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Short Communication

High-performance liquid chromatography of inorganic mercury and organomercury with 2-mercaptobenzothiazole

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ABSTRACT

A reversed-phase liquid chromatographic method is described for the determination of inorganic mercury and organomercury in aqueous solution. Using an eluent of methanol–10 m*M* sodium acetate buffer (80:20, pH 6.2) containing 0.1 m*M* 2-mercaptobenzothiazole (MBT), methylmercury, ethylmercury, phenylmercury and inorganic mercury can be separated on a C_{18} column in less than 9 min. UV detection was carried out at 285 nm. Calibration graphs were linear ($r \geqslant 0.997$) over three orders of magnitude of concentration for the three organomercury species. The linear range of inorganic mercury was smaller. Detection limits (3σ) ranged from 0.3 ng of Hg for methylmercury to 0.5 ng of Hg for inorganic mercury. Interference due to metal ions can be eliminated by inclusion of a low concentration (*ca.* 50 μM) of EDTA in the eluent.

INTRODUCTION

Conventionally, speciation and determination of mercury in biological and environmental samples have been performed by gas chromatographic (GC) methods (usually based on that developed by Westöö [1,2]) and cold-vapour atomic absorption spectrometry (CVAAS, originally developed by Poluektov and co-workers [3,4]). In GC analysis, it is essential to form strong, thermally stable derivatives, whereas in CVAAS, complete reduction and quantitative collection of mercury are necessary.

In the past decade, several high-performance liquid chromatography (HPLC) procedures for the determination of inorganic and organomercury have also been developed. In comparison with GC and CVAAS methods, the use of HPLC for mercury speciation generally has the advantage of simplified sample preparation. However, the detection of mercury(II) compounds is problematic because of their lack of chromophores, which precludes the direct use of simple ultraviolet (UV)–visible detection. Several methods have been employed to overcome this problem, such as on-column derivatization with 2-mercaptoethanol followed by electrochemical [5–7], AAS [8,9], inductively coupled plasma atomic emission spectrometric (ICP-AES) [10] and ICP mass spectrometric (ICP-MS) detection [11]. Most of these methods require the use of expensive and specialized detectors. An alternative approach to the use of simple UV–visible detection in HPLC of mercury(II) compounds is to incorporate an on- or off-column derivatization procedure with organ-

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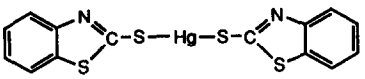
ic complexing agents. The commonly used complexing agents include dithizone [12,13], 6-mercaptopurine [14] and various dithiocarbamates [15–18].

In this paper, we describe an HPLC procedure for the separation and determination of inorganic mercury and organomercury as their 2-mercaptobenzothiazole (MBT) complexes. MBT can form stable complexes with both inorganic mercury and organomercury ions. The structures of the mercury–MBT complexes have been determined by infrared spectrometry [19] and X-ray diffraction analysis [20], and can be represented as



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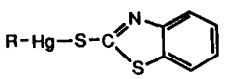

where R = alkyl or phenyl. A preconcentration method for inorganic mercury and organomercury in sea water with MBT supported on silica gel also has been described [21], but to our knowledge there has been no previous report on the use of MBT in the HPLC of mercury(II) compounds. In this study, the mercury–MBT complexes were formed on-column by direct injection of the analyte into a mobile phase containing the complexing agent and detection was performed with a UV detector operated at 285 nm.

EXPERIMENTAL

Apparatus

The HPLC system consisted of an Eldex 9600 solvent-delivery system, a Rheodyne Model 7125 injector with a 100-μl sample loop, a Brownlee Labs. RP-18 column (220 × 4.6 mm I.D., 5 μm), a Soma S-3702 variable-wavelength UV detector operated at 285 nm and a Pantos U-228 strip-chart recorder. A 15 × 3.2 mm I.D. guard column packed with 7-μm RP-18 particles was placed in front of the analytical column.

Reagents

All chemicals were of analytical-reagent grade. Distilled deionized water was obtained using a Nanopure II system.

Inorganic mercury and organomercury chlorides were obtained from Merck. Stock standard solutions (1000 μg ml^{-1} of mercury) were prepared in HPLC-grade methanol and stored in glass bottles below 4°C. Working standard solutions of lower concentration were freshly prepared by appropriate dilution with methanol before use.

The chromatographic eluent consisted of methanol–10 m*M* sodium acetate buffer (80:20) (pH 6.2) containing 0.1 m*M* MBT. This solution was filtered through a 0.45-μm membrane filter and degassed ultrasonically before use.

RESULTS AND DISCUSSION

HPLC of mercury–MBT complexes

MBT can form stable complexes with inorganic mercury and organomercury. The neutral compounds formed can be separated by reversed-phase liquid chromatography using a Brownlee Labs. C_{18} column. Fig. 1 shows a typical chromatogram for

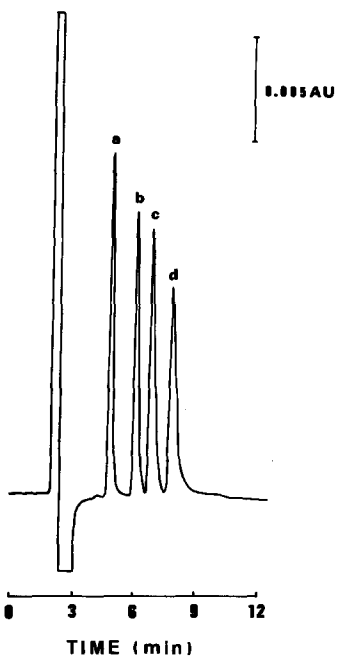

Fig. 1. Chromatogram of (a) methylmercury(I), (b) ethylmercury (I), (c) phenylmercury(I) and (d) inorganic mercury(II) complexes of MBT. Conditions: Brownlee Labs. RP-18 column; eluent, methanol–10 m*M* sodium acetate buffer (80:20) (pH 6.2) containing 0.1 m*M* MBT; flow-rate, 1.0 ml min^{-1}; UV detection, 285 nm; sample amount, 45 ng of Hg for each species.

TABLE I

CALIBRATION DATA FOR DETERMINATION OF INORGANIC MERCURY AND ORGANOMERCURY BY HPLC

Injection volume, 100 μl; other conditions as in Fig. 1.

Compound	Linear range[a] (μg Hg)	r[b]	D.L.[c] (ng Hg)	R.S.D. (%)
Methylmercury	0.001–3.0	0.9989	0.3	2.1
Ethylmercury	0.002–2.5	0.9986	0.4	2.6
Phenylmercury	0.002–2.5	0.9995	0.4	3.4
Inorganic mercury	0.002–0.5	0.9969	0.5	3.1

[a] Range between the limit of quantification (LOQ) and the limit of linearity (LOL) [22].
[b] Correlation coefficient.
[c] Detection limit.

methylmercury, ethylmercury, phenylmercury and inorganic mercury. Complete separation of all four mercury–MBT complexes was obtained in less than 9 min. The elution order was the same as observed for dithizonate complexes [13] and diethyldithiocarbamate chelates [15].

Calibration

A series of spiked, distilled water samples containing known amounts of the four mercury species were used for construction of calibration graphs. The results are summarized in Table I. A linear correlation between the peak height and the amount injected was obtained for each species. The correlation coefficients of the calibration graphs were $\geqslant 0.997$ in all instances. For inorganic mercury, linearity was observed only up to about 0.5 μg, above which the calibration graph gradually approached a plateau. This phenomenon may be explained by the fact that each Hg(II) needs two MBT molecules to form a neutral complex whereas other organomercury ions just only one [$Hg(MBT)_2$ *versus* RHg (MBT)]. At a certain concentration of MBT in the eluent, *e.g.*, 0.1 m*M* in the present instance, the linear range of the calibration graph for inorganic mercury will be smaller than that for organomercury, assuming all inorganic mercury– and organomercury–MBT complexes have similar stability constants. Increasing the MBT concentration in the eluent may extend the linear range of the calibration graph for inorganic mercury. However, a higher MBT concentration and accompanying higher background noise levels were found to deteriorate both the resolution and detection of the four mercury species.

The detection limits listed in Table I were calculated from peak heights corresponding to three times the average standard deviation of the baseline noise [23]. The baseline noise was calculated from the height of the largest noise fluctuation measured in a preselected chart interval. The detection limits range from 0.3 ng of Hg for methylmercury to 0.5 ng of Hg for inorganic mercury.

The reproducibility of the method was tested with seven replicate injections of 5 ng of each mercury species. The relative standard deviations ranged from 2.1% for methylmercury to 3.4% for phenylmercury.

Interferences

Possible interferences of Ca(II), Mg(II), Cu(II), Cd(II), Pb(II), Zn(II), Co(II), Ni(II), Mn(II), Fe (III), Al(III) and Cr(III) in the HPLC of mercury were studied. The results indicated that, except for Ca(II), Mg(II), Co(II), Ni(II), Fe(III) and Al(III), the metals tested at a level of five times the amount of mercury species also showed detectable signals. Under the optimum HPLC conditions for mercury, most of these interfering ions were eluted at *ca.* 4 min, which is close to the elution time of methylmercury (4.9 min). This interference can be effectively eliminated by inclusion of a low concentration (*e.g.*, 50 μ*M*) of EDTA in the eluent. The EDTA apparently forms stronger complexes with the

metals than does the MBT, but organomercury does not. In addition, the peak size of inorganic mercury was not affected by the presence of EDTA in the eluent. The complex formed between Hg(II) and MBT is obviously much stronger than the Hg(II)–EDTA complex.

Applications

In order to validate the method, mercury was determined in the standard reference material NIST SRM 1641b (mercury in water). The sample was diluted 5:1 with distilled, deionized water before injection. The value obtained for inorganic mercury was 1.43 ± 0.07 μg ml^{-1}, based on six replicate injections, which compares favourably with the certified mercury content of 1.52 μg ml^{-1}. No organomercury species could be found in this sample.

A commercial contact lens cleaning solution containing thimerosal [ethyl (sodium *o*-mercaptobenzoato)mercury(I)] was analysed. Thimerosal was known to degrade rapidly in aqeuous solution, forming an ethylmercury salt and thiosalicylic acid [24]. The sample analysed had been stored at ambient temperature for about 1 month after purchase. The solution was diluted 2:1 with water before injection. Fig. 2 shows a typical chromatogram obtained for this sample. The presence of free ethylmercury is evident. Results of quantitative measurement indicated that the ethylmercury content in this particular sample corresponds to 15% of the original concentration of thimerosal (10 mg l^{-1}) marked on the sample container. However, it had been reported that by forming a more stable complex, a thiol-containing complexing agent (*e.g.*, dithiocarbamate) can easily displace the bound ethylmercury group from the undecomposed thimerosal [25]. Therefore, in the present instance, the measured ethylmercury content may not represent the true degree of degradation of thimerosal in the original sample. Further study in this respect is needed.

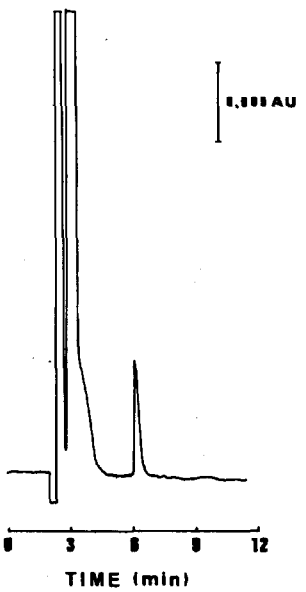

Fig. 2. Chromatogram of a contact lens cleaning solution. Conditions as in Fig. 1, except that the eluent also contained 0.1 m*M* EDTA.

CONCLUSIONS

A simple HPLC procedure for the determination of inorganic mercury and organomercury in aqueous solution has been developed. On-column complexation between mercury species and MBT permits the use of a UV detector for rapid and sensitive detection. Interferences from metal ions can be avoided by the inclusion of a low concentration of EDTA in the eluent. This method is applicable to the determination of mercury species in aqueous samples of a relatively simple matrix, such as pharmaceutical formulations. On the other hand, the detection limits of the method are probably insufficient for the direct determination of trace mercury in natural waters. In combination with a suitable means of sample pretreatment, *e.g.*, solvent extraction or preconcentration, this method should be useful for environmental and/or biological applications. An on-line preconcentration procedure for trace mercury using a short enrichment column and a switching valve directly connected to the HPLC column [26] is under study.

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