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APPLICATION NOTE 44371

Determination of lead in powdered milk using the Thermo Scientific iCE 3500 AAS

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Keywords

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Goal

The goal of this application note is to demonstrate a simple method for the analysis of lead in powdered milk without the need for sample digestion.

Introduction

Powdered milk is produced by the dehydration of milk and is used for a variety of products such as, standard milk powder, powdered infant formula and food supplements. Powdered infant formula is used as substitute for breast milk and as a follow on food product once the infant is weaned. For this reason the production and quality of the powdered milk is closely monitored to ensure product safety. In many countries not only does the powdered milk have to meet food quality standards, it must also meet certain nutraceutical and drug safety standards. For example in China the following standards must be met:

GB 23790-2010 National food safety standard – Good manufacturing practice for powdered formulae for infants and young children

GB 10765-2010 National food safety standard – Infant formula

GB 10767-2010 National food safety standard – Infant formula

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One of the toxic elements specified in both GB 10765-2010 and GB 10767-2010 is lead, which must not be present in infant formula at concentrations greater than 0.15 mg·kg^{-1}. If the infant is exposed to concentrations greater than this then possible effects will include damage to the central nervous system, digestive system as well as the hematopoietic function of bones.

Instrumentation

The Thermo Scientific™ iCE™ 3500 Atomic Absorption Spectrometer (AAS) was used for the analysis. The iCE 3500 AAS is a dual atomizer with flame and graphite furnace option, for this analysis the graphite furnace was used. The graphite furnace has both Zeeman and deuterium background correction and is capable of heating the sample at rates of up to 3000 ˚C per second, regardless of cuvette age. The instrument parameters are shown in Table 1 and Figure 1.

Table 1. Instrument parameters.

Parameter	Setting
Analysis Wavelength	282.2 nm
Band Pass	0.5 nm
Signal Measurement Mode	Peak height, high-speed signal acquisition mode
Background Correction	Zeeman
Type of Graphite Tube	Normal electro graphite tube

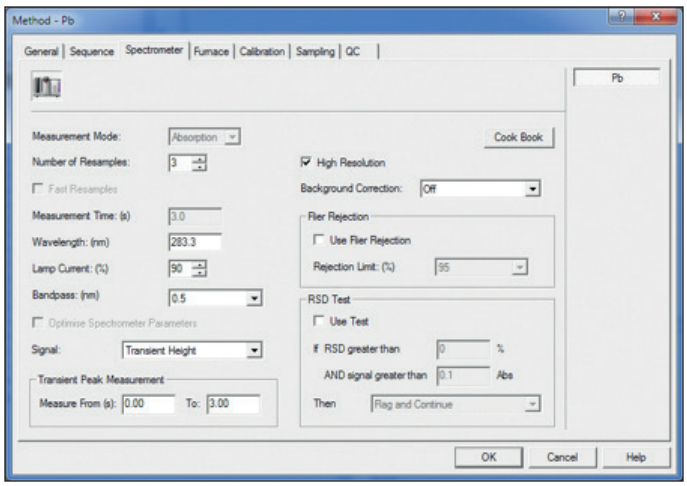

Figure 1. Spectrometer parameters.

Sample and standard preparation

The powdered milk samples were accurately weighed (1.0 g) in to a 15 mL centrifuge tube and Triton™ X-100 solution (approximate 5 mL of 0.2% (m/v)) was added. The sample tubes were sealed and mixed by vortex oscillation. The tubes containing the samples were then placed in a sonic bath and sonicated for a period of 1 hour after which the samples were made to volume (10 mL) with Triton X-100 solution (0.2% (m/v)). The samples were then mixed a final time by vortex oscillation prior to analysis. The same procedure was repeated with just Triton X-100 solution to act as a reagent blank.

Triton X-100 solution (0.2% m/v) was used to make 0 ng·mL^{-1}, 4 ng·mL^{-1}, 8 ng·mL^{-1}, 12 ng·mL^{-1}, 16 ng·mL^{-1} and 20 ng·mL^{-1} Pb standard solutions. Since the iCE 3500 AAS is equipped with a high-precision autosampler with an auto-dilution functionality, only one standard solution of 20 ng·mL^{-1} had to be prepared manually and other standards were diluted from it automatically to the corresponding concentration by using the Thermo Scientific™ SOLAAR™ Software (Figure 2).

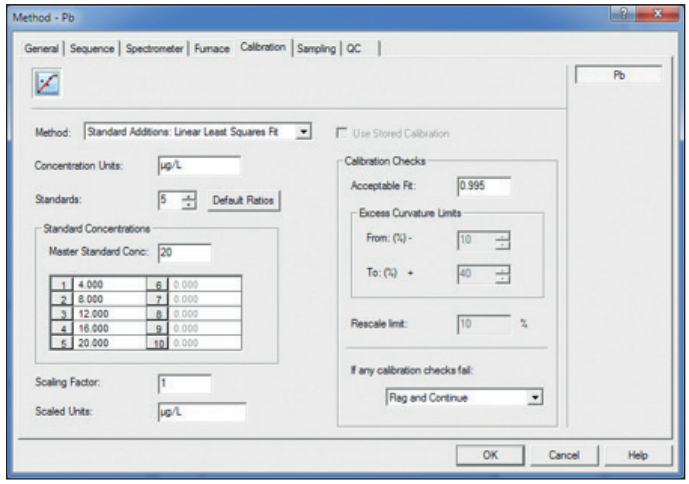

Figure 2. Calibration setup in SOLAAR Software.

Reagents

- Pure HNO_3, 65% v/v (Fisher Scientific)
- Triton X-100, made to 0.2% m/v solution (CAS9002-93-1, Sigma Aldrich)
- Lead (Pb) standard solution, 1000 µg·mL^{-1} (National Research Center for Certified Reference Materials, China; NRCCRM)
- Ammonium dihydrogen phosphate (APD)
- Oxalic acid
- Palladium chloride, made to 1% palladium nitrate solution (m/v)
- Matrix modifier: 0.5% APD (m/v) + 0.1% palladium nitrate (m/v), 0.2% m/v Triton X-100 solution medium; or 0.5% Oxalic acid + 0.1% palladium nitrate (m/v), 0.2% m/v Triton X-100 solution medium
- Cleaning solution, 2%-5% v/v made from 0.2% m/v Triton X-100, to clean the autosampler needle

Method development

For initial method development, a mixture containing ammonium dihydrogen phosphate (APD) and palladium nitrate was used as matrix modifier, which avoids the influence of the matrix effectively as well as the loss of lead during the ashing procedure in all kinds of milk powder.

For maximum extension of the number of uses of the graphite tube, a certain amount of compressed air was added during the pre-ashing procedure to remove the deposition of carbon in the graphite tube.

The following temperature program was used for this method development (Figure 3):

1. Sample injection temperature: 0 ˚C
2. First step drying temperature: 85 ˚C, keep for 15 s, heating rate: 5 ˚C·s^{-1}, inert gas: 0.2 L·min^{-1}
3. Second step drying temperature: 105 ˚C, keep for 15 s, heating rate: 10 ˚C·s^{-1}, inert gas: 0.2 L·min^{-1}
4. Pre-ashing temperature: 450 ˚C, keep for 5 s, heating rate: 50 ˚C·s^{-1}, compressed air: 0.2 L·min^{-1}
5. Ashing temperature: 600 ˚C, keep for 15 s, heating rate: 150 ˚C·s^{-1}, inert gas: 0.2 L·min^{-1}
6. Atomization temperature: 1400 ˚C, atomization time 3 s, heating rate: reach the point directly, inert gas: 0.0 L·min^{-1}
7. Cleaning residual temperature: 2600 ˚C, keep for 3 s, inert gas: 0.2 L·min^{-1}

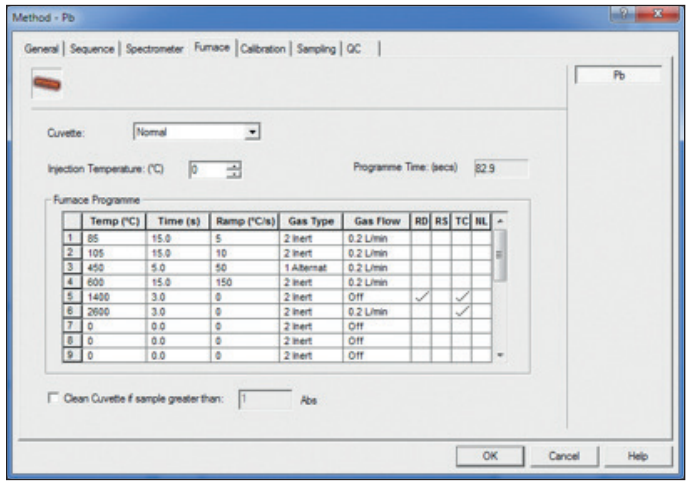

Figure 3. Furnace temperature program.

Results

GBW08509a national standard skimmed milk powder was selected as reference material which was used to control the accuracy of the developed analysis' method. Two kinds of real milk powder samples were spiked and analyzed to verify the recovery of the method.

The analysis results of GBW08509a indicate that the reproducibility of the measurement between 100.4% – 101.3% for lead is quantitative and close to the standard recommended values. Furthermore, the precision of the two parallel reference materials is 0.5%.

The spike recoveries for each repeat of the two real samples were 97.8% and 107.4%, they all easily meet the method validation requirement using the iCE 3500 AAS (Table 2).

Table 2. Analysis results.

Sample	Weight (g)	Volume (mL)	Absorbance (Abs)	Concentration (µg·L^{-1})	Measured value (mg·kg^{-1})	Expected value (mg·kg^{-1})	Accuracy (%)
GBW08509a	1.0000	10	0.0468	2.4104	0.0241	0.024 ± 0.001	100.4
GBW08509a	1.0000	10	0.0469	2.4286	0.0243	0.024 ± 0.001	101.3
Infants	1.0000	10	0.0484	2.7009	0.0270	---	---
Infants	1.0000	10	0.0477	2.5829	0.0258	---	---
German Brand	1.0000	10	0.0461	2.2773	0.0228	---	---
German Brand	1.0000	10	0.0453	2.1381	0.0214	---	---
Infants + 2 µg·kg^{-1} spike	1.0000	10	0.0582	4.4891	---	Recovery: 97.8%	
Infants + 2 µg·kg^{-1} spike	1.0000	10	0.0595	4.7070	---		
German Brand + 2 µg·kg^{-1} spike	1.0000	10	0.0575	4.3590	---	Recovery: 107.4%	
German Brand + 2 µg·kg^{-1} spike	1.0000	10	0.0575	4.3530	---		

Discussion

Due to its material properties, the prepared milk powder sample solutions should stand for more than 5 hours to create a stable emulsion. Once a stable emulsion has been formed, an appropriate temperature needs to be maintained in order to avoid fermentation. The solutions can be retained for over 48 hours without significant changes.

Besides, during the preparation of the sample solution, heating and adding acidic solution is not recommended. This prevents solidification and precipitation of the proteins, but it can affect the stability of the solution and the accuracy of the measurement results.

To prove stability of the sample solutions, the sample was analyzed after 24, 48 and 72 hours. The measurement results in Table 3 show, that the standard deviation of the three measurements was less than 10%, which indicates good stability of the solution (Table 3).

During direct injection analysis of milk powder samples the first problem which needs to be solved is the deposition of carbon in the graphite tube during the ashing procedure. Otherwise there will be more and more carbon with a porous structure deposited in the tube and this will finally lead to a foaming effect of the solution when heated to the next step, which seriously influences the measurement reproducibility (see Figure 4). Because of the deposition in the graphite tube, the effective volume will keep decreasing until the light pass is blocked and the measurement is terminated completely.

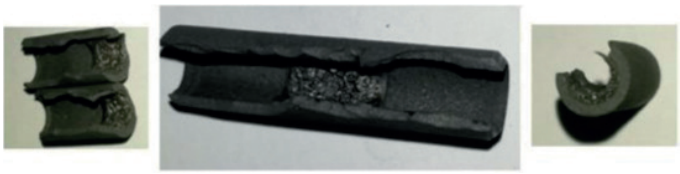

Figure 4. Deposition of carbon in the graphite tube without adding compressed air during pre-ashing step (after 70 runs).

By inserting a pre-ashing procedure at 450 °C while letting compressed air flow through the graphite tube at 0.2 $L \cdot min^{-1}$ for 5 s duration time, all residual carbon can be burned of effectively. However, an over-introduction of compressed air inside the tube should be avoided because the tube could over-oxidize and lifetime will be decreased. The GFTV images in Figure 5 show that after 300 injections no carbon deposition or over-oxidation in the graphite tube occurred during the pre-ashing procedure.

Furthermore, an appropriate amount of Triton X-100 solution has to be added into the rinse solution to avoid blockage of the autosampler needle during direct injection analysis of milk powder sample solutions.

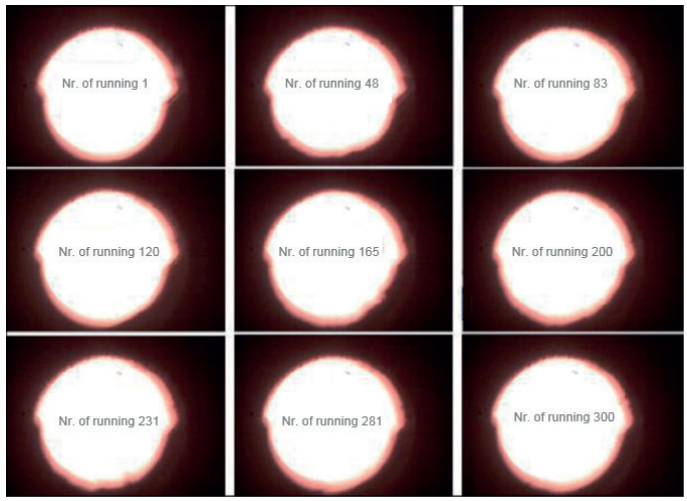

Figure 5. GTFV pictures showing that after 300 injections no carbon deposition or over-oxidation occurred in the graphite tube.

Table 3. Results of repeated measurements of one sample to show stability of solution.

Time (h)	Measurement time	Absorbance	Concentration ($mg \cdot kg^{-1}$)	Average ($mg \cdot kg^{-1}$)	Recommended value ($mg \cdot kg^{-1}$)	Matrix modifier
24	2015/3/13 11:00	0.0468	0.0241	0.0242	0.0240	0.5% APD (m/v) + 0.1% palladium nitrate (m/v)
	2015/3/13 11:26	0.0469	0.0243			
48	2015/3/14 13:42	0.0465	0.0246	0.02485	0.0240	0.5% APD (m/v) + 0.1% palladium nitrate (m/v)
	2015/3/14 14:22	0.0468	0.0251			
72	2015/3/15 12:15	0.0225	0.0260	0.0263	0.0240	0.5% Oxalic acid + 0.1% palladium nitrate (m/v)
	2015/3/15 12:42	0.0229	0.0266			

Conclusion

This application note has shown that the Thermo Scientific iCE 3500 AAS is an ideal tool for analysis of Pb in milk powder samples with direct injection. With its high sensitivity and robustness, the instrument is easily capable of accurately and precisely measuring Pb in a single analysis method. This method has a very simple sample pre-treatment process, a direct injection process and rapid as well as high sensitivity performance at the same time. In summary, determination of Pb in a wide range of milk powder sample types can be efficiently and rapidly performed using the iCE 3500 AAS.

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