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Determination of Lead, Cadmium, Chromium, and Arsenic in 13 Herbs of Tocolysis Formulation Using Atomic Absorption Spectrometry

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ABSTRACT

The contents of Pb, Cd, Cr, and As in 13 herbs of tocolysis formulations were studied using three kinds of sample pretreatment and atomic absorption spectrometry. Thirteen herbs were pretreated with either incineration, wet digestion, or microwave digestion, and the results of recoveries and precision about these elements were compared. The average recoveries of 13 herbs via incineration, wet digestion, and microwave digestion pretreatments were 80 ± 12%, 85.7 ± 3.3%, and 94.5 ± 2.1%, respectively. Among them, microwave digestion pretreatment had the highest recovery and precision. The average relative standard deviations for the four measured elements (Pb, Cd, Cr, and As) from incineration, wet digestion, and microwave digestion pretreatments were 8.8 ± 1.7%, 7.1 ± 3.3%, and 3.4 ± 0.8%, respectively. The detection limits for Pb, Cd, Cr, and As using microwave digestion pretreatment were 0.45 ppb, 0.03 ppb, 0.20 ppb, and 0.64 ppb, respectively. Concentrations of Pb, Cd, Cr, and As measured from the tocolysis formulation using microwave digestion pretreatment were 28.0 ± 0.7 ppb, 1.11 ± 0.03 ppb, 8.5 ± 0.3 ppb, and 1.52 ± 0.04 ppb, respectively. Lead was found to have the highest concentration among the four determined elements in 13 herbs. Schizonepetae Herba contained the highest Pb (60.5 ± 2.1 ppb), Cr (23.3 ± 0.5 ppb), and As (8.8 ± 0.1 ppb) among the 13 herbs. Ligustici Rhizoma contained the highest Cd (3.76 ± 0.04 ppb) among the 13 herbs.

Key words: tocolysis formulation, sample pretreatments, atomic absorption spectrometry

INTRODUCTION

Use of herbal medicines to relieve and treat many human diseases is increasing around the world due to their mild features and low side effects[1-7]. Herbal medicines are very commonly used in Asia. However, herbs may be contaminated easily during growing and processing. It is important to have a good quality control for herbal medicines in order to protect consumers from contamination. Pb, Cd, Cr, and As are widely considered as potential contaminants in our environment due to their toxicities to human[8-15]. Reasonable regulations and accurate measurements for Pb, Cd, Cr, and As and other toxic heavy metals in herbal medicines are really needed in Taiwan and in other countries to which most herbal samples are imported. Combination regimens are common and widely used in prescriptions for herbal medicines. Therefore, it is important to use combination regimens for herbal medicinal studies and analyses. Tocolysis formulation is one popular combination regimen commonly used to stabilize the embryo for pregnant women. Therefore, a tocolysis formulation containing 13 herbs was investigated for its contents of Pb, Cd, Cr, and As using three kinds of sample pretreatment and a graphite furnace atomic absorption spectrometer. The 13 herbs studied were Citri Immaturus Fructus, Astragali Radix, Fritillaria Cirrhosae Bulbus, Dodder, Glycyrrhizae Radix, Schizonepetae Herba, Paeoniae Lactiflorae Radix, Ligustici Rhizoma, Notopterygii Rhizoma, Mugwort, Angelicae Sinensis Rhizoma, Magnoliae Cortex, and Zingiberis Soccatum Rhizoma. In the present work, concentrations of Pb, Cd, Cr, and As in 13 herbs by using three kinds of sample pretreatment method including incineration, wet digestion, and microwave digestion together with electrothermal atomic absorption spectroscopy were obtained and compared. The recoveries and precision for these four elements were also discussed.

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METHODS

I. *Instrumentation*

A Perkin-Elmer (Überlingen, Germany) Analyst 300 atomic absorption spectrometer equipped with a deuterium-arc background corrector and an HGA 800 graphite furnace atomizer were used for the atomic absorption measurement of Pb, Cd, and Cr. A flow injection analysis system (FIAS-100, Perkin-Elmer) was also used for the measurement of As. A model Neytech 3-550 (Bloomfield, Connecticut, USA) incineration oven was used for incineration pretreatment. A model O-I-Analytical 7165 (College station,

Texas, USA) microwave digestion system was used for microwave digestion pretreatment. A hot plate stirrer from the Jii Tsann Co., (Taipei, Taiwan), was used for wet digestion pretreatment.

II. *Reagents and samples*

Deionized water used was from Milli-Q sp (Millipore, Bedford, Massachusetts, USA). Lead, cadmium, arsenic, and chromium standard solutions (1000mg/l) were from Merck (Darmstadt, Germany). Nitric acid, hydrochloric acid, potassium iodide, sodium borohydride, and ascorbic acid were of analytical grade and also purchased from Merck (Darmstadt, Germany). The 13 herbs(Citri Immaturus Fructus, Astragali Radix, Fritillaria Cirrhosae Bulbus, Dodder, Glycyrrhizae Radix, Schizonepetae Herba, Paeoniae Lactiflorae Radix, Ligustici Rhizoma, Notopterygii Rhizoma, Mugwort, Angelicae Sinensis Rhizoma, Magnoliae Cortex, and Zingiberis Soccatum Rhizoma) were imported from mainland China and obtained from a local herbal shop in Taichung, Taiwan. Herbal samples were cut into small pieces then ground into powder using a coffee-bean grinder before weighing. One gram of each herbal sample powder was used in each sample pretreatment experiment.

III. *Procedure*

(I) *Incineration pretreatment*

One gram of each herbal sample powder was heated at 10˚C/min in a crucible from room temperature to 250˚C, held for 1 h, then ramped up at 15˚C/min to 600˚C and held for 3 h. After cooling to room temperature in a dessicator, samples were dissolved in 10 mL of 4 N HNO_3 and then diluted to 100 mL with 0.2% (v/v) HNO_3 for Pb, Cd, and Cr analysis.

(II) *Wet digestion pretreatment*

One gram of herbal samples was digested with 5 ml of 16 N HNO3 in the covered beakers to near dryness. If necessary, another 5 mL portion of 16 N HNO_3 was further added each time until the sample solutions became clear. Five milliliters of 12 N HCl were then added to ensure complete digestion. After cooling to room temperature, the digested solutions were diluted to 100 mL with deionized water for Pb, Cd, and Cr analysis.

(III) *Microwave digestion pretreatment*

One gram of each herbal sample was mixed with 4 ml of H_2O_2 and 10 mL of 16 N HNO_3 before being placed in separate Teflon sample cells. A four-stage power program was used as follows: 70% power at a pressure of 20 PSI at 25˚C; 80% power at a pressure of 40 PSI at 35˚C; 90% power at a pressure of 80 PSI at 45˚C ; and 100% power at a pressure of 120 PSI at 55˚C. The digested samples were diluted to 100 mL with deionized water and were ready for analysis.

For arsenic analysis, 10 mL of 10% KI and 5% ascorbic acid solutions were added after cooling to room temperature in three sample pretreatments. Each solution was then diluted to 100 mL with 10% (v/v) HCl solution. Hydride generation method was used for As analysis. Mixed solutions of potassium iodide and ascorbic acid were used to reduce As (V) to As (III) for better hydride absorbance.

Hollow cathode lamps were used as light sources for Pb, Cd, Cr and a electrodeless discharge lamp was used for As. The wavelengths used for Pb, Cd, Cr, and As measurements were 283.3 nm, 228.8 nm, 357.9 nm, and 193.7 nm, respectively. The lamp currents used for Pb, Cd, Cr, and As measurements were 10 mA, 4 mA, 25 mA, and 5 mA respectively. The optimal incineration temperatures of the graphite furnace used for Pb, Cd, and Cr were studied in the range of 250 – 750˚C at an interval of 100˚C, 100 – 500˚C at 100˚C interval, and 800 – 1600˚C at 200˚C interval, respectively. The optimal atomization temperatures of the graphite furnace used for Pb, Cd, and Cr were studied in the range of 1400 – 2200˚C, 1000–1800˚C, and 2100 – 2600˚C at an interval of 200˚C, respectively. Twenty microliters of sample volumes were injected into graphite furnace for Pb, Cd, and Cr analyses. In the arsenic hydride experiment, 500 µL of each sample solution containing 10% HCl was added 1.3 mL of 0.2% $NaBH_4$ reduction solution before being injected into a quartz tube atomizer (900˚C). Three standard solutions within their linear concentration ranges were added in the standard addition method for the four elemental absorbance measurements. The recovery was calculated by the following equation,

$$\text{Recovery (\%)} = [(C_{tot} - C_{samp})/C_{std}] \times 100 \quad (1),$$

where C_{tot} is the total concentration measured for the specified element in the sample together with the standard solution added, C_{samp} is the concentration measured for the specified element in the sample, and C_{std} is the concentration of standard solution of the specified element added. Detection limit was calculated at a signal-to-noise ratio 3 : 1. Standard solutions of calibration line for each element were used for detection limit determinations. Eight, six, ten, and five standard concentration solutions within each linear range were used for Pb, Cd, Cr, and As study respectively.

RESULTS AND DISCUSSIONS

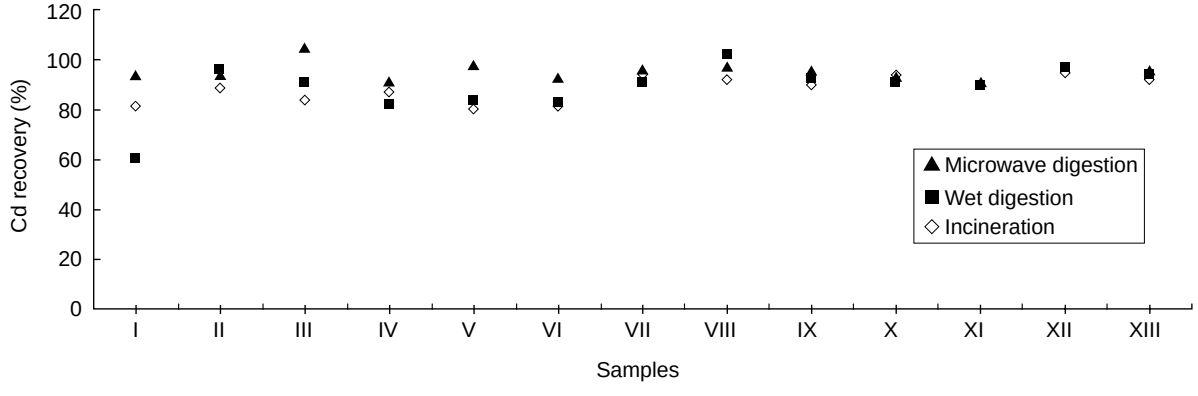
The heating programs of incineration and atomization temperatures in graphite furnace atomic absorption spectrometry were very important. The optimal temperatures of incineration and atomization for Pb, Cd, Cr, and As were determined by the measured absorbances of standard solutions at various temperature ranges specified in the experi-

mental section. The optimal temperatures were independent of elemental concentration within their linear concentration ranges. The optimal incineration and atomization temperatures for the graphite furnace were 550˚C, 250˚C, 1250˚C, and 1600˚C, 1200˚C, 2300˚C for Pb, Cd, and Cr respectively, as shown in Table 1. These optimal temperatures were used for the rest of the Pb, Cd, and Cr studies.

Recoveries of Pb, Cd, Cr, and As in tocolysis formulations containing 13 herbs using three different sample pretreatments were studied and compared. The recoveries obtained for Cd and As using three kinds of sample pretreatment are shown in Figure 1 and 2. The results indicated that Cd and As had higher recoveries with microwave digestion pretreatment than with incineration and wet digestion pretreatments. The recoveries for Cd were roughly equal with incineration and with wet digestion pretreatments. The standard deviation bars are not shown for Cd in Figure 1 because recoveries may overlap. In Figure 2, the wet digestion pretreatment had a better recovery for As than the incineration pretreatment. The recoveries for Pb and Cr using the three kinds of sample pretreatment were similar to that for Cd. Overall, recoveries obtained by the microwave digestion pretreatment are about 10–15% higher than other sample pretreatments for Pb, Cd, Cr, and As using atomic absorption spectrometry. Concentrations measured for Cr, As, Cd, and Pb in the tocolysis formulations containing 13 herbs using three kinds of pretreatment are shown in Figure 3 to 6. In general, the concentrations of these four elements obtained with microwave digestion pre-

Table 1. Optimal incineration and atomization temperatures for Pb, Cd, Cr, and As in herbs of tocolysis formulation by graphite furnace atomic absorption spectrometry

Element	Incineration temperature	Atomization temperature
Pb	550˚C（30sec）	1600˚C（5sec）
Cd	250˚C（30sec）	1200˚C（5sec）
Cr	1250˚C（30sec）	2300˚C（5sec）



* I: Citri Immaturus Fructus II: Astragali Radix III: Fritillaria Cirrhosae Bulbus IV: Dodder V: Glycyrrhizae Radix
VI: Schizonepetae Herba VII: Paeoniae Lactiflorae Radix VIII: Ligustici Rhizoma IX: Notopterygii Rhizoma X: Mugwort
XI: Angelicae Sinensis Rhizoma XII: Magnoliae Cortex XIII: Zingiberis Soccatum Rhizoma

Figure 1. Cadmium recovery from 13 herbs using three different sample pretreatments

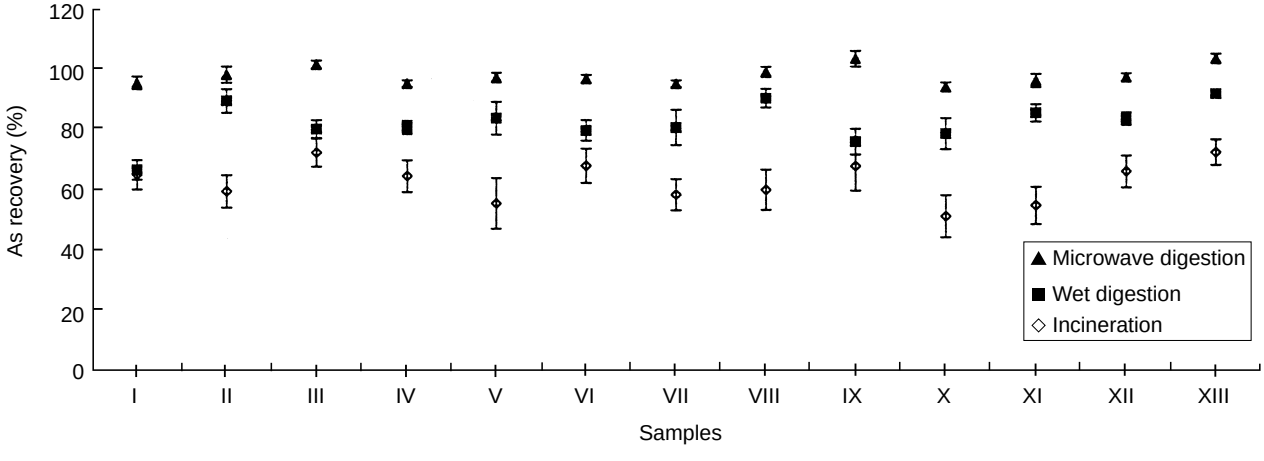


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Figure 2. Arsenic recovery from 13 herbs using three different sample pretreatments

treatment were higher than the other two pretreatment methods. This trend is roughly consistent with the recovery experiments. The results indicate that the relative amounts of Pb, Cr, As, and Cd decrease in that order. The relatively high amounts of Pb found in the herbal samples should be examined carefully and regulated by food and drug administrators for consumers' safety.

Table 2 shows concentrations of Pb, Cd, Cr, and As in the tocolysis formulations containing 13 herbs by using microwave digestion pretreatment together with atomic

Table 2. Concentrations of Pb, Cd, Cr, and As measured in 13 herbs of tocolysis formulation using microwave digestion pretreatment together with atomic absorption spectrometry

Samples	Pb (ppb)	Cd (ppb)	Cr (ppb)	As (ppb)
I	33.88 ± 0.38	0.62 ± 0.02	9.44 ± 0.36	0.98 ± 0.04
II	15.76 ± 0.38	0.76 ± 0.02	4.24 ± 0.12	1.46 ± 0.02
III	19.30 ± 0.78	0.86 ± 0.02	1.64 ± 0.12	ND
IV	28.74 ± 0.16	0.44 ± 0.02	9.76 ± 0.36	3.31 ± 0.08
V	59.70 ± 1.08	1.50 ± 0.04	4.32 ± 0.10	0.78 ± 0.05
VI	60.46 ± 2.06	0.86 ± 0.06	23.32 ± 0.52	8.78 ± 0.13
VII	22.34 ± 0.52	1.02 ± 0.02	8.48 ± 0.17	ND
VIII	20.24 ± 1.10	3.76 ± 0.04	11.16 ± 0.62	1.83 ± 0.06
IX	21.52 ± 0.54	2.14 ± 0.06	13.64 ± 0.12	3.54 ± 0.11
X	26.34 ± 0.54	0.94 ± 0.04	9.64 ± 0.38	ND
XI	17.36 ± 0.30	0.38 ± 0.02	5.16 ± 0.32	0.78 ± 0.03
XII	37.14 ± 0.60	1.18 ± 0.02	8.60 ± 0.38	ND
XIII	1.87 ± 0.02	ND	0.52 ± 0.03	ND

* I: Citri Immaturus Fructus II: Astragali Radix III: Fritillaria Cirrhosae Bulbus IV: Dodder
V: Glycyrrhizae Radix VI: Schizonepetae Herba VII: Paeoniae Lactiflorae Radix VIII: Ligustici Rhizoma
IX: Notopterygii Rhizoma X: Mugwort XI: Angelicae Sinensis Rhizoma
XII: Magnoliae Cortex XIII: Zingiberis Soccatum Rhizoma

**n = 3 ND: not detected (< 0.64 ppb for As and < 0.03 ppb for Cd)

Table 3. Results obtained in tocolysis formulation using microwave digestion pretreatment and atomic absorption spectrometry

	Pb	Cd	Cr	As
Amount (ppb) (mean ± SD)	28.05 ± 0.65	1.11 ± 0.03	8.46 ± 0.28	1.52 ± 0.04
Precision (%)	2.3	2.7	3.3	2.6
Recovery (%)	94.1 ± 2.7	95.4 ± 3.6	92.6 ± 6.6	97.5 ± 3.1
Liner range (ppb)	0.5 – 50	0.1 – 3.0	0.5 – 10.0	1.0 – 10.0
Detection limit (ppb)	0.45	0.03	0.20	0.64

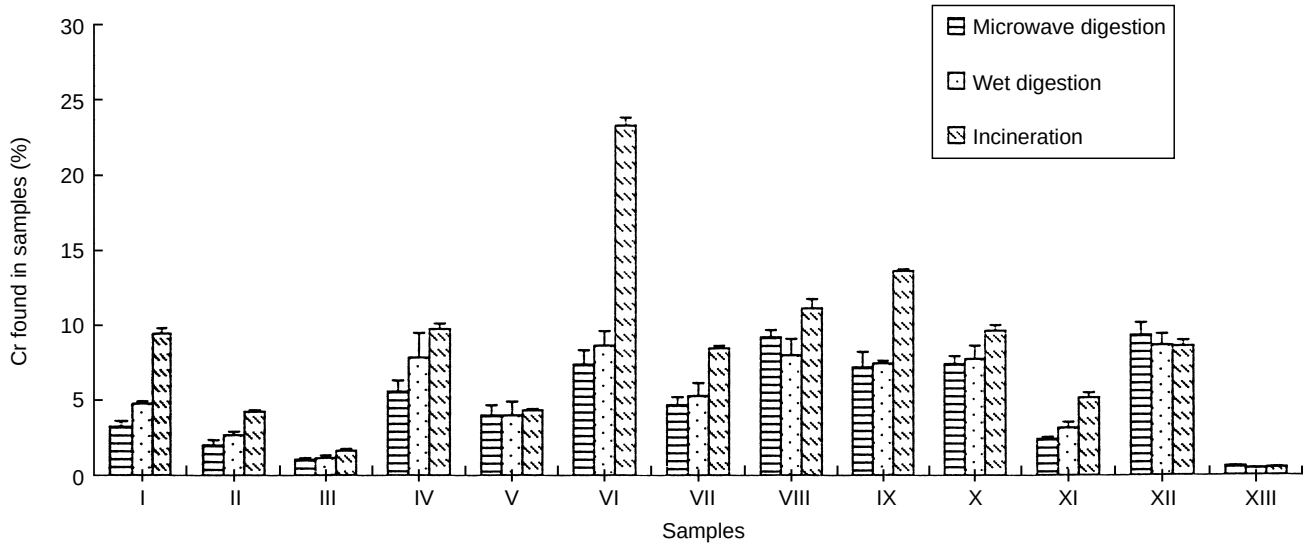

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Figure 3. Experimentally determined chromium from 13 herbs using three different sample pretreatments

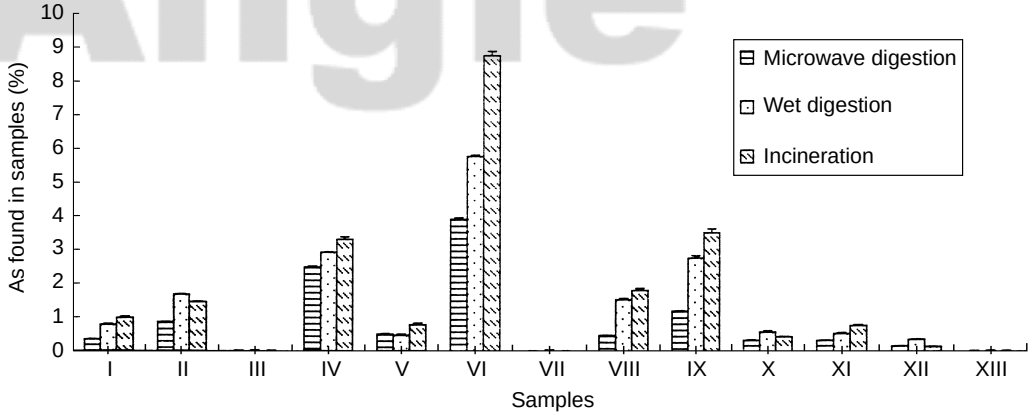

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Figure 4. Experimentally determined arsenic from 13 herbs using three different sample pretreatments

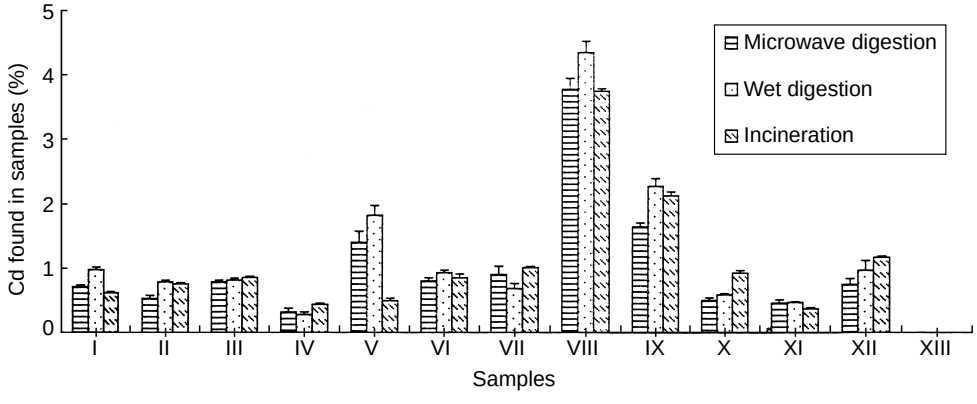

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Figure 5. Experimentally determined cadmium from 13 herbs using three different sample pretreatments

absorption spectrometry. The results indicated that Schizonepetae Herba contained the highest Pb, Cr, and As among the 13 herbs studied. Schizonepetae Herba in herbal medicinal applications should be examined carefully in the future due to its highly toxic elements. Ligustici Rhizoma contained the highest Cd among the 13 herbs. Table 3 shows these four elements found in tocolysis formulation with microwave digestion pretreatment and atomic absorption spectrometry. Recoveries of the four elements studied using microwave digestion pretreatment varied from 92.6% to 97.5%, with an average of 94.9%. Two elements (Pb and Cr) are shown in Table 4 as examples. Precision values in term of relative standard deviation in this study were all within 3.5%. Average concentrations of Pb, Cd, Cr, and As measured in tocolysis formulations were 28.05 ± 0.65 ppb, 1.11 ± 0.03 ppb, 8.46 ± 0.28 ppb, and 1.15 ± 0.04 ppb, respectively. The relatively high concentration of Pb in herbal samples should be made available to consumers, and should be investigated further to identify the sources of contamination. The linear ranges for Pb, Cd, Cr, and As were roughly within one order of magnitude except Pb, as shown in Table 5. The correlation coefficients of all calibration curves from these four elements were greater than or equal to 0.999. The detection limits were all in the sub-ppb range.

The time required for pretreatment was 8 h, 10 h, and 0.8 h, for incineration, wet digestion, and microwave digestion respectively. Microwave digestion pretreatment was much faster than the other two. The relative cost for the three kinds of sample pretreatment was approximately 4:1:60 for incineration, wet digestion, and microwave digestion. Though the cheapest, wet digestion pretreatment is not as easily automated as its incineration and microwave counterparts. Sample capacities available for the three pretreatment methods were approximately 5 g, 1 g, and 5 g for incineration, wet digestion, and microwave digestion, respectively. At present, incineration and microwave digestion pretreatments can handle more samples than wet digestion pretreatment. A higher sampling capacity for the microwave digestion is possible at higher pressure with advanced instrumentation.

Proper regulations and accurate measurements of Pb,

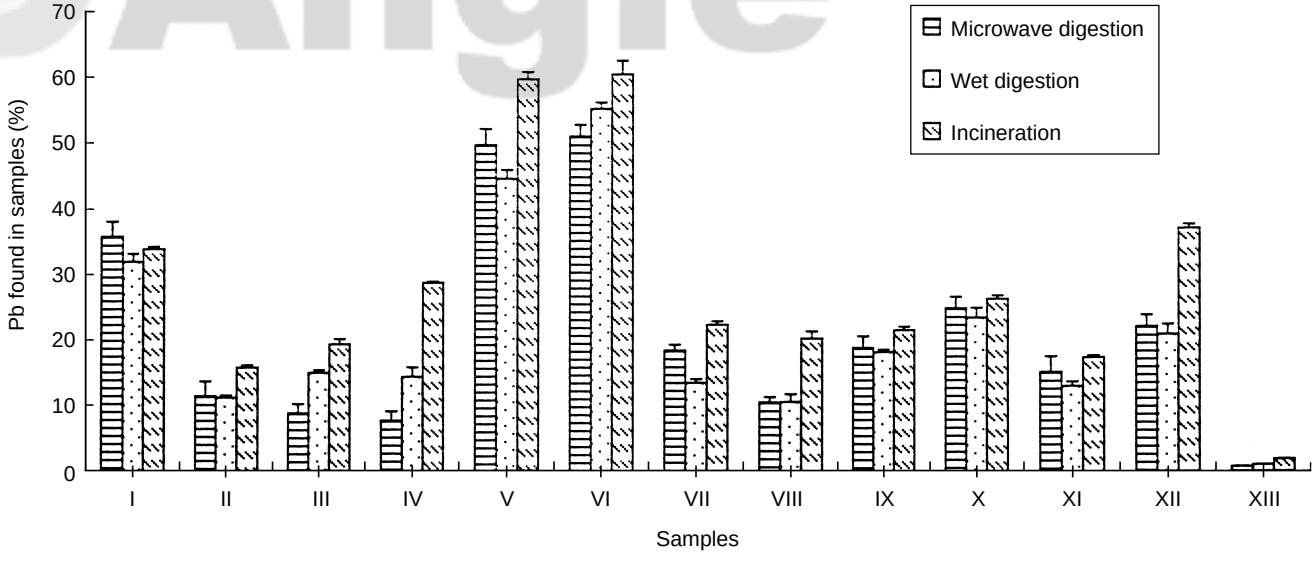

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Figure 6. Experimentally determined lead from 13 herbs using three different sample pretreatments.

Table 4. Recovery tests for Pb and Cr in tocolysis formulation using microwave digestion pretreatment and atomic absorption spectrometry

Sample	Element	Concentrations（ppb）(n = 3)			Recovery (%)
		In the sample	added	found	
Citri Immaturus Fructus	Pb	33.88 ± 0.38	3.00	36.66 ± 1.12	92.7
	Cr	9.44 ± 0.36	3.00	12.24 ± 0.20	93.3
Astragali Radix	Pb	15.76 ± 0.38	3.00	18.55 ± 0.17	93.0
	Cr	4.24 ± 0.12	3.00	7.15 ± 0.14	97.0
Fritillaria Cirrhosae Bulbus	Pb	19.3 ± 0.78	3.00	22.11 ± 0.89	93.7
	Cr	1.04 ± 0.02	3.00	3.86 ± 0.05	94.0
Dodder	Pb	28.74 ± 0.16	3.00	31.63 ± 1.09	96.3
	Cr	9.76 ± 0.36	3.00	12.47 ± 0.21	90.3
Glycyrrhizae Radix	Pb	59.7 ± 1.08	3.00	62.58 ± 1.81	96.0
	Cr	4.32 ± 0.10	3.00	7.21 ± 0.18	96.3
Schizonepetae Herba	Pb	60.46 ± 2.06	3.00	63.21 ± 1.72	91.7
	Cr	23.32 ± 0.52	3.00	26.02 ± 0.36	90.0
Paeoniae Lactiflorae Radix	Pb	22.34 ± 0.52	3.00	25.17 ± 0.83	94.3
	Cr	8.48 ± 0.17	3.00	11.16 ± 0.16	89.3
Ligustici Rhizoma	Pb	20.24 ± 1.10	3.00	23.06 ± 1.21	94.0
	Cr	11.16 ± 0.62	3.00	13.90 ± 0.17	91.3
Notopterygii Rhizoma	Pb	21.52 ± 0.54	3.00	24.23 ± 0.82	90.3
	Cr	13.64 ± 0.12	3.00	16.66 ± 0.28	101
Mugwort	Pb	26.34 ± 0.54	3.00	29.08 ± 0.78	91.3
	Cr	9.64 ± 0.38	3.00	12.41 ± 0.21	92.3
Angelicae Sinensis Rhizoma	Pb	17.36 ± 0.30	3.00	20.19 ± 0.65	94.3
	Cr	5.16 ± 0.32	3.00	7.97 ± 0.16	93.7
Magnoliae Cortex	Pb	37.14 ± 0.60	3.00	40.17 ± 1.11	101
	Cr	8.60 ± 0.38	3.00	11.57 ± 0.23	99.0
Zingiberis Soccatum Rhizoma	Pb	1.87 ± 0.02	3.00	4.72 ± 0.12	95.0
	Cr	0.52 ± 0.03	3.00	2.87 ± 0.06	95.7

Table 5. Calibration equation and linear ranges of Pb , Cd , Cr and As measurements

Element	Calibration equation	Linear range(ppb)	Correlation coefficient (R2)
Pb	Y = 0.0067X - 0.0018	0.5 – 50.0	0.9999
Cd	Y = 0.2074X - 0.0063	0.25 – 3.0	0.9996
Cr	Y = 0.0308X - 0.0145	1.5 – 10.0	0.9997
As	Y = 0.0455X + 0.0042	1.0 – 10.0	0.9999

Cd, Cr, and As and other toxic heavy metals in herbal medicines are really needed in Taiwan and countries where herbal samples are mostly imported. It is very important to ensure the safety of the fetus and the mother taking tocolysis formulations and other combination regimens. The results of this study may provide a useful reference for analysis of toxic elements in herbal medicines.

ACKNOWLEDGMENT

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