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Reversed-phase high-performance liquid chromatographic separation of inorganic mercury and methylmercury driven by their different coordination chemistry towards thiols

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Abstract

Since mercuric mercury (Hg^{2+}) and methylmercury (CH_3Hg^+) display different toxicological properties in mammals, methods for their quantification in dietary items must be available. Employing Hg-specific detection, we have developed a rapid, isocratic, and affordable RP-HPLC separation of these mercurials using thiol-containing mobile phases. Optimal separation was achieved with a 50 mM phosphate-buffer containing 10 mM L-cysteine at pH 7.5. The separation is driven by the on-column formation of complexes between each mercurial and L-cysteine, which are then separated according to their different hydrophobicities. The developed method is compatible with inductively coupled plasma atomic emission spectrometry and was applied to analyze spiked human urine.

Keywords: Mercuric mercury; Methylmercury; Thiols; Separation mechanism; HPLC; Mercury-specific detection; Optimization

1. Introduction

Natural and anthropogenic emissions of elemental mercury (Hg^0) followed by long-range atmospheric transport render this heavy metal an omnipresent global pollutant [1,2]. In addition to the inhalation of Hg^0-containing air, the ingestion of CH_3Hg^+-containing marine fish [3,4] represents another major source of exposure for humans. Since inhaled Hg^0 is rapidly oxidized in the bloodstream to Hg^{2+} – which exhibits a notably different toxicity (many Hg^{2+} compounds are nephrotoxic) compared to CH_3Hg^+ (many organomercury compounds are neurotoxic) [5] – analytical methods for the identification and quantification of these Hg compounds in dietary items must be readily available.[1] This task is usually accomplished by the extraction of the Hg compounds [6,7] and their subsequent chromatographic separation using GC [8,9], CE [10,11], or HPLC [12,13] followed by their detection with Hg-specific detectors, such as inductively coupled plasma (ICP)-MS.

Even though GC has been used to separate Hg^{2+} and CH_3Hg^+, this separation technique requires laborious and time-consuming sample preparation procedures in order to derivatize the mercurials to non-polar, volatile and thermally stable, dialkyl derivatives [8,14]. A GC-based separation of these mercurial derivatives, however, can lead to erroneous results due to species conversion at elevated column temperatures. Conversely, separation in the liquid domain, such as CE and HPLC, evade derivatization altogether and allow to directly analyze extracts or filtered aqueous samples at ambient temperature. In comparison to HPLC, CE utilizes smaller sample volumes (nL rather than μL) and entails longer analysis times (CE ∼15 min versus HPLC ∼9 min) [10–13]. HPLC therefore represents the method that is least prone to species conversion and that allows the most expedient separation of these mercurials. With regard to the HPLC separation mechanism, anion-exchange chromatography (AEC) [15], cation-exchange chromatography (CEC) [16], and RP chromatography (RPC) [17–42] have been employed in the isocratic mode. In addition, a RP-HPLC method in the gradient mode has also been reported [43].

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[1] Throughout the manuscript, the use of Hg^{2+} and CH_3Hg^+ implies a generic meaning and does not indicate that these species are present as cations.

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In AEC, separation was achieved with chloride-containing mobile phases and was based on the on-column formation of $[HgCl_4]^{2-}$ and CH_3HgCl, with the former species being electrostatically retained and the latter species demonstrating negligible retention on the anionic resin [15]. CEC has also been used to separate Hg^{2+} and CH_3Hg^+ with mobile phases containing Cys (5.0 mM) and 1.0 mM sodium perchlorate in 1.0 mM acetate buffer (pH 4.4) [16]. The separation, however, was likely achieved by the interaction of mercurial-Cys complexes (formed on the column head) with the hydrophobic stationary phase and not the positively charged mercurial-Cys complexes with the cation-exchange sites, as noted by the authors, since the complexes are zwitterionic.

With regard to RPC, the incorporation of an ion-pairing reagent, such as tetrabutylammonium bromide (TBAB, 10 mM), into mobile phases containing methanol (50–60%) and sodium chloride (0–150 mM) has been employed [19,26,28,35]. The separation is based on the on-column formation of ion-pairs between the TBAB cations and $[HgCl_4]^{2-}$ which results in the observed stronger retention of Hg^{2+} compared to CH_3Hg^+. With TBAB-containing mobile phases, however, adsorption phenomena of Hg^{2+} on stainless steel HPLC columns have resulted in considerable peak tailing and concomitant quantification problems [28]. This undesirable effect could be eliminated by the substitution of steel columns by polyetheretherketone (PEEK) columns and/or the introduction of thiols (e.g. 2-mercaptoethanol) into the mobile phase, which suppressed adsorption, but still allowed a separation of Hg^{2+} and CH_3Hg^+ [28].

Alternatively, RPC separations based on the introduction of complexing agents, such as sodium/ammonium pyrrolidine dithiocarbamate (0.5–1.5 mM) or sulfhydryl-containing modifies, such as Cys (0.2–41.3 mM) and/or 2-mercaptoethanol (0.06–12.8 mM), to the mobile phase in the pH range between 3.5 and 6.8 have been reported [17,18,20–25,27,29–34,36–43]. The vast majority of these separations involved silica-based C_{18} stationary phases and mobile phases which contained considerable amounts of methanol (3–95%) and/or acetonitrile (1–75%) [18,21–23,25,27,30,31,33,34,36–39,41–43]. These mobile phases, however, are not only expensive due to the cost to purchase and to dispose of, but some of them are also incompatible with ICP-based detectors, since ~10% of an organic solvent in the mobile phase has been reported to destabilize [44] and >20% to extinguish the plasma [44,45]. In view of the fact that Cys and 2-mercaptoethanol are cheaper than TBAB and sodium/ammonium pyrrolidine dithiocarbamate, aqueous thiol-based mobile phases will result in a more affordable separation of these mercurials.

Although only six studies have reported a RP-HPLC separation of Hg^{2+} and CH_3Hg^+ with 100% aqueous mobile phases containing Cys or 2-mercaptoethanol as the complexing agent [17,20,24,29,32,40], none of these studies specified the t_0 of their chromatographic system. Judging from their chromatograms – either Hg^{2+} or CH_3Hg^+ – eluted in, or close to, t_0 [20,29,32,40], which is from a chromatographic point of view undesirable since only species that are sufficiently retained can be accurately identified. In addition, the reported chromatographic peaks are broad (w_b for Hg^{2+} ~ 68 s, w_b for CH_3Hg^+ ~ 84 s) which unnecessarily increases the achievable detection limits.

A systematic optimization of a thiol-containing 100% aqueous mobile phase-driven RP-HPLC separation of Hg^{2+} and CH_3Hg^+, with regard to the nature of the thiol and the pH of the mobile phase, has not been conducted. This, however, would represent an important prerequisite to establish conditions at which both mercurials are sufficiently retained, baseline separated, produce narrow chromatographic peaks, and elute in the shortest possible analysis time. In addition, the structural characterization of eluting mercurial-thiol complexes could provide valuable insight into the underlying molecular separation mechanism. Neither of these aforementioned points, however, have been addressed.

In order to achieve an on-column complex formation-driven HPLC separation of Hg^{2+} and CH_3Hg^+, three prerequisites must be met: (a) complex formation between each mercurial and the mobile phase thiol (RSH) must occur rapidly; (b) each RS-mercurial complex must remain intact throughout the chromatographic separation; and (c) each RS-mercurial complex must exhibit dissimilar physicochemical properties from each other (e.g. size, charge, hydrophobicity). Chemically, Hg^{2+} and CH_3Hg^+ both have extremely strong affinities for sulfhydryl-containing ligands [46] which satisfies prerequisite (a). Even though RS–Hg–SR bonds and RS–$HgCH_3$ bonds have been demonstrated to be thermodynamically stable – which seems to fulfill prerequisite (b) – they are kinetically labile [47]. In the context of developing a chromatographic separation, however, this kinetic lability can be circumvented by introducing a RSH into the mobile phase, which will shift the chemical equilibria (Eqs. (1) and (2)) during the chromatographic separation process to the right.

$$Hg^{2+} + 2RSH \rightleftharpoons RS\text{–}Hg\text{–}SR + 2H^+ \quad (1)$$

$$CH_3Hg^+ + RSH \rightleftharpoons RS\text{–}HgCH_3 + H^+ \quad (2)$$

Thus, the RS-mercurial complexes are "preserved" throughout the separation, which consequently fulfills prerequisite (b). With regard to prerequisite (c), it has been demonstrated that, in aqueous solution and between pH 4 and 13, Hg^{2+} and CH_3Hg^+ react with monothiols to form RS–Hg–SR and RS–$HgCH_3$ [48–51]. Thus, the fundamentally different coordination chemistries of Hg^{2+} and CH_3Hg^+ toward thiols [52] can be utilized to drive their separation, which satisfies prerequisite (c).

Following this separation strategy, we have investigated the pH-dependent retention behaviour of Hg^{2+} and CH_3Hg^+ on RP-HPLC columns with 100% aqueous mobile phases containing various monothiols and dithiols (pH range 5.0–8.0) using a flame atomic absorption spectrometer (FAAS) as the Hg-specific detector. The obtained results provided the basis for the development of a rapid, isocratic, and affordable RP-HPLC separation method.