ION LABS	Standard Operating Procedure **Determination of L-Carnitine by HPLC/UV**	**SOP Number** **D-718**	**Revision** **1**
		Effective Date 04/25/22	**Page** **Page 1 of 6**
Written by/ Date SAS 04/05/22	**Reviewed by/ Date** 04/06/22	**Approved by/ Date** 04/06/22	
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1.0 Purpose

The purpose of this procedure is to define the method for the quantification and identification of L-Carnitine in raw materials and finished products by HPLC/UV.

2.0 Scope

This procedure applies to the quantification and identification of L-Carnitine in raw materials and finished products by HPLC/UV.

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 Analytical Development personnel to keep this procedure aligned with current practices.

4.0 Definitions

4.1 **HPLC/UV** – High Pressure Liquid Chromatography with Ultraviolet Detection

4.2 **QC** – Quality Control

4.3 **$NaH_2PO_4 \cdot 2H_2O$** – Sodium Phosphate Monobasic Dihydrate

4.4 **H_3PO_4** – Phosphoric Acid 85%

5.0 References

5.1 MV-LAB-19-028, Protocol, Validation of an Analytical Method for the Determination of L-Carnitine and O-Acetyl-L-Carnitine by HPLC/UV

6.0 Supplies

6.1 Chemicals: All reagents are HPLC grade or better.

6.1.1 Carnitine Reference Standard

6.1.2 Sodium-1-Nonanesulfonate

6.1.3 $NaH_2PO_4 \cdot 2H_2O$

6.1.4 H_3PO_4

6.2 Glassware

6.2.1 Volumetric glassware as required for standard and sample preparations

6.3 Disposables (as required for standard and sample preparations)

6.3.1 10mL Pipette Tips

6.3.2 1mL Pipette Tips

6.3.3 200µL Pipette Tips

6.3.4 Microcentrifuge tubes

6.3.5 16mL Test Tubes

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

6.3.7 Nylon Syringe Filters, 0.45 µm

6.3.8 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 Centrifuge

6.4.4 Adjustable Pipette

7.0 Procedure

7.1 Mobile Phase Preparation

7.1.1 Mobile Phase

7.1.1.1 5 mM sodium-1-nonanesulfonate, 20 mM NaH_2PO_4, pH 2.5 / methanol (70/30).

7.1.1.2 Combine 0.806 g of sodium-1-nonanesulfonate, 2.184 g of $NaH_2PO_4 \cdot 2H_2O$, and 700 mL of water. Begin stirring and adjust the pH to 2.5 using H_3PO_4. Add 300 mL of methanol and mix well.

7.1.1.3 Other hydration states of sodium-1-nonanesulfonate and NaH_2PO_4 may be used provided that correction is made for molecular weight.

7.1.2 Diluent

7.1.2.1 Use water as the diluent.

7.2 Standard Preparation

7.2.1 Accurately weigh and transfer about 30 mg of L-carnitine reference standard (or 37 mg of the hydrochloride salt) into a 50-mL volumetric flask.

7.2.2 Dissolve in and dilute to volume with Water.

7.3 Sample Preparation

7.3.1 The linear range of the method for L-carnitine is 0.16 – 0.80 mg/mL. The sample concentration must be within the linear range of the method.

7.3.2 For raw materials: weigh no less than 20 mg into a suitably sized volumetric flask of no less than 50 mL volume to generate an analyte concentration that is within the validated linearity range. Dissolve in and dilute to volume with water.

7.3.3 For solid dose finished products: Combine and homogenize no less than ten dosage units. Based on the label claim and fill weight (for capsules) or tablet weight per dose, weigh no less than 20 mg of the pooled dosages into a suitably sized volumetric flask of no less than 50 mL to generate an analyte concentration that is within the validated linearity range. Dilute the sample to 2/3 of the flask volume with water and swirl to dissolve. Shaking or sonication can also be used to assist dissolution. After the sample is dissolved, equilibrate the sample to room temperature (if sonicated), and dilute to volume using water.

7.3.4 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 water collecting the rinses in the volumetric flask. Dilute to volume using water.

7.3.5 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 suitably sized volumetric flask to generate an analyte concentration that is within the validated linearity range. Dilute the sample to 2/3 of the flask volume with water and shake for 20 minutes. Sonicate for 5 minutes, and then shake again for an additional 15 minutes. Dilute to volume with water, and mix well. Filter the solution through a 0.45 µm membrane discarding the first 3-4 mL. Alternatively, the solution may be centrifuged to remove particulates provided that the final solution is clear.

7.3.6 If particulates remain in the final sample preparation, a portion may be centrifuged at 10,000 rpm for 200 seconds prior to HPLC analysis. Alternatively, the sample may be filtered through a 0.45 µm membrane discarding the first 3 – 4 mL.

7.3.7 For finished products or raw materials being analyzed for the first time using this method, an in process validation is required to demonstrate spectral purity, baseline separation of peaks, and extraction efficiency as a part of system suitability before data can be reported using this method.

7.4 HPLC Parameters

7.4.1 Column: Inertsil ODS-3, 5 µm, 4.6 mm X 150 mm or equivalent

7.4.2 Column Temperature: 40 °C

7.4.3 Flow rate: 1.0 mL/min

7.4.4 Wavelength: 210 nm

7.4.5 Injection Volume: 10 µL

7.4.6 Run Time: 10 minutes

7.4.7 Spectral Range (for Identification)- 200 nm to 400 nm

7.5 Retention Time

7.5.1 Carnitine – 4.9 min

7.6 Recommended Sequence

7.6.1 Make at least 2 injections of the diluent.

7.6.2 Make five (5) injections of Standard Solution.

7.6.3 Make a single injection of each Sample Preparation.

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

7.7 System Suitability Requirements

7.7.1 The %RSD of the first five (5) standard injections is NMT 5.0%.

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

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

7.8.1 $$\%\ \text{assay} = \frac{R_u}{R_s} \times \frac{Wt_{std} \times P}{V_{std}} \times \frac{V_{spl}}{SA} \times \frac{SS}{LA} \times 100$$

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

7.9 Column Wash and Storage

7.9.1 Rinse and store the column with Water / MeOH (70/30)

8.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	05/28/20	New	N/A	S. Sassman
1	03/23/22	Minor edits for clarity. Add bracketing standard requirement for system suitability.	CC-22-0118	S. Sassman