1.0 Purpose

The purpose of this procedure is to define the method for the quantitative analysis and/or identification of Coenzyme Q10 and its acid form in raw materials and finished products using HPLC with UV/VIS detection.

2.0 Scope

This procedure applies to the quantification and identification of Coenzyme Q10. Some excipients and dietary ingredients used in finished products can interfere with the analysis of Coenzyme Q10.

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 implement this procedure and to ensure that the procedure is being followed.

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

4.0 Definitions

4.1 **HPLC** – High Performance Liquid Chromatography

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

4.3 **Ubidecarenone** – Coenzyme Q10

4.4 **$FeCl_3$** – Ferric Chloride

4.5 **H_2O** – Water ($\geq$ 18.2 MΩ·cm)

4.6 **CofA** – Certificate of Analysis

5.0 References

5.1 Phenomenex, Application ID# 1070

5.2 Determination of Coenzyme Q10 Content in Raw material and Dietary Supplements by High-Performnce Liquid chromaography-UV: Collaborative Study. *J AOAC Int.* 2008; 91(4): 702-708

5.3 MV-LAB-15-035, Protocol, Coenzyme Q10 (Ubidecarenone) Determination using HPLC coupled with UV/VIS Spectroscopy

6.0 Reagents, Supplies, Glassware and Equipment

6.1 Chemicals: All reagents are HPLC grade or better.

6.1.1 Millipore Water

6.1.2 Denatured Ethanol

6.1.3 Methanol

6.1.4 $FeCl_3$

6.1.5 H_2O

6.2 Glassware and Supplies

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 50mL Volumetric Flask

6.2.5 100mL Volumetric Flask

6.3 Disposables

6.3.1 10mL Pipette Tips

6.3.2 1mL Pipette Tips

6.3.3 200µL Pipette Tips

6.3.4 16mL Test Tubes

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

6.3.6 Nylon Syringe Filters, 0.45µm

6.3.7 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 Stir Plate

6.4.4 Wrist Action Shaker

6.4.5 Vortex

6.4.6 Sonicator Bath

6.4.7 200µL, 1mL, and 10mL Pipettes- adjustable

6.4.8 Eppendorf Centrifuge

7.0 Preparation of Mobile Phase, Diluent, Samples and Standards

7.1 Mobile Phase A – Ethanol

7.2 Mobile Phase B – Methanol

7.3 Diluent – 1% $FeCl_3$ in Ethanol

7.3.1 Add 1.0 g of $FeCl_3$ to 1000mL of Ethanol.

7.3.2 Sonicate for 30min to dissolve.

7.3.3 Scale as necessary.

7.4 Standard Preparation

7.4.1 The linear range of the method is 0.0025 mg/mL – 0.25 mg/mL. The standard and sample preparations must be within the linear range of the method.

7.4.2 Use the actual purity from the CofA or the standard certification for Coenzyme Q10 reference material for calculations. The stock standard preparation reflects 100% content for the analyte assayed.

7.4.3 The standard is prepared by weighing no less than the minimum weight of the analytical balance, then suspended in the final volume and sonicated for 25 minutes. Then the sample is allowed to cool to RT before use.

7.4.4 Dilutions are prepared using diluent. Dilutions can be made using volumetric flasks or using 10mL, 1mL, and 200µL variable 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.

7.5 Sample Preparation

7.5.1 At least 10 dosage units are pooled and/or ground by mortar and pestle as necessary.

7.5.2 Based on the fill weight, tablet weight, or raw material potency, weigh a portion of the pooled dosages to generate an analyte concentration that is within the validated linearity and solubility range.

7.5.3 Samples can be dissolved in diluent at any volume starting from 10mL and any weight greater than the minimum weight of the analytical balance.

7.5.4 The sample is suspended in the final volume and sonicated for 25 minutes. Then the sample is allowed to cool to RT before use.

7.5.5 Before injection, insoluble matter should be removed via filtration using a nylon syringe filter. Discard at least 2mL of the filtrate before collecting a portion in an HPLC vial for analysis.

7.5.5.1 Alternatively, samples and standards can also be centrifuged at 5000 RPM for 3 minutes in an Eppendorf centrifuge to pellet insoluble matter.

7.5.6 For Coenzyme Q10 materials being analyzed for the first time using this method, in-process verification is required to demonstrate spectral purity and extraction efficiency before the method can be implemented.

8.0 Test Conditions

8.1 Gradient – Isocratic

Time	%A	%B	Gradient Type
0.00	65	35	0
15.0	65	35	0

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

8.3 Flow Rate – 1.0mL/min

8.4 UV Detection – 275nm

8.5 Injection Volume - 20µL

8.6 Column Temperature – 28°C

8.7 Retention Time – about 11 min

8.8 Recommended Sequence

8.8.1 Make at least 2 injections of the diluent.

8.8.2 Make five (5) injections of Standard Solution.

8.8.3 Make a single injection of each Sample Preparation.

8.8.4 Make a single injection of the Standard Solution after every six (6) samples and at the end of the run.

8.9 System Suitability Requirements

8.9.1 The %RSD of the first five (5) standard injections 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 Column wash is not required.

8.10.2 Store the column with mobile phase.

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

8.11.1 $$\%\ \text{assay} = \frac{R_u}{R_s} \times \frac{Wt_{std} \times P}{V_{std}} \times \frac{V_{spl}}{SA} \times \frac{SS}{LA} \times 100$$

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)

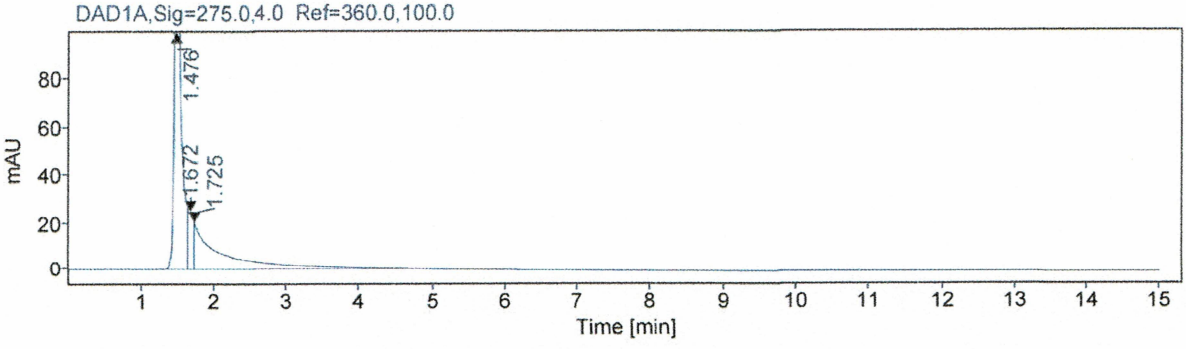
V_{spl} Volume of the sample preparation accounting for dilutions in mL

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.

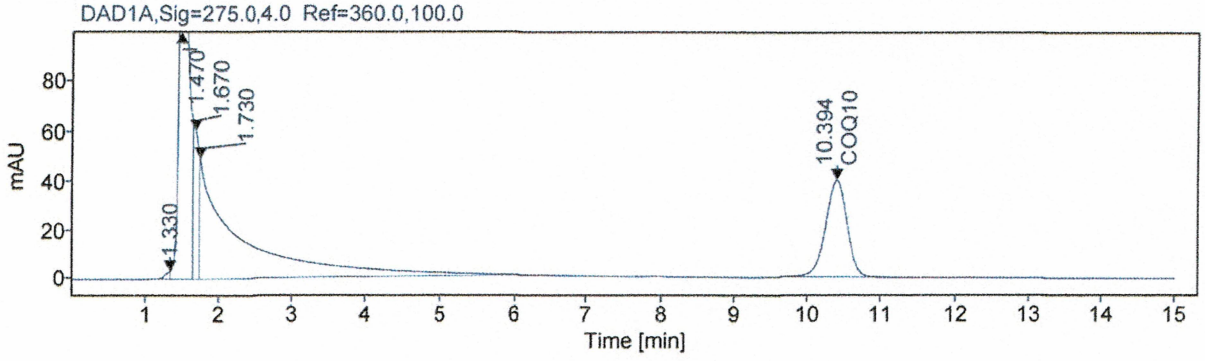
LA Label amount in mg per dose or 1 for raw materials

8.12 Example Chromatography

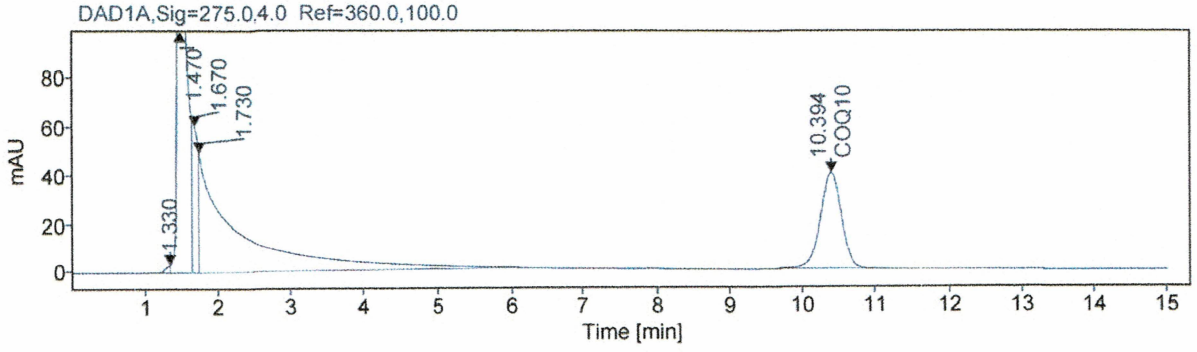
8.12.1 Blank



8.12.2 Standard



8.12.3 Sample



9.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	02/05/16	New	16-0104	X. Shao
1	01/29/19	Scheduled review: updated responsibilities and fixed typos.	19-0107	J. Maignan
2	04/15/22	Update to reflect current practices, add linear range, add recommended sequence, add system suitability section, add column wash and storage, add example chromatography.	CC-22-0181	S. Sassman