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Analytical method validation report for assay of ascorbic acid by RP- HPLC

S Muneer Banu[1], M Prasad Naidu[2*], N Mallikarjuna Rao[3],P Anusha[4], Madhu Sudan Reddy[5], S V Prasad[6]

1. Msc, S V University, Thirupathi, Andhra Pradesh, India.
2. Msc Medical, Narayana Medical College & Hospital, Andhra Pradesh, India
3. MSc, Acharya Nagarjuna University, Andhra Pradesh, India
4. MSc Medical, Narayana Medical College & Hospital, Andhra Pradesh, India
5. Department of Pharmacology, Narayana Medical College & Hospital, Andhra Pradesh, India.
6. PhD, S V University, Andhra Pradesh, India

Abstract

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the determination of assay of ascorbic acid tablet dosage forms. A reversed- phase C-18 column (4.6x250mm, 5µl, make :waters) column with mobile phase consisting of methanol and phosphate buffer 40:60 (v/v) was used. The flow rate was 1.0 ml/ min and effluents were monitored at 2249nm. The retention time of ascorbic acid is 2.5. The method was validated in terms of linearity, range, specificity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ). By using this method, the retention time of the ascorbic acid in method development is reduced.

Keywords: Ascorbic acid, RP-HPLC, Tablet dosage forms

*Corresponding Author: M Prasad Naidu, M.Sc Medical, Department of Biochemistry, Narayana Medical College, Nellore, Andhra Pradesh, India. E.mail: m.prasadnaidu@ymail.com

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Objective

To validate for determination of Assay of Ascorbic acid in Ascorbic acid Tablets 0.5mg by HPLC method.

Reagents and Standard – Ascorbic acid tablets:

a. Water HPLC grade.
b. Ascorbic acid working standard.
c. Ortho phosphoric acid.

Chromatographic Parameters

Equipment: High performance liquid chromatography equipped with auto Sampler and DAD or UV detector.

Column	: Symmetry C18 (4.6 x 250mm, 5 µm, Make: Waters) or equivalent
Flow rate	: 1.0mL per min
Wavelength	: 249 nm
Injection volume	: 20 µl
Column oven	: Ambient
Run time	: 6 min

Preparation of Phosphate buffer:

Weigh 7.0 grams of Potassium dihydrogen phosphate into a 1000ml beaker, dissolve and diluted to 1000ml with HPLC water. Adjusted the pH to 3 with ortho phosphoric acid.

Preparation of mobile phase:

Mix a mixture of above buffer 600mL (60%) and 400 mL of Methanol HPLC (40%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45µ filter under vacuum filtration.

Diluent Preparation: Mobile phase as diluent.

Preparation of the Ascorbic acid Standard & Sample Solution:

Standard Solution Preparation:

Accurately weigh and transfer 10mg of Ascorbic acid working standard into a 10mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Sample Solution Preparation:

Weigh 5 Ascorbic acid Tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10 mg of Ascorbic acid into a 10 mL volumetric flask. Add about 7 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45µm filter. Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Procedure: Inject 20 µL of the standard, sample into the chromatographic system and measure the area for the ascorbic acid peak and calculate the %Assay by using the formulae.

System Suitability: Tailing factor for the peak due to Ascorbic acid in Standard solution should not be more than 2.0. Theoretical plates for the Ascorbic acid peak in Standard solution should not less than 2000.

Calculation:

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where:

AT = Peak Area of Ascorbic acid obtained with test preparation
AS = Peak Area of Ascorbic acid obtained with standard preparation
WS = Weight of working standard taken in mg
WT = Weight of sample taken in mg
DS = Dilution of Standard solution
DT = Dilution of sample solution
P = Percentage purity of working standard

Results

System Suitability Results:

1). Tailing factor Obtained from the standard injection is 1.3
2). Theoretical Plates Obtained from the standard injection is 3066.15

Assay Results

Weight of 5 tablets: 1.2630 grams
Average Weight : 0.631 grams

$$\frac{1199057}{1231305} \times \frac{10}{10} \times \frac{0.4}{10} \times \frac{10}{12.5} \times \frac{10}{0.4} \times \frac{99.8}{100} \times \frac{631}{500} \times 100 = 98.1\%$$

Sample and standard details

S. No.	Samples	B.NO
1	Ascorbic acid Tablets 0.5mg	_
2	Ascorbic acid working standard	--

Method validation summary

Preparation of stock solution:

Accurately weigh and transfer 10 mg of Ascorbic acid Working standard into a 10mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stocksolution)

Preparation of 40 µg/ml solution: Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Procedure: The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was

The results are summarized

Injection	Peak Area
Injection-1	982224
Injection-2	981218
Injection-3	980263
Injection-4	990143

Injection-5	989230
Average	984615
Standard Deviation	4691.5
%RSD	0.47

Acceptance Criteria: The % RSD for the area of five standard injections results should not be more than 2%.

Intermediate Precision/Ruggedness: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions.

Preparation of stock solution:

Accurately weigh and transfer 10 mg of Ascorbic acid working standard into a 10 mL volumetric flask add about 7 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Preparation of 40 µg/ml solution: Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

The results are summarized in

Injection	Peak Area
Injection-1	971015
Injection-2	972356
Injection-3	970045
Injection-4	971156
Injection-5	971234
Average	971234
Standard Deviation	821.60
%RSD	0.08

Acceptance Criteria:

The % RSD for the area of five standard injections results should not be more than 2%.

Accuracy:

Preparation of stock solution:

Accurately weigh and transfer 10 mg of Ascorbic acid Working standard into a 10 mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stocksolution).

Preparation of 40 µg/ml solution:

Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):

Accurately weigh and transfer 4.10mg of Ascorbic acid API sample into a 10 mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stocksolution)

Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

For preparation of 100% solution (With respect to target Assay concentration):

Accurately weigh and transfer 8.60mg of Ascorbic acid API sample into a 10 mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

For preparation of 150% solution (With respect to target Assay concentration):

Accurately weigh and transfer 13.21 mg of Ascorbic acid API sample into a 10mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Procedure:

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy - 150% Solutions. Calculate the Amount found and Amount added for Ascorbic acid and calculate the individual recovery and mean recovery values.

The results are summarized

%Conc. (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	506773	4.15	4.10	98.9%	99.6%
100%	1061329	8.75	8.60	99.3%	
150%	1618209	13.30	13.11	98.6%	

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102.0%.

Linearity:

Preparation of stock solution:

Accurately weigh and transfer 10mg of Ascorbic acid API sample into a 10 mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (20µg/ml):

0.2ml of stock solution has taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (30µg/ml):

0.3ml of stock solution taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (40µg/ml):

0.4 ml of stock solution taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (50µg/ml):

0.5ml of stock solution taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (60µg/ml):

0.6ml of stock solution taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Procedure:

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Linearity Results:

S.No	Linearity Level	Concentration	Peak Area
1	I	20µg/ml	512346
2	II	30µg/ml	768519
3	III	40µg/ml	1024692
4	IV	50µg/ml	1280865
5	V	60µg/ml	1537038
Correlation Coefficient			1.000

Acceptance Criteria: Correlation coefficient should be not less than 0.999.

Limit of detection:

Preparation of 40µg/ml solution:

Accurately weigh and transfer 10mg of Ascorbic acid Working standard into a 10 ml Volumetric flasks add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to

the mark with diluent. Mix well and filter through 0.45µm filter.

Preparation of 0.15% solution At Specification level (0.06µg/ml solution):

Pipette 1mL of 10µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent. Further pipette 0.15mL of above diluted solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank: 48µV

Signal Obtained from LOD solution (0.15% of target assay concentration): 146µV

S/N = 146/48 = 3.04

Acceptance Criteria:

S/N Ratio value shall be 3 for LOD solution.

Limit of quantification:

Preparation of 40µg/ml solution:

Accurately weigh and transfer 10mg of Ascorbic acid Working standard into a 10 mL Volumetric flasks add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Preparation of 0.4% solution At Specification level (0.16µg/ml solution):

Pipette 1mL of 10µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent. Further pipette 0.4mL of above diluted solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank: 46µV

Signal Obtained from LOD solution (0.4% of target assay concentration): 476 µV

S/N = 476/48 = 9.91

Acceptance Criteria:

S/N Ratio value shall be 10 for LOQ solution.

Robustness:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

a). the flow rate was varied at 0.9 to 1.1ml/min. Standard solution 40 µg/ml was prepared and analysed using the varied flow rates along with method flow rate

The results are summarized

On evaluation of the above results, it can be concluded that the variation in flow rate do not affect the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	2915.1	1.5
2	1.0	2352.4	1.2
3	1.1	2867.9	1.6

***** Results for actual flow (1.0 ml/min) have been considered from Assay standard.

b). The Organic composition in the Mobile phase was varied from55% to 65%. Standard solution 40 µg/ml was prepared and analysed using the varied. Mobile phase composition along with the actual mobile phase composition in the method.

The results are summarized

On evaluation of the above results, it can be concluded that the variation in 10% Organic composition in the mobile phase do not affect the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ±10%.

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3017.3	1.4
2	*Actual	2352.4	1.2
3	10% more	2907.0	1.4

Results for actual Mobile phase composition (40:60 methanol: Buffer) have been considered from Assay standard.

Conclusion:

The retention time of the ascorbic acid is reduced by using the mobile phase whose composition is 40:60 methanol:buffer by maintaining the above parameters.

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