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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-0073 Analytical Method of Analysis: Guanidine Thiocyanate, MOPS, and Urea via ICP-MS.
- 4.3. BSI-ATM-0081 Urea 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:

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

- 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:**

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- 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:

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

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  $25 \pm 2^\circ\text{C}$ .
- 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 ( $\leq 5\text{ppm}$ ):**

- 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.