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Determination of Methylmercury Salts in Various Kinds of Biological Material

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The cysteine acetate modification of the method for determining methylmercury salts in foods,[1] which was useful for analysis of fish, egg white, and meat, was not efficient when applied to egg yolk with low methylmercury content, liver, sediments in aquaria, or sludge. Therefore some modifications of the procedure have been investigated. A combination of the mercuric chloride [1] and cysteine acetate procedures gave good results for sediments in aquaria and sludge and could also be used for, *e.g.*, fish, egg white, bile, kidney, blood, meat, and moss. Precipitation of the proteins with molybdic acid at the first extraction improved the results for liver but not for egg yolk. For egg yolk an increase of the concentration of the cysteine acetate solution from 1 to 10 % gave 90 % recovery of added methylmercury, repeated extractions 100 % recovery.

When known amounts of methylmercury dicyandiamide were added to samples of liver before the analyses for methylmercury compounds by the cysteine acetate procedure, low recoveries were obtained.[1] This might be due to the SH-groups in the proteins or related compounds in the liver, competing with the cysteine for binding the methylmercury ions at the purification. Four procedures were tried to overcome difficulties of this type:

1) Addition of excess mercuric ions to bind the SH-groups of the liver proteins. 2) Precipitation of the proteins with molybdic acid. 3) Increase of the cysteine concentration. 4) Repeated extractions with cysteine solution.

1. Excess mercuric ions were added to an aqueous liver suspension containing known amounts of a methylmercury salt. The analysis was performed according to the cysteine acetate modification.[1] More than 100 % of the methylmercury was recovered. When the acidified liver suspension containing mercuric ions was kept at room temperature overnight, the recovery increased. This indicated a synthesis of methylmercury ions from mercuric ions by the liver under the conditions used. Thus, this combined mercuric ion-cysteine acetate procedure for analysis of methylmercury could not be applied to liver. Some results obtained without addition of methylmercury compounds are seen in Table 1.

Table 1.

Sample	Total mercury mg/kg	Methylmercury compounds analysed by combined mercuric ion-cysteine acetate procedure, mg of Hg/kg	
		Reaction time 0.5 h	Reaction time 20 h
Ox liver	0.006	0.029	0.045
Ox liver, boiled	0.006	0.031	0.047

For egg yolk with low content of methylmercury the cysteine acetate procedure gave less than 90 % recovery.[1] With the combined method the recovery of methylmercury salt decreased almost to zero. But for sediments in aquaria and sludge, which similarly could not be analysed by the original cysteine acetate modification, the combined method gave good results (Table 2).

The combined method has also been applied to fish muscles with good recoveries, and it is now often used in this laboratory as a control procedure in fish analysis[2,3] with the cysteine acetate procedure as the main method. In 10 samples of fish (pike, cod) analysed by these two methods 98 ± 3 % of added methylmercury (0.2—0.6 mg of Hg/kg of fish muscles) was recovered. Egg white, kidney, blood, meat, bile, and moss have also been analysed using the combined method (>90 % recovery).

2. Precipitation of the proteins in liver by molybdic acid increased the recovery of added methylmercury salt to about 90 %. In egg yolk with low content of methylmercury compounds, however, neither molybdic acid nor phosphomolybdic acid improved the results. A washing of the homogenized liver with organic solvent (benzene was used in this laboratory) before the acidification and extraction of the methylmercury as used by Kitamura *et al.*[4] also improved the results, but required more time.

Table 2.

Sample	Total mercury mg/kg	Methylmercury compounds found, mg of Hg/kg		Methylmercury compounds found after addition of methylmercury dicyandiamide, 0.1 mg of Hg/kg			
		Cysteine acetate procedure	Combined procedure	Cysteine acetate procedure		Combined procedure	
				mg of Hg/kg	% recovery	mg of Hg/kg	% recovery
Egg yolk	0.004	0.000	0.000	0.082	82	0.001	1
Sediment	0.063	0.000	0.017	0.004	4	0.109	92
Sludge	0.52	0.01	0.041	0.028	18	0.125	84

3, 4. As to egg yolk with low methylmercury content repeated extractions with 1 % cysteine acetate solution were used with success (100 % recovery), but one single extraction with 10 % cysteine acetate was more rapid and also gave good results (about 90 % recovery).

In both the original and the modified cysteine acetate methods a smaller aliquot of the cysteine extract was used than earlier to save time at the centrifugation. For the same reason sodium chloride was added at the first extraction.

The cysteine modifications for analysis of methylmercury compounds — except the combined mercuric ion-cysteine procedure — are only slightly influenced by the presence of dimethylmercury (about 1 % of added dimethylmercury (20 μg) was transformed to methylmercury during the analysis). The mercuric chloride [1] and the mercuric chloride-cysteine procedures transform the dimethylmercury to methylmercury compound and thus give high methylmercury contents, if dimethylmercury is present. Several hundred samples of fish have been analysed for methylmercury both with the cysteine procedure and the mercuric ion or the mercuric ion-cysteine procedure without significant differences in the results. Accordingly these samples of fish did not contain any dimethylmercury.

EXPERIMENTAL

Chemicals and cleaning of glassware: See the preceding report.[1]

A. *Rapid determination of methylmercury salt in fish, eggs, meat, bile, and algae by the cysteine acetate procedure.* Homogenize 10.00 (or 2 × 25.0) g of the sample with water in a 250 ml centrifuge flask (two 500 ml) and rinse the homogenizer quantitatively. Use a total of 55 (2 × 135) ml of water for these procedures. Add 14 (2 × 35) ml of concentrated hydrochloric acid and 10 (2 × 25) g of sodium chloride and mix. Add 70 (2 × 175) ml of benzene, and shake the mixture for 15 min in a shaking-machine or for 5 min by hand. Centrifuge. Transfer 50.0 (250.0) ml of the benzene extract to a separating funnel. Add 6.00 ml of a 1.0 % (10.0 % for egg yolk) solution of cysteine acetate saturated with sodium sulphate and shake vigorously for 2 min. Transfer 2.00 ml of the clear, aqueous phase (a disturbing precipitate in the water layer can easily be removed by centrifugation, stirring and recentrifugation) to a separating funnel, and acidify with 1.2 ml of 6 N hydrochloric acid. Extract the solution with 4.00 ml of benzene by shaking for 2 min. Dry the extract with anhydrous sodium sulphate, and submit it to gas chromatography and, after concentration, to thin-layer chromatography.

B. *Rapid determination of methylmercury salt in fish, egg white, kidney, blood, meat, bile, algae, sediments, moss, and sludge by combined mercuric chloride-cysteine acetate procedure.* Follow the above procedure, only adding 2.0 (10.0 for 50 g sample) ml of a purified,[1] aqueous 5 % mercuric chloride solution before the first extraction with benzene.

C. *Rapid determination of methylmercury salt in liver.* Follow the above procedure A only adding 1 g of molybdic acid/10 g of sample to the liver suspension and shake for ½ min. Then add the sodium chloride, hydrochloric acid and benzene at once. Shake and centrifuge immediately. When preparing the calibration curve according to this procedure, add the methylmercury salt immediately before the first extraction with benzene.

D. *Determination of methylmercury salt in egg yolk containing <0.1 mg of methylmercury/kg.* Follow the procedure A using 50 g of sample up to and including the transfer of 250 ml of the first benzene extract to a separating funnel. Shake this extract with 4+3+3 ml of 1.0 % cysteine acetate solution. Collect the water layers in a separating funnel and acidify with 5.5 ml of 6 N hydrochloric acid. Extract the solution with 10.00 ml of benzene by shaking for 2 min. Dry the extract with anhydrous sodium sulphate and submit to gas chromatography and, after concentration, to thin-layer chromatography.

Blank. Proceed according to the above descriptions, only exchanging the sample for water.

Calibration curves. Run known amounts of methylmercury chloride or methylmercury dicyandiamide through the whole procedures, only exchanging the sample for the same amount of water.

Gas chromatography and thin-layer chromatography. See "Determination of Methylmercury Compounds in Foodstuffs I".[5] During the last year glass columns were used instead of stainless steel columns and phenyl diethanolamine succinate (PDEAS) as stationary phase as well as Carbowax 20 M.

Sample solutions, which contained hydrogen sulphide or sulphur, poisoned the gas chromatographic system, so that no peaks or too small and broadened peaks of methylmercury compounds were obtained. In such case the system was improved by injection of benzene solutions of methoxyethylmercury iodide or mercuric chloride.

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