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RP-HPLC Method Development and Validation for the Simultaneous Estimation of Etodolac and Paracetamol in Tablet Dosage Form

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ABSTRACT
A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous determination of Etodolac And Paracetamol in pharmaceutical tablet dosage form. Chromatographic analysis was performed on a Symmetry X-terra C8 (4.6mm x 150mm, 5µm)column at ambient temperature with a mixture of mixed ortho phosphoric acid buffer and Acetonitrile in the ratio 50:50 v/v (ortho phosphoric acid buffer preparation; Take 1000ml of HPLC grade water. Dissolve 2.72 grams of Potassium di hydrogen phosphate salt and Adjusted the pH to 3.0 with orthophosphoric acid. as mobile phase, at a flow rate of 0.9 mL min-1. UV detection was performed at 260 nm. The method was validated for accuracy, precision, linearity and sensitivity. The retention times of Etodolac And Paracetamol were 2.190 and 3.373 min, respectively.
Calibration plots were linear over the concentration ranges 4–20 µg mL-1 to 5-25 µg mL-1 for Etodolac And Paracetamol, respectively. The Limit of detection was 0.361 and 0.559µg mL-1 and the quantification limit was 1.203 µg mL-1 and 1.864µg mL-1 for Etodolac And Paracetamol, respectively. The accuracy of the proposed method was determined by recovery studies and found to be 99.23% to 99.93%. Commercial tablet formulation was successfully analyzed using the developed method and the proposed method is applicable to routine analysis of determination of Etodolac And Paracetamol in pharmaceutical tablet dosage form.

Keywords:
Etodolac, Paracetamol, RP-HPLC,Validation

INTRODUCTION:

Paracetamol {N-(4-hydroxyphenyl) acetamide} is analgesic and antipyretic . Etodolac {(RS)-2-(1, 8-Diethyl- 4,9-dihydro-3H-pyrano[3,4-b]indol-1- yl)acetic acid} is an NSAID. Paracetamol is thought to act primarily by increasing the pain threshold by inhibiting both isoforms of cyclooxygenase, COX-1 and COX-2, enzymes involved in prostaglandin (PG) synthesis. Unlike NSAIDs, it does not inhibit cyclooxygenase in peripheral tissues and, thus, has no peripheral anti-inflammatory effects whereas Etodolac inhibits the biosynthesis of prostaglandins by selectively inhibiting COX-2 enzyme, resulting in lower concentrations of prostaglandins. As a consequence, inflammation, pain and fever are reduced .Analytical method for Paracetamol is official in IP, BP and USP and Etodolac is official in BP and USP.

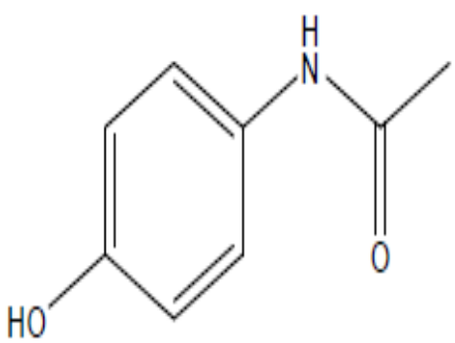


Fig 1: Structure of Paracetamol

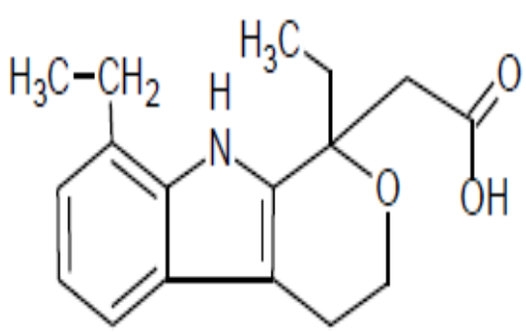


Fig 2: Structure of Etodolac

MATERIALS AND METHODS

Etodolac -300mgand Paracetamol-425mg were obtained from, IPCA Laboratories. Ltd. Mumbai. Double Distilled Water (HPLC grade), Methanol(HPLC grade), Acetonitrile (HPLC grade), orthophosphoric acid and Potassium-dihydrogen phosphate were of reagent grade. The pharmaceutical preparations of combination of Etodolac and paracetamol that is

ETOVA-P tablet (IPCA Laboratories. Ltd. Mumbai). Chemicals/ Reagents and Solvents.

Instrumentation and Equipments:

The HPLC analysis was accomplished on WATERS high pressure liquid chromatograph outfitted with 515 reciprocating dual column HPLC pump, a manually operating Rheodyne injector with 20µL sample loop, X-terra C8 4.6mm x 150mm analytical column reversed-phase material of 5µ size and a 2487 model UV-Visible detector. All the parameters of HPLC were controlled by N 2000 chromatographic system software. Other instruments used were TECHCOMP UV-Vis spectrophotometer of model 2310, Shimadzu electronic balance of model XEX-200, ADWA of model AD102U digital pH meter and ENERTECH of model SE60US ultrasonic bath sonicator.

ANALYTICAL METHOD DEVELOPMENT

Optimization of UV conditions:

A X-terra symmetry C8 (4.6mm x 150mm, 5µm) was used for chromatographic separation . Ther mobile phase composed of pH3Buffer(Orthophosphoricacid):Acetonitrile (50 : 50) at flow rate 0.9 mL/min with run time 6mins. Mobile phase and sample solution were filtered through a 0.45µm membrane filter and degassed.The detection of both drugs ws carried out at 260nm.

Figure-1.Isobestic point of Etodolac and Paracetamol

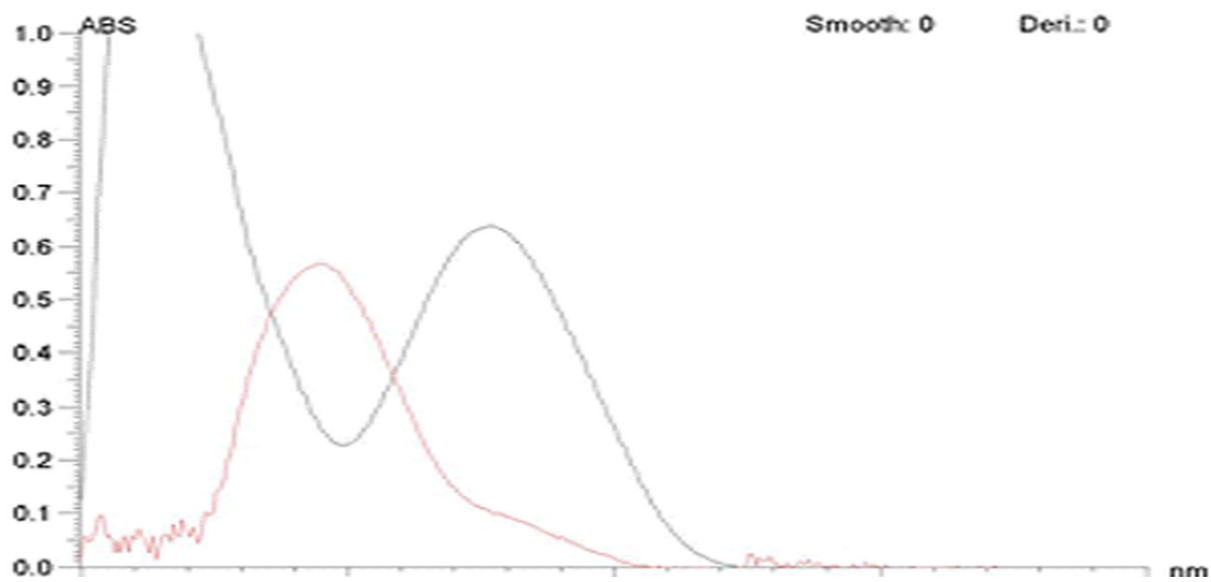
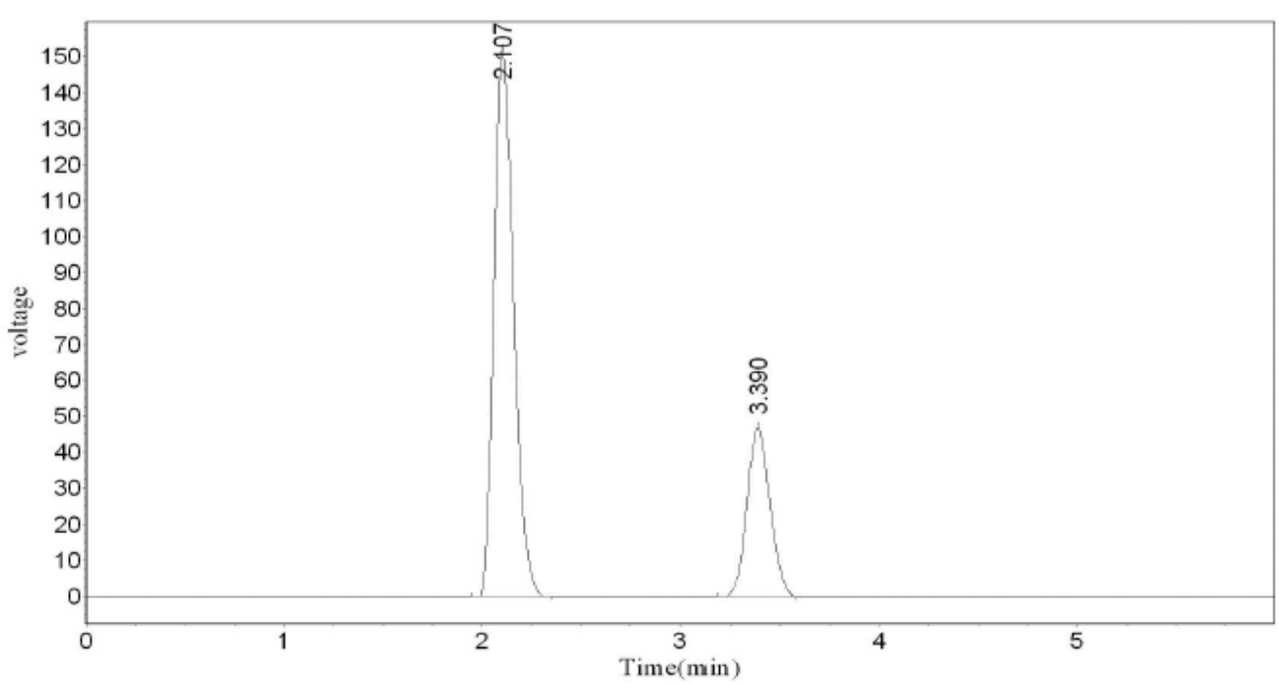


Figure 1.1.Optimized chromatogram



Optimized Method Parameters

MobilePhase : Phosphate buffer (3.0 pH): Acetonitrile(50:50)
Column (Stationary Phase): X-terra(C8) (4.6mm x 150mm, 5µm)
Flow rate (ml/min): 0.9
Column temperature (°C): Ambient
Volume of injection loop (µl): 20
Detection wavelength (nm):260
Drug RT (min): Etodolac- 2.1, Paracetamol- 3.3

PROCEDURE FOR PREPARATION OF SOLUTION:

Preparation of buffer:

Take 1000ml of HPLC grade water. Dissolve 2.72 grams of Potassium di hydrogen phosphate salt and Adjusted the pH to 3.0 with orthophosphoric acid.
Preparation of mobile phase:
A mixture of above prepared buffer 500 ml (50%),and 100 ml of HPLC grade Acetonitrile (50%) were mixed and degassed in ultrasonic water bath for 5 minutes. The mobile phase was filterred through 0.45 µ filter under vacuum.

Diluent Preparation:

Use Mobile phase Diluent Phase

ASSAY:

Preparation of the Etodolac and Paracetamol standard & sample solution:

Preparation of Standard Solution:

Accurately weighed and transferred 10 mg of Etodolac and 12.5 mg of Paracetamol working standard into a 10ml clean dry volumetric flask and added about 7.0ml of diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluent. (Stock solution) From this, 5 ml of the solution was pipette into another 50ml volumetric flask and diluted up to the mark with diluent
From this, 2.4 ml of the solution was pipetted into another 20 ml volumetric flask and diluted up to the mark with diluent.

Sample Solution Preparation:

Accurately weighed and transferred tablet powder equivalent to 8 mg of Etodolac and 10 mg of Paracetamol working standard into a 100 ml clean dry volumetric flask and added about 70ml of diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluent. (Stock solution)
From this, 3 ml of the solution was pipetted into another 10ml volumetric flask and diluted up to the mark with diluent..
Procedure:
20 µL of the standard and sample solutions were injected into the chromatographic system and areas for the Etodolac and Paracetamol peaks were measured. %Assay was calculated by using the formulae.

Calculation:

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where:
AT = Average area counts of sample preparation.
AS = Average area counts of standard preparation.
WS = Weight of working standard taken in mg.
P = Percentage purity of working standard
LC = LABEL CLAIM mg/ml.

ANALYTICAL METHOD VALIDATION

The HPLC method was validated in accordance with ICH guidelines.

Accuracy:

Accuracy was carried out by % recovery studies at three different concentration levels. To the pre-analyzed sample solution of ETO and PARA a known amount of standard drug powder of ETO and PARA were added at 50, 100 and 150 % level.

Precision:

The system precision of the method was verified by five replicate injections of standard solution containing ETO and PARA. The method precision was carried out the analyte five times using the proposed method. Repeatability was measured by multiple injections of a homogenous sample of ETO and PARA.

Linearity:

The linearity was determined separately for ETO and PARA. Linearity of the method was studied by injecting 5 concentrations of both drugs prepared in methanol and calibration curves were constructed by plotting peak area against the respective concentrations.

Limit of detection and Limit of quantitation:

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD = 3.3 x ASD/S and LOQ = 10 x ASD/S, Where, 'ASD' is the average standard deviation and 'S' is the slope of the line.

Robustness:

Robustness was evaluated by making deliberate variations in few method parameters such as variation of wave length; flow rate and change in mobile phase

composition. The robustness of the method was studied for ETO and PARA.

RESULTS:

Selection of Chromatographic Conditions and Optimization of Mobile Phase:

Mobile phase was optimized to separate ETO and PARA using Symmetry C8 column (150 mm x 4.6 mm i.d., 5µm). Initially, ACN and phosphate buffer in the Equal proportions were tried as mobile phase but the splitting of the peaks for both these drugs was observed. Therefore, after adjustment of pH of mixed phosphate buffer to 3.0 with ortho-phosphoric acid, and mobile phase composition (ACN and phosphate buffer in 50:50 % v/v) was tried for resolution of both drugs. Good resolution and symmetric peaks were obtained for both drugs when the pH of the mobile phase (buffer) was adjusted to 3.0. The flow rate of the mobile phase was 0.9 mL min-1. Under optimum chromatographic conditions, the retention time for ETO and PARA was found to be 2.1 and 3.3 min, respectively when the detection was carried out at 260 nm. A typical chromatogram of two drugs is shown in (Figure 1).

Table-1: ACCURACY DATA

Drugs	Amount Added (mg)	Amount Found (mg)	% Recovery	% of mean recovery
Etodolac	12.0	5.28	99.5 %	99.25 %
	18.0	10.0	100.25 %	
	30.0	14.9	98.00 %	
Paracetamol	22.5	5.12	100.00 %	100.00%
	30.0	10.0	100.00 %	
	37.5	15.0	100.00 %	

Table2: SYSTEM PRECISION

	ETODOLAC		PARACETAMOL	
S.NO	RT	AREA	RT	AREA
1	2.098	1931909	3.373	667961
2	2.090	1885618	3.348	669145
3	2.090	1853618	3.365	653218
4	2.082	1857934	3.340	647193
5	2.098	1864418	3.373	642990
Average	2.0916	1878699	3.3598	656101.4
Standard Deviation	0.0066993	32184.49	0.015057	11941.19
% RSD	0.31999	1.713126	0.45627	1.82

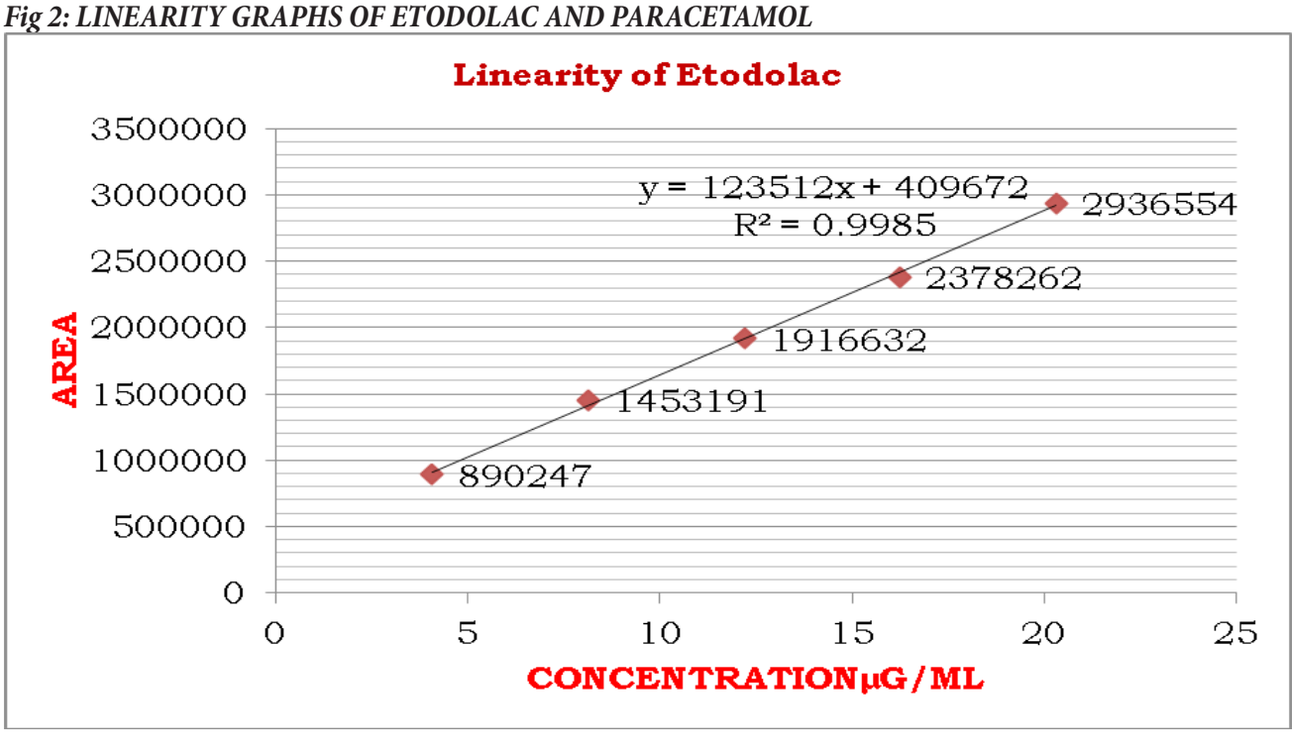
Table3: METHOD PRECISION

	ETODOLAC		PARACETAMOL	
S.NO	RT	AREA	RT	AREA
1	2.115	1786036	3.39	681699
2	2.09	1828867	3.348	685004
3	2.09	1848587	3.357	691862
4	2.098	1783285	3.373	687733
5	2.115	1816632	3.382	685330
Average	2.1016	1812681.4	3.37	686325.6
Standard Deviation	0.012661	28021.94976	0.017364	3767.485
% RSD	0.602	1.545883891	0.51525	0.55

Table 4: Linearity results of Etodolac and Paracetamol

ETODOLAC		PARACETAMOL	
Conc(mcg/ml)	Mean Area	Conc(mcg/ml)	Mean Area
4 ppm	8902478	5 ppm	293111
8 ppm	1453191	10 ppm	506467
12 ppm	1916632	15 ppm	685330
16 ppm	2378262	20 ppm	863014
20 ppm	2936554	25 ppm	1085942

Fig 2: LINEARITY GRAPHS OF ETODOLAC AND PARACETAMOL



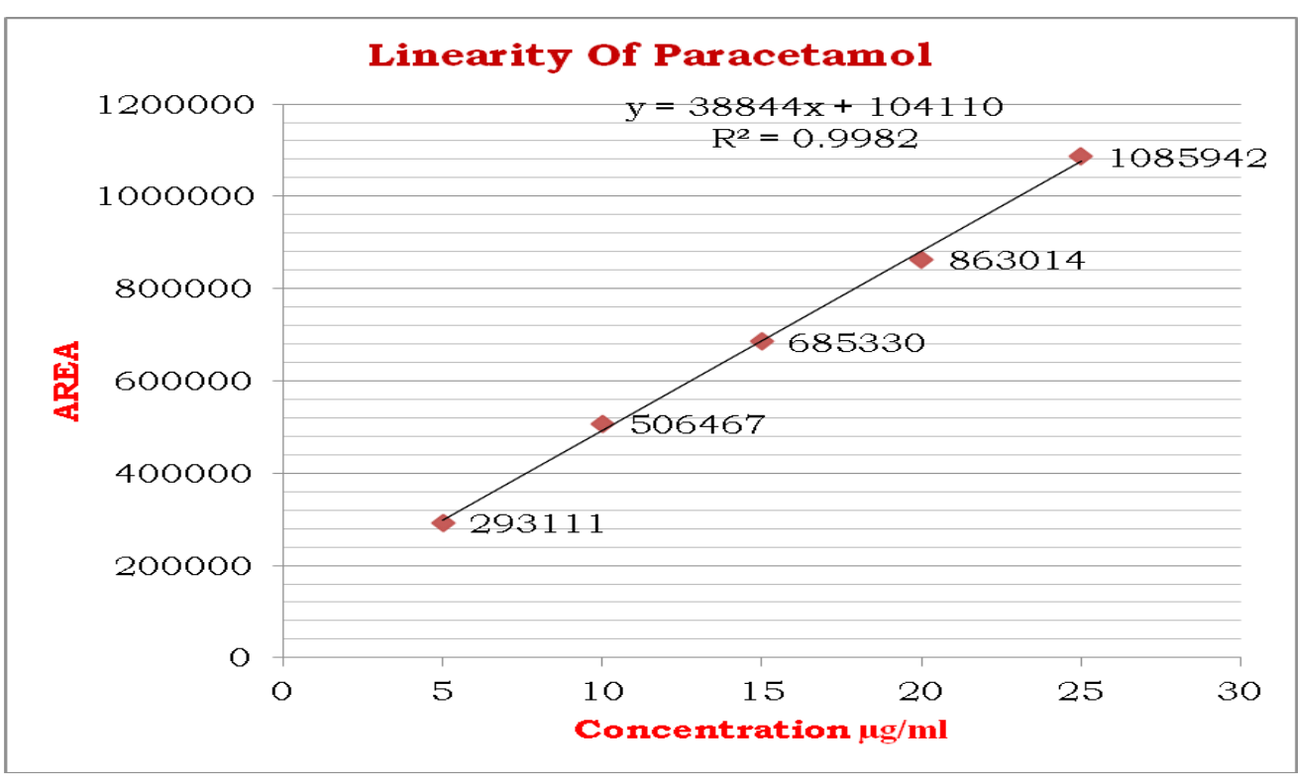


Table 5: LOD AND LOQ RESULTS

S.No	Drug name	Standard deviation	Slope	LOD	LOQ
1	Etodolac	14864	123512	0.361034	1.203446
2	Paracetamol	7244	38844	0.559469	1.864895

Table 6 Robustness Result For Etodolac And Paracetamol At Different Condition

S.No	Parameter	Etodolac			Paracetamol		
		Theoretical plates per column	Tailing factor	Resolution	Theoretical plates per column	Tailing factor	Resolution
1	Less flow(0.8ml/ min)	2244	1.144	-	4308	1.081	5.6
2	More flow(0.98 ml/min)	1952	1.222	-	3859	1.104	5.3
3	%10 Less organic	2261	1.167	-	4204	1.077	5.6
4	%10 More organic	1965	1.236	-	3653	1.114	5.263

RESULTS AND DISCUSSION:

Accuracy:

The accuracy of the method studied at three different concentration levels i.e. 50 %, 100 % and 150 % showed acceptable % recoveries in the range of 99.25% % for ETO and 100 % for PARA. The results are shown in Table 1.

Precision:

The precision study was evaluated on the basis of % RSD value was found to be in the range 0.3 – 1.82 and 0.5 – 1.5 %, respectively. As the RSD values were < 2% therefore developed method was precise. Results of precision study are shown in Table 2 & 3.

Linearity:

The linearity was determined separately for ETO and PARA.Linearity of the method was studied by injecting 5 concentrations of both drugs prepared in methanol and calibration curves were constructed by plotting peak area against the respective concentrations. The ETO and PARA followed linearity in the concentration range of 4– 20 µg mL-1 and 5-25µg mL-1; respectively. The results are shown in Table 4.and Fig no 2.

Limit of detection and Limit of quantitation:

The LOD for ETO and PARA was found to be 0.361034 and 1.203446µg, respectively. The LOQ for ETO and PARA was found to be 0.559469 and 1.864895 µg, respectively. The low values of LOD and LOQ indicates high sensitivity of the method. The results are shown in Table 5.

Robustness study:

Robustness of the method was studied by making de¬liberate changes in the chromatographic conditions and the effects on the results were examined. The low value changes of theoretical plates, tailing factor in-dicating robustness of the method. The results are shown in Table 6.

Analysis of marketed tablet formulation:

3 replicates of the samples solutions (20 µL) were injected for quantitative analysis. The amounts of ETO and PARA estimated were found to 99.09 % and 98.42 %, respectively. A good separation and resolution of both drugs indicates that there was no interference from the excipients commonly present in pharmaceutical formulations. The results are shown in Table 7.

System Suitability Test:

The system suitability parameters such as resolution, number of theoretical plates and tailing factor were studied and were summarized in Table 8.

Table 7:ASSAY RESULTS		
Assay Results Drug	**Amount present/tablet**	**% of Assay**
Etodolac	325mg	99.09
Paracetamol	400mg	98.42

Table 8:SYSTEM SUITABILITY PARAMETERS

System suitability parameters Parameters	Etodolac	Paracetamol
Tailing Factor	1.273	1.095
Theoritical plates	2094	4034
Resolution	-	5.423

CONCLUSION:

The developed RP-HPLC method is simple, precise, accurate, selective and reproducible. The method has been found to be adequately in linearty and robustness can be used for simultaneous determination of Etodolac and Paracetamol in tablet formulation. The method was validated as per ICH guidelines

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