Electronic supplementary information for

A broad-applicable method for mercury speciation in various seafood using microwave-assisted extraction and ion chromatography-inductively coupled plasma mass spectrometry

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1. Chemicals, materials and apparatus used in the experiment

The standard solutions of Hg^{2+} (mercuric chloride, 1000 μg/mL), CH_3Hg^+ (methylmercury chloride, 100 μg/mL) and $C_2H_5Hg^+$ (ethylmercury chloride, 100 μg/mL) were all purchased from National Institute of Metrology, China (Beijing, China). The standards of each mercury species with different concentrations used in the experiments were prepared by directly diluted above standards with pure water. Ultra-pure HNO_3 and analytical grade of L-Cysteine (Cys) were obtained from Sangon Biotech Co. Ltd (Shanghai, China). The water used in the experiment is Milli-Q water (18.2 MΩ·cm), which was prepared with Millipore pure system. Strong cation exchange guard column (Zorbax 300SCX, 4.6×12.5 mm) was purchased from Agilent Technology (Shanghai, China).

The IC-ICP-MS system, which couples an Agilent 7800 inductively coupled plasma mass spectrometry (ICP-MS, Agilent of USA) with a Dionex ICS-900 ion chromatography (ThermoFisher Scientific, China), was used in this study.

Table S1: Optimal running parameters of IC-ICP-MS system

Parameter	Value
IC system	
IC separation column	Two consecutive strong cation guard column (Zorbax 300SCX, 4.6×12.5 mm)
IC eluent	5.0 mmol/L HNO_3-1.0 mmol/L Cys
IC elution mode	iso-elution
Sample injection volume	10 μL
Flow rate of IC eluent	1.0 mL/min
Temperature of IC separation column	30 ℃
ICP-MS system	
ICP-MS RF power	1350 W
ICP-MS cool gas flow rate	15 L/min
ICP-MS auxiliary gas flow rate	0.90 L/min
ICP-MS nebulizer gas flow rate	0.80 L/min
ICP-MS makeup gas flow rate	0.20 L/min
Monitored isotope (m/z)	^{202}Hg

Table S2: The effect of extracting solvent volume and extraction mode on mercury extraction efficiency of shellfish (*Morula uva*) sample

Sample	0.10 g shellfish (*Morula uva*)	
Extracting solvent volume	Extraction mode	Extraction efficiency of Hg
8 mL	4.0 mL for previous soaking and first extraction, 4.0 mL for second extraction	72.4%
10 mL	5.0 mL for previous soaking and first extraction, 5.0 mL for second extraction	83.6%
12 mL	6.0 mL for previous soaking and first extraction, 6.0 mL for second extraction	89.8%
	7.0 mL for previous soaking and first extraction, 5.0 mL for second extraction	91.2%
14 mL	7.0 mL for previous soaking and first extraction, 7.0 mL for second extraction	90.8%
	8.0 mL for previous soaking and first extraction, 6.0 mL for second extraction	91.5%

Table S3: The comparison on analytical performance among previous ICP-MS-based methods for mercury speciation in seafood and our method

Method	Separation column	Analyte	Extraction method	Applicable sample	Instrument LOD	Method LOD in seafood	Ref.
HPLC-ICP-MS	C8 column	Hg^{2+} and CH_3Hg^+	Enzymatic extraction and microwave-assisted extraction	Fish	0.4 ng/mL	10 ng/g dried weight	1
Ion chromatography-ICP-MS	Anion guard column	Hg^{2+}, CH_3Hg^+ and $C_2H_5Hg^+$	Ultrasonic extraction	Fish	0.008-0.029 ng/mL	Not provided	2
Ion-pairing reversed-phase HPLC-ICP-MS	C18 guard columns	Hg^{2+}, CH_3Hg^+ and $C_2H_5Hg^+$	Ultrasonic extraction	Fish	0.014-0.028 ng/mL	0.18-0.33 ng/g fresh weight	3
HPLC-ICP-MS	C18 column	Hg^{2+}, CH_3Hg^+ and $C_2H_5Hg^+$	Ultrasonic extraction together with magnetic solid phase pre-concentration	Water and fish	0.49-0.76 ng/L	Not provided	4
Ion chromatography-ICP-MS	Cation guard columns	Hg^{2+}, CH_3Hg^+ and $C_2H_5Hg^+$	Ultrasonic extraction	Water and fish	Not provided	1.9-3.1 ng/g fresh weight	5
HPLC-ICP-MS	ZORBAX SB-Aq C18 column	Hg^{2+} and CH_3Hg^+	Microwave-assisted extraction with protease	Fish	0.013–0.015 ng/mL	Not provided	6
HPLC-isotope dilution ICP-MS	Symmetry Shield RP C18 column	Hg^{2+} and CH_3Hg^+	Microwave-assisted extraction	Marine biota	Not provided	Not provided	7
IC-ICP-MS	Strong cation guard column	Hg^{2+}, CH_3Hg^+ and $C_2H_5Hg^+$	Microwave-assisted extraction without protease	Various seafood (seaweeds, fishes and shellfishes)	0.02-0.05 ng/mL	2.4-6.0 ng/g dried weight (about 0.48 – 1.2 ng/g fresh weight)	This study

Reference:

(1) R. Jagtap, F. Krikowa, W. Maher, S. Foster, M. Ellwood, *Talanta*, 2011, 85, 49-55

(2) X. P. Chen, C. Han, H. Y. Cheng, J. H. Liu, Z. G. Xu, X. F. Yin, *Anal. Chim. Acta*, 2013, 796, 7-13.

(3) H. Y. Cheng, X. P. Chen, L. H. Shen, Y. C. Wang, Z. G. Xu, J. H. Liu, *J. Chromatogr. A*, 2018, 1531, 104-111.

(4) S. Q. Zhu, B. B. Chen, M. He, T. Huang, B. Hu, *Talanta*, 2017, 171, 213-219.

(5) X. P. Chen, C. Han, H. Y. Cheng, Y. C. Wang, J. H. Liu, Z. G. Xu, L. Hu, *J. Chromatogr. A*, 2013, 1314, 86-93.

(6) Y.-C. Chen, S.-J. Jiang, *J. Anal. At. Spectrom.*, 2021, 36, 938-945.

(7) A. A. Krata, E. Vassileva, *Talanta*, 2020, 217, 121113.

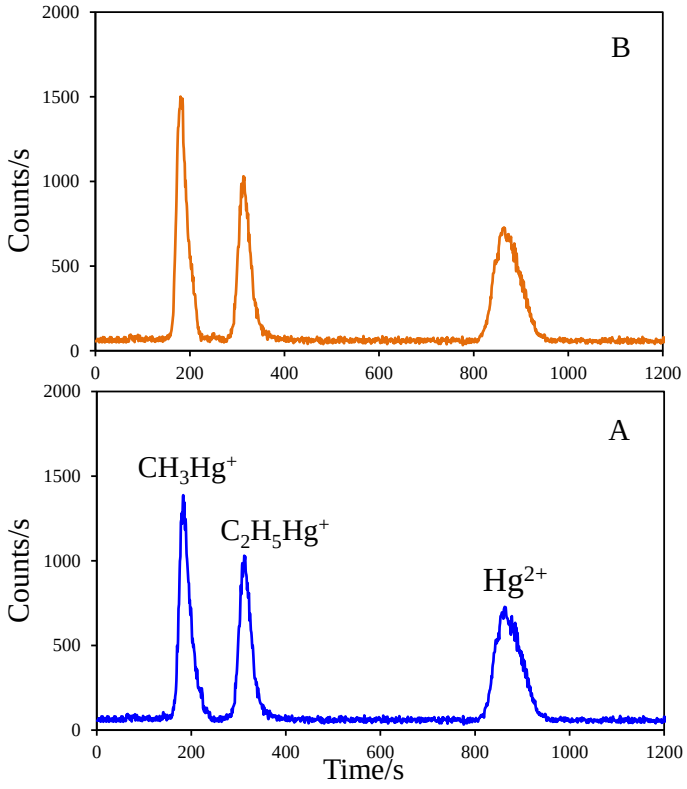

Figure S1: The IC-ICP-MS chromatograms of the mixed standard of mercury species. The concentrations of each mercury species are all 10.0 ng/mL. (A): Original mixed standard; (B) The mixed standard pretreated with pre-soaking microwave-assisted extraction.

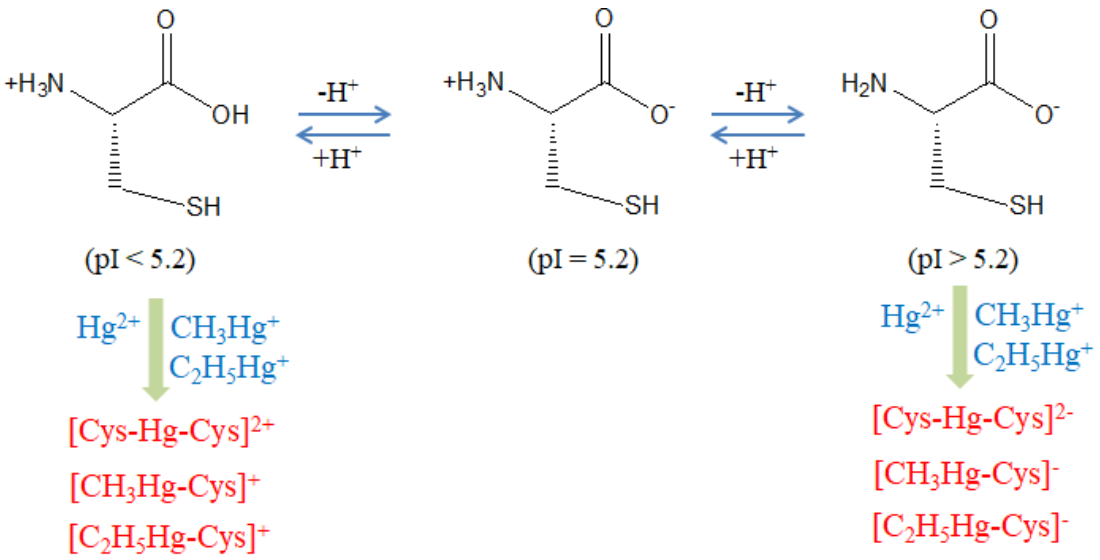

Figure S2: The structure and protonation state of Cys and the charge state of the Cys-mercury species complexes.

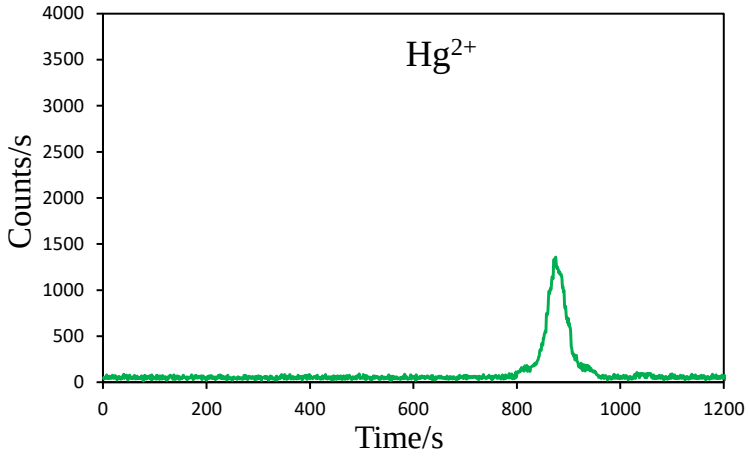

Figure S3: The IC-ICP-MS chromatogram for determining Hg^{2+} after $Hg^{2+}$was pre-chelated with 6.0 mM of L-Cys. The concentrations of $Hg^{2+}$is 10 ng/mL, and the IC-ICP-MS chromatogram was obtained under Table S1 conditions.

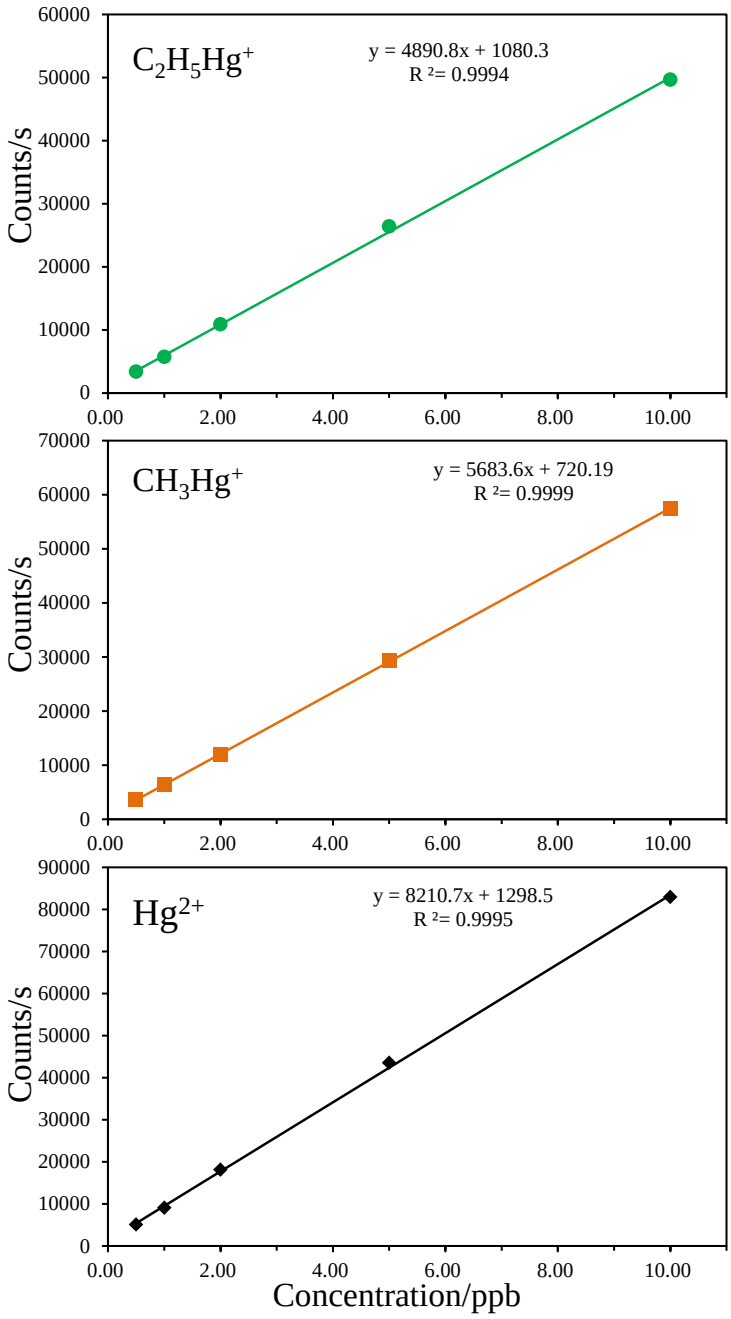

Figure S4: The linear relationship between IC-ICP-MS signal (peak area) and the concentrations of mercury species (calibration curves) for determining Hg^{2+}, CH_3Hg^+ and $C_2H_5Hg^+$ with IC-ICP-MS under Table S1 conditions.