Talanta 133 (2015) 162–169

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Uncertainty contributions to the measurement of dissolved Co, Fe, Pb and V in seawater using flow injection with solid phase preconcentration and detection by collision/reaction cell—quadrupole ICP–MS

Robert Clough [a], Hagit Sela [a,b], Angela Milne [a], Maeve C. Lohan [a], Serife Tokalioglu [a,c], Paul J. Worsfold [a,*]

[a] *Biogeochemistry Research Centre, School of Geography, Earth and Environmental Sciences, Plymouth University, Plymouth PL4 8AA, United Kingdom*
[b] *Nuclear Research Centre, Negev, PO Box 9001, Beer-Sheva, Israel*
[c] *Erciyes University, Faculty of Science, Chemistry Department, TR 38039 Kayseri, Turkey*

ARTICLE INFO

Article history:
Received 10 June 2014
Received in revised form
14 August 2014
Accepted 16 August 2014
Available online 23 August 2014

Keywords:
Flow injection
Quadrupole ICP–MS
Seawater
Preconcentration
Trace metals

ABSTRACT

A flow injection manifold incorporating a solid phase chelating resin (Toyopearl AF-Chelate-650) is reported for the preconcentration of dissolved metals from seawater, with a focus on investigating the effect of the loading pH, wash solution composition and wash time. Cobalt, iron, lead and vanadium have been used as target analytes with contrasting oceanographic behaviour. Quadrupole ICP–MS has been used for detection to make the approach accessible to most laboratories and a collision/reaction cell has been incorporated to minimise polyatomic interferences. Results for the seawater CRM NASS-6 and two GEOTRACES reference materials were in good agreement with the certified/consensus values, demonstrating the suitability of the approach for the determination of trace metals in seawater. The experimental design used allowed a thorough investigation of the uncertainty contribution from each method parameter to the overall expanded uncertainty of the measurement. The results showed that the parameters making the largest contributions were the precision of the peak area measurement and the uncertainty associated with the slope of the calibration curve. Therefore, these are the critical parameters that should be targeted in order to reduce the overall measurement uncertainty. For iron, the wash blank also gave a measureable contribution.

1. Introduction

Trace metal micronutrients such as iron (Fe) and cobalt (Co) are involved in regulating primary production in the marine environment and therefore play a key role in controlling global climate via their impact on the global carbon cycle [1]. Iron is arguably the most important trace element for cell metabolism in marine photosynthetic organisms as it acts as a vital electron mediator in photosynthetic apparatus [2]. Despite Fe being the fourth most abundant element in the earth's crust, its solubility in seawater is low ($\sim$0.1 nmol L^{-1}). The availability of Fe (and other trace metal micronutrients) regulates the growth, biomass and species composition of phytoplankton over large areas of the surface ocean and CO_2 drawdown has been observed in iron fertilisation studies.

The marine biogeochemistry of trace metal micronutrients therefore has great scientific, ecological and political significance [3]. It follows that to improve our understanding of important issues such as the linkages between trace metal micronutrients and marine phytoplankton, and associated biological feedback mechanisms that may influence global climate, requires a detailed knowledge of the oceanic distribution, sources, sinks and cycling of these elements.

More reliable determinations of dissolved trace metal micronutrients at sub-nM concentrations in marine waters are therefore needed to enhance our understanding of their impact on ocean productivity and processes such as ocean acidification. However, they are analytically challenging to measure due to their very low concentrations, the presence of labile and complexed forms of the elements and the potential for contamination. Hence methods need to be sufficiently validated to allow intercomparison between data sets from different spatial and temporal regimes.

Progress towards reliable measurements of dissolved trace elements in seawater has been made in recent years with increasing emphasis on laboratory [4–6] and shipboard [7] intercalibration and

---

[*] Corresponding author. Tel.: +44 1752 584579; fax: +44 1752 584999.
E-mail address: pworsfold@plymouth.ac.uk (P.J. Worsfold).

http://dx.doi.org/10.1016/j.talanta.2014.08.045
0039-9140/

the use of consensus mean reference materials [8,9]. A key aspect of intercalibration is the determination of the uncertainty of replicate measurements. A logical extension of this is to undertake a structured approach to the measurement of combined uncertainties [10–15]. There are two distinct approaches to the estimation of the uncertainty associated with a measurement result, often termed the 'bottom-up' and 'top-down' approaches, with both requiring a measurement model to be constructed [12].

In the bottom-up approach, standard uncertainties associated with each stage of the entire measurement procedure are estimated and combined using uncertainty propagation laws. In this approach the effect of different aspects of the measurement procedure, such as blank correction and sample manipulation, on the combined uncertainty estimate can be calculated. Subsequently, the analytical method can be refined to minimise these effects and reduce the combined standard uncertainty.

The top-down approach uses internal quality control (QC) data from QC samples that cover the entire analytical process. This approach is much simpler than the bottom-up approach and, provided that long term QC data have been acquired, can be readily implemented. However, as part of the initial method development and validation it is prudent to investigate the individual uncertainty sources of the method and attempt to minimise them so that subsequent QC data can account for and reflect the method uncertainty.

This paper presents an investigation of the uncertainty sources associated with a flow injection (FI) method with detection by inductively couple plasma–mass spectrometry (ICP–MS) for the determination of dissolved trace metals in seawater using the bottom-up approach. The FI manifold incorporated an immobilised iminodiacetate chelating ligand for on-line preconcentration and matrix removal. ICP–MS was used for detection due to its multi-elemental capabilities and ability to acquire the time resolved data necessary for capturing transient signals. Toyopearl AF-Chelate-650, containing the iminodiacetate functional group, was used as the chelating resin as it has been used for the preconcentration of various dissolved metals from seawater [16–22] and therefore allows direct comparison of the results as well as providing relevant information for the user community on the uncertainty sources associated with using this resin. A target of 10% was set for the expanded uncertainty (expressed in relative terms) associated with each analyte as determined by FI-ICP–MS.

Four contrasting metals were chosen for this study; Co and Fe as they are biologically significant trace metals essential for phytoplankton growth, lead (Pb) as it is a scavenged anthropogenic element and therefore present at very low concentrations and vanadium (V) as it exhibits conservative behaviour with depth. Vanadium is also present at much higher concentrations than the other three elements in seawater (typically low ng L^{-1} concentrations) which presents less of a measurement challenge and is therefore likely to result in a lower uncertainty estimate. The method used to estimate the combined uncertainty follows that outlined in a recent review [15] and was undertaken using on-line preconcentration with quadrupole ICP–MS detection, i.e. the most widely available ICP–MS technology, to maximise the general applicability of the principles described herein but also incorporating a collision/reaction cell to minimise polyatomic interferences.

2. Experimental

2.1. Reagents

Concentrated nitric acid (HNO_3, UpA grade), hydrochloric acid (HCl), ammonia (NH_3, 20–22%) and glacial acetic acid ($C_2H_4O_2$), all SpA grade, were purchased from Romil (Cambridge, UK). Individual 10,000 mg L^{-1} ICP–MS standards of Co, Fe, Pb and V were purchased from Fisher Scientific (Loughborough, UK) and used to prepare a multi-element working stock solution of 10 mg L^{-1} in 2% HNO_3. A 2 M ammonium acetate stock solution was prepared from concentrated ammonia and glacial acetic acid and used to prepare a series of 0.05 M ammonium acetate solutions with the pH adjusted in the range 3.5–7.0 in half unit steps and spiked to a concentration of 10 ng L^{-1} with the multi-element stock solution. The FI eluent, 1 M HNO_3, was prepared from concentrated nitric acid by dilution with high purity water. The various wash solutions were prepared from concentrated nitric and hydrochloric acids and a 2 M ammonium acetate stock solution. Hydrogen peroxide, Merck Suprapur grade, was obtained from VWR (Lutterworth UK). All high purity water (HPW), 18.2 MΩ cm, was drawn from an ElgaStat Maxima system (Marlow, UK). The seawater CRM (NASS-6) was supplied by LGC Promochem (Teddington, UK) and the GEOTRACES surface (GS) and deep (GD) reference materials (RMs) were supplied free of charge by Professor Ken Bruland, UCSC, California, USA.

To minimise blank concentrations all sample and reagent handling prior to analysis was undertaken in an ISO 14644-1 Class 5 laminar flow hood (Bassaire, Southampton, UK) situated within an ISO 14644-1 Class 5 clean room. For the analytical sessions sample handling was again undertaken in an ISO 14644-1 Class 5 laminar flow hood (Envirco, Cleanroomshop, UK) situated in an ISO 14644-1 Class 7 clean room which also housed the ICP–MS instrument. Reagent and sample containers were made of low density polyethylene (LDPE; Nalgene, Fisher Scientific, UK) and acid cleaned. Containers were immersed in ~1.1 M trace metal grade HCl (Fisher Scientific) for at least seven days. Subsequently, the containers were rinsed in copious amounts of HPW, filled with 0.01 M HCl and stored in double re-sealable plastic bags until use.

All weighing was performed using an analytical balance (OH1602/C, Ohaus, Thetford, UK). The accuracy of the balance was checked daily before use using F1 Class certified weights (KERN, Albstadt, Germany). All pipettes used were calibrated prior to use using HPW and the Ohaus balance. All facilities are managed under ISO 9001:2008 certification.

The seawater used as a calibration matrix (denoted SWC) was a surface sample collected by a tow fish at 40°S 48°W during research cruise JC068 on board RRS *James Clark Ross*. The bulk sample was filtered through a dual 0.8/0.2 μm cartridge filter (Acropak, Pall, UK) into 25 L LDPE carboys (Nalgene, Fisher, UK). One litre sub-samples were acidified in the laboratory with 2 mL of Romil UpA grade HCl per L of seawater at least one month before use. Calibration standards were prepared by gravimetric dilution of the ICP–MS standards and final spiking into SWC to give calibration ranges appropriate to the concentration of the element being determined. These were prepared at least 12 h in advance of analysis to allow complete equilibration of the added metals with the seawater matrix components. The pH of all calibration standards and samples was adjusted off-line to pH 5.0 by the addition of the appropriate amount of a 0.4 M ammonium acetate solution adjusted to pH 7.2. This solution was passed through a ‘clean up’ column in the FI manifold containing Toyopearl AF-Chelate-650 resin to minimise the blank contribution.

2.2. Instrumentation

The flow injection (FI) manifold, depicted schematically in Fig. 1, was designed so that any of the three flow streams could be directed to the ICP–MS instrument at any point in the analytical cycle. It consisted of three peristaltic pumps (Minipuls 3, Gilson, Luton, UK), and three six port electronically actuated valves (Valves 1, 2 and 3; VICI, Thames Restek, UK). The peristaltic pump

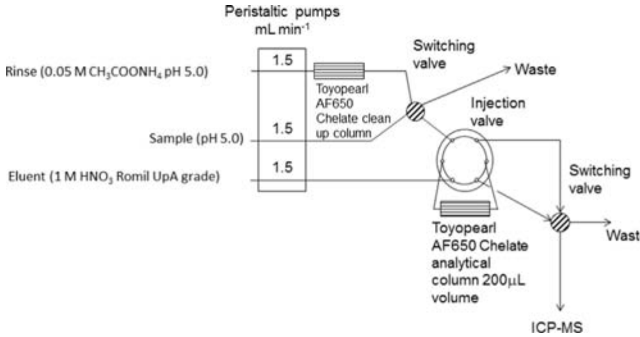

Fig. 1. The FI manifold for analyte (trace metal) pre-concentration and matrix separation in seawater matrices.

Table 1
The Vcom settings for the switching valve (V_1) toggling between the wash solution and the sample and the injection valve (V_2) toggling between loading and elution. cc = Clockwise and cw = Counter clockwise.

Time (min:s)	Valve position	Operation
0:00	V_1cc	Wash solution
0:00	V_2cw	Loading
0:15	V_1cw	Sample
5:15	V_1cc	Wash solution
6:15	V_2cc	Elution
8:15	V_1cc	Wash solution
8:15	V_2cw	Loading

tubing used was two stop accu-rated™ PVC (Elkay, Basingstoke, UK), all other manifold tubing was 0.8 mm i.d. PTFE. The system used two poly(methyl methacrylate) columns (1 cm long; 1.5 mm i.d.), loaded with Toyopearl AF-Chelate-650 resin (Tosoh Bioscience, Stuttgart, Germany) retained with HDPE frits (BioVyon F, 0.75 mm thick, 22–57 µm pore size), one to clean the rinse solutions and one as the analytical column. To minimise compaction and/or 'channelling' effects in the analytical column, the directional flow of the load/elute cycle was alternated. After each analytical session all fluid paths were flushed with 0.3 M HNO_3 for at least 10 min, with the 'clean up' column being flushed with a reverse flow, and then for 15 min with HPW which was left in the lines. As the flow direction through the clean-up column was one way during analytical sessions the cleaning solution flow was reversed to minimise resin compaction in this column. Valve control was by Vcom software (Valco Instruments, Schenkon, Switzerland). The timing sequences used for sample loading, washing and elution are given in the relevant results sections and the Vcom settings for the final conditions are shown in Table 1.

The eluent from the FI manifold was directly coupled to an X Series 2 ICP–MS instrument (Thermo Scientific, Hemel Hempstead, UK) via a Conikal high flow nebuliser (Glass Expansion, Melbourne, Australia) and a PC3 spray chamber (Elemental Scientific, Omaha, USA) cooled to 5 °C. A high flow nebuliser was used to be compatible with the flow rates from the FI manifold. Cooling the spray chamber increases stability by reducing solvent loading on the plasma and avoiding temperature fluctuations. Before each analytical session the instrument was tuned in standard mode, using a 10 µg L^{-1} solution of Ba, Ce, Co, Li, In and U, and the performance checked to ensure that it was operating to the manufacturers specifications. Subsequently, to minimise the effect of polyatomic interferences, particularly the $^{40}Ar^{16}O$ signal which is a polyatomic interferent for Fe, the instrument was tuned and operated in collision/reaction cell mode, with a cell gas of 7% H in He flowing at 3.6 mL min^{-1}, to give a $^{140}Ce^{16}O$:^{140}Ce ratio of $\leq$ 0.07%. The potential for other polyatomic interferences was further reduced by efficient washing of the column to remove sea salt ions, as discussed in Section 3.2. Operating in collision/reaction cell mode had minimal effect on the sensitivity for Pb and a tenfold loss of sensitivity for Co, Fe and V compared with operating in standard mode. The most abundant masses were used for all elements except for Pb, for which 208Pb was used to avoid the potential for detector overload. Data acquisition was by the PlasmaLab software of the ICP–MS instrument which was operated in time resolved analysis mode with a dwell time of 50 ms. This dwell time was selected as a compromise between speed (the fastest dwell time setting is 0.1 ms), spectral noise and skew and gave suitable resolution for the peak widths obtained with a 120 s analyte elution. The operation of the Vcom software was synchronised with the Plasmalab software to allow consistent flow injection traces to be produced. Peak area measurements were made by integrating the recorded transient signals using the Plasmalab software and the raw data exported to allow further off-line processing.

2.3. *Measurement uncertainty*

Uncertainty is defined as "A parameter associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the measurand" [13]. The uncertainty of a measurement generally comprises multiple components, with each of these components termed a standard uncertainty. For experimental measurements the standard uncertainty is given by the standard deviation of those measurements. For other parameters the standard uncertainty can be taken from data supplied by the instrument manufacturers. The individual standard uncertainties are subsequently combined using uncertainty propagation rules to give the combined standard uncertainty, which is then multiplied by an appropriate 'coverage factor' to give the expanded uncertainty of the measurement result. A coverage factor of 2 is usually applied, which gives a level of confidence of approximately 95%. For a more detailed explanation of measurement uncertainty see [12,13,15].

The expanded uncertainty of each analytical result was estimated using the numerical differentiation method of Kragten [23]. This approach, which is easy to adopt, estimates the effect each parameter in the measurement equation has on the analytical result using a simple spreadsheet, for which a worked example is given in the Eurachem guide [12]. The equation used to calculate the analyte concentration in seawater was used as the model for these calculations and is shown in Eq. (1). This model takes into account the two potential sources of blank contamination, namely the pH adjustment buffer and the column wash solution used for removal of the residual seawater matrix from the analytical column.

$$C_S = \frac{\left(\frac{(I_S - I_{WB}) \times V_1}{M}\right) - (B_C \times B_V)}{V_2} \qquad (1)$$

where C_S is the analyte concentration in the sample, I_S is the signal (area measurement) of the analyte; I_{WB} is the signal (area measurement) of the wash blank; V_1 is the volume of the sample + pH adjustment buffer; M is the slope of the calibration curve; B_C is the analyte concentration in the pH adjustment buffer; B_V is the volume of the pH adjustment buffer and V_2 is the initial sample volume.

The standard uncertainties for I_S and I_{WB} were calculated from the precision of the respective peak areas (n = 3 and 10 respectively). The standard uncertainty of B_C was taken as the standard deviation of five replicate measurements of the pH adjustment buffer. The uncertainty of the slope of the calibration curve was calculated using regression statistics [24]. For the volume measurements, V_1, V_2 and B_V, the standard uncertainties were taken from the manufacturers certificates for the pipettes and plastic laboratory ware used.

3. Results and discussion

3.1. Effect of pH on sample loading

The optimal loading pH for each element of interest was determined using 10 μg L^{-1} solutions of Co (170 nM), Fe (179 nM), Pb (48 nM) and V (196 nM) in 0.05 M ammonium acetate with the pH adjusted in the range 3.5–7.0 in half unit steps. Solutions were left to equilibrate for at least 2 h prior to use. The approach used is similar to that reported by Willie et al. [25] and Sohrin et al. [9]. For these experiments the analytical column was equilibrated with an unspiked ammonium acetate solution of the same pH as the spiked solution for 30 s followed by loading of the sample for 120 s (30 ng of each element in absolute terms) and analyte elution from the column for 120 s. There was no column wash step because seawater was not used. The FI system was operated such that the flow of each reagent was directed to the ICP–MS instrument and ammonium acetate was used as the matrix (rather than seawater) to allow continuous monitoring of the entire extraction procedure. The amount of analyte extracted was calculated as a percentage of the total analyte signal recorded in the resulting fiagram, i.e. the sum of the peak areas from all the steps in the analytical cycle shown in Table 1 (washing, loading, elution, washing). The results of these experiments are shown in Fig. 2, with each solution analysed in triplicate. Blank contributions were determined by analysis of unspiked portions of the pH adjusted ammonium acetate solutions. At a sample pH of 5.0 a retention factor of $\geq 97\%$ was calculated for all of the elements under study. The plots for Co, Pb and V showed clear trends with pH which were commensurate with their speciation behaviour whereas the plot for Fe showed an irregular loss of retention above pH 5.0. This effect, which was consistent over several experimental runs, may be due to pH induced changes in Fe speciation [2] and/or the formation of more stable iron complexes with the ammonium (acetate), rendering it less readily available for chelation.

3.2. Effect of wash solution on sample elution

For seawater samples it is necessary to remove the major cations, Ca, Mg, K and Na, which can be retained on the iminodiacetate column and/or remain in the void spaces [25,26], prior to analysis by ICP–MS. The relative order of the stability constants for the major seawater cations to bind to this resin are $Ca^{2+} > Mg^{2+} > K^+ = Na^+$ and they will compete non-selectively with the ammonium ions in the carrier stream for binding sites, thus potentially lowering the effective capacity of the resin for the trace metals of interest. Effective removal of these matrix cations also prevents excess deposits building up on the ICP–MS sample and skimmer cones which would otherwise lead to signal instability due to alteration of the thermal characteristics of the cones and signal suppression through the easily ionisable element effect. For some elements, e.g. Cu, it also removes elements such as Na that can lead to polyatomic interferences arising from the seawater matrix. The column wash step prior to elution is therefore critical. A number of different reagents were considered for use as wash solutions, namely H_2O, 0.05 M ammonium acetate, HCl (0.012, 0.006, 0.003 M) and HNO_3 (0.008 M). For these experiments the elements under study were added (at 5 μg L^{-1}) into seawater (SWC), which was then adjusted to pH 5.0. The FI-ICP–MS procedure involved conditioning of the analytical column with 0.05 M ammonium acetate (pH 5.0) for 30 s followed by sample loading for 120 s, column washing for 120 s and elution with 1 M HNO_3 for 120 s, with the eluent flow continuously directed to the ICP–MS instrument.

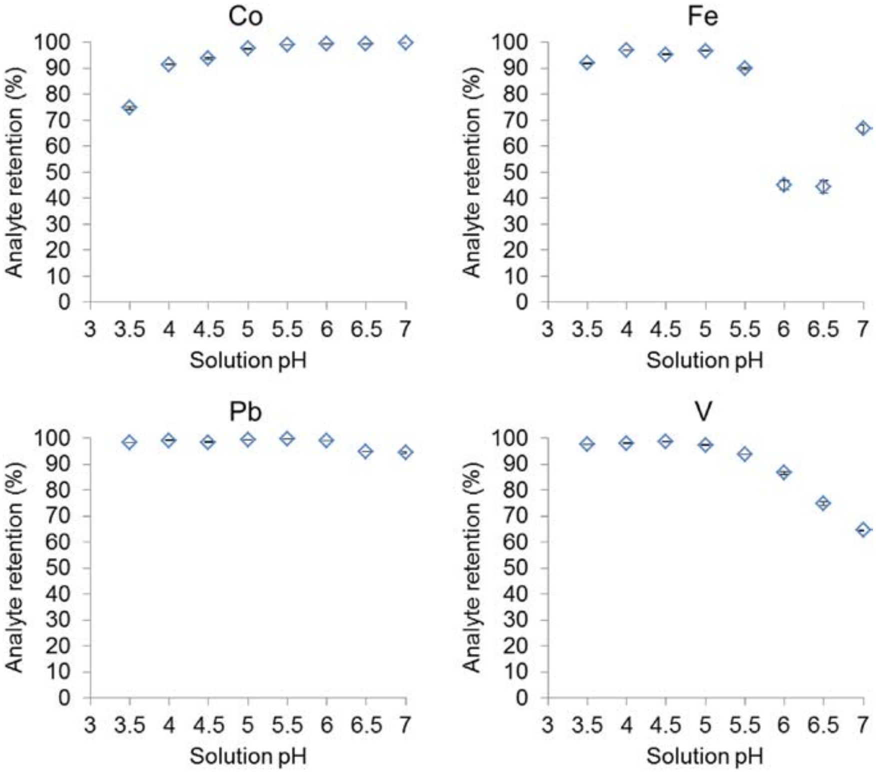

Fig. 2. Analyte retention on the Toyopearl AF-Chelate-650 resin as a function of pH. The uncertainties shown are one standard deviation of three replicate measurements.

Blank contributions were assessed by analysing the conditioning and wash solutions only, i.e. by omitting the sample loading step. Four different patterns were observed for analyte retention with the different wash solutions and representative plots are shown in Fig. 3. The least effective wash solution for Na was H_2O, with all of the other wash solutions removing $\geq 80\%$ of the Na retained with a H_2O wash. For the four elements under study (Co, Fe and V shown) quantitative retention was observed for the 0.05 M ammonium acetate (pH 5.0) wash solution. All of the HCl and HNO_3 wash solutions removed $>99\%$ of the Co and Pb, 35–40% of the Fe and up to 18% of the V.

Five different wash times, 20, 40, 60, 90 and 120 s, were then used to determine the amount of time needed to efficiently remove the major cations when washing with 0.05 M ammonium acetate, with data expressed as a percentage of the signal obtained for the 20 s wash time and shown in Fig. 4. For a wash time of 40 s the Ca, Mg and Na signals were reduced to 11.9, 6.7 and 2.4% respectively of the signal obtained for a 20 s wash time. These values were further reduced for a 60 s wash time; with Ca, Mg and Na being 4.2, 1.1 and 0.4% respectively of the signal obtained for a 20 s wash time. The further reduction obtained for Ca is important due to the potential $^{40}Ca^{16}O$ isobaric interference on ^{56}Fe. There was minimal further reduction from these values for longer wash times of 90 and 120 s.

For a 60 s wash time the Fe and V signals were reduced to 74 and 71% respectively of the 20 s wash time signal. For Co and Pb the signal obtained was $\geq 90\%$ of the 20 s wash signal for all other wash times evaluated. A 40 s wash time gave similar recoveries for the four elements of interest but it is important to ensure that all matrix cations are removed. Thus, the optimal conditions for the separation and pre-concentration of Co, Fe, Pb and V and efficient removal of the major matrix cations from seawater were a sample loading pH of 5.0 and a wash solution of 0.05 M ammonium acetate adjusted to pH 5.0 pumped over the column for 60 s.

3.3. Analysis of reference seawater samples

The FI-ICP–MS method was validated using NASS-6 CRM (NRCC, Canada) and the optimum load and wash conditions stated in Section 3.2. For these and subsequent analyses the FI timings were, column conditioning 15 s, sample loading (for analyte extraction and matrix removal) 300 s, column washing 60 s and analyte elution 120 s, giving a total analytical time of 8.25 min per replicate. The analyte peak width at the detector was 20–30 s, depending on the analyte concentration and the wash out characteristics of the ICP–MS spray chamber. With an eluent flow rate of 1.5 mL min^{-1} the preconcentration factor was 10 to 15 fold.

Blank contributions from the wash solution were measured by omitting the sample loading stage and were performed 10 times before sample analysis to ensure that blank estimations were under control. Blank signals arising from the conditioning/wash solution were only measureable for the determination of Fe (0.393, 0.150 and 0.101 nmol L^{-1} for NASS-6 and the GEOTRACES GS and GD RMs respectively) and Pb (0.321, 1.42 and 1.22 pmol L^{-1} for NASS-6 and the GEOTRACES GS and GD RMs respectively). It should be noted that these values are probably overestimated due to an increase in analyte extraction efficiency for a simple ammonium acetate matrix compared with the seawater matrix used to obtain the calibration curve slope. However, as the raw wash signal is subtracted from the raw sample signal, the actual concentration values are not used in any calculations. Blank signals for Co and V were indistinguishable from the baseline, which was

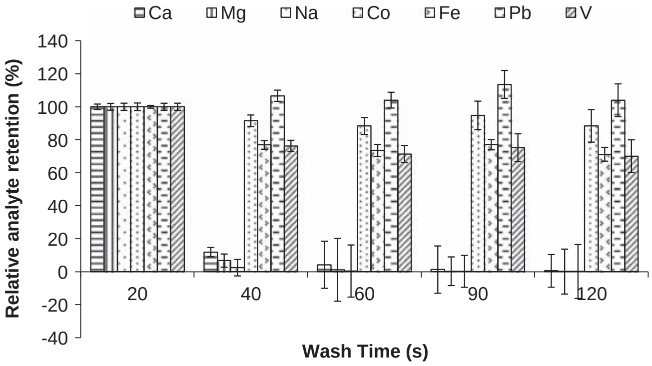

Fig. 4. Analyte retention with different column wash times with a 0.05 M ammonium acetate wash solution normalised to 100% for a 20 s wash time. For each plot the data are expressed as a percentage of the value obtained for the H_2O wash for that element. The uncertainties shown are the combined standard uncertainty of the H_2O and individual wash solution measurements ($n=3$) in each case.

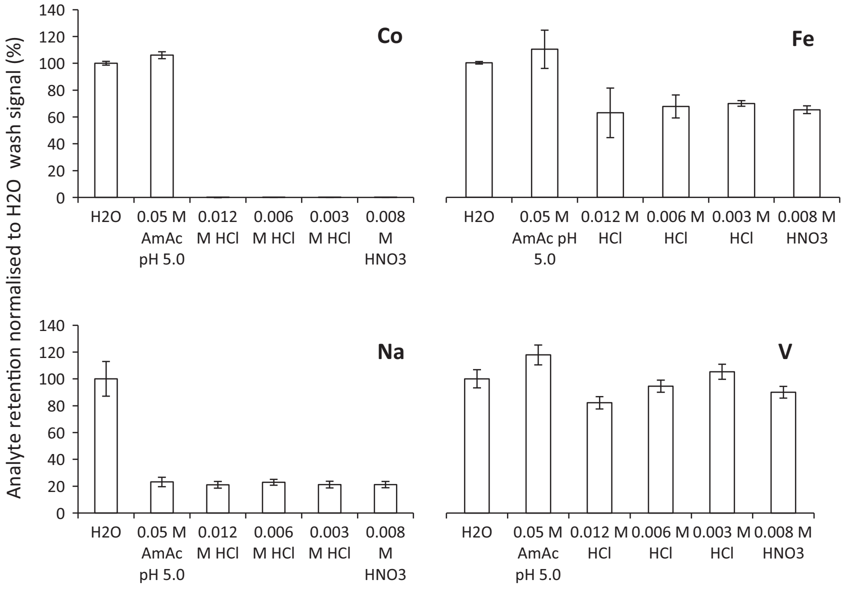

Fig. 3. Analyte retention with different column wash solutions normalised to 100% for H_2O. All samples were eluted with 1 M HNO_3. For each plot the data are expressed as a percentage of the value obtained for the H_2O wash for that element. The uncertainties shown are the combined standard uncertainty of the H_2O and individual wash solution measurements ($n=3$) in each case.

≤ 100 counts s^{-1}. The limits of detection were calculated from three times the combined standard uncertainty of the elemental determinations in the wash solution and pH adjustment buffer and the data are presented in Table 2. Reported limits of detection using magnetic sector ICP–MS are generally one order of magnitude lower compared with the quadrupole ICP–MS reported here [18,27,28], due in part to the increased sensitivity, and hence improved count rates and precision for extremely low elemental concentrations. In the former the ion beam undergoes focussing by both an electrostatic sector and a magnetic field, whereas for quadrupole ICP–MS only single focussing of the ion beam occurs. In addition, collisional defocussing of the ion beam occurs, with the effect increasing as m/z decreases, further decreasing sensitivity when a collision/reaction cell is used to mitigate polyatomic interferences. Nonetheless, the limit of detection values obtained were sufficient to allow detection of Co, Fe, Pb and V in all of the samples, suggesting that on-line preconcentration with quadrupole ICP–MS detection and a collision cell attachment is a suitable approach for the determination of trace metals in seawater. Calibration curve correlation coefficients were >0.99 for all measurements except for Fe in NASS-6 which was 0.98.

Statistical agreement with the certified values for NASS-6 was evaluated by comparing the difference between the found and certified values, Δm, and the expanded uncertainty of Δm, $U_{\Delta m}$, which was estimated by combining the standard uncertainties for the found and certified values. If $\Delta m \leq U_{\Delta m}$ then the two values are in agreement [29]. Results for Fe, Pb and V were in agreement with the certified values. For Co, NASS-6 only gives an indicative value, without a stated uncertainty. If the typical relative standard uncertainty (4.5%) for the certified elements in NASS-6 was applied to the stated value for Co then the found value reported here would also be in agreement.

The two GEOTRACES RMs, GS and GD [30] were also analysed. For these seawaters, which are open ocean samples and typically have trace metal concentrations an order of magnitude lower than those found in NASS-6, statistical agreement was achieved for all measurements, except for Co in GEOTRACES GD, as shown in Table 3. The two RMs did not undergo UV oxidation to avoid the potential risk of Fe contamination. UV oxidation is however required to release Co from strong organic ligands with a greater affinity for Co than the iminodiacetate functional groups of the extraction resin. The Co measured in these samples can therefore be considered as the 'labile Co fraction' [28], because non-UV irradiated samples were used and the values obtained, 0.0301 ± 0.0035 and 0.0495 ± 0.0027 nmol L^{-1} for GS and GD respectively, are in statistical agreement with those reported in reference 28 of 0.0233 ± 0.0028 and 0.0425 ± 0.0042 nmol L^{-1} for non-irradiated GS and GD respectively.

In most cases the target relative expanded uncertainty of 10% or less was achieved, which compares favourably with reported data for on-line FI-ICP–MS using a sector field instrument [31]. Expanded uncertainties of around 5% have been reported for off-line extractions using isotope dilution ICP–MS or standard additions [18,27,28] but it should be noted that these latter data only reported precision in terms of standard deviations rather than accounting for all of the uncertainties in the method, such as the precision of the measured isotope ratios and the mass bias correction factor for isotope dilution ICP–MS, and hence are not truly comparable with the data presented here. In two cases, Co and Fe in the GS RM, the expanded uncertainty was significantly above the target value, 23 and 18% relative, and the reasons for this are discussed in Section 3.4.

Table 2
Limits of detection for the measurement of dissolved Co, Fe, Pb and V in seawater by FI-ICP–MS.

Analytical measurement	Co (nmol L^{-1})	Fe (nmol L^{-1})	Pb (nmol L^{-1})	V (nmol L^{-1})
NASS-6	0.0058	0.34	0.0053	0.032
GD RM	0.0021	0.071	0.0024	0.011
GS RM	0.014	0.23	0.011	0.036

3.4. Relative contributions to the expanded uncertainties

In order to determine which method parameters contributed the most uncertainty to the overall expanded uncertainty of the measurement, the expanded uncertainties of seven components of the method were determined. The raw data used for these calculations are shown in Table 4 whilst the results for the relative contributions (%) of each parameter (defined in Eq. (1) are shown in Table 5. For almost all of the analyses undertaken the major contribution, typically $\geq 75\%$, to the expanded uncertainty of each result was the precision of the signal intensity of the analyte, i.e. the peak area measurement parameter I_S in Eq. (1). When the precision of I_S improved, e.g. at higher analyte concentrations, the relative contribution of the uncertainty associated with the slope of the calibration curve, i.e. measurement parameter M in Eq. (1), increased. These two uncertainties generally contributed $\geq 98\%$ of the expanded uncertainty of the measurement. As the uncertainty associated with the calibration curve slope is in part dependent on the precision of the analyte peak area measurements, as well as the repeatability of the analyte pre-concentration step, these are the two key parameters that should be targeted for improvement to reduce the overall expanded uncertainty of the measurement.

The standard uncertainty for peak area measurements in all three sample types and for all of the elements under study was typically $\leq 5\%$, which compares with that obtained for FI-CL measurements in our laboratory of 3–6%, and was not concentration dependent. Rhodium was added as an internal standard to the eluent, for monitoring purposes only rather than signal intensity correction, and this signal was stable during each individual analytical run and for the longer period required for all data acquisition (8 h). This suggests that the peak area standard uncertainty is due to a variable extraction efficiency of analytes onto the analytical column. Thus, in order to minimise uncertainties associated with sample peak areas and the slope of the calibration curve, it is essential to ensure that the analyte extraction part of the procedure is optimised and under full control. One reason for the uncertainties observed could be loose packing of the columns. The use of monolithic columns, which are compatible with low pressure pumping systems, may provide a more repeatable extraction and elution profile than loose packed columns. However, no suitable monolithic columns are readily available and therefore in-house production would be required. Longer sample loading times, e.g. 10 min, could also improve the stability of the analyte extraction efficiency but would not be a cost effective use of instrument time and in this case an off-line approach would be recommended.

For one element, Fe, the uncertainty associated with the wash blank also contributed significantly to the overall expanded uncertainty of the measurement, 8, 17 and 11% for the determinations in NASS-6, GS and GD respectively, with the relative contribution increasing with decreasing analyte concentration. This was due to two effects. Firstly the relative magnitude of the wash blank signal compared with the analyte signal, 4.2, 20 and 2.8% for NASS-6, GS and GD respectively, and secondly the poor precision obtained when measuring the blank signal, 28% for NASS-6, 11% for GS and 36% for GD. Thus, although the data can be corrected for blank contamination, the wash blank does impact on the expanded uncertainty and it is therefore recommended

Table 3
Analytical data for the measurement of dissolved Co, Fe, Pb and V in seawater. Uncertainties for the certified or consensus means represent ± 2 s.d.

Element	Sample	[Analyte] (nmol L^{-1})	Expanded uncertainty (U) (nmol L^{-1})	Relative Expanded uncertainty (%)	Certified or consensus value[c] (nmol L^{-1})	Statistical agreement
Co	NASS-6[a]	0.284	0.0144	5	0.255	N/A
	GS	0.0301	0.0069	23	0.0318 ± 0.0022	Yes
	GD	0.0495	0.0053	11	0.0652 ± 0.0024	No
Fe	NASS-6	9.64	0.84	9	8.86 ± 0.82	Yes
	GS	0.505	0.089	18	0.546 ± 0.92	Yes
	GD	1.035	0.079	8	1.00 ± 0.20	Yes
Pb	NASS-6	0.0239	0.0022	9	0.0290 ± 0.010	Yes
	GS	0.0278	0.0018	7	0.0286 ± 0.002	Yes
	GD	0.0442	0.0019	4	0.0427 ± 0.003	Yes
V	NASS-6	28.9	2.1	7	28.7 ± 3.3	Yes
	GS[b]	28.9	1.9	6		N/A
	GD[b]	36.1	1.8	5		N/A

[a] An indicative value only is given for Co in NASS-6 and no uncertainty is stated for this element.
[b] No values for V have been reported for either of the GEOTRACES RMs.
[c] Any data originally quoted as nmol kg^{-1} have been converted to nmol L^{-1} for consistency.

Table 4
Raw data for the measurement of dissolved Co, Fe, Pb and V in seawater. The symbols used for the method parameters investigated are defined in Eq. (1).

	Sample peak area (counts) (I_S)	u (counts)	Slope (counts/nmol L^{-1}) (M)	u (counts)	Wash blank (counts) (I_{WB})	u (counts)	Buffer blank concentration (nmol L^{-1}) (B_C)	u (nmol L^{-1})	Buffer Volume (mL) (B_V)	u (mL)	Sample Volume (mL) (V_2)	u (mL)
NASS-6												
Co	49,023	854	186,906	3435	0	0	0.0063	0.0019	2.13	0.0094	25	0.06
Fe	836,898	11,187	89,967	3692	35,386	9846	0.0066	0.0020	2.13	0.0094	25	0.06
Pb	67,297	2,928	3013,897	46,462	967	321	0.021	0.0018	2.13	0.0094	25	0.06
V	93,867	2,777	3,517	71	0	0	0.034	0.011	2.13	0.0094	25	0.06
GD												
Co	7,173	379	15,7522	726	0	0	0.00479	0.00070	4.19	0.021	43	0.060
Fe	71,277	1,544	60,061	1154	2028	725	2.36	0.020	4.19	0.021	43	0.060
Pb	35,529	133	836,791	17,835	1018	89	0.0105	0.00079	4.19	0.021	43	0.060
V	1924,878	14,990	58,603	1456	0	0	0.044	0.0037	4.19	0.021	43	0.060
GS												
Co	2,980	317	101,945	2268	0	0	0.0179	0.0046	1.88	0.0094	25	0.06
Fe	42,034	2,020	56,936	1197	8563	980	1.69	0.074	1.88	0.0094	25	0.06
Pb	41,918	1,097	1470,593	16,908	2082	83	0.0382	0.0038	1.88	0.0094	25	0.06
V	11,0222	2,983	4,104	71	0	0	0.0303	0.012	1.88	0.0094	25	0.06

Table 5
Uncertainty contributions for the measurement of dissolved Co, Fe, Pb and V in seawater. The symbols used for the method parameters investigated are defined in Eq. (1).

Element	Sample	Relative uncertainty contribution (%)						
		Sample peak area (I_S)	Calibration curve slope (M)	Wash blank (I_{WB})	Sample volume (V_2)	Sample+buffer volume (V_1)	Buffer blank concentration (B_C)	Buffer volume (B_V)
Co	NASS-6	47	51	0	1	1	0	0
	GS	95	4	0	0	0	1	0
	GD	99	1	0	0	0	0	0
Fe	NASS-6	10	81	8	0	0	0	0
	GS	73	8	17	0	0	2	0
	GD	51	37	11	0	0	0	0
Pb	NASS-6	88	10	1	0	0	0	0
	GS	75	14	0	1	1	9	0
	GD	96	3	0	0	0	1	0
V	NASS-6	69	31	0	0	0	0	0
	GS	71	28	0	1	0	0	0
	GD	9	90	0	0	0	0	0

that it should be accounted for in all similar methods. For Co in the GS RM the peak area uncertainty was 11% relative, which is the cause of the high expanded uncertainty for this particular measurement. Finally, there were minor (< 2%) contributions from the wash blank signal, sample volume, (sample+buffer) volume, buffer blank concentration and the buffer volume apart from the measurement of Pb in GS where the buffer blank contributed 9% relative.

In summary, provided blank contributions are sufficiently low, a realistic uncertainty estimate for the determination of trace metals in seawater can be made by combining the uncertainties associated with the signal intensities and the slope of the calibration curve.

4. Conclusion

Preconcentration using a flow injection manifold coupled with collision/reaction cell—quadrupole ICP–MS detection can be used for the determination of dissolved trace metals (specifically Co, Fe, Pb and V) in seawater. The optimum FI preconcentration/matrix removal procedure using the Toyopearl AF-Chelate-650 iminodiacetate resin involved conditioning of the analytical column with 0.05 M ammonium acetate (pH 5.0) for 15 s followed by sample loading for 300 s, column washing for 60 s and elution in the reverse direction with 1 M HNO_3 for 120 s, with the eluent flow continuously directed to the ICP–MS instrument. This gave a total analysis time for one replicate of 8.25 min. Good agreement with certified/consensus values was obtained for all four elements using NASS-6 and the GEOTRACES GS and GD reference materials (except for Co in GD). The expanded uncertainty contributions of seven components of the method were determined and compare favourably with previously reported data; the sample peak area measurement and the calibration curve slope contributed the majority of the uncertainty. There were minor contributions from the wash blank (except for Fe), sample volume, buffer volume and the buffer blank concentration. In order to further reduce the measurement uncertainty the stability of the analyte extraction efficiency needs to be reduced. This warrants the investigation of functionalised monolithic columns for the extraction and preconcentration of trace metals from seawater.

Acknowledgements

Dr. Robert Clough acknowledges funding by the EMRP via JRP-ENV05-REG1 (Metrology for ocean salinity and acidity). The EMRP is jointly funded by the EMRP participating countries within EURAMET and the European Union. Dr. Serife Tokalioglu is grateful for the financial support of The Council of Higher Education in Turkey.

References

[1] K.A. Hunter, P. Boyd, Mar. Freshwater Res. 50 (1999) 739.
[2] S.J. Ussher, E.P. Achterberg, P.J. Worsfold, Environ. Chem. 1 (2004) 67.
[3] Y. Sohrin, K.W. Bruland, TrAC, Trends Anal. Chem. 30 (2011) 1291.
[4] A.R. Bowie, E.P. Achterberg, P.L. Croot, H.J.W. De Baar, P. Laan, J.W. Moffett, S. Ussher, P.J. Worsfold, Mar. Chem. 98 (2006) 81.
[5] G.A. Cutter, Limnol. Oceanogr. Methods 11 (2013) 418.
[6] K. Pahnke, T. van de Flierdt, K.M. Jones, M. Lambelet, S.R. Hemming, S.L. Goldstein, Limnol. Oceanogr. Methods 10 (2012) 252.
[7] A.R. Bowie, E.P. Achterberg, S. Blain, M. Boye, P.L. Croot, H.J.W. De Baar, P. Laan, G. Sarthou, P.J. Worsfold, Mar. Chem. 84 (2003) 19.
[8] K.S. Johnson, E. Boyle, K. Bruland, K. Coale, C. Measures, J. Moffett, A. Aguilar-Islas, K. Barbeau, B. Bergquist, A. Bowie, K. Buck, Y. Cai, Z. Chase, J. Cullen, T. Doi, V. Elrod, S. Fitzwater, M. Gordon, A. King, P. Laan, L. Laglera-Baquer, W. Landing, M. Lohan, J. Mendez, A. Milne, H. Obata, L. Ossiander, J. Plant, G. Sarthou, P. Sedwick, G.J. Smith, B. Sohst, S. Tanner, S. Van den Berg, J. Wu, Eos 88 (2007) 131.
[9] Y. Sohrin, S. Urushihara, S. Nakatsuka, T. Kono, E. Higo, T. Minami, K. Norisuye, S. Umetani, Anal. Chem. 80 (2008) 6267.
[10] M. Borecka, A. Białk-Bielińska, G. Siedlewicz, K. Kornowska, J. Kumirska, P. Stepnowski, K. Pazdro, J. Chromatogr. A 1304 (2013) 138.
[11] E.R.M. Druffel, S. Griffin, B.D. Walker, A.I. Coppola, D.S. Glynn, Radiocarbon 55 (2013) 1135.
[12] S.L.R. Ellison, A. Williams (Eds.), Eurachem/CITAC Guide: Quantifying Uncertainty in Analytical Measurement, Eurachem, 2012.
[13] JCGM, Evaluation of Measurement Data—Guide to the Expression of Uncertainty in Measurement (GUM), Joint Committee for Guides in Metrology, Paris, 2008.
[14] S. Rigaud, V. Puigcorbé, P. Cámara-Mor, N. Casacuberta, M. Roca-Martí, J. Garcia-Orellana, C.R. Benitez-Nelson, P. Masqué, T. Church, Limnol. Oceanogr. Methods 11 (2013) 561.
[15] P.J. Worsfold, R. Clough, M.C. Lohan, P. Monbet, P.S. Ellis, C.R. Quetel, G.H. Floor, I.D. McKelvie, Anal. Chim. Acta 803 (2013) 15.
[16] A.M. Aguilar-Islas, J.A. Resing, K.W. Bruland, Limnol. Oceanogr. Methods 4 (2006) 105.
[17] N.G. Beck, R.P. Franks, K.W. Bruland, Anal. Chim. Acta 455 (2002) 11.
[18] A. Milne, W. Landing, M. Bizimis, P. Morton, Anal. Chim. Acta 665 (2010) 200.
[19] R.U. Shelley, B. Zachhuber, P.N. Sedwick, P.J. Worsfold, M.C. Lohan, Limnol. Oceanogr. Methods 8 (2010) 352.
[20] K.W. Warnken, D. Tang, G.A. Gill, P.H. Santschi, Anal. Chim. Acta 423 (2000) 265.
[21] N.J. Wyatt, A. Milne, E.M.S. Woodward, A.P. Rees, T.J. Browning, H.A. Bouman, P.J. Worsfold, M.C. Lohan, Global Biogeochem. Cycles 28 (2014).
[22] C.M. Zurbrick, C. Gallon, A.R. Flegal, Anal. Chim. Acta 800 (2013) 29.
[23] J. Kragten, Analyst 119 (1994) 2161.
[24] J.N. Miller, J.C. Miller, Statistics and Chemometrics for Analytical Chemistry, Pearson Education, London, England, 2005.
[25] S.N. Willie, J.W.H. Lam, L. Yang, G. Tao, Anal. Chim. Acta 447 (2001) 143.
[26] R.A. Nickson, S.J. Hill, P.J. Worsfold, Anal. Chim. Acta 351 (1997) 311.
[27] D.V. Biller, K.W. Bruland, Mar. Chem. (2012) 130–131 (12).
[28] M.E. Lagerström, M.P. Field, M. Séguret, L. Fischer, S. Hann, R.M. Sherrell, Mar. Chem. 155 (2013) 71.
[29] T. Linsinger, Comparison of a Measurement Result with the Certified Value, European Commission—Joint Research Centre (IRMM), Geel, Belgium (2005) 2.
[30] G.M. Henderson, R.F. Anderson, J. Adkins, P. Andersson, E.A. Boyle, G. Cutter, H. de Baar, A. Eisenhauer, M. Frank, R. Francois, K. Orians, T. Gamo, C. German, W. Jenkins, J. Moffett, C. Jeandel, T. Jickells, S. Krishnaswami, D. Mackey, C.I. Measures, J.K. Moore, A. Oschlies, R. Pollard, M.R.D. van der Loeff, R. Schlitzer, M. Sharma, K. von Damm, J. Zhang, P. Masque, S.W. Grp, Chem. Erde 67 (2007) 85.
[31] F. Queroue, A. Townsend, P. Van Der Merwe, D. Lannuzel, G. Sarthou, E. Bucciarelli, A. Bowie, Anal. Methods 6 (2014) 2837.