ION LABS QUALITY FIRST	Standard Operating Procedure Monacolin K Determination by HPLC with UV/Vis Spectroscopy	SOP Number D-764	Revision 0
		Effective Date 06/03/21	Page Page 1 of 6
Written by/ Date 04-16-21	Reviewed by/ Date 04/16/21	Approved by/ Date 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 Monacolin K (MK) in raw materials and finished products.

2.0 Scope

This procedure applies to the identification and quantification of Monacolin K in raw materials and finished products. Monacolin K can be present in two forms: (1) the unhydrolyzed form interchangeably referred to as, among many other synonyms: Monacolin K, Monacolin K Lactone and Lovastatin, and (2) the hydrolyzed acid form referred to as Monacolin K Acid. Monacolin K Acid may or may not be present. If Monacolin K Acid is present, its result is expressed as equivalents Monacolin K Lactone. MK (Total) may also be reported, and is expressed as the sum of Monacolin K and Monacolin K Acid. This method was validated under Protocol PRTCL-20-0029 and reported in RPT-20-0034.

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 MK – Monacolin K

4.4 ACN – Acetonitrile

4.5 MeOH – Methanol

4.6 H3PO4 – Phosphoric Acid

4.7 NaOH – Sodium Hydroxide

4.8 ACS – American Chemical Society

4.9 HPLC – High Performance Liquid Chromatography

4.10 UV/Vis – Ultraviolet & Visible Electromagnetic Spectra

5.0 References

5.1 PRTCL-20-0029 – Monacolin K Determination by HPLC Using UV/Vis Spectroscopy

5.2 RPT-20-0034 – Monacolin K 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

6.1.3 ACN

6.1.4 H3PO4

6.1.5 1N NaOH

6.1.6 MeOH

6.1.7 MK (Lovastatin) 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 Sonicator

6.3.4 pH Meter

7.0 Procedure

7.1 Mobile Phase & Diluent Preparation

7.1.1 Mobile Phase

7.1.1.1 Mobile Phase A: Add 1000 µL of H3PO4 to 1000 mL of water and mix well.

7.1.1.2 Mobile Phase B: ACN

7.1.2 Extraction Solvent / Diluent

7.1.2.1 pH 4.0 Buffer: Add 950 µL of H3PO4 to 950 mL of water and mix well, then pH to 4.0 with 1N NaOH.

7.1.2.2 Diluent / Extraction Solvent: Add 250 mL of the pH 4.0 Buffer to 750 mL of MeOH and mix well.

7.1.3 Preparations may be scaled as necessary

7.2 Standard Preparation

7.2.1 Accurately weigh and transfer about 25 mg Lovastatin reference standard into a 100 mL volumetric flask. Add ~50 mL of Diluent and sonicate for five minutes. Add ~ 45 mL of Diluent and sonicate for an additional five minutes.

7.2.2 Cool to ambient then QS to volume with Diluent and mix well – this is the Lovastatin Stock. Dilute the Lovastatin Stock 4:100 with Diluent – this is the Lovastatin Working Standard.

7.3 Sample Preparation

7.3.1 The validated range for the analytical method is 0.17 – 10.86 µg/mL.

7.3.2 For finished products, extract sufficient sample with Diluent in order to generate an MK concentration that is within the validated linear range.

7.3.3 Prepare raw materials like standards, remembering that red yeast rice powders generally contain approximately 0.2% MK (Total).

7.3.4 Finished product samples can be dissolved in Diluent at any volume starting from 100mL. The volume chosen must be in the solubility range of MK (validated at approximately 16 µg/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 to bring the MK concentration into the linear range.

7.3.5 Fill the flask to about 50% of the chosen volume with Diluent and sonicate for 5 minutes. Then fill the flask to about 95% of the chosen volume with Diluent and sonicate for an additional 5 minutes. Cool to ambient then QS to volume with Diluent.

7.3.6 Perform further dilutions as required using Diluent. Filter a 5ml aliquot for analysis, discarding the first 3-4ml of filtrate.

7.3.7 For finished products or 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: Waters Cortecs Shield RP18, 4.6 x 150mm, 2.7µm (Or Equivalent)

7.4.2 Column Temperature: 40°C

7.4.3 Flow rate: 1.0 mL/min

7.4.4 Mobile Phase: Isocratic 55% A / 45% B

7.4.5 Wavelength: 239 nm

7.4.6 Injection Volume: 10 µL [LOQ = 0.17 µg/ml. For peaks presenting with S/N <10, report as < (0.17 µg/ml / Sample Conc in µg/ml) * 100%.]

7.4.7 Run Time: 20 minutes

7.4.8 3-D Spectral Range (for Identification) - 210nm to 350nm

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 MK Working Standard.

7.5.3 Make a single injection of each Sample Preparation.

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

7.6 System Suitability Requirements

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

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

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

7.7 Example calculations for determining % Analyte:

7.7.1 $$\%\ \text{MK (Lactone)} = \frac{R_u}{R_s} \times \frac{Wt_{std} \times P}{V_{std}} \times \frac{V_{spl}}{SA} \times 100$$

R_u Sample peak area

[**Note:** To calculate % MK Acid, use the area of the Acid peak. To calculate % MK (Total), add the peak areas due to both the Acid (if present) + Lactone forms.]

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

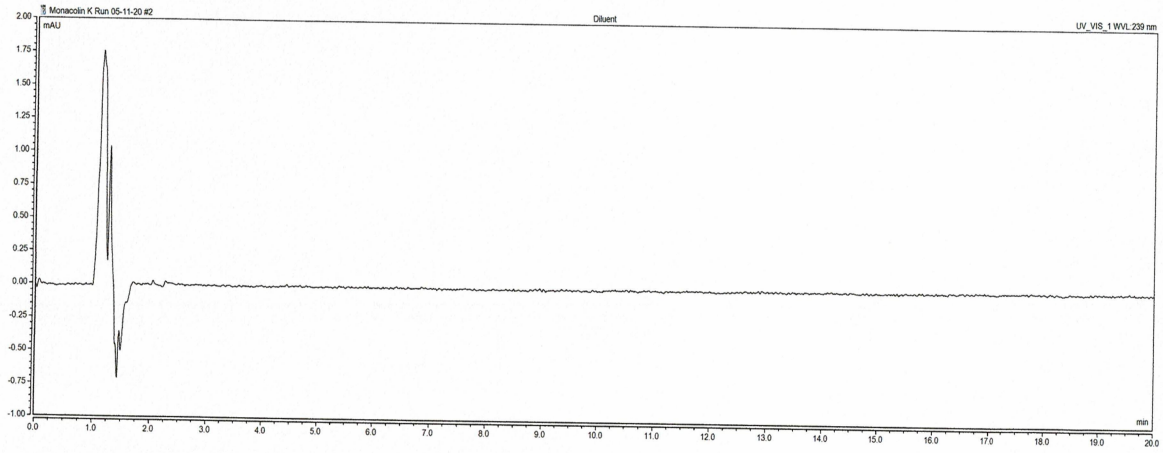
V_{spl} Volume of the sample preparation accounting for dilutions in mL

7.8 System Wash, Column Wash and Column Storage

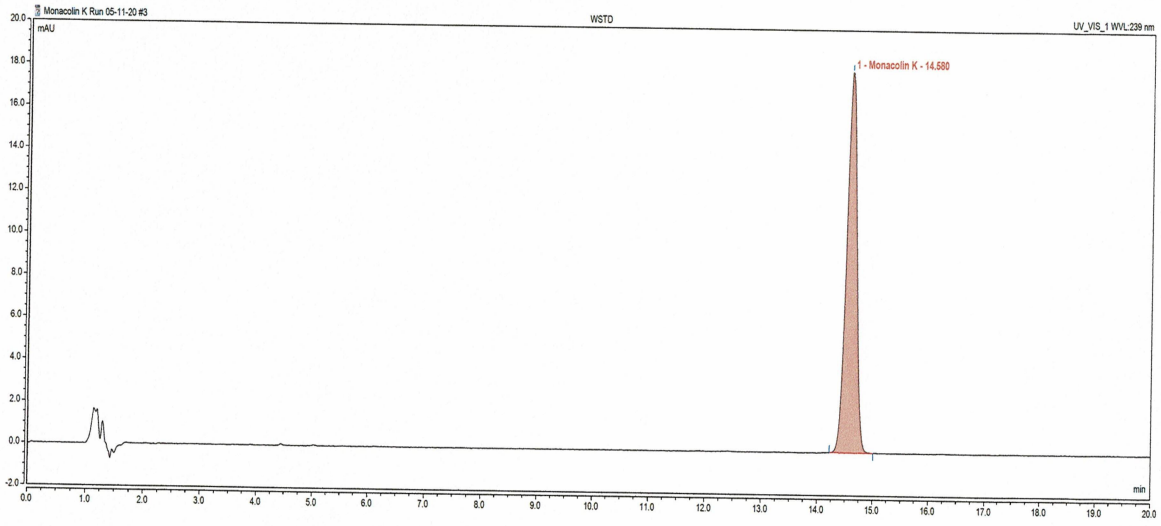
7.8.1 Wash and store the column in 50:50 ACN / Water.

8.0 Chromatograms

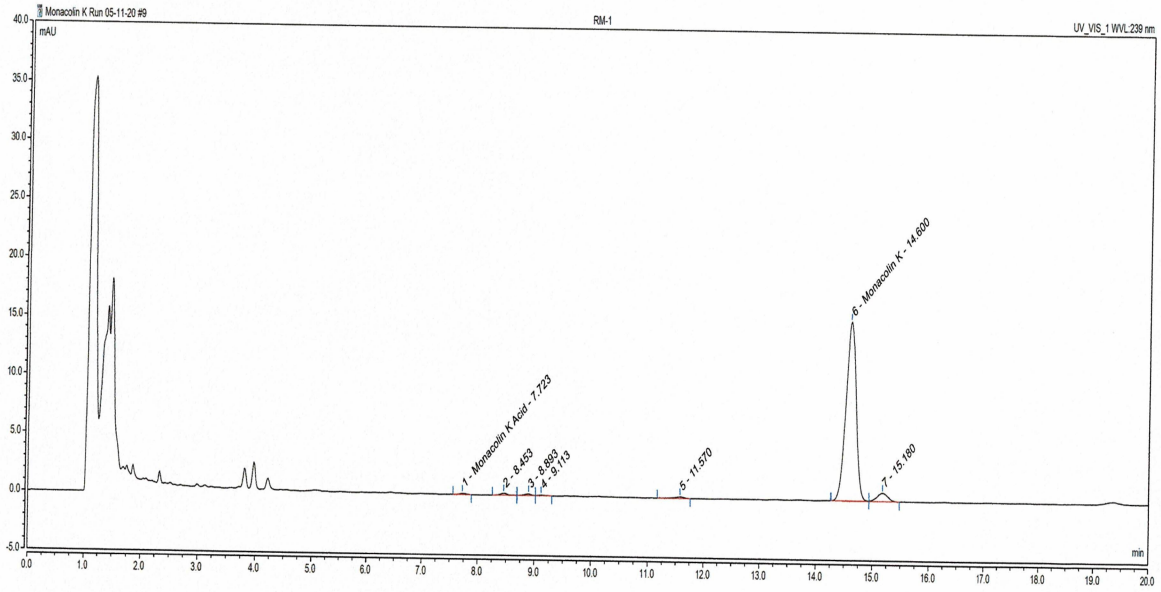
8.1 Typical Diluent Chromatogram



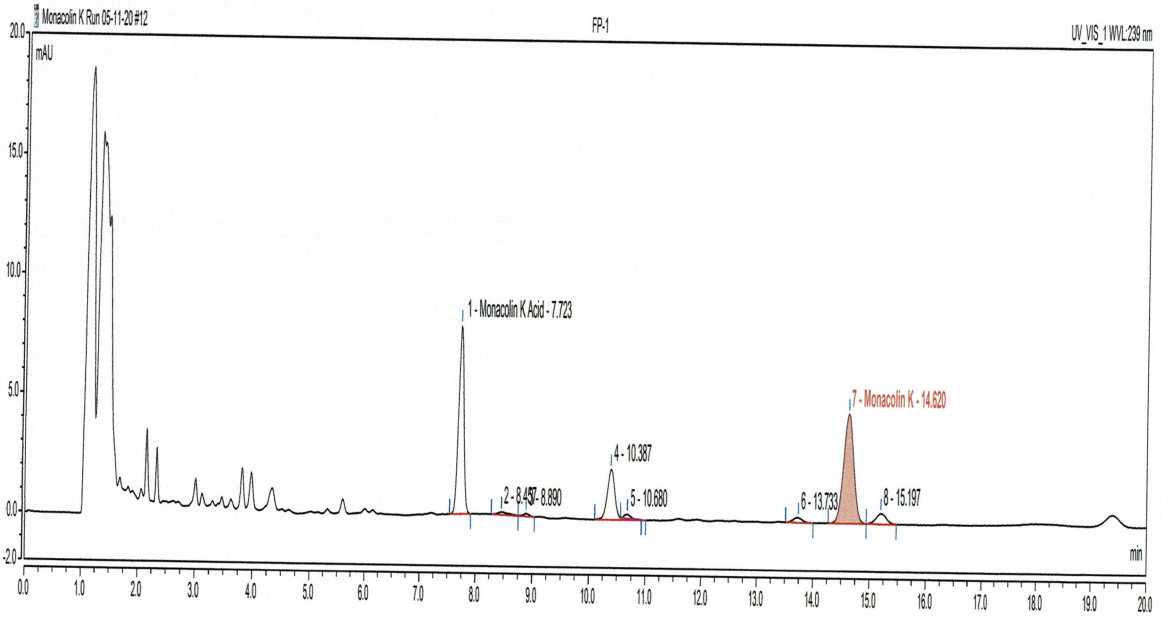
8.2 Typical Working Standard Chromatogram



8.3 Typical Raw Material Chromatogram



8.4 Typical Finished Product Chromatogram



9.0 Revision History

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