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ANALYTICAL METHOD VERIFICATION REPORT: GALACTOSE ASSAY AND RELATED SUBSTANCES VIA LIQUID CHROMATOGRAPHY WITH RI DETECTION

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1. PURPOSE:

1.1. The purpose of this protocol is to:

1.1.1. Summarize the results obtained for Galactose and Related Substances verification, which was performed on the Waters Alliance HPLC, and ensure the method was adequately evaluated.

2. SCOPE:

2.1. This Analytical Method Verification Report applies to the Galactose USP Assay and USP Related Substances analytical methods using BioSpectra's Waters Alliance HPLC.

2.2. Assay specification: 98.0% – 102.0%

2.3. Related Substances:

USP Galactose Acceptance Criteria		
Name	Approximate Relative Retention Time	Acceptance Criteria
Lactose and 1,6-galactosyl-galactose	0.79	NMT 0.6 %
Galacturonic acid	0.89	NMT 0.6 %
Dextrose	0.93	NMT 0.6 %
Tagatose	0.96	NMT 0.6 %
Dulcitol	1.06	NMT 0.6 %
Arabinose	1.10	NMT 0.6 %
Any unspecified impurity	----	NMT 0.2%
Total impurities	----	NMT 1.0%

3. RESPONSIBILITIES:

3.1. The Senior Chromatography Specialist, analysts and/or the Associate director of Product Life Cycle, if necessary, are responsible for completing the Method Verification Report using conclusions made from the results obtained from testing.

4. REFERENCE:

4.1. BSI-PRL-0586, Analytical Method Verification: Galactose Assay and Related Substances via Liquid Chromatography with RI Detection

4.2. BSI-SOP-0098, Balance SOP

4.3. BSI-SOP-0134, Pipette SOP

4.4. BSI-SOP-0436, Analytical Methods Validation Master Plan.

4.5. *USP Galactose*

4.6. *USP <621> Chromatography*

4.7. *USP <1225> Validation of Compendial Procedures*

4.8. *USP <1226> Verification of Compendial Procedures*

4.9. Waters 2414 Refractive Index Detector Operator's Guide

4.10. Waters 2695 Separations Module Operator's Guide

5. PRE-VERIFICATION REQUIREMENTS:

5.1. Equipment

5.1.1. All equipment used in this verification was in proper working order and within calibration. Serial numbers, date of last calibration, and the calibration due date for each instrument and equipment, where applicable, are included in the report.

5.2. Personnel

5.2.1. All personnel who executed this verification were properly trained in accordance with the Analytical Methods Validation Master Plan.

5.3. Supplies

5.3.1. All supplies used in the verification were clean and appropriate for their intended use. Suppliers and part numbers of all supplies are included in this report.

5.4. Reagents

5.4.1. All reagents were current and suitable for their intended use. The reagent name, lot number, manufacturer, date of opening, date of expiration, and part number are included in this report

5.5. Reference Standards

5.5.1. All reference standards that were used in this verification are listed in Section 6 and are traceable to nationally recognized standards, if available. The name of the reference standard, lot number, manufacturer, date of opening, date of expiration, and part number for reference standards used are included in this report.

6. MATERIALS AND EQUIPMENT:

6.1. All materials and equipment utilized in this Validation are outlined in this section.

6.2. Analytical Balance

6.2.1. Manufacturer: Sartorius
6.2.2. Model: Secura 124-1S
6.2.3. Serial Number: 29212172
6.2.4. Last Serviced: 04/20/22
6.2.5. Next Service: 10/2022

6.3. Waters Alliance HPLC

6.3.1. 30cm Column Compartment
6.3.1.1. SN: K21SMC188G
6.3.1.2. Last PM: 03/03/22
6.3.1.3. Next PM due: 03/2023

6.3.2. e2695 Separations Module
6.3.2.1. SN: M21SM4744A
6.3.2.2. Last PM: 03/03/22
6.3.2.3. Next PM due: 03/2023

6.3.3. 2414 Refractive Index Detector
6.3.3.1. SN: M21214125M
6.3.3.2. Last PM: 03/03/22
6.3.3.3. Next PM due: 03/2023

6.4. Reagents
- 6.4.1. UPLC Grade Water (Milli-Q Purified Water)
  - 6.4.1.1. Manufacturer: Millipore Sigma
  - 6.4.1.2. Serial Number: F9SA14284H
- 6.4.2. 1 N Sulfuric Acid, Certified Grade
  - 6.4.2.1. Manufacturer: LabChem
  - 6.4.2.2. Part Number: LC257704
  - 6.4.2.3. Lot Number: M077-16
  - 6.4.2.4. Date of Opening: Not Applicable
  - 6.4.2.5. Expiration: 03/25/24

6.5. Supplies
- 6.5.1. Disposable Polypropylene Weighing Funnels
  - 6.5.1.1. Supplier: TWD Scientific, LLC
  - 6.5.1.2. Part Number: DPWF-PP1-S
- 6.5.2. Micropipettes
  - 6.5.2.1. Model: 100 µL – 1000 µL
    - 6.5.2.1.1. Supplier: Eppendorf
    - 6.5.2.1.2. Serial Number: Q28940G
    - 6.5.2.1.3. Last Service: 07/14/22
    - 6.5.2.1.4. Next Service: 01/31/23
  - 6.5.2.2. Model: 100 µL – 1000 µL
    - 6.5.2.2.1. Supplier: Eppendorf
    - 6.5.2.2.2. Serial Number: O39512B
    - 6.5.2.2.3. Last Service: 06/13/22
    - 6.5.2.2.4. Next Service: 12/31/22
  - 6.5.2.3. Model: 500 µL – 5000 µL
    - 6.5.2.3.1. Supplier: Eppendorf
    - 6.5.2.3.2. Serial Number: L21310F
    - 6.5.2.3.3. Last Service: 07/14/22
    - 6.5.2.3.4. Next Service: 01/31/23
- 6.5.3. Transfer pipettes
  - 6.5.3.1. Supplier: Fisher
  - 6.5.3.2. Part Number: 13-711-9AM
- 6.5.4. Screw top glass autosampler vials and caps
  - 6.5.4.1. Supplier: Fisher
  - 6.5.4.2. Part Number: 03-391-18

6.6. Reference Standards
- 6.6.1. USP Traceable Galactose
  - 6.6.1.1. Supplier: Sigma Aldrich
  - 6.6.1.2. Lot: LRAC4019
  - 6.6.1.3. Expiration Date: 10/23
  - 6.6.1.4. Purity: 99.5%
  - 6.6.1.5. Part Number: PHR1206-500MG
  - 6.6.1.6. Date of Opening:10/17/22; 10/21/22
  - 6.6.1.7. CAS Number:59-23-4
- 6.6.2. USP Traceable Arabinose
  - 6.6.2.1. Supplier: Sigma-Aldrich
  - 6.6.2.2. Lot: LRAB9850
  - 6.6.2.3. Expiration Date: 05/23
  - 6.6.2.4. Purity: 97.5%

6.6.2.5. Part Number: PHR2100-500mg
6.6.2.6. Date of Opening10/17/22
6.6.2.7. CAS Number: 10323-20-3
6.6.3. Galacturonic Acid Monohydrate (NLT 97.0%)
6.6.3.1. Supplier: Sigma-Aldrich
6.6.3.2. Lot: BCCD0001
6.6.3.3. Expiration Date: 04/28/26
6.6.3.4. Purity: 98.8%
6.6.3.5. Part Number: 48280-25-G-F
6.6.3.6. Date of Opening: 04/28/21
6.6.3.7. CAS Number: 91510-62-2
6.6.4. USP Traceable Dextrose (Glucose)
6.6.4.1. Supplier: Sigma-Aldrich
6.6.4.2. Lot: LRAC4110
6.6.4.3. Expiration Date: 11/23
6.6.4.4. Purity: 99.9%
6.6.4.5. Part Number: PHR1000-1G
6.6.4.6. Date of Opening: 05/03/21
6.6.4.7. CAS Number: 50-99-7
6.6.5. USP Traceable Anhydrous Lactose
6.6.5.1. Supplier: Sigma Aldrich
6.6.5.2. Lot: LRAC0303
6.6.5.3. Expiration Date: 10/23
6.6.5.4. Purity: 99.3%
6.6.5.5. Part Number: PHR1025-1G
6.6.5.6. Date of Opening: 05/03/21
6.6.5.7. CAS Number: 63-42-3

6.7. Galactose Authentic Sample:
6.7.1. Supplier: Biospectra, Inc.
6.7.2. Lot: GALP-0121-00004-PV

6.8. LC Columns
6.8.1. Two (2) SUPELCOGEL C-610H, 6% Crosslinked HPLC Columns in Tandem
6.8.2. Dimensions: 9 µm particle size, 30 cm x 7.8 mm
6.8.3. Supplier: Supelco
6.8.4. Part Number: 59320-U
6.8.5. Serial Numbers: 263080-06, 263081-06

7. TESTING PROCEDURE:

7.1. The following testing procedure was performed on each day of analysis.

7.2. Solution Preparation

7.2.1. Note: All solutions are to be thoroughly mixed after being prepared. Solutions may be scaled as needed.

7.2.2. Mobile Phase (0.009 N Sulfuric Acid)
- 7.2.2.1. Dilute 9 mL of 1 N Sulfuric Acid to a final volume of 1000 mL using purified water.
- 7.2.2.2. Mix thoroughly.
- 7.2.2.3. Expires one week (7 days) after preparation.

7.2.3. Stock Impurity Reference Standard Solution (1.0 mg/mL Related Substances)
- 7.2.3.1. Note: The amount of reference standard to be used may require adjustment based off CoA values. The final concentration for each analyte must be within ±10% of 1.0 mg/mL.
- 7.2.3.2. Accurately weigh 25 mg of each impurity (See Below) and transfer into a 25 mL volumetric flask.
  - 7.2.3.2.1. Arabinose RS
  - 7.2.3.2.2. Galacturonic Acid
  - 7.2.3.2.3. Dextrose (Glucose) RS
  - 7.2.3.2.4. Anhydrous Lactose RS
- 7.2.3.3. Fill the flask ~3/4 full with mobile phase and swirl to dissolve.
- 7.2.3.4. Dilute to volume with mobile phase and mix well.

7.2.4. System Suitability Solution (10.0 mg/mL Galactose CRS, 0.2 mg/mL Related Substances)
- 7.2.4.1. Note: The amount of USP Traceable Galactose Reference Standard to be used may require adjustment based off CoA values. The final concentration of Galactose must be within ±10% of 10.0mg/mL.
- 7.2.4.2. Accurately weigh 250 mg (±10%) of USP Traceable Galactose Reference Standard and transfer to a 25.0 mL volumetric flask.
- 7.2.4.3. Pipette 5.0 mL of the above *Stock Impurity Reference Standard Solution*.
- 7.2.4.4. Fill the flask ~3/4 full with mobile phase and swirl to dissolve.
- 7.2.4.5. Fill to volume with mobile phase and mix thoroughly.
- 7.2.4.6. Solution Stability: Four (4) days when stored, sealed with a fitted stopper, in clear glassware, at normal laboratory conditions

7.2.5. Sensitivity Solution (5.0 µg/mL (0.05%) Related Substances)
- 7.2.5.1. Pipette 0.5 mL above *Stock Impurity Reference Standard Solution* into a 100 mL volumetric flask.
- 7.2.5.2. Dilute to volume with mobile phase and mix well.
- 7.2.5.3. Solution Stability: Four (4) days when stored, sealed with a fitted stopper, in clear glassware, at normal laboratory conditions

7.2.6. Assay Standard Solution (10.0 mg/mL Galactose CRS)
- 7.2.6.1. Note: The amount of USP Traceable Galactose Reference Standard to be used may require adjustment based off CoA values. The final concentration of Galactose must be within ±10% of 10.0 mg/mL.
- 7.2.6.2. Accurately weigh 250 mg (±10%) of USP Traceable Galactose Reference Standard and transfer to a 25.0 mL volumetric flask.
- 7.2.6.3. Fill the flask ~3/4 full with mobile phase and swirl to dissolve.

7.2.6.4. Dilute to volume with mobile phase and mix well.
7.2.6.5. Prepare in duplicate.
7.2.6.6. Label AS1 and AS2, respectively.
7.2.6.7. Solution Stability: Four (4) days when stored, sealed with a fitted stopper, in clear glassware, at normal laboratory conditions

7.2.7. Sample Solution (10.0 mg/mL Galactose)
7.2.7.1. Accurately weigh 250 mg (±10%) of Galactose sample and transfer to a 25.0 mL volumetric flask.
7.2.7.2. Fill the flask ~3/4 full with mobile phase and swirl to dissolve.
7.2.7.3. Dilute to volume with mobile phase and mix thoroughly.
7.2.7.4. Solution Stability: Four (4) days when stored, sealed with a fitted stopper, in clear glassware, at normal laboratory conditions

7.3. Instrument Setup

7.3.1. Waters Alliance HPLC Method Parameters

Parameter	Setting
Flow Type	Isocratic
Diluent	0.009N Sulfuric acid
Mobile Phase A	0.009N Sulfuric acid
Needle Wash	Water
Flow Rate	0.25 mL/min
Run Time	70 minutes
Injection Volume	25 µL
Stroke Volume	25 µL
Syringe Draw Rate	Normal
Pre-Column Volume	0.0
Needle Wash Time	Normal
Column Temperature (°C)	35 ± 1.0
Sample Temperature (°C)	Ambient
RI Detector Settings	
Detector	Refractive Index
Detector Temperature	40 °C
Sampling Rate	5
Filter Time	1.0
Sensitivity	4
Polarity	Positive

7.3.2. Chromatographic System:

7.3.2.1. Flush HPLC system with purified water, place 0.009 N Sulfuric Acid in the mobile phase reservoir (A) and prime the lines.
7.3.2.2. Install Two (2) Supelcogel C610H, 7.8mm x 30cm columns in tandem and slowly bring flow up to 0.25 mL/min. Allow the column to equilibrate until a consistent pressure is observed.
7.3.2.3. Turn on the RI detector and allow to warm and stabilize at 40°C. It is recommended to allow the RI detector to stabilize for a few hours prior to initiating the analysis
7.3.2.4. Purge RID reference cell for at least 30 minutes prior to initiating the injection sequence.

7.3.3. Injection Sequence:

Sample ID	Number of Injections
System Suitability	
Diluent	≥1
Sensitivity	1
System Suitability	1
AS1	6
AS2	1
Sample Injections	
Diluent	1
Samples	≤ 6
AS1 (QC Check)	1

- Repeat the sample injection sequence if additional samples are to be analyzed
- Samples may be substituted with diluent injections
- The Sensitivity injection may be omitted if performing Assay only
- The AS2 injection may be omitted if performing Related Substances only

7.3.4. System Suitability Criteria:

System Suitability Parameter	Acceptance Criteria
The relative standard deviation of the galactose peak from the first six (6) injections of the AS1 solution.	NMT 1.0%
The relative standard deviation of the galactose peak from all injections of the AS1 solution.	NMT 1.0%
USP Resolution between Lactose and Galacturonic acid in the *System Suitability Solution* injection.	NLT 3.0
USP Resolution between Galacturonic acid and Dextrose in the *System Suitability Solution* injection.	NLT 1.5
USP Resolution between Dextrose (Glucose) and Galactose in the *System Suitability Solution* injection.	NLT 2.0
USP Resolution between Galactose and Arabinose in the *System Suitability Solution* injection.	NLT 3.0
[1]Signal-to-noise Ratio for Lactose, Galacturonic Acid, Dextrose, and Arabinose peaks in the *Sensitivity Solution* injection.	NLT 10
Standard %Agreement between the first six (6) AS1 injections and the AS2 injection.	99% - 101%
[1]The noise value should be determined from a stable region of the baseline within the *Sensitivity Solution* injection chromatogram.	

7.4. Calculations

7.4.1. The following equations will be calculated in the Empower software:

7.4.2. Percent Agreement = (R_{AS2}/R_{AS1}) x (C_{AS1}/C_{AS2}) x 100

7.4.2.1. R_{AS1} = average peak response of galactose from the first six (6) AS1 injections

7.4.2.2. R_{AS2} = peak response of Galactose from the AS2 injection

7.4.2.3. C_{AS1} = Concentration of Galactose in AS1 x Purity Factor

7.4.2.4. C_{AS2} = Concentration of Galactose in AS2 x Purity Factor

7.4.3. Assay = (R_u/R_{AS1}) x (C_{AS1}/C_u) x 100

7.4.3.1. R_{AS1} = average peak response of galactose from all AS1 injections

7.4.3.2. R_u = peak response of Galactose from the sample

7.4.3.3. C_{AS1} = Concentration of AS1 x Purity Factor

7.4.3.4. C_u = Concentration of the sample

7.4.4. Related Substances (Area%) = $R_u/(R_{SPL}$ x RRF) x 100

7.4.4.1. R_u = peak response of each individual impurity in the sample solution

7.4.4.2. R_{SPL} = peak response of Galactose in the sample solution

7.4.4.3. RRF = Empower Relative Response Factor

7.4.4.3.1. See below for the response factors for the known Related Substances

7.4.4.3.2. Disregard any peak due to the solvent and the peak at the relative retention time of approximately 0.64.

Relative Response Factors	
Name	USP Relative Response Factor
Lactose and 1,6-galactosyl-galactose	0.95
Galacturonic acid	0.88
Dextrose	0.99
Tagatose	0.96
Dulcitol	0.96
Arabinose	0.95
Any unspecified impurity	1.0

8. PERFORMANCE PARAMETERS:

8.1. System Suitability:

8.1.1. Refer to the injection sequence (7.3.3) and the system suitability acceptance criteria (7.3.4)

8.1.2. System suitability was carried out on each day of analysis per the analytical procedure is Section 7. All acceptance criteria were met. See Table 1 below for a summary of results.

8.1.2.1. Note: The USP S/N calculation utilized a noise value determined from a stable region of the baseline within the *Sensitivity Solution* chromatogram.

Table 1: System suitability summary for each analysis

System Suitability Parameter	Results (notebook reference, date)	
	MV9P3, 10/17/22	MV9P11, 10/21/22
The relative standard deviation of the Galactose peak from the first six (6) injections of the AS1 solution. Criterion: NMT 1.0%	0.05%	0.05%
The relative standard deviation of the Galactose peak from all injections of the AS1 solution. Criterion: NMT 1.0%	0.04%	0.05%
USP Resolution between Lactose and Galacturonic acid in the *System Suitability Solution* injection. Criterion: NLT 3.0	4.0	4.0
USP Resolution between Galacturonic acid and Dextrose in the *System Suitability Solution* injection. Criterion: NLT 1.5	1.7	1.7
USP Resolution between Dextrose (Glucose) and Galactose in the *System Suitability Solution* injection. Criterion: NLT 2.0	3.2	3.1
USP Resolution between Galactose and Arabinose in the *System Suitability Solution* injection. Criterion: NLT 3.0	3.9	3.9
Signal-to-noise Ratio for Lactose, Galacturonic Acid (GLTA), Dextrose, and Arabinose peaks in the Sensitivity Solution injection. Criterion: NLT 10	Lactose: 21 GLTA: 20 Dextrose: 20 Arabinose: 20	Lactose: 22 GLTA: 21 Dextrose: 21 Arabinose: 20
Standard %Agreement between the first six (6) AS1 injections and the AS2 injection. Criterion: NLT 99% - 101%	100	100

8.2. Specificity:

8.2.1. The chromatograms from the following injections were overlaid: one (1) Diluent injection, one (1) Sensitivity Solution, one (1) System Suitability injection, one (1) Galactose blank injections, one (1) Spiked Sample Injection.

8.2.2. Acceptance Criteria:

8.2.2.1. All analytes of interest showed enough resolution to allow for unequivocal identification and accurate quantitation per section 8.3. The resolution of the analytes of interest in the spiked sample met the resolution requirements outlined in the system suitability criteria (section 7.2.4). See Table 2 and Figure 1 for results.

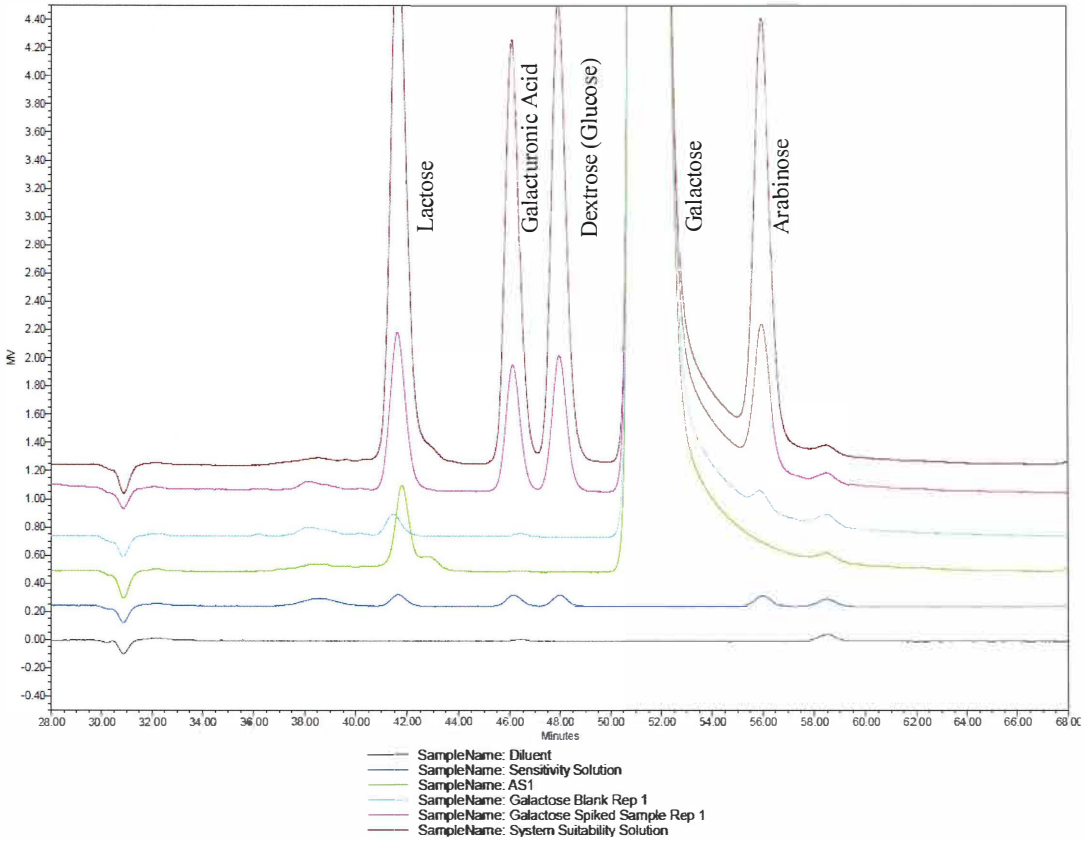

Figure 1: Specificity Overlay

Table 2: Resolution summary for the spiked sample

Sample ID	USP Resolution			
	Lactose and Galacturonic Acid	Galacturonic Acid and Dextrose	Dextrose and Galactose	Galactose and Arabinose
Spiked Sample Replicate 1	4.0	1.7	3.2	4.0
Acceptance Criteria	NLT 3.0	NLT 1.5	NLT 2.0	NLT 3.0
Pass/Fail	Pass	Pass	Pass	Pass

8.3. Accuracy and Precision

8.3.1. Accuracy and precision were performed with neat, authentic samples and authentic samples spiked with known impurities at 100% of the impurity specification (0.6%) The Samples were analyzed per the Testing Procedure (section 7). Assay and the %RSD for Galactose, and the %Recovery and the %RSD of the Related Substances were calculated. All acceptance criteria were met for both assay and related substances. See Tables 3 through 8.

8.3.2. Blank Samples (10 mg/mL Galactose)

8.3.2.1. Accurately weighed 250 mg (±10%) of Galactose sample and transferred to a 25.0 mL volumetric flask.

8.3.2.2. Filled the flask ~3/4 full with mobile phase and swirled to dissolve

8.3.2.3. Diluted to volume with mobile phase and mixed thoroughly.

8.3.2.4. Prepared in duplicate.

8.3.3. Stock Impurity Reference Standard Solution (1.0 mg/mL Related Substances)

8.3.3.1. Accurately weighed 100 mg (±10%) of each impurity (See Below) and transferred into a 100 mL volumetric flask.

8.3.3.1.1. Arabinose RS

8.3.3.1.2. Galacturonic Acid

8.3.3.1.3. Dextrose (Glucose) RS

8.3.3.1.4. Anhydrous Lactose RS

8.3.3.2. Filled the flask ~3/4 full with mobile phase and swirled to dissolve.

8.3.3.3. Diluted to volume with mobile phase and mixed well.

8.3.4. Spiked Samples (10 mg/mL Galactose, 0.06 mg/mL (0.6%) Related Substances)

8.3.4.1. Accurately weighed 250 mg (±10%) of Galactose Authentic Sample and transferred to a 25.0 mL volumetric flask.

8.3.4.2. Pipetted 1.5 mL of the *Stock Impurity Solution* (sec. 8.3.3) into the flask.

8.3.4.3. Filled the flask ~3/4 full with mobile phase and swirled to dissolve.

8.3.4.4. Diluted to volume with mobile phase and mixed thoroughly.

8.3.4.5. Prepared six (6) replicates.

8.3.5. Calculation:

8.3.5.1. % Recovery = $A_M/(A_{Added}+A_0)$ x 100

8.3.5.1.1. A_M = amount of related substance measured

8.3.5.1.2. A_{Added} = amount of related substance added

8.3.5.1.3. A_0 = average amount of analyte measured in the Blank samples

Table 3: Summary of assay values for the spiked samples

Precision of Authentic Samples				
Preparation	Amount weighed (mg)	Concentration (mg/mL)	Assay (%)	Acceptance Criteria
1	250.2	10.0	99.3	• %RSD is NMT 1.0% • All assay values are between 98.0% and 102.0%
2	249.8	10.0	99.3	
3	250.1	10.0	99.0	
4	250.0	10.0	99.4	
5	250.0	10.0	99.4	
6	250.2	10.0	99.2	
		Mean	99.3	**Pass/Fail**
		% RSD	0.2	Pass

Table 4: Average amount of Impurity found in neat galactose samples

Galactose Blanks	
Analyte	Average Amount Measured (%Area)
Lactose	0.09
Galacturonic Acid	None Detected
Dextrose	None Detected
Arabinose	0.06

Table 5: Summary of results for Lactose in the spiked samples

Lactose - Accuracy and Precision (0.6%)				
		Average amount of Lactose in the Galactose blanks (%Area)		0.09460
Preparation	Amount Added (%wt)	Amount Found (%Area)	% Recovery	Acceptance Criteria
1	0.59520	0.73065	106	• %RSD is NMT 20.0% • The %Recovery for all analytes in each spiked sample is between 80% and 120%.
2		0.73848	107	
3		0.72953	106	
4		0.72780	106	
5		0.74288	108	
6		0.73556	107	
			Mean 106	**Pass/Fail**
			% RSD 0.8	Pass

Table 6: Summary of results for Galacturonic Acid in the spiked samples

Galacturonic Acid - Accuracy and Precision (0.6%)				
Average amount of Galacturonic Acid in the Galactose blanks (%Area)			None Detected	
Preparation	Amount Added (%wt)	Amount Found (%Area)	% Recovery	Acceptance Criteria
1	0.59102	0.60417	102	• %RSD is NMT 20.0% • The %Recovery for all analytes in each spiked sample is between 80% and 120%.
2		0.60394	102	
3		0.59028	100	
4		0.59647	101	
5		0.60073	102	
6		0.59758	101	
		Mean	101	**Pass/Fail**
		% RSD	0.9	Pass

Table 7: Summary of results for Dextrose in the spiked samples

Dextrose (Glucose) - Accuracy and Precision (0.6%)				
Average amount of Dextrose in the Galactose blanks (%Area)			None Detected	
Preparation	Amount Added (%wt)	Amount Found (%Area)	% Recovery	Acceptance Criteria
1	0.60060	0.59893	100	• %RSD is NMT 20.0% • The %Recovery for all analytes in each spiked sample is between 80% and 120%.
2		0.59978	100	
3		0.59574	99	
4		0.59191	99	
5		0.59572	99	
6		0.59472	99	
		Mean	99	**Pass/Fail**
		% RSD	0.5	Pass

Table 8: Summary of results for Arabinose in the spiked samples

Arabinose - Accuracy and Precision (0.6%)				
Average amount of Arabinose in the Galactose blanks (%Area)			0.05857	
Preparation	Amount Added (%wt)	Amount Found (%Area)	% Recovery	Acceptance Criteria
1	0.58500	0.63394	99	• %RSD is NMT 20.0% • The %Recovery for all analytes in each spiked sample is between 80% and 120%.
2		0.63599	99	
3		0.63638	99	
4		0.63378	98	
5		0.63364	98	
6		0.63402	99	
		Mean	99	**Pass/Fail**
		% RSD	0.2	Pass

8.4. Solution Stability:

8.4.1. One (1) *Spiked Sample*, one (1) AS1 Solution, and one (1) *Sensitivity* solution were stored in the original glassware at normal lighting and laboratory conditions. The aged solutions were analyzed against freshly prepared standards. % Agreement was calculated for standards, including the LOQ, whereas the %Recovery and Absolute difference was calculated for the samples (Related substances and assay). Additionally, the USP S/N was calculated for all analytes of interest in the *Aged Sensitivity Solution*. All acceptance criteria were met through 4 days. Refer to tables 9 through 14.

8.4.2. Calculations:

8.4.2.1. Absolute Difference = |Day 0 – Day X|

8.4.2.1.1. Day 0 = fresh solution Assay/%Recovery value

8.4.2.1.2. Day X = aged solution Assay/%Recovery value

Table 9: Solution Stability – The % Agreement and S/N values of the aged Sensitivity solution.

Sensitivity Solution Stability						
Timepoint (day)	% Agreement				Acceptance Criteria	Pass/Fail
	Lactose	Galacturonic Acid	Dextrose	Arabinose		
4	100%	116%	106%	111%	The % Agreement is between 75% and 125%	Pass

Table 10: Solution Stability – The S/N values of the aged Sensitivity solution.

Sensitivity Solution Stability						
Timepoint (day)	USP S/N				Acceptance Criteria	Pass/Fail
	Lactose	Galacturonic Acid	Dextrose	Arabinose		
4	23	24	23	20	NLT 10	Pass

Table 11: Solution Stability – The % Agreement value of the aged Assay Standard solution.

Assay Standard Solution Stability			
Timepoint (day)	% Agreement	Acceptance Criteria	Pass/Fail
4	101	The % Agreement is between 99% and 101%	Pass

Table 12: Solution Stability – Summary of the % Recovery values for Related Substances in the Spiked Sample.

Related Substances - Sample Solution Stability - %Recovery						
Timepoint (day)	%Recovery				Acceptance Criteria	Pass/Fail
	Lactose	Galacturonic Acid	Dextrose	Arabinose		
0	106%	102%	100%	99%	The assay of the aged solution is between 80% and 120%	NA
4	107%	101%	99%	99%		Pass
Note: equivalent solution stability assumed for the *System Suitability Solution* and *Spiked Sample*						

Table 13: Solution Stability – Summary of the absolute difference in % Recovery values for the Related Substances in the Spiked Sample

Related Substances - Sample Solution Stability – Absolute Difference						
Timepoint (day)	Absolute Difference				Acceptance Criteria	Pass/Fail
	Lactose	Galacturonic Acid	Dextrose	Arabinose		
4	0.8%	1.2%	0.9%	0.1%	The absolute difference between Day 0 and Day X and aged solution is NMT 20.0%	Pass

Table 14: Solution Stability – Summary of the %Assay and absolute difference in % Assay values for galactose in the Spiked Sample

Assay - Sample Solution Stability				
Timepoint (day)	Measured % Assay	Absolute Difference	Acceptance Criteria	Pass/Fail
0	99.3%	NA	• The assay of the aged solution is between 98.0% and 102.0% • The absolute difference between Day 0 and Day X and aged solution is NMT 2.0%	NA
4	99.8%	0.5%		Pass

8.5. Empower Custom Fields Verification:

8.5.1. In order to fully automate calculations within Empower software, the following equations required custom fields. The following custom fields were verified using the data from Accuracy and Precision. All acceptance criteria were met. Refer to Tables 15 through 17.

8.5.2. Standard % Agreement Calculation

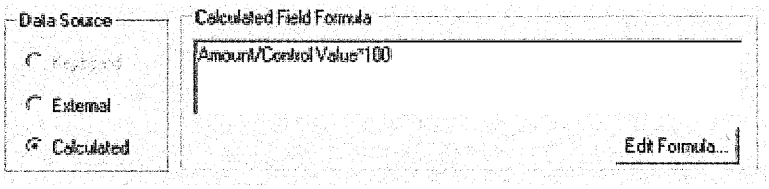

Table 15: Empower Verification Summary of % Agreement

% Agreement - Custom Field Verification			
Empower Calculated Result (%)	Hand Calculated Result (%)	Acceptance Criterion	Pass/Fail
100.13266	100.13266	Hand calculated results and Empower results must be identical out to 5 decimal places	Pass

8.5.3. Assay Calculation:

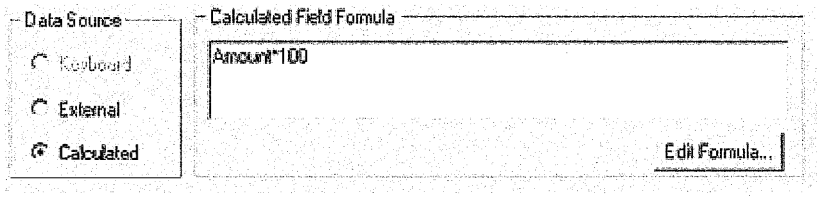

Table 16: Empower Verification Summary of Assay

Assay - Custom Field Verification			
Empower Calculated Result (%)	Hand Calculated Result (%)	Acceptance Criterion	Pass/Fail
99.33702	99.33702	Hand calculated results and Empower results must be identical out to 5 decimal places	Pass
99.26338	99.26338		
98.95642	98.95642		
99.41330	99.41330		
99.40968	99.40968		
99.21969	99.21969		

8.5.4. Related Substances (%Area) Calculation:

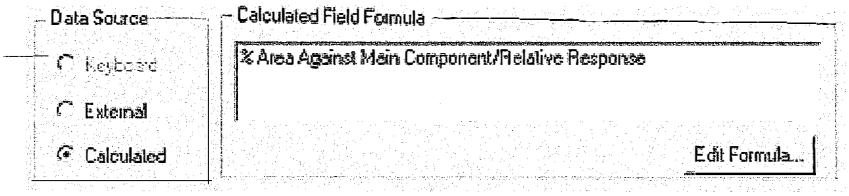

Table 17: Empower Verification Summary of % Area

%Area Glucose – Custom Field Verification			
Empower Calculated Result (%)	Hand Calculated Result (%)	Acceptance Criterion	Pass/Fail
0.59893	0.59893	Hand calculated results and Empower results must be identical out to 5 decimal places	Pass
0.59978	0.59978		
0.59574	0.59574		
0.59191	0.59191		
0.59572	0.59572		
0.59472	0.59472		

9. VALIDATION STATUS:

9.1. The method "Galactose Assay and Related Substances via Liquid Chromatography with RI Detection" is considered verified and suitable for use at the BioSpectra Bangor, PA facility. All acceptance criteria for system suitability, accuracy, precision, and specificity were met. The sample solution, System Suitability Solution, Sensitivity solution, and Assay Standard solution are considered stable for 4 days when stored stoppered in clear glassware at normal laboratory conditions

9.2. The instrument injection precision (System Suitability) and repeatability (Accuracy and Precision) showed suitable performance to justify the use of a single sample preparation for assay and related substances determination. The RSD system suitability requirement for injection precision will be reduced from 1.0% to 0.85% to ensure reliable results for the single preparation replicate strategy.

9.2.1. Note: The maximum permitted RSD of 0.85% was referenced from Table 1 in USP <621>.

9.3. Laboratory Notebook References:

MV10, P3 - Accuracy and Precision

MV10, P11 - Solution Stability

MV10, P16 - Empower Custom Field Verification