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UV-Spectrophotometric method for quantification of ascorbic acid in bulk powder

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Abstract
Ascorbic acid is a potent reducing and antioxidant agent that functions in fighting bacterial infections, in detoxifying reactions. In the present research work an attempt has been made to develop and validate spectrophotometric method for determination of ascorbic acid in bulk powder form using UV-1900 model. The method was developed using methanol: water (50:50 v/v) as a solvent in which ascorbic acid shows maximum absorbance wavelength at 258nm. In order to optimize the developed method, validation of ascorbic acid were performed as per ICH guidelines parameters such as Linearity, Selectivity, Specificity, Precision, Robustness, Ruggedness, LOD & LOQ, Solution stability were performed. Ascorbic acid Showed linear response between concentration range of 3, 6, 9, 12, 15µg/mL with r^2 value 0.997 all the validation report of Precision, robustness, ruggedness and solution stability were found to be in acceptance limit. Newly developed and validated spectrophotometric method was found to be selective, specific, linear, precise, robust, rugged and stable for determination of ascorbic acid in bulk form using UV-1900.

Keywords: Ascorbic acid, anti-oxidant, UV-1900, ICH-guideline, stability

1. Introduction
Ascorbic acid is a six carbon compound related to glucose. It is found naturally in citrus fruits and many vegetables. (Drug bank) L-Ascorbic acid is a white to very pale yellow crystalline powder with a pleasant sharp acidic taste almost odourless. Ascorbic acid is a potent reducing and antioxidant agent that functions in fighting bacterial infections, in detoxifying reactions, and in the formation of collagen in fibrous tissue, teeth, bones, connective tissue, skin, and capillaries [1]. It is available in synthetic form and well as found naturally in many medicinal plants which includes Indian gooseberry, amla citrus fruits like limes, oranges and lemons, green leafy vegetables [2] which is responsible for potent antioxidant activity.
Amla is one of the major source of Ascorbic acid. Literature survey has been done for the content estimation of ascorbic acid in amla. Analytical methods such as UPLC (Klimczak I *et al.*, 2015) [3] HPLC (Saikh M. *et al.*, 2013) [4] were reported for estimation of Ascorbic acid in bulk. No UV-Spectrophotometric method was reported for content estimation of ascorbic acid in amla as well as in bulk form. Hence in this research work an attempt has been made to develop and validate UV-Spectrophotometric method by using standard ascorbic acid and apply the same for content estimation of ascorbic acid in amla extract.

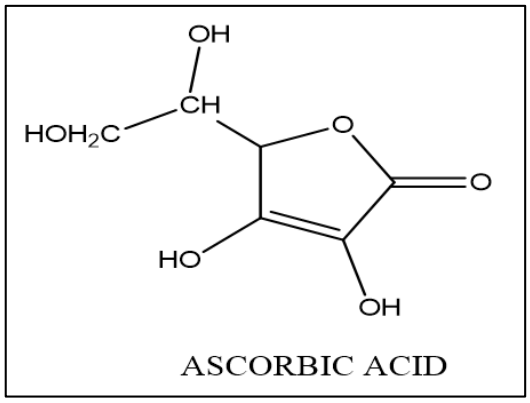

Fig 1: Chemical Structure of Ascorbic acid

2. Material and Methods

2.1 Instruments and apparatus

UV-Spectrophotometer of Shimadzu-1900 with Lab solutions software& Shimadzu-1800 with UV probe software was used for determination of Ascorbic acid.

2.2 Reagents and chemicals

All the chemicals and reagents used for the experiment were analytical grade. Methanol was obtained from Molychem, Mumbai.

2.3 Drug samples

Ascorbic acid was obtained as gift sample from Spectrochem Pvt Ltd. Mumbai.

2.4 Development of UV-Spectrophotometric technique

Development of UV-Spectrophotometric method involves the two steps, mainly selection of solvent system and selection of wavelength of detection for determination of Ascorbic acid. Solubility profile of Ascorbic acid in different solvents was obtained by literature review and by practical analysis. Literature survey revealed that Ascorbic acid is soluble in mixture of methanol & Acetic acid (95:5), pH-2.1(Phosphoric acid), Acetonitrile and ammonium acetate buffer 6.8(78:22) (Novokova L *et al.*,2005) [5]. Several trials were made to obtain suitable wavelength of detection by utilizing various mixtures of solvents. Mixture of Methanol: Water (50: 50v/v) was chosen as the best solvent. In order to obtain UV-spectrum of the analyte, solution containing Ascorbic acid in solvent was scanned in UV-spectrophotometer between the range of 400-200 nm, showed maximum absorbance wavelength at 258 nm.

2.5 Validation of UV-Spectrophotometric Technique [9, 10]

(Shailendra S *et al.*, 2015, Pancham Y. *et al.*, 2019)

According to ICH guidelines the newly developed UV-Spectrophotometric method was validated. Ascorbic acid was validated in terms of specificity, selectivity, linearity, range, limit of detection, limit of quantification, precision, ruggedness, robustness and solution stability [6-8].

2.5.1 Specificity and selectivity

UV-spectrum of blank solvent (Methanol: Water 50:50v/v) and solution containing Ascorbic acid was scanned between the range of 400-200 nm and observed for interference of any absorbance at 258 nm.

2.5.2 Linearity and range

100mg of Ascorbic acid was weighed and transferred into composed 100 ml of volumetric flask and volume was made up to the mark using solvent system of methanol: water (50:50v/v) to obtain 1000 µg/ml. From this stock solution, serial dilutions were made to obtain 3, 6, 9, 12, 15µg/mL solutions of Ascorbic acid. The resulted solution was prepared in triplicates and absorbance was measured at 258 nm.

2.5.3 Limit of Detection and Limit of Quantification [11]

Limit of detection and quantification was calculated by using statistical calculations using following formulas:

$$LOD = \frac{3.3 \times \text{standard deviation of y-intercept}}{\text{Slope of the calibration curve}}$$

$$LOQ = \frac{10 \times \text{standard deviation of y-intercept}}{\text{Slope of the calibration curve}}$$

2.5.4 Precision

Precision was performed by using system precision, intraday precision and Interday precision.

2.5.4.1 System Precision

Six replicates of solution containing 3µg/mL of Ascorbic acid were prepared and absorbance of each was measured at 258 nm and %RSD was calculated.

2.5.4.2 Intraday Precision

Solutions containing 9µg/mL of Ascorbic acid were analyzed in six replicates and %RSD for absorbance obtained was calculated at different time intervals on same day.

2.5.4.3 Interday Precision

Solutions containing 12µg/mL of Ascorbic acid were analyzed in six replicates and %RSD for absorbance obtained was calculated on three different days.

2.5.5 Ruggedness

Ruggedness of the method was proved by obtaining consistent results by different analyst, employing different instruments on different days. 15 & 3 µg/mL solutions containing Ascorbic acid was prepared in six replicates by different analyst and absorbance was measured at 258 nm, analyzed on different instrument and %RSD was calculated.

2.5.6 Robustness

Stock solutions of analyte were prepared using solvent system composed of Methanol: Water (51:49v/v) and Methanol: Water (49:51 v/v) separately. Using the above solvent systems six replicates of solutions containing Ascorbic acid were prepared. The absorbance was measured at 258 nm &%RSD was calculated.

2.5.7 Solvent and standard stock solution stability

Stability of solvent and stock solution was determined by comparing the absorbance between fresh stock dilutions and old stock dilutions. Stock solution of Ascorbic acid and solvent system was prepared and stored at room temperature for 2 days. On 2nd day dilutions in triplicates were prepared from old stock solution and fresh stock solution. %RSD was calculated for the absorbance obtained.

3. Results and Discussion

3.1 Development

The UV-spectrum of Ascorbic acid in Methanol: Water (50:50v/v) solvent showed maximum absorbance at 258 nm and hence it was selected as wavelength of detection. Developed method parameters are reported in Table. 1

3.2 Validation

3.2.1 Specificity and selectivity

No interference was showed by the solvent spectrum at the maximum wavelength of absorbance of Ascorbic acid. Maximum wavelength was selectively exhibited by the analyte at 258 nm. Thus specificity and selectivity of the method was validated. UV spectrum of Ascorbic acid is presented in Fig 2 and overlay spectrum of Ascorbic acid is presented in Fig 3.

3.2.2 Linearity and range
Standard calibration curve was plotted using concentration vs absorbance obtained by Ascorbic acid. Analyte showed linear response between the concentration range of 3, 6, 9, 12, 15µg/mL with regression equation of 0.997. Linearity data is reported in Table. 2 and standard calibration curve is presented in Fig. 4

3.2.3 Limit of Detection and Limit of Quantification
LOD value of Ascorbic acid was found to be 0.96µg/ml and LOQ value was found to be 2.91 µg/ml respectively. LOD & LOQ values are presented in Table. 2.

3.2.4 Precision
The %RSD values calculated for all six replicates of the respective solution of Ascorbic acid at each level of precision was found to be less than 2%, proving the precision of method. Data of system precision study is reported in Table. 3

3.2.5 Ruggedness and Robustness
Method was found to be rugged with respect to change in the analyst and change in the instrument with %RSD less than 2% also it was found to be robust with slight change in the percent composition of solvent system with %RSD less than 2%. Ruggedness and robustness is reported in Table 6 and Table 7 respectively.

3.2.6 Solvent and standard stock solution stability
%RSD for absorbance obtained by fresh and old dilutions containing Ascorbic acid was found to be within the acceptance and data obtained showed the standard stock solution and solvent system showed stability of 2 working days at room temperature. Solution and standard stock solutions stability is reported in Table. 8
The validation report was presented in Table. 9
Literature survey revealed that analytical method such as UPLC, HPLC were reported for estimation of ascorbic acid. The reported methods were more costly and time consuming also uses the large amount of organic solvents which are hazardous to the health. To overcome these disadvantages of reported methods we have developed a new spectrophotometric method for estimation of ascorbic acid in bulk powder form. The presented method was simple, precise, accurate, less time consuming, cost effective and economic.

Table 1: Developed method parameters

Parameters	Specifications
Analyte	Ascorbic acid
Solvent	Methanol: Water (50:50% v/v)
Maximum wavelength of Ascorbic acid	258 nm

Table 2: Linearity data of Ascorbic acid

Sr. No.	Concentration	*Absorbance at 258nm
1	3µg/ml	0.193
2	6µg/ml	0.393
3	9µg/ml	0.606
4	12µg/ml	0.750
5	15µg/ml	0.983
Correlation Coefficient		0.997
LOD		0.96 µg/ml
LOQ		2.91 µg/ml

*Replicates of three concentrations.

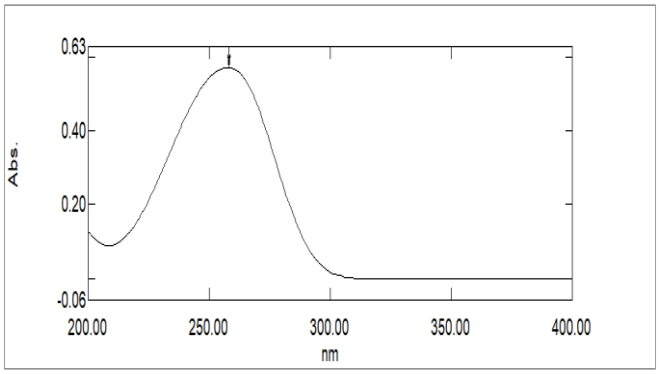

Fig 2: UV-Spectrum of Ascorbic acid

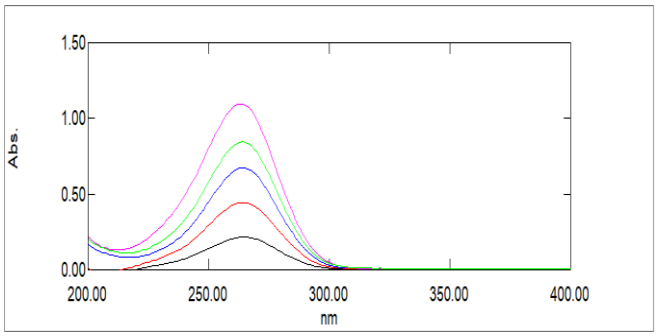

Fig 3: Overlay Spectrum of ascorbic acid

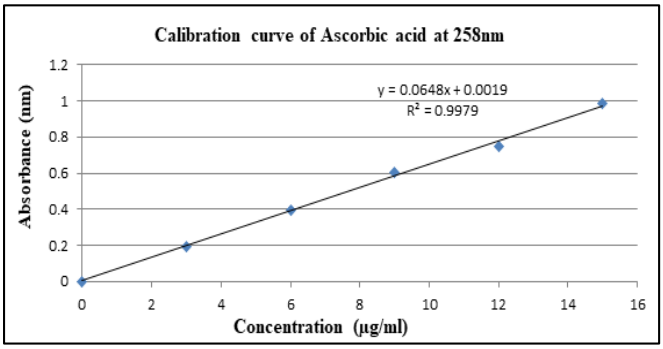

Fig 4: Standard Calibration Plot of Ascorbic acid

Table 3: System precision data of Ascorbic acid

Replicates	Concentration	Absorbance at 258 nm
1	3 µg/ml	0.191
2	3 µg/ml	0.192
3	3 µg/ml	0.193
4	3 µg/ml	0.193
5	3 µg/ml	0.192
6	3 µg/ml	0.193
%RSD		**0.425**

Table 4: Intraday Precision data of Ascorbic acid

Intraday Precision		Absorbance at 258 nm		
Replicates	Concentration	Initial hour	1st Hour	5 th Hour
1	9 µg/ml	0.588	0.589	0.589
2	9 µg/ml	0.589	0.588	0.589
3	9 µg/ml	0.590	0.589	0.591
4	9 µg/ml	0.591	0.591	0.591
5	9 µg/ml	0.589	0.587	0.590
6	9 µg/ml	0.587	0.591	0.591
%RSD		0.240	0.272	0.167

Table 5: Interday precision data of Ascorbic acid

Interday Precision		Absorbance at 258 nm		
Replicates	**Concentration**	**Day-1**	**Day-2**	**Day-3**
1	12 µg/ml	0.743	0.753	0.778
2	12 µg/ml	0.744	0.753	0.779
3	12 µg/ml	0.746	0.757	0.783
4	12 µg/ml	0.745	0.756	0.784
5	12 µg/ml	0.746	0.757	0.781
6	12 µg/ml	0.742	0.757	0.782
%RSD		0.219	0.261	0.297

Table 6: Ruggedness data of Ascorbic acid

Replicates	Concentration	Absorbance at 258 nm	
		Change in instrument	**Change in analyst**
1	15 µg/ml	1.002	1.001
2	15 µg/ml	1.001	1.002
3	15 µg/ml	1.005	1.003
4	15 µg/ml	1.004	1.005
5	15 µg/ml	1.004	1.004
6	15 µg/ml	1.005	1.002
%RSD		0.164	0.147

Table 7: Robustness data of Ascorbic acid

Replicates	Absorbance at 258 nm		
	Concentration	**Solvent composition-1**	**Solvent composition-2**
1	6 µg/ml	0.381	0.383
2	6 µg/ml	0.379	0.383
3	6 µg/ml	0.379	0.384
4	6 µg/ml	0.381	0.383
5	6 µg/m	0.383	0.384
6	6 µg/ml	0.383	0.381
%RSD		0.470	0.286

Table 8: Solution stability of Ascorbic acid

Concentration	Absorbance at 321 nm	
	Fresh	**Old**
12 µg/ml	0.739	0.749
12 µg/ml	0.740	0.748
12 µg/ml	0.741	0.748
%RSD	0.621	

Table 9: Validation parameters report

Sr. No.	Validation parameters		Values obtained
1	**Linearity range**		3-15 µg/ml
2	Precision	System Precision	0.425%
		Interday Day-1	0.219%
		Interday Day-2	0.261%
		Interday Day-3	0.297%
		Initial hour	0.240%
		Intraday 1 st hour	0.272%
		Intraday 5 th hour	0.167%
3	Robustness	Change in solvent composition-I	0.470%
		Change in solvent composition-II	0.286%
4	Ruggedness	By change in analyst	0.164%
		By change in instrument	0.664%
5	LOD		0.96 µg/ml
6	LOQ		2.91 µg/ml
7	Solution Stability		2 days at room temperature (0.621%)

4. Conclusion

The newly developed UV-Spectrophotometric analytical method is specific and selective for the determination of Ascorbic acid in bulk. The developed method is subjected for the validation as per ICH Guidelines. The developed method was found to be linear, simple, precise, robust, rugged, stable and economic for routine use in the synthetic drug industry.

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