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ANALYTICAL METHOD FOR THE QUANTIFICATION OF SULFUR BY INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY (ICP-OES) IN DEXTRAN SULFATE

TABLE OF CONTENTS

1. PURPOSE:

1.1. To provide a procedure for the quantification of sulfur via the Avio 500 S/N 081S1905062 ICP-OES. This procedure was assessed as a full quantitative procedure as per validation report BSI-RPT-0999 v1.0 and follows the validation parameters for quantitation procedures as outlined in Analytical Method Validation Plan.

2. SCOPE:

2.1. Applies to Dextran Sulfate (molecular weight 8000) and related products 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. QC management, 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-SOP-0362, Operation and Maintenance of the Perkin Elmer Avio 500 ICP-OES Instrument

4.2. BSI-SOP-0436, Analytical Method Validation Master Plan

4.3. BSI-PRL-0538, Analytical Method Validation Protocol: Sulfur Quantification in Dextran Sulfate

4.4. BSI-RPT-0999, Analytical Method Validation Report: Sulfur Quantification in Dextran Sulfate

4.5. USP <730> Plasma Spectrochemistry

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

5. MATERIALS AND EQUIPMENT:

5.1. Equipment

- 5.1.1. Analytical Balance
- 5.1.2. Avio 500 ICP-OES S/N 081S1905062
- 5.1.3. CEM Mars 6 Digestion Microwave S/N MY2255
- 5.1.4. Micropipettes, Rainin and 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

TABLE 1: REFERENCE STANDARDS

Identification*	Manufacturer	Concentrations / Elements
Manganese Stock Standard	Perkin Elmer	Mn (1,000 µg/mL)
Scandium Stock Standard	Perkin Elmer	Sc (1,000 µg/mL)
Sulfur Stock Standard	Perkin Elmer	S (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 Solution

6.2.1. Pipette 100 µL of 1,000 µg/mL Scandium Stock Standard and 100 µL of 1,000 µg/mL Yttrium Stock Standard into a 15 mL Digitube®.

6.2.2. Dilute to a final volume of 10 mL with deionized water and mix well.

6.2.3. Scale proportionally as needed for use.

6.3. Calibration Standard Preparation

6.3.1. Prepare calibration standards as per Table 2 below in 5.0% HNO_3 matrix with Sc and Y internal standards.

6.3.2. Add intermediate standard and internal standard to separate 50 mL Digitube® followed by addition of approximately 45 mL of deionized water.

6.3.3. Add 2.5 mL of Nitric Acid.

6.3.4. Dilute to final volume using deionized water. (Standards are stable for 6 days)

TABLE 2: CALIBRATION STANDARDS

Identification	Element	Intermediate Standard (mL)	Internal Standard (mL)	Nitric Acid (mL)	Final Volume (mL)	Final Concentration (µg/L)
Cal Blank	S	0.00	0.100	2.5	50	0.0
2.5 ppm Std		0.125				2.5
5.0 ppm Std		0.250				5.0
10 ppm Std		0.500				10
15 ppm Std		0.750				15

6.4. Method Blank Preparation

6.4.1. Pipette 4.0 mL of nitric acid into a clean 20 mL digestion vessel, place a plug on the vessel, and properly torque the vessel cap. Place vessel in the microwave carousel then digest and complete sample preparation according to Section 6.6 below.

6.5. Sample Preparation

6.5.1. Weigh approximately 100 mg of the sample into a clean 20 mL digestion vessel and add 4.0 mL of nitric acid.

6.5.2. Place a plug on the vessels and properly torque vessel cap before placing in the microwave carousel. Digest and complete sample preparation according to Section 6.6 below.

6.6. Microwave Digestion Procedure

6.6.1. Sample solutions are stable for a total of 6 days.

6.6.2. Refer to BSI-SOP-0426 for general usages and guidelines for the Mars 6 Digestion System.

6.6.3. Prepare at least one method blank per digestion run. Method blank is prepared in the same manner following microwave digestion as the actual samples without addition of sample.

6.6.4. Digest the sample using the program listed in Table 3 below.

TABLE 3: TEMPERATURE CONTROLLED MICROWAVE DIGESTION PROGRAM

Power (Watts)	Percent Power	Ramp (Minutes)	Temperature (°C)	Hold (Minutes)
1800	100	15:00	150	10:00
1800	100	6:00	175	5:00

6.6.5. If fewer than 4 samples are to be digested with the CEM digestion vessels, use extra place holder "dummy" samples to ensure that there are at least 4 microwave vessels placed evenly around the carousel. The extra place holder "dummy samples" can be discarded after the digestion is complete.

6.6.6. After digestion, place the digestion vessels in an ice bath and allow the vessels to cool for approximately 40 minutes. Before opening, turn the vessels sideways and slowly rotate in order to collect the condensation on the inside walls of the vessels.

6.6.7. Quantitatively transfer the vessel contents into a 50 mL Digitube® containing 5 mL of deionized water. Rinse the bottom of the plug into the same 50 mL Digitube® using deionized water.

6.6.8. Rinse the vessel an additional two times with deionized water and transfer each rinse to the 50 mL Digitube®. Dilute to final volume of 50 mL using deionized water and mix well.

6.6.9. Perform an additional dilution for each sample by pipetting 1.0 mL of the digested sample into another 50 mL Digitube®.

6.6.10. Pipette 100 µL of internal standard and dilute to 45 mL using deionized water.

6.6.11. Pipette 2.5 mL of nitric acid then dilute to final volume of 50 mL using deionized water and mix well.

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 4.

7.4. Confirm the calibration by analyzing the 10 ppm standard after the calibration. The calibration check must recover ± 10% of the calculated theoretical concentration 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 standards must recover ± 10% of the calculated theoretical concentration for single element determinations and the drift between the bracketing standard checks must be NMT 10% for each Target wavelength.

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 4: EXAMPLE SAMPLE ANALYSIS SEQUENCE

ID	Type	Level
Cal Blank	Cal Blank	Level 1
2.5 ppm Std	Cal Std	Level 2
5.0 ppm Std	Cal Std	Level 3
10 ppm Std	Cal Std	Level 4
15 ppm Std	Cal Std	Level 5
Cal Blank Check	QC Check	N/A
10 ppm Check Std 1	QC Check	N/A
Method Blank	Sample	N/A
Sample(s) 10 or less	Sample	N/A
10 ppm Check Std 2	QC Check	N/A

8. INSTRUMENT SETUP AND PARAMETERS

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

8.2. The instrument method is stored under the Test Method Folder labelled as "Sulfur_Quant_DextranSulfate.mth" for quantification of sulfur.

TABLE 5: ICP-OES PARAMETERS

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

TABLE 6: LINEAR RANGE AND CORRESPONDING MODE

Element	Mode	Wavelength (nm)	Linear Range (µg/mL)
S	Axial	181.975	1.0-20
		180.669	

9. REPORTING

9.1. Results will be displayed in calibration units (mg/mL) and sample units (ppm). The value to report is the calculated value in ppm from the dilution factor used in the software sample info table. For sulfur quantification, the average of the two wavelengths will be used for reporting.