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RESEARCH ARTICLE

METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF LYCOPENE AND UBIDECARENONE BY RP-HPLC IN COMBINED PHARMACEUTICAL DOSAGE FORM

Saravanan VS*, Revathi R, Meera Nadhini V

The Erode College of Pharmacy, Perundurai Main Road, Vallipurathanpalayam (Po), Veppampalayam, Erode – 638112, Tamilnadu, India

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ABSTRACT

Lycopene is used for the treatment of cancer and cardiovascular disease and Ubidecarenone is used as a dietary supplement and categorized as cardiovascular agent used for the treatment of heart failure and cardiac disorder. A Reverse Phase Liquid Chromatography (RP–HPLC) method was developed and validated for the simultaneous estimation of Lycopene and Ubidecarenone in combined pharmaceutical dosage form. The different analytical parameters such as linearity, range, precision, accuracy, and robustness were determined according to the International Conference on Harmonization ICH Q2 R (1) guidelines. Chromatography was carried out by Isocratic technique at a flow rate of 1.5 ml/min on Waters Reliant C18 (150 X 4.6mm I.D., 5µm particle size) column at 50. The mobile phase consists of mixture of methanol:tetrahydrofuran:acetonitrile:water in the ratio of 25:15:58:2 % v/v and optimized depending upon the polarity. The UV detection wavelength was 400 nm and 50 µL of sample was injected. The retention times of Lycopene and Ubidecarenone were found to be 11.126 min and 20.504 min respectively. The calibration curves were linear over the concentrations 13-38 mcg/mL and 188-563 mcg/mL with correlation coefficient of 0.9986 and 0.9989, for Lycopene and Ubidecarenone. Percentage recovery obtained 98.58-100.15 % for Lycopene and 99.30-100.64 % for Ubidecarenone. The % RSD for Precision and Accuracy of the method was found to be NMT 2%. The proposed method was highly sensitive, precise and accurate. Hence, the method was successfully applied for the reliable quantification of active pharmaceutical ingredient content in in-house prepared tablet formulation.

Key words: Lycopene, Ubidecarenone, RP - HPLC, Validation, Simultaneous estimation, % RSD.

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INTRODUCTION

Lycopene is chemically 2, 6, 10, 14, 19, 23, 27, 31- Octamethyldotriaconta- 2, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30- tridecaene (Figure 1). Lycopene does not have a Pro-vitamin A activity[1]. Lycopene is regarded as a strong antioxidant and have a protective effect against Prostate cancer[2,3]. Lycopene is the only carotenoid associated with cancer risk reduction and its plasma concentration is also considered as a useful clinical parameter associated with myocardial infarction[4]. Ubidecarenone is chemically 2- [(2E, 6E, 10E, 14E, 18E, 22E, 26E, 30E, 34E)- 3,7,11,15,19, 23, 27, 31, 35, 39- Decamethyltetraconta- 2, 6,10,14,18, 22, 26, 30, 34, 38- decaenyl]- 5, 6- dimethoxy-3-methylcyclohexa- 2, 5- diene-1,4- dione (Figure 2). It is also known as Coenzyme Q10 and used as a dietary supplement. It is categorized as a cardiovascular agent used in the treatment of congestive cardiac failure and angina pectoris[5]. It increases the contractive force of the heart through positive inotropic action to improve cardiac output.

The Literature survey shows that there are few analytical methods reported for the determination of Lycopene and Ubidecarenone in pharmaceutical dosage forms either as a single drug or in combination with some other drugs. Lycopene has been determined in food, biological samples and pharmaceutical dosage form by various analytical methods, such as, Supercritical Fluid Chromatography[6] (SFC), RP-HPLC[7-10], HPLC with UV-Detection[11,12].Ubidecarenone has been determined by analytical methods either individually[13,14] or in combination with other drugs by UV[15], RP-HPLC[16-19].

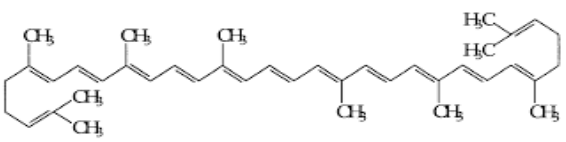

Figure 1: Chemical structure of Lycopene

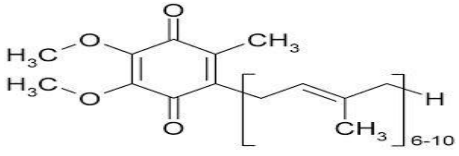

Figure 2: Chemical structure of Ubidecarenone

However, there is no HPLC method reported for the simultaneous estimation of Lycopene and Ubidecarenone in Pharmaceutical dosage forms. The present paper describes a simple, rapid, economical and accurate RP-HPLC method for the simultaneous estimation of Lycopene and Ubidecarenone in Pharmaceutical dosage form. The method was validated[20] in compliance with ICH Q2 R (1) guidelines.

EXPERIMENTAL

Chemicals and reagents

Methanol HPLC Grade, Tetrahydrofuran HPLC Grade, Acetonitrile HPLC Grade, Water HPLC Grade, and Ethanol HPLC Grade were used. The standard samples of Lycopene and Ubidecarenone were obtained as gift samples from Sai Mirra Innopharm Pvt. Ltd., Chennai with purity of 10.21 % for Lycopene and 99.98 % for Ubidecarenone. The tablet formulation (Blesspa) is also obtained from Sai Mirra Innopharm pvt. Ltd., Chennai. Each table contains about 20 mg of Lycopene and 300 mg of Ubidecarenone.

Apparatus and Instrument

The analysis was carried out using Shimadzu LC 2010A HT system with LC solution software. The system was equipped with LC-20 ATVP series pump, Autosampler, and UV - Visible detector SPD 20A. Other apparatus and instruments used were UV-Visible Spectrophotometer (UV-1601 SHIMADZU) with UV-Probe software, Analytical balance (Uni Bloc), pH meter (Spectrum Tek), Vaccum pump (Gelchem science), Sonicator (Spectrum Tek). All the instruments and glass wares were calibrated.

Mobile Phase

The mobile phase consists of mixture of methanol:tetrahydrofuran:acetonitrile:water in the ratio of 25:15:58:2 % v/v. The mobile phase was filtered through Millipore filter paper type HV (0.45 µm) and degassed by ultrasonication for 10 minutes.

Chromatographic conditions

Chromatographic analysis was carried out on a Waters reliant C18, (150 mm x 4.6 mm I.D., 5µm) was used for separation. The mobile phase consisted of mixture of methanol:tetrahydrofuran:acetonitrile:water in the ratio of 25:15:58:2 % v/v. The flow rate was delivered at 1.5 mL/min with detection wavelength at 400 nm. A 50 µL sample solution was injected to the chromatographic system with column temperature of 50. Under these conditions the run time was 35 min.

Preparation of standard stock solution of Lycopene and Ubidecarenone

Stock solution was prepared by taking 25 mg of Lycopene and 37.5 mg of Ubidecarenone working standard in a 50 mL volumetric flask. 15 ml of DMF was added and sonicated for about 20 min until all the contents has been dissolved completely. Then, the remaining volume was made up to the mark with ethanol and filtered through 0.45 PVDF filter. The further dilutions were done using diluent ACN:Methanol (60:40) v/v.

Preparation of combined working standard of Lycopene and Ubidecarenone

The standard stock solution was diluted further to get the concentrations in the range of 12.5, 20, 25, 30, 37.5 µg/mL Lycopene and 187.5, 300, 375, 450, 562.5 µg/mL Ubidecarenone. The calibration curve was plotted by taking six different concentrations from the stock solution and all the six injections were injected into the chromatographic system and chromatograms were recorded.

Assay Procedure[21]

Accurately about 20 tablets were weighed and the average weight was taken. The tablets were powdered finely and tablet powder equivalent to 25 mg of Lycopene and 37.5 mg of Ubidecarenone were taken into 100mL volumetric flask. About 50mL of ethanol was added and sonicated for about 20 min until all the contents were dissolved which was made up to the mark with mobile phase. The above solution was filtered through 0.45 PVDF filter with discarding first 10mL of the filtrate. With optimized chromatographic condition a steady baseline was recorded with mobile phase. About 50µL of the sample solution was injected and the chromatogram was recorded.

Table 1: Results of Assay

Drug Name	Label Claim (mg)	Amount found (mg)	% Content*
Lycopene	20	20.25	101.25
Ubidecarenone	300	294.62	98.43

(n = 3)*

Method Validation

Precision

Precision is the degree of repeatability of an analytical method under normal conditions. System precision was performed by injecting six replicate injections of Lycopene and Ubidecarenone (25 ppm of Lycopene and 37.5 ppm of Ubidecarenone) working standard. The precision of test method was evaluated by performing assay for six individual test preparations of 20/300 mg strength as per test method. About 50 µL of the solution was injected and the chromatograms were

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recorded. The procedures for system and method precision were repeated for 6 times. The peak areas were measured and the % relative standard deviation was calculated.

Linearity

The linearity of measurements was evaluated by analyzing six different concentrations of the standard solutions of Lycopene and Ubidecarenone in the range of 13-38 mcg/mL and 188-563 mcg/mL for both drugs respectively and a calibration plot was constructed. The linearity was evaluated by linear regression analysis.

Accuracy

The accuracy was confirmed by recovery studies by adding known amount of placebo to the pure API of Lycopene and Ubidecarenone from about 50 % to 150 % of the initial assay concentration. Sample solutions was prepared in triplicate for each level and analyzed as per test method.

Robustness

Influence of deliberate change in the chromatographic conditions such as change in flow rate of 1.4 and 1.6 mL/min, change in wavelength of 398 and 402 nm, and change in mobile phase composition of methanol:tetrahydrofuran:acetonitrile:water (23:15:60:2) % v/v and methanol:tetrahydrofuran:acetonitrile:water (27:15:56:2) % v/v was made to evaluate the impact of the method. The mixed standard solution is injected in six replicate and % RSD was calculated.

Ruggedness

The ruggedness of the method was demonstrated by conducting the precision study on different HPLC system (1 & 2) and performed by different analyst on different day. Assay was performed for six individual test preparations of 20/300 mg strengths as per test methods. From the peak of the chromatograms, the % RSD was calculated.

Specificity

Specificity is the ability to assess unequivalently the analyte in the presence of components, which may be expected to be present. Typically these might include impurities, matrix, degradants etc. Specificity is evaluated for blank and placebo interference by injecting the blank and the control sample solution prepared as per the proposed method. It is used to check for the interference of any peak at the retention time of Lycopene and Ubidecarenone.

System suitability

System suitability study of the method was carried out by six replicate analysis of solution containing 100% target concentration of Lycopene and Ubidecarenone. The system suitability parameters like USP plate count, asymmetry factor, retention time, resolution, tailing factor were evaluated from standard chromatogram.

Stability of solution

A study to establish the stability of standard and test preparation on bench top was conducted for 1, 2, 4, 8, 10 Hours.

RESULTS AND DISSCUSSION

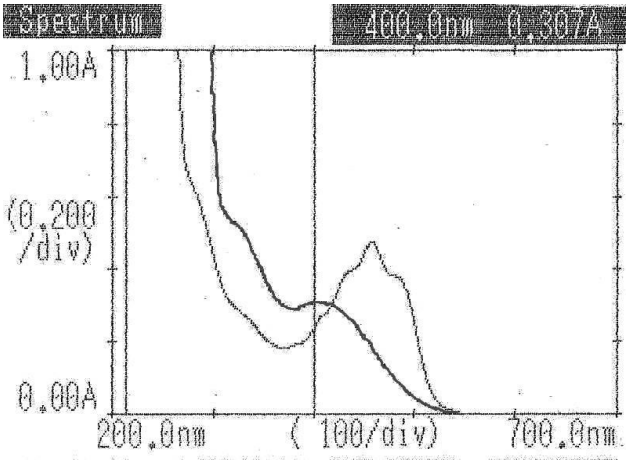

Figure 3: UV spectrum of Lycopene and Ubidecarenone

Selection of Detection wavelength

The wavelength selected was from the overlay of Lycopene and Ubidecarenone. Both the drugs showed typical peak nature and the peaks were symmetrical at 400 nm. Hence the wavelength has been selected as the detection wavelength.

Development and optimization of the HPLC method

For getting an optimized chromatographic condition a Waters Reliant C18 (150 X 4.6mm I.D., 5µm particle size) column was selected as a stationary phase. The mobile phase composition of a mixture of methanol:tetrahydrofuran:acetonitrile:water in the ratio of 25:15:58:2 % v/v was selected which gave good resolution and asymmetry. The flow rate of 1.5 mL and column temperature of 50was selected. The retention time for Lycopene and Ubidecarenone was found to be 11.126 min and 20.504 min respectively.

Validation

Linearity

The linearity study was conducted for the Lycopene and Ubidecarenone by preparation of the stock solution using the ethanol. Calibration curve was plotted by taking six concentrations in the range of 13 mcg/mL- 38 mcg/mL for Lycopene 188 mcg/mL-563 mcg/mL for Ubidecarenone. The slope, intercept, and correlation coefficient of Lycopene was found to be 4065, 846, 0.9989 and for Ubidecarenone was found to be 1254, 734, 0.9986. The data regarding linearity was shown in Table 2. Calibration curves of Lycopene and Ubidecarenone were shown in Figure: 4 and 5.

Table 2: Linearity data

	Lycopene		Ubidecarenone	
% Level	Concentration (mcg/mL)	Peak area	Concentration (mcg/mL)	Peak area
50	12.5	51733	187.5	242066
80	20.0	83593	300	375082
100	25.0*	100730	375*	460885
120	30.0	122172	450	567228
150	37.5	154205	562.5	711012

** Operating concentration*

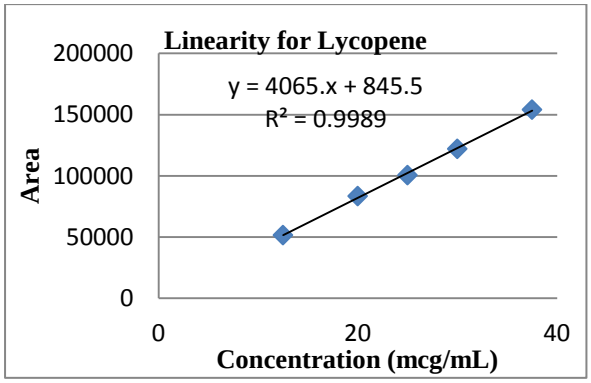

Figure 4: Calibration curve of Lycopene

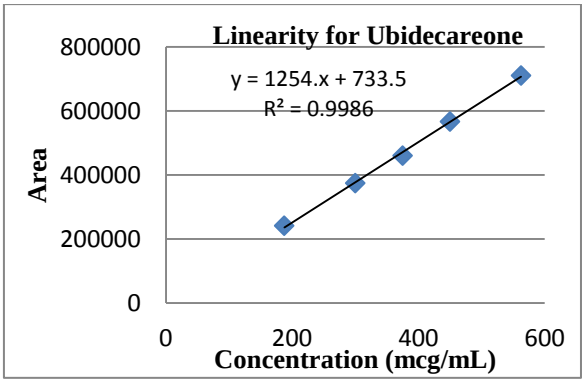

Figure 5: Calibration curve of Ubidecarenone

Precision

System precision was performed using six replicated injections of Lycopene (25 ppm) and Ubidecarenone (37.5 ppm) working standard into the HPLC system from the stock solution. The % RSD for peak area of Lycopene and Ubidecarenone from six replicate injections of standard solution was found to be NMT 2.0. The precision of test method was evaluated by performing assay for six individual test preparations of 20/300 mg strength as per test method. The % RSD of the six assay values should was found to be not more than 2.

Table 3: System precision

Injection Number	Lycopene Peak area	Ubidecarenone Peak area
1	115029	477434
2	115372	480062
3	115086	481810
4	115765	477844
5	115565	480530
6	115435	481519
Average	**115375.3**	**479866.5**
SD	**256.5947951**	**1682.9519**
% RSD	**0.22**	**0.35**

Table 4: Method precision

S. No	Area	% Assay	Area	% Assay
	Lycopene	Label claim 20mg	Ubidecarenone	Label claim 300mg
1	115601	20.28 mg (101.40%)	478700	295.97 mg (98.66%)
2	116292	20.24 mg (101.20%)	483642	295.16 mg (98.39%)
3	116929	20.28 mg (101.40%)	485875	291.81 mg (97.27%)
4	116320	19.98 mg (99.90%)	479028	291.63 mg (98.54%)
5	116040	20.22 mg (101.10%)	483614	296.94 mg (98.98%)
6	117720	20.50 mg (102.50%)	486878	296.23 mg (98.74%)
	Mean	**20.25 mg (101.25%)**	**Mean**	**294.62mg (98.43%)**
	%RSD	**0.83%**	**%RSD**	**0.61%**

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Accuracy:

The accuracy was confirmed by recovery studies by adding known amount of placebo to the pure API of Lycopene and Ubidecarenone at about three levels. Sample solution was prepared in triplicate for each level and analyzed as per test method. The amount spiked and the mean recovery values were calculated. The % RSD was found to be NMT 2%.

Table 5: Accuracy data

Drug	Amount of Lycopene spiked (mg)	Amount recovered (mg)	% Recovery (98% to 102%)	Mean % Recovery and % RSD
Lycopene	2.5545	2.5553	100.02	**Mean = 99.59%** **% RSD**=0.67%
		2.5525	99.92	
		2.5245	98.82	
Ubidecarenone	37.5225	38.0979	101.53	**Mean = 100.64%** **% RSD**=0.77%
		37.5558	100.09	
		37.6370	100.31	

Robustness

As part of the robustness, deliberate change in the flow rate at 1.4 and 1.6 mL/min, wavelength at 402 nm and 398 nm, and mobile phase composition such as methanol:tetrahydrofuran:acetonitrile:water in the ratio of 23:15:60:2 % v/v and 27:15:56:2 % v/v was made to evaluate the impact of the method. The mixed standard solution was injected in six replicate. The % RSD and the system suitability parameters were calculated. The % RSD of areas of Lycopene and Ubidecarenone for 6 replicate standard injections was found to be NMT 2.0%. The tailing factor for both the peaks was found to be NMT 2.0. The column efficiency was found to be NLT 2000 theoretical plates and the resolution between the two peaks was found to be NLT 5.0.

Table 6: Robustness data for Lycopene

Parameters	Variation	Lycopene		
		% RSD	Tailing factor	Theoretical plate
Flow rate (mL/min)	1.4	0.94	1.420	6504.166
	1.6	0.82	1.436	8080.108
Wavelength (nm)	398	1.25	1.423	6044.404
	402	1.23	1.420	6218.216
Mobile phase ratio	23:15:60:2	0.64	1.195	8459.057
	27:15:56:2	0.75	1.203	9295.619

Table 7: Robustness data for Ubidecarenone

Parameters	Variation	Ubidecarenone			
		% RSD	Tailing Factor	Theoretical Plate	Resolution
Flow rate (mL/min)	1.4	0.85	1.229	6577.089	13.164
	1.6	0.12	1.224	8104.396	14.833
Wavelength (nm)	398	0.49	1.242	6524.340	12.952
	402	0.52	1.249	6531.517	13.024
Mobile phase ratio	23:15:60:2	0.22	1.197	8252.140	14.381
	27:15:56:2	0.35	1.193	9162.936	14.909

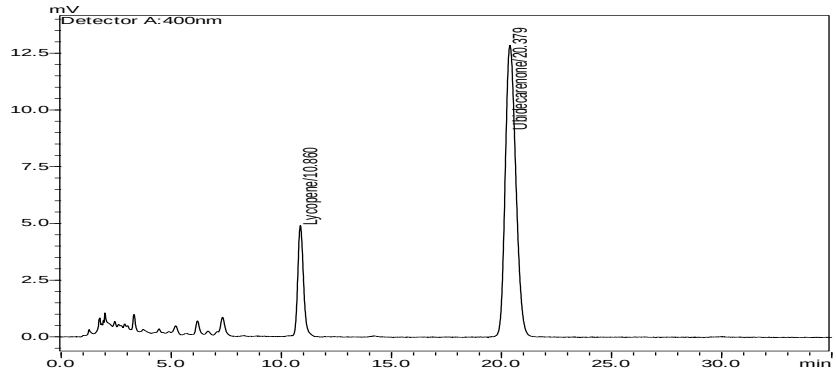

Figure 6: Optimized chromatogram

System suitability

The System suitability parameters were calculated, and the values were found to be within the limit. From the system suitability parameters the method was found to be linear with good resolution and symmetry.

Table 8: System suitability parameters

System Suitability Parameters	Observed Values	
	Lycopene	Ubidecarenone
Retention time	12.455	24.875
USP plate count	10818.229	12521.263
Resolution	-	16.672
Tailing factor	1.474	1.282
Linearity (µg/mL)	13- 38	188 -563
Correlation coefficient (R^2)	0.9989	0.9986
Slope (m)	4065	1254
Intercept (c)	845.5	733.5

CONCLUSION

A precise RP–HPLC method was developed for the simultaneous estimation of Lycopene and Ubidecarenone showed good precision and accuracy. The low % RSD values in the recovery studies for the method shows that there are no interferences due to excipients during formulation. Hence it was concluded that the developed method was simple, precise and rapid for the analysis of combination of Lycopene and Ubidecarenone. The method was validated as per the ICH Q2 (R1) guidelines.

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Address for Correspondence:* *Dr. Saravanan VS, Department of Pharmaceutical Analysis, The Erode College of Pharmacy, Perundurai Main Road, Vallipurathanpalayam (Po), Veppampalayam, Erode – 638112, Tamilnadu, India,* ***E-mail:* *saravecp@yahoo.co.in,* ***Phone:*** *+91-9443838566*

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