Determination of Methyl Mercury in Fish and Shellfish

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A simple and rapid method for the determination of methyl mercury in fish and shellfish using a gas chromatograph with electron capture detection (GC/ECD) was developed.

Lipids and other organic interference in the test materials were eliminated by pretreatment with acetone and benzene, and then the methyl mercury extracted with the acidified benzene was analyzed by GC/ECD.

Good recoveries (tuna, $90.3 \pm 2.3\%$; shark, $105.5 \pm 3.2\%$; sail-fish, $87.5 \pm 2.7\%$; and shrimp meat, $88.4 \pm 2.6\%$) were obtained from samples spiked with 1 ppm of methyl mercury.

The detection limit of methyl mercury was approximately 0.0016 ppm.

Keywords — GC/ECD; methyl mercury; fish; shellfish

Introduction

“Minamata disease,” which was caused by methyl mercury passing through the food chain from industrial waste water in Japan, is well known throughout the world.¹⁻⁴⁾

For the purpose of evaluating the safety of methyl mercury in seafood, there have been many reports discussing analytical methods, such as gas chromatography,⁵⁻¹⁶⁾ atomic absorption spectrometry,¹⁷⁻²⁰⁾ high performance liquid chromatography and a micro emission system,^{22,23)} but all of them are rather complicated and not easy to practice.

In this study, a simple and rapid method for the determination of methyl mercury in fish and shellfish has been developed.

Experimentals

Instruments — Glassware: All glassware was cleaned with 25% NaOH aqueous solution and then rinsed successively with water, 10% HCl,

water and acetone.

Gas Chromatograph: A Hewlett Packard Co. Model 5890 series II gas chromatograph equipped with an electron capture detector (⁶³Ni, GC/ECD) and a 2 m × 2 mm i.d. glass column, packed with 5% diethylene glycol succinate (DEGS) polyester on 80–100 mesh (Supelco Inc., U.S.A.).

Centrifuge Tubes: 50 ml with ground-glass stoppers.

Homogenizer: Model PT 3000 (Kinematica Co., Switzerland).

Conditions of GC/ECD: Column temperature 170°C, detector 300°C, injector 210°C; carrier gas N₂; 50 ml/min, injection volume, 5 μl.

Materials and Reagents — Samples: All samples were purchased from Wellcome Supermarket at Tei-Mu, Taipei.

Hydrochloric Acid Solution: Concentrated HCl was added to an equal volume of deionized water and mixed. Potential interference in the

hydrochloric acid solution was eliminated by extraction with benzene equal to 1/4 volume of the above HCl solution. This was repeated for additional four time's. Extraction must be performed quickly before using the HCl solution in order to avoid the formation of electron capturing compounds which produced extraneous peaks in GC/ECD.

Methyl Mercuric Chloride Standard Solutions: a) Stock solution (1000 $\mu\text{g-Hg/ml}$) : 125.2 mg methyl mercury chloride was dissolved with deionized water to make up to 100 ml. b) Intermediate solution (10.0 $\mu\text{g/ml}$ as Hg) : 1.0 ml of the stock solution was dissolved with deionized water to make up to 100 ml. c) Working solution (100 ng/ml as Hg) : 1.0 ml of the intermediate solution was dissolved with deionized water to make up to 100 ml.

Pretreatment of Mercuric Chloride Column: 100.0 mg of mercuric chloride was dissolved in 100 ml of benzene.

Solvents: Acetone, toluene and benzene were all of pesticide grade, (J.T. Baker Co., U.S.A.).

Biological Standards: The reference materials of trot and drom containing 0.128 ± 0.014 and 0.731 ± 0.069 mg/kg of methyl mercury, respectively, as Hg were purchased from the National Research Council, Canada.

Conditioning of Column — Prior to use, a 5% DEGS-PS column was conditioned by injecting it with the mercuric chloride solution and heating it at 200°C.

Analytical Procedure — To 2.0 g of a homogenized (30000 rpm, 15 min) sample in a 50 ml centrifuge tube was added 25 ml of acetone which was shaken vigorously for 15 s and centrifuged at 2000 rpm for 5 min. The acetone layer was carefully discarded. After the acetone washing was repeated twice more, 20 ml of benzene was added, the mixture was shaken vigorously for 30 s and then centrifuged 2000 rpm for 5 min. The benzene layer was carefully discarded.

Next 2.5 ml of 6 N HCl solution and 20 ml of benzene were added to the centrifuge tube, after which it was shaken gently for 5 min and centrifuged at 2000 rpm for 5 min. If an emulsion

was formed, 1 ml of isopropanol was added and the benzene layer was gently stirred, to destroy the emulsion. Care was taken not to disturb the aqueous phase, which was recentrifuged with a dropping pipet.

The benzene layer was transferred to a graduated cylinder carefully. Then 10 g of anhydrous sodium sulfate was added and mixed again. The filtrate was then analyzed by GC/ECD.

Results and Discussion

Calibration Curve

The calibration curve of methyl mercuric chloride at a concentration range of 0.1–1.0 $\mu\text{g/ml}$ was constructed. It provided good linearity, with a relation coefficient of 0.9981.

Column Temperature

Since the peak tailing appeared when column temperatures were held below 160°C, the column temperature was maintained at 170°C throughout this study.

Recovery of Methyl Mercury in Reference Materials

Good recoveries of methyl mercury from the reference materials (trot, $82.8 \pm 2.7\%$, and drom, $86.4 \pm 3.1\%$) were obtained as summarized in Table I.

Recovery Rate of Methyl Mercury by Different Methods

The recovery rate of methyl mercury from tuna, shark, sailfish and shrimp by means of AOAC,²⁴ JSPHC,²⁵ and the present method are summarized in Table II. The method we developed was much simpler than the other two methods, and the recoveries ($88.4 \pm 2.8\%$ – $105.5 \pm 3.2\%$) were better than with AOAC ($82.6 \pm 2.8\%$ – $90.5 \pm 3.3\%$)

TABLE I. Recovery of Methyl Mercury From Standard of Fish – Shell

Standards	Methyl mercury added (mg/kg)	Methyl mercury found (mg/kg)	Recovery (%)
Tort	0.128 ± 0.014	0.106 ± 0.017	82.8 ± 2.7
Drom	0.731 ± 0.060	0.632 ± 0.082	86.4 ± 3.1

Each value is the mean $\pm$ S.D. of 3 runs.

TABLE II. Recovery of Methyl Mercury by Different Methods

Sample	Recovery (%)		
	This method	AOAC ^{a)}	JPSHC ^{b)}
Tuna	90.3±2.3	85.5±2.7	72.2±3.7
Shark	105.5±3.2	90.5±3.3	74.8±6.3
Sailfish	87.5±2.7	80.3±3.1	79.2±3.4
Shrimp	88.4±2.6	82.6±2.8	69.4±5.2

a) AOAC, American official methods of analysis.

b) JPSH, standard methods of analysis for hygienic chemists. Each value is the mean±S.D. of 3 runs.

and JSPH ($69.4 \pm 5.7\%$ — $79.2 \pm 3.4\%$).

Conclusion

A simple and rapid method for the deter-

mination of methyl mercury in fish and shellfish was developed. Good recoveries and reproducibility of the method were performed by pre-washing with acetone and benzene, followed by extraction with acidified benzene. The final extracts were clear enough to be directly injected into to the GC/ECD.

This simple and rapid method may be suitable for quick analysis of methyl mercury in seafood for the safety evaluation of food.

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