

Simultaneous Determination of Four Heavy Metal Ions in Tobacco and Tobacco Additive by Online Enrichment Followed by RP-HPLC and Microwave Digestion

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Abstract

A new method for the simultaneous determination of tin, lead, cadmium, and mercury in tobacco and tobacco additive by reversed-phase high-performance liquid chromatography combined with microwave digestion and an online enrichment technique is developed. The tin, lead, cadmium, and mercury ions are precolumn derivatized with tetra-(4-dimethylaminophenyl)-porphyrin (T₄-DMAPP) to form color chelates. The Sn-T₄-DMAPP, Hg-T₄-DMAPP, Cd-T₄-DMAPP, and Pb-T₄-DMAPP chelates are absorbed onto the front of the enrichment column using a buffer solution of 0.05 mol/L pyrrolidine-acetic acid (pH = 10.0) as the mobile phase. After the concentration is finished (by switching the six-port switching valve) the retained chelates are back-flushed by the mobile phase and move to the analytical column. The chelate separation on the analytical column is satisfactory using gradient elution with methanol (containing 0.05 mol/L pyrrolidine-acetic acid buffer salt, pH = 10.0) and tetrahydrofuran (containing 0.05 mol/L pyrrolidine-acetic acid buffer salt, pH = 10.0). The linearity range is 0.01–120 µg/L for each metal ion. The detection limits (S/N = 3) of tin, lead, cadmium, and mercury are 0.6, 0.8, 0.5, and 0.6 ng/L, respectively. This method is applied to the determination of tin, lead, cadmium, and mercury in tobacco and its additive with good results.

Introduction

Tin, lead, cadmium, and mercury ions are toxic heavy metal. The quality standards of the Chinese Tobacco Company for tobacco and tobacco additive state that the concentration of tin, lead, cadmium, and mercury should not exceed 0.5, 1.0, 0.2, and 0.1 µg/g, respectively, in tobacco and its additive (1). Therefore, the determination of trace tin, lead, cadmium, and mercury ions in tobacco and its additive is important (2,3). The reversed-phase (RP) high-performance liquid chromatographic (HPLC) tech-

nique with precolumn derivatization has been proven to be a reliable technique for the separation and determination of trace amount of metal ions. Many reagents (such as 8-quinolinol derivatives, azo dyes, β-diketones, hydrazones, dithiocarbamate, cyclohexanediamine, porphyrin, and the like) have been examined as precolumn derivatization reagent (4–10). Among the various reagents, porphyrins showed the advantages of having strong complexing ability and high sensitivity and stability of metal chelates. The molar absorptivity of metal porphyrin chelates above 10⁵ L/mol/cm is often found. Porphyrins can form 1:1 chelates with many metal ions and these chelates are stable during HPLC separation (7–10). Because of these facts, porphyrin reagents have received considerable attention and are widely applied to the simultaneous determination of metal ions (9–19). However, for the determination of tin, lead, cadmium, and mercury ions, the separation of the chelates using the traditional RP-HPLC techniques have some disadvantages. The silica-bond RP columns are not suitable for the separation the porphyrin chelates of tin, lead, mercury, and cadmium, because they can be used only in the range of pH 2–8, whereas the metal chelates are stable in weak alkaline medium. Also the metal-porphyrin chelates have a narrow absorption band, and using a single wavelength detector for the simultaneous monitoring of several metal chelates cannot achieve high sensitivity, especially for the lead ion. For the mentioned reasons, the simultaneous determination of tin, lead, cadmium, and mercury has not been reported yet. In this paper, a Waters (Milford, MA) XTerra RP18 chromatographic column (pH range 1–12) was recommended for the enrichment and separation of Sn-tetra-(4-dimethylaminophenyl)-porphyrin (T₄-DMAPP), Pb-T₄-DMAPP, Hg-T₄-DMAPP, and Cd-T₄-DMAPP chelates using a mobile phase pH of 10.0. The injection of a large volume of sample solution was possible by a valve-switching HPLC system equipped with a photodiode array detector. With a photodiode array detector, each of the metal chelates could be monitored at its maximum absorption wavelength and the peak purity could be identified. The tin, lead, cadmium, and mercury in tobacco and its additive could be simultaneously determined with good results by this method.

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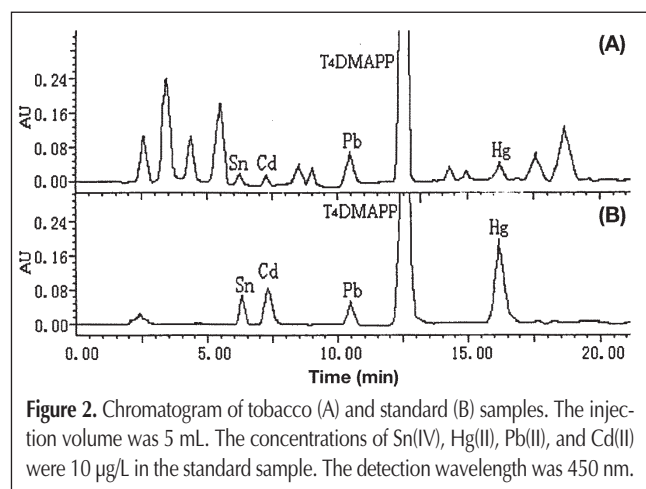
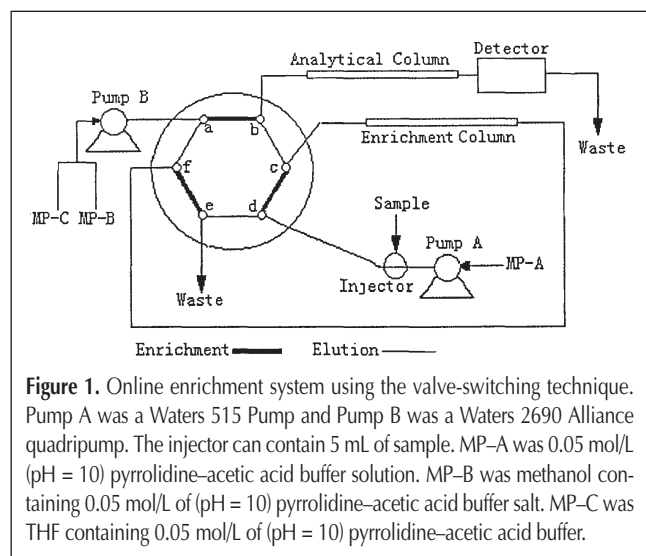
Experimental

Apparatus

The online column enrichment system used is shown in Figure 1. This system included a Waters 2690 Alliance quadripump, 515 pump, 996 photodiode array detector, six-port switching valve, large volume injector (which can contain a 5.0-mL sample) and column. The enrichment column was a Waters XTerra RP18 (5- μ m, 3.9- \times 20-mm) and the analytical column was a Waters XTerra RP18 (5- μ m, 3.9- \times 150-mm). The pH value was determined with a Beckman Φ -200 pH meter (Beckman Instruments, Fullerton, CA).

Chemicals

All of the solutions were prepared with ultrapure water obtained from a Milli-Q50 SP Reagent Water system (Millipore, Bedford, MA). The tin(IV), lead(II), cadmium(II), and mercury(II) standard solution consisted 1.0 mg/mL (obtained from Fisher Scientific, Atlanta, GA), and a working solution of 0.2 μ g/mL was prepared by diluting this standard solution. HPLC-grade methanol and tetrahydrofuran (THF) was acquired from Fisher. The pyrrolidine-acetic acid buffer solution consisted of 0.5 mol/L and a pH of 10. T_4 -DMAPP was synthesized by our laboratory to be



used in accordance with the literature (20) and was dissolved with THF to make a 1.0×10^{-4} mol/L solution. Mobile phase A consisted of 0.05 mol/L pyrrolidine-acetic acid buffer solution (pH = 10). Mobile phase B was methanol containing 0.05 mol/L pyrrolidine-acetic acid buffer salt (pH = 10). Mobile phase C was THF containing 0.05 mol/L pyrrolidine-acetic acid buffer salt (pH = 10). All other reagents used were of analytical reagent grade. The glass and Teflon ware was used often and soaked in 5% of nitric acid overnight and then thoroughly washed with pure water.

Standard procedure

Amounts of 0–15 mL of a 0.2- μ g/mL standard or digested sample solution was transferred into a 25-mL volumetric flask. Six milliliters of a 1.0×10^{-4} mol/L T_4 -DMAPP THF solution and 4.0 mL of a 0.5-mol/L pyrrolidine-acetic acid buffer solution (pH = 10) were added. The solution was diluted to volume with water and mixed well. The mixture was heated in a boiling water bath for 15 min. After cooled, the solution was diluted to volume with THF for subsequent analysis. A 5.0-mL solution was introduced into the injector and sent to the enrichment column with mobile phase A at a flow rate of 2.0 mL/min. After the concentration was finished, by switching the valve of the six ports switching valve, the metal- T_4 DMAPP chelates, which absorbed onto the front of enrichment column, were eluted by mobile phase B and mobile phase C at a flow rate of 1.0 mL/min in the opposite direction. This was sent to the analytical column and separated on it. A tridimensional chromatogram was recorded from 350–600 nm with photodiode array detector and the chromatogram of 450 nm is shown in Figure 2. In the course of the separation, the composition of mobile phase was 0 min (100% B and 0% C) and 10 min (90% B and 10% C) in linear ramp. Each of the metal- T_4 DMAPP chelates was detected at its maximum absorption wavelength.

Result and Discussion

Precolumn derivation

In a weak alkaline medium of pH 8.5–12, Sn(IV), Hg(II), Pb(II), and Cd(II) can form stable and colored chelates with T_4 -DMAPP, therefore, 0.5 mol/L of pyrrolidine-acetic acid buffer solution (pH = 10) was recommended to control the pH. It was found that 0.5 mL of 1.0×10^{-4} mol/L T_4 -DMAPP THF solution was sufficient to complex 3.0 μ g of Sn(IV), Hg(II), Pb(II), and Cd(II). But in real samples, the foreign ions (such as Mg^{2+} , Cu^{2+} , Pd^{2+} , Ni^{2+} , Co^{2+} , Fe^{3+} , Mn^{2+} , Ru^{3+} , and Zn^{2+}) can form complexes with T_4 -DMAPP and consume the reagents. Thus, the amount of T_4 -DMAPP must be in excess. In this experiment, A 6.0-mL of 1.0×10^{-4} mol/L T_4 -DMAPP was recommended. The reaction of Sn(IV), Hg(II), Pb(II), and Cd(II) with T_4 -DMAPP was slow at room temperature. Heating can accelerate the reaction. The reaction was complete when the heating was done in a boiling water bath for 15 min and the complex remained stable for 4 h after cooling. Good results were obtained with 15 min of heating. Metal- T_4 -DMAPP chelates have poor solubility in the water solution. It was necessary to add an organic solvent to improve their solubility. Various organic solvents enhance the solubility in the following sequence: THF > acetone > acetonitrile > ethanol > methanol. THF was selected as

being the best solvent. The volume of THF within 5.4–8 mL had a good effect on improving the solubility and do not affect the chromatographic separation. Therefore, a volume of 6.0 mL T₄-DMAPP THF solution was recommended.

Online enrichment

Because the Sn-T₄-DMAPP, Hg-T₄-DMAPP, Pb-T₄-DMAPP and Cd-T₄-DMAPP chelates were stable in weak alkaline medium, to avoid the chelates decomposing during the elution, a 0.05 mol/L of pH = 10 pyrrolidine–acetic acid buffer solution (mobile phase A) was selected as mobile phase to send the chelates to the enrichment column and a Waters XTerra RP18 chromatographic column (5- μ m, 3.9- \times 20-mm) with a pH range of 1–12 was selected as the enrichment column. In order to determine the trace metal ions, a large volume of sample should be injected. An injected sample with a volume of 5 mL was large enough to determine Sn(VI), Hg(II), Pb(II), and Cd(II) in tobacco and its additive, and therefore a 5-mL sample injection was recommended.

Spectrophotometric properties

The absorption spectrum of metal-T₄-DMAPP was obtained from the chromatogram that was recorded by the photodiode array detector. The maximum absorption wavelengths of Sn-T₄-DMAPP, Hg-T₄-DMAPP, Pb-T₄-DMAPP, and Cd-T₄-DMAPP were 435, 450, 462, and 438 nm, respectively. To get maximum sensitivity, each metal-T₄-DMAPP chelate was monitored at its maximum absorption wavelength.

Chromatographic separation

Chelate decomposition can be avoided and a good peak shape can be achieved with a mobile phase that has a pH between 8.5 and 12. Therefore, two weak alkaline solutions were recommended: mobile phase B of methanol [containing 0.05 mol/L pyrrolidine–acetic acid buffer salt (pH = 10)] and mobile phase C of THF [containing 0.05 mol/L pyrrolidine–acetic acid buffer salt (pH = 10)]. Because a routine silica-bonded RP chromatographic column is not stable at pH 10, a Waters XTerra RP18 chromatographic column (5- μ m, 3.9- \times 150-mm) was selected as the analytical column in this experiment. XTerra RP18 columns have good stability at pH 1–12. The relative proportion of mobile phase B and C was varied to effect the separation. The proper composition of mobile phase during gradient elution was selected as follows: 0 min (100% B and 0% C), 10 min (90% B and 10% C) in linear ramp.

Calibration graphs

Under the optimum conditions, regression equations of metal-T₄-DMAPP chelates were established based on the standard samples injected and its peak areas. Limits of detection of a signal-to-noise ratio (S/N = 3) were calculated.

Table I. Regression Equation, Coefficient, and Detection Limit for Various Components

Components	Regression equation	Linear range (μ g/L)	Coefficient	Detect limit (ng/L)	%RSD* (n = 10)
Sn-T ₄ -DMAPP	$A = 1.85 \times 10^6 C + 113$	0.01–120	$r = 0.9996$	0.6	1.8
Cd-T ₄ -DMAPP	$A = 2.78 \times 10^6 C + 106$	0.01–120	$r = 0.9996$	0.5	2.2
Pb-T ₄ -DMAPP	$A = 2.06 \times 10^6 C + 154$	0.01–120	$r = 0.9995$	0.8	2.8
Hg-T ₄ -DMAPP	$A = 2.12 \times 10^6 C - 121$	0.01–120	$r = 0.9994$	0.6	2.1

* RSD, relative standard deviation.

Table II. Tolerance Amounts of Foreign Ions for the Determination of 10 μ g/L of Cd(II), Pb(II), Hg(II), and Sn(IV)

Ions for determination	Tolerance amount of foreign ions (μ g/L)											
	Mg ²⁺	Cu ²⁺	Pd ²⁺	Ni ²⁺	Co ²⁺	Fe ³⁺	Mn ²⁺	Zn ²⁺	Pt ²⁺	Ba ²⁺	Ru ³⁺	Ag ⁺
Cd(II)	50000	2500	1000	3000	2500	2500	1500	2000	1000	3000	500	100
Pb(II)	40000	3000	500	2500	1500	2500	3000	2500	1500	2500	800	500
Sn(IV)	2000	2000	800	1500	2000	2500	3000	1500	2000	800	1000	1000
Hg(II)	60000	2000	1000	2500	2500	2000	2500	2000	1000	3000	800	1000

Table III. Determination Results (ng/g) of the Sample with This Method

Components	Samples (ng/g)							%RSD*	%Recovery*
	Propylene glycol	Glycerol	Sorbitol	Tobacco sauce	Tobacco leaf	Cigarette			
Cd	11.8	48.4	128	94.5	36.2	68.4	2.1–2.8	94–103	
Hg	11.2	18.6	52.6	46.3	14.5	23.5	1.8–3.1	95–104	
Pb	102	212	916	820	205	532	1.8–2.6	93–102	
Sn	86.5	156	435	235	138	218	2.2–3.2	96–103	

* n = 5.

The results are shown in Table I. The reproducibility of this method was also examined for 10 µg/L of Sn(IV), Pb(II), Cd(II), and Hg(II). The relative standard deviations ($n = 10$) were also shown in Table I.

Interference

Under the precolumn derivatization conditions, the foreign ions of Mg²⁺, Cu²⁺, Pd²⁺, Ni²⁺, Co²⁺, Fe³⁺, Mn²⁺, Zn²⁺, Pt²⁺, Ba²⁺, Ag⁺, Ru³⁺, as well as others can react with T₄-DMAPP to form color chelates. To examine the selectivity of this method, the interference of foreign ions was investigated. When 6.0 mL of 1.0 × 10⁻⁴ mol/L T₄-DMAPP was used with 10 µg/L of Sn(IV), Pb(II), Cd(II), and Hg(II), respectively, the tolerance amount of each ion with an error of ± 5% was shown in Table II. The results show that most foreign ions do not interfere with the determination.

Application to tobacco and its additive sample

A sample of 0.20 g was weighed accurately into the Teflon high-pressure microwave acid-digestion bomb (Fei Yue Analytical Instrument Factory, Shanghai, China). A 2.5-mL sample of concentrated nitric acid and 2.5 mL of 30% hydrogen peroxide were added. The bombs were sealed tightly and then positioned in the carousel of the microwave oven (Model WL 5001, 1000-W, Fei Yue Analytical Instrument Factory). The system was operated at full

power for 6.0 min. The digest was evaporated to near dryness. The residue was dissolved with 5 mL of 5% of nitric acid and quantitatively transferred to a 50-mL calibrated flask. The volume was diluted to 50 mL with water. The Sn, Hg, Pb, and Cd contents were analyzed using a proper volume of this solution according to the general procedure. The results were shown in Table III together with the results of a recovery test by the appropriate added amount of Sn, Hg, Pb, and Cd in tobacco or its additive sample. A reference method using inductively coupled plasma-mass spectrometry (ICP-MS) was also used. The results are shown in Table IV. For each of the tobacco and additive samples, the peak purities of Sn-T₄-DMAPP, Hg-T₄-DMAPP, Pb-T₄-DMAPP, and Cd-T₄-DMAPP chelates were identified by a photodiode array detector to ensure that no foreign peaks overlapped with them.

Conclusion

The comparison of the proposed method with another method using porphyrin reagents for the determination of heavy metal by HPLC is given in Table V. The proposed method has the following advantages. (1) Four heavy metal ions, Sn(IV), Pb(II), Cd(II), and Hg(II) can be successfully separated. (2) Through the use of an

Components	Samples (ng/g)						%RSD*	%Recovery*
	Propylene glycol	Glycerol	Sorbitol	Tobacco sause	Tobacco leaf	Cigarette		
Cd	12.5	49.2	122	93.2	35.4	67.3	2.5–3.6	91–107
Hg	10.3	20.1	52.9	45.8	15.3	22.8	2.4–3.5	90–105
Pb	108	217	912	815	212	521	2.8–2.8	89–106
Sn	87.6	148	416	218	132	225	2.6–3.4	91–108

* $n = 5$.

Reagents	Metal ions	Column	Mobile phase	Detection limit	Ref
TPP	Cd, Cu, Pb, Zn	Micropak MCH-5 (150 × 4)	Methanol–water–chloroform (94:1:5, v/v)	Cd (10 ng/mL), Cu (40 ng/mL), Pb (30 ng/mL)	10
Br-TPPS ₄	Mn, Fe, Zn, Pd, Cu	Lichrosorb ODS (5 µm, 200 × 4.6)	Acetonitrile–water (45:55), Contain 0.01 mol/L TBABr	Mn (16 ng/mL), Fe (7.9 ng/mL), Zn (3.1 ng/mL), Pd (7.0 ng/mL), Cu (3.6 ng/mL)	12
TPPS ₄	Cd, Hg, Zn	Silica-gel cation Exchanger (200 × 4)	NaCl-Tartaric solution	Cd (20 ng/mL), Hg (50 ng/mL), Zn (10 ng/mL)	13
T ₄ HPP	Cd, Pb, Cu, Zn	Prodigy-ODS Yilite-ODS (250 × 4)	Methanol–pH 8.0 borate–sodium Hydroxide buffer solution	Cd (80 ng/mL), Pb (80 ng/mL), Cu (80 ng/mL), Zn (500 ng/mL)	14
T ₄ MPPS ₄	Cu, Zn	ODS-80Ts (250 × 4.6)	Acetonitrile–water (38:62), Contain 0.01 mol/L TEAI	Cu (30 ng/mL), Zn (50 ng/mL)	15
TPyP	Cu, Pd	(250 × 4.6)	Methanol	Cu (0.32 ng/mL), Pd (8.5 ng/mL)	16
T ₄ -DMAPP	Sn, Pb, Hg, Cd	Xterra RP18 (150 × 3.9)	Methanol–THF (containing 0.05 mol/L pH 10 buffer)	Sn (0.6 pg/mL), Pb (0.8 pg/mL), Hg (0.6 pg/mL), Cd (0.5 pg/mL)	This work

* Abbreviations: TPP, tetraphenylporphyrin; Br-TPPS₄, meso-tetrakis-(3-bromo-4-sulfophenyl)-porphyrin; TPPS₄, meso-tetra-(4-sulfophenyl)-porphyrin; T₄HPP, (meso-tetra-(4-hydroxyphenyl))-porphyrin; T₄MPPS₄, meso-tetrakis-(4-methyl-3-sulfophenyl)-porphyrin; and TpyP, 5,10,15,20-tetrakis-(4N-pyridyl) porphyrin.

online enrichment system, a large volume of sample (5 mL) can be injected. (3) With a photodiode array detector, each metal chelate can be monitored at its maximum absorption wavelength to get a maximum sensitivity. The peak purity can be identified to avoid the interference of foreign peaks. It is more sensitive than single wavelength detector and the results are more reliable. This method is highly sensitive and selective and can be applied to the determination of tin, lead, cadmium, and mercury in tobacco and its additive.

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