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Sensitive analysis of selenium speciation in natural seawater by isotope-dilution and large volume injection using PTV-GC-ICP-MS

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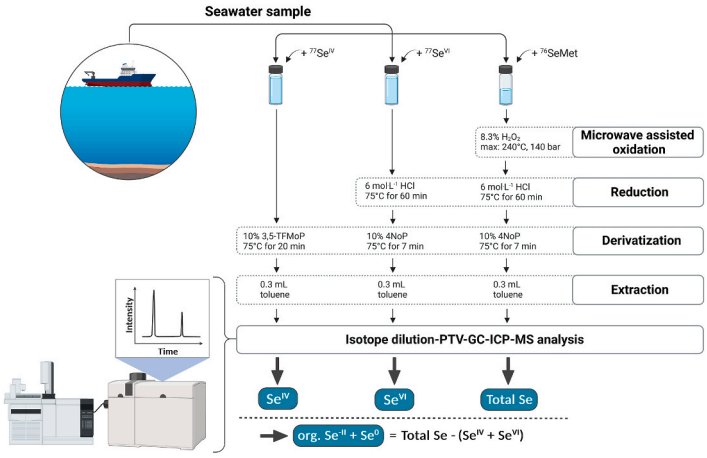
HIGHLIGHTS

- Determination of Se chemical fractions (SeIV, SeVI, reduced and total Se) in seawater.
- Direct SeIV derivatization with 3,5-TFMoP and extraction in toluene.
- Microwave oxidation of total and reduced Se using 8.3% v/v hydrogen peroxide.
- Reduction of SeVI to SeIV using 6 M HCl followed by derivatization with 4-NoP.
- Accuracy and sensitivity through isotope dilution combined with LVI-GC-ICP-MS.

GRAPHICAL ABSTRACT



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ABSTRACT

Although oceans play a key role in the global selenium (Se) cycle, there is currently very little quantitative information available on the distribution of Se concentrations and Se speciation in marine environments. In general, determining Se concentration and speciation in seawater is highly challenging due to very low Se levels ((sub)ng·L^{-1}), whereas matrix elements interfering Se pre-concentration and detection are up to the g·L^{-1} levels. In this study, we established a sensitive method for the determination of the various Se chemical fractions present in natural seawater, i.e. selenite (Se^{IV}), selenate (Se^{VI}), organic Se^{-II} + Se^{0} and total Se, using species-specific isotope dilution gas chromatography coupled to inductively coupled plasma mass spectrometry (ID-GC-ICP-MS). We compared different derivatization reagents and optimized specific pre-treatment protocols, including a

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microwave assisted oxidation protocol for the determination of total Se and organic Se^{-II} + Se^0 using H_2O_2. To increase sensitivity, we developed an online pre-concentration method based on large volume injection (LVI) using a programmed temperature vaporization (PTV) inlet. Eventually, the developed method achieved low absolute and methodological detection limits, i.e., respectively, 0.1–0.3 pg and 0.9–3.1 ng.L^{-1} for the different fractions. The accuracy of our method was of 2% for a certified reference material (CRM) diluted in artificial seawater while the precision was better than 4% for a freshwater CRM in artificial seawater matrix as well as two common seawater CRMs certified for trace elements excluding Se. As a proof-of-concept, we quantified the various Se fractions in a large number of natural water samples from the Baltic and North Seas, encompassing a wide range of salinity (7–35 psu), which shows that its detection limits are sufficient to determine total Se, Se^{IV}, Se^{VI} and organic Se^{-II} + Se^0 concentrations in brackish and marine systems.

1. Introduction

Selenium (Se) is an essential trace element for many organisms [1,2], including humans as well as other terrestrial and aquatic organisms, with many marine algae in culture presenting obligate Se requirements or strongly benefiting from acquiring it [3,4]. Furthermore, marine environments play a key role in the global Se cycle by supplying substantial amounts of Se to the atmosphere via volatilization, which eventually will feed terrestrial ecosystems [5–8]. However, there is currently little quantitative information available on the distribution of Se concentrations and speciation in marine environments. Se can exist under different oxidation states, including the most oxidized, inorganic Se species, selenate ($Se^{VI}O_4^{2-}$), which is the thermodynamically favoured species in oxic seawaters [9]. The presence of inorganic selenite ($Se^{IV}O_3^{2-}$), reduced organic selenides (Se^{-II}) and/or elemental selenium (Se^0) may be attributed to different abiotic and/or biotic transformation processes [9–13]. According to present understanding, Se follows major nutrient profiles in marine systems, i.e., dissolved Se is depleted at the surface due to uptake by marine bacteria and/or phytoplankton and is then regenerated with depth [9,13]. However, due to a general lack of environmental data, Se cycling in marine environments is largely unknown. Particularly, information on the distribution of Se and its species in marine waters may offer insights on Se pathways in these environmental systems, such as biological uptake, volatilization and immobilisation in sediments.

Quantifying Se and its species in seawater is highly challenging. Se concentration levels are very low, in the (sub)ng·L^{-1} ranges, whereas matrix elements interfering with its pre-concentration and separation (e.g., sulfur and chlorine) and/or with its detection (e.g., bromine with inductively coupled plasma mass spectrometry; ICP-MS; [14,15]) are highly concentrated, i.e., in the mg·L^{-1} up to g·L^{-1} ranges [16]. In the last few years, the method of choice for Se speciation at low ng·L^{-1} levels in water samples has been liquid chromatography (LC) coupled to ICP-MS (e.g., Refs. [17–19]). However, to minimize interferences, seawater analysis by LC-ICP-MS, requires substantial dilution [19] to ensure chromatographic separation (i.e., complete loss of retention is observed in undiluted seawater due to severe ion competition). Due to the high salt matrix, conventional pre-concentration methods such as lyophilisation and evaporation are also not feasible. Pre-concentration of ions and hydrophilic species by solid phase extraction (SPE) faces the same challenge of strong ionic competition as described above for LC-ICP-MS. Recently, Chang et al. [15] reported a SPE method for Se pre-concentration in a seawater matrix, which requires highly acidic conditions to elute Se from the cartridge and thus again implies large dilution (~100 times) before analysis by (LC-)ICP-MS. Moreover, removal of matrix elements such as Br (~60–150 mg·L^{-1}) in seawater with SPE is highly challenging. With their SPE method, Chang et al. [15] could remove 98% of Br. However, when considering natural Se levels (~100 ng·L^{-1}), the 2% remaining Br still account for concentrations that are 4 orders of magnitude higher than Se concentrations, and Se analysis by ICP-MS thus remains very challenging.

Most available data on Se in natural seawater has been obtained by Se separation from the matrix through selective conversion of Se^{IV} to Se hydride (hydride generation, HG) in combination with atomic absorption spectrometry (AAS; e.g., Refs. [9–12,20]), atomic fluorescence spectrometry (AFS; e.g., Refs. [7,21–23]) or high-resolution sector-field-ICP-MS [24,25]. Apart from HG based techniques, Measures and Burton [13,26] used gas chromatography (GC) coupled to electron capture detection (ECD) after selective derivatization of Se^{IV} into a volatile piazselenol compound. As the formation of both Se hydride and derivatization to volatile Se compounds are selective for Se^{IV}, the determination of different chemical Se fractions requires specific pre-treatments (oxidation and/or reduction) to convert Se^{VI} as well as reduced Se^{-II} and elemental selenium (Se^0) into Se^{IV} [9–12]. Although these methods can be sensitive, with reported detection limits for Se^{IV} ranging between 0.2 and 34 ng·L^{-1} ([9–12,20,24,25]), the relatively large sample volumes (up to 1 L) required, substantially limit the number of samples that can be collected during oceanographic cruises and analysed. Furthermore, AAS and AFS are mono-elemental techniques that are less common in research laboratories nowadays. On the other hand, GC in combination with ICP-MS is a highly sensitive, multi-elemental and isotopic technique that has been used for diverse applications, such as the speciation analysis of tin, lead and mercury species in seawater at ultra-trace levels (pg·L^{-1} to sub-ng·L^{-1}, e.g., Refs. [27–30]). Furthermore, GC-ICP-MS represents a more effective sample introduction technique (due to dry plasma conditions) with higher separation efficiencies (i.e., peak sharpness and resolution) compared to LC-based methods [31,32]. Respective detection limits can be further improved by (on-line) pre-concentration methods, including e.g., dynamic headspace in-tube extraction [27] or large-volume injection using a programmed temperature vaporization (PTV) inlet [28,30]. It can also be combined with species-specific isotope dilution (ID) allowing for higher accuracy and precision compared to other calibration techniques as well as the correction of potential losses during sample preparation (e.g., correction of non-quantitative derivatization) and/or other matrix effects [32].

Apart from the GC-ECD method of Measures and Burton [26], other studies have developed methods based on GC coupled to electron impact mass spectrometry (MS) to quantify Se in different water samples. These methods applied various derivatization reagents and protocols to form volatile alkylselenides using sodium tetraethylborate [33–35] or sodium tetrapropylborate, and volatile piazselenol using 4-Chlor-1,2-phenylendiamin [36,37], 4,5-Dichlor-o-phenylendiamin [35,36], 4-Nitro-o-phenylenediamine [13,26,38,39] and 3,5-Bis(trifluoromethyl)-1,2-phenylenediamine [38]. Among these studies, only Gómez-Ariza et al. [36] and Campillo et al. [35] compared the efficiency of derivatization with four of the reagents listed above as well as the extractability of the Se derivatives and potential matrix effects, however their methods were specifically optimized for GC-MS analysis after solid phase (micro) extraction. So far, there is not systematic comparison of different derivatization reagents, extraction of formed derivatives and matrix effects for seawater samples. Similarly, various procedures have been employed to convert Se^{VI} and reduced Se species into Se^{IV} in order to quantify these chemical Se fractions. For example, sample oxidation to determine total Se concentrations has been done using specific reagents (e.g., potassium persulfate [9,40], sodium borate under UV-irradiation [13,26]) or microwave assisted oxidation with nitric acid (HNO_3) and/or hydrogen peroxide (H_2O_2) [41,42]. Moreover, most

previous methods were developed and tested on a small number of contaminated and/or spiked (sea-)waters (up to 270 μg(Se)·L^{-1}), and were thus not specifically designed to address environmentally relevant concentrations of Se in seawater.

Here, we developed a method based on programmed temperature vaporization (PTV)-GC-ICP-MS and species-specific isotope dilution, which enables a sensitive and precise quantification of total Se as well as Se chemical fractions (i.e., Se^{IV}, Se^{VI}, and organic $Se^{-II} + Se^{0}$) in brackish water and seawater using very low sample volumes, i.e., 3 mL for total Se and 16 mL for Se chemical fractions. We tested 7 different derivatization reagents and compared their suitability in terms of derivatization efficiency and formation of spectral interferences when applied to seawater as well as to the complex matrix resulting from pre-treatments required to determine Se^{VI} and total Se. We also optimized a new protocol of microwave-assisted oxidation for the determination of total Se and organic $Se^{-II} + Se^{0}$ in seawater. To enable the simultaneous analysis of the extracts of two Se chemical fractions (i.e., Se^{IV} and Se^{VI}) at trace levels, we optimized the operating conditions for large volume injections using a PTV inlet as well as the GC temperature program. Finally, we demonstrated the performance of the developed analytical workflow in terms of detection limits, accuracy and precision (using certified reference materials), as well as its applicability by investigating the distribution of Se concentrations and speciation in 168 seawater samples collected in the Baltic and North Seas.

2. Materials and methods

2.1. Overview of the analytical workflow

Fig. 1 shows the analytical workflow used to determine the concentrations of total Se and its various chemical fractions in seawater. The performed optimization and/or used operating conditions for the different steps of this workflow are described thereafter. Overall, the workflow consists of spiking isotopically enriched Se species ($^{77}Se^{IV}$, $^{77}Se^{VI}$, ^{76}SeMet) to seawater samples, followed by specific pre-treatments (to determine different chemical Se fractions in three sub-samples), derivatization of Se, organic solvent extraction, which entails pre-concentration of derivatized Se compounds, and finally analysis by GC-ICP-MS. Specific sample pre-treatments are used to quantify Se^{VI} and total Se because only Se^{IV} can be derivatized and thus be detected by GC-ICP-MS. A reduction of the sample is used to determine Se^{VI} concentration, and an oxidation followed by a reduction of the sample are used to determine total Se concentrations. The organic $Se^{-II} + Se^{0}$ fraction is calculated as the difference between total Se concentration and the sum of Se^{IV} and Se^{VI} concentrations.

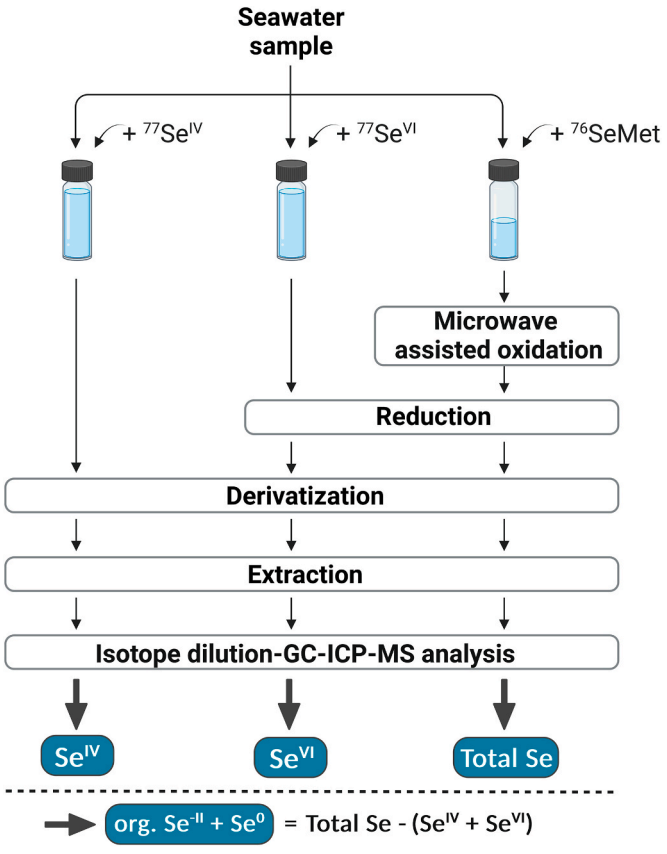

Fig. 1. Analytical workflow used to determine the concentrations of total Se and of Se chemical fractions (shown in cyan boxes) in seawater. Created using BioRender.com.

All materials and vials used for sampling, standards and sample preparation were acid-cleaned. Digestion tubes and glass vials used for derivatization were cleaned in successive acid baths with 10% nitric acid (HNO_3, 65%, Suprapur®, Merck) and 10% hydrochloric acid (HCl, 35%, ROTIPURAN®supra, Roth) for 24 h each and rinsed with ultrapure water.

2.2. Seawater pre-treatments (oxidation and reduction to Se^{IV})

The determination of total Se and organic $Se^{-II} + Se^{0}$ (Fig. 1) requires oxidative pre-treatment of seawater, for which we tested different oxidizing reagents using a microwave oven (UltraClave IV, MLS). Different mixtures of HNO_3 (65%, Suprapur®, Merck) and H_2O_2 (30%, for ultra-trace analysis, Sigma-Aldrich), ranging from 1.3 to 25% and 8.3–25% (compared to total digestion volume), respectively, and with ratios of 0.1–3.0 (HNO_3:H_2O_2) were tested. Besides HNO_3–H_2O_2 mixtures, we tested single application of H_2O_2, which has been shown to be sufficient to extract (trace) elements in carbohydrate-rich foods [43,44]. Single application of H_2O_2 were tested with H_2O_2 ranging from 8.3 to 50% (compared to total digestion volume). The microwave oven program included a first temperature increase to 160 °C (15 min ramp) followed by a final increase to 240 °C (9 min ramp, temperature held for 10 min). To evaluate the efficiency of tested microwave assisted Se oxidation procedures, we used (i) single solutions of selenite (Se^{IV}, 99% Sigma-Aldrich), as well as (ii) mixed standard solutions containing the isotopically enriched Se species selenomethionine (^{76}SeMet), selenite ($^{78}Se^{IV}$) and selenate ($^{77}Se^{VI}$), each prepared at 50 μg·L^{-1} in artificial seawater (always prepared according to the protocol of Luxem et al. [45] without nutrient enrichment solutions, unless stated otherwise). The isotopically enriched standards of elemental Se ($^{77}Se^{0}$, $^{78}Se^{0}$) and SeMet (^{76}SeMet) were purchased from Isoflex USA and LGC, respectively. Isotopically enriched standards of $^{78}Se^{IV}$, $^{77}Se^{IV}$ and $^{77}Se^{VI}$ were prepared at 1000 mg·L^{-1} from elemental Se following the protocol of Dael et al. [46]. Once produced, the $^{78}Se^{IV}$, $^{77}Se^{IV}$ and $^{77}Se^{VI}$ standards were characterized in terms of isotopic composition (by ICP-MS), concentration (by reverse isotope dilution combined with ICP-MS analysis), and speciation (by HPLC-ICP-MS described in the following), and stored at 4 °C. We analysed single and mixed standard solutions before and after microwave assisted oxidation using high pressure liquid chromatography (Agilent 1260 Infinity II Bio-inert HPLC System) coupled to an Agilent 8900 ICP-MS/MS in order to verify the completeness of Se oxidation into Se^{VI} under the different tested conditions. Standard solutions prepared in artificial seawater with concentrations of 50 μg·L^{-1} were diluted by a factor of 100 to avoid column overloading.

Chromatographic separation of Se^{IV}, Se^{VI} and SeMet was done by adapting the method published by Tolu et al. [17]. The optimized chromatographic method includes separation by anion exchange using a PRPX-100 column (Hamilton, 2.1 × 150 mm, 5 μm) equipped with an in-line filter (Titanium Frit 0.5 μm, 10–32 Waters type, BGB). Se separation was done by gradient elution with ammonium citrate (5.2–13 mmol·L^{-1}, 2% MeOH, pH 5.2) delivered at 0.5 mL ·min^{-1} using an injection volume of 20 μL. The ICP-MS/MS operated in MS/MS mode with

H_2 as a reaction gas (5 mL min^{-1}) and an acquisition time of 100 ms for selected Se isotopes: (*m/z* 76, 77, 78, 80 and 82). To check for potential interferences, Br was monitored during all Se analyses (acquisition time: 50 ms, *m/z* 79 → 79, 81 → 81).

For the reductive pre-treatment of Se^{VI} into Se^{IV}, which is needed to determine concentrations of Se^{VI} as well as total Se/organic Se^{-II} + Se^0 (Fig. 1), the protocol developed by Bordash et al. [38] for Se^{VI} concentrations in mining wastewater was tested. This involved adding HCl to the seawater to reach 6 mol·L^{-1} HCl and heating this mixture for 60 min at 75 °C. Since the sample aliquot that is used to determine Se^{VI} potentially contains Se^{IV}, we tested the following treatments (1) quantitative reduction of Se^{VI} into Se^{IV}, and (2) stability of Se^{IV} throughout the reduction process (i.e. no further reduction) to ensure correct quantification. These two treatments were tested in solutions prepared in ultrapure water and artificial seawater. Finally, after quantification of the Se^{VI} + Se^{IV} fraction, Se^{VI} is calculated as the difference between Se^{VI} + Se^{IV} and Se^{IV} concentrations.

2.3. Derivatization of Se^{IV} and extraction of Se compound derivatives

Different reagents were tested for the derivatization of Se^{IV}, included three tetraalkylborate compounds: sodium tetrapropylborate ($NaBPr_4$, ≥85.0%), sodium tetraethylborate ($NaBEt_4$, 97%), tetrabutylammonium-tetrabutylborat ($Bu_4NH_4BBu_4$, 97%); as well as four different phenylendiamine compounds: 4-Chlor-1,2-phenylendiamin (4CloP, 97%), 4,5-Dichlor-o-phenylendiamin (4,5-CloP, 97%), 3,5-Bis(trifluoromethyl)-1,2-phenylenediamine (3,5-TFMoP), and 4-Nitro-o-phenylenediamine (4NoP, 98%). All tested derivatization reagents were purchased from Sigma-Aldrich, and their chemical structures are shown in Fig. S1. $NaBPr_4$ and $NaBEt_4$ were prepared as 5% (w/w) solutions as commonly used for speciation analysis of mercury in seawater [47] and organo-tin in sediments [48]. $Bu_4NH_4BBu_4$ was prepared as a 0.2% (w/w) solution in 1% methanol (≥99.9%, VWR) as previously described for organo-lead species [49]. 4CloP and 4,5-CloP were prepared as 1% (w/w) solutions in 0.1 mol·L^{-1} HCl, with the addition of ethanol (99.8%, VWR) for 4,5-CloP according to the protocols by Gomez-Ariza et al. [36] and Campillo et al. [35], respectively. According to the protocol of Bordash et al., which was previously applied to determination of Se^{IV} and Se^{VI} in mining wastewater, 0.5% and 0.2% (w/w) solutions of 4NoP and 3,5-TFMoP were prepared in 10% HCl and 10% HNO_3, respectively [38]. To further increase solubility of 3,5-TFMoP, 25% ethanol was added (adapted from Ref. [38]) before sonication for approximately 1 h at 20–30 °C. The derivatization reaction of phenylendiamine compounds required heating at 70–75 °C with varying reaction times: 7 min for 4CloP and 4NoP [36,38], 10 min for 4,5-CloP [35], and 20 min for 3,5-TFMoP [38].

Different derivatization parameters, including pH, choice of extraction solvent as well as reagent concentration relative to sample volume, were optimized with 1 µg·L^{-1} Se^{IV} standard solutions prepared in ultrapure water (18.2 mΩ·cm, Milli-Q® Advantage A10) and artificial seawater. Potential effects of the highly acidic matrix necessary for reduction and seawater matrix were tested with 1 µg·L^{-1} standard solutions of Se^{IV} and Se^{VI} prepared in ultrapure water and artificial seawater. Buffer solutions were prepared with sodium acetate trihydrate (≥99.5%, Merck) and acetic acid (≥99.7%, Sigma-Aldrich). Further pH adjustments were done with HCl. The amount of derivatization reagent necessary to carry out the derivatization step was studied by adding different volumes ranging between 25 µL and 500 µL of different derivatization reagents to 1 mL buffered standard solutions, corresponding to a ratio of reagent to sample of $V_{reagent}/V_{sample}$ = 2.5–50%.

For the extraction of the different Se compound derivatives in seawater, we tested isooctane, hexane and toluene, which have been previously used to extract Se compounds produced from Se^{IV} derivatization with $NaBPr_4$ and $NaBEt_4$ [47,48] or with 4NoP and 4CloP [26,36]. The conducted tests aimed to find one suitable organic solvent for all different tested derivatization reagents in order to combine specific extracts of different Se chemical fractions for their simultaneous analysis by GC-ICP-MS and thus drastically reduce measurement time for a seawater sample. After the addition of organic solvent, suspensions were shaken for at least 10 min to ensure sufficient mixing.

2.4. GC-ICP-MS analysis

Derivatized Se compounds were analysed by gas chromatography (GC, Agilent 7890B) coupled to an ICP-MS (Agilent 7900) via a heated interface (250 °C). The GC was equipped with a 7693 autosampler, a G4513A multi-mode injector and fitted with a J&W HP-5 column (30 m; i.d. 0.32 mm; 0.25 m film thickness, Agilent) and a 25 µL syringe (ALS syringe, fixed needle, 23–26/42/cone, PTFE-tip plunger, Agilent). The GC inlet was operated with O_2 (20% O_2 in Ar) at 3 mL·min^{-1} to limit organic matrix introduction. Furthermore, the inlet was packed with glass wool (approximatively 1.5 cm), which supports retention of derivatives during venting and provides column protection from heavy matrix introduction. The optimization of the instrument's performance was made daily with Xe. All operating parameters of optimized GC inlet conditions, oven program and ICP-MS are presented in Table 2.

2.5. Isotopic dilution

Concentrations in the different Se fractions were determined by isotope dilution (ID), which entails the addition of a precise amount of an isotopically labelled Se species ($^{77}Se^{IV}$, $^{77}Se^{VI}$, or ^{76}SeMet) to the sample prior to sample preparation (Fig. 1). The amount of $^{77}Se^{IV}$, $^{77}Se^{VI}$, or ^{76}SeMet added was adjusted to reach isotopic $^{78}Se/^{77}Se$ or $^{78}Se/^{76}Se$ ratios by GC-ICP-MS close to unity and thus obtain minor error propagation during ID calculation [50]. The concentration of Se in the sample (c_{sample}) was calculated with the following equation (1):

$$c_{sample} = \frac{c_{spike}\, w_{spike}\, AW_{sample} \left(RA^{a}_{spike} - A^{b}_{spike} \right)}{w_{sample}\, AW_{spike} \left(A^{b}_{spike} - RA^{a}_{sample} \right)} \quad (1)$$

with the concentrations of Se in the spike (c_{spike}, in ng·L^{-1}), the volume of the spike (w_{spike}, in L) and of the sample (w_{sample}, in L); the atomic weight of Se in the sample (AW_{sample} = 78.96) and in the spike (AW_{spike}: $^{77}Se^{IV}$ and $^{77}Se^{VI}$ = 77.00, ^{76}SeMet = 76.00). Relative isotopic abundances (atom-%) refer to the isotopes "a" (^{77}Se or ^{76}Se) and "b" (^{78}Se). A^{a}_{sample} is the abundance of Se in the sample, i.e. natural abundance of ^{77}Se (7.63%) or ^{76}Se (9.73%), respectively. A^{a}_{spike} is the abundance of isotopes "a" in the used spike solutions, i.e. 99.93% ($^{77}Se^{IV}$), 99.79% ($^{77}Se^{VI}$) or 99.80% (^{76}SeMet, certified by LGC). A^{b}_{spike} is the abundance of ^{78}Se in the spike, i.e. 0.002% ($^{77}Se^{IV}$), 0.085% ($^{77}Se^{VI}$) or 0.002% (^{76}SeMet) in this study. R is the measured $^{78}Se/^{77}Se$ or $^{78}Se/^{76}Se$ ratios after their correction for mass bias (2.8%, n = 30) as described in Tolu et al. [14].

2.6. Proof of concept: detection limit and application to reference materials and natural samples

Calibration linearity and detection limits were determined by analysing Se species spiked in artificial seawater. Absolut (ADLs, in pg) and methodological (MDLs, in ng·L^{-1}) detection limits were calculated for each detected Se chemical fractions according to IUPAC recommendations [51] as shown in equation (2).

$$DL = \frac{3 \times s.d.}{S} \quad (2)$$

with *s.d.*, the standard deviation of the background signal (8 blank measurements) and *S*, the calibration slope, which is calculated from calibration curves either based on peak height versus mass injected (ADLs) or peak height versus concentration (MDLs).

Because no certified reference material (CRM) is available for Se in seawater, the analytical accuracy of the procedure was determined using a CRM for (trace) elements in surface waters (i.e., SRM, NIST 1643f), which was analysed after diluting it 100 times using artificial seawater. Furthermore, we applied the method to two other CRMs for trace elements (excluding Se) in seawater (i.e., CASS-6, NRC, Government of Canada, and NASS-7, NRC, Government of Canada) as well as to natural samples collected in the Baltic and North Seas. Water samples (n = 168) were monthly collected between March 2020 and November 2021 within the framework of the marine monitoring program conducted by the Swedish Meteorological and Hydrological Institute (SMHI). Samples were taken at four depths (surface, chlorophyll-*a* maximum, O_2 minimum and sulfidic conditions up to 250 m depth) at five stations in the Baltic and North Seas along a gradient of salinity (7–35 psu), nutrient and O_2 content as well as algae distribution.

3. Results and discussion

3.1. Optimization of the derivatization and extraction protocol

3.1.1. Derivatization reagents and organic extraction solvents

We first tested the conditions for the formation of different volatile and thermally stable Se derivatives based on the reaction of Se^{IV} present in seawater with different reagents. The intensities of Se derivatives obtained with the three tetraalkylborate reagents ($NaBPr_4$, $NaBEt_4$, $Bu_4NH_4BBu_4$) were overall 1 order of magnitude lower compared to the phenylendiamine ones (4CloP, 4,5-CloP, 4NoP, 3,5-TFMoP) for the same conditions (~20–100 counts versus 1000–2000 counts for a 1 μg ·L^{-1} Se^{IV} solution extracted in isooctane). Furthermore, we noticed a potential source of Se contamination when handling $NaBPr_4$ with plastic spatulas. This reagent seemed to partially dissolve the plastic material leading to a substantial increase in Se blank values. Accordingly, tetraalkylborate reagents were excluded from further investigations due to insufficient derivatization yields for an application to seawater analysis where Se concentrations are in the low ng·L^{-1} range. On the other hand, the extraction of Se derivatives with 4CloP, 4,5-CloP and 3,5-TFMoP was found to be 3.2, 2.8, and 1.2 times more efficient in toluene than in hexane, respectively (Fig. S2). Higher extraction efficiencies of 4NoP in toluene compared to hexane have been previously reported [36,52]. Overall, given the observed better extraction efficiencies and the relatively high boiling points of the Se phenylendiamine derivatives, toluene was chosen as extraction solvent to allow for a higher injection temperature, which in turn reduces the analysis time by GC-ICP-MS.

3.1.2. pH and amounts of derivatization reagents

The derivatization efficiency of the two chlorinated phenylendiamine reagents, 4CloP and 4,5-CloP, was tested for pH values ranging from 0.5 to 2.4 as previous studies already evidenced the importance of acidic conditions [38]. The derivatization efficiency did not vary significantly for the pH range tested (Mann-Whitney $p > 0.05$, Fig. S3A), although a general trend of higher derivatization efficiencies for pH values > 1.5 was observed. For the four phenylendiamine reagents, the intensities of reagent to sample volumes ($V_{reagent}/V_{sample}$) increased from 2.5 to 25%, and then remained the same until 50% (Fig. 2A for 4NoP and 3,5-TFMoP, and Fig. S3B for 4CloP and 4,5-CloP). To limit the amount of reagent and thus the analytical costs, a reagent to sample volume of 25% was selected.

3.1.3. Interferences from the reducing and seawater matrices

We investigated the effects of the highly acidic matrix (6 mol·L^{-1} HCl, boiled at 75 °C for 60 min) used to reduce Se^{VI} into Se^{IV} (required to determine Se^{VI} + Se^{IV} and total Se) as well as those of the seawater matrix on Se derivatization and subsequent detection of Se derivatives by GC-ICP-MS. First, we observed that the Se derivative with 3,5-TFMoP was degraded by the highly acidic matrix as indicated by two Se peaks (Fig. S4). Similarly, the 4CloP-Se derivative was degraded and found as 2 or 3 peaks when derivatization was performed in 6 mol·L^{-1} HCl (see ^{78}Se chromatograms in Fig. 3B and C versus 3A). Secondly, we observed large ^{79}Br-peaks for Se derivatization with 4CloP and 4,5-CloP in the reducing matrix coming from organo-Br compounds already present in the HCl or derivatization reagents, and/or from the addition of Br to the derivatization reagents. These ^{79}Br-peaks strongly interfere the ^{80}Se signal due to the formation of BrH^+ in the plasma (Fig. 3A–B for 4CloP and Figs. S5A–B for 4,5-CloP). For 4,5-CloP, one of these ^{79}Br compounds eluted at the retention time of the Se derivative (Fig. S5B). When derivatization with 4CloP and 4,5-CloP was performed on the combined reducing and seawater matrix, large ^{79}Br peaks eluted at the retention times of both the Se derivatives of 4CloP (Figs. 3C) and 4,5-CloP (Fig. S5C), strongly interfering ^{80}Se and thus resulting in $^{80/78}Se$ ratios that are 50–60 times higher than natural abundance. Finally, no clear separation of the organic phase could be achieved for 4,5-CloP derivatives when using the minimal pre-concentration factor required for the analysis of Se in natural seawater, i.e., 20 (6 mL of sample into 0.3 mL solvent). Adding a centrifugation step did not improve the separation of the organic phase. Therefore, 3,5-TFMoP was considered not suitable for derivatization of Se after the reduction step, while 4CloP and 4,5-CloP were not considered suitable reagents for seawater analysis and were thus excluded from further investigation in this study.

As the 6 mol·L^{-1} HCl matrix was found to not degrade the Se derivative with 4NoP (only one peak in the reducing matrix; Figs. S6A–B), we then tested the suitability of 4NoP for Se derivatization in seawater after the reduction step. Similar to the other tested derivatization

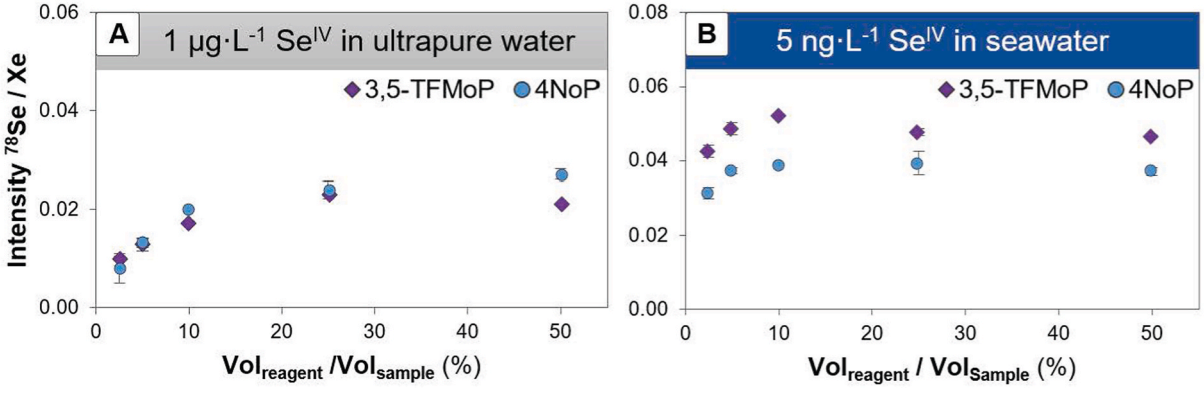

Fig. 2. Comparison of Se intensities normalized by Xe for different ratios of reagent volumes of 3,5-TFMoP (violet diamonds) and 4NoP (blue circles) to sample volume ($Vol_{reagent}$: Vol_{sample}) for Se^{IV} standard prepared in ultrapure water (1 μg·L^{-1}; panel A) and for Baltic seawater samples spiked with 5 ng ·L^{-1} Se^{IV} (panel B). For the results shown in panel A, the sample was not pre-concentrated during the extraction step, while for the results given in panel B, the sample was pre-concentrated from an initial volume of 8 mL–0.3 mL in the final extract.

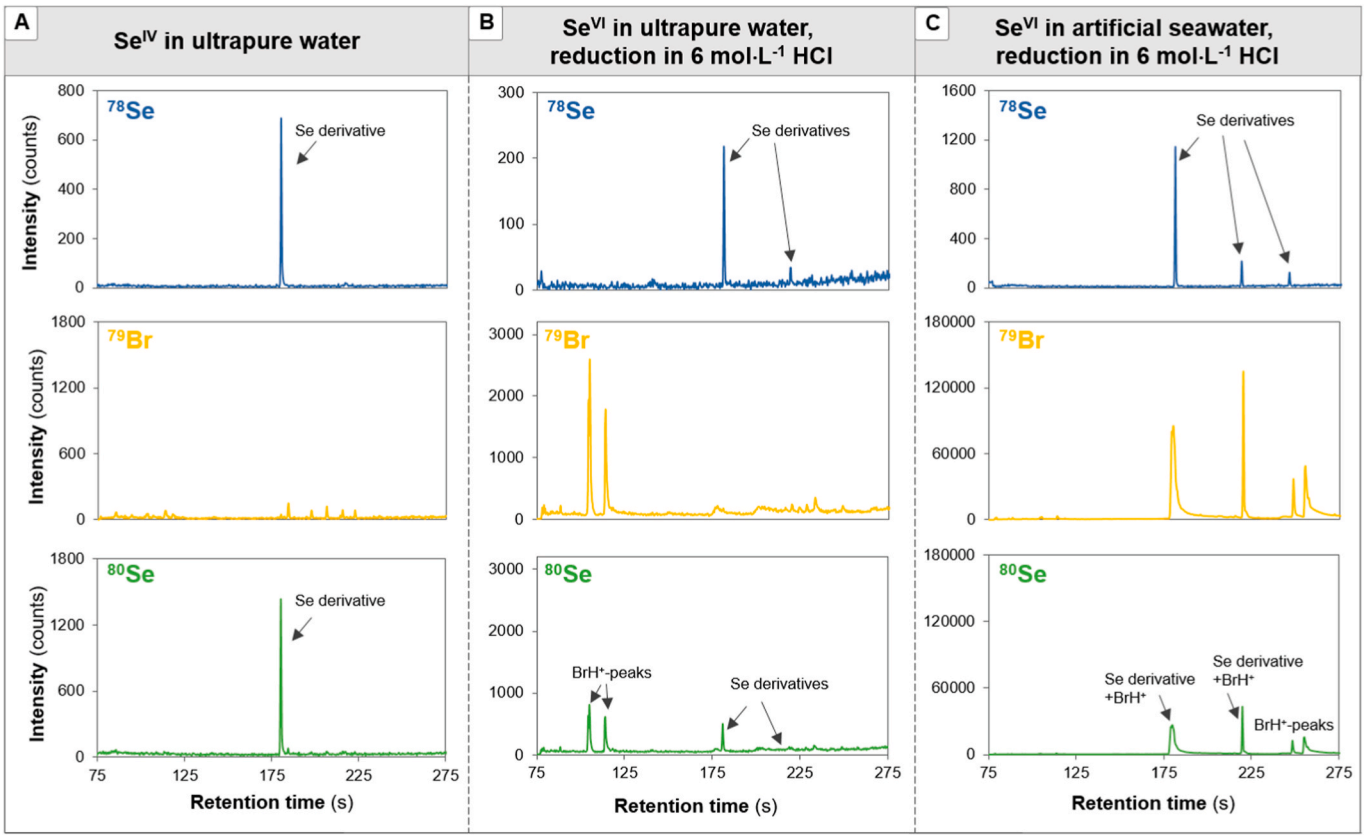

Fig. 3. Effect of the highly acidic (6 mol·L^{-1} HCl) and seawater matrices on derivatization with 4CloP and detection by GC-ICP-MS. Panel A show the chromatograms of ^{78}Se (green line), ^{79}Br (brown line) and ^{80}Se (blue line) obtained after derivatization with 4CloP of a Se^{IV} standard (1 μg·L^{-1}) prepared in ultrapure water. Panels B and C show ^{78}Se, ^{79}Br and ^{80}Se chromatograms obtained after reduction and subsequent derivatization with 4CloP of a Se^{VI} standard (1 μg·L^{-1}) prepared in ultrapure water and artificial seawater, respectively. It should be noted that ^{78}Se intensities are shown with different y-axes than ^{79}Br and ^{80}Se for respective matrices.

Table 1
Optimized derivatization protocol using the 4NoP and 3,5-TFMoP derivatization reagents for the analysis of different Se chemical fractions in seawater.

Target Se chemical fractions	Se^{IV}	Se^{IV} + Se^{VI} (to calculate Se^{VI})	total Se (to calculate orgSe + Se^{0})
Isotopically labelled spike	^{77}Se^{IV}	^{77}Se^{VI}	^{76}SeMet
Volume of sample fraction	8 mL	8 mL	3 mL
Pre-treatment(s)	Buffered acidic conditions (pH 0.5–2)	Reduction in 6 mol·L^{-1} HCl (75 °C for 60 min)	1) Microwave assisted oxidation 2) Reduction in 6 mol·L^{-1} HCl (75 °C for 60 min)
Derivatizing reagent	3,5-TFMoP 0.2%	4NoP 0.5%	4NoP 0.5%
$V_{reagent}/V_{sample}$	10%	10%	10%
Derivatization temperature & time	75 °C for 20 min	75 °C for 7 min	75 °C for 7 min
Extractant	toluene	toluene	toluene
Extraction volume	0.3 mL	0.3 mL	0.3 mL
Retention time of derivative	2.8 min	4.3 min	4.3 min
Monitored Se ratios	78/77	78/77	78/76

reagents, Br peaks were also present in the combined reducing/seawater matrices, including a small BrH^{+} interference on ^{80}Se that eluted close to the retention time of the 4NoP-Se derivative (Fig. S6C). We observed no significant differences (Mann-Whitney $p > 0.05$) for the 4NoP-Se derivative intensities for a Se^{IV} standard derivatized directly and a Se^{VI} standard derivatized after the reduction step in both ultrapure water and artificial seawater (see Fig. S7, treatment 1 and 3). Furthermore, we demonstrated that Se^{IV} remained stable throughout the reduction step, which is necessary for correct quantification of Se^{VI} + Se^{IV} and thus Se^{VI} (see Fig. S7, treatment 2). Overall, 4NoP was found as the most suitable to derivatize Se after reduction of seawater samples (to determine Se^{VI} + Se^{IV}) and of oxidized seawater sub-samples (to determine total Se and organic Se^{-II} + Se^{0}). Due to the small BrH^{+} interference on ^{80}Se that elutes close to the retention time of the 4NoP-Se derivative, ^{78}Se was selected for quantification instead of ^{80}Se.

3,5-TFMoP showed no significant differences in Se signals in artificial seawater compared to ultrapure water (Fig. S8), and was thus considered as the most suitable reagent for Se^{IV} quantification (direct derivatization of seawater samples without reduction).

3.1.4. Optimized derivatization and extraction procedures

Table 1 provides a summary of the optimized derivatization protocol. Once the two suitable reagents (4NoP and 3,5-TMFoP) for Se derivatization of the different Se chemical fractions were selected, we re-tested ratios of reagent to sample volumes between 2.5 and 50% using 8 mL of natural seawater collected in the Baltic Sea, spiked with 5 ng L^{-1} Se^{IV} and extracted into 0.3 mL toluene (pre-concentration of 26.7). Similarly to previous tests performed with ultrapure and artificial seawater with higher Se concentrations, intensities of Se derivatives generally increased from 2.5 to 10% but then remained the same until

Table 2
Operating conditions of optimized PTV-GC-ICP-MS.

GC	
PTV inlet conditions	
Mode	Solvent vent
Initial temperature	90 °C for 0.2 min
Ramp rate 1	600 °C·min^{-1}
Final temperature 1	300 °C for 2 min
Injection volume	5 μL
Vent flow	150 mL·min^{-1} at 5 psi for 0.15 min
Additional gas	20% O_2 in Ar at 3 mL·min^{-1}
Oven program	
Initial temperature	75 °C for 0.5 min
Ramp rate 1	50 °C·min^{-1}
Final temperature 1	250 °C for 0.5 min
Carrier gas flow (He)	3 mL·min^{-1}
ICP-MS	
Forward power	900 W
Make-up gas flow (Ar)	0.45–0.55 L·min^{-1} (tuned daily with Xe)
CRC H_2 flow	4.5 L·min^{-1}
CRC He flow	0.5 L·min^{-1}
Isotopes (mass)/dwell times	Se (76, 77, 78, 80, 82)/30 ms SeH (83)/30 ms Br (79, 81)/10 ms Xe (124)/10 ms

50% (Fig. 2B). Therefore, a ratio of 10% reagent to sample was eventually selected for the derivatization of natural seawater samples with low Se concentrations.

3.2. Microwave assisted oxidation of Se for determination of total concentrations

In a first set of experiments, we tested different ratios of HNO_3 and H_2O_2 for the microwave assisted oxidation of reduced Se species that is needed to determine total Se concentrations and so the organic Se^{-II} + Se^0 fraction. Generally, H_2O_2 contents above 12.5% with HNO_3:H_2O_2 ratios ≤1.0 were found to be sufficient to fully oxidize Se^{IV} and SeMet (each at 50 μg·L^{-1}) into Se^{VI} in artificial seawater based on HPLC-ICP-MS/MS analysis (Table S1). We also checked that Se^{VI} is stable, and not associated with transformation or losses, during the oxidation for all tested conditions (Table S1). However, when using a mixture of HNO_3:H_2O_2 for the microwave assisted oxidation of artificial seawater, we observed the formation of a distinct orange colour (Fig. S9A) during the subsequent reduction step. This orange colour is likely caused by nitrogen compounds (nitrate, nitrite, nitrogen-oxides) and was associated with significant degradation of the performance of the derivatization. The injection of those extracts also led to a quick and irreversible degradation of the GC-column and the objective thereafter was thus to avoid the use of HNO_3. All tested single applications of H_2O_2, ranging between 8.3 and 50% (compared to total digestion volume), were found sufficient to oxidize Se^{IV} and SeMet (each at 50 μg·L^{-1}) into Se^{VI} in artificial seawater based on HPLC-ICP-MS/MS analysis (Table S2 and Fig. S10). The same tested H_2O_2 contents were then applied to Se^{IV} spiked in artificial seawater at three environmentally relevant concentrations (10, 50 and 100 ng·L^{-1} Se^{IV} spiked with ^{76}SeMet) and analysed using the full optimized protocol, i.e., subsequent reduction, derivatization, and ID-GC-ICP-MS analysis using the isotopically labelled ^{76}SeMet for quantification and correction of potential losses during sample preparation. Fig. S11 shows high linearity between added and determined Se by ID-GC-ICP-MS for all tested H_2O_2 conditions with correlation coefficients of 0.993. Since no significant differences were observed, 8.3% was selected as final H_2O_2 content for the microwave assisted oxidation of natural seawater.

3.3. Optimization of large volume injections for GC-ICP-MS analysis

Preliminary experiments to test the various derivatization-extractions conditions were carried out with an initial GC oven temperature of 70 °C (held for 0.5 min) followed by an increase to 220 °C at 50 °C·min^{-1} and an injection volume of 0.5 μL in a normal split mode (split ratio 2:1, inlet temperature: 250 °C). Once 3,5-TFMoP and 4NoP were chosen as derivatization reagents, the oven temperature program was slightly increase to an initial temperature of 75 °C (held for 0.5 min) and a final temperature of 250 °C (ramp 50 °C·min^{-1}, held for 0.5 min) to reduce the analysis time. To further improve detection limits, we tested and optimized large volume injections using a programmed temperature vaporization (PTV) inlet. The optimized parameters for 5 μL injections are presented in Table 2.

Considering the boiling point of toluene (111 °C), the initial temperature of the inlet was set to 90 °C and held for 0.2 min with a flow rate of 150 mL·min^{-1} (held for 0.15 min) to obtain an optimal elimination of the solvent. Under these conditions, the perturbation of the plasma by the solvent elution (assessed by drop in Xe) lasted 0.5 min (Fig. S12). To achieve the transfer of the volatile piazelenols of 3,5-TFMoP and 4NoP, the temperature of the inlet was increased at a rate of 600 °C·min^{-1} and maintained at 300 °C for 2 min. To minimize the inlet cooling time in-between injections, its decrease to the initial temperature (90 °C) was triggered after 2.55 min and not at the end of the acquisition. The duration for one analysis (including oven and inlet cooling) increased to 12 min compared to 7 min with conventional split injection. A typical chromatogram of the optimized method is presented in Fig. 4A, while Fig. S13A shows a comparison between the large volume injection and the conventional split injection. As seen in the chromatograms, the separation of the two species remained consistent, except for the retention time shift. However, the need for solvent venting led to a slightly distorted peak shape for the more volatile Se derivative with 3,5-TFMoP, i.e. a slight fronting that indicates either column saturation and/or more likely that the derivative is not totally retained during venting. Overall, we tested injection volumes ranging between 2.5 and 10 μL, for which solvent elimination was individually adjusted with the venting flow. For 2.5, 7.5 and 10 μL, venting flows of respectively 15, 300 and 800 mL·min^{-1} were found optimal (based on the duration of the Xe signal perturbation, Fig. S12). Fig. 4B presents Se intensities of 3,5-TFMoP and 4NoP for different tested large volume injections, showing a linear increase with increasing injection volumes (corresponding chromatograms shown in S13B). For injection volumes larger than 5 μL, more frequent liner and cone cleaning would be necessary due to carbon built-up from the solvent. Routine analysis of larger volumes would require further optimization of the O_2 flow, which we optimized for 5 μL injection volumes. At a gas flow rate of 3 mL·min^{-1} of O_2, Se sensitivity remained stable (precision within ±2%) over at least 300 injections, demonstrating the suitability of our new large volume injection method for the analysis of a large number of environmental samples with low concentrations.

3.4. Proof of concept: application to reference materials and natural samples

Very good linearity was achieved between 0.03 and 10 pg(Se) for different chemical fractions of Se, with correlation coefficients of 0.999 (Fig. S14). We reached absolute detection limits (ADLs) of 0.1 pg for Se^{IV}, 0.2 pg for Se^{VI} + Se^{IV}, and 0.3 pg for total Se (Table 3). Higher ADLs for Se^{VI} + Se^{IV} and total Se compared to Se^{IV} are due to higher background levels coming from the microwave assisted oxidation and reduction steps as well as to generally lower sensitivity of the detection of 4NoP-Se compared to 3,5-TFMoP-Se derivatives (as indicated in lower slopes of respective calibration curves, Fig. S14). Our ADL for Se^{IV} is four orders of magnitude lower than previously reported using GC-MS after pre-concentration and selective derivatization of Se^{IV} with 4NoP and 4-CloP [36], highlighting the substantial advancement in sensitivity of our optimized approach. Using a sample volume of 8 mL for Se^{IV} and Se^{VI} + Se^{IV} (used for the determination of Se^{VI}) and 3 mL for total Se, we reached method detection limits (MDLs) of 0.9 ng·L^{-1} for Se^{IV}, 1.9

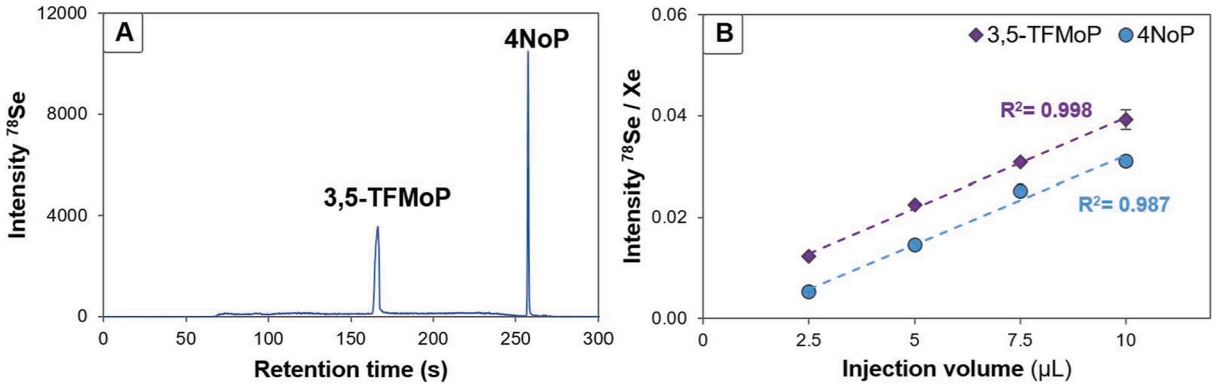

Fig. 4. Performance of the optimized PTV-GC-ICP-MS detection. Panel (A) shows a chromatogram obtained from a combined extract of both derivatization reagents 3,5-TFMoP and 4NoP from a 100 ng·L^{-1} Se standard solution. Panel (B) compares the intensities of ^{78}Se normalized by Xe obtained with different injection volumes (2.5, 5, 7.5 and 10 µL) for both reagents 3,5-TFMoP and 4NoP, showing good linearity (correlation coefficients shown in B).

Table 3

Comparison of absolute (ADL) and methodological detection limits (ADLs) for different Se fractions obtained by optimized ID-PTV-GC-ICP-MS method (highlighted in blue) and other previous applied methods. Additional information on different methods includes pre-concentration method(s), necessary samples volumes as well as application to different water matrices (respective sample number in brackets) with measured Se concentration ranges in seawater and all other water matrices. Listed pre-concentration methods include liquid-liquid extraction (LLE), large volume injection (LVI), solid phase extraction (SPE), solid phase micro-extraction (SPME), dispersive liquid–liquid micro-extraction (DLLME), ultrasound-assisted emulsification micro-extraction (USAEME) and cryogenic trapping (CT).

						Application	Measured Se concentrations[c]		
Species	**Method**	**Pre-conc. method**	**Sample volume** (mL)	**ADLs[a]** (pg)	**MDLs[b]** (ng·L^{-1})	**Matrix** (*sample number*)	**In seawater** (ng·L^{-1})	**In other water matrices** (ng·L^{-1})	**Reference**
Se^{IV}	**ID-PTV-GC-ICP-MS**	**LLE, LVI**	**8**	**0.1**	**0.9**	**seawater (*168*)**	**0.9-19.7**	**-**	**this study**
	GC-MS	LLE	2.5	-	560	mining water (*5*)	-	2'910-75'500	[38]
	GC-MS	SPE, LLE	500 [36], 1000 [37]	1000 [36]	1.6 [37]	fresh- (*3* [36, 37]), coastal seawater (*1* [36], *5* [37])	5'500-6'000 [36] 340-270'000 [37]	17-270'000 [37]	[36, 37]
	GC-MS	SPME	4-17 [34], 5-7 [35]	-	81-166 [34], 3-7 [35]	fresh-(*9-10* [34, 35]), mining-(*2* [35]), seawater (*2* [35])	270-1'020 [35]	<LOD [34], 190-1'020 [35]	[34, 35]
	GC-ECD	LLE	100	-	2 [13, 26]	seawater (*3* [26] *200* [13])	2-40 [13], 3-152 [26]	-	[13, 26]
	GC-ECD	DLLME	5	-	5	spiked freshwater (*3*)	-	210-5'900	[39]
	GC-FID	USAEME/ DLLME	12	-	50-110	spiked fresh- (*4*), waste- (*1*), seawater (*1*)	11'600	11'370-15'000	[52]
	GC-AAS	-	5	-	8	freshwater (*2*)	-	<LOD	[33]
	HG-AAS	-	~100-1000[d]	-	0.8 [9-12], 5 [20]	fresh- (*3* [20]), seawater (*7* [20], ~26-130 [9-12])	5-70 [20], 2-81 [9-12]	17-52 [20]	[7, 9-12, 20]
	HG-AFS	-	35 [21]	-	5 [21, 22], 6 [23], 34 [7]	seawater (5 [21], *~100* [22], *40* [23], *35* [7])	13-44 [21], 16-95 [22], 18-105 [23], <LOD [7]	-	[7, 21-23]
	HG-CT-AFS	CT	10	4	0.4	spiked fresh- (*1*), coastal (*1*), estuary water (*2*)	4-50	6-50	[53]
	HG-HR-ICP-MS	-	n/a	-	0.2 [24], 2 [25]	estuary water (*~60* [24],*~90* [25])	0.2-132 [24], 10-153 [25]	-	[24, 25]
Se^{VI} + Se^{IV}	**ID-PTV-GC-ICP-MS**	**LLE, LVI**	**8**	**0.2**	**1.9**	**seawater (*168*)**	**3-73**	**-**	**this study**
	GC-MS	LLE	2.5	-	1670	mining water (*5*)	-	4'310-68'600	[38]
	GC-MS	SPE, LLE	1000	-	1.4	fresh- (*3*), seawater (*5*)	310-81'000	25-81'000	[37]
	HG-AAS	-	n/a	-	12	seawater (*35*)	17-38	-	[7]
	HG-AFS	-	35 [21]	-	4 [21], 8 [22]	seawater (5 [21], *~100* [22])	[ci]41-63 [21], [ci]28-195 [22]	-	[21, 22]
	HG-HR-ICP-MS	-	n/a	-	0.5 [24], 2 [25]	estuary water (*~60* [24],*~90* [25])	12-376 [24], 28-375 [25]	-	[24, 25]
total Se	**ID-PTV-GC-ICP-MS**	**LLE, LVI**	**3**	**0.3**	**3.1**	**seawater (*168*)**	**25-208**	**-**	**this study**
	HG-HR-ICP-MS	-	n/a	-	0.8	estuary water (*~60*)	84-551	-	[24]

[a,b] both calculated as 3 x sd / slope, where "sd" refers to baseline variations, and the "slope" is calculated from calibration curves either based on [a]peak height vs. mass injected or [b]peak height vs. concentration.
[c] measured Se concentrations in tested water matrices corresponding to specific Se chemical fraction for which MDLs were reported. For Se^{VI} + Se^{IV}, listed studies reported either concentrations of Se^{VI} (calculated by difference of Se^{IV} from Se^{VI} + Se^{IV}) or of Se^{VI} + Se^{IV} (two studies labelled with [ci]).
[d] these values are to the best of our understanding of information given in respective studies.

ng·L^{-1} for Se^{VI} + Se^{IV}, and 3.1 ng·L^{-1} for total Se. Our MDL for Se^{IV} (0.9 ng·L^{-1}) is also generally lower than previously reported MDLs in seawater and/or other water matrices (Table 3) using GC coupled to different detection systems (MS: 1.6–560 ng·L^{-1} [34–38]; ECD/AAS: 2–8 ng·L^{-1} [13,26,33,39]; FID: 50–110 [52]), as well as hydride generation coupled to AAS (0.8–5 ng·L^{-1} [9,11,12,20]) and AFS (5–34 ng·L^{-1} [7,21–23]). In general, low MDLs were achieved with specific pre-concentration methods, including liquid-liquid (micro) extraction (LLE/dispersive-LLME), and solid phase (micro) extraction (SPE/SPME), and/or by using high sample volumes. Previous studies that were applied to natural seawaters used sample volumes that are 4–125 times larger than the volume we used with our method (i.e., 35–1000 mL in Refs. [7,9–13,20,21,26,37] versus 8 mL in our study), while obtaining similar or up to 34 times higher MDLs (0.8–34 ng·L^{-1} versus 0.9 ng·L^{-1}). Although some studies did report concentrations of Se^{VI} and total Se (e.g. Refs. [9,11–13]), respective MDLs were not indicated and thus those reported concentrations are not listed in Table 3. According to our data and other studies that did report MDLs for Se^{VI} + Se^{IV} and total Se, differences in MDLs for different chemical Se fraction are expected for all considered methods. It should also be noted that many previous

studies validated their methods on spiked seawater and/or contaminated waters (up to μg(Se)·L^{-1} levels). Apart from reaching generally lower MDLs, an important advantage of our developed method is the use of enriched Se isotopes, which enables the quantification of Se species by species-specific isotope dilution and correction of potential losses during the sample pre-treatment (oxidation/reduction) and derivatization.

Two recent studies also applied ICP-MS technology to determine Se speciation in estuary waters, using HG coupled to high-resolution sector field-ICP-MS (HG-HR-ICP-MS; [24,25]). Our MDL are within the same range to the ones they obtained, i.e., respectively, 0.9–3.1 and 0.2–2 ng·L^{-1} depending on the Se chemical fractions. However, it should be noted that necessary sample volumes for different Se chemical fractions were not indicated in these two studies, thus making a direct comparison difficult. Altogether, our method involving single-quad ICP-MS represents an efficient alternative in regard to robustness and handling of HG-sector-field ICP-MS for the sensitive analysis of Se speciation in seawater.

To check for the accuracy of our method, we analysed total Se concentrations in different reference materials. Firstly, for the freshwater CRM NIST 1643f that was diluted 100 times in artificial seawater (certified Se concentration considering dilution: 117 ng·L^{-1}), we obtained a recovery of 102 ± 2%. The relative standard deviation (RSD) for the determined total Se concentration was 3% (standard deviation of independent triplicates injected each two times, so a total of 6 analytical replicates). For the NASS-7 and CASS-6 seawater reference materials, for which neither certified, recommended nor previously reported values are available (to the best of our knowledge), we measured total Se concentrations of respectively 68 ± 1 ng·L^{-1} (RSD, 2%) and 70 ± 1 ng·L^{-1} (RSD, 1%), with standard deviation including independent triplicates injected each two times. We found Se^{IV} concentrations of 7.1 ± 0.3 and 11.4 ± 0.4 ng·L^{-1} (both RSD, 4%, n = 6) in NASS-7 and CASS-6, respectively, which are very much in the range of those reported for Se^{IV} in NASS-5 (16.8 ± 0.5 ng·L^{-1} [53]) and CASS-3 (17 ± 2 ng·L^{-1} [37]), which were previously collected at the same places, however, are not commercially available anymore.

To further demonstrate the applicability of the developed method involving ID-PTV-GC-ICP-MS detection, we analysed dissolved concentration of the different Se chemical fractions in 168 water samples collected in the Baltic and North Seas (Fig. 5). Typical Se chromatograms obtained for these samples are shown in Fig. S15. Total dissolved Se concentrations could be quantified in all analysed samples, and ranged from 25.4 to 208.4 ng·L^{-1} (median, 63.9 ± 17.0 ng·L^{-1}; n = 168), demonstrating that a MDL for total Se of 3.1 ng·L^{-1} is sufficient to investigate the distribution of Se in marine environments. A MDL for Se^{IV} of 0.9 ng·L^{-1} is also suitable to study the distribution of the inorganic Se species, which is considered as the most bioavailable species for marine algae [54]. Indeed, Se^{IV} could be detected in the large majority of analysed seawater samples and ranged between 0.9 and 19.7 ng·L^{-1} (median, 7.0 ± 4.1 ng·L^{-1}) with only 13 out of 168 samples found below MDL. For Se^{VI}, despite the low achieved MDL (1.9 ng·L^{-1}), 67 out of the 168 analysed samples were found below MDL with dissolved Se^{VI} concentrations ranging from 1.9 to 73.2 ng·L^{-1} (median, 9.2 ± 15.9 ng·L^{-1}). Our method also captures the strong variability in the reduced organic Se^{-II} + Se^{0} fraction, covering 3.1–164.5 ng·L^{-1} (median, 48.1 ± 21.8 ng·L^{-1}), in the Baltic and North Sea samples. The concentrations of different chemical Se fractions in our measurements are substantially lower than previous concentrations determined in contaminated waters (up to μg·L^{-1} levels) as well as natural seawaters and estuary waters for Se^{IV} (~2–150 ng·L^{-1} [9–12,22–26]), Se^{VI} (~10–375 ng·L^{-1} [7,21,22,24,25]) and total Se (~85–550 ng·L−1 [24]; Table 3).

Interestingly, we detected a Se peak that did not match the retention times of known Se-derivatives when measuring inorganic Se species (Se^{IV} and Se^{IV} + Se^{VI}) in sulfidic seawater samples (see example chromatogram in Fig. S16). This suggests the presence of an unknown Se–S species formed under sulfidic conditions, and although it would require further analysis by e.g. high-resolution mass spectrometry, this finding illustrates another advantage of using an ICP-MS based method compared to other commonly used detectors through identification as Se species of unknown peaks based on isotopic information.

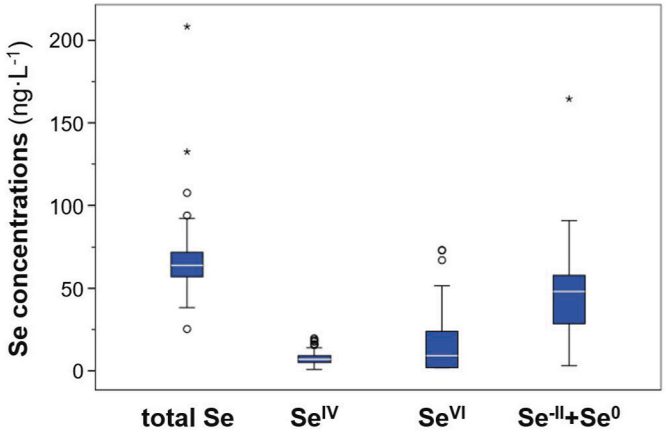

Fig. 5. Variability in concentrations of total Se and Se species in natural seawater samples. Data shown from Baltic and North seawater samples (n = 168; taken at four depths: surface, chlorophyll-*a* maximum, O_2 minimum and sulfidic conditions up to 250 m depth) collected monthly between March 2020–November 2021. Boxplots show the interquartile range, representing the middle (50%) of the data, which fall between the upper quartile (75% data below that score) and the lower quartile (less than 25% below that score).

4. Conclusions

A sensitive method was developed for the determination of Se speciation in natural seawater, including the thorough optimization of isotope dilution, programmed temperature vaporization coupled to GC-ICP-MS (ID-PTV-GC-ICP-MS) as well as sample pre-treatments that are specific for the different Se chemical fractions (Se^{IV}, Se^{VI} + Se^{IV}, total Se) and subsequent Se derivatization and extraction of formed derivatives. We first demonstrated the applicability of our method with a certified reference material diluted in seawater matrix and then applied it to a large number of natural samples collected in the Baltic and North Seas. Detection limits of the optimized method could be further improved by increasing pre-concentration factors through larger sample volumes, using larger injection volumes (e.g. 7.5 or 10 μL) or individual injections of Se species derivatives of Se^{IV} and Se^{VI}. Altogether, our method provides a promising alternative to previous methods for the analysis of Se in seawater and will help to better understand the Se cycling in marine environments.

CRediT authorship contribution statement

Esther S. Breuninger: Conceptualization, Investigation, Methodology, Validation, Formal analysis, Visualization, Writing – original draft. **Julie Tolu:** Conceptualization, Methodology, Validation, Writing – review & editing, Funding acquisition, and, Supervision. **Sylvain Bouchet:** Conceptualization, Methodology, Validation, Writing – review & editing, Funding acquisition, and, Supervision. **Lenny H.E. Winkel:** Conceptualization, Writing – review & editing, Funding acquisition, and, Supervision.

Declaration of competing interest

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2023.341833.

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