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## UR3220 UREA TEST METHODS

**INTRODUCTION:** The following specifications and testing methods for Urea, Bio Excipient Grade, Product Code UR3220, were obtained from DCN: 16-001182 v.6.0 Certificate of Analysis and Urea Testing Methods DCN: 16-000495 v.3.0.

### **1.0. ALCOHOL INSOLUBLE MATTER** **USP & JP (0.04% max.):**

- 1.0.1. Dissolve 5 g of sample in 50mL of warm alcohol, and if any insoluble residue remains, filter the solution on a tared filter, wash the residue and the filter with 20mL of warm alcohol, and dry at 105 ±2°C for 1 hour. The weight of the residue does not exceed 2 mg (0.04%).
- 1.0.2. Calculate the % Insoluble Matter as follows:

$$\% \text{ Insoluble Matter} = \frac{\text{Residue Weight(g)}}{\text{Sample Weight (g)}} \times 100$$

### **1.1. APPEARANCE & COLOR** **White / Crystals:**

- 1.1.1. Place 25-50g of the sample in a clean, dry glass beaker. Record the sample weight used.
- 1.1.2. In an area with sufficient lighting, view the sample from all sides.
- 1.1.3. The sample should be white in color and characteristic of crystals.

### **1.2. ASSAY (As Is)** **:**

- 1.2.1. USP 98.0-102.0%  
1.2.1.1. Refer to Urea Assay via HPLC SOP.

### **1.3. ENZYME ACTIVITY** **None Detected:**

- 1.3.1. Follow RNase (Ribonuclease) Assay, DNase (Endonuclease) Assay, DNase (Exonuclease) Assay, and Protease Assay for sample preparation and analysis.

### **1.4. HEAVY METALS** **EP/BP (10 ppm/0.001% max):**

- 1.4.1. EP/BP Test Sample preparation:  
1.4.1.1. In a Nessler Color Comparison Tube, dissolve 2.0g Urea in 20mL of USP purified water. Add 5mL of 0.1N hydrochloric acid.
- 1.4.2. Standard Lead Solution:  
1.4.2.1. On the day of use, dilute 10.0 mL of Lead Nitrate Stock Solution with USP Purified Water to 100 mL in a volumetric flask.
- 1.4.3. Standard Preparation:  
1.4.3.1. Into a Nessler Color Comparison Tube, pipette 2 mL of Standard Lead Solution, and dilute with USP Purified Water to 25 mL. Adjust to a pH between 3.0 and 4.0 using 1N acetic acid or 6N ammonium hydroxide, using a pH meter or short-range pH indicator paper as an external indicator. Dilute with USP Purified Water to 40mL and mix.

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**1.4.4. Monitor Preparation:**

1.4.4.1. Place 40mL of a solution prepared as directed for Test Preparation and add 2.0mL of Standard Lead Solution.

**1.4.5. Procedure:**

1.4.5.1. Adjust all Nessler Color Comparison tubes to a pH between 3.0 and 4.0 using a 1N acetic acid or 6N ammonium hydroxide, using a pH meter or short range pH indicator paper as an external indicator.

1.4.5.2. Dilute each with USP purified water to 45mL and mix.

1.4.5.3. To each tube add 2 mL of pH 3.5 Acetate Buffer and 1.2 mL of thioacetamide-glycerin base TS (mix 1mL of glycerin TS and 0.2mL of thioacetamide TS, heat in a boiling water bath for approximately 20 seconds use immediately). Dilute with USP Purified Water to 50 mL mix and allow to stand for 2 minutes.

1.4.5.4. View downward over a white surface. The color of the Test Preparation must not be darker than the Standard Preparation, and the color of the Monitor Preparation is equal to or darker than the Standard Preparation.

**1.5. IDENTIFICATION TEST – USP (A) EP/BP (B) Passes Test:**

1.5.1. Follow Spectrum Two UATR SOP.

**1.6. IDENTIFICATION TEST – USP (B) Passes Test:**

1.6.1. The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay via HPLC.

**1.7. IMPURITIES Urea RCA <0.1%; Total <2.0%; Unspecified <0.1%:**

1.7.1. Refer to the Urea Assay via HPLC. Acceptance Criteria are as follows:

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Urea Related Compound A	0.9	0.1
Urea	1.0	N/A
Any individual unspecified impurity	N/A	0.1
Total impurities	N/A	2.0

**1.8. INSOLUBLE MATTER 0.010% max.:**

1.8.1. Accurately weigh 20.0 g of sample and transfer to a 250mL beaker.

1.8.2. Add 150 mL of USP Purified Water and utilize a Teflon encapsulated magnetic stirring bar and electric stir plate to dissolve sample.

1.8.2.1. If cloudiness is observed immediately notify the appropriate personnel.

1.8.3. Heat to boiling and digest on a hotplate in a covered beaker for 1 hour.

1.8.4. Prepare a Gooch filtering crucible and 10-15µm filter by drying at 105°C ± 2°C for 1 hour. Allow to cool in ambient air for 15 minutes and weigh.

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- 1.8.5. Filter sample solution through conditioned filtering crucible and 10-15µm filter. Rinse thoroughly with at least 150mL of hot USP purified water. If needed, rinse with Hot USP Purified Water until all soluble crystal residue is removed.
- 1.8.6. Dry the crucible at 105°C ± 2°C for 1 hour.
- 1.8.7. Cool in ambient air for 15 minutes and reweigh.
- 1.8.8. Calculate the % Insoluble Matter as follows:

$$\% \text{ Insoluble Matter} = \frac{\text{Residue Weight (g)}}{\text{Sample Weight (g)}} \times 100$$

### **1.9. LOSS ON DRYING** **EP/BP (1.0% max.):**

- 1.9.1. Dry an LOD vial in an oven at 105 ± 2°C for 30 minutes.
- 1.9.2. Cool for 15 minutes in a desiccator, weigh the LOD vial on the analytical balance, and record results.
- 1.9.3. Tare the dried vial and weigh 1g of sample and record. By gentle, sidewise shaking, distribute the sample as evenly as possible in the LOD vial.
- 1.9.4. Place the LOD vial containing the sample into the oven and dry at 105°C ± 2°C for 1 hour.
- 1.9.5. Cool for 15 minutes in desiccator.
- 1.9.6. Reweigh and calculate the % LOD.

$$\% \text{ LOD} = \frac{[\text{initial sample weight (g)} - \text{final sample weight (g)}]}{\text{initial sample weight (g)}} \times 100$$

### **1.10. MELTING RANGE** **EP/BP Identification A (132 – 135°C):**

- 1.10.1. Refer to MP50 Melting Range Operation and Calibration SOP to analyze and record results.

### **1.11. RESIDUE ON IGNITION/SULFATED ASH** **(0.010% max):**

- 1.11.1. Turn on muffle furnace and allow it to stabilize at 600°C. Follow Muffle Furnace SOP and Calibration for operation of the muffle furnace.
- 1.11.2. Inspect a quartz crucible for cracks, chips and discoloration.
- 1.11.3. Utilize the 10 inch forceps to insert and remove the crucible from the furnace.
- 1.11.4. Ignite quartz crucible at 600 ± 50 °C for 30 minutes. Cool in a desiccator and weigh on an analytical balance.
- 1.11.5. For specification of 0.01% max:
  - 1.11.5.1. Weigh 4.0 g sample in the previously ignited quartz crucible. Moisten the sample with small amount (usually 1mL) of sulfuric acid.
- 1.11.6. Volatilize the sample with a Bunsen burner until the sample is thoroughly charred. Keep the sample an appropriate distance from the flame, so that the sample does not boil over and sample is not lost.
  - 1.11.6.1. The rate of heating should be such that from ½ to 1 hour is required to volatilize the sample.
  - 1.11.6.2. Continue using the Bunsen burner to heat the sample until all the excess sulfuric acid has been volatilized.

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- 1.11.7. Allow the sample to cool, and then moisten with small amount (usually 1mL) of sulfuric acid.
- 1.11.8. Volatilize the sample with a Bunsen burner until the sample is thoroughly charred and white fumes are no longer evolved. Keep the sample an appropriate distance from the flame, so that the sample does not boil over and sample is not lost.
  - 1.11.8.1. The rate of heating should be such that from ½ to 1 hour is required to volatilize the sample.
  - 1.11.8.2. Continue using the Bunsen burner to heat the sample until all the excess sulfuric acid has been volatilized.
- 1.11.9. Ignite in the muffle furnace at 600 ± 50 °C for 15 minutes or until all carbon has been removed.
- 1.11.10. Cool in a desiccator for the same amount of time employed in the preparation of the crucible and weigh on an analytical balance.
- 1.11.11. Calculate the %ROI as follows:
 
$$\%ROI = \frac{\text{Residue Weight (g)}}{\text{Sample Weight (g)}} \times 100$$
- 1.11.12. If the amount of the residue exceeds the limit specified, repeat the moistening with sulfuric acid using up to 1mL, heat via Bunsen burner and ignite at 600 ± 50 °C for 30 minutes until two consecutive weighing's of the residue do not differ by more than 0.0005g or until the specification is met.

**1.12. TRACE METALS**

**As, Cu, Fe, & Pb (5ppm max.):**

- 1.12.1. Refer to NexION 350X ICP-MS SOP.

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