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Determination of trimethylselenonium iodide, selenomethionine, selenious acid, and selenic acid using high-performance liquid chromatography with on-line detection by inductively coupled plasma mass spectrometry or flame atomic absorption spectrometry

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Abstract

An analytical method has been developed for the determination of selenious acid, selenic acid, trimethylselenonium ion, and selenomethionine. The four selenium compounds were separated by HPLC on a column (25 cm×4 mm I.D.) of the anion-exchanger ESA Anion III with a mobile phase (1.5 ml/min) of 0.0055 *M* ammonium citrate (pH 5.5). Detection was carried out using an on-line inductively coupled plasma mass spectrometer (ICP-MS) or a flame atomic absorption spectrometer (FAAS) as the selenium-specific detector. The chromatographic parameters and the chemical factors affecting the separation of the selenium species were optimized. The four selenium compounds could be separated within 8 minutes. The detection limits of the coupled HPLC–FAAS system were approximately 1 mg Se/l for each compound (100 μl injection), estimated as three times the base-line noise of the chromatograms. More powerful selenium detection was achieved with an ICP-MS. Selenium was measured at *m/z* 78. To increase the nebulization efficiency, the Meinhard concentric glass nebulizer was replaced by an ultrasonic nebulizer. The ICP-MS signal intensity was increased with the ultrasonic nebulization by a factor of 7 times for selenious acid and 24 to 31 times for trimethylselenonium ion, selenomethionine, and selenic acid compared to that with the Meinhard nebulization. The detection limits achieved by the HPLC–ICP-MS with the ultrasonic nebulization were 0.08 μg Se/l for trimethylselenonium ion, 0.34 μg Se/l for selenious acid, 0.18 μg Se/l for selenomethionine, and 0.07 μg Se/l for selenic acid, respectively.

Keywords: Selenium compounds

1. Introduction

In recent years, the chemistry and biology of selenium and its various species have been the subject of increasing attention, due to the importance of selenium both as an essential and toxic element. However, the identification and determination of the many chemical forms of selenium in environmental and biological systems is still a major challenge for analytical chemists, a prerequisite to investigate its pathways in the environment and its mechanisms of action in living organisms.

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The analytical chemistry of selenium with a focus on its speciation has been reviewed recently [1]. Compared with the extensive investigations on inorganic selenium, little speciation work has been done for organic selenium compounds.

The inorganic selenium species, selenious acid and selenic acid (selenite and selenate), are very important in the biochemical cycle of selenium. Because of their difference in oxidation state, these two species exhibit quite different chemical and biological properties. Organic species of selenium, such as selenoamino acids, take part in the biological selenium cycle and are incorporated into proteins. The main selenoamino acid evidenced in plants is selenomethionine (SeMet) [2], which is used as selenium supplement in the diet of man and animals. Trimethylselenonium ion (TMSe) has been identified in urine [3] and is used as a tracer of Se levels of humans.

Since the toxicity, bioavailability and transport of selenium depend on different selenium forms and their concentrations, it is essential to selectively determine selenium species present in the studied samples.

The use of atomic spectrometry as a detection system in high performance liquid chromatography entails the introduction of element-specific detectors coupled with this separation technique [4]. Among the atomic spectrometric techniques, ICP-MS is a powerful element-specific detector. ICP-MS allows on-line detection of the separated selenium species at biological sample concentrations [5].

This study presents an analytical method for the separation and determination of selenious acid, selenic acid, TMSe, and SeMet by anion-exchange HPLC with on-line specific detection of selenium by ICP-MS or FAAS.

2. Experimental

2.1. Chemicals and reagents

All commercial chemicals were of analytical grade and were used without further purification. Sodium selenate was purchased from Fluka, sodium selenite pentahydrate from Merck, and seleno-DL-methionine from Sigma. Trimethylselenonium iodide was prepared according to literature procedure [6].

Stock solutions were prepared with NANOpure water (18.0 MΩ cm) from anhydrous sodium selenate (1196.4 mg to 500 ml, 1000 mg Se/l), from sodium selenite pentahydrate (1665.5 mg to 500 ml, 1000 mg Se/l), from trimethylselenonium iodide (63.6 mg to 20 ml, 1000 mg Se/l), and from selenomethionine (24.8 mg to 20 ml, 500 mg Se/l). The stock solutions were stored in the refrigerator at −20°C before use. No degradation of the compounds was observed over 3 months of storage.

Solutions of the selenium compounds with concentrations in the range 1.00–50.0 μg Se/l were prepared daily by appropriate dilution of the stock solutions with NANOpure water.

2.2. Mobile phase

A citrate solution (0.0055 *M*) was prepared by dissolving 1.13 g di-ammonium hydrogen citrate (Merck analytical grade) to 1000 ml with NANOpure water. The pH of the solution was adjusted by drop-wise addition of aqueous ammonia solution (25%, Merck analytical grade). The pH was measured with a pH meter. Aliquots (50 μl) of a rubidium chloride solution (1000 mg Rb/l) were added to the mobile phases as an internal standard for ICP-MS.

2.3. Instrumentation

Separations were performed on a strong basic anion-exchange column, ESA Anion III (Reno, USA; 25 cm×4 mm I.D., 10 μm spherical poly-(styrene-divinylbenzene) particles with trimethylammonium exchange sites). A PRP X-100 precolumn (25 mm×4 mm I.D.) filled with the same stationary phase protected the analytical column.

The HPLC–FAAS system consisted of a Waters 600E multisolvent delivery system (Waters Division of Millipore, Milford, USA), a 100-μl injection loop in conjunction with a Rheodyne 9125 six-port injection valve (Rheodyne, Cotati, USA), and a Hitachi 6100 flame polarized Zeeman atomic absorption spectrophotometer as the selenium-specific detector. The analyte solutions were injected with a microliter

syringe (250 μl, Hamilton 1000 series). The outlet of the ESA Anion III was connected via a 50-mm long plastic capillary tube (1.0 mm I.D.) to the FAAS nebulizer. The instrumental operating conditions are described in the Table 1. The chromatograms were recorded by a PC. The retention times and peak areas determined with software written in house [7].

The HPLC–ICP-MS system consisted of a Hewlett Packard 1050 solvent delivery unit (Hewlett Packard, Waldbronm, Germany), a 100 μl injection loop in conjunction with a Rheodyne 9125 six-port injection valve (Rheodyne, Cotati, USA), and an ICP-MS (VG Plasma Quad 2 Turbo Plus) as the selenium-specific detector. The outlet of the ESA Anion III exit was connected to the SB-30-A3 Meinhard concentric nebulizer or to the U-6000 AT^+ ultrasonic nebulizer via a 0.5 m 1/16 in. PEEK (polyether ether ketone) capillary tube (1 in.=2.54 cm). The column for the separation of selenium compounds was operated under the same condition as in the HPLC–FAAS experiments. The ICP-MS operating conditions are described in Table 1. The ion intensity at m/z 78 was monitored using the 'time-resolved' analysis software© Version 1a (Fisons Scientific Equipment Division, Middlesex, UK). Prior to each HPLC–ICP-MS run, the ion

Table 1
Operating conditions of the chromatographic system and the FAAS and ICP-MS detectors

HPLC		
Mobile phase		0.0055 *M* ammonium citrate, pH 5.5
Flow rate		1.5 ml/min
Injected sample volume		100 μl
FAAS		
Selenium lamp current		12.5 mA
Wavelength		196.0 nm
Slit width		1.3 nm
Burner height		7.5 mm
C_2H_2 pressure		15 kPa
Air pressure		160 kPa
Measurement mode		Absorption
Background correction		on
ICP-MS		
Plasma rf power	forward	1.40 kW
	reflected	<5 W
Argon gas flows	cooling	13.5 l/min
	auxiliary	1.1 l/min
Ion sampling	sample cone	Nickel, orifice 1.0 mm diameter
	skimmer cone	Nickel, orifice 0.7 mm diameter
Vacuum	expansion	1.6 mbar
	intermediate	$<1.0\times10^{-4}$ mbar
	analyzer	2.1×10^{-6} mbar
Mass of selenium monitored		78
Time/slice		0.51 s
Slices		700
Total analysis time		500 s
Meinhard nebulizer gas		0.84 l/min
Ultrasonic nebulizer		
Nebulizer gas		0.84 l/min, optimized on ^{87}Rb
Heater temperature		140°C
Condenser temperature		2°C
Membrane desolvator	Heater temperature	160°C
	Sweep gas flow	2.6 l/min

intensity at m/z 87 (^{87}Rb) was checked at the rate meter while aspirating the mobile phase containing rubidium at a concentration of 50 μg/l. The lens settings were adjusted for optimal response of the instrument (typically 3×10^6 Hz for 50 μg Rb/l). The chromatograms were exported and the retention times and peak areas determined with software written in house [7].

3. Results and discussion

3.1. Selection and optimization of the chromatographic system

Selenious acid, selenic acid, TMSe, and SeMet can be present in solution as anions, cations, or zwitterions. The distribution diagrams for selenious acid, selenic acid, SeMet, and citric acid are shown in Fig. 1. Selenic acid, a strong acid, and selenious acid, a weak acid, can be present in aqueous solution as anions with one or two negative charges. At pH values below 4.0, selenious acid may remain undissociated. SeMet will carry a positive charge at relatively low pH localized to the protonated amino group, but will be zwitterionic (ammonium group, carboxylate group) at intermediate pH, and becomes anionic (carboxylate group) at higher pH. TMSe is a cation irrespective of pH.

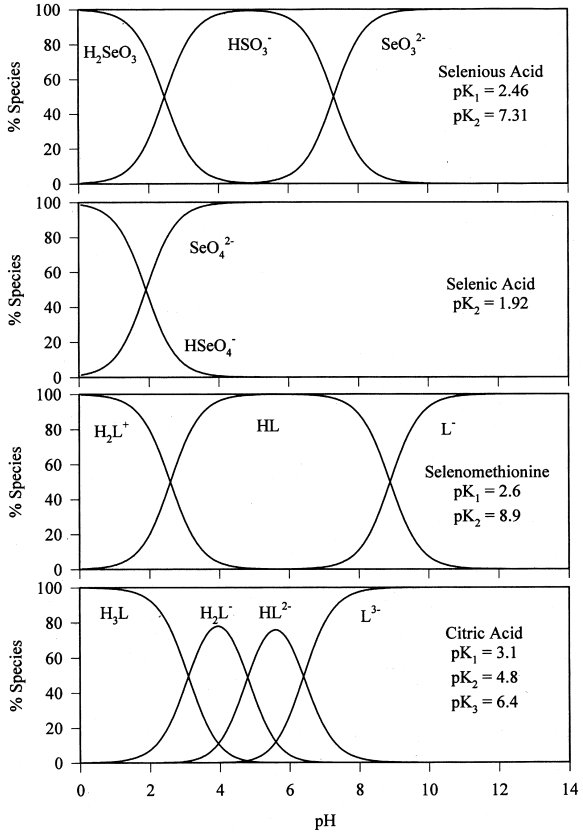

Fig. 1. Species distribution diagrams for selenious acid, selenic acid, SeMet, and citric acid in the pH range from 0 to 14.

An anion-exchange HPLC was chosen to separate the four selenium compounds, because it utilizes the pH-dependent anionic character of the species and allows the use of an aqueous mobile phase. The ESA Anion III column used in this study allows a wide pH range from 1 to 13. An aqueous solution of the ammonium citrate used in combination with ammonia for pH adjustment proved successful as the chromatographic mobile phase, because it not only has the buffer capabilities in the range of the pK_a of the anionic selenium compounds but also avoids an organic solvent load, which is often used in mobile phases and could destabilize or extinguish the argon plasma of the ICP-MS.

3.2. Retention behavior of selenium compounds

The column void volumes of the chromatographic systems including the ESA Anion III column were determined by passing a solution of LiCl (10 mg/l) through the column at various flow rates. The total effluent was monitored for lithium by FAAS. The void volume of the HPLC–FAAS system was found to be 2.05±0.01 ml.

3.2.1. Effect of pH

Dependence of retention behavior of the four selenium compounds on the pH of the mobile phase (0.0055 *M* aqueous solution of ammonium citrate) in the pH range 3.0–7.0 was investigated using the ESA Anion III column. The capacity factors for the four selenium compounds in the pH range 3.0–7.0 are shown in Fig. 2. In the pH range 3.0–7.0, TMSe was eluted in the dead volume, because of its cationic nature. The retention time of SeMet was

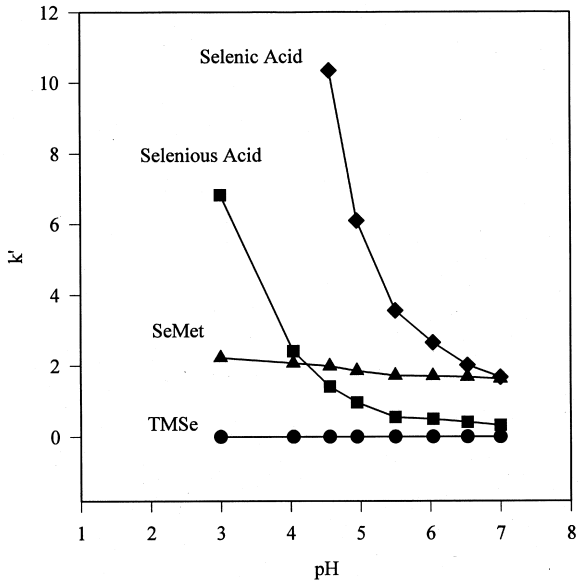

Fig. 2. Dependence of k'-values of the selenium compounds on the pH (3.0–7.0) of the mobile phase (flow rate 1.5 ml/min).

almost constant, because it is neutral in the pH range 3.0–7.0. The capacity factor for selenious acid decreased from 6.8 at pH 3.0 to 0.3 at pH 7.0. In the pH range 4.6–7.0 the capacity factor for selenic acid decreased quickly from 10.3 to 1.7. The retention behavior of selenious acid and selenic acid on the anion-exchange column is governed by the pH-controlled protonation of the selenium oxo-anions and the citrate anion. These anions compete for the ammonium groups of the stationary phase. At a pH of the mobile phase higher than 4.0, selenious acid eluted before SeMet. Because between pH 3.0–7.0 selenious acid is present as $HSeO_3^-$ ($pK_1=2.3$ and $pK_2=7.8$) and selenic acid as SeO_4^{2-} ($pK_2=2.1$), selenic acid has the strongest electrostatic interaction with the stationary phase and was eluted last. From pH 5 to pH 6 of the mobile phase, the retention times of the species were reasonably different and in a convenient range. The pH of the mobile phase for the further work was chosen as 5.5.

3.2.2. Effect of flow rate

To be compatible with the flow rate of ICP nebulization, the flow rates of the mobile phase in the range 1.0–2.0 ml/min were tested. The four selenium compounds were fully separated when the flow rate of the mobile phase (0.0055 *M* ammonium citrate, pH 5.5) was 1.0–2.0 ml/min. The flow rate of 1.5 ml/min was used in further experiments.

3.2.3. Performance characteristics of HPLC–FAAS system

A typical chromatogram for a solution containing selenious acid, selenic acid, SeMet, and TMSe with on-line FAAS detection is shown in Fig. 3. The four selenium species were fully resolved and the separation was complete in less than 8 min. The peak areas of absorbance of the four selenium species (100 mg Se/l for each selenium compound) are almost identical. This indicates that the FAAS-response of selenium does not depend on the molecular forms of selenium compounds.

The detection limits of the coupled HPLC–FAAS system were approximately 1 mg Se/l for each compound, estimated as three times of the base-line noise of the chromatograms. These relatively high

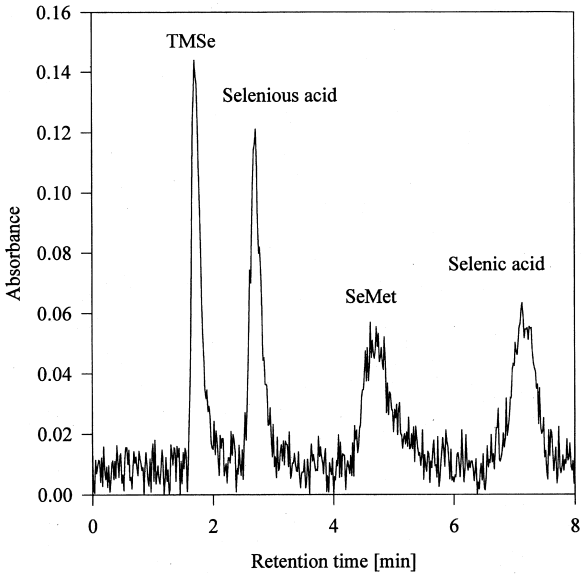

Fig. 3. HPLC–FAAS chromatogram of a solution (100.0 μl) containing selenious acid, selenic acid, SeMet, and TMSe (100.0 mg Se/l of each).

detection limits may be sufficient for certain applications, e.g. selenium speciation in selenized yeast or in selenium-rich plants. In this study, the HPLC–FAAS system was primarily used for chromatographic development.

For the detection of the individual selenium species in biological samples, a much more powerful chromatographic detector ICP-MS is required.

3.3. Performance characteristics of HPLC–ICP-MS system

To improve the detection limits, an ICP-MS was used as the selenium-specific detector instead of the FAAS detector. For the detection of selenium with ICP-MS, six selenium isotopes with natural abundances between 0.96% and 49.96% are available. The major selenium isotope ^{80}Se (49.96%) suffers from a severe $^{40}Ar_2$ interference. The relative abundance of ^{74}Se is only 0.96%. With this isotope, low detection limits are not achievable. From the remaining isotopes (^{76}Se 9.12%, ^{77}Se 7.5%, ^{78}Se 23.61%, and ^{82}Se 8.84%), ^{78}Se was chosen because of its higher relative abundance.

Table 2
Determination of selenium in an aqueous solution containing four selenium compounds (100 μg Se/l, each) by HPLC–ICP-MS at m/z 78 and comparison of the signal areas between ultrasonic nebulization (USN) and Meinhard nebulization (100 μl injection)

Se Compound	Signal Area		
	USN (counts)	Meinhard (counts)	USN/Meinhard (relative)
TMSe	5.22×10^5	1.67×10^4	31
Selenious acid	1.07×10^5	1.61×10^4	7
SeMet	3.39×10^5	1.40×10^4	24
Selenic acid	4.83×10^5	1.86×10^4	26

3.3.1. Comparison of ultrasonic and Meinhard nebulization

The sample introduction into the ICP is a critical part in ICP-MS. Pneumatic nebulizers are commonly used in ICP-MS. The ultrasonic nebulizer (USN) is used to increase the nebulization efficiency and to improve detection limits of the ICP-MS. The chromatograms obtained with the on-line detection by ICP-MS with a Meinhard or an ultrasonic nebulizer under the optimized conditions are shown in Fig. 4.

The peak areas (100 μg Se/l for each selenium

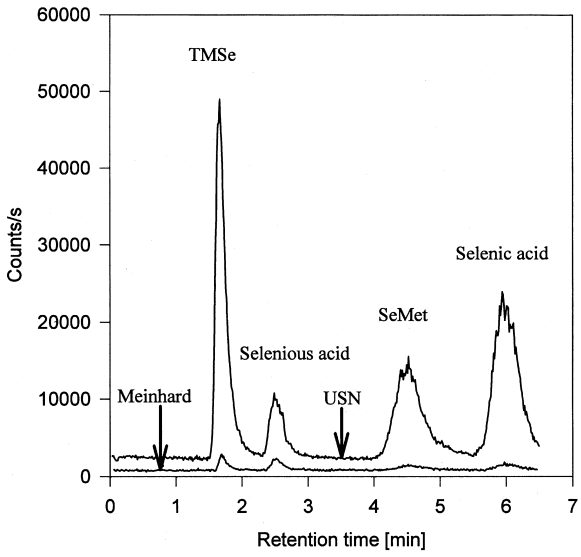

Fig. 4. HPLC–ICP-MS chromatogram of a solution (100 μl) containing selenious acid, selenic acid, SeMet, and TMSe (100.0 μg Se/l of each) with <Meinhard or ultrasonic nebulization (USN).

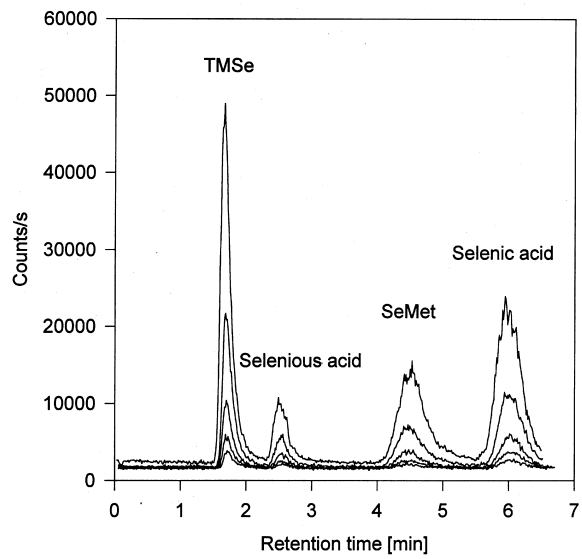

Fig. 5. HPLC–USN-ICP-MS chromatograms of solutions (100 μl) containing 5.00, 10.0, 20.0, 50.0, or 100 μg Se/l of selenious acid, selenic acid, SeMet, and TMSe.

Table 3
Calibration curves for the selenium compounds

Compound	Calibration curve $y=kx+d$[a]	Regression r^2	Determination limit μg Se/l
Selenic acid	$y=4222x+5412$	0.9997	0.07
Selenious acid	$y=1033x+1393$	0.9944	0.34
SeMet	$y=2727x-2477$	0.9996	0.18
TMSe	$y=4215x+412$	0.9999	0.08

[a] y=peak area (counts); x=concentration of selenium (μg/l).

compound) of the four selenium species were almost identical (Table 2) when the Meinhard nebulizer was used. This indicates that the responsibility of the ICP-MS with the Meinhard nebulizer for detection of selenium does not depend on the molecular forms of selenium compounds. When Crews et al. [8] investigated an ICP-MS with a fixed cross-flow nebulizer and a water-cooled double-pass Scott-type spray chamber, they found that the responses of Se from selenomethionine and selenocystine were approximately 40% and 55% of that from selenite or selenate.

In this work, the responsibility of the ICP-MS with the ultrasonic nebulizer for detection of selenium was species dependent (Table 2). Compared with the Meinhard nebulizer, the ultrasonic nebulizer has a higher nebulization efficiency. The signals increased about 7 times for selenious acid and 24–31 times for TMSe, SeMet, and selenic acid. However, during the desolvation process of ultrasonic nebulization, which is necessary to reduce the water loading in the aerosol to avoid extinguishing the plasma, a compound may have different properties, such as chemical reactivity and stability, from those under normal conditions.

The response of the HPLC–USN-ICP-MS for selenious acid was found to be lower than that for selenic acid in aqueous solutions at the same concentration. Yang et al. [9] observed the similar behavior of selenite during thermospray nebulization and ICP atomic emission spectrometry (ICP-AES) detection. They found that the low sensitivity for selenite resulted from its reduction during the thermospray process to metallic selenium, which deposited on the vaporizer and, therefore, was not transported to the ICP.

3.3.2. Calibration and detection limits of HPLC–USN-ICP-MS

Calibration curves were obtained from the areas of the HPLC–USN-ICP-MS signals at m/z 78 by chromatographing aliquots (100 μl) of solutions containing 5.00, 10.0, 20.0, 50.0, or 100 μg Se/l of selenious acid, selenic acid, TMSe, and SeMet on the ESA Anion III anion-exchange column (Fig. 5). The calibration curves based on peak areas were linear for each selenium compound in this concentration range (Table 3). The determination limits were 0.08 μg Se/l for TMSe, 0.34 μg Se/l for selenious acid, 0.18 μg Se/l for SeMet, and 0.07 μg Se/l for selenic acid, respectively, calculated as the concentration of selenium required to reach a signal of three times of the baseline noise.

This isocratic anion-exchange method with the on-line USN-ICP-MS detection has potential for the determination of selenium compounds in biological samples.

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