



APPLICATION NOTE

Atomic Absorption

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Determination of Lead and Cadmium in Foods by Graphite Furnace Atomic Absorption Spectroscopy

Background

Humans can be exposed to heavy metals through a variety of means, including consumption of contaminated food. Although heavy metals are usually present in foods at very low levels, long-

term exposure can have negative health impacts. Two of the more important toxic elements that must be monitored are cadmium (Cd) and lead (Pb), which can enter food either through environmental processes or through contamination in processing and/or packaging. As a result, it is very important to accurately measure low levels of Cd and Pb in a variety of food matrices.

A major challenge in the analysis of food samples is the extremely low analyte levels and the very high matrix levels. For many years, graphite furnace atomic absorption spectroscopy (GFAAS) has been a reliable technique and the preferred method for this analysis, especially for the determination of Cd and Pb.

In the past few years, a number of instrumental developments have contributed to providing more reliable results and better detection limits for trace determination of lead and cadmium by GFAAS. These include improved electrodeless discharge (EDL) and hollow cathode (HCL) lamps for increased light output, and improved wet ashing sample preparation techniques (e.g., microwave digestion).

This work will focus on the use of GFAAS for the determination of lead and cadmium in a variety of food samples.

Experimental

Atomic Absorption Instrumentation

A PerkinElmer PinAAcle™ 900H atomic absorption (AA) spectrometer (Figure 1) was used for all analyses. This instrument was equipped with a Massman-type/HGA graphite furnace and deuterium continuum source background correction, AS900 autosampler, water re-circulator system, high-speed automatic wavelength drive, automatic lamp selection, and EDL power supply. The use of cutting-edge fiber optics in the PinAAcle 900 spectrometers maximizes light throughput for improved detection levels. Syngistix™ for AA software was used and includes a Method Development module which automates the optimization of the temperature program for each element in a specific matrix (previous-generation WinLab™ for AA software would provide equivalent capabilities and results). The instrument's TubeView™ furnace camera (Figure 2) is extremely useful for the user in adjusting the pipette tip to the most appropriate depth in the graphite tube and also for monitoring any residue buildup on the platform during the progress of the analysis. The furnace camera was also used during method development of the temperature program to verify the drying steps, ensuring that sample boiling or spattering does not occur.



Figure 1. PerkinElmer's PinAAcle 900H atomic absorption spectrometer with AS900 furnace autosampler.



Figure 2. TubeView furnace camera on PerkinElmer's PinAAcle 900H, showing the optimized sampler tip depth inside the tube.

Reagents

All solutions were prepared in polypropylene volumetric flasks using ultra-pure deionized (DI) water. Other reagents used include:

1. Nitric acid Ultrapure 10 (TAMAPURE, Tama Chemicals Co., Kanagawa Japan)
2. Hydrogen peroxide: Ultrapure (30%), (Kanto Chemical Co., Tokyo, Japan)
3. Lead (Pb) and cadmium (Cd) stock solutions, 1000 mg/L (PerkinElmer). Cadmium and lead working solutions were prepared fresh daily by dilution of the cadmium and lead stock solutions with 2% (v/v) nitric acid.
4. Matrix modifier: mixtures of palladium [Pd(NO₃)₂] and magnesium nitrate [Mg (NO₃)₂ · 6H₂O] in 10 % nitric acid solution for Pb analysis and ammonia phosphate [NH₄H₂PO₄] and magnesium nitrate [Mg (NO₃)₂ · 6H₂O] in 10% nitric acid solution for Cd analysis.

Sample Preparation

The samples were conveniently and rapidly digested in a microwave oven using standard pressure vessels which were pre-cleaned by rinsing with ultrapure nitric acid prior to use.

All sample weighings were carried out in a Class 100 laminar flow cabinet. Samples of 300 mg each were accurately weighed in the digestion vessels, followed by the addition of 7 mL concentrated double-distilled nitric acid. Eight vessels were placed into the rotor and heated in the microwave oven according to the temperature program shown in Table 1.

Table 1. Temperature program for microwave.

Step	Temp (°C)	Pressure (Bar)	Ramp (min)	Hold (min)	Power (%)
1	150	30	5	10	80
2	200	30	5	10	100
3	50	30	0	20	0

The rotor was removed from the microwave oven and allowed to cool to room temperature. The vessels were carefully opened in a fume cupboard and the inner walls rinsed with DI water. The final volume of each sample was made up to 20 mL with 1 mL hydrogen peroxide and water.

GFAAS Determination of Lead and Cadmium

The wavelength and GFAAS instrument parameters for the determination of lead and cadmium are listed in Table 2. Pyrolytically coated integrated platform HGA graphite tubes were longitudinally heated. Calibrations were performed using external standards (blank, five calibration standards). All analyses were performed with triplicate firings. During an analytical series, a mid-range QC standard solution was injected every 10 analytical sample solutions to verify the calibration slope. QC readings can be plotted to monitor the trend using the software's built-in QC Charting Wizard, as shown in Figure 3. Recovery checks were carried out using system spikes on every sample.

Table 2. Instrument settings used on the PinAAcle 900H.

Element	Wavelength (nm)	Slit (nm)	Lamp Type	Lamp Current (mA)	Read Delay (sec)	Read Time (sec)
Cd	228.8	0.7	HCL	4	0.5	2.5
Pb	283.3	0.7	HCL	10	0.5	3.5

Baseline offset correction (BOC) was five seconds for all analytes.

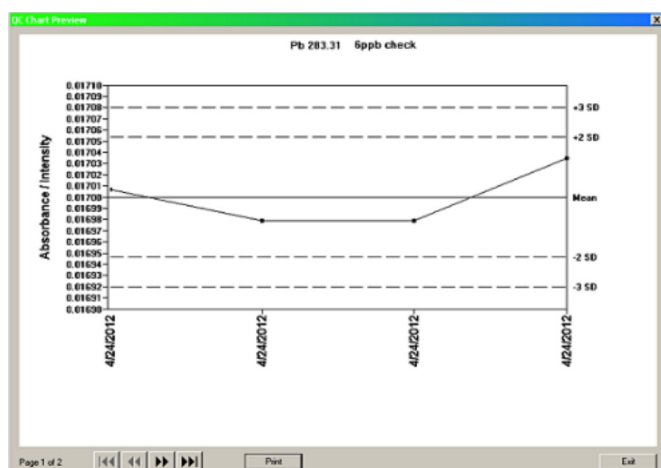


Figure 3. QC Charting of lead (Pb) QC samples.

Results and Discussion

Graphite Furnace Method Development

The aim is to use a single matrix modifier for both elements to simplify the analytical process. The main purpose of a matrix modifier is to volatilize the matrix during the pyrolysis while increasing the stability of the element. This ensures almost interference-free analysis whereby the element is separated from the potential interference. As a result, aqueous calibration standards can be used; the method of standard additions is not required. The best results obtained in terms of peak profile and recoveries were with a 5 μ L mixture of 0.06% magnesium nitrate and 0.1% palladium for Pb mixture, while a 5 μ L mixture of 0.1% ammonia phosphate and 0.06% magnesium nitrate gives better peak profile and recovery for Cd. In both cases, the non-specific background was somewhat higher than without modifier. The peak profiles for Pb and Cd standards are shown in Figures 4 and 5.

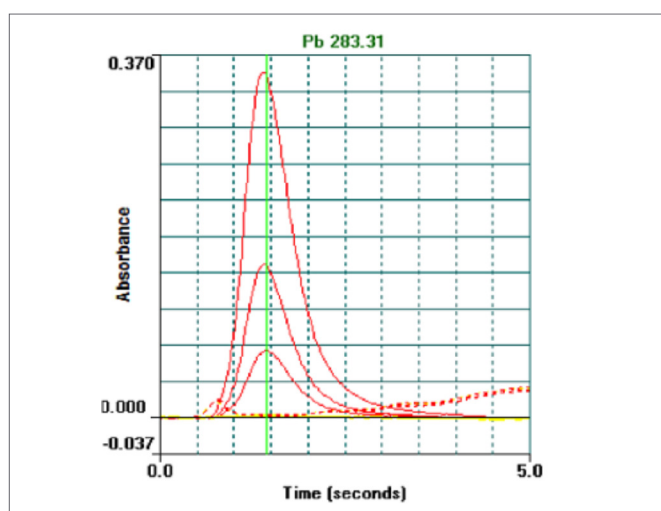


Figure 4. Overlay of spectral profiles of Pb standard solutions at 6 μ g/L, 16 μ g/L, and 40 μ g/L.

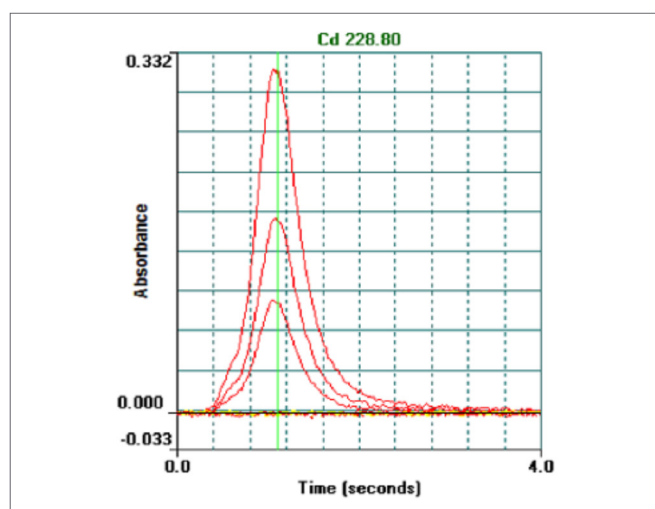


Figure 5. Overlay of spectral profiles of Cd standard solutions at 0.5 μ g/L, 1 μ g/L, and 2 μ g/L.

With respect to the GFAAS temperature programs, the final pyrolysis and atomization temperatures adopted are listed for lead and cadmium in Tables 3 and 4.

Table 3. Furnace temperature program for lead (Pb).

Temp ($^{\circ}$ C)	Ramp (sec)	Hold (sec)	Internal Flow (mL/min)
110	5	25	250
130	15	20	250
800	10	20	250
2000	0	5	0
2600	1	3	250

Table 4. Furnace temperature program for cadmium (Cd).

Temp ($^{\circ}$ C)	Ramp (sec)	Hold (sec)	Internal Flow (mL/min)
110	5	25	250
130	15	25	250
850	10	20	250
1650	0	5	0
2600	1	5	250

Calibration Range

Lead was calibrated at 6 μ g/L (ppb), 16 μ g/L (ppb) and 40 μ g/L (ppb) with 0.003 mg $\text{Mg}(\text{NO}_3)_2$ + 0.005 mg Pd, resulting in a calibration correlation coefficient of > 0.995, as shown in Figure 6a.

Cadmium was calibrated at 0.5 μ g/L (ppb), 1 μ g/L (ppb) and 2 μ g/L (ppb) with 0.05 mg $\text{NH}_4\text{H}_2\text{PO}_4$ + 0.003 mg $\text{Mg}(\text{NO}_3)_2$, resulting in a calibration correlation coefficient of > 0.995, as shown in Figure 6b.

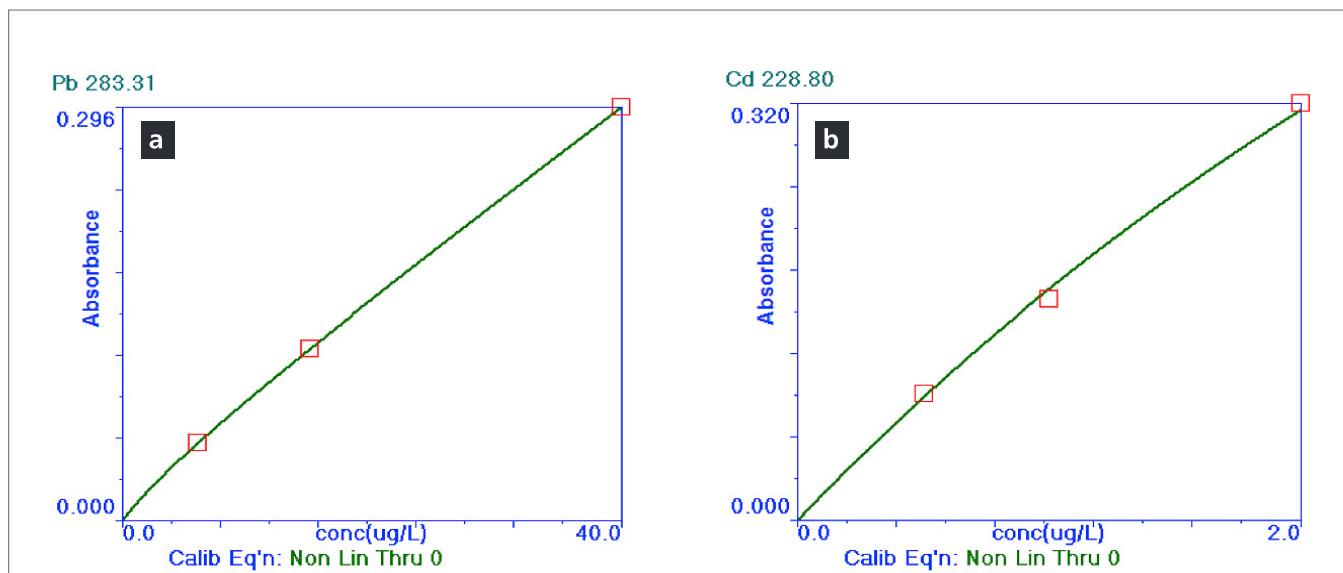


Figure 6. Calibration curves for Pb (a) and Cd (b).

Limits of Detection and Quantification

An important aspect of the method performance evaluation is the calculation of the limits of detection. The limits of detection, based on the repeated analysis of blank solutions, were calculated as instrument detection limit (IDL), while the average standard deviation of repeated analysis of sample blanks (or samples containing very low concentration of the analytes) were calculated as the method detection limit (MDL). All detection limits were obtained by analyzing 10 blank/samples each; the results are shown in Table 5.

Table 5. IDL, MDL and Linear Range using the PinAAcle 900H.

Element	IDL (µg/L)	MDL (µg/L)	Linear Range (µg/L)
Cd	0.03	0.08	2.50
Pb	0.3	0.4	100

Result and Recovery

To ascertain the accuracy of the results obtained with the developed method, spike recoveries were carried out on the samples. An 8 µg/L system spike was used for Pb, while a 0.5 µg/L system spike was performed on Cd. The results and recoveries are tabulated in Tables 6 and 7 for Pb and Cd, respectively. Both show recoveries of 100% ± 15%, well within the tolerance for complex food sample matrices.

Table 6. Sample recoveries for Pb.

Sample	Sample Result (µg/L)	Sample Spike (µg/L)	% Recovery
Milk	1.42	8.68	90.7
Mushroom	5.72	13.9	95.7
Coffee 1	2.37	9.73	92.0
Coffee 2	2.57	9.59	87.8
Soybean	4.37	12.4	99.8
Cooking Oil	1.24	9.11	98.5

Table 7. Sample recoveries for Cd.

Sample	Sample Result (µg/L)	Sample Spike (µg/L)	% Recovery
Milk	1.98	3.75	88.6
Mushroom	3.80	5.85	102
Coffee 1	1.56	2.07	103
Coffee 2	1.69	2.17	94.9
Soybean	1.97	2.44	94.9
Cooking Oil	<MDL	0.44	87.8

Conclusion

The PerkinElmer PinAAcle 900H AA spectrometer features a closed-furnace design that is sealed at both ends with easily removable bayonet-mount windows. The closed design is a prerequisite for controlled, reproducible analytical conditions. The separate, independently controlled external and internal gas streams provide maximum flexibility, tube life and sensitivity.

The PinAAcle 900H system uses enhanced power control circuitry to maintain a uniform heating rate, so no matter where a system is located, it provides outstanding and consistent performance. The PinAAcle 900H, equipped with an HGA graphite furnace, has demonstrated its capabilities in handling different digested food sample matrices. The system provides the superior sensitivity required for heavy-metal testing in food matrices, with excellent accuracy, as observed from the recovery studies.

The TubeView furnace camera is a handy tool to determine the optimized drying temperature and holding time for complete dryness prior to the pyrolysis step. Through the aid of Method Development software and TubeView, the PinAAcle 900H AA spectrometer makes it faster and easier to get from sample to results by reducing the time required for method development.

The AS900 furnace autosampler was used to ensure accurate delivery of the sample, and the probe did not require any cleaning or maintenance between analytical runs to prevent carryover from high-level samples. The autosampler can also provide automated spiking of samples for recovery checks. Results from QC samples, standards, or any sample can be plotted using the software's QC Charting Wizard. Limit ranges, means, or expected values can be included on the chart. Quality control charts can be quickly and easily prepared.

Consumables Used

Component	Description	Part Number
Cadmium lamp	Electrodeless discharge lamp (EDL)	N3050615
Lead 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)
Autosampler cups	1.2 mL polypropylene (2000)	B0510397
Cadmium standard	1000 ppm	N9300176 (125 mL) N9300107 (500 mL)
Lead standard	1000 ppm	N9300175 (125 mL) N9300128 (500 mL)
Mg(NO ₃) ₂	1% Mg, 100 mL	B0190634
Pd(NO ₃) ₂	1% Pd, 50 mL	B0190635
NH ₄ H ₂ PO ₄	10%, 100 mL	N9303445

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