

Quantitative Determination of Trace Elements in Some Oriental Herb Products

Nguyen Thi Kim Dzung, Pham Ngoc Khai, and Rainer Ludwig

Abstract—The quantitative determination of several trace elements (Cr, As, Se, Cd, Hg, Pb) existing as inorganic impurities in some oriental herb-products such as Lingzhi Mushroom capsules, Philamin powder, etc using ICP-MS has been studied. Various instrumental parameters such as power, gas flow rate, sample depth, as well as the concentration of nitric acid and thick background due to high concentration of possible interferences on the determination of these above-mentioned elements was investigated and the optimum working conditions of the sample measurement on ICP-MS (Agilent-7500a) were reported. Appropriate isotope internal standards were also used to improve the accuracy of mercury determination. Optimal parameters for sampling digestion were also investigated. The recovery of analytical procedure was examined by using a Certified Reference Material (IAEA-CRM 359). The recommended procedure was then applied for the quantitative determination of Cr, As, Se, Cd, Hg, Pb in Lingzhi Mushroom capsule, and Philamine powder samples. The reproducibility of sample measurement (average value between 94 and 102%) and the uncertainty of analytical data (less than 20%) are acceptable.

Keywords—Oriental herbal product, trace elements, ICP-MS, biochemistry, medical chemistry.

I. INTRODUCTION

AS in many other countries of Asia, traditional Vietnamese medicines based on herbal products and extracts play an ever-increasing role among the human community. Lingzhi Mushroom is one of these common herbal products, which was recently produced by DOPHARMA and present in market as capsules, while Philamin was a product of Azolla microphylla, which has been produced under the name of mediphyllamin by MEDIPLATEX. The medicinal herb products have been used for strengthening the body immune system, and are rich source of many essential nutrient elements in bioavailable form [1]. The heavy metal content of medicinal herb products is commonly studied from the viewpoint of toxicity and bioavailability. Heavy metals such as lead, cadmium, mercury, and arsenic might be introduced into herbs in a variety of ways, including contamination during cultivation, processing, and storage [2]. High contents of Cd, Pb and As in herbs may be attributed to the uptake of these elements from polluted soil due

to industrial and anthropogenic activities. In China, it was found that commercial scientific Chinese medicine contains higher trace element concentrations than those of herbs used in the prescription, which may indicate that possible contamination could be caused by unknown ingredients added in the process [3]. In general, all medicinal products for human and animal use must meet regulatory guidelines for quality, safety, and efficacy. The European Union (EU) recently established a Traditional Herbal Medicinal Products Directive for controlling the quality of imported products. As a result, manufacturers who export herbal medicines to the EU must ensure that their product quality complies with requirements of the European Pharmacopoeia. In Vietnam this issue has not been much interested in and there is a lack of national regulatory on quality of traditional herbal products except for only some basic studies on the substance and formula composition.

The investigation of heavy metals in herbal products is widely interested in many countries over the world (China, India, Turkey, Poland, Germany, Australia...), where the herbal products have been used as “scientific medicines”. The organic compositions and accompanied inorganic elements in several herbal products have been well determined using different advanced analytical techniques [4-13]. Among them, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) proved to be the technique of choice to analyze herbal medicines for metals. This technique can simultaneously determine a large number of heavy metals in a wide range of samples [2].

This work was carried out in order to study of the simultaneous determination of trace level of some heavy metals in herbal products by ICP-MS. Isotope internal standards were also used to improve accuracy for determination of mercury concentrations. The analytical procedure was then established and applied for the quantitative determination of Lingzhi Mushroom capsule and Philamin powder samples.

II. EXPERIMENTS

A. Reagents and standard solutions

All chemicals were analytical grade. The experiment was performed at room temperature. Water was purified till the resistant value reached $18 \text{ M}\Omega\cdot\text{cm}^{-1}$ in SG water purification system (German made).

Standard stock solutions of individual element (Cr, As, Se, Cd, Hg, Pb, and others) were specially prepared in HNO_3 for ICP use. Further dilution of these standard solutions is

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necessary before the use in order to obtain the solution of 0.1, 1, 5, 10, 50, 100 ng/ml each element or higher concentration for the mixture of multi-element solution. The final HNO_3 concentration for all solution was fixed at 0.4 M.

B. Apparatus

The Agilent (USA) model 7500a inductively coupled plasma mass spectrometer that was interfaced to an IBM computer system and controlled by Chemstation software was used for the measurement of sample solutions.

The microwave sampling system (Q45 ENVIROPREP), which is equipped with microwave power from 0 to 100% (1000 W) has been used for the decomposition of herb samples. This system has a rotary table, on which a maximum of 12 100-ml vessels can be set. Vessels are made of PFA Teflon and designed to release at 220 psi and 200°C.

C. Sample Preparation

Samples of Lingzhi Mushroom (capsules), Curcumin and Philamin (powder) were obtained from pharmaceutical manufacturers. Accurate weights of sample (0.100 to 0.200 grams) were added in to 100 ml Teflon vessel and moisten with some drops of pure water. Then, the volume of 2 ml of the concentrated HNO_3 and 2 ml of the concentrated H_2O_2 were added. A total 12 samples was placed on the rotary table and the program for the microwave oven was set as listed in Table I.

TABLE I
OPERATING CONDITION OF MICROWAY OVEN

Step	Power (%)	Time (min.)
1	60	3
2	40	3
3	40	3

After being cooled to room temperature, the content in each Teflon vessel was transferred quantitatively into a certain volumetric flask and made up the volume with dilute HNO_3 in order to get the final acid concentration at 0.4 M. The sample solution was filtered through 0.45 μm membrane then take for the measurement on ICP-MS under the optimized conditions (see Table II).

The calibrations of these above mentioned elements were prepared from single solution, and that of multi-element mixed solution for the correction of the interferences.

III. RESULTS AND DISCUSSION

A. Optimization of instrumental parameters for simultaneous determination of trace elements

In order to optimize the instrumental parameters, the mixture of studied elements (Cr, As, Se, Cd, Hg, Pb) with fixed individual concentration of 2 ng/ml (ppb) each element was prepared in 0.4 M HNO_3 and the measurement was carried out

at the certain combination of various parameters on 7500a Agilent ICP-MS.

The optimizing instrumental operating conditions are summarized in Table II and these parameters were fixed for further study and quantitative analysis of samples.

TABLE II
ICP-MS OPERATING CONDITION

ICP-MS System	Optimized parameter
RF Power	1550
RF Matching	1.55 V
Reflected Power	0 W
Sample Uptake Time	30 sec.
Sample Uptake Rate	0.4 r/sec.
Sample Depth	5.0 - 5.5 mm
Coolant Argon Flow Rate	15 l/min.
Carrier Gas Flow Rate	1.2 l/min.
Auxiliary Gas Flow Rate	0.9 l/min.
Water RF/TP Flow Rate	2.4 l/min.
Water RF/TP Temperature	20°C
Peristaltic Pump	0.1 r/sec.
Analyzer Press	3.10^{-4} – 2.10^{-3} Pa

B. Selection of Isotopes for Quantitative Analysis and Detection Limits

The isotope of each element (Cr, As, Se, Cd, Hg, Pb) for quantitative analysis would be generally selected by the typical characters such as no spectral overlapping, higher isotopic abundance, and more ion accounts within an integration time. Each element consists of isotope variety, which is corresponding to the various abundances. Table III shows an example of Chromium isotopes corresponding to their abundances and the isotope index for quantitative analysis. Although the abundance at the position of ^{53}Cr is poorer than at ^{52}Cr , this isotope (53) has been selected as index atomic number because ^{52}Cr isotope is favorable for cooling plasma.

TABLE III
ISOTOPIC ABUNDANCE AND INDEX ATOMIC NUMBER

Cr				
Isotope	50	52	53	54
Abundance	4.345	83.79	9.501	2.365

According to the natural abundance percentage and taking into account of the interference caused by the formation of MO^+ , MOH^+ , double charge ion of the lower atomic number elements on the analyzed element, and from the results of experimental test on 7500a Agilent ICP-MS, the isotopes written in Table IV were found to be sufficient for quantitative analysis. However, when analyzing arsenic with the only atomic number 75 (100% abundance), the interference due to $^{40}\text{Ar}^{35}\text{Cl}$ to As was eliminated by introducing the calculation from a mathematic equation related to the atomic number of 77, which was corrected from the concentration of ^{82}Se .

Limits of detection (LOD) were calculated as 3σ standard deviation of at least seven determinations of a 0.4M HNO_3

blank solution (as ISO 17025 guidance) and data were shown in Table IV. The sensitivity of each element was also calculated as counts per second (cps) of a certain amount of individual element standard solution. The results were also given in Table IV.

This result indicates that trace elements can be simultaneously determined at concentrations about hundreds ppt (pg/g) using ICP-MS and therefore this method is useful for analyzing trace levels of heavy metals in herbal product samples.

C. Linearity and standard calibration

The wide range of linearity is the advantage of ICP-MS, which can be valid from blank value up to hundreds $\mu\text{g/ml}$ (ppm) of each studied element. However, due to the low concentration of the investigated elements in the studied sample, the standard calibration was measured in the range from 0.1 ppb (ng/ml) up to 10 or 20 ppb. The following Figure shows an example of standard calibration curves for quantitative determination of Cd and As.

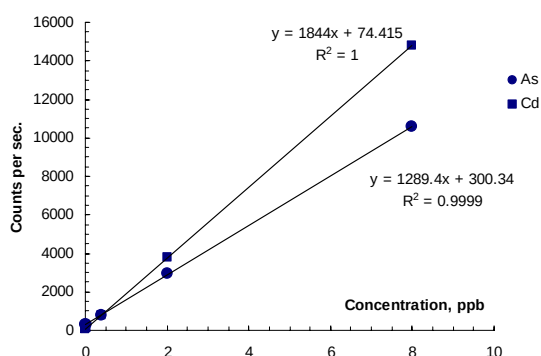


Fig. 1 Standard Calibration Curves for determination of As and Cd

D. Enhancement of accuracy for Hg determination using the internal standard

Mercury is typically difficult element to analyze in solution as it tends to adhere to the surfaces of the sample introduction system and exists at low level in the studied samples. This may cause the low reproducibility of measuring data and result in the large error of the analyzed result. In order to overcome this problem, an internal standard was introduced. According to the general principles for choosing an internal standard to measure Hg, four different elements, which rarely contain in the studied samples, have similar ionization potentials as of Hg, and their isotopes closer to four isotopes of Hg (199, 200, 201 and 202) were proposed. They were Pt, Au, Tl and Bi with their isotope number of 195, 197, 205 and 209, respectively.

The standard calibration curves for Hg were established from the measurement of a set of various Hg concentrations in the respective presence of Pt, Au, Tl and Bi, 10 ppb of each. Fig. 2 shows an example of Hg standard calibration curve using Au as an internal standard.

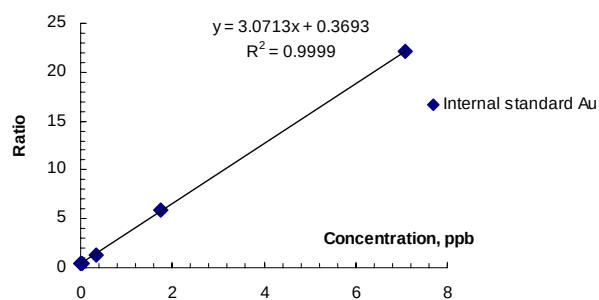


Fig.2 Hg external calibration curve using Au as an internal standard

A synthesized sample consisting of all investigated elements with their concentration similar to those in studied samples, in which Hg concentration was 0.35 ppb was prepared for recovery experiment from external calibration using different internal standards. From the experimental tests the higher recovery of Hg concentration was obtained when ^{195}Pt or ^{197}Au was introduced as the internal standard. The use of 100 ppm Au for stabilizing the measured signal when ^{89}Y was mixed with calibrators and samples was also confirmed by the US researcher [14]. The recovery of Hg determination using conventional external calibration and that of using internal standards was given in Table V.

TABLE V
RECOVERY OF MERCURY DETERMINATION USING INTERNAL STANDARDS

Studied Element	Internal standard	Correlation Factor (r^2)	Recovery (%)	
			Using Internal Standard	Conventional Calibration
Hg	^{195}Pt	0,9998	95.66	84.23
Hg	^{197}Au	0,9999	98.23	

E. Analysis of the Certified Reference Material

Certified Reference Material (IAEA-CRM359) with matrix of cabbage powder was used for quality assurance and quality control (QA/QC). This CRM sample was analyzed using the same method described above with 7 replicates and the result is shown in Table VI. The absolute error shows high accuracy of the method, suggesting that this method can be use as routine analysis of heavy metals in herbal products.

TABLE VI
ANALYSIS OF THE CERTIFIED REFERENCE MATERIAL (IAEA-CRM359)

Element	Found concentration ($\mu\text{g/g}$)	Certified concentration ($\mu\text{g/g}$)	Relative Error (%)	Absolute Error ($\mu\text{g/g}$)
Cr	1.219 ± 0.082	1.30	6.75	-0.0810
As	0.102 ± 0.011	0.1	10.64	0.0020
Se	0.119 ± 0.020	0.12	16.50	-0.0010
Cd	0.118 ± 0.021	0.12	17.28	-0.0020
Hg	0.0125 ± 0.0014	0.013	11.50	-0.0005
Pb	0.684 ± 0.018	nd	2.62	

F. Analysis of herbal product samples

The recommended procedure has been applied for the determination of Cr, As, Se, Cd, Hg, Pb in some samples. Samples of Lingzhi Mushroom (powder contained in capsule) and Philamin powder were digested in Microwave Oven as mentioned in experimental part. The samples solutions were injected into the nebulizer of ICP-MS under the optimum operating condition and the results were given in Table VII.

TABLE VII
ANALYSIS OF SOME HERBAL PRODUCT SAMPLES

Sample name	Content (µg/g or ppm)					
	Cr	As	Se	Cd	Hg	Pb
LingZhi 1	0.821	0.211	0.137	0.058	0.065	1.422
LingZhi 2	1.172	0.173	0.038	0.084	0.044	4.226
LingZhi 3	0.980	0.502	0.158	0.102	0.050	4.487
LingZhi 4	0.886	0.255	0.149	0.050	0.044	5.489
LingZhi 5	0.430	0.190	0.130	0.016	0.062	1.108
Philamin 1	0.804	0.396	1.168	0.252	0.047	3.351
Philamin 2	0.942	0.720	0.153	0.055	0.024	1.833
Philamin 3	5.042	15.162	0.446	0.108	0.062	2.68
Philamin 4	3.808	10.563	0.343	0.083	0.508	2.383
Philamin 5	0.934	0.347	0.306	0.051	0.085	6.242
Limited range for safety*	-	0.100	-	0.300	0.050	10.00

(*Given by WHO for herbal medicines [15] or by Standards applied for Vietnamese Food Additives, No. 10/2006/TCCS-TP).

In general the content of heavy metal impurities in these samples meets the requirement of safety limit, except for arsenic content in some samples. The samples with higher content of arsenic are raw materials (LingZhi 3, Philamin 3 and Philamin 4), and the others are treated. However, the further purification is necessary for these products before sending them to the market as commercial products due to the safety reason for consumers.

IV. CONCLUSION

ICP-MS was proved to be the powerful tool for trace element analysis. Our study on simultaneous determination of Cr, As, Se, Cd, Hg and Pb trace in some herbal products (Lingzhi Mushroom capsules and Philamin powder) provided a sensitive and reliable method to control the quality of herbal products, which were so called food additives in Vietnam and became more common among the human community. The efficacy of using different internal standard for the enhancement of accuracy to determine mercury by ICP-MS has also been demonstrated. The recommended procedure was successfully applied for routine analysis of Cr, As, Se, Cd, Hg and Pb in several herbal products with high precision and accuracy. The study would contribute to the quality control (QC) system of processing the herbal products in Vietnam.

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REFERENCES

- [1] Garg A., Kumar A., Nair A., Reddy A., "Analysis of some Indian medicinal herbs by INAA" *J. Radioanal. and Nucl. Chem.*, Vol. **271**, No 3, Mar. 2007, pp. 611-619 (9).
- [2] U.Bober, D.Chen, "A powerful tool for analysis of heavy metals in traditional Chinese medicines", *Agilent ePharmaceutical Analysis*, Issue 15, 2005.
- [3] Chu-Fang Wang, Ming-Jenq Duo, E.E.Chang and Jenq Yann Yang, "Essential and toxic trace elements in the Chinese medicine". *J.Radioanal. and Nucl. Chem.*, Vol. **211**, No 2 Dec. 1996. pp. 333-347.
- [4] S.Başgel and S.B. Erdemoğlu, "Determination of mineral and trace elements in some medicinal herbs and their infusions consumed in Turkey". *Sci. of the total Environ*, Vol. **359**, Issue 1-3, 15 Apr. 2006, pp 82-89.
- [5] Han-wen Sun, Feng-xia Qiao, Ran Suo, Li-xin Li and Shu-xuan Liang, "Simultaneous determination of trace arsenic(III), antimony(III), total arsenic and antimony in Chinese medicinal herbs by hydride generation-double channel atomic fluorescence spectrometry", *Analytica Chimica Acta*, Vol. **505**, Issue 2, 10 Mar. 2004, pp 255-261.
- [6] Tianlong Deng, Mengxia Liao, Minru Jia, "Arsenic Speciation in Chinese Herb *Ligusticum chuanxiong* Hort by Hydride Generation Atomic Fluorescence Spectrometry", *Spectroscopy Letters*, Vol. **38**, Issue 2 Mar. 2005, pp 109 – 119.
- [7] N. Efe, H. Yilmaz, A. Zararsiz and P. Arikan "Application of calibration standardization method to the analysis of diuretic pharmaceutical herbs", *J.Radioanal. and Nucl. Chem.*, Vol. **238**, No 1-2 Dec. 1998, pp 97-99.
- [8] Musa Ozcan, "Determination of Mineral Contents of Turkish Herbal Tea (*Salvia aucheri* var. *canescens*) at Different Infusion Periods", *Journal of Medicinal Food*, 2005, **8**(1) pp 110-112.
- [9] Silvia Arce, Soledad Cerutti, Roberto Olsina, María R. Gomez, Luis D. Martinez, "Determination of Metal Content in Valerian Root Phytopharmaceutical Derivatives by Atomic Spectrometry" *Journal of AOAC international*, January 2005, Vol. **88**, Issue: 1, pp 221-225.
- [10] Nazim Sekeroglu, Faruk Ozkutlu, S. Metin Kara, Mensure Ozguven, "Determination of cadmium and selected micronutrients in commonly used and traded medicinal plants in Turkey", *Journal of the Science of Food and Agriculture* (Research Article).
- [11] I. Queralt, M. Ovejero, M.L. Carvalho, A.F. Marques, J.M.Llabrés, "Quantitative determination of essential and trace element content of medicinal plants and their infusions by XRF and ICP techniques", *X-Ray Spectrometry*, Vol. **34**, Issue 3, pp 213 – 217.
- [12] Kalny P., Fijalek Z., Daszczyk A., Ostapczuk P., "Determination of selected microelements in polish herbs and their infusions", *Sci Total Environ*, 2007, Aug 1;381(1-3):99-104. E-publication 2007 Apr. 30.
- [13] U.Divrikli, N.Horzum, M.Soylak, L.Elci, "Trace heavy metal contents of some spices and herbal plants from western Anatolia. Turkey". *Int. J.of Food Sci. & Technol.*, Vol. **41**, Issue 6 pp. 712-716 (June 2006).
- [14] Agilent "Determination of Heavy Metals in Whole Blood by ICP-MS Printed 3/2001 Publication number 5988-0533EN.
- [15] Quality control methods for medicinal plant materials, World Health Organization, Geneva-1998.