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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE QUANTITATIVE DETERMINATION OF DICLOFENAC SODIUM IN PHARMACEUTICAL DOSAGE FORM

N. Sivakumar[1], A. Chenthilnathan[1*] and A.Vairamani [2]

[1]Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli – 627 012, Tamil Nadu, India.

[2] Caplin Point Laboratories Ltd., Gummidipoondi Taluk, Chennai – 601 201, Tamil Nadu, India.

***Corresponding author E-mail: ala.chenthil@gmail.com**

ABSTRACT

This paper deals with a simple, feasible and sensitive reverse-phase high-performance liquid chromatographic method for the quantitative determination of diclofenac sodium in suppositories. The chromatography was carried out by using HPLC system (Shimadzu LC2010HT) with UV- Visible dual absorbance detector (PDA), Phenomenex C18, 25 cm X 4.6 mm, 5 µm column. The mobile phase consisting of Acetonitrile and Buffer (1.0 g of Potassium dihydrogen orthophosphate in 40 ml of water and adjusted the pH to 3.0 with dilute orthophosphoric acid) in the ratio of 60:40. The mobile phase flowed at 1.0 ml/min and the detection was made at 254 nm. The retention time of diclofenac sodium was 5.91 min. Validation parameters such as system suitability, specificity, linearity, accuracy, precision robustness, ruggedness and stability were performed according to the ICH guidelines for the proposed method and the results obtained were within the limits. Hence, the method could be successfully applied for routine analysis of diclofenac sodium in pharmaceutical dosage forms.

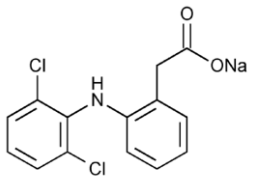
KEY WORDS

Diclofenac sodium, Suppositories, RP-HPLC, Validation.

INTRODUCTION

Diclofenac sodium (**Fig. 1**) chemically, mono sodium salt of 2- (2- (2, 6-dichlorophenylamino) phenyl) acetic acid, is a non-steroidal anti-inflammatory agent with analgesic and antipyretic properties. It is used for the treatment of mild to moderate pain, fever, inflammation such as rheumatoid arthritis, gout and osteoarthritis as a result of the blockade of synthesis of prostaglandin by inhibition of the enzyme, cyclooxygenase [1-4].

Figure 1: Structure of Diclofenac sodium



Literature survey reveals that diclofenac sodium is estimated individually or in combination with other drugs in the formulations by UV Spectrophotometry [5], HPTLC [6], Atomic absorption spectroscopy [7], Capillary electrophoresis [7] and HPLC [8, 9] methods. In this present work, an attempt was made to develop a simple, economic, feasible and sensitive reverse-phase high-performance liquid chromatographic method for the quantitative determination of diclofenac sodium in suppositories and to validate the proposed method as per ICH guidelines.

MATERIALS AND METHODS

Experimental

Chemicals and reagents

Acetonitrile, Potassium dihydrogen phosphate of HPLC grades were purchased from E.Merck (India) Ltd., Mumbai. Orthophosphoric acid of AR grade was obtained from Qualigens Fine Chemicals Ltd., Mumbai. Diclofenac sodium was a gift sample by Caplin Point Laboratories Ltd., Gummidipoondi Taluk, Chennai – 601 201, Tamil Nadu, India. The commercially available suppositories containing Diclofenac sodium 100mg were procured from the local market.

Instrumentation and chromatographic conditions

The chromatography was carried out by using HPLC system (Shimadzu LC2010HT) with UV-Visible dual absorbance detector (PDA), Phenomenex C18, 25 cm X 4.6 mm, 5 µm column. The mobile phase consisting of Acetonitrile and Buffer (1.0 g of Potassium dihydrogen orthophosphate in 40 ml of water and adjusted the pH to 3.0 with dilute orthophosphoric acid) in the ratio of 60:40. The mobile phase was pumped into the column at a flow rate of 1.0 ml/min. The chromatographic detection was monitored at 254 nm. The volume of injection loop was 20 µl prior to the injection of the drug solution; the column was equilibrated for at least 15 min. with the mobile phase flowing through the system.

Preparation of Standard solution

Weighed accurately 20 mg of diclofenac sodium WS and transferred into a 100 ml volumetric flask. Added 50 ml of mobile phase and Sonication was done for 5 minutes. Dissolved, cooled and diluted up to the volume with mobile phase. Filtered the solution through 0.45µ Nylon filter and collected the solution in an HPLC vial after discarded first 2 ml of filtrate (0.2 mg/ml of diclofenac sodium).

Preparation of Sample solution

Randomly collected 5 diclofenac sodium suppositories were weighed, mashed and then mixed. Accurately weighed the portion of the mass contain 20 mg equivalent of diclofenac sodium and transferred into a 100 ml volumetric flask. Added 50 ml of mobile phase and Sonication was done for 10 minutes. Dissolved, cooled and diluted up to the volume with mobile phase. Filtered the

solution through 0.45µ Nylon filter and collected the solution in an HPLC vial after discarded first 2 ml of filtrate (0.2 mg/ml of diclofenac sodium).

CALCULATIONS:

Content of Diclofenac sodium in mg

$$= \frac{\text{Spl Area}}{\text{STD Area}} \times \frac{\text{STD wt in mg}}{100} \times \frac{100}{\text{Spl Weight}} \times \frac{\text{STD Purity in ASB}}{100} \times \text{Average weight.}$$

Content of Diclofenac sodium in %

$$= \frac{\text{Diclofenac Sodium in mg}}{\text{The Label claim of the each suppository contains in mg}} \times 100$$

RESULTS AND DISCUSSION

All of the analytical validation parameters for the proposed method were determined according to International Conference on Harmonization (ICH) guidelines [10].

System Suitability

It is essential for the assurance of the quality performance of chromatographic system. Six injections of standard drug solutions, diclofenac sodium was given separately to the system. The system suitability parameters such as retention time, area, tailing factor and no. of theoretical plates were calculated for the standard drug solutions and mentioned in **Table 1**. It was observed that all the values are within the limits.

Table 1: System suitability for Diclofenac sodium

S. No.	Diclofenac Sodium Retention time	Area	Tailing factor	No. of Theoretical plates
1	5.91	70066681	1.13	23285
2	5.91	69997783	1.11	23124
3	5.91	69967366	1.15	23079
4	5.91	69955546	1.13	23282
5	5.91	70039804	1.13	23203
6	5.91	70016607	1.14	23112
Mean	**5.91**	**70007298**	**1.15**	**23181**
SD	**0.0027**	**42504.13522**		
RSD (%)	**0.0454**	**0.061**		

Specificity

The specificity of the HPLC method is illustrated in **Fig. 2**, where a complete separation of diclofenac sodium was noticed in presence of other inactive excipients used in suppositories. In addition, there was no any interference at the retention time of in the chromatogram of placebo solution. In peak purity analysis with PDA, purity angle was always less than purity threshold for the analyte. This shows that the peaks of analyte were pure and excipients in the formulation does not interfere the analyte. The data were presented in the **Table 2**.

Table 2: Specificity for Diclofenac sodium

S.No.	Name	No. of Injections	Area
1.	Blank	1	Nil
2.	Placebo	1	Nil
3.	Standard	1	69967366
4.	Sample	1	69955546

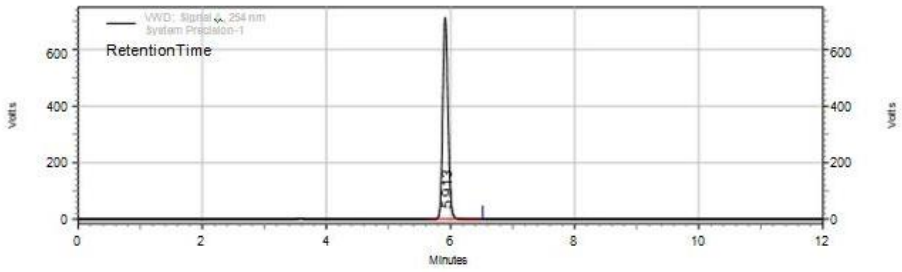


Figure 2 : Typical HPLC Chromatogram of Sample Suppositories (Diclofenac sodium)

Linearity

The Linearity of this method was determined at five levels from 10%– 200% of operating concentrations for diclofenac sodium and it was shown in **Table 3**. The plots of peak area of each sample against respective concentrations of diclofenac sodium were found to be linear (Figure 3) in the range of 10%– 200% of operating concentrations. Beer's law was found to be obeyed over this concentration range. The linearity was evaluated by linear regression analysis using least square method. The regression equations were found to be Y= 356157x +287664 for diclofenac sodium and correlation coefficient of the standard curves were found to be 0.9998 for diclofenac sodium. It observed that correlation

coefficient and regression analysis are within the limits.

Table 3: Linearity of response for Diclofenac sodium

Linearity level concentration (%)	Concentration of Diclofenac Sodium (µg/ml)	Linearity Sample Area		
		Area-1	Area-2	Average of area
10	20	7232643	7240908	7236776
20	40	14294558	14475615	14385087
50	100	34429502	34451308	34440405
100*	200	69899134	69802361	69850748
120	240	86130049	86339176	86234613
160	320	113758211	113900531	113829371
200	400	142073521	142199928	142136725

* Operating concentration

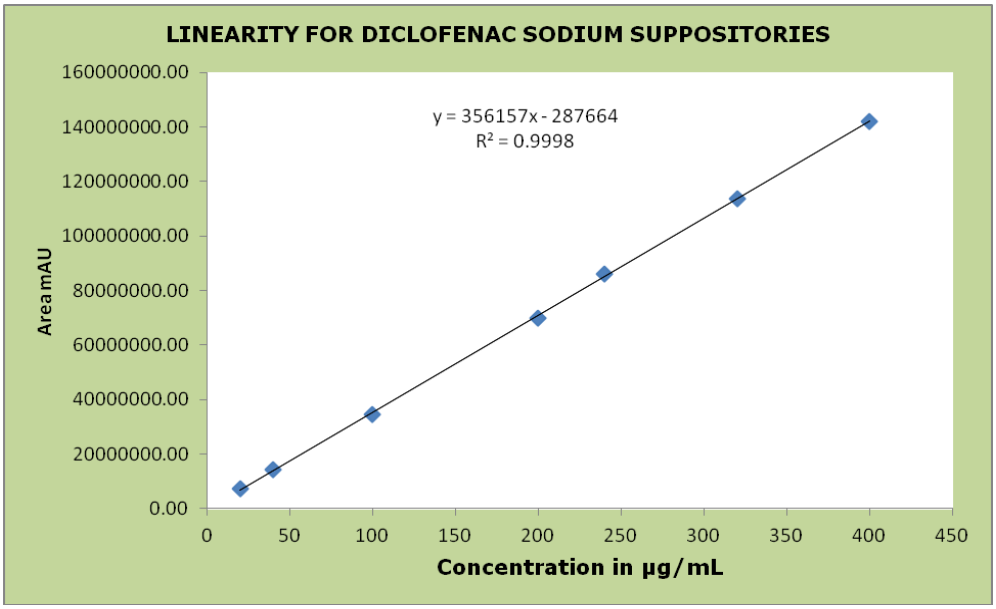


Figure 3: Linearity of response for Diclofenac sodium

Accuracy

Accuracy of the method was found out by recovery study by standard addition method. The known amounts of standard, diclofenac sodium was added to pre-analysed samples at a level from 80% up to 120% and then subjected to the proposed HPLC method individually. The results of recovery studies were shown in **Table 4**. It was observed that the mean percentage recoveries were found to be for diclofenac sodium which demonstrated that the method was highly accurate.

Table 4: Accuracy for Diclofenac sodium

S.No.	Target level	Area	Drug Recovery (%)
1.	80%	0.1612	0.1617
2.	80%	0.1622	0.1611
3.	80%	0.1640	0.1627
4.	100%	0.2027	0.2033
5.	100%	0.2012	0.2013
6.	100%	0.2058	0.2049
7.	120%	0.2425	0.2416
8.	120%	0.2413	0.2414
9.	100%	0.2417	0.2406
		Mean	**0.2020**
		SD	**0.034**
		RSD %	**17.02**

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the homogenous sample under the prescribed conditions.

Repeatability

Repeatability is the precision of a method under the same operating conditions over a short period of time. One aspect of this is instrumental precision and a second aspect is sometimes termed intra-assay precision and involves multiple measurements of the same sample by the same analyst under the same condition. The repeatability data for diclofenac sodium was shown in **Table 5**. This indicated that method was highly precise.

Table 5: Precision – Repeatability for Diclofenac sodium

No. of Sample preparation	Samples Area			Results obtained	
	Sample -1	Sample -2	Average	Amount of drug present (mg)	Percentage of drug
1	72226327	72203005	72214666	102.21	102.21
2	71857802	71796078	71826940	102.81	102.81
3	71032187	71277787	71154987	102.35	102.35
4	71126605	71200417	71163511	102.12	102.12
5	71772324	71719686	71746005	102.41	102.41
6	71358330	71290139	71324235	101.65	101.65
				Mean	**102.26**
				STD	**0.38**
				RSD (%)	**0.37**

Robustness

Measure of method's capacity to remain unaffected by small, but deliberate variations in method. A deliberate plus and minus changes in the analytical method parameters in wavelength, mobile phase composition and flow rate were altered and the assay was done as per the procedure. It was shown in **Table 6**. It was observed that there were no marked changes in the chromatograms, which demonstrated that the proposed method was robust.

Table 6: Robustness data for Diclofenac sodium

S.No.	Change of parameter	Amount of drug present (mg)	Drug Recovery (%)
1.	Wavelength (- 2) 252 nm	102.65	102.65
2.	Wavelength (+2) 256 nm	103.17	103.17
3.	Flow rate (- 2) 0.8mL	102.32	102.32
4.	Flow rate (- 2) 1.2mL	102.67	102.67
5.	Mobile phase Composition (-5 %)	102.49	102.49
6.	Mobile phase Composition (+5 %)	103.63	103.63

Ruggedness

Six sample preparations were analyzed as per the methodology by a different analyst on a different instrument on a different day. The robustness data for diclofenac sodium was shown in **Table 7**. It was observed that there were no marked changes in the chromatograms, which demonstrated that the proposed method was ruggedness.

Table 7: Ruggedness data for Diclofenac sodium

S.No.	Samples Area			Amount of drug present (mg)	Drug Recovery (%)
	Sample -1	Sample -2	Average		
1.	70736869	70524549	70630709	102.94	102.94
2.	70542890	70390433	70466662	102.38	102.38
3.	69620965	69687204	69654085	102.22	102.22
4.	70384778	70422318	70403548	102.98	102.98
5.	70316123	70331184	70323654	102.58	102.58
6.	69481555	69547881	69514718	101.72	101.72
				Mean	**102.47**
				SD	**0.4745**
				RSD (%)	**0.46**

Stability

Standard and sample solutions to be used in the analytical method were scrutinized for their solution's stability. This study was performed by injecting standard and sample solution for the period of 24 hours and results were presented in the **Table 8 and 9**. It was found that there were no remarked changes in the system suitability parameters.

Table 8: Stability results obtained for Diclofenac sodium standard solution

S. No.	Time point	Standard solution area	Results obtained		
			Cumulative % RSD	Tailing factor	Theoretical plate
1.	0 hour	68037663		1.127	23055
2.	1st hour	68024122	0.014	1.127	23049
3.	2nd hour	68116463	0.014	1.153	23059
4.	3rd hour	68224588	0.073	1.138	23303
5.	4th hour	68232102	0.145	1.112	23330
6.	5th hour	68303775	0.168	1.144	23226
7.	6th hour	68342963	0.185	1.111	23360
8.	7th hour	68404836	0.206	1.141	23313
9.	8th hour	68462007	0.228	1.114	23391
10.	9th hour	68491214	0.228	1.133	23353
11.	10th hour	68563270	0.267	1.143	23321

12.	11th hour	68616173	0.288	1.131	23327
13.	12th hour	68694483	0.316	1.136	23357
14.	13th hour	68734892	0.339	1.131	23400
15.	14th hour	68789661	0.362	1.153	23254
16.	15th hour	68809725	0.380	1.127	23387
17.	16th hour	68846297	0.380	1.152	23169
18.	17th hour	68994939	0.428	1.147	23331
19.	18th hour	69036106	0.455	1.155	23417
20.	19th hour	69253222	0.504	1.114	23392
21.	20th hour	69338300	0.552	1.129	23398
22.	21st hour	69496218	0.608	1.129	23360
23.	22nd hour	69564244	0.658	1.139	23408
24.	23rd hour	69813509	0.728	1.156	23179
25.	24th hour	69860130	0.786	1.135	23045

Table 9: Stability results obtained results obtained for Diclofenac sodium sample solution

S. No.	Time point	Standard solution area	Results obtained		
			Cumulative % RSD	Tailing factor	Theoretical plate
1.	0 hour	73625865		1.138	22886
2.	1st hour	73510774	0.111	1.135	23104
3.	2nd hour	73379313	0.168	1.155	23091
4.	3rd hour	73804580	0.245	1.122	23243
5.	4th hour	73110288	0.356	1.133	23268
6.	5th hour	73619447	0.327	1.126	23361
7.	6th hour	73713397	0.317	1.134	23273
8.	7th hour	73803628	0.320	1.132	23339
9.	8th hour	73053114	0.380	1.144	23296
10.	9th hour	73514203	0.359	1.127	23308
11.	10th hour	73999020	0.358	1.131	23360

12.	11th hour	74299078	0.475	1.125	23368
13.	12th hour	74575159	0.579	1.149	23312
14.	13th hour	74710023	0.667	1.151	23306
15.	14th hour	74842195	0.744	1.127	23411
16.	15th hour	74982725	0.816	1.141	23267
17.	16th hour	75078649	0.878	1.155	23392
18.	17th hour	75213118	0.937	1.143	23373
19.	18th hour	75325049	0.992	1.120	23381
20.	19th hour	75463248	1.047	1.123	23349
21.	20th hour	75587085	1.100	1.133	23379
22.	21st hour	75699912	1.151	1.157	23338
23.	22nd hour	75866295	1.205	1.151	23316
24.	23rd hour	76180462	1.276	1.122	23236
25.	24th hour	76107790	1.325	1.124	23075

CONCLUSION

The Proposed study describes a simple, feasible and sensitive reverse-phase high-performance liquid chromatographic method for the quantitative determination of diclofenac sodium in suppositories dosage form. The method was validated as per ICH guidelines and found to be simple, sensitive, accurate and precise. Therefore the proposed method can be successfully used for the routine analysis of diclofenac sodium in solid dosage form without interference.

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REFERENCES

[1]. Indian Pharmacopoeia, Vol. II, The Controller Publication. Govt. of India, New Delhi, 2010. p. 1199.

[2]. British Pharmacopoeia, Vol. I, The British Pharmacopoeia Commission, London, 2010. p. 672.

[3]. Sundaram PS, Phanindra SS, Thiyagarajan SN, Aanandhi MV (2008) Validated RP-HPLC Method for the Simultaneous Determination of Diclofenac Potassium and Tizanidine Hydrochloride in Tablet Dosage Form. Trade Sci Inc 7:6.

[4]. Emami J, Ghassami N, Talari R. A rapid and sensitive modified HPLC method for

determination of diclofenac in human plasma and its application in pharmacokinetic studies. DARU 2007; 15 suppl 3: 132-138.

[5]. Khaskheli AR, Abro K, Sherazi ST, Afridi HI, Mahesar SA, Saeed M. Simpler and faster spectrophotometric determination of diclofenac sodium in tablet, serum and urine samples. Pak J Anal Environ Chem 2009; 10:53-58.

[6]. Dhaneshwar SR, Bhusari VK. Validated HPTLC method for simultaneous quantitation of diclofenac sodium and misoprostol in bulk drug and formulation. Asian J Pharm Bio Res 2011; 1:15-21.

[7]. Wei X, Ding L and Gao J. Rapid and sensitive chromatography, atomic absorption spectroscopy and capillary electrophoresis method for the determination of diclofenac sodium in human plasma: method and clinical applications. Journal on Chromatograph. 2004; 39:309–311.

[8]. Chan KKH, Vyas KH, Wnuck K. A rapid and sensitive method for the determination of diclofenac sodium in plasma by HPLC. Anal Lett 1982; 15:1649-1663.

[9]. Gowramma B, Rajan S, Muralidharan S, Meyyanathan SN, Suresh B. Validated HPLC method for simultaneous estimation of paracetamol and diclofenac in pharmaceutical formulation. Int J ChemTech Res 2010; 2:676-680.

[10].ICH guidelines, *analytical method validation* (Q3). Geneva, July 2000.