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Identification and Determination of Methylmercury Compounds in Fish Using Combination Gas Chromatograph-Mass Spectrometer

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The identification and determination of methylmercury compounds in fish by the use of a combined gas chromatograph-mass spectrometer and a standard gas chromatograph with an electron capture detector are described. Samples containing 0.15–3.2 mg Hg/kg fish flesh were studied. The results of the two methods are compared.

Methylmercury compounds in biological material have been studied by different methods. Some of these methods were designed for mercury levels higher than those usually found in foods. When gas chromatography and thin-layer chromatography were used, this led to more rapid analytical procedures [1-5] and the required concentration of methylmercury compounds was reduced to 1 μg Hg/kg. In several gas chromatographic methods the methylmercury was extracted with benzene as methylmercury chloride from a homogenate of the sample acidified with hydrochloric acid and was purified by reextraction into an aqueous solution of ammonia, cysteine or glutathione. Finally, after acidification with hydrochloric acid, it was again transferred into benzene. Added methylmercury could be recovered to more than 90 % from, *e.g.*, fish, meat, liver, kidney, egg with the procedures described by Westöö.[1-3]

This paper will describe how a combined instrument, gas chromatograph-mass spectrometer, is used for identification and determination of the methylmercury compounds in extracts from fish.

Table 1 shows the results obtained by GLC with electron capture and mass spectrometric detection for 8 samples of fish. For 7 of the fish the total mercury

Table 1. Comparison between results for mercury levels in fish flesh, determined by combination gas chromatograph-mass spectrometer, gas chromatograph with electron capture detector, and activation analysis.

	Methylmercury, mg Hg/kg fish flesh		Total Hg, mg/kg fish flesh
	GLC-mass spectrometric measurement of $^{202}Hg^{+}$	Gas chromatography with electron capture detector	Activation analysis
Pike 1	0.14	0.17	Not determined
Pike 2	0.55	0.54	0.59
Pike 3	2.53	2.57	2.70
Pike 4	0.43	0.41	0.39
Pike 5	0.49	0.55	0.54
Pike 6	0.75	0.66	0.63
Pike 7	0.72	0.70	0.66
Perch 8	3.19	3.29	3.12

was determined by activation analysis at the Isotope Techniques Laboratory, Stockholm. The results are calculated on the bases of reference compounds and the three methods coincide with a deflection of less than ± 10 % from the average value.

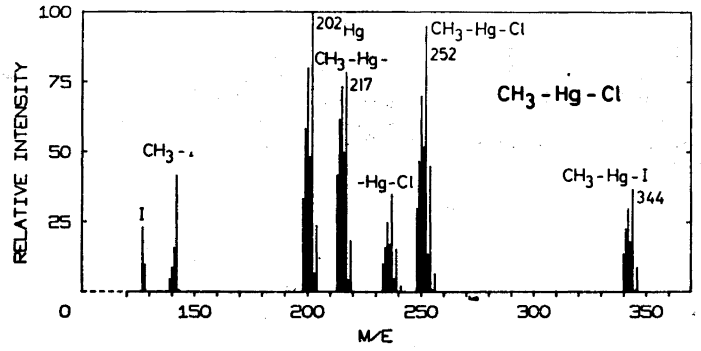

Fig. 1. Mass spectrum of methylmercury chloride obtained by the combined GC-MS instrument.

Fig. 1 shows the mass spectrum of the purified and distilled extracts of methylmercury compounds from fish obtained by the combined GC-MS instrument. The spectrum is similar to the reference mass spectrum of methylmercury chloride analysed by the direct inlet. Both spectra indicate the existence of methylmercury iodide, and no separation could be observed between the chloride and iodide on the total ion current recorder using a column packed with Carbowax 20 M.

Nishi and Horimoto [6] supposed that thermal degradation of alkylmercury compounds occurs if a stainless steel column is used, packed with 5 % polydiethyleneglycol succinate on Chromosorb W, 60 – 80 mesh, for all quantities

of sample injected. For glass columns they observed the same effect only when the quantity of the injected sample was below 10^{-8} g. They assume that in the compound RHgX (X = I, Br, Cl, CH_3CO_2, SO_4, OH, dithizonate) the X group was destroyed and another compound was formed. In our experiments with the Carbowax 20 M in glass column we have injected CH_3HgCl in quantities from 5 to 2000 ng ($5 \times 10^{-9} - 2 \times 10^{-6}$ g). These mass spectra indicated also that the CH_3HgCl was mixed with CH_3HgI.

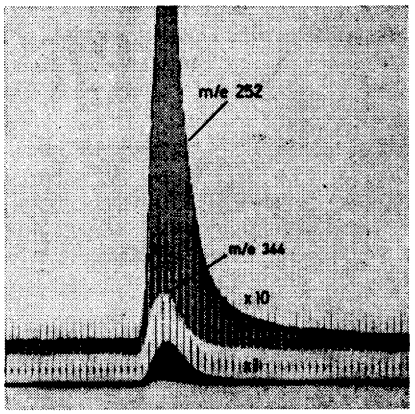

Fig. 2. Continuous recording of *m/e* 252 (CH_3HgCl) and 344 (CH_3HgI) during elution of methylmercury compounds.

Since GLC separation was not obtained, the AVA unit was used to measure and compare the intensities of $CH_3{}^{202}Hg^{35}Cl$ and $CH_3{}^{200}Hg^{37}Cl$ at mass number 252 and $CH_3{}^{202}Hg^{127}I$ at mass number 344. Fig. 2 shows a continuous recording of *m/e* 252 for CH_3HgCl and *m/e* 344 CH_3HgI from an injected amount of 300 ng of CH_3HgCl into the GLC-MS instrument. A slight difference in retention time can be observed between these compounds for all injections of 5 to 2000 ng.

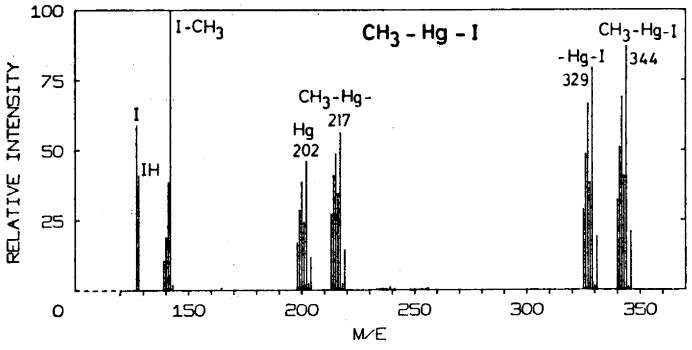

Fig. 3. Mass spectrum of methylmercury iodide obtained by using the direct probe inlet of the mass spectrometer.

It was found that if pure CH_3HgI was injected in a newly packed Carbowax column, the mass spectrum showed a mixture of CH_3HgCl and CH_3HgI. Since the purity of the injected iodide compound was tested by using the direct probe inlet system and found to be pure (see Fig. 3) the CH_3HgCl found in the mass spectrum must have been caused by a reaction in the column where a substitution of Cl for I occurred.

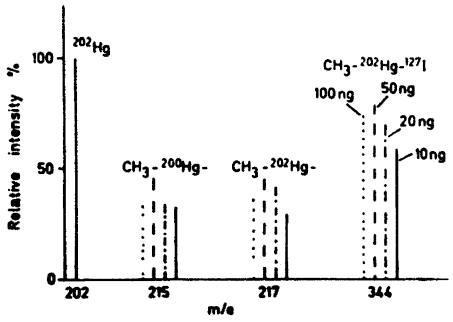

Fig. 4. The relative intensities of *m/e* 344, 217, 215, and 202 in the mass spectrum of methylmercury iodide when different amounts of the compound were injected.

Fig. 4 shows that the relative intensity of *m/e* 344 does not change radically as compared to *m/e* 202, when different amounts of pure CH_3HgI is injected into the column. The mass range from 202 to 344 is too extensive to be used by the modified AVA unit, and for this reason mass spectra were taken. The mass range 127 – 346 was studied and the structures of the ions found are given in Table 2.

The rearranged ions CH_3I^+ and HI^+ are probably formed by the ionization process. It is of interest to note that the *m/e* 128 corresponding to HI^+ is much more intense when the sample is introduced by the direct probe than when using the combination GLC-MS. The reason for this effect must be that the CH_3HgI compound is mixed with helium of about 10^{-4} mm Hg when using GLC-MS. The probability that $CH_3HgI^+ \rightarrow CH_2 = Hg + HI^+$ occurs is much less under such circumstances than when the ionization chamber is at high vacuum (5×10^{-6} mm Hg).

The combined gas chromatograph-mass spectrometer can with advantage be used to measure the amount of methylmercury halogenides and, by using the GLC-MS instrument in combination with the AVA unit, it has about the same sensitivity as the electron capture detector. The results show that the mass spectrometer provides a positive identification and a good quantitative determination of the methylmercury compounds studied. If pure methylmercury iodide is injected into the column, the methylmercury chloride is also shown on the mass spectra, which means that the iodide is replaced by chloride on the column. With the exception of this exchange no destruction or transformation to other molecules of the RHgX (X = I, Br, Cl) compounds were observed.

Table 2. Structure of the ions found in the mass range 127–346.

m/e	Ion	*m/e*	Ion
127	I^+	239	$^{202}Hg-^{37}Cl^+$
128	$*IH^+$		$^{204}Hg-^{35}Cl^+$
139	$*IC^+$	241	$^{204}Hg-^{37}Cl^+$
140	ICH^+		
141	ICH_2^+	248	$CH_3-^{198}Hg-^{35}Cl^+$
142	ICH_3^+	249	$CH_3-^{199}Hg-^{35}Cl^+$
		250	$CH_3-^{198}Hg-^{37}Cl^+$
198	$^{198}Hg^+$		$CH_3-^{200}Hg-^{35}Cl^+$
199	$^{199}Hg^+$	251	$CH_3-^{199}Hg-^{37}Cl^+$
200	$^{200}Hg^+$		$CH_3-^{201}Hg-^{35}Cl^+$
201	$^{201}Hg^+$	252	$CH_3-^{200}Hg-^{37}Cl^+$
202	$^{202}Hg^+$		$CH_3-^{202}Hg-^{35}Cl^+$
204	$^{204}Hg^+$	253	$CH_3-^{201}Hg-^{37}Cl^+$
		254	$CH_3-^{202}Hg-^{37}Cl^+$
213	$^{198}Hg-CH_3^+$		$CH_3-^{204}Hg-^{35}Cl^+$
214	$^{199}Hg-CH_3^+$	256	$CH_3-^{204}Hg-^{37}Cl^+$
215	$^{200}Hg-CH_3^+$		
216	$^{201}Hg-CH_3^+$	325	$^{198}Hg-I^+$
217	$^{202}Hg-CH_3^+$	326	$^{199}Hg-I^+$
219	$^{204}Hg-CH_3^+$	327	$^{200}Hg-I^+$
		328	$^{201}Hg-I^+$
233	$^{198}Hg-^{35}Cl^+$	329	$^{202}Hg-I^+$
234	$^{199}Hg-^{35}Cl^+$	331	$^{204}Hg-I^+$
235	$^{198}Hg-^{37}Cl^+$		
	$^{200}Hg-^{35}Cl^+$	340	$CH_3-^{198}Hg-I^+$
236	$^{199}Hg-^{37}Cl^+$	341	$CH_3-^{199}Hg-I^+$
	$^{201}Hg-^{35}Cl^+$	342	$CH_3-^{200}Hg-I^+$
237	$^{200}Hg-^{37}Cl^+$	343	$CH_3-^{201}Hg-I^+$
	$^{202}Hg-^{35}Cl^+$	344	$CH_3-^{202}Hg-I^+$
238	$^{201}Hg-^{37}Cl^+$	346	$CH_3-^{204}Hg-I^+$

* The presence of ^{13}C and ^{2}H was disregarded.

EXPERIMENTAL

Preparation and purification of the extracts of methylmercury compounds

1. The extracts of methylmercury from about 50 samples of fish, purified for GLC according to Westöö,[1] were combined and most of the benzene solvent distilled off. The concentrated solution was again purified by extraction with ammonium hydroxide solution, acidification with hydrochloric acid and reextraction with benzene. Heptane (0.3 ml) was added, and the solution was distilled to almost dryness. The distillate was analysed.

2. Purified methylmercury extracts from fish have also been concentrated, using extraction with a small volume of cysteine solution, acidification with hydrochloric acid, and reextraction into benzene.

3. Finally, the purified extracts of methylmercury used in the GLC analysis of methylmercury according to Westöö [2] (cysteine procedure) were analysed without further purification or concentration (Table 1).

All the samples were submitted to a combined gas chromatograph-mass spectrometer LKB 9000, and both the direct and gas chromatograph inlets were used for these analyses. The samples were in both cases compared with equivalent amounts of methylmercury standards.

Data of the combination gas chromatograph-mass spectrometer for analysis of methylmercury compounds

Gas chromatograph	
Glass column	3 m × 2.5 mm
Liquid phase	Carbowax 20 M
Carrier	Chromosorb W, 80—100 mesh
Carrier gas and flow rate	Helium 30 ml/min
Column temperature	140°C
Injection port temperature	200°C
Molecular separator temperature	200°C
Mass spectrometer	
Accelerating voltage	3500 V
Electron energy	70 eV
Electron current	60 μA

For extract concentrations of 1 ng Hg/μl or lower, 10 μl of the solution were injected into the column of the combination instrument.

In the first two methods the distillate, resp. solutions, were subjected to the standard combined instrument. In the third method a quantitative mass spectrometric analysis of methylmercury was performed using the combined instrument supplied with an accelerating voltage alternator (AVA unit).[7,8]

While eluents from the column pass the ionization chamber in the mass spectrometer, the galvanometers in the UV-recorder for ion current detection monitor 1, 2, or 3 chosen mass numbers. If the peak heights of these masses are of the same relative intensities as found for a known compound, then the possibility that the sample contains this compound is very great. In the investigation of methylmercury, the mass numbers 202, 215, and 217 were chosen corresponding to the ion fragments ^{202}Hg, $^{200}HgCH_3$, and $^{202}HgCH_3$. However, when small quantities of sample were injected, only the mercury peak at ^{202}Hg was studied. In order to measure the molecular ions at *m/e* 252 and *m/e* 344 of the chloride and iodide compounds, it was necessary to modify the AVA unit by increasing the drop of the high voltage to 30 % (standard 10 %).

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REFERENCES

1. Westöö, G. *Acta Chem. Scand.* **20** (1966) 2131.
2. Westöö, G. *Acta Chem. Scand.* **21** (1967) 1790.
3. Westöö, G. *Acta Chem. Scand.* **22** (1968) 2277.
4. Kitamura, S., Sumino, K. and Hayakawa, K. *Igaku To Seibutsugaku* **72** (1966) 274.
5. Sumino, K. *Kobe J. Med. Sci.* **14** (1968) 115.
6. Nishi, S. and Horimoto, Y. *Bunseki Kagaku* **17** (1968) 75.
7. Sweeley, C. C., Elliott, W. H., Fries, I. and Ryhage, R. *Anal. Chem.* **38** (1966) 1549.
8. Ryhage, R. and Wikström, S. *Science Tools* **14** (1967) 1.

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