BIOSPECTRA
100 Majestic Way, Bangor, PA 18013 / www.biospectra.us

TR3220 AND TR4220 TESTING METHODS

1.1. ABSORBANCE (40% Solution)

1.1.1. Prepare a 40% solution of the specified sample.
- 1.1.1.1. Accurately weigh 10.00g of sample.
- 1.1.1.2. Transfer accurately weighed sample to a 50mL graduated cylinder and q.s. to 25mL with purified water.
- 1.1.1.3. Swirl to dissolve completely
  - 1.1.1.3.1. Sonicate if necessary to accelerate dissolution. Allow to cool to room temperature before analysis, if applicable.

1.1.2. Refer to Lambda 25 UV/Vis Operation and Calibration to measure the Absorbance of the sample.

1.1.3. **TR3220 and TR4220 Specification: 0.2 a.u. maximum @ 290nm**

1.2. APPEARANCE AND COLOR

1.2.1. Place 25-50g of the sample in a clean, dry glass beaker.

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

1.2.3. The sample should be white in color and characteristic of crystals. If the sample does not conform to these specifications, notify the QC Manager immediately.

1.2.4. **TR3220 and TR4220 Specification: White / Crystals**

1.3. ASSAY (USP)

1.3.1. Perform standardization of the 0.1*N* HCl per Standardization of Titrants utilizing NIST Tromethamine.

1.3.2. Accurately weigh 0.25g ± 0.0005g of Tris previously dried at 105°C for 3 hours (use LOD sample).

1.3.3. Transfer accurately weighed sample to a suitable clean, glass beaker. Add 100mL of purified water and swirl to dissolve.

1.3.4. Titrate with 0.1*N* HCl VS to a potentiometric endpoint using the Metrohm 907 Auto Titrator.

1.3.5. Each mL of 0.1*N* HCl is equivalent to 12.11mg of Tris:

$$\% \text{ Tris} = \frac{(mL\ x\ N\ of\ 0.1N\ HCL) \cdot 12.11\ mg}{Sample\ Weight\ (g)}$$

1.3.6. **TR3220 and TR4220 Specification: (99.0 – 101.0%)**

1.4. ENZYME ACTIVITY

1.4.1. Refer to DNase (Endonuclease) Assay, DNase (Exonuclease) Assay, Protease Assay and RNase (Ribonuclease) Assay SOPs.

1.4.2. **TR3220 and TR4220 Specification: DNase, Protease, Rnase : None Detected**

1.5. HEAVY METALS – USP <231>

1.5.1. USP– Test sample preparation:
- 1.5.1.1. Prepare the sample solution by dissolving 4.0g of Tris in 20mL of purified water and neutralize with 2.4mL of concentrated HCl in a 50-mL color-comparison tube. Dilute to 25 mL with purified water.

Printed On:	19-Oct-2020 10:53:29 AM	Ledergerber, Alissa	: Printed By
Authored By:	Fisher, Nicole	24-Aug-2017 08:55:23 AM	
Approval:	Hamelburg, Crystal	24-Aug-2017 12:38:23 PM	
Notice:	CONFIDENTIAL: The information contained herein is the property of BioSpectra. The recipient is responsible for its safe-keeping and the prevention of unauthorized appropriation, use, disclosure and copying.		

1.5.2. Standard Lead Solution – On the day of use, dilute 10.0mL of Lead Nitrate Stock Solution with purified water to 100.0mL in a volumetric flask. .

1.5.3. Standard Preparation – Into a 50-mL color-comparison tube, pipette 2mL of Standard Lead Solution prepared above, and dilute with purified water to 25mL.

1.5.4. Monitor Preparation – Into a third 50-mL color-comparison tube, place 25mL of a solution prepared as directed for Test Preparation and add 2.0mL of Standard Lead Solution.

1.5.5. Procedure:

1.5.5.1. Adjust all 50-mL color-comparison tubes to a pH between 3.0 and 4.0 using 1*N* acetic acid or 6*N* ammonium hydroxide. Use a pH meter or short-range pH indicator paper as an external indicator.

1.5.5.2. Dilute each with purified water to 40 mL and mix.

1.5.5.3. To all tubes add 2 mL of pH 3.5 Acetate Buffer and 1.2 mL of thioacetamide-glycerin base TS (1mL of glycerin TS and 0.2mL of thioacetamide TS gently heated for about 20 seconds). Dilute with purified water to 50 mL, parafilm and mix by inversion.

1.5.5.4. Allow to stand for 2 minutes.

1.5.5.5. View downward over a white surface. The color of the Test Preparation is not darker than the Standard Preparation, and the Monitor Preparation is equal to or darker than the Standard Preparation.

1.5.6. **TR3220 and TR4220 Specification: 5 ppm maximum**

1.6. IDENTIFICATION B

1.6.1. To 4.5mL of a saturated solution of Salicylaldehyde, add 0.5mL of glacial acetic acid and mix.

1.6.2. Dissolve 1g of sample in 5mL of purified water.

1.6.3. Transfer 4.0mL of the sample solution to the above 50mL beaker and mix. A yellow color should be produced.

1.6.4. **TR3220 and TR4220 Specification: Passes Test**

1.7. IDENTIFICATION C

1.7.1. Prepare a 4 in 10 solution of Ceric Ammonium Nitrate in 2 *N* Nitric Acid. Transfer 0.5mL of the resulting solution to a 50mL beaker and add 3mL of purified water.

1.7.2. Dissolve 1g of sample in 5mL of purified water.

1.7.3. Transfer 0.5mL of the sample solution to the above 50mL beaker and mix. The color should change from light yellow to orange.

1.7.4. **TR3220 and TR4220 Specification: Passes Test**

1.8. IDENTIFICATION (IR)

1.8.1. Follow Spectrum Two UATR SOP to obtain the IR spectrum. Compare to a previously calibrated IR Reference Standard. A 0.95 correlation coefficient must be achieved in order to report as Passes Test.

1.8.2. **TR3220 and TR4220 Specification: Passes Test**

1.9. INSOLUBLE MATTER

1.9.1. Accurately weigh 20.0g of sample and transfer to a 600mL beaker.

1.9.2. Add 200mL of purified water and utilize a Teflon encapsulated magnetic stirring bar and electric stir plate to dissolve sample.

1.9.3. Heat to boiling and digest on a hot plate in a covered beaker for 1 hour.

Printed On:	19-Oct-2020 10:53:29 AM	Ledergerber, Alissa	: Printed By
Authored By:	Fisher, Nicole 24-Aug-2017 08:55:23 AM		
Approval:	Hamelburg, Crystal 24-Aug-2017 12:38:23 PM		
Notice:	CONFIDENTIAL: The information contained herein is the property of BioSpectra. The recipient is responsible for its safe-keeping and the prevention of unauthorized appropriation, use, disclosure and copying.		

1.9.4. Prepare a Gooch filtering crucible and 10-15µm filter by drying at 105°C ± 2°C for 1 hour. Allow to cool in ambient air for 15 minutes and weigh.
1.9.5. Filter sample solution through conditioned filtering crucible and 10-15µm filter. Rinse thoroughly with at least 3 crucible volumes of hot purified water.
1.9.6. Dry the crucible at 105° ± 2°C for 1 hour.
1.9.7. Cool in ambient air for 15 minutes and reweigh.
1.9.8. Calculate the % Insoluble Matter as follows:

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

1.9.9. **TR3220 and TR4220 Specification: 0.005% maximum**

1.10. **WATER (by Karl Fischer Titration)**

1.10.1. Perform a standardization of the titrant (Composite 5) as per Standardization of Titrants utilizing the Hydranal Water Standard.
1.10.2. Grind the sample in a dry mortar into a fine powder utilizing a pestle.
1.10.3. Immediately weigh ~0.800g of sample into the glass weighing spoon and tare it.
1.10.4. Transfer the sample to the KF vessel by removing the rubber septum and adding the sample into the titration vessel.
1.10.4.1. Do not leave the rubber septum open for long periods of time as this will allow moisture to enter the titration vessel.
1.10.5. 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.
1.10.6. Check to make sure there is no residual sample stuck to the sides of the titration vessel.
1.10.6.1. If there is any sample stuck to the side, stop the stir bead from spinning before swirling the vessel to rinse the sides.
1.10.7. Once the method begins, check to ensure the sample is fully dissolved before the titration begins (i.e. before the stir command completes).
1.10.8. The moisture content will then be determined by the Metrohm Auto Titrando 907.

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

1.10.9. **TR3220 and TR4220 Specification: 2.0% maximum**

1.11. **LOSS ON DRYING**

1.11.1. Dry an LOD vial in the oven at 105 ± 2˚C for 30 minutes.
1.11.2. Cool for 15 minutes in a desiccator, weigh the LOD vial, and record results.
1.11.3. If the substance to be tested is in the form of large crystals, reduce the particle size to about 2mm by quickly crushing.
1.11.4. Transfer approximately 1- 2g 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.
1.11.5. Place the LOD vial containing the sample into the oven and dry at 105°C ± 2°C for 3 hours.
1.11.6. Remove LOD vial from the oven and allow to cool in the desiccator for 15 minutes.
1.11.7. Reweigh the LOD vial and sample and retain the dried sample to perform the Assays.
1.11.8. Calculate the %LOD as follows:

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

1.11.9. **TR3220 and TR4220 Specification: 1.0% max.**

1.12. **MELTING RANGE**

1.12.1. Reduce the sample to a fine powder in a mortar and pestle and dry sample in the desiccator for a minimum of 16 hours.
1.12.2. Fill capillary tube with an adequate amount of sample to be tested. Use the capillary packing rod to push the sample down until the sample packs into the bottom of the tube. The final height of the packed sample in the capillary should be between 2.5-3.0mm.
1.12.3. Place the capillary tube containing the sample in the melting point apparatus.
1.12.4. Do not force the capillary tube(s) into the apparatus; they should drop right in.
1.12.5. Select the Tris method on the home screen.
1.12.6. The instrument will beep once the initial temperature is reached.
1.12.7. Press the start button.
1.12.8. Results will print upon completion.
1.12.9. Up to four Tris samples may be ran at the same time.
1.12.10. **TR3220 and TR4220 Specification: 168-172°C**

1.13. **pH of a 5% SOLUTION**

1.13.1. Accurately weigh 2.5g of sample. Transfer to a 50mL graduated cylinder.
1.13.2. Q.S. to 50mL with purified water. Cover with parafilm and mix by inversion.
1.13.3. Calibrate the pH meter prior to pH measurement.
1.13.3.1. Follow the Metrohm Titrando 907 Auto-Titrator SOP or XL200 pH/mV/Conductivity S/N XL94102869 Instrument SOP or Metrohm Titrando 907 Auto-Titrator SOP to determine the pH of the sample.
1.13.4. **TR3220 and TR4220 Specification: 10.0-11.5**

1.14. **RESIDUE ON IGNITION/SULFATED ASH**

1.14.1. Turn on muffle furnace and allow the temperature to stabilize at 800 degrees Celsius. Follow muffle furnace calibration procedure operation of the furnace.
1.14.2. Utilize the10 inch forceps to insert and remove the crucible in the furnace.
1.14.3. Ignite quartz crucible at 800 ± 50 degrees Celsius for 30 minutes. Cool in a desiccator for one hour and thirty minutes and weigh.
1.14.4. Weigh 1.0 g sample in the previously ignited quartz crucible. Moisten the sample with 0.2 mL of sulfuric acid.
1.14.5. Volatilize the sample with a Bunsen burner. Keep the sample an appropriate distance from the flame, so that the sample does not boil over and sample is not lost.
1.14.5.1. The rate of heating should be such that from ½ to 1 hour is required to volatilize the sample.
1.14.5.2. Continue using the Bunsen burner to heat the sample until all the excess sulfuric acid has been volatilized.
1.14.6. Ignite in the muffle furnace at 800 ± 50 degrees Celsius for 15 minutes or until all carbon has been removed.
1.14.7. Cool in a desiccator for an hour and a half and reweigh.

Printed On:	19-Oct-2020 10:53:29 AM	Ledergerber, Alissa	: Printed By
Authored By:	Fisher, Nicole	24-Aug-2017 08:55:23 AM	
Approval:	Hamelburg, Crystal	24-Aug-2017 12:38:23 PM	
Notice:	CONFIDENTIAL: The information contained herein is the property of BioSpectra. The recipient is responsible for its safe-keeping and the prevention of unauthorized appropriation, use, disclosure and copying.		

1.14.8. The weight of residue should not exceed 0.001 grams (0.1 %). Calculate the %ROI as follows:

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

1.14.9. **TR3220 and TR4220 Specification: 0.1% maximum**

1.15. **TRACE METALS**

1.15.1. Use the NexION 350X ICP MS SOP for the Operation and preparation of all samples and standards.

1.15.2. General Sample Preparation:

1.15.2.1. Weigh 0.10 g of sample on an analytical balance. Add 100µL of Environmental Standard 6 and Q.S. to 50.0g with 1% Nitric Acid in a 50mL pre-rinsed centrifuge tube.

1.15.3. **TR3220 and TR4220 Specification:**

Arsenic (As)- 5 ppm maximum
Calcium (Ca)- 5 ppm maximum
Copper (Cu)- 5 ppm maximum
Iron (Fe)- 5 ppm maximum
Lead (Pb)- 5 ppm maximum
Magnesium (Mg)- 5 ppm maximum

Printed On:	19-Oct-2020 10:53:29 AM	Ledergerber, Alissa	: Printed By
Authored By:	Fisher, Nicole 24-Aug-2017 08:55:23 AM		
Approval:	Hamelburg, Crystal 24-Aug-2017 12:38:23 PM		
Notice:	CONFIDENTIAL: The information contained herein is the property of BioSpectra. The recipient is responsible for its safe-keeping and the prevention of unauthorized appropriation, use, disclosure and copying.		