Determination of Nine Organoselenium Compounds Using High-performance Liquid Chromatography Coupled with Electrospray Mass Spectrometry

Georges-Marie Momplaisir*, Leon D. Betowski, and Witold Winnik‡

US Environmental Protection Agency, National Exposure Research Laboratory, Environmental Sciences Division, P.O. Box 93478, Las Vegas, NV 89193-3478, USA

* U.S. National Research Council Fellow

‡Present address: Environmental Medicine, New York University Medical Center, Tuxedo Park, NY 10987

1. Introduction

Selenium is an essential ultratrace element to most mammalian species, including man. It is an integral component of the enzyme glutathione peroxidase (GSHPx), which protects cells against oxidative damage.[1] Although essential to these organisms, the element can also be highly toxic. A scant tenfold increase in the recommended daily intake of selenium to man (50-70 µg/day)[2] converts overt signs of deficiency into overt signs of toxicity. Both the nutritional bioavailability[3] and toxicity[4,5] of selenium were found to be species dependent. It is therefore important to identify and quantify the individual compounds of selenium present in a sample.

A popular approach for the identification/quantification of selenium compounds has been to couple a chromatographic separation with on-line detection. Atomic absorption spectrometry, inductively coupled plasma atomic emission spectrometry or inductively coupled plasma mass spectrometry are the most commonly used detectors. These detection systems do not provide any structural information about the analytes, and therefore make it difficult to identify compounds for which synthetic standards are not available.

This paper presents an analytical method for the separation of nine organoselenium compounds, by reversed phase high-performance liquid chromatography (HPLC) with on-line detection by electrospray mass spectrometry (ES-MS). The HPLC-ES-MS method was then applied to the determination of organoselenium compounds in aqueous extracts of a selenium-enriched food supplement, a plant sample and a urine reference material (NIST SRM 2670).

2. The Organoselenium Compounds

The analytical work included nine selenium species that were selected because of their reported or suspected occurrence in environmental/biological materials. The name acronyms and formulae of the selenium compounds are presented in Table 1.

Table 1. Acronyms, Names and Formulae of Nine Selenium Compounds

Selenoamino Acids		Formula
SeM	Selenomethionine	$CH_3SeCH_2CH_2CHCOO^-$ NH_3^+
SeC	Selenocystine	$^-OOCCHCH_2Se\text{-}SeCH_2CHCOO^-$ NH_3^+ NH_3^+
SeMC	Methyl Selenocysteine	$CH_3SeCH_2CHCOO^-$ NH_3^+
SeAC	Allyl Selenocysteine	$CH_2{=}CHCH_2SeCH_2CHCOO^-$ NH_3^+
SePC	Propyl Selenocysteine	$CH_3CH_2CH_2SeCH_2CHCOO^-$ NH_3^+
SeE	Selenoethionine	$CH_3CH_2SeCH_2CH_2CHCOO^-$ NH_3^+
SeCt	Selenocystamine	$H_2CCH_2Se\text{-}SeCH_2CH_2$ NH_3^+ NH_2
Sechol	Selenoniumcholine	$(CH_3)_2Se^+CH_2CH_2OH$
TMSe	Trimethylselenonium-ion	$(CH_3)_3Se^+$

3. Electrospray Ionization of the Organoselenium Compounds

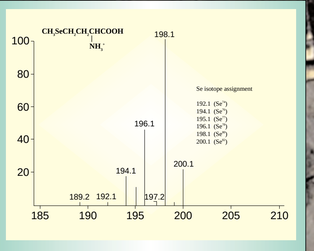

Figure 1. The protonated molecular ion's region of Selenomethionine, showing the relative abundance of the Selenium isotopes.

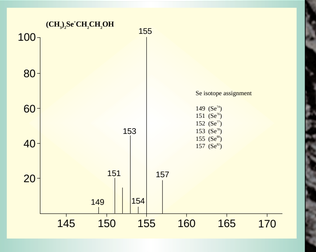

Figure 2. The molecular ion's region of Selenoniumcholine, showing the relative abundance of the Selenium isotopes.

A Finnigan MAT TSQ 700 triple quadrupole mass spectrometer equipped with an electrospray source was used to characterize the synthetic organoselenium compounds. The mass spectrometer was operated in the positive ion mode. The spray voltage was increased to 5.2 kV and the capillary temperature was adjusted to 220°C. Protonated molecular ions were the most abundant ions for selenocystamine and all the selenoamino acids studied. As an example, the mass spectrum of selenomethionine showing the molecular ion's region is reproduced in **Figure 1**. Selenium has six stable isotopes (74, 76, 77, 78, 80 and 82), most of which appeared in the mass spectrum of the individual Se-compounds.

For selenoniumcholine and the trimethylselenonium-ion, which exist as cations in solution, intense (100%) molecular ions were observed [**Figure 2** (molecular ion's region of selenoniumcholine)]. The selected selenium compounds were used as standards for the development of chromatographic separation procedures. The peak corresponding to the most intense ion of each Se-compound was monitored during HPLC-ES-MS analyses.

4. HPLC-ES-MS Analysis of the Organoselenium Standards

Operating parameters, such as the flow rates of gas (sheath gas and auxiliary gas) and HPLC mobile phases, the spray voltage and capillary temperature of the hyphenated system, have been optimized for maximum selenium analytes detection signals. The use of ion-pairing reagents in the HPLC mobile phase was avoided because of the analyte signal suppression they cause during ES ionization. Taking into consideration this limitation, three chromatographic separation procedures have been developed.

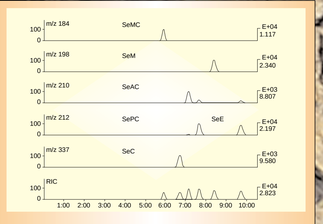

Figure 3. HPLC-ES-MS chromatogram of 20 ng each (as Se) of Se-methylselenocysteine (t_R, 5.55 min), Selenocystine (t_R, 6.42), Se-allylselenocysteine (t_R, 709), Se-propylselenocysteine (t_R, 7.39), Selenomethionine (t_R, 8.24), and Selenoethionine (t_R, 9.44). The analytes were eluted from the cyanopropyl column with an aqueous mobile phase which contained methanol 30% (w/w) and acetic acid 1% (w/w).

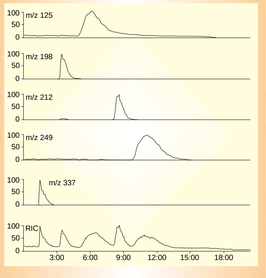

Figure 4. HPLC-ES-MS chromatogram of 20 ng each (as Se) of Selenocystine (t_R, 2.00 min), Selenomethionine (t_R, 4.204), Trimethylselenonium-ion (t_R, 6.38), Selenoethionine (t_R, 8.27) and Selenocystamine (t_R, 11.02). The analytes were separated on a C_{18} column with 1% (w/w) acetic acid.

A mixture of the six selenoamino acids (selenocystine, Se-methylselenocysteine, Se-allyl selenocysteine, Sepropylselenocysteine, selenomethionine, and selenoethionine) was separated on a silica-based cyanopropyl column (5 µm silica support, 4.6 mm i.d. x 15 cm, LC-CN, Supelco, INC.; Bellefonte, PA), with a methanol-rich mobile phase, which contained acetic acid. As shown in the chromatogram of **Figure 3,** SeMC was the first compound to elute from the chromatographic column, followed by SeC, SeAC, SePC, SeM, and SeE. Selenocystamine, selenoniumcholine and the trimethylselenonium ion, when added to the mixture of selenoamino acids, were strongly retained on the cyanopropyl column and would not elute without addition of an ion-pairing reagent to the mobile phase. Another chromatographic approach involved the use of a C_{18} column (Spherisorb 3 µm C_{18} material 1 mm i.d. x 15 cm, Isco Inc.) and dilute acetic acid as eluent. With these conditions (**Figure 4**), SeC, SeM, TMSe, SeE, and SeCt were separated to base line in less than 15 minutes. Using the same C_{18} column and an aqueous mobile phase which contained trifluoroacetic acid as a modifier for amino acids and a small amount of methanol, SeC, SeMC, SeM, SeAC, Sechol, SePC and TMSe were effectively separated from each other **(Figure 5)**. The addition of methanol to the mobile phase was necessary in order to improve the chromatographic separation of the analytes.

Chromatographic limits of detection (LODs), defined as the amount of analyte that would produce a signal 3 times the peak to peak noise in the base line, range from 2 ng to 5 ng. Relative standard deviations were less than 10% at the 10 ng level. The detection signals of the Se-analytes did not vary appreciably with the composition of the mobile phase. The limits of detection of the selenium compounds were considered low enough to allow their detection/identification in many natural matrices.

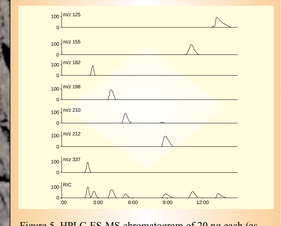

Figure 5. HPLC-ES-MS chromatogram of 20 ng each (as Se) of Selenocystine (t_R, 2.08 min), Se-methylselenocysteine (t_R, 2.59), Selenomethionine (t_R, 4.08), Se-allylselenocysteine (t_R, 5.22), Se-propylselenocysteine (t_R, 8.48), Selenoniumcholine (t_R, 11.52) and Trimethylselenonium ion (t_R, 13.32). The analytes were separated on a C_{18} column with 0.01% (v/v) trifluoroacetic acid.

5. Determination of Organoselenium Compounds in Natural Matrices by HPLC-ES-MS

The applicability of the HPLC-ES-MS system for the determination of selenium analytes in natural matrices is still under investigation. The samples that have been analyzed so far included a selenium-enriched yeast food supplement, a sample of plant collected from an abandoned manganese mine, and a human urine reference material (NIST SRM 2670). Prior to HPLC-ES-MS analysis, the urine sample was diluted three-fold with water and acidified with acetic acid. The plant sample and the selenium-enriched yeast food supplement were extracted according to the procedure reported by Gilon et al.[6], with slight modifications. Plant or yeast product (0.5 g) and protease (40 mg) were added to 6 ml H_2O in a 15-ml propylene centrifuge tube, sonicated for one hour, and kept in the dark for 24 hours. Protease XIV is a non specific protease, which breaks peptide bonds of any protein present in the materials. The use of a large excess of enzyme appeared to be efficient in cleaving the major parts of these bonds. The mixtures were then centrifuged for 20 minutes. The supernatant was removed and filtered through a 0.2 µm polypropylene filter and analyzed.

Selenomethionine, and Se-methylselenocysteine were identified in the selenium enriched food supplement extract **(Figure 6),** selenomethionine being the predominant selenium compound in the sample. The plant sample contained Se-methylselenocysteine as a major selenium species, as well as selenocystine and other selenium constituents whose identities are under investigation **(Figure 7).** Trimethylselenonium-ion, a metabolite of selenium that has been identified in human urine, was not found in the urine reference material. Other selenium compounds such as selenite and selenate, might have been present as well in the samples but could not be analyzed in the positive-ion mode. The determination of selenium compounds by ES-MS using a negative-ion mode will be the subject of another study.

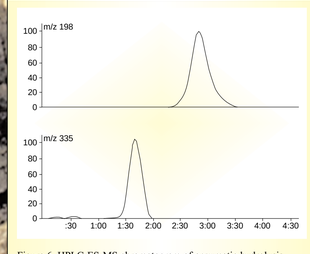

Figure 6. HPLC-ES-MS chromatogram of enzymatic hydrolysis water extract of the Selenium-enriched yeast food supplement, indicating the presence of Selenocystine (t_R, 1.40 min) and Selenomethionine (t_R, 2.51).

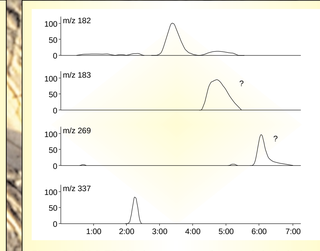

Figure 7. HPLC-ES-MS chromatogram of enzymatic hydrolysis water extract of the Plant sample, showing the presence of Selenocystine (t_R, 2.15 min), Se-methylselenocysteine (t_R, 3.26).

6. Conclusion

It has been demonstrated that ES-MS can be used for the efficient determination of selenium compounds. The combination of HPLC-ES-MS provides a very selective and yet sensitive tool to conduct successfully the separation, identification, and quantification of selenium species in many environmental and biological samples. The selenium analytes were identified based on their chromatographic retention times and the structural information provided by the mass spectrometer. The urine sample did not contain any detectable amount of selenoamino acids or selenonium compounds. Several species of selenoamino acids have been identified in the yeast supplement and the plant sample. Identification of other selenium compounds present in the yeast supplement and the plant extracts is currently being made.

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