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Notes

Complementary Use of LC-ICP-MS and LC-ESI-Q-TOF-MS for Selenium Speciation

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We demonstrated the complementary use of inductively coupled plasma–mass spectrometry (ICP-MS) and electrospray ionization quadrupole time-of-flight mass spectrometry (ESI-Q-TOF-MS) for the analysis of Se-containing compounds, such as selenate, selenomethionine (SeMet), and trimethylselenonium ion (TMSe), found in biological samples. The sensitivity of ESI-Q-TOF-MS for Se-containing compounds was strongly dependent on the chemical species. ICP-MS exhibited higher sensitivity than ESI-Q-TOF-MS, and had no species dependency. On the other hand, ESI-Q-TOF-MS enabled easy and robust identification of Se-containing compounds.

Keywords Selenium, speciation, ICP-MS, Q-TOF-MS, selenate, selenomethionine, trimethylselenonium

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Introduction

Selenium (Se) belongs to group 16 on the periodic table, the same group as oxygen, sulfur, and tellurium. It is an essential element in animals because Se is required as the active center of selenoproteins that function as an antioxidant and participate in thyroid hormone production, DNA synthesis, and fertilization.[1-4] Se is utilized in the metabolic pathways of animals and plants to form Se-containing compounds/metabolites having carbon-Se covalent bond(s). Therefore, the speciation and identification of Se-containing metabolites is necessary to reveal the metabolic pathway of Se and to understand the biological, pharmacological, and toxicological effects of Se-containing metabolites.[5]

The hyphenated technique is the most frequently used analytical technique for Se speciation. The hyphenated technique for metal/metalloid speciation consists of two analytical techniques: the separation technique and the detection technique.[6] As for the separation technique, chromatography,[7] capillary electrophoresis,[8] and gel electrophoresis[9] are usually used. Regarding the detection technique, inductively coupled plasma–mass spectrometry (ICP-MS) is primarily used due to its sensitivity and robustness for Se detection. Because of easy hyphenation with HPLC, LC-ICP-MS is the technique of choice for Se speciation in biological samples. However, LC-ICP-MS has one crucial disadvantage: since ICP-MS provides only elemental information about Se-containing metabolites, identification by LC-ICP-MS is effective only when the retention times of samples match those of some certified or authentic Se species (standard compounds). As a complementary technique to LC-ICP-MS, electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI)–mass spectrometry was used for the identification of unknown Se-containing metabolites in biological samples.[10-15] Since ESI is a softer technique than ICP, it can provide molecular information.[16] Hence, ICP-MS and ESI/APCI-MS are occasionally called inorganic and organic mass spectrometry, respectively. Today, organic mass spectrometers equipped with several types of mass filter/sector, such as quadrupole (QMS), time-of-flight (TOF-MS), and ion trap (MS^n), are available. In addition, combinations of those mass spectrometers, *i.e.*, a tandem QMS and a hybrid of QMS and TOF-MS (Q-TOF-MS), are available, which enable more precise structure elucidation than single mass spectrometers. However, we speculate that organic mass spectrometry has two weak points compared with inorganic mass spectrometry when it comes to Se speciation. As the major disadvantage, the detection limit of organic mass spectrometry for Se-containing compounds is inferior to that of ICP-MS.[17] Therefore, the complementary use of inorganic and organic mass spectrometry is desired for the speciation and identification of Se-containing metabolites.

In this study, we evaluated both the advantages and disadvantages of organic and inorganic mass spectrometry, and showed appropriate analytical conditions for the speciation of Se compounds in biological samples by organic mass spectrometry.

Experimental

Reagents

Sodium selenate and ammonium acetate were purchased from Wako (Osaka, Japan). L-Selenomethionine (SeMet) and trimethylselenonium iodide (TMSe) were purchased from Sigma (St. Louis, MO) and Tri Chemical Laboratories, Inc.

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Table 1 Analytical conditions for ICP-MS and ESI-Q-TOF-MS

	ICP-MS
RF power/W	1450
Nebulizer type	MicroMist
Nebulizer gas flow/L min^{-1}	1.15
Make-up gas flow/L min^{-1}	0.11
Plasma gas flow/L min^{-1}	15.0
	ESI-Q-TOF-MS
Polarity	positive and negative
Drying gas (N_2) flow/L min^{-1}	12
Drying gas temp./°C	200
Nebulizer pressure/psi	55
Sheath gas (N_2) flow/L min^{-1}	12
Sheath gas temp./°C	350
Fragmentor voltage/V	260
Capillary voltage/V	3500
Mass range	40 – 1000
References	purine: 121.050873 (positive) 119.036320 (negative) hexakis(2,2,3,3-tetrafluoropropoxy) phosphazine: 922.009798 (positive) 980.016375 (negative, acetate adduct)

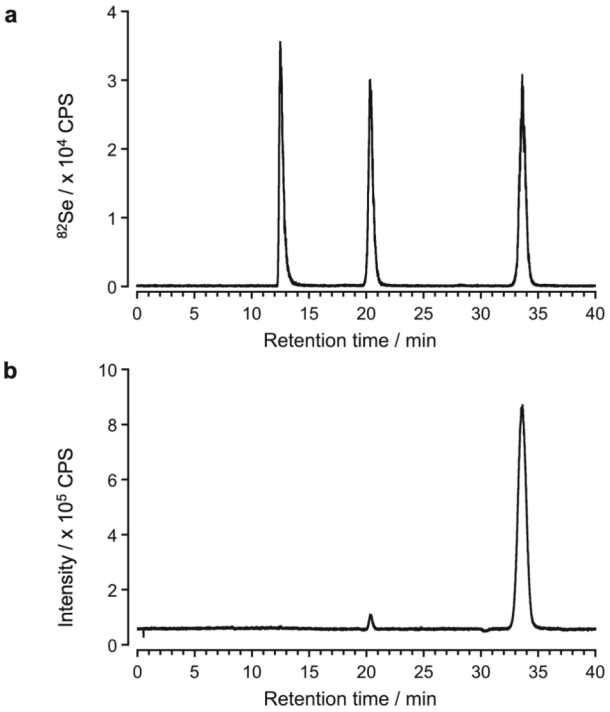

Fig. 1 Chromatograms of Se-containing compounds as measured by ICP-MS and ESI-Q-TOF-MS. Se compounds, such as selenate, SeMet, and TMSe, were applied on a GS-320HQ column, and the column was eluted with 10 mM ammonium acetate. The eluate was introduced into an ICP-MS or an ESI-Q-TOF-MS. The elution of Se was monitored at *m/z* 82 by ICP-MS (a). Total ion counts detected in the positive-ion mode are depicted (b).

(Yamanashi, Japan), respectively. All reagents used in this study were of the highest or analytical grade. Deionized water (18.3 MΩ cm) was used throughout.

LC-ICP-MS and LC-ESI-Q-TOF-MS analyses

An Agilent 7700 ICP-MS (Agilent Technologies, Tokyo, Japan) equipped with an octopole reaction system (ORS) and an Agilent 6550 ESI-Q-TOF-MS (Agilent Technologies) were used. Both mass spectrometers were tuned daily and calibrated with a standard solution provided by the manufacturer. The ICP-MS or the ESI-Q-TOF-MS was coupled to an HPLC system (Shimadzu Prominence or Agilent 1200, respectively). A Shodex GS-320HQ multi-mode gel filtration column (7.5 i.d. × 300 mm, with a guard column, 7.5 i.d. × 75 mm, Showa Denko, Tokyo, Japan) was used. A 20 or 10 µL aliquot of the sample was injected into the column for LC-ICP-MS or LC-ESI-Q-TOF-MS, respectively, and then eluted with 10 mmol L^{-1} ammonium acetate, pH 6.5, at a flow rate of 0.6 mL min^{-1}. The operating conditions are summarized in Table 1.

Results and Discussion

Speciation of Se-containing compounds by LC-ICP-MS

Selenate, SeMet, and TMSe, each at 1.0 µg Se/mL, were eluted at retention times of 12.5, 20.4, and 33.6 min, respectively (Fig. 1a). The Shodex GS-320HQ column is a multi-mode size exclusion column that has additional separation modes aside from the size-exclusion mode. Under the elution conditions, the cation-exchange mode appeared. Indeed, TMSe, a cation, was mostly retained and selenate, an anion, was most rapidly eluted from the column. The peak sizes of selenate, SeMet, and TMSe were almost identical, suggesting that ICP-MS could detect Se-containing compounds with identical sensitivity. The total ion counts detected by LC-ESI-Q-TOF-MS in the positive-ion mode are depicted in Fig. 1b. Although all Se compounds were eluted at the same concentration, *i.e.*, 5.0 µg Se/mL, their intensities varied, as compared to Fig. 1a. Namely, TMSe was detected with the highest sensitivity in the positive-ion mode, followed by SeMet, and no selenate was detected (Fig. 1b). TMSe, a cation, was more sensitively detected than neutral and negatively charged compounds in the positive-ion mode, indicating that the sensitivity of the Se compounds to the detection by ESI-MS was dependent on their chemical forms. Contrary to the positive-ion mode, selenate was detected with the highest sensitivity in the negative-ion mode, followed by SeMet, and no TMSe was detected (data not shown). To confirm the identification of Se-containing compounds, the mass spectra at retention times of 11.9 – 12.5, 20.1 – 20.7, and 33.0 – 34.2 min, which contained peaks for selenate, SeMet, and TMSe, respectively, were measured. Se consists of six isotopes: ^{74}Se (0.89%), ^{76}Se (9.36%), ^{77}Se (7.63%), ^{78}Se (23.8%), ^{80}Se (49.6%), and ^{82}Se (8.73%). The isotopic pattern appeared at around *m/z* 144.9050 in the eluate at a retention time of 11.9 – 12.5 min in the negative-ion mode (Fig. 2a). The theoretical mass of ^{80}Se-containing selenate is 144.9040. The mass accuracy was +6.9 ppm; thus, the Se peak at a retention time of 12.5 min was assigned to selenate. Likewise, SeMet and TMSe were also assigned by LC-ESI-Q-TOF-MS (Figs. 2b and 2c). The mass accuracies of SeMet and TMSe were +1.0 and +2.4 ppm, respectively. This easy and robust identification is the major advantage of ESI-Q-TOF-MS over ICP-MS.

Comparison of sensitivity for detection of Se-containing compounds between ICP-MS and ESI-Q-TOF-MS

The sensitivity for the detection of Se-containing compounds by ICP-MS and ESI-Q-TOF-MS under our conventional conditions was evaluated. The elution profile of Se detected by

ICP-MS at *m/z* 82 is depicted in Fig. 1a. Likewise, the elution profiles of selenate, SeMet, and TMSe detected by ESI-Q-TOF-MS at the selected *m/z* were obtained for a sensitivity comparison. Selenate was monitored at *m/z* 146.9052 ± 0.1000 in the negative-ion mode as a ^{82}Se-containing molecule (Fig. 3a). The peak at a retention time of 12.5 min in Fig. 3a seemed to be narrower than that in Fig. 1a. This is because the eluate is more easily diffused in the flow route of ICP-MS than ESI-Q-TOF-MS, since ICP-MS is not optimized for use as an HPLC detector. The peak height of selenate was 684×10^3 counts per second (CPS) at a concentration of 5.0 µg/mL. The background equivalent concentrations (BECs) of selenate were 2.58 ng/L and 314 µg/L for ICP-MS and ESI-Q-TOF-MS, respectively (Table 2). Under our conventional conditions, the signals of $[SeMet+H–CH_3]^+$ were larger than those of $[SeMet+H]^+$ in the positive-ion mode. From this, it can be speculated that the methyl group in SeMet is readily cleaved during ionization under the condition. Thus, SeMet was monitored at *m/z*

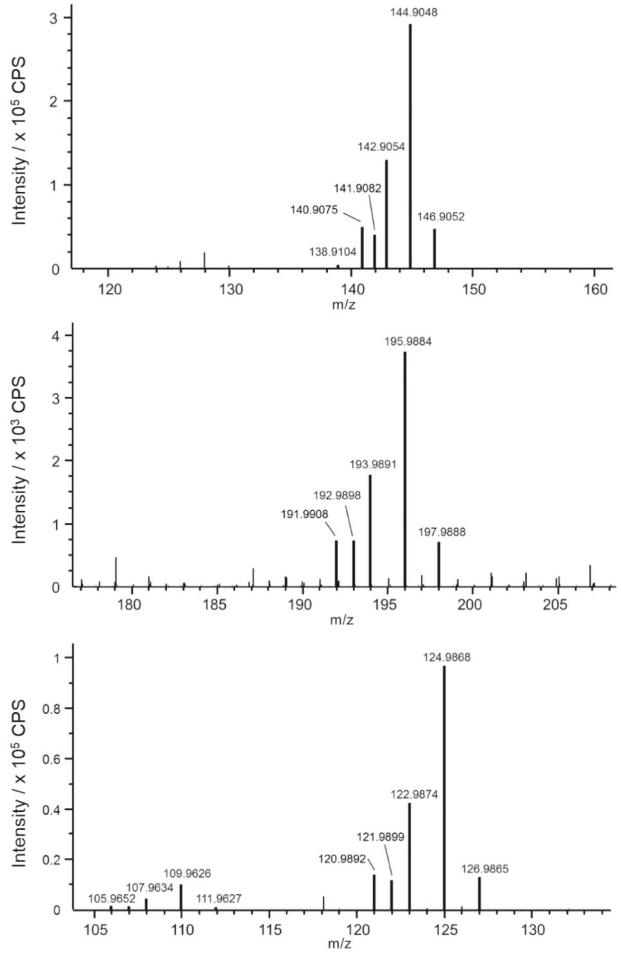

Fig. 2 Mass spectra of Se-containing compounds. Selenate (a) and SeMet (b) were monitored in the negative-ion mode, and TMSe (c) was monitored in the positive-ion mode.

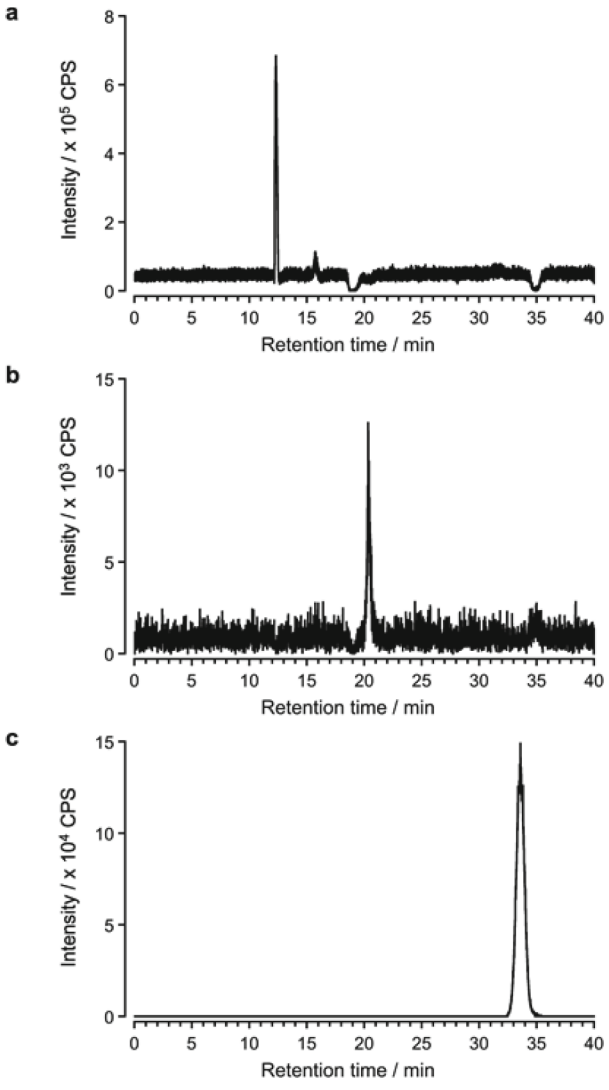

Fig. 3 Single-ion monitoring of Se-containing compounds by ESI-Q-TOF-MS. Selenate (a) and SeMet (b) were monitored in the negative-ion mode at *m/z* 146.9052 ± 0.1000 and 197.9895 ± 0.1000, respectively. TMSe (c) was monitored in the positive-ion mode at *m/z* 126.9868 ± 0.1000.

Table 2 Comparison of the sensitivity for the detection of Se-containing compounds between ICP-MS and ESI-Q-TOF-MS

Ion source	Selenate		SeMet			TMSe	
	ICP	ESI	ICP	ESI	ESI	ICP	ESI
m/z	82	146.9052 ± 0.1000	82	182.9756 ± 0.1000	197.9895 ± 0.1000	82	126.9868 ± 0.1000
Mode	positive	negative	positive	positive	negative	positive	positive
Concentration (µg Se/mL)	1.0	5.0	1.0	5.0	5.0	1.0	5.0
Peak height (CPS × 10^3)	35.6	684	30.1	10.8	12.6	29.5	149
BEC[a]	2.58 ng/L	314 µg/L	3.05 ng/L	< 220 µg/L	337 µg/L	3.15 ng/L	< 16.0 µg/L

a. Background equivalent concentration.

182.9756 ± 0.1000 in the positive-ion mode (Fig. 3b). In addition, SeMet was monitored at *m/z* 197.9895 ± 0.1000 in the negative-ion mode (Fig. 3b). BECs of SeMet obtained by ESI-Q-TOF-MS were < 220 and 337 µg/mL in the positive and negative modes, respectively. BEC of SeMet, obtained by ICP-MS, was 3.05 ng/L (Table 2). The peak height of TMSe was 149×10^3 cps at *m/z* 126.9868 ± 0.1000 at a concentration of 5.0 µg/mL in the positive-ion mode (Fig. 3c). BECs of TMSe obtained by ICP-MS and ESI-Q-TOF-MS were 3.15 ng/L and < 16.0 µg/L, respectively. From the results, it is obvious that the sensitivity of Se-containing compounds to ESI-Q-TOF-MS analysis is strongly dependent on their chemical species. More easily ionized compounds, such as TMSe and selenate, were more sensitive than the zwitterion, SeMet. ICP-MS showed higher sensitivity for the detection of Se compounds than ESI-Q-TOF-MS without any species dependency. This is the advantage of ICP-MS over ESI-Q-TOF-MS.

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