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Food Chemistry 91 (2005) 557–570
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Determination of total mercury in seafood by cold vapor-atomic absorption spectroscopy (CVAAS) after microwave decomposition

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Received 9 August 2004; received in revised form 15 September 2004; accepted 10 October 2004

Abstract

A method was developed for determination of total Hg in seafood using 10% w/v $SnCl_2 \cdot 2H_2O$ and continuous flow CVAAS after microwave decomposition in closed vessels. Seafoods were decomposed with 5 mL HNO_3 and 1 mL 1% w/v NaCl at 200 °C, transferred to polypropylene tubes containing 3.5 mL HCl, and diluted to 50 mL with H_2O. Standards were prepared in diluent containing 10% v/v HNO_3, 7% v/v HCl, and 0.02% w/v NaCl. Potential interference by 22 elements was evaluated. Interference by Se and Au was observed. Stability of standard solutions in HNO_3, HCl and NaCl was evaluated. Stabilizing effect of chloride was demonstrated. Results for six reference materials containing 0.0371–1.59 mg/kg Hg were 86–106% of certificate values. Concentrations in 11 varieties of seafood were 0.015–1.78 mg/kg. Average recoveries of inorganic and organic Hg added to seafood were 102% and 99%, respectively; average recoveries from fortified method blanks were 100% and 97%, respectively. The limits of quantitation were 0.0022 and 0.011 mg/kg in seafoods and reference materials, respectively.
Published by Elsevier Ltd.

Keywords: Total Hg; Seafood; CVAAS; Microwave decomposition

1. Introduction

Toxic effects of Hg and its compounds are well documented. The most important source of non-occupational exposure to Hg is dietary intake of fish and fish products. Hg from geologic and human activity enters the environment mainly as Hg vapor, is converted to methylmercury in aquatic environments by bacteria and phytoplankton, and is retained by sulfhydryl groups in tissues of aquatic animals. Mercury is present in highest concentrations in species high in the food chain, such as tuna, shark, and swordfish (Agency for Toxic Substances & Disease Registry, 1999).

The US Food and Drug Administration (FDA) is responsible for ensuring that seafoods sold in US interstate commerce do not contain excessive levels of Hg (FDA, 1995) and has monitored Hg levels in seafood and other foods since the 1970s. In the 1970s, FDA chemists developed several analytical methods for determination of total Hg in regulatory samples. In these methods, seafood is decomposed by a mixture of hot nitric and sulfuric acids in glass flasks fitted with condensers packed with glass beads that prevent vaporization losses; sodium molybdate (Munns & Holland, 1971) or vanadium pentoxide (Munns & Holland, 1977) catalysts are added. In the method of Holak, Krinitz, and Williams (1972), seafood is decomposed by oven heating at 150 °C $\geqslant$ 30 min with nitric acid in a sealed, stainless

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0308-8146/$ - see front matter. Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2004.10.004

steel crucible lined with Teflon®. In these methods, Hg^{+2} ion in solution is determined by the CVAAS technique described by Hatch and Ott (1968). Hg^{+2} is reduced to Hg^0 vapor by mixing stannous tin and decomposition solutions in a reaction flask that is connected to a flow-through atomic absorption cell. A diaphragm pump recirculates Hg^0 vapors through the system. Atomic absorption is measured at wavelength 253.7 nm. The lowest concentration that can be determined with good precision (±5 relative standard deviation, RSD) by these methods, calculated from the concentration of the lowest standard solution, volume of decomposition solution, and weight of fish decomposed, is approximately 0.2 mg/kg.

Modern Hg analyzers make use of numerous technological improvements that provide more precise, accurate, and sensitive Hg determinations and less reagent consumption than apparatus used in the 1970s. Improvements include continuous flow (Oda & Engle, 1981) or flow injection techniques (Saraswati, Beck, & Epstein, 1993), small-volume and thin-film gas liquid separators, solid state detectors, dual beam optics, Hg^0 vapor generation in precisely controlled, flowing inert gas, selective membranes that separate water vapor from the Hg^0 stream (Corns, Ebdon, Hill, & Stockwell, 1992) and miniaturized systems that are fully automated and computer controlled. Improved instrumentation for decomposition of biological materials is also available. Microwave heating in closed, Teflon® vessels is highly effective when temperature feedback control and high pressure vessels are used. In addition, contamination is extremely low (<1 ng/L Hg) when closed, Teflon® vessels, ultra-pure reagents, and microwave cleaning cycles between samples are used. A thorough review of modern methods for determination of Hg is presented by Clevenger, Smith, and Winefordner (1997).

The goal of this project was to develop a new method that utilizes modern, continuous flow CVAAS instrumentation for determination of $\geqslant$0.1 mg/kg total Hg in seafood collected in FDA's regulatory program. The goal included development of a decomposition procedure for seafood and reference materials that produces solutions suitable for analysis by continuous flow CVAAS, evaluation of stability of standard solutions in various acids, optimization of reagent concentrations for the CVAAS determination, and evaluation of potential interference by concomitant elements. Results of this project are the subject of this report.

2. Materials and methods

2.1. Mercury analyzer

Model QuickTrace M-7500 equipped with autosampler model ASX-510, 4-channel, 12-roller, peristaltic pump, and computer controller (CETAC Technologies, Omaha, NE 68144, USA) was used. Stannous chloride solution is pumped continuously through channel 1 of the pump. Solutions of standards, decomposed seafood, or autosampler rinse solution are pumped through channel 2. Waste solution is pumped out of the gas–liquid separator (GLS) through channels 3 and 4. Santoprene® tubing is used in all four channels. Solutions from channels 1 and 2 are combined in a mixing tee and transported through Viton® tubing to the GLS where mixture flows in a thin film over a frosted glass post. Hg^0 vapors are carried by argon from the GLS through a Nafion® tube to remove H_2O vapor and into the absorption cell. A separate argon stream flows through a reference cell. Atomic absorbance (μAbs) is measured in the thermally equilibrated, 220 mm reference and absorption cells by dual, thermally stabilized, solid state silicon detectors. Hg emission spectrum is produced by an electro-optically regulated Hg lamp. Wavelength 254 ± 2 nm is isolated by fixed optical interference filters. Liquid flows, gas flows, and data acquisition parameters are entered into the software and controlled by the analyzer. Concentration in decomposition solutions is calculated by standard curve. Long term (2–3 h) precision is $\leqslant$2% RSD for Hg concentrations $\geqslant$0.010 μg/L when operating conditions in Table 1 are used.

2.2. Microwave heating system

Model MARS-5 equipped with XP-1500 Plus vessels, EST-300 Plus temperature sensor, and ESP-1500 Plus pressure sensor (CEM Corporation, Matthew, NC 28106, USA) was used. System includes microwave power source that generates up to 1200 ± 15% watts, alternating rotation carousel for holding decomposition vessels, Teflon® TFM decomposition vessels with capacity of approximately 100 mL, and Teflon® PFA vessel covers with Teflon® safety membranes that withstand pressure up to 800 psi. System has temperature feedback control that applies ramped microwave energy to achieve user selected temperature program and pressure monitoring that prevents pressure buildup in excess of vessel rating.

2.3. Seafoods and reference materials

Seafoods were purchased at local retail establishments. Inedible shells, heads, tails, scales, viscera, bones, and skin were removed and discarded. Packing oil, broth, or water were discarded. Products were processed in a food processor until a visually homogeneous paste was obtained. Addition of reagent water $\leqslant$20% the weight of product was necessary to adequately homogenize some products. Results are presented on the basis of product before addition of water. Homogenized prod-

Table 1
Operating conditions and figures of merit for determination of Hg[a]

Condition/figure of merit	Concentration range (μg/L)	
	0.050–5.00	0.005–0.500
Gas flow (mL/min)	125	50
Pump speed (% of full speed)	80	100
Sample uptake rate (mL/min)	7.4	9.3
Reducing solution uptake rate (mL/min)	2.4	3.0
Sample uptake time (s)	25	42
Sample uptake per determination (mL)	3.1	6.5
Rinse time (s)	65	90
Total time per determination	90	132
Baseline read start time (s)	15	10
Baseline read end time (s)	20	18
Hg signal read delay (s)	43	54
Hg signal replicate read time (s)	1	1
Hg signal read replicates on the plateau (number)	5	8
Precision of read replicates on the plateau (%RSD)	⩽1	⩽1
Sensitivity (slope) (μAbs/(μg/L))	18,400	45,000
Range of quantitation and linear response (μg/L)	0.050–5.00	0.005–0.500
Range of standard solutions (μg/L)	0.05–0.5	0.01–0.1
Precision of solution analyses (n = 2–6)		
%RSD (μg/mL)	1 (2 μg/L)	1 (0.03 μg/L)
%RSD (μg/mL)	1 (0.2 μg/L)	1 (0.02 μg/L)
%RSD (μg/mL)	2 (0.05 μg/L)	2 (0.01 μg/L)
Instrument detection limit[b] (μg/L)	0.001	0.0004

Conditions that are the same for both ranges: absorption cell heater set = 60 °C (actual gas temperature = approximately 37 °C); Instrumental zero before first sample = no, periodically = yes before each calibration; calibration settings algorithm = linear, through blank = no, weighted fit = no, recalibration and reslope rates = 0.

[a] Hg^{+2} in diluent (10% v/v HNO_3, 7% v/v HCl, 0.02% w/v NaCl) determined using 10% $SnCl_2 \cdot 2H_2O$.

[b] 3 × standard deviation of concentration determined in standard blank ($n \geqslant 3$).

ucts were stored at −30 ± 2 °C in 50-mL polypropylene tubes with screw caps. Thawed products were re-homogenized with laboratory spatula immediately before analysis to mix liquid that separated upon thawing. Reference materials were obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD 20899, USA). Reference material results are reported on the basis of weight analyzed and are not corrected for moisture content.

2.4. Reagents

Water and acids used for analyses and autosampler rinse solution were ultrapure. Water was ASTM type I. HNO_3 and HCl were Ultrex II grade (catalog nos. 6901 and 6900, respectively, J. T. Baker, Div. Mallinkrodt, Phillipsburg, NJ 08865, USA). HNO_3 used for cleaning microwave vessels was trace metals grade (Catalog No. A509-212, Fisher Scientific, Pittsburgh, PA 15205, USA). $SnCl_2 \cdot 2H_2O$ and NaCl (Catalog Nos. 3980–01 and 3624–01, respectively, J. T. Baker, Div. Mallinkrodt, Phillipsburg, NJ 08865, USA) were A.C.S. grade. Single-element, stock standard solutions of Hg and other elements, 1000 mg/L in dilute HNO_3, were atomic emission or mass spectroscopy grade (Inorganic Ventures, Inc., Lakewood, NJ 08701, USA and High Purity Standards, Charleston, SC 29423, USA). Stock standard solutions of P, S, and B were 1000 mg/L in water; Au was 1000 mg/L in 3.3% v/v HCl; Sb, 1000 mg/L, was stabilized in a mixture of 0.7% v/v HNO_3 and 3% v/v tartaric acid (Inorganic Ventures, Inc., Lakewood, NJ 08701, USA). Methylmercury chloride solution, 1000 mg/L, (Catalog No. 33553, Alfa Aesar, Ward Hill, MA 01835, USA) was used to determine recovery of organic Hg. Argon used for Hg analyzer and preparation of reducing solution was ultra high purity grade compressed gas.

Diluent containing 10% v/v HNO_3, 7% v/v HCl and 0.02% w/v NaCl was prepared by adding 200 mL HNO_3, 140 mL HCl, and 0.4 g NaCl to approximately 1700 mL H_2O in an acid-cleaned, 2-L Teflon® container that had been marked on the outside at 2000-mL level; mixture was diluted to mark with H_2O. Reducing solution containing 10% or 20% w/v $SnCl_2 \cdot 2H_2O$ and 7% v/v HCl was prepared by mixing 35 ml HCl with approximately 300 mL H_2O in a 1-L glass bottle marked at 100-mL increments and adding 50 or 100 g $SnCl_2 \cdot 2H_2O$. Mixture was shaken to dissolve $SnCl_2 \cdot 2H_2O$, diluted to 500 mL with H_2O, and gently sparged ⩾15 min with argon to expel Hg^0. Reducing solution that contained undissolved or precipitated material, was discolored, or was >3 days old was discarded. Only clear, colorless reducing solution was used. Autosampler rinse solution containing 1% v/v HNO_3 and 1% v/v HCl was

prepared by adding 20 mL portions of HNO_3 and HCl to H_2O in an acid-cleaned, 2-L Teflon® container; mixture was diluted to mark with H_2O. NaCl solution used for decomposition was 1% w/v in H_2O.

2.5. Miscellaneous equipment

A commercial food processor with metal blade and plastic work bowl was used to homogenize seafoods (Laboratory Micronizer, Waring Products Div. Dynamics Corp., New Hartford, CT, 06057, USA). Teflon® and glass bottles for holding reagents were new, acid-cleaned, and dedicated for Hg analyses. Motorized and manual adjustable pipettes with capacity of 100 and 1000 μL and 5 and 10 mL with colorless, virgin polypropylene tips were used (Rainin Instrument Co., Emeryville, CA, 12345, USA). Polypropylene centrifuge tubes, 15 and 50 mL capacity, with volume marks at 0.5 and 2.5 mL increments, respectively, and screw caps were used (Falcon nos. 37–2067 and 35–2097, respectively, Becton Dickinson and Co., Franklin Lakes, NJ 12345, USA). Fifteen milliliter tubes were used in the autosampler; 50-mL tubes were used to hold decomposition solutions and measure reagents. Accuracy of 35-, 40-, and 50-mL marks was determined for several tubes by filling to marks with H_2O of known temperature and calculating volume as H_2O weight/H_2O density. Thirty five-milliliter and 40-mL marks were <1% in error. Fifty-milliliter marks were >1% in error. Actual volume at 50-mL mark, determined for every 25th tube, was 48.96 ± 0.11 mL (n = 20).

2.6. Contamination control

Hg contamination from reagents, miscellaneous equipment, and laboratory air was minimized by using disposable, plastic labware that did not need acid-cleaning whenever possible, acid-cleaning non-disposable labware, putting caps in place as much as possible, using ultrapure acids and water, cleaning microwave vessels by heating with HNO_3 in microwave oven before use, and using gravimetric procedures to prepare standards and reagents. Volumetric glassware was not used because potential for contamination was too great.

2.7. Gravimetric preparation of standard solutions

Standards with Hg concentrations of approximately 0, 0.05, 0.1, 0.2, 0.5, and 2 μg/L were prepared by placing 0, 0.5, 1, 2, 5, and 20 g portions of 5 μg/L Hg solution in tared tubes, weighing the portions (⩾4 significant figures), and diluting to 53.000 ± 0.001 g with diluent. Concentration in final solution was calculated as $C_2 = C_1 \times W_1/W_2$ where C_1 and C_2 are concentrations (μg/L) and W_1 and W_2 are weights (g) in high standard (subscript 1) and final solution (subscript 2). Hg solution with 5 μg/L was prepared by pipetting 50 μL of a 5 mg/L Hg solution into 53.000 ± 0.001 g diluent from which 50 μL diluent had been removed. Hg solution with 5 mg/L was prepared by pipetting 250 μL of a 1000 mg/L Hg solution into 53.000 ± 0.001 g diluent from which 250 μL had been removed. Density of diluent was determined by adding 5.0 ± 0.05 mL HNO_3, 3.5 ± 0.05 mL HCl and 1.0 ± 0.01 mL 1% v/v NaCl to H_2O in a tared, glass 50-mL volumetric flask, diluting to mark with H_2O, cooling to room temperature, and weighing the flask. Density of diluent at room temperature was 1.060 ± 0.002 g/mL. This solution was discarded.

2.8. Cleaning microwave vessels

Vessels were cleaned before each use with 10 mL HNO_3 and the following 2-step microwave program: (1) apply 1200 W to heat from ambient to 200 °C over a period of 10 min and (2) apply 1200 W and hold at 200 °C for 3 min. Vessels were disassembled when contents in control vessel indicated ⩽50 psi and ⩽50 °C and cleaning acid was discarded. Vessels were rinsed with H_2O and dried in chemical fume hood.

2.9. Preparation of decomposition solutions

Reference materials and seafood were weighed to nearest 0.1 mg in tared decomposition vessels. 500 μL H_2O was pipetted into vessels for method blanks. 1.0 ± 0.01 mL 1% w/v NaCl and 5.0 ± 0.1 mL HNO_3 were added to each vessel. Hg fortification solution (⩽1 mL) was added as needed for recovery experiments. Vessels were immediately assembled, gently swirled, and placed in the microwave apparatus. Materials were decomposed using temperature feedback control and the following 3-step program: (1) apply 300 W to heat from ambient to 130 °C over a period of 5 m, (2) apply 1200 W to heat from 130 °C to maximum temperature in experiments over a period of 20 m, and (3) apply 1200 W and hold at maximum temperature for time in experiments. Contents were cooled to ⩽50 psi and ⩽50 °C before vessel disassembly and then quantitatively transferred to 50-mL tube containing 5 mL H_2O and 3.5 ± 0.1 mL HCl. Contents were diluted to 50-mL mark with H_2O, mixed, and cooled to room temperature by setting them in chemical fume hood for approximately 10 min. Caps were off during cooling to release dissolved gases. Decomposition solutions were analyzed within 3 days of preparation.

2.10. Determination of Hg and calculation of results

Analyzer was optimized as in Section 3.1 and was standardized with solutions prepared in Section 2.7. One reading per solution was made. Solutions containing known Hg concentrations in diluent (quality control

(QC) solutions) were analyzed periodically during experiments. When QC results differed >5% from actual values, results were rejected, analyzer was re-optimized and re-standardized, and all solutions were re-analyzed. Decomposition solutions were analyzed undiluted or were diluted with diluent to eliminate signal suppression by residual components or to reduce concentrations that were greater than those in the linear range. Concentration in decomposition solutions (C, μg/L) was calculated as $C = D_F \times [(R\text{-intercept})/\text{slope}]$ where D_F and R are dilution factor (v/v) and instrument response (μAbs), respectively, of solution analyzed and intercept (μAbs) and slope ([μAbs/(μg/L])) are quantities calculated by linear least squares regression of instrument responses and concentrations of standards. Concentration in seafood and reference materials (M, mg/kg) was calculated as $M = (C-B) \times V/W$ where C is defined above and B is concentration (μg/L) determined in decomposition solutions of method blank. V is volume (L) of decomposition solution. W is weight (kg) of material decomposed. Percent recovery of Hg added to analytical portions was calculated as $100 \times (F-M)/(N \times O/P)$ where F is concentration (mg/kg) determined in fortified portions, M is concentration (mg/kg) determined in unfortified portions, N is concentration (mg/L) in fortification solution, O is volume of fortification solution (L), and P is weight (kg) of portion decomposed.

3. Results and discussion

3.1. Optimization of mercury analyzer

The analyzer used in this project determines Hg concentrations from approximately 50–<0.001 μg/L. Different concentration ranges are determined by using different gas and liquid flow rates. Low liquid and high gas flows are used to analyze high concentrations; high liquid and low gas flows are used for low concentrations. The signal generated by the analyzer is a peak with a flat top (plateau). The signal is approximately 0 μAbs when autosampler rinse solution flows through the GLS and rises quickly when Hg solution is introduced. The peak plateau forms when production of Hg^0 vapor reaches a steady state and falls rapidly when rinse solution is reintroduced. Operating conditions were optimized to give (1) precision ⩽1% RSD for 5, 1-s readings on the plateau, (2) precision ⩽2% RSD for results obtained by repeated analysis of standard solutions (with autosampler returning to rinse station after each analysis), (3) reduction of Hg carry-over from immediately preceding analysis to ⩽0.1 of the concentration in lowest standard in a relatively short time (⩽135 s total analysis time), and (4) sensitivity (μAbs/μg/L) that agreed ±20% with manufacturer specifications. One point baseline correction, immediately before signal rise, was used. Optimized operating conditions for the two concentration ranges used in this project are presented in Table 1. Concentration ranges that are lower and higher than those in Table 1 are determined by this instrument but were not needed for analysis of products analyzed in this study.

Figures of merit in Table 1 were determined for Hg in diluent containing 10% v/v HNO_3, 7% v/v HCl, and 0.02% w/v NaCl. Reducing solution contained 10% w/v $SnCl_2 \cdot 2H_2O$ in 7% HCl as recommended in manufacturer specifications. Instrument sensitivities were expressed as slope of standard curve (μAbs/μg/L) and agreed well (±20%) with manufacturer specifications for Hg sensitivity in 7% HCl. The lower boundary of the range of quantitation and linear response was the lowest concentration determined with precision ⩽2% RSD for repeated analysis of the same standard solution during 2–3 h. The upper boundary of the range of quantitation and linear response was the highest concentration that deviated ⩽5% relative difference from linearity. Upper boundaries of the ranges decreased approximately 15% when a more stringent criteria for deviation from linearity, ⩽2% relative difference, was used.

3.2. Initial evaluation of decomposition conditions

Heating with acid is the most often used practice for decomposing biological materials for determination of total Hg. HNO_3 in combination with H_2SO_4, $HClO_4$, HCl, H_2O_2, $KMnO_4$, $K_2Cr_2O_7$, or $KBr/KBrO_3$ mixtures are acids and oxidizing agents used (Evans et al., 2003; Taylor, Branch, Halls, Patriarca, & White, 2003). The first decomposition procedure evaluated in this project was a modification of the procedure of Munns and Holland (1977) recommended by Louie (1983) and Louie et al. (1985) who showed that when HCl was added to a mixture of $HNO_3 + H_2SO_4$, precision, recovery, and decomposition time improved and packed condensers were not needed to prevent Hg loss. Complexation of Hg^{+2} ions as $Hg_2Cl_4^{-2}$ was proposed as the mechanism for Hg retention. Similar observations were reported by others when HCl was added to acid mixtures in open vessels (Adeloju & Mann, 1987; Haas & Krivan, 1984; Vibhakar, Krishnarajpet, Nagaraja, & Kapur, 1983). Improved results were also reported when chlorine-containing acid was added to mixtures in closed vessels. Zhou, Wong, Koh, and Wee (1996) improved results by adding $HClO_4$ or HCl to HNO_3 when determining Hg in sediments; aqua regia ($HCl + HNO_3$, 3 + 1) was recommended because it is safer to use than $HClO_4$. Hepp, Cargill, and Shields (2001) reported improved results using a less corrosive mixture of $HCl + HNO_3$ (1 + 3) to decompose FDA color additives. Dabeka, Bradley, and McKenzie (2002) decomposed foods by heating with HNO_3, HCl, and H_2O_2 in capped polypropylene tubes and reported that stability of Hg in solutions was better with HCl than with Au^{+3} or $K_2Cr_2O_7$.

Dabeka et al. (2002) also reported that when HCl was omitted from the decomposition mixture, recovery from method blanks fortified with Hg was erratic, sometimes as low as 3%, even though recovery from foods was 90–100% and that adding HCl to the mixture gave equivalent recoveries from fortified foods and method blanks. Initial experiments in the authors' laboratory showed that addition of 1 mL 1% w/v NaCl to $HNO_3 + H_2SO_4$ in open glass flasks provided adequate chloride ion to achieve 100% recovery of Hg added to method blanks, seafood, and reference materials and that recovery of Hg from method blanks was unacceptable when NaCl was omitted. However, corrosive HNO_3, HCl, and Cl_2 fumes generated during heating rapidly damaged equipment inside the chemical fume hood. Containment of corrosive fumes by using glass-packed condensers was successful, but unacceptably high carry-over contamination in the condensers could not be removed by acid cleaning. Experiments with $HNO_3 + H_2SO_4 + NaCl$ in glass vessels therefore were discontinued.

FDA's decomposition procedure for determination of elements in food by inductively coupled plasma-atomic emission spectroscopy (ICP-AES, Dolan & Capar, 2002) was investigated next. In the ICP-AES procedure, 9 mL HNO_3 and $\leqslant$10 g food are heated 3 min at 200 °C in closed Teflon® vessels by microwave energy. H_2O_2 is added after heating to remove residual yellow color and decomposition products are diluted to 50 mL with H_2O. To obtain Hg concentrations that are appropriate for the CVAAS analyzer used in this project, 0.5 g seafood and 0.1 g reference material were decomposed. To conserve reagents, 5 mL HNO_3 was used for decomposition. One mL 1% w/v NaCl was added to decomposition vessels to prevent Hg loss. HCl was added to decomposition products after microwave heating so that HCl concentrations in decomposition solutions and $SnCl_2 \cdot 2H_2O$ reducing solution matched. Reducing solution recommended by the instrument manufacturer containing 10% w/v $SnCl_2 \cdot 2H_2O$ in 7% v/v HCl was used. Decomposition products were diluted to 50 mL with H_2O. H_2O_2 added to solutions after heating produced poorly shaped Hg signals; peak shapes were irregular (not flat-topped), non-reproducible, and precision of replicate readings on the poorly shaped plateau was >1% RSD. Addition of H_2O_2 therefore was discontinued. Good precision and recovery were obtained using these modifications. In addition, carry-over contamination from previous analyses did not occur. Seafood heated with HNO_3 and NaCl in closed, Teflon® vessels was used throughout the remainder of the project.

3.3. Stability of standard solutions

Previous researchers reported that oxidizing agents such as $K_2Cr_2O_7$ (Feldman, 1974), $KMnO_4$ (Zhou et al., 1996), Au (Allibone, Fatemian, & Walker, 1999), and BrCl (Hall, Pelchat, Pelchat, & Vaive, 2002), complexing agents such as HCl (Dabeka et al., 2002; Saraswati et al., 1993), and sufficiently high acid concentrations were needed to prevent Hg losses by reduction of Hg^{+2} to Hg^0 vapor and adsorption of Hg^{+2} on container walls (Sturman, 2000). Experiments therefore were done to determine stability of Hg in solutions that contain reagents to be used in this method. Experimental solutions were prepared to contain 1 μg/L Hg and 10% v/v HNO_3 with or without 5% v/v HCl and 0.02% w/v NaCl. Solutions were held in 50-mL tubes and portions taken for analysis $\leqslant$10 m, 2, 4, 6, and 23 h, and 2, 3, 6, and 7 d after preparation. The analyzer was standardized immediately before analysis. New standard solutions were prepared daily and contained 10% v/v HNO_3, 5% v/v HCl, and 0.02% w/v NaCl. Ten percent w/v $SnCl_2 \cdot 2H_2O$ reducing solution was used. Results obtained at $\leqslant$10 min, 2, 4, 6, and 23 h are presented in Fig. 1. Trends demonstrated in Fig. 1 continued on days 2–7.

Experimental results demonstrate the stabilizing effect of chloride. Hg concentration was nearly constant at all times in solutions that contained chloride. Concentration varied $\leqslant$2% relative difference in solutions that contained 5% v/v HCl. Concentration decreased $\leqslant$5% when the source of chloride was 0.02% w/v NaCl. Hg concentration decreased significantly, however, in the solution that contained only 10% v/v HNO_3; concentration decrease was 6–28% in 4–23 h, and 54–94% in 2–7 d. Results in Fig. 1 also demonstrate that instrument response is slightly higher when NaCl is present and HCl is absent than when HCl is present with or without NaCl. HCl therefore must be present in both decomposition and standard solutions. To determine whether 5% v/v HCl added to decomposi-

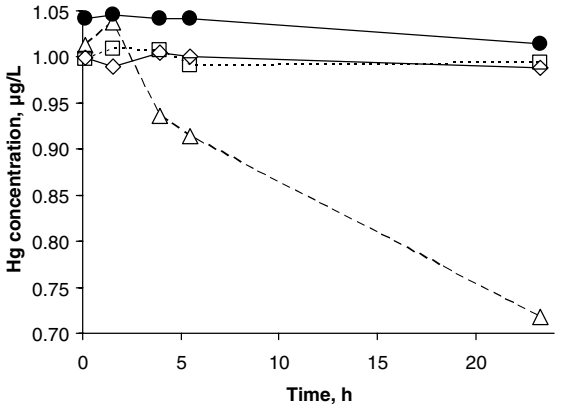

Fig. 1. Hg^{+2} concentration vs. time in solutions containing (1)10% v/v HNO_3, 0% v/v HCl, 0% w/v NaCl (–△–), (2)10% v/v HNO_3, 5% v/v HCl, 0% w/v NaCl (–◇–), (3)10% v/v HNO_3, 5% v/v HCl, 0.02% w/v NaCl (–□–), and (4)10% v/v HNO_3, 0% v/v HCl, 0.02% w/v NaCl (–●–).

tion and standard solutions was adequate when variable amounts of chloride expected in seafood were present, triplicate portions of homogenized, canned tuna were fortified with 0, 10, and 30 mg NaCl (giving additional 0, 0.02, and 0.06% w/v NaCl in 50 mL decomposition solutions) and analyzed using standards that contained 10 v/v HNO_3, 5% v/v HCl, and 0.02% w/v NaCl. Average Hg concentrations ±1 standard deviation ($n = 3$) were 0.060 ± 0.001, 0.060 ± 0.001 and 0.061 ± 0.002 mg/kg, respectively, and were considered equivalent. Standards, diluent, and decomposition solutions were prepared to contained HNO_3, HCl, and 0.02% w/v NaCl in the remainder of the experiments. Concentrations of HNO_3 and HCl are selected in Section 3.4.

3.4. Effect of reagent concentrations on instrument response

Good instrument sensitivity (μAbs/μg/L), precision, and recovery were obtained in Section 3.2 from decomposition solutions that contained 10% v/v HNO_3, 7% v/v HCl, 0.02% w/v NaCl and were analyzed using 10% w/v $SnCl_2 \cdot 2H_2O$. Experiments were done to determine the effect of varying HNO_3, HCl, and $SnCl_2 \cdot 2H_2O$ concentrations on instrument sensitivity. Standard solutions containing 1 μg/mL Hg^{+2} and various concentrations of HNO_3 (5, 10 and 20% v/v) and HCl (0, 0.5, 1, 2, 3, 5, 7, 10 and 15% v/v) were analyzed using various concentrations of $SnCl_2 \cdot 2H_2O$ (2.5, 5, 10, and 20% w/v). Apparent Hg^{+2} concentration determined vs. HCl concentration for various HNO_3 and $SnCl_2 \cdot 2H_2O$ concentrations is presented in Fig. 2.

Instrument sensitivity increased up to 10% for almost all combinations of $SnCl_2 \cdot 2H_2O$ and HNO_3 when HCl concentration increased from 0 to 1% v/v. These results indicate that variable amounts of HCl <1% v/v are unsuitable for use in the method. Increased sensitivity in the presence of HCl agrees with observations of Adeloju and Mann (1987) and Louie (1983) and may be due to increased concentrations of Hg^{+2} ions available for reduction as $Hg_2Cl_4^{-2}$.

When 20% w/v $SnCl_2 \cdot 2H_2O$ was used, instrument sensitivity was constant (varied ⩽5% relative difference) for all HNO_3 concentrations in solutions that contained ⩾1% v/v HCl (Fig. 2(a)). When 10% w/v $SnCl_2 \cdot 2H_2O$ was used, sensitivity was constant for solutions containing 5 and 10% v/v HNO_3 and ⩾1% v/v HCl; sensitivity declined for solutions containing 20% v/v HNO_3 and ⩾7% v/v HCl (Fig. 2(b)). Sensitivity declined rapidly for all HNO_3 and HCl concentrations when 5% w/v $SnCl_2 \cdot 2H_2O$ was used (Fig. 2(c)) and very rapidly when 2.5% w/v $SnCl_2 \cdot 2H_2O$ was used (Fig. 2(d)). Reduced sensitivity in the presence of high HNO_3 and low $SnCl_2 \cdot 2H_2O$ concentrations was observed by Adeloju and Mann (1987) and may be caused by the concentration of reducible components exceeding the reducing capacity of $SnCl_2 \cdot 2H_2O$.

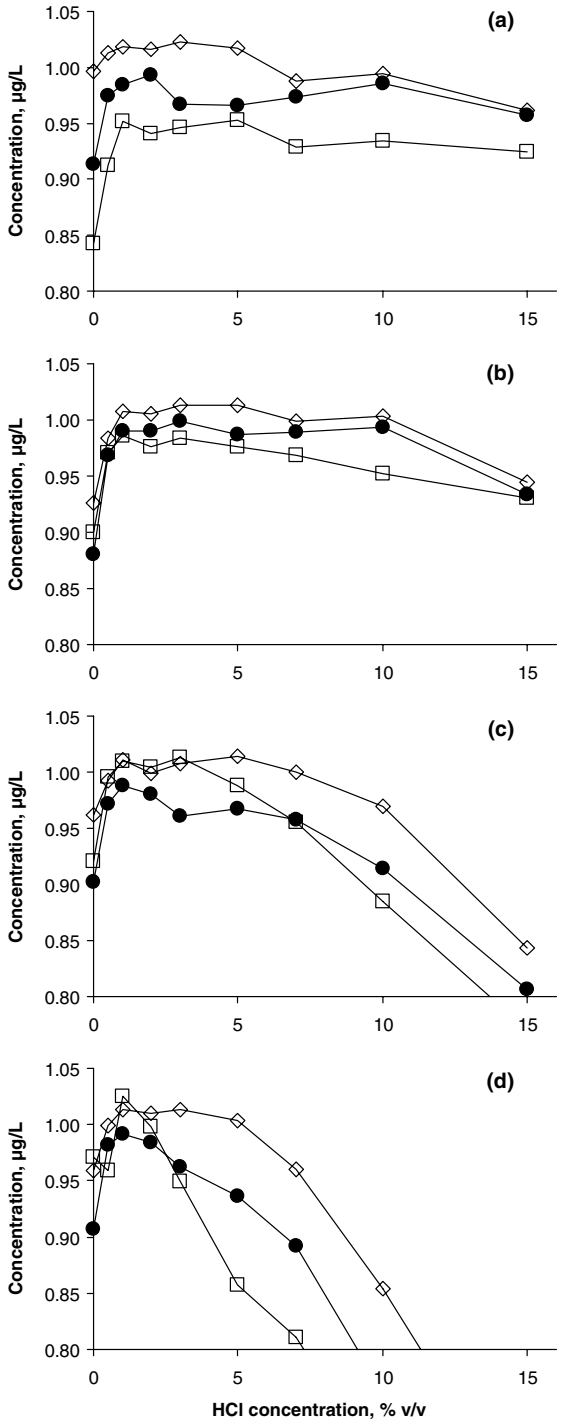

Fig. 2. Apparent concentration (μg/L) of 1 μg/L Hg^{+2} determined using various concentrations of HCl, HNO_3, and $SnCl_2 \cdot 2H_2O$. Concentration of $SnCl_2 \cdot 2H_2O$ is 20% w/v in (a), 10% w/v in (b), 5% w/v in (c), and 2.5% w/v in (d). Concentration of HNO_3 is 5% v/v (–◇–), 10% v/v (–●–), and 20% v/v (–□–).

Results in Fig. 2 demonstrate that instrument sensitivity is relatively constant when the following

combinations of $SnCl_2 \cdot 2H_2O$, HNO_3, and HCl are used: (a) 20% w/v $SnCl_2 \cdot 2H_2O$, 5–20% v/v HNO_3, and 1–15% v/v HCl, and (b) 10% w/v $SnCl_2 \cdot 2H_2O$, 5–10% v/v HNO_3, and 1–10% v/v HCl. Other practical considerations were used to make the final selection of concentrations for use in the method. Ten percent w/v $SnCl_2 \cdot 2H_2O$ reducing solution is recommended for the method because the potential for Hg contamination is less, stannic tin precipitates less quickly (>3 d vs. <3 d), and analyses cost less when the more dilute reducing solution is used. Note that this concentration is high enough to ensure that a sufficient level of stannous tin remains in reducing solution that is up to 3 d old and has degraded. Stannous tin is rapidly oxidized to stannic tin by oxygen and light. In the authors' laboratory, 5% w/v $SnCl_2 \cdot 2H_2O$ that was >5 days old produced results that were similar to those obtained with 2.5% w/v $SnCl_2 \cdot 2H_2O$; 10% w/v $SnCl_2 \cdot 2H_2O$ that was $\leqslant$3 days old produced results equivalent to fresh solution. Seven percent v/v HCl is recommended for use in decomposition solutions, standards, and diluent because it is adequately above minimum HCl concentration (1% v/v) needed for constant sensitivity in Fig. 2, matches minimum HCl concentration needed to keep $SnCl_2 \cdot 2H_2O$ solutions free of colloidal precipitates for several days, and matches HCl concentration recommended by the manufacturer to obtain performance specifications. Ten percent v/v HNO_3 is recommended for use in decomposition solutions, standards, and diluent because solutions containing 20% v/v HNO_3 and 7% v/v HCl require the use of 20% w/v $SnCl_2 \cdot 2H_2O$ reducing solution; the potential for Hg contamination, cost of analyses, and degradation of Santoprene® tubing is less when using the more dilute HNO_3 concentration; and preparation of decomposition solutions containing 10% v/v HNO_3 is much less time-consuming than preparing 5% v/v HNO_3 solutions. Decomposition solutions having 5% v/v HNO_3 are prepared by taring an acid-cleaned bottle, adding H_2O and HCl, transferring microwave vessel contents (5 mL HNO_3 and decomposition products) to the bottle, and adding H_2O until 106.0 g total weight is obtained. Decomposition solutions having 10% v/v HNO_3 are more easily prepared by placing H_2O and HCl in 50-mL tubes that do not need acid-cleaning, transferring microwave vessel contents to the tube, and diluting to the mark with H_2O. The reason for not using 5% v/v HNO_3 solutions prepared in 50-mL disposable tubes is that this concentration limits the amount of HNO_3 used for decomposition to 2.5 mL; this volume is less than the minimum required for safe and effective use of the microwave vessels. Five milliliter HNO_3 in microwave vessels and decomposition solutions diluted to 50-mL in disposable tubes were used in the remainder of this work.

3.5. Interference by concomitant elements

Numerous elements that interfere with CVAAS determination of Hg^{+2} have been reported. Suppression of Hg signal by Au, Co, Cu, Fe, Mn, Ni, Pb, Sb, and Se and enhancement of signal by Cu, Mn, Ni, Pb, Sb, and Sn were reported by Krata, Pyrzynska, and Bulska (2003), Murphy et al. (1996), DeAndrade, Pasquini, Baccan, and Van Loon (1988), DeAndrade et al. (1983), Rooney (1976), and Toffaletti and Savory (1975). Experiments therefore were done to determine if elements present in seafoods, reference materials, and foods routinely analyzed by FDA interfere with determination of Hg^{+2} by this method.

Concentrations (mg/L) selected for this experiment were 2–2000 times those that would be present in solutions prepared by decomposing 0.1 g seafood reference material or 0.5 g seafood or food and diluting decomposition solutions to 50 mL. Concentrations (mg/kg) used to calculate mg/L were taken from reference material certificates and FDA publications on levels of elements in foods (FDA, 2003). The Hg concentration selected for experiment, 0.020 μg/L, is very low but is determined with good precision ($\leqslant$2% RSD). Operating conditions for the 0.005–0.500 μg/L Hg quantitation range were used. Solutions were prepared in 10% v/v HNO_3, 7% v/v HCl, and 0.02% v/v NaCl and were analyzed with 10% w/v $SnCl_2 \cdot 2H_2O$. Solutions containing concomitant element but not Hg were analyzed as controls. Elements, concentrations evaluated, and results are shown in Table 2. Error $\leqslant$5% relative difference from actual was considered not significant because it was indistinguishable from imprecision of instrument readings and preparing solutions with known concentrations of Hg^{+2}. No interference was observed for Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, S, Sb, V, and Zn at concentrations studied.

No interference was observed for Se concentrations $\leqslant$0.025 mg/L when present alone or in mixtures with other elements. Se concentrations of 0.050, 0.10, 0.25. 0.5, 1, and 5 mg/L suppressed Hg response 6, 11, 23, 35, 44, and 67%, respectively. Hg response returned to expected levels when subsequent solutions containing $\leqslant$0.025 mg/L Se were analyzed. The maximum tolerable Se concentration (concentration that does not interfere with this method) depends on the quantitation range (analyzer operating conditions) used, weight of product decomposed, Hg concentration in product, and dilution factor of the solution analyzed. When the 0.05–5 μg/L range is used to analyze undiluted decomposition solutions of 0.5 g product containing 0.005 mg/kg Hg (undiluted decomposition solutions containing 0.05 μg/L Hg), the maximum tolerable Se concentration is 2.5 mg/kg (0.025 mg/L Se × 0.05 L decomposition solution/0.0005 kg product). For products containing 0.025, 0.05, 0.5, or 1 mg/kg Hg, decomposition solutions may be diluted

Table 2
Determination of 20.0 ng/L Hg^{+2} in the presence of concomitant elements[a]

Concomitant element	Concomitant concentration (mg/L)	Apparent Hg^{+2} concentration (ng/L)	Error[b] (%)
None	0	19.7	−2[c]
Na^{+1}	500	19.8	−1
K^{+1}	500	19.9	−0.5
Ca^{+2}	500	19.8	−1
P^{+5} as PO_4^{-3}	250	19.6	−2
Mg^{+2}	100	19.8	−1
S^{+6} as SO_4^{-2}	100	19.7	−2
Fe^{+2}	100	20.5	3
Al^{+3}	50	19.8	−1
As^{+3}/As^{+5}	50	19.7	−2
Co^{+2}	50	20.1	0.5
Cu^{+2}	50	20.0	0
Mn^{+2}	50	20.0	0
Sb^{+3}	50	19.7	−2
Zn^{+2}	50	19.8	−1
B^{+3} as $B(OH)_3$	5	19.7	−2
Cd^{+2}	5	19.9	−0.5
Cr^{+3}	5	19.7	−2
Ni^{+2}	5	20.1	0.5
Pb^{+2}	5	19.3	−4
V^{+5}	5	19.6	−2
Au^{+3}	0.001	20.2	1
Au^{+3}	0.010	20.4	2
Au^{+3}	0.10	19.7	−2
Au^{+3}	5.0	12.9	−35
Se^{+4}	0.010	19.8	−1
Se^{+4}	0.025	19.8	−1
Se^{+4}	0.050	18.9	−6
Se^{+4}	0.10	17.8	−11
Se^{+4}	0.25	15.5	−23
Se^{+4}	0.50	13.1	−35
Se^{+4}	1	11.1	−44
Se^{+4}	5	6.8	−66
Se^{+4}	5	33[d]	−67
Se^{+4}	5	160[e]	−67
Se^{+4}	5	333[f]	−67
Mix A + Se^{+4}, Au^{+3}	Various[g]	19.1	−4
Mix B + Se^{+4}, Au^{+3}	Various[h]	18.9	−5
Mix C + Se^{+4}, Au^{+3}	Various[i]	19.7	−1

[a] Solutions were analyzed once.
[b] Error was calculated as $100*(A - B)/20$. A is Hg^{+2} concentration determined in solution containing Hg^{+2} and concomitant element. B is Hg^{+2} concentration determined in solution containing concomitant element only.
[c] Error was calculated as $100*(C - D)/20$. C is Hg^{+2} concentration determined in solutions containing Hg^{+2} and no concomitant. D is Hg^{+2} concentration determined in solution containing neither Hg nor concomitant. Solution was analyzed eight times at periodic intervals during experiment. Hg^{+2} concentrations ranged 19.1–20.1 ng/L and were within 5% relative difference of 20.0 ng/L.
[d] Hg^{+2} concentration is 100 ng/L.
[e] Hg^{+2} concentration is 500 ng/L.
[f] Hg^{+2} concentration 1000 ng/L.
[g] Na, K = 500 mg/L; P = 250 mg/L; Ca, Mg, S = 100 mg/L; Se = 0.025 mg/L; Au = 0.005 mg/L.
[h] Al, Co, Cu, Fe, Mn, Sb, Zn = 50 mg/L; Se = 0.025 mg/L; Au = 0.005 mg/L.
[i] As, B, Cd, Cr, Ni, Pb, V = 5 mg/L; Se = 0.025 mg/L; Au = 0.005 mg/L.

for analysis by factors of 5, 10, 100, or 200, and Se concentrations of 12.5, 25, 250, and 500 mg/kg are tolerated. When 0.1 g reference material is decomposed or when the 0.005–0.5 μg/L concentration range is used, maximum tolerable Se concentration (mg/kg) in product is 5 or 10 times greater, respectively. Se concentrations generally are ⩽2 mg/kg in foods (FDA, 2003), ⩽6 mg/kg in seafood-related reference materials, and often concomitant with sufficiently high Hg concentrations that decomposition solutions may be diluted for analysis. When Se interference is suspected (recovery of Hg from fortified portions of product is low), it can be eliminated by diluting decomposition solutions. Se in foods and reference materials therefore will not adversely affect

Table 3
Determination of total Hg in unfortified and fortified portions of chunk light tuna, canned in oil, using various maximum heating temperatures, hold times at maximum temperature, weights of tuna, and stannous chloride concentrations

Stannous chloride concentration and fortification species	Maximum heating temperature, hold time at maximum temperature, weight of tuna				
	200 °C, 3 min, 2 g	200 °C, 10 min, 2 g	210 °C, 10 min, 2 g	210 °C, 10 min, 1 g	210 °C, 10 min, 0.5 g
	Average concentration ±1 SD (mg/kg) in unfortified portions, (average recovery ±1 sd (%) in fortified portions)[a]				
20% $SnCl_2$ solution					
Unfortified tuna	0.061 ± 0.003	0.061 ± 0.001	0.061 ± 0.001	0.064 ± 0.002	0.062 ± 0.001
Tuna fortified with inorganic Hg[b]	0.118 ± 0.004 (94 ± 7)	0.119 ± 0.001 (96 ± 2)	0.117 ± 0.001 (93 ± 1)	0.119 ± 0.004 (92 ± 2)	0.122 ± 0.000 (100 ± 1)
Tuna fortified with organic Hg[c]	0.116 ± 0.002 (92 ± 3)	0.118 ± 0.001 (94 ± 1)	0.118 ± 0.001 (94 ± 1)	0.119 ± 0.002 (92 ± 3)	0.121 ± 0.001 (98 ± 1)
10% $SnCl_2$ solution					
Unfortified tuna	0.062 ± 0.001	0.061 ± 0.002	0.061 ± 0.002	0.062 ± 0.001	0.061 ± 0.001
Tuna fortified with inorganic Hg[b]	0.118 ± 0.002 (94 ± 4)	0.118 ± 0.001 (94 ± 2)	0.117 ± 0.003 (93 ± 4)	0.118 ± 0.004 (93 ± 6)	0.120 ± 0.001 (97 ± 2)
Tuna fortified with organic Hg[c]	0.118 ± 0.001 (94 ± 2)	0.118 ± 0.002 (95 ± 2)	0.116 ± 0.004 (92 ± 6)	0.117 ± 0.001 (92 ± 2)	0.119 ± 0.001 (95 ± 1)
	Ratio (C_u/C_d) of concentrations determined in undiluted (C_u) and diluted (C_d) solutions from one of the three portions of tuna[d]				
20% $SnCl_2$ solution					
Unfortified tuna	0.91[e]	0.99[f]	1.02[f]	0.99[f]	1.00[f]
Tuna fortified with inorganic Hg[b]	0.92[e]	0.94[e]	1.02[f]	1.01[f]	0.99[f]
Tuna fortified with organic Hg[c]	0.92[e]	0.96[e]	1.02[f]	0.98[f]	1.00[f]
10% $SnCl_2$ solution					
Unfortified tuna	0.95[e]	0.99[f]	1.04[f]	0.99[f]	0.99[e]
Tuna fortified with inorganic Hg[b]	0.95[e]	0.97[e]	1.00[f]	0.98[e]	0.98[e]
Tuna fortified with organic Hg[c]	0.97[e]	0.99[e]	0.98[f]	0.97[e]	0.97[e]

[a] Decomposition solutions were not diluted for analysis; $n = 3$.
[b] Source of inorganic Hg was Hg^{+2} in a mixture of dilute HNO_3 and HCl. Concentration of Hg added was 0.060 mg/kg.
[c] Source of organic Hg was CH_3HgCl in water. Concentration of Hg added was 0.060 mg/kg.
[d] Dilution factor was 8 for unfortified tuna and 16 for fortified tuna; $n = 1$.
[e] Indicates apparent concentration (mg/kg) was a smooth function of dilution factor (DF) of the solution analyzed. Apparent concentration (mg/kg) was erroneously low when DF = 1 and increased as DF increased to 8 (unfortified portions) or 16 (fortified portions).
[f] Indicates that apparent concentration (mg/kg) was independent of dilution factor.

results obtained by this method if recoveries of Hg from fortified portions of product are monitored and solutions are diluted and reanalyzed when recoveries determined in undiluted solutions are low.

High concentrations of Au^{+3} interfered with Hg determination in these experiments. Hg signal was suppressed 35% when determined in the presence of 5 mg/L Au^{+3} and remained suppressed approximately 20% in subsequent analyses of solutions containing 0 mg/L Au^{+3}. Rinsing with copious volumes of diluent, 25% v/v HNO_3, or 25% v/v HCl did not restore instrument sensitivity. Sensitivity was restored by soaking the GLS 24 h in aqua regia. Signal suppression and permanently reduced instrument sensitivity may have been caused by chemically reduced, insoluble Au^0 that precipitated in the system, thereby trapping Hg^{+2} or Hg^0 by occlusion or amalgamation. Occlusion of Hg with finely dispersed trace elements (Ag, Cu, Ni, Se) that precipitated upon reduction by stannous chloride has been proposed to explain suppression observed in previous studies (DeAndrade et al., 1983; Krata et al., 2003; Welz & Schubert-Jacobs, 1988) and may have caused 30% suppression that occurred in research using cold vapor atomic fluorescence spectroscopy (Fatemian, Allibone, & Walker, 1999). The maximum tolerable Au^{+3} concentration for this method was not determined because another GLS would have been desensitized. Instead, several solutions that contained up to 30 times the maximum Au^{+3} concentration present in decomposition solutions of food-related reference materials were analyzed. Concentrations (mg/kg) used to calculate μg/L in experimental solutions were taken from reference material certificates or published reference material results (Roelandts & Gladney, 1998). Au concentrations in foods were not available at the time of this experiment. Au concentrations in NIST 1641 d (natural water preserved with Au) and other reference materials are 1 mg/kg and $\leqslant$0.003 mg/kg and produce 3.18 and $\leqslant$0.006 μg/L Au in decomposition solutions, respectively (mg/kg Au in material $\times$ 0.0001 kg material/0.05 L). No suppression was observed for Au^{+3} concentrations $\leqslant$0.10 mg/L when present alone or in mixtures with other elements. Au interference therefore will not adversely affect results obtained by this method for reference materials routinely analyzed by FDA. Solutions to which 5 mg/L Au is added, as is sometimes done to preserve solutions or reduce Hg washout time by inductively coupled plasma-mass spectroscopy methods, cannot be analyzed by this method unless Hg concentrations are high enough to allow analysis of solutions that are diluted so that Au^{+3} concentration is $\leqslant$0.10 mg/L.

3.6. Decomposition conditions: temperature, time, weight of test portion

Oxidation of organic matter by heating with HNO_3 is not complete; residual carbon containing compounds such as nitrobenzoic acid may remain. Kingston, Walter, Chalk, Lorentzen, and Link (1997) summarized research that showed mixtures of HNO_3 and biological materials must reach temperatures of 140, 150, and 165 °C to adequately decompose carbohydrates, proteins, and fats, respectively, in materials that are analyzed by inductively coupled plasma and flame spectroscopies and that higher temperatures may be needed to minimize interference from incompletely decomposed components when less robust detection techniques such as polarography and graphite-furnace atomic absorption spectroscopy are used. Analyses therefore were done to evaluate the effect of residual components on results when various maximum heating

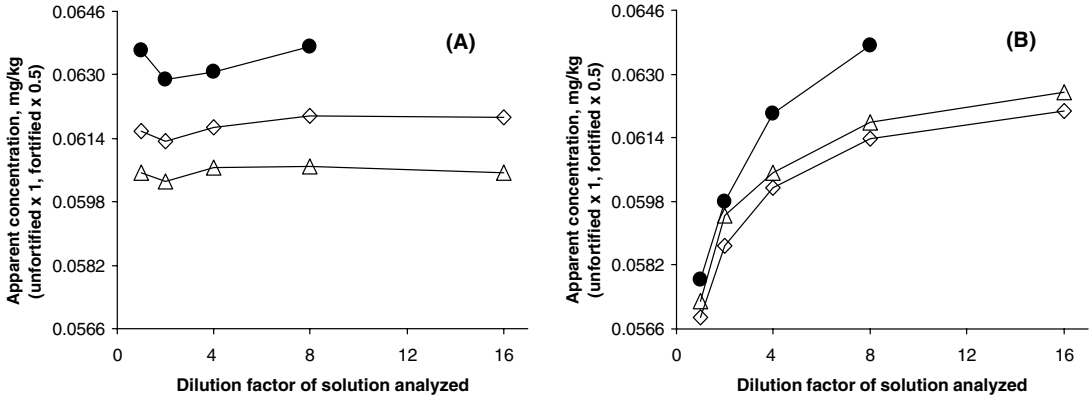

Fig. 3. Apparent concentration (mg/kg) vs. dilution factor (D_F) of the solution analyzed for decomposition solutions that were prepared by heating (A) 0.5 g tuna 10 min at 210 °C and (B) 2 g tuna 3 min at 200 °C. In A, apparent concentrations are independent of D_F. In B, apparent concentration is erroneously low when $D_F = 1$ and increases as D_F increases to 8 or 16 indicating that residual components suppress Hg^{+2} signal in undiluted solutions. Decomposition solutions were analyzed with 20% w/v $SnCl_2 \cdot H_2O$. Circle (–●–) indicates results for unfortified tuna. Diamond (–◇–) indicates results for tuna fortified with inorganic Hg^{+2}. Triangle (–△–) indicates results for tuna fortified with organic Hg. Concentration of Hg added was +0.060 mg/kg. Fortified results are multiplied by 0.5 for presentation.

temperatures, hold times at maximum temperature, weights of material, and stannous chloride concentrations are used. A simple dilution test was used to determine whether residual components in decomposition solutions interfered with the CVAAS determination of Hg. Weights of seafood were limited to 0.5, 1, and 2 g because preliminary evaluation of decomposition conditions showed that heating 4 g seafood to 200 °C opened the pressure release mechanism of the microwave vessels. Heating conditions in Table 3 were used. Three portions of each test material were prepared as described in Section 2.9 and mg/kg results were calculated from analyses of undiluted decomposition solutions. The solution that gave the lowest mg/kg result was used for the dilution test. For the dilution test, undiluted and serially diluted (2-, 4-, 8-, and 16-fold) decomposition solutions were analyzed. Apparent concentration vs. dilution factor was plotted. Apparent concentrations that increase as a smooth function of dilution factor indicate that residual components suppress Hg signals in undiluted solutions. Examples of concentrations (mg/kg) that were and were not a function of dilution factor are presented in Fig. 3. Solutions for which apparent concentrations (mg/kg) increased as dilution factor increased are indicated in Table 3 by footnote e; solutions for which concentration (mg/kg) was independent of the dilution factor are indicated by footnote f. Ratios (C_u/C_d) of concentrations determined in undiluted (C_u) and diluted (C_d) decomposition solutions (unfortified portions diluted eightfold, fortified portions diluted 16-fold) also were calculated. Ratios <1 indicate that Hg signals from undiluted solutions are less than those from diluted solutions and that residual components in undiluted solutions suppress the Hg signal.

Concentrations (mg/kg), recoveries (%), and ratios (C_u/C_d) for canned tuna are presented in Table 3. Concentrations (mg/kg) and recoveries (%) indicate that the decomposition conditions in Table 3 apparently give equivalent results for all conditions used. Average total Hg in unfortified tuna was 0.061–0.064 mg/kg; precision calculated as standard deviation was 0.001–0.003 mg/kg (2–5% relative). Recovery of inorganic and organic Hg from fortified portions was 92–100%. No major differences in concentration (mg/kg) or recovery (%) were observed for analyses using 20 or 10% w/v $SnCl_2 \cdot 2H_2O$. Concentration ratios (C_u/C_d) and plots of apparent concentration vs. dilution factor, however, indicate that using 210 °C has the advantage of eliminating signal suppression in undiluted solutions. For solutions prepared using 210 °C, all concentration ratios (C_u/C_d) were $\geqslant 0.97$ and apparent concentrations (mg/kg) generally were independent of dilution factor (footnote f). For solutions prepared using 200 °C, concentration ratios (C_u/C_d) were as low as 0.91 and apparent concentrations (mg/kg) generally were a function of dilution factor (footnote e). Presence of residual components in solutions prepared by heating at 200 °C was also indicated by poorly shaped peaks (precision of readings on the plateau >1%) that were produced when undiluted solutions were analyzed. Peak shapes were excellent when 200 °C solutions were diluted for analysis. Peak shapes for solutions heated at 210 °C, however, were excellent when undiluted solutions were analyzed. The effect of decomposition conditions on results for reference materials (Table 4), fortified method blanks (Table 5), and a variety of seafoods (Table 6) was also evaluated. Results in Tables 4–6 show that concentrations and recoveries decreased slightly as decomposition time and temperature increased. Although heating at 210 °C provided better breakdown of residual components than heating at 200 °C, some Hg was lost and results were low using the higher temperature. Heating at 200 °C for 3 or 10

Table 4
Determination of total Hg in certified reference materials decomposed by heating 3 and 10 min at 200 °C and 10 min at 210 °C[a]

Reference material ID	Certified concentration mg/kg	This work, concentration (mg/kg)			This work (% of certified value)		
		200 °C, 3 min	200 °C, 10 min	210 °C, 10 min	200 °C, 3 min	200 °C, 10 min	210 °C, 10 min[b]
1566-b Oyster tissue	0.0371 ± 0.0013	0.034	0.032	0.032	92	86	86
2976 Mussel tissue	0.061 ± 0.0036	0.063	0.064	0.059	103	105	97
2977 Mussel tissue	0.101 ± 0.004	0.107	0.098	0.096	106	97	95
1946 Whitefish	0.433 ± 0.009	NA[c]	0.414	0.427	NC[d]	96[e]	99[e]
RM-50 Albacore tuna	0.95 ± 0.1	0.945	0.899	0.872	99	95	92
1641d Natural water	1.59 ± 0.018	NA	1.54	1.53	NC	97[e]	96[e]
Average of selected materials					100	96	93
Average of all materials					100	96	94

[a] Results are for one portion. Weight of material 1946 (frozen seafood) was 0.5 g. Weight of all other materials (freeze-dried) was 0.1 g.
[b] Analyses were repeated. Results of second analysis were 93, 93, 92, 104, 90, and 94% of certificate values for materials 1566-b, 2976, 2977, 1946, RM-50, and 1641 d, respectively. Average for all materials was 94% of certificate values.
[c] Not analyzed.
[d] Not calculated.
[e] Not used in calculation of average of selected materials to allow comparison of 200 and 210 °C results.

Table 5
Recovery of total Hg from fortified method blanks[a] (%)

	200 °C, 3 min[b]	200 °C, 10 min[c]	210 °C, 10 min[d]
Inorganic Hg[e]	100	94	96
Organic Hg[f]	97	98	93

[a] Methods blanks were fortified before decomposition. Amount of Hg added was 0.025–0.5 μg (0.5–10 μg/L in decomposition solutions).
[b] Result is average of one determination from each of six analytical batches ($n = 6$).
[c] Result is for one determination.
[d] Result is average of one determination from each of seven analytical batches ($n = 7$).
[e] Source of inorganic Hg was Hg^{+2} ion in diluent.
[f] Source of organic Hg was CH_3HgCl in H_2O.

min and diluting decomposition solutions in which suppression of Hg signal by residual components is suspected (in which recovery of fortified Hg is low) therefore is recommended for this method.

Minimum weights recommended for decomposition by this method are 0.1 g of freeze-dried materials that contain approximately 100% dry solids (e.g., references materials and powders) and 0.5 g of non-dried foods that contain 20–30% dry solids (e.g. homogenized seafoods). Lower weights were not evaluated in this project. Up to 0.4 g freeze-dried materials and 2 g non-dried seafood may be decomposed but are not recommended because time-consuming dilution is required to eliminate signal suppression and prevent erroneously low results caused by residual components in decomposition solutions.

3.7. Analysis of seafood, method blanks, and reference materials

Total Hg was determined using 3 min hold time at 200 °C in fortified and unfortified seafoods (Table 6) and method blanks (Table 5). Concentrations determined in a variety of seafoods were 0.015–1.78 mg/kg. Average (and range) of recoveries of inorganic and organic Hg added to the seafoods were 102% (87–115%) and 99% (91–108%), respectively. Average concentration ±1 standard deviation (and range of concentrations) determined in six method blanks prepared in six different batches of analyses was 0.0014 ± 0.0022 μg/L (−0.0020 to 0.0055 μg/L). Average (and range) of recoveries of inorganic and organic Hg added to method blanks before microwave decomposition were 100% (93–106%) and 97% (86–102%), respectively. The limit of quantitation (LOQ), calculated as 10 × standard deviation of concentration (μg/L) in method blanks × 0.05 L/0.5 g or 0.1 g, was 0.0022 mg/kg for seafoods and 0.011 mg/kg for dry reference materials. Total Hg in certified reference materials was determined using 3 min and 10 min hold times at 200 °C (Table 4). Concentrations determined using 10 min hold time were 0.032–1.54 mg/kg. The average (and range) of these results, as % of certificate value, was 96% (86–105%). The average (and range) of results determined using 3 min hold time were 100% (92–106%) and were slightly greater than those determined using 10 min.

Table 6
Determination of total Hg in unfortified and fortified portions of seafood decomposed by heating 3 min at 200 °C and 10 min at 210 °C[a]

	Concentration (mg/kg)		Relative difference of concentrations (%)	Recovery inorganic Hg[b] (%)		Recovery organic Hg[c] (%)	
	200 °C, 3 min	210 °C, 10 min		200 °C, 3 min	210 °C, 10 min	200 °C, 3 min	210 °C, 10 min
Trout	0.015	0.013	−13	98	88	96	81
Catfish	0.058	0.050	−14	98	94	91	90
Tuna (light), canned in oil	0.060	NA[d]	NC[e]	100[f]	NA[d]	98[f]	NA[d]
Tilapia	0.065	0.057	−12	97	93	95	91
Orange roughy	0.137	0.148	8	103	94	99	91
Swordfish A	0.189	0.176	−7	100	95	97	93
Bluefish	0.353	0.365	3	102	93	104	91
Tuna steak, fresh	0.364	NA	NC[e]	115[f]	NA[d]	104[f]	NC[e]
Grouper	0.463	0.443	−4	105	96	101	92
Shark	1.74	1.80	3	87	90	NA[d]	96[f]
Swordfish B	1.78	1.66	−7	113	92	108	86
Average of selected seafoods			−5	100	93	99	90
Average of all seafoods			−5	102	93	99	90

[a] Results are for one portion. Amount of Hg added for recovery experiments was 0.1, 0.5 or 1 mg/kg, depending on incurred level. Hg was added before decomposition.
[b] Source of inorganic Hg was Hg^{+2} ion in diluent.
[c] Source of organic Hg was CH_3HgCl in H_2O.
[d] Not analyzed.
[e] Not calculated.
[f] Not included in calculation of average of selected seafoods to allow comparison of 200 and 210 °C result.

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