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An ICP-MS procedure to determine Cd, Co, Cu, Ni, Pb and Zn in oceanic waters using in-line flow-injection with solid-phase extraction for preconcentration

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ABSTRACT

An automated procedure including both in-line preconcentration and multi-element determination by an inductively coupled plasma mass spectrometer (ICP-MS) has been developed for the determination of Cd, Co, Cu, Ni, Pb and Zn in open-ocean samples. The method relies on flow injection of the sample through a minicolumn of chelating (iminodiacetate) sorbent to preconcentrate the trace metals, while simultaneously eliminating the major cations and anions of seawater. The effectiveness of this step is tested and reliability in results are secured with a rigorous process of quality assurance comprising 36 calibration and reference samples in a run for analysis of 24 oceanic seawaters in a 6-h program. The in-line configuration and procedures presented minimise analyst operations and exposure to contamination. Seawater samples are used for calibration providing a true matrix match. The continuous automated pH measurement registers that chelation occurs within a selected narrow pH range and monitors the consistency of the entire analytical sequence. The eluent (0.8 M HNO_3) is sufficiently strong to elute the six metals in 39 s at a flow rate of 2.0 mL/min, while being compatible for prolonged use with the mass spectrometer. Throughput is one sample of 7 mL every 6 min. Detection limits were Co 3.2 pM, Ni 23 pM, Cu 46 pM, Zn 71 pM, Cd 2.7 pM and Pb 1.5 pM with coefficients of variation ranging from 3.4% to 8.6% ($n=14$) and linearity of calibration established beyond the observed concentration range of each trace metal in ocean waters. Recoveries were Co 96.7%, Ni 102%, Cu 102%, Zn 98.1%, Cd 92.2% and Pb 97.6%. The method has been used to analyse ~800 samples from three voyages in the Southern Ocean and Tasman Sea. It has the potential to be extended to other trace elements in ocean waters.

1. Introduction

The international GEOTRACES [1], SOLAS [2] and IMBER [3] projects each have a focus on trace elements in the oceans. This has brought a fresh emphasis to their measurement at natural oceanic background levels and with unprecedented spatial and temporal resolution. Multi-element analytical methods are needed that can deliver results with high sensitivity (picomolar and lower), efficiency, and in the absence of contamination.

Key breakthroughs with regard to contamination control in sampling and analysis for trace metals in ocean waters occurred in the 1970s [4,5], providing pioneering results. These methods were often laborious with demanding preconcentration steps followed by single element measurements, and many months could be spent analysing samples from a single voyage.

Advances in automation and new generations of multi-element instruments—especially inductively coupled plasma mass spectrometer (ICP-MS) have significantly reduced analysis times and allowed for a large number of elements to be determined simultaneously. Limitations of ICP-MS instrumentation in handling high salt matrices, combined with determining elements at ultra-trace levels, have instigated the incorporation of a pre-treatment step; examples are listed in Sohrin and Bruland [6]. It brings together both preconcentration and matrix elimination in the same step, which improves limits of detection for analyte elements and removes interfering salts. Earlier work with preconcentration before ICP-MS has used various techniques, such as chelate solvent extraction followed by back-extraction [7,8] and co-precipitation [9,10]. These methods satisfied the requirement for suitable

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preconcentration factors coupled with matrix elimination, but they were still protracted batch procedures.

Progress in the synthesis of solid phases with metal-chelating functional groups and low susceptibility to changes in bead volume associated with variations in pH or ionic strength [11,12], and their availability in suitable commercial resins has led to sorbent extraction of trace metals from seawater becoming more viable. Encapsulation of the sorbents in small columns allows for flow-through procedures with improved contamination control and the option for automation.

Chelating sorbent extraction as a preliminary to ICP-MS has proceeded down two paths. The first pursues an off-line batch procedure [13–15] with separate collection of acid eluates (containing the analyte trace metals) from the sorbent cartridge or column for each sample. Once sufficient numbers of samples have been processed, a set of eluates are analysed by ICP-MS. The preconcentration step is often semi-automated using peristaltic pumps and switching valves, although this process is still demanding of analyst attention if simultaneous preconcentration systems are used to minimise sample preparation time. The alternative is to use flow injection (FI) analysis for the preconcentration step in-line with ICP-MS detection [12,16–19], whereby the eluent from the chelating sorbent is fed directly to the spectrometer. By necessity, these in-line methods are fully automated, under control of the ICP-MS software. These systems can be plumbed to allow column wash with diluted buffer; incorporate autosampler functions; enable flushing of sample lines whilst eluting; and provide reproducible timing to guarantee adequate reproducibility. Having a closed system also affords easier control of contamination and the speed and efficiency of this system can counteract the additional time required if reanalysis under different pH or ICP-MS conditions is needed.

The method presented here is an automated in-line preconcentration procedure for the simultaneous determination of six trace metals (Co, Ni, Cu, Zn, Cd, Pb) in seawater. Preconcentration was achieved using FI with a minicolumn of commercially available Toyopearl AF-chelate-650M, an iminodiacetate (IDA) sorbent. In comparison to earlier methods, we have incorporated a wash solution via the autosampler enabling a simplified FI sequence and the removal of 'T' junctions [12,19]; introduced an in-line pH monitoring system; used a seawater sample for a matrix-matched calibration instead of the laborious preparation of artificial seawater [17,18]; designed the sample and solution preparations to minimise operations and reduce potential contamination; used peak area processing to improve reproducibility; and introduced rigorous quality controls providing numerous monitoring tools. These tools provide the evidence for effective resolution of polyatomic interferences, addressing the questions raised in the limitations of quadrupole ICP-MS and seawater analysis [19] and provide confidence in the reliability of results. We report on the results from daily analysis of certified and consensus reference samples over multiple days. The method has improved levels of detection in comparison with earlier work using in-line FI procedures [12,17–19] and comparable results to the batch-analysis alternatives [15]. It has been used for the routine daily analysis of hundreds of open-ocean seawater samples. Reliability is shown in our figures of merit and method validation, especially where validation is representative of a full analysis set of 300 samples.

2. Experimental

2.1. Instrumentation

A PerkinElmer SCIEX (Concord, Ontario, Canada) ELAN DRC II ICP-MS with a quadrupole mass spectrometer and a dynamic reaction cell (DRC) was custom-installed into a Clean Room facility (ISO Class 5). The ELAN DRC II ICP-MS was operated using ELAN v3.4 software, which also controlled the CETAC (Omaha, NE, USA) ASX-520 autosampler and the PerkinElmer (Shelton, CT, USA) FIAS 400MS flow-injection analysis system (FIAS). A TPS SmartChem-pH system (Springwood, QLD, Australia) was incorporated to monitor the pH in-line during the analysis. Sampling and interface components, and typical conditions for ICP-MS operation, are listed in Table S1.

In addition to local control, remote control of the ICP-MS software from outside the Clean Room facility was set up using DameWare (Covington, LA, USA; v6.2) on an external office computer. A 'webcam' was installed in the Clean Room to enable remote viewing of the autosampler.

The schematic of the FI manifold used for preconcentration and matrix elimination is shown in (Fig. 1). All reagent transfer lines

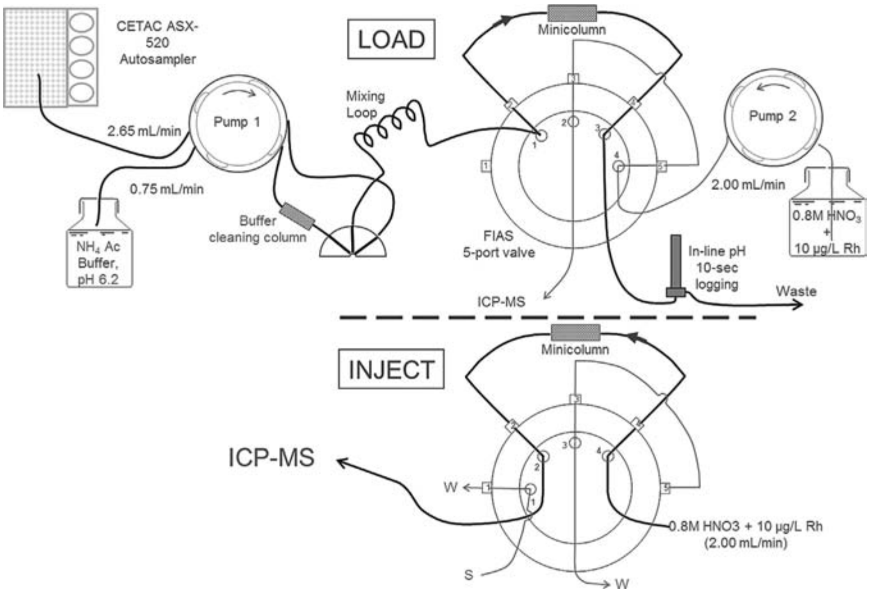

Fig. 1. Schematic of automated flow-injection system assembled from Perkin Elmer FIAS-400 five port valve and CETAC ASX-520 autosampler. Upper portion depicts load sequence; lower portion depicts inject (elution, in reverse direction) sequence with transfer of eluted trace metals to ICP-MS.

were either polytetraflurorethylene (PTFE) or perfluoroalkoxy (PFA) (1 mm i.d., 1/16" o.d.) from VICI Jour (Schenkon, Switzerland) and GlobalFIA (Fox Island, WA, USA). Peristaltic pump tubing was Tygon 3-stop tubing red/red/red 1.143 mm i.d. (TACS, Sydney, NSW, Australia) and flanged Tygon 3-stop orange/yellow/orange 0.508 mm i.d. tubing (Choice Analytical, Sydney, NSW, Australia).

A slurry of Toyopearl AF-chelate-650M (particle size: 40–90 μm; Tosoh Bioscience GmbH, Stuttgart Germany) was packed into metal-free minicolumns (GlobalFIA, Fox Island, WA, USA). A 1-cm minicolumn was used for sample preconcentration, while a 2-cm minicolumn was used for in-line buffer cleaning.

2.2. *Plasticware and cleaning*

Plasticware used was either low-density polyethylene (LDPE) (Nalgene Labware, Rochester, NY, USA) or natural polypropylene (Sarstedt, Ingle Farm, SA, Australia; Azlon, Stone, Staffordshire, UK). Plasticware was cleaned, in an ISO Class 5 clean room, for a minimum of two days in a 2.5% Extran detergent bath and one week in 10% HCl. De-ionised water (18 MΩ/cm; hereafter MQ water) from a Milli-Q analytical-reagent-grade water purification system (Millipore, Bedford, MA, USA) was used to thoroughly rinse plasticware of cleaning acids.

2.3. *Gas, reagents, standards and Mo correction samples*

The argon gas supplied to the ICP-MS was delivered from a cylinder (Alphagas N50 grade, Air Liquide Australia Ltd., Melbourne, VIC, Australia). Initially the argon had been supplied from the headspace over liquid Argon (FL734, BOC Australia, North Ryde, NSW, Australia).There was no increase observed in background signals with the change.

All reagent and standard solutions were prepared in an ISO Class 5 clean room with MQ water as the diluent. Ultra-pure acetic, hydrochloric and nitric acids (BASELINE grade, SEASTAR CHEMICALS Inc., Sidney, BC, Canada) were employed throughout. Ammonia solution, 25% analytical grade, (AnalaR, BDH, Kilsyth, VIC, Australia) was purified by isothermal distillation before use. All primary standards were Single-Element High Purity 1000-μg/mL solutions (Choice Analytical, Sydney, NSW, Australia; QCD Analysts Englewood, FL, USA). The stock ICP-MS Setup Solution (Elemental Standards, Everton Park, QLD, Australia) was 10 ng/mL in each of Ba, Be, Ca, Cd, Ce, Co, Cu, Fe, In, Mg, Pb, Rh, Sc, Tl, U in 2% HNO_3.

Dedicated reagent bottles were marked to indicate target volume (calibrated by weight), enabling easy preparation of solutions, and minimising handling and the risk of contamination. The eluent and minicolumn rinse solutions were prepared within one day of analysis. The 0.8 M HNO_3 eluent solution was spiked with 10 μg/L rhodium as an internal standard. The rinse solutions included one of MQ water and two of 0.02 M HCl (0.01 M for early samples acidified to 0.01 M HCl). Bulk near-surface seawater collected from the pooling of previously analysed samples, was used as a rinse/conditioning sample during the analysis sequence (Table S2).

The buffer solution of ~1.0 M ammonium acetate was also made up by weight. For the analysis of samples acidified to 0.01 M HCl, the buffer pH was 6.14 ± 0.07 and for the analysis of samples acidified to 0.02 M HCl, the buffer pH was 6.26 ± 0.13.

Six elements were determined Co, Ni, Cu, Zn, Cd and Pb. A multi-element, intermediate substandard solution was made up in 2% (v/v) HNO_3 in a 250-mL LDPE bottle and subsampled into a 10-mL polypropylene tube for easy dispensing. This substandard contained 10 μg/L Co (≡170 nM), 20 μg/L Pb (≡96.5 nM), 50 μg/L Cd (≡445 nM), 100 μg/L Cu (≡1.57 μM) and 200 μg/L Ni (≡3.41 μM) and Zn (≡3.06 μM) and was prepared by serial dilution of 1000-μg/mL primary standard solutions of individual elements (see above). On each day of analysis, a daily working substandard solution was prepared by the 1-in-10 (v/v) dilution of the multi-element intermediate substandard with MQ water. This was an important step to dissipate the effect of the acid in the intermediate substandard solution on the extraction pH of the calibration standards to ensure that any difference between the extraction pH of calibration standards and samples was trivial and did not affect recovery. From the set of seawater samples to be analysed on the day, the sample collected one depth beneath the surface sample was selected for calibration purposes. It was used to prepare four multi-element calibration standards via additions of the daily working substandard solution (Table S3), two unamended ('zero') samples and a duplicate top standard. The second zero seawater sample was run immediately after the first, since they are analysed after the top standard (Table S2) by default using the ELAN software. The calibration was obtained using the first zero sample. Comparison of the evaluated concentration between the first and second zero samples provided a check on carryover.

Molybdenum concentration, even as a trace metal, in seawater is sufficiently high and constant (105 nmol/kg [20]) to interfere as a polyatomic species in ICP-MS detection (viz. $^{95/98}Mo^{16}O$ on $^{111/114}Cd$) [19,21]. The interference was evaluated by adding a 10 μg/L (104 nM) Mo spike to a subset of samples (four near-surface samples, NASS 5 and SAFe S reference seawaters) for each analysis sequence. The ^{111}Cd results were corrected by the increment attributable to $^{95}Mo^{16}O$. As with the calibration standards, a daily working substandard was prepared by the 1-in-10 (v/v) MQ water dilution of an intermediate Mo substandard to reduce the effect of acid on the extraction pH.

2.4. *Blanks and reference seawaters*

Field blanks were acid-washed sample bottles filled with MQ water; these were prepared with the sample bottles and taken to sea. Two field blanks were included with each batch of samples when acidified—one at the start of the process and one at the end. The addition of ultra-pure HCl in early voyages was to a concentration of 1.0 mL/L, but this was adjusted post-2007 to 2.0 mL/L, in line with the GEOTRACES protocols. (e.g., GEOTRACES—⟨www.obs-vlfr.fr/GEOTRACES/libraries/documents/Intercalibration/Cookbook.pdf⟩, Version 1.0: December 2010, accessed on 13 March 2013).

In addition to the field blanks, analytical blanks were included with the sample set for analysis. These included MQ water and 0.2% HCl (≡concentration of acid in acidified samples) blanks, and an 'analytical procedure' blank. The last evaluated the specific blank contribution from buffer, column rinse and elution solutions for our FIAS program. It was determined using an abbreviated loading step in the FIAS program analysing the buffer solution. The buffer alone was loaded onto the chelating sorbent from both the sample and buffer lines (Fig. 1) for a reduced time (30 s) equivalent to the amount of buffer introduced during the loading time of a regular sample (159 s) from the buffer line.

Reference seawaters (certified—e.g., NASS-5 from the National Research Council of Canada and community consensus—e.g., SAFe [22,23]) were used to assess method performance and analysed in each sample set.

2.5. *Analytical procedure*

The ICP-MS performance was assessed daily using a 1 μg/L Daily Performance Solution (1-in-10 dilution of stock ICP-MS Setup Solution, adjusted to 0.5% HNO_3). The sensitivity and relative standard deviations for Mg, In and U, and the oxide (CeO^+/Ce^+) and doubly charged ion (Ba^{2+}/Ba^+) formation ratios were monitored. For each analysis, the required specifications (Table S4) were achieved performing nebuliser and autolens optimisations, as necessary. The daily performance program was also run on a

MQ water sample to check the blank readings of the instrument. In addition, mass calibration tuning, detector voltages and dual detector optimisations were performed at the start of a set of analyses (e.g., the full quota from a voyage, 200–400 individual samples), and then every 1–2 months, depending on usage.

Before each analysis sequence, both minicolumns (1-cm sample preconcentration and 2-cm buffer cleaning, Fig. 1) were washed for 6 min with 10% HCl and then rinsed for 6 min with MQ water. The eluent line was rinsed with 5% HNO_3. During these steps, the opportunity was taken to confirm flow rates of sample/buffer and eluent lines. The calibration checks were carried out by weight, with three 1-min volumes collected. The peristaltic pump tubing was changed after every second sample set.

With calibration standards and Mo spike samples prepared (see Section 2.3), the only sample manipulation required for the seawater samples, blanks and reference seawaters was the transfer into acid-cleaned, 10-mL polypropylene tubes filled to the 10-mL mark. The tubes containing the samples were weighed before loading onto a 60-position autosampler rack. The arrangement and sequence of these in a typical batch set for analysis is shown in Table S2.

Once preliminaries were completed, the automated FI preconcentration/matrix-elimination sequence (Table 1) was initiated with samples mixed in-line with ammonium acetate buffer and passed through the chelating sorbent column for 159 s. After sample loading, the autosampler probe was sent to a MQ water rinse solution resulting in a flushing of the column for 60 s with diluted buffer solution. This rinse was continued for a further 15 s with the eluent flow-rate (Pump 2) increased in preparation for elution. The FIAS valve was switched to its second position (Fig. 1, lower panel) for elution direct to the ICP-MS. Meanwhile, the autosampler probe and supply tubing were flushed with 0.02 M HCl in readiness for the final step of 30 s of column conditioning with the same dilute acid.

With our set-up, the buffer was mixed with the sample in-line immediately before loading onto the 1-cm minicolumn. Throughout the analysis, the pH of the sample was monitored with 10-s readings of the column waste line. An acrylic (PMMA) flow-cell, made in-house, was used with a flat-ended pH electrode (TPS, Springwood, QLD, Australia) (Fig. 2). The pH readings from the electrode were logged to a smartCHEM-pH meter (TPS, Springwood, QLD, Australia). At the end of a day's analysis, the logged pH data was downloaded to a laptop for further processing and review if required.

For transient analysis, only one sweep/reading is possible, so the number of reading/replicates selected is important to create an accurate picture of the transient peak. Typical dwell time values for peak hopping are 20–100 ms, with the use of shorter dwell times recommended for transient analysis when determining a large number of analytes [24]. To match an elution profile time of 54 s, using a 40-ms dwell time and analysing 22 isotopes (Table 2), 55 readings was selected, with an estimated sample time of 54.4 s.

The peak area of each transient isotope peak registered by the ICP-MS, from elution of the chelating sorbent, was calculated using the 'sum' processing of the signal profile acquired by the ELAN software. A five-point baseline was used to eliminate any background readings. A macro (ICPMS Data Summary v2 (2006), Robinson Scientific Ltd., Cambridge, New Zealand) used the information in the ELAN output report file (∗.rep) and created

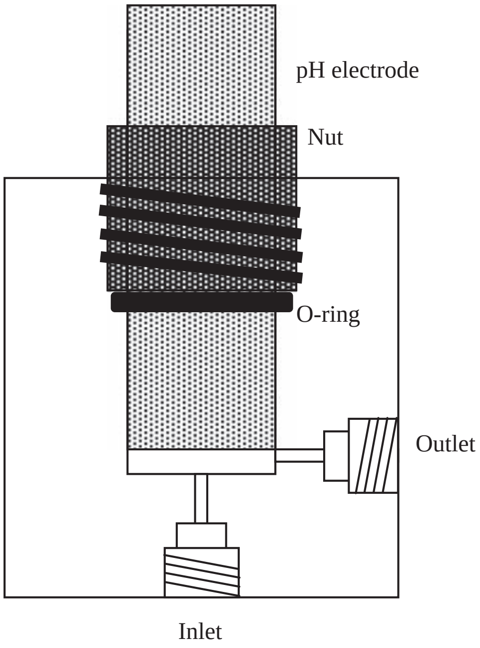

Fig. 2. Flow cell for pH measurement of the column waste from the FIAS system.

Table 2
List of isotopes monitored by ICP-MS detector; basis is the reason for measurement.

Isotope	Basis
^{59}Co, ^{60}Ni, ^{63}Cu, ^{65}Cu, ^{66}Zn, ^{68}Zn, ^{111}Cd, ^{206}Pb, ^{207}Pb, ^{208}Pb, (^{27}Al, ^{28}Si, ^{55}Mn)	A
^{13}C, ^{23}Na, ^{24}Mg, ^{35}Cl, ^{39}K, ^{43}Ca	M
^{23}Na, ^{24}Mg, ^{43}Ca, ^{98}Mo	I
^{103}Rh	S
220 amu	B

A: analyte elements (Al, Si and Mn residual elements from previous studies); B: background; I: interference; M: matrix; and S: internal standard.

Table 1
FIAS-400 program for the in-line preconcentration.

Step	Read	Duration (s)	Pump 1 (rpm)	Pump 2 (rpm)	Valve	A/S[a]	Operation
Pre	–	60	60	20	1	Sample	Load sample
Step 1		99	60	20	1	Sample	Load sample
Step 2		60	60	20	1	MQ	Rinse column (buffered MQ)
Step 3		15	60	35	1	MQ	Rinse column (buffered MQ)
Step 4	X	54	60	35	2	0.02 M HCl (Bottle 1)	Elute and read[b] (0.8 M HNO_3+10 μg/L Rh)
Step 5		30	60	20	2	0.02 M HCl (Bottle 2)	Wash column (0.8 M HNO_3+10 μg/L Rh)
Step 6		30	60	20	1	0.02 M HCl (Bottle 2)	Condition column (0.02 M HCl)
Post-run	–	–	0	20	1	0.02 M HCl (Bottle 2)	Standby

[a] Autosampler solution.

[b] Probe and sampling tubing also being washed (to waste) during this step.

three Excel spreadsheets: (1) raw data with the summary report data; (2) output intensities for each sample and each isotope; and (3) output concentration in the sample unit designated in the method file.

After sample analysis, the sample tubes were weighed so the volume aliquot sampled could be calculated and the reproducibility monitored for the analysis run.

2.6. Buffer pH investigation

For the investigation into the selection of the buffer pH, seawater samples with HCl added (to 0.01 M) were used as zero samples and also for the preparation of top standard addition samples (Table S3). The seawater zero samples were analysed in duplicate and the top standard addition samples in triplicate at each test pH. The samples were adjusted to the different pH values by merging in-line with buffers of pH values of 4, 6, 8.5 and 9, or MQ water (unbuffered) for pH 2. The buffers were made from solutions of either 1.0 M acetic acid (for buffers pH 4 and 6) or 1.0 M HCl (for buffers pH 8.5 and 9). The buffers were then adjusted to the selected pH using ammonia solution. The averaged corrected output intensity and ratios of standard addition to seawater zero were calculated for each trace metal over the pH range.

2.7. Estimation of efficiency of loading and recovery of trace metals on the chelating sorbent

The efficiency of loading was determined by comparing the peak area intensity of a top standard addition to a 'zero' seawater sample measured by the in-line FI preconcentration procedure, to an expected peak area intensity of a standard in MQ water measured by direct analysis. The peak area (intensity) from the standard addition was interpolated from the integrated counts determined by the ELAN software. The time required to produce an equivalent volume to the preconcentration sample using the direct analysis flow rate of 1 mL/min was determined. This time was used to extrapolate the integrated intensity determined by the ELAN software and to calculate the expected peak area intensity. The resulting efficiency of loading takes into account not only the extraction itself but also influences from all components of the FI system and ICP-MS.

Recoveries were rigorously assessed by first establishing the calibration curve using the recommended sequence of standard additions to the zero sample. Then, the concentration increment for the top standard addition was established independently by running the zero sample again in duplicate followed by the top standard addition (see Table S3) in duplicate. This was followed by a 'test' sample analysed in duplicate, and then with the top standard addition also in duplicate. Percentage recovery was evaluated as the concentration increment for the top standard addition to the test sample relative to that for the zero (reference) sample. Both samples were natural oceanic water subsurface from the mixed layer of the Subantarctic Zone, with a zero (calibration) sample from the south-east of Tasmania (SAZ-Sense voyage [25], Station 17j), and the 'test' sample to the south of Tasmania (CASO-GEOTRACES SR3 [26], Station N23).

2.8. Analytical quality assurance

The set up of the sample introduction and its ongoing reproducibility; and the value of the extraction pH at 10-s intervals were monitored for each analysis (Section 2.5).

The daily analysis included several quality control samples: various blank solutions from MQ water, buffer and acid reagents; multiple certified (NASS-5) and consensus (SAFe) reference seawaters; replicate samples; replicate seawater samples with a 10 μg/L Mo spike added; preconditioning and rinse samples for both 0.2% HCl and seawater solutions (Table S2).

Of the 22 isotopes measured (Table 2), several were included for quality assurance purposes. The efficiency of the rinse steps in removing the seawater matrix from the minicolumn, before elution, was evaluated by monitoring the residual intensities of major seawater ions (Na, Mg, K, Ca and Cl) in the eluent. Rhodium was added to the eluent and measured to track detector sensitivity and instrument stability. Carbon was measured as an indicator of any buffer delivery problems. Two isotopes of Cu and Zn were measured to identify potential polyatomic interferences, particularly those that might arise from ineffective removal of major seawater cations Ca and Na. The background signal was monitored by measuring at 220 amu.

The 'real time' display in the ELAN software was used to view and monitor the elution profile for six selected isotopes (^{59}Co, ^{60}Ni, ^{65}Cu, ^{66}Zn, ^{111}Cd, ^{208}Pb) during the analyses, remotely if convenient. The file of intensity as a function of time was saved at the end of each analysis. The data was used to monitor Rh in the eluent and a macro was used to create profile charts for the elements of interest for further investigation if required.

3. Results and discussion

3.1. ICP-MS and FI set up

When analysing the daily performance solution at the beginning of a run, the Mg signal could be elevated from previous analysis of seawater. This was traced to Mg adsorbed to the CETAC sample probe and sample introduction system. Although sitting the probe overnight in MQ water reduced this problem, the incorporation of a 3-way valve enabling direct delivery (excluding the CETAC autosampler probe) of the daily performance solution to the ICP-MS improved it further so that Mg values were within an acceptable range (9000–15000 cps with a slightly elevated precision of, $<2.5\%$). The 3-way valve also enabled easy interchange between analyses by direct injection and those using the FI set-up for preconcentration/matrix elimination.

Using an in-line FI system with continuous ICP-MS detection (i.e., transient peaks in eluent), it was not feasible to switch between standard (STD) and Dynamic Reaction Cell (DRC) modes for recording peaked profiles. Therefore, a split was made between trace metals amenable to STD-mode detection (this method) and trace metals suffering polyatomic interferences with a quadrupole ICP-MS. During our early pH investigations using both STD and DRC modes, $^{54/56}$Fe (^{40}Ar^{14}N, ^{40}Ar^{16}O), ^{51}V (^{35}Cl^{16}O, ^{37}Cl^{14}N) and ^{55}Mn (^{39}K^{16}O) all showed effects of interferences, although ^{55}Mn to a lesser degree.

When processing of the signal profile acquired by the ICP-MS software small irregularities in peak shape from analysis to analysis adversely affected quantitation by peak height. Peak-area processing yielded results that were less variable, and it was consequently implemented in our method in comparison to others [17–19].

Our initial method development began with a similar FI configuration to that described in Hirata et al. [17,18], who also used a FIAS 400 system. We incorporated in-line buffering [12,19] a dilute buffer wash solution [18,19]; an internal standard (10 μg/L Rh) [19] in the nitric acid eluent and Toyopearl IDA sorbent in a tapered minicolumn [12,19]. In addition, we utilised the autosampler to introduce the wash solutions instead of using T junctions [12,19] which could result in dead volume and problems with reagent mixing; introduced a mixing loop for the buffer and sample; in-line pH monitoring; flow-rate monitoring and matrix-matched standards. By including the buffer in-line, the acidified seawater samples could be neutralised immediately before analysis, resulting in an absolute minimum interval conceded at neutral pH for refractory dissolved organic matter to complex or recomplex trace metals [27]. The tapered design of GlobalFIA minicolumn, and the eluent in countercurrent flow, minimises dispersion and focuses the preconcentrated zone. Countercurrent elution also averts the sorbent packing becoming ever tighter and minimises its blocking [Fang et al. [11]]. The mixing loop assisted the merging of the buffer and sample prior to loading; the in-line pH enabled the FI analytical sequence to be monitored; flow-rate checks ensured the system lines were maintained and the matrix-matched standards assisted in retaining a similar matrix to that of the samples to promote reproducibility in the analytical sequence. A summary of method optimisation steps are documented in Table 3.

The selection of FI flow rates was constrained by physical (e.g., back-pressure, mixing efficiency of intersecting sample and buffer streams) and operational factors (e.g., maximum flow rate to nebuliser of 3 mL/min). Our loading of the merged buffer and seawater sample onto the IDA sorbent in the minicolumn was at 3.40 mL/min (Pump 1: 2.65+0.75 mL/min, to waste). The loading flow rate was within the operational bounds recommended by the manufacturer (2.0–8.0 mL/min, for larger columns [28]), and within the range of other published reports using the same Toyopearl IDA sorbent for trace metals from seawater (1.0–4.0 mL/min [12,19,21,29]). The full optimisation of this step was not investigated although an alternative configuration (with FI transfer lines of 0.5-mm i.d. tubing) to improve sample throughput was tested with a 36-s read time, but back-pressure causing leakages within the FIAS system made this unworkable.

The choice of eluent flow rate was influenced strongly by ICP-MS requisites—sample introduction and analyte peak measurement. Pump 2 delivered 2.00 mL/min in the step immediately preceding and during elution. At other times, it operated at 1.15 mL/min to conserve eluent and concurrently satisfy standby operation of the ICP-MS. The elution flow rate is again consistent with other published methods using Toyopearl IDA sorbent, and the same eluting acid (1.0—2.4 mL/min; HNO_3 [19,21,29]).

Table 3
Method optimisation steps.

- **Analytical set up**
  - Minimise analyst handling with sample/standard preparation
  - Matrix-matched standard addition
  - Buffer mixing in-line
  - Buffer cleaning in-line
  - In-line pH monitoring
  - Monitoring of flow rates in FIAS system
  - Processing of output signal peak—peak area vs. peak height
  - 'Real-time' display monitor (remote)
  - Blank controls
- **Sorbent and matrix elimination**
  - Rinse solution selection and volume
  - Effects on major seawater cations removal
- **Buffer selection and pH monitoring:**
  - Response output for buffer pH 2, 4, 6, 8.5 and 9
  - In-line pH monitoring
- **Loading efficiency and trace metal recovery tests**
  - **QA and QC**
    - Analysing multiple isotopes Cu and Zn
    - Multiple blanks, conditioning and references solutions
    - Mo spike for Cd correction
    - Rh internal standard
    - Monitoring major seawater ions isotopes
- **Interference effects**
  - Investigate results of multiple isotope analyses and subsequent ratios
  - Determine sensitivity to major seawater ions
- **Figures of merit and method validation**
- **Application of method to open ocean waters**

The step durations in Table 1, at the chosen flow rates, were verified to adequately flush the dead volume of the FI manifold (tubing, mixing loop, etc.) and propel the analytes through the ICP-MS sample introduction system.

The routine monitoring of flow rates showed that the variability in flow rates over a complete set of analysis was $<4\%$ for both the eluent and sample/buffer flows when tubing was changed regularly after two sets of analysis.

Nitric acid was chosen as the eluent in preference to HCl to avoid adding chloride that would lead to additional polyatomic interferences. A similar concentration as described in Hirata et al. [17] was used. A 0.8 M concentration was used in preference to their 0.7 M, simply for ease in preparation of a 5% solution; others have used 0.1–2.0 M HNO_3 to elute IDA sorbents [12,16,19,21,29–31]. A higher concentration was not used here, because 0.8 M was the maximum recommended by the instrument manufacturer for high-volume, continuous flow to the ICP-MS system.

With an eluent flow of 2.0 mL/min and the described FI set-up, all six trace metals Cd, Co, Cu, Ni, Pb and Zn, preconcentrated from an oceanic sample, eluted baseline to baseline within 39 s (Fig. 7). The five baseline readings recorded during the initial 11–13 s were used as a baseline for the ELAN software processing of peak area. During the method development, the read time was reduced to remove unnecessary trailing background readings and minimise the analysis time per sample. A 54-s read time satisfied these objectives, while still capturing the elution profile fully.

3.2. Chelating sorbent and matrix elimination

When the choice of sorbent was considered, it came down to the available IDA or 8-hydroxyquinoline (oxine) as chelating

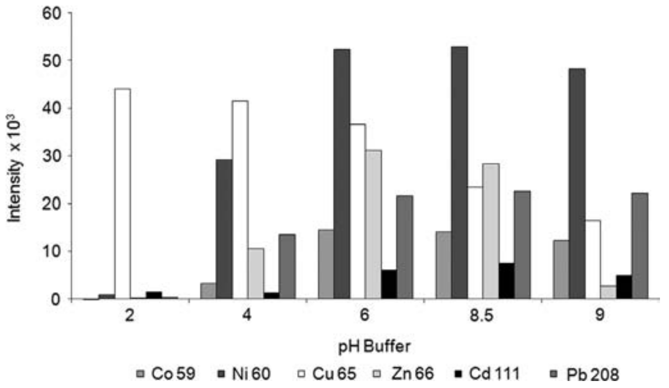

Fig. 3. The averaged ($n=3$) output intensity of standard addition seawater samples using different pH buffer loadings. Intensities corrected with 'zero' seawater reading.

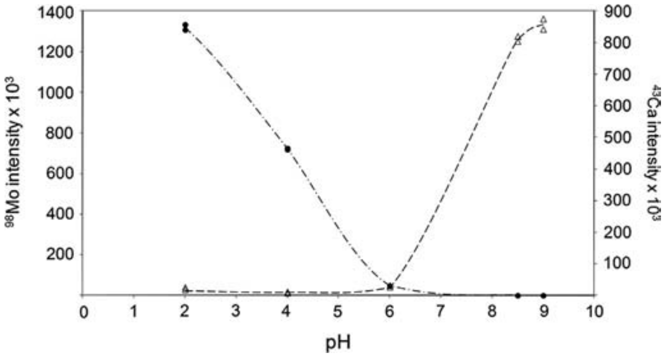

Fig. 4. The pH dependence of ^{98}Mo (●) and ^{43}Ca (Δ) intensities, as potential interferences, retained from oceanic seawater ($n=2$).

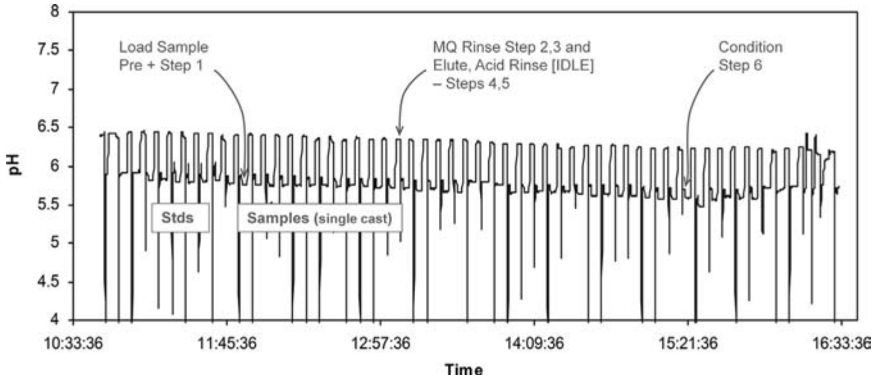

Fig. 5. pH trace of an analytical run (standards, samples, blanks and quality control tests) as recorded by in-line pH probe in waste stream.

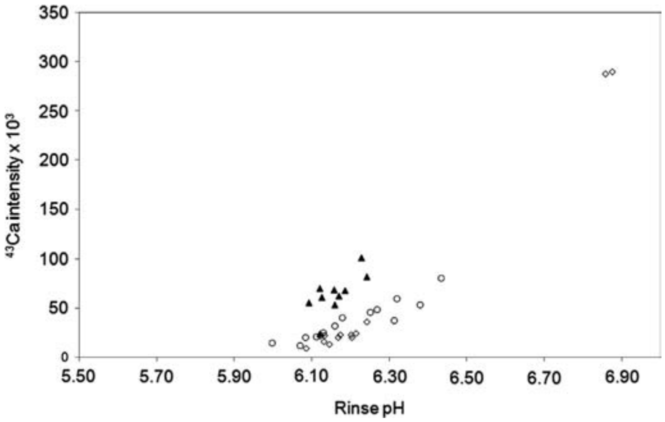

Fig. 6. The relation of the pH of the rinse solution (dilute ammonium acetate buffer), and the output intensity of ^{43}Ca retained from oceanic seawater. Voyage samples: SAZ-Sense (2007, ▲), CASO-GEOTRACES SR3 (2008, ○) and PINTS (2010, ◇).

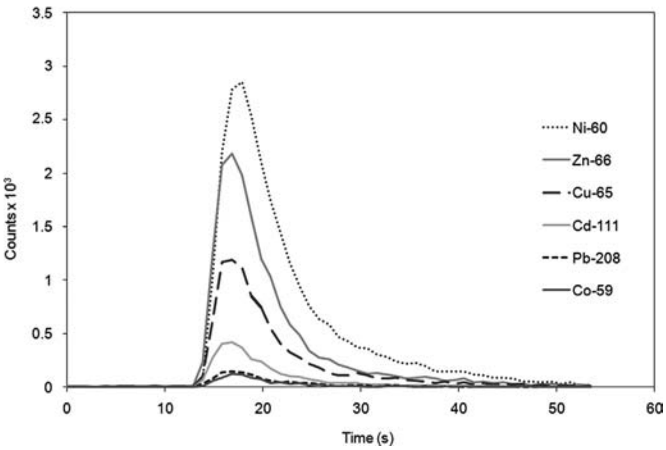

Fig. 7. Real-time elution profile of six trace metals from a sample collected at a depth of 1000 m in the Southern Ocean (51° 32.37′ S, 142° 59.73′ E).

functional groups. From the IDA sorbents, Toyopearl AF-chelate-650M was selected because of its high capacity (35 ± 10 μeq/mL, ligand density≅20 μmol/mL; ⟨www.separations.eu.tosohbioscience.com/Products/ProcessMedia/ByMode/AFC/ToyopearlAF-Chelate-650.htm⟩, accessed on 14 March 2013); inert, hydrophilic methacrylate polymer support; and its lack of swelling/shrinking associated with changes in pH or ionic strength [11,12]. The Toyopearl IDA sorbent is regularly used in analyses of seawater for trace metals (e.g., [12,14,19,29]). The test oxine sorbent was synthesised in the laboratory of Prof. William Landing [32], and has proved reliable for determination of trace metals in open-ocean samples (e.g., [33]).

Early experiments (results not shown) established that for the trace metals Co, Cu, Ni, Pb and Zn, in regard to analytical sensitivity, blanks and precision, the IDA sorbent at neutral pH performed better than oxine. Cadmium performed best at alkaline pH for both IDA and oxine—with IDA the better of the two. At neutral pH, Cd gave comparable results for both IDA and oxine.

The chelating sorbent is required to retain trace metals as universally as possible and to efficiently exclude seawater salts, particularly Group 1 and 2 cations that could give rise to interferences. The subject has been considered extensively by Beck et al. [19]. Their information for potential polyatomic interferences arising from the seawater matrix for our six trace metals and their monitored isotopes is incorporated in Table 4 with additional information sourced from [34,35].

The major cations of seawater (e.g., $\mathrm{Na^{+}}$, $\mathrm{K^{+}}$, $\mathrm{Mg^{2+}}$ and $\mathrm{Ca^{2+)}}$ causing matrix effects or contributing to polyatomic interferences [36] are bound by ion exchange and chelation, although the latter is negligible for the alkali metals $\mathrm{Na^{+}}$ and $\mathrm{K^{+}}$ and weak for $\mathrm{Mg^{2+}}$ and $\mathrm{Ca^{2+}}$; the order of binding onto IDA is $\mathrm{Ca^{2+}} > \mathrm{Mg^{2+}} > > \mathrm{K^{+}} \approx \mathrm{Na^{+}}$ [37]. We found, in common with previous reports on IDA sorbents [12,19,21,38] that effective removal of the major seawater cations ensued with the rinsing solution containing the ammonium acetate buffer (with the $\mathrm{NH_4}^{+}$ ion as a weak eluent displacing $\mathrm{Ca^{2+}}$ and $\mathrm{Mg^{2+}}$). Using our recommended conditions (with ICP-MS intensity as a means of quantitation), 99.995% of $\mathrm{Na^{+}}$, $\mathrm{K^{+}}$, and $\mathrm{Mg^{2+}}$, and 99.98% of $\mathrm{Ca^{2+}}$ were excluded by the rinsing step.

The effect of the volume of rinsing solution is shown for $\mathrm{Ca^{2+}}$ in (Fig. 6). For the analysis of (SAZ-Sense samples) 3.4 mL of 0.22 M ammonium acetate was used, which proved sufficient to remove all major cations to levels that did not interfere. In later analyses (CASO-GEOTRACES SR3 and PINTS [39] voyages), to give further leeway the volume was increased to 4.25 mL with a matching depression in ^{43}Ca intensity at ICP-MS detector observed.

The ^{63}Cu/^{65}Cu ratio was monitored to gauge the effectiveness of the elimination of $\mathrm{Na^{+,}}$ and $\mathrm{Mg^{2+}}$ If neither are present to cause interference (see Table 4), the ratio should be 2.24—matching natural abundance. For 302 oceanic samples (from the CASO-GEOTRACES voyage), a mean ratio of intensities of 2.16 ± 0.04 (range for 14 analytical runs 2.12–2.22) was recorded. In addition, no relation with ^{23}Na/^{25}Mg was observed, and results from either Cu isotope were equivalent for the complete sample set. It appears that the slightly low value for the ^{63}Cu/^{65}Cu ratio is an artefact of the ICP-MS and our operating conditions, rather than a minor residual interference from $\mathrm{Mg^{2+}}$. Greenway et al. [40] reported a ratio of 2.17 ± 0.12, in the absence of matrix effects, for the ratio from spiked seawater using a similar FI-quadrupole ICP-MS method with IDA groups immobilised on a controlled pore glass support. They attributed the marginally low value to "mass discrimination effects and mass bias, owing to sequential scanning of the transient peak".

Table 4
Potential polyatomic interferences on analyte trace metals analysing seawater by quadrupole ICP-MS with Ar plasma. Information sourced from [19,34,35].

Mass	Isotopes	Isotopic abundance (%)	Potential polyatomics[a]	Interference isotopic abundance (%)[b]
59	^{59}Co	100	$^{24}Mg^{35}Cl$	59.9
			$^{42}Ca^{16}OH$	0.6
			$^{23}Na^{36}Ar$	0.3
			$^{40}Ar^{18}OH$	0.2
			$^{43}Ca^{16}O$	0.1
60	^{60}Ni	26.1	$^{23}Na^{37}Cl$	24.2
			$^{44}Ca^{16}O$	2
			$^{24}Mg^{36}Ar$	0.2
			$^{43}Ca^{16}OH$	0.1
63	^{63}Cu	69.2	$^{23}Na^{40}Ar$	99.9
			$^{31}P^{16}O_2$	99.8
			$^{23}Na^{16}OH$	99.8
			$^{23}Na^{40}Ca$	96.9
			$^{24}Mg^{23}Na^{16}O$	79.0
			$^{16}O^{12}C^{35}Cl$	75.5
			$^{14}N_2{}^{35}Cl$	75.5
			$^{14}N^{12}C^{37}Cl$	24.1
			$^{26}Mg^{37}Cl$	2.7
			$^{36}Ar^{27}Al$	0.3
65	^{65}Cu	30.8	$^{32}S^{16}O_2H$	94.8
			$^{32}S_2H$	90.3
			$^{33}S^{16}O_2$	74.8
			$^{12}C^{16}O^{37}Cl$	24.2
			$^{37}Cl^{14}N_2$	24.1
			$^{12}C^{18}O^{35}Cl$	15
			$^{25}Mg^{40}Ar$	10
			$^{32}S^{16}O^{17}O$	3.6
			$^{32}S^{33}S$	0.7
			$^{36}Ar^{14}N_2H$	0.3
			$^{31}P^{16}O^{18}O$	0.2
			$^{48}Ca^{16}OH$	0.2
66	^{66}Zn	27.9	$^{26}Mg^{40}Ar$	11
			$^{50}Cr^{16}O$	4.3
			$^{34}S^{16}O_2$	4.2
			$^{32}S^{34}S$	4
			$^{33}S^{16}O_2H$	0.7
			$^{32}S^{16}O^{18}O$	0.2
68	^{68}Zn	18.8	$^{51}V^{16}OH$	99.5
			$^{40}Ar^{14}N_2$	99.4
			$^{40}Ar^{12}C^{16}O$	98.5
			$^{40}Ar^{28}Si$	91.9
			$^{52}Cr^{16}O$	83.6
			$^{35}Cl^{16}O_2H$	75.6
			$^{34}S^{16}O^{18}O$	0.8
			$^{36}Ar^{16}O_2$	0.3
			$^{36}Ar^{32}S$	0.3
			$^{34}S_2$	0.2
111	^{111}Cd	12.8	$^{95}Mo^{16}O$	16
			$^{39}K_2{}^{16}O_2H$	93

[a] Potential polyatomic interferences—few, if any, are expected for ^{206}Pb, ^{207}Pb or ^{208}Pb in seawater.

[b] Interference isotopic abundance is the product of the individual isotope abundances calculated using IUPAC.

The IDA functional group carries a net negative charge at slightly acidic to neutral pH; it is rigorously described as a chelating ion exchange sorbent. One advantage of this net negative charge is that the major anions of seawater (Cl^-, $SO_4{}^{2-}$, etc.) will be excluded from the sorbent, and should emerge with the minicolumn void volume. Polyatomic interferences suggested arising from Cl (or potentially S) in Table 4 can be overlooked; nonetheless, ^{35}Cl (Table 2) continued to be monitored as an indicator of successful matrix elimination. The results for the $^{63}Cu/^{65}Cu$ ratio also support the effective removal of the chloride interference on ^{63}Cu (see Table 4).

3.3. pH optima and monitoring

The effect of the buffer pH on extraction efficiency of the IDA sorbent for our selected six trace metals is shown in Fig. 3. Copper was retained best under acidic conditions, declining with increasing pH, until at pH 9 it was less than half the response at pH 2. Co, Ni and Zn gave their best response at near neutral pH (6 and 8.5). Cadmium was retained most strongly at pH ~8.5, with a decreased response at pH 9. Lead was retained most strongly at pH 8.5 with slightly lower response for pH 6.5 and 9. The most suitable condition for all six metals was pH ~6. Sumida et al. [41] found very similar results with pH profiles for trace metal extraction from seawater on Muromac A-1—another IDA sorbent. They selected an optimum pH of 5.5 for a set of 11 trace elements (Al, V, Fe, Co, Ni, Cu, Zn, Cd, Pb, Th and U), including our six. Hirata [17] also selected in a similar range with pH 5.0 for simultaneous determination of multiple elements in seawater using Muromac A-1.

Another factor in refining the choice was the pH-dependent response of interferences. These included major seawater ions, as well as other trace elements (e.g., Mo—as $^{95/98}Mo^{16}O$ on $^{111/114}Cd$) causing polyatomic interferences. In Fig. 4, the retention of Ca (monitored as ^{43}Ca) on the Toyopearl IDA sorbent rises sharply for pH > 6. Whereas, Mo (as ^{98}Mo) declines from acidic pH; it is undetectable for pH≥8.5 and low but measurable at pH 6. Once more, the effective compromise pH was ~6.

An indirect measure of interferences was also gained by evaluating the response ratios of the top standard addition to zero seawater samples (results not shown). For example, the increased retention of Ca at alkaline pH caused decreased response ratios for ^{59}Co, and to a lesser degree ^{60}Ni, at pH 8.5 and 9 with the emergence of polyatomic interferences from $^{43}Ca^{16}O$ and $^{44}Ca^{16}O$.

The pH buffering affects the efficiency of extraction of trace metals as reported here and by others [17,41] and the removal of interferents, also identified here and previously [5,19,21,38]. Therefore, the reliability and reproducibility of the preconcentration procedure, and also as a direct consequence—the consistency of ICP-MS detection—depend vitally on maintaining a constant pH (or operationally, in a very narrow range). A narrow pH range was also found to minimise the variation in Mo signal and consequently the variability of the Cd correction value. The average difference over the complete set of analyses was < 5 pM.

A representative trace of 10-s readings during a full analytical sequence (Fig. 5) demonstrates the efficacy of this arrangement in monitoring the pH of the buffered sample during loading. For samples acidified to 0.01 M HCl (SAZ-Sense voyage), the buffer constrained the sample pH to 5.75 ± 0.08; for those latterly acidified to 0.02 M HCl, and with a different buffer, it was 5.58 ± 0.08.

An additional benefit of continuous pH monitoring was that other steps in the preconcentration process could be monitored. For example, the only two occasions, during a single analytical run, that Ca^{2+} interference (as polyatomics) was observed, was when the rinse pH was aberrant at 6.8, instead of < 6.25 (Fig. 6). The resulting ~10-fold increase in ^{43}Ca intensity over the mean background exceeded the threshold and created a signal equivalent to ~17 pM ^{59}Co and 0.27–0.50 nM ^{60}Ni. With a pH increase for the rinse buffer solution from 5.3 to 8.5 for the same Toyopearl IDA sorbent, Willie et al. [21] observed a 150-fold increase in Ca^{2+} intensity (consistent with Fig. 4) and a 15-fold increase in Mg^{2+} intensity.

3.4. Interferences

The suppression of interferences arising from the major seawater cations that are retained residually by the IDA sorbent has already being dealt with in earlier Sections 3.2 and 3.3.

Many of the remaining polyatomic interferences are generated by plasma gas argon and ubiquitous elements hydrogen, oxygen, carbon and nitrogen. The major plasma interferences (Table 4) are seen on ^{68}Zn with ^{40}Ar^{14}N$_2$ and ^{40}Ar^{12}C ^{16}O both at 99% interference isotopic abundance. Our general observation was that ^{68}Zn response was greater and more variable than that of ^{66}Zn. In addition to the plasma gas interferences on ^{68}Zn, Cr and V are present in seawater at nanomolar and tens of nanomolar concentrations, respectively [20]. ^{51}V^{16}OH and ^{52}Cr^{16}O, with interference isotopic abundance of 99.5% and 83.6% respectively, could also have an effect although ^{53}Cr was not evident in the eluent of seawater samples analysed at pH 6 when comparing MQ and seawater samples analysed in an earlier test (results not shown). The isotope ^{66}Zn was used, because it is in greater abundance, and less prone to polyatomic interference. Of the other plasma interferences ^{40}Ar^{18}OH, ^{36}ArN$_2$H and ^{36}Ar^{12}C^{14}N H on ^{59}Co, ^{63}Cu and ^{65}Cu respectively, all have relatively low interference isotopic abundance between 0.2% and 0.3%. Detectable interference from these polyatomics was not observed in our blanks. The interferences from the formation of metal argide and metal oxide species have been addressed earlier with the significant reduction of Ca, Mg, K and Na in the preconcentration process.

Of the Cu isotopes, 63 and 65, ^{65}Cu was monitored. Although it is the copper isotope in lesser abundance, its polyatomic interferent (Table 4) is proportionally much less abundant than that affecting ^{63}Cu. It has been demonstrated operationally in Section 3.2 that either isotope can be used.

Both isotopes of Cd, 111 and 114, are affected by Mo interference to a similar degree [19,21]. At pH 6, we found that Mo retention on the IDA sorbent had declined sharply from its peak at lower pH (Fig. 4). Beck et al. [19] have reported that MoO amounted to ≈0.5% of the Mo signal for their operating conditions. We evaluated that the extent of the interference of the polyatomic ^{95}Mo^{16}O on ^{111}Cd was sufficiently diminished at our chelation pH (5.5–6.0) for us to include Cd among analyte trace metals. However, as a precaution Mo was included in the set of isotopes measured; and standard addition of Mo to a subset of samples (see Section 2.3) enabled direct correction of Cd data. The Mo interference did not affect Cd results for the majority of samples analysed (e.g., 91.3% from CASO-GEOTRACES SR3 voyage unaffected) but minor corrections were evident on low-level concentrations (typically <50 pM).

Isobaric interferences (isotopes of different elements having the same mass) are not manifest in seawater for the six trace metals that are the focus of this work.

3.5. Blanks and contamination control

The field blank effectively emulates the treatment and exposure of seawater samples to contamination. Field blanks of acidified MQ water (0.2% HCl) varied between nanomolar and picomolar depending on trace metal: Zn > Cu > Ni > Co≈Cd≈Pb. Results for assays covering the CASO-GEOTRACES voyage are depicted in Fig. 8. The main aggregation of blank values for each trace metal fell within an order of magnitude; occasional outliers were outside this interval. Cobalt blanks were effectively below the limit of detection (3 × standard deviation of blanks). Others were above the limit of detection, but generally below the concentration range of the metal in oceanic waters. Zinc and Cd were exceptions; in low-latitude surface waters (e.g,. subtropical SW Pacific), the blank concentration in 0.2%-HCl acidified MQ water exceeded that in samples.

No strong correlation was found between any of the trace metals in the blank data (results not shown), which suggests that they were not derived from a common source, but rather several unrelated and haphazard sources. The blanks for all metals in acidified MQ water exceeded the analytical procedure blanks (see Section 2.4) by factors of 1.14 (for Zn) to 5.34 (for Cd), suggesting that both MQ water itself and acid (HCl) contributed. We discerned that Cu and possibly Ni were higher in acidified than unacidified MQ water, and also higher again for 0.2% HCl (acid blanks for CASO-GEOTRACES SR3 and PINTS voyages) compared with 0.1% HCl (acid blanks for SAZ-Sense). This indicates that HCl used for seawater sample preservation contributes a background for at least Cu, and possibly Ni, but it is only one of several sources. With a coefficient of determination, $r^2 = 0.299$ for the relation between Cu and Ni, only about 30% of variation of Cu is explained by Ni.

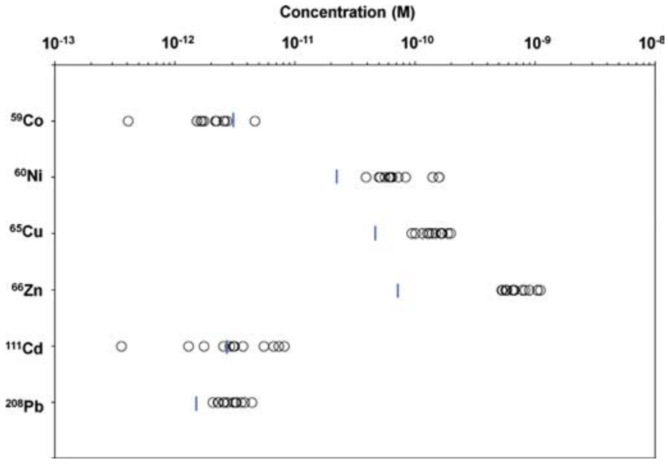

Fig. 8. Field blank concentrations (0.2% HCl). for six trace metals Co, Ni, Cu, Zn, Cd and Pb, no corrections were made. (|) Detection Limit (3 s.d. of blanks see Table 6).

Table 5
Loading efficiency of spiked seawater samples onto IDA sorbent, relative to direct ICP-MS analysis of standards in MQ water; and recoveries of top standard addition to Southern Ocean seawater.

	Co	Ni	Cu	Zn	Cd	Pb
Loading efficiency (%)	80	58	83	53	43	60
Recovery (%)	96.7	102	102	98.1	92.2	97.6

In comparing the blank data with Ho et al. [31], who used ion chromatography on an IDA sorbent before detection with sector field ICP-MS, the same blank order for the six trace metals (i.e., Zn > Cu > Ni > Co≈Cd≈Pb) was found. Similar acidified water blanks for Zn were determined, but our blank concentrations for the other five metals were lower by up to an order of magnitude. Using a clean room environment and minimised sample handling would have been factors in this improvement.

3.6. Loading efficiency and recovery of trace metals on IDA sorbent

Loading efficiencies were determined by comparing intensities of spiked seawater samples from elution profiles from the IDA sorbent (after preconcentration/matrix elimination) with the intensities from the direct ICP-MS analysis of standards in unacidified MQ water. The efficiencies (Table 5) are a factor of both thermodynamics and kinetics of adsorption and elution for each trace metal onto the sorbent from the seawater matrix, and also of the physics of the ICP-MS introduction system (minor differences in matrix and flow rates for the two types of measurement). The efficiencies ranged from 43% for Cd to 83% for Cu. Little difference was seen in these for seawater samples acidified to 0.1% HCl compared to those acidified to 0.2% HCl. The variations noted among elements for loading efficiencies were an impetus to standardise and make the procedure as rigorously repeatable as possible—for example, in-line calibration by standard addition was chosen.

Recoveries of the six trace metals for the top standard addition to an oceanic seawater are also listed in Table 5. They were quantitative for all metals within precisions reported in Table 6. Although loading efficiencies suggest incomplete sorption of metals for the loading flow rates and adsorption kinetics (i.e., not reaching equilibrium), the excellent repeatability of the fully automated flow-injection procedure and the standard-addition calibration assured quantitative recoveries of the six trace metals.

3.7. Quality assurance and quality control

The final FIAS program selected ran for 6 min per sample which enabled the in-line preconcentration and ICP-MS determination of up to 60 samples in a day. Of the 60, 24 were actual oceanic water samples and the remainder were calibration standards or quality control samples (blanks, reference seawaters, interference test samples or preconditioning solutions—Table S2). The duplicate zero seawater sample provided a check of any carry-over from the top standard addition immediately preceding the first zero seawater, but this was rarely found. Using a sample from the set of seawater samples, to be analysed on the day, for the standard additions calibration provided a true matrix match for the run.

Preliminary flow checks and measurements of sample aliquots loaded (by mass) showed the reproducibility of the FI set up and throughout the analysis run.

The in-line pH probe provided continuous monitoring of the preconcentration procedure. The stored pH plot enabled confirmation of correct chelation pH for each sample. However, it also had the unanticipated benefit of tracking the complete flow-injection sequence. The trace proved very useful in identifying and rectifying problems, and discriminating between 'good' and 'bad' data.

Real-time viewing of the elution profile was a further surveillance tool covering multiple aspects of the analysis, including the preconcentration process, sensitivity, blanks and baseline. The exported intensity versus time plots were available post analyses for detailed review, if required.

The output intensities of the isotopes of Na, Mg, K, Ca and Cl gauged the effective exclusion of alkali and alkaline earth metals and chloride during the preconcentration and rinsing steps. The multiple isotopes for Cu (63, 65) and Zn (66, 68) allowed interferences to be examined. The repeat top standard, analysed later in a run, provided an initial check on drift in sensitivity. However, it was the intensity record of ^{103}Rh in the eluent that provided a definitive assessment of the stability and reproducibility of an individual analytical run, and between runs. We were guided by Beck et al. [19] to correct for sensitivity drift only if the ^{103}Rh record varied by greater than 5%. Such correction was not required in our experience. During analysis of the sample set for the CASO GEOTRACES SR3 voyage (runs, $n=14$), the average variance was 1.6%, with a maximum observed of 3.5%.

The quality control measures incorporated in each sample set, as well as operating procedures universally, played an important role in the reproducibility and reliability of this method.

3.8. Figures of merit and method validation

The performance of the recommended procedure is demonstrated using the figures of merit from the analyses of seawater samples from the CASO GEOTRACES SR3 voyage (Table 6). Data from two other voyages were similar; some differences for the early voyage SAZ-Sense accrue from a more dilute acid preservation of samples and the lower volume of ammonium acetate rinse solution.

3.8.1. Calibration curves and linearity

The calibration curves were assessed by both coefficient of determination (r^2) and a test of linearity. Table 6 shows the average r^2 values and standard deviations for 13 sets of analyses. The row beneath presents the maximum concentration for each trace metal up to which a linear calibration has been established. The linearity was evaluated from plots of standardised residuals (with a threshold of ±2 [42]). Our results demonstrate linearity of at least two to three orders of magnitude for the in-line FI procedure.

3.8.2. Method limits of detection

The limits of detection (LOD) for the six trace metals were estimated from the blank values obtained with a field blank acidified during the CASO GEOTRACES voyage (see Sections 2.4 and 3.5). Seven measurements were made during a single analysis run; the limits of detection are reported in Table 6 as 3 × s.d. of the blank measurements. An improved LOD compared with earlier work using in-line FI procedures [12,17–19] was achieved, including an improvement greater than an order of magnitude for Ni, Cu and Zn [17–19]. The improvement to the LOD may be attributed to the selection and combination of previous FI processes used in conjunction with the wash solution via the autosampler, the sample and standard preparation procedures and the monitoring tools used. Warnken et al. [29] had improved levels of detection but their method was not automated and they replaced the FIAS switching valve with a Teflon valve (an arrangement that is likely to minimise contamination). In comparison to batch procedures, LODs were comparable to [15] apart from the result for Co, but our LOD for Co was comparable to those obtained in [13,14] using a batch procedure.

3.8.3. Precision

Estimates of precision were obtained from repeat analyses of the reference seawater SAFe D2 (Table 6). The replicate determinations were between analysis runs, rather than from within a single run. The coefficients of variation (CVs) were estimated from 14 replicates obtained over the period 22 April–23 July 2010 during the complete analysis of the sample set from the

Table 6
Figures of merit for analytical method for samples analysed from CASO GEOTRACES SR3 voyage.

	Co	Ni	Cu	Zn	Cd	Pb
r^2, Calibration curve[a]	0.9997 (0.0004)	0.9995 (0.0005)	0.9991 (0.0004)	0.9996 (0.0004)	0.9997 (0.0003)	0.9997 (0.0006)
Linearity of calibration curve (nM)[b]	0.89	24	9.6	20	2.8	0.59
Limit of detection (pM)	3.2	23	46	71	2.7	1.5
Precision (CV, %)[c]	8.6	3.4	6.5	5.4	4.1	5.5

[a] Average ($n=14$; each from a separate analysis run) and standard deviation (in parentheses) of calibration curves.
[b] Maximum concentration demonstrated for linear calibration curve, using a linearity test described in the body of text [42].
[c] Precision is estimated from 14 replicates of SAFe D2 reference seawater (Bottle 344), each from a separate analysis run on a different day. The concentrations at which the precision is reported can be found in Table 7.

CASO-GEOTRACES SR3 voyage. This is a more demanding estimate of precision than is typically used, where the replicates would all come from a single analytical sequence. Even so, all CV results were under 10%; the range was Ni 3.4% to Co 8.6%. SAFe D2 were subsurface samples from 1000 m; they had higher concentrations for the nutrient-like trace metals Cd, Cu, Ni and Zn (Table 7). SAFe S1 surface water ($\sim$2 m) had much lower concentrations of these same elements. Cadmium and Zn were below, and Co verged on, the limit of detection. The lower concentration of Cu (0.51 nM) resulted in greater imprecision with a CV of 13.5% ($n=26$). However, CVs for Ni and Pb in SAFe S1 were comparable with SAFe D2 at 4.3% and 5.1%, respectively. Procedures to maintain a reproducible system were a key emphasis of the method presented, using matrix-matched calibration, peak-area processing, and the use of rigorous quality controls and monitoring tools. The level of precision obtained over the full set of analyses shows the success in maintaining a reproducible system.

3.8.4. *Reference seawaters*

Two sets of reference seawaters (Table 7) were analysed. The first was the certified reference material NASS-5. This was a surface water from the North Atlantic Ocean collected off Nova Scotia. Its sampling and preservation were not up to the latest non-contaminating standards, so its concentrations for some trace metals are above what is expected in surface waters. Nonetheless, it is a useful reference, because it has been rigorously analysed by a range of analytical techniques and certified in a standards programme. The SAFe shallow and deep samples were collected more recently using the highest standards of clean, non-contaminating procedures. Trace metal concentrations in these are consensus values with results obtained by analysts specialised in trace metals in seawater. The SAFe reference samples are being used in the current global GEOTRACES programme for intercalibrations between different laboratories and countries.

Our results for the NASS-5 and SAFe D2 samples agree with the certified/consensus concentrations within the bounds of experimental uncertainty, apart from Co in SAFe D2. Without pretreatment of seawaters by UV irradiation, it should be remembered that our measurements are of the dissolved *labile* metal. Higher concentrations are reported for dissolved Co and Cu after UV photo-oxidation [15]. This additional increment is attributed to the decomposition of refractory organic complexes of these two metals to measurable forms. In fact, our results for Co and Cu are in good agreement with earlier SAFe consensus reports [43,44], where non-UV-irradiated results were reported: Co 26.9 ± 4.7 pM and Cu 2.02 ± 0.17 nM.

The very low concentrations of trace metals in the surface SAFe S1 reference water meant that Cd and Zn were undetectable, and that Co was verging on its limit of detection. Our determined Co, Ni, Cu and Pb concentrations are, within experimental error, the same as the SAFe S1 consensus values.

3.9. *Application of method to open ocean waters*

The method as described has been applied so far to trace metal determination in about 800 samples from three voyages—SAZ-Sense [45], CASO GEOTRACES SR3 (Butler et al., unpublished results) and PINTS ([39] and Butler et al., unpublished results)—in the South-West Pacific and Southern Oceans. It has only been at the surface in highly oligotrophic, subtropical waters that the limit of detection has been reached, with Cd, Co and Zn. Further improvements in blanks (with better contamination control and clean-up of reagents) and signal-to-noise ratios, as well as preconcentration factors (through increased sample loading and/or elution efficiency) should see even these waters become fully measurable.

To this point, we have determined dissolved labile metals (i.e., free inorganic species and trace metal that can be displaced from the organic complexes by the IDA ligand). We have yet to settle on a UV-irradiation procedure and apparatus that we are convinced will not cause contamination. UV photo-oxidation to release Co and Cu from refractory organic complexes is now a recommendation for analysis of ocean waters collected on GEOTRACES voyages.

4. Conclusions

The method described and results obtained demonstrate a reliable, reproducible and effective automated procedure using in-line FI preconcentration chelating (IDA) sorbent extraction and multi-element determination by an ICP-MS for the determination of Cd, Co, Cu, Ni, Pb and Zn in open-ocean samples.

The key features of this method are the incorporation of multiple monitoring systems to maintain reproducibility, rigorous interference assessments, contamination controls and method validation. The combination of these features with the adaptation and modification of previous instrumental set-ups and operational conditions, reviewed at each step, and incorporation of new devices (e.g., in-line pH sensor) has delivered enhanced performance over earlier reports of similar in-line, FI-preconcentration ICP-MS determination of trace metals in seawater. It has limits of detection improved by up to an order of magnitude compared with earlier works [17–19], more power to discern flawed results, and greater reproducibility. Reappraisal of polyatomic interferences (after Beck et al. [19]) and their avoidance, coupled with monitoring measures, has shown that reliable, interference-free results can be obtained for quadrupole ICP-MS determination of

Table 7
Concentrations determined in reference seawaters, and compared with consensus or certified values.

	Units	SAFe S1	SAFe S1 consensus value (November 2011)	SAFe D2	SAFe D2 consensus value (November 2011)	NASS-5	NASS-5 certified value
		$n=26$		$n=14$		$n=12$	
Co	pM	4.1[a] ± 1.7	5.4 ± 2.2	32.1 ± 2.8	45.4 ± 3.8	201 ± 10	187 ± 5
Ni	nM	2.51 ± 0.11	2.31 ± 0.10	9.15 ± 0.31	8.56 ± 0.22	4.78 ± 0.19	4.31 ± 0.48
Cu	nM	0.51 ± 0.07	0.51 ± 0.05	1.99 ± 0.13	2.25 ± 0.11	4.91 ± 0.25	4.67 ± 0.72
Zn	nM	< 0.07[b]	0.064 ± 0.019	7.29 ± 0.39	7.2 ± 0.5	1.53 ± 0.37	1.56 ± 0.60
Cd	pM	< 2.7[b]	1.0 ± 0.2	934 ± 38	986 ± 27	197 ± 20	205 ± 3
Pb	pM	47.3 ± 2.4	47.6 ± 2.4	26.8 ± 1.5	27.7 ± 1.8	31 ± 3	39 ± 2

SAFe bottles numbers used: SAFe S1—254; SAFe D2—344.

[a] Close to detection limit (3.2 pM), calculated as 3 × s.d. of 0.2% HCl, $n=7$.

[b] Below detection limit, calculated as 3 × s.d. of 0.2% HCl, $n=7$.

Co, Cu, Ni, Pb and Zn in oceanic waters. Only for Cd in some surface oceanic waters did polyatomic interference ensue from Mo. This can be corrected by Mo standard addition using present conditions, or it can possibly be avoided altogether by preconcentrating Cd at a higher pH in a variant of this method.

The method can be enhanced with a goal of improved limits of detection and a larger range of analytes in seawaters. This may be achieved through the use of different sorbents (e.g., NTA, nitrilotriacetate; EDTrA, ethylenediaminetriacetate). Replacement of the peristaltic pumps by syringe and/or piston pumps, and changes to more compact forms of flow analysis (e.g., sequential injection analysis) will improve system noise, and reduce dead volumes and possibly reduce blank readings further. Finally, the detection step might be improved by data collection and analysis systems more suited to the measurement of transient peaks, and possibly by alternative calibration techniques (e.g., stable isotope dilution).

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.06.054.

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