RESEARCH PAPER

Development and Validation of a Stability-Indicating HPLC Method for Levocarnitine Oral Solution Impurity Profiling (USP SUGAR and SUGAR-FREE)

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ABSTRACT
Levocarnitine Oral Solution (0.1 g/10 mL), an orphan drug product official in the USP monograph, lacks a compendial method for organic impurity determination. This study develops and validates a simple, cost-effective, high-performance liquid chromatography (HPLC) method for quantifying levocarnitine and related impurities in both sugar and sugar-free formulations, addressing this critical gap in quality control. The reversed-phase HPLC method employed a C18 column with gradient elution, achieving baseline resolution of levocarnitine (RT 4.6 min) and key impurities, including Levocarnitine Impurity-A (RT 4.9 min). Relative retention times (RRTs) and relative response factors (RRFs) were established for principal impurities. Per ICH Q2(R1) guidelines, comprehensive validation assessed specificity, system precision (RSD <1.0%), method precision (RSD <2.0%), LOQ precision (RSD <10%), linearity (r^2 >0.999 over LOQ to 300% specification level), accuracy (recovery 98-102%), robustness, ruggedness, and solution stability. Forced degradation studies under acidic, basic, oxidative, thermal, and photolytic conditions confirmed method specificity, demonstrating no interference from placebo, diluent, or degradation products. Solution stability at 25°C for 45 hours showed peak area variation <2.5%, affirming suitability for routine analysis. This sensitive, precise, and stability-indicating method provides a reliable tool for impurity profiling and quality assurance of levocarnitine oral solutions when compendial methods are unavailable, ensuring therapeutic product integrity.
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INTRODUCTION

Orphan drugs represent a vital class of pharmaceuticals developed to treat rare diseases affecting a small fraction of the population, posing unique challenges to drug development and regulatory approval [1]. The critical need for safe and effective therapies for these underserved patient groups makes the development of robust, reliable analytical methods paramount, not only for regulatory compliance but also to ensure patient safety during long-term treatment.

Levocarnitine (L-carnitine) is a naturally occurring quaternary ammonium compound essential for mitochondrial transport of long-chain fatty acids, thereby playing a key role in cellular energy metabolism [2]. It is clinically indicated for managing primary systemic carnitine deficiency as well as secondary deficiencies linked to conditions such as renal disease, valproic acid therapy, and neuromuscular disorders. Due to its indispensable physiological function, Levocarnitine has been granted orphan drug designation, underscoring its therapeutic significance in rare metabolic disorders [2].

While Levocarnitine has been prescribed for decades, emerging clinical demands have led to wider use of oral formulations, particularly sugar-based and sugar-free suspensions to accommodate pediatric, diabetic, and other special populations. The introduction of these formulations elevates the importance of stringent quality control measures that include detailed impurity profiling to safeguard product safety, efficacy, and stability over shelf life. Notably, process- and degradation-related impurities such as Levocarnitine Impurity-A pose risks that require sensitive and specific detection techniques [3,4].

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Despite scattered reports on Levocarnitine assay methods, a notable gap exists in validated, stability-indicating analytical procedures tailored for both sugar and sugar-free oral solutions. The significance of this study lies in its holistic approach, developing and validating a simple, reliable HPLC method capable of separating Levocarnitine from its primary impurity and extensive degradation products. This capability is crucial to meet regulatory expectations and evolving therapeutic needs, especially given the increasing preference for sugar-free formulations in vulnerable populations [5,6].

This research further contributes by thoroughly examining method robustness, filter compatibility, solution stability, and forced degradation under multiple stress conditions, including acid/base hydrolysis, oxidation, thermal, and photolytic exposure, that are often underreported in routine impurity studies. By demonstrating that the method maintains accuracy and specificity under these stringent conditions, it stands as both a quantitative and confirmatory technique. This aligns rigorously with ICH guidelines that mandate validated methods to exhibit specificity in the presence of degradation products [7,8].

In sum, the uniqueness of the present work derives from its comprehensive validation of a stability-indicating method for an orphan drug across multiple oral formulations, thereby filling a critical analytical void. The developed procedure not only supports routine quality control testing but also extends its utility to stability studies, fulfilling dual regulatory and therapeutic objectives. Ultimately, the study highlights the critical role of robust analytical validation in ensuring that orphan drugs like Levocarnitine remain safe, effective, and reliable therapies for their specialized patient populations.

MATERIALS AND METHODS:

Materials

Levocarnitine Impurity-A reference standard was purchased from EP reference standards. Acetonitrile (HPLC grade) was purchased from Merck, and Orthophosphoric acid (HPLC grade) was purchased from Merck (Germany). Sodium lauryl sulfate and Monobasic Ammonium phosphate were purchased from Sigma-Aldrich. Milli-Q water was obtained by reverse osmosis using a Millipak system (0.22 µm; Millipore, USA).

Preparation of Mobile Phase

The mobile phase was prepared by combining 300 mL of acetonitrile, 700 mL of water, and 1 mL of phosphoric acid in a suitable container. Subsequently, 2.88 g of sodium lauryl sulfate and 2.3 g of monobasic ammonium phosphate were added to the mixture. The solution was sonicated until complete dissolution, and the pH was adjusted to 2.40 using dilute phosphoric acid. The final mixture was homogenized and filtered through a 0.45 µm nylon membrane filter before use.[9]

Standard Preparation

Approximately 5 mg of Levocarnitine Reference Standard and RC-A were weighed and transferred into a 50 mL volumetric flask. About 30 mL of diluent was added, followed by sonication until complete dissolution. The solution was then diluted to volume with diluent and mixed well, resulting in a concentration of approximately 100 µg/mL for both compounds. For the working standard solution, 3.0 mL of the stock solution was pipetted into a 50 mL volumetric flask, diluted to volume with diluent, and mixed, yielding a concentration of about 6 µg/mL for Levocarnitine and RC-A.[10]

Sample Preparation

For the analysis, an accurately measured 10.0 mL of Levocarnitine oral solution, corresponding to 1000 mg of Levocarnitine, was transferred into a 200 mL volumetric flask. The solution was diluted to approximately three-fourths of the flask volume with the diluent and sonicated to ensure complete dissolution. The volume was then made up to the mark with the diluent, followed by thorough mixing. The prepared solution was filtered through a 0.45 µm PVDF membrane filter, discarding the initial portion of the filtrate. The final concentration of Levocarnitine in the resulting solution was approximately 5000 µg/mL.

Chromatographic Parameters

Chromatographic separation was carried out using a Waters X-Bridge C18 column (4.6 × 150 mm, 3.5 µm; P/N 186003034). The mobile phase was composed of acetonitrile, water, and phosphoric acid in the proportion of 300:700:1 (v/v/v), further supplemented with 10 mM sodium lauryl sulfate and 20 mM ammonium phosphate, and the pH was adjusted to 2.40. The mobile phase was delivered at a flow rate of 1.2 mL/min. The column oven temperature was maintained at 30°C, while the autosampler temperature was set at 25°C. A UV detector was employed at a wavelength of 205 nm. The injection volume was 10 µL, and the total run time was 20 minutes. [11] System suitability was assessed by injecting six replicates of the standard solution. The relative standard deviation (%RSD) for the peak area response of Levocarnitine Impurity-A was required to be not more than 6.0%. [12]

VALIDATION OF ANALYTICAL METHOD

System Precision

System precision was evaluated by preparing a standard solution of Levocarnitine Impurity-A according to the test method. The solution was injected six times into the chromatographic system under the specified conditions. The peak area responses were recorded, and the relative standard deviation (%RSD) was calculated.[13]

Linearity:

Linearity was established by preparing solutions of Levocarnitine at concentrations corresponding to 0.002–0.80% of the expected impurity level of Levocarnitine Impurity-A. Each solution was injected into the HPLC system under the specified chromatographic conditions. A calibration curve was constructed by plotting Levocarnitine Impurity-A peak area against the

corresponding concentration in µg/mL, covering the specified range. [14]

Limit of Quantitation (LOQ):
The limit of quantitation (LOQ) for Levocarnitine Impurity-A was determined by serially diluting the solution to progressively lower concentration levels and evaluating the signal-to-noise (S/N) ratio. Precision at the LOQ level was assessed by preparing and injecting the LOQ solution of Levocarnitine Impurity-A in six replicates under the specified chromatographic conditions. [15]

Method Precision:
The precision of the organic impurity method was determined by preparing six (6) individual sample solutions, each of sugar-based and sugar-free formulations of Levocarnitine Oral Solution USP (1.0 g/10 mL). All sample solutions were prepared according to the validated analytical procedure and injected under the established chromatographic conditions.[15]

Method Accuracy (Recovery):
To evaluate the accuracy of the proposed method, placebo solutions (both sugar-based and sugar-free formulations) were taken. Known and varying amounts of Levocarnitine Impurity-A were quantitatively spiked into the placebo matrix at levels representing 0.002% to 1.44%, with respect to the sample concentration.

The spiked samples were prepared in triplicate in accordance with the validated analytical method and subsequently injected under the chromatographic conditions described. The recovery of Levocarnitine Impurity-A was calculated at each concentration level to determine the accuracy of the method across the studied range. [16,17]

Solution Stability:
Standard and sample solutions of Levocarnitine Oral Solution USP (both sugar and sugar-free formulations) were prepared in accordance with the validated analytical method. Prepared standard and sample solutions were stored at ambient room temperature (25°C) and injected at designated time intervals:

For standards: Initial (0 hr), 23 hr, and 47 hr.

For samples: Initial (0 hr), 21 hr, and 45 hr.

For the standard solution, the percentage difference in peak area was calculated between the initial and subsequent time points to evaluate stability. For the sample solutions, the percentage difference in individual impurity levels (% impurity) was determined between initial and time interval measurements.[18]

Intermediate Precision:(Ruggedness)
To assess the ruggedness (intermediate precision) of the organic impurity method, standard and six (6) individual sample solutions of sugar-based and sugar-free Levocarnitine Oral Solution USP (1.0 g/10 mL) were prepared by a second analyst on a different day using a different HPLC system and a different column. All samples were prepared as per the validated method and injected under established chromatographic conditions.

Precision and ruggedness were evaluated by comparing the impurity results obtained from the two analysts, instruments, and columns. The %RSD for the % impurity of individual components was calculated across all sample preparations.[19]

Forced Degradation and Specificity Study
Blank, standard, control, stress, and spiked sample solutions of both sugar and sugar-free formulations of Levocarnitine Oral Solution USP (1.0 g/10 mL) were prepared and injected into the chromatographic system to assess peak identification and confirm the absence of interference at the Levocarnitine retention time.

Forced degradation studies were performed to demonstrate that the analytical method is stability-indicating. The samples were subjected to the following stress conditions:

Acid stress, Base stress, Peroxide (oxidative) stress, UV light stress, and Heat stress

Control Sample Preparation
Approximately 10.0 g of each formulation (equivalent to 1000 mg Levocarnitine) was accurately weighed into individual 200-mL volumetric flasks. Diluent was added to about three-fourths of the flask volume, followed by sonication to dissolve the sample. The solution was diluted to volume with diluent, mixed well, and filtered through a 0.45 µm PVDF filter, discarding the initial filtrate volume. The final concentration of Levocarnitine was approximately 5000 µg/mL. [20]

Acid Degradation
Approximately 10.0 g of each formulation was weighed and dissolved using about 25 mL of diluent with sonication in a 200-mL volumetric flask. To this, 5.0 mL of 3N HCl was added, and the solution was heated at 70°C for 14 hours. After cooling to room temperature, the solution was neutralized with 5.0 mL of 3N NaOH. Additional diluent was added to about three-fourths volume with sonication, diluted to volume, mixed, and filtered similarly.

Base Degradation
Similar to acid degradation, 10.0 g of sample was dissolved in 25 mL diluent, sonicated, and 5.0 mL 3N NaOH was added. The solution was heated at 70°C for 14 hours, then cooled and neutralized with 5.0 mL of 3N HCl. Dilution, mixing, and filtration were as described above.

Peroxide Degradation
Ten grams of the sample was dissolved in 25 mL of diluent with sonication. Five milliliters of 3% hydrogen peroxide (H_2O_2) were added, and the solution was heated at 70°C for 14 hours. After cooling, the solution was diluted, mixed, and filtered similarly.

Heat Degradation
Samples were exposed to dry heat in an oven at 80°C for 24 hours. After cooling, approximately 10 g of the treated sample was dissolved in diluent with sonication, diluted, mixed, and filtered as above.

UV-Light Degradation
Samples were exposed to UV light at 254 nm in a UV chamber for 24 hours. Post-exposure, approximately 10 g of the sample was accurately weighed and prepared as per control sample procedure.

Impurity Stock Solution Preparation
Approximately 7.5 mg of Levocarnitine Impurity-A standard was accurately weighed into a 50-mL volumetric flask, diluted with diluent to about three-fourths volume, sonicated to dissolve, and diluted to volume for a stock concentration around 150 µg/mL.

Individual Impurity Solution Preparation
2.5 mL of the impurity stock solution was further diluted to 25 mL with diluent to prepare a working solution of approximately 15 µg/mL.

Sample Preparation
Accurately weighed and transferred 10.0 mL of Levocarnitine oral solution (equivalent to 1000 mg of Levocarnitine) into a 200-mL volumetric flask. Diluent was added to approximately three-fourths of the flask volume, and the solution was sonicated to ensure complete dissolution. The solution was then diluted to volume with diluent and mixed thoroughly. The final solution was filtered through a 0.45 µm PVDF filter after discarding the initial few milliliters of filtrate. The concentration of Levocarnitine in the prepared solution was approximately 5000 µg/mL.

Spiked Sample Preparation
Approximately 1.0 g each of the sugar-containing and sugar-free Levocarnitine oral solution samples, equivalent to 100 mg of Levocarnitine, were weighed accurately and transferred into separate 10 mL volumetric flasks. To each flask, 2.0 mL of Levocarnitine Impurity-A stock solution was added, followed by sonication to ensure complete dissolution. The solutions were then made up to volume with diluent and mixed thoroughly. The resulting solutions were filtered through 0.45 µm PVDF membrane filters, after discarding the initial portion of the filtrate. From these solutions, 5.0 mL was pipetted into a 10 mL volumetric flask, diluted to volume with diluent, and mixed well before analysis.

Control and stressed placebo samples (both sugar and sugar-free) were prepared similarly. All stock solutions, including control, stress, and spiked samples, were injected into the chromatographic system to determine % impurity levels and assess peak purity to confirm method specificity and stability-indicating capability.

Filter Study
For both sugar and sugar-free samples, the test solutions were filtered through a 0.45 µm PVDF membrane filter. During filtration, 2 mL, 4 mL, 6 mL, and 8 mL of the initial filtrate portions were discarded, and the subsequent filtrates were collected and injected for analysis. In parallel, an unfiltered solution was prepared, centrifuged, and injected under identical conditions for comparison.

Relative Response Factor (RRF) and Relative Retention Time (RRT)
The relative response factors (RRFs) for known impurities were determined using a spiked sample containing Levocarnitine and its associated impurities. RRF values were calculated based on the results of the linearity study.

Robustness serves as a metric for a method's capacity to remain unaffected by minor, intentional variations in method parameters, thereby providing a reliable indication of the method's performance during routine applications. For this study, standard and sample solutions were prepared and injected into the chromatographic system under the specified method conditions. To evaluate robustness, the same standard solution was re-injected by varying one chromatographic parameter at a time, while keeping all other parameters constant. The system suitability parameters generated under the altered conditions were then compared with those obtained under the normal conditions. [21]

The robustness of the method was specifically evaluated by studying two parameters. First, the effect of variation in column operating temperature was assessed by altering the temperature by ±5°C from the procedural temperature of 30°C, resulting in two test conditions: a low temperature of 25°C and a high temperature of 35°C. Secondly, the effect of variation in the organic phase composition was evaluated by adjusting the ratio of acetonitrile to water by ±5% from the procedural composition of 300:700 (v/v). This yielded two additional test conditions: a low organic phase composition of 285:715 (v/v) acetonitrile to water, and a high organic phase composition of 315:685 (v/v) acetonitrile to water. Chromatograms of blank, standard, sample, and placebo were mentioned in figures 1 to 5a.

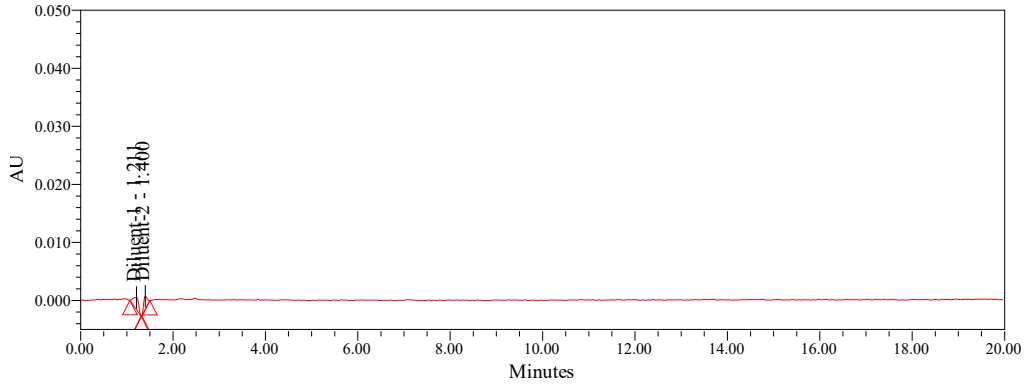

Fig.1: Typical Chromatogram of Blank

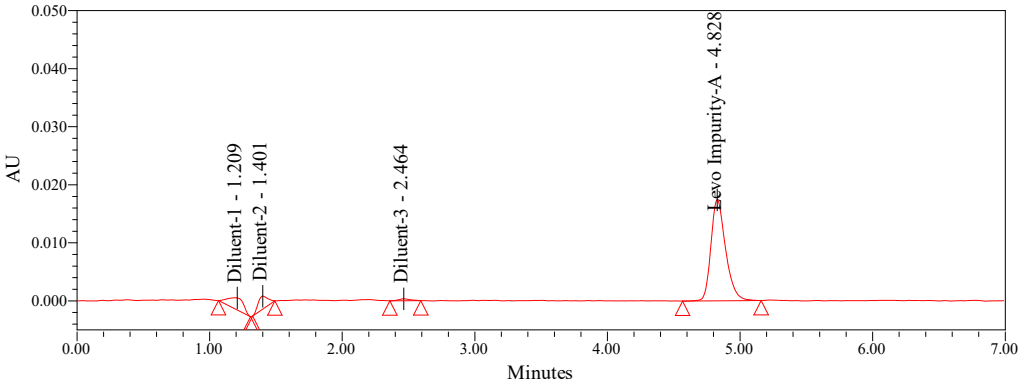

Fig.2: Typical Chromatogram of Standard

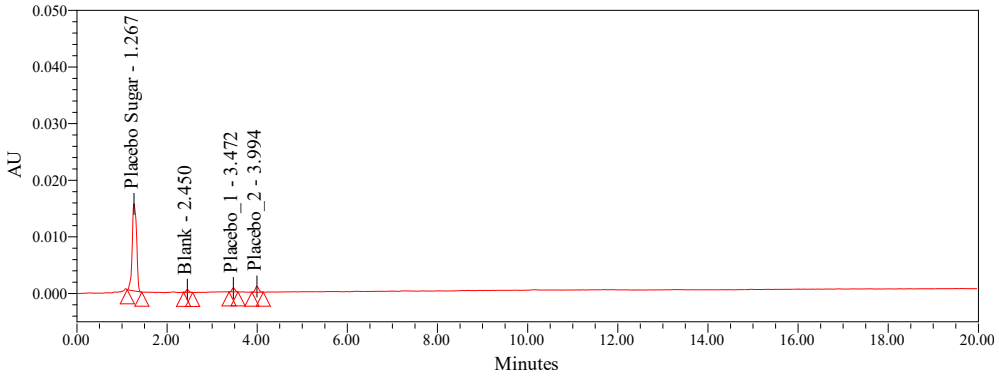

Fig.3: Typical Chromatogram of Placebo (with Sugar-No Preservatives)

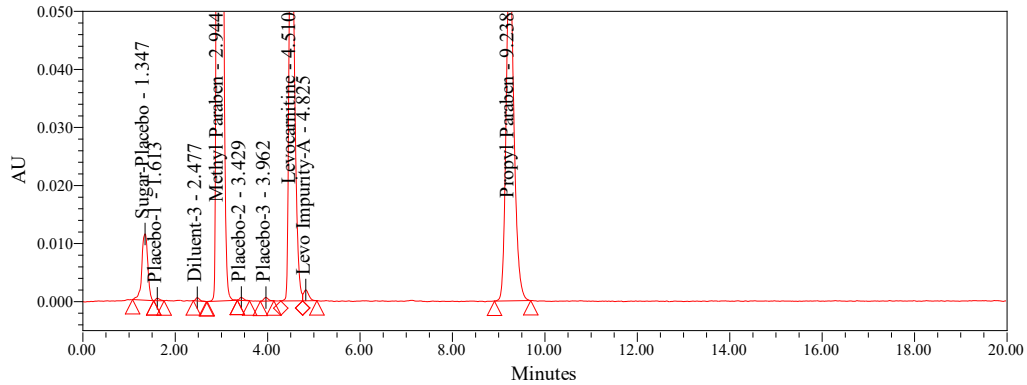

Fig.4: Typical Chromatogram of Control Sample (Sugar)

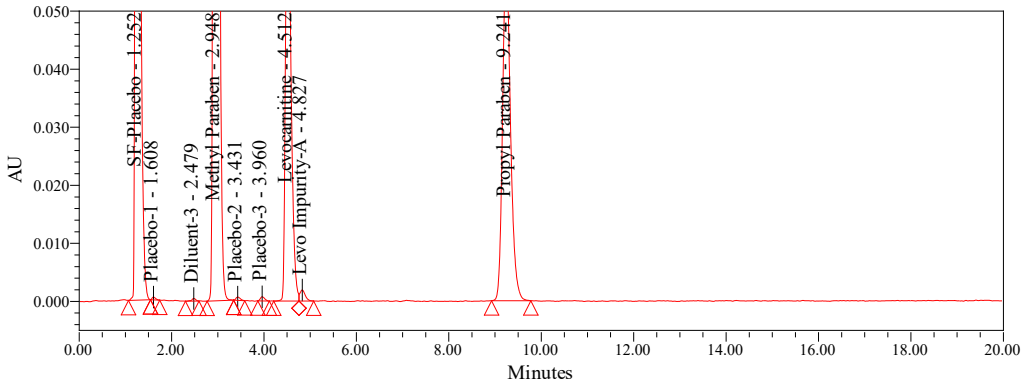

Fig.5 a: Typical Chromatogram of Control Sample (Sugar- Free)

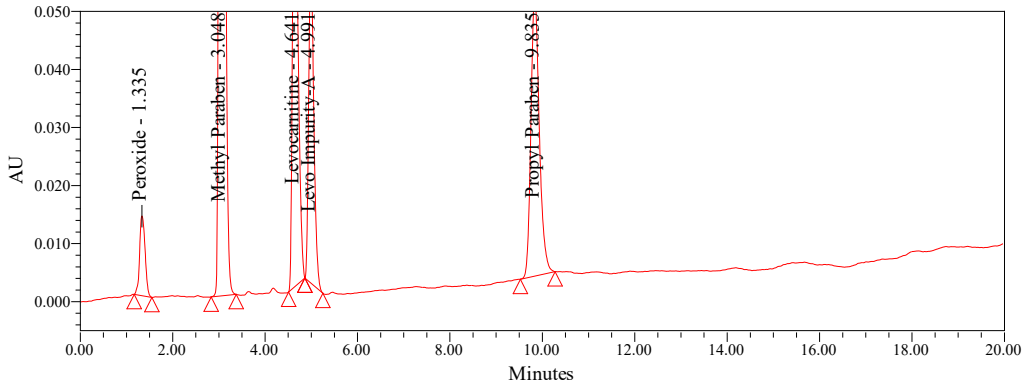

Fig.6: Typical Chromatogram of Spiked Sample (Sugar)

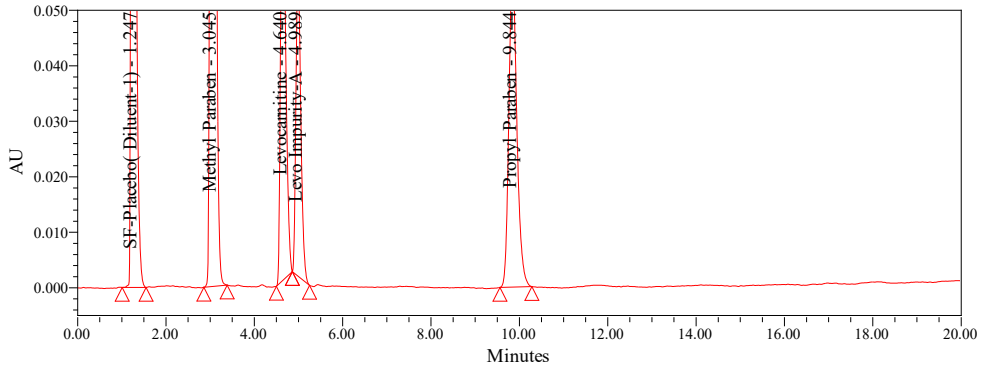

Fig.7a: Typical Chromatogram of Spiked Sample (Sugar-Free)

LINEARITY

All tested analytes, including Levocarnitine Impurity-A, showed linearity over a broad concentration range (0.02–0.80%), level of Levocarnitine Impurity-A with a correlation coefficient (r^2) of 1.000. Beyond the threshold criterion of $r^2 \geq 0.98$, this remarkable linearity validates the method's ability to produce precise and proportionate responses over a broad range of impurity levels from 0.002% to 0.8% of the specification, ensuring appropriateness for detecting both trace and elevated impurity concentrations during batch release and stability testing. The linearity table is mentioned in Table 1.

Table 1: Linearity data for Levocarnitine and Impurities

S.No.	Sample Name	Levocarnitine Impurity A	
		Amount (µg/mL)	Area
1	0.002	0.1115	4446
2	0.05	2.7886	108921
3	0.10	5.5772	219080
4	0.20	11.1545	435984
5	0.32	18.5908	725741
6	0.40	22.3089	867739
7	0.80	46.4769	1831560

Sensitivity

Sensitivity parameters underwent a thorough evaluation. Each analyte's limit of quantification (LOQ) was set at a signal-to-noise ratio greater than 10, and accuracy at LOQ was deemed acceptable when the percentage RSD values fell within the 25% limit. LOQ results are tabulated in Table 2.

Table 2: LOQ Precision & RRT and RRF for Levocarnitine and Impurities

Name	LOQ Level (%)	USP S/N	% RSD	RRT	RRF
Levocarnitine RC-A	0.002	21	2	4.989	1.08

Method Precision and System Precision

The robustness of the method was further validated by studies on method precision and intermediate precision. Excellent repeatability was demonstrated by precision assessments, which produced %RSD values for individual impurities ranging from 0.2% to 5.1%. With inter-assay %RSD values below 15% and the mean % assay value difference between runs maintained within 25%, intermediate precision, assessed across various days and analysts, also satisfied acceptance criteria. This satisfies the ruggedness requirements for validated analytical procedures and validates the method's reproducibility under varying analytical conditions. The results are mentioned in Tables 3 and 4.

Table 3: Method Precision and Intermediate Precision for Levocarnitine and Impurities

S.No.	Sample Name (Sugar Free)	Levocarnitine RC-A Peak Area	% Impurity
1	Method Precision-1	15090	0.008
2	Method Precision-2	13621	0.008
3	Method Precision-3	15577	0.009
4	Method Precision-4	15707	0.009
5	Method Precision-5	16975	0.009
6	Method Precision-6	15303	0.009
		Mean	0.008
		% RSD	7
		Intermediate Precision Analyst-1	0.008
		Intermediate Precision Analyst-2	0.008
		% Difference	0

Table 4: System Precision of for Levocarnitine and Impurity Standard

Injection No.	Levocarnitine RC-A
1	134323
2	133813
3	133501
4	132591
5	133143
6	133784
Mean	133526
% RSD	0.4

Accuracy

Recovery studies at various concentration levels, including the LOQ, were used to assess accuracy. With corresponding percentage RSD values that satisfied guideline requirements, recoveries ranged from 70% to 130% at the LOQ and from 80% to 120% for all spiked levels. These findings confirm that there are no matrix interferences from tablet excipients and validate the precision of the method for quantifying known and unknown impurities. The accuracy results are mentioned in Table 5.

Table 5: Accuracy Study for Levocarnitine Tablets Impurities

Levocarnitine RC-A (Sugar)					
S.No.	**Sample Name**	**Area**	**Amount Added (µg/mL)**	**Amount Found (µg/mL)**	**% Recovery**
1	Accuracy LOQ	3938	0.1202	0.1075	89
2	Accuracy 50%	446742	12.0202	12.1953	101
3	Accuracy 100%	901959	24.0404	24.6220	102
4	Accuracy 300%	2665352	72.1212	72.4057	100
Levocarnitine RC-A (Sugar Free)					
1	Accuracy LOQ	3490	0.1202	0.0953	79
2	Accuracy 50%	459592	12.0202	12.5461	104

3	Accuracy 100%	897828	24.0404	24.5093	102
4	Accuracy 300%	2689544	72.1212	73.4203	102

Solution Stability

According to studies on solution stability, both the standard and sample preparations held up well under refrigeration (5°C) for long periods of time (48 hours for the standards and 24 hours for the samples), with impurity levels exhibiting a change of less than 25% from the initial measurements. This shows enough stability for real-world laboratory operations and allows for postponed analysis when necessary. The results are depicted in Tables 6 and 7.

Table 6: Solution Stability of Standard and Sample at 25°C Temperature

Standard			Levocarnitine Impurity-A Sample				
Levocarnitine			Sugar			Sugar-free	
Time point	Peak Area	%Difference	Time point	% Impurity	%Difference	% Impurity	%Difference
Initial	131889	NA	Initial	0.008	NA	0.008	NA
23h	131578	0	21h	0.008	0	0.008	0
47h	133393	1	45h	0.008	0	0.008	0

Table 7: Filter Study for Levocarnitine Impurities

0.45µm PVDF Filter	%Difference in peak area Levocarnitine Impurity-A	
	Sugar	Sugar-Free
Centrifuge	NA	NA
2mL Discard	0	0
4mL Discard	4	1
6mL Discard	8	2
8mL Discard	3	8

Forced Degradation Studies

The forced degradation studies provided important information about the method's ability to indicate stability. While mild to moderate degradation was noted under oxidative and alkaline conditions, Levocarnitine demonstrated notable stability under thermal and photolytic conditions. Acidic stress resulted in the highest impurity formation (4.5%), which is consistent with amino acid derivatives' known hydrolytic susceptibility. Crucially, under all stress conditions, mass balance was preserved, and peak purity analysis with a photodiode array detector verified that there were no co-eluting degradation products at the main analyte's retention time. This supports the method's suitability as a stability-indicating assay and validates its specificity. The results are depicted in Tables 8 and 9.

Table 8: Forced Degradation Results (Sugar sample)

Sample Name/Condition	**% Impurity of Levocarnitine Impurity-A**	**% Impurity of Unknown* Impurities**	**Total %Impurities (Levocarnitine Impurity A and unknown impurities)**
Control Sample/NA	0.004	ND	NA
Acid Deg. Spl./3N HCl/70°C/14-hrs.	0.01	1.825	1.835
Base Deg. Spl./3N NaOH/70°C/14-hrs.	0.078	1.360	1.438
Peroxide Deg. Spl./3% H_2O_2/70°C/14-hrs.	0.004	ND	0.004
Heat Deg. Spl./80°C/24-hrs.	0.011	ND	0.011
UV light Deg. Spl./UV light/24-hrs.	0.004	ND	0.004

*Unknown impurities were calculated by using area normalization with respect to the control sample area.

Table 9: Forced Degradation Results (Sugar free sample)

Sample Name/Condition	**% Impurity of Levocarnitine Impurity-A**	**% Impurity of Unknown* Impurities**	**Total %Impurities (Levocarnitine Impurity A and unknown impurities)**
Control Sample/NA	0.004	ND	NA
Acid Deg. Spl./3N HCl/70°C/14-hrs.	0.01	ND	0.01
Base Deg. Spl./3N NaOH/70°C/14-hrs.	0.085	1.500	1.585
Peroxide Deg. Spl./3% H_2O_2/70°C/14-hrs.	0.004	ND	0.004
Heat Deg. Spl./80°C/24-hrs.	0.007	ND	0.007
UV light Deg. Spl./UV light/24-hrs.	0.004	ND	0.004

*Unknown impurities were calculated by using area normalization with respect to the control sample area

Robustness:
Eventually, robustness testing showed that small intentional changes to chromatographic parameters, such as flow rate, column temperature, and mobile phase pH, had no discernible effects on retention behavior or system suitability. The method's resilience to normal operational fluctuations was highlighted by the fact that all crucial parameters stayed within the established system suitability criteria.

All of these results together confirm that the developed HPLC method satisfies all analytical performance requirements needed for pharmaceutical impurity profiling and is precise, accurate, linear, robust, and stability-indicating. Its suitability for use in quality control labs for both routine release and stability testing is confirmed by its successful application to the analysis of Levocarnitine tablets. The results are depicted in Table 10.

Table 10: Robustness Study

Method Parameters		Retention Time in Min. (1st Injection)	%RSD Levocarnitine Impurity-A
Normal Condition Column Temp.: 30°C Acetonitrile: water; 300:700		4.828	0.4
Column Temp. Minus	25°C	5.200	0.9
Column Temp. Plus	35°C	4.727	0.6
Organic Minus	285:715	5.768	0.2
Organic Plus	315:685	4.288	0.3

DISCUSSION

This study presents the development and ICH Q2(R1)-compliant validation of a reversed-phase HPLC (RP-HPLC) method for quantifying Levocarnitine Impurity-A in sugar and sugar-free Levocarnitine Oral Solution (0.1 g/10 mL). Validation encompassed system precision, method precision, linearity, accuracy, LOD/LOQ, robustness, ruggedness, solution stability, and filter compatibility. [3,7].

Chromatographic optimization employed a Waters X-Bridge C18 column (150 × 4.6 mm, 5 µm) with isocratic elution using acetonitrile-phosphate buffer (pH 3.0) containing 0.1% sodium lauryl sulfate, at 1.0 mL/min flow and 205 nm UV detection. This achieved baseline resolution of levocarnitine (RT 4.6 min), Impurity-A (RT 4.9 min), and degradants/excipients (resolution >2.0; tailing <1.5; N >5000).[7,18]. System suitability was consistently achieved. Method precision showed intra-day %RSD <1.0% (n=6); intermediate precision across analysts/instruments/columns yielded %RSD <1.5%. Linearity exhibited perfect correlation (r^2=1.000) from LOQ (0.002%) to 0.80%, with robust LOQ precision (%RSD<5%) and recovery (98–102%). Accuracy recovery at 50–150% levels averaged 99.5–101.2% (%RSD<1.5%), matrix-independent.[19,21].

Solutions remained stable for 47 hours at 25°C (%area variation <1.5%); PVDF filters showed no adsorption (>99% recovery). Forced degradation (ICH Q1B: 1N HCl/NaOH, 3% H_2O_2, thermal 80°C, photolytic) confirmed specificity—acid hydrolysis generated maximum degradants, with PDA peak purity verifying no co-elution for Impurity-A. Robustness testing (±2°C temperature, ±2% acetonitrile, ±0.1 pH) maintained RT variation <0.2 min and %RSD <2.0%.[22-24].This precise, accurate, sensitive, robust, stability-indicating method suits routine quality control and stability studies for levocarnitine formulations, ensuring regulatory compliance.

CONCLUSION

A streamlined, precise, and robust reversed-phase HPLC (RP-HPLC) method was developed and comprehensively validated per ICH Q2(R1) for trace quantification of Levocarnitine Impurity-A in USP-compliant Levocarnitine Oral Solution (1.0 g/10 mL), encompassing both sugar-containing and sugar-free variants. The assay demonstrated superior linearity (r^2 > 0.999, LOQ to 150% specification), precision (%RSD < 1.5% intra-/inter-day), accuracy (recovery 98.5–101.5%), sensitivity (LOD/LOQ signal-to-noise >10:1), robustness (±0.1 pH, ±2% organic phase), and solution stability (47 h at 25°C, %area variation <2%). Employing a C18 column with phosphate buffer-acetonitrile mobile phase (pH 3.0, 0.1% SLS) and 205 nm detection, the method resolved Impurity-A (RT 4.9 min) from levocarnitine (RT 4.6 min) and degradants (resolution >2.5). ICH Q1B forced degradation (hydrolytic, oxidative, thermal, photolytic) affirmed specificity, with peak purity (PDA) confirming no co-elution, establishing stability-indicating attributes. This innovation addresses the absence of compendial impurity profiling for levocarnitine oral solutions, integrating filter compatibility (PVDF, >99% recovery) and ruggedness across instruments/analysts—features underexplored in prior reports. Tailored for sugar-free formulations critical in pediatric/diabetic cohorts, it enables reliable shelf-life monitoring and quality assurance. As a cost-effective, transferable assay, it bolsters regulatory compliance, ensuring impurity control (<0.5% specification) and therapeutic integrity for this orphan drug across diverse laboratory settings.

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