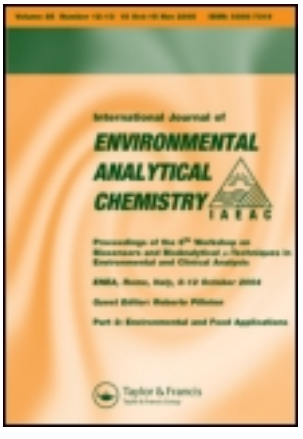

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/geac20

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Available online: 04 Nov 2011

To cite this article: Jacqueline L. Stroud, Steve P. McGrath & Fang-Jie Zhao (2012): Selenium speciation in soil extracts using LC-ICP-MS, International Journal of Environmental Analytical Chemistry, 92:2, 222-236

To link to this article: http://dx.doi.org/10.1080/03067310903111661

Intern. J. Environ. Anal. Chem.
Vol. 92, No. 2, 15 February 2012, 222–236

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Selenium speciation in soil extracts using LC-ICP-MS

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(*Received 15 December 2008; final version received 11 June 2009*)

Selenium (Se) speciation in soil affects its bioavailability to crops. An analytical procedure for the determination of inorganic Se species (selenite and selenate) in soil extracts by anion-exchange liquid chromatography (LC) with ICP-MS detection has been developed, with 10-fold higher sensitivity than existing HGAAS-based soil Se measurements. A comparison of phosphate extraction solutions on agricultural soils amended with 20 $\mu g\,kg^{-1}$ selenate or selenite was carried out, and a 0.016 M KH_2PO_4 extraction solution is recommended. Recovery of selenate was >91%; however, selenite recovery ranged between 18.5% and 46.1%, due to rapid binding to the soil. Soil preparation did not have a significant ($p > 0.05$) effect on the extractability of the selenate or selenite amendments. The stability of Se species in the phosphate extracts was variable, depending on temperature and storage time. Therefore, immediate (<1 h) analysis of the soil extracts is preferable. The method developed was applied to the determination of extractable Se from six arable soils in the UK. Extractable Se levels in these soils ranged between 6 and 13 $\mu g\,kg^{-1}$ consisting of selenite and some soluble organic Se.

Keywords: selenate; selenite; selenium speciation; LC-ICP-MS; soil

1. Introduction

Selenium (Se) is an essential micronutrient for humans and animals, needed for hormone regulation, the immune system and defence against oxidative stresses. There is also evidence that Se helps prevent some cancers [1]. It has been estimated that 0.5–1 billion people worldwide may have insufficient intake of Se [2]. The average Se intake by the UK population is below the recommended level [3] as a consequence of generally low Se supply from the soil, with >95% of the UK soils containing <1 mg Se kg^{-1} [4]. Agronomic fortification of staple crops using Se fertilisers is a proposed solution [4,5], as it has been practiced successfully in Finland since the mid-1980s [4].

Plant-available Se in soil is strongly influenced by Se speciation, with selenate being more available for uptake than selenite [6]. Data reporting available Se concentrations and its speciation in soil would be invaluable to agronomic biofortification strategies

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ISSN 0306–7319 print/ISSN 1029–0397 online

http://dx.doi.org/10.1080/03067310903111661
http://www.tandfonline.com

currently being considered in an effort to improve UK Se status [4]. However, there is no method for the quantitative determination of plant-available of Se species in low Se soils with low Se concentrations ($<20\,\mu g$ soluble Se kg^{-1} soil), with most determinations carried out on high Se soils (>50–9000 μg soluble Se kg^{-1} soil; Table 1).

Plant-available Se is usually estimated using a phosphate (e.g. KH_2PO_4) extraction [7] (Table 1) which removes the soluble and adsorbed Se in soil [8]. A $0.016\,M\,KH_2PO_4$ extraction has been optimised for the extraction of available sulphate [9], and as S and Se are chemical analogues, the same extraction may be used for both elements. Phosphate exchanges with inorganic Se species adsorbed by clay minerals and oxides [7]. In aerobic soils, Se is present as either selenite or selenate. Thermodynamic calculations show that selenite is the dominant Se species under moderate redox soil conditions [10], which is adsorbed by strong inner-sphere ligand exchange mechanisms [11]. This results in limited plant availability and low potential for leaching. Selenate is found in soils under high redox conditions [10] and is weakly adsorbed by outer-sphere mechanisms [11], resulting in high plant availability and potential for leaching. The sensitivity of Se speciation to redox changes means that Se speciation needs to be determined by methods that do not alter the chemical form of Se during extraction and analysis.

Soil preparation methods can change oxidative conditions in soil, altering Se speciation and extractability [12]. Methods of soil preparation for Se analysis vary. They include the analysis of fresh soil, oven and air-dried soil (Table 1). Oven-drying soils before extraction has been shown to decrease selenate concentrations [12]. The stability of extracted Se species is affected by storage temperature, characteristics of the solution and properties of the storage container [13]. Soil extract solutions are stored at a range of temperatures before analysis (Table 1) and there is great variability in the reported stability of Se in solutions. Cobo *et al*. [13] reported the highest stability of inorganic Se in aqueous samples stored at −20°C, whereas Lindemann *et al*. [14] reported that instability and the formation of new peaks occurred in water extracts of soil stored at −20°C. No studies have investigated the influence of storage conditions on the analysis of Se species in phosphate extracts.

Selenium speciation of soil-phosphate extracts has been routinely carried out by hydride generation atomic absorption spectroscopy (HGAAS) [7,8,12,15]. However, this technique has numerous disadvantages, particularly that selenate cannot be directly measured and is calculated by the difference between analysed total Se and selenite concentrations after a lengthy treatment procedure on the extracts. Furthermore, this method has a sensitivity reported at $10\,\mu g\,Se\,kg^{-1}$ [8,12], which is not suitable for speciation analysis of soils with low extractable Se concentrations. Inductively coupled plasma mass spectrometry (ICP-MS) provides highly sensitive quantification of trace elements including Se, and when coupled with liquid chromatography (LC), is capable of Se speciation at the sub parts per billion (ppb) level. LC-ICP-MS has been used to determine Se speciation in a variety of samples such as plants, beverages and grain samples; however, no studies have been reported on its use to soils with very low levels of Se. LC-ICP-MS has been applied to water [11] and microwave [16] extractions of Se-contaminated soil and sediments (>250 μg soluble Se kg) using gradient or ion-pairing chromatography conditions. The advantage of LC-ICP-MS is the direct measurement of selenite and selenate; however, in the past the best Instrument Detection Limit (IDL) for these species was $0.497\,\mu g\,L^{-1}$ and $0.660\,\mu g\,L^{-1}$ respectively [11] (although this may not be sensitive enough to measure extractable Se concentrations in low Se soils occurring commonly in the UK and other regions). Further, both of these methods [11,16] analysed Se concentrations using the low abundant isotopes ^{77}Se and ^{82}Se. ICP-MS instruments

Table 1. Selenite (SeIV) and selenate (SeVI) analysis in soils and sediments from the literature.

	Mild extraction solution					Extractant Storage					
Sample preparation	Conc. (M)	pH	Extractant	Dry w/v ratio ($g\,ml^{-1}$)	Se fraction	Temp	Time	Method of Detection	Se species in soil extract ($\mu g\,kg^{-1}$)	IDL	Ref.
Fresh, air dried or oven dried. Sieved (2 mm)	0.016	4.8	KH_2PO_4	1 : 3	Soluble Exchangeable	−18°C to 20°C	<12 h –28 d	LC-ICP-MS (anion exchange)	1–5 *selenite*	SeIV: 0.15 $\mu g\,L^{-1}$ SeVI: 0.16 $\mu g\,L^{-1}$	This exp.
Air dried, ground (0.25 mm)	1	4.8	KH_2PO_4*[s]	1 : 5	Exchangeable	–	–	HGAAS	10–240	n.d.	[7]
Air dried, ground (150 µm)	0.1	7	K_2HPO_4-KH_2PO_4*[s]	1 : 5	Exchangeable	4°C	–	HGAAS	170–3390	SeIV: 0.01 $mg\,kg^{-1}$	[8]
Not reported	n/a	n.d.	dH_20,	1 : 20	Soil solution	–	<12 h	LC-ICP-MS (anion exchange)	250–8070	SeIV: 0.497 $\mu g\,L^{-1}$ SeVl: 0.660 $\mu g\,L^{-1}$	[11]
Fresh, air dried or oven dried. Sieved (2 mm)	0.1	7	K_2HPO_4-KH_2PO_4*[s]	1 : 5	Exchangeable	–	–	HGAAS	770–1670	SeIV: 0.01 $mg\,kg^{-1}$	[12]
Air dried, ground (1 mm)	0.33	4.8	KH_2PO_4	1 : 6	Soluble Exchangeable	–	–	HGAAS	26–337	n.d.	[15]
Not reported	Low power microwave digestion				Total	4°C	24 h	LC-ICP-MS (ion pair)	530–3220 *selenite*	SeIV: 0.77 $\mu g\,L^{-1}$ SeVI: 0.63 $\mu g\,L^{-1}$	[16]
Not reported	n/a	n.d.	dH_2O	1 : 10	Soluble	–	–	a) DPCSV b) HG-QFAAS	32.7–78	a) SeIV: 25 $ng\,L^{-1}$ b) SeIV: 30 $ng\,L^{-1}$	[30]
Sieved (2–5 mm), frozen (−18°C), defrosted	0.2	7.2	KH_2PO_4*[s]	1 : 10 (wet w)	Exchangeable	−18°C	–	ICP-MS (Total Se)	n/a	70–300 $ng\,L^{-1}$	[31]

Notes: – Not reported; n/a: Not applicable; n.d. Not determined; *[s]: Sequential extractions including named extractant.

equipped with a collision/reaction cell to remove the polyatomic interferences caused by argon ($^{40}Ar^{40}Ar^{+}$, $^{40}Ar^{38}Ar^{+}$) enable the quantitative analysis of Se on its abundant isotopes ^{78}Se (23.8% abundance) and ^{80}Se (49.6% abundance), and enhances Se detection by a factor of 2–5 fold [17]. The presence of hydrogen (the reaction gas) causes an interference on ICP-MS ^{78}Se detection due to the hydride formation $^{77}Se^{1}H^{+}$. However, this is corrected automatically by the calibration of Se standards. Interferences during LC-ICP-MS could form from bromine in the soil extracts, causing a chromatographic peak when signal m/z 80 is monitored, which could be $^{79}Br^{1}H^{+}$ or ^{80}Se. Therefore, more than one Se isotope needs to be monitored during LC-ICP-MS analysis, to ensure the correct identification of peaks.

The aim of this work was to evaluate a method for the extraction and analysis of low concentrations of Se in soils. The method was applied to agricultural soils in the UK to determine the concentrations of inorganic Se species extracted with phosphate, as an indication of Se plant-availability.

2. Experimental

2.1 *Chemicals*

All solutions were prepared in ultrapure (>18 MΩ) deionised water. Sodium selenate, sodium selenite, Se-methyl-selenocysteine and selenomethionine were obtained from Sigma (St Louis, MO, USA). Selenomethioinine Se-oxide was prepared as described in [6]. Stock standard solutions containing 1000 mg L^{-1} were stored in the dark at 4°C. The stability of stock solutions was checked periodically using ICP-MS and LC-ICP-MS. No changes were detected during the experiment. Working standards were serially diluted from the stock solutions. Methanol (HPLC grade), sodium carbonate, nitric acid (trace analysis grade), hydrogen peroxide (trace analysis grade) and potassium bromide (analytical reagent grade) were obtained from Fisher Scientific (Loughborough, UK). There are no certified reference materials for extractable Se speciation in soil; therefore, an in-house reference material (Woburn soil) was used because of its low levels of extractable Se (5.5 ± 0.2 μg kg^{-1}). Glassware was decontaminated by soaking overnight in 10% (v/v) HNO_3, rinsed thoroughly with ultrapure water and air dried, as any traces of HNO_3 could lead to the oxidation of Se species [18].

2.2 *Instrumentation*

2.2.1 *ICP-MS analysis for extractable Se concentrations*

Analysis was carried out using a 7500ce Octopole Reaction System ICP-MS (Agilent Technologies, Palo Alto, CA, USA). The sample introduction system consisted of a Micromist glass concentric nebuliser, Quartz Scott type double pass spray chamber at 2°C and nickel sample (1.0 mm) and skimmer (0.4 mm) cones. Operating parameters are shown in Appendix 1, optimised with consideration to the CeO^{+} to Ce^{+} ratio, which was less than <1.0% on H_2 mode. Calibrations were performed using external standards and quantification was carried out using the data analysis program ChemStation Plus (August 2004 edition). Duplicate samples were analysed with calibration standards, reagent blanks and in-house reference material (Woburn Soil) in each batch for quality control.

2.2.2 *LC-ICP-MS analysis for selenium speciation*

Operating parameters are shown in Appendix 1. The LC-ICP-MS interface consisted of a minimal length of polyetheretherketone (PEEK) tubing. Chromotagraphic separation was carried out using an Agilent 1100 series HPLC system (Palo Alto, CA, USA). The anion exchange analytical column was a Dionex Ion Pack AS14 (9 μm particle size, 4 mm × 250 mm id), fitted with a guard column (AG14). The polymer based Dionex AS14 column is functionalised with quaternary ammonium groups which interact with the negatively charged selenite and selenate ions. The mobile phase was 6 mM Na_2CO_3 (pH 9.5) which is the maximum buffer strength to minimise analysis time without causing a significant salt build-up on ICP-MS sample and skimmer cones which could reduce analytical precision. Se has a low ionisation in argon plasma (33%) [19], 2% methanol was pre-mixed with the mobile phase to enhance Se ionisation, as Se has a lower ionisation energy (9.25 eV) than C (11.25 eV) and the transfer of electrons to the carbon atoms enhances Se detection by up to 3-fold [20]. The injection volume was 50 μL and the mobile phase was delivered at 1 mL min^{-1} isocratically. The identification of Se species was determined by comparison with retention times of standard compounds. Quantification of Se species was determined using external calibration curves and peak area measurements. Duplicate samples were measured with calibration, reagent blanks and calibration standards analysed at the start of the run were repeated at the end of the run for quality control.

2.2.3 *Performance of the method*

Method performance is described in terms of: LC-ICP-MS calibration curve (1–100 μg L^{-1}) and ICP-MS calibration curve (1–20 μg L^{-1}); Instrument Detection Limits (IDL) and Background Equivalent Concentration (BEC). All were determined using ChemStation Plus (August 2004 edition).

2.3 *Procedures*

2.3.1 *Soil sampling and property analysis*

Soils were sampled in April 2007, from an arable field at Rothamsted, Harpenden (Hertfordshire). The remaining soils were sampled in August 2007 from Pickwell (Leicestershire), Sutton Bonington, Loughborough (Leicestershire) and Rothamsted from arable fields. Soil properties are shown in Table 2. Ten soil cores were sampled to a depth of 30 cm, bulked, air-dried for 7–10 d and passed through a 2 mm sieve to remove stones and roots. Soil pH was determined in a soil : water suspension (10 g soil : 25 mL H_2O) using a calibrated pH meter. Total nitrogen (N) and carbon (C) were determined

Table 2. Selected properties of soils used for selenium amendments.

Soil	Texture	pH	Organic C (%)	Total N (%)	Total Se (μg kg^{-1})
Pickwell	Clay loam	6.38	2.48	0.22	527
Sutton Bonington	Sandy loam	7.04	1.97	0.16	300
Rothamsted	Silt loam	8.23	1.32	0.10	394

using the combustion method (LECO CNS 2000). Particle size analysis was determined gravimetrically. Total soil Se concentrations were determined using an *aqua regia* digest [21] followed by ICP-MS analysis.

2.3.2 *Recovery of Se species added to soil*

Air-dried soils were amended with (1) 20 μg kg^{-1} selenate; (2) 20 μg kg^{-1} selenite, delivered in 30 mL water to bring the soil to 30% moisture content, mixed using a plastic spatula for 2 minutes and the samples were incubated overnight at 4°C. Each Se addition was carried out in triplicate. In the investigation of soil preparation methods we tested: fresh (no drying), air-dried for 7–10 d at 20°C and oven-dried at 40°C for 18 h. The moisture content of the soil samples was determined after preparation and used to calculate Se concentrations on a dry weight basis. Samples were extracted as detailed in Section 2.3.3. Non-amended soil samples were also prepared in the same way to allow calculations of the recovery of the Se added to soils. To determine the impact of sample storage time and temperature on Se speciation analysis, extracts were analysed by LC-ICP-MS immediately after extraction (<1 h) and within 48 h for ICP-MS analysis. Extracts were further stored in the freezer (−18°C), fridge (4°C) or room temperature (20°C) for 7, 14 and 28 d before analysis of Se species using LC-ICP-MS.

2.3.3 *Soil extraction*

Prepared soil (10 g) was placed in a 50 mL polypropylene bottle and 30 mL extraction solution of either 0.016 M KH_2PO_4 (pH 4.5), 0.1 M KH_2PO_4 (pH 4.7) or 0.1 M K_2HPO_4.KH_2PO_4 (pH 7.1). The bottle was capped and shaken at 120 oscillations min^{-1} on a horizontal shaker at 20°C for 1 h. The solution was filtered through Whatman no. 42 paper. A portion (1.5 mL) was syringe filtered (0.4 μm) into a screwtop clear borosilicate glass vial for Se speciation analysis by LC-ICP-MS. The remaining filtrate (9.5 mL) was acidified with 0.5 mL HNO_3 for analysis of total extractable Se by ICP-MS. LC-ICP-MS analysis was carried out immediately (<1 h) following sample extraction, while ICP-MS analysis was carried out within 48 h.

2.3.4 *Application of the method to agricultural soils*

Using the results from Section 2.3.3, the method was applied to soil samples taken from the 6 arable fields. The soils were analysed for extractable Se using 0.016M KH_2PO_4 extraction solution. Samples for the ICP-MS were analysed within 7 d, and samples for the LC-ICP-MS were further syringe filtered (0.45 μm) and analysed within 1 h of the extraction.

2.3.5 *Investigating the organic selenium fraction in agricultural soil extracts*

A portion (2 mL) of the filtered, acidified (5%) solution was added to a 25 mL sterilin vial. One millilitre of H_2O_2 was added, and the vial gently agitated. The vial was placed in a water bath and heated to 80°C for 1 h. The purpose of this treatment was to provide evidence of the existence of Se-containing amino-acids which may be released by peroxide treatment, and further be oxidised to LC-ICP-MS detectable forms such as Se-oxides.

LC-ICP-MS analysis was carried out immediately (<1 h) following sample preparation, and the peaks were compared to standard solutions of organic and inorganic Se ions for identification purposes only.

2.3.6 *Data analysis*

The concentrations of Se in soil samples were expressed on the dry weight basis. ANOVA were performed to test the difference between treatments using Genstat® (10th edition, VSN International, UK).

3. Results

3.1 *Performance of the analytical method*

Linear calibration was precise ($r^2 = 0.99$) for ICP-MS and LC-ICP-MS for the range of Se concentrations tested (Figure 1). Throughout analysis, total Se was quantified using ^{78}Se to avoid a $^{79}Br^1H^+$ interference potentially affecting quantitation of ^{80}Se due to the significant amounts of Br in the soils. The in-house Woburn soil standard showed good

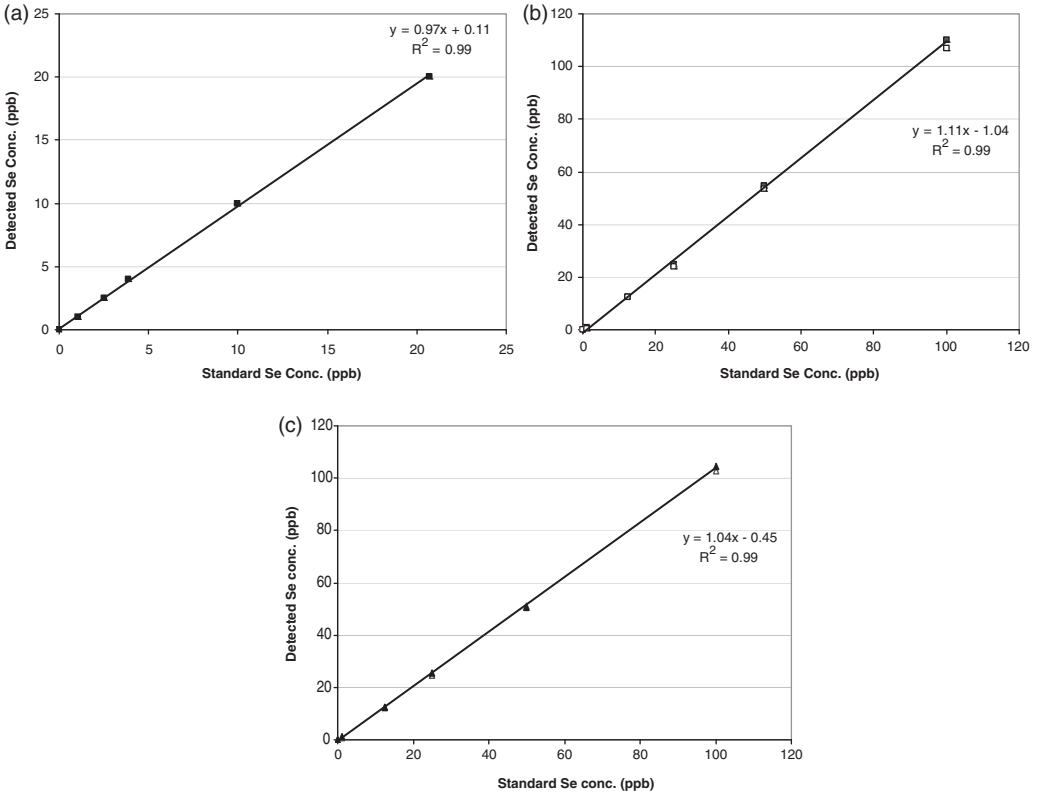

Figure 1. Calibration curves for selenium analysis using different instruments (a) selenium using ICP-MS, (b) Selenite using LC-ICP-MS, (c) Selenate using LC-ICP-MS with empty symbols detection for on ^{78}Se, filled symbols for detection on ^{80}Se.

agreement to the target Se concentration ($5.5 \pm 0.2\,\mu g\,kg^{-1}$) in the 0.016 M KH_2PO_4 extraction. The Se signal during LC-ICP-MS analysis was improved two-fold by the addition of 2% methanol to the mobile phase; this was used throughout analysis. The IDL for ^{78}Selenite was $0.15\,\mu g\,L^{-1}$ and BEC was $0.1\,\mu g\,L^{-1}$. Similarly, the IDL for ^{78}Selenate was $0.16\,\mu g\,L^{-1}$ and BEC was $0.1\,\mu g\,L^{-1}$. Figure 2(a) shows a typical chromatogram of mixed Se standards ($12.5\,\mu g\,L^{-1}$). The detection limit of the method was determined to be $0.46\,\mu g$ Se kg^{-1} soil. The retention time was 7.10 ± 0.2 min and 9.50 ± 0.2 min for selenite

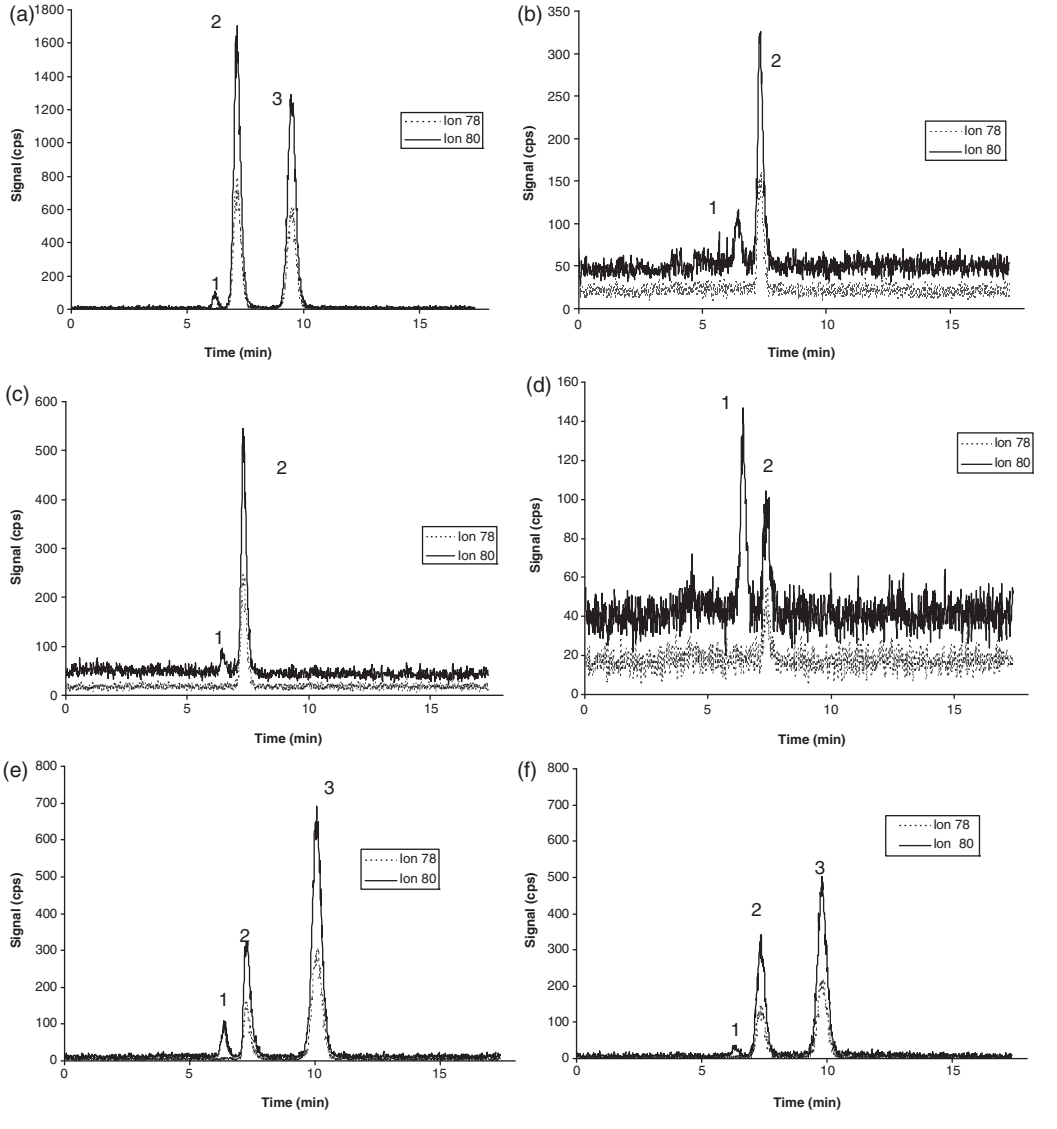

Figure 2. Anion exchange chromatograms LC-ICP-MS detection for (a) a mixture of selenium standards ($12.5\,mg\,L^{-1}$) and $100\,\mu g$ Br L^{-1}: 1 = 79Br1H + KBr: 1 = KBr, 2 = Selenite, 3 = Selenate; and Se speciation in 0.016 M KH_2PO_4 Rothamsted soil extract after: (b) Rothamsted, (c) Sutton Bonington, (d) Pickwell, (e) 0.016 M Rothamsted extract of $20\,\mu g\,L^{-1}$ selenate and selenite at 0 d, (f) Rothamsted soil extract after E after 28 d storage at 4°C. Note different scales on y-axis.

and selenate, respectively. As expected, chromatograms from signal m/z 80 showed an extra peak with a retention time of 6.20 ± 0.1 min and was identified as the $^{79}Br^{1}H^{+}$ interference using a 100 μg L^{-1} Br standard. Figures 2(b)–2(d) show chromatograms from phosphate extractions of three UK agricultural soils. A small matrix effect was found, delaying the retention time for selenite slightly to 7.2 ± 0.1 min and selenate to 9.79 ± 0.25 min in the presence of phosphate. Figures 2(e) and 2(f) show chromatograms from phosphate extractions of soils amended with selenite and/or selenate.

3.2 *Performance of soil extraction*

3.2.1 *Phosphate extraction solutions*

The recovery of selenite added to each of the 3 soils at 20 μg Se kg^{-1} by 0.016 M KH_2PO_4 was low, ranging between 18.5% and 46.1% for Pickwell and Rothamsted soils, respectively (Table 3). Similarly, different phosphate extraction solutions (in terms of ionic strength and pH) showed similar ($p > 0.05$), low recoveries ranging between 38.5–46.1% of the selenite amendment (Table 4). Further investigations into extraction time (data not shown) showed that 1 h shaking was sufficient, with no additional selenite extracted after 3, 6 or 24 h shaking. Excellent agreement between the Se concentrations in the 0.016 M KH_2PO_4 soil extracts determined by ICP-MS compared to LC-ICP-MS was found ($93.8 \pm 4\%$, Table 4), meeting analytical detection objectives.

The recovery of 20 μg Se kg^{-1} added as selenate to each of the soils and extracted by 0.016 M KH_2PO_4 was 92.5–109% (data not shown). Different phosphate extraction solutions (in terms of ionic strength and pH) showed similar ($p > 0.05$) recoveries (Table 4). The 0.016 M KH_2PO_4 soil extracts showed good agreement between ICP-MS total Se and LC-ICP-MS selenate determination with mean recoveries (±SD) of 19.4 ± 0.7 μg kg^{-1} and 18.2 ± 0.8 μg kg^{-1} respectively (Table 4). The pH of the soil slurry in the 0.1 M $KH_2PO_4 \cdot K_2HPO_4$, 0.1 M KH_2PO_4 and 0.016 M KH_2PO_4 extractions were 7.26 ± 0.03, 5.35 ± 0.01 and 6.21 ± 0.02, respectively.

3.2.2 *Influence of soil preparation on Se speciation in amended soils*

The recovery of selenate and selenite after Se spiking was not significantly ($p > 0.05$) different in the fresh, air or oven dried soils. The recovery of selenate and selenite was very similar (within 6%) between oven-drying and air-drying treatments indicating that soil drying did not result in the conversion of either of the two inorganic Se species.

3.2.3 *Stability of Se species during storage*

A decrease in selenate concentrations in the amended soil extracts between 12% and 39% occurred over 28 days in all of the storage conditions. Decreases of 1–3 μg kg^{-1} in selenite

Table 3. Extraction efficiency of 0.016M KH_2PO_4 for 20 μg kg^{-1} selenite added to soils (±SD, $n = 3$).

Soil	Selenite recovery (μg kg^{-1})	Recovery (%)
Rothamsted	9.21 ± 0.2	46.1
Sutton Bonington	8.7 ± 0.4	43.5
Pickwell	3.7 ± 0.1	18.5

Table 4. Detection of Se species from Rothamsted soil amended with selenate or selenite using different extraction solutions (±SD, $n = 3$).

Soil amendment	20 μg kg^{-1} Selenate			20 μg kg^{-1} Selenite		
Extraction solution	0.016 M KH_2PO_4	0.1 M KH_2PO_4	0.1 M $KH_2PO_4 \cdot K_2HPO_4$	0.016 M KH_2PO_4	0.1 M KH_2PO_4	0.1 M $KH_2PO_4 \cdot K_2HPO_4$
Extractable Se[1] (μg kg^{-1})	19.4 ± 0.7	20.4 ± 0.5	15.7 ± 1.4	9.80 ± 0.4	12.0 ± 0.7	10.4 ± 1.0
Se species[2] (μg kg^{-1})	18.2 ± 0.8	16.2 ± 0.6	13.1 ± 0.0	9.21 ± 0.2	10.1 ± 0.0	7.70 ± 0.1
% Detection[3]	93.8 ± 4	79.4 ± 3	83.4 ± 0	93.9 ± 1	84.2 ± 0	74.0 ± 1

Notes: [1]Extractable selenium determined by ICP-MS analysis; [2]Selenium species determined by LC-ICP-MS analysis; [3]Detection is calculated as the Se species concentration compared to the extractable Se concentration, expressed as a percentage.

were found for all soil extracts stored in the fridge (4°C) or at room temperature. Storing the extracts in the freezer (–20°C) resulted in the best preservation of selenite, although an increase in the concentration from 6.6 to 9.6 μg kg^{-1} was detected in the Rothamsted soil extracts. No new peaks of Se species were detected in the stored samples (Figure 2f). Therefore, to avoid potential changes that may occur during storage, measurements of Se concentration and speciation should preferably be carried out immediately (<1 h) following extraction, as no storage technique adequately preserved Se speciation.

3.3 *Concentrations and speciation of extractable Se in agricultural soils*

Soils were collected from fields around England used for growing wheat. The soils were air-dried, sieved to 2 mm and extracted using 0.016 M KH_2PO_4 solution. Analysis using LC-ICP-MS was carried out immediately after extraction (<1 h), and ICP-MS within 48 h. Extractable Se levels varied between 6 and 13 μg Se kg^{-1}. Speciation of these extracts showed that only selenite was present, with concentrations ranging between <1 and 5 μg Se kg^{-1}. This accounted for 17.1–47.6% of the extractable Se (Table 5), so further investigation into the Se speciation of the extracts was carried out.

3.4 *Investigating the organic selenium fraction in the agricultural soils*

As detailed above (Section 3.3), the inorganic Se species did not account for all of the Se extracted in the soils, suggesting the presence of other Se species, probably in organic forms. This was investigated by treating soil extracts (0.016 M KH_2PO_4) with hydrogen peroxide. Selenite was the only species of Se detected before the peroxide treatment. The selenite peak disappeared after the peroxide treatment, and selenomethionine Se oxide, selenate and several unidentified peaks appeared (Figure 3).

4. Discussion

Current Se speciation analysis of soil extracts does not have the sensitivity required for the analysis of low Se soils, which occur widely in the world including the UK. Routine analysis of Se in soil extracts is by HGAAS (Table 1), a technique that can only

Table 5. Extractable selenium concentrations and inorganic species in six agricultural soils in the UK.

Location	Extractable selenium[1] (μg kg^{-1})	Selenite concentration[2] (μg kg^{-1})	Organic species detected[2] after peroxide treatment
Stamford, Lincolnshire	8.35 ± 0.24	1.14 ± 0.07	Yes
Dover, Kent	13.0 ± 0.42	5.36 ± 0.63	Yes
Pickwell, Leicestershire	6.36 ± 0.28	1.10 ± 0.20	Yes
Loughborough, Leicestershire	9.53 ± 0.63	3.57 ± 0.92	Yes
Royston, Hertfordshire	9.28 ± 0.35	0.95 ± 0.10	Yes
Harpenden, Hertfordshire	10.7 ± 0.24	5.10 ± 0.10	Yes

Notes: [1]Extractable selenium determined by ICP-MS analysis;
[2]Selenium species determined by LC-ICP-MS analysis.

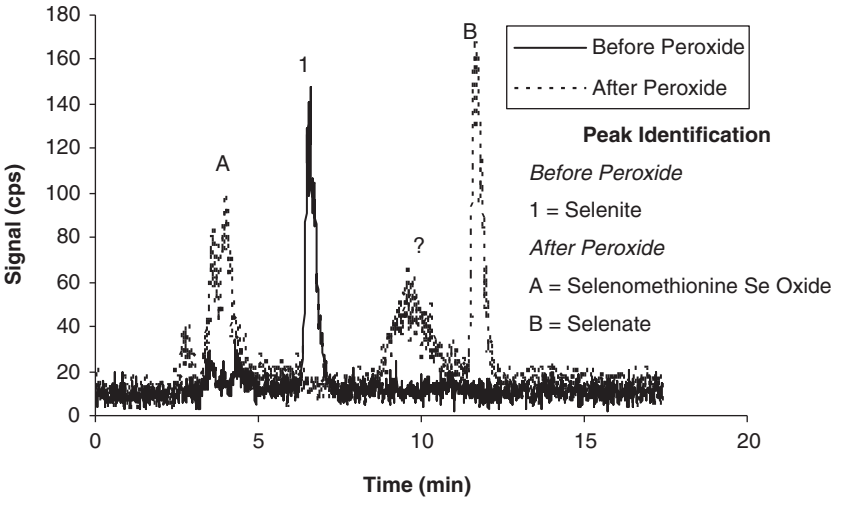

Figure 3. Anion exchange chromatograms from LC-ICP-MS before and after oxidation of the Rothamsted soil extract with peroxide.

indirectly measure selenate. As a result of improved ICP-MS technology coupled to anion-exchange LC, we were able to analyse low Se concentrations in soil extracts and determine the inorganic Se species present. Selenate and selenite were separated by anion-exchange chromatography with a 6 mM sodium carbonate buffer in 2% methanol with a total run time of 15 minutes. Bromine was a source of interference in the chromatographic detection of Se species on isotope ^{80}Se, particularly in soils samples from Rothamsted, however this was easily resolved as a separate peak before the Se ions. This analytical method for measuring extractable Se speciation concentrations has approximately 10-times improved sensitivity compared to existing methods based on HGAAS, and 3-times improved sensitivity compared to LC-ICP-MS soil Se speciation methods (Table 1). This method was applied to a range of soil extraction solutions from optimisation experiments designed to determine inorganic Se concentrations and speciation in UK soils.

Currently, little is known about the plant-availability of Se in UK soils and there is no established method for the measurement of extractable Se and speciation in soils (Table 1). Phosphate extractions have been proposed as an estimate of plant Se availability because phosphate solutions solubilise Se weakly adsorbed to soil surfaces [8]. However, the extraction of soil Se species may be affected by the pH of the phosphate extraction solution. This study compared the three phosphate extraction solutions which have been proposed for estimating plant Se availability [7] on soils that were amended with 20 $\mu g\,kg^{-1}$ selenate or selenite. The results showed that there was little difference between these solutions in terms of the recovery of selenate or selenite added to the soil, indicating that solution pH did not change Se speciation. The recovery of selenate was 91% in the 0.016 M KH_2PO_4 extraction solution, indicating the efficiency of the phosphate extraction solution for selenate recovery. The recovery of soil-amended selenate for the three soils ranged between 92.5% and 109% (data not shown), demonstrating the weak absorption of selenate to soils across a range of physico–chemical properties. In comparison, recoveries of selenite amended to the three soils ranged between 18.5% and 50.5% for the 0.016 M KH_2PO_4 extractions. Extraction solutions with different ionic strength and pH

did not improve selenite recovery, neither did extraction shaking time (data not shown) while analytical detection of selenite in the extracts was excellent (Table 3). These results indicate that a significant proportion of the amended selenite rapidly bound to soil components, rendering it unextractable by phosphate. Pickwell soil showed the lowest recovery of selenite. This soil has a higher clay content, higher organic carbon content and most acidic pH compared to the other soils tested; these soil properties – may explain the low recovery of amended selenite. Higher recoveries of added selenite (>71%) have been reported by Martens and Suarez [8]; however, they noted that selenite binding was not significant in the semi-arid soils tested.

Preparation of soil can cause changes in Se speciation. Intense oven-drying (>75°C) has been reported to significantly decrease selenate concentrations in soils (determined indirectly by HGAAS) due to a change in oxidation state [12]. No difference in Se extractability in differently treated soils was found in the soils that were amended with $20\,\mu g\,kg^{-1}$ selenate or selenite, indicating that the gentle heating (40°C) did not alter the oxidation state.

The optimised method, 0.016 M KH_2PO_4 extraction was applied to air-dried soils, and total Se extracted was 6.4–13 $\mu g\,kg^{-1}$ Se from a number of arable soils in the UK (Table 4). These results are similar to the concentrations of extractable Se determined in Finnish soils by hot-water extractions, which ranged between 2 and 27 $\mu g\,kg^{-1}$ and were considered to be too low to produce crops containing sufficient Se for human nutrition [22]. Seleniferous soils have much higher Se concentrations; Martens and Suarez [12] found 60–595 $\mu g\,kg^{-1}$ phosphate extractable Se in eight soils from California, USA.

The phosphate extraction solutions were analysed by LC-ICP-MS to determine selenate and selenite concentrations. No selenate was found in any of the samples from the agricultural soils, and selenite concentrations ranged between 0.95 and 5.36 $\mu g\,kg^{-1}$. Selenite concentrations were less than the total Se extracted, suggesting that some organic Se of microbial or plant origins was also extracted, as it is known that phosphate extractions can extract seleniferous plant proteins that are present in undecomposed plant material in soil [8]. Peroxide is a powerful and non-discriminating oxidising agent which may decompose soluble proteins of microbial or plant origins. This releases Se-containing amino-acids, which may further be oxidised into Se-oxides or other inorganic Se species, enabling their detection by LC-ICP-MS. Therefore, extract solutions were treated with peroxide (Figure 3) and analysed using LC-ICP-MS. Several Se peaks including one corresponding to the retention time of selenomethionine Se oxide were found, confirming the presence of organic Se species in the extracts. Selenomethionine Se oxide is the principal oxidation product of selenomethionine, an amino acid of microbial or plant origin. Assuming the difference between extractable Se and selenite concentrations was organic Se species, the proportion of organic Se in the total extract varied among different soils (potentially 52–83%), likely to be caused by different amounts of soluble Se amino acids of microbial or plant origins in differing soils. Similar to the Se data reported here, it has been reported that organic S accounted for 30–60% of the total S extracted by KH_2PO_4 [9], S and Se being chemical analogues.

In all of the soils analysed in this study, selenite was the only inorganic Se species present in the phosphate extracts. This is a new finding, as it was thought that selenate is likely to be the dominant inorganic Se species in well-drained alkaline soils [10]. If selenate existed in the soils used in this study, it would be either below the detection limit of the method used ($0.47\,\mu g\,kg^{-1}$ soil) or present in the fractions not extracted by phosphate. The lack of detection of selenate can be explained by the weak adsorption

of this Se species by soil, which means that selenate can be easily leached under the UK climatic conditions with significant winter rainfall. Furthermore, the biogeochemical cycle of Se involves the reduction of selenate to selenite by microorganisms [23], which may affect the selenate concentrations in soil. Selenite is much more strongly adsorbed by soil than selenate [24,25] therefore it is less bioavailable to plants than selenate [26,27]. Further, there are competitive interactions between selenite and phosphate ions during uptake in plant roots [6]. The results from this study suggest that the generally low Se status in the UK crops [28,29] is attributable not only to low concentrations of extractable Se but also to inorganic Se speciation in soil being dominated by selenite. However, further studies are required to investigate the potential seasonal variation and effect of low (potentially sub ppb) concentrations of plant-available selenate and organic Se in UK soils on crop Se concentrations.

5. Conclusions

We have established a direct and sensitive method for the analysis of inorganic Se species in soil extracts using LC-ICP-MS and investigated the factors affecting extraction and determination of Se species. Based on the results of this study, we recommend an extraction of air-dried soil with 0.016 M KH_2PO_4, followed by immediate analysis using LC-ICP-MS. Inorganic Se ions and bromine interferences were rapidly detected and identified as separate peaks when both ^{78}Se and ^{80}Se were monitored. This study showed that the concentrations of extractable Se were low in six UK agricultural soils, and Se in the extracts was present as selenite and organic Se.

Acknowledgements

Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council. This project was sponsored by Defra through the Sustainable Arable LINK Programme (LK0974 grant). We thank Martin Broadley and Keith Norman for providing soils from Sutton Bonington and Pickwell from the LK0974 project.

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Appendix 1: Operating parameters for selenium analysis

Parameter	Value	
Power	1550 W	
Plasma gas flow	15 L min^{-1}	
Carrier gas flow	0.95 L min^{-1}	
Make-up gas flow	0.10 L min^{-1}	
Reaction cell gas flow	H_2, 4.5 L min^{-1}	
Sampling depth	8 mm	
Ion lens voltages	Optimised daily using 1 μg L^{-1} tune solution (Ce, Li, Te, Y)	
Octopole Bias	−18 V	
Quadropole Bias	−15 V	
Data acquisition	ICP-MS	LC-ICP-MS
Nebuliser pump speed	0.1 rps	0.2 rps
Integration time per isotope	0.3 s	0.3 s
Points per peak	5	1
Integration time per mass	1.5 s	0.3 s
Isotopes monitored	78, 80, 82	78, 80
Internal standard	500 μg kg^{-1} Ge	500 μg kg^{-1} Ge