1.0 Purpose

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

2.0 Scope

This procedure applies to the identification and quantification of mogroside V in raw materials and finished products. This method was validated under Protocol MV-LAB-19-016.

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 aligned with current laboratory practices.

4.0 Definitions

4.1 **QC** – Quality Control

4.2 **AD** – Analytical Development

4.3 **MGV** – Mogroside V

4.4 **ACN** – Acetonitrile

4.5 **MeOH** – Methanol

4.6 **HPLC** – High Performance Liquid Chromatography

5.0 References

5.1 MV-LAB-19-016, Protocol, Determination of Mogroside V using HPLC with UV/Vis Detection

5.2 RPT-23-0058, Report, Change of Mobile Phase for D-773

6.0 Supplies

6.1 Chemicals – All reagents are HPLC grade or better

6.1.1 ACN

6.1.2 MeOH

6.1.3 MGV 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 1.5mL or 2.0mL micro centrifuge tubes

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 Centrifuge

7.0 Procedure

7.1 Mobile Phase Preparation

7.1.1 Mobile Phase

7.1.1.1 Combine 700 mL of ACN with 300 mL of water.

7.1.2 Diluent

7.1.2.1 MeOH

7.1.3 May be scaled as necessary

7.2 Standard Prep

7.2.1 Accurately weigh and transfer about 22 mg of MGV reference standard into a 100-mL volumetric flask.

7.2.2 Dissolve in and dilute to volume with Diluent.

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 0.06 – 0.29 mg/mL.

7.3.3 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 90% of the calculated volume with Diluent and sonicate for 10 minutes. Equilibrate to room temperature, and bring up to volume with Diluent.

7.3.4 For solid and liquid dose finished products: Combine and homogenize no less than ten dosage units. 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 90% of the calculated volume with Diluent and sonicate for 10 minutes. Equilibrate to room temperature, and bring up to volume with Diluent.

7.3.5 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 400 mg of the pooled and homogenized dosages into a suitably sized beaker. Add a volume of Diluent equivalent to 50% of the desired flask volume, cover the beaker opening, and sonicate for 10 minutes or until dissolved. Transfer the 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 to rinse any remaining residue from the beaker into the volumetric flask ensuring complete transfer, and dilute to volume using Diluent.

7.3.6 To manage large volumes, the sample can be initially dissolved in a smaller volume and a portion further diluted using Diluent 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.

7.3.7 Centrifuge an aliquot of the final sample at 10,000 rpm for 5 min to remove particulates. Alternatively, the sample may be filtered through a 0.45 µm membrane discarding the first 3 – 4 mL before collecting a portion for analysis.

7.4 HPLC Parameters

7.4.1 Column: Hypersil Gold Amino Column 5µm x 4.6mm x 250mm

7.4.2 Column Temperature: Not controlled

7.4.3 Flow rate: 1.0 mL/min

7.4.4 Wavelength: 210 nm

7.4.5 Injection Volume: 10 µL

7.4.6 Run Time: 15 minutes

7.4.7 Recommended 3-D Spectral Range (for Identification): 200 nm - 400 nm

7.5 Recommended Sequence

7.5.1 Make at least 2 injections of the diluent.

7.5.2 Make five (5) injections of Standard Solution.

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 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 3.0%.

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

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 finished product % label or raw material % purity

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

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

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, capsules, and gummies; weight of a single serving from the theoretical formula in mg for liquids and powders, or 1 for raw materials.

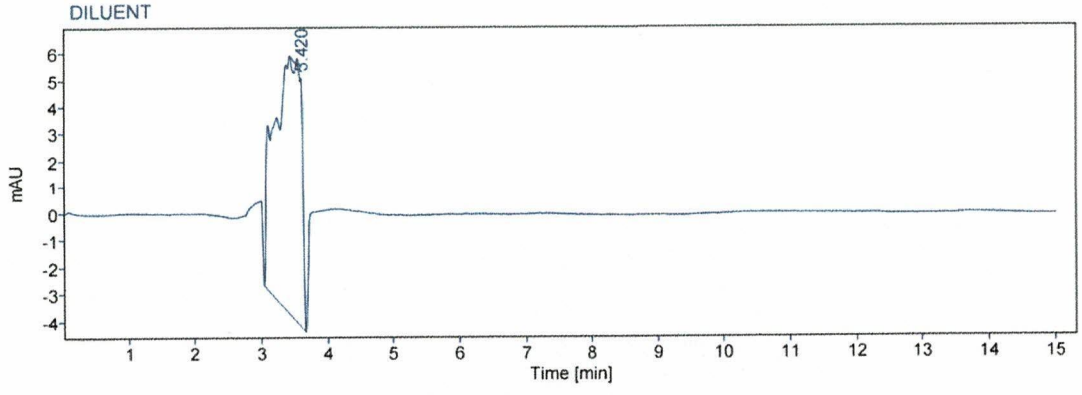
LA Label amount in mg of mogroside V (use 1 for raw materials)

7.8 Column Wash and Storage

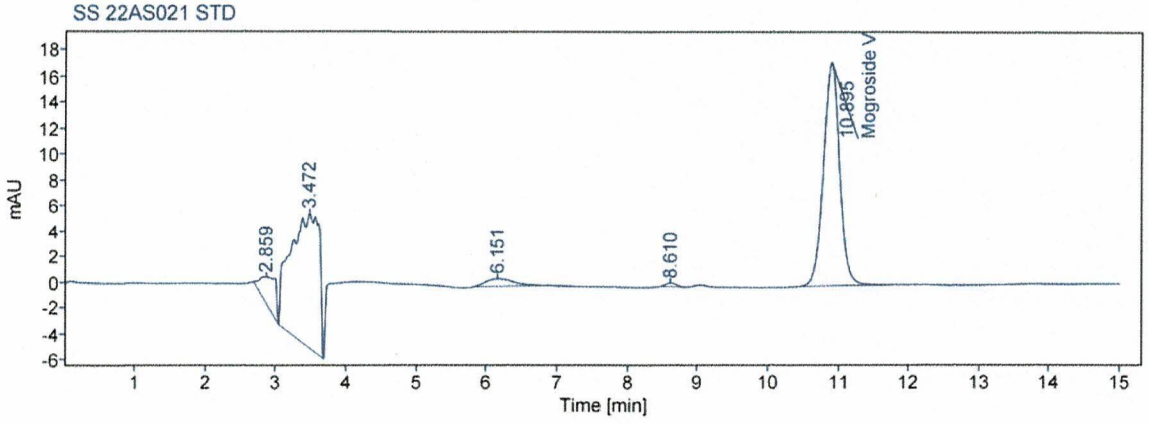
7.8.1 Store the column in 100% ACN.

8.0 Chromatograms

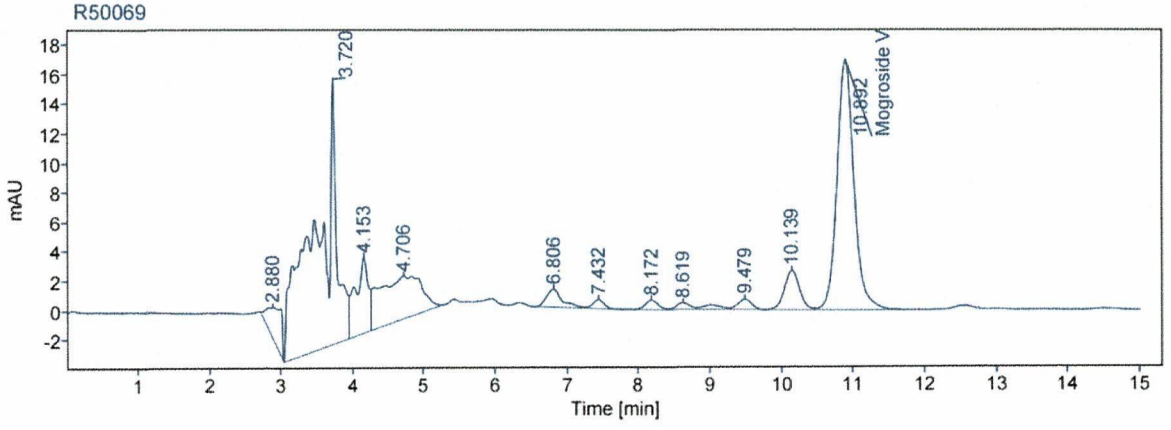
8.1 Typical Diluent Chromatogram



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	S. Sassman
1	06/22/22	Updated logo and format.	CC-22-0291	K. Burris
2	10/20/23	Change mobile phase as discussed in RPT-23-0058, update example chromatograms, update sample prep section for consistency with current methods, change column storage to be more appropriate for amino column.	CC-23-0522	S. Sassman