Development of Methylmercury Analysis by Ultra-High Performance Liquid Chromatography Coupled with ICP-MS and Its Application on Sharks' Meat Measurement

Suratno Suratno[1,2], Satriyo Krido Wahono[2], Dwi Siswanta[1], and Nurul Hidayat Aprilita[1*]

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada, Sekip Utara, Yogyakarta 55281, Indonesia*

[2]*Research Center for Food Technology and Processing (PRTPP), National Research and Innovation Agency (BRIN), Gunungkidul, Yogyakarta 55861, Indonesia*

* ***Corresponding author:***

email: nurul.hidayat@ugm.ac.id

Received: February 22, 2023
Accepted: March 24, 2023

DOI: 10.22146/ijc.82512

Abstract: *This study analyzed MeHg in a fast, simple, low-waste, and accurate by using ultra-high liquid chromatography coupled with inductively coupled plasma mass spectrometry. Simple preparation by liquid extraction with sonication at room temperature was effective extract MeHg from Certified Reference Material (CRM) and shark meat samples. Effective MeHg separation was achieved in less than 300 s using a C18 Hypersil Gold analytical column with a mobile gradient phase of 0.5% (w/v) L-cysteine in 2% (v/v) HNO_3 and 100% methanol. The MeHg was extracted from 100 mg of shark meat using 1 mL of 0.5% (w/v) L-cysteine in 2% (v/v) HNO_3 and sonicated for 30 min. Analysis of certified reference material (DORM-4) showed values between the experimental and certified values. The observed limit of detection and quantification MeHg were 0.86 and 2.85 pg/L, respectively. This method was applied to measure MeHg in shark meat from Binuangeun areas. The MeHg concentration in* Rhizoprionodon acutus *was 0.22–0.63 mg/kg wet weight (w.w.),* Squalus hemipinis *0.68–1.14 mg/kg w.w., and 0.29–1.22 mg/kg w.w. for* Sphyrna lewini. *This study provides a quick and easy method to evaluate MeHg in shark meat or other seafood products and applies to many samples in a single assay.*

Keywords: *UHPLC-ICP-MS; methylmercury; sonication-assisted extraction; shark meat*

■ INTRODUCTION

In particular, methylmercury (MeHg) is the most toxic food web and bioaccumulating mercury. It is the predominant chemical form, accounting for 80–90% of the total mercury present in fish muscle tissue [1-2]. Mercury concentrations of up to 4,000 mg/kg and MeHg concentrations of up to 95% have been found in large carnivorous fish such as sharks, swordfish, and some tuna [2]. As a result, fish and other organisms at the end of the food chain constitute a significant source of MeHg in the human diet [3]. MeHg causes non-fatal effects, including impaired reproductive function [4], decreased liver function and metabolism [5], and neurological damage [6-7].

The most effective instrumental methods of mercury speciation analysis are based on chromatography, such as gas (GC) [8] or liquid chromatography (LC) [9-11] and combined with specific and sensitive detectors (such as inductively coupled plasma mass spectrometry (ICP-MS)). Compared to GC, mercury speciation does not need to be converted to volatile compounds prior to High Performance Liquid Chromatography (HPLC) separation, so LC is a suitable separation technique for mercury speciation [12]. Several methods have been developed to determine mercury speciation using HPLC-ICPMS on biological samples. However, they all use many solvents in the extraction methods and

produce large amounts of waste products containing MeHg [9,12-13].

Therefore, this article's objective was to evaluate a method for determining the MeHg concentration in shark meat by incorporating a more straightforward and economical extraction process using ultra-high-performance chromatography combined with ICP-MS with a fast procedure and less waste-containing MeHg.

■ EXPERIMENTAL SECTION

Materials

The materials used in this study were MS Grade for water (Thermo Fisher, USA), HNO_3 65% (Merck), methylmercury chloride (CH_3HgCl) standard (Sigma, USA), methanol for LC grade (Merck, Germany), DORM-4 as CRM for MeHg and L-cysteine (99% purity Sigma, USA). All glassware was washed by soaking in 10% (v/v) HNO_3 for 12 h, rinsed three times with double-distilled deionized water and air drying for 12 h. A 1,000 mg/L standard solution for CH_3HgCl was prepared by weight 1 mg CH_3HgCl diluted in 1 mL with 0.5% L-cysteine 2% HNO_3. A series of standard solutions (1, 5, 10, 25, 50, 75, 100 µg/L) were prepared by dilution of 1,000 mg/L MeHg stock solution with 0.5% L-cysteine 2% HNO_3. The standard curve was prepared in duplicate (Fig. 1).

Instrumentation

The instrumentations used in this study were a UHPLC-ICPMS (Thermo Scientific) with PFA Cyclonic Spray Chamber, PFA Micro mist Nebuliser and ICAP Q/Qnova Quartz Torch Organics. A Thermo Scientific™ Vanquish™ UHPLC Quaternary Pump with reverse-phase column (C18, Hypersil GOLD™, 250 mm × 4.6 mm ID, 5 µm particle size, 175 Å pore size). The mobile phase used was 0.5% L-cysteine in 2% HNO_3 (A) and methanol 100% (B), and the flow rate of the gradient technique was 1.0 mL/min. The mobile phase B was adjusted to 2% and gradually increased to 90% in 132 s. After that, hold at 90% for 20 s and continue at the initial state until 180 s. The temperature of the column was set at 40 °C, and the volume of injection was 50 µL. The optimal experimental conditions for UHPLC and ICPMS are shown in Table 1.

Procedure

Sample preparation and extraction MeHg

Shark samples were collected by direct purchase on September 11-16, 2019, from Binuangeun Fish Auction, Lebak, Banten, Indonesia (Southern Java Sea areas). Sixteen sharks were identified as *Rhizoprionodon acutus* (RA), six as *Squalus hemipinis* (SH), and eight as *Sphyrna lewini*. The total weight (TW) was measured using a digital balance, and the total length (TL) was measured using a rolling meter. Shark muscles for mercury analysis were cut from an area near the dorsal fin, placed in a zip-lock plastic bag, and frozen (–20 °C) until further analysis. In the laboratory, shark meat was

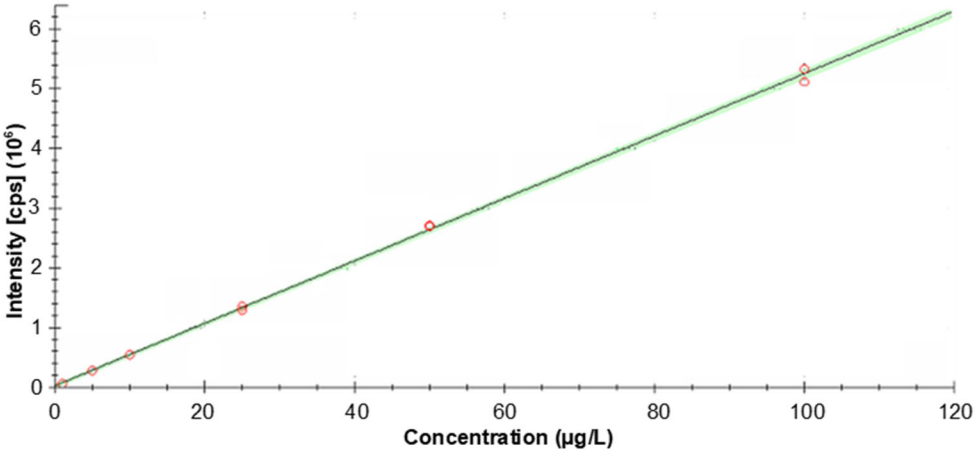

Fig 1. MeHg concentration calibration curve (µg/L) in duplicates with $R^2 = 0.999$, slope = 52.336.311, intercept = 15.501.082 and RSE = 12.34%

Table 1. Liquid chromatography and ICPMS operating condition for mercury speciation (MeHg)

UHPLC condition	
Column	C18, Hypersil GOLD™, 250 mm × 4.6 mm ID, 5 µm particle size, 175 Å pore size
Mobile phase	A: 0.5% L-cysteine in 2% HNO_3 B: MeOH 100%
Mobile phase flow rate	1.0 mL/min
Elution	Gradient flow with a quaternary pump 0–132 s (2–90%B) 132–144 s (90% B) 145–180 s (2% B)
Volume injection	50 µL
ICP-MS condition	
Plasma power (W)	1550
Auxiliary flow (L/min)	0.8
Nebulizer flow (L/min)	0.6179
Isotopes	^{202}Hg
$^{140}Ce.^{16}O/^{140}Ce$ ratio	0.0161
Spray chamber	PFA cyclonic spray chamber
Torch	iCAP Q/Qnova torch for organics (FI475-01W)
Quartz injector	Quartz injector (1.0 mm ID) iCAP Q/Qnova

oven-dried for 24 h at 60 °C for MeHg analysis and moisture content to convert the dry weight to wet weight. Dried samples were ground using mortar and pestle to get fine powder for further analysis.

The development method of MeHg extraction was based on de Souza et al. [9] with modifications for the volume of solvent extraction, sonication time, mobile phase, and elution conditions. The use of L-cysteine in acid conditions as the solvent and mobile phase and also the use of gradient elution was the development method compared to de Souza et al. [9]. A 100 mg of the dried sample was placed in 2 mL microtubes containing 1 mL of 0.5% L cysteine in 2% HNO_3 and applied sonication (Elmasonic S60H, f: 50/60 Hz, 550 W) for 30 min at room temperature. The microtube was then centrifuged at 3,000 rpm for 5 min, and the supernatant was filtered through a 0.20 µm nylon syringe filter and placed in a 2 mL HPLC vial.

Method validation

A CRM DORM4 (n = 10) was used as sample preparation to validate the accuracy of the method. The sample was performed in duplicate, and blanks were used with 0.5% L-cysteine in 2% HNO_3. Two replicates of MeHg standard solutions with concentrations (as mercury) in the range of 1–100 µg/L were used to form linear calibration plots with r^2 values of 0.999. The limit of detection (LOD) and limit of quantification (LOQ) were measured based on [14] with the equation; LOD = $3.9 \times \frac{SDb}{Sb}$ with SDb was the standard deviation measurement of blank/pseudo blank (n = 10), Sb was the slope of the calibration curve, and the LOQ was 3.33 of LOD.

Data analysis

Graphical from UPLC was analyzed with Qtegra and Chrom Control software (Thermo Scientific, USA), and all graphic plot was based on the open source program platform R (package 4.12 for windows) [15].

■ RESULTS AND DISCUSSION

Extraction and Validation Method with DORM-4

The development method showed good extraction of MeHg in DORM4 with 1 mL of L-cysteine in acid condition compared to previous studies from Vallant et al. [13] used 5 mL solvent for MeHg extraction, while de

Souza et al. [9] used 10 mL solvent for CRM extraction, and Rodrigues et al. [12] used 4.75 mL solvent. Previous studies commonly use two compounds, such as L-cysteine combined with 2-mercaptoethanol [9-10,16]. Vallant et al. [13] use HCl and NaOH for MeHg extraction while our method shows good results only using L-cysteine in acidic conditions. L-cysteine was used in acid conditions as solvent extraction. We combined L-cysteine with methanol as the mobile phase to increase the sensitivity of Hg on the complex compound of MeHg-L-cysteine [9]. L-cysteine was less toxic than the mercapto compound, and the complexing ability was poor [10]. The development method gives less solvent for extraction, less chemical compound is used in analysis and less waste containing MeHg.

The development method used gradient elution in a UHPLC system and compared it to a previous study [9,11-13] and showed good separation with mobile phase 0.5% L-cysteine and 2% HNO_3 and 100% methanol under gradient conditions of less than 300 s. Inorganic mercury (InHg) was showed a peak at 196.5 s and MeHg around 266 s (Fig. 2). Gradient elution shows low signal noise at a low concentration compared to isocratic elution [9]. The use of gradient elution can reduce 20% of the time of MeHg analysis by comparing isocratic elution [9].

The recovery of MeHg concentration with this method shows good result measurement (Table 2) with values of 96.90%. Our method uses less solvent in this study because solvent extraction is the same as for the mobile phase. The development method shows good results in reducing the uses of solvent extraction. Our results can reduce 80% on the use of solvent compared with Vallant et al. [13] and reduce by almost 90% compared to de Souza et al. [9]. Compared to previous studies [4,9,12-13,16-17], our method uses a small volume (1 mL) of extraction to reduce organic waste containing MeHg (Table 3). The observed limit of detection and limit of quantification MeHg in this method was 0.86 and 2.85 pg/L, respectively.

Method Application for Methylmercury Measurement on Shark Meat

The total length of milk sharks ranged from 34.5–46.8 cm, 49.5–62.3 cm for Squalus sharks, and 56.4–68 cm for hammerhead sharks. The total weight of milk sharks was 195–450 g, 485–1190 g for Squalus shark, and 825–1335 g for hammerhead shark, respectively (Table 4).

The concentration of MeHg in the meat/muscle tissue ranged between 0.22 and 0.63 mg/kg w.w. (mean concentration of MeHg: 0.41 ± 0.13 mg/kg w.w.) for *R. acutus*, 0.68 to 1.14 mg/kg w.w. (0.81 ± 0.17 mg/kg w.w.)

Table 2. Recovery of MeHg concentration in DORM-4 by UHPLC-ICP-MS (n = 10) extracted with 0.5% (m/v) L-cysteine in 2% (v/v) HNO_3 using gradient elution condition

CRM	Analyte	Certified value (mg/kg)	Measurement value (mg/kg)
DORM-4	MeHg	0.355 ± 0.028	0.344 ± 0.028
Recovery (%)			96.90

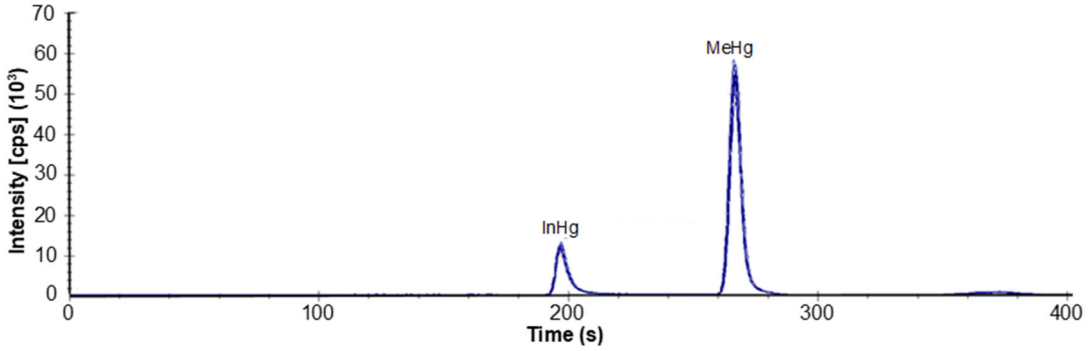

Fig 2. Chromatogram showing the separation of inorganic mercury (InHg) and methyl mercury (MeHg) in DORM-4 (n = 10) with mobile phase 0.5% (m/v) L-cysteine in 2% (v/v) HNO_3, Methanol 100% on gradient condition

Table 3. Comparison MeHg analysis using HPLC-ICPMS on biological samples with previous research

Type of samples	Mobile phase and type of elution	Solvent extraction	Weight of samples	Volume of extraction	Time of extraction	LOD	Ref.
Human hair	0.05% v/v mercaptoethanol; 0.4% m/v L-cysteine; 5% v/v methanol and 0.06 mol/L ammonium acetate. *Isocratic elution*	0.10% v/v HCl + 0.05% m/v L-cysteine + 0.10% v/v 2-mercaptoethanol	50 mg	10 mL	10 min sonication	MeHg: 10.0 ng/g InHg: 15.0 ng/g EtHg: 38.0 ng/g	[9]
DORM-2 DOLT-3	50 mmol/L pyridine, 0.5% w/w L-cysteine, 5% v/w MeOH, at pH 2. *Isocratic elution*	5 M HCl; 10 M NaOH	250 mg	5 mL	15 min sonication; 20 min centrifugation	InHg: 0.05 µg/L MeHg: 0.08 µg/L	[13]
Human plasma NIST 966	3% v/v methanol + 97% v/v (0.5% v/v 2-mercaptoethanol + 0.05% v/v formic acid). *Isocratic elution*	0.10% v/v HCl + 0.05% m/v L-cysteine + 0.10% v/v 2-mercaptoethanol	250 µL	2.75 mL	15 min sonication	InHg: 12 ng/L EtHg: 5 ng/L MeHg: 4 ng/L	[16]
Blood SRM 966	0.05% v/v mercaptoethanol; 0.4% m/v L-cysteine; 5% v/v methanol and 0.06 mol/L ammonium acetate. *Isocratic elution*	0.10% (v/v) HCl + 0.05% (m/v) L-cysteine + 0.10% (v/v) 2-mercaptoethanol	250 µL	4.75 mL	15 min sonication	InHg: 0.25 µg/L MeHg: 0.1 µg/L	[12]
ERM CE-464	50 mM pyridine, 0.5% (w/v) L-cysteine, 5% (v/v) MeOH, pH 3. *Isocratic elution*	25% (w/v) KOH in MeOH.	300 mg	9 mL (3 mL × 3)	30 min waterbath, 30 min sonication, 10 min centrifugation	InHg: 0.46 µg/L MeHg: 0.78 µg/L	[17]
		25% (w/v) TMAH in MeOH	300 mg	9 mL (3 mL × 3)	30 min waterbath, 30 min sonication, 10 min centrifugation		
		5% (w/v) TMAH in MeOH	200 mg	10 mL	20 min microwave digestion		
		5 M HCl	300 mg	5 mL	5 min sonication, 10 min centrifugation		
		4 M HNO_3	500 mg	10 mL	20 min microwave digestion		
		Glacial acetic acid	300mg	9 mL	10 min microwave digestion		
		1% (w/v) L-cysteine hydrochloride hydrate	200 mg	20 mL	2 h waterbath, 2 h heating		
		20 mg protease type XIV, 0.1 M phosphate buffer pH 7.5 containing 0.05% (w/v) cysteine	200 mg	8 mL	2 h enzyme digestion, 20 min centrifugation		
Sea cucumber	8% MeOH; 92% H_2O containing 0.12% L-cysteine + 0.01 mol/L ammonium acetate. *Isocratic elution*	0.10% HCl (v/v), 0.12% L-cysteine (m/v), and 0.10% 2-mercaptoethanol (v/v)	500 mg	10 mL	30 min sonication	InHg: 0.12 µg/L MeHg: 0.08 µg/L EtHg: 0.20 µg/L	[10]
DORM-4 Shark meat	0.5% (m/v) L-cysteine in 2% (v/v) HNO_3 + MeOH 100%. *Gradient elution*	0.5% (m/v) L-cysteine in 2% (v/v) HNO_3	100 mg	1 mL	30 min sonication	MeHg: 0.86 pg/L	This study

for *S. hemipinis* and 0.29 to 1.22 mg/kg w.w. (0.56 ± 0.32 mg/kg w.w.), and for *S. lewini* as described in Fig. 3. The mercury concentration on hammerhead sharks in this study is relatively lower compared to previous research by Mohammed and Mohammed [18]. The difference in size and weight of hammerhead sharks from this study were correlated to the mercury concentration. Based on the size, hammerhead sharks from Binuangeun are still in the juvenile phase compared to previous research in Trinidad, the Gulf of California and the Korean coast [18-20]. Based

Table 4. Morphometric of samples *R. acutus*, *S. hemipinis* and *S. lewini.* M: male; F: female

Common name	N	Scientific name	The total weight (g)	Total length (cm)	Sex
Milk shark	16	*Rhizoprionodon acutus*	195–450	34.5–46.8	M: 11; F: 5
Squalus shark	6	*Squalus hemipinis*	485–1190	49.5–62.3	M: 1; F:5
Hammerhead shark	8	*Sphyrna lewini*	825–1335	56.4–68.0	M:4; F:4

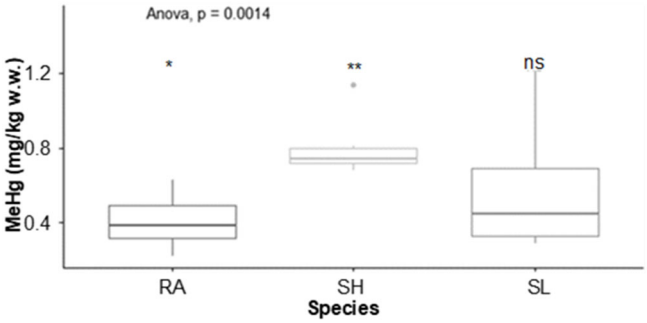

Fig 3. Methylmercury (MeHg) concentration (mg/kg w.w.) on shark samples. SH: *S. hemipinis*, SL: *S. lewini*, and RA: *R. acutus*

on our results, there are no differences MeHg concentration between sex on *R. acutus* and *S. lewini* (*p*-value 0.93 and 0.2, respectively). The chromatogram peak of MeHg from each sample of sharks showed in Fig. 4.

MeHg concentrations in milk sharks from the Binuangeun area were higher than in previous research on the Korean coast [20]. MeHg concentration in Squalus sharks from Binuangeun was lower than in Brazil [21] and Southeast Australia [22]. Due to limited studies on *S. hemipinis*, mercury concentrations in these sharks compared to *S. acanthias* and *S. albicaudus* indicate the same habitat on the shelf and upper slopes of the ocean (from 0–600 m) [23]. Some studies have shown that several factors influence mercury accumulation in sharks, such as age, body length, habitat, sex and local pollution [24-25].

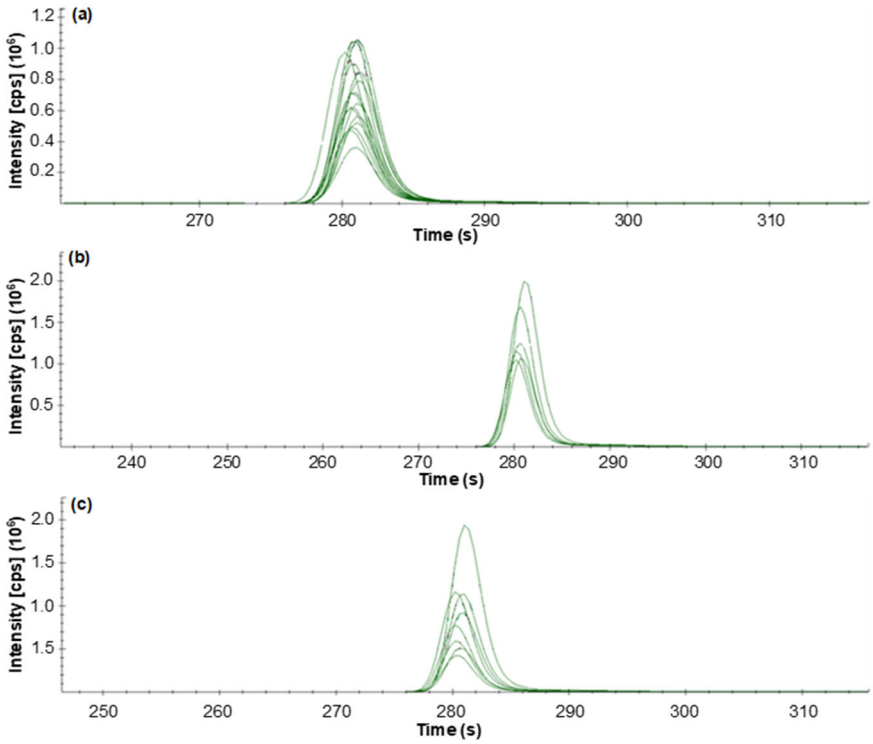

Fig 4. Chromatogram showing the separation methylmercury (MeHg) with mobile phase 0.5% (m/v) L-cysteine in 2% (v/v) HNO_3, Methanol 100% on gradient condition in (a) *R. acutus* (n = 16), (b) *S. hemipinnis* (n = 6), and (c) *S. lewini* (n = 8)

■ CONCLUSION

A quick and easy method has been developed for analyzing MeHg using UHPLC-ICPMS. Gradient elution applied using a C18 reversed-phase column with 0.5% L cysteine in 2% HNO_3 and methanol as mobile phase can separate inorganic Hg and MeHg on CRM (DORM-4) with an accuracy of fewer than 300 s. The small amount of solvent used for extraction produces small amounts of waste containing MeHg. Finally, this method was successfully applied to analyze MeHg in shark meat samples.

■ ACKNOWLEDGMENTS

This research was fully funded by the Final Recognition Program (RTA) from Universitas Gadjah Mada, Yogyakarta, contract no: 3143/UN1.P.III/DIT-LIT/PT/2021. The authors would like to thank Aristya B.N. Jati for assisting during the analysis. The authors also thank the National Research and Innovation Agency (BRIN), Gunungkidul, Yogyakarta, for promoting the UHPLC-ICPMS.

■ AUTHOR CONTRIBUTIONS

Suratno wrote the original draft, conceptualization, formal analysis, sample analysis, and writing review and editing. Dwi Siswanta performed the statistical analysis, investigation, and data curation. Nurul Hidayat Aprilita supporting for funding acquisition, project administration and writing review and editing. Satriyo Krido Wahono wrote and reviewed the manuscript.

■ REFERENCES

[1] Nalluri, D., Baumann, Z., Abercrombie, D.L., Chapman, D.D., Hammerschmidt, C.R., and Fisher, N.S., 2014, Methylmercury in dried shark fins and shark fin soup from American restaurants, *Sci. Total Environ.*, 496, 644–648.

[2] Brombach, C.C., Manorut, P., Kolambage-Dona, P.P.P., Ezzeldin, M.F., Chen, B., Corns, W.T., Feldmann, J., and Krupp, E.M., 2017, Methylmercury varies more than one order of magnitude in commercial European rice, *Food Chem.*, 214, 360–365.

[3] Karimi, R., Silbernagel, S., Fisher, N.S., and Meliker, J.R., 2014, Elevated blood Hg at recommended seafood consumption rates in adult seafood consumers, *Int. J. Hyg. Environ. Health*, 217 (7), 758–764.

[4] Huang, X., Liu, Z., Xie, Z., Dupont, S., Huang, W., Wu, F., Kong, H., Liu, L., Sui, Y., Lin, D., Lu, W., Hu, M., and Wang, Y., 2018, Oxidative stress induced by titanium dioxide nanoparticles increases under seawater acidification in the thick shell mussel *Mytilus coruscus*, *Mar. Environ. Res.*, 137, 49–59.

[5] Nogara, P.A., Madabeni, A., Bortoli, M., Teixeira Rocha, J.B., and Orian, L., 2021, Methylmercury can facilitate the formation of dehydroalanine in selenoenzymes: Insight from DFT molecular modeling, *Chem. Res. Toxicol.*, 34 (6), 1655–1663.

[6] Man, Y.B., Wu, S.C., and Wong, M.H., 2014, Shark fin, a symbol of wealth and good fortune may pose health risks: the case of mercury, *Environ. Geochem. Health*, 36 (6), 1015–1027.

[7] Yu, X., Khan, S., Khan, A., Tang, Y., Nunes, L.M., Yan, J., Ye, X., and Li, G., 2020, Methyl mercury concentrations in seafood collected from Zhoushan Islands, Zhejiang, China, and their potential health risk for the fishing community: Capsule: Methyl mercury in seafood causes potential health risk, *Environ. Int.*, 137, 105420.

[8] Kim, T.H., Cho, M.J., Lee, Y., Kim, J.H., Hwang, J.Y., Lee, H.E., Kim, S.H., Choi, J.D., and Kang, G.J., 2020, Methylmercury determination in fish by direct mercury analyzer, *J. AOAC Int.*, 103 (1), 244–249.

[9] de Souza, S.S., Rodrigues, J.L., de Oliveira Souza, V.C., and Barbosa, F., 2010, A fast sample preparation procedure for mercury speciation in hair samples by high-performance liquid chromatography coupled to ICP-MS, *J. Anal. At. Spectrom.*, 25 (1), 79–83.

[10] Liu, H., Luo, J., Ding, T., Gu, S., Yang, S., and Yang, M., 2018, Speciation analysis of trace mercury in sea cucumber species of *Apostichopus japonicus* using high-performance liquid chromatography conjunction with inductively coupled plasma mass

spectrometry, *Biol. Trace Elem. Res.*, 186 (2), 554–561.

[11] Anual, Z.F., Maher, W., Krikowa, F., Hakim, L., Ahmad, N.I., and Foster, S., 2018, Mercury and risk assessment from consumption of crustaceans, cephalopods and fish from West Peninsular Malaysia, *Microchem. J.*, 140, 214–221.

[12] Rodrigues, J.L., de Souza, S.S., de Oliveira Souza, V.C., and Barbosa, F., 2010, Methylmercury and inorganic mercury determination in blood by using liquid chromatography with inductively coupled plasma mass spectrometry and a fast sample preparation procedure, *Talanta*, 80 (3), 1158–1163.

[13] Vallant, B., Kadnar, R., and Goessler, W., 2007, Development of a new HPLC method for the determination of inorganic and methylmercury in biological samples with ICP-MS detection, *J. Anal. At. Spectrom.*, 22 (3), 322–325.

[14] Wenzl, T., Haedrich, J., Schaechtele, A., Robouch, P., and Stroka, J., 2016, *Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food,* EUR 28099, Publications Office of the European Union, Luxembourg.

[15] R Core Team, 2021, *R: A Language and Environment for Statistical Computing*, R Foundation for Statistical Computing, Viena, Austria.

[16] de Souza, S.S., Campiglia, A.D., and Barbosa, F., 2013, A simple method for methylmercury, inorganic mercury and ethylmercury determination in plasma samples by high performance liquid chromatography-cold-vapor-inductively coupled plasma mass spectrometry, *Anal. Chim. Acta*, 761, 11–17.

[17] Reyes, L.H., Mizanur Rahman, G.M., Fahrenholz, T., and Skip Kingston, H.M., 2008, Comparison of methods with respect to efficiencies, recoveries, and quantitation of mercury species interconversions in food demonstrated using tuna fish, *Anal. Bioanal. Chem.*, 390 (8), 2123–2132.

[18] Mohammed, A., and Mohammed, T., 2017, Mercury, arsenic, cadmium and lead in two commercial shark species (*Sphyrna lewini* and *Caraharinus porosus*) in Trinidad and Tobago, *Mar. Pollut. Bull.*, 119 (2), 214–218.

[19] Bergés-Tiznado, M.E., Márquez-Farías, F., Lara-Mendoza, R.E., Torres-Rojas, Y.E., Galván-Magaña, F., Bojórquez-Leyva, H., and Páez-Osuna, F., 2015, Mercury and selenium in muscle and target organs of scalloped hammerhead sharks *Sphyrna lewini* of the se gulf of California: Dietary intake, molar ratios, loads, and human health risks, *Arch. Environ. Contam. Toxicol.*, 69 (4), 440–452.

[20] Kim, S.J., Lee, H.K., Badejo, A.C., Lee, W.C., and Moon, H.B., 2016, Species-specific accumulation of methyl and total mercury in sharks from offshore and coastal waters of Korea, *Mar. Pollut. Bull.*, 102, 210–215.

[21] Hauser-Davis, R.A., Pereira, C.F., Pinto, F., Torres, J.P.M., Malm, O., and Vianna, M., 2020, Mercury contamination in the recently described Brazilian white-tail dogfish *Squalus albicaudus* (Squalidae, Chondrichthyes), *Chemosphere*, 250, 126228.

[22] Elsayed, H., Yigiterhan, O., Al-Ansari, E.M.A.S., Al-Ashwel, A.A., Elezz, A.A., and Al-Maslamani, I.A., 2020, Methylmercury bioaccumulation among different food chain levels in the EEZ of Qatar (Arabian Gulf), *Reg. Stud. Mar. Sci.*, 37, 101334.

[23] Wosnick, N., Niella, Y., Hammerschlag, N., Chaves, A.P., Hauser-Davis, R.A., da Rocha, R.C.C., Jorge, M.B., de Oliveira, R.W.S., and Nunes, J.L.S., 2021, Negative metal bioaccumulation impacts on systemic shark health and homeostatic balance, *Mar. Pollut. Bull.*, 168, 112398.

[24] Teffer, A.K., Staudinger, M.D., Taylor, D.L., and Juanes, F., 2014, Trophic influences on mercury accumulation in top pelagic predators from offshore New England waters of the northwest Atlantic Ocean, *Mar. Environ. Res.*, 101, 124–134.

[25] Lyons, K., and Lowe, C.G., 2013, Mechanisms of maternal transfer of organochlorine contaminants and mercury in the common thresher shark (*Alopias vulpinus*), *Can. J. Fish. Aquat. Sci.*, 70 (12), 1667–1672.