	Standard Operating Procedure Determination of Gingerols, Gingerdiones, and Shogaols by HPLC-UV		SOP Number D-732	Revision 1
			Effective Date 05/24/23	Page Page 1 of 9
Written by/ Date SAS 05/24/23	Reviewed by/ Date CJey 05-24-23	Approved by/ Date SSB 05/25/23		
Title: Analytical Development Scientist	Title: Analytical Development Scientist	Title: Quality Control Director		

1.0 Purpose

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

2.0 Scope

This procedure applies to the determination of gingerols, gingerdiones, and shogaols 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 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

5.0 References

- 5.1 PRTCL-20-0098, Protocol, Validation of an Analytical Method for the Determination of Gingerols by HPLC-UV
- 5.2 D-793, SOP, Cryogenic Grinding of Chewable Gels

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6.0 Supplies

6.1 Chemicals

- 6.1.1 Capsaicin Reference Standard
- 6.1.2 USP Ginger Constituent Mixture Reference Standard or equivalent
- 6.1.3 Acetonitrile (HPLC grade)
- 6.1.4 Methanol (HPLC grade)
- 6.1.5 Phosphoric acid (ACS reagent or HPLC grade)
- 6.1.6 Water (>18 M Ω •cm)

6.2 Glassware

- 6.2.1 Volumetric glassware as required for standard and sample preparations
- 6.2.2 Luer-lock syringe and 0.45 μ m 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
- 6.3.3 Wrist action shaker
- 6.3.4 Centrifuge

7.0 Mobile Phase

7.1 Mobile Phase A

- 7.1.1 Transfer 550 mL of acetonitrile to a 1-L media bottle.
- 7.1.2 Add 10.0 mL of methanol.

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7.1.3 Add 440 mL of water.

7.1.4 Add 0.44 mL of phosphoric acid.

7.1.5 Mix well.

7.2 Mobile Phase B

7.2.1 Use acetonitrile.

7.3 Diluent

7.3.1 Use methanol.

8.0 Stock Standard

8.1 The concentration of capsaicin in the *Stock Standard* is 0.3 mg/mL.

8.2 The purity of commercially available capsaicin reference standards may be much lower than 100%. Use the certified purity listed on the certificate of analysis to calculate the amount of reference standard required to generate a capsaicin concentration of 0.3 mg/mL.

8.3 Accurately weigh and transfer a quantity of capsaicin reference standard containing about 30 mg of capsaicin to a 100-mL volumetric flask.

8.4 Dissolve and dilute to volume with *Diluent*.

9.0 Working Standard

9.1 Transfer 5.0 mL of the *Stock Standard* to a 25-mL volumetric flask.

9.2 Dilute to volume with *Diluent*.

9.3 Alternatively, dilution may be performed directly into an HPLC vial using an adjustable pipet.

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10.0 System Suitability Solution

- 10.1 The System Suitability Solution may be stored at 2°C - 8°C for up to one year if the chromatogram obtained from the solution is similar in appearance to that provided with the CoA for USP Ginger Constituent Mixture Reference Standard.
- 10.2 Transfer the contents of one vial of USP Ginger Constituent Mixture Reference Standard to a 2-mL HPLC vial.
- 10.3 Add 1.0 mL of *Working Standard*.
- 10.4 Mix by vortex to dissolve.

11.0 Sample Solution

- 11.1 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.
- 11.2 For raw materials: weigh no less than 20 mg into a suitably sized volumetric flask of no less than 25 mL volume to generate a total gingerols concentration of about 0.35 mg/mL, add Diluent to 2/3 flask capacity, shake mechanically for 20 min, and dilute to volume using Diluent.
- 11.3 For solid and liquid dose finished products: Combine and homogenize no less than ten dosage units. Based on the label claim and fill weight, serving size or tablet weight per dose, weigh no less than 50 mg of the pooled dosages into a suitably sized volumetric flask of no less than 25 mL to generate a total gingerols concentration of about 0.35 mg/mL, add Diluent to 2/3 flask capacity, shake mechanically for 20 min, and dilute to volume using Diluent.
- 11.4 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 a portion of the pooled and homogenized dosages into a beaker. Use several small portions of Diluent to completely transfer the sample into a suitably sized volumetric flask to generate a total gingerols concentration of about 0.35 mg/mL, add Diluent to 2/3 flask capacity, shake mechanically for 20 min, and dilute to volume using Diluent.

- 11.5 To manage large volumes, the sample can be initially dissolved in a smaller volume and a portion further diluted using Diluent to bring the total gingerols concentration to 0.35 mg/mL.
- 11.6 Filter through a 0.45 µm nylon syringe filter, discarding the first 2-3 mL before collecting a portion of the filtrate in an HPLC vial. Alternatively, the sample can be centrifuged at 10,000 rpm for 5 min to remove particulates prior to analysis.

12.0 HPLC Parameters

- 12.1 Column: Acclaim 120 C18, 5µm, 4.6 mm x 250 mm, or equivalent
- 12.2 Column Temperature: 30 °C
- 12.3 Flow rate: 1.0 mL/min
- 12.4 Wavelength: 282 nm
- 12.5 Injection Volume: 15 µL
- 12.6 Run Time: 94 minutes (samples, blank, and system suitability solution)
- 12.7 Run Time: 15 minutes (working standard)
- 12.8 Gradient

Time (min)	% A	% B
0	100	0
65	100	0
67	0	100
77	0	100
79	100	0
94	100	0

- 12.9 Recommended Spectral Range (for Identification)- 200 nm to 400 nm

13.0 Recommended Sequence

- 13.1 Make two injections of *Diluent*
- 13.2 Make one injection of *System Suitability Solution*
- 13.3 Make five injections of *Working Standard*

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13.4 Make a single injection of each Sample Preparation

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

14.0 System Suitability Requirements

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

14.2 The %RSD of the peak area in five consecutive injections of the Working Standard is NMT 2.5%.

14.3 The %RSD of the peak area in all injections of the Working Standard is NMT 3%.

14.4 The USP resolution between 6-gingerol and capsaicin in the injection of *System Suitability Solution* is NLT 3.0.

14.5 The USP resolution between capsaicin and 6-shogaol in the injection of *System Suitability Solution* is NLT 10.0.

14.6 The tailing factors for 6-gingerol, capsaicin, and 6-shogaol in the injection of *System Suitability Solution* is NMT 2.0.

15.0 Retention Times

15.1 6-Gingerol = 7.3 min

15.2 Capsaicin = 9.2 min (do not include capsaicin in the total)

15.3 8-Gingerol A = 15.0 min

15.4 6-Shogaol = 18.3 min

15.5 8-Gingerol B = 21.5 min

15.6 6-Gingerdiol = 25.1 min

15.7 6-Gingerdione = 26.7 min

15.8 10-Gingerol = 36.1 min

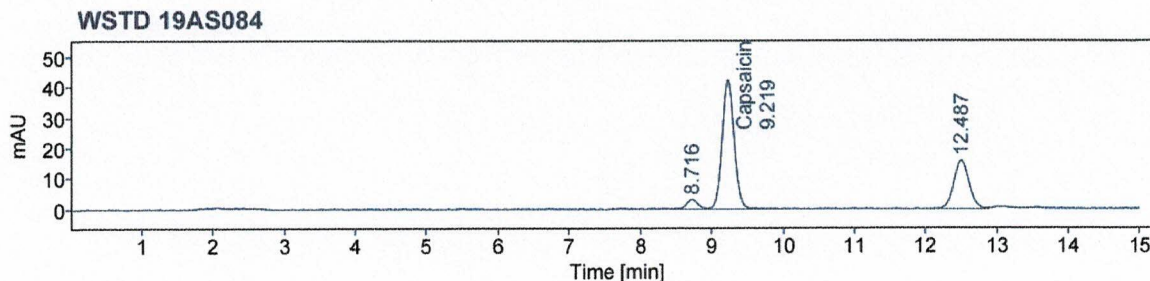
15.9 8-Shogaol = 38.9 min

15.10 8-Gingerdione = 44.8 min

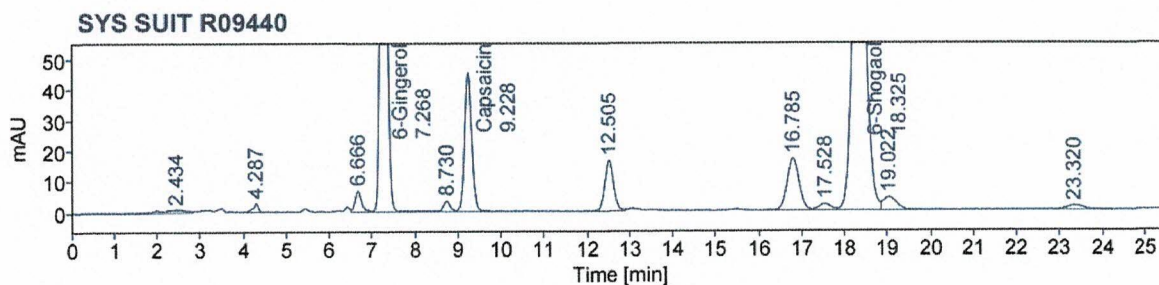
15.11 10-Shogaol = 53.7 min

16.0 Example Chromatograms

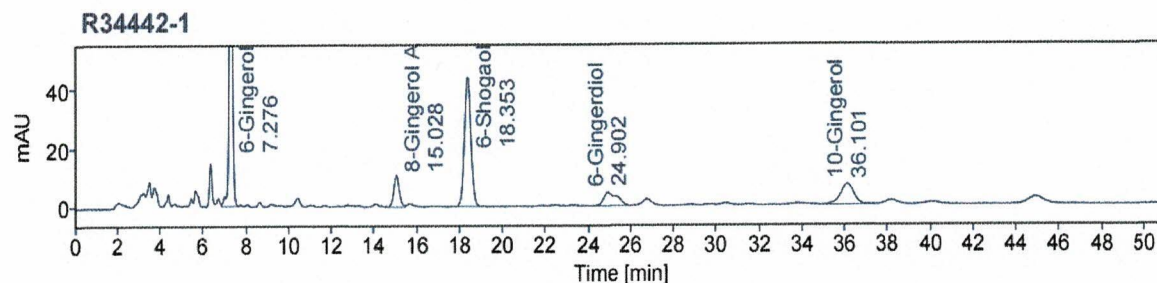
16.1 Working Standard



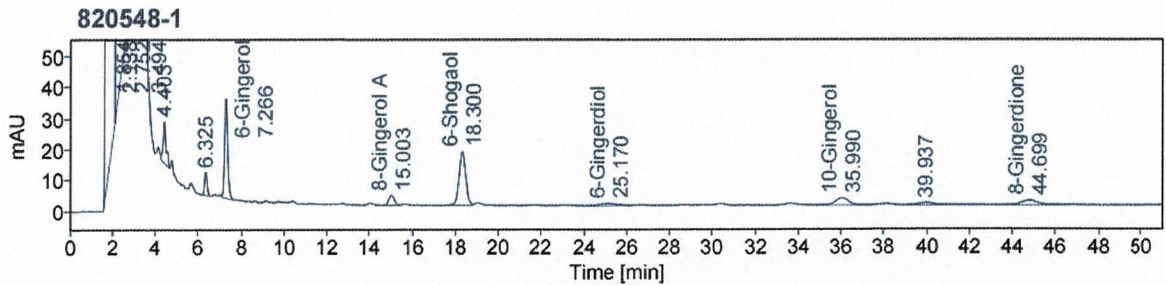
16.2 System Suitability Solution



16.3 Raw Material Sample



16.4 Finished Product Sample



17.0 Example Calculations

Note: Consult the raw material or finished product specification to see if shogaols should be included in the total gingerols. Gingerdiones are always included in the total.

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

- R_t Peak area for each gingerol present in the injection of *Sample Solution*
- R_s Mean *Working Standard* peak area (5 injections)
- Wt_{std} Weight of reference standard used to prepare the *Stock Standard* (mg)
- P Purity of reference standard (% w/w)
- 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)

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18.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	11/16/20	New	N/A	S. Sassman
1	05/17/23	Added reference to D-793 for gummy sample prep. Added instruction to refer to test details for product specific sample preparation. Added specific sample preparation instruction for different dosage forms Added requirement for bracketing standard. Changed logo.	CC-23-0249	S. Sassman