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RESEARCH ARTICLE

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METHOD DEVELOPMENT, VALIDATION AND FORCED DEGRADATION STUDIES OF TOLPERISONE HYDROCHLORIDE BY RP-HPLC METHOD IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A simple, reproducible and efficient reverse phase high performance liquid chromatographic method was developed for estimation of Tolperisone hydrochloride in tablet dosage form. Formulation containing Tolperisone hydrochloride is used as centrally acting muscle relaxant. Chromatographic separations were carried out isocratically by reverse phase C_{18} column (Symmetry C_{18}, 5µ, 250nm x4.6mm). The sample was analyzed using Acetonitrile: water in the ratio of 45:55(pH adjusted to 3.0 with Orthophosphoric acid) as mobile phase at flow rate of 1ml/min and detection at 260nm. The drug was subjected to oxidation, acid, alkali and photolytic degradation condition and the stressed samples were analyzed by proposed method. The retention time for Tolperisone hydrochloride was found to be 1.847min and the recoveries for dosage form were between 99.01 to 101.86 for brand I and 101.65 to 102.86 for brand II. Linearity was obtained in concentration range of 2-10µg/ml with correlation coefficient of 0.99628. The drug substance was found to be susceptible to stress condition of oxidation and more stable to acid, alkali and photolytic condition attempted. The result of the analysis was validated statistically and recovery studies confirmed the accuracy and precision of the proposed method.

KEY WORDS

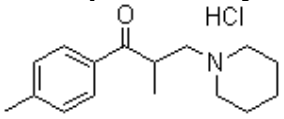
Tolperisone hydrochloride, Rp-HPLC, validation, Forced degradation studies.

INTRODUCTION

Tolperisone hydrochloride chemically 2- methyl – 1- (4 –methyl phenyl) – 3- (1- piperidyl) propane -1 one is a piperidine derivative[1] and the structure was shown in fig-1.It is a centrally acting muscle relaxant which is used in the treatment of different pathological conditions like multiocular sclerosis ,myelopathy , encephalomyelitis , spondylosis , spondylarthrosis , cervical and lumbar syndrome , Arthrosis of the large joints obliterating artherosclerosis of the extremity vessels , Diabetical angiopathy , thromboangitis obliterans , raynauds syndrome[2]. Tolperisone hydrochloride is official in japan pharmacopoeia[3].The literature survey revealed that there are some analytical methods reported for tolperosine hydrochloride either individually like visible spectrophotometric method [4-6], HPTLC[7] or in combination with other drugs by Rp-HPLC[8] and also reported on biological fluids[9].

Literature survey did not reveal any reported methods for the analysis of Tolperisone hydrochloride in tablet dosage form and degradation studies. Present work emphasizes on the quantitative estimation of Tolperisone hydrochloride in bulk and tablet dosage form by Rp-HPLC and their degradation studies.

Figure 1
Structure of Tolperisone hydrochloride



EXPERIMENTAL

Instrumentation and chromatographic conditions:

A high performance liquid chromatographic system (WATERS ALLIANCE 2695seperation module) with an auto sampler was used for analysis. The data was recorded using WATERS EMPOWER software. The purity determination performed on a stainless column (symmetry) 250mm long 4.6mm internal diameter filled with octadecyl silane chemically bonded to porous silica particles of 5µm diameter (250x4.6mm) Optimized chromatographic conditions were listed in table -1.

Table 1
Optimized chromatographic conditions

Parameter	Optimized conditions
Instrument	Waters Alliance 2695seperation module
Column	C_{18},5µ,250mmx4.6mm
Mobile phase *	Acetonitrile :water (45:55) pH 3.0 (dilute Orthophosphoric acid)
Flow rate	1.0ml/min
Detection	260nm
Injection volume	20µl
Temperature	Ambient

Filtered through a Whatman filter pape, degassed and sonicated .

Materials and Chemicals

Pure sample of Tolperisone hydrochloride was obtained from Amanath pharmaceuticals – Puducherry. The purity range was 99.79% w/w. Tablet formulations containing Tolperisone hydrochloride MYO MR-150mg (Brand I) Grandix pharmaceuticals Chennai and Tolfree -100mg (Brand II) Zydus Cadila, Headband were used for the estimation. HPLC water prepared by using Millipore Q_3 purification system. HPLC grade Acetonitrile were procured from Merck (Mumbai, India) Analytical grade Orthophosphoric acid,HCl,NaoH,H_2O_2 were purchased from Nice chemicals (Mumbai ,India) , Digital balance (Sartorius BT 224S) ,Digital pH meter MK-VI were employed for the estimation.

Preparation of Mobile Phase

The mobile phase was prepared by mixing of Acetonitrile with water (pH 3.0) (45:55v/v) and the water pH adjusted to 3.0by using O-phosphoric acid. The mobile phase was sonicated for 15min and then it was filtered through a 0.45membrane filter paper.

Preparation of standard solution:

A stock solution (1000µg/ml) of the standard drug was prepared by taking 25mg of Tolperisone hydrochloride in a 25ml volumetric flask containing a mixture of Acetonitrile : water (pH 3.0) (45:55v/v) ,sonicated for about 10min and then made up to the volume. The stock solution was suitably diluted to produce a concentration of 10µg/ml of Tolperisone hydrochloride.

Preparation of Sample Solution

Twenty tablets of two brands were weighed separately to obtain the average weight and finely powdered. Tablet powder equivalent to 50mg of each brand of Tolperisone HCl was transferred into a separate 50ml standard flask and dissolved in 30ml of mobile phase. The solution was kept in a ultrasonicator bath for 10min and the volume was adjusted upto the mark with mobile phase .The sample solution was filtered through 0.45µ membrane filter paper. The sample solution was further diluted with mobile phase to obtain the final concentration of 10µg/mland injected into the HPLC system. The result of the tablet analysis are given in table 2.

Table 2

Statistical evaluation of tablet analysis

Tablet formulation	Amount Present*	%Label claim*	% purity	SD	%RSD
Brand 1	98.69 mg	100 mg	99.69	0.3273	0.3316
Brand 2	153.008 mg	150 mg	100.72	0.5104	0.3335

**** Mean of six determination (n=6)***

FORCED DEGRADATION STUDIES

In order to establish whether the analytical method was specific and stability indicating for pure active ingredient Active Pharmaceutical Ingradient Forced degradation studies were Carriedout under various stressed conditions like acidic, alkali, photolytic and hydrogen peroxide induced oxidation. Tolperisone HCl is freely soluble in water, ACN, methanol, 0.1N HCl, phosphate buffer pH6.8, acetate buffer and slightly soluble in 0.1N NaOH. All solutions prepared for use in forced degradation studies were prepared by dissolving API in small volume of ACN and later diluted with either aqueous hydrogen peroxide, distilled water, aqueous HCl or aqueous NaoH to achieve a final concentration of 100 mcg/ml of tolperisone HCl by using mobile phase. The result of stress degradation studies are shown in Table 7.

Acid and alkali degradation studies

Solutions for acid degradation studies were prepared in ACN initially to get the concentration of 1 mg/ml (Stock soln). Then 10

ml of the above stock solution was taken in 50ml standard flask and 10ml of 0.01N HCl or 0.01N NaOH were added then the volume made up by mobile phase. The flask was kept in the dark place to exclude the possible degradative effect of light. The resultant solution pH was adjusted to 7.0 and analyzed every 1 hrs, 2 hrs after preparation.

Oxidation degradation studies

The 10 ml of the above stock solution was taken in 50ml standard flask and 10ml of 3 % H_2O_2 was added then the volume made up by mobile phase. The flask was kept in the dark place to exclude the possible degradative effect of light. The resultant solution pH was adjusted to 7.0 and analyzed every1 hrs, 2 hrs after preparation.

Photolytic degradation studies

The API powder solution was prepared and exposed to light to determine the effect of irradiation on the stability of the drug in solution. 10 ml of stock solution of API (1 mg/ml) and 10ml of HPLC grade water were mixed in 50ml standard flask and the volume made up by mobile phase. Control sample was protected from light with aluminium foil. All samples for photo stability testing were placed under UV light. Following removal from UV light, all samples were prepared for analysis as previously described.

VALIDATION

Linearity and Range

Transfer 0.2ml to 1ml of aliquots of standard stock solution (1000µg/ml) of Tolperisone HCl into five individual 100ml standard flask and diluted upto the mark with mobile phase to get the final concentration ranging from about 2-10µ/ml of both the drugs. Triplicate injections of 20µl were made two times for each concentration and chromatographed under the conditions as described above. Peak areas of the drug were recorded with the UV detector set at 260nm. Plot the peak area Vs respective concentration of Tolperisone HCl were found to be linear in the range of 2-10µg/ml with correlation co-efficient (r2) 0.99628. The system suitability parameters given in table 3 and plotted in fig 2.

Figure 2
Linearity of Tolperisone HCl

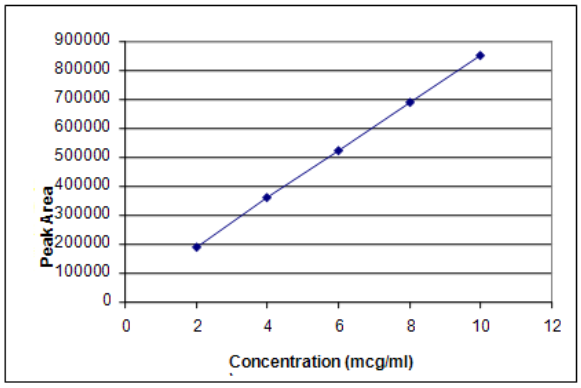
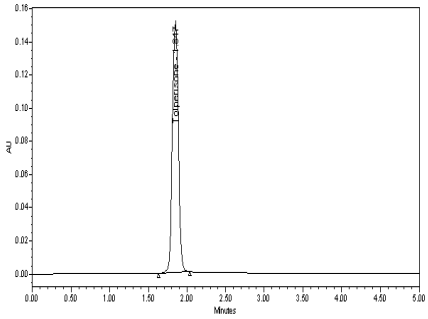


Figure 3
A typical chromatogram of standard Tolperisone HCl



Specificity

The specificity of the Rp-HPLC method was determined by elution of Tolperisone HCl shown in fig 3 Here, tailing factor for peak obtained was less than 2% and satisfactory. The retention time for Tolperisone HCl was found to be 1.85for six replicates. The peak obtained for Tolperisone HCl was sharp with clear baseline. Result of the method validation experiments are given in table3.

Linearity and Range

Table 3
Validation and System suitability Parameters

PARAMETER	TOLPERISONE HCl
Linearity	2-10µg/ml
Slope	1.25615
Intercept	0.49583
Correlation co-efficient (r2)	0.99628
Retention time (min)	1.847
Tailing factor	1.02
LOD(µg/ml)	0.009
LOQ(µg/ml)	0.03
Theoretical plates (USP)	2734

Precision

A standard and sample (tablet) solution containing Tolperisone HCl of 10µg/ml were prepared. The standard and sample solution (n=6) were injected and the peak area of Tolperisone HCl present in pure and formulation were determined. Statistical evaluations of tablet analysis were showed in table 2. The intra day and inter day precision were determined and results are given in table 4.

Precision

. **Table 4**
Intraday and Interday Precision

Tablet Formulation	Intraday Precision *	Interday Precision *	%RSD	
	Mean%±S.D	Mean%±S.D	Intraday	Interday
Brand 1	99.15±0.4913	99.07±0.6047	0.4957	0.61045
Brand 2	101.30±0.5886	102.53±0.1555	0.5811	0.1517

Mean of six determination (n=6)

Accuracy

Accuracy of the method was carried by recovery studies at three different levels to cover both above and below (50%to 150%) the normal levels expected in the sample .The accuracy studies were carried out six times at each level of recovery. The results of studies along with its evaluation are given in table 5.

Accuracy

Table 5

Recovery studies of Tolperisone HCl in tablet dosage form

Formulation	%taken	%Recovered	%Recovery± S.D	% R.S.D
Brand 1	50	49.98	101.86±0.9713	0.9695
	100	100.05	99.01 ± 0.3273	0.3316
	150	150.35	99.25 ± 0.6973	0.6921
Brand 2	50	49.99	102.86±0.3259	0.3256
	100	100.08	101.65±0.5104	0.3335
	150	150.12	102.84±0.0529	0.0521

Mean of six determination (n=6)

Detection Limit and Quantification Limit

The LOD and LOQ were separately determined (table 1) based on the standard calibration curve. The residual standard deviation of the regression line or standard deviation of y- intercepts of regression lines may be used to calculate LOD and LOQ. LOD = 3.3xD/S and LOQ = 10x D/S , where , D is the SD of the y-intercepts of regression line and S is the slope of calibration curve.

Robustness

Robustness of the method was studied by deliberate variation of the analytical parameter such as flow rate (1± 0.2ml\min) pH of the water in mobile phase (pH 3±0.2) and the composition of mobile phase (45:55) ± 2.The results of robustness studies are give in table 6.

Robustness

Table 6

Results for Robustness study

Factor	Level	%RSD
Flow rate	1.2	0.5552
	0.8	0.1955
pH of mobile phase	2.8	0.4756
	3.2	0.5669
% of Acetonitrile	43	0.2029
	57	0.2799

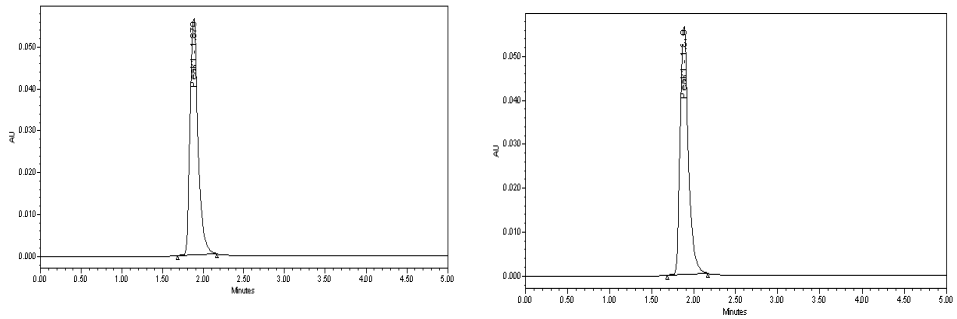
RESULT AND DISCUSSION

Optimization of mobile phase was performed based on asymmetric factor and peak area obtained for Tolperisone HCl .The optimized mobile phase containing acetonitrile: water (pH 3) (45:55 v/v) and water pH adjusted to 3 with

Ortho phosphoric acid was found to be satisfactory and gave symmetric resolved peak for Tolperisone HCl.The elution of Tolperisone HCl was found to be 1.847min. The flow rate was 1ml/min with UV detection at 260 nm. The calibration curve was found to be linear in the range of 2-10µg/ml for Tolperisone HCl with a correlation coefficient of 0.99628. The data of regression analysis of the calibration curves and the validation parameters are summarized in table 2.The quantification limit for Tolperisone HCl was 0.009µg/ml. The low % RSD value for intra day and inter day precision revealed that the proposed method is robust and rugged. The result obtained by the proposed method was close to the label claim of both brands. The lower values of %RSD in table 5, 6 indicate that the method is precise and accurate. The mean % recoveries of Tolperisone HCl for brand -1 and brand 2 ranging from 99.01 to 101.86 and 101.65 to 102.86 respectively. No interfering peaks were found in the chromatogram indicating that the excipients used in tablet formulations did not interfere with the estimation of drug by the proposed HPLC method. Forced degradation study was carried out by subjecting the drug to acid and alkali hydrolysis, chemical oxidation and photolytic conditions and its chromatograms were showed in fig:4-7.The chromatograms of oxidation degraded sample showed complete degradation product peaks at retention time (Rt)1.82, 2.18(after1hrs) 0.71, 1.06(after2hrs). The tolperisone HCl was found to be stable to rest of the conditions like photolytic stress degradation, acid and alkali degradation (table: 7).

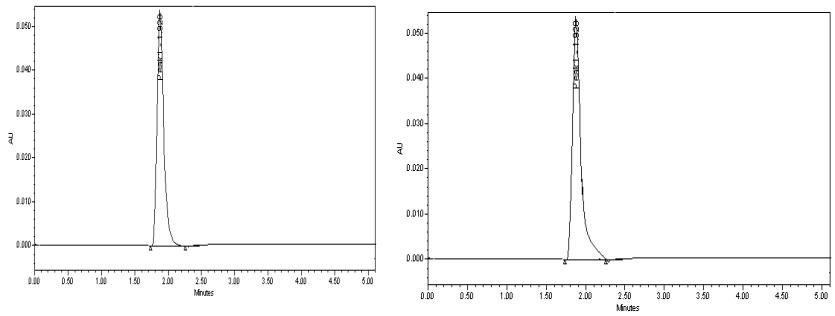
Forced degradation studies

Figure 4
Chromatogram for acid degradation studies after 1hrs (a) & 2hrs (b)



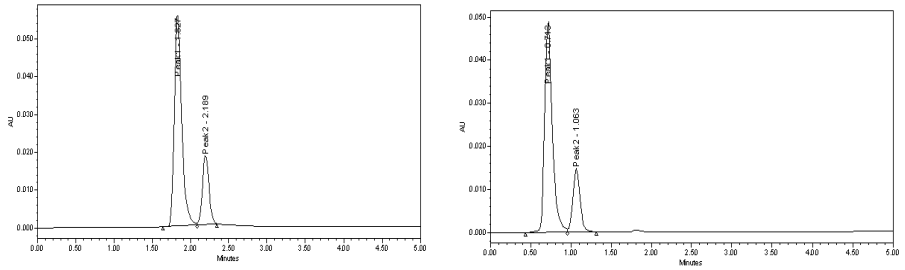
(a) (b)

Figure 5
Chromatogram for alkali degradation studies after1hrs (a) & 2hrs (b)



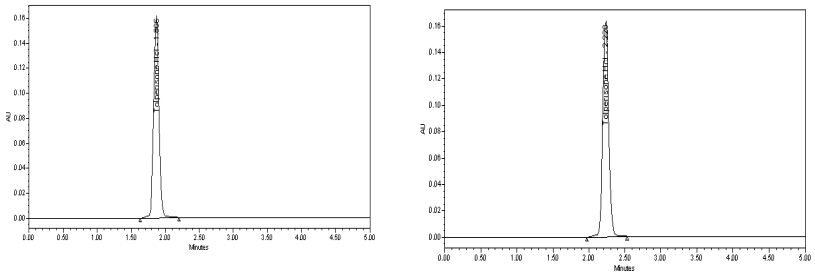
(a) (b)

Figure 6
Chromatogram for Oxidation degradation studies after 1hrs (a) & 2hrs (b)



(a) (b)

Figure 7
Chromatogram for photolytic degradation studies after1hrs (a) & 2hrs (b)



(a) (b)

Forced Degradation Studies

Table 7
Summary of result of stress degradation studies

Condition	Time	%Degradation
0.01N NaOH (10ml)	1 hr	99.95
	2 hr	99.02
0.01N HCl (10ml)	1 hr	98.01
	2 hr	102.24
3% Hydrogen Peroxide(10ml)	1 hr	61.36
	2 hr	52.53
Photolytic	1 hr	99.55
	2 hr	99.63

CONCLUSION

The study describes a new RP-HPLC method for the estimation of Tolperisone HCl in bulk and pharmaceutical formulation which is simple, accurate, sensitive and reproducible. The proposed method has the use of inexpensive solvent where it has the ability to separate these drugs from their degradation products, related substance, excipients found in tablet dosage forms and can be applied for routine analysis in quality control laboratories.

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