STANDARDIZED METHYLMERCURY ANALYTICAL PROCEDURE

FOR

FISH AND SEDIMENTS

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FOREWORD

The method presented in this manual is a result of research performed at Vanderbilt University and described in "Standardization of Methylmercury Analysis", by P. A. Krenkel and W. D. Burrows, which was the final report of the Environmental and Water Resources Engineering Program to the Tennessee Wildlife Resources Agency and the National Marine Fisheries Service. (Contract N-042-56-72NFA, RC-2052-11)

Since preparing the report both Dr. Krenkel and Dr. Burrows have left Vanderbilt University. Questions concerning the analytical procedure described in this manual should be sent to Drs. Krenkel or Burrows at the following addresses:

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Standardized Methylmercury Analytical Procedure for Fish and Sediments

Introduction

The problem of methylmercury determination falls naturally into three steps: (1) liberation of the alkylmercury moiety from the environmental sample; (2) isolation of methylmercury from other liberated constituents; and (3) identification and estimation of the methylmercury derivative. Gas-liquid chromatographs are readily available in most locations and are the most widely used means of measuring methylmercury. Therefore, the method presented in this manual involves gas-liquid chromatography (GLC) in the third step.

The analytical method chosen is a modification of the procedure of TW Beak Associates, which is, in turn, an adaptation of methods developed in Japan and Sweden by analytical pioneers Sumino and Westöö. Refinements have been made to produce the present scheme to increase the efficiency and simplicity of each step by variation of techniques and reagents. The advantages of a proteolytic enzyme to digest samples, different organic solvents, different halide reagents, varied volumes of reagents, peak height versus peak area measurement, and different methods of standard curve preparation; the stability of compounds for potential use as a standard; efficiencies of the extractions into the various solvents; and the possibility of increasing sensitivity by evaporating the final extract were evaluated in the report by Krenkel and Burrows. For routine laboratory analysis of methylmercury in fish

and sediments, this manual presents a straightforward, simple approach. For greater sensitivity or to review the rationale for steps chosen in this method, the report of Vanderbilt University should be consulted.

The procedure starts with liberation of methylmercury from the sample by treatment with aqueous HBr and $CuSO_4$. Mineral acid treatment is absolutely essential to cleave methylmercury from the thiol groups of proteinaceous material. The copper salt is added to assist in cleavage of alkylmercury-sulfur bonds by binding tightly to the protein sulfide groups liberated. The CH_3HgBr formed by this treatment and methylmercury which is dispersed through fatty tissue is extracted from the aqueous solution with benzene. Methylmercury is separated from benzene-soluble impurities by extraction into aqueous sodium thiosulfate solution. Treatment of this solution with a small volume of benzene produces the final test sample, which is analyzed by GLC using an electron capture detector. The detection limit for the method is 0.05 ng of Hg in the original sample.

Apparatus

1. Varian Aerograph 2400 gas chromatograph with Tritium electron capture detector (H^3) and recorder.
2. Centrifuge tubes, 35 ml with Quickfit ground stoppers, or equivalent.
3. Centrifuge tubes, 15 ml with Teflon lined screw caps and conical bottom, graduated in 1/10 ml.
4. Dispensing pipets, 1.0 ml, 2.0 ml and 5.0 ml.
5. Test tubes, 8 ml with ground stoppers.
6. Pasteur capillary pipets, 2 ml.
7. Hamilton CR700-20µl spring-loaded syringe (injections reproducible to ± 1%).
8. Thomas 30 ml. Teflon pestle homogenizer.

Reagents

The quantities mentioned below will be sufficient for about 200 analyses. All chemicals used are of Analytical Reagent grade and water is deionized.

Benzene- Anagraphic or Pesticide Grade solvent.

Bromide Solution, 3M - Mix carefully 110 ml of H_2SO_4 (sp. gr. 1.840 with an equal volume of water and cool. Dissolve 310 g of NaBr in approximately 700 ml of water. Mix the two solutions and dilute to 1000 ml in a 1-liter volumetric flask.

Copper Sulfate, 1M - Dissolve 50 g of $CuSO_4 \cdot 5H_2O$ in water to make 2000 ml of solution.

Sodium Thiosulfate - (Stock solution) Dissolve 21.6 g of $Na_2S_2O_3$ in water and make up to 1000 ml in a volumetric flask.

Sodium Thiosulfate, 0.1M - Dilute 50 ml of the thiosulfate stock solution to 500 ml with water.

Cupric Bromide, 3M - Dissolve 67 g of $CuBr_2$ in water and dilute to 100 ml. It is important to extract this solution with benzene until the benzene phase shows no contaminants when injected on the GLC.

Methylmercury Hydroxide - (Stock solution) The stock solution contains 11.6 mg methylmercury hydroxide per 100 ml of water, equivalent to 10 mg of Hg per 100 ml.

Methylmercury Hydroxide - (Standard solution) Dilute 10 ml of the methylmercury hydroxide stock solution to 100 ml with water in a volumetric flask. 1 µl of this solution contains 10 ng of Hg. Prepare daily.

Methylmercury Bromide Solutions -

a. Stock solution: Weigh accurately an amount of methylmercury bromide crystals (around 30-60 mg) and dissolve in benzene to make up to 100 ml. Calculate the number of ng Hg/µl (rather than ng CH_3HgBr/µl) in that solution (200-400 ng/µl)

b. Working stock solution: Dilute an appropriate number of ml of stock solution in benzene to 100 ml to give an approximately 10 ng/µl solution.

c. Standard solutions: Prepare 2 standards by diluting 10 ml working stock to 100 ml with benzene (~1ng/µl) and by diluting 1 ml working stock to 100 ml with benzene (~0.1 ng/µl).

Conditions of GLC

Chromatograph:	Varian Aerograph 2400
Detector:	Electron capture, Tritium
	6-ft. glass with I.D.
	0.20 cm, 5% carbowax 20M
	TPA on acid-washed 100/120
	mesh Chromosorb W pretreated
	with dimethyldichlorosilane
Carrier gas:	N_2 (O_2 free), purified by
	passing through molecular sieve
Flow rate:	30 - 40 ml per minute
Temp. of column:	140-160°C
Temp. of injector:	195°C
Temp. of detector:	195°C
Chart speed:	0.5 in/min or 1 in/min
Standing current:	1.28×10^{-8} to 3×10^{-8} amps
Retention time:	approximately 3 minutes

PTFE coated septa should be used to decrease bleed and increase the number of injections possible before changing septa. Higher column temperature leads to a shorter detention time and sharper peaks. Higher flow rate also decreases detention time, however, to a lesser extent.

Extraction Procedure

1. From the homogenized sample, weigh 1-2 g in a 35 ml centrifuge tube and add water to make 10 ml.
2. Add 1 ml of 1M $CuSO_4$ solution, mix and let stand for 2 minutes.
3. Add 5 ml of 3M NaBr solution, mix and let stand for 10-15 minutes.
4. Add 10 ml benzene; extract for 2 minutes and centrifuge for at least 5 minutes.
5. Transfer an exactly measured volume (around 7 ml) of clear benzene solution to a 15 ml graduated centrifuge tube. Note the volume transferred.
6. Add 2 ml of 0.01M $Na_2S_2O_3$ solution and extract for 2 minutes. Centrifuge to obtain a clear phase. With biological samples an emulsion may form, and centrifugation may need to be repeated after gentle shaking.
7. Transfer carefully the aqueous layer to an 8 ml test tube, using a disposable Pasteur pipet. Add 1 ml of 3M $CuBr_2$ with a pipet, and 0.5-1.0 ml of benzene with a graduated syringe.
8. Shake the tube and let it stand until the benzene and aqueous layer are completely separated.
9. Use the benzene layer for GLC analysis.

GLC Analysis

1. Wash the clean syringe with benzene 4-5 times then with sample 4-5 times.
2. Withdraw the required volume (1 - 10 µl) and inject the sample to obtain a peak in the standard curve range.
3. Note the amount retained in the needle for volume correction if a constant rate syringe is not being used.
4. Allow the pen to return to the base line after the methylmercuric bromide is eluted before the next sample is injected.

Calibration Curve

Prior to each set of determinations, a calibration curve is prepared by injecting known volumes of a standard, CH_3HgBr, as shown in Figure 1. Sample injections are then alternated with standards using the same volume of one of the standards to make any change in sensitivity of the instrument readily apparent. If the sensitivity (peak height) changes more than 10%, a complete new calibration series should be injected. Under conditions of rapidly changing sensitivity, it may be easier to use the method of injecting a volume of sample and varying the volume of standard to get a peak higher and one lower than that of the sample. A mathematical interpolation is then performed to find the concentration of methylmercury in the sample.

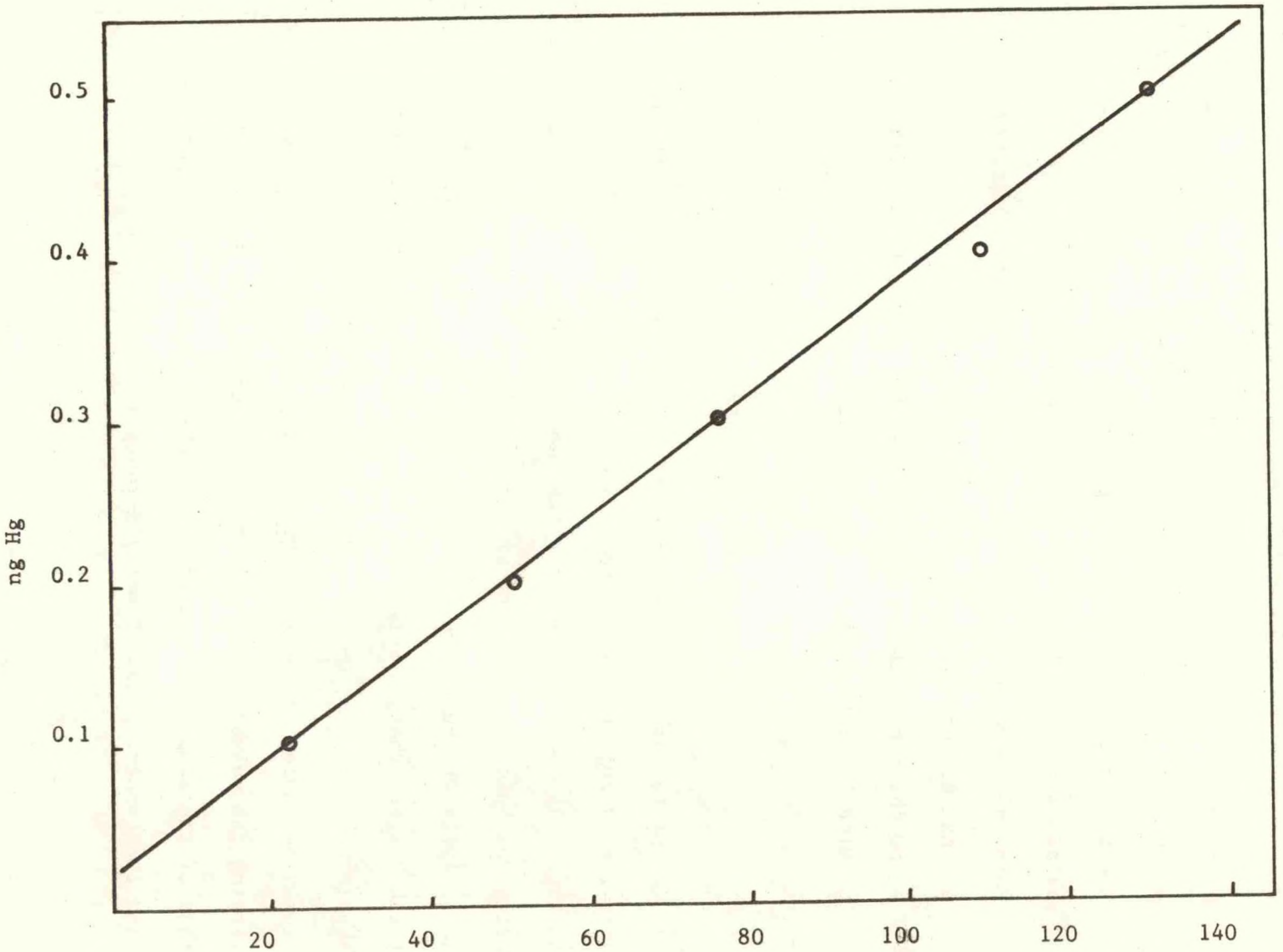

FIGURE 1 - STANDARD CURVE

Extraction Efficiency

After the conversion of CH_3Hg complex to CH_3HgBr, there are small losses during extraction. The correction factor for this is evaluated by treating replicate samples of standard CH_3HgOH solutions under conditions exactly identical to the CH_3Hg complex in unknown samples and calculating the yield from the chromatogram. Knowing the amount of Hg in the original CH_3HgOH solutions, the per cent recovery is calculated. The following steps are carried out:

1. Add two different amounts (µl) of standard CH_3HgOH solution to correspond with the ng Hg expected in 1-2 g of the unknown samples to a 40 ml centrifuge tube.
2. Perform steps 1-8 of the extraction procedure for both samples of CH_3HgOH, keeping all conditions identical with the unknown samples being determined.
3. Take a known volume of the benzene extract (1-10µl) and inject into the chromatograph.
4. Measure the height of the peak from the chromatogram obtained and vary the volume of sample injected to obtain a peak height in the range of the calibration curve.
5. From the calibration curve determine the amount of Hg in the CH_3HgOH standard recovered and calculate the per cent extracted as shown below.

$$\text{Per cent Recovery} = \left(\frac{V_2 \times q}{V_1}\right)\left(\frac{10}{V}\right)\left(\frac{100}{w}\right)$$

Where: q = wt of Hg computed from calibration curve (ng)
V = volume of benzene transferred in Step 5 (μl)
V_1 = volume of CH_3HgBr solution injected in column (μl)
V_2 = volume of benzene in final extraction (Step 7) (μl)
w = wt. of Hg introduced in standard sample as CH_3HgOH

The recovery in standard samples is of the order of 90%

Calculation of Results

The concentration of mercury (Hg) in an unknown sample in ng Hg/gram sample is given by:

$$\text{Mercury Concentration} = \left(\frac{q_1 \times V_2}{V_1}\right)\left(\frac{10}{V}\right)\left(\frac{100}{\%\ \text{Recovery}}\right)\left(\frac{1}{w}\right)$$

Where: q_1 = wt. of Hg corresponding to sample peak (ng)
V = volume of benzene transferred in Step 5 (μl)
V_1 = volume of CH_3HgBr solution injected in column (μl)
V_2 = volume of benzene in final extraction (Step 7) (500-1000μl)
% recovery = the % recovery in standard samples calculated (Extraction efficiency)
w = wt. of sample taken (grams)

Cleaning

a. Glassware - The glassware is cleaned by boiling or soaking in aqua regia followed by rinsing with copious amounts of tap water, followed by distilled water.

b. Syringe - Before injection of each sample, rinse the syringe with benzene and then with the sample solution 4 to 5 times. After a run, the syringe should be rinsed with benzene until no methylmercury remains.

c. Column - Check the column efficiency by obtaining a peak for a standard sample of CH_3HgBr. From the peak obtained calculate the column efficiency as follows:

$$N = 5.54 \frac{(d)^2}{(h)}$$

Where: 5.54 is a constant
d = distance between the injection point and the peak, mm
h = width of the peak at half height, mm

If N is less than 1300, the column should be repacked.

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