Atomic Absorption

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Fast Digestion Analysis of Lead and Cadmium in Rice Using Graphite Furnace Atomic Absorption

Introduction

Lead (Pb) and cadmium (Cd) are common pollutants in grains and are extremely toxic. Pb is harmful to human organs even at trace levels, and once it accumulates in the body, it causes inhibition of hemoglobin formation and neurological disorders.[1] Cd is even classified as human carcinogen [Group 1 – according to International Agency for Research on Cancer].[2] It is reported that Cd leads to severe kidney problems which can be fatal,[3] and is also associated with brittle bones and liver problems. Rice, as the most widely consumed cereal grain in Asia/China, can quickly pick up Pb and Cd from toxins, pesticides and fertilizers in the soil, thereby endangering the health of millions of people through their diet. Therefore, it is extremely important to develop a simple, reliable method to monitor the levels of Pb and Cd in rice.

According to Chinese national standard GB 2715-2016 Hygienic Standard for Grain, the maximum concentrations of Pb or Cd in grains must be below 0.2 mg/kg; the allowable level in the European Union is the same [EC 1881/2006]. The official technique for the determination of heavy metals in both cases is graphite furnace atomic absorption spectroscopy (GFAAS, GB/T 5009. 12-2017, GB/T 5009-2017. 15 and EN 14083:2003). Samples can be pretreated using various methods, including microwave digestion, hot block digestion, dry ashing, and hot plate digestion. It is found that these conventional digestion procedures are always complicated and time-consuming (two-four hours or even longer). Plus, conventional sample preparation techniques require large quantities of corrosive and oxidizing reagents, increasing the chance for contamination which could lead to inaccurate results. Special PTFE vessels are needed for microwave digestion, however, reusable utensils might also cause cross contamination.

The pretreatment method applied in this experiment is 'fast digestion', a modified version of hot block digestion. Compared to the aforementioned digestions, fast digestion simplifies the pretreatment process with only polypropylene tubes and nitric acid being utilized (Table 1). In this work, a rapid, accurate method for trace-level analysis of Pb and Cd in rice grains is established and verified using fast digestion with GFAAS.

Experimental Conditions

Instrumentation

The measurements were performed using a PerkinElmer PinAAcle 900T atomic absorption (AA) spectrometer (PerkinElmer Inc., Shelton, CT, USA), equipped with the intuitive Syngystix™ for AA software for sample analysis, data reporting, and archiving results. (A PinAAcle 900Z may also be used.) The PinAAcle 900T is equipped with the TubeView™ furnace camera, designed for aligning the autosampler tip in the graphite tube and monitoring residue buildup during sample analysis. The PinAAcle 900T is also equipped with an advanced longitudinal Zeeman furnace for accurate background correction. The unique design of the transversely heated graphite atomizer (THGA) provides uniform temperature distribution across the entire length of the graphite tube, unlike with longitudinal-heating where only certain regions in the tube are uniformly heated. THGA also ensures a faster heating process in the graphite tube. Plus, THGA has been proven to eliminate memory effects and potential interferences that may occur with high-matrix sample analyses, leading to highly accurate results.

Sample Preparation

Microwave digestion, pressure digestion, and wet digestion are popular sample preparation methods for rice analysis. Table 1 shows the reagents, quantities, time, and vessel type used in these sample preparation techniques, as well as in fast digestion. As shown in Table 1, only 1.5 mL of nitric acid is necessary in fast digestion; the pretreatment takes place in disposable plastic vials, which also effectively minimize potential contamination. The use of lower reagent volumes ensures fast digestion and lower costs compared to conventional techniques. Sample preparation can be completed within 0.5 hours by fast digestion method.

In a typical fast digestion cycle, approximately 0.5 g of each sample was accurately weighed in duplicate and transferred to 50 mL polypropylene autosampler tubes for fast digestion. Next, 1.5 mL concentrated nitric acid was added, and the loosely capped vial was then heated in the sample preparation block digestion system (SPB series, PerkinElmer) at 120 °C for 30 minutes. The digested samples were then diluted with DI water and brought up to 25 mL.

Three samples (Samples A, B and C) from proficiency tests in the Shanghai area were measured in this experiment. The certified reference material (CRM) NIST 1568 was also analyzed to validate the digestion and analysis methods. Each sample was subjected to three replicates: two by fast digestion and one by traditional wet digestion. Pb and Cd determination in CRM was replicated three times by fast digestion. The GFAAS analytical conditions were the same throughout the whole process. The instrumental conditions are given in Table 2, and the optimized graphite furnace temperature programs are listed in Tables 3 and 4.

Table 1. Various sample digestion techniques; reagents volume based on 0.2 - 0.5 gram sample.

Digestion Method	Reagent	Time (h)	Vessel
Microwave	5 mL HNO$_3$, 2 mL H$_2$O$_2$	1-2	Reusable PTFE vessel and volumetric flask
Wet	10 mL HNO$_3$, 0.5 mL HClO$_4$	4-5	Reusable beakers and volumetric flask
Fast	1.5 mL HNO$_3$	0.5	Disposable volumetric tubes

Table 2. Instrumental conditions for analyzing Pb and Cd in rice grains on the PinAAcle 900T spectrometer.

Analyte	Lead (Pb)	Cadmium (Cd)
Wavelength (nm)	283.31	228.80
Slit Width (nm)	0.7	0.7
Lamp Type	EDL	EDL
Measurement Type	Peak Area	Peak Area
Read Time (sec)	4	4
Sample Volume (µL)	16	16
Diluent Volume (µL)	16	16
Matrix Modifier	0.05% Pd	0.05% Pd
Matrix Modifier Volume (µL)	5	5
Calibration Equation	Standard Addition	Standard Addition
Standard Concentration (µg/L)	0, 12.5, 25, 37.5	0, 1.25, 2.5, 3.75

Table 3. Optimized temperature programs for analyzing Pb in rice grains on the PinAAcle 900T spectrometer.

Lead (Pb)				
Temp. (°C)	Ramp (s)	Hold (s)	Internal Gas Flow (mL/min)	Gas Type
120	5	30	250	Normal
150	30	30	250	Normal
700	10	20	250	Normal
1800	0	4	0	Normal
2500	1	5	250	Normal

Table 4. Optimized temperature programs for analyzing Cd in rice grains on the PinAAcle 900T spectrometer.

Cadmium (Cd)				
Temp. (°C)	Ramp (s)	Hold (s)	Internal Gas Flow (mL/min)	Gas Type
120	5	30	250	Normal
150	30	30	250	Normal
500	10	20	250	Normal
1500	0	4	0	Normal
2500	1	5	250	Normal

Results and Discussion

The standard addition calibration curves of Pb and Cd are presented in Figure 1, showing an R^2 value ≥ 0.999, demonstrating the linearity of the analysis. The peaks for all the samples and calibration standards are shown in Figure 2. The same peak appearance time and peak shape demonstrates that matrix effects were effectively eliminated by using the standard addition method.

Method detection limits (MDLs) were calculated (Table 5) based on the standard deviation of eleven replicates of the reagent blank and accounted for the dilution factor used in sample preparation. The MDLs are well below the regulated levels and show the capability of the PinAAcle 900T system to measure low concentrations in difficult matrices.
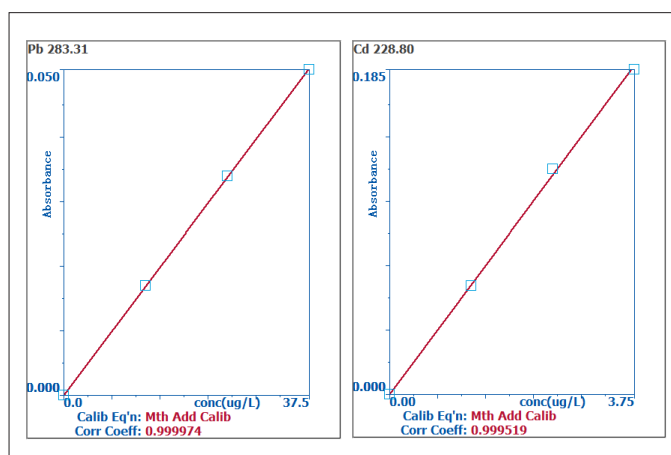

Figure 1. Standard addition curve of Pb (left) and Cd (right) in rice.
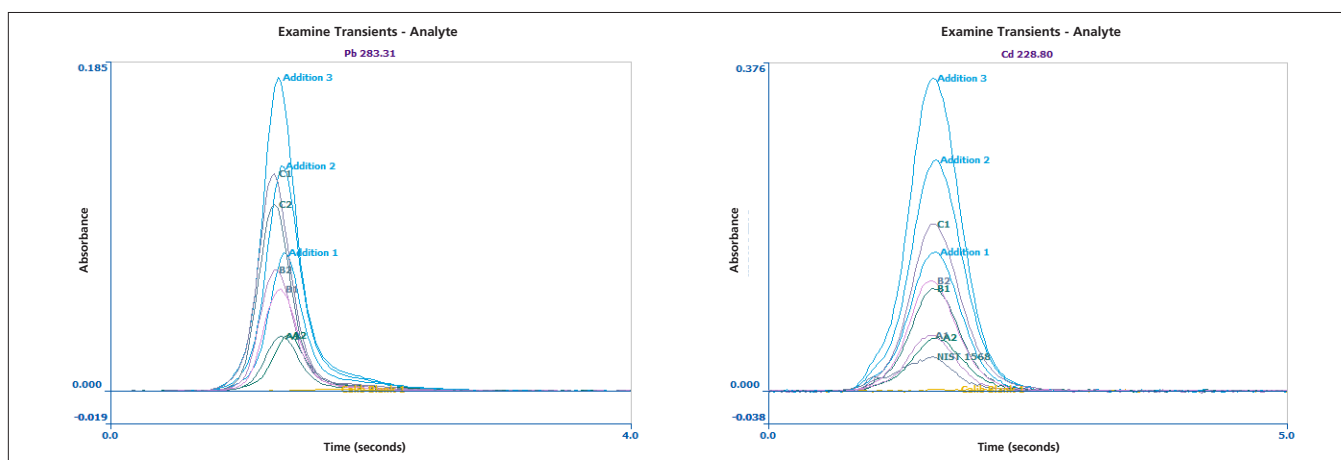

Figure 2. Overlay of Pb (left) and Cd (right) signals in three samples and NIST 1568 and their replicates.

The accuracy and reliability of the methodology was established with the analysis of NIST 1568. Although Pb was not certified in NIST 1568, the measured value of Cd in CRM is 0.028 µg/L, which falls within the confidence limit given by NIST as provided in Table 6.

The results from the analysis of three samples are summarized in Table 7. For the fast digestions (FD), samples were digested and analyzed twice to evaluate reproducibility. The samples were also measured by wet digestion (WD), so that the results of the fast digestion could be compared to a conventional sample preparation technique. For all three samples and concentration ranges, the results agree, demonstrating the accuracy and reproducibility of the fast digestion method. The precision of the results meets the requirement in GB 5009.12 and GB 5009.15 (Deviation ≤ 20%) and comply with (EC) 333/2007 (HorRat$_r$ or HorRat$_R$ values of less than 2 – please find detailed calculation in Reference 4).

Table 5. MDLs using the PinAAcle 900T spectrometer.

Analyte	MDL (3σ, µg/kg)		
	Calculated	Regulated	
Pb	5.4	20[a]	20[c]
Cd	0.8	1.0[b]	20[c]

(0.5 grams sample diluted to 25 mL; [a]: regulated in GB 5009.12-2017; [b]: regulated in GB 5009.15-2014; [c]: regulated in (EC) No 1881/2006).

Table 6. Analysis of NIST 1568 pretreated by fast digestion on the PinAAcle 900T spectrometer.

Analyte	Certified Value (mg/kg)	Measured Value (mg/kg)
Pb	n.a.	n.a.
Cd	0.029 ± 0.004	0.028

Table 7. Results for the detection of Pb and Cd in rice grains (mg/kg).

Sample	Pb (mg/kg)				Cd (mg/kg)			
	FD1	FD2	WD	Deviation*%	FD1	FD2	WD	Deviation*%
A	0.38	0.39	0.40	3.8	0.37	0.36	0.37	1.4
B	0.82	0.81	0.81	0.6	0.71	0.71	0.71	0
C	1.33	1.32	1.20	9.9	1.14	1.15	1.20	4.7

*: Deviation (%) = $\dfrac{|N_1 - N_2|}{N_{avg}} \times 100\%$, where N_1: average of FD1 and FD2; N_2: WD; N_{avg}: average of N_1 and N_2.

Conclusions

Fast digestion followed by GFAAS analysis has been applied to the analysis of Pb and Cd in rice grains. The simplicity of fast digestion can effectively shorten sample preparation time and avoid potential contamination from additional reagent use and reusable vessels. Combined with the automated PinAAcle 900T GFAAS to reduce chemical and matrix interferences, accurate determination of trace-level Pb and Cd is successfully obtained by the standard addition method. This application has the potential to be extended to heavy metal analysis in a variety of other food types, including cereal grains/flour, corn, beans, and milk powder.

References

1. WHO: http://www.who.int/mediacentre/factsheets/fs379/en/

2. IARC: http://monographs.iarc.fr/ENG/Classification/latest_classif.php

3. U.S. ATSDR: https://www.atsdr.cdc.gov/csem/csem.asp?csem=6&po=12

4. Horwitz W., Albert R., J. AOAC Int. 2006 Jul-Aug; 89(4):1095-109. 'The Horwitz ratio (HorRat): A useful index of method performance with respect to precision'

Consumables Used

Component	Description	Part Number
Sample Preparation Block	SPB 50-24, 24 position 50 mL 115/230 V SPB 50-48, 48 position 50 mL 115/230 V	N9300802 N9300803
Cd Lamp	Electrodeless Discharge Lamp (EDL)	N3050615
Pb Lamp	Electrodeless Discharge Lamp (EDL)	N3050657
Graphite Tubes	Pyrocoated graphite tubes with advanced platform	N9307834 (5-pack) N9307835 (20-pack)
	Pyrocoated graphite tubes with integrated platform	B3001262 (5-pack) B3001264 (20-pack) N9300651 (40-pack)
Cd Standard	1000 ppm, matrix 2% HNO_3	N9300176 (125 mL) N9300107 (500 mL)
Pb Standard	1000 ppm, matrix 2% HNO_3	N9300175 (125 mL) N9300128 (500 mL)
Modifier Pd $(NO_3)_2$	1% Pd, 50 mL	B0190635
Conical Centrifuge Tube	50 mL - Qty 500	B0193234

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