

	Standard Operating Procedure		SOP Number D-771	Revision 1
	Ribose Determination by UV/VIS Spectroscopy		Effective Date 03/21/23	Page Page 1 of 7
Written by/ Date SAS 03/16/23		Reviewed by/ Date CJL 03-16-23		Approved by/ Date SS 03/20/23
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1.0 Purpose

The purpose of this procedure is to define the method for the quantitation of Ribose content in raw materials and finished products using UV/VIS spectrophotometry.

2.0 Scope

This procedure applies to the quantification of ribose 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 chromophore. 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 personnel to keep this procedure aligned with current practices.

4.0 Definitions

- 4.1 **UV/VIS** – Ultraviolet and Visible Electromagnetic Spectrums
- 4.2 **H₂SO₄** – Sulfuric Acid
- 4.3 **CofA** – Certificate of Analysis

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4.4 **RT** – Room Temperature

4.5 **H₂O** – Millipore Water

4.6 **STD** – Standard

4.7 **cGMP** – Current Good Manufacturing Practices

4.8 **Ribose** – a pentose class sugar used in nature in nucleosides, vitamins and enzymes

4.9 **QS** – Quantity Sufficient

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5.4 Manufacturers Internal test method (unpublished)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 H₂SO₄

6.1.4 Ribose

6.2 Glassware

6.2.1 GC headspace vials, with crimp cap enclosures

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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

6.3.3 200 μ L 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, 0.2 μ m

6.3.8 Weigh paper

6.4 Equipment

6.4.1 Lambda 45 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

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6.4.7 Vortex

6.4.8 Stir Plate

6.4.9 Eppendorf Centrifuge

6.4.10 10mL Pipette

6.4.11 1mL Pipette

6.4.12 200 μ L 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 ribose reference material for calculations. The stock standard preparation reflects 100% content for the analyte assayed.

7.2.2 Accurately weigh about 100mg of Ref. standard into a 100mL volumetric and QS with DI H₂O 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 H₂O. **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 H₂O.

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7.3.2 Pipet 1.0mL of the above solution into a 50mL volumetric and QS with DI H₂O.

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, 20uL, 40uL, 80uL, 120uL, and 160uL into separate GC headspace vials (or other appropriate vials) and dilute each to 400uL with DI H₂O. Add 200uL of the 5% phenol solution to each vial.

8.1.1.2 In a fume hood, carefully add 1mL of concentrated H₂SO₄ to each vial. **Be careful with H₂SO₄ 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 400uL of the solution into a GC headspace vial (or other appropriate vial) and add 200uL of the 5% phenol solution to the vial.

8.2.2 In a fume hood, carefully add 1mL of concentrated H₂SO₄. Be careful with H₂SO₄ as it is corrosive and when mixed with water will make the vials very hot.

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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 (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 ribose in the sample can be determined by the equation

$$\% \text{ content} = [C]_{\text{sam}} * D / SW * 100$$

[C]_{sam} = Concentration of sample from step 10.1.2 (mg/mL)

D = Sample dilution volume (mL)

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SW = Sample weight (mg)

11.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	01/18/19	New.	N/A	J. Maignan
1	03/16/23	Minor edits for clarity.	CC-23-0142	S.Sassman