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ANALYTICAL METHOD FOR ESTIMATION OF LYCOPENE (POWDER) IN MULTIVITAMIN AND MULTIMINERAL SYRUP

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ABSTRACT

An RP-HPLC method was developed and validated for the Lycopene (Powder form or water suspend able form) in Multivitamin and Multimineral Syrup. The chromatographic system was equipped with C18 a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5µm) and wavelength set at 475 nm, in conjunction with a mobile phase of Methanol, Water and Tetrahydrofuran in the ratio of 66:4:30 % v/v at a flow rate of 1.5 ml/min. The retention time of Lycopene was found to be 6 min ±1 min. The separation was performed at ambient temperature. Linearity was observed in the concentration range of 80-120% with correlation coefficient 0.9999 and slope 75145.63 Percentage recovery obtained 99.06-101.83 %. The percentage Assay was found to be 100.25 to 102.61 %.The proposed method is precise, accurate, selective and rapid for the determination of lycopene (Powder form) in Multivitamin and Multimineral Syrup. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines.

KEYWORDS

RP-HPLC, Lycopene and Validation.

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INTRODUCTION

Lycopene powder is a red-coloured 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 No.1. Chemically, carotenes are polyunsaturated hydrocarbons containing 40 carbon atoms per molecule, 56 numbers of hydrogen atoms and no other elements. Molecular weight of lycopene is

156.86. IUPAC name of lycopene is (6E, 8E, 10E, 12E, 14E, 16E, 18E, 20E, 22E, 24E, 26E) -2, 6, 10, 14, 19, 23, 27, 31-Octamethyldotriaconta -2, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30 -tridecaene and other name is Ψ, Ψ-Carotene[4]. Lycopene is insoluble in water, Methanol and Ethanol, and soluble in carbon disulfide, Chloroform, Tetrahydrofuran, Ether, Hexane and Vegetable oils[5].

Carotene is an orange photosynthetic pigment important for photosynthesis[6]. Lycopene does not have the pro-vitamin A activity[7] 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[8]. Lycopene is psi-carotene, antioxidant also used as anticancer agent[9].

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.

MATERIAL AND METHODS

Materials

Lycopene Powder Working Standard, Placebo, HPLC Water, Methanol HPLC Grade and Tetrahydrofuran HPLC Grade.

Equipments

Weighing Balance (Satorius), Sonicator and HPLC (Hitachi).

Instrumentation and Chromatographic conditions

The analysis was performed by using C18 a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5µm) and wavelength set at 475 nm, in conjunction with a mobile phase of Methanol, Water and Tetrahydrofuran in the ratio of 66:4:30 % v/v at a flow rate of 1.5 ml/min. The retention time of Lycopene was found to be 6 min ±1 min. The injection volume was 20µl. Reagents and Solutions Methanol, Water and Tetrahydrofuran of HPLC grade and double distilled water were used in analysis.

Mobile Phase Preparation

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

Preparation of Solvent Mixture

Prepared a mixture of Tetrahydrofuran: Water, (80: 20) mixed, filtered and sonicated.

Preparation of standard Solution

Transfer an accurately weighed quantity containing about 100mg of sample Lycopene to a 100 ml volumetric flask, add 20ml water and dissolve and make up the volume with tetrahydrofuran. Transfer 1ml to a 50ml volumetric flask and mix well and make up the volume with solvent mixture. Filter through a 0.45 micron membrane filter and inject.

Preparation of test solutions

Measure accurately Eq. to 1000 mcg of Lycopene in syrup and transfer to a 50 ml volumetric flask and add 7ml water and dissolve and make up the volume with tetrahydrofuran. Filter through a 0.45 micron membrane filter and inject.

Method validation

The method was validated for Precision- intra-day (Repeatability) and inter-day precision (Intermediate Precision), linearity, Accuracy, Specificity, robustness and ruggedness in accordance with ICH guidelines.

Precision

One set of 20 mcg concentrations of standard solutions of Lycopene was prepared. The solutions was analyzed in order to record any intraday variations in the results. For Inter day variations study 20 mcg concentrations of the standard solution was analyzed. The peak area was recorded and Relative standard deviation (RSD) was calculated for both series of analyses.

The result of Intraday was shown in Table No.1 and result of Inter day was shown in Table No.2.

Linearity

Prepared a Standard stock solution of Lycopene 100%. A series of concentration of 80 %, 90 %, 100 %, 110 % and 120 % solutions were prepared by dissolving sample in 20 ml water first and volume was adjusted with tetrahydrofuran. Five replicates per concentration were injected and chromatograms were recorded. Evaluation was performed with PDA detector at 475 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 No.2 and the value obtained was shown in Table No.3.

Recovery studies (Accuracy)

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to preanalyzed sample solution at three different levels 80%, 100% and 120%. Each level was injected 3 times. The percentages of recoveries were calculated.

The value obtained was shown in Table No.4.

Specificity

Is the ability to assess unequivocally the analyst in the presence of components which may be expected to be present. Typically these include matrix (placebo). To check specificity diluents, Placebo, Standard Solution, Test Solution and Standard with placebo solution were prepared and were injected and chromatograms were recorded. There are no any interference of diluents, Placebo, Standard Solution, Test Solution and Standard with placebo solution on active peak.

The results obtained were shown in Table No.5.

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 (475 ± 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.

The results obtained were shown in Table No.6.

Limit of Detection and Limit of Quantification

LOD and LOQ were calculated as 3.4 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.

RESULTS AND DISCUSSION

The percentage Assay was found to be 100.25 to 102.61 %. The proposed method was validated as per ICH parameter. Precision of the proposed HPLC method was carried out by injecting replicate of six injections of concentration of 20µg/ml and the % RSD for precision was found to be 0.84 for intra-day and 1.13 for Inter-day. The RSD values indicate that the proposed method had good precision. Linearity of the method was found to be in the range of 800-1200 µg/ml. The correlation co-efficient was found to be 0.9999 with slope 75145.63. LOD and LOQ was found to be 3.4 σ/S and 2.88 σ/S respectively. The average recovery of Lycopene was found to be 99.06-101.83%. 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.

Table No.1: Repeatability of Lycopene (Intraday)

S.No	Sample Name	Assay in mcg (Lycopene)
1	Sample – 1	1067.57
2	Sample – 2	1054.60
3	Sample – 3	1044.21
4	Sample – 4	1045.85
5	Sample – 5	1061.02
6	Sample – 6	1054.23
Mean		1054.58
Standard Deviation		8.87
RSD %		0.84

Table No.2: Repeatability of Lycopene (Inter day)

Parameters		Day: 1 Chemist : 1 Equipment: 1 Lycopene	Day: 2 Chemist : 2 Equipment: 2 Lycopene
S.No	Sample Name	Assay in mcg	Assay in mcg
1	Sample – 1	1067.57	1044.15
2	Sample – 2	1054.60	1052.75
3	Sample – 3	1044.21	1043.79
4	Sample – 4	1045.85	1071.36
5	Sample – 5	1061.02	1040.60
6	Sample – 6	1054.23	1076.04
Mean		1054.58	1054.78
Standard Deviation		8.87	15.27
RSD%		0.84	1.44
Total RSD% for 12 Samples		1.13	

Table No.3: Linearity of Lycopene

S.No	Solution	Concentration in %	Results	Mean
1	Solution 1	80%	5935.854	5935.483
			5935.111	
2	Solution-2	90%	6682.697	6683.156
			6683.614	
3	Solution-3	100%	7447.798	7429.515
			7411.231	
4	Solution-4	110%	8187.049	8192.479
			8197.909	
5	Solution-5	120%	8931.295	8938.103
			8944.910	
Linear regression coefficient				0.9999

Table No.4: % Recovery of Lycopene

S.No	% Recovery/ Concentration	Placebo in ml	Standard Weight in mg	% of Recovered
1	80	50	80	100.02
				99.35
				101.74
2	100	50	100	99.43
				100.05
				99.06
3	120	50	120	100.37
				101.83
				100.95
Mean				100.31
Standard Deviation				1.01
% RSD				1.01

Table No.5: Specificity of Method

S.No	Sample Name	Interference of the any peak with the active peak
1.	Diluents	NO
2.	Placebo	NO
3.	Standard	NO
4.	Sample	NO
5	Standard Placebo	NO

Table No.6: Robustness of Method

S.No	Change in parameter	Result in mcg	Variatio in %	% RSD
1	Actual	1062.91	---	0.11%
2	Change in flow rate	1065.55	0.12%	
3	Change in wavelength	1052.55	0.49%	

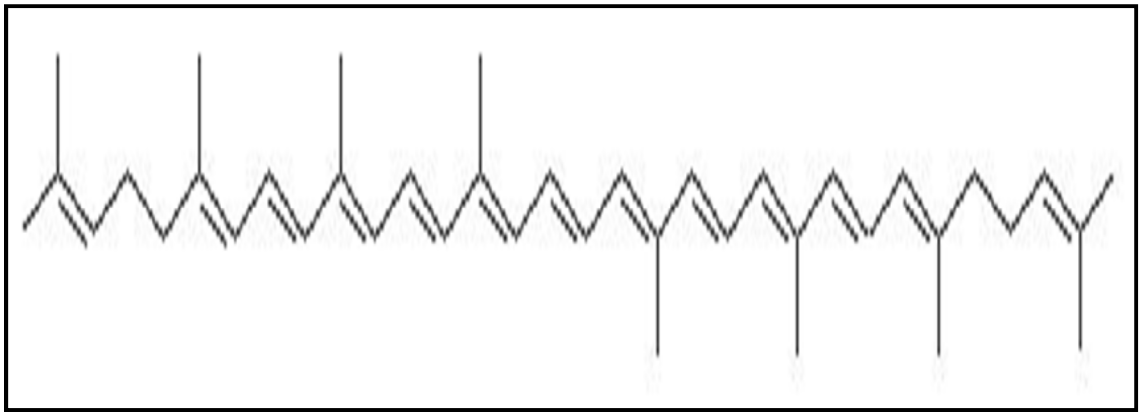

Figure No.1: Molecular Structure of Lycopene

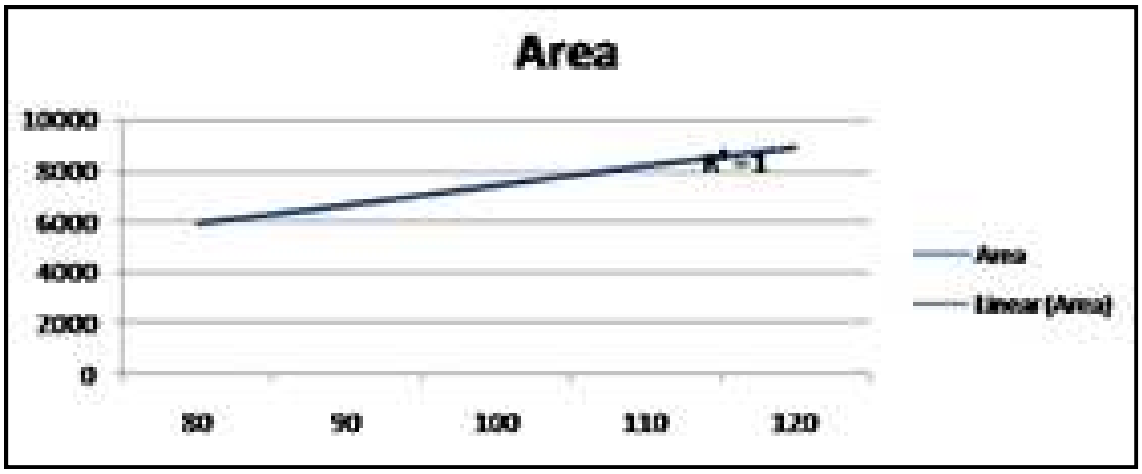

Figure No.2: Linear Regression coefficient Graph

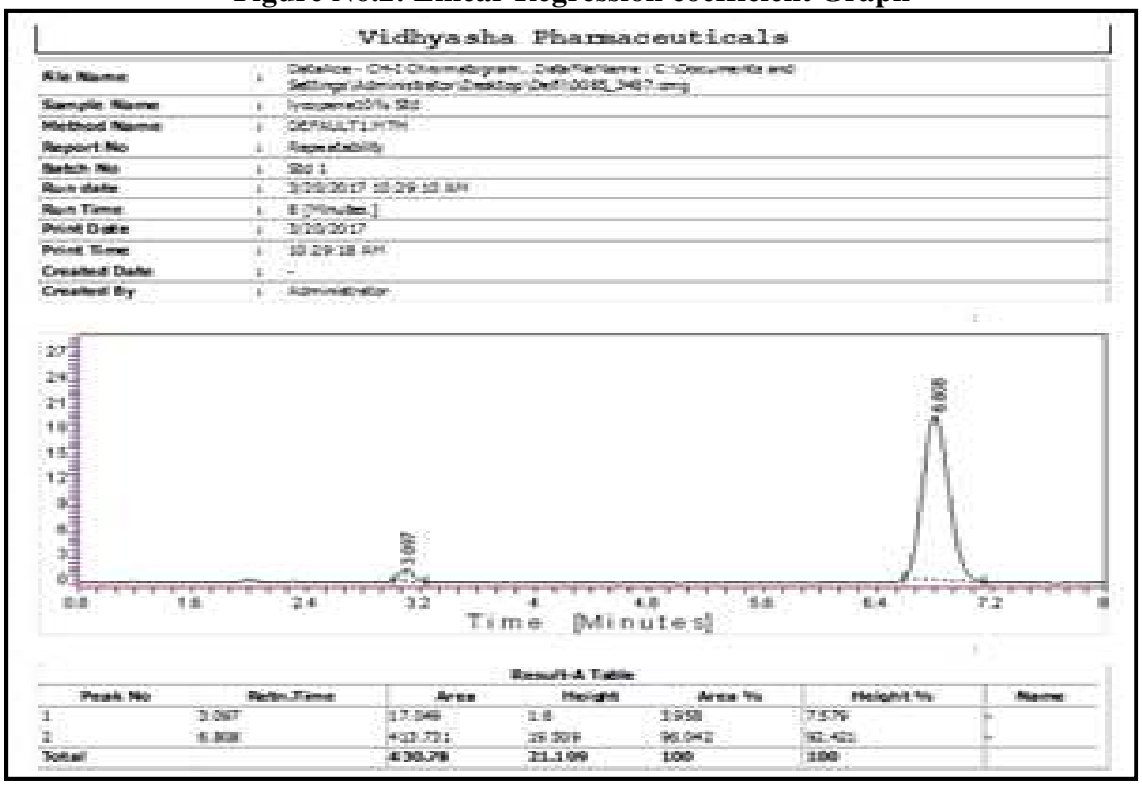

Figure No.3: Chromatogram of Standard Lycopene

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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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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