DETERMINATION OF SELENIUM COMPOUNDS BY HPLC WITH ICP-MS OR FAAS AS SELENIUM-SPECIFIC DETECTOR

Li Fangshi[1], Walter Goessler and Kurt J Irgolic

[1] (*Department of Applied Chemistry, Nanjing University of Chemical Technology, Nanjing, 210009*)

(*Institute for Analytical Chemistry, Karl-Franzens-University Graz, Universitaetsplatz 1, A-8010 Graz, Austria*)

Abstract A speciation method was developed for selenious acid, selenic acid, trimethylselenonium ion (TMSe) and selenomethionine (SeMet). Separation of the four selenium species was achieved by HPLC on an ESA Anion III anion-exchange column using aqueous mobile phase of 5.5 mmol/L ammonium citrate at pH 5.5 with a flow rate of 1.5 mL/min. Under the optimal conditions, the four selenium species were separated within 8 minutes. On-line selenium-specific detection was carried out with an inductively coupled plasma mass spectrometer (ICP-MS) or a flame atomic absorption spectrometer (FAAS). The detection limits of HPLC-FAAS were approximately $\rho(Se) = 1$ mg/L for each compound (100 μL injection). To increase the nebulization efficiency of the ICP-MS, the Meinhard concentric nebulizer was replaced by an ultrasonic nebulizer (USN). The ICP-MS signal intensity was increased by a factor of 7 for selenious acid and 24 to 31 for TMSe, SeMet and selenic acid with the USN compared to that with the Meinhard nebulizer. The detection limits of the HPLC-USN-ICP-MS were $\rho(Se) = 0.08$ μg/L for TMSe, $\rho(Se) = 0.34$ μg/L for selenious acid, $\rho(Se) = 0.18$ μg/L for SeMet and $\rho(Se) = 0.07$ μg/L for selenic acid.

Key words high performance liquid chromatography, inductively coupled plasma-mass spectrometer, flame atomic absorption spectrometer, selenium compound, speciation

Classification number O658

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 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.

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

The inorganic 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 SeMet[2], which is used as selenium supplement in the diet of man and animals. 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 are depending on different selenium forms

本文收稿日期:1998-07-17,修回日期:1998-10-25

and their concentrations, it is essential to selectively determine selenium compounds present in the studied samples.

The use of atomic spectrometry as a detection system in HPLC entails the introduction of element-specific detectors coupled with this separation technique[4]. Among the atomic spectrometric techniques, ICP-MS seems to be the ideal selenium-specific detector, because it allows on-line detection of the separated selenium compounds 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 by ICP-MS or FAAS.

2 Experimental

2.1 Chemicals and reagents

All reagents were of analytical grade or higher purity from Fluka, Merck, or Sigma, except trimethylselenonium iodide which was prepared according to the literature procedure[6]. NANO pure water (18.0 MΩ · cm) was used throughout. Stock solutions [ρ(Se) = 100 mg/L, each] of selenic acid, selenious acid, SeMet and TMSe were prepared with NANOpure water and stored at −20 ℃ before use. Dilute solutions for analysis were prepared daily with NANOpure water.

The mobile phases were prepared by dissolving di-ammonium hydrogen citrate in NANOpure water and the pH was adjusted with 25% aqueous ammonia. Fifty μL rubidium chloride solution [ρ(Rb) = 1 g/L] was added to 1 000 mL mobile phase as internal standard for ICP-MS.

2.2 Instrumentations

The optimized operation conditions of the HPLC, the FAAS (Hitachi Z-6100) and the ICP-MS (VG Plasma Quad 2 Turbo Plus) are described in Table 1. The chromatograms were recorded by a PC. The retention times and peak areas were determined with the software written in house[7].

Prior to each HPLC-ICP-MS run, the ion intensity at m/z 87 (^{87}Rb) was checked at the rate meter while aspirating the mobile phase containing ρ(Rb) = 50 μg/L. The lens settings were adjusted for optimal response of the instrument (typically 3×10^6 Hz).

3 RESULTS AND DISCUSSION

3.1 Retention behavior of selenium compounds

Selenic acid, a strong acid ($pK_1 < 1$, $pK_2 = 1.92$), and selenious acid, a weak acid ($pK_1 = 2.46$, $pK_2 = 7.31$), 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($pK_1 = 2.6$, $pK_2 = 8.9$) 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.

Dependence of retention behavior of the four selenium compounds on the pH of the mobile phase in the pH range 3.0-7.0 was investigated (Fig. 1). In the pH range 3.0-7.0, TMSe was eluted in the dead volume, because of its cationic nature. The retention

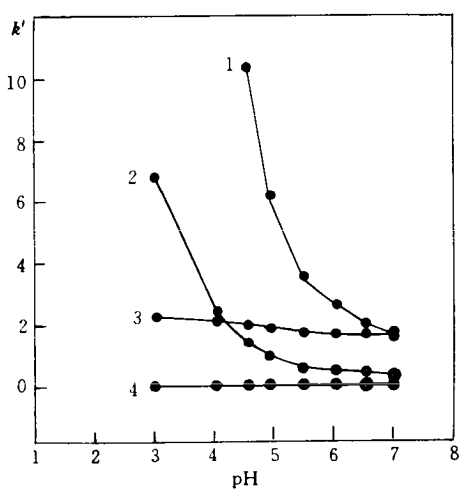

Fig. 1 Dependence of the k'-values of selenious acid, selenic acid, TMSe, and SeMet on the pH of the mobile phase

1. Selenic acid, 2. selenious acid, 3. SeMet, 4. TMSe.

Table 1 Operating conditions of HPLC-FAAS and HPLC-ICP-MS

HPLC	
Solvent delivery unit	Waters 600E(for HPLC-FAAS)
	Hewlett Packard 1050 (for HPLC-ICP-MS)
Pre-column	Hamilton PRP X-100, 25 mm×4 mm i. d., the same stationary phase as the column
Column	ESA Anion III(Reno, Nevada, USA), 250 mm×4 mm i. d., 10 μm spherical poly (styrene-divinylbenzene) particles with trimethylammonium exchange sites
Mobile phase	5. 5 mmol/L ammonium citrate, pH 5. 5
Flow rate	1. 5 mL/min
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
Vacuum	expansion:0. 16 kPa
	intermediate:$<0.1\times10^{-4}$ kPa
	analyzer:0.21×10^{-6} kPa
Mass of selenium monitored	78
Time/slice	0. 51 s
Slices	700
Total analysis time	500 s
Meinhard nebulizer (SB-30-A3)gas	0. 84 L/min
Ultrasonic nebulizer (U-6000 AT$^+$)	
Nebulizer gas	0. 84 L/min, optimized on ^{87}Rb
Heater temperature	140 ℃
Condenser temperature	2 ℃
Membrane desolvator	heater temperature:160 ℃
	sweep gas flow:2. 6 L/min

time of SeMet was 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 $HSeO_3^-$ and selenic acid as SeO_4^{2-}, selenic acid has the strongest electrostatic interaction with the stationary phase and will be 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.

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 was in the tested range. The flow rate of 1.5 mL/min was used in further experiments.

3.2 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. 2. All four species studied were fully resolved and the separation was completed in less than 8 min. The peak areas of absorbance of the four selenium species [ρ(Se) = 100 mg/L for each selenium compound] were 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 ρ(Se) = 1 mg/L for each compound, estimated as three times of the base-line noise of the chromatograms. These relatively high 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.

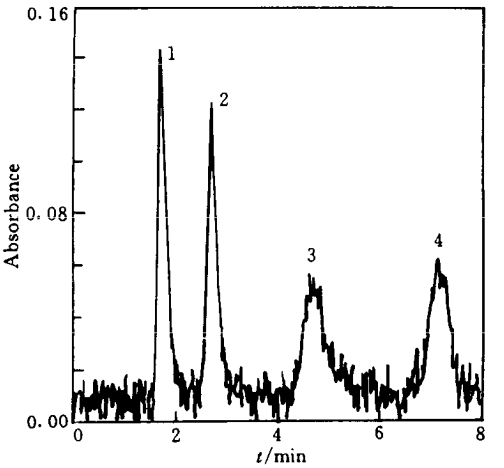

Fig. 2 HPLC-FAAS chromatogram of selenious acid, selenic acid, TMSe and SeMet

1. TMSe, 2. selenious acid, 3. SeMet, 4. selenic acid.

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, ^{78}Se was chosen because of its higher relative abundance and because the major selenium isotope ^{80}Se suffers from a severe $^{40}Ar_2$ interference.

The sample introduction into the ICP is a critical part in ICP-MS. The chromatograms obtained with the on-line ICP-MS detector and Meinhard or USN nebulizer under the optimized conditions are shown in Fig. 3.

The peak areas [ρ(Se) = 100 μg/L for each selenium compound] of the four selenium species were almost identical (Table 2), when the Meinhard nebulizer was used. This indicates that the response of the ICP-MS with Meinhard nebulizer does not depend on the molecular forms of the selenium compounds.

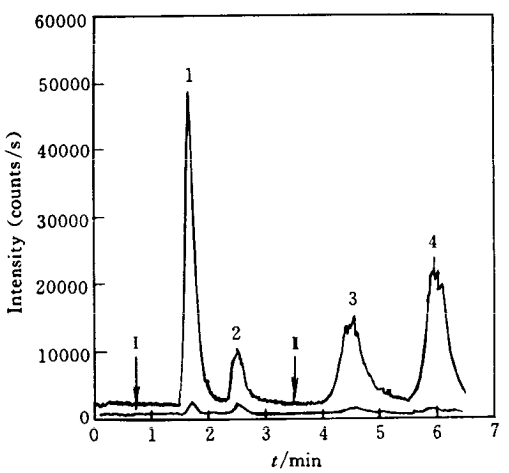

Fig. 3 HPLC-ICP-MS chromatogram of selenious acid, selenic acid, TMSe and SeMet

1. TMSe, 2. selenious acid, 3. SeMet, 4. selenic acid.
I. Meinhard nebulizer, I. USN nebulizer.

The response of the ICP-MS with USN nebulizer was selenium species dependent (Table 2). The ICP-MS signal intensity was increased by a factor of 7 for selenious acid and 24 to 31 for TMSe, SeMet and selenic acid with the USN compared to that with the Meinhard nebulizer.

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. The similar behavior of selenite was observed by Yang et al[8], when an ICP atomic emission spectrometer with a thermospray nebulizer was used for detection of selenium.

The calibration curves of the HPLC-USN-ICP-MS for determination of selenious acid, selenic acid, TMSe and SeMet were linear for each selenium compound in ρ(Se) = 0-100 μg/L (Table 3). The determination limits were ρ(Se) = 0.08 μg/L for TMSe, ρ(Se) = 0.34 μg/L for selenious acid, ρ(Se) = 0.18 μg/L for SeMet and ρ(Se) = 0.07 μg/L for selenic acid, calculated as the concentration of selenium required to obtain a signal of 3 times of the baseline noise.

Table 2 The response of HPLC-ICP-MS with USN or Meinhard nebulizer for selenium compounds

Se compound [ρ(Se) = 100 μg/L]	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

Table 3 HPLC-USN-ICP-MS calibration curves for Se compounds [ρ(Se)=0-100 μg/L]

Compound	Calibration curve $Y = kX + d$*	Regression r^2	Detection limit [ρ(Se), μg/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

* Y = peak area (counts); X = mass concentration of selenium (μg/L).

REFERENCES

1 Koelbl G, Kalcher K, Irgolic K J et al. Appl Organomet Chem, 1993, 7:443-466

2 Mcneal J M, Balistrieri L S. In: Jacobs L W ed. Selenium in agriculture and environment. Madison, WI: American Society of Agronomy and Soil Science Society of America, 1989. 1

3 Shibata Y, Morita M, Fuwa K. Adv Biophys, 1992,28:31-80

4 Munoz Olivas R, Donard O F X. Anal Chim Acta, 1994, 286:357-370

5 Goessler W, Kuehnelt D, Schlagenhaufen C et al. J Chromatogr A, 1997, 789:233-246

6 Koelbl G. Determination of selenium compounds in environmental and biological samples with high performance liquid chromatography using selenium-specific detector. (PhD dissertation). Austria: Institute for Analytical Chemistry, Karl-Franzens-University Graz, 1994

7 Koelbl G, Kalcher K, Irgolic K J. J Automat Chem, 1993, 15:37-45

8 Yang J, Conver T S, Koropchack J A. Anal Chem, 1996, 68:4064-4071

HPLC-ICP-MS 或 HPLC-FAAS 法分离测定硒化合物

李方实[1] Walter Goessler Kurt J Irgolic

[1](南京化工大学应用化学系 南京 210009)

(Institute for Analytical Chemistry, Karl-Franzens-University Graz, A-8010 Graz, Austria)

提 要 提出了一种用高效液相色谱(HPLC)分离和用电感偶合等离子体质谱仪(ICP-MS)或火焰原子吸收光谱仪(FAAS)作元素专一检测器在线测定硒的化学形态的方法。在优化的 HPLC 条件下,用 ESA III 阴离子色谱柱(250 mm×4.6 mm),以柠檬酸铵为流动相(5.5 mmol/L, pH 5.5, 流速 1.5 mL/min),进样量 100 μL,分离和测定三甲基硒离子、硒代蛋氨酸、亚硒酸和硒酸盐只需 8 min。HPLC-FAAS 在线分析 4 种硒化合物的检测限为 ρ(Se) = 1 mg/L。用超声雾化器作 ICP-MS 的接口,HPLC-ICP-MS 在线分析 4 种硒化合物的检测限分别为 ρ(Se) = 0.34 μg/L (亚硒酸),0.18 μg/L(硒代蛋氨酸),0.08 μg/L(三甲基硒离子)和 0.07 μg/L(硒酸盐)。与气动雾化器接口相比,信号强度增加 7 至 31 倍。

关键词 高效液相色谱,电感偶合等离子体质谱,火焰原子吸收光谱,硒化合物,化学形态

分类号 O658