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ORIGINAL ARTICLE **OPEN ACCESS**

Area Under Curve by UV Spectrophotometric Method for Determination of Ascorbic Acid in Bulk

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ABSTRACT
The aim of present work is to establish simple, precise, and rapid Spectrophotometric method for the quantification of Ascorbic acid in Active Pharmaceutical Ingredient. In this, work is carried out to for estimation of Ascorbic acid bulk by utilizing an Area under Curve (AUC) method using UV – Visible Spectrophotometry. The study is designed to validate the developed methods as per ICH guidelines. For this purpose the wavelength range between 200-400 nm was selected. Distilled water was used as a solvent throughout the work. Linearity was obtained in concentration range 2 to 10 µg/ml (r2 =0.991) for the method. The developed method was found to be simple, linear, accurate, precise and highly sensitive and which can be used for routine quality control analysis for Spectrophotometric estimation of Active Pharmaceutical Ingredient.
KEYWOERDS: *Ascorbic acid, AUC, distilled water, Spectrophotometer, linearity.*

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INTRODUCTION

Ascorbic Acid also called as (vitamin C) is a freely water-soluble vitamin indicated for the prevention and treatment of scurvy, as ascorbic acid deficiency results in scurvy. Collagens like structures are primarily affected, and lesions produced in bones and blood vessels.[1] Administration of ascorbic acid completely reduced the symptoms of ascorbic acid (vitamin) deficiency. Ascorbic acid contains six carbon compound related to glucose nutraceutical compound. It is chemically described as (5R)-5-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxy-2,5-dihydrofuran-2-one. Ascorbic acid (Vitamin C) is an essential nutrient in human diets. And which is important to maintain connective tissue and bone. Its biologically active Vitamin C is considered an antioxidant. The aim of this present work is to develop simple, precise and accurate Spectrophotometric method for the routine determination of Ascorbic acid in bulk.[2]

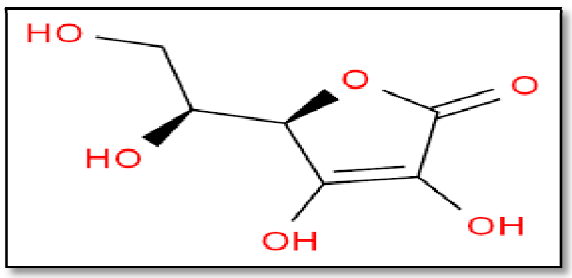

Fig No 1: Chemical Structure of Ascorbic acid

MATERIAL AND METHODS

Chemicals:

Ascorbic acid was obtained at collage sample of Vishal institute pharmaceuticals education and research Ale, Pune. Distilled water was used as solvent throughout the experimentation.

Instrumentation:

A Shimadzu (Kyoto, Japan) model UV- 1800 double beam UV- Visible spectrophotometer attached with computer operated by software UV probe 2.33 with spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells was used to measure absorbance of the resulting solutions. Digital Analytical balance, Mettler Toledo (Model JL 1503-C) was used for weighing purpose.

METHOD

Experimental Work:

A) To check the solubility of Ascorbic acid:

Qualitative solubility analyses of drugs were done by dissolving 5 mg of Ascorbic acid in 5 ml solvent such as distilled water, methanol and ethanol.[3]

B) To identify the ʎ max of Ascorbic acid:

Weigh10 mg of the pure drug and dissolve it in small portion of Distilled water and make up the volume upto 10 ml using distilled water to obtained a standard stock solution of 1000µm/ml. From above solution 50 ml of the standard solution was withdrawn involumetric flask and diluted to 50 ml distilled water to prepare 100ppm solution. Suitable serial dilutions were made with distilled water to get standard solutions of concentrations: 2,4,6,8 and 10µm/ml.[4, 5] Spectrum peak details are shown in Figure No 2.

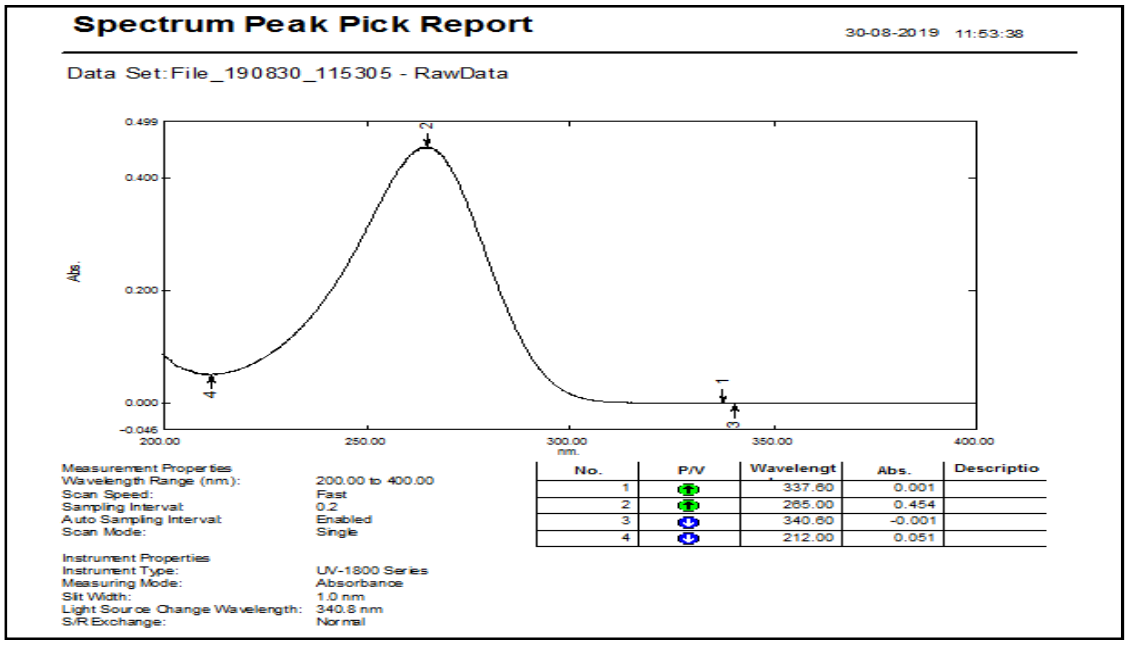

Fig No 2: ʎmax of Ascorbic acid, Spectrum peak pick.

C) Analytical Method Development and Validation:

Linearity / calibration curve:

The linearity of an analytical procedure is the interval between the upper and lower concentration of Analyte in the sample. For which demonstrated that the analytical procedure is of linearity. The standard solution of Ascorbic acid (2, 4, 6, 8 and 10 µm/ml) 0.5, 1, 1.5, 2, and 2.5 ml solution was pipette out in a separated series of 25 ml volumetric flask. Make up the volume with distilled water and mixedwell. The absorbance maxima and area under curve for the solutions was measured at 265 nm and range of nm for two methods respectively against distilled water as blank. Calibration Curve table of Ascorbic acid is shown in table no 1. Calibration curve of Ascorbic acid.[6, 8]

Table 1: Calibration curve of Ascorbic acid

Conc. µg/ml	Absorbance
2	0.049
4	0.065
6	0.084
8	0.111
10	0.133

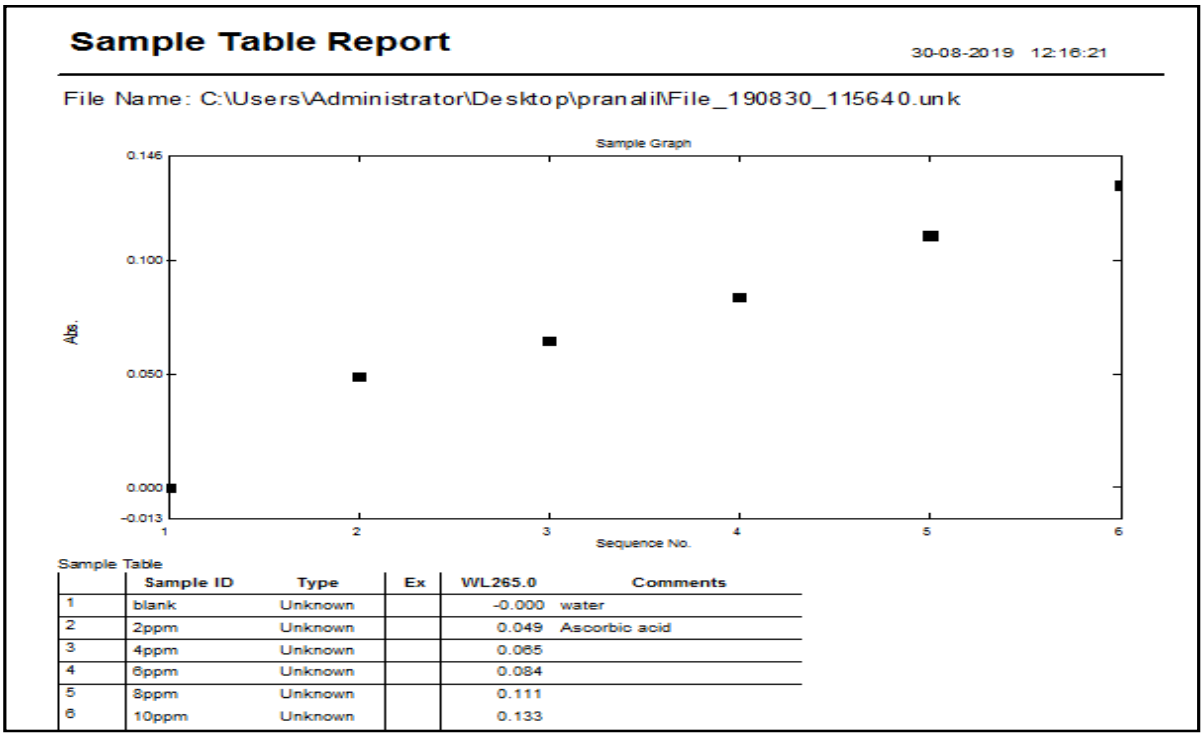

Fig No 3: linearity of Ascorbic acid

D) Area Under Curve Method

In case of AUC (Area under Curve) method is applicable for there is sharp peak or broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths $\lambda 1$ and $\lambda 2$. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by the entering the wavelength ranges over which area has to be calculated. This wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. The above mentioned spectrums were used to calculate AUC. Thus, the calibration curve can be constructed by plotting concentration Vs AUC.[6, 7]

RESULTS AND DISCUSSION

The AUC (Area under Curve) spectra for Ascorbic acid were recorded at the wavelength of 265 nm.

A] Calibration Curve for Drug:

Absorbance maxima method:

The absorbance maxima of ascorbic acid were found to 265 nm in distilled water. Under the Experimental conditions described, the graph obtained for the absorbance maxima for pure drug showed linear relationship (Figure 4). Regression analysis was made for the slope, intercept and correlation coefficient values. The regression equations of calibration curve were y = 0.010x + intercept 0.024 R^2 = 0.991 at 265 nm for absorption maxima the range was found to be 2 to 10µm/ml by the UV Spectrophotometric analysis. Calibration Curve is shown in Table. 1. Calibration Curve of Ascorbic acid. Calibration curve of Ascorbic acid is shown in Figure. 4. Calibration Curve of Ascorbic acid.

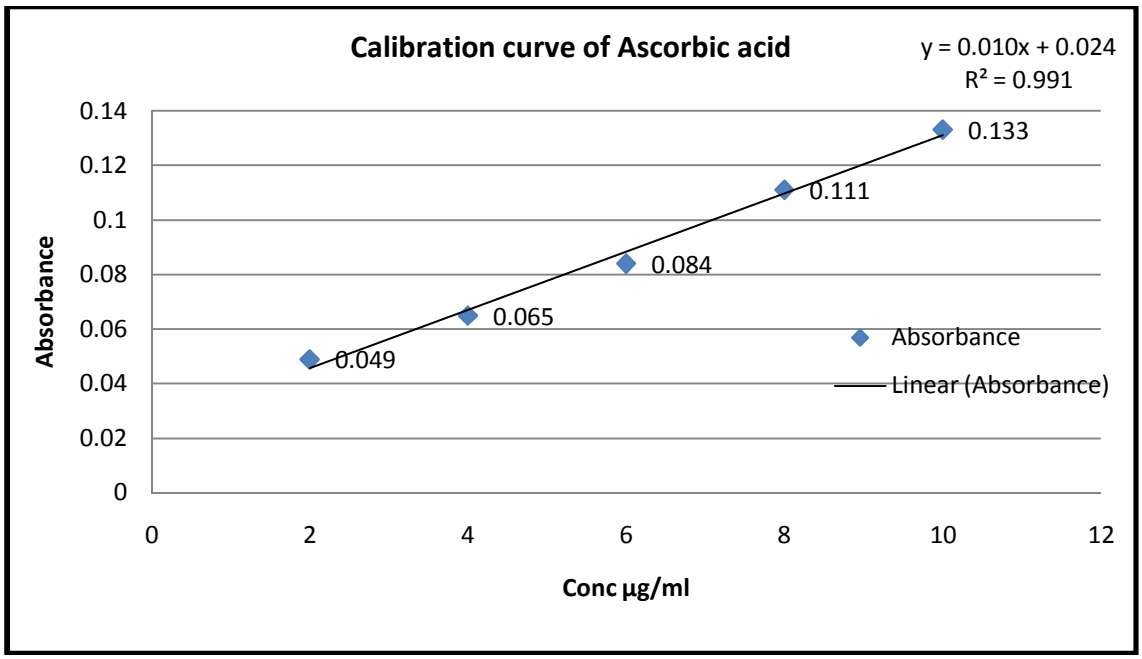

Fig No 4: Calibration curve of Ascorbic acid

B] Area Under Curve Method :

In the Experimental conditions described, the graph obtained of the Area Under Curve (AUC) spectra shows linear relationship (Figure 5). Regression analysis was made of the slope, intercept and R^2 values. The equation is Y = 0.010x + intercept 0.024 R^2 = 0.991 at 265 nm in between range 200 – 400 nm for Area Under Curve Spectrophotometry analysis. The range was found to be 2 to 10µm/ml for the Area Under Curve UV Spectrophotometric analysis.

Table 2: Area Under curve of Ascorbic acid:

Parameter	AUC
Wavelength Range (nm)	200 – 400
Concentration Range (µm/ml)	2 -10
Slope (m)	0.010
Intercept (c)	0.024
Correlation Coefficient (r2)	0.991

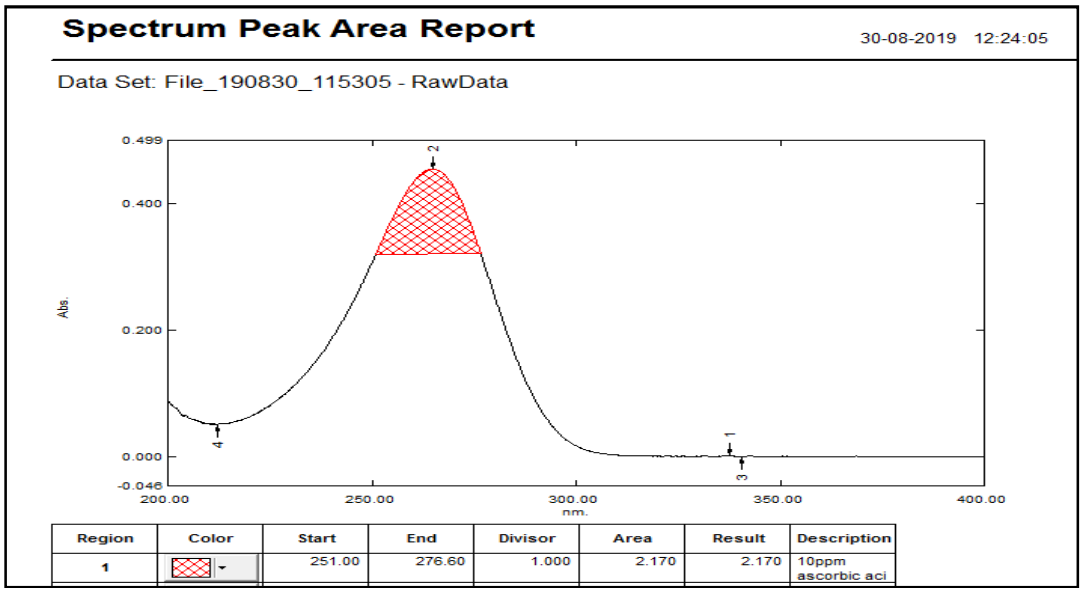

Fig no 5: Area Under Curve of Ascorbic acid

CONCLUSION

There is no any Spectrophotometric methods have been described for AUC determination of Ascorbic acid. Therefore simple, fast and precise method for area under curve was developed by UV spectrophotometrically for the routine analysis of Ascorbic acid. The developed
Method can be concluded as simple, accurate, sensitive and precise and can be easily applicable in the pharmaceutical formulation.

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