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APPLICATION NOTE 43263

Analysis of trace elements in Traditional Chinese Medicine (TCM)

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Keywords

Herbal medicine, Trace elements, Traditional Chinese Medicine

Goal

To demonstrate the ability of the Thermo Scientific iCAP 7000 Plus Series ICP-OES to determine trace elements in materials of plant origin that are used for health benefits.

Introduction

Traditional Chinese Medicine (TCM) are therapeutic practices that have been developed over the past 2000 years and are used for the prevention and treatment of diseases. The administration of natural drugs is one of the five pillars of TCM that additionally encompasses acupuncture, massage techniques, kinesiatrics and dietetics. Over 90% of the used drugs are herbal products, mineral and animal products play a less important role. Typically, the herbal drugs are administered in the form of teas or infusions. Less used dosage forms include granulates, hydrophilic concentrates or even pills and capsules of ready-made mixtures of the herbal components.

As TCM was the only practiced medicine in the region before the arrival of western medicines, its field of application is diverse. The tradition is still widely practiced in many Asian countries and recently the usage has also increased in the western countries. However, the safety of these remedies has been questioned due to cases of illness and fatalities. Ongoing environmental pollution leads to enrichment of toxic substances in the soils and accumulation of toxic substances in the harvestable parts of the plants used as medicine.

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The potential danger of contamination can only be minimized by control from the responsible drug authorities and sale of the products via pharmacies or drug stores rather than non-regulated outlets. A first step to securing the quality of these products is being achieved with the recommendations of the World Health Organization (WHO) for maximum allowances of heavy metals and other toxic elements in herbal materials and the introduction of a new classification of TCMs within the United States Food and Drug Administration (FDA) draft guidance for Complementary and Alternative Medicine products (CAM). Table 1 gives an overview of existing regulations and recommendations.

Table 1. Recommendations and regulations for trace element concentrations in TCMs in mg·kg^{-1}.

Element	Hong Kong (HKCMMS, 2010)	China, Mainland (CP, 2015)	USA (NSF/ANSI 173 – 2010)	WHO (2005)	UK (BP)	Korea (KP X, 2012)
As	2	2	5	-	5	3
Cd	1	0.3	0.3	0.3	-	0.3
Cr	-	-	2	-	-	-
Cu	-	20	-	-	-	-
Hg	0.2	0.2	0.2	-	-	0.2
Pb	5	5	10	10	5	5

HKCMMS: Hong Kong Chinese Materia Medica Standards, CP: Chinese Pharmacopoeia, NSF/ANSI: National Science Foundation/American National Standards Institute, BP: British Pharmacopoeia, KP: Korean Pharmacopoeia

In this application note Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES) was used to determine six elements in three different TCM products purchased in China.

Instrumentation

For the sample analysis, the Thermo Scientific™ iCAP™ 7400 ICP-OES Duo was used together with an aqueous sample introduction kit, consisting of a concentric glass nebulizer and a cyclonic glass spray chamber as well as a 2 mm injector tube, aqueous pump tubing and an internal standard kit for online addition of the internal standard. The duo configuration was chosen for its low detection capability that is necessary when working in a pharmaceutical environment. A Teledyne CETAC ASX-560 autosampler was used to transfer the sample to the introduction system of the ICP-OES. The Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software simplifies method development and provides easy options for post-analysis data manipulation.

Standard preparation

All solutions were prepared from 1000 mg·kg^{-1} single element solutions provided by Spex CertiPrep (SPEX CertiPrep Group, Metuchen, US). The individual solutions were made up with 18 MΩ ultra-pure water and TraceMetal™ grade HNO_3 (Fisher Chemical, Loughborough, UK) to a final concentration of 7.8% HNO_3. A spike solution and an internal standard solution of yttrium (10 mg·kg^{-1}) were prepared in the same way. All concentrations are displayed in Table 2.

Table 2. Concentrations of calibration standards and spiked sample solution in µg·kg^{-1}.

	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Spike in sample
Cd, Cr, Pb	2.5	5	10	25	50	25
As, Hg	5	10	20	50	100	50
Cu	10	20	40	100	200	100

Sample preparation

The three different TCM samples (plant roots) were: *Huang Qi, Dan Shen* and *Dang Shen*. The samples were dried, ground and digested, each in three replicates of which one was spiked with the concentration of analytes in the middle of the calibration range (Table 1). Two duplicates were analyzed to show reproducibility of the digestion. For the digestion, the ground plant root material was weighed (~0.5 g) into a PTFE high pressure vessel and 6 mL of concentrated nitric acid were added. If material adhered to the walls of the vessel, it was washed down carefully with the acid. For better oxidation of the organic matrix, 2 mL of concentrated hydrogen peroxide (Primar™ grade, Fisher Chemical, Loughborough, UK) were added as well. The digestion was conducted in a Milestone Ethos EZ microwave, equipped with an SK-10 segmented rotor and a temperature sensor, according to the protocol in Figure 1. After digestion the digest was transferred to a 50 mL volumetric flask. The digestion vessels were rinsed with 18 MΩ ultra-pure water and after transferring this to the flask as well, it was filled up with ultra-pure water to the measured mark. Additionally, for each digestion cycle a duplicate of a digestion blank was run. This contained only acid and hydrogen peroxide and after digestion was treated the same way as the samples.

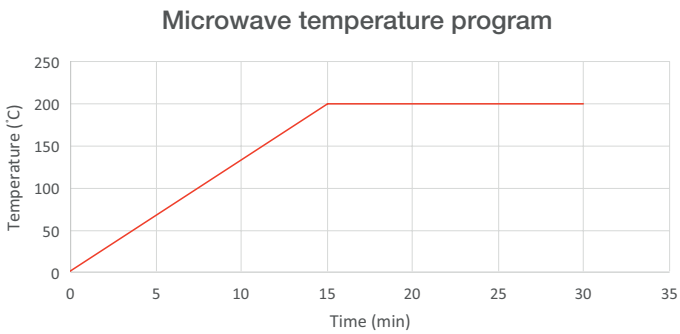

Figure 1. Temperature program of the digestion.

Method development and analysis

A method was created in Qtegra ISDS Software. Due to the low concentration range (µg·kg^{-1}), wavelengths were only viewed axially. The wavelengths used for the analysis are shown in Table 4, these were selected as they were free from interferences and provided the sensitivity to quantify the elements of interest in the expected concentration range. The parameters used for the method can be found in Table 3. The plasma was ignited and the instrument was allowed to warm up for a period of 15 minutes. A spectrometer optimization was performed directly before analysis.

Following method development, the instrument was calibrated and the samples were analyzed. A method detection limit study was carried out by analyzing the digestion blank with ten replicates and multiplying the standard deviation of this analysis by three. This was repeated three times and the average values for detection limits were calculated.

Table 3. Method parameters.

Parameter	Setting
Pump Tubing (Standard Pump)	Sample Tygon® orange/white Drain Tygon® white/white Internal standard Tygon® orange/blue
Pump Speed	50 rpm
Spray Chamber	Glass cyclonic
Nebulizer	Glass concentric
Nebulizer Gas Flow	0.55 L·min^{-1}
Coolant Gas Flow	12 L·min^{-1}
Auxiliary Gas Flow	0.5 L·min^{-1}
Center Tube	2 mm
RF Power	1150 W
Plasma View	Axial
Exposure Time	UV 15 s, Vis 5 s

Results

After digestion, the digested solutions were clear and only some small white particulates remained in the solutions of *Huang Qi* and *Dan Shen* (possibly silicates that cannot be digested by nitric acid). The results for the spike recovery test prove that none of the target elements were lost during digestion. With the exception of mercury, all correlation coefficients of the calibration were greater than 0.9994, indicating very good linearity. A reason for the slightly worse calibration of mercury may be the adhesion of mercury to the walls of the LDPE vials at low concentrations. This effect could be minimized by the addition of gold (in the form of $AuCl_3$) to the standard solutions. The detection limits for all elements are in the single digit or sub µg·kg^{-1} concentration range.

Spike recoveries were calculated from the concentration values in Table 4, not taking into account values that were below the detection limit (<DL). The recoveries for the three samples were within ±10% of the spiked concentration for all elements, most of them being within ±5% of the expected value, showing very good accuracy of the method.

The corrected concentrations of elements in the original plant root material are shown in Table 5. They are calculated by applying the factor derived from the dilution of the sample aliquot in 50 mL sample solution. The two replicates for each sample are in good agreement with each other, demonstrating the repeatability of the method. When comparing the concentrations of the measured elements in the TCM samples to the limits and recommendations of various pharmacopoeias and the World Health Organization (see Table 1), it is obvious that all concentrations are around one order of magnitude below the recommended limits and therefore considered as safe for human consumption.

Table 4. Concentration of elements in solution. The first and second replicate as well as the measured spiked concentration and calculated recovery in percentage for each TCM sample: *Huang Qi, Dan Shen* and *Dang Shen*. Also given are correlation coefficients and detection limits for each element wavelength. Concentrations in µg·kg^{-1}.

Element wavelength (nm)	Huang Qi				Dan Shen				Dang Shen				R2	Detection limit
	Replicate		Spike	Recovery (%)	Replicate		Spike	Recovery (%)	Replicate		Spike	Recovery (%)		
	1	2			1	2			1	2				
As 193.759	<DL	<DL	51.1	**102.1**	<DL	<DL	53.1	**106.1**	<DL	<DL	50.8	**101.5**	0.99995	3.6
Cd 214.438	0.2	0.2	25.5	**101.1**	0.4	0.4	25.3	**99.7**	0.3	0.3	25.3	**100.1**	1.00000	0.1
Cr 205.560	1.4	1.7	26.2	**98.6**	7.4	7.5	32.3	**99.5**	2.2	2.5	26.9	**98.4**	0.99997	0.3
Cu 224.700	64.6	66.5	171.4	**105.9**	128.2	129.2	228.6	**99.9**	60.9	60.5	156.6	**96**	0.99999	0.9
Hg 184.950	0.7	<DL	49.5	**97.4**	<DL	<DL	50.1	**100.2**	<DL	1.5	47.0	**91.1**	0.99665	0.6
Pb 220.353	2.6	2.8	25.5	**91.3**	6.2	7.2	30.6	**95.4**	3.2	3.2	27.7	**98**	0.99942	1.9

Table 5. Calculated concentration of six elements in the TCM samples in mg·kg^{-1}.

Element and wavelength (nm)	Huang Qi (Replicate)		Dan Shen (Replicate)		Dang Shen (Replicate)	
	1	2	1	2	1	2
As 193.759	<0.4*	<0.4*	<0.4*	<0.4*	<0.4*	<0.4*
Cd 214.438	0.02	0.02	0.04	0.03	0.03	0.03
Cr 205.560	0.1	0.2	0.7	0.7	0.2	0.2
Cu 224.700	6	6	13	12	6	6
Hg 184.950	0.07	<0.06*	<0.06*	<0.06*	<0.06*	0.14
Pb 220.353	0.3	0.3	0.6	0.7	0.3	0.3

*Concentrations were <DL

Conclusion

The analysis shows that the Thermo Scientific iCAP 7000 Plus Series ICP-OES delivers excellent accuracy and sensitivity for analysis of trace elements in herbal products used as TCM in conformity with the present recommendations for concentration limits. The detection limits obtained are comparable to those in drinking water proving the excellent ability of the instrument of handling complex acidic matrices. Moreover, very good spike recoveries indicate that no target elements are lost during the sample preparation demonstrating microwave digestion is an excellent choice for sample preparation.

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