ION LABS QUALITY FIRST	Standard Operating Procedure Mangiferin Determination by HPLC-UV	SOP Number D-739	Revision 0
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Written by/ Date	Reviewed by/ Date	Approved by/ Date
04-16-21	04/16/21	04/16/21
Title: Analytical Development Scientist	Title: Analytical Development Scientist	Title: Analytical Development Manager

1.0 Purpose

This document describes the analytical procedure for the determination of Mangiferin in raw materials.

2.0 Scope

This procedure applies to the identification and quantification of Mangiferin in raw materials. This method was validated under protocol PRTCL-21-0013.

3.0 Responsibility

3.1 It is the responsibility of QC and Analytical chemists who have verified their ability to execute this procedure to follow this procedure.

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

3.3 It is the responsibility of the QC Laboratory Management and AD Personnel to keep this procedure current with the associated monographs and laboratory practices.

4.0 Definitions

4.1 QC – Quality Control

4.2 AD – Analytical Development

4.3 DMSO - Dimethylsulfoxide

4.4 MeOH – Methanol

4.5 H_3PO_4 – Phosphoric Acid

4.6 ACS – American Chemical Society

4.7 HPLC – High Performance Liquid Chromatography

4.8 UV – Ultraviolet (Detection)

5.0 References

5.1 PRTCL-21-0013 – Validation of an Analytical Method for the Determination of Mangiferin by HPLC-UV

6.0 Supplies

6.1 Chemicals – All reagents are ACS grade or better.

6.1.1 Milli-Q Water

6.1.2 MeOH

6.1.3 DMSO

6.1.4 H_3PO_4

6.1.5 Mangiferin Reference Standard

6.2 Supplies and Glassware

6.2.1 HPLC vials, 12mm X 32mm with screw cap enclosures w/ septa

6.2.2 Volumetric glassware and/or adjustable pipettes and tips

6.2.3 Weigh paper and/or funnels

6.2.4 Syringes with 0.45µ Nylon Syringe Filters

6.3 Equipment

6.3.1 Suitable gradient HPLC system consisting of a pump, autosampler, column oven and UV detector with a chromatographic data handling system

6.3.2 Analytical Balance

6.3.3 Wrist Action Shaker

6.3.4 Sonicator Bath

7.0 Procedure

7.1 Mobile Phase & Diluent Preparation

7.1.1 Mobile Phase

7.1.1.1 Mobile Phase A: Add 2000 µL of H_3PO_4 to 1000 mL of water and mix well.

7.1.1.2 Mobile Phase B: MeOH

7.1.2 Extraction Solvent

7.1.2.1 Combine equal volumes of MeOH and DMSO and mix well.

7.1.3 Diluent

7.1.3.1 MeOH

7.1.4 Preparations may be scaled as necessary

7.2 Standard Preparation

7.2.1 Accurately weigh and transfer about 25 mg of Mangiferin reference standard into a 100 mL volumetric flask. Add ~50 mL of Extraction Solvent and shake mechanically for ten minutes.

7.2.2 QS to volume and sonicate for five minutes. Cool to ambient and mix well – this is the Mangiferin Stock. Dilute the Mangiferin Stock 1:5 with Diluent – this is the Mangiferin Working Standard.

7.3 Sample Preparation

7.3.1 The validated range for the analytical method is 0.0306 – 0.0713 mg/mL.

7.3.2 Extract sufficient sample (based on the manufacturer assay value) with Extraction Solvent in order to generate a concentration that is within the validated linear range.

7.3.3 Samples can be dissolved in Extraction Solvent at any volume starting from 100mL. The volume chosen must be in the solubility range of Mangiferin (validated at ~0.25 mg/ml). To manage large volumes, the sample can be initially dissolved in a smaller volume that is within the solubility range and a portion further diluted with Diluent to bring the Mangiferin concentration into the linear range.

7.3.4 Fill the flask to about 50% of the chosen volume with Extraction Solvent and shake mechanically for 10 minutes. QS to volume then sonicate for 5 minutes. Cool to ambient, perform further dilutions as required using Diluent then filter a 5ml aliquot for analysis, discarding the first 3-4ml of filtrate.

7.3.5 For raw materials being analyzed for the first time using this method, an in process validation is required to demonstrate spectral purity, baseline separation of peaks, and extraction efficiency as a part of system suitability before data can be reported using this method.

7.4 HPLC Parameters

7.4.1 Column: Restek Raptor ARC-18, 4.6 x 150mm, 2.7µm (Or Equivalent)

7.4.2 Column Temperature: 45°C

7.4.3 Flow rate: 0.5 mL/min

7.4.4 Mobile Phase: Gradient

7.4.4.1

Time, min	%B (MeOH)
0.00	10
2.00	10
14.00	80
14.10	10
20.00	10

7.4.5 Wavelength: 258 nm

7.4.6 Injection Volume: 5 µL

7.4.7 Run Time: 20 minutes

7.4.8 3-D Spectral Range (for Identification) - 220nm to 400nm

7.5 Recommended Sequence

7.5.1 Make at least 2 injections of the Diluent.

7.5.2 Make at least five (5) injections of Mangiferin Working Standard.

7.5.3 Make a single injection of each Sample Preparation.

7.6 System Suitability Requirements

7.6.1 The %RSD of five (5) consecutive standard injections is NMT 2.0%

7.6.2 If present, any interference in the diluent should be subtracted out of the sample and standard peak areas.

7.7 Example calculations for determining raw material % assay:

7.7.1 $$\% = \frac{R_u}{R_s} \times \frac{Wt_{std} \times P}{V_{std}} \times \frac{1}{SA} \times \frac{V_{spl}}{1} \times 100$$

R_u Sample peak area

R_s Mean (n=5) standard peak area

Wt_{std} Weight of the reference standard

V_{std} Volume of the standard preparation accounting for dilutions in mL

P Purity of the reference standard in decimal format

SA Sample amount

V_{spl} Volume of the sample preparation accounting for dilutions in mL

7.8 System Wash, Column Wash and Column Storage

7.8.1 Wash and store the column in 75:25 ACN / Water.

8.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	04/14/21	New	N/A	C. Perry