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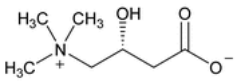
Levocarnitine



$C_7H_{15}NO_3$ 161.20

(*R*)-3-Carboxy-2-hydroxy-*N*,*N*,*N*-trimethyl-1-propanaminium, inner salt;

(*R*)-(3-Carboxy-2-hydroxypropyl)trimethylammonium, inner salt CAS RN®: 541-15-1; UNII: 0G389FZZ9M.

DEFINITION

Levocarnitine contains NLT 97.0% and NMT 103.0% of levocarnitine ($C_7H_{15}NO_3$), calculated on the anhydrous basis.

IDENTIFICATION

• **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197K

Analysis: Dry the sample and USP Levocarnitine RS under vacuum at 50° for 5 h.

Acceptance criteria: Meets the requirements

• **B.** The retention time of the major peak of the derivatized *Sample solution* corresponds to that of the levocarnitine peak of the derivatized *System suitability solution*, as obtained in the test for *Enantiomeric Purity*.

ASSAY

• **Procedure**

Sample: 100 mg of Levocarnitine

Blank: Mix 3 mL of formic acid with 50 mL of glacial acetic acid.

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in a mixture of 3 mL of formic acid and 50 mL of glacial acetic acid. Add 2 drops of crystal violet TS, and titrate with the *Titrant* to an emerald green endpoint. Perform the blank determination.

Calculate the percentage of levocarnitine ($C_7H_{15}NO_3$) in the portion of Levocarnitine taken:

$$\text{Result} = \{[(V_S - V_B) \times N_A \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)

V_B = *Titrant* volume consumed by the *Blank* (mL)

N_A = actual normality of the *Titrant* (mEq/mL)

F = equivalency factor, 161.2 mg/mEq

W = *Sample* weight (mg)

Acceptance criteria: 97.0%–103.0% on the anhydrous basis

IMPURITIES

• **Residue on Ignition ⟨281⟩:** NMT 0.5%

• **Chloride and Sulfate ⟨221⟩, *Chloride***

Standard solution: 0.50 mL of 0.020 N hydrochloric acid

Sample: 0.090 g of Levocarnitine

Acceptance criteria: NMT 0.4%

• **Enantiomeric Purity**

Buffer solution: Mix thoroughly 2000 mL of water with 5 mL of phosphoric acid, and add accurately 13.6 mL of triethylamine dropwise while stirring.

Solution A: Mix 1500 mL of *Buffer solution* and 500 mL of acetonitrile. Adjust the solution with phosphoric acid to a pH of 2.6.

Solution B: Acetonitrile

Carbonate buffer solution: Transfer 338 mg of sodium carbonate and 152 mg of sodium bicarbonate to a 100-mL volumetric flask, and dissolve in and dilute with water to volume.

Sodium hydroxide solution: 30% solution of sodium hydroxide in water

Acetate buffer solution: Transfer 0.3 mL of glacial acetic acid to a 100-mL volumetric flask, add 90 mL of water to dissolve, adjust with *Sodium hydroxide solution* to a pH of 4.2, and dilute with water to volume.

Derivatization reagent: (+)-1-(9-Fluorenyl)ethyl chloroformate solution ((+)-FLEC)

System suitability solution: 1.25 mg/mL of USP Levocarnitine RS in water

Sample solution: 1.25 mg/mL of Levocarnitine in water

Blank: Water

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: Fluorescence

Excitation wavelength: 260 nm

Emission wavelength: 315 nm

Column: 4.6-mm × 7.5-cm; 2.7-µm packing L1

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 20.0 µL

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	100	0
7.4	100	0
7.5	2	98
9.1	2	98
9.3	100	0
11.0	100	0

After each sequence of samples, rinse the column with water for 10 min and then with acetonitrile and water (98:2) for another 10 min.

System suitability

Sample: Derivatized *System suitability solution*. Prepare as directed in *Analysis*.

[Note—The relative retention times for the (+)-FLEC derivatives of D-carnitine and L-carnitine are about 0.87 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between (+)-FLEC derivatives of D-carnitine and L-carnitine

Analysis

Samples: *System suitability solution*, *Sample solution*, and *Blank*

Transfer 30.0 µL of the *Blank*, *System suitability solution*, and *Sample solution* to separate 10-mL test tubes. Add 30 µL of *Carbonate buffer solution* and 80 µL of *Derivatization reagent* to each test tube and mix by vortex mixer. Allow the solutions to react for 1 h at 45° over a water bath. Cool the test tubes to room temperature, add 5.0 mL of *Acetate buffer solution* to each test tube, mix by vortex mixer, and transfer to vials for chromatographic analyses.

Separately inject and analyze equal volumes of derivatized *Blank*, derivatized *System suitability solution*, and derivatized *Sample solution*. Identify the two diastereomer peaks due to (+)-FLEC derivatives of D-carnitine and L-carnitine from the derivatized *System suitability solution* and derivatized *Sample solution*. Depending on the enantiomeric purity of the *Derivatization reagent*, these two peaks may contain co-eluting enantiomers of (−)-FLEC derivatives with D- and L-carnitines, which are accounted in the percentage calculations below. There should be no peaks observed at the retention times of D- and L-carnitine derivatives from the derivatized *Blank*.

Calculate the percentage of L-carnitine derivative (R_L) from the derivatized *Sample solution*:

$$\text{Result} = r_L/(r_L + r_D) \times 100$$

r_L = peak response of the L-carnitine derivative from the derivatized *Sample solution*

r_D = peak response of the D-carnitine derivative from the derivatized *Sample solution*

Calculate the corrected percentage of L-carnitine ($C_7H_{15}NO_3$) (C_L) in the portion of Levocarnitine taken:

$$\text{Result} = (R_L - P_B)/(P_A - P_B) \times 100$$

R_L = percentage of L-carnitine derivative, calculated previously

P_B = percentage of (−)-FLEC as determined for (+)-1-(9-Fluorenyl)ethyl chloroformate solution

P_A = percentage of (+)-FLEC as determined for (+)-1-(9-Fluorenyl)ethyl chloroformate solution

Calculate the corrected percentage of D-carnitine in the portion of Levocarnitine taken:

$$\text{Result} = 100 - C_L$$

C_L = corrected percentage of L-carnitine, calculated previously

Acceptance criteria: NMT 0.2% of D-carnitine

- **Limit of Potassium**

[Note—The *Standard solution* and the *Sample solutions* may be modified, if necessary, to obtain solutions of suitable concentrations adaptable to the linear or working range of the instrument.]

Standard solution: 31.25 µg/mL of potassium in water, prepared from potassium chloride previously dried at 105° for 2 h

Sample stock solution: 0.625 mg/mL of Levocarnitine in water

Sample solution A: Transfer 20.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, and dilute with water to volume. This solution contains 500 µg/mL of Levocarnitine and 0 µg/mL of added potassium from the *Standard solution*.

Sample solution B: Transfer 20.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, add 2.0 mL of the *Standard solution*, and dilute with water to volume. This solution contains 500 µg/mL of Levocarnitine and 2.5 µg/mL of added potassium from the *Standard solution*.

Sample solution C: Transfer 20.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, add 4.0 mL of the *Standard solution*, and dilute with water to volume. This solution contains 500 µg/mL of Levocarnitine and 5.0 µg/mL of added potassium from the *Standard solution*.

Blank: Water

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 766.7 nm

Lamp: Potassium hollow-cathode

Flame: Air–acetylene

Analysis

Samples: *Sample solution A*, *Sample solution B*, *Sample solution C*, and *Blank*

Determine the absorbances of the solutions against the *Blank*. Plot the absorbances of the three *Sample solutions* versus their added potassium concentrations, in µg/mL. Draw the straight line best fitting the three points, and extrapolate the line until it intercepts the concentration axis. From the intercept determine the concentration, in µg/mL, of potassium in *Sample solution A*.

Calculate the percentage of potassium in the portion of Levocarnitine taken:

$$\text{Result} = (C_K/C_U) \times 100$$

C_K = concentration of potassium in *Sample solution A* (µg/mL), determined from the intercept of the linear regression line

C_U = concentration of Levocarnitine in *Sample solution A* (µg/mL)

Acceptance criteria: NMT 0.2%

- **Limit of Sodium**

[Note—The *Standard solution* and the *Sample solutions* may be modified, if necessary, to obtain solutions of suitable concentrations adaptable to the linear or working range of the instrument.]

Standard solution: 250 µg/mL of sodium in water, prepared from sodium chloride previously dried at 105° for 2 h

Sample stock solution: 40.0 mg/mL of Levocarnitine in water

Sample solution A: Transfer 20.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, and dilute with water to volume. This solution contains 32 mg/mL of Levocarnitine and 0 µg/mL of added sodium from the *Standard solution*.

Sample solution B: Transfer 20.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, add 2.0 mL of the *Standard solution*, and dilute with water to volume. This solution contains 32 mg/mL of Levocarnitine and 20 µg/mL of added sodium from the *Standard solution*.

Sample solution C: Transfer 20.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, add 4.0 mL of the *Standard solution*, and dilute with water to volume. This solution contains 32 mg/mL of Levocarnitine and 40 µg/mL of added sodium from the *Standard solution*.

Blank: Water

Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 589.0 nm

Lamp: Sodium hollow-cathode

Flame: Air–acetylene

Analysis

Samples: *Sample solution A*, *Sample solution B*, *Sample solution C*, and *Blank*

Determine the absorbances of the solutions against the *Blank*. Plot the absorbances of the three *Sample solutions* versus their added sodium concentrations, in µg/mL. Draw the straight line best fitting the three points, and extrapolate the line until it intercepts the concentration axis. From the intercept determine the concentration, in µg/mL, of sodium in *Sample solution A*.

Calculate the percentage of sodium in the portion of Levocarnitine taken:

$$\text{Result} = (C_{Na}/C_U) \times 100$$

C_{Na} = concentration of sodium in *Sample solution A* (µg/mL), determined from the intercept of the linear regression line

C_U = concentration of Levocarnitine in *Sample solution A* (µg/mL)

Acceptance criteria: NMT 0.1%

SPECIFIC TESTS

• **pH** ⟨791⟩

Sample solution: 50 mg/mL

Acceptance criteria: 5.5–9.5

Change to read:

• **Water Determination** ⟨921⟩▲, *Method I, Method Ia*▲ (USP 1-Dec-2021) : NMT 4.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers at temperatures between −15° and 35°. Protect from light.

• **USP Reference Standards** ⟨11⟩

USP Levocarnitine RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
LEVOCARNITINE	Fatkhulla K Tadjimukhamedov Associate Scientific Liaison	NBDS2020 Non-botanical Dietary Supplements

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
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