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Speciation of trace mercury impurities in fish oil supplements

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ABSTRACT

Fish oil supplement is becoming increasingly popular worldwide because of beneficial long-chain omega-3 polyunsaturated fatty acids. However, mercury (Hg) impurity causes considerable concern because of its toxicity and bioaccumulation in the food chain. In this work, Hg impurities were extracted from fish oil by liquid-liquid partitioning. The sample solution was then mixed with a reductant (0.4% anthranilic acid-20% formic acid) and sequentially exposed to 311 and 254 nm UV radiation. The resulting Hg^0 vapor was detected by atomic fluorescence spectrometry. Speciation was fulfilled by solving a set of two linear equations. Recovery of $MeHg^+$ was 73%; total Hg was validated by ICP-MS. This method achieved 0.50 and 0.63 ng mL^{-1} limits of detection for Hg^{++} and $MeHg^+$, respectively. Average Hg^{++} and $MeHg^+$ contents in fish oil samples (n = 38), 0.67 ± 0.45 and 1.1 ± 1.3 ng mL^{-1}, respectively, were 2-3 orders of magnitude lower than those in fish.

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1. Introduction

Benefits of long-chain omega-3 polyunsaturated fatty acids (LCn3PUFAs) in fish oil were recognized four decades ago based on the correlation of high fish diet and low coronary artery disease (CAD) rate in the Greenland Eskimo population (Dyerberg, Bang, & Hjorne, 1975). Numerous studies since have shown that in addition to cardiovascular health (Kris-Etherton, Harris, & Appel, 2002; Mozaffarian & Wu, 2011; Nestel et al., 2015). LCn3PUFAs also benefit eye (Yashodhara et al., 2009), gastrointestinal (Yashodhara et al., 2009), brain and neurological (Burca & Watson, 2014), as well as dozens of other conditions. These benefits were ascribed to rich presence of eicosapentaenoic acid (EPA, 20:5n-3) and decosahexanoic acid (DHA, 22:6n-3). In comparison, benefits of alpha-linolenic acid (ALA, 18:3n-3), another LCn3PUFA derived from plant oils or seeds such as flaxseed, remains inconclusive (Wang et al., 2006). Pathways of endogenous conversion to EPA and DHA exist but at low rates: <8% to EPA *vs.* <4% to DHA (Pawlosky, Hibbeln, Novotny, & Salem, 2001). Worldwide, fish oil production reached 1–1.25 million tonnes in 2010 (Pike & Jackson, 2010); LCn3PUFA supplements are becoming increasingly popular reaching $1.1 billion annual sale in 2011 (FN Media Group, 2015).

$MeHg^+$ is known to bioaccumulate along the aquatic food chain and accounts for 75–98% of total Hg presence in fish. Fish oil supplements are produced from anchovy, mackerel, herring, sardines, tuna, salmon, cod, krill, etc., among which long-life, piscivorous species can accumulate Hg up to several μg g^{-1}. For human, consumption of fish and shell fish is the main pathway of Hg exposure. Hg impurities in fish oil thus become legitimate concerns besides other lipophilic environmental pollutants such as polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) (Burca & Watson, 2014; Oh, 2005). Once ingested, lipophilic and hydrophilic $MeHg^+$ can easily pass cell membranes and blood-brain barrier, bind to sulfhydryl and selenohydryl groups, alter 3-D structures of proteins, and interrupt cell functions (Farina, Aschner, & Rocha, 2011). It is well documented that $MeHg^+$ manifests a wide spectrum of adverse effects to mammal, collectively known as Minamata disease (National Research Council, 2000). Neurotoxicity of this compound is extremely harmful to children from utero stage to early childhood (Castoldi et al., 2008; Trasande, Landrigan, & Schechter, 2005). Losses of intelligence and productivity caused by Hg emission from US coal-fired power plants alone,

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which accounts for 41% of US anthropogenic emission, are estimated to reach $8.7 billion per year (Hylander & Goodsite, 2006). To protect public health, the United Nations Food and Agriculture Organization/World Health Organization (FAO/WHO) Joint Expert Committee on Food Additives (JECFA) has established provisional tolerable weekly intakes (PTWI) at 1.6 μg $MeHg^+$/kgbw and 4 μg iHg/kgbw for the general population, but warned a greater risk for pregnant and lactating women. Hg impurities in fish oil supplements is not regulated by the US Food and Drug Administration (FDA); the fish oil industry follows the 100 parts per billion (ppb) total Hg safe level set by the Global Organization for EPA and DHA Omega-3s (GOED), the European Pharmacopoeia, Norwegian Medicinal Standard, and the Council for Responsible Nutrition, etc. To boost consumer confidence and uphold public health, Hg impurities in fish oil supplement must be closely monitored.

Ashing coupled to atomic absorption spectrometry (AAS) achieved 6 ng mL^{-1} limit of detection (LOD) for tHg impurity (Foran, Flood, & Lewandrowski, 2003). Such sensitivity is marginal for fish oil samples. To enhance sensitivity, amalgamation was implemented to enrich elemental Hg after ashing and catalytic conversion (Levine et al., 2005). Speciation analysis of Hg impurities in fish oil has recently been fulfilled by HPLC coupled to inductively-coupled plasma (ICPMS) that improved LOD to 0.5–1 ng g^{-1} (Yao, Jiang, Sahayam, & Huang, 2017). Speciation analysis is not only important to consumers due to species-dependent toxicity, but also to quality control and quality assurance. Speciation data render extra insight to guide purification practice. HPLC-ICPMS is a powerful technique for sensitive speciation analysis, but expensive instrumentation and high operation cost become obstacles to its availability in smaller laboratories especially in developing countries. Described in this manuscript are the speciation results of trace Hg impurities in fish oil by differential photoreduction under two UV wavelengths, 311 vs. 254 nm. The resulting Hg^0 vapor was detected by atomic fluorescence spectrometry (AFS). Average contents of Hg^{++} and $MeHg^+$ (n = 38) were 2-3 orders of magnitude lower than fish and hence much safer.

2. Materials and methods

2.1. Chemicals and solutions

$Hg(NO_3)_2$ standards in 12% HNO_3 (1000 μg mL^{-1}) and 99.9% solid methylmercury chloride (MeHgCl) were purchased from Fluka (Milwaukee, WI, USA). $HgCl_2$ powder (99.999%) and methylmercury chloride standard in water (1000 μg mL^{-1}) were purchased from Alfa Aesar (Ward Hill, MA, USA). ACS reagent grade anthranilic acid (AA), 96% formic acid (FA), and nitric acid were purchased from Sigma-Aldrich (Milwaukee, WI, USA), from which a 0.4% (w/v) AA–20% (v/v) FA reductant solution was prepared daily. Stock standards at 0.5 μg mL^{-1} were made weekly by serial dilution in deionized water (DIW) and stored at 4 °C; working standard solutions at 0–5 ng mL^{-1} were prepared daily. Spiked fish oil samples were prepared from MeHgCl and serially diluted to 10–100 ng mL^{-1} using a fish oil with low (<LOQ) intrinsic Hg content. Used glassware was soaked in 15% nitric acid overnight and rinsed thoroughly with deionized water (DIW). A Barnstead E-pure system (Dubuque, IA) was used to make DIW.

2.2. Liquid-liquid extraction

Fish oil samples in capsule or liquid form were purchased from local stores in the Philadephia area (PA, USA) or online. Capsules were punctured with a clean syringe needle; the fish oil was squeezed to a glass vial, from which 2 mL was pipetted to a 50 mL polypropylene centrifuge tube using a displacement pipette. After addition of 40 mL of DIW, the tubes were capped tightly and arranged vertically in a tube rack. The tubes were secured between a base and a cover of a LC1012 vortex mixer (Glas-Col, Terre Haute, IN, USA); both were lined with a 0.5" sheet of polymeric rubber. Vigorous agitation, set at motor speed 80, lasted for 10 min with pulsing. Centrifugation followed at 4000 rpm for 10 min; the upper oil layer was discarded while the aqueous layer was kept for analysis.

2.3. Photochemical vapor generation

A custom-made, synthetic silica coil was installed between a mixing valve and a gas/liquid separator (G/L) of a Millennium Merlin atomic fluorescence spectrometer (AFS) (P S Analytical, Kent, UK) as a UV-photoreactor (Chen, Lai, Mei, Liu, & Mao, 2017). A 254 nm low-pressure Hg lamp was inserted into the center of the coil while two 311 nm fluorescent lamps were installed by the coil with a 10 mm gap. The spectrometer, under the control of the Millennium software, was operated in flow-injection (FI) mode. The aqueous sample solution and the 0.4% AA–20% FA reductant solution were delivered by a peristaltic pump at 9 and 4.5 mL min^{-1} flow rates, respectively, and mixed in the reactor coil. The mixture was exposed to either 311 nm or 254 nm UV light. The resulting Hg^0 vapor was swept by high-purity argon at 300 mL min^{-1} to the G/L where liquid was drained. The vapor reached a Perma-Pure dryer where most moisture permeated across a Nafion membrane and evaporated into a counter-flowing (2500 mL min^{-1}) nitrogen stream. The Hg^0 vapor finally entered the AFS detector.

2.4. Atomic fluorescence spectrometry

Detection of Hg^0 vapor was performed in the detection chamber of the Millennium Merlin AFS. Dry Hg^0 vapor was excited by a 254 nm beam from a hollow cathode lamp. The resonance fluorescence signal, collected at 90°, passed a 254 nm filter and was detected by a photomultiplier tube.

2.5. Hg^{++} vs. $MeHg^+$ speciation

Concentrations of both analytes in a sample were solved from a set of two linear equations obtained at 311 or 254 nm, respectively. The AFS signal intensity was a linear function of the concentrations of both analytes:

$$I_\lambda = m_\lambda\left[Hg^{++}\right] + n_\lambda\left[MeHg^+\right] \quad (\lambda = 254 \text{ or } 311 \text{ nm})$$

where I_λ was AFS peak height at wavelength λ; m_λ and n_λ are slopes of standard curves at wavelengths λ; $[Hg^{++}]$ and $[MeHg^+]$ are the concentrations of Hg^{++} and $MeHg^+$ expressed in Hg.

2.6. Microwave digestion and ICP-MS validation

Digestion of fish oil was performed using a MARS 6 microwave digestion system (CEM, Matthews, NC, USA). Aliquots of 0.50 mL fish oil were delivered to 55 mL Xpress vessels and added with 5 mL of concentrated HNO_3. Digestion was carried out at 120 °C for 2 min, 160 °C for 8 min, and 190 °C for 30 min; with 2 min ramps. After cooled down to room temperature, the contents were filled with DIW to 50 mL. Total Hg (tHg) was measured using an Agilent 7900 ICP-MS (Santa Clara, CA, USA) under the operation parameters listed in our previous report (Chen et al., 2017).