APPLICATION NOTE

Atomic Absorption

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# Accurate Determination of Lead in Different Dairy Products by Graphite Furnace Atomic Absorption Spectrometry

## Introduction

Milk is one of the basic food groups in the human diet, both in its original form and as various dairy products. The Chinese contaminated baby formula scandal in 2008 has increased public awareness of contamination possibilities, and has lead to tighter supervision of dairy products as China is faced with demands – both from home and abroad – to improve its food safety record. It is well-known that lead (Pb) is toxic and causes damage to the nervous system; it has a particularly detrimental effect on young children[1] and it has become a cause of major concern since the 1970s. As per World Health Organization (WHO) standards, the permissible limit of lead in drinking water is 10 µg/kg (parts per billion, ppb). Following an in-depth review of the toxicological literature, the Chinese guideline for maximum levels of lead content is set at 20 µg/kg (ppb wet weight) in infant formula (use of milk as a raw material measured by fluid milk diluted from powder, referring to the product ready-to-use) and at 50 µg/kg (ppb) in fresh milk, respectively.[2]

Lead analysis has traditionally been one of the major applications of graphite furnace atomic absorption spectrometry (GFAAS) worldwide. Currently, the Chinese regulatory framework approved standard methods for lead analysis has set GFAAS as the technique for the compulsory arbitration in food testing.[3] In order to ensure protection of consumers, analysis should be sensitive, efficient, and cost-effective so that more effective monitoring can be accomplished. Because GFAAS is a mature technique, it is well-understood and routinely used by technicians and suitable for this determination. Sample preparation is an important part of an analysis and yet can be time consuming.

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Generally, milk is an emulsion or colloid of butterfat globules within a water-based fluid. The exact components of raw milk vary by different animal species, but it contains significant amounts of lactose, fat, protein and minerals as well as vitamins. Due to the relative interference resulting from such a complex matrix, complete decomposition of milk samples prior to instrumental measurement by microwave or heating block acid digestion is generally recommended. This approach, however, is more time-consuming and poses a more rigorous requirement on quality assurance than simple dilution when concentrations of lead are to be determined at µg/kg level in the final solution which is extremely sensitive to reagent blank contribution and environmental contamination.

To overcome these issues, this work describes a simple and direct dilution method for sample preparation, followed by automated analysis using GFAAS. This method minimizes sample preparation, and also reduces potential contamination while still maintaining the speed of analysis.

## Experimental Conditions

### Instrumentation

A PerkinElmer® PinAAcle™ 900T flame and longitudinal Zeeman atomic absorption spectrometer (Figure 1) was used for the GFAAS measurements of lead (Pb) in different milk samples. The PinAAcle 900T spectrometer's transversely heated graphite atomizer (THGA) with Longitudinal AC Zeeman background correction provide a constant uniform temperature distribution across the entire length of the graphite tube. This allows a full implementation of the Stabilized Temperature Platform Furnace™ (STPF) technique in graphite furnace analysis where we can analyze complex sample matrices using aqueous standard solutions as calibration for suspended sample solutions to get accurate and precise results. Maximum atomic signals can be obtained with minimum memory effect and potential interference.

*Figure 1.* PinAAcle 900T atomic absorption spectrometer with AS 900 furnace autosampler.

The spectrometer was equipped with an AS 900 autosampler and a PerkinElmer Lumina™ single-element Pb hollow cathode lamp (Part No. N3050157) was used as the light source. A standard THGA tube (Part No. B0504033) and 1.2 mL polypropylene autosampler cups (Part No. B0510397) were used throughout for all measurement. The instrument was controlled by WinLab32™ for AA software running under Microsoft® Windows® 7 operating system. A summary of the PinAAcle 900T instrument settings is listed in Table 1.

***Table 1.*** **Instrument settings for the PinAAcle 900T spectrometer.**

| Parameter | Value |
|---|---|
| Wavelength: | 283.3 nm |
| Slit Width: | 0.7 nm |
| Lamp Current: | 10 mA |
| Signal Measurement: | Peak Area |
| Measurement Type: | AA-BG |
| Integration Time: | 5 s |
| Replicates: | 3 |
| Calibration Standard: | 12.5, 25, 37.5, 50 µg/L |
| Sample Volume: | 16 µL |

### Sampling

A total of 15 samples of six different dairy products were investigated in this study, representing all the main types of milk commercially available in China, including milk powder, skimmed milk powder, whole milk, low-fat milk, children's milk and yogurt. All the samples collected from the original packaging in a sealed clean polyethylene bag, were labeled and taken to the laboratory then kept refrigerated until analysis.

### Sample Preparation

For the preparation of all solutions, ultrapure deionized (DI) water from a MiliQ-Element system (Millipore®, Milford, MA, USA) was used throughout. Concentrated nitric acid (69-70%), $HNO_3$, and hydrogen peroxide (30%), $H_2O_2$, were trace-metal grade or better (Jingrui Chemical Co., Ltd., Jiangsu, China). Metal-free polypropylene vials and pipette tips were pre-cleaned with diluted nitric acid (~5% $HNO_3$) and rinsed thoroughly with DI water before use.

For the subsequent GFAAS analysis, a solution containing 0.5% $HNO_3$ with 0.1% Triton X-100 (Part No. N9300260), a non-ionic detergent, was prepared daily both as a diluent and as a blank.

A 1-g sample of liquid milk or solid milk powder was accurately weighed and transferred into a 15-mL conical polypropylene tube (Part No. B0193233) which was subsequently diluted to make up the volume of 10 mL, and shaken vigorously for a few minutes to ensure homogeneity. The obtained suspension solution was immediately ready for GFAAS measurement using the autosampler. These suspensions were stable for more than 2 days. Even the more challenging total fat milk powder prepared by this rapid dilute-and-shoot procedure can be stable for this duration, which is sufficient for the inter-day variability check. The same procedure was used to prepare the blanks, and all the samples were prepared in duplicate on a routine analysis basis, unless stated otherwise.

For the validation by ICP-MS determination, a Multiwave™ 3000 high-pressure microwave digestion system (PerkinElmer, Inc., Shelton, CT) was employed to completely decompose the milk sample matrix using an acid mixture of $HNO_3$ and $H_2O_2$.

### Calibration

As the concentration of Pb in milk samples is generally very low, all the reagents used must be of ultra-pure grade. Thus, Single-Element PerkinElmer Pure Plus Grade Standards (Part No. N9303748, lead in 2% $HNO_3$) and Matrix Modifiers (Part No. B0190635, 10% Pd as nitrate and Part No. B0190634, 1% Mg as nitrate) were recommended to be used. Calibration curves were constructed using online auto-dilution of a working stock lead standard solution of 50 µg/kg (ppb) by the AS 900 autosampler.

### Method Validation

The performance of the procedure using GFAAS measurement was assessed by spike recovery and the evaluation of the Standard Reference Materials (SRMs) from National Institute of Standards and Technology (NIST®), NIST® 1549 Non-Fat Milk Powder, and China National Institute of Metrology (NIM), GBW08509a Skimmed Milk Powder. These two commercial lyophilized SRMs were treated as any dairy product sample.

In addition, these results were also compared to that obtained by the conventional mineralization-based procedures, followed by analysis using the NexION® 300X ICP-MS (PerkinElmer, Inc., Shelton, CT). The complete mineralization was carried out with the Multiwave 3000 microwave digestion system. Instrumental operating parameters for the ICP-MS measurements followed the routinely established protocols.

## Results and Discussion

The temperature program for the analysis of lead is optimized to provide maximum matrix decomposition without loss of analyte. The furnace temperature program is given in Table 2.

Due to the challenging characteristics of the sample matrix, an additional drying step, using a special gas of dry compressed air, is recommended to eliminate the carbonaceous residues left after analyzing more than 50 samples in one single batch. The PinAAcle 900T spectrometer's TubeView™ color furnace camera is of great advantage in checking the position of the tip in the furnace, relative to the platform, which brings benefits in optimizing the drying and pyrolysis steps for the complex undigested milk matrix to ensure that no sample boiling or splattering occurred (Figure 2).

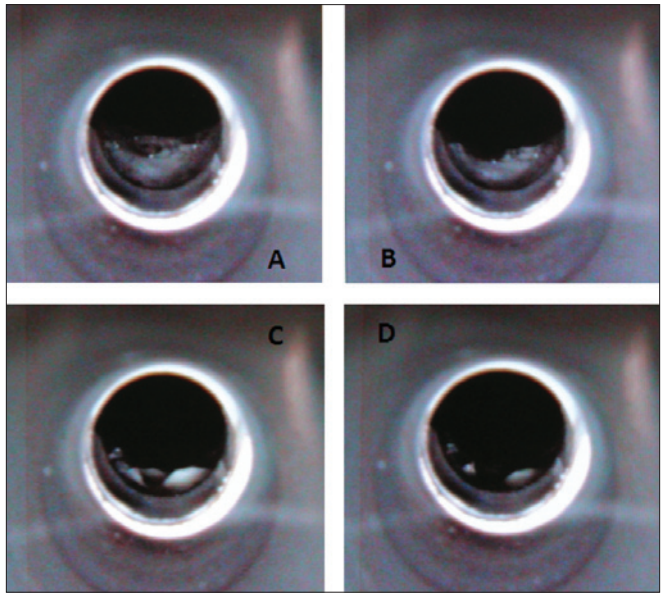


*Figure 2.* The drying steps of a complex undigested milk sample in the graphite tube, as seen using the TubeView color furnace camera.

***Table 2.*** **Furnace temperature program for the direct measurement of lead in milk samples using the PinAAcle 900T spectrometer with THGA tubes.**

| Step | | Temp. (°C) | Ramp Time (sec) | Hold Time (sec) | Internal Flow | Read Step | Gas Type |
|---|---|---|---|---|---|---|---|
| 1 | Drying | 130 | 5 | 30 | 250 | | Normal |
| 2 | Drying | 150 | 15 | 30 | 250 | | Normal |
| 3 | Drying | 450 | 15 | 15 | 50 | | Dried Air |
| 4 | Pyrolysis | 600 | 10 | 20 | 250 | | Normal |
| 5 | Atomization | 1600 | 0 | 3 | 0 | X | Normal |
| 6 | Clean-out | 2500 | 1 | 5 | 250 | | Normal |

Therefore, it helped in simpler and faster furnace (temperature) method development.

For Pb determination, complete mineralization of the milk components is not necessary when using the proven and established STPF technique with the patented THGA design which ensures uniform and consistent heating and high atomization efficiency, significantly reducing matrix interferences. All data were calculated from 3 replicate readings for each solution using peak-area (integrated absorbance) integration. Figure 3 depicts the overlay of typical peak profiles of the various solutions. One of the unique benefits of the STPF technique is clearly demonstrated here: even though the peaks may not appear at exactly the same time, the peak-area calculation still provides consistently accurate results.

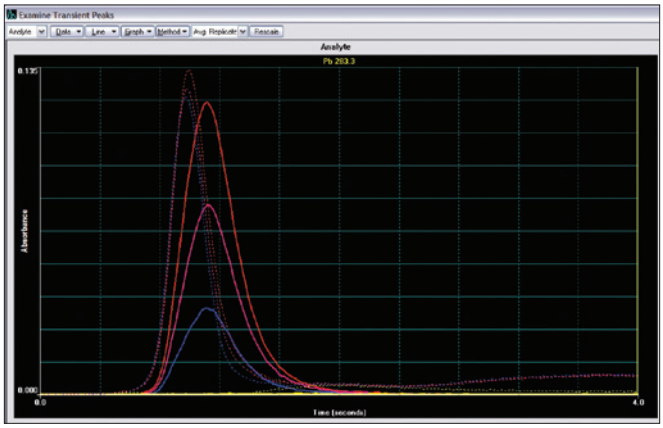


*Figure 3.* Overlay of typical lead atomic and background signal for the control material of skimmed milk powder. The solid blue line is from the control material of skimmed milk powder, the solid purple line is from the spiked control material, and the solid red line is from the standard at a concentration of 25 µg/kg, while the solid yellow line at the bottom is the reagent blank signal. Dashed lines represent the background absorption profiles.

To test the accuracy of the method, Pb was analyzed in the control material of non-fat milk powder from NIST® 1549 and skimmed milk powder from NIM GBW08509a. The high level of accuracy of the direct method is demonstrated by the good agreement of the results obtained in the analysis of the two SRMs with the certified values, as shown in Table 3. An estimation of analyte recovery was also obtained by spiking one of the SRM samples (GBW08509a) at the 50, 100, and 200% levels with the Pb single-element standard working stock solution, and the data, also collated in Table 3, demonstrates quantitative recovery.

***Table 3.* Results for the direct measurement of NIST® 1549 and GBW08509a by GFAAS (all in µg/kg).**

| Sample | Certified Value | Spike Level | Expected Mean | Found Mean | Recovery (%) |
|---|---|---|---|---|---|
| NIST® 1549 | 19 ±3 | 0 | 19 | 19 | 101 |
| GBW08509a | 24 ±6 | 0 | 24 | 23 | 95 |
| GBW08509a | 24 ±6 | 12 | 36 | 35 | 96 |
| GBW08509a | 24 ±6 | 24 | 48 | 48 | 99 |
| GBW08509a | 24 ±6 | 48 | 72 | 71 | 98 |

Method detection limits (MDLs), defined as the analyte concentration in micrograms per kilogram (ppb) of dairy products which provides an absorbance reading statistically different from that of the blank, are calculated by dividing 3 times the standard deviation (SD) of the absorbance readings of the reagent blanks by the sensitivity. An impressive characteristic of this method, which uses a sample volume at 16 µL with 10-fold dilution factor, provides the MDL of 0.25 µg/kg (ppb). Thus, the MDL measured in the original dairy products is about two orders of magnitude below the expected level in the typical control materials (around 20 µg/kg). It indicates that this method could prove highly suitable for determining Pb in dairy products.

For additional independent comparative data against GFAAS analysis using this simple method, all collected dairy products were mineralized by conventional microwave total acid digestion, then analyzed for lead by ICP-MS. Table 4 (Page 5) shows the concentrations of Pb found in each dairy product sample.

*Table 4.* **Lead levels in commercially available dairy products determined by direct GFAAS analysis and conventional ICP-MS measurement (values are means ± SD, all in µg/kg).**

| No. | SRMs/Samples | Certified Value | Measured Results GFAAS | ICP-MS |
|---|---|---|---|---|
| 1 | GBW08509a (Skimmed milk powder) | 24 ±6 | 23.3 ±0.7 | 23.9 ±1.7 |
| 2 | GBW10017 (Milk powder) | 70 ±20 | 23.9 ±2.7 | 25.7 ±8.7 |
| 3 | NIST® 1549 (Non-fat milk powder) | 19 ±3 | 19.1 ±1.3 | 19.3 ±6.5 |
| 4 | Milk powder | – | 40.2 ±1.8 | 42.1 ±1.9 |
| 5 | Skimmed milk powder | – | 25.7 ±1.3 | 23.3 ±6.1 |
| 6 | Whole milk (Brand 1) | – | 4.46 ±0.32 | 4.57 ±0.60 |
| 7 | Whole milk (Brand 2) | – | 2.75 ±0.07 | 2.73 ±0.09 |
| 8 | Whole milk (Brand 3) | – | 6.13 ±0.07 | 6.78 ±0.49 |
| 9 | Whole milk (Brand 4) | – | 5.65 ±0.11 | 5.85 ±0.37 |
| 10 | Low-fat milk (Brand 1) | – | 2.34 ±0.09 | 2.39 ±0.38 |
| 11 | Low-fat milk (Brand 2) | – | 0.53 ±0.02 | 0.58 ±0.21 |
| 12 | Drinkable children's milk (Brand 1) | – | 1.70 ±0.09 | 1.73 ±0.22 |
| 13 | Drinkable children's milk (Brand 2) | – | 0.22 ±0.01 | 0.54 ±0.15 |
| 14 | Drinkable yogurt (Brand 1) | – | 1.89 ±0.16 | 2.02 ±0.18 |
| 15 | Drinkable yogurt (Brand 2) | – | 1.36 ±0.02 | 1.61 ±0.33 |

It is important to emphasize that there are no significant differences between the two independent testing methods, which further demonstrates the accuracy of the overall methods. However, the relative standard deviation (RSD) was generally higher for data obtained by ICP-MS analysis after conventional mineralization. This is most likely due to the dilution introduced during the digestion step used in the ICP-MS sample preparation. Even though the ICP-MS technique is more sensitive than GFAAS, the dilution of the extremely low levels of Pb present in the samples introduces additional uncertainty. Based on the results, it clearly appears that total digestion of matrix components is unnecessary with all these types of dairy-product samples, and it is more rapid and economical to run the samples with minimal preparation.

As is also shown in Table 4, the Pb concentration in one of the tested SRMs issued by State General Administration of the People's Republic of China for Quality Supervision and Inspection and Quarantine (AQSIQ), GBW10017 milk powder found in this study, is 23.9 ±2.7 µg/kg by direct GFAAS method and 25.7 ±8.7 µg/kg by total digested ICP-MS method, which are both significantly lower than the certified value (70 ±20 µg/kg). This difference has also been observed by other laboratories purchasing this reference material. Based on the higher value of standard deviation (20 µg/kg, 29% of error), the actual certified Pb result in this GBW10017 SRM issued by AQSIQ has yet to be ascertained and needs further investigation.

For an intuitive and illustrative comparison, the differences in Pb concentration and analytical precision are also presented in Figure 4 as a plot with error bar. Our results clearly affirm the great advantage of easy handling and precise analysis using direct determination of Pb concentration by GFAAS, since the need to measure Pb at such a low level (in µg/kg range) in the original dairy product samples requires extremely strict control of reagents, environment and process. This is very challenging, even for experienced professionals, due to the large dilution factor if undergoing the time-consuming and labor-burdened total digestion procedure, taking the poor match of experimental value with the certified value in the SRMs of GBW10017 as an additional proof.

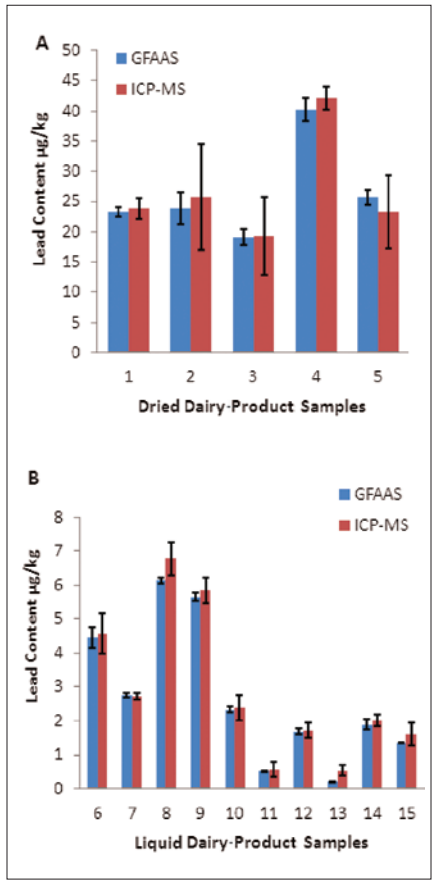


*Figure 4.* Comparison of lead levels in different dairy-product samples obtained by two independent test methods: A) dried milk powder samples; B) liquid milk samples.

## Conclusions

In conclusion, a method involving simple sample dilution and automated PinAAcle 900T GFAAS detection can be successfully applied to the accurate measurement of Pb in different dairy products. Reduced sample handling minimizes the potential for losses or contamination. The advanced THGA technique keeps the risk of chemical interferences to a minimum, which provides a method detection limit well below the normal range of Pb that might be encountered. This method should also be applicable for analysis of samples with equivalent content of fat and complex matrices.

ICP-MS provides multi-element analysis and very high sensitivity. However, the high initial investment and more costly cost of ownership when compared with GFAAS may not offer the best choice for a simple single-element analysis. GFAAS offers not only high selectivity, sensitivity, and ease of operation, but also high tolerance to complex matrices. When coupled with simple sample preparation, it is consequently more appropriate for the trace level determination of a few toxic elements in dairy products as a routine monitoring technique in protecting human health.

## References


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