BIOSPECTRA
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URACIL TESTING METHODS

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1. PURPOSE:

1.1. To provide Laboratory personnel with a procedure for examining Uracil In-Process, Raw Materials, and Finished Goods.

2. SCOPE:

2.1. Applies to the examination of Uracil In-Process, Raw Materials, and Finished Goods in the Laboratory. Methods include testing for all types of Uracil sold by BioSpectra; only the specific tests required for the desired type must be tested.

2.2. This document applies to both the Bangor, PA and Stroudsburg, PA BioSpectra facilities.

3. RESPONSIBILITIES:

3.1. The Director of Laboratory Testing is responsible for training, maintenance and implementation of this procedure.

3.2. The Laboratory Analysts are responsible for compliance with the terms of this procedure. This includes notifying the QA/Laboratory Management if any analyses fail to meet their respective specifications.

4. REFERENCES:

4.1. BSI-ATM-0023, Uracil In-Process Testing Methods and Specifications
4.2. BSI-ATM-0105, Uracil Assay via Liquid Chromatography with UV Detection
4.3. BSI-ATM-0118, Uracil Assay via Waters Alliance HPLC with UV Detection
4.4. BSI-FRM-0334, Bangor Outside Testing Samples Log Book
4.5. BSI-SOP-0098, Balance SOP
4.6. BSI-SOP-0126, Laboratory Notebooks
4.7. BSI-SOP-0254, Spectrum Two UATR SOP
4.8. BSI-SOP-0256, MP50 Melting Range Operation and Calibration SOP
4.9. BSI-SOP-0303, NexION 350X ICP-MS SOP
4.10. BSI-SOP-0345, Endosafe nexgen-PTS Endotoxin Reader SOP
4.11. BSI-SOP-0422, Empower 3 General Procedure
4.12. *ACQUITY UPLC TUV Detector Operator's Overview and Maintenance Guide*
4.13. *ACQUITY UPLC Quaternary Solvent Manager PLUS Series*
4.14. *ACS, Reagent Chemicals*, current edition.
4.15. Client Method T405, proprietary.
4.16. *Current USP*
4.17. *Waters 2489 UV/Visible Detector Operator's Guide*
4.18. *Waters 2695 Separations Module Operator's Guide*

5. EQUIPMENT:

5.1. Analytical Balance
5.2. Spectrum Two UATR
5.3. Endosafe nexgen-PTS Endotoxin Reader
5.4. NexION 350X ICP-MS
5.5. MP50 Melting Point Apparatus 5
5.6. Water Alliance HPLC or Acquity UPLC with UV-Vis Detector

5.7. Perkin Elmer Flexar HPLC

6. REAGENTS:

6.1. **0.02 N Hydrochloric Acid (HCl)** – Slowly add 20 mL of 0.1 N Hydrochloric Acid (HCl) to 80 mL of purified water to make a total volume of 100 mL.

6.2. **0.02 N Sulfuric Acid (H_2SO_4)** – Slowly add 20 mL of 0.1 N Sulfuric Acid (H_2SO_4) to 80 mL of purified water to make a total volume of 100 mL.

6.3. **0.1 M Sodium Hydroxide (NaOH)** – Purchased commercially.

6.4. **0.1 N Hydrochloric Acid (HCl)** - Purchased commercially.

6.5. **0.1 N Silver Nitrate ($AgNO_3$)** – Weigh 1.7 g of $AgNO_3$ and dilute to 100 mL with purified water. Or purchased commercially.

6.6. **0.1 N Sulfuric Acid (H_2SO_4)** - Purchased commercially.

6.7. **0.1 N Tetrabutylammonium Hydroxide (TBAH) solution** – Purchased commercially.

6.8. **0.5 – 0.005 EU/mL High Sensitivity Cartridge** – Purchased commercially.

6.9. **1 in 100 Solution Thymol Blue** – Weight 1 g of Thymol Blue, transfer to a 100 mL volumetric flask, dilute to volume with N,N-dimethylformamide, cap, and mix thoroughly.

6.10. **3 N Hydrochloric Acid (HCl)** – Pipette 25.75 mL of concentrated Hydrochloric Acid (HCl) and transfer to a 100 mL volumetric flask that contains a small amount of purified water. Dilute to volume with purified water, cap, and mix thoroughly.

6.11. **Barium Chloride TS** – Dissolve 30 g of Barium Chloride dihydrate in water to make 250 mL.

6.12. **Benzoic Acid, Traceable Reference** Material – Purchased commercially.

6.13. **LAL Reagent Water (LRW)** – Purchased commercially.

6.14. **Nitric Acid (HNO_3), concentrated**– Purchased commercially.

6.15. **Nitrogen gas** – Purchased commercially.

6.16. **N,N-dimethylformamide** – Purchased commercially.

6.17. **Sulfuric Acid (H_2SO_4), concentrated** – Purchased commercially.

6.18. **Thymol Blue (solid)** – Purchased commercially.

7. PROCEDURE:

IN-PROCESS ANALYSIS

7.1. **MOTHER LIQUOR PH** :

7.1.1. If the sample has fallen out of solution, decant the entire sample into a clean, dry beaker. Gently heat and stir the solution until it becomes clear but do not exceed 95°C.
7.1.2. Note: Solution will not be colorless.
7.1.3. Measure and record the pH of the solution following the appropriate SOP.
7.1.4. Record the result in the appropriate batch record and analytical documentation.

7.2. **DRY/WET CRYSTAL UATR** :

7.2.1. Refer to the Spectrum Two UATR SOP.
7.2.2. Dry crystals should be analyzed as-is.
7.2.3. Wet crystals or crystal washes should be dried if correlation does not meet specification as-is, refer to Loss on Drying for drying parameters.

NOTE: Samples dried to a constant weight prepared in section 6.3 are considered equivalently dried to Loss on Drying samples.

7.2.4. If sample fails to meet requirements, additional samples will be submitted by production.
7.2.5. Record the results in the appropriate batch record and analytical documentation.

7.3. **ASSAY (WET CRYSTAL)** :

NOTE: Sample and Standard solutions must be prepared at the same time.

7.3.1. Dry wet crystals to a constant weight at 105°C.
7.3.1.1. Transfer approximately 1- 2 g of the wet crystal sample to a suitable glass vessel (a shallow vial, or watch glass) and accurately weigh the vessel and contents. Distribute the sample as evenly as possible in the vessel.
7.3.1.2. Place the vessel containing the sample into the oven and dry at 105°C ± 2°C for 1-3 hours.
7.3.1.3. Remove LOD vial from the oven and allow to cool in the desiccator for 15 minutes.
7.3.1.4. Reweigh.
7.3.1.5. Place the sample back into oven for 1-3 hours.
7.3.1.6. Remove LOD vial from the oven and allow to cool in the desiccator for 15 minutes.
7.3.1.7. Reweigh.
7.3.1.8. Continue drying and reweighing using steps 7.3.1.5.-7.3.1.7. until the sample achieves a constant weight NMT than 0.0005 g difference between weighings.
7.3.1.9. Grind dried sample if necessary to help facilitate solubility.
7.3.1.10. Uracil is relatively insoluble in diluent and sonication will be required to ensure complete dissolution.
7.3.2. Refer to 7.5 or 7.6 for Assay standard operating procedures for sample analysis and calculations.

FINISHED GOOD ANALYSIS

7.4. APPEARANCE & COLOR:

7.4.1. Place a suitable amount of sample in a clean and dry glass beaker.
7.4.2. In an area with sufficient lighting, view the sample from all sides.
7.4.3. The sample should conform to the specification detailed on the summary sheet.

7.5. ASSAY (HPLC – DRIED BASIS):

7.5.1. Refer to BSI-ATM-0105 for instrument setup if using the Waters Acquity UPLC, sample preparation, and analysis.
7.5.2. Refer to BSI-ATM-0118 for instrument setup if using the Waters Alliance HPLC, sample preparation, and analysis.

7.6. ASSAY (TITRIMETRIC):

7.6.1. Standardize 0.1N TBAH VS on day of use.
7.6.1.1. Dissolve about 400 mg of traceable benzoic acid reference material, accurately weighed, in 80 mL of N,N-dimethylformamide.
7.6.1.2. Add 3 drops of a 1 in 100 solution of thymol blue in N,N-dimethylformamide, and titrate to a blue endpoint with the tetrabutylammonium hydroxide solution.
7.6.1.3. Under constant nitrogen flow, deliver the titrant from a 50 mL burette.
7.6.1.4. Perform a blank determination, and make any necessary correction.
7.6.1.5. Each mL of 0.1 N tetrabutylammonium hydroxide is equivalent to 12.21 mg of benzoic acid.

$$N = \frac{mg\ of\ Benzoic\ Acid}{122.1\ x\ EP1(mL)}$$

7.6.2. Weigh 150 mg (+/- 0.2 mg) of uracil sample into a 150 mL beaker in duplicate.
7.6.3. Add 75 mL of *N,N*-Dimethylformamide and dissolve the sample.
7.6.4. Add 3 drops of 1 in 100 solution of thymol blue in dimethylformamide.
7.6.5. Titrate with 0.1N TBAH until a pure blue endpoint is reached.
7.6.5.1. Note: The color change to blue is gradual and indicated by the absence of any green present in the titration. The change to a pure blue color is the complete end point (EP1).
7.6.5.2. Incomplete EP: 
7.6.5.3. Complete EP: 
7.6.6. Calculate the % w/w Uracil using the following equation:
7.6.6.1. Where:
7.6.6.1.1. EPB = Titration volume, Blank (mL)
7.6.6.1.2. $EP1$ = Titration volume, Sample (mL)
7.6.6.1.3. N = Normality of TBAH (N)
7.6.6.1.4. w = Sample Weight (mg)

$$\%\ Uracil = \frac{(EP1 - EPB)(N)(112.09)}{w}\ x\ 100$$

7.7. **CHLORIDE** :

7.7.1. Standard Preparation 100 ppm (0.01%) Cl: Pipette 0.072 mL of 0.02N HCl in to a 100 mL Nessler Color Comparison Tube. Dilute to 100 mL with purified water.
7.7.2. Sample Preparation: 0.50 g dissolved in 100 mL of purified water in a Nessler color comparison tube. Heat or sonicate if necessary.
7.7.3. Procedure: Using a heated water bath, keep the standard and sample tubes in the water bath throughout the analysis. Add 1 mL of concentrated nitric acid and 1 mL of 0.1N Silver Nitrate to each tube. Cover and mix by inversion.
7.7.4. After 5 minutes, the turbidity in the sample solution should not exceed the turbidity in the standard solution to report at < 0.01% or < 100 ppm.

7.8. **ENDOTOXIN** :

7.8.1. Sample Preparation: Weigh 25 mg of sample into a sterile tube. Dilute to 10mL with LAL Reagent Water (LRW) and dissolve completely.
7.8.2. Procedure: Analyze sample with 0.5-0.005 EU/mL high sensitivity cartridge following the Endosafe Nexgen-PTS Reader SOP for instrument operation.

7.9. **HEAVY METALS** :

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

7.10. **IDENTIFICATION TEST** :

7.10.1. Follow Spectrum Two UATR SOP. Analyze the sample after it has been dried for 3 hours at 105°C. The LOD sample may be utilized for this test.

7.11. **LOSS ON DRYING** :

7.11.1. Dry a Loss on Drying (LOD) vial in the oven at 105 ± 2°C for 30 minutes.
7.11.2. Cool for 15 minutes in a desiccator, weigh the LOD vial, and record results.
7.11.3. If the substance to be tested is in the form of large crystals, reduce the particle size to about 2 mm by quickly crushing.
7.11.4. Transfer approximately 1- 2 g of the sample to the LOD vial, and accurately weigh the vial and contents. By gentle, sidewise shaking, distribute the sample as evenly as possible in the LOD vial.
7.11.5. Place the LOD vial containing the sample into the oven and dry at 105°C ± 2°C for 3 hours.
7.11.6. Remove LOD vial from the oven and allow to cool in the desiccator for 15 minutes.
7.11.7. Reweigh the LOD vial and sample and retain the dried sample to perform the IR and Assay.
7.11.8. Calculate the %LOD as follows:

$$\%LOD = \frac{[Initial\ Sample\ Weight\ (g) - \ Final\ Sample\ Weight\ (g)]}{Initial\ Sample\ Weight\ (g)} x\ 100$$

7.12. **MELTING POINT** :

7.12.1. Refer to MP50 Melting Range Operation and Calibration SOP for general instrument guidelines, sample preparation and operation.
7.12.1.1. Manually set the method to a max range of 300°C, start ~3°C below the limit.
7.12.1.2. Verify the sample does not melt at 300°C to report as ≥ 300°C.

7.13. **MICROBIAL ANALYSIS (TAMC)** :

7.13.1. Package at least 20 g of testing sample into a sterile container and send to EMSL or other qualified microbial testing provider for analysis.

7.13.2. Record applicable sample data in outside testing log book for reference.

7.14. **REACTION** :

7.14.1. Note: If a pH meter is utilized for analysis, document the pH readout on the notebook page.

7.14.2. Product Codes URAC-4202 and URAC-4250:

7.14.2.1. The 1% solution prepared for solubility must be neutral to faintly basic or acidic when tested with broad range pH paper or a calibrated pH meter.

7.14.2.1.1. The target pH for the sample solution is 5-8.

7.14.3. Product Codes URAC-4201 and URAC-4301:

7.14.3.1. The 1% solution prepared for solubility must be neutral to faintly basic when tested with broad range pH paper or a calibrated pH meter.

7.14.4. Raw Material:

7.14.4.1. The 1% solution prepared for solubility may be neutral to faintly acidic when tested with broad range pH paper or a calibrated pH meter.

7.14.5. Stability:

7.14.5.1. The 1% solution prepared for solubility must be neutral to faintly basic, basic or acidic when tested with broad range pH paper or a calibrated pH meter.

7.14.5.2. For the result Document the result as Passes Test. Then specify if the result was neutral, basic, faintly basic, or acidic.

7.14.5.3. Example: Passes Test-Basic

7.15. **RESIDUE ON IGNITION** :

7.15.1. Turn on muffle furnace and allow temperature to stabilize at 600°C. Follow muffle furnace SOP and calibration procedure for operation.

7.15.2. Inspect a quartz crucible for cracks, chips and discoloration.

7.15.3. Utilize the 10-inch forceps to insert and remove a crucible into the furnace.

7.15.4. Ignite the quartz crucible at 600 ± 50°C for 30 minutes. Cool in a desiccator for one hour and 30 minutes and weigh.

7.15.5. Tare crucible and weigh 1 g of sample directly into the crucible. Record sample weight.

7.15.6. Add 1 mL of concentrated sulfuric acid to the sample.

7.15.7. Volatilize using an appropriate heat source until fumes are no longer produced. Ensur that flames are not produced at any time during the procedure.

7.15.8. Ignite the crucible in the muffle furnace for a minimum of 30 minutes or until no residue remained.

7.15.9. Remove crucible from the muffle furnace, and cool for 1.5 hours in a desiccator.

7.15.10. Weigh the final crucible weight and calculate residue on ignition.

7.15.11. If the amount of residue obtained exceeds the limit specified in the individual summary sheet, repeat steps 7.15.6. to 7.15.8. until two consecutive weighings of the residue do not differ by more than 0.5 mg or until the residue complies with the limit in the individual summary sheet.

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

7.16. **SOLUBILITY** :

7.16.1. Weigh 1 g of sample and dissolve in 99 mL of boiling water.

7.16.2. In an area with sufficient lighting, view the sample from all sides.

7.16.3. The solution must be clear or faintly hazy with no more than a light-yellow color in order to pass test.

7.17. **SULPHATES** :

7.17.1. Standard Preparation 400 ppm (0.0400%): Pipette 0.20 mL of 0.02N Sulfuric acid in to a 100mL Nessler Color Comparison Tube. Dilute to 100 mL with purified water.

7.17.2. Sample Preparation: 0.50 g dissolved in 100 mL of purified water in a Nessler color comparison tube. Heat or sonicate if necessary.

7.17.3. Procedure: Add 1 mL of 3N HCl and 3 mL of Barium Chloride TS to each tube. Cover and mix by inversion.

7.17.4. Allow sample and standard to stand for 10 minutes.

7.17.5. The turbidity of the sample preparation should not exceed the turbidity of the standard solution to report as $\leq$ 400 ppm (0.0400%).