BIOSPECTRA
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UREA 6M SOLUTION TESTING METHODS

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1. PURPOSE:

1.1. To provide the Quality Control (QC) Laboratory personnel with a procedure for analyzing Urea 6M In-Process, Finished Goods, and Stability.

2. SCOPE:

2.1. Applies to the analysis of Urea 6M In-Process, Finished Goods, and Stability in the QC Laboratory at all BioSpectra facilities.

3. RESPONSIBILITIES:

3.1. The Executive Director of Quality Control is responsible for training, maintenance and implementation of this procedure.
3.2. The QC Analysts are responsible for compliance with the terms of this procedure. This includes notifying the appropriate personnel if any analyses fail to meet their respective specifications.

4. REFERENCES:

4.1. *ACS, Reagent Chemicals*, current edition.
4.2. BSI-ATM-0073Analytical Method of Analysis: Guanidine Thiocyanate, MOPS, and Urea via ICP-MS.
4.3. BSI-ATM-0081Urea 6M Molarity Testing Method via UPLC
4.4. BSI-SOP-0098 Balance SOP.
4.5. *Current USP*
4.6. *Current EP*
4.7. BSI-SOP-0126 Laboratory Notebooks
4.8. BSI-SOP-0254 Spectrum Two UATR.
4.9. BSI-SOP-0255 XL200 pH/mV/Conductivity Meter SOP.
4.10. BSI-SOP-0303 NexION 350X ICP-MS SOP..
4.11. BSI-SOP-0345 Endosafe Nexgen-PTS Endotoxin Reader SOP.
4.12. BSI-SOP-0350 Anton Paar DMA 35 Portable Density Meter Operation and Calibration SOP.

5. EQUIPMENT:

5.1. Analytical Balance
5.2. Perkin Elmer NexION 350X ICP-MS
5.3. Perkin Elmer Spectrum Two UATR
5.4. XL200 pH/mV/Conductivity Meter or equivalent
5.5. Anton Paar DMA 35 Portable Density Meter
5.6. HPLC/UPLC with 195nm Detection Capability

6. REAGENTS:

6.1. LAL reagent water is purchased commercially.
6.2. Purified Water is produced by the qualified water systems at each BioSpectra facility.
6.3. Primary or Secondary Urea Reference standards are purchased commercially.
6.4. Qualified In-house Urea material for Reference standard use.
6.5. pH 4, 7, and 10 buffers are purchased commercially.

7. ANALYTICAL PROCEDURES:

IN PROCESS TESTING

7.1. **MOLARITY** **5.85 – 6.15M:**

7.1.1. Refer to the Urea 6M Molarity Testing Method via UPLC, DCN BSI-ATM-0081, for sample preparation, instrumental method parameters and instrument operation for Molarity determination.

7.1.2. The weight % will determined via the UPLC analysis, molarity is calculated by the following calculation:

7.1.2.1. $\text{Molarity} = \frac{WPs\ (g)}{100g} x \frac{Density\ (g)}{mL} x \frac{1000mL}{1L} x \frac{mol}{60.056g}$

7.1.2.1.1. WP_s= Calculated weight percent of the Sample

7.1.2.1.2. Density= Measured of the sample

7.2. DENSITY **Report:**

7.2.1. Analyze the density of the as-is sample utilizing the Anton Paar DMA 35 Portable Density Meter.

7.2.2. Perform a water check prior to analysis.

7.2.3. Follow the Anton Paar DMA 35 Portable Density Meter Operation and Calibration SOP for instrument operation and calibration, BSI-SOP-0350

FINISHED GOOD TESTING

7.3. **APPEARANCE** **Colorless Liquid:**

7.3.1. Add 50 mL of sample into a Nessler Color Comparison Tube.

7.3.2. Add 50 mL of *Purified Water* into a second Nessler Color Comparison Tube.

7.3.3. Compare the colors in diffused daylight, viewing vertically against a white background.

7.3.4. In order for the sample solution to be colorless, it must have the appearance of *Purified Water.*

7.4. **ENDOTOXIN** **Report:**

7.4.1. Pipette 0.140mL of 6M Urea sample into a sterile vessel.

7.4.1.1. Note: Total dissolved urea equivalent to 50mg.

7.4.1.2. Calculation: 0.140mL*360mg urea /mL = 50mg Urea

7.4.2. Dilute sample aliquot to 10mL with LAL reagent water and mix thoroughly.

7.4.3. Refer to Endosafe nexgen-PTS Endotoxin Reader SOP, BSI-SOP-0345 for instrument operating instructions.

7.4.4. Input a dilution factor of 71.4 into the instrument when prompted.

7.5. **IDENTIFICATION (IR)** **Conforms to Standard:**

7.5.1. Prepare a Urea 6M reference standard from a certified reference standard at the time of use.

7.5.1.1. Urea 6M= 360mg/mL (360g/L)

7.5.2. Sample will be analyzed as-is

7.5.3. Refer to Spectrum Two UATR SOP, BSI-SOP-0254 for instrument operation and standard and sample analysis and comparison.

7.6. **TAMC/TYMC USP <61>** **Report:**

7.6.1. Aseptically decant and package no less than 50mL of sample into a sterile container and send to MPL laboratories for analysis. The analysis request form should include TAMC/TYMC USP <61> and designate which suitability method to be used on the Analysis Request Form (ARF). A new suitability should be requested if a reference suitability is unavailable.

7.7. **MOLARITY** **5.8 – 6.2M:**

7.7.1. Refer to the Urea 6M Molarity Testing Method via UPLC, DCN BSI-ATM-0081, for sample preparation, instrumental method parameters and instrument operation for Molarity determination.

7.7.2. The weight % will determined via the UPLC analysis, molarity is calculated by the following calculation:

7.7.2.1. $\text{Molarity} = \frac{WPs\ (g)}{100g} \times \frac{Density\ (g)}{mL} \times \frac{1000mL}{1L} \times \frac{mol}{60.056g}$

7.7.2.1.1. WP_s= Calculated weight percent of the Sample

7.7.2.1.2. Density= Measured of the sample

7.8. DENSITY **Report:**

7.8.1. Analyze the density of the as-is sample utilizing the Anton Paar DMA 35 Portable Density Meter.

7.8.2. Perform a water check prior to analysis.

7.8.3. Follow the Anton Paar DMA 35 Portable Density Meter Operation and Calibration SOP for instrument operation and calibration, BSI-SOP-0350.

7.9. **pH @ 25°C** **Refer to Summary Sheet:**

7.9.1. Calibrate the XL200 pH/mV/Conductivity meter with pH 4, 7, and 10 buffer standards.

7.9.2. Measure the neat sample and record the results at 25 ± 2°C.

7.9.3. Refer to XL200 pH/mV/Conductivity SOP for instrument operation and sample analysis, BSI-SOP-0255.

7.10. **TRACE ELEMENTS** **As, Cu, Fe, & Pb (≤5ppm):**

7.10.1. Refer to Analytical Method of Analysis: Guanidine Thiocyanate, Mops, and Urea via ICP-MS (BSI-ATM-0073) for sample preparation and analysis.