1.0 Purpose

The purpose of this procedure is to define the method for the determination of ascorbyl palmitate in raw materials and finished products by HPLC-UV.

2.0 Scope

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

3.0 Responsibility

3.1 It is the responsibility of QC 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 ensure that this procedure is being followed.

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 **QC** – Quality control

4.2 **HPLC-UV** – High Performance Liquid Chromatography with Ultraviolet Detection

4.3 **ACN** – Acetonitrile

4.4 **H_3PO_4** – Phosphoric acid

4.5 **H_2O** – Deionized water (>18MΩ·cm)

5.0 References

5.1 PRTCL-24-0038, Protocol, Validation of a Method for the Determination of Ascorbyl Palmitate by HPLC/UV

5.2 D-903, SOP, Conversion Factors Used in Analytical Determinations and New Product Formulations

6.0 Supplies

6.1 Chemicals

6.1.1 Ascorbyl palmitate reference standard

6.1.2 ACN

6.1.3 H_3PO_4

6.1.4 Methanol

6.1.5 Citric acid

6.1.6 Ascorbic acid

6.2 Glassware and Disposables

6.2.1 Volumetric glassware as required for standard and sample preparations

6.2.2 HPLC vials, 2mL with screw-cap enclosures and septa

6.2.3 Tips for adjustable pipettes

6.2.4 0.45 µm nylon or PVDF syringe filters

6.2.5 10-mL plastic syringes with luer lock fitting

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 Micro balance

6.3.4 Wrist action shaker

6.3.5 Adjustable pipettes

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

7.1 Mobile Phase A (0.1% H_3PO_4)

7.1.1 Transfer 1000 mL of H_2O to a suitable container.

7.1.2 Add 1.0 mL of H_3PO_4, and mix well.

7.1.3 Scale as necessary. Mobile phase A has an expiry of one month.

7.2 Mobile Phase B

7.2.1 Use 100% ACN.

7.2.2 Mobile phase B has an expiry of three months.

7.3 Diluent A (1 g/L citric acid + 1g/L ascorbic acid in methanol)

7.3.1 Transfer 1.0 g of citric acid to a suitable container.

7.3.2 Add 1.0 g of ascorbic acid.

7.3.3 Add 1000 mL of methanol.

7.3.4 Sonicate or stir until completely dissolved.

7.3.5 Equilibrate to room temperature before use.

7.3.6 Store at 4°C. The diluent has an expiry of one month.

7.4 Diluent B (1 g/L citric acid + 1g/L ascorbic acid in DMSO/methanol 50/50)

7.4.1 Diluent B is used for chewable gels (gummies) only.

7.4.2 Transfer 1.0 g of citric acid to a suitable container.

7.4.3 Add 1.0 g of ascorbic acid.

7.4.4 Add 500 mL of methanol.

7.4.5 Add 500 mL of DMSO

7.4.6 Sonicate or stir until completely dissolved.

7.4.7 Equilibrate to room temperature before use.

7.4.8 Store at 4°C. The diluent has an expiry of one month.

7.5 Stock Standard (~500 mcg/mL)

7.5.1 Accurately weigh and transfer about 25 mg of reference standard into a 50-mL low-actinic (red) volumetric flask.

7.5.2 Dissolve in and dilute to volume using Diluent A.

7.6 Working Standard (~100 mcg/mL)

7.6.1 Transfer 10.0 mL of Stock Standard to a 50-mL low-actinic (red) volumetric flask.

7.6.2 Dilute to volume with Diluent A.

7.7 Sample Preparation

7.7.1 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 of this method.

7.7.2 The linear range is 5 – 500 mcg/mL of ascorbyl palmitate.

7.7.3 Ensure that the sample is homogeneous prior to weighing.

7.7.3.1 For capsules, combine the fill material from at least 10 dosage units and homogenize in a mortar and pestle if necessary.

7.7.3.2 For tablets, combine at least 10 dosage units and homogenize in a mortar and pestle.

7.7.3.3 For chewable gels (gummies), homogenize at least 10 dosage units as outlined in D-793.

7.7.4 For raw materials: Weigh no less than 25 mg into a suitably sized volumetric flask of no less than 25 mL volume to generate an analyte concentration that is within the validated linearity range. Fill the flask to about 65% of the calculated volume with Diluent A, shake mechanically for 20 minutes, and dilute to volume using Diluent A.

7.7.5 For solid and liquid dose finished products: Based on the label claim and fill weight (capsules), serving size (powders and liquids) or tablet weight per dose, weigh no less than 100 mg of the pooled dosages into a suitably sized volumetric flask of no less than 25 mL to generate an analyte concentration that is within the validated linear range. Fill the flask to about 65% of the calculated volume with Diluent A, shake mechanically for 20 minutes, and dilute to volume using Diluent A.

7.7.6 For chewable gels (gummies): Homogenize at least 10 dosage units according to the procedure outlined in D-793 Cryogenic Grinding of Chewable Gels. Quickly weigh no less than 200 mg of the homogenized sample into a suitably sized beaker. Add a volume of Diluent B equivalent to 50% of the desired flask volume, add a stir bar, protect from light, and stir for 30 min. Transfer the resulting solution to a volumetric flask of size suitable to generate an analyte concentration that is within the validated linear range. Use several small portions of Diluent B to rinse any remaining residue from the beaker into the volumetric flask ensuring complete transfer, then sonicate for 10 min. Equilibrate to room temperature, and dilute to volume using Diluent B.

7.7.7 To manage large volumes, the sample can be initially dissolved in a smaller volume and a portion further diluted using Diluent A to bring the analyte concentration into the linear range. Dilutions can be made using volumetric glassware and/or adjustable pipettes. Dilutions can be prepared in HPLC vials.

7.7.8 Filter through a 0.45 µm membrane discarding the first 2 – 3 mL before collecting a portion for analysis. Alternatively, centrifuge an aliquot of the final sample at 10,000 rpm for 5 min to remove particulates.

7.8 Instrument Method Parameters

7.8.1 Column: Ascentis Express C8, 2.7 µm, 4.6 mm x 100 mm

7.8.2 Mobile phase: Isocratic A/B (33/67)

7.8.3 Flow Rate: 1.0 mL/min

7.8.4 Injection volume: 10 µL

7.8.5 Column temperature: 30 °C

7.8.6 Wavelength: 242 nm

7.8.7 Recommended Spectral Range (for Identification): 200 – 400 nm

7.9 Recommended Sequence

7.9.1 Make at least 2 injections of Diluent.

7.9.2 Make 5 injections of the Working Standard.

7.9.3 Make a single injection of each Working Sample.

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

7.10 System Suitability Requirements

7.10.1 The %RSD for five consecutive injections of Working Standard is NMT 2%.

7.10.2 The %RSD for all injections of Working Standard is NMT 3%.

7.10.3 No significant (>0.5%) interferences are present in the diluent injection.

7.11 Column Cleaning

7.11.1 It is recommended to clean the column after every run.

7.11.2 Clean the column at 0.2 mL/min with Mobile Phase A / Mobile Phase B (20/80) for at least 30 min.

7.12 Column Storage

7.12.1 Wash and store the column in ACN/H_2O (50/50).

8.0 Example Calculation

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

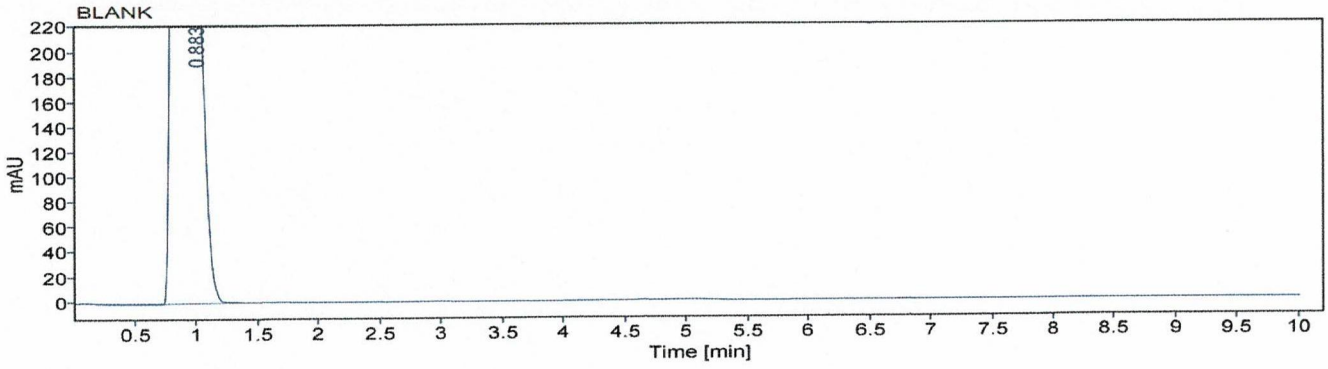
R_u Sample peak area

R_s Mean standard peak area

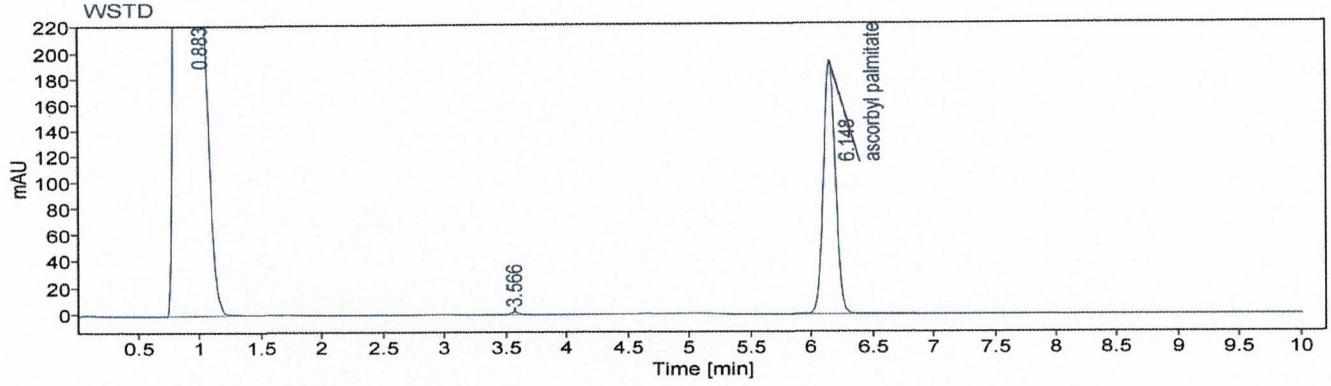
Wt_{std}	Weight of reference standard in mg
V_{std}	Volume of the standard preparation accounting for dilutions in mL
P	Purity of the reference standard in decimal format
SS	Serving size: Weight of a single dosage unit in mg or 1 for raw materials.
Spl_{wt}	Sample weight in mg
V_{spl}	Volume of the sample preparation accounting for dilutions in mL
LA	Label amount in mg per dose or 1 for raw materials
MULT	Multiplier (from SOP D-903 Conversion Factors Used in Analytical Determinations and New Product Formulations)

9.0 Example Chromatograms

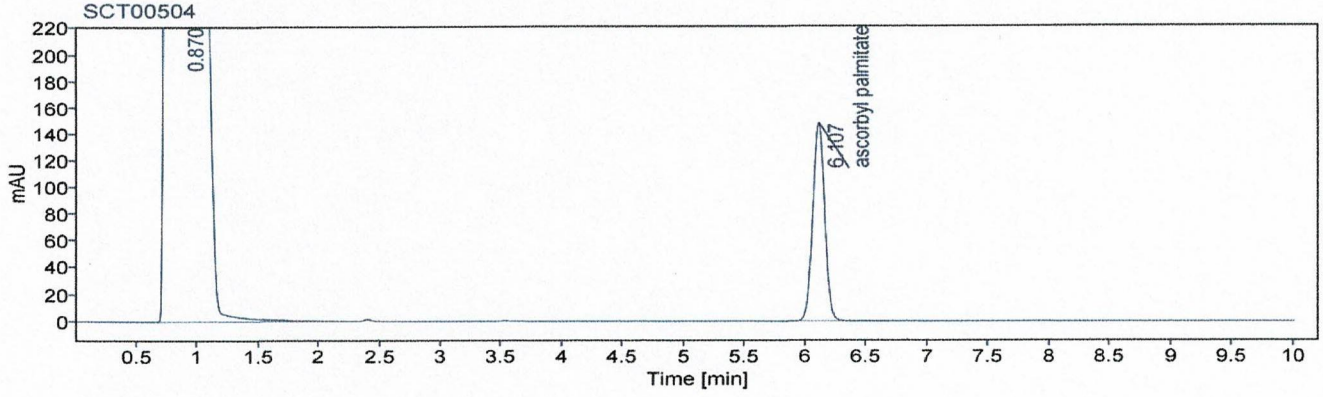
9.1 Blank



9.2 Standard



9.3 Sample



10.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	05/02/24	New procedure.	N/A	S. Sassman