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Research Article

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF DICLOFENAC AND TOLPERISONE IN TABLET DOSAGE FORM

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ABSTRACT

Objective: To develop a simple and cost effective reverse phase high performance liquid chromatography method for simultaneous estimation of Diclofenac and Tolperisone in tablet dosage form.

Methods: Chromatography was carried out isocratically at 30°C ± 0.5°C on an XDB C-18 column (4.6 x 150mm, 5µ particle size) with a mobile phase composed of acetonitrile -phosphate buffer pH-3.4 (30:70% v/v) at a flow rate of 1.0 mL/min. Detection was carried out using a PDA detector at 260 nm. Validation parameters were studied as per ICH guidelines.

Results: The retention times for Diclofenac and Tolperisone are 2.2 min. and 4.7 min. respectively. The linearity range for Diclofenac and Tolperisone are 12.5-125µg/mL and 37.5-375 µg/mL respectively. The percentage recoveries of Diclofenac and Tolperisone are 100.75% and 100.84% respectively. The correlation coefficients for both components are close to 1.

Conclusion: This method is fast, accurate, precise and sensitive hence it can be employed for routine quality control of tablets containing both drugs in industries.

Keywords: RP-HPLC, Diclofenac, Tolperisone, Simultaneous estimation.

INTRODUCTION

Diclofenac is chemically named as 2-(2-(2,6-dichlorophenylamino) phenyl)acetic acid(Figure 2). Diclofenac belongs to a class of drugs called non-steroidal anti-inflammatory drugs (NSAIDs) that are used for the treatment of mild to moderate pain, fever, inflammation such as rheumatoid arthritis, gout, pyrophosphate arthropathy, osteoarthritis, and ankylosing spondylitis. Diclofenac sodium acts by potent cyclo-oxygenase inhibition, reduction of arachidonic acid release, and enhancement of arachidonic acid uptake. It thereby results in a dual inhibitory effect on both the cyclo-oxygenase and lipoxygenase pathways. The recommended dose for most conditions is 100-200 mg daily. Diclofenac (DIC) tablets can cause side effects like stomach pain, indigestion, heartburn and nausea [1-4]. Tolperisone (Figure 2) is chemically named as 2-methyl-1-(4-methylphenyl)-3-piperidin-1-ylpropan-1-one. Tolperisone is a centrally acting muscle relaxant (muscle relaxant acting on spasticity by interaction with upper motor neurons), which is also used for the treatment of chronic pain. It is mainly used for treating muscle spasticities of neurological origin and painful muscle spasms due to rheumatologic conditions. Besides being an effective antispastic agent, tolperisone (TOL) also has analgesic activity in rodents and in humans [5-6]. Various HPLC assay methods are reported in the literature for the estimation of Diclofenac [7-13] and Tolperisone [14-17] individually and in-combination with other drugs. According to literature survey there is no official method for the simultaneous estimation of Diclofenac and Tolperisone by RP-HPLC in combined tablet dosage forms. In this study, an HPLC method was optimized and validated for simultaneous estimation and validation of Diclofenac and Tolperisone in tablet formulation in accordance with the ICH guidelines [12-14].

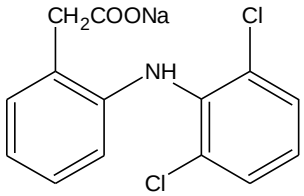

Figure 1: Structure of Diclofenac

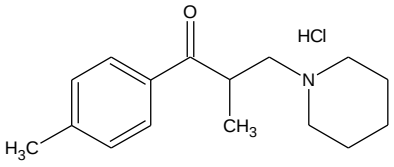

Figure 2: Structure of Tolperisone

MATERIALS AND METHODS

Instrumentation: Chromatography was performed with Water's 2695 HPLC system provided with Hamilton Syringe, auto sampler and 2996 Photodiode array detector. All HPLC systems were equipped with a column compartment with temperature control and an on-line degasser. Sample acquisition, analysis, and reporting were performed by Empower2 (Waters) chromatography software.

Reagents and chemicals: Pharmaceutically pure sample of Diclofenac and Tolperisone were obtained from Spectrum Pharma Research Solutions, Hyderabad as gift samples along with their analytical reports. Acetonitrile and Methanol of HPLC grade was obtained from Merck chemical division, Mumbai and Commercial tablets of Diclofenac (50mg), and Tolperisone (150mg); TOLPIDOL-D was procured from the local drug market.

Chromatographic condition: The isocratic mobile phase consisted of acetonitrile: phosphate buffer (pH-3.4) in the ratio of 30:70v/v at a flow rate of 1.0 ml min-1. XDB C-18 column (4.6 x150mm, 5µ particle size) was used as the stationary phase. Although the Diclofenac and Tolperisone have different λ max, but considering the chromatographic parameter, sensitivity and selectivity of method for both drugs, 260 nm was selected as the detection wavelength for PDA detector.

Preparation of standard stock solution: Standard stock solutions were prepared by dissolving 50 mg of Diclofenac drug and 150 mg of Tolperisone into a clean and dry 100 ml volumetric flask, 70ml of diluent was added, sonicated for 5 minutes and volume was made up to 100 ml with diluent to get Stock Solution.

Preparation of Working Standard Solutions: Aliquot of 0.25ml, 0.5ml, 1.0ml, 1.25ml and 1.5ml and 2.5ml were pipette out from

stock-A into 10 ml volumetric flask separately and volume was made up to 10ml with diluent. This gives the solutions of 12.5µg/ml, 25µg/ml, 50µg/ml, 62.5µg/ml, 75µg/ml and 125µg/ml respectively for Diclofenac, and 37.5µg/ml, 75µg/ml, 150µg/ml, 187.5µg/ml, 225µg/ml and 375µg/ml respectively for Tolperisone.

Sample preparation: Twenty tablets of TOLPIDOL-D containing Diclofenac and Tolperisone (50mg & 150mg respectively) were weighed and crushed into fine powder. Powder equivalent to weight of one tablet was weighed and dissolved in 1 0 0 ml diluent, sonicated for 20 min and filtered through PVDF 0.45µ filter. From the filtrate, 1 ml was pipetted and transferred into a 10ml volumetric flask and the solution was made up to the volume with diluent.

Method validation: Parameters like system suitability, linearity, accuracy, precision, LOD, LOQ, solution stability and robustness were estimated as per ICH guidelines.

RESULTS AND DISCUSSION

Method development

Various mobile phase combinations were tried initially to separate diclofenac and tolperisone on C18 column. Preliminary experiments indicated that using different concentrations of acetonitrile or methanol with water was not able to separate the peaks of Diclofenac and Tolperisone or to obtain suitable retention and peak shape. In order to achieve acceptable peak shapes and perform the separation on a suitable run time, various buffer systems were tried systematically. The retention time of Diclofenac and Tolperisone obtained for different phosphate buffer: acetonitrile ratios (40:60, 45:55, 50:50, 55:45, 60:40 v/v) indicated that the resolution between Diclofenac and Tolperisone increased using higher phosphate buffer ratio Thereafter, acetonitrile-phosphate buffer (pH-3.4) (30:70 v/v) at a flow rate of 1.0 ml/min. XDB C-18 column (4.6 x150mm, 5µ particle size) was used as the stationary phase was selected to improve resolution, short run time and the tailing of both peaks were reduced close to 1. To analyze both drugs detection were tried at various wavelengths from 215nm to 280nm. The wavelength at which both Diclofenac and Tolperisone showed maximum absorption at 260nm was selected as the detection wavelength for PDA detector. The retention time was found to be about 2.2 min and 4.7 min for Diclofenac and Tolperisone, respectively. The chromatogram obtained was shown in the figure 3.

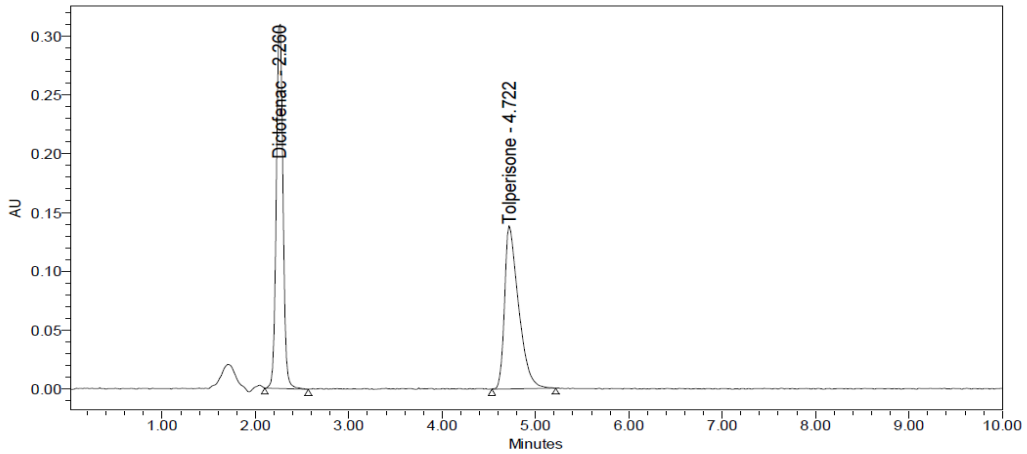

Fig. 3: Representative chromatogram of Diclofenac and Tolperisone

System Suitability Tests: To ensure the validity of the analytical procedure, a system suitability test was established. Data from six injections of 10µL of the working standard solutions of DIC and TOL were used for the evaluation of the system suitability parameters like tailing factor, the number of theoretical plates and retention time. The results obtained are shown in table-1.

Table 1: System suitability of DIC and TOL

PARAMETERS	DIC	TOL
No of theoretical plates	5311	4621
Tailing Factor	1.0	1.6
Mean Area	1544817	1442973

Linearity: The solutions for linearity were prepared at six concentration levels ranging from 25 to 250% of the target concentration. Each experiment was performed in triplicate according to optimized chromatographic conditions. The peak areas of the chromatograms were plotted against the concentrations and the correlation coefficients, slopes and Y-intercepts of the calibration curve were determined. These results were summarized Figure 4 and Figure 5.

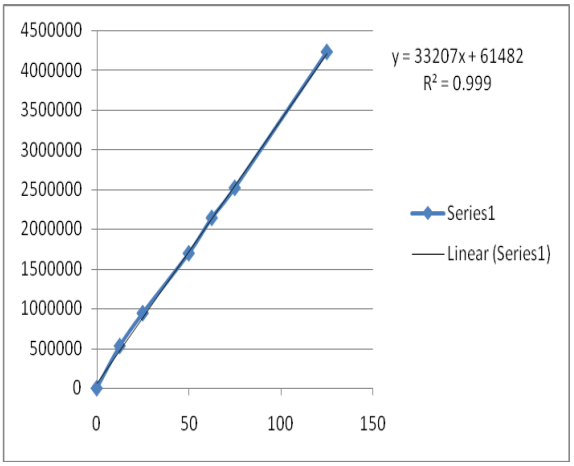

Fig. 4: Calibration Curve for Diclofenac

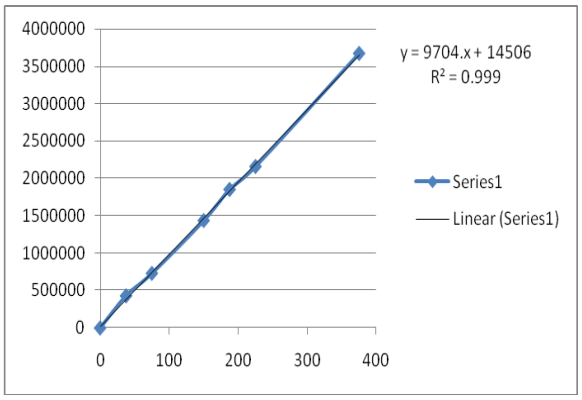

Fig. 5: Calibration Curve for Tolperisone

Accuracy: Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analyzed samples of DIC and TOL to which known amounts of standard DIC and TOL corresponding to 50%, 100% and 150% of label claim were added. The accuracy expressed as the percentage of analyte recovered by the proposed method. These results are summarized in table- 2.

Table 2: Results of Recovery Experiments of DIC and TOL

Preanalysed sample solution conc. (ug/ml)		**Standard drug conc. (ug/ml)**		**% Recovered**	
DIC	TOL	DIC	TOL	DIC	TOL
50	150	25	75	101.38	101.17
50	150	25	75	101.33	100.90
50	150	25	75	102.42	102.92
50	150	50	150	101.44	100.38
50	150	50	150	101.57	101.28
50	150	50	150	100.55	99.32
50	150	75	225	99.70	100.38
50	150	75	225	99.12	100.99
50	150	75	225	99.25	100.23
MEAN				100.75	100.84
SD				1.082	0.985
%RSD				1.07	0.97

Precision: Precision was determined as repeatability and intermediate precision, in accordance with ICH guidelines. The intra-day and inter-day precision were determined by analyzing the samples of DIC and TOL. Determinations were performed on the same day as well as well as on consequent days. Probability value (P) for DIC and TOL at 5% significance level is found to be 0.27and 0.93 and which are greater than 0.05 and hence no significant difference is observed in the precision results carried out on two consecutive days and the results are shown in table 3 & 4.

Table 3: Results of Precision of DIC and TOL

Validation parameter	**Sample no.**	**DIC**	**TOL**
Repeatability (Day1, Analyst 1)	1.	1536430	1439107
	2.	1506312	1440512
	3.	1546873	1439593
	4.	1541826	1447510
	5.	1531184	1436482
	6.	1516676	1433833
	Mean	1529884	1439506
	SD	15529.8	4616.7
	%RSD	1.02	0.32
Intermediate precision (Day 2, Analyst 2)	1.	1543107	1445957
	2.	1524902	1450682
	3.	1552268	1424338
	4.	1548671	1459622
	5.	1548552	1436911
	6.	1520664	1423068
	Mean	1539694	1440096
	SD	13488.9	14670.3
	%RSD	0.88	1.02
Global statistics (Inter day precision)	Overall Mean	1534789	1439801
	SD	6936.718	417.3109
	Overall % RSD	0.45	0.03

SD= Standard deviation RSD= Relative standard deviation

Table 4: t- test (statistical test) results for Precision results of DIC and TOL

Validation parameter	**DIC Mean response**	**Probability P (≥0.05)**	**TOL Mean response**	**Probability P (≥0.05)**
Repeatability -Day 1	1529884	0.27	1439506	0.93
Intermediate precision - Day 2	1539694		1440096	

Robustness: The change was made in the ratio of mobile phase, instead of ACN- Phosphate buffer (30:70 V/V), ACN- Phosphate buffer (25:75V/V) & (35:65 V/V) were used as Mobile Phases, Column temperature was changed to 25 & 30^{0}c, Flow rate was changed to 0.9 & 1.1 ml/min. Results of analysis were summarized in table- 5.

Table 5: Results of Robustness of DIC and TOL

	Changed value	**Retention time**		**Tailing factor**		**% Assay**	
		DIC	TOL	DIC	TOL	DIC	TOL
Column Temperature	25	2.2	5.5	1.23	1.07	101.56	101.10
	35	2.2	5.4	1.17	1.06	98.68	101.20
Flow Rate	0.9	2.4	6.3	1.19	1.08	98.75	98.61
	1.1	2.0	5.0	1.21	1.07	100.05	101.95
Mobile Phase Composition	25:75	2.0	5.3	1.15	1.06	100.26	99.74
	35:65	2.4	5.9	1.18	1.06	100.60	98.92
					Mean	99.98	100.25
					SD	1.111	1.359
					%RSD	1.11	1.36

Stability of sample solution: The sample solution injected after 24 hr did not show any appreciable change. Results are shown in table-6.

Table 6: Stability data of DIC and TOL

Drug	%Assay at 0 hr	%Assay at 24hr	%Deviation
DIC	99.65	101.59	1.94
TOL	99.00	100.03	1.03

LOD and LOQ: LOD and LOQ of DIC and TOL were determined by calibration curve method. Solutions of both DIC and TOL were

prepared in the range of 12.5-125µg/ml and 37.5-375 µg/ml respectively and injected in triplicate. Average peak area of three analyses was plotted against concentration. LOD and LOQ were calculated by using following equations. LOD=(3.3 ×Syx)/b, LOQ=(10.0×Syx)/b. Where Syx is residual variance due to regression; b is slope. LOD and LOQ for DIC were 1.117799 and 3.38727 µg/ml respectively and for TOL were 3.370641 and 10.21406 µg/ml, respectively.

Tablet Analysis: Content of DIC and TOL found in the tablets by the proposed method are shown in Table-7.

Table 7: Results of HPLC Analysis of Tablets

Sample No	Peak Area		%Assay	
	DIC	TOL	DIC	TOL
1	1536430	1439107	100.04	99.23
2	1506312	1440512	98.08	99.33
3	1546873	1439593	100.72	99.27
4	1541826	1447510	100.39	99.81
5	1531184	1436482	99.70	99.05
6	1516676	1433833	98.75	98.87
		AVG	99.61	99.26
		SD	1.011	0.318
		%RSD	1.02	0.32

CONCLUSION

RP-HPLC method was developed and validated for simultaneous estimation of Diclofenac and Tolperisone in tablet dosage form. The resolution between two peaks was always more than 2. The system suitability tests revealed that numbers of theoretical plates were above 2000 and tailing factor is less than 2. DIC and TOL showed a linearity of response between 12.5-125µg/ml and 37.5-375 µg/ml. The peak areas of the chromatograms were plotted against the concentration of DIC and TOL to obtain the calibration curve. The linearity's were represented by a linear regression equation as follows: y(DIC)=33207.x+61482 (r^2=0.999); y(TOL)=9704.x+14506 (r^2=0.999). The percentage recoveries of Diclofenac and Tolperisone are 100.75 % and 100.84 % respectively and it shows the accuracy of method. The regression value is 0.999 for both DIC and TOL and the response is linear. Repeatability and intermediate precision values were within the acceptable limits. This indicates that the method is precise. Selectivity experiment shows that there is no interference or overlapping of the peaks either due to excipients or diluents with the main peak of DIC and TOL. The lowest values of LOD and LOQ as obtained by the proposed method indicate that the method is sensitive. The solution stability studies indicate that both the drugs were stable up to 24 hours. Change in flow rate, temperature and mobile phase composition doesn't cause any significant change in results shows stability of the development method.The percentage RSD for precision is <2 which confirms that method is sufficiently precise. The total run time required for the method is only 10 minutes for eluting both Diclofenac and Tolperisone. So, this method is fast, accurate, precise and sensitive hence it can be employed for routine quality control of tablets containing both drugs in industries.

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