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METHOD DEVELOPMENT FOR ESTIMATION OF DICLOFENAC SODIUM IN A CHOCOLATE DOSAGE FORM

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RP-HPLC method, validation

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ABSTRACT: A simple, rapid, precise, sensitive and reproducible Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method has been developed for the quantitative analysis of Diclofenac sodium in the chocolate dosage form. Chromatographic separation of Diclofenac sodium was achieved on waters alliance-e2695, by using waters symmetry C18, 150 mm × 4.6 mm, 3.5 µm, column and the mobile phase containing 0.1% formic acid & ACN in the ratio of 30:70% v/v. The flow rate was 1.0 ml/min; detection was carried out by absorption at 275 nm using a photodiode array detector at ambient temperature. The calibration curve was linear over the range of 5-75 µg/ml. The number of theoretical plates and tailing factor for Diclofenac sodium was NLT 2000 and were not more than two, respectively. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate & robust method for quantitative analysis of Diclofenac sodium and study of its stability.

INTRODUCTION: Diclofenac sodium (sodium; 2-[2-(2, 6-dichloroanilino)phenyl]acetate) **Fig. 1** is one of the potential NSAIDS which is commonly used as an anti-inflammatory, analgesic and antipyretic. It is used for the long term symptomatic treatment of several alignments such as osteoporosis, rheumatoid arthritis, ankylosing spondylitis. Diclofenac is rapidly and completely absorbed after oral administration, and peak plasma concentration is reached within 2-3 h. It undergoes extensive first-pass metabolism; hence only 50% of Diclofenac is available systemically. Its half-life in plasma is 1-2 h.

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It is also used for acute musculoskeletal injury, acute painful shoulder postoperative pain; dysmenorrheal [1, 2, 3]. A literature survey revealed that a number of analytical methods have been developed for the determination of Diclofenac sodium alone and in combination in various dosage forms and biological samples using HPLC, HPTLC and spectrophotometry techniques [4-13].

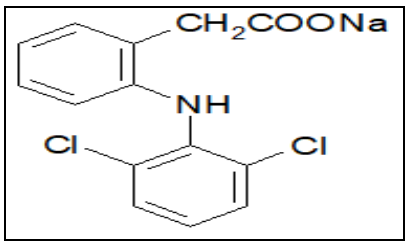

FIG. 1: STRUCTURE OF DICLOFENAC SODIUM

We have developed a new accurate and precise RP-HPLC method for the determination of Diclofenac sodium in a chocolate dosage form. The developed method is validated as per ICH guidelines [15].

MATERIALS AND METHODS:

Drugs, Chemicals, and Solvents: The reference sample of Diclofenac sodium was obtained from Glenmark pharmaceuticals. Ltd. HPLC grade water was purchased from Rankem. HPLC grade acetonitrile, triethanolamine and formic acid, pure hydrochloric acid, sodium hydroxide, hydrogen peroxide, and sodium bisulfate were purchased from Merc.

Equipment and Chromatographic Conditions: A waters alliance liquid chromatography (model 2695) monitored with empowering 2 data handling system and fitted with a Waters X-Bridge C18, (150 mm × 4.6 mm, 3.5 µm) and a diode array was used for this study. The mobile phase used consisted of 0.1% Formic acid & ACN in the ratio of 30:70% v/v. All the chromatographic runs were carried out in isocratic elution mode with a flow rate of 1ml/min, and the sample injection volume was 10 µL, detector wavelength was set at 275 nm.

Preparation of the Mobile Phase and Diluent: 0.1% formic acid and acetonitrile were mixed together in the ratio of 30:70 v/v and employed as the mobile phase. The same solution was also used as the diluents for preparing drug dilutions.

Preparation of Working Standard Solution of Diclofenac Sodium: About 50 mg of Diclofenac sodium was weighed accurately and transferred into a 100 ml volumetric flask. 70 ml of the diluents were added to it and sonicated to dissolve the drug. The volume was made up to the quantity with the diluents and mixed well. This was used as a standard stock solution. 5.0 ml of the stock solution was transferred to 50ml volumetric flask and made empowering the volume using diluents to get a 50 µg/ml of Diclofenac sodium. This was used as a working standard solution.

Calibration Curve: Solutions of various concentrations of Diclofenac sodium were prepared from the standard solution including the working standard. A volume of 10 microlitres of each concentration was injected into the HPLC system. Six replicate runs were performed at each concentration level. The response was read at 275nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak area at each concentration level was calculated. The relevant linearity graph of the mean peak area over concentration was plotted.

Estimation of the Drug from the Chocolate Dosage Form: Ten chocolates were weighed and crushed in a mortar. An amount of equivalent to about 50 mg of Diclofenac was transferred into a 10 ml volumetric flask and diluent was added to it and sonicated. The solution is filtered through a 0.45µ nylon syringe filter. 5 ml of the filtrate was transferred to 50 ml volumetric flask and made up the volume using diluents and mixed well. Five replicate injections of the sample were analyzed on the column. The mean of the peak areas of the drug was found out and the drug content in the formulation was calculated by using the regression equation obtained for the pure drug.

RESULTS AND DISCUSSION: The method development was preceded with the determination of the wavelength of Diclofenac sodium by the photodiode array. The wavelength was found to be 275 nm and is shown in **Fig. 2**. During the method optimization studies trails were carried out for an ideal separation of the drug-using different mobile phases and different chromatographic conditions. Finally, the following conditions were found to be optimum after evaluating the column efficiency by parameters like theoretical plates and tailing factor.

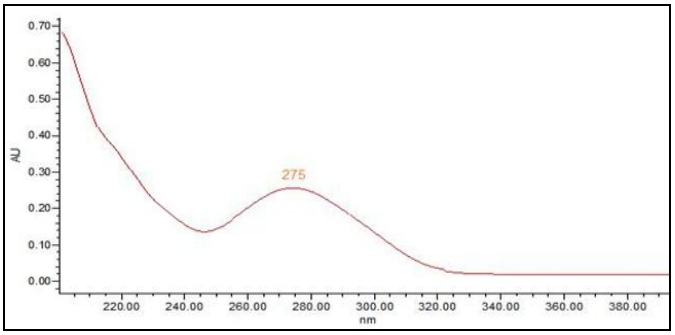

FIG. 2: PDA SPECTRUM OF DICLOFENAC SODIUM

TABLE 1: OPTIMIZED CONDITIONS FOR THE PROPOSED HPLC METHOD

Stationary phase	Waters, Symmetry C18, 150 mm × 4.6 mm, 3.5 µm
Mobile phase	ACN : 0.1% Formic acid (70: 30)
Flow rate	1.0 ml/min
Column temperature	25 °C
Injection volume	10 µl
Detection wavelength	275nm
Run time	5 min
Retention time of the drug	3.49 min

Optimum wavelength was selected by injecting a standard solution of the drug into HPLC with PDA-detector and the wavelength which gives higher response for the compound is selected. The wavelength was found to be 275 nm. Under the optimized conditions, the retention time of diclofenac sodium was found to be 3.49 min.

Specificity: The chromatograms obtained for the samples of chocolate dosage form demonstrate that no interfering peaks were found at the retention time of Diclofenac sodium due to excipients.

Linearity: The regression of the plot was computed by least squares method and is shown in **Fig. 5**. The calibration curve of the drug was linear over the concentration range of 5-75 µg/ml with the correlation coefficient 0.999 and the % RSD for each component was less than 2.

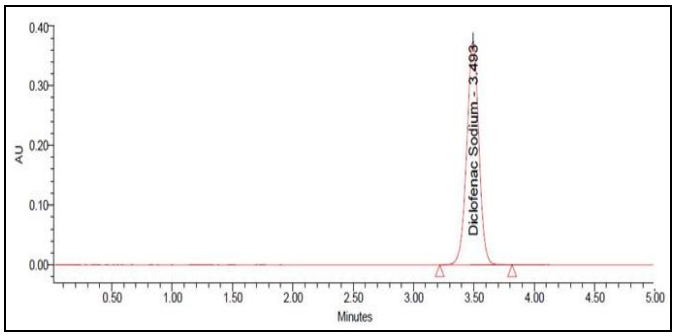

FIG. 3: CHROMATOGRAM OF STANDARD DICLOFENAC SODIUM

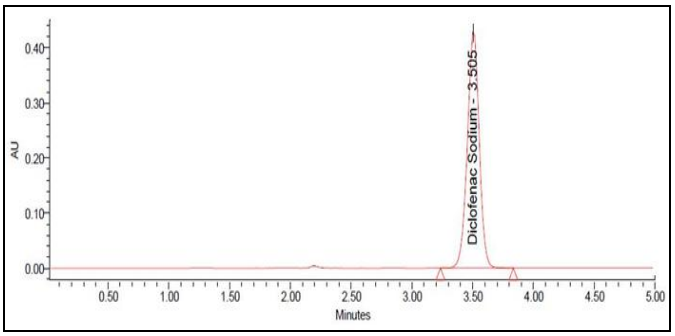

FIG. 4: CHROMATOGRAM OF DICLOFENAC SODIUM IN CHOCOLATE DOSAGE FORM

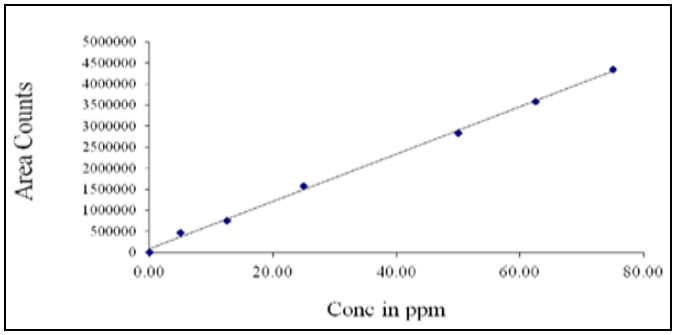

FIG. 5: LINEARITY OF DETECTOR RESPONSE GRAPHS FOR DICLOFENAC SODIUM

Accuracy and Precision: The accuracy of the method was determined by recovery experiments. Individual percentage recovery, mean percentage recovery, percentage RSD and squares correlation coefficient for linearity of the test method were calculated and the results were presented in **Table 2**. The high percentage recovery indicates that the developed method is highly accurate. The precision was studied by injecting the sample solution in six replicates and the percentage RSD was calculated and presented in **Table 3**. From the results, it was found that the developed method is precise.

TABLE 2: ACCURACY DATA OF DEVELOPED METHOD

Recovery level	Accuracy of Diclofenac sodium				
	Amount taken	Area	Avg. area	% recovery	% RSD
50%	9.6	1390583	1394534	100.2	0.28
	9.5	1398385			
	9.6	1394635			
100%	19.1	2570991	2535026	100.5	1.25
	19.2	2523255			
	19.1	2510832			
150%	28.7	3900434	3912157	99.9	0.43
	28.8	3931334			
	28.7	3904702			

TABLE 3: PRECISION DATA OF DEVELOPED METHOD

S. no.	Retention time	Area
1	3.505	2517262
2	3.495	2599401
3	3.498	2526996
4	3.499	2586363
5	3.497	2518923
6	3.507	2519405
Avg. area		2544725
St. dev		37679.086
%RSD		1.48

System Suitability: System suitability parameters were studied with six replicates of the standard sample solution and the corresponding values are presented in **Table 4**.

TABLE 4: SYSTEM SUITABILITY PARAMETERS OF DEVELOPED METHOD

Parameter	Value
Retention time (min)	3.47
Tailing factor	1.15
Theoretical plates	5417

Limit of Detection (LOD) and Limit of Quantification (LOQ): LOD and LOQ in the sample were determined with acceptable precision and accuracy. The results were presented in **Table 5**.

TABLE 5: LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION DATA

S. no.	Sample name	LOD		LOQ	
		Conc (µg/ml)	S/N	Conc (µg/ml)	S/N
1	Diclofenac sodium	0.05	7	0.5	25

Robustness: Robustness of the proposed analytical method was determined by varying flow rate and mobile phase composition. Percentage RSD was given in **Table 6**.

TABLE 6: ROBUSTNESS OF PROPOSED METHOD

Variability		% RSD
Flow rate	1.2 ml/min	0.44
	0.8 ml/min	0.61
Organic phase	77:23	0.29
	63:37	0.64

CONCLUSION: The developed RP-HPLC method is simple, sensitive, precise and accurate and can be used for the estimation of Diclofenac sodium in the chocolate dosage form for quality control analysis.

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CONFLICTS OF INTEREST: Nil

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