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METHOD DEVELOPMENT AND VALIDATION OF DICLOFENAC SODIUM BY USING UV SPECTROSCOPY

Dr. Sanjeev Kumar Subudhi *, T. Ramya Krishna, D. Snidgha, N.Mounika,T. Hareesh Kalyan, N. Suresh

Department of Pharmaceutical Chemistry, Talla Padmavathi Pharmacy College, Orus, Kareemabad, Warangal, Telangana, India

ABSTRACT

The proposed method is simple, precise, accurate, and selective for the estimation of diclofenac sodium in bulk and in tablet dosage forms. The method is economical, rapid and do not require any sophisticated instruments. Hence it can be effectively used for the routine analysis of diclofenac sodium in bulk and in tablet dosage forms.

Key Words: Uv Spectroscopy, diclofenac sodium

***Corresponding Author:**
Dr. Sanjeev Kumar Subudhi,
Talla Padmavathi Pharmacy College,
Orus, Kareemabad,
Warangal,Telangana,India.

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INTRODUCTION

Pharmaceutical analysis is a branch of practical chemistry that involves a series of process for identification, determination, quantification and purification of a substance, separation of the components of a solution or mixture or determination of structure of chemical compounds. Drug analysis and assays play important role in the development, manufacture and therapeutic usage of drugs. This includes identification, characterization and determination of drugs in mixtures such as dosage forms. The drug analysis procedures play essential roles in pharmacokinetics of drugs in human and animals[1]. Bulk drugs are obtained by chemical synthesis, biosynthesis, isolation from plants or animals or biotechnological source.

EXPERIMENTATION

Instrumentation: UV/visible double beam spectrophotometer (Jasco Model V530) was employed with spectral bandwidth of 1nm and wavelength accuracy of ±0.3 nm, with a pair of 1 cm matched quartz cells(Optigalss).

Reagents and Chemicals: Analytical pure standard samples of diclofenac sodium were supplied as gift sample by Torque Pharma. Pvt. Ltd. Baddi (H.P.), India and used without further purification. The Pharmaceutical dosage form used in Cipla pvt. Ltd. Methanol LR grade was purchased from CDH, Rankem and Qualigens, and inbuilt distilled water was used. 20% v/v aqueous methanol was used as solvent.

Preparation of Standard Stock Solution: The stock solution of standard diclofenac sodium was prepared by dissolving approximately 5mg diclofenac sodium in 10ml 20% v/v aqueous methanol the suspension was quantitatively transferred into a 25ml calibrated volumetric flask and volume was made up to 25ml with solvent. The strength of the resulting solution will be approx.200 µg/ml.

Preparation of Calibration Curve: In a series of 10ml volumetric flasks, sufficient aliquot of the standard stock solution (200 µg /ml) were transferred and diluted with 20% v/v aqueous methanol so as to give several dilutions of 4.64 – 27.84 µg/ml. The absorbance was measured against 20% v/v aqueous methanol at 277.5nm (λmax of diclofenac sodium).

Preparation of Sample Stock Solution: Twenty tablets were powdered and weight equivalent to approx. 10 mg of diclofenac sodium was dissolved in 20 ml of 20% v/v aqueous methanol. The suspension was sonicated vigorously for 5 min to completely dissolve the remaining drug in powder. The solution after filtrations through Whattman filter paper no. 41 was quantitatively transferred to 100ml calibrated volumetric flask and the volume was then made up to 100 ml with 20% v/v aqueous methanol by

continuously washing filter paper to quantitatively transfer the total amount of drug. The strength resulting solution will be of approx.100 µg/ml.

Validation: The developed method for the estimation of DIC was validated as per ICH guidelines (ICH 1996). The described method has been validated for linearity, precision, accuracy, specificity, and robustness.

Linearity: Least square regression analysis was carried out for the slope, intercept and correlation coefficient (Table I). The linear fit of the system was illustrated graphically. The linearity range was found to be 4.08- 32.64 µg/ml Regression equation for DIC was

$$\mathbf{y = 0.0406\ x + 0.0129\ (r^2 = 0.9992).}$$

Accuracy: This experiment was performed at three levels in which sample stock solutions were spiked with standard drug solution containing 80, 100 and 120% of sample solution of the diclofenac sodium. Three replicate samples of each concentration level were prepared and the % recovery at each level (n = 3), and mean % recovery (n=9) were determined. The means of %recovery (%RSD) were found to be low values <1 (Table III). These results revealed that any small change in the drug concentration in the solution could be accurately determined by the proposed analytical methods.

Precision: The precision of the proposed method was evaluated by carrying out nine independent assays of test sample (10, 15, 20µg/ml). RSD (%) of nine assay values obtained was calculated. Intermediate precision was carried out by analyzing the samples by a different analyst with deferent reagent on same instrument. No statistically significant difference was observed. The resultant data was presented in table IV. %RSD values were not more than 2.0% in all the cases. RSD values found for all three analytical methods were well with in the acceptable range indicating that these all methods have excellent repeatability and intermediate precision.

Specificity: Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to be present in the sample matrix. It was found that the proposed method was specific because there is no interference of other active ingredients and excipients, ensuring that the peak response is due only to a single component. Based on the results, obtained from the analysis of standard drug and samples using the described method, it can be concluded that the method is specific for estimation of DIC in presence of degradants.

Robustness: The percentage recovery of DIC was good under most conditions and did not show any significant change when the critical parameters (day, time, reagent, analyst) were modified. Thus the method conditions were robust.

Assay: The validated method was applied to the determination of DIC in commercially available Reactin® (tab). Appropriate dilution of diclofenac sodium was prepared and absorbance was recorded and concentration of the drug was determined from the regression equation of standard drug. Figure 3 illustrates overlaid spectra obtained from DIC standard solution and from the assay of Reactin®. The observed concentration of DIC was found to be 50.5±0.241mg (mean±SD) for Reactin®. The results of the assay (*n* = 9) undertaken yielded 101 % (%RSD = 0.24) of label claim for DIC in Reactin®. The results of the assay indicate that the method is selective for the estimation of DIC without interference from the excipients used to formulate and produce these tablets.

Ultraviolet visible spectrum of diclofenac sodium

The ultraviolet visible spectrum (200 – 350 nm) of diclofenac sodium in acidic (0.1 N HCl), alkaline (0.1 N NaOH) and neutral (methanol) solvent systems are shown in Fig.. The maximum ultraviolet absorption of diclofenac was found at 273, 275, 279 nm in acidic (0.1 N HCl), alkaline (0.1 N NaOH) and neutral (methanol) solvent systems, respectively. The obtained spectra and maximum absorption wavelength for diclofenac sodium in acidic and alkaline solvent system was compared with the reference spectra of diclofenac sodium.

RESULTS AND DISCUSSION:

The proposed method utilizes UV/visible double beam spectrophotometer (Jasco Model V530) with spectral bandwidth of 1nm and wavelength accuracy of ±0.3 nm. The optical characteristics such as absorption maxima, beer's law limit, absorptivity, correlation coefficient (r), slope (m), y- intercept (c) were calculated are shown in table 2. The repeatability, reproducibility were found to be good as evident by the low standard deviation value (less than 2) in all cases reported in table 4. The percentage recovery values obtained were 98.339 reported in table 3. This shows that there is no interference of the excipients in the analysis. The analysis result of tablet formulations are in good agreement with the official standard reported,

S.no	Concentration (µg/ml)	Absorbance at 277.5nm
1.	4.64	0.20465
2.	9.28	0.38656
3.	13.92	0.56009
4.	18.56	0.72635
5.	23.2	0.90894
6.	27.56	1.10823

Table 1: Calibration curve of Diclofenac Sodium

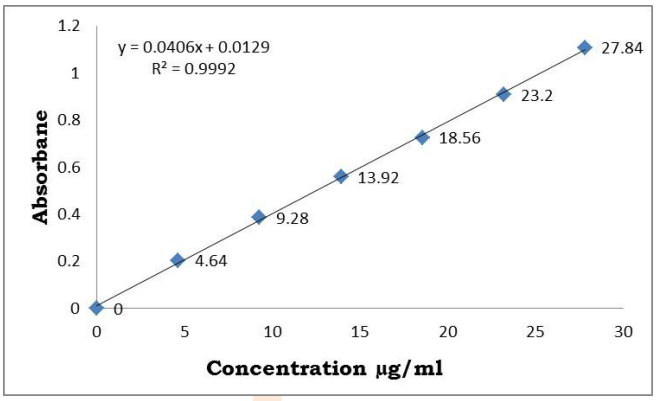


Fig 1: Calibration curve of Diclofenac Sodium.

Method development: Standard solution of diclofenac sodium was dissolved in 20 ml of 20% v/v aqueous methanol. The drugs showed maximum absorbance at 277.5 nm.

Method Validation: The method was validated according to the ICH guidelines. The following validation Characteristics were addressed: linearity, accuracy, precision, specificity and robustness.

Linearity studies: The standard curves were determined for the diclofenac sodium. The linear fit of the system was illustrated graphically. The linearity range was found to be 4.08- 32.64 µg/ml Regression equation for DIC was **y= 0.0406 x + 0.0129 (r^2= 0.9992)** (Table I)**.**

Accuracy: The validity and reliability of proposed method was assessed by recovery studies by standard addition method. Known concentration of working standard of diclofenac sodium was added to the fixed concentration of the pre-analyzed tablet solution. Percent recovery was calculated. The recovery studies were performed in triplicate. This standard addition method was performed at 80%, 100%, 120% level and the percentage recovery was calculated. The mean of %recovery (%RSD) were found to be low values (<1) for proposed method (Table III). These results revealed that any small change in the drug concentration in the solution could be accurately determined by the proposed analytical methods.

S. No.	Parameters	Result obtained
1	Beer's law limit	4.64 – 27.84 µg/ml
2	Absorption maximum	277.5 nm
3	Molar absorptivity	12935.659
4	Percent absorptivity	406.654
5	Slope	0.0406
6	Intercept	0.0129
7	Regression coefficient	0.999

Table 2: Results of accuracy experiment using proposed method

Level of % recovery	% mean estimated	S.D.	% R.S.D.
80	97.699	0.4755	0.4852
100	98.301	0.4832	0.4941
120	97.017	0.4875	0.4965

Precision: Precision study was performed to find out intra-day and inter -day variations. The precision of the proposed method was evaluated by carrying out nine independent assays of test sample. RSD (%) of nine assay values obtained was less than 2%. Intermediate precision was carried out by analyzing the samples by a different analyst on another instrument and the results are reported in terms of relative standard deviation

Table 3: Data of precision study

% R.S.D intraday	% R.S.D interdays	% R.S.D intermediate	Error
1.0455	1.4721	1.0095	0.4801

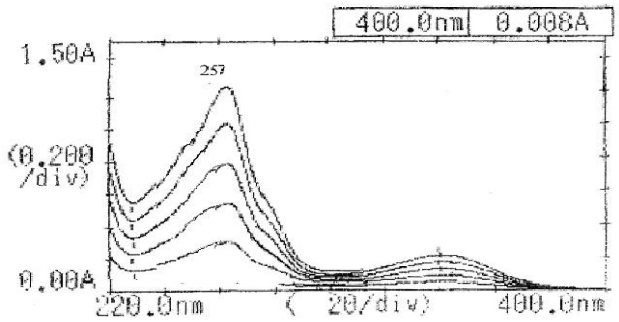
R.S.D. is relative standard deviation

Table 4: Results of commercial formulation analysis:

Drug	Label claim (Mg/tab)	% of label claim estimate data	S.D.	% R.S.D.	S.E.
DIC	50	101	0.2403	0.2414	0.0801

Average of nine determination, S.D.: Standard deviation, R.S.D. : Relative standard deviation, S.E.: Standard error

Fig 2: Ultraviolet visible spectrum of diclofenac sodium



CONCLUSION :

The proposed method is simple, precise, accurate, and selective for the estimation of diclofenac sodium in bulk and in tablet dosage forms. The method is economical, rapid and do not require any sophisticated instruments. Hence it can be effectively used for the routine analysis of diclofenac sodium in bulk and in tablet dosage forms.

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