BIOSPECTRA
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INTRODUCTION: The following analyses are conducted for Sucrose, Beet Derived, Bio Excipient Grade product code SB3250, in accordance with the Sucrose Testing Methods DCN: 18-002551 v.2.2 and Certificate of Analysis DCN: 18-002571 v.2.1. Specific details for the procedures were also obtained from Spectrum Two UATR SOP DCN: 16-001330 v.3.0 and NexION 350X ICP-MS SOP DCN: 16-001923 v.2.0.

1. SOLUTION S :

1.1. Dissolve 50.0g of sample in purified water and dilute to 100mL with the same solvent.
  1.1.1. Note: Specification of Solution S is clear. A liquid is considered clear if its clarity is the same as that of purified water or of the solvent used, or if its opalescence is not more pronounced than that of reference suspension I when examined.
1.2. Solution S may be saved to use in the following analyses: Appearance of Solution, Clarity of Solution, Dextrins, Reducing Sugars, Identification C.

2. APPEARANCE White to Off-White Crystalline Powder or Crystals:

2.1. Place a suitable amount of sample in a clean, dry glass beaker.
2.2. In an area with sufficient lighting, view the sample from all sides.
2.3. The sample should be white to off white in color, colorless crystals or white crystalline powder and free of foreign matter.
2.4. If the sample does not conform to these specifications, notify the QC Manager immediately.

3. APPEARANCE OF SOLUTION(NF)(EP) Passes Test (NF); Clear (EP):

3.1. Clear *(2.2.1.)* Turbidimetry
  3.1.1. Rinse the sample bottle with the sample solution twice.
  3.1.2. Fill sample bottle with the sample Solution S to the white line.
  3.1.3. Coat outside of bottle with a thin coat of silicon oil.
  3.1.4. Remove any air bubbles from the solution by using a syringe.
  3.1.5. Allow the sample to sit capped for 2-3 minutes.
  3.1.6. Follow the appropriate SOP as follows:
    3.1.6.1. Stroudsburg- Measure and record the turbidity of the sample according to Portable Turbidimeter Operation and Calibration.
    3.1.6.2. Bangor- Measure and record the turbidity of the sample according to Bangor Portable Turbidimeter SOP.
  3.1.7. The sample solution must be ≤ 3 NTU.
3.2. Colorless (*2.2.2, Method II*)
  3.2.1. Add 10mL of Solution S into a Nessler Color Comparison Tube.
  3.2.2. Add 10 mL of *USP Purified Water* into a second Nessler Color Comparison Tube.
  3.2.3. Compare the colors in sufficient lighting, viewing vertically against a white background.

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3.2.4. In order for the sample solution to be colorless, it must have the appearance of *USP Purified Water*.

4. COLOR VALUE ≤ 45:

4.1. Part 1:

4.1.1. Prepare two Sample Solutions in separate beakers by dissolving 50.0 grams of sample in 50.0 mL of purified water in each.

4.1.1.1. Mix, filter (0.45µm pores) and degas.

4.1.2. Refer to the Lambda 25 UV/Vis Operation and Calibration SOP to measure the absorbance of each sample solution at 420nm, using a cell length of 10cm.

4.1.3. In order for results to be considered suitable for report the absolute difference between two results is NMT 3.

4.2. Part 2:

4.2.1. Measure the refractive index of one of the sample solutions. Refer to Fisherbrand™ Handheld Digital Brix/RI Refractometer SOP for operation.

4.2.2. Calculate the Concentration of the solution (g/mL) using the refractive index of the solution and Table 1 below:

Table 1:

n^{20}_{D}	c (g/mL)
1.4138	0.570
1.4159	0.585
1.4179	0.600
1.4200	0.615
1.4221	0.630
1.4243	0.645
1.4264	0.661

4.3. Calculate the color value using the following equation:

4.3.1. Result = (A x 1000) / (b x c)

4.3.1.1. A = absorbance measured at 420 nm

4.3.1.2. b = cell path length (cm)

4.3.1.3. c = concentration of the solution (g/mL)

5. CONDUCTIVITY ≤ 35 µS/Cm:

5.1. *Sample Solution (C1):*

5.1.1. Dissolve 15.65g of sample in recently boiled and cool purified water, dilute to a final volume of 50mL for a final sample solution concentration of 313mg/mL.

5.2. *Sample Solution (C2):*

5.2.1. Freshly boiled and cooled purified water.

5.3. Calibrate the conductivity meter prior to sample measurement with 25µS KCl standard.

5.3.1. Follow the appropriate SOP:

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

5.3.1.2. Bangor: XL200 pH/mV/Conductivity SOP

5.4. Rinse the electrode thoroughly with purified water that has been freshly boiled and cooled.

5.5. Measure the conductivity of the *Sample solution* (*C1*), while gently stirring with a magnetic stirrer. Readings must be stable within 1% over a period of 30 seconds.

5.6. Measure the conductivity of the water used for preparing the *Sample solution* (*C2*).

5.6.1. Calculate the conductivity of the *Sample solution* from the expression:

$$\text{Result} = C_1 - (0.35 \times C_2)$$

6. DEXTRINS **Passes Test:**

6.1. To 2 mL of the Solution S, add 8 mL of purified water.
6.2. Add 0.05 mL of dilute hydrochloric acid (73g/L of HCl).
6.3. Add 0.05 mL of 0.05M (0.1N) iodine.
6.4. To pass the solution must remain yellow.

7. ELEMENTAL IMPURITIES **Refer to Certificate of Analysis:**

7.1. NOTE: The USP General Chapter will be followed for <232>, <233> testing and ICHQ3D.
7.2. Refer to NexION 350X ICP-MS SOP for Instrument Set-Up and Use.
7.2.1. Sample Preparation:
7.2.2. General Notes:
7.2.2.1. Before use, all plasticware that is not rated as "metal-free", should first be rinsed with purified water, rinsed with 15% Nitric Acid, and then rinsed again with purified water. Plasticware rated as "metal-free" may be used as-is.
7.2.2.2. Glass should be avoided as it has high potential for metal and mineral contaminations.
7.2.2.3. Standard and sample solutions should be prepared in 50mL centrifuge tubes.
7.2.3. 1% Nitric Acid
7.2.3.1. Measure 14.5 mL of Trace Metal Grade Nitric Acid and transfer to a rinsed plastic 1000 mL volumetric flask. QS to 1000 mL with purified water.
7.2.4. 15% Nitric Acid
7.2.4.1. Dilute approximately 110 mL of Trace Metal Grade Nitric Acid to 500 mL with purified water.
7.2.4.2. The solution is only used to rinse glassware and plasticware.
7.2.5. BioSpectra Daily Method:
7.2.5.1. Sample Solutions:
7.2.5.1.1. Weigh 0.10g of sample on an analytical balance. Add 100 uL of Environmental Standard Mix 6 and QS to 50.0 with 1% Nitric Acid.
7.2.5.2. Standard Curve Preparation:
7.2.5.2.1. 2 ppm Stock
7.2.5.2.1.1. Weigh 1.00 g of Instrument Calibration Standard 2 and QS to 50.0 g with 1% Nitric Acid.
7.2.5.2.2. 100 ppb Stock
7.2.5.2.2.1. Weigh 2.50 g of 2 ppm Stock and QS to 50.0 with 1% Nitric Acid.
7.2.5.2.3. Blank
7.2.5.2.3.1. Pipette 100 uL of Environmental Standard Mix 6 into the centrifuge tube. QS to 50.0 g with 1% Nitric Acid.
7.2.5.2.4. 1 ppb Standard
7.2.5.2.4.1. Pipette 0.50 mL of the 100 ppb Stock, add 100 uL of Environmental Standard Mix 6 and QS to 50.0 g with 1% Nitric Acid.

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7.2.5.2.5. 2 ppb Standard (also used as a Continuing Check Verification Sample (CCV))
7.2.5.2.5.1. Pipette 1.00 mL of the 100 ppb Stock, add 100 uL of Environmental Standard Mix 6 and QS to 50.0 g with 1% Nitric Acid.
7.2.5.2.6. 4 ppb Standard
7.2.5.2.6.1. Pipette 2.00 mL of the 100 ppb Stock, add 100 uL of Environmental Standard Mix 6 and QS to 50.0 g with 1% Nitric Acid.
7.2.5.2.7. 6 ppb Standard
7.2.5.2.7.1. Pipette 3.00 mL of the 100 ppb Stock, add 100 uL of Environmental Standard Mix 6 and QS to 50.0g with 1% Nitric Acid.

8. ENDOTOXINS ≤ 0.6 EU/g:

8.1. Accurately weigh 0.30g of sample.
8.2. Hygienically transfer to a sterile tube with a capacity of greater than 10mL.
8.3. Dilute to 10mL with LAL reagent water, dissolve and mix thoroughly.
8.4. Refer to Endosafe nexgen-PTS Endotoxin Reader SOP for instrument operation and sample analysis.

9. IDENTIFICATION A (IR) Passes Test:

9.1. For UATR analysis, follow Spectrum Two UATR SOP for Instrument Set-Up and Use.
9.1.1. Perform a background scan prior to use each day and after every ten samples.
9.1.2. Each analyst must run a Reference Standard prior to analyzing a product. A Reference Standard may be compared to multiple lots of the corresponding product on that day.
9.1.3. Enter the Lot Number, Expiration Date, Date of Analysis, and Analyst Initials in the Sample ID.
9.1.4. Place the Sample on the UATR crystal using a static free scoop.
9.1.5. Align the swinging arm with the crystal and apply force by turning the green arm clockwise.
9.1.6. Press “Scan” on the top Toolbar. The program will preview the sample. Turn the green arm until the Force Gauge is approximately 125, or until the noise has subsided.
9.1.7. Once the Force Gauge is adjusted, press “Scan”.
9.1.8. Once the scan is complete, release the swinging arm by turning it counterclockwise.
9.1.9. Clean the UATR crystal and the swinging arm with methanol and a Kim Wipe.
9.1.10. If the correlation is above 0.95. the comparison will be reported with Pass as the result.

10. IDENTIFICATION B Passes Test:

10.1. Primary method: HPLC Identification
Refer to DCN: 20-003502 v.1.1 Analytical Method Validation Protocol: Sucrose Chromatographic Identity for instrumental parameters and sample analysis.
10.2. Alternate Method:
10.2.1. Thin-Layer Chromatography:

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10.2.1.1. Test Solution: dissolve 10mg of sample in a mixture of 8 ml of purified water and 12 ml of methanol r.
Reference Solution a: dissolve 10mg of sucrose crs in a mixture of 8 ml of purified water and 12 ml of methanol r.
Reference Solution b: dissolve 10mg of fructose r, 10mg of glucose r, 10mg of lactose monohydrate r and 10mg of sucrose r in a mixture of 8 ml of purified water and 12 ml of methanol r.
10.2.1.2. Plate: TLC silica gel G plate R.
10.2.1.3. Mobile Phase: cold saturated boric acid solution R, 60 percent v/v solution of glacial acetic acid R, anhydrous ethanol R, acetone R, ethyl acetate R
10.2.1.4. Application: 2µl
10.2.1.5. Development: in an unsaturated tank, over ¾ of the plate.
10.2.1.6. Drying: in a current of warm air.
10.2.1.7. Detection: spray with a solution of 0.5g of thymol r in a mixture of 5ml of sulfuric acid r and 95ml of ethanol (96% per cent) r. heat the plate at 130°c for 10 min.
10.2.1.8. System Suitability: the chromatogram obtained with reference solution (b) shows 4 clearly separated spots.
10.2.1.9. Results: the principal spot in the chromatogram obtained with the test solution is similar in position, colour, and size to the principal spot in the chromatogram obtained with reference solution (a).

11. IDENTIFICATION C — Passes Test:

11.1. Dilute 1mL of Solution S to 100mL with purified water. To 5mL of the solution add 0.15mL of freshly prepared copper sulfate solution R and 2mL of freshly prepared dilute sodium hydroxide solution R. The solution is blue and clear and remains so after boiling. To the hot solution add 4mL of dilute hydrochloric acid R and boil for 1 min. Add 4mL of dilute sodium hydroxide solution R. An orange precipitate is formed immediately.

12. LOSS ON DRYING — ≤ 0.1%:

12.1. Dry an LOD vial in the oven at 105 ± 2˚C for 30 minutes.
12.2. Cool for 15 minutes in a desiccator, weigh the LOD vial, and record results.
12.3. If the substance to be tested is in the form of large crystals, reduce the particle size by quickly crushing before weighing.
12.4. Transfer 2.000 grams of the sample to the LOD vial, and accurately weigh the vial and contents. By gentle, sidewise shaking, distribute the sample as evenly as possible in the LOD vial.
12.5. Place the LOD vial containing the sample into the oven and dry at 105°C ± 2°C for 3 hours.
12.6. Remove LOD vial from the oven and allow it to cool in a desiccator for 15 minutes.
12.7. Reweigh the LOD vial and sample.
12.8. Calculate the %LOD as follows:

$$\%\boldsymbol{LOD} = \frac{[\textbf{initial sample weight (g)} - \textbf{final sample weight (g)}]}{\textbf{initial sample weight (g)}} \times 100$$

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13. MICROBIAL CONTENT **Refer to Certificate of Analysis:**

13.1. Microbial analysis will be performed by an approved Outside Testing Laboratory.

13.2. Analyses:

13.2.1. Total Aerobic Microbial Count (TAMC)

13.2.2. Total Yeasts and Molds Count (TYMC)

13.2.3. Absence of *Salmonella species, Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa*

14. REDUCING SUGARS **Passes Test:**

14.1. Place 5 mL of Solution S in a test tube.

14.2. Add 5 mL of purified water.

14.3. Add 1.0 mL of 1M sodium hydroxide.

14.4. Add 1.0 mL of methylene blue solution (1g/L).

14.4.1. Prepare by dissolving 0.1g of methylene blue to a final volume 100mL with purified water.

14.5. Mix and place the test tube in a water bath and allow to stand for 2 minutes using a timer.

14.6. Removed from the bath and examine the solution immediately.

14.7. To pass, the blue color must not disappear completely, ignoring any blue color at the air/solution interface.

15. RESIDUAL SOLVENTS **Refer to Certificate of Analysis:**

15.1. Residual Solvents analysis may be performed by an outside testing laboratory, or on a validated in-house method.

16. SPECIFIC (OPTICAL) ROTATION @ 20°C **+66.3° to +67.0°:**

16.1. Prepare a sample solution (260mg/mL) by weighing 26.0 grams of sample on an analytical balance and dissolving to 100 mL with purified water.

16.2. Analyze the sample following the operation portion of the MCP 300 Polarimeter SOP.

17. SULFITES **≤ 10 ppm:**

17.1. Solution Preparation

17.1.1. Sample Solution Preparation (400 mg/mL): Accurately weigh 4.00 grams of sample. Quantitatively transfer aliquot to a 10 mL volumetric flask and dilute to volume with purified water.

17.1.2. Sulfite Standard Solution Preparation (80 ppm SO_2): Accurately weigh 0.0158 grams of anhydrous sodium sulfite. Quantitatively transfer aliquot to a 100 mL volumetric flask and dilute to volume with purified water.

17.1.3. 4 ppm Controlled Reference Solution Preparation: Accurately weigh 4.0 grams of sample. Quantitatively transfer aliquot to a 10 mL volumetric flask and add approximately 8 mL of purified water. Add 0.5 mL of Sulfite Standard Solution and dilute to volume with purified water.

17.1.4. Test Kit Solution 1: Bottle 1 of Test Kit is used undiluted as Test Kit Solution 1.

17.1.5. Test Kit Solution 2: Dissolve 1 tablet of bottle 2 per mL of Test Kit Solution 1. One mL of solution is needed for each preparation. Prepare as needed.

17.1.6. Test Kit Solution 3: Bottle 3 of Test Kit is used undiluted as Test Kit Solution 3.

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17.1.7. Test Kit Solution 4: Bottle 4 of Test Kit is used undiluted as Test Kit Solution 4.

17.2. Sulfite Measurement

17.2.1. Measure the absorbance of each solution against a blank of purified water.

17.2.2. System Blank Measurement

17.2.2.1. Pipette 1.000 mL of Test Kit Solution 2 into a cuvette.

17.2.2.2. Add 2.000 mL of water.

17.2.2.3. Add 0.010 mL of Test Kit Solution 3.

17.2.2.4. Thoroughly mix.

17.2.2.5. Using the Lambda 25 UV/Vis, read absorbance at 340 nm after approximately 5 minutes. The result is A_1.

17.2.2.6. Add 0.050 mL Test Kit Solution 4.

17.2.2.7. Thoroughly mix.

17.2.2.8. Using the Lambda 25 UV/Vis, read absorbance at 340 nm after the completion of the reaction (about 30 minutes). The result will be A_2.

17.2.2.8.1. If the reaction has not stopped after 30 minutes, continue to read the absorbance at 5 minute intervals until the absorbance decreases constantly over 5 minutes.

17.2.2.8.2. If the absorbance at A_2 decreases constantly, extrapolate the absorbance to the time of the addition of Test Kit Solution 4.

17.2.3. Sample Measurement

17.2.3.1. Pipette 1.000 mL of Test Kit Solution 2 into a cuvette.

17.2.3.2. Add 2.000 mL Sample Solution.

17.2.3.3. Add 0.010 mL of Test Kit Solution 3.

17.2.3.4. Thoroughly mix.

17.2.3.5. Using the Lambda 25 UV/Vis, read absorbance at 340 nm after approximately 5 minutes. The result will be A_1.

17.2.3.6. Add 0.050 mL Test Kit Solution 4.

17.2.3.7. Thoroughly mix.

17.2.3.8. Using the Lambda 25 UV/Vis, read absorbance at 340 nm after the completion of the reaction (about 30 minutes). The result is A_2.

17.2.3.8.1. If the reaction has not stopped after 30 minutes, continue to read the absorbance at 5 minute intervals until the absorbance decreases constantly over 5 minutes.

17.2.3.8.2. If the absorbance at A_2 decreases constantly, extrapolate the absorbance to the time of the addition of Test Kit Solution 4.

17.2.4. 4 ppm Controlled Reference Solution (CRS) Measurement

17.2.4.1. Follow steps 6.20.2.3 - 6.20.2.3.8.2, adding 4 ppm Controlled Reference Solution instead of Sample Solution.

17.3. Calculations

17.3.1. Determine the absorbance difference (A_1-A_2) for all measured solutions.

17.3.1.1. If the absorbance difference of the sample is higher than 1.000 (measured at 340 nm), the concentration of sulfite in the sample solution is too high. The sample should be diluted and a corresponding dilution factor must be accounted for in the final calculation.

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17.3.2. Subtract the absorbance difference of the blank from the absorbance difference of the measured solution.

$$\Delta A = (A_1 - A_2)_{sample} - (A_1 - A_2)_{blank}$$

$$\Delta A = (A_1 - A_2)_{4ppm\ CRS} - (A_1 - A_2)_{blank}$$

17.4. Acceptance Criteria: The ΔA of the Sample Solution is NMT half the ΔA of the 4 ppm Controlled Reference Solution.

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