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ANALYTICAL METHOD OF ANALYSIS: TRACE METALS IN FINISHED GOODS PRODUCTS BY INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY (ICP-OES)

TABLE OF CONTENTS

1. PURPOSE:

1.1. To provide a procedure for the assessment of Trace Metals in Finished Good Products, specifically Guanidine Hydrochloride (GHCl), GHCl 6M solution, Guanidine Thiocyanate (G. Thio), HEPES, MES Monohydrate (MESM), MOPS, TRIS, TRIS Hydrochloride (THCL), Urea, and Urea 6M solution, via the Avio 500 S/N 081S1905062 ICP-OES. This procedure was assessed using a 100% target concentration in order to encompass multiple products as per validation reports BSI-RPT-0959 and BSI-RPT-1012 and follows the validation parameters for quantitation procedures as outlined in USP <233>.

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

1.2.1. Class 1: As, Cd, and Pb

1.2.2. Class 2A: Ni

1.2.3. Class 3: Sb and Cu

1.2.4. Class 4: Ca, Fe, Mg, Mn, and Zn

2. SCOPE:

2.1. Applies to Finished Good Products, specifically the ten products listed above in the purposes, manufactured at BioSpectra.

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

3. RESPONSIBILITIES:

3.1. The Executive Director of Quality Control or other qualified designated individual, is responsible for the control, implementation, training, and maintenance of this method.

3.2. The QC Staff is 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, the QC Managers 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-0527, Determination of Trace Metals by ICP-OES in Finished Goods

4.2. BSI-PRL-0547, Analytical Method Validation Protocol Addendum: Determination of Trace Metals by ICP-OES in Finished Goods

4.3. BSI-RPT-0959, Analytical Method Validation Report: Determination of Trace Metals by ICP-OES in Finished Goods Products

4.4. BSI-RPT-1012, Analytical Method Validation Addendum Report: Determination of Trace Metals by ICP-OES in Finished Goods Products

4.5. BSI-SOP-0362, Avio 500 ICP-OES SOP

4.6. ICH Guideline for Elemental Impurities Q3D Current

4.7. USP <730> Plasma Spectrochemistry

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

4.9. USP <232>, <233>

TABLE 1: LIMITS FOR FINISHED GOODS

Elements	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
As	1	0.45	0.75	1.5	2.25
Cd	1	0.30	0.50	1.0	1.5
[1]Pb	1	0.30	0.50	1.0	1.5
Ni	2A	0.75	1.25	2.5	3.75
Sb	3	0.45	0.75	1.5	2.25
Cu	3	0.15	0.25	0.50	0.75
Ca	4	0.60	1.0	2.0	3.0
Fe	4	0.30	0.50	1.0	1.5
Mg	4	0.60	1.0	2.0	3.0
Mn	4	0.60	1.0	2.0	3.0
Zn	4	0.60	1.0	2.0	3.0

[1]For all MOPS finished good products analyzed by this method, the LOQ for Pb will be the 100% target concentration or 1.0 ppm.

5. MATERIALS AND EQUIPMENT:

5.1. Equipment
  5.1.1. Analytical Balance
  5.1.2. Avio 500 ICP-OES S/N 081S1905062
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*	Manufacturer	Concentrations / Elements
Arsenic Stock Standard	Perkin Elmer	As (1,000 µg/mL)
Cadmium Stock Standard	Perkin Elmer	Cd (1,000 µg/mL)
Lead Stock Standard	Perkin Elmer	Pb (1,000 µg/mL)
Nickel Stock Standard	Perkin Elmer	Ni (1,000 µg/mL)
Antimony Stock Standard	Perkin Elmer	Sb (1,000 µg/mL)
Copper Stock Standard	Perkin Elmer	Cu (1,000 µg/mL)
Calcium Stock Standard	Perkin Elmer	Ca (1,000 µg/mL)
Iron Stock Standard	Perkin Elmer	Fe (1,000 µg/mL)
Magnesium Stock Standard	Perkin Elmer	Mg (1,000 µg/mL)
Manganese Stock Standard	Perkin Elmer	Mn (1,000 µg/mL)
Zinc Stock Standard	Perkin Elmer	Zn (1,000 µg/mL)
Scandium Stock Standard	Perkin Elmer	Sc (1,000 µg/mL)
Yttrium Stock Standard	Perkin Elmer	Y (1,000 µg/mL)

* 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 Preparation**

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 a final volume of 50 mL with deionized water and mix well.

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 stock standards.

6.3.2. Prepare by adding stock standards to a 15 mL Digitube®.

6.3.3. Add DI Water to approximately 8 mL and pipette 1.0 mL nitric acid.

6.3.4. Dilute to volume using DI Water.

TABLE 3: INTERMEDIATE STANDARD

Identification	Element	Stock Identification	Amount Added (mL)	Nitric Acid (mL)	Final Volume (mL)	Final Concentration (µg/mL)
Intermediate Standard	As	1,000 µg/mL As Std	0.150	1.0	10	15
	Cd	1,000 µg/mL Cd Std	0.100			10
	Pb	1,000 µg/mL Pb Std	0.100			10
	Ni	1,000 µg/mL Ni Std	0.250			25
	Sb	1,000 µg/mL Sb Std	0.150			15
	Cu	1,000 µg/mL Cu Std	0.050			5.0
	Ca	1,000 µg/mL Ca Std	0.200			20
	Fe	1,000 µg/mL Fe Std	0.100			10
	Mg	1,000 µg/mL Mg Std	0.200			20
	Mn	1,000 µg/mL Mn Std	0.200			20
	Zn	1,000 µg/mL Zn Std	0.200			20

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 matrix.
6.4.2. Add 0.050 mL of intermediate standard to 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 5 days after preparation)

TABLE 4: 50% CALIBRATION STANDARD

Identification	Element	Intermediate Standard (mL)	Nitric Acid (mL)	Internal Standard Solution (mL)	Final Volume (mL)	Final Concentration (µg/L)
50% Calibration Standard	As	0.050	2.50	1.0	50	15
	Cd					10
	Pb					10
	Ni					25
	Sb					15
	Cu					5.0
	Ca					20
	Fe					10
	Mg					20
	Mn					20
	Zn					20

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 matrix.
6.5.2. Add 0.150 mL of intermediate standard to 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 5 days after preparation)

TABLE 5: 150% CALIBRATION STANDARD

Identification	Element	Intermediate Standard (mL)	Nitric Acid (mL)	Internal Standard Solution (mL)	Final Volume (mL)	Final Concentration (µg/L)
150% Calibration Standard	As	0.150	2.50	1.0	50	45
	Cd					30
	Pb					30
	Ni					75
	Sb					45
	Cu					15
	Ca					60
	Fe					30
	Mg					60
	Mn					60
	Zn					60

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 matrix.
6.6.2. Add 0.200 mL of intermediate standard to 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 5 days after preparation)

TABLE 6: 200% CALIBRATION STANDARD

Identification	Element	Intermediate Standard (mL)	Nitric Acid (mL)	Internal Standard Solution (mL)	Final Volume (mL)	Final Concentration (µg/L)
200% Calibration Standard	As	0.200	2.50	1.0	50	60
	Cd					40
	Pb					40
	Ni					100
	Sb					60
	Cu					20
	Ca					80
	Fe					60
	Mg					80
	Mn					80
	Zn					80

6.7. Calibration Blank

6.7.1. Prepare a solution containing 5.0% HNO_3 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 Solution (mL)	Final Volume (mL)
Cal Blank	2.50	1.0	50

6.8. Method Blank Preparation

6.8.1. Refer to Calibration Blank

6.9. **Sample Preparation**

6.9.1. All HEPES finished good samples are to be prepared fresh each time for analysis. All other finished good products are stable for 24 hours.

6.9.2. Weigh approximately 1,000 mg of sample into a 50 mL Digitube®.

6.9.3. Add 20 mL of deionized water and swirl solution to dissolve solid.

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 Solution.

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 as per 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 for multi-element analysis and ± 10% for single element determinations.

7.5. The check standard must be verified after the calibration. 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 ± 20% of the calculated theoretical concentration for multi-element analysis and ± 10% for single element determinations. Additionally, the drift (calculated as absolute difference) between the bracketing standard checks must be NMT 20% for each target wavelength (NMT 10% for single element determination).

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	N/A
150% Check Std 1	QC Check	N/A
Method Blank	Sample	N/A
Sample(s) 10 or less	Sample	N/A
150% Check Std 2	QC Check	N/A

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 instrument method is stored under the Test Method Folder labelled as "FG_TraceMetal" for trace metal testing.

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	Argon
Shear Gas	Compressed Air + Nitrogen
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 TUNE MODE

Element	Internal Standard	Mode	Wavelength	Linear Range (µg/L)
As	Sc 357.253	Axial	228.812	9.0-60
Cd	Sc 357.253	Axial	214.440	6.0-40
Pb	Sc 357.253	Axial	220.353	6.0-40
Ni	Sc 357.253	Axial	227.022	15-100
Sb	Sc 357.253	Axial	206.836	9.0-60
Cu	Sc 357.253	Axial	324.752	3.0-20
Ca	Sc 357.253	Axial	396.847	12-80
Fe	Sc 357.253	Axial	259.939	6.0-40
Mg	Sc 357.253	Axial	279.553	12-80
Mn	Sc 357.253	Axial	257.610	12-80
Zn	Sc 357.253	Axial	202.548	12-80

8. REPORTING

8.1. Any result below the 30% target concentration will be reported as less than the corresponding LOQ value listed in Table 1. For MOPS samples, the LOQ for lead is 100% target concentration or 1.0 ppm. Results above the LOQ concentration will be reported in µg/g and to 2 decimal places. Report the average result for multiple wavelengths of the same element that are above the LOQ concentration.