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UREA TESTING METHODS

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1. PURPOSE:

1.1. To provide the Laboratory personnel with a procedure for analyzing Urea Raw Materials, In-Process, Finished Goods, and Stability.

2. SCOPE:

2.1. Applies to the analysis of Urea Raw Materials, In-Process, Finished Goods, and Stability in the Laboratory. Methods include testing for all codes of each grade of Urea sold by BioSpectra; only the specific tests required for the desired code must be tested for. This document applies all BioSpectra facilities.

3. RESPONSIBILITIES:

3.1. The Laboratory Manager, or qualified designee, is responsible for training, maintenance, and implementation of this procedure.

3.2. Laboratory Personnel 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.

3.3. Laboratory Personnel are responsible for referring to the applicable batch record or summary sheet for specifications.

4. REFERENCES:

4.1. BSI-ATM-0073, Analytical Method for the Determination of ICH Q3D Elemental Impurities (Class 1, 2A, 2B, 3 & 4) by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Guanidine Thiocyanate, MOPS, and Urea

4.2. BSI-ATM-0089, Analytical Method of Analysis: Trace Metals in Finished Goods Products by ICP-OES

4.3. BSI-ATM-0090, Urea Assay and Organic Impurity determination by liquid chromatography with UV detection

4.4. BSI-ATM-0131, Analytical Method for the Determination of Trace Metals in BioTech Products

4.5. BSI-MEM-0130, Endosafe NexGen PTS Endotoxin Reader: Qualified Products

4.6. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration

4.7. BSI-SOP-0091, Portable Turbidimeter SOP and Calibration

4.8. BSI-SOP-0094, Muffle Furnace SOP and Calibration

4.9. BSI-SOP-0095, DNase (Endonuclease) Assay

4.10. BSI-SOP-0096, RNase (Ribonuclease) Assay

4.11. BSI-SOP-0098, Balance SOP

4.12. BSI-SOP-0126, Laboratory Notebooks

4.13. BSI-SOP-0138, DNase (Exonuclease) Assay

4.14. BSI-SOP-0139, Protease Assay

4.15. BSI-SOP-0140, Standardization of Titrants

4.16. BSI-SOP-0141, MF-50 Moisture Balance Operation and Calibration

4.17. BSI-SOP-0144, Metrohm 914 pH Conductometer Operation and Calibration

4.18. BSI-SOP-0242, Portable Turbidimeter Operation and Calibration

4.19. BSI-SOP-0254, Spectrum Two UATR SOP

4.20. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP

4.21. BSI-SOP-0256, MP50 Melting Range Operation, Verification and Calibration SOP

4.22. BSI-SOP-0303, NexION 350X ICP-MS SOP
4.23. BSI-SOP-0345, Endosafe nexgen-PTS Endotoxin Reader SOP
4.24. BSI-SOP-0348, Waters Acquity UPLC H-Class Plus SOP
4.25. BSI-SOP-0573, MP90 Melting Range Operation, Verification, and Calibration SOP
4.26. BSI-SOP-0668, OPI-180 OD Handheld Colorimeter SOP
4.27. ACS, Reagent Chemicals, current edition
4.28. Current EP/BP
4.29. Current JP
4.30. Current *USP*

5. EQUIPMENT:

5.1. Analytical Balance
5.2. Calibrated Oven
5.3. Calibrated Muffle Furnace
5.4. Endosafe nexgen-PTS Endotoxin Reader, or equivalent
5.5. Hach Portable Turbidimeter, or equivalent
5.6. Lambda 25 UV/Vis Spectrophotometer
5.7. MF-50 Moisture Balance
5.8. MP50 Melting Point Apparatus
5.9. MP90 Melting Point Apparatus
5.10. OPI-180 OD Handheld Colorimeter SOP
5.11. Perkin Elmer NexION 350X ICP-MS
5.12. Perkin Elmer Avio 500 ICP-OES
5.13. Perkin Elmer Spectrum Two UATR
5.14. Waters Acquity UPLC
5.15. XL200 pH/mV/Conductivity Meter or equivalent

6. REAGENTS:

6.1. **1413 µS/cm Conductivity Standard:** Purchased Commercially.
6.2. **Reagent Alcohol:** purchased commercially.
6.3. **Methyl Red Solution R:** Can be purchased commercially. In house: Dissolve 50 mg of Methyl Red R in a mixture of 1.86 mL of 0.1M sodium hydroxide and 50 mL of ethanol (~96%). Then, dilute to 100 mL with water R.
6.4. **0.01N Hydrochloric Acid:** dilute 97 µL of concentrated hydrochloric acid (37.5% or 10.29M) to 100 mL with water R.
6.5. **Dilute Sodium Hydroxide R:** Dissolve 8.5 g of sodium hydroxide R in USP purified water and dilute to 100 mL with USP purified water.
6.6. **Alkaline Potassium tetraiodomercurate solution R:** Dissolve 11 g of potassium iodide R and 15 g of mercuric iodide R in USP purified water and dilute to 100 mL with USP purified water.
6.7. **Sodium Hydroxide R:** Purchased Commercially.
6.8. **Potassium Iodide R:** Purchased Commercially.
6.9. **Mercuric Iodide R:** Purchased Commercially.
6.10. **250 g/L Sodium Hydroxide R:** Dissolve 25.0 g of Sodium Hydroxide R in water R and dilute to 100 mL with water R.

6.11. **Ammonium Standard Solution (2.5 ppm NH_4) R:** Immediately before use, dilute with USP purified water to 100 times its volume a solution containing ammonium chloride R equivalent to 0.741 g of NH_4Cl in 1000 mL.
6.12. **Ammonium Chloride R:** Purchased Commercially.
6.13. **Ammonium Standard Solution (1 ppm NH_4) R:** Immediately before use, dilute ammonium standard solution (2.5 ppm NH4) to 2.5 times its volume with USP purified water.
6.14. **1000 ppm Biuret Standard:** Dissolve 250 mg of Biuret R in 250 mL of water R.
6.15. **Biuret R, (0.2 g/L):** Weigh 0.02 g of biuret and dissolve in a total volume of 100 mL of water R.
6.16. **0.5% Copper Sulfate:** Dissolve 1.0 g cupric sulfate in 200 mL purified water.
6.17. **42% Sodium Hydroxide:** Dissolve 42.0 g of sodium hydroxide in purified water and dilute to 100 mL with purified water.
6.18. **Biuret Test Solution:** Dissolve 1.73 g of copper sulfate pentahydrate in about 10 mL of hot water. Dissolve 17.3 g of sodium citrate and 10 g of anhydrous sodium carbonate in about 80 mL of water by heating. When both solutions are cool, pour the copper sulfate solution into the other while stirring. Dilute to 100 mL with water. NOTE: Prepare fresh at time of use. Solution may be scaled, as necessary.
6.19. **0.02N HCl:** Slowly add 20 mL of 0.1N Hydrochloric acid to 80 mL of purified water to make a total volume of 100 mL.
6.20. **Concentrated Nitric Acid:** Purchased Commercially.
6.21. **0.1N Silver Nitrate:** Purchased Commercially.
6.22. **Potassium Cyanate:** Purchased Commercially.
6.23. **LAL Reagent Water:** Purchase Commercially.
6.24. **0.1N Hydrochloric acid:** Purchased commercially.
6.25. **Lead Nitrate Stock Solution:** Weigh exactly 159.8 mg of lead (II) nitrate and dissolve in 10 mLof dilute (10%) nitric acid and add water to make exactly 1000 mL. Prepare and store this solution using glass containers, free from soluble lead salts.
6.26. **1N Acetic Acid:** Slowly add 5.74 mL of glacial acetic acid to a small amount of purified water. Adjust the final volume to 100 mL with purified water.
6.27. **6N Ammonium Hydroxide:** Slowly add 41.28 mL of ammonium hydroxide to a small amount of purified water. Adjust the final volume to 100 mL with purified water.
6.28. **pH 3.5 Acetate Buffer:** Dissolve 62.5 g of ammonium acetate in 62.5 mL of purified water, and add 47.0 mL of concentrated hydrochloric acid. Adjust, if necessary, with 6N ammonium hydroxide or 6N hydrochloric acid to a pH of 3.5, dilute with purified water to 250 mL.
6.29. **Thioacetamide TS:** Dissolve 4.0 g of thioacetamide in 100 mL purified water.
6.30. **Glycerin Base TS:** To 200 g of glycerol, add purified water to bring the total weight to 235 g. Add 140 mL of 1N sodium hydroxide and 50 mL purified water.
6.31. **Sodium Hydroxide (1 in 10):** Dilute 20 mL of 50% Sodium Hydroxide Solution to 100 mL with purified water.
6.32. **Cupric Sulfate TS:** Dissolve 12.5 g of cupric sulfate in purified water to make 100 mL.
6.33. **Nitric Acid R:** See concentrated nitric acid.

6.34. **Urea IR Reference Standard:** Weigh approximately 5 grams of Urea from an outside source into a clean LOD vial. Dry the vial in an oven at 105°C for 1 hour. Allow the standard to cool, cap, and store in a desiccator. Perform a UATR analysis on the Reference Standard and compare it to a previously approved reference scan. The correlation must be 0.95 or greater between the two scans.

6.35. **Concentrated Sulfuric Acid:** Purchased Commercially.

6.36. **Concentrated Hydrochloric Acid:** Purchased Commercially.

6.37. **0.020N H_2SO_4:** Slowly add 20 mL of 0.1N sulfuric acid to 80 mL of purified water to make a total volume of 100 mL.

6.38. **3N HCl:** Pipette 25.75 mL of concentrated hydrochloric acid and transfer to a 100-mL volumetric flask that contains a small amount of purified water. Dilute to volume with purified water.

6.39. **Barium Chloride TS:** Dissolve 30 g of barium chloride dihydrate in water to make 250 mL.

7. ANALYTICAL PROCEDURES:

IN PROCESS TESTING

7.1. **ML ABSORBANCE :**

7.1.1. Transfer the entire Mother Liquor sample to a 150 mL beaker and gently heat until all crystals are back in solution, if necessary.

7.1.2. Prepare 40 mL of a 1:1 dilution with purified water of the specified Mother Liquor sample. Swirl to dissolve completely.

7.1.3. Measure and record the absorbance of the sample solution. Refer to Lambda 25 UV/Vis Operation and Calibration.

7.2. **CONDUCTIVITY :**

7.2.1. Calibrate the conductivity meter prior to sample measurement using the 1413µS/cm Conductivity standard.

7.2.2. Follow the appropriate SOP:

7.2.2.1. Stroudsburg: BSI-SOP-0144, Metrohm 914 pH/Conductometer Operation and Calibration or BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP

7.2.2.2. Bangor: BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP

7.2.3. Rinse the electrode and temperature probe, if necessary, thoroughly with purified water.

7.2.4. Use the previously prepared 1:1 dilution sample.

7.2.5. Measure the conductivity of the sample solution according to the appropriate SOP.

7.3. **BIURET UV :**

7.3.1. To the sample beaker, pipette 1.0 mL of Mother Liquor sample and 4.0 mL of Biuret Test Solution.

7.3.2. To the reference beaker, pipette 2.0 mL of purified water and 8.0 mL of Biuret Test Solution.

7.3.2.1. Note: Prepare Biuret Test Solution fresh at the time of use.

7.3.3. After 15-20 minutes, measure the absorbance of the sample against the reference in a 1.0 cm cell at 540 nm. Refer to Lambda 25 UV/Vis Operation and Calibration.

7.3.3.1. To perform a 100% / 0A Baseline (Autozero), utilize the reference beaker solution to blank the instrument by placing the solution in both the front and back cuvette.

FINISHED GOOD TESTING

7.4. Solution S (EP) - Weigh 10.0 g of sample and dissolve in Purified Water. Dilute to a total volume of 50 mL with Purified Water.

7.4.1. The Solution S preparation may be used for multiple analyses.

7.5. **ABSORBANCE (5M) :**

7.5.1. Prepare a 5*M* solution of the specified sample.

7.5.1.1. Accurately weigh 7.5 g of sample.

7.5.1.2. Transfer accurately weighed sample to a graduated cylinder and dilute to 25 mL with purified water. Dissolve completely.

7.5.2. Refer to Lambda 25 UV/Vis Operation and Calibration to measure and record the absorbance of the sample.

7.6. **ALCOHOL INSOLUBLE MATTER (USP) :**

7.6.1. Dissolve 5 g of sample in 50 mL of warm alcohol. If any insoluble residue remains, filter the solution on a tared filter, wash the residue and the filter with 20 mL of warm alcohol, and dry at 105 ± 2°C for 1 hour. The weight of the residue does not exceed 2 mg (0.04%).

7.6.2. Calculate the % Insoluble Matter as follows:

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

7.7. **ALCOHOL INSOLUBLE MATTER :**

7.7.1. Dissolve 10 g of sample in 100 mL of warm alcohol. If any insoluble residue remains, filter the solution on a tared filter, wash the residue and the filter with 40 mL of warm alcohol, and dry at 105 ± 2°C for 1 hour. The weight of the residue does not exceed 1 mg (0.01%).

7.7.2. Calculate the % Insoluble Matter as follows:

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

7.8. **ALKALINITY (EP) :**

7.8.1. To 2.5 mL of Solution S, add 7.5 mL of Purified Water.

7.8.2. Add 0.1 mL of methyl red solution R and 0.4 mL of 0.01N hydrochloric acid (0.01M hydrochloric acid).

7.8.3. The solution must be red to orange to pass.

7.9. **AMMONIUM - METHOD A (EP) :**

7.9.1. To 0.1 mL of Solution S, add 14 mL of USP Purified Water in a test tube. Make alkaline, if necessary, by the addition of dilute sodium hydroxide solution R and dilute to 15 mL with USP Purified water. To the solution, add 0.3 mL of alkaline potassium tetraiodomercurate solution R.

7.9.1.1. Dilute sodium hydroxide solution R: Dissolve 8.5 g of sodium hydroxide R in USP purified water and dilute to 100 mL with USP purified water.

7.9.1.2. Alkaline potassium tetraiodomercurate solution R: Dissolve 11 g of potassium iodide R and 15 g of mercuric iodide R in USP purified water and dilute to 100 mL with USP purified water.

7.9.1.2.1. Immediately before use, mix 1 volume of this solution with an equal volume of a 250 g/L solution of sodium hydroxide R. Use this solution for analysis.

7.9.2. Prepare a standard by mixing 10 mL of ammonium standard solution (1ppm NH_4) R, 5 mL of USP Purified water and 0.3 mL of alkaline potassium tetraiodomercurate solution R.

7.9.2.1. Ammonium standard solution (2.5ppm NH_4): Immediately before use, dilute with USP purified water to 100 times its volume a solution containing ammonium chloride R equivalent to 0.741 g of NH_4Cl in 1000 mL.

7.9.2.2. Ammonium standard solution (1ppm NH_4): Immediately before use, dilute ammonium standard solution (2.5ppm NH_4) to 2.5 times its volume with USP purified water.

7.9.3. Stopper the test-tubes. After 5 min, any yellow color in the test solution is not more intense than that in the standard.

7.10. **APPEARANCE & COLOR :**

7.10.1. Place 25-50 g of the sample in a clean, dry glass beaker. Record the sample weight used.

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

7.10.3. The sample should be white in color and characteristic of needles or crystals as required.

7.10.4. If the appearance and color result is unable to be definitively determined visually, the sample may be analyzed using the Colorimeter. Refer to BSI-SOP-0668, OPI-180 OD Handheld Colorimeter SOP.

7.11. **APPEARANCE OF SOLUTION (EP) :**

7.11.1. Clear *(2.2.1.)* Turbidimetry

7.11.1.1. Pipette 5 mL of Solution S into a beaker and add 15 mL of Purified Water.

7.11.1.2. Rinse the sample bottle with the sample solution twice.

7.11.1.3. Fill sample bottle with the sample solution to the white line.

7.11.1.4. Coat outside of bottle with thin coat of silicon oil.

7.11.1.5. Remove any air bubbles from the solution by using a syringe.

7.11.1.6. Allow the sample to sit capped for 2-3 minutes.

7.11.1.7. Follow the appropriate SOP as follows:

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

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

7.11.1.8. The sample solution must be < 3 NTU, reference suspension I to pass as clear.

7.11.2. Colorless (*2.2.2, Method II*)

7.11.2.1. Pipette 2.5 mL of Solution S into a Nessler Color Comparison Tube and add 7.5 mL of Purified Water.

7.11.2.2. Add 10 mL of Purified Water into a second Nessler Color Comparison Tube.

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

7.11.2.4. In order for the sample solution to be colorless, it must have the appearance of Purified Water or is not more intensely colored than reference solution B9.

7.12. **ASSAY (As Is) and Organic Impurities (USP) :**

7.12.1. Refer to BSI-ATM-0090, Urea Assay and Organic Impurity determination by liquid chromatography with UV detection, for sample preparation and analysis.

7.12.1.1. The verified Method of Analysis for Assay and Organic Impurities is in accordance with the USP monograph and will be the official value reported for Assay and Organic Impurities for all specifications.

7.12.1.2. Action Limit for results obtained is 99.0 - 100.5% for Urea Excipient Finished goods. This limit is derived from the most stringent quality specifications for this analysis. If a result obtained is lower or higher than the action limit but within USP specification 98.0 - 102.0%, contact QA management to see if further action is required.

7.13. **ASSAY (Dried Basis / Dried Substance) :**

7.13.1. Utilize the Assay (as-is) result reported from section 7.12 and LOD result reported from section 7.27 to calculate on a dried basis.

Assay (dried substance) calculation: $\frac{Assay\ (as{-}is)\%}{100{-}LOD}$ *x 100*

7.14. **BIURET (Urea Related Compound A) :**

7.14.1. Primary Method is to refer to BSI-ATM-0090 for sample preparation and analysis.

7.14.2. Alternative methods of analysis:

7.14.2.1. EP:

7.14.2.1.1. Test Solution:

7.14.2.1.1.1. Add 5 mL of Purified Water to 10 mL of Solution S.

7.14.2.1.2. Standard Solution:

7.14.2.1.2.1. Dilute 10 mL of a 0.2g/L solution of Biuret R to 15 mL with Purified Water.

7.14.2.1.3. Procedure:

7.14.2.1.3.1. To the standard and sample, add 0.5 mL of 0.5% copper sulfate solution (5 g/L of copper sulfate pentahydrate R) and 0.5 mL of 42% sodium hydroxide solution (strong sodium hydroxide solution R).

7.14.2.1.3.2. Allow to stand for five minutes.

7.14.2.1.3.3. In order to pass, any reddish violet color in solution must not be more intense than the standard.

7.14.2.2. Raw Material:

7.14.2.2.1. Test Solution:

7.14.2.2.1.1. Weigh 2 g of sample and dissolve with 10 mL of purified water.

7.14.2.2.2. Standard Solution:

7.14.2.2.2.1. Prepare standard by diluting 6 mL of 1000 ppm Biuret Standard to 10 mL with purified water.

7.14.2.2.3. Procedure:

7.14.2.2.3.1. To the standard and sample, add 0.5 mL of 0.5 % copper sulfate and 0.5 mL of 42% sodium hydroxide solution.

7.14.2.2.3.2. Allow to stand for five minutes.
7.14.2.2.3.3. In order to pass, any reddish violet color in solution must not be more intense than the standard.

7.15. **BIURET-UV :**

7.15.1. Ensure the cuvettes are clean prior to analysis.
7.15.2. Weigh 24 g of sample and transfer into a 100 mL volumetric flask. Dissolve and dilute to the mark with purified water. Mix well.
7.15.3. To the sample beaker, add 1.0 mL of the sample solution and 4.0 mL of the biuret test solution.
7.15.4. To the reference beaker, add 1.0 mL of USP Purified Water and 4.0 mL of the Biuret Test Solution.
7.15.4.1. Note: Prepare Biuret Test Solution fresh at the time of use.
7.15.5. After 15-20 minutes, measure the absorbance of the sample against the reference in a 1.0 cm cell at 540 nm. Refer to Lambda 25 UV/Vis Operation and Calibration.
7.15.5.1. To perform a 100% / 0A Baseline (Autozero), utilize the reference beaker solution to blank the instrument by placing the solution in both the front and back cuvette.
7.15.6. The absorbance must not exceed 0.01 a.u.

7.16. **CHLORIDE :**

7.16.1. Standard preparation (Specification 0.0001% max): (Prepare standard using 0.01 mg chloride ion)
7.16.1.1. Pipette 1.410 mL of 0.02*N* HCl and QS to 100 mL with purified water.
7.16.1.2. Pipette 0.200 mL of the above 100 mL HCl solution into a Nessler Color Comparison Tube and dilute to approximately 40 mL with purified water.
7.16.2. Standard preparation (Specification 0.0005% max): (Prepare standard using 0.01 mg chloride ion)
7.16.2.1. Pipette 1.410 mL of 0.02N HCl and QS to 100 mL with purified water.
7.16.2.2. Pipette 1.0 mL, of the above 100 mL HCl solution into a Nessler Color Comparison Tube and dilute to approximately 40 mL with purified water.
7.16.3. JP Standard preparation (Specification 0.007% max):
7.16.3.1. Pipette 0.2 mL of 0.02*N* HCl into a Nessler Color Comparison Tube and dilute to approximately 40 mL with purified water.
7.16.4. Procedure:
7.16.4.1. Weigh 2.0 g of sample and dissolve in approximately 40 mL of purified water. If necessary, neutralize the solution with nitric acid to litmus.
7.16.4.2. 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.
7.16.4.3. After 5 minutes, the turbidity of the sample preparation does not exceed that produced by the 1 ppm standard. View against a dark background.
7.16.4.4. 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:

7.16.4.4.1. Stroudsburg - Measure and record the turbidity of the sample according to BSI-SOP-0091, Portable Turbidimeter Operation and Calibration.

7.16.4.4.2. Bangor - Measure and record the turbidity of the sample according to BSI-SOP-0242, Bangor Portable Turbidimeter SOP.

7.17. **CONDUCTIVITY OF AN 8.5*M* SOLUTION :**

7.17.1. Calibrate the conductivity meter prior to sample measurement.

7.17.1.1. Follow the appropriate SOP:

7.17.1.1.1. Stroudsburg - BSI-SOP-0144, Metrohm 914 pH/Conductometer Operation and Calibration.

7.17.1.1.2. Bangor - BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP.

7.17.2. Rinse the electrode thoroughly with purified water.

7.17.3. Prepare an 8.5*M* solution of Urea.

7.17.3.1. Pre-rinse all glassware using USP purified water.

7.17.3.2. Accurately weigh 12.8 g of sample and transfer to a 50 mL graduated cylinder.

7.17.3.3. Q.S. to 25 mL with purified water.

7.17.3.4. Dissolve completely and allow solution to reach room temperature.

7.17.4. Measure the conductivity per the appropriate SOP immediately after the sample dissolves and reaches room temperature in order to reduce interaction with carbon dioxide in the air. Ensure the probe is adequately submerged when measuring conductivity.

7.17.5. After measurement is completed, the electrode should be rinsed with, and then stored in, purified water.

7.18. **CYANATE (CNO) :**

7.18.1. Cyanate Working Standard Solution (1.0 mg/mL):

7.18.1.1. Immediately before use, weigh 0.1931 g of Potassium Cyanate and transfer to a 100 mL volumetric flask. Dilute to volume with purified water.

7.18.2. Sample Preparation:

7.18.2.1. Prepare a 10% solution by dissolving 1.00 g of sample in 10 mL of purified water in a single use sterile tube.

7.18.3. 300 ppm Standard Preparation:

7.18.3.1. Pipette 0.3 mL of the Cyanate Working Standard Solution into a single use sterile tube and add 9.7 mL of purified water.

7.18.4. Procedure:

7.18.4.1. Add 5 mL of 0.1N Silver Nitrate to each sterile tube and mix.

7.18.4.2. Measure and record the turbidity of the standard and sample within 5 minutes of the addition of Silver Nitrate using the Portable Turbidimeter and appropriate SOP.

7.18.4.2.1. Stroudsburg - Measure and record the turbidity of the sample according to BSI-SOP-0091, Portable Turbidimeter SOP and Calibration.

7.18.4.2.2. Bangor - Measure and record the turbidity of the sample according to BSI-SOP-0242, Bangor Portable Turbidimeter Operation and Calibration.

7.18.4.3. The turbidity of the sample should be less than that of the 300ppm standard.

7.19. **ENDOTOXIN :**

7.19.1. This analysis can be performed in-house or by an approved outside laboratory.

7.19.1.1. In-House Analysis: Solution may be scaled, as necessary.

7.19.1.1.1. Accurately weigh 50-60 mg of sample into a sterile tube. Dilute with LAL reagent water to 10 mL, dissolve, and mix thoroughly for a final concentration of 0.005-0.006g/mL.

7.19.1.1.2. Refer to BSI-SOP-0345, Endosafe nexgen-PTS Endotoxin Reader SOP for instrument use.

7.19.2. Outside lab Analysis:

7.19.2.1. If Endotoxin analysis will be performed by an outside laboratory, package and send NLT 10 grams of sample to an approved outside testing facility.

7.20. **ENZYME ACTIVITY :**

7.20.1. Follow BSI-SOP-0096, RNase (Ribonuclease) Assay, BSI-SOP-0095, DNase (Endonuclease) Assay, BSI-SOP-0138, DNase (Exonuclease) Assay, and BSI-SOP-0139, Protease Assay for sample preparation and analysis.

7.21. **HEAVY METALS :**

7.21.1. Primary method of Analysis, refer to BSI-ATM-0073, Analytical Method of Analysis: Guanidine Thiocyanate MOPS and Urea via ICP-MS for sample preparation and Lead analysis result.

7.21.2. Alternate method of analysis:

7.21.3. USP&JP Test Sample preparation:

7.21.3.1. In a Nessler Color Comparison Tube, dissolve 1.0 g Urea in 20 mL of USP purified water. Add 5 mL of 0.1*N* hydrochloric acid. Dilute with purified water to 40 mL.

7.21.4. EP Test Sample preparation:

7.21.4.1. In a Nessler Color Comparison Tube, dissolve 2.0 g Urea in 20 mL of USP purified water. Add 5 mL of 0.1*N* hydrochloric acid.

7.21.5. Standard Lead Solution:

7.21.5.1. 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.

7.21.6. Standard Preparation:

7.21.6.1. 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 with 1*N* acetic acid or 6*N* ammonium hydroxide, using a pH meter or short-range pH indicator paper as an external indicator. Dilute with USP Purified Water to 40 mL and mix.

7.21.7. Monitor Preparation:

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

7.21.8. Procedure:

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

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

7.21.8.3. To each tube, add 2 mL of pH 3.5 Acetate Buffer and 1.2 mL of thioacetamide-glycerin base TS (mix 1 mL of glycerin TS and 0.2 mL 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.

7.21.8.4. 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 must be equal to or darker than the Standard Preparation.

7.22. **IDENTIFICATION TEST - JP (1), EP (D) :**

7.22.1. In a well-ventilated hood, heat 0.5 g of sample in a glass vial: it liquefies, and ammonia is evolved.

7.22.2. Continue heating the sample until the liquid becomes turbid, then cool.

7.22.3. Dissolve the fused mass in a mixture of 10 mL of USP Purified Water and 1 mL of sodium hydroxide solution (1 in 10).

7.22.4. Add 0.05 mL of cupric sulfate TS and mix. The sample solution should become a reddish violet color.

7.23. **IDENTIFICATION TEST –JP (2), EP/BP (C) :**

7.23.1. Weigh 0.1 g of sample and dissolve in 1 mL of USP Purified Water.

7.23.2. Add 1 mL of nitric acid R. A white crystalline precipitate should be formed.

7.24. **IDENTIFICATION TEST – USP (A) EP/BP (B) :**

7.24.1. Follow BSI-SOP-0254, Spectrum Two UATR SOP.

7.25. **IDENTIFICATION TEST – USP (B) :**

7.25.1. 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.

7.26. **INSOLUBLE MATTER :**

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

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

7.26.2.1. If cloudiness is observed, immediately notify the appropriate personnel.

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

7.26.4. 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.

7.26.5. Filter sample solution through conditioned filtering crucible and 10-15µm filter. Rinse thoroughly with at least 150 mL of hot USP purified water. If needed, rinse with Hot USP Purified Water until all soluble crystal residue is removed. Dry the crucible at 105°C ± 2°C for 1 hour.

7.26.6. Cool in ambient air for 15 minutes and reweigh.

7.26.7. Calculate the % Insoluble Matter as follows:

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

7.27. **LOSS ON DRYING :**

7.27.1. Dry an LOD vial in an oven at 105 ± 2˚C for 30 minutes.

7.27.2. Cool for 15 minutes in a desiccator, weigh the LOD vial using an analytical balance, and record results.

7.27.3. Tare the dried vial and weigh 1 g of sample and record. By gentle, sidewise shaking, distribute the sample as evenly as possible in the LOD vial.

7.27.4. Place the LOD vial containing the sample into the oven and dry at 105˚C ± 2˚C for 1 hour.

7.27.5. Cool for 15 minutes in desiccator.

7.27.6. Reweigh and calculate the % LOD.

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

7.28. **MELTING RANGE (USP) :**

7.28.1. Refer to BSI-SOP-0256, MP50 Melting Range Operation, Verification, and Calibration SOP or BSI-SOP-0573, MP90 Melting Range Operation, Verification, and Calibration SOP to analyze and record results.

7.28.2. JP Melting Point:

7.28.2.1. For reporting Melting Point according to the JP, record the temperature at which the sample is completely liquified (end of melt).

7.29. **MOISTURE :**

7.29.1. Refer to BSI-SOP-0141, MF-50 Moisture Balance Operation SOP for the operation of the moisture balance.

7.29.2. Ensure all settings are correct on the moisture balance prior to use:

7.29.2.1. Program - 1 (Standard Mode)

7.29.2.2. Accuracy - Hi and 10 g sample

7.29.2.3. Temperature - 100°C

7.29.2.4. Measurement Unit - % MOIST/w

7.29.3. Place a clean, room temperature weighing pan on the scale and press “Reset” to zero.

7.29.4. Weigh 10.000 g of sample onto the pan, to the nearest 0.01 g.

7.29.5. Record the initial weight of the sample.

7.29.6. Close the cover and press “Start”.

7.29.7. Record the displayed moisture.

7.29.8. Record the final weight of the sample by pressing “Select” once.

7.29.9. Calculate the percent moisture:

$$\%\ Moisture = \frac{Inital\ Weight\ (g)\ -\ Final\ Weight\ (g)}{Initial\ Weight\ (g)}\ x\ 100$$

7.30. **pH (5M) @ 20°C :**

7.30.1. Weigh 15.0 grams of sample into a suitable beaker.

7.30.2. Add 50mL of purified water and dissolve.

7.30.3. Follow the appropriate SOP to measure and record the pH @ 20°C (19.5-20.4°C).

7.31. **RESIDUE ON IGNITION/SULFATED ASH (USP) :**

7.31.1. Turn on muffle furnace and allow it to stabilize at 600°C. Follow BSI-SOP-0094, Muffle Furnace SOP and Calibration, for operation of the muffle furnace.

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

7.31.3. Utilize the 10-inch forceps to insert and remove the crucible from the furnace.

7.31.4. Ignite quartz crucible at 600 ± 50°C for 30 minutes. Cool in a desiccator and weigh on an analytical balance.

7.31.5. For specification of 0.1% max:

7.31.5.1. Weigh 1.0 g sample in the previously ignited quartz crucible. Moisten the sample with small amount (usually 1 mL) of sulfuric acid.

7.31.6. For specification of 0.01% max:

7.31.6.1. Weigh 4.0 g sample in the previously ignited quartz crucible. Moisten the sample with small amount (usually 1 mL) of sulfuric acid.

7.31.7. Volatilize the sample with a suitable heating apparatus until the sample is thoroughly charred. Ensure that the rate of heating is such that the sample does not boil over and sample is not lost.

7.31.7.1. The rate of heating should be such that from ½ to 1 hour is required to volatilize the sample.

7.31.7.2. Continue using the heating apparatus to heat the sample until all the excess sulfuric acid has been volatilized.

7.31.8. Allow the sample to cool, and then moisten with a small amount (usually 1 mL) of sulfuric acid.

7.31.9. Volatilize the sample with a suitable heating apparatus until the sample is thoroughly charred and white fumes are no longer evolved.

7.31.9.1. The rate of heating should be such that from ½ to 1 hour is required to volatilize the sample.

7.31.9.2. Continue using the heating apparatus to heat the sample until all the excess sulfuric acid has been volatilized.

7.31.10. Ignite in the muffle furnace at 600 ± 50°C for 15 minutes or until all carbon has been removed.

7.31.11. Cool in a desiccator for the same amount of time employed in the preparation of the crucible and weigh on an analytical balance.

7.31.12. Calculate the %ROI as follows:

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

7.32. If the amount of the residue exceeds the limit specified, repeat the moistening with sulfuric acid using up to 1 mL, heat utilizing an appropriate heating apparatus and ignite at 600 ± 50°C for 30 minutes until two consecutive weighings of the residue do not differ by more than 0.0005 g or until the specification is met.

7.33. **SOLUTIONS TEST :**

7.33.1. Dissolve 50 g of sample in 200 mL of USP purified water.

7.33.2. Filter the solution through a filter no larger than a 10 µm Millipore filter, with the aid of vacuum.

7.33.3. Rinse the beaker with USP Purified Water or solvent and pour into the filter funnel, rinsing the funnel walls carefully without disturbing the particles on the filter surface.

7.33.4. Remove the funnel from the holder, remove the filter using forceps and place on a watch glass.

7.33.5. There should be no visible particulate matter present on filter for test to Pass Test.

7.33.6. If particulate matter is present, run a blank on the same volume of USP Purified Water or solvent with the same filter used with the sample preparation.

7.33.7. Undissolved crystals (product) are disregarded, and must not be mistaken for glass or metal.

7.34. **SULFATE :**

7.34.1. JP Test sample preparation:

7.34.1.1. Weigh 9.6 g of sample and transfer to a Nessler Color Comparison tube. Dissolve in approximately 40 mL of purified water. If necessary, neutralize the solution with hydrochloric acid to litmus.

7.34.2. JP Standard preparation (Specification 0.010% max):

7.34.2.1. Pipette 1.0 mL of 0.020*N* H_2SO_4 into a Nessler Color Comparison tube and add approximately 40 mL of purified water.

7.34.3. Standard preparation (Specification 0.001% max):

7.34.3.1. Pipette 0.1 mL of 0.020*N* H_2SO_4 into a Nessler Color Comparison tube and add approximately 40 mL of purified water.

7.34.4. Procedure:

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

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

7.34.4.3. Allow to stand for 10 minutes.

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

7.34.4.5. 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:

7.34.4.5.1. Stroudsburg - Measure and record the turbidity of the sample according to BSI-SOP-0091, Portable Turbidimeter SOP and Calibration.

7.34.4.5.2. Bangor - Measure and record the turbidity of the sample according to BSI-SOP-0242, Bangor Portable Turbidimeter Operation and Calibration.

7.35. **TRACE ELEMENTS :**

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

7.35.2. Alternate method: Refer to BSI-ATM-0089, Analytical Method of Analysis: Trace Metals in Finished Goods Products by ICP-OES, for sample preparation and analysis.

7.35.3. For BioTech Product Analysis: Refer to BSI-ATM-0131, Analytical Method for the Determination of Trace Metals in BioTech Products.

8. COMPENDIAL DIFFERENTIATIONS:

Table 1: Compendial Analysis Overview

USP Compendia	EP Compendia	JP Compendia
Assay	Appearance	Assay
Identification A	Assay	Identification 1
Identification B	Identification A	Identification 2
Residue on Ignition	Identification B	Melting Point
Organic Impurities	Identification C	Chloride
Alcohol Insoluble Matter	Identification D	Sulfate
Bacterial Endotoxins	Appearance of Solution	Heavy Metals
	Alkalinity	Ethanol-Insoluble Substances
	Ammonium	Residue on Ignition
	Loss on Drying	
	Sulfated Ash	

Table 2: One Method Utilized for Analysis and Reporting

Analysis Name
Alcohol Insoluble Matter (USP), Ethanol-Insoluble Substances (JP)
Assay (USP), (JP), (EP)
Identification A (USP), Identification B (EP/BP)
Identification C (EP/BP), Identification 2 (JP)
Identification D (EP), Identification 1 (JP)
Melting Range (USP), Melting Point (JP)
Residue on Ignition (USP), (JP), Sulfated Ash (EP)

8.1. <1225> Validation of Compendial Procedures using a guidance document

Table 3: In-House Validated Methods in Accordance with USP General Chapters

Analysis Name
Endotoxin
Enzyme Activity
Heavy Metals (by ICP-MS)

Table 4: In-House Methods for Product Quality Description

Analysis Name
Appearance and Color
Appearance of Solution
pH of 5M Solution 20°C

Table 5: Outside Approved Laboratory Testing (if required):

Analysis Name
Endotoxin
Microbial Analysis