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Retention behavior of inorganic and organic selenium compounds on a silica-based strong-cation-exchange column with an inductively coupled plasma mass spectrometer as selenium-specific detector

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Abstract

The retention behavior of eight selenium compounds (selenous acid, selenic acid, selenocystine, selenohomocystine, selenomethionine, selenoethionine, trimethylselenonium iodide, and dimethyl(3-amino-3-carboxy-1-propyl)selenonium iodide) with aqueous solutions of pyridine (20 mmol/l) in the pH range 2.0–5.7 on a Supelcosil LC-SCX cation-exchange column was investigated. An inductively coupled plasma mass spectrometer was employed as the selenium-specific detector. To increase the nebulization efficiency, the Meinhard concentric glass nebulizer was replaced by a hydraulic high-pressure nebulizer. At pH 5.0, seven selenium compounds could be separated within 400 s, but selenohomocystine and selenomethionine had the same retention time. Selenomethionine can be separated from selenohomocystine with an aqueous solution of pyridine (20 mmol/l) adjusted with formic acid to pH 2.0. At 1 ng Se ml^{-1}, the relative standard deviations ($n=5$) of the signal area for the eight selenium compounds ranged from 7 to 11%, and at 50 ng Se ml^{-1} from 0.6 to 2.6%.

Keywords: Selenium compounds; Seleno amino acids

1. Introduction

Selenium is recognized as an essential trace element for plants, animals, and humans [1]. Adequate supplementation with selenium in the form of sodium selenate has been reported to reduce risks of heart disease and cancer [2]. Selenium compounds are also able to diminish or prevent the toxic effects caused by excesses of toxic elements such as arsenic and mercury [3].

Several inorganic and organic selenium compounds have been identified in biological samples over the past four decades. Among these compounds are selenous acid, selenic acid, dimethyl selenide, dimethyl diselenide, dimethyl selenide sulfide [9], trimethylselenonium salts, selenoamino acids, selenium-containing carbohydrates [4], proteins [5], and nucleosides [6]. Selenous acid and selenic acid are the predominant selenium species in the aquatic environment [7]. Volatile dimethyl selenide, dimethyl diselenide and dimethyl selenide sulfide are both released by bacteria [8,9]. Several selenoamino acids have been reported to occur in organisms. Selenocysteine is a constituent of the enzymes glutathione peroxidase and glycine reductase [10]. Selenomethionine was found to be present in plants [11]. The trimethylselenonium ion, identified in human urine, is thought to indicate excessive intake

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of selenium [12]. Because of the chemical similarity between sulfur and selenium, compounds with Se–S bonds may also exist in biological systems and were shown to be formed in reactions of appropriate selenium compounds with glutathione [13].

The chemical form and not the total concentration determines bioavailability, the beneficial effects, and toxic actions of a trace element. For the determination of selenium compounds, an efficient separation for instance by liquid chromatography should be followed by an adequate detection step. Recent developments in the determination of selenium compounds were reviewed by Pyrzynska 1995 [14]. A review published by Kölbl et al. [15] covers the literature from 1974 to 1993. Flame atomic absorption spectrometry (FAAS) [12], graphite furnace atomic absorption spectrometry (GFAAS) [12,17–19], and inductively coupled plasma mass spectrometry (ICP-MS) [12,20–24] have served as selenium-specific detection methods. The detection limits obtained with FAAS as a selenium-specific detector at a few mg Se/l are too high for practical use. Adding hydride-generation, that requires conversion of all selenium species to selenite, to FAAS lowers the detection limits to a few ng/ml. GFAAS requires fraction collection or a sophisticated interface [16] to connect the HPLC system to the GFAAS instrument. ICP-MS seems to be the ideal selenium-specific detector because of its excellent detection limits and its ready connectability to the chromatographic system.

Groups of selenium compounds have been successfully separated by anion-exchange chromatography [12,19,21,24,25], cation-exchange chromatography [12], vesicle-mediated reversed-phase chromatography [18], ion-pair reversed-phase chromatography [12,16,17,19,20,22,23], and by capillary electrophoresis [25]. For instance, three selenium compounds (selenocystine, selenomethionine, trimethylselenonium iodide) are separated by reversed-phase chromatography [20], four selenium compounds (selenous acid, selenic acid, selenocystine, selenomethionine) [19,21,23], (selenous acid, selenocystine, selenomethionine, selenoethionine) [18] by reversed-phase [18,19,23] and by anion-exchange chromatography [21], six selenium compounds (selenous acid, selenic acid, selenocystine, methylselenocysteine, allylselenocysteine, selenomethionine) by anion-exchange chromatography [24], and seven selenium compounds (selenous acid, selenic acid, selenocystine, selenohomocystine, selenoethionine, selenomethionine, trimethylselenonium iodide) by anion-exchange chromatography with solutions of potassium hydrogen phthalate as mobile phases (2 m*M* switched to 12 m*M* after 2.5 min) [35].

To simplify the chromatography and evaluate cation-exchange chromatography for the separation of anionic, cationic, and zwitterionic selenium compounds, the retention behavior of eight selenium compounds (selenous acid, selenic acid, selenocystine, selenohomocystine, selenomethionine, selenoethionine, trimethylselenonium iodide, dimethyl(3-amino-3-carboxy-1-propyl)selenonium iodide) on a silica-based LC-SCX cation-exchange column (Supelcosil) was investigated, and an optimal procedure for the separation and quantification of these selenium compounds developed with an inductively coupled plasma mass spectrometer as selenium-specific detector. Experiments to extend these investigations to more complex organic compounds containing selenium or a Se–S bond are planned and will be carried out when sufficient amounts of synthetic standards have been prepared.

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, seleno-DL-ethionine, seleno-DL-methionine and seleno-DL-cystine from Sigma. Selenohomocystine [26] and trimethylselenonium iodide [12] were prepared according to literature procedures. Dimethyl(3-amino-3-carboxy-1-propyl)selenonium iodide was synthesized as described for *S*-methylmethionine iodide [27].

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^{-1}), from sodium selenite pentahydrate (1665.5 mg to 500 ml, 1000 mg Se l^{-1}), from trimethylselenonium iodide (63.6 mg to 20 ml, 1000 mg Se l^{-1}), from

dimethyl(3-amino-3-carboxy-1-propyl)selenonium iodide (21.4 mg to 10 ml, 500 mg Se l^{-1}), from selenomethionine (24.8 mg to 20 ml, 500 mg Se l^{-1}), from selenoethionine (26.6 mg to 20 ml, 500 mg Se l^{-1}), from selenocystine (5.3 mg to 20 ml, 125 mg Se l^{-1}), and from selenohomocystine (5.7 mg to 20 ml, 125 mg Se l^{-1}). 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 1–50 ng Se ml^{-1} were prepared by appropriate dilution of the stock solutions with NANOpure water.

2.2. Mobile phases

Pyridine solutions (0.020 mol l^{-1}) were prepared by dissolving 1.58 g pyridine (Merck analytical grade) to 1000 ml with NANOpure water and adjusting the pH of these solutions to 5.7, 5.0, 4.0, 3.0, 2.8, 2.5, 2.3, or 2.0 by addition of 0.06, 0.480, 1.08, 5.08, 9.83, 16.8, 29.8, or 58.8 ml formic acid (~98%, Fluka puriss. analytical-reagent grade). Aliquots (50 μl) of a rubidium chloride solution (1000 mg Rb l^{-1}) were added to the mobile phases as internal standard for ICP–MS.

2.3. Instrumentation

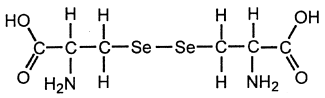
The high-performance liquid chromatography system consisted of a Hewlett-Packard 1050 solvent delivery unit (Hewlett-Packard, Waldbronn, Germany) and a Rheodyne 9125 six-port injection valve (Rheodyne, Cotati, USA) with a 100-mm^3 injection loop. The separations were performed on a Supelcosil LC-SCX cation-exchange column (25 cm×4.6 mm I.D., 5-μm silica-based particles with propylsulfonic acid exchange sites).

The outlet of the HPLC column was connected via a 60-cm, 1/16-in. PEEK (polyether ether ketone) capillary tubing (0.25 mm I.D.) to a hydraulic high-pressure nebulizer (HHPN) (Knauer, Berlin, Germany) (1 in.=2.54 cm). The desolvation unit was set to 140°C heating temperature and 2°C cooling temperature. A back pressure of ~200 bar was observed during the experiments.

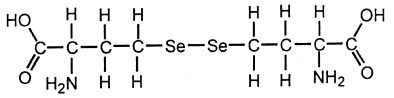
The VG Plasma Quad 2 Turbo Plus inductively coupled argon-plasma mass spectrometer (ICP-MS, VG Elemental, Winsford, UK) served as the selenium-specific detector. The instrumental operating conditions are the same as described previously [31,34]. The ion intensities at m/z 76, m/z 77, m/z 78, and m/z 82 were monitored using the 'time-resolved' analysis software©Version 1a (Fisons Scientific Equipment Division, Middlesex, UK). 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 rubidium at a concentration of 50 ng Rb ml^{-1}. The lens settings were adjusted for optimal response of the instrument (typically 3×10^6 Hz for 50 ng Rb ml^{-1}). The chromatograms were exported and the retention times and peak areas determined with software written in house [28].

3. Results and discussion

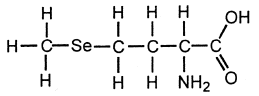
Selenium compounds (Fig. 1) (selenous acid (selenite), selenic acid (selenate), selenocystine (Secys), selenomethionine (Semet), selenohomocystine (Sehcys), dimethyl(3-amino-3-carboxy-1-propyl)-selenonium iodide (DmpSe), selenoethionine (Seet), and trimethylselenonium iodide (TmSe)) can be present in solution as cations, anions or zwitterions. The Supelcosil LC-SCX strong cation-exchange column has strongly acidic sulfonic acid groups as exchange sites. The surface of the functionalized silica particles is covered with siloxane groups, free silanol groups, geminal silanol groups, and associated silanol groups that may interact with the analyte. The sulfonate exchange sites will interact with the cationic selenium compounds, the hydrophobic backbone of the column material with uncharged lipophilic selenium compounds, and the compounds with carboxylic acid groups may be retained via hydrogen bonds to siloxane or silanol groups. The column can be operated from pH 2.0 to 7.0. An aqueous solution of pyridine was chosen as mobile phase, because this mobile phase is completely volatilized in the argon plasma and does not clog the cones during a 12-h working period [31,32]. Additionally, carbon-containing mobile phases may en-



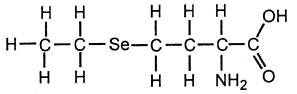
Selenocystine (Secys)



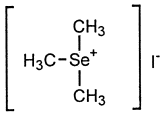
Selenohomocystine (Sehcys)



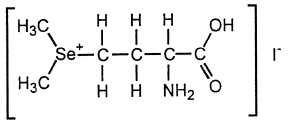
Selenomethionine (Semet)



Selenoethionine (Seet)



Trimethylselenonium iodide (TmSe)



Dimethyl(3-carboxy-3-amino-1-propyl)selenonium iodide (DmpSe)

Fig. 1. Formulas for organic selenium compounds.

hance the signal intensities for selenium by a factor of ~4 [33].

3.1. Dissociation of the selenium compounds in aqueous solution

Selenic acid, a strong acid, and selenous acid, a weak acid, can be present in aqueous solution as anions with one or two negative charges. At pH values below 4.0 selenous acid may remain undissociated. Selenoethionine and selenomethionine (Fig. 1) 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. Selenocystine and selenohomocystine (Fig. 1) carries two positive charges at very low pH localized to the two protonated amino groups, will carry one positive charge at low pH (two ammonium groups, one carboxylate group), but becomes zwitterionic (two ammonium groups, two carboxylate groups) at intermediate pH. It carries one negative charge at higher pH (ammonium group, two carboxylate groups) and converts to divalent anions (two carboxylate groups) at high pH. Dimethyl(3-amino-3-carboxy-1-propyl)selenonium iodide carries two positive charges at low pH localized to the protonated amino groups and the selenium atom, carries a positive charge at intermediate pH values (ammonium group, selenonium group, carboxylate group), but will be zwitterionic at high pH (selenonium group, carboxylate group). The theoretical distributions of charges for the compounds where dissociation constants are known can be found in Fig. 2. Trimethylselenonium iodide is a cation irrespective of pH.

3.2. Retention behavior of selenium compounds

Dependence of retention times on the pH of the mobile phase (20 m*M* aqueous solution of pyridine) in the pH range 2.0–5.7 was investigated using the Supelcosil LC-SCX silica-based cation-exchange column. Where chromatographic separations were repeated three times, the relative standard deviations of the retention times did not exceed 1%.

With a solution of 10 mg/l chloride (as sodium chloride), the void volume of the system was determined. Surprisingly, the selenate dianion (SeO_4^{2-}) always eluted 4 s before the chloride ion. The shortest elution time obtained for selenic acid was 85 s at pH 5.7. With a flow-rate of 1.5 ml/min, the void volume is calculated to be 2.13 ml.

3.2.1. Retention behavior of selenic acid

The retention time of selenic acid increases monotonic from 85 s at pH 5.7 to 124 s at pH 2.0 (Fig. 3). Since the selenate ion will not interact with the

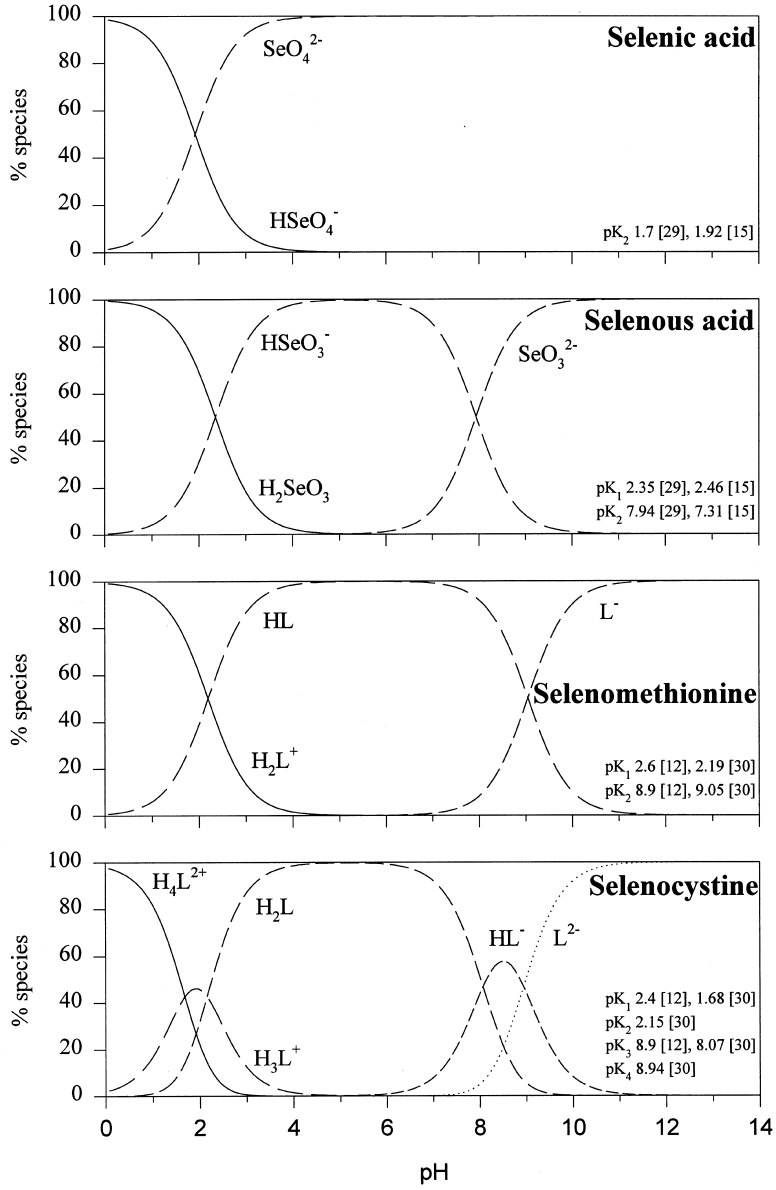


Fig. 2. Species distribution diagrams for selenic acid, selenous acid, selenomethionine, and selenocystine in the pH range from 0 to 14. p*K* values taken from Refs. [12,15,29,30].

negatively charged sulfonic acid groups, the prolonged retention time can only be caused by the interactions with the silanol groups of the stationary phase. According to the manufacturer of the Supelco column, the pK_a value of the silanol groups is in the range of 2.5–3.0. Around pH 5.0 all of the silanol groups are deprotonated and carry a negative charge. These anionic repulsions are therefore responsible for the shorter retention time at higher pH values. At lower pH values the stationary phase is neutral, but the R_3-SiOH groups may form hydrogen bonds with the negatively charged SeO_4^{2-} ions. The formation of hydrogen bonds may be responsible for the retention of selenate at lower pH values.

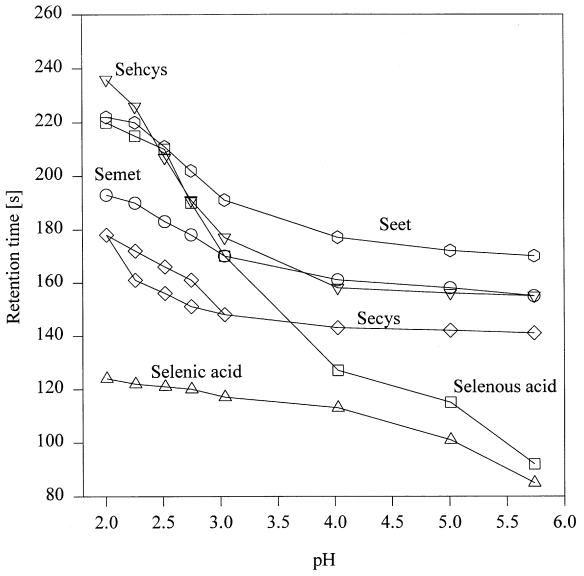


Fig. 3. Dependence of the retention times for selenic acid, selenous acid, selenocystine, selenomethionine, selenoethionine, and selenohomocystine on pH (Supelcosil LC-SCX column; 20 ng Se ml^{-1} each; 100 μl injected; aqueous 20 m*M* pyridine as mobile phase; flow rate of 1.5 ml/min; ICP-MS as selenium-specific detector, unless otherwise specified).

3.2.2. Retention behavior of selenous acid

Chromatograms of selenous acid obtained with mobile phases of pH 2.0–3.0 contain only very broad signals with retention times between ~250 and ~200 s (Fig. 4). Sharp signals were only obtained with mobile phases between pH 4.0 and 5.7. The retention time for selenous acid decreased with increasing pH much more (from 220 s at pH 2.0 to ~90 s at pH 5.7) than for selenic acid (Fig. 3). The retention of selenous acid at low pH may be attributable to hydrogen bonding between the silanol groups and the neutral H_2SeO_3 and the $HSeO_3^-$ species. With increasing pH the silanol groups and selenous acid become deprotonated and negatively charged. The repulsion between the surface silanolates and the monohydrogen selenite anion leads to shorter retention times, so that at pH 5.7 it is eluted approximately with the solvent front.

3.2.3. Retention behavior of trimethylselenonium iodide and dimethyl(3-amino-3-carboxy-1-propyl)selenonium iodide

The trimethylselenonium cation remains positively charged irrespective of mobile-phase pH. The retention time of the trimethylselenonium cation changes little from pH 2.0 (~220 s) to pH 4.0 (~250 s), then increases to 320 s at pH 5.0, and to ~1180 s at pH 5.7 (Fig. 5). Although the dimethyl(3-amino-3-carboxy-1-propyl)selenonium compound could be present as a divalent cation at low pH, as a cation with one positive charge and zwitterionic amino acid groups, and as a zwitterion at high pH (dissociation constants have not been determined for this molecule), the behavior of this selenium compound is very similar to the trimethylselenonium cation (Fig. 5). This similarity suggests that dimethyl(3-amino-3-carboxy-1-propyl)selenonium iodide is also present as a unipositively charged cation. A chromatographic separation of these two cationic selenium compounds should be possible between pH 3.0 and 5.7.

3.2.4. Retention behavior of selenocystine

The chromatograms obtained for selenocystine at pH values from 2.0 to 5.7 are shown in Fig. 6. At pH 2.0 selenocystine generates a signal with a shoulder at 178 s. At pH 2.3 two signals are present in the chromatogram. The signal at the lower retention time increases in intensity with a concomitant decrease of the intensity for the signal at the higher retention time. At pH 3.0 only one major signal at a retention time of ~150 s is present (Fig. 6). Over the entire pH range investigated (pH 3.0–5.7) the retention time decreases from ~180 to ~140 s (Fig. 3).

3.2.5. Retention behavior of selenohomocystine

Selenohomocystine has two more side-chain carbons than selenocystine (Fig. 1). Therefore, a similar retention behavior was expected. Surprisingly the two signals observed for selenocystine were not present in the chromatograms for selenohomocystine (Fig. 7). Selenohomocystine has its longest retention times at pH 2.0 (236 s) and its shortest at pH 5.7 (155 s). At all pH values, selenohomocystine has a larger retention time than selenocystine (Fig. 3). The difference between the retention times for selenocystine and selenohomocystine is ~58 s at pH 2.0, 29 s at pH 3.0, and ~15 s from pH 4.0 to 5.7. Above pH 4.0, selenocystine as well as selenohomocystine would be present as zwitterions, and the retention of these compounds cannot be attributed to a cation-exchange mechanism. The

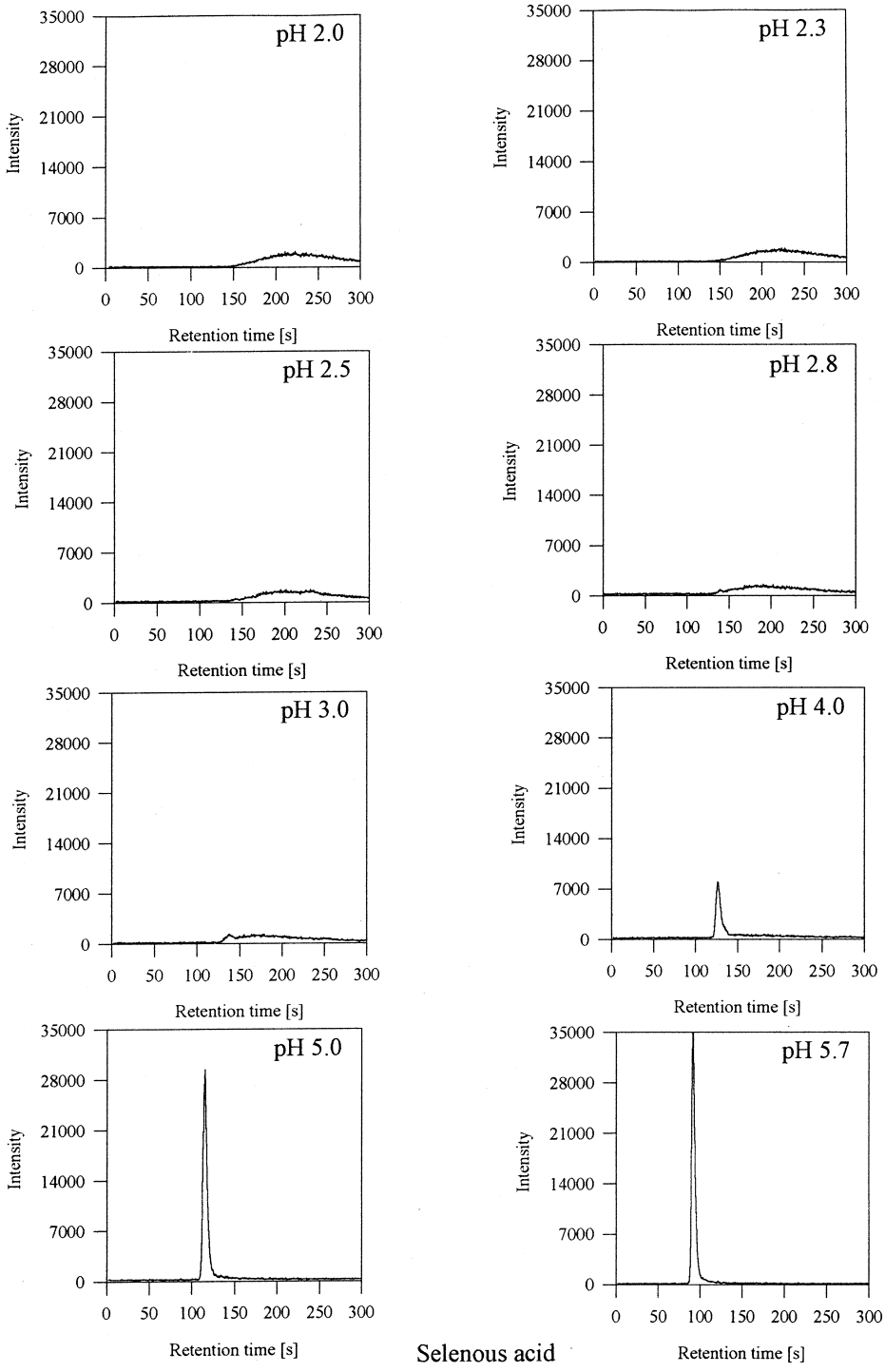


Fig. 4. Chromatograms obtained for selenous acid with mobile phases in the pH range of 2.0–5.7.

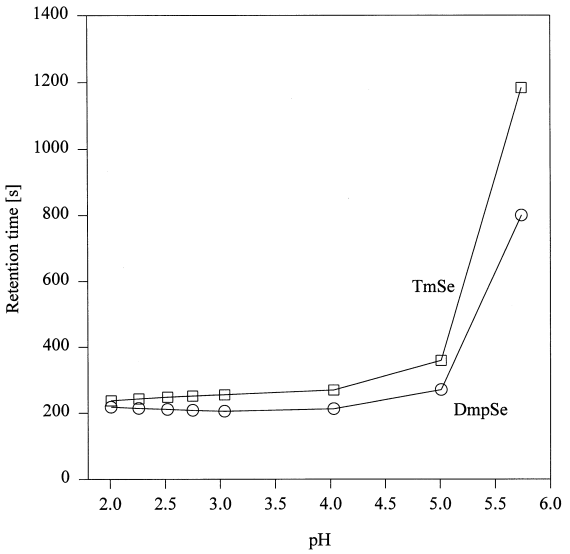


Fig. 5. Dependence of the retention times of trimethylselenonium iodide (TmSe) and dimethyl(3-amino-3carboxy-1propyl)-selenonium iodide (DmpSe) on the pH of the mobile phase.

longer side chains in selenohomocystine increase the hydrophobic character of the molecule. Hydrophobic interactions of the propyl groups in the Supelcosil LC-SCX column may be responsible for the longer retention times of selenohomocystine compared to selenocystine.

3.2.6. Retention behavior of selenomethionine and selenoethionine

Selenomethionine (methyl(3-amino-3-carboxy-1-propyl) selenide) and selenoethionine (ethyl(3-amino-3-carboxy-1-propyl) selenide) are both dialkyl selenides (Fig. 1). The retention times of these two selenoamino acids decrease similarly with increasing pH (Fig. 3). At pH 2.0, the retention time for selenoethionine is 30 s longer than for selenomethionine. At pH 5.7 this difference is only 15 s.

In Table 1 the retention time differences of the homologous pairs selenoethionine–selenomethionine and selenohomocystine–selenocystine are displayed. At pH values ≥4.0 selenocystine and selenomethionine are present as zwitterions. No dissociation constants are available for selenomethionine and selenohomocystine. Both selenium compounds have the amino acid moiety in the molecule and the dissociation constants for these two compounds should be similar to selenocystine and selenomethionine. Under this assumption selenomethionine and selenohomocystine are also present as zwitterions at pH values ≥4.0. Although selenohomocystine has two more CH_2 groups in the molecule than selenocystine, the difference in the retention time in the pH range 4.0–5.7 is the same as for selenoethionine, which has just one more CH_2 group in the molecule than selenomethionine. The retention time differences in the pH range 2.0–4.0 may be attributed to the different charges on the homologous pairs selenoethionine–selenomethionine and selenohomocystine–selenocystine.

3.3. Optimal conditions for the separation of the selenium compounds

Ideally, the inorganic and organic selenium compounds should be separable to baseline with reasonably short retention times. On the Supelcosil LC-SCX cation-exchange column with 20 m*M* pyridine solution as mobile phase (pH 2.0–5.7) only seven of the eight investigated selenium compounds can be simultaneously identified and quantified. At pH values below 4.0, the very broad signal for selenous acid (Fig. 4) prevents identification of selenous acid and reliable quantification of the other selenium compounds. In the pH range 4.0–5.0, all eight investigated selenium compounds generate sharp signals. However, signals for selenohomocystine and selenomethionine overlap (Fig. 8). At pH values above 5.0, the retention times of trimethylselenonium iodide and dimethyl(3-amino-3-carboxy-1-propyl)selenonium iodide are prolonged unacceptably. At pH 2.0, selenomethionine (a selenoamino acid frequently encountered in biological samples) is well separated from selenic acid and the other organic selenium compounds, and can be easily identified and quantified. Under these conditions selenoethionine and dimethyl(3-amino-3-carboxy-1-propyl)selenonium iodide have the same retention time and the signals for selenocystine and trimethylselenonium iodide overlap (Fig. 9).

To quantify all eight selenium compounds two chromatographic experiments are needed. With 20 m*M* pyridine solution (pH 5.0) as mobile phase, selenic acid, selenous acid, selenocystine, selenoethionine, dimethyl(3-amino-3-carboxy-1-pro-

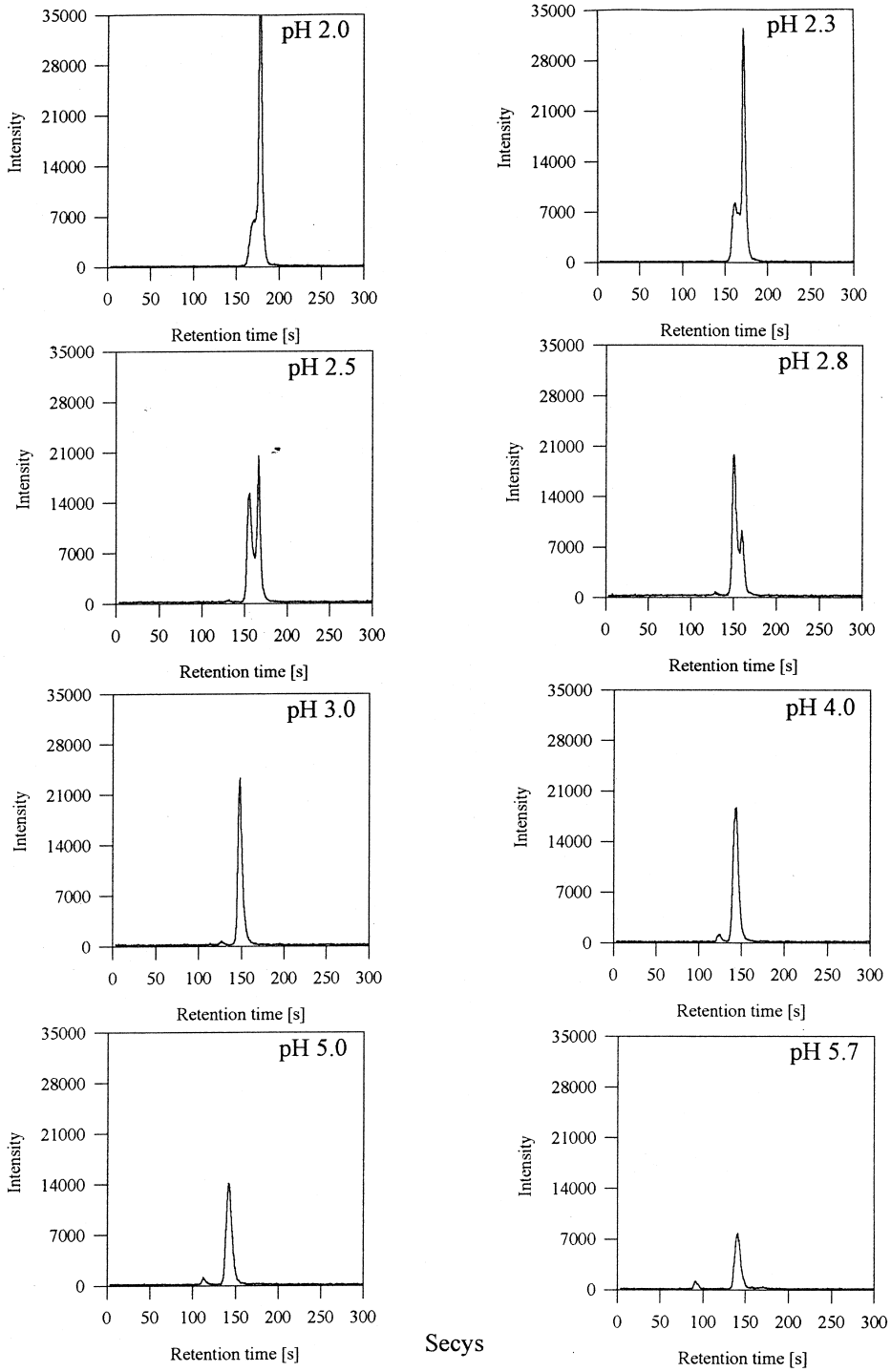


Fig. 6. Chromatograms obtained for selenocystine with mobile phases in the pH range of 2.0–5.7.

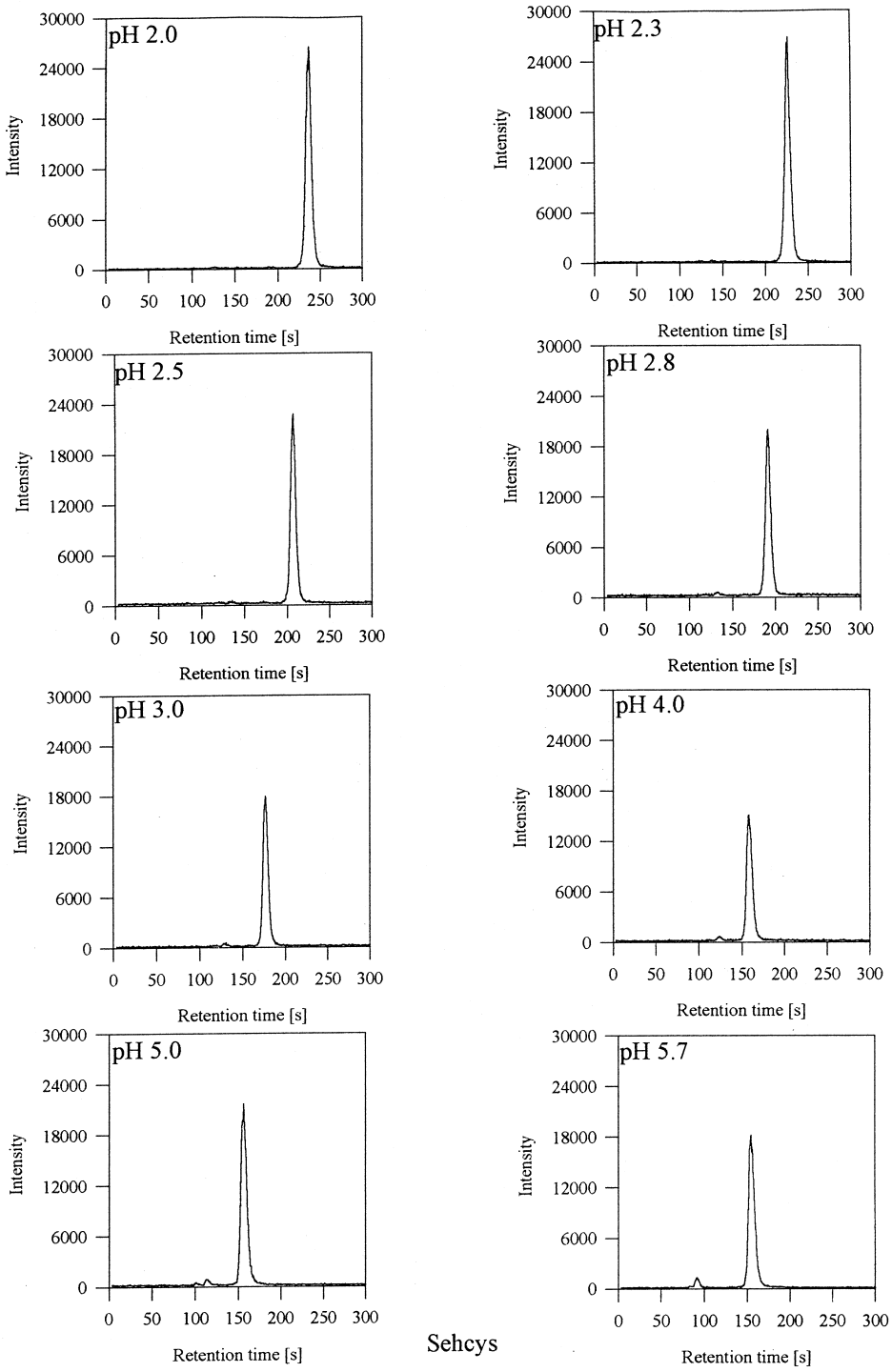


Fig. 7. Chromatograms obtained for selenohomocystine with mobile phases in the pH range of 2.0–5.7.

Table 1
Differences in the retention times for the homologous pairs selenohomocystine–selenocystine (Sehcys–Secys) and selenoethionine–selenomethionine (Seet–Semet)

pH	Sehcys–Secys (s)	Seet–Semet (s)
2.0	58	29
2.3	54	30
2.5	41	28
2.8	30	24
3.0	29	21
4.0	15	16
5.0	15	15
5.7	14	15

pyl)selenonium iodide, and trimethylselenonium iodide are separable and quantifiable within 400 s. If selenohomocystine is not present, selenomethionine can also be determined (Fig. 8). Selenomethionine can be quantified in the chromatogram obtained with 20 m*M* pyridine solution (pH 2.0) (Fig. 9). The presence of selenohomocystine can be deduced from the difference between the intensity for the signal selenohomocystine/selenomethionine (Fig. 8) and the intensity of the signal selenomethionine (Fig. 9), or from the difference between the intensity for the signal trimethylselenonium iodide/selenohomocystine (Fig. 9) and the intensity for the signal trimethylselenonium iodide (Fig. 8). The presence of selenohomocystine can be best ascertained by chromatographing the solution at pH 2.1.

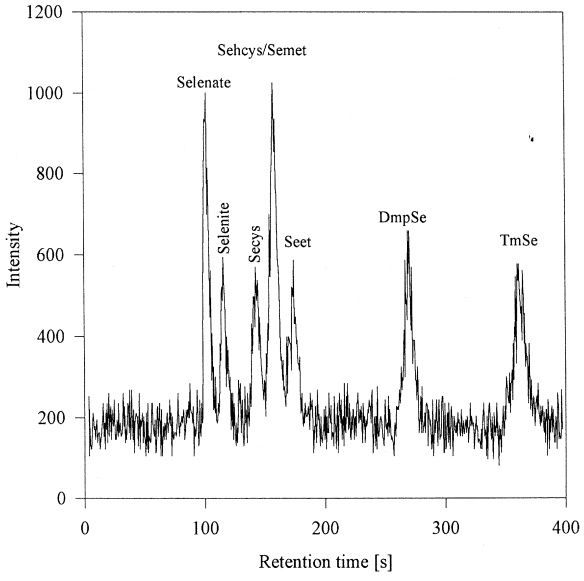


Fig. 8. Chromatogram of a standard solution containing selenic acid, selenous acid, Secys, Semet, Seet, Sehcys, TmSe and DmpSe at a concentration of 1 ng Se ml^{-1} each (20 m*M* pyridine at pH 5.0).

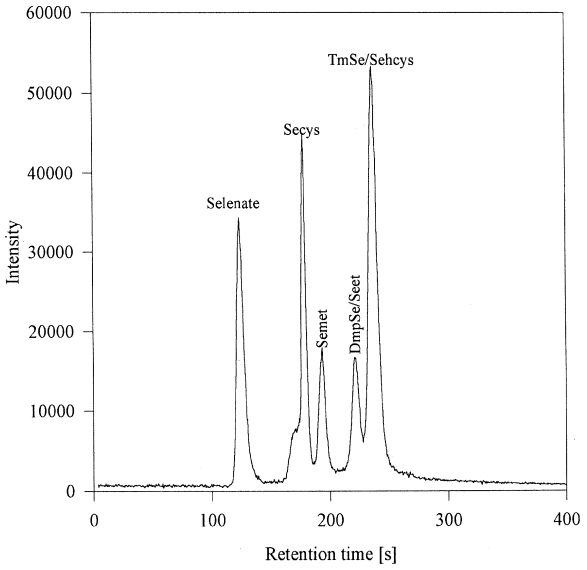


Fig. 9. Chromatogram of a standard solution containing selenic acid, selenous acid, Secys, Semet, Seet, Sehcys, TmSe and DmpSe at a concentration of 20 ng Se ml^{-1} each (20 m*M* pyridine at pH 2.0).

Selenohomocystine is not a common selenium compound in biological samples. Should selenohomocystine not be present in the sample, then the quantification of selenomethionine with 20 m*M* pyridine at pH 5.0 will not cause any problems.

Thus, this method allows the determination of seven selenium compounds (selenic acid, selenous acid, selenocystine, selenoethionine, selenomethionine, dimethyl(3-amino-3-carboxy-1-propyl)selenonium iodide, trimethylselenonium iodide) under isocratic conditions by cation-exchange chromatography in one chromatographic experiment.

3.4. Figures of merit

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

Table 2
Figures of merit

Compound	Reproducibility (R.S.D., %) $n=5$ at ng Se ml^{-1}:					Calibration $y=kx+d$[a]		
	1.00	5.00	10.0	25.0	50.0	k	d	r^2
Selenic acid	10.7	1.6	3.1	1.9	0.6	3669	532	0.9998
Selenous acid	6.3	4.4	3.2	3.2	1.7	1401	2067	0.9959
Secys	9.4	1.4	3.6	1.4	1.9	2482	214	0.9999
Sehcys/Semet	8.1	2.1	1.6	2.1	1.9	5114	3816	0.9989
Seet	7.1	6.0	3.0	3.0	2.6	2381	1336	0.9995
DmpSe	8.2	1.4	3.0	1.8	2.1	3369	1312	0.9997
TmSe	10.8	5.6	2.8	0.7	1.4	3609	1494	0.9997

[a] y=peak area (counts/s); x=concentration of selenium (ng/ml).

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 high relative abundance.

Calibration curves were obtained from the areas of the HPLC–ICP-MS signals at m/z 78 (five replicate injections) in chromatograms obtained with standard solutions containing selenous acid, selenic acid, selenocystine, selenohomocystine, selenomethionine, selenoethionine, dimethyl(3-amino-3-carboxy-1-propyl)selenonium iodide, and trimethylselenonium iodide at concentrations of 1.0, 5.0, 10.0, 25.0, 50.0 ng Se ml^{-1} (each). The curves were linear in this concentration range. Higher concentrations were not investigated, but the linear range is expected to be much wider [34]. The relative standard deviations of the peak areas do not exceed 11% even at the low concentration of 1.0 ng Se ml^{-1} (each species). At a concentration of 10.0 ng Se ml^{-1} the highest relative standard deviation was 3.6% for selenocystine. The relative standard deviations never exceed 2.6% at a concentration of 50 ng Se ml^{-1} (Table 2).

This isocratic cation-exchange method has great promise for the determination of selenium compounds in biological samples.

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