1.0 Purpose

The purpose of this procedure is to define the method for the trace analysis and/or identification of monacolin K and its acid form in raw materials and dietary supplements using HPLC and UV/VIS spectrophotometry.

2.0 Scope

This procedure applies to the quantification and identification of monacolin K and its acid form. This procedure applies to the identification of monacolin K forms in raw materials and finished products using spectral analysis and quantitation of monacolins using monacolin K as the standard.

3.0 Responsibility

3.1 It is the responsibility of QC Chemists to understand and work within the guidelines of this procedure.

3.2 It is the responsibility of QC Laboratory Management to ensure compliance with this procedure.

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 **HPLC** – High Performance Liquid Chromatography

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

4.3 **Lovastatin** – Monacolin K

4.4 **H_3PO_4** – Phosphoric Acid

4.5 **NaOH** – Sodium Hydroxide

4.6 **ACN** – Acetonitrile

4.7 **CofA** – Certificate of Analysis

4.8 **H_2O** – Water

5.0 References

5.1 MV-LAB-14-002, Protocol, Monocalin K Determination by HPLC

5.2 Lovastatin USP Monograph

6.0 Supplies

6.1 Chemicals: All reagents are HPLC grade or better.

6.1.1 H_2O ($\geq$ 18.2 MΩ·cm)

6.1.2 ACN

6.1.3 H_3PO_4

6.1.4 NaOH

6.2 Glassware

6.2.1 HPLC vials, 12mm x 32mm with screw cap enclosures with septa

6.2.2 Scintillation Vials

6.2.3 1L Mobile Phase Container

6.2.4 Volumetric Flasks

6.2.5 Volumetric Pipets

6.3 Disposables

6.3.1 200µL, 1mL and 10mL Pipette Tips

6.3.2 16mL Test Tubes

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

6.3.4 Nylon Syringe Filters, 0.45µm

6.3.5 Weigh paper

6.4 Equipment

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

6.4.2 Analytical Balance

6.4.3 Vortex

6.4.4 Stir Plate

6.4.5 Eppendorf Micro-Centrifuge

6.4.6 200µL, 1mL and 10mL Pipettes

7.0 Preparation of Mobile Phase, Diluent, Samples, and Standards

7.1 Mobile Phase A (0.1% H_3PO_4 in H_2O)

7.1.1 Transfer 1000 mL of H2O to a mobile phase bottle.

7.1.2 Add 1.0 mL of H_3PO_4, and mix well.

7.2 Mobile Phase B (100% ACN)

7.3 Diluent (MeOH:H_2O:H_3PO_4 775:225:0.225)

7.3.1 Transfer 775mL of MeOH to a mobile phase bottle.

7.3.2 Add 225mL of H2O.

7.3.3 Add 225 µL of H_3PO_4, and mix well.

7.4 Stock Standard Preparation

7.4.1 Accurately weigh and transfer about 25 mg of reference material into a 100-mL volumetric flask.

7.4.2 Dilute to volume with Diluent.

7.4.3 Sonicate for 10 minutes to dissolve.

7.5 Working Standard Preparation

7.5.1 Transfer 2.0 mL of the Stock Standard to a 50-mL volumetric flask.

7.5.2 Dilute to volume with Diluent, and mix well.

7.6 Sample Preparation

7.6.1 The linear range of the method is 1 µg/mL – 30 µg/mL. The analyte concentration in all working sample preparations must be within this range.

7.6.2 Samples can be dissolved in Diluent at any volume starting from 50mL and any weight ≥ 50mg.

7.6.3 The sample is suspended in one third the final volume and sonicated for four minutes. The sample is cooled to room temperature before bringing to volume. At volume, the sample is sonicated for an additional six minutes and allowed to cool to room temperature before use.

7.6.4 Before injection, insoluble matter should be removed via filtration using a nylon syringe filter. Discard at least 0.5mL of the sample before collecting filtrate. Dilute filtrate as needed then add 1mL of the final dilution to an HPLC vial for analysis.

7.6.4.1 Alternatively, samples and standards can also be centrifuged at 6000 RPM for 5 minutes in an Eppendorf centrifuge to pellet insoluble matter.

7.6.5 For non-Red Yeast Rice raw materials being analyzed for the first time using this method, in-process verification is required to demonstrate spectral purity and extraction efficiency before the method can be implemented.

8.0 Test Conditions

8.1 Isocratic

Time	%A	%B	Gradient Type
0.00	35	65	0
12.00	35	65	0

8.2 Column – Luna C18 (2), 5µm, 100Å, LC column, 150mm x 4.6mm or equivalent.

8.3 Flow Rate – 1.0mL/min

8.4 UV Detection – 238nm

8.5 3-D Spectral Range – 200nm to 300nm

8.6 Injection Volume - 20µL

8.7 Column Temperature – 40°C

8.8 Recommended Sequence

8.8.1 Make at least 2 injections of a Blank (Diluent).

8.8.2 Make five injections of the Working Standard.

8.8.3 Make a single injection of each Sample Preparation.

8.8.4 Make a single injection of the Working Standard after every six samples and at the end of the run.

8.9 System Suitability

8.9.1 The %RSD of five consecutive injections of Working Standard is NMT 5.0%.

8.9.2 The %RSD of all Working Standard injections is NMT 5%.

8.10 Column Wash and Storage

8.10.1 Rinse the column with H_2O / ACN (50/50) at 1 mL/min for at least 15 min.

8.10.2 Store the column with H_2O / ACN (50/50).

9.0 Example Calculation

9.1 $$\% \text{ assay} = \frac{R_u}{R_s} \times \frac{Wt_{std} \times P}{V_{std}} \times \frac{V_{spl}}{SA} \times \frac{SS}{LA} \times 100$$

R_u Peak area of monacolin peak in the Sample Preparation

R_s Mean standard peak area

Wt_{std} Weight of 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 (solids) or mL (liquids)

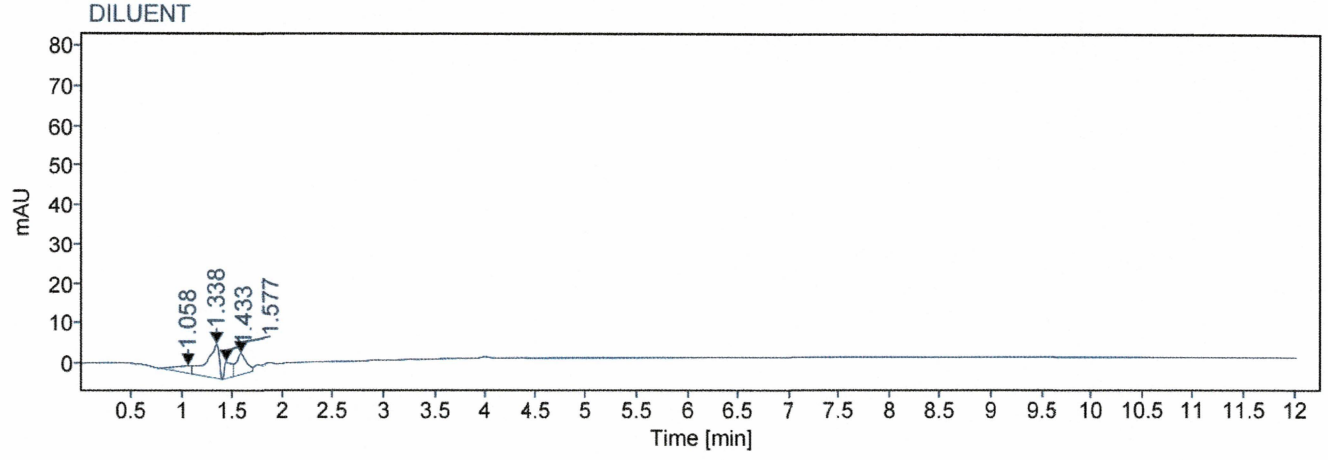
V_{spl} Volume of the sample preparation accounting for dilutions in mL

SS Serving size: Weight of a single dosage unit in mg for tablets and capsules, volume of a single serving from the theoretical formula in mL for liquids, or 1 for raw materials.

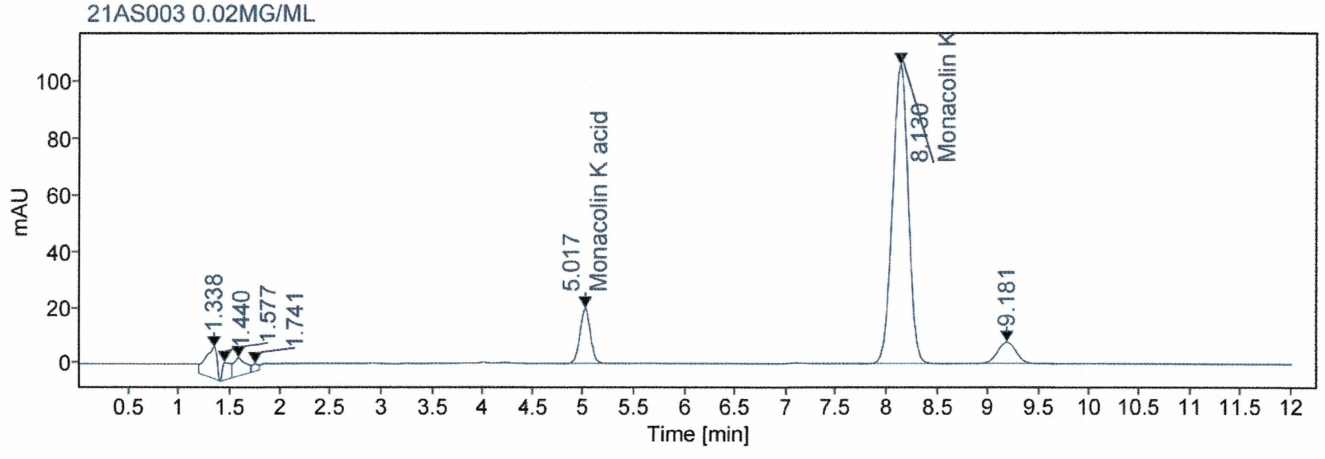
9.2 Total monacolins are calculated by summing all peaks that were determined to be monacolins by retention time and/or spectral match.

10.0 Example Chromatography

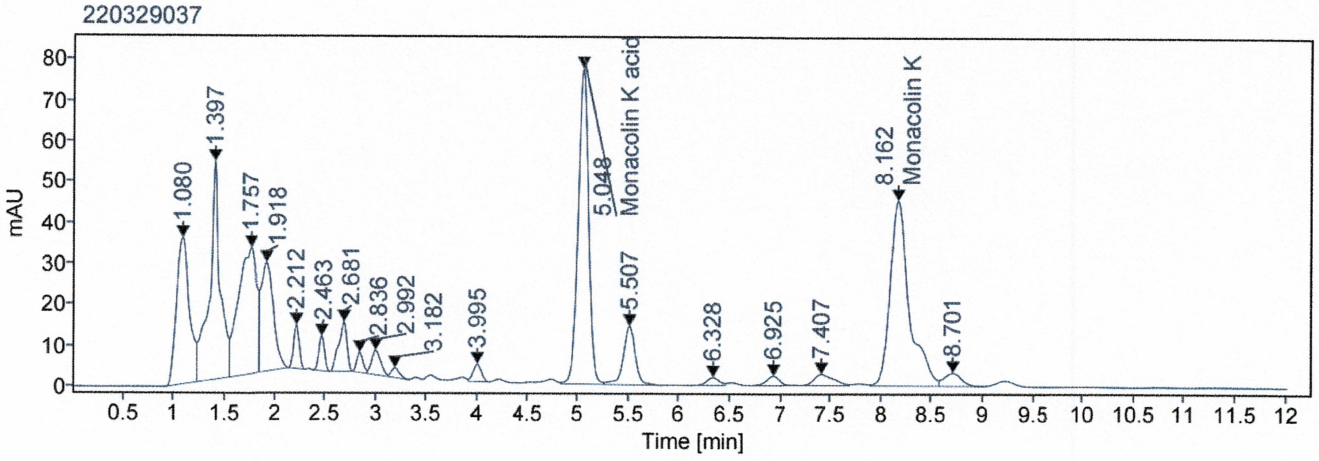
10.1 Blank



10.2 Working Standard



10.3 Sample



11.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	05/07/14	New	14-0402	B. Johns
1	09/16/16	Biennial review: Updated SOP format.	16-0849	N. Zhang
2	11/26/19	3 Year review – Updated to be in line with current procedure and practices.	19-0903	W. Gonzales
3	08/05/22	Update for consistency with current methods and lab practices, add recommended sequence section, replace requirements with system suitability section, add column wash and storage, add example chromatography	CC-22-0342	S. Sassman