Quantification of Selenium Compounds from Soil Samples by HPLC-ICP-MS

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Speciation determination can be obtained by solid analysis techniques for samples with high Se concentrations (mg/kg). Such results are not transposable to samples containing low selenium concentrations (μg/kg), in particular in a radiological context or biosphere monitoring. In this paper, it is highlighted the development of some methods regarding the determination of selenium content from soil samples, presuming a preliminary sample preparation. Traditionally, wet acid digestion has been used which involves digestion/heating with strong acids to destroy the organic matter and dissolve the metal ions. The proposed procedure involves microwave sample preparation by using a mixture of HNO_3/ Ultrapure water. HPLC-ICP-MS (liquid chromatography-inductively coupled plasma mass spectrometry) was applied to assess the selenium concentrations in some soil samples from Romanian Plain, Central and South Dobrogea. For this purpose, data regarding selenium total content in soil, as well as selenium species from soil are presented, the contents being determined by chemical methods and analytical techniques. For extracting the selenium species existing in the studied soil samples, a 0.1M NaOH solution has been used because it had the greatest efficiency for selenium speciation from soil sample with low content of selenium. The major extractable species was selenite.

Keywords: Selenium speciation, environment, hyphenated technique, separation

The development of a methodology to determine selenium speciation in soils at ultra-trace level was studied. Analytical procedure was based on liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP-MS). A special attention was paid on selenium species extraction optimisation. The extractants were selected on the basis of reagents used in Se sequential extraction schemes. The conservation of the original Se speciation in soil during extraction was checked for each extractant. After extraction, total Se content was quantified in dissolved (extracts) and solid phases. Extraction efficiencies increased in order waters << citric acid ≈ phosphate buffers < nitric acid << sodium hydroxide. Speciation analyses indicate the occurrence of selenite (SeIV) in all extracts, whiles selenate (SeVI) and Se containing compound, with retention time close to the one of selenocystine species (SeCys2), were detected only in water extracts [1]. Separation, quantification, and characterization continue to be the biggest problems associated with selenium speciation; most of the recent researches have been accomplished using techniques that couple the separation capability of chromatography to the sensitive detecting ability of inductively coupled plasma mass spectrometry (ICP-MS). Liquid chromatography, particularly the high performance one, is well suited for speciation analysis, as it requires little sample preparation of liquid samples, it could be performed at ambient temperatures, it could achieve maximum separation capability by varying both stationary and mobile phase compositions, it could achieve low detection limits, and it is relatively fast [2]. The speciation determination can be obtained by solid analysis techniques for samples with high Se concentrations (mg/kg). Such results are not transposable to samples containing low selenium concentrations (μg/kg), in particular in a radiological context or biosphere monitoring. In this case, inductively coupled plasma mass spectrometry (ICP-MS) is one of the most often used detection systems for total and speciation analyses due to its high sensitivity and its easy coupling to HPLC [3-5]. However, studies on Se speciation in soils using these hyphenated techniques are scarce in literature. Two complementary chromatographic separations to confirm identity of selenium species have been used. An extraction step is necessary before analysis of dissolved species [6]. Different extractants have been compared, selected based on sequential extraction scheme. Ultrapure water, 0.1 mol/L phosphate buffer (KH_2PO_4/K_2HPO_4) at *p*H 7 and 0.1 mol/L NaOH, have been chosen because of their efficiency in selenium extraction and their compatibility with stable species of selenium. These extractants also allow the assessment of soluble fraction in water (i.e. most mobile fraction of Se), of exchangeable fraction (i.e. the soil surface absorbed) and selenium fraction related to organic matter. Thus, this methodology provides information on selenium mobility related to its distribution in soil maintaining the original species. Detection limits are between 3-29 ng/l allowing the determination of selenium concentrations in extracts from soils with a native selenium content trace [7-9]. A simple method for the inorganic selenium determination in mg/L domain has been developed, using ion chromatography coupled with atomic absorption spectrometry with hydride generation. Because

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of differences in toxicity and absorption behaviour, it is important to determine the valences of selenite Se(IV) and selenate Se(VI). Anion exchange chromatography is used to separate Se (IV) and Se (VI) based on differences in retention time. Adding Se (IV) and (VI) to the extracts of samples, it was showed that the procedure was more accurate. Average recovery was 93.1% for Se (IV) and 108% for Se (VI). This technique was used to determine Se (IV) and Se (VI) in deionised water and current synthetic irrigation water [10].

This paper is focused on the South-Eastern part of the Romanian Plain, as well as on the Central and South Dobrogea, where a study was done regarding low level of selenium in soil, these areas being characterized by a natural handicap, selenium deficiency [11, 12]. For this purpose, data regarding selenium total content in soil, as well as selenium species from soil are presented, the contents being determined by chemical methods and analytic techniques.

For extraction of selenium species existing in the studied soil samples, 0.1M NaOH solution has been used because it had the greatest efficiency for selenium speciation from soil sample with low content of selenium [13]. An anion exchange chromatographic system was coupled to an Agilent 7500 inductively coupled plasma mass spectrometer (ICP-MS) for the identification and quantification of selenium compounds in soil samples. Separation of selenite, selenate, and selenomethionine was achieved by high performance liquid chromatography (HPLC) on a Hamilton PRP-X100 column using a 10 mM ammonium citrate buffer (*p*H 5.2), 2 % methanol as mobile phase and a flow rate of 1 mL min^{-1}. Three baseline separated chromatographic peaks were obtained within 2-4 min.

Experimental part

Reagents

All chemicals used were of analytical grade (Merck, Germany). High quality water, obtained using a Milli-Q system (Purelab ELGA, Anglia) was used exclusively.

Apparatus

Determination of total selenium content and speciation

Total selenium in the soil was determined by ICP-MS (Agilent 7500ce, Agilent, Waldbronn, Germany) using He as collision cell gas to remove politatomics interference and ^{74}Ge, ^{115}In as internal standards. The internal standards (500 µg/L Ge and In for 10% HNO_3) were added online through T tube. Measurements were made using He as collision gas cell to eliminate interference from argon chloride (isobaric interference of $^{40}Ar^{35}Cl$ on ^{78}Se).

Selenium speciation was determined by a High Performance Liquid Chromatography (Agilent Series 1100) coupled to ICP-MS.

Chromatographic separation of SeIV, SeMet and SeVI was carried out on an anionic exchange column (Hamilton PRP-X100, 25 cm x 4.1 mm) using a 10 mmol/L ammonium citrate buffer at *p*H 5.2. The mobile phase was isocratically delivered at 1 mL/min. The HPLC-ICP-MS interface consisted simply in a polyetheretherketone (PEEK) capillary.

Soil samples mineralization and extractions procedures

For the determination of total selenium content, about 0,25 g of finely ground soil sample was weighed with a precision of 0.1mg and digested in a microwave digestion system Ultraclave III (MLS, Leutkirch, Germany) in quartz tubes with 2 mL nitric acid and 2mL. Ultrapure water. The tubes were transferred to a Teflon support and covered with Teflon caps, being settled then into the microwave. After closing the system, a pressure of argon for 4x 10^6Pa was applied, the mixture being heated then to 250°C for 30 min , following a specific temperature schedule: (a) from room temperature to 80°C in 5', (2) -150°C in 15', (3) 150 - 250°C in 15', (4) 250°C for 30' (the approximately total time including ventilation of 60': 2:05h). After mineralization, samples were diluted with 50 mL Ultrapure water in polypropylene tubes (Greiner Bio-One, Frickenhausen, Germany). Total selenium calibration curve is obtained from standard solution of selenium single element: Se in 2%HNO_3 1000 ±3µg/mL, Lot 0CF092.

For extracting the selenium species from soil samples sodium hydroxide (0.1 mol/L) was used, with following operating conditions: 150 mg of soil was placed in polypropylene tubes with 5 mL of the extractant, the mixture was shaken at 250 rpm during 24h. Then, the suspension was centrifuged at 1500 rpm for 30 min. The supernatant was taken up and stored in polypropylene tubes at 4°C until analysis.

Results and discussions

Total selenium content determination

Ten soil samples from Romania (South-Eastern Romanian Plain and Central and Southern Dobrogea) were analysed for the assessment of total selenium content by ICP-MS following microwave-assisted acid digestion (fig. 1). Data regarding total selenium content in soil, as well as selenium species existing in soil samples, contents being determined by chemical methods and analytic techniques, are presented in tables 1 and 2.

Based on data collected in the field, data/existing maps on ICPA Bucharest, observations and analysis of field data, was created a map with using a geographic information system (GIS) called ESRI software ArcViewGis Version 3.1.

Table 1

TOTAL SELENIUM CONTENT (mg/kg) IN ANALYSED SOIL SAMPLES FROM SOUTH-EASTERN ROMANIAN PLAIN AND CENTRAL AND SOUTHERN DOBROGEA

Sample number	Sample code	Weight (g)	Dilution factor (10/g)	µg/L	in 10 mL	Se (mg/kg)	Se (µg/kg)
1	461.D	0.250	40.0	0.96	0.010	0.04	38.4
	462.D	0.250	39.9	0.98	0.010	0.04	39.0
	463.D	0.251	39.9	0.97	0.010	0.04	38.5
2	471.D	0.251	39.9	1.01	0.010	0.04	40.1
	472.D	0.250	40.0	1.04	0.010	0.04	41.4
	473.D	0.251	39.9	1.01	0.010	0.04	40.5
3	481.D	0.251	39.9	1.59	0.016	0.06	63.2
	482.D	0.251	39.9	1.78	0.018	0.07	71.1
	483.D	0.251	39.9	1.62	0.016	0.06	64.5
4	491.D	0.251	39.9	0.91	0.009	0.04	36.4
	492.D	0.250	40.0	0.88	0.009	0.03	35.0
	493.D	0.251	39.9	0.86	0.009	0.03	34.3
5	501.D	0.250	39.9	0.90	0.009	0.04	35.8
	502.D	0.251	39.9	0.88	0.009	0.04	35.3
	503.D	0.251	39.9	0.88	0.009	0.04	35.1
6	511.D	0.251	39.9	1.09	0.011	0.04	43.4
	512.D	0.251	39.9	1.07	0.011	0.04	42.6
	513.D	0.251	39.8	1.05	0.011	0.04	41.8
7	521.D	0.251	39.9	0.97	0.010	0.04	38.8
	522.D	0.251	39.9	1.00	0.010	0.04	39.8
	523.D	0.251	39.9	1.01	0.010	0.04	40.3
8	541.D	0.251	39.9	0.80	0.008	0.03	31.9
	542.D	0.251	39.9	0.80	0.008	0.03	31.8
	543.D	0.251	39.9	0.79	0.008	0.03	31.6
9	551.D	0.251	39.9	0.75	0.008	0.03	30.1
	552.D	0.251	39.9	0.77	0.008	0.03	30.8
	553.D	0.251	39.9	0.71	0.007	0.03	28.4
10	561.D	0.250	39.9	0.61	0.006	0.02	24.4
	562.D	0.251	39.9	0.64	0.006	0.03	25.4
	563.D	0.251	39.9	0.65	0.006	0.03	25.8

Table 2
DATA REGARDING SELENIUM SPECIES

Sample number	Sample code	Weight (g)	Dilution factor (10/g)
1	461.D	0.151	33.1
	462.D	0.150	33.3
	463.D	0.151	33.1
3	481.D	0.152	32.9
	482.D	0.151	33.0
	483.D	0.151	33.2
6	511.D	0.151	33.2
	512.D	0.150	33.3
	513.D	0.150	33.2

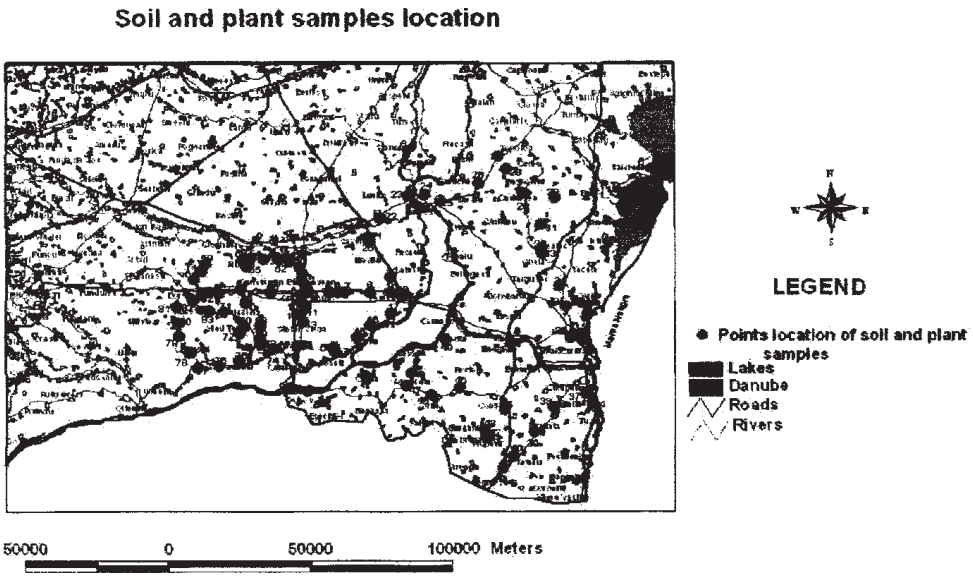

Fig. 1. The study area: 1. South-Eastern Part of Romanian Plain; 2. Central and South Dobrogea

Plasma parameters	*Reaction parameters*	*Data acquisition parameters*
1550 RF/W Power	3.5 l/min H_2 discharge	Monitored isotopes: 77, 78, 80, 82
The discharge of gas injection in plasma: 0.82 l/min	QP bas/V -17 - 19	Time of integration: 0.1s
Debit of auxiliar gas, 1-1.5 min/l	Focus lens: QP 4 V	Total retention time: 10 min
Sample chamber depth: 6-8 mm		RSD < 5%
Nebuliser pump 0.5 rpm		

Table 3
OPERATING CONDITIONS FOR ICP-MS AND DATA ACQUISITION PARAMETERS

Table 4
CHROMATOGRAPHIC CONDITIONS USED IN THE SPECIATION OF SELENIUM COMPOUNDS BY HPLC WITH ANION EXCHANGER

Flow rate (mL/min)	1
Injection volume (µL)	20
*p*H of the mobile phase	5.2
Mobile phase concentration – buffer solution of ammonium citrate	10 mM
Anion exchange column (MeOH/H_2O)	Hamilton PRP X-100
Column temperature (°C)	30

Since soil samples analysed by ICP-MS presents similar total selenium contents, for selenium speciation three samples have been considered, taken in triplicate, as showed in table 2. Table 3 shows operating conditions for ICP-MS and data acquisition parameters.

Extraction of selenium species

The extraction of selenium species from soil samples was done with 0.1 M NaOH solution, because had the greatest efficiency for selenium speciation in soil samples with low content in selenium [5].

For the extraction of soluble species of selenium, a certain amount of sample was added to a certain volume of 0.1 M NaOH, being stirred after that for 24 h. Before HPLC analysis, extracts were centrifuged, taking an aliquot for determination. The selenium species in the samples are separated by HPLC due to their different affinities of the column, so they elute at different retention times.

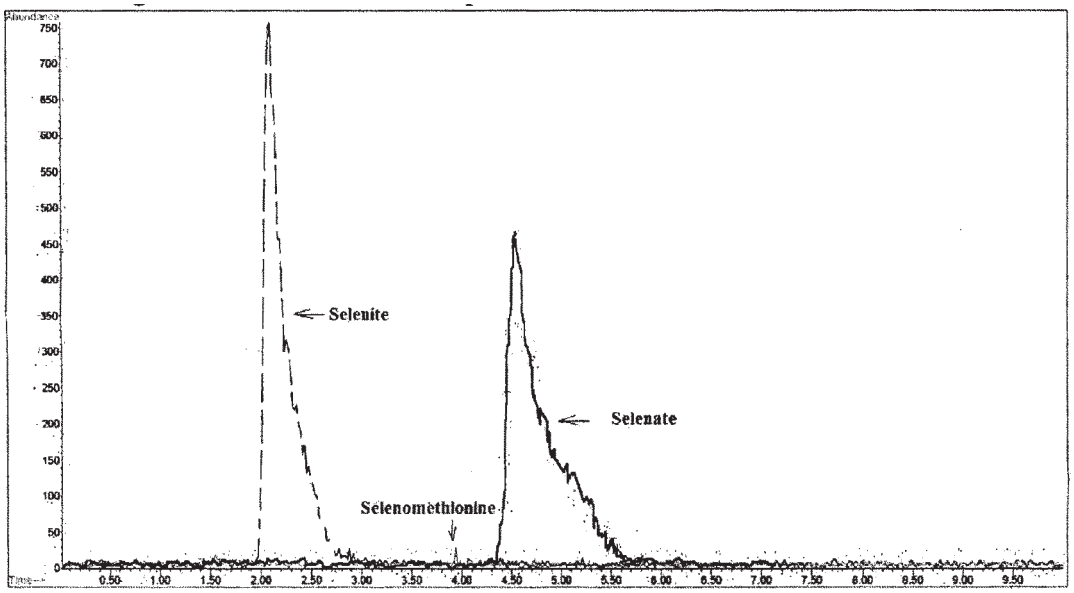

Fig.2. Chromatogram of selenium standard solutions, 0.1 – 5 µg Se/L concentration range

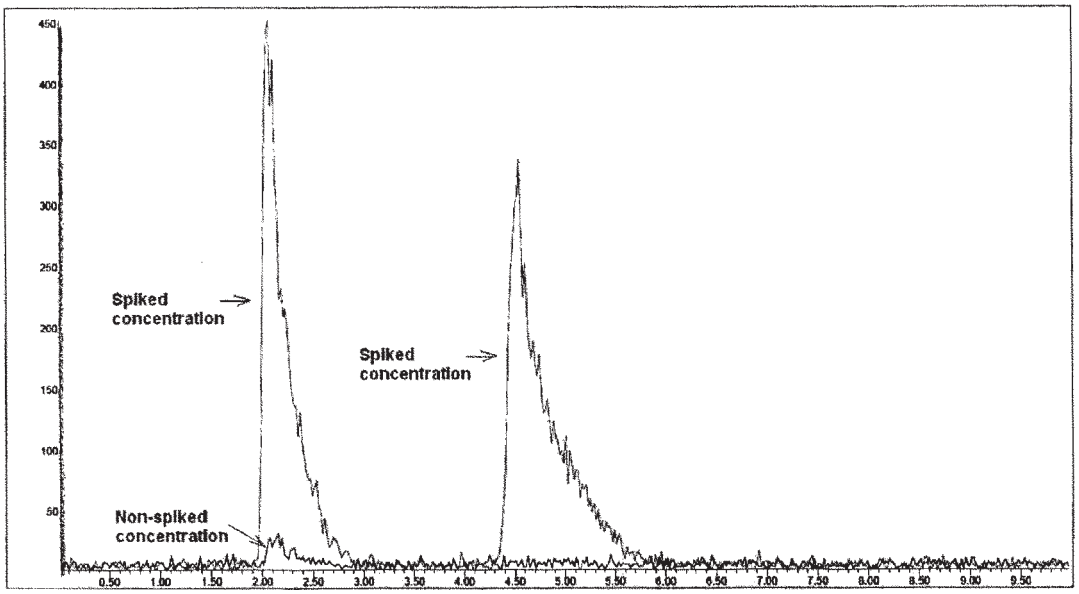

Fig. 3. Chromatogram of Se (IV) and Se (VI) species from one soil samples

Inductively coupled plasma mass spectrometry is used for post-column element-selective detection of selenium.

In table 4 chromatographic conditions used in speciation of selenium compounds by HPLC with anion exchanger are presented.

Figure 2 presents the chromatogram of three standard solutions of selenium, selenite (IV), selenate (VI) and selenomethionine (SeMet), the latter having no signal in selenium speciation, being undetectable in soil samples.

In a first phase of separation, it could not get any signal for selenium species existing in one of three samples. Subsequently, it was decided to add known concentrations of selenium standards solution in analysed samples, resulting in obtaining selenite, Se (IV) and selenate Se (VI) in samples. To correct the concentration of selenium, 19 μL of sample extract have been taken and adding 1 μL of 60 ppb standard containing selenite and selenite. Elution order of peaks in the chromatogram is: Se (IV), Se (VI) at different retention times (2 min; 4.5 min) (fig. 3).

The method selected for speciation analysis is the HPLC-ICP-MS method. Extraction of selenium species from soil samples was performed with 0.1M NaOH. The major extractable species was selenite.

Conclusions

Inductively coupled plasma mass spectrometry (ICP-MS) is one of the most often used method for detecting the total content and speciation analyses, due to its high sensitivity and its easy coupling to HPLC. No losses by volatility through microwave digestion system were detected, which represents a great advantage in comparison to the traditional reduction methodologies.

Ten soil samples from Romania (South-Eastern Romanian Plain and Central and Southern Dobrogea) were analysed for the determination of total selenium content by ICP-MS following microwave-assisted acid digestion. These soil samples have very low selenium contents. The samples were also analysed for selenium species by HPLC-ICP-MS. Extraction was performed with 0.1M NaOH because it had the greatest efficiency for selenium speciation in soil samples with low content in selenium. The major extractable species was selenite.

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