1.0 Purpose

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

2.0 Scope

This procedure applies to the determination of ginsenosides in raw materials in the QC Laboratory at Ion Labs.

3.0 Responsibility

3.1 It is the responsibility of QC Chemists 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 **H_2O** – Water

4.4 **ACN** – Acetonitrile

5.0 References

5.1 PRTCL-22-0060, Protocol, Validation of an Analytical Method for the Determination of Ginsenosides by HPLC-UV

6.0 Supplies

6.1 Chemicals

6.1.1 Ginsenoside Rb1 Reference Standard

6.1.2 Ginsenoside Rg1 Reference Standard

6.1.3 USP Powdered Asian Ginseng Extract Reference Standard

6.1.4 Acetonitrile (HPLC grade)

6.1.5 Methanol (HPLC grade)

6.1.6 H_2O (>18 MΩ•cm)

6.2 Glassware

6.2.1 Volumetric glassware as required for standard and sample preparations

6.2.2 Luer-lock syringe and 0.22µm nylon filter

6.2.3 Adjustable pipette and tips

6.2.4 1.5-mL or 2-mL Eppendorf 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 with a minimum weight of no less than 40 mg

6.3.3 Microbalance with a minimum weight of no less than 3 mg

6.3.4 Wrist action shaker

6.3.5 Centrifuge

7.0 Mobile Phase

7.1 Mobile Phase A – H_2O

7.2 Mobile Phase B – ACN

7.3 Diluent – Methanol:H_2O (70:30), equilibrate to room temperature before use

8.0 Working Standard

8.1 Accurately weigh and transfer about 3.75 mg of ginsenoside Rb1 reference standard to a 25-mL volumetric flask.

8.2 Accurately weigh and transfer about 3.75 mg of ginsenoside Rg1 reference standard to the same 25-mL volumetric flask

8.3 Dissolve in and dilute to volume with *Diluent*.

9.0 System Suitability Solution

9.1 Accurately weigh and transfer about 100 mg of USP Powdered Asian Ginseng Extract reference standard to a 10-mL volumetric flask.

9.2 Add 7.0 mL of methanol and swirl to disperse the powder.

9.3 Slowly dilute to volume with H_2O while gently swirling. Do not shake or invert.

9.4 Equilibrate to room temperature for at least 10 min.

9.5 Adjust to volume using H_2O.

9.6 Sonicate for 10 min with occasional shaking.

9.7 Pass through a 0.22 µm syringe filter discarding the first 2 – 3 mL before collecting a sample for analysis.

10.0 Sample Solution

10.1 Accurately weigh and transfer about 100 mg of raw material sample to a 10-mL volumetric flask.

10.2 Add 7.0 mL of methanol and swirl to disperse the powder.

10.3 Slowly dilute to volume with H_2O while gently swirling. Do not shake or invert.

10.4 Equilibrate to room temperature for at least 10 min.

10.5 Adjust to volume using H_2O.

10.6 Sonicate for 10 min with occasional shaking.

10.7 Pass through a 0.22 µm syringe filter discarding the first 2 – 3 mL before collecting a sample for analysis.

11.0 HPLC Parameters

11.1 Column: Agilent EclipsePlus C18 RRHD 1.8µm, 2.1 mm x 50 mm, or equivalent

11.2 Column Temperature: 40 °C

11.3 Flow rate: 0.4 mL/min **(do not increase above 0.4 mL/min)**

11.4 Wavelength: 203 nm

11.5 Injection Volume: 5 µL

11.6 Run Time: 45 minutes

11.7 Gradient

Time (min)	% A	% B
0.0	84	16
15.0	80	20
15.1	73	27
35.0	68	32
35.1	5	95
40.0	5	95
40.1	84	16
45.0	84	16

11.8 Spectral Range (for Identification)- 200 nm to 400 nm

12.0 Recommended Sequence

12.1 Make two injections of *Diluent*

12.2 Make five injections of *Working Standard*

12.3 Make a single injection of *System Suitability Solution*

12.4 Make a single injection of each Sample Preparation

12.5 Make a single injection of *Working Standard* after every six samples and/or at the end of the run.

13.0 System Suitability Requirements

13.1 No significant (>0.5%) interfering peaks are present in the blank (Diluent) injection.

13.2 The %RSD of the *Working Standard* is NMT 2.0% for ginsenosides Rg1 and Rb1.

13.3 The USP resolution between ginsenoside Rg1 and Re in the injection of *System Suitability Solution* is NLT 1.3.

13.4 The USP resolution between ginsenoside Ro and Rb1 in the injection of *System Suitability Solution* is NLT 1.5.

13.5 The USP resolution between ginsenoside Rd and the peak before it in the injection of *System Suitability Solution* is NLT 1.3.

13.6 The tailing factors for ginsenosides Rg1 and Rb1 in the injections of *Working Standard* is NMT 2.0.

13.7 For materials being analyzed for the first time using this method, non-conforming results may trigger execution of a product specific method optimization per D-126/D-103.

14.0 Retention Times

Note: Retention times are approximate. Peak identification should be accomplished by comparison of the chromatogram obtained from the *System Suitability Solution* to the chromatogram provided with the USP Powdered Asian Ginseng Extract reference standard.

14.1	Ginsenoside Rg1	15.6
14.2	Ginsenoside Re	16.3
14.3	Ginsenoside Ro	26.4
14.4	Ginsenoside Rb1	26.9
14.5	Ginsenoside Rc	28.7
14.6	Malonyl ginsenoside Rc	29.3
14.7	Ginsenoside Rb2	30.8
14.8	Malonyl ginsenoside Rb2	31.6

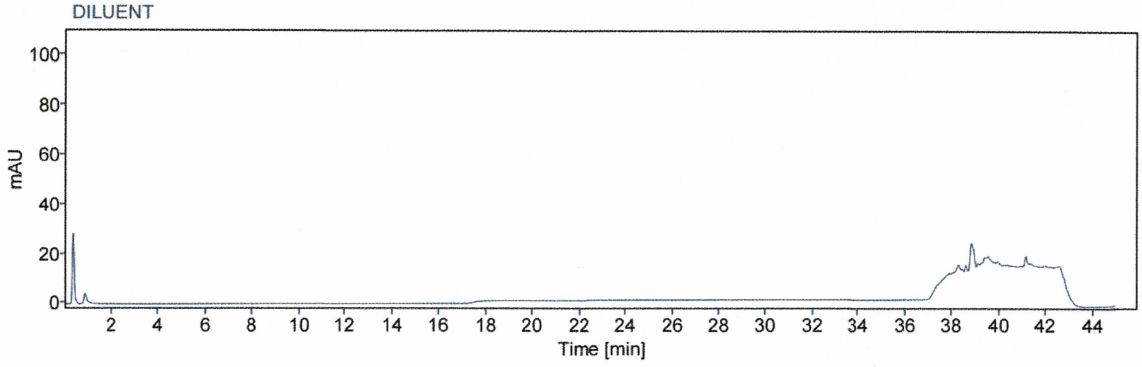
14.9 Ginsenoside Rd 34.9

15.0 Relative Response Factors

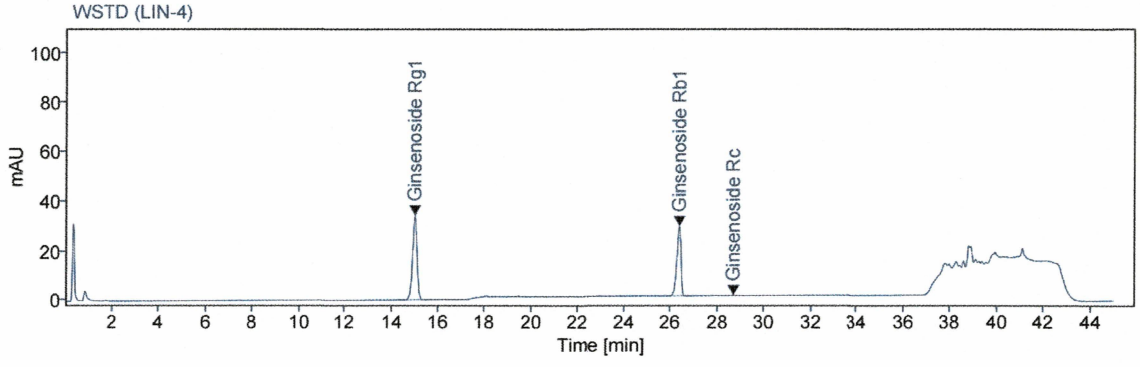
Analyte	RRF	Ref Std
Ginsenoside Rg1	1.00	*Rg1*
Ginsenoside Re	1.00	*Rg1*
Ginsenoside Rb1	1.00	Rb1
Ginsenoside Rc	0.96	Rb1
Ginsenoside Rb2	0.96	Rb1
Ginsenoside Rd	0.80	Rb1

16.0 Example Chromatograms

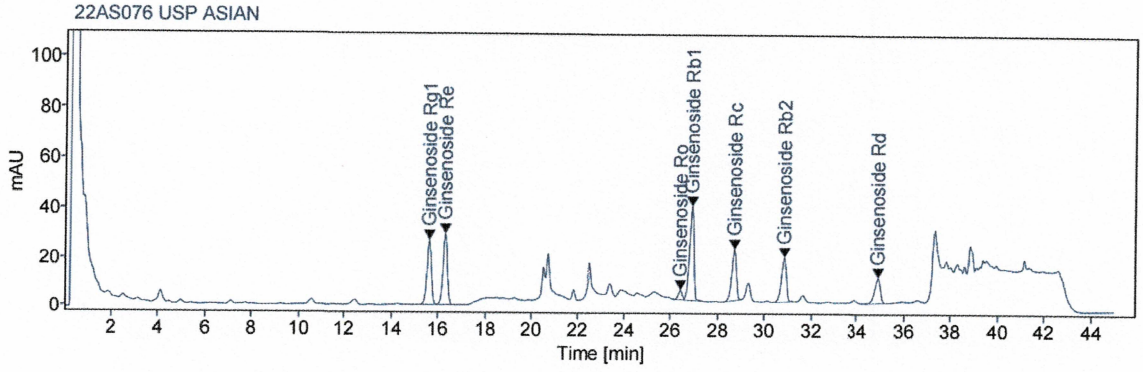
16.1 Blank



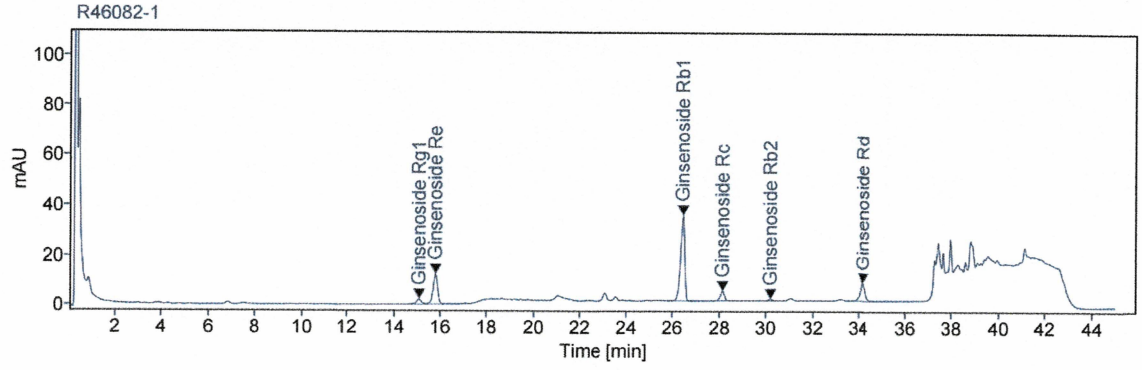
16.2 Working Standard



16.3 System Suitability Solution



16.4 Raw Material Sample



17.0 Example Calculations

$$\text{Assay (\%)} = \frac{R_t}{R_s} \times \frac{Wt_{std} \times P}{V_{stock}} \times \frac{Aq_{stock}}{V_{std}} \times \frac{V_{spl}}{Spl_{wt}} \times \frac{SS}{LA} \times RRF \times 100$$

R_t	Peak area for each gingerol present in the injection of *Sample Solution*
R_s	Mean *Working Standard* peak area ratio (5 injections)
Wt_{std}	Weight of reference standard used to prepare the *Stock Standard* (mg)
P	Purity of reference standard (decimal)
V_{stock}	Volume of the *Stock Standard* (mL)
Aq_{stock}	Aliquot of the *Stock Standard* used to prepare the *Working Standard* (mL)
V_{std}	Volume of the *Working Standard* (mL)
V_{spl}	Volume of *Sample Solution* (mL)

Spl_{wt}	Sample weight (mg)
SS	Serving size: Average weight of ten dosage units in mg for tablets and capsules, weight of a single serving from the theoretical formula in mg for liquids, or 1 for raw materials
LA	Label amount in mg (use 1 for raw materials)
RRF	Relative response factor for the individual ginsenoside

18.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	11/09/22	New procedure.	N/A	S. Sassman