Simple interface of high-performance liquid chromatography–atomic fluorescence spectrometry hyphenated system for speciation of mercury based on photo-induced chemical vapour generation with formic acid in mobile phase as reaction reagent

Yongguang Yin, Jingfu Liu, Bin He, Jianbo Shi, Guibin Jiang*

*State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences,
Chinese Academy of Sciences, P.O. Box 2871, Beijing 100085, China*

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Abstract

Photo-induced chemical vapour generation (CVG) with formic acid in mobile phase as reaction reagent was developed as interface to on-line couple HPLC with atomic fluorescence spectrometry for the separation and determination of inorganic mercury, methylmercury (MeHg), ethylmercury (EtHg) and phenylmercury (PhHg). In the developed procedure, formic acid in mobile phase was used to decompose organomercuries and reduce Hg^{2+} to mercury cold vapour under UV irradiation. Therefore, no post-column reagent was used and the flow injection system in traditional procedure is omitted. A number of operating parameters including pH of mobile phase, concentration of formate, flow rate of mobile phase, length of PTFE reaction coil, flow rate of carrier gas and $\text{Na}_2\text{S}_2\text{O}_3$ in sample matrix were optimized. The limits of detection at the optimized conditions were 0.085, 0.033, 0.029 and $0.038 \mu\text{g L}^{-1}$ for inorganic mercury, MeHg, EtHg and PhHg, respectively. The developed method was validated by determination of certified reference material DORM-2 and was further applied in analyses of seafood samples from Yantai port, China. The UV-CVG with formic acid simplifies the instrumentation and reduces the analytical cost significantly.

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1. Introduction

Compared with direct liquid-sample introduction, chemical vapour generation (CVG), as a liquid-sample introduction technique, including hydride generation, alkylation, halide generation, metal carbonyls or cold elemental vapour generation, can separate the analyte from the complex matrix, reduce interferences and improve detection sensitivity and selectivity [1]. Thus, CVG have many advantages in real sample analysis. CVG is widely used as a liquid-sample introduction technique for atomic spectroscopy and as interface for HPLC and atomic spectroscopy such as atomic absorption spectrometry (AAS) [2–4], inductively coupled plasma mass spectrometry (ICP-

MS) [5,6], atomic fluorescence spectrometry (AFS) [7–14] and atomic emission spectrometry (AES) [15]. Hydride or cold elemental vapour generation, as interface of HPLC and atomic spectroscopy is widely used for speciation of Hg, Sn, Se, As and other metals. The mostly used reagent for hydride or cold elemental vapour generation is potassium or sodium borohydride. But reduction by potassium or sodium borohydride has some disadvantages such as unstable reagent and interference of transitional metal ions. In the past years, photo-induced reduction or CVG was reported as a technique for remediation of heavy metal contamination [16–19]. This chemical vapour generation, in presence of organic acid under UV irradiation, was also coupled with atomic spectrometry for the analysis of selenium, mercury and other metal or non-metal element [20,21], which proved that the green, simple and selective UV-CVG is a useful new alternative to the conventional KBH_4 system. Further, a UV/nano- TiO_2 vapour generation device with formic acid

* Corresponding author. Fax: +86 10 62849179.
E-mail address: gbjiang@rcees.ac.cn (G. Jiang).

was adopted for coupling HPLC with ICP-MS for the determination of inorganic Se(IV) and Se(VI) [22]. Also, direct vapour generation of mercury species on nano-TiO₂ under UV irradiation in the presence of a formic acid and sodium formate mixture was developed as interface of HPLC–AFS for speciation of mercury [23]. Based on photo-induced CVG with formic acid instead of the conventional K₂S₂O₈/KBH₄ system, we recently developed an on-line coupled HPLC–AFS procedure for the speciation of inorganic mercury, methylmercury (MeHg), ethylmercury (EtHg) and phenylmercury (PhHg) [24]. This procedure was based on a post-column mix and reaction with formic acid. Under UV irradiation, the decomposition of organic mercury species and reduction of Hg²⁺ could be completed in one step [25] with this proposed photo-induced CVG system without nano-TiO₂ catalyst.

In this present work, we describe an on-line HPLC–AFS hyphenated system for mercury speciation with formic acid in mobile phase as reaction reagent for UV-CVG. Mercury species separated by HPLC was decomposed and reduced to mercury cold vapour under UV irradiation with the formic acid added in mobile phase. Because formic acid in the mobile phase was used as reaction reagent for UV-CVG, no other post-column reagent was used and the flow injection system in conventional procedure is not necessary, which dramatically simplified the hyphenated system. Optimum conditions for the new photo-chemical vapour generation procedure were studied, and the developed method was validated by determination of certified reference material.

2. Experimental

2.1. Instrumentation

Samples were injected by a Rheodyne model 7725i injection valve with a 100 µL sample loop (Rheodyne, Cotati, CA, USA) and the mobile phase was delivered by a P680 HPLC pump (Dionex, Sunnyvale, CA, USA). The HPLC separation of mercury species was achieved by using a Shim-pack CLC-ODS column (15 cm length, 6 mm I.D., 5 µm diameter particles, Shimadzu, Japan).

Chromatographic eluent entered into a polytetrafluoroethylene (PTFE) tube (0.5 m length, 0.8 mm I.D., 1.2 mm O.D.) wrapped around a 20 W mercury UV lamp, where the organomercuries were digested to Hg²⁺ and further reduced to cold mercury vapour. Then by using argon as carrier gas, the mercury vapour generated in the reaction was separated in a gas–liquid separator and brought to the AFS (AF-610A, Beijing Raileigh Analytical Instrument Company) for determination.

The experimental conditions for the developed UV-CVG with formic acid in mobile phase as reaction reagent are listed in Table 1.

2.2. Chemicals and reagents

Stock solution of standard inorganic mercury (1 mg mL^{−1} as Hg) was prepared by dissolving appropriate amount of HgCl₂ (≥99.5%, Beijing Chemical Factory, Beijing, China) in 5%

Table 1
Operating conditions for the developed HPLC–AFS system

Parameter	Optimized value
HPLC	
Column	Shim-pack CLC-ODS (Shimadzu), 15 cm length, 6.0 mm I.D., 5 µm
Mobile phase	A: 3% (v/v) CH ₃ CN, 240 mmol L ^{−1} HCOONH ₄ –HCOOH (pH 2.8), 0.01% 2-mercaptoethanol; B: 30% (v/v) CH ₃ CN, 240 mmol L ^{−1} HCOONH ₄ –HCOOH (pH 2.8), 0.01% 2-mercaptoethanol; 0–11 min: 100% A; 11–12 min: 100% A → 100% B; 12–30 min: 100% B; 30–31 min: 100% B → 100% A
Flow rate of mobile phase	1.4 mL min ^{−1}
Sample injection	100 µL
UV digestion and vapour generation	
Power of UV lamp	Mercury lamp, 20 W
Reaction coil	PTFE tube, 0.5 m length, 0.8 mm I.D., 1.2 mm O.D.
AFS	
Lamp	Mercury hollow cathode lamp, 253.7 nm
PMT voltage	280 V
Primary current	40 mA
Carrier gas	Ar, 500 mL min ^{−1}

(v/v) HNO₃. Individual stock solutions of standard organomercury chloride (1 mg mL^{−1} as Hg) were prepared by dissolving appropriate amounts of methylmercury chloride, ethylmercury chloride and phenylmercury chloride in methanol, respectively. All the organomercury compounds were purchased from Merck (≥98%, Darmstadt, Germany). The mixture standard of mercury species for injection was diluted from bulk solution daily and was dissolved in 10 mmol L^{−1} sodium thiosulfate.

Analytical grade formic acid, ammonia solution and sodium thiosulfate, as well as GR grade sodium hydroxide, potassium hydroxide and hydrochloric acid were from Beijing Chemical Factory (Beijing, China). Methanol and acetonitrile were HPLC solvent (J.T. Baker, Phillipsburg, NJ, USA). All other chemicals were analytical grade.

Mobile phase and standard solutions were prepared with de-ionized water from a Barnstead ultra-pure water system (Barnstead International, Dubuque, IA, USA).

2.3. Preparation of sample extracts

Certified reference material DORM-2 (from Institute for National Measurement Standards, National Research Council of Canada) and seafood samples were prepared with an alkaline digestion method [8]. Briefly, into a 50 mL glass centrifuge tube was added accurately weighed lyophilized and ground sample (~0.2 g) and 2 mL 25% (m/v) KOH (in methanol), and the tube was shaken overnight. Then, 6 mL of CH₂Cl₂ was added, and the solution was titrated by 1.5 mL concentrated HCl. The resulting solution was shaken for 15 min. After centrifuging at 3000 rpm for 15 min, the CH₂Cl₂ phase was accurately transferred into a 10 mL glass centrifuge tube and 1 mL of 10 mmol L^{−1} sodium thiosulfate was added. The mixture was shaken for 45 min.

After centrifuging at 3500 rpm for 15 min, the water phase was taken as sample for the determination of organomercuries by HPLC–AFS.

3. Results and discussion

3.1. Effect of mobile phase pH

HCOONH₄–HCOOH buffer was adopted in the chromatographic separation. Keeping the concentration of formate at 300 mmol L^{−1}, the pH of mobile phase was investigated in the range from 2.0 to 3.0. Mobile phase with pH 2.8 was found have relatively high sensitivities for detection and appropriate buffer capacity. Therefore, a mobile phase with pH 2.8 was used for further experiments. The elution order of four mercury species is MeHg, inorganic mercury, EtHg and PhHg. Both inorganic mercury and MeHg can form complex with 2-mercaptoethanol (ME) in mobile phase. Hg(II) and MeHg bind with two and one molecule of 2-mercaptoethanol to form complexes of Hg(II)–(ME)₂ and MeHg–ME, respectively. Hg(II)–(ME)₂ is more hydrophobic than MeHg–ME and has longer retention time on a C18 column. Therefore, inorganic mercury elutes after MeHg. Lower sensitivity was observed for Hg(II) than other organomercurial species in this UV–CVG system. Similar phenomena were observed in UV–CVG system with formic acid [24] and mercaptoethanol [26]. The reason is not known and further investigation is required to elucidate these phenomena.

3.2. Effect of formate concentration

The influence of formate concentration on the separation and detection of mercury species was studied in the range of 0–420 mmol L^{−1}, and the results were shown in Fig. 1. With no formate in the mobile phase, the four mercury species still can be detected with low sensitivity. This perhaps can be ascribed to

Fig. 1. Effect of formate concentration on the fluorescence intensities of mercury compounds. pH of mobile phase: 2.8. Length of PTFE reaction coil: 1.0 m. Flow rate of mobile phase: 1.2 mL min^{−1}. Concentration of mercury species: 100 μg L^{−1}. Other conditions were given in Table 1.

the generation of mercury vapour resulted from the UV irradiation of mercaptoethanol [26]. The signal intensity of inorganic mercury increased with the increasing of formate concentration, while the sensitivity of organic mercury species increased firstly and then decreased slightly. Considering the sensitivities of four mercury species, a formate concentration of 240 mmol L^{−1} was selected as a compromise.

3.3. Effect of mobile phase flow rate

The influence of mobile phase flow rate on separation was investigated in the range from 0.8 to 1.4 mL min^{−1}, with the gradient program shown in Table 1. When the mobile phase flow rate was lower than 1.2 mL min^{−1}, the signal intensities of mercury species were low and MeHg cannot be separated from inorganic mercury effectively. With a mobile phase flow rate of 1.4 mL min^{−1}, baseline separation of four mercury species was achieved in 30 min with acceptable column pressure (130 bar). Therefore, a flow rate of 1.4 mL min^{−1} was employed for later research.

3.4. Effect of length of PTFE reaction coil

Fig. 2 shows the influence of the length of PTFE reaction coil on the signal intensities of mercury species. For all the mercury species except for EtHg, the signal intensities increased with the reaction coil length in the range of 0.25–0.5 m. By using a 0.25 m PTFE reaction coil, the signal intensities were low, owing to the insufficient conversion efficiencies of organomercuries or Hg²⁺ to Hg⁰. With a PTFE reaction coil longer than 0.5 m, the signal intensities decreased due to further photo-oxidation of Hg⁰ [27]. For EtHg, however, the signal intensity decreased with the reaction coil length in the entire studied range, which might suggest EtHg is more easily converted to Hg²⁺ than the other species. Accordingly, in the following study, a 0.5 m PTFE tube as the reaction coil was employed to achieve maximum signal intensities for analyte. The irradiation time was about 11 s, as

Fig. 2. Effect of length of PTFE reaction coil on the fluorescence intensities of mercury compounds. Concentration of mercury species: 100 μg L^{−1}. Other conditions were given in Table 1.

Fig. 3. Effect of carrier gas flow rate on the fluorescence intensities of mercury compounds. Concentration of mercury species: $100 \mu\text{g L}^{-1}$. Other conditions were given in Table 1.

calculated from the flow rate and the inner volume of the PTFE reaction coil. The shorter reaction time than post-column mix [24] perhaps ascribed to a higher energy mercury UV lamp used.

3.5. Effect of carrier gas flow rate

The effect of argon gas flow rate on signal intensities of mercury species was studied in the range of $200\text{--}800 \text{ mL L}^{-1}$ (Fig. 3). The baseline noise was very high when the flow rate of argon gas was lower than 300 mL min^{-1} . Better baseline was observed with the increase of argon gas flow. However, the signal intensity decreased slowly with the flow rate of argon gas increasing. Low baseline noise and satisfactory signal intensities of mercury species were obtained at an argon carrier gas flow rate of 500 mL min^{-1} , which was chosen in the following study.

3.6. Effect of $\text{Na}_2\text{S}_2\text{O}_3$ in sample matrix

Since the final sample for injection contained $10 \text{ mmol L}^{-1} \text{Na}_2\text{S}_2\text{O}_3$, the influence of $\text{Na}_2\text{S}_2\text{O}_3$ in sample matrix on separation and detection was investigated. The result revealed that with the addition of $\text{Na}_2\text{S}_2\text{O}_3$ in the sample, the resolution of MeHg and inorganic mercury was improved and signal intensities of mercury species increased. The improvement of sensitivity was possibly induced by on-column concentration. $\text{Na}_2\text{S}_2\text{O}_3$ can form water soluble complex with organic or inorganic mercury

Fig. 4. Chromatogram of four mercury species using the developed HPLC–UV–CVG–AFS system. Concentration of mercury species: $10 \mu\text{g L}^{-1}$. Experimental conditions were given in Table 1.

species, which is weakly retained on a C18 column. When the injected sample was mixed with the mobile phase, however, 2-mercaptoethanol competed with $\text{Na}_2\text{S}_2\text{O}_3$ for binding mercury species to form mercury–mercaptoethanol complex, which is retained on a C18 column more strongly than $\text{Na}_2\text{S}_2\text{O}_3$ –mercury complex. Therefore, the migration velocity of mercury species was decreased which induced on-column stacking. Another advantage of the addition of $\text{Na}_2\text{S}_2\text{O}_3$ in sample is that it avoided the adsorption of mercury species on the injection loop. The separation and signal intensities of mercury species have no apparent change when the $\text{Na}_2\text{S}_2\text{O}_3$ concentration was varied from 2 to 12 mmol L^{-1} . Considering that the final sample for injection contained $10 \text{ mmol L}^{-1} \text{Na}_2\text{S}_2\text{O}_3$, all standard solution for injection was prepared in $10 \text{ mmol L}^{-1} \text{Na}_2\text{S}_2\text{O}_3$.

3.7. Analytical performance

Fig. 4 shows the typical chromatogram of MeHg, Hg, EtHg and PhHg standards at $10 \mu\text{g L}^{-1}$ levels, obtained with the optimized conditions. Table 2 shows the analytical features of the proposed method under optimized conditions. The detection limits based on three levels of the background were $0.033 \mu\text{g L}^{-1}$ for MeHg, $0.085 \mu\text{g L}^{-1}$ for Hg, $0.029 \mu\text{g L}^{-1}$

Table 2
Analytical performance of the developed HPLC–AFS system

Compound	Calibration curve ^a	Relation coefficient	Detection limit ($\mu\text{g L}^{-1}$)	RSD (%) ^b	Recovery (%) ^c
MeHg	$Y = 261.4X - 646.3$	0.9990	0.033	1.7	103
Hg(II)	$Y = 86.1X - 50.6$	0.9975	0.085	4.6	–
EtHg	$Y = 265.1X - 454.4$	0.9988	0.029	1.9	87.1
PhHg	$Y = 227.6X - 816.6$	0.9969	0.038	2.2	74.9

^a Y, peak area; X, concentration ($\mu\text{g L}^{-1}$).

^b Standard concentration, $20 \mu\text{g L}^{-1}$, $n = 3$.

^c Recovery for spiking with 20 ng (as Hg) of each individual mercury species.

Table 3

Comparison of method detection limits for mercury speciation using various HPLC-hyphenated techniques

	Detection limits (as Hg, pg)				Reference
	MeHg	EtHg	PhHg	Hg(II)	
HPLC–CV–AFS	3.3	2.9	3.8	8.5	This work
HPLC–CV–AFS	16.2	4.0	17.4	20.2	[24]
HPLC–CV–AFS	200	170	140	300	[8]
HPLC–CV–AFS	27	26	21	19	[9]
HPLC–CV–AFS	8	10	10	10	[11]
HPLC–CV–AFS	18	18	20	16	[12]
HPLC–CV–AFS	10	–	–	–	[14]
HPLC–CV–ICP–MS	6	5	–	9	[6]
HPLC–USN–ICP–MS	140	160	–	80	[28]
HPLC–ICP–MS	1.6	–	–	1.0	[29]
HPLC–CV–AAS	80	80	80	80	[7]
HPLC–CV–MIP–AES	35	–	–	15	[15]

for EtHg and $0.038 \mu\text{g L}^{-1}$ for PhHg, which correspond to an absolute detection limit of 3.3, 8.5, 2.9 and 3.8 pg (as Hg), respectively. A comparison of the detection limits obtained by other HPLC-hyphenated techniques for mercury speciation is given in Table 3. The detection limits of the present method are comparable with other HPLC-hyphenated techniques. The recoveries of the spikes of each organomercurial species of seafood sample ranged from 74.9 to 103%. The relatively lower recoveries of EtHg and PhHg might be ascribed to the dealkylation during extraction [30].

3.8. Validation of the method and application

In order to validate the method developed in the present study, MeHg content in certified reference material DORM-2 (dogfish muscle) was determined. As shown in Table 4, the determined result was in good agreement with the certified value. The proposed method was also applied to determine MeHg in seafood collected from Yantai port, Shandong Province of China, and results were also shown in Table 4. These results indicate that the developed method is applicable for the determination of MeHg in biological samples.

Table 4

Results of MeHg contents in DORM-2 and seafood samples

Sample	MeHg concentration (mean ^a ± SD ^b , ng g ⁻¹)	Certified value (mean ± SD ^b , ng g ⁻¹)
DORM-2	$4.19 \times 10^3 \pm 0.07 \times 10^3$	4470 ± 320
<i>Ruditapes philippinarum</i> (Yantai)	16.3 ± 1.1	–
<i>Sinonovacula constricta</i> (Yantai)	33.2 ± 3.0	–
<i>Neverita didyma</i> (Yantai)	71.1 ± 1.7	–
<i>Saxidomus purpuratus</i> (Yantai)	78.9 ± 7.9	–
<i>Scapharca subcrenata</i> (Yantai)	16.8 ± 1.1	–

^a $n = 3$.

^b Standard deviation.

4. Conclusions

A novel photo-induced chemical vapour generation method with formic acid in mobile phase was developed as interface of HPLC–AFS hyphenated system for the speciation of inorganic mercury, MeHg, EtHg and PhHg. In the developed hyphenated systems, formic acid in mobile phase was used as reaction reagent for UV–CVG, therefore no other post-column reaction reagent and pump system were needed, which simplified instrumentation and decreased analytical cost significantly.

The developed UV–CVG system with formic acid in mobile phase as reaction reagent was expected to be applicable in speciation of other organometallic compounds. Also, the novel CVG system developed could be applied as interface for HPLC with other analytical atomic spectrometric techniques. Moreover, other energy irradiations besides UV, such as microwave [8], heat [31] and supersonic energy [31,32] were also expected to be applied in the hyphenated system based on CVG with formic acid.

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