Shivaji D. et al. / Vol 5 / Issue 1 / 2015 /30-35.

International Journal of Pharmaceutical Research & Analysis

e-ISSN: 2249 – 7781
Print ISSN: 2249 – 779X

www.ijpra.com

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUS ESTIMATION OF TOLPERISONE AND DICLOFENAC SODIUM BY RP-HPLC

Shivaji D*, Usha Sree G, Ajitha A , Uma Maheswara Rao V

Department of Pharmaceutical Analysis & Quality Assurance, CMR College of Pharmacy, JNTU (H) University, Hyderabad, Andhra Pradesh, India.

ABSTRACT

A new, simple, precise, accurate and reproducible RP-HPLC method for Simultaneous estimation of Tolpersione and Diclofenac sodium in bulk and pharmaceutical formulations. Separation of Tolpersione and Diclofenac sodium was successfully achieved on an Eclipse XDB C18 (150mm X 4.6mm X 5µ Make: Waters) or equivalent in an isocratic mode utilizing KH_2PO_4 buffer (pH 4.5): Methanol (60:40%v/v) at a flow rate of 0.8 mL/min and eluate was monitored at 258nm, with a retention time of 3.523 and 4.766 minutes for Diclofenac sodium and Tolpersione. The method was validated and the response was found to be linear in the drug concentration range of 50µg/Ml to150 µg/mL for Diclofenac sodium and 50µg/mLto150 µg/mL for Tolpersione. The value of the correlation coefficient was found to be 0.999 and 0.999 for Diclofenac sodium and Tolpersione. The LOD and LOQ for Tolpersione were found to be 0.334 , 1.113 respectively. The LOD and LOQ for Diclofenac sodium were found to be 0.1301, 0.4338 respectively. This method was found to be good percentage recovery for Diclofenac sodium and Tolpersione were found to be 98.00 and 100.00 respectively indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard with the sample so, the method specifically determines the analyte in the sample without interference from excipients of tablet dosage forms. The method was extensively validated according to ICH guidelines for linearity, range, accuracy, precision, specificity and robustness.

Keywords: Diclofenac sodium and Tolpersione, High performance liquid chromatography.

INTRODUCTION

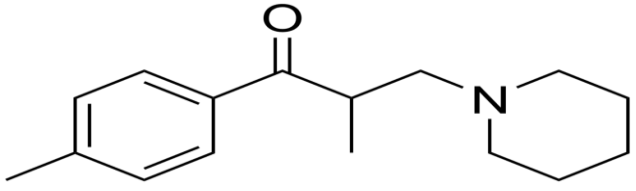
Tolperisone is indicated for use in the treatment of pathologically increased tone of the cross-striated muscle caused by neurological diseases (damage of the pyramidal tract, multiple sclerosis, myelopathy, encephalomyelitis) and of spastic paralysis and other encephalopathies manifested with muscular dystonia [1-4].

Diclofenac sodium exact mechanism of action is not entirely known, but the primary mechanism responsible for its anti-inflammatory, antipyretic, and analgesic action is thought to be inhibition of prostaglandin synthesis by inhibition of cyclooxygenase (COX) [5-8].

DRUG PROFILE

TOLPERISONE

Chemical structure:



TOLPERSIONE

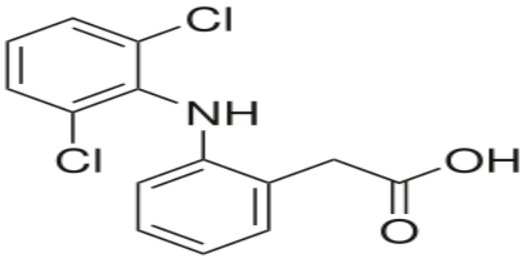
IUPAC Name :2-Methyl-1-(4-methylphenyl)-3-piperidin-1-ylpropan-1-one hydrochloride

Molecular formula: $C_{16}H_{24}ClNO$

Corresponding Author:-Shivaji D. Email:- shivajidharmulad@gmail.com

Molecular Weight:281.82 g/mol
Category: Antispastic agent Voltage-gated Na+ and Ca2+ channel blocker.

DICLOFENAC SODIUM
Structure :



DICLOFENAC SODIUM
Chemical name: [2-{2-[(2,6-dichlorophenyl)amino]phenyl}acetic acid
Molecular formulae: $C_{14}H_{11}Cl_2NO_2$
Molecular Weight : 296.149 g/mol
Category : Anti-inflammatory [9-13]

MATERIALS AND METHODS

Instrumentation

The separation was carried out on HPLC system with WATERS, software: Empower 2, 2695 separation module. 996 PDA detector with binary HPLC pump, and C18 column, Symmetry C18 (4.6 x 250mm, 5µm, Make: X-terra).

Chemicals

TOL (50mg Diclofenac sodium and 150mg Tolperisone) manufactured by Dr. Reddy's Laboratories Ltd. All chemicals and reagents used were of AR grade. Standard sample was taken from Surapharma training lab.

HPLC Conditions

The mobile phase consisting of Methanol, Phosphate buffer and (HPLC grade) were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 65:30:5v/v was pumped into the column at a flow rate of 1.0ml/min. The column temperature was 30°C. The detection was monitored at 275nm and the run time was 5 min. The volume of injection loop was 10µl prior to injection of the drug solution the column was equilibrated for at least 30 min. with the mobile phase flowing through the system.

Preparation of standard solution

Accurately weigh 50 mg of Diclofenac sodium and 150mg of Tolperisone into a 10ml of volumetric flask and dissolve the sample using diluent and sonicate it for 15min then finally make up the volume to 10 ml. Now pipette out 0.3ml of this solution into 10 ml of volumetric flask and make up the volume up to mark using same diluent.

Preparation of sample solution

Accurately weighed 10 tablets and calculated average weight of those tablets and crushed. Transfer the tablet powder weigh about 10mg of sample into 10ml of volumetric flask added with diluent and sonicated for 30 mins and make up the volume with diluent and filtered through the0.45µm millipore filter paper Transfer above solution 0.3ml into 10ml volumetric flask and make up the volume with diluent.

METHOD VALIDATION

System Suitability Studies

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 % standard deviation range during routine performance of the method

Specificity

Specificity was checked for the interference of impurities in the analysis of blank solution and injecting sample solution under optimized chromatographic conditions to demonstrate separation of both Diclofenac sodium and Tolperisone from impurities.

Accuracy

Accuracy was determined by the recovery studies at three different concentrations (corresponding to 50, 100 and 150% of the test solution concentration) by addition of known amounts of standard to pre-analysed sample preparation. For each concentration, three sets were prepared and injected. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated. From the data obtained in added recoveries of standard drugs were found to be accurate as shown in table 2(a) & 2(b)

Precision

Method Precision was determined by injecting six replicates of drug sample solution. The retention times and peak areas of six replicates are recorded. The precision is expressed as the % RSD of Peak areas and it should not be more than 2% shown in table 3.

Linearity

Linearity of the method was determined by constructing calibration curves. Standard solutions of Tolperisone and Diclofenac sodium different concentration level (10ppm, 20ppm, 30ppm, 40ppm, 50ppm) were used for this purpose. Each measurement was carried out in six replicates to verify the reproducibility of the detector response at each concentration level. The peak areas of the

chromatograms were plotted against the concentration of Tolperisone and Diclofenac sodium to obtain the calibration curves. The five concentrations of the standard were subjected to regression analysis to calculate equation and correlation coefficients as shown in Fig4(a),(b).

Limit of detection and limit of quantitation

Limit of detection and limit of quantitation represent the concentration of analyte that would yield signal to noise ratio of 3 for LOD and 10 for LOQ respectively. To determine LOQ and LOD serial dilutions of mixed standard solution of Tolperisone and Diclofenac sodium was made from standard solution. The samples were injected in the system and measured signal from the samples was compared with those of blank samples. LOD and LOQ was calculated from linear curve using formulae LOD= 3.3 * σ / slope, LOQ= 10 * σ / slope (Where σ = the standard deviation of the response and S = Slope of calibration curve) shown in table 5,6.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP HPLC method developed, are rugged and robust shown in table 7(a) and 7(b).

Table 1. System Suitability parameters

S. No	Parameter	Tolperisone	Diclofenac sodium
1	Retention time	3.505	4.744
2	Theoretical plates	4926	6748
3	Tailing factor	1.69	1.66
4	Resolution	-	6.197
5	Regression factor	0.9989	0.9999

Table 2(a). Accuracy Observation of Tolperisone

Spike level	Sample weight	Sample area	µg/ml Added	µg/ml Found	% Recovery	% Mean
50%	291.50	2047216	147.00	148.50	101.33	
50%	291.50	2047893	147.00	148.21	99.33	
50%	291.50	2058741	147.00	148.26	100.66	100.44
100%	583.00	4085210	294.00	297.00	99.3	
100%	583.00	4078963	294.00	297.93	102.33	100.76
100%	580.00	4076328	294.00	297.60	100.66	
150%	874.50	6048951	441.00	446.05	99.77	
150%	874.50	6014735	441.00	446.93	100.66	100.21
150%	847.50	6012591	441.00	445.75	100.22	

Table 2(b). Accuracy Observation of Diclofenac sodium

Spike level	Sample weight	Sample area	µg/ml Added	µg/ml Found	% Recovery	% Mean
50%	291.50	2045783	50.00	49.87	99.33	
50%	291.50	2014587	50.00	49.63	102	
50%	291.50	2069853	50.00	49.76	100.66	100.66
100%	583.00	4175263	100.00	99.30	100.66	
100%	583.00	4157832	100.00	100.34	100.66	100.21
100%	583.0	4158321	100.00	99.54	99.33	
150%	874.50	6251304	150.50	149.99	100.22	
150%	874.50	6225696	150.50	143.56	99.55	100.07
150%	874.50	6227322	150.50	149.25	100.44	

Table 3(a). Results of precision for Tolperisone

S.no	RT	Area	%Assay
injection1	3.538	4053948	99
injection2	3.519	4027748	98
injection3	3.524	4020804	98
injection4	3.523	4039574	99

Injection 5	3.517	4026138	98
Injection 6	3.507	4021502	98
Mean			98
Std. Dev.			0.31
% RSD			0.31

Precision data for Diclofenac sodium

Table 3(b). Results of precision for Diclofenac sodium

S.no	RT	Area	%Assay
injection1	4.782	4156198	100
injection 2	4.767	4150101	100
injection 3	4.774	4141902	99
injection 4	4.779	4157002	100
injection 5	4.768	4146181	99
injection 6	4.759	4192719	101
Mean			100
Std. Dev.			0.44
%RSD			0.44

Table 5. LOD results of the method

Drug	Amount (µg/mL)
Tolperisone	3.504
Diclofenac sodium	4.770

Table 6. LOQ results of the method

Drug	Amount (µg/mL)
Tolperisone	3.503
Diclofenac sodium	4.758

Table 7(a). Flow Rate Observation of Rupatadine

Flow Rate (ml/min)		System Suitability Results		
		USP Plate Count	USP Tailing	Area
Low	0.9	4479	0.9	1104154
Actual*	1.0	4759	1.1	1245977
High	1.1	3072	1.1	1408920

Table 7(b). Flow Rate Observation of Diclofenac sodium

Flow Rate(ml/min)		System Suitability Results		
		USP Plate Count	USP Tailing	Area
Low	0.8	4508	0.9	2104921
Actual*	1.0	3695	0.9	1517199
High	1.2	3072	1.0	1408920

Table 8(a). Variation of Mobile phase composition of Tolperisone

Change in M.P organic composition	System Suitability Results		
5%more	2028	0.9	1012763
Actual*	4759	0.9	1245977
5%less	3002	1.0	912635

Table 8(b). Variation of Mobile phase composition of Diclofenac sodium

Change in M.P organic composition	System Suitability Results		
5%more	3035	1.0	1501336
Actual*	3695	0.9	1517199
5%less	3002	1.0	1415632

Fig 1. Chromatogram of standard preparation

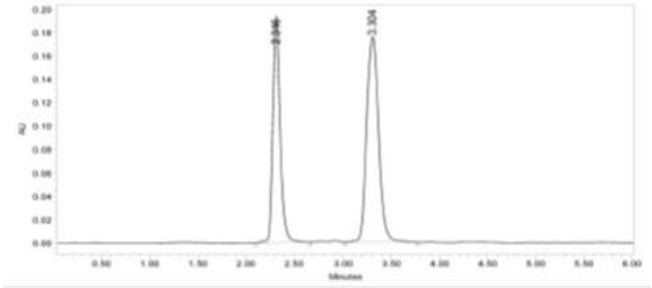


Fig 2. Chromatogram of sample preparation

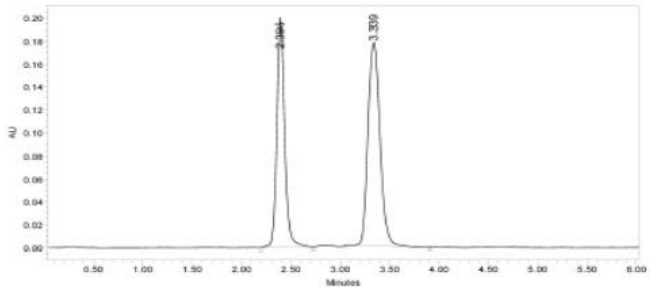


Fig 3. Blank chromatogram

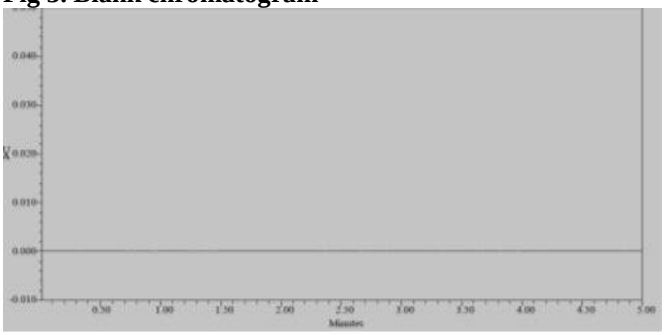
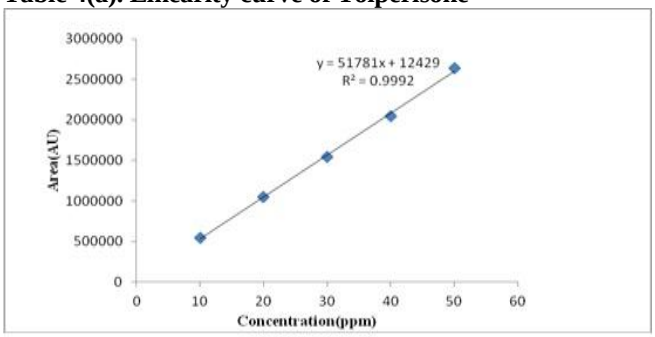


Table 4(a). Linearity curve of Tolperisone



RESULTS AND DISCUSSION

System suitability results were given by table1 and system suitability parameters are retention time, resolution, tailing and plate count were shown uniformity and %RSD was less than 1 so we can say system is suitable for analysis method specificity was concluded by fig-1 are Tolperisone and Diclofenac sodium standard chromatogram and other one is formulation, they were not observed placebo and excipients peaks interference with standard and analytic peak so it proves method is selective. The result given in table 2 says that the method accuracy passed for both Tolperisone and Diclofenac sodium evaluated by recovery studies and the percentage mean recovery was found to be 100.47 and 100.31 for Tolperisone and Diclofenac sodium respectively. The method precision was passed for both the drugs given in table 3. Linearity calibration curve was given below fig: 4the regression coefficient of Rupatidine fumarate is 0.9989 and Diclofenac sodium is 0.9999.

The LOD values of Tolperisone and Diclofenac sodium are 3.504 and 4.770 respectively and LOQ values of Tolperisone and Diclofenac sodium are 3.503 and 4.758 respectively.

CONCLUSION

The proposed RP-HPLC method was validated as per International Conference on Harmonization (ICH) guidelines, and found to be applicable for routine quality control analysis for the simultaneous estimation of Tolperisone and Diclofenac sodium using isocratic mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The proposed method is highly sensitive, reproducible, reliable, rapid and specific. Hence, this method can easily and conveniently adopt for routine quality control analysis of Tolperisone and Diclofenac sodium in its pharmaceutical dosage forms.

ACKNOWLEDGEMENT

The authors are thankful to Rainbows Pharma Training Lab, Hyderabad.

REFERENCES

1. Sharma BK. Instrumental Methods of Chemical Analysis, GOEL Publication House, Meerut, 1999, 133-161, 68-80, 114-165, 286-320.

2. Sethi PD. HPLC-Quantitative analysis of pharmaceuticalformulations; 3rd ed., CBS publishers & distributors, 1997, 182-184.
3. Green C. A step-by-step approach to establishing a method validation program, *Validation Technol*,6, 2000, 622-633.
4. ICH harmonised tripartite guideline validation of analytical procedures Q2(R1) http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf.
5. Rang & Dale's. Pharmacology, 6th ed., Churchill Livingstone Publishers, 303-318.
6. Indian Pharmacopoeia. Indian Pharmacopoeia commission, Ghaziabad, II, 2010, 1250.
7. British Pharmacopoeia. British Pharmacopoeia Commission Office, London, I, 2011, 758- 759.
8. Martindale. The Extra Pharmacopoeia; 31st Edn; The Royal Pharmaceutical Society, London, 1996, 310.
9. The Merck Index. An Encyclopedia of Chemicals, Drugs and Biological; 14th ed., Merck Co. Inc., whitehouse, 2001, 3429.
10. Skoog DA. Introduction of analytical chemistry. Sounder college of publishing, Harcourt Brace college publishers, 1994, 1-5.
11. Introduction to analytical method validation (online), available from: URL: http://www.standardbase.hu/tech/HPLC%20validation%20PE.pdf.
12. Data elements required for assay validation, (online) available from: URL: http://www.labcompliance.com/tutorial/methods/default.aspx.
13. Introduction to Column. (Online), URL:http://amitpatel745.topcities.com/index_files/study/column care.pdf.