

## Development and Validation of UV Spectrophotometric Estimation of Diclofenac Sodium Bulk and Tablet Dosage form using Area under Curve Method

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### ABSTRACT

A simple, precise, accurate and economical UV visible spectrophotometric method has been developed for estimation of Diclofenac sodium drug by AUC method. The standard and sample solutions were prepared by using double distilled water as a solvent. Quantitative determination of the drug was performed at wavelength range 270-282 nm. The linearity was established over the concentration range of 05, 10, 15, 20 & 25 µg/ml for Diclofenac sodium with correlation coefficient value of 0.9981. Precision studies showed that % relative standard deviation was within range of acceptable limits. The mean percentage recovery was found to be 99.38%. The proposed method has been validated as per ICH guidelines.

Keywords: Diclofenac sodium, UV visible spectrophotometry, AUC, Method Validation

### INTRODUCTION

Diclofenac Sodium (DS) is chemically Sodium salt of 2-[[2,6-dichlorophenyl]amino]benzene acetic acid. It is having anti-inflammatory and analgesic properties<sup>[1]</sup>. Literature survey revealed several analytical methods UV spectrophotometry<sup>[2]</sup> and HPLC<sup>[3]</sup> have been reported in bulk, pharmaceutical dosage form for determination of Diclofenac sodium. To our notice so far no UV- spectrophotometric method using Area Under Curve (AUC) has been reported for the determination of Diclofenac sodium in bulk and tablets. Hence an attempt has been made to develop new UV-spectrophotometry(AUC) method for estimation of Diclofenac sodium in bulk and pharmaceutical formulations with good accuracy simplicity, precision and economy.

**Molecular Formula:** C<sub>14</sub>H<sub>10</sub>Cl<sub>2</sub>NNAO<sub>2</sub>.

**Molecular weight:** 318.130g/mole

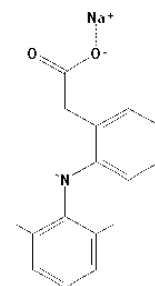


Fig. 1: Chemical Structure of Diclofenac sodium.

### MATERIALS AND METHODS

#### Apparatus and instrumentation:

A shimadzu1800 UV/VIS double beam spectrophotometer with 1cm matched quartz cells was used for all spectral measurements. Single Pan Electronic balance (CONTECH, CA 223, India) was used for weighing purpose. Sonication of the solutions was carried out using an Ultrasonic Cleaning Bath (Spectra lab UCB 40, India). Calibrated volumetric glassware (Borosil®) was used for the validation study.

**Materials:**

Reference standard of Diclofenac sodium API was supplied as gift sample by Marksan Pharmaceutical Ltd., Verna, Goa. Tablet sample with label claim 500mg per tablet were purchased from local market Pune.

**Method development:****Determination of Wavelength Range:-**

For the selection of analytical wavelength range for area under curve method 20 $\mu$ g/ml solution of

Diclofenac sodium was scanned in the spectrum mode from 400nm to 200nm against distilled water as blank. Wavelength range was selected around wavelength maxima (276nm). Different working standards were prepared between 05, 10, 15, 20 & 25  $\mu$ g/ml. Various wavelength range were tried and final wavelength range between 270-282 nm was selected on the basis of linear relationship between area and corresponding concentration (Figure 2).

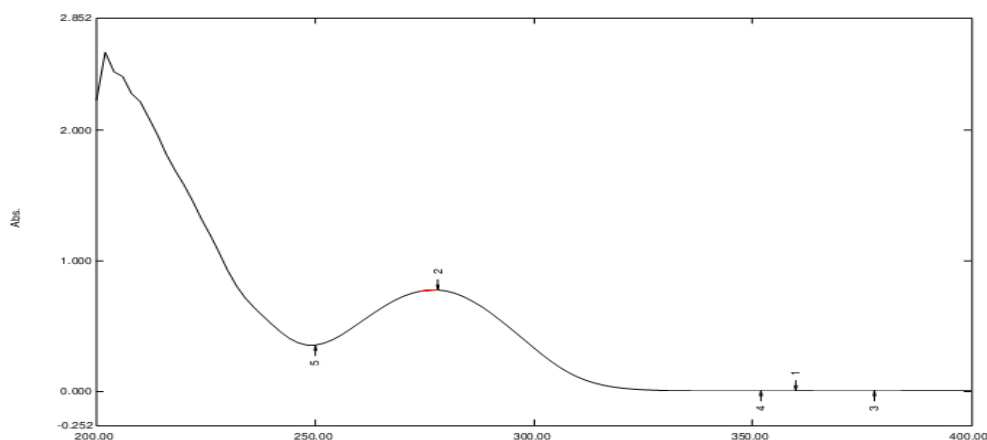


Fig:- 2 UV AUC spectrum of Diclofenac sodium (20 $\mu$ g/ml)

**Area under curve (Area calculation):-**

Area under curve method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths such as  $\lambda_1$  and  $\lambda_2$  representing start and end point of curve region. The area under curve between  $\lambda_1$  and  $\lambda_2$  was calculated using UV probe software. In this study area was integrated between wavelength ranges from 227 to 282 nm.

$$\text{Area calculation: } (\alpha + \beta) = \int_{\lambda_2}^{\lambda_1} A d\lambda$$

Where,  $\alpha$  is area of portion bounded by curve data and a straight line connecting the start and end point  $\beta$  is the area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis  $\lambda_1$  and  $\lambda_2$  are wavelength range start and end point of curve region<sup>[4]</sup>.

**Preparation of standard solution:-**

The standard stock solution of Diclofenac sodium was prepared by accurately weighing & transferring, 10 mg of API to 100 ml of volumetric flask. Then take from that 2ml and add to 10ml volumetric flask and make up with distilled water to get final standard stock solution (20 $\mu$ g/ml) was further diluted with distilled water to obtain 05-25  $\mu$ g/ml Diclofenac sodium solutions.

**Calibration curve for Diclofenac sodium:-**

The dilutions were made from Standard Stock solution to get concentration of 05, 10, 15, 20, and 25 $\mu$ g/ml respectively. These solutions were scanned from 400 to 200 nm and area under curve (AUC) values was integrated in the range of 270-282nm. The calibration curve was plotted between areas under curve values against concentration (Fig. 3).

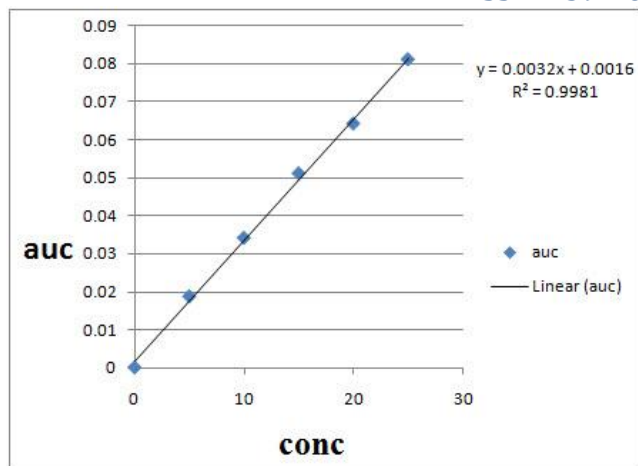


Fig-3 Linearity of Diclofenac sodium.

**Assay of tablet formulation:-**

Twenty tablets each containing 500mg of Diclofenac sodium were weighed crushed to powder and average weight was calculated. Powder equivalent to 10 mg of Diclofenac sodium was transferred in 100 ml of volumetric flask. A 50ml of distilled water was added and sonicated for 15 minutes. Then solution was further diluted up to the mark with distilled water. The solution was filtered using Whatmann filter paper no.41, first 5ml of filtrate was discarded. This solution was further diluted to obtain 15µg/mL solution with water, subjected for UV analysis using distilled water as blank. This procedure was repeated three times (Table 1).

**Table 1:-Assay of tablet dosage form.**

Sr. No.	Sample Solution Concentration (µg/ml)	Amount found (%)*	Mean % found	%RSD
1	15	100.22	99.25	0.5672
2	15	99.49		
3	15	98.04		

\*n=3, SD=Standard Deviation, % RSD = % Relative Standard Deviation.

**Method validation:**

The above method was validated for various parameters such as Accuracy, Linearity, Precision, Limit of detection (LOD) and Limit of Quantitation (LOQ) according to ICH guideline.

**Accuracy:**

The accuracy for the analytical method was evaluated at 80%, 100% and 120% levels of 20µg/ml standard solution. Area under curve (AUC) was measured in wavelength range 270-282 nm and results were obtained in terms of percent recovery. Three determinations at each level were performed and % RSD was calculated for each level.

**Table 2:-Accuracy results for Diclofenac sodium.**

Accuracy level	Sample conc (µg/ml)	Std. conc	Total amount. Added (µg/ml)	% Recovery	Mean % Recovery	% RSD
80	15	12	27	99.13		
100	15	15	30	100.77	99.38	1.8073
120	15	18	33	99.26		

**Precision:** The precision of an analytical procedure expresses the closeness of an agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous

sample under the prescribed conditions intraday precision was studied by integrating area of standard solution of 20µg/ml concentration at six independent series in the same day. Inter-day

precision studies were performed by integrating area of standard solution of 20 $\mu\text{g/ml}$  concentration on three consequent days. The % RSD was calculated.

Table 3:-Precision results for Diclofenac sodium.

Parameter	Intra-day	Inter-day
Sample sol conc	20	20
AUC (mean)	0.2181	0.3254

#### Linearity and Range:-

The linearity was determined by using working standard solutions between 05, 10, 15, 20 & 25  $\mu\text{g/ml}$ . The areas under curve (AUC) of these solutions were recorded. Calibration curve of area under curve to concentration plotted on excel sheet and linear regression was performed. The correlation coefficient, regression Equation was calculated.(Fig. 3)

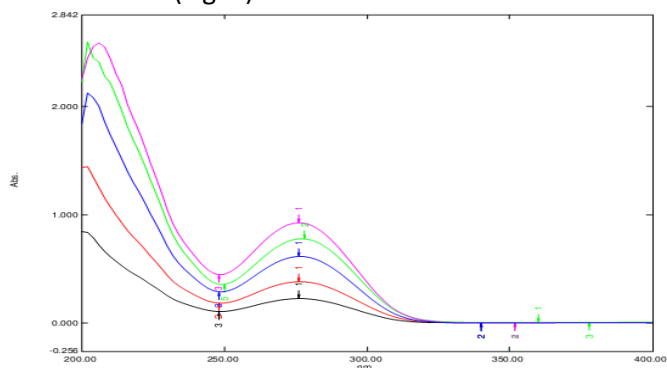


Fig:-4 Overlay of Diclofenac sodium spectra at diff. Concentration.

**Note :** (1) Spectra ----- shows 05 $\mu\text{g/ml}$ .  
 (2) Spectra - - - - - shows 10 $\mu\text{g/ml}$ .  
 (3) Spectra - . . . . . shows 15 $\mu\text{g/ml}$ .  
 (4) Spectra - - - - - shows 20 $\mu\text{g/ml}$ .  
 (5) Spectra - - - - - shows 25 $\mu\text{g/ml}$ .

#### Limit of Detection and Limit of Quantification:-

The Limit of Detection(LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula  

$$\text{LOD} = 3.3 \sigma / S$$

The Limit of Quantification(LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified. LOQ was calculated using the following formula  

$$\text{LOQ} = 10 \sigma / S$$

Where,  $\sigma$  is standard deviation of the response and  
 $S$  is the slope of the calibration curve.

LOD& LOQ of Diclofenac sodium found to be 0.9741 $\mu\text{g/ml}$  &2.9311/ml respectively.

Five sets of known concentrations (05-25 $\mu\text{g/ml}$ ) were prepared and scanned. By using these spectras, regression equations were obtained. By taking average of slopes and standard deviation of y-intercept, LOD and LOQ were calculated. The values of LOD and LOQ are given in table 4.

Table 4:-Summary of validation parameters:-

Parameter	Result
$\lambda$ range	270-282nm
Regression Equation (y=mx+c)	Y = 0.0032x+ 0.0016
Measured wavelength	276nm
Linearity range	5-25 $\mu\text{g/ml}$
Slope	0.0032
Intercept	0.0016
Correlation coefficient ( $R^2$ )	0.9981
Limit of Detection (LOD) $\mu\text{g/ml}$	0.9741
Limit of Quantitation (LOQ) $\mu\text{g/ml}$	2.9311
Accuracy (Mean % Recovery)	99.38
Precision (%RSD)	1.8073

#### RESULTS AND DISCUSSION

The UV visible spectroscopic method for the Diclofenac sodium by area under curve was found to be simple, accurate, economical and reproducible. The drug concentrations were found to be linear in the range of 05-25  $\mu\text{g/ml}$  and the correlation coefficient value of 0.9981 indicates that developed method was linear. For Precision the percent relative standard deviation (% RSD) was found to be 1.8073 while, intra-day and inter-day precision results in

terms of percent relative standard deviation values were found to be 0.21811 and 0.3254 respectively thus the method is observed as precise. The accuracy of the method was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The values of standard deviation were satisfactory and the recovery studies were close to 100%. The % RSD value is  $\leq 2$  indicates the accuracy of the method. The Limit of Detection and Limit of Quantitation values were found to be  $0.9741\mu\text{g/ml}$  &  $2.9311\mu\text{g/ml}$  respectively. The result of the analysis for pharmaceutical formulation by the developed

method was consistent with the label claim, highly reproducible and reliable. The validation parameters are summarized in Table 4. The method can be used for routine quality control analysis of Diclofenac sodium in bulk and pharmaceutical formulations.

#### CONCLUSION

The UV spectroscopic AUC method for the analysis of Diclofenac sodium was found to be simple, precise, and accurate can be used for assay of bulk drug and pharmaceutical dosage formulations.

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