Original Article

Estimation Of Coq10 In Bulk And Formulation By High Performance Thin Layer Chromatography

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Abstract

High performance thin layer chromatography (HPTLC) gives many advantages over HPLC. It reduces the total cost of analysis as compared to HPLC. The mobile phase consumption per sample is extremely low in HPTLC, hence reducing the acquisition and disposal cost. Considering the cost and suitability of analysis for estimation of CoQ10 in bulk and its marketed capsule formulation, HPTLC method was developed and validated. The Camag HPTLC system, employed with software win CATS was used for proposed analytical work. Planar chromatographic development was carried out with the help of Pre-coated silica 60F254 TLC plates. Sample application was facilitated by Linomat 5 applicator. After sample application plates were subjected for ascending development in twin trough chamber of 10x10 cm dimension, using 10 ml of solvent system. The optimized mobile phase was composed of Toluene: ethyl acetate: chloroform (10:1:1 v/v/v). In post development, the plates were air dried and then scanned densitometrically using a UV detector at 550 nm in absorbance mode. In HPTLC densitogram well defined peak was obtained for CoQ10 with optimal Rf value of 0.71. Performance characteristics of HPTLC method for estimation of CoQ10 in bulk and its marketed capsule dosage form were statistically validated as per recommendations of ICH guidelines of analytical method validation. The HPTLC method was found to be linear across the range 50-250 ng/band. The LOD and LOQ values were found to be 0.0480 and 0.1467 µg respectively. The method was found to be accurate, precise, robust and economical for the analysis of CoQ10 from bulk and its capsule formulation.

Keywords: CoQ10, HPTLC, ICH Guidelines, Validation

INTRODUCTION

HPTLC is a well-known and versatile separation method which is type of planar chromatography, involves principle of adsorption. It is a flexible enough to analyse a wide variety of samples. It is useful in many ways as it is simple to handle and requires short analysis time to analyse the simple or complex samples. Nowadays, HPTLC serves as a preferred analytical tool for quantitative analysis of drug substances in bulk, from their formulations, from biological matrix, analysis of herbal extracts and standardization of herbal drugs (Sethi, 2013) Coenzyme Q 10 (CoQ10; ubidecarenone) is a biologically active compound that is similar in chemical structure to menaquinones (vitamin K2). Part of a family of quinone compounds known as coenzyme Q, CoQ10 is characterized by a quinone ring attached to a repeating series of side-chain isoprene units (Figure 1). The number of isoprene units is denoted by the coenzyme-X designation. In the case of CoQ10, there are 10 repeating isoprene units. (Lunetta and Roman, 2008)

CoQ10 was first discovered by researchers at the University of Wisconsin in 1957 (Crane et al, 1957). Later the chemical structure of the compound was reported. (Wolf et al, 1958). The reduced biologically active forms of CoQ10, QH, and QH2 are the result of protonation at the carbonyl moieties of the quinone ring (Figure 2). CoQ10 is lipophilic and highly water-insoluble.

CoQ10 is found in numerous cellular structures within the body, including the endoplasmic reticulum, lysosomes, other vesicles, and mitochondria, where it is an important part of the electron transport chain. Other purported beneficial effects of CoQ10 include the prevention of lipid peroxidation initiation in plasma membranes (Alleva et al, 1997), prevention of low-density lipoprotein oxidation (Raitakari et al, 2000) , antihypertensive functions (Langsjoen et al, 1994), migraine headache treatment, neurodegenerative disease treatment and cardiovascular disease (Kendler ,1997). There are no known toxicity

factors.A large number of dietary supplements containing CoQ10 are currently on the market. These products include soft gels, 2-piece hard-shell capsules, and chewable tablets. CoQ10 may be present as a single entity or in combination with other active ingredients, such as fish oil, vitamins, or botanicals.

There are HPLC methods (Tang et al, 2001) and FT NIR method (Anita et al, 2015) reported for estimation of CoQ10. The objective of research work was to develop accurate, precise, specific and economic HPTLC method for the estimation of CoQ10 in bulk and marketed formulation. Considering the predefined objective of the research work, cost and suitability of analysis for estimation of CoQ10 in bulk and its marketed formulation, HPTLC method was developed and then validated as per the recommendations of ICH guidelines of analytical method validation.(ICH Q2 (R1), 2005)

Materials And Methods

Materials and marketed formulation

CoQ10 was purchased from Bulk Supplements, 7511, Eastgate Road, Henderson, NV. Commercial Capsules containing CoQ10 (100 mg) were used for the study. Merck HPTLC Aluminium plates precoated with silica gel 60 F254were procured from local scientific and chemical supplier.

Reagents: Chemicals of (A.R. and HPLC grade) were purchased from S.D. Fine Chemicals, Mumbai, Maharashtra, India.

Instrumentation: Details of HPTLC instrument are given in Table 1.

Experimental:

Analytical Method Development

Preparation of the standard solution

About 10 mg of COQ10 STD was weighed into 100 mL volumetric flask and the volume was made up to the mark with diluent to make 100 ppm stock solution then 1.5 ml of stock solution was diluted to 10 ml to make 15 ppm solution.

Preparation of the sample solution

As each capsule formulation contains 100 mg of COQ10 and average weight of Capsule was found to be around 299.6 mg. Hence 30 mg of formulation was taken and dissolved in 100 ml of diluent to make 100 ppm sample solution. Then 1.5 ml of stock solution was diluted to 10 ml to make 15 ppm solution.

Selection of stationary phase

HPTLC Aluminium plates pre-coated with silica gel 60 F254 were selected as the stationary phase.

Layer pre-washing

Precoated TLC plates were prewashed with methanol to remove adsorbed material, impurities which include water vapours and other volatile substances from the atmosphere when they get exposed in the lab environment.

Layer preconditioning

Prewashed plates were placed in oven at 105°C for 5 minutes prior to the sample application.

Selection of detection wavelength

10µg/ml (10 ppm) solution of CoQ10 was applied on HPTLC plate (suitable dimension), scanned densitometrically over the range of 200- 700 nm using Camag HPTLC scanner 3.

Optimisation of chromatographic conditions

Many preliminary trials were carried out for selection and optimisation of Mobile phase composition and Chamber saturation time.

Analytical Method Validation

Specificity

The specificity of the method for Assay was demonstrated by Applying 10 µL band of STD, blank and sample solutions on the HPTLC Plates for rectification of specificity of method.

Linearity

The linearity of peak area response for COQ10 was determined from 50 % to 150 % level of working concentration for COQ10. The stock solutions of COQ10 were diluted in five different known concentrations (5ppm – 25 ppm). Graphs of concentration (as x-value) versus area (as y-value) were plotted. The correlation coefficient, y-intercept and slope of the regression were calculated.

Precision

System precision

System precision was evaluated from five replicate bands of standard as per proposed method. The Peak area, average and % RSD were calculated.

Method precision

The six sample solutions were prepared separately. Each sample solution was analysed as per proposed procedure. The % assay, average and % RSD were calculated.

Intermediate precision

The Intermediate precision was determined by comparison of two independent analysis on 2 different days.

Robustness

The influence of slightly changed parameters of the chromatographic conditions was tested according to ICH guidelines to demonstrate sufficient robustness of the method. The tests are carried out by Applying Standard solution by varying some of the parameters of chromatography mentioned in table 2.

Accuracy

The accuracy was determined from recovery studies. A known but varying amount of STD was spiked into pre-analysed formulation sample solution at 80%, 100% and 120% recovery levels of working concentration in triplicate. The spiked sample solution was analysed according to the proposed procedure. The percentage recoveries were calculated against respective levels.

Stability in standard and test solution

The standard and sample solutions were prepared as per the proposed method and kept at room temperature. The standard and sample solutions were analysed at initial and at different time (2 hrs) intervals till 6 hr.

Limit of detection and Limit of Quantitation

The values of Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined based on the standard deviation of the response and the slope of calibration graph. The quantitation was done with the help of following equations.

$$\mathrm{LOD} = \frac{3.3\,\sigma}{S} \quad , \quad \mathrm{LOQ} = \frac{10\,\sigma}{S}$$

where σ = Standard Deviation of replication, S = Slope of calibration curve

Results And Discussion

Analytical Method Development

The experimental conditions selected are shown in table 3.

Selection of detection wavelength

The detection wavelength selected was 550 nm after spraying with derivatizing reagent Anisaldehyde. (Figure 3)

Analytical Method Validation

Specificity

The chromatograms of blank, Standard CoQ10 and Sample CoQ10 are shown in Figure 4, Figure 5 and Figure 6.

The observations for Rf value and tailing effects are listed in table 4.

By comparing the Chromatograms of the Blank solution, Standard solution and sample solution of CoQ10, it was observed that no peak was co-eluted with the analyte band from Blank solution. Purity of COQ10 in STD and sample solution was observed OK. Hence the method is considered to be specific as per the above mentioned observations.

Linearity

The observations for linearity are listed in table 5 and a depiction in the form of 3D graph is shown in the figure 7. The calibration curve for linearity is drawn and correlation coefficient calculated as shown in figure 8.

The Correlation coefficient (r2) > 0.9995. Method is considered to be linear as the Correlation coefficient was found to be within acceptance criteria.

Precision

System Precision

Observations for system precision are shown in table 6 and repeatability of Rf is depicted in figure 9.

The relative standard deviation should be within the following limits % RSD for Area of COQ10< 2.0%. The % RSD observed within acceptable limit indicates the precision of the system.

Method precision

Observations for method precision are shown in table 7 and repeatability of Rf is depicted in figure 10 and 11.

The relative standard deviation should be within the limits. % RSD for % Assay of COQ10 should be less than 2.0%. The % RSD was observed within the limit indicates that the method has an acceptable level of precision.

Intermediate precision

Observations for intermediate precision are shown in table 8.

The relative standard deviation from Day 1 analysis and Day 2 analysis should be within the limits i.e. the overall % RSD for % assay of COQ10 from Day 1 analysis and Day 2 analysis should be < 2.0%. % RSD of % assay results from 6 determinations are within acceptance criteria for day 1 analysis & day 2 analysis. Hence the method of assay for COQ10 from formulation is rugged.

Robustness

The observations for robustness are listed in table 9.

The % RSD of peak area response due to COQ10 in three replicate application of standard solution should be less than 2.0 % and system suitability parameters should be passed. The % RSD and system suitability parameters for results obtained with varied chromatographic conditions are within the limits. Hence, the method is robust.

Accuracy

The observations for Accuracy are shown in Table 10.

% Average recovery should be in the range of 98-102%. The % Average recovery of COQ10 formulation observed within acceptance criterion of 98-102% indicates the accuracy of the method.

Stability in standard and test solution

The Percentage Relative change of COQ10 with respect to initial in Standard and formulation, with respect to initial test solutions should be less than 5.0 %. As the Percentage Relative changes of COQ10 and formulation were within limit, Standard solution and Test solution is stable up to 6 hours at Room temperature.

Limit of detection and Limit of Quantitation

Values of LOD and LOQ calculated using slope of calibration plot (135.24) and standard deviation (1.97) for CoQ10. (Table 11)

Conclusion

The developed HPTLC method was found to be fast, simple, sensitive and economic. The method was validated and found to be specific, linear, accurate, precise and robust. Hence the HPTLC method can be conveniently adopted for routine analysis of the CoQ10 in bulk and in capsule formulation.

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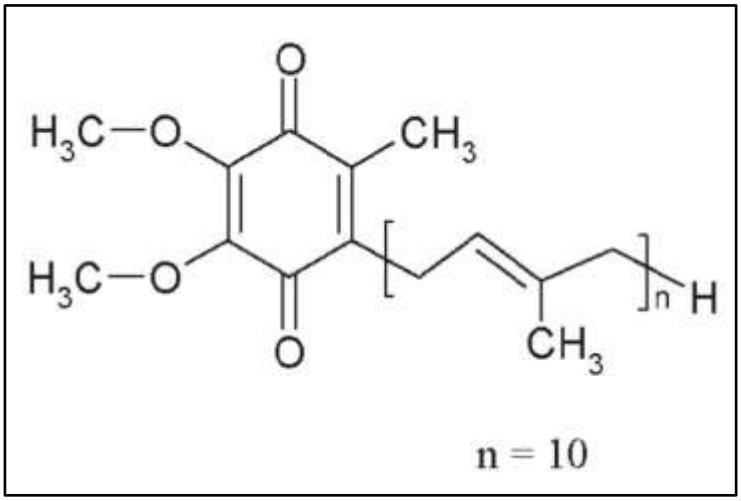

Fig 1: Structure of CoQ10

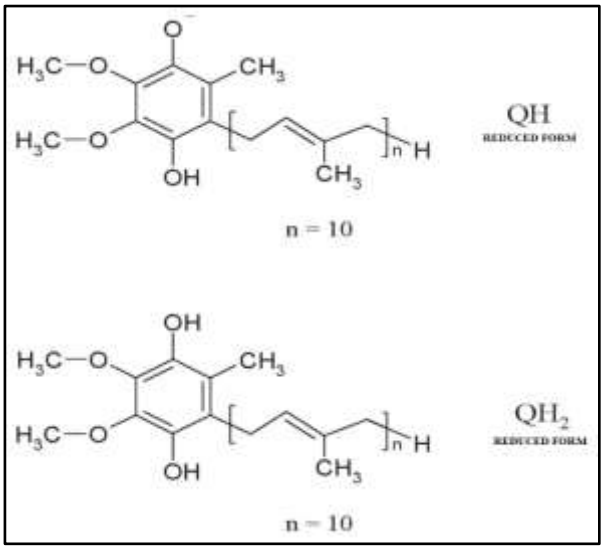

Fig. 2. Chemical structure of reduced CoQ10

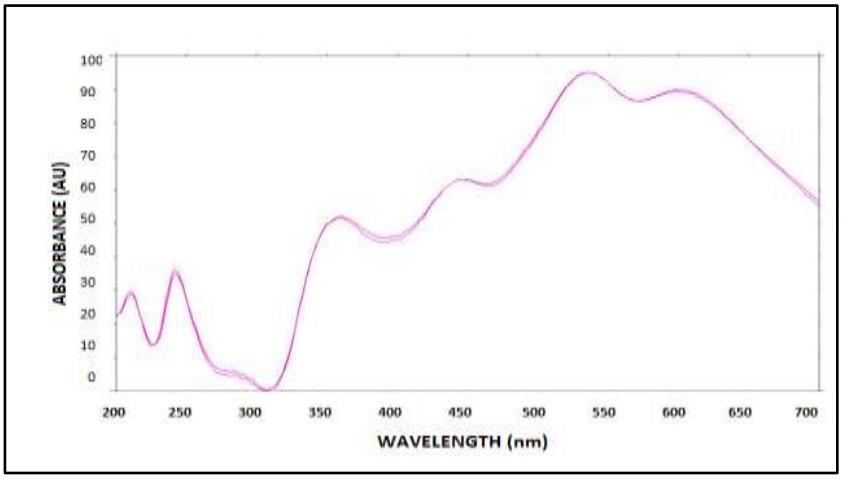

Fig 3: UV spectra of CoQ10 std and sample

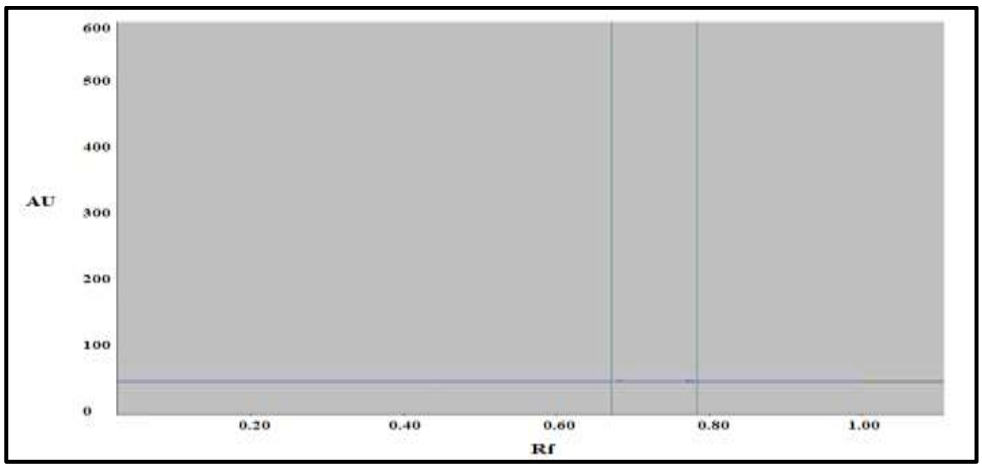

Fig. 4: HPTLC chromatogram of Blank solution

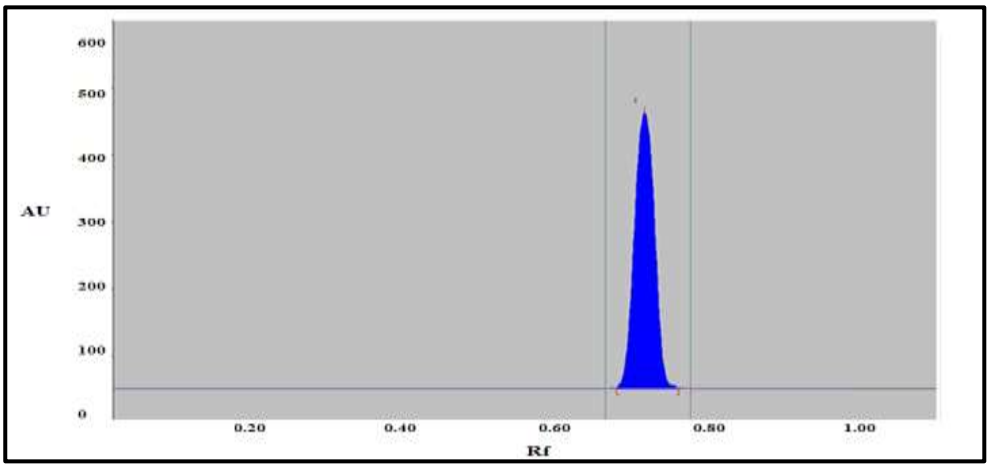

Fig 5: HPTLC chromatogram of Standard solution

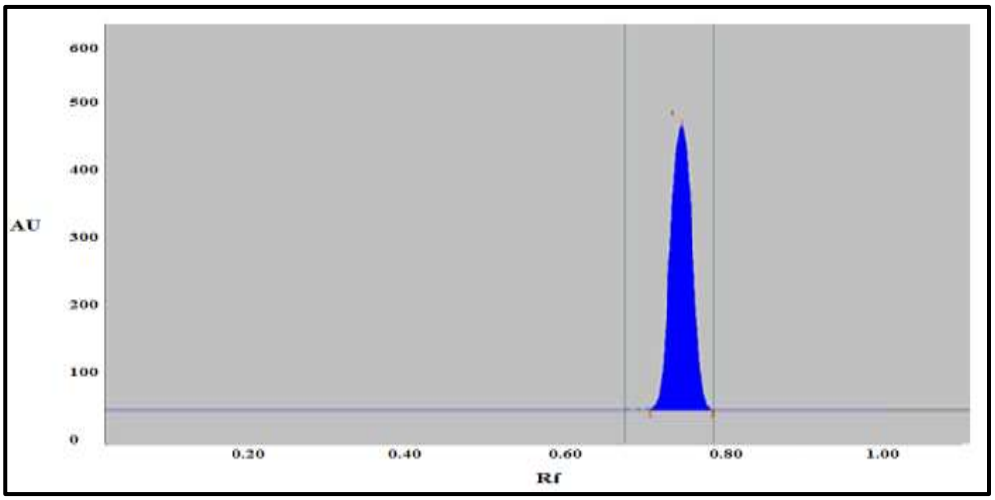

Fig 6: HPTLC chromatogram of Sample solution

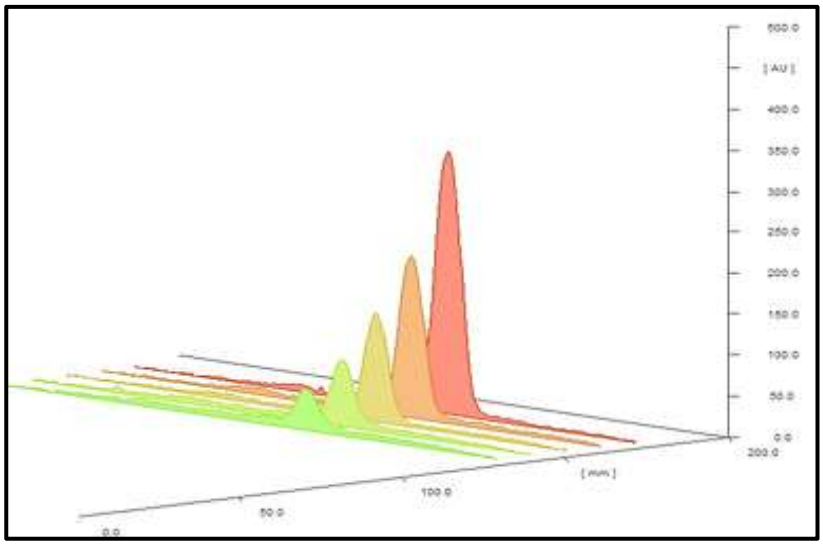

Fig. 7: Linearity of CoQ10

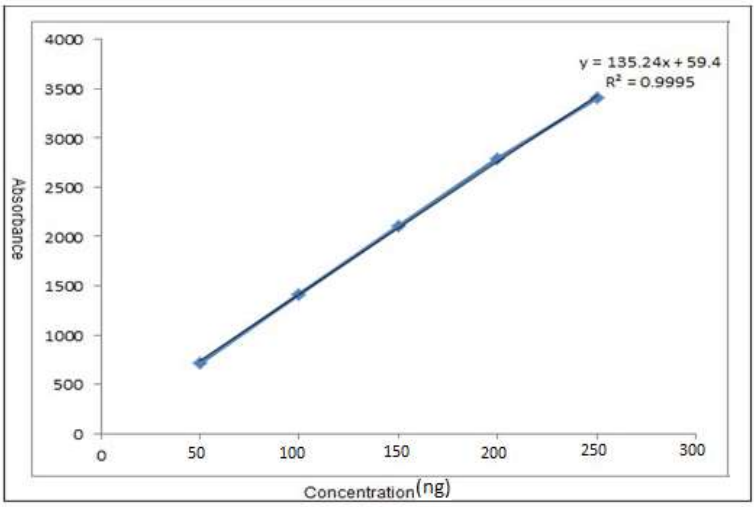

Fig 8: Calibration Curve for COQ10

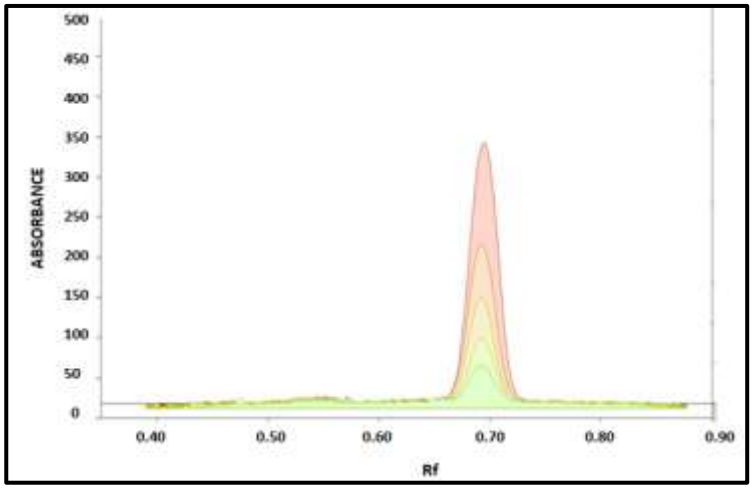

Fig 9: Precision of System giving reproducible Rf Value

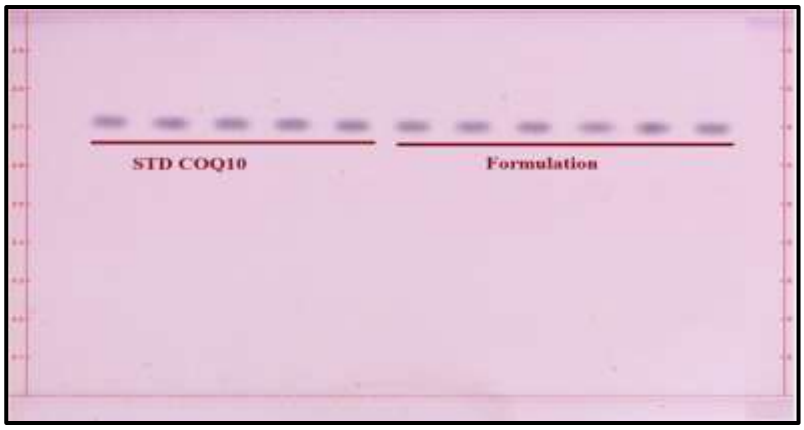

Fig 10: Method Precision Observation

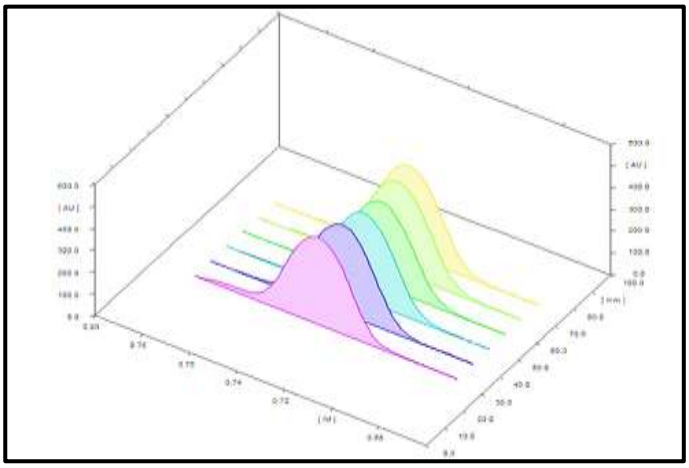

Fig 11: Method Precision

Table 1: HPTLC Instrument Specifications

Parameter	Specification
HPTLC instrument	Camag HPTLC
Applicator	Linomat 5
Detection by	Scanner 3
Visualizer	Camag TLC visualizer
HPTLC Syringe	100 and 500 µL
TLC Plates	Pre-coated silica 60F254
Software	Win CATS

Table 2: Robustness Parameters

Sr. No.	Parameter	Level 1	Level 2	Level 3
1	Saturation Time (minute)	4	5	6
2	Polar Solvent Volume (MeOH)	0.9 ml	1.0 ml	1.1 ml
3	Mobile Phase Volume (ml)	9	10	11

Table 3: Optimized Experimental Conditions

Parameter	Specification
Plate activation	at 105°C for 5 minutes
Saturation time	5 minutes
Mobile phase chamber	Twin through Chamber (10x10 and 20x10)
Derivatisation reagent	Anisaldehyde reagent
Photo documentation	white R Light
Application volume	10µL
Retention Factor	0.71
Room temperature	22°C at the time of experimentation
Type of Application	Band Type
Storage conditions of sample and STD	at 2-8°C and in dark chamber
Diluent	HPLC Grade Methanol

Table 4: Specificity Observation

Sr. No.	**Sample Name**	**Rf**	**Tailing Effect**	**Identity**	**Purity**
1.	COQ10 STD	0.71	NO	OK	OK
2.	Blank	No band is observed	NO	OK	OK
3.	Sample Solution	0.71	NO	OK	OK

Table 5: Linearity Observation

Conc. Of Coq10 (Ppm)	Average Peak Area Of Coq10
5	720
10	1412
15	2103
20	2796
25	3409

Table 6: System Precision Observation

Band Number	**Peak Area Of Coq10**
1	2102
2	2110
3	2025
4	2134
5	2098
Mean	2094
SD	40.91
%RSD	1.95

Table 7: % Assay readings for method precision

Sample No.	% Assay Of Coq10
1	101.60
2	99.67
3	101.39
4	100.50
5	99.53
6	96.19
Mean	99.81
SD	1.97053
% RSD	1.97

Table 8 : Intermediate precision observation

Name of analyte	Sr. No.	Assay (% w/w) (day 1)	Assay (% w/w) (day 2)
COQ10	1	101.60	100.84
	2	99.67	100.71
	3	101.39	98.49
	4	100.50	101.71
	5	99.53	101.27
	6	96.19	99.43
	Average	99.81	100.41
	% RSD	1.97	1.21
	Overall % RSD	1.59	

Table 9: Robustness observations

Robustness parameter	Level	% RSD	RT	Peak purity	Remark
Saturation Time (minute)	4	0.88	0.70	OK	PASS
	5	1.45	0.70	OK	PASS
	6	1.69	0.69	OK	PASS
Polar Solvent Volume (MeOH)	0.9	1.77	0.70	OK	PASS
	1.0	1.40	1.71	OK	PASS
	1.1	1.01	0.72	OK	PASS
Mobile Phase Volume	9	1.82	0.71	OK	PASS
	10	1.18	0.71	OK	PASS
	11	1.23	0.71	OK	PASS

Table 10: Accuracy Observations

Band	Recovery Levels (%)	Sample Wt.	Std Wt. (Spiked)	Amount Recovered	% Recovery	Avg % Recovery
1	80%	30.1	8.12	8.20	100.94	
2	80%	30.2	8.16	8.04	98.58	99.60
3	80%	30.2	8.05	7.99	99.28	
4	100%	30.1	10.10	9.99	98.87	
5	100%	30.3	10.20	10.10	99.02	99.06
6	100%	30.1	10.05	9.98	99.27	
7	120%	30.1	12.10	11.99	99.05	
8	120%	30.1	12.06	12.04	99.82	99.63
9	120%	29.9	12.05	12.05	100.02	

Table 11: LOD and LOQ Values

Sr. No.	Parameter	Values Obtained
1	LOD	0.0480 µg/band
2	LOQ	0.1467 µg/band