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ORIGINAL PAPER

Mercury speciation by a high performance liquid chromatography—atomic fluorescence spectrometry hyphenated system with photo-induced chemical vapour generation reagent in the mobile phase

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Abstract Speciation of mercury was accomplished by using a simple interface with photo-induced chemical vapour generation in a high performance liquid chromatography—atomic fluorescence spectrometry (HPLC-AFS) hyphenated system. Acetic acid and 2-mercaptoethanol in the mobile phase were used as photochemical reagent. The operating parameters were optimized to give limits of detection of 0.53 μg L^{-1}, 0.22 μg L^{-1}, 0.18 μg L^{-1} and 0.25 μg L^{-1} for inorganic mercury, methylmercury, ethylmercury and phenylmercury, respectively. The method was validated with the certified reference material DORM-2 and applied to the analysis of seafood samples. The HPLC-AFS hyphenated system is simple, environmentally friendly, and represents an attractive alternative to the conventional peroxothiosulfate-borohydride method.

Keywords UV-induced chemical vapour generation · Speciation · Mercury · High performance liquid chromatography · Atomic fluorescence spectrometry · Mercaptoethanol

Introduction

Photo-induced chemical vapour generation (CVG), introduced by Guo et al. [1, 2] is a new alternative sample introduction technique to traditional chemical vapour generation [3, 4]. In this novel sample introduction method, volatile species were generated under UV irradiation with low molecular weight organic acid. It has many advantages such as avoiding the use of unstable potassium or sodium borohydride and decreasing interference of transitional metal ions. This technique can be applied to the analysis of conventional hydride-forming elements transition metals, noble metals, Hg and nonmetals such as I and S [2]. Low molecular weight organic acid such as formic, acetic and propionic acid, were the mostly used photo-reduction reagents, but other chemicals such as aldehyde and alcohol were also reported [5].

Photo-induced chemical vapour generation was also used as a novel interface of liquid chromatography and atomic spectrometry such as atomic fluorescence spectrometry (AFS) and inductively coupled plasma- mass spectrometry (ICP-MS) for elemental speciation analysis. An on-line HPLC-UV/nano-TiO_2-ICP-MS system was developed for the determination of Se (IV) and Se (VI) in water samples [6]. Nano- TiO_2 can catalyze the reduction of Se(VI) by formic acid under UV irradiation, which significantly improves the detection sensitivity of Se(VI) in comparison with conventional hydride generation ICP-MS and MCN-ICP-MS. Moreover, UV-induced photocatalytic reactions can not only reduce metal cation to its zero or low-valent species, but also degrade organometallic species to inorganic species [7]. Therefore, photo-induced chemical vapour generation was coupled with HPLC and AFS for the speciation analysis of mercury [8, 9]. Under UV irradiation, chromatographic eluent was mixed with formic acid in the presence [8] or absence [9] of TiO_2 photo-catalysis. Organic mercury species were decomposed and reduced to elemental mercury vapour in one step. Further, we developed a simple photo-induced chemical vapour generation interface for HPLC-AFS hyphenated system [10].

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In this system, formic acid in mobile phase was used as photochemical reaction reagent, which significantly simplified the instrumentation as no post-column flow injection system was need.

Recently, mercaptoethanol was reported to be an effective photo-chemical reagent in UV-CVG for determination of total mercury and methylmercury [11]. Also, mercaptoethanol was usually used as an effective mobile phase component to improve the HPLC separation of mercury species [12]. Therefore, for the purpose of simplified instrumentation, it is a good choice to use UV-CVG based on mercaptoethanol as interface of HPLC and atomic spectrometry. Using acetate and mercaptoethanol in mobile phase as photochemical reagent, a novel UV-CVG interface was developed for HPLC-AFS in this study. Speciation analysis of four mercury species, *viz.* Hg(II), methylmercury, ethylmercury and phenylmercury, was demonstrated and operation parameters were optimized.

Experimental

Instrumentation

Atomic fluorescence spectrometer (AFS 610A) (Raileigh Analytical Instrument Corporation, Beijing, China) was coupled with a P680 HPLC pump (Dionex, Sunnyvale, USA) for speciation analysis of mercury. The HPLC separation was carried out by using a Shim-pack CLC-ODS column (15 cm×6 mm I.D.×5 μm, Shimadzu, Kyoto, Japan).

Chromatographic eluent entered into a polytetrafluoroethylene (PTFE) tube (1.0 m×0.8 mm I.D.×1.2 mm O.D.) wrapped around a 20 W mercury UV lamp (253.7 nm, ZW20S26W, Beijing Lighting Research Institute). Then by using argon as carrier gas, the mercury vapor generated in the photo-reaction was separated in a gas-liquid separator and brought to the AFS for the determination. The operational parameters of the HPLC-AFS hyphenated system are shown in Table 1.

Table 1 Operating conditions for the developed HPLC-AFS system

Parameter	Optimized value
HPLC	
Column	Shim-pack CLC-ODS (Shimadzu), 15cm× 6.0mm ID×5μm
Mobile phase	A: 3% (*v*/*v*) CH_3CN, 60mmolL^{-1} NH_4Ac (pH 6.9), 0.02% 2-mercaptoethanol; B: 30% (*v*/*v*) CH_3CN, 60mmolL^{-1} NH_4Ac (pH6.9), 0.02% 2-mercaptoethanol
	0–11min: 100% A; 11–12min: 100%A→100% B; 12 –30min: 100%B; 30–31min: 100% B→100% A
Flow rate of mobile phase	1.4mLmin^{-1}
Sample injection	100μL
UV digestion and vapor generation	
Power of UV lamp	Mercury lamp, 20W
Reaction coil	PTFE tube, 1.0m×0.8mm I.D.×1.2mm O.D.
AFS	
Lamp	Mercury hollow cathode lamp, 253.7nm
PMT voltage	280V
Primary current	40mA
Carrier gas	Ar, 500mLmin^{-1}

Chemicals and reagents

Stock solution of standard inorganic mercury (1 mg mL^{-1} as Hg) was prepared by dissolving appropriate amount of $HgCl_2$ (≥99.5%, Beijing Chemical Factory, Beijing, China) in 5% (*v*/*v*) HNO_3 (Merck, Darmstadt, Germany). Individual stock solutions of standard organomercury chloride (1 mg mL^{-1} as Hg) were prepared by dissolving appropriate amounts of methylmercury chloride (MeHg), ethylmercury chloride (EtMg) and phenylmercury chloride (PhHg) 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. 2-mercaptoethanol (≥98%) was from Alfa Aesar (Ward Hill, MA, USA). Analytical grade ammonia acetate, 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 from J. T. Baker (Phillipsburg, NJ, USA). All other chemicals were analytical grade.

Safety note: Organic mercury species are very toxic. Wearing appropriate protective clothing and glove is necessary in the solution preparation procedure. Elevated levels of mercury should be handled in a glovebox with precaution.

Preparation of sample extracts

An alkaline digestion method based on the procedure reported by Cai et al. [13] was used to extract MeHg from fish and mollusks sample collected from Weihai port. Briefly, 0.1–0.2 g pooled, homogenized and freeze-dried sample or DORM-2 (from Institute for National Measurement Standards, National Research Council of Canada, Ottawa, Ontario, Canada) was accurately weighed into a 50 mL glass vial, and 2 mL of 25% (*w*/*v*) KOH in methanol

were added. The vial then was shaken overnight, and 6 mL of CH_2Cl_2 was added. The 1.5 mL concentrated HCl was added into the resulting solution drop by drop slowly. After centrifuging at 3,000 rpm for 15 min, the CH_2Cl_2 phase was accurately taken into a 10 mL glass vial and 1 mL of 10 mmol L^{-1} sodium thiosulfate was added. The mixture was shaken for 45 min and after centrifuging at 3,500 rpm for 15 min, the water phase was injected for the determination of organomercurils by HPLC-AFS.

Results and discussion

Effect of mobile phase pH

A typical chromatogram of MeHg, Hg(II), EtHg and PhHg standard at 100 μg L^{-1} (as Hg) levels was given in Fig. 1. The effect of mobile phase pH on the signal intensities of mercury species was investigated from 3.2 to 7.4 with 60 mmol L^{-1} acetate, as shown in Fig. 2. The signal intensities of mercury species increased significantly with pH up to 6.8, and then decreased with the further increase of pH. Thus, pH 6.8 was selected as the optimum of mobile phase.

Effect of acetate concentration

Effect of acetate concentration, in the range of 0–80 mmol L^{-1}, on the signal intensities of four mercury species was investigated. Owning to the photo-degradation and reduction by 2-mercaptoethanol [11], four mercury species still could be detected even though no acetate was present in mobile phase. With the increasing of acetate concentration, the signal intensities and column pressure increased slightly. Sixty mmol L^{-1} ammonia acetate was selected as a compromise of the signal intensities and column pressure.

Effect of mercaptoethanol concentration

In this method, concentration of mercaptoethanol in mobile phase play a very important role as it function as a reagent for both separation and photo-induced chemical vapour generation. The effect of mercaptoethanol concentration on separation and detection was studied in the range from 0–0.2% (*v*/*v*) and the result was shown in Fig. 3. When mobile phase contained no mercaptoethanol, the four mercury species can not be separated effectively. While, the signals of Hg(II) and organomercurials still could be observed owning to photoreduction by acetic acid. The separation of four mercury species was not apparently influenced within the mercaptoethanol concentration range of 0.005–0.2% (*v*/*v*). However, the signal intensities increased and then decreased significantly with the increasing mercaptoethanol concentration except for Hg(II). A similar phenomenon was also observed by Yin et al. [11] in a vapor generation system for total mercury and MeHg analysis based on the UV irradiation of mercaptoethanol, although a much lower

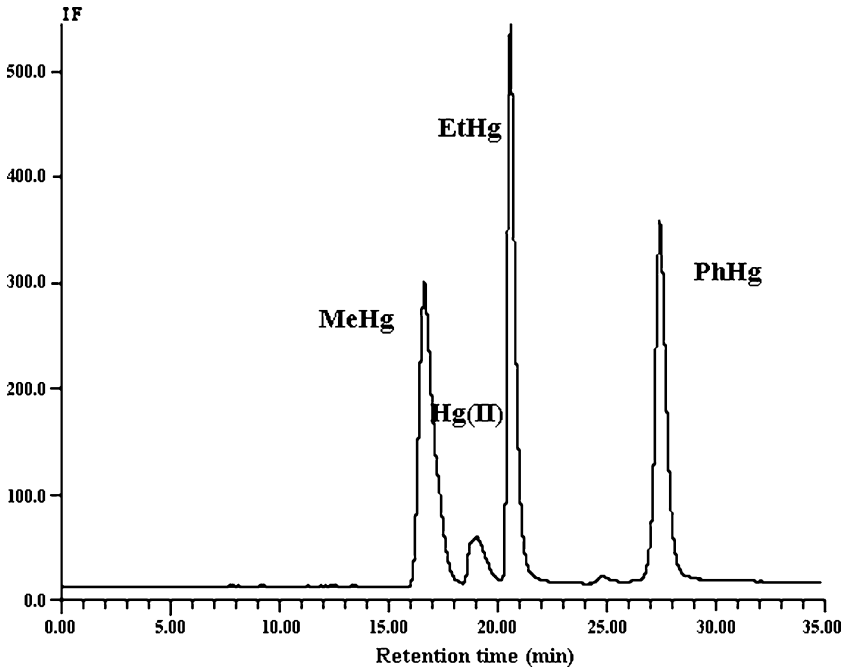

Fig. 1 Typical chromatogram of four mercury species using the HPLC-UV-CVG-AFS system. Concentration of mercury species: 100 μg L^{-1}. Experimental conditions were given in Table 1

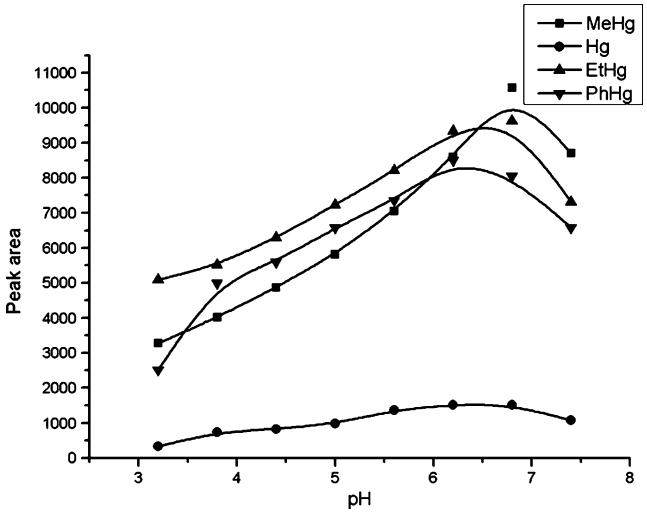

Fig. 2 Effect of mobile phase pH on the fluorescence intensities of mercury compounds. The concentration of acetic acid was 60 mmol L^{-1}. Concentration of 2-mercaptoethanol was 0.05% (v/v). Concentration of mercury species: 100 μg L^{-1}. Other conditions were given in Table 1

mercaptoethanol concentration was applied in the present research. Therefore, 0.02% mercaptoethanol was applied as an optimized condition in later studies.

Effect of mobile phase flow rate

The influence of mobile phase flow rate on separation was investigated using the same gradient dilution program, as shown in Fig. 4. The signal intensities of mercury species increased with the mobile phase flow rate, perhaps owing to

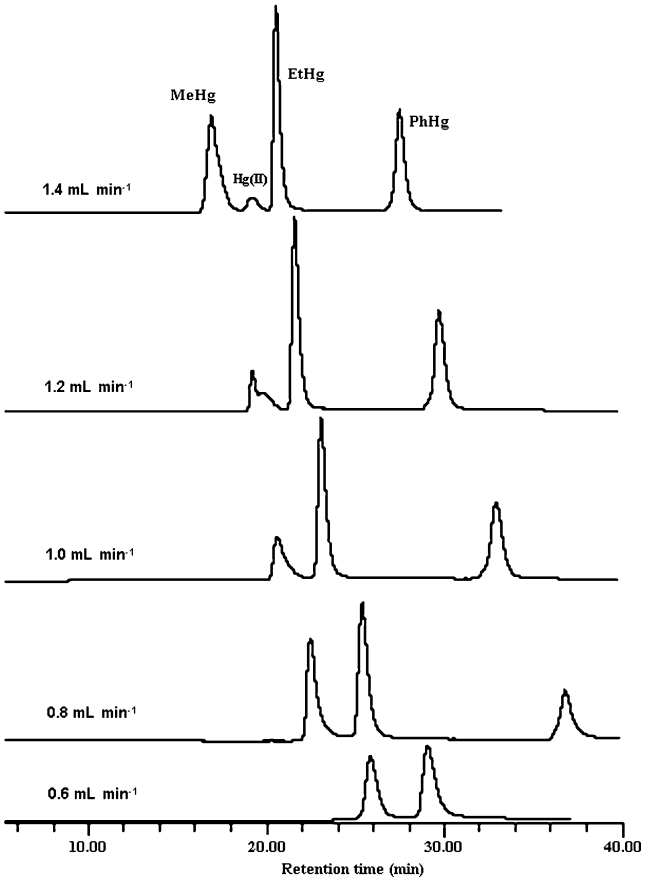

Fig. 4 Effect of mobile phase flow rate on the separation of mercury compounds. Concentration of mercury species: 100 μg L^{-1}. From bottom to up, flow rate of mobile phase was 0.6 mL min^{-1}, 0.8 mL min^{-1}, 1.0 mL min^{-1}, 1.2 mL min^{-1} and 1.4 mL min^{-1}, respectively. Other conditions were given in Table 1

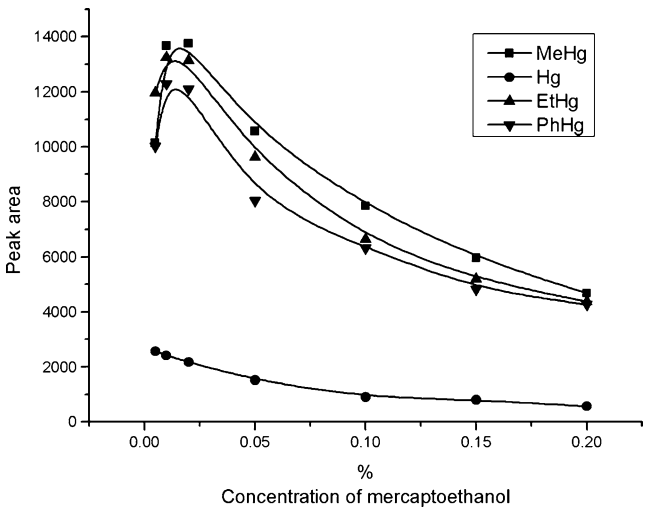

Fig. 3 Effect of 2-mercaptoethanol concentration on the fluorescence intensities of mercury compounds. Concentration of mercury species: 100 μg L^{-1}. Other conditions were given in Table 1

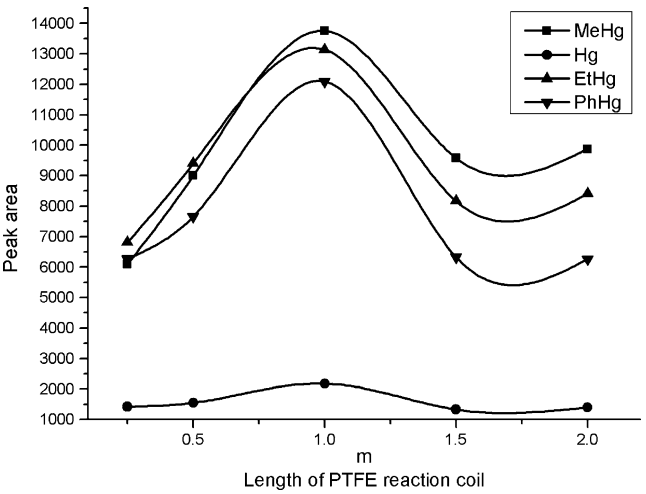

Fig. 5 Effect of length of PTFE reaction coil on the fluorescence intensities of mercury compounds. Concentration of mercury species: 100 μg L^{-1}. Other conditions were given in Table 1

Table 2 Analytical performance of the developed HPLC-AFS system

[a] Standard concentration, 20 μg L^{-1}, n=3

[b] Peak aera, mAU s

[c] Concentration, μg L^{-1} (as Hg)

Compound	Calibration curve	Relation coefficient	Detection limit (μgL^{-1})/(pg)	RSD%[a]
MeHg	Y^{b}=149.8X^{c}−530.6	0.9983	0.22/22	3.84
Hg	Y=18.3X−384.3	0.9767	0.53/53	8.74
EtHg	Y=126.6X+343.5	0.9990	0.18/18	4.74
PhHg	Y=113.1X−32.4	0.9971	0.25/25	6.70

a better pulverization and gas-liquid separation in the gas-liquid separator at higher flow rate. Moreover, MeHg and Hg(II) can be baseline separated only when the flow rate of mobile phase was higher than 1.2 mL min^{-1}. A flow rate of 1.4 mL min^{-1} was selected in the following experiments.

Effect of length of PTFE reaction coil

The length of PTFE reaction coil determines the UV irradiation time, which is very important for rapid transformation of Hg(II) or organic mercury to Hg(0). Figure 5 shows the effect of length of PTFE reaction coil on signal intensities of four mercury species. The signal intensities of all the mercury species firstly increased with the increasing of reaction coil length in the range of 0.25–1.0 m, then decreased with reaction coil length of 1.0–2.0 m. The signal intensities were low when using PTFE reaction coil shorter than 1.0 m, owning to the incomplete conversion of organomercuries or Hg^{2+} to Hg^{0}. With a PTFE reaction coil longer than 1.0 m, the signal intensities decreased. The concentration of Hg(II) in the liquid phase of the gas-liquid separator was determined using reaction coil with different length. Results showed that concentration of Hg (II) decreased with the increasing reaction coil length. Therefore, the decrease of signal should not be ascribed to photo-oxidation but to the raising dead volume. Therefore, in the later research, PTFE tube with 1.0 m length was used as the reaction coil. The irradiation time was about 22 s, as calculated from the inner volume of the PTFE reaction coil and the flow rate of mobile phase. The optimized irradiation time is consistent with the reported UV-CVG based on mercaptoethanol [11], but longer than the UV-CVG based on formic acid [10], which indicates relatively slow reaction kinetics of UV-CVG based on mercaptoethanol.

Effect of carrier gas flow rate

The effect of argon carrier gas flow rate on detection of four mercury species was studied from 200 mL min^{-1} to 800 mL min^{-1}. The baseline was not stable when the flow rate of argon gas was lower than 300 mL min^{-1}, which perhaps induced by the instability of the argon gas flow rate. Better baseline could be obtained with the increasing of argon gas flow. While the signal intensity decreased slowly with the increase of argon gas flow rate, owning to dilution and short residence time of volatile mercury vapour in the atomizer. Therefore, an argon carrier gas flow rate of 500 mL min^{-1} was chosen as an optimized condition to maintain a high sensitivity.

Mechanism of photo-induced chemical vapour generation

UV irradiation induces the transformation of organomercuiral and inorganic mercury compounds to Hg(0). Acetate and mercaptoethanol play the role of reaction reagent in the photo-chemical reaction. However, the mechanism of this process is not clearly understood.

Table 3 Comparison of method detection limits for mercury speciation using various HPLC-AFS hyphenated techniques

	Detection limits (as Hg)/pg				Reference
	MeHg	EtHg	PhHg	Hg(II)	
HPLC-UV-CVG-AFS	22	18	25	53	This work
HPLC-UV-CVG-AFS	16.2	4.0	17.4	20.2	9
HPLC-microwave-CVG-AFS	200	170	140	300	16
HPLC- knotted reactor-CVG-AFS	27	26	21	19	17
HPLC-UV-CVG-AFS	8	10	10	10	18
HPLC-Ar/H_2 flame-CVG-AFS	18	18	20	16	19
HPLC-UV-CVG-AFS	10	–	–	–	20

Table 4 Results of MeHg contents in DORM-2 and seafood samples

Sample	MeHg concentration (mean[a] ± S.D.[b], ngg^{-1})	Certified value (mean ± S.D.[b], ngg^{-1})
DORM-2	4150±50	4470±320
Meretix meretrix	62.0±11.9	–
Scapharca subcrenata	17.1±3.0	–
Neverita didyma	24.4±1.9	–
Mytilus edulis	30.1±0.2	–
Chlamys farreri	71.4±1.7	–

[a] n=3

[b] Standard deviation

Guo et al. [1] proposed a mechanism for photo-reduction of Hg(II) by acetic acid based on free radical generation:

$$CH_3COOH + h\upsilon \rightarrow CH_3CO\cdot + \cdot OH \rightarrow CO + CH_3OH \quad (1)$$

The generated CO radical reduces Hg(II) to Hg(0). While, Bendl et al. [14] suggested reducing species CH_2CO generated in the photo-reduction process according to (2).

$$CH_3COOH + h\upsilon \rightarrow CH_3COOH^* \rightarrow H_2O + CH_2CO \quad (2)$$

The resulting CH_2CO reduces Hg(II) to Hg(0). While, Takatani et al. [15] applied computational methods to propose a possible mechanism in the absence of free radicals for photoreduction of Se(IV) by formic acid. Significant work still should be done to further elucidate the mechanism of photoreduction by low molecular weight organic acids.

Under UV irradiation, mercaptoethanol can transform Hg(II) and methylmercury to Hg(0). Yin et al. [11] found that both mercapto and hydroxyl group in the mercaptoethnol molecule play important role in the photochemical reaction. Based on the product analysis by GC-MS, a possible mechanism for the generation of Hg vapor via UV irradiation of mercaptoethanol was proposed [11]. Under UV irradiation, mercapotoethnol was firstly transformed to $HOCHCH_2SH$ radical, then to $HOCH_2CH_2S$, which is further oxidized by Hg(II) to $HOOCCH_2S$, while Hg(II) was reduced to Hg(0).

Analytical performance

Analytical features of the method were showed in Table 2. The RSD (n= 3) was lower than 7% for MeHg, EtHg and PhHg species, whereas the RSD of Hg(II) was slightly higher. The concentration detection limits based on three levels of the background were 0.22 μg L^{-1} for MeHg, 0.53 μg L^{-1} for Hg(II), 0.18 μg L^{-1} for EtHg, and 0.25 μg L^{-1} for PhHg (as Hg), respectively. Comparison of the detection limits of different HPLC-AFS hyphenated systems for mercury speciation analysis is given in Table 3. The detection limits of the new methods are comparable with traditional $K_2S_2O_8$/KBH_4 system.

Validation of the method and application

The accuracy of the method was evaluated by analyzing MeHg content in certified reference material DORM-2 (dogfish muscle). The results are summarized in Table 4, which shows good agreement between the determined result and the certified value. MeHg in mollusks from Weihai, Shandong Province was also determined and results were shown in Table 3. There was no certified reference material for EtHg and PhHg. Therefore, spiking recoveries were investigated for organomercurial species. The recoveries of MeHg, EtHg, and PhHg by spiking organomercurials to mollusks samples were 98.7%, 89.3%, and 79.5%, respectively. These results indicated that the method is suitable for determination of organomercurial species in biological samples.

Conclusions

A simple interface of HPLC-AFS without using any post-column reagent and flow injection system was constructed for speciation analysis of mercury. Species of mercury were decomposed and reduced to Hg(0) by acetic acid and 2-mercaptoethanol in mobile phase under UV irradiation, which dramatically decreased reagent cost, simplified instrumentation and minimized pollution to environment. The simple UV-CVG system was a good alternative to conventional $K_2S_2O_8$/KBH_4 system and was expected to be used for speciation analysis of other element and coupled with other atomic spectrometric techniques.

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References

1. Guo X, Sturgeon RE, Mester Z, Gardner GJ (2003) UV vapor generation for determination of selenium by heated quartz tube atomic absorption spectrometry. Anal Chem 75:2092
2. Guo X, Sturgeon RE, Mester Z, Gardner GJ (2004) Vapor generation by UV irradiation for sample introduction with atomic spectrometry. Anal Chem 76:2401
3. Su YY, Xu KL, Gao Y, Hou XD (2008) Determination of trace mercury in geological samples by direct slurry sampling cold vapor generation atomic absorption spectrometry. Microchim Acta 160:191
4. He Y, Hou X, Zheng C, Sturgeon R (2007) Critical evaluation of the application of photochemical vapor generation in analytical atomic spectrometry. Anal Bioanal Chem 388:769
5. Han C, Zheng C, Wang J, Cheng G, Lv Y, Hou X (2007) Photo-induced cold vapor generation with low molecular weight alcohol, aldehyde, or carboxylic acid for atomic fluorescence spectrometric determination of mercury. Anal Bioanal Chem 388:825
6. Sun YC, Chang YC, Su CK (2006) On-line HPLC-UV/nano-TiO_2-ICPMS system for the determination of inorganic selenium species. Anal Chem 78:2640
7. Zheng C, Li Y, He Y, Ma Q, Hou X (2005) Photo-induced chemical vapor generation with formic acid for ultrasensitive atomic fluorescence spectrometric determination of mercury: potential application to mercury speciation in water. J Anal At Spectrom 20:746
8. Yin Y, Liang J, Yang L, Wang Q (2007) Vapour generation at a UV/TiO_2 photocatalysis reaction device for determination and speciation of mercury by AFS and HPLC-AFS. J Anal At Spectrom 22:330
9. Yin Y, Liu J, He B, Gao E, Jiang G (2007) Photo-induced chemical vapour generation with formic acid: novel interface for high performance liquid chromatography-atomic fluorescence spectrometry hyphenated system and application in speciation of mercury. J Anal At Spectrom 22:822
10. Yin Y, Liu J, He B, Shi J, Jiang G (2008) 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. J Chromatogr A 1181:77
11. Yin Y, Qiu J, Yang L, Wang Q (2007) A new vapor generation system for mercury species based on the UV irradiation of mercaptoethanol used in the determination of total and methyl mercury in environmental and biological samples by atomic fluorescence spectrometry. Anal Bioanal Chem 388:831
12. Harrington CF (2000) The speciation of mercury and organomercury compounds by using high-performance liquid chromatography. Trends Anal Chem 19:167
13. Cai Y, Tang G, Jaffé R, Jones R (1997) Evaluation of some isolation methods for organomercury determination in soil and fish samples by capillary gas chromatography: Atomic fluorescence spectrometry. Int J Environ Anal Chem 68:331
14. Bendl RF, Madden JT, Regan AL, Fitzgerald N (2006) Mercury determination by cold vapor atomic absorption spectrometry utilizing UV photoreduction. Talanta 68:1366
15. Takatani T, Fitzgerald N, Galbraith JM (2007) Proposed reaction mechanisms for selenium UV photolysis vapor generation by computational methods. Anal Bioanal Chem 388:859
16. Liang L, Jiang G, Liu J, Hu J (2003) Speciation analysis of mercury in seafood by using high-performance liquid chromatography on-line coupled with cold-vapor atomic fluorescence spectrometry via a post column microwave digestion. Anal Chim Acta 477:131
17. Li Y, Yan X, Dong L, Wang S, Jiang Y, Jiang D (2005) Development of an ambient temperature post-column oxidation system for high-performance liquid chromatography on-line coupled with cold vapor atomic fluorescence spectrometry for mercury speciation in seafood. J Anal At Spectrom 20:467
18. Falter R, Ilgen G (1997) Coupling of the RP C18 preconcentration HPLC-UV-PCO system with atomic fluorescence detection for the determination of methylmercury in sediment and biological tissue. Fresenius J Anal Chem 358:407
19. Bramanti E, Lomonte C, Onor M, Zamboni R, D'Ulivo A, Raspi G (2005) Mercury speciation by liquid chromatography coupled with on-line chemical vapour generation and atomic fluorescence spectrometric detection (LC-CVGAFS). Talanta 66:762
20. Ramalhosa E, Segade SR, Pereira E, Vale C, Duarte A (2001) Simple methodology for methylmercury and inorganic mercury determinations by high-performance liquid chromatography-cold vapour atomic fluorescence spectrometry. Anal Chim Acta 448:135