Development and Validation of Hplc-Dad Method for Diclofenac Sodium Assay from New Orodispersible Tablets with Co-Processed Excipients

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The aim of this study is to develop and validate a reliable, fast, and precise High-performance liquid chromatography (HPLC) method for the assay of diclofenac sodium (DIC) from previously optimized new orodispersible tablets (ODTs) developed with co-processed excipients.The method was conducted on an HPLC Agilent 1200, Zorbax C18 column, mobile phase of orthophosphoric acid solutions 0.1%, acetonitrile and methanol in the ratio (40:50:10 v/v/v) with a flow rate of 1.5 mL/min with isocratic elution and a total run time of 5 min. Detection of diclofenac sodium was carried out at 276 nm. The method was validated for linearity, precision, accuracy, robustness as per international guidelines. The developed method was found to be accurate, precise, fast, without interference from the co-processed excipients and can be useful for routine quality control analysis of diclofenac sodium in ODTs.

Keywords: HPLC-DAD, validation, diclofenac sodium, co-processed excipients, orodispersible tablets

Diclofenac sodium (DIC) is sodium 2-[(2,6-dichlorophenyl) amino]phenil-acetate is among the most commonly prescribed non-steroidal anti-inflammatory drug (NSAIDs) due to its anti-inflammatory, antipyretic, and analgesic effects [1,2]. Contrary to the action of many traditional NSAIDs, DIC inhibits cyclooxygenase (COX)-2 enzyme with greater potency than it does COX-1 [3, 4] *in vitro* and *in vivo,* thus decreasing the production of prostaglandins, prostacyclines and thromboxanes. It is widely used in management of mild to moderate pain, when inflammatory is also present as in cases of rheumatoid arthritis, osteoarthritis [5], ankylosing spondylitis, gouty arthritis [6], tendinitis, and bursitis [7], and certain nonrheumatic conditions, and in other inflammatory painful conditions such as postoperative pain, treatment of dysmenorrhea, back pain, sciatica [8, 9].

As for the existing dosage forms for diclofenac sodium there are tablets, suppository, injections and orodispersible tablets. Orally disintegrating tablets (ODTs) are dosage forms which disintegrate in mouth within seconds without need of water. This type of quality in dosage form can be attained by addition of different varieties of excipients, co-processed excipients system which allows rapid disintegration and low adhesion to punches [10]. As a result of optimizing the formulation of orodispersible tablets (ODTs) with diclofenac sodium and co-processed excipients, Pharmaburst™ 500 and Prosolv® ODT [10,11] the following obtained formulas (table 1) were further subjected to *in vitro* qualitative and quantitative diclofenac sodium evaluation.Pharmaburst™500 is a mixture of co-processed excipients consisting of crospovidone, mannitol, sorbitol and precipitated silicon or Aerosil 200. It is easy to use, it dissolves rapidly.

Pharmaburst™ 500 has been specifically developed for optimal performance in terms of robustness (compactness and friability), superior organoleptic characteristics and rapid disintegration time [10].

Prosolv® ODT, co-processed excipient, high functionality is a mixture of microcrystalline cellulose, colloidal silicon dioxide, mannitol, fructose and crospovidone. It performs the functions of disintegrant, due to crospovidone considered as a super-disintegrant, diluent, sweetener (mannitol), sliding (silicon dioxide), agglutinate (through fructose) [11].There is not a single chromatographic method of separation and assay for diclofenac sodium in the literature. Some of the published methods describe separations of mixtures of diclofenac sodium with substances from other therapeutic classes,assay of diclofenac from pharmaceutical raw materials, from pharmaceutical dosage forms, and from biological samples (especially blood, plasma, urine).For this purpose,spectrophotometric [12-13], potentiometric [14,15], capillary zone electrophoresis [16,17], liquid chromatography with UV detection [18-20], with electrochemical detection [21],

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liquid chromatography- mass spectrometry [22], spectrofluorometry [23], thin layer chromatography [24], gas chromatography - mass spectrometry techniques are used [25]**.** In this regard, the aim of this study was to develop and validate a reliable, fast, and precise HPLC method for the determination and assay of DIC in optimized new orodispersible tablets (ODTs) with co-processed excipients.

Table 1
OPTIMAL FORMULATIONS OF ORODISPERSIBLE TABLETS WITH DICLOFENAC SODIUM 25 mg

Components	**Amount (mg)**	
	A (Pharmaburst^{TM} 500)	B (Prosolv® ODT)
Diclofenac sodium	25	25
Pharmaburst^{TM} 500	100	-
Prosolv® ODT	-	113
Avicel PH 102	13,6	-
Magnesium stearate	1,4	0,04
Total tablet mass	140	138,04
Compression force (kN)	10	15

Experimental part

Materials and methods

Reagents

All solvents were HPLC grade and all reagents were analytical grade. Diclofenac sodium (DIC) pure drug was obtained from Amoli Organics Pvt. Ltd. India, Methanol, acetonitrile, orthophosphoric acid were obtained from Lab Scan (Germany), bidistilled water obtained with a Milli-Q water purification system (Millipore, Watford, England) with a minimum resistivity of 18.2 MΩ and a maximum content of organic compounds TOC maximum 30 ppb; 240 mm quantitative filter paper (Schleicher & Schuell, Germany).

HPLC method

The method was conducted on an HPLC Agilent 1200, with quaternary pump (G1311A), degassing system (G1322A), thermostat (G1316A), autosampler (G1329A), C18 type column (150 x 4.6) 5µm XDB-C18 Agilent (Zorbax Eclipse XDB-C18); pore size 80Å; surface area 180m2 / g; boundary = 60 ° C, pH = 2-9; double-headed ends; 10% bound carbon and DAD detector (G1315), Germany; Chem Station data acquisition and processing program; software for statistical data processing Microsoft Excel 2010 software.

Chromatographic conditions

The mobile phase was ortophosphoric acid solution 0.1%, acetonitrile and methanol in the ratio (40:50:10, v/v/v) under isocratic conditions. The oven temperature was controlled at 25°C. The flow rate was 1.5 mL/min, and the injection volume of the standard and sample solutions were set at 20 µL. UV detection was performed at 276 nm and DAD was employed for determination of peak purity.

Standard solution preparation

A stock solution of DIC was prepared in methanol HPLC-grade to obtain a concentration of 400 µg/mL by dissolving 20 mg DIC in a 50 mL volumetric flask. The solution was kept in a refrigerator at 5°C. Working DIC standard solutions within the range of (6-200) µg/mL were prepared by diluting different volumes of the stock solution into 100 mL volumetric flask with methanol.

Different working standard solutions of DIC, were filtered through 0.45µm membrane filter (Millipore, Milford, MA, USA) before injection into HPLC system.

Validation

The optimized HPLC method was validated as per ICH, FDA, E.P.9.0 guidelines for specificity, linearity, accuracy, precision, and robustness [26-32].

Specificity

The specificity was demonstrated by the HPLC chromatograms recorded for the blank, standard solution, sample solution of DIC in pharmaceutical formulation. Peak purity was checked in all the chromatograms using the diode array detector. Peak purity analysis is an evaluation for detecting the presence of coeluting impurities in HPLC data.

Linearity, LOD, and LOQ: The linearity of the proposed HPLC method was evaluated by analysing three different standard calibration curves prepared daily for three consecutive days. The linearity was determinated at 8 concentration levels measured in triplicate ranging from 6-200 μg/mL. Each set of reference solutions was injected into the column from lowest to highest concentration. Peak area of DIC was plotted against their respective concentrations on a Cartesian axis. The results were processed in Microsoft Excel. The linearity was established by linear regression analysis (least square regression method) and the validity of the method was verified by means of the one-way ANOVA (α=0.05).

The limit of detection (LOD) and the limit of quantification (LOQ) were determined from three standard calibration curves and were calculated using the equations :

LOD = SE/s × 3 (1),

LOQ = SE/s × 10, (2),

where SE is standard error of the intercept, *s* is regression slope.

Precision

The precision of the method was performed at two levels, repeatability and intermediate precision. To determine the intra-day precision, 50 μg/mL of DIC was prepared six times separately and analyzed on the same day and corresponding responses of six times were evaluated. Mean, standard deviation and % RSD (relative standard deviation) were calculated to evaluate repeatability. The inter-day precision was determined by analysing 50 μg/mL of DIC from fresh sample solutions. The samples were injected 6 times in three different days under the same chromatographic conditions. We determined mean, standard deviation and %RSD.

Accuracy

The accuracy of the proposed method was determined by recovery studies. The percent recovery of DIC was established on three spiked concentration levels (80%, 100%, and 120%) around the test concentration (50 μg/mL) of DIC by standard addition method. Each concentration samples were analysed in triplicate. The percent recovery and RSD were calculated for each of the replicate samples.

Recovery solutions were prepared by taking 2.0, 2.5, and 3.0 mL aliquots of working standard solutions (100 μg/mL) into three separate 10 mL volumetric flasks, each containing 2.5 mL (100 μg/mL) of sample solutions. Volumes were made up using methanol to obtain total concentration of 45, 50, and 55 μg/mL for 80%, 100%, and 120% solutions, respectively.

Robustness

Robustness of the HPLC-DAD method was demonstrated by evaluation of the effect of different chromatographic parameters on the concentration of DIC. Factors such as flow rate, proportions of mobile phase and injection volume were studied. Working solutions and working samples (concentration of DIC 50 μg/mL) were analysed for each change.

HPLC Assay of diclofenac sodium from orodispersible tablets (ODTs)

After method validation, in order to prove suitability of the method, were analyzed samples from new orodispersible tablets with final concentration of 25 μg/mL DIC.

The test was performed according to the noncompendial HPLC method, modified and validated and the admissibility limits for diclofenac sodium in new orodispersible tablets ranged between 90.0 - 110.0%, from the claimed content [32].

HPLC determination of diclofenac sodium from ODTs tablets with diclofenac sodium and Pharmaburst ^{TM500} (formula A) was performed under the same chromatographic conditions as for tablet B (Prosolv ODT).

Twenty new orodispersible tablets were individually weighed and ground to obtain a homogeneous mixture. An amount of powder equivalent to 1.44 mg of diclofenac sodium was transferred to a 10 mL volumetric flask, dissolved in 2 mL methanol and were completed with the same solvent, resulting a theoretical concentration of 25 μg / mL.

A number of 6 such solutions have been prepared. Using the working procedure described above, after equilibrating the chromatographic column with the mobile phase for 60 minutes, 20 μL of each sample was injected and the chromatograms were detected with ultraviolet detection at 276 nm.

HPLC assay of diclofenac sodium from ODTs with diclofenac sodium and Prosolv ODT (formula B)

Preparation of solutions

Preparation of standard diclofenac sodium solution (0.6 mg / 100mL): 0.6 mg diclofenac sodium (CRS according to the European Pharmacopoeia) [32] is brought to a 100 mL volumetric flask and dissolved in 20 mL methanol, then completed with the same solvent.

Sample preparation

Twenty new orodispersible tablets wereindividually weighed and ground to obtain a homogeneous mixture. An amount of powder equivalent to 2 mg of diclofenac sodium was transferred to a 10 mL volumetric flask, dissolved in 2 mL methanol and were completed with the same solvent, resulting a theoretical concentration of 25 μg / mL.

A number of 6 such solutions have been prepared using the working procedure described above, after balancing the chromatographic column with the mobile phase for 60 minutes, 20 μL of each sample was injected and the ultraviolet detection chromatograms were recorded at 276 nm.

Diclofenac sodium assay from ODTs, formulas coded A and B was done by UV spectrophotometric method, at the concentration of 0.001% diclofenac sodium solution in 96% ethyl alcohol at λ = 276 nm.

Results and discussions

HPLC method optimization for diclofenac assay

During preliminary investigations, mobile phase composition and flow rate of the mobile phase were optimized. Several mobile phases containing orthophosphoric acid solutions 0.1%, acetonitrile and methanol were investigated where the composition of the organic phase varied from 60–80 %. The best result was obtained using mobile phase containing orthophosphoric acid solutions 0.1%, acetonitrile and methanol 40:50:10 (v/v/v). The flow rate was investigated in the range from 0.7–2.0 mL/min and the final flow rate was set at 1.5 mL/min.

Method validation

The following method validation characteristics were addressed for DIC: linearity, limit of detection, limit of quantification, accuracy, precision, specificity, and robustness.

Specificity

The specificity was carried out by injecting blank, standard solution, sample solution of DIC. A representative chromatogram (fig. 1) was generated to show that the other components, which could be present in the sample matrix, showed no interference of standard and sample in the blank preparation. The retention time was 4.444 for samples with DIC in pharmaceutical formulation, 4.511 for standard solution with DIC pure. No significant changes in retention times of the drugs in the presence and the absence of excipients clearly indicated the specificity of the method**.**

Peak homogeneity or purity of DIC in all samples was checked by using a diode array detector for the HPLC method. The purity angle was within the purity threshold limit in all samples, indicating that no additional peaks were coeluting with each of the analytes and evidencing the ability of the method to assess the analytes of interest in the presence of potential interferences (fig. 2). Baseline resolution was achieved for all investigated compounds.

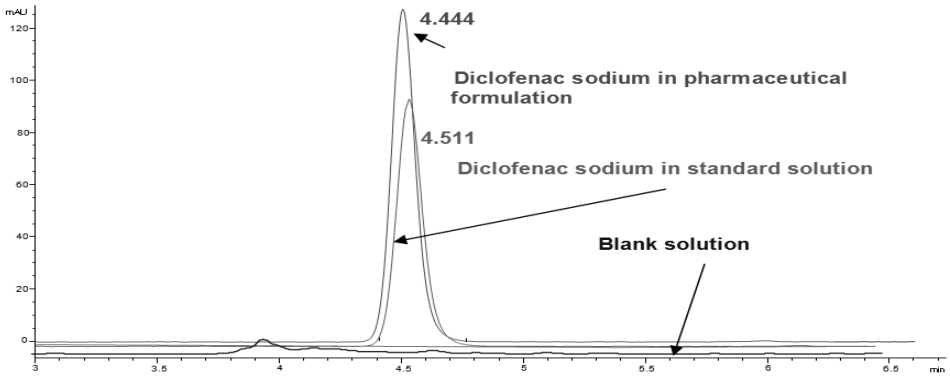

Fig. 1. The chromatograms of blank, standard solution, and DIC in pharmaceutical formulation

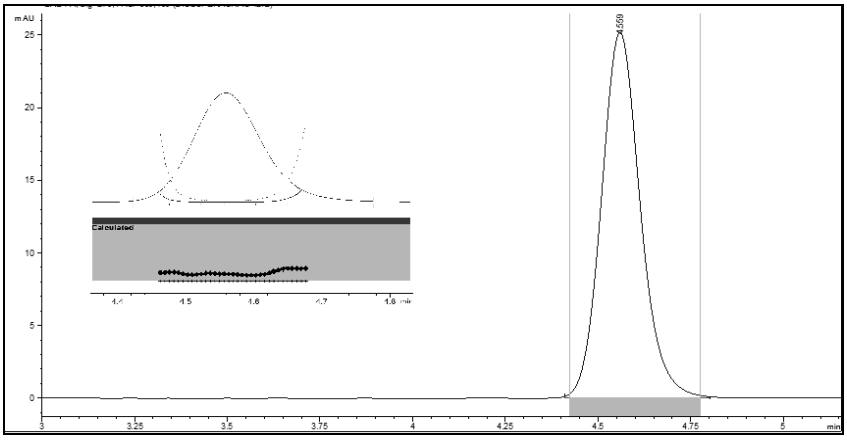

Fig. 2. Purity plot of DIC

Linearity, LOD, and LOQ

The calibration curve of DIC showed good linearity over the concentration range with a correlation coefficient (r) of 0.9993. The statistical data of the regression equations is presented in table 1.

The LOD is the smallest concentration of the analyte that gives a measurable response. The LOD for DIC was 8.04 µg/mL. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified. The LOQ was found to be 26.81 µg/mL.

These results showed adequate sensitivity for the analytical assay and this method could be used for de determination of very small concentrations of DIC in pharmaceutical formulation.

Precision

The precision of an analytical method is defined as the closeness of agreement between a series of measurements obtained from multiple sampling under the prescribed conditions, and it is normally expressed as the relative standard deviation.

The results of repeatability and intermediate precision testing showed that the suggested method is precise within the acceptable limits. The RSD were calculated for DIC, all the results are within limits. The precision was not more than 2%RSD, as are summarized in table 3.

Accuracy

The results of accuracy testing showed that the method is accurate within the acceptable limits. The percentage recovery and RSD were calculated, all the results are within limits. Acceptable accuracy was within the range of 98.31% to 102.52% recovery and not more than 2.0% RSD, as shown in table 4.

Table 2
STATISTICAL DATA OF THE REGRESSION ANALYSIS, LOD, AND LOQ

Parameter	**Values for Diclofenac sodium**
n	8
λ (nm)	276
Calibration range (µg/mL)	6.0 – 200.0
Slope (s)	1.4685
Intercept (i)	2.7617
Regression coefficient (r)	0.9993
Coefficient of determination (r^2)	0.9987
Standard error of the intercept (SE)	3.99
LOD (µg/mL)	8.043
LOQ (µg/mL)	26.81

n represents number of total measurements

Table 3
RESULTS OF INTRA-DAY AND INTER-DAY PRECISION OF DICLOFENAC SODIUM BY THE PROPOSED METHOD

Standard concentration (µg/mL)	**intra-day precision**		**inter-day precision**					
			1st day		**2nd day**		**3rd day**	
	Ammount Measured (µg/mL)	Recovery (%)	Ammount Measured (µg/mL)	Recovery (%)	Ammount Measured (µg/mL)	Recovery (%)	Ammount Measured (µg/mL)	Recovery (%)
50	51.22	102.44	50.52	101.04	51.47	102.95	49.81	99.63
	51.80	103.60	50.77	101.54	51.22	102.45	50.99	101.98
	51.83	103.66	52.07	104.14	51.54	103.09	50.61	101.22
	52.07	104.14	51.87	103.75	51.30	102.60	49.55	99.11
	52.42	104.84	52.04	104.08	51.67	103.35	49.21	98.42
	52.26	104.52	52.11	104.22	49.30	98.61	49.32	98.65
Mean (µg/mL)	51.93		51.56		51.09		49.91	
SD	0.42		0.72		0.88		0.72	
RSD (%)	0.82		1.40		1.74		1.45	

Table 4
RESULTS OF THE ACCURACY OF THE PROPOSED METHOD

Replicate number	Actual concentration (µg/mL)	Calculated concentration (µg/mL	Recovery (%)	Mean recovery (%)	SD (n=3)	RSD (%)
1	45	43.75	97.22	98.31	1.09	1.11
2		44.24	98.32			
3		44.73	99.40			
1	50	51.86	103.73	102.52	1.04	1.02
2		51.01	102.02			
3		50.91	101.82			
1	55	54.81	99.66	101.06	1.54	1.52
2		56.50	102.72			
3		55.44	100.80			

Table 5
RESULTS OF THE ROBUSTNESS OF THE PROPOSED METHOD

Parameters	Value	Recovery (%)	Mean recovery (%)	RSD (%)
Mobile phase (v/v/v)	38:51:11	97.22	101.78	1.39
	40:50:10	98.32	99.57	1.14
	41:49:9	99.40	98.77	3.28
Flow rate (mL/min)	1.4	103.73	102.65	1.57
	1.5	102.02	102.26	1.22
	1.6	101.82	102.87	1.81
Injection volume (µg/mL)	15	99.66	101.59	1.46
	20	102.72	100.91	1.07
	25	100.80	98.26	1.19

Robustness

The robustness of proposed method was performed by applying little deliberately changes of different chromatographic parameters on the concentration of DIC: flow rate, proportions of mobile phase and injection volume. Obtained data were evaluated by calculating percent of recovery and %RSD. Significant differences were not observed in chromatographic parameters. The robustness results for the proposed method are reported in table 5.

Assay of DIC from ODTs A and B formulas

The proposed validated method was successfully applied to determine DIC in diclofenac sodium ODT.

The sample concentration was calculated using the calibration equation, and the results are presented in table 6 and analyzed statistically in table 7 (formula A) and tables 8 and 9, for formula B.

Recovery, standard deviation and % RSD (relative standard deviation) were calculated. As shown in figure 2, no interfering peaks were obtained in the chromatogram from the tablet dosage excipients.

Table 6
DICLOFENAC SODIUM ASSAY FROM ODTs – FORMULA A

Nr.	Theorethical concentration µg/mL	Peak area (mAU x sec)	Calculated concentration		Recovery %
			mg diclofenac sodium/ ODT - A	(µg/mL sample)	
1	25	41.5965	0.026445	26.4452	105.7809
2		40.3864	0.025621	25.6212	102.4847
3		40.8794	0.025957	25.9569	103.8276
4		41.9518	0.026687	26.6872	106.7487
5		41.0587	0.026079	26.0790	104.3160
6		40.2372	0.025520	25.5196	102.0783

Table 7
STATISTIC ANALYSIS (FORMULA A)

n	6
ν	5
$\overline{x}$	104.2060
Minimum	102,0783
Maximum	106,7487
Standard deviation (SD)	0.7436
$\overline{x} \pm t_{\alpha/2} \times_{SD_x}$	102.0783± 0.76
Relative standard deviation (RSD%)	0.2975

Table 8
DICLOFENAC SODIUM ASSAY FROM ODTs – FORMULA B

Nr.	Theorethical concentration µg/mL	Peak area (mAU x sec)	Calculated concentration		Recovery %
			mg diclofenac sodium/ ODT - A	(µg/mL sample)	
1	25	40.5360	0.025723	25.7231	102.8922
2		39.4461	0.024981	24.9809	99.9235
3		40.9533	0.026007	26.0072	104.0289
4		38.4944	0.024333	24.3328	97.3312
5		40.3982	0.025629	25.6292	102.5169
6		39.1699	0.024793	24.7928	99.1711

Table 9
STATISTIC ANALYSIS (FORMULA B)

n	6
ν	5
$\overline{x}$	100.9773
Minimum	97.3312
Maximum	104.0289
Standard deviation (SD)	2.5696
$\overline{x} \pm t_{\alpha/2} \times_{SD_x}$	100.9773± 0.91
Relative standard deviation (RSD%)	0.3642

Compared to the claimed content of active substance on the tablet, a standard deviation of 0.29 (formula A)and of 0.36% (formula B) were detected, values that range between allowed deviation of ± 10%.

The identification of diclofenac sodium by HPLC method was positive for both formulas and the content was between 90-110% range.

The method turned out to be sensitive and suitable for quality control and assay of diclofenac sodium ODTs.

Conclusions

In recent times, HPLC-DAD is an analytical technique not only in drug development but also in the routine quality control laboratory. As the method was validated based on international guidelines, it can be used in quality control laboratories for the routine pharmaceutical analysis of DIC from new orodispersible tablets with co-processed tablets.

With a run time of 5 min, the proposed method allows a relatively high sample throughput. The short run time of this method will significantly reduce the analysis time and cost.

The proposed RP-HPLC-UV analytical method presents a series of advanges: more accessible detection with lower costs, no derivatization step, small volume of organic solvents, without interference from the co-processed excipients and therefore suitable for DIC analysis in solid dosage forms, as ODTs.

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