	Standard Operating Procedure α-Mangostin Determination by HPLC with UV/Vis Spectroscopy	SOP Number D-799	Revision 1
		Effective Date 04/24/24	Page Page 1 of 8
Written by/ Date SAS 04/08/23	Reviewed by/ Date CJL 04-09-24	Approved by/ Date AJS 04/21/24	
Title: Analytical Development Scientist	Title: Analytical Development Scientist	Title: QC Laboratory Manager	

1.0 Purpose

This document describes the analytical procedure for the determination of α -Mangostin in raw materials.

2.0 Scope

This procedure applies to the identification and quantification of α -Mangostin in raw materials.

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 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 Management and/or Analytical Development 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 **ACN** – Acetonitrile

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- 4.4 **H3PO4** – Phosphoric Acid
- 4.5 **ACS** – American Chemical Society
- 4.6 **HPLC** – High Performance Liquid Chromatography
- 4.7 **UV/Vis** – Ultraviolet & Visible Electromagnetic Spectra

5.0 References

- 5.1 PRTCL-20-0083, Protocol, α -Mangostin Determination by HPLC Using UV/Vis Spectroscopy
- 5.2 RPT-20-0045, Report, -Mangostin Determination by HPLC Using UV/Vis Spectroscopy

6.0 Supplies

- 6.1 Chemicals – All reagents are ACS grade or better.
 - 6.1.1 Milli-Q Water
 - 6.1.2 ACN
 - 6.1.3 H3PO4
 - 6.1.4 α -Mangostin 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 0.45 μ Nylon Syringe Filters

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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 1000 μ L of H₃PO₄ to 1000 mL of water and mix well.

7.1.1.2 Mobile Phase B: ACN

7.1.2 Extraction Solvent = Diluent = ACN

7.1.3 Preparations may be scaled as necessary

7.2 Stock Standard

7.2.1 Accurately weigh and transfer about 25 mg of α -Mangostin reference standard into a 50 mL volumetric flask.

7.2.2 Add 30 mL of Diluent, and sonicate for five minutes.

7.2.3 Equilibrate to ambient temperature, dilute to volume with Diluent, and mix well.

7.3 Working Standard

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7.3.1 Transfer 5.0 mL of the Stock Standard to a 25-mL volumetric flask.

7.3.2 Dilute to volume with Diluent, and mix well.

7.4 Sample Preparation

7.4.1 The validated range for the analytical method is 0.049 – 0.247 mg/mL.

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

7.4.3 Samples can be dissolved in Diluent at any volume starting from 100mL. The volume chosen must be in the solubility range of α -Mangostin (validated at ~100 μ g/ml).

7.4.4 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 to bring the α -Mangostin concentration into the linear range.

7.4.5 Fill the flask to about 60% of the chosen volume with Diluent and shake mechanically for 10 minutes. Dilute to volume, then sonicate for 5 minutes. Equilibrate to ambient temperature, and perform further dilutions as required using Diluent.

7.4.6 Filter a 5ml aliquot for analysis, discarding the first 3-4ml of filtrate. Alternatively, centrifuge a portion at 10,000 rpm for 5 min to remove particulates.

7.5 HPLC Parameters

7.5.1 Column: Phenomenex Luna C₁₈ (2), 4.6 x 250mm, 5 μ m (Or Equivalent)

7.5.2 Column Temperature: 35°C

7.5.3 Flow rate: 1.5 mL/min

7.5.4 Mobile Phase: Gradient

7.5.4.1	Time (min)	%A	%B
	0	40	60
	5	40	60
	7	30	70
	20	25	75
	21	5	95
	26	5	95
	28	40	60
	35	40	60

7.5.5 Wavelength: 318 nm

7.5.6 Injection Volume: 5 μ L

7.5.7 Run Time: 35 minutes

7.5.8 Recommended 3-D Spectral Range (for Identification) - 205nm to 350nm

7.6 Recommended Sequence

7.6.1 Make at least 2 injections of the Diluent.

7.6.2 Make at least five (5) injections of α -Mangostin Working Standard.

7.6.3 Make a single injection of each Sample Preparation.

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7.6.4 Make a single injection of the Standard Solution after every ten (10) sample injections and/or at the end of a run.

7.7 System Suitability Requirements

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

7.7.2 The %RSD of all standard injections is NMT 3.0%.

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

7.8 Example calculations for determining finished product raw material % assay:

$$7.8.1 \quad \% = \frac{R_u}{R_s} \times \frac{Wt_{std} \times P}{V_{std}} \times \frac{1}{SA} \times \frac{V_{spl}}{1} \times 100$$

R_u Sample peak area

R_s Mean (n=5) standard peak area

Wt_{std} Weight of the reference standard in mg

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

P Purity of the reference standard in decimal format

SA Sample amount in mg

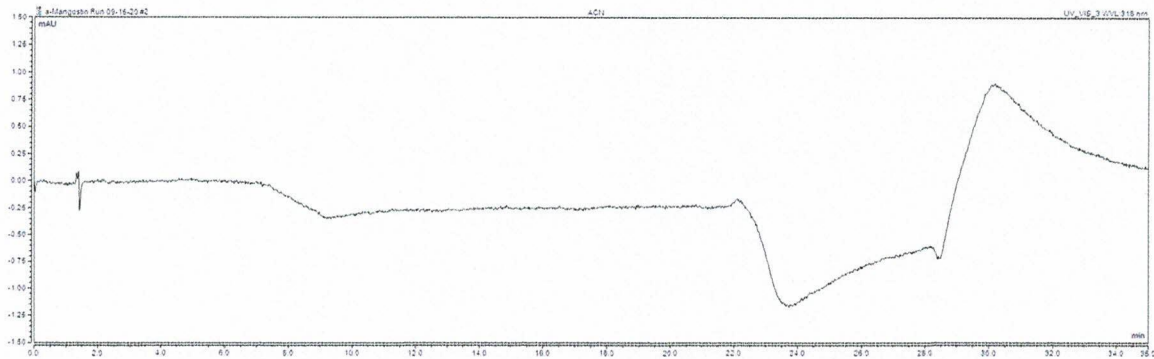
V_{spl} Volume of the sample preparation accounting for dilutions in mL

7.9 System Wash, Column Wash and Column Storage

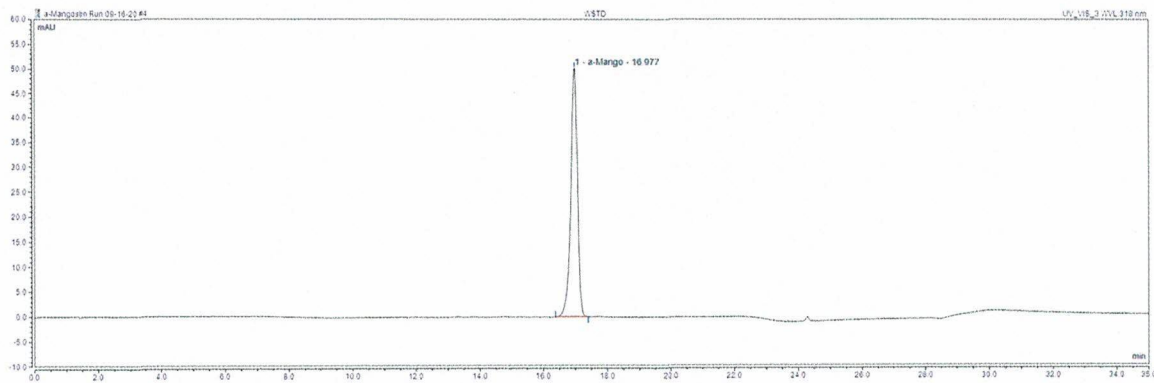
7.9.1 Wash and store the column in 60:40 ACN / Water.

8.0 Example Chromatograms

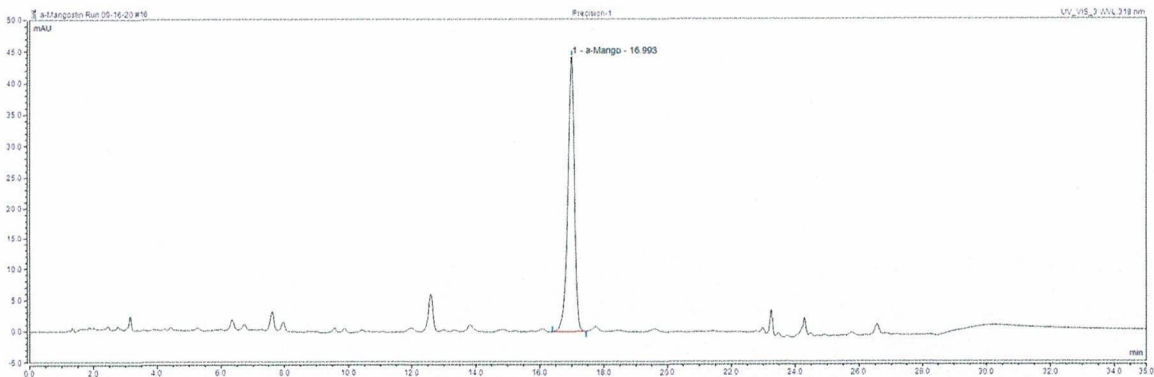
8.1 Typical Diluent Chromatogram



8.2 Typical Working Standard Chromatogram



8.3 Typical Raw Material Chromatogram



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9.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	10/08/20	New	N/A	C. Perry
1	04/03/24	Scheduled review: edit for consistency with current methods	CC-24-0130	S. Sassman