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RESEARCH ARTICLE

Development and Validation of Novel RP-HPLC Method for Simultaneous Estimation of Tolperisone HCl and Diclofenac Sodium in Pharmaceutical Dosage Form

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ABSTRACT

A simple Reverse phase liquid chromatography method has been developed and subsequently validated for the tablet dosage form. Here solvent used was Methanol and mobile phase consisting Acetonitrile: Water: Triethylamine (95:5:0.01) pH set to 3.2 with O-Phosphoric acid gave resolution of peaks. C-18(250mm × 4.6mm i.d with particle size of 5 µm) used with flow rate 1.2 ml/min using UV detection at 270 nm. The retention time of Diclofenac sodium and Tolperisone HCl were found at 3.1min and 2.4min respectively. In Which Linearity for DIC and TOL was found to be y=36565x+66101, R^2 = 0.999 and y=23278x+1040 R^2 = 0.998 in concentration range of 10-90 µg/ml for 30-270 µg/ml respectively. Recovery was found to be 99.16-100.5% for DIC and 98.62-100.32% for TOL. Other all the data (Precision, LOD and LOQ, Assay, Robustness) were within the specified criteria of ICH guideline.

KEYWORDS

HPLC, Diclofenac sodium, Tolperisone HCl

INTRODUCTION

Tolperisone HCl is chemically 1 - (4-methyl-phenyl) - 2-methyl - 3- (1-piperidine) - 1-propanone -hydrochloride. Generally piperidine derivative is centrally acting muscle relaxant which is used in the treatment of acute and chronic muscle spasm, In back pain, arthritis of large joints, spastic muscle cramps, paralysis and muscle pain, myelopathy, encephalomyelitis, arthrosis of the large joints obliterating artherosclerosis of the extremity vessels. TOL is official in Japanese pharmacopoeia. Chemically Diclofenac sodium is, sodium {2-[(2, 6-dichlorophenyl)-amino] phenyl} acetate, used as analgesic and anti-inflammatory drug used in the treatment of Rheumatoid arthritis, osteoarthritis, acute musculoskeletal disorders (e.g. tendinitis, sprains and dislocations), ankylosing spondylitis, acute gout, postoperative pain, renal colic and control of pain and inflammation in orthopedic, dental and other minor surgery. Diclofenac sodium is official in IP 2010, BP 2009, and USP 30.

The review of literature[1-11] stated that various analytical methods involving spectrophotometry, HPLC, HPTLC have been reported for TOL in single form and in combination with other drugs. Several analytical methods have been reported for DIC in single form and in combination with other drugs including spectrophotometry, HPLC, HPTLC Methods. However no references have been found for simultaneous estimation of

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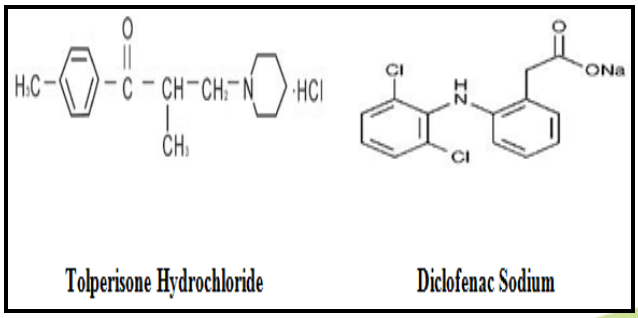
Tolperisone Hydrochloride and Diclofenac Sodium in their combined tablet dosage form by Area under curve method. So, the objective is to develop simple, accurate and precise method for estimation of Tolperisone HCl (TOL) and Diclofenac sodium (DIC) in combined tablet dosage form. The developed method was validated as per ICH Guidelines and successfully applied for the assay of TOL and DIC in their combined tablet dosage form.



MATERIALS AND METHOD

Instrumentation

A Shimadzu HPLC system equipped with LC-20 AT VP pump, DGU-14 AM on-line degasser, Rheodyne manual injector fitted with a 20 µl loop, C18 (250mm × 4.6mm i.d with particle size of 5 µm) column and UV–VIS detector was utilized.

Materials

All solvents and reagents were of an HPLC analytical grade (Methanol, Water, Ortho-phosphoric acid, Acetonitrile, Triethylamine)

Preparation of Stock Solution of TOL (T_A) (3000 µg/mL)

Accurately weighed TOL (150 mg) was transferred into 50 mL volumetric flask, dissolved and volume made up to mark with methanol.

Preparation of Stock Solution of DIC (D_A) (1000 µg/mL)

Accurately weighed DIC (50 mg) was transferred into 50 mL volumetric flask, dissolved and volume made up to mark with methanol.

Preparation of Mixture of Tolperisone and Diclofenac

Accurately weighed TOL (150 mg) and Diclofenac (50 mg) were transferred to 50 mL volumetric flask, dissolved and diluted up to mark with methanol.

Preparation of Calibration Curve

Pipette out 0.1, 0.3, 0.5, 0.7and 0.9 ml from the mixture of TOL and DIC, transferred into a series of 10 ml volumetric flasks, diluted up to the mark with solvent methanol. Thus final solutions of mixture of DIC and TOL obtained contain 10 & 30 µg/ml, 30 & 90 µg/ml, 50 &150 µg/ml, 70& 210 µg/ml and 90 & 270 µg/ml respectively.

Validation of Proposed Method

Linearity (n=5)

The linearity for DIC and TOL were assessed by determining peak area of combined standard solution in range of 10-90 µg/ml and 30-270 µg/ml respectively.

Precision

Repeatability (n=6)

The peak area of same concentration of DIC (50 µg/ml) & TOL (150 µg/ml) were measured six times and %RSD was calculated. 0.5 ml of mixture of DIC and TOL were transferred in 10 ml volumetric flask and diluted up to the mark with methanol. Resulting solution was prepared six times.

Intermediate precision (n=6)

Intraday precision was determined by analyzing DIC and TOL in combined solution for six times in the same day. Inter-day precision was determined by analyzing DIC and TOL in for three days.

Reproducibility

Reproducibility was performed by preparing the standard solution of DIC (50µg/ml) and TOL (150µg/ml) for six times and analyzed as per the proposed method.

Accuracy (n=3)

Accuracy of the method was carried out at three levels (80%, 100% and 120%). Known amount standard of DIC and TOL was added to a pre

analyzed sample solution. The amount of DIC and TOL were estimated from straight line equation of calibration curve.

Specificity

A solution of placebo in mobile phase was prepared as common tablet excipient like starch, lactose, talc were dispersed in mobile phase, filtered and injected. The chromatogram of placebo were compared with those acquired from DIC (50 µg/ml) and TOL (150 µg/ml) standards, correlation in terms of interference at retention time and peak area was evaluated to indicate the specificity of method.

LOD (Limit of Detection)

The LOD was estimated from the set of 5 calibration curves used to determine method linearity.

LOQ (Limit of Quantification)

The LOQ was estimated from the set of 5 calibration curves used to determine method linearity.

Ruggedness

Ruggedness of the proposed method was determined by analysis of aliquots of sample solution (50 µg/ml DIC and 150 µg/ml TOL) by two analyst using same operational and environmental conditions.

Robustness

Typical changes include flow rate changed to 1.2 ± 0.2 ml/min, pH changed ± 0.2 and detection wavelength changed to 270 ± 2 nm.

Quantification of DIC and TOL in Combined Capsule Dosage Form (n=6)

Twenty tablets were weighed and average weight of content was determined & the content of tablets was powdered. The powder equivalent to 150 mg of TOL or 50 mg of DIC was transferred to a 50 ml volumetric flask, dissolved and diluted up to the mark with methanol. The solution was filtered through Whatman filter paper no. 41 and first few ml of filtrate were discarded. Aliquots of 0.5 ml of this solution were diluted to 10 ml with methanol six times.

RESULTS AND DISCUSSION

The proposed RP-HPLC shows good linearity in the concentration range of 10 to 90 µg/ml for DIC and 30 to 270 µg/ml for TOL with correlation co-efficient, slope and intercept 0.9993, 36565 and 66101 for DIC and 0.998, 23278 and 10401 for TOL respectively which proved that method is linear. Repeatability for RP-HPLC method was measured in terms of %RSD. The average %RSD of measurements for determination of DIC was found to be 0.502 % and for TOL was found to be 0.589% depicted in Table 2. Reproducibility is given in Table 3. % RSD of DIC and TOL was found to be 0.981, 0.978 and 0.956, 1.011 respectively. The values of % Recovery for analysis of formulations are found within 98-102 %. The results are shown in Table 4 and 5. Recovery greater than 98 % with low SD justifies the accuracy of the method. The values of LOD and LOQ are given in Table 6. LOD were found to be 1.7867µg/ml and 1.36µg/ml and LOQ were 5.41436µg/ml and 4.14436µg/ml for TOL and DIC respectively. %RSD for robustness and ruggedness parameter was less than 2% for DIC and TOL shown in table 7-9. Assay value was found to be 99.96% for TOL and 98.30% shown in table 10.

Table 1: System Suitability Test Parameter

Parameters	Proposed Method		Standard Values
	DIC	TOL	
Retention times (tR)	3.148	2.401	-
Theoretical plates (N)	3236.768	2635.855	Greater than 2000
Resolution (RS)	2.0	-	Greater than 2
Tailing factor (Tf	1.771	1.298	Not greater than 2.0

Linearity and Range

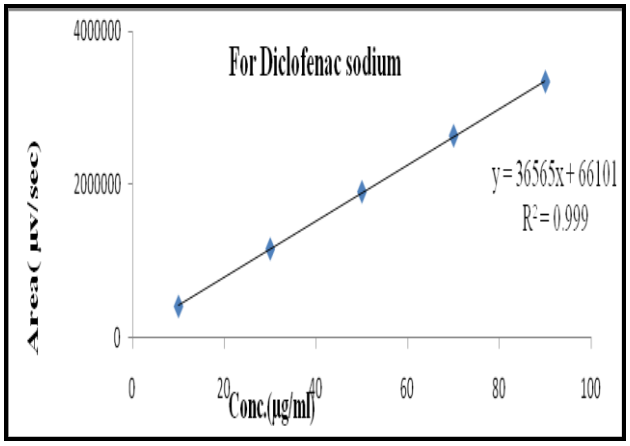


Figure 1: Calibration curve for DIC in ACN: Water (95: 05 v/v) with Triethylamine (0.01%) pH 3.2

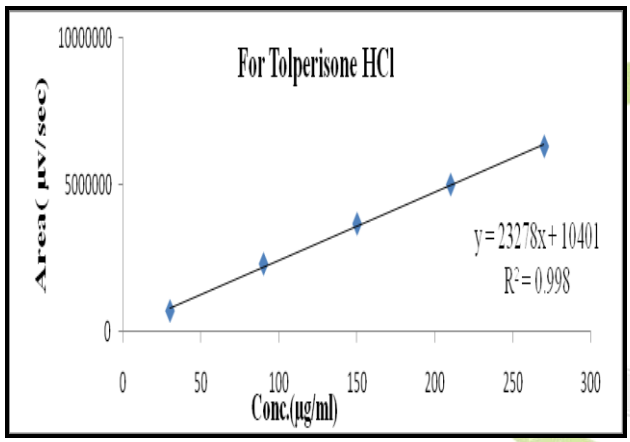


Figure 2: Calibration curve for TOL in ACN: Water (95: 05 v/v) with Triethylamine (0.01%) pH 3.2

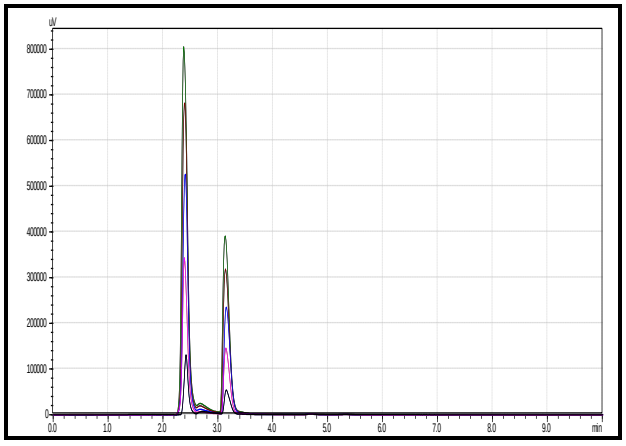


Figure 3: Chromatogram of calibration curve for DIC (10-90 µg/ml) and TOL (30-270 µg/ml)

Precision

Table 2: Repeatability data for estimation of TOL and DIC

Sr. No.	Con.(µg/ml)		Peak Area	
	TOL	DIC	TOL	DIC
1	150	50	3379545	1917777
2	150	50	3377559	1908569
3	150	50	3365668	1924558
4	150	50	3365579	1905483
5	150	50	3352584	1929423
6	150	50	3325956	1924891
Mean			1918450	3361149
S.D.			19797.24	9648.12
% RSD			0.589	0.502

Table 3: Reproducibility by HPLC

Sr. No	DIC AREA		TOL AREA	
	Lab.1	Lab.2	Lab.1	Lab.2
Avg.	1916454	1916422	3343622	3340267
SD	18816.82	18759.89	31974.96	33782.24
% RSD	0.981	0.978	0.956	1.011

Table 4: Recovery of DIC from formulation (TOLPIDOL D)

DIC	Test (100%) (µg/ml)	Std. Added (µg/ml)	Total Area	Conc. (µg/ml)	% Recover	Avg.	SD	% RSD
80%	50	40	3323288	39.63	99.08	99.14	0.354	0.357
	50	40	3314903	39.53	98.83			
	50	40	3339388	39.82	99.56			
100 %	50	50	3704841	49.71	99.41	99.10	0.449	0.453
	50	50	3674281	49.29	98.59			
	50	50	3701487	49.66	99.32			
120 %	50	60	4149117	60.72	101.20	100.35	0.746	0.746
	50	60	4099513	60.00	100.01			
	50	60	4092953	59.90	99.84			

Table 5: Recovery of TOL from formulation (TOLPIDOL D)

TOL	Test (100%) (µg/ml)	Std. Added (µg/ml)	Total Area	Conc. (µg/ml)	% Recover	Avg.	SD	% RSD
80%	150	120	6214554	118.34	98.62	98.74	0.3194	0.323
	150	120	6245931	118.93	99.11			
	150	120	6207622	118.21	98.51			
100%	150	150	6897358	147.76	99.51	99.46	0.4216	0.424
	150	150	6933067	148.53	99.02			
	150	150	6991881	149.79	99.86			
120%	150	180	7630994	178.34	99.08	99.34	0.8793	0.88
	150	180	7595566	177.51	98.62			
	150	180	7701852	180.11	100.32			

Table 6: LOD and LOQ data of DIC and TOL

Parameter	TOL	DIC
Standard Deviation	19797.24	9648.12
Slope	36565	23278
LOD (µg/ml)	1.7867	1.36
LOQ (µg/ml)	5.414	4.144

Table 7: Ruggedness Data

Ruggedness Study				
Parameter	**TOL**		**DIC**	
	Analyst – I	**Analyst – II**	**Analyst – I**	**Analyst – II**
Mean*	3367815	3344603	1920430	1920431.5
SD	27598.58	30615.67	11777.62	11712.15
% RSD	0.819	0.915	0.613	0.609

Table 8: Robustness Study for TOL 150µg/ml

Parameter	**Normal**	**Change Condition 1**	**Change Condition 2**
Flow Rate & pH	**Flow Rate1.2 ml/min & pH 3.2**	**Flow Rate 1 ml/min**	**pH 3**
Average	3361277	4031025	3555602
STDEV	7528.49	33385.7	28818.07
%RSD	0.31	0.828	0.81

Table 9: Result of Robustness Study for DIC 50µg/ml

Parameter	**Normal**	**Change Condition 1**	**Change Condition 2**
Flow Rate & pH	**FlowRate1.2 ml/min & pH 3.2**	**Flow Rate 1 ml/min**	**pH 3**
Average	1912870	2354730	2061643
STDEV	10239.04	36882.45	31380.51
%RSD	0.535	1.566	1.522

Table 10: Analysis of marketed formulation (Tolpidol D)

Drug Amount (µg/ml)		**Obtained Peak Area**		**Drug recovered (µg/ml)**		**Assay (% Estimated (n=3)**	
TOL	DIC	TOL	DIC	TOL	DIC	TOL	DIC
150	50	3500842	1863415	149.94	49.15	99.96	98.30

CONCLUSION

The proposed RP-HPLC method used for the simultaneous estimation of Tolperisone HCl and Diclofenac Sodium was found to be sensitive, accurate, precise, simple, and rapid. Hence the present RP-HPLC method may be used for routine analysis of the raw materials, in vitro dissolution study of combinational dosage formulations containing Tolperisone HCl and Diclofenac Sodium.

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