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DETECTION OF MERCURY SPECIES BY HPLC USING 2-MERCAPTOPROPIONIC ACID AS COMPLEX AGENT

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Abstract

A new HPLC method to detect mercurial species, useful in the treatment of phenylmercuric acetate and chloride by TiO_2-heterogeneous photocatalysis under UV irradiation, has been developed. The method uses simple mercury complexation with 2-mercaptopropionic acid and UV detection. The initial phenylmercury salt, together with inorganic mercury (II) and phenol, products of the photocatalytic process, as well as other organomercurials such as methylmercury, and ethylmercury, were detected and quantified. The method is rapid and can be used for general mercury speciation. Linearity was observed in the 0.1-50 mg L^{-1} range, except for inorganic mercury, for which it was in

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the 0.1-25 mg L^{-1} range. The detection limits were 108 μg L^{-1} for Hg^{2+}, 39 μg L^{-1} for CH_3Hg^+, 36 μg L^{-1} for C_6H_5OH, 42 μg L^{-1} for $C_2H_5Hg^+$ and 50 μg L^{-1} for $PhHg^+$. Possible interferences have been also analyzed.

Keywords: mercury speciation; phenylmercury salts; high performance liquid chromatography; 2-mercaptopropionic acid; heterogeneous photocatalysis

Resumen

Se desarrolló un nuevo método de cromatografía líquida de alto rendimiento (HPLC) útil en el tratamiento de acetato y cloruro de fenilmercurio por fotocatálisis heterogénea sobre TiO_2 bajo irradiación UV. El método utiliza la complejación del mercurio (II) con ácido 2-mercaptopropanoico y detección UV. Se pueden detectar y cuantificar la sal inicial de fenilmercurio, mercurio (II) inorgánico y fenol, estos dos últimos, productos de la degradación fotocatalítica, como así también otros compuestos organomercúricos como metilmercurio y etilmercurio. El método es rápido y puede ser utilizado para la especiación de mercurio en general. Se observó linealidad en el ámbito de 0,1-50 mg L^{-1}, excepto para el mercurio inorgánico, para el cual el ámbito fue 0,1-25 mg L^{-1}. Los límites de detección fueron 108 μg L^{-1} para Hg^{2+}, 39 μg L^{-1} para CH_3Hg^+, 36 μg L^{-1} para C_6H_5OH, 42 μg L^{-1} para $C_2H_5Hg^+$ y 50 μg L^{-1} para $PhHg^+$. Se analizaron también posibles interferencias.

Palabras clave: especiación de mercurio; sales de fenilmercurio; cromatografía líquida de alto rendimiento; ácido 2-mercaptopropanoico; fotocatálisis heterogénea

INTRODUCTION

The impact of mercury compounds in the natural environment represents nowadays a very important matter [1]. Toxicity of organomercurials such as methyl- or phenylmercuric salts is higher than that of inorganic species [2]. Some mercury compounds have been used in agricultural activities and, particularly, phenylmercuric acetate (PMA) has been widely used in Argentina as a pesticide two decades ago. Although it is now forbidden, rests of this toxic pollutant can be still present in soil and water. In a recent paper [3], UV/TiO_2 photocatalysis of phenylmercuric salts (acetate, $C_6H_5HgCH_3CO_2$, PMA, and chloride, C_6H_5HgCl, PMC) in aqueous solutions was tested as an innovative method to treat mercury species in water. In order to determine the evolution of the initial organomercuric compound and to identify possible intermediates for the elucidation of the photocatalytic mechanism, an analytical method capable of determining mercury species at low concentrations was necessary.

Methods for mercury determination include colorimetry, atomic absorption spectroscopy (AAS), atomic emission spectroscopy (AES), cold vapor atomic absorption spectroscopy (CVAAS), cold vapor atomic fluorescence spectroscopy (CVAFS), polarography, potentiometric titration, neutron activation analysis, gas chromatography (GC) and high-performance liquid chromatography (HPLC) [4-7]. The assessment of environmental impact, predictions of mobility of mercury in the environment or evaluation of concentrations in removal technologies require the speciation of the element. For this purpose, inorganic and organic mercury species have been separated by GC or HPLC [8, 9 and references therein]. GC techniques require long clean-up procedures, and several organomercury compounds are thermally stable or strongly retained by the columns [10]. In contrast, HPLC techniques are simpler, but detection systems as, for example, mass spectrometry (MS), inductively coupled plasma techniques (ICP-AES, ICP-MS), etc., require costly instrumentation [10, 11].

Photometry is a less expensive detection technique, but mercury species are poorly- or non-absorbing compounds; this precludes direct UV-Vis detection. However, Hg^{2+} and organomercurials have strong affinities with thiols, yielding complexes of high stability with elevated complexation constants [12-14]. These complexes can be especially useful for UV/visible

spectroscopy detection in HPLC, gathering excellent features such as fast complex formation and physicochemical properties different enough to allow column separation. Particularly, thiols form monomercaptides with organomercurials and dimercaptides with free Hg^{2+}, a good chemical property for separation [14]. Several sulfur-containing ligands have been already tested [7, 8, 10, 15-17 and references therein], and recently, in order to study phytochelatin extracts, a method for determination of thiol-containing peptides has been adapted to the analysis of mixtures of glutathione (GSH) and some related peptides with their Hg(II) complexes [18]. Specifically, phenylmercury has been detected by complexation with different thiols: 6-mercaptopurine was used for eye-drop products [4], 2-mercaptoethanol was used for river sediments [17], and 2-mercaptobenzothiazole for contact lens solutions [19].

However, some of the complexing agents present drawbacks. For example, dithizone needs extreme purification and is sensitive to oxidants [10, 20], and dithiocarbamates are unselective because their detection must be performed at 254 nm [16, 21-23]. In some cases, time consuming and complicated extractions or concentration steps must be done before injection [7, 10, 11, 15, 24]. Another observed drawback is the kinetic lability (decomposition) of the complexes during the chromatographic run, for example, with glutathion, cysteine, 2-mercaptoethanol, 2,3-dimercapto-1-propanesulfonic acid sodium salt monohydrate, dithioerythritol, etc. [14]. This problem can be solved by addition of the complex agent directly to the mobile phase, as done in the case of 6-mercaptopurine [4]. However, dithizone decomposes even in these conditions [10]. Thioglycolic acid was discarded because its Hg(II) complex absorbs close to 196 nm, the maximal absorption of phenylmercuric species. In this article, a new HPLC technique to determine mercury that uses complexation with 2-mercaptopropionic acid (thiolactic acid, 2-MPA) is described. The method presents good advantages such as short retention time, economy of eluent and good separation of species.

EXPERIMENTAL

Chemicals

2-Mercaptopropionic acid (2-MPA, Fluka, Steinheim, Germany), $HgCl_2$ (Merck, Darmstadt, Germany), ethylmercury chloride (C_2H_5HgCl, Alfa Aeser, Ward Hill, USA), methylmercury chloride (CH_3HgCl, Alfa Aeser, Ward Hill, USA), PMA (Lennox, Buenos Aires, Argentina), PMC (Fluka, Steinheim, Germany), phenol, hydroquinone and catechol (Mallinckrodt, Griesheim, Germany) of the highest purity were used. Methanol and acetonitrile (Merck, Darmstadt, Germany) were HPLC grade. Standards aqueous solutions of mercury species were freshly prepared from stock solutions stored in glass bottles at 4 ºC before use. Standards solutions of Hg(II) and Ga(III) for total reflection X-ray fluorescence (TXRF) determination were prepared by appropriate dilution of stock solutions (1000 µg mL^{-1}) of CertiPur Merck (Germany) and Chem-Lab NV (Zedelgem, Belgium), respectively. Ethylenediaminetetracetic acid disodic salt (Na_2EDTA) from Carlo Erba was used to complex mercury in TXRF analysis. All other reagents were at least of reagent grade and used without further purification. Solutions were prepared with Milli-Q water (resistivity = 18 MΩ.cm).

Instrumentation and analytical procedure

The chromatographic system consisted of an Alltech 301 HPLC pump (Deerfield, IL, USA), equipped with a Rheodyne 7125NS injection valve (100-µL loop, Cotati, USA). The separation was carried out in a Thermo C18 column (5 µm, 15 cm × 4.6 mm) (Bellefont, USA) and a Spectra SYSTEM UV1000 variable-wavelength detector was used (Thermo Electron Corporation, San Jose, CA, USA).

The mobile phase was composed of CH_3OH-CH_3CN-5 mM NaH_2PO_4 (1:4:5) containing 0.1 mM 2-MPA (its concentration, as complex agent, has to be higher than the mercuric analytes). It was filtered through 0.45 μm Millipore membrane filters.

A flow rate of 0.8 mL min^{-1} was employed, and detection was performed at 220 nm. Data acquisition was processed with a Konikrom software (Konik, Barcelona, Spain). All chromatographic runs were performed at room temperature. Peak height was employed to quantify the concentration of the different analytes. This method was chosen because during the photocatalytic reaction intermediates of structure similar to the standards could be formed, yielding very close peaks, which would difficult the integration if area is employed for quantification.

To avoid any degradation, the aqueous solutions of the standards were prepared daily, stored at 4 ºC and protected from light. Samples of the photocatalytic treatment were analyzed similarly, immediately after sampling.

At the end of each chromatographic run, the column was washed with a 40% water-methanol solution at 0.4 mL min^{-1} for at least 40 min to assure that no deposits of salts coming from the mobile phase were formed. The syringe of the equipment was also washed with the same solution.

Total mercury analysis of stock solutions was carried out using an X-ray fluorescence system with total reflection geometry. The spectrometer consists of a Seifert X-ray generator and a fine focus diffraction molybdenum anode X-ray tube with a Mo anode and a cut-off-filter. The detection and data acquisition system consists of a 80 mm^2 Si(Li) detector with 166 eV FWHM for 5.9 keV, a 0.008 mm thick Be window, an Ortec 672 fast spectroscopy amplifier and a PCA2 ADC Nucleus. Excitation conditions were 50 kV and 30 mA in all cases. The acquisition time for each spectrum was 300 s. For quantification, a Ga(III) internal standard was selected [25] and EDTA was added (1:5 Hg/EDTA molar ratio) to avoid losses of the metallic species before to the quantification, according to a previous report [26].

For molecular absorption measurements, a HP 8453 diode-array UV-Vis spectrometer was used.

Chromatographic runs

For calibration of the method, 15 mg L^{-1} 2-MPA complexes of mercury standards (inorganic mercury, methylmercury, ethylmercury and phenylmercury) were run.

TiO_2-photocatalytic experiments starting from PMA and PMC were performed as described in ref. [3]. Samples (3 mL) were periodically withdrawn and filtered through 0.22 μm Millipore membrane filters. A 3/10 dilution was made in all cases, and 2-MPA (in the same concentration as in the eluent) was added to the sample before injection, to ensure the complex stability. Then, samples were run similarly to the standards.

RESULTS AND DISCUSSION

2-MPA forms stable complexes with inorganic mercury and organomercury compounds. These compounds are neutral, $Hg(2\text{-}MPA)_2$ and RHg(2-MPA), and can be separated by reversed-phase liquid chromatography. 2-MPA has a poorly UV absorbance (ε_{220nm} = 53.9 m^2 mol^{-1}) in the 0.05-5 mM range.

The mobile phase was the same used by Parkin [4], composed of CH_3OH-CH_3CN-5 mM aqueous NaH_2PO_4 (1:4:5), and this eluent was selected because it offers a good solubility of PMA and PMC in the column. As stated before for the case of 6-mercaptopurine [4], to prevent decomposition of the complex during the run, the complexing agent, 0.1 mM 2-MPA, was added to the eluent.

A good and fast separation of the 2-MPA complexes of mercury standards (inorganic mercury, methylmercury, ethylmercury and phenylmercury), as described in the experimental section, was possible in a very short time, less than 5 minutes. This time was shorter than the one found with other chelates, and even shorter than the reported with 2-mercaptoethanol using a multiinjection procedure [27].

Figure 1 shows the evolution of the peaks in the chromatogram of samples taken during a photocatalytic experiment starting from PMA as described in ref. [3]; samples corresponding to the beginning of the photocatalytic run and after 120 min under irradiation were injected. The decrease of the peak corresponding to phenylmercury during the photocatalytic reaction can be seen. By comparison with standards, phenol was identified as one of the products of the reaction. The same result was obtained in photocatalytic experiments starting from PMC.

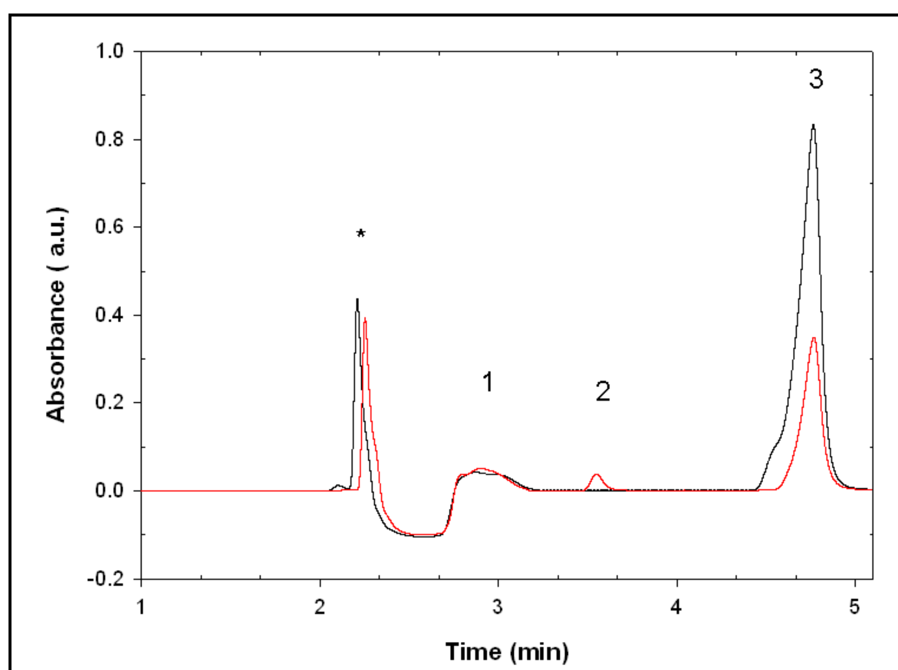

Figure 1. Chromatogram of samples taken at 0 and 120 min (black and red lines, respectively) of irradiation of 0.75 mM (252 mg L^{-1}) PMA in the presence of TiO_2 (1g L^{-1}) with the reactor open to air, pH 3.9. Injection peak (*), $Hg(2\text{-}MPA)_2$ (1), phenol (2) and $C_6H_5Hg(2\text{-}MPA)$ (3). Chromatographic conditions: eluent: CH_3OH-CH_3CN 5 mM NaH_2PO_4 (1:4:5) containing 0.1 mM 2-MPA; flow rate: 0.8 mL min^{-1}; UV detection at 220 nm.

A chromatogram of a solution containing 15 mg L^{-1} standards of Hg^{2+}, C_6H_5OH, CH_3Hg^+, $C_2H_5Hg^+$ and $PhHg^+$ (as PMA), carried out in the same conditions, is shown in Figure 2. As can be seen, possible interactions between phenol and mercury species are discarded, because no changes in the elution time and/or peak height of phenol were produced in the presence of the other two compounds, with a good separation of the peaks. As no signals at 3.3 and 3.9 min corresponding to methyl- and ethylmercury were registered, it can be concluded that alkylmercurials were not formed in the photocatalysis of PMA [3]. Positive and negative peaks present at 2-3 min were assigned to

the injection peaks, belonging to the solvent in which the sample was dissolved and the components of the sample not retained by the stationary phase.

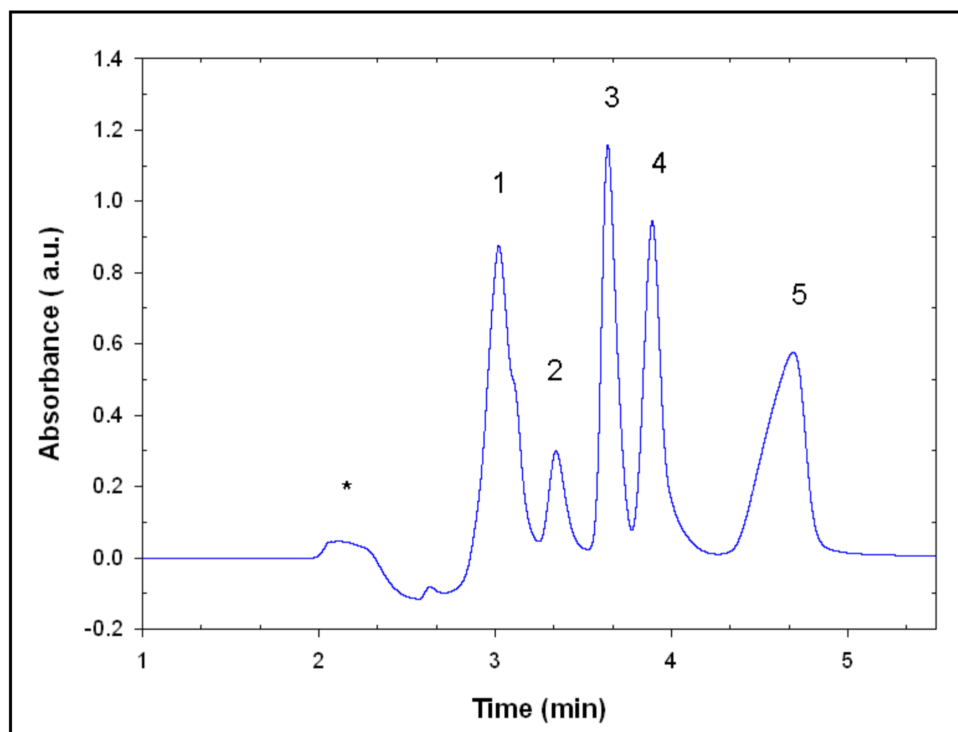

Figure 2. Chromatogram of a solution of standards of mercury species (15 mg L^{-1}) as their 2-MPA complexes and phenol. Injection peak and solvent peak (*), inorganic mercury (1), methylmercury (2), phenol (3), ethylmercury (4) and phenylmercury (5). Chromatographic conditions as in Figure 1.

For calibration, phenylmercury acetate and chloride standard solutions were used and no chromatographic differences between these solutions were observed. A good linearity (correlation coefficients ≥ 0.995) with concentrations in the 0.1-50 mg L^{-1} range for all species was found, except for inorganic mercury, which was linear in the more restricted 0.1-25 mg L^{-1} range. This lower range of lineality, also obtained when using 2-mercaptobenzothiazole as the complexing agent, can be explained by the fact that each Hg^{2+} needs two molecules of the complexant to form a neutral complex, in contrast with other organomercurials that need only one [19]. At concentrations higher than 25 mg L^{-1}, monomercaptides begin to be formed.

In Table 1, the limits of detection (LODs, three times signal-to-noise ratio) and the retention times obtained in this work for the different mercury species and phenol are indicated. The values are in the range attained by other methods. For example, Wang [19] obtained LODs in the 30-50 µg L^{-1} range for the same mercury species, but separation of the four species in that case was achieved in more than 8 min. The LODs obtained in the present work were enough to follow the concentration the mercury species of the photocatalytic process under investigation. The

reproducibility of the method was proved by replicating seven injections of each standard solution, with agreement among the signals and retention times.

Table 1. Retention times (RT), limits of detection (LODs) and standard deviations of RT (SD) (for n = 7) obtained for the different mercury species and phenol.

Chemical species	Retention times (min)	SD (%)	LOD (μg L^{-1})
Hg^{2+}	2.8	3.1	108
CH_3Hg^+	3.3	2.7	39
C_6H_5OH	3.7	3.0	36
$C_2H_5Hg^+$	3.9	2.5	42
$PhHg^+$	4.7	3.2	50

Concerning possible interferences, it was found that Co^{2+}, Mn^{2+}, Cd^{2+}, Cu^{2+}, Fe^{3+} and Al^{3+} (at 10 mg L^{-1}) produced detectable signals which appear close to that of Hg^{2+}. In contrast, Pb^{2+}, Zn^{2+}, Ca^{2+}, Mg^{2+}, and Cr^{3+} did not interfere, in accordance with results obtained before with 2-mercaptobenzothiazole [19]. No interferences were observed between the tested cations and the other organomercurials.

In addition, a complex matrix was simulated by injecting a 1/10 dilution of a commercial mineral water (containing Na^+, Ca^{2+}, Mg^{2+}, HCO_3^-, Cl^-, SO_4^{2-}) spiked or not with mercury species at 15 mg L^{-1} (Hg^{2+}, $C_2H_5Hg^+$, $PhHg^+$). Neither differences in the elution time of Hg^{2+}, $C_2H_5Hg^+$ or $PhHg^+$ complexes nor interferences with their peaks were observed in the chromatograms although again a change in the elution time of the injection peak due to the more complex matrix was observed. However, it was concluded that the matrix of the mineral water has no influence on the chromatographic method.

CONCLUSIONS

Complexation of mercury species with 2-mercaptopropionic acid is a suitable method to evaluate mercury species in simple matrices by HPLC. The procedure is simple, rapid and with low waste generation and low reagent consumption. Neither complicated extraction procedures nor concentration steps before injection are needed and the resulting elution time is satisfactory, being less than 5 min. No expensive detection equipment is needed and clean-up procedures are kept at a minimum. It was found that the method can be used for simultaneous determination of inorganic and organic forms of mercury and could be extended to a general speciation of the element.

The developed analytical method allowed the determination of products during the TiO_2-photocatalytic degradation of PMA and PMC. The evolution of the different species and the assessment of their concentration permitted us to postulate the underlying reaction mechanism described in ref. [3]. Our results indicate that the chromatographic procedure could be useful to follow the evolution of mercury species in similar treatment systems of organomercuric compounds.

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