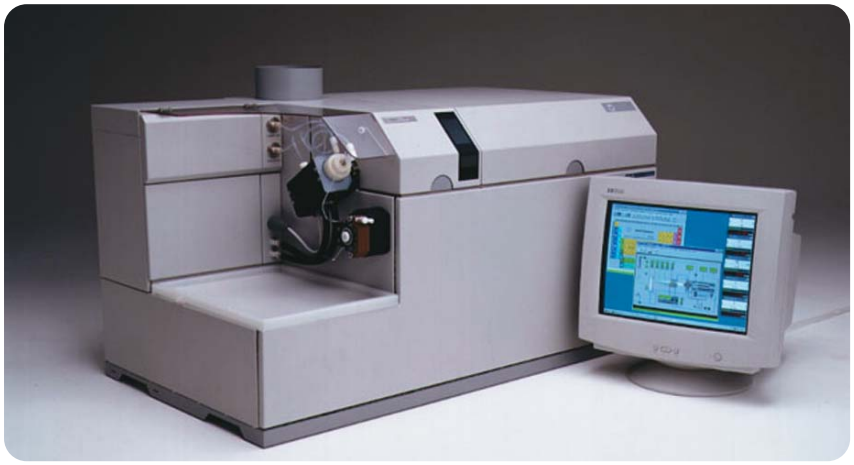
Determination of Toxic Elements in Traditional Chinese Medicine Using Inductively Coupled Plasma Mass Spectrometry

Application Note

Hua Zhang
Yan-zhi Shi
Yu-hong Chen
Ying-feng Wang



Abstract

This Application Note describes a method for the analysis of Be, Cr, Mn, Ni, Cu, Zn, As, Ag, Cd, Ba, Hg, Tl and Pb in Traditional Chinese Medicines using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The samples were dissolved by microwave digestion. To validate the method, two certified reference materials were digested and measured. The method detection limits for all target elements were between 0.1-7.2 $ng \cdot g^{-1}$.

Agilent Technologies

Introduction

Traditional Chinese Medicines (TCMs) and their related products have been widely used in China for centuries. The Chinese government as well as academic research scientists are paying greater attention to the safety issues of TCM in clinical use. This is a key issue which needs to be resolved to improve the development of TCM to meet international standards and advance its worldwide acceptance. With the recent developments in science and technology, people are becoming more aware of the risks associated with heavy metals such as mercury (Hg), arsenic (As), lead (Pb) and cadmium (Cd), which are present in some TCMs. After these elements enter the human body, they will severely damage the hemopoietic, immune, nervous and reproductive systems. Because these substances cannot be completely excreted from the body, they will accumulate and finally have an impact on health. Therefore the analysis of toxic heavy metals is crucial in quality control of TCMs and regulations have been implemented to restrict their levels[1]. The export of TCMs is under intense pressure due to strict regulations, and the high content of heavy metals in traditional Chinese medicine has resulted in international response.

Limiting other toxic or harmful elements, such as beryllium (Be), chromium (Cr), nickel (Ni), silver (Ag), barium (Ba) and thallium (Tl), have not been clearly specified in current hygiene rules and regulations yet. However, with the improvement of living standards and a desire for good health, people now care more about whether these elements offer advantages or disadvantages for their wellbeing. Therefore, strict control of the content of these elements in TCM is required.

In addition, manganese (Mn) is good for the human endocrine, nervous, and enzyme systems and is the key element of most enzymes as well as supporting normal myocardial metabolism. Zinc is good for the immune system. Lack of zinc (Zn) will harm normal cell metabolism and lack of copper (Cu) is one of the reasons for coronary heart disease[2]. However, if the level of these elements is too high, they will place human health at risk.

By nature TCM is very complex and contains many elements which have different concentration levels – high and low. In the past, they have been analyzed by a combination of ASS, AFS and AES technology. These procedures are time-consuming and costly[3]. ICP-MS is fast becoming the technique of choice for the determination of elements in a wide range of samples. Additionally, it has the widest linear range (nine orders of magnitude, from 1 $ng \cdot mL^{-1}$ to 1000 $\mu g \cdot mL^{-1}$), the highest sensitivity and lowest detection limit for metals, as well as the ability to rapidly and accurately measure multiple elements simultaneously. The latest revision of the Pharmacopoeia of the People's Republic of China (Chinese Pharmacopoeia 2005) now includes ICP-MS as one of the standard methods for the determination of heavy metals in herbal medicines[4].

In this study, microwave digestion is used for sample preparation. High temperature and a closed system assure fast and complete digestion and avoid the loss of those elements which can easily evaporate, such as arsenic and mercury. Thirteen toxic elements including Be, Cr, Mn, Ni, Cu, Zn, As, Ag, Cd, Ba, Hg, Tl and Pb were analyzed using the microwave digestion-ICP-MS method. The method is validated by using two certified reference materials: shrub leaves and tea leaves. The results obtained agree with the certified values. The recovery experiment of standard addition was applied in the study to evaluate the proposed method. The recovery percentages are between 90.3 -109.7 %.

Experimental

Instrumentation

An Agilent 7500 Series c ICP-MS was used in this study (table 1). The samples were prepared by microwave digestion with a CEM

Nebulizer:	Babington nebulizer
Spray chamber:	Quartz scott-type, Peltier-thermostatted to 2 ± 0.1 °C
Torch:	Quartz, 2.5 mm ID
Interface:	Ni cone

Table 1
Agilent 7500c ICP-MS instrument details.

MARS5 microwave digestion system (CEM Co. Ltd, USA), including microwave oven, PTFE-TFE high pressure vessel and fixed tray. Deionized water (Milli-Q ultrapure water system) was used.

Reagents

Nitric acid (HNO_3), trace metal grade (Merck); hydrogen peroxide (H_2O_2), MOS grade;standard stock solution: 10 µg·mL^{-1} mixed standard solution including Be, Cr, Mn, Ni, Cu, Zn, As, Ag, Cd, Ba, Hg, Tl and Pb. Calibration standards were prepared by using applicable dilutions of standard stock solution with 5 % HNO_3; Hg stock solution: 1000 µg·mL^{-1} (National Analytical Center for Iron & Steel, China). Calibration standards were prepared by using applicable dilutions of standard stock solution with 5 % HNO_3, internal standard solution: 1.0 µg·mL^{-1}. Diluted from 10 µg·mL^{-1} Li, Sc, Ge, Y, Tb and Bi mixed standard solution. (Agilent part number 5183-4680) with 5 % HNO_3, Tune solution: 10 ng·mL^{-1} ^{7}Li, ^{59}Co, ^{89}Y, ^{140}Ce and ^{205}Tl mixed standard solution (2 % HNO_3) (Agilent part number. 5184-3566). Deionized water (18.2 MΩ) produced with the Milli-Q® ultrapure water purification system (Milllipore Corp.) was used in all standard solution and sample preparations. Certified reference materials: GBW07602 (shrub leaves) and GBW08513 (tea leaves).

Sample pretreatment

Exactly 0.5000 g of sample were weighed and placed into the PTFE vessel. 6 mL HNO_3 and 1 mL H_2O_2 were added. Because of the large organic content of TCM and the large amount of sample, pre-digestion is recommended. To prevent sample loss due to an explosion caused by gas and pressure build-up produced in the digestion process, the sample was dissolved in the solution and placed in the microwave oven. First, the temperature was raised and set at 120 °C for approximately 5 minutes, then the sample was cooled completely and the pressure was allowed to drop to normal. Subsequently, the digestion program specified in table 2 was followed. After digestion the vessel was cooled to room temperature and the contents poured into a 50-mL PET bottle. The vessel and cap were washed with a small amount of distilled water several times and this mixture was added to the PET bottle. Water was added to bring the exact weight to 50.00 g. The procedure for reagent blanks is identical to that for test samples and is carried out concurrently without a sample.

ICP-MS parameters and target element isotopes

The sample solution was analyzed under the optimized condition. The target element isotopes are summarized in table 3. ^{72}Ge was selected as the internal standard element for Be, Cr, Mn, Ni, Cu, Zn and As. ^{115}In was selected as the internal standard element for Ag, Cd and Ba and ^{209}Bi was selected as the internal standard element for Hg, Tl and Pb. ICP-MS parameters were automatically optimized by the instrument. All of the specifications meet the installation requirements including sensitivity, background, oxide, doubly charge, stability, etc. The parameters are listed in table 4.

Stage	Power		Ramp (min)	T (°C)	Hold (min)
	Max (W)	%			
1	1200	100	5:00	120	5:00
2	1200	100	6:00	180	20:00

Table 2
Microwave digestion program.

Parameter	Set value
Power	1350 W
Flow rate of plasma gas	15.0 L·min^{-1}
Flow rate of auxiliary gas	1.0 L·min^{-1}
Flow rate of carrier gas	1.12 L·min^{-1}
Sampling rate	0.4 mL·min^{-1}
Sampling depth	7 mm
Orifice of sampling cone	1.0 mm
Orifice of skimmer cone	0.4 mm
Data acquisition mode	Quantitative analysis
Integration time	0.3 s /isotope
Cerium oxide/Cerium	<0.5 %
Doubly charge	<2 %

Table 4
ICP-MS operating parameters.

Element	Be	Cr	Mn	Ni	Cu	Zn	As	Ag	Cd	Ba	Hg	Tl	Pb
Isotope	9	52	55	60	63	66	75	107	114	137	202	205	208

Table 3
Target element isotopes.

Calibration curves

The mixed stock solution and Hg stock solution were diluted to 0.1, 0.5, 2,10 ng·mL^{-1} using 5 % HNO_3 respectively. The blank solution is 5 % HNO_3. The blank and calibration solutions were measured under optimized conditions. The calibration curve was automatically plotted by the instrument. Linear correlation coefficients (r) in all calibration curves were better than 0.9999.

Results and discussion

Method detection limit

The blank sample was analyzed 11 times under optimized conditions. The method detection limits (MDL) for each element were calculated (table 5).

Element	MDL (ng·g^{-1})
Be	0.1
Cr	7.2
Mn	1.1
Ni	1.9
Cu	2.5
Zn	3.4
As	3.5
Ag	0.6
Cd	0.8
Ba	2.4
Hg	1.1
Tl	0.1
Pb	0.9

Table 5
Method detection limits (MDL).

Determination of standard materials

To evaluate reliability and accuracy, the microwave digestion ICP-MS method was applied to the determination of two certified reference materials: GBW07602 (shrub leaves) and GBW08513 (tea leaves). The results are in strong agreement with the certified values, a comparison is shown in table 6.

Recovery

The percent recovery for each element was determined using the standard addition method to evaluate the reliability and accuracy of the method. Percent recoveries of all elements were between 90.3 %-and 109.7 %. The results were considered satisfactory (table 7).

Sample analysis

This study analyzed 13 toxic elements in seven TCMs purchased on the market. Each sample was analyzed eight times and the accuracy (RSD %) varied between 0.3 % and 6.8 % (table 8). According to the results in Table 8, not all TCMs meet the minimum legal requirements. The concentrations of some elements highly exceed the limit allowed. However, in most TCMs the amount of all toxic elements is low.

Element	GBW07602 (shrub leaves)		GBW08513 (tea leaves)	
	Certified value µg·g^{-1}	Found value µg·g^{-1}	Certified value µg·g^{-1}	Found value µg·g^{-1}
Be	0.056 ± 0.014	0.046	/	0.088
Cr	2.3 ± 0.3	2.000	/	2.200
Mn	58 ± 6	54.000	2170 ± 110	2074.000
Ni	1.7 ± 0.4	1.700	5.09 ± 0.76	4.940
Cu	5.2 ± 0.5	4.600	8.96 ± 0.59	8.230
Zn	20.6 ± 2.2	19.800	22.6 ± 1.5	22.400
As	0.95 ± 0.12	0.790	0.180 ± 0.049	0.134
Ag	0.027 ± 0.006	0.024	/	0.022
Cd	0.14 ± 0.06	0.180	0.023 ± 0.004	0.022
Ba	19 ± 3	17.000	120 ± 10	112.000
Hg	/	42.500	0.017	0.018
Tl	/	0.015	/	0.016
Pb	7.1 + 1.1	6.100	1.00 ± 0.05	0.910

Table 6
Comparison of found value and certified value.

Element	Spiked value (ng·mL^{-1})	Found value (ng·mL^{-1})	Recovery percentage (%)
Be	10	9.37	93.7
Cr	10	10.63	106.3
Mn	10	10.97	109.7
Ni	10	10.24	102.4
Cu	10	10.68	106.8
Zn	10	10.36	103.6
As	10	10.89	108.9
Ag	10	9.20	92.0
Cd	10	9.25	92.5
Ba	10	9.16	91.6
Hg	5	4.87	97.3
Tl	10	9.58	95.8
Pb	10	9.03	90.3

Table 7
Results of the recovery experiments.

Name of sample / Element		Gegen Soup	Zhike San	Guifudihuang Pill	Huanglianshangqing Pill	Jinsangsanjie Pill	Naodesheng Pill	Shugan Pill
Be	Found value	0.020	0.009	0.032	0.034	0.060	0.037	0.013
	RSD%	5.2	2.6	1.0	5.1	2.0	1.4	5.2
Cr	Found value	0.34	0.46	1.40	1.58	4.89	1.74	4.76
	RSD%	2.0	2.0	1.9	2.6	2.3	2.5	0.8
Mn	Found value	34.77	17.80	54.78	30.37	87.23	23.96	124.4
	RSD%	0.7	2.4	1.1	2.20	1.21	2.62	3.10
Ni	Found value	1.09	1.17	1.24	1.64	3.09	1.50	3.15
	RSD%	0.6	0.9	1.4	0.8	2.2	1.8	1.8
Cu	Found value	1.49	1.68	4.08	8.85	15.23	3.56	5.38
	RSD%	0.6	0.6	0.6	2.5	2.3	2.38	0.46
Zn	Found value	5.66	4.82	21.27	18.57	37.07	15.33	22.59
	RSD%	0.6	1.1	2.4	1.3	1.5	1.7	1.7
As	Found value	N.D.	0.26	0.46	0.79	0.89	0.56	29.14
	RSD%	1.6	1.4	3.9	3.5	5.7	3.3	1.5
Ag	Found value	0.0009	0.0008	0.005	0.007	0.079	0.006	0.007
	RSD%	5.3	6.3	2.1	5.8	3.5	5.6	4.3
Cd	Found value	0.022	0.042	0.10	0.11	0.15	0.077	0.082
	RSD%	5.5	1.5	1.2	2.7	1.9	3.6	2.0
Ba	Found value	5.86	8.94	15.32	44.76	167.8	136.7	12.98
	RSD%	1.1	1.7	1.4	2.1	2.8	0.3	0.9
Hg	Found value	0.003	0.002	0.85	0.088	0.027	0.076	5.05
	RSD%	6.8	5.8	3.3	5.5	5.0	3.5	0.6
Tl	Found value	0.008	0.027	0.025	0.020	0.033	0.036	0.014
	RSD%	1.3	2.4	2.7	3.6	3.2	1.1	4.1
Pb	Found value	0.15	0.16	0.93	1.54	2.14	0.68	1.69
	RSD%	0.7	1.1	2.6	2.1	1.7	1.5	1.3

Table 8
Analytical results of TCMs (n = 8). Unit: μg g^{-1}.

Conclusion

The evidence suggests that the current TCM market is complex and strict management and quality controls are needed to ensure that the maximum allowable limits of toxic elements set by regulations are not exceeded. National and local drug administrations also require regulations to manage and control the production and sale of TCMs. The study uses microwave digestion for sample preparation, With optimized working parameters all elements in different medicines are analyzed simultaneously with an Agilent 7500 Series c ICP-MS. This method is validated with certified reference materials by conducting recovery experiments using known standard additions. It offers many advantages over other alternative techniques, such as precision and accuracy, simplicity, rapidity, low limits of detection and multiple element determination.

References

1.
Wang Xiao-ru, "Application of Inductively Coupled Plasma Mass Spectrometry." *Chemical Industry Press. Bei Jing.*, *102*, **2005.**

2.
Dong Hong-bo, Han Li-qing, Dong Shun-fu, "Instrumentation & Analysis Monitoring" *(3): 29~30*, **2002.**

3.
Yu Zi-li, Guang Lei, "Analytical Technique of Metal Ion". *Chemical Industry Press. Bei Jing*, *225~226*, **2004.**

4.
Determination of Lead (Pb), Cadmium (Cd), Arsenic (As), Mercury (Hg) and Copper (Cu), Ch. P 2005 (English version), Appendix IX B, p. 54-56.

Hua Zhang is apostgraduate student in the chemistry department of the Capital Normal University, Yan-zhi Shi and Ying-feng Wang are professors at the Analytical Center of the Capital Normal University, Beijing, China. Yu-hong Chen is Application Engineer at Agilent Technologies Co. Ltd, China.

www.agilent.com/chem

© Agilent Technologies, 2006

Published August 1, 2006
Publication Number 5989-5591EN

Agilent Technologies