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Determination of Methylmercury Compounds in Foodstuffs

II. Determination of Methylmercury in Fish, Egg, Meat, and Liver

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The combined gas chromatographic and thin-layer chromatographic method for the identification and determination of methylmercury compounds in fish (Westöö [1]) has been modified in order to render it applicable to a wider range of foods. In animal foodstuffs methylmercury is probably to a great extent attached to thiol groups. When these foods are extracted according to Gage,[2] methylmercury chloride is formed and dissolves in the benzene together with varying amounts of thio compounds. Purification of the methylmercury in the benzene extracts of, *e.g.*, egg yolk or liver by extraction with aqueous alkali solution did not work, probably because the thio compounds were not volatile and could form alkali-insoluble methylmercury salts. Evidently methylmercury-S-compounds were reformed at high pH and prevented the formation of the water-soluble methylmercury hydroxide. Addition of excess mercuric ions, which expelled the methylmercury from the thio compounds, solved this clean-up problem. Samples of fish, egg-white, egg yolk, meat, and liver have been analysed with the modified method.

Extraction of the methylmercury into an aqueous phase was also possible with the aid of a water solution of cysteine. This led to a more rapid analytical procedure.

In the earlier method [1] for the identification and determination of methylmercury in fish by gas chromatography and thin-layer chromatography, the methylmercury was extracted with benzene from a homogenate of the fish acidified with hydrochloric acid (*cf.* Gage [2]). It was then taken up into ammonium hydroxide solution and finally reextracted into benzene after acidification with hydrochloric acid. The extraction with alkali was incomplete unless the benzene extract was previously concentrated by distillation. The distillation procedure was assumed to remove volatile thio compounds binding part of the methylmercury and preventing its uptake into ammonia. Any methylmercury attached to a sulphur atom of nonvolatile compounds giving rise to alkali-insoluble methylmercury salts at the purification stage would not be determined. In fish from Swedish lakes and the Baltic, with total

mercury contents above 0.15 mg/kg, 52–97 % (mean value 76 %) of the total mercury was recovered as methylmercury.

When small amounts of methylmercury dicyandiamide (<0.05 mg/kg) were added to meat, liver or egg yolk and analysed according to the above method, the methylmercury was completely lost in liver and egg yolk, and only partly recovered from meat. After addition of 10 mg/kg of methylmercury to meat or liver, most of it was recovered from meat, but only 5 % from liver. Such a failure of the procedure can be expected, if the methylmercury in the neutralized extracts from these special foodstuffs is firmly attached, exclusively or to a considerable extent, to thiol groups of non-volatile compounds, but only if the methylmercury salts formed are insoluble in alkali solutions.

Model experiments were therefore performed with methylmercury solutions containing excess methanethiol or thiophenol. As expected, shaking of benzene solutions of these compounds with 2 N ammonium or sodium hydroxide solutions left the methylmercury in the benzene layers.

Cysteine cannot be the disturbing factor in the foods, because its S-methylmercury derivative is alkali-soluble on account of the free carboxyl group.

Two ways were tried to bring about an extraction of the methylmercury into an aqueous phase: A) a binding of the disturbing thiols by excess mercuric ions and B) a synthesis of a water-soluble methylmercury-S-compound. A combination of these two ways is now being studied and will be published later.

A. ADDITION OF MERCURIC IONS

By adding excess mercuric chloride to the acidified homogenate of egg yolk, meat, or liver before the extraction with benzene, or by shaking the first benzene extract with a 5 % mercuric chloride solution, it was possible to extract mercuric ions with the benzene solution in amounts sufficient to bind the disturbing thio compounds at the clean-up stage. With any of these changes of the procedure, added methylmercury could be recovered to more than 90 %.

The question was now: is the methylmercury extracted from the acidified homogenates of the foods linked to the sulphur atoms of the thiols, or combined with the chloride ions? In the latter case the thiols must also be extracted by the benzene to be able to disturb the extraction with alkali solution in the absence of excess mercuric ions.

If the methylmercury is linked to the thiols already at the first extraction, this extraction is not imitated in the preparation of the calibration curve. However, in view of the large excess of hydrochloric acid and the small dissociation constants of thiols, it seems most likely that methylmercury is extracted as the chloride. The ratio $[CH_3HgCl]:[CH_3HgSR]$ in an aqueous phase has been estimated by using the constants given by Schwarzenbach and Schellenberg [3] for CH_3HgCl, $CH_3HgSCH_2CH_2OH$ and $HOCH_2CH_2SH$.

$$\frac{[CH_3HgCl]}{[CH_3Hg^+]\,[Cl^-]} = 10^{k_1} \qquad (I)$$

$$\frac{[CH_3HgSR]}{[CH_3Hg^+]\,[RS^-]} = 10^{k_2} \qquad (II)$$

$$\frac{[CH_3HgCl]}{[CH_3HgSR]} = \frac{[Cl^-]}{[RS^-]} \times 10^{k_1-k_2} \qquad (I, II)$$

$$\frac{[RSH]}{[H^+]\,[RS^-]} = 10^{k_3} \qquad (III)$$

$$\frac{[CH_3HgCl]}{[CH_3HgSR]} = \frac{[Cl^-][H^+] \times 10^{k_1-k_2+k_3}}{[RSH]} \qquad (I, II, III)$$

Assume $[RSH] < 10^{-4}$ and $[H^+] = [Cl^-] = 2$

$$\frac{[CH_3HgCl]}{[CH_3HgSR]} > \frac{2 \times 2 \times 10^{k_1-k_2+k_3}}{10^{-4}}$$

If $k_1 = 5.25$, $k_2 = 16.12$ and $k_3 = 9.52$

$$\frac{[CH_3HgCl]}{[CH_3HgSR]} > 4 \times 10^{2.65}$$

From the above it is evident that CH_3HgCl is likely to dominate heavily over CH_3HgSR in the aqueous phase at the low pH prevailing at the first extraction. Furthermore, the partition coefficient for methylmercury chloride in benzene-aqueous 2 N hydrochloric acid is > 5. The dissociation constants of the compounds in benzene are not known, but a noteworthy transition of methylmercury chloride into a methylmercury-S-compound seems unlikely in the presence of hydrochloric acid. Accordingly CH_3HgCl, not CH_3HgSR, should be partitioned at the first extraction, even though the methylmercury is probably attached to thiol groups at the low hydrogen and chloride ion concentrations in the untreated tissues.

The above conclusions were verified by experiments. When equal volumes of a benzene solution of methylmercury chloride (1 μg Hg/ml) and a water solution of cysteine hydrochloride (2 g/100 ml) were shaken with each other, the methylmercury went into the aqueous phase, evidently as a S-cysteine salt. When this aqueous phase was acidified with 1/5 of its volume of concentrated hydrochloric acid and again shaken with an equal volume of benzene, the main part of the methylmercury went into the benzene layer as methylmercury chloride (verified by thin-layer chromatography on silica gel).

Further evidence was obtained by adding excess sulphide and thiols to the methylmercury solution when preparing the calibration curve (according to the mercuric chloride procedure A in Table 1). The curve did not change, when 0.05 g of crystallized sodium sulphide, 1.2 g of cysteine hydrochloride, or 1.2 g of glutathione was added. Neither did the addition of 0.25 g of sodium

sulphide to the homogenates of 50 g samples of fish, meat, liver, or egg change the results (procedure A). Thus both theoretical estimations and model experiments were in accordance with an extraction of methylmercury chloride from the suspensions of the foods in hydrochloric acid.

The interference of thiols on the extraction of the benzene extract with ammonium hydroxide solution can also be estimated theoretically.

Divide equation $$\frac{[CH_3HgOH]}{[CH_3Hg^+]\ [OH^-]} = 10^{k_4} \quad \text{(IV)}$$

with equation II, and assume $[OH^-] = 0.1$, $[CH_3Hg_{total}] = 10^{-7}$, $[RS_{total}] = 10^{-6}$, $k_2 = 16.12$ and $k_4 = 9.37$

$$\because 10^{-6} > [RS^-] > 10^{-6} - 10^{-7}$$

$$\frac{[CH_3HgOH]}{[CH_3HgSR]} \simeq 10^{-1.7} \quad \text{(V)}$$

From equation V is seen that even at the above small RS^- concentration, CH_3HgOH is not likely to form from CH_3HgSR to any appreciable extent in the absence of excess mercuric ions.

Model experiments were performed with methylmercury chloride, thiophenol, and mercuric chloride solutions. The methylmercury was determined by several modifications of the original method (Table 1).

Table 1. Recovery of 5.00 μg of mercury as methylmercury from solutions containing 21.4 mg of thiophenol.

Procedure	Description of procedures	Methylmercury found μg of Hg	Methylmercury found %
Original	Original method [1] for 10 g of fish	0	0
A	Original method modified: first benzene extract shaken with 2 ml of 5 % $HgCl_2$ solution and washed with 5 ml of 6 N hydrochloric acid before the distillation	3.6	72
B	Procedure A modified: distillation omitted	4.5	90
C	Procedure A modified: washing with hydrochloric acid omitted	4.9	98
D	Procedure A modified: $HgCl_2$ added before the first extraction	0	0
E	Procedure C modified: $HgCl_2$ added before the first extraction	3.2	64
F	Original method modified: distillation omitted and the ammonium hydroxide solution containing 0.4 % of $HgCl_2$	4.8	96

From procedure F in Table 1 is evident that excess mercuric ions present at the extraction with ammonium hydroxide solution expelled the methylmercury from the thiol so that methylmercury hydroxide could be formed and migrate into the aqueous phase. From procedure C is seen that a benzene solution of thiophenol, when shaken with a 5 % mercuric chloride solution, dissolved mercuric ions in amounts large enough to take care of a considerable amount of thiol at the neutralization. The washing with hydrochloric acid (procedures A and D) removed part of the mercuric ions from the benzene extracts. Accordingly less thiol could be present without disturbing.

When the procedures A—F were applied to foods, an additional clean-up by chromatography through acid aluminium oxide before the extraction with ammonium hydroxide solution was carried out for egg yolk, meat, and liver.

The procedures B and F were tested with fish and egg-white, but gave low recoveries. Procedure F was tried also for liver, meat, and egg yolk. It was efficient only for meat. For liver and egg yolk a treatment with mercuric ions before the clean-up with aluminium oxide was necessary for good results. The mercuric ions probably prevented methylmercury-S-compounds to be reformed and adsorbed on the alumina.

With samples of fish, egg, meat, and liver, the procedures A and E gave an average recovery of 91—92 % of added methylmercury. Procedure C gave about 5 % higher recovery. Procedure D has not been tested.

The high recoveries in procedures A, C, and E compared to B or F, when applied to some foods, were not caused by a synthesis of methylmercury from mercuric ions at the distillation. Thus good recoveries were obtained when procedure B was modified by passing the benzene extracts of fish or egg-white through acid aluminium oxide before the treatment with ammonium hydroxide solution (procedure G, Table 2). The disturbing thio compounds were evidently removed, and the result was more than 90 % recovery — even

Table 2. Recovery of mercury as methylmercury from fish and egg-white.

Sample	Methyl-mercury content mg Hg/kg	Procedure	Methyl-mercury found mg Hg/kg	Recovery %
10 g of fish	0.42	A	0.39	93
—»—	0.42	B with aluminium oxide column before the clean-up with ammonium hydroxide solution (procedure G)	0.38	90
—»—	2.21	A	2.13	96
—»—	2.21	G	2.05	93
10 g of egg-white	0.121 (0.100 added)	A	0.121	100
—»—	0.121 (0.100 added)	G	0.121	100

though the extracts were not heated. Furthermore, when the concentrated benzene extracts in procedure A were diluted with benzene before the extraction with ammonia, the recoveries of methylmercury were low.

Because the procedures A, C, and E were successful for all the foods tested and could be used for egg-white and fish without a clean-up by chromatography, one of them was chosen for further control. A was preferred, because in some cases it gave cleaner extracts than C and E. The procedures C, E, or G might as well have been chosen.

The recovery of methylmercury added to foods when analysed according to procedure A was 91 ± 6 % (Table 3).

Table 3. Recovery of methylmercury added to foods when analysed by procedure A.

Foods	Sample g	μg of mercury/g of food, added as methylmercury dicyandiamide	μg of mercury/g of food as methylmercury compound found		Recovery %
			before the addition	after the addition	
Meat of chicken	10.00	0.700	0.003	0.61	87
» » »	10.00	0.500	0.002	0.46	92
» » »	10.00	0.500	0.003	0.49	97
» » »	10.00	0.200	0.003	0.196	97
» » »	50.0	0.100	0.005	0.094	89
» » »	50.0	0.100	0.017	0.114	97
Liver	10.00	0.700	0.000	0.61	87
»	10.00	0.500	0.002	0.45	90
»	10.00	0.200	0.005	0.204	100
»	10.00	0.100	0.000	0.102	102
»	50.0	0.100	0.012	0.104	92
»	50.0	0.100	0.002	0.088	86
»	50.0	0.060	0.002	0.051	82
»	50.0	0.020	0.000	0.018	90
Egg yolk	10.00	0.500	0.001	0.47	94
» »	10.00	0.500	0.001	0.42	84
» »	50.0	0.100	0.005	0.083	78
» »	50.0	0.100	0.005	0.087	82
Egg-white	150.0	0.100	0.0106	0.102	91
» »	150.0	0.0333	0.0106	0.0423	95
» »	150.0	0.0333	0.0166	0.0454	86
Muscle tissue of haddock	10.00	0.600	0.030	0.548	86
»	10.00	0.500	0.030	0.471	88
»	10.00	0.400	0.030	0.396	92
»	10.00	0.300	0.030	0.330	100
»	10.00	0.200	0.030	0.211	91
»	50.0	0.020	0.042	0.059	85
Muscle tissue of cod	50.0	0.100	0.040	0.129	89
»	50.0	0.060	0.040	0.098	97
		Mean value ± standard deviation			91 ± 6

B. EXTRACTION OF THE FIRST BENZENE EXTRACT WITH AQUEOUS SOLUTIONS OF SODIUM SULPHIDE, 2-MERCAPTOETHANOL, OR CYSTEINE

A simpler method was first elaborated for fish and egg-white: From the first benzene extract methylmercury could be quantitatively extracted by an aqueous solution of sodium sulphide, 2-mercaptoethanol, or cysteine. After acidification with excess hydrochloric acid, the methylmercury could in all cases be reextracted into benzene. However, some hydrogen sulphide and sulphur also went into the benzene layer in the first case and disturbed the gas chromatography. Gas chromatography disturbances also occurred with 2-mercaptoethanol. In the cysteine case good results (with 0.1—1.0 % cysteine hydrochloride solutions identical with those obtained using procedure A) were obtained with fish and egg-white.

Also meat and egg yolk could be analysed, if the cysteine concentration was not too low and the clean-up on aluminium oxide was omitted (*cf.* p. 1794). With 50 g samples of egg yolk lower yields (80—90 %) were obtained than with fish, egg-white and meat (> 90 %).

The elimination of the distillation step in this procedure saves considerable time.

Liver gave lower yields than egg yolk and could not be analysed using one, single extraction with 1 % cysteine acetate solution. Instead of using repeated extractions, a combination of the mercuric chloride and cysteine procedures is now tried.

RESULTS

Table 4 shows some methylmercury contents found in foods, and Figs. 1 and 2 gas chromatograms of foods.

The meat and liver samples in Table 4 have been chosen because of their exceptionally high mercury contents. The other samples are ordinary Swedish food samples. All the foodstuffs contained high percentages of methylmercury

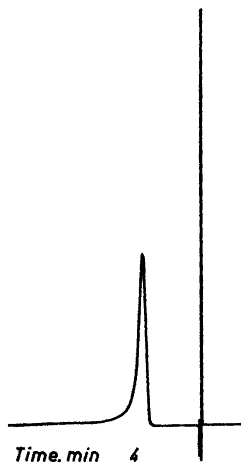

Fig. 1. Gas chromatogram of a purified cod extract (10 g sample).

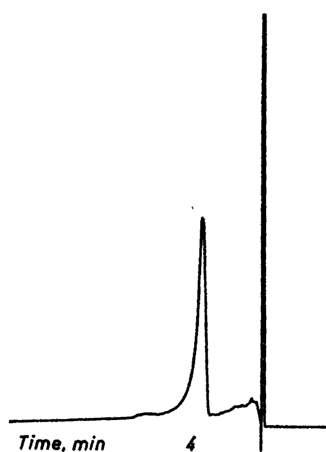

Fig. 2. Gas chromatogram of a purified fillet of beef extract (50 g sample).

Table 4. Some methylmercury contents in foods, analysed by procedure A.

Foods	μg of total mercury/g of food	Methylmercury in foods mg Hg/kg	% of total Hg
Meat (ox)	0.074	0.068	92
» (hen)	0.051	0.037	73
» »	0.023	0.017	74
Liver (pig)	0.130	0.095	73
» »	0.140	0.095	68
» »	0.096	0.075	78
Egg yolk	0.010	0.005	50
» »	0.010	0.009	90
Egg white	0.023	0.020	87
» »	0.025	0.019	76
» »	0.012	0.011	92
» »	0.025	0.024	96
» »	0.012	0.011	92
Muscle tissue of perch	0.75	0.70	93
» » » »	0.22	0.20	91
» » » »	0.30	0.25	83
» » » »	0.42	0.38	90
» » » »	0.29	0.25	86
» » » »	3.25	2.99	92
» » » »	3.10	2.81	91
Muscle tissue of pike	3.35	3.11	93
» » » »	2.67	2.57	96
» » » »	0.68	0.60	88
» » » »	1.90	1.81	95
» » » »	0.56	0.55	98
» » » »	0.79	0.72	91
Muscle tissue of haddock	0.052	0.043	83
» » » »	0.033	0.025	76
Muscle tissue of cod	0.036	0.028	78
» » » »	0.026	0.022	85

referred to the total mercury. The lowest methylmercury percentage (50 %) was found in a sample of egg yolk.

In samples of fish with mercury contents above 0.15 mg/kg an average of 16 % more of the mercury was recovered as methylmercury with the new method (procedure A) than with the method described earlier.[1] For fish with low mercury contents, *e.g.* marine fish and salmon, much lower methylmercury concentrations were found with the old method, indicating that larger proportions of the methylmercury were attached to non-volatile thio compounds (Norén and Westöö [4]) forming alkali-insoluble methylmercury salts at the clean-up.

In Swedish fresh-water fish (perch, pike, pike-perch, eelpout, whitefish) an average of 92 % of the total mercury content was recovered as methylmercury with the mercuric chloride procedure A.[4] In marine fish (haddock, cod, plaice) an average of 82 % was found as methylmercury.[4] With this method the recovery of added methylmercury was 91 % (Table 3). Therefore, in the studied samples of fish from the Swedish coast of the Baltic and from Swedish

lakes and rivers practically all the mercury was present in the form of methylmercury. This was the case also for fish with very high mercury contents (> 2 mg Hg/kg) caught downstream of factories, which until a few years earlier had used phenylmercury compounds or were still using inorganic mercury compounds.[4]

About 200 samples of fish have been analysed both by the mercuric chloride and the cysteine procedures.[5] So far the two methods have given results identical within the methodical errors, when applied to samples of pike, *Esox lucius*, bream, *Abramis brama*, pike-perch, *Lucioperca sandra*, eelpout, *Lota vulgaris*, perch, *Perca fluviatilis*, eel, *Anguilla vulgaris*, plaice, *Pleuronectes platessa*, whitefish, *Coregonus lavaretus* and *Coregonus albula*, char, *Salmo salvelinus*, salmon-trout, *Salmo trutta*, and cod, *Gadus morrhua*.

EXPERIMENTAL

Purity of chemicals. See Westöö.[1] When benzene is purified by distillation, protect the distillate from taking up impurities from the air by a short tube with silica gel. Control the purity of the hexane and benzene used by gas chromatography. The benzene, even after concentration from 150 ml to 5 ml, should not give rise to any peaks except the front peak.

Cleaning of glassware. Clean all glassware first by ordinary laboratory procedures and then by shaking with 1 N ammonium hydroxide solution, with deionized water, and with ethanol.

Mercuric chloride solution. Dissolve 50 g of mercuric chloride and 170 ml of concentrated hydrochloric acid in water, and make up to 1000 ml with water. Extract any methylmercury or other impurities present by shaking four times with 500 ml of benzene for 3 min.

Purify 10 ml of the fourth benzene extract by extraction with ammonium hydroxide solution, acidification with hydrochloric acid and re-extraction into benzene as described below. Gas chromatograph the benzene solution. If a methylmercury peak is found, repeat the extraction of the mercuric chloride solution until the test for methylmercury is satisfactory.

Aluminium oxide for column chromatography. Heat acid aluminium oxide in an oven at 800° for 4 h. After cooling, mix the oxide well with 5 % of water by shaking and rotating for 2 h in a sealed flask.

Ammonium hydroxide-sodium sulphate solution. Add a solution of 150 g of anhydrous sodium sulphate in 600 ml of water to 165 ml of concentrated ammonium hydroxide solution, and dilute to 1 l with water.

Cysteine acetate solution 1 %. Dissolve 1.00 g of cysteine hydrochloride (1 H_2O), 0.775 g of sodium acetate (3 H_2O) and 12.5 g of anhydrous sodium sulphate in water and make up to 100 ml.

Determination of methylmercury in fish, egg-white, egg yolk, meat, and liver (mercuric chloride modification A and C)

A) *Samples containing more than 0.1 mg of total mercury/kg.* Homogenize 10.0 g of the sample with water, and transfer the homogenate quantitatively to a 500 ml separatory funnel. A total of 55 ml of water is used for these procedures. Add concentrated hydrochloric acid (14 ml) and mix. Add benzene (70.0 ml), and shake the mixture vigorously for 5 min. Centrifuge and then shake 55—60 ml of the clear benzene extract for 5 min in a separatory funnel with 2.0 ml of purified, 5 % solution of mercuric chloride in dilute hydrochloric acid. Either centrifuge and discard the aqueous phase and the precipitate (procedure C), or discard the aqueous phase and as much of the precipitate as possible

without centrifugation, shake the benzene layer with 5 ml of hydrochloric acid (1+5) for 1 min, and finally centrifuge and discard the aqueous phase (procedure A).

For extracts other than of fish and egg-white, pass 50.0 ml of the benzene phase through a column with aluminium oxide (a slurry of 35 g of aluminium oxide in benzene should be poured into a column (Ø 20 mm) and allowed to settle under flow). Elute with 75 ml of benzene. Transfer the eluate (for fish and egg-white 50.0 ml of the extract) to a 200 ml (150 ml) flask containing 5 ml of a 0.1 N heptane solution of acetic acid. Distil off the benzene at a moderate rate, until 5 ml remain in the flask. For fish with high fat content, 7 ml may remain. The space saving Soxhlet apparatus was used for the distillation, the extraction part acting as receiver. Cool the apparatus before removing the flask. Transfer the heptane solution quantitatively to a separatory funnel with the aid of 5 ml of hexane.

If the analysis has to be interrupted, it should be done after the first centrifugation or between the second centrifugation 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. Transfer 5.00 ml of the clear, aqueous phase (centrifuge, if necessary) to a 60 ml 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 and, after concentration, to thin-layer chromatography.

B) *Samples containing up to 0.1 mg of total mercury/kg.* Take 50.0 g of the sample and follow the procedure described above, only multiplying all volumes by five. This should be done up to and including the second centrifugation. For samples other than fish and egg-white transfer 250.0 ml of the benzene extract to a 500 ml flask. Distil off the benzene at a moderate rate until 25 ml remain. Pass these 25 ml through a 35 g aluminium oxide column followed by 15 ml of benzene, used for rinsing the flask. Elute with 85 ml of benzene. Transfer the eluate (for fish and egg-white 250 ml of the extract) to a 250 ml (500 ml) flask containing 5 ml of a 0.1 N heptane solution of acetic acid, and proceed according to the description for 10 g samples, without changing the volumes.

Determination of methylmercury in fish, egg, and meat (cysteine modification)

A) *Samples containing more than 0.1 mg of total mercury/kg.* Homogenize 10.0 g of the sample with water and transfer the homogenate quantitatively to a 500 ml separatory funnel. A total of 55 ml of water is used for these procedures. Add concentrated hydrochloric acid (14 ml) and mix. Add benzene (70 ml), and shake the mixture vigorously for 5 min. Centrifuge. Transfer 50.0 ml of the extract to a separatory funnel. Add 6.00 ml of a 1.0 % solution of cysteine acetate saturated with sodium sulphate and shake vigorously for 2 min. Transfer 5.00 ml of the clear, aqueous phase (a disturbing precipitate in the water layer can easily be removed by centrifugation, stirring and recentrifugation) to a 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 and, after concentration, to thin-layer chromatography.

B) *Samples containing up to 0.1 mg of total mercury/kg.* Take 50.0 g of the sample and follow the procedure described above, only multiplying all volumes by five. This should be done up to and including the first centrifugation. Transfer 250.0 ml of the extract to a separatory funnel, and proceed according to the description for 10 g samples, without changing the volumes.

Gas chromatography and thin-layer chromatography. See the description for determination of methylmercury in fish.[1]

Standard curve. The standard curve is a straight line through the origin, if the areas of the peaks are plotted.

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

Determination of total mercury. The contents of total mercury have been determined by activation analysis [6,7,8] at the Isotope Techniques Laboratory in Stockholm.

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