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Rapid speciation analysis of mercury in seawater and marine fish by cation exchange chromatography hyphenated with inductively coupled plasma mass spectrometry

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ABSTRACT

In this work, a hybrid method for the rapid speciation of mercury compounds by cation exchange chromatographic separation and inductively coupled plasma mass spectrometry (ICP-MS) detection is reported. Effective separation of inorganic mercury (Hg^{2+}), methylmercury (MeHg), ethylmercury (EtHg) and phenylmercury (PhHg) within 2–2.5 min was achieved on two consecutive 12.5-mm strong cation exchange guard columns with 2.0 mM L-cysteine or thiourea (pH 2.0) as the mobile phase. This separation met the requirements of green analytical chemistry such as the prevention of toxic waste, safer HPLC mobile phases, and short separation times to reduce operating costs. The detection limits for Hg^{2+}, MeHg, EtHg and PhHg were 0.019, 0.027, 0.031 and 0.022 μg L^{-1}, each, and the repeatabilities of peak height and peak area (5.0 μg L^{-1} for each Hg species) were all lower than 3%. Contents of Hg species and total mercury in certified reference materials of seawater (GBW(E) 080042) and fish tissue (GBW 10029) were in good accordance with the certified values, and satisfactory recoveries (96–102% for GBW(E) 080042 and 94–101% for GBW 10029) validated the developed method. The developed method was applied for the speciation of mercury in five seawater sample and five marine fish samples. The concentrations of mercury species in all analyzed fish samples did not exceed the permissible levels of the National Standard of China.

1. Introduction

Mercury is one of the most hazardous inorganic pollutants due to its bioaccumulative and persistent properties in the environment and ecosystem. Mercury from global anthropogenic sources released into the ecosystem undergoes biogeochemical transformation processes, and is converted into many forms such as inorganic mercury, monomethylmercury and dimethylmercury which makes this metal a true global pollutant [1]. The toxicity, metabolism and bioavailability of mercury depend not only on the total concentration but also on the chemical forms [2]. For example, organic mercury species, especially in the form of monomethylmercury, are more toxic to marine animals than inorganic mercury owing to their easy penetration of biological membranes, high stability and biomagnification effects [3]. Hence, speciation analysis of mercury rather than total mercury quantification is critical to assess health risks of mercury and to better understand its biogeochemical cycling of mercury in the global environment.

The analytical techniques that are frequently employed for the speciation of mercury in environmental samples involve a chromatographic separation system and an element-selective detection system. High performance liquid chromatography (HPLC), gas chromatography (GC) and capillary electrophoresis (CE) are the prevailing separation techniques, and have been widely used for mercury speciation analysis in environmental and biological samples [4]. In comparison with GC and CE, high performance liquid chromatography offers several advantages including comparatively simple sample pretreatment, ease of interface to analytical atomic spectrometries and the capability to easily analyze both inorganic and organometallic species. Consequently, HPLC is appealing to researchers in mercury speciation analysis and HPLC-based separations are therefore more frequently used. The most commonly used mode for HPLC is reversed phase chromatography mode, which has been generally employed to separate Hg species in environmental samples (water [5–11], sediment [7,8,12], soil [13], *etc.*), food [14–22], fluids and tissues from human [23–26] and animals [27], and cosmetic samples [28]. In addition, several methods

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have been developed using the ion exchange chromatography (IC) for mercury speciation analysis in water [29], soil [30], seafood [31], and certified reference materials [32]. In these methods, different detection techniques were employed including ultraviolet-visible spectroscopy (UV, by detection of the dithiozone complexes of the mercury species) [9,14], atomic absorption spectrometry (AAS) [5], atomic fluorescence spectrometry (AFS) [7,12,15–20], inductively coupled plasma atomic emission spectroscopy (ICP-AES) [33,34] and inductively coupled plasma mass spectrometry (ICP-MS) [6,8,10,11,13,21–28]. Among the above detection techniques, atomic fluorescence spectrometry and inductively coupled plasma mass spectrometry offer the highest sensitivity. The derivatization process for AFS detection, however, produces large amounts of waste (oxidant and reductant), which is in violation of the principles of green analytical chemistry [35]. ICP-MS detection on the other hand is adopted more frequently for mercury speciation owing to its ultra-sensitive element-specific detection capabilities. However, there is very little literature on the detection of inorganic mercury and organomercurial species using ion exchange chromatography coupled with ICP-MS.

In recent years, an increased interest has arisen in the analytical community for the implementation of the principles of green chemistry such as the prevention of wastes, safer solvents and reagents, energy efficiency, *etc.* [35]. However, two green chemistry principles are frequently violated by HPLC–ICP-MS methods. First, the use of toxic organic solvents contained in the mobile phase and secondly long analysis times to elute the mercury species from the column. One strategy to dramatically shorten the analysis time of HPLC is the utilization of gradient elution and/or ultra performance liquid chromatography. However, these may unnecessarily increase the overall instrumentation costs. Another effective way is to employ short analytical columns or even guard columns. Recently, Jia et al. reported on the development of a method for the rapid speciation of mercury in seawater by HPLC–ICP-MS based on the use of a guard column for the separation [11]. Separation of inorganic mercury, monomethylmercury and ethylmercury was achieved in only 3 min. However, an extra sampling pump at a flow rate of 9 mL min^{-1} was used for loading 30 mL sample through the preconcentration column, increasing the total analytical time. In addition, the mobile phase for this separation contained 4% methanol [11].

The objective of this work is to develop a green method for the speciation of mercury compounds by coupling IC to ICP-MS (IC-ICP-MS) using an aqueous mobile phase that contains low concentrations of reagents. We here report on the development of a hyphenated method for mercury speciation by cation exchange chromatography with ICP-MS detection. Using L-cysteine (or thiourea) as the complexing agent in the aqueous mobile phase, baseline separation of four mercury species (Hg^{2+}, MeHg, EtHg and PhHg) was achieved in 2.5 min by using two consecutive 12.5-mm strong cation exchange (SCX) guard columns. Key factors of the mobile phase such as pH, flow rate and complexing reagent concentration were further investigated. To validate this method, the analysis of certified reference materials of seawater (GBW(E) 080042) and fish tissue (GBW 10029) was conducted and the recovery of the mercury species was also performed. Finally, the proposed method was applied to the mercury speciation in five seawater samples and five marine fish samples.

2. Materials and methods

2.1. Chemicals and reagents

All reagents used were of analytical or chromatographic grade. Ultrapure water with a resistivity of 18.2 MΩ cm, obtained from a Milli-Q Plus water purification system (Millipore, Bedford, MA, USA) was used throughout the experiment. A stock standard solution of 1000 mg L^{-1} Hg^{2+} in 5% nitric acid was obtained from National Standard Material Center (GSBG 62069–90, Beijing, China). Methylmercury chloride (≥95%) and ethylmercury chloride (≥95%) were both purchased from Alfa Aesar (A Johnson Matthey Company, MA, USA) while phenylmercuric acetate (98%) was supplied by Acros Organics (New Jersey, USA). Stock standard solutions of MeHg, EtHg and PhHg at 1000 mg L^{-1} (as Hg) were prepared by individually dissolving appropriate amounts of methylmercury chloride, ethylmercury chloride and phenylmercuric acetate in methanol, respectively. All stock solutions were stored in amber glass bottles and kept at 4 °C in the dark. Standard solutions of the four mercury species were prepared by successive dilution of the above stock solutions in the mobile phase. 1000 mg L^{-1} bismuth atomic absorption standard solution purchased from Sigma–Aldrich (St. Louis, MO, USA) was employed to prepare the internal standard solution. L-Cysteine (Cys, 99% m/m) from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and thiourea (TU, ≥99.0% m/m) from Aladdin Chemistry Co., Ltd. (Shanghai, China) as complexing reagents were used to prepare the mobile phase. Two mobile phases (2.0 mM Cys and 2.0 mM TU both at pH 2.0) were investigated. 20% v/v nitric acid (Jiangyin Chemical Regent, Jiangyin, China) and 20% v/v ammonia (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) were used to adjust the pH of the mobile phase. High-purity nitric acid (65–68% m/m, Jiangyin Chemical Regent, Jiangyin, China) was used in the microwave assisted digestion process of fish samples for total mercury determination by ICP-MS. Certified reference materials (CRM) of seawater (GBW(E) 080042) and fish tissue (GBW 10029) from National Standard Material Center (Beijing, China) were used to validate the accuracy for mercury speciation analysis in seawater and fish samples. All solutions were filtered through 0.45 μm membranes prior to use.

Warning: Due to high toxicity of organomercurial solutions (especially the extremely toxic MeHg), they must be prepared in a well-ventilated hood, and extreme caution should be exercised to avoid a direct skin contact with these solutions. Disposal of all mercury-containing wastes from the experiment should be treated in accordance with facility guidelines.

2.2. Instrumentation

Mercury speciation analysis was performed by IC-ICP-MS. The cation exchange chromatography system consisted 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 strong cation exchange guard columns (Zorbax SCX 5 μm, 4.6 mm i.d. × 12.5 mm long, Agilent Technologies, Santa Clara, CA, USA) connected in series as the analytical column. Each strong cation exchange guard column is packed with 5 μm, spherical silica particles which contained surface bound aromatic sulfonate groups. Measurements of pH were made with a HI 98128 pH-meter (Hanna World Instruments (Beijing) Co., Ltd., Beijing, China). The columns were equilibrated with the mobile phase at a flow rate of 1.0 mL min^{-1} for at least 0.5 h before sample injection. PTFE tubing (200 mm long, 0.4 mm i.d.) with appropriate fittings was used to connect the SCX column directly to the concentric nebulizer (TR-30-A1, Meinhard Glass Products, Golden, Colorado, USA) of the argon ICP-MS (X Series^{II}, Thermo Fisher Scientific Inc., Waltham, MA, USA). A conical spray chamber (cooled to 3 °C, Thermo Fisher Scientific Inc., Waltham, MA, USA) of the X Series^{II} ICP-MS instrument was used to remove the coarsest aerosol droplets. The dead time of the chromatographic system was determined to be 17.4 s by monitoring the ^{75}As intensity following the injection of a 10 μg L^{-1} As standard solution (as