1.0 Purpose

The purpose of this procedure is to define the method for the analysis and/or identification of Hydroxycitric Acid in raw materials and dietary supplements using HPLC and UV/VIS spectrophotometry.

2.0 Scope

This procedure applies to the identification of Hydroxycitric Acid forms in raw materials and finished products using spectral analysis and quantitation of Hydroxycitric Acid using a traceable Calcium (-)-Hydroxycitrate reference standard.

3.0 Responsibility

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

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

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

4.0 Definitions

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

4.2 **HCA** – Hydroxycitric Acid

4.3 **H_3PO_4** – Phosphoric Acid

4.4 **KH_2PO_4** – Potassium dihydrogen phosphate (potassium phosphate monobasic)

4.5 **NaOH** – Sodium Hydroxide

4.6 **CofA** – Certificate of Analysis

4.7 **H_2O** – Water

4.8 **QC** – Quality Control

5.0 References

5.1 MV-LAB-14-029, Protocol, Hydroxycitric Acid Determination by HPLC and UV/Vis Spectroscopy

5.2 Garcinia Hydroxycitrate USP Monograph

5.3 D-793, SOP, Cryogenic Grinding of Chewable Gels

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 KH_2PO_4

6.1.3 H_3PO_4

6.1.4 NaOH

6.1.5 Calcium (-)-hydroxycitrate 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 Volumetric glassware as required by standard and sample preparations

6.3 Disposables

6.3.1 Tips for Adjustable Pipettes

6.3.2 Microfuge tubes

6.3.3 16mL Test Tubes

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

6.3.5 Nylon Syringe Filters, 0.45µm

6.3.6 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 Vortex

6.4.4 Stir Plate

6.4.5 Eppendorf Centrifuge

6.4.6 Adjustable Pipettes

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

7.1 Mobile Phase (1.36g/L aqueous KH_2PO_4 pH 2.5)

7.1.1 Transfer 1.36g of KH_2P0_4 to a 1000-mL mobile phase bottle.

7.1.2 Add 900mL of water.

7.1.3 Adjust with H_3PO_4 to a pH of 2.5.

7.1.4 Transfer the resulting solution to a 1000-mL volumetric flask.

7.1.5 Dilute to volume with H_2O, and mix well.

7.2 Diluent (3% H_3PO_4 in H_2O)

7.2.1 Transfer 970ml of H_2O to a 1000-mL mobile phase bottle.

7.2.2 Slowly add 30mL of H_3PO_4, and mix well.

7.3 Standard Preparation

7.3.1 The linear range of the method is 0.1 mg/mL – 1.0 mg/mL. All standard and sample preparations must be within the linear range.

7.3.2 The standard is prepared by weighing no less than the minimum allowed on the scale at the time of use. Accurately weigh and transfer the reference standard to an appropriately sized volumetric flask, dissolve in Diluent, and dilute to volume using Diluent.

7.3.3 Dilutions are prepared using Diluent. Dilutions can be made using volumetric glassware and/or adjustable pipettes. Specific standard concentrations will approximate the concentration expected to be found in the product being tested based on the sample dilution and calculated from the label. Dilutions can be prepared in HPLC vials.

7.4 Sample Preparation

7.4.1 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 above.

7.4.2 For raw materials: weigh no less than 20 mg into a suitably sized volumetric flask of no less than 25 mL volume to generate an analyte concentration that is within the validated linearity range. Dilute to volume with Diluent, and sonicate for 10 min.

7.4.3 For solid and liquid dose finished products: Combine and homogenize no less than ten dosage units. Based on the label claim and fill weight (capsules), serving size (powders) 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 an analyte concentration that is within the validated linear range. Dilute to volume with Diluent, and sonicate for 10 min.

7.4.4 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 an analyte concentration that is within the validated linear range. Dilute to volume, and sonicate for 10 min.

7.4.5 To manage large volumes, the standard can be initially prepared at a higher concentration and further diluted into the linear range using Diluent. **Equilibrate to room temperature prior to performing further dilution.** Dilutions can be made using volumetric glassware and/or adjustable pipettes. Dilutions can be prepared in HPLC vials

7.4.6 If particulates remain in the final sample preparation, a portion may be centrifuged at 10,000 rpm for 5 min prior to HPLC analysis. Alternatively, the sample may be filtered through a 0.45 µm membrane discarding the first 3 – 4 mL.

8.0 Test Conditions

8.1 Gradient – Isocratic

Time	%A	%B	Gradient Type
0.00	100	0	0
10.00	100	0	0

8.2 Column – XB C18, 5µm, 100Å, LC column, 250mm x 4.6mm

8.3 Flow Rate – 1.0mL/min

8.4 UV Detection – 215nm

8.5 Injection Volume – 20µL

8.6 Column Temperature – 28°C

8.7 3-D Spectral Range – 200nm to 300nm

8.8 Recommended Sequence

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

8.8.2 Make five injections of the Working Standard.

8.8.3 Make a single injection of each Sample Preparation.

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

8.9 System Suitability

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

8.9.2 The %RSD of all standard injections is NMT 5%.

8.10 Column Wash and Storage

8.10.1 Rinse the column with H_2O / methanol (90/10) at 1 mL/min for at least 15 min.

8.10.2 Rinse the column with H_2O / methanol (50/50) at 1 mL/min for at least 10 min.

8.10.3 Store the column with H_2O / methanol (50/50).

9.0 Example Calculations

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

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)

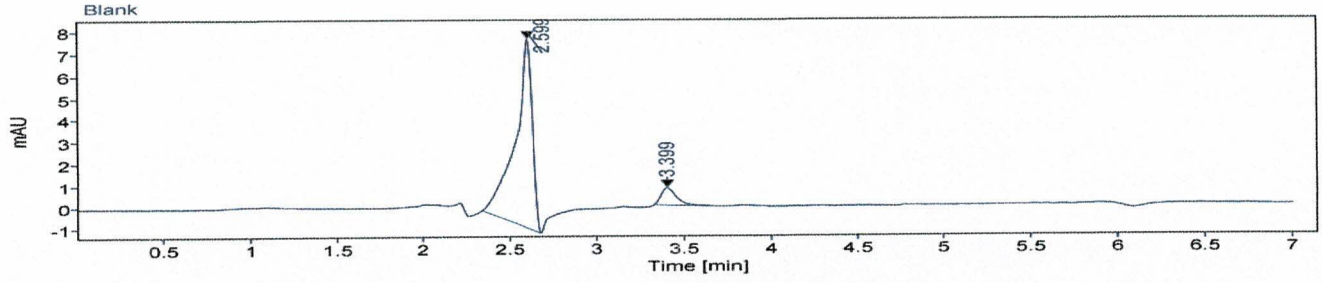
V_{spl} Volume of the sample preparation accounting for dilutions in mL

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.

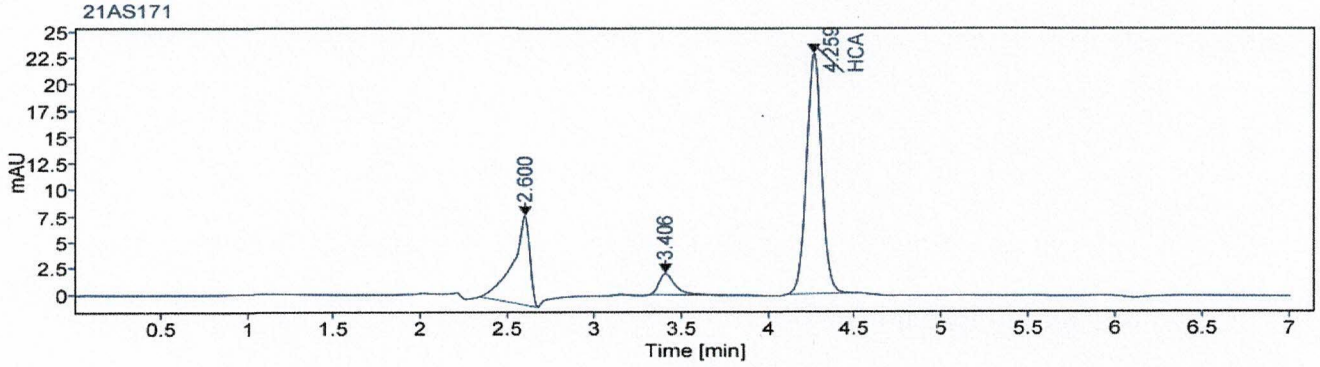
LA Label amount in mg per dose or 1 for raw materials

10.0 Example Chromatography

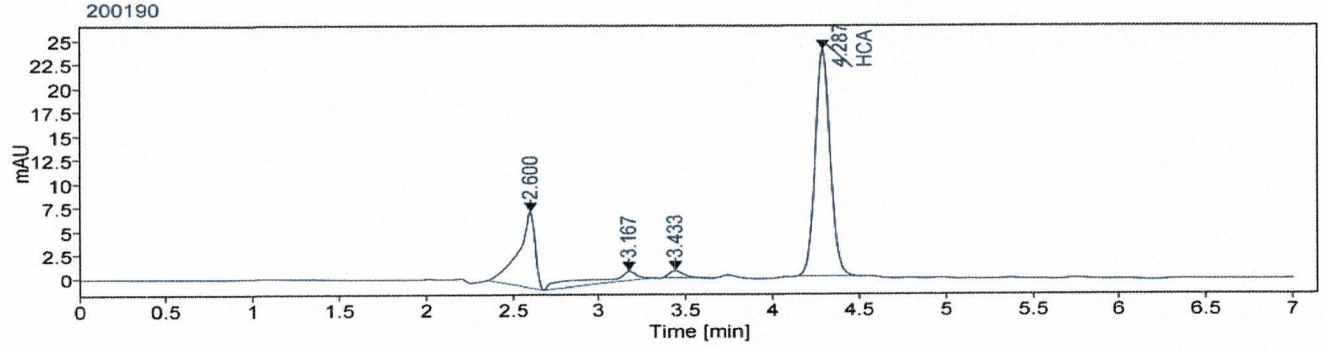
10.1 Diluent



10.2 Working Standard



10.3 Sample



11.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	10/16/14	New	14-0822	X. Shao
1	02/24/17	Biennial review: fixed typographical errors. Changed stability requirements to 100%	16-1138	J. Maignan
2	07/20/20	Scheduled Review: Removed requirements, updated responsibilities. Updated scope.	CC-20-0514	J. Maignan
3	06/01/22	Update for consistency with current methods. Add reference to method validation. Add recommended sequence section. Replace requirements section with system suitability. Add example chromatography.	CC-22-0252	S. Sassman
4	05/02/23	Remove unnecessary information and align with current SOP format, add instruction to follow test details containing product specific sample preparation, add specific sample prep instructions for different dosage forms. Changed logo.	CC-23-0226	S. Sassman