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Analysis of fish otoliths by electrothermal vaporization inductively coupled plasma mass spectrometry: aspects of precipitating otolith calcium with hydrofluoric acid for trace element determination

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Abstract

A method is developed for determination of trace elements, including Ag, As, Cd, Co, Cr, Cu, Mn, Ni, Se, Tl and Zn, in fish otoliths by electrothermal vaporization inductively coupled plasma mass spectrometry (ETV-ICP-MS). Hydrofluoric acid was used to precipitate calcium resulting from acid dissolution of otolith calcium carbonate. Initial acidity of the sample solution influenced the precipitation efficiency of calcium fluoride. Up to 99.5% of Ca was precipitated in solutions that contained less than 2% (v/v) HNO_3. Recoveries of the elements obtained from spiked artificial otolith solutions were between 90 and 103%. Stabilization of the elements within the ETV cell was achieved with 0.3 μg Pd/0.2 μg Rh chemical modifier that also afforded optimum sensitivity for multielement determination. The method was validated by the analysis of a fish otolith reference material (CRM) of emperor snapper, and then applied to the determination of the trace elements in otoliths of several fish species captured in Raritan Bay, New Jersey. Results indicated that fish physiology and biological processes could influence the levels of Cu, Mn, Se and Zn in the otoliths of fish inhabiting a similar aqueous environment. Otolith concentrations of Cr and Ni did not show any significant differences among different species. Concentrations for Ag, As, Cd, Co and Tl were also not significantly different, but were very low indicating low affinity of otolith calcium carbonate to these elements.

Keywords: Fish otoliths; Trace elements; Calcium fluoride precipitation; Electrothermal vaporization; Inductively coupled plasma mass spectrometry

1. Introduction

Otoliths of a teleost fish are the paired calcified structures located in the inner ear. The composition of otoliths is primarily calcium carbonate (ca. 96% aragonite) in a proteinaceous matrix that makes up about 3% of the total mass of an otolith [1,2]. The remaining 1% assay of the otoliths is occupied by trace and minor elements. Over the last two decades, otolith chemistry has been applied increasingly for discrimination of fish stocks [3–5], identification of natal origins [6,7] and migration routes of fish [8,9], constructing life history [10] and temperature history of fish [11], determination of age [12] and anadromy [13,14], and chemical composition of fish habitats [15]. These potential applications of fish otoliths in fisheries related research comes from the fact that otoliths grow daily throughout the life of fish and are metabolically inert. Because calcium carbonate and trace metals are primarily derived from the surrounding water, temporal changes in the elemental chemistry of the resident waters are (as modified by physiological processes) permanently stored in the radially growing layers of otoliths that integrate over the entire lifetime of the fish when a whole otolith is dissolved [1,2].

Inductively coupled plasma mass spectrometry (ICP-MS) has become a powerful technique in the analysis of fish otoliths for trace elements because of its high sensitivity and

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multielement capability. Difficulties in solution-based ICP-MS analysis of fish otoliths mainly arise from the saline nature of otolith solutions that substantially diminish the detection capabilities of conventional ICP-MS instrument. Spectral overlaps from polyatomic ions of calcium oxides and hydroxides may also lead to inaccuracy in the results of analyte of interest at the same nominal mass-to-charge ratio (m/z) (e.g., $^{44}Ca^{16}O^{+}$ on $^{60}Ni^{+}$). Although there are several articles [3,6,16] that report successful determinations of Cu, Ni, Pb and Zn, the information of trace elements has not been, to date, fully utilized to population studies using otolith chemistry as the data are often sporadic. It is because spectral and matrix interferences are more pronounced for the determination of trace transition elements due to their low concentrations in saline fish otoliths. One approach to overcome these problems is to extract the transition metals from solution and preconcentrate by means of liquid–liquid or solid–liquid extraction procedures. Initiative studies performed by several independent groups [17–20] have recently demonstrated the ability of the extraction/preconcentration procedures for determination trace elements reliably in fish otoliths by ICP-MS.

Another difficulty associated with the solution-based ICP-MS in trace element determinations is the low efficiency of the nebulization process (less than 2% for pneumatic nebulizers). Electrothermal vaporization (ETV) as a sample introduction technique, on the other hand, offers advantages over the conventional solution nebulization. The most universal advantage is the greater transport efficiency (usually greater than 20%) due to the gaseous nature of the sample introduced [21,22]. This allows better sensitivity and detection to be achieved since a larger portion of the sample reaches the plasma. Additional advantages of the ETV sample introduction come from the fact that before atomization solvents are evaporated with the aid of a temperature-controlled heating program. Not only does this add to the sensitivity since plasma is not required to vaporize the sample, but also polyatomic species of oxides and hydroxides originating from the solvent vapors are reduced at least by an order magnitude in comparison to solution nebulization [22]. Non-volatile matrix components (e.g., calcium for otoliths), however, mostly remain in the sample to be analyzed unless they are removed prior to ETV processing. Upon atomization and introduction, vapors of large amount of matrix components entering the plasma could cause severe interferences and drift in instrument sensitivity due to the salt deposition on the instrument interface.

In this study, we developed a procedure for analysis of fish otoliths by ICP-MS. An ETV device was used for sample introduction. Calcium in the sample solution resulting from acid dissolution of the otoliths was precipitated as insoluble calcium fluoride using hydrofluoric acid (HF). Effects of the precipitation on the recoveries of transition metals were examined in artificial otolith solutions. Operating parameters of ETV sample introduction system were examined to find optimum settings. The method was validated by analysis of a fish otolith certified reference material (CRM No. 22), and then applied to otolith samples of fish captured in Raritan Bay, NJ.

2. Experimental

2.1. Materials and solutions

Distilled deionized water (18 MΩ cm) produced by a commercial mixed-bed ion exchange system (Barnstead, B-pure, Boston, MA) was used throughout. Optima® grade nitric acid (Fisher Scientific, Pittsburg, PA) was used for sample dissolution and preparation of standard solutions. Ultrex® grade hydrofluoric acid (J.T. Baker, Jackson, TN) was used for precipitation of calcium. Solutions of 10 mg ml^{-1} palladium nitrate (Perkin-Elmer) and 1 mg ml^{-1} rhodium chloride (High Purity Standards, Charleston, SC) were used for chemical modification. Multielement standard solutions (10 μg ml^{-1}, SPEX CertiPrep, Metuchen, NJ) were further diluted by deionized water to make up daily stock solutions (0.1 or 1.0 μg ml^{-1}) that were used for preparation of calibration standards and spiked artificial otolith solutions.

An artificial otolith solution containing 10 mg ml^{-1} Ca (as nitrate) was prepared by dissolving 12.5 g of Suprapur® grade calcium carbonate (EM Science, Gibbstown, NJ) in concentrated HNO_3 in a Teflon beaker. Excess acid was removed by heating to dryness on a hot plate. The residue was then dissolved with deionized water and diluted to 500 ml. A fish otolith certified reference material (CRM No. 22) obtained from the National Institute of Environmental Studies (NIES) of Japan was used for method validation. The material was prepared from saggittal otoliths of Pacific emperor snapper (*Lutjanus sebae*).

Otolith samples were obtained from fish species (striped bass, winter flounder and spotted hake) captured during 1996–1997 cruises in Raritan Bay of New Jersey, USA. Otoliths were removed with plastic forceps and immersed in deionized water to remove the attached tissue mechanically. The remainder of the tissue was removed by soaking for 5 min first in 1% (v/v) H_2O_2, then in 1% (v/v) HNO_3. Otoliths were then flooded with deionized water to rinse off the excess acid and surface contamination. After air-drying under a laminar-flow hood, they were weighed to nearest 0.01 mg and stored in pre-cleaned plastic vials at room temperature until analysis.

2.2. Instrumentation and data collection

A Perkin-Elmer Sciex Elan® 5000 ICP-MS (Norwalk, CT, USA) instrument was used for measurements. Signal measurements were performed by ELAN software (Version 2.2). Solutions were introduced to the instrument by two methods: solution nebulization and electrothermal vaporization (ETV). Solution nebulization was performed through a MCN-100 micro-nebulizer (Cetac Technologies, Omaha,

NE) and was used only during the optimization of the conditions for calcium fluoride precipitation and consequently for the determination of elemental recoveries in artificial otolith solutions (Section 2.3). Because of the lower sensitivity, measurements with micro-nebulization enabled spiking the artificial otolith solutions with relatively higher concentrations (e.g., 20 ppb) than those usually present in fish otoliths. In addition, micro-nebulization enabled the measurement of levels of calcium that remained soluble in the artificial otolith solution when calcium fluoride precipitation had reached completion.

In the case of ETV measurements, a Perkin-Elmer HGA-600MS electrothermal vaporization (ETV) unit with a Perkin-Elmer Model AS-60 autosampler attachment was used for introduction. The ETV unit was connected to the ICP-MS instrument by a 65 cm long (0.6 cm i.d.) PTFE tubing. Pyrolytically coated platformless graphite tubes were used. Integrated analyte signals were measured at transient peak hopping mode by using Graphics program of the ELAN software package. The operating parameters of ICP-MS instrument are summarized in Table 1 for nebulization and ETV introductions.

2.3. Examination of precipitation conditions by using micro-nebulization

The detector was desensitized at $m/z = 44$ to achieve simultaneous measurement of calcium remaining in solution soluble after precipitation with HF. Germanium was used for internal standardization. The effects of HF and HNO_3 on the precipitation of calcium were examined by a univariate method. Initially, artificial otolith solutions were acidified to 2% (v/v) HNO_3 and the volume of HF was varied from 50 to 150 μl to examine the effect of HF concentration (e.g., fluoride ion concentration) on the efficiency of the calcium precipitation. To a 15 ml centrifuge tube, 2.5 ml of the artificial otolith solution (10 mg ml^{-1} Ca), 60 μl of concentrated HNO_3 and 60 μl of 1.0 μg ml^{-1} multielement solution were placed. The volume was completed to 3 ml yielding an artificial otolith solution of 8.3 mg ml^{-1} Ca in 2% (v/v) HNO_3 that also contained 20 ng ml^{-1} multielement spike. Calcium was precipitated by adding 50, 75, 100, 125 or 150 μl of concentrated HF.

To affect the solution acidity from 0 to 10% (v/v) HNO_3 before precipitation, 0, 30, 60, 150 or 300 μl of concentrated HNO_3 was added to a 15 ml centrifuge tube containing 2.5 ml of the artificial otolith solution. The volume was completed to 3 ml after adding 60 μl of 1.0 μg ml^{-1} multielement standard. Contents were precipitated with 100 μl of concentrated HF. In another set of experiments, spike concentrations were increased to 100 ng ml^{-1} to investigate whether the recoveries of trace elements were influenced by rigorous precipitation of calcium fluoride or not.

Three replicate artificial otolith solutions were prepared for each set. Solutions were centrifuged upon addition of HF. About 1.5 ml from each solution was then transferred to a 2 ml microcentrifuge tube and was analyzed by micro-nebulization ICP-MS after adding 15 μl of 1.0 μg ml^{-1} internal standard solution (^{74}Ge). Results obtained from analysis were corrected for the appropriate dilution factor due to the addition of HF and internal standard solution.

Table 1
Operation of conditions of ICP-MS instrument

	Nebulization	ETV
Rf power (W)	1150	1150
Plasma gas flow (l min^{-1})	15	15
Nebulizer gas flow (l min^{-1})	0.93	0.95
Auxiliary gas flow (l min^{-1})	0.80	0.80
Scanning mode	Peak hop	Peak hop transient
Dwell time (ms)	200	5
Sweeps/reading	1	1
Number of replicates	3	3
Readings/replicate	1	20
Points/spectral peak	1	1
Resolution	Normal	Normal
Measurement mode	Peak height	Peak area

2.4. Examination of ETV parameters and method validation

The operation of the ETV system was examined for optimum conditions by direct atomization of 5.0 ng ml^{-1} aqueous multielement solution that also contained 5.0 ng ml^{-1} Ge as internal standard. A quadrupole dwell time of 5 ms was used. The effects of nebulizer gas flow rate, chemical modifiers and drying temperature on integrated signals were studied for triplicate firings of 50 μl of the standard solution at 2600 °C. Nebulizer gas flow rate was varied from 0.6 to 1.3 l min^{-1}. Palladium and rhodium were studied as chemical modifiers for analyte stabilization. About 1.5 μg of each element was applied either individually or in combination. For optimum medium (Pd/Rh), the total mass of each element in the composition was further examined from 0 to 0.5 μg by univariate method so as to minimize the contribution from reagent blanks while maintaining the sensitivity.

In the last stage of the method development, the drying temperature of the furnace was raised from 120 to 500 °C to examine the effect on analyte signals. The method was then validated by analysis of the fish otolith CRM by ETV-ICP-MS. External calibration was performed with aqueous standard solutions in 1% (v/v) HNO_3. The multielement calibration standards were between 0 and 2 ng ml^{-1}. For Cu and Zn, the external standards were 10-fold concentrated. Three replicate firings were made for each solution by injecting 50 μl of sample and 10 μl of Pd/Rh modifier solution into the furnace.

2.5. Dissolution and precipitation procedures

Dissolution of otolith samples was carried out in 15 ml Teflon beakers with concentrated HNO_3. Approximately 50–60 mg sample of the fish otolith CRM was weighed into

a Teflon beaker and dissolved with 1 ml HNO_3 in two steps. In the first step, 0.5 ml of the acid was added, and the contents were warmed on a hot plate (ca. 2–3 min) to facilitate the dissolution and gas (CO_2) evolution. Following another addition of 0.5 ml of the acid, the solution was heated slowly to dryness to get rid of the excess acid. This enabled almost complete removal of the acid vapors not to affect the final acidity significantly. After cooling to room temperature, the residue in the beaker was redissolved with 1 ml of 1% (v/v) HNO_3 and transferred to a 15 ml centrifuge tube. The beaker was rinsed twice with 0.5 ml of 1% (v/v) HNO_3 and the washings were added to the centrifuge tube to give a volume of 2 ml before precipitation.

Calcium in the sample solution was precipitated by two additions of 50 μl concentrated HF (44.6% (m/v)). After each addition, the solution was centrifuged for 5 min at 1500 rpm. At the end of the second centrifugation, 1.5 ml of the solution was transferred to a 2 ml autosampler cup for analysis without disturbing the precipitate.

Otoliths of the Raritan Bay fish were dissolved using the same procedure in either 2 or 1 ml concentrated HNO_3 depending on the mass range. Otolith masses for striped bass ranged from 120.5 to 216.4 mg for which a total of 2 ml HNO_3 was used. For the dissolution of smaller winter flounder (16.1–38.3 mg) and spotted hake otoliths (34.1–46.0 mg), 1 ml of the acid was sufficient. After dissolution, volumes were completed to 2 ml with 1% (v/v) HNO_3. Total volume of HF used to precipitate Ca in winter flounder and spotted hake otolith solutions was 100 μl, and was 300 μl for those of striped bass. Blank solutions were prepared similarly for CRM and each set of otolith samples with the appropriate volumes of HNO_3 and HF.

3. Results and discussion

3.1. Micro-nebulization ICP-MS studies

3.1.1. Precipitation of calcium

In an aqueous solution, the precipitation of calcium to form sparingly soluble calcium fluoride, $K_{sp} = 1.46 \times 10^{-10}$ [23], occurs according to the reaction, $Ca^{2+} + 2HF \equiv CaF_2(s) + 2H^+$, where Ca is in the form of calcium nitrate in the solution. The 3 ml otolith solution (8.3 mg ml^{-1} Ca) contains 25 mg Ca that stoichiometrically requires 47 μl of the concentrated HF (44.6% (m/v), $d = 1.18$ g ml^{-1}) for complete precipitation. The precipitation profile observed for calcium fluoride is shown in Fig. 1(a) with respect to the volume of HF used. A steep decrease in calcium concentration from 8.3 to 326 μg ml^{-1} occurred when precipitation was performed with 50 μl of HF. Although the precipitation was as efficient as 96%, the amount of calcium remained soluble in the solution was sufficiently high to cause inaccuracy by suppressing the ion signals. For 100 μl of HF, approximately 39 μg ml^{-1} Ca remained in the solution that was significantly lower than that for 50 μl of HF. Above 100 μl of HF, relative decrease in calcium concentration was small indicating that 100 μl was the optimum volume. When this result is related to otoliths that are approximately 96% calcium carbonate, a minimum volume of 150–160 μl of HF would be required for efficient precipitation of calcium when a 0.1 g otolith sample is dissolved.

Fig. 1(b) illustrates the effect of solution acidity on the precipitation of calcium using 100 μl HF. Precipitation efficiency decreased with increasing acidity (% HNO_3) of the artificial otolith solution. Lowest Ca concentration was measured as 21 μg ml^{-1} from solutions that were virtually in water (no HNO_3 added). Those from 1 and 2% (v/v) HNO_3 conditions were about 24 and 33 μg ml^{-1}, respectively, that were also found to be suitable to successfully precipitate at least 99.5% of initial calcium (8.3 mg ml^{-1}). In the application to otolith samples, acidity prior to precipitation was adjusted with 1% (v/v) HNO_3 to improve the stability of the elements in the solution.

3.1.2. Recoveries of trace elements

Artificial otolith solutions (8.3 mg ml^{-1} Ca) that contained 20 ng ml^{-1} multielement spike were analyzed by micro-nebulization ICP-MS when the precipitation was complete. The results are summarized in Table 2. Losses were observed for Al, Bi, Fe, Mo, Pb, Sb, Sn and V via precipitation whereas trace elements, including Ag, As, Cd, Co, Cr, Cu, Mn, Ni, Se, Tl and Zn, did not show any sign of precipitation despite the presence of HF in excess. Recoveries for ^{59}Co, ^{60}Ni and ^{205}Tl were higher in acidic solutions (≥5% (v/v) HNO_3) as well as when the volume of HF was inadequate (e.g., 50 μl). Negligible levels of blank concentrations indicate that high recoveries for ^{59}Co and ^{60}Ni are mainly due to the spectral interferences of $^{43}Ca^{16}O^+$ on $^{59}Co^+$, and $^{44}Ca^{16}O^+$ and $^{43}Ca^{16}OH^+$ on $^{60}Ni^+$. Lowering the acidity (≤2% (v/v) HNO_3) and adding excess HF (≥100 μl) resulted in better accuracy for both elements as the contributions of the polyatomic ion signals were eliminated (Table 2). Thallium at $m/z = 205$ is virtually not influenced by polyatomic ion interferences of Ca. Thus, high recoveries for ^{205}Tl observed for 50 μl HF, and for 5 and 10% (v/v) HNO_3 conditions were attributed to non-spectral interferences arising from high levels of calcium remained in the solutions. This calcium matrix (~300 μg ml^{-1}) caused suppression on ion signals of the elements that ^{74}Ge internal standard could not compensate for on $^{205}Tl^+$ accurately. It is, however, clear from the data in Table 2 that the depressive effect of calcium became insignificant as the acidity of the solutions was reduced (≤2% (v/v) HNO_3) and in the presence of excess HF (≥100 μl) that consequently enabled more efficient precipitation.

The precipitation of calcium as calcium fluoride occurred instantaneously and very vigorously. Such vigorous precipitation, however, did not lead to any precipitation of Ag, As, Cd, Co, Cr, Cu, Mn, Ni, Se, Tl and Zn even at elevated concentrations Table 2 (last column). Recoveries for 100 ng ml^{-1} multielement spike ranged from 92 ± 2% for As to 103 ± 2%

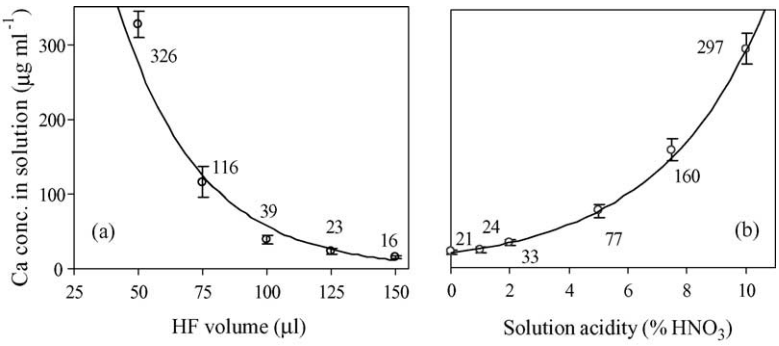

Fig. 1. Effects of HF volume (a) and solution acidity (b) on the precipitation of calcium in artificial otolith solution (8.3 mg ml^{-1} Ca).

for Tl. This result demonstrates that the method is highly robust when applied to the analysis of fish otoliths since possible losses of the trace elements (Ag, As, Cd, Co, Cr, Cu, Mn, Ni, Se, Tl and Zn) via precipitation would be unlikely during the precipitation of calcium by HF in fish otoliths. It is because the concentrations of these elements in fish otoliths are usually between ng g^{-1} and low μg g^{-1} levels yielding solution concentrations between pg ml^{-1} (ppt) and a few ng ml^{-1} (ppb) [3,17,19].

The precipitation for Al, Bi, Pb and V was strong; therefore, recoveries were less than 1% (Table 2). It is likely that these elements coprecipitated with calcium fluoride since increasing the acidity of the artificial otolith solution up to 10% HNO_3 did not provide any improvement to achieve quantitative results. For other trace transition elements (Fe, Mo, Sb and Sn), recoveries were less than 50% (excluding Fe) and were strongly dependent on both initial HNO_3 concentration and the volume of HF added. Apparently Fe did precipitate upon addition of HF. The anomalous high recoveries for Fe most likely originate from high Fe blanks in the artificial otolith solution as well as the spectral overlap of the broad $^{40}Ca^{16}OH^+$ peak on $^{57}Fe^+$. During the ETV-ICP-MS studies, determinations were not performed for these precipitating trace elements.

Table 2
Effects of HF volume, solution acidity and spike concentration on elemental recoveries

Element/isotope	Artificial otolith solution blank[a] (ng ml^{-1})	Recovery (for 20 ng ml^{-1} spike) (%)								Recovery (%) (100 ng ml^{-1} spike), 1% HNO_3 and 100 μl HF
		Volume of HF added for precipitation (μl)			Solution acidity prior to precipitation (% HNO_3)					
		50	100	150	0	1	2	5	10	
Ag/107	0.027	102 ± 5	103 ± 2	98 ± 2	103 ± 1	101 ± 1	101 ± 1	98 ± 1	96 ± 1	102 ± 2
Al/27	0.054	<1	<1	<1	<1	<1	<1	<1	<1	<1
As/75	0.38	102 ± 6	92 ± 2	94 ± 2	104 ± 2	97 ± 1	96 ± 1	96 ± 2	93 ± 2	92 ± 2
Bi/209	0.097	<1	<1	<1	<1	<1	<1	<1	<1	<1
Cd/114	0.057	103 ± 5	102 ± 1	99 ± 3	102 ± 3	103 ± 1	104 ± 3	104 ± 3	102 ± 2	93 ± 2
Co/59	0.78	120 ± 6	98 ± 2	93 ± 2	98 ± 2	96 ± 1	94 ± 2	105 ± 2	118 ± 3	98 ± 1
Cr/53	1.3	94 ± 3	94 ± 6	91 ± 6	101 ± 5	104 ± 1	101 ± 2	100 ± 2	103 ± 2	97 ± 1
Cu/63	0.51	104 ± 6	96 ± 2	92 ± 2	97 ± 2	101 ± 1	97 ± 2	92 ± 1	94 ± 5	95 ± 2
Fe/57	8.5	303 ± 26	78 ± 14	34 ± 3	33 ± 3	40 ± 1	50 ± 4	107 ± 5	261 ± 9	60 ± 4
Mn/55	0.31	102 ± 5	90 ± 1	91 ± 3	88 ± 2	93 ± 2	93 ± 4	96 ± 1	99 ± 2	93 ± 2
Mo/95	0.19	12 ± 1	20 ± 1	24 ± 1	14 ± 1	18 ± 1	25 ± 4	32 ± 1	41 ± 1	20 ± 2
Ni/60	1.1	116 ± 5	97 ± 1	92 ± 3	99 ± 1	96 ± 2	93 ± 5	103 ± 2	124 ± 5	97 ± 3
Pb/208	0.015	∼2	∼1	<1	<1	∼1	∼	5	8	∼2
Sb/121	0.14	25 ± 1	24 ± 1	22 ± 1	19 ± 1	20 ± 1	22 ± 1	25 ± 1	27 ± 1	19 ± 1
Se/82	0.10	102 ± 2	90 ± 2	100 ± 4	101 ± 4	93 ± 3	91 ± 2	89 ± 5	95 ± 2	92 ± 3
Sn/120	0.40	10 ± 1	29 ± 2	44 ± 2	28 ± 1	32 ± 2	41 ± 2	45 ± 2	50 ± 3	27 ± 3
Tl/205	0.004	121 ± 4	102 ± 7	99 ± 2	100 ± 5	101 ± 5	105 ± 4	112 ± 6	119 ± 4	103 ± 2
V/51	0.057	<1	<1	<1	<1	<1	<1	<1	<1	<1
Zn/66	1.1	102 ± 4	96 ± 2	90 ± 4	104 ± 3	103 ± 2	96 ± 1	96 ± 5	96 ± 4	92 ± 4
Ca[b]/44	n.d.	326	39	16	21	24	33	77	297	n.d.

[a] Blank concentrations are for the artificial otolith solution (8.3 mg ml^{-1} Ca) in 2% (v/v) HNO_3 that is precipitated with 100 μl HF.
[b] Values are the concentrations of Ca remaining in the solution after precipitation with HF.

3.2. ETV-ICP-MS studies

3.2.1. ETV conditions and chemical modification

All elements (Ag, As, Cd, Co, Cr, Cu, Mn, Ni, Se, Tl and Zn) that were quantitatively remained in solution were measured in each ETV firing. Five milliseconds dwell time (scan speed) was used throughout to collect as many data points as possible from fast transient signals generated by ETV sample introduction system. Optimum range of nebulizer gas flow rate was between 0.9 and 1.0 l min^{-1} when 50 μl of 5 ng ml^{-1} multielement solution was atomized at 2600 °C. At lower flow rates, sensitivity was lower because of the loss of the atom vapor via condensation within the transfer line and on the cooler parts of the ETV unit. Signals were also reduced at higher flow rates because of the shift of the sampling zone in plasma.

In ETV-ICP-MS, chemical modifier element also contributes to more efficient transport of atomized analyte to plasma in addition to stabilization in the furnace to avoid losses during the drying steps. This is because of the higher concentration of modifier particles (stable nuclei) in vapor phase that condenses on particulate matter (e.g., analyte atoms) to form condensation nuclei [21,24]. Not only does this process reduce the condensation of analyte vapor on the cooler surfaces within the ETV cell and transfer line, but also the modifier of the condensation nuclei acts as physical carrier for the transport of small amounts of analyte to the plasma. The results from initial examination of chemical modifiers (Pd, Rh and Pd/Rh) for a drying temperature of 120 °C are illustrated in Fig. 2. Integrated analyte signals obtained for each modifier were normalized against those without any modifier. For Cr, Mn and Tl, all three modifiers appeared to be equally suitable since the effects on signals did not differ significantly. Palladium (0.5 μg) provided the highest signals for As and Se, and for other elements signals increased to an extent. Signals were improved with 0.5 μg Rh modifier for Ag, Cd, Co, Cu, Ni and Zn suggesting a better stabilization and more efficient transport of analytes to the plasma. A further increase in the signals (except that for Cd) with 0.5 μg Pd and 0.5 μg Rh indicated that Pd/Rh composition was the optimum modifier. In the case of As and Se, signals obtained with 0.5 μg Pd/0.5 μg Rh were similar to those observed with Rh only, most likely because of the overstabilization of As and Se by Rh in the furnace. Further examination of the composition of Pd and Rh modifier to minimize the contribution from reagent blanks indicated that 0.3 μg Pd and 0.2 μg Rh were adequate to achieve similar stability and precision (% R.S.D. < 4% for 5 ng ml^{-1} standard solution).

The effect of the ETV cell (drying) temperature on the stability of the elements is illustrated in Fig. 3 for 0.3 μg Pd/0.2 μg Rh modifier injected onto 50 μl of 5 ng ml^{-1} standard solution. Results are relative to those obtained at 120 °C. Maximum drying temperature was approximately 200 °C based on the less stable Cd and Tl. There was not any significant loss for Zn (mp 419 °C) up to 250 °C, while Ni (mp 1455 °C) was found to be stable up to 300 °C. A gradual increase in the signals of As (mp 813 °C) and Se (mp 217 °C) was observed with increasing drying temperature. This result supports the hypothesis above with regard to stabilizing action of Rh on As and Se such that for low drying temperature atomization was less efficient due to the relatively longer time required for the cell temperature to reach 2600 °C. Increasing the duration of the initial atomization time (4 s) to 6 s eliminated this problem. For other elements, including Ag, Co, Cu, Cr and Mn, the signals were almost flat, indicating that up to 500 °C no significant losses occurred. The program of the ETV sample introduction system is summarized in Table 3.

3.2.2. Analytical performance and method validation

The data obtained from analysis of fish otolith reference material (CRM) of emperor snapper (*Lutjanus sebae*) are given with the detection limits in Table 4. The detection limits are expressed as the analyte concentrations that give signals equivalent to three times the standard deviation of the blank signals ($3s_{blank}$) obtained from triplicate firings of 1% (v/v) HNO_3 solution ($n = 10$). These detection limits are at least an order of magnitude lower than the analyzed mass of the

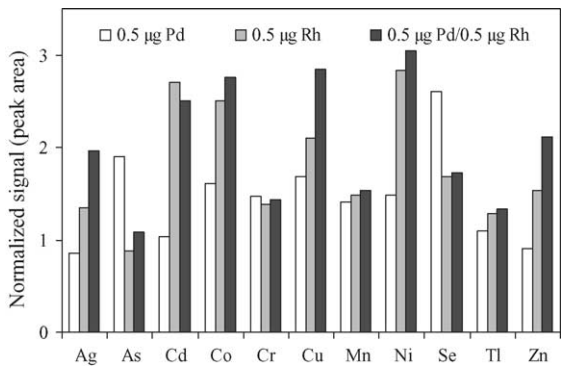

Fig. 2. Effects of Pd, Rh and Pd/Rh modifiers on analyte signals. Signals are relative to those without modifier.

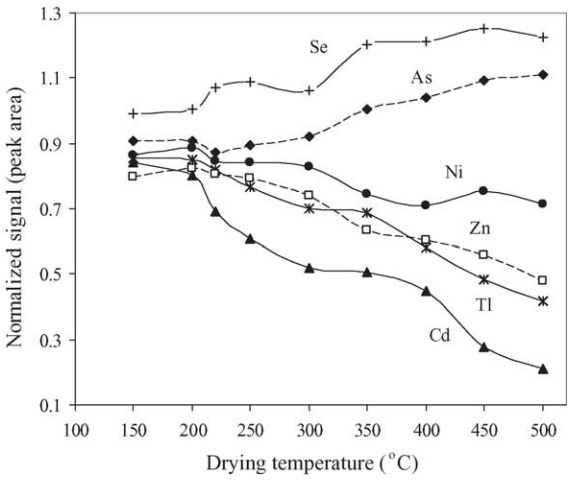

Fig. 3. Effect of ETV furnace drying temperature on analyte signals. Signals are normalized against those obtained at 120 °C.

Table 3
Temperature–time program of HGA 600MS

Step	Remarks	Temperature (°C)	Ramp (°C s^{-1})	Hold time (s)	Ar flow rate (ml min^{-1})	Gas to vent	Gas to ICP
1	Sample (50 μl) and modifier (10 μl) injection	20	–	–	–	–	–
2	Drying	100	5	20	300	√	
		120	2	15	300	√	
		150	2	15	300	√	
3	Atomization/read	2600	0	6	20		√
4	Cool	20	3	10	20		√

individual elements in 50 μl of the otolith CRM solution, indicating that the sensitivity of ETV-ICP-MS is sufficiently high to achieve accurate determination of trace elements in otoliths when calcium matrix is eliminated.

Otoliths contain some proteinaceous material (∼3%). Attacking the sample with concentrated HNO_3 enables the oxidation of the majority of organics with the aid of the heat-assisted evaporation on a hot-plate. The remaining portion, however, may cause inaccuracies in the results by altering analyte transport and generating spectral interferences of argon carbides and nitrides (e.g., $^{40}Ar^{13}C^+$ on $^{53}Cr^+$), unless they are destroyed during the drying stage in the furnace. The consistency between the results obtained by external calibration and standard additions method clearly demonstrates that such interferences from undestroyed organic components were insignificant even at ng g^{-1} levels (Table 4).

The results in Table 4 also agreed with the data reported for the analysis of the fish otolith CRM for Cd, Cu and Zn by off-line solvent extraction isotope-dilution ICP-MS [17] and for Cd, Co, Cu, Mn, Ni and Zn by on-line solid phase extraction ICP-MS [19]. At the 95% confidence limit, there were no significant differences between the results of this study and the reported values ($0 \leq t_{exp} \leq 1.87 < t_{crt} = 2.31$, d.f. = 7, $P = 0.05$). Most importantly no significant interferences were observed from $^{43}CaO^+$ and $^{42}CaOH^+$ on $^{59}Co^+$ and from $^{44}CaO^+$ on $^{60}Ni^+$ as indicated by the agreement between the results for external calibration and standard additions methods. The results for $^{60}Ni^+$ were also supported by the agreement with those of $^{62}Ni^+$ that is relatively free from the interference of $^{46}CaO^+$ due to low abundance of ^{46}Ca isotope (0.004%). This is because of the elimination of water and acid vapors (e.g., sources of oxides) by temperature-controlled heating program. For analysis of the same solutions by ICP-MS using MCN-100 micronebulizer, on the other hand, the concentrations ranged from 48 to 57 ng g^{-1} for ^{59}Co and 36–40 ng g^{-1} for ^{60}Ni despite the fact that the nebulizer afforded low solvent throughput (0.1–0.2 ml min^{-1}).

3.2.3. Analysis of Raritan Bay fish otoliths

Chemistry of resident habitats (e.g., salinity, metal and nutrient concentration) is an important factor that influences the uptake levels of trace elements into fish otoliths [1,3]. For different fish families inhabiting similar aqueous and benthic environment, incorporation of metals into otoliths may be influenced by the physiological state of fish in addition to the concentrations in the surrounding water. Certain elements may preferentially be taken up and metabolized by particular fish resulting in greater accumulation in the otoliths, whereas some elements (usually toxic elements) are excreted after conversion into biologically inactive

Table 4
Detection limits and results from analysis of fish otolith CRM

Element	Absolute detection limit (pg)	Detection limit[a] (pg g^{-1})	CRM results (ng g^{-1})			
			This method (ETV-ICP-MS)[b]		Reported	
			External calibration	Standard additions	ID-ICP-MS[c]	SPE-ICP-MS[d]
^{107}Ag	0.14	3.0	2.1 ± 0.5	2.3 ± 0.7	–	–
^{75}As	0.12	2.4	2.3 ± 0.3	2.9 ± 0.4	–	–
^{114}Cd	0.20	4.1	2.9 ± 0.3	2.7 ± 0.7	2.8 ± 0.2	3.0 ± 0.4
^{59}Co	0.19	3.8	2.8 ± 0.4	2.9 ± 0.3	–	2.8 ± 0.8
^{53}Cr	0.72	14.5	18 ± 2	21 ± 3	–	–
^{63}Cu	0.61	12.3	700 ± 69	689 ± 25	742 ± 7	686 ± 59
^{55}Mn	0.24	4.9	43 ± 4	41 ± 6	–	43 ± 8
^{60}Ni	0.48	9.7	25 ± 3	23± 3	–	22 ± 5
^{62}Ni	0.47	9.5	27 ± 3	24 ± 5	–	19 ± 1
^{82}Se	0.41	8.3	33 ± 5	31 ± 4	–	–
^{205}Tl	0.06	1.2	0.60 ± 0.07	0.70 ± 0.2	–	–
^{66}Zn	0.9	18	468 ± 32	461 ± 15	471 ± 2	448 ± 69

[a] For injection of 50 μl of 1% (v/v) HNO_3 solution.
[b] Results are mean ± S.D. for four separate analyses.
[c] Off-line solvent extraction and isotope dilution ICP-MS determination (Ref. [17]).
[d] On-line solid phase extraction and ICP-MS determination (Ref. [19]).

Table 5
Trace element concentrations (ng g^{-1}) from otoliths of fish captured in Raritan Bay, NJ

Element		Raritan Bay, New Jersey			
		Striped bass (n = 6)	Winter flounder (n = 6)	Spotted hake (n = 6)	Approximate seawater concentrations[a] (ng l^{-1})
^{107}Ag	Average ± S.D. (range)	2.9 ± 0.3 (2.6–3.2)	4.6 ± 1.2 (3.1–6.2)	2.2 ± 0.6 (1.5–3.0)	n.a.
^{75}As	Average ± S.D. (range)	2.4 ± 0.6 (2.0–2.9)	3.2 ± 1 (1.6–4.2)	2.7 ± 0.5 (2.0–3.2)	n.a.
^{114}Cd	Average ± S.D. (range)	3.5 ± 0.7 (2.6–4.5)	5.4 ± 2.3 (2.8–8.7)	5.9 ± 1.8 (3.9–8.7)	55 (45–65)
^{59}Co	Average ± S.D. (range)	2.7 ± 1.1 (1.5–4.8)	7.2 ± 2.5 (5.9–11.2)	5.0 ± 1.1 (3.8–6.2)	147 (107–205)
^{53}Cr	Average ± S.D. (range)	43 ± 9 (29–52)	30 ± 7 (25–40)	52 ± 23 (29–80)	n.a.
^{63}Cu	Average ± S.D. (range)	180 ± 28 (139–203)	260 ± 50 (189–315)	157 ± 13 (143–174)	720 (610–860)
^{55}Mn	Average ± S.D. (range)	1943 ± 1158 (920–3080)	1534 ± 247 (1156–1856)	12220 ± 3260 (8750–17480)	57000 (33000–83500)
^{60}Ni	Average ± S.D. (range)	76 ± 33 (44–126)	131 ± 37 (66–165)	88 ± 39 (42–135)	1270 (1140–1570)
^{62}Ni	Average ± S.D. (range)	79 ± 28 (55–126)	112 ± 34 (53–150)	80 ± 31 (33–114)	1270 (1140–1570)
^{82}Se	Average ± S.D. (range)	72 ± 14 (51–88)	77 ± 12 (59–91)	20 ± 7 (15–33)	n.a.
^{205}Tl	Average ± S.D. (range)	2.1 ± 1.1 (0.9–3.3)	1.1 ± 0.9 (0.6–2.1)	2.8 ± 0.8 (2.2–3.4)	n.a.
^{66}Zn	Average ± S.D. (range)	375 ± 97 (313–415)	270 ± 35 (222–305)	245 ± 77 (162–302)	n.a.

[a] Results were obtained by FI-ICP-MS preconcentration (A.J. Paulson, personal communication).

forms, therefore, there may not be significant deposition in the otoliths.

The results for the otoliths of Raritan Bay fish (spotted hake, winter flounder and striped bass) are given in Table 5. Differences were observed in the otolith concentrations of Cu, Mn, Se and Zn among the fish species. Average Mn concentration in the otoliths of spotted hake was about an order of magnitude greater than in those of striped bass and winter flounder, whereas Se was about three-fold higher in striped bass and winter flounder otoliths than in those of spotted hake. Levels of Cu in winter flounder otoliths appeared to be significantly higher since there was little overlap of Cu values of striped bass and spotted hake. Likewise, Zn concentrations in striped bass appeared to be significantly greater. These results suggest that within similar aqueous environment uptake levels of certain trace elements into otoliths could vary among different fish species because of the physiological differences that affect the exchange rates of these metals between external and internal environment. Selective uptake through biological processes could be an alternative pathway for higher deposition rates observed for Cu, Mn, Se and Zn in the otoliths of certain fish. Because otoliths contain some proteinaceous matrix, these biological processes concentrate the metals to higher levels in the organic matter of blood fluid of the fish and, in turn, may increase the levels in the otoliths. For Cr and Ni, it is not evident if there are any influences of fish physiology and/or biological processes on the otolith concentrations because of the close overlap among the concentration ranges in the otoliths of the fish species studied. Thus, it could be speculated that incorporation of Cr and Ni is dependent mostly on the concentrations available in surrounding water.

Otolith concentrations of other elements, Ag, As, Cd, Co and Tl, were very low. One explanation for this behavior could be that these elements have no biological utility to the fish and hence are not assimilated by the fish to influence the levels in otoliths. In this case, differences observed for the elemental concentrations in otoliths would mainly be due to the concentrations available in the surrounding water. However, there was an overlap among the concentration ranges of Ag, As, Cd, Co and Tl in the otoliths of all fish species, despite the noticeable differences in the concentrations of these elements in Raritan Bay seawater (e.g., Cd and Co, Table 5, last column). This could be an indicator of low affinity of otolith calcium carbonate to these metals. Under such circumstances, the information from these elements could be marginal in otolith elemental fingerprinting for stock discrimination.

4. Conclusion

This study has shown that the removal of calcium matrix via calcium fluoride precipitation allows accurate determination of trace elements from fish otoliths to be achieved by ETV-ICP-MS. The concentration of calcium in solution was reduced substantially to levels as low as 21 μg ml^{-1}. Although non-spectral interferences were not observed from the remaining soluble calcium matrix, the levels were still sufficiently high to cause spectral interferences on several elements such as Co and Ni that, due to very low concentrations in otoliths, suffered from the overlaps of calcium oxides and hydroxides for determinations by conventional solution nebulization ICP-MS. The use of ETV sample introduction afforded not only better sensitivity but also eliminated the interferences of calcium oxides and hydroxides enabling accurate determinations.

Incorporation of trace elements into fish otoliths is a complex process. It is reported that osmo-regulation controlling the uptake levels of major elements such as Na and K from surrounding water to the blood stream of the fish and subsequently to its otoliths may not affect the levels of trace elements in the otoliths as their concentrations in freshwater and seawater are significantly lower than those of major elements whilst trace element concentrations in otoliths are primarily determined by the concentrations available in resident waters [1]. The data obtained from Raritan Bay fish, however, indicates that family or physiological differences

can also influence the otolith concentrations of certain trace elements, especially of those that are utilized by the fish (e.g., Cu, Mn, Se and Zn). Accumulation to higher levels in otoliths by biological means suggests that the protein matrix of the otolith is involved in the overall uptake process besides the direct incorporation from the water. The results also suggest that certain heavy elements including Ag, As, Cd, Co and Tl are not accumulated significantly in the otoliths regardless of the family differences, either because of their toxicity to the fish or inaffinity of otolith calcium carbonate to these metals.

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