fold prior to HPLC-ICP-MS analysis.

The digested yeast samples and the four reverse spike ID calibration samples were analyzed by HPLC-ICP-MS on the same day. The HPLC was coupled to the ELAN 6000 and data acquisition manually triggered after injection of the sample (100 µl) onto the HPLC. Isotopes of ⁷⁴Se, ⁷⁸Se and ⁸²Se were monitored during every run. Mass bias correction was implemented based on the 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 in-house software to yield background corrected peak areas in order to generate ⁷⁸Se/⁷⁴Se or ⁸²Se/⁷⁴Se ratios, from which the analyte concentrations in the yeast samples were calculated.

Sample preparation and procedure for dried-droplets LA-ICP-MS analysis

Sample digests and reverse spike isotope dilution calibration samples were injected into the HPLC. Only that fraction (0.75 ml) of HPLC effluent containing SeMet at retention times between 180 and 210 seconds was collected. Dried droplets of the collected eluent were prepared as described previously¹⁹ for the quantitation of SeMet using LA-ICP-MS. Because of the high natural salt content of the HPLC eluents, no additional LA matrix was added. Four replicates of 20 µl of each collected eluent were pipetted onto the surface of a polystyrene weighing boat and located within the confines of a square previously marked on the transverse surface. The weighing boats were then placed in a class-10 fume hood on a hot plate (at 70 °C) under an IR lamp for drying. The bottom of the weighing boat was then excised and placed into the LA sampling chamber for analysis.

Daily optimization of the ELAN 6000 was achieved using a standard liquid sample introduction system. The plasma was then extinguished and the spray chamber and nebulizer assembly replaced with the LA transfer line and its adapter. The final optimization of lens voltage and Ar carrier gas flow for dry plasma conditions was performed by monitoring the ⁶³Cu intensity during continuous ablation of a penny coin. Typical operating parameters for LA-ICP-MS are summarized in Table 1.

Results and discussion

HPLC-ICP-MS analysis

HPLC coupled to ICP-MS is a popularly used technique for speciation of Se in yeast. Anion exchange chromatography was used for the separation of Se species in the yeast extract.²⁶ As is shown in Fig. 1, chromatographic separation of several species in a standard solution was readily achieved. It is evident from Fig. 2 that the major Se species in this yeast sample is SeMet, confirmed by comparison of retention time with the standard and also from the elevated ⁷⁴Se signal obtained from processing a ⁷⁴Se enriched SeMet spiked yeast. This conclusion is in agreement with observations obtained by others.^{23,24,26–28}

Fig. 1 Chromatogram obtained by processing a standard solution containing $10 \mu\text{g ml}^{-1}$ SeCys, SeMet and SeEt by HPLC-ICP-MS.

Isotope dilution and reverse isotope dilution were used for the quantitation of SeMet, based on the following equation:

$$C = C_z \cdot \frac{m_y}{m_x} \cdot \frac{m_z}{m'_y} \cdot \frac{A_y - B_y \cdot R_n}{B_{xz} \cdot R_n - A_{xz}} \cdot \frac{B_{xz} \cdot R'_n - A_{xz}}{A_y - B_y \cdot R'_n} - C_b \quad (1)$$

where C is the blank corrected SeMet concentration ($\mu\text{g g}^{-1}$) in the yeast; C_z is the concentration of primary assay SeMet standard ($\mu\text{g g}^{-1}$); m_y is the mass (g) of spike used to prepare the blend solution of sample and spike; m_z is the mass (g) of primary assay SeMet 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; A_y is the abundance of the reference isotope in the spike; B_y is the abundance of the spike isotope in the spike; A_{xz} is the abundance of the reference isotope in the sample or primary assay standard; B_{xz} is the abundance of the spike isotope in the sample or primary assay standard; R_n is the measured reference/spike isotope ratio (mass bias corrected) in

Fig. 2 Chromatogram of a $^{74}\text{SeMet}$ spiked yeast extract obtained using HPLC-ICP-MS.

Fig. 3 Reconstructed chromatogram obtained by dried-droplet LA-ICP-MS using collected HPLC fractions generated by injection of the mixed standard used in Fig. 1.

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_b is the analyte concentration in the blank ($\mu\text{g g}^{-1}$) normalized to a sample weight of 0.20 g .

SeMet concentrations of 3309 ± 19 and $3305 \pm 26 \mu\text{g g}^{-1}$ (one standard deviation, $n = 4$, with RSDs of 0.58% and 0.79%) were obtained based on measurement of $^{78}\text{Se}/^{74}\text{Se}$ and $^{82}\text{Se}/^{74}\text{Se}$ ratios, respectively. These values are in good agreement with a concentration of $3275 \pm 13 \mu\text{g g}^{-1}$ (one standard deviation, $n = 8$, RSD of 0.40%) obtained using previously developed GC-MS methodology.¹⁴

LA-ICP-MS analysis

The optimization of sample deposition and drying as well as the LA-ICP-MS system have been reported in detail previously.¹⁹ Parameters used in this study are summarized in Table 1. Fig. 3 shows a reconstructed chromatogram obtained with dried droplet LA-ICP-MS sampling of all collected fractions (10 seconds per fraction, 0.25 ml per fraction) during HPLC separation of a $10 \mu\text{g ml}^{-1}$ mixed standard solution. The peak area (5 s integration time) intensity for ^{78}Se arising from ablation of a dried $20 \mu\text{l}$ volume of solution taken from each fraction was used to construct this chromatogram, which is comparable to the continuous HPLC-ICP-MS chromatogram presented in Fig. 1.

The retention time for SeMet remained very stable during the study period. Thus, only one fraction in the elution interval 180–210 seconds was collected off-line from the HPLC system for each yeast extract and reverse ID calibration sample for the final quantitation of SeMet by dried droplet LA-ICP-MS. Fig. 4 (upper) shows a typical dried residue from a collected yeast sample fraction, with a spot diameter of $600 \mu\text{m}$ ($20 \mu\text{l}$ deposit). It is evident from Fig. 4 (lower) that one single LA shot from a large diameter beam ($400 \mu\text{m}$) only achieves about 50% ablation of the sample. In order to achieve complete ablation of the dried sample, a raster mode of LA was used. As is shown in Fig. 5, one measurement can be completed in 20 s, providing Se isotope signal intensities adequate for quantitative analysis.

Fig. 4 Video images of a 20 µl deposit of a yeast extract: upper, before LA; lower, after LA.

Using eqn. (1), concentrations of 3301 ± 18 and 3309 ± 24 $\mu\text{g g}^{-1}$ (one standard deviation, $n = 4$) were obtained for SeMet based on $^{78}\text{Se}/^{74}\text{Se}$ and $^{82}\text{Se}/^{74}\text{Se}$ ratios, respectively. These results are in agreement with those obtained by direct HPLC-ICP-MS analysis. Precisions of 0.55% and 0.73% RSD in measured SeMet concentrations obtained by dried droplet LA-ICP-MS are comparable to values of 0.58% and 0.78% RSD obtained using direct HPLC-ICP-MS.

Method detection limits (LODs, 3SD) for direct HPLC-ICP-MS and dried droplet LA-ICP-MS were estimated based on

Fig. 5 LA-ICP-MS transient signal from a 20 µl deposit of the collected HPLC SeMet fraction of a yeast extract.

response from four spiked blank measurements. Values of 17 and $6.6 \mu\text{g g}^{-1}$ (normalized to a 0.20 g subsample) obtained based on $^{78}\text{Se}/^{74}\text{Se}$ and $^{82}\text{Se}/^{74}\text{Se}$, respectively, were estimated using the direct HPLC-ICP-MS approach. Poorer LODs of 110 and $36 \mu\text{g g}^{-1}$ based on $^{78}\text{Se}/^{74}\text{Se}$ and $^{82}\text{Se}/^{74}\text{Se}$, respectively, were obtained using the dried droplet LA-ICP-MS approach.

Conclusions

A fast and quantitative species-specific isotope dilution ICP-MS method, based on the LA introduction of dried droplets of HPLC fractions, was developed for the determination of SeMet in yeast. Although the detection limit is degraded compared with direct HPLC-ICP-MS, it is adequate for the determination of SeMet in Se-enriched yeast. Detection limits could potentially be improved by use of a more powerful LA system. The LA approach can significantly reduce analysis time on a costly ICP-MS (one measurement can be made every 30 s) and eliminates continuous plasma loading of HPLC eluent, which often contains undesirable organic solvents, thereby avoiding the need for frequent ICP-MS maintenance.

Use of both dried droplet LA-ICP-MS and MALDI MS is currently under investigation in our laboratory to study metal containing proteins and peptides in biological samples for their structure identification and quantitation. Since the dried droplet is not fully ablated by the LA with use of a small size laser beam (e.g., 5 µm), a single sample can be used in both systems.

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