Research Article

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METHOD DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF TOLPERISONE HCl AND ETODOLAC IN BULK AND ITS PHARMACEUTICAL FORMULATIONS

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Abstract: A rapid and sensitive Reverse Phase High Performance Liquid Chromatographic [RP-HPLC] method was developed for the estimation of Tolperisone and Etodolac[1,2,3] in pure and its tablet dosage forms. The method was validated as per International Conference on Harmonization [ICH] guidelines[35]. A C18 column (250×4.6mm, 5µm) was used with a mobile phase containing a mixture of potassium phosphate monohydrate buffer (pH-2.6) and Acetonitrile in the ratio of 70:30% v/v. The analysis was performed with run time of 6 minutes at a flow rate of 1ml/min. The Tolperisone and Etodolac was monitored at 263nm with UV detection and Tolperisone and Etodolac was eluted at 2.8 min and 4.2 min. The method was linear (r2 =0.999) at concentration ranging from 7.5 to 25µg/ml for Tolperisone and 100-300µg/ml for Etodolac, precise (intra-day relative standard deviation [RSD] and inter-day RSD values < 1.0%), accurate (99.3 to 100.9 for Tolperisone and 100.1 to 100.6 for etodolac), specific and robust. Detection limit of 1.30 for Tolperisone and 1.88 µg/ml for Etodolac. Similarly quantification limits were 3.93 for Tolperisone and 5.70 for Etodolac µg/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: Liquid Chromatography; Tolperisone, Etodolac, Combined dosage forms; Simultaneous estimation, Validation

INTRODUCTION

Tolperisone Hydrochloride (TOL) chemically (R, S) 2-methyl-1-(4-methyl phenyl)-3- (1-piperidyl) propan-1 one is a piperidine derivative1 [Figure-1]. It is a centrally 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, Diabetical angiopathy, thromboangitis obliterans, raynauds syndrome2,3. Tolperisone Hydrochloride is official in Japanese pharmacopoeia1

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] (**Figure 1**) 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 3.

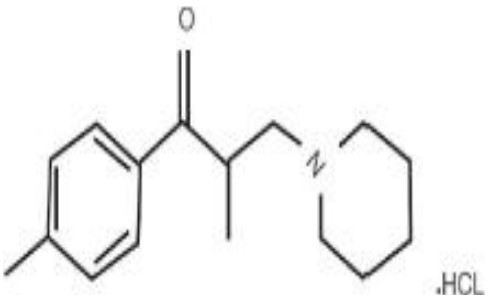

Figure 1A: Structure of Tolperisone

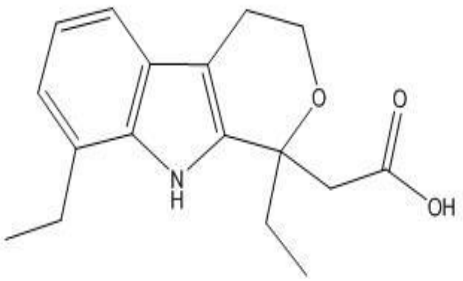

Figure 1B: Structure of Etodolac

Literature survey reveals that tolperisone can be estimated by spectrophotometry [4,5], HPLC[7,8,9] and by HPTLC methods individually or in combination with other drugs. Etodolac is reported to be estimated by spectrophotometry and HPLC[10,11,12]. The reported methods are applicable for the estimation of either for

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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.

MATERIALS AND METHODS

Materials

HPLC grade potassium dihydrogen phosphate (KH_2PO_4), ortho phosphoric acid, acetonitrile and water were procured from Merck India. All dilutions were performed in standard class-A, volumetric glassware. For the estimation of commercial formulation, Combination tablets of Etodolac 400mg and Tolperisone 150mg Tolpirisone manufactured by West Coast in Pharmaceuticals works ltd were procured from the local market.

Instrumentation

Agilent 1120 compact LC chromatographic system, with DAD detector and a fixed injector equipped with 20µL loop was used for the chromatographic separation. The chromatogram was recorded at and peaks quantified by means of Ezchrome software. Chromatographic separation was carried out on a C18 column [Sunsil, 250mm x4.5mm 5µ]. Sartorius electronic balance was used for weighing the samples. Ultra-sonic bath sonicator was used for degassing and mixing of the mobile phase.

Chromatographic conditions

The mobile phase used in this study was a mixture of Acetonitrile and potassium dihydrogen phosphate monohydrate buffer (pH-2.6) in the ratio of 70:30% v/v. Stationary phase was Sunsil C18 reverse phase column (250×4.6mm, 5µm) dimensions at ambient temperature. 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 6min. The elute was monitored at 263nm using UV-detector. The retention time of the drug was found to be 2.8 min and 4.2 min for Tolperisone and Etodolac.

Method development

Preparation of Standard Stock Solutions: Standard stock solutions was prepared by dissolving 50 mg of Tolperisone and 40 mg Etodolac working standard in two separate 100mL and 50mL volumetric flasks using 15mL of mobile phase and made up to the mark with mobile phase to obtain a final concentration of 500µg/mL and 400µg/mL of each Tolperisone and Etodolac. From the above stock solutions, 5 and 10 ml aliquots each was pipette in to a 100mLvolumetric flask and dissolved in 25mL of the mobile phase and made up to the mark with the solvent to obtain a final concentration of 30µg/mL and 80µg/mL for Tolperisone and Etodolac respectively.

Preparation of Sample solutions: Weighed and finely powdered 20 Tablets. Accurately weighed and transferred equivalent to 150mg of Tolperisone and 400mg of Etodolac into a 200 mL volumetric flask, added 150 mL of diluent, and sonicated for 30minutes with intermittent shaking at controlled temperature and diluted to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transferred 4.0 mL of the above solution into a 100 mL volumetric flask and diluted to volume with diluent to obtain a concentration of 30 and 80µg/mL of Tolperisone and Etodolac respectively.

Method validation

The developed HPLC method for the simultaneous determination of Tolperisone and Etodolac was validated as per the ICHguidelines[13, 14].

System suitability and System Precision: System suitability for chromatographic separation was checked on each day of validation to evaluate the components of the analytical system in order to show that the performance of the system meet the standards required by the method. System suitability parameters established for the developed method include number of theoretical plates (efficiency), Resolution, Tailing factor. The HPLC system was equilibrated using the initial mobile phase composition, followed by 5 injections of the standard solution of 100% concentration containing 30 µg/mL tolperisone and 80 µg/ml etodolac. These 5 consecutive injections were used to evaluate the system suitability on each day of method validation. The result was given in the Table 1.

Table 1: System suitability parameters for Tolperisone and Etodolac by proposed method

Name of the Compound	Retention Time	Tailing factor	Theoretical plates	USP Resolution
Tolperisone	2.957	1.39	5183	-
Etodolac	4.193	1.44	4143	5.82

Specificity

Blank interference: A study to establish the interference of blank was conducted. Diluent was injected into the chromatograph in the defined above chromatographic conditions and the blank chromatograms were recorded. Chromatogram of Blank solution **(Fig.2)** showed no peaks at the retention time of Tolperisone and Etodolac peak. This indicates

that the diluent solution used in sample preparation do not interfere in estimation of Tolperisone and Etodolac in Tolperisone and Etodolac tablets. Similarly typical representative chromatogram of standard is also shown (**Fig.3**)

Figure 2: A typical HPLC Chromatogram showing the no interference of diluent for Tolperisone and Etodolac

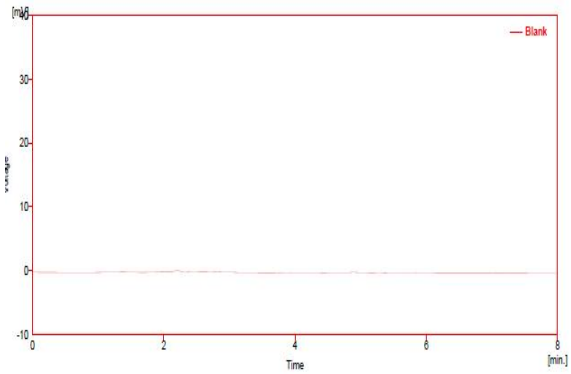
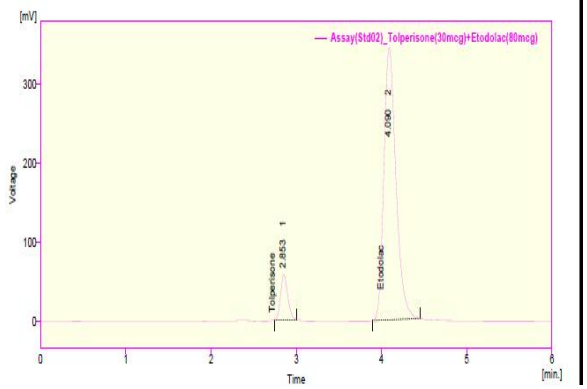


Figure 3: A typical HPLC Chromatogram showing the peak of Tolperisone and Etodolac



Forced Degradation

Control Sample: Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 150.mg of tolperisone and 400mg of etodolac into a 200 mL volumetric flask, add 150 mL of diluent, and sonicate for 30 minutes with intermittent shaking at controlled temperature and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 4.0 mL of the above solution into a 100 mL volumetric flask and dilute to volume with diluent. Refer **(Fig.4A)**

Acid Degradation Sample: Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 150.mg of tolperisone and 400mg of etodolac into a 200 mL volumetric flask, add 150 mL of diluent, and sonicate for 30 minutes with intermittent shaking at controlled temperature. Then add 5mL of 1N acid, refluxed for 30min at 60°C, then cooled to room temperature, neutralize with 1N NaoH and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 4.0 mL of the above solution into a 100 mL volumetric flask and dilute to volume with diluent. Refer **(Fig.4B)**

Base Degradation Sample: Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 150.mg of tolperisone and 400mg of etodolac into a 200 mL volumetric flask, add 150 mL of diluent, and sonicate for 30 minutes with intermittent shaking at controlled temperature. Then add 5mL of 1N NaoH, refluxed for 30min at 60°C, then cooled to room temperature, neutralize with 1N HCl and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 4.0 mL of the above solution into a 100 mL volumetric flask and dilute to volume with diluent. Refer **(Fig.4C)**

Peroxide Degradation Sample: Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 150.mg of tolperisone and 400mg of etodolac into a 200 mL volumetric flask, add 150 mL of diluent, and sonicate for 30 minutes with intermittent shaking at controlled temperature. Then add 5mL of Hydrogen Peroxide, refluxed for 30min at 60°C, then cooled to room temperature, and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 4.0 mL of the above solution into a 100 mL volumetric flask and dilute to volume with diluent. Refer **(Fig.4D)**

Thermal Degradation Sample: Powder collected from 20 tablets are exposed to heat at 105°C for about 5days. Accurately weigh and transfer equivalent to 150.mg of tolperisone and 400mg of etodolac into a 200 mL volumetric flask, add 150 mL of diluent, and sonicate for 30 minutes with intermittent shaking at controlled temperature and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 4.0 mL of the above solution into a 100 mL volumetric flask and dilute to volume with diluent. Refer **(Fig.4E)**

Similarly Humidity, UV-Light exposure, Sunlight exposure and Water hydrolysis stress samples are prepared and checked for their purity by proposed method.

Figure 4A: A typical HPLC Chromatogram showing the Control Sample profile of Tolperisone and Etodolac by proposed method.

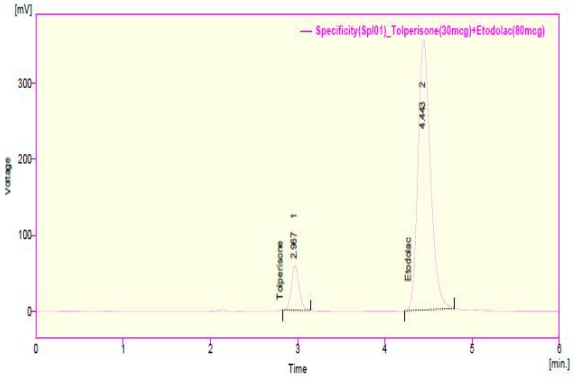


Figure 4B: A typical HPLC Chromatogram showing the profile of Tolperisone and Etodolac in Acidic hydrolysis by proposed method.

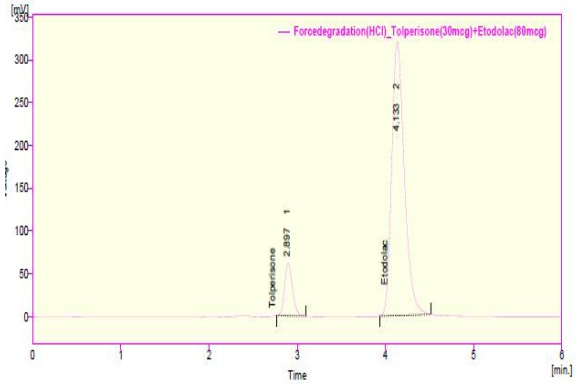


Figure 4C: A typical HPLC Chromatogram showing the profile of Tolperisone and Etodolac in Base hydrolysis by proposed method.

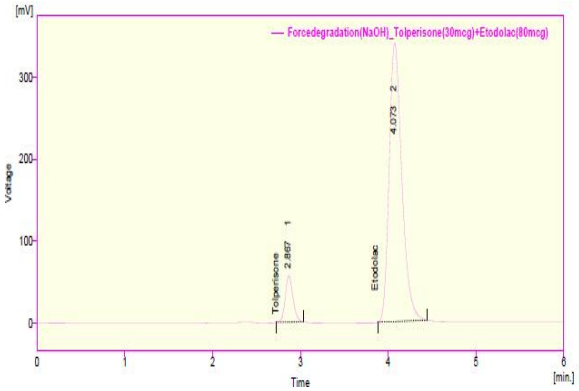


Figure 4D: A typical HPLC Chromatogram showing the profile of Tolperisone and Etodolac in Peroxide hydrolysis by proposed method.

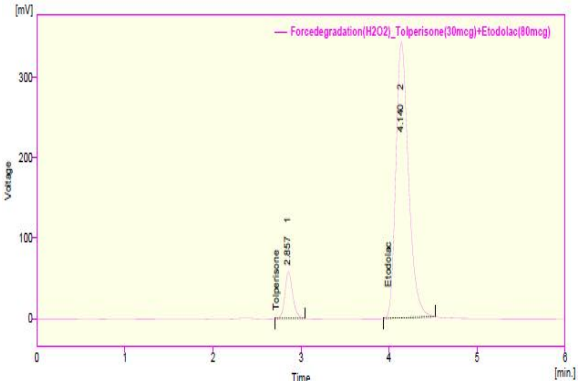
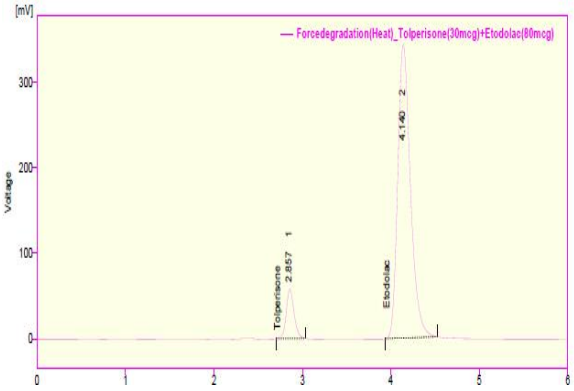


Figure 4E: A typical HPLC Chromatogram showing the profile of Tolperisone and Etodolac in Thermal hydrolysis by proposed method.



Linearity and range

The standard curve was obtained in the concentration range of 18-42µg/ml for tolperisone and 48-112 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-5A (For tolperisone) and Figure-5B (For etodolac) to demonstrate the linearity of the proposed method. The result of regression analysis was given in the Table 2.

Table 2: Linearity studies for Tolperisone and Etodolac by proposed method

Linearity Study for Tolperisone			**Linearity Study for Etodolac**	
% Level	**Conc. µg/mL**	**Area**	**Conc. µg/mL**	**Area**
60	18.00	212.082	48.00	2124.641
80	24.00	284.081	64.00	2834.196
100	30.00	349.659	80.00	3456.154
120	36.00	413.469	96.00	4105.076
140	42.00	472.695	112.00	4759.097
Slope		10.8		40.874
Intercept		21.1		185.94
% Y-Intercept		194.5		454.9
Residual Sum of Squares		4.3		23.312
CC(r)		0.9994		0.9998
RSQ(r2)		0.9987		0.9996
LLD		1.30		1.88
LLQ		3.93		5.70

From the data obtained which given in Table-2 (For Tolperisone and Etodolac) the method was found to be linear within the proposed range.

Figure 5A: Calibration curve for Tolperisone

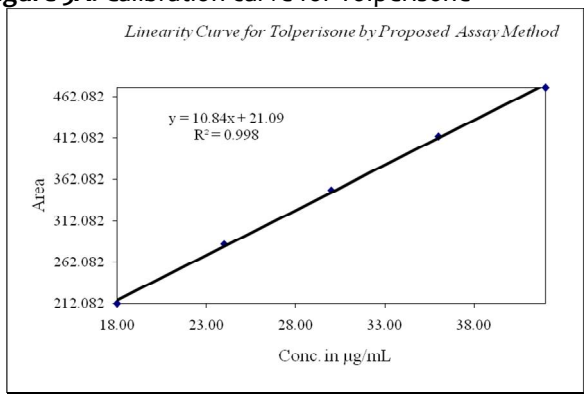
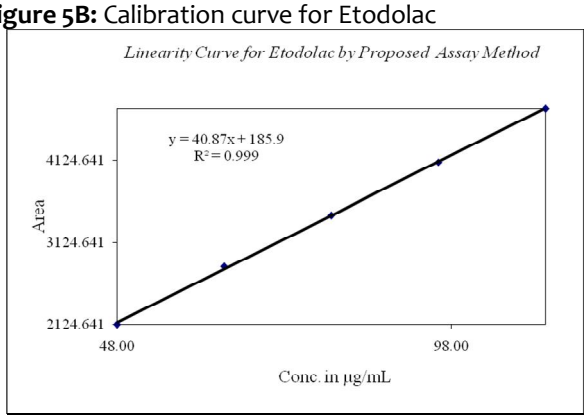


Figure 5B: Calibration curve for Etodolac



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 3.

Table 3A: Recovery studies for Tolperisone by proposed method

% Level	Recovery Range	% RSD at each level	Over all %RSD
50	97.8-98.9	0.6	
100	98.2-99.8	0.8	0.7
150	97.8-98.9	0.6	

Table 3B: Recovery studies for Etodolac by proposed method

% Level	Recovery Range	% RSD at each level	Over all %RSD
50	98.1-98.5	0.2	
100	98.6-99.6	0.5	0.8
150	99.2-100.5	0.7	

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 4.

Table 4: Method Precision (Inter and Intraday) studies for Tolperisone and Etodolac by proposed method

Summary showing Method Precision by Proposed Method			
For Tolperisone		**For Etodolac**	
Method Precision (Inter &Intra Day)		**Method Precision (Inter &Intra Day)**	
99.1	100.1	98.9	98.6
99.2	100.5	98.6	98.4
99.5	99.5	98.6	98.6
99.4	99.7	98.1	98.1
98.5	98.9	98.5	98.9
99.4	98.4	98.8	98.4
Overall Avg.	99.35		98.52
Overage Std Dev.	0.66		0.40
Over all %RSD	0.66		0.41

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 Table5.

Robustness

The robustness of the method was determined by assessing the ability of the developed method to remain unaffected by the small changes in the parameters such as percent organic content, pH of the mobile phase, buffer concentration, temperature, injection volume and flow rate. A deviation of ± 2nm in the detection wavelength, ± 0.1 mL/min in the flow rate, ± 5%change in the organic phase were tried individually. The result was given in the Table 5.

Table 4: Robustness studies for Tolperisone and Etodolac by proposed method

Parameter		% RSD	
		Tolperisone	Etodolac
Wavelength ±2	261 nm	0.22	0.36
	265 nm	0.34	0.57
Flow Rate mL /min	0.8 mL/min	0.68	0.49
	1.2mL.min	0.54	0.31

RESULTS AND DISCUSSION

Column chemistry, solvent selectivity, solvent strength (volume fraction of organic solvent(s) in the mobile phase), detection wavelength and flow rate were varied to determine the chromatographic conditions for giving the best separation. Several mobile phase compositions were tried to resolve the peaks of Tolperisone and etodolac. The optimum results were attained with acetonitrile: phosphate buffer (pH 2.6)in the ratio 30:70(v/v) because it could resolve the peaks of thiocolchicoside with retention time at 2.957 min and Aceclofenac retention time at 4.193 min. The two peaks were symmetric and sufficiently resolved. System suitability was carried out by injecting 5 replicate injections of 100%concentration of Tolperisone and etodolac. The resolution was found to be greater than 2 and the other parameters are presented in Table 1.

Specificity of the chromatographic method was tested by injecting mobile phase as blank and sample concentration prepared from marketed formulation. The response was compared with that obtained from the standard drug. The chromatogram confirms the presence of Tolperisone and Etodolac at 2.957min and 4.193min respectively without any interference. Thus the developed method was specific for analyzing the commercial formulations for Tolperisone and etodolac. An optimized chromatogram with the retention times of Tolperisone and Etodolac was shown in the Figure 2.

The peak areas corresponding to the concentration range of tolperisone 18-42 µg/mL and etodolac 48-112 µg/ml prepared in triplicate were plotted against the respective concentrations. The calibration curves were linear in the range studied for Tolperisone and etodolac, respectively, with mean correlation coefficients (n=3) of 0.999 and higher, the representative calibration curve is shown in Figure3. The regression analysis was given in Table 2.

Accuracy of the proposed method was assessed by standard addition method at 50%, 100% and 150% levels of recovery to the pre analyzed sample in triplicate. The recovery of the added standard to the sample was calculated and it was found to be 97.8-99.8 %w/w for tolperisone and 98.1-100.5%w/w for etodolac respectively and the % RSD was less than 2 for both the drugs which indicates good accuracy of the method. The result of recovery was given in table 3.

LOD and LOQ were calculated from the average slope and standard deviation of y intercepts of the calibration curve. Limit of detection for Tolperisone and Etodolac were 1.30 µg/mL and 1.88µg/mL respectively whereas limit of quantitation of Tolperisone and Etodolac were 3.93 µg/mL and 5.70 µg/mL respectively indicating high sensitivity of the method. LOD and LOQ value was given in table 2. The method is precise with a %RSD of less than 2 for both Tolperisone and Etodolac respectively. The results of intraday and inter day precision was given in table 4. Robustness was carried out by change in the flow rate (±1mL/min), mobile phase variation (±5%) and variation in wavelength (± 2 nm).Solution of 100% concentration is prepared and injected in triplicate for each varied operational condition and % R.S.D was found to be less than 2. The result was given in table 5. The proposed method was applied for the assay of commercial formulation containing Tolperisone and etodolac. Each sample was analyzed in triplicate. The mean recovery values were 99.43 and 100.45 for Tolperisone and etodolac. The result of estimation was given in table 6.

Table 6: Robustness studies for Tolperisone and Etodolac by proposed method

Drug	Amount Claimed in mg per Tablet	Tolperisone HCl	Etodolac
Tolperisone HCl	150	148.12	98.73
Etodolac	400	389.28	97.25

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 no interference 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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