

Study on the Solid Phase Extraction of Hg(II)-SBDTR Chelate with C₁₈ Disks and Its Application to the Determination of Mercury in Tobacco and Tobacco Additive

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A sensitive, selective and rapid method has been developed for the determination of mercury based on the rapid reaction of mercury(II) with *p*-sulfobenzylidenethiorhodanine (SBDTR) and the solid phase extraction of the colored chelate with C₁₈ disks. In the presence of pH 3.5 sodium acetate-acetic acid buffer solution and Emulsifier-OP medium, SBDTR reacts with mercury(II) to form a red chelate of a molar ratio 1 : 2 (mercury to SBDTR). This chelate was preconcentrated by solid phase extraction with C₁₈ disks. An enrichment factor of 50 was achieved. The molar absorptivity of the chelate is $1.28 \times 10^5 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ at 545 nm in measured solution. Beer's law is obeyed in the range of 0.01-3 $\mu\text{g/mL}$. The relative standard deviation for eleven replicates sample of 0.01 $\mu\text{g/mL}$ is 1.65%. This method was applied to the determination of mercury in tobacco and tobacco additive with good results.

Key Words : Mercury, Solid phase extraction, Spectrophotometry, *p*-Sulfobenzylidenethiorhodanine

Introduction

Mercury is a toxic heavy metal ion. The Quality Standards of Tobacco and Tobacco Additives in Chinese states that the concentration of mercury should not be exceed 0.2 $\mu\text{g/g}$ in tobacco and tobacco additives.¹ Many sensitive instruments (such as spectrofluorimetry, X-ray fluorescence spectrometry, neutron activation analysis, atomic absorption spectrometry, chemiluminescence, electrochemical analysis, and the like) have widely been applied to the determination of mercury.²⁻⁸ Especially spectrophotometric methods have gained popularity for mercury determination as advantageous in respect of simplicity and low operating costs. For this reasons, a wide variety of spectrophotometric methods for the determination of mercury have been reported.⁹⁻²¹ Each chromogenic system has its advantages and disadvantages with respect to sensitivity, selectivity and rapidity.

However, the routine spectrophotometric methods are often not sensitive enough to determine low concentrations of mercury ion in tobacco and tobacco additives directly, when the mercury ion may be only $\mu\text{g/L}$ level. Consequently, a preconcentration step is usually required. Solid phase extraction is an attractive technique because it presents notable advantages.²²⁻²⁷ In this work, a new chromogenic reagent *p*-sulfobenzylidenethiorhodanine (SBDTR) was synthesized. The color reaction of SBDTR with mercury and the solid phase extraction of the colored chelate with C₁₈ disks were thoroughly studied. Based on this, a highly sensitive, selective and rapid method for the determination of mercury in tobacco and tobacco additives was developed.

Experimental Section

Apparatus. A UV-2401 spectrophotometer (Shimidzu, Japanese), equipped with a 1 cm microcells (0.5 mL) was used for all absorbance measurements. The pH measurements were measured with a Beckman Φ -200 pH meter. The solid phase extraction was performed on a Waters Solid Phase Extraction (SPE) Device (The device can prepare twenty samples simultaneously). Zorbax C₁₈ membrane disks [47 mm (diameter) \times 0.5 mm (thickness), 8 μm , 50 mg] (Agilent Technologies, USA) were used.

Chemicals. The SBDTR was synthesized according to the following procedure: 40 mL of acetic, 1.5 g of thiorhodanine acid and 1.2 g of *p*-sulfobenzaldehyde were added into a round bottom flask. The mixture was heated gently to dissolve thiorhodanine and *p*-sulfobenzaldehyde completely, and the solution was refluxed for about 1.5 h. In the course of refluxing, 1.0 mL of concentrated sulfuric acid was added

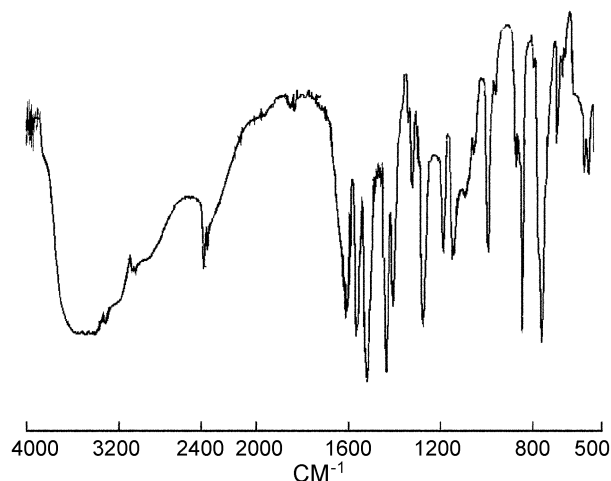


Figure 1. The infrared spectrum of SBDTR.

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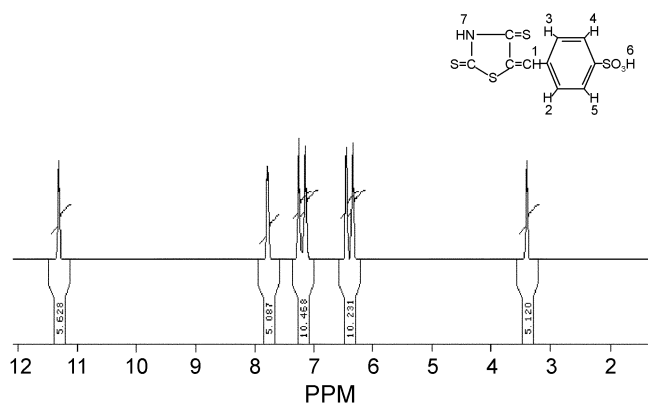


Figure 2. The ^1H nuclear magnetic resonance spectrum of QADMAB SBDTR.

dropwise. After the color of the solution turned red, the refluxing was stopped and the precipitant was separated by filtration. It was recrystallized twice with alcohol, and the SBDTR was obtained with a yield of 31%. The structure of SBDTR was verified by elemental analysis, IR (Figure 1), ^1H NMR (Figure 2). Elemental analysis: $\text{C}_{10}\text{H}_7\text{NO}_3\text{S}_4$, found (calculated) C 37.38 (37.84), N 4.21 (4.41), H 2.47 (2.22), S 39.76 (40.41).

All of the solutions were prepared with ultra-pure water obtained from a Milli-Q50 SP Reagent Water System (Millipore Corporation). High purity dimethyl formamide (DMF) (Fisher Corporation, USA) was used. A 3.0×10^{-4} mol/L of SBDTR solution was prepared by dissolving SBDTR with ethanol. A stock standard solution of mercury (1.0 mg/mL) was obtained from Chinese Material Standard Center, and a work solution of 0.5 $\mu\text{g}/\text{mL}$ was prepared by diluting this solution. A 0.5 mol/L of pH = 3.5 sodium acetate-acetic acid buffer solution containing 0.2 mol/L of pyrophosphoric acid was used. Emulsifier-OP solution (2.0% (v/v)) was prepared by dissolving Emulsifier-OP with water. All chemical used were of analytical grade unless otherwise stated.

Standard procedure. To a standard or sample solution containing no more than 3.0 μg of Hg(II) in a 50 mL of calibrated flask, A 5 mL of pH 3.5 sodium acetate-acetic acid containing 0.2 mol/L pyrophosphoric acid, 3.0 mL of 3.0×10^{-4} mol/L SBDTR solution and 2.0 mL of 2.0% Emulsifier-OP solution were added. The mixture was diluted to volume of 50 mL and mixed well. After 10 min, the solution was passed through the C_{18} disks at a flow rate of 50 mL/min, the colored chelate would be retained on the disks. After the enrichment finished, the retained chelate was eluted from the disks with 1.0 mL of DMF at a flow rate of 5 mL/min in reverse direction. The absorbance of this solution was measured at 545 nm in a 1 cm microcells (0.5 mL)

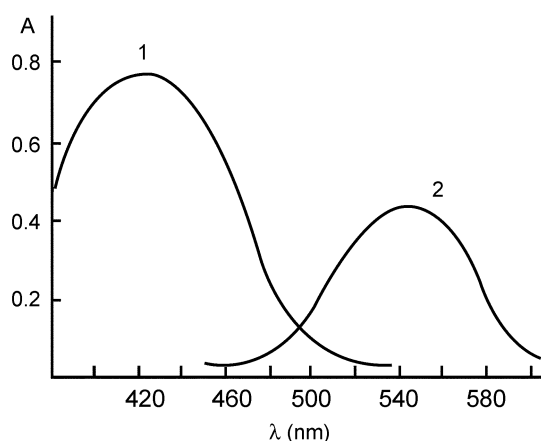


Figure 3. Absorption spectra of SBDTR and its Hg(II) complex: 1 SBDTR-Emulsifier-OP blank against water; 2 SBDTR-Emulsifier-OP-Hg(II) chelate against reagent blank.

against a reagent blank prepared in a similar way without mercury.

Results and Discussion

Absorption spectra. The absorption spectra of SBDTR and its Hg(II) chelate are shown in Figure 3. The absorption peaks of SBDTR and its complex in DMF medium are located at 420 nm and 545 nm.

Effect of acidity. The results showed that the optimal pH for the reaction of Hg(II) with SBDTR is 1.2-4.2. A sodium acetate-acetic acid buffer solution of pH 3.5 was recommended to control pH. As the use of 4-6 mL of the buffer solution (pH 3.5) per 50 mL of final solution was found to give a maximum and constant absorbance. The use of 5 mL buffer solution was recommended. The buffer solution containing a 0.15-0.25 mol/L of pyrophosphoric acid could greatly increase the selectivity of this system and do not affect the sensitivity. (Without pyrophosphoric in the buffer solution, the tolerance limits of foreign ions were 0.005 mg for Cu(II), Ag(I); 0.002 mg for Pd(II), Pb(II). However, the tolerance limits of foreign ions reaches 0.5 mg for Cu(II); 0.2 mg For Pb(II); 0.1 mg for Pd(II), Ag(I) when pyrophosphoric was present in the buffer solution). Therefore, 0.2 mol/L pyrophosphoric in the buffer was recommended.

Effect of surfactants. The effects of surfactants on Hg(II)-SBDTR system were studied (Table 1). In the absence of surfactants, as well as anionic surfactants or cationic surfactants medium, the Hg(II)-SBDTR chromogenic system gives a low absorption, whereas, in the presence of nonionic surfactants medium, the absorption of the chromogenic system increased markedly. Various nonionic surfactants

Table 1. The Effect of Surfactants on Hg(II)-SBDTR Chromogenic System

Surfactant	Absence	Emulsifier-OP	Tween-80	Tween-20	Tween-60	SDS	CTMAB	CPB
λ_{max} (nm)	520	545	540	535	536	525	520	520
ϵ ($\times 10^4$) $\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$	6.32	12.8	11.6	9.92	8.98	6.96	6.87	7.21

enhance the absorbance in the following order: Emulsifier-OP > Tween-80 > Tween-20 > Tween-60. Accordingly, Emulsifier-OP was the best additive and the use of 0.5-3 ml. of Emulsifier-OP solution gives a constant and maximum absorbance. The use of 2.0 ml. was recommended.

Effect of SBDTR concentration. For up to 3.0 μg of Hg(II), the use of about 3 ml. of 3.0×10^{-1} mol/L. of SBDTR solution has been found to be sufficient for a complete reaction. Accordingly, 3.0 ml. of SBDTR solution were added in all further measurement.

Stability of the chromogenic system. After the components are mixed, the absorbance reaches its maximum within 5 min at room temperature and remains stable for at least 8 h. When extracted into the DMF medium, the chelate remain stable for at least 12 h.

Solid phase extraction. Both the enrichment and the elution were carried out on a Waters SPE device. The flow rate was set to 50 ml./min during enrichment and 5 ml./min during elution.

Some experiments were carried out in order to investigate the retention of SBDTR and its Hg(II) chelate on the disks. It was found that the SBDTR and its Hg(II) chelate could be retained on disks quantitatively when they pass the disks as aqueous solution. The capacity of the disks for SBDTR was 32 mg and for its Hg(II) chelate was 28 mg in 50 ml. solution. In this experiment, the disks has adequate capacity to enrich the Hg(II)-SBDTR chelate.

In order to choose a proper eluant for the retained SBDTR and its Hg(II) chelate, we studied various organic solvents. The effect was in following order: DMF > acetonitrile > acetone > ethanol > methanol. So DMF was selected as eluant. Experiment show that it is easier to elute the retained SBDTR and its Hg(II) chelate from the disks in reverse direction than in forward direction, so it is necessary to invert the disks when eluting. 1.0 ml. of DMF was sufficient to elute the SBDTR and its Hg(II) chelate from the disks at a flow rate of 5 ml./min. The volume of 1.0 ml. DMF was selected.

Calibration curve and sensitivity. The calibration curve shows that beer's law is obeyed in the concentration range of 0.01-3 μg Hg(II) per ml in the measured solution. The linear regression equation obtained was: $A = 0.642 C (\mu\text{g}/\text{mL}) + 0.0108$ ($r = 0.9992$). The molar absorptivity was calculated

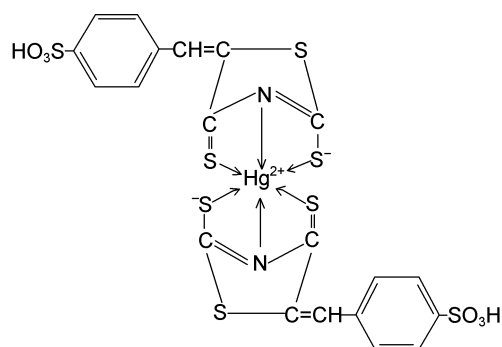


Figure 4. The structure of Hg(II)-SBDTR chelate.

Table 2. Tolerance Limits for the Determination of 1.0 mg of Hg(II) with SBDTR (relative error $\pm 5\%$)

Ion added	Tolerance (mg)
NO_3^- , K^+ , borate, Na^+	50
Li^+ , Al^{3+} , PO_4^{3-} , NO_2^- , SO_4^{2-} , ClO_4^-	20
Ca^{2+} , Mg^{2+} , SO_3^{2-} , S^{2-} , Ba^{2+} , IO_3^- , BrO_3^- , ClO_3^-	10
Mn^{2+} , Ce(IV) , W(VI) , Mo(VI) , U(IV) , Fe^{3+}	4
Ti(IV) , Bi(III) , V(V) , Cr(VI) , Zr(IV) , F^- , Fe^{2+} , Cl^-	1
Cd^{2+} , Cr^{3+} , La^{3+} , Sn(IV) , Zn^{2+} , Zr(IV) , Co^{2+} , Ni^{2+} , Cu^{2+}	0.5
Ru(III) , Bi(III) , Pb^{2+} , Sb^{3+} , Th(IV) , Br^- , Os(VIII) , I^-	0.2
Se(IV) , Te(IV) , $\text{S}_2\text{O}_3^{2-}$, Pd^{2+} , Ag^+	0.1
Ir(IV) , Rh(III) , Ru(III)	0.05
Pt(IV) , Au^{3+}	0.01
CN^- , SCN^-	0.005

to be $1.28 \times 10^5 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ at 545 nm. The relative standard deviation at a concentration level of 0.01 $\mu\text{g}/\text{ml}$. of Hg(II) (11 repeats determination) was 1.65%.

Composition of the complex. The composition of the complex was determined by continuous variation and molar ratio method. Both show that the molar ratio of Hg(II) to SBDTR is 1 : 2. The chelate probably has the structure gives in Figure 4.

Interference. The selectivity of the proposed method was investigated by the determination 1.0 $\mu\text{g}/50 \text{ ml}$. of Hg(II) in the presence of various ions within a relative error of $\pm 5\%$ are given in Table 2. The results show that most routine ions do not interfere with the determination. This method is high selectivity.

Application. The proposed method has been successfully applied to the determination of mercury in tobacco and tobacco additive. A 0.50 g of sample was accurately weighted into the Teflon high-pressure microwave acid-digestion bomb (Fei Yue, Analytical Instrument Factory, Shanghai, China). A 3.0 ml. of concentrated nitric acid and 5.0 ml. of 30% hydrogen peroxide were added. The bombs were tightly sealed and then positioned in the carousel of a microwave oven (Model WL 5001, 1000W, Fei Yue Analytical Instrument Factory, Shanghai, China). The system was operated at full power for 8.0 min. The digest was evaporated to near dryness. The residue was dissolved with 5 mL of 5% of nitric acid and transferred into a 50 mL of calibrated flask quantitatively, and then analyzed by general

Table 3. Determination Results of Mercury in Real Samples

Samples	Mercury found ($\mu\text{g}/\text{g}$)		RSD% (n=5)	Recovery %
	This Method	Reference Method		
Tobacco leaf (SA1)	0.135	0.132	2.1	97
Tobacco leaf (SA2)	0.198	0.204	2.3	102
Glycerol (AL)	0.204	0.198	2.5	96
Tobacco sauce (AM)	0.167	0.175	2.1	105
Cigarette (SF)	0.201	0.208	2.1	104
Tobacco essence (AG)	0.193	0.183	2.5	95

Table 4. The comparison reagents for the spectrophotometric determination of mercury

Reagents	λ_{\max} (nm)	ϵ (10^4)	Linear range ($\mu\text{g/mL}$)	Detect limit (ng/mL)	Conditions	Interference ion	Ref
H ₂ Dz	485	7.1	0.1 - 2	1	pH 2-5, extraction CCl ₄	Cu, Zn, Cd, Pb	1
CPDAAB	540	22.2	0.08 - 0.8	20	alkaline medium, pH 10	Co, Zn, Cd, Ni	11
DAA	514	22	0 - 0.8	2	pH 9.0, Triton X-100	Cd, Ag, Pb, Ni	13
BG	625	59.6	0.004 - 0.5	—	pH 5, flotation of Hg-I ⁻ -BG with cyclohexane	Ni	14
CMPQ	512	8.0	0.08 - 2	40	pH 4-4.8, Triton X-100	Co, Cu, Pb, Zn, Ni	15
TPPS ₄	413	27.6	0 - 0.6	0.5	pH 8.0, heated in 100 °C water bath for 30 min	Mn, Cu, Ag, Pb, Fe, Co, Ni, Zn, Cd, Mg	16
PAR	500	6.8	0.1 - 2	10	pH 10,	Cu, Ni, Co, Fe, Zn, Ag, Cr, V, W, Mo, Mn	17
NBS	430	—	1 - 10	0.2	nitric acid medium	Cu, Zn, Cu	18
SBDTR	545	12.8	0.01 - 3	0.02	pH 3.5, Emulsifier-OP	Pt(IV), Au ³⁺ , CN ⁻ , SCN ⁻	This Work

H₂Dz (Diphenylthiocarbazone); CPDAAB (o-carboxyphenyl diazoamino *p*-azobenzene); DAA (Diazoaminoazobenzene); BG (Brilliant green); (CMPQ) 5-(2'-Carbomethoxyphenyl)-azo-8-quinolinol; TPPS₄ (tetra-(4-aminophenyl)-porphyrin); PAR (2-pyridylazo)-naphthol; NBS (*p*-Nitrobenzenesulfonate)

procedure. The recovery test of mercury by added 0.2 μg of mercury in samples was carried out. A standard method using atomic absorption spectrometry was also been used as reference method. The results are shown in Table 3.

Conclusion

A comparison of the possibilities of the SBDTR with those of other methods (Table 4) shows that SBDTR is a sensitive and selective spectrophotometric reagent for mercury. The molar absorptivity of the chelate reaches $1.28 \times 10^5 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$. When masked with pyrophosphoric acid, most of the routine foreign ions do not interfere the determination. By solid phase extraction of the SBDTR-Hg(II) chelate with C₁₈ disks, the enrichment factor of 50 was achieved, and the detection limit reaches 0.02 mg/L in the original samples. The consuming of organic solvents in this method is much lower than those consumed in liquid-liquid extraction method. By using Waters SPE device, twenty samples can be prepared simultaneously. This method is rapid for simultaneously preparing large amount of sample. In a word, for determination of mercury, this method is high sensitivity, selectivity and rapidity.

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