BIOSPECTRA

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URIDINE TESTING METHODS

TABLE OF CONTENTS

1. PURPOSE:

1.1. To provide Laboratory personnel with a procedure for analyzing Uridine.

2. SCOPE:

2.1. Applies to testing of Uridine Raw Materials, In Process, Stability, and Finished Goods in the Laboratory. Methods include testing for all types of Uridine sold by BioSpectra; only the specific tests required for the desired type must be tested. This document applies to all BioSpectra facilities.

3. RESPONSIBILITIES:

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

3.2. Laboratory Analysts are responsible for compliance with the terms of this procedure. This includes notifying the Quality Assurance and Laboratory Managers, or qualified designees, if any analyses fail to meet their respective specifications.

3.3. It is the responsibility of all personnel to read and understand the SDS and don the appropriate PPE for handling and disposing of chemicals in a safe manner.

3.4. It is the responsibility of all personnel to refer to the applicable batch record or summary sheet for applicable specifications.

4. REFERENCES:

4.1. BSI-ATM-0092, Uridine Assay and Related Substances Determination by UPLC with UV Detection

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

4.3. BSI-PRL-0762, Analytical Method Validation Protocol: Uridine UV/VIS Assay

4.4. BSI-RPT-1015, Analytical Method Validation Report: Residual Solvents by Head Space GC FID (Uridine)

4.5. BSI-RPT-1595, Analytical Method Validation Report: Uridine UV/VIS Assay

4.6. BSI-SOP-0069, Preparation of Samples for Outside Testing

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

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

4.9. BSI-SOP-0098, Balance SOP

4.10. BSI-SOP-0126, Laboratory Notebooks

4.11. BSI-SOP-0133, Blue M Convection Oven Operation and Calibration SOP

4.12. BSI-SOP-0134, Pipette SOP

4.13. BSI-SOP-0135, Laboratory Chemicals

4.14. BSI-SOP-0140, Standardization of Titrants

4.15. BSI-SOP-0143, Metrohm Titrando 907 Auto-Titrator SOP

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

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

4.18. BSI-SOP-0244, VWR Gravity Convection Oven Operation and Calibration (Model Number 414005-106)

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-0350, Anton Paar DMA 35 Portable Density Meter Operation and Calibration
4.26. BSI-SOP-0353, Densito Handheld Density Meter Operation
4.27. BSI-SOP-0420, 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 Cytidine, Uridine, L-Arginine HCL, and L-Glutamine
4.28. BSI-SOP-0422, Empower 3 General Procedure
4.29. *ACS, Reagent Chemicals,* current edition
4.30. *Current EP/BP*
4.31. *Current USP*
4.32. *Current USP General Chapter <791> pH*

5. EQUIPMENT:

5.1. Analytical Balance
5.2. Blue M Oven, or equivalent
5.3. Calibrated Pipettes
5.4. Calibrated Timer
5.5. Endosafe Nexgen-PTS Endotoxin Reader
5.6. Hach Portable Turbidimeter
5.7. Lambda 25 UV/Vis Spectrophotometer
5.8. Metrohm 907 Titrando Auto-Titrator
5.9. MP50 Melting Point Apparatus
5.10. Muffle Furnace
5.11. Perkin Elmer NexION 350X ICP MS
5.12. Perkin Elmer Spectrum Two UATR
5.13. XL200 pH/Conductivity Meter or equivalent
5.14. Waters H Class HPLC/ UPLC or equivalent
5.15. Shimadzu QP2010 GC-MS w/ Headspace Sampler or equivalent
5.16. Density Meter

6. REAGENTS:

6.1. **1 – 0.01 EU/mL LAL Test Cartridge:** Purchased Commercially.
6.2. **4-(methylamino) Phenol Sulfate Reagent Solution:** Purchased Commercially.
6.3. **Alkaline Potassium Tetraiodomercurate:** Dissolve 11 g of Potassium Iodide and 15 g of Mercury (II) Iodide in purified water and dilute to 100 mL.
6.4. **Ammonium Chloride:** Purchased Commercially.
6.5. **Ammonium Molybdate-Sulfuric Acid Solution (5%):** Dissolve 5.0 g of Ammonium Molybdate Tetrahydrate in 50 mL of 10% Sulfuric Acid and dilute to 100 mL with purified water.
6.6. **Ammonium Molybdate Tetrahydrate:** Purchased Commercially.
6.7. **Ammonium Stock Solution (248.9 ppm):** Weigh 0.0741 g of Ammonium Chloride and dilute to 100 mL with Water R.
6.8. **Composite 5:** Purchased Commercially.

6.9. **Formamide, Dry:** Purchased Commercially.
6.10. **Hydrochloride Acid (0.02N)**: Slowly add 20mL of 0.1N Hydrochloric acid to 80mL of purified water to make a total volume of 100mL. May also be purchased commercially.
6.11. **LAL Reagent Water:** Purchased Commercially.
6.12. **Mercury (II) Iodide:** Purchased Commercially.
6.13. **Methanol, Dry:** Purchased Commercially.
6.14. **Methanol Reference Standard:** Purchased Commercially.
6.15. **Nitric Acid, Concentrated:** Purchased Commercially.
6.16. **Potassium Iodide:** Purchased Commercially.
6.17. **Potassium Phosphate, Monobasic:** Purchased Commercially.
6.18. **SDA 3C Standard Solution:** Purchased Commercially.
6.19. **Silver Nitrate (0.1N):** Purchased Commercially
6.20. **Sodium Hydroxide Solution R, Dilute (8.5% w/v or ~2M):** Weigh 8.5 g of Sodium Hydroxide R and dilute to 100 mL with water R.
6.21. **Sodium Hydroxide (1N):** Purchased Commercially or prepared in-house.
6.22. **Sodium Hydroxide (25%):** Weight 25 g of Sodium Hydroxide R and dilute to 100 mL with water R.
6.23. **Sulfuric Acid (10%):** In a well-ventilated fume hood, slowly add 30 mL of Sulfuric Acid to 375 mL of water, cool, and dilute with water to 500 mL.
6.24. **Sulfuric Acid, Concentrated:** Purchased Commercially.

7. ANALYTICAL PROCEDURES:

7.1. MOTHER LIQUOR COMPOSITION (IN-PROCESS):

7.1.1. Determine mother liquor variables A, B, C, and density (ρ).

7.1.1.1. Determination of A (Mass % Uridine): Refer to section 7.2

7.1.1.2. Determination of B (Mass % Water): Refer to section 7.3.

7.1.1.3. Determination of C (Mass % IPA) = 100.00% - (A + B)

7.1.1.4. Density, (ρ): Refer to Anton Paar DMA 35 Portable Density Meter Operation and Calibration (BSI-SOP-0350) or Densito Handheld Density Meter Operation (BSI-SOP-0353).

7.1.1.5. Transmittance/Transparency: Refer to Lambda 25 UV/Vis Operation and Calibration to measure the Transparency of the sample, neat, at 430 nm using 1cm pathlength cuvette.

7.1.2. Report results in to the applicable batch record and notify manufacturing of completion.

7.2. MOTHER LIQUOR ASSAY (UV):

7.2.1. Assay Solution (~24 mg/L):

7.2.1.1. Utilizing the density, calculate the volume of solution needed to get a solution concentration of ~24 mg/L

7.2.1.1.1. $Volume(mL) = \frac{0.024\ g}{Density}$

7.2.1.2. Pipette the volume of solution calculated above into a 1000 mL volumetric flask.

7.2.1.3. Dissolve and dilute to 1000 mL with purified water and mix completely.

7.2.1.4. Calculate *Assay Test Solution* concentration in mg/L.

7.2.1.4.1. $Concentration = Volume(mL) x\ Density\ x\ 1000$

7.2.2. Refer to Lambda 25 UV/Vis Operation and Calibration to measure the absorbance of the Assay Test Solution at 262 nm using 1cm pathlength cuvette.

7.2.3. Calculate % Assay using the following equation:

$$\%\ Assay\ Uridine = \frac{Abs\ @\ 262\ nm\ (a.u.)}{1.000\ a.u.} \times \frac{24.18\ (\frac{mg}{L})}{Sample\ Concentration\ (\frac{mg}{L})} \times 100$$

Where:

Abs @ 262 nm = Absorbance Measured of *Assay Test Solution* at 262 nm; 1 cm pathlength.

Sample Concentration (mg/L) = Concentration of the *Assay Test Solution*; mg/L.

24.18 mg/L = Concentration of 1.00 a.u. absorbance Uridine Solution based on Molar absorptivity coefficient of 10100 a.u. per mole.

1.00 a.u. = Absorbance of 24.18 mg/L of Uridine based on 10100 molar absorptivity coefficient and 1 cm pathlength.

7.3. MOTHER LIQUOR WATER (by Karl Fischer Titration):

7.3.1. Perform a standardization of the titrant (Composite 5) as per Standardization of Titrants.

7.3.2. Pipette 0.050 mL of sample into the vessel. This may be reduced if titration exceeds burette volume (10 mL).

7.3.2.1. Calculate the sample weight utilizing the density

7.3.2.1.1. $Sample\ Weight\ (g) = Density * Volume\ (mL)$

7.3.3. Transfer the sample to the KF vessel by removing the rubber septum and adding the sample into the titration vessel.
  7.3.3.1. Do not leave the rubber septum open for long periods of time as this will allow moisture to enter the titration vessel.
7.3.4. Check to make sure there is no residual sample stuck to the sides of the titration vessel.
  7.3.4.1. If there is any sample stuck to the side, stop the stir bead from spinning before swirling the vessel to rinse the sides.
7.3.5. Once the method begins, check to ensure the sample is fully dissolved before the titration begins (i.e. before the stir command completes).
7.3.6. The moisture content will then be determined by the Metrohm Auto Titrando 907.

$$\%\ Moisture = \frac{(mL\ of\ Composite\ 5)\ x\ \left(\frac{mg}{mL}\ of\ Composite\ 5\right)\ x\ (0.1)}{Sample\ weight\ (g)}$$

7.4. **AMMONIUM:**
7.4.1. Reagent Preparations:
  7.4.1.1. Alkaline Potassium Tetraiodomercurate Solution R
    7.4.1.1.1. Prepare *alkaline potassium tetraiodomercurate solution R* immediately before use.
    7.4.1.1.2. Make a 1:1 solution of alkaline potassium tetraiodomercurate solution and 25% NaOH. Scale as needed.
  7.4.1.2. Ammonium Standard Solution (1 ppm NH_4^+):
    7.4.1.2.1. Immediately before use, dilute 0.1 mL of a solution containing ammonium chloride R equivalent to 0.741 g of NH_4Cl in 1000 mL to 25mL with purified water.
  7.4.1.3. Ammonium Standard Test Solution
    7.4.1.3.1. Pipette 15 mL of the *Ammonium Standard Solution (1 ppm NH_4^+)* into a 100 mL Nessler Tube.
    7.4.1.3.2. Dilute standard solution to ~70 mL with purified water.
    7.4.1.3.3. Make alkaline if necessary, using dilute sodium hydroxide solution R (~8.5% w/v or ~2M).
7.4.2. Sample Test Preparation:
  7.4.2.1. Dissolve 0.01 g of sample into ~70 mL of purified water in a 100 mL Nessler Tube.
  7.4.2.2. Make alkaline if necessary, using dilute Sodium Hydroxide Solution R (~8.5% w/v or ~2M).
7.4.3. Analysis:
  7.4.3.1. To both sample and standard test solutions, add 4.5 mL of *alkaline potassium tetraiodomercurate solution R.*
  7.4.3.2. Dilute both the sample and standard test solutions to 100 mL using purified water.
  7.4.3.3. Cover the Nessler Tubes and mix.
  7.4.3.4. After 5 minutes any yellow color in the test solution is not more intense than any yellow color in the standard solution to report as < 1500 ppm.

7.5. **APPEARANCE AND COLOR:**
7.5.1. Place ~25 g of the sample in a clean, dry glass beaker.
7.5.2. Observe the sample under diffuse daylight or well illuminated area and note the physical appearance and structure of the material as well as the material color.
7.5.3. The sample should conform to specification on summary sheet. If the sample does not conform to these specifications, notify the Laboratory Manager immediately.

7.6. **ARSENIC:**

7.6.1. Refer to Elemental Impurities via ICP-MS in Cytidine, Uridine, L-Arginine HCl, and L-Glutamine; DCN: BSI-SOP-0420 for method parameters and sample preparation for Elemental Impurity assessment via ICP-MS.

7.7. **ASSAY (HPLC):**

7.7.1. Refer to Uridine Assay and Related Substances Determination by UPLC with UV Detection; DCN: BSI-ATM-0092 for method parameters, system suitability and sample preparation for HPLC (UPLC) Assay % w/w, calculated on the anhydrous basis.

7.8. **ASSAY (UV):**

7.8.1. Note: Solutions may be scaled appropriately provided the weight is within the minimum and maximum of the balance.

7.8.2. Assay Solution (~24 mg/L):

7.8.2.1. Accurately weigh 2.4g (± 0.2 g) of sample on an analytical balance. Quantitatively transfer aliquot to a 1000 mL volumetric flask.

7.8.2.2. Dissolve and dilute to 1000 mL with purified water and mix completely.

7.8.2.3. Pipette 1.0 mL of the Uridine solution to a 100 mL volumetric flask.

7.8.2.4. Dilute to volume with purified water, cap, and mix thoroughly.

7.8.2.5. Calculate *Assay Test Solution* concentration in mg/L.

$$Assay\ Test\ Solution\ (\frac{mg}{L}) = \frac{Sample\ Weight\ (g)}{0.001\frac{g}{mg}x\ 1L} \times \frac{1\ (mL)}{100\ (mL)}$$

7.8.3. Refer to Lambda 25 UV/Vis Operation and Calibration to measure the absorbance of the Assay Test Solution at 262 nm using 1 cm pathlength cuvette.

7.8.4. Calculate % Assay using the following equation:

$$\%\ Assay\ Uridine = \frac{Abs\ @\ 262\ nm\ (a.u.)}{1.000\ a.u.} \times \frac{24.18(\frac{mg}{L})}{Sample\ Concentration\ (\frac{mg}{L})} \times 100$$

Where:

Abs @ 262 nm = Absorbance Measured of *Assay Test Solution* at 262 nm; 1 cm pathlength.

Sample Concentration (mg/L) = Concentration of the *Assay Test Solution*; mg/L.

24.18 mg/L = Concentration of 1.00 a.u. absorbance Uridine Solution based on Molar absorptivity coefficient of 10100 a.u. per mole.

1.00 a.u. = Absorbance of 24.18 mg/L of Uridine based on 10100 molar absorptivity coefficient and 1 cm pathlength.

7.9. **BIOBURDEN (TAMC/TYMC):**

7.9.1. Package no less than 35 g of sample into a sterile container and send to MPL Laboratories. The Analysis Request form should include TAMC, TYMC.

7.9.2. If required, request specified microorganisms. Package no less than 65 g if requesting additional IDs.

7.9.2.1. *Escherichia coli* Test for Absence per 1 g

7.9.2.2. *Salmonella* Test for Absence per 10 g

7.9.2.3. *Pseudomonas aeruginosa* Test for Absence per 1 g

7.9.2.4. *Staphylococcus aureus* Test for Absence per 1 g

7.10. **CHLORIDES:**

7.10.1. Sample Solution:

7.10.1.1. Weigh 0.2 grams of sample and dissolve in approximately 40mL of purified water.

7.10.1.2. If necessary, neutralize the solution with nitric acid to litmus.

7.10.2. 500ppm Chloride Standard Solution:

7.10.2.1. Pipette 0.141 mL of 0.02N HCl into a Nessler Color Comparison Tube and dilute to approximately 40 mL with purified water.

7.10.3. Procedure:

7.10.3.1. To each solution add 1 mL of Concentrated Nitric Acid and 1 mL of 0.1N Silver Nitrate.

7.10.3.2. Q.S. to 50 mL with purified water. Cover with parafilm and mix by inversion.

7.10.3.3. After 5 minutes, the turbidity of the sample preparation does not exceed that produced by the 500ppm Chloride standard when viewed against a dark background.

7.10.3.4. If a visible difference in turbidity is not observed, then utilize the Turbidimeter to measure the turbidity of the standard and the sample solutions. Follow the appropriate Portable Turbidimeter SOP.

7.11. **DRY SUBSTANCE:**

7.11.1. Use the result from Loss on Drying to calculate Dry Substance result.

7.11.2. Calculate the % Dry Substance as follows:

$$\%\ Dry\ Substance = 100.00\% - \ Loss\ on\ Drying\ \%$$

7.12. **ENDOTOXINS:**

7.12.1. Accurately weigh 100 mg of sample into a sterile tube. Add 10 µL of 1N NaOH. Dilute to 10 mL with LAL reagent water, dissolve, and mix thoroughly for a final concentration of 0.0100 g/mL (10 mg/mL).

7.12.2. Refer to Endosafe Nexgen-PTS Endotoxin Reader SOP, BSI-SOP-0345, for instrument operation.

7.13. **ENDOTOXINS (BIOBUFFER SOLUTIONS RM):**

7.13.1. Accurately weigh 100 mg of sample into a sterile tube. Add 10 µL of 1N NaOH. Dilute to 10 mL with LAL reagent water, dissolve, and mix thoroughly. Pipette 1 mL of the resulting solution into a sterile tube and dilute to 10 mL with LAL reagent water for a final concentration of 1 mg/mL.

7.13.2. Refer to Endosafe Nexgen-PTS Endotoxin Reader SOP, BSI-SOP-0345, for instrument operation.

7.14. **HEAVY METALS (ELEMENTAL IMPURITIES) Refer to Summary Sheet:**

7.14.1. Refer to Elemental Impurities via ICP-MS in Cytidine, Uridine, L-Arginine HCl, and L-Glutamine, BSI-SOP-0420, for method parameters and sample preparation for Elemental Impurity assessment via ICP-MS.

7.15. **HPLC PURITY:**

7.15.1. Refer to Uridine Assay and Related substances Determination by UPLC with UV Detection, BSI-ATM-0092, for method parameters, system suitability and sample preparation for HPLC (UPLC) Purity.

7.16. **IDENTIFICATION TEST B (HPLC):**

7.16.1. Refer to Section 7.15. for method of analysis.

7.16.2. The retention time of the largest peak in the sample solution corresponds to the principle peak in the standard solution for HPLC Purity.

7.17. **IDENTIFICATION TEST A (IR)** :

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

7.18. **INSOLUBLE MATTER** :

7.18.1. Accurately weigh 5.0 g of sample. Transfer to a suitable beaker.

7.18.2. Add 100 mL of purified water and utilize a Teflon encapsulated magnetic stirring bar and electric stir plate to dissolve sample.

7.18.3. Warm solution and digest on a hot plate in a covered beaker for 1 hour.

7.18.4. Prepare a Gooch filtering crucible and 6-15-micron filter by drying at 105°C ± 2°C for 1 hour. Allow to cool in ambient air for 15 minutes and weigh.

7.18.5. Filter sample solution through conditioned filtered crucible and 6-15-micron filter. Rinse thoroughly with at least 3 crucibles volumes of hot purified water.

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

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

7.18.8. Calculate the % Insoluble Matter as follows:

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

7.19. **LOSS ON DRYING** :

7.19.1. Dry an LOD vial in the oven at 105 ± 2°C for 30 minutes.

7.19.2. Cool for 15 minutes in a desiccator, weigh the LOD vial, and record results.

7.19.3. If the substance to be tested is in the form of large crystals, reduce the particle size to about 2mm by quickly crushing before weighing.

7.19.4. Transfer approximately 1 - 2 g of the sample to the LOD vial, and accurately weigh the bottle and contents. By gentle, sidewise shaking, distribute the sample as evenly as possible in the LOD vial to a depth of about 5 mm.

7.19.4.1. For mother liquor samples: Pipette 1.000 mL using a 100 - 1000 µL pipette.

7.19.5. Place the LOD vial containing the sample into the oven and dry at 105°C ± 2°C for 3 hours.

7.19.6. Remove LOD vial from the oven and allow to cool in the desiccator for 15 minutes.

7.19.7. Reweigh the LOD vial and sample and retain the dried sample to perform the Assays.

7.19.8. Calculate the %LOD as follows:

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

7.20. **MELTING RANGE** :

7.20.1. Refer to MP50 Melting Range Operation and Calibration SOP, BSI-SOP-0256.

7.21. **pH of a 5% or 1 in 20 SOLUTION @ 25 +/-2°C** :

7.21.1. Accurately weigh 5.0 g of sample. Transfer to a suitable beaker.

7.21.2. Add 100 mL of purified water and dissolve.

7.21.3. Follow the appropriate SOP to measure and record the pH.

7.22. **PHOSPHATES** :

7.22.1. Phosphate Stock Standard preparation: Dissolve 0.143 g of monobasic potassium phosphate in water, and dilute with water to 100 mL.

7.22.2. Phosphate Standard Solution Preparation (0.02 mg/mL PO_4): Dilute 2 mL of the *phosphate stock standard* and dilute with water to 100 mL. Scale as needed.

7.22.3. Standard preparation: Pipet 1mL of the 0.02 mg/mL *Phosphate standard solution* and add 10 mL of water.

7.22.4. Sample preparation: To 0.02 g of sample, add 10 mL of water.

7.22.5. To both solutions, add 1 mL of ammonium molybdate-Sulfuric Acid Solution (5%) and 1 mL of 4-(methylamino) phenol sulfate reagent solution. Allow to stand at room temperature for 2 hr. Any blue color should not exceed that produced by 0.02 mg of phosphate ion in an equal volume of solution containing the quantities of reagents used in the test to report as < 1000 ppm (0.1%).

7.23. **RELATED SUBSTANCES:**

7.23.1. Refer to Uridine Assay and Related Substances Determination by UPLC with UV Detection, BSI-ATM-0092, for Primary Method UPLC.

7.24. **RESIDUAL SOLVENTS:**

7.24.1. Log in and ensure the correction parameters are set on the GC-FID. Check consumables before use including all required gases.

7.24.1.1. Refer to BSI-RPT-1015 for GC-FID configuration details.

7.24.2. Pre-Requisite Solutions:

7.24.2.1. Residual Solvent Stock Solutions:

7.24.2.1.1. Prepare individually a 10,000 mg/L* (ppm) solution of each residual solvent LTBP in purified water by weighing approximately 0.50 g of standard directly into a 50 mL volumetric flask. Mix thoroughly. Calculate actual concentrationbased off CoA/purity. Include calculations in notebook.

7.24.2.1.2. Mix thoroughly.

7.24.3. Calibration Standards and Spike Diluent Preparation:

7.24.3.1. NOTE: Addition of solutions or reagents to head space vial may be done in any order.

7.24.3.2. 0 ppb (Blank)

7.24.3.2.1. Purified water or equivalent.

7.24.3.2.2. Calibration Level 1 (50% Level)

7.24.3.2.3. In a 100.0 mL volumetric flask add the following:

7.24.3.2.3.1. 0.50 mL of 10,000 ppm *Methanol Stock Solution*

7.24.3.2.3.2. 2.50 mL of 10,000 ppm *Ethanol (SDA 3C) Stock Solution.*

7.24.3.2.3.3. 2.50 mL of 10,000 ppm *2-Propanol Stock Solution.*

7.24.3.2.4. Dilute to 100.0 mL with water.

7.24.3.2.5. Mix thoroughly.

7.24.3.3. Calibration Level 2 (80% Level)

7.24.3.3.1. In a 100.0 mL volumetric flask add the following:

7.24.3.3.1.1. 0.80 mL of 10,000 ppm *Methanol Stock Solution*

7.24.3.3.1.2. 4.00 mL of 10,000 ppm *Ethanol (SDA 3C) Stock Solution.*

7.24.3.3.1.3. 4.00 mL of 10,000 ppm *2-Propanol Stock Solution.*

7.24.3.3.2. Dilute to 100.0mL with water.

7.24.3.3.3. Mix thoroughly.

7.24.3.4. Calibration Level 3 (100% Level)

7.24.3.4.1. In a 100.0 mL volumetric flask add the following:

7.24.3.4.1.1. 1.00 mL of 10,000 ppm *Methanol Stock Solution*

7.24.3.4.1.2. 5.00 mL of 10,000 ppm *Ethanol (SDA 3C) Stock Solution.*

7.24.3.4.1.3. 5.00 mL of 10,000 ppm *2-Propanol Stock Solution.*

7.24.3.4.2. Dilute to 100.0 mL with water.

7.24.3.4.3. Mix thoroughly.

7.24.3.5. Calibration Level 4 (120% Level)

7.24.3.5.1. In a 100.0 mL volumetric flask add the following:

7.24.3.5.1.1. 1.20 mL of 10,000 ppm *Methanol Stock Solution*

7.24.3.5.1.2. 6.00 mL of 10,000 ppm *Ethanol (SDA 3C) Stock Solution.*

7.24.3.5.1.3. 6.00 mL of 10,000 ppm *2-Propanol Stock Solution.*
7.24.3.5.2. Dilute to 100.0 mL with water.
7.24.3.5.3. Mix thoroughly.
7.24.3.6. Calibration Level 5 (150% Level)
7.24.3.6.1. In a 100.0 mL volumetric flask add the following:
7.24.3.6.1.1. 1.50 mL of 10,000 ppm *Methanol Stock Solution*
7.24.3.6.1.2. 7.50 mL of 10,000 ppm *Ethanol (SDA 3C) Stock Solution.*
7.24.3.6.1.3. 7.50 mL of 10,000 ppm *2-Propanol Stock Solution.*
7.24.3.6.1.4. Dilute to 100.0 mL with water.

7.24.4. Test Sample Preparation:
7.24.4.1. Weigh and add 1.0 g of sample to head space vial.
7.24.4.2. Add 10 mL of purified water to headspace vial.
7.24.4.3. Dissolve.
7.24.4.4. Crimp to seal, mix thoroughly.

7.24.5. Calibration and System Suitability:
7.24.5.1. Calibrate the GC-FID instrument using calibration levels 1, 2, 3, 4 and 5 and a diluent blank (Standard 0 ppm) by pipetting 10 mL of each standard to a headspace vial. Crimp to seal, mix thoroughly.
7.24.5.2. An r^2 of NLT 0.95 is required for each solvent of interest.
7.24.5.3. Add test samples to the sequence following the calibration / system suitability injections.
7.24.5.4. Print and initial and date sequence/batch file.
7.24.5.5. Data processing should be automated and performed within the method during data acquisition, notify a supervisor is any changes to the integration parameters or chromatographic issues are noted.

7.25. **RESIDUE ON IGNITION/SULFATED ASH :**

7.25.1. NOTE: The USP General Chapter will be followed for USP, EP/BP, and JP testing, unless a dispute arises in which case the appropriate compendia chapter will be followed.
7.25.2. Turn on muffle furnace and allow it to stabilize at 600°C.Follow muffle furnace calibration procedure for operation of furnace.
7.25.3. Inspect a quartz crucible for cracks, chips and discoloration.
7.25.4. Utilize forceps to insert and remove the crucible from the furnace.
7.25.5. Ignite quartz crucible at 600 ± 50 °C for 30 minutes. Cool in a desiccator and weigh on an analytical balance.
7.25.6. Weigh 1.0 g sample in the previously ignited quartz crucible. Moisten the sample with 0.2 mL of sulfuric acid.
7.25.7. Volatilize the sample until the sample is thoroughly charred. Heat the sample slowly, so that the sample does not boil over and sample is not lost.
7.25.7.1. The rate of heating should be such that from ½ to 1 hour is required to volatilize the sample.
7.25.7.2. Continue to heat the sample until all the excess sulfuric acid has been volatilized.
7.25.8. Allow the sample to cool, and then moisten with 0.2 mL of sulfuric acid.
7.25.9. Volatilize the sample until the sample is thoroughly charred and white fumes are no longer evolved. Heat the sample slowly, so that the sample does not boil over and sample is not lost.
7.25.9.1. The rate of heating should be such that from ½ to 1 hour is required to volatilize the sample.
7.25.9.2. Continue to heat the sample until all the excess sulfuric acid has been volatilized.
7.25.10. Ignite in the muffle furnace at 600 ± 50 °C for 15 minutes or until all carbon has been removed.

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

7.25.12. Calculate the %ROI as follows:

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

7.25.13. If the amount of the residue exceeds the limit specified, repeat the moistening with sulfuric acid using up to 1 mL, heat to char, then ignite at 600 ± 50 °C for 30 minutes until two consecutive weighing's of the residue do not differ by more than 0.0005 g or until the specification is met.

7.26. **SOLUBILITY** :

7.26.1. Sample Preparation:

7.26.1.1. Dissolve 5 grams of sample in purified water and dilute to 100mL with purified water. (Solution prepared for Insoluble Matter may be utilized)

7.26.2. Turbidity:

7.26.2.1. Analyze the *Sample Preparation* for turbidity using a calibrated turbidimeter.

7.26.2.2. Acceptance Criteria:

7.26.2.2.1. The turbidity result may not exceed 3NTU to report as clear.

7.26.3. Color:

7.26.3.1. In an area with sufficient lighting, compare the color of the *Sample Preparation* to Purified Water.

7.26.3.2. Acceptance Criteria:

7.26.3.2.1. The color of the *Sample Preparation* may not be more intense than the color of purified water to report as colorless.

7.26.4. To report as Passes Test, the solution must be both Clear and Colorless.

7.27. **TRACE METALS** :

7.27.1. Refer to BSI-ATM-0131, Analytical Method for the Determination of Trace Metals in BioTech Products, for standard and sample preparation.

7.28. **TRANSPARENCY 1%w/v SOLUTION** :

7.28.1. Accurately weigh 1.0 g of sample. Transfer to a volumetric flask.

7.28.2. Dilute to 100 mL of purified water and dissolve and mix completely.

7.28.3. Refer to Lambda 25 UV/Vis Operation and Calibration to measure the Transparency of the sample 430 nm using 1 cm pathlength cuvette.

7.29. **TRANSMITTANCE 5%w/v SOLUTION** :

7.29.1. Accurately weigh 5.0 g of sample. Transfer to a volumetric flask.

7.29.2. Dilute to 100 mL of purified water and dissolve and mix completely.

7.29.3. Refer to Lambda 25 UV/Vis Operation and Calibration to measure the Transmittance of the sample 430 nm using 1 cm pathlength cuvette.

7.30. **WATER (by Karl Fischer Titration)** :

7.30.1. Perform a standardization of the titrant (Composite 5) as per Standardization of Titrants.

7.30.2. Grind the sample in a dry mortar into a fine powder utilizing a pestle.

7.30.3. Immediately weigh 1 g of sample into the glass weighing spoon and tare it.

7.30.3.1. Mother Liquor Samples: Pipette ~50 µL of sample into the vessel. This may be reduced if titration exceeds burette volume (10 mL).

7.30.4. Transfer the sample to the KF vessel by removing the rubber septum and adding the sample into the titration vessel.

7.30.4.1. Do not leave the rubber septum open for long periods of time as this will allow moisture to enter the titration vessel.

7.30.5. Return the weighing spoon to the balance, making sure not to lose any sample that was left behind. Once the weight stabilizes, press the print button on the balance.
7.30.6. Check to make sure there is no residual sample stuck to the sides of the titration vessel.
    7.30.6.1. If there is any sample stuck to the side, stop the stir bead from spinning before swirling the vessel to rinse the sides.
7.30.7. Once the method begins, check to ensure the sample is fully dissolved before the titration begins (i.e. before the stir command completes).
7.30.8. The moisture content will then be determined by the Metrohm Auto Titrando 907.

$$\%\ Moisture = \frac{(mL\ of\ Composite\ 5)\ x\ \left(\frac{mg}{mL}\ of\ Composite\ 5\right)\ x\ (0.1)}{Sample\ weight\ (g)}$$