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

# D-GALACTOSE TESTING METHODS

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## 1. PURPOSE:

1.1. To provide the Laboratory personnel with procedures for analyzing all D-Galactose samples.

## 2. SCOPE:

2.1. Applies to the testing of D-Galactose in the Laboratory at the Bangor, PA location. Methods include testing for all types of D-Galactose sold by BioSpectra; only the specific tests required for the requested type must be tested.

## 3. RESPONSIBILITIES:

3.1. The Laboratory Manager, or qualified designee, is responsible for control, training, maintenance, and implementation of this procedure.
3.2. The Laboratory Technicians are responsible for compliance with the terms of this procedure. This includes notifying the Laboratory Manager, or qualified designee, if any analyses fail to meet their respective specifications.

## 4. EQUIPMENT:

4.1. Analytical Balance
4.2. Calibrated Oven
4.3. Endosafe Nexgen PTS reader, or equivalent
4.4. Lambda 25 UV/Vis Spectrophotometer, or equivalent
4.5. Metrohm 907 Titrando Auto-Titrator.
4.6. Muffle Furnace
4.7. Perkin Elmer NexION 350X ICP-MS
4.8. Perkin Elmer Spectrum Two UATR
4.9. Waters Alliance HPLC equipped with RI detector and two 7.8mm x 30-cm columns in tandem, 9-µm packing L17 or equivalent.
4.10. XL200 pH/Conductivity Meter or equivalent.
4.11. MCP 5300 Polarimeter
4.12. MCP 300 Polarimeter
4.13. OPI-180 OD Handheld Colorimeter SOP

## 5. REAGENTS:

5.1. **0.01-1.0EU/mL Endotoxin Cartridges:** Purchased Commercially.
5.2. **0.01N Sodium Hydroxide:** Transfer 10mL of 1N Sodium Hydroxide to a 1000 mL volumetric flask and dilute to volume.
5.3. **Alkaline cupric tartrate TS (Fehling's Solution):**
5.3.1. **The Alkaline Tartrate Solution (B):** Dissolve 173 g of crystallized potassium sodium tartrate and 50 g of sodium hydroxide in water to make 500 mL. Store this solution in small, alkali-resistant containers.
5.3.2. **The Copper Solution (A):** Dissolve 34.66 g of carefully selected, small crystals of cupric sulfate, showing no trace of efflorescence of adhering moisture, in water to make 500 mL.Store this solution in small, tight containers.
5.3.3. For use, mix exactly equal volumes of Solutions A and B at the time required.
5.4. **Ammonia TS (9.5-10.5%):** Prepare by diluting 350 mL of Ammonia 29% (purchased commercially) with water to make 1000 mL.
5.5. **Anhydrous Lactose RS:** Purchased Commercially.
5.6. **Arabinose RS:** Purchased Commercially.
5.7. **Cobaltous Chloride CS:** Purchased Commercially.
5.8. **Composite 5:** Purchased Commercially.
5.9. **Cupric Sulfate CS:** Purchased Commercially.

5.10. **Dextrose RS:** Purchased Commercially.
5.11. **Dilute Hydrochloric Acid (10mg/mL, 10%):** Prepare by mixing 226 mL of hydrochloric acid 37% (purchased commercially) with sufficient water dilute with water to make 1000 mL.
5.12. **Dilute Sulfuric Acid:** Transfer 57 mL of sulfuric acid 96% to a 1000 mL volumetric flask containing about 500 mL of water. Cool and dilute with water to volume.
5.13. **Ethanol:** Purchase commercially; denatured ethanol may be used based on local legislation.
5.14. **Ferric Chloride CS:** Purchased Commercially.
5.15. **Formamide:** Purchased Commercially.
5.16. **Galactose RS:** Purchased Commercially.
5.17. **2-Propanol:** Purchased Commercially.
5.18. **LAL Reagent Water:** Purchased Commercially.
5.19. **Methanol:** Purchased commercially.
5.20. **Methyl IsoButyl Ketone (MIBK):** Purchased Commercially.
5.21. **Phenolphthalein TS:** Dissolve 1 g of phenolphthalein in 100 mL of alcohol.
5.22. **Purified Water:** Type 1 Ultra-pure from Milli-Q system.
    5.22.1. Alternate Source: USP/EP WFI D10DI01 Located in Zone D.
5.23. **Sulfuric Acid 0.009N:** Dilute 9 mL of 1N Sulfuric Acid to 1000 mL with purified water.
    5.23.1. Other sulfuric acid concentrations may be used for preparation; include calculations during solution preparation.
5.24. **Sulfuric Acid 96%:** Purchased Commercially.

## 6. REFERENCES:

6.1. BSI-ATM-0069, Analytical Method: Determination of Elemental Impurities by ICP-MS in Galactose
6.2. BSI-ATM-0101, Residual Solvents Method for D-Galactose
6.3. BSI-ATM-0103, Galactose Assay and Related Substances via Liquid Chromatography with RI Detection
6.4. BSI-SOP-0019, Result Reporting
6.5. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration
6.6. BSI-SOP-0094, Muffle Furnace SOP and Calibration
6.7. BSI-SOP-0098, Balance SOP
6.8. BSI-SOP-0126, Laboratory Notebooks
6.9. BSI-SOP-0140, Standardization of Titrants
6.10. BSI-SOP-0143, Metrohm Titrando 907 Auto-Titrator SOP
6.11. BSI-SOP-0254, Spectrum Two UATR SOP
6.12. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP
6.13. BSI-SOP-0257, MCP 300 Polarimeter SOP
6.14. BSI-SOP-0303, NexION 350X ICP-MS SOP
6.15. BSI-SOP-0490, MCP 5300 Polarimeter SOP
6.16. BSI-SOP-0668, OPI-180 OD Handheld Colorimeter SOP
6.17. *Current ACS, Reagent Chemicals*
6.18. *Current EP*
6.19. *Current USP*

## 7. ANALYTICAL PROCEDURES:

# In-Process

7.1. **Rinse Water Endotoxin :**

7.1.1. Pipette 25 µL of sample into each well on a 0.01-1.0 EU/mL Endotoxin cartridge. Measure endotoxin levels using the Endosafe nexgen PTS reader, or equivalent. If the dilution required is greater than 1, LAL Reagent water will be used for dilution.

7.1.1.1. Refer to Endosafe nexgen-PTS Endotoxin Reader SOP for instrument analysis.

7.1.1.2. If system suitability does not meet requirements- check the pH of the sample to ensure that is within 6-8. If pH is not 6-8, adjust with LAL grade buffers, bases, and acids to pH of 6-8 and re-analyze.

7.2. **ML Endotoxin :**

7.2.1. Pipette 0.3 mL of sample into a sterile tube and dilute to 10 mL with LAL Reagent water. Mix thoroughly.

7.2.1.1. Measure the pH of the sample to ensure that the sample is neutral (pH 6-8). If pH is not 6-8, adjust with LAL grade buffers, bases, and/or acids to a pH of 6-8.

7.2.2. Pipette 25 µL of sample into each well on a 0.01-1.0EU/mL Endotoxin cartridge.

7.2.3. Measure endotoxin levels using the Endosafe nexgen PTS reader, or equivalent.

7.2.3.1. Refer to Endosafe nexgen-PTS Endotoxin Reader SOP for instrument analysis.

7.3. **ML Assay :**

7.3.1. Refer to the Assay Section 7.7 for analysis.

# Finished Goods

7.4. **ACIDITY or ALKALINITY :**

7.4.1. Sample solution: 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. [Note—Use this solution for the *Barium* test.]

7.4.2. Analysis: To 30 mL of the Sample solution add 0.3 mL of phenolphthalein TS, the solution should be clear.

7.4.3. Titrate with 0.01 N sodium hydroxide to a pink color.

7.4.4. Acceptance Criteria: The solution is colorless upon addition of phenolphthalein TS, and NMT 1.5 mL of 0.01N NaOH is required to reach the pink endpoint.

7.5. **APPEARANCE :**

7.5.1. Place a suitable amount of sample in a clean, dry glass beaker.

7.5.2. In an area with sufficient lighting, view the sample from all angles.

7.5.3. The sample should be white to almost white in color and characteristic of crystalline or finely granulated powder.

7.5.4. If the appearance and color result is unable to be definitively determined visually, the sample may be analyzed using the Colorimeter. Refer to BSI-SOP-0668, OPI-180 OD Handheld Colorimeter SOP.

7.5.5. If the sample does not conform to these specifications, notify the Laboratory Manager, or designee, immediately.

7.6. **APPEARANCE OF SOLUTION :**

7.6.1. Clear (2.2.1) Turbidimetry:

7.6.1.1. **Pre-Rinse all glassware prior to use.**

7.6.1.2. **Sample Solution**: Prepare sample solution by dissolving 10.0 g of sample while heating in a water bath at 50°C in 50 mL of purified water. Swirl sample to dissolve.

7.6.1.3. Rinse the sample bottle with the sample solution twice.

7.6.1.4. Fill the sample bottle with the solution to the white line.

7.6.1.5. Remove any air bubbles from the solution using a syringe or pipette.

7.6.1.6. Follow the appropriate SOP as follows:

7.6.1.6.1. Stroudsburg: Measure and record the turbidity of the sample according to the Portable Turbidimeter Operation and Calibration.

7.6.1.6.2. Bangor: Measure and record the turbidity of the sample according to the Portable Turbidimeter Operation and Calibration.

7.6.1.7. Turbidity of the sample solution should not exceed 3 NTU.

7.6.2. Color (2.2.2) Degree of Coloration of Liquids:

7.6.2.1. **Control Solution:** Prepare the control solution immediately before use by pipetting 3.0 mL of ferric chloride CS, 3.0 mL of cobaltous chloride CS, and 2.4 mL of cupric sulfate CS into a 10 mL volumetric flask. Q.S. to the line with dilute HCl(10mg/mL) to make 10 mL. Mix thoroughly. Dilute 1.5 mL of this solution with dilute HCl to 100 mL.

7.6.2.2. Procedure: Decant the sample solution and 50 mL of the control solution into separate Nessler tubes and view downward against a white surface. The sample solution is not more intense in color than the $B_8$ control solution in order to report passes test.

7.7. **ASSAY/RELATED SUBSTANCES :**

7.7.1. ***Note: This section will cover the results for the following:***

7.7.1.1. *Identification B*

7.7.2. Refer to BSI-ATM-0103 for instrument, sample preparation, and analysis.

7.7.2.1. Assay Reporting:

7.7.2.1.1. Finished Goods will be reported on the Anhydrous basis (Refer to BSI-CC25-0012. BSI-ATM-0103 updated to reflect this change and set effective 3/3/25).

7.7.2.1.2. Stability lots placed on the program in 2024 or prior will be reported as-is.

7.7.2.1.3. Stability lots placed on the program in 2025 to present will be reported on the Anhydrous basis.

7.8. **BARIUM :**

7.8.1. Prepare the *standard solution* by adding 6 mL of purified water to 5 mL of the sample solution prepared for the ***acidity test.***

7.8.2. Prepare the *sample solution* by adding 5 mL of water and 1 mL of 10% sulfuric acid to 5 mL of the *sample solution* prepared for the acidity test. Allow to stand for 1 hour.

7.8.3. Observe sample solution and standard solution against a dark surface. Any opalescence in the *sample solution* is not more intense than that in the *standard solution* in order to report as passes test.

7.9. **ENDOTOXINS :**

7.9.1. Accurately weigh 100 mg of sample into a sterile tube. Dilute with LAL reagent water to 10 mL, dissolve, and mix for a final concentration of 0.0100g/mL.

7.9.2. Measure the pH to ensure it is between 6-8 prior to loading sample onto 0.01-1.0EU/mL endotoxin cartridge. If pH is not 6-8, adjust with LAL grade buffers, bases, and/or acids to a pH of 6-8.

7.9.3. Refer to Endosafe nexgen-PTS Endotoxin Reader SOP for instrument analysis.

7.10. **IDENTIFICATION (A) :**

7.10.1. Follow Spectrum Two UATR SOP.

7.10.2. Note: Disregard any peaks at about 875 and 889$cm^{-1}$.

7.11. **IDENTIFICATION (B) :**

7.11.1. Primary method Refer to Assay section 7.7.

7.11.2. Acceptance Criteria: The retention time of the major peak of the Sample solution corresponds to the galactose peak of the Standard solution, as obtained in the Assay.

7.11.3. Alternate method:

7.11.3.1. Prepare Standard Solution A by weighing out 10 mg ± 0.5 mg of USP Galactose Reference Standard (RS) and dissolving in 20 mL of Solution A[1].

7.11.3.2. EP Standard Solution B:

7.11.3.2.1. Weigh out 10 mg ± 0.5 mg each of Galactose CRS, Glucose CRS, and Lactose Monohydrate CRS and dissolving in 20 mL Solution A[1].

7.11.3.3. USP Standard Solution B:

7.11.3.3.1. Weigh out 10 mg ± 0.5 mg each of Dextrose CRS, Galactose CRS, and Lactose Monohydrate CRS and dissolving in 20 mL Solution A[1].

7.11.3.4. Prepare sample Solution by weighing out 10 mg ± 0.5 mg of Galactose sample and dissolving in 20 mL of Solution A[1].

7.11.3.5. Draw a line parallel to and 1 cm above the bottom edge of the TLC plate. Pipette 2 µL of each standard solution and sample solution directly above the line with at least 10 mm between the centers of the spots. While the applied spots dry, decant the developing solvent[1] into developing chamber such that the depth is 3 – 5 mm from the bottom of the plate. Once the sample spots have dried, place the TLC plate into the developing chamber and close with glass lid.

7.11.3.6. After the solvent front has moved over 15 cm, remove the plate from the chamber. Dry the plate with warm air and then spray the entire plate with the Thymol spray reagent[3] **IN THE FUME HOOD! MOVE PLATE DIRECTLY INTO OVEN.** Dry for 10 minutes in oven at 130°C.

7.11.3.6.1. [1]60 parts methanol to 40 parts water, 60:40.

7.11.3.6.2. [2]85 parts propanol to 15 parts water, 85:15.

7.11.3.6.3. [3] Prepared by dissolving 0.5g of thymol in a mixture of 95 parts alcohol and 5 parts sulfuric acid (95:5).

7.11.3.7. Remove plate from oven and transfer to fume hood. There must be three clearly resolved spots in the chromatogram for Standard Solution B. The principal spot of the sample solution must be similar in size and intensity to that of Standard Solution A to pass. Use laboratory camera to document developed plate and save picture in respective TLC folder located in QC files.

7.12. **IDENTIFICATION (C) :**

7.12.1. Accurately weigh out 0.1 g of sample and dissolve in 10 mL of purified water.

7.12.2. Prepare cupric-tartrate solution R (Alkaline cupric sulfate TS) immediately before use by mixing equal parts of Fehling's solution A and Fehling's B.

7.12.3. Pipette 3.0 mL of cupric-tartrate solution prepared in preceding step into sample solution prepared in step 7.12.1.

7.12.4. Gently heat solution. An orange or red precipitate is formed for sample to pass test.

7.13. **LIMIT OF LEAD :**

7.13.1. Refer to Analytical Method: Determination of Elemental Impurities by ICP-MS in Galactose, DCN: BSI-ATM-0069 for sample preparation and analysis.

7.14. **MICROBIAL CONTENT :**

7.14.1. Package a microbial sample during composite of the finished good sample and send to MPL (Or other approved service provider) for TAMC/TYMC as well the following specified microorganisms:

7.14.1.1. *Escherichia coli*

7.14.1.2. *Pseudomonas aeruginosa*

7.14.1.3. *Salmonella*

7.14.1.4. *Staphylococcus aureus*

7.15. **OPTICAL ROTATION, SPECIFIC ROTATION :**

7.15.1. 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.

7.15.2. Refer to MCP 5300 Polarimeter, DCN: BSI-SOP-0490 for instrument analysis.

7.15.2.1. Select the following method in the software: BSI-Specific Rotation (USP/NF, 20°C).

7.15.2.2. The following information will be required to be entered into the software

7.15.2.3. Volume (dryness) (mL), Mass (dryness) (g), Drying Loss (%).

7.15.2.3.1. Drying loss is either the sample's Loss on drying result or KF water result.

7.15.2.4. The software will perform the following calculation:

7.15.2.4.1. Specific Rotation (anhydrous basis): (Raw result) *(100/(100-KF Result)).

7.15.3. Refer to the MCP 300 Polarimeter, DCN: BSI-SOP-0257 for instrument analysis.

7.15.3.1. Select the following method in the software: Specific Rotation @ 20°C – BioSpectra (LOD).

7.15.3.2. Calculate the result using the following calculation:

7.15.3.2.1. Specific Rotation = (Raw Result) * (100 / (100-Drying Loss))

7.15.3.2.2. Drying loss is either the sample's Loss on drying or KF water result.

7.15.4. Acceptance criteria: +78.0° to +81.5°

7.16. **PROTEINS :**

7.16.1. Accurately weigh out 1.000 g of sample and dissolve in 10.0 mL of purified water.

7.16.2. Measure the absorbance of the solution at 260nm and 280nm and calculate the protein content, in milligrams per milliliter, using the following calculation:

$$(A_{280} \times 1.45) - (A_{260} \times 0.74) = \text{Protein Content mg/mL}$$

7.16.3. $A_{280}$= absorbance at 280nm

7.16.4. $A_{260}$= absorbance at 260nm

7.17. **RESIDUAL SOLVENTS :**

7.17.1. Refer to Residual Solvents Method for D-Galactose, DCN: BSI-ATM-0101 for sample preparation and analysis.

7.18. **RESIDUE ON IGNITION :**

7.18.1. Turn on muffle furnace and allow it to stabilize at 600 °C.

7.18.2. Use long forceps to place the crucible in the furnace and to remove the crucible out of the furnace. Ignite quartz crucible at 600 ± 50 °C for 30 minutes, cool in a desiccator for 1.5 hours and weigh.

7.18.3. Accurately weigh 1 - 2 g of the sample and moisten with a small amount (1 mL) of sulfuric acid then heat gently until the sample is thoroughly charred.

7.18.4. Cool, then moisten the residue with 1mL of sulfuric acid and heat gently until white fumes are no longer evolved.

7.18.5. Ignite in a muffle furnace at 600 ± 50 °C until all carbon has been removed and a constant mass is obtained.

7.18.6. Cool in a desiccator for 1.5 hours and reweigh.

7.18.7. Dry until two consecutive weighings of the residue do not differ by more than 0.5 mg.

7.18.8. The weight of residue weighs a maximum of 1 mg.

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

7.19. **SULFATED ASH :**

7.19.1. Turn on muffle furnace and allow it to stabilize at 600 °C.

7.19.2. Use long forceps to place the crucible in the furnace and to remove the crucible out of the furnace. Ignite quartz crucible at 600 ± 50 °C for 30 minutes, cool in a desiccator for 1.5 hours and weigh.

7.19.3. Pipette 5 mL of sample solution prepared for the *acidity test* into the previously ignited quartz crucible. Moisten the sample with 2.0 mL of sulfuric acid. Evaporate to dryness on a water-bath.

7.19.4. Ignite in a muffle furnace at 600 ± 50 °C until all carbon has been removed and a constant mass is obtained.

7.19.5. Cool in a desiccator for 1.5 hours and reweigh.

7.19.6. The weight of residue weighs a maximum of 1 mg.

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

7.20. **TRACE METALS :**

7.20.1. Refer to Analytical Method: Determination of Elemental Impurities by ICP-MS in Galactose, DCN: BSI-ATM-0069.

7.21. **WATER** **Refer to Summary Sheet:**

7.21.1. Standardize Composite 5 as per Standardization of Titrants.

7.21.2. Immediately weigh 1.0 g of sample into a glass weighing spoon and tare the balance.

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

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

7.21.4. 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.

7.21.5. Galactose will 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.

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

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

## 8. COMPENDIAL DIFFERENTIATIONS:

**Table 1: Compendial Analyses**

| USP-NF Compendia | EP Compendia |
|---|---|
| Analysis Name | Analysis Name |
| Barium | Proteins |
| Optical Rotation | Sulfated Ash |
| Residue on Ignition | |

**Table 2: Harmonized Methods**

| Analysis Name |
|---|
| Acidity or Alkalinity (EP), Acidity (NF) |
| Appearance of Solution (EP), (NF) |
| Identification A (EP), (NF) |
| Identification C (EP), (NF) |
| Microbial Content TAMC (EP), Microbial Content, and species (NF) |

**Table 3: In-House Validated Methods in accordance with USP General Chapters:**

| Analysis Name |
|---|
| Assay/Identification B |
| [1]Endotoxins |
| [1]Elemental Impurities/Trace Metals/Limit of Lead |
| [1]Glucose |
| Related Substances |
| [1]Residual Solvents (Ethanol, IPA, Methanol, MIBK) |
| Water |

[1]Customer Requested specifications

**Table 4: In-House methods for Product Quality Description**

| Analysis Name |
|---|
| Appearance |