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A Simple and Sensitive RP-HPLC Method for Estimation of Lycopene in Pharmaceutical Solid Dosage Forms

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ABSTRACT:

An RP-HPLC method was developed and validated for the estimation of Lycopene in bulk drug and solid dosage forms. The chromatographic system was equipped with Inertsil ODS-3V, C-18, 150 X 4.6mm internal diameter with 5 micron particle size column and PDA detector set at 472 nm, in conjunction with a mobile phase of Methanol, Tetra Hydro Furan and Water in the ratio of 66:30:4 % v/v at a flow rate of 1.5 ml/min. The retention time of Lycopene was found to be 6.805 minute. The separation was performed at ambient temperature. Linearity was observed in the concentration range of 10-80µg/ml with correlation co-efficient 0.9997 and slope 78219.2.Percentage recovery obtained 99.92-100.14 %. The percentage Assay was found to be 100.51 to 102.64 %.The proposed method is precise, accurate, selective and rapid for the determination of lycopene in bulk drug and solid dosage forms. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines.

KEYWORDS: RP-HPLC; Lycopene; Validation,; Solid Dosage Forms.

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INTRODUCTION:

Lycopene is a red-colored, fat-soluble carotenoid, which gives tomatoes and several other fruits their deep red color. [1,2] The molecular structure of lycopene [3], which belongs to the carotenoids and occurs widely in nature, is shown in figure 1.Chemically, carotenes are polyunsaturated hydrocarbons containing 40 carbon atoms per molecule, variable numbers of hydrogen atoms and no other elements. Carotene is an orange photosynthetic pigment important for photosynthesis [4] .Lycopene does not have the pro-vitamin A activity [5] and its various benefits on human health can be explained based on its properties of antioxidant activity, inhibition of cancer cell proliferation, interference with growth factor stimulation, inducing phase II enzymes, regulation of transcription and restoration of gap junctions.Lycopene was very effective in the management of oral lichen planus and oxidative stress may have a role in disease pathogenesis.[6]

Although several analytical methods are available such as HPLC [7, 8,] LC-MS [9] and SFC [10], it remains unclear whether they are suitable to the analysis of lycopene in pharmaceutical solid dosage form.

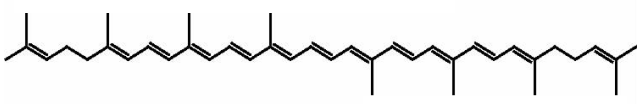

Figure 1 Molecular structure of lycopene.

We have decided to estimate Lycopene by RP-HPLC method. This paper presents simple, rapid and reproducible and an economical RP- HPLC method for estimation of Lycopene in bulk drug and pharmaceutical dosage forms. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines.[11]

MATERIAL AND METHOD

Instrumentation and Chromatographic conditions

The analysis was performed by using Inertsil ODS-3V, C-18, 150 X 4.6mm internal diameter with 5 micron particle size column and PDA detector set at 472 nm, in conjunction with a mobile phase of Methanol, Tetra Hydro Furan and Water in the ratio of 66:30:4 % v/v at a flow rate of 1.5 ml/min. The retention time of Lycopene was found to be 6.805 minute. The injection volume was 10µl.

Reagents and Solutions

Methanol, Water and Tetra Hydro Furan, Dimenthylformamide of HPLC grade and double distilled water were used in analysis.

Mobile Phase Preparation

Prepared a mixture of Methanol (HPLC Grade), Tetra Hydro Furan (HPLC Grade) and Water (HPLC Grade) in the ratio of 66:30:4 % v/v mixed and sonicated.

Preparation of standard Solution

Accurately weigh 166.66 mg (6%) beadlets of Lycopene Working Standard and transferred into a 100 ml dried volumetric flask. Dissolved with 50 ml of Dimethylformamide, sonicated for 15 minutes with occasional shaking. Added 20-30 ml of mobile phase sonicated for 5 minutes and diluted to volume with mobile phase unto 100ml. Further diluted 3ml above solution upto 10ml with mobile phase. Filter through 42 no. filter paper and inject

Procedure for analysis of tablet formulation

Weigh 10 tablets and triturate in mortar and pestle. Weigh powdered equivalent to 10 mg of Lycopene in a 100 ml dried volumetric flask. Dissolved with 50 ml of Dimethylformamide, sonicated for 15 minutes with occasional shaking. Added 20-30 ml of mobile phase sonicated for 5 minutes and diluted to volume with mobile phase. Filter the solution and further diluted 3ml above solution upto 10ml with mobile phase and inject.

Method validation

The method was validated for linearity, accuracy, intra-day and inter-day precision, robustness and ruggedness in accordance with ICH guidelines.

Linearity

Prepared a Standard stock solution of Lycopene 100 µg/ml. Several aliquots of standard solution were taken into different 10ml calibrated volumetric flasks and diluted up to mark with mobile phase such that final concentration of Lycopene was 10-80 µg/ml . Five replicates per concentration were injected and chromatograms were recorded. Evaluation was performed with PDA detector at 472 nm, peak areas were recorded for all the peaks. The peak areas show excellent correlation between peak area and concentration range. The linearity graph is shown in the Figure 2 and the value obtained was shown in table 1.

Precision

One set of three different concentrations of standard solutions of Lycopene was prepared. All the solutions were analyzed thrice in order to record any intra day variations in the results. For Inter day variations study three different concentrations of the standard solutions in linearity range were analyzed on three consecutive days. The peak area was recorded and Relative standard deviation (RSD) was calculated for both series of analyses.

Recovery studies

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100% and 150%.Each level was injected 3 times. The percentages of recoveries were calculated.

The value obtained was shown in table 2.

Ruggedness and Robustness:

In the robustness study, the influence of small, deliberate variations of the analytical parameters on retention time of the drug was examined. The following two factors were selected for change: flow rate of the mobile phase (1.5 ± 0.1 ml/min) and a wavelength at which the drugs were recorded (472 ± 2 nm). One factor at the time was changed to estimate the effect. Ruggedness of the method was determined by carrying out the assay by different analysts on different days. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust and rugged.

Limit of Detection and Limit of Quantification

LOD and LOQ were calculated as 3.3 σ /S and 10 σ /S respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

RESULT AND DISCUSSION

The percentage Assay was found to be 100.51 to 102.64 %.The proposed method was validated as per ICH parameter. Linearity of the method was found to be in the range of 10-80 µg/ml. The correlation co-efficient was found to be 0.9997 with slope 78219.2.LOD and LOQ was found to be 0.95 µg/ml and 2.88 µg/ml respectively. Precision of the proposed HPLC method was carried out by injecting replicate of six of concentration of 30 µg/ml and the %RSD for precision was found to be 0.250 for intra-day and 0.328 for Inter-day. The RSD values indicate that the proposed method had good precision. The average recovery of Lycopene was found to be 99.92-100.14 %.High percentage recovery showed that the method was free from interferences of the excipients used in the formulations. Ruggedness and Robustness test results were found to be with percentage RSD not more than 2.

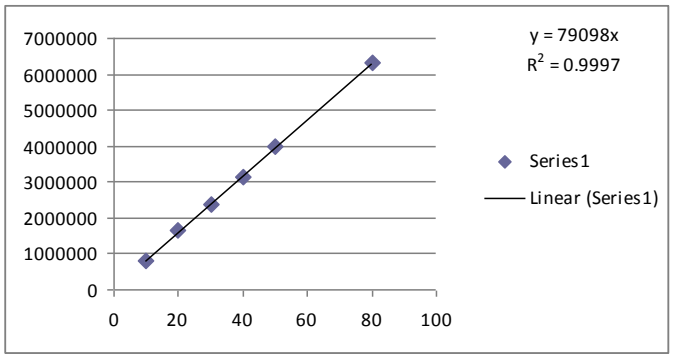

Figure 2: Linearity of Lycopene

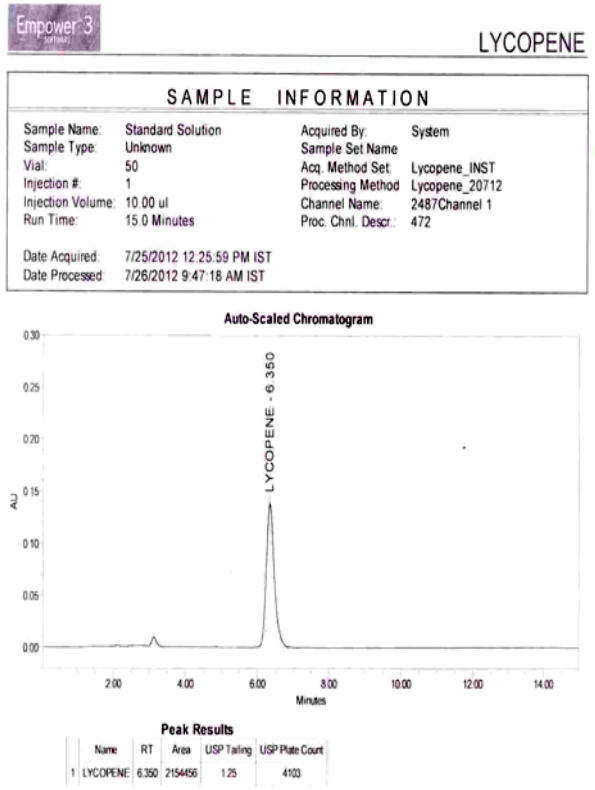

Figure 3: Chromatogram of Standard Lycopene.

Table 1: Data for Linearity of Lycopene

Sr.No.	Concentrations	Area
1	10µg/ml	826722
2	20 µg/ml	1643346
3	30 µg/ml	2383899
4	40 µg/ml	3151673
5	50 µg/ml	3969812
6	80 µg/ml	6324206

Table 2: Data for Recovery study

Level of % Recovery	Mean Recovery*	Standard Deviation	% R.S.D.
50	100.14	0.091	0.183
100	100.06	0.085	0.087
150	99.92	0.054	0.031

*Average of three determinations, R.S.D. is relative standard deviation.

Table 3: Summary of validation parameters of proposed RP-HPLC method

Sr.No.	Parameters	Value founds
1	Linearity and Range µg/ml	10-80
2	Correlation coefficient	0.9997
3	Accuracy (% Recovery)	99.92-100.14
	Precision (% RSD)*	
4	Intra- Day	0.250
5	Inter- Day	0.328
6	Ruggedness(% RSD)*	0.516
	Robustness(% RSD)*	
7	Change in Wavelength	0.015
8	Change in Flow Rate	0.031
9	LOD[a] µg/ml	0.95
10	LOQ[b] µg/ml	2.88

*All the values expressed as a mean Six Determination
[a]LOD = Limit of detection.
[b]LOQ =Limit of quantitation.

CONCLUSION

Proposed study describes a new and simple RP-HPLC method for the estimation of Lycopene. The method validated was according to ICH guidelines, it is found to be simple, sensitive, accurate and precise. Therefore the proposed method was used for the routine analysis of the pharmaceutical dosage forms.

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