1.0 Purpose

This document describes the analytical procedure for the determination of Quercetin in raw materials and finished products.

2.0 Scope

This procedure applies to the identification and quantification of Quercetin in raw materials and finished products in the QC laboratory at Ion Labs.

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 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 **H_3PO_4** – Phosphoric Acid

4.4 **MeOH** – Methanol

4.5 **HPLC** – High Performance Liquid Chromatography

4.6 **UV/Vis** – Ultraviolet & Visible Electromagnetic Spectra

5.0 References

5.1 MV-LAB-19-118, Protocol, Quercetin Determination Using HPLC with UV/Vis Spectroscopy

5.2 RPT-21-0026, Report, Estimation of Uncertainty

6.0 Reagents, Supplies, Glassware, and Equipment

6.1 Chemicals – All reagents are HPLC grade or better

6.1.1 Milli-Q Water

6.1.3 MeOH

6.1.4 H_3PO_4

6.1.4 Quercetin 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 or funnels

6.2.4 10ml Syringes with 17mm x 0.45u 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 bath

6.3.4 Wrist Action Shaker

7.0 Procedure

7.1 Mobile Phase & Diluent Preparation

7.1.1 Mobile Phase

7.1.1.1 Combine 500 mL of MeOH, 500 mL of Milli-Q Water and 5mL of H3PO4. Mix well.

7.1.2 Diluent

7.1.2.1 MeOH.

7.1.3 Preparations may be scaled as necessary.

7.2 Standard Prep

7.2.1 Stock Std A: Accurately weigh and transfer about 20 mg of Quercetin reference standard into a 100-mL volumetric flask. Add 50mL of MeOH. Shake mechanically for 10min then sonicate for 5min. Let cool to ambient then dilute to volume with MeOH and mix well.

7.2.2 Working Std A: Transfer 5.0 mL of Stock Std A to a 50-mL volumetric flask. Dilute to volume with MeOH.

7.2.3 Prepare a second standard, Std B, as a standard recovery check.

7.3 Sample Preparation

7.3.1 Specific sample testing details are provided in each products profile. If a specific testing details section is not available, then follow preparation procedure as described below, maintaining concentration within the linear range listed below.

7.3.2 The validated range for the analytical method is 2 - 93 µg/mL.

7.3.3 For finished products, if not a powder, at least 10 dosage units are pooled and ground by mortar and pestle. Based on the dosage weight (for powders), fill weight (for capsules) or tablet weight per dose and the label claim, weigh no less than 190 mg of the pooled dosages into a suitably sized volumetric flask to generate an analyte concentration that is within the validated linearity range for the analyte being tested.

7.3.4 Prepare raw materials like standards. (However, be sure to consult the specification for expected potency, as raw material samples may not be 100%.)

7.3.5 Samples can be dissolved in MeOH at any volume starting from 50mL. The volume chosen must be in the solubility range of Quercetin (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 to bring the analyte concentration into the linear range.

7.3.6 Fill the flask to about 70% of the calculated volume with MeOH and shake mechanically for 10 minutes. Sonicate for 10 minutes, cool to room temperature and bring up to volume with MeOH. Filter a portion for use in subsequent dilutions.

7.3.7 Perform further dilutions as required using MeOH.

Note: Sample preparations must be injected on the same day as the preparation. Degradation of Quercetin may occur in extended hold times.

7.4 HPLC Parameters

7.4.1 Column: Agilent InfinityLab Poroshell 120 EC-C18, 4.6 x 100mm, 2.7u or equivalent

7.4.2 Column Temperature: 30°C

7.4.3 Flow rate: 0.7 mL/min

7.4.4 Wavelength: 370 nm

7.4.5 Injection Volume: 5 µL

7.4.6 Run Time: 15 minutes.

7.4.7 Recommended 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 five (5) injections of Working Std A.

7.5.3 Make two (2) injections of Working Std B.

7.5.4 Make a single injection of each Sample Preparation.

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

7.6 System Suitability Requirements

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

7.6.2 The % recovery of Working Standard A, using Working Standard B is 98-102%.

7.6.3 The %RSD of all Working Std A injections is NMT 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 finished product % label or raw material % purity

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

R_u	Sample peak area
R_s	Mean 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 (solids) or mL (liquids)
V_{spl}	Volume of the sample preparation accounting for dilutions in mL
SS	Serving size: Average weight of ten dosage units in mg for tablets, fill weight for capsules, mass of a single serving in mg for powders, volume of a single serving from the theoretical formula in mL for liquids, or 1 for raw materials.

LA Label amount in mg of Quercetin (use 1 for raw materials)

7.8 Reporting Results

7.8.1 The expanded uncertainty of the method is 1.1% with a coverage factor of 2.

7.8.2 Results should include the expanded uncertainty of the method along with the coverage factor in the following format:

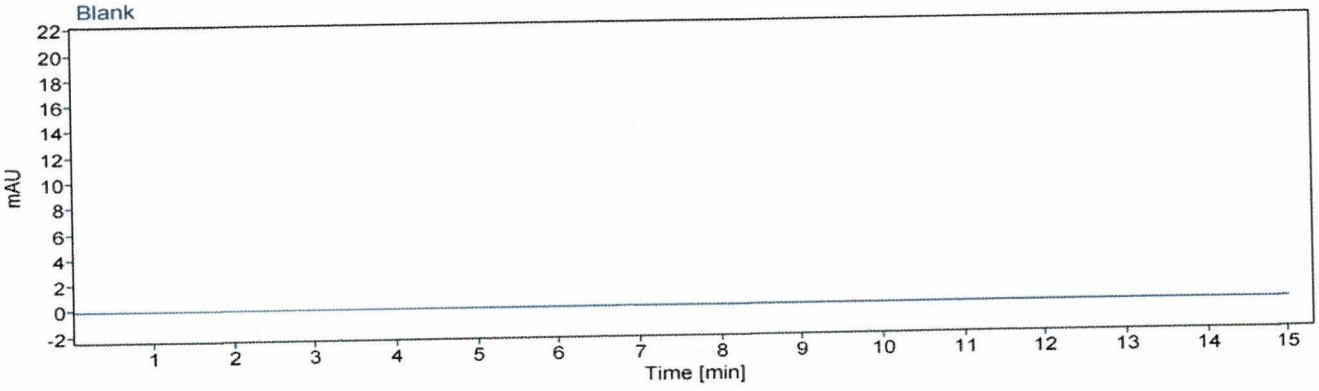
7.8.2.1 102% of Label Claim, U = ± 1.1% k = 2

7.9 Column Wash and Storage

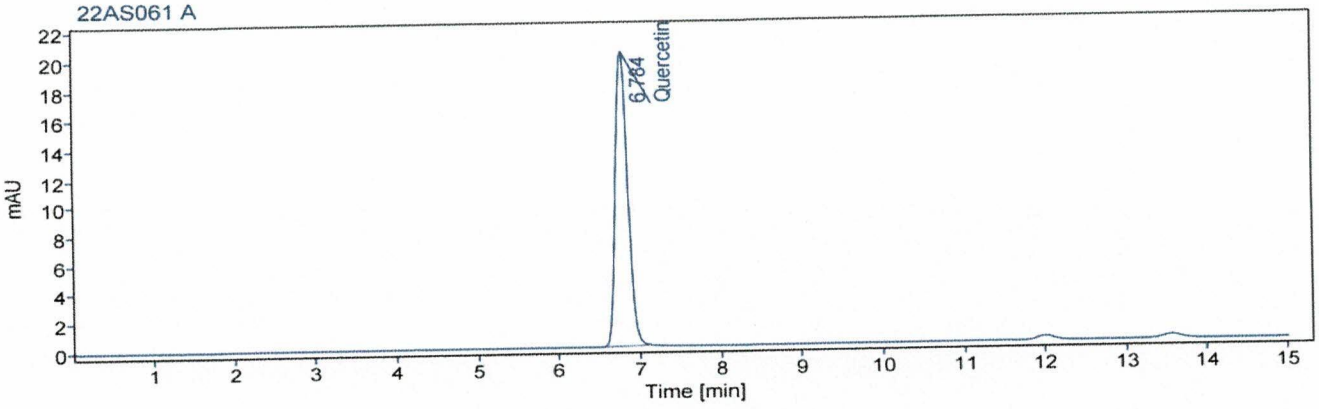
7.9.1 Wash and store the column in 50:50 MeOH / Milli-Q Water.

8.0 Chromatograms

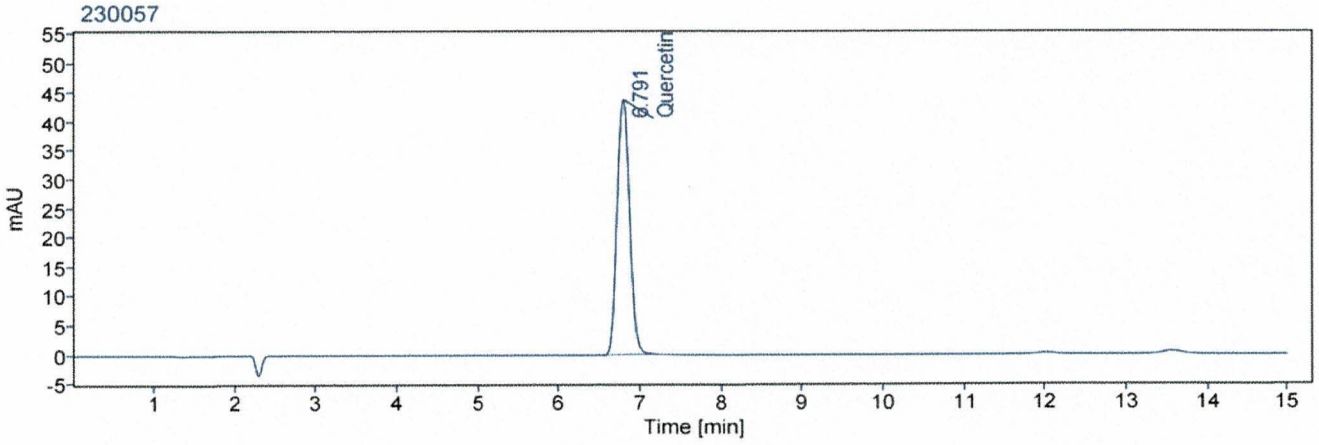
8.1 Typical Diluent Chromatogram



8.2 Typical Working Standard Chromatogram



8.3 Typical Sample Chromatogram



9.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	06/26/19	New.	N/A	C. Perry
1	11/09/21	Updated for ISO 17025 requirements.	CC-21-0426	J. Sassman
2	02/23/23	Change Std B prep to be separate weigh off, add instruction to check the product profile for test details, remove language requiring in-process validation for new products, make chromatograms look better, correct the linear range.	CC-23-0093	S. Sassman
3	12/12/23	Added statement regarding sample stability.	CC-23-0598	J. Sassman