BIOSPECTRA
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ANALYTICAL METHOD VALIDATION REPORT: TRACE METAL ANALYSIS IN BIOTECH PRODUCTS

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1. PURPOSE:

1.1. The purpose of this validation report is to establish documented evidence that the validation protocol, BSI-PRL-0853 v. 1.0, for Trace Metals in BioTech products performs according to USP and BioSpectra requirements.

1.1.1. Elements under USP <232> will be considered and are as follows:

1.1.1.1. Class 2A: Co

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

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

1.1.1.4. Other: Bi, P, and Sr

2. SCOPE:

2.1. Applies to BioTech Line products manufactured at BioSpectra, which include Guanidine Hydrochloride, HEPES, MOPS, Bis-Tris, Guanidine Thiocyanate, MES Monohydrate, TRIS, Urea, and Uridine.

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

2.3. This report applies to the validation protocol for trace metals in BioTech Products by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) performed at BioSpectra Inc.

3. REFERENCES:

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

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

3.3. BSI-SOP-0436, Analytical Methods Validation Master Plan

3.4. ICH Guideline for Elemental Impurities Q3D

3.5. USP <232> Elemental Impurities-Limits

3.6. USP <233> Elemental Impurities-Procedures

3.7. USP <730> Plasma Spectrochemistry

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

4. BACKGROUND:

4.1. This method validation protocol was executed for multiple products. The linearity and specificity sections were executed once as the standard preparation was the same regardless of the product, while the rest of the validation attributes were executed by the validation procedure below and held to same validation criteria for each BioTech Line product.

4.2. The 100% Target Concentration was set by the lowest specification for most elements in all products associated with the validation. Bismuth, molybdenum, and phosphorous are set by detectability of the instrument where the proposed limit of quantitation is less than the lowest specification of products under the validation.

4.3. The test protocol validation report includes the following parameters:

4.3.1. Specificity

4.3.2. Linearity and Range

4.3.3. Limit of Quantification (LOQ)

4.3.4. Accuracy by “Spiked Recovery”

4.3.5. Precision (Repeatability)

4.3.6. Intermediate Precision (Ruggedness)

4.3.7. Standard and Sample Solution Stability

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:

TABLE 2: EQUIPMENT				
Type	**Supplier**	**Model**	**Serial Number**	**Cal. Due**
Analytical Balance	Sartorius	MSE224S	36707108	04/30/25
Automatic Pipette	Rainin	E4-XLS (20-200 µL)	C249353515	06/30/25
Automatic Pipette	Rainin	E4-XLS (100-1000 µL)	C244197408	06/30/25
Automatic Pipette	Rainin	E4-XLS (0.5-5 mL)	C238841856	06/30/25
ICP-OES	Perkin Elmer	Avio 500	081S1905062	09/2025
Deionized water system	Millipore	IQ-7005/ Element POD	F9SA14284H	05/17/25

TABLE 3: REAGENTS					
Type	**Grade**	**Supplier**	**Catalog Number**	**Lot Number**	**Expiration**
70% Nitric Acid	PlasmaPure	SCP Science	250-038-175	24010022	02/25/26
70% Nitric Acid	PlasmaPure	SCP Science	250-038-175	24010022	02/28/26
Deionized water	Type 1 Ultrapure	In-House	Not Applicable	Not Applicable	Not Applicable
Manganese Stock Standard	PlasmaCal	SCP Science	140-051-251	S231010001	04/30/25

5.1. Consumable Supplies
- 5.1.1. SCP Digitubes® 15 mL, 50 mL, and 100 mL
- 5.1.2. Pipette Tips of various sizes

5.2. Reagent Lots for validation analysis
- 5.2.1. Bis-Tris; BTRI-E04-1124-0025
- 5.2.2. Guanidine Hydrochloride (GHCL); GHCL-0224-00025
- 5.2.3. Guanidine Thiocyanate; GTHI-0124-00005
- 5.2.4. HEPES; HEPE-E03-1024-0097
- 5.2.5. MES Monohydrate; MESM-0124-00042
- 5.2.6. MOPS; MOPS-S02-0924-0210
- 5.2.7. Tris; TRIS-S01-1024-0177
- 5.2.8. Urea; UREA-0124-00020
- 5.2.9. Uridine; URID-0123-00005-PV

TABLE 4: REFERENCE STANDARDS					
Identification	**Part Number**	**Manufacturer**	**Lot Number**	**Expiration**	**Concentrations / Elements**
Cobalt Stock Standard	140-051-271	SCP Science	S240408028	09/25/25	Co (1,000 µg/mL)
Barium Stock Standard	140-051-561	SCP Science	S230623019	07/2025	Ba (1,000 µg/mL)
Chromium Stock Standard	140-052-241	SCP Science	S240306018	11/02/25	Cr (1,000 µg/mL)
Copper Stock Standard	140-051-291	SCP Science	S230721030	03/04/25	Cu (1,000 µg/mL)
Lithium Stock Standard	140-051-031	SCP Science	S231116003	10/02/25	Li (1,000 µg/mL)
Molybdenum Stock Standard	140-050-421	SCP Science	S240408002	03/30/26	Mo (1,000 µg/mL)
Aluminum Stock Standard	140-051-131	SCP Science	S240416015	11/02/25	Al (1,000 µg/mL)
Calcium Stock Standard	140-051-201	SCP Science	S230427001	02/03/25	Ca (1,000 µg/mL)
Iron Stock Standard	140-051-261	SCP Science	S230504001	01/31/25	Fe (1,000 µg/mL)
Potassium Stock Standard	140-061-191	SCP Science	S240805018	04/03/26	K (10,000 µg/mL)
Magnesium Stock Standard	140-051-121	SCP Science	S230607025	03/04/25	Mg (1,000 µg/mL)
Manganese Stock Standard	140-051-251	SCP Science	S231010001	04/30/25	Mn (1,000 µg/mL)
Sodium Stock Standard	140-061-111	SCP Science	S240619012	04/03/26	Na (10,000 µg/mL)
Zinc Stock Standard	140-051-301	SCP Science	S230619001	06/05/25	Zn (1,000 µg/mL)
Bismuth Stock Standard	140-051-831	SCP Science	S230523001	02/21/25	Bi (1,000 µg/mL)
Phosphorous Stock Standard	CGP10	Inorganic Ventures	V2-P742630	01/03/26	P (10,000 µg/mL)
Strontium Stock Standard	140-051-381	SCP Science	S240531005	02/01/26	Sr (1,000 µg/mL)
Scandium Stock Standard	140-051-211	SCP Science	S230824024	07/30/25	Sc (1,000 µg/mL)
Yttrium Stock Standard	140-051-391	SCP Science	S240404001	09/03/25	Y (1,000 µg/mL)

6. PROCEDURE:

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

6.2. Internal Standard Solution

6.2.1. Added 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. Diluted to 50 mL final volume with deionized water.

6.2.3. Scaled proportionally as needed for use.

6.3. Intermediate Standard Preparation

6.3.1. Prepared a standard solution containing the elements listed in Table 5, using the individual single source 1,000 µg/mL and 10,000 µg/mL stock standards. Prepared by adding stock standards to a 15 mL Digitube®. Diluted to final volume using DI Water.

TABLE 5: 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. Prepared a solution containing the elements listed in Table 6 below in 5.0% HNO_3. Intermediate standard was not allowed to contact concentrated acid while preparing solutions. Added intermediate standard to separate 50 mL Digitube® followed by addition of approximately 35 mL of DI Water. Added nitric acid then diluted to 45 mL using DI Water. Added internal standard solution and diluted to final volume using DI Water.

TABLE 6: 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. Prepared a solution containing the elements listed in Table 7 below in 5.0% HNO_3. Intermediate standard was not allowed to contact concentrated acid while preparing solutions. Added intermediate standard to separate 50 mL Digitube® followed by addition of approximately 35 mL of DI Water. Added nitric acid then diluted to 45 mL using DI Water. Added internal standard solution and diluted to final volume using DI Water.

TABLE 7: 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. Prepared a solution containing the elements listed in Table 8 below in 5.0% HNO_3. Intermediate standard was not allowed to contact concentrated acid while preparing solutions. Added intermediate standard to separate 50 mL Digitube® followed by addition of approximately 35 mL of DI Water. Added nitric acid then diluted to 45 mL using DI Water. Added internal standard solution and diluted to final volume using DI Water.

TABLE 8: 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. Prepared a solution containing 5.0% HNO_3 acid matrix as per Table 9 below. To a separate 50 mL Digitube®, added approximately 35 mL of DI Water. Added nitric acid then diluted to 45 mL using DI Water. Added Internal Standard and diluted to volume using DI Water.

TABLE 9: 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. Added approximately 35 mL of deionized water to a 50 mL Digitube®.

6.8.2. Added 2.50 mL of nitric acid and swirled to mix.

6.8.3. Added deionized water to approximately 45 mL and then transferred 1.0 mL of Internal Standard.

6.8.4. Diluted to a final volume of 50 mL using deionized water and mixed well.

6.9. Sample Preparation

6.9.1. Weighed approximately 1.0 gram of sample into a 50 mL Digitube®.

6.9.2. Transferred approximately 20 mL of deionized water and swirled the solution to mix thoroughly.

6.9.3. Added 1.0 mL of nitric acid for guanidine thiocyanate samples or 2.50 mL of nitric acid to all other product samples and allowed to react with gentle swirling.

6.9.4. Added deionized water to approximately 45 mL and then transferred 1.0 mL of Internal Standard.

6.9.5. Diluted to a final volume of 50 mL with deionized water and mixed thoroughly.

7. INSTRUMENT PROCEDURE:

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

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

7.3. Set up the sequence as per the example sequence in Table 10.

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

7.5. A re-analysis of the 150% check standard was performed at a minimum of once every 10 samples and at the end of the analytical run.

7.6. Bracketing standard checks must recover ±20% of the calculated theoretical concentration for each bracketing standard. Additionally, the drift (calculated as absolute difference) between the bracketing standard checks must be NMT 20% for each 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 10: 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.

TABLE 11: 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 12: LINEAR RANGE			
Element	**Mode**	**Wavelength (nm)**	**Linear Range (µg/L)**
Co	Axial	228.616 230.786	30-200
Ba	Axial	233.527 455.403 493.408	30-200
Cr	Axial	267.716 205.560	30-200
Cu	Axial	324.752 327.393	30-200
Li	Axial	670.784	30-200
Mo	Axial	203.845 204.597	60-400
Al	Axial	396.153	30-200
Ca	Axial	396.847	30-200
Fe	Axial	259.939	30-200
K	Axial	766.490	300-2,000
Mg	Axial	279.553	30-200
Mn	Axial	257.610	30-200
Na	Axial	589.592	300-2,000
Zn	Axial	202.548	30-200
Bi	Axial	206.170 223.061	60-400
P	Axial	177.434 178.221 214.914	600-4,000
Sr	Axial	407.771	30-200

7.9. Linearity and Range

7.9.1. The ICP-OES linearity study included standards equivalent to the concentrations shown in Table 14 and encompassed the following standards: 30%, 50%, 100%, 150%, and 200% of the Target Concentration. Each standard was prepared in triplicate and analyzed against the calibration curve described in Section 6.4 to Section 6.7. The average standard recovery for each level of the three replicates was then determined.

7.9.2. For all replicates of the linearity standards, intensity was plotted against concentration and correlation coefficients were determined for each wavelength. The data is uploaded as supporting information with the report.

7.9.3. The preparation for linearity standards is described in Table 13 and percent recovery values for each wavelength are tabulated in Table 15 below.

7.9.3.1. Acceptance Criteria:

7.9.3.1.1. The mean standard recovery for each element at each of the spike levels, as per USP <233> requirement, must be in the range of 70% - 150%.

TABLE 13: LINEARITY STANDARD PREPARATION

Description	Intermediate Standard (mL)	Nitric Acid (mL)	Internal Standard (mL)	Final Volume Deionized Water (mL)
Cal Blank Reference	Not Applicable	2.50	1.0	50
30% Standard	0.030	2.50	1.0	50
50% Standard	0.050	2.50	1.0	50
100% Standard	0.100	2.50	1.0	50
150% Standard	0.150	2.50	1.0	50
200% Standard	0.200	2.50	1.0	50

TABLE 14: LINEARITY STANDARD CONCENTRATIONS					
Element	**30% Standard (µg/L)**	**50% Standard (µg/L)**	**100% Standard (µg/L)**	**150% Standard (µg/L)**	**200% Standard (µg/L)**
Co	30	50	100	150	200
Ba	30	50	100	150	200
Cr	30	50	100	150	200
Cu	30	50	100	150	200
Li	30	50	100	150	200
Mo	60	100	200	300	400
Al	30	50	100	150	200
Ca	30	50	100	150	200
Fe	30	50	100	150	200
K	300	500	1,000	1,500	2,000
Mg	30	50	100	150	200
Mn	30	50	100	150	200
Na	300	500	1,000	1,500	2,000
Zn	30	50	100	150	200
Bi	60	100	200	300	400
P	600	1,000	2,000	3,000	4,000
Sr	30	50	100	150	200

TABLE 15: LINEARITY PERCENT RECOVERY RESULTS											
Wavelength	**30% Mean**	**50% Mean**	**100% Mean**	**150% Mean**	**200% Mean**	**Wavelength**	**30% Mean**	**50% Mean**	**100% Mean**	**150% Mean**	**200% Mean**
Fe 259.939	101%	101%	99%	100%	100%	Ba 455.403	101%	101%	101%	101%	101%
Mn 257.610	100%	99%	98%	98%	98%	Ba 493.408	99%	100%	100%	100%	101%
Zn 202.548	100%	101%	100%	100%	100%	Li 670.784	87%	89%	93%	97%	99%
Ca 396.847	101%	100%	99%	100%	101%	Mo 203.845	83%	90%	94%	98%	99%
Mg 279.553	101%	101%	100%	100%	100%	Cu 324.752	98%	98%	98%	99%	98%
Al 396.153	96%	95%	96%	98%	100%	Cu 327.393	100%	99%	98%	100%	100%
Cr 267.716	99%	100%	99%	100%	100%	Bi 206.170	100%	102%	101%	101%	102%
Cr 205.560	101%	101%	99%	100%	99%	Bi 223.061	94%	98%	98%	99%	99%
Na 589.592	89%	90%	93%	97%	100%	P 177.434	104%	102%	100%	100%	100%
K 766.490	89%	90%	94%	97%	101%	P 178.221	98%	99%	98%	97%	97%
Co 228.616	100%	100%	99%	99%	99%	P 214.914	100%	102%	100%	100%	100%
Co 230.786	100%	99%	100%	100%	100%	Sr 407.771	100%	100%	100%	100%	101%
Ba 233.527	100%	100%	99%	100%	100%						

All analytes meet Linearity acceptance criteria of 70% - 150%.

7.10. Accuracy

7.10.1. Three (N=3) unspiked samples were prepared for analysis. The unspiked sample preparations were used for spike recovery calculations. Samples were prepared in triplicate at three spiking levels (50%, 100%, and 150% of the 100% Target Concentration) as shown in Table 1. The solutions were analyzed by ICP-OES, as per the method, by a single analyst. Results are shown in Tables 17 to 19 for spike recoveries.

$$\% \text{ Recovery} = \frac{(\text{Conc. of spiked replicate} - \text{Average Conc. of 3 unspiked samples}) \times 100}{\text{Expected spiked concentration}}$$

7.10.1.1. Acceptance Criteria

7.10.1.1.1. The mean spike recovery for each element at each of the three spike levels, as per USP <233> requirement, must be in the range of 70% - 150%.

7.10.2. Spiked Reference (Unspiked) Solution Preparation

7.10.2.1. Prepared as per Section 6.9.

7.10.3. Spike Recovery Sample Preparation

7.10.3.1. Weighed approximately 1.0 gram of sample as per Table 16 into a 50 mL Digitube®.

7.10.3.2. Pipetted appropriate intermediate standard spike amount as per Table 16.

7.10.3.3. Added approximately 20 mL of deionized water and swirled to mix sample thoroughly.

7.10.3.4. Pipetted 1.0 mL of nitric acid for guanidine thiocyanate samples or 2.50 mL of nitric acid to all other product samples and allowed to react with gentle swirling.

7.10.3.5. Added deionized water to 45 mL and transferred 1.0 mL Internal Standard Solution.

7.10.3.6. Diluted to final volume of 50 mL using deionized water and mixed well.

7.10.3.7. Prepared spiked sample solutions in triplicate and three preparation of unspiked sample solutions.

TABLE 16: ACCURACY/LOQ SAMPLE SPIKES

Description	Sample Amount (mg)	Intermediate Standard Spike (mL)	Nitric Acid (mL)	Internal Standard (mL)	Final Volume (mL)
Method Blank	None	None	1.0/2.50	1.0	50
Unspiked	1.0	None	1.0/2.50	1.0	50
30% Spiked Sample	1.0	0.030	1.0/2.50	1.0	50
50% Spiked Sample	1.0	0.050	1.0/2.50	1.0	50
100% Spiked Sample	1.0	0.100	1.0/2.50	1.0	50
150% Spiked Sample	1.0	0.150	1.0/2.50	1.0	50

TABLE 17: ACCURACY RESULTS FOR MESM, BTRI, AND TRIS
(Mean percent recovery of triplicate preparations)

Wavelength	MESM			BTRI			TRIS		
	50% Mean	100% Mean	150% Mean	50% Mean	100% Mean	150% Mean	50% Mean	100% Mean	150% Mean
Fe 259.939	100%	100%	100%	103%	103%	102%	102%	102%	103%
Mn 257.610	95%	94%	93%	96%	96%	95%	96%	96%	96%
Zn 202.548	100%	101%	102%	105%	106%	105%	104%	105%	105%
Ca 396.847	100%	101%	101%	101%	102%	100%	101%	102%	102%
Mg 279.553	101%	101%	102%	105%	105%	104%	104%	104%	104%
Al 396.153	93%	95%	97%	93%	95%	95%	92%	94%	95%
Cr 267.716	102%	101%	103%	106%	105%	104%	104%	104%	104%
Cr 205.560	100%	100%	101%	104%	104%	103%	104%	104%	103%
Na 589.592	84%	87%	90%	80%	83%	85%	80%	83%	87%
K 766.490	96%	100%	103%	94%	97%	99%	93%	96%	100%
Co 228.616	96%	96%	96%	99%	100%	99%	100%	100%	100%
Co 230.786	98%	98%	99%	102%	102%	101%	102%	102%	102%
Ba 233.527	100%	100%	100%	102%	102%	101%	101%	102%	102%
Ba 455.403	101%	100%	101%	102%	101%	100%	101%	100%	101%
Ba 493.408	102%	102%	103%	104%	103%	102%	103%	102%	103%
Li 670.784	83%	86%	89%	80%	84%	87%	80%	84%	88%
Mo 203.845	95%	100%	103%	100%	101%	103%	97%	100%	104%
Cu 324.752	93%	93%	92%	91%	92%	91%	92%	93%	93%
Cu 327.393	96%	96%	96%	95%	95%	95%	94%	96%	97%
Bi 206.170	105%	106%	108%	112%	111%	109%	110%	109%	110%
Bi 223.061	88%	91%	93%	94%	93%	92%	89%	92%	92%
P 177.434	104%	105%	106%	111%	111%	110%	110%	109%	110%
P 178.221	100%	99%	98%	104%	104%	103%	102%	103%	104%
P 214.914	106%	106%	107%	113%	112%	111%	112%	110%	111%
Sr 407.771	105%	104%	105%	107%	106%	104%	106%	105%	105%

All elements meet Accuracy acceptance criteria of 70% - 150%.

TABLE 18: ACCURACY RESULTS FOR HEPE, UREA, URID
(Mean percent recovery of triplicate preparations)

Wavelength	HEPE			UREA			URID		
	50% Mean	100% Mean	150% Mean	50% Mean	100% Mean	150% Mean	50% Mean	100% Mean	150% Mean
Fe 259.939	99%	99%	99%	99%	99%	99%	101%	100%	101%
Mn 257.610	97%	96%	95%	94%	93%	93%	94%	94%	94%
Zn 202.548	100%	101%	101%	100%	102%	102%	102%	103%	102%
Ca 396.847	99%	100%	100%	98%	99%	101%	103%	101%	101%
Mg 279.553	100%	100%	100%	102%	103%	103%	103%	103%	103%
Al 396.153	94%	96%	102%	92%	93%	96%	93%	93%	95%
Cr 267.716	100%	100%	100%	101%	102%	102%	104%	103%	103%
Cr 205.560	99%	99%	99%	101%	101%	101%	102%	101%	102%
Na 589.592	83%	86%	88%	78%	80%	83%	78%	81%	83%
K 766.490	94%	98%	100%	87%	90%	93%	91%	94%	97%
Co 228.616	95%	95%	95%	97%	99%	98%	97%	98%	98%
Co 230.786	97%	96%	96%	98%	100%	100%	99%	99%	100%
Ba 233.527	99%	98%	99%	100%	101%	101%	100%	100%	100%
Ba 455.403	99%	100%	99%	99%	99%	98%	100%	99%	99%
Ba 493.408	100%	101%	101%	97%	98%	98%	101%	100%	100%
Li 670.784	81%	86%	88%	78%	81%	84%	79%	82%	85%
Mo 203.845	96%	100%	100%	86%	92%	95%	93%	97%	100%
Cu 324.752	93%	93%	92%	90%	90%	91%	90%	90%	92%
Cu 327.393	95%	95%	95%	93%	93%	95%	94%	93%	95%
Bi 206.170	100%	102%	102%	107%	109%	109%	109%	109%	109%
Bi 223.061	89%	91%	90%	82%	85%	89%	83%	85%	88%
P 177.434	104%	103%	104%	100%	100%	101%	103%	103%	105%
P 178.221	100%	100%	99%	90%	90%	91%	93%	94%	96%
P 214.914	103%	105%	104%	99%	100%	100%	107%	105%	106%
Sr 407.771	101%	103%	103%	104%	104%	104%	105%	105%	105%

All elements meet Accuracy acceptance criteria of 70% - 150%.

TABLE 19: ACCURACY RESULTS FOR GHCL, GTHI, MOPS
(Mean percent recovery of triplicate preparations)

Wavelength	GHCL			GTHI			MOPS		
	50% Mean	100% Mean	150% Mean	50% Mean	100% Mean	150% Mean	50% Mean	100% Mean	150% Mean
Fe 259.939	93%	93%	95%	104%	102%	101%	96%	95%	95%
Mn 257.610	98%	98%	96%	97%	95%	93%	93%	93%	93%
Zn 202.548	97%	98%	99%	99%	100%	101%	103%	102%	101%
Ca 396.847	98%	98%	99%	99%	102%	103%	91%	94%	98%
Mg 279.553	101%	101%	101%	103%	103%	103%	102%	102%	102%
Al 396.153	92%	93%	93%	101%	98%	99%	94%	95%	95%
Cr 267.716	99%	99%	100%	102%	103%	103%	103%	102%	101%
Cr 205.560	98%	98%	98%	100%	101%	101%	100%	100%	99%
Na 589.592	80%	85%	87%	91%	93%	95%	81%	83%	84%
K 766.490	85%	88%	89%	94%	97%	100%	92%	95%	97%
Co 228.616	98%	98%	97%	98%	97%	97%	97%	96%	95%
Co 230.786	98%	98%	98%	99%	99%	99%	101%	99%	98%
Ba 233.527	99%	99%	99%	101%	101%	101%	101%	100%	99%
Ba 455.403	100%	101%	100%	102%	102%	102%	100%	100%	100%
Ba 493.408	98%	99%	98%	102%	103%	103%	100%	101%	100%
Li 670.784	84%	88%	90%	86%	90%	92%	79%	82%	84%
Mo 203.845	93%	99%	101%	88%	96%	100%	96%	99%	99%
Cu 324.752	91%	92%	92%	89%	90%	89%	92%	90%	90%
Cu 327.393	96%	94%	95%	95%	95%	95%	94%	93%	94%
Bi 206.170	100%	101%	103%	109%	112%	114%	110%	109%	108%
Bi 223.061	95%	95%	94%	88%	90%	92%	88%	86%	85%
P 177.434	96%	95%	94%	102%	104%	105%	98%	98%	97%
P 178.221	95%	95%	93%	104%	102%	100%	95%	93%	91%
P 214.914	97%	97%	97%	103%	106%	108%	101%	100%	99%
Sr 407.771	100%	101%	101%	108%	109%	109%	104%	105%	105%

7.11. Specificity

7.11.1. Specificity was demonstrated by using a calibration blank and spiked calibration blank for ICP-OES analysis. The calibration blank was prepared as per the analytical method protocol. A separate blank was spiked with a mixed standard solution which produced a spiked solution at a concentration equivalent to the 200% calibration standard.

7.11.2. The solutions were analyzed as per the analytical method and the intensities for the calibration blank, 200% calibration standard, and method blank are reported in Table 20.

7.11.2.1. Acceptance Criteria:

7.11.2.1.1. The lack of a significant interference (as demonstrated by the spike recovery of 70% to 150%, as per the Accuracy requirement from USP <233>) or by any other element in the spiked blank solution or the solution matrix itself will indicate the specificity of the method.

TABLE 20: SPECIFICITY RESULTS

Wavelength	Blank (CPS)	200% STD (CPS)	Method Blank (CPS)	Wavelength	Blank (CPS)	200% STD (CPS)	Method Blank (CPS)
Fe 259.939	-11	26475	17	Ba 455.403	213	1066351	-74
Mn 257.610	608	126841	-5	Ba 493.408	-443	1319296	48
Zn 202.548	23	8863	-22	Li 670.784	423	2078262	267
Ca 396.847	3579	1142005	487	Mo 203.845	292	2775	48
Mg 279.553	157	566085	1	Cu 324.752	1231	32591	-22
Al 396.153	-68	20898	73	Cu 327.393	-2984	21865	29
Cr 267.716	-26	12635	37	Bi 206.170	35	5099	8
Cr 205.560	-43	5531	8	Bi 223.061	-153	1854	11
Na 589.592	5180	654589	-2115	P 177.434	-19	2138	2
K 766.490	14319	598386	-6777	P 178.221	28	3317	1
Co 228.616	-257	8586	4	P 214.914	176	4358	11
Co 230.786	-136	7078	1	Sr 407.771	-341	3154311	44
Ba 233.527	-86	20147	1				

7.12. Precision

7.12.1. All solutions for the Precision (Repeatability) experiments were prepared by a single analyst for all BioTech samples and reported in Table 21 to Table 23.

7.12.2. The value of the unspiked sample preparations from Section 7.10, "Accuracy," was used for spike recovery calculations. Six sample solutions were prepared at the 100% Target Concentration as shown in Table 1. For ICP-OES analysis, the Target Concentration spiked samples and the unspiked samples were used for the accuracy experiment.

7.12.3. Precision results are reported to three decimal places and %RSD results are reported to the whole number, but values are calculated from data that contains nine decimal places.

7.12.3.1. Acceptance Criteria:

7.12.3.1.1. The %RSD for the spike recovery concentration must be NMT 20% for each element in each sample.

TABLE 21: PRECISION RESULTS FOR MESM, BTRI, TRIS
(Mean concentration of 6 preparations)

Wavelength	MESM		BTRI		TRIS	
	100% Mean Conc. N=6 (ppm)	%RSD N=6	100% Mean Conc. N=6 (ppm)	%RSD N=6	100% Mean Conc. N=6 (ppm)	%RSD N=6
Fe 259.939	5.071	0%	5.133	0%	5.111	1%
Mn 257.610	4.708	0%	4.787	0%	4.829	0%
Zn 202.548	4.982	1%	5.231	0%	5.169	1%
Ca 396.847	5.212	0%	5.306	0%	5.179	1%
Mg 279.553	5.093	0%	5.245	0%	5.214	0%
Al 396.153	4.858	1%	5.420	1%	4.981	1%
Cr 267.716	5.126	1%	5.287	0%	5.246	0%
Cr 205.560	4.929	1%	5.024	0%	5.042	0%
Na 589.592	44.048	0%	42.062	0%	42.376	1%
K 766.490	49.163	0%	47.644	0%	47.886	0%
Co 228.616	4.816	0%	4.962	0%	4.964	0%
Co 230.786	4.860	0%	5.005	0%	5.007	1%
Ba 233.527	4.998	0%	5.089	0%	5.078	0%
Ba 455.403	5.028	1%	5.054	0%	5.027	0%
Ba 493.408	5.146	1%	5.187	0%	5.129	0%
Li 670.784	4.446	1%	4.325	0%	4.348	0%
Mo 203.845	10.874	2%	10.815	1%	10.751	1%
Cu 324.752	4.607	0%	4.508	0%	4.580	1%
Cu 327.393	4.951	0%	4.938	1%	4.938	1%
Bi 206.170	10.661	1%	11.108	0%	10.950	0%
Bi 223.061	9.101	1%	9.279	1%	9.315	2%
P 177.434	117.915	1%	110.046	1%	108.317	0%
P 178.221	96.875	1%	102.945	0%	102.665	1%
P 214.914	109.556	1%	117.002	0%	114.641	0%
Sr 407.771	5.267	1%	5.338	0%	5.263	0%

All analytes meet Precision RSD% acceptance criteria of NMT 20%.

TABLE 22: PRECISION RESULTS FOR HEPE, UREA, URID
(Mean concentration of 6 preparations)

Wavelength	HEPE		UREA		URID	
	100% Mean Conc. N=6 (ppm)	%RSD N=6	100% Mean Conc. N=6 (ppm)	%RSD N=6	100% Mean Conc. N=6 (ppm)	%RSD N=6
Fe 259.939	4.918	1%	4.960	1%	4.999	1%
Mn 257.610	4.772	1%	4.651	0%	4.687	0%
Zn 202.548	4.993	0%	5.050	0%	5.095	1%
Ca 396.847	5.187	1%	5.250	0%	5.682	1%
Mg 279.553	5.029	0%	5.217	0%	5.342	0%
Al 396.153	4.844	1%	4.773	1%	4.901	0%
Cr 267.716	5.053	0%	5.200	1%	5.229	1%
Cr 205.560	4.819	0%	5.000	0%	4.936	1%
Na 589.592	44.321	1%	41.715	1%	41.772	1%
K 766.490	50.091	1%	51.126	1%	48.232	1%
Co 228.616	4.755	1%	4.847	1%	4.833	1%
Co 230.786	4.758	1%	4.893	0%	4.872	1%
Ba 233.527	4.934	0%	5.026	1%	5.002	1%
Ba 455.403	4.967	1%	4.961	1%	4.973	1%
Ba 493.408	5.059	1%	4.931	1%	5.037	1%
Li 670.784	4.411	1%	4.199	1%	4.232	1%
Mo 203.845	10.728	1%	10.470	2%	10.979	2%
Cu 324.752	4.622	1%	4.424	1%	4.436	0%
Cu 327.393	4.842	1%	4.771	0%	4.787	1%
Bi 206.170	10.351	0%	11.082	1%	11.016	1%
Bi 223.061	8.666	2%	8.215	1%	8.332	2%
P 177.434	113.540	0%	98.333	1%	103.505	1%
P 178.221	98.673	1%	89.690	1%	96.287	1%
P 214.914	108.189	1%	108.613	1%	113.482	1%
Sr 407.771	5.171	1%	5.233	1%	5.248	1%

All anal ytesmeet Precision RSD% acceptance criteria of NMT 20%.

TABLE 23: PRECISION RESULTS FOR GHCL, GTHI, MOPS
(Mean concentration of 6 preparations)

Wavelength	GHCL		GTHI		MOPS	
	100% Mean Conc. N=6 (ppm)	%RSD N=6	100% Mean Conc. N=6 (ppm)	%RSD N=6	100% Mean Conc. N=6 (ppm)	%RSD N=6
Fe 259.939	4.709	1%	4.776	2%	4.742	2%
Mn 257.610	4.874	0%	4.752	0%	4.634	1%
Zn 202.548	4.956	1%	5.067	1%	5.020	2%
Ca 396.847	5.574	1%	5.182	1%	6.122	1%
Mg 279.553	5.082	0%	5.160	0%	5.337	1%
Al 396.153	4.759	1%	4.714	0%	4.784	1%
Cr 267.716	5.005	1%	5.182	1%	5.166	2%
Cr 205.560	4.873	1%	4.984	1%	4.896	2%
Na 589.592	51.547	1%	51.266	1%	43.449	2%
K 766.490	46.649	1%	48.287	0%	52.306	1%
Co 228.616	4.878	0%	4.828	1%	4.739	1%
Co 230.786	4.896	1%	4.901	1%	4.828	1%
Ba 233.527	4.975	1%	5.041	1%	5.000	2%
Ba 455.403	5.041	0%	5.064	1%	5.017	2%
Ba 493.408	4.929	1%	5.064	1%	5.044	2%
Li 670.784	4.517	1%	4.553	1%	4.205	2%
Mo 203.845	10.655	1%	11.029	3%	11.296	2%
Cu 324.752	4.577	1%	4.502	1%	4.436	2%
Cu 327.393	4.779	0%	4.789	1%	4.766	1%
Bi 206.170	10.282	1%	10.803	1%	10.957	2%
Bi 223.061	9.627	3%	7.602	4%	8.002	2%
P 177.434	96.482	0%	121.977	1%	111.596	2%
P 178.221	94.737	1%	94.241	1%	90.401	2%
P 214.914	101.353	1%	111.183	1%	111.031	2%
Sr 407.771	5.069	1%	5.212	1%	5.247	2%

All analytes meet Precision RSD% acceptance criteria of NMT 20%.

7.13. Intermediate Precision (Ruggedness)

7.13.1. A second analyst, on a different day from the performance of the Repeatability experiments, prepared and analyzed the Intermediate Precision solutions. Six sample solutions were prepared at the 100% Target Concentration level found in Table 1 for ICP-OES analysis (this fulfilled two events as "different day" and "different analyst").

7.13.2. Ruggedness results are reported to three decimal places and %RSD results are reported to the nearest whole number, but values are calculated from data that contains nine decimal places. Results are reported in Table 24 to Table 26.

7.13.2.1. Acceptance Criteria:

7.13.2.1.1. The %RSD for the spike recovery concentration from both analysts (N=12) must be NMT 25% for each element.

TABLE 24: RUGGEDNESS RESULTS FOR MESM, BTRI, TRIS
(Mean concentration of 12 preparations)

Wavelength	MESM		BTRI		TRIS	
	100% Mean Conc. N=12 (ppm)	%RSD N=12	100% Mean Conc. N=12 (ppm)	%RSD N=12	100% Mean Conc. N=12 (ppm)	%RSD N=12
Fe 259.939	5.062	0%	5.072	2%	5.093	1%
Mn 257.610	4.754	1%	4.826	1%	4.857	1%
Zn 202.548	4.994	1%	5.150	2%	5.129	1%
Ca 396.847	5.191	1%	5.254	2%	5.172	1%
Mg 279.553	5.078	1%	5.171	2%	5.169	1%
Al 396.153	4.860	1%	5.353	1%	4.962	1%
Cr 267.716	5.129	1%	5.215	2%	5.207	1%
Cr 205.560	4.929	1%	4.96	1%	4.996	1%
Na 589.592	44.780	2%	42.905	2%	43.044	2%
K 766.490	49.802	1%	47.895	1%	48.449	1%
Co 228.616	4.829	1%	4.912	1%	4.943	1%
Co 230.786	4.853	0%	4.941	1%	4.972	1%
Ba 233.527	4.998	0%	5.020	2%	5.041	1%
Ba 455.403	5.049	1%	5.061	1%	5.068	1%
Ba 493.408	5.132	1%	5.127	1%	5.119	0%
Li 670.784	4.451	1%	4.310	1%	4.345	0%
Mo 203.845	10.905	2%	10.759	1%	10.754	1%
Cu 324.752	4.663	1%	4.563	1%	4.646	2%
Cu 327.393	4.956	0%	4.906	1%	4.954	1%
Bi 206.170	10.556	1%	10.743	4%	10.714	2%
Bi 223.061	9.148	1%	9.172	2%	9.231	2%
P 177.434	117.839	1%	108.711	2%	108.113	1%
P 178.221	98.921	2%	104.805	2%	104.434	2%
P 214.914	109.883	1%	114.88	2%	113.291	2%
Sr 407.771	5.224	1%	5.221	2%	5.215	1%

All analytes meet the Ruggedness %RSD acceptance criteria of NMT 25%.

TABLE 25: RUGGEDNESS RESULTS FOR HEPE, UREA, URID
(Mean concentration of 12 preparations)

Wavelength	HEPE		UREA		URID	
	100% Mean Conc. N=12 (ppm)	%RSD N=12	100% Mean Conc. N=12 (ppm)	%RSD N=12	100% Mean Conc. N=12 (ppm)	%RSD N=12
Fe 259.939	5.061	10%	4.986	1%	5.013	1%
Mn 257.610	4.751	1%	4.692	1%	4.726	1%
Zn 202.548	4.978	1%	5.033	1%	5.020	2%
Ca 396.847	5.181	1%	5.255	1%	5.695	1%
Mg 279.553	5.021	0%	5.202	0%	5.309	1%
Al 396.153	4.815	1%	4.814	2%	4.917	1%
Cr 267.716	5.058	1%	5.205	1%	5.214	1%
Cr 205.560	4.805	1%	4.997	1%	4.920	1%
Na 589.592	44.778	1%	43.248	4%	42.99	3%
K 766.490	49.960	1%	52.068	2%	49.029	2%
Co 228.616	4.752	1%	4.871	1%	4.838	1%
Co 230.786	4.759	1%	4.927	1%	4.873	1%
Ba 233.527	4.933	1%	5.031	1%	4.979	1%
Ba 455.403	5.032	2%	5.071	2%	5.061	2%
Ba 493.408	5.080	1%	4.995	2%	5.087	1%
Li 670.784	4.385	1%	4.256	2%	4.266	1%
Mo 203.845	10.726	0%	10.588	2%	11.063	2%
Cu 324.752	4.623	1%	4.494	2%	4.544	3%
Cu 327.393	4.871	1%	4.833	2%	4.874	2%
Bi 206.170	10.305	1%	10.967	1%	10.826	2%
Bi 223.061	8.714	2%	8.697	6%	8.675	4%
P 177.434	113.872	1%	99.933	2%	104.086	1%
P 178.221	98.882	1%	92.562	3%	98.956	3%
P 214.914	108.657	2%	108.923	1%	112.996	1%
Sr 407.771	5.156	1%	5.222	1%	5.229	1%

All analytes meet the Ruggedness %RSD acceptance criteria of NMT 25%.

TABLE 26: RUGGEDNESS RESULTS FOR GHCL, GTHI, MOPS
(Mean concentration of 12 preparations)

Wavelength	GHCL		GTHI		MOPS	
	100% Mean Conc. N=12 (ppm)	%RSD N=12	100% Mean Conc. N=12 (ppm)	%RSD N=12	100% Mean Conc. N=12 (ppm)	%RSD N=12
Fe 259.939	4.886	4%	4.914	3%	4.866	3%
Mn 257.610	4.881	0%	4.717	1%	4.707	2%
Zn 202.548	4.945	1%	5.047	1%	4.974	1%
Ca 396.847	5.641	2%	5.195	1%	6.182	1%
Mg 279.553	5.087	0%	5.159	0%	5.307	1%
Al 396.153	4.821	1%	4.744	1%	4.828	1%
Cr 267.716	5.024	1%	5.192	1%	5.126	2%
Cr 205.560	4.897	1%	4.992	1%	4.875	1%
Na 589.592	52.289	2%	51.883	1%	44.602	3%
K 766.490	47.760	3%	48.953	1%	53.483	2%
Co 228.616	4.887	0%	4.818	1%	4.755	1%
Co 230.786	4.893	0%	4.880	1%	4.796	1%
Ba 233.527	4.984	1%	5.040	0%	4.974	1%
Ba 455.403	5.055	0%	5.087	1%	5.018	1%
Ba 493.408	4.969	1%	5.104	1%	5.066	1%
Li 670.784	4.610	2%	4.576	1%	4.299	3%
Mo 203.845	10.588	1%	10.986	2%	11.095	3%
Cu 324.752	4.635	2%	4.471	1%	4.520	2%
Cu 327.393	4.829	1%	4.823	1%	4.826	2%
Bi 206.170	10.411	2%	11.100	3%	10.799	2%
Bi 223.061	9.586	2%	8.176	8%	8.521	7%
P 177.434	97.611	1%	123.149	1%	113.699	2%
P 178.221	96.008	2%	95.753	2%	95.836	6%
P 214.914	102.079	1%	112.407	1%	110.072	2%
Sr 407.771	5.105	1%	5.345	3%	5.230	1%

All analytes meet the Ruggedness %RSD acceptance criteria of NMT 25%.

7.14. Limit of Quantitation (LOQ)

7.14.1. The limit of quantitation (LOQ) is demonstrated from spike recovery performed at the 30% Target Concentration spiking levels as shown in Table 1.

7.14.2. Samples were prepared in triplicate following Section 7.10.3. Results for all BioTech Products are reported in Table 27 to Table 28 below.

7.14.2.1. Acceptance Criteria:

7.14.2.1.1. The mean percent spike recovery for each wavelength at the 30% Target Concentration spiking levels, as per the USP <233> accuracy guideline, must be in the range of 70% - 150%.

TABLE 27: LIMIT OF QUANTITATION RESULTS FOR MESM, BTRI, TRIS, HEPE, UREA
(MEAN PERCENT RECOVERY OF 3 PREPARATIONS)

Wavelength	MESM 30% Recovery	BTRI 30% Recovery	TRIS 30% Recovery	HEPE 30% Recovery	UREA 30% Recovery
Fe 259.939	100%	104%	104%	100%	100%
Mn 257.610	96%	97%	98%	97%	95%
Zn 202.548	101%	104%	105%	101%	99%
Ca 396.847	102%	99%	102%	99%	97%
Mg 279.553	102%	105%	105%	100%	103%
Al 396.153	95%	95%	94%	96%	93%
Cr 267.716	101%	106%	106%	101%	102%
Cr 205.560	101%	104%	106%	100%	102%
Na 589.592	84%	79%	80%	82%	78%
K 766.490	95%	93%	93%	93%	87%
Co 228.616	97%	99%	102%	96%	98%
Co 230.786	98%	103%	104%	96%	99%
Ba 233.527	100%	102%	103%	99%	100%
Ba 455.403	101%	102%	102%	100%	100%
Ba 493.408	103%	104%	103%	100%	98%
Li 670.784	82%	79%	80%	81%	77%
Mo 203.845	92%	91%	90%	94%	91%
Cu 324.752	94%	91%	92%	94%	92%
Cu 327.393	96%	95%	97%	94%	94%
Bi 206.170	105%	112%	111%	99%	107%
Bi 223.061	91%	91%	87%	86%	82%
P 177.434	106%	112%	111%	103%	103%
P 178.221	100%	105%	106%	100%	92%
P 214.914	106%	114%	112%	105%	101%
Sr 407.771	104%	107%	107%	102%	104%

All analytes meet LOQ acceptance criteria of 70% - 150%.

TABLE 28: LIMIT OF QUANTITATION RESULTS FOR URID, GHCL, GTHI, MOPS
(MEAN PERCENT RECOVERY OF 3 PREPARATIONS)

Wavelength	URID 30% Recovery	GHCL 30% Recovery	GTHI 30% Recovery	MOPS 30% Recovery
Fe 259.939	101%	91%	106%	98%
Mn 257.610	96%	99%	99%	94%
Zn 202.548	102%	96%	98%	103%
Ca 396.847	101%	98%	98%	88%
Mg 279.553	103%	102%	104%	102%
Al 396.153	92%	96%	100%	96%
Cr 267.716	103%	101%	103%	103%
Cr 205.560	102%	99%	102%	102%
Na 589.592	78%	79%	90%	82%
K 766.490	90%	85%	95%	92%
Co 228.616	97%	100%	99%	98%
Co 230.786	99%	100%	102%	101%
Ba 233.527	99%	100%	102%	103%
Ba 455.403	100%	102%	103%	102%
Ba 493.408	101%	99%	104%	102%
Li 670.784	78%	84%	87%	79%
Mo 203.845	85%	87%	79%	94%
Cu 324.752	91%	93%	91%	94%
Cu 327.393	94%	98%	98%	94%
Bi 206.170	107%	100%	108%	112%
Bi 223.061	81%	91%	93%	91%
P 177.434	102%	95%	100%	101%
P 178.221	94%	97%	110%	97%
P 214.914	105%	99%	99%	101%
Sr 407.771	105%	102%	107%	106%

All analytes meet LOQ acceptance criteria of 70% - 150%.

7.15. Sample and Standard Stability

7.15.1. The 50% and 200% Target Concentration level calibration standards were analyzed as samples against calibration curves constructed from freshly prepared calibration standards at T=1 day and T=8 days from the date of preparation.

7.15.2. A spiked sample solution prepared at the 100% Target Concentration level from the Precision or Ruggedness experiments were used for sample stability. The spiked sample solution was analyzed against calibration curves constructed from freshly prepared calibration standards at time point T=1 (1 day from date of preparation).

7.15.3. Both sample and standard solution stability results for T=1 day and 8 days are reported in Table 29 and Table 30.

7.15.3.1. Acceptance Criteria:

7.15.3.1.1. The recovery of each element must be within the range of 80% to 120% recovery of the T = 0 results for the calibration standard.

7.15.3.1.2. The recovery of each element must be within the range of 80% to 120% recovery of the T = 0 results for the spiked sample solution.

TABLE 29: SAMPLE AND STANDARD STABILITY (% RECOVERY)

Wavelength	50% Std T=1 (%)	200% Std T=1 (%)	50% Std T=8 (%)	200% Std T=8 (%)	MESM T=1 (%)	BTRI T=1 (%)	TRIS T=1 (%)
Fe 259.939	99%	100%	97%	102%	99%	99%	99%
Mn 257.610	99%	99%	97%	99%	101%	99%	101%
Zn 202.548	97%	97%	99%	100%	97%	97%	98%
Ca 396.847	101%	100%	102%	100%	100%	96%	105%
Mg 279.553	99%	99%	100%	100%	98%	99%	99%
Al 396.153	95%	99%	96%	99%	100%	93%	107%
Cr 267.716	99%	98%	100%	100%	98%	99%	99%
Cr 205.560	99%	98%	98%	99%	99%	100%	99%
Na 589.592	100%	100%	99%	99%	100%	98%	98%
K 766.490	99%	99%	98%	99%	99%	100%	98%
Co 228.616	97%	98%	99%	99%	99%	99%	98%
Co 230.786	99%	98%	98%	99%	99%	98%	98%
Ba 233.527	99%	98%	99%	100%	98%	99%	99%
Ba 455.403	100%	100%	99%	99%	98%	99%	97%
Ba 493.408	100%	100%	98%	99%	99%	100%	99%
Li 670.784	98%	99%	97%	99%	99%	99%	98%
Mo 203.845	111%	98%	116%	99%	103%	98%	99%
Cu 324.752	100%	99%	97%	98%	102%	101%	99%
Cu 327.393	100%	99%	97%	98%	100%	101%	99%
Bi 206.170	100%	99%	110%	108%	97%	101%	99%
Bi 223.061	99%	98%	103%	100%	101%	100%	100%
P 177.434	96%	96%	99%	99%	95%	95%	98%
P 178.221	98%	96%	98%	99%	100%	96%	100%
P 214.914	96%	97%	104%	100%	96%	95%	96%
Sr 407.771	100%	100%	102%	101%	99%	99%	99%

TABLE 30: SAMPLE AND STANDARD STABILITY (% RECOVERY)						
Wavelength	**HEPE T=1 (%)**	**UREA T=1 (%)**	**URID T=1 (%)**	**GHCL T=1 (%)**	**GTHI T=1 (%)**	**MOPS T=1 (%)**
Fe 259.939	100%	100%	100%	105%	106%	105%
Mn 257.610	99%	102%	100%	102%	103%	106%
Zn 202.548	99%	100%	99%	99%	100%	97%
Ca 396.847	101%	100%	100%	99%	100%	100%
Mg 279.553	99%	99%	100%	100%	98%	99%
Al 396.153	101%	99%	99%	102%	103%	104%
Cr 267.716	99%	99%	99%	100%	99%	97%
Cr 205.560	101%	101%	99%	100%	100%	99%
Na 589.592	98%	98%	97%	101%	106%	106%
K 766.490	101%	99%	98%	102%	107%	107%
Co 228.616	100%	100%	99%	101%	101%	101%
Co 230.786	99%	100%	99%	99%	99%	99%
Ba 233.527	100%	100%	99%	100%	100%	99%
Ba 455.403	99%	98%	98%	99%	100%	100%
Ba 493.408	101%	100%	100%	100%	101%	101%
Li 670.784	100%	100%	99%	103%	106%	107%
Mo 203.845	100%	99%	98%	97%	103%	101%
Cu 324.752	101%	103%	100%	103%	107%	107%
Cu 327.393	100%	100%	99%	101%	104%	103%
Bi 206.170	102%	98%	101%	99%	97%	93%
Bi 223.061	101%	101%	99%	96%	119%	112%
P 177.434	98%	100%	99%	103%	101%	104%
P 178.221	98%	105%	101%	103%	106%	115%
P 214.914	100%	98%	99%	100%	96%	96%
Sr 407.771	101%	100%	101%	99%	99%	97%

8. DEVIATIONS:

8.1. During the validation, Mo 204.597 nm was listed as a wavelength to be analyzed. While this wavelength was analyzed during each analysis, the calibration standard correlation coefficient was never above 0.99 and thus results were not included for this wavelength. The validation protocol concentration changed slightly from feasibility studies and thus this wavelength was not compatible for this method. This is deemed acceptable as Mo 203.845 nm met all validation parameters set forth in the validation report and will be the wavelength apart of final test method.

8.2. Due to contamination, two additional MOPS samples at the unspiked level were re-prepared during the MOPS validation study. This is justified as the percent recoveries were inflated and due to salted roads during winter months.

8.3. During standard stability, the standard solutions were analyzed for solution stability at T=8 days instead of T=7 days as specified in the protocol. This is deemed justifiable as the solution stability passed for the standards at T=8 days and would have been stable at T=7 days.

9. CONCLUSION:

9.1. The test method for Trace Metals in BioTech products, specifically MESM, BTRI, TRIS, HEPE, URID, UREA, GHCL, GTHI, and MOPS, has been validated on the ICP-OES. The Method was found to be:

9.1.1. Specific: The method blank did not show any significant interference for all analyzed masses.

9.1.2. Linear: 30% to 200% of working standard solution. Mean percent recovery ranged from 83% to 104% and met acceptance criteria for all wavelengths.

9.1.3. Sensitive: LOQ recoveries were within 77% to 114% between all nine products. All analytes met acceptance criteria established at the 30% level, thus the LOQ will be 30% in the final method.

9.1.4. Accurate: From 50% to 150% of working standard concentration level with mean percent recoveries ranging from 78% to 114% between all nine products. All wavelengths analyzed met acceptance criteria within the specified range.

9.1.5. Precise: Closeness of agreement demonstrated between six sample preparations by percent RSD's ranging from 0% to 4% for between all nine products.

9.1.6. Rugged: Satisfactory precision was demonstrated between two sets of six sample preparations performed on different days and by different analysts. The percent RSDs ranged from 0% to 10% between all nine products.

9.1.7. Stable: With respect to stability of solutions, the sample solutions are shown to be stable for 1 day for all elements analyzed using this protocol. The working standard preparations were shown to be stable for all analytes under the protocol for 8 days. The samples are to be noted as stable for 1 day and the standards to be noted as stable for 8 days in the final analytical testing method.

9.1.8. All wavelengths met all validation parameters set forth in the validation protocol with the exception of Mo 204.597 nm. In order to simplify the method and reduce the number of total wavelengths, the final method will incorporate one wavelength per element as shown below in Table 31. For elements having multiple wavelengths, the wavelength chosen was selected based on factors including intensity, baseline resolution, and possible sample interference. Wavelengths could be reintroduced at later date if needed.

TABLE 31: FINAL METHOD WAVELENGTHS

Element	Wavelength (nm)	Element	Wavelength (nm)
Co	230.786	K	766.490
Ba	493.408	Mg	279.553
Cr	267.716	Mn	257.610
Cu	324.752	Na	589.592
Li	670.784	Zn	202.548
Mo	203.845	Bi	206.170
Al	396.153	P	177.434
Ca	396.847	Sr	407.771
Fe	259.939		

10. NOTEBOOK REFERENCE:

TABLE 32: NOTEBOOK REFERENCE		
STUDY		**NOTEBOOK REFERENCE**
Specificity		BEIV01/ pages 23-31
Linearity and Range		BEIV01/ pages 23-31
LOQ by "Spiked" recovery		BEIV01/ pages 23-31, 32-38, 50-55, 56-61
Accuracy/ Precision by "Spiked" recovery		BEIV01/ pages 23-31, 32-38, 50-55, 56-61
Intermediate Precision (Ruggedness)		BEIV01/ pages 39-44, 56-61
Standard Solution Stability	Day-0	BEIV01/ pages 39-44
	Day-1	BEIV01/ pages 45-49
	Day-8	BEIV01/ pages 62-64
Sample Solution Stability	Day-0	BEIV01/ pages 39-44, 50-55
	Day-8	BEIV01/ pages 45-49, 56-61