

Full Length Research Paper

Determination of trace elements of *Melicope patulinervia* from different part by microwave digestion-inductively coupled plasma-mass spectrometry (ICP-MS)

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This paper is to determine the contents of zinc (Zn), manganese (Mn), iron (Fe), copper (Cu), chromium (Cr), Arsenic (As), cadmium (Cd), lead (Pb) in the stems, leaves and the flower of *Melicope patulinervia* simultaneously by microwave-digestion and inductively coupled plasma-mass spectrometry (ICP-MS). The average sample-added recovery rate is between 98.14 - 103.37% and its relative standard deviation (RSD) is between 1.34- 2.69%. The study shows that this method is simple, fast, sensitive and it is suitable for the simultaneous determination of the trace elements and the heavy metals in *M. patulinervia*. The experiment results indicate that in every part of *M. patulinervia* the contents of trace elements Mn, Fe, Zn which are beneficial to the human body are all very high. Among its heavy metals which are harmful to the human body, the contents of heavy metals Cu, Cr, As, Cd in every part of the plant are all lower than the standard amount restricted in Chinese pharmacopoeia and the import and export Green trade standard of the medicinal plants and their products, while the content of Pb is a little higher. This experiment result provides the scientific data for further study of the relationship between the content of the trace elements, the heavy metals and their medical therapy, and it is also useful to development and application of this natural medicinal resource.

Key words: Microwave digestion, inductively coupled plasma-mass spectrometry (ICP-MS), *Melicope patulinervia*, trace elements, heavy metals.

INTRODUCTION

Trace elements are the important part of the material basis of medical effects (Guo et al., 2005). Through many years of systematic research for the physiological and pharmacological activities, we find that the medical effect of the Chinese medicine is not only related with its organic matters but also with the content of trace elements (Gong et al., 2002). Therefore, with further research into the chemical components of the Chinese

medicine, the research of the inorganic matters especially the trace elements and the heavy metals is being considered more and more important (Zhang et al., 2005; Wang et al., 2004). Heavy metal pollution is an important factor in the present environmental deterioration. The heavy metals can be absorbed by the medicinal plants and into our human bodies, which can cause great harm. So it is very important for us to determine the content of these heavy metals in the Chinese medicine. As one of the most important quality safety checking items of the Chinese medicine, the analysis of the heavy metals and some beneficial trace elements has become the important prescript in the international common statute. *Melicope patulinervia* (Merr. and Chun) Huang, shrub or arbor species, belongs to the Rutaceae family. There are about 50 types, mainly on the Pacific Islands and Australia. There is very rare in Chinese mainland with

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Abbreviations: Zn, zinc; Mn, manganese; Fe, iron; Cu, copper; Cr, chromium; As, arsenic; Cd, cadmium; Pb, lead; RSD, relative standard deviation; ICP-MS, inductively coupled plasma-mass spectrometry; PTFE, polytetrafluoroethylene; HNO₃/H₂SO₄, nitric/sulphuric acids;

Table 1. Optimized parameters of ICP-MS system.

ICP-MS (ICP system) parameters	Measure parameters	Mass Spectrum (MS system) parameters
Power/W: 1200	Resolution(peak high)/amu: 0.02	Vacuum of analytical room / mbar: $<1 \times 10^{-6}$
Flow of cooling air /(L•min ⁻¹): 13.02	Scanning mode: jumping peak	Analytical time / s: 20
Flow of assisting air /(L•min ⁻¹): 0.70	Scanning time: 100	Sampling depth/mm: 7.8
Flow of vaporized air /(L•min ⁻¹): 0.84	Staying time/(μs): 10000	Pulse voltage/V: 3200
Promoting rate of sample /(mL•min ⁻¹): 1.0	Time of sampling collection /s: 49	
Temperature of atomization room /°C:2	Time of sample-feeding /s: 45	

one in Taiwan and another in Hainan Island and both grow in the open forests of the sloping fields with the altitude of 900~1200 m. "Flora of China" classifies *M. patulinervia* (Merr. and Chun) Huang as the "endan-gered species". It is reported in the literature that the the chemical compositions contained in this herb mainly are flavonoids, alkaloids and terpenoids, all of which have the functions of antioxidation, insecticidal bacteriostasis, heat-clearing and detoxification, dampness-eliminating and anti-itching. It is really a popular herb used in treating sore throat, hot swollen boils, rheumatism and numbness, bone pain and all kinds of traumatic injuries. Surgically, it is used in treating skin sores and rashes, itchy skins and piles (Guangdong institute of botany, 1977). There are very few researches on the plant of *M. patulinervia* (Merr. and Chun) Huang at home or abroad. Through medical literature searching, no report has ever been made about the study on the trace elements and heavy metals contained in *M. patulinervia* (Merr. and Chun) Huang in Hainan Island, China.

At present, the main methods used in determining the trace elements and the heavy metals in the Chinese medicines are atomic absorption spectrometry (Liang et al., 2007; Zhang et al., 2007; Zheng et al., 2006; Yin et al., 2005; Yi et al., 2004; Shen et al., 2006), inductively coupled plasma atomic emission spectrometry (Kou et al., 2007; Chen et al., 2005; Deng et al., 2005; Zhang et al., 2004), inductively coupled plasma-mass spectrometry (ICP-MS) (Wu et al., 2005; Shi et al., 2007; Li et al., 2008; Cheng et al., 2008) and atomic fluorescent spectroscopy (Chen, 2003; Kou et al., 2009), etc. ICP-MS technique was developed in the 1990s, and now one of the latest kinds of instruments was used in detecting and analyzing inorganic trace and micro elements. Compared with the other methods, it can almost instantly detect all the chemical elements with high sensibility and accuracy, having the characteristic of good selectivity, low detection limit, wide linear dynamic range, etc. These advantages enable it to play a very important role in the fields of biology, food, environment and medicine. Microwave digesting technique is an advanced and efficient sample digesting technology developed recently, with the merits of fast digestion, complete separation, no volatile loss of elements, and little pollution. For these advantages, it has been widely used in the sample analysis in all fields.

In order to explore the distribution of the trace elements and the heavy metals in different parts of *M. patulinervia*, provide scientific data for its quality evaluation, in this paper, the contents of zinc (Zn), manganese (Mn), iron (Fe), copper (Cu), chromium (Cr), arsenic (As), cadmium (Cd), lead (Pb) in different parts of *M. patulinervia* were determined simultaneously by microwave digestion (Zhou et al., 2008; Xu et al., 2008) and ICP-MS. It is helpful to further study of the relationship between the content of the trace elements, the heavy metals and their medical therapy, and to further development and application of this natural medicinal resource.

MATERIALS AND METHODS

Instrument and working parameters

X-series ICP-MS (American Thermoelectricity Company); MARS-5 microwave digesting equipment (America CEM company), RTP-300 plus temperature controlling device; Milli-Q academic super purified water treatment device (American Millipore Company); Nitric acid is excellent purification grade; all the others reagent are analytical purity. The instrument working parameters are presented in Table 1.

Sources of samples

The stems, leaves and flower of *M. patulinervia* in this experiment were collected in Sanya city, Hainan province, PR China, in October, 2008 and identified by botanist professor Shi-man Huang of Hainan University. The samples were washed once by double boiled water, dried up and then put in the oven with 60°C for 1 h. After completely dried, they were taken out and ground to powder by agate mortar. Later, the powder is screened through the 60-hole sieve for later use.

Standard solution

Zn, Mn, Fe, Cu, Cr, As, Cd, Pb standard storing solution (American SPEX CertiPrep Inc.), with its concentration of 10 mg • L⁻¹, and store them respectively in polythene plastic bottles. Then according to the needs of determination, confect them into mixed standard solution with suitable concentration (containing 1% of nitric acid).

Microwave digestion of samples

Take the samples of leaves, stems and the whole plant of *M. patulinervia*, respectively and dry them under the infrared lamp to constant weight. Precisely weigh each sample powder for 0.1 g (precision to 0.0001 g). Put these powders into the inner pot of

Table 2. Procedure of microwave digestion.

The digestion procedure	Power (W)	Temperature rising time (min)	Controlled pressure (kPa)	Temperature (°C)	Time duration (min)
1	1200	5	340	120	2
2	1200	3	689	150	3
3	1200	3	1000	180	6

microwave digestion made of polytetrafluoroethylene (PTFE) and add 5 mL thick nitric acid, then give it a shake, finally keep it static for half an hour. After this, put them into the microwave digesting device to digest according to the procedures listed in Table 2. After digesting, cool them to room temperature. Take out the inner pot and transfer the digesting solution to another bottle of 50 mL. At the same time, prepare a bottle of blank solution for contrast.

RESULTS

The optimizing of the digesting conditions

Pressure, temperature and time are the most important parameters in the process of sample microwave digesting. Within the safety limits of digesting, we choose the best digesting pressure, temperature and the warming time inside the digesting pot. It is found that by using the first two procedures in the Table 2 to digest, although the trace elements in the samples have been completely melted out, there is a small amount of dregs left in the solution and the digesting solution is muddy. Meanwhile, by adopting the third procedure with gradual rising of the temperature and pressure in Table 2, we get the transparent digesting solution without dregs, which only takes about 11 min during the whole microwave process. Two systems were tried in this experiment to digest sample: nitric acid and sulphuric acid (HNO_3 and $\text{HNO}_3/\text{H}_2\text{SO}_4$). It is clear that HNO_3 system has the best effect while $\text{HNO}_3/\text{H}_2\text{SO}_4$ can cause carbonization of the sample easily. As for the volatile elements like As and Pb, we must strictly control the temperature during the process of sample digesting. The experiment proved that the temperature should be at about 150°C , which is just near the boiling state of the solution and the boiling point of HNO_3 . At the same time, in order to prevent carbonization, the nitric acid should be added dropwise. And pay attention to control the temperature after adding the nitric acid to keep the solution at the boiling point, thus try to ensure minimum loss of the elements in the samples.

Linear range of the method

Take out part of storing solution from the standard storing solution, and then dilute it by high purified water into the standard solution by the method of gradual diluting. The concentrations of the eight elements Zn, Mn, Fe, Cu, Cr,

As, Cd, Pb in this experiment are respectively 2.0、5.0、10.0、20.0 $\mu\text{g} \cdot \text{L}^{-1}$ and the standard solution contains 1% of HNO_3 . Then add the inner standard solution, respectively and determine them by ICP-MS method under the chosen optimizing condition. After the standard solution flows into the ICP-MS device, the device shows the working curves and its linear relationship of each element. The linear regression chart can be seen in Figure 1, the linear regression equation, linear correlation coefficient, linear range and detection limits of these eight elements can be seen in Table 3.

The accuracy experiment of the method

In order to evaluate the accuracy of this method, we adopted the standard adding method to determine the recovery rate of each element. Take the leaves of *M. patulinervia* as the example. We repeated the experiment for three times under the chosen condition and determined the average recovery rate and relative standard deviation (RSD) of each element. The experiment results are shown in Table 4. The average recovery rate is between 98.14-103.37% and the RSD is between 1.34-2.69%. This shows that the method used here is reliable, accurate and can be used to determine each element.

Determination results of the sample

Ignite the device, pump the high purified water, examine the blank count of each element, pump into the mixed standard solution of $2 \mu\text{g} \cdot \text{L}^{-1}$ and then check the accuracy of the instrument. Make sure that the above steps are correct and the devices are stable. Then begin to determine according to the working parameter. Pump the digested sample solution into ICP-MS device, respectively, determine the contents of each element, and determine the blank sample solution and its standard solution. The results are shown in Table 4.

DISCUSSION

In the growth of the Chinese medicinal herbs, they will selectively absorb some elements which are essential to the human bodies and are the basis for preventing certain

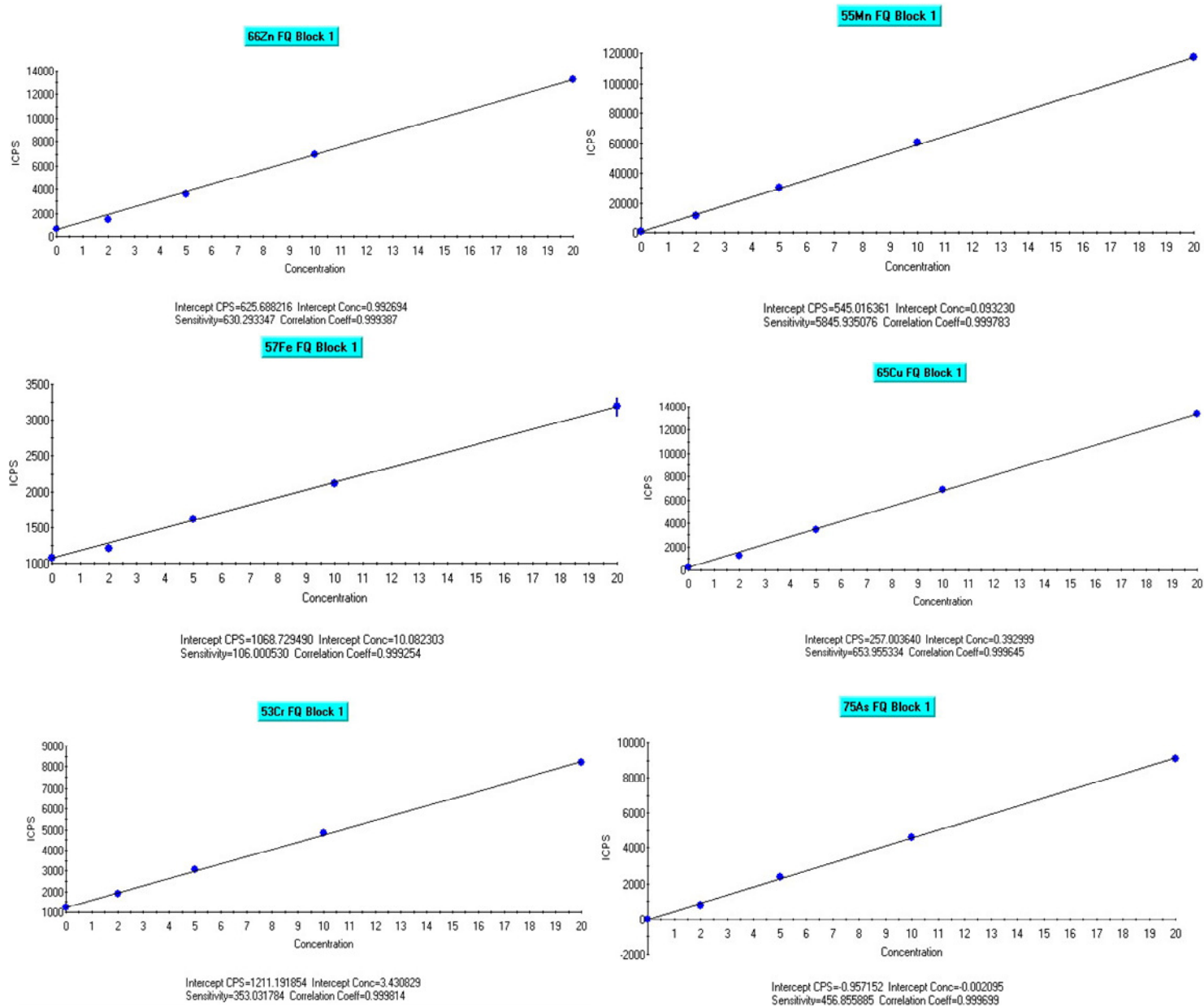


Figure 1. Linear curves of 8 trace elements.

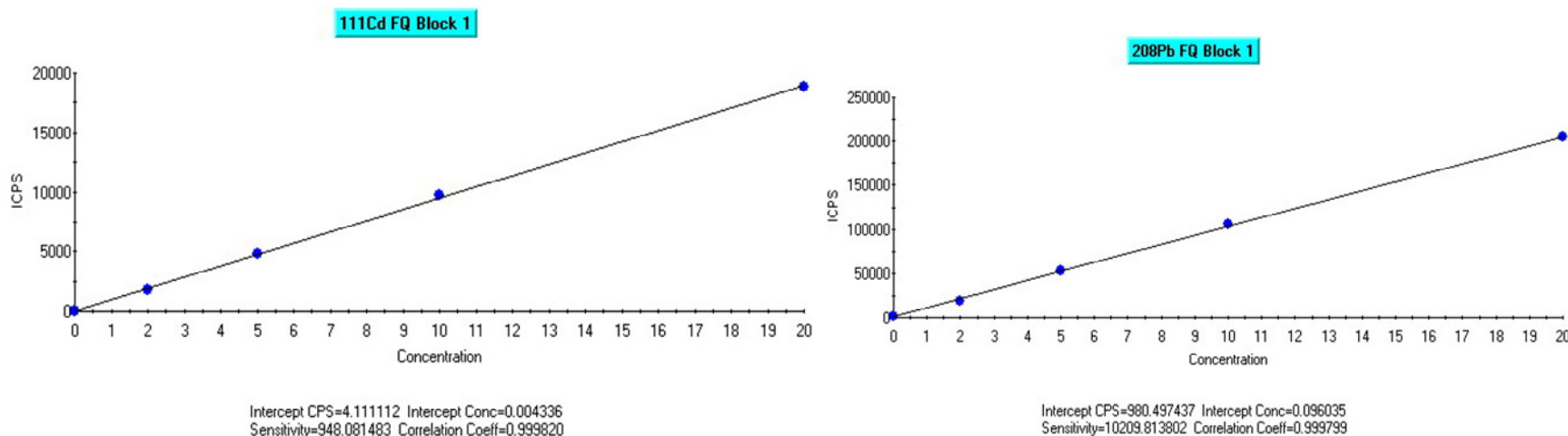


Figure 1. Contd.

diseases. There are some trace elements which are essential to the human bodies like Mn, Fe and Zn in *M. patulinervia*, which have important functions for people's growth, blood-making and immune system. In addition, *M. patulinervia* has some functions of healthcare and nutritious value. The results show that in the different parts of *M. patulinervia*, the content of Mn is the highest, next are Fe and Zn. The content distribution of the three elements in the different parts of *M. patulinervia* is flower>leaf>stem.

These years, people are more and more concerned about the over standard of the heavy metals and harmful elements in Chinese medicinal herbs. The over standard of the heavy metals can not guarantee the quality and the safety of the Chinese medicinal herbs, it will directly influence the safety use of the Chinese traditional patent medicine and will accordingly limit its exportation. In the standardized planting of the Chinese medicinal herbs, we must strictly

control the contents of heavy metals and arsenic salt. Cu, Cr, As, Cd and Pb are harmful elements which can cause progressive poisoning once absorbed by human bodies.

The permitted amount of these heavy metals and arsenic salt should be assessed according to the medical book of Chinese pharmacopoeia and the import and export green vocation standard of the medicinal plants and their preparations. This paper consults the following standards, that is, $Cu \leq 20.0 \mu\text{g} \cdot \text{g}^{-1}$, $Cd \leq 0.3 \mu\text{g} \cdot \text{g}^{-1}$, $Pb \leq 5.0 \mu\text{g} \cdot \text{g}^{-1}$, $As \leq 2.0 \mu\text{g} \cdot \text{g}^{-1}$, $Cr \leq 1.0 \mu\text{g} \cdot \text{g}^{-1}$. This experiment determined the contents of five heavy metals and arsenic salt in the different parts of *M. patulinervia* and found that the contents of Cr, Cd and As were a little lower, while the content of Pb was a little higher in the leaves, reaching $5.86 \mu\text{g} \cdot \text{g}^{-1}$ which approximates the permitted amount of Pb listed in the medicine encyclopedia Chinese pharmacopoeia. The content of the trace element Cu is a little higher, but it does not surpass the

permitted standard. The high content of Pb is perhaps related to its growing environment and the soil condition. The detailed cause needs to be researched further. The experiment results indicate that the contents of the beneficial trace elements Mn, Fe and Zn in every part of *M. patulinervia* are all very high. Among its harmful heavy metals, the contents of heavy metals Cu, Cr, As and Cd in every part of the plant are all lower than the standard amount listed in Chinese pharmacopoeia while the content of Pb is a little higher. In order to meet the standard requirement, we must reduce the content of Pb by changing growing environment, adopting proper process, and so on. This experiment result can provide the scientific data for further study of the relationship between the content of the trace elements, the heavy metals and their different effects. And it can also provide reference for its quality evaluation; promote development and application of the natural resource.

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