1.0 Purpose

The purpose of this procedure is to define the method for the quantitation and/or identification of salidroside in raw materials and finished product dietary supplements using HPLC and UV/VIS spectrophotometry.

2.0 Scope

This procedure applies to the quantification and identification of salidroside in raw materials and finished products. Salidroside is a good chromophore and was measured at 205, other wavelengths should not be used without justification.

3.0 Responsibility

3.1 It is the responsibility of QC Chemists to follow this procedure.

3.2 It is the responsibility of the QC Laboratory Management to ensure that this procedure is being followed.

3.3 It is the responsibility of the QC Laboratory and Analytical Development Management to keep this procedure aligned with current practices.

4.0 Definitions

4.1 **HPLC** – High Performance Liquid Chromatography

4.2 **UV/VIS** – Ultraviolet and Visible Electromagnetic Spectrums

4.3 **H_3PO_4** – Phosphoric Acid

4.4 **KH_2PO_4** – Potassium phosphate monobasic

4.5 **ACN** – Acetonitrile

4.6 **CofA** – Certificate of Analysis

4.7 **H_2O** – Millipore Water

4.8 **Salidroside** – 2-(4-hydroxyphenyl)ethyl β-D-glucopyranoside

5.0 References

5.1 USP43-NF38 USP Monograph for Rhodiola rosea

6.0 Supplies

6.1 Chemicals: All reagents are HPLC grade or better.

6.1.1 H_2O

6.1.2 ACN

6.1.3 H_3PO_4

6.1.4 KH_2PO_4

6.1.5 Methanol

6.1.6 Salidroside reference standard

6.2 Glassware

6.2.1 HPLC vials, 12mm x 32mm with screw cap enclosures with septa

6.2.2 Scintillation Vials

6.2.3 1L Mobile Phase Container

6.2.4 50mL Volumetric Flask

6.2.5 100mL Volumetric Flask

6.3 Disposables

6.3.1 10mL Pipette Tips

6.3.2 1mL Pipette Tips

6.3.3 200µL Pipette Tips

6.3.4 1.5mL microfuge tubes

6.3.5 16mL Test Tubes

6.3.6 Disposable Plastic Luer Lock Syringe – 3mL, 6mL, or 10mL

6.3.7 Nylon Syringe Filters, 0.2µm

6.3.8 Weigh paper

6.4 Equipment

6.4.1 Suitable gradient HPLC system consisting of a pump, autosampler, column oven and UV detector with a chromatographic data handling system

6.4.2 Analytical Balance

6.4.3 Ultrasonic bath

6.4.4 Vortex

6.4.5 Stir Plate

6.4.6 Eppendorf Centrifuge

6.4.7 10mL Pipette

6.4.8 1mL Pipette

6.4.9 200µL Pipette

7.0 Preparation of Mobile Phase, Dissolution Buffer, Samples, and Standards

7.1 Mobile Phase A – H_2O

7.2 Mobile Phase B – ACN

7.3 Diluent–100% methanol

7.4 Working Standard Preparation

7.4.1 Accurately weigh and transfer about 30 mg of Salidroside reference standard into a 100-mL volumetric flask.

7.4.2 Dissolve in and QS with Diluent.

7.5 Sample Preparation

7.5.1 Raw materials can be dissolved in Diluent at any volume starting from 50 mL and any weight greater than the minimum weight of the analytical balance.

7.5.2 Accurately weigh and transfer an amount of sample sufficient to generate a salidroside concentration of 0.3 mg/mL into a suitably sized volumetric flask.

7.5.3 Dilute to volume using Diluent, and sonicate for 10 minutes.

7.5.4 Before injection, insoluble matter should be removed via filtration using a 0.45µm nylon syringe filter. Discard at least 0.5 mL of the filtrate before collecting a portion in an HPLC vial for analysis.

7.5.4.1 Alternatively, samples and standards can also be centrifuged at 5000 RPM for 3 minutes in an Eppendorf centrifuge to pellet insoluble matter.

7.5.5 For raw materials or finished products being analyzed for the first time using this method, in-process verification is required to demonstrate spectral purity and extraction efficiency before the method can be implemented.

8.0 Test Conditions

8.1 Gradient

Time	%A	%B	Gradient Type
0.00	94	6	Initial
6.00	83	17	Linear
7.00	80.3	19.7	Linear
9.00	80.3	19.7	0
10.0	0	100	Linear
12.0	94	6	Linear
17.0	94	6	0

8.2 Column – ZORBAX Eclipse AAA. 3.5 µm, 120Å, 4.6 X 250 mm, or equivalent

8.3 Flow Rate – 1.0 mL/min

8.4 UV Detection – 205 nm

8.5 3D Spectral Range – 210 nm – 320 nm

8.6 Injection Volume – 1 µL

8.7 Column Temperature – 40 °C

8.8 Recommended Sequence

8.8.1 Make at least 2 injections of the diluent.

8.8.2 Make five (5) injections of Standard Solution.

8.8.3 Make a single injection of each Sample Preparation.

8.8.4 Make a single injection of the Standard Solution after every six (6) samples and at the end of the run.

8.9 System Suitability Requirements

8.9.1 The %RSD of five (5) injections of Working Standard is NMT 2%.

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

8.9.3 The spectral match over the range 210 nm – 320 nm is NLT 900.

8.9.4 The retention time of the sample is within 0.3 min of the standard.

8.10 Column Wash and Storage

8.10.1 Rinse and store the column with H_2O/ACN (50/50).

9.0 Calculations

9.1 Example calculations for determining finished product % label or raw material % purity

9.1.1 $$\%\ \text{assay} = \frac{R_u}{R_s} \times \frac{Wt_{std} \times P}{V_{std}} \times \frac{V_{spl}}{SA} \times \frac{SS}{LA} \times 100$$

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

SA Sample amount in mg (solids) or mL (liquids)

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

SS Serving size: Weight of a single dosage unit in mg for tablets and capsules, volume of a single serving from the theoretical formula in mL for liquids, or 1 for raw materials.

LA Label amount in mg per dose or 1 for raw materials

10.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	01/02/19	New	N/A	J. Maignan
1	04/11/22	Update for consistency with current practices and clarity. Fix standard concentration to match USP since linearity is not validated. Add system suitability section. Add recommended sequence. Add column wash and storage. Add requirement for spectral match and set range.	CC-22-0175	S. Sassman