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Ion-pairing reversed-phase chromatography coupled to inductively coupled plasma mass spectrometry as a tool to determine mercurial species in freshwater fish

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ABSTRACT

Most of analytical community is focused on reversed phase high performance liquid chromatography (RP-HPLC) for mercury speciation by employing mobile phases comprising of high salts and moderate amounts of organic solvents. This study aims at rapid mercury speciation analysis by ion-pairing RP-HPLC with inductively coupled plasma mass spectrometry (ICP-MS) detection only using low salts for the sake of green analytical chemistry. Two ion-pairing HPLC methods were developed on individual usage of positively and negatively charged ion-pairing reagents (tetrabutylammonium hydroxide –TBAH and sodium dodecylbenzene sulfonate –SDBS), where sodium 3-mercapto-1-propysulfonate (MPS) and L-cysteine (Cys) were individually added in mobile phases to transform mercury species into negative and positive Hg-complexes for good resolution. Addition of phenylalanine was also utilized for rapid baseline separation in combination of short C_{18} guard columns. Optimum mobile phases of 2.0 mM SDBS + 2.0 mM Cys + 1.0 mM Phe (pH 3.0) and 4.0 mM TBAH + 2.0 mM MPS + 2.0 mM Phe (pH 6.0) both achieved baseline separation of inorganic mercury (Hg^{2+}), methylmercury (MeHg), ethylmercury (EtHg) and phenylmercury (PhHg) on two consecutive 12.5-mm C_{18} columns. The former mobile phase was selected for mercury speciation in freshwater fish because of short separation time (3.0 min). Detection limits of 0.015 for Hg^{2+}, 0.014 for MeHg, 0.028 for EtHg and 0.042 $\mu g\,L^{-1}$ for PhHg were obtained along with satisfactory precisions of peak height and area (1.0-2.8% for 5.0 $\mu g\,L^{-1}$ Hg-mixture standard). Good accordance of determined values of MeHg and total mercury in certified reference materials of fish tissue (GBW 10029) and tuna fish (BCR-463) with certified values as well as good recoveries (91–106%) proved good accuracy of the proposed method. An example application to freshwater fish indicated its potential in routine analysis, where MeHg was presented at 3.7–20.3 $\mu g\,kg^{-1}$ as the dominate species.

1. Introduction

Mercury is highly toxic in environment and ecosystems which can readily biomagnify to harmful concentrations in marine fish and other living creature. Mercury arises from global anthropogenic sources and undergoes biogeochemical transformation into many different organic forms such as monomethylmercury (MeHg), dimethylmercury, ethylmercury (EtHg) and phenylmercury (PhHg) [1]. It is well-known that organic mercury species (especially MeHg) are more toxic to marine fish than inorganic mercury owing to their efficient trophic transfer, high stability and biomagnification effects [2]. In other words, toxicity and bioavailability of mercury is not just of correlation with total content but more dependent on its chemical form. Considering fish as one of the most important food resources for human beings, speciation analysis of mercury rather than total mercury quantification is more reasonable to assess health risks.

Over the past decades, high performance liquid chromatography (HPLC) [3,4], gas chromatography (GC) [5,6] and capillary electrophoresis (CE) [7,8] have become the powerhouse of speciation analysis of mercury in food, environmental and biological samples [9–11]. In comparison with GC and CE, HPLC has several important advantages including ease of sample derivatization, ease of interface to analytical atomic spectrometries and the ability to analyze

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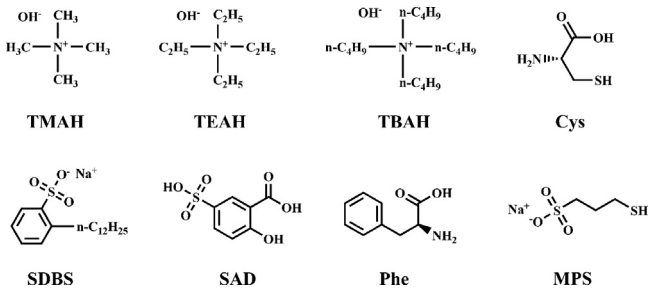

Fig. 1. Chemical structures of TMAH, TEAH, TBAH, Cys, MPS, SDBS, SAD and Phe.

both inorganic an organometallic species. Hence, HPLC is appealing to researchers in mercury speciation analysis. Apart from several reports for mercury speciation by ion exchange chromatography [12–17], most of analytical community devoted to mercury speciation is focused on reversed phase chromatography (RPC), where mobile phases comprising of high salts and moderate amounts of organic solvents were adopted. Detectors following HPLC separation included ultraviolet-visible spectroscopy (UV) [18,19], atomic absorption spectrometry (AAS) [20], atomic fluorescent spectrometry (AFS) [15,21], inductively coupled plasma mass spectrometry (ICP-MS) [3,4,10,12,14,17,20,22–33]. Of these detection technologies for mercury speciation, ICP-MS detection is adopted more frequently because of its powerful capability in highly sensitive and metal-specific and multi-element detection. Applicability of RPC methods for analysis of mercury speciation has been extended from environmental samples (water [4,14,27,28,31,33–35], sediment and soil [10,13], *etc*), food [4,12,14,24,26,29,30,32] to fluids and tissues [3,5,11,22,23] from human and animals. However, these mobile phases were not only environment-toxic but also deleterious to some particular detectors such as ICP-MS. Another adverse effect of HPLC methods is large amount of mobile phase waste due to long separation time. Long separation time also deteriorated analytical efficiency and throughput. Our group achieved rapid elution of four common mercury species during 3–5 min on cation and anion exchange columns [12,14], indicating potential of ion-pairing (IP) RPC in mercury speciation. Although separation of four mercury species was achieved in 13 min with IP-RPC and AAS/AFS detection using an ion-pairing agent of tetrabutylammonium bromide by Ríio-Segade and Bendicho [36], and Jiang group [37], from long time for separation and low sensitivity as well as utilization of high salts and organic solvents were still suffered. To the best of our knowledge, there is no literature reporting rapid mercury speciation by IP-RPC-ICP-MS.

This work reports a novel approach for speciation analysis of mercury by ion-pairing reversed phase HPLC coupled with ICP-MS detection using an aqueous mobile phase which contains small amounts of reagents. Positively charged ion-pairing reagent –tetrabutylammonium hydroxide (TBAH) and negatively charged ion-pairing reagent –sodium dodecylbenzene sulfonate(SDBS) were individually added in aqueous mobile phases for baseline separation of four mercury species (Hg^{2+}, MeHg, EtHg and PhHg) by using short C_{18} guard columns, where two thiol-containing compounds *i.e.* sodium 3-mercapto-1-propysulfonate (MPS) and L-cysteine (Cys), were separately added to coordinate with mercury species and improve separation (Chemical structures shown in Fig. 1). The presence of ion-pairing reagents, complexing reagents and eluting reagents in mobile phases was studied to obtain fast baseline separation apart from pH optimization. Mercury speciation in fish was thereafter analyzed to demonstrate the feasibility of the proposed method.

2. Materials and methods

2.1. Chemicals and reagents

Chemicals and solvents of analytical or chromatographic grade were used throughout experiment. Ultrapure water of 18.2 MΩ cm resistivity was also throughout used, which was newly prepared from a Milli-Q Plus water purification system (Millipore, Bedford, MA, USA) prior to experiment. All the utensils were soaked in 10% HNO_3 for 24 h, followed by thorough rinsing with ultrapure water before use. Sodium dodecylbenzene sulfonate and 5-sulfosalicylic acid dihydrate (SAD) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). L-Cysteine was obtained from Sigma-Aldrich (St. Louis, MO, USA) and tetrabutylammonium hydroxide (25% v/v, ~0.8 M) and phenylalanine (Phe) were supplied by Aladdin Chemistry Co., Ltd. (Shanghai, China). High-purity nitric acid (65% v/v), ammonia (28% v/v), tetramethylammonium hydroxide (TMAH, 25% v/v, ~0.8 M), tetraethylammonium hydroxide (TEAH, 25% v/v, ~0.8 M) were acquired from Jiangyin Chemical Regents (Jiangyin, China). Ammonia, TMAH and TEAH of 4 mM, and TBAH of 0.5–8.0 mM were individually added in mobile phases as positively charged ion-pairing reagents whereas SAD of 2 mM and SDBS of 0.1–5.0 mM were individually added in mobile phases as negatively charged ion-pairing reagents. MPS of 0.2–8.0 mM and Cys of 0.1–5.0 mM were individually added in mobile phases as chelating reagents whereas Phe of 0.1–8.0 mM was added in mobile phases as the eluting reagent. pH values of mobile phases were adjusted in the range of 1.5–4.5 for SDBS + Cys based mobile phases and 3.0–8.0 for TBAH + MPS based mobile phases. A stock standard solution of 1000 mg L^{-1} Hg^{2+} in 5% nitric acid was supplied by National Standard Material Center (GSBG 6206-90, Beijing, China). Methylmercury chloride (≥95%) and ethylmercury chloride (≥95%) from Alfa Aesar (A Johnson Matthey Company, MA, USA) and phenylmercuric acetate (98%) from Acros Organics (New Jersey, USA) were utilized for the preparation of 1000 mg L^{-1} stock standard solutions of MeHg, EtHg and PhHg (as Hg) in methanol, respectively. All these stock solutions were stored in amber glass bottles and kept at 4° C in the dark. The standard mixture solutions of the four mercury species were prepared by successive dilution of the above stock solutions in mobile phases. 1000 mg L^{-1} bismuth stock solution obtained by dissolving bismuth(III) nitrate pentahydrate from Sigma-Aldrich in 1% HNO_3 was employed to prepare the internal standard solution (5 μg L^{-1}). Two certified reference materials (CRMs) of fish tissue (GBW 10029) and tuna fish (BCR-463) from National Standard Material Center (Beijing, China) were used to validate the accuracy for mercury speciation analysis in fish samples. All solutions were filtered through membranes of 0.45 μm pore size before analysis.

2.2. Instrumentation

The determination of four mercury species was performed on a homemade ion-pairing reversed phase chromatography system consisting of a HPLC pump (Jasco PU-985, Jasco International Co. Ltd., Hachioji, Tokyo, Japan), a six-port injection valve with a 5 μL sample loop (Rheodyne 7175, Rheodyne, LP, Rohnert Park, CA, USA) and two consecutive C_{18} guard columns (Zorbax Eclipse C_{18}, 5 μm, 12.5 mm × 2.1 mm i.d., Agilent Technology, Shanghai, China). Measurements of pH were made with a HI 98128 pH-meter (Hanna World Instruments (Beijing) Co., Ltd., Beijing, China). The columns were equilibrated with mobile phases at a flow rate of 1.5 mL min^{-1} for at least 0.5 h before sample injection. PEEK tubing (150 mm long, 0.25 mm i.d.) with appropriate fittings was used to connect the guard columns directly to the concentric nebulizer (*TR*-30-A1, Meinhard Glass Products, USA) of the argon ICP-MS (X Series^{II}, Thermo Fisher Scientific Inc., Waltham, MA, USA). A conical spray