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A rapid and sensitive IC-ICP-MS method for determining selenium speciation in natural waters

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Abstract

Selenium (Se) is an element monitored by water quality agencies world-wide. The challenge of assessing its presence in aquatic systems is its low concentrations (parts-per-trillion) and the need for determining its chemical speciation. A method was developed using an ion chromatograph (IC) paired with a quadrupole inductively coupled plasma mass spectrometer (ICP-MS) equipped with a hydrogen reaction cell to provide analysts with a rapid and sensitive method to measure Se speciation with suitable accuracy and precision. The Se species selenite (Se^{IV}) and selenate (Se^{VI}) were separated within a 5-min span using dilute nitric acid as a mobile phase in a step-wise gradient (50-400mM), and quantified using ^{80}Se isotope that yielded low limits of detection ($< 10 \text{ ng L}^{-1}$). Spectral interference from plasma generated diatomic argon ions ($^{40}\text{Ar}_2^+$; $m/z = 80$) on ^{80}Se was eliminated by hydrogen gas (H_2) in the reaction cell. Polyatomic $^{79}\text{Br}^1\text{H}^+$ ($m/z = 80$) did not interfere with ^{80}Se for quantification of common aquatic Se species (Se^{VI} and Se^{IV}) due to different column retention times. Two organic species (methylselenocysteine and selenomethionine) commonly found in aquatic and terrestrial plant tissues were also tested to rule out possible chromatographic interference and explore the potential application to biological samples. Urban rain water and Canadian river water samples were analyzed for Se species to demonstrate the applicability of the method. Owing to its ability to rapidly determine Se species in water samples at environmentally relevant concentrations, the method may be useful for monitoring agencies to routinely measure Se species in freshwater aquatic systems.

Key Words:

Selenium
Chemical speciation
Water monitoring
Ion chromatography

1.0 Introduction

The chemistry of selenium (Se) in the environment has long captured the interest of researchers from a variety of disciplines. Given that it is an essential trace element for humans and animals¹ and is also sometimes an environmental toxin,² understanding the complex behavior of Se in environmental systems is of great importance. Depending on biogeochemical processes and redox conditions, Se can exist in a variety of oxidation states (-II, -I, 0, IV, VI) with greater mobility at higher redox potentials.³ Owing to its ability to bio-accumulate in the food chain, Se concentrations in water deemed harmful to aquatic life are low relative to other elements (e.g., Fe, Mo, Zn, Cu) essential to life.^{2,4} Surface water (oxic to suboxic environments) where Se concentrations exceed guideline values (typically $\geq 1 \mu\text{g L}^{-1}$) are generally attributed to a direct geologic or anthropogenic Se source, with the resulting predominant forms being the oxyanions selenite (SeO_3^{2-} or Se^{IV}), and selenate (SeO_4^{2-} or Se^{VI}).^{5,6} Reports of other inorganic and organic forms, in addition to the ubiquitous Se^{IV} and Se^{VI} , have been described in aquatic systems that are biologically productive or impacted by industrial effluents (e.g., natural organic matter containing Se, Se-NOM; selenocyanate, SeCN^-).^{6,7} Selenium species are also known to differ with respect to bioavailability.⁸ Therefore, the fundamental challenge to understand Se in the aquatic environment is the low concentration combined with the need to understand its chemical speciation.

Numerous methods have been developed to determine Se species,^{9,10} with the bulk of conventional methods utilizing the hydride forming ability of Se^{IV} . Following the pioneering work of Cutter,^{11,12} a large portion of Se speciation analyses of water are based on a selective sequential hydride generation (SSHG) approach.⁶ That technique takes advantage of the fact that only Se^{IV} forms a hydride, therefore, samples can be analyzed directly for Se^{IV} and then

chemically altered through online or offline oxidation and reduction reactions to obtain Se^{VI} and reduced Se species by difference.

The SSHG method is a first step towards the chemical speciation and has some advantages: i) it can be used to incorporate colloidal and particulate Se^{13} ii) it is effective for saline waters (e.g., sea water) to bypass chromatographic interferences and iii) when coupled with appropriate detection technique such as atomic fluorescence, it has excellent sensitivity, even at low concentrations ($\text{LOD typically} < 10 \text{ ng L}^{-1}$). Modifications to this method have also been made to limit bias resulting from the decomposition of organo-Se molecules during heat-acid reflux,¹⁴ but there is still considerable potential for large errors to be made when determining speciation by difference.⁶ SSHG procedures also call for large quantities of sample with considerable time and effort to perform the necessary redox reactions, with each step and sample type requiring detailed quality control. Fundamentally, the greatest disadvantage of any version of the SSHG approach is the inability to identify species within the “reduced Se” (primarily organic) fraction.

Ion chromatography (IC) coupled with inductively coupled plasma mass spectrometry (ICP-MS) is a powerful analytical tool that has been used successfully for identifying a wide variety of Se containing compounds based on their retention times.¹⁵⁻¹⁷ For Se analyses, however, conventional quadrupole ICP-MS operation suffers from inadequate sensitivity for concentrations typical of natural waters. This is due to the high ionization potential of Se in the plasma as well as isobaric and polyatomic interferences.^{9,18} Selenium has six stable isotopes (^{74}Se [0.87%], ^{76}Se [9.02%], ^{77}Se [0.58%], ^{78}Se [23.52%], ^{80}Se [49.82%], and ^{82}Se [9.19%]), which adds complexity to the analysis.¹⁹ The greatest sensitivity is achieved by monitoring the most abundant isotope (^{80}Se), however the use of argon (Ar) gas in ICP-MS operation results in the dimer $^{40}\text{Ar}_2^+$ ($m/z=80$) interference.^{9,20} Using a reaction gas such as hydrogen (H_2) or

methane (CH_4), eliminates the interference by $^{40}\text{Ar}_2^+$ and greatly increases sensitivity by reliably quantifying ^{80}Se .²⁰

While using a chromatographic approach offers greater detail to identify the species that are present, methods for coupling these instruments and achieving the necessary detection limits (parts per trillion or lower) for natural waters are still limited in the literature and may not be practical for routine monitoring due to long run times. Adequate sensitivity is achievable with ICP-MS, but appropriate mobile and stationary phases must also be considered. To provide analysts with a rapid and sensitive method for Se speciation in freshwater, we tested the use of dilute nitric acid (HNO_3) as a mobile phase. This offers some advantages to other mobile phases with basic or near-neutral pH: i) it prevents precipitation of iron (Fe) and aluminum (Al) (hydr)-oxides in samples taken from acidic²¹ or anoxic waters,²² ii) eliminates the need for salt or organic solvent-based eluents that can also be undesirable for interfacing IC with ICP-MS, thus improving detection limits as well as reducing maintenance costs²³ and iii) HNO_3 is already commonly used for ICP-MS analyses and can be distilled and purified to the extent necessary to achieve the desired background concentrations (blank values).

2.0 Experimental

2.1 Chemicals and reagents

To prepare standard solutions for selenium speciation, Se compounds were purchased from Sigma-Aldrich and dissolved in high purity ($18.2\Omega\text{ cm}$) water (Barnstead Nanopure, Thermo ScientificTM). Individual stock solutions had concentrations of 2000 mg L^{-1} for sodium selenite (Se^{IV} ; 99%; Cat. #S3876) and sodium selenate decahydrate (Se^{VI} ; 99.999% - trace metal basis; Cat. #450294), while organic Se species were prepared with a concentration of 500 mg L^{-1} using

seleno-DL-methionine (SeMet; $\geq 99\%$; Cat. #S3876) and Se-(methyl)selenocysteine hydrochloride (MeSeCys; $\geq 95\%$; Cat. #M6680). Working standards (20 to 400 ng L⁻¹) were prepared daily from the stock solutions by diluting with high purity water inside a HEPA filtered clean air cabinet. Mass calibration standard solutions for total Se analysis were prepared by diluting SPEX CertiPrep Instrument Calibration Standard 2 (100 mg Se L⁻¹ stock) with high purity water and acidifying final calibration solutions (0.1 to 2 µg L⁻¹) to 1% HNO₃. A multi-element Internal Standard 1 was also diluted from its original concentration (10 mg L⁻¹) to 2 µg L⁻¹ with high purity water and acidified to 1% HNO₃.

TraceMetal™ grade HNO₃ purchased from Fisher Scientific (Cat # A509P212) was used to prepare the mobile phase and acidify solutions analyzed for total concentration of Se. Hydrochloric acid (HCl) used for preserving samples and determining blank values was either TraceSELECT® (Sigma-Aldrich, Cat. #72787) or Optima™ (Fisher Scientific, Cat. #A466-500) HCl.

2.2 Instrument set-up for Se speciation and total Se analysis

A high performance ion chromatograph (HPIC; Thermo Scientific™ Dionex ICS-5000⁺) equipped with a Dionex IonPac™ AS7 anion exchange column (4 mm ID x 250 mm length) and AG7 guard column (4 mm ID x 50 mm length) was used to separate different Se species. The system included a single gradient pump, temperature controlled oven module, degasser, and AS-AP auto-sampler equipped with a 25 µL sample loop. The mobile phase was prepared in a step-wise concentration gradient as follows: 0-2 min (50 mM HNO₃), 2-4 min (400 mM HNO₃) and 4-5 min (50 mM HNO₃). It's important to note that the potential corrosion of metallic parts by a dilute HNO₃ mobile phase is not problematic in this system due to the use of PEEK™

(polyetheretherketone) instead of steel, thus allowing for a 'metal-free' flow path. This IC system was connected to a single quadrupole ICP-MS (iCap Q, Thermo Scientific™) operated in CRC (chemical reaction cell) mode. After passing through the column, the mobile phase was introduced to the ICP-MS at a fixed flow rate (1.0 mL min^{-1}) through a MicroFlow PFA-LC zero dead volume nebulizer into a quartz cyclonic spray chamber cooled to $\sim 3^\circ\text{C}$. Peak areas of Se species were integrated using Dionex Chromeleon® 7 software.

Preliminary work for the chromatographic conditions presented above is also described by Javed.²⁴ Two important modifications were made to that method which are included here: i) the flow rate of the mobile phase was kept constant (1 mL min^{-1}) to keep the optimized aerosol size and introduction rate consistent with tuned parameters and ii) H_2 gas was used as a reaction gas in place of He (collision gas) in the pressurized cell to enable the accurate quantification of ^{80}Se . The flow rate of H_2 was adjusted until low background signals were achieved for ^{80}Se , without any substantial loss of sensitivity. The isotopes ^{77}Se and ^{78}Se were monitored during analysis to compare differences in the accuracy and precision, and also to assess the role of relevant polyatomic interferences, such as $^{79}\text{Br}^1\text{H}^+$ (see Results and Discussion).

The same ICP-MS was used for total Se analyses, but in this case the instrument was equipped with a CETAC ASX-520 auto-sampler and PFA-ST MicroFlow Nebulizer. Total Se in water samples (described below) was determined under kinetic energy discrimination (KED) mode with He as a collision gas. Quantification was based on the average of 3 main runs with 25 sweeps using ^{78}Se . The internal standard solution was introduced continuously online through a PEEK™ T-mixing piece. Scandium (Sc), indium (In) and holmium (Ho) from the internal standard solution were monitored and used to account for any instrumental drift on the basis of interpolation. Corrections made by software to account instrument drift were minor, with no

substantial changes in the internal standard observed during analyses ($\sim \pm 10\%$). Gases (Ar, He, H₂) used for (IC)-ICP-MS analysis were of ultra-high purity (Praxair, purity 5.0). A summary of relevant operating information for both total Se and Se speciation is provided in Table 1.

2.3 Quality control and method validation

Method accuracy for Se speciation and total Se concentrations was evaluated by analyzing certified standard reference material (SRM; NIST 1640a: Trace Elements in Natural Water; Se = $20.13 \pm 0.17 \mu\text{g L}^{-1}$), whereas precision was assessed by analyzing samples in triplicate. For routine ICP-MS quality control, instrument blanks (1% HNO₃) and check standards were used. Multiple blanks containing either high purity water only or a dilute mixture of HCl and high purity water ($\sim 0.8\%$ HCl) were also included to identify any possible contamination and evaluate spectral interference related to the increased chloride concentration from HCl. To further study any interconversion of Se species during separation in the IC, or potential chromatographic interferences (e.g., competition from competing ions), an acidified river water sample was also chosen and spiked with Se^{IV} and Se^{VI}.

As an additional method for verification of Se^{IV} and Se^{VI}, acidified blank ($\sim 0.8\%$ HCl) samples ($n=3$) were spiked with Se^{IV} and analyzed using both IC-ICP-MS and HG-AFS. Details for HG-AFS analysis are provided in the supplementary information. Briefly, samples spiked at $200 \text{ ng Se}^{\text{IV}} \text{ L}^{-1}$ were acidified with HCl to 3.6M, diluted (5 times dilution), and analyzed directly with a PS Analytical 10.055 (Millennium Excalibur) HG-AFS system. During the on-line chemical reduction with sodium borohydride, only Se^{IV} is capable of forming a gaseous hydride and therefore any Se signal is the result of Se^{IV} in the sample.

2.4 Method application: River and rain water sampling

The method was applied to natural water samples collected from three different rivers, and one sample of urban rain water. The purpose was to demonstrate the applicability of the method on chemically diverse natural waters containing low concentrations of Se. The determined chemical speciation is compared with the total concentrations and with the results available in the literature where different Se speciation methods have been applied to similar water types (e.g., snow, rain, river water).¹⁰

For river water, samples from the shore of the North Saskatchewan River in the city of Edmonton, (Alberta, Canada) were collected in June 2016. Four samples were syringe filtered into polypropylene (PP) centrifuge tubes (15 mL) on-site using 30 mL all plastic syringes (Thermo Scientific™, Cat. #S7510) and 0.22 µm polytetrafluoroethylene (PTFE) disk filters (Thermo Scientific™; 30mm; Cat. # 033911E). Samples were analyzed on the same day; samples for chemical speciation of Se ($n=3$) were analyzed directly (i.e., filtered only) without any further treatment, while the sample ($n=1$) for total Se was acidified with HNO₃ (1% v/v) before analysis. A water sample was also collected from the shore of Maligne River in Jasper National Park, Alberta, Canada and was immediately syringe filtered using 30 mL plastic syringes (see above) and 0.45 µm PTFE disk filters (Thermo Scientific™; 30mm; Cat. #03-391-1C) into 125 mL acid-cleaned fluorinated polyethylene (FLPE) bottle containing 1 mL of HCl as preservative. The sample was stored at ~4°C until analysis was performed. The Athabasca River in northern Alberta, Canada was also sampled in October 2015. Water sample collection and handling used the same materials and procedure as described above for Maligne River, but were collected from the bow of a small boat anchored in the middle of the river. This sample is unique in that it was collected relatively close to the large surface mines of the Athabasca bituminous (oil) sands.

Urban rain water was collected in an acid-cleaned 125 mL borosilicate glass bottle (Wheaton™; 219815) with Teflon™ lined lid during a rain event at the University of Alberta, Edmonton on June 30, 2016. The sample was immediately filtered (0.22 µm) using a 30 mL plastic syringe (see above) and PTFE disk filters into 3 separate vials for analysis on the same day (< 30 min from collection). Only a small portion of the original sample was saved for Se speciation analysis and total Se was not analyzed.

3.0 RESULTS AND DISCUSSION

3.1 Peak resolution of Se species and differences in isotopic sensitivity with corresponding detection limits

Inorganic Se species (Se^{IV} and Se^{VI}) maintained excellent peak shape and resolution at low concentrations when a standard solution containing inorganic Se species (200 ng L⁻¹) was injected into the IC-ICP-MS (Figure 1A). Similar to the behavior of arsenite (As^{III}) in a method using a HNO_3 mobile phase and AS7 column for arsenic speciation,²² the low mobile phase pH and acid dissociation constant of Se^{IV} ($\text{pK}_{\text{a}1}=2.46$)²⁵ explains its quick movement through the column with little retention. Selenate required a stronger HNO_3 eluent concentration (400 mM) to elute in a suitable amount of time. An additional 60 s of run time using 50 mM HNO_3 mobile phase was added following the elution of Se^{VI} to re-condition the column and prepare for the next sample. After adequate separation and sensitivity was achieved for the inorganic Se species, the method was thoroughly tested for potential interferences (see below). Because the predominant species in aquatic systems tend to be Se^{IV} and Se^{VI} , the method was optimized for those species. However, to evaluate its performance in samples that contain organo-Se species, two selenoamino acids (SeMet and MeSeCys) were also tested. This is important because Se^{IV} is passing through the column with little interaction with the stationary phase (presumably in the

void volume) and an analytical artefact is plausible if there are other Se species which do not interact with the stationary phase. With recent evidence of organo-Se species in ultra-trace quantities in biologically productive waters,^{26,27} two selenoamino acids (SeMet and MeSeCys) were also tested using the same instrumental conditions. Although effective for the oxyanions Se^{IV} and Se^{VI} , the analysis of selenoamino acids by ion exchange chromatography is significantly more challenging; this is in part due to the zwitterionic nature of amino acids. For example, at the low pH (< 2) of mobile phase used here, SeMet would be present as positively charged cation^{28,29} and may not be suitable for analysis with an anionic exchange column. However, despite being developed as a strong anion exchange column for polyvalent species,³⁰ the IonPac[™] AG7 and AS7 are known for both cation and anion exchange capability due to a sulfonic surface coating and an outer layer of submicron anion-exchange MicroBeads[™] with alkyl quaternary ammonium functional groups;³¹ this is in addition to other retention mechanisms such as molecule and stationary phase polarity, as the stationary phase is hydrophobic. With these processes in mind, simultaneous determination of cationic and anionic Se species using this set-up was deemed possible.

The results showed that SeMet and MeSeCys had a relatively high affinity for the stationary phase and eluted in a short time (<5 minutes) with consistent peak shapes and good separation (Figure 1B), however baseline separation between Se^{VI} and SeMet was not obtained. In an attempt to gain additional resolution between Se^{VI} and SeMet, the eluent strength was decreased to 300 mM and then to 100 mM. These manipulations did not yield greater peak resolution and instead showed increased retention times and produced an undesirable peak shape compared to the desired Gaussian peak shape (data not shown). Analysis of calibration standards with different concentrations yielded consistent but considerable differences in sensitivity for all

species among the three measured isotopes, with the most sensitive to least sensitive being $^{80}\text{Se} > ^{78}\text{Se} > ^{77}\text{Se}$ (see Figure 1 for example). The difference in signal intensity of ^{80}Se from ^{77}Se and ^{78}Se was approximately 2 and 5 times, respectively (Table 2). As mentioned above, this sensitivity was directly related to the natural abundance of each isotope. The limit of detection (LOD) and limit of quantification (LOQ) were also calculated based on 3 and 10 times the standard deviation of eight blanks ($n=8$), respectively. These values were assessed experimentally by analyzing a 20 ng L^{-1} and a 50 ng L^{-1} standard 5 times each. Overall, the accuracy and precision of the measured standards were representative of the calculated LOD/LOQs (Table 2). Between the three isotopes, ^{80}Se had the lowest overall LODs and produced excellent accuracy and precision at 20 and 50 ng L^{-1} concentrations. Isotope ^{78}Se also produced good overall results and is beneficial to include in the Se analysis for critically evaluating the data for potential interferences (see below). Due to the relatively poor results of ^{77}Se , this isotope will not be discussed in detail, however it is still useful in the data evaluation stages to distinguish Se from interferences.

The importance of using a reaction cell gas (H_2) is further highlighted by an observed increase in sensitivity as compared to the previous method that utilized He collision cell performed on the same IC-ICP-MS.²⁴ Using reaction gas and the method described here, the sensitivity was approximately 28,000 and 24,000 cps ppb⁻¹ for Se^{IV} and Se^{VI} , respectively. Using He as a collision gas (and ^{78}Se for quantification) the sensitivity was approximately 1400 and 800 cps ppb⁻¹ for Se^{IV} and Se^{VI} , respectively.²⁴

3.2 Bromine interference and influence of HCl preservation on Se species determination

Arguably one of the most critical challenges to overcome when using H_2 in a reaction cell of ICP-MS for Se measurements is the polyatomic interferences created by samples containing

253 bromine (Br). Hydrogen gas reacts to form $^{79}\text{Br}^1\text{H}^+$ ($m/z=80$) and $^{81}\text{Br}^1\text{H}^+$ ($m/z=82$) and these
254 polyatomic ions overlap with the commonly measured ^{80}Se and ^{82}Se isotopes. In this study, we
255 observed the interference caused by $^{79}\text{Br}^1\text{H}^+$ on ^{80}Se , but since Br (presumably as ionic bromide;
256 Br^-) was effectively separated from three of the species in question there was no influence on the
257 results (Figure 2). SeMet quantified using ^{80}Se was an exception to this, as it was observed to
258 elute at approximately the same time as Br^- ; therefore, ^{78}Se must be used for accurate
259 quantification of SeMet. If the extremely high sensitivity offered by ^{80}Se is critical for the
260 application, the use of deuterium (D_2) in place of H_2 as the reaction gas has been successful in
261 overcoming the Br interference³² and might be a suitable alternative in this method as well.
262 Silver cartridges are commonly used for removing unwanted Cl^- , Br^- and I^- from samples but
263 should not be used in this particular scenario as it has been reported that these cartridges remove
264 SeMet along with other halogen ions.²⁶

265 The potential influence of HCl on this method was also evaluated for two main reasons: i) it
266 has been described as a preservative (in addition to refrigerated storage) used to stabilize Se^{IV}
267 and Se^{VI} in water samples³³ and ii) it is known to contain trace amounts of Br. To test the
268 potential influence from Br contamination, a solution of high purity water and HCl
269 (TraceMetal™ grade) was analyzed (final HCl concentration ~0.8%). This concentration was
270 chosen because it maintains a $\text{pH} < 2$ required for preserving a wide variety of samples and is
271 also appropriate for reliable ICP-MS analysis. Even with the high purity acid, Br contamination
272 was evident from Br^- peaks (Figure 2A) and is enough to affect quantification of SeMet using
273 ^{80}Se . The effect of HCl used to acidify water containing Se^{IV} and Se^{VI} speciation was assessed by
274 adding the same proportion of HCl (~0.8%). The change in sample pH did not affect retention
275 times of either species and Br^- eluted after Se^{VI} , without interfering with Se^{VI} (Figure 2B). Based

on these results, it is confirmed that HCl does not impact the analysis of Se^{IV} or Se^{VI} and samples preserved with $\leq 0.8\%$ HCl can be analyzed reliably using this method for Se^{IV} or Se^{VI} determination.

3.3 Method validation

The accuracy of measurements was partially determined by measuring certified standard reference material NIST 1640a (Trace Elements in Natural Water; $\text{Se} = 20.13 \pm 0.17 \mu\text{g L}^{-1}$) because there are currently no certified standard reference water samples for Se species. The SRM was diluted 50 times prior to analysis to bring the concentration into the range of the calibration standards. It was found that all measurable Se was present as Se^{IV} and the recovery for every measurement was within 10% of the certified total Se value. An independent method check of triplicate samples containing 200 ng L^{-1} of Se^{IV} measured by IC-ICP-MS and HG-AFS also yielded no observable difference (Figure S1). To evaluate the recovery of spiked Se species from a natural water sample, an aliquot of the Athabasca River water (deemed to be the most chemically complex of the river samples) was spiked with 100 ng L^{-1} of Se^{IV} and Se^{VI} . Full recovery ($> 96\%$) was obtained for both species and repeatability of triplicate measurements had $< 3\%$ RSD (Table 3). Accuracy of total Se measurements was assessed using NIST 1640a (diluted 50 times). Full details for all QA/QC are provided in Table 3.

3.4 Se speciation in natural waters

Water samples from three rivers and one rain event were used to evaluate the method. Figure 3 displays the Se speciation in those water samples, with each river having slightly different proportions of Se^{IV} and Se^{VI} relative to the total Se. Compared to the North Saskatchewan River (261 ng L^{-1}) and the Athabasca River (187 ng L^{-1} ; Table 4), Maligne River contained the highest

concentration of total Se (314 ng L^{-1}) and the most Se^{VI} . This observation is most likely a reflection of the local geology as well as input from snow melt, which has been found to contain predominantly Se^{VI} .³⁴⁻³⁶

The sum of inorganic species (Se^{IV} and Se^{VI}) accounted for most of the Se dissolved in North Saskatchewan River water (77%) and the Maligne River (88%), but in the Athabasca River there was no detectable Se^{IV} and Se^{VI} was only 43% of the total Se (Table 4). The differences in proportion of inorganic Se could be related to the natural organic matter (NOM) content of the waters and the known ability of complex organic structures to incorporate Se.⁶ Dominated by snow melt, the Maligne River likely contains low concentrations of NOM, whereas the Athabasca River is well known for its complex dissolved organic matter consisting of humic material as well as bitumen derived compounds from bituminous sand outcrops.^{37,38}

Rain water was selected for analysis using this method because concentrations are usually low and therefore requires the level of sensitivity demonstrated here. Moreover, because Se speciation in rain water has been documented using various analytical techniques, it offers some additional insight into performance relative to other methods using a similar matrix. Unlike snow, Se^{IV} is commonly the predominant oxidation state in rain water.^{11,34,35,39} The results for rain water from Edmonton (Table 4) yielded similar chemical speciation results as those obtained by others which also employed a chromatography based Se speciation approach.³⁹ Rain water ($n=3$) from Edmonton contained $72 \pm 5 \text{ ng L}^{-1} \text{ Se}^{\text{IV}}$ and $24 \pm 5 \text{ ng L}^{-1} \text{ Se}^{\text{VI}}$, while Seattle rain ($n=3$) contained $59.4 \pm 2.6 \text{ ng L}^{-1} \text{ Se}^{\text{IV}}$ and $16 \pm 0.4 \text{ ng L}^{-1} \text{ Se}^{\text{VI}}$.³⁹

3.5 Method limitations

The method was found to be effective for water samples with a very simple matrix (low salinity and NOM concentrations), but its performance with more complex matrices was not critically evaluated. A comprehensive study is necessary to fully evaluate the effect of competing ions (e.g. sulfate) and any necessary sample clean-up steps. This would help expand its application toward samples with greater ionic strength, such as seawater or industrial effluents. There is also an inherent disadvantage associated with using a low pH mobile phase on water samples containing abundant colloidal (ie., $< 0.45\mu\text{m}$) organic and mineral particles. A more representative determination of the chemical speciation of Se (or other elements) requires methods of collection and analyses, including selection of the mobile phase, to mimic as closely as possible the environmental conditions at the time of sampling. Mobile phases used in chromatography typically are not tuned for site-specific parameters (e.g., pH, ionic strength), so there are risks of potential changes to the colloids such as partial dissolution, desorption, or dissociation. Therefore, a combination of other techniques for Se speciation such as asymmetrical flow-field flow fractionation⁴⁰ would be worth investigating. To this point, we offer an example from some preliminary work on Se speciation in surface water of a peatbog in Northern Saskatchewan, situated near a flooded uranium mine (Figure 4). The total concentration of Se was 140 ng L^{-1} , with $40\text{ ng L}^{-1}\text{ Se}^{\text{VI}}$ and no detectable Se^{IV} . As in the case of the Athabasca River, the form(s) of the unidentified Se fraction in these waters remains unknown; however, given the complex chemistry and array of organic compounds present in peat bog water, it may be possible that a significant portion of the Se was incorporated into NOM.

4.0 CONCLUSIONS

The purpose of this study was to develop a method which can quickly analyze Se species in natural water samples with high sensitivity using a dilute HNO_3 acid mobile phase. While

originally intended for Se^{IV} and Se^{VI} , it was also found to be effective for at least two organic species and therefore may also have applications for studying biological (e.g. plant or animal tissue) samples. The use of an acidic mobile phase may also be useful for anoxic groundwater or sediment pore water samples that require acid preservation to prevent the precipitation of Fe (hydr)-oxides. When coupled with single quadrupole ICP-MS using hydrogen in the reaction cell the method has excellent sensitivity and allows for accurate and precise measurements below 50 ng Se L⁻¹. Given the simple mobile phase, low cost of consumables and rapid elution/detection of species of interest, this method will be a useful analytical option for monitoring agencies tasked with routinely determining Se speciation in freshwater environments.

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Tables and figures

Table 1. Instrumental parameters and conditions for IC-ICP-MS and ICP-MS operations

Instrument	Parameter	Optimized Setting
IC-ICP-MS	R.F. power (W)	1548
	Ar flow rate (L min ⁻¹)	0.8
	H ₂ flow rate (mL min ⁻¹)	9
	Dwell time (s)	0.1 (Se)
	Eluent flow rate (mL min ⁻¹)	1.0
	Mobile phase A	50 mM HNO ₃
	Mobile phase B	400 mM HNO ₃
	Gradient program	0-2 min 100% A 2-4 min 100% B 4-5min 100% A
	Wash volume	250 (μL)
	Column temperature	30 (°C)
ICP-MS	Skimmer cone	Ni
	Sample cone	Ni/Cu
		0.2 (Se)
	Dwell time (s)	0.02 (Sc)
		0.01 (In)
		0.01 (Ho)
	Auxiliary gas flow rate	0.8 (L min ⁻¹)
	He flow rate	5.0 (mL min ⁻¹)

Table 2. Comparison of method accuracy, precision and sensitivity between isotopes for each of the studied species. Standard deviations for measurements are shown in parentheses. Bold values indicate analyses with > 20% RSD.

Isotope	Speciation	Calibration Linear Coefficient	LOD (ng L ⁻¹)	LOQ (ng L ⁻¹)	Recovery of 20 ng L ⁻¹ standard (n=5)	Recovery of 50 ng L ⁻¹ standard (n=5)	Instrument sensitivity (cts) at 200 ng L ⁻¹
⁷⁷ Se	Se ^{IV}	0.961	24	110	11 (±8)	33 (±12)	1056
	MetSeCys	0.967	41	100	2 (±5)	11 (±16)	1050
	Se ^{VI}	0.984	10	149	ND	28 (±12)	620
	SeMet	0.970	38	147	6 (±8)	20 (±16)	498
⁷⁸ Se	Se ^{IV}	1.000	9	28	18 (±3)	52 (±7)	2462
	MetSeCys	0.996	20	64	6 (±4)	39 (±6)	2697
	Se ^{VI}	0.999	3	23	18 (±2)	54 (±5)	2192
	SeMet	0.996	12	36	20 (±8)	44 (±3)	1586
⁸⁰ Se	Se ^{IV}	0.997	4	14	21 (±3)	49 (±7)	5354
	MetSeCys	0.999	5	12	21 (±3)	51 (±2)	5868
	Se ^{VI}	0.999	4	15	20 (±3)	49 (±4)	4673
	SeMet	0.998	9	38	18 (±4)	46 (±4)	3154

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Table 3. Method validation using standard reference water and spiked river water. Results are based on quantification of ⁸⁰Se. Athabasca River water was spiked with Se^{IV} and Se^{VI}.

Water Type	Analysis	Number of measurements (n)	Certified or expected value (µg L ⁻¹)	Measured value	Recovery (%)	Relative standard deviation (%)
NIST 1640a*	Speciation	4	20.13 (±0.17)	20.35	101	5.7
Se(IV) spike**	Speciation	3	0.100	0.099	99	2.5
Se(VI) spike**	Speciation	3	0.177	0.170	96	2.6
NIST 1640a	Total	1	20.13 (±0.17)	20.20	100	NA

* NIST 1640a is used as an indicator of method accuracy for speciation by comparing sum of species with certified total concentration, ** spiked solutions diluted the original sample by 5%

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Table 4. Total Se and its chemical species in natural water samples. Results for chemical speciation were quantified using ^{80}Se and total Se analysis was ^{78}Se .

Water Type	GPS Location	Sample number (<i>n</i>)	Se^{IV} (ng L ⁻¹)	SeMetCys (ng L ⁻¹)	Se^{VI} (ng L ⁻¹)	SeMet (ng L ⁻¹)	Total Se (ng L ⁻¹)	Sum of species relative to total (%)
Maligne River	52.921591 -118.016796	1	24	ND	252	ND	314	88
Athabasca River	56.8971667 -111.419116	1	<LOD	ND	77	ND	187	43
North Sask. River	53.529857 -113.517937	3	45 (±6)	ND	155 (±55)	ND	261	77
Edmonton Rain	53.526103 -113.523896	3	72 (±5)	ND	24 (±5)	ND	NA	NA

ND: not detected; NA: not available

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Figure 1. Peak resolution of Se species and the sensitivity difference of measured isotopes. Chromatogram A represents a standard solution containing 200 ng L^{-1} each of Se^{IV} and Se^{VI} ; Chromatogram B represents a standard solution containing 200 ng L^{-1} of each Se species. Both standards were prepared using high purity water without any acidification of solutions. Chromatography method uses a Dionex IonPacTM AS7 anion exchange column (4 mm ID, 250 mm length, $10 \mu\text{m}$ particles size), AG7 guard column (4 mm ID x 50 mm length) at 30°C , with 1 mL min^{-1} flow rate and $25 \mu\text{L}$ sample injection.

Figure 2. The potential influence of bromine (Br) and HCl on Se quantification. Chromatogram A represents a solution of high purity water and HCl (acidified to $\sim 0.8\%$). Two isotopes of Br were monitored to confirm that Br (presumably as ionic Br^-) was present in HCl, and due to H_2 reaction with ^{79}Br , the resulting $^{79}\text{Br}^1\text{H}^+$ is responsible for the observed artefact of ^{80}Se . Chromatogram B represents a standard solution (200 ng L^{-1} each of Se^{IV} and Se^{VI}) prepared using high purity water and acidified with HCl. The results indicate that the Br^- peak does not interfere with Se^{VI} and the acidification of the solution has no influence on Se^{IV} and Se^{VI} retention times.

Figure 3. General depiction of Se speciation in river and rain water samples obtained using the developed method. Chromatograms represent (A) Athabasca River, (B) North Saskatchewan River, (C) Maligne River and (D) urban rain water. A false Se peak was expected in chromatograms A and C due to preservation with HCl, but trace Br was still detected in un-acidified samples (B, D). See Table 4 for the corresponding concentrations.

Figure 4. Selenium speciation of chemically complex peatbog surface water. A signal response from all three Se isotopes appear at 300s, which suggests that it is Se being detected, but ^{80}Se signal had the lowest response and therefore it's likely that a different spectral interference is at play here. A second Br peak was also observed ($\sim 225\text{s}$), possibly indicating the presence of another form or column artifact due to the very high concentration of Br^- .

Figure 1.0

Figure 2.0

Figure 3.0

Figure 4.0