



APPLICATION NOTE

Atomic Absorption

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Analysis of Pb, Cd and As in Spice Mixtures using Graphite Furnace Atomic Absorption Spectrophotometry

Introduction

The toxicity and effect of trace heavy metals on human health and the environment has attracted considerable attention and concern in recent years. With an inherent toxicity, a tendency to accumulate in the food chain and a particularly low removal rate through excretion,¹ lead (Pb), cadmium (Cd) and arsenic (As) cause harm to humans even at low concentrations. Exposure to trace and heavy metals above the permissible level affects human health and may result in teratogenicity (reproductive effects). Individuals may also experience high blood pressure, fatigue, as well as kidney and neurological disorders.

Spices, the dried parts of plants, grow widely in various regions of the world, are produced either on small farmlands or naturally grown, and have been used for several purposes since ancient times. Most are fragrant and flavorful and are used for culinary purposes to improve the quality of food.² Natural food spices, such as pepper, have been reported to contain significant quantities of some heavy metals, including Pb, Cd and As. Contamination with heavy metals may be accidental (e.g. contamination of the 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.^{3,4} 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 concentrations in them.⁵

The major challenge in performing spice analyses is the extremely low analyte concentrations combined with high matrix levels. Graphite furnace atomic absorption spectrophotometry (GFAAS) has been one of the more reliable techniques used for many years and is therefore the preferred analytical method. The use of longitudinal Zeeman background correction and the use of matrix modifiers provides accurate results and low detection limits in samples with high matrices. This makes GFAAS an indispensable tool in carrying out such analyses.

The objective of this work is two-fold: (1) to use GFAAS to accurately analyze the levels of Pb, Cd, and As present in some major spices commonly available on the market; and (2) to cross reference these measured levels to the recommended limits specified by the U.S. FDA.

Experimental Conditions

Instrumentation

The measurements were performed using a PerkinElmer® PinAAcle™ 900T atomic absorption spectrophotometer (Shelton, CT, USA) equipped with the intuitive WinLab32™ for AA software running under Microsoft® Windows™ 7, which features all the tools to analyze samples, report and archive data and ensure regulatory compliance (Figure 1). The high-efficiency optical system and solid-state detector used in the PinAAcle 900T spectrometer provide outstanding signal-to-noise ratios. The longitudinal Zeeman-effect background correction for graphite furnace analysis provides accurate background correction without the loss of light that usually occurs in transverse Zeeman systems. The use of a transversely heated graphite atomizer (THGA) provides uniform temperature distribution across the entire length of the graphite tube, eliminating memory effects and potential interferences that may occur with high-matrix sample analysis. Pyrolytically coated THGA tubes with end caps (Part No. B3000655) were used for all measurements. The optimized instrumental conditions for furnace experiments are given in Table 1 (Page 3), and the graphite furnace temperature programs are listed in Appendix I (Page 5). A heated injection was used for lead; it can also be used for cadmium and arsenic.



Figure 1. PerkinElmer PinAAcle 900T atomic absorption spectrophotometer.

A microwave sample preparation system was used for the microwave-assisted digestion. This is an industrial-type microwave oven, which is equipped with various accessories to optimize the sample digestion. The samples were digested using ten 100 mL high-pressure vessels made of PTFE. The microwave digestion program is listed in Table 2 (Page 3).

Standards, Chemicals and Certified Reference Materials

PerkinElmer Pure single-element calibration standards for Pb, Cd, and As were used as the stock standards for preparing the working standards (Part Nos. Pb: N9300128; Cd: N9300107; As: N9300102). Working standards were prepared by serial volume/volume (v/v) dilution in polypropylene vials (Part Nos. B0193233 15 mL Conical; B0193234 50 mL Conical Freestanding) ASTM® Type I deionized water (Millipore® Corporation, Billerica, Massachusetts, U.S.) acidified with 0.2% nitric acid (HNO₃) (Tamapure®, TAMA Chemicals, Japan) was used as the calibration blank and for all dilutions. 30% hydrogen peroxide (H₂O₂) (Kanto Chemicals, Tokyo, Japan) was used for digestion along with nitric acid.

Matrix modifiers were prepared from 10% NH₄H₂PO₄ (Part No. N9303445), 1% Mg as Mg(NO₃)₂ (Part No. B0190634) and 1% Pd (Part No. B0190635) stock solutions, by diluting with the 0.2% HNO₃ made above. Matrix modifiers were added automatically to each standard, blank and sample by the AS 900 autosampler, an integral part of the PinAAcle 900T spectrometer.

NIST® 1568a Certified Reference Material (CRM) for Trace Metals in Rice Flour was used to validate the method. Quality control (QC) check standards were prepared at the calibration curve midpoint concentration for each individual element.

Sample and Certified Reference Material Preparation

Plastic bottles were cleaned by soaking with 10% v/v HNO₃ for at least 24 hours and rinsed abundantly in deionized water before use. The polypropylene autosampler cups (Part No. B3001566) 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 each analyte and the calibration curve correlation coefficient was examined to ensure a value better than 0.998 before the start of the sample analysis (Appendix II – Page 6).

Five branded powdered spice samples available in local supermarkets in India (coriander powder, ginger powder, asafetida, black pepper powder and red chili powder) were analyzed. Approximately 0.5 g of each sample or CRM, accurately weighed in duplicate, was transferred to the vessel of the microwave digestion system and the sample digestion method (Table 2) was performed in accordance with U.S. Environmental Protection Agency (EPA) Method 3052. The digested samples were diluted with 0.2% HNO₃ and brought up to 25 mL in polypropylene vials.

Table 1. Optimized experimental conditions of PinAAcle 900T.

Analyte	Pb	Cd	As
Wavelength (nm)	283.3	228.8	193.7
Lamp Type	EDL (Part No. N3050657)	HCL (Part No. N3050115)	EDL (Part No. N3050605)
Standards (µg/L)	5, 10, 15, 20	0.5, 0.75, 1.0, 2.0	10, 20, 30, 40
Correlation Coefficient	0.9991	0.9996	0.9989
Read Time (sec)	3	5	3
Injection Temp (°C)	90	20	20
Matrix Modifier (mg)	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		
Slit Width (nm)	0.7		
Sample Volume (µL)	20		
Measurement Type	Peak Area		
Calibration	Linear through zero		

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

Sequence	1	2
Power (watts)	1000	0
Ramp Time (min)	10	0
Hold Time (min)	10	20
Weight Taken (mg)	~500	
H ₂ O ₂ (mL)	1.0	
HNO ₃ (mL)	7.0	
Temperature (°C)	180	

Results and Discussions

In the analysis of complex samples with the graphite furnace, obtaining reproducible results can be a challenging task as one has to deal with analytes present at the low µg/L ppb level and the potential for matrix interferences. With Stabilized Temperature Platform Furnace (STPF) conditions (including: pyrolytically coated graphite tubes, platform atomization, rapid heating, internal gas stop during atomization, fast signal processing and Zeeman background correction) the analysis of low level analytes in spice mixtures is an easy task.

The graphite furnace sample introduction system is of paramount importance in optimizing the short-term stability of signals. The PinAAcle 900T spectrometer uses a unique built-in camera to monitor sampler tip alignment and sample introduction into the graphite tube. With the TubeView™ furnace camera, it is simple to position the AS 900 autosampler sample capillary tip at the correct depth inside the tube so as to achieve highly reproducible pipetting.

Table 3. Analysis of certified reference material by GFAA.

NIST® 1568a Rice Flour		
Analyte	Certified Value (µg/g)	Measured Value (µg/g)
Pb	<0.010	0.0093
Cd	0.022 ±0.002	0.020 ±0.004
As	0.29 ±0.03	0.24 ±0.02

Table 4. Estimated method detection limits (MDLs).

Analyte	MDL (µg/L)
Pb	9.5
Cd	2.35
As	9.5

Table 5. Spike recovery studies.

Analyte	Sample	Post-digestion Recovery (%)	Spike Level (µg/L)	Pre-digestion Recovery (%)	Spike Level (µg/L)
Pb	Coriander powder	91	5.00	111	5.00
	Ginger powder	90		*	
Cd	Coriander powder	104	0.625	119	1.00
	Ginger powder	96		*	
As	Coriander powder	108	8.33	100	5.00
	Ginger powder	106		*	

*Pre-digestion spike recovery not performed

The developed method has been validated by incorporating various QC checks and by the analysis of a CRM. The agreement between the certified and measured values was good (Table 3), which demonstrates the accuracy of the direct calibration being used as well as the overall accuracy of the developed method. Method detection limits (MDLs) were calculated (Table 4) based on the standard deviation of seven replicates of the reagent blank (t-value = 3.14) and took into account the dilution factor for the samples. The low method detection limits obtained show the capability of the PinAAcle 900T spectrometer in analyzing difficult matrices at the measured concentrations.

Incomplete mineralization of samples during the microwave-digestion process, due to high organic content, may cause difficulty in transferring analytes into solution. These impurities may also affect the analytical measurements.⁶ Application of concentrated HNO₃ along with H₂O₂ for mineralization of spices and herbs leads to the complete digestion of samples, which is proven by the determination of the values of the analytes in the CRM (Table 3). Both a pre-digestion and an automatic post-digestion recovery study were performed. The recoveries obtained for the pre-digestion spike easily meet the U.S. EPA recovery guidelines of ±30% and indicate that neither analyte loss nor contamination was of major concern throughout the analytical procedure (Table 5). The recoveries obtained for the post-digestion spike indicate that no matrix interference towards the analyte signal was seen. The results of the sample analysis are summarized in Table 6. The level 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.

Table 6. Analysis of spices and herbs samples using GFAAS (µg/kg).

Analyte	U.S. FDA						
	Limit	Replicate	Coriander Powder	Ginger Powder	Asafetida	Black Pepper Powder	Red Chili Powder
Pb	10,000	Sample	455	551	124	9.8	286
Cd	300	Sample	48	89	8.2	25	57
		Duplicate	47	92	8.2	28	59
As	10,000	Sample	20	49	41	49	<MDL
		Duplicate	24	42	38	32	<MDL

Conclusions

A complete method for the accurate determination of Pb, Cd and As in various spice mixtures using the PinAAcle 900T atomic absorption spectrophotometer in the GFAAS mode was developed. The results confirmed that the determination of arsenic, cadmium and lead in spice mixtures, after acid solubilization by microwave digestion, can be performed by GFAAS without any interference. The PinAAcle 900Z (Longitudinal Zeeman Furnace only) spectrometer can also be used for this application.

References

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Appendix I – Graphite Furnace Temperature Program

Table 7. Furnace temperature program for Pb.

Analyte	Step	Temp °C	Ramp Time (sec)	Hold Time (sec)	Internal Gas Flow (mL/min)	Gas Type
Pb	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	850	10	20	250	Argon
	4	1600	0	5	0	–
	5	2450	1	3	250	Argon

Table 8. Furnace temperature program for Cd.

Analyte	Step	Temp (°C)	Ramp Time (sec)	Hold Time (sec)	Internal Gas Flow (mL/min)	Gas Type
Cd	1	110	10	30	250	Argon
	2	130	15	30	250	Argon
	3	500	15	35	250	Argon
	4	1500	0	3	0	–
	5	2450	1	3	250	Argon

Table 9. Furnace temperature program for As.

Analyte	Step	Temp (°C)	Ramp Time (sec)	Hold Time (sec)	Internal Gas Flow (mL/min)	Gas Type
As	1	110	5	30	250	Argon
	2	130	20	30	250	Argon
	3	800	15	40	250	Argon
	4	1200	15	30	250	Argon
	5	2200	0	5	0	–
	6	2450	1	3	250	Argon

Appendix II – Calibration Graphs and Linearity

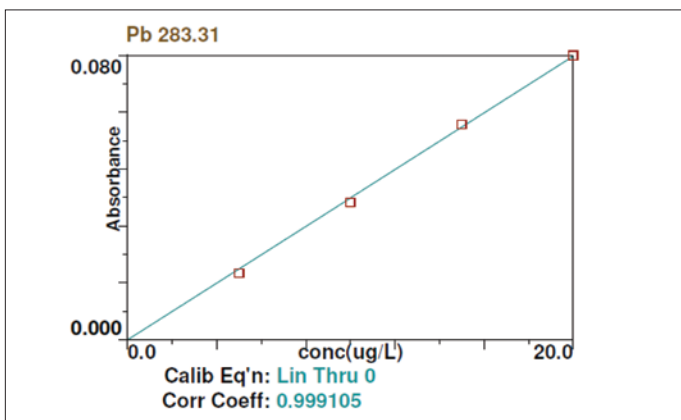


Figure 2. Calibration curve for the determination of Pb using GFAAS.

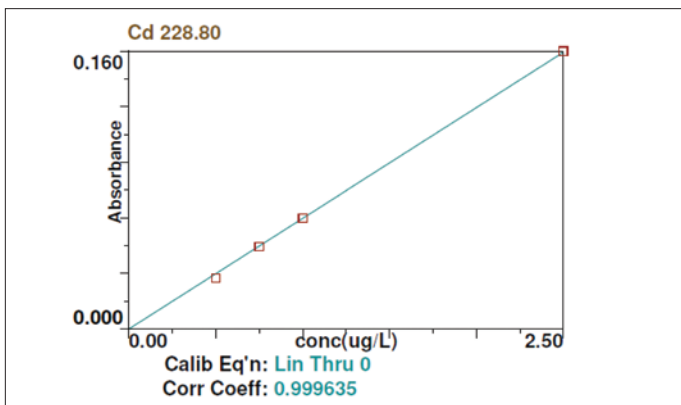


Figure 3. Calibration curve for the determination of Cd using GFAAS.

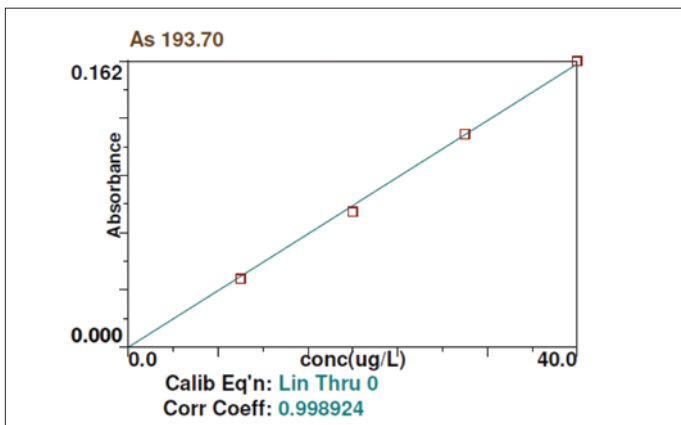


Figure 4. Calibration curve for the determination of As using GFAAS.