1.0 Purpose

The purpose of this procedure is to define the method for the quantitation of total polysaccharide content in raw materials using UV/VIS spectrophotometry.

2.0 Scope

This procedure applies to the quantification of polysaccharides in raw materials and straight fill finished products. This method is not appropriate as an identification for individual sugars and all sugars will react to form a chromaphore. Therefore, care must be taken in the use of this method to quantify one sugar in the presence of others as all other present sugars will be quantitated.

3.0 Responsibility

3.1 It is the responsibility of QC and Analytical Chemists to follow this procedure.

3.2 It is the responsibility of QC Laboratory Management to ensure that this procedure is being followed.

3.3 It is the responsibility of QC Laboratory Management and/or Analytical Development to keep this procedure aligned with current practices.

4.0 Definitions

4.1 **UV/VIS** – Ultraviolet and Visible Electromagnetic Spectrums

4.2 **H_2SO_4** – Sulfuric Acid

4.3 **CofA** – Certificate of Analysis

4.4 **RT** – Room Temperature

4.5 **H_2O** – Millipore Water

4.6 **STD** – Standard

4.7 **cGMP –** Current Good Manufacturing Practices

4.8 **Polysaccharide** – a polymeric carbohydrate molecule comprised of long chains of monosaccharide units

4.9 **QS –** Quantity Sufficient

4.10 **QC** – Quality Control

5.0 References

5.1 Journal of Applied Pharmaceutical Science 01 (03); 2011; 93-95

5.2 Int. J. Modern Biol. Med. 4 (3); 2013; 204-215

5.3 Analytical Chemistry 28 (3); 1956; 350-356

5.4 Manufacturers Internal test method (unpublished)

6.0 Supplies

6.1 Chemicals: All reagents are ACS grade or better.

6.1.1 Millipore Water

6.1.2 Phenol

6.1.3 H_2SO_4

6.1.4 Sucrose

6.2 Glassware

6.2.1 GC headspace vials, with crimp cap enclosures

6.2.2 Scintillation Vials

6.2.3 50mL Volumetric Flask

6.2.4 100mL Volumetric Flask

6.3 Disposables

6.3.1 10mL Pipette Tips

6.3.2 1mL Pipette Tips

6.3.3 200µL Pipette Tips

6.3.4 1.5mL microfuge tubes

6.3.5 16mL Test Tubes

6.3.6 Disposable Plastic Luer Lock Syringe – 3mL, 6mL, or 10mL

6.3.7 Nylon Syringe Filters, 0.2µm

6.3.8 Weigh paper

6.4 Equipment

6.4.1 Lambda 365 Spectrophotometer, Perkin Elmer, with Win-Lab Software

6.4.2 Crimper

6.4.3 Decrimper

6.4.4 Analytical Balance

6.4.5 Hot water bath

6.4.6 Ultrasonic bath

6.4.7 Vortex

6.4.8 Stir Plate

6.4.9 Eppendorf Centrifuge

6.4.10 10mL Pipette

6.4.11 1mL Pipette

6.4.12 200µL Pipette

7.0 Preparation of Reagents, Samples, and Standards

7.1 5% Phenol Solution

Prepared by mixing phenol stock and water to a concentration of 5% (v/v)

7.2 Standard Preparation

7.2.1 Use the actual purity from the CofA or the standard certification for sucrose reference material for calculations. The stock standard preparation reflects 100% content for the analyte assayed.

Example: Sucrose, 99% purity

Prepare 50mL of a 1mg/mL solution

1mg/mL x50mL = 50mg

50mg/0.99 = 50.5mg

Dissolve 50.5mg up to 50mL = 1.0mg/mL

7.2.2 Accurately weigh about 100mg of Ref standard into a 100mL volumetric and QS with DI H_2O and dissolving fully to make a 1mg/mL solution.

7.2.3 Pipet 10.0mL of the above solution into a 100mL volumetric and QS to with DI H_2O. **This is the standard stock.**

7.3 Sample Preparation

7.3.1 Accurately weigh about 50mg of sample into a 50mL volumetric and QS with DI H_2O.

7.3.2 Pipet 1.0mL of the above solution into a 50mL volumetric and QS with DI H_2O. This is the sample stock.

7.3.3 Sample preparation amounts and dilutions may need to be adjusted to ensure sample concentration is within the standard calibration curve.

8.0 Test Procedure

8.1 Make a calibration curve

8.1.1 A calibration curve should include a blank and 3-5 points. An example standard curve is listed below.

8.1.1.1 Using the standard stock, pipet 0.0, 1mL, 2mL, 4mL, 6mL, and 8mL into separate GC headspace vials (or other appropriate vials) and dilute each to 20mL with DI H_2O. Transfer 800uL of each of the standard stock into separate glass vials and add 400uL of the 5% phenol solution to each vial.

8.1.1.2 In a fume hood, carefully add 2mL of concentrated H_2SO_4 to each vial. Be careful with H_2SO_4 as it is corrosive and when mixed with water will make the vials very hot.

8.1.1.3 Carefully and gently swirl the vials and crimp to seal. Heat in a water bath at 80°C for 30min.

8.2 Preparing Sample

8.2.1 Using the sample stock, pipet 800uL of the solution into a GC headspace vial (or other appropriate vial) and add 400uL of the 5% phenol solution to the vial.

8.2.2 In a fume hood, carefully add 2mL of concentrated H_2SO_4. **Be careful with H_2SO_4 as it is corrosive and when mixed with water will make the vials very hot.**

8.2.3 Carefully and gently swirl the vial and crimp to seal. Heat in a water bath at 80°C for 30min.

9.0 Measurement

9.1 After the 30 minutes of heating standard and samples is complete, allow to cool for 15min.

9.2 De-crimp vials and transfer to cuvettes for analysis.

9.3 Measure each standard and sample vial at 490nm being sure to use the blank to zero the spectrophotometer and write the values obtained in a laboratory notebook.

10.0 Calculations

10.1 Calculations for Determining Quantity

10.1.1 Plot the concentration (ppm) (Y-axis) vs absorbance (X-axis) for the standard points.

10.1.2 Use the slope of the standard line to obtain the concentration (Y) of the sample solution from the absorbance (X) from the equation $y = mx + b$.

10.1.2.1 Y = value on the y-axis which is concentration of the sample

10.1.2.2 X = value on the x-axis which is the absorbance of the sample

10.1.2.3 b = y-intercept

10.1.2.4 m = slope of the line obtained from standard calibration curve

10.1.3 The % content of polysaccharide in the sample can be determined by the equation

% content = $[C]_{sam}$ * [Dilution amount (mL) / Sample Weight (mg)] * 100

$[C]_{sam}$ = Concentration of sample in mg/mL.

11.0 System Suitability

11.1 The coefficient of determination (R^2) for the calibration is NLT 0.98.

12.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	01/18/19	New.	N/A	J. Maignan
1	04/11/23	Change instrument to Lambda 365 (new instrument), edit calculation for clarity, change requirements section to system suitability.	CC-23-0184	S. Sassman
2	08/08/24	Increased the volume used to make the standards for the standard curve in order to utilize glass pipettes. Doubled standard and sample test volumes to fill more of the cuvettes. Specified concentration units for the graph.	CC-24-0352	W. Davis