International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Analytical Method Development and Validation of Lycopene Present in Multivitamin Tablets

Anand Kumar[1], Roshan Zehra[2], Saurabh Kumar[3]

[1]Student, Innovative College of Pharmacy, Greater Noida
[2]Associate Professor, Innovative College of Pharmacy
[3]QA Manager, Grampus Laboratories, HP

ABSTRACT

Lycopene is an organic compound belonging to tetraterpene and a carotene category. Lycopene is a brick red carotenoid hydrocarbon found in tomatoes and other red fruits and vegetables. Lycopene is extracted from red fruit & vegetables and found in two physical form Oily liquid & granular solid powder form. Lycopene is also used as supplement with multivitamin formulated as tablets. An RP-HPLC method was developed and validated for the Lycopene (Powder form or water suspendable form) in Multivitamin Tablets. 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 micro particles (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 tablet. The proposed method is optimized and validated as per guidelines of WHO TRS 937 & the International Conference on Harmonization (ICH) guidelines.

Keywords: RP HPLC, Development, Analytical method Validation, Lycopene.

INTRODUCTION

The dosage forms are the physical form of dose of a pharmaceutical compound used as a medicine as prescribed by physician intended for administration or consumption. Common dosage forms are tablets, pills, capsules, syrup, aerosol, inhaler, liquid injection, dry Injection, Ointment, Lotion, Suspension. Dosage Form decides the route of administration of drug. various dosage forms may exist for a single particular drug as above mentioned, but among them solid dosage form (Tablets & Capsules) covers 80% of drugs formulations (Bankar & Anderson, 1986a). Even other dosage formulations options are available “Tablets most commonly used among all dosage forms,” Major advantages of tablets are simplicity, low cost & speed of production. (Mehta, 2002a)

TABLETS

Tablets are unit solid pharmaceutical dosage forms containing one or more than one drug substances with or without suitable diluents and prepared by either direct compression or moulding methods. A

tablet offers several advantages compared to other forms of drug preparations. These advantages include patient convenience and the stability of the drug substance within the dosage form. It is considered the unit dosage form with the highest potential for precise dosing and minimal content variability (Bankar & Anderson, 1986b). In the market, various types of tablets are available, and they can be classified based on their drug release profiles. This classification is commonly used (Mehta, 2002b).

Types of tablets:

1. Sugar-coated tablets
2. Film-coated tablets
3. Enteric-coated tablets
4. Layered tablets
5. Controlled release tablets
6. Immediate Release Tablets
7. Buccal or sublingual tablets
8. Fast dissolving / disintegrating tablets. *(Mehta, 2002c).*

The product under transfer is a film coated tablet.

1.1.02 Film Coated Tablets:

In pharmaceutical industries final appearance of product (tablets) is important for marketing point of view. There are some other reasons of film coating as to protect tablets from physicochemical damage during handling to mask smell or taste etc. The initial film coating composition employed one or more polymers, which is usually includes a plasticizers for the polymers and possibly a surfactant to facilitate spreading. ***(US-FDA, CDER, Guidance for Industry, 2015)***

The non-functional film coating process is an attractive tablet coating method when aqueous-based coating process applied. Drug like as Metoprolol Succinate having daily high dosing quantity requirements & satisfactory plasma half- life, which is the basis for selection of Immediate Release tablets. The coating basic formula of film coating is obtained from past experience or from various literatures ***(Patel, et al., 2009a).***

OBJECTIVES OF THE STUDY

To development of analytical method of Lycopene in Multivitamin tablets and its validation.

The primary goal of the current study is to establish a stable and resilient analytical method for the determination of Lycopene in Multivitamin tablets. This endeavor involves the optimization of critical variables. The overarching objective is to develop an analytical method for quantifying Lycopene in Multivitamin tablets that aligns with the predefined specifications. Quantitative testing of the active ingredient in drug substance or drug product samples or specific components within the drug product is conducted. The objective of validating an analytical method is to demonstrate its suitability for its intended purpose. A tabular summary of the characteristics relevant to identification, control of impurities and assay methods is provided. Additional analytical methods may be considered in future updates to this document.

DRUG PROFILE

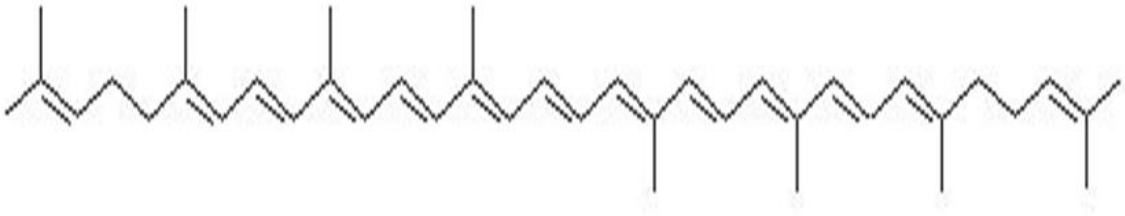
The following section gives brief description about chemical, physicochemical & pharmacological properties of Lycopene.

CHEMICAL PROPERTIES

Name of drug : Lycopene

Molecular Formula: C40H56. ***(Lycopene Monograph., 2003).***

- **Category:**Lycopene is psi-carotene, antioxidant also used as anticancer agent. ***(www.wikipedia.org).***
- **Chemical structure:** ***(www.wikipedia.org)***



- **IUPAC name:**

(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. ***(www.wikipedia.org).***

- **Other Name:**

ψ,ψ-Carotene. ***(www.wikipedia.org).***

- **CAS registry:**Lycopene- 502-65-8 ***(www.pharmatech.com).***
- **Appearance:**Deep red coloured granular powder. ***(USP-36, NF-31, 2013)***

.

PHYSICOCHEMICAL PROPERTIES

- **Molecular weight:** 536.88, ***(USP-36, NF-31, 2013).***
- **Solubility:** Lycopene is characterized by its insolubility in water, methanol, and ethanol. In contrast, it exhibits solubility in carbon disulfide, chloroform, tetrahydrofuran, ether, hexane, and various vegetable oils. ***(USP-36, NF-31, 2013).***

  Solubility behavior of drug & its salt form is most important parameter in Drug analysis and it affects *in vivo* & *in vitro* drug release profile. The BSC classification also depends on drug solubity parameter.
- **Melting point:** Lycopene having melting point between 172–173 °C (342–343 °F; 445–446 K). ***(www.wikipedia.org).***

This physicochemical property of drug substance is mainly used in identifying drug & to access effect of temperature variation on drug stability during various operations of dosage form manufacturing.

- **Loss on drying:** Not more than 0.2 % of Lycopene. ***(www.wikipedia.org).***

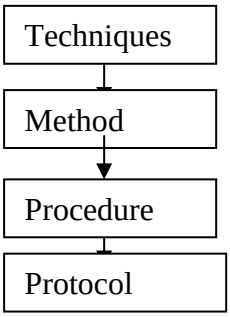
  These physicochemical properties mainly considered during wet granulation, tablets compression because it directly affects powders flow behaviors, compression, tablets coating & dissolution of tablets
- **Residue after ignition:** NMT 0.2 %. ***(www.wikipedia.org).***
- **Heavy metals:** NMT 10 µg per g. ***(www.wikipedia.org).***

- **Spectral range:** 300 to 700 nm.Ratio: A476 / A508, between 1.10 and 1.14.
-

METHODOLOGY

Pharmaceutical analysis plays an important role in quality assurance and quality control of APIs and pha rma products. The rapid growth of the pharma industry and global drug production has led to an increased demand for new testing tools in the pharmaceutical industry. ***(Corner, K.A., 2001)***.
Therefore, developing the analysis method has become an important part of the analysis.
Recent advances in analytical methods result from advances in measurement techniques.
Improvement in analytical method and improvement in measurement has reduced analysis time increased accuracy and precision and reduced analytical costs *(Ravisankar, P., et. al., 2014).*

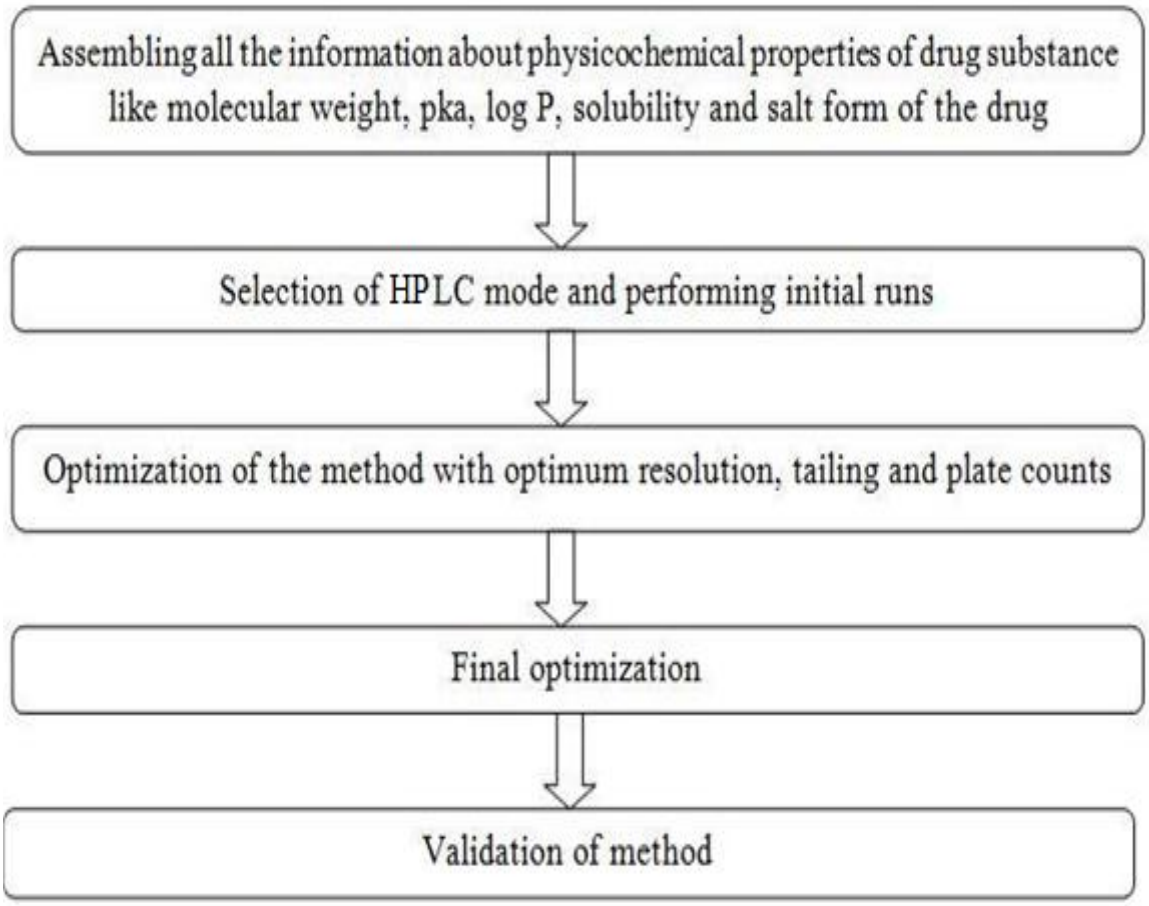
Analytical method development consists of followings:-



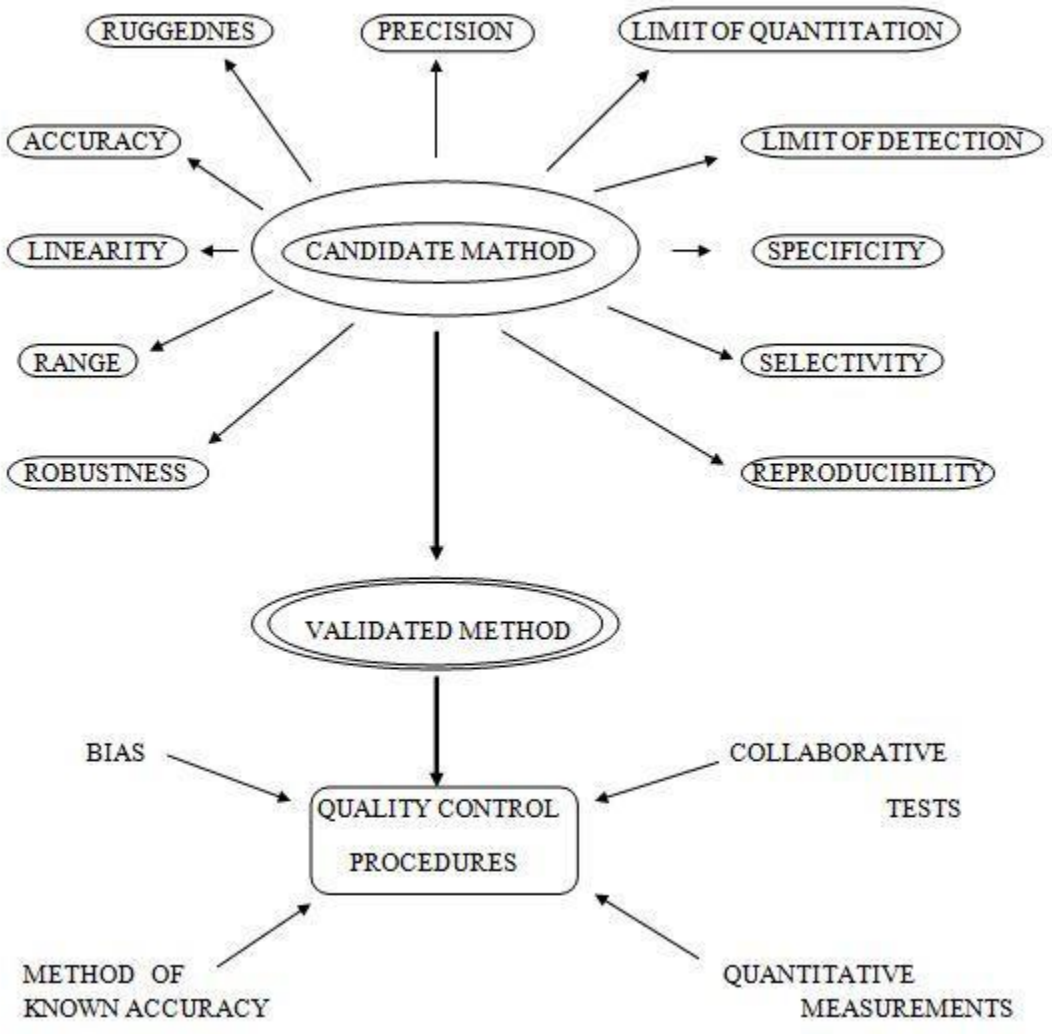
Method validation or evaluation involves the process of documenting or demonstrating that an analytical method can supply the necessary analytical data for its intended use.
The validation of an analytical method typically requires the following steps or considerations:

- Assuring quality
- Achieving acceptance of pharmaceutical products by the international agencies.
- Mandatory requirements for accreditation as per NABL ISO 17025 guidelines.
- Mandatory required documents for registration of any drug product or pesticide formulation.
- Validation methods are only acceptable for under taking proficiency testing.
- Validated analytical method undergoes quality control department for further evaluation. ***(ICH Guidelines Q2 (R1), 2005)***

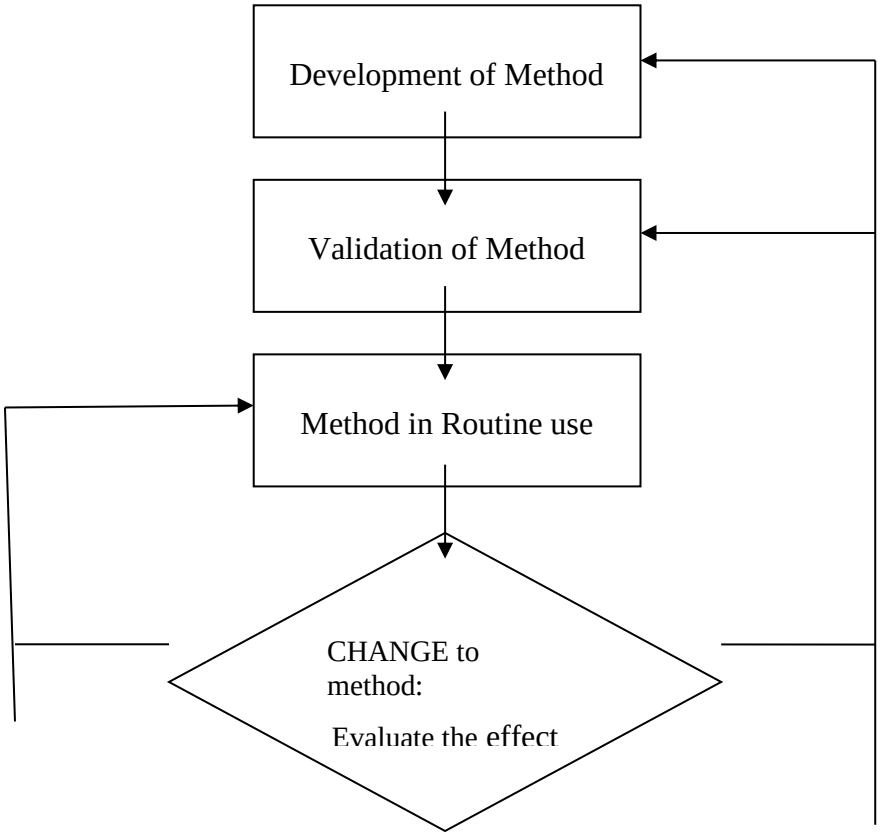
DESCRIPTION OF ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF LYCOPENE IN MULTIVITAMIN TABLET:



FLOW CHART OF METHOD DEVELOPMENT



FLOW CHART OF METHOD DEVELOPMENT



THE LIFECYCLE OF ANALYTICAL METHOD

QUIPMENT USED

The following equipment were used during tablets manufacturing process in present study.

NAME OF THE EQUIPMENT	MANUFACTURER
Electronic balance	Sartorius
HPLC	Merck-Hitachi
Sonicator	Spectralab

LIST OF EQUIPMENTS USED DURING ANALYSIS

Following an understanding of the flow chart and the equipment used for analytical method development and method validation, the subsequent section outlines the standard testing procedure for lycopene in multivitamin tablets. This procedure delineates the specifications and parameters in a stepwise manner.

STANDARD TESTING PROCEDURE

Analytical Method: Assay determine by liquid chromatography.

Solvent Buffer: Tetrahydrofuran : Water (80 : 20)

Standard solution: Weigh accurately about 100 mg of standard of lycopene and transfer to a 100 ml volumetric flask, add 20ml water and sonicate to dissolve & make up the volume with tetrahydrofuran. Transfer 1ml to a 50ml volumetric flask and make up the volume with solvent buffer. Filter through a 0.45 micron membrane filter.

Test solution: Transfer an accurately weighed quantity of crushed sample containing about 100mg of sample Lycopene to a 100 ml volumetric flask, add 20ml water and dissolve & make up the volume with tetrahydrofuran. Transfer 1ml to a 50ml volumetric flask and make up the volume with solvent buffer. Filter through a 0.45 micron membrane filter.

Chromatography system:

Column: -C18 a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5µm).

Mobile phase: Prepare a suitable filtered mixture of methanol : water : Tetrahydrofuran, (660 : 40 : 300).

Flow Rate: 1.5 ml per minute.

Injection Volume: 20µl.

Wave length: 475 nm.

The resolution, R of Lycopene peaks should not less than 3.5; the column efficiency determined from each analyte peak should not less than 550 theoretical plates; the tailing factor for each analyte peak should not more than 1.5; and the RSD for replicate injections Should not more than 2.0%.

Procedure: Separately inject equal volumes (about 20µl) of the standard solution and the sample solution into HPLC to obtain the chromatograph, record the chromatograms, and measure the responses for the major peaks. The average retention times are about 5 min for Lycopene. Calculate the quantity of Lycopene, in mcg, in each tablet.

Calculation:

$$\text{Lycopene (in mg)} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{1}{50} \times \frac{100}{WT} \times \frac{50}{1} \times \frac{P}{100} \times 100 \times \text{Avg. Weight}$$

Where,

AT= Test Area

AS= Standard Area

WS= Weight of standard in mg.

WT= Measure of Sample in ml.

P = Potency of Standard.

Mechanism of action

Lycopene possesses the capacity to mitigate cell damage inflicted by free radicals generated through reactive oxygen species. It functions as a crucial antioxidant, both in vitro and in humans, by reducing the vulnerability of lymphocyte DNA to oxidative harm. Lycopene also deactivates hydrogen peroxide and nitrogen dioxide, shielding lymphocyte membranes from damage caused by nitrogen oxide. Furthermore, it serves to protect against cell death, offering protective effects comparable to those of beta-carotene.

Uses of Lycopene

Used as antioxidant. **(*Cantuti, C.I., et. al., 2000*).**

Lycopene had anti-carcinogenic activities in mammary gland, liver, skin, prostate, lung, bladder, ovaries, colon, and pancreas. ***(Sharoni, Y., et. al. 2002; Schunemann, H.J., et. al., 2002; Sook-Lee, H., et. al., 2003; Kitade, Y. et. al., 2002).***

Lycopene used for preventing heart disease, "hardening of the arteries (atherosclerosis). ***(Rao, A. V., 2002).***

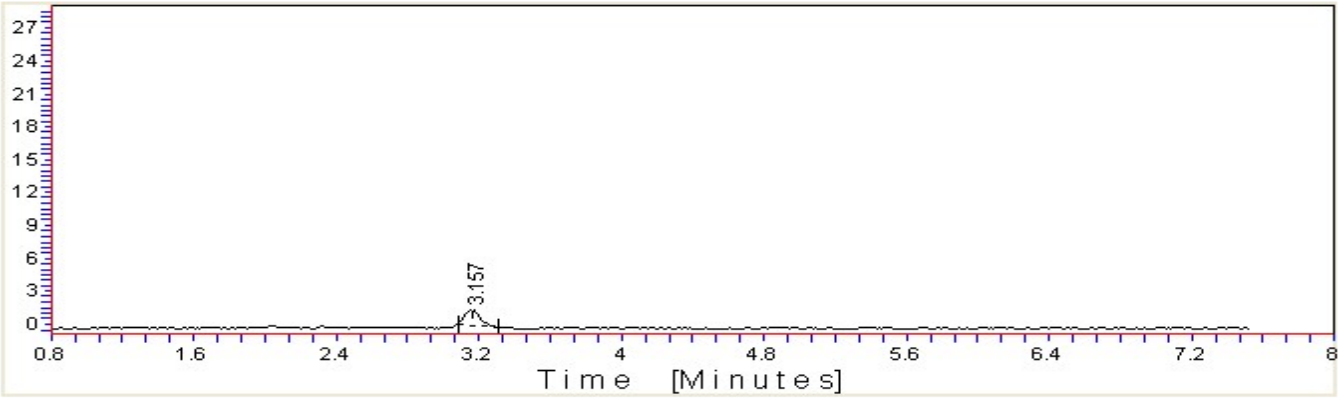
Use of lycopene appeared to minimize risk of preeclampsia, a dangerous complication of pregnancy. In addition, use of lycopene appeared to prevent inadequate growth of the foetus. ***(Sharma, J.B., et. al.,2003; Stahl, W., & Sies, H., 1996).***

Lycopene is also commonly used in treatment of human papilloma virus (HPV) infection, which is a main cause of uterine cancer. ***(Sedjo, R.L., et. al., 2002).***

Some people also use lycopene for cataracts and asthma. **(Gerster, H., 1997).**

Result: The Chromatogram obtained in Repeatability test are following:

GRAMPUS LABORATORIES		
File Name	**:**	DataAce - CH-I Chromatogram...DataFileName : C:\Documents and Settings\Administrator\Desktop\Delfi\0085_3484.cmg
Sample Name	**:**	Precision of lycopene10% Blank
Method Name	**:**	DEFAULT1.MTH
Report No	**:**	Repeatability
Batch No	**:**	Blank
Run date	**:**	3/20/2023 1:54:10 AM
Run Time	**:**	7 [Minutes.]
Print Date	**:**	3/20/2023
Print Time	**:**	1:55:46 AM
Created Date	**:**	-
Created By	**:**	Administrator



Result-A Table						
Peak No	**Retn.Time**	**Area**	**Height**	**Area %**	**Height %**	**Name**
1	3.157	13.5	1.329	100	100	-

Total		**13.5**	**1.329**	**100**	**100**	

PerformanceB Table										
Pk.No	**RT**	**Asymmetry**	**Tailing**	**Capacity**	**Efficiency**	**Efficiency/L**	**Resolution**	**Selectivity**	**HETP**	**RRT**
1	3.157	2.25	1.4	2.157	27272.541	27272.541	Nil	2.761	0.367	2.157

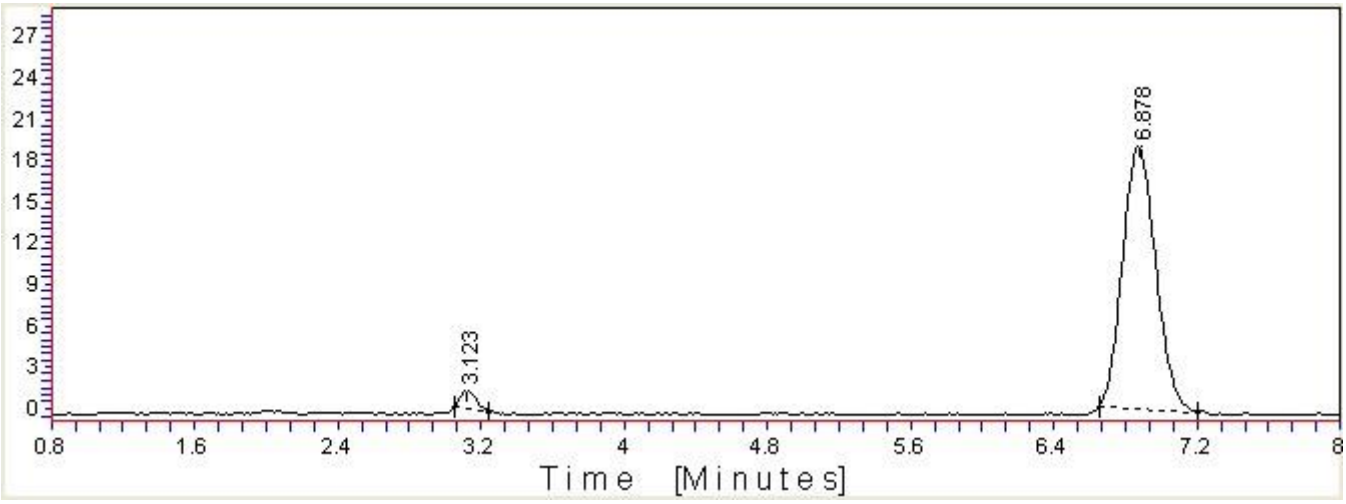
Date

Signature

GRAMPUS LABORATORIES

File Name	**:**	DataAce - CH-I Chromatogram...DataFileName : C:\Documents and Settings\Administrator\Desktop\Delfi\0085_3485.cmg
Sample Name	**:**	Precision of lycopene10% Std 1
Method Name	**:**	DEFAULT1.MTH
Report No	**:**	Repeatability
Batch No	**:**	Std 1
Run date	**:**	3/20/2023 2:06:20 AM
Run Time	**:**	11 [Minutes.]
Print Date	**:**	3/20/2023
Print Time	**:**	2:06:49 AM
Created Date	**:**	-
Created By	**:**	Administrator

:



:

Result-A Table						
Peak No	**Retn.Time**	**Area**	**Height**	**Area %**	**Height %**	**Name**

1	3.123	13.484	1.35	3.172	6.594	-
2	6.878	411.559	19.124	96.828	93.406	-
Total		**425.043**	**20.474**	**100**	**100**	

PerformanceB Table

Pk.No	RT	Asymmetry	Tailing	Capacity	Efficiency	Efficiency/L	Resolution	Selectivity	HETP	RRT
1	3.123	1.75	1.2	2.123	26688.331	26688.331	Nil	2.769	0.375	2.123
2	6.878	1.498	1.249	5.878	129422.29	129422.29	49.228	Nil	0.077	5.878

Date

Signature

CONCLUSION

An analytical method for the quantification of lycopene in a multivitamin tablet was developed and validated within the laboratory. This study followed a systematic approach, wherein critical parameters were fine-tuned to create a reliable and resilient analytical method.

This project encompasses the development of an analytical method, which was established at Grampus Laboratories, Trilokpur Road, Kala Amb, (HP). The data generated through method development underwent thorough analysis to assess its suitability. Furthermore, the method's feasibility was confirmed using the existing facilities and equipment. The study also delved deeply into critical process variables during method validation. Firstly we were determined the solubility of lycopene powder because without determining the solubility the analysis is impossible and we found the lycopene powder is suspendable in water and soluble in tetrahydrofuran. Then we were determined the UV wavelength at the lycopene shows maximum absorption by scanning the lycopene solution in tetrahydrofuran at different wavelength and we found that maximum absorption of lycopene at 475 nm. The selection of mobile phase is also very necessary and by experiment this is found that filtered mixture of methanol : water : tetrahydrofuran, (660 : 40 : 300) is suitable for optimum separation.

The choice of column is a crucial parameter. After experimenting with both C18 and C8 columns, it was determined that the C18 column, specifically a stainless steel column measuring 30 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5µm), yielded the best results.

Then by experimental data it is found that on the flow rate 1.5 ml and injection volume 20 µl shows maximum result.

Then this method is validated on following parameter:

- Precision
- Repeatability
- Linearity
- Accuracy

- Specificity
- Robustness

The method's stability was confirmed through validation and ultimately, it was successful in accurately determining the lycopene content within the multivitamin tablet.

In summary, the outcome of this project indicates that this method is suitable for the analysis of lycopene content within multivitamin tablets.

REFERENCES

1. Agalloco, J., Carleton J., (2008), Validation of Pharmaceutical process. Why Validation. *Informa Healthcare: NY*, 3rd Edition, P.P.-3-14.
2. Arias, R., Lee, T.C., Logendra, L., Janes, H., (2000), Correlation of lycopene measured by HPLC with the L*, a*, b * colour readings of a hydroponic tomato and the relationship of maturity with color and lycopene content. *J. Agric. Food Chem.* 48, 1697-1702.
3. Adsule, P.G., Dan, A., (1979), Simplified extraction procedure in the rapid spectrophotometric method for lycopene estimation in tomato, *J. Food Sci. Technol*., 16, 216-216.
4. D'Souza, M.C., Singha, S., Ingle, M., (1992). Lycopene content of tomato fruit can be estimated from chromaticity values. *Hort Science* 27, 465-466.
5. Dr. Chauhan Komal et.al, (2011) Lycopene of tomato fame: its role in health and disease, *International Journal of Pharmaceutical Sciences Review and Research*, 47, 951-955.
6. Guidance for Industry, Q2 (R1) Validation of Analytical Procedures: Methodology, (2005), U.S. Department of Health and Human Services, Food and Drug Administration, Centre for Drug Evaluation and Research (CDER), Centre for Biologics Evaluation and Research (CBER), *International Conference on Harmonizatio*n, Geneva, P.P.-623-635.
7. Konings, E.J.M., & Roomans, H.H.S. (1997). Evaluation and validation of an LC method for the analysis of carotenoids in vegetables and fruit. *Food Chemistry*, 59, 599–603.
8. US FDA, Technical Review Guide: Validation of chromatographic Methods, 1993, P.-726-731.
9. Van-Breemen, R. B., Xu, X. Y., Viana, M. A., Chen, L. W., Stacewicz-Sapuntzakis, M., Duncan, C., et al. (2002). Liquid chromatography- mass spectrometry of cis- and all-trans-lycopene in human serum and prostate tissue after dietary supplementation with tomato sauce. *Journal of Agricultural and Food Chemistry*, 50, 2214–2219.
10. www.wikipedia.org
11. www.pharmatech.com
12. Remington, J.P.; Beringer, P., (2005), The Science and practice of pharmacy, *Lippincott Williams & Wilkins, Washington*, 21st edition, P-754-757.
13. Ravisankar, P., Gowthami, S., Devlala-Rao, G., (2014), A review on analytical method development, *Indian Journal of Research in Pharmacy and Biotechnology*, 15, 1183-1193.
14. Rao, A.V., & Honglei, S., (2002). Effect of low dose lycopene intake on lycopene bioavailability and oxidative stress. *Nutrition Research*, 22(10), 1125–1131.
15. Rane, M., Parmar, J., (2009). Tablet Formulation design and Manufacturing: Oral Immediate Release Application. *Pharma Times*, 41, 21-24.
16. Rao, A. V., & Agarwal, S. (2000). Role of antioxidant lycopene in cancer and heart disease. *Journal of the American College of Nutrition*, 19, 563–569.

17. Rao, A. V. (2002). Lycopene, tomatoes, and the prevention of coronary heart disease. *Experimental Biology and Medicine*, 227(10), 908–913.
18. Richelle, M., Bortlik, K., Liardet, S., Hager, C., Lambelet, P., Baur, M., et al. (2002). A food-based formulation provides lycopene with the same bioavailability to humans as that from tomato paste. *Journal of Nutrition*, 132, 404–408.