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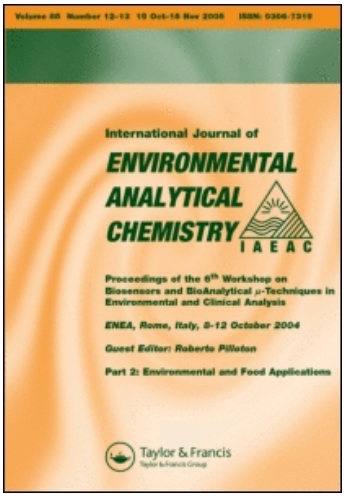

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Mohammad Sadiq[a]; Tahir H. Zaidi[a]
[a] Water and Environment Division, Research Institute, University of Petroleum and Minerals, Dhahran, Saudi Arabia

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A Study of Various Factors Affecting Digestion of Fish Tissue Prior to Mercury Determination

MOHAMMAD SADIQ and TAHIR H. ZAIDI

Water and Environment Division, Research Institute, University of Petroleum and Minerals, Dhahran, Saudi Arabia

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The effect of temperature on digestion, the acid combinations and their quantities, and the time required for digestion of fish tissue were investigated using Hamour (Epinephelus tauvina) body tissue.

Concentrations of Hg in fish tissue digested for four hours at $80\pm2°C$ and $95\pm2°C$ were statistically similar and significantly higher than in tissue digested at $60\pm2°C$. Eight acid combinations were investigated as digestion media and a 1:2 mixture of concentrated HNO_3:H_2SO_4 proved to be the best. A quantity of 15 ml of this digestion media were found to be sufficient to digest approximately two grams of wet fish tissue. The use of 25 ml of digestion media resulted in significantly reduced Hg concentration whereas 10 ml was not sufficient to digest two grams of fish tissue. A digestion period of four to six hours at 80°C was sufficient to oxidize the fish tissue. However, a two hour digestion resulted in reduced Hg values. Mercury determinations made from the samples prepared by the best combination of all the experimental conditions showed a good agreement with those of samples prepared in Teflon Acid Digestion Bombs. This study has pointed the necessity of developing a uniform standard procedure for digesting fish tissue prior to Hg determinations.

KEY WORDS: Temperature effect, acid combinations, mercury in fish, duration of digestion.

INTRODUCTION

Total mercury in fish muscle have been determined by many investigators.[1-10] The procedure for determining Hg using cold vapor technique[11] or flameless atomic absorption is well established. But, unfortunately, standard procedure for digesting fish tissue prior to Hg determinations has received little consideration. In general, the temperature during digestion, the composition of oxidizing acids, the quantity of acids and the duration of digestion are some of the parameters which need to be standardized.

Powell *et al.*[5] digested fish tissue in concentrated HNO_3, at room temperature to avoid Hg volatilization. Whereas, Duve *et al.*[12] performed the digestion at 95–100°C in an HNO_3-H_2SO_4 mixture (1:1). Other investigators [Bull *et al.*;[13] Aronson *et al.*;[14] Hoover *et al.*;[15] Walting *et al.*;[16] Zook *et al.*[10]] preferred 50–60°C digestion temperature for Hg determinations. Taguchi *et al.*[17] determined Hg in shark muscles which were digested at 200°C. Many investigators had not mentioned the temperature at which digestion was carried out [Brown and Chow;[18] Palmer and Rand;[4] Parvaneh;[19] Phillips *et al.*[20]].

Different acid combinations have been used for digesting fish tissue. Aronson *et al.*,[14] Akielaszek and Haines[21] and Hoover *et al.*[15] digested fish tissue in concentrated HNO_3 only. Walting *et al.*[16] and Wobester *et al.*[9] used only concentrated H_2SO_4 in the digestion. Mixture of HNO_3-H_2SO_4 was the most common acid combination used in the digestion of fish muscles for Hg determination [Brown and Chow.;[18] Palmer and Rand;[4] Duve *et al.*;[12] Taguchi *et al.*;[17] Phillips *et al*[20]]. The proportion of HNO_3 to H_2SO_4 varied in each investigation. Bull *et al.*[13] and Soha *et al.*[7] used HNO_3-$HClO_4$ combination for oxidizing fish tissue. Parvaneh[19] digested fish tissue in HNO_3-$HC10_4$-H_2SO_4 mixture.

The time of digestion also varied significantly. Watling *et al.*[16] carried digestion for two hours at 55°C as compared with Aronson *et al.*[14] and Zook *et al.*[10] who performed digestion for 45 minutes and overnight, respectively, at 55°C temperature. Many of the above investigators have not mentioned the time required to complete digestion.

In light of the above discussion, the objectives of this study were to investigate the effect of: (a) temperature during digestion, (b) the acid combinations, (c) the quantity of acids, and (d) the duration of digestion on Hg determination in fish tissue.

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MATERIALS AND METHODS

Four to five hundred grams of muscle filet from Hamour (Epinephelus tauvina) weighing 5.5 kilograms were cut from the ventral region of first dorsal fin. The skin was removed and the muscle was homogenized thoroughly.

To study the effect of temperature during digestion on Hg determination, approximately 2 grams of homogenized tissue were taken into 39 BOD bottles (cap. 250 ml). Nine of the bottles were spiked with 0.5 μg of Hg as $HgCl_2$. Ten ml of concentrated H_2SO_4 and 5 ml of concentrated HNO_3 were added to each bottle, and the bottles were left overnight. On the following day, 10 non-spiked and 3 spiked bottles (for each temperature) were heated for four hours in a water bath at $60\pm2°C$, $80\pm2°C$ and $95\pm2°C$. After colling the bottles, 20 ml of %5 $KMnO_4$, 5 ml of 5% $K_2S_2O_8$ were added to each bottle while the bottles were in ice cold water. The bottles were heated for another two hours on their respective temperatures. After cooling, 5–10 ml of 12% hydroxylamine hydrochloride was added to reduce excess of $KMnO_4$. Mercury ions in the acid digest were reduced to metallic Hg by using 5 ml of 10% $SnCl_2$. Mercury was determined using the Perkin Elmer Mercury Analyzer 50A. Some of the results were verified using atomic absorption spectrophotometer and MHS-20.

To investigate the effectiveness of acid combination, approximately 2 grams of homogenized fish tissue were taken into 104 BOD bottles. Twenty-four bottles were spiked with 0.5 μg Hg as $HgCl_2$. Fifteen ml of concentrated either HNO_3 only or HNO_3-H_2SO_4 (4:1), or HNO_3:H_2SO_4 (2:1) or HNO_3:H_2SO_4 (1:2) or HNO_3:H_2SO_4 (1:4) or H_2SO_4 only or HNO_3:$HC10_4$ (2:1) or HNO_3:$HC10_4$ (4:1) were added to 10 non-spiked and 3 spiked bottles. The bottles were left overnight. On the following day, all the bottles were heated for four hours in a water bath at $80\pm2°C$. The remaining procedure was similar to that described above.

The optimum amount of acid required for the fish tissue digestion was studied by taking approximately 2 grams of homogenized tissue into 40 BOD bottles. To each set of 10 BOD bottles, 10 ml, 15 ml, 20 ml or 25 ml of concentrated HNO_3-H_2SO_4 (1:2) was added. After overnight digestion at room temperature, the bottles were heated at $80\pm2°C$ for 4 hours. The remaining procedure was similar to as descirbed above.

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The effect of duration of preliminary digestion was investigated by taking approximately 2 grams of homogenized tissue into 40 BOD bottles. To each bottle, 15 ml of HNO_3-H_2SO_4 (1:2) was added. The remaining procedure was similar to as described above except that a set of 10 BOD bottles was heated for 2 hours, and the other sets for 4 and 6 hours, respectively.

RESULTS AND DISCUSSIONS

The results of the temperature effect study are given in Table I. The concentrations of Hg were significantly lower when the fish tissue was digested at 60°C as compared with 80°C or 95°C digestion. There was no significant difference between Hg concentrations in the tissue digested at 80°C and 95°C. The observations of this study are in line with the recommendation of USEPA[22] who suggests a temperature of 95°C for digesting organic Hg.

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TABLE I

Effect of digestion on Hg concentration in Hamour body tissue.

	Hg (μg/g wet tissue)		
Replication	Digestion at 60°C	Digestion at 80°C	Digestion at 95°C
1	0.55	0.58	0.58
2	0.53	0.57	0.56
3	0.57	0.56	0.63
4	0.53	0.57	0.58
5	0.55	0.57	0.62
6	0.54	0.63	0.61
7	0.50	0.57	0.57
8	0.53	0.59	0.56
9	0.57	0.61	0.58
10	0.55	0.57	0.57
Mean	0.54a	0.58b	0.59b
Standard deviation	0.02	0.02	0.02
Least significant difference = 0.03			

It seems that Powell *et al.*,[5] who digested fish at room temperature, and Aronson *et al.*;[14] Bull *et al.*,[13] Hoover *et al.*,[15] Watling *et al.*,[16] and Zook *et al.*,[10] who prepared their samples at 50–60°C might have underestimated Hg contents in their fish samples. The results of this study emphasize that the temperature at which initial digestion is carried out should be mentioned. The recovery of the spiked Hg was not affected by digestion temperature (90–106% recovery).

The results of acid combinations used to digest the fish tissue prior to Hg determination are given in Table II. This table shows that HNO_3-H_2SO_4 (1:2) was the best acid combination and determined the highest concentration of Hg in the fish tissue.

It was followed by HNO_3:H_2SO_4 (1:4). There was no significant difference between HNO_3-H_2SO_4 (2:1) and HNO_3 only. Significantly lower concentrations of Hg were determined in the fish tissue digested in HNO_3:H_2SO_4 (4:1), H_2SO_4 only, HNO_3:$HC10_4$ (2:1) and HNO_3:$HC10_4$ (4:1), combinations.

As mentioned under introduction section, different acid combinations have been used by different investigators. Those digesting fish in concentrated HNO_3 only were probably significantly under-estimating Hg concentrations. It may be pointed out that HNO_3-$HC10_4$ combination was not any better as shown in Table II. The results of this study suggest that serious efforts may be exerted to develop a standard procedure for digesting fish tissue prior to Hg determinations.

The effect of HNO_3-H_2SO_4 (1:2) quantities on Hg concentration in Hamour body tissue is shown in Table III. The maximum concentration of Hg was observed in the fish tissue digested in 15 ml HNO_3-H_2SO_4 (1:2). The quantity of Hg determined in the fish tissue digested in 25 ml acids was significantly lower than 15 ml but was statistically similar to Hg concentrations in 10 and 20 ml acid digest. It is obvious that 10 ml of HNO_3-H_2SO_4 (1:2) was not sufficient to oxidize fish tissue.

The effect of duration of preliminary digestion of the fish tissue on Hg concentration is shown in Table IV. The digestion of fish tissue at 80°C for two hours resulted in significantly lower concentrations of Hg suggesting that two hours may not be sufficient for oxidizing organic Hg in the fish. There was no significant difference between the digestion of the fish tissue for four hours and for six hours.

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TABLE II

Effect of acid combination on Hg concentration (μg/g wet tissue) in the Hamour body tissue.

Replication	HNO_3 only	HNO_3:H_2SO_4 (4:1)	HNO_3:H_2SO_4 (2:1)	HNO_3:H_2SO_4 (1:2)	HNO_3:H_2SO_4 (1:4)	H_2SO_4 only	HNO_3:$HC10_4$ (2:1)	HNO_3:$HC10_4$ (4:1)
1	0.56	0.51	0.46,	0.63	0.63	0.43	0.48	0.42
2	0.54	0.52	0.52	0.64	0.59	0.49	0.47	0.43
3	0.50	0.50	0.42	0.59	0.58	0.40	0.53	0.45
4	0.52	0.52	0.46	0.64	0.56	0.51	0.41	0.44
5	0.51	0.50	0.44	0.60	0.56	0.46	0.46	0.49
6	0.51	0.54	0.41	0.67	0.60	0.44	0.40	0.41
7	0.50	0.53	0.45	0.68	0.61	0.48	0.39	0.47
8	0.48	0.50	0.47	0.61	0.59	0.40	0.51	0.42
9	0.50	0.48	0.45	0.64	0.57	0.45	0.45	0.45
10	0.51	0.52	0.43	0.65	0.61	0.44	0.45	0.43
Mean	0.51b	0.51b	0.45a	0.64d	0.59c	0.45a	0.46a	0.42a
Standard deviation	0.02	0.02	0.03	0.02	0.02	0.04	0.05	0.07

Least significant difference = 0.04

TABLE III

Effect of acid quantities on Hg concentration (μg/g wet tissue) in Hamour body tissue.

Replication	$HNO_3:H_2SO_4$ (1:2)			
	10 ml	15 ml	20 ml	25 ml
1	0.51	0.57	0.56	0.53
2	0.46	0.59	0.54	0.53
3	0.54	0.62	0.59	0.54
4	0.57	0.56	0.60	0.55
5	0.58	0.63	0.53	0.53
6	0.55	0.57	0.56	0.53
7	0.52	0.63	0.58	0.52
8	0.52	0.62	0.55	0.53
9	0.53	0.61	0.55	0.51
10	0.54	0.59	0.57	0.54
Mean	0.53a	0.60b	0.56ab	0.53a
Standard deviation	0.03	0.04	0.02	0.02
Least significant difference = 0.04				

TABLE IV

Effect of digestion time on Hg concentration in the Hamour body tissue.

Replication	Hg (μg wet tissue)		
	Digestion time		
	2 hour	4 hour	6 hour
1	0.48	0.59	0.57
2	0.54	0.59	0.58
3	0.55	0.56	0.59
4	0.51	0.58	0.61
5	0.55	0.56	0.54
6	0.56	0.63	0.58
7	0.55	0.58	0.57
8	0.50	0.55	0.55
9	0.48	0.57	0.57
10	0.56	0.56	0.56
Mean	0.53a	0.58b	0.57ab
Standard deviation	0.03	0.02	0.02
Least significant difference = 0.03			

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Based on the results of this study, a best combination of digestion conditions was selected (temperature—80°C, acid combination—$HNO_3 : H_2SO_4$ (1:2), acid quantity—15 ml, digestion time—4 hours). Several fish specimens were digested under these conditions and Hg was determined in the acid digest. These results are compared in Table V with Hg concentrations in the same fish tissue digested in "Teflon Acid Digestion Bomb" (TADB) under similar experimental conditions. Table V shows no significant difference between Hg concentrations determined in $HNO_3 : H_2SO_4$ (1:2) with and without the use of the TADB. Although digestion of organic matter in TADB is ideal, this technique is slow and cumbersome.

TABLE V

Comparison of Hg concentrations in the fish body tissue samples prepared by using Teflon acid digestion bomb (TADB) and $HNO_3 : H_2SO_4$ combination.

		Hg (μg/g wet tissue)*	
Fish species	Specimen wt. (g)	TADB	$HNO_3 : H_2SO_4$ (1:2)
(1:2)			
Hamour,	500	0.06	0.05
Epinephelus	900	0.18	0.18
tauvina	1500	0.28	0.31
	2500	0.25	0.23
	3900	0.47	0.50
	4500	0.81	0.75
Hamra,	1000	0.10	0.08
Lutjanus	2400	0.30	0.31
sanguineus	2600	0.16	0.19
	3400	0.15	0.16
	3600	0.41	0.45
	3700	0.59	0.59
Shaeri,	1000	0.26	0.28
Lethrinus	1500	0.27	0.29
choerorhynchus	1600	0.44	0.41
	1800	0.41	0.45
	1900	0.47	0.41

*Mean of three determinations.

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CONCLUSION

Based on the results of this study, it may be concluded that:

—Digestion of fish tissue at 80–90°C may produce best results.

—$HNO_3 : H_2SO_4$ (1 : 2) was the best acid combination for fish tissue digestion.

—Four hours digestion at 80°C was optimum time for oxidizing organic and inorganic Hg in fish tissue.

—Fifteen ml of HNO_3-H_2SO_4 (1 : 2) was sufficient to digest fish tissue.

—No significant differences were found in Hg concentrations in the fish tissue digested in $HNO_3 : H_2SO_4$ (1 : 2) and and without the use of the "Teflon Acid Digestion Bomb".

—The significant contribution of the study is that it emphasizes the need for developing a uniform standard procedure for digesting the fish tissue prior to Hg determination.

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