BIOSPECTRA

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UREA TESTING METHODS

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TABLE OF CONTENTS

1. PURPOSE:

1.1. To provide the Quality Control (QC) 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 QC 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 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. Balance SOP
4.3. Bangor Portable Turbidimeter Operation and Calibration
4.4. *Current USP*
4.5. *Current EP/BP*
4.6. *Current JP*
4.7. DNase (Endonuclease) Assay
4.8. DNase (Exonuclease) Assay
4.9. Endosafe PTS Endotoxin Reader SOP
4.10. Laboratory Notebooks
4.11. Lambda 25 UV/Vis Operation and Calibration
4.12. Metrohm 914 pH Conductometer Operation and Calibration
4.13. MF-50 Moisture Balance Operation and Calibration
4.14. MP50 Melting Range Operation and Calibration SOP
4.15. Muffle Furnace SOP and Calibration
4.16. NexION 350X ICP-MS SOP
4.17. Portable Turbidimeter SOP and Calibration
4.18. Protease Assay
4.19. RNase (Ribonuclease) Assay
4.20. Spectrum Two UATR SOP
4.21. Standardization of Titrants
4.22. Urea Assay via HPLC
4.23. XL200 pH/mV/Conductivity Meter SOP
4.24. Urea Mother Liquor Testing and Specifications

5. EQUIPMENT:

5.1. Analytical Balance
5.2. Calibrated Oven

5.3. Hach Portable Turbidimeter, or equivalent
5.4. Lambda 25 UV/Vis Spectrophotometer
5.5. MF-50 Moisture Balance
5.6. MP50 Melting Point Apparatus
5.7. Calibrated Muffle Furnace
5.8. Perkin Elmer NexION 350X ICP-MS
5.9. Perkin Elmer Spectrum Two UATR
5.10. XL200 pH/mV/Conductivity Meter or equivalent
5.11. Endosafe PTS Endotoxin Reader, or equivalent

6. REAGENTS:

6.1. Solution S (EP/BP) -Weigh 10.0g of sample and dissolve in *USP Purified Water*. Dilute to a total volume of 50 mL with *USP Purified Water*.
6.2. *USP Purified Water* is produced by the qualified water system at each BioSpectra facility.

7. ANALYTICAL PROCEDURES:

IN PROCESS TESTING

7.1. **ML ABSORBANCE 0.2 a.u. max @ 260 nm; Action Limit >0.18 a.u. @ 260 nm:**
- 7.1.1.1. Transfer the entire Mother Liquor sample to a 150 mL beaker and gently heat until all crystals are back in solution, if necessary.
- 7.1.1.2. Prepare 40 mL of a 1:1 dilution with purified water of the specified Mother Liquor sample. Swirl to dissolve completely.
- 7.1.1.3. Measure and record the absorbance of the sample solution. Refer to Lambda 25 UV/Vis Operation and Calibration.

7.2. CONDUCTIVITY 0-2000µS/cm; Action Limit 1900-2000µS/cm:
- 7.2.1.1. Calibrate the conductivity meter prior to sample measurement using the 1413 µS/cm Conductivity standard.
- 7.2.1.2. Follow the appropriate SOP:
  - 7.2.1.2.1. Stroudsburg: Metrohm 914 pH/Conductometer Operation and Calibration
  - 7.2.1.2.2. Bangor: XL200 pH/mV/Conductivity Meter SOP
- 7.2.1.3. Rinse the electrode and temperature probe, if necessary, thoroughly with purified water.
- 7.2.1.4. Use the previously prepared 1:1 dilution sample.
- 7.2.1.5. Measure the conductivity of the sample solution according to the appropriate SOP.

7.3. BIURET UV Action Limit 0.14 -0.16 a.u.:
- 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 1.0 mL of purified water and 4.0 mL oc Biuret Test Solution.
- 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.

FINISHED GOOD TESTING

7.4. **ABSORBANCE (5M) 0.03 max. @ 280 nm, 0.05 max. @ 260 nm:**

7.4.1. Prepare a 5M solution of the specified sample.

7.4.1.1. Accurately weigh 7.5 g of sample.

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

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

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

7.5.1. 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%).

7.5.2. Calculate the % Insoluble Matter as follows:

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

7.6. **ALCOHOL INSOLUBLE MATTER (0.01% max.):**

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

7.6.2. Calculate the % Insoluble Matter as follows:

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

7.7. **ALKALINITY EP/BP (Passes Test):**

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

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

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

7.8. **AMMONIUM - METHOD A EP/BP (500 ppm max.):**

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

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

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

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

7.8.2. Prepare a standard by mixing 10mL of ammonium standard solution (1ppm NH_4) R, 5mL of USP Purified water and 0.3mL of alkaline potassium tetraiodomercurate solution R.
7.8.2.1. 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 0.741g of NH_4Cl in 1000mL.
7.8.2.2. 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.
7.8.3. Stopper the test-tubes. After 5 min, any yellow color in the test solution is not more intense than that in the standard.

7.9. **APPEARANCE & COLOR White / Needles or White / Crystals:**
7.9.1. Place 25-50g of the sample in a clean, dry glass beaker. Record the sample weight used.
7.9.2. In an area with sufficient lighting, view the sample from all sides.
7.9.3. The sample should be white in color and characteristic of needles or crystals as required.

7.10. **APPEARANCE OF SOLUTION EP/BP (Passes Test):**
7.10.1. Clear *(2.2.1.)* Turbidimetry
7.10.1.1. Pipette 5 mL of Solution S into a beaker and add 15 mL of *USP Purified Water*.
7.10.1.2. Rinse the sample bottle with the sample solution twice.
7.10.1.3. Fill sample bottle with the sample solution to the white line.
7.10.1.4. Coat outside of bottle with thin coat of silicon oil.
7.10.1.5. Remove any air bubbles from the solution by using a syringe.
7.10.1.6. Allow the sample to sit capped for 2-3 minutes.
7.10.1.7. Follow the appropriate SOP as follows:
7.10.1.7.1. Stroudsburg- Measure and record the turbidity of the sample according to Portable Turbidimeter Operation and Calibration.
7.10.1.7.2. Bangor- Measure and record the turbidity of the sample according to Bangor Portable Turbidimeter SOP.
7.10.1.8. The sample solution must be < 3 NTU, reference suspension I to pass as clear.
7.10.2. Colorless (*2.2.2, Method II*)
7.10.2.1. Pipette 2.5 mL of Solution S into a Nessler Color Comparison Tube and add 7.5 mL of *USP Purified Water.*
7.10.2.2. Add 10 mL of *USP Purified Water* into a second Nessler Color Comparison Tube.
7.10.2.3. Compare the colors in diffused daylight, viewing vertically against a white background.
7.10.2.4. In order for the sample solution to be colorless, it must have the appearance of *USP Purified Water* or is not more intensely colored than reference solution B_9.

7.11. **ASSAY (As Is) :**
7.11.1. USP 98.0-102.0%
7.11.1.1. Refer to Urea Assay via HPLC SOP.

7.11.2. EP 98.5- 101.5% or JP 99.0% minimum.

7.11.2.1. Package and send NLT 10 grams of sample to an approved outside testing facility.

7.12. **BIURET** **EP/BP (0.1% max.), Raw Material (0.3% max.):**

7.12.1. EP/BP:

7.12.1.1. Test Solution:

7.12.1.1.1. Add 5 mL of USP Purified Water to 10 mL of Solution S.

7.12.1.2. Standard Solution:

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

7.12.1.3. Procedure:

7.12.1.3.1. 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)

7.12.1.3.2. Allow to stand for five minutes.

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

7.12.2. Raw Material:

7.12.2.1. Test Solution:

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

7.12.2.2. Standard Solution:

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

7.12.2.3. Procedure:

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

7.12.2.3.2. Allow to stand for five minutes.

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

7.13. **BIURET-UV 0.01 a.u. max. @ 540nm:**

7.13.1. Ensure the cuvettes are clean prior to analysis.
7.13.2. Weigh 24 g of sample and transfer into a 100 mL volumetric flask. Dissolve and dilute to the mark with purified water. Mix well.
7.13.3. To the sample beaker, add 1.0 mL of the sample solution and 4.0 mL of the biuret test solution.
7.13.4. To the reference beaker, add 1.0 mL of USP Purified Water and 4.0 mL of the biuret test solution.
7.13.5. After 15-20 minutes, measure the absorbance of the sample against the reference in a 1.0 cm cell at 540 nm.
7.13.6. The absorbance must not exceed 0.01 a.u.

7.14. **CHLORIDE 0.0001% max, 0.0005% max, JP (0.007% max.):**

7.14.1. Standard preparation (Specification 0.0001% max): (Prepare standard using 0.01 mg chloride ion)
7.14.1.1. Pipette 1.410 mL of 0.02 N HCl and QS to 100 mL with purified water.
7.14.1.2. 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.

7.14.2. Standard preparation (Specification 0.0005% max): (Prepare standard using 0.01 mg chloride ion)
7.14.2.1. Pipette 1.410 mL of 0.02 N HCl and q.s to 100 mL with purified water.
7.14.2.2. 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.

7.14.3. JP Standard preparation:
7.14.3.1. Pipette 0.2 mL of 0.02 N HCl into a Nessler Color Comparison Tube and dilute to approximately 40 mL with purified water.

7.14.4. Procedure:
7.14.4.1. 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.
7.14.4.2.
7.14.4.3. 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.
7.14.4.4. After 5 minutes, the turbidity of the sample preparation does not exceed that produced by the 1 ppm standard. View against a dark background.
7.14.4.5. 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 as follows:
7.14.4.5.1. Stroudsburg- Measure and record the turbidity of the sample according to Portable Turbidimeter Operation and Calibration.Bangor- Measure and record the turbidity of the sample according to Bangor Portable Turbidimeter SOP.

7.15. **CONDUCTIVITY OF AN 8.5M SOLUTION 30µS/cm max.:**

7.15.1. Calibrate the conductivity meter prior to sample measurement.

7.15.1.1. Follow the appropriate SOP:

7.15.1.1.1. Stroudsburg: Metrohm 914 pH/Conductometer Operation and Calibration.

7.15.1.1.2. Bangor: XL200 pH/mV/Conductivity Meter SOP.

7.15.2. Rinse the electrode thoroughly with purified water.

7.15.3. Prepare an 8.5M solution of Urea.

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

7.15.3.2. Accurately weigh 12.8 g of sample and transfer to a 50 mL graduated cylinder. Q.S. to 25 mL with purified water.

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

7.15.4. 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.

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

7.16. **CYANATE (CNO) 300 ppm max.:**

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

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

7.16.2. Sample Preparation:

7.16.2.1. Prepare a 10% solution by dissolving 1.00g of sample in 10 mL of purified water.

7.16.3. 300 ppm Standard Preparation:

7.16.3.1. Pipette 0.3 mL of the Cyanate Working Standard Solution into a 50 mL beaker and add 9.7 mL of purified water.

7.16.4. Procedure:

7.16.4.1. Add 5 mL of 0.1N Silver Nitrate to each beaker and mix.

7.16.4.2. 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.

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

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

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

7.17. **ENDOTOXIN 1.3 EU/g max:**

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

7.17.2. If Endotoxin analysis will be performed in house, reference appropriate Endosafe SOP for sample preparation and analysis.

7.18. **ENZYME ACTIVITY None Detected:**

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

7.19. **HEAVY METALS USP&JP (0.002% max.), EP/BP (0.001% max):**

7.19.1. USP&JP Test Sample preparation:

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

7.19.2. EP/BP Test Sample preparation:

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

7.19.3. Standard Lead Solution:

7.19.3.1. 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.

7.19.4. Standard Preparation:

7.19.4.1. 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 40mL and mix.

7.19.5. Monitor Preparation:

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

7.19.6. Procedure:

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

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

7.19.6.3. 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 water bath for approximately 20 seconds use immediately). Dilute with USP Purified Water to 50 mL mix and allow to stand for 2 minutes.

7.19.6.4. 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.

7.20. **IDENTIFICATION TEST - JP (1), EP/BP (D) Passes Test:**

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

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

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

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

7.21. **IDENTIFICATION TEST –JP (2), EP/BP (C)** **Passes Test:**

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

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

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

7.22.1. Follow Spectrum Two UATR SOP.

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

7.23.1. 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.

7.24. **INSOLUBLE MATTER** **0.005% max.:**

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

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

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

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

7.24.4. 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.

7.24.5. 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. Dry the crucible at 105°C ± 2°C for 1 hour.

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

7.24.7. Calculate the % Insoluble Matter as follows:

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

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

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

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

7.25.3. 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.

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

7.25.5. Cool for 15 minutes in desiccator.

7.25.6. Reweigh and calculate the % LOD.

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

7.26. **MELTING RANGE EP/BP *Identification A* (132 – 135°C), JP *Melting Point* (132.5 – 134.5°C):**

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

7.27. **MOISTURE 0.5% max.:**

7.27.1. Refer to MF-50 Moisture Balance Operation for the operation of the moisture balance.

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

7.27.2.1. Program-1 (Standard Mode)

7.27.2.2. Accuracy- Hi and 10g sample

7.27.2.3. Temperature- 100 °C

7.27.2.4. Measurement Unit- % MOIST/w

7.27.3. Place a clean, room temperature weighing pan on the scale and press "Reset" to zero.

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

7.27.5. Record the initial weight of the sample.

7.27.6. Close the cover and press "Start".

7.27.7. Record the final weight of the sample by pressing "Select" once.

7.27.8. Calculate the present moisture:

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

7.28. **ORGANIC IMPURITIES USP:**

7.28.1. 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

7.29. **RESIDUE ON IGNITION/SULFATED ASH USP, EP/BP, JP (0.1% max.),(0.010% max):**

7.29.1. Turn on muffle furnace and allow it to stabilize at 600°C. Follow Muffle Furnace SOP and Calibration for operation of the muffle furnace.

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

7.29.3. Utilize the10 inch forceps to insert and remove the crucible from the furnace.

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

7.29.5. For specification of 0.1% max:

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

7.29.6. For specification of 0.01% max:

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

7.29.7. 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.

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

7.29.7.2. Continue using the Bunsen burner to heat the sample until all the excess sulfuric acid has been volatilized.

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

7.29.9. 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.

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

7.29.9.2. Continue using the Bunsen burner to heat the sample until all the excess sulfuric acid has been volatilized.

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

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

7.29.12. Calculate the %ROI as follows:

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

7.29.13. 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.

7.30. **SOLUTIONS TEST** **Passes Test:**

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

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

7.30.3. 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.

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

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

7.30.6. 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.

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

7.31. **SULFATE** **JP (0.010% max.), (0.001% max):**

7.31.1. JP Test sample preparation:

7.31.1.1. Weigh 2.0 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.

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

7.31.2.1. Pipette 0.2 mL of 0.020 *N* H_2SO_4 into a Nessler Color Comparison tube and add approximately 40 mL of purified water.

7.31.3. Standard preparation (Specification 0.001% max):

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

7.31.4. Procedure:

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

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

7.31.4.3. Allow to stand for 10 minutes.

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

7.31.4.5. 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:

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

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

7.32. **TRACE ELEMENTS** **As, Cu, Fe, & Pb (1ppm max.):**

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