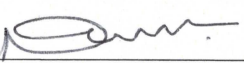
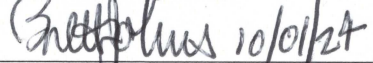

	Standard Operating Procedure		SOP Number D-1026	Revision 0
	Total Saponins Assay (Vanillin-Sulfuric Acid TSC)		Effective Date 10/03/24	Page Page 1 of 4
Written by/ Date  10/01/24		Reviewed by/ Date  10/01/24		Approved by/ Date  10/03/24
Title: QC Analytical Services, Manager		Title: Quality Control, Director		Title: Regulatory Affairs Supervisor

1.0 Purpose

The purpose of this procedure is to define the process used for the determination of Total Saponins using UV/Vis.

2.0 Scope

This procedure applies to the determination of Total Saponins as percentage for powdered samples. This procedure follows “Improving the Vanillin-Sulphuric Acid Method for Quantifying Total Saponins” from MDPI Technologies, Method <2.2.1> *The Usual Vanillin-Sulphuric Acid TSC Assay*.

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 implement this procedure and to ensure that the procedure is being followed.
- 3.3 It is the responsibility of QC Laboratory to keep this procedure current with latest Ion Labs Practice.

4.0 Definitions

- 4.1 QC – Quality Control

5.0 References

- 5.1 MDPI Technologies, 2018, 6, 84, Method <2.2.1>

6.0 Procedure

6.1 Equipment

- 6.1.1 UV/VIS Spectrophotometer

- 6.1.2 Analytical Balance
- 6.1.3 Thermometer
- 6.1.4 Sonicator
- 6.1.5 Auto Pipette
- 6.1.6 0.45- μ m nylon membrane syringe filter
- 6.1.7 Scintillation Vials
- 6.1.8 Hot water bath

6.2 Reagents

- 6.2.1 DI Water – Fresh
- 6.2.2 Saponin (Sapogenin content 20-35%), Sigma-Aldrich (Quillaja sp.)
- 6.2.3 Vanillin
- 6.2.4 Ethyl Alcohol, Sigma-Aldrich, Anhydrous Reagent grade
- 6.2.5 Sulfuric Acid, Sigma-Aldrich, ACS grade

6.3 Solution Preparation

6.3.1 Vanillin Reagent Preparation

- 6.3.1.1 To a 10-mL Volumetric flask add approximately 1-gram of Vanillin.
- 6.3.1.2 Add 5-7 mL of Ethanol and handshake until all the solids dissolve.
- 6.3.1.3 Dilute to volume with Ethanol and mix well.
- 6.3.1.4 Prepare fresh.

6.3.2 72% Sulfuric Acid

- 6.3.2.1 In a beaker containing 6.6 mL of DI H₂O slowly add and mix 18.4 mL of H₂SO₄.
- 6.3.2.2 Manually stir for at least 30-seconds or until the solution is uniformly dissolved.
- 6.3.2.3 Prepare fresh.

6.3.3 Standard Preparation

- 6.3.3.1 Approximately weigh 0.1-gram of Saponin (containing 30% Sapogenin) into a 50-mL volumetric flask.
- 6.3.3.2 Dissolve and dilute to volume with Ethanol.
- 6.3.3.3 Sonicate for 20-minutes.
- 6.3.3.4 Filter with 0.45-µm syringe filter.

$$\text{Standard Conc} \left(\frac{\text{mg}}{\text{mL}} \right) = \frac{\text{Weight of Saponin (mg)}}{\text{Dilution volume (mL)}} \times \frac{\text{Saponin Purity}}{100}$$

6.3.4 Sample Preparation

- 6.3.4.1 Approximately weigh 0.1 gram of Sample into a 50 mL volumetric flask.
- 6.3.4.2 Dissolve and dilute to volume with Ethanol.
- 6.3.4.3 Sonicate for 20-minutes.
- 6.3.4.4 Filter with 0.45-µm nylon membrane syringe filter.

$$\text{Sample Conc} \left(\frac{\text{mg}}{\text{mL}} \right) = \frac{\text{Weight of Sample (mg)}}{\text{Dilution volume (mL)}}$$

6.4 Testing

- 6.4.1 Three separate scintillation vials are taken and labelled Blank, Standard, and Sample respectively.
- 6.4.2 Into each vial with an auto pipette add solutions as per the table listed below;

Component	Reagent Blank (mL)	Standard (mL)	Sample (mL)
Extraction Solvent	0.25	-	-
Standards in Extraction Solvent	-	0.25	-
Sample in Extraction Solvent	-	-	0.25
Vanillin 8% (w/v) in Ethanol	0.25	0.25	0.25
72% Sulfuric Acid (v/v) in H ₂ O	2.5	2.5	2.5

- 6.4.3 Handshake the above solutions for 20-seconds to mix.

- 6.4.4 The solutions are kept on shaking hot water bath for 15-minutes at 60°C.
- 6.4.5 Cool to room temperature.
- 6.4.6 Measure and record the absorbance of the solutions at 560-nm using a UV/VIS Spectrophotometer.

6.5 Calculation

$$\% \text{Total Saponins} = \frac{\text{Standard Conc}}{\text{Sample Conc}} \times \frac{\text{Sample Absorbance}}{\text{Standard Absorbance}} \times 100$$

7.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	9/17/24	New Procedure	N/A	Naseem M.