Speciation of Selenium(IV) and Selenium(VI) using Coupled Ion Chromatography—Hydride Generation Atomic Absorption Spectrometry

Sabine Goldberg,* D. A. Martens, H. S. Forster, and M. J. Herbel

ABSTRACT

A simple method was developed to speciate inorganic Se in the $\mu g\ L^{-1}$ range using coupled ion chromatography-hydride generation atomic absorption spectrometry. Because of the differences in toxicity and adsorption behavior, determination of the redox states selenite, Se(IV), and selenate, Se(VI), is important. We used anion exchange chromatography to separate Se(IV) and Se(VI) based on differences in retention times. Samples were then mixed with concentrated HCl and passed through a 130°C sand bath to reduce Se(VI) to Se(IV) for Se determination as the hydride. Detection limits were 0.68 μg L^{-1} for Se(IV) and 0.55 $\mu g\ L^{-1}$ for Se(VI). Spiking of actual sample solutions with Se(IV) and Se(VI) showed the procedure to be accurate for solutions with Se(IV)/Se(VI) ratios ranging from 1:4 to 4:1. Average recovery was 93.1% for Se(IV) and 108% for Se(VI). The technique was used to determine Se(IV) and Se(VI) in deionized water and actual and synthetic irrigation waters.

SELENIUM is an essential element in animal nutrition that is toxic at elevated concentration (Girling, 1984). Elevated solution Se concentrations can occur as a result of mining operations and drainage of seleniferous soils (Sharma and Singh, 1984). The range between Se deficiency and toxicity is narrow (Gupta and Watkinson, 1985). The major inorganic Se species found in soil solution are selenite, Se(IV), and selenate, Se(VI) (Adriano, 1986). Selenium toxicity is dependent on its oxidation state (Harr, 1978). Selenite is generally considered to be more toxic than selenate (Cobo Fernandez et al., 1993). The transformation rates of selenite to selenate and selenate to selenite are slow so that both redox states can coexist in soil solution (Masscheleyn et al., 1990). Adsorption reactions with soil mineral surfaces attenuate solution Se concentrations. Selenite adsorbs strongly on soil surfaces while selenate generally adsorbs weakly or not at all and is readily leached (Neal and Sposito, 1989). Because of the differences in toxicity and adsorption behavior, speciation of inorganic Se is important. Understanding the speciation of Se in agricultural drainage waters is extremely important as these waters can impact drinking water quality and wildlife habitat (Schuler et al., 1990).

The method of choice for Se analysis in aqueous solution is usually hydride generation atomic absorption spectrometry (HGAAS) because of its relative sensitivity and the general availability of atomic absorption spectrometers in most laboratories (Huang and Fujii, 1996). While inductively coupled plasma mass spectrometry (ICP–MS) exhibits superior sensitivity, it suffers from interferences between the dominant isotope of Se and the argon dimer, Ar_2 (both mass 80), unless reaction cell technology is used, which is not yet standard in most laboratories. In Se speciation analysis by HGAAS, two separate analyses are performed (Huang and Fujii, 1996). The Se(IV) redox state is determined directly. Total inorganic Se is determined after quantitative reduction of selenate to selenite by digestion with HCl at elevated temperature. The Se(VI) redox state is subsequently determined by difference. This procedure is necessary because chemical species must be fully protonated to form hydrides (Howard, 1997). This is the case for selenite, $pK_{a1} = 2.5$, but not for selenate whose pK_{a1} value is approximately -3. An inherent disadvantage in this difference method is that any error in either the measurement of selenite or of total inorganic Se will automatically compromise the accuracy of the selenate analysis.

Direct simultaneous analysis of Se(IV) and Se(VI) redox states requires separation of the Se species. The preferred species separation method has been ion chromatography (e.g., Kölbl et al., 1993; Lei and Marshall, 1995; Vassileva et al., 2001). The Se species are separated by differences in retention time since the higher charged selenate anion is retained longer on the chromatography column than the selenite anion. Subsequent to separation, the Se redox states are detected using nonsuppressed conductivity (Mehra and Frankenberger, 1988), flame atomic absorption spectroscopy (FAAS) (Lei and Marshall, 1995), graphite furnace atomic absorption spectroscopy (GFAAS) (Kölbl et al., 1993), or ICP–MS (Jackson and Miller, 1999; Wallschläger and Roehl, 2001). To avoid the interference of the argon dimer, Ar_2, Jackson and Miller (1999) and (Wallschläger and Roehl, 2001) measured the less abundant [82]Se isotope. Although easy to employ, ion chromatography with conductivity detection is usually not the method of choice for analyzing agricultural drainage waters because of the high level of anions such as SO_4^{2-} and NO_3^-, which can elute near selenite and selenate, respectively, masking the proportionally smaller Se peaks. As such, conductivity detection is prone to interferences, while FAAS lacks the sensitivity of HGAAS and ICP–MS, and GFAAS is not

S. Goldberg and H.S. Forster, USDA-ARS, George E. Brown Jr., Salinity Lab., 450 W. Big Springs Rd., Riverside, CA 92507; D.A. Martens, USDA-ARS Southwest Watershed Research Center, Tucson, AZ 85719; M.J. Herbel, Dep. of Environmental Sciences, Univ. of California, Riverside, CA 92521. Contribution from the George E. Brown Jr., Salinity Lab. Received 3 May 2005. *Corresponding author (sgoldberg@ussl.ars.usda.gov).

Abbreviations: FAAS, flame atomic absorption spectroscopy; HGAAS, hydride generation atomic absorption spectrometry; ICP-MS, inductively coupled plasma mass spectrometry; SRM, standard reference material.

Table 1. HPLC-HGAAS System and Operating Parameters.

	HPLC
Pump	Dionex DQP-1
Switching valve gas	90 psi N_2
Guard Column (size)	Dionex IonPac Guard AG11 (4 × 50 mm)
Column (size)	Dionex IonPac Analytical AS11 (4 × 250 mm)
Mobile phase (flow rate)	10 mM NaOH (1 mL min^{-1})
Sample inject volume	200 μL
	Hydride generation
Model	Varian VGA-76
Argon flow rate	40 mL min^{-1}
0.16 M NaBH$_4$ flow rate	1 mL min^{-1}
12 M HCl flow rate	1 mL min^{-1}
	Atomic absorption spectrometer
Model	Perkin Elmer 3030B
Lamp	Perkin Elmer EDL System 2
EDL System 2 power	230 milliamps
Wavelength	196.0 nm
Bandwidth	2.0 nm
Flame	Air + Acetylene
	Integrator
Model	Hewlett Packard 3393A
Zero setting	0
Attenuation (ATT2∧)	8
Chart speed	0.4 cm min^{-1}
Area reject	3 000 000
Threshhold	8
Peak width	0.30

a flow-through technique. Selenium speciation has also been achieved by coupling ion chromatography with microwave digestion and HGAAS (Ellend et al., 1996; Johansson et al., 2000).

The objective of our study was to develop a sensitive analytical technique for direct, simultaneous determination of inorganic selenite and selenate compatible with the selective nature of HGAAS. We optimized ion chromatography conditions for Se species separation and employed a heated (12 M HCl) flowthrough conversion of selenate to selenite for HGAAS detection of Se species in agricultural drainage waters.

MATERIALS AND METHODS

Chemicals used for the Se speciation were reagent grade sodium salts: Se(IV) solutions were made from Na_2SeO_3 obtained from Sigma[1] (St. Louis, MO) and Se(VI) solutions were made from Na_2SeO_4 obtained from Aldrich (Milwaukee, WI). Laboratory water was further purified using an absorber ion-exchange cartridge and a research ion-exchange cartridge from Illinois Water Treatment (Rockford, IL). These cartridges remove organics, free chlorine, and essentially all ions. All water used as an eluent in the mobile phase was degassed under vacuum for 1 h before use.

Experimentation found that an eluent concentration of 10 mM NaOH as the mobile phase resulted in very good separation of the Se(IV) and Se(VI) peaks. The eluent was made by pipetting 1.0 mL of 50% (w/w) NaOH solution (Fisher, Phillipsburg, NJ) into degassed water to a total volume of 2.0 L. The eluent was then vacuum filtered through a 0.2-μm nylon filter and poured into a polyethylene container. The hydride solution consisting of 0.16 M NaBH$_4$ and 0.12 M

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[1] Trade names and company names are included for the benefit of the reader and do not imply any endorsement or preferential treatment of the product listed by the U.S. Department of Agriculture.
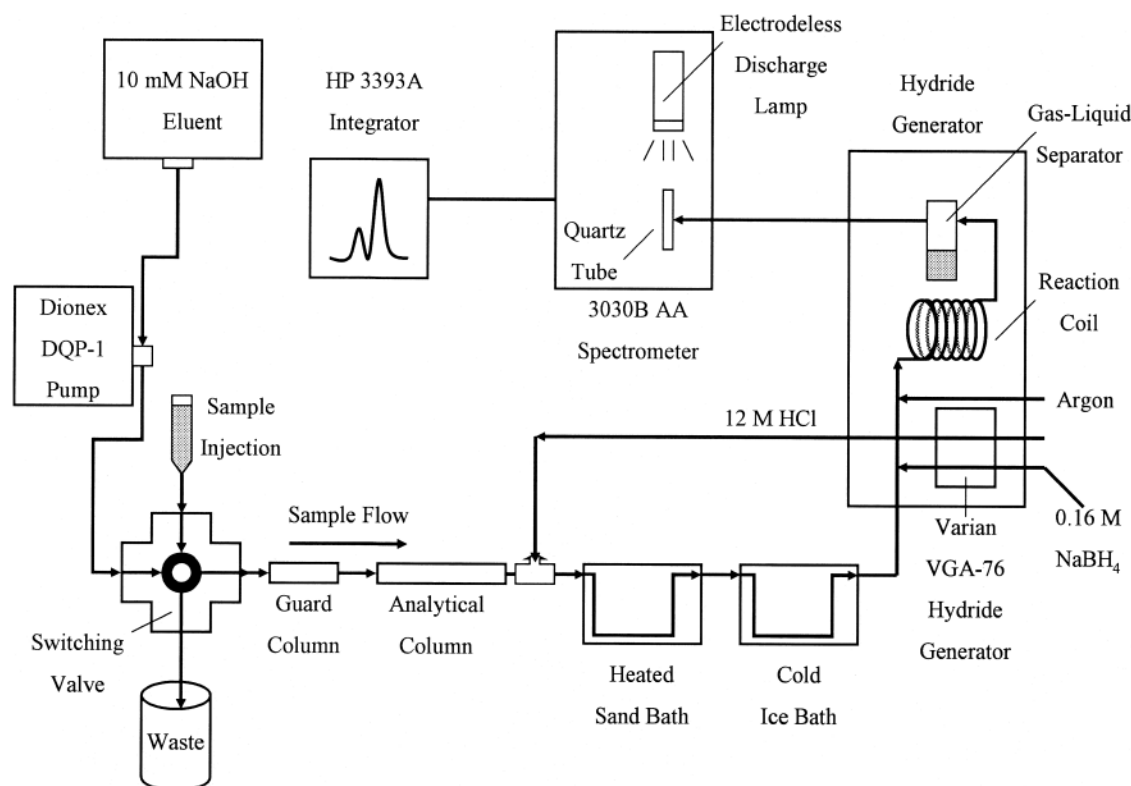

Fig. 1. Schematic of the analytical equipment for the ion chromatography-hydride generation atomic absorption spectrometry method. DQP = Dionex Quick Pump, VGA = Vapor Generation Accessory.

NaOH was also vacuum filtered. The acid used for Se reduction and hydride generation was concentrated (12.0 M) HCl (38% Instra-Analyzed, Baker, Pittsburgh, PA).

A 1000 mg L^{-1} stock solution of Se(IV) was made by dissolving 0.22 g of Na$_2$SeO$_3$ into 100 mL of ion exchange water; a 1000 mg L^{-1} stock solution of Se(VI) was made by dissolving 0.24 g of Na$_2$SeO$_4$ into 100 mL of ion exchange water. Standards were prepared each day just before analysis. The standards ranged from 5 to 30 μg L^{-1} and were either single ion standards of Se(IV) or Se(VI) or mixed ion standards containing both Se(IV) and Se(VI). We utilized two standard reference materials (SRM) from the National Institute of Standards and Technology (Gaithersburg, MD). These SRMs are intended for use in evaluating methods for determining trace elements in fresh water. SRM 1640 is a natural fresh water collected from Clear Creek, CO and certified as 22.0 ± 0.5 μg Se L^{-1}. SRM 1643e is a simulated fresh water certified as 12.0 ± 0.1 μg Se L^{-1}. In addition, an irrigation water obtained from a shallow ground water source from Section 4-2 of the Broadview Water District in the San Joaquin Valley of California and a synthetic irrigation water containing Na$^+$, K$^+$, Mg^{2+}, Ca^{2+}, Cl$^-$, and SO$_4^{2-}$, EC = 4.77 dS m^{-1} were analyzed. The ground water was diluted to an EC = 0.55 dS m^{-1} and the synthetic irrigation water was diluted to an EC = 0.95 dS m^{-1}.

The analytical equipment needed for the separation and detection of Se(IV) and Se(VI) is listed in Table 1. A schematic drawing of the experimental set-up is shown in Fig. 1. To achieve separation of the inorganic Se species, the sample and eluent were first passed through a Dionex IonPac AG11 (4 × 50 mm) guard column and then passed through a Dionex IonPac AS11 analytical column (4 × 250 mm) (Dionex Corp., Sunnyvale, CA). The sample and eluent were then passed through a PEEK tee (0.127 cm through hole, 3.06 μL swept volume) (Upchurch Scientific, Oak Harbor, WA). 12 M HCl was also pumped into the tee and mixed with the sample to begin the reduction of Se(VI) to Se(IV). The acidified sample and eluent were passed from the tee into a 1.5 m length of PEEK tubing (0.16 cm OD by 0.10 cm ID) coiled to a diameter of 15 cm so as to fit inside an aluminum sand bath box (17.8 by 17.8 by 10.2 cm). The sand in the bath was 5 cm deep. The sand bath box rested on a hotplate (Thermolyne, Nova, IL) that maintained the sand at a temperature of 130°C. Heating the acidified sample greatly enhanced the reduction of Se(VI) to Se(IV). The heated acidified sample was passed from the sand bath into a 1 m length of PEEK tubing (0.16 cm OD by 0.05 cm ID) coiled inside a 15-cm diameter reservoir 7.6 cm in height. The reservoir was filled with ice and served as an ice bath to cool the heated acidified sample. The cooled acidified
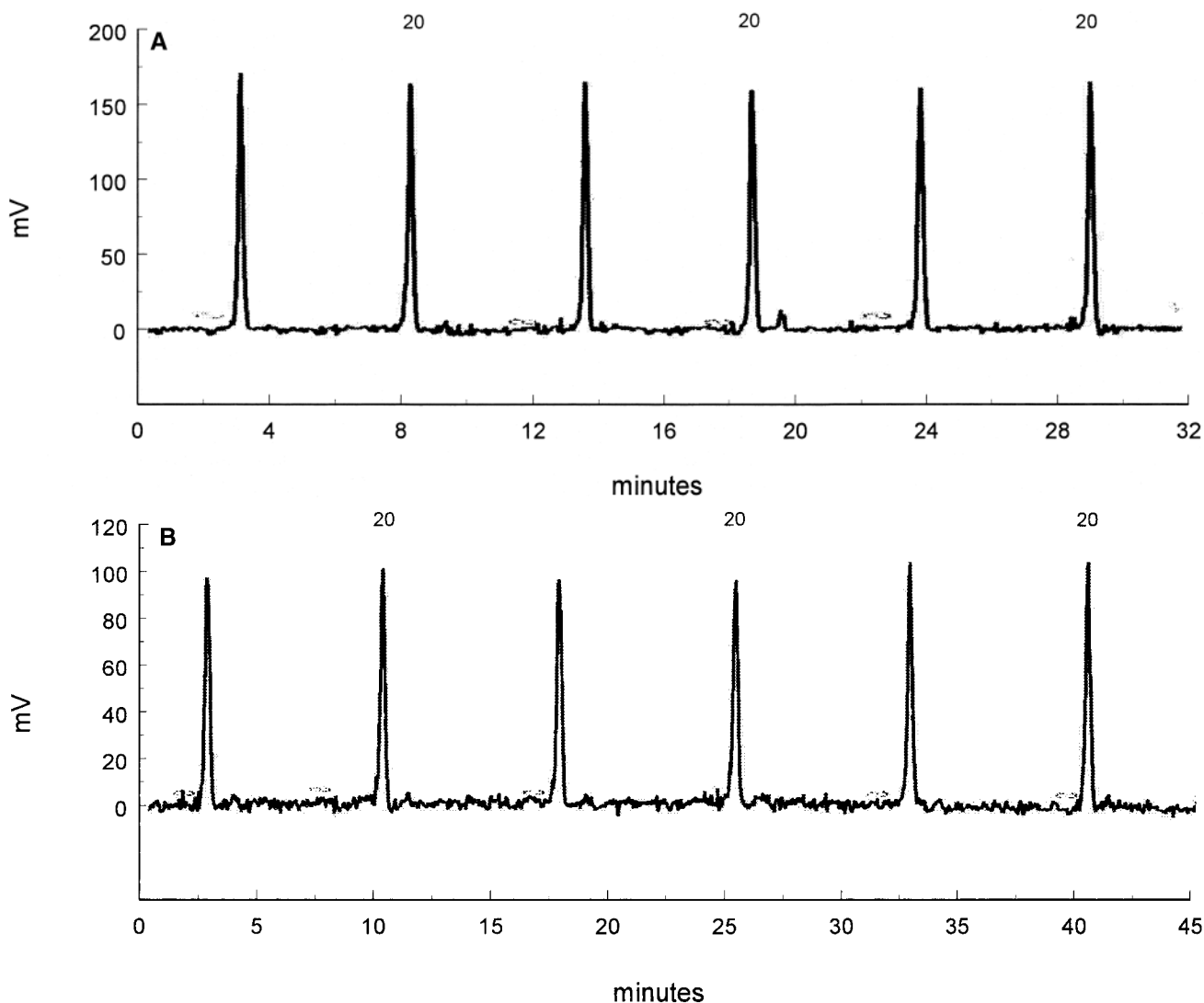

Fig. 2. Reproducibility of the IC-HGAAS method for multiple injections of a 20 μg L^{-1} standard: A) Se(IV); B) Se(VI).
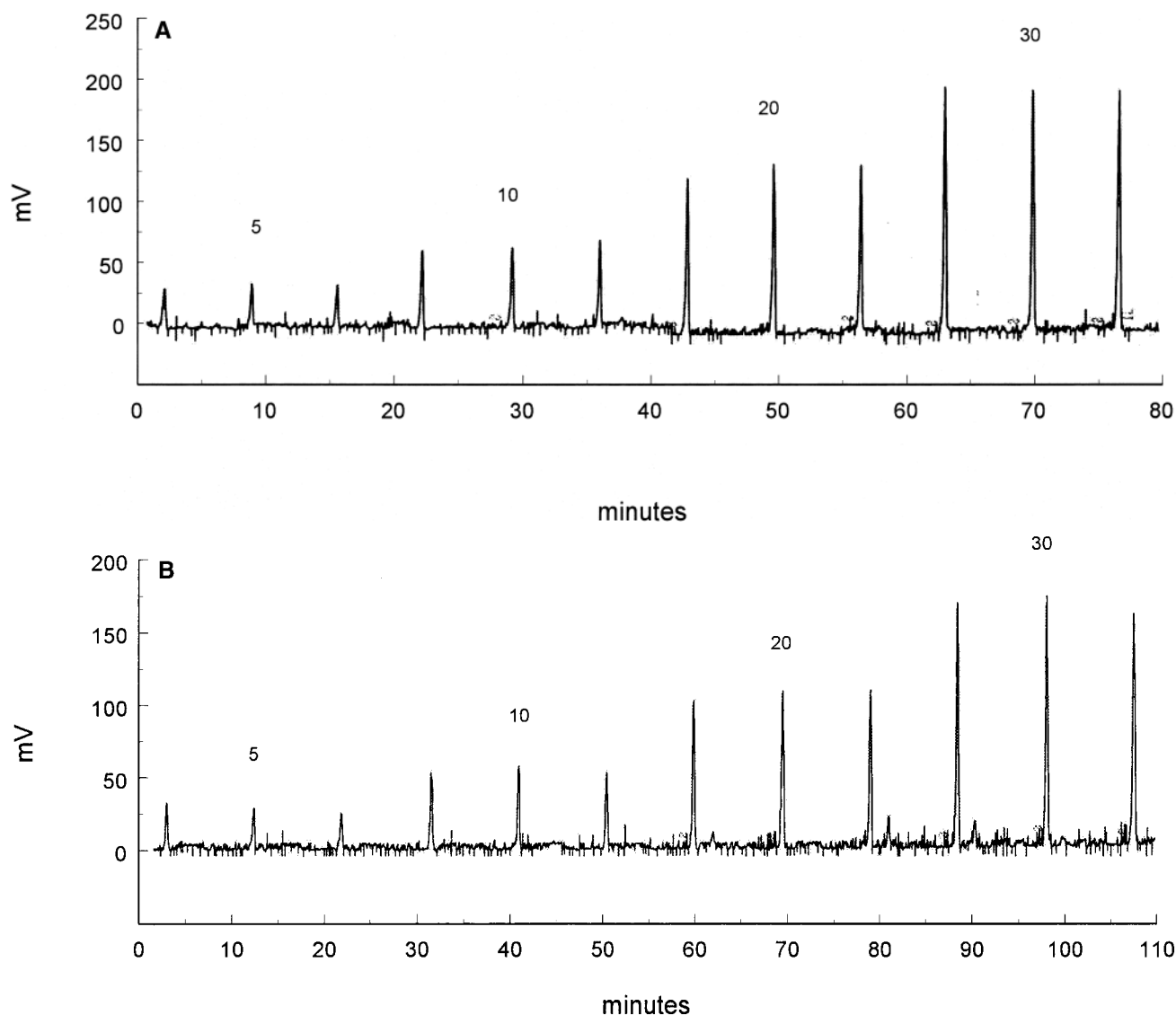
Fig. 3. Reproducibility of the IC-HGAAS method for triplicate injections of standards ranging from 5 to 30 μg L^{-1}: (A) Se(IV); (B) Se(VI).

sample passed from the ice bath into a polypropylene tee (0.16 by 0.16 by 0.16 cm) linked to the hydride generator. The hydride solution containing 0.16 M NaBH$_4$ and 0.12 M NaOH was also pumped into the tee and mixed with the sample to form the Se hydride gas. The Se hydride gas was then stripped from the solution using Ar gas and swept into an air/acetylene flame heated quartz tube in the light path of the atomic absorption spectrometer. The wavelength used to determine Se was 197.3 nm. A Hewlett-Packard 3393A integrator was coupled to the 1 V output of a PerkinElmer 3030B atomic absorption spectrometer to record the absorbance signals in peak areas.

RESULTS AND DISCUSSION

Standard Se solutions were used to test the sensitivity of the speciation technique. The reproducibility of multiple injections of a 20 μg L^{-1} Se standard is shown in Fig. 2a for Se(IV) and Fig. 2b for Se(VI). Coefficient of variation for these measurements was 2.2% for Se(IV) and 5.1% for Se(VI). The results of triplicate injections of standards ranging from 5 to 30 μg L^{-1} Se are shown

in Fig. 3a for Se(IV) and Fig. 3b for Se(VI). Mixed standards containing equimolar amounts of Se(IV) and Se(VI) were analyzed in the range of 1 to 30 μg L^{-1} Se and the results presented in Fig. 4. Our method provides complete resolution of the two redox states while minimizing the overall run time to <8 min per sample. The detection limits, calculated as three times the standard deviation of 20 blank readings were 0.68 μg L^{-1} for Se(IV) and 0.55 μg L^{-1} for Se(VI) using a 200 μg L^{-1} injection loop. Advantages of the coupled methodology include shorter analysis time as both species can be analyzed in one chromatographic run and less use and exposure to acids as the digestion step in a fume hood is eliminated.

To evaluate the ability of our method to accurately determine a known concentration of Se, we analyzed two SRMs from the NIST (Gaithersburg, MD) that are intended for use in evaluating methods for determining trace elements in fresh water. SRM 1640 is certified as 22.0 ± 0.5 μg Se L^{-1}. Sextuplicate analyses with our
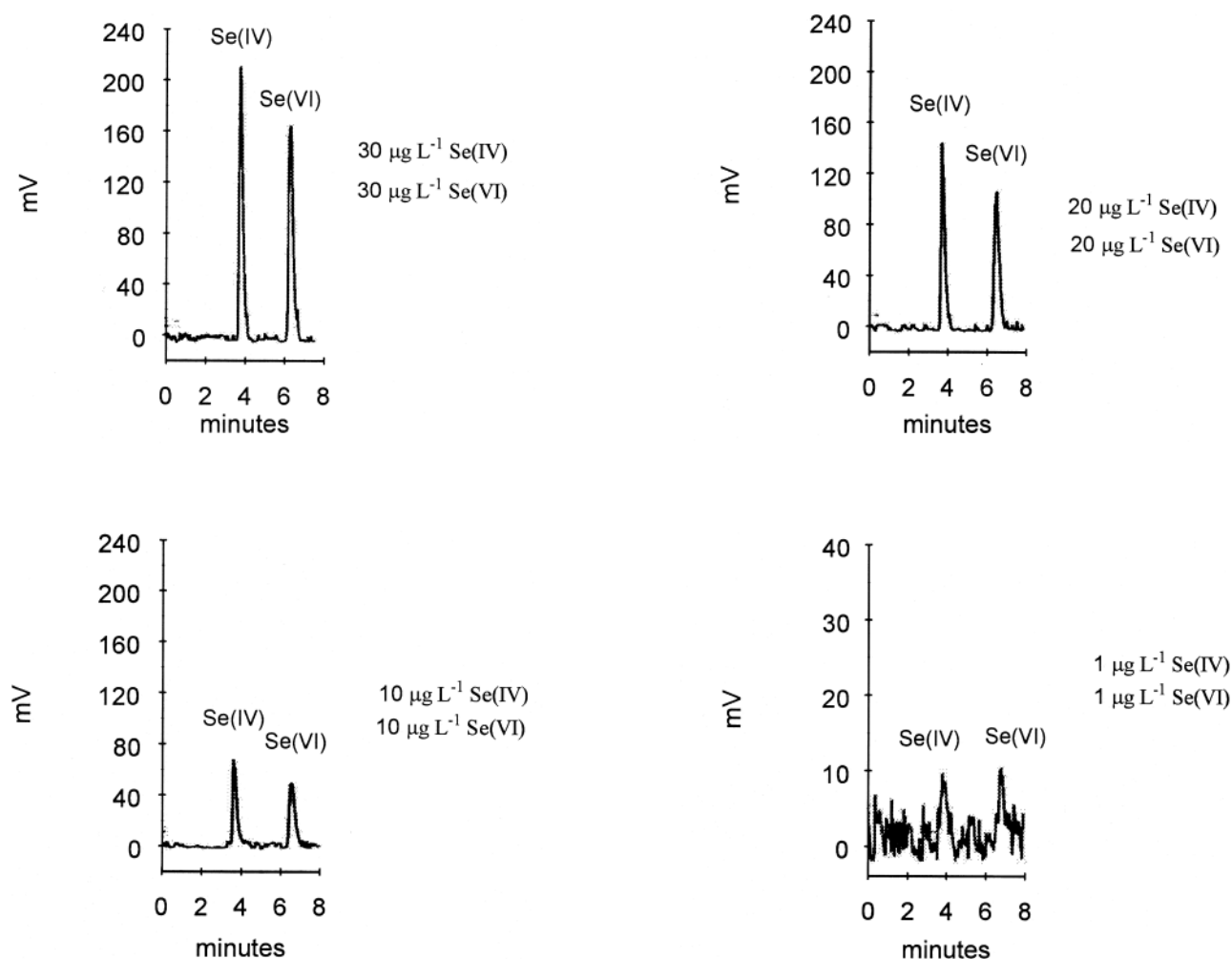
Fig. 4. Ability of the IC-HGAAS method to analyze mixed standards in the range of 1 to 30 μg L^{-1}. Parameters are those given in Table 1 except for 1 μg L^{-1} where attenuation was set at 5 and area reject at 1 000 000.
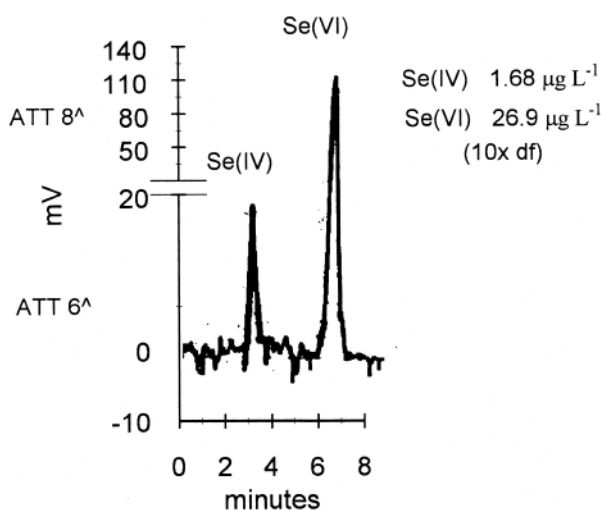
method determined the concentration of SRM 1640 to be 21.0 ± 1.0 μg Se L^{-1} (96% recovery) with all Se being present as Se(VI). SRM 1643e is certified as 12.0 ± 0.1 μg Se L^{-1}. Sextuplicate analyses with our method determined the concentration of SRM 1643e to be 12.0 ± 1.1 μg Se L^{-1} (100% recovery) with all Se being present as Se(IV). Our results for both SRMs are not significantly different than the NIST results at the 95% level of confidence.

Selenium concentrations in agricultural drainage waters such as those of the western San Joaquin Valley of California, are highly variable, with Se levels above 10 μg L^{-1} being common (Sylvester, 1990). In the Grassland region, which includes the Broadview Water District, Se concentrations in drainage waters averaged 82 μg L^{-1} from 1986 to 1995 (Presser and Piper, 1998). The capability of the procedure to determine Se in actual and simulated drainage waters of varying Se(IV)/Se(VI) ratio was tested by spiking sample solutions. Three solutions were prepared for this study: (1) a shallow seleniferous ground water from Section 4-2 of the Broadview Water District in the San Joaquin Valley of California diluted by a factor of 16.7 to attain an electrical conductivity, EC = 0.55 dS m^{-1}; (2) a Se-free syn-thetic irrigation water containing Na$^+$, K$^+$, Mg^{2+}, Ca^{2+}, Cl$^-$, and SO$_4^{2-}$, EC = 4.77 dS m^{-1}; (3) the synthetic irrigation water diluted to an EC = 0.95 dS m^{-1}. The three spikes added to each solution varied in Se(IV)/Se(VI) ratio from 1:4 to 4:1 and are shown in Table 2. Recoveries of Se(IV) and Se(VI) were calculated as the differences between concentrations found in spiked and unspiked samples. The values in Table 2 for the synthetic irrigation waters "sample + treatment" and "treatment spike recoveries" are identical. Spike recoveries deviated by ≤ 1.8 μg L^{-1} for Se(IV) and ≤ 1.5 μg L^{-1} for Se(VI) from expected values at all addition ratios for the Broadview ground water and the diluted synthetic irrigation water. Recoveries of Se(IV) were consistently below the spike concentrations by an average of 6%, while recoveries of Se(VI) were consistently above the spike concentrations by an average of 6%. For the undiluted synthetic irrigation water, spike recoveries were very poor, ranging from 36 to 53%. We believe this is due to saturation of exchange sites on the column, since chromatographic resolution was compromised. Peak height was reduced and peak width was broadened. These results indicate that the present method should not be used on sample solutions having EC values > 1 dS m^{-1}.

Table 2. Selenium concentrations and recoveries of spiked sample solutions.

Sample	Treatment spike		Sample + treatment spike		Treatment spike recoveries (% recoveries)	
	Se(IV)	Se(VI)	Se(IV)	Se(VI)	Se(IV)	Se(VI)
			$\mu g\ L^{-1}$			
Broadview	0.0	0.0	1.67	16.2	–	–
ground	7.5	22.5	8.43	41.3	6.76 (90.1%)	25.1 (112%)
water	15.0	15.0	16.1	32.7	14.4 (96.0%)	16.5 (110%)
	22.5	7.5	22.4	24.6	20.7 (92.0%)	8.4 (112%)
Average recovery					(92.7%)	(111%)
Synthetic	0.0	0.0	–	–	–	–
irrigation	7.5	22.5	4.0	9.1	4.0 (53.3%)	9.1 (40.4%)
water	15.0	15.0	6.8	6.7	6.8 (45.3%)	6.7 (44.7%)
	22.5	7.5	9.6	2.7	9.6 (42.7%)	2.7 (36.0%)
Average recovery					(47.1%)	(40.4%)
Diluted	0.0	0.0	–	–	–	–
synthetic	7.5	22.5	7.0	23.6	7.0 (93.3%)	23.6 (105%)
irrigation	15.0	15.0	14.0	15.3	14.0 (93.3%)	15.3 (102%)
water	22.5	7.5	21.7	7.8	21.7 (96.4%)	7.8 (104%)
Average recovery					(94.3%)	(104%)

Fig. 5. Ability of the IC-HGAAS method to analyze a natural sample containing both Se redox species.

The ability of the speciation method to analyze a natural sample is depicted in Fig. 5 for a $10\times$ dilution of the seleniferous Broadview ground water. A concentration of 1.7 $\mu g\ L^{-1}$ Se(IV) was accurately detected highlighting the sensitivity of the technique. Peak resolution for the two Se species is complete even in this natural water mixed ion system.

Ion chromatography couple with HGAAS is a sensitive, direct method for simultaneously determining inorganic Se(IV) and Se(VI) for laboratories that do not have ICP–MS reaction cell technology. The optimized method has advantages over traditional HGAAS methodologies as it saves time, reduces use of costly purified reagents (HCl), and reduces technician exposure to concentrated acid. The technique was able to quantify these Se redox states in natural and synthetic waters.

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