Trace Metals in Traditional Chinese Medicine: A Preliminary Study Using ICP-MS for Metal Determination and As Speciation

Xiaoru Wang, Zhixia Zhuang, Dahai Sun, Jiangxing Hong, and Xihong Wu
Chemistry Department, Xiamen University, Xiamen, P.R. China
and
Frank Sen-Chun Lee, Mildred S. Yang, and H.W. Leung
Hong Kong Baptist University, Hong Kong

ABSTRACT

The metal content in several TCM drugs was determined by ICP-MS. The efficiencies of different sample digestion methods were compared. Since one of the products studied is known to contain arsenic sulfides as a main ingredient, a solvent fractionation scheme was developed and applied to speciate As in the product. The metal content in the same TCM drug produced by different manufacturers was compared. The concentration of some metals such as Pb and Cd differs widely with different manufacturers, suggesting that their origin is primarily from external contamination. The high sensitivity and precision of the ICP-MS technique offers considerable advantages over conventional ICP-OES techniques for the analysis of complex samples such as TCM materials. Standardized analytical protocols based on ICP-MS are being developed for the determination and characterization of metals and trace elements in TCM materials for product quality assessment.

INTRODUCTION

Recently, the importance of Traditional Chinese Medicinal Material (TCM) as a natural medicinal material has been gaining increasing recognition worldwide (1). However, the safety, quality, and efficacy of TCM must be critically evaluated before they can be put into clinical trials or placed on the market (2). Many of these parameters are closely related to the concentration levels of toxic and/or heavy metals in the TCM material. Some of these metals, e.g., As, Hg, Cd, Pb, etc., are present only at extremely low concentrations, making their quantitative analysis and speciation a challenging task. At present, inductively coupled plasma optical emission spectrometry (ICP-OES) is usually the method of choice for the determination of trace metals because of its speed and wide availability. However, for the determination of these elements in complex matrices such as TCM, ICP-OES often lacks the high sensitivity needed, and may be prone to problems of background interferences from the organics in the sample matrices. The rapidly developing ICP-MS technique is becoming an attractive alternative because of its ultra-high sensitivity and specificity (3). In toxicity assessment, high sensitivity and specificity is of particular importance because it helps to reduce the risk of false negatives in trace contaminant analysis.

*Corresponding author.

Our laboratory has a comprehensive program aimed at the development of analytical techniques for the determination of metals in TCM for quality control and standardization. TCM consists of a complex array of materials in different forms, including raw herbs, herbal or animal extracts, minerals, and finished products in powder, pill, tablet or syrup forms. The latter products are usually a mixture of different products each derived from different raw materials. This is quite different from western drugs which normally consist of a single or a few well-defined chemicals with specific formulations. Since the biological raw materials in TCM show natural variations in composition, the final products also vary, sometimes greatly, even under standardized manufacturing processes. Thus, the standardization of the quality of TCM and the development of reliable analytical techniques to standardize the products are very challenging tasks indeed.

In the present work, the metal content in several TCM drug products was determined. Since one of the products is known to have a high As content, a solvent fractionation scheme was used to speciate its As species. Our objective was to develop an ICP-MS-based analytical protocol to determine trace metals in several TCM drug products. Sample preparation is a key step in analysis and thus the efficiency of different sample treatment procedures was compared. Our goal was the development of standardized analytical procedures to measure and characterize trace elements in TCM for quality assessment and regulatory purposes.

EXPERIMENTAL

Instrumentation

ICP-OES

A Model PS-4 multi-channel ICP-OES (Baird Co., Bedford, MA, USA) was used for the sample preparation study. The optimized operating conditions and the analytical lines used are given in Table I.

TABLE I
ICP-OES Operating Conditions

Power	1.15 kW
RF frequency	27.12 MHz
Coolant gas flow rate	10 L/min
Plasma gas flow rate	1 L/min
Carrier gas flow rate	0.8 L/min
Observation height	15 mm
Integration time	4 s

Wavelength of Analytical Lines

Elements	Wavelength (nm)
Zn	213.8
Fe	259.9
Mn	257.6
Mg	279.5
Na	589.5
Ca	317.9
Ba	455.4
K	766.4

ICP-MS

A Model ELAN® 6000 ICP-MS (Perkin Elmer SCIEX, Concord, Ont., Canada) was used for the metal determination in real TCM samples. Aqueous standards of 10 ng/mL Rh, Mg, Pb, Cd and Cu in 2% HNO_3 were used to calibrate the instrument. Mass discrimination and auxiliary argon and coolant gas flow rates were controlled automatically by the instrument. The operating conditions are listed in Table II.

Microwave Oven

A Model MK-1 MWO (Institute of Microwave Technology, Shanghai, PR of China) was used for the TCM digestion study.

Chemicals and Standards

Standard stock solutions of metals studied (1000 µg/mL) were prepared with the individual Spectra-pure oxides, which were dissolved by HCl/HNO_3, and diluted to volume with 5% HCl/HNO_3. The standard solutions for the calibration were prepared daily with the stock solution, diluted with purified water (Milli-Q™ purification system, Millipore, Bedford, MA, USA).

The standard reference materials of peach leaves (GBW-08501) and tea leaves (GBW-08505), both obtained from the Institute of Standard Material, Beijing, China, were used to verify the analytical results.

The TCM samples used for this study were oven-dried at 120°C for one hour. After cooling, the samples were ground to pass through an 80-mesh sieve before further treatment as described below.

Sample Digestion

Wet digestion by HNO_3-$HClO_4$

0.5–1.0 g of ground TCM samples were each accurately weighed into a 50-mL beaker. Several drops of purified water were added to the sample, followed by the addition of 10 mL of concentrated HNO_3. After gentle shaking, the sample was heated on a hot plate at 150°C for two hours. The sample was frequently mixed by shaking during heating. Six mL 60% $HClO_4$ was then added into the beaker and the heating process continued for five minutes. (**Caution:** $HClO_4$ acid is highly corrosive to skin and potentially explosive during heating. Add to water slowly and always wear gloves and goggles during operation.) When the sample solution became clear, the heating was stopped, and the sample cooled to room temperature. The solution was then diluted with purified water to 50 mL for analysis.

TABLE II
ICP-MS Operating Conditions

RF generator power	1.0 kW
RF frequency	40 MHz (nominal)
Carrier gas flow rate	0.91 L/min
Sampling orifice	1.0 mm Ni
Skimmer orifice	0.8 mm Ni
Ion lens	7.25 V
Analog stage voltage	– 2237.5 V
Pulser stage voltage	1650 V
Discriminator threshold	70 V
AC rod offset	– 6 V
Resolution	0.7 amu
Integration time	300 ms
Scanning time	6 s
Replicates	3
Sample uptake rate	1.0 mL/min

Wet digestion with HNO_3-H_2O_2

The same procedure as described above was carried out except that 8 mL of H_2O_2 was used instead of $HClO_4$ to stop excessive foaming, and was added in three aliquots of 2 mL, 3 mL, and 3 mL volumes.

Microwave oven (MWO) digestion with HNO_3-H_2O_2

The containers were first cleaned with detergent solutions, followed by rinsing with 5% HNO_3. Four mL of concentrated HNO_3 was put into the cleaned containers. The containers were heated in the MWO for five minutes. The containers were cooled, then opened and rinsed with purified water. This nitric acid cleaning step was repeated twice. The containers were air-dried before use. For digestion, 0.5 – 1.0 g of ground sample was weighed into the MWO sample containers, then digested in the MWO following the procedure listed in Table III.

Arsenic Speciation Analysis

The analysis was carried out for a TCM drug Lushen Wan (manufactured by Dong Shan pharmaceutical factory, Wuhan, lot number 001184, 97/03/07). Six ground powder samples of about 1.0 g of Lushen Wan were accurately weighed individually into six 150-mL beakers. The flow chart illustrated in Figure 1 shows the fractionation process used for different arsenic species of the samples.

TABLE III
MWO Digestion Process (100% Power)

Step	Time (min)	Pressure setting	Reagent
1	1	1	5mL HNO_3
2	2	2	Without addition of reagent
3	8	3	Without addition of reagent
4	4	2	2 mL H_2O_2
5	2	2	0.5 mL H_2O_2

*Between the changes of the pressure the samples in the containers were kept stable for a few minutes without heating. Pressure setting numerical values are set by the manufacturer: the higher the number, the higher the pressure.

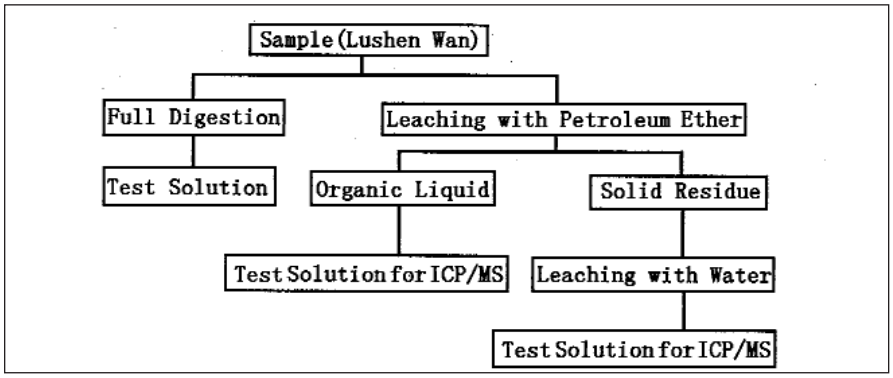

Fig. 1. Flow chart for arsenic speciation analysis.

The sequential steps illustrated in the scheme are described as follows:

(a) Three of the samples were digested by MWO using procedures described in the previous section. The digested samples were used to determine the total amounts of arsenic in the sample.

(b) The other three samples were leached individually with 10 mL of petroleum ether. The samples were continuously stirred for four hours, then left undisturbed overnight. Afterwards, the clear supernatant in each sample was decanted into a clean beaker. The leaching process was repeated twice with 5 mL of petroleum ether each time. The three supernatants were combined and 5 mL of HNO_3 was added. The solution was then heated to boiling with petroleum ether on a hot plate for five minutes. After cooling, the solution was transferred into a 50-mL flask, and diluted with purified water to volume for ICP-MS analysis. The extracted fraction represents the organic arsenic portion of the sample.

(c) The residues after the leaching step (b) were extracted overnight with purified water, stirring slowly at the beginning. The extract was then filtered and the extract collected. The extracted residue was again extracted twice following the same procedure. The three filtered extracts were combined, diluted with purified water to 50-mL volume for ICP-MS analysis. This fraction represents the water-extractable arsenic species.

(d) The final residues from step (c) were each MWO digested with 5 mL concentrated HNO_3 acid for 10 minutes, and then with 4 mL H_2O_2 for four minutes for ICP-MS analysis. The As found represents the residue portion of the total As in the sample.

RESULTS AND DISCUSSION

Comparison of Digestion Methods

Using the three different digestion methods, the results obtained for the two reference materials (GBW-08501 and GBW-08505) are listed and compared in Table IV, along with the certified values. Both reference standards are herbal material similar to TCM products. A similar comparison was also carried out for a real TCM sample (Huang Lian Su) and the results are listed in Table V. The concentrations of the eight metals determined vary from a fraction to several hundred μg/g. All samples were run in triplicate and the RSD of the measurements are in general better than 10%.

The statistical *F* tests show that the precision values of the measurements for the two reference samples were not significantly different from each other, and also not different from those of the drug samples at the 95% confidence level. For the three digestion methods, statistical analysis of variance and the student's *t* tests suggest that at 95% confidence, the measured metal concentrations are not significantly different for the individual metals or the overall metal distributions as a whole. Compared with the certified values, however, most of the metal measurements show a small negative bias. These biases are in general less than 3% relative, except for Mg, for which both the precision and the bias are relatively poorer, probably because of its presence in very low (sub-ppm) concentrations.

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TABLE IV
Comparison of Three Sample Digestion Methods (µg/g)

Element	HNO_3+$HClO_4$	HNO_3+H_2O_2	MWO	Certified
(GBW-08501)				
Zn	21.1±1.2	22.0±2.3	21.2±0.9	22.8 ±1.3
Fe	395±5	393±23	406±19	431±15
Mn	72.3±3.0	68.2±3.5	68.6±1.8	75.4±2.7
Mg	0.44±0.02	0.43±0.02	0.44±0.04	0.47±0.02
K	1.96±0.01	1.99±0.11	2.01±0.05	2.17±0.08
Ba	17.9±1.1	17.9±0.8	17.2±0.7	18.4±0.9
(GBW-08505)				
Zn	35.2±4.6	34.9±2.2	36.1±1.4	38.7±3.9
Fe	392±15	349±20	367±10	373±23
Mn	744±33	697±37	739±13	766±28
Mg	0.214±0.025	0.206±0.013	0.208±0.061	0.224±0.019
Ca	0.291±0.035	0.274±0.034	0.279±0.025	0.284±0.021
K	1.79±0.24	1.82±0.15	1.94±0.24	1.97±0.13
Na	131±20	143±19	139±5	142±13
Ba	14.8±2.9	14.2±1.3	13.9±1.4	15.7±1.9

The results are the mean value of three repeated sample analysis.

TABLE V
Metals in TCM Huang Lian Su Determined with ICP-AES (µg/g)

Element	HNO_3+$HClO_4$	HNO_3+H_2O_2	MWO
Zn	164.8±5.7	170.7±4.9	162.7±2.6
Fe	384±30	379±24	385±9
Mn	166.4±5.5	170.1±4.3	165.2±2.3
Mg	1810±74	1847±20	1761±55
Ca	2296±114	2321±57	2323±42
K	2189±31	2223±36	2297±28
Na	58.4±5.0	56.4±4.3	54.5±0.6
Ba	84.6±6.2	79.8±1.4	80.9±2.5

The results are the mean value of three repeated sample analysis.

The results in Table VI indicate that satisfactory metal extraction efficiency can be obtained for TCM material when strong acids accompanied by oxidants are used for digestion. Despite the shorter digestion time involved in the MWO procedure, its extraction efficiency is as good as the conventional hot plate procedures. The MWO method is advantageous because of its speed, lower reagent consumption, and less potential contamination (4,5).

As Speciation by Solvent Fractionation

Quite often, toxic metals are intentionally included in TCM as part of the active ingredients. In Lushen Wan, for instance, arsenic in the form of realgar, a crystal form of AS_4S_4 or AS_2S_2, is presented as a part of the drug formulation rather than a contaminant. The drug has been used for years in the Chinese community because of its demonstrated effectiveness in treating a swollen throat and other related throat infection diseases. However, the exact function of reaglar in the formulation remains unclear. Chemically, the arsenic in realgar, when mixed with other components in the drug, may very well lead to some other chemical forms through chemical reactions. As is true for many of the other TCM drugs, studies of this type have not been reported for Lushen Wan.

Using the fractionation scheme given in Figure 1, our interest in this preliminary investigation was to find out whether and how much of the As species in the drug can be extracted by water, leading to the release of highly toxic inorganic arsenic ions when taken orally.

Results from the analysis of the fractions obtained by the fractionation scheme are tabulated in Table VII and are graphically illustrated in the pie chart in Figure 2. The data clearly show that the extractable arsenic species accounts for only 6.6% of the total arsenic in the drug. Of the extractable, 0.5% is in the organic phase and only 6.1% is in the toxic inorganic arsenic ions in aqueous phase.

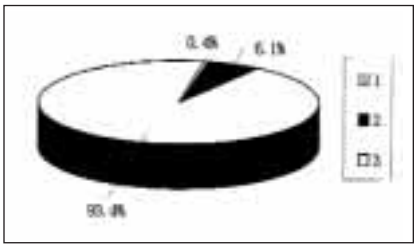

Fig. 2. The fraction of each species of arsenic.
1: Ether-extractable
2: Water-extractable
3: Residue

TABLE VI
Metals in Some TCM Determined with ICP-MS (µg/g)

Element	1	2	3	4	5	6
Fe	19727	2562	ND	1510	456	816
Mo	1520	144	688	197	54.6	15.7
Zn	275	291	ND	75.6	144	251
As	168	541	10.2	0.15	115	9.2
Cu	161	19.5	2.2	9.8	4.4	4.9
Ni	138	24.9	1.86	11.9	4.3	6.4
Pb	47.9	99.5	ND	ND	ND	ND
Cr	40.5	46.8	35.9	12.2	13.5	15.7
Co	3.93	1.69	4.49	0.57	0.70	0.72
Cd	0.90	2.39	10.5	ND	0.05	0.008

The samples were digested with MWO method, the results are the mean value of three replicate analysis.

1 = Lushen Wan made in PR China ND = not detected.
2 = Lushen Wan made in Hong Kong
3 = Pearl 70Wan made in Tibet
4 = Ba Bao JinFen San made in Hong Kong
5 = NiuHuang JieDu Pian made in Beijing
6 = NiuHuang JieDu Wan made in Beijing

TABLE VII
Arsenic Speciations in Lushen Wan (µg/g)[a]

Ether-extractable As (in organic form)	Water-extractable As (in inorganic form)	Residue[b]	Total As
0.499±0.115	7.1±0.12	108.2±17.0	115.8±17.2

[a]The results are the mean values of three repeated analysis.
[b]Arsenic in residue is the differences of total arsenic subtract ether- and water-extractables.

TABLE VIII
Comparison of Analytical Results for the Same Drugs From Different Manufactures (µg/g)

Element	Lu Shen Wan Source A	Lu Shen Wan Source B	Nu Huang Jiedu Wan Source A	Nu Huang Jiedu Wan Source B
Cr	40.5	46.8	13.5	15.8
Fe	19727	2562	456	816
Ni	138	24.9	1.86	11.9
Co	3.93	1.69	0.70	0.71
Cu	161	19.5	4.37	4.86
Zn	276	291	144	251
As	168	541	115	9.2
Mo	1520	143.9	54.6	15.7
Cd	0.9	2.4	0.048	0.008
Pb	47.9	99.5	ND	ND

ND = Not detected in this work.

The As in realgar is known to be more soluble in acidic solutions. In Ref. (6), it was reported that up to 82% of the As in realgar can be readily extracted by artificial stomach fluid. Such extraction was carried out in our study for comparison. The artificial stomach fluid with a pH of 1.3 was prepared by mixing ultrapure HCl in purified water (7). The total extractable As from the artificial stomach fluid so prepared was 39–40%, which is considerably lower than reported for realgar. Thus, although realgar is originally mixed only physically in the drug, a process quite common in the formulation of TCM drugs, such physical mixing processes could actually affect the solubilities of the species significantly. Whether such change in solubility behavior is due to matrix interactions, or chemical transformations during processing, remains unclear. More in-depth investigation of the exact forms of As in the drug and the possible reactions leading to their formation is continuing in our laboratory.

Trace Metals in TCM Drugs

The trace metals in several selected TCM drugs were determined by ICP-MS following MWO extraction using the analytical protocol described. The results of two drugs, each produced from two different manufacturing sources, are listed and compared in Table VIII. The metal concentra-tions in the samples vary widely from sub-µg/g (e.g., Cd) to thousands of µg/g (e.g., Fe). However, the ICP-MS technique is able to perform simultaneous multielemental analysis in one run with high precision (3–10% RSD) because of the wide linear dynamic range of the technique, typically 5 to 6 orders of magnitude. Some of the ultratrace metals such as Cd and Co, with their concentrations in the 0.005- to 5-µg/g range, would be difficult to quantify using convenient sample sizes by ICP-OES, because of its relatively poorer detection limits.

A comparison of the metals in the two pairs of samples shows that the metal contents in the same drug vary widely with their source of manufacturing. It is probable that such variations for some of the toxic metals such As, Cu, Ni, and Cd are due to external contamination during manufacturing or in the raw herbal material. A study of the positive identification of the sources of contamination for metals and pesticides in these TCM drugs through chemometric techniques and ICP-MS isotopic ratio analysis is in progress.

CONCLUSION

Three digestion methods for TCM samples: wet digestion with $HNO_3/HClO_4$, wet digestion with HNO_3/H_2O_2 and MWO digestion with HNO_3/H_2O_2, were found to give comparable metal results by ICP-MS analysis with satisfactory recovery of herbal standards. The MWO digestion method is recommended due to its speed and lower reagent consumption.

The As species in a TCM drug (Lushen Wan) were characterized by a sequential leaching and solvent extraction procedure. The drug contains physically mixed realgar (As_4S_4 and AS_2S_2) as part of the formulation. Aqueous leaching showed that only a small portion (6.1%) of the total As is highly toxic inorganic arsenic ions; and extraction with artificial stomach fluid showed that only about half of the As was in its original realgar form. Detailed chemical speciation is therefore necessary for complex TCM drug products in order to obtain meaningful toxicity information for risk assessment.

The metal content in the same TCM drug varies widely with manufacturers, due likely to external contamination either at the herbal sources or in the processing. Thus, contamination control for raw herbs and during processing is important for TCM manufacturing.

ICP-MS, because of its sensitivity, precision, and wide dynamic range, is a viable tool for the determination of trace toxic elements or metals in TCM. Its high sensitivity offers opportunities for detailed speciation analysis which is urgently needed in TCM development.

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