APPLICATION NOTE

Atomic Absorption

Author:

Lee Davidowski, Ph.D.

PerkinElmer, Inc.
Shelton, CT 06484 USA

Determination of Aluminum in Serum in Customer-Validated Applications using THGA and Longitudinal Zeeman Atomic Absorption

Introduction

Determinations of aluminum (Al) in serum are performed routinely by laboratories through customer-validated applications. Graphite furnace atomic absorption spectroscopy (GFAAS) is one of only a few routine techniques with a dynamic range sensitive enough to be used clinically in customer-validated applications to measure Al in serum. There are a few specific analytical challenges that an analyst must consider in the determination of Al in serum by GFAAS. The serum matrix contains an organic carbohydrate fraction as well as a considerable level of inorganic salts, all of which can interfere with an accurate GFAAS measurement. Some means of reducing these interferences must be considered. Aluminum is also a commonly occurring element such that pronounced external contamination of the sample can occur during the sample-handling phases of the analytical method. Minimal analyst interaction with the sample prior to analysis is needed in order to reduce the chance and degree of contamination.

This work will describe a simple, direct procedure for the analysis of aluminum in serum with aqueous calibration, using small sample volumes, STPF concepts and Zeeman background correction, over a wide range of serum concentrations and with minimal sample interaction.

PerkinElmer®
For the Better

Experimental

Reagents

The sample diluent/modifier solution consisted of 0.1% nitric acid (v/v), 0.01% Triton X-100 and 0.2% $Mg(NO_3)_2$. It was prepared from ASTM® Type I deionized water (18MΩ), 1% $Mg(NO_3)_2$ modifier solution (Part No. B0190634), and Triton® X-100 nonionic detergent (Part No. N9300260). Instrument calibration standards were prepared from an aluminum stock standard (PerkinElmer Pure, Single-element Al standard, Part No. N9300184). Bi-Level Trace Element Control freeze-dried Serum samples were from UTAK® Laboratories, Inc. (Valencia, CA).

Instrumental Conditions

All measurements were made using a PerkinElmer® PinAAcle™ 900T flame and longitudinal Zeeman furnace atomic absorption spectrometer (Figure 1) which includes a transversely heated graphite atomizer and longitudinal Zeeman-effect background correction. Standard tubes (Part No. B0504033) were used for all analyses. The spectrometer was equipped with an AS 900 autosampler and 2.5 mL polypropylene autosampler cups (Part No. B3001566). The system was controlled by WinLab32™ for AA software running under Microsoft® Windows® 7 operating system.

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

Particularly for aluminum analyses, the graphite tube design plays a vital role in the overall sensitivity and stability of the analysis. Aluminum is volatilized as a stable oxide which only partially dissociates when atomization is from the tube wall due to the low gas-phase temperatures at the time of volatilization. With platform atomization, however, volatilization is delayed until the gas phase has reached a higher, steady-state temperature, thereby increasing atomization efficiency three-fold. Additionally, the patented THGA tube design provides a uniform temperature distribution over its entire length, ensuring not only maximum sensitivity, but also freedom from interferences by eliminating gas-phase analyte recombinations that normally occur at the cold ends of non-THGA tubes.

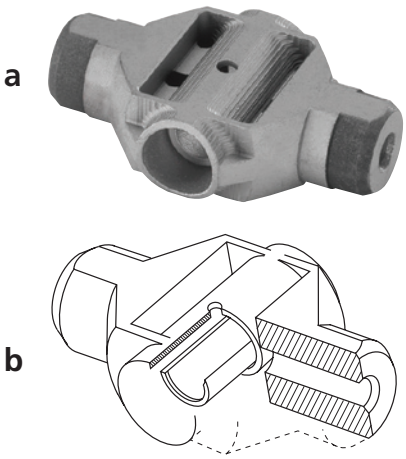

Figure 2. a) Standard THGA tube with integrated L'vov platform. b) Cutaway view of THGA tube.

The THGA tube, with its unique integrated platform (Figure 2), is machined from a single block of graphite and is pyrolytically coated to ensure maximum performance and lifetime.

Sample Analysis

The commercial freeze-dried bi-level serum control materials were reconstituted with laboratory DI water as per the manufacturer's directions. Thereafter, the control samples are treated the same as any serum patient sample. One mL of serum was transferred with a mechanical pipette into a pre-rinsed autosampler cup. One mL of the diluent/modifier solution was then added (yielding a 1+1 diluted serum). The sample was mixed by repeated pipetting until the diluted sample appeared totally mixed. A "diluent blank" was prepared by mixing one mL DI water with one mL of the diluent/modifier. The aluminum calibration standards were diluted from a 100 µg/L Al standard which was prepared in 50% diluent/modifier solution. The instrument then constructed a multi-point calibration curve by diluting the "stock" 100 µg/L standard with appropriate volumes of the diluent blank solution to levels of 100, 50 and 25 µg/L (Figure 3).

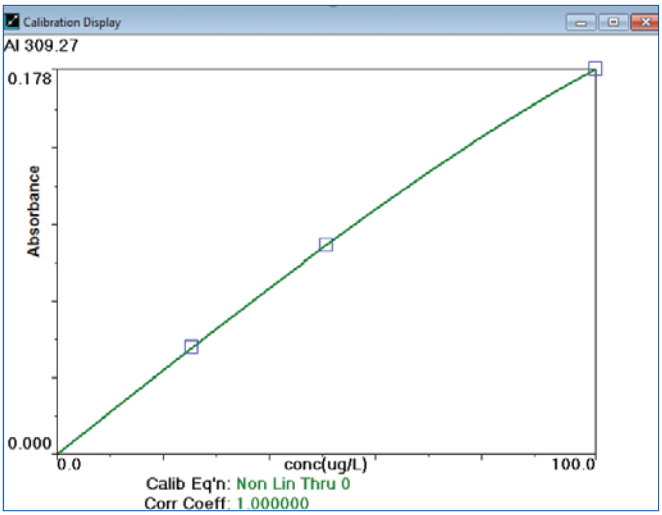

Figure 3. Calibration curve for aluminum in serum using peak area (Abs-sec).

The instrumental parameters and furnace heating program are given in Tables 1 and 2. Due to the slightly more challenging drying characteristics of the of the 1+1 serum sample matrix, 3 drying steps were used. The Triton® X-100 aids in releasing the serum cleanly and reproducibly from the plastic pipette tip. The PinAAcle 900T's TubeView™ furnace camera (Figure 4) is a great benefit in checking the position of the pipette tip and in optimizing the drying times and temperatures for the serum matrix.

***Table 1.* Instrumental parameters of PinAAcle 900T for measurement of aluminum in serum.**

Component/Parameter	Type/Value/Mode
Wavelength (nm)	309.27
Source Lamp (current)	Al HCL (25 mA) (Part No. N3050103)
Slit Width (nm)	0.7
Background Correction	Longitudinal AC Zeeman-effect
Measurement Mode	Peak Area, 3 Replicates
Calibration Algorithm	Non-Linear Through Zero
Integration Time (s)	5.0
Sample Volume (µL)	12
Calibration Stds. (µg/L)	25, 50, 100
THGA	Standard THGA Tube

***Table 2.* Temperature program for the measurement of aluminum in serum using THGA.**

Step		Temp. (˚C)	Ramp Time (sec)	Hold Time (sec)	Argon Gas (mL/min)
1	Drying	120	1	10	250
2	Drying	140	5	10	250
3	Drying	200	5	5	250
4	Pyrolysis	1200	5	15	250
5	Atomization	2300	0	5	0
6	Clean-out	2450	1	3	250

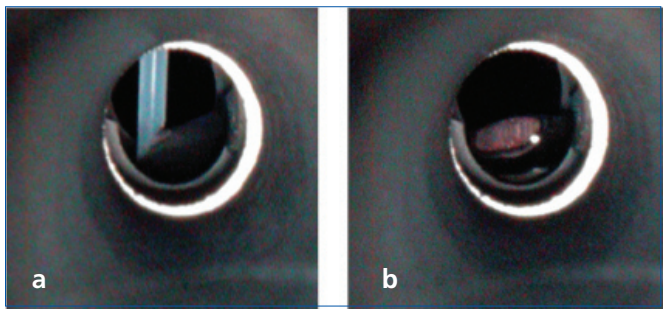

Figure 4. TubeView furnace camera captures of AS 900 pipette in graphite tube during sample deposition.

Results

The sensitivity of a method can be defined as the characteristic mass (M_0), the mass of the element injected into the furnace that yields a background-corrected signal of 0.0044 Abs.sec. A characteristic mass of 29 pg was found for Al, which is in very close agreement with the manufacturer's recommended M_0 value of 31 pg. Due to serum matrix components, a slight difference in the appearance time of the atomic signal and the peak shape was found as compared to the aqueous standard (Figure 5). This, however, is not a problem when using STPF conditions. Peak area signal processing is used here so that the entire Al signal is measured and quantified, regardless of appearance time and peak shape. This method of quantification was used for all analyses and samples.

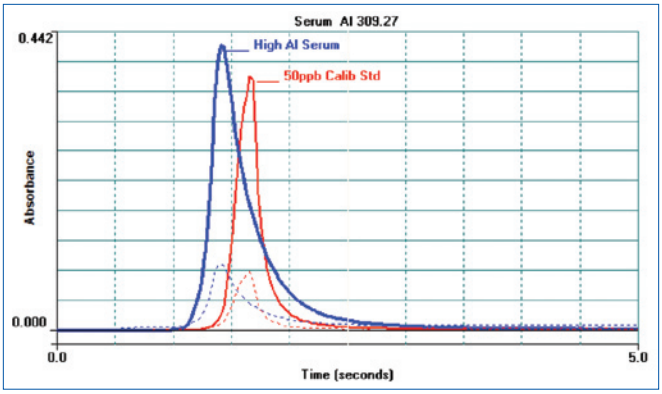

Figure 5. Solid lines represent Al atomic absorption profile signals for 50 µg/L Calibration Standard (Red) and High Range Serum Control (Blue). Dashed lines represent the background absorption profiles.

The results of the serum analyses are shown in Table 3. The concentration values are corrected for the in-lab dilution. The serum control samples show very good agreement with the expected values. Using the STPF principles of platform atomization and proper matrix modification, it is shown that a simple aqueous calibration scheme can be used for detection of Al in serum samples. The standard deviation and relative standard deviation (%RSD) values (Table 3) show relatively good reproducibility for these 1+1 serum solutions. Based on the standard deviations of the normal level serum and/or the diluent blank determinations, this method yields a method detection limit of less than 1 µg/L (<0.04 µmol/L) of Al in serum.

***Table 3.* Corrected results for the analyses of aluminum in serum (µg/L).**

Sample ID	Verified Value	Expected Range	Found Mean	Std Dev	%RSD
Normal Range	12	7.2 – 16.8	11.1	0.4	3.6%
High Range	176	141 - 211	198	3	1.6%
Diluent Blank			0.52	0.03	

Conclusion

Using the PinAAcle 900T atomic absorption spectrometer, aqueous calibration standards, and a simple 1+1 dilution, this method has been successfully applied to the determination of aluminum in serum. Bi-level serum controls showed the method to be simple, reliable, accurate and precise. The reduced sample handling keeps the risk of contamination and sample preparation time to a minimum. This application procedure has a wide dynamic range with a method detection limit well below the normal range of aluminum in serum. It also covers the higher aluminum concentrations found in medically exposed samples. By employing the latest analytical concepts of the stabilized temperature platform (STPF) technique and the instrumental advances of transversely heated graphite atomizer (THGA) tubes, chemical interferences are overcome allowing for faster, simpler direct calibration. The PinAAcle 900T atomic absorption spectrometer is suitable for clinical use in customer-validated applications for the measurement of aluminum in serum.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

009637_01