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## Abstract

A methodology based on coupling isocratic high-performance liquid chromatography (HPLC) and inductively coupled plasma mass spectrometry (ICP-MS) with optimized collision/reaction cell conditions has been developed for the simultaneous analysis of organic and inorganic selenium species in natural water samples. Selenium concentrations found in total and speciation analysis of a number of water samples showed good agreement. Because HPLC-ICP-MS coupling is easily automated, the method can be considered robust and applicable to the routine monitoring of selenium species in environmental and nutritional samples.

## Introduction

In the last 20 years, there has been increasing interest in the determination of the different

chemical forms in which an element can exist, that is, in the determination of its speciation. Indeed, knowledge of total concentrations of elements is not sufficient to assess their effects on human health or the environment. Among the elements of concern, there is a growing interest in selenium. Selenium is a very important element from an ecotoxicological point of view due to the narrow concentration range between its essential and toxic effects. Selenium compounds are distributed throughout the environment as a result of human activities (industrial and agricultural uses) and natural processes (weathering of minerals, erosion of soils, and volcanic activity). In waters, concentrations can vary from 2 ng/L to 1,900  $\mu$ g/L depending on the system [1]. The natural cycle of selenium shows its existence in four oxidation states (-II, selenide; 0, elemental selenium; +IV, selenite; and +VI, selenate) and in a variety of inorganic and organic compounds. The organically bound Se(-II) compounds include seleno-amino acids and volatile forms (dimethylselenide and dimethyldiselenide), which are less toxic relative to other species and result from various detoxification pathways. The toxic dose of selenium as a function of its chemical form is shown in Table 1.



#### Table 1. Selected Selenium Compounds and Their Toxicity

Compound	Formula	Lethal dose-LD-50*	Ref.	
Dimethylselenide (-II)	(CH₃)₂Se	1600 mg <b>/</b> kg (Int.)	[2]	
Hydrogen selenide (–II)	H <sub>2</sub> Se	0.02 mg/L (Resp.)	[3]	
Trimethylselenonium (–II)	(CH₃)₃Se⁺	49 mg <b>/</b> kg (Int.)	[3]	
Selenocystine (-I)	[HO <sub>2</sub> CCH(NH <sub>2</sub> )CH <sub>2</sub> Se] <sub>2</sub>	35.8 mg/kg (Or.)	[4]	
Selenomethionine (-II)	CH <sub>3</sub> Se(CH <sub>2</sub> ) <sub>2</sub> CH(NH <sub>2</sub> )CO <sub>2</sub> H	4.3 mg/kg (Int.)	[3]	
Selenite (+IV)	SeO <sub>3</sub> <sup>2-</sup>	3.5 mg <b>/</b> kg (Int.)	[5]	
Selenate (+VI)	SeO42-	5.8 mg/kg (Int.)	[5]	

\*Lethal doses obtained on mice or rats by intraperitoneal (Int.), oral (Or.), or respiratory (Resp.) absorption.

A number of analytical procedures exist for the determination of selenium and its various species in samples from different environmental sources. Existing methods can be divided in three groups, depending on selenium concentration:

- Total selenium
- Selenite species
- Species including inorganic and organic forms of selenium

Various redox reactions are often used to determine selenite species. However, the series of required reagents and pretreatment steps increases the possibility of element loss and contamination. Speciation results can also be distorted as back-oxidation of selenite to selenate may occur during sample pretreatment. Moreover, selenite and selenate are distinguished by two separate analyses, which is not the case for individual organic selenium species that remain unidentified. Hence, methods able to separate and quantify different selenium species simultaneously, in a single analysis, are preferred and are becoming more widespread.

In this application, the coupling of high-performance liquid chromatography (HPLC) with inductively coupled plasma mass spectrometry (ICP-MS) is presented for selenium speciation analysis with emphasis on its application to natural water samples.

#### Instrumentation

A 7500ce ICP-MS from Agilent Technologies (Tokyo, Japan), equipped with an Octopole Reaction System (ORS) cell, was used for this study; see Table 2 for operating parameters. The sample introduction system consisted of a concentric nebulizer (Meinhard Associates, California, USA) and a Scott double-pass spray chamber cooled to 2 °C. Nickel sampler and skimmer cones were used.

Table 2.	Instrumental Parameters for Agilent 7500ce ORS
	ICP-MS

Parameter	Value
RF power	1590 W
Ar plasma gas flow	15.0 L <b>/</b> min
Ar auxiliary gas flow	0.86 L <b>/</b> min
Ar nebulizer gas flow	1–1.1 L <b>/</b> min
Spray chamber temperature	2 °C
Integration time per isotope for speciation analysis	400 ms
m∕z ratio monitored	77 to 82
Integration time per isotope for elemental analysis	100 ms

Chromatographic separation was carried out using the Agilent 1100 Series HPLC pump, equipped with an autosampler and variable volume sample loop. The analytical column was a Hamilton PRPX-100, 10  $\mu$ m particle size, 25 cm length × 4.1 mm internal diameter (id). The chromatographic separation of selenocystine (SeCyst), selenomethionine (SeMet), selenite (SeIV), and selenate (SeVI) was adapted from Ge et. al. [6] and performed using a 5 mmol/L ammonium citrate buffer with pH adjusted to 5.2. Injection volume was fixed at 100  $\mu$ L. Methanol (2% v/v) was added to the mobile phase to improve sensitivity [7]. The mobile phase was delivered at 1 mL/min isocratically. The HPLC-ICP-MS interface consisted simply of polyetheretherketone (PEEK) tubing.

#### **Polyatomic Interference Removal**

ICP-MS is the detector of choice for trace element analysis due to its high sensitivity and selectivity. It is also one of the most often used detection systems for total and speciation analyses of selenium. Nevertheless, selenium detection limits obtained with a conventional ICP-MS (quadrupole filter without collision/reaction cell system) are not sufficient when dealing with selenium determinations in natural waters. Difficulties in Se determination by ICP-MS are mainly due to its high first ionization potential (9.75 eV) compared to argon (15.75 eV) and, as a consequence, its low ionization in an Ar plasma (around 33% [8]). Secondly, argon polyatomic interferences, especially <sup>40</sup>Ar<sup>40</sup>Ar<sup>+</sup> and <sup>40</sup>Ar<sup>38</sup>Ar<sup>+</sup> dimers, prevent selenium determination from its most abundant isotopes <sup>80</sup>Se (49.6% abundance) and <sup>78</sup>Se (23.8% abundance). Hence, the less interfered and less abundant <sup>82</sup>Se isotope (9.2% abundance) is generally monitored. The problem of argon-based polyatomic interferences can be solved with the use of ICP-MS systems equipped with a collision/reaction cell (CRC). A 10- to 20fold improvement in total Se and speciation analysis detection limits was observed using the ORS cell of the Agilent 7500ce. Speciation analysis detection limits are below 15 ng/L based on monitoring <sup>80</sup>Se (see Table 3). Better detection limits were achieved for <sup>80</sup>Se compared to <sup>78</sup>Se because the 7500ce was optimized on <sup>80</sup>Se.

#### Table 3. Optimization of ORS Operating Conditions

Instrument	Agilent 7500ce	
Cell gases	5.5 mL/min H <sub>2</sub> 0.5 mL/min He*	
Elemental Analysis		
	<sup>78</sup> Se	<sup>80</sup> Se
Detection limit (ng/L)	6	4
Repeatability (%)	2	2
HPLC Coupling		
	<sup>78</sup> Se	<sup>80</sup> Se
Detection limit (ng/L)	14–30	7–15
Repeatability (%)	2	2

\*Addition of He is optional. Similar detection limits should be achievable without He.



Figure 1. Chromatogram of standard, 1  $\mu$ g(Se)/L per species; 100  $\mu$ L injected, Hamilton PRP X-100 column, citrate buffer pH 5.2 and 2% methanol as mobile phase.

The use of CRC technology allows efficient removal of argon-based interferences, resulting in improved ICP-MS detection power for selenium by permitting monitoring of its most abundant isotope, <sup>80</sup>Se. However, such improvements are mitigated, in some cases, by reaction cell induced interferences. Indeed, hydrogen, or impurities contained in gases, can cause hydride formation from elements such as bromine, selenium, or arsenic [9-11]. Therefore, in samples containing bromine, as in the case of natural waters, there would be an interference on <sup>80</sup>Se and <sup>82</sup>Se from bromine hydride. As a result, the <sup>78</sup>Se signal should be monitored to avoid misinterpretation of the results and alleviate the need for correction equations.

Selenium concentrations determined in different mineral and spring waters, under the ICP-MS operating conditions described in Table 3, are summarized in Table 4. Results for certified simulated rain water (TM-Rain 95 from National Water Research Institute, [Ontario, Canada]) are also given. Total Se was established by measuring the <sup>78</sup>Se isotope without correction equations.

### Experimental

Figure 1 shows a chromatogram of 1  $\mu$ g(Se)/L per species standard obtained using HPLC-ICP-MS. The method was then applied to the mineral and spring water samples previously analyzed for their total selenium content. The results of selenium species concentrations are summarized in Table 4, together with the total selenium data.

Table 4.	Selenium Concentrations Determined in Different Natural Waters	[units: ng	g(Se)/L	]
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	Elemental Analysis	HPLC Coupling			
	-	<sup>78</sup> Se		<sup>80</sup> Se	
Natural Water	<sup>78</sup> Se	SelV	SeVI	SelV	SeVI
TM-Rain 95	622 ± 19*	629 ± 7	< DL	615 ± 8	< DL
Α	67 ± 1	< DL	69 ± 2	< DL	72 ± 6
В	142 ± 24	< DL	140 ± 9	< DL	143 ± 4
С	240 ± 20	< DL	232 ± 13	< DL	267 ± 13
D	467 ± 17	< DL	475 ± 4	< DL	492 ± 5
E	1890 ± 160	55 ± 2	1840 ± 30	57 ± 6	1920 ± 20

\*Certified value 740  $\pm$  290 ng(Se)/L

Concentrations found in total and speciation analyses are in complete agreement, showing the suitability of the method when applied to natural water samples. Although the bromine hydride interference on m/z 80 is present, it is separated chromatographically without overlapping with the selenium species. The chromatogram of water sample "C" (Figure 2) shows bromine elutes after the selenate peak. Selenate, commonly found in oxygenated waters, was determined in commercial waters A-D. Selenite was identified in TM-Rain 95 water, which is only certified for its total selenium content. Only water "E," a noncommercial ground water, contained both inorganic selenite and selenate species (see Figure 3).



Figure 2. Chromatogram of natural water "C" showing reaction cell induced interference from bromine hydride elutes after the selenate peak.



Figure 3. Chromatogram of natural water "E," the only sample to contain both inorganic species. First peak is SeIV, second peak is SeVI.

## Conclusions

Interest in selenium speciation has grown in recent years due to its characteristics as both an essential and toxic element. However, the complete speciation of selenium, including organic and inorganic forms, is still a major challenge. This is particularly true when exploring selenium speciation in natural waters due to the low levels of Se present. A hyphenated technique consisting of isocratic HPLC coupled to ICP-MS with optimized collision/reaction cell conditions allows for a quick and precise simultaneous analysis of organic and inorganic selenium species. Moreover, as HPLC-ICP-MS coupling is easily automated, it can be considered a robust routine method to monitor selenium species levels in environmental and nutritional samples.

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