

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF LYCOPENE IN MULTIVITAMIN & MULTIMINERALS SYRUP

Authors Name:¹Vijay Chandel ²Dr. Ishab Kumar
 SBS Polytechnic and Pharmacy College, Patti, India
Corresponding E-Mail: vijay1986chandel@gmail.com
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Abstract	<i>The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formulae for the calculation. Validation protocol is very necessary in which the objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated. Analytical method of lycopene present in multivitamin & multimineral syrup was developed and validated in lab. This study was carried out through a systematic plan; critical parameters were optimized to produce a stable & robust analytical method. This project involves analytical method development, The data provided by Method development was studied extensively to understand the eligibility of method also verified feasibility of method on available sets of facilities and equipments. The critical process variables studied extensively during method validation. The stability of this method was validated and at last this is found that the content of lycopene present in multivitamin & multimineral syrup was successfully determined.</i>
Keywords	<i>lycopene. Analytical Method development, validation, syrup</i>

INTRODUCTION

“A dosage form is the physical form of a dose of a chemical compound used as a drug or medication intended for administration or consumption.”

Common dosage form includes tablets, pill, capsules, syrup, aerosol, inhaler, liquid injection. The route of administration for drug delivery is dependent on the dosage form of substance.

SYRUPS

“Syrups may be defined as Liquid pharmaceutical oral dosage forms containing drug substances made by dissolving sugar in water or glycerine or Sorbitol with or without excipients and flavours”. Syrup has numerous advantages over other dosage form, among which are patient convenience of a drug substance in a drug dosage form. Syrup is better for patients who have trouble swallowing. Syrup has faster absorption than solid and semisolid dosage forms. Syrup has more flexibility in achieving the proper dosage of the medication. There are various types of syrups are available in market amongst them commonly used types of syrup classified as per their drug & base of syrup are as follows (Mehta, R.M., 2002).

Multivitamin and Multimineral syrup is widely used to treatment of weakness and also used as nutraceuticals. Lycopene is widely used in the composition of multivitamin and Multimineral syrup.

- SUGAR → MIXING ← WATER
- EXCIPIENTS → MIXING ← PRESERVATIVES
- ACTIVE INGREDIENTS → MIXING ← FLAVOURS
- Ph ADJUSTMENTS
- MIXING
- SYRUP
- FAIL AND REJECTED
- SAMPLING FOR ANALYSIS → ANALYSIS
- PASS AND APPROVED



PACKING

Fig. No. 1.01: Syrups Manufacturing Flow Chart.

ANALYTICAL PROCEDURE

The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formulae for the calculation, etc.

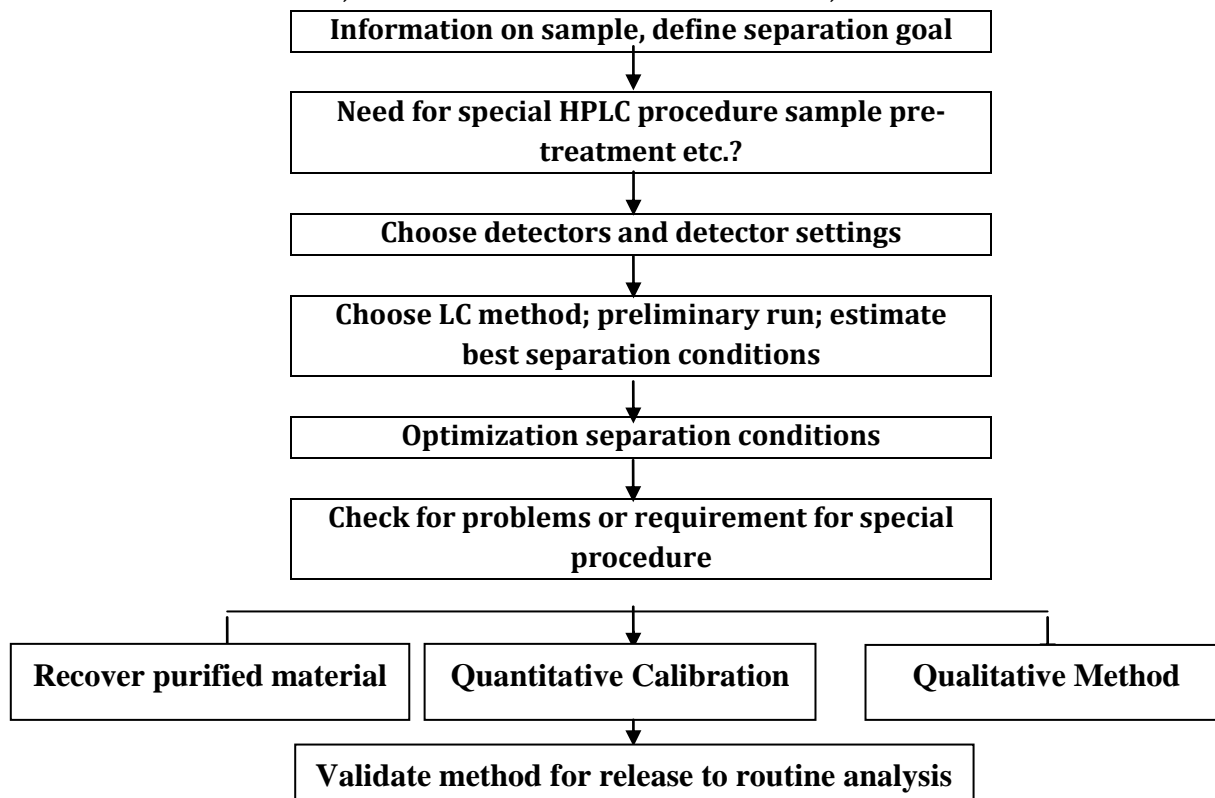


Fig. No.: 1.02 STEPS IN HPLC METHOD DEVELOPMENT

VALIDATION PROTOCOL & REPORT

As US FDA defines validation protocol is a written stating how validation will be conducted, including test parameters, product characteristics, production equipment& decision points on what constitutes the acceptable test results.

The validation results are documented in a Analytical Method validation report (AMVR). The validation report should include, a description of the process, and detailed summery of the results obtained form in process and final testing. The current project involves international technology transfer of tablets manufacturing process.

Typical validation characteristics which should be considered are listed below:

- Accuracy
- Precision
- Repeatability
- Intermediate Precision
- Specificity
- Detection Limit
- Quantization Limit
- Linearity
- Range

Validation should be performed in accordance with the validation protocol. The protocol should include procedures and acceptance criteria for all characteristics. The results should be documented in the validation report.

Standard test methods should be described in detail and should provide sufficient information to allow properly trained analysts to perform the analysis in a reliable manner.

As a minimum, the description should include the chromatographic conditions (in the case of chromatographic tests), reagents needed, reference standards, the formulae for the calculation of results and system suitability tests.

OBJECTIVES OF THE STUDY

The objective of present study is to develop a stable and robust analytical method for Lycopene present in multivitamin & Multiminerals syrup. Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product is done. The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. A tabular summation of the characteristics applicable to identification, control of impurities and assay procedures is included. Other analytical procedures may be considered in future additions to this document.

DRUG PROFILE

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

CHEMICAL PROPERTIES

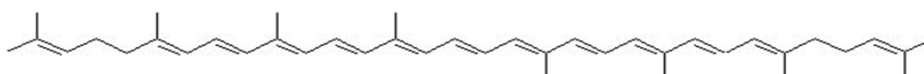
(Brennall, A. E., Clarke, G. S., 1998; EP 2006).

Name of drug :Lycopene

Molecular Formula: C₄₀H₅₆

Category:Lycopene is psi-carotene, antioxidant also used as anticancer agent.

Chemical structure:



IUPAC name:

(6E,8E,10E,12E,14E,16E,18E,20E,22E,24E,26E)-2,6,10,14,19,23,27,31--Octamethyldotriacont-2,6,8,10,12,14,16,18,20,22,24,26,30-tridecaene.

Other Name:ψ,ψ-Carotene

MATERIAL AND METHODS

The following section briefly explains materials, equipment's & standard testing procedure used for the analysis of Lycopene.

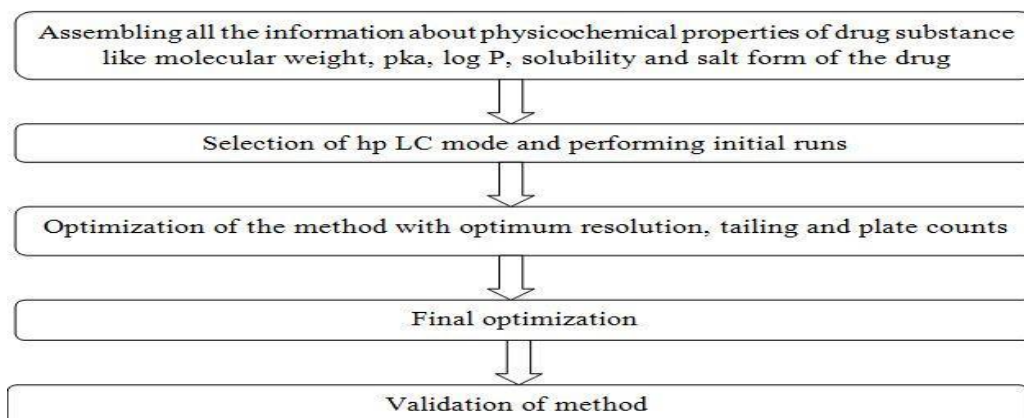


Figure.1. Flow chart of method development

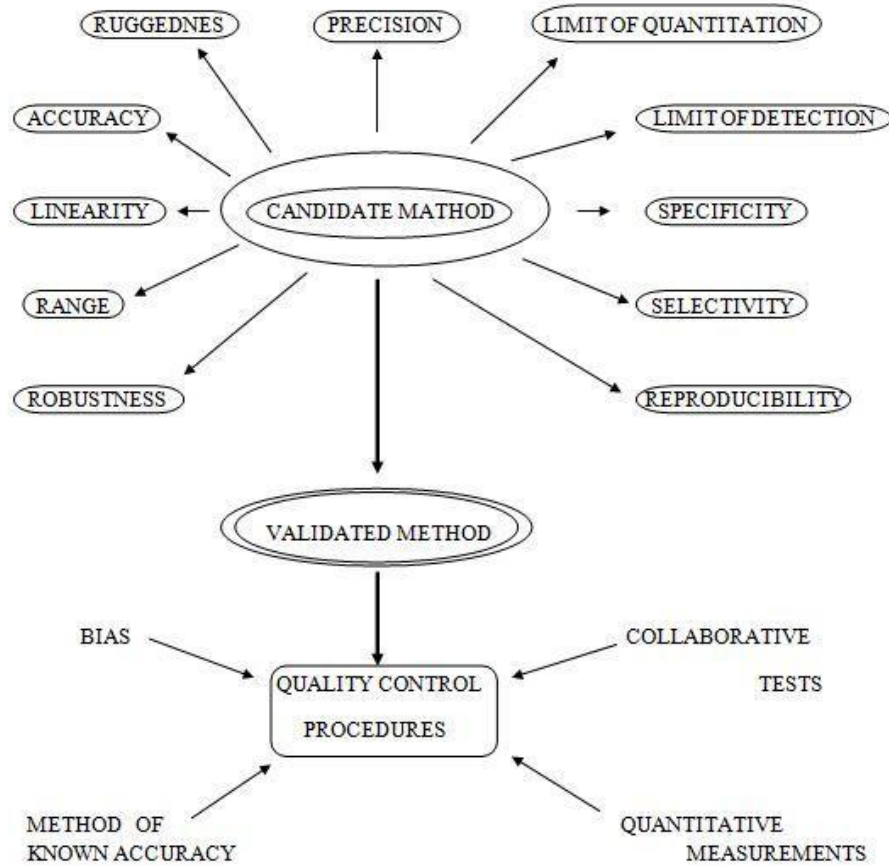


Figure.2. Flow chart of method validation

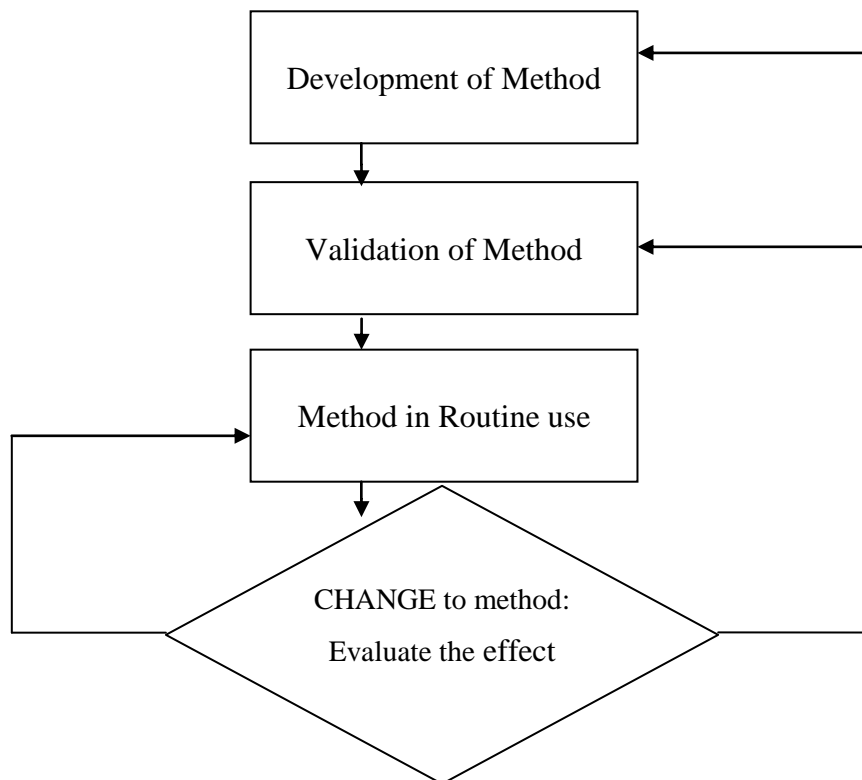


Figure.2. The Lifecycle of Analytical Method

After understanding flow chart & equipments used for analytical method development & method validation the following section explains standard testing procedure of lycopene in multivitamin &

multiminerals syrups. The specifications and parameters were given stepwise.

STANDARD TESTING PROCEDURE

Analytical Method:

Assay: Determine by liquid chromatography.

Solvent Buffer: Tetrahydrofuran: Water, (80: 20).

Standard solution: Transfer an accurately weighed quantity 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 mix well and make up the volume with solvent buffer. Filter through a 0.45-micron membrane filter.

Test solution: Transfer 15 ml to a 50 ml volumetric flask and add 7ml water and dissolve & make up the volume with tetrahydrofuran. 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 micro particles (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 is not less than 3.5; the column efficiency determined from each analyte peak is not less than 550 theoretical plates; the tailing factor for each analyte peak is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

PROCEDURE

Separately inject equal volumes (about 20µl) of the standard preparation and the assay preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 5 for Lycopene. Calculate the quantity, in mcg, of Lycopene in each ml of the Lycopene, Multivitamin & Multiminerals Syrup taken by the formula.

CALCULATION

$$\text{Lycopene (in mg)} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{1}{50} \times \frac{50}{WT} \times \frac{P}{100} \times 15 \times 100$$

RESULTS AND CONCLUSION

Analytical Method Development of Lycopene:

Solubility of Lycopene:

Solubility is the main parameter in case of analytical method Development by HPLC.

The result of solubility is given below:

Solvent	Solubility Result
Water	Insoluble
Methanol	Insoluble
Ethanol	Insoluble
Carbon disulfide	Soluble
Chloroform	Soluble
Tetrahydrofuran	Soluble
Ether	Soluble
Hexane	Soluble
Vegetable oils.	Soluble

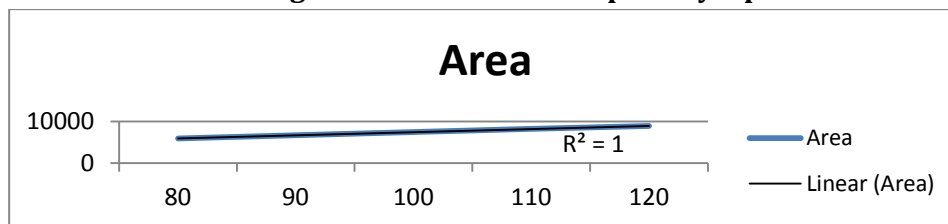
Mobile phase: Prepare a suitable filtered mixture of methanol: water: Tetrahydrofuran, (660: 40 : 300) is suitable for optimum separation.

Validation, Precision

Repeatability: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same Homogeneous sample under the prescribed conditions. It comes within prescribed limit under 2%RSD

Linearity: The linearity of an analytical procedure is its ability (within a given range) to obtain test Results which are directly proportional to the concentration (amount) of analyst in the sample (Range of 80% to 120 %). Each concentration to be analyzed as duplicates.

Linear Regression coefficient Graph of Lycopene



Accuracy: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Each concentration to be analyzed as triplicates.

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

Acceptance criteria: Recovery at all concentration level should be within 98.0% to 102% and RSD should not be more than 2.0%

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).

Sr. 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

Acceptance Criteria: There should not be any interference due to placebo in sample and standard preparation

Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal range.

Sr. No.	Change in parameter	Result in mcg	RSD	Variation in %
1	Actual	1062.91	0.11%	31.48%
2	Change in flow rate	1065.55		
3	Change in Column	1052.55		

CONCLUSION

Analytical method of lycopene present in multivitamin & multiminerals syrup was developed and validated in lab. This study was carried out through a systematic plan; critical parameters were optimized to produce a stable & robust analytical method. This project involves analytical method development, which been developed within Radisson Pharmaceutical Jharmajri Baddi (HP). The data provided by Method development was studied extensively to understand the eligibility of method also verified feasibility of method on available sets of facilities and equipment's. The critical process variables studied extensively during method validation.

The stability of this method was validated and at last this is found that the content of lycopene present in multivitamin & multiminerals syrup was successfully determined.

The overall result of this project is this method can be used for analysis of lycopene present in multivitamin and multiminerals syrups.

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