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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF DICLOFENAC SODIUM IN BULK AND TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT
A new RP-HPLC method for the quantitative determination of Diclofenac sodium was developed and validated as per ICH guidelines. The drugs were injected into Inertsil C18 column (250×4.6, 5 µm), maintained at ambient temperature and effluent monitored at 283 nm. The mobile phase consisted of Methanol: Acetonitrile: Water (60:20:20 V/V). The flow rate was maintained at 1.0 ml/min. The calibration curve for Diclofenac sodium was linear from 2-10 µg/ml (r^2 for Diclofenac sodium = 0.99). The proposed method was adequate, sensitive, reproducible, accurate and precise for the determination of Diclofenac sodium in bulk and pharmaceutical dosage forms.

KEYWORDS: Diclofenac sodium, Linearity, Accuracy, Validation.

INTRODUCTION

Diclofenac is a nonsteroidal anti-inflammatory drug (NSAID) taken or applied to reduce inflammation and as an analgesic reducing pain in certain conditions. In the United Kingdom, United States, India, and Brazil diclofenac may be supplied as either the sodium or potassium salt; in China, it is most often supplied as the sodium salt, while in some other countries it is only available as the potassium salt. It has a molecular formula of $C_{14}H_{10}Cl_2NNaO_2$. The IUPAC name is sodium 2-[2-(2,6-dichloroanilino)phenyl]acetate with a molar mass of 318.129 g/mol. The primary mechanism responsible for its anti-inflammatory, antipyretic and analgesic action is thought to be inhibition of prostaglandin synthesis by inhibition of the transiently expressed prostaglandin-endoperoxide synthase-2 (PGES-2) also known as Cycloxygenase-2 (COX-2). It also appears to exhibit bacteriostatic activity by inhibiting bacterial DNA synthesis. Diclofenac has a low to moderate preference to block the constitutively expressed COX-1 isoenzyme (approximately 10-fold) and is said to have, therefore, a somewhat lower incidence of gastrointestinal complaints than noted with aspirin which irreversibly inhibits COX-1. Besides the COX-inhibition, a number of other molecular targets of diclofenac possibly contributing to its pain-relieving actions like Blockage of voltage-dependent sodium channels, Blockage of acid-sensing ion channels that have recently been identified.[1-6] Various analytical methods have been reported for the estimation of Diclofenac sodium, including spectrophotometric methods and HPLC. The suggested HPTLC and HPLC methods for assay of Diclofenac sodium are quite expensive and need complex and sophisticated instrumentation. HPLC is the most widely used technique for the estimation of Diclofenac sodium in human plasma, saliva, cerebrospinal fluid, and human blood cells, as well as for studying the drug metabolites in the urine. The present research work describes a HPLC and UV spectrophotometric method for estimation of Diclofenac sodium, in API.[7-10] The present method aims at developing a simple, accurate and precise RP-HPLC method for its estimation in bulk and Pharmaceutical dosage forms.

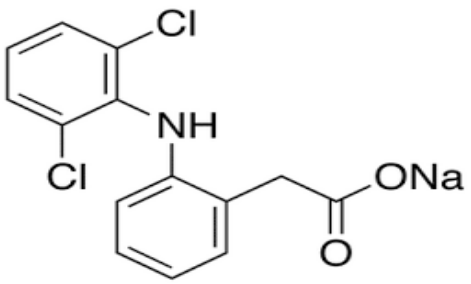

Fig. 1: Chemical structure of Diclofenac Sodium.

MATERIALS AND METHODS

Instrumentation: A YL- instrument 9300 module equipped with a UV spectrophotometer for finding out the λmax values of the drugs was used throughout this study. An Inertsil ODS 18(250×4.6, 5 mm) column was employed for the method development. The chromatographic system was monitored by Autochrome software. Analytes were monitored by UV detection at 283 nm using an isocratic mode with Methanol: Acetonitrile: water in the ratio 60:20:20 was used as mobile phase. The flow rate was set at 1.0 ml/min and effluent was monitored at 283 nm.The temperature and run time were maintained at 25°C and 5 min. respectively. Solubility of the compounds was enhanced by sonication on an ultrasonicator.

Chemicals and solvents: The reference sample of Diclofenac sodium was obtained from Yarrow chemicals, India. HPLC grade water (prepared by using 0.45 Millipore Milli –Q) was procured from Standard Reagents, Hyderabad. HPLC grade Acetonitrile, from Merck, Mumbai.

Selection of mobile phase: The objective of this experiment was to optimize the method for estimation of Diclofenac sodium based on the literature survey. Various mobile phases were tested to select the best possible system. The various mobile phases used included Acetonitrile : water (40:60) methanol and water (50:50). water: methanol (70:30), Better peak resolution and adequate retention time were obtained with the ratio of Methanol: Acetonitrile : water (60:20:20).

Preparation of Mobile Phase: The mobile phase was prepared by mixing 600 ml of Methanol, 200ml of Acetonitrile and 200 ml of water in a 1000 ml clean and dry flask. The mobile phase was then degassed using Ultra-Sonicator to remove dissolved gases and the resultant solution was filtered through a 0.45 µm membrane filter under vacuum.

Preparation of standard solution: Standard solution was prepared by accurately weighing 100 mg of Diclofenac sodium and transferring them into a 100ml clean dry volumetric flask containing mobile phase. The solution was sonicated for about 10 mins. and then made upto volume with the solution. The resultant solution was filtered through a 0.45 µm membrane filter under vacuum. From this 1 ml of solution was taken & made upto 10 ml with mobile phase. The solution was sonicated for about 10 mins and then injected.

Preparation of sample solution: Sample solution was prepared by taking 10 tablets and triturating into fine powder and accurately weighing the quantity of drug powder equivalent 100mg of Diclofenac sodium, and transferring them into a 100 ml clean dry volumetric flask containing solution. The solution was sonicated for about 10 mins. and then solution. The resultant solution was filtered through a 0.45 µm membrane filter under vacuum. From this 1 ml of solution was taken & made upto 10 ml with mobile phase. The solution was sonicated for about 10 mins and then injected.

VALIDATION

Prior to validation studies blank solution was injected and chromatogram was noted. Optimized conditions maintained were the drug was eluted with good retention time and peak area which was shown in the fig. 3.

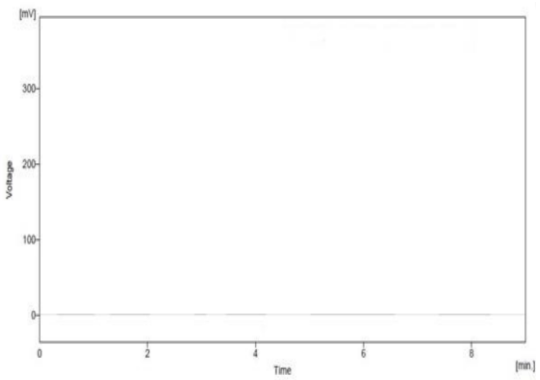

Fig. 2: Blank chromatogram

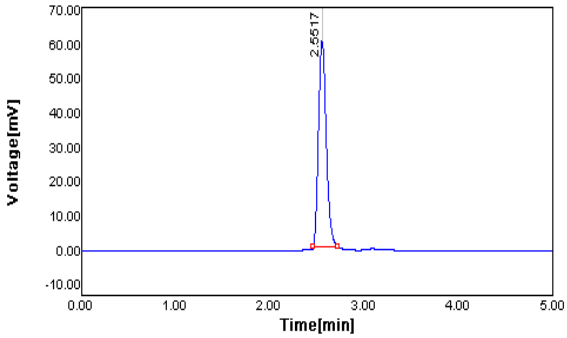

Fig. 3: Optimized Chromatogram of Diclofenac Sodium.

Linearity: The linearity of the method was established by determining the absorbance of different concentrations of Diclofenac sodium over a range of 2-10µg/ml respectively. The linearity data was given in table 1.

Table 1: Linearity data of Diclofenac Sodium.

S.No	Concentration of Diclofenac Sodium (mcg/ml)	Peak area
1	0	0
2	2	71
3	4	145
4	6	248
5	8	330
6	10	396

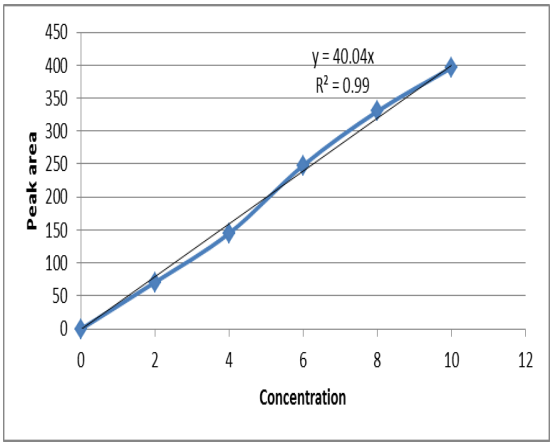

Fig. 4: Calibration curve of Diclofenac sodium.

Accuracy: To determine the accuracy of the proposed method, recovery studies were carried out by analyzing the samples were carried out by analyzing the measured concentration and the added concentration of the drug. Each sample was injected thrice. The percent recoveries of the drugs were estimated. The accuracy data was represented in table 2.

Table 2: Accuracy data of Diclofenac Sodium.

S. No	Accuracy level	% Recovery	Avg.% Recovery
1	80%	100.45	100.58
2	80%	100.85	
3	80%	100.46	
4	100%	97.65	97.62
5	100%	97.24	
6	100%	97.99	
7	120%	99.68	99.34
8	120%	99.16	
9	120%	99.20	

Precision: Precision is one of the important factors which determine the reliability of an analytical method. The precision of the developed method was tested and was found to be suitable. Both system and method precision were performed and are given in table 3,4.

Table 3: Method precision data of Diclofenac sodium.

S.No	Injection number	Retention time of Diclofenac sodium	Area of Diclofenac sodium
1	Injection 1	2.551	5316
2	Injection 2	2.548	5247
3	Injection 3	2.558	5489
4	Injection 4	2.551	5316
5	Injection 5	2.549	5285
	Average	2.551	5330.6
	Standard deviation		92.98
	% RSD		1.74

Table 4: System precision data of Diclofenac sodium

S.No	Injection number	Retention Time of Diclofenac sodium	Area of Diclofenac sodium
1	Injection 1	2.551	5316
2	Injection 2	2.548	5247
3	Injection 3	2.558	5400
4	Injection 4	2.551	5330
5	Injection 5	2.549	5285
	Average	2.551	5315.6
	Standard deviation		56.94
	% RSD		1.07

Robustness: The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like volume of injection, wavelength which may differ but the responses were still within the limits of the assay.

Table 5: Robustness data of Diclofenac sodium.

Proposed variations		Retention time	Theoritical plates	Assymetric factor
Variation in flow rate	0.9 ml	2.549	5300	1.52
	1.1ml	2.551	5316	1.67
Variation in mobile phase	50:30:20	2.551	5316	1.67
	40:20:60	2.549	5264	1.48

Ruggedness: Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions. It was checked that the results were reproducible under different analysts.

Table 6: Ruggedness data of Diclofenac sodium.

	Retention time of Diclofenac sodium	Area of Diclofenac sodium
Analyst(1)100%	2.552	5489
Analyst(2)100%	2.551	5320

Assay: Assay of different formulations available in the market was carried by injecting sample corresponding to equivalent weight into HPLC system and recovery studies were carried out.

Table 7: Assay data of Diclofenac sodium marketed formulations

Drug	Labelled claim(mg)	Drug found	% Purity
Sample 1	100mg of Diclofenac Sodium	99.6 mg of Diclofenac Sodium	99.6%
Sample 2	100mg of Diclofenac Sodium	98.5mg of Diclofenac Sodium	98.5%

DISCUSSION

In the present work, an attempt was made to provide a newer, sensitive, simple, accurate and economical RP-HPLC method. It was successfully applied for the determination of Diclofenac sodium in pharmaceutical dosage forms without the interferences of other constituents in the formulations. Different mobile phase compositions were tried, to get good optimum results. Mobile phase and flow rate selection was done based on peak parameters (height, tailing, theoretical plates, capacity factor), run time etc. The system with Methanol: Acetonitrile:Water (60:20:20) with 1.0 ml/min flow rate was quite robust.

The optimum wavelength for detection was 283 nm at which better detector response for drug was obtained. The average retention time of Diclofenac sodium was found to be 2.551mins.The calibration was linear in concentration range of 2-10 mcg/ml for Diclofenac Sodium**.** The low values of % RSD indicate the method was precise and accurate.

Ruggedness of the proposed methods was determined by analysis of aliquots from homogeneous slot by different analysts, using similar operational and environmental conditions; the % RSD. reported was found to be less than 2%. Sample to sample precision and accuracy were evaluated using, three samples of five and three different concentrations respectively, which were prepared and analyzed on same day. These results show the accuracy and reproducibility of the assay. The proposed method was validated in accordance with ICH parameters and the results of all methods were very close to each other as well as to the label value of commercial pharmaceutical formulation. There was no significant difference in the results achieved by the proposed method.

CONCLUSION

The proposed method for the assay of the Diclofenac Sodium in the commercially available dosage formulation was simple, accurate, economical, and rapid. It can be easily adopted for routine quality control for monitoring the assay in the API, in-process samples, and the finished tablet formulation.

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