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Determination of Methylmercury Compounds in Foodstuffs

I. Methylmercury Compounds in Fish, Identification and Determination

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A combined gas chromatographic and thin-layer chromatographic method for the identification and determination of methylmercury compounds in fish is presented. For the identification five derivatives of the supposed methylmercury compound from three samples of fish were studied. In thirty samples of fish from the Baltic, Swedish lakes and rivers two or three derivatives were analysed using both chromatographic methods. The samples contained 0.07–4.45 mg Hg/kg as methylmercury compounds. Also in marine fish methylmercury compounds were found, but only in small amounts. Any methylmercury attached to a sulphur atom of nonvolatile compounds will not be determined with this method.

With sensitive methods mercury compounds can be detected and analysed everywhere in our surroundings, even in food. Stock *et al.*[1] in 1934 determined mercury in ppb concentrations by reducing mercuric chloride to mercury and measuring the size of the drops. Today activation analysis [2] offers a more rapid means to analyse mercury in this range.

Until recently very little was known about the chemical state of the mercury in foods, and there were no specific methods to investigate this question. Consequently, a toxicological evaluation of the mercury content in foods has been difficult. This fact is especially unsatisfactory as to fish from the Baltic, Swedish lakes and rivers, in view of the comparatively high mercury levels found by Westermark.[3]

Several authors (*e.g.* Polley and Miller,[4] Gage [5]) have presented methods for the determination of organic mercury compounds, but these methods either do not separate different compounds, *e.g.* methylmercury from phenylmercury compounds, or are designed for mercury contents higher than those usually met with in foods.

In the present paper methods are given for the purification, identification and determination of methylmercury extracted from fish, using gas chromatography and thin-layer chromatography. The methods have also been used with success for analysis of a few samples of egg white.

EXTRACTION AND PURIFICATION

Muscle tissue of fish was homogenized with water and acidified with concentrated hydrochloric acid (1/5 of the volume of the suspension). Organomercuric compounds were extracted in one step with benzene according to Gage.[5] Methylmercury, originally present or added to the fish, could be extracted, though with difficulty, also when only a small amount of acid was added, *e.g.* at pH 1. From an aliquot of the benzene solution organomercury could be extracted with ammonium or sodium hydroxide solution, saturated with sodium sulphate, for elimination of lipids. The yields were low and variable, but could be improved as described below.

In 1961 Uchida *et al.*[6] showed that the mercury compound in the shellfish that caused the Minamata disease was methyl(methylthio)mercury. It is reasonable to assume that methylmercury, if present in Swedish fish, should at least to some extent be a methylthio derivative. The Hg—S bond is stronger than Hg—NH or Hg—OH bonds (Schwarzenbach [7]). Accordingly it prevents the formation of these bonds, which should be produced by the ammonium hydroxide solution and increase the solubility in water. Any methylthio group present should therefore be removed before the extraction with alkali.

Distillation of the benzene extract at reduced pressure at room temperature or at ordinary pressure at 80° to 1/10 of the original volume removed the factor that prevented an acceptable extraction by ammonium or sodium hydroxide solution (probably methanethiol and maybe hydrogen sulphide). After the distillation and subsequent extraction with ammonium hydroxide solution the extract was acidified with hydrochloric acid, and the organomercury compound was extracted once with benzene. After drying with anhydrous sodium sulphate, the benzene solution was ready for gas chromatography and, after concentration, also for thin-layer chromatography.

In the above procedure about 30 % of the methylmercury was lost, mainly by unfavourable partition coefficients. In a model experiment of the benzene extraction of methylmercury from a hydrochloric acid solution, for instance, 14 % of the methylmercury was left in the water layer. The losses by partition are, however, characteristic of the compounds involved and reproducible. Consequently they can be included in the calibration curve (Fig. 1), thus disturbing the results only slightly. The yields can be increased by repeated extractions, but good results are obtained with the above simple procedure. The calibration curve is based on the partition laws for methylmercury

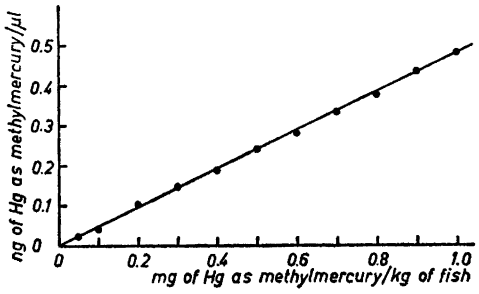

Fig. 1. Calibration curve for 10 g samples.

chloride, though some methylmercaptide and maybe sulphide are probably present in fish. However, when hydrogen sulphide or methanethiol was added (30 μg per 5 μg of mercury as methylmercury) to the aqueous phase before the first extraction, when preparing the calibration curve, the 5 μg point was unaltered. (Large amounts of these compounds disturbed the analysis because they were not completely removed by the distillation).

When known amounts of methylmercury dicyandiamide were added to salt-water fish (frozen cod, *Gadus morrhua*, or haddock, *Gadus aeglefinus*), 82—95 % of the additions were recovered (Table 1).

Table 1.

μg of Hg/g of fish added as methylmercury dicyandiamide	μg of Hg/g of fish as methylmercury compound found before the addition	μg of Hg/g of fish as methylmercury compound found after the addition	Recovery %
0.300	0.008	0.294	95
0.400	0.016	0.384	92
0.500	0.008	0.419	82
0.600	0.023	0.542	87
0.800	0.010	0.757	93
1.600	0.023	1.484	91

THIN-LAYER AND GAS CHROMATOGRAPHY STUDIES OF METHYLMERCURY DERIVATIVES

Methylmercury dithizonate and phenylmercury dithizonate could be separated from each other by thin-layer chromatography on aluminum oxide (limit of detection: 0.2 μg) (Fig. 2). Methylmercury cyanide, chloride, bromide

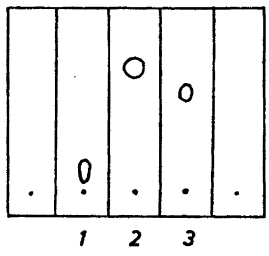

Fig. 2. Thin-layer chromatogram on aluminum oxide of mercury dithizonate (1), methylmercury dithizonate (2), and phenylmercury dithizonate (3).

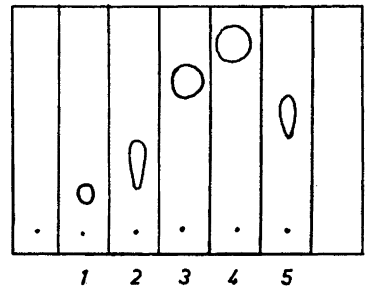

Fig. 3. Thin-layer chromatogram of methylmercury cyanide (1), chloride (2), bromide (3), and iodide (4), and of phenylmercury chloride (5) on silica gel.

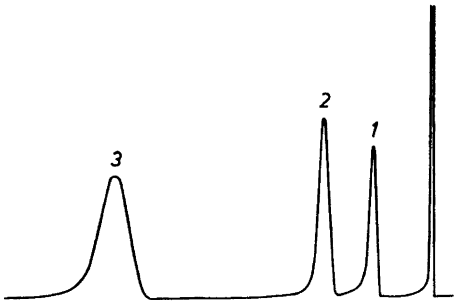

Fig. 4. Gas chromatogram of methylmercury (1), ethylmercury (2), and methoxyethylmercury (3) compounds.

and iodide were separated by thin-layer chromatography on silica gel (limit of detection of the chloride and bromide: 0.02 μg on spraying with an ethanol solution of Michler's thioketone) (Fig. 3).

Methylmercury cyanide, chloride, bromide, iodide and dithizonate, but not dimethylmercury, when gaschromatographed at 130°, all gave the same peak (Fig. 4). The corresponding ethylmercury derivatives behaved similarly, but had a longer retention time (Fig. 4). Also methoxyethylmercury compounds gave only one peak, with still longer retention time (Fig. 4). Phenylmercury chloride had an extremely long retention time on the column used.

IDENTIFICATION OF METHYLMERCURY IN FISH

Three pikes (*Esox lucius*), from the southern part of the Baltic, lake Mälaren and the river Dalälven, were used for the main part of the identification of the methylmercury compounds. With gas chromatography, purified benzene extracts of the samples gave peaks with the same retention time as did methylmercury chloride. Likewise the samples and methylmercury chloride, when thin-layer chromatographed on silica gel, gave rise to spots with identical R_F-values.

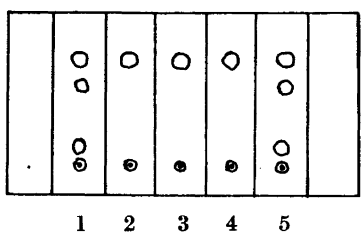

Fig. 5. Thin-layer chromatogram on aluminum oxide of authentic methylmercury dithizonate, phenylmercury dithizonate, and mercury dithizonate (1,5) together with dithizonates prepared from methylmercury extracted from pikes (2, 3, 4).

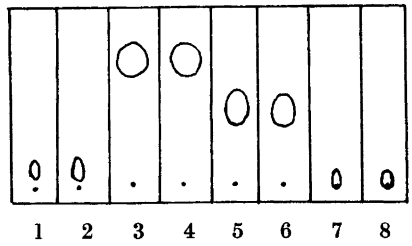

Fig. 6. Thin-layer chromatogram on silica gel of authentic methylmercury chloride (1), iodide (3), bromide (5), and cyanide (7) together with the corresponding compounds prepared from methylmercury extracted from pike (2, 4, 6, 8).

Different derivatives were prepared from portions of the purified extracts by treatment with dithizone, hydrobromic acid, potassium iodide or potassium cyanide. The dithizonates formed had the same R_F-value as methylmercury dithizonate using thin-layer chromatography on aluminum oxide (Fig. 5). Furthermore, when the dithizonates from the fish samples were eluted from the spots on the developed chromatographic plates and gas chromatographed, peaks with the same retention time as for methylmercury chloride were obtained. Standard methods for the determination of mercury showed that the spots contained this element.

The bromide, iodide, and cyanide from the fish extracts moved as methylmercury bromide, iodide, and cyanide (Fig. 6) when thin-layer chromatographed on silica gel. These spots, too, were eluted and proved to contain mercury. The eluates were gas chromatographed under the same conditions as authentic methylmercury chloride. In all cases peaks were obtained with the same retention time as when authentic methylmercury chloride or the simply purified extracts were gas chromatographed.

Thus, from the purified extracts of three pikes there have been prepared a mercury-containing chloride, bromide, iodide, cyanide and dithizonate, each with its own R_F-value on thin-layer chromatography, identical with the R_F-value of the corresponding authentic methylmercury derivative. Furthermore, when gas chromatographed on a column, which well separated, *e.g.*, methylmercury compounds from ethylmercury compounds, they all gave peaks with one and the same retention time identical with that obtained with authentic methylmercury compounds.

Addition of 2 ppm of an inorganic mercury salt, metallic mercury, or phenylmercury to the fish samples before extraction did not increase the amount of methylmercury compound found. Thus there is no doubt that methylmercury compounds are originally present in these samples of fish. The amounts found calculated as mercury were 2.11, 0.76, and 0.50 mg/kg.

Extracts from thirty samples of fish from the Baltic and from Swedish lakes and rivers (pike, *Esox lucius*, bream, *Abramis brama*, pike-perch, *Lucioperca sandra*, eelpout, *Lota vulgaris*, and perch, *Perca fluviatilis*) have so far been analysed for methylmercury contents by gas chromatography of the chlorides and thin-layer chromatography of the dithizonates on aluminium oxide, which by routine analysis should be sufficient for identification. In some samples the iodides or bromides have also been studied. In all cases methylmercury compounds were found in comparatively large amounts, varying from 0.07 to 4.45 calculated as mg Hg/kg.[8] In some of the fish samples about 90 % of the total mercury was methylmercury, but about 75 % was a more common result. Boiling of the fish did not eliminate the methylmercury. In marine fish, too, methylmercury compounds have been found, but only in small amounts.[8]

Any methylmercury attached to a sulphur atom of a nonvolatile compound will not be determined by this method. A method with which all the methylmercury can be determined is being developed.

EXPERIMENTAL

The water used was distilled and deionized. All chemicals were of analytical grade or for scientific use. The concentrated ammonium hydroxide solution was prepared by dissolving ammonia gas in deionized water. The organic solvents used were distilled.

Cleaning of glassware. Clean all glassware first by ordinary laboratory procedures and then by shaking or rinsing with a mixture of 10 parts of 0.04 % dithizone in carbon tetrachloride and 25 parts of 1 N ammonium hydroxide solution, until the carbon tetrachloride solution does not show any trace of red colour. Finally rinse with carbon tetrachloride and deionized water.

Extraction of muscle tissues of fish and purification of the extract. Homogenize 10.0 g of muscle tissue of fish with water, and transfer the homogenate quantitatively to a separatory funnel. For the homogenization and transfer a total of 60 ml of water is used. Add concentrated hydrochloric acid (14 ml) and mix. Add benzene (70.0 ml), and shake the mixture vigorously for 5 min. After centrifugation transfer 50.0 ml of the clear benzene extract to a 200 ml flask together with 5 ml of a 0.1 N heptane solution of acetic acid. Distil off the solvents at a moderate rate until 5 ml remain. The space saving Soxhlet apparatus was used for the distillation, the extraction part being receiver.

Cool the apparatus before removing the flask. Transfer the heptane solution in the flask quantitatively to a separatory funnel with the aid of 5 ml of hexane. If the analysis has to be interrupted, do this any time between the centrifugation above and the addition of ammonium hydroxide solution below.

Shake the heptane-hexane solution vigorously for 3 min with 6.00 ml of a 2 N ammonium hydroxide solution saturated with sodium sulphate. (The ammonium hydroxide-sodium sulphate solution can be prepared by adding a solution of 150 g of anhydrous sodium sulphate in 600 ml of water to 165 ml of concentrated ammonium hydroxide solution and diluting to 1 l with water). Transfer 5.00 ml of the clear aqueous phase (centrifuge, if necessary) to a small separatory funnel and acidify with 3.0 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 it to gas chromatography. Calculate the methylmercury concentration of the samples using the calibration curve (Fig. 1).

From marine and other fish, in which low mercury contents are expected, take 50.0 g for the analyses. Multiply all volumes given in the procedure above by five. If the resulting 50.0 ml of purified benzene extract gives too small a peak with gas chromatography, add 5 ml of heptane to 40.00 ml of the solution and distil off 40 ml. Transfer the remainder quantitatively to a 10 ml volumetric flask, make up to the mark with hexane and analyse by gas chromatography.

Calibration curve. For the calibration curve (Fig. 1) run known amounts of methylmercury dicyandiamide through the whole procedure, only exchanging the fish for the same amount of water.

Gas chromatography. Apparatus: Wilkens Aerograph, Moduline, model 202 with electron capture detector. Column: 5′ × 1/8″ stainless steel column with Carbowax $\geqslant$ 1500 (polyethylene glycol, average mol. wt $\geq$ 1500) 10 % on Teflon 6, 35/60 mesh or on Chromosorb W, acid-washed DMCS, 60/80 mesh. Carbowax 20 M has been used for most of the samples. Gas flow rate: 65 ml of nitrogen/min. Temperature of column: 130°—145°. Temperature of injector: 150°—170°. Recorder: Texas Instruments, model PWS-IMVC-05-A 25-BT. The peak of each sample solution has been compared with the peak of a standard solution with about the same concentration of methylmercury.

Transformation of methylmercury chloride to methylmercury dithizonate for thin-layer chromatography. Concentrate 1—10 ml of a benzene solution of methylmercury chloride to 0.1 ml by evaporation of part of the solvent under reduced pressure. There is an obvious loss of the mercury compound by this concentration. Add a 0.4 % dithizone solution in benzene until green coloration.

Thin-layer chromatography on aluminum oxide. Developing solvent: light petroleum + diethyl ether (70 + 30). Activation of the plates: 105° for 1 h.

Transformation of methylmercury chloride to bromide, iodide, or cyanide. Shake a benzene solution of methylmercury chloride with excess hydrobromic acid, potassium iodide, or potassium cyanide in dilute aqueous solution. Dry the benzene layers with sodium sulphate and concentrate them by evaporation under reduced pressure for thin-layer chromatography.

Thin-layer chromatography on silica gel. Developing solvent: light petroleum + diethyl ether (70 + 30). Spraying agent: Saturated solution of Michler's thioketone in ethanol.

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REFERENCES

1. Stock, A. and Cucuel, F. *Naturwiss.* **22** (1934) 390.
2. Sjöstrand, B. *Anal. Chem.* **36** (1964) 814.
3. Westermark, T. *Kvicksilverfrågan i Sverige* (Stockholm 1965), 25 (1964 års naturresursutredning, Jordbruksdepartementet, Kvicksilverkonferensen 1965).
4. Polley, D. and Miller, V. L. *J. Agr. Food Chem.* **2** (1954) 1030.
5. Gage, J. C. *Analyst* **86** (1961) 457.
6. Uchida, M., Hirakawa, K. and Inoue, T. *Kumamoto Med. J.* **14** (1961) 181.
7. Schwarzenbach, G. and Schellenberg, M. *Helv. Chim. Acta* **48** (1965) 28.
8. Norén, K. and Westöö, G. *Vår föda* **19** (1967). *To be published.*

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