Development of an analytical methodology for ultra-trace selenium speciation determination in soils

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Abstract
This study aimed to develop a methodology to determine selenium speciation in soils at ultra-trace level. This methodology was optimised with a soil containing 431 ± 44 µg/kg of native selenium. 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. Extractants were chosen on the basis of reagents used in Se sequential extraction schemes. Conservation of the original Se speciation in soil during extraction was checked for each extractant. After extraction, total Se was quantified in dissolved (extracts) and solid phases. Extraction efficiencies were in order waters<< citric acid ≈ phosphate buffers< nitric acid<<sodium hydroxide. Speciation analyses indicate the occurrence of selenite (SeIV) in all extracts whereas selenate (SeVI) and a Se containing compound, with retention time close to the one of selenocystine specie ($SeCys_2$), were detected only in water extracts.

Key Words
Metalloid, environment, solid matrix, hyphenated technique, separation, biogeochemistry

Introduction

Selenium is a very important trace element because of the small difference between its essential and toxic levels (Barceloux 1999). Its long-lived radioisotope ^{79}Se is found in high- and intermediate level and long-lived nuclear wastes for which a geological disposal in deep clay formations is considered to be a safe and feasible option (ANDRA 2005). The safety assessment of nuclear waste disposal involves the determination of potential radiological consequences. That implies the understanding of Se transfers in biosphere. In that context, Se speciation data in the different soil components (solid phase, soil solution and associated colloids) are important because the various Se species have different toxicity and different behaviours in the environment (Séby *et al.* 1998). 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 (Darrouzès *et al.* 2005; Montes-Bayon *et al.* 2003; Ponce De León *et al.* 2002). However, studies on Se speciation in soils using these hyphenated techniques are scarce in literature. An extraction step is necessary before analysis of dissolved species (Séby *et al.* 1997). In this study the extraction step was optimised to ensure sample integrity, i.e. original distribution of the Se species. Different reagents were selected on the basis of sequential extractions schemes and tested (Coppin *et al.* 2006; Ponce de Léon *et al.* 2003). Extractant efficiencies were calculated from total Se determinations in supernatant and residual solid phase. HPLC-ICP-MS analyses were performed allowing determination of selenite (SeIV), selenate (SeVI), selenomethionine (SeMet) and selenocystine ($SeCys_2$) depending on extraction solution (Darrouzès *et al.* 2005).

Material and method

Reagents and samples

All reagents used were analytical grade. Ultrapure water was obtained from Milli-Q System (18,2 Ω.cm). An English meadow clay loam soil (Rothamsted) was used for method optimisation. It was air dried, sieved (<2 mm) and grinded in zirconium bowl during 7 minutes at 30 Hz before mineralization and extraction.

Soil mineralization and extractions procedures

The soil was mineralized by microwave-assisted acid digestion (Milestone). To validate the mineralization, 3 reference soil materials (ZC73001, BCR-143R and GBW 07405) were digested in the same manner. According to the literature, seven selective chemical solutions were tested: nitric acid (1 mol/L), citric acid (0.1 mol/L), ultra-pure water, calcium chloride ($5.\ 10^{-4}$ mol/L), phosphate buffer (KH_2PO_4/K_2HPO_4; 0.1 mol/L) with pH 7 and 8, and sodium hydroxide (0.1 mol/L). Each extraction was realized in triplicate with

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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, suspension was centrifuged at 15000 g for 30 min at 4°C. The supernatant was taken up and stored in polypropylene tubes at 4°C until analysis. Residual solid phase was mineralized.

Total and speciation selenium determination
Total selenium was determined with an Agilent 7500ce ICP-MS instrument equipped with an octopole collision/reaction cell. The sample introduction system was constituted of a Micromist nebuliser and a Scott spray chamber. Operating conditions were optimised daily and m/z monitored with ^{78}Se. Selenium speciation was determined by a High Performance Liquid Chromatography (Agilent Series 1100) hyphenated to ICP-MS. Chromatographic separation of $SeCys_2$, SeIV, SeMet and SeVI, was carried on anionic exchange column (Hamilton PRP-X100, 25cm x 4.1 mm i.d) using a 5 mmol/L ammonium citrate buffer at pH 5.2 (Darrouzès *et al.* 2005). The mobile phase was delivered at 1 mL/min isocratically. The HPLC-ICP-MS interface consisted simply in a polyetheretherketone (PEEK) capillary.

Results
Analytical performances and matrix effects studies
Analytical performances were determined for mineralization and extractions procedures according to IUPAC recommendations (IUPAC 1987):

$D.L. = (3\sigma)/p$ with σ = standard deviation of 10 blanks (i.e. recreated matrix) (1)
$Q.L. = (10\sigma)/p$ with p = matrix calibration slope (2)

Detection and quantification limits are, respectively from 0.2 to 23 and 0.7 to 77 µg/kg depending on the extraction matrix. For speciation, they are from 0.002 to 0.2 and 0.01 to 0.7 µg/kg in function of matrix and Se species. Moreover, extern calibration realized in a recreated matrix (extraction or mineralization) and standards additions were performed the same day in order to control matrix effect during Se quantifications.

Selenium speciation stability during extractions
Individual Se species standards were prepared in the matrix extraction. They were analysed by HPLC-ICP-MS just after preparation and after 7 days of storage at 4°C. Speciation conservation was evaluated using the ratio R_s, which is the mean value ($[Se\ specie]_{at\ 7\ days}$) of 3 determinations made after 7 days storage divided by the mean value for 3 initial measurements ($[Se\ specie]_{at\ 0\ days}$) (Equation 3). The uncertainty measurement (U) was obtained from the variation coefficient (CV) calculated for 3 measurements (Equation 4). No instability can be concluded when the value 1 is comprised between the range $R_s \pm U$ (Kramer *et al.* 2001).

$$R_s = [Se\ specie]_{at\ 7\ days} / [Se\ specie]_{at\ 0\ days} \quad (3)$$

$$U = R_s.(CV^2_{at\ 7\ days} + CV^2_{at\ 0\ days})^{1/2}/100 \quad (4)$$

Only results obtained for Se species observed in soil extracts (i.e. SeIV, SeVI and $SeCys_2$) are presented in this paper (Table 1). Results indicate that selenium species are stable in extractant solutions during 7 days.

Table 1. Selenium speciation stability during extractions (n.d. = No Determined)

	SeIV: $R_s \pm U$	SeVI: $R_s \pm U$	$SeCys_2$: $R_s \pm U$
Nitric acid and citric acid	**n.d. – strong matrix effect on chromatographic separation**		
Ultrapure water	**0.99** ± 0.05	**1.00** ± 0.03	**0.97** ± 0.04
Phosphate buffer pH 7	**0.98** ± 0.02	**0.99** ± 0.04	**1.00** ± 0.02
Phosphate buffer pH 8	**0.98** ± 0.06	**1.00** ± 0.05	**0.99** ± 0.02
Sodium hydroxide	**1.00** ± 0.04	**0.99** ± 0.04	**0.98** ± 0.03

Validation of the selenium total determination protocol and Se_{total} concentration in the Rothamsted soil
Total selenium concentrations of soil reference materials obtained after mineralization and ICP-MS analyses were in complete agreement with theoretical values (Table 2). Thus, the protocol of total Se determination in soil was validated.

Table 2. Selenium total concentrations in the studied soil and reference soil materials (in µg(Se)/kg, mean values on 12 replicates, s.d. = standard deviation)

	Rothamsted soil	NCS ZC73001	BCR-143R	GBW-07405
Theoretical values	423 (no s.d.)*	210 ± 20	600 (no s.d.)	1560 (no s.d.)
Obtained values	431 ± 44	225 ± 30	532 ± 54	1536 ± 123

** value obtained by Arras Laboratory (INRA, France)*

Calculated mass balances (Extracted Se + residual Se) resulting from tested extractions indicate that no contamination or losses occurred during extraction (Figure 1) allowing validation of the extraction protocol.

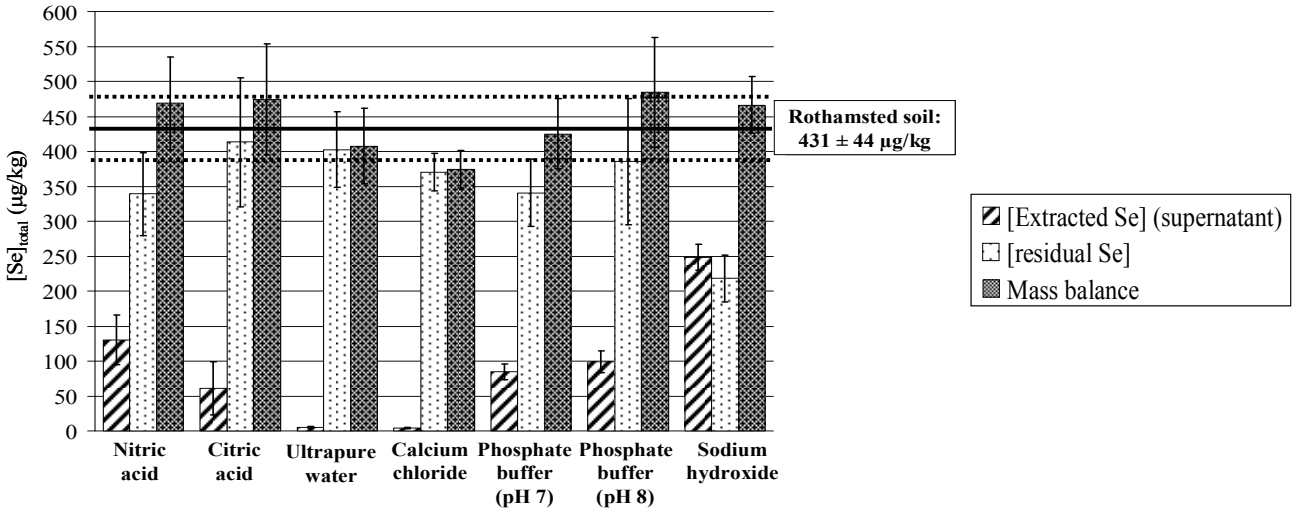

Figure 1. Mass balances for each extraction (— :$[Se]_{total}$ in Rothamsted soil; – · : standard deviation of $[Se]_{total}$)

Extraction efficiencies and selenium speciation

The efficiencies of tested extractants increase following: ultrapure water ≈ calcium chloride << citric acid ≈ phosphate buffer pH 7 ≈ phosphate buffer pH 8 < nitric acid << sodium hydroxide (Table 3), which is consistent with their successive use in sequential extraction scheme for selenium (Coppin *et al.* 2006; Martens and Suarez 1997; Ponce de Léon *et al.* 2003; Wright *et al.* 2003; Zhang and Moore 1996).

Table 3. Efficiencies of extractions and Se speciation in extracts (n.d. = No Determined)

Extractant	Efficiency (%)	Se speciation (% of $[extracted\ Se]_{total}$)
Ultrapure water	1.1 ± 0.3	SeIV = 30 ± 1; SeVI = 15 ± 1; $SeCys_2$ = 19 ± 1; Unknown species ≈ 36
Calcium chloride	1.9 ± 0.4	SeIV = 28 ± 2; SeVI = 9 ± 1; $SeCys_2$ = 34 ± 3; Unknown species ≈ 29
Citric acid	18 ± 3	n.d. (strong matrix effect on chromatographic separation)
Phosphate buffer pH 7	20 ± 1	SeIV = 94 ± 9
Phosphate buffer pH 8	22 ± 3	SeIV = 94 ± 3
Nitric acid	30 ± 7	n.d. (strong matrix effect on chromatographic separation)
Sodium hydroxide	58 ± 3	SeIV = 78 ± 9; Unknown species ≈ 22

Obtained extraction efficiencies are in the order of magnitude of those reported in the literature (Coppin *et al.* 2006; Kang *et al.* 1991; Martens and Suarez 1997; Séby *et al.* 1997; Zhang and Moore 1996). In the case of Se spiked soils, extraction efficiencies may be higher (Darcheville *et al.* 2008) because spiked Se is usually easier to extract than native Se. For phosphate buffer extractant, pH adjustment to 8 does not increase extraction efficiency contrary to Wright et al. (2003) study. But these authors have worked on synthetic model sediments spiked with Se. Selenium speciation was not determined in acidic extractions due to their strong matrix effect on chromatographic separation. Selenite was detected in all extracts (i.e. other than acidic ones), as single Se species in phosphate buffers (94 ± 9% of total Se) and sodium hydroxide (78 ± 9%) extracts. Selenate was quantified only in ultrapure water and calcium chloride extracts. A Se-containing compound with retention time close to the one of selenocystine ($SeCys_2$) specie was also detected in these samples. The sum of species concentrations is in the range 64 - 78% of total Se, excepted for phosphate buffer extractants. These results could indicate i) the presence of dissolved selenium species that are not eluted by the chromatographic separation chosen. This can be checked by the use of complementary chromatographic mechanisms (e.g., reverse phase); ii) the presence of colloidal selenium species (inorganic or organic SeIV and/or SeVI or colloidal Se(0)). To confirm this last hypothesis, extract analyses with Flux Force Fractionation (FFF) hyphenated to Multi-Angle Laser Light Scattering and UV would allow identification and characterisation of the different colloid families. Corresponding total selenium concentrations can be obtained by FFF - ICP-MS coupling.

Conclusion

For each extractant, ultra-trace total and speciation selenium quantification was carefully controlled by matrix effect studies. Repeatability and reproducibility of developed analytical methodology are below 10%. In this study, relevance of the different extractants was evaluated on the basis of three key factors: i) sample

integrity preservation (i.e. original distribution of Se species in soil sample); ii) quantitative Se extraction; iii) Se binding phase assessment (i.e mobility data). Selenium species stability was controlled for all extractants excepted for acidic ones due to their strong matrix effect on chromatographic separation. Three extractants showed to be of particular interest: sodium hydroxide, phosphate buffer at pH 7 and ultrapure water. Indeed, highest extraction efficiencies (about 60%) were obtained with sodium hydroxide, a reagent usually used to dissolve organic solid phase. Both phosphate buffers (pH 7 and 8) showed similar results with an extraction efficiency of about 21%; a pH 7 buffer is generally used in literature to access Se fraction bounded to Fe, Al and Mn (oxy)hydroxides and clays. The amount of Se extracted with ultrapure water was low (about 1%) but such determination is very important since water soluble selenium is considered as mobile and available fraction for living organisms (Gissel-Nielsen and Bisbjerg 1970), especially with regards to native Se. For the Rothamsted soil, three Se species (SeIV, SeVI and a Se containing compound, probably $SeCys_2$) were observed in the water extracts whereas SeIV was the single extracted Se species in phosphate buffers and sodium hydroxide.

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