

Solid-Phase Extraction and Spectrophotometric Determination of Mercury with 6-Mercaptopurine in Tobacco and Tobacco Additive

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A new method for the determination of trace amounts of mercury based on the reaction of Hg(II) with 6-mercaptopurine (6-MP) and the solid phase extraction of the complex on C₁₈ membrane disks was developed. The 6-MP selectively reacts with Hg(II) to form a complex in the pH range of 5-8. This complex was preconcentrated by solid phase extraction with C₁₈ disks. An enrichment factor of 50 was achieved. The molar absorptivity of the complex is $4.17 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ measured at 315 nm. The Beer's law is obeyed in the concentration range of 0.05-5.4 $\mu\text{g mL}^{-1}$. The relative standard deviation for eleven-replicated measurement of $0.024 \mu\text{g mL}^{-1}$ is 1.5 %. The detection limit is $0.4 \mu\text{g L}^{-1}$ in the original samples. The advantage of the method is that the determination of Hg(II) is free from interference of the almost all cations and anions found in samples. The determination of Hg(II) in tobacco and tobacco additive was carried out by the present method and cold vapour atomic absorption spectrometry (CVAAS). The obtained results by the present procedure were in good agreement with those of the CVAAS, so that the applicability of the proposed method was confirmed to the real samples.

Key Words: Mercury, 6-Mercaptopurine, Preconcentration, Solid phase extraction.

INTRODUCTION

Mercury is a toxic heavy metal. Thus, trace mercury determination in tobacco and tobacco additives is a very important issue. The Quality Standards of Tobacco in Chinese Tobacco Company says that the concentration of mercury should not exceed $0.2 \mu\text{g g}^{-1}$ in tobacco and tobacco additives¹. Many sensitive instrumental techniques, such as spectrofluorimetry, X-ray fluorescence spectrometry, neutron activation analysis, atomic absorption spectrometry, chemiluminescence, electrochemical analysis and other have been widely applied to the determination of mercury²⁻⁸. However, the spectrophotometric method has still the advantage of its simplicity and

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accessibility, not needing expensive or complicated equipments. For this reason, a wide variety of spectrophotometric methods for the determination of mercury have been reported⁹⁻¹⁹.

Nevertheless, for the routine spectrophotometric determination of mercury trace, a preconcentration step is usually required. The most widely used preconcentration methods are coprecipitation, ion exchange, solvent extraction, flotation and solid phase extraction (SPE)²⁰⁻²³. Solid phase extraction is an attractive technique that reduces solvent consumption and exposure, disposal costs and extraction time for sample preparation²⁴. 6-Mercaptopurine is a biologically active molecule containing sulfur and nitrogen donor sites that can form stable complex with mercury^{25,26}. This study describes a procedure for the determination of mercury in tobacco and tobacco additive using the solid phase extraction technique. Several significant advantages of the present method includes, simplicity of the operation, least interferences, excellent detection and avoiding the use of harmful organic solvents.

EXPERIMENTAL

A UV-2401 Spectrophotometer (Shimadzu, Japan) was used for all absorbance measurements with a 1 cm quartz cell. Solid phase extractions were conducted on C₁₈ membrane disks, ENVI-18DSKTM [47 mm (diameter) × 0.6 mm (thickness) 30 μm (particles), 70 Å (pore size)] obtained from Supelco (Bellefonte, USA), in conjunction with a standard Millipore 47 mm filtration apparatus equipped with a desktop vacuum pump. A pH meter Metrohm 744 A model was used for pH measurements.

Analytical reagent grade chemicals were employed for the preparation of all solutions. Solutions were prepared using deionized water from a Nanopure water system (Millipore corporation, USA). Methanol Merck (Darmstadt, Germany) was used. A stock solution of mercury (1000 μg mL⁻¹, Hg(II) in 0.5 mol L⁻¹ HNO₃) was prepared from mercury chloride (Darmstadt, Germany). Working Hg(II) standards were prepared daily by appropriate dilution of the stock solution. The selected reagent, 6-mercaptopurine was provided by Sigma-Aldrich (Steinheim, Germany). A solution of 1.0 × 10⁻³ mol L⁻¹ 6-MP was prepared daily by dilution with the buffer solution. The 0.1 mol L⁻¹ phosphate buffer solution was prepared by dissolving appropriate amount of sodium dihydrogen phosphate in 500 mL water, then adjusting the pH to 6 with sodium hydroxide solution and diluting to a volume of 1000 mL with water. Special care was taken in the preparation and handling of solutions and containers to minimize any possible risk of mercury contamination. Calibration flasks were left overnight in 10 % (v/v) HNO₃ and then rinsed thoroughly with ultra-pure mili-Q water before use to minimize exogenous metal contamination.

Sample preparation: The samples (0.50 g) were accurately weighted into the PTFE high-pressure microwave acid-digestion vessels and 3.0 mL of concentrated nitric acid plus 5.0 mL of 30 % hydrogen peroxide were added. The vessels were sealed tightly and then positioned in the carousel of the microwave oven. The system was operated at full power for 8 min. The digest was evaporated to near dryness. The residue was dissolved with 5 mL 1 % (m/v) nitric acid and quantitatively transferred to a 50 mL volumetric flask for further analysis.

General procedure: To a standard or sample solution containing *ca.* 5.4 µg of Hg(II) in a 50 mL of calibrated flask, 5 mL of sodium dihydrogen phosphate-disodium hydrogen phosphate buffer solution (containing 0.1 mol L⁻¹ Na₂EDTA) and 2 mL of 1.0 × 10⁻³ mol L⁻¹ of 6-MP solution were added. The mixture was diluted to the volume of 50 mL and mixed well. After 10 min, the solution was passed through the C₁₈ disk at flow rate of 50 mL min⁻¹. The mercury complex was retained on the disk. After the enrichment finished, it was desorbed from the disk with 1.0 mL of methanol (contain 0.5 % KOH) at the flow rate of 5 mL min⁻¹ in reverse direction. The absorbance of this solution was measured at 315 nm in a 1 cm cell against a reagent blank prepared in a similar way without mercury.

CVAAS Analysis: The CVAAS analysis was carried out with a Varian (Spectra AA-220) atomic absorption spectrometer equipped with mercury hollow cathodic lamp and a vapour generator accessory (VGA 77) in a continuous system. The experimental conditions were as slit width, 0.5 mm; lamp current, 4 mA; wavelength, 253.7 nm; time constant, 5s; PMT voltage, 290 V.

RESULTS AND DISCUSSION

Absorbance spectra: The absorption spectra of 6-mercaptopurine and its Hg(II) complex under the optimum conditions are shown in Fig. 1. As seen, the spectra of the Hg(II) 6-MP complex have two maximums that overlap with the maximum of the ligand. However, it does not interfere in determination of mercury because the unreacted 6-MP would not retain on the C₁₈ disk. The peak at 315 nm is more practicable in real sample. Thus, the wavelength of 315 nm was used in all subsequent absorbance measurements.

Effect of the pH: The result indicated that the optimal pH for the reaction of Hg(II) with 6-MP was 5.0-8.0 (Fig. 2). In acidic pHs formation of complex between Hg(II) and 6-MP is not fast enough and in basic pH the complex would be solved easily in aqueous medium, In acidic pH the selectivity is improved noticeably, therefore, the pH 6.0 was selected as the optimum. A sodium dihydrogen phosphate-disodium hydrogen phosphate buffer solution of pH 6 was recommended to control pH. The use of

2-10 mL of the buffer solution (pH 6) per 50 mL of the final solution was found to give the maximum and stable absorbance. The use of 5 mL of the buffer solution is recommended.

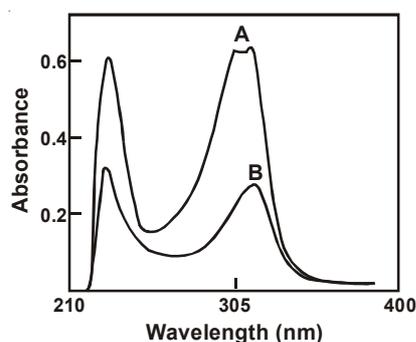


Fig. 1. Absorption spectra of 6-mercaptapurine and its mercury complex
(A) Hg (II)-6-MP complex against reagent blank
(B) 6-MP in methanol (contain 0.5 % KOH)

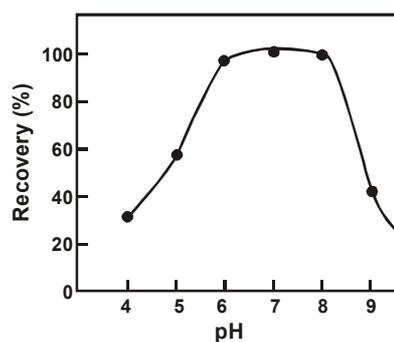


Fig. 2. Effect of pH on the percentage of recovery

Effect of 6-mercaptapurine concentration: The optimum amount of 6-MP for the quantitative extraction of Hg(II) was also investigated. Fig. 3. From these results, the addition of about 2.0 mL of 1×10^{-3} mol L^{-1} of 6-MP solution has been found to be sufficient for a complete reaction. Accordingly, 2.0 mL of 6-MP solution was added in all further measurements.

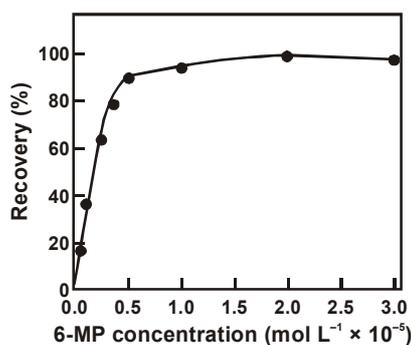


Fig. 3. Effect of 6-mercaptapurine (6-MP) concentration on the percentage of recovery

Stability of complex: After mixing the reactants, the absorbance reaches its maximum within 6 min at room temperature and remains stable for 12 h in aqueous solution. The complex is stable for at least 24 h if extracted into the methanol (contain 0.5 % KOH).

Effect of surfactant: The effect of surfactants was tested on the recovery of extraction by a cationic surfactant (CTAB) an anionic surfactant (SDS) and a non-anionic surfactant (Triton-X-100). None of them increase the absorbance markedly. Therefore, no surfactants were added in the final procedure developed.

Solid phase extraction: Some experiments were carried out in order to investigate the retention of 6-MP and its Hg(II) complex on the disks. It was found that the Hg(II) 6-MP complex was retained on the disks quantitatively when it passes the disk as aqueous solution. The capacity of the disk for the Hg(II) 6-MP complex was determined as 18 mg in 50 mL of solution. In this experiment, the disks have adequate capacity to enrich the Hg(II) 6-MP complex. In order to choose a suitable eluent for the retained Hg(II) 6-MP complex, various organic solvents were examined. It was found that pure organic solvents could not elute the Hg(II) 6-MP complex from the disk quantitatively. Regardless of two bonding sites of 6-MP (N,S), it acts as a monodentate ligand coordinating through sulfur with mercury^{27,28}, therefore the NH site is inactive in case of mercury. Moreover, in basic medium, the polarity of complex increases owing to H releasing then the solubility of complex increase in polar organic solvents like methanol. So, methanol (contain 0.5 % of KOH) was selected as eluent. Experiment showed that it was easier to elute the retained complex in reverse direction. 1.0 mL methanol (contain 0.5 % of KOH) was sufficient to elute the complex from the disk at flow rate of 5 mL min⁻¹. Therefore, the volume 1.0 mL of eluent was chosen.

Calibration curve and sensitivity: The calibration curve showed that the Beer's law is obeyed in the concentration range of 0.05-5.4 µg Hg(II) per mL in the measured solution. The linear regression equation obtained was: $A = 0.1883 C (\mu\text{g mL}^{-1}) + 0.0077$ ($r = 0.9995$). The molar absorptivity was calculated to be $4.17 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ at 315 nm. The relative standard deviation at a concentration level of 0.024 µg Hg(II) per mL (11 replicate determinations) was 1.5 %.

Interferences: The selectivity of the proposed method was investigated by the determination 0.024 mg L⁻¹ of Hg(II) in the presence of various ions within a relative error of ± 5 %. The tolerance limits are listed in Table-1. According to these results, the method is highly selective. The interference of Cu, Pb and Cd were eliminated successfully by the use of EDTA, Furthermore, the formation of Ag(I) 6-mercaptopurine complex also could not interfere in the determination of mercury. Because the molar ratio of Ag complex is 1:1 while that of mercury is 1:2 therefore, it would not retain on the C₁₈ disk. In almost all spectrophotometric methods for determination of Hg(II), Ag(I) is a serious interfering cation, but this method presents a selective spectrophotometric method for determination of Hg(II) without Ag(I) interference.

TABLE-1
TOLERANCE LIMITS IN THE DETERMINATION OF 0.024 mg L⁻¹
OF Hg(II) WITH 6-MERCAPTOPYRINE (RELATIVE ERROR \pm 5 %)

Ion added	Tolerated (g L ⁻¹)
Na ⁺ , K ⁺ , CH ₃ COO ⁻ , SO ₄ ²⁻ , PO ₄ ³⁻	40.00
Ca ²⁺ , Ba ²⁺ , Mg ²⁺ , Bi ³⁺	3.00
Pb ²⁺ , Cu ²⁺ , Cd ²⁺ , Fe ³⁺ , Ni ²⁺ , Cr ³⁺ , Co ²⁺	0.20
Fe ²⁺ , Al ³⁺ , CO ₃ ²⁻ , Cl ⁻ , Ag ⁻ , Br ⁻ , I ⁻ , CN ⁻	0.01

Application and validation: The proposed method has been successfully applied to the determination of mercury in tobacco and tobacco additives. The digested sample was quantitatively transferred to a 50 mL of volumetric flask and analyzed by the proposed procedure. The validity of the proposed method was confirmed by comparing the results obtaining from the sample analysis with those obtained by CVAAS. The results of various sample analysis are tabulated in Table-2.

TABLE-2
DETERMINATION OF MERCURY IN DIGESTED TOBACCO AND
TOBACCO ADDITIVE SAMPLES

Sample	Hg(II) added (μ g L ⁻¹)	Measured (μ g L ⁻¹)	
		Proposed method	CVAAS
Tobacco sauce (AM)	0	5.20 \pm 0.11	5.12 \pm 0.07
	16	21.3 \pm 0.17	21.16 \pm 0.05
	32	37.4 \pm 0.15	37.1 \pm 0.06
Cigarette (SF)	16	16.14 \pm 0.14	16.03 \pm 0.04
	32	32.18 \pm 0.13	32.06 \pm 0.09
	0	N.D.	N.D.
Tobacco leaf (SA1)	0	N.D.	N.D.
	16	16.07 \pm 0.07	16.02 \pm 0.04
	32	32.09 \pm 0.11	32.04 \pm 0.09

Conclusions

The proposed solid phase extraction method is a simple, rapid and high selective method for separation, preconcentration and determination of mercury in different tobacco samples. Practically any of applied cations interferes with the proposed method; this showed that the complexing agent is very selective toward Hg(II) in presence of other metal ions. This could be considered as an important advantage of both the ligand and the

proposed method. Furthermore, the enrichment factor of 50 was achieved with solid phase extraction by C₁₈ disks. The detection limit of proposed method reaches 0.4 µg L⁻¹, therefore the low concentration of mercury could be determined in tobacco samples with good results.

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