COMMUNICATIONS

Determination of Methylmercury in Fish and in Cereal Grain Products

Methylmercury was determined in fish by gas–liquid chromatography following a modification of existing extraction procedures. The method obviated centrifugation to assist phase separation and yielded overall recoveries of 94% ± 6% for whitefish and 98% ± 6% for cod of methylmercury. Methylmercury was also determined in wheat flour and ground oats by extraction with a benzene–formic acid mixture followed by purification and gas–liquid chromatography. Interfering substances were removed from the extracts by column chromatography on silicic acid and partitioning with cysteine acetate solution. The method is sensitive in the 0.01–0.90 ppm range, with a mean recovery generally greater than 95%.

It has been shown that mercuric chloride may be methylated by aquatic sediments (Jensen and Jernelöv, 1969) and the resulting accumulation of methylmercury in fish tissues has been well documented (Backstrom, 1969). In addition, organomercurials are often used as fungicidal seed dressings for cereals. In veiw of the potential hazard which methylmercury poses to human health, it is necessary to monitor the levels of methylmercury appearing in the edible portion of fish and in cereal grains.

Methods presently available for the determination of methylmercury in fish (Sumino, 1968; Westöö, 1968) involve acidification of an aqueous homogenate of the tissue and extraction of the homogenate with benzene. The mixture is then centrifuged with subsequent recovery and purification of the methylmercury in the benzene layer. In our hands these methods were unsatisfactory, due to the formation of gelatinous emulsions and incomplete recoveries, particularly in samples with a high lipid content. Intractable emulsions resulted when the methods were applied to products derived from cereal grains. Accordingly, the existing methods for fish were modified by incorporating a filtration step and by the use of hydrobromic rather than hydrochloric acid to enhance the partition ratio (Simpson, 1961), and facilitate the extraction of methylmercury from the aqueous phase. A different procedure involving the use of an organic solvent was developed for wheat flour or ground oats, and, presumably, is applicable to the determination of methylmercury in other similar materials.

EXPERIMENTAL

Methylmercuric chloride was purchased from Alfa Inorganics, Beverly, Mass. It was homogeneous by thin-layer chromatography on silica gel using a solvent system of hexane:diethyl ether (1:1) and employing a saturated solution of Michler's thioketone in ethanol for visualization. The compound gave a single peak on gas–liquid chromatography. The mercurial was dissolved in absolute ethanol or acetone to give a stock solution and was added to the samples as a spike in volumes of less than 1.0 ml. Methylmercuric chloride added to the samples was not extractable with benzene or benzene:ethanol (2:1), and was considered to be a valid spike.

Commercial 48% hydrogen bromide contained significant impurities which were removed before use by exhaustive extraction with benzene. Cysteine acetate solution used to determine methylmercury in fish was prepared by dissolving cysteine hydrochloride monohydrate (2.00 g), sodium acetate trihydrate (4.00 g), and anhydrous sodium sulfate (12.5 g) in water, and making to volume (100 ml). The cysteine solution used with cereal extracts was prepared in essentially the same manner, but contained 1.00 g/100 ml of cysteine hydrochloride monohydrate.

Gas–Liquid Chromatography. Gas–liquid chromatography

Table I. Determination of Methylmercury in Cod and Whitefish Fillets

Sample	Methylmercuric Chloride Added (ppm)		Methylmercuric Chloride Found (ppm)		Methylmercuric Chloride Found Corrected (for blank, ppm)		Mean Recovery (%)	
	Cod	Whitefish	Cod	Whitefish	Cod	Whitefish	Cod	Whitefish
A_1	0	0	0.068	0	...	0	...	...
A_2	0	0	0.074	0	...	0	...	...
B_1	0.080	0.10	0.147	0.103	0.076	0.103	101	100
B_2	0.080	0.10	0.156	0.096	0.085	0.096		
C_1	0.10	0.30	0.162	0.296	0.091	0.296	94	95
C_2	0.10	0.30	0.168	0.269	0.097	0.269		
D_1	0.30	0.50	0.340	0.502	0.269	0.502	91	100
D_2	0.30	0.50	0.346	0.497	0.275	0.497		
E_1	0.50	0.70	0.633	0.592	0.562	0.592	110	85
E_2	0.50	0.70	0.615	0.593	0.544	0.593		
F_1	0.70	0.90	0.775	0.790	0.704	0.790	99	88
F_2	0.70	0.90	0.755	0.782	0.684	0.782		
G_1	0.90		0.930		0.859		95	
G_2	0.90		0.915		0.844			
					Overall Recovery		98% ± 6%	94% ± 6%

Table II. Recovery of Methylmercury from Wheat Flour and Ground Oats

Sample	Methylmercuric Chloride added (ppm)		Methylmercuric Chloride found (ppm)		Mean Recovery (%)	
	Wheat Flour	Ground Oats	Wheat Flour	Ground Oats	Wheat Flour	Ground Oats
A_1	0.010	0.10	0.013	0.10	130	105
A_2	0.010	0.10	0.013	0.11		
B_1	0.050	0.30	0.048	0.29	104	102
B_2	0.050	0.30	0.055	0.32		
C_1	0.080	0.50	0.096	0.52	111	103
C_2	0.080	0.50	0.081	0.51		
D_1	0.10	0.70	0.09	0.71	95	99
D_2	0.10	0.70	0.10	0.68		
E_1	0.30	0.90	0.29	0.79	95	88
E_2	0.30	0.90	0.28	0.79		
F_1	0.50		0.50		99	
F_2	0.50		0.49			
G_1	0.70		0.72		102	
G_2	0.70		0.71			
H_1	0.90		0.95		108	
H_2	0.90		0.99			

was performed on an Aerograph 705 fitted with a glass injection insert and tritium foil electron capture detector. The detector was connected directly to the column outlet. The glass column was 40 cm × 4 mm and packed with 2% butanediol succinate on 100–120 mesh Chromosorb W, AW, DMCS. The column was conditioned by heating for 24 hr at 200° C under a flow of nitrogen carreir gas at 60–80 ml/min. The conditioning time could be reduced to 2 hr using Chromosorb W, HP as a support. For the determination of methylmercury, typical operating temperatures were: injection port 110° C; oven 120° C; and detector 200° C. The flow of carrier gas was maintained at 80–100 ml/min. Under these conditions methylmercuric chloride had a retention time of approximately 1 min.

Standards and samples were injected as benzene solutions in 2.0 μl aliquots. Solutions of methylmercuric bromide, prepared by equilibrating a benzene solution of the chloride with excess hydrobromic acid (Simpson, 1961), were found to give a gas-chromatographic response equivalent to the chloride on a molar basis. Each sample was preceded by the injection of a standard of methylmercuric chloride, since small changes in the column temperature or flow rate of carrier gas resulted in slightly altered peak heights. The samples were quantitated by comparing the peak height with that of a standard and expressed in terms of methylmercuric chloride. The lower limit of detection was approximately 0.02 ng of methylmercuric chloride, using injection of the standard solutions or final extracts of food samples.

Extraction and Purification. Fish. Fish fillet (10.0 g) was homogenized in a Virtis 23 homogenizer for 10 min with a solution of 1.0 *N* hydrobromic acid and 2.1 *N* potassium bromide (60.0 ml). The homogenate was filtered through glass wool under gravity on a Buchner funnel, and the residue and glass wool rehomogenized with a further portion (60.0 ml) of hydrobromic acid–potassium bromide solution. The filtrates were combined and extracted twice with benzene (1 × 100 ml, 1 × 50 ml). After removing as much of the aqueous phase as possible, the benzene layers were combined. Any emulsions remaining in the organic layer were broken by shaking with solid anhydrous sodium sulfate. The combined benzene layers were then extracted with cysteine acetate solution (8.0 ml). An aliquot (5.0 ml) of the cysteine layer was recovered and acidified with 48% hydrobromic acid (1.0 ml). The mercury was then extracted with benzene (10.0 ml) and the benzene extract subjected to analysis by gas–liquid chromatography.

Cereal Products. Flour or ground oats (10.0 g) was extracted for 5 min in a homogenizer with benzene (50 ml) and 90% formic acid (5.0 ml). The homogenate was filtered through Whatman No. 1 paper and aliquot of the filtrate (30.0 ml) transferred to a 10 × 1.6 cm column of silicic acid (Mallinckrodt, 100 mesh) in benzene. The column was run

under nitrogen pressure at a flow rate of approximately 1 ml/min and the 15–55 ml fraction was eluted with benzene collected. The eluate was shaken with cysteine acetate (6.0 ml) and a portion (5.0 ml) of the aqueous layer recovered. After acidifying the cysteine layer with 48% hydrobromic acid (1.0 ml), the mercurial was extracted with benzene (10.0 ml) and subjected to analysis by gas–liquid chromatography.

RESULTS AND DISCUSSION

A preliminary removal of lipid from the fish by extraction with benzene:ethanol (2:1) or acetone was not sufficient to prevent emulsion formation in the subsequent steps, probably due to the presence of lipoprotein components. This problem was circumvented by homogenizing with aqueous acid, followed by filtration to remove insoluble residue. It was found, using hydrochloric acid to acidify the cysteine extract, that a threefold extraction with benzene was necessary to remove all the mercury. Using hydrobromic acid, essentially all of the methylmercury was obtained by a single extraction. As shown in Table I, the recoveries of methylmercury from whitefish and cod were 94% and 98% ± 6%, respectively. Although some emulsions are formed, they are easily broken and the constituent phases obtained without centrifugation.

The recoveries of methylmercury from wheat flour and ground oats are shown in Table II. The data in the 0.010–0.080 ppm range were obtained by extracting the acidified aliquot of cysteine with 1.0 ml instead of 10.0 ml of benzene. In most cases the yield of methylmercury was greater than 95%.

The extraction procedure used for flour and oats is not applicable to the determination of methylmercury in foods containing a high proportion of protein, such as fish or liver, presumably because of poor solvent penetration. However, the method should serve as a useful adjunct to total mercury determinations in foodstuffs containing a relatively high proportion of carbohydrate.

LITERATURE CITED

Backstrom, J., *Acta Pharmacol. Toxicol.* **27,** Suppl. 3 (1969).
Jensen, S., Jernelöv, A., *Nature (London)* **223,** 753 (1969).
Simpson, R. B., *J. Amer. Chem. Soc.* **83,** 4711 (1961).
Sumino, K., *Kobe J. Med. Sci.* **14,** 115 (1968).
Westöö, G., *Acta Chem. Scand.* **22,** 2277 (1968).

William H. Newsome

Research Laboratories
Food and Drug Directorate
Department of National
Health and Welfare
Ottawa, Canada

Received for review November 6, 1970. Accepted January 25, 1971.