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Primary speciation analysis of metal trace elements in *Radix Scutellariae* using inductively coupled plasma-mass spectrometry

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ABSTRACT

Radix Scutellariae from different producing areas were processed by wet digestion, and a total of 28 metal trace elements, which may be essential or harmful to human being, were determined using inductively coupled plasma-mass spectrometry (ICP-MS) firstly. The results indicate that the kind of metal elements in *Radix Scutellariae* from different area were basically the same, but the contents maybe different and the contents of different metal elements change in a wide range with Se 0.1 $\mu g \cdot g^{-1}$~Mg 5277 $\mu g \cdot g^{-1}$. Furthermore, take the leaching rate as index, the primary speciation analysis of metal trace elements in *Radix Scutellariae* were performed. The results show that the leaching rates of different metals are different, *i.e.*, the majority are lower than 30% while several are higher than 85%. The higher or lower leaching rates of different metal elements show that the metal elements can form complexes with other organic active ingredient in *Radix Scutellariae,* and the stabilities of the complex is determined by its solubility and the size of the organic ligand. Among the higher soluble metal elements, they are maybe existed as free ions or binding with lower molecular weight and all of them are formed in small particles, they are easier to pass through the filter membrane and easily to be absorbed. The experimental results can provide theoretical basis for decoction of TCM to a certain extent.

KEYWORDS

Radix Scutellariae;
Inductively coupled plasma-mass spectrometry (ICP-MS);
Metal trace element;
Primary speciation analysis.

INTRODUCTION

The term “trace element” is applied to a group that is extremely small in quantity and plays a vital part in the metabolism of plants and animals[1]. In the human body, the trace element is defined as that which makes up 0.01% of the body’s mass. Each element has made a different contribution in order to make the human body function. Essential trace elements are needed for optimal function of the mammalian organism, for growth, healing and the activity of many metabolic processes[2]. Trace elements have both a curative and a preventative role in combating diseases. However, non-essential metals such as cadmium and chromium lead to adverse effects, even though they are only present in trace amounts. Elements, in one form or another, play an important role in the field of medicine, including the trace elements present in Traditional Chinese Medicine (TCM). TCM has gained people's satisfaction with its therapeutic outcomes[3,4] and there are perceptions that herbal medicines are inherently safe[5]. Despite the claimed and wide belief in TCM as being beneficial, there have been reports of acute and chronic intoxications resulting from its use[6-13]. The consumption of TCM contributes to the intake of both essential and non-essential trace elements by the human body[14]. While many investigations of the quality of TCM have been reported in the current literature[15], there is less emphasis on the trace element content and its speciation analysis of TCM products[16]. The main reason for trace element monitoring is due to an increase in contamination of the general environment[17,18].

It is that te environmental behavior, bioavailability and physiology, toxicology of trace element is bound up with its chemical nature and chemical speciation mode. In the study of the TCM relationship between trace elements and human health and disease, it is not enough that determination of the total trace elements in the TCM, and it is absolutely essential to specify their chemical forms[19-22].

Huang Qin (*Radix Scutellariae*) is a medicinal plant officially listed in the Chinese Pharmacopoeia, and has been widely used in Chinese and Japanese. It is traditionally used for the treatment of various inflammatory diseases, hepatitis, tumours and diarrhea[23]. *Radix Scutellariae* contains four major flavones: wogonin, wogonoside, baicalein and baicalin, which make up about 1.3%, 3.55%, 5.41% and 10.11%, respectively, of the dry material[24]. Baicalein (5,6,7-trihydroxy-2-phenyl-4H-1-benzopyran-4-one) shows a variety of biological activities, including anti-thrombotic[25], antiviral[26], anti-cancer[27], anti-oxidant, antibacterial, antiangiogenic effects[28] and anti-inflammatory[29] activities. For a long time, people only pay more attention to the study of chemical constituents of *Radix Scutellariae*. In recent years, with the deepening of the research on trace elements in TCM, it is found that the contents of trace elements and their forms in TCM are directly related to their pharmacological activities of TCM. Therefore, the study of trace elements in TCM and their forms has attracted more and more concern[30-33], and the efficacy of *radix scutellariae* is studied from the view of trace elements have been reported[34]. Inductively coupled plasma mass spectrometry (ICP-MS), as a method of highly sensitive and simultaneous determination of trace elements in TCM or natural product, is applied more and more extensively[35]. Herbal decoction is the most commonly used forms of administration, in general, the water-soluble metal element with the other ingredients is effective. In order to understand biological utilization of *radix scutellariae* and medicinal mechanism of essential trace elements content and its primary speciation analysis of relevant information, the samples of *radix scutellariae* from different areas were first processed by wet digestion process, the scutellaria decoction process were simulated in this paper, the multiple trace elements content in *radix scutellariae* and water decoction were determined by ICP-MS respectively, and then their dissolution rates can be calculated.

MATERIALS AND METHODS

Instruments and parameters

ELAN 9000 ICP-MS: PerkinElmer, US; Millipore ultrapure water equipment: Shanghai Renhe Scientific Instrument Co., Ltd, China; METTLER electronic balance: Hangzhou Fuda photoelectric technology company, China; Vacuum filtration unit: Tianjin Jinteng Experiment Equipment Co., Ltd, China. Optimization of the ICP-MS instruments operating conditions and parameters are shown in TABLE 1.

TABLE 1 : Working parameters of ICP-MS

Working Parameter	Setting Value
Atomizer Flow/($L \cdot min^{-1}$)	0.85
ICP Gas Flow/($L \cdot min^{-1}$)	14.5
Assistant Gas Flow/($L \cdot min^{-1}$)	1.40
Atomizer Flow/($L \cdot min^{-1}$)	0.92
Lens Voltage/V	6
Scan Mode	Peak Hopping
Stop Time/ms	50
Integration Time /ms	1000
Curve Type	Linear Thru Zero
Data Acquisition Repetitions	3
Detector Model	Dual

Reagents and samples

Concentrated HNO_3: Luoyang Chemical reagent

Co., Ltd., China; H_2O_2: Tianjin Kemiou Chemical Reagent Co., LTD, China; HNO_3 and $HClO_4$ were both in excellent purity. Standard solution of 29 element, Such as Li, Na, K, Mg, Al, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Zr, Mo, Ag, Sn, Ba, W, Hg, Pb, were provided by National Center of Analysis and Testing for Nonferrous Metals and Electronic Materials, and which were diluted to different concentrations of standard step by step. Radix scutellariae originated from Henan province and Gansu province, were purchased in Zhengzhou. All utensils before used in the experiment were immersed in 3% HNO_3 overnight, then cleaned and dried.

Experiment

Determination of metal trace elements in radix scutellariae

Samples of radix scutellariae from different areas were first weighed, cleaned with distilled water and ultrapure water in order, dried in drying oven at 60 ℃for 24 h, and then crushed, sifted (40 mesh), and saved in the dryer before use.

0.3 g prepared sample of radix scutellariae was accurately weighed and put into PTFE beaker, 5mL concentrated HNO_3 was poured in and put overnight, then 5 mL concentrated nitric acid was additional added on the next day. The mixture was heated to boiling and kept it, 2 mL 30% H_2O_2 solution was added slowly drop by drop until the red brown smoke disappeared. The procedure above were repeated until the solution was colorless and transparent or light yellow, then the sample was heated for removing of the acid to nearly dry. Cooled the residue to room temperature and transferred to volumetric flask, then it was adjusted to 50 mL with 3% HNO_3 and mixed for measurement evenly. According to the scan result of semi-quantitative analysis using ICP-MS, the determined 29 metal elements can be divided into three categories of common metal elements, essential trace elements and harmful metal elements. The blank solution, which were used in the determination of metal content, by ICP-MS, can be obtained using the same method above.

Analysis of primary speciation of metal trace elements

25g prepared sample of *radix scutellariae* was accurately weighed and put into 150 mL ultrapure water, heated to be boiling for 0.5h, then cooled and filtrated. The procedure were repeated four times, crude filtrate were gathered and mixed throughly. Filtrated with 0.45 μm filter membrane again and the filtrate was put for use. The filtrate can be divided into crude filtrate and filtrate by 0.45μm filter membrane.

According to the same procedure as 2.3.1, the contents, soluble metal elements, extraction yield of selected 29 metal elements in the crude filtrate and filtrate were respectively determined by ICP-MS. The leaching rate can be calculated by the ratio of the content of metal elements in decoction and total contents of metal elements in samples. Soluble metal elements can be calculated by the ratio of total contents of metal elements in filtrate and in crude filtrate.

RESULTS AND DISCUSSION

Determination of mental trace elements in radix scutellariae from different areas

According to the method of 2.3.1 above mentioned, the contents of mental elements in the radix scutellariae from different areas were determined and the result were shown in TABLE 2. ("/" in TABLE 2 is indetectable).

It can be seen from TABLE 2 that the contents of the metal elements in *radix scutellariae* from two areas are different. The content of 10 elements in sample from Henan province are arranged according to the order from high to low is Mg, Ca, Fe, Cr, Al, Ba, Cu, Sr, Zn, Mn, and it is different from thet of the Gansu province. Results of the experiment indicated that factor of content of metal elements in soil and its physical and chemical properties, water may have great influence on the content of metal trace elements in sample.

It also should be noted that there are some harmful metal elements in *radix scutellariae*, such as as, Hg, Pb. Therefore it is necessary to take some measures to reduce the content of harmful metal elements in the *radix scutellariae* decoction process.

The content of essential metal trace elements in samples, such as Fe, Cu, Zn, Mn, is relatively higher. These metal trace elements can interact with other active ingredients and would have a synergistic effect on the pharmacological effect of *radix scutellariae*. Metal element K is not detected, it may be due to the interference caused by other metal elements or matrix.

TABLE 2 : Contents of metal elements in the radix scutellariae from different areas

Metal element	Content (Henan)/ (μg/g^{-1})	Content (Gansu)/ (μg/g^{-1})	Metal element	Content (Henan)/ (μg/g^{-1})	Content (Gansu)/ (μg/g^{-1})
Mg	5277.0	4353.8	Pb	2.7	1.5
Ca	3248.8	2600.1	W	2.1	5.5
Na	932.1	868.3	Hg	2.1	2.8
Fe	182.1	144.2	Ga	1.9	1.9
Cr	107.5	93.9	Zr	1.9	4.3
Al	75.4	84	Ni	1.5	3
Ba	71.6	74.1	Mo	0.9	2.1
Cu	54.9	23.4	V	0.8	0.7
Sr	16.6	31.9	Li	0.6	1.1
Zn	15.7	14.5	As	0.6	0.9
Mn	11.2	9.8	Sc	0.1	0.1
Ti	10.4	7.5	Co	0.1	0.1
Ag	9.9	11	Se	0.1	0.2
Rb	7.2	3.2	K	/	/
Sn	5.3	4.1			

Determination of leaching rates of metal trace elements in radix scutellariae from different areas

According to the procedure presented in subsection 2.3.2, the contents of mental elements in the radix scutellariae from different areas were determined and leaching rate of 28 kinds of metal trace elements can be calculated. The result can be seen from TABLE 3.

TABLE 3 : Leaching rates of metal trace elements in the *radix scutellariae* from different areas

Metal element	Leaching rate (Henan)/ %	Leaching rate (Gansu)/ %	Metal element	Leaching rate (Henan)/ %	Leaching rate (Gansu)/ %
Li	46.0	30.0	Zn	26.7	31.6
Na	30.1	19.7	Ga	7.3	9.2
Mg	27.9	25.2	As	15.9	15.3
Al	4.9	7.5	Se	0.6	5.0
Ca	14.7	15.0	Rb	17.1	27.8
Sc	2.4	5.4	Sr	28.3	17.2
Ti	8.9	12.0	Zr	6.4	8.2
V	16.9	19.1	Mo	6.7	5.1
Cr	30.0	33.9	Sn	14.3	15.9
Mn	16.0	16.3	Ba	8.5	9.6
Fe	5.3	8.2	W	6.1	6.2
Co	11.6	10.7	Ag	1.2	3.1
Ni	16.9	6.1	Hg	12.3	14.8
Cu	2.3	4.9	Pb	12.0	22.2

It can be seen that the leaching rates of most of the metal trace elements are lower than 30%, and and that of 28 metal trace elements differs greatly from each other. It is known that the organic components in *radix scutellariae* are mainly hydroxyl, amino, carboxyl and carbonyl ligands, which can form complexes with the metal elements.

Some metal trace elements in *radix scutellariae*, such as Ca, presents in abundance, but their leaching rate have no obvious difference with others. The experiment results indicate that the leaching rate of a metal trace element is not directly related to its contents, whereas it is mainly depend on the chemical speciation.

The metal trace elements, such as As, Hg, Pb in samples, are harmful to human body, and their leaching rates are lower than others. The dissolution of metal trace element in samples is often accompanied by the stripping of active ingredient, and the level of metal trace element stripping can reflect the level of stripping of active ingredient. The results also indicate that the TCM processing is also a kind of efficacy enhancing and toxicity reducing.

Analysis of soluble metal trace elements in radix scutellariae from different areas

According to the procedure of primary speciation analysis of metal elements described in 2.3.2, the soluble of metal trace elements in *radix scutellariae* from different areas are calculated and shown in TABLE 4.

There are no significant differences of the soluble metal trace elements in *radix scutellariae* between the two producing areas, and some of soluble metal trace elements, Li, Na, Mg, Ca, Ti, V, Cr, Rb, Sr, Mo, Sn, W, Hg, are at the range of 85% ~ 110%.

It can be seen that the measured values of several soluble metal trace elements in *radix scutellariae* maybe higher than the actual value. This may be caused by the sample contamination of elements from environment or reagent, or the combination between trace elements and organic ligands *in radix scutellariae* is weak, which leads elements or ligand exchange adsorption occurred during the process of treatment and separation of *radix scutellariae*. Practically, that the sample contamination and element loss is a common problem in the process of determination and speciation analysis of metal trace element in TCM. There are advantages of high efficiency and sensitivity, meanwhile there are disadvantages of sample blank, contamination and element loss when using ICP-MS applied for determination of metal trace elements in TCM by. Therefore, It should be pay attention to and improve the pretreatment procedure for determination of trace elements in natural products using ICP-MS.

TABLE 4 : Comparison of soluble of metal trace elements in *radix scutellariae* from different areas

Metal element	Soluble (Henan)/ %	Soluble (Gansu)/ %	Metal element	Soluble (Henan)/ %	Soluble (Gansu)/ %
Li	92.6	94.2	Zn	90.1	177.2
Na	93.4	92.9	Ga	95.9	122.8
Mg	86.9	91.2	As	111.9	128.1
Al	106.8	183.3	Se	300.0	35.4
Ca	100.8	88.6	Rb	91.2	90.6
Sc	209.4	218.3	Sr	91.3	89.0
Ti	99.1	107.3	Zr	119.8	123.5
V	95.2	102.2	Mo	86.4	102.5
Cr	97.7	97.5	Sn	86.0	86.3
Mn	157.7	90.0	Ba	92.1	114.7
Fe	99.5	153.1	W	93.0	107.3
Co	126.6	156.9	Ag	119.8	84.5
Ni	93.3	147.8	Hg	90.4	84.1
Cu	102.2	107.4	Pb	111.5	247.6

CONCLUSION

The information about the contents of metal trace elements in radix scutellariae and its primary form can be got from simulation of radix scutellariae decoction with wet digestion process and determination of metal trace element in radix scutellariae by ICP-MS. Result shows that metal trace elements can form complexes with other ingredients in radix scutellariae, and its dissolution is not isolated. Metal trace elements of the dissolution rates can reflect the dissolution of effective components in radix scutellariae; Soluble state molecules in radix scutellariae can be in the form of free ions or small molecular complexes, and it is easy to be absorbed by human body. The results of metal trace elements content in radix scutellariae and its primary speciation analysis, can provide the theoretical basis for decoction of TCM to a certain extent.

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