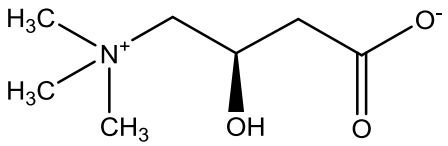
# Certificate of Analysis

**ISO GUIDE 34**
**ANAB Cert# AR-1470**

**ISO/IEC 17025**
**ANAB Cert# AT-1467**

## LEVOCARNITINE

***CERTIFIED REFERENCE MATERIAL***




**CERTIFIED PURITY: 98.9%,** $U_{crm}$ = ±0.2% k = 2.1
(Mass Balance/anhydrous basis)

**NOMINAL PACKAGE SIZE:** 1g

**CATALOG #:** PHR1569 **LOT #:** LRAB7787

**CERTIFICATE VERSION:** LRAB7787.1 **ISSUE DATE:** 29 June 2018
*Note: Certificates may be updated due to Pharmacopeial Lot changes or the availability of new data. Check our website at:* www.sigma-aldrich.com *for the most current version.*

**CRM EXPIRATION:** 31 December 2022 (Proper Storage and Handling Required).

**RECEIPT DATE:** _____
Note: this space is provided for convenience only and its use is not required.

**STORAGE:** Store at Room Temperature, keep container tightly closed. Attachment of a 20 mm aluminum crimp seal recommended for unused portions.

**CHEMICAL FORMULA:** $C_7H_{15}NO_3$ **MW:** 161.2

**PHYSICAL DESCRIPTION:** White powder in amber vial **CAS #:** 541-15-1

**HAZARDS:** Read Safety Data Sheet before using. All chemical reference materials should be considered potentially hazardous and should be used only by qualified laboratory personnel.

**INSTRUCTIONS FOR USE:** Determine water content at the time of use. Use on the Anhydrous Basis. The internal pressure of the container may be slightly different from the atmospheric pressure at the user's location. Open slowly and carefully to avoid dispersion of the material. This material is intended for Laboratory Use only. Not for drug, household or other uses.

## TRACEABILITY ASSAY

Comparative assay demonstrates direct traceability to Pharmacopeial Standards

**METHOD: HPLC (ref.: Levocarnitine Oral Solution, Current Compendial Monographs)**

### *ASSAY vs. USP REFERENCE STANDARD (anhydrous basis)*

| ASSAY VALUE | vs. USP LOT |
|---|---|
| **99.96%** | **G1J095**<br>Labeled Content = 0.999 mg/mg |

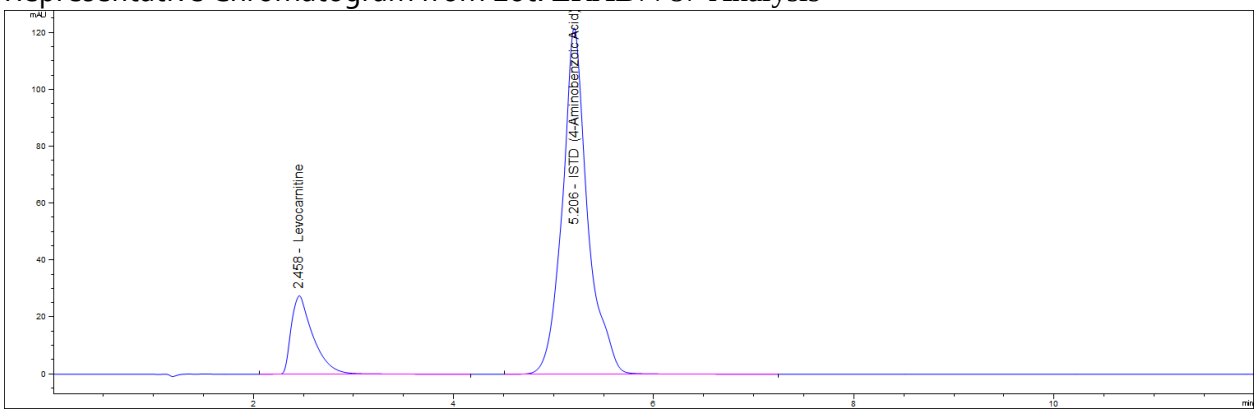
### *ASSAY vs. EP CRS (anhydrous basis)*

| ASSAY VALUE | vs. EP BATCH |
|---|---|
| **99.96%** | **3.0**<br>Labeled Content = None<br>Assigned Content = 99.2%* |

*The assigned content of the EP CRS was determined by assay against the USP Reference Standard

Column: Supelcosil LC-18-DB, 4.6 x 150mm, 5µm
Mobile Phase: 0.555mg/mL Sodium-1-heptanesulfonate in Buffer, Methanol (98:2)
Buffer: 0.05M $H_3PO_4$ in Water (pH 2.4)
Flow Rate: 1.5 mL/min
Column Temperature: 30 ºC
Injection: 40 µL
Detector: 225 nm
Internal Standard: 4-Aminobenzoic Acid

Representative Chromatogram from Lot: LRAB7787 Analysis




## PURITY DETERMINATION BY MASS BALANCE

### CHROMATOGRAPHIC IMPURITY ANALYSIS

**METHOD: HPLC (ref.: Levocarnitine, Current Compendial Monographs)**

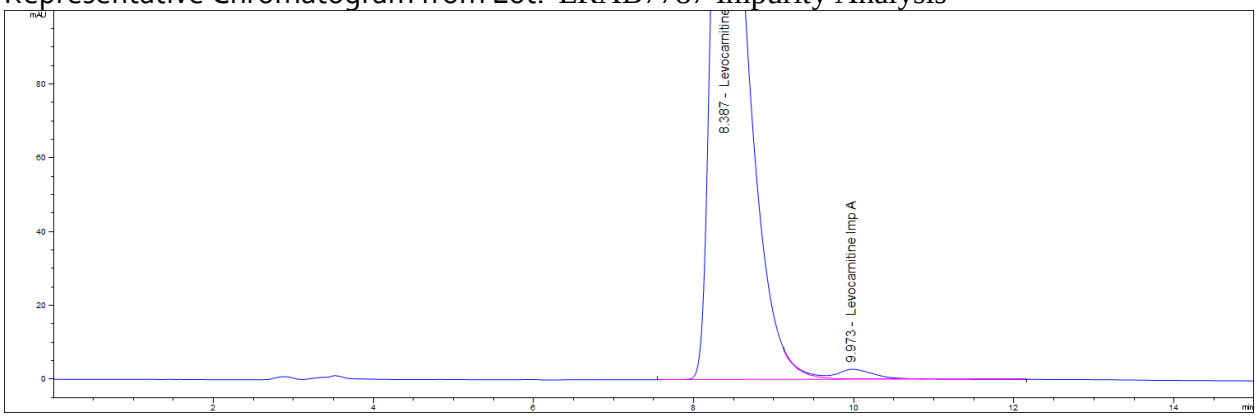
Column: Supelcosil $NH_2$, 4.6 x 250mm 4.6µm
Mobile Phase: 6.81 g/L $KH_2PO_4$ in H2O (pH 4.69), Acetonitrile (35:65)
Flow Rate: 1.0 mL/min
Column Temperature: 30 °C
Injection: 25 µL
Detector: 205 nm

Impurities Detected:

Levocarnitine Impurity A: 0.023%

Total Impurities: **0.023%**

Representative Chromatogram from Lot: LRAB7787 Impurity Analysis



## RESIDUAL SOLVENTS

Method: GC-MS Headspace (ref.: Adapted from Residual Solvents USP <467>)
Column: SPB-624, 30 m x 0.25 mm, 1.4 mm
Carrier gas: He
Flow: 1.2 mL/min
Split Ratio: 1:5
Injection/Temperature: 1 mL/220 °C
Temperature Program: 40 °C for 5 min, 8 °C/min to 200 °C, hold 5 min

Solvents Detected: **None**

## WATER DETERMINATION

Method: Karl Fisher titration (ref.: Current Compendial Monographs)
Mean of three measurements, Water Content = **0.952%**

## RESIDUE ANALYSIS

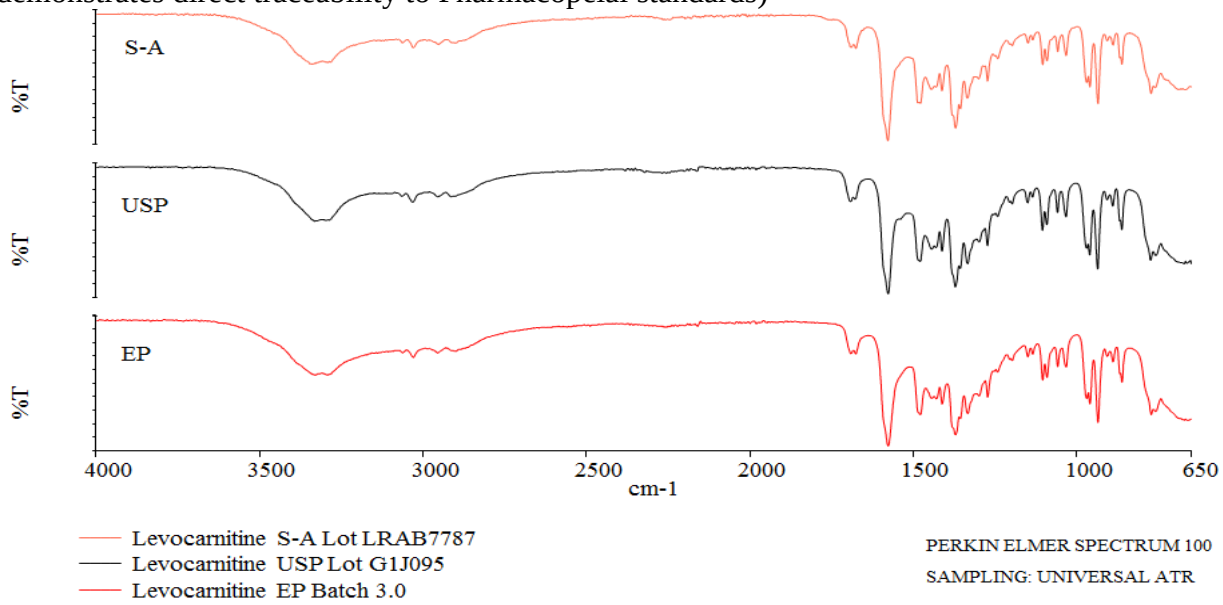
Method: Sulfated Ash (ref.: Current Compendial Monographs)
Sample Size: ~100mg
Mean of three measurements, Residue = **0.071%**

## CERTIFIED PURITY BY MASS BALANCE [100% - Impurities (normalized)]

**98.9%** $U_{crm}$ = ±0.2%, k = 2.1
(anhydrous basis)

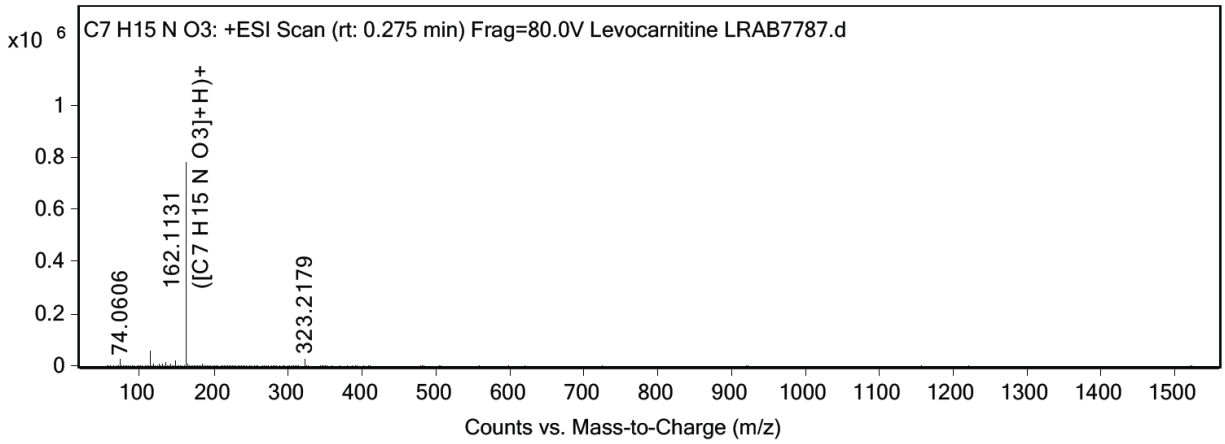
## IDENTIFICATION TESTS

### INFRARED SPECTROPHOTOMETRY (Comparative identification analysis demonstrates direct traceability to Pharmacopeial standards)



## MASS SPECTRUM

Method: HR-QTOF; 4.0 kV ESI+; temperature: 325 °C




Theoretical value: 162.1130 m/z

The signal of the MS spectrum is consistent with the theoretical value and its interpretation is consistent with the structural formula.

## OPTICAL ROTATION

Specification: Between -29° and -32° (USP)
Perkin Elmer Polarimeter 343
Wavelength: 589 nm
Concentration: 100mg/mL in Water
Cell Path: 100 mm
Mean of three Measurements = **-30.9°**

HOMOGENEITY ASSESSMENT

Homogeneity was assessed in accordance with ISO Guide 35. Completed units were sampled using a random stratified sampling protocol. The results of chemical analysis were then compared by Single Factor Analysis of Variance (ANOVA). The uncertainty due to homogeneity was derived from the ANOVA. Heterogeneity was not detected under the conditions of the ANOVA.

Analytical Method: HPLC Sample size: ~50 mg

UNCERTAINTY STATEMENT

Uncertainty values in this document are expressed as Expanded Uncertainty ($U_{crm}$) corresponding to the 95% confidence interval. $U_{crm}$ is derived from the combined standard uncertainty multiplied by the coverage factor k, which is obtained from a *t*-distribution and degrees of freedom. The components of combined standard uncertainty include the uncertainties due to characterization, homogeneity, long term stability, and short term stability (transport). The components due to stability are generally considered to be negligible unless otherwise indicated by stability studies.

STABILITY ASSESSMENT

Significance of the stability assessment will be demonstrated if the analytical result of the study and the range of values represented by the Expanded Uncertainty do not overlap the result of the original assay and the range of its values represented by the Expanded Uncertainty. The method employed will usually be the same method used to characterize the assay value in the initial evaluation.

Long Term Stability Evaluation - An assessment, or re-test, versus a Compendial Reference Standard may be scheduled, within the 3 year anniversary date of a release of a Secondary Standard. The re-test interval will be determined on a case-by-case basis.

Short Term Stability Study - It is useful to assess stability under reasonably anticipated, short term transport conditions by simulating exposure of the product to humidity and temperature stress. This type of study is conducted under controlled conditions of elevated temperature and humidity.

QC Manager

QA Supervisor

APPENDIX

Original Release Date: 29 June 2018

Manufactured and certified by Sigma-Aldrich RTC, Inc.
2931 Soldier Springs Rd, Laramie WY, USA 82070
(Phone): 1-307-742-5452 (Fax): 1-855-831-9212
email: RTCTechGroup@sial.com

ANAB ACCREDITED ISO/IEC 17025 TESTING LABORATORY

ANAB ACCREDITED ISO GUIDE 34 REFERENCE MATERIAL PRODUCER