J A A S

Journal of Analytical Atomic Spectrometry

www.rsc.org/jaas

Volume 25 | Number 6 | June 2010 | Pages 749–904

Hg

HCl & KBH₄

L-Cysteine

ISSN 0267-9477

RSC Publishing

HOT PAPER

Jiang *et al.*

L-cysteine-induced degradation of organic mercury as a novel interface in the HPLC-CV-AFS hyphenated system for speciation of mercury

TUTORIAL REVIEW

Barwick and Wood

Achieving metrological traceability in chemical and bioanalytical measurement

HOT PAPER

Santos Jr. *et al.*

Evaluation of laser induced breakdown spectrometry for the determination of macro and micronutrients in pharmaceutical tablets

L-cysteine-induced degradation of organic mercury as a novel interface in the HPLC-CV-AFS hyphenated system for speciation of mercury†

Zhen-hua Wang, Yong-guang Yin, Bin He, Jian-bo Shi, Jing-fu Liu and Gui-bin Jiang*

Received 18th November 2009, Accepted 12th February 2010

First published as an Advance Article on the web 15th April 2010

DOI: 10.1039/b924291k

By adding L-cysteine into the mobile phase of chromatography, a simple on-line high performance liquid chromatography-cold vapor generation-atomic fluorescence spectrometry (HPLC-CV-AFS) method for separation and determination of divalent mercury (Hg^{2+}), monomethylmercury (MMC) and ethylmercury (EMC) was developed. Compared with the conventional HPLC-UV-CV-AFS hyphenated systems, the proposed HPLC-CV-AFS system is very simple and low cost as no strong oxidant, no additional post-column interface, such as ultraviolet or microwave, and no organic solvent were needed. Parameters influencing mercury separation and determination were optimized in detail. The detection limits for MMC, EMC and Hg^{2+} , based on three folds of baseline noise and 100 μL injection, were 0.05, 0.07, and 0.1 $\mu\text{g L}^{-1}$, respectively. The relative standard deviation (RSD) for three parallels determinations varied from 1.4 to 2.5% at a concentration level of 50 $\mu\text{g L}^{-1}$. The proposed method was successfully applied to analyze two certified reference material, DORM-2 (dogfish muscle) and TORT-2 (lobster hepatopancreas), and biological samples.

Introduction

Mercury is harmful to human beings and biological bodies with high bioaccumulation and toxicity. The toxicity and bioavailability of mercury are strongly dependent on its species. Monomethylmercury (MMC), a well-documented neurotoxicant, may cause adverse effects on the development of brain and is difficult to be expelled from biological bodies. Neurological and behavioral disorders in humans have also been observed after inhalation of elemental mercury vapor. Therefore, it is important to analyze the species when assessing the toxicity and health risks of mercury.^{1–3}

In recent years, many methods have been developed for mercury speciation based on high performance liquid chromatography (HPLC) hyphenated with element specific detectors. Among these methods, reversed phase HPLC with a mobile phase containing organic modifier, chelating, ion-pair reagent, or pH buffer was mostly used for separation of different mercury species. However, organic solvents such as methanol and acetonitrile used in the mobile phase are harmful to operators and may contaminate the environment when disposed of.³

Element specific detectors coupled with HPLC included inductively coupled plasma mass spectrometry (ICP-MS),^{1,4–6} atomic absorption spectrometry (AAS)^{7,8} and atomic fluorescence spectrometry (AFS).^{9–11} Because of its high sensitivity and selectivity, as well as low running cost, atomic fluorescence spectrometry (AFS) has been widely used as the elemental specific detector in speciation of Hg, As and Se.^{12–15}

Elemental mercury vapor can be generated by borohydride¹⁰ or stannous chloride.⁶ Recently many new methods have been developed to generate mercury vapor for determination by AFS including photo-induced chemical vapor generation such as low molecule weight alcohol, aldehyde, carboxylic acid, mercaptoethanol^{15–20} and coating of thin layer of NaBH_4 solution,¹¹ intermittent flow electrochemical cold vapor generation,²¹ ultrasound-assisted vapor generation.^{22,23}

However, before mercury vapor generation organic mercury species are usually necessary to be converted to divalent mercury (Hg^{2+}) and then produce fluorescence signal in AFS. Therefore, post-column oxidation using strong oxidant, with the assistance of UV,^{7,8,24} microwave²⁵ or external heating sources,²⁶ was adopted to realize this on-line conversion. The strong oxidants used include potassium dichromate,²⁷ acidic potassium persulfate,²⁸ bromine generated by KBr/KBrO_3 in HCl medium,²⁹ and formic acid.²⁰ However, these post-column interfaces or strong oxidants could cause the complication and instability of the HPLC-CV-AFS system.

In this work, a simple HPLC-CV-AFS hyphenated system was developed for the separation and determination of divalent mercury, monomethylmercury and ethylmercury. In this HPLC-CV-AFS system, the organic mercury compounds could be on-line converted to elemental Hg in the presence of L-cysteine, HCl and KBH_4 without thus post-column interface, strong oxidants and organic solvents. The proposed method has been successfully applied to analyze mercury species in the certified reference material (DORM-2 (dogfish muscle) and TORT-2 (lobster hepatopancreas)).

Experimental

Instrumentation

The on-line HPLC-CV-AFS hyphenated system employed was shown in Fig. 1. The HPLC system consisted of an Elite P200II

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. E-mail: gbjiang@rcees.ac.cn; Fax: +86-10-62849179

† Electronic supplementary information (ESI) available: Fig. S1–4. See DOI: 10.1039/b924291k

Fig. 1 Schematic illustration of HPLC-CV-AFS system.

pump (Dalian Elite Analytical Instruments Co., Ltd., Dalian, China) and a Rheodyne model 7725i injector (Rheodyne, Cotati, CA, USA) equipped with a 100 μL sample loop. The separation was performed on a Shim-pack CLC-ODS column (15 cm $\times$ 6 mm I.D. $\times$ 5 μm , Shimadzu, Japan). The determination was performed on an AF-610D2 atomic fluorescence spectrophotometer (Beijing Rayleigh Analytical Instrument Corporation, Beijing, China) with mercury hollow cathode lamp (HCL) (General Research Institute of Non-Ferrous Metals, Beijing, China) employed as the radiation source. A laboratory microwave system (MARSSX, CEM, NC, USA) was used for sample preparation in determination of total mercury.

The HPLC system was coupled with AFS instrument using PEEK (0.1 mm I.D. $\times$ 1/16 mm O.D.) and polytetrafluoroethylene (PTFE) tube (0.8 mm I.D. $\times$ 1.2 mm O.D.). The effluent from separation column was introduced into AFS directly. The experimental conditions for the developed HPLC-CV-AFS system are listed in Table 1.

A conventional HPLC-UV-CV-AFS instrumentation which was detailed in other literature¹⁰ was constructed to compare with this new method.

Reagents

The stock solutions of Hg^{2+} , MMC and EMC (1000 mg L^{-1} as Hg) were prepared from an appropriate amount of mercury chloride ($\geq 99.5\%$, Beijing Chemical Reagents Company, Beijing,

China), methylmercury chloride and ethylmercury chloride ($\geq 98\%$, Merck, Darmstadt, Germany), respectively. Divalent mercury stock solution was dissolved in 5% HNO_3 (65%, Merck, Darmstadt, Germany). Stock solutions of MMC and EMC were dissolved in HPLC-grade methanol. All stock solutions were stored in the dark at 4 $^\circ\text{C}$. Diluted working solutions were prepared from stock solutions using 10 mmol L^{-1} of sodium thiosulfate daily. L-cysteine was obtained from Beijing Solarbio Science & technology Co., Ltd. (Beijing, China), and KBH_4 was purchased from Tianjin Refined Chemical Institution (Tianjin, China). Mercaptoethanol was purchased from Alfa Aesar (Ward Hill, MA, USA). HPLC-grade methanol and acetonitrile were obtained from J. T. Baker (Phillipsburg, NJ, USA). All other chemicals were obtained from Beijing Chemical Reagents Company (Beijing, China) and were of analytical grade or better. De-ionized water (18.2 M Ω) produced with a Millipore ultra-pure water system (Millipore; Bedford, USA) was used throughout.

The mobile phase for the HPLC-CV-AFS system consisting of 1 g L^{-1} L-cysteine, 0.06 mol L^{-1} ammonium acetate and water was prepared daily, whereas the mobile phase for the HPLC-UV-CV-AFS system was composed of 5% (v/v) acetonitrile, 0.06 mol L^{-1} ammonium acetate and 0.1% (v/v) mercaptoethanol.

Sample preparation

Organic mercury compounds were extracted from the biological samples using an alkaline extraction method:²⁰ (1) 0.1–0.2 g of freeze-dried sample was added into 2 mL of 25% (w/v) KOH in methanol held in a 40 mL glass centrifuge tube, which was shaken overnight; (2) 6 mL of CH_2Cl_2 was added to the solution which was then neutralized by addition of 1.5 mL of concentrated HCl dropwise; (3) The resulting solution was shaken for 15 min to extract organic mercury into the CH_2Cl_2 phase; (4) After centrifuging at 3000 rpm for 15 min, the CH_2Cl_2 phase was transferred into a 10 mL glass centrifuge tube and 1 mL of 10 mmol L^{-1} sodium thiosulfate solution was added; (5) The mixture was shaken for 45 min to extract the organic mercury into the water phase. After centrifuging at 3500 rpm for 15 min, the water phase was taken and determined with HPLC-CV-AFS under optimized conditions.

In the above mentioned method for sample preparation inorganic mercury cannot be extracted quantitatively. Therefore, an ultrasound-aided acid leaching method was employed for extracting inorganic and organic mercury simultaneously and the procedure was detailed elsewhere.⁹

The total Hg concentrations of samples were determined using microwave-assisted digestion method. Briefly, (1) 0.1–0.2 g samples were exactly weighed into PTFE digestion containers and 3 mL HNO_3 , 3 mL H_2O_2 were added into each container. (2) The microwave containers were irradiated for 10 min after temperature was raised to 120 $^\circ\text{C}$ in 8 min, then held 20 min after raising to 180 $^\circ\text{C}$ in 10 min. Finally, the microwave containers were cooled down to room temperature. (3) The samples were diluted with Milli-Q water to 50 mL, and the solutions were passed through 0.45 μm filter prior to analysis. The total mercury concentrations were determined with AFS.

Table 1 Optimized conditions of the HPLC-CV-AFS for mercury speciation

Parameters	Optimized value
HPLC	
Column	Shim-pack CLC-ODS column (15 cm $\times$ 6 mm I.D. $\times$ 5 μm)
Mobile phase	1 g L^{-1} L-cysteine, 0.06 mol L^{-1} ammonium acetate in water
Flow rate of mobile phase	1.0 mL min^{-1}
Injection volume	100 μL
Cold vapor generation	
Speed of HCl and KBH_4	1.7 mL min^{-1}
Flow rate of carrier gas	300 mL min^{-1}
Flow rate of auxiliary gas	300 mL min^{-1}
Concentration of KBH_4	0.5% (w/v), in 0.2% (w/v) KOH solution
Concentration of HCl	10% (v/v)
AFS	
PMT voltage	320 V
Atomizer temperature	Room temperature
Atomizer height	8.0 mm
Lamp current	40 mA

Results and discussion

Preliminary study on the role of L-cysteine

AFS only responds for elemental mercury vapor. Therefore, in previous HPLC-CV-AFS hyphenated system, organic mercury species were usually digested with post-column ultraviolet light and/or strong oxidants and converted to Hg^{2+} , followed by reduction to Hg^0 to produce fluorescence signals in AFS. In this work, it was found for the first time that by adding L-cysteine into the mobile phase, high fluorescence signals of organic mercury can be produced without post-column ultraviolet or strong oxidants digestion. Since organic mercury should be transformed to divalent mercury and then to elemental mercury (Hg^0) to emit fluorescence signals, we envisioned that it was L-cysteine alone or together with HCl and KBH_4 that converted organic mercury into divalent mercury.

In order to identify whether digestion of organic mercury was induced by L-cysteine, a flow injection-atomic fluorescence spectrometry (FI-CV-AFS) system was used. Deionized water was used to convey the samples. HCl and KBH_4 were employed to produce cold vapor (the concentrations of HCl and KBH_4 were listed in Table 2). Organic mercury standards prepared in water and L-cysteine solution were injected into the FI-CV-AFS system, respectively. Only small peaks were found when organic mercury standards prepared in water was injected. This may be resulted from organic mercury reduction by KBH_4 directly.^{30,31} On the contrary, much higher peaks were obtained when organic mercury standards prepared in L-cysteine solution were injected. These results indicated that L-cysteine still played an important role in digestion of organic mercury.

If L-cysteine alone converted organic mercury into inorganic mercury, injection of organic mercury standard dissolved in L-cysteine solution into the conventional HPLC-UV-CV-AFS system should give a divalent mercury peak, and significant decrease of organic mercury peaks. However, the peak of divalent mercury was not found when injecting organic mercury standards prepared in L-cysteine solution and water, and only high peaks of organic mercury were obtained. The experiments suggested that the reduction of organic mercury happened after the separation, and L-cysteine alone can not degrade organic mercury species. That is, HCl and KBH_4 also contribute to the conversion of organic mercury to divalent mercury.

Based on the above discussion, we speculated that L-cysteine could weaken the C-Hg bond by forming complexes with organic mercury, then in the presence of KBH_4 and HCl C-Hg bond was broken and organic mercury was reduced to elemental mercury which can produce fluorescence signal in AFS. Further

studies should be done to find out the exact mechanism of this phenomenon.

Effect of L-cysteine and ammonium acetate on chromatographic separation and fluorescence signal production

To separate the three species of mercury, a mobile phase consisting of water, ammonium acetate and L-cysteine was employed. L-cysteine functioned as a complex reagent to form hydrophilic chelates with inorganic and organic mercury that are dissolvable in the water, whereas the ammonium acetate was added in the mobile phase to assure a neutral environment.

The retention time of all of the three mercury species reduced slowly with the increase of L-cysteine concentration. Nevertheless, baseline separation of the three mercury species was obtained at the studied range of L-cysteine concentration, and retention times of the three mercury species was constant in the range of 0.5–2.0 g L⁻¹ L-cysteine.

Another role of L-cysteine in this novel method is to weaken the C-Hg bond of organic mercury by chelating with them.³² As shown in Fig. 2(A), the intensity of MMC and EMC fluorescence signals at 0.05–2 g L⁻¹ L-cysteine was similar. No fluorescence signal was found for MMC and EMC without L-cysteine in the

Fig. 2 Effects of concentration of L-cysteine and ammonium acetate on fluorescence signal intensity. Each mercury species was present at 10 $\mu\text{g L}^{-1}$. Other conditions were given in Table 1. (A) The concentration of ammonium acetate was 0.06 mol L⁻¹. (B) The concentration of L-cysteine was 1 g L⁻¹.

Table 2 Analytical performance of the developed HPLC-CV-AFS system

Compound	Linearity equation	Correlation coefficient	Detection limit/ $\mu\text{g L}^{-1}$	RSD (%) ^a
MMC	$y = 2292.22x + 196.35$	0.9997	0.05	1.4
Hg^{2+}	$y = 1965.75x + 95.91$	0.9989	0.10	2.5
EMC	$y = 2535.935x - 82.628$	0.9994	0.07	1.9

^a Standard concentration, 10 $\mu\text{g L}^{-1}$, $n = 5$.

mobile phase. This probably is due to the fact that organic mercury can not be eluted from the separation column with the absence of L-cysteine. For divalent mercury, however, the fluorescence signal strengthened with the increase of L-cysteine concentration up to 1 g L^{-1} , then leveled off. Therefore, 1 g L^{-1} of L-cysteine was chosen in the subsequent studies.

The role of ammonium acetate concentration on the chromatographic separation and the fluorescence signals was investigated in the range of $0\text{--}0.2 \text{ mol L}^{-1}$. The experiments showed that the retention times of divalent mercury and MMC were almost constant, whereas the retention time of EMC increased slowly with the increase of ammonium acetate concentration. Baseline separation could be obtained at different concentrations of ammonium acetate. However, ammonium acetate concentration had a significant influence on the fluorescence signals (Fig. 2(B)). The signal intensities of mercury species increased with the increase of ammonium acetate concentration from 0 to 0.06 mol L^{-1} and then decreased with the further increase of concentration. Therefore, 0.06 mol L^{-1} of ammonium acetate was selected in this work.

The effects of methanol and acetonitrile as additives on the chromatographic separation were also studied. The results showed that the retention times of mercury species reduced when they were added into the mobile phase. In addition, methylmercury and divalent mercury could not be baseline separated. In view of this point, methanol and acetonitrile were not used in this method.

Effects of concentrations and flow rates of KBH_4 and HCl

In CV-AFS method, divalent mercury is commonly reduced to elemental mercury by reacting with KBH_4 and HCl . Appropriate amounts of KBH_4 and HCl can increase the sensitivity of Hg . Therefore, the concentrations and flow rates of KBH_4 and HCl were also optimized.

As shown in Fig. S1 (ESI[†]), in the range from 0.25% to 1.5% (w/v), the fluorescence signals increased at first and then decreased with the increase of KBH_4 concentration. The optimized concentration of 0.5% was used. The flow rate of KBH_4 was investigated in the range of $0.8\text{--}5.0 \text{ mL min}^{-1}$. As showed in Fig. S1 (ESI[†]), the highest signal intensities of mercury species were obtained at 1.7 mL min^{-1} which was adopted as optimum in the following studies.

The concentration and flow rate of HCl were optimized in the range from 1 to 20% (v/v) and from 0.8 to 5.0 mL min^{-1} , respectively. The results were shown in Fig. S2 (ESI[†]). The signal intensities of mercury species increased at first and then decreased with the increase of the concentration of HCl , and the highest signal appeared at the concentration of 10% (v/v). The

flow rate of HCl had little influence on signal intensity of inorganic mercury but significant effect on organic mercury. When the flow rate of HCl was higher than 1.7 mL min^{-1} , the signal intensities of organic mercury decreased sharply. Thus, the flow rate of 1.7 mL min^{-1} was selected.

Flow rate of argon gas

Argon is usually employed as both carrier gas and auxiliary gas in AFS. The role of the carrier gas is bringing element mercury, produced in the gas mixture, into the atomizer. If carrier gas is excessive, the concentrations of element mercury in atomizer will be diluted and its residence time would be shortened, which resulted in low sensitivity. However, lower argon flow rate can not bring element mercury into atomizer timely thus decreased the sensitivity.

The role of auxiliary gas is dehumidification. It can prevent aqueous vapor from entering into the atomizer, which could influence efficiency of atomic fluorescence production. According to instrument settings, flow rates of carrier gas and auxiliary gas were investigated in the range of $200\text{--}900 \text{ mL min}^{-1}$, respectively. The results shown in Fig. S3 (ESI[†]) revealed that the best results were obtained by using carrier gas and auxiliary gas flow rates of 300 and 300 mL min^{-1} , respectively.

Analytical performance

The signal intensities obtained with this new system and conventional HPLC-UV-CV-AFS were compared under respective optimized conditions (Fig. S4 (ESI[†])). The MMC and EMC peak heights of the proposed method are slightly lower than the HPLC-UV-CV-AFS method, but the inorganic mercury peak height of this method is higher. Based on peak height, these two methods have comparable sensitivity. For inorganic mercury, the higher sensitivity may be caused by the shorter pipeline used in this new method, which could depress the diffusion of chromatographic peaks.

The analytical features of the present method for speciation of three mercury species under the optimized conditions are listed in Table 2. For a mixture solution of $10 \mu\text{g L}^{-1}$ (as Hg), the precisions (RSD, %) of five replicate injections were 1.4% for MMC, 2.5% for Hg^{2+} , and 1.9% for EMC, respectively. The detection limits (3σ) of MMC, EMC and Hg^{2+} were 0.05 , 0.07 and $0.1 \mu\text{g L}^{-1}$, respectively. Linear regression coefficients ranged from 0.9989 to 0.9997 . Fig. S4 (ESI[†]) includes a typical chromatogram of three mercury species at $50 \mu\text{g L}^{-1}$ levels obtained at the optimized conditions.

Table 3 Analysis results of MMC in the certified reference materials

Samples	MMC		Hg^{2+}		Total Mercury Certified value
	Concentration found	Certified value	Concentration found	Certified value	
DORM-2 ^a	4.384 ± 0.171	4.470 ± 0.320	—	—	4.64 ± 0.26
TORT-2 ^b	0.146 ± 0.014	0.152 ± 0.013	0.114 ± 0.007	—	0.27 ± 0.06

^a alkaline digestion method. ^b acid leaching method.

Table 4 Analysis results of MMC and total mercury in fish samples (means $\pm$ SD of three determinations, $\mu\text{g g}^{-1}$, as Hg)

Samples	MMC		Total Hg
	This method	HPLC-UV-CV-AFS	
Fish sample 1 ^a	0.402 $\pm$ 0.023	0.397 $\pm$ 0.019	0.476 $\pm$ 0.032
Fish sample 2 ^a	0.151 $\pm$ 0.014	0.153 $\pm$ 0.026	0.181 $\pm$ 0.045
Fish sample 3 ^a	0.610 $\pm$ 0.052	0.624 $\pm$ 0.048	0.686 $\pm$ 0.074

^a alkaline digestion method.

Validation and application

In order to evaluate the accuracy of the developed method, two certified reference materials, DORM-2 (dogfish muscle) and TORT-2 (lobster hepatopancreas), were analyzed. Good agreement was obtained between the certified value and the determined value of MMC, also the sum of Hg^{2+} and MMC concentration was well consistent with the total mercury certified value of TORT-2 (Table 3). The method was also applied to determine MMC in fish samples collected from Qinghai and Tibet plateau, and the results were comparable with the concentrations determined by HPLC-UV-CV-AFS (Table 4). These results indicated that the proposed HPLC-CV-AFS method can be applied with confidence for determination of organic mercury in biological samples.

Conclusions

By using a mobile phase consisting of L-cysteine and ammonium acetate, a novel HPLC-CV-AFS hyphenated system was developed for the separation and determination of three mercury species. The effluence of mercury species from HPLC separation column can be introduced directly to the AFS without any post-column oxidation process and additional interface. Organic solvent and strong oxidants were not needed in this system. In comparison with other methods reported in literature, this method has many additional advantages such as simplified instrumentation, low instrumental and operating costs, easy operation, reduced damage to instrumental pipeline and less use of harmful reagents. The proposed method has been successfully applied to analyze the certified reference material (DORM-2 and TORT-2) and fish samples.

Acknowledgements

This work was jointly supported by the National Basic Research Program of China (2009CB421605), Chinese Academy of Science (KJCX2-YW-H04), Key Project in the National Science & Technology Pillar Program (2006BAK03A14) and National

Natural Science Foundation of P. R. China (20677069, 20890110 and 20807047).

References

- 1 L. Y. Lin, L. F. Chang and S. J. Jiang, *J. Agric. Food Chem.*, 2008, **56**, 6868–6872.
- 2 L. Ebdon, M. E. Foulkes, S. Le Roux and R. Munoz-Olivas, *Analyst*, 2002, **127**, 1108–1114.
- 3 C. F. Harrington, *TrAC, Trends Anal. Chem.*, 2000, **19**, 167–179.
- 4 S. S. de Souza, J. L. Rodrigues, V. C. D. Souza and F. Barbosa, *J. Anal. At. Spectrom.*, 2010, **25**, 79–83.
- 5 Y. F. Li, C. Y. Chen, B. Li, Q. Wang, J. X. Wang, Y. X. Gao, Y. L. Zhao and Z. F. Chai, *J. Anal. At. Spectrom.*, 2007, **22**, 925–930.
- 6 J. L. Mann, S. E. Long and W. R. Kelly, *J. Anal. At. Spectrom.*, 2003, **18**, 1293–1296.
- 7 R. Falter and H. F. Scholer, *J. Chromatogr., A*, 1994, **675**, 253–256.
- 8 R. Falter and H. F. Scholer, *Fresenius J. Anal. Chem.*, 1995, **353**, 34–38.
- 9 Y. G. Yin, Z. H. Wang, J. F. Peng, J. F. Liu, B. He and G. B. Jiang, *J. Anal. At. Spectrom.*, 2009, **24**, 1575–1578.
- 10 E. L. Gao, G. B. Jiang, B. He, Y. G. Yin and J. B. Shi, *J. Anal. At. Spectrom.*, 2008, **23**, 1397–1400.
- 11 Y. L. Yu, Z. Du, M. L. Chen and J. H. Wang, *J. Anal. At. Spectrom.*, 2007, **22**, 800–806.
- 12 N. Bloom and W. F. Fitzgerald, *Anal. Chim. Acta*, 1988, **208**, 151–161.
- 13 J. L. Gómez-Ariza, D. Sanchez-Rodas, I. Giraldez and E. Morales, *Talanta*, 2000, **51**, 257–268.
- 14 Y. Cai, *TrAC, Trends Anal. Chem.*, 2000, **19**, 62–66.
- 15 Y. M. Yin, J. Liang, L. M. Yang and Q. Q. Wang, *J. Anal. At. Spectrom.*, 2007, **22**, 330–334.
- 16 C. B. Zheng, Y. Li, Y. H. He, Q. Ma and X. D. Hou, *J. Anal. At. Spectrom.*, 2005, **20**, 746–750.
- 17 Y. M. Yin, J. H. Qiu, L. M. Yang and Q. Q. Wang, *Anal. Bioanal. Chem.*, 2007, **388**, 831–836.
- 18 Y. Li, C. B. Zheng, Q. Ma, L. Wu, C. W. Hu and X. D. Hou, *J. Anal. At. Spectrom.*, 2006, **21**, 82–85.
- 19 C. F. Han, C. B. Zheng, J. Wang, G. L. Cheng, Y. Lv and X. D. Hou, *Anal. Bioanal. Chem.*, 2007, **388**, 825–830.
- 20 Y. G. Yin, J. F. Liu, B. He, E. L. Gao and G. B. Jiang, *J. Anal. At. Spectrom.*, 2007, **22**, 822–826.
- 21 X. Li and Z. H. Wang, *Anal. Chim. Acta*, 2007, **588**, 179–183.
- 22 A. S. Ribeiro, M. A. Vieira, S. Willie and R. E. Sturgeon, *Anal. Bioanal. Chem.*, 2007, **388**, 849–857.
- 23 S. Gil, I. Lavilla and C. Bendicho, *Anal. Chem.*, 2006, **78**, 6260–6264.
- 24 R. Falter and H. F. Scholer, *Chemosphere*, 1994, **29**, 1333–1338.
- 25 L. N. Liang, G. B. Jiang, J. F. Liu and J. T. Hu, *Anal. Chim. Acta*, 2003, **477**, 131–137.
- 26 X. F. Yin, W. Frech, E. Hoffmann, C. Ludke and J. Skole, *Fresenius J. Anal. Chem.*, 1998, **361**, 761–766.
- 27 J. C. G. Wu, *Spectrosc. Lett.*, 1991, **24**, 681–697.
- 28 Y. Li, X. P. Yan, L. M. Dong, S. W. Wang, Y. Jiang and D. Q. Jiang, *J. Anal. At. Spectrom.*, 2005, **20**, 467–472.
- 29 E. Bramanti, C. Lomonte, M. Onor, R. Zamboni, A. D'Ulivo and G. Raspi, *Talanta*, 2005, **66**, 762–768.
- 30 H. M. Li, Y. Zhang, C. B. Zheng, L. Wu, Y. Lv and X. D. Hou, *Anal. Sci.*, 2006, **22**, 1361–1365.
- 31 S. R. Segade and J. F. Tyson, *Spectrosc. Acta Pt. B-Atom. Spectr.*, 2003, **58**, 797–807.
- 32 X. Gaona and M. Valiente, *Anal. Chim. Acta*, 2003, **480**, 219–230.