

Appendix B. Analytical Method for the Speciation of Dissolved Inorganic Selenium in Natural Water¹

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Introduction

Dissolved selenite [Se(IV)] and selenate [Se(VI)] in natural water samples were determined by the U.S. Geological Survey National Water Quality Laboratory using a strong anion exchange column and malonate/acetate mobile phase for separation, and inductively coupled plasma–mass spectrometry (ICP-MS) for detection. Analysis time is about 3 minutes, method detection limits are 0.9 and 0.8 micrograms-selenium per liter ($\mu\text{g-Se/L}$) for selenite and selenate, respectively, and the calibration range extends to 100 $\mu\text{g-Se/L}$ for both species. The natural distribution of Se(IV) and Se(VI) species is preserved in the field by adding ethylenediaminetetraacetic acid (EDTA) to the samples; the maximum holding time prior to analysis has been established at 90 days.

The short-term variability over a period of about 5 hours for reagent-blank solutions having 1 and 100 $\mu\text{g-Se/L}$ averaged 23 and 0.3 percent for Se(IV), and 20 and 0.7 percent for Se(VI), relative standard deviation, respectively ($n=5$). The long-term variability over a period of about 3 months for a reagent-blank solution having 5 and 60 $\mu\text{g-Se/L}$ averaged 2.7 and 2.1 percent for Se(IV), and 3.0 and 1.6 percent for Se(VI), relative standard deviation, respectively ($n=8$). Spike recoveries at 25 $\mu\text{g-Se/L}$ for more than 100 filtered natural-water samples from 22 states averaged 100.7 ± 0.02 percent and 100.5 ± 0.03 percent, for Se(IV) and Se(VI), respectively.

Inorganic Selenium-Speciation Method

The Se(IV) and Se(VI) species are separated rapidly by using a 50-mm column packed with a strong anion exchange resin (table B1). This column is identical to the one used for separating arsenic (As) species (Garbarino and others, 2002). Furthermore, if required, Se(IV), Se(VI), trivalent arsenic [As(III)], and pentavalent arsenic [As(V)] can be determined in a single injection using this column. A malonate/acetate mobile phase is used to separate the species in about 3.5 minutes, as shown in figure B1.

The column was prepared by dry-packing about 500 mg of Supelclean LC-SAX strong anion exchange quaternary amine media (Supelco, Bellefonte, Penn., part number 57203) into the barrel of a 4- mm by 50-mm column made of polyetheretherketone (PEEK) capped with a 10–32 threaded end fitting with a bed-support disc (Dionex, Sunnyvale, Calif., p/n 035393). As the media is added, the column is tapped lightly. When full, the column is capped with another bed-support disc and end fitting, and deionized water is pumped through the column at a rate of 1–2 mL/min for about 5 minutes. The end cap from the high-pressure side of the column is removed so that more media can be added if necessary.

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Table B1. Method parameters for the determination of selenite and selenate in filtered natural water using high-performance liquid chromatography/inductively coupled plasma–mass spectrometry.

[LC-SAX, Supelclean quaternary amine strong anion exchange packing; mm, millimeter; SAX, quaternary amine strong anion exchange packing; °C, degrees Celsius; mM, millimole per liter; mL/min, milliliter per minute; min, minute; kPa, kilopascal; lb/in², pound per square inch; μL, microliter; L/min, liter per minute; W, watt; ms, millisecond; *m/z*, mass-to-charge ratio; ICP-MS, inductively coupled plasma-mass spectrometer]

Chromatographic parameters (Agilent 1100 Series)	
Analytical column	User packed LC-SAX 4 by 50 mm
Guard column	Phenomenex SecurityGuard SAX 4 by 3 mm
Column temperature	25 °C
Mobile phase, isocratic	12.5 mM malonate and 17.5 mM acetate, pH 4.8
Mobile phase flow rate	1.0 mL/min
Nominal column pressure	2.1 x 10 ³ kPa (300 lb/in ²)
Injection volume	100 μL
Selenite [Se(IV)] retention time	1.10 min
Selenate [Se(VI)] retention time	3.31 min
Sample introduction and detector parameters (PE Sciex Elan 6100 ICP-MS)	
Pneumatic cross-flow nebulizer flow rate	0.8 L/min
Power	1100 W
Dwell time	500 ms
Readings per replicate	215 (enough time to elute analytes)
Masses measured (<i>m/z</i>)	77, 81, and 82

Instrumentation

The instrumentation used for the selenium speciation method with corresponding operating conditions is listed in table B1. The system is composed of three major components: (1) a high-performance liquid chromatograph (HPLC) with an anion exchange column, (2) a sample introduction system, and (3) a selenium-specific detector. The HPLC consists of a commercially available controller, fluid unit, and autosampler. The column type, column temperature, mobile-phase composition and flow rate, and injection volume are listed in table B1. The column effluent was introduced directly into the nebulizer of the ICP-MS. The ICP-MS was used as a selenium-specific detector by measuring mass-to-charge (*m/z*) ratios 77, 81, and 82.

Interferences

Chemical and spectral interferences can affect the accuracy of determining selenium species unless appropriate procedures are followed. Speciation methods can be affected by cations, such as iron, manganese, or aluminum, or by anions, such as sulfate or carbonate, which often compose the sample matrix.

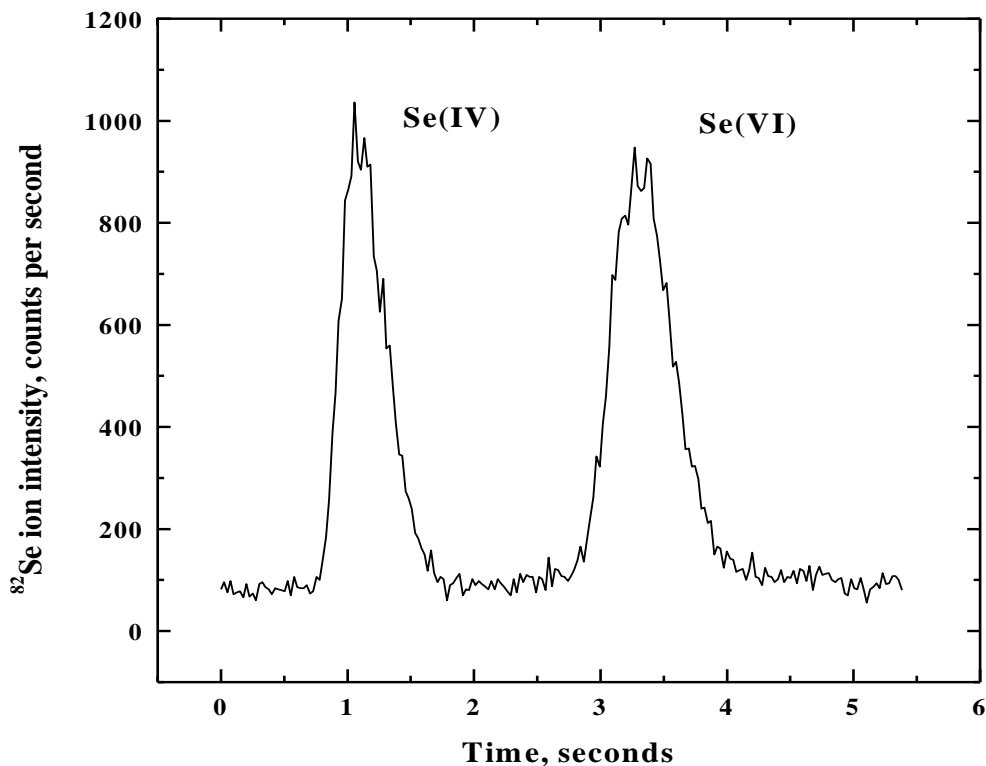


Figure B1. A typical chromatogram obtained using a short LC-SAX column, a gradient mobile phase of malonate and acetate, and a pneumatic nebulizer for sample introduction. The peaks represent a 100-microliter injection of a standard containing 10 micrograms-selenium per liter of selenite [Se(IV)] and selenate [Se(VI)].

Dissolved iron, manganese, or aluminum can affect the inorganic selenium speciation method. These metal cations are problematic in matrices with low redox potential (Eh) and dissolved-oxygen concentration, conditions that usually are more common in groundwater than in surface water. When groundwater is pumped to the surface during sampling and interacts with atmospheric oxygen, iron and manganese oxyhydroxides precipitate to provide sorption sites for dissolved selenium species (Raven and others, 1998) that might negatively bias selenium speciation results. Formation of the precipitate is minimized by either decreasing the pH to stabilize metal cations or by complexation of the metal cations. Results indicate that EDTA works well to preserve the selenium speciation by sequestering problematic dissolved metal cations for a wide range of sample matrices. Typically, 100 microliters of 250 millimolar EDTA is added to approximately 10 milliliters of filtered sample (see Arsenic Speciation chapter in Wilde and others, 2004). In addition to sequestering interfering metal cations, EDTA also buffers the sample solution. The chromatographic separation of the selenium species is not normally affected by concomitant anions found in natural-water samples because of their relatively low concentration levels as well as the small injection volume used. It is rare for the capacity of the column

to be exceeded when analyzing natural-water samples. Typical bromide concentrations found in natural-water samples do not interfere with the quantitation of selenium because of a different retention time.

Method Performance

Method detection limits achieved were 0.9 and 0.8 microgram-selenium per liter ($\mu\text{g-Se/L}$) for Se(IV) and Se(VI), respectively, and the calibration range extended to 100 $\mu\text{g-Se/L}$ for both species (table B2).

The short-term analytical variability over a period of about 5 hours for reagent-blank solutions having 1 and 100 $\mu\text{g-Se/L}$ averaged 23 and 0.3 percent for Se(IV), and 20 and 0.7 percent for Se(VI), relative standard deviation respectively (n=5, table B3). The long-term variability over a period of about 3 months for a reagent-water, groundwater, and surface-water matrices spiked with 5 $\mu\text{g-Se/L}$ averaged 2.7, 3.5, and 11 percent for Se(IV), and 3.0, 1.6, and 4.5 percent for Se(VI), relative standard deviation, respectively (n=8, table B4). There was an average bias of about 1 percent for Se(IV) and -1 percent for Se(VI) in data for the 5 $\mu\text{g-Se/L}$ spike in reagent water. The bias for groundwater and surface-water spikes was somewhat greater (note that the groundwater test matrix had about 3 $\mu\text{g-Se/L}$ selenate naturally present). Spike recoveries at 25 $\mu\text{g-Se/L}$ for more than 100 filtered natural-water samples having a wide range of water chemistry from 21 states (table B5) averaged 100.7 ± 0.02 and 100.5 ± 0.03 percent for Se(IV) and Se(VI), respectively (table B6).

Table B2. Short-term method detection limits for inorganic selenium species using high-performance liquid chromatography/inductively coupled plasma-mass spectrometry

[$\mu\text{g-Se/L}$, microgram-selenium per liter; pg, picogram (when using an injection volume of 100 microliters)]

Selenium specie	Short-term method detection limits	
	($\mu\text{g-Se/L}$)	(pg)
Selenite [Se(IV)]	0.9	90
Selenate[Se(VI)]	0.8	80

Table B3. Short-term analytical variability for spiked reagent water.

[Reagent-water spikes randomly analyzed over a 5-hour period after instrument calibration; statistics based on five replicate determinations at each spike concentration; SD, standard deviation; % RSD, percent relative standard deviation; $\mu\text{g-Se/L}$, micrograms-selenium per liter]

Nominal spike concentration ($\mu\text{g-Se/L}$)	Selenite [Se(IV)]			Selenate [Se(VI)]		
	Mean ($\mu\text{g/L}$)	SD	%RSD	Mean($\mu\text{g/L}$)	SD	%RSD
1	1.5	0.3	23	1.7	0.3	20
10	10.2	0.4	3.9	10.2	0.2	2.2
25	26.4	0.6	2.1	25.0	0.2	0.8
50	49.9	0.8	1.5	50.2	0.2	0.5
100	98.0	0.3	0.3	98.8	0.7	0.7

Table B4. Long-term bias and variability for selenite and selenate determinations in reagent-water, groundwater, and surface-water matrices spiked at three concentrations.

[$\mu\text{g-Se/L}$, micrograms-selenium per liter; Se(IV), selenite; Se(VI), selenate; sd, standard deviation; %RSD, percent relative standard deviation]

Nominal spike of 5 $\mu\text{g-Se/L}$						
Date	Reagent water		Groundwater		Surface water	
	Se(IV)	Se(VI)	Se(IV)	Se(VI)	Se(IV)	Se(VI)
02-05-2004	5.02	4.94	6.12	8.18	5.35	5.48
2-06-2004	5.23	5.03	5.93	8.15	5.67	5.57
2-09-2004	5.03	4.85	5.96	7.96	5.70	5.66
2-17-2004	5.02	5.03	5.84	8.04	5.38	5.88
3-02-2004	4.93	4.95	5.62	8.09	4.41	5.78
3-22-2004	4.87	4.64	5.60	8.09	4.78	5.79
4-20-2004	5.15	5.08	5.65	7.85	4.99	6.05
5-12-2004	5.25	5.06	5.61	8.26	4.24	6.29
Mean	5.06	4.95	5.79	8.08	5.06	5.81
sd	0.138	0.147	0.201	0.129	0.556	0.264
%RSD	2.7	3.0	3.5	1.6	11.0	4.5

Nominal spike of 30 $\mu\text{g-Se/L}$						
Date	Reagent water		Groundwater		Surface water	
	Se(IV)	Se(VI)	Se(IV)	Se(VI)	Se(IV)	Se(VI)
2-05-2004	29.2	29.8	30.6	32.8	30.4	30.9
2-06-2004	29.4	30.0	31.1	32.6	29.9	30.6
2-09-2004	29.8	29.8	30.5	32.8	30.1	30.4
2-17-2004	29.6	29.7	30.4	32.7	29.2	30.4
3-02-2004	29.8	30.4	31.0	32.9	29.0	31.4
3-22-2004	31.2	30.4	31.2	33.5	29.0	31.9
4-20-2004	31.6	30.7	29.9	32.2	27.0	31.6
5-12-2004	32.5	32.7	32.4	34.5	26.7	34.5
Mean	30.4	30.4	30.9	33.0	28.9	31.5
sd	1.20	0.974	0.726	0.707	1.38	1.35
%RSD	4.0	3.2	2.3	2.1	4.8	4.3

Nominal spike of 60 $\mu\text{g-Se/L}$						
Date	Reagent water		Groundwater		Surface water	
	Se(IV)	Se(VI)	Se(IV)	Se(VI)	Se(IV)	Se(VI)
2-05-2004	59.3	60.4	61.04	63.2	59.8	61.2
2-06-2004	59.7	60.4	61.66	63.7	60.1	61.4
2-09-2004	59.8	60.5	61.48	64.0	59.2	61.1
2-17-2004	59.8	60.0	60.25	63.1	59.2	61.0
3-2-2004	60.9	59.6	60.62	62.0	57.6	61.0
3-22-2004	60.8	60.5	63.24	64.9	61.7	63.8
4-20-2004	59.2	60.1	60.63	62.8	61.9	66.9
5-12-2004	63.1	62.8	65.21	66.2	62.8	71.8
Mean	60.3	60.5	61.77	63.7	60.3	63.5
sd	1.27	0.971	1.671	1.30	1.72	3.96
%RSD	2.1	1.6	2.7	2.0	2.9	6.2

Table B5. Sampling sites and matrix composition for the test samples analyzed.

[SC, specific conductance in microsiemens per centimeter at 25 degrees Celsius; mg/L, milligram per liter; Br-, bromide; SO₄²⁻, sulfate; Cl-, chloride; NO₃⁻, nitrate; CaCO₃, calcium carbonate;--, not determined; numbers in parentheses are median values for the parameter listed in the corresponding column heading]

State, number of test samples	pH	SC	Br- (mg/L)	SO ₄ ²⁻ (mg/L)	Cl- (mg/L)	NO ₃ ⁻ (mg/L)	CaCO ₃ (mg/L)
Alaska, 1	7.73	241	--	40	0.6	--	87.6
Arizona, 1	8.18	928	0.05	209	86	--	128
Arkansas, 1	8.15	555	--	45	83	0.03	112
California, 5	7.20-8.78 (7.49)	124-955 (685)	0.28-1.6 (0.39)	5.7-76 (13)	0.9-23 (12)	1.5-17 (1.8)	--
Colorado, 39	6.35-8.14 (7.21)	185- 16,580 (1,210)	0.02-7.4 (0.34)	3.1-13,280 (378)	0.5-1,260 (50)	0.1-24.2 (4.4)	86-486 (207)
Connecticut, 4	5.88-7.13 (6.89)	218- 1,417 (296)	0.03-0.17 (0.034)	8.7-29 (14)	22-400 (38)	0.003-3.2 (1.8)	--
Florida, 5	6.72-7.29	364-967	0.03-0.11 (0.05)	30-355 (120)	8.9-19 (10)	0.001-4.8 (0.6)	--
Georgia, 2	4.69-6.99	231-472	--	2.6-4.0	2.7-28	--	--
Idaho, 2	7.61-8.03	320-910	--	26-116	17-112	--	--
Iowa, 2	8.35-8.61	376-699	--	21-188	13-19	0.006-0.17	163-168
Kentucky, 3	7.69-7.87	272-438	--	42-56	17-20	1.1-3.0	--
Louisiana, 1	8.04	412	--	49	28	0.58	122
Maryland, 5	4.64-6.60 (6.07)	98-366 (152)	0.036-0.13 (0.05)	5.7-68 (10)	11-36 (16)	3.4-18 (11)	--
Missouri, 10	7.14-8.27 (7.58)	205-855 (628)	--	12-157 (61)	7.7-99 (28)	0.3-5.8 (1.1)	141-163 (152)
Montana, 3	7.92-8.19 (8.09)	576- 2,627 (984)	--	132-943 (241)	6.6-13 (10)	--	--
Nevada, 1	7.87	2,900	--	805	406	1.5	258
New England, 2	6.92-7.05	683-740	0.073-0.084	67-110	5.8-7.1	13	--
New Mexico, 5	6.79-7.92 (7.69)	450- 8,240 (1,470)	0.1-0.4 (0.2)	25-2,180 (54)	4.9-1,610 (106)	--	--
North Dakota, 2	8.01-8.05	1,200- 1,830	--	287-378	27-292	--	--
Oklahoma, 2	8.08-8.16	1,509- 1,586	--	318-443	146-178	--	--
Oregon, 2	7.37-7.62	98-126	--	6.3-8.8	3.1-4.2	--	--
Summary							
Total number of test samples = 101							
Median	7.41	98	0.008	110	24	2.0	166
Minimum	4.64	16,580	7.4	2.6	0.48	0.001	86
Maximum	8.78	712	0.13	13,280	1,606	24	486
Count	87	89	51	100	101	49	25

Table B6. Percent spike recovery statistics for selenite and selenate in 100 filtered natural-water samples.

[Both species spiked at 25 µg-Se/L; water chemistry for test samples is given in table B5; µg-Se/L, micrograms-selenium per liter; Se(IV), selenite; Se(VI), selenate; sd, standard deviation; %RSD, percent relative standard deviation]

	Percent recovery	
	Se(IV)	Se(VI)
Mean	100.7	100.5
sd	0.02	0.03
%RSD	2.27	2.72
Median	100.6	100.8
Minimum	92.7	86.7
Maximum	107.6	105.2

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