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A reliable method to determine methylmercury and ethylmercury simultaneously in foods by gas chromatography with inductively coupled plasma mass spectrometry after enzymatic and acid digestion

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ABSTRACT

A reliable and sensitive method for determination simultaneously of monomethylmercury (MeHg) and monoethylmercury (EtHg) in various types of foods by gas chromatography inductively coupled plasma mass spectrometry (GC-ICP/MS) was developed and validated. Samples were digested with pancreatin and then hydrochloric acid. MeHg and EtHg in the extract were derivatized in an aqueous buffer with sodium tetraphenylborate. After phase separation, the extract was directly transferred to analysis. The analyses were conducted by GC-ICP/MS with monopropylmercury chloride (PrHgCl) as surrogate standard. Concentrations of 254 ± 5.1, 13.7 ± 0.69 and $162 \pm 6.2\ \mu g\, Hg\, kg^{-1}$ (one standard deviation, $n = 3$) were obtained for MeHg in NIST SRM 1947 (Superior Lake fish), SRM 1566b (oyster tissue) and NRC Tort-2 (lobster Hepatopancreas), respectively. These are in good agreement with the certified values of 233 ± 10, 13.2 ± 0.7 and $152 \pm 13\ \mu g\, Hg\, kg^{-1}$ (as 95% confidence interval), respectively. The method detection limits (3σ) for MeHg and EtHg are $0.3\ \mu g\, Hg\, kg^{-1}$. The method detection limit was estimated by using a 0.5 g of subsample, sufficiently low for the risk assessment of MeHg and EtHg in foods. The spiked recoveries of MeHg and EtHg in different food matrices were between 87 and 117% and the RSDs were less than 15%. When isotopic dilution mass spectrometry (IDMS) analysis was performed with a commercial available ^{201}Hg-enriched monomethylmercury ($Me^{201}Hg$) solution as internal standard, concentrations of 244 ± 13.4, 13.9 ± 0.25 and $161 \pm 1.3\ \mu g\, Hg\, kg^{-1}$ were obtained for MeHg in NIST SRM 1947, SRM 1566b and NRC Tort-2, respectively. It shown clearly that IDMS analysis got improvement in precision and accuracy, however, EtHg cannot be analyze simultaneously and the cost of analysis is higher.

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1. Introduction

Food is usually the main source of human exposure to heavy metals. Amongst the heavy metals present in foods, methylmercury (MeHg) is of particular concern in terms of food safety and public health. Mercury exists naturally in abundance in the environment. It enters the environment by both natural and human means. Mercury in the environment can be oxidized to inorganic bivalent mercury with the presence of organic matters in waters. Inorganic mercury can also be converted to methylated form by microorganisms especially in aquatic systems [1].

In food, mercury can exist in inorganic form and the more toxic organic forms such as MeHg in fish and shellfish. Mercury present in other foods mainly in inorganic form. Dietary inorganic mercury is of little toxicological concern [2]. Fish and other seafood products are the main source of MeHg, especially large predatory species such as tuna and swordfish. Chung et al. [3] reported MeHg levels of different fish species and generally confirmed that more than 75% (w/w) of the total mercury content in the edible portion of fish is in form of MeHg. However, fish provide a healthy source of dietary protein and are relatively low in cholesterol and high in omega-3 fatty acids [4].

As Hong Kong is going to conduct its first total diet study on different chemicals including MeHg and EtHg, a sensitive method for testing the content of MeHg and EtHg in different food matrices, including mixed food, is required.

A number of analytical methods have been developed for monitoring organo-mercury compounds, especially MeHg, in the marine environment. However, there was few literature reported the determination of MeHg and EtHg simultaneously in foods. The most frequently used procedures for the extraction of mercury species from solid samples were based on alkaline [5–10], acidic leaching [11–14], aqueous distillation [15–19], ultrasonic extraction [20],

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supercritical fluid extraction (SFE) [21] and microwave-assisted extraction (MAE) [22–26]. Though MAE can shorten the sample extraction time, extreme care about the extraction condition is required as significant amount of MeHg could be lost. Lemes and Wang [27] recently reported that enzymatic hydrolysis with trysin could release methylmercuric cysteinate or glutathionate from fish protein.

Amongst the reported literatures, different analytical techniques have been developed for speciation of mercury [28]. They usually combined of a separation and a detection technique, such as gas or liquid chromatography coupled with element-selective detectors, such as atomic emission, atomic absorption, atomic fluorescence, or inductively coupled plasma mass spectrometry (ICP/MS). Amongst the methods mentioned, the coupling of GC to ICP/MS combines high sensitivity with the possibility of speciated isotope dilution measurements [29].

In view of the difficulties of fast extraction methods and the possibility of determining MeHg and EtHg simultaneously in foods at background level for risk assessment, a new method was developed. This present work describes the development and validation of an analytical method for the determination of MeHg and EtHg at parts-per-billion level simultaneously in various types of food items to be tested in the total diet study. For the analysis of both MeHg and EtHg, monopropylmercury chloride (PrHgCl) was used as surrogate standard. Good recovery and precision values were obtained for analyte in certified reference samples. For more accurate measurement of MeHg, a commercial available isotopic-labelled standard, was used as internal standard. Better precision, accuracy and recovery data were obtained for MeHg.

2. Experimental

2.1. Reagents and solutions

All the chemicals and solvents used were of analytical-reagent grade or higher, unless otherwise specified. Ultra-pure deionized water (18.2 MΩ cm; Milli-Q, Millipore Corp., Bedford, MA) was used throughout. Pancreatin solution of 1.5% (w/v) was prepared by dissolving pancreation (Sigma–Aldrich, St. Louis, USA) in water. A 0.08 mol L^{-1} phosphate buffer was prepared by dissolving appropriate amount of sodium phosphate dibasic and sodium phosphate monobasic in water and adjusted the pH value to 7.5 with sodium hydroxide solution. Sodium tetraphenylbroate (1.5%, w/v) solution, prepared by dissolving $NaBPh_4$ (Sigma–Aldrich) in water, was used as a derivatization reagent. A 2 mol L^{-1} sodium acetate buffer was prepared by dissolving an appropriate amount of sodium acetate in water and adjusted the pH to 4.5 with acetic acid.

MeHg and EtHg chloride were purchased from Acros (Geel, Belgium). PrHgCl was purchased from Fluka (Buchs, Switzerland). Individual stock solutions of 100 mg Hg L^{-1} were prepared in methanol with 1% (v/v) hydrochloric acid (1% HCl) and kept refrigerated until used. Working solutions were obtained by dilution of the stock solution with 1% HCl and prepared daily before use. A 250 μg Hg L^{-1} PrHgCl solution was prepared daily by diluting the stock solution in 1% HCl for use as surrogate standard.

^{201}Hg-enriched MeHg in acetic acid/methanol (3:1), 5.494 mg Hg kg^{-1}, was purchased from ISC Science (Oviedo, Spain). ^{201}Hg-enriched MeHg spike solution at a nominal concentration of 1.5 μg Hg kg^{-1} was prepared by diluting the stock solution with a solution of 12% (v/v) methanol in 1% HCl. All dilutions were achieved by mass.

The SRM 1947 (Superior Lake fish) and SRM 1566b (oyster tissue), obtained from the NIST (Boulder, USA), were used for method validation. The CRM Tort-2 (lobster Hepatopancreas) was obtained from the National Measurement Standards of the Research Council of Canada (Ottawa, Ontario, Canada).

Calibration standard solutions, 0.0, 0.01, 0.05, 0.1, 0.5, 1.0 and 5.0 μg Hg L^{-1} were prepared by transferred appropriate volume of mixed intermediate MeHg and EtHg standard solution into individual 50 mL centrifuge tube. Pipetted 0.13 mL of working surrogate/internal standard solution, 3 mL pancreatin solution, 7 mL water and 5 mL phosphate buffer solution into each centrifuge tube. Diluted to the volume of 30 mL with concentrated HCl.

Table 1
GC-ICP/MS operating conditions.

GC	
Column	DB-5MS, 30 m × 0.25 mm, 0.25 μm film thickness
Injection	Splitless
Injection volume	1 μL
Injector temperature	220 °C
Oven temperature programme	50 °C hold 1 min; ramp to 280 °C at 20 °C/min; hold 3 min
Carrier gas	1% Xenon in Helium
Flow rate	1 mL/min
Transfer line temperature	280 °C
ICP-MS	
Rf power	1440 W
Plasma Ar gas flow rate	15 L min^{-1}
Carrier gas (1% Xenon in Helium) flow rate	1.2 L min^{-1}
Sampling cone	Nickel
Skimmer cone	Nickel
Acquisition mode	Full quantitation
Dwell time	100 ms

2.2. Instrumentations

An Agilent 6890 gas chromatograph (Agilent Technologies, Santa Clara, CA), with autosampler, fitted with a DB-5MS column (Agilent Technologies) was used for the separation of the mercury species. The 7500ce inductively coupled plasma-mass spectrometer (Agilent Technologies) equipped with ChemStation software (Version B.03.02) was used for the detection of the mercury species. Helium containing 1% (w/w) Xenon (Linde Canada Limited, Alberta, Canada) was used as carrier gas. The isotopic masses of mercury of 200, 201 and 202 were measured. Typical GC-ICP/MS operating conditions are summarized in Table 1.

2.3. Sample extraction

Weighed approximately 0.5–1 g of a sample in a 40 mL-glass vial. Added 25 mL acetone, capped tightly and shook vigorously for 15 s. Centrifuged (Falcon 6/300, Measuring and Scientific Equipment, London, UK) at 2000 rpm (or 900 × g) for 5 min and then discarded acetone carefully with a Pasteur pipette. If the tissue formed clots, broke up with a glass rod. Repeated the fat removal procedure two more times with acetone and toluene respectively. Spiked 0.06 mL of surrogate standard or weighted appropriate amount of internal standard of ^{201}Hg-enriched MeHg spike solution into the sample for mercury speciation and isotopic dilution analysis respectively. Added 3 mL 1.5% (w/v) pancreatin solution and 5 mL phosphate buffer to the residue. Screwed the cap and kept the solution in a shaking water bath at 37 ± 2 °C overnight. Transferred the extract to a 50 mL centrifuge tube. Added appropriate amount of water to 15 mL and finally made up to 30 mL with concentrated HCl. Screwed the cap tightly. Shook vigorously to disperse the solid into the solution and maintained shaking for 3 h with a shaker. Centrifuged the tubes at 3000 rpm (or 2000 × g) for 20 min.

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