umn. The eluent from this column was concentrated to 1 ml in a 250-ml Kuderna-Danish apparatus. For further concentration, it was transferred to a Chromaflex sample tube (K-422560) and carefully brought to 100 μl under a gentle stream of nitrogen. A proximate concentration of this extract was 10 ng/μl. Ten microliters of the extract was injected on the GC–MS column.

Figure 5 shows the analysis performed using a chemical ionization source with methane as the reagent gas. Chemical ionization offered the advantages of an intense protonated molecular ion 75^+ and a simpler fragmentation pattern. Specific ion monitor of the suspected 75^+ peak in the chromatogram established its retention time with that of the DMNA standard. In this case, the 6000 Interactive GC–MS data system subtracted the background caused by an interfering peak and recalled the spectral information stored on a high speed disk.

In view of the above findings, it can be concluded that less than 10 ppb of *N*-nitrosodimethylamine was found in cold-smoked sablefish, that this value was not increased when nitrite was used up to 550 ppm, and that no other nitrosamines were identified.

ACKNOWLEDGMENT

The authors wish to thank Judith Krzynowek and Jim Knight of Finnigan Corporation for analyzing the samples by GC–MS.

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Received for review January 20, 1975. Accepted April 11, 1975.

Rapid Method for the Determination of Mercury in Fish Tissue by Atomic Absorption Spectroscopy

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A simple, rapid method for the determination of mercury in fish tissue by flameless atomic absorption spectroscopy is described. Digestion of the sample is done with sulfuric–nitric–hydrochloric acids. A digestion time of 15 min is sufficient to convert the mercury to an inorganic form so that it can be reduced to the elemental state and determined by flameless atomic absorption spectroscopy. Results using this method compare very favorably with those obtained using the Food and Drug Administration Official First Action Method for the determination of mercury in fish.

Several methods have been used for the decomposition of fish samples for mercury analysis (Holak et al., 1972; Malaiyandi and Barrette, 1970; Munns and Holland, 1971; Saha and Lea, 1972; Uthe et al., 1970). Problems associated with prevention of mercury losses, use of potentially explosive reagents, length of digestion time, reproducibility, excessive fumes, interference of fat in the tissue samples, and varying mercury values obtained when varying sample weights are used showed a pressing need for a convenient method to determine microquantities of mercury in biological materials. This paper describes a simple method for determination of mercury in fish tissue using a mixture of acids for digesting the samples. It also describes the optimum conditions for the digestion. The total mercury content in the digested samples is determined by the flameless atomic absorption spectroscopy technique.

EXPERIMENTAL SECTION

Apparatus. The equipment for this method is the same as that utilized in the Food and Drug Administration Official First Action Method for the determination of mercury in fish (Munns and Holland, 1971).

Reagents used included: (a) hydrochloric acid, 6 *N;* (b) sulfuric acid, 6 and 1 *N;* (c) dilute nitric–sulfuric acid mixture, 1 part nitric, 9 parts sulfuric, in 8 parts water; (d) reducing solution (in a 1000-ml volumetric flask containing 600 ml of 6 *N* sulfuric acid, 30 g of sodium chloride, 30 g of hydroxylamine sulfate, and 50 g of stannous chloride were dissolved; solution was diluted to mark with distilled water); (e) mercury standard solution: (1) 1000 μg/ml, 0.1354 g of mercuric chloride ($HgCl_2$) was dissolved in 100.0 ml of water; (2) 1 μg/ml, 1 μg/ml standard in 1 *N* sulfuric acid from standard solution was prepared daily.

Determination of Mercury. About 1 g of wet fish sample (about 400 mg of dry fish sample) was weighed into a digestion flask. To this was added 10 ml of dilute nitric–sulfuric acid mixture and 3–4 boiling chips. Condenser was connected and cold water circulated through it. To this was added 1 ml of 6 *N* HCl through condenser and gentle heat was applied for 15 min (about 10 min for dissolution of the sample and 5 min of gentle boil). The heat was removed and the contents were allowed to stand 15 min. Distilled water (90 ml) was added through the condenser while the contents of the flask were swirled. The flask was disconnected from the condenser and cooled to room temperature. Residual fumes from the flask were blown off using a

U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Pacific Utilization Research Center, Seattle, Washington 98112.

Table I. Effect of Digestion Time upon Recovery of Mercury from Halibut and Sablefish Flesh

Digestion time, min	Mercury found, μg/g	
	Halibut	Sablefish
2	0.66, 0.65	0.51, 0.51
5	0.69, 0.67	0.51, 0.52
10	0.67, 0.68	0.51, 0.51
15	0.67, 0.67	0.53, 0.52
30	0.64, 0.63	0.51, 0.50
60	0.64, 0.66	0.52, 0.52

Table II. Comparison of Mercury Recovered from Halibut, Sablefish, and FPC[a]

Sample	Proposed method, ppm		FDA method, ppm
	Presence of HCl	Absence of HCl	
Halibut	0.67, 0.66	0.63, 0.50	0.65, 0.66
Sablefish	0.53, 0.52	0.53, 0.53	0.48, 0.47
FPC No. 1	0.44, 0.44	0.26, 0.29	0.42, 0.42
FPC No. 2	0.63, 0.63	0.43, 0.34	0.64, 0.66

[a] Digested with dilute nitric-sulfuric acid mixture with and without hydrochloric acid, as compared to mercury recovered from similar samples using the FDA method.

Table III. Effect of Sample Weight upon Recovery of Mercury from Halibut and Sablefish and from Fish Protein Concentrate (FPC)

Sample wt, g	Mercury found, μg/g			
	Halibut	Sablefish	Herring FPC	Hake FPC
0.10			0.39, 0.39	0.58, 0.62
0.30			0.43, 0.43	0.60, 0.61
0.50	0.64, 0.66	0.50, 0.50	0.46, 0.42	0.63, 0.58
0.75				Foaming
1.00	0.65, 0.64	0.49, 0.50	0.44, 0.41	Foaming
1.50	0.64, 0.65	0.50, 0.51		
2.00	0.64, 0.63	0.51, 0.51		
3.00	0.61, 0.61	0.46, 0.44		

Table IV. Recovery of Mercury Added to Sablefish and Halibut Using the Proposed Method

Mercury added, ppm	Mercury found,[a] ppm		Recovery, %	
	Sablefish	Halibut	Sablefish	Halibut
0.3	0.304	0.301	101	100
	0.301	0.299	100	100
0.6	0.589	0.579	98	97
	0.594	0.582	99	97
0.9	0.889	0.879	99	98
	0.872	0.864	97	96
1.2	1.178	1.162	98	97
	1.191	1.175	99	98

[a] Mercury values of 0.46 and 0.55 ppm initially found in sablefish and halibut flesh, respectively, had been subtracted.

Table V. Effect of Fish Oil upon Recovery of Mercury from Fish Protein Concentrate

Fish protein concentrate, mg	Added menhaden oil, mg	Mercury content, ppm
250	0	0.30, 0.27
250	60	0.29, 0.29
250	125	0.28, 0.27
250	200	0.29, 0.28
250	250	0.28, 0.27
250	350	0.27,

Table VI. Mercury Content of Several Samples of Fish Muscle and Hair as Determined by the Proposed and FDA Methods

Tissue	Mercury found, μg/g	
	Proposed method	FDA method
Salmon	0.11, 0.11	0.09, 0.11
	0.03, 0.03	0.03, 0.02
Green-striped rockfish	0.75, 0.77	0.75, 0.75
	0.18, 0.17	0.16, 0.16
Halibut	0.65, 0.64	0.64, 0.66
	0.87, 0.88	0.84, 0.86
Sablefish	0.48, 0.50	0.47, 0.49
Tuna	0.29, 0.29	0.30, 0.31
	0.26, 0.25	0.26, 0.24
	0.25, 0.27	0.23, 0.24
Fish protein concentrate	0.41, 0.42	0.42, 0.42
	0.65, 0.66	0.66, 0.64
Oysters	0.03, 0.03	0.04, 0.04
Ladyfish	0.98, 1.00	0.92, 0.96
	0.47, 0.49	0.46, 0.45
Human hair	4.33	4.25

Table VII. Collaborative Results for Analysis of Mercury in Several Samples of Fish Tissue

Sample no.	Proposed method, ppm	Other methods	
		Mean ± std. dev., ppm	Ranges, ppm
1	0.26, 0.27	0.28 ± 0.05	0.19–0.41
2	0.56, 0.59	0.63 ± 0.09	0.46–0.81
3	0.79, 0.89	0.93 ± 0.11	0.73–1.18
4	0.45, 0.47	0.52 ± 0.08	0.33–0.67
5	0.42, 0.43	0.45 ± 0.06	0.30–0.55
6	0.34, 0.33	0.39 ± 0.15	0.20–0.89
7	0.71, 0.73	0.77 ± 0.11	0.51–0.98
8	0.49, 0.50	0.54 ± 0.08	0.34–0.74
9	0.56, 0.55	0.61 ± 0.10	0.49–0.95
10	0.34, 0.34	0.40 ± 0.10	0.30–0.67
11	1.02, 1.04	1.13 ± 0.18	0.92–1.66
12	0.42, 0.44	0.49 ± 0.08	0.38–0.73

gentle stream of air. Reducing solution (20 ml) was added to the digest, and immediately the gas adapter was connected and the solution aerated for about 2.5 min (adjust aeration time to obtain maximum absorption). The absorption of metallic mercury was measured using an atomic absorption spectrophotometer set at 253.7 nm. The gas

Table VIII. Mercury Values Found in Halibut and Sablefish Flesh and Fish Protein Concentrate Analyzed by the Proposed Method Separately by Four Chemists at the Research Center and at a Commercial Laboratory

	ppm				
Species	Chemist 1	Chemist 2	Chemist 3	Chemist 4	Mean ± std. dev.
Halibut	0.66, 0.67	0.67, 0.66	0.69, 0.67	0.77, 0.69	0.69 ± 0.04
Sablefish	0.52, 0.53	0.56, 0.59	0.55, 0.56	0.47, 0.56	0.54 ± 0.04
FPC	0.65, 0.66	0.68, 0.70	0.70, 0.70	0.66, 0.65	0.68 ± 0.02

adapter was disconnected from the flask and this system flushed before analyzing the next sample (Munns and Holland, 1971).

A blank and a standard curve were prepared by adding 0, 0.1, 0.3, 0.5, and 1.0 μg of mercury to a series of digestion flasks containing 10 ml of nitric–sulfuric acid, 1 ml of 6 *N* HCl, and enough water for a total volume of 101 ml. Analysis of standards was carried out the same way as the samples. Absorbance reading was plotted vs. micrograms of mercury. The micrograms of mercury in the sample were determined from the curve: parts per million of mercury = micrograms of Hg/weight of sample (grams).

RESULTS AND DISCUSSION

This digestion method was evaluated in terms of the following criteria: accuracy of the data, reproducibility from day to day, safety, speed, and versatility (high fat, low fat; dry and wet samples). For evaluation, we used halibut and sablefish flesh and fish protein concentrate (FPC) previously analyzed for mercury by the FDA method. This method was also utilized to determine the mercury present in several other samples some of which were used in a collaborative study with several other laboratories.

Effect of Digestion Time. Time of digestion (after dissolution of samples) was varied between 2 and 60 min. The results (Table I) showed the mercury values to be practically the same whether a 2-, 5-, 10-, 15-, 30-, or 60-min digestion time was utilized. This indicates that available mercury was converted to reducible form even after 2 min of gentle boil. A total digestion time of 15 min (about 10 min for sample dissolution followed by 5 min of gentle boil) was chosen as the digestion time in this work.

Effect of the Various Acids. The fish samples were digested with a dilute nitric–sulfuric acid mixture with and without hydrochloric acid. Results of the test (Table II) show that hydrochloric acid was essential for high mercury recovery. In the absence of hydrochloric acid, mercury recovery from halibut flesh and FPC was either erratic or significantly lower than was found using the FDA method. Higher concentrations of HCl had no effect upon mercury results.

Digesting the samples with a nitric–sulfuric acid mixture of different acid ratios in the presence of 6 *N* hydrochloric acid resulted in large variations in the mercury values, fume evolution during digestion, foaming during aeration of the mercury, and viscosity and stickiness of the fatty portion of the digested samples. Results of this study show the following. (1) The samples digested in nitric acid (no sulfuric acid) foamed excessively during aeration of the mercury and necessitated the cessation of aeration to prevent the foam from fouling up the system. (2) The samples digested in sulfuric acid (no nitric acid) resulted in large variations in the mercury values; also, the fatty portion of the digested samples turned into a dark, viscous mass that clung to the air dispersion glass frit and partially plugged the pores which, in turn, made the operation inefficient. The same type of product was observed in the samples digested in 1:24 and 1:49 nitric–sulfuric acid mixtures. The formation of viscous and sticky material was the basis for rejecting the 1:24 and 1:49 acid ratios. (3) Nitric–sulfuric acid ratios of 1:1 and 1:4 were rejected on the basis of excessive fumes during the digestion procedure. (4) A nitric–sulfuric acid ratio of 1:9 was the acid of choice based on consistency of results, low fume evolution during digestion, and absence of the sticky and viscous mass such as that mentioned above.

Dilution of the nitric–sulfuric acid mixture with water prevented charring during digestion and prevented foaming during aeration of the digested samples. Best results were obtained when the ratio of acid to water was 5:4.

Ten milliliters of the dilute nitric–sulfuric acid mixture in the presence of 1 ml of 6 N hydrochloric acid was adequate to digest up to 2 g of fish tissue and 500 mg of FPC without a significant change in mercury recoveries (Table III). Higher weights resulted in lower recoveries and in foaming.

No change in mercury recoveries was observed when the amount of the nitric–sulfuric acid in the digest was increased by up to 3 times; here, however, a buildup in gas pressure during aeration of the mercury was observed. This can result in leaks in the system and loss of mercury.

Recovery Studies. Mercuric chloride was added to halibut and sablefish at levels of 0.3, 0.6, 0.9, and 1.2 ppm and digested, and mercury values calculated. Results (Table IV) showed recoveries of 96 to 101%. These recoveries compare favorably with those of other methods.

The addition of up to 350 mg of fish oil to fish protein concentrate resulted in no significant drop in mercury recoveries (Table V). Similar results were obtained when fish oil was added to solutions of standards.

Several samples of fish, shellfish, and human hair were analyzed for mercury using the described and FDA methods. Results of the study (Table VI) showed a good agreement between the two methods. Several other fish samples previously analyzed for mercury by up to 20 different laboratories were analyzed at this Center using the described method. Our results (Table VII) were well within the mean mercury values found by all participating laboratories. [Our laboratory participated with about 20 other laboratories in a study to determine the mercury content of several samples of fish. The work was coordinated by the Inspection Branch, Department of the Environment, Fisheries and Marine Service, Winnipeg, Manitoba. Table VII includes a summary of the results of all laboratories including our own. The results we submitted in this study were those found using the FDA method.]

A number of chemists at this Center and at a commercial laboratory used this method to determine the mercury content of several fish samples previously analyzed by the author. All results were in good agreement (Table VIII).

CONCLUSION

A digestion method for the determination of mercury in biological samples by flameless atomic spectroscopy technique was developed. The proposed method is simple and rapid, and avoids the use of potentially explosive reagents. Air and water pollution and energy requirements are minimized. Day-to-day reproducibility is excellent, and recov-

ery studies show the method to be quite acceptable. A comparison of the mercury data using this method and the FDA Official Method shows the two methods to be in agreement within experimental error.

ACKNOWLEDGMENT

The author wishes to express his thanks to M. Hendzel of the Department of the Environment, Fisheries Service, Winnipeg, Manitoba for permission to use a part of the mercury data gathered in a collaborative study on mercury in fish. Thanks are due also to Erich Gauglitz, Alice Hall, and Clifford Houle of this Center and to Lee Johnson of Food, Chemical, and Research Laboratories Inc., Seattle, Wash., for their independent evaluation of the method.

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Received for review December 27, 1974. Accepted March 13, 1975.

Submicrogram Level Determination of Mercury in Seeds, Grains, and Food Products by Cold-Vapor Atomic Absorption Spectrometry

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A cold-vapor atomic absorption spectrometric technique has been employed for the determination of total mercury in several biological samples such as seeds, grains, fruits, vegetables, fish, and meat. The biological sample was digested with a mixture of concentrated sulfuric and nitric acids. Organically bound sample mercury was converted to the divalent form by a 3–5 hr 60° digestion with sulfuric and nitric acids using erlenmeyer flasks. Sample digestates were subsequently reduced with stannous chloride for determination by recirculating cold-vapor AAS down to 0.01 μg. The maximum amounts of mercury, 9.45 and 7.15 ppm, were found in hothouse tomato seeds and treated wheat seeds, respectively.

In the United States, in spite of the past extensive use of mercury and mercury compounds, little has been known about the extent of contamination of food and of other biological material (Basely, 1971; Newsome, 1971; Bache and Lisk, 1971; Pillay et al., 1971; Rottschafer et al., 1971; Moffitt and Kupel, 1970; Kalb, 1970; Fishman, 1970; Mayer, 1970; Cranston and Buckley, 1972). In contrast, much work has been done in Sweden, Japan, and Canada on mercury and methylmercury determination in biological materials (Lindstedt, 1970; Lindstedt and Skare, 1971; Malaiyandi and Barrette, 1970; Hatch and Ott, 1968; Takizawa, 1970; Suzuki et al., 1970, 1971; Sumino, 1968a,b; Fagerstrom and Jernelov, 1971; Westoo, 1966, 1967, 1968, 1969; Noren and Westoo, 1968; Johnasson et al., 1970). Although federal regulations establish a zero tolerance for mercury pesticides, no residue at all may appear in food samples sold in interstate commerce. Research in Japan, Sweden, and Canada reveals that the widespread use of these materials may result in some residues in many food products.

It was, therefore, decided that several biological samples, such as fruits, vegetables, meat, seeds, and grains, etc., be systematically analyzed for total mercury content, so that information may be available about the mercury content in various biological samples in Western Kentucky.

Various methods have been employed for the determination of mercury in atmospheric, aquatic, and biological samples (Basely, 1971; Pillay et al., 1971; Rottschafer et al., 1971; Moffitt and Kupel, 1970; Kalb, 1970; Fishman, 1970; Mayer, 1970; Cranston and Buckley, 1972; Lindstedt, 1970; Lindstedt and Skare, 1971; Malaiyandi and Barrette, 1970; Hatch and Ott, 1968; Suzuki et al., 1970, 1971; Sjostrand, 1964; Sandell, 1959; Nobel, 1961; Rathje, 1969; Gage and Watten, 1970; Bucknell, 1951). The colorimetric dithizone method (Basely, 1971; Mayer, 1970; Sandell, 1959; Nobel, 1961; Bucknell, 1951), which is considered to be the classical analytical procedure for the determination of trace amounts of mercury, is not very sensitive and is subject to a large number of chemical interferences. The method of neutron activation analysis (Pillay et al., 1971; Rottschafer et al., 1971; Sjostrand, 1964) has been employed for a variety of samples, but this method is expensive. Recently, several methods involving the use of cold-vapor atomic absorption spectrometry for mercury determinations have been published (Moffitt and Kupel, 1970; Kalb, 1970; Fishman, 1970; Cranston and Buckley, 1972; Lindstedt, 1970; Lindstedt and Skare, 1971; Malaiyandi and Barrette, 1970; Hatch and Ott, 1968; Suzuki et al., 1970, 1971; Rathje, 1969; Gage and Watten, 1970).

Kimura and Miller (1962) were the first to demonstrate the well-known reaction (eq 1) to liberate mercury from a digested sample and transport it to an absorption vessel by an air current in connection with dithizone determination. The same principle was later utilized by Poluektov et al. (1964) in liberating mercury vapor using cold-vapor atomic absorption spectrometry. This principle has since been applied for the analysis of biological samples for total mercury content. Presently, the cold-vapor atomic absorption spectrometric technique is preferred over less sensitive colorimetric dithizone and more expensive neutron activation techniques.

$$Hg^{2+} + Sn^{2+} \rightarrow Hg^{0} + Sn^{4+} \quad (1)$$

A relatively simple digestion procedure has been developed in the present study which can be applied to various biological samples. The biological sample was digested for 3 to 5 hr in a mixture of concentrated sulfuric and nitric acids in a water-bath shaker at 60°. The chemically bound

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