

Using Lead Isotope Ratios to Distinguish between Samples of the Traditional Chinese Medicine Dan-shen

Geological

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Abstract

Quadrupole Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used to determine lead (Pb) isotope ratios in Dan-shen, a type of herb used in Traditional Chinese Medicines (TCM), and in water and soil samples all taken from the same geographical location. The precision obtained for the $^{208}\text{Pb}/^{206}\text{Pb}$ ratio, the $^{207}\text{Pb}/^{206}\text{Pb}$ ratio and the $^{204}\text{Pb}/^{206}\text{Pb}$ ratio values was considerably lower than 0.5% demonstrating the applicability of the technique for Pb isotope ratio studies. The results show that it is possible to distinguish Dan-shen samples originating from different geographical areas using Pb isotope ratio measurements. As the medicinal effectiveness of a TCM is highly dependant on the source of origin of its herb components, it is useful to have a reliable, routine means of “fingerprinting” the components grown in different habitats.

Introduction

Most Traditional Chinese Medicines (TCMs) are a mixture of several different herbs that undergo special treatment to make them useful. The amounts of the effective components in the plants are influenced by the soil, water, weather conditions etc in the area where they are grown. Experienced practitioners of traditional Chinese healing know that the quality of herb medicines is strongly related to their source of origin. It is therefore important to know the specific location of a TCM herb component so ensure its effectiveness.

Many techniques have been applied to characterize various herb medicines and to correlate them with their place of origin. High Performance Liquid Chromatography, Mass Spectrometry, Nuclear Magnetic Resonance, Infrared Spectroscopy and X-ray Fluorescence have all been used to produce spectra that can be used to “fingerprint” the TCM habitat.

Lead isotope ratio measurements provide analytical information relating to the source of lead contamination in naturally occurring samples. Concentration measurements cannot provide this information. Studies of the isotopic composition of lead are therefore commonly used in environmental science as well as geological and anthropological studies. Among all the naturally occurring lead isotopes, only ^{204}Pb is non-radiogenic, whereas, ^{206}Pb , ^{207}Pb and ^{208}Pb are the daughter products from the radioactive decay of ^{238}U and ^{235}U and ^{232}Th respectively. As a consequence, small Pb isotope abundance variations occur in nature and the isotopic composition of lead in the environment is dependent on local ore deposits. If lead is present in the soil, a plant will take up small amounts and subsequent isotope ratio studies might provide a unique



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means of differentiating between different plant sources of origin. Of course, local lead levels may become mixed with external sources of contamination (e.g. automobile exhaust) that vary with time depending on the anthropogenic activity. These mixing processes can be quantified as long as each lead source shows a different lead isotope abundance. The work described in this application note was undertaken to investigate if lead isotope ratios can be used to “fingerprint” the Dan-shen herb grown in different habitats. While isotope ratio studies have traditionally been undertaken with a multicollector mass spectrometer such as Thermal Ionization Mass Spectrometry (TIMS), the technique is relatively slow and the instrumentation is expensive. These experiments were carried using quadrupole Inductively Coupled Plasma Mass Spectrometry (ICP-MS) that provides a fast, convenient and precise method to determine isotope ratios.

The Agilent 7500 Series ICP-MS is an excellent tool for routine isotope ratio measurements. It is easy to operate with its fully automated optimization system (Auto-tune function) and its user-friendly software. Because of these advantages, Agilent ICP-MS systems have been widely used for isotope ratio studies for environmental monitoring applications and nuclear research projects, e.g., U isotope ratios in human urine ^[2], in the cooling water of nuclear plants etc., the certified values of some certified reference materials such as rock samples ^[3], biological samples ^[3,4] by isotope dilution. In Encinar et al.'s work^[4,5], an older model Agilent ICP-MS (HP-4500) was used, and the performance was as good as the more expensive multi-collector ICP-MS and double focusing sector field ICP-MS especially for long term stability and isotope dilution applications.

Experimental

Instrumentation

An Agilent 7500i ICP-MS, (Agilent Technologies, Palo Alto, CA, USA), fitted with a PFA micro flow nebulizer (100 uL/min) in self-aspiration mode, a quartz spray chamber and a one-piece quartz torch was used throughout the study.

A MK-II microwave sample digestion system (Shang Hai Xin Ke Factory, China) was used for sample digestion.

Reagents

The following reagents were used during the course of the study:

- SRM981 (National Institute of Standards & Technology, USA) - for mass bias correction.
- HBr, HCl, HF, and HNO₃, (GR grade, after sub-boiling purification) - for sample digestion and dilution.
- Anion exchange resin (DOWEX 1×8, 200-400 mesh, USA), - for separating Pb from the matrix in the samples.
- Ultra Pure Water, supplied by Milli-Q water system (18.2 MOhms)

Sample preparation

Dry samples and filtered water samples were digested using a HNO₃/HF acid mixture in a microwave oven then heated to dryness in a fume cupboard to remove the HF. 1.0 mL HBr (0.5N) was used to redissolve the residue. Samples were then passed through a DOWEX 1×8 column to remove the matrix. The Pb containing eluant was dried and diluted with 3% HNO₃. In order to avoid memory effects and to keep the ICP-MS detector working in pulse counting mode only, the concentrations were controlled between 1 and 80 ug/L.

Optimization of the ICP-MS operation parameters Since the sample volumes collected after the column pretreatment step were limited, most of them were less than 1 mL, the Agilent PFA micro flow nebulizer (100 uL type) was selected for sample introduction. Self-aspiration mode was used and the carrier gas flow rate was controlled to maintain the sample introduction flow rate at about 50 uL/min. The 1 mL sample was sufficient for 2 or 3 individual measurements so that repeat analyses could be undertaken in case of error during the determination.

Instrument sensitivity and the other parameters, such as oxide level and doubly charged ions, were optimized automatically using the AutoTune feature of ChemStation on a 10 ppb tuning solution containing Li, Y, Ce, Tl. The carrier gas was then set to 0.4 L/min to obtain a sample uptake rate of about 50 uL/min. Finally, the make-up gas was adjusted to produce the best sensitivities and lowest interferences.

Instrument operating parameters are given in Table 1.

Table 1. ICP-MS operating conditions**Plasma Conditions:**

RF power:	1250 W
RF Matching:	1.7V
Sample Depth:	7 mm
Torch-H:	0 mm
Torch-V:	0.3 mm
Carrier Gas:	0.4 L/min
Make-up Gas:	0.9 mL/min
Peripump1:	0.1 rps
Spray Chamber Temperature:	2.0 degC

Data Acquisition Parameters:

“Isotope Ratio Analysis” mode, 1000 scan/sec, minimum dwell time 100 us. The integration times were 20sec for ²⁰⁴Pb, 10sec for ²⁰⁶Pb and ²⁰⁷Pb, 5 sec for ²⁰⁸Pb. Each data point is the average of 3 repetitions.

Results

Before beginning the analysis, the instrument was allowed 30 minutes following plasma ignition to reach thermal stability. A 20ppb solution of NIST SRM 981 isotope ratio standard was measured after every 5 unknowns. This data provided the 7500i short-term and long-term stability information and provided a means of making the small mass bias corrections required. The instrument demonstrated excellent precision. See Tables 2 and 3. Note that the instrument consistently delivered %RSD's at less than 0.1% over the short and long term.

Table 2: Short-term Stability of Pb Isotope Ratio Determinations of 20 ppb SRM981 using the Agilent 7500i ICP-MS (20min, 5 measurements)

File Name	PB-206A.D	PB-206B.D	PB-206C.D	PB-206D.D	PB-206E.D	RSD(%)
Acq Date	Jun 9 2001	Jun 9 2001	Jun 9 2001	Jun 9 2001	Jun 9 2001	
Acq Time	3:45 PM	3:50 PM	3:55 PM	4:00 PM	4:06 PM	
204/Total	0.01427	0.01426	0.01426	0.01426	0.01425	0.05
206/Total	0.2413	0.2414	0.2414	0.2415	0.2416	0.05
207/Total	0.2211	0.2208	0.2209	0.2209	0.2209	0.05
208/Total	0.5233	0.5235	0.5235	0.5233	0.5232	0.03
204/206	0.05913	0.05904	0.05907	0.05906	0.05897	0.10
207/206	0.9163	0.9146	0.9149	0.9146	0.9144	0.08
208/206	2.169	2.168	2.169	2.167	2.165	0.08

Table 3. Long-term Stability (10 hours) of Pb Isotope Ratio Determinations of 20 ppb SRM981 using the Agilent 7500i ICP-MS

File Name	Acq Date	Acq Time	206/Total	207/Total	208/Total	207/206	208/206
PB20-A.D	Jun 9 2001	6:14 AM	0.2411	0.2209	0.5235	0.9162	2.171
PB20-B.D	Jun 9 2001	6:26 AM	0.2412	0.2209	0.5236	0.9159	2.171
PB20-C.D	Jun 9 2001	6:33 AM	0.2412	0.2209	0.5235	0.9158	2.170
PB20-D.D	Jun 9 2001	6:40 AM	0.2411	0.2210	0.5236	0.9167	2.172
PB20_1A.D	Jun 9 2001	7:59 AM	0.2411	0.2211	0.5236	0.9172	2.172
Pb-202A.D	Jun 9 2001	9:12 AM	0.2410	0.2211	0.5236	0.9173	2.172
PB_202B.D	Jun 9 2001	9:18 AM	0.2414	0.2210	0.5234	0.9153	2.168
PB-203A.D	Jun 9 2001	11:17 AM	0.2413	0.2211	0.5233	0.9161	2.168
PB-204A.D	Jun 9 2001	12:52 PM	0.2412	0.2212	0.5233	0.9168	2.169
PB-204B.D	Jun 9 2001	12:59 PM	0.2410	0.2214	0.5234	0.9185	2.172
PB_205A.D	Jun 9 2001	2:27 PM	0.2412	0.2210	0.5236	0.9163	2.171
PB_205B.D	Jun 9 2001	2:32 PM	0.2415	0.2210	0.5232	0.9153	2.167
RSD(%)			0.06	0.07	0.03	0.10	0.08

The excellent results from the NIST quality assurance sample suggest that the data from the unknown samples is also very reliable and can be reported with a high degree of confidence. In this study, three types of samples were measured:

- Four samples of surface water taken from different areas in Zhong-Jiang, Si-Chuan Province
- Soil samples came from Er-Mei Mountain (2), Zhong-Jiang, Si-Chuan Province (5), Jiang-Xi Province (1)

- Dan Shen plant samples were from Zhong-Jiang, Si-Chuan Province (3), Er-Mei Mountain (2), Bei-Jing (1), Jiang-Xi Province (1), Tai Mountain, Shan-Dong Province (1), Shang-Luo, Shan-Xi Province (1), Xin-Jiang Province (1), He-Nan Province (1)

All the samples were taken from different areas even within the same province. The measured results and the standard deviations are listed in Table 4 a-c.

Table 4a: Results of Pb Isotope Ratio Measurements of Dan-shen Plant Samples

Sample Name	$^{207}\text{Pb} / ^{206}\text{Pb} \pm \text{sd}(n=3)$	$^{208}\text{Pb} / ^{206}\text{Pb} \pm \text{sd}(n=3)$
Jiang-Xi Province	0.8485 ± 0.0005	2.079 ± 0.002
Shan-Luo, Shan-Xi Province	0.8502 ± 0.0014	2.100 ± 0.002
Bei-Jing	0.8674 ± 0.0019	2.159 ± 0.003
Shan Dong Province	0.8570 ± 0.0018	2.107 ± 0.002
Xin-Jiang Province	0.8495 ± 0.0007	2.095 ± 0.001
He-Nan Province	0.8576 ± 0.0007	2.115 ± 0.002
ErMei Mountain, D1	0.8467 ± 0.0003	2.093 ± 0.001
ErMei Mountain, D2	0.8469 ± 0.0004	2.095 ± 0.004
Zhong-Jiang, D1	0.8529 ± 0.0022	2.105 ± 0.004
Zhong-Jiang, D2	0.8524 ± 0.0012	2.105 ± 0.002
Zhong-Jiang, D3	0.8537 ± 0.0003	2.103 ± 0.002

Table 4b: Results of Pb Isotope Ratio Measurements of Soil Samples

Sample Name	$^{207}\text{Pb} / ^{206}\text{Pb} \pm \text{sd}(n=3)$	$^{208}\text{Pb} / ^{206}\text{Pb} \pm \text{sd}(n=3)$
Jiang-Xi Province	0.8463 ± 0.0017	2.090 ± 0.001
ErMei Mountain, T1	0.8520 ± 0.0016	2.101 ± 0.004
ErMei Mountain, T2	0.8523 ± 0.0007	2.103 ± 0.001
Zhong-Jiang, T1	0.8395 ± 0.0007	2.084 ± 0.001
Zhong-Jiang, T2	0.8417 ± 0.0004	2.089 ± 0.001
Zhong-Jiang, T3	0.8411 ± 0.0015	2.084 ± 0.002
Zhong-Jiang, T4	0.8400 ± 0.0007	2.090 ± 0.001
Zhong-Jiang, T5	0.8391 ± 0.0014	2.083 ± 0.002

Table 4c: Results of Pb Isotope Ratio Measurements of Water Samples

Sample Name	$^{207}\text{Pb} / ^{206}\text{Pb} \pm \text{sd}(n=3)$	$^{208}\text{Pb} / ^{206}\text{Pb} \pm \text{sd}(n=3)$
Zhong-Jiang, w1	0.8702 ± 0.0015	2.140 ± 0.003
Zhong-Jiang, w2	0.8735 ± 0.0005	2.137 ± 0.002
Zhong-Jiang, w3	0.8675 ± 0.0002	2.136 ± 0.002
Zhong-Jiang, w4	0.8693 ± 0.0014	2.138 ± 0.003

Discussion

Since ^{206}Pb , ^{207}Pb and ^{208}Pb are the daughter products from the radioactive decay of ^{238}U and ^{235}U and ^{232}Th respectively, the Pb isotope abundance varies in nature. In most of the samples, the $^{208}\text{Pb}/^{206}\text{Pb}$ ratio values are between 1.95 and 2.15, the $^{207}\text{Pb}/^{206}\text{Pb}$ ratio values are between 0.78 and 0.86 and the $^{204}\text{Pb}/^{206}\text{Pb}$ ratio values are between 0.05 and 0.06^[1]. In order to distinguish between the different types of samples by their lead isotope ratio, the measurement precision is extremely important, both over the short and long term. The RSD

values should be at most 0.5% in order to make a clear differentiation between the samples. As indicated in

Table 4a-c, the SD values for the determinations are considerably better (lower) than 0.5%, so it is possible to use lead isotope ratio measurements to distinguish between the different samples.

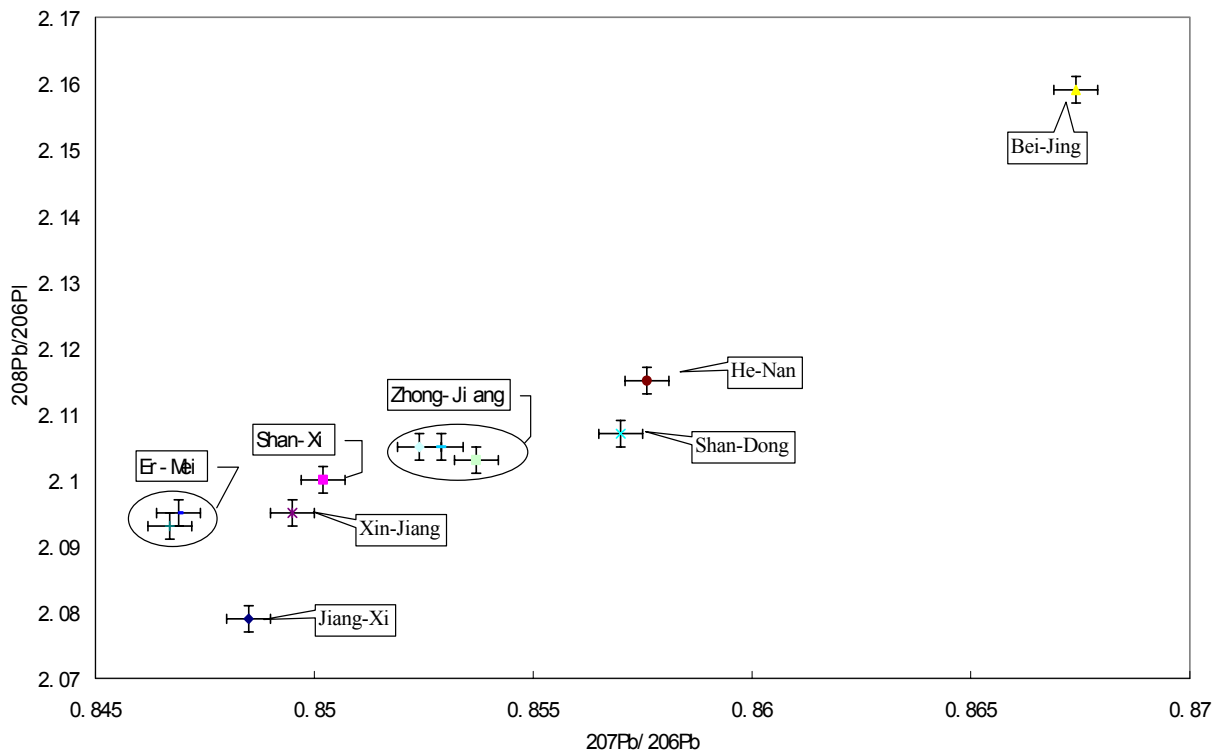


Figure 1: Pb Isotope Ratio Distribution of the Herb Medicine, Dan-Shen, from Different Sources

As a means of looking for variations/similarities in the measurements, the $^{208}\text{Pb}/^{206}\text{Pb}$ ratio values were plotted on the y-axis vs the $^{207}\text{Pb}/^{206}\text{Pb}$ ratio values on the x-axis. Figure 1 illustrates the data from the analyses of the Dan-shen plant and shows very interesting results. Obviously, the Pb isotope ratio values show their own special pattern for the Dan-shen samples from different sources. For example, Dan-shen samples grown in the Zhong-Jiang area have similar Pb isotope ratio values, although the

samples were taken from different sampling areas. When compared to the Pb isotope values of the Dan-shen samples from the Er-Mei Mountain region (the two samples also have similar Pb isotope ratio values), the difference is distinct. In conclusion, it is possible to distinguish the Dan-shen samples from different sources.

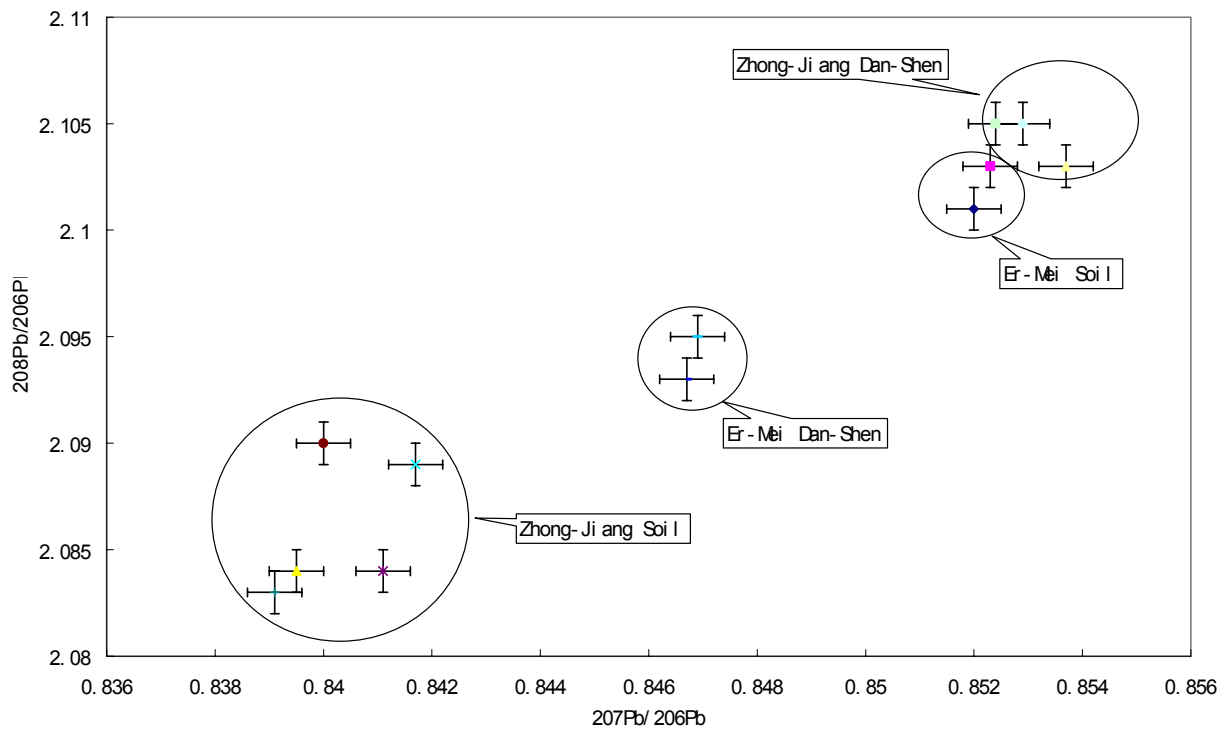


Figure 2: Pb Isotope Ratio Distribution of Soil and Dan-Shen Samples taken from Different Sources

When the soil samples from different sources are considered, it is found that the isotope values of the soil samples from different sources also have their own special pattern, as shown in Figure 2. But, interestingly, the isotope values of the soils are different to the Dan-shen samples from the same place. This suggests that soil isn't the only source of lead within the plant.

When the water samples are considered, it is obvious that the Pb isotope ratio values are a combination of those found in the soil and in the water, as shown in Figure 3.

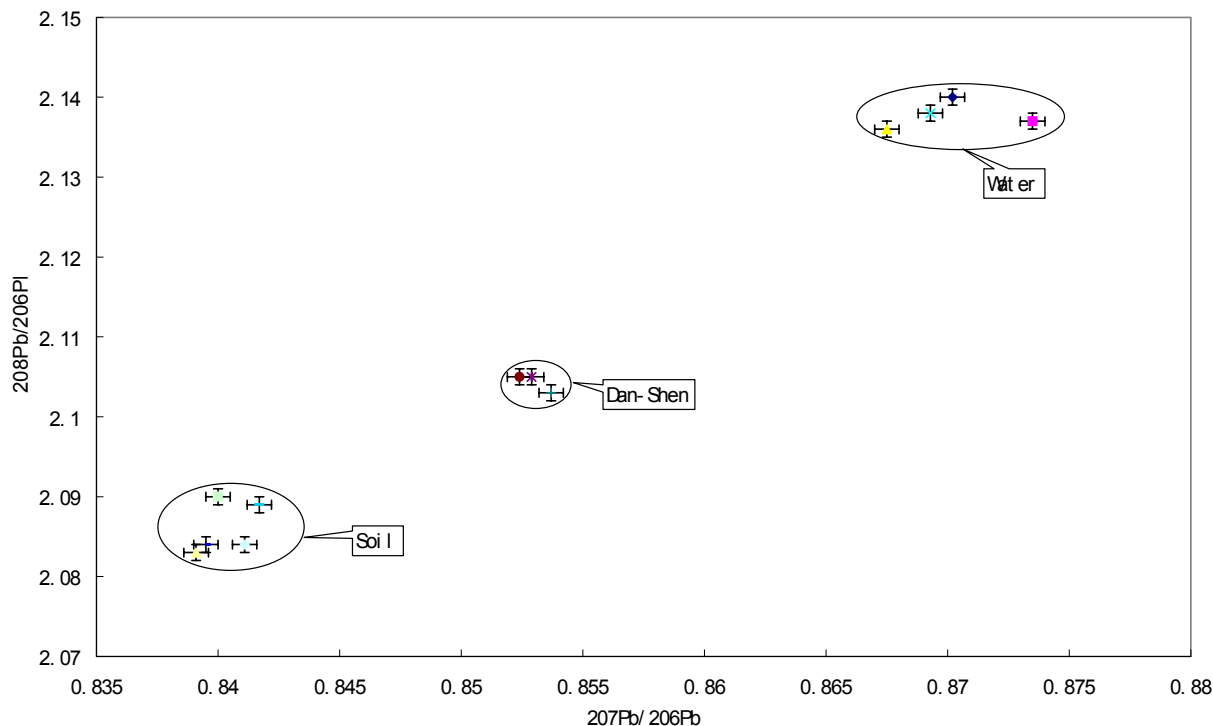


Figure 3: Pb Isotope Ratio Distribution of the Soil, Dan-Shen and Water Samples Taken from similar Sampling Area (Zhong-Jiang, Si-Chuan Province)

Conclusions

The Agilent PFA micro flow nebulizer is a good choice for isotope ratio analysis since it has very good nebulization efficiency and stability, especially when the sample amount is small. Self-aspiration mode avoids small amounts of pulsation from the peristaltic pump that could affect the precision of the isotope ratio analysis.

As this data suggests, the Agilent 7500 Series ICP-MS is well suited for routine isotope ratio analysis. Further improvements in precision may be obtained by modifying the method used in this study slightly. For instance, Tl may be added to the samples as an internal standard for lead isotope ratio analysis^[5]. In addition, optimization of the dead-time correction, the stand-by mass selection, may also improve the performance of Agilent 7500 ICP-MS so that the theoretical minimum %RSD of 0.03 can be obtained. Future work at Xiamen University will look in to these potential improvements.

This preliminary research indicates that it is possible to distinguish Dan-shen samples from different areas using

Pb isotope ratio measurements. Continuing these studies by running more types of samples will allow a large database to be set-up, and a chemometric model to be built to provide a convenient way to distinguish herb medicines from different sources.

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