

Investigation of Distribution for Trace Lead and Cadmium in Chinese Herbal Medicines and Their Decoctions by Graphite Furnace Atomic Absorption Spectrometry

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Abstract

Lead and cadmium in herbal medicines are highly toxic to living organisms even in low concentrations. An effective method is developed for analysis of trace lead and cadmium in Chinese herbal medicines and their decoctions by graphite furnace atomic absorption spectrometry (GFAAS). The effects of analytical conditions on absorbance were investigated and optimized. A water-dissolving capability for Pb and Cd was investigated, and the contents of different species in five Chinese herbal medicines and their decoctions were analyzed. The content ratios (k_{ow}) of n-octanol-soluble Pb or Cd to water-soluble Pb or Cd were evaluated, and the distribution of Pb and Cd in water decoction at stomach and intestine acidities was developed, in the first time. The contents of water-soluble Pb and Cd, n-octanol-soluble Pb and Cd, and their content ratios were related with the kind of medicine and the acidity of the decoction. The proposed method has the advantages of simple operation, high sensitivity and high speed, with 3 σ detection limits of 4.2 μg for Pb and 0.1 μg for Cd.

Keywords: Distribution, Lead, Cadmium, Herbal Medicines, Decoctions, Graphite Furnace Atomic Absorption Spectrometry

1. Introduction

Chinese herbal medicine has been used as a form of treatment for a multitude of ailments ranging from acute infections to chronic fatigue. Study on the trace elements in Chinese herbal medicine can provide science providence for pharmacology and toxicology. Lead has well-documented toxic effects on plants, animals, and micro-organisms. Cadmium exposure can produce a wide variety of acute and chronic effects in humans, leading to a build-up of cadmium in the kidneys that can cause kidney disease. Some cases of intoxication with Pb and Cd resulting from the ingestion of Chinese herbal medicines have been reported [1,2]. Therefore, the analysis of trace Pb and Cd in herbal medicines and their decoctions is very important for pharmacological and toxicological evaluations.

A series of atomic spectrometric methods were reported for the determination of Pb and/or Cd in herbal medicines. Atomic absorption spectrometry is the ana-

lytical technique, most employed for metal analysis due to its low interference level and reasonable sensitivity. A pre-concentration flow injection analysis-flame atomic absorption spectrometry (FAAS) method was reported for determination of Pb content in medicinal plants with the limit of detection (LOD) of 19 $\mu\text{g}\cdot\text{L}^{-1}$ [3]. A solid sampling-FAAS method was used for determination of Cd and Pb in medicinal plants, relative standard deviations (RSDs) were below 10% ($n = 5$) and LODs were 0.1 $\mu\text{g}\cdot\text{kg}^{-1}$ and 12 $\mu\text{g}\cdot\text{kg}^{-1}$ for Pb and Cd, respectively [4]. To increase method sensitivity graphite furnace atomic absorption spectrometry (GFAAS) was frequently used to determine heavy metals for the quality control in herbal medicines. Several GFAAS methods had been reported with higher sensitivity, such as for the determination of Pb in Chinese herbal medicine with the LOD of 0.1 $\mu\text{g}\cdot\text{kg}^{-1}$ [5], and for the determination of Pb and Cd in Chinese crude drugs with the LOD of 11.6 μg and 2 μg [6], and 6 $\mu\text{g}\cdot\text{kg}^{-1}$ and 0.208 $\mu\text{g}\cdot\text{kg}^{-1}$ [7] for Pb and Cd, respectively. GFAAS was utilized to the determination

of 4 metal element in 13 herbs of tocolysis formulation with the LOD of 0.45 ppb ($\mu\text{g}\cdot\text{L}^{-1}$) for Pb and 0.03 ppb ($\mu\text{g}\cdot\text{L}^{-1}$) for Cd [8], and to the quality control in Argentinian herbal medicines with the LOD of $0.06\mu\text{g}\cdot\text{L}^{-1}$ for Pb and $0.008\mu\text{g}\cdot\text{L}^{-1}$ for Cd [9]. GFAAS and inductively coupled plasma mass spectrometry (ICP-MS) had been used for the determination of arsenic, cadmium and lead for quality control monitoring purposes of Bulgarian herbs and their infusions, and the LODs using GFAAS were $0.5\mu\text{g}\cdot\text{L}^{-1}$ for Pb and $0.01\mu\text{g}\cdot\text{L}^{-1}$ for Cd [10]. Hydride generation-atomic fluorescence spectrometry (HGAFS) method was described for simultaneous determination of trace Cd and arsenic in biological samples with the LOD of $0.01\mu\text{g}\cdot\text{L}^{-1}$ for Cd [11]. Otherwise, a-reversed-phase high-performance liquid chromatography (RP-HPLC) method, was developed for the simultaneous determination of heavy metal ions in Chinese herbal medicine. The method had low LOD value of $0.004\mu\text{g}\cdot\text{L}^{-1}$ for Pb and $0.003\mu\text{g}\cdot\text{L}^{-1}$ for Cd, but analytical procedure was complicated, and required pre-column derivatized with tetra-(4-chlorophenyl)-porphyrin ($\text{T}_4\text{-CPP}$) to form the colored chelates [12]. All the methods reported above were used for effective determination of total content of analyte. However, because the action of trace elements in Chinese herbal medicine on human body is mostly depended on the combination forms of elements, so the distribution analysis of trace elements is more important than total content analysis.

The n-octanol system was used to study the distribution of major-element or micro-element in water decoction in our stomach and intestine, and to appraise the medicine affection in pharmacology [13]. The combination forms of Zn and Mn in some Chinese herbal medicines was investigated by FAAS using n-octanol system, but since FAAS normally allows the quantification of elements only at $\mu\text{g}\cdot\text{g}^{-1}$ levels [14,15]. A new method was developed for simultaneous determination of trace arsenic and antimony in Chinese herbal medicines by HGAFS with a Soxhlet extraction system and n-octanol-water extraction system [16]. Lead and cadmium in herbal medicines are highly toxic to living organisms, however there was no report for analysis of trace Pb and Cd in Chinese herbal medicines and their decoctions up to now.

The main purpose of our work is to study the distribution of Pb and Cd at stomach and intestine acidities. Chemical modification technique was used for GFAAS to increase the analytical sensitivity and to overcome matrix interference. The n-octanol-water extraction system was used for the analysis of trace Pb and Cd in the decoctions of Chinese herbal medicines. The content of water-soluble and n-octanol-soluble analyte and its ratio were investigated. The proposed method was used to ex-

amine the distribution of Pb and Cd at stomach and intestine acidities. The method was used for the determination of total Pb/Cd, water-soluble and n-octanol-soluble Pb/Cd in Chinese herbal medicines and their decoctions with satisfactory results.

2. Materials and Methods

2.1. Instrumentation

A PE-1100B graphite furnace atomic absorption spectrometer (Perkin Elmer Co.) equipped with Pb and Cd hollow cathode lamps (Beijing Haiguang Instrument Co., China) was used for all the measurements. Pyrocoated graphite tubes were used. The instrument operating parameters are given in **Table 1**.

2.2. Reagents and Samples

All reagents were of analytical-reagent grade except when indicated otherwise. De-ionized water was used throughout this work. The working solutions of Pb and

Table 1. The operating parameters of GFAAS.

Parameters	Pb	Cd
Measurement wavelength/nm	283.3	228.8
Lamp current/mA	6	6
Slit/nm	0.7	0.7
Charring temperature/ $^{\circ}\text{C}$	60	120
Ramp time/s	5	10
Hold time/s	10	10
Pyrolysis temperature/ $^{\circ}\text{C}$	800	800
Ramp time/s	10	15
Hold time/s	10	15
Atomization temperature/ $^{\circ}\text{C}$	1700	1800
Ramp time/s	0	0
Hold time/s	5	4
Cleaning temperature/ $^{\circ}\text{C}$	2500	2500
Ramp time/s	1	1
Hold time/s	3	3
Background correction	D-lamp	D-lamp
Measurement model	Peak area	Peak area
Integral time/s	3	3
Carries gas flow/ $\text{mL}\cdot\text{min}^{-1}$	300	300

Cd were prepared by diluting appropriate aliquots from the stock solution ($1 \text{ mg}\cdot\text{mL}^{-1}$, provided by the National Steel Material Testing Center, China). $\text{Mg}(\text{NO}_3)_2$, NH_4NO_3 , $\text{NH}_4\text{H}_2\text{PO}_4$ and PdCl_2 were used to study modification effects. A n-octanol solution was used for analysis.

Samples of Chinese herbal medicines, *Common anemarrhena rhizome*, *Liquorice*, *Dioscorea nipponica makino*, *Radix salviae miltiorrhizae* and *Fructus ligustri lucidi*, were bought from a market.

2.3. Procedure

For the analysis of total Pb/Cd in herbal medicines, complete digestion of a herbal sample was performed by wet digestion with a mixture of nitric acid and hydrochloric acid. A 1.000 g sample of Chinese herbal medicine was digested with 8 mL of nitric acid and 2 mL of perchloric acid in a beaker covered with a watch glass on an electric hot-plate for about one hour, then each digest solution was gently heated, and any excess acid was removed. After cooling to room temperature (25°C), the residues were dissolved with 1% nitric acid. The content was transferred to a volumetric flask and diluted to 50 mL with 1% nitric acid for the determination of total Pb/Cd. The sample blank preparation and recovery test were carried out in the same way.

For the analysis of total water-soluble Pb/Cd in water decoctions, a 20.00 g sample of Chinese herbal medicines in a 400 mL beaker was extracted with triplet deionized water (200 mL, each) and heated on an electric hot-plate to slight boiling of 1 h. The obtained decoctions were filtered with a $0.45 \mu\text{m}$ membrane. The water decoction was obtained by concentrating the filtrate to 100 mL. An aliquot of the water decoction (30.0 mL) was used for the determination of total water-soluble Pb/Cd. The 30.0 mL solution was heated until about 5 mL of solution was obtained, then 8 mL of nitric acid and 2 mL of perchloric acid were added and gently heated on a hot plate to dryness. After cooling, the residue was dissolved with 1% (v/v) nitric acid and diluted to 25 mL of volume with de-ionized water.

For the analysis of Pb and Cd in water decoctions at stomach and intestine acidity, two aliquots of the above water decoction (30.0 mL, each) were added into two beakers, respectively. One was adjusted to the acidity of the stomach (pH 1.3) with 2.0 mol/L hydrochloric acid and 30% (v/v) $\text{NH}_3\cdot\text{H}_2\text{O}$, the other was adjusted to the acidity of intestine (pH 7.6). They were translated into a separatory funnel, and then 10.0 mL of n-octanol was added, oscillating for extraction for 2 h. The water phase obtained was diluted to 25.0 mL with 1% (v/v) nitric acid for the determination of water-soluble Pb/Cd in the water

decoctions. Then contents of n-octanol-soluble Pb/Cd were obtained by subtracting the content of water-soluble Pb/Cd from that of total Pb/Cd in the water decoctions, respectively.

3. Results and Discussion

3.1. Effect of Acid Medium and Acidity

The effects of hydrochloric acid, nitric acid, phosphoric acid and sulfuric acid acidity from 1% to 2% (v/v) on the absorbance of Pb and Cd were investigated. Used hydrochloric acid or phosphoric acid as a medium, the background interferences would be increased, and the absorbance would be lower than using sulfuric acid as a medium. Nitric acid in the range of 0.7% - 1.5% (v/v) was selected as a medium with higher sensitivity and stability for the determination of Pb and Cd. A 1% nitric acid was used in this work.

3.2. Matrix Modification Effect

The modification effects of several chemicals, such as NH_4NO_3 , $(\text{NH}_4)_2\text{SO}_4$, $\text{NH}_4\text{H}_2\text{PO}_4$ and PdCl_2 as well as $\text{NH}_4\text{H}_2\text{PO}_4 + \text{Mg}(\text{NO}_3)_2$, on the determinations of Pb and Cd were investigated comparatively. The absorbance-pyrolysis temperature curves are shown in Figure 1.

Using $\text{NH}_4\text{H}_2\text{PO}_4$ as a modifier, the critical pyrolysis temperature for the determination of Pb was raised from 300°C to 900°C with a higher sensitivity and lower background absorption because of the formations of more stable lead phosphate and easy volatile ammonium chloride in the presence of $\text{NH}_4\text{H}_2\text{PO}_4$. $(\text{NH}_4)_2\text{H}_2\text{PO}_4$ was used as a matrix modifier for Cd determination [8]. In our work, use of $\text{NH}_4\text{H}_2\text{PO}_4$ plus $\text{Mg}(\text{NO}_3)_2$ as a matrix

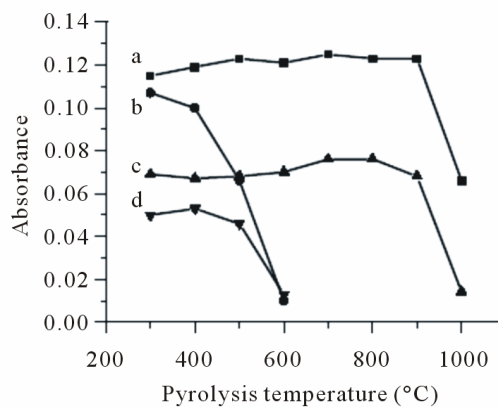


Figure 1. The absorbance- pyrolysis temperature curves. a. Pb + $\text{NH}_4\text{H}_2\text{PO}_4$, b. Pb, c. Cd + $\text{NH}_4 \text{H}_2\text{PO}_4 + \text{Mg}(\text{NO}_3)_2$, d. Cd. Data point is the average value of three measurements with < 2% of RSD.

modifier made the critical pyrolysis temperature of Cd raised from 400°C to 800°C, and obtained higher sensitivity than $\text{NH}_4\text{H}_2\text{PO}_4$ alone. Thus, the optimum pyrolysis temperature of 800°C was chosen for the determination of Pb and Cd in the presence of the modifiers.

The effects of modifier amounts on the sensitivity were investigated. The sensitivity increased obviously with the increase of the modifier concentration from 0 to 200 μg . The result showed that the best sensitivities were achieved using 100 μg $\text{NH}_4\text{H}_2\text{PO}_4$ and 120 μg $\text{NH}_4\text{H}_2\text{PO}_4 + 5 \mu\text{g}$ $\text{Mg}(\text{NO}_3)_2$ for the determination of Pb and Cd, respectively. Plateaus of the atomization temperature were observed over 1700°C - 2000°C using $\text{NH}_4\text{H}_2\text{PO}_4$ and $\text{NH}_4\text{H}_2\text{PO}_4 + \text{Mg}(\text{NO}_3)_2$ modifiers, respectively. Therefore, the optimal atomization temperatures were selected as 1700°C for Pb and 1800°C for Cd. In addition, the "maximum power" and gas stop modes were used for the determination of Pb and Cd in order to obtain high sensitivity.

3.3. Linearity, Detection Limit and Precision

The calibration graph was linear up to 70 $\mu\text{g}\cdot\text{L}^{-1}$ for Pb and up to 4 $\mu\text{g}\cdot\text{L}^{-1}$ for Cd. Linearity relation between absorbance (A) and concentrations (C) can be described by a regression equation: for Pb, $A = 454.802C - 2.600$, and for Cd, $A = 37.068C - 0.123$, with a correlation coefficient of > 0.999 . The detection limit calculated according to the IUPAC rules on the basis of 3σ criterion for 11 replicate measurements of the blank signal was 0.21 $\mu\text{g}\cdot\text{L}^{-1}$ for Pb and 0.005 $\mu\text{g}\cdot\text{L}^{-1}$ for Cd (4.2 pg Pb and 0.1 pg Cd for sampling 20 μL solutions). For 1 g sample and 50 mL final solution, the method LOD was 10.5 $\mu\text{g}\cdot\text{kg}^{-1}$ for Pb and 0.25 $\mu\text{g}\cdot\text{kg}^{-1}$ for Cd. The relative standard deviation (RSD, $n = 7$) was 1.54% for Pb at 40 $\text{ng}\cdot\text{mL}^{-1}$ level and 2.4% for Cd at 0.4 $\text{ng}\cdot\text{mL}^{-1}$ level. It is indicated that the proposed method has good linearity, high sensitivity and precision, and can permit the accurate detection of trace Pb and Cd in herbs and their decoctions.

3.4. Determination of Pb and Cd in Herbs and Their Decoctions

A water decoction is the one used for people to treat disease, and the content of Pb and Cd in the water decoction was the actual contribution to the toxicity. The preparation of water decoctions and determination of total Pb/Cd in herbs and total water-soluble Pb/Cd in water decoctions were carried out according to the method described in the procedure. The results are listed in **Table 2**.

The contents of Pb in the five herbs were higher than for Cd in the same herbs. Though the content of total Pb in *Common anemarrhena rhizome* was the highest in the five herbs, but the content of water-soluble Pb was the lowest. In contrast, the contents of total Pb in *Liquorice* was the lowest, but the contents of water-soluble Pb were near to that in *Common anemarrhena rhizome*. It was indicated that the dose of trace element taken in human body could not be evaluated with total content of the element in herb.

The practical feasibility of the proposed system was tested on the five kinds of Chinese herbal medicines. Since standard reference materials with certified Pb and Cd values were not available, the selectivity and accuracy of the method were tested by a standard spiking method. The recovery test was carried out in the same way for spiked samples in the range of 0.15 - 10 $\mu\text{g}\cdot\text{g}^{-1}$ for Pb, and 0.01 - 0.2 $\mu\text{g}\cdot\text{g}^{-1}$ for Cd (each added content nears real content in the sample). The recoveries of analyte from *Common anemarrhena rhizome*, *Liquorice*, *Dioscorea nipponica makino*, *Radix salviae miltiorrhizae* and *Fructus ligustri lucidi* were 98.6, 99.3, 96.9, 102 and 98.4% for total Pb, and 97.3, 98.7, 95.8, 97.7 and 98.9% for total water-soluble Pb, and 97.9, 100, 99.3, 98.8 and 96.9% for total Cd, and 98.9, 97.3, 98.6, 101 and 98.5% for total water-soluble Cd, respectively. The data showed that recovery was in the range of 95.8% - 102% for Pb and 96.9% - 101% for Cd. The relative standard deviations (R.S.D., $n = 3$) were in the range 1.7% - 3.4%, demonstrating the general reproducibility of this method.

Table 2. Determination of total Pb/Cd and total water-soluble Pb/Cd in herbs and their decoctions ($n = 3$).

Sample	Total Pb ($\mu\text{g g}^{-1}$)	Total Cd ($\mu\text{g g}^{-1}$)	Total water-soluble Pb ($\mu\text{g}\cdot\text{g}^{-1}$)	Total water-soluble Cd ($\mu\text{g}\cdot\text{g}^{-1}$)	Dissolving ratio	
					Pb (%)	Cd (%)
<i>Common anemarrhena rhizome</i>	9.29	0.19	0.17	0.01	1.83	5.26
<i>Liquorice</i>	1.54	0.15	0.18	0.01	11.7	6.04
<i>Dioscorea nipponica makino</i>	4.77	0.14	0.38	0.03	7.97	21.4
<i>Radix salviae miltiorrhizae</i>	4.94	0.19	0.25	0.02	5.06	10.5
<i>Fructus ligustri lucidi</i>	5.11	0.21	1.21	0.02	23.7	9.52

3.5. Dissolving Capability

Based on above result, water-dissolving capability of the analytes was examined. **Table 2** showed the dissoluble ratios (total water-soluble Pb/Cd contents to total Pb/Cd content). The dissoluble ratio was related to the kind of herb and element. For *Fructus ligustri lucidi*, the dissolving ratio of Pb was the highest. Although the content of Pb in *Common anemarrhena rhizome* was the highest, the dissolving ratio was the lowest. The content of Cd in *Dioscorea nipponica makino* was the lowest, and the dissolving ratio also was the highest. For *Common anemarrhena rhizome* the dissolving ratio of Cd was the lowest. Otherwise, except for *Liquorice* and *Fructus ligustri lucidi*, the dissolving ratios of Cd were higher than for Pb in the same herbs. The dissolving capability of Pb and Cd in each of the five herbs has their own distributed regularity. The kind of herb has a strong relationship to the dissolving ratios.

3.6. Distribution of Pb and Cd in Water Decoction at Stomach and Intestine Acidities

A decoction is one of the well known herbal preparations that are used for healing many diseases and ailments. The stomach and intestine are the main absorption organs in the human body. The n-octanol-soluble Pb and Cd have stronger lipophilic and biological activity. Since the structure of n-octanol is similar to aqua-carbon compound and ipide's structure, so the distribution of Pb and Cd in water decoction at stomach and intestinal acidity in the presence of n-octanol is represented by their distribu-

tion in the stomach and intestine.

The content ratio k_{ow} ($k_{ow} = c_o/c_w$, where c_o is n-octanol-soluble content; c_w is water-soluble content) was employed to evaluate the lipophilic and biological behaviors of organic compounds [17]. In this study, the concentrations of water-soluble and n-octanol-soluble Pb and Cd under gastric acidity (pH = 1.3) and intestinal acidity (pH = 7.6) conditions were investigated. The results are listed in **Table 3**.

For the studied herbs, k_{ow} for Pb and Cd under intestinal acidity was higher than that under gastric acidity, it showed that analyte would be removed from water-soluble to n-octanol-soluble Pb with raising pH of water decoction. Otherwise, k_{ow} values for the five herbs also had some differences. It is indicated that the content of water-soluble and n-octanol-soluble analyte and its ratio were related with the kind of herbal medicine and the acidity of the decoction.

When $k_{ow} > 1$, the quantum of n-octanol-soluble Pb and Cd taken in gastric and intestinal part would be higher than that of water-soluble Pb and Cd. When $k_{ow} < 1$, it was reverse. Therefore, when pharmacology actions of herbal medicines were studied, medicine effects of water decoction would not be evaluated only with total content of Pb or Cd in water decoction. The component of medicines, acted target, acidity of target and compatibility of medicines should be taken into account when studying toxicology of herbal medicine.

4. Conclusions

It was indicated that the dose of trace element taken in human body could not be evaluated with total content of

Table 3. Distribution of water-soluble and n-octanol-soluble Pb and Cd at gastric and intestinal acidity (n = 3)

Sample	Element	Stomach acidity (pH 1.3)			Intestine acidity (pH 7.6)		
		Water-Soluble	n-octanol-soluble	k_{ow}	Water-Soluble	n-octanol-soluble	k_{ow}
<i>Common anemarrhena rhizome</i>	Pb	0.098	0.083	0.85	0.059	0.123	2.10
	Cd	0.008	0.004	0.50	0.004	0.009	1.78
<i>Liquorice</i>	Pb	0.073	0.097	1.33	0.060	0.111	1.82
	Cd	0.007	0.003	0.42	0.007	0.003	0.45
<i>Dioscorea nipponica makino</i>	Pb	0.360	0.017	0.05	0.246	0.131	0.53
	Cd	0.010	0.004	0.40	0.008	0.007	0.82
<i>Radix salviae miltiorrhizae,</i>	Pb	0.226	0.022	0.10	0.215	0.034	0.16
	Cd	0.009	0.004	0.45	0.007	0.007	1.00
<i>Fructus ligustri lucidi</i>	Pb	0.878	0.333	0.40	0.806	0.406	0.50
	Cd	0.015	0.009	0.62	0.017	0.011	0.65

the element in herb. A n-octanol system can be used to study the distribution of Pb and Cd in water decoction in our stomach and intestine. By using $\text{NH}_4\text{H}_2\text{PO}_4$ as a modifier for Pb and $\text{NH}_4\text{H}_2\text{PO}_4$ plus $\text{Mg}(\text{NO}_3)_2$ as a matrix modifier for Cd, the critical pyrolysis temperature could be raised to 900°C and 800°C , respectively, assuring high analytical sensitivity and lower background absorption. The detection limits of the proposed method for Pb and Cd were lower than that of FAAS [3,4], GFAAS [5,6,8,10] and HGAFS methods[11]. The proposed method is a reliable and effective for the determination of Pb and Cd in Chinese herbal medicines and their decoctions. The distribution of Pb and Cd in water decoction at stomach and intestine acidities was developed in the first time. The proposed method can help us make a further study on the chemical and biological behaviors, regularity of elements movement and toxicity of Pb and Cd in Chinese herbal medicines, and gives some advice on Chinese traditional herbs' plant and use.

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6. References

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