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STABILITY INDICATING RP HPLC METHOD FOR DETERMINATION AND VALIDATION OF TOLPERISONE AND ETODOLAC IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

This present study reports the simultaneous estimation of Tolperisone hydrochloride and Etodolac in tablet dosage form employing simultaneous equation and absorbance ratio method. The method was validated as per International Conference on Harmonization [ICH] guidelines. A kromasil column (150mm x 4.6mm, 5µ) was used with a mobile phase containing a mixture of ammonium acetate with ortho phosphoric acid solution buffer (pH-3.7) and Acetonitrile in the ratio of 52:48% v/v. The analysis was performed with run time of 10 minutes at a flow rate of 1ml/min. The retention time of Tolperisone HCl and etodolac were found to be 2.50 and 5.02 respectively. The validation of the proposed method was carried out for linearity, accuracy, recovery, precision, limit of detection and limit of quantification and robustness. Detection limit of for Tolperisone and Etodolac were 0.53 similarly quantification limits for Tolperisone and Etodolac were 1.62µg/ml, estimated from linearity by regression respectively. The results showed that the proposed method is suitable for the precise, accurate and rapid determination of Tolperisone and Etodolac in bulk, its combined dosage forms.

KEYWORDS

Tolperisone Hydrochloride, Etodolac, Kromasil Clumn, ICH and Validation.

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INTRODUCTION

Tolperisone Hydrochloride (TOL) chemically (R, S) 2-methyl-1-(4-methyl phenyl)-3- (1-piperidyl) propan-1 one is a piperidine derivative[1]. It is acentrally acting muscle relaxant which is used in the treatment of different pathological conditions like acute and chronic muscle spasm, electroconvulsive therapy, neurological conditions and orthopedic manipulation - multiocular sclerosis,

myelopathy, encephalomyelitis, spondylosis, spondylarthrosis, cervical and lumbar syndrome, Arthrosis of the large joints obliterating artherosclerosis of the extremity vessels, Diabeticalangiopathy, thromboangitisobliterans, raynauds syndrome[2,3]. Tolperisone Hydrochloride is official in Japanese pharmacopoeia. Non-steroidal anti-inflammatory drugs (NSAIDs) are a group of drugs of diverse chemical composition and different therapeutic potentials having a minimum of three common features: identical basic pharmacological properties, similar basic mechanism of action as well as similar adverse effects[1]. Etodolac [1, 8-Diethyl-1, 3, 4, 9-tetrahydropyrano (3, 4- b)-indole-1-acetic acid] is a non-steroidal anti-inflammatory drug, that it is used for treatment of postoperative pain and inflammation, for rheumatoid arthritis and osteoarthritis[2]. It is rapidly metabolized in the liver, followed by renal elimination as the primary route of excretion Literature survey reveals that tolperisone can be estimated by spectrophotometry HPLC and by HPTLC methods individually or in combination with other drugs. Etodolac is reported to be estimated by spectrophotometry and HPLC. The reported methods are applicable for the estimation of either for TPS or ETD individually or in combination with other drugs from pharmaceutical dosage forms or biological fluids. But all those methods are not reported any degradation studies to prove that the method is stability indicating method. The present work describes the development of a validated stability indicating analytical RP-HPLC method, which can quantify these Components simultaneously from a combined dosage form.

MATERIAL AND METHODS

Materials

Etogesic-T (Tolperisone and Etodolac) was manufacture by cadila health care ltd, containing 400mg of Etodolac and 150mg of Tolperisonehydrochloride were procured from a local pharmacy. And methanol, water and acetonitrile were used as diluent.

Instrumentation

A HPLC system Alliance (2695), photodiode array detector (2998) with Empower-2 software, manufactured by waters, used in isocratic elution of mobile phase. Ammonium acetate and disolved in 1000ml of water and pH was adjusted to 3.7 with Ortho Phosphoric acid solution Mobile phase Buffer and Acetonitrile are taken in the ratio of 52:48 (v/v) with flow rate of 1ml/min was performed on Kromasil 150mm x 4.6 mm, 5µ. The run time was set at 10 min and column temperature was maintained at 30^0C. The volume of injection was 10 µl, prior to injection of analyte, the column was equilibrated for 30-40 min with mobile phase. The eluents were monitored at 210nm using PDA detector, data were acquired, stored and analysed with Empower-2 software.

Chromatographic Conditions

The mobile phase used in this study was a mixture of ammonium acetate, water, orthophosphate and Acetonitrile buffer Ammonium acetate, Ortho Phosphoric acid solution and Acetonitrile (pH-3.7) in the ratio of 52:48% v/v. Stationary phase was Kromasil 150mm x 4.6 mm, 5µ. The contents of the mobile phase were filtered before use through a 0.45µ membrane. The mobile phase was pumped from the solvent reservoirs to the column at a flow rate of 1.0ml/min for 10min. The elute was monitored at 210nm using UV-detector. The retention time of the drug was found to be 2.5 min and 5.2 min for Tolperisone and Etodolac (Table No.1).

METHOD DEVELOPMENT[4-8]

Preparation of Standard Solutions (150µg/ml Tolperisone and 400µg/ml Etodolac)

Accurately weighed and transferred 15mg and 40mg of Tolperisone and Etodolac working standards into a 10 ml clean dry volumetric flask, add 7ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solutions, 1ml was pipeted out in to a 10ml volumetric flask and then make up to the final volume with diluent.

Preparation of Sample Solutions

5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 5 tablets was transferred into a 100 ml volumetric flask, 30ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipeted out into a 10 ml volumetric flask and made up to 10ml with diluent. Label Claim: 150mg Tolperisone + 400mg of Etodolac.

Method Validation

The developed HPLC method for the simultaneous determination of Tolperisone and Etodolac was validated as per the ICH guidelines.

Degradation Studies

Oxidation

To 1 ml of stock solution of Paroxetine and Clonazepam, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^0C. For HPL Cstudy, there sultant solution was diluted to obtain 500µg/ml and 10µg/ml solution and10µl were injected in to the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies

To 1 ml of stocks solution Paroxetine and Clonazepam, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0C. The resultant solution was diluted to obtain 500µg/ml and 10µg/ml solution and10µl solutions were injected in to the system and the chromatograms were recorded to assess the stability of sample (Figure No.2).

Alkali Degradation Studies

To 1 ml of stock solution Paroxetine and Clonazepam, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0C. There sultant solution was diluted to obtain 500µg/ml and 10µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies

The standard drug solution was placed in oven at 105^0C for 6h to study dry heat degradation. For HPL Cstudy, the resultant solution was diluted to 500µg/ml and 10µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability Studies

The photochemical stability of the drug was also studied by exposing the 300µg/ml and 10µg/ml and 25µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 500µg/ml and 10µg/ml solutions and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample (Figure No.1, 3-6).

Neutral Degradation Studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at temperature of 60º C. For HPLC study, the resultant solution was diluted to 500µg/ml and 10µg/ml solution and 10µl were injected in to the system and the chromatograms were recorded to assess the stability of the sample.

Linearity and Range

The standard curve was obtained in the concentration range of 37-225µg/ml for Tolperisone and 100-600 µg/mL for Etodolac. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r2] of standard curve were calculated and given in Figure No.7 (For Tolperisone) and Figure No.8 (For Etodolac) to demonstrate the linearity of the proposed method. The result of regression analysis was given in the Table No.2.

Accuracy

The accuracy of an analytical method is the closeness of results obtained by that method to the true value for the sample. It is expressed as recovery (%), which is determined by the standard addition method. In the current study recovery at three spike levels 50%, 100% and 150% were carried out. The % recovery at each spike level was calculated and was given in Table No.3. Recovery studies for Tolperisone by proposed method showed in the Table No.3. Recovery studies for Etodolac by proposed method showed in the Table No.4.

Precision

The precision of an analytical method is the closeness of replicate results obtained from analysis

of the same homogeneous sample. Precision was considered at different levels, i.e. method, system, Inter day and intraday. Precision of the developed method was assessed by measuring the response on the same day (intraday precision) and next two consecutive days (inter day precision). The precision of the method was assessed by six replicate injections of 100% test concentration. Intra and inter-day precision of the method was assessed by determination of standard deviation and % RSD for the analyte response. The result was given in Table No.5.

LOD and LOQ

LOD and LOQ values were determined by the formulae LOD = 3.3 σ/S and LOQ = 10 σ/S (Where, σ is the standard deviation of the responses and S is the slope of the calibration curves). In the present method σ is the mean of standard deviation of y intercepts of the three calibration curves and S is the mean of slopes of the calibration curves. The result was given in Table No.6.

Table No.1: System Suitability and Precision

S.No	Peak Name	RT	Area	% Area	Theoretical plates	Tailing factor
1	Tolperisone	2.509	2960049	25.75	2060	1.21
2	Etodolac	5.025	8534843	74.25	2555	0.96

Table No.2: Regression Analysis Results

S.No	Linearity study for Tolperisone			Linearity study for Etodolac	
	% Level	Conc. (µg/ml)	Area	Conc. (µg/ml)	Area
1	25	37.5	740090	100	2201061
2	50	75	1513160	200	4180360
3	75	112.5	2241172	300	6504834
4	100	150	2906048	400	8617580
5	125	187.5	3627055	500	10914979
6	150	225	4503986	600	12777177

Table No.3: Recovery Studies for Tolperisone

S.No	% Level	Amount Added	Amount Found	% Recovery
1	50%	750mg	747mg	99.6
2	100%	1495mg	1494mg	99.8
3	150%	2244mg	2241mg	99.8

Table No.4: Recovery studies for Etodolac

S.No	% level	Amount added	Amount found	% Recovery
1	50%	1980	1992	100
2	100%	3960	3984	100
3	150%	5940	5979	100

Table No.5: Method Precision (Inter and Intraday) studies for Tolperisone and Etodolac by proposed method

S.No	Summary showing Method Precision by Proposed Method	
	For Tolperisone	For Etodolac
	Method Precision	Method Precision
1	100.0409	99.20415
2	99.7957	99.5407
3	100.2392	100.4455
4	100.3904	99.95533
5	99.92416	100.906
6	99.6596	99.47771
Mean	100.01	99.92157
SD	0.2736	0.648315
% RSD	0.27	0.648824

Table No.6: LOD and LOQ Results

S.No	Tolperisone		Etodolac	
	LOD	LOQ	LOD	LOQ
1	0.5368742	1.6268914	1.0644914	3.2257314

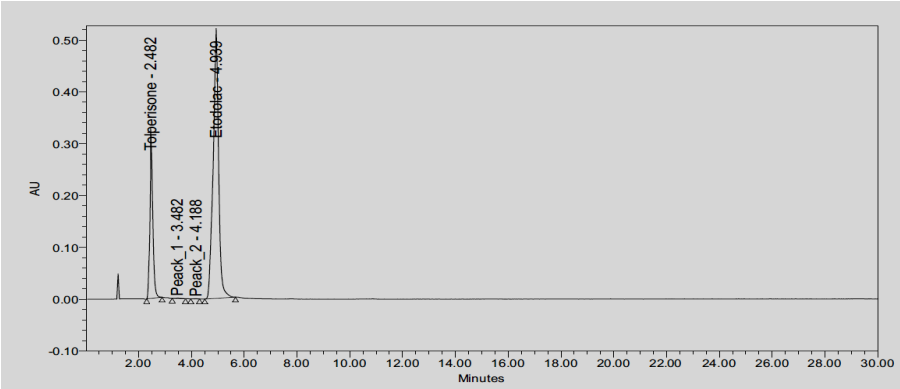


Figure No.1: A typical HPLC Chromatogram showing the profile of Tolperisone and Etodolac in Photo Stability by proposed method

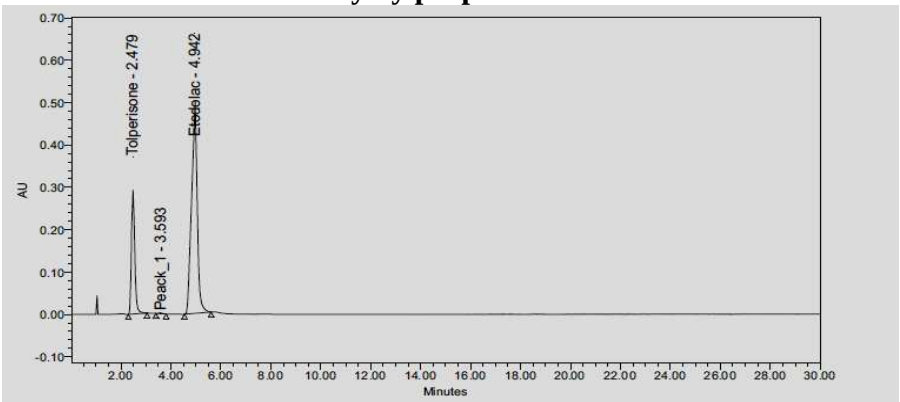


Figure No.2: A typical HPLC Chromatogram showing the profile of Tolperisone and Etodolac in Acidic hydrolysis by proposed method

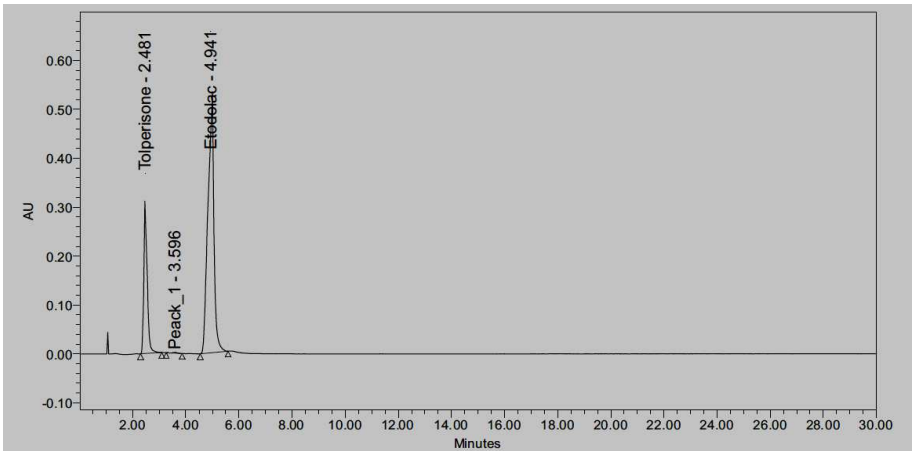


Figure No.3: A typical HPLC Chromatogram showing the profile of Tolperisone and Etodolac in Photo Stability by proposed method

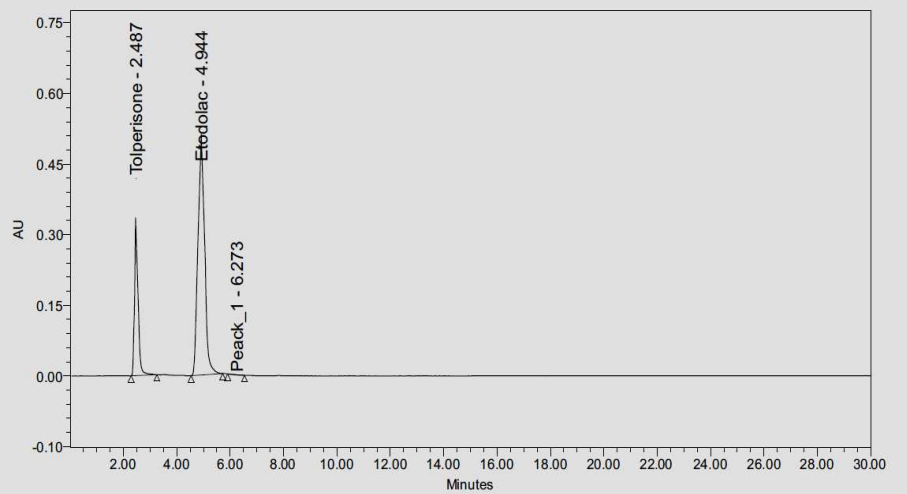


Figure No.4: A typical HPLC Chromatogram showing the profile of Tolperisone and Etodolac in Photo Stability by proposed method

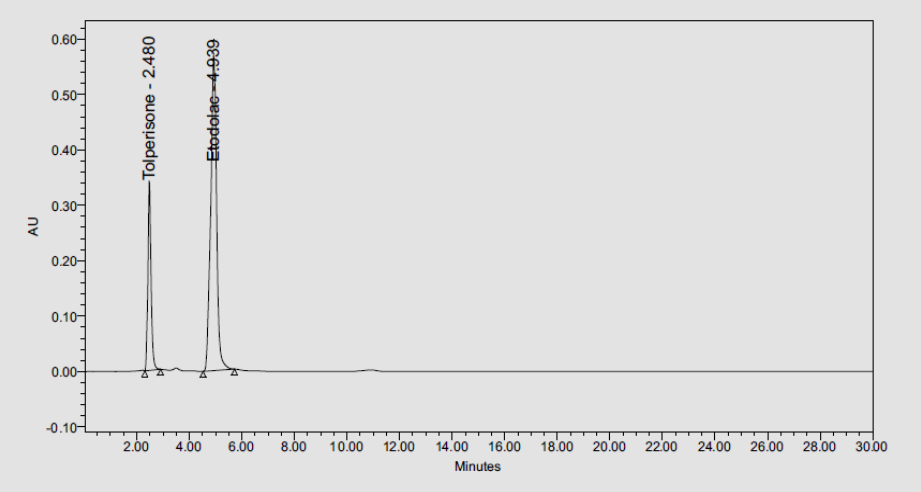


Figure No.5: A typical HPLC Chromatogram showing the profile of Tolperisone and Etodolac in Photo Stability by proposed method

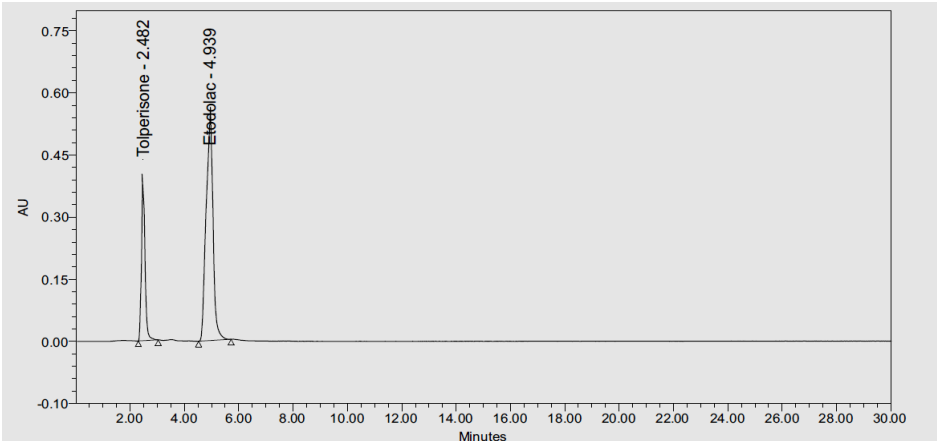


Figure No.6: A typical HPLC Chromatogram showing the profile of Tolperisone and Etodolac in Photo Stability by proposed method

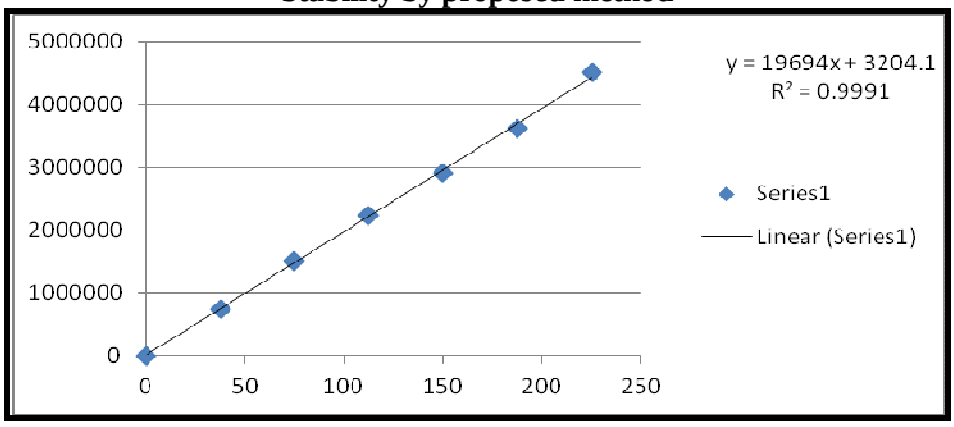
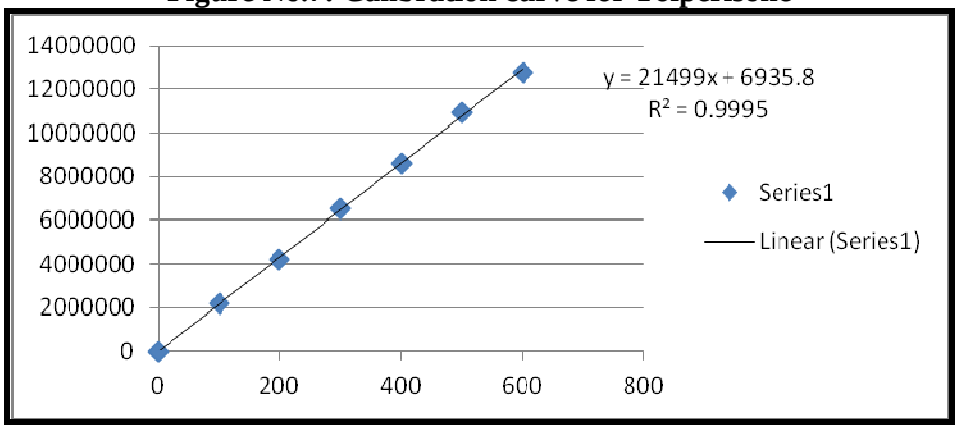


Figure No.7: Calibration curve for Tolperisone



FigureNo.8: Calibration curve for Etodolac

CONCLUSION

The proposed RP-HPLC method for simultaneous assay Tolperisone and Etodolac in combined dosage forms was validated, and found to be applicable for routine quantitative analysis of Tolperisone and Etodolac. The results of linearity, precision, accuracy and specificity, were proved to be within the limits. The method provides selective quantification of Tolperisone and Etodolac with noninterference from other formulation excipients. Therefore, this method can be employed for the routine analysis for simultaneous estimation Tolperisone and Etodolac in quality control of formulations and also in the dissolution studies.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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