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A Simple and Rapid Procedure for the Gas-Chromatographic Determination of Methylmercury in Biological Samples

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INTRODUCTION

Widespread environmental distribution and high toxicity of methylmercury demand rapid and sensitive analytical methods applicable to a variety of biological samples. Electron-capture gas chromatography has been extensively used for this purpose, and its application has been reviewed by MUSHAK (1973). WESTOO (1968) developed the first practical procedure for methylmercury analysis. Several specific variations of this procedure have since appeared for fish, sediment, urine, hair, and blood. Most involve lengthy sample extraction and clean-up, and employ different instrumental conditions. More important, not all of these procedures are routinely applicable to all sample types. This paper describes a simplified, rapid, and routine procedure for a variety of samples which include blood, grain, feces, fish, hair, sediment, soft tissue, urine, and water. The procedure is also applicable to ethylmercury analysis.

EXPERIMENTAL

Reagents. All chemicals are of Analytical Reagent grade and deionized water is used for reagent preparation. Cysteine·HCl was obtained from Sigma Chemical Company. The H_2SO_4-NaBr reagent is prepared according to LONGBOTTOM et al. (1973). Benzene (thiophene-free and spectrograde) was obtained from Mallinckrodt Chemicals. All reagents are extracted three times with 1/5 their volume of benzene to remove potential GC contaminants. Florisil (Florindin Company) was 60/100 mesh.

Standards. Methylmercuric bromide, used for preparing gas-chromatographic (GC) standards, was purchased from K and K Laboratories. A stock solution (1 ppm CH_3HgBr) is prepared in spectrograde benzene and appropriate dilutions are made to prepare working standards ranging

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from 0.05 to 2.0 ng/10 µl. Working standards are prepared fresh weekly in glass vials and stored in the dark. $CH_3{}^{203}HgCl$ in 0.02 N Na_2CO_3 was obtained from New England Nuclear. The radiometric purity is >99%, and the range of specific activity is 0.5-3.0 mCi/mg Hg. Appropriate stock dilutions are made to prepare a working (spike) solution containing approximately 6000 dpm and less than 1 ng Hg/10 µl. The spike solution is stored at 0°C inside a lead container. $CH_3{}^{203}HgCl$ liquid scintillation spectrometric (LSS) standards are prepared fresh in triplicate for each analysis. An amount of spike solution equal to that added to samples (10 µl) is placed in a 20 ml glass scintillation counting vial (Packard Instrument Co., Inc.) and 20 ml of Aquasol Universal LSC Cocktail (New England Nuclear) is added. For ethylmercury analysis, ethylmercuric bromide is purchased from the same source. $C_2H_5{}^{203}HgCl$ is obtained in solid form from Amersham-Searle Corporation and must be dissolved in 0.02 N Na_2CO_3.

Sample Preparation. Whole samples, aqueous homogenates, or alkaline digests of samples can be used for analysis. To prepare aqueous homogenates, a 0.5-2.0 g portion of sample and an equal volume of distilled water are uniformly homogenized with a Brinkman Polytron Model 3675 homogenizer (Kinematica) and diluted to a known final volume with water. For alkaline digests, a 0.5-2.0 g portion of sample is placed in a 20 ml glass vial. Two ml of 45% NaOH and 1 ml of 1% cysteine·HCl are added. The contents are gently heated until the sample has dissolved. Boiling must be avoided. The mixture is cooled and diluted to a known final volume with 1% NaCl.

These preparation procedures are only general. Because the original sample is diluted, the optimum amount of sample and dilution are dependent upon sample methylmercury concentration and instrumental sensitivity. Unprocessed grain samples are usually analyzed whole since most of the mercury is on the surface from fungicidal treatment. Processed grain, being impregnated with mercury, can be digested. Sediment samples do not digest completely and must be analyzed whole or as homogenates.

Procedure. The sample (0.5-2.0 g, ml) is placed in a 50 ml Nalgene polypropylene Oak Ridge centrifuge tube (Nalge Co.) and incubated with 10 µl of $CH_3{}^{203}HgCl$ spike and 2 ml of 8 M urea for 10 minutes. One ml of 0.5 M $CuBr_2$ and 3 ml of H_2SO_4-NaBr reagent are added. The contents are mixed 10 seconds on a Vortex mixer (Scientific Products) and let stand 5 minutes. Ten ml of thiophene-free benzene is added, the mixture shaken 5 minutes on a Thomas-Borner shaker (Arthur H. Thomas Co.), and centrifuged 5 minutes at 3000 x g in an IEC

Model C centrifuge (International Equipment Co.). Emulsified mixtures are centrifuged 10 minutes at 15,000 x g in a Sorval Model RC2-B superspeed centrifuge (Dupont Co., Instrument Div.). The benzene layer is removed and placed in a second Oak Ridge tube. One ml of 0.01 M $Na_2S_2O_3$ is added, the contents are mixed vigorously 15 seconds on the Vortex mixer, and centrifuged 5 minutes at 3000 x g. The benzene layer is discarded. If the aqueous layer becomes emulsified, 1 ml of 95% ethanol is added at this point. To the aqueous layer, 0.5 ml of 0.5 M $CuBr_2$ is added and the contents are mixed 10 seconds. Spectrograde benzene (usually 1-2 ml) is added, the mixture shaken 30 seconds and centrifuged 5 minutes at 3000 x g, and the benzene transferred to a glass test tube containing 0.1 g of 1:1 (w/w) anhydrous Na_2SO_4-Florisil. After mixing 10 seconds, the benzene is transferred to a glass scintillation vial and submitted for GC assay. Upon completing an analysis, the Oak Ridge tubes are cleaned using a sulfuric acid solution of Chromerge Labware Cleaner (Monostat Corp.) to remove any traces of electron-capturing contaminants.

Instrumentation. Instrument operating conditions for gas chromatography are given in Table I, along with corresponding retention times for methyl- and ethylmercuric bromide. The detector cell and probe, ^{3}H foil, and syringes are cleaned periodically according to CAPPON and SMITH (1976). For determining methylmercury recovery, a Packard Model 2450 liquid scintillation spectrometer is used to measure ^{203}Hg beta-activity in sample extracts. Upon completion of GC analysis, the extract is diluted to 20 ml with Aquasol Universal LSC Cocktail and counted 10 minutes in the spectrometer along with $CH_3{}^{203}HgCl$ spike standards.

Calculations. Methylmercury is assessed by measuring peak heights from 10 µl sample injections and obtaining concentration values from standard calibration curves prepared daily. Calculation of methylmercury content in samples using ^{203}Hg recovery data is described by VON BURG et al. (1974).

RESULTS AND DISCUSSION

Alkaline digestion, first developed for hair (GIOVANOLI-JAKUBCZAK, 1974), is advantageous for blood, fish, feces, and soft tissue. Sodium hydroxide digests are very homogeneous and do not emulsify during the initial acid extraction step as do aqueous homogenates. Consequently, high-speed centrifugation is not required,

and methylmercury recovery is more efficient due to breakdown of proteinaceous material during sample digestion. Final benzene extracts are cleaner. Although cysteine serves to complex methylmercury and prevent breakdown to inorganic mercury, it gradually oxidizes in the strong alkaline solution and methylmercury slowly breaks down after a few days. Therefore, the sample must be analyzed soon after digestion.

TABLE I

Gas Chromatography

Instrument:	Packard Model 7401
Detector:	^{3}H Electron Capture (150 mCi), DC Mode
Column:	Glass length = 1.22 m, id. = 4 mm; 1.5% OV-17 + 1.95% QF-1 on 80/100 Chromosorb W-HP (Alltech Associates)

Instrument Settings

Temperature (°C)	Inlet	Column	Detector
	130	110	150

Carrier Flow Rate:	120 cm^3/min, nitrogen
Sensitivity:	3 x 10^{-9} A full-scale (AFS)
Suppression Current:	1-2 x 10^{-7} A
Potential:	5 V
Chart Speed:	12 inches/hr
Retention times, min:	MeHgBr, 0.6; EtHgBr, 1.6

The analytical procedure is outlined in Figure 1. In the first extraction step, the highly acidic medium and excess bromide ions permit more efficient methylmercury extraction into the benzene layer. Urea (VON BURG, 1974) and cupric ions (WESTOO, 1968) also enhance the extraction. Urea uncoils proteins and exposes mercury-binding sulfhydryl sites for acid cleavage, while cupric ions bind any free sulfhydryl groups and displace mercury bound to sulfur. Cleanup of the initial benzene extract is quantitatively achieved with $Na_2S_2O_3$, which rapidly complexes methylmercury. In the final extraction step, bromide allows rapid partitioning of methylmercury between the organic and aqueous phases. Cupric ions aid in liberating methylmercury from thiosulfate. High overall methylmercury recovery (75-90%) is achieved while minimizing solvent and reagent volumes and extraction times.

Table II reveals good accuracy, precision, and recoveries obtained with this procedure for samples containing added methylmercury. Mean deviation and relative accuracy averaged 1.3 and 3.8%, respectively. The validity of analytical results obtained with this GC method was verified by examining the correlation with atomic absorption (AA) data. Several environmental samples previously analyzed for total and inorganic mercury by an AA procedure (MAGOS and CLARKSON, 1972) were obtained for this purpose. GC-AA comparison results given in Table III show good agreement between both methods for samples containing methyl- and ethylmercury, and this is expressed in terms of GC/AA ratios.

HARTUNG (1972) successfully applied 11% OV-17 + QF-1 to methylmercury analysis. 1.5% OV-17 + 1.95% QF-1, used for chlorinated pesticide analysis by THOMPSON (1969), was used in this study. The packing permits column operation at a lower temperature, and provides excellent resolution and very short retention times for methyl- and ethylmercury, while minimizing column bleed. Standard calibration curves, expressed as peak height vs organomercury bromide concentration, are linear up to at least 2.0 ng/10 µl injection. For the given instrument settings, the minimum detectable organomercury bromide concentration is 0.02 ng/10 µl, or 2 ppb RHgBr. Expressed as mercury concentration, this corresponds to 1.36 and 1.30 ppb Hg for methyl- and ethylmercury, respectively. For a 2 g whole sample containing methylmercury, assuming 1 ml of final extract and 80% recovery, this translates to a sample concentration of 0.85 ppb Hg. Benzene extracts from this procedure are quite clean, mercury peaks are well resolved from any contaminants, and any late peaks elute within three minutes from sample injection (Figure 2).

Liquid scintillation spectrometry is a valuable method for determining methylmercury recovery. Monitoring of stepwise and overall recovery with ^{203}Hg isotope is straightforward. This technique greatly simplifies GC analyses and calculations by eliminating the need for internal mercury standards. Since samples are spiked with less than 1 ng of Hg before extraction, no correction is required for added spike except for samples of very low mercury content.

Although this specific procedure is very useful for methylmercury analysis, a more general routine gas-chromatographic procedure for both organic and inorganic mercury has recently been developed in this laboratory (CAPPON and SMITH, 1976).

	% Recovery
Sample (MeHg-S-protein)	
↓ 1. $Me^{203}HgCl$, 8 M Urea, 0.4 M $CuBr_2$, H_2SO_4-NaBr. 2. Benzene. 5 min.	
Benzene (MeHgBr)	90-100
↓ 0.01 M $Na_2S_2O_3$. 15 sec.	
Aqueous (MeHg-Thiosulfate)	100
↓ 1. 0.5 M $CuBr_2$, Benzene. 30 sec. 2. 1:1 Na_2SO_4-Florisil	
Benzene (MeHgBr)	80-90
↓ GC, LSS	Overall % recovery: 75-90

Figure 1. Outline of MeHg procedure

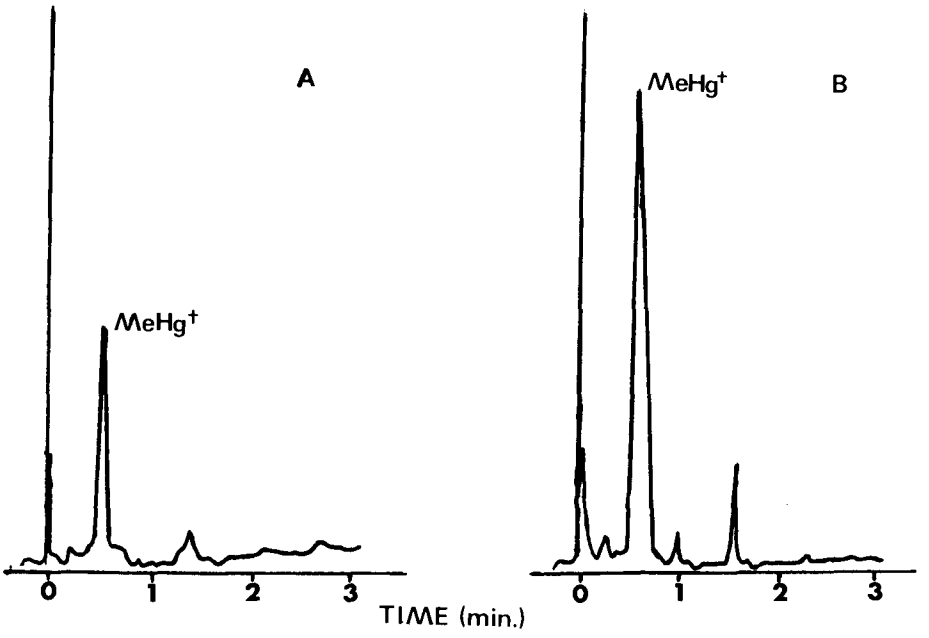

Figure 2. (A) Chromatogram of blood sample (whole) containing 0.19 ppm methylmercury. (B) Chromatogram of fish sample (alkaline digest) containing 3.24 ppm methylmercury.

TABLE II

Analytical and Recovery Data

Sample	Added MgHgCl[a] (ng/g)	Mean[b]	% Mean deviation	% Relative accuracy[c]	% Recovery, avg.
Blood (whole)	197.8	198.7	1.0	0.5	80.7
Feces (digest)	12.7	11.5	1.7	9.4	87.4
Liver (digest)	39.6	41.5	1.5	4.7	84.1
Urine	27.9	28.7	1.2	2.9	91.3
Water	105.5	107.0	0.9	1.4	89.3

[a]Whole sample.

[b]Mean values are the average of three analyses. All have been corrected for recovery.

[c]Percent relative accuracy of the mean.

TABLE III

GC-AA Intercomparison Study

Sample	ppm Hg, as MeHg		
	GC	AA	GC/AA
Blood, human[a]	0.13	0.12	1.08
Egg, yolk[b]	3.02	3.23	0.93
Fish, pike[c]	1.08	1.06	1.02
Flour[c]	4.55	4.63	0.98
Hair, human[c]	258.6	272.9	0.95
Sediment, river[b]	0.023	0.028	0.82
Wheat[a]	7.74	7.50	1.03
Urine, human[a]	1.66 ppb	1.60 ppb	1.04
	ppm Hg, as EtHg		
Blood, human[c]	0.79	0.77	1.03
Kidney, human[c]	0.67	0.68	0.99

[a]Whole sample. [b]Aqueous homogenate.

[c]Alkaline digest.

CONCLUSIONS

The relative simplicity of this procedure allows easy routine application to a wide range of environmental samples. Up to 32 samples daily can be analyzed by a skilled technician, almost twice the number possible with most other published procedures. In addition, smaller amounts of reagents and organic solvent are required, and high levels of sensitivity and reliability are achieved.

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