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Determination of total mercury in biological tissue by isotope dilution ICPMS after UV photochemical vapor generation

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ABSTRACT

A method is developed for the determination of trace mercury in biological samples using photo chemical vapor generation (PVG) and isotope dilution inductively coupled plasma mass spectrometry (ID ICPMS) detection. Biological tissues were solubilized in formic acid. Subsequently, the sample solutions were exposed to an ultraviolet (UV) source for the reduction of mercury into vapor species prior to ICPMS measurements. The formic acid served not only as a tissue solubilizer in the sample preparation procedure, but also as a photochemical reductant for mercury in the PVG process. The problem arising from the opaque formic acid digested solution was efficiently solved by using ID method. The optimum conditions for sample treatment and PVG were investigated. A limit of detection (LOD) of 0.5 pg g^{-1}, based on an external calibration, provided 350-fold improvement over that obtained by utilizing conventional pneumatic nebulization sample introduction. Method validation was demonstrated by the determination of total mercury in several biological tissue certified reference materials (CRMs). The results were in good agreement with the certified values.

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1. Introduction

Mercury is a global pollutant due to its potent neurotoxicity as well as its bioaccumulation in the food chain [1]. The toxicity, bioavailability, and mobility of mercury depend not only on its total concentration but also significantly on its chemical forms. Generally, methyl mercury (MeHg) is more toxic than inorganic mercury and elemental mercury because of its high liposolubility and high biomagnification factor in the food chain [1–3]. The released inorganic mercury in the ecosystem undergoes biogeochemical transformation processes and can be converted into MeHg by microorganisms and microalgae in aquatic environments. About 75–100% of mercury exists as MeHg in fish. Fish consumption is regarded as the main source of human MeHg exposure [4,5]. The total concentration of mercury in human hairs is widely used as a biological indicator for the assessment of MeHg exposure [6].

Consequently, many studies have been devoted to the development of sensitive and accurate methods for the determination of mercury in biological samples. Sample preparation is a crucial step in mercury measurements [7–12], in order to achieve accurate results. Alkaline dissolution methods using tetramethylammonium hydroxide (TMAH) [13] or formic acid based solubilization techniques [14] have been successfully applied to the analysis of biological tissues. Compared to the conventional acid digestion, these sample preparation procedures are simple, cost-efficient, and less labor intensive. For the determination of mercury, chemical vapor generation (CVG) in combination with atomic spectrometry techniques remains the most successful approach, which can easily separate analytes from the troublesome sample matrix and improve the sample introduction efficiency, compared to that of traditional pneumatic nebulization [15–17]. Among CVG techniques, cold vapor generation using tetrahydroborate (THB) or $SnCl_2$ as a reductant is the most widespread one for the determination of trace/ultratrace mercury in many matrices such as natural waters and biological samples [18,19]. Unfortunately, interferences from transition metals often decrease the sensitivity and reproducibility, leading to poor analytical results. Moreover, contamination from THB reagent and the instability of its solution limit the dynamic linear concentration range. Additionally, the toxicity and low vapor generation efficiency restrict the wide application of the $SnCl_2$ system.

Photo chemical vapor generation (PVG), utilizing free radicals produced by photo-redox reactions as reductants, appears as an ideal alternative technique for conventional CVG methods [20–23].

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Hg and its species can be efficiently converted into Hg^0 in the presence of low molecular weight organic compounds (e.g., alcohol, formic acid, acetic acid or formaldehyde) under UV irradiation [24]. This technique retains the principle advantages of conventional CVG but further provides simpler reactions, greener analytical chemistry, and cost-effective approach [8,25,26]. The use of formic acid as the solubilizer for biological sample analysis facilitates the determination procedure of mercury with the PVG system, which provides the possibility of analyzing mercury in biological sample with minimal reagent. However, the opaque sample solution after formic acid solubilization can reduce the penetration efficiency of UV radiation, which in turn influence the mercury vapor generation efficiency in sample solution, leading to poor analytical results by external calibration detection [21,27,28].

In this paper, a new method was proposed for the quantitation of ultratrace mercury in biological samples based on ID–PVG–ICPMS after ultrasonic-assisted acid solubilization of samples. Once isotopic equilibrium is achieved between the added spike and the endogenous analyte in the sample [20,29–31], ID–ICPMS is capable of compensating any subsequent loss of analyte during sample preparation, matrix effect, and instrument drift. Therefore, coupling ID–ICPMS with PVG after formic acid digestion will provide a simple, sensitive and reliable method for mercury determination in biological samples. Moreover, only single reagent, namely formic acid, was involved in the analytical procedure. It was served as both the solubilizer for biological samples in sample preparation procedure and the reductant for PVG of mercury, decreasing the risks of sample contamination. To our knowledge, this is the first report of using PVG coupled with ID–ICPMS for mercury quantitative analysis.

2. Experimentation

2.1. Instrument

An ELAN DRC™-e ICPMS (Perkin-Elmer, Inc., Shelton, CT, USA) equipped with quartz torch and alumina sample injector tube was used. The fitted Gem Tip cross-flow nebulizer and the corrosion resistant double pass Rytons spray chamber mounted outside the torch box were replaced by a PVG-system for mercury determination in this work. Optimization of the ELAN DRC™-e was performed as recommended by the manufacturer. Optimum detection conditions for PVG were investigated independently. The instrument dead time was obtained by measuring the $^{204}Pb/^{206}Pb$ ratio in standard solutions at different concentration levels, and was found to be 50 ns. Isotopes of ^{201}Hg and ^{202}Hg were simultaneously monitored. Operating conditions are summarized in Table 1.

A schematic of the PVG system hyphenated to the ICPMS is shown in Fig. 1. A model FIA-3110 flow injection pump system (Vital Instruments Co. Ltd., Beijing, P.R. China) was used for the introduction of sample solutions to the PVG system. A low-pressure Hg vapor UV lamp (15 W, Philips Co., Holland), fitted with a quartz tube (15 cm × 1.8 mm i.d. × 2.8 mm o.d.), was employed as the PVG reactor. For convenience, the PVG reactor was wrapped with aluminum foil to protect the operator from UV radiation and minimize ozone formation. After reduction by photo reactor, sample solution was pumped through a tandem set of two gas–liquid separators (GLSs). The generated mercury vapor was stripped from sample solution and directed to the ICPMS using an argon carrier gas from sample gas channel of ELAN DRC™-e. The tandem set of GLSs were immersed in ice bathes to prevent liquid droplets derived from condensation of water vapor or coexisting volatile organic compounds being transported to the ICP torch. After each analytical process, the analytical system was repeatedly washed at least 2 min with a 40% solution of formic acid to remove the residual mercury.

Table 1
ICPMS Instrumental Operating Parameters.

Parameters	Values
RF power/W	1300 W
Cool gas flow	15 L min^{-1}
Auxiliary gas flow	0.8 L min^{-1}
Nebulizer gas flow	1.0 L min^{-1}
Scanning mode	Peak hopping
Resolution	0.7 amu
Dwell time	30 ms
Dead time	50 ns
Sweeps per reading	5
Readings per replicate	1
Number of replicates	4
Sample flow rate	4.5 mL min^{-1}
Isotope monitored	^{202}Hg, ^{201}Hg

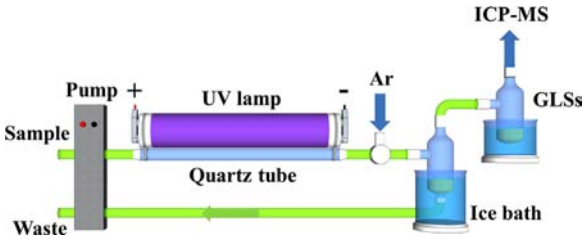

Fig. 1. The schematic of the PVG–ICPMS system.

2.2. Reagents and solutions

All reagents were of analytical reagent grade or better, and deionized water (DIW) was used throughout. ACS grade formic acid was obtained from Sinopharm Chemicals Reagent Company (Shanghai, China). A 1000 mg g^{-1} stock solution of Hg was purchased from the National Research Center for Standard Materials (NRCSM) of China. Working standard solution prepared by dilution of the stock solution with 2% HNO_3 to the concentration of 10 μg g^{-1} Hg was used for the reverse spike isotope dilution of the enriched spike solution.

The ^{201}Hg enriched spike material (95.5% atom fraction of ^{201}Hg and 2.8% atom fraction of ^{202}Hg) was obtained from Oak Ridge National Laboratory (Oak Ridge, TN) as solid mercuric oxide. The isotopic composition of the spike material was verified by ICPMS measurement. A stock solution of the spike was prepared by dissolution of the oxide in high-purity nitric acid and serial dilutions with a preservative solution containing 0.05% (w/v) potassium dichromate and 3% (v/v) nitric acid. Working spike solution of 8.3 μg g^{-1} ^{201}Hg was prepared by dilution with 2% HNO_3 solution. Certified biological reference materials including GBW 07601a (GSH-1) (hair) and GBW 09101b (hair) from National Research Center for Standard Materials (NRCSM, Beijing, China), DORM-2 (dogfish muscle), and DORM-3 (fish protein) from the National Research Council Canada (NRCC, Ottawa, Canada), were used for method valuation.

2.3. Sample preparation

The ultrasonic-assisted acid solubilization procedure was used for the sample treatment of biological certified materials. Approximately 0.1 g sample tissue was weighed into 50 mL dark polyethylene centrifuge tube. Subsequently, appropriate amounts of enriched spikes (leading to ratios of $^{201}Hg/^{202}Hg$ near 1 for the final solution), and 10 mL of formic acid were added. Then the centrifuge tube was

placed in an ultrasonic bath at 40 °C for 2 h to dissolve sample tissues. For Hg determination, a volume ranging from 0.2 to 2.5 mL of the sample solution containing the solubilized tissue was added into a 40 mL calibrated flask and diluted with DIW. If needed, additional formic acid solution was added to keep the sample solution containing the final concentration of 20% (v/v) formic acid. Three sample blanks (spiked with 10% of the amount of enriched spikes used in the samples) were processed along with samples.

For reverse ID, 0.1 g of ^{201}Hg working solution was accurately weighed into four 50 mL pre-cleaned polyethylene screw-capped bottles and known masses of natural abundance standard solutions were followed, yielding an isotope ratio of ^{201}Hg/^{202}Hg near 1. Following the addition of 10 mL of concentrated formic acid to each vial, the content was diluted to mark with DIW. To achieve optimum results, the samples and reverse spike ID calibration samples were subjected to online UV-PVG with ID ICPMS detection on the same day, as mentioned by Yang et al. [20] The mass bias per mass unit in percentage (MB [%]) was calculated according to the report from Xie et al. [32] which was found to be 0.31% for Hg^{201}/Hg^{202} based on 12 measurements. Mass bias correction was implemented on the basis of the IUPAC natural abundance ratios for ^{201}Hg/^{202}Hg, divided by their mean value determined in the natural abundance standards.

3. Results and discussion

3.1. Optimization of sample preparation procedure and PVG for ICPMS detection

The effect of ultrasonic treatment time on the recovery was investigated in order to achieve quantitative digestion. The argon carrier gas flow rate, the formic acid concentration, and the sample flow rate through the UV–PVG system, comprised three basic parameters, which determined CVG efficiency of analyte and transport efficiency to the ICPMS, were investigated. These parameters were examined in order to optimize the response on mercury determination. Due to the different sample matrix between standard solutions and sample digests, the optimal experimental conditions for mercury determination in standard solutions may not in accordance with sample digests [21]. Therefore, the CRM of tuna fish tissue, namely DORM-2, was used for the optimization of PVG and sample preparation procedures instead of using standard solutions to eliminate the matrix effect from biological samples.

The effect of the ultrasonic irradiation time on the extraction efficiencies of mercury using concentrated formic acid was evaluated from 1 to 8 h. As shown in Fig. 2, similar signal intensities of Hg in digested solution were observed during 1 to 5 h ultrasonic irradiation. A slight decrease in the signal intensities was found at longer time. When ultrasonic irradiation time of less than 1 h was used, incomplete dissolution of sample tissue occurred and tissue particles were evident in these digests, resulting in a poor analytical precision. No significant improvement in the extraction efficiency was observed when further increase the irradiation time to longer than 2 h. In addition, the loss of analyte due to the conversion of inorganic mercury to mercury vapor in the presence of formic acid was observed when irradiation time used was longer than 6 h. Therefore, the ultrasonic irradiation time of 2 h was selected for all subsequent measurements.

The argon carrier gas flow rate through the gas/liquid separators (GLSs), which also served as the sample gas of ICPMS, is an important parameter affecting the efficiency of gas–liquid separation and transport of analyte vapor, as well as the depth of sampling in the plasma. The effect of Ar carrier gas flow rate is presented in Fig. 3. The maximal signal intensity was observed at

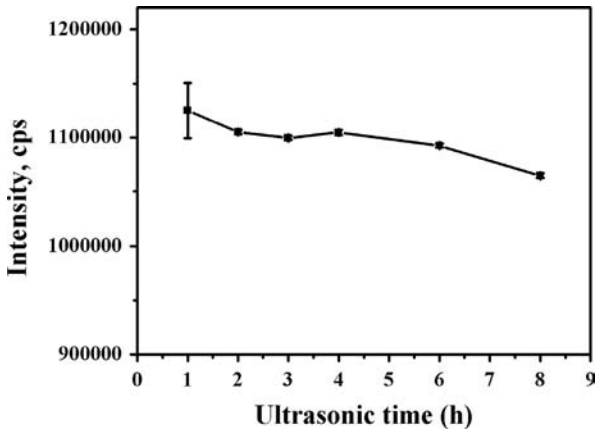

Fig. 2. Effect of the ultrasonic time on the extraction efficiencies of mercury with concentrated formic acid.

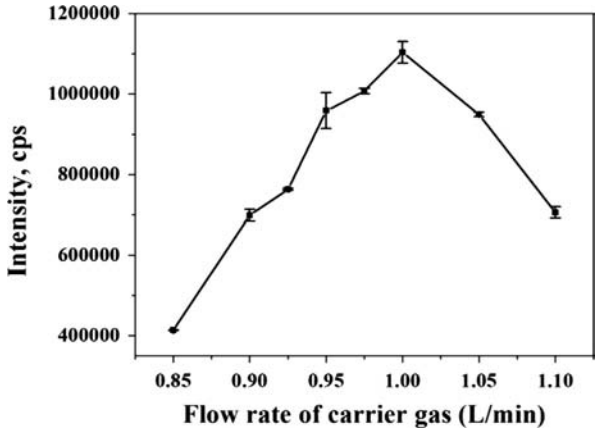

Fig. 3. Effect of argon carrier flow rate on ^{202}Hg response in DORM-2 digested solution. Experimental conditions: 20% (v/v) formic acid and 4.5 mL min^{-1} sample flow rate.

1.0 L min^{-1} gas flow rate. Lower carrier gas flow may result in inefficient stripping and transport of analyte from the solution, whereas higher flow may lead to significant dilution of analyte. Therefore, argon flow rate of 1.0 L min^{-1} was selected for all subsequent measurements.

The effect of formic acid concentration in a range of 5–40% on the response of Hg in DORM-2 digested solution is shown in Fig. 4. The intensity of ^{202}Hg increased with the increase of formic acid concentrations from 5% to 20% (v/v), and remained constant at higher concentrations. This observation contrasts with the results observed by Vieira et al. [14] wherein an efficient reduction of mercury was achieved even in the present of very low concentration of formic acid. This is maybe due to different sample matrices studied. At low formic acid concentration, the co-existing compounds in sample digest many react with and quench the radicals generated by photo decomposition of formic acid, which consequently reduced the available radicals for mercury reduction [33]. With increased concentration of formic acid, the amount of available radicals greatly increased, resulting in an increase in efficiency of mercury reduction. Considering the best response for mercury and reagents consumption, a formic acid concentration of 20% was selected for all subsequent measurements.

The dose of UV radiation received by sample solution mainly determines the efficiency of radical formation and the vapor generation efficiency of mercury [21,25,34]. With a specific photo reactor system, the received UV radiation dose is primarily influenced by the residence time of the sample in the flow-through reactor,

namely irradiation time. The effect of sample introduction flow rate in the range of 2.5–8 mL min^{-1} was thus investigated for the determination of Hg in DORM-2, as shown in Fig. 5. Optimum sensitivity was obtained at the flow rate of 4.5 mL^{-1} and the response decreased at both lower and higher flow rates. In the range of 2.5–4.5 mL min^{-1}, the sensitivity increased almost linearly with the increase of sample introduction flow rate, because of the increased sample introduction volume for ICPMS determination in a given sampling time. Above 4.5 mL min^{-1}, the increased sample introduction speed led to reduced irradiation time for mercury, resulting an inefficient reduction of mercury in sample solution [27]. A flow rate of 4.5 mL min^{-1} was thus selected for all subsequent measurements.

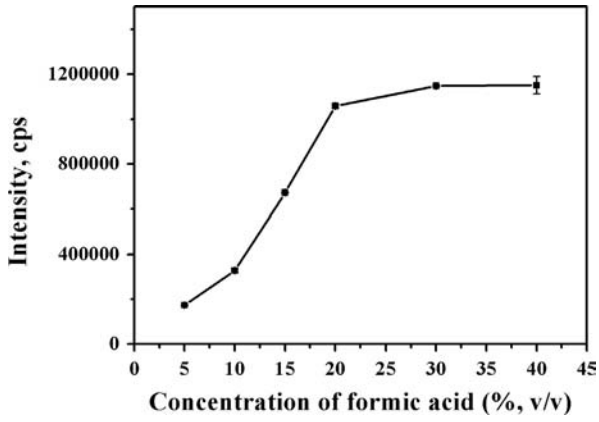

Fig. 4. Effect of the concentration of formic acid on ^{202}Hg response from DORM-2 digested solution. Experimental conditions: 1.0 L min^{-1} carrier gas flow rate and 4.5 mL min^{-1} sample flow rate.

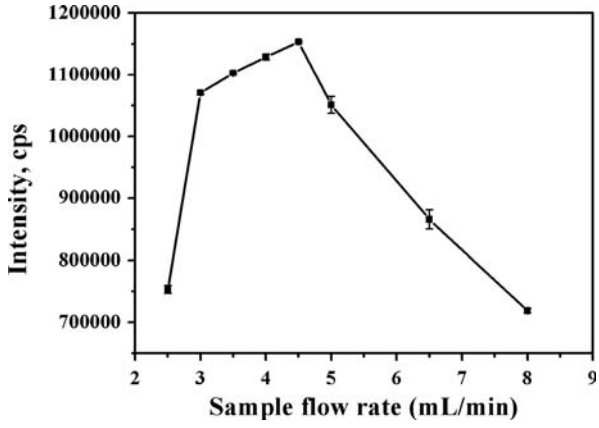

Fig. 5. Effect of sample introduction flow rate on ^{202}Hg response from DORM-2 digested solution. Experimental conditions: 1.0 L min^{-1} carrier gas flow rate and 20% (v/v) formic acid.

3.2. Analytical performance

The analytical performance of the present method was evaluated. Precision of replicate measurements, expressed as relative standard deviation (RSD, $n=7$) was 1.4% for mercury standard solution at a concentration of 0.5 ng g^{-1}. The procedure blank was assessed by adding a very small amount of ^{201}Hg spike and 10 mL HCOOH to a calibrated flask, and processing through the entire analytical procedure. The average blank contribution was 0.15 pg g^{-1} (1σ) for a set of 11-times separated measurements. The equation for the external calibration using peak height for the atomic mass ^{202}Hg signal intensity was as following: $y==523213c++1354$, $R^2=0.999$, where y is ^{202}Hg signal intensity (expressed as cps) and c is the concentration of mercury (expressed as ng mL^{-1}). The limit of detection (LOD) of 0.5 pg g^{-1}, based on an external calibration, provided 350-fold improvement by using vapor generation compared to that using pneumatic liquid introduction. The improved transfer efficiency of mercury from the sample to the plasma source and the ionization processes taking place under "dry" plasma conditions attribute to the significant improvement in sensitivity. Furthermore, the LOD of the proposed method is superior to values obtained using different chemical vapor generation techniques, including $NaBH_4$ or $SnCl_2$ based CVG, as summarized in Table 2.

For the final quantitation of mercury in biological samples, the following ID and reverse ID equation was used [20]:

$$C = C_z \cdot \frac{m_y}{w \cdot m_x} \cdot \frac{m_z}{m_y'} \cdot \frac{A_y - B_y}{B_x \cdot R_n - A_x} \cdot \frac{B_z \cdot R_n' - A_z}{A_y - B_y \cdot R_n'} - C_b \quad (1)$$

where C is the blank corrected Hg concentration (μg g^{-1}) of analyte in the sample; C_z is the concentration of natural abundance Hg standard (μg g^{-1}); m_z is the mass of natural abundance Hg standard solution used (g); m'_y is the mass of enriched spike used to prepare the mixture of spike and natural abundance Hg standard solution (g); A_y is the abundance of the reference isotope (^{202}Hg) in the spike; B_y is the abundance of the spike isotope (^{201}Hg) in the spike; A_{xz} is the abundance of the reference isotope in the sample or natural abundance standard; B_{xz} is the abundance of the spike isotope in the sample or natural abundance standard; R_n is the measured reference/spike isotope ratio (mass bias corrected) in the blend solution of sample and spike; R'_n is the measured ratio of reference/spike isotopes (mass bias corrected) in the mixtures of spike and natural abundance standard; and C_b is the analyte concentration (μg g^{-1}) in the blank concentration normalized to sample mass m_x.

Ratios of $^{202}Hg/^{201}Hg$ measured in unspiked DORM-2, DORM-3 GBW09101b, and GBW07601a (GSH-1) solutions agreed with expected natural abundance values for these ratios, confirming the absence of any detectable polyatomic interferences arising from sample matrices. Analytical results obtained for the Hg concentrations in four CRMs by ID PVG–ICPMS detection are in good agreement with the certified values, as shown in Table 3. A subsequent comparative analysis was undertaken using external calibration method with PVG–ICPMS. As shown in Table 3, it is

Table 2
Comparison of the LODs for mercury using different techniques.

Sample matrix	Method	Reductant	LODs	Reference
Rice	CV–AFS	$SnCl_2$	5 ng g^{-1}	[35]
Cereals	CV–ID–ICP MS	$NaBH_4$	0.07 ng g^{-1}	[36]
Biological tissues	PVG–AAS	Formic acid	0.025–0.06 μg g^{-1}	[8]
River water	PVG–OES	Formic acid	250 pg mL^{-1}	[37]
Fish muscle tissues	UV-light emitting diode (LED)–PVG-AFS	Formic acid	0.01 μg L^{-1}	[24]
Biological samples	PVG–AAS	Formic acid	6 ng g^{-1}	[27]
Water samples	PVG–AFS	Formic acid	3 ng L^{-1}	[38]
Biological samples	PVG–ID–ICP MS	Formic acid	0.5 pg g^{-1}	This issue

Table 3
Analytical results of biological samples by the proposed method.

Samples	Detected[a] ($\mu g\ g^{-1}$)		Certified ($\mu g\ g^{-1}$)
	ID method	EC method	
DORM-2 (dogfish muscle)	4.38 ± 0.31	3.9 ± 0.21	4.64 ± 0.26
DORM-3 (fish protein)	0.403 ± 0.026	0.313 ± 0.036	0.382 ± 0.060
GBW07601a(GSH-1) (hair)	0.66 ± 0.05	0.43 ± 0.06	0.67 ± 0.10
GBW09101B (hair)	0.97 ± 0.03	0.78 ± 0.15	1.08 ± 0.28

[a] Average $\pm$ SD, $n=3$.

evident that the proposed PVG–ID–ICPMS method is superior to the external calibration method in terms of analytical accuracy and precision, especially for hair samples. This is because the PVG efficiency of sample solution is dependent on the received dose of UV irradiation, which is influenced by the residence time in the UV field as well as the sample matrix [21,27]. Clearly, the penetration of UV into the solution is more efficient for colorless standard solution than that for the opaque biological digests. Biological samples, especially the hair samples, produced brown or even black solution after formic acid digestion. However, the use of ID calibration can efficiently eliminate the matrix effect for the determination of mercury and obtain satisfactory analytical results. A *t*-test was applied to obtained results and confirmed no significant difference between the results obtained using the proposed method and the certified values at the confidence level of 95%.

4. Conclusion

An accurate and sensitive PVG ID–ICPMS method is developed for the accurate determination of total mercury in biological samples. The sample preparation and determination are quite simple in comparison with conventional sample digestion procedure. Furthermore, the use of ID efficiently eliminates the problem of low transmittance of UV irradiation in opaque sample solutions for PVG. The new technique is expected to have wide application for the determination of total mercury in biological samples, and the evaluation of human mercury exposure and the mercury pollution in the environment. In addition, species-specific ID in combination with liquid chromatography (LC)–PVG–ICP MS should be explored for its suitability for the speciation of mercury species.

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