Simultaneous Determination of Tin, Nickel, Lead, Cadmium and Mercury in Cigarette Material by Solid Phase Extraction and HPLC

Qun Hu,[†,‡,*] Guangyu Yang,[‡] Jing Ma,[‡] and Jikai Liu[†]

[†]*Institute of Kunming Botany, Chinese Academy of Science, Kunming 650223, P.R. China*
[‡]*Institute of Industry, Yunnan Academy of Tobacco Science, Kunming 650106, P.R. China*
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A new method for the simultaneous determination of heavy metal ions in cigarette material by microwave digestion and reversed-phase high-performance liquid chromatography (RP-HPLC) has been developed. The cigarette material was digested by microwave digestion. Lead, cadmium, mercury, nickel and tin ions in the digested samples were pre-column derivatized with tetra-(2-chlorophenyl)-porphyrin (T_2-CPP) to form color chelates, which were then enriched by solid phase extraction with a C_{18} cartridge. The chelates were separated on a Waters Xterra[TM] RP_{18} column by gradient elution with 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 mobile phase at a flow rate of 0.5 mL/min and analyzed with a photodiode array detector from 350-600 nm. The detection limits of lead, cadmium, mercury, nickel and tin were 4, 3, 3, 8, and 5 ng/L, respectively, in the original samples. This method was afforded good results.

Key Words : RP-HPLC, Tetra-(2-chlorophenyl)-porphyrin, Solid phase extraction, Heavy metal

Introduction

The RP-HPLC technique with pre-column derivatization has been proved to be a reliable technique for the separation and determination of trace amounts of metal ions.[1-6] Among the various kinds of reagents, porphyrin ligands are useful because of their high molar absorptivity and high stability. Porphyrin reagents have, therefore, been widely applied to the simultaneous determination of metal ions.[5-13] However, for the simultaneous determination of heavy metal ions, the traditional RP-HPLC techniques have some disadvantages. On the one hand, the routine reserved phase chromatographic column has a narrow pH range of 2-8. The column is not suitable for the separation of porphyrin chelates of heavy metal ions, which are only stable in a weak alkaline medium.[5-7] On the other hand, the metal-porphyrin chelates have a narrow absorption band. Using a single wavelength detector for the simultaneous monitoring of several metal chelates will not produce high sensitivity, least of all for lead ion.

Determination of trace lead, cadmium, mercury, nickel and tin in cigarette material is very important because of their biological significance.[14,15] The present paper describes the development of a simple, sensitive and selective method for the simultaneous determination of lead, cadmium, mercury, nickel and tin ions in cigarette material. The method is based on the solid phase extraction and the RP-HPLC separation of the metal-T_2-CPP chelates.

Experimental Section

**Apparatus.** The HPLC system consisted of a Waters 2690

Alliance separation model and a 996 photodiode array detector (Waters Corporation, USA). The pH values were determined with a Beckman Φ-200 pH meter. The absorbance was measured with a Shimidzu UV-2401 spectrophotometer. The atomic absorption spectrometry analysis was made with a Shimidzu AA-6701F atomic absorption spectrophotometer.

The separation column was a Waters Xterra[TM] RP_{18} column (3.9×150 mm, 5 μm) (Waters Corporation, USA). The cartridge was a Zorbax C_{18} solid phase extraction cartridge (1 cc/50 mg, 30 μm) (Agilent Corporation, USA). The solid phase extraction was conducted on a Waters Solid Phase Extraction (SPE) Device (Waters Corporation, USA)which can prepare twenty samples simultaneously.

Chemicals. All of the solutions were prepared with ultra-pure water obtained from a Milli-Q50 SP Reagent Water System (Millipore Corporation, USA). A 1.0 mg/mL Pb(II), Cd(II), Hg(II), Ni(II) and Sn(IV) standard solution was obtained from Chinese Standards Center, and a working solution of 0.2 μg/mL was prepared by diluting this standard solution. HPLC grade methanol and acetone were obtained from Fisher Corporation, USA. Pyrrolidine-acetic acid buffer solution was 0.5 mol/L and pH=10. TritonX-100 solution was 2.0% (v/v). T_2-CPP was synthesized by our laboratory as described in the literature[16] and 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)) and mobile phase B was acetone (containing 0.05 mol/L pH=10 pyrrolidine-acetic acid buffer salt). All other reagents were analytical reagent-grade. The glass and Teflon ware was soaked in 5% nitric acid overnight, and then thoroughly washed with pure water.

Standard procedure. An appropriate volume of 0.2 μg/ mL standard or the digested sample solution was transferred in a 50 mL volumetric flask. To this were added 5.0 mL of

*Corresponding Author. Fax: +86-871-8319128; E-mail: qun_hu@163.com
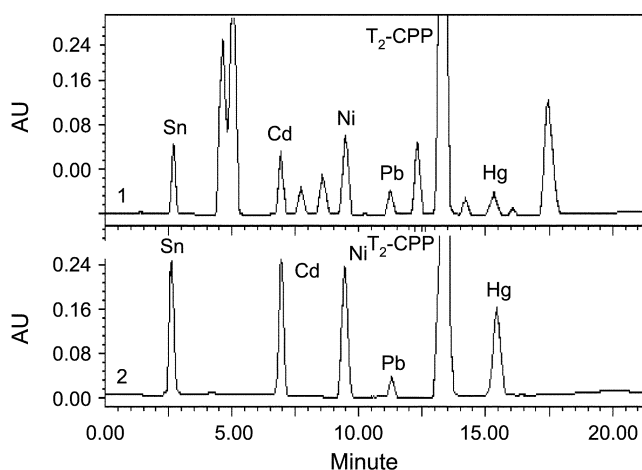
Figure 1. Chromatogram of standard sample and cigarettes material sample: 1) cigarettes material sample, 2 Standard sample. The Injection volume is 20 μL. Detection wavelength i 435 nm. Other conditions as in standard procedure.

T_2-CPP 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 resulting mixture was diluted to volume with water and stirred well. The mixture was then heated in a boiling water bath for 10 min. After cooling, the solution was passed through the C_{18} cartridge at a flow rate of 10 mL/min. After the enrichment, the retained chelates were eluted from the cartridge with 2.5 mL of THF at a flow rate of 5 mL/min in the opposite direction. The solution was filtered with 0.45 μm filters and volatilized to 0.6 mL, then diluted to 1.0 mL with methanol. A 20 μL of sample was injected for HPLC analysis. A tri-dimensional chromatogram was recorded from 350-600 nm with a photodiode array detector. The chromatogram of 435 nm is shown in Figure 1. In the course of separation, the composition of the mobile phase was: 0 min (A 100% and B 0%), 15 min (A 80% and B 20%) in linear ramp. To get maximum sensitivities, each metal-T_2-CPP chelate was detected at its maximum absorption wavelength.

Results and Discussion

Precolumn derivation. According to the literature,[16-18] the optimal pH is 8.2-11.8 for the reaction of Ni(II), Sn(IV), Hg(II), Pb(II) and Cd(II) with T_2-CPP. Therefore, a 0.5 mol/L of pH=10 pyrrolidine-acetic acid buffer solution was used to control pH. It was found that 1.0 mL of 1.5×10^{-4} mol/L T_2-CPP THF solution was sufficient to complex 3.0 μg of Ni(II), Sn(IV), Hg(II), Pb(II) and Cd(II). But in real samples, the foreign ions, such as Mg^{2+}, Pd^{2+}, Fe^{3+}, Mn^{2+}, Zn^{2+}, Bi^{3+}, Co^{2+}, Ba^{2+}, Cu^{2+}, Ag^+, can complex with T_2-CPP to consume reagents. Therefore, more T_2-CPP was needed. In this experiment, 5.0 mL of 1.5×10^{-4} mol/L T_2-CPP solution was recommended.

The reaction of Ni(II), Sn(IV), Hg(II), Pb(II) and Cd(II) with T_2-CPP was slow at room temperature. Heating accelerated the reaction. The reaction was completed after

heating in a boiling water bath for 10 min, and the chelate was stable for 5 h after cooling, so heating 10 min was selected.

Metal-T_2-CPP chelates have poor solubility in water. The addition of a suitable amount of surfactants is required to improve their solubility. Various surfactants enhanced the absorbance in the following order: Triton X-100 > Tween-80 > Tween-20 > CTMAB > CPB > SDS > SDBS. Hence, Triton X-100 was selected as co-solvent. Experiments show that an amount 2.0% of Triton X-100 solution used within 1-4 mL have a positive effect on solubility and did not affect the chromatographic peak. Thus, 2.0 mL of Triton X-100 solution was recommended.

Solid phase extraction. Both the enrichment and the elution were carried out on a Waters SPE device, which can prepare twenty samples simultaneously. The flow rate was set to 10 mL/min for enrichment and 5 mL/min for elution.

Some experiments were carried out in order to investigate the retention of metal-T_2-CPP chelates on the cartridge. It was found that the Sn-T_2-CPP, Ni-T_2-CPP, Hg-T_2-CPP, Pb-T_2-CPP and Cd-T_2-CPP chelates could be retained on the cartridge quantitatively when they passed through the cartridge as aqueous solution. The capacity of the cartridge for Sn-T_2-CPP, Ni-T_2-CPP, Hg-T_2-CPP, Pb-T_2-CPP and Cd-T_2-CPP chelates was 26, 28, 30, 30 and 36 mg, in a 50 mL of solution. In this experiment, the concentration of metal ions is on the μg level. Hence, the cartridge has adequate capacity to enrich the metal-T_2-CPP chelates.

To choose a proper eluent for the retained T_2-CPP and its metal-chelates, various organic solvents were evaluated. It was found that the THF, acetone and chloroform could elute the metal-T_2-CPP chelates from cartridge quantitatively. Quantitative elution of the metal-T_2-CPP chelates from the cartridge required the following volume of organic solvents: 2.5 mL for THF, 3.0 mL for chloroform and 5.0 mL for acetone. So THF was selected. The metal-T_2-CPP chelate has good stability in a weak alkaline medium. A 0.05 mol/L pyrrolidine-acetic acid buffer salt (pH=10) in THF increased the stability of the metal-T_2-CPP chelate during elution. It was selected as the eluent. Experiments show that it is easier to elute the retained T_2-CPP and its metal-chelate on cartridge in reverse direction than in forward direction. Therefore, it was necessary to upturned the cartridge, with 2.5 mL of THF (containing 0.05 mol/L pyrrolidine-acetic acid buffer salt (pH=10)) being sufficient to elute the metal-T_2-CPP chelate from cartridge quantitatively at a flow rate of 5 mL/min. An eluent volume of 2.5 mL was selected.

Spectrophotometric properties. The absorption spectrum of metal-T_2-CPP chelates was obtained from the tridimensional chromatogram recorded by a photodiode array detector. The maximum absorption wavelengths of Ni-T_2-CPP, Sn-T_2-CPP, Hg-T_2-CPP, Pb-T_2-*CPP* and Cd-T_2-CPP are 435, 432, 452, 465 and 440 nm. To get maximum sensitivity, each metal-T_2-CPP chelate was monitored at its maximum absorption wavelength.

Chromatographic separation. The Ni-T_2-CPP, Sn-T_2-CPP, Hg-T_2-CPP, Pb-T_2-CPP and Cd-T_2-CPP chelates were
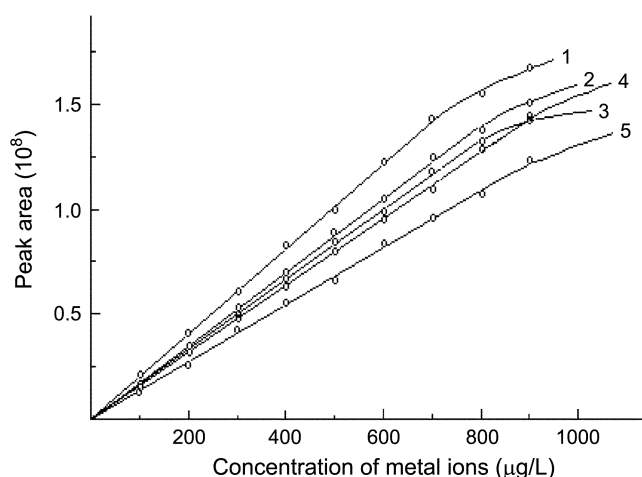
Figure 2. The calibration curves of metal ions 1) Cd; 2) Ni; 3) Pb; 4) Sn; 5) Hg.

stable in weak alkaline medium. The pH of the mobile phase within 8.5-11.8 prevents the chelates from decomposing and produces a good peak shape. Hence, 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) are recommended. Since common silica bonds reserved phase chromatographic columns are not stable in pH 10, a Waters Xterra^{TM} RP$_{18}$ chromatographic column (5 μ, 3.9 × 150 mm) was selected as the analytical column in this experiment. It has good stability in pH 1-12. The relative proportion of mobile phase A and B varies in effecting the separation. Gradient elution can get a good result. The proper composition of mobile phase during gradient elution was selected as follows: 0 min (100% A and 0 B), 15 min (80% A and 20 B) in linear ramp.

Calibration graphs. The calibration curves are shown in

Figure 2. Under optimum conditions, the regression equations of metal-T$_2$-CPP chelates were established based on the standard samples injected and their peak areas, and the limits of detection were calculated by the ratio of signal to noise (S/N=3). The results are given in Table 1. The reproducibility of this method was also examined by determination 10 μg/L of Ni(II), Sn(IV), Pb(II), Cd(II) and Hg(II) for 10 trials. The relative standard deviations (n=10) are also shown in Table 1.

Interference. Under the pre-column derivatization condition, foreign ions of Mg^{2+}, Pd^{2+}, Rh^{3+}, Fe^{3+}, Mn^{2+}, Zn^{2+}, Pt^{2+}, Ba^{2+}, Bi^{3+}, Cu^{2+}, Ru^{3+} and Ag^+ can react with T$_2$-CPP to form color chelates. To examine the selectivity of this method, the interference of foreign ions was investigated. When 5.0 mL of 1.5×10^{-4} mol/L T$_2$-CPP was used with 10 μg/L of Ni(II), Sn(IV), Pb(II), Cd(II) and Hg(II), respectively, the tolerance amount with an error of ± 5% was 2000 μg/mL for Fe(III), Mg(II), Cu(II), 1000 μg/mL for Co(II), Bi(III), Zn(II), Ba(II), Ag$^+$ and 800 μg/mL for Pd(II), Pt(II), Ru(III), Rh(III). The results show that most foreign ions do not interfere with the determination.

Application to the samples. The cigarette material sample (0.20 g) was weighed accurately into the Teflon high-pressure microwave acid-digestion bomb (Fei Yue Analytical Instrument Factory, Shanghai, China). Concentrated nitric acid (2.5 mL) 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, 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% of nitric acid and quantitatively transferred into a 50 mL of calibrated flask. The solution was diluted to volume with water. The Ni, Sn, Hg, Pb and Cd contents were analyzed by using a proper volume of this

Table 1. Regression Equation, Coefficient and Detect limit

Components	Regression Equation	Linear Range (μg/L)^a	Coefficient	Detect limit (ng/L)^b	RSD % ($n = 10$)^c
Cd-T$_2$-CPP	$A = 2.02 \times 10^5$ C+121	0.5-720	r = 0.9992	3	2.1
Pb-T$_2$-CPP	$A = 1.65 \times 10^6$ C−113	0.6-820	r = 0.9995	4	1.8
Hg-T$_2$-CPP	$A = 1.41 \times 10^6$ C+76.2	0.4-880	r = 0.9993	3	2.2
Sn-T$_2$-CPP	$A = 1.53 \times 10^6$ C+83.6	0.8-920	r = 0.9996	8	2.3
Ni-T$_2$-CPP	$A = 1.76 \times 10^6$ C−115	0.6-820	r = 0.9991	5	1.6

^{a}In the measured solution. ^{b}In the original digested sample. ^{c}RSD was obtained from the determination of 10 μg/L of Ni(II), Sn(IV), Pb(II), Cd(II) and Hg(II) solution for 10 times.

Table 2. Determination results (μg/g) of the sample with this method

Components	Samples (μg/g)					RSD % ($n = 5$)^a	Average recovery % ($n = 5$)
	Glycerol	Cigarette Paper	Tobacco sause	Fiber tow	Tobacco essence		
Ni	1.52	1.23	1.26	2.68	1.42	2.1	97
Sn	1.11	1.26	1.28	1.12	1.25	2.6	104
Cd	0.138	0.205	0.312	0.125	0.183	2.2	98
Pb	0.421	0.572	0.432	0.536	0.232	1.8	97
Hg	0.052	0.048	0.036	0.024	0.066	2.2	102

^{a}RSD was obtained from the determination of the same samples for 5 times.

Table 3. Determination results (μg/g) of the sample with reference method

Components	Samples (μg/g)					RSD % $(n = 5)^a$	Average recovery % $(n = 5)$
	Glycerol	Cigarette Paper	Tobacco sause	Fiber tow	Tobacco essence		
Ni	1.47	1.36	1.31	2.54	1.51	2.2	96
Sn	1.22	1.32	1.33	1.25	1.36	3.3	105
Cd	0.142	0.213	0.324	0.136	0.178	2.9	94
Pb	0.426	0.564	0.442	0.521	0.227	2.6	95
Hg	0.049	0.051	0.038	0.029	0.067	2.7	103

^{a}RSD was obtained from the determination of the same samples for 5 times.

solution, according to general procedure. The results (deducted the reagents blank) are given in Table 2, together with the results of a recovery test by adding 0.2 *μ*g of Ni, Sn, Hg, Pb and Cd into sample. Atomic absorption spectrometry was used as a reference method. The results are shown in Table 3.

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