

ARTICLES

Study on the Determination of Heavy-Metal Ions in Tobacco and Tobacco Additives by Microwave Digestion and HPLC with PAD Detection

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Abstract—A new method for the simultaneous determination of heavy-metal ions in tobacco and tobacco additive by microwave digestion and reversed-phase high-performance liquid chromatography (RP-HPLC) was developed. The tobacco and tobacco additive samples were digested by microwave digestion. The lead, cadmium, mercury, nickel, copper, and tin ions in the digested samples were precolumn derivated with tetra-(4-aminophenyl)-porphyrin (T₄-APP) to form color chelates; the Hg-T₄-APP, Cd-T₄-APP, Pb-T₄-APP, Ni-T₄-APP, Cu-T₄-APP, and Sn-T₄-APP chelates were then enriched by solid-phase extraction with C₁₈ disks and the retained chelates were eluted from the disks using tetrahydrofuran (THF). The chelates were separated on a Waters Xterra™ RP₁₈ column by gradient using methanol (containing 0.05 mol/L pyrrolidine–acetic acid buffer salt, pH 10.0) and acetone (containing 0.05 mol/L pyrrolidine–acetic acid buffer salt, pH 10.0) as a mobile phase at a flow rate of 0.5 mL/min and detected with a photodiode array detector in the range 350–600 nm. The detection limits of lead, cadmium, mercury, nickel, copper, and tin were 5, 4, 2.5, 5, 8, and 4 ng/L, respectively, in the original samples. The method was applied to the determination of lead, cadmium, mercury, nickel, copper, and tin in tobacco and tobacco additive with good results.

INTRODUCTION

The RP-HPLC technique with precolumn derivation has proven to be an effective and reliable technique for the separation and determination of trace amounts of metal ions. Many kinds of reagents (e.g., 8-quinolinol derivatives [1–3], azo dyes [4–8], β-diketones [9, 10], hydrazones [11–13], dithiocarbamate [14–17], cyclohexanediamine [18, 19], porphyrin [20–22], etc.) have been examined as precolumn derivation reagents. A number of articles on this approach have been published [20–21]. Among the various kinds of reagents, porphyrin ligands are useful because of their high molar absorptivity and high stability. The molar absorptivity of metal–porphyrin chelates above is often 10⁵. Porphyrins can form very stable 1 : 1 chelates with many metal ions, and these chelates are very difficult to decompose during HPLC separation [20–25]. Porphyrin reagents have therefore been the subject of growing attention and are widely applied to the simultaneous determination of metal ions [32–42]. However, for the simultaneous determination of heavy-metal ions, traditional RP-HPLC techniques have a number of disadvantages. On the one hand, the routine reversed-phase chromatographic column has a narrow pH range of 2–8. The column is not suitable for separating the porphyrin chelates of heavy-metal ions, which are stable only in a weak alkaline medium [20, 24, 25]. On the other

hand, metal–porphyrin chelates have a narrow absorption band, and using a single wavelength detector for the simultaneous monitoring of several metal chelates cannot provide high sensitivity, especially for the lead ion.

Determination of trace lead, cadmium, mercury, copper, nickel, and tin in tobacco and tobacco additives is important because of the metals' biological significance [43, 44]. In this paper, a simple, sensitive, and selective method for the simultaneous determination of lead, cadmium, mercury, nickel, copper, and tin ions in tobacco and tobacco additives was developed on the basis of solid-phase extraction and the RP-HPLC separation of the metal–T₄-APP chelates using a pH 10.0 mobile phase on a Waters Xterra™ RP₁₈ chromatographic column.

EXPERIMENTAL

Apparatus. The HPLC system consisted of an HP 1100 pump and an HP 1100 photodiode array detector (Agilent Technologies, United States). The pH values were determined using a Beckman Φ-200 pH meter. The absorbance measurement was measured with a Shimadzu UV-2401 spectrophotometer. The atomic absorption spectrometry analysis was made with a Shimadzu AA-6701F atomic absorption spectrophotometer.

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The separation column used was a Waters Xterra™ RP₁₈ column (3.9 × 150 mm, 5 μm) (Waters Corporation, United States). The extraction was performed on a Waters solid-phase extraction (SPE) device (which can prepare twenty samples simultaneously), and Zorbax C₁₈ membrane disks (47 mm in diameter 0.5 mm thick, 8 μm, 50 mg) (Agilent Technologies, United States) were used.

Chemicals. All of the solutions were prepared with ultrapure water, obtained from a Milli-Q50 SP Reagent Water System (Millipore Corporation, United States). Pb(II), Cd(II), Hg(II), Ni(II), Cu(II), and Sn(IV) standard solutions of 1.0 mg/mL were obtained from the Chinese Standards Center; working solutions of 0.2 μg/mL were prepared by diluting the standard solutions with HPLC grade methanol and acetone (Fisher Corporation, United States). The pyrrolidine–acetic acid buffer solution was 0.5 mol/L, pH 10. The triton X-100 solution was 2.0% (v/v). T₄-APP was synthesized by our laboratory as described in [45] and was dissolved with THF to make a 1.5 × 10⁻⁴ mol/L solution. Mobile phase A was methanol (containing 0.05 mol/L pyrrolidine–acetic acid buffer salt (pH 10)), while mobile phase B was acetone (containing 0.05 mol/L pH 10 pyrrolidine–acetic acid buffer salt). All of the other reagents used were of analytical reagent grade. The glass- and Teflonware used were soaked in 5% nitric acid for a prolonged period and then thoroughly washed with pure water.

Standard procedure. We transferred the appropriate volume of 0.2 μg/mL standard or digested sample solution to a 50 mL volumetric flask. To this were added 5.0 mL of T₄-APP THF solution, 2 mL of Triton X-100 solution, and 5 mL of 0.5 mol/L pH 10 pyrrolidine–acetic acid buffer solution. The solution was diluted to volume with water and mixed well. The mixture was heated in a boiling water bath for 10 min. After it cooled, the solution was passed through the C₁₈ disks at a flow rate of 50 mL/min. After enrichment was complete, the retained chelates were eluted from disks with 2.5 mL of THF at a flow rate of 5 mL/min in the opposite direction. The solution was passed through filters of 0.45 μm and volatilized to 0.6 mL, then diluted to a volume of 1.0 mL with methanol. We then injected 20 μL of sample for HPLC analysis. A three-dimensional chromatogram was recorded in the range 350–600 nm with a photodiode array detector; the chromatogram of 435 nm is shown in the figure. During separation, the composition of the mobile phase took 0 min (A 100% and B 0%) and 10 min (A 90% and B 10%) in the linear ramp. To get maximum sensitivities, each metal–T₄-APP chelate was detected at its maximum absorption wavelength.

RESULTS AND DISCUSSION

The selection of porphyrin reagent. In this paper, seven porphyrin reagents—tetra-(4-bromophenyl)-por-

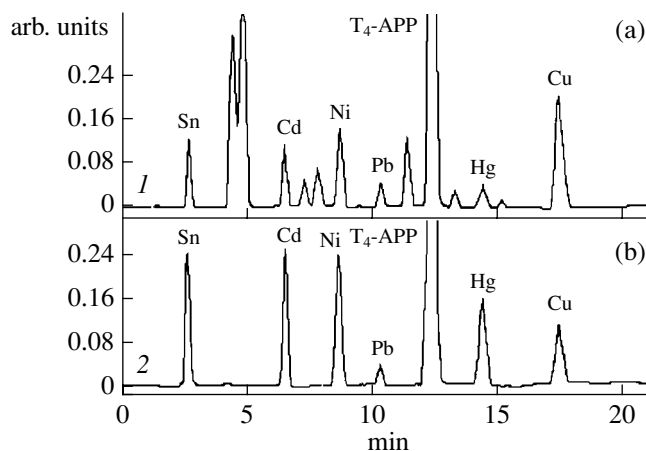


Fig. 1. Chromatogram of standard sample and tobacco sample: (a) tobacco sample, (b) standard sample. The injection volume is 20 μL. Detection wavelength is 435 nm. Other conditions are as in the standard procedure.

phyrin (T₄-BPP), tetra-(4-chlorophenyl)-porphyrin (T₄-CPP), tetra-(4-methoxyphenyl)-porphyrin (T₄-MOPP), tetra-(4-methylphenyl)-porphyrin (T₄-MPP), tetra-(4-sulfophenyl)-porphyrin (T₄-SPP), tetra-(4-hydroxyphenyl)-porphyrin (T₄-HPP), and tetra-(4-aminophenyl)-porphyrin (T₄-APP)—were studied as precolumn derivation reagents for Cu(II), Ni(II), Sn(IV), Hg(II), Pb(II), and Cd(II) ions. The experiment showed that all seven reagents can form colored chelates with Cu(II), Ni(II), Sn(IV), Hg(II), Pb(II), and Cd(II). T₄-MPP, T₄-CPP, T₄-MOPP, T₄-BPP, and their metal chelates have very poor solubility in water. To get a stable solution, a large amount of organic solvents must be added to improve the chelates' solubility. This is inconvenient. T₄-SPP and T₄-HPP can form soluble chelates with metal ions. In an alkaline medium, however, the chelates have poor retention on the reversed-phase column because both the sulfonic group and the hydroxy group on the porphyrin reagents can ionize. Thus, T₄-SPP and T₄-HPP were unsuitable for use as precolumn derivation reagents in this experiment. T₄-APP can form soluble chelates in an aqueous medium when Triton X-100 is present, and the chelates have good retention on a reverse-phase column. T₄-APP was thus selected as the precolumn derivation reagent in this experiment.

Precolumn derivation. According to the literature [46–48], the optimal pH is 8.2–11.8 for the reaction of Cu(II), Ni(II), Sn(IV), Hg(II), Pb(II), and Cd(II) with T₄-APP, so a 0.5 mol/L buffer solution of pH 10 pyrrolidine–acetic acid was recommended to control pH. It was found that 1.0 mL of 1.5 × 10⁻⁴ mol/L T₄-APP THF solution was sufficient to complex 3.0 μg of Cu(II), Ni(II), Sn(IV), Hg(II), Pb(II), and Cd(II). In real samples, however, foreign ions (e.g., Mg²⁺, Pd²⁺, Fe³⁺, Mn²⁺, Zn²⁺, Bi³⁺, Co²⁺, Ba²⁺, Ag⁺, etc.) can complex with T₄-APP to consume reagents. More T₄-APP was thus

needed. In this experiment, a 5.0 mL of 1.5×10^{-4} mol/L T_4 -APP solution was used.

The reaction of Cu(II), Ni(II), Sn(IV), Hg(II), Pb(II), and Cd(II) with T_4 -APP was slow at room temperature. Heating can accelerate the reaction; the reaction was complete upon heating in a boiling water bath for 10 min. The chelate remains stable for 5 h after cooling, so a 10 min period of heating was selected.

Metal- T_4 -APP chelates have poor solubility in water. A suitable amount of surfactants must be added to improve their solubility. Various surfactants enhance the absorbance in the following sequence: Triton X-100 > Tween-80 > Tween-20 > Tween-60 > CTMAB > CPB > SDS > SDBS. Triton X-100 was thus selected as cosolvent. Our experiments showed that 2.0% Triton X-100 solution in 1–4 mL improved solubility and did not affect the chromatographic peak. We thus recommend 2.0 mL of Triton X-100 solution.

Solid-phase extraction. Both enrichment and elution were done on a Waters SPE device, which can prepare twenty samples simultaneously. The flow rate was set at 50 mL/min for enrichment and 5 mL/min for elution.

A number of experiments were performed in order to investigate the retention of metal- T_4 -APP chelates on the disks. It was found that the Cu- T_4 -APP, Sn- T_4 -APP, Ni- T_4 -APP, Hg- T_4 -APP, Pb- T_4 -APP, and Cd- T_4 -APP chelates were retained quantitatively on the disks when they passed through them in aqueous solution. The capacity of the disks for metal- T_4 -APP chelates was 40 mg in 50 mL of solution. In this experiment, the disks had a capacity adequate to enrich the metal- T_4 -APP chelates.

Various organic solvents were studied to choose a proper eluent for the retained T_4 -APP and its metal chelates. It was found that THF, acetone, and chloroform could quantitatively elute the metal- T_4 -APP chelates from the disks. The effect of the various eluents for the retained metal- T_4 -APP chelates was in following sequence: THF > chloroform > acetone > ethanol > methanol. Thus, THF was selected. The metal- T_4 -APP chelate has good stability in a weak alkaline medium. Adding 0.05 mol/L pyrrolidine-acetic acid buffer salt (pH 10) to the THF could increase the stability of the metal- T_4 -APP chelate during elution. Thus, THF (containing 0.05 mol/L pyrrolidine-acetic acid buffer salt (pH 10)) was selected as the eluent. Our experiment showed that it was easier to elute the retained T_4 -APP and its metal chelate on the disks in the reverse direction than in the forward direction; it was therefore necessary to turn the disks up during elution. We found that 2.5 mL of THF (containing 0.05 mol/L pyrrolidine-acetic acid buffer salt (pH 10)) was sufficient to elute the metal- T_4 -APP chelate from the disks quantitatively at a flow rate of 5 mL/min. A 2.5 mL volume of eluent was selected.

Spectrophotometric properties. The absorption spectrum of the metal- T_4 -APP chelates was obtained from the three-dimensional chromatogram recorded by the photodiode array detector. The maximum absorption wavelengths of Cu- T_4 -APP, Ni- T_4 -APP, Sn- T_4 -APP, Hg- T_4 -APP, Pb- T_4 -APP, and Cd- T_4 -APP were 430, 431, 435, 452, 466, and 440 nm. To get maximum sensitivity, each metal- T_4 -APP chelate was monitored at its maximum absorption wavelength.

Chromatographic separation. The Cu- T_4 -APP, Ni- T_4 -APP, Ag- T_4 -APP, Hg- T_4 -APP, Pb- T_4 -APP, and Cd- T_4 -APP chelates were stable in a weak alkaline medium. A pH of 8.5–11.8 in the mobile phase can keep the chelates from decomposing and obtain a good peak shape. Thus, two weak alkaline solutions, mobile phase A (methanol containing 0.05 mol/L of pH 10 pyrrolidine-acetic acid buffer salt) and mobile phase B (acetone containing 0.05 mol/L of pH 10 pyrrolidine-acetic acid buffer salt) were used. Because of the routine silica bonds, the reversed-phase chromatographic column was unstable at pH 10, and a Waters XterraTM RP₁₈ chromatographic column (5 μ m, 3.9 \times 150 mm) was selected as the analytical column in this experiment. The XterraTM RP₁₈ column has good stability at pH 1–12. The relative proportions of mobile phases A and B were varied to effect the separation. Gradient elution can provide good results. The proper composition of the mobile phase during gradient elution was 100% A and 0% B at 0 min, and 90% A and 10% B at 10 min in the linear ramp.

Calibration graphs. Under optimum conditions, the regression equations of the metal- T_4 -APP chelates were established on the basis of the standard samples injected and their peak areas. The limits of detection were calculated by the ratio of signal to noise ($S/N = 3$). The results are shown in Table 1. The reproducibility of this method was also examined for 10 μ g/L of Ni(II), Cu(II), Sn(IV), Pb(II), Cd(II), and Hg(II). The relative standard deviations ($n = 10$) are also shown in Table 1.

Interference. Under the conditions of precolumn derivation, the foreign ions of Mg^{2+} , Pd^{2+} , Rh^{3+} , Fe^{3+} , Mn^{2+} , Zn^{2+} , Pt^{2+} , Ba^{2+} , Bi^{3+} , Ru^{3+} , and Ag^+ can react with T_4 -APP to form colored chelates. To examine the selectivity of this method, the interference of the foreign ions was investigated. When 5.0 mL of 1.5×10^{-4} mol/L T_4 -APP was used, with 10 μ g/L of Cu(II), Ni(II), Sn(II), Pb(II), Cd(II), and Hg(II), respectively, the tolerance was (with an error of ~5%) 2000 μ g/mL for Fe(III), Mg(II), and Mn(II), 1000 μ g/mL for Bi(III), Zn(II), Ba(II), and Ag^+ , and 800 μ g/mL for Pd(II), Pt(II), Ru(III), and Rh(III). The results show that most foreign ions do not interfere with determination.

Application. We accurately weighed 0.20 g of sample into a Teflon high-pressure microwave acid-digestion bomb (Fei Yue Analytical Instrument Factory, Shanghai, China). Next, 2.5 mL of concentrated nitric acid and 2.5 mL of 30% hydrogen peroxide were

Table 1. Regression equation, coefficient, and detection limit

Components	Regression equation	Linear range, $\mu\text{g/L}^*$	Coefficient	Detection limit, ng/L^{**}	RSD, % ($n = 10$)
Cd-T ₄ -APP	$A = 2.25 \times 10^5 C + 101$	0.5~520	$r = 0.9996$	4	1.8
Pb-T ₄ -APP	$A = 1.86 \times 10^6 C + 127$	0.6~620	$r = 0.9993$	5	2.1
Hg-T ₄ -APP	$A = 1.52 \times 10^6 C - 88.4$	0.4~980	$r = 0.9995$	2	2.2
Cu-T ₄ -APP	$A = 3.06 \times 10^6 C - 115$	1.2~1560	$r = 0.9994$	8	1.9
Sn-T ₄ -APP	$A = 1.72 \times 10^6 C + 108$	0.8~620	$r = 0.9996$	4	1.6
Ni-T ₄ -APP	$A = 1.89 \times 10^6 C - 121$	0.6~620	$r = 0.9995$	5	2.3

* In measured solution.

** In original digested sample.

Table 2. Results using this method for the determination of samples ($\mu\text{g/g}$)

Components	Samples, $\mu\text{g/g}$					RSD, % ($n = 5$)	Average recovery, % ($n = 5$)
	glycerol	tobacco leaf	tobacco paste	cigarette	tobacco essence		
Cu	2.01	11.5	3.08	4.13	5.16	2.5	97.1
Ni	1.26	1.72	2.08	2.23	2.46	2.1	97.1
Sn	1.31	1.46	2.24	1.22	1.32	2.8	104
Cd	0.138	0.205	0.322	0.176	0.273	2.2	98.2
Pb	0.425	0.586	0.853	0.636	0.235	1.8	97.3
Hg	0.052	0.125	0.113	0.181	0.076	2.2	102

Table 3. Results using the reference method for the determination of samples ($\mu\text{g/g}$)

Components	Samples, $\mu\text{g/g}$					RSD, % ($n = 5$)	Average recovery, % ($n = 5$)
	glycerol	tobacco leaf	tobacco paste	cigarette	tobacco essence		
Cu	2.12	10.5	3.26	4.28	5.25	2.6	96.8
Ni	1.43	1.85	2.12	2.34	2.53	2.2	96.8
Sn	1.23	1.42	2.36	1.28	1.47	3.4	105
Cd	0.145	0.212	0.315	0.186	0.278	2.8	94.1
Pb	0.421	0.568	0.842	0.621	0.224	2.6	95.6
Hg	0.049	0.112	0.126	0.192	0.082	2.5	103

added. The bombs were sealed tightly and then positioned in the carousel of a microwave oven (Model WL 5001, 1000 W, Fei Yue Analytical Instrument Factory, Shanghai, China). 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% nitric acid and transferred quantitatively to a 50 mL calibrated flask. It was then diluted to volume with water. The Cu, Ni, Sn, Hg, Pb, and Cd contents were analyzed using an appropriate volume of this solution and fol-

lowing the common procedure. The results (with the reagents blank deducted) are shown in Table 2, together with the results of a recovery test with 0.5 μg of Cu, Ni, Sn, Hg, Pb, and Cd added to the samples. Atomic absorption spectrometry was also used as a reference method. The results are shown in Table 3. For each sample, the peak purities of the Cu-T₄-APP, Ni-T₄-APP, Sn-T₄-APP, Hg-T₄-APP, Pb-T₄-APP, and Cd-T₄-APP chelates were identified using the photodiode

Table 4. Comparison of porphyrin reagents for the determination of metals by HPLC

Reagents	Metal ions	Column	Mobile phase	Detection limit	Ref.
TPP	Cd, Cu, Pb, Zn	Micro-pak MCH-5 (150 × 4)	Methanol–water–chloroform (94 : 1 : 5, v/v)	Cd (10 ng/mL), Cu (40 ng/mL), Pb (30 ng/mL)	9
Br-TPPS ₄	Mn, Fe, Zn, Pd, Cu	Lichrosorb ODS (5 μm, 200 × 4.6)	Acetonitrile–water (45 : 55), 0.01 mol/L TBABr	Mn (160 pg), Fe (79 pg), Zn (31 pg), Pd (70 pg), Cu (36 pg)	10
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)	11
T ₄ HPP	Cd, Pb, Cu, Zn	Prodigy–ODS	Methanol–pH 8.0 borate–sodium hydroxide buffer solution	Cd (8 ng), Pb (8 ng), Cu (8 ng), Zn (50 ng)	12
T ₄ MPPS ₄	Cu, Zn	Yilite–ODS (250 × 4)	Acetonitrile–water (38 : 62), 0.01 mol/L TEAI	Cu (30 ng/mL), Zn (50 ng/mL)	13
TPyP	Cu, Pd	ODS–80Ts (250 × 4.6)	Methanol	Cu (5 × 10 ⁻⁹ M), Pd (8 × 10 ⁻⁹ M)	14
T _m -APP	Cu, Co, Ag, Ni, Pb, Hg, Cd	Xterra TM RP ₁₈ (150 × 3.9)	Methanol–acetone (containing 0.05 mol/L pH 10 buffer salt)	Cu (8 pg/mL), Sn (4 pg/mL), Ni (5 pg/mL), Pb (5 pg/mL), Hg (2 pg/mL), Cd (4 pg/mL)	This work

Notes: 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; TPyP—5,10,15,20-tetrakis-(4N-pyridyl)-porphyrin

array detector to avoid the interference of foreign peaks.

CONCLUSIONS

A comparison of the proposed method with others that employ porphyrin reagents for the determination of heavy metals by HPLC is given in Table 4. The results show that the method is highly selective and sensitive. The metal chelate was preconcentrated by the C₁₈ disks, and an enrichment factor of 50 was achieved. The detection limits of this method reach the ng/L level. Most foreign ions do not interfere with the determination. This is one of the most sensitive and selective methods yet for the simultaneous determination of Cu, Ni, Sn, Pb, Cd, and Hg. With solid-phase extraction, the consumption of organic solvents in this method is much lower than that in the liquid–liquid extraction method and is environmentally friendly. Using microwave digestion, only a small amount of sample (0.20 g) is needed, and little time is consumed (the digestion of a sample set takes only 6.0 min). The Waters SPE device can prepare twenty samples simultaneously. The method allows the rapid simultaneous preparation of a large number of samples. With the photodiode array detector, each metal chelate can be monitored at its maximum absorption wavelength and its peak purity can be identified. The results are more reliable than those obtained using a single wavelength detector. To sum up, this method is highly sensitive, selective, and rapid for the simultaneous determination of Cu, Ni, Sn, Pb, Cd, and Hg in tobacco and tobacco additives.

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