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# STABILITY INDICATING PROTOCOL: GALACTOSE

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# TABLE OF CONTENTS



| | | | |
|---|---|---|---|
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## 1. PURPOSE:

1.1. The purpose of this Protocol is to gather insight into which Quality Control (QC) analyses are stability indicating through assessment of results on chemically and physically stressed material.

1.2. To determine which tests are stability indicating for Galactose when the product is stressed in extreme conditions.

1.2.1. The first developmental lot of Galactose will be stressed in the following ways:

1.2.1.1. Temperature
1.2.1.2. Humidity
1.2.1.3. Light
1.2.1.4. pH
1.2.1.4.1. Acid
1.2.1.4.2. Base
1.2.1.5. Oxidative

1.2.2. The analyses to be performed after manipulation are as follows:

1.2.2.1. Appearance of Solution (NF)
1.2.2.2. Appearance and Color
1.2.2.3. Acidity/Alkalinity (NF)
1.2.2.4. Assay % w/w (NF)
1.2.2.5. Chromatographic Purity/ Related Substances (NF)
1.2.2.6. Identification A, B, C (NF)
1.2.2.7. Specific/Optical Rotation
1.2.2.8. Water (KF)

1.2.3. The following tests will not be performed after material manipulation:

1.2.3.1. Microbial Enumeration Tests / Tests for Specified Microorganisms
1.2.3.2. Residual Solvents (Excluding Water, via Karl Fischer)
1.2.3.3. Heavy Metals /Elemental Impurities / Limit of Pb or Fe
1.2.3.4. Residue on Ignition /Sulfated Ash

1.2.4. After testing is complete, the results will be analyzed and stability indicating tests will be established in a Stability Indicating Report. The report will include a clear statement determining which analyses are considered to be stability indicating. The results will be integrated into the current stability program at BioSpectra to give the most pertinent information.

## 2. SCOPE:

2.1 This Protocol applies to the stability testing of BioSpectra manufactured Galactose.

## 3. RESPONSIBILITIES:

3.1. The Executive Director of Quality Control is responsible for the control, implementation, training and maintenance of this procedure.

3.2. The QC Analysts are responsible for performing the testing stated in this Protocol and recording all results in current laboratory documentation.

3.3. The QC Analysts are responsible for utilizing a developmental lot for any new product as the stability indicating sample. If developmental lots are unavailable, the first lot from process validation will be used from the stability sample submitted by production during validation as per the Stability Indication Protocol.

3.4. The Executive Director of Quality or qualified personnel is responsible for completing the Stability Indicating Report at the conclusion of the stability indicating testing before Product

Validation samples are placed into Long Term or Accelerated Stability as per the Stability Indication Protocol.

3.5. Standard laboratory safety regulations apply. Before working with any chemical, read and understand the Safety Data Sheet (SDS).

## 4. REFERENCES:

4.1. Balance SOP, DCN: 16-000368
4.2. Blue M Convection Oven Operation and Calibration SOP, DCN: 16-000502
4.3. *Current USP/ NF*
4.4. Fisher Scientific Isotemp Water Bath Operation Calibration SOP, DCN: 16-001355
4.5. Laboratory Notebooks, DCN: 16-000482
4.6. Muffle Furnace SOP and Calibration, DCN:16-000364
4.7. NexION 350X ICP-MS SOP, DCN: 16-001923
4.8. Stability Indication Protocol, DCN: 16-001384
4.9. VWR Gravity Convection Oven Operation and Calibration (Model Number 414005-106), DCN: 16-001319
4.10. ICH Q1A

## 5. EQUIPMENT:

5.1 Analytical Balance
5.2 Blue M Oven, or equivalent
5.3 Calibrated Timer
5.4 Desiccator
5.5 Fisher Scientific Isotemp Water Bath
5.6 Hach Portable Turbidimeter
5.7 Lambda 25 UV/Vis Spectrophotometer
5.8 Lux Meter
5.9 MCP Polarimeter 300 or equivalent
5.10 Muffle Furnace
5.11 Perkin Elmer Spectrum Two UATR
5.12 Perkin Elmer Flexar HPLC or Equivalent
5.13 XL200 pH/Conductivity Meter or equivalent

## 6. PROCEDURE:

6.1 Stress Procedures:
- 6.1.1. Control:
  - 6.1.1.1. Unstressed Material
- 6.1.2. Thermal Stress:
  - 6.1.2.1. Transfer the crystal to a suitable tray and evenly spread to increase exposure area.
  - 6.1.2.2. Heat sample between 110-130°C for 12-24 hours to degrade.
  - 6.1.2.3. Freeze sample for 12-36 hours after heat exposure.
  - 6.1.2.4. Bring sample back to room temperature before analysis.
- 6.1.3. Acid Stress:
  - 6.1.3.1. Apply 2.5mL of hydrochloric acid per 100g of sample; mix the acidic mixture thoroughly in a suitable container.

6.1.3.2. Dry the sample using a well-ventilated tray and use a mortar and pestle to homogenize after drying. Note: HCl is very caustic and corrosive, use proper PPE while handling.

6.1.4. Basic Stress:

6.1.4.1. Apply 2.5mL of 50% sodium hydroxide per 100g of sample; mix the basic mixture thoroughly in a suitable container.

6.1.4.2. Dry the sample using a well-ventilated tray and use a mortar and pestle to homogenize after drying. Note: NaOH is very caustic, use proper PPE while handling.

6.1.5. Oxidative Stress:

6.1.5.1. Apply 2.5mL of 30% hydrogen peroxide per 100g of sample. Mix the mixture thoroughly and allow to react in an open container or vessel. Do not seal.

6.1.5.2. Once reaction (if any) has ceased, transfer material to a well-ventilated tray and use a mortar and pestle to homogenize after drying. Note: Hydrogen peroxide is very reactive, read and understand the SDS before use and understand proper disposal and hazards associated with strong oxidizers.

6.1.6. Humidity/Hydrolytic Stress:

6.1.6.1. Transfer material to a suitable tray and evenly spread to increase exposure area.

6.1.6.2. Contact Stability management to place the material, covered loosely, into accelerated stability chamber and record conditions.

6.1.6.3. Allow sample 24-48 hours exposure to accelerated conditions before removing for test.

6.1.7. Photolytic Stress:

Expose sample to approx. 1.2 million lux hours of light. Lux hours = (Average Lux Intensity * Hours of Exposure).

## 7. ANALYTICAL PROCEDURES:

7.1. **APPEARANCE AND COLOR** **White to off white crystalline powder:**

7.1.1. Observe a suitable amount of sample under diffuse daylight or well illuminated area and note the physical appearance and structure of the material as well as the material color.

7.2. **ACIDITY/ALKALINITY** **Passes Test:**

7.2.1. Sample solution:

7.2.1.1. Dissolve 10.0 g of Galactose, with heating at 50°C, in 40 mL of carbon dioxide-free water. Dilute with carbon dioxide-free water to 50 mL.

7.2.2. Analysis:

7.2.2.1. To 30 mL of the Sample solution add 0.3 mL of phenolphthalein TS.

7.2.2.1.1. If sample solution is colorless, titrate with 0.01 N sodium hydroxide to a pink color.

7.2.2.1.2. If sample solution is pink, titrate with 0.01N hydrochloric acid to a colorless endpoint.

7.2.3. Acceptance criteria:

7.2.3.1. NMT 1.5 mL of 0.01 N sodium hydroxide or 0.01N Hydrochloric Acid is required for neutralization.

7.3. **ASSAY** **98.0-102.0%:**

7.3.1. *Mobile phase*: 0.009 N sulfuric acid

7.3.2. *System suitability solution*:10 mg/mL of Galactose RS and 0.2 mg/mL each of Arabinose RS, galacturonic acid, Dextrose RS, and Anhydrous Lactose RS in *Mobile phase*

7.3.3. *Standard solution*:10 mg/mL of USP Galactose RS in *Mobile phase*

7.3.4. *Sample solution*:10 mg/mL of Galactose in *Mobile phase*
7.3.5. Chromatographic system
7.3.5.1. Mode: LC
7.3.5.2. Detector: Refractive index
7.3.5.3. Column: Two 7.8-mm × 30-cm columns in tandem; 9-µm packing L17
7.3.5.4. Temperatures
7.3.5.4.1. Column:35°
7.3.5.4.2. Detector:40°
7.3.5.5. Flow rate:0.25 mL/min
7.3.5.6. Injection volume:25 µL
7.3.5.7. Run time:70 min
7.3.6. System suitability
7.3.6.1. Samples: System suitability solution and Standard solution
7.3.6.1.1. [Note—The relative retention times for lactose, galacturonic acid, dextrose, galactose, and arabinose are listed in Table 1.]
7.3.6.2. Suitability requirements
7.3.6.2.1. Resolution: NLT 3.0 between the lactose and galacturonic acid peaks; NLT 1.5 between the galacturonic acid and dextrose peaks; NLT 2.0 between the dextrose and galactose peaks; NLT 3.0 between the galactose and arabinose peaks, System suitability solution
7.3.6.2.2. Relative standard deviation: NMT 1.0%, Standard solution
7.3.7. Analysis
7.3.7.1. Samples: *Standard solution* and *Sample solution*
7.3.8. Calculate the percentage of galactose in the portion of Galactose taken:
7.3.8.1. Result = $(r_U/r_S) \times (C_S/C_U) \times 100$
7.3.8.2. Where:

$r_U$ = peak area of galactose from the Sample solution
$r_S$ = peak area of galactose from the Standard solution
$C_S$ = concentration of USP Galactose RS in the Standard solution (mg/mL)
$C_U$ = concentration of Galactose in the Sample solution (mg/mL)

7.3.9. Acceptance criteria:98.0%–102.0% on the anhydrous basis

7.4. **APPEARANCE OF SOLUTION** **Refer to Summary Sheet:**

7.4.1. Sample solution:
7.4.1.1. Dissolve, with heating at 50°C, 10.0 g of Galactose in 50 mL of carbon dioxide-free water.
7.4.2. Control solution:
7.4.2.1. Prepare immediately before use by mixing 3.0 mL of ferric chloride CS, 3.0 mL of cobaltous chloride CS, and 2.4 mL of cupric sulfate CS with dilute hydrochloric acid (10 mg/mL) to make 10 mL, and diluting 1.5 mL of this solution with the dilute hydrochloric acid to 100 mL.
7.4.3. Analysis:
7.4.3.1. Compare by viewing the Sample solution and the Control solution downward in matched color-comparison tubes against a white surface.
7.4.4. Acceptance criteria:
7.4.4.1. The Sample solution is not more intensely colored than the Control solution.

7.5. **IDENTIFICATION TEST A** **Conforms to Standard:**

7.5.1. Follow Spectrum Two UATR SOP for sample preparation and analysis.

7.6. **IDENTIFICATION TEST B** **Corresponds to Standard:**

7.6.1. Refer to Assay for method of analysis.

7.6.2. The retention time of the largest peak in the sample solution corresponds to the largest peak in the standard solution for Assay.

7.7. **IDENTIFICATION TEST C** **Passes Test:**

7.7.1. Sample solution:10 mg/mL

7.7.2. Analysis: To 10 mL of the Sample solution add 3 mL of alkaline cupric tartrate TS and heat.

7.7.3. Acceptance criteria: An orange or red precipitate is formed.

7.8. **RELATED SUBSTANCES** **Refer to Summary Sheet:**

7.8.1. *Mobile phase, System suitability solution, Sample solution*, and Chromatographic system: Proceed as directed in the Assay.

7.8.2. *Sensitivity solution*:5 µg/mL each of Arabinose RS, galacturonic acid, Dextrose RS, and Anhydrous Lactose RS in Mobile phase.

7.8.3. System suitability

7.8.3.1. Samples: *System suitability solution* and *Sensitivity solution*

7.8.3.2. Suitability requirements:

*7.8.3.2.1.* Resolution: NLT 3.0 between the lactose and galacturonic acid peaks; NLT 1.5 between the galacturonic acid and dextrose peaks; NLT 2.0 between the dextrose and galactose peaks; NLT 3.0 between the galactose and arabinose peaks, *System suitability solution*

7.8.3.2.2. Signal-to-noise ratio: NLT 10 for the lactose, galacturonic acid, dextrose, and arabinose peaks, *Sensitivity solution*

7.8.4. Analysis

7.8.4.1. Sample: *Sample solution*

7.8.4.2. Record the chromatograms and measure the area response of each peak in the chromatogram of the Sample solution. Disregard any peak due to the solvent and the peak at the relative retention time of approximately 0.64.

7.8.5. Calculate the percentage of each individual impurity in the portion of Galactose taken:

$$\text{Result} = r_U/(r_S \times F) \times 100$$

Where:

$r_U$ = peak area of each individual impurity from the Sample solution

$r_S$ = peak area of galactose from the Sample solution

F = relative response factor (see Table 1)

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**Table 1**

| Name | Relative Retention Time | Relative Response Factor | Acceptance Criteria, NMT (%) |
|---|---|---|---|
| Lactose and 1,6-galactosylgalactose | 0.79 | 0.95 | 0.6 |
| Galacturonic acid | 0.89 | 0.88 | 0.6 |
| Dextrose | 0.93 | 0.99 | 0.6 |
| Tagatose | 0.96 | 0.96 | 0.6 |
| Dulcitol | 1.06 | 0.96 | 0.6 |
| Arabinose | 1.10 | 0.95 | 0.6 |
| Any unspecified impurity | — | 1.0 | 0.2 |
| Total impurities | — | — | 1.0▲ (NF 1-Aug-2020) |

7.9. **SPECIFIC ROTATION/OPTICAL ROTATION +78.0° to +81.5@ 20°C:**

7.9.1. Sample solution: Transfer 10.0 g to a 100-mL volumetric flask, and dissolve in 80 mL of water. Add 0.2 mL of ammonia TS, allow to stand for 30 min, then dilute with water to volume.

7.9.2. Analysis: Perform at 20°.

7.9.3. Acceptance criteria:+78.0°C to +81.5°C

7.10. **WATER (By Karl Fischer Titration) 1.0% Max:**

7.10.1. Standardize Composite 5:

7.10.1.1. Standardize Karl Fischer titrant (Composite 5) using Standardization of Titrants, DCN: 16-000513 as guidance as well as Metrohm instrument operation manual (electronic or print).

7.10.2. Galactose Test Procedure:

7.10.2.1. Finely grind the sample with a mortar and pestle, this is an important step to make water trapped in larger crystals available for titration.

7.10.2.2. Weigh 1.0g of sample into a glass weighing spoon and tare the balance.

7.10.2.3. Transfer the sample to the Karl Fischer vessel by removing the rubber septum and adding the sample into the titration vessel.

7.10.2.4. Do not leave the rubber septum open for longer than 20 seconds as this will allow moisture to enter the titration vessel.

7.10.2.5. Return the weighing spoon to the balance, making sure not to lose any sample that was left behind. Once the weight stabilizes, record the weight in the Tiamo Software.

7.10.2.6. Galactose may not fully dissolve in the 50/50 Methanol/Formamide mix. Ensure that all sample that was added to the KF vessel is suspended in the solution.

7.10.2.7. The moisture content will then be determined by the Metrohm Titrando 907.

$$Water = \frac{(mL\ of\ Composite\ 5)(\frac{mg}{mL}\ of\ Composite\ 5)(0.1)}{Sample\ Weight\ (g)}$$

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