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Environmental

Determination of selenite and selenate in environmental waters by ion chromatography-mass spectrometry (IC-MS)

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Keywords

Single quadrupole mass spectrometer, Dionex Integrion HPIC, Dionex IonPac AS11-HC column, ISQ-EC, ADRS 600 suppressor

Goal

To develop a method to determine selenite and selenate in spiked environmental water by coupling ion chromatography (IC) with single quadrupole mass spectrometry

Introduction

Selenium is an essential trace mineral for the human body. It is a constituent of selenoproteins that play critical roles in reproduction, thyroid hormone metabolism, DNA synthesis, and protection from oxidative damage and infection.[1] Selenium is also an important element in environmental research due to the narrow window differentiating its presence as an essential trace element and its toxic effect upon exposure.[2] Due to this potential toxicity, there is a regulatory need to reduce selenium contamination of environmental waters. The U.S. EPA has set the maximum contaminant level (MCL) for selenium in drinking water at 50 µg/L.[3] The EPA only regulates total selenium in drinking water. However, the speciation of selenium in environmental waters has long captured the interest of researchers.

Selenium is often found in the inorganic forms of selenite and selenate in environmental samples, and the toxicity of selenium compounds greatly depends on their speciation. Consequently, selenium in environmental samples should be determined not only as total selenium, but also as species-specific when possible. Selenite is more toxic than

its selenate form.[4] Though the U.S. EPA has defined the MCL for selenium in drinking water using inductively coupled plasma mass spectrometry (ICP-MS), the method can only determine total selenium concentration, not its speciation. Therefore, a method using ion chromatography (IC) paired with ICP-MS (IC-ICP-MS) was developed to measure selenium speciation in natural waters.[5] The method uses nitric acid as the IC mobile phase, which must be manually prepared. This IC-ICP-MS method is very sensitive, with a limit of detection below 10 ng/L, offering the most sensitive method developed to determine the selenium speciation in clean environment waters. Previously, a method was developed that coupled ion chromatography with conductivity detection (IC-CD) for the determination of selenate and selenite species in processed wastewater.[6] The IC mobile phase was KOH that was automatically generated by an Eluent Generator Cartridge (EGC) using DI water for Reagent-Free™ Ion Chromatography (RFIC). With this method, the detection limits were 20 µg/L and 40 µg/L for selenate and selenite, respectively. Therefore, this method exceeds the MCL limit for total selenium in drinking water required by the EPA, creating a need for additional IC methods that capitalize on RFIC technologies.

Here, we sought to develop a method that can determine whether environmental water has been contaminated with selenite and selenate at the MCL level that is required by the EPA for screening purposes. We developed a more sensitive method to quantitate selenite and selenate in spiked environmental waters by coupling ion chromatography with mass spectrometry (IC-MS). A Thermo Scientific™ Dionex™ Integrion™ IC system coupled to an economical and simple-to-use single quadrupole MS (Thermo Scientific™ ISQ-EC MS system) was used to screen and confirm the presence of selenite and selenate. The ISQ EC single quadrupole mass spectrometer seamlessly integrates IC with MS, taking advantage of the strengths of both techniques. Anion exchange chromatography using eluent generation and suppressed conductivity detection provides chromatographic selectivity and analytes in the ionic form, allowing for the possibility of downstream MS detection. Electrospray ionization (ESI) is used to introduce the liquid IC stream, after suppression, as a fine spray into the MS source. The HESI-II probe improves the ESI interface by allowing high temperatures and voltage to deliver better desolvation and enhanced sensitivity; thus, a make-up solvent is not needed. The mass spectrometer was operated in selected ion monitoring (SIM) mode, allowing minimum sample cleanup and ensuring sensitive and selective quantification. Isotope labeled chlorate ^{18}O was used as the internal standard to ensure quantitation accuracy. Performance data for the method, such as recovery, precision, sensitivity, and calibration range, were also reported. These data show that IC-MS can successfully determine the two targeted selenium species in spiked environmental water samples, allowing for the identification of the species due to their *m/z* ratio.

Experimental

Equipment

- A Thermo Scientific™ Dionex™ Integrion™ HPIC™ system (P/N 22153-60305) including:*
  - Eluent generator
  - Pump
  - Degasser
  - Conductivity detector
  - Second 6-port injection valve (P/N 22153-62027) used as diverter valve
  - Thermo Scientific™ Dionex™ IC Viper™ fitting tubing assembly kit (P/N 088798)
  - Column oven temperature control
  - Detector-suppressor compartment temperature control

*This method can also be run on a Thermo Scientific™ Dionex™ Inuvion™ IC system or a Thermo Scientific™ Dionex™ ICS-6000 dual IC system using the second pump channel to deliver suppressor external water instead of the AXP-MS auxiliary pump.

- Thermo Scientific™ Dionex™ AS-AP Autosampler with 250 µL syringe and 1.2 mL buffer line assembly (P/N 074921), 10 µL injection loop (P/N 302895), and 10 mL vial trays (P/N 074938)
- Thermo Scientific ISQ EC single quadrupole mass spectrometer (P/N ISQEC000IC) including Thermo Scientific™ HESI-II probe (P/N 70005-60155)
- Thermo Scientific™ Dionex™ AXP-MS auxiliary pump (used to deliver suppressor external water) (P/N 060684)
- Nitrogen generator with capacity for 3 L/min flow at 100 psi (110 V: P/N 1R77606-3120); 230V: P/N 1R77606-3230)

Software

Thermo Scientific™ Dionex™ Chromeleon™ Data System (CDS) Version 7.3 1

Consumables

- Thermo Scientific™ Dionex™ EGC 500 KOH Cartridge (P/N 075778)
- Thermo Scientific™ Dionex™ CR-ATC 600 Continuously Regenerated Anion Trap Column (P/N 088662)
- Thermo Scientific™ Dionex™ ADRS 600 Anion Dynamically Regenerated Suppressor, 2 mm (P/N 088667)
- Dionex AS-AP Autosampler Vials 10 mL (P/N 074228)
- Fisherbrand™ Narrow-mouth field sample bottles, high density polyethylene (HDPE), 125 mL, 250 mL sizes for storage of standards and samples (Fisher Scientific P/N 02-895A, B)

Reagents and standards

- Deionized (DI) water, Type 1 reagent grade, 18 MΩ·cm resistivity or better
- Sodium and potassium salts, A.C.S. reagent grade or better, for preparing anions standards
- Sodium selenite, anhydrous, 99% (Fisher Scientific™ P/N AA1258522)
- Sodium selenate decahydrate, 99% (Fisher Scientific™ P/N AA1423914)
- Potassium chlorate (90-95% chemical purity) (18O3, 98%) 100 µg/mL (Cambridge Isotope Laboratories P/N OLM-10485-1.2)

Samples

Environmental water samples were collected from the San Francisco Bay Area (Sample #1: Wastewater, Sample #2: Lake water, Sample #3: River water).

There are no selenate and selenite isotope standards available commercially. Therefore, in this study, a chlorate isotope standard was selected as an internal standard because the retention time of chlorate is close to our analytes of interest. Table 1 shows the mass scan conditions for selenate, selenite, and the chlorate internal standard.

Chromatographic conditions

Parameter	Setting
Columns	Thermo Scientific™ Dionex™ IonPac™ AS11-HC guard column, 2 × 50 mm (P/N 052963) Thermo Scientific™ Dionex™ IonPac™ AS11-HC analytical column, 2 × 250 mm (P/N 052961)
Eluent	12–20 mM KOH, 0–10 min; 20–50 mM KOH, 10–14 min; 50 mM KOH, 14–16 min; 12 mM KOH, 16–20 min
Eluent source	Dionex EGC 500 KOH cartridge with CR-ATC 600
Flow rate	0.38 mL/min
Injection volume	5 µL in Push-Full mode
Column temp.	30 ˚C
Detection 1	Suppressed conductivity
Suppressor	Dionex ADRS 600 (2 mm) Suppressor, external water mode (flow 0.38 mL/min), 48 mA current
Detection/Suppressor compartment	20 °C
Cell temp.	35 ˚C
Background conductance	<1 µS/cm
System backpressure	~2,600 psi (100 psi = 689.5 kPa)
Noise	<1 nS/cm
Run time	20 min
Detection 2	Mass spectrometry
MS detector	ISQ-EC single quadrupole MS
Ionization interface:	Electrospray ionization (ESI), negative mode
Diverter valve switch time	0–6 min to waste, 6–20 min to MS
Sheath gas pressure	56.4 psi
Aux gas pressure	5.7 psi
Sweep gas pressure	1 psi
Source voltage	-2,500 V
Vaporizer temp.	316 ˚C
Ion transfer tube temp.	250 ˚C
Chrom. filter peak width	Off
Scan mode	Table 1

Table 1. MS scan mode

Scan name	Mass list (amu)	Dwell or Scan time (s)	SIM width (amu)	Ion polarity	Spectrum type	Source CID voltage (V)
Selenate	144.91	0.5	0.1	Negative	Centroid	40
Selenite	128.91	0.5	0.1	Negative	Centroid	40
Chlorate internal standard	88.96	0.5	0.1	Negative	Centroid	40

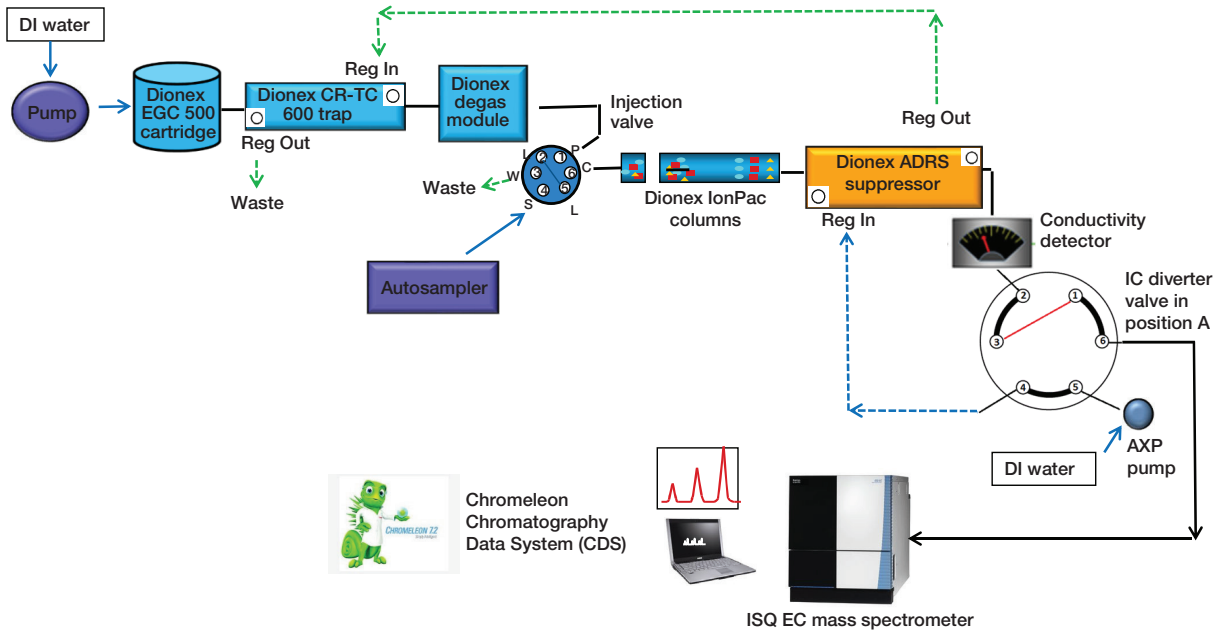

Figure 1. Flow diagram for IC-CD/MS with diverter valve in "A" position

System preparation and setup

Figure 1 shows the flow diagram of the IC-MS system. The Integrion HPIC system is plumbed as a Reagent-Free IC™ (RFIC™) system using eluent generation following the Dionex Integrion Operator's Manual.[7] The suppressor is installed in external water mode using a Dionex AXP-MS pump to provide the DI water regenerant.[8] The Dionex AXP-MS pump can be added in the instrument configuration, and thus be controlled by Chromeleon CDS. The ISQ-EC is installed according to the installation guide.[9]

A 6-port diverter valve is placed between the conductivity detector (CD) and the mass spectrometer. The diverter valve can be operated in two positions (Figure 2). A small piece of red PEEK tubing called a "jumper" is installed in the IC diverter valve connecting port 1 to port 3. In position A, eluent flows from the CD to the mass spectrometer, and the AXP delivers water to the suppressor Regen In. In position B, eluent flow is in recycle mode for the suppressor, and the AXP delivers water to the mass spectrometer. Configure the diverter valve in the instrument method script editor to divert everything to waste except the compounds of interest. Detailed instructions for configuring the IC-MS system are in Technical Note 72611.[10]

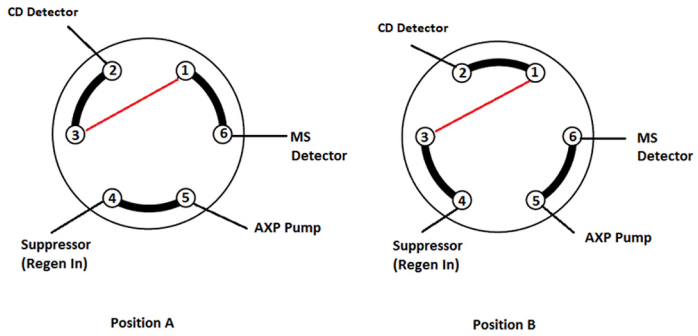

Figure 2. Divert valve positions

Precautions

Allow the system to equilibrate at the column Quality Assurance Report condition until the total conductivity is <1.5 µS/cm. This is done to ensure that the suppressor is fully equilibrated and that the eluent is suppressed sufficiently before sending it downstream to the MS. At this point, it is safe to connect the IC flow to an operating mass spectrometer. While doing this, keep the divert valve in position B with flow from the Dionex AXP-MS auxiliary pump to the mass spectrometer until the background conductivity is below 1.5 µS/cm. This can prevent the non-volatile eluent from precipitating inside the ESI capillary.

Preparation of solutions and reagents

Common anions stock standard solutions

Stock standard solutions (1,000 mg/L) can be prepared by dissolving the appropriate amounts of the required analytes in 100 mL of DI water according to Table 2.

Table 2. Masses of compounds used to prepare 100 mL of 1,000 mg/L ion standards

Analyte	Compound	Amount (mg)
Fluoride	Sodium fluoride (NaF)	221.0
Chlorite	Sodium chlorite ($NaClO_2$), 80%	167.6
Bromate	Sodium bromate ($NaBrO_3$)	118.0
Chloride	Sodium chloride (NaCl)	164.9
Nitrite	Sodium nitrite ($NaNO_2$)	150.0
Chlorate	Sodium chlorate ($NaClO_3$)	127.5
Bromide	Sodium bromide (NaBr)	128.8
Nitrate	Sodium nitrate ($NaNO_3$)	137.1
Sulfate	Sodium sulfate (Na_2SO_4)	147.9
Phosphate	Potassium phosphate, monobasic (KH_2PO_4)	143.3
Carbonate	Sodium carbonate (Na_2CO_3)	176.6

Selenite stock standard solutions (1,000 mg/L)

Selenite stock standard solutions (1,000 mg/L) can be prepared by dissolving 136.2 mg of sodium selenite, anhydrous, in 100 mL of DI water.

Selenate stock standard solutions (1,000 mg/L)

Selenate stock standard solutions (1,000 mg/L) can be prepared by dissolving 258.2 mg of sodium selenate, decahydrate, in 100 mL of DI water.

Calibration stock working solution mixture (10 mg/L)

Prepare 10 mg/L of standard working solution mixture (selenite and selenate) by diluting the standard stock solution with DI water.

Working calibration standard solutions

Diluted working calibration standard solutions were prepared using the calibration stock working solution mixture (10 mg/L). The mixed calibration standard solutions were 10, 50, 100, 200, 250 µg/L.

Laboratory Synthetic Sample Matrix (LSSM)

Additional anions listed in Table 2 were used to prepare a LSSM containing 250 mg/L chloride, 20 mg/L nitrate, 150 mg/L carbonate, and 250 mg/L sulfate.

Standard and sample with internal standard (ISTD)

Add 5 µL of potassium chlorate $^{18}O_3$ (100 mg/L) to each 5 mL of calibration standard or sample. This results in 100 µg/L of potassium chlorate $^{18}O_3$ as internal standard in both standard and samples.

Results and discussion

Separation

The Dionex IonPac AS11-HC columns are specifically designed to resolve many inorganic anions and organic acid anions in one run using hydroxide eluents. The high-capacity Dionex IonPac AS11-HC column allows the injection of more concentrated samples without overloading and peak broadening.[11] High capacity is a critical factor for determining selenite and selenate at low µg/L concentrations in environmental water samples containing high concentrations of common anions such as chloride, nitrate, and sulfate. Figure 3 shows a separation of common anions, selenite, and selenate within 20 min using the Dionex IonPac AS11-HC column. The top chromatogram displays the CD profiles of all anions. The bottom chromatogram displays the MS profile of selenite and selenate. As Figure 3 shows, selenite and selenate were resolved from common inorganic anions.

A delay time of 0.2 min is applied to the MS profile to match the CD profile. The delay time results from the delay caused by the additional connection path from the first detector (in our case the conductivity detector) and the second detector (in this case the MS detector). The delay time can be compensated in Chromeleon CDS so that the comparison of chromatograms from both detectors is simplified.

Limits of detection (LOD)

Several approaches are possible for determining the detection limit of selenite and selenate in environmental samples. The LOD method is based on the MS signal-to-noise (S/N) ratio, which is determined by comparing a measured signal from a low-concentration standard and establishing the minimum concentration at which the analyte can be reliably detected. A S/N of 3, aligned with the industry standard, is used to estimate the LOD.[12] In this study, the baseline noise was first determined by measuring the peak-to-peak noise in a representative 1-minute segment of the baseline where no peaks elute but close to the peak of interest. The signal was determined using selenite standard (5 µg/L) and selenate standard (2.5 µg/L).

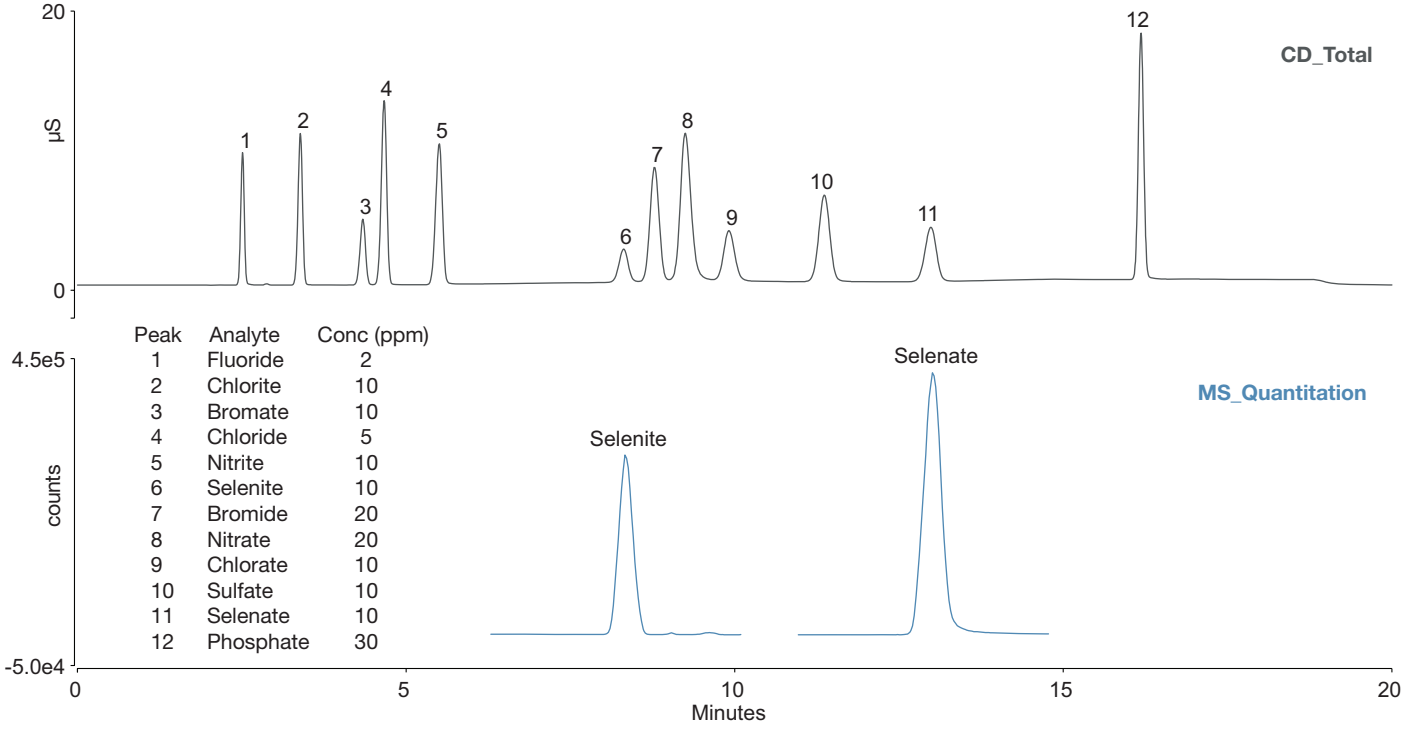

Figure 3. Separation of selenite and selenate from common anions

To examine the influence of a high-concentration anion matrix on the measurements, a laboratory synthetic sample matrix (LSSM) was prepared. The LSSM is a solution of common anions prepared at high concentrations (250 mg/L chloride, 20 mg/L nitrate, 150 mg/L carbonate, 250 mg/L sulfate). Selenite standard (5 µg/L) and selenate standard (2.5 µg/L) were prepared in LSSM matrix. LSSM has minimal impact on the LOD value. For both matrices, water and LSSM, the LODs were identical; the selenite LOD was 4 µg/L, and the selenate LOD was 2 µg/L. Retention times of selenite and selenate also do not shift in LSSM matrix. Retention times of selenite in H_2O and LSSM were both 8.22 min, and retention times of selenate in H_2O and LSSM were both at 12.81 min. This is due to the excellent separation of selenite and selenate from chloride and sulfate, which are present in higher concentrations in the sample.

Calibration

Calibration standard mixtures (selenite and selenate) in the 10–250 µg/L range were prepared in DI water. Potassium chlorate ^{18}O standard was spiked to each calibration standard at 100 µg/L. The use of the internal standard offers two advantages. The first is that the internal standard provides a means to account for losses in ionization efficiencies due to components of the matrix that may compete for ion formation in the source that are not accounted for using an external standard. The second is that using isotopically labeled internal standard ensures that compound quantification is exact and accurate due to the lack of the isotopically labeled internal standard compound in the sample prior to spiking. Table 3 summarizes the calibration results. Calibration curves were generated using internal standard calibration (Figure 4). The coefficient of determination is greater than 0.999 for all components.

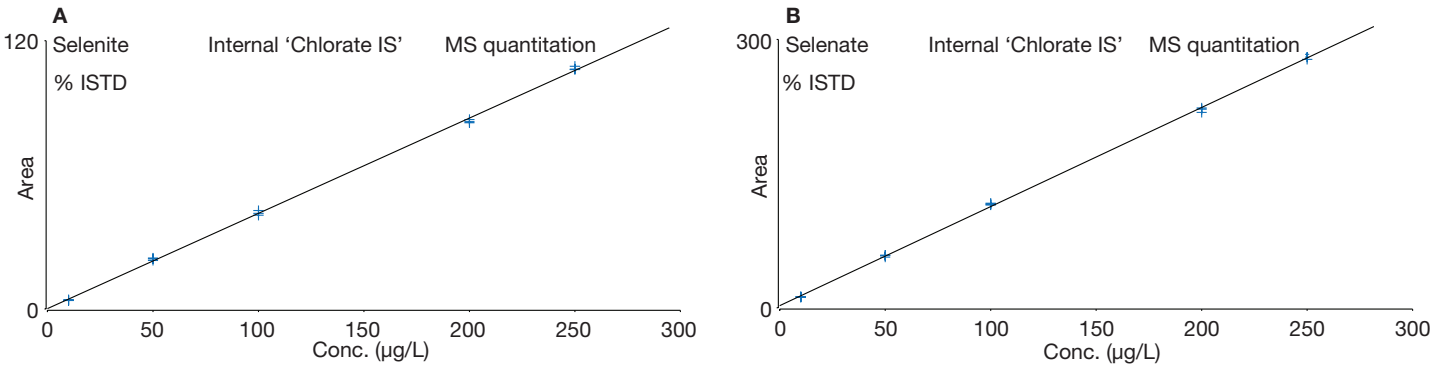

Figure 4. MS calibration curves for (A) selenite and (B) selenate

Table 3. Calibrations

	Range (µg/L)	Calibration type	Internal standard	Internal standard concentration (µg/L)	Coefficient of Determination (r^2)
Selenite	10–250	Linear	Chlorate isotope standard	100	0.9992
Selenate	10–250	Linear	Chlorate isotope standard	100	0.9994

Sample analysis

Environmental waters, including wastewater, lake water, and river water, were collected from the San Francisco Bay Area in California. Selenite and selenate were undetected in any of the three environmental water samples. This agrees with previous reports where selenite and selenate were detected at ng/L levels in environmental waters using the more sensitive IC-ICP-MS techniques.[5] The objective of this study was to create a reagent-free ion chromatography method coupled with mass spectrometry to efficiently detect and confirm the presence of selenite and selenate in environmental waters at elevated concentrations of µg/L via their different *m/z*-ratios.

Therefore, we spiked selenite and selenate into real environmental waters and created simulated contaminated environmental water samples. Figure 5 shows the chromatographic profiles (MS and CD) of wastewater spiked with the 50 µg/L selenite and selenate mixture.

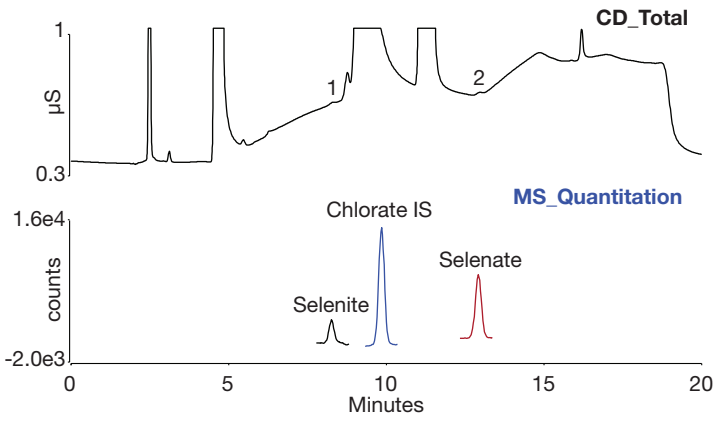

Figure 5. Sample #1 (wastewater) spike with 50 µg/L selenite and 50 µg/L selenate

Method accuracy

Method accuracy was evaluated through recovery studies using environmental water samples. Table 4 shows recoveries of selenite and selenate standards spiked into environmental samples. The recoveries for selenite and selenate in the three samples were in the range of 90–110%.

Table 4. Recoveries of selenite and selenate spiked in environmental water samples

Sample	Spike level (µg/L)	Selenite recovery (%)	Selenate recovery (%)
Sample #1 (Wastewater)	20	100.8	93.6
	50	91.8	99.8
	100	91.6	98.3
Sample #2 (Lake water)	20	94.7	96.4
	50	94.9	101.3
	100	96.9	102.2
Sample #3 (River water)	20	97.7	94.1
	50	98.3	97.0
	100	99.6	102.1

Precision

The method's intraday precision was determined by injecting the 200 µg/L calibration standard on five separate days. The relative peak area (analyte peak area/internal standard peak area) precision varied from 2.3% to 2.8%, with retention time precision <0.5% for all target anions (Table 5).

Table 5. Retention time and peak area precisions

Component	Retention time (RSD)	MS relative peak area to internal standard (RSD)
Selenite	0.46	2.29
Selenate	0.37	2.77

Conclusion

An IC-MS method that enables the determination of selenium species in simulated contaminated environmental waters was developed. The reagent-free ion chromatography system provides excellent reproducibility, yielding exceptional quantification accuracy and consistently reliable results. Recoveries for selenite and selenate in the three water samples ranged between 90–110%, with a method LOD of 4 and 2 µg/L for selenite and selenate, respectively. This method is helpful for highly selective screening of selenium species at µg/L levels in environmental waters. Although not with the same sensitivity as IC-ICP-MS, it offers the advantage of selectively identifying selenium species through their *m/z* ratio. Moreover, this method enables determining whether drinking water exceeds the EPA's outlined MCL of 50 ppb for selenium contamination.

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