

Determination of Heavy Metal Ions in Tobacco and Tobacco Additives

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ABSTRACT

This paper describes a new method for the simultaneous determination of heavy metal ions in tobacco and tobacco additives by microcolumn high-performance liquid chromatography. The samples were digested by microwave digestion. The lead, cadmium, mercury, nickel, cobalt and tin ions in the digested samples were pre-column derivatized with tetra-(*o*-aminophenyl)-porphyrin (To-APP) to form coloured chelates. The Hg-To-APP, Cd-To-APP, Pb-To-APP, Ni-To-APP, Co-To-APP and Sn-To-APP chelates were enriched by solid phase extraction with a C₁₈ cartridge resulting in an enrichment factor of 50. The chelates were separated on a Waters Xterra™ RP₁₈ microcolumn (50 mm × 1.0 mm, 2.5 μm) with a mixture of methanol-tetrahydrofuran (95:5, v/v, containing 0.05 mol L⁻¹ pyrrolidine-acetic acid buffer salt, pH = 10.0) as mobile phase at a flow rate of 0.2 mL min⁻¹ and identified with a photodiode array detector at 350–600 nm. The metal chelates were fully separated in 2.0 min. The detection limits for lead, cadmium, mercury, nickel, cobalt and tin in the original digested samples were 4 ng L⁻¹, 3 ng L⁻¹, 6 ng L⁻¹, 5 ng L⁻¹, 5 ng L⁻¹ and 4 ng L⁻¹, respectively. The relative standard deviation for five replicate samples was 2.65–3.24%. The standard recoveries were 95.6–108%. The method was applied with good results to the determination of lead, cadmium, mercury, nickel, cobalt and tin in tobacco and tobacco additives.

KEYWORDS

Microcolumn high-performance liquid chromatography, tetra-(*o*-aminophenyl)-porphyrin, heavy metal ions.

1. Introduction

Reversed-phase high performance liquid chromatography with pre-column derivatization has been proven to be a convenient and reliable technique for the separation and determination of trace amounts of metal ions. Numerous reagents have been examined as pre-column derivatization reagents and several review articles have appeared on this approach.^{1–8} Among the various kinds of reagents, porphyrin ligands were found to be useful because of their high molar absorptivity and high stability.^{1,3,5} Porphyrin reagents have therefore received increasing attention and are widely applied for the simultaneous determination of metal ions.^{9–19}

The determination of trace amounts of lead, cadmium, mercury, nickel, cobalt and tin in tobacco and tobacco additives is important because of the biological significance of these metals.^{20,21} In previous investigations high performance liquid chromatography has been described as a method for the determination of metal ions using derivatization and proven to be a convenient and reliable technique.^{7,11,22–25} Current routine chromatographic methods have, however, the disadvantage of being time-consuming with separation times of more than 10 min.

This paper describes the use of tetra-(*o*-aminophenyl)-porphyrins (To-APP) as new derivatization agents that show improved sensitivity, selectivity and convenience for the determination of heavy metal ions (lead, cadmium, mercury, nickel, cobalt, tin) in tobacco and tobacco additives. To shorten separation time and reduce mobile phase consumption, a Waters Xterra™ RP₁₈ microcolumn (50 mm × 1.0 mm, 2.5 μm, pH range 1–12) was

used for the separation of the metal chelates Hg-To-APP, Cd-To-APP, Pb-To-APP, Ni-To-APP, Co-To-APP and Sn-To-APP at a mobile phase pH of 10. The six chelates were completely separated in 2 min using a flow rate of only 0.2 mL min⁻¹. This represents a significant improvement in terms of separation time and solvent consumption compared to routine chromatographic methods.

2. Experimental

2.1. 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 measurements were measured with a Shimadzu UV-2401 spectrophotometer. The atomic absorption spectrometry analysis was carried out on a Shimadzu AA-6701F atomic absorption spectrophotometer.

A Waters Xterra™ RP₁₈ microcolumn (50 mm × 1.0 mm, 2.5 μm) (Waters Corporation, USA) with a Zorbax C₁₈ solid phase extraction cartridge (1 cm³/50 mg, 30 μm) (Agilent Corporation, USA) was used as separation column. Extractions were carried out on a Waters Solid Phase Extraction (SPE) Device (the device can extract twenty samples simultaneously).

2.2. Chemicals

All solutions were prepared using ultra-pure water, which was obtained from a Milli-Q50 SP Reagent Water System (Millipore Corporation, USA). Pb(II), Cd(II), Hg(II), Ni(II), Co(II) and Sn(IV) standard solutions (1.0 mg mL⁻¹) were obtained from the

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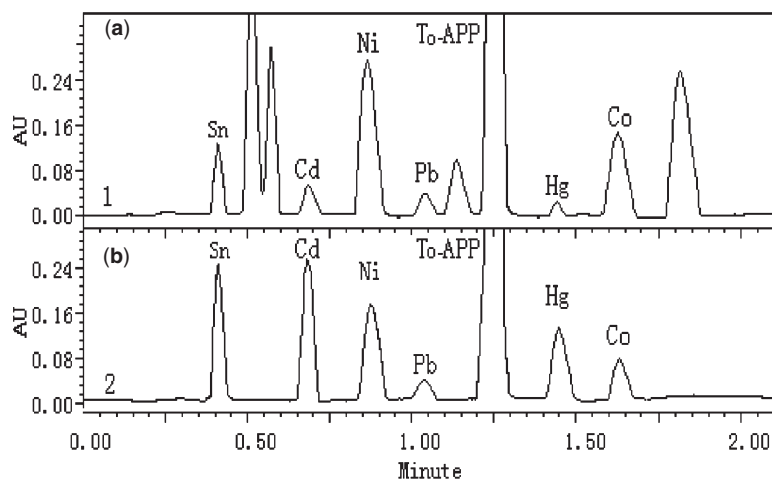


Figure 1 Chromatogram of standard sample and tobacco sample: (a) tobacco sample, (b) standard sample. The injection volume is 2.0 μL . Detection wavelength is 435 nm. Other conditions as in standard procedure.

Chinese Standards Center, and a working solution of 0.2 $\mu\text{g mL}^{-1}$ was prepared by diluting this standard solution. HPLC grade methanol and THF were obtained from Fisher Corporation, USA. A solution of 0.5 mol L^{-1} pyrrolidine-acetic acid buffer solution pH = 10 and 2% (v/v) Triton X-100 was used. To-APP was synthesized according to the literature²⁶ and used to prepare a 1.5×10^{-4} mol L^{-1} stock solution in THF. Glass- and Teflonware was soaked for 24 h in 5% nitric acid and washed with pure water (obtained from a Milli-Q50 SP reagent water system) before use.

2.3. Standard Procedure

An appropriate volume (0–38 mL) of a standard or a digested sample solution was transferred into a 50 mL volumetric flask and 5.0 mL To-APP THF solution, 2 mL Triton X-100 solution and 5 mL pyrrolidine-acetic acid buffer solution were added. The solution was diluted with water to a volume of 50 mL and mixed well. The mixture was heated in a boiling water bath for 15 min, cooled to room temperature and passed through a C_{18} cartridge at a flow rate of 10 mL min^{-1} . After the enrichment was completed, the retained chelates were eluted from the cartridge with 1.0 mL of THF at a flow rate (reverse direction) of 5 mL min^{-1} . The solution was filtered with 0.45 μm filters and adjusted to a volume of 1.0 mL. 2.0 μL of that solution were then injected for HPLC analysis. A three-dimensional chromatogram was recorded from 350–600 nm with a photodiode array detector. A typical chromatogram at 430 nm is shown in Fig. 1.

2.4. Sample Preparation for Tobacco and Tobacco Additives

0.20 g sample were weighed accurately into a Teflon high-pressure microwave acid-digestion bomb (Fei Yue Analytical Instrument Factory, Shanghai, China) and 2.5 mL of concentrated nitric acid and 2.5 mL of 30% hydrogen peroxide were added. The bombs were tightly sealed 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 obtained solution was evaporated to near dryness. The residue was dissolved with 5 mL of 5% nitric acid, transferred into a 50 mL calibrated flask and diluted with water.

3. Results and Discussion

3.1. Selection of Porphyrin Reagent

The following porphyrin reagents were studied as pre-column derivatization reagents for Co(II), Ni(II), Sn(IV), Hg(II), Pb(II)

and Cd(II): tetra-(*p*-bromophenyl)-porphyrin (To-BPP), tetra-(*p*-chlorophenyl)-porphyrin (Tp-CPP), tetra-(*p*-methoxyphenyl)-porphyrin (Tp-MOPP), tetra-(*p*-methylphenyl)-porphyrin (Tp-MPP), tetra-(*p*-sulphophenyl)-porphyrin (Tp-SPP), tetra-(*p*-hydroxyphenyl)-porphyrin (Tp-HPP) and tetra-(*o*-aminophenyl)-porphyrin (To-APP). All reagents formed coloured metal complexes with all the metals under investigation, but Tp-MPP, Tp-CPP, Tp-MOPP, Tp-BPP and their corresponding metal-chelates were only sparingly soluble in water and could only be used if large amounts of organic solvents were added to improve solubility. Tp-SPP and Tp-HPP in contrast formed soluble chelates with all metal ions, but the complexes showed poor retention on the reversed-phase column in alkaline medium. This may be a result of the presence of the sulphonate or hydroxy group on the porphyrin reagent both of which are able to ionize under basic conditions. The addition of an ion-pair reagent (quaternary ammonium salt) greatly increased the retention of the metal chelates, but resulted in peak tailing and incomplete separation in the pH range tolerable for the ion pair reagent and further experiments using Tp-SPP and Tp-HPP were therefore abandoned. To-APP in contrast formed water soluble chelates with metal ions in the pH region where Triton X-100 is stable and the chelates showed good retention and peak shape on the reverse-phase column. To-APP was therefore selected as the pre-column derivatization reagent of choice in all further experiments.

3.2. Precolumn Derivatization

According to the literature^{26–28} the optimal pH for the reaction of Co(II), Ni(II), Sn(IV), Hg(II), Pb(II) and Cd(II) with To-APP is pH 8.2–11.8 and a 0.5 mol L^{-1} pyrrolidine-acetic acid buffer solution of pH = 10 was recommended to control the pH. It was found that a 1.0 mL 1.5×10^{-4} mol L^{-1} To-APP THF solution was sufficient to complex pure solutions of 3.0 μg of Ni(II), Sn(IV), Co(II), Hg(II), Pb(II) and Cd(II). In real samples foreign ions such as Mg^{2+} , Pd^{2+} , Fe^{3+} , Mn^{2+} , Zn^{2+} , Bi^{3+} , Ba^{2+} , Cu^{2+} , Ag^{+} and others can form complexes with To-APP and consume reagents and an excess of To-APP is therefore needed. 5.0 mL of a 1.5×10^{-4} mol L^{-1} To-APP solution was found to give good results.

The reaction of Co(II), Ni(II), Sn(IV), Hg(II), Pb(II) and Cd(II) with To-APP was very slow at room temperature (more than 2 h were needed to complete the reaction) but could be accelerated by heating. It was shown that the complexes were formed after heating the reagents in a boiling water bath for 10 min and that they remained stable for 5 h after cooling to room temperature.

Table 1 Regression equation and detection limits for metal ions.

Components	Regression equation	Linear range ($\mu\text{g L}^{-1}$) ^a	Coefficient	Detection limit (ng L^{-1}) ^b	RSD% ($n = 10$)
Cd-To-APP	$A = 2.38 \times 10^5 C + 125$	0.5–520	$r = 0.9994$	3	1.8
Pb-To-APP	$A = 1.98 \times 10^6 C + 107$	0.6–620	$r = 0.9992$	4	2.1
Hg-To-APP	$A = 1.45 \times 10^6 C - 92.7$	0.9–980	$r = 0.9995$	6	2.2
Co-To-APP	$A = 1.92 \times 10^6 C - 116$	0.6–830	$r = 0.9993$	5	2.1
Sn-To-APP	$A = 1.72 \times 10^6 C + 89.2$	0.5–620	$r = 0.9996$	4	1.6
Ni-To-APP	$A = 1.89 \times 10^6 C - 103$	0.6–620	$r = 0.9995$	5	2.3

^a In the measured solution; ^b in the original digested sample.

3.3. Solid Phase Extraction

Enrichment and elution were both carried out with a Waters SPE device (the device can extract twenty samples simultaneously). The flow rate was set to 10 mL min^{-1} for enrichment and 5 mL min^{-1} for elution.

In order to choose the best eluant for the retained To-APP and its metal-chelates various organic solvents were studied. It was found that THF, isopentyl alcohol, acetonitrile, acetone, ethanol and methanol could quantitatively elute the metal-To-APP chelates from the cartridge. The required volumes to elute the metal-To-APP chelates from the cartridge were 0.8 mL THF, 1.3 mL isopentyl alcohol, 1.6 mL acetonitrile, 1.6 mL acetone, 2.2 mL ethanol and 2.4 mL methanol. Maximal enrichment was achieved when THF was selected as eluant in weakly alkaline medium. Addition of a 0.05 mol L^{-1} pyrrolidine-acetic acid buffer ($\text{pH} = 10$) in THF increased the stability of the metal-To-APP chelates during the elution and THF (containing 0.05 mol L^{-1} pyrrolidine-acetic acid buffer, $\text{pH} = 10$) was therefore selected as eluant. Further experiments showed that it was preferable to elute the retained To-APP and its metal-chelate from the cartridge in reverse direction rather than in forward direction (only 0.8 mL of eluant was needed for elution in reverse direction, but 2.2 mL for elution in forward direction). 1.0 mL of THF containing buffer was sufficient for eluting the metal-To-APP chelates quantitatively from the cartridge at a flow rate of 5 mL min^{-1} .

3.4. Spectrophotometric Properties

The absorption spectrum of the metal To-APP chelates was obtained from a three-dimensional chromatogram recorded by photodiode array detector. The wavelengths of maximum absorption for Ni-To-APP, Sn-To-APP, Co-To-APP, Hg-To-APP, Pb-To-APP and Cd-To-APP were 428 nm, 432 nm, 434 nm, 451 nm, 466 nm and 438 nm, respectively. For maximum sensitivity the following detection wavelength were selected: 428 nm for Ni-To-APP, 432 nm for Sn-To-APP, 434 nm for Co-To-APP, 451 nm for Hg-To-APP, 466 nm for Pb-To-APP and 438 nm for Cd-To-APP.

3.5. Chromatographic Separation

The Co-To-APP, Sn-To-APP, Ni-To-APP, Hg-To-APP, Pb-To-APP

and Cd-To-APP chelates were stable in weakly alkaline medium and a pH of 8.5–11.8 in the mobile phase avoided the decomposition of the chelates while retaining a good peak shape. A methanol-tetrahydrofuran mixture (95:5, v/v, containing 0.05 mol L^{-1} pyrrolidine-acetic acid buffer salt, $\text{pH} = 10.0$) was therefore selected as mobile phase. As standard Silica bonded reversed phase chromatographic columns were not stable at $\text{pH} 10$, a Waters Xterra™ RP₁₈ microcolumn (50 mm \times 1.0 mm, 2.5 μm) which is stable from $\text{pH} 1\sim 12$ was selected as analytical column in this experiment.

3.6. Calibration Graphs

Regression equations for the metal-To-APP chelates were obtained from the peak areas of standard samples. Limits of detection were calculated as the ratio of signal to noise ($S/N = 3$). The results are shown in Table 1. The reproducibility of this method was examined for $10 \mu\text{g L}^{-1}$ samples of Ni(II), Sn(IV), Co(II), Pb(II), Cd(II) and Hg(II) and relative standard deviations ($n = 10$) are shown in Table 1.

3.7. Interference

Under pre-column derivatization conditions foreign ions such as Mg^{2+} , Cu^{2+} , Pd^{2+} , Rh^{3+} , Fe^{3+} , Mn^{2+} , Zn^{2+} , Pt^{2+} , Ba^{2+} , Bi^{3+} , Ru^{3+} and others react with To-APP to form coloured chelates and are likely to interfere with the determination. To examine the selectivity of the method, the interference of common foreign ions was investigated. When 5.0 mL of $1.5 \times 10^{-4} \text{ mol L}^{-1}$ To-APP were used for $10 \mu\text{g L}^{-1}$ of Ni(II), Sn(IV), Pb(II), Co(II), Cd(II) and Hg(II), respectively, the tolerance (error of $\pm 5\%$) was $6000 \mu\text{g mL}^{-1}$ for Fe(III), $1000 \mu\text{g mL}^{-1}$ for Cu(II), Bi(III), Zn(II), Ba(II) and $500 \mu\text{g mL}^{-1}$ for Ag(I), Pd(II), Pt(II), Ru(III), Rh(III) indicating the high selectivity of the method.

3.8. Application

This method was applied to the determination of Co, Ni, Sn, Hg, Pb and Cd in the glycerol, tobacco leaf, tobacco sauce and cigarette samples. The samples were prepared according to the sample preparation section. The concentration of Co, Ni, Sn, Hg, Pb and Cd was analysed using an appropriate volume of the sample solution according to the general procedure. A blank test (all reagents were added according to the general procedure in

Table 2 Results ($\mu\text{g g}^{-1}$) for tobacco samples.

Components	Samples ($\mu\text{g g}^{-1}$)				RSD% ($n = 5$)	Average recovery% ($n = 5$)
	Glycerol	Tobacco leaf	Tobacco sauce	Cigarette		
Co	4.25	3.85	5.26	3.28	1.85	99.2
Ni	6.14	4.84	6.25	4.18	2.34	103
Sn	1.46	1.23	1.58	1.06	3.16	104
Cd	0.126	0.258	0.335	0.122	2.25	97.4
Pb	0.812	0.763	1.462	0.946	2.12	102
Hg	0.124	0.135	0.213	0.153	2.25	98.4

Table 3 Results ($\mu\text{g g}^{-1}$) for tobacco samples using reference method (AAS method)

Components	Samples ($\mu\text{g g}^{-1}$)				RSD% ($n = 5$)	Average recovery% ($n = 5$)
	Glycerol	Tobacco leaf	Tobacco sauce	Cigarette		
Co	4.13	3.61	5.12	3.12	3.12	106
Ni	6.43	4.65	6.13	4.32	2.98	96.8
Sn	1.58	1.42	1.36	1.23	3.24	108
Cd	0.115	0.248	0.352	0.143	2.87	94.1
Pb	0.825	0.778	1.453	0.936	2.65	95.6
Hg	0.136	0.146	0.238	0.167	2.87	105

addition to the sample or standard) was out carried out. The results (reagent blank is deducted) are shown in Table 2 together with the results of a recovery test when $0.5 \mu\text{g}$ of Co, Ni, Sn, Hg, Pb and Cd were added to the sample. Atomic absorption spectrometry was used as a reference method and the results are shown in Table 3. The relative standard deviations for five replicate samples are 2.65–3.24%. The standard recoveries are 94.1–108%. The results agree well with the reference method (AAS method) and show that the new method is a satisfactory alternative.

4. Conclusion

The proposed method has the following characteristics: (1) To-APP was selected as pre-column derivatization reagent as it rapidly forms stable coloured chelates with lead, cadmium, mercury, nickel, cobalt and tin and is a sensitive and convenient pre-column derivatization reagent for the above metal ions. (2) A Waters Xterra™ RP₁₈ microcolumn (50 mm × 1.0 mm, 2.5 μm) with a pH range of 1–12 was shown to completely separate the metal complexes Hg-To-APP, Cd-To-APP, Pb-To-APP, Ni-To-APP, Co-To-APP and Sn-To-APP within 2.0 min at a flow rate of the mobile phase of only 0.2 mL min⁻¹. This represents a significant shortening of separation time and a reduction in mobile phase consumption as compared to routine chromatographic methods. (3) An enrichment factor of 50 was achieved with detection limits on the ng L⁻¹ level.

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