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Chapter

Chromatographic Analysis of Selenium Species

Aleksandra Sentkowska

Abstract

Selenium is an important element in environmental and living organisms that is being essential in very narrow concentration range, while deficiency or toxicity occurs outside this range. However, its toxicity depends not only on its dose but also on its chemical form. In environmental samples, selenium can exist in inorganic forms (as elemental selenium, metal selenides, selenite, or selenate anions) and as organic species with direct C-Se bonds (methylated compounds, selenoaminoacids, and selenoproteins). Thus, the development of reliable techniques to study the speciation of selenium in environmental samples is necessary. The main purpose of this chapter is to provide an update on the recent literature concerning the strategies for selenium speciation in environmental samples. Liquid chromatography coupled with sensitive detector is a commonly used technique for selenium separation. Gas chromatography can also be applied for such purpose; however derivatization step is usually required before analysis. Direct determination of selenium species at the concentration levels present in natural samples is very often difficult or even impossible. For this, several preconcentration/separation procedures for selenium have been proposed, including coprecipitation, extraction into an organic solvent, or application of solid sorbents.

Keywords: selenium, speciation, chromatography, gas chromatography, liquid chromatography

1. Introduction

Selenium and its several species have been demonstrated to be essential for living organisms [1, 2]. It plays a key role in many important metabolic pathways such as thyroid hormone metabolism and antioxidant defense systems [3]. For these reasons, it should be present in human diet. The range between the deficiency and toxicity of selenium is very narrow. The nutritionally required daily uptake of selenium is 55 μg; however some studies suggest that it should be 100 μg [4]. It is estimated that the diets of 1 billion people might lack sufficiency for their well-being [5, 6]. The toxic dose of selenium is very much dependent on its chemical form, with different toxicity for organic and inorganic forms [7]. Selenoaminoacids are principal dietary forms of selenium, and selenomethionine (SeMet) is derived from plants, while selenocysteine (SeCys) from animals [8, 9].

Selenium naturally exists in many different inorganic (elemental selenium, selenide, selenite, and selenate ions) and organic forms (methylated compounds, selenoaminoacids, and selenoproteins). Inorganic selenium, present in water and soil, can be easily transformed into volatile compounds by plants and fungi.
<table>
<thead>
<tr>
<th>IUPAC name</th>
<th>Abbreviation</th>
<th>Chemical formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diethylselenide</td>
<td>DESe</td>
<td>CH₂₃CH₂-Se-CH₂CH₃</td>
</tr>
<tr>
<td>Soil and water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selenite</td>
<td>Se(IV)</td>
<td>SeO₃²⁻</td>
</tr>
<tr>
<td>Selenate</td>
<td>Se(VI)</td>
<td>SeO₄²⁻</td>
</tr>
<tr>
<td>Plants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimethylselenide</td>
<td>DMSe</td>
<td>CH₂-Se-CH₂</td>
</tr>
<tr>
<td>Dimethyldiselenide</td>
<td>DMDSe</td>
<td>CH₂-Se-Se-CH₂</td>
</tr>
<tr>
<td>Dimethylseleniumsulfide</td>
<td>DMSSe</td>
<td>CH₂-Se-S-CH₂</td>
</tr>
<tr>
<td>Selenocysteine</td>
<td>SeCys</td>
<td>HSe-CH₂CH(NH₂)-COOH</td>
</tr>
<tr>
<td>Selenocystine</td>
<td>SeCys₂</td>
<td>HCOOH-CH(NH₂)CH₂-Se-CH₂CH(NH₂)-COOH</td>
</tr>
<tr>
<td>Se-Methylselenocysteine</td>
<td>SeMC</td>
<td>CH₃Se-CH₂CH(NH₂)-COOH</td>
</tr>
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<td>Selenomethionine</td>
<td>SeMet</td>
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</tr>
<tr>
<td>Se-Methylselenomethionine</td>
<td>MeSeMet</td>
<td>(CH₂)₃Se-CH₂CH(NH₂)-COOH</td>
</tr>
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<td>γ-Glutamyl-Se-methylselenocysteine</td>
<td>γ-Glu-SeMC</td>
<td>H₂NCH₂CH₂-CO-NGCH(COOH)CH₂-Se-CH₂</td>
</tr>
<tr>
<td>Selenocystathionine</td>
<td>SeCysth</td>
<td>HCOOH-CH(NH₂)-CH₂-Se-CH₂CH(NH₂)COOH</td>
</tr>
<tr>
<td>Selenocystamine</td>
<td>SeCystm</td>
<td>H₂NCH₂CH₂-Se-Se-CH₂CH₂NH₂</td>
</tr>
<tr>
<td>Se-Adenosylselenohomocysteine</td>
<td>AdoSeHCy</td>
<td>NH₃(CH(COOH))CH₂-CH₂-Se-CH₂CH₂H₂OCS₂N₂-NH₂</td>
</tr>
</tbody>
</table>

Table 1. Principal selenium species present in environmental samples.
Organic species of selenium form covalent C-Se bonds. SeCys is included into selenoproteins and participates in redox reactions. The metabolic pathway of selenium in the human body is complicated [10]. In general it can be divided into three groups including reduction of inorganic species by glutathione (GHS) to selenide, cleavage reaction of organic species by β-lyase; utilization according to the UGA codon leading the synthesis of selenoproteins; and finally excretion after being metabolized to methylated species.

Apart from the importance of the selenium in living organisms, this element is also spread throughout the environment. Sulfur-containing minerals are natural sources of selenium, but it is also produced by combustion of fossil fuels. It should be noticed that selenium is used in electronic industries and agriculture as a component of fertilizers.

Several analytical procedures for determination of selenium at low concentration levels in environmental samples have been proposed and recently reviewed [11, 12]. For selenium speciation analysis, the coupling of chromatographic techniques such as gas chromatography (GC) or high-performance liquid chromatography (HPLC) with a highly sensitive and selective detector is very useful [13]. Even though GC exhibits high efficiency and simplicity, HPLC has the ability of dealing with non-volatile compounds, extending the range of application and avoiding a derivatization step. This chapter will focus on the recent progress in the application of HPLC in different modes for selenium speciation analysis in water, soil, and plants. Sample pretreatment procedures will be also considered. The principal selenium species present in environmental samples are summarized in Table 1.

2. Sample preparation

Sample preparation step is crucial in every analysis where analytes are present at very low concentration levels. In the speciation analysis, there is also another difficulty that should be overcome. An important requirement for reliable speciation is to retain the concentration and structure of the original chemical forms in the sample. In general, aquatic samples such as rain, ground- or surface water, tap and drinking water, seawater, and soil solutions do not require any pretreatment procedures other than filtration through 0.45 μm filter.

Extraction of selenium species from the solid samples with the highest recovery is quite challenging. According to Peachey et al. [14], selection of the extraction method, which provides high extraction efficiency while preventing the integrity of selenium species, is essential for the accurate measurement of its species. The most used method can be divided into three main groups:

- Extraction using aqueous solution (water, water-methanol, and buffers).
- Acid or alkali hydrolysis (hydrochloric acid and methanesulfonic acid).
- Enzymatic hydrolysis (proteinase, protease, and mixture of enzymes).

Since selenoaminoacids are water-soluble, extraction with hot water is extensively used [14–17]. However, the efficiency of water extraction from yeast was only 10% [17]. To release bounded selenoaminoacids, enzymatic or acidic hydrolysis was necessary [18–20]. The addition of methanesulfonic acid was used for selenomethionine extraction from yeast when heated under reflux [21]. Casiot et al. [22] reported that extraction of selenium species from yeast with water and ethanol led only to 10–20% recoveries of selenium and not allowed to extract
selenomethionine. The addition of pectinolytic enzymes released additional 20% of selenomethionine, while the addition of dodecyl sulfate solution allowed solubilization of a selenoprotein that accounted for 30% of total selenium. On the other hand, using tetramethylammonium hydroxide solubilized the sample completely, but the extracted selenium species were entirely degraded to selenomethionine and inorganic selenium [22]. It should be noticed that this type of extraction strictly depends on the choice of an enzyme, pH of the extraction solution, as well as temperature and time of the extraction. The most commonly used enzymes for this purpose are proteinase K or proteolytic enzymes (protease XIV), which were used for water-insoluble selenium fraction in many complicated matrices [23]. To reduce the extraction time, also ultrasonic hydrolysis can be used as the breakdown of Se-containing proteins (peptide bonds) into selenoaminoacids occurs [24]. The in vitro digestion with gastric juice was also used for selenium extraction from fish samples [25]. This procedure allowed SeMet determination, but the whole process takes few hours.

The sequential procedure developed by Chassaigne et al. [26] consisted of three steps: first, Tris-HCl buffer was used for extraction of water-soluble fractions, then also Tris-HCl with the addition of SDS for solubilization of protein fraction, and, finally, concentrated HNO₃ was used for dissolving of the remaining solid residue. A similar three-step procedure was used for extraction of selenium from mushrooms with 89% extraction efficiency [27]. It should be noticed that sequential treatment was also applied for dietary supplements [28, 29].

Cuderman et al. [30] examined different extraction media to identify selenium species in buckwheat. The optimal extraction efficiencies were obtained by hydrolysis with HCl, followed by breaking the cells with liquid nitrogen and then enzymatic hydrolysis with protease XIV. Ammonium acetate [31], sodium hydroxide [32], and enzymatic hydrolysis with pronase E [33] have been proposed for extraction of selenium species from green onion leaves.

3. Total selenium determination

Determination of the total selenium content is still the first step of its analysis. This procedure requires that organic forms must be transferred into inorganic selenium that is usually achieved with digestion using strong mineral acids or UV irradiation after addition of hydrogen peroxide [12, 34]. For this purpose, fluorometry, electrochemical detectors, atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), mass spectrometry (MS), and neutron activation analysis have been used (Table 2).

Hydride generation coupled to AAS or AFS detectors is specific for Se(IV) determination, where these species are selectively reduced to volatile SeH₂, usually by sodium tetrahydroborate in hydrochloric medium. This technique can be applied for the determination of total inorganic selenium (e.g., sum of selenite and selenate) after quantitative reduction. The content of Se(VI) is then obtained by the difference between two determinations. This technique can be fully automatic by connection with flow injection analysis system. The advantage of such system is minimum sample and reagent consumption as well as short time of single run.

Stripeikis et al. [44] determined selenite and selenate in drinking water using fully automatic online separation/pre-concentration system coupled to electrochemical atomic spectrometry. Preconcentration of both selenium forms was carried out onto a microcolumn packed with an anionic resin (Dowex 1X8) that was placed in the robotic arm of the autosampling device. Selenite and selenate were then sequentially eluted with HCl at concentration of 0.1 and 4 mol L⁻¹,
respectively. The interference of large amounts of chloride ions during selenium atomization was prevented by using iridium as a permanent chemical modifier.

Kocot et al. [36] proposed a dispersive micro-solid-phase extraction with graphene as a solid adsorbent and ammonium pyrrolidine dithiocarbamate as a chelating agent for Se(IV) in analysis of inorganic selenium by the energy-dispersive X-ray fluorescence spectrometry; the concentration of Se(VI) was calculated as the difference between the concentration of selenite after and before reduction of selenate.

Due to the high selectivity and sensitivity, wide linear range, as well as multielement and multi-isotope detection, inductively coupled plasma mass spectrometry (ICP-MS) is a great tool for selenium analysis. However, some difficulties can be found when conventional ICP-MS is used for this purpose. To avoid the spectra interferences with 80Se isotope (the largest natural abundance of 49.6%), 82Se and 77Se are often monitored. The use of collision/reaction cell, operating with hydrogen gas, lowers argon dimer interferences. This technique was applied by Reyes et al. to determine the selenium in biological reference materials and serum samples [45] and yeast [46].

### 4. Separation techniques in selenium speciation analysis

The occurrence of selenium at very low concentration levels as well as the dependence of its toxicity on the form in which it occurs resulted in the need for reliable analytical procedures for identification and quantification of its species. The coupling of separation of selenium species with sensitive detection has become a powerful technique for Se speciation. Liquid chromatography operating in different modes is the most used analytical technique for this purpose; however electrophoresis and gas chromatography also were used in selenium analysis. That is why these two methods will be also described in this chapter, which will mainly focus on liquid chromatography.

#### 4.1 Gas chromatography

Selenium species can be divided into volatile and nonvolatile compounds. The first group of compounds can be directly analyzed using gas chromatography,
for example, dimethylselenide was determined in human breath after ingestion of Se-enriched selenite [47]. For other nonvolatile compounds, derivatization is required.

In the literature many derivatization procedures can be found. Pelaez et al. [48] tested two methods of derivatization of selenomethionine and selenomethionine in selenium supplements. The first method consisted of esterification of the carboxylic acid group using propan-2-ol and then acylation of amino group. The second described procedure used ethanol-ethyl chloroformated for one step derivatization. Then detection using ICP-MS was performed.

In general, many types of detectors were coupled with GC for speciation analysis of selenium. Yang et al. [49] successfully used ICP detection for the determination of selenium in yeast. Organoselenium species in plants were determined using GC-MIP-AES detection [50]. Because of the requirement of the derivatization, GC is not so widely used in selenium speciation analysis in comparison to liquid chromatography.

4.2 Capillary electrophoresis

Due to the high resolving power, capillary electrophoresis (CE) has a potential to be used in speciation analysis as an alternative or complementary technique to HPLC. CE coupled with ICP-MS was used in the analysis of selenium in yeast extract after SEC separation [51]. The obtained limit of detection for organic and inorganic species was in range 7–18 μg L$^{-1}$. The main difficulty in such separation can be big sizes of selenopolypeptides resulting in their slow migration. In such case, the predigestion of selenoprotein fraction is recommended.

It should be highlighted that gel electrophoresis offers better resolution than liquid chromatography in the analysis of high molecular weight selenoproteins, which was used in such analysis by Chassaigne et al. [26], and in Se-enriched yeast analysis as well by Chery et al. in blood sample analysis [52].

4.3 High-performance liquid chromatography

4.3.1 Size-exclusion chromatography

Separation in size-exclusion chromatography (SEC) strictly depends on the size of separated analytes. That is why this technique is widely used as a preliminary step to sample purification or to separate selenoproteins from the matrix. Ayouni [53] observed high molecular weight selenium compounds in the extract of dietary supplements after separation by SEC. Conjunction of SEC with ICP-MS was used for analysis of the products of enzymatic digestion of selenoproteins fraction [54, 55]. Size-exclusion chromatography was also used in selenium analysis in human plasma [56] and extracts from rats’ internal organs [57].

4.3.2 Ion-exchange chromatography

Anion-exchange chromatography has been mainly employed in selenium speciation analysis [58–63]. Mobile phases used in this mode usually contain small content of organic modifier (e.g., 2–5% methanol) and buffered salt solution (e.g., acetate, phosphate, and citrate). During the separation process, the equilibria between the charged solute ion and the oppositely charged surface of the stationary phase are established. The separation is achieved based on the differences of the strength of such interaction between analytes. Anion-exchange
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DOI: http://dx.doi.org/10.5772/intechopen.87053

Chromatography was used in the selenium speciation analysis in garlic, sunflowers, and radish sprouts [64]. In addition to well-known compounds like SeMet or MeSeCys, several unidentified signals were obtained. The application of high-resolution mass spectrometry enabled identification of additional selenocompounds as inorganic metabolites, such as deamino-hydroxy-seleno-homolanthionine, N-acetylcyesteine-selenomethionine, methylseleno-pentose-hexose, methylselenogluthathione, 2,3-dihydroxy-propionyl selenocysteine-cysteine, methylthio-selenoglutathione, 2,3-dihydroxy propionyl-seleno-lanthionine, and two Se-containing compounds with proposed molecular formula C_{10}H_{18}N_{2}O_{6}Se and C_{10}H_{13}N_{5}O_{3}Se.

Cation-exchange chromatography was used to analyze selenium-enriched yeast in a human adsorption study [65]. As a mobile phase, pyridinium formate buffer with 3% of methanol was used. This method was suitable for separation of organic selenium species, however not suitable to separate selenite and selenate.

4.3.3 Reversed-phase chromatography

Both, simple reversed-phase [66–68] and ion-pair (IP) reversed-phase chromatography [15, 28, 69], are widely used for analysis of ionic and neutral selenium species. The mobile phases are aqueous with small amount of polar organic solvent (usually methanol or acetonitrile). Because of their hydrophilicity, selenoamino-acids are not retained onto typical reversed-phase columns. The use of ion-pairing reagents as mobile phase additives allows their separation. The ion-pairing reagent is usually an alkyl sulfonate, an alkyl sulfate, or an alkylammonium salt. Its non-polar chain interacts with hydrophobic stationary phase (e.g., C8 or C18), while ionizable group is neutralized by oppositely charged analyte. Hexanesulfonic acid has been used as anion-pairing reagent in the speciation analysis of selenium in Brazil nuts, using C8 column for separation [70]. Obtaining separation was satisfied for organic compounds but poor for selenite and selenate. For separation of organic and inorganic forms of selenium, tetrabutylammonium acetate was proposed [71]. Also mixed ion-pairing reagents (butanesulfonic and tetramethylammonium hydroxide) were also used to simultaneously separate inorganic and organic species with satisfactory separation efficiencies [72].

New mobile phase additives are still developed, for example, room temperature ionic liquids [73]. Their mechanism of action is based on bilayer formulation onto stationary phase. It gives the possibility of additional interactions between the analyte and the bed, which significantly affects the retention and shape of the recorded signals. The effects of several imidazolium chlorides on the separation of selenium species mixture was described in details [74]. In all cases, SeMeCys was the first species eluted indicating its weak retention in the column, while the retention time of Se(VI) was increasing with the increase of alkyl chain.

4.3.4 Hydrophilic interaction liquid chromatography

Hydrophilic interaction liquid chromatography (HILIC) is a complementary technique to reversed-phase mode. The separation mechanism is mainly based on the partition of the analyte between the thin water layer adsorbed onto the stationary surface and the eluent, which contain high content of organic solvent usually acetonitrile. It is known that also other interactions such as hydrogen bonding dipole-dipole interactions and electrostatic forces may play an important role in the retention mechanism in HILIC [75]. The governed retention mechanism strictly depends on the type of used stationary phase and the buffer conditions (content of
organic solvent, concentration of salt, and pH). TSKgel Amide-80 stationary phase with covalently bound carbamoyl groups is frequently used in the analysis of selenium in HILIC mode [76, 77]. According to the characterization of amide stationary phase, the achieved separation is not pH dependent [78]. Also zwitterionic and silica stationary phases have been also used in HILIC separation of selenium [79, 80]. It should be highlighted that in selenium separation in HILIC mode methanol is recommended instead of acetonitrile as a main component of mobile phase [79] which is shown in Figure 1.

The use of methanol enhances peak intensity, improve the separation of SeMet and SeMeSeCys, and shorten time of the single run. The best separation conditions

![Figure 1](image-url)

*Figure 1.* The chromatogram of selenium compounds obtained on silica column using (A) MeOH and (B) ACN in the mobile phase [79]. Reprinted with permission from Elsevier.
have been obtained for silica column and mobile phase consisted of 85% of methanol and 8 mM of ammonium acetate. Using the zwitterionic column (ZIC-HILIC) instead of silica stationary phase resulted in recording of very asymmetric peaks.

The potential of two orthogonal chromatographic modes—RP and HILIC—was examined in the analysis of onion leaf extracts [79]. Higher separation efficiency (mainly for inorganic selenium species) and shorter retention times were obtained when HILIC mode was used (Figure 2).

Figure 2.
The chromatographic separation of selenium species present in onion leaf extracts in (A) HILIC mode and (B) RP mode. (A) Atlantis HILIC (silica) column, mobile phase: 85% MeOH and 8 mM CH₃COONH₄, pH 7. (B) Luna C8 column, mobile phase: 99.5% HCOOH and 0.5% ACN [79]. Reprinted with permission from Elsevier.
5. Conclusions

The determination of selenium is of great importance from the point of view of understanding its metabolism and its potential benefits for human health. Due to the variety of selenium species and low concentrations in which it occurs in environmental samples, liquid chromatography coupled to ICP-MS is the most powerful method for speciation analysis of selenium. However, other techniques, for example, HILIC-MS/MS, have also been successfully applied in the speciation analysis of selenium. It should be highlighted that for such analysis all the analytical steps like selection of separation and detection method and the optimization of separation parameters (eluent type and composition, mobile phase additives) should be optimized to avoid the coelution of selenium species.

The recent application of liquid chromatography for selenium separation in plants (vegetables samples) is shown in Table 3. For sure new chromatographic strategies will be described in the literature in the nearest future.

<table>
<thead>
<tr>
<th>Sample (determined selenium species)</th>
<th>Mobile phase</th>
<th>Stationary phase</th>
<th>Detection</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green leafy vegetables (SeCys₂, SeMet)</td>
<td>50% MeOH + 1.5% HCOOH</td>
<td>C18 (250 × 4.6 mm, 5 μm)</td>
<td>ICP-MS</td>
<td>[69]</td>
</tr>
<tr>
<td>Garlic (MeSeCys, SeMet, Se(IV), Se(VI))</td>
<td>Gradient phosphate buffer pH 6.0, 5% MeOH, 0.1% [C6mim]Cl</td>
<td>Agilent Zorbax SB-C8 (150 × 4.6 mm)</td>
<td>HG-AFS</td>
<td>[58]</td>
</tr>
<tr>
<td>Onion (MeSeCys, SeMet, Se(IV), g-glu-MeSeCys, Se(VI))</td>
<td>Gradient phosphate buffer, pH 4.7</td>
<td>Hamilton PRP X100 (250 × 4.1 mm, 30 mm)</td>
<td>ICP-MS</td>
<td>[69]</td>
</tr>
<tr>
<td>Carrot (inorganic Se, SeMet, g-glu-MeSeCys)</td>
<td>Gradient: ammonium formate, pH 3.0, 5% MeOH</td>
<td>Chrompack IonoSpher 5C (150 × 2.0 mm, 5 mm)</td>
<td>ICP-MS</td>
<td>[75]</td>
</tr>
<tr>
<td>Onion leaves (Se(IV), Se(VI), MeSeCys, SeMet)</td>
<td>Gradient: MeOH and ammonium acetate, pH 7</td>
<td>Atlantis HILIC (silica) (100 × 2.1 mm, 3.0 mm)</td>
<td>MS/MS</td>
<td>[81]</td>
</tr>
</tbody>
</table>

Table 3. Examples of the recent HPLC application for selenium speciation in plant materials.

Conflict of interest

The author declares no conflict of interest.

Appendices and nomenclature

AAS  atomic absorption spectrometry
AFS  atomic fluorescence spectrometry
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ACN</td>
<td>acetonitrile</td>
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<tr>
<td>C8</td>
<td>octylsilane column</td>
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<tr>
<td>CE</td>
<td>capillary electrophoresis</td>
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<tr>
<td>CH₃COONH₄</td>
<td>ammonium acetate</td>
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<td>ETAAS</td>
<td>electrothermal atomic absorption</td>
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<td>GC</td>
<td>gas chromatography</td>
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<td>HCOOH</td>
<td>formic acid</td>
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<td>HCl</td>
<td>hydrochloric acid</td>
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<tr>
<td>HGAAS</td>
<td>hydride generation atomic absorption spectrometric</td>
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<tr>
<td>HILIC</td>
<td>hydrophilic interaction liquid chromatography</td>
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<td>HNO₃</td>
<td>nitric acid</td>
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<td>HPLC</td>
<td>high-performance liquid chromatography</td>
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<td>inductively coupled plasma-mass spectrometry</td>
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<td>ionic liquids</td>
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<td>ion pair</td>
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<td>RP</td>
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<td>selenocysteine</td>
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<td>SEC</td>
<td>size-exclusion chromatography</td>
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<td>SeMet</td>
<td>selenomethionine</td>
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**Author details**

Aleksandra Sentkowska  
Heavy Ion Laboratory, University of Warsaw, Warsaw, Poland  

*Address all correspondence to: sentkowska@slcj.uw.edu.pl*
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Chromatographic Analysis of Selenium Species
DOI: http://dx.doi.org/10.5772/intechopen.87053

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