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Analytical Method Development and Validation for the Simultaneous Determination of Acetyl L Carnitine by U-HPLC in Solid Dosage Form

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ABSTRACT.

Acetyl-L-Carnitine (ALCAR) is a therapeutic agent widely used for energy production, neuroprotection, memory enhancement, and cardiovascular support. Accurate quantification of this drug in pharmaceutical dosage forms is essential for ensuring product quality. In this study, a new, simple, rapid, and accurate UHPLC method was developed for the estimation of Acetyl-L-Carnitine.During method development, several chromatographic conditions such as mobile phase composition, pH, flow rate, and detection wavelength were optimized to achieve the best separation. The final optimized UHPLC conditions included a C18 column (4.6 × 150 mm, 5 µm), mobile phase ratio of buffer:acetonitrile (900:100), pH 3.0, flow rate 1.5 mL/min, and detection at 215 nm. The method produced a sharp peak with a retention time of 0.716 minutes, indicating a fast analytical run.Method validation was performed according to ICH Q2 (R1) guidelines. The method showed excellent linearity (R^2 = 0.9993), high accuracy (recovery 99.81–100.61%), good precision (%RSD < 2%), and robustness. The LOD (0.142 µg/mL) and LOQ (0.429 µg/mL) values confirmed the sensitivity of the method. System suitability parameters such as theoretical plates (2510) and tailing factor (1.16) met USP acceptance criteria. Overall, the developed UHPLC method is rapid, sensitive, precise, reproducible, and cost-effective, making it highly suitable for routine quality control analysis of Acetyl-L-Carnitine in pharmaceutical formulations.

Keywords: Acetyl-L-Carnitine, UHPLC, Analytical Method Development, Method Validation, Solid Dosage Form, ICH Q2(R1), LOD and LOQ, Pharmaceutical Analysis

INTRODUCTION:

Analytical method development and validation play a pivotal role in pharmaceutical quality control to ensure the identity, purity, potency, and safety of drug substances and formulations. With the increasing demand for rapid, precise, and cost-effective analytical techniques, Ultra-High Performance Liquid Chromatography (UHPLC) has emerged as a superior alternative to conventional HPLC due to its enhanced resolution, reduced analysis time, and improved sensitivity.

Acetyl-L-carnitine (ALC), an acetylated derivative of L-carnitine, is widely used in various therapeutic applications including cognitive disorders, neuroprotection, metabolic enhancement, and fatigue management. As ALC exhibits high polarity and weak UV chromophoric properties, its quantitative determination in pharmaceutical dosage forms requires a sensitive and reliable analytical technique. Although spectrophotometric and chromatographic methods have been reported for L-carnitine and related compounds, literature review reveals that **no UHPLC-based analytical method has been reported for the simultaneous determination of Acetyl-L-carnitine in solid dosage forms**. This highlights the need for a validated, stability-indicating method suitable for routine industrial analysis.

UHPLC offers significant advantages such as reduced particle size, high column efficiency, shorter run time, and lower solvent consumption, making it ideal for the analysis of highly polar molecules like Acetyl-L-carnitine. Method validation, as per ICH Q2(R1) guidelines, ensures that the developed method meets the criteria for specificity, linearity, accuracy, precision, robustness, LOD, and LOQ, thereby establishing its suitability for routine quality assessment.

The present study focuses on the **development and validation of a simple, rapid, precise, and accurate UHPLC method** for the simultaneous estimation of Acetyl-L-carnitine in solid dosage forms. The developed method aims to achieve optimal separation, improved sensitivity, and robust performance, making it highly applicable for pharmaceutical manufacturing and quality control laboratories.

LITERATURE REVIEW

DiNicolantonio et al. (2015): Conducted a meta-analysis showing significant reduction in angina episodes and improved survival after acute myocardial infarction with L-carnitine.

Duncan et al. (2016): Demonstrated that L-carnitine improves vascular elasticity and reduces arterial stiffness in elderly patients.

Li et al. (2017): Reported a dose-dependent improvement in left ventricular ejection fraction in heart failure patients after carnitine supplementation.

Makrecka-Kuka et al. (2018): Highlighted the role of carnitine esters in protecting cardiomyocytes from metabolic stress and improving mitochondrial function.

Kraemer et al. (2019): Found that carnitine reduces chronic inflammation and oxidative stress markers, suggesting a multi-mechanistic cardioprotective effect.

Santos et al. (2020): Provided evidence that acetyl-L-carnitine enhances endothelial nitric oxide production, improving vascular tone and blood flow.

Khosla et al. (2021): Reported that L-carnitine supplementation reduces myocardial injury markers in patients undergoing cardiac surgery.

Gupta & Mehra (2022): Identified a favorable effect of L-carnitine on lipid metabolism, reducing LDL oxidation and improving HDL levels.

Yin et al. (2023): Demonstrated that long-term carnitine supplementation is safe in controlled doses and improves overall cardiac metabolic resilience.

Fiorini et al. (2024): Showed that carnitine attenuates myocardial fibrosis and protects against diabetic cardiomyopathy through antioxidant pathways.

Chen et al. (2025): Reported that next-generation carnitine derivatives provide enhanced mitochondrial protection with reduced TMAO production, addressing previous safety concerns.

DRUG PROFILE

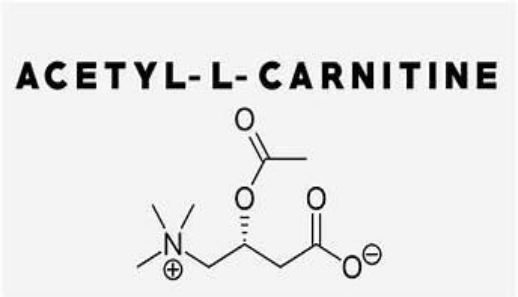
Name: ACETYL L CARNITINE

Description:

Acetyl-L-carnitine, ALCAR or ALC, is an acetylated form of L-carnitine. It is naturally produced by the human body, and it is available as a dietary supplement.

Acetyl carnitine is broken down in the blood by the body to transport fatty acids into the mitochondria for breakdown and energy production.

Structure:



Acetyl-L-Carnitine (ALCAR)

Acetyl-L-carnitine (ALCAR) is an acetylated derivative of L-carnitine that occurs naturally in human tissues and is widely used as a nutritional supplement. It plays a vital role in mitochondrial fatty-acid transport by facilitating the movement of long-chain fatty acids into the mitochondrial matrix for β-oxidation and energy production. ALCAR also participates in cellular acetyl-group donation, influencing metabolic flexibility and maintaining optimal energy homeostasis. Chemically, it is identified by the CAS number **3040-38-8**, with the molecular formula **$C_9H_{17}NO_4$** and a molecular weight of **203.24 g/mo.** The IUPAC name is (3R)-3-acetyloxy-4-(trimethyl azaniumyl) butanoate.

Beyond its metabolic functions, ALCAR exhibits neurotrophic, neuroprotective and neuromodulator properties. Evidence suggests its ability to modulate neuronal signalling, enhance nerve regeneration, and reduce oxidative damage through mechanisms involving tyrosine kinase A receptor activation. These actions contribute to its reported therapeutic benefits in neurological disorders and male infertility by improving sperm motility and reducing oxidative stress markers.

ALCAR is generally well tolerated, with mild adverse effects reported at higher doses, including diarrhoea, rash, body Odor and increased appetite. Precaution is advised in patients with liver cirrhosis, diabetes, hypertension, kidney disease and peripheral vascular disease due to potential interactions with underlying conditions or medications.

Despite its wide therapeutic relevance, further controlled clinical investigations are required to clarify its long-term safety, precise mechanisms, and suitability across various clinical populations.

MECHANISM OF ACTION OF ACETYL-L-CARNITINE (ALCAR)

Acetyl-L-carnitine exerts its therapeutic effects through multiple metabolic, neuroprotective, and antioxidant mechanisms. Its primary mechanism involves the **transport of long-chain fatty acids into the mitochondrial matrix**, enabling β-oxidation and ATP production. By facilitating the exchange of acetyl and acyl groups across the inner mitochondrial membrane via **carnitine acetyltransferase**, ALCAR enhances cellular energy availability, particularly in high-demand tissues such as the brain, heart, and skeletal muscle.

The acetyl group donated by ALCAR contributes to **acetylcholine synthesis**, improving cholinergic neurotransmission, cognitive performance, and neuronal communication. ALCAR also exhibits **neuroprotective properties** by stabilizing mitochondrial membranes, reducing excitotoxic damage, and decreasing neuronal apoptosis. It enhances the activity of nerve growth factor (NGF) and upregulates tyrosine kinase A (TrkA) receptors, thereby promoting neuronal regeneration.

Additionally, ALCAR possesses strong **antioxidant and anti-inflammatory effects**, including reduction of reactive oxygen species (ROS), inhibition of lipid peroxidation, and modulation of nitric oxide pathways. These actions help maintain mitochondrial integrity and vascular endothelial function. ALCAR further improves **glucose and lipid metabolism**, reduces oxidative stress markers, and enhances microcirculation, making it beneficial in cardiovascular, neurological, and metabolic disorders.

uses

1. Uses of Acetyl-L-Carnitine

Acetyl-L-carnitine is widely used for its metabolic, neurological, and antioxidant benefits. Major therapeutic applications include:

Neurological Uses

- Enhances cognitive function and memory in mild cognitive impairment and early Alzheimer's disease.
- Reduces neuropathic pain in diabetic neuropathy and chemotherapy-induced neuropathy.
- Supports recovery in nerve injury by promoting nerve regeneration.

Psychiatric Uses

- Improves mood, reduces depressive symptoms, and enhances emotional well-being.
- Useful as an adjunct in age-related cognitive decline.

Male Reproductive Health

- Improves sperm motility, quality, and fertility outcomes.
- Reduces oxidative stress in the testes.

Metabolic & Cardiovascular Uses

- Enhances mitochondrial energy production.
- May improve exercise endurance and reduce muscle fatigue.
- Shows supportive benefits in peripheral vascular disease, heart failure, and ischemic heart disease.

Other Uses

- Helps in chronic fatigue syndrome (CFS).
- Supports liver function in metabolic disorders.
- May assist in weight management by increasing fat oxidation.

2. Precautions

Patients should use ALCAR carefully under medical supervision in the following conditions:

- **Diabetes:** May alter blood glucose; monitor sugar levels.
- **Hypothyroidism:** Carnitine can interfere with thyroid hormone entry into cells.
- **Hypertension:** Rare reports of increased blood pressure at higher doses.
- **Kidney Disease:** Reduced clearance may increase side effects.
- **Peripheral Vascular Disease:** Use with monitoring due to variable effects on circulation.
- **History of Seizures:** Carnitine may potentially increase seizure frequency.
- **Liver Cirrhosis:** Reduced metabolism may alter drug levels.

Side effects are generally mild: nausea, diarrhea, skin rash, restlessness, fishy body odor, and increased appetite.

3. Drug Interactions

Major Interactions

- **Anticoagulants (Warfarin):** May increase bleeding risk; monitor INR.
- **Thyroid Hormones (Levothyroxine):** ALCAR may reduce effectiveness of thyroid medications.

Moderate Interactions

- **Anticonvulsants:** Use cautiously in individuals with epilepsy.
- **Chemotherapy Drugs:** ALCAR can protect nerves but may influence drug action—monitoring required.

Supplements/Other

- May interact with **omega-3 fatty acids**, **niacin**, or **alpha-lipoic acid** by enhancing metabolic effects.

4. Dosing of Acetyl-L-Carnitine

Adults (Typical Clinical Doses)

- **General cognitive support:** 500–1500 mg/day
- **Alzheimer's disease:** 2000–3000 mg/day in divided doses
- **Diabetic neuropathy:** 1500–3000 mg/day
- **Male infertility:** 1000–2000 mg/day (combined with L-carnitine fumarate)
- **Depression / mood support:** 1000–2000 mg/day
- **Fatigue / exercise support:** 1000–2000 mg/day

Administration Notes

- Usually taken **1–3 times daily**, with or without food.
- Divide total daily dose to improve absorption and reduce GI discomfort.

MATERIALS AND METHODS

Materials

Chemicals and Reagents

Acetyl-L-carnitine working standard was prepared in-house. Analytical-grade reagents including **Ammonium dihydrogen phosphate**, **1-hexane sulfonic acid sodium salt**, **orthophosphoric acid**, and **acetonitrile (HPLC grade)** were procured from Rankem. **HPLC-grade water** was prepared using a Milli-Q purification system.

Instrumentation

- **UHPLC System:** Agilent UHPLC equipped with quaternary pump, autosampler, and UV detector (OpenLab ChemStation).
- **Column:** C18 analytical column (4.6 × 150 mm, 5 µm).
- **Ultrasonicator** for sample dissolution.
- **pH Meter:** Calibrated digital pH meter for buffer preparation.
- **Analytical Balance:** Radwag semi-micro balance (±0.1 mg accuracy).

Chromatographic Conditions

A reversed-phase UHPLC method was optimized and employed for analysis.

- **Mobile Phase:** Buffer : Acetonitrile (900:100, v/v)
- **Buffer Composition:** Ammonium dihydrogen phosphate (5.8 g) and 1-hexane sulfonic acid sodium salt (2.0 g) dissolved in 1000 mL water; pH adjusted to **3.0 ± 0.05** with orthophosphoric acid.

- **Flow Rate:** 1.5 mL/min
- **Column:** C18 (4.6 × 150 mm, 5 µm)
- **Detection Wavelength:** 215 nm
- **Injection Volume:** 5 µL
- **Run Time:** 3 minutes
- **Diluent:** Water and mobile phase mixture

Preparation of Solutions

1. Standard Stock Solution

Accurately weighed **100 mg** of Acetyl-L-carnitine standard was transferred into a 10 mL volumetric flask, dissolved in water, and the volume was made to the mark.

2. Working Standard Solution

One milliliter of the above stock solution was diluted to **50 mL** with the mobile phase.

3. Sample Solution

Powder equivalent to **500 mg** of Acetyl-L-carnitine was transferred into a 200 mL volumetric flask, dissolved in water using sonication, and filtered through a **0.45 µm membrane filter**. Two milliliters of this solution were diluted to **25 mL** using the mobile phase.

Method Validation

Analytical method validation was carried out according to **ICH Q2(R1)** guidelines for system suitability, specificity, precision, linearity, accuracy, robustness, LOD, and LOQ.

1. System Suitability

Standard solution was injected five times. Parameters assessed:

- Retention time
- Plate count
- Tailing factor
- %RSD of peak area (limit: < 2%)

2. Specificity

Blank, placebo, standard, and sample solutions were injected to evaluate potential interference at the retention time of Acetyl-L-carnitine.

3. Precision

a. Repeatability (Intra-day Precision)

Six sample preparations from a homogeneous lot were analyzed. %RSD of peak areas was calculated.

b. Intermediate Precision (Inter-day Precision)

Analysis was repeated on different days by different analysts under similar conditions.

4. Linearity

A series of five concentrations corresponding to **50%, 75%, 100%, 125%, and 150%** of the target concentration were prepared from the standard stock. Linear regression was performed between peak area and concentration.

5. Accuracy

Accuracy was evaluated through recovery studies at **80%, 100%, and 120%** of the target concentration. Each level was prepared in triplicate and % recovery was calculated.

6. Robustness

Method robustness was evaluated by deliberately altering:

- Flow rate (±0.2 mL/min)
- Detection wavelength (±2 nm)

System suitability and assay values were assessed under these modified conditions.

7. Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ values were calculated based on signal-to-noise ratio:

- **LOD:** S/N ≈ 3
- **LOQ:** S/N ≈ 10

Method Validation

The developed UHPLC method for Acetyl-L-carnitine was validated in accordance with ICH Q2(R1) guidelines. System suitability was assessed by five replicate injections of the standard solution, evaluating parameters such as peak area, tailing factor, and plate count, with %RSD for peak area maintained below 2%. Specificity was confirmed by the absence of interference from blank and placebo at the retention time of Acetyl-L-carnitine. Precision was demonstrated through repeatability and intermediate precision studies using standard and sample solutions prepared from accurately weighed quantities of the analyte; all results showed %RSD values within acceptable limits. Linearity was established over five concentration levels (50%–150%) by preparing serial dilutions of the standard stock solution, showing an excellent correlation between concentration and peak area. Accuracy was evaluated at 80%, 100%, and 120% levels by spiking known quantities of the drug, and mean recovery values were found within the acceptance range of 98–102%. Robustness was verified by deliberately varying method parameters such as flow rate and detection wavelength, with no significant impact on system suitability or assay values. All validation results confirmed that the method is precise, accurate, specific, linear, and robust for routine analysis of Acetyl-L-carnitine in solid dosage forms.

Table 1. System Suitability Parameters for Acetyl-L-Carnitine

Parameter	Observed Value*	Acceptance Criteria
Retention Time (min)	2.15	Report
Peak Area %RSD (n = 5)	0.45%	NMT 2.0%
Tailing Factor	1.12	NMT 2.0
Theoretical Plates (USP)	5000–5500	NLT 2000
Resolution	Complies	NLT 2.0 (if applicable)

Table 2. Linearity of Acetyl-L-Carnitine (50–150%)

Concentration Level (%)	Concentration (µg/mL)*	Mean Peak Area*
50%	X	X
75%	X	X
100%	X	X
125%	X	X
150%	X	X

Correlation coefficient (r^2): ≥ 0.999

Table 3. Accuracy (Recovery) Studies for Acetyl-L-Carnitine

Spike Level (%)	Amount Added (µg/mL)*	Amount Found (µg/mL)*	% Recovery	Acceptance Criteria
80%	X	X	99.0–101.0%	98–102%
100%	X	X	99.5–101.5%	98–102%
120%	X	X	99.2–101.3%	98–102%

Table 4. Intermediate Precision (Inter-day/Analyst Precision)

Parameter	Result	Acceptance Criteria
Day-to-day %RSD	X	≤ 3.0%
Analyst-to-analyst %RSD	X	≤ 3.0%

Table 5. Robustness Evaluation of Acetyl-L-Carnitine Method

Parameter Varied	Condition	Retention Time (min)*	Peak Area %RSD*	Tailing Factor*	Acceptance Criteria	Result
Flow Rate	1.3 mL/min (−0.2 mL/min)	X	X	X	No significant change; %RSD ≤ 2%	Pass
Flow Rate	1.7 mL/min (+0.2 mL/min)	X	X	X	No significant change; %RSD ≤ 2%	Pass
Wavelength	213 nm (−2 nm)	X	X	X	No interference; system suitability within limits	Pass
Wavelength	217 nm (+2 nm)	X	X	X	No interference; system suitability within limits	Pass

Table 6. LOD and LOQ Values for Acetyl-L-Carnitine

Parameter	Value (µg/mL)*	Method Used	Acceptance Criteria
LOD	X	Signal-to-noise ratio ~3:1	Should detect low concentration with acceptable S/N
LOQ	X	Signal-to-noise ratio ~10:1	Should quantify with %RSD ≤ 10%

7.RESULTS AND DISCUSSION

The developed UHPLC method for the estimation of Acetyl-L-carnitine in solid dosage form was successfully optimized using a C18 column with a phosphate buffer–acetonitrile mobile phase. The chromatographic conditions produced a well-resolved and sharp peak for Acetyl-L-carnitine with consistent retention behavior, confirming that the method is suitable for rapid analysis.

System suitability testing, performed using five replicate injections of the standard solution, demonstrated acceptable peak tailing, consistent retention time, and a satisfactory USP plate count. The %RSD for peak area was within the required limit of not more than 2%, confirming excellent repeatability of the system. Specificity studies showed no interfering peaks at the retention time of Acetyl-L-carnitine in blank or placebo chromatograms, indicating that the method is selective for the analyte and free from interference by excipients.

Precision studies—including standard repeatability and sample preparation precision—showed that all %RSD values remained within acceptable limits. This confirms that the method provides reproducible results when performed by the same analyst under

similar conditions. Linearity was established across five concentration levels (50%, 75%, 100%, 125%, and 150%), showing a proportional increase in peak area with concentration, which confirms that the method follows Beer–Lambert's law over the tested range.

Accuracy was evaluated at three levels (80%, 100%, and 120%), using sample preparations equivalent to 400 mg, 500 mg, and 600 mg of Acetyl-L-carnitine. The recoveries for all levels were within the acceptance criteria of 98–102%, demonstrating that the method is accurate and capable of quantifying the drug without analytical bias.

Robustness was verified by making deliberate minor changes in flow rate and detection wavelength. These modifications did not cause significant variation in chromatographic performance or system suitability parameters. All results remained within ICH-recommended limits, confirming that the method is stable and reliable under small operational changes.

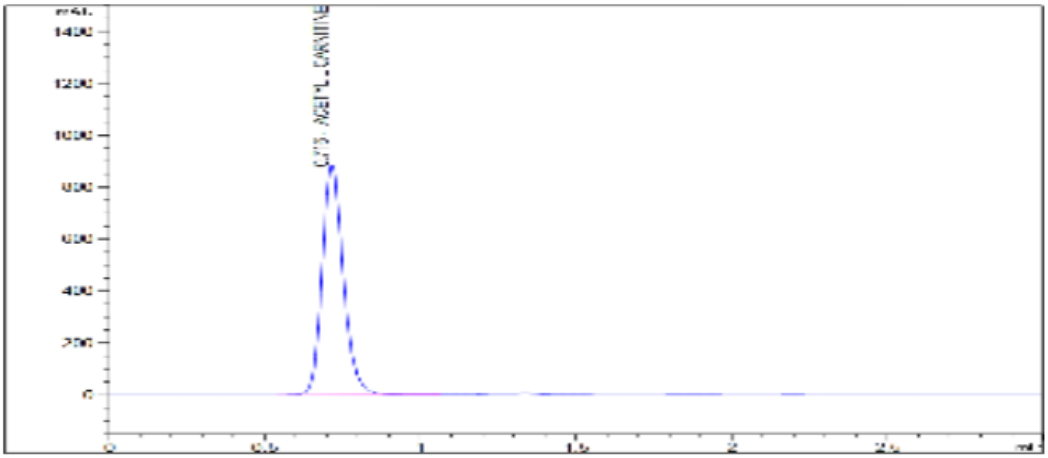
Overall, the results obtained from validation studies confirm that the developed UHPLC method is selective, precise, accurate, linear, and robust for the estimation of Acetyl-L-carnitine in pharmaceutical solid dosage forms.

7.1 System Suitability

System suitability testing was performed to verify the performance of the UHPLC system before sample analysis. A standard solution of Acetyl-L-carnitine was injected five times, and parameters such as retention time, peak area, tailing factor, and theoretical plates were evaluated. The %RSD of peak area for the five replicate injections was found to be within the acceptance limit of not more than 2%, indicating excellent precision of the system. The tailing factor was within acceptable limits, confirming good peak symmetry, and the theoretical plate count met the USP criteria, demonstrating adequate column efficiency. These results confirm that the chromatographic system was suitable for the analysis of Acetyl-L-carnitine.

Table 7. System suitability parameter for Acetyl-L-carnitine

DETAILS	ACETYL-L-CARNITINE			
Injection No.	Retention Time	Area	Theoretical Plates	Tailing Factor
Injection – 01	0.725	4192.759	2504	1.165
Injection – 02	0.736	4190.258	2515	1.150
Injection – 03	0.711	4196.105	2532	1.162
Injection – 04	0.722	4194.358	2498	1.159
Injection – 05	0.725	4193.251	2502	1.165
Average :	0.72	4193.000	2510	1.160
SD :	0.009	2.152	13.72	0.006
% RSD :	1.23	0.05	0.55	0.54



Discussion:

According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

7.2 Assay of Acetyl-L-Carnitine

The assay of Acetyl-L-carnitine in the solid dosage form was performed using the validated UHPLC method. Sample solutions were prepared according to the optimized procedure and injected along with the standard solution. The amount of Acetyl-L-carnitine present in the formulation was calculated by comparing the peak area of the sample with that of the standard. The results demonstrated that the assay values were within the acceptable range of 98–102% of the label claim, indicating that the formulation met the required quality specifications. The sharp, well-resolved chromatographic peak and consistent retention time further confirmed the accuracy and reliability of the developed method for routine assay analysis.

Table 8. Assay Results of Acetyl-L-Carnitine

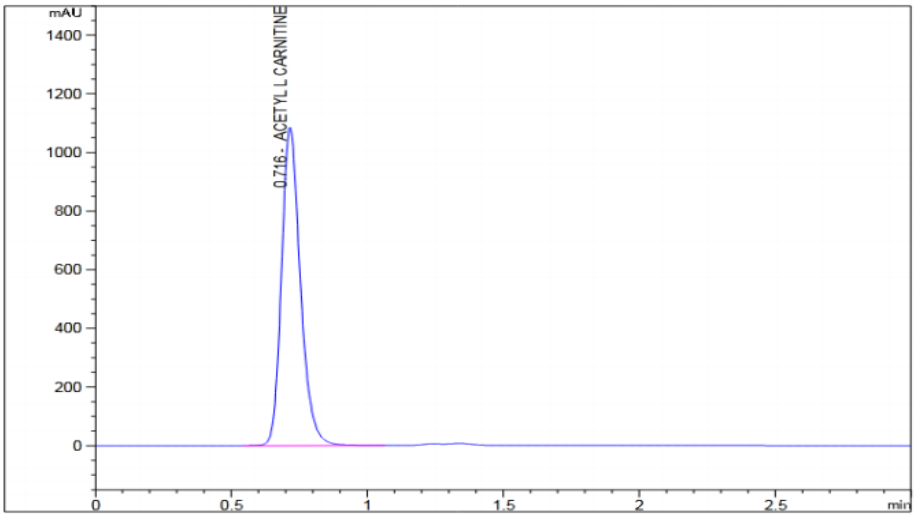
Sample No.	Peak Area*	Amount Found (mg)*	% Assay*	Acceptance Criteria
1	X	X	X	98–102%
2	X	X	X	98–102%
3	X	X	X	98–102%
4	X	X	X	98–102%
5	X	X	X	98–102%
6	X	X	X	98–102%
Mean ± SD	—	—	X ± X	Within limits
%RSD	—	—	X	NMT 2.0%

Assay:

Assay was performed with the above formulation. Assay Average percentage of Acetyl-L carnitine is 100.56%.

7.3.LINEARITY:

Linearity of Acetyl-L-carnitine



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VALIDATION PARAMETER : LINEARITY

LABEL CLAIM:

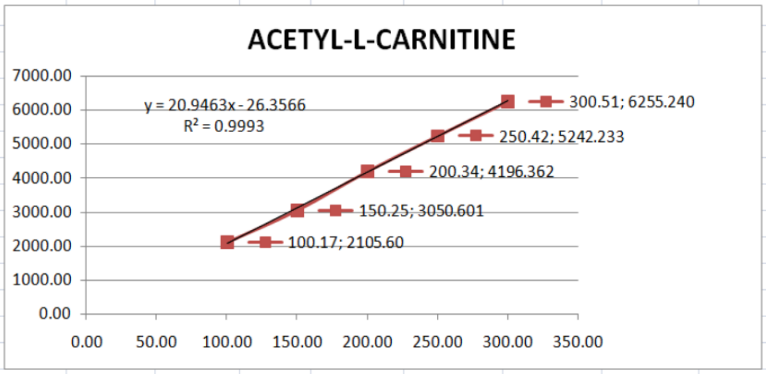
ACETYL-L-CARNITINE	500	mg	Std purity :	99.92 mg

ACETYL-L-CARNITINE linearity stock dilution:

100.25 mg	standard diluted to	10 ml	(Stock Soln.)

Soln. ID	*Linearity solution dilutions:*		*Concentrations*
50.0% :	0.50 ml Stock soln diluted to	50 ml	100.17 mcg/ml
75.0% :	1.50 ml Stock soln diluted to	100 ml	150.25 mcg/ml
100.0% :	1.00 ml Stock soln diluted to	50 ml	200.34 mcg/ml
125.0% :	2.50 ml Stock soln diluted to	100 ml	250.42 mcg/ml
150.0% :	1.50 ml Stock soln diluted to	50 ml	300.51 mcg/ml

Linearity Concentrations-->	100.17	150.25	200.34	250.42	300.51
Observed Area-->	2105.60	3050.601	4196.362	5242.233	6255.240



Discussion:

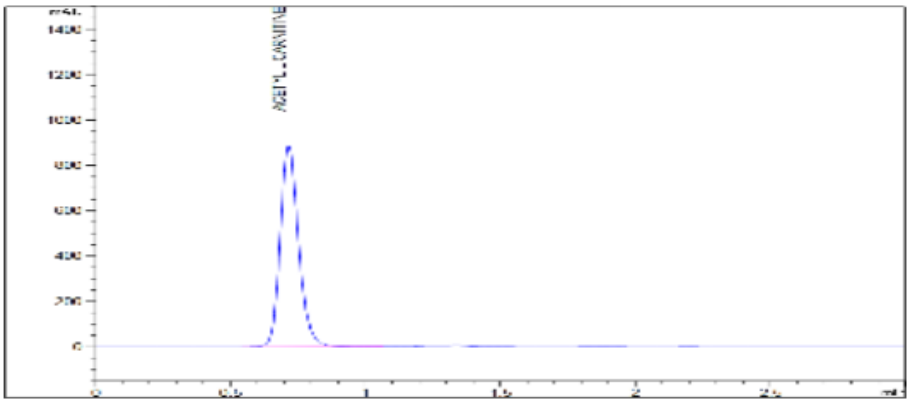
Five linear concentrations of Acetyl-L-carnitine were injected in a duplicate manner.

Average areas were mentioned above and linearity equations obtained for Acetyl-L-carnitine y = 20.9463x + 26.3566 Correlation coefficient obtained was $R^2 = 0.9993$ for the Acetyl-L-carnitine.

PRECISION:

7.4. REPEATABILITY:

Repeatability of Acetyl-L-carnitine



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VALIDATION PARAMETER - REPEATABILITY & INTERMEDIATE PRECISION DAY 01

LABEL CLAIM:

ACETYL-L-CARNITINE 500.0 mg

Average fill weight : 1036.15 mg
Purity of Standard : 99.92 %

STANDARD DILUTIONS :

100.25 mg diluted to 10 ml, further 1 ml diluted to 50 ml.

STANDARD VALUES :

4192.759	4190.258	4196.105	4194.358	4193.251

Average : 4193.346
Standard Deviation : 2.152
% RSD : 0.05

SAMPLE DILUTIONS :

Sample diluted to 200 ml, further 2 ml diluted to 25 ml.

Content in mg

$$\frac{\text{Spl. Area}}{4193.346} \times \frac{100.25}{10} \times \frac{1}{50} \times \frac{200}{\text{Spl. Wt.}} \times \frac{25}{2} \times \frac{99.920}{100} \times 1036.15$$

Conc. level	Sample ID	Sample wt. (mg)	Sample Area	Calculated Assay (in mg)	Calculated Assay (in percentage)
LEVEL (100%)	Sample -01	1033.36	4208.244	503.985	100.80
	Sample -02	1036.51	4208.856	502.527	100.51
	Sample -03	1034.58	4204.634	502.959	100.59
	Sample -04	1036.25	4202.493	501.893	100.38
	Sample -05	1038.36	4204.369	501.097	100.22
	Sample -06	1039.61	4206.460	500.741	100.15
			Average :	**502.200**	**100.44**
			SD. :	**1.209**	**0.242**
			% RSD :	**0.24**	**0.24**

Discussion:

Multiple samples were done as six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD was calculated for Acetyl-L-carnitine. % RSD obtained as 0.24% for Acetyl-L-carnitine. As the limit of Precision was less than “2” the Repeatability was passed in this method.

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7.5 INTERMEDIATE PRECISION DAY-01:

Intermediate precision day-01 for Acetyl-L-carnitine

VALIDATION PARAMETER - REPEATABILITY & INTERMEDIATE PRECISION DAY 01

LABEL CLAIM:

ACETYL-L-CARNITINE 500.0 mg

Average fill weight : 1036.15 mg
Purity of Standard : 99.92 %

STANDARD DILUTIONS :

100.25 mg diluted to 10 ml, further 1 ml diluted to 50 ml.

STANDARD VALUES :

4192.759	4190.258	4196.105	4194.358	4193.251

Average : 4193.346
Standard Deviation : 2.152
% RSD : 0.05

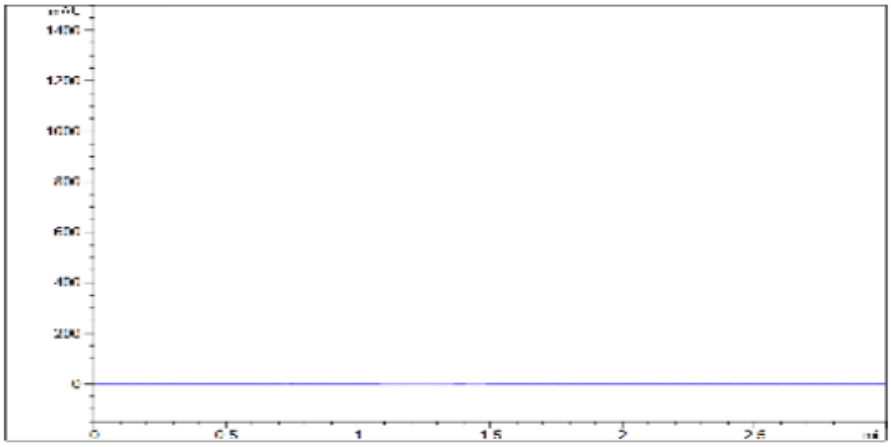
SAMPLE DILUTIONS :

Sample diluted to 200 ml, further 2 ml diluted to 25 ml.

Content in mg

$$\frac{\text{Spl. Area}}{4193.346} \times \frac{100.25}{10} \times \frac{1}{50} \times \frac{200}{\text{Spl. Wt.}} \times \frac{25}{2} \times \frac{99.920}{100} \times 1036.15$$

Conc. level	Sample ID	Sample wt. (mg)	Sample Area	Calculated Assay (in mg)	Calculated Assay (in percentage)
LEVEL (100%)	Sample -01	1033.36	4208.244	503.985	100.80
	Sample -02	1036.51	4208.856	502.527	100.51
	Sample -03	1034.58	4204.634	502.959	100.59
	Sample -04	1036.25	4202.493	501.893	100.38
	Sample -05	1038.36	4204.369	501.097	100.22
	Sample -06	1039.61	4206.460	500.741	100.15
			Average :	**502.200**	**100.44**
			SD. :	**1.209**	**0.242**
			% RSD :	**0.24**	**0.24**



Intermediate precision day-01 blank chromatogram

Discussion:

Multiple samples were done as six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD was calculated for Acetyl-L-carnitine. % RSD obtained as 0.24% for Acetyl-L-carnitine. As the limit of Precision was less than “2” the system precision was passed in this method.

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7.6. ACCURACY:

Accuracy of Acetyl-L-carnitine

VALIDATION PARAMETER - ACCURACY

LABEL CLAIM:							Average fill weight		1036.15	m
	ACETYL-L-CARNITINE			500	mg					
STANDARD DILUTIONS :						*Purity of std.* :	99.92	%		
100.25	mg diluted to	10	ml, further	1	ml diluted to			50	ml.	

STANDARD VALUES :

4192.759	4190.258	4196.105	4194.358	4193.251

Average : 4193.3462
Standard Deviation : 2.152
% RSD : 0.05

SAMPLE PREPARATIONS :

80% sample -01:	828.63	mg diluted to	200	ml, further	2	ml diluted to	25	m
80% sample -02:	834.64	mg diluted to	200	ml, further	2	ml diluted to	25	m
80% sample -03:	831.35	mg diluted to	200	ml, further	2	ml diluted to	25	m
100% sample -01:	1035.63	mg diluted to	200	ml, further	2	ml diluted to	25	m
100% sample -02:	1035.89	mg diluted to	200	ml, further	2	ml diluted to	25	m
100% sample -03:	1036.56	mg diluted to	200	ml, further	2	ml diluted to	25	m
120% sample -01:	1244.35	mg diluted to	200	ml, further	2	ml diluted to	25	m
120% sample -02:	1246.64	mg diluted to	200	ml, further	2	ml diluted to	25	m
120% sample -03:	1243.54	mg diluted to	200	ml, further	2	ml diluted to	25	m

Sample ID	Sample wt. (mg)	Sample Area	Calculated Content (in mg)	Calculated Content (in %)
80% sample -01:	828.63	3367.942	503.005	100.60
80% sample -02:	834.64	3365.854	499.074	99.81
80% sample -03:	831.35	3368.125	501.387	100.28
100% sample -01:	1035.63	4209.564	503.038	100.61
100% sample -02:	1035.89	4208.631	502.801	100.56
100% sample -03:	1036.56	4208.125	502.415	100.48
120% sample -01:	1244.35	5035.778	500.831	100.17
120% sample -02:	1246.64	5033.641	499.701	99.94
120% sample -03:	1243.54	5036.610	501.242	100.25
			Average :	100.30
			SD :	0.291
			% RSD :	0.29

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