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METHOD DEVELOPMENT OF VITAMIN 'C' ON RP-HPLC

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ABSTRACT

Few spectraphotometric and chromatographic methods have been reported for the estimation of Vitamin C in bulk and tablet dosage form. But the there is not much more methods for assay of effervescent tablet dosage form. The following method had been developed for estimation of Vitamin C in bulk drug and effervescent formulation. Development and Validation of RPHPLC method for the estimation of Vitamin C in Bulk Drug and Dosage Form. A new RP-HPLC method was developed for assay of Vitamin C in bulk drug and formulation. The separation was achieve on C18 ODS (Hypersil), 250 nm x 4.6 mm, 5 µ using Buffer and Methanol (90:10) use as a mobile phase for estimation of Vitamin C. Detection was carried out in a UV detector at 244 nm. The validation of method was carried out using ICH guidelines. The developed method was accurate, precise, selective, and economical. The method provides sharp and proper peak hence can be employed for the estimation of Vitamin C in bulk and formulation.

INTRODUCTION:

Vitamin C [Ascorbic acid, Ascorbate AA] is a water soluble organic compound involved in many biological processes. Although all function of AA have not been fully elucidated, it is likely that it also involved in a maintaining the reduced state of metal cofactor.

Vitamin C has been widely employed in Pharmaceutical and Cosmetic preparation to protect them against oxidation and to exert

physiological / Biological activities. In view of the fact that Pharmaceutical dosage forms usually contain a variety of excipients that may appear as interferants as well as the likelihood of presence of degradation products and / or stabilizing antioxidant agent of Vitamin C HPLC method possesses advantages.

Drug Profile:

Name	**Vitamin C**
Chemical formula	**$C_6H_8O_6$**
Molar mass	**171.12 g/mol**
Solubility	**Freely soluble in water, difficult in alcohol and insoluble in chloroform, ether and benzene.**
Melting point	**190°C**
Category	**Antioxidant**
BCS class	**Ascorbic acid is classified as a BCS class I active substance by the WHO**

Chemical and Reagent:

S. No.	Name of Chemical
1.	HPLC Water
2.	Methanol
3.	Potassiuam Dihydrogen Phosphate
4.	N- Heptane Sulphonic Acid
5.	Orthophosphoric Acid

Preparation of Mobile Phase:

Weigh accurately 6.8 gm Potassium Dihydrogen Phosphate and 2.02 gm of n-Heptane, Sulphonic Acid, Sodium Salt Anhydrous and take in 1000 ml of volumetric flask and make up the volume by using water mix well and sonicate for 5 minute with intermediate shaking and then adjust thepH up to 3.0 by using orthophosphoricacid.

Stock Solution Preparation (A):

An accurately weighed quantity of 25 mg standard Vitamin C was transferred into a 50 mL of volumetric flask and 30 mL of mobile phase (i.e. Buffer : Methanol 90:10 v/v) was added into it, then resultant solution was sonicate for 10 min for complete dissolution of the drug. The final volume was made up to the mark with selected mobile phase.

Standardpreparation (B):

5.0 mL of stock solution of Vitamin C (A) was pipette out and transfers to a 50 mL volumetric flask and made volume up to the mark with mobile phase (Concentration of Vitamin C is 50 ppm).

RP-HPLC Method Development and optimization:

The different trials were taken to develop method and optimize the method for the analysis of vitamin C. For the trials of the method development, 50 ppm standard Vitamin C solution was used with a stationary phase ODS Hypersil C18 and 1.0 ml /min flow rate.

Selection of stationary phase:

On the basis of reversed phase HPLC mode and number of carbon present in the molecule stationary phase with C18 bounded phase i.e. ODS Hypersil C18, 5µ was selected.

Selection of Mobile Phase:

The selection of mobile was done after assessing the solubility of drug in different solvent as well as basis of survey and trials taken during work.The mobile phase was selected for the mixture of buffer and methanol in the ratio (90:10) v/v.

Selection of Detector and Detection Wavelength:

For selection of wavelength , the 50 ppm of standard Vitamin C solution was prepared in mixture of mixture of Buffer: Methanol (90:10v/v). It was scanned over wavelength range of 190-780 nm using a double beam UV spectrophotometer with Buffer: Methanol (90:10v/v) was founded to be 244 nm.was selected. This is optimum wavelength as shown maximum absorption at this wavelength.

Method Validation:

1. Linearity study:

The linearity was carried out to find out the linear relationship between concentrations of drug to the peak area. The range of 30 – 70 µg/ml was selected for the linearity of a standard Vitamin C. The solution wasprepared by diluting known volume of stock solution with mobile phase. Then 30-70 µg/ml solution was injected sequentially with intermediate column equilibrium and the chromatogram were required.

Linearity range PPM	Volume taken from linearity stock solution	Dill. Upto (ml)	Concentration Ratio
Linearity			
3 0	1 5 0 0	5 0	3 0
4 0	2 0 0 0	5 0	4 0
5 0	2 5 0 0	5 0	5 0
6 0	3 0 0 0	5 0	6 0
7 0	3 5 0 0	5 0	7 0

Acceptance criteria:

The co-relation coefficient for Vitamin C must be 0.999 over a selected concentration range.

2. Accuracy study:

The recovery study was perform to evaluate the developed method was accurate for the analysis of Vitamin C. The 80%, 100% and 120% level of recovery study.

Procedure:

Prepared three samples with a different concentration of analyse with respect to label like 80%, 100%, 120% and analyzed three samples.

3. Precision:

The precision of a method was a carried out Intraday precision. The 50 ppm standard Vitamin C solution was suitably selected for method repeatability.

Intraday Precision:

The intraday precision was carried out by preparing six different sample solutions which 50 PPM by using uniform mixed blend and run to replicate of each sample on different time.

The % RSD of the peak areas of the six chromatogram were reported for the fulfilment of accepted criteria.

4. Specificity:

For the specificity study, solution of placebo, sample and standard solution were used, the standard and sample solution was injected into system and chromatogram was recorded. It was found that no interference from purity and excipients.

Identification test:

Identification test should be able to differentiate compound of closely related structure which are expected to be present. I.e. to assure identity of an analyte.

Purity test:

To ensure that analytical procedure performed allows an accurate statement of content of the impurity of an analyte i.e. related substances, residual solvent content, heavy metals etc.

5. Robustness:

The robustness of an analytical method was carried to confirm the analytical method remains unaffected by small variation in optimized method parameter. The 50 PPM of standard solution were injected for each varied condition like change in flow rate ±2 mL/min, change in wavelength ±2nm and change in column temperature ±3 and the chromatogram were recorded , then result were obtained by calculating the %RSD of peak area for each varied condition

1) 6. Ruggedness:

The ruggedness was assessed to check the effect of change in instrument on different day on analysis of Vitamin C and % RSD was calculated.

1. For different column on same instrument on same day:

In which ruggedness assessed by using different column. First column is ODS Hypersil C18 on same instrument Agilent 1260 LS infinitely on same day.

2. For different instrument by same column on different day:

In which ruggedness was assessed by using different HPLC instrument on different days. First HPLC Instrument was agilent 1260 LS infinitely and second is Shimadzu HPLC by using same column on both instrument.

7. System Suitability:

System suitability testing originally believed by the industry of pharmaceuticals to decide whether a chromatographic system is being utilized day today routine manner in pharmaceutical laboratories where quality of result is most important which is suitable for definite analysis.

The parameters used in the system suitability test report are as follows:

1. Number of theoretical plate or Efficiency (N)
2. Capacity factor (K)
3. Separation or Relative retention (α)
4. Resolution (Rs)
5. Tailing factor(T)
6. Relative Standard Deviation (RSD)

8. Limit Of Detection:

LOD is a determined by the analysis of sample with known concentration of analyte and by establishing that minimum level at which the analyte can reliably detected, but not necessarily quantitated as precise value, under the stated experimental condition. The detection limit is generally expressed in the concentration of analyte (PPM) in the sample. A number of approaches are recommended by ICH for determining the detection limit of sample, depending on instrument used for analysis, nature of analyte and suitability of method.

The acceptable approaches are:

1. Visual evaluation
2. Signal –to–noise ratio
3. Standard deviation of the response.
4. Standard deviation of the slope of linearity plot.

The formula for calculating LOD is LOD =3.3δ/S

Where, δ= Standard deviation of intercepts of calibration curves.

S= the slope of linearity plot.

9. Limit of Quantitation:

Limit of Quantitation is the least concentration of drug in a sample which is estimated with appropriate precision and accuracy under experimental condition.

A review on step-by-step Analytical Similar to LOD, ICH recommends the following four methods for estimation of LOQ. The acceptable approaches are:

- Visual evaluation
- Signal-to-noise ratio.
- Standard deviation of the response
- Standard deviation of the slope of linearity plot.

The formula for calculating LOQ is LOQ = 10 δ / S

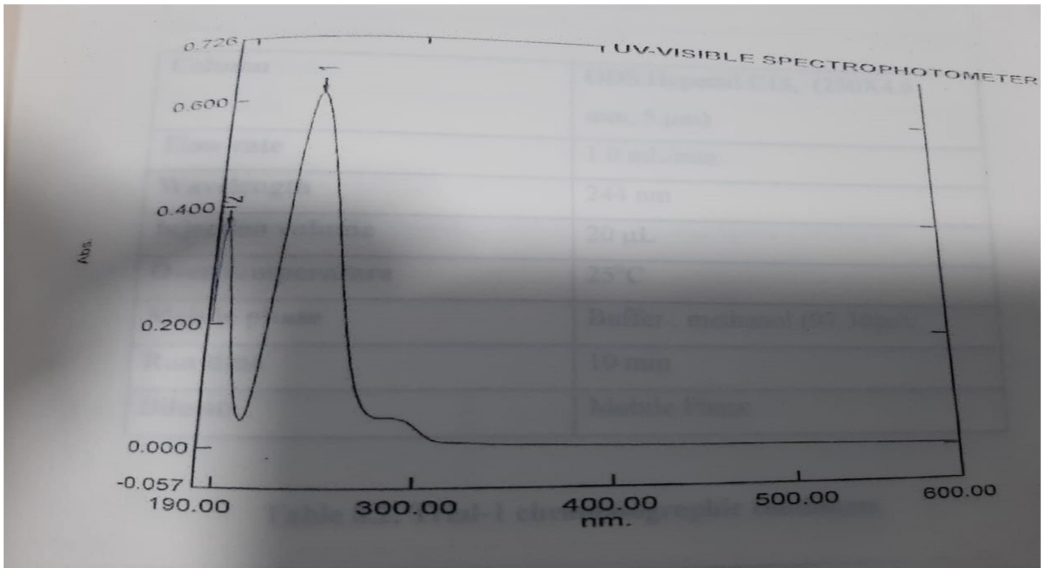
Where, δ = Standard deviation of response.

S Mean of slopes of the calibration curves.

Characteristics	Acceptance Criteria
Accuracy/ Truness	Recovery 98-102% (individual)
Precision	RSD < 2%
Repeatability	RSD < 2%
Intermediate Precision	RSD < 2%
Specificity/ Selectivity	No interference
Detection Limit	S/N > 2 or 3
Quantitation Limit	S/N > 10

Determination of Analytical Wavelength (λ max):

Vitamin C standard solution of 50 ppm in Buffer: Methanol (90:10) was scanned in the range of 190-790 nm in 1.0 cm cell against Buffer: Methanol blank and spectrum was recorded. The spectrum of Vitamin C standard solution was shown in figure below. The λ max of Vitamin C was found to be 244 nm which would be further used for the experimental studies.



Sr. No.	Wavelength	Absorbance
1	244.00	0.612

The spectrum of Vitamin C standard solution was shown in figure. The λ max of Vitamin C was found to be 244 nm which would be further used for experimental studies.

RESULT AND DISCUSSION:

The solubility study of Vitamin C was carried out in different solvent such as water, methanol and acetonitrile. An accurately weighed 500 mg of Vitamin C was dissolved in water;methanol, acetonitrile respectively and the resultant solution were sonicated for 10 min to dissolve the drug. The result was visually observed. It was found that the Vitamin C were soluble methanol, water and acetonitrile.

S. No.	Solvent	Solubility
1.	Water	Soluble
2.	Methanol	Soluble
3.	Acetonitrile	Soluble

From the above observation, Methanol and water shows a good solubility for Vitamin C. Hence, methanol and water selected as a common solvent for proper elution in RP-HPLC.

CONCLUSION:

The RP-HPLC method validated for parameter like Precision, accuracy, linearity, robustness, ruggedness, system suitability, limit of detection, limit of quantification etc. And use of determining the assay of Vitamin C and method found to be accurate, precision, linear, robust and rugged according to guideline of USP and ICH. Further result was compared with the acceptance criteria of stability protocol of

product to find out its degree of stability, and products were found to be stable.

Therefore the developed method was found to be simple, sensitive, accurate and precise. There is no interference with excipients used in the formulation. Therefore it can be used for routine analysis of estimation of Vitamin C.

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