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DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR ESTIMATION OF UBIDECARENONE

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ABSTRACT

A new, simple, and rapid high-performance thin-layer chromatographic method was developed and validated for quantitative determination of Ubidecarenone. Ubidecarenone also known as Q10 was chromatographed on silica gel 60 F254 TLC plate using toluene as mobile phase. The method was found to give compact spot for the drug (Rf= 0.2) The method was validated for Linearity, precision, repeatability, and robustness as per the International Council on Harmonization guidelines. Statistical analysis of the data showed that the method is precise, accurate, reproducible, and selective for the analysis of Q10.

KEYWORDS: Ubidecarenone, Validation, linear, Selective.

INTRODUCTION

Analysis of Ubidecarenone (Also known as Q10) is done by HPTLC method and could be considered as a good alternative for analysis, as it can serve as an important tool in routine drug analysis. Major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phase. This reduces time and cost of analysis. HPTLC also facilitates repeated detection of chromatogram with same or different parameters. The present paper describes the development and validation of HPTLC method for routine estimation of analyte from the formulation.

Chemically Coenzyme Q10 is 1, 4-benzoquinone where Q refers to quinone group and 10 refers to the number of isoprenyl chemical subunits in its tail. Q10 is also known as (Ubidecarenone/ Ubiquinone).[2,5]

Importance of Coenzyme Q10: It is physiologically important vitamin like naturally occurring compound. It acts as an electron shuttle in mitochondrial respiratory chain as a stabilizing agent in cellular membranes functions like a rechargeable battery in the transfer of energy.[11,13] It is necessary for proper functioning of many organs and for basic functioning of cells. Coenzyme Q10 is for which a range of potential health benefits has been postulated. But according to European Food Safety Authority (EFSA) a cause-and-effect relationship has not been established between the consumption of Coenzyme Q10 and the health claims by the industry.[14] Coenzyme Q10 levels are reported to decrease with age and to be low in cardiac conditions, Parkinson's disease, cancer, diabetes, muscular dystrophies, HIV/AIDs, etc. Some drugs also lower Coenzyme Q10 levels. Coenzyme Q10 is fairly safe and well tolerated.[3,6]

Experimental

Materials and Method

Coenzyme Q10 was obtained from Samex enterprises Surat. All reagents were of analytical grade (E Merc). All the solvents used for mobile phase preparation were of analytical grade. A CAMAG HPTLC system equipped with a simple applicator Linomat V twin trough plate developing chamber, TLC scanner III and an integration software Win CATS V.1.2.3 was used. Silica gel GF60 aluminium sheets (Merck) was used as a stationary phase.[4,8]

Apparatus

The HPTLC system (Camag, Muttenz, Switzerland) consisted of Limomat V autosprayer connected to a nitrogen cylinder, a twin trough chamber (10 × 10 cm), a derivatization chamber, and a plate heater. Precoated silica gel 60 F254 TLC plates (10 × 10 cm, layer thickness 0.2 mm (E. Merck KGaA, Darmstadt, Germany) was used as stationary phase. Analysis was carried out using a TLC scanner III with winCATS software.

- **Standard solution of coenzyme Q10**

A stock solution of coenzyme Q10 was prepared by dissolving 5mg of accurately weighed Co Q10 in solvent toluene and making up the volume to 5 ml with toluene to get the final concentration of 1mg/ml.[10]

The stock solution was appropriately diluted with toluene to give a final concentration of 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 µg/µl of the standard solution was applied 1cm edge of the HPTLC PLATE using a band width of 8mm and distance between tracks of 15mm. The chromatogram was developed and scanned at 280nm. Before and after derivatisation with spray reagent.

- **Sample preparation**

Test Formulation of Aloe vera health drink was prepared.

To test formulation three times by volume methanol was added was sonicated and the residue was diluted with methanol for analysis. and applied along with standards at two levels 5µl and 2 µl.

HPTLC Method and Chromatographic Conditions

Chromatography was performed on a pre-activated (1100C) silica gel HPTLC plate 60F254, 10x10 cm. Samples and standards were applied to the plate as 6 mm wide bands with an automatic TLC applicator, Linomate IV, with Nitrogen flow (Camag, Muttenz, Switzerland). The application parameters were identical for all the analyses performed.

The TLC plates were developed using a Camag twin-trough glass tank which was pre-saturated with the mobile phase. Plate was developed in the solvent system of toluene.

After development, the plate was removed and dried and spots were visualized in UV light (UV cabinet, Camag, Switzerland). The Rf, peak areas and absorption spectra were recorded.

Sr No	Parameters	Conditions
1	Test plate	Normal phase HPTLC precoated plates. Silica gel 60 F254 (Merck)
2	Format	20X10cm plate
3	Spotting volume	0.2,0.3,0.4,0.5,0.6,0.7,0.8,0.9 µl
4	Seperation technique	Ascending
5	Mobile phase	Toluene
6	Spray reagent	Phosphomolybdic acid
7	Detection	At 280nm
8	Densitometric scanning	CAMAG TLC SCANNER III
9	Mode	absorbance
10	Wavelength:	280nm (LAMBDA MAX of coenzyme Q10)
11	HPTLC model	Linomat V

Method validation

Validation of the developed HPTLC method was carried out as per the International Council on Harmonization (ICH) guidelines Q2 (R1) for linearity, specificity, accuracy, precision, repeatability, LOD and LOQ , ruggedness, robustness.[9,12]

- **Linearity of the standard curve:** Linearity of the method was evaluated by constructing calibration curves at six concentration levels. The extent to which the relationship between the experimental response value (or a mathematical manipulation of this) and the

concentration of the analyte approximates to a straight line. This relationship may also be called the calibration curve. It is proportionality of measured value to concentration.

- **Specificity*:*** Ability to measure desired analyte in a complex mixture. Specificity generally refers to a method that produces a response for a particular analyte only. The specificity was confirmed by overlaying the spectra of std Q10 with spectra of a sample recorded on TLC scanner in UV range. UV spectrum showed lambda max at 280nm.[1]

- **Accuracy:** Compare the results of the method with results from an established reference method. Accuracy is determined by replicate analysis of samples containing known amounts of the analyte.

- ***Precision:*** Agreement between a series of measurements. Precision was evaluated in terms of Intraday and Interday precisions.

- **Repeatability:** Precision under the same operating conditions.

- **Quantification range:** Sensitivity of the method was determined with respect to limit of detection (LOD) and limit of quantification (LOQ). Formula to calculate LOD= 3.3 σ/S *L*OQ= 10 *σ*/*S*

- **Ruggedness**-The degree of reproducibility of results obtained under a variety of conditions, such as different laboratories, analysts, instruments, environmental conditions, operators and materials. Ruggedness is a measure of reproducibility of test results under normal, expected operational conditions from laboratory to laboratory and from analyst to analyst. Ruggedness is determined by the analysis of aliquots from homogeneous lots in different laboratories.

- **Robustness:** The ability of the method to deliver accurate, precise results under normal variations of operating conditions. Such variations may include performance by different analysts, the use of reagents from different suppliers, or of reagents with differing storage times. Also, different types of analytical equipment, perhaps with different sensitivity or accuracy, may be in use in the test development laboratory and in those laboratories that will perform routine quality control (QC) testing. The more robust the assay, the less the effect of these factors on its accuracy and precision.
- All readings are Average of three to six determinations

RESULTS AND DISCUSSION

1) Linearity Studies of Coenzyme Q10

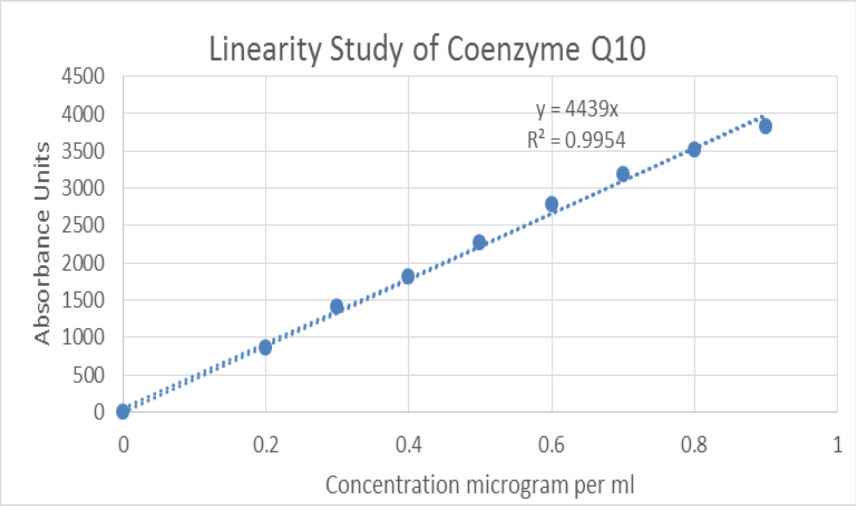


Figure 1.1: Linearity studies of coenzyme Q10.

Table 1.1: Linearity studies of coenzyme Q10.

S. no.	Concentration(mcg/ml)	Peak Area
1	0.2	857
2	0.3	1402
3	0.4	1807
4	0.5	2267
5	0.6	2790
6	0.7	3118
7	0.8	3523
8	1	3824

2) Specificity

Identification: to ensure the identity of an analyte. Rf=0.2

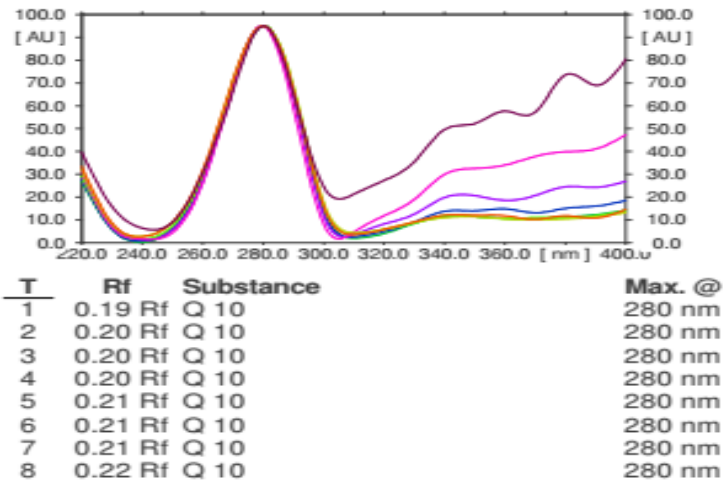
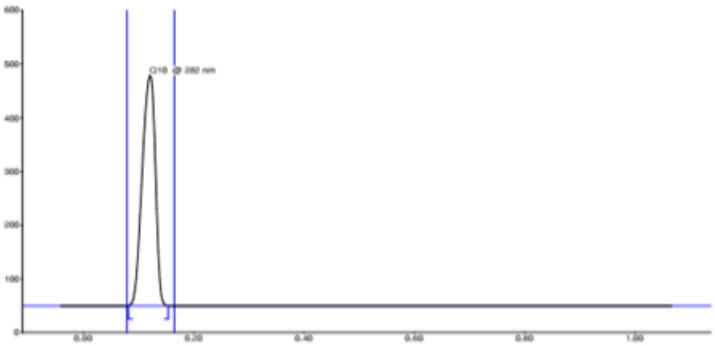


Figure 2.1: Identity of Analyte at different Rf value.

3) Accuracy

Accuracy is established across the specified range of the analytical procedure. With same Rf and peak area.

Track 1, ID: std



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.08	1.2	0.12	429.7	100.00	0.15	0.8	8580.8	100.00	Q10

Track 2, ID: sample

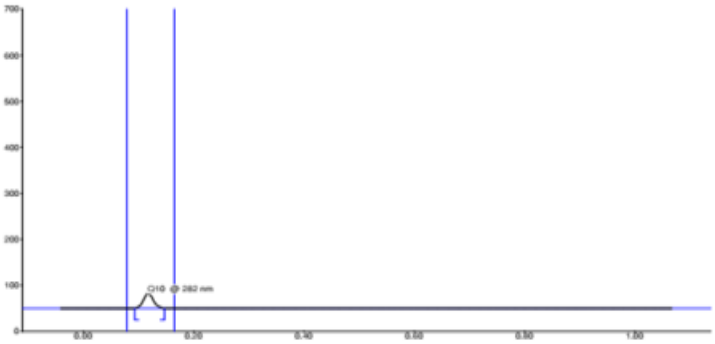
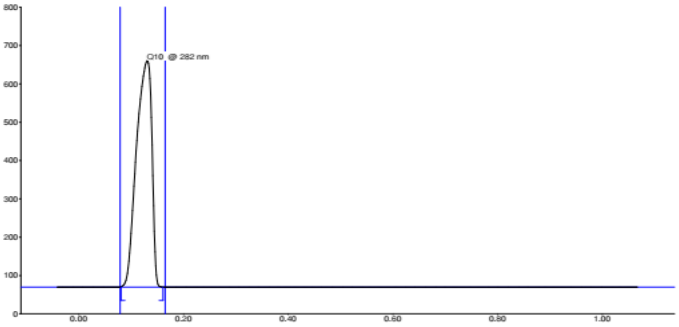


Figure 3.1: Accuracy studies.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.09	0.7	0.12	31.7	100.00	0.15	0.0	504.4	100.00	Q10

Track 3, ID: std



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.08	1.0	0.13	590.4	100.00	0.16	0.0	14547.9	100.00	Q10

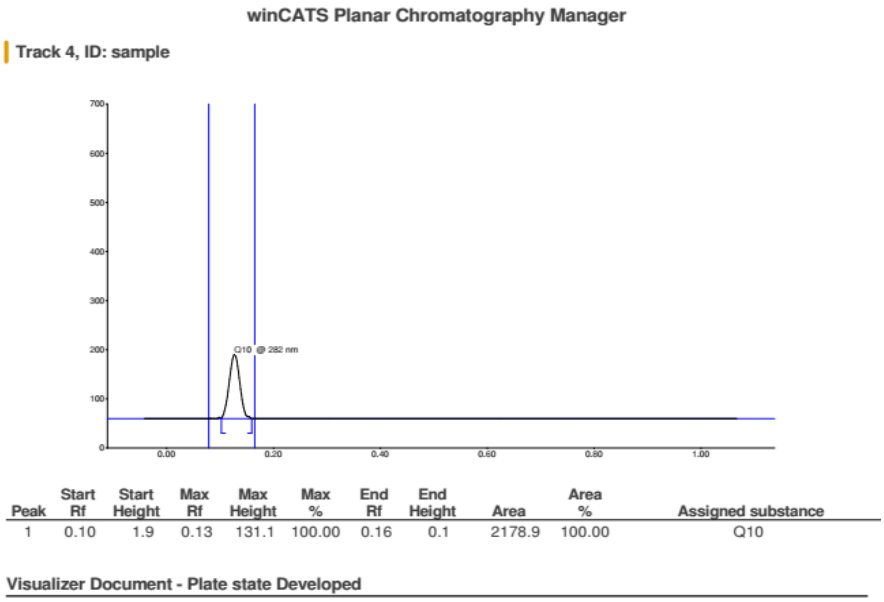


Figure 3.1: Accuracy studies.

4) Precision studies

i) Intraday precision

Table 4.1: Intraday precision.

Component	Concentration µl	Amount found AU	% Recovered (Mean ± SD)	Intra-day Precision %RSD
Test Formulation	2 µl	500	100.26	0.018
	5 µl	2000	99.37	
MEAN ± SD			99.81 ± 0.44	

ii) Inter day precision

Table 4.1: Interday precision.

Component	Concentration µl	Amount found AU	% Recovered (Mean ± SD)	Inter-day Precision %RSD
Test Formulation	2 µl	500	100.46	0.028
	5 µl	2000	99.04	
MEAN ± SD			99.75 ± 0.71	

5) Repeatability

Table 5.1: Repeatability.

Component 1	Amount taken in µl	Amount Found AU	Amount found (%)	Mean ± SD
Test Formulation	5	2000	99.37	99.81 ± 0.44

6) Limit of detection (LOD) and Limit of Quantification (LOQ)

Table 6.1: LOD and LOQ.

LOD µl / spot	LOQ µl / spot
0.03	0.15

7) Ruggedness

Table 7.1: Ruggedness.

	% Amount found	% RSD
Analyst I	97.45	0.0018
	97.54	
Analyst II	97.43	0.00530
	97.24	

8) Robustness

Table 8.1: Robustness.

Development distance(cm)	% Amount found	% RSD
2	98.32	0.0022
	98.21	
5	98.37	0.0037
	98.24	

HPTLC study of coenzyme Q10

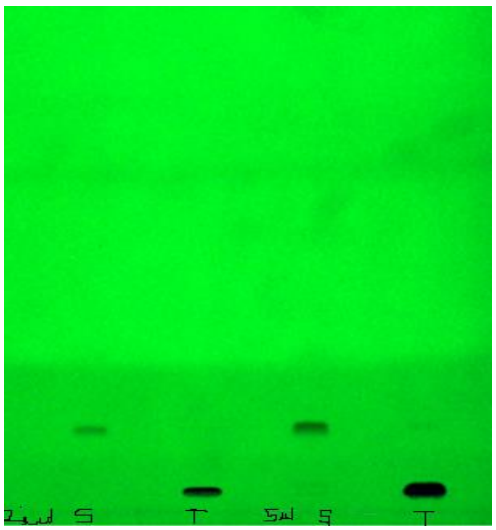


Figure 8.1: HPTLC of coenzyme Q10 at 2 and 5 µl.

Application pattern

Track1	STD	2 µl	2µg	8500Au
Track2	Test formulation	2 µl	2000µg	500Au
Track3	STD	5µl	5µg	14000Au
Track4	Test Formulation	5µl	5000µg	2000Au

CONCLUSION

HPTLC method has been developed for the identification and quantification of analyte. Low cost, faster speed, and satisfactory precision and accuracy are the main features of this method. Method was successfully validated as per ICH guidelines and statistical analysis proves that method is Linear, specific, and repeatable.

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