BIOSPECTRA
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Urea UR4220 Test Methods

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:

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

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 needles or crystals as required.

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. **CHLORIDE** **0.0005% max, :**

1.3.1. Standard preparation (Specification 0.0005% max): (Prepare standard using 0.01 mg chloride ion)

1.3.1.1. Pipette 1.410 mL of 0.02 N HCl and q.s to 100 mL with purified water.

1.3.1.2. Pipette 1.0 mL, of the above 100 mL HCl solution into a Nessler Color Comparison Tube and q.s to about 40 mL with purified water.

1.3.2. Procedure:

1.3.2.1. Weigh 2.0 g of sample and dissolve in 40 mL of purified water. If necessary, neutralize the solution with nitric acid to litmus.

1.3.2.2. Add to each solution, 1 mL concentrated nitric acid and 1 mL 0.1N Silver Nitrate. Q.S. to 50 mL with purified water. Cover with parafilm and mix by inversion.

1.3.2.3. After 5 minutes, the turbidity of the sample preparation does not exceed that produced by the 1 ppm standard. View against a dark background.

1.3.2.4. If a visible difference in the turbidity is not observed, utilize the Turbidimeter to measure the turbidity of the standard and the sample solutions. Follow the appropriate SOP as follows:

1.3.2.4.1. Stroudsburg- Measure and record the turbidity of the sample according to Portable Turbidimeter Operation and Calibration.

1.3.2.4.2. Bangor- Measure and record the turbidity of the sample according to Bangor Portable Turbidimeter SOP.

1.4. **ENZYME ACTIVITY** **None Detected:**

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

Printed On:	22-Sep-2020 09:58:59 AM	Ledergerber, Alissa	: Printed By
Authored By:	Baun, Cassie	17-Oct-2019 09:24:08 AM	
Approval:	Hamelburg, Crystal	18-Oct-2019 10:32:56 AM	
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1.5. **HEAVY METALS EP/BP (10 ppm/0.001% max):**

1.5.1. EP/BP Test Sample preparation:
1.5.1.1. In a Nessler Color Comparison Tube, dissolve 1.0g Urea in 20mL of USP purified water. Add 5mL of 0.1N hydrochloric acid.
1.5.2. Standard Lead Solution:
1.5.2.1. 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.
1.5.3. Standard Preparation:
1.5.3.1. Into a Nessler Color Comparison Tube, pipette 2 mL of Standard Lead Solution, and add 2mL of dilute acetic acid and water to make 50mL. Adjust to a pH between 3.0 and 4.0 using 1*N* acetic acid or 6*N* ammonium hydroxide. Use a pH meter or short-range pH indicator paper as an external indicator. Dilute with *USP Purified Water* to 40mL and mix.
1.5.4. Test Preparation:
1.5.4.1. In the Nessler Color Comparison Tube prepared above, diluted with purified water to 40mL.
1.5.5. Monitor Preparation:
1.5.5.1. Place 40mL of a solution prepared as directed for Test Preparation and add 2.0mL of Standard Lead Solution.
1.5.6. Procedure:
1.5.6.1. Adjust all Nessler Color Comparison tubes to a pH between 3.0 and 4.0 using a 1N acetic acid or 6N ammonium hydroxide. Use a pH meter or short range pH indicator paper as an external indicator.
1.5.6.2. Dilute each with USP purified water to 45mL and mix.
1.5.6.3. 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 *USP Purified Water* bath for approximately 20 seconds use immediately). Dilute with USP Purified Water to 50 mL mix and allow to stand for 2 minutes.
1.5.6.4. View downward over a white surface. The color of the Test Preparation is not darker than the Standard Preparation, and the color of the Monitor Preparation is equal to or darker than the Standard Preparation.

1.6. **IDENTIFICATION TEST – USP (A) EP/BP (B) Passes Test:**
1.6.1. Follow Spectrum Two UATR SOP.

1.7. **IDENTIFICATION TEST – USP (B) Passes Test:**
1.7.1. 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.

1.8. **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 QC and QA Managers.
1.8.3. Heat to boiling and digest on a hotplate in a covered beaker for 1 hour.

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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.
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 add additional hot USP purified water rinse. If needed add additional hot USP purified water rinse to ensure 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:

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

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.

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

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

1.10.1. Reduce the sample to a fine powder in a mortar and pestle and dry sample in the desiccator for a minimum of 16 hours.
1.10.2. Fill capillary tube with an adequate amount of sample to be tested. Use the capillary packing rod to push the sample down until the sample packs into the bottom of the tube. The final height of the packed sample in the capillary should be between 2.5-3.0mm.
1.10.3. Place the capillary tube containing the sample in the melting point apparatus.
1.10.4. Do not force the capillary tube(s) into the apparatus; they should drop right in.
1.10.5. Select the Urea method on the home screen.
1.10.6. The instrument will beep once the initial temperature is reached.
1.10.7. Press the start button.
1.10.8. Results will print upon completion.
1.10.9. Up to four Urea samples may be ran at the same time.
1.10.10. The melting range breadth should be between 0.5-1.5°C.

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 calibration procedure for operation of 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.

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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.

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:

$$\%ROI = \frac{Residue\ Weight\ (g)}{Sample\ Weight\ (g)} x\ 100$$

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 weighings of the residue do not differ by more than 0.0005g or until the specification is met.

1.12. **SULFATE (0.001% max):**

1.12.1. Standard preparation (Specification 0.001% max):

1.12.1.1. Pipette 0.02 mL of 0.020 *N* H_2SO_4 into a Nessler Color Comparison tube and add 40 mL purified water.

1.12.2. Procedure:

1.12.2.1. Add 1 mL of 3*N* HCl, 3 mL of Barium Chloride TS to each tube.

1.12.2.2. Q.S. to 50 mL with *USP Purified Water*, parafilm and mix by inversion.

1.12.2.3. Allow to stand for 10 minutes.

1.12.2.4. Any turbidity produced in the sample solution should not exceed that produced by the standard.

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1.12.2.5. If a visible difference in the turbidity is not observed, utilize the Turbidimeter to measure the turbidity of the standard and the sample solutions. Follow the appropriate SOP as follows:

1.12.2.5.1. Stroudsburg- Measure and record the turbidity of the sample according to Portable Turbidimeter Operation and Calibration.

1.12.2.5.2. Bangor- Measure and record the turbidity of the sample according to Bangor Portable Turbidimeter SOP.

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

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

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