Determination of K, Na, and Zn in Albumin Using Flame AAS

Application Note

Area Identifier

Author

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Introduction

The highly soluble protein, albumin, has many functions within the body. The primary role of albumin is maintenance of blood plasma's osmotic pressure although it also acts as a transport protein for drugs, metals, fatty acids, hormones and enzymes. Sodium is a major cation in extra-cellular fluids and participates in many physiological functions. Potassium is the primary intracellular cation found in the human body. The concentration of ions in intracellular and extra cellular fluids is maintained by an active transport mechanism. Zinc is essential for growth of body cells and the functioning of enzymes and exists at trace levels in blood plasma and albumin.

Agilent Technologies

Instrumentation

All sample analyses were performed using the Agilent 240 FS double-beam AA spectrometer, which has the following specifications:

Optics

- All reflective optics for optimum transmission efficiency
- Fully sealed double-beam optics with hard quartz over-coated mirrors
- A single beam splitter with a rotating beam combining mirror for maximum light transmission and low noise

Monochromator

- Czerny-Turner 0.25 m focal length monochromator with microstepping driver for enhanced resolution
- Holographic grating with 1200 lines/mm
- Wavelength range 185-900 nm
- Computer controlled wavelength and slit selection with automatic peaking on each analytical wavelength
- Wavelength repeatability ± 0.04 nm or better

Lamp Control and Power Supplies

- The power supply runs in two modes. The first mode is a two-lamp power supply; one for the operating lamp and another for the automatic warm-up of the next lamp in sequence. The second mode powers four lamps simultaneously, enabling fast, sequential, multi-element per sample operation
- User selectable option to leave lamps on or automatic switch off at the end of an auto run
- Lamps are electronically modulated for better sensitivity and extended lifetime
- Four fixed lamp positions with fast automatic lamp selection (conventional lamp turret is slow) to enable fast sequential multi-element determinations sample by sample
- Lamps fit directly into the lamp sockets without the need for corrosion prone restraining clamps or leads and electrical connectors

Background Correction

- High speed deuterium background corrector for accurate correction of background signals
- Correction range 185 to 425 nm to a total 2.5 absorbance
- Electronic modulation with automatic gain attenuation for improved beam balance

Flame Control

- Fully programmable flame controller with safety interlocks
- Fuel and oxidant gas flows set from PC
- Gas control provides instantaneous gas flow change for fast sequential operation
- Automatic changeover between air and nitrous oxide flames as well as automatic recall and setting of optimum gas flows for each element.
- Separate flame-on and flame-off buttons
- Eight safety interlocks and separate upper and lower flame shields

Spraychamber

- External manual adjustment of impact bead (without tools), for complete control over sensitivity and atomization interferences
- PTFE impact bead available for applications where hydrofluoric acid is used
- Impact bead is available for use with both air-acetylene and nitrous oxide-acetylene flames
- Fluorinated high density polyethylene construction capable of handling acids, alkalis and organic solvents
- Fitted with an adjustable nebulizer with inert platinum/iridium capillary and PEEK venture for corrosion resistance

Burner

- Designed for high solids operation with minimal carbon build-up and burner blockage
- Universal design suitable for both nitrous oxide-acetylene and air-acetylene
- PTFE corrosion shield

Atomizer Mounting and Adjustment

- Atomizer mounting mechanism suitable for flame, vapor and furnace AAS with corrosion resistant construction
- Burner rotation through a full 90 degrees without having to remove flame shields or extinguish the flame

Materials and Reagents

Trace metal grade acetic acid (trace metal acetic acid, glacial (99.4%), Fisher Scientific, USA) and deionized water (18.2 MΩ Barnstead, Dubuque, IA, USA) were used for the sample and standards preparations. The standards were prepared by diluting multi-element stock standards (K, Na, and Zn, 1000mg/L, Inorganic Ventures, Lakewood, NJ USA). A stock QC (6020, 20mg/L, Inorganic Ventures, Lakewood, NJ, USA) was used for calibration verification. All samples, calibration standards, and QC's were diluted with 5% v/v acetic acid.

Sample Preparation

Calibration and QC solutions were prepared as summarized in Table 1. The albumin samples and dilution factors are detailed in Table 2.

Table 1. Calibration and QC Solutions

Standard Name	Element	Concentration (mg/L)
Blank	K, Na, Zn	0
Standard 1	K, Na, Zn	0.5
Standard 2	K, Na, Zn	1.0
QC1	Na, K	0.5
QC2	Zn	0.2

Table 2. Sample Preparations

Sample id	Element	Dilution
LA 05D	Na	5000x
LA 05D	K	10x
LH	Na	5000x
LH	Zn	2x
AHF	Na	5000x

The samples listed were 25% albumin and were submitted by a customer for analysis on the Agilent 240 FS. The sample dilutions used in this work were based upon the customer-provided estimates of the concentration of K, Na, and Zn.

Conditions

The analysis conditions used in this work are described in Table 3.

Table 3. AA Analysis Conditions

	Na	K	Zn
Wavelength (nm)	589.6	766.5	213.9
Gas used	Air/Acetylene	Air/Acetylene	Air/Acetylene
Bandpass (nm)	0.2	1	1
Fuel flow (L/min)	2	2	2
Mode	Emission	Emission	Absorption
D2 correction	No	No	Yes
Replicates	3	3	3

Results and Discussion

Table 4 details the original estimates of sample concentration provided by the customer as well as the sample concentration results obtained with the Agilent 240 FS AA spectrometer.

Table 4. Customer-Provided Estimates and Agilent 240 FS Spectrometer Sample Results

Sample id	Element	Customer-provided concentration (mg/L)	240 FS concentration (mg/L)	%RSD
LA 05D	Na	2500–3500	3090	0.3
LA 05D	K	3–5	4.15	0.5
LH	Na	3000–4000	3570	0.1
LH	Zn	0.2–0.5	0.267	0.8
AHF	Na	2500–3500	3080	0.1

Several samples were spiked to verify the instrument performance in the albumin matrix. Sample LA 05D was spiked at 0.3 mg/L for Na. Sample AHF was spiked at 0.2 mg/L for Na. Sample LH was spiked at 0.5 mg/L for Zn. The spike recovery results are listed below in Table 5. Duplicates of sample LH were analyzed for Na and sample LA 05D for analyzed for K as a further indication of the stability of the Agilent 240FS.

Table 5. Spike and Duplicate Results (on Diluted Sample)

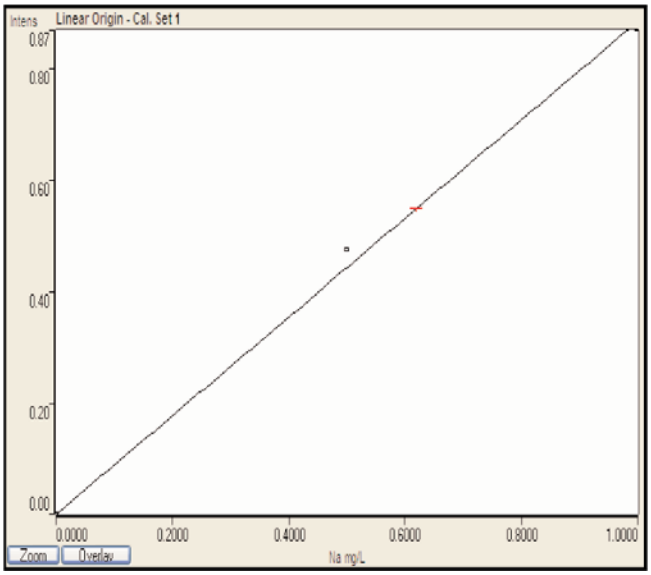
Sample id	Na (mg/L)	K (mg/L)	Zn (mg/L)
LA 05D 5000x Na	0.619		
LA 05D 5000x Na 0.3 ppm spk	0.939		
Spike recovery %	106.7		
LA 05D 10x K		0.415	
LA 05D 10x K dup		0.409	
LH 5000x Na	0.715		
LH 5000x Na dup	0.719		
LH 2x Zn			0.134
LH 2x Zn 0.5ppm spk			0.635
Spike recovery %			100.3
AHF 5000x Na	0.615		
AHF 5000x Na 0.2 ppm spk	0.828		
Spike recovery %	106.4		

The QC samples were analyzed after the calibration and at the end of the run. The results for the QC samples are as follows.

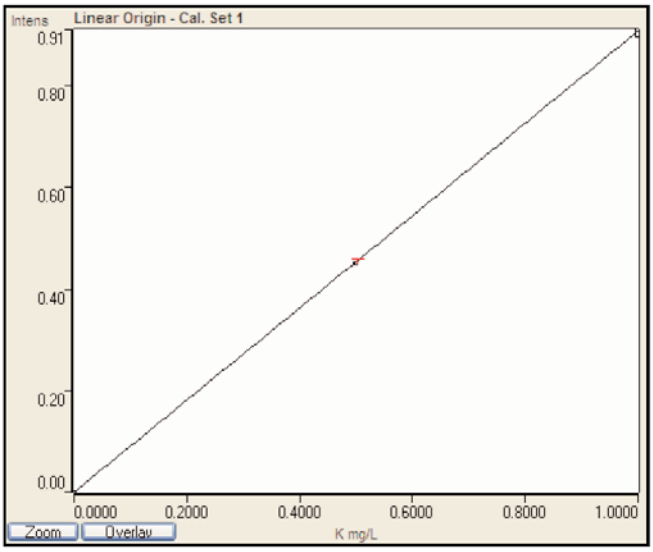
Table 6. QC Results (mg/L)

Sample id	Na	K	Zn
QC sample after calibration	0.509	0.503	0.199
% Recovery	101.8	100.5	99.4
QC sample at the end of run	0.517	0.51	0.201
% Recovery	103.4	100.8	100.6

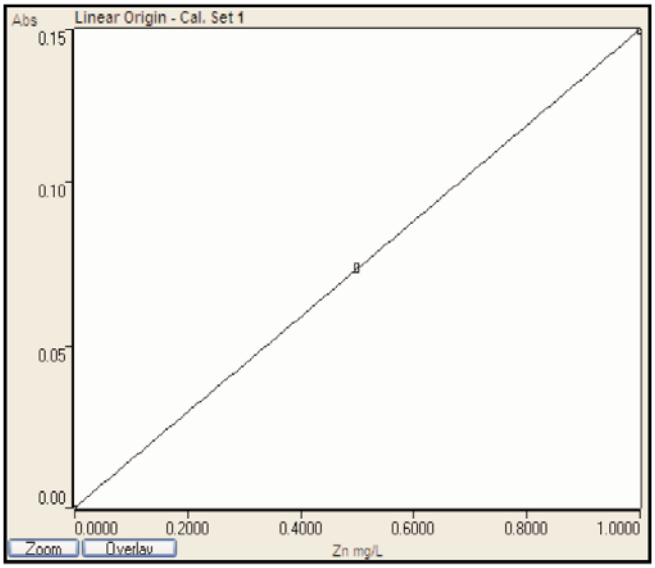
The calibration curves are detailed below; the curves are linear fits.



Curve fit linear origin
Characteristic conc: 0.0050 mg/L
Correlation coefficient 0.9984



Curve fit linear origin
Characteristic conc: 0.0048 mg/L
Correlation coefficient 1.000



Curve fit linear origin
Characteristic conc: 0.0300 mg/L
Correlation coefficient 1.000

Method detection limits were determined by analyzing seven replicates of the low standard (0.5 mg/L) and multiplying the standard deviation of the seven replicates by three. The method detection limits are listed in Table 7.

Table 7. Method Detection Limits

Element	Method Detection Limit (mg/L)
Na	0.008
K	0.009
Zn	0.001

Conclusion

The results obtained illustrate that the Agilent 240 FS AA system excels at providing a simple and effective solution for the determination of K, Na, and Zn in albumin. The method has shown to be accurate and repeatable.

The QC samples are within ± 10% of the true value. The spike recoveries and duplicate samples show recoveries from 90–110%. The duplicates samples are within 5% of each other.

The cookbook settings provided in the software were used for the analysis of Na, K, and Zn. All of the sample results fall within the expected ranges.

References

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Printed in the USA
August 21, 2015
SI-00409

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