

Atomic Absorption

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Analysis of Arsenic, Cadmium and Lead in Chinese Spice Mixtures using Graphite Furnace Atomic Absorption Spectrophotometry

ulcers and as diuretics, depuratives and vermifuges. Also, some species are used as tea-flavoring agents in several regions. Natural food spices, such as pepper and mustard, have been reported to contain significant quantities of some heavy metals, including cadmium, lead and arsenic. Exposure to trace and heavy metals above the permissible affects human health and may result in illness to human fetus, abortion and preterm labor, as well as mental retardation to children. Adults also may experience high blood pressure, fatigue and kidney and neurological disorders.¹ Contamination with heavy metals may be accidental (e.g. contamination of environment during plant cultivation) or deliberate – in some cultures, according to traditional belief, specially treated heavy metals are associated with health benefits and are thus an intentional ingredient of traditional remedies. Spices and herbal plants may contain heavy-metal ions over a wide range of concentrations.^{2,3}

Introduction

Spices are dried parts of plants. Together with herbs, these plants grow widely in various regions of the world and have been used for several purposes since ancient times. Most are fragrant, aromatic and pungent and are used for culinary purposes to improve color, aroma, palatability and acceptability of food.¹ In addition, they are also used in folk medicine as antiscorbutic, antispasmodic, tonic, carminative agents against bronchitis,

India and China have a high diversity of plants used as spices, herbs, and traditional medicines. Several herbs and spices are either produced on small farmlands or naturally grow in different regions. There is often little information available about the safety of those plants and their products in respect to heavy metal contamination. Due to the significant amount of spices consumed, it is important to know the toxic metal contents in these spices.⁴

The objective of this work is two-fold: (1) to accurately analyze the levels of toxic heavy metals like lead, cadmium and arsenic that may be present in some major spice brands available in the local markets in China, by using graphite furnace atomic absorption spectrophotometry (GFAAS); (2) to cross-reference these measured levels to the recommended limits specified by the U.S. FDA.

Experimental

The measurements were performed using a PerkinElmer® AAnalyst™ 800 Atomic Absorption Spectrophotometer (Shelton, CT, USA) equipped with the intuitive WinLab32™ for AA (Version 6.5) software, which features all the tools to analyze samples, report and archive data and ensure regulatory compliance. The high-efficiency optical system and solid-state detector used in this spectrophotometer provide outstanding signal-to-noise ratios. This solid-state detector is also highly efficient at low UV and high wavelengths at one time. It also features longitudinal Zeeman-effect background correction for graphite furnace analysis. The use of a transversely heated graphite atomizer (THGA) provides uniform temperature distribution across the entire length of the graphite tube. This eliminates the memory effect inherent with high-matrix sample analysis. The THGA features an integrated L'vov platform which is useful in overcoming potential chemical interference effects common to the GFAAS technique. High-energy electrodeless discharge lamps (EDLs) were used for all the elements.



Figure 1. PerkinElmer AAnalyst 800 Atomic Absorption Spectrophotometer.

A Multiwave™ 3000 Microwave Sample Preparation System (PerkinElmer/Anton-Paar) was used for the microwave-assisted digestion (Figure 2). This is an industrial-type microwave oven which is equipped with various accessories to optimize the sample digestion. The samples were digested in the 8XF100 rotor using eight 100 mL high-pressure vessels made of PTFE-TFM protected with its individual ceramic jackets. TFM is chemically modified PTFE that has enhanced mechanical properties at high temperatures compared to the conventional PTFE. This vessel has a working pressure of 60 bars (870 psi) and temperatures of up to 260 °C.



Figure 2. PerkinElmer/Anton-Paar Multiwave 3000 Microwave Sample Preparation System.

Standards, Chemicals and Certified Reference Materials

PerkinElmer single-element calibration standards for Atomic Spectroscopy were used as the stock standards for preparing the working standards. Working standards were prepared by serial volume/volume dilution in polypropylene vials (Sarstedt®, Germany)

ASTM® Type I water (from a Millipore® filtration system – Millipore® Corporation, Billerica, Massachusetts, USA) acidified with 0.2% Suprapur® nitric acid, from Merck® (Darmstadt, Germany) was used as the calibration blank and for all dilutions. Chemical modifiers were prepared from stock solutions, by diluting with acidified Millipore® water and were added automatically to each standard, blank and sample by the AS-800 autosampler, an integral part of the AAnalyst 800. Micropipettes (Eppendorf®, Germany) with disposable tips were used for pipetting solutions.

NIST® 1573e certified reference material for trace metals in tomato leaves, GBW 10016 certified reference material for trace metals in tea leaves, and GBW 09101 certified reference material for trace metals in human hair were used to validate the method developed. Multi-element ICP standards for trace-metal ions in nitric acid (Spex. Certiprep.®, New Jersey, USA), prepared at concentrations of the midpoint of the calibration curves for different elements, were used as quality control (QC) check standards.

Sample and Certified Reference Material Preparation

Four branded powdered spice and herb samples available in China (five-spice mix, ground szechuan, five-spice powder and curry powder), bought from a local Chinese supermarket, were used for the analysis.

Approx. 0.5 g of each sample, accurately weighed in duplicate, was transferred to the digestion vessels of the microwave digestion system and the sample digestion was done in accordance with the program given in Table 3 (Page 4). The digested samples were diluted with 0.2% HNO₃ and made up to 20 mL in polypropylene vials. The certified reference materials were also digested in a similar manner.

Plastic bottles were cleaned by soaking with 10% volume/volume HNO₃ for at least 24 hours and rinsed abundantly in de-ionized water before use. The instrumental conditions for furnace experiments are given in Table 1, and the graphite furnace temperature programs are listed in Appendix I (Page 6). Heated injection at 90 °C was used for all the furnace experiments. Pyrolytically-coated graphite tubes with integrated platforms were used. The autosampler cups were soaked in 20% nitric acid overnight to minimize sample contamination, and thoroughly rinsed with 0.5% HNO₃ acid before use. Five-point calibration curves (four standards and one blank) were constructed for all the metal ions and the calibration-curve correlation coefficient was ensured to be better than 0.999 before the start of the sample analysis.

Element	Cd	Pb	As
Wavelength (nm)	228.8	283.3	193.7
Slit (nm)	0.7	0.7	0.7
Mode	AA-BG	AA-BG	AA-BG
Calibration	Linear with calculated intercept	Linear with calculated intercept	Linear with calculated intercept
Lamp	EDL	EDL	EDL
Current (mA)	230	440	380
Standards (µg/L)	0.2, 0.5, 1.0	5.0, 10, 25	5.0, 10, 25
Correlation Coefficient	0.9997	0.9991	0.9992
Read Time (sec)	5	5	5
Measurement	Peak Area	Peak Area	Peak Area
Injection Temp (°C)	90	90	90
Sample Volume (µL)	20	20	20
Matrix Modifier	0.05 mg NH ₄ H ₂ PO ₄ and 0.003 mg Mg(NO ₃) ₂	0.05 mg NH ₄ H ₂ PO ₄ and 0.003 mg Mg(NO ₃) ₂	0.005 mg Pd and 0.003 mg Mg(NO ₃) ₂
Modifier Volume (µL)	5	5	5

Table 2. Program used for the digestion of spices and herbs with the Multiwave.

Sequence	Power	Ramp Time (min)	Hold Time (min)	Fan
1	1200	15	15	1
2	0		15	3
Weight Taken	~500 mg	HNO ₃	5.0 mL	
H ₂ O ₂	1.0 mL	Rate	0.5 bar/sec	
Pressure	55 Bars			

Results and Discussions

The validity of the developed method has been ensured by incorporating various quality control (QC) checks and analysis of certified reference materials (CRMs). The agreement between the certified values and the measured values were excellent, which demonstrates the accuracy of the generated calibration as well as the overall accuracy of the developed method (Table 3 – Page 4). The slightly higher values obtained for Cd in NIST® CRM 1573e is due to the fact that the container of the CRM was opened a few years ago and the reference material was left in a polyethylene bag where contamination for Cd had occurred. This was confirmed by analyzing the same CRM using an ICP-MS (PerkinElmer ELAN® DRC-e) where a value of 2.37 µg/g was obtained. The QC standard gave excellent recovery with a variation of less than 10% usually prescribed by the regulatory bodies (Table 4 – Page 4). There was virtually no difference between the QC standard which was performed immediately after calibration and the QC standard which was analyzed at the end of the analysis with a time difference of more than three hours. This shows the long-term stability of the instrument. Method detection limits (MDLs) were calculated (Table 5 – Page 4) based on the standard deviation of seven replicates of the reagent blanks (student t-value of 3.14 for a confidence interval of 98%). These limits were obtained under routine operating conditions, and this is not reflective of the optimum detection limits achievable by the system. The extremely lower detection limits obtained show the capability of the AAnalyst 800 spectrometer in analyzing difficult matrices at the measured concentrations.

Table 3. Analysis of certified reference materials.

Analyte	NIST® 1573e		GBW 09101		GBW 10016	
	Certified Value (µg/g)	Measured Value (µg/g)	Certified Value (µg/g)	Measured Value (µg/g)	Certified Value (µg/g)	Measured Value (µg/g)
Pb	*	–	3.83 ± 0.18	3.83 ± 0.54	1.5 ± 0.2	1.3 ± 0.5
Cd	1.52	2.24	0.072 ± 0.010	0.075 ± 0.01	0.062 ± 0.01	0.06 ± 0.02
As	0.112	0.15	0.198 ± 0.023	0.23 ± 0.03	0.09 ± 0.01	0.07 ± 0.02

**Not a reference element*

Table 4. Results of QC recovery studies.

Analyte	Prepared Concentration (µg/L) QC	Measured Concentration (µg/L) QC	% QC Recovery	Prepared Concentration (µg/L) QC	Measured Concentration (µg/L) QC	% QC Recovery
	(analyzed at 10:00 am)	(analyzed at 10:00 am)		(analyzed at 1:30 pm)	(analyzed at 1:30 pm)	
Pb	12.5	12.6	101	12.5	12.6	101
Cd	0.50	0.50	100	0.50	0.50	100
As	12.5	12.1	97	12.5	13.3	106

Table 5. Method detection limits (MDLs).

Analyte	MDL (µg/kg)
Pb	9
Cd	2
As	6

Table 6. Analysis of spices and herbs samples using GFAAS.

Analyte (µg/g)	Five-Spice Mix	Ground Szechuan	Five-Spice Powder	Five-Spice Powder Duplicate	Curry Powder	Curry Powder Duplicate
Pb	2.42	4.48	6.30	6.93	1.21	1.22
Cd	0.25	0.07	0.25	0.23	0.38	0.35
As	0.12	0.07	0.16	0.15	0.14	0.14

Table 7. Analysis of spices and herbs samples using ICP-MS.*

Analyte(µg/g)	Five-Spice Mix	Ground Szechuan	Five-Spice Powder	Curry Powder
Pb	2.72	4.69	6.04	1.28
Cd	0.11	0.06	0.27	0.26
As	0.19	0.16	0.18	0.31

**Different set of similar samples*

Conclusions

An accurate and reliable microwave-assisted sample pretreatment procedure for the determination of arsenic, cadmium and lead in spices using GFAAS is described. The spices contain a number of organic substances of different stability and impurities of sparingly soluble mineral components. Incomplete mineralization of samples during the microwave-digestion process may cause difficulty in transferring analytes into solution and this also disturbs spectrochemical measurements.⁵ Application of concentrated HNO₃ along with hydrogen peroxide for mineralization of spices and herbs leads to the complete digestion of samples, which is proven by determined values of the analytes in various CRMs. Toxicity of medicinal spices and herbs is of much greater concern today than ever before. In recent

years, much emphasis is being laid on toxic-element contents, as several European Union countries have banned many varieties of ayurvedic drugs. The results in Table 6 and 7 (Page 4) show that the levels of arsenic, cadmium and lead in all the samples analyzed were well within the permissible limits of 10, 0.3 and 10 mg/kg respectively, as specified by the U.S. FDA. The results confirmed that the determination of arsenic, cadmium and lead after acid solubilization of spice-mixture samples by microwave digestion can be performed by GFAAS without any interference and the same has been cross-checked by analyzing a different set of similar samples with ICP-MS analysis (using a PerkinElmer ELAN DRC-e ICP-MS). The good agreement of the values obtained with standard ICP-MS and GFAAS analysis further confirmed the accuracy of the method developed.

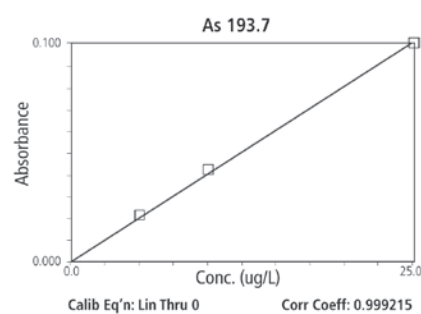
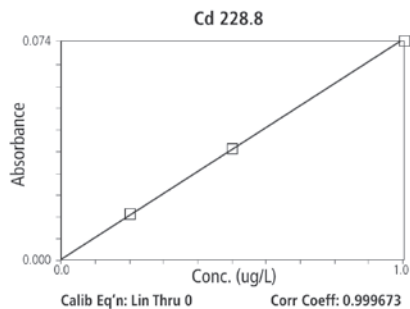
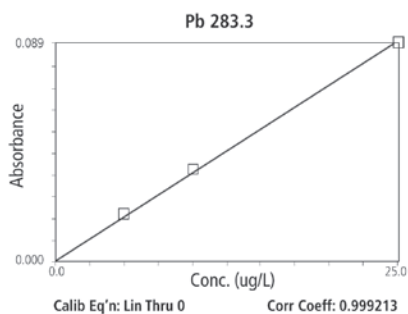
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Appendix I. Graphite Furnace Temperature Program.

Element	Step	Temp °C	Ramp Time (sec)	Hold Time (sec)	Internal Gas Flow (mL/min)	Gas Type
Cd	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	500	10	20	250	Argon
	4	1500	0	5	0	Argon
	5	2450	1	3	250	Argon
As	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	1200	10	20	250	Argon
	4	2000	0	5	0	Argon
	5	2450	1	3	250	Argon
Pb	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	850	10	20	250	Argon
	4	1600	0	5	0	Argon
	5	2450	1	3	250	Argon

Appendix II. Calibration Graphs for Different Analytes.



Appendix III. Atomization Profiles of Lower Standard and Some Samples.

