1.0 Purpose

The purpose of this procedure is to define the method for the quantitation and/or identification of (-)Epigallocatechin-3-*O*-gallate (EGCG) in raw materials and finished product dietary supplements using HPLC-UV.

2.0 Scope

This procedure applies to the quantification and identification of EGCG 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 **HPLC-UV** – High Performance Liquid Chromatography with Ultraviolet Detection

4.2 **QC** – Quality Control

4.3 **H_3PO_4** – Phosphoric Acid

4.4 **ACN** – Acetonitrile

4.5 **H_2O** – Water

4.6 **PVDF** – Polyvinylidene fluoride

4.7 **EGCG** – (-)Epigallocatechin-3-*O*-gallate

5.0 References

5.1 PRTCL-23-0001, Protocol, Validation of an Analytical Method for the Determination of EGCG by HPLC-UV

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 (≈85%)

6.1.4 Citric Acid

6.1.5 EGCG reference standard

6.2 Glassware

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

6.2.2 Volumetric glassware as required for standard and sample preparations

6.2.3 Mobile Phase Container

6.3 Disposables

6.3.1 Pipet Tips

6.3.2 Centrifuge tubes

6.3.3 Disposable Plastic Luer Lock Syringe and 0.45 µm PVDF Syringe Filters

6.3.4 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 Eppendorf Centrifuge

6.4.5 Adjustable Pipet

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

7.1 Mobile Phase A – 0.1% H_3PO_4 (aq)

7.1.1 Transfer 1000 mL of H_2O to a 1-L bottle.

7.1.2 Add 1.0 mL of H_3PO_4, and mix well.

7.2 Mobile Phase B – ACN

7.2.1 Transfer 1000 mL of ACN to a 1-L bottle.

7.3 Diluent – 0.1% H_3PO_4 (aq)

7.3.1 Use Mobile Phase A.

7.4 Extraction Solution – Ethanol / H_2O / Citric Acid / H_3PO_4 (70/30/0.1/0.25)

7.4.1 Transfer 700 mL of ethanol to a 1-L bottle.

7.4.2 Add 300 mL of H_2O.

7.4.3 Add 1.0 g of citric acid.

7.4.4 Add 2.5 mL of phosphoric acid, and mix until dissolved.

8.0 Preparation of Standard and Sample Solutions

8.1 Stock Standard

8.1.1 Accurately weigh and transfer about 25 mg of reference standard into a 100-mL volumetric flask.

8.1.2 Dissolve in and dilute to volume using *Extraction Solution.*

8.2 Working Standard

8.2.1 3.0 mL of the *Stock Standard* into a 25-mL volumetric flask.

8.2.2 Dilute to volume using *Diluent.*

8.3 Stock Sample

8.3.1 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 of this method.

8.3.2 The linear range of the method is 8 – 252 mcg/mL, all working standard and sample preparations must be within the linear range.

8.3.3 Accurately weigh and transfer a quantity of sample containing about 25 mg of EGCG into a 100-mL volumetric flask.

8.3.3.1 For finished products:

$$\text{Sample Wt (mg)} = 25\text{mg} \times \frac{\text{Dosage Unit (mg)}}{\text{Label Claim (mg)}}$$

8.3.3.2 For raw materials:

$$\text{Sample Wt (mg)} = 25\text{mg} \times \frac{100}{\text{Raw Material Potency (\%)}}$$

8.3.4 Dilute to volume with *Extraction Solution*.

8.3.5 Sonicate for 20 minutes.

8.3.6 **Equilibrate to room temperature before further dilution.**

8.4 Working Sample Solution

8.4.1 Transfer 3.0 mL of *Stock Sample* to a 25-mL volumetric flask.

8.4.2 Dilute to volume using *Diluent*.

8.4.3 Pass through a 0.45 µm PVDF syringe filter discarding the first 2 – 3 mL before collecting a sample for analysis. **Do not use nylon syringe filters for EGCG since they will absorb the analyte resulting in an inaccurate result.**

8.4.3.1 Alternatively, centrifuge at 6,000 rpm for 5 minutes to remove particulates.

9.0 Test Conditions

9.1 Column – Agilent InfinityLab Poroshell 120 EC-C18, 2.7 µm, 4.6 mm x 100 mm, or equivalent

9.2 Flow Rate – 1.0 mL/min

9.3 UV Detection – 278nm

9.4 Recommended Spectral Range – 200 nm – 400 nm

9.5 Injection Volume – 5µL

9.6 Column Temperature – 35 °C

9.7 Gradient

Time (min)	% A	% B
0.0	92	8
10.0	84	16
10.1	0	100
13.0	0	100
13.1	92	8
17.0	92	8

9.8 Recommended Sequence

9.8.1 Make at least two injections of *Diluent*.

9.8.2 Make five injections of *Working Standard*.

9.8.3 Make a single injection of each *Working Sample*.

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

9.9 System Suitability Requirements

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

9.9.2 The %RSD of five consecutive injections of *Working Standard* is NMT 2.0%.

9.9.3 The %RSD of all injection of *Working Standard* is NMT 2%.

9.10 Column Wash and Storage

9.10.1 Wash and store the column with H_2O/ACN (50/50).

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

9.11.1 $$\%\ \text{assay} = \frac{R_u}{R_s} \times \frac{Wt_{std} \times P}{V_{std}} \times \frac{V_{spl}}{SA} \times \frac{SS}{LA} \times 100$$

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)

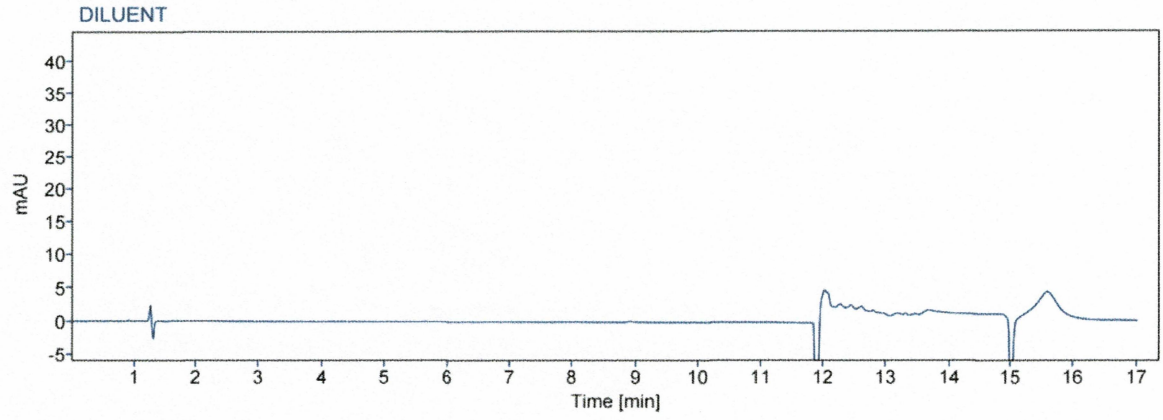
V_{spl} Volume of the sample preparation accounting for dilutions in mL

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.

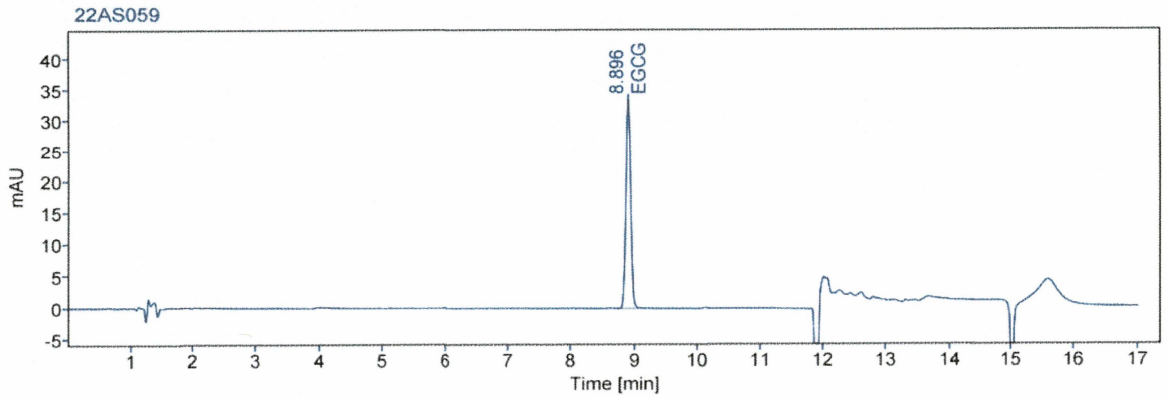
LA Label amount in mg per dose or 1 for raw materials

10.0 Example Chromatography

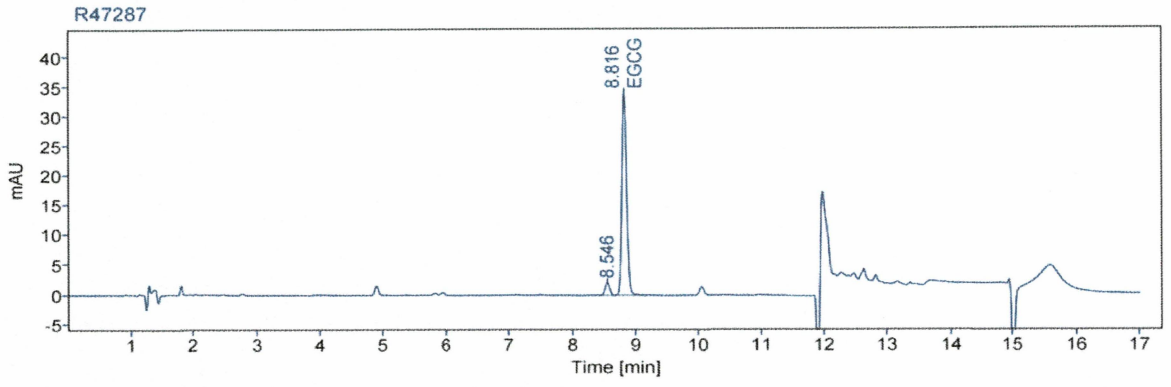
10.1 Blank



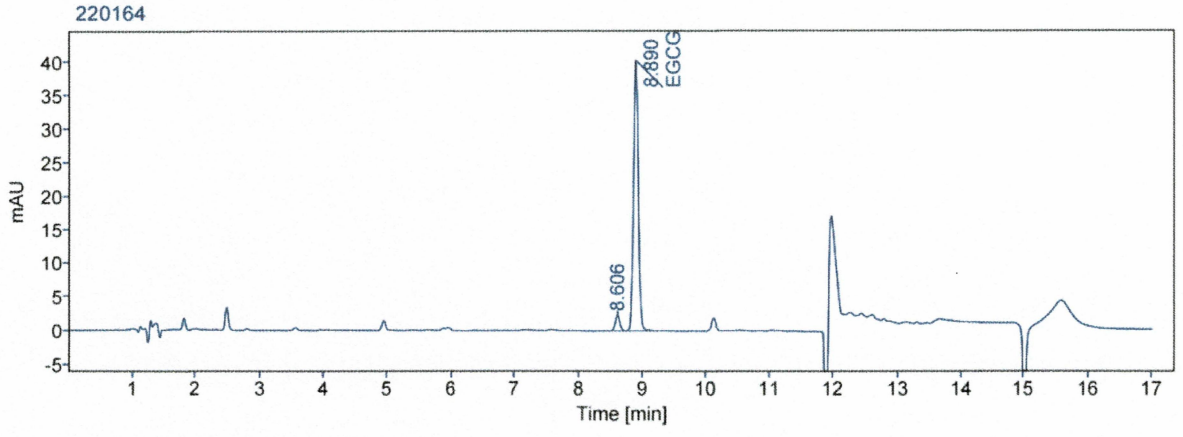
10.2 Working Standard



10.3 Raw Material Sample



10.4 Finished Product Sample



11.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	01/02/19	New	N/A	J. Maignan
1	04/11/22	Update to reflect current practices, minor edits for clarity, add reference to USP monograph, fix concentration at 0.1mg/mL since method is not validated, add recommended sequence, add system suitability section, add column wash and storage, add example chromatography.	CC-22-0174	S. Sassman
2	01/30/23	A new method was developed and validated according to PRTCL-23-0001. This revision has been updated to reflect the new method.	CC-23-0050	S. Sassman