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METHOD DEVELOPMENT AND SIMULTANEOUS ESTIMATION FOR SKELETAL MUSCLE RELAXANT IN SOLID DOSAGE FORM USING RP-HPLC

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ABSTRACT

A reversed-phase liquid chromatography (RP-HPLC) method was developed for the simultaneous determination of tolperisone hydrochloride (TOLP) and etodolac (ETD) in a solid dosage form. The analysis was performed using a Grace C18 column. The optimal composition of the mobile phase was found to be Methanol: Buffer 55:45 (v/v). The flow rate was set at 1.4 ml/min and UV detection was carried out at 254 nm. Retention times of Etodolac 8.30 and Tolperisone (9.36). The method showed a good linearity range between 10-50ug/ml for etodolac and 2-10 ug/ml for tolpresione. The LOD was found to be 0.17µg/mL and 0.08µg/mL for Etodolac and Tolperisone respectively and LOQ was found to be 0.51µg/mL and 0.25µg/mL for Etodolac and Tolperisone respectively. % Recovery study for Etodolac and Tolpresione was found to be 100.57,99.94 respectively. The RSD for the precision of the method was found to be less than 2%.

KEYWORDS: Etodolac, tolperisone, simultaneous estimation, validation.

INTRODUCTION

Chemically speaking, TOL is 2RS) -2-Methyl-1-(4-methylphenyl)-3-piperidin-1-yl propan-1-one monohydrochloride (Fig 1a). A centrally acting muscle relaxant piperidine derivative that is used to treat many kinds of pathological conditions, involving myelopathy,

encephalomyelitis, spondylosis, spondylarthrosis, cervical and lumbar syndrome, arthrosis of the large joints obliterating artherosclerosis of the extremity vessels, diabetical angthromboangitis obliterans, Raynaud's syndrome, and neurological illnesses. (IJPS 2012) Etodolac is non steroidal anti-inflammatory drug. Chemically speaking ETD, (R, S)-2-[1, 8-Diethyl-4, 9-dihidro-3H-pyrano (3, 4-b) indol-1-yl] acetic acid is a cox inhibitor, used as an analgesic to reduce pain in arthritis or acute injuries and to relieve moderate pain. Paper 2

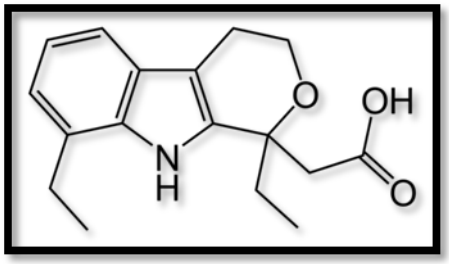

Fig. 1: Etodolac structure.

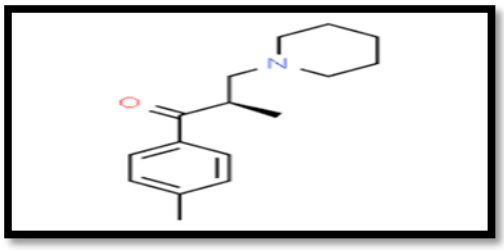

Fig. 2: Tolpresione structure.

EXPERIMENTAL WORK

1. MATERIALS

Etodolac and tolperisone were kindly supplied by Zydus Cadila Healthcare, Ltd.. Acetonitrile, water (HPLC grade, Merck), and all other reagents of AR grade were purchased from M R Enterprisers. A tablet ETOGESIC T (ZydusCadilaHealthcare Ltd.) containing 400 mg of etodolac and 150 mg of tolperisone was used. All the chemicals and reagents used were of HPLC grade.

2. Instruments and Equipments used

Table No. 2: List of Instrument and Equipment.

Sr. No.	Name of Instrument	Company Name
1	HPLC Instrument	YOUNGLIN ACME 9000 (Youngline Autochro 3000)
2	UV Spectrophotometer	Analytical Technological Ltd.
3	Column(C_{18})	C18 Grace smart (250mm X 4.6mm, 5µ)
4	pH meter	VSI pH meter(VSI 1-B)
6	Sonicator	FRONTLINE FS 4
7	Degases	Perkin Elmer Series 200S

3. Selection of Analytical Wavelength

We have screened both the standard solution over 190 nm to 400 nm using the advantage of photo diode array detector. On the basis of peak absorption maxima and peak purity index of

both the analyte, the 254 nm was decided as the detection wavelength which also gives the maximum chromatographic compatibility to the method.

4. Optimization of HPLC Method

The HPLC procedure was optimized with a view to develop method. Pure drug products were injected and run in different solvent systems. Initially different combinations of mobile phases such as in Methanol andBuffer (Accurately weighed 0.77gm of ammonium acetate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then pH adjusted to 3.7 with dil. Orthophosphoric acid solution)70: 30 (v/v), Methanol: Buffer 50: 50 (v/v), Methanol: Buffer 60: 40 (v/v), Methanol: Buffer 55: 45 (v/v) with at flow rate 1.4 ml/min gives acceptable retention time, theoretical plates and theoretical factor of the drugs.

4. Chromatographic Conditions for HPLC Method:

Different mobile phases were tried in order to find the best condition for separation of Etodolac and Tolperisone. The optimal composition of mobile phase was optimized to be Methanol :Buffer 55: 45 (v/v). The flow rate was set at 1.4 ml/min and UV detection was carried out at 254 nm. The mobile phase and samples was filtered using 0.45 µm membrane filter before injecting in HPLC system. Mobile phase was degassed by Ultrasonicator FRONTLINE FS 4. All determinations were performed at ambient temperature.

Diluent: Water and acetonitrile (50:50).

5. Preparation of Stock Solutions and Calibration Curves

5.1 Stock solution of ETO (400 µg/mL)

An accurately weighed quantity of about 400 mgTOL was transferred into 100 mL volumetric flask, add 70ml of diluent, sonicated for 30 minutes and make up to the final volume with diluent. From the above stock solution, 1ml was pipetted out in to a 10ml volumetric flask and then make up to the final volume with diluents to get concentration of 400µg/ml.

5. 2 Stock solution of TOL (150 µg/mL)

An accurately weighed quantity of about 150 mgTOL was transferred into 100 mL volumetric flask, add 70ml of diluent, sonicated for 30 minutes and make up to the final volume with diluent. From the above stock solution, 1ml was pipetted out in to a 10ml volumetric flask and then make up to the final volume with diluents to get concentration of 150µg/ml.

5.3 Sample preparation

About 20 tablets were taken and their average weight was calculated. The tablets were crushed to a fine powder and drug equivalent to 400mg of Etodolac and 150mg of Tolperisone were transferred to a 100ml volumetric flask, dissolved in diluent. Transfer 1ml from the above solution into 10ml volumetric flask and filtered through 0.45µ membrane filter to get concentration of 400µg/ml and 150µg/ml for Etodolac and Tolperisone.

5.4 Mobile phase preparation

The mobile phase consisted of a mixture of Methanol and buffer (Accurately weighed 0.77gm of ammonium acetate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then pH adjusted to 3.7 with dil. Orthophosphoric acid solution).

5.6 Analysis of Marketed Formulation

For the present work we selected a tablet ETOGESIC T (ZydusCadilaHealthcare Ltd.,) containing 400mg of Etodolac and 150mg of Tolperisonewere used.

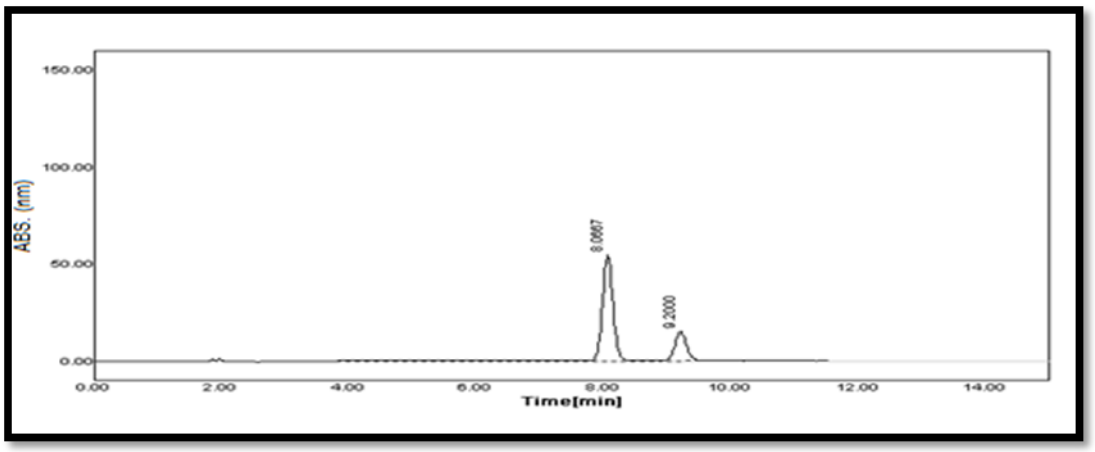

Fig. 1: Chromatogram of sample solution at optimized protocol.

Table No. 3: Analysis of Marketed Formulation of ETO and TOL by Proposed Method.

Parameters	Etodolac	Tolperisone
Label Claim(gm/tablet)	0.400gm	0.150gm
Drug content (%)/ ±SD	99.63/ ± 1.27	98.75/ ±0.94
%RSD	0.20	0.48

6. Method Validation

(A) System suitability

System suitability was performed and calculated at the start of study of each validation parameter. The values of system suitability results obtained during the entire study were recorded (Table 12).

Table No. 4 Mean values of system suitability parameters (n=5).

Parameters	ETO ± RSD (n = 5)	TOL± RSD (n = 5)
Retention time (min)	8.5± 0.14	9.76± 0.35
Tailing factor	1.04 ± 1.71	1.07± 0.51
Theoretical plates	8503.6 ± 1.21	11258.7 ± 1.06
Resolution	2.84 ± 1.48	

(B) Linearity

Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solutions of Etodolac and Tolperisone at different concentration levels. Absorbance of resulting solutions was measured and the calibration curve was plotted between absorbance vs concentration of the drug.A 20 µl volume of each sample solutions were injected into HPLC, five times.

Table No. 5: Linear regression data for calibration curves for Etodolac.

Con.(µg/mL)	Average area	Parameters	Etodolac
10	304.58	Linearity range	10-50 µg/mL
20	644.41	Correlation ± SD coefficient	0.998± 1.58
30	963.36	Slope	31.05
40	1259.09	Intercept	12.72
50	1549.76		

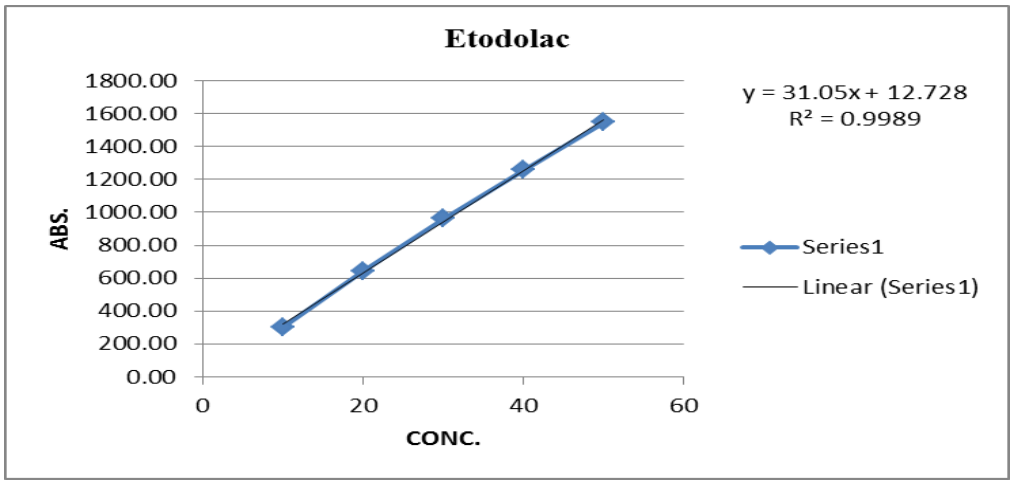

Fig. No. 2: Calibration curve of Etodolac.

Table No. 6: Linear regression data for calibration curves for Tolperisone.

Con.(µg/mL)	Average area	Parameters	Tolperisone
2	92.35	Linearity range	2-10 µg/mL
4	196.50	Correlation ± SD coefficient	0.999± 1.28
6	300.54	Slope	51.61
8	409.17	Intercept	9.517
10	502.12		

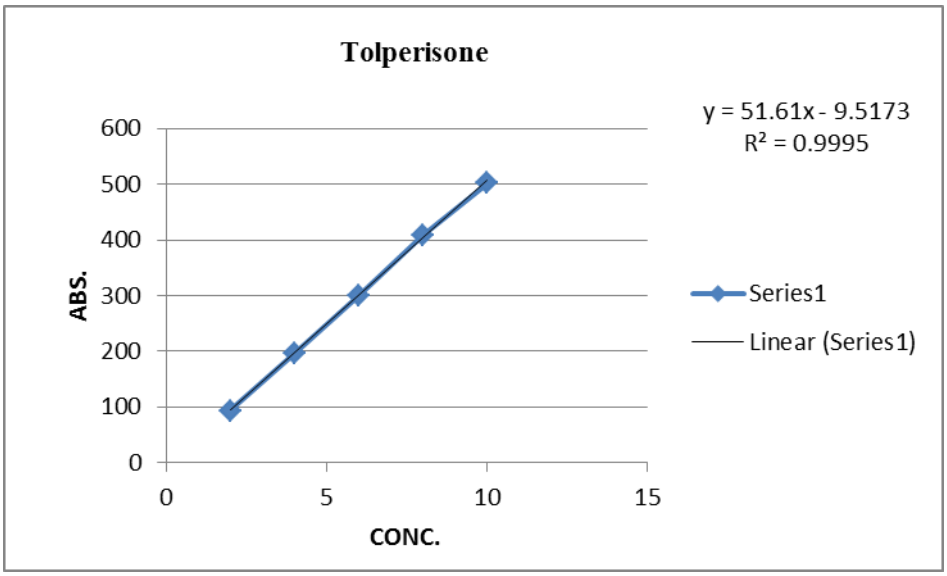

Fig. 4: Calibration curve of Tolperisone.

(C) Precision

The intraday and interday precision of the proposed method was determined by analyzing on 2 different days over a period of 1 week for 3 different concentrations of standard solutions of ETO (20, 30 and 40 µg/mL) and TOL (4, 6 and 8 µg/mL). The results were reported in terms of relative standard deviation (RSD). Precision data was shown in Table no. 7 and 8.

Table No. 7: Intra and inter-day precision of Etodolac.

Intra-day precision		Inter-day precision	
SD of areas	%RSD	SD of areas	%RSD
1.96	0.30	1.58	0.24
0.86	0.09	0.79	0.08
1.46	0.12	2.57	0.20

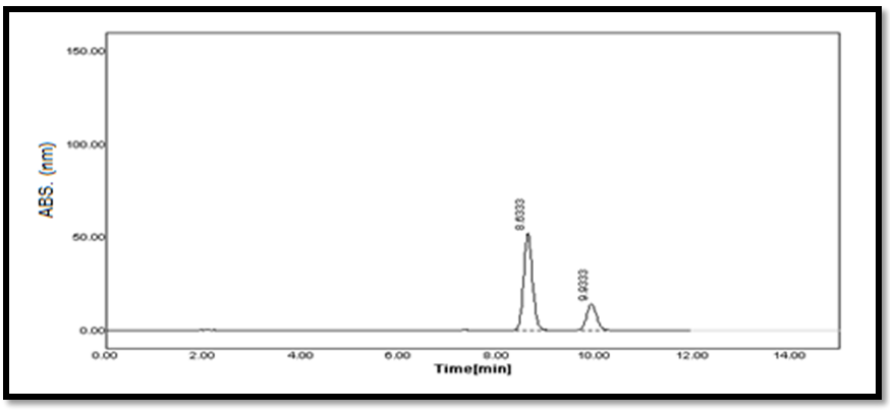

Fig No. 5: Chromatograms of solution using MeOH: Buffer: TEA (55:45:0.1).

At last, it was considered that methanol is best eluting agent for both drug. TOL and ETO are freely soluble in methanol. TEA was used to optimize retention time. So Methanol: Buffer : 0.1 TEA (55: 45: 0.1 v/v/v) was finalized as mobile phase.

Table No. 8: Chromatogram report of Etodolac and Tolperisone.

Sample	Area	Rt[min]	Tp	Tf	Resolution
Etodolac	643.49	8.63	10332.7	1.04	3.12
Tolperisone	195.18	9.93	11655.3	1.03	

Table No. 9: Intra and inter-day precision of Tolperisone.

Intra-day precision		Inter-day precision	
SD of areas	%RSD	SD of areas	%RSD
1.20	0.61	1.53	0.77
1.35	0.45	1.47	0.49
1.19	0.29	1.82	0.44

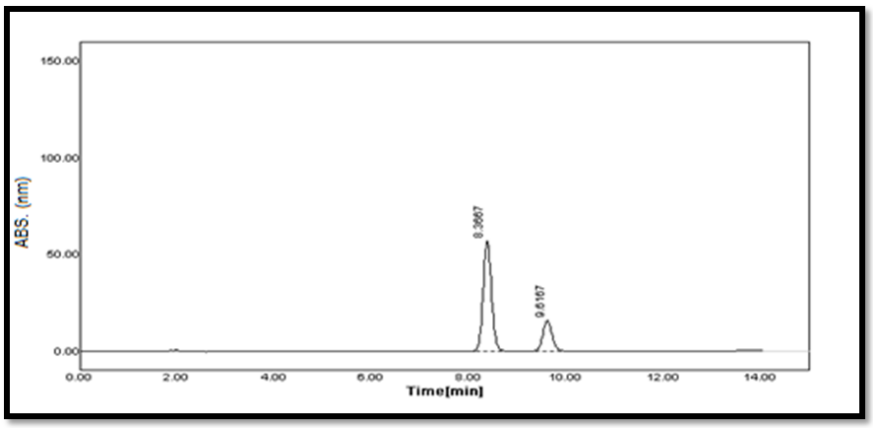

Fig. No. 6: Chromatograms of solution using MeOH: Buffer: TEA (55:45:0.1).

At last, it was considered that methanol is best eluting agent for both drug. ETO and TOL are freely soluble in methanol. TEA was used to optimize retention time. So Methanol: Buffer: 0.1 TEA (55: 45: 0.1 v/v/v) was finalized as mobile phase.

Table No. 10: Chromatogram report of Etodolac and Tolperisone.

Sample	Area	Rt[min]	Tp	Tf	Resolution
Etodolac	647.11	8.36	11548.9	1.13	3.12
Tolperisone	197.40	9.61	10924.0	1.03	

(D) Robustness of the method

To evaluate robustness of the developed method, few parameters were deliberately varied. These parameters included variation in flow rate, Percentage of mobile phase, Change of Wavelength. Robustness of the method was done at concentration level of 40µg/mL and 8µg/mL for Etodolac and Tolperisone. Each factor selected was changed at three levels (-1, 0, +1). One factor was changed at one time to estimate the effect.

Acceptance Criteria: The % RSD should NMT 2%

Result: The low % RSD value (< 2%) reveal that the proposed method is robust for this variation as shown in the Table 11 and 12.

Table No. 11: Change the flow rate of mobile phase.

Standard repetitions (n=3)	1.3mL/min		1.4mL/min		1.5mL/min	
	ETO	TOL	ETO	TOL	ETO	TOL
Mean Area ± %RSD	1565.10± 0.45	470.05± 1.08	1255.09± 0.12	409.17± 0.29	1393.39± 0.10	432.99± 0.32
Theoretical Plates ±% RSD	11643.8 ± 0.55	10785.8 ± 1.87	9749.60 ± 1.67	10849.7 ± 0.90	10450.2 ± 0.71	11111.17 ± 0.77
Ret. Time ±% RSD	8.31 ± 1.04	9.35 ± 0.99	8.39 ± 0.76	9.62 ± 0.78	7.64 ± 0.98	8.57 ± 0.88
Resolution	2.64 ± 1.53		2.99 ± 0.77		2.67 ± 0.65	

Table No. 12: Change the wavelength.

Standard repetitions (n=3)	253 nm		254 nm		255 nm	
	ETO	TOL	ETO	TOL	ETO	TOL
Mean Area ± % RSD	1365.74± 0.53	413.80± 1.28	1255.09± 0.12	409.17± 0.29	1434.12± 0.35	418.88± 1.19
TheoreticalPlates ± % RSD	9756.27± 1.61	11000.4± 1.95	9749.60± 1.67	10849.73± 0.90	9360.27± 1.18	10567.87± 1.50
Ret. Time ± % RSD	8.39± 0.76	9.62± 0.78	8.39± 0.76	9.62± 0.78	8.24± 0.70	9.44± 0.67

Resolution	2.97 ± 0.78	2.99 ± 0.77	2.85 ± 0.41

Table No. 13: Change the mobile phase ratio.

Standard repetitions (n=3)	M:B:T (54:46:0.1)		M:B:T (55:45:0.1)		M:B:T (56:44:0.1)	
	ETO	TOL	ETO	TOL	ETO	TOL
Mean Area ± % RSD	1268.77 ± 0.81	417.07 ± 0.83	1255.09± 0.12	409.17± 0.29	1332.37 ± 0.10	407.50 ± 0.21
Theoretical Plates ± % RSD	9812.00 ± 1.40	10992 ± 1.39	9749.60± 1.67	10849.73± 0.90	9801.70 ± 0.65	10307.57 ± 0.25
Ret. Time ± % RSD	8.42 ± 0.75	9.67 ± 0.78	8.39± 0.76	9.62± 0.78	7.03 ± 0.41	7.87 ± 0.73
Resolution	2.99 ± 0.77		2.99 ± 0.77		2.53 ± 0.91	

(E) Limit of detection and limit of quantitation

Limit of detection is lowest concentration of the analyte in the sample that the method can detect but not necessarily quantify under the stated experimental condition simply indicates that the sample is below or above certain level. Limit of quantification is the lowest concentration of the substance (analyte) in the sample that can be estimated quantitatively with acceptable precision, accuracy, and reliability by given method under stated experimental condition.

LOD= 3.3(SD)/S

LOQ = 10 (SD)/ S

Where, SD = Standard Deviation of response

S = the slope of the calibration curve

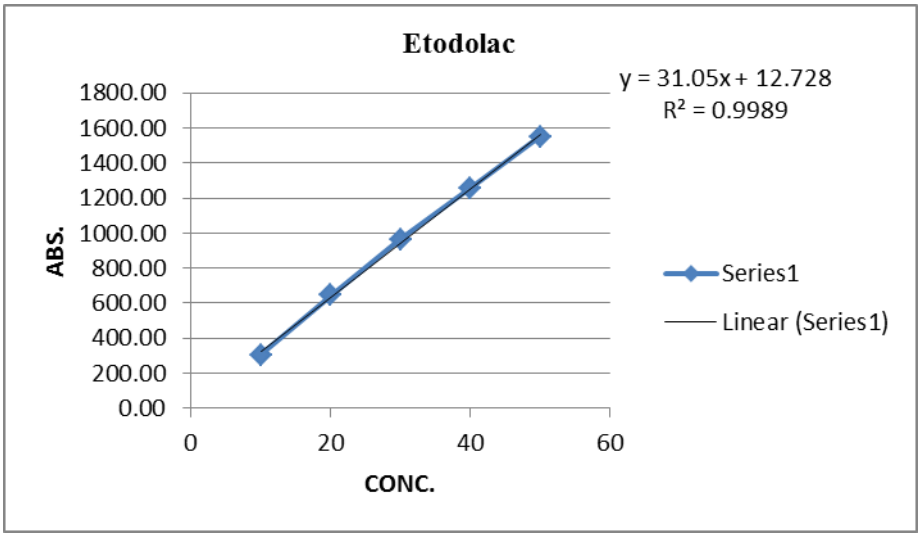

Fig. 7: Calibration curve for Etodolac.

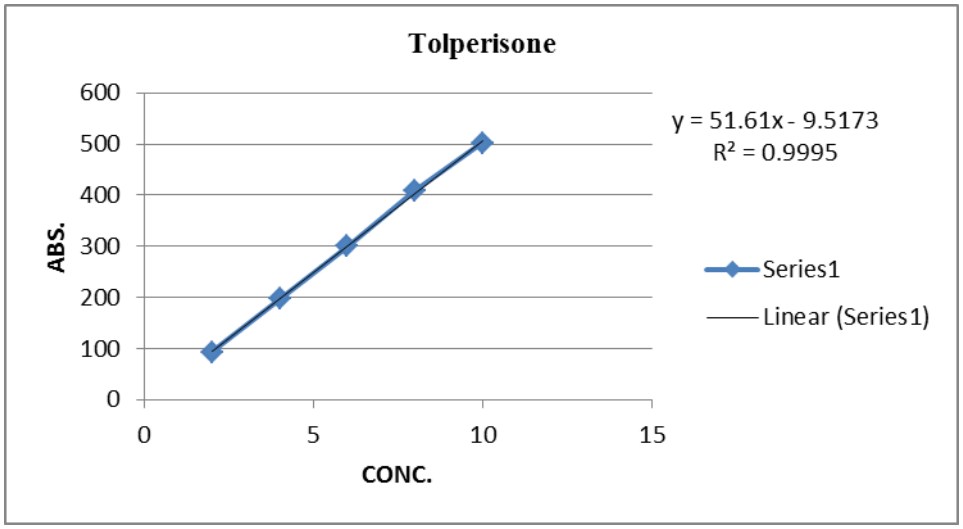

Fig. 8: Calibration curve for Tolperisone.

(F) Recovery studies (Accuracy)

For both drugs recovery studies were carried out by applying the method to drug sample to which known amount of Etodolac and Tolperisone corresponding to 80%, 100% and 120% of label claim had been added (Standard addition method). At each level three determinations were carried out and results obtained were compared with expected results. Recovery for individual and mean value (n=3) at each level should be between 98.0% to 102.0% with RSD not more than 2.0%.

Table No. 14: Standard addition technique for recovery determination Etodolac by HPLC.

Conc.	Amount added (µg/mL)	Amount found(µg/mL)	Amount recover(µg/mL)	% Recovery	Mean % recovery	Mean added (µg/mL)	Mean found(µg/mL)
80%	16	36.03	15.69	98.05	98.46	16	36.09
	16	36.06	15.72	98.22			
	16	36.19	15.85	99.09			
100%	20	40.53	20.19	100.96	100.92	20	40.52
	20	40.46	20.12	100.61			
	20	40.58	20.24	101.20			
120%	24	44.74	24.40	101.66	101.44	24	44.68
	24	44.63	24.29	101.21			
	24	44.69	24.35	101.46			

Table No. 15: Standard addition technique for recovery determination Tolperisone by HPLC.

Conc.	Amount added (µg/mL)	Amount found (µg/mL)	Amount recover (µg/mL)	% Recovery	Mean % recovery	Mean added (µg/mL)	Mean found(µg/mL)
80%	3.2	7.14	3.15	98.67	98.88	3.2	7.15
	3.2	7.17	3.18	99.48			
	3.2	7.14	3.15	98.49			
100%	4.0	8.00	4.01	100.44	100.73	4.0	8.01
	4.0	8.01	4.02	100.71			
	4.0	8.03	4.04	101.05			
120%	4.8	8.84	4.85	101.04	101.22	4.8	8.84
	4.8	8.83	4.84	100.99			
	4.8	8.86	4.87	101.63			

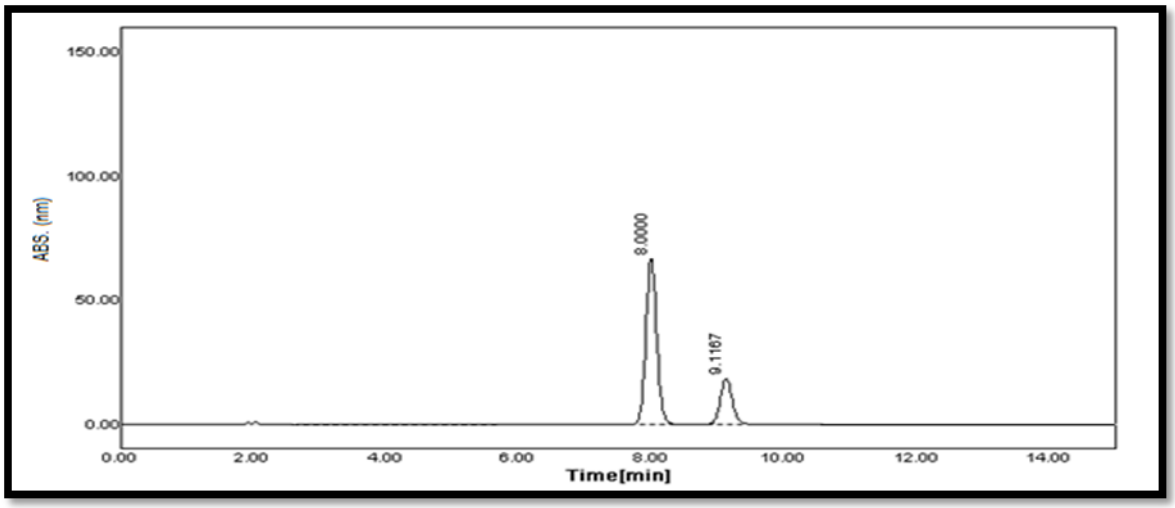

Fig. 8: Chromatogram of ETO and TOL [80%] (MeOH: Buffer (55:45, v/v), 1.4mL/min).

Table No. 16: Chromatogram report of ETO and TOL [80%].

Sample	Area	Rt[min]	Tp	Tf	Resolution
Etodolac	1201.09	8.00	10558.8	1.00	2.79
Tolperisone	359.37	9.11	9817.6	1.08	

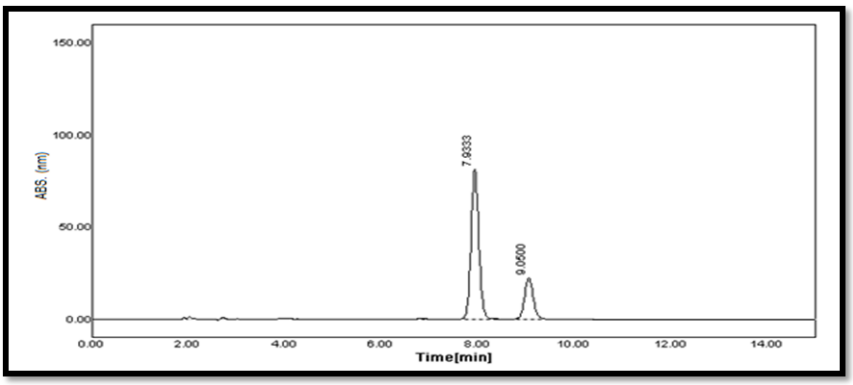

Fig. 9: Chromatogram of ETO and TOL [100%] (MeOH: Buffer (55:45, v/v), 1.4mL/min).

Table No. 17: Chromatogram repot of ETO and TOL [100%].

Sample	Area	Rt[min]	Tp	Tf	Resolution
Etodolac	1271.24	7.93	8725.1	1.09	2.79
Tolperisone	403.76	9.05	11354.1	1.08	

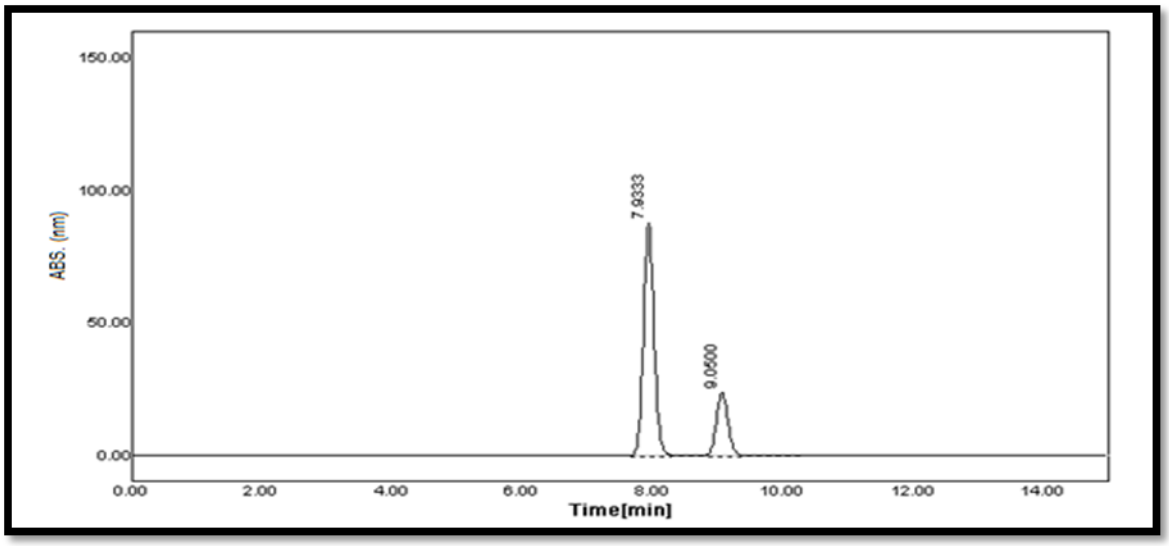

Fig. 10: Chromatogram of ETO and TOL [120%] (MeOH: Buffer (55:45, v/v), 1mL/min).

Table No. 18: Chromatogram repot of ETO and TOL [120%].

Sample	Area	Rt[min]	Tp	Tf	Resolution
Etodolac	1401.91	7.93	8725.1	1.09	2.68
Tolperisone	447.22	9.05	9674.5	1.12	

RESULT AND DISCUSSION

The results of analysis in all the method were validated in terms of Accuracy, Precision, Robustness, Linearity, Limit of Detection, Limit of Quantitation and System Suitability. The

methods were found to be sensitive, reliable, reproducible, rapid and economic also for the simultaneous estimation of Etodolac and Tolperisone in marketed formulation by RP-HPLC method and it can be used for routine analysis. For development of analytical method for simultaneous estimation of Etodolac and Tolperisone in marketed formulation by RP-HPLC method, various mobile phases like methanol and buffer are tried in various proportions at different flow rate and finally optimized chromatographic condition are shown below;

Column : Grace C_{18} (250mm X 4.6mm,5µm)

Particle size packing: 5 µL

Stationary phases: C_{18} (Grace)

Mobile phase : Methanol: Buffer:TEA (0.1 %), (55: 45 mix pH 3.0)

Detection wavelength :254 nm

Flow rate : 1.4 ml/min.

Temperature: Ambient

Sample size: 20 µL

Above developed method was then validated by Linearity, system suitability, Precision, Accuracy, Robustness like validation parameters and their results complies with official standards, are summarized in Table No.19.

Table 19: Summarized Result of Development and Validation for simultaneous estimation of Etodolac and Tolperisoneby RP-HPLC method.

PARAMETERS		ETODOLAC	TOLPERISONE	ACCEPTANCE CRITERIA
Linearity (µg/mL)		10 – 50	2 – 10	-
Regression equation		y=31.05x+12.72	y=51.61x– 9.517	-
Correlation coefficient (R)		0.998	0.999	R^2> 0.999
System Suitability	**R_t (min)**	8.50	9.76	-
	Tailing factor	1.04	1.07	Less Than 2
	N	8503.6	11258.7	More Than 2000
	R_S	0.00	2.84	More Than 2
LOD (µg/mL)		0.17	0.08	-
LOQ (µg/mL)		0.51	0.25	-
%Recovery (n=3)		100.57	99.94	98 - 102 %
Interday precision (%RSD) (n = 3)		0.18	0.56	RSD < 2 %
Intraday precision (%RSD) (n = 3)		0.17	1.45	RSD < 2 %
Robustness		Complies	Complies	-
% Assay ± SD (n= 3)		99.63 ± 1.27	98.75 ± 0.94	-

CONCLUSION

An attempt was made to develop HPLC method for estimation of Etodolac and Tolperisonein tablet dosage form. Method development included so many trials of pure drug sample by varying the chromatographic condition depending upon the properties of drug and result obtained from initial trial. Developed method was successively applied to pharmaceutical formulation. No chromatographic interferences were found from the excipients present in tablet. The present study was undertaken with an objective of developing suitable, sensitive and simple analytical method like RP-HPLC method for simultaneous estimation of Etodolac and Tolperisonein marketed formulation. The results of analysis in all the method were validated as per ICH guidelines in terms of System suitability, Accuracy, Precision, Robustness, Linearity, Limit of Detection and Limit of Quantitation . The methods were found to be sensitive, reliable, reproducible, rapid and economic also. Hence, this method can be employed for routine quality control analysis of Etodolac and Tolperisone in solid dosage form.

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