1.0 Purpose

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

2.0 Scope

This procedure applies to the quantification of total chlorogenic acids in raw materials and finished products. This procedure applies to the identification of chlorogenic acid forms in raw materials and finished products by retention time and spectral matching.

3.0 Responsibility

3.1 It is the responsibility of QC and Analytical 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 **UV/VIS** – Ultraviolet and Visible Electromagnetic Spectrums

4.3 **ACN** – Acetonitrile

4.4 **CofA** – Certificate of Analysis

4.5 **H_2O** – Water

4.6 **CGA** – Chlorogenic Acid or 3CQA or 3-O-Caffeoylquinic acid

5.0 References

5.1 MV-LAB-14-040, Protocol, Chlorogenic acid Determination by HPLC and UV/Vis Spectroscopy

5.2 RPT-20-0044, Report, Chlorogenic Acid: Assigning 3-CQA and 5-CQA in Raw Material and Finished Products

5.3 TN-1134, Chlorogenic Acids from Green Coffee by HPLC, Z. Aqeel, D. Truong, J. Preston, S. Lazzaro, and S. Baugh. Joint Phenomenex and Chromadex.

6.0 Supplies

6.1 Chemicals: All reagents are HPLC grade or better.

6.1.1 H_2O ($\geq$ 18.2 MΩ·cm)

6.1.2 ACN

6.1.3 Chlorogenic acid 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 Mobile phase containers

6.2.4 Volumetric glassware as required by standard and sample preparations

6.3 Disposables

6.3.1 Tips for adjustable pipets

6.3.2 Centrifuge tubes

6.3.3 Disposable plastic luer lock syringe – 3mL, 6mL, or 10mL

6.3.4 Nylon syringe filters, 0.45µm

6.3.5 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 Adjustable pipettes

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

7.1 Mobile Phase A – 0.1% Formic acid in 25% ACN (aq)

7.1.1 Transfer 250 mL of ACN to a suitable container.

7.1.2 Add 1.0 mL of formic acid.

7.1.3 Add 750 mL of H2O, and mix well.

7.2 Mobile Phase B – 0.1% Formic acid in 10% ACN (aq)

7.2.1 Transfer 100 mL of ACN to a suitable container.

7.2.2 Add 1.0 mL of formic acid.

7.2.3 Add 900ml of H_2O, and mix well.

7.3 Diluent–60% MeOH (aq)

7.3.1 Transfer 600 mL of methanol to a suitable container.

7.3.2 Add 400 ml of H_2O, and mix well.

7.3.3 **Equilibrate to room temperature before use.**

7.4 Standard Preparation

7.4.1 Accurately weigh and transfer about 25 mg of CGA reference standard into a 250-mL volumetric flask.

7.4.2 Dilute to volume using Diluent.

7.4.3 Sonicate for 10 minutes or until completely dissolved.

7.5 Sample Preparation

7.5.1 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.

7.5.2 The linear range of the method is 0.025 mg/mL – 0.2 mg/mL. All working sample preparations must be within this range.

7.5.3 For raw materials: Accurately weigh and transfer a portion into a suitably sized volumetric flask of no less than 10 mL volume to generate an analyte concentration that is within the validated linearity range. Dilute to volume with Diluent, and sonicate for 10 min.

7.5.4 For solid dose finished products: Combine and homogenize no less than 20 dosage units. Based on the label claim and weight per dose, accurately weigh and transfer a portion of the homogenized sample into a suitably sized volumetric flask of no less than 10 mL to generate an analyte concentration that is within the validated linearity range. Dilute to volume with Diluent, and sonicate for 10 min.

7.5.5 For liquid dose finished products: Use a TC pipet to transfer no less than 2.0 mL of the product into a suitably sized volumetric flask of no less than 25 mL to generate an analyte concentration that is within the validated linearity range. Wipe the outside of the pipet, and rinse the pipet three times with Diluent collecting the rinses in the volumetric flask. Dilute to volume using Diluent.

7.5.6 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 a portion of the homogenized sample into a volumetric flask of no less than 10 mL to generate an analyte concentration that is within the validated linearity range. Dilute to volume with Diluent, and sonicate for 10 minutes.

7.5.7 To manage large volumes, the sample can be initially dissolved in a smaller volume that is within the solubility range and a portion further diluted to bring the

analyte concentration into the linear range of measurement. Ensure that the stock sample is equilibrated to room temperature prior to performing further dilution.

7.5.8 The final sample must be filtered or centrifuged before analyzing by HPLC.

7.5.8.1 For filtration: filter a portion through a 0.45µm nylon membrane discarding the first 2-3 mL of filtrate before collecting an aliquot for analysis.

7.5.8.2 For centrifugation: centrifuge for 5 min at 10,000 rpm.

8.0 Test Conditions

8.1 Gradient

Time	%A	%B	Gradient Type
0.00	0	100	0
20.00	100	0	0
20.10	0	100	0
30.00	0	100	0

8.2 Column – Phenomenex Luna, C18 (2), 5µm, 100Å, 150mm x 4.6mm, or equivalent

8.3 Flow Rate – 1.0mL/min

8.4 UV Detection – 327nm

8.5 Recommended 3-D Spectral Range – 200nm – 400nm

8.6 Injection Volume - 20µL

8.7 Column Temperature – 30°C

8.8 Retention Times

8.8.1 Peak 1 – 4.7 min

8.8.2 CGA – 6.9 min

8.8.3 Peak 3 – 7.5 min

8.8.4 Peak 4 – 11.5 min

8.8.5 Peak 5 – 18.2 min

8.8.6 Peak 6 – 19.0 min

8.8.7 Peak 7 – 20.7 min

8.9 Recommended Sequence

8.9.1 Make at least 2 injections of a Blank (Diluent).

8.9.2 Make five injections of the Working Standard.

8.9.3 Make a single injection of each Sample Preparation.

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

8.10 System Suitability

8.10.1 The %RSD of five consecutive injections of Working Standard is NMT 5.0%.

8.10.2 The %RSD of all Working Standard injections is NMT 5%.

8.11 Column Wash and Storage

8.11.1 Rinse the column with H_2O/ACN (50/50) at 1 mL/min for at least 15 min.

8.11.2 Store the column with H_2O/ACN (50/50).

9.0 Calculations

9.1 $$\% \text{ assay} = \frac{R_u}{R_s} \times \frac{Wt_{std} \times P}{V_{std}} \times \frac{V_{spl}}{SA} \times \frac{SS}{LA} \times 100$$

R_u Sample peak area for chlorogenic acid peak

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)

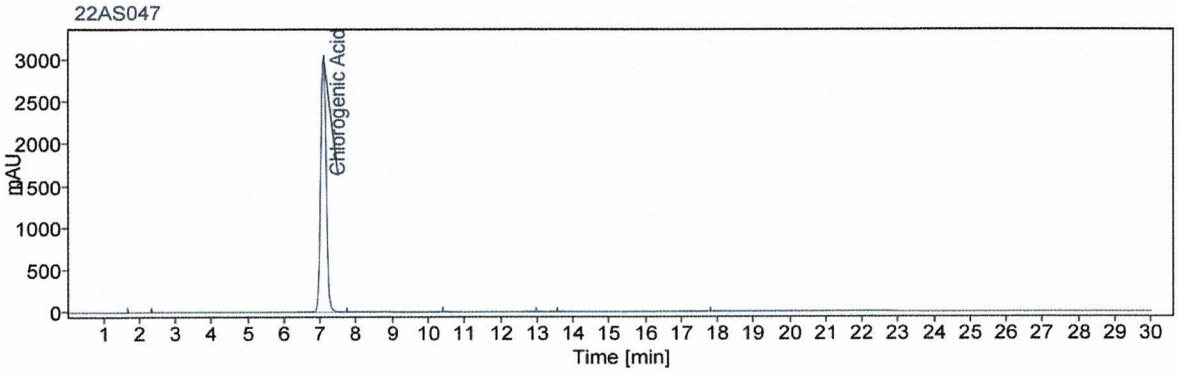
V_{spl} Volume of the sample preparation accounting for dilutions in mL

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.

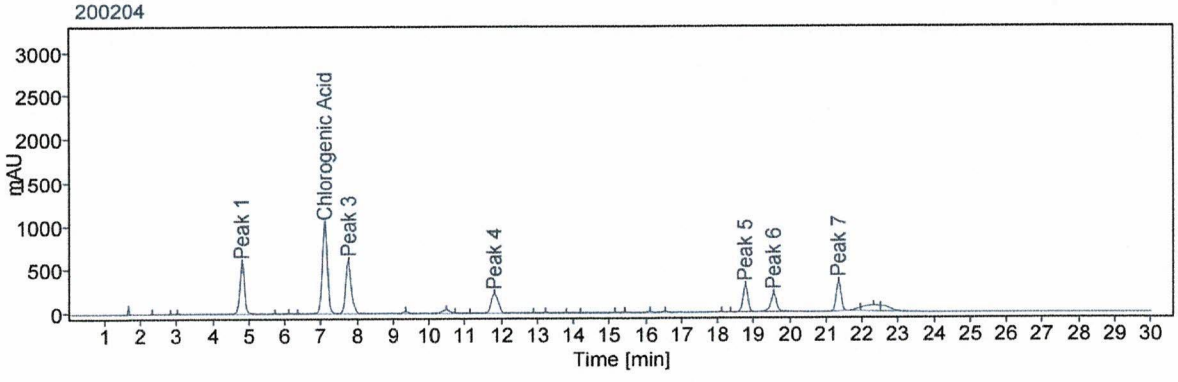
LA Label amount in mg per dose or 1 for raw materials

10.0 Example Chromatography and Spectrum

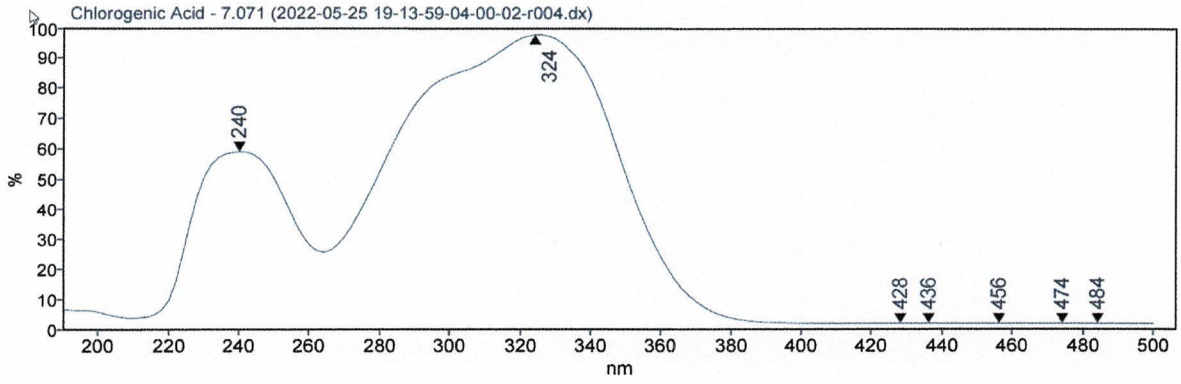
10.1 Working Standard



10.2 Sample



10.3 CGA UV Spectrum



11.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	12/17/14	New	14-1036	X. Shao
1	02/09/15	Incorporation of validation sub-analysis for the determination of total chlorogenic acids in finished product.	15-0105	X. Shao
2	01/14/19	Scheduled review: updated format to match current Ion practices.	19-0037	J. Maignan
3	06/24/21	Added example chromatography, system suitability requirements. Strengthened SOP details.	CC-21-0242	J. Sassman
4	08/05/22	Update for consistency with current methods and lab practices, add column wash and storage, edit for clarity and ease of use.	CC-22-0335	S. Sassman
5	04/05/23	Add reference to report explaining naming of CGA isomers, edit for consistency with current SOPs, add instruction to follow test details in the product profile, modify sample preparation to outline specific instructions for different sample types.	CC-23-0178	S. Sassman