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Publisher's version / Version de l'éditeur:
https://doi.org/10.1016/j.scitotenv.2020.140877
Science of The Total Environment, 15 July 2020, 2020-07-15

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PII: S0048-9697(20)34406-5

DOI: https://doi.org/10.1016/j.scitotenv.2020.140877

Reference: STOTEN 140877

To appear in: *Science of the Total Environment*

Received date: 17 April 2020

Revised date: 24 June 2020

Accepted date: 8 July 2020

Please cite this article as: M.G. Bordash, E. Pagliano, K.L. LeBlanc, et al., Determination of selenocyanate, selenate, and selenite in mining wastewater by GC–MS using sequential derivatization and extraction, *Science of the Total Environment* (2020), https://doi.org/10.1016/j.scitotenv.2020.140877

Determination of selenocyanate, selenate, and selenite in mining wastewater by GC-MS using sequential derivatization and extraction

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Abstract

Selenium speciation analysis is usually carried out using complex hyphenated analytical systems such as LC-ICP-MS. Here we present a novel selenium speciation approach based on a sequential derivatization and extraction combined with gas chromatography mass spectrometry for the simultaneous determination of selenite, selenate, and selenocyanate in aqueous mine wastewater samples. Selenocyanate was derivatized with triethyloxonium tetraflouroborate to ethylselenocyanate, which was extracted into chloroform, following which the sample was split into two aliquots. One aliquot was acidified and 3,5-bis(trifluoromethyl)-*o*-phenylenediamine was used for the novel derivatization of selenite to 4,6-bis(trifluoromethyl)-2,1,3-benzoselenadiazole, for the determination of selenite. For the second aliquot, concentrated hydrochloric acid was added along with 4-nitro-*o*-phenylenediamine to simultaneously reduce selenate to selenite and derivatize the combined "selenite + selenate" fraction to 5-nitro-2,1,3-benzoselenadiazole. The benzoselenadiazoles were extracted with chloroform and all extracts were combined for GC-MS analysis. Low ng g^{-1}

detection limits were reported for all three species. The method is unhindered by concentrations of chloride and sulphate up to 3%, as well as nitrate concentrations up to 3% for selenocyanate and selenite analysis, with minor losses in sensitivity for selenate up to 100 ppm nitrate, making the method particularly suitable for aqueous mine waste characterization. Quantitative trace selenium speciation was achieved using cost-effective materials and apparatus on a simple-to-operate benchtop instrument. The novel methodology was tested on gold mine wastewater samples; comparing to total selenium, a 63-149% recovery as the sum of species was observed. Additionally, this novel speciation approach was compared to LC-ICP-MS based selenium speciation and a reasonable agreement was found in the species distribution.

Keywords: selenium speciation; gas chromatography; derivatization; piazselenol; triethyloxonium; wastewater

1. Introduction

Environmental selenium (Se) contamination is a major issue in some North American aquatic ecosystems, where elevated concentrations can cause teratogenic effects in oviparous vertebrates.[1] Se is mobilized through various anthropogenic activities including mining, metallurgical processes, and the burning of high-sulfur coal. In most scenarios, at roughly-neutral pH, Se enters aquatic ecosystems in the form of selenite (Se(IV), $HSeO_3^-$) or selenate (Se(VI), SeO_4^{2-}), with the two oxyanions present in various proportions depending on redox conditions.[2] Certain environments promote the production of various other Se species, including selenocyanate ($SeCN^-$) – this species is thought to be formed via the combination of free cyanide and elemental $Se^{(0)}$ [3, 4] in a process analogous to the formation of thiocyanate observed during the treatment of sulphide ores with cyanide.[5] The presence of $SeCN^-$ has also been noted in in flue-gas scrubber waters produced at coal-fired power plants,[6] as well as in eutrophic Se-contaminated natural waters.[7]

The United States Environmental Protection Agency (USEPA) has recently tightened regulations surrounding allowable Se concentrations in aquatic environments,[8] which has led to the development and implementation of improved wastewater treatment technologies. In almost all cases, the success of the treatment process depends on the chemical speciation of Se present in the influent. This is particularly relevant for systems employing biological treatment protocols as the organisms involved tend to preferentially uptake one form of Se.[9, 10] Chemical pretreatment can be used to convert Se to the desired species, with varying degrees of efficiency depending on the specific conditions, but to ensure these reactions are quantitative it is necessary to have a thorough understanding of the species present in wastewater samples.

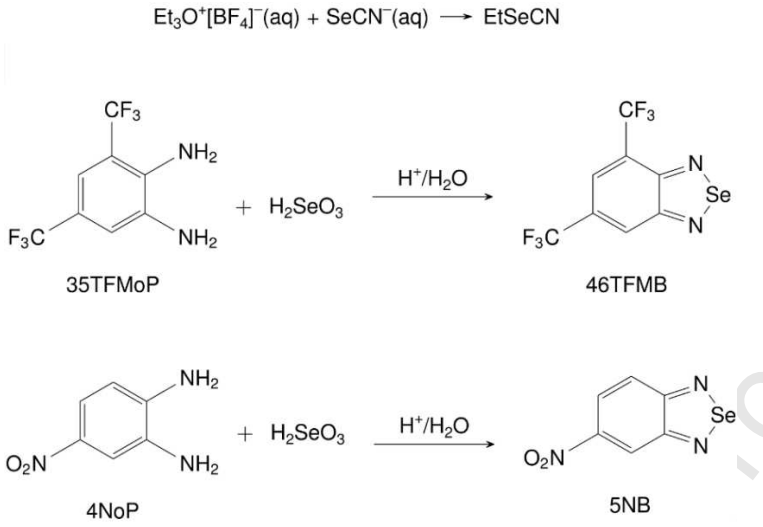
Se speciation analysis has been conducted using fractionation methods such as selective sequential hydride generation (SSHG), where Se species must be selectively converted to Se(IV) to be detected.[11, 12] Unfortunately, following this method $SeCN^-$ is indistinguishable from other reduced (i.e. organic) Se species.[13] Anion exchange chromatography (AEC) employing an inductively-coupled plasma mass spectrometer (ICP-MS) for highly sensitive element-specific detection has therefore become the standard method for inorganic Se speciation analysis.[14] While AEC-ICP-MS has the advantage of being able to measure these analytes directly, without prior derivatization steps, it is a complex instrumental arrangement not commonly available in testing laboratories. In an effort to make $SeCN^-$ analysis more accessible, we recently developed a simple aqueous phase derivatization of $SeCN^-$ to volatile ethyl-SeCN for analysis by gas chromatography mass spectrometry (GC-MS) with a detection limit of 0.1 ng g^{-1} Se;[15] though no other Se species were targeted in this study.

However, Se(IV) and Se(VI) can also be analyzed by GC after converting the ionic Se species to volatile analytes. In a derivatization method that was first introduced in the 1940's for use with UV/Vis spectrophotometry due to the yellow colour of the derivatized analytes, Se(IV) is selectively

reacted with an *o*-phenylenediamine under acidic conditions and gentle heating to produce a benzoselenadiazole. When this derivatization was first applied in conjunction with GC, it was typically used with electron capture detection (ECD), as halogenated and nitrated *o*-phenylenediamines could react with Se(IV) for trace-level GC-ECD determination.[16, 17] The reaction mechanism is dependent on the existence in solution of the singly-protonated form of the *o*-phenylenediamine and the undissociated form of the selenious acid (H_2SeO_3).[18] The optimal pH range is usually between 0 and 2.5; depending on the substitution around the diamine, the pK_{a1} value and optimal reaction pH can shift slightly.[17] 4-nitro-*o*-phenylenediamine has historically been the most popular *o*-phenylenediamine for benzoselenadiazole formation due to the relatively high sensitivity of the derivative in ECD (compared to other singly-substituted reagents).[17] Other popular alternatives have been halogenated (Br, Cl, F) and methylated *o*-phenylenediamines, as well as a variety of mono- and poly-substituted *o*-phenylenediamines which have been investigated previously.[17] To the best of our knowledge benzoselenadiazole formation with 3,5-(trifluoromethyl)-*o*-phenylenediamine and selenite has not been researched, though the diamine is commercially available.

Se(VI) is reduced to Se(IV) in high concentrations of HCl (i.e. 6 mol L^{-1}) under high temperature conditions. This reaction is common in fractionation methods of Se speciation which involve a preliminary reduction step.[14] The pK_{a2} value for 4-nitro-*o*-phenylenediamine is about -2.49,[17] so it was hypothesized that benzoselenadiazole formation with this diamine would still proceed in concentrated HCl. Here, we present a novel method for the speciation of three Se compounds, carried out via sequential derivatization and extraction through the ethylation of $SeCN^-$ followed by benzoselenadiazole formation using *o*-phenylenediamine derivitizing agents for the quantification of Se(IV) and Se(VI); these reactions are shown in Scheme 1. The target analyte matrix of this method

was mine waste waters characterized by high dissolved salt content, enriched with sulphate up to 3500 mg kg^{-1} and nitrate up to 800 mg kg^{-1} (Unpublished data).



Scheme 1: Reaction mechanisms for the derivatization of $SeCN^-$ to EtSeCN, Se(IV) to 46TFMB, and Se(IV) to 5NB. Here, the last of these reactions is based on the [Se(IV) + Se(VI)] fraction, where Se(VI) is reduced to Se(IV) for the reaction to proceed.

2. Experimental Section

2.1. Reagents and materials

Potassium selenocyanate (KSeCN, >99%), sodium selenate decahydrate ($Na_2SeO_4 \cdot 10H_2O$, 99.999%), sodium selenite (Na_2SeO_3, 99%), sodium bicarbonate ($NaHCO_3$, 99.7%), anhydrous sodium sulphate (Na_2SO_4, 99%), chloroform ($CHCl_3$, GC grade), hexane (C_6H_{14}, HPLC grade), acetonitrile (CH_3CN, HPLC grade), triethyloxonium tetrafluoroborate (Et_3OBF_4, 97%), 4-nitro-*o*-phenylenediamine (4NoP, 98%, CAS 99-56-9), and 3,5-bis(trifluoromethyl)-1,2-phenylenediamine (35TFMoP, CAS 367-65-7) were purchased from Sigma-Aldrich (Oakville, Ontario). Ultra-pure water was generated in-house with a Thermo Scientific GenPure UV xCAD plus system (18.2 MΩ cm at 25 °C). Hydrochloric acid (HCl) and nitric acid (HNO_3) were purified in-house by sub-boiling distillation. A solution of 1:1 w/w of Et_3OBF_4:CH_3CN was prepared by weighing out approximately

10 g of Et_3OBF_4 into a PTFE vessel and dissolving it in an equivalent mass of CH_3CN precooled to -20 °C. The Et_3OBF_4 solution was stored at -20°C where it was stable for over one month, and was hydrolyzed in water before disposal. 2000 µg g^{-1} solutions of 4NoP and 35TFMoP were prepared in 10% HCl and 10% HNO_3 respectively. 35TFMoP was sparingly soluble, so the solution was sonicated for 1h at 25 °C to assist in dissolution. Table 1 lists the reagents used during these experiments. All reusable glassware were cleaned by soaking for 24 h in a 10% HCl acid bath, then rinsed 3 times with both deionized water and ultra-pure water prior to use.

Several samples of treated wastewaters from Canadian gold mine sites were characterized in this study. After collection, these samples were stored in the dark in a cool environment (~4°C) without any additional treatment. Samples were analyzed for dissolved and total Se using ICP-MS. For AEC-ICP-MS analysis (Section 2.3.2), samples were diluted 2x in water and injected without further pretreatment. Samples were analyzed directly (undiluted) when following the sequential derivatization and extraction GC-MS protocol.

2.2. Sample preparation

2.2.1. Derivatization and extraction of $SeCN^-$

$SeCN^-$ derivatization roughly followed the method of Pagliano *et al.*,[15, 19] but was scaled up from a 1 mL sample size. 5 mL of sample was measured into a conical-bottom 15 mL glass centrifuge tube with a PTFE-faced screw cap. 50 mg of $NaHCO_3$ was added before dispensing a 200 µL aliquot of Et_3OBF_4:CH_3CN solution into the sample. The cap was secured tightly and the tube was shaken before allowing the reaction to proceed for 1 h at room temperature. The EtSeCN derivative was extracted using 500 µL of $CHCl_3$ and dried over 1 g of anhydrous Na_2SO_4 in a small glass sample vial.

2.2.2. Derivatization and extraction of Se(IV)

After EtSeCN extraction, the aqueous phase was divided into two aliquots by pipetting half of the sample into another glass centrifuge tube. To one aliquot 250 µL of 35TFMoP solution was added. The sample was capped and shaken to mix before being placed in a 75 °C water bath for 20 minutes. The sample was then removed from the bath and allowed to cool to room temperature before the Se(IV) derivative 4,6-bis(trifluoromethyl)-2,1,3-benzoselenadiazole (46TFMB) was extracted into 500 µL of $CHCl_3$. This extract was added into the vial containing the EtSeCN extract.

2.2.3. Derivatization and extraction of [Se(IV) + Se(VI)]

3 mL of concentrated HCl was added to the remaining aqueous sample aliquot, along with 250 µL of 4NoP solution. After mixing, the tube was placed in a 75 °C water bath for 1 h to simultaneously reduce Se(VI) to Se(IV) and yield the Se(IV) derivative 5-nitro-2,1,3-benzoselenadiazole (5NB). The sample was removed from the bath and was cooled to room temperature before extraction with 500 µL of $CHCl_3$. This extract was combined with the EtSeCN and 46TFMB extracts being dried over Na_2SO_4 then transferred to an autosampler vial for analysis by GC-MS.

2.2.4. $SeCN^-$ Stability Study

A composite wastewater sample was prepared by combining various volumes of the individual treated gold mine wastewaters. A portion of this was then spiked with a known amount of $SeCN^-$, homogenized, and then split into multiple aliquots in 15 mL HDPE bottles, adjusted as necessary, and stored in various environments, as follows. In the pH adjustment test, the pH of an aliquot was adjusted (using HCl or NaOH to within ± 0.1) to 2, 4, 6, 8, 10, or left untreated at pH 8.9 and stored in the dark at 4 °C. To determine the effect of temperature, samples were stored in the dark in the refrigerator (4 °C), at room temperature (21 °C), or in the oven (37 °C). The effect of light on $SeCN^-$ stability was examined by placing samples in an environmental chamber with a UV lamp, either

wrapped in aluminum foil or exposed to the light; the chamber could not be cooled to 4 °C so it was held at 20 °C. Finally, the effect of the headspace gas was tested by capping one bottle under nitrogen; it was stored in the dark at 4 °C. Aliquots from each bottle were collected after 1 day, 4, and 7 days, and were analyzed for $SeCN^-$ by GC-MS.

2.3. Instrumentation

2.3.1. GC-MS instrumentation

GC separation of the derivatives was performed using the Agilent Technologies 7890A GC System equipped with an Agilent J&W DB-1701 column (30 m x 0.250 mm inner diameter, 0.25 µm film thickness). 1 µL of extract was injected in pulsed splitless mode using a Gooseneck splitless liner (2 mm x 6.5 mm x 78.5 mm; RK20796, Restek Corporation, Bellefonte, Pennsylvania) at 235°C. The temperature program started with a 2 min hold at 40 °C, ramped to 250 °C at 20 °C/min, then held for a further 4.5 min. The eluting analytes were ionized in EI mode and detected by the Agilent Technologies 7000 Triple Quadrupole MS. EtSeCN was measured in Multiple Reaction Monitoring (MRM) mode (*m/z* 134.96>106.93 and 136.96>108.93, collision energy 5 eV). Single Ion Monitoring (SIM) was used for the detection of 46TFMB (at *m/z* 319.9 and 321.9) and 5NB (at *m/z* 228.9 and 230.9).

2.3.2. AEC-ICP-MS instrumentation

Reaction completion was monitored throughout the method development by analyzing the aqueous phase by AEC-ICP-MS after derivatization and extraction. The same AEC-ICP-MS method was also used to analyze the wastewater samples. Chromatographic separation of Se(IV), Se(VI), and $SeCN^-$ was based on the method of Wallschläger and Roehl[3] and was performed on a Thermo Scientific Dionex ICS5000+ High Performance Ion Chromatograph with a Thermo Scientific Dionex AS16 guard (4 x 50 mm) and analytical (4 x 250 mm) anion exchange column. The mobile phases

were deionized water (DIW) and 100 mmol L^{-1} NaOH, both containing 2% methanol to increase ICP-MS signal for Se, and followed the gradient: 17 mmol L^{-1} 0-4 min, 100 mmol L^{-1} 4-12 min, 17.5 mmol L^{-1} 12-15 min. The flow rate was constant at 1.5 mL/min. An injection volume of 500 µL was used, and a 4 mm AERS suppressor set to 250 mA was used to remove excess salt ions from the eluant. A Perkin Elmer Elan DRC II ICP-DRC-MS was used for detection, adding H_2 as cell gas at 3 mL/min. ^{78}Se, ^{80}Se, and ^{82}Se were monitored with a dwell time of 50 ms per isotope.

A second AEC-ICP-MS method was employed for the mine wastewater samples. This utilized a Hamilton PRP-X100 anion exchange column (Chromatographic Specialties, Brockville, Ontario) on an Agilent 1200 Series HPLC coupled to an Agilent 7900 ICP-MS (Agilent Technologies, Mississauga, Ontario). The ICP-MS was operated in collision reaction cell (CRC) mode, using hydrogen as a cell gas to eliminate polyatomic interferences on Se. Chromatographic conditions included a gradient elution at 1.0 mL/min of mobile phase A (ultra-pure water) and B (200 mmol L^{-1} ammonium acetate / 200 mmol L^{-1} acetic acid in water, adjusted to pH 8.5 with NH_4OH): 0.5% B for 3 minutes, step to 60% then ramp to 100% B over 7 minutes, hold at 100% B for 5 minutes, then re-equilibrate at 0.5% B for 5 minutes. The injection volume was 10 µL.

3. Results and discussion

3.1. Optimization of the $SeCN^-$ derivatization

The $SeCN^-$ derivatization procedure was adapted from that of Pagliano *et al.*[15] The sample volume was scaled up to ensure adequate quantities for the splitting of the sample involved in the second steps of the sequential derivatization, so some adjustments were made to the $SeCN^-$ method to account for this. After $CHCl_3$ extraction following the reaction with various volumes of 50% Et_3OBF_4 solution, the unreacted $SeCN^-$ in the aqueous phase was measured using AEC-ICP-DRC-MS. No detectable $SeCN^-$ remained in the aqueous phase following a one hour reaction with 200 µL

of the Et_3OBF_4 solution, as is displayed in Figure S1 in the Supplementary Information (SI). The reaction is buffered by $NaHCO_3$ to prevent the acidic ethylating agent from decreasing the pH below 4, at which point the $SeCN^-$ molecule degrades into elemental Se^0 and a cyanide ion.[7] The amount of $NaHCO_3$ was also optimized to maximize the GC-MS signal as it was observed that adding excess $NaHCO_3$ reduced the GC-MS signal. An optimal value of 50 mg (±10 mg) $NaHCO_3$ was chosen based on the results presented in Figure S2 in the SI. Measuring the pH after reaction completion resulted in a neutral (~7) pH.

3.2. Optimization of the Se(IV) and [Se(IV) + Se(VI)] derivatizations

The 2.5 mL aliquot of aqueous sample was treated with 250 µL of the 35TFMoP reagent. The reaction proceeded for 20 minutes at pH 2 and 75 °C. As noted in Figure 1, ICP-MS determination of the reaction progress (by measuring the Se remaining in the aqueous phase) found that more than 90% of the Se(IV) reacted practically immediately, and only 0.2% of the Se(IV) remained in the aqueous phase after 20 minutes at 75 °C, with negligible increases in reaction completion at reaction time > 20 minutes. This fast reaction time was not unexpected and similar results have been noted with the use of other *o*-phenylenediamines.[20-22]

To ensure that the Se(IV) fraction was not overestimated by the reduction of Se(VI) during derivatization and heating, the 35TFMoP reaction was tested on a Se(VI) standard solution. While a small 46TFMB signal was observed during GC-MS analysis, comparison to AEC-ICP-MS data showed that this was consistent with the small Se(IV) impurity present in the Se(VI) standard. Therefore, it was concluded that the presence of Se(VI) does not interfere with the accurate detection and quantitation of Se(IV). The use of dilute HNO_3 in the TFMoP solution (rather than HCl) was likely an important factor contributing to the stability of Se(VI) under the high temperature conditions experienced during the derivatization.

Derivatization of the Se(IV) + Se(VI) aliquot required the use of HCl (about 6.6 mol L^{-1}) to reduce all of the Se(VI) to Se(IV) to enable the reaction with 4NoP. It has previously been established that the pK_a range between the singly- and doubly-protonated forms of 4NoP was of the largest among the studied *o*-phenylenediamines,[17] so it was hypothesized that 4NoP could successfully react with Se(IV) in concentrated acid, and the need for separate reduction, dilution, and derivatization steps could be eliminated. Different reaction times and volumes of HCl were tested, and the results are presented in Table S1. Considering the most complete reaction, in the shortest time, while also minimizing dilution, the optimized conditions included the addition of 3 mL HCl (37%, concentrated stock) to the ~2.5 mL sample, followed by a 60 minute reaction with 4NoP at 75°C. Following extraction, it was noted that less than 0.2% of the initial Se remained in the aqueous phase, indicating near-complete reduction of Se(VI) to Se(IV) and subsequent conversion to 5NB.

3.3. GC-MS

An example GC-MS chromatogram is shown in Figure 2. The DB-1701 column was selected because it was observed to allow for separation between 46TFMB and the excess derivatizing agent, 35TFMoP, which was also extracted. This was particularly important during method development when full scan mass spectra were being collected, and also helped to ensure optimal peak shapes for the analyte. The signal-to-noise ratios and peak shapes for each analyte in both select ion monitoring (SIM) and multiple reaction monitoring (MRM) modes were compared. For EtSeCN, MRM gave a more symmetrical peak shape compared to SIM, and also provided favourable signal-to-noise ratios. To the contrary, SIM mode offered better limits of detection for the benzimidazole derivatives, likely because of low noise levels in this higher molecular mass range, noted in the standard solutions. Following this method, the limits of detection (LOD), defined as 3 times the signal to noise ratio (S/N = 3), were 0.35, 0.56, and 1.67 µg L^{-1} Se (in the original aqueous sample), for $SeCN^-$, Se(IV), and Se(VI), respectively.

The EI mass spectra for 46TFMB and 5NB are provided in Figure 3; our previous work[15] reported the mass spectrum for EtSeCN. Figure 3 provides the first report of the mass spectrum of 46TFMB – the molecular ion of *m/z* 320 is also the base peak, and fragments *m/z* 301 $[M-F]^+$ and 251 $[M-CF_3]^+$ are consistent with the identity of this novel derivative of Se(IV). Due to the nature of the fluorinated substituents of the 35TFMoP, the derivative produced has a distinct advantage over those generated from more commonly used *o*-phenylenediamines – namely, it can be detected with much higher sensitivity. Here, we demonstrate an approximately 35-fold increase in sensitivity, measured as peak area, over 5NB (for equivalent Se concentration). Additionally, it is likely that the use of 35TFMoP will be particularly beneficial when using ECD, which is highly sensitive to halogens.[17]

3.4. Robustness Test

The method was designed for conditions present in a mine waste water (pH 4.5 to 9.0, solids up to 6000 mg kg^{-1}, and high levels of sulphate, chloride, and nitrate), so particular attention was paid to concomitant interferences. The robustness of the method was determined by spiking standard solutions with salts to mimic wastewater profiles. It was observed that the analytical protocol could tolerate chloride and sulphate concentrations up to 3% w/v without any loss of reaction or extraction efficiently. Nitrate concentrations up to 3% had no effect on SeCN or Se(IV) measurements, but did significantly reduce analytical performance for the derivative (Se(VI)) – at 3% nitrate, no 5NB signal was observed. Pagliano *et al.*[15] previously demonstrated this robustness for the $SeCN^-$ portion of the method (for SO_4^{2-} and Cl^-), and additional results can be found in Figure S3 of the SI.

While high concentrations of sulphate and chloride are common in Se-contaminated waters, nitrate is often not present in such large quantities. Se-contaminated natural waters typically contain NO_3^- in concentrations less than 5 ppm.[7, 23] Agricultural drainage waters – specifically those in the San Joaquin Valley, California, which are infamous for their historical Se-contamination – tend to contain more NO_3^-, averaging around 47 ppm.[24] Conversely, nitrogen in gold mine wastewater is

usually present as ammonia, but can be oxidized during initial treatment steps to result in up to 200 ppm N as NO_3^-.[25, 26]

Testing the effect of smaller amounts of nitrate on the derivatization and extraction demonstrated that there is a minor suppression in the 5NB signal for concentrations above 10 ppm NO_3^- (Figure 4). For many types of samples of interest, this interference could be corrected by a simple dilution – elevated concentrations of NO_3^- and Se tend to occur simultaneously,[27] so this would not typically result in detection limit issues. However, this interference with NO_3^- does highlight the benefit of advanced quantification strategies such as standard addition or isotope dilution analysis.[28, 29]

Another important factor to consider during this analysis is the fact that natural and industrial waters might contain organic Se species.[12, 30] Organic Se species will not directly react with the *o*-phenylenediamine reagents, and typical selective sequential analysis of the operationally-defined "organic Se" fraction in a water sample involves the use of UV irradiation for photo-oxidation to Se(VI) before subsequent reduction to Se(IV).[12] However, it has been observed that under certain conditions, some organic Se species can decompose following heating in HCl,[31] which could cause potential over-estimation of Se(IV) and/or Se(VI). Standard solutions (50 µg L^{-1} Se in water) of each selenomethionine, Se-methylselenocysteine, and selenocystine were subjected to this sequential derivatization and extraction protocol and no false-positive signals were observed, as noted in Figure S4.

3.5. Analysis of Mine Water Samples

In addition to testing synthetic samples, real wastewater samples were examined. These samples were wastewaters collected from gold mining operations and contained Se in a range of concentrations. Initially, Se speciation analysis was conducted by two AEC-ICP-MS methods (one described by Wallschläger and Roehl[3], and the other method commonly applied to Se speciation in

mine waters), as these is known to be a fairly robust method for the analysis for industrial waters.Applying the sequential derivatization and GC-MS method described here, including spiking with isotopically-labelled internal standards for quantification by isotope dilution mass spectrometry (IDMS, see section 3.7), arrived at results in agreement with those determined by AEC-ICP-MS, as noted in Table 2.

In some cases there were small discrepencies between the results of the different analysis methods, likely due to the presence of interfering ions. For example, Sample B, which shows the largest discrepancy between Se(VI) concentrations measured by AEC-ICP-MS and GC-MS, contains about 820 ppm nitrate, while Sample C, where Se(VI) values are in better agreement, contains only 48 ppm. As noted in Table 2, the sum of species did not always match the measured total Se content; this is most apparent for Sample D. This will be due to the presence of Se in a form that is not detected by either of the two analysis methods – elemental Se, for example.

Spike recovery tests were also conducted for these wastewater samples. Known amounts of Se(IV), Se(VI) and $SeCN^-$, individually and combined, were added to aliquots of the wastewaters prior to any sample preparation steps. The whole sequential derivatization and extraction procedure was carried out and recoveries were determined. When employing isotope dilution analysis (see section 3.7), recoveries were high for all analytes, as noted in Table 3, though lowest for Se(VI), potentially due to nitrate inferference in the samples, as discussed in above and in Section 3.4. Additionally, since concentrations were well above the detection limit, matrix effects could be reduced by dilution of the samples prior to analysis.

3.6.$SeCN^-$ Stability

When the treated gold mine wastewater samples were analyzed for Se speciation as discussed in Section 3.5, only Se(IV) and Se(VI) were detected. The observed absence of $SeCN^-$ was unexpected

based on the known use of cyanide in the mine's processing. Since the samples had been collected several months prior to this analysis, we investigated to determine if the preservation conditions of the samples was a potential cause of the loss of $SeCN^-$.

A stability study was performed on a composite wastewater sample by spiking it with $SeCN^-$ and subjecting aliquots to different environmental conditions to investigate various factors causing degradation of $SeCN^-$. It was noted that exposure to UV irradiation and limiting oxygen in the sample's headspace had negligible effects on the stability of $SeCN^-$ in these samples over the one week period studied. Conversely, significant differences were noted in samples with varied pH, and which were stored under various temperature conditions. Degradation of $SeCN^-$ was much faster at higher temperatures. Additionally, when held at 4°C, $SeCN^-$ was noted to degrade more quickly in samples with lower pH. Both of these scenarios are outlined in Figure 5. Based on these results, it can be concluded that for the best determination of $SeCN^-$, samples should be kept in alkaline condition upon collection, kept cool, and analyzed as soon after collection as possible. These conditions may require the collection of additional aliquots specifically for Se speciation analysis as increasing the pH of samples with high dissolved metal content (i.e. Ca, Mg) can promote the precipitation of the metal hydroxides. Additionally, spiking samples in the field (with a natural, or ideally an isotopically labelled standard) will also help to ensure that any degradation can be taken into account.

3.7. Use of IDMS for Quantitation

Like any GC-MS method, the procedure described herein will greatly benefit from the use of an internal standard, ideally an isotopically-labelled form of the analyte such that more accurate and precise quantification strategies may be employed.[28, 29] A $Se^{13}C^{15}N^-$ standard is commercially available, as is metallic Se – the latter can be gently dissolved in HNO_3 to generate Se(IV), or undergo further heating for oxidation to Se(VI). We tested this method using a more streamlined

approach to isotope dilution, where $Se^{13}C^{15}N^-$ and $^{82}Se(IV)$ were spiked into the sample prior to any manipulation and were used for quantification of all three fractions. Since it had been rigorously tested and confirmed that heating the sample in 6.6 mol L^{-1} HCl was sufficient to convert all Se(VI) to Se(IV) this approach was successful for the quantification of the [Se(VI) + Se(IV)] fraction. It is important to note, however, that if using a single labelled-Se(IV) spike for both Se(IV) and [Se(IV) + Se(VI)] fractions, it is vital to test the efficiency of the Se(VI) to Se(IV) reduction in the specific matrix being investigated as certain co-existing components can interfere with this reaction.[32] Conversely, one could prepare a Se(VI) spike from a different isotope, allowing for a full ID analysis.

The IDMS approach was noted to significantly improve data quality. For example, in a spike recovery test on a mine water sample, quantification by external calibration resulted in a 37% recovery of Se(VI), but when this same experiment was performed using IDMS for quantitation, the recovery was 105%. This clearly demonstrated the ability of IDMS to account for matrix interference.

Additionally stable isotope enriched Se spikes could potentially deployed at the field sampling stage to compensate and correct species loss / conversion.

4. Conclusions

Herein, we presented a method for the simultaneous GC-MS analysis of Se(IV), Se(VI), and $SeCN^-$ in aqueous samples following a sequential derivatization and extraction protocol. This robust method provides detection limits similar to those achieved by AEC-ICP-MS but uses analytical equipment that is significantly more cost effective and available to laboratories wishing to conduct routine analysis of Se speciation in contaminated water samples. The performance of the method described is not affected by sulphate and chloride in concentrations up to 3%. Nitrate does interfere with Se(VI)

measurements when present above 10 ppm, though concentrations up to 3% do not impact results for Se(IV) or $SeCN^-$. Upon examination of the stability of $SeCN^-$ in a sample of gold mine wastewater, we determined that although storage in cold alkaline conditions provide the best recovery, field spiking is probably necessary to ensure accurate $SeCN^-$ determination.

Acknowledgements

Author M. Bordash would like to thank the faculty of Trent University's M.ICA graduate program and NRC's ICM group for the opportunity to participate in the summer internship program.

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Table 1: Reagents used during sample preparation for GC-MS

Acronym	Full Name	Purpose / Notes
Et_3OBF_4	triethyloxonium tetrafluoroborate	$SeCN^-$ derivatizing agent
35TFMoP	3,5-bis(trifluoromethyl)-1,2-phenylenediamine	Se(IV) derivatizing agent
4NoP	4-nitro-*o*-phenylenediamine	[Se(IV) + Se(VI)] derivatizing agent
EtSeCN	ethyl selenocyanate	Derivatized $SeCN^-$
46TFMB	4,6-bis(trifluoromethyl)-2,1,3-benzoselenadiazole	Derivatized Se(IV)
5NB	5-nitro-2,1,3-benzoselenadiazole	Derivatized [Se(IV) + Se(VI)]

Table 2: Concentrations of Se(IV), Se(VI) in mine water samples as determined using various analysis methods; Total Se is given for each sample and sum of species[(a)] is compared to this value as a % recovery by each method, shown in brackets. Quantification approach is noted for each method as IDMS for isotope dilution or Ext. Cal. for external calibration.

Sample ID	Total Dissolved Se (µg L^{-1})	Measurand	Sequential Derivatization / Extraction GC-MS [IDMS]	AEC-ICP-MS[(b)] [Ext. Cal.]
A	163 ± 2	Se(IV)	75.5	95.3
		Se(VI)	68.6	83.7
		Sum (% of TSe)	144 (88%)	178 (110%)
B	94.6 ± 2.6	Se(IV)	6.10	4.9
		Se(VI)	67.0	86.3
		Sum (% of TSe)	73.1 (77%)	91.2 (96%)
C	36.7 ± 0.9	Se(IV)	2.91	1.40
		Se(VI)	32.1	36.0
		Sum (% of TSe)	35.0 (95%)	37.4 (102%)

D	70.8 ± 21.5	Se(IV)	13.8	17.2
		Se(VI)	30.6	29.7
		Sum (% of TSe)	44.4 (63%)	46.9 (66%)
E	5.39 ± 0.41	Se(IV)	3.71	2.37
		Se(VI)	4.31	2.83
		Sum (% of TSe)	8.02 (149%)	5.20 (96%)

NOTE: SeCN was below the detection limit for both all methods.
(a) Sum of species includes unidentified compound(s) and recovery is determined by dividing this value by the total Se; (b) Data is an average of results from the two AEC-ICP-MS methods

Table 3: Spike recoveries for each analyte spiked individually (~ 50 µg Se L^{-1}) and combined (each ~ 30 µg Se L^{-1}) into mine water samples, following the sequential derivatization and extraction GC-MS method, Quantification by IDMS

	% Recovery		
	SeCN	Se(IV)	Se(VI)
SeCN Spike	130	-	-
Se(IV) Spike	-	128	-
Se(VI) Spike	-	-	105
SeCN + Se(IV) + Se(VI) Spike	136	133	93

Figure 1: Efficiency of the reaction between Se(IV) and 35TFMoP as a function of reaction time

Figure 2: Total ion chromatogram of a standard mixture of 200 µg Se L^{-1} each as $SeCN^-$, Se(IV), and Se(VI) following sequential derivatization and extraction into $CHCl_3$. GC-MS parameters are summarized in Section 2.3.1.

Figure 3: EI mass spectrum of 4,6-bis(trifluoromethyl)-2,1,3-benzoselenadiazole (top) and 5-nitro-2,1,3-benzoselenadiazole (bottom)

Figure 4: The effect of NO_3^- on the GC-MS signal for derivatives of $SeCN^-$, Se(IV), and Se(VI) following the sequential method described herein; 100% represents the signal for samples without any added ions. The black rectangle in (a) is expanded into (b).

Figure 5: $SeCN^-$ remaining in solution after spending time under various (a) pH and (b) temperature conditions

Scheme 1: Reaction mechanisms for the derivatization of $SeCN^-$ to EtSeCN, Se(IV) to 46TFMB, and Se(IV) to 5NB. Here, the last of these reactions is based on the [Se(IV) + Se(VI)] fraction, where Se(VI) is reduced to Se(IV) for the reaction to proceed.

Declaration of competing interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Credit Author Statement

Mitchell G. Bordash: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Roles/Writing - original draft

Enea Pagliano: Conceptualization; Data curation; Methodology; Resources; Software; Supervision; Validation; Visualization; Writing - review & editing.

Kelly L. LeBlanc: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing - review & editing.

Paramee Kumkrong: Methodology; Resources; Writing - review & editing.

Dirk Wallschläger: Supervision; Writing - review & editing.

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Graphical abstract

Highlights:

- Selenocyanate derivatized to ethylselenocyanate
- Selenite derivatized to 4,6-bis(trifluoromethyl)-2,1,3-benzoselenadiazole
- Selenate reduced to selenite and derivatized to 5-nitro-2,1,3-benzoselenadiazole
- Three extractions combined for simultaneous GC-MS analysis
- GC-MS method applied to analysis of selenium species in mine wastewater