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Application News

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Spectrophotometric Analysis

Measurement of Heavy Metals (Cd, Pb) in Pet Food by AAS

Introduction

In 2007, an incident occurred in which pet food was found to contain melamine that had been mixed in with the pet food in the country where it was produced. However, this was discovered only after many pet cats and dogs died as a result of melamine poisoning. As a result of this incident, the Japanese Ministry of the Environment and the Ministry of Agriculture, Forestry and Fisheries established a study group to address this situation, and in 2008, the Act on Ensuring of Safety of Pet Animals Feed was enacted. Based on this law, an ordinance pertaining to standards of pet animal feed components was promulgated (Amendment, September 1, 2011, Ministry of Agriculture, Forestry and Fisheries, Ministry of Environment, Ordinance No. 3), and has been in effect since March 1, 2012. Also cited in this ordinance are mycotoxins, organochlorine compounds, and three heavy metal elements, cadmium, lead and arsenic. The concentrations of heavy metals in pet foods for sale are required to meet the criteria of Table 1, assuming a 10 % moisture content.

Table 1 Regulated Values for Heavy Metals in Pet Food

Cd	1 ppm or less
Pb	3 ppm or less
As	15 ppm or less

Here, we introduce an example of analysis of Cd and Pb in pet food using the AA-7000 Atomic Absorption Spectrophotometer.

Sample Preparation

The sample consisted of dry pet food that was finely ground in a food processor. Sample digestion for electrothermal atomic absorption was conducted using the ETHOS One microwave sample preparation system (Milestone Srl). The digestion process flow is shown in Fig. 1. For validity assessment, the same preparation process was conducted on a sample spiked with standard solution prior to digestion. Preparation was conducted so that the spiked solid concentrations were 0.5 ppm of Cd and 1 ppm of Pb.

Transfer 0.5 g sample to digestion container.
↓
Add small amount of distilled water and 8 mL nitric acid, then mix.
↓
Seal container, set in ETHOS One, digest for approx. 1 hour.
↓
After cooling, transfer to a vessel, add distilled water to a volume to 50 mL.
↓
Conduct measurement by electrothermal atomic absorption.

Fig. 1 Flow Chart of Sample Digestion for ETAAS (Electrothermal Atomic Absorption Spectrometry)

Processing for flame measurement was conducted according to the Test Method for Pet Animal Feed (established by the Food and Agricultural Materials Inspection Center, the Ministry of Agriculture, Forestry and Fisheries of Japan, No. 1764, September 1, 2009). Fig. 2 shows the flow chart for sample digestion, and Fig. 3 the flow chart for solvent extraction for Flame AA, respectively. The solvent extraction process is intended for removal of coexisting substances and the concentration of analyte elements.

Transfer 10 g of sample to 100 mL tall beaker.
↓
Ashing by electric furnace (up to 500 °C maximum)
↓
Let cool, then add 10 mL HCl, and water to about 30 mL.
↓
Heat for several minutes.
↓
Let cool, adjust volume to 100 mL.
↓
Collect filtrate (six filter paper) and use as sample.

Fig. 2 Flow Chart of Sample Digestion for Flame AA

Transfer 30 mL sample solution to separatory funnel containing 14 mL phosphoric acid.
↓
Add 5 mL potassium iodide solution (68 w/v%) and purified water to bring volume to about 50 mL.
↓
Gently shake to mix, and let stand 5 minutes.
↓
Accurately add 10 mL MIBK, and after shaking vigorously, let stand.
↓
Collect MIBK layer in test tube and measure by Flame AA.

Fig. 3 Flow Chart of Sample Extraction for Flame AA

Analytical Method and Conditions

The standard solution for electrothermal measurement by atomic absorption analysis was prepared by diluting a 1000 mg/L standard solution to obtain 2 ppb (µg/L) of Cd and 20 ppb of Pb (µg/L). A calibration curve was generated using an autosampler to prepare stepwise increasing injection volumes. In addition, 5 µL of a palladium nitrate solution (100 ppm (mg/L) palladium content) was added as a matrix modifier to all of the samples.
The standard solution used for the flame AA method was prepared using solvent extraction similarly as for the sample solution. The main conditions that were used for the spectrometer and atomization are shown in Tables 2 - 4.

Table 2 Optics Parameters

	Cd	Pb
Analytical wavelength	228.8 nm	283.3 nm
Slit width	0.7 nm	
Ignition mode	BGC-D2	

Table 3 Atomizing Conditions for ETAAS

	Cd	Pb
Ashing temperature	600 °C	900 °C
Atomizing temperature	2200 °C	2400 °C
Standard solution oncentration (ppb)	0.5, 1.0, 2.0	5, 10, 20
Tube type	Platform	
Matrix modifier	5 µL of 100 ppm palladium nitrate	

Table 4 Atomizing Conditions for Flame AA

	Cd	Pb
Flame type	Air – Acetylene	
Acetylene flowrate	0.8 L/min	
Standard solution concentration (ppm)	0.05, 0.10, 0.20	0.25, 0.50, 1.00

The standard solution concentration refers to the concentration in the solvent.

Results and Conclusion

Table 5 shows the sample measurement results. The values were converted to indicate the concentrations in the solid sample. Table 6 shows the lower limits of quantitation. The calculated concentrations in the solid sample are based on the absorbance values of 0.01 Abs and 0.004 Abs obtained using the Electrothermal and Flame methods, respectively.
Fig. 4 and 5 show the calibration curves and measurement solution peak profiles by the Electrothermal method.

Table 5 Measurement Results for Cd and Pb in Dog Food

Element	Cd	Pb
Reference value	1 ppm or less	3 ppm or less
Electrothermal method	0.19 ppm (94 %)	0.26 ppm (106 %)
Flame method	0.20 ppm	< 0.3 ppm

Values in parentheses indicate spike/recovery rate.

Table 6 Limit of Quantitation of Cd and Pb

Element	Cd	Pb
Electrothermal method	0.003 ppm	0.07 ppm
Flame method	0.01 ppm	0.3 ppm

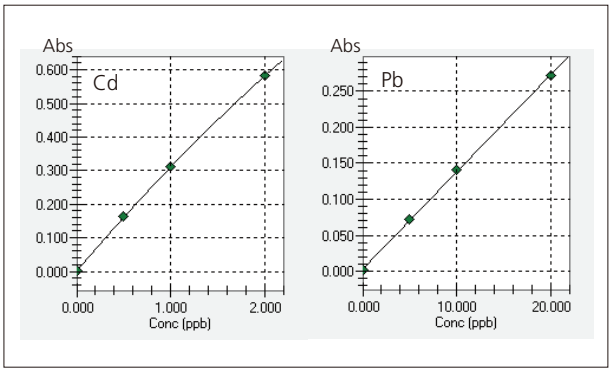

Fig. 4 Calibration Curves by ETAAS

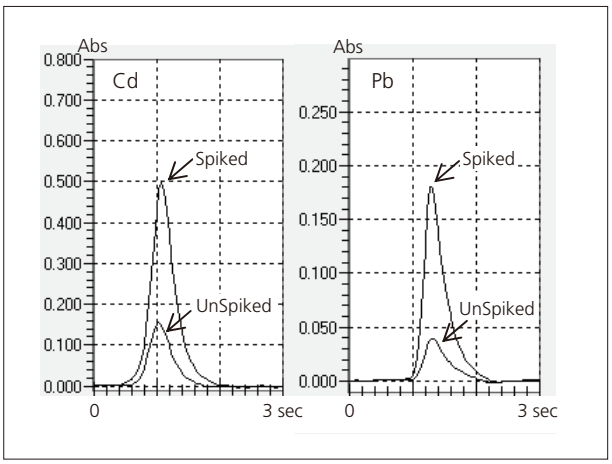

Fig. 5 Peak Profiles of Sample and Spiked Sample by ETAAS

The measurement results obtained using microwave digestion – electrothermal atomic absorption were in good agreement with the measurement results obtained by the method prescribed in the Test Method for Pet Animal Feed, and excellent spike and recovery results were also obtained. Further, compared to the dry ashing – solvent extraction pretreatment operations, preparation of the measurement solution by microwave digestion can be accomplished more quickly, and combined with the electrothermal absorption method using the AA-7000, heavy metals could be analyzed with high sensitivity.
Not only does the AA-7000 support analysis by the flame and electrothermal methods, the lineup includes a model that permits automated switching between both atomization methods to accommodate a wide range of requirements.

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