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Development &Validation of Rp-Hplc Method for Estimation of L-Carnitine and Caffeine as a Energy Boosting Agents in Their Synthetic Mixture

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ABSTRACT:

Over-the-counter energy boosting agents tablet formulation consist of L-carnitine and caffeine and garciniacumbogia one of the common used. In this research paper we have proposed RP-HPLC method for L-carnitine and Caffeine in synthetic mixture combination. As both the drugs shows synergistic effect as a energy boost which additionally used to reduce triglycerides level with no harmful effect on lipid profile.The HPLC method employed at thermo scientific, hypersil C18column (250mm× 4.6mm, 5µm) with an isocratic mixture of acetonitrile and water (20:80 v/v) as the mobile phase. The column temperature was kept at 30°C.The flow rate was1.0 mL/min and detection was by means of a UV detector at wavelength of 252 nm. Both the components were successfully eluted with mean retention times of 3.2 min &4.5 min for caffeine &L-carnitine respectively. The method was found to be linear (R^2> 0.99), precise (RSD < 0.7 %), accurate (recoveries 97.9–100.8 %), specific, simple, sensitive, rapid and robust. The validated method can be used in routine quality control analysis without any interference by excipients.

Keywords: L-carnitine, Caffeine, RP-HPLC method, Quality control analysis, Energy boosting agents

1. Introduction

Combining caffeine and L-carnitine in a dosage form, such as a supplement or medication, may serve several purposes due to the individual properties of these compounds.

1. **Energy Boost**: Caffeine is a well-known stimulant that can enhance alertness and reduce fatigue by blocking the action of adenosine, a neurotransmitter that promotes sleep. When combined with L-carnitine, which plays a role in energy metabolism by transporting fatty acids into the mitochondria where they can be burned for energy, the combination may provide a synergistic effect in boosting energy levels.

2. **Enhanced Exercise Performance**: Both caffeine and L-carnitine have been studied for their potential to improve exercise performance. Caffeine can increase endurance and reduce the perception of effort during physical activity. L-carnitine, on the other hand, may help

improve the utilization of fat as an energy source during exercise. Combining the two could theoretically enhance the benefits on exercise performance, particularly for endurance activities.

3. **Fat Loss**: There is some evidence to suggest that both caffeine and L-carnitine may aid in fat loss. Caffeine can increase metabolism and promote fat oxidation, while L-carnitine plays a role in the transportation of fatty acids into the mitochondria for energy production. Therefore, combining the two may support weight loss efforts by increasing fat burning and energy expenditure.

4. **Cognitive Function**: Caffeine is known to improve cognitive function, including alertness, attention, and concentration. L-carnitine has been investigated for its potential cognitive benefits as well, though the evidence is less robust. Combining the two may provide a comprehensive approach to supporting cognitive function and mental clarity.

There is no study investigating the combined effect of caffeine and carnitine to make weight-loss interventions as effective as possible, with no side effect on cardiovascular status and performance. Therefore, we combined caffeine supplementation with carnitine to investigate the alleviation of possible negative consequences on lipid profile. In market available product of these combination is “Muscle Blaze Tablet” which contains caffeine, L-carnitine ,garciniacombogia& green coffee bean extract but in these combination caffeine and L-carnitine is the active constituents which shows energy boosting activity but there is no any formulation available in the market for these two drugs combinations that’s why I prepared synthetic mixture of these combination and then studied the HPLC parameters.

Most multicomponent drug formulations usually contain two or more active ingredients which are responsible for a combined therapeutic activity of the drug. This concept is beneficial when the selective agents have different mechanisms of action that provide additive or synergistic efficacy. There is increased production of multicomponent drugs formulation due to increased efficacy, increased resistance of microorganisms to single component formulations and dependency and/or tolerance, and this has further led to increased drug counterfeiting and adulteration.

Official pharmacopoeias typically provide monographs for single-component drugs. Consequently, local pharmaceutical manufacturers often employ methods involving multiple and repeated extractions to isolate each active component before quantification using spectrophotometry or titrimetry. However, these methods can be laborious and cumbersome. Consequently, researchers have endeavored to develop techniques to streamline the analysis of multi-component drugs. High-Performance Liquid Chromatography (HPLC) has emerged as a preferred method for this purpose. Numerous researchers have focused on developing Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) methods to enable the simultaneous estimation of various active components in multi-component drugs.

Both synthetic mixtures and extractions have their advantages and limitations in HPLC studies of multicomponent dosage forms. The choice between the two approaches should be based on factors such as the availability of standards, the complexity of the dosage form matrix, accuracy requirements, cost considerations, and method validation needs. The use of synthetic mixtures offers greater precision, accuracy, and reliability in pharmaceutical analysis compared to natural extracts, making them preferable for many analytical applications.

2. Materials & Methods

Chemical and Reagents-

Pure Drugs procured from Yarrow chem products, Ghatkopar, Mumbai.All HPLC grade solvents were used, including water and acetonitrile. AR-grade Starch, magnesium stearate & talc were used for synthetic mixture.

Instruments-

HPLC- Schimadzu 228-45041-91 with software Lab solutions and Thermo scientific, gold, C18 (250mm x 4.6 mm, 5 µm) with UV detector. Digital Analytical Balance- Contech CA223. UV visible spectrophotometer- Jasco V-530. FTIR- Schimadzu 8400S.

Methoddevelopmentandoptimization

Mobilephase selection

Preliminary studies with several solvent systems were performedtoselectthemosteffectivesolventsystem for the separation of the two APIs. The selection ofthesesolventsaspossiblemobilephase(s)dependedon factorssuchascostofsolvent(s),polaritiesofsolvent(s) andthatoftheanalyte(s)ofinterestandthesolubilityoftheanalyte(s)Solvents such as ethanol, Methanol & acetonitrile aswellascombinationsofthesesolvents were tried. The mobile phase of acetonitrile and water was tried in different proportions. However, an isocratic mixture of acetonitrile and water in the ratio of (20:80; v/v) was chosen as the mobile phase becauseitproducedthebestresolutionofpeaks,peaksymmetry and separation of all components within the leastretentiontimes.Meanretentiontimesof3.2 minutes & 4.5 minuteswererecordedforcaffeine & L-carnitine respectively.

Stationaryphaseselection-

The polarities of the analytes of interest were takeninto consideration when choosing the stationary phase. As the drug molecules are polar or moderately polar, reversedphasestationaryphaseswereTried. A thermo scientific, hypersil gold, C18 column was choseninordertoreducethetimeofinteractionbetween the stationary phase and the analytes. This helped to reduceanalysistimeasthereisreducedaffinityoftheanalytes for the stationary phase, and increased interaction of the analytes with the mobile phase.

Optimized Chromatographic conditions are listed in table-

Method Development Trails

Table1- Trail 1

Chromatographic Conditions	
Stationary Phase	Hypersil gold, C18 Column (250mm× 4.6mm, 5µm)
Mobile Phase	Methanol:Water (50:50%v/v)
Detected Wavelength	252 nm
Flow Rate	1.0 ml/min

Injected Volume	10µl
Run Time	30 min

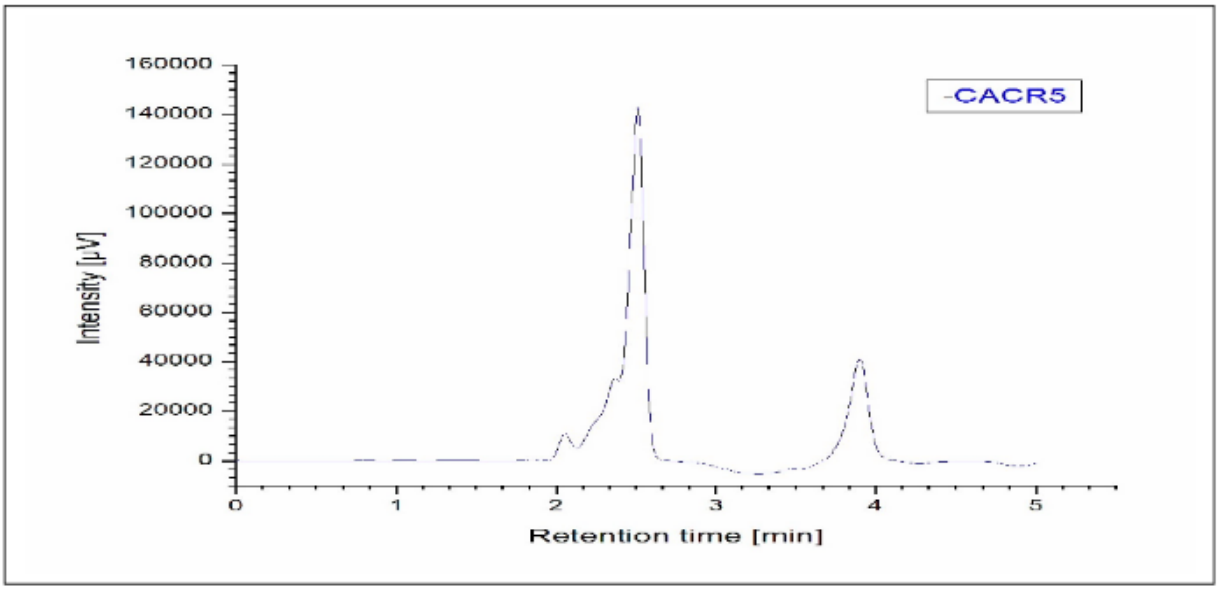

Figure 1 - Chromatogram of combination of Caffeine & L-carnitine

Conclusion- Extra peaks was shown in trial 1.

Table 2- Trail2

Chromatographic Conditions	
Stationary Phase	Hypersil gold, C18 Column (250mm× 4.6mm, 5µm)
Mobile Phase	Acetonitrile:Water (50:50%v/v)
Detected Wavelength	227 nm
Flow Rate	1.0 ml/min
Injected Volume	10µl
Run Time	30 min

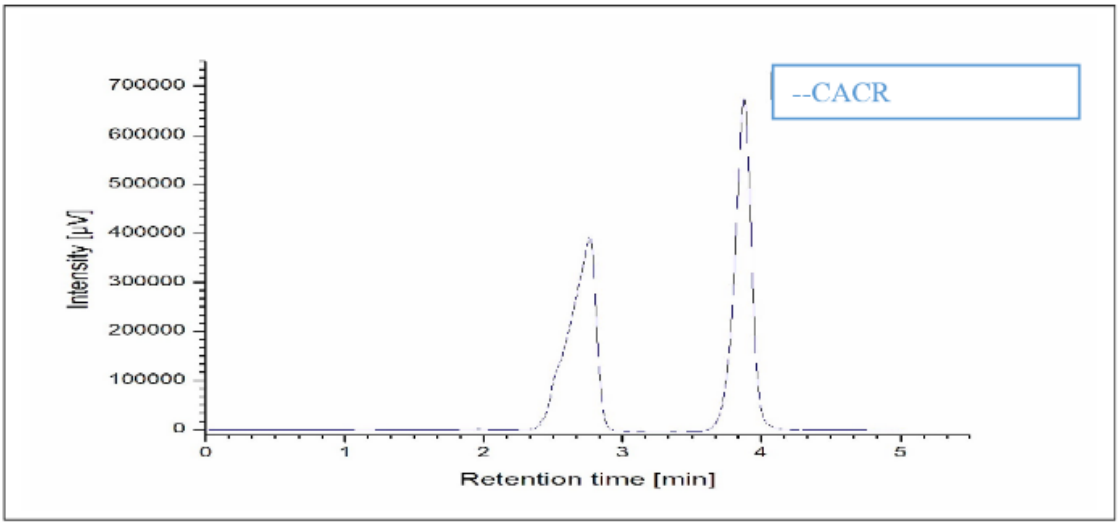

Figure 2- Chromatogram of combination of Caffeine & L-carnitine

Conclusion-The wavelength used (227 nm) might not be ideal for caffeine and L-carnitine detection. Although caffeine absorbs reasonably well at 254 nm, L-carnitine has a very low absorbance at this wavelength. Using a wavelength closer to the absorbance maxima of the target compounds might improve the peak intensity.

Table 3- Trail 3

Chromatographic Conditions	
Stationary Phase	Hypersil gold, C18 Column (250mm× 4.6mm, 5µm)
Mobile Phase	Acetonitrile:Water (70:30%v/v)
Detected Wavelength	252 nm
Flow Rate	1.0 ml/min
Injected Volume	10µl
Run Time	15 min

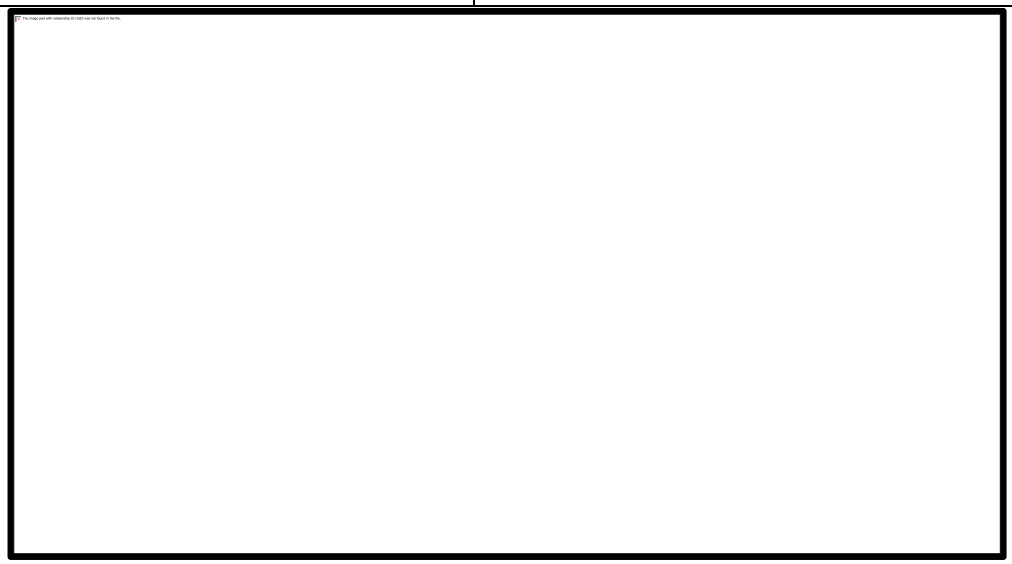

Figure 3- Chromatogram of combination of Caffeine & L-carnitine

Conclusion-peak does not meet the required criteria for resolution or shape (e.g., tailing, fronting, or broadening), it might be considered unresolved or not suitable for integration. Chromatographic peaks should ideally be Gaussian or symmetrical to ensure accurate quantification.

Table 4- Trail 4

Chromatographic Conditions	
Stationary Phase	Hypersil gold, C18 Column (250mm× 4.6mm, 5µm)
Mobile Phase	Acetonitrile:Water (20:80%v/v)
Detected Wavelength	252 nm
Flow Rate	1.0 ml/min
Injected Volume	10µl
Run Time	10 min

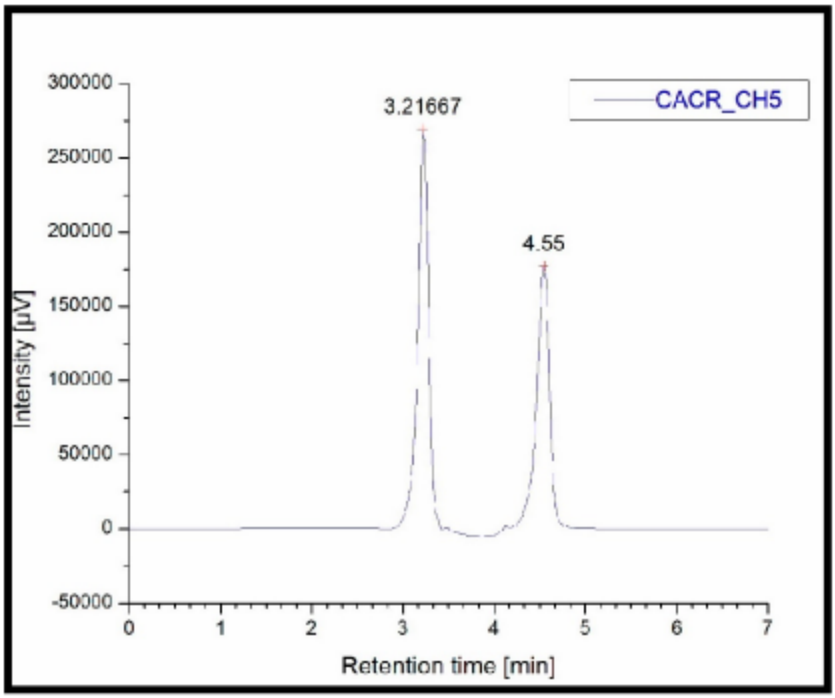

Figure 4- Chromatogram of Optimized condition of combination of Caffeine & L-carnitine

Conclusion- The peak of both drugs was good.An extra peak was not seen.all values are within the acceptable range.

3. Resultsanddiscussion

Methoddevelopmentandoptimization

System suitability

System suitability tests are crucial for ensuring the reproducibility of any chromatographic system. These tests play a vital role in the chromatographic method. The HPLC method is considered suitable when the theoretical plates > 2000, the tailing factor < 2 and resolution and the results are shown in table 5

In accordance with International Council for Harmonization (ICH), the proposed HPLC method was validated by evaluating a number of parameters.

Table 5: Data of system suitability test

Marker	Number of theoretical plate (N)	Tailing factor (T)	Resolution (Rs)
Caffeine	5541	1.021	12.47
L Carnitine	4954	1.217	NA

2. Linearity

Within the context of linearity, the determination of the linear relation between the marker concentrations and their associated peak area responses was carried out. For Caffeine and L Carnitine linear correlation was achieved at range of concentrations of 2–10µg/ml; and, 10–30µg/ml respectively. It has been noted that the peak area is directly proportional to Concentration $R^2 = 0.9996$ & $R^2 = 0.9992$ for Caffeine and L-carnitine respectively. All linearity data for Caffeine, L-carnitine & overlay of both as shown in Fig. 5,6 & 7 Respectively. All statistical data is shown in table 6.

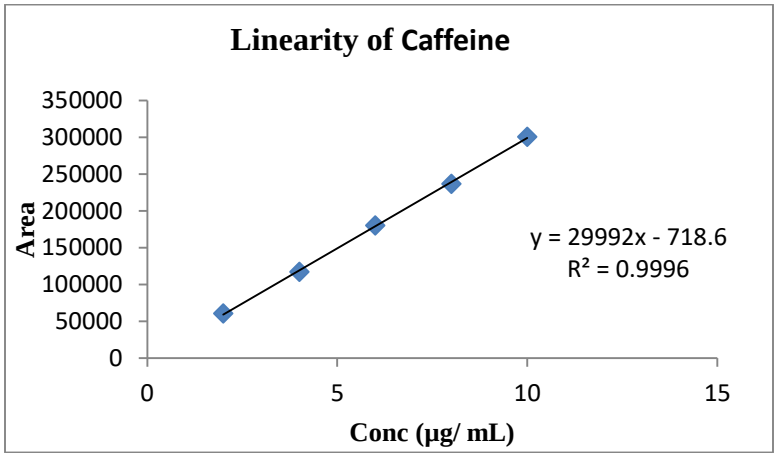

Figure 5- Linearity of Caffeine

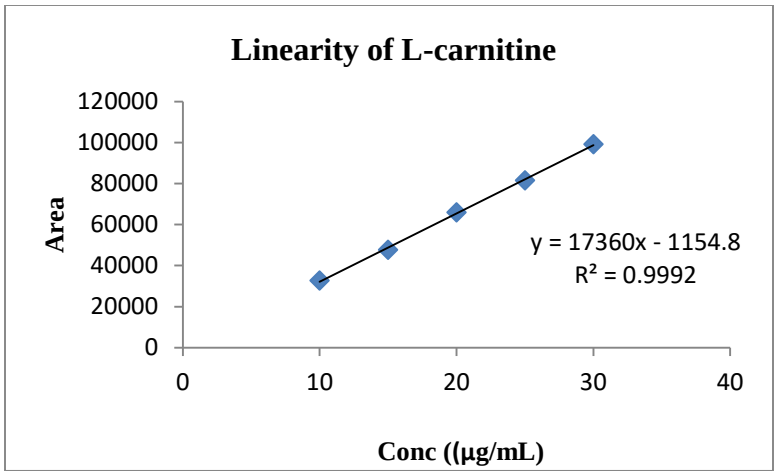

Figure 6- Linearity of L-carnitine

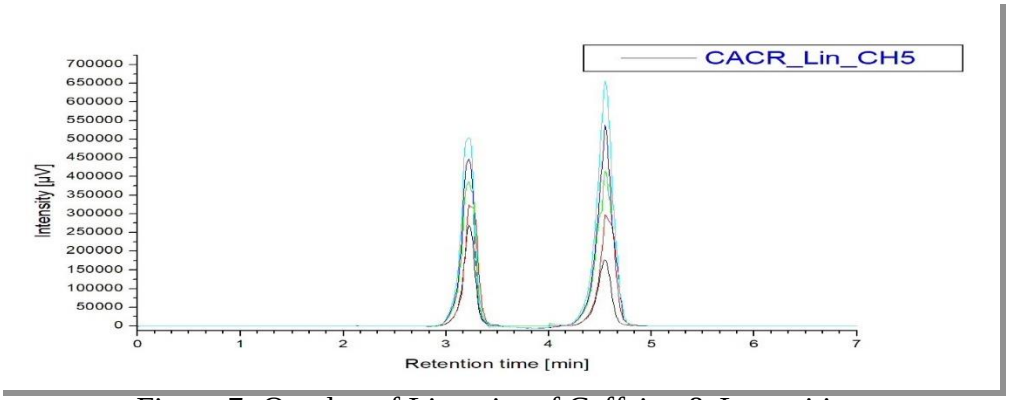

Figure 7- Overlay of Linearity of Caffeine & L-carnitine

3. Limit of detection and limit of quantification

The calculation of the limit of detection (LOD) and limit of quantification (LOQ) for the markers involved using the standard deviation of the intercept and the slope of the calibration curve. LOD and LOQ for Caffeine, and L-carnitine are shown in table 3. & in Figure 7 respectively.

Table 6: Data of Linear, Regression, LOD & LOQ

Marker	RT	Linearity Range	Equation	R^2	LOD	LOQ
Caffeine	3.21±0.019	2-10 µg/ Ml	y = 29992x - 718.6	0.9996	0.55	1.68
L -carnitine	4.55± 0.021	10-30 µg/ mL	y = 17360x - 1154.8	0.9992	2.65	4.97

4. Precision

The intra-day precision and inter-day precision for the peak area for all markers were determined by repeated assessment. The % RSD for each marker was found to be less than 2, indicating a high degree of precision in the developed HPLC method and shown in table 7.

Table 7: Data of Precision

Parameters/ Markers	Intra-day				Inter-day		
	Conc. (µg/ mL)	Mean Area (n=3)	SD	%RSD	Mean Area (n=3)	SD	%RSD
Caffeine	4 µg/mL	111564	346.41	0.7161	111342	348.15	0.7412
	6 µg /mL	167149	672.22	0.7821	168113	677.73	0.7427
	8 µg /mL	212767	789.21	0.8384	217698	796.75	0.7407
L-carnitine	15 µg /mL	45136	271.71	0.7687	45776	281.93	0.7551
	20 µg /mL	63522	423.75	0.6311	63548	431.31	0.7660
	25 µg /mL	78757	381.14	0.6424	78142	337.10	0.7414

5. Robustness

The developed HPLC method was found to be robust in terms of variations in the column temp (30 ±2 ^{0}C) and flow rate (1 ±0.1 milliliter/min). The results obtained for robustness studies, and % RSD shown in table 8.

Table 8: Data of Robustness

Parameters/ Markers	Conc. (ug/ mL)	Column temp. (^{0}C)	Mean Area	%RSD	Flow rate (mL/min)	Mean Area	%RSD
Caffeine	6 µg /mL	28 ^{0}C	119719	1.025	0.9 mL/min	117917	1.167
	6 µg /mL	30 ^{0}C	110942	0.7465	1.0 mL/min	118342	0.7654
	6 µg /mL	32 ^{0}C	111943	1.107	1.1mL/min	118015	1.121
L-carnitine	20 µg /mL	28 ^{0}C	63543	1.025	0.9 mL/min	62981	1.215
	20 µg /mL	30 ^{0}C	63981	0.7424	1.mL/min	62971	0.7214
	20 µg /mL	32 ^{0}C	63279	1.112	1.1mL/min	63107	1.167

4.12.6. Accuracy

The accuracy of the developed HPLC method was evaluated using the standard addition method. The formulations were analyzed by adding a known quantity of standards to the formulation. The percent recovery of the standard was calculated. Results obtained were shown in table. The % recovery of Caffeine and L-carnitine were found within a range of 99.92 to 100.10% and 99.87 to 100.02% respectively.

Table 9: Data of Recovery studies

Parameters/ Markers	Amount of Standard Added (µg/ mL)	% Recovery ± SD
Caffeine	50 %	99.96±0.037
	100 %	99.92±0.029

	150 %	100.10±0.065
L-carnitine	50 %	99.92±0.034
	100 %	99.87±0.035
	150 %	100.02±0.041

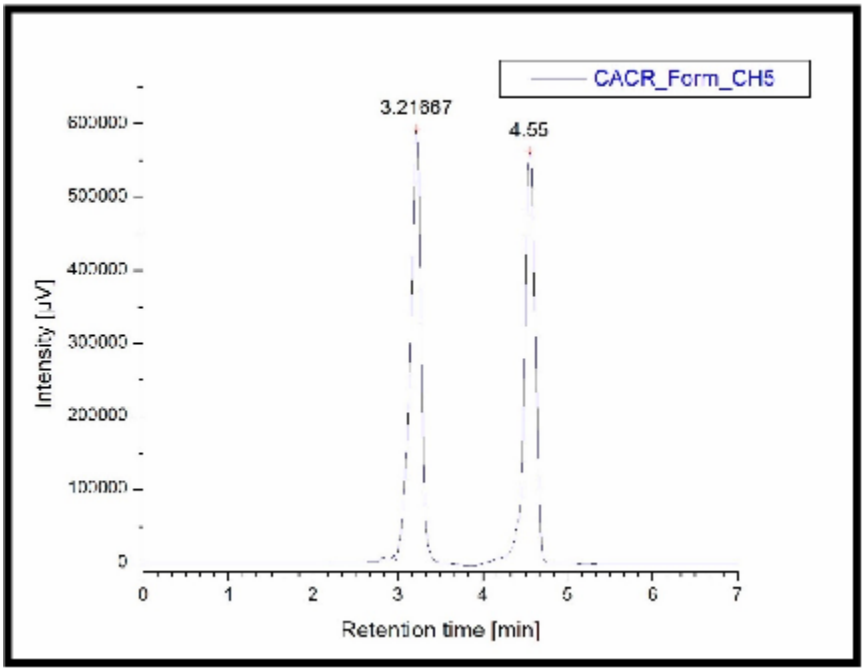

Figure 8- Linearity Overlay graph of Caffeine (50 µg/ml) & L-carnitine (250 µg/ml)

4. Conclusion

A rapid, sensitive HPLC method was developed and validated for the simultaneous estimation of Caffeine and L-carnitine.

The method development was carried out by using a mobile phase consisting of of Acetonitrile: Water (20:80 % v/v)

The detection was carried out by using UV detector at 252 nm. The column was Thermo scientific, hypersilgold,synchornis C18The flow rate was selected as 1ml/min.

The retention time of Caffeine and L-carnitine was found to be 3.21 min and 4.55 min respectively.

The linearity range of Caffeine 2 to 10 µg/ml and L-carnitine 10 to 30 µg/ml was found to be obeying linearity with the correlation coefficient $R^2 = 0.9996$ & $R^2 = 0.9992$ for Caffeine and L-carnitine respectively. The proposed and validated method was successfully applied to determine Caffeine and L-carnitine in their combined synthetic mixture. The results obtained for Caffeine and L-carnitine were comparable with the corresponding labeled amounts.

The tailing factor of Caffeine and L-carnitine was found 1.021 and 1.217 and the number of theoretical plates was found 5541 and 4954 respectively, indicating efficiency of column and these parameters represent the specificity of the method.

The recovery experiment was performed by the standard addition method. The % recovery of Caffeine and L-carnitine were found to be in the range of 99.92 to 100.10% and 99.87 to 100.02% respectively. The results indicate that proposed method is accurate. The RSD values of Caffeine and L-carnitine 0.7427 and 0.7414 %, revealed that proposed method is precise.
% RSD values of Caffeine and L-carnitine0.7654 & 0.7214 revealed that the developed HPLC method was found to be robust in terms of variations in the column temp (30 ±2 ^{0}C) and flow rate (1 ±0.1 mL/min).
LOD and LOQ values for Caffeine (0.55 µg/ml and 1.68 µg/ml) and (2.65 µg/ml and 4.97 µg/ml) showed that the method is sensitive for the determination of Caffeine and L-carnitine.
So we can conclude that proposed method can be used for routine purpose.

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