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ANALYTICAL METHOD FOR THE DETERMINATION OF TRACE METALS BY INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROSCOPY (ICP-OES) IN BIOTECH PRODUCTS

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1. PURPOSE:

1.1. To provide a procedure for the assessment of Trace Metals BioTech products via ICP-OES. This procedure was assessed as a full quantitative option-1 procedure as per validation report BSI-RPT-2110 and follows the validation parameters for quantitation procedures as outlined in USP <233>.

1.2. Elements under USP <232> validated for this test method are as follows:

1.2.1. Class 2A: Co

1.2.2. Class 3: Ba, Cr, Cu, Li, and, Mo

1.2.3. Class 4: Al, Ca, Fe, K, Mg, Mn, Na, and Zn

1.2.4. Other: Bi, P, and Sr

2. SCOPE:

2.1. Applies to BioTech Line products, specifically Guanidine Hydrochloride, Guanidine Thiocyanate, HEPES, Bis-Tris, MOPS, MES Monohydrate, Tris, Urea, and Uridine, along with related products manufactured at BioSpectra.

2.2. Applies to the Perkin Elmer Avio 500 S/N 081S1905062 ICP-OES, or qualified ICP-OES, located in the Quality Control (QC) Laboratory at the BioSpectra Bangor, PA facility.

3. RESPONSIBILITIES:

3.1. The Laboratory Technology Manager, or other designated individual, is responsible for the control, implementation, training, and maintenance of this method.

3.2. The Laboratory Services Staff are responsible for complying with the requirements of this procedure

3.3. If any abnormalities are determined during routine use of the ICP-OES or during calibration, Management shall be promptly notified. If necessary, the ICP-OES will be serviced and recalibrated by Perkin Elmer before being approved for use.

4. REFERENCES:

4.1. BSI-PRL-0853, Analytical Method Validation Protocol: Trace Metals in BioTech Products

4.2. BSI-RPT-2110, Analytical Method Validation Report: Trace Metals in BioTech Products

4.3. BSI-SOP-0362, Operation and Maintenance of the Perkin Elmer Avio 500 ICP-OES

4.4. ICH Guideline for Elemental Impurities Q3D Current

4.5. NexION Operation with Syngistix Software Guide

4.6. USP <232> Elemental Impurities- Limits

4.7. USP <233> Elemental Impurities- Procedures

4.8. USP <730> Plasma Spectrochemistry

4.9. USP <1730> Plasma Spectrochemistry—Theory and Practice

TABLE 1: LIMITS FOR BIOTECH PRODUCTS

Element	ICH Class	30% LOQ (µg/g) in sample	50% Target (µg/g) in sample	100% Target (µg/g) in sample	150% Target (µg/g) in sample
Co	2A	1.5	2.5	5.0	7.5
Ba	3	1.5	2.5	5.0	7.5
Cr	3	1.5	2.5	5.0	7.5
Cu	3	1.5	2.5	5.0	7.5
Li	3	1.5	2.5	5.0	7.5
Mo	3	3.0	5.0	10	15
Al	4	1.5	2.5	5.0	7.5
Ca	4	1.5	2.5	5.0	7.5
Fe	4	1.5	2.5	5.0	7.5
K	4	15	25	50	75
Mg	4	1.5	2.5	5.0	7.5
Mn	4	1.5	2.5	5.0	7.5
Na	4	15	25	50	75
Zn	4	1.5	2.5	5.0	7.5
Bi	Not Applicable	3.0	5.0	10	15
P	Not Applicable	30	50	100	150
Sr	Not Applicable	1.5	2.5	5.0	7.5

5. MATERIALS AND EQUIPMENT:

5.1. Equipment
- 5.1.1. Analytical Balance
- 5.1.2. Perkin Elmer Avio 500 ICP-OES S/N 081S1905062, or qualified ICP-OES
- 5.1.3. Micropipettes, Rainin or Eppendorf

5.2. Reagents
- 5.2.1. Nitric Acid, Trace metals grade or equivalent
- 5.2.2. Deionized (DI) water (Type 1 Ultrapure)

5.3. Consumable Supplies
- 5.3.1. SCP Digitubes® 15 mL, 50 mL, and 100 mL
- 5.3.2. Pipette Tips of various sizes

5.4. Personnel
- 5.4.1. All personnel that executed the protocol are trained on ICP-OES or are considered Subject Matter Experts. This test method will be assigned a mark as read training to QC analysts involved with the execution.

TABLE 2: REFERENCE STANDARDS

Identification[1]	Manufacturer	Concentrations / Elements
Cobalt Stock Standard	SCP Science	Co (1,000 µg/mL)
Barium Stock Standard	SCP Science	Ba (1,000 µg/mL)
Chromium Stock Standard	SCP Science	Cr (1,000 µg/mL)
Copper Stock Standard	SCP Science	Cu (1,000 µg/mL)
Lithium Stock Standard	SCP Science	Li (1,000 µg/mL)
Molybdenum Stock Standard	SCP Science	Mo (1,000 µg/mL)
Aluminum Stock Standard	SCP Science	Al (1,000 µg/mL)
Calcium Stock Standard	SCP Science	Ca (1,000 µg/mL)
Iron Stock Standard	SCP Science	Fe (1,000 µg/mL)
Potassium Stock Standard	SCP Science	K (10,000 µg/mL)
Magnesium Stock Standard	SCP Science	Mg (1,000 µg/mL)
Manganese Stock Standard	SCP Science	Mn (1,000 µg/mL)
Sodium Stock Standard	SCP Science	Na (10,000 µg/mL)
Zinc Stock Standard	SCP Science	Zn (1,000 µg/mL)
Bismuth Stock Standard	SCP Science	Bi (1,000 µg/mL)
Phosphorous Stock Standard	Inorganic Ventures	P (10,000 µg/mL)
Strontium Stock Standard	SCP Science	Sr (1,000 µg/mL)
Scandium Stock Standard	SCP Science	Sc (1,000 µg/mL)
Yttrium Stock Standard	SCP Science	Y (1,000 µg/mL)

[1] Additional standards/custom standards can be used as long as the concentration remains the same in final preparations.

6. PROCEDURE:

6.1. All standards will be prepared volumetrically from stock solutions purchased from certified vendors. If the vendor supplied stock standard is within 2% of the nominal value as per the certificate of analysis, then the nominal value will be used to calculate the concentration of the standard. If the stock standard certificate of analysis value is greater than or less than 2% of the nominal value, then the certificate of analysis value will be used for the stock standard concentration.

6.2. Internal Standard Solution

6.2.1. Add 0.500 mL of Sc (1,000 µg/mL) and 0.500 mL of Y (1,000 µg/mL) to a 50 mL Digitube®.

6.2.2. Dilute to 50 mL final volume with deionized water.

6.2.3. Scale proportionally as needed for use.

6.3. Intermediate Standard Preparation

6.3.1. Prepare a standard solution containing the elements listed in Table 3, using the individual single source 1,000 µg/mL and 10,000 µg/mL stock standards. Prepare by adding stock standards to a 15 mL Digitube®. Dilute to final volume using DI Water.

TABLE 3: INTERMEDIATE STANDARD

Identification	Element	Stock Identification	Amount Added (mL)	Final Volume (mL)	Final Conc. (µg/mL)
Intermediate Standard	Co	1,000 µg/mL Co Std	0.500	10	50
	Ba	1,000 µg/mL Ba Std	0.500		50
	Cr	1,000 µg/mL Cr Std	0.500		50
	Cu	1,000 µg/mL Cu Std	0.500		50
	Li	1,000 µg/mL Li Std	0.500		50
	Mo	1,000 µg/mL Mo Std	1.000		100
	Al	1,000 µg/mL Al Std	0.500		50
	Ca	1,000 µg/mL Ca Std	0.500		50
	Fe	1,000 µg/mL Fe Std	0.500		50
	K	10,000 µg/mL K Std	0.500		500
	Mg	1,000 µg/mL Mg Std	0.500		50
	Mn	1,000 µg/mL Mn Std	0.500		50
	Na	10,000 µg/mL Na Std	0.500		500
	Zn	1,000 µg/mL Zn Std	0.500		50
	Bi	1,000 µg/mL Bi Std	1.000		100
	P	10,000 µg/mL P Std	1.000		1,000
	Sr	1,000 µg/mL Sr Std	0.500		50

6.4. 50% Calibration Standard Preparation

6.4.1. Prepare a solution containing the elements listed in Table 4 below in 5.0% HNO_3 acid matrix.

6.4.2. Add 0.050 mL of intermediate standard to a separate 50 mL Digitube® followed by addition of approximately 35 mL of deionized water.

6.4.3. Add 2.50 mL of nitric acid then dilute to 45 mL using deionized water.

6.4.4. Add 1.0 mL of internal standard solution and dilute to volume using deionized water.

6.4.5. Do not allow intermediate standard to contact concentrated acids while preparing solutions. (Standards are stable for 8 days).

TABLE 4: 50% CALIBRATION STANDARD

Identification	Element	Intermediate Standard (mL)	Nitric Acid (mL)	Internal Standard Solution (mL)	Final Volume (mL)	Final Conc. (µg/L)
50% Calibration Standard	Co	0.050	2.50	1.0	50	50
	Ba					50
	Cr					50
	Cu					50
	Li					50
	Mo					100
	Al					50
	Ca					50
	Fe					50
	K					500
	Mg					50
	Mn					50
	Na					500
	Zn					50
	Bi					100
	P					1,000
	Sr					50

6.5. 150% Calibration Standard Preparation

6.5.1. Prepare a solution containing the elements listed in Table 5 below in 5.0% HNO_3 acid matrix.

6.5.2. Add 0.150 mL of intermediate standard to a separate 50 mL Digitube® followed by addition of approximately 35 mL of deionized water.

6.5.3. Add 2.50 mL of nitric acid then dilute to 45 mL using deionized water.

6.5.4. Add 1.0 mL of internal standard solution and dilute to volume using deionized water.

6.5.5. Do not allow intermediate standard to contact concentrated acids while preparing solutions. (Standards are stable for 8 days).

TABLE 5: 150% CALIBRATION STANDARD

Identification	Element	Intermediate Standard (mL)	Nitric Acid (mL)	Internal Standard Solution (mL)	Final Volume (mL)	Final Conc. (µg/L)
150% Calibration Standard	Co	0.150	2.50	1.0	50	150
	Ba					150
	Cr					150
	Cu					150
	Li					150
	Mo					300
	Al					150
	Ca					150
	Fe					150
	K					1,500
	Mg					150
	Mn					150
	Na					1,500
	Zn					150
	Bi					300
	P					3,000
	Sr					150

6.6. 200% Calibration Standard Preparation

6.6.1. Prepare a solution containing the elements listed in Table 6 below in 5.0% HNO_3 acid matrix.

6.6.2. Add 0.200 mL of intermediate standard to a separate 50 mL Digitube® followed by addition of approximately 35 mL of deionized water.

6.6.3. Add 2.50 mL of nitric acid then dilute to 45 mL using deionized water.

6.6.4. Add 1.0 mL of internal standard solution and dilute to volume using deionized water.

6.6.5. Do not allow intermediate standard to contact concentrated acids while preparing solutions. (Standards are stable for 8 days).

TABLE 6: 200% CALIBRATION STANDARD

Identification	Element	Intermediate Standard (mL)	Nitric Acid (mL)	Internal Standard Solution (mL)	Final Volume (mL)	Final Conc. (µg/L)
200% Calibration Standard	Co	0.200	2.50	1.0	50	200
	Ba					200
	Cr					200
	Cu					200
	Li					200
	Mo					400
	Al					200
	Ca					200
	Fe					200
	K					2,000
	Mg					200
	Mn					200
	Na					2,000
	Zn					200
	Bi					400
	P					4,000
	Sr					200

6.7. Calibration Blank

6.7.1. Prepare a solution containing 5.0% HNO_3 acid matrix as described in Table 7 below.

6.7.2. To a separate 50 mL Digitube®, add approximately 35 mL of DI Water.

6.7.3. Add 2.50 mL of nitric acid then dilute to 45 mL using DI Water.

6.7.4. Add 1.0 mL of internal standard solution and dilute to volume using DI Water.

6.7.5. Do not allow Internal Standard Solution to contact concentrated acids.

TABLE 7: CALIBRATION BLANK

Description	Nitric Acid (mL)	Internal Standard (mL)	Final Volume (mL)
Cal Blank	2.50	1.0	50

6.8. Method Blank Preparation

6.8.1. Add approximately 35 mL of deionized water to a 50 mL Digitube®.

6.8.2. Add 2.50 mL of nitric acid and swirl to mix.

6.8.3. Add deionized water to approximately 45 mL and then transfer 1.0 mL of Internal Standard.

6.8.4. Dilute to a final volume of 50 mL using deionized water and mix well.

6.9. Sample Preparation

6.9.1. Samples are stable for 24 hours.

6.9.2. Weigh approximately 1.0 gram of sample into a 50 mL Digitube®.

6.9.3. Transfer approximately 10 mL of deionized water and swirl the solution to mix thoroughly.

6.9.4. Add 1.0 mL of nitric acid to guanidine thiocyanate sample solutions and swirl to mix. For all other products, add 2.50 mL of nitric acid and swirl to mix.

6.9.5. Add deionized water to approximately 45 mL and then transfer 1.0 mL of Internal Standard.

6.9.6. Dilute to a final volume of 50 mL with deionized water and mix thoroughly.

7. INSTRUMENT PROCEDURE:

7.1. Perform the ICP-OES daily performance check prior to beginning the analytical sequence. Refer to Avio 500 ICP-OES SOP, BSI-SOP-0362 for Daily Check procedures.

7.2. A calibration curve of no less than two standards and a blank must be used. The calibration correlation coefficient (R) must be ≥ 0.99.

7.3. Set up the sequence similar to that shown in Table 8.

7.4. Confirm the calibration by analyzing the 150% standard after the calibration. The calibration check must recover ± 20% of the calculated theoretical concentration following calibration.

7.5. A re-analysis of the check standard will be performed a minimum of once every 10 samples and at the end of the analytical run.

7.6. Bracketing standard checks must recover NMT 20% of the calculated theoretical concentration for multi-element analysis. Additionally, the drift (calculated as absolute difference) between the bracketing standard checks are to be NMT 20% for each target element.

7.7. The sample concentration is calculated as:

$$\text{Conc. } (\mu g/g) = \frac{\text{Solution Conc. } (\mu g/L) \times \text{Solution vol. (L)} \times \text{Dilution Factor}}{\text{Sample Mass (g)}}$$

TABLE 8: EXAMPLE SAMPLE ANALYSIS SEQUENCE

ID	Type	Level
Cal Blank	Cal Blank	Level 1
50% Cal Std	Cal Std	Level 2
150% Cal Std	Cal Std	Level 3
200% Cal Std	Cal Std	Level 4
Cal Blank Check	QC Check	Not Applicable
150% Check Std 1	QC Check	Not Applicable
Method Blank	Sample	Not Applicable
Sample(s) 10 or less	Sample	Not Applicable
150% Check Std 2	QC Check	Not Applicable

7.8. Instrument Setup and Parameters

7.8.1. Instrument settings are only listed as guidelines. Settings may be changed in order to accommodate changes in sample matrix or hardware configurations.

7.8.2. The gas flows for Plasma, Auxiliary, and Nebulizer can be set at 12 mL/min, 0.20 mL/min, and 0.70 mL/min, respectively.

7.8.3. The instrument method is stored under the Test Methods labelled as "BioTech_TraceMetal" for trace metal analysis

7.8.4. Instrument method can be truncated in order to selectively analyze metals as long as parameters match the full methods.

7.8.5. All wavelengths are tied to Sc 357.253 as the internal standard and performed in axial mode for detection. Other wavelengths that met validation parameters, as described in validation report BSI-RPT-2110, can be substituted at a later date if instrument conditions change over time.

TABLE 9: ICP-OES PARAMETERS

ICP-OES System	Perkin Elmer Avio 500 Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)
Points per Peak	4
Replicates	3
Viewing Distance	15.0
Nebulizer Gas	Argon
Shear Gas	Compressed Air
Sample Rinses	Rinse-1: 30 sec at 1.0 mL/min 5.0% HNO_3 (or as applicable to mitigate carry over)

TABLE 10: LINEAR RANGE AND CORRESPONDING WAVELENGTH

Element	Wavelength (nm)	Linear Range (µg/L)	Element	Wavelength (nm)	Linear Range (µg/L)
Co	230.786	30-200	K	766.490	300-2,000
Ba	493.408	30-200	Mg	279.553	30-200
Cr	267.716	30-200	Mn	257.610	30-200
Cu	324.752	30-200	Na	589.592	300-2,000
Li	670.784	30-200	Zn	202.548	30-200
Mo	203.845	60-400	Bi	206.170	60-400
Al	396.153	30-200	P	177.434	600-4,000
Ca	396.847	30-200	Sr	407.771	30-200
Fe	259.939	30-200			

8. REPORTING:

8.1. Any result below the 30% LOQ concentration will be reported as less than the corresponding LOQ value listed in Table 1. Results above the LOQ concentration will be reported in µg/g (ppm) according to Table 12 below.

TABLE 11: RESULT REPORTING

Result	Reporting
If < LOQ	Report as < LOQ
If ≥ LOQ and < 1.0 ppm	Report to two (2) decimal places
If ≥ LOQ and ≥ 1.0 ppm	Report to whole number