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Analytical method development and validation of Carotene in multivitamin & Multiminerals syrup

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Abstract
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 Carotene 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, 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 Carotene present in multivitamin & Multiminerals syrup was successfully determined.

Keywords: Carotene analytical method development, validation, syrup

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".
Syrups 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 Multiminerals syrup is widely used to treatment of weakness and also used as nutraceuticals. Carotene is widely used in the composition of multivitamin and Multiminerals syrup.

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.

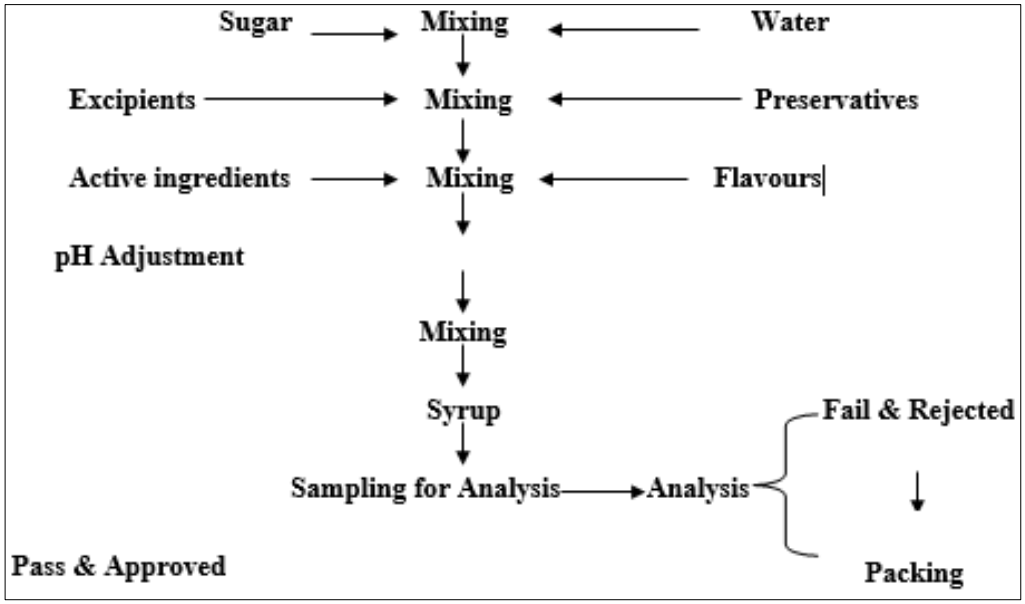

Fig 1: Syrups manufacturing flow chart.

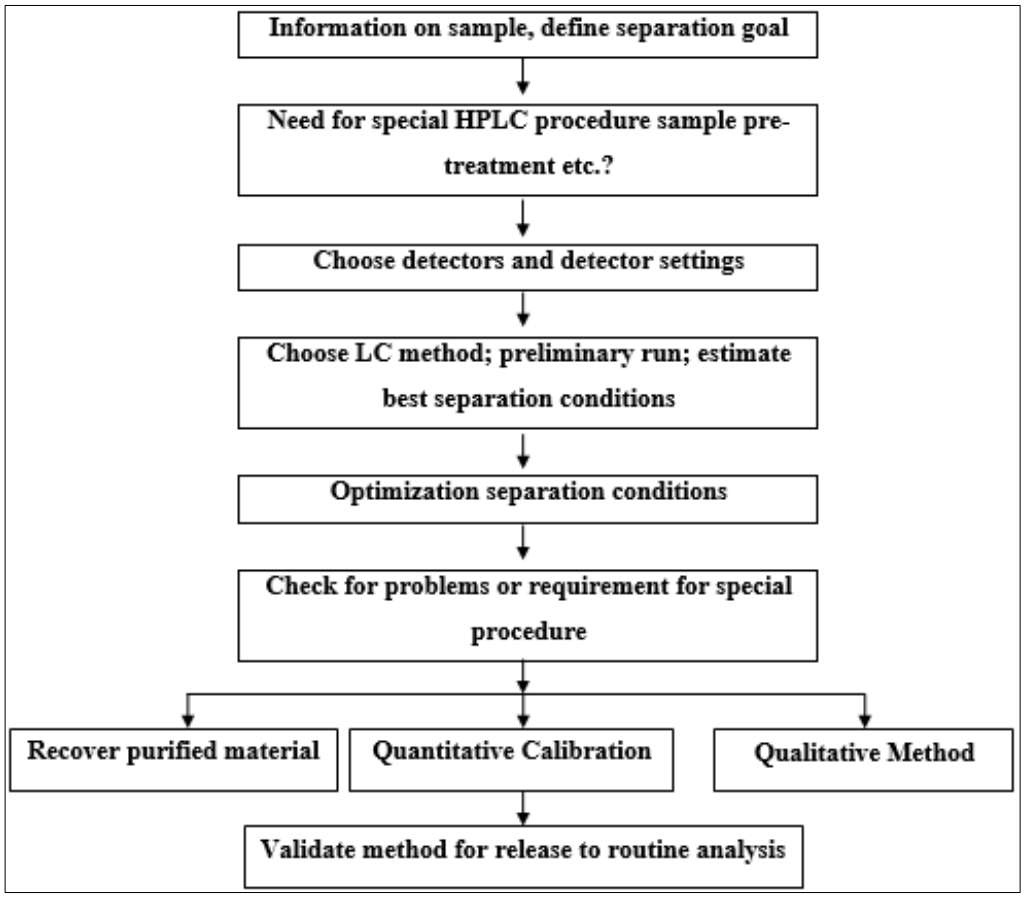

Fig 2: Steps in HPLC method development

Validation protocol & report

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

The validation results are documented in an 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 Carotene present in multivitamin & Multiminerals syrup. The critical variables were optimized. The present study was aimed to produce analytical method for Carotene present in multivitamin &Multiminerals syrup meeting their predetermined specification. 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 Carotene.

Chemical Properties (Bretnall, A. E., Clarke, G. S., 1998; EP 2006).

Name of drug: Carotene

Molecular Formula: C40H56

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

Chemical structure

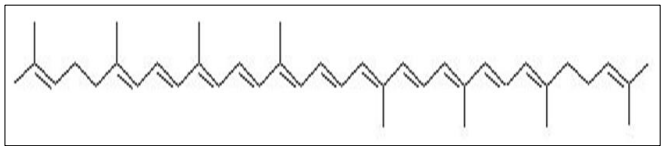

Fig 3: Chemical structure

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.

Other Name
ψ, ψ-Carotene

Material and Methods
The following section briefly explains materials, equipments & standard testing procedure used for the analysis of Carotene.

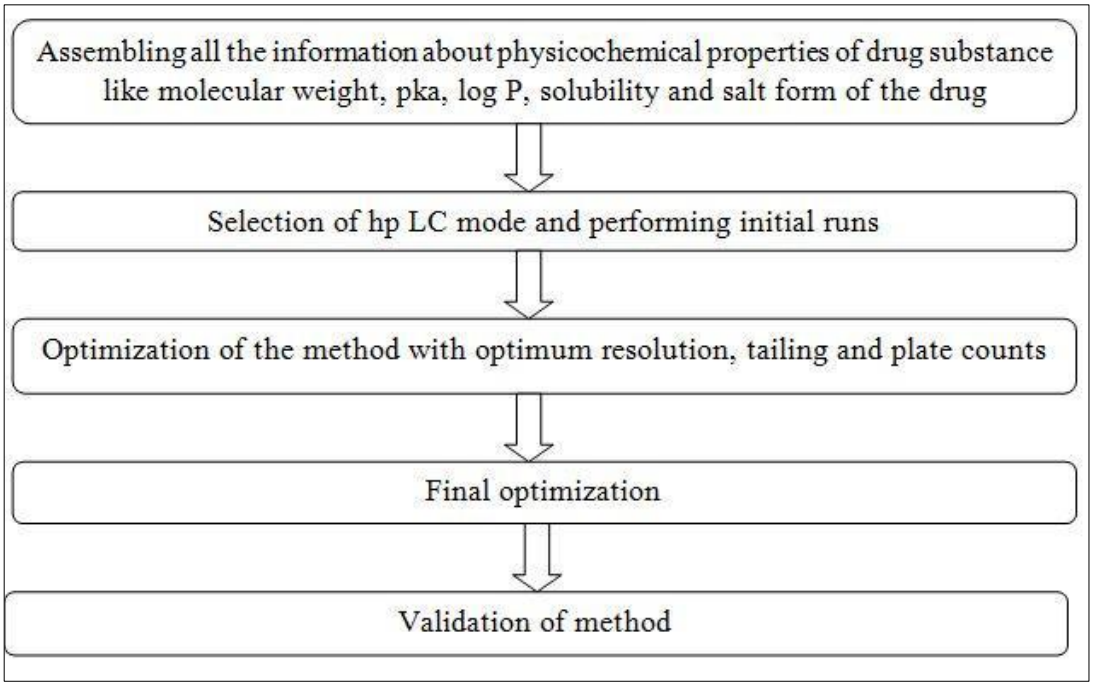

Fig 4: Flow chart of method development

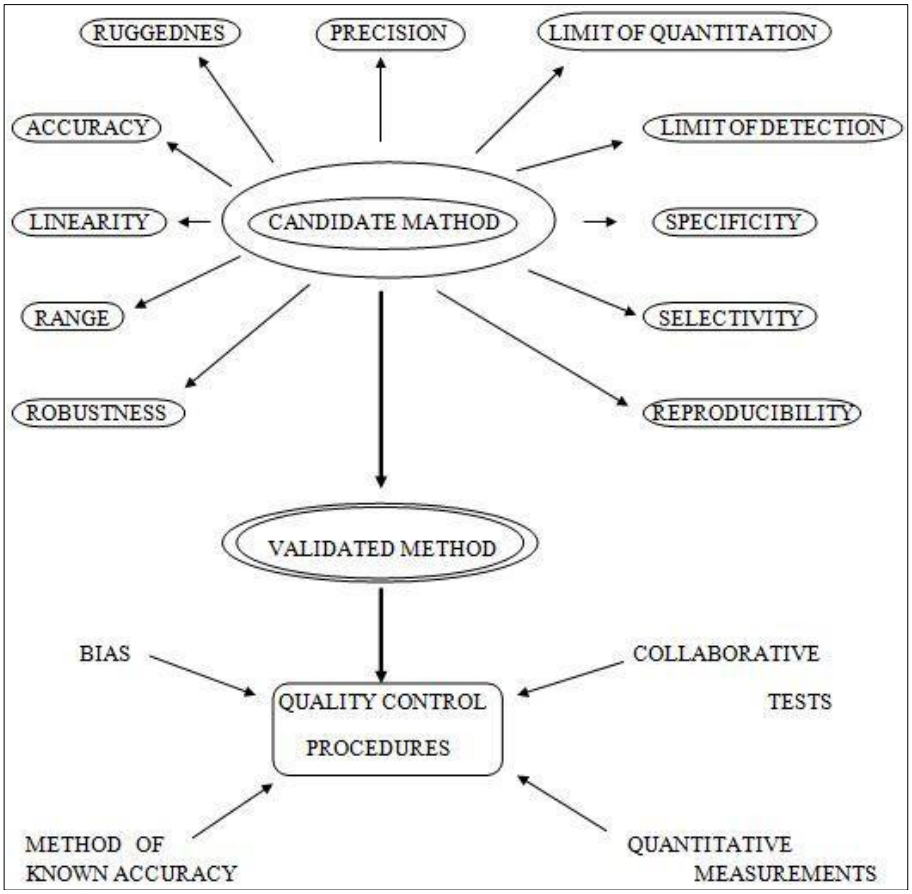

Fig 5: Flow chart of method validation

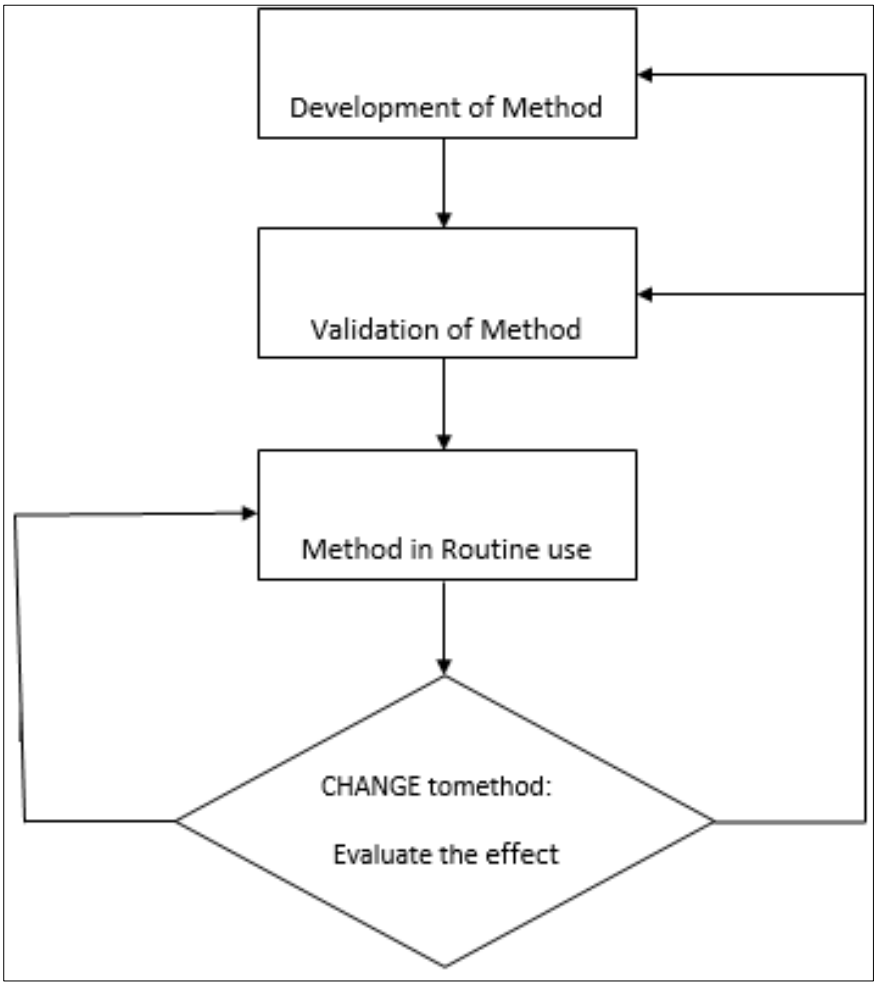

Fig 6: The Lifecycle of Analytical Method

After understanding flow chart & equipments used for analytical method development & method validation the following section explains standard testing procedure of Carotene 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 Carotene 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 orceramic 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 Carotene peaks is not less than 3.5; the column efficiency determined from each analyte peak is not less than 550theoretical 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 Carotene. Calculate the quantity, in mcg, of Carotene in each ml of the Carotene, Multivitamin &Multiminerals Syrup taken by the formula.

Calculation

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

Results and Conclusion
Analytical Method Development of Carotene
Solubility of Carotene
Solubility is the main parameter in case of analytical method Development by HPLC.

Table 1: 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.

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 with in 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.

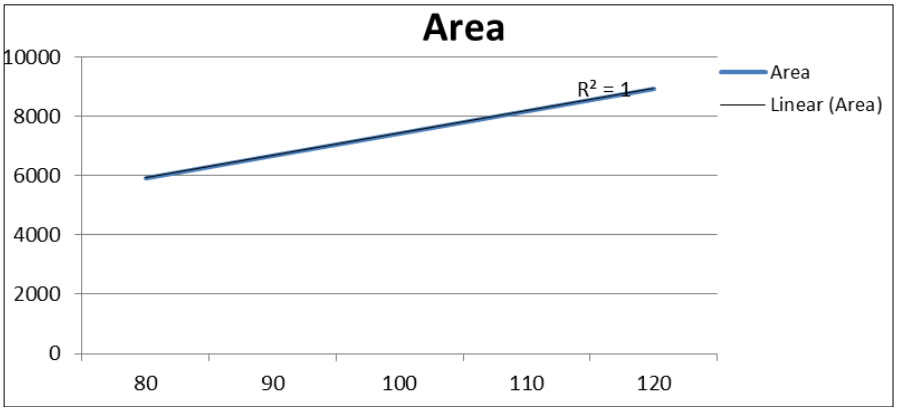

Fig 7: Linear Regression coefficient Graph of Carotene

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.

Table 2: Shows the recovery and concentration

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).

Table 3: Shows sample name and Interference of the any peak with the active peak

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.

Table 4: Shows Change in parameter Result in mcg RSD and its variation

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 Carotene 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 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 Carotene present in multivitamin & Multiminerals syrup was successfully determined.

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

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