1.0 Purpose

This document describes the analytical procedure for the determination of Vitamin K1 in raw materials.

2.0 Scope

This procedure applies to the identification and quantification of Vitamin K1 in raw materials. This method was validated under protocol PRTCL-23-0019.

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 **ACN** – Acetonitrile

4.3 **EtOH** – Ethanol

4.4 **ACS** – American Chemical Society

4.5 **HPLC** – High Performance Liquid Chromatography

4.6 **UV-Vis** – Ultraviolet-Visible (Detection)

5.0 References

5.1 PRTCL-23-0019, Protocol, Validation of an Analytical Method for the Determination of Vitamin K1 by HPLC-UV

6.0 Supplies

6.1 Chemicals – All reagents are ACS grade or better

6.1.1 ACN

6.1.2 EtOH

6.1.3 Vitamin K1 (Phytonadione) Reference Standard

6.2 Supplies and Glassware **(Use Red Glassware!)**

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

6.2.2 **Red** Volumetric glassware

6.2.3 Weigh paper

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 compartment and UV-Vis detector with a chromatographic data handling system

6.3.2 Analytical Balance

6.3.3 Wrist Action Shaker

6.3.4 Sonicator

7.0 Procedure

7.1 Mobile Phase and Extraction Solvent / Diluent Preparation

7.1.1 Mobile Phase

7.1.1.1 ACN (Isocratic)

7.1.2 Extraction Solvent / Diluent

7.1.2.1 Extraction Solvent = Diluent = EtOH

7.2 Standard Preparation **(Use Red Glassware!)**

7.2.1 Prepare standard stock at ~0.6 mg/mL analyte in Extraction Solvent. Mechanically shake stock for 15 minutes at ~ ½ volume then QS to volume. Sonicate for 10 minutes then let cool to room temperature. Shake vigorously then dilute stock 2:50 with Diluent. Shake vigorously then filter a 5mL aliquot for analysis, discarding the first 3-4mL of filtrate.

7.3 Sample Preparation **(Use Red Glassware!)**

7.3.1 The validated linear range for the analytical method is 0.0097 – 0.0341 mg/mL.

7.3.2 Extract sufficient sample (based on the raw material manufacturer assay value) with Extraction Solvent in order to generate a sample stock that is ~0.6 mg/mL analyte. Mechanically shake stock for 15 minutes at ~ ½ volume then QS to volume. Sonicate for 10 minutes then let cool to room temperature. Shake vigorously then dilute stock 2:50 with Diluent. Shake vigorously then filter a 5mL aliquot for analysis, discarding the first 3-4mL of filtrate.

7.4 HPLC Parameters

7.4.1 Column: Supelco Ascentis Express RP-Amide, 2.7µm (SPP), 4.6 x 150mm

7.4.2 Column Temperature: 30°C

7.4.3 Flow rate: 1.5 mL/min

7.4.4 Run Time: 10 minutes

7.4.5 Mobile Phase: Isocratic

7.4.6 Wavelength: 248 nm

7.4.7 Injection Volume: 10 µL

7.4.8 Suggested 3-D Spectral Range (for Identification): 210nm - 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 Working Standard.

7.5.3 Make a single injection of each Sample Preparation.

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

7.6 System Suitability Requirements

7.6.1 The %RSD of five (5) consecutive standard injections is NMT 2%.

7.6.2 The %RSD of all standard injections is NMT 3%.

7.7 Example calculations for determining % Assay:

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

R_u Sample peak area

R_s Mean (All) standard peak area

Wt_{std} Weight of the reference standard, mg

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

P Purity of the reference standard in decimal format

SA Sample amount, mg

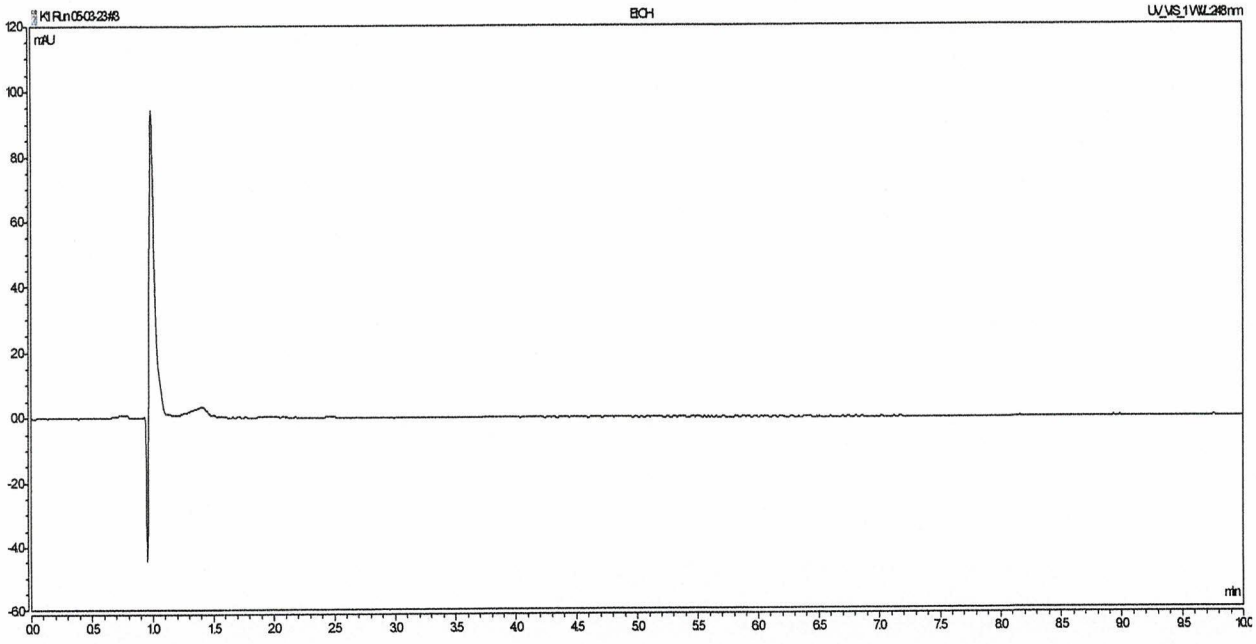
V_{spl} Volume of the sample preparation accounting for dilutions, mL

7.8 System Wash, Column Wash and Column Storage

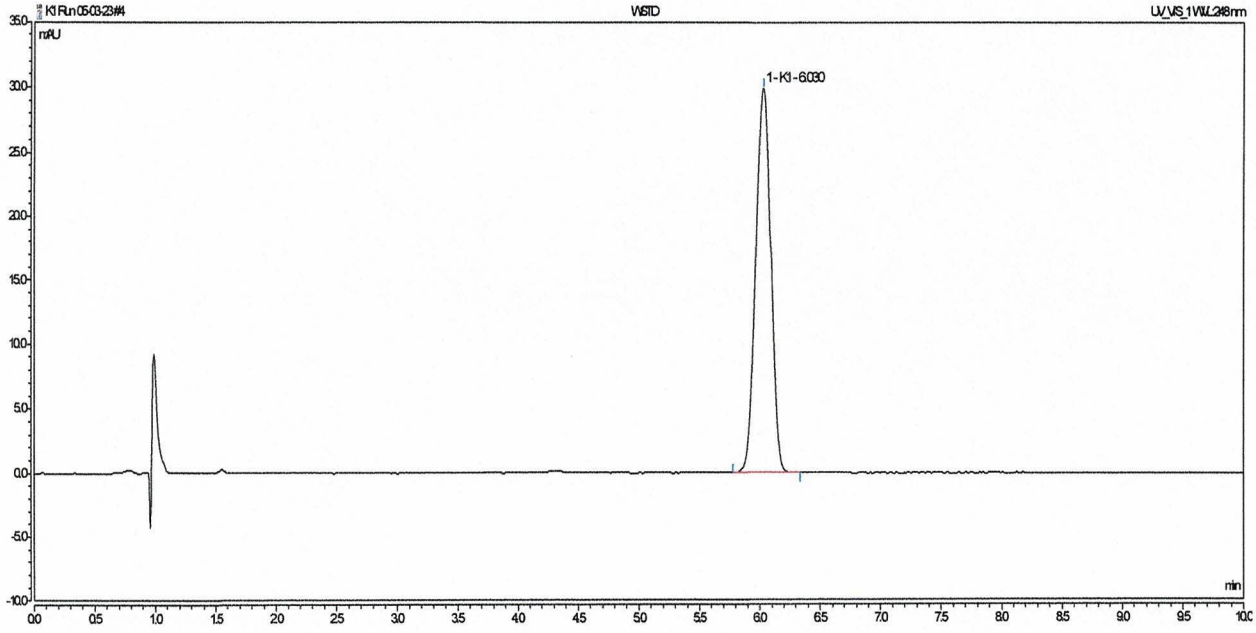
7.8.1 Wash and store the column in 100% ACN.

8.0 Chromatograms

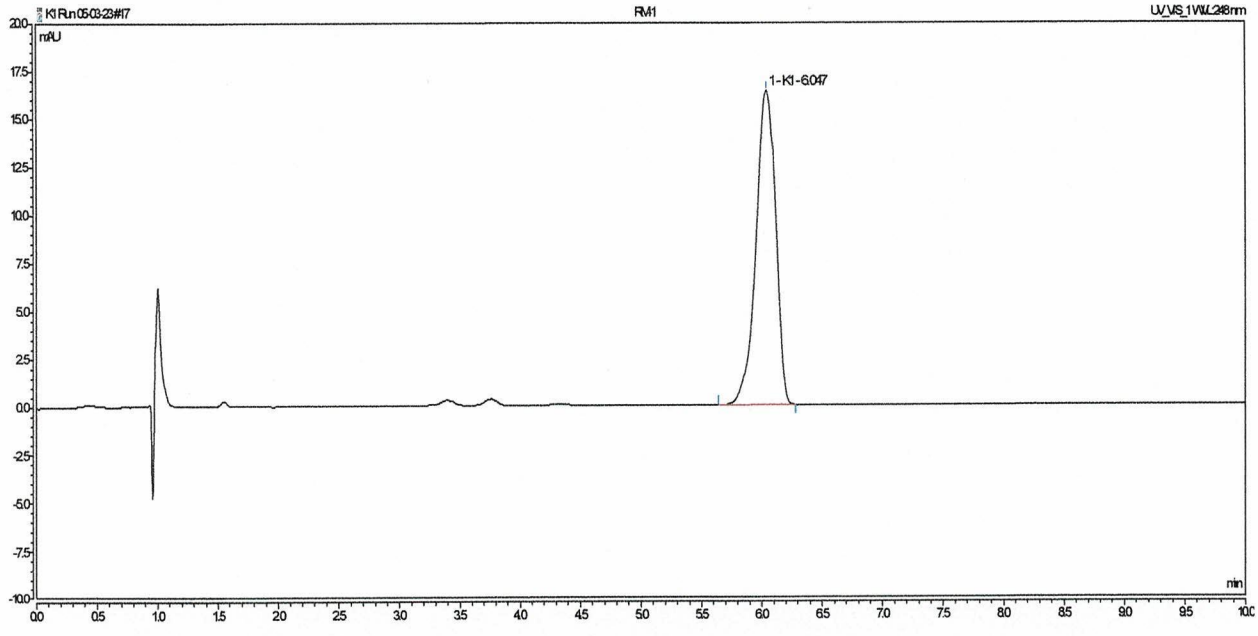
8.1 Typical Diluent Chromatogram



8.2 Typical Standard Chromatogram



8.3 Typical Raw Material Chromatogram



9.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	05/22/23	New procedure.	N/A	C. Perry