APPLICATION NOTE

Atomic Absorption

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# The Determination of Toxic, Trace, and Essential Elements in Food Matrices using THGA Coupled with Longitudinal Zeeman Background Correction

## Introduction

Ingestion of trace elements from food can be linked to nutrition, disease, and physiological development. Whether they are needed for proper nutritional value or contain toxic elements, the presence of major and minor elements in food needs to be verified to help determine health effects for the consumer. Contamination of food products may result from metals present during cultivation and/or processing. Acute or chronic exposure to heavy metals can lead to damaged nervous system function and have detrimental effects on vital organs. Food safety laboratories performing these analyses are often high-throughput facilities and require a detection tool that is efficient and cost effective.

Unlike flame atomic absorption spectrophotometry (FAAS) where the ground state atoms quickly diffuse into surrounding air, graphite furnace atomic absorption spectrophotometry (GFAAS), being a total consumption technique, offers the ability to dry and atomize the entire pipetted sample in a more controlled environment within the graphite tube. This significantly increases sensitivity and provides superior detection limits with microliter (µL) sample volumes. Only ICP-MS can provide the same level of detection as GFAAS, however GFAAS is more cost efficient, simpler to operate and has fewer laboratory facility requirements.

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The PerkinElmer® PinAAcle™ 900T atomic absorption spectrophotometer (Figure 1) uses the unique transversely heated graphite atomizer (THGA) design which provides a uniform temperature profile over the entire length of the graphite tube, unlike longitudinally heated systems which have only a small constant temperature zone in the center of the tube (Figure 2). The transverse design also provides exceptionally fast heating. A direct benefit of the fast, uniform temperature distribution in the THGA tube is a major reduction or elimination of condensation interferences caused by cooler temperatures at the tube ends. This significantly improves analytical accuracy and reduces memory effects. The PinAAcle 900T spectrometer has the ability to provide full implementation of the Stabilized Temperature Platform Furnace™ (STPF) technique which is paramount in providing nearly interference-free analysis. This eliminates the need for the method of additions and allows for direct calibration with simple aqueous standards, independent of the sample matrix. The curved, integrated platform inside of the THGA tube provides the ability to determine any element (including refractory elements) under full STPF conditions.

*Figure 1.* PerkinElmer PinAAcle 900T atomic absorption spectrophotometer.

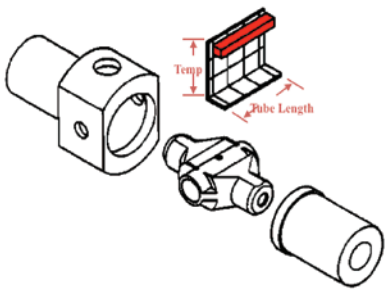


*Figure 2.* Uniform temperature distribution profile with THGA tube and heating assembly.

## Experimental Conditions

### Sample Preparation

Five different NIST® Standard Reference Material (SRM) food samples, which represent a typical cross-section of food types for human consumption, were chosen. The foods included non-fat milk powder (dairy), spinach (vegetable), mussel tissue (shellfish), bovine muscle (meat), and corn bran (grain).

The samples were digested using a Multiwave™ 3000 microwave digestion system equipped with a Rotor 16HF100. Details of the procedure are provided in Table 1. Approximately 0.5 g of each SRM was digested in duplicate with 5 mL of nitric acid ($HNO_3$) (Fisher Optima grade) and 2 mL of hydrogen peroxide ($H_2O_2$) (Fisher Optima grade) in pre-cleaned HF100 PTFE vessels. The 16-position rotor was equipped with a pressure/temperature sensor to monitor a single sample to ensure the program was running properly. In addition, an external infrared (IR) sensor measured the temperature for every location in the rotor throughout the analysis. All samples were completely digested and diluted to a final volume of 50 mL with deionized water (≥18 MΩ). Samples were further diluted as necessary in the same manner. Preparation blanks, consisting of the acid mixture, were taken through the same digestion and preparation process as the samples and analyzed accordingly.

***Table 1.*** **Microwave digestion heating program for all six NIST® food SRMs and preparation blanks.**

| Step | Power (W) | Ramp (min) | Hold (min) | Fan |
|---|---|---|---|---|
| 1 | 500 | 1 | 4 | 1 |
| 2 | 1000 | 5 | 5 | 1 |
| 3 | 1400 | 5 | 10 | 1 |
| 4 (cooling) | 0 | — | 15 | 3 |

### Instrumentation

All data were generated with the PinAAcle 900T atomic absorption spectrophotometer in graphite furnace mode and based on a 20 µL sample + 5 µL modifier injection volume (Table 2 – Page 3). For arsenic (As) and selenium (Se), an end-capped tube (Part No. B3000653) was employed instead of the standard, open-ended tubes (Figure 3 – Page 3). End-capped tubes provide a longer atom residence time, thereby improving sensitivity. If desired, the type of tube can easily be switched and does not require major changes to the existing furnace program. End-capped tubes can be used for all elements except for refractory elements such as V, Ti, Mo, etc. With these high-temperature elements, there is little to no increase in sensitivity, but there is an increase in memory/carryover (Figure 4 – Page 3). Electrodeless discharge lamps (EDLs) were used for As (Part No. N3050605) and Se (Part No. N3050672) to provide lower detection limits and better light output compared to standard hollow cathode lamps (HCLs).

*Table 2.* **GFAAS conditions for the analysis of trace metals in food.**

| Analyte | Wavelength | Matrix Modifier | Tube Type | Lamp |
|---|---|---|---|---|
| As | 193.7 | $Pd/Mg(NO_3)_2$ | End-Capped | EDL |
| Cd | 228.8 | $PO_4/Mg(NO_3)_2$ | Standard | HCL |
| Cr | 357.9 | $Mg(NO_3)_2$ | Standard | HCL |
| Cu | 324.8 | $Pd/Mg(NO_3)_2$ | Standard | HCL |
| Fe | 248.3 | $Mg(NO_3)_2$ | Standard | HCL |
| Mn | 279.5 | $Pd/Mg(NO_3)_2$ | Standard | HCL |
| Ni | 232.0 | $Pd/Mg(NO_3)_2$ | Standard | HCL |
| Pb | 283.3 | $PO_4/Mg(NO_3)_2$ | Standard | HCL |
| Se | 196.0 | $Pd/Mg(NO_3)_2$ | End-Capped | EDL |

*Figure 3.* Standard THGA tube (left) (Part No. B3000641) and end-capped THGA tube (right) (Part No. B3000653).

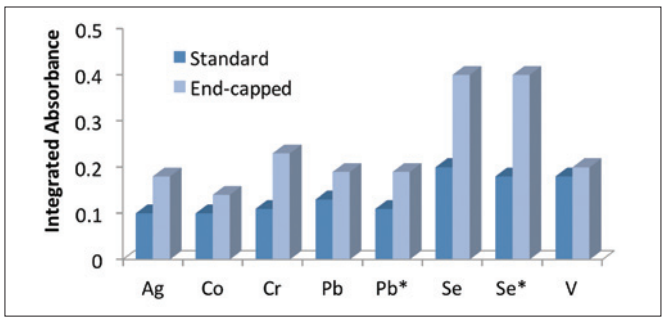


*Figure 4.* Comparison of integrated absorbances for end-capped vs. standard THGA tubes. *Denotes use of the recommended matrix modifier.

## Furnace Program

All elements except for selenium (Se) used a conventional program that included two drying steps, a pyrolysis or pretreatment step to remove unwanted matrix components, an atomization step with gas-stop conditions, and a brief clean-out step (Table 3). The Se program was modified to include pre-reduction of the Pd modifier using hydrogen. This results in lower background levels during the atomization step, reducing the potential for vapor-phase interferences that could degrade data quality (Table 4).

*Table 3.* **Conventional furnace program, using argon, for the analysis of trace metals in food products using GFAAS.**

| Step | Temp (˚C) | Ramp Time(s) | Hold Time(s) | Internal Flow (mL/min) | Gas Type |
|---|---|---|---|---|---|
| 1 | 110 | 1 | 30 | 250 | Normal |
| 2 | 140 | 15 | 35 | 250 | Normal |
| 3 | 1200 | 10 | 40 | 250 | Normal |
| 4* | 2100 | 0 | 4 | 0 | Normal |
| 5 | 2450 | 1 | 3 | 250 | Normal |

**Read step*

*Table 4.* **Furnace program incorporating an alternate gas type ($Ar/H_2$) for the analysis of Se.**

| Step | Temp (˚C) | Ramp Time(s) | Hold Time(s) | Internal Flow (mL/min) | Gas Type |
|---|---|---|---|---|---|
| 1 | 120 | 1 | 40 | 250 | Special |
| 2 | 1000 | 10 | 30 | 250 | Special |
| 3 | 110 | 1 | 30 | 250 | Normal |
| 4 | 140 | 15 | 40 | 250 | Normal |
| 5 | 1200 | 10 | 45 | 250 | Normal |
| 6* | 2100 | 0 | 5 | 0 | Normal |
| 7 | 2450 | 1 | 3 | 250 | Normal |

**Read step*

The PinAAcle 900T spectrometer's TubeView™ furnace camera (Figure 5) was used to check the position of the pipette tip and in optimizing the drying times and temperatures for the food matrix.

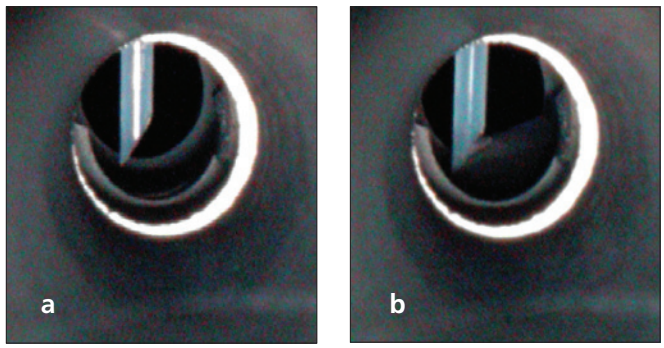


*Figure 5.* TubeView furnace camera captures of AS 900 autosampler pipette in graphite tube during sample deposition.

## Results

### Calibration

Calibration standards were chosen to ensure all samples fell within range. A linear calibration equation, forced through zero, was used and the calibration statistics showed excellent correlation (Table 5). The instrument design and software provided for easy implementation, selection and use of the special $Ar/H_2$ gas for the determination of Se. Preparation blanks were determined to contain negligible levels as compared to reported data.

***Table 5.*** **Calibration data for the detection of trace elements in food.**

| Analyte | Wavelength | Matrix Modifier | Tube Type | Lamp |
|---|---|---|---|---|
| As | 193.7 | $Pd/Mg(NO_3)_2$ | End-Capped | EDL |
| Cd | 228.8 | $PO4/Mg(NO_3)_2$ | Standard | HCL |
| Cr | 357.9 | $Mg(NO_3)_2$ | Standard | HCL |
| Cu | 324.8 | $Pd/Mg(NO_3)_2$ | Standard | HCL |
| Fe | 248.3 | $Mg(NO_3)_2$ | Standard | HCL |
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| Ni | 232.0 | $Pd/Mg(NO_3)_2$ | Standard | HCL |
| Pb | 283.3 | $PO4/Mg(NO_3)_2$ | Standard | HCL |
| Se | 196.0 | $Pd/Mg(NO_3)_2$ | End-Capped | EDL |

### Standard Reference Materials

Quantitative results for the five different SRMs are shown in Tables 6-10. Recovery data is based on the mean reference value provided by NIST®. All of the experimental values with concentrations sufficient for this technique had recoveries of 90-110% and/or fell within the confidence limit given by NIST®. Even at the very low level of lead and selenium in the non-fat milk powder reference material, with a solution concentration of 0.158 µg/L and 1.26 µg/L respectively, the PinAAcle 900T spectrometer was able to achieve excellent recoveries with respect to the reference range.

Non-specific background, caused by high concentrations of concomitant molecules, small particles, or smoke that may absorb or scatter light from the light source, is the most common type of interference. This can be noteworthy in high-matrix samples such as food digests. Longitudinal Zeeman background correction is especially suited to correct for high or structured background. The absorption and background signal are measured with the same light source and at the same wavelength, with identical resolution. This ensures an exceptionally accurate, background-corrected measurement. In addition, with the longitudinal Zeeman design of the PinAAcle 900T spectrometer, the need for an optical polarizer is eliminated. Compared to the transverse Zeeman designs of other systems, this provides a greater than 50% increase in light throughput. The accuracy of the method incorporating longitudinal Zeeman background correction is shown in Tables 6-10 with the recoveries of trace metals from food reference materials.

Standard programs with the recommended matrix modifiers were used to successfully analyze arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), and lead (Pb). A custom program was successfully used to analyze Se. The custom analysis of Se in WinLab32™ for AA software indicates the ability of the system to easily implement an alternate gas with a custom AS 900 autosampler sequence whereby the modifier was pipetted and furnace steps 1 and 2 were performed; then the sample plus diluent were added and furnace steps 3 through 7 were run.

***Table 6.*** **Analysis of NIST® 1549 Non-Fat Milk Powder.**

| Analyte | Reference Value (mg/kg) | Experimental Value (mg/kg) | Recovery (%) |
|---|---|---|---|
| Cd | 0.0005 ±0.0002 | * | |
| Cu | 0.7 ±0.1 | 0.627 | 90 |
| Pb | 0.019 ±0.003 | 0.0158 | 83 |
| Mn | 0.26 ±0.06 | 0.268 | 103 |
| Se | 0.11 ±0.01 | 0.126 | 115 |

**Concentration was not sufficient for this technique.*

***Table 7.*** **Analysis of NIST® 1570a Spinach Leaves.**

| Analyte | Reference Value (mg/kg) | Experimental Value (mg/kg) | Recovery (%) |
|---|---|---|---|
| Cd | 2.89 ±0.07 | 2.61 | 90 |
| Cu | 12.2 ±0.6 | 12.5 | 102 |
| Pb | (0.20) | 0.208 | 104 |
| Mn | 75.9 ±1.9 | 74.8 | 99 |
| Ni | 2.14 ±0.10 | 2.21 | 103 |

*The value in parentheses ( ) in the "Reference Value" column is not a certified value – it is included for information only.*

***Table 8.*** **Analysis of NIST® 2976 Mussel Tissue.**

| Analyte | Reference Value (mg/kg) | Experimental Value (mg/kg) | Recovery (%) |
|---|---|---|---|
| As | 13.3 ±1.8 | 13.1 | 98 |
| Cd | 0.82 ±0.16 | 0.869 | 106 |
| Cr | 0.50 ±0.16 | 0.501 | 100 |
| Cu | 4.02 ±0.33 | 3.97 | 99 |
| Fe | 171.0 ±4.9 | 182 | 106 |
| Pb | 1.19 ±0.18 | 1.07 | 90 |
| Mn | 33 ±2 | 34.2 | 104 |
| Ni | 0.93 ±0.12 | 0.958 | 103 |
| Se | 1.80 ±0.15 | 1.74 | 97 |

*Table 9.* **Analysis of NIST® 8414 Bovine Muscle Powder.**

| Analyte | Reference Value (mg/kg) | Experimental Value (mg/kg) | Recovery (%) |
|---|---|---|---|
| Cd | 0.013 ±0.011 | 0.0162 | 125 |
| Cu | 2.84 ±0.45 | 2.69 | 95 |
| Fe | 71.2 ±9.2 | 70.9 | 99 |
| Pb | 0.38 ±0.24 | 0.334 | 87 |
| Mn | 0.37 ±0.09 | 0.343 | 93 |
| Ni | 0.05 ±0.04 | 0.0631 | 126 |

*Table 10.* **Analysis of NIST® 8433 Corn Bran.**

| Analyte | Reference Value (mg/kg) | Experimental Value (mg/kg) | Recovery (%) |
|---|---|---|---|
| Cd | 0.012 ±0.005 | * | |
| Cu | 2.47 ±0.40 | 2.61 | 106 |
| Fe | 14.8 ±1.8 | 14.8 | 100 |
| Pb | 0.14 ±0.034 | 0.120 | 86 |
| Mn | 2.55 ±0.29 | 2.44 | 96 |
| Ni | 0.158 ±0.054 | 0.158 | 100 |

**Concentration not sufficient for this technique.*

## Conclusion

The analysis of the various SRM materials clearly demonstrates the ability of the PinAAcle 900T spectrometer to generate quality data incorporating THGA tubes, longitudinal Zeeman background correction and STPF conditions for interference-free, accurate quantitation. In addition, the ability of the Multiwave 3000 system to perform complete dissolution in a closed-vessel microwave digestion was shown. The overall agreement of the results compared to the various SRM matrices demonstrates the versatility and ruggedness of the Multiwave 3000 digestion system using a single program with the same reagents for several food types. This work demonstrates the ability of the PinAAcle 900T spectrometer to quantitate in the low-ppb range, thereby reducing the need for an ICP-MS to successfully measure this suite of elements in foods. The PinAAcle 900Z (Longitudinal Zeeman Furnace only) spectrometer can also be used for this application.

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