Simultaneous multi-elemental speciation of As, Hg and Pb by inductively coupled plasma mass spectrometry interfaced with high-performance liquid chromatography

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ABSTRACT

This work establishes a hyphenated methodology coupling HPLC with ICP-MS for simultaneous speciation analysis of arsenic, mercury and lead for the first time. Four arsenicals (As(III), DMA, MMA and As(V)), four mercurials (Hg(II), MeHg, EtHg and PhHg) and three lead compounds (Pb(II), TML and TEL) were simultaneously analyzed within only 8 min with acceptable resolution (2.0–8.2 for As, 1.6–6.1 for Hg and 2.7–4.0 for Pb). The detection limits were 0.036–0.20 for As-species, 0.023–0.041 for Hg-species, and 0.0076–0.14 μg L^{-1} for Pb-species. The developed method was applied for the measurement of five lotus seed samples, indicating the presence of DMA (19.6–28.2 μg kg^{-1}), TML (1.4–2.9 μg kg^{-1}), MeHg (1.2–4.8 μg kg^{-1}) and EtHg (0.8–2.2 μg kg^{-1}). This method provides a promising tool for studying the toxic, metabolic and bioavailable behaviors of arsenic, mercury and lead.

1. Introduction

Arsenic is a universal element that appears in the environment from anthropogenic and natural sources (Sadee, Foulkes, & Hill, 2015), leading to human exposure to high doses of arsenic in food and various environmental media (Reyes et al., 2009). Apart from biological activities, microbiological and physico-chemical processes also determine the biogeochemical cycles of As (Lièvremont, Bertin, & Lett, 2009). There are also enormous differences in the hazardous properties of individual arsenic forms. Inorganic arsenic species are generally more hazardous than organoarsenic forms. In particular, arsenite has stronger solubility and mobile capabilities, and higher toxicity, than arsenate (Komorowicz & Barałkiewicz, 2011).

As one common environmental pollutant, mercury is found in biological samples in both inorganic (Hg(II)) and monomethylmercuric (MeHg) forms. All mercury-containing substances are extremely poisonous, and organomercuric compounds in particular are more harmful than inorganic forms. The inorganic mercuric forms in organisms inhibit enzyme activity and ultimately obstruct cellular processes. High affinity of organomercurials to L-cysteine (Cys) deactivates the protein (Jia et al., 2019; Zhang, Miró, & Kolev, 2018). A bacterium facilitates the methylation process of Hg(II) to readily generate MeHg (Thongsaw, Sananmuang, Udnan, Ross, & Chaiyasith, 2019). As a result, mercury (both elemental and mercuric forms) is identified by the United States in the "Priority List of Hazardous Substances" (Leopold, Foulkes, & Worsfold, 2010).

Lead is another widely distributed heavy metal that is readily accumulated but difficultly excreted from the living body. Lead compounds are commonly used as pigments, auxiliaries and additives. For instance, tetraethyl lead has been used as an anti-knock agent for gasoline. Tetraethyl lead is now banned from fuel in all countries (i.e., the U.S. Clean Air Act in 1996 and the regulations of the 25th/1998 State Council of the People's Republic of China in 2000) other than Algeria (United Nations Environment Programme. (2017), 2017). Many researchers have shown that organic lead is more harmful than inorganic lead, and tri- and tetraethyl lead are the two most hazardous forms to humans (Verity, 1990). Tetraethyl lead is metabolized into triethyl lead in the liver, which hinders the oxidation of the energy-supplying substance, glucose, in the brain (Gidlow, 2004). Long-term exposure to even very small amounts of lead compounds may damage digestive, nervous, circulatory and reproductive systems and increase the risks of cancer and mutagenicity (Ahamed, Akhtar, & Alhadlaq, 2019).

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As an important subject in analytical science, the speciation of trace elements (physical and chemical forms existing in the environment) plays an essential role in eventual toxicity, bioavailability and mobility (Terán-Baamonde, Bouchet, Tessier, & Amouroux, 2018). Considering the above species-dependence of toxic properties, and metabolic and bioavailable behaviors, speciation analysis of As, Hg and Pb is reasonable and essential.

Recently, individual speciation of As, Hg and Pb has been widely analyzed by many hybrid analytical techniques integrating gas and liquid chromatography (GC and HPLC) and capillary electrophoresis with element-sensitive detectors including atomic absorption and emission spectroscopy, atomic fluorescence spectroscopy, and inductively coupled plasma mass spectrometry (ICP-MS) (Cheng, Shen, Liu, Xu, & Wang, 2018; Lai, Chen, & Chen, 2016; Xia et al., 2017). GC is generally favorable for volatile species or species capable of forming volatile compounds without uncontrolled changes in the derivatization/separation processes. However, the use of HPLC simplifies sample processing, eliminates the derivatization step, and is extensible to various analytes with distinct properties based on many available separation mechanisms (such as reversed-phase chromatography, ion exchange chromatography, ion-pairing chromatography, size exclusion chromatography, etc.) (Castillo, Roig-Navarro, & Pozo, 2006). ICP-MS is presently preferred for speciation analysis due to its high sensitivity, wide linear range and simultaneous detection of multiple elements (Zhu, Chen, He, Huang, & Hu, 2017). For routine speciation analysis of arsenic, mercury and lead, sample pretreatment requires special attention with respect to consideration of low concentrations and complex matrices (Wang et al., 2017). Microwave/ultrasonic-assisted digestion is a widely adopted strategy for sample decomposition (Verity, 1990). Nitric acid and tetramethylammonium hydroxide are typically used for extracting arsenic species (Kroukamp, Godeto, & Forbes, 2019; Mao Tseng, De Diego, Martin, Amouroux, & Donard, 1997; Sadee, Foulkes, & Hill, 2016). Nitric/hydrochloric acid mixed with 2-mercaptoethanol (ME) or L-cysteine are commonly employed to extract mercury species (Chen, Chen, Jin, & Wei, 2009; Liu et al., 2018). For lead species, a mixed solution of ethylenediaminetetraacetic acid sodium salt (EDTA) and methanol is preferred (Chen et al., 2014; Xia et al., 2017).

To this point, most methodologies that allow speciation analysis involve the species of a single element. In this sense, every separation and detection method corresponding to each element should be developed, and the samples should be consecutively extracted by different reagents for several trials if speciation analysis of several metal(loid)s is required. This single-elemental strategy is time-consuming, labor-intensive and highly operation-expensive, which does not conform to the concept of green chemistry (Sun, Yang, Lee, & Wang, 2015). Particularly, common preservation and storage methods may be effective in ensuring the stability of all elements for a short time (Wolf, Morman, Hageman, Hoefen, & Plumlee, 2011), where the single-elemental strategy is unsuitable. Accordingly, shortening the total analysis time and enhancing the overall analytical throughput represent desirable choices. A multi-elemental strategy achieves simultaneous speciation analysis of several metal(loid)s in one trial. The multi-elemental strategy can significantly reduce analysis time, reagent consumption and waste discharge compared to the conventional single-elemental methodologies (Marcinkowska & Baralkiewicz, 2016). In addition, multi-elemental speciation analysis is also a valuable tool to study complex metabolic processes, and possible interactions with each other (Castillo, Roig-Navarro, & Pozo, 2008). Therefore, multi-elemental speciation analysis draws extensive interest from the analytical community.

To date, most multi-elemental studies have involved the analysis of two-element species, with approximately 20% involving three elements and only a few studies involving four, five or more elements in one trial (Marcinkowska & Baralkiewicz, 2016). Among these multi-element speciation studies, arsenic and selenium are the two most popular elements, accounting for more than half of the multi-elemental methodologies. In comparison, there are only a few multi-elemental studies for mercury, and extremely rare reports for lead (accounting for 5% or less of the total). Chang et al. described an HPLC-ICP-MS method for Hg and Pb speciation (Chang, Jiang, & Sahayam, 2007). A mixed solution of 0.2% (v/v) ME, 1 mg L^{-1} EDTA, 174.2 mg L^{-1} sodium 1-pentane-sulfonate and 12% (v/v) methanol at pH 2.8 was used for the separation on a C$_{18}$ column. Recently, two studies achieved simultaneous measurements of mercury, lead and tin by using GC and ICP-MS (Moscoso-Pérez et al., 2016; Terán-Baamonde et al., 2018). Additionally, there were also studies regarding simultaneous arsenic and mercury speciation (Fang et al., 2016; Li et al., 2019; Zhang, Yang, Cheng, Wang, & Liu, 2019). However, to the best of our knowledge, there is no study conducting simultaneous measurements of arsenic, mercury and lead species in one run.

The objective of this research is to develop a new HPLC-ICP-MS method for simultaneous speciation analysis of arsenic, mercury and lead. To separate the forms of the three elements in one trial, the composition and pH of the mobile phase were carefully optimized. Lotus seed is a delicious, nutritious and healthy component in food courses, porridge, tea, and Chinese traditional medicine, etc. However, lotus seed may be at high risk of metal(loid) pollution during the cultivation of lotus in ponds and seed processing (polishing using bleach) in food plants. Therefore, we demonstrated the applicability of the proposed method to lotus seed for estimating food safety.

2. Experimental

2.1. Chemicals and reagents

All chemicals and solvents used were of analytical or HPLC grade. Standard stock solutions of 1 g L^{-1} As(V), MMA and DMA (as As) were obtained by individually dissolving Na$_3$AsO$_4$, CH$_4$AsNaO$_3$ (MMA) and C$_2$H$_6$AsNaO$_4$ (DMA) from J&K Scientific Ltd. (Beijing, China) in ultra-pure water. A stock standard of 1 g L^{-1} arsenic for atomic spectroscopy from Sigma-Aldrich (St. Louis, MO, USA) was directly utilized as an As (III) standard. Standard stock solutions of 1 g L^{-1} MeHg, ethylmercury (EtHg) and phenylmercury (PhHg) (as Hg) were individually dissolved with CH$_3$HgCl and C$_2$H$_5$HgCl from Alfa Aesar (A Johnson Matthey Company, MA, USA), as well as C$_6$H$_5$HgOCOCH$_3$ (PhHg) from Acros Organics (New Jersey, USA) in methanol in amber glass bottles. A 1 g L^{-1} Hg(II) standard in 5% HNO$_3$ (GSBG 62069-90) was purchased from Reference Materials of China (Beijing, China). Two stock solutions of 1 g L^{-1} trimethyl lead (TML) and triethyl lead (TEL) (as Pb) were dissolved with (CH$_3$)$_3$ClPb and (C$_2$H$_5$)$_3$ClPb from LGC Science (Shanghai) Ltd. (Shanghai, China) in methanol, respectively. A 1 g L^{-1} Pb(II) standard was obtained from J&K Scientific. Two 1 g L^{-1} stock standards of yttrium (GBW(E)080576) and bismuth (GBW(E)080589) from Reference Materials of China were used to prepare internal standards. NH$_4$Cl, KBr, EDTA-2Na, Cys, ME, HS(CH$_2$)$_3$SO$_3$Na (MPS) and (C$_4$H$_9$)$_4$NOH (TBAH, 25% v/v in water) were supplied by Aladdin Chemistry (Shanghai, China) for preparing mobile phases. Concentrated HNO$_3$ was provided by Jiangyin Chemical Reagent (Jiangyin, China) for sample extraction. A reference material of brown rice (NMIJ 7532-a) containing arsenic species was obtained from Sigma-Aldrich for method validation. Two certified materials of tuna fish (ERM-CE464) for mercury species and freeze-dried oyster tissue (SRM 1566b) for lead from Sigma-Aldrich were also used, considering the non-availability of certified plant-based materials for mercury and lead speciation. Ultrapure water (18.2 MΩ·cm) was produced from a Milli-Q Plus system (Millipore, Bedford, MA, USA). All tubes were soaked with 10% HNO$_3$ and thoroughly washed with ultrapure water. Membrane filters of 0.45 μm pore size were obtained from Aladdin Chemistry for the filtration of all used solutions.
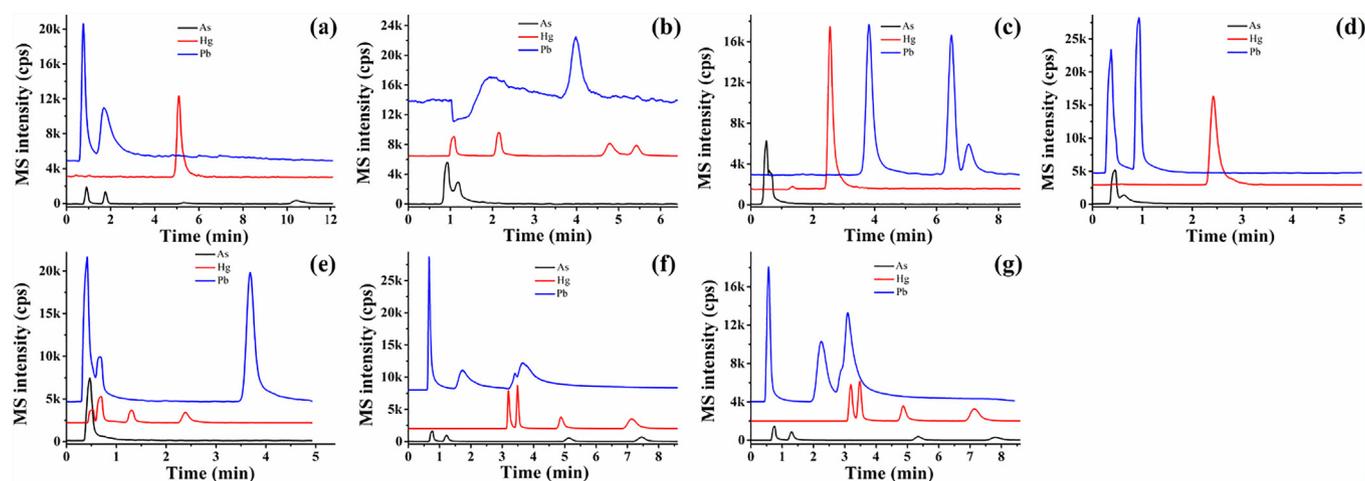
Fig. 1. HPLC separation and ICP-MS detection of As, Hg and Pb species in one run by using 2 mM TBAH at pH 5.0 (a), 20 mM Cys at pH 5.0 (b), 1 mM SPS at pH 3.0 (c), 2 mM TBAH + 1 mM SPS at pH 3.0 (d), 20 mM Cys + 1 mM SPS at pH 3.0 (e), 2 mM TBAH + 20 mM Cys at pH 5.0 (f) and 2 mM TBAH + 20 mM Cys + 1 mM SPS at pH 5.0 (g) as mobile phases. A standard solution of 100 µg L^{-1} As-species, 10 µg L^{-1} Hg-species and 50 µg L^{-1} Pb-species was used.

2.2. Instrumentation

The three metal(loid)s were detected by an X^{II} ICP-MS (Thermo Fisher Scientific Inc., USA) at ^{75}As, ^{202}Hg and ^{208}Pb with internal calibration at ^{89}Y and ^{209}Bi. All separations were carried out on an HPLC system comprising a PU-985 pump (Jasco, Japan), a 7175 injector with a 20 µL loop (Rheodyne, LP, Rohnert Park, CA, USA) and a 15 cm Diamonsil (2) C$_{18}$ column of 4.6 mm i.d. × 5 µm (Acchrom Inc., Beijing, China). The column was conditioned with each mobile phase at 1.0 mL min^{-1} for 0.5 h before analysis. A 10 cm PEEK tube of 0.25 mm i.d. was used to connect the separation column with ICP-MS. ICP-MS parameters were optimized using a 10 µg·L^{-1} tune solution at ^{7}Li, ^{59}Co, ^{115}In, ^{208}Pb and ^{238}U before experiment, and the optimal parameters are listed in Table S1. pH adjustment was performed with an HI 98,128 pH meter (Hanna Instrument, Italy). Sample extraction was carried out with an SCQ-2201B ultrasonic cleaner (40 kHz frequency and 120 W ultrasonic power, Shengyan Ultrasonic Instrument, Shanghai, China) and an 80–1 centrifuge (Jiangsu Zhenji Instruments, Jintan, Jiangsu, China). Sample digestion for total quantification was performed on a MARS 5 microwave system (CEM Corp., Matthews, NC, USA).

2.3. Pretreatment of lotus seed

Fresh lotus seed was collected from local supermarkets in Hangzhou. The lotus seed shells were removed to obtain the white contents, followed by homogenization, bottling (plastic) and storage in a refrigerator at −20 °C before pretreatment. Arsenic, mercury and lead species in lotus seed were simultaneously extracted following a previous procedure (Xia et al., 2017; Zhang et al., 2019). Briefly, three aliquots of 5.0 g of each homogenized sample and 10 mL extraction solution (10 g L^{-1} EDTA + 2% methanol + 5 M nitric acid) were consecutively added into three 50 mL plastic centrifuge tubes. The tubes were subjected to ultrasonic extraction at 40 °C for 1 h, followed by centrifugation at 3500 rpm for 10 min to isolate each supernatant into a 20 mL glass flask. The residues were extracted again following the above procedure. Both supernatants were combined and neutralized with 10 M NH$_3$·H$_2$O, and then filtered through a 0.45 µm membrane. For spike recovery testing, 10 µL of 50 mg L^{-1} As-species (equal to 100 µg kg^{-1}) and 5 mg L^{-1} Hg- and Pb-species (equal to 10 µg kg^{-1}) were added into each 5.0 g sample, and then the spiked samples were pretreated according to the above described procedure. For certified reference materials, 0.25 g of ERM-CE464 and SRM 1566b, and 0.5 g of NMIJ 7532-a, were individually mixed with 2 mL of the extract solution and processed according to the same extraction procedure described

above. In addition, 10 µL of 5 mg L^{-1} As-species (equal to 100 µg kg^{-1}) and 0.5 mg L^{-1} Hg- and Pb-species (equal to 10 µg kg^{-1}) were added into 0.5 g brown rice (NMIJ 7532-a), and then the spiked reference rice was pretreated with two 2-mL aliquots of the extract solution according to the above described procedure. Because of the high mercury content, the extracted ERM-CE464 solution was diluted 10 times before injection. Total contents of As, Hg and Pb in lotus seed (1.0 g) were measured by ICP-MS after microwave decomposition using 5 mL HNO$_3$ according to the following procedure: stepping to 120 °C in 7 min and incubating for 3 min; stepping to 150 °C in 3 min and incubating for 3 min; stepping to 180 °C in 3 min and incubating for 5 min; ultimately cooling to 25 °C. The remaining content was collected in a glass flask, with H$_2$O added to 25 mL.

3. Results and discussion

3.1. Consideration of the simultaneous tri-elemental speciation of As, Hg and Pb

Most HPLC-ICP-MS methodologies for single-elemental speciation of As, Hg and Pb have been achieved on the basis of reversed-phase chromatography (RPC) (Chen, Guo, & Liu, 2017; Döker & Boşgelmez, 2015; Jeong, Lee, Kim, & Yoon, 2017; Peng et al., 2015; Xia et al., 2017; Yao, Jiang, Sahayam, & Huang, 2017). Positively charged alkyl ammonium bases/salts such as TBAH and hexadecyl trimethyl ammonium bromide are typically used in mobile phases for separating anionic arsenic species based on the ion-pairing RPC mechanism. Negatively charged alkyl sulfonic acids/salts (sodium 1-pentanesulfonate, sodium dodecyl sulfate, etc.) are commonly adopted for HPLC separation of cationic lead forms based on the ion-pairing RPC mechanism. For mercury speciation, thiol compounds (Cys and 2-mercaptoethanol, etc.) are typically utilized for complexation with mercurials. Because of the considerable differences of the mobile phases, it is necessary to investigate the compatibility of each mobile phase for single-elemental speciation with RPC separation of the species of the other two metal(loid)s in tri-elemental speciation analysis. As shown in Fig. 1a-c, mobile phases individually comprising TBAH, Cys and SPS alone are capable of isolating the species of only one metal(loid), but incapable of distinguishing the species of the other two metal(loid)s. A further experiment was then performed by utilizing mobile phases comprising any two-chemical mixed solutions of the three compounds (TBAH, Cys and SPS). The results (Fig. 1d–f) demonstrate that only a mixed solution of TBAH and Cys is suitable for simultaneous separation of all species of the three metal(loid)s. A solution containing TBAH, Cys and SPS is also
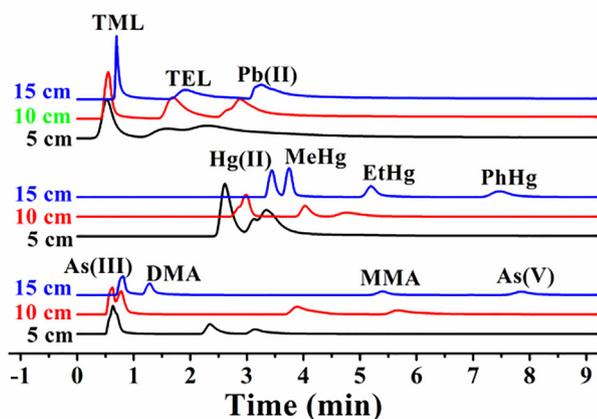
Fig. 2. The separation of arsenic, mercury and lead species by HPLC-ICP-MS on C_{18} columns of different lengths. A standard solution of 100 µg L^{-1} As-species, 10 µg L^{-1} Hg-species and 50 µg L^{-1} Pb-species was used. Other chromatographic conditions are listed in Table S1.

able to simultaneously separate the species of As, Hg and Pb (Fig. 1g), but the chromatogram shows no observable resolution improvement in comparison with the usage of TBAH and Cys as the mobile phase (Fig. 1f). Cys has high affinity for the chemical forms of both lead and mercury, thereby enabling the retention of Hg and Pb species on the C_{18} column. These results indicate that the selection of TBAH and Cys in mobile phases is sufficient to separate all species of the three metal (loid)s. It should be noted that Cys is incompatible with arsenite because of As(III)-Cys interaction. Therefore, a gradient elution procedure was adopted, similarly to our previous study (Zhang et al., 2019), where a TBAH solution alone was introduced for the initial elution of As (III).

Based on the compatibility experiment, a 5-cm Diamonsil (2) C_{18} column was preliminarily used for the tri-elemental speciation by using two mobile phases **A** (4 mM TBAH) and **B** (20 mM Cys + 4 mM TBAH) under gradient elution (see the gradient procedure in Table S1), according to our previous study (Zhang et al., 2019). The result shows complete separation of the four As forms, but peak tailing of PhHg and peak overlapping of the three lead forms (Fig. 2). The three lead species could not be baseline separated no matter how the mobile phase (the concentrations of TBAH and Cys, and pH) was optimized. We then attempted to strengthen the retention behaviors of these target species on long columns. As shown in Fig. 2, the separation resolution is apparently improved with the use of 10 and 15 cm C_{18} columns, and baseline separation of As, Hg and Pb species can be achieved on the 15 cm C_{18} column. Therefore, the 15 cm C_{18} column was selected for the next experiments.

3.2. Selection of the mobile phase

Apart from the separation column, the mobile phase is also crucial for the successful separation of As, Hg and Pb species simultaneously by HPLC-ICP-MS. Accordingly, the concentrations of TBAH and Cys in mobile phases and their pH values were optimized before method estimation and application.

The influence of the Cys concentration on the separation of arsenic, mercury and lead compounds was first optimized in the 0.1–25 mM range. As illustrated in Fig. S1a, the retention times of the arsenic compounds are slightly shortened upon increasing to 2 mM but level off with further increase. Baseline separation of the four arsenicals can be achieved with high Cys concentrations above 5 mM. As(III) is eluted from the column by using TBAH alone in the first gradient step, therefore, its retention time is independent of the Cys concentration. It should be noted that the trend of As(V) versus Cys in the mobile phase is different from our previous work (Zhang et al., 2019). We speculate

that different pH values of the used mobile phases generated different As(V) trends. Cys carries positive charges at pH 5.0, and As(V) is more likely present as a neutral at pH 5.0 in this study. Therefore the retention of As(V) may be insensitive to Cys concentration. In comparison, Cys carries negative charges at pH 6.0 in the previous work (Zhang et al., 2019), where As(V) is also possibly negatively charged. It means that Cys may compete for TBAH cations with As(V) to expedite the elution of As(V). The elutions of MeHg and Hg(II) are hastened, whereas the retentions of EtHg and PhHg are significantly strengthened with 0.1–2.0 mM Cys in the mobile phase (Fig. S1b). Although further addition of more Cys induces slight alteration of the retention time, the resolution, in particular between Hg(II) and MeHg, is gradually improved. Baseline separation of the four mercurials can only be achieved by using Cys concentrations as high as 15 mM. In addition, peak shapes of the four mercury forms are also gradually improved by enhancing the Cys concentration. For lead speciation (Fig. S1c), low amounts of Cys (0.1–5.0 mM) in the mobile phase accelerate the elution of Pb(II) and TEL. No observable alteration in the retention time of Pb(II) results from the usage of 5–25 mM Cys. However, the retention of TEL gradually improves when 10–25 mM Cys was adopted. TML elution is almost independent of the Cys concentration. 15 mM Cys was chosen as a good compromise between short retention time, baseline separation and good peak profile.

The concentration of TBAH was thereafter investigated in the 0.1–6.0 mM range for As, Hg and Pb speciation. The increase of the TBAH concentration first strengthens the retention of MMA and As(V) but then expedites the elution (Fig. S2a). The retention time of DMA is slowly reduced over the concentration range. Arsenite is independent of all tested TBAH concentrations because it is neutral at pH < 7.3. However, baseline separation of the four arsenicals can only be obtained with low TBAH concentrations below 2 mM. For mercury speciation (Fig. S2b), the retention of EtHg and PhHg is gradually weakened as the concentration of TBAH increases. The retention times of Hg(II) and MeHg are slightly decreased over the TBAH concentration range, and the baseline separation is always obtained. Cys (isoelectric point pI 5.2) is positively charged at pH 5.0. The Hg-Cys complexes are positively charged, and electrostatically expelled by the TABH cations to facilitate the elution. We also notice that the relationship of EtHg with TBAH in the mobile phase is different from our previous work (Zhang et al., 2019), which may be attributed to different pH values of the used mobile phases. Similarly to Cys (pI 5.2), the EtHg-Cys complexes are positively charged at pH 5.0 in this study, and they may compete for the C_{18} stationary phase with TBAH cations, leading to accelerated elution of EtHg by increased TBAH. In comparison, the EtHg-Cys complexes carry negative charges at pH 6.0 in the previous work (Zhang et al., 2019), which may be electrostatically attracted by TBAH cations, leading to improved retention on the C_{18} stationary phase. The increase of the TBAH concentration over the 0.1–2.0 mM range leads to a drastic decrease in the retention time of TEL but a slight decrease for TML, and even reverses the elution order (Fig. S2c). The retention times of TEL and TML remain nearly constant with further increasing TBAH concentration. However, the TBAH concentration exerts no observable influence on the inorganic lead form. Lead compounds can moderately coordinate with Cys to form Pb-Cys complexes (i.e., logβ = 13.2 for Pb(II)-Cys interaction (Corrie, Touche, & Williams, 1973)). The Pb-Cys complexes are also positively charged at pH 5.0, similarly to the Hg-Cys complexes, and are favorably eluted by the TBAH cations with high concentrations. The different elution behaviors of the Pb-complexes induced by TBAH may be plausibly rooted in the affinity ability although the formation constants for organolead species with Cys are unavailable. By taking into account rapid separation and good resolution of arsenic, mercury and lead speciation, 2 mM TBAH was selected as optimal.

The effect of the pH value of the mobile phase on the separation of all As, Hg and Pb species was finally estimated in the pH range of 4.0–5.5 (Fig. S3). It is observed that the retention times of arsenic

(except As(III)) and mercury species are gradually prolonged at high pH values, whereas the retention time of As(III) is stable around the dead time (Figs. S3a & b). As(III) and DMA are baseline separated at high pH values above 5.0 whereas Hg(II) can be distinguished from MeHg at low pH values below 5.0. DMA, MMA and As(V) may carry more negative charges under basic conditions, whereas As(III) cannot be dissociated at any of the test pH values, improving the electrostatic attraction by TBAH cations. The Hg-Cys complexes may be more stable under basic conditions, strengthening the retention. Similarly, enlarging the pH value also gives rise to gradual retention improvement of Pb(II) and TML and significantly strengthened retention of TEL (Fig. S3c). The resolution between TML and TEL is apparently improved, whereas the resolution between Pb(II) and TEL is conversely deteriorated upon increasing the pH value. The elution order of Pb(II) and TEL is even reversed at pH > 5.4. Metal-ligand interactions (Pb-Cys coordination here) are generally enhanced under high pH values because of reduced acidic effects. The stable Pb-Cys complexes can be strongly retained by the C_{18} column, leading to retention improvement. We speculate that the stability of the TEL-Cys complex was improved to larger degrees than the Pb(II)-Cys and TML-Cys complexes, considering little knowledge of the interaction of organolead compounds with Cys. Accordingly, the retention time of the TEL-Cys complex was dramatically prolonged. By trading off good resolution and rapid separation, an optimal pH of 5.0 was considered for the mobile phase.

3.3. Analytical characteristics of the tri-elemental speciation method

Under the optimal conditions (Table S1), typical chromatograms showing the simultaneous speciation analysis of As, Hg and Pb are illustrated in Fig. 3a. The repeatabilities of the retention time, peak height and area were calculated from the replicated determinations of the As, Hg and Pb mixture standards. A series of arsenic, mercury and lead mixture standards were also measured three times for estimating the linear coefficients and the detection limits of the method (Table 1). Peak areas of arsenic, mercury and lead forms are linearly related to their concentrations over the 1–500 µg L^{-1} range for As, the 0.2–100 µg L^{-1} range for Hg and the 0.5–100 µg L^{-1} range for Pb. The precisions of retention time, peak height and area of the eleven target analytes are 0.2–2.1%, 0.5–3.8% and 1.4–4.4%, respectively. The resolutions between As(III), DMA, MMA and As(V) are 2.0, 8.2 and 2.5, respectively. The resolutions between Hg(II), MeHg, EtHg and PhHg are 1.6, 6.1 and 5.5, respectively, and the resolutions between TML, TEL and Pb(II) are 4.0 and 2.7, respectively. The limits of detection (LODs, S/N = 3) based on three times the standard deviations for the signals of the sample blank are 0.036–0.20 for As species, 0.023–0.041 for Hg species, and 0.0076–0.16 µg L^{-1} for Pb species. The limits of quantification of the method (LOQs, S/N = 10) are obtained as 0.49–2.68 for As species, 0.31–0.56 for Hg species, and 0.11–1.34 µg kg^{-1} for Pb species considering the sample pretreatment (approximately 5.0 g

extracted in 20 mL extractant). The above data verify the reliability of the method.

In comparison with conventional single-elemental speciation methodologies based on HPLC-ICP-MS (Table S2), the proposed tri-elemental speciation method offers wide linear range, low detection limit and precision, and satisfactory recovery. More importantly, the proposed method exhibits great advantages in operational cost and time. Among the single-elemental methodologies, for instance, it took 9–65 min for arsenic speciation (Chen et al., 2017; Cheng, Zhang, Wang, & Liu, 2018; Jeong et al., 2017; Son, Lee, Kim, Lee, & Nam, 2019), 4–12 min for mercury speciation (Cheng et al., 2018; Döker & Boşgelmez, 2015; Yao et al., 2017), and 4–22 min for lead speciation (Peng et al., 2015; Xia et al., 2017). Total times (separation and re-equilibration) of 20 and 11 min were consumed for the four arsenic and the three mercury species by the methodologies of the Fang group (Fang et al., 2016; Li et al., 2019) and Zhang et al (Zhang et al., 2019), respectively, while the separation time for the three mercury and the three lead forms was 17 min (Chang et al., 2007). However, < 8 min was consumed for the speciation of As, Hg and Pb in this work, even though an additional time of 2 min was consumed only for the elution of PhHg, which is commonly difficult to elute from C_{18} columns. In other words, the net time for each species was 0.7 min for the proposed method in contrast with 1.6 min by Zhang et al. (Zhang et al., 2019), and 2.8 min by the Fang group (Fang et al., 2016; Li et al., 2019) and Chang et al. (Chang et al., 2007). This indicated that our method was 2–4 times more efficient than the above di-elemental methodologies. In addition, inorganic salts and organic solvents in moderate to high amounts were employed in mobile phases for most of the HPLC-ICP-MS methodologies, which are deleterious to the environment and incompatible with ICP-MS instruments. However, organic solvents were excluded from our method. This implied that the proposed method was simple and green. Furthermore, we developed a novel HPLC separation strategy for lead speciation based on the typical reversed-phase retention mechanism, where lead compounds first formed Pb-Cys complexes, and then the complexes were retained on C_{18} columns. However, alkyl sulfonates are typically adopted in current HPLC methods based on the ion-pairing retention mechanism.

3.4. Arsenic, mercury and lead species in lotus seed

Arsenic, mercury and lead compounds in the three reference materials (ERM-CE464, SRM 1566b and NMIJ 7532-a) were determined by the tri-elemental speciation method to validate its accuracy (Fig. 3b). Peak tailing of Pb(II) was encountered with SRM 1566b, which may be due to baseline shifting from the sample matrix. As(III), DMA, As(V) and total arsenic of 0.229 ± 0.004, 0.019 ± 0.002, 0.049 ± 0.005 and 0.329 ± 0.012 mg kg^{-1} are present in NMIJ 7532-a, respectively, which agree well with the certified contents (0.289 ± 0.008 mg kg^{-1} for As(III) + As(V), 0.0186 ± 0.0008 mg kg^{-1} for DMA, and
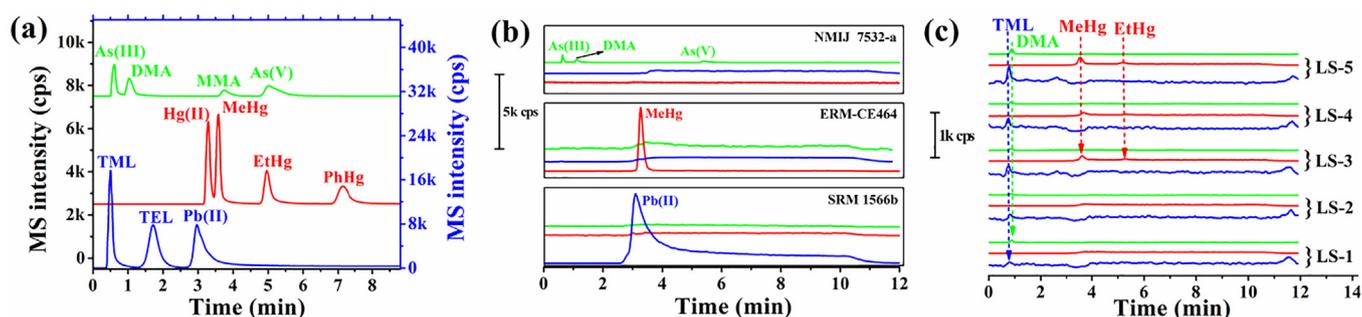

Fig. 3. Representative chromatograms of a standard solution (100 µg L^{-1} for As species, 10 µg L^{-1} for Hg species and 50 µg L^{-1} for Pb species) (a), three reference materials (ERM-CE464, SRM 1566b and NMIJ 7532-a) (b) and five lotus seed samples (c) by HPLC-ICP-MS. Chromatograms showing speciation analysis of arsenic, mercury and lead are labelled by green, red and blue, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Linearities, detection limits and precisions of arsenic, mercury and lead speciation by HPLC-ICP-MS.

Analyte	Linear range (µg L^{-1})	Correlation coefficient (R^2)	Detection limit (µg L^{-1})	Precision (%, n = 5)		
				Retention time	Peak height	Peak area
As(III)	1–500	0.9965	0.036	0.6	3.3	2.8
DMA	1–500	0.9971	0.043	0.3	3.7	4.4
MMA	1–500	0.9966	0.13	0.5	1.6	2.5
As(V)	1–500	0.9967	0.20	0.2	1.8	3.1
Hg(II)	0.2–100	0.9967	0.023	0.2	3.3	2.7
MeHg	0.2–100	0.9942	0.025	0.2	2.9	2.4
EtHg	0.2–100	0.9934	0.036	0.2	1.4	1.4
PhHg	0.2–100	0.9929	0.041	0.5	2.9	1.7
TML	0.5–100	0.9961	0.0076	1.9	3.8	4.2
TEL	0.5–100	0.9942	0.016	2.1	1.7	1.8
Pb(II)	0.5–100	0.9973	0.10	0.5	0.5	1.7

Table 2

Contents and recoveries of arsenic, mercury and lead species in lotus seed (µg kg^{-1}, n = 3).

Analyte		Contents				
		LS-1	LS-2	LS-3	LS-4	LS-5
As	As(III)	< 0.5 [a] (98) [b]	< 0.5 (97)	< 0.5 (1 0 1)	< 0.5 (93)	< 0.5 (97)
	DMA	28.2 ± 0.2 (98)	19.6 ± 0.2 (97)	22.4 ± 0.5 (1 0 2)	26.8 ± 0.4 (99)	21.2 ± 0.4 (91)
	MMA	< 1.7 (94)	< 1.7 (96)	< 1.7 (97)	< 1.7 (98)	< 1.7 (94)
	As(V)	< 2.7 (94)	< 2.7 (95)	< 2.7 (94)	< 2.7 (95)	< 2.7 (92)
	Total	31.4 ± 0.6	21.8 ± 0.5	25.0 ± 0.8	28.5 ± 0.9	22.8 ± 0.6
Hg	Hg(II)	< 0.3 (95)	< 0.3 (93)	< 0.3 (95)	< 0.3 (98)	< 0.3 (93)
	MeHg	< 0.3 (96)	< 0.3 (95)	4.8 ± 0.1 (98)	1.2 ± 0.2 (97)	3.4 ± 0.2 (99)
	EtHg	< 0.5 (95)	< 0.5 (97)	1.7 ± 0.1 (99)	0.8 ± 0.1 (1 0 3)	2.2 ± 0.1 (96)
	PhHg	< 0.6 (97)	< 0.6 (96)	< 0.6 (99)	< 0.6 (96)	< 0.6 (98)
	Total	1.6 ± 0.0	1.8 ± 0.1	9.3 ± 0.4	3.1 ± 0.2	10.2 ± 0.4
Pb	TML	1.4 ± 0.1 (97)	1.6 ± 0.1 (97)	2.9 ± 0.1 (97)	1.4 ± 0.2 (97)	2.0 ± 0.2 (97)
	TEL	< 0.2 (99)	< 0.2 (95)	< 0.2 (95)	< 0.2 (98)	< 0.2 (94)
	Pb(II)	< 1.3 (97)	< 1.3 (95)	< 1.3 (95)	< 1.3 (98)	< 1.3 (94)
	Total	2.2 ± 0.1	2.8 ± 0.1	3.4 ± 0.2	1.7 ± 0.1	2.6 ± 0.1

[a] As the method quantification limit (S/N = 10) on the basis of the pretreatment procedure.

[b] Recoveries (%, n = 3) given in parentheses at a spiked level of 100 µg kg^{-1} for As species and 10 µg kg^{-1} for Hg and Pb species.

0.320 ± 0.010 mg kg^{-1} for total arsenic). MeHg and total mercury of 5.33 ± 0.20 and 5.05 ± 0.17 mg kg^{-1} are found in ERM-CE464, respectively, which are fairly consistent with the certified contents (5.50 ± 0.17 mg kg^{-1} for MeHg and 5.24 ± 0.10 mg kg^{-1} for total mercury). The measured value of the only lead species (Pb(II)) in SRM 1566b is 0.32 ± 0.01 mg kg^{-1}, which accords well with the authentication value (0.308 ± 0.009 mg kg^{-1}) for total lead. NMIJ 7532-a was selected for a further spike recovery test at a spiked level of 100 µg kg^{-1} for As-species, and 10 µg kg^{-1} for Hg- and Pb-species, considering that its plant matrix is similar to that of lotus seed. Recovery values of 92–98% for the four As species, 96–112% for the four Hg species and 90–97% for the three Pb species were obtained. These data demonstrate species integrity (no interspecies transformation) in the sample pretreatment. Overall, all of the results prove the high accuracy of the developed method.

The feasibility of the tri-elemental method in routine speciation analysis was finally demonstrated by simultaneous speciation analyses of arsenic, mercury and lead in five lotus seed samples (Fig. 3c). As shown in Table 2, only DMA, TML, MeHg and EtHg are detected in these samples. The contents of DMA, MeHg, EtHg and TML are variable in the ranges of 19.6–28.2, 1.2–4.8, 0.8–2.2 and 1.4–2.9 µg kg^{-1}, respectively, which are far below the limitation contents in fresh vegetables (0.5 mg kg^{-1} for As, 10 µg kg^{-1} for Hg and 0.3 mg kg^{-1} for Pb), according to GB 2762-2017. The results demonstrate the safety of the five lotus seed samples. The determined contents of DMA and MeHg, as well as total arsenic and total mercury in lotus seed, are 2–10 times higher than the contents of As and Hg (3.5–7.9 µg kg^{-1} for DMA and

5.4–9.8 µg kg^{-1} for total As, and 0.6–1.0 µg kg^{-1} for MeHg and 0.9–1.4 µg kg^{-1} for total Hg, with other As and Hg species below the LOQs) in lotus root (Zhang et al., 2019). EtHg content of no more than 2.2 µg kg^{-1} is detected in particular in lotus seed. These results demonstrate that As and Hg are more possibly accumulated in seeds than in other organs, but this demonstration needs further confirmation based on more samples analyzed in a statistical sense. In addition, the sum amounts of the As/Hg/Pb species are slightly lower than the total contents determined by ICP-MS alone. Good recoveries which are variable in the ranges of 91–102% for As species, 93–103% for Hg species and 94–99% for Pb species are also obtained. These results demonstrate high accuracy and satisfactory applicability of the proposed tri-elemental speciation method in routine analysis.

4. Conclusions

Simultaneous separation and detection of As, Hg and Pb compounds within 8 min using HPLC-ICP-MS is demonstrated for the first time. Compared with conventional single-elemental speciation analysis, the proposed method improves the analytical efficiency by at least three times. This method also conforms to green chemistry goals because it reduces toxic waste and operation cost. Successful measurement of lotus seed demonstrates its feasibility for routine speciation analysis in the food field. The developed method will be used to analyze more samples in food, environmental and biological fields in the near future. Furthermore, multi-elemental speciation (more than four metal(loid)s in one trial, such as Hg, Cd, Pb, Cr, As, etc.) by HPC-ICP-MS is also

anticipated to be developed based on the present method.

CRediT authorship contribution statement

Danyu Zhang: Methodology, Data curation, Writing - original draft. **Shiwei Yang:** Methodology, Data curation. **Qingfang Ma:** Investigation, Resources. **Jiannan Sun:** Investigation, Resources. **Heyong Cheng:** Conceptualization, Supervision, Writing - original draft, Writing - review & editing, Visualization. **Yuanchao Wang:** Funding acquisition, Project administration. **Jinhua Liu:** Funding acquisition, Project administration, Writing - review & editing, Visualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.126119.

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