

Atomic Absorption

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Analysis of Pb, Cd and As in Tea Leaves Using Graphite Furnace Atomic Absorption Spectrophotometry

application of pesticides, fertilizers or industrial activities. There is often little information available about the safety of tea leaves and finished tea products with respect to heavy metal contamination. Due to the significant amount of tea consumed, it is important to know the toxic metal contents.

The toxicity and effect of trace heavy metals on human health and the environment has attracted considerable attention and concern in recent years. Among the heavy metals, lead (Pb), cadmium (Cd) and arsenic (As) are especially toxic and are harmful to humans even at low concentrations. They have an inherent toxicity with a tendency to accumulate in the food chain and a particularly low removal rate through excretion.³ Exposure to heavy metals above the permissible level can cause high blood pressure, fatigue, as well as kidney and neurological disorders. Heavy metals are also known to cause harmful reproductive effects.⁴

A major challenge in the analysis of tea leaves is the extremely low analyte levels and the very high matrix levels. For many years, graphite furnace atomic absorption spectrophotometry (GFAAS) has been a reliable technique and the preferred method for this analysis. The use of longitudinal Zeeman background correction and matrix modifiers help to achieve extremely low detection limits in high matrix samples such as tea leaves, making GFAAS an indispensable tool for carrying out such analyses.

Introduction

Tea is drunk by about half of the world's population. It is widely cultivated and consumed in Southeast Asia. Tea is rich in many trace inorganic elements.^{1,2} In addition to many essential elements required for human health, some toxic elements may also be present in tea leaves. This could be due to polluted soil,

Experimental Conditions

Instrumentation

The measurements were performed using a PerkinElmer® PinAAcle™ 900T atomic absorption (AA) 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. 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 in other 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 analyses. Pyrolytically coated THGA tubes with end caps (Part No. B3000655) were used for all measurements. The instrumental conditions are given in Table 1, and the graphite furnace temperature programs are

listed in Appendix I (Page 5). Heated injection was used for lead; it can also be used for cadmium and arsenic, if better precision is desired. A high-performance microwave sample preparation system was used for the microwave-assisted digestion (Table 2). The samples were digested using ten 100 mL high-pressure vessels made of PTFE.



Figure 1. PerkinElmer PinAAcle 900T atomic absorption spectrophotometer.

Table 1. Optimized parameters for the analysis of tea leaves using the PinAAcle 900T in GFAAS mode.

Analyte	Pb	Cd	As
Wavelength (nm)	283.3	228.8	193.7
Slit (nm)	0.7	0.7	0.7
Mode	AA-BG	AA-BG	AA-BG
Calibration	Linear through zero	Linear through zero	Linear through zero
Lamp	EDL	HCL	EDL
Current (mA)	440	3	380
Standards (µg/L)	5, 10, 15, 20	0.5, 0.75, 1, 2	10, 20, 30, 40
Correlation Coefficient	0.9991	0.9996	0.9989
Read Time (sec)	3	5	3
Measurement	Peak Area	Peak Area	Peak Area
Injection Temp (°C)	90	20	20
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. Microwave digestion program.

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	
Temp (°C)	180	

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 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. Thirty percent 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% volume/volume 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. The calibration curve correlation coefficient was examined to ensure an $r^2 \geq 0.998$ before the start of the sample analysis.

Three branded tea leaf samples available in Singapore markets (Tieguanyin tea leaves, Japanese green tea leaves and Loong Jin green tea leaves) 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.

Results and Discussions

In GFAAS experiments, obtaining reproducible results is a challenging task, as one has to deal with analytes present at low levels in high matrix samples. The role of the 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 sample introduction into the graphite tube. With the furnace camera, it is easier and simpler to position the tip of the injector to the correct depth inside the tube so as to achieve highly reproducible pipetting. The capability to use full Stabilized Temperature Platform Furnace (STPF) conditions along with longitudinal Zeeman background correction and automatic matrix modification made the analysis of low-level analytes in tea leaves an almost effortless task with little to no influence by the concomitant elements in the sample matrix.

The developed method has been validated by incorporating various quality control (QC) checks (Table 3) and Certified Reference Materials (CRMs) (Table 4). The QC standard gave excellent recovery with a variation over 4 hours of less than the limit of 10% usually prescribed by the regulatory bodies (Table 4). The QC standard analyzed immediately after calibration and the QC standard analyzed at the end of the analysis were in close agreement with each other, demonstrating the long-term stability of the instrument. Method detection limits (MDLs) obtained under routine operating conditions were calculated based on the standard deviation of seven replicates of the reagent blank (Student's t-value = 3.14, $p = 0.02$) (Table 5). The detection limits obtained show the capability of the PinAAcle 900T spectrometer in analyzing difficult matrices at the measured concentrations.

Table 3. Results of a QC sample run over time.

Analyte	T ₀ Measured (µg/L)	T _{+4 hours} Measured (µg/L)	% Recovery
Pb	9.62	10.0	104
Cd	1.08	1.09	100
As	20.2	21.6	107

Table 4. Analysis of certified reference material by GFAAS.

Analyte	NIST® 1568a Rice Flour	
	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 ± 0.01

Table 5. Method detection limits (MDLs).

Analyte	MDL (µg/L)
Pb	0.19
Cd	0.047
As	0.19

Tea leaves 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, which can disturb spectrochemical measurements.⁵ Application of concentrated HNO₃ along with H₂O₂ for mineralization of tea leaves leads to the complete digestion of samples, which is proven by determination of the values of the analytes in the CRM (Table 4). A post-digestion recovery study was done and the results are summarized in Table 6. The recoveries obtained for the post-digestion spikes indicate there was no interference from the matrix towards the analyte signals.

The results in Table 7 show that the level of lead, cadmium and arsenic 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 for edible plant parts. The results confirmed that the determination of arsenic, cadmium and lead in tea leaves, after acid solubilization by microwave digestion, can be performed by GFAAS without any interference.

Table 6. Post-digestion spike recoveries (%).

Analyte	Pb	Cd	As
Tieguanyin tea leaves	102	96	98
Japanese green tea leaves	92	100	99

Table 7. Results for the detection of toxic metals in tea leaf mixtures (mg/kg).

Analyte	Pb		Cd		As	
	1	2	1	2	1	2
Tieguanyin tea leaves	0.68	0.88	0.032	0.026	0.038	0.047
Japanese green tea leaves	0.23	0.27	0.021	0.025	<DL	<DL
Loong Jin green tea leaves	0.88	0.95	0.058	0.064	<DL	<DL
U.S. FDA limit	10		0.3		10	

Conclusions

Toxicity of food materials is of much greater concern today than ever before. In recent years, greater emphasis has been given to toxic-element contents. A method for the accurate determination of arsenic, cadmium and lead in tea leaves using the PinAAcle 900T atomic absorption spectrophotometer in the GFAAS mode after utilizing microwave-assisted sample digestion was developed. Spike recoveries and the analysis of CRM and QC samples showed the method to be accurate, while the MDLs proved the method to be robust and precise. 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 8. Furnace program for lead (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 9. Furnace program for cadmium (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 10. Furnace program for arsenic (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 for Different Analytes

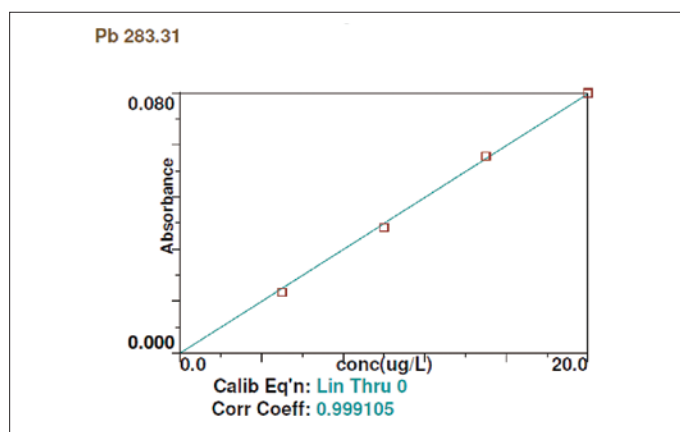


Figure 2. Calibration curve for lead (Pb).

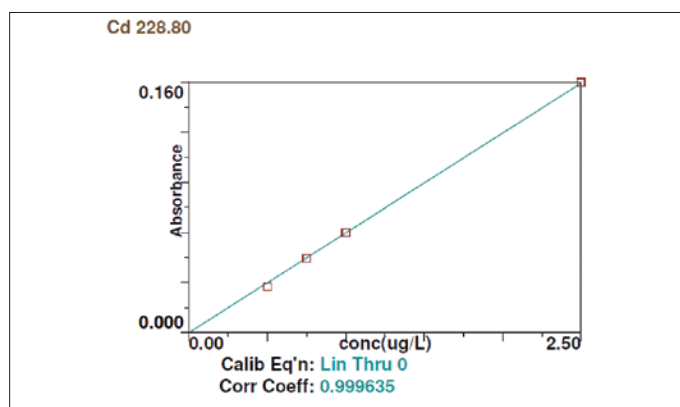


Figure 3. Calibration curve for cadmium (Cd).

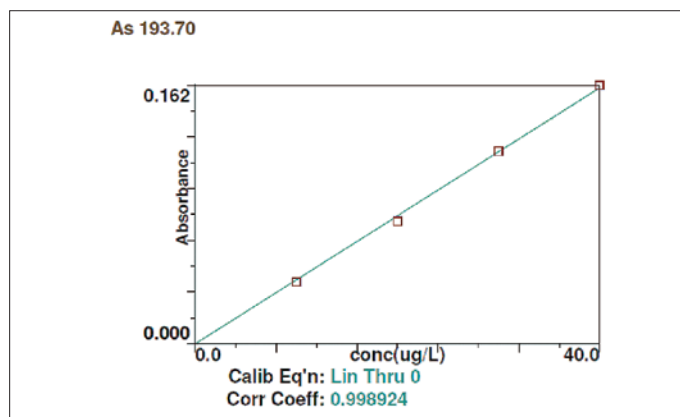


Figure 4. Calibration curve for arsenic (As).