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"Development and Validation of Analytical Method for the Estimation of NSAID Drugs"

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ABSTRACT
NSAIDs are among the most often prescribed pain relievers. NSAIDs are a highly successful medicine class for pain and inflammation due to the ever-increasing number of medications and drug combinations on the market necessitates the development of analytical tools for monitoring their quality. The procedures must be developed in such a way that they take less time to design and produce the most accurate and robust results possible. The current study's combined dosage form comprises Paracetamol and etoricoxib in solid oral dosage forms, which was recently approved by the USFDA.This research consistsof method validation developmentwith simple, precise, accurate, and economical RP-HPLC method according to ICH criteria for the analysis of Paracetamol and Etoricoxib in bulk and different dosage forms.In this research study, it was tried to develop a stability-indicating RP-HPLC method to determine possible degradation products of Paracetamol and Etoricoxib. Gradient system GradientSystem UV Detector and C_{18} column with 250mm x4.6 mm i.d and 5µm particle size Acetonitrile: phosphate Buffer (80:20v/v) For the technique, the mobile phase was pH 3. The flow rate was 0.9 ml/min and the detecting wavelength was 237 nm. The retention times of paracetamol and etoricoxib were determined to be 4.77 minutes and 11.97 minutes, respectively, in the described approach. The linearity, precision, range, and robustness were all within the ICH recommendations' parameters.

Keywords:NSAID's, Validation, Etoricoxib, Paracetamol, HPLC, Gradient

I. INTRODUCTION

Non-steroidal anti-inflammatory drugs, or NSAIDs, are among the most given as pain relievers. NSAIDs are a highly successful medicine class for pain and inflammation, but they are also known to cause gastrointestinal bleeding, side effects in the cardiovascular system, and NSAID-induced nephrotoxicity **[1,2].** In general practice, NSAID use is prevalent in patients over the age of 65, with a prevalence of up to 96 percent. **[3]**.In one year, around 7.3 percent of elderly individuals for the age group of 60 years filled at least one NSAID prescription **[4].**NSAIDs containantipyretic and analgesic qualities in addition to their anti-inflammatory activities. These drugs block cyclooxygenases (Coxs), which are rate-determining enzymes in the production of prostaglandins and other prostanoids like thromboxane**[5].** The capacity of NSAIDs to stop the manufacture of specific prostaglandins (PGs)its action is inhibition of the cyclooxygenase enzymes (COX-1 and COX-2) is the primary therapeutic activity of these drugs. COX-1 is responsible for the production of prostaglandins and thromboxane A2, which regulate the mucosal barrier in the GI tract, and renal balance. COX-2 creates prostaglandins (PGs), which are linked to inflammation, pain, and fever. Normal cells produce COX-1, whereas inflammatory cells produce COX-2 **[1, 6].**NSAIDs are classified into three classes as per thestructural properties and selectivity: acetylated salicylates (aspirin), non-acetylated salicylates (aspirin), and non-acetylated salicylates (aspirin).propionic acids (naproxen, ibuprofen, acetic acids (diclofenac, indomethacin), and selective COX-2 inhibitors (celecoxib, etoricoxib**) [7-9].**

Etoricoxib is sold under the brand name Ebov 60 mg, 90 mg, and 120 mg (Glenmark pharmaceuticals Ltd) available in India. It is prescribed for osteoarthritis, rheumatoid arthritis as well as in gouty arthritis. It acts by inhibiting the enzyme cyclo-oxigenase-2**[10, 11].** Acetaminophen is used to provide temporary analgesia for curing mild to moderate pain. Acetaminophen also is used in fixed combination with other agents Paracetamol is available in different dosage forms like syrup, tablets, capsules, cream, ointments, liquid, or

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injection. Calpol (500 mg) is the brand name of the Paracetamol tablet dosage form manufactured by GlaxoSmithKline Pharmaceuticals Ltd**[12].**

Pharmaceutical analysis is the application of analytical procedure which is essential for the determination of the purity, safety, and quality of drugs and chemicals. Any technique creation for HPLC should be founded on this understanding of the chromatographic process. In the vast majority of circumstances, achieving the necessary separation is simple with very few experiments. In other circumstances, a significant amount of trial and error may be required **[13]**. Analytical chemistry is a branch of chemistry whose goal is to understand the chemical composition of all matter and to develop different tools and analytical procedures that may be used to make qualitative or quantitative measurements **[13, 14].**

Many procedures are purposefully maintained static once they've been developed so that data can be compared across time. Apart from QA, analytical chemistry is becoming significant in the pharmaceutical business, where It is used in the discovery of novel medication candidates and in clinical applications where understanding the medication-patient interaction is critical. Method validation is used to ensure that the analytical approach used for a specific test is suitable for its intended application. The findings of method validation can be used to evaluate the quality, consistency, and reliability of analytical results; it is an important part of any good analytical practice. It is the process of defining an analytical requirement and ensuring that the method under consideration meets the application's performance requirements. The method validation procedure requires the use of equipment that is within specification, performing well, and properly calibrated. Similarly, the operator performing the studies must be knowledgeable about the analysis being performed and have enough knowledge of the procedure to draw inferences from the data as the validation work progresses. validation of methods are frequently followed method development, and the two operations are often intertwined, with the validation study using the same tools and steps in the analysis as the method creation **[15, 16].**There are two types of analysis, qualitative and quantitative. The qualitative analysis represents the presence of the functional group in organic as wellas inorganic compound samples.Whereas, the quantitative analysis produces or represents the amount of given compound in the sample **[17, 18].**

Analytical methods are classified as non-instrumental and instrumental. Different instrumental methods involve spectroscopy, chromatography, mass spectroscopy, Calorimetry, microscopy, electrochemistry, environmental analysis, forensic, crystallography, etc. The process of chromatography is applied for the application of separation, purification of the test substances. The mixture which is to be applied for separations is placed on a stationary phase (solid or liquid), and a pure solvent which includes water or any kind of gas is permitted slowlyto travel over the stationary phase, transporting the components separately according to their solubility in the pure solvent. Chromatography differs from most other physical and chemical separation processes in that two mutually incompatible phases are brought into contact; one is stationary and the other is mobile. A sample is conveyed through a column (manifold) containing a dispersed stationary phase after being put into a mobile phase **[17-19].**The ever-increasing number of medications and drug combinations on the market necessitates the development of analytical tools for monitoring their quality. The procedures must be developed in such a way that they take less time to design and produce the most accurate and robust results possible.The current study's combined dosage form comprises paracetamol and etoricoxib in solid oral dosage forms, which was recently approved by the USFDA.The goal of this project is to develop and validate a simple, precise, accurate, and cost-effective RP-HPLC technique for the estimation of paracetamol and etoricoxib in bulk and pharmaceutical dosage forms that follows ICH recommendations.

II. MATERIALS AND METHODS:

2.1 Chemicals and reagents

Paracetamol and Etoricoxib were procured from the Alembic pharma, Ahmedabad. Other analytical reagents includeorthophosphoric acid (OPA) of HPLC grade was purchased from the Avantor Performance material India Ltd. Thane, Maharashtra. MEOH andwater of HPLC grade were purchased from the Merck Specialities Pvt.Ltd.Shiv Sager Estate 'A' Worli, Mumbai. Analytical grade reagents and chemicals were used in this study.

2.2 Method Development

2.2.1 Chromatographic conditions and instruments

The analytical method development and validation were performed by using Agilent

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1100with an autosampler (Chemstation software) which includes the Agilent C18 (100mmX 4.6mm,5µm) having particle size packing 5 µm. The stationary phase used in this system is C-18 (Agilent) and the mobile phase was water (0.05% with OPA): MEOH in the ratio of 60:40. Optimization was done at the wavelength of 250 nm. The temperature of the system was maintained at 26^0C and pH was 3. The sample size used for this method was 20 µl with a flow rate of 0.7 ml/min.The different mobile phases with run times tried are presented in Table 1.

Table 1: List of trials

Sr.No.	Mobile Phase
1	[80% MEOH +20% plane Water Flow 0.7 ml/min abs at 250, 284 ,242 & 235 nm (column 100 mm X 4.6, 5.0 µm)
2	[60% MEOH +40% plane Water Flow 0.7 ml/min abs at 250, 284 ,242 & 235 nm (column 100 mm X 4.6, 5.0 µm)
3	[50 % MEOH + 50 % plane Water Flow 1.0 ml/min abs at 250 nm (column 100mm X 4.6, 5.0 µm)
4	[30 % MEOH + 70 % plane Water Flow 1.0 ml/min abs at 250 nm (column 100mm X 4.6, 5.0 µm)
5	[40 % MEOH + 60 % plane Water Flow 1.0 ml/min abs at 250 nm (column 100mm X 4.6, 5.0 µm)

2.2.2 Selection of wavelength by UV-Visible Spectrophotometry [20]

2.2.2.1 Preparation of standard stock solution

Paracetamol standard stock solution:(Stock I)

About 20 milligrams of Paracetamol (PARA) was carefully weighed and dissolved in methanol.in a 10 ml volumetric flask and adjust the volume up to 10 ml to solve 2000 ug/ml.

Etoricoxib standard stock solution: (StockII)

About 10 milligrams of Etoricoxib (ECXB) was correctly weighed and dissolved in methanol in a 10 ml volumetric flask and adjusted the volume up to 10 ml to get the concentration of 1000 ug/ml.

Preparation of Stock Standard Combination Solution :(Stock III) {PARA+ECXB]

Weight and transfer with precision In a 10 mL volumetric flask, combine 20 mg paracetamol and 10 mg Etoricoxib as a working standard as about diluent methanol completely and adjust the volume upto the given mark to get the concentration of 1000 &2000 µg/ml standard (stock solution). Sonication is to be done for about 15 min. for proper dissolving contents and removing the unwanted gas from the solution. A volumetric flask of capacity 10 ml was taken and the uppermost portion of the solution of Paracetamol and Etoricoxib stock solution in the ratio of 1:2 were taken and volume was adjusted up to the given mark by using mobile phase. About 0.1 ml solution from the resulting solution was transferred to the volumetric flask of capacity 10 ml and adjusted the volume with the help of MEOH:Water (0.1% OPA) which was prepared in (75 ml MEOH: 25 ml Water (0.1% OPA))solvent

2.2.2.2HPLC used for chromatographic condition applies on the Preparation of standard solution:-

Preparation of standardParacetamol solution: (StockI)

About 20 milligrams of Paracetamol (PARA) was carefully weighed and dissolved in methanol ina volumetric flask of capacity 10 ml its volume was adjusted up to the given mark to get a solution of 2000 ug/ml.Previously prepared fresh standard stock solution (2000ug/ml), about 0.1ml stock solution was taken out with the help of pipette and transferred into the volumetric flask which is having capacity of 10 ml and by using the mobile phase adjusted the volumeto prepare the final concentration of 20 ug/ml.

Preparation of standard Etoricoxib solution: (Stock II)

About 10 milligrams of Etoricoxib (DFS) was correctly weighed and dissolved in methanol in a volumetric flask of 10 ml capacity adjusted the volume up to the mark to get the final concentration of 40 ug/ml. From previously prepared fresh standard stock solution (1000ug/ml), 0.1 ml stock solution taken out with the help of the pipette in volumetric flask and volume adjusted up to the 10 ml by using mobile phase to prepare final concentration 0.1 ug/ml.

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Preparation of std. Paracetamol and Etoricoxib solution :(Stock III)

About 0.1 ml stock solution was taken out by pipette into a 10 ml volumetric flask from freshly made standard stock solution (1000 & 2000 ug/ml) and its volume adjusted with 10 ml with mobile phase to produce concentration 10 & 20 ug/ml.

2.2.3. Selection of mobile phase:

Each mobile phase was vacuum degassed and filtered through a membrane filter with 0.45-micron pore size.The mobile phase was allowed to equilibrate until OPA was achieved by baseline.To obtain a satisfactory separation and stable peak, the standard solution containing a mixture of paracetamol and etoricoxib was run with various individual solvents as well as combinations of solvents. The mobile phase containing MEOH and Water (0.1 percent OPA) was chosen from the several mobile phases tested because it produced crisp, well-resolved peaks with symmetry. For paracetamol and etoricoxib, it was within the limits and had a considerable repeatable retention time. (Table 1) shows the chromatograms of paracetamol and etoricoxib, respectively.

2.3 Studies of the Calibration plot

2.3.1 Procedure for calibration curve of Paracetamol and Etoricoxib

The mobile phase and stationary phase were allowed to equilibrate until OPA by baseline was achieved. Pipette 20 mg paracetamol and 20 mg etoricoxib into a 10 ml volumetric flask from the freshly made standard stock solution. It was then diluted with the mobile phase. To reach the final concentration, 0.1, 0.2, 0.3, 0.4, and 0.5 of the solution were pipette out into a 10 ml volumetric flask, and volume was brought up to 10 ml with the mobile phase. Etoricoxib (10, 20, 30, 40, and 50 g/ml) and Paracetamol (20, 40, 60, 80, and 100 g/ml).Samples were injected and peaks were recorded at 275 nm, as shown in the graph plotting drug concentration versus peak area.

2.3.2 Study of system suitability parameters:

The system suitability test is used to determine whether the chromatographic system's resolution and reproducibility are acceptable.These are adequate for analysis to be done. The data was collected from five replicate injections of reference solution during the test.

2.3.2.1 Calibration experiment [21]

Preparation of Calibration curve standard:

Five calibration curve (cc) standards were created by diluting the aforementioned standard stock solution (1: 2 g/ml) of Paracetamol and Etoricoxib with mobile phase with concentrations of 10, 20, 30, 40and 50 µg/ml of Etoricoxib and 20, 40, 60, 80 and 100 µg/ml of Paracetamol (Table 1) Figures 3 and 4 show the calibration curves for paracetamol and etoricoxib.

Selection of detection Wavelength:

Scanning of the standard solutions was done in the range of 200-400 nm, as a comparison to 10 mL MEOH and a volume made with a water solvent system The absorbance maxima (lambda max) of Paracetamol and etoricoxib were found to be at 230 nm and 271 nm, respectively.(Figure 3 and 4).This site is called isosbestic if two paracetamol and etoricoxib samples interact with itthen wavelength detection in the Isobestic point is 275 nm.

2.4 Validation of a method for analysis of Paracetamol and Etoricoxib

Linearity:

The mathematical treatment of test findings acquired by analysis of samples with analyte concentrations across the specified range determines the analytical method's linearity. The area as a function of analyte concentration is visually shown. (Figure3 & 4). Fittings of percentage curves are calculated.

Accuracy (recovery):

A known amount of analyte is added in the system is used for the analysis of the accuracy.The percentage of analyte recovered by the assay is used to compute the accuracy from the test findings.

Repeatability:

It was analyzed with the sample of the RP-HPLC method. Six replicates of a sample solution containing 80 µg/ml of Paracetamol and 40 µg/ml Etoricoxib.Peak regions were measured after the injectionsand %RSD was calculated.

Precision:

Intra-day precision:

Sample solutions containing 20 mg of Paracetamol and 10 mg of Etoricoxib three different concentration (20 µg/ml, 60 µg/ml, 100 µg/ml) Paracetamol and (10 µg/ml, 30 µg/ml, 50 µg/ml) Etoricoxib. Paracetamol and Etoricoxib weredetermined three times on the same day and %R.S.D was calculated.

Inter-day precision:

Sample solutions containing 20 mg of Paracetamol and 10 mg of Etoricoxib three

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different concentration (20 µg/ml, 60 µg/ml, 100 µg/ml) Paracetamol and (10 µg/ml, 30 µg/ml, 50 µg/ml) Etoricoxib. Paracetamol and Etoricoxib were determined three times on the same day and % RSD was calculated.

Robustness:

It was performed using 15 µg/ml and 30 µg/ml solution of Paracetamol and Etoricoxib in duplicate.

Detection Limit: By using the below formula, it can be calculated

$$DL = \frac{3.3\sigma}{S}$$

Where,

σ = the S.D. of the y-intercepts of regression lines.

S = the slope of the calibration curve.

The calibration curve can be used to estimate the slope S and S.D. was used should be calculated from the y-intercepts of the regression line in the calibration curve.

Quantitation Limit:

It was calculated by the below formula.

$$QL = \frac{10\sigma}{S}$$

Where,

σ = the S.D. of the y-intercepts of regression lines.

S = the slope of the calibration curve.

The calibration curve can be used to estimate the slope S and S.D. was used should be calculated from the y-intercepts of the regression line in the calibration curve.

2.5 Marketed formulation and its analysis

To determine the content of Paracetamol and Etoricoxib in marketed tablets (label claim 20 mg of Paracetamol and 10 mg Etoricoxib), 20 tablets powder weighed in 9.860 Gms and an average weight of powder was calculated in 0.493 Tablets were undergone trituration to make powder or tablet which should be equivalent to the weight in 49.3 mg. Extraction of the drug from tablet powder was done with help of 10 mL MEOH. It was sonicated for 15 minutes to achieve thorough extraction. The supernatant was then diluted with mobile phase to a final concentration of 0.3 mL.The resultant solution was put into an HPLC column, and the area of the drug peak was measured (Table 10). Peak regions of standard solutions were used to create the regression equation.The amount of Paracetamol and etoricoxib in the sample was determined using the regression equation and the sample's peak area. The amount of Paracetamol and Etoricoxib Per tablet was obtained from the regression equation of the calibration curve as described in the analysis of Tablet formulation.

III. RESULT AND DISCUSSION

3.1 method development and optimized chromatographic conditions

During analytical method development, the selection of stationary phase is very crucial and it mainly depends on the molecular weight and solubility of the molecule.In this study,Agilent C18 (100mmX 4.6mm,5µm) column was utilized which was having the particle size packing 5 µm. Optimization was done at 250 nm. UV absorption of 20 mcg solution of Paracetamol and Etoricoxib in MEOH was generated and absorbance was taken in the range of 200-400 nm. The maximum wavelength of Paracetamol and Etoricoxib in MEOH was observed to be 271 nm and 239 nm respectively. Figure 1 and 2 shows the chromatograms of Paracetamol and Etoricoxib respectively. Table 2 shows the studies on the chromatographic behavior of Paracetamol and Etoricoxib. Table 3 shows the optimized chromatographic conditions selected for Paracetamol and Etoricoxib.

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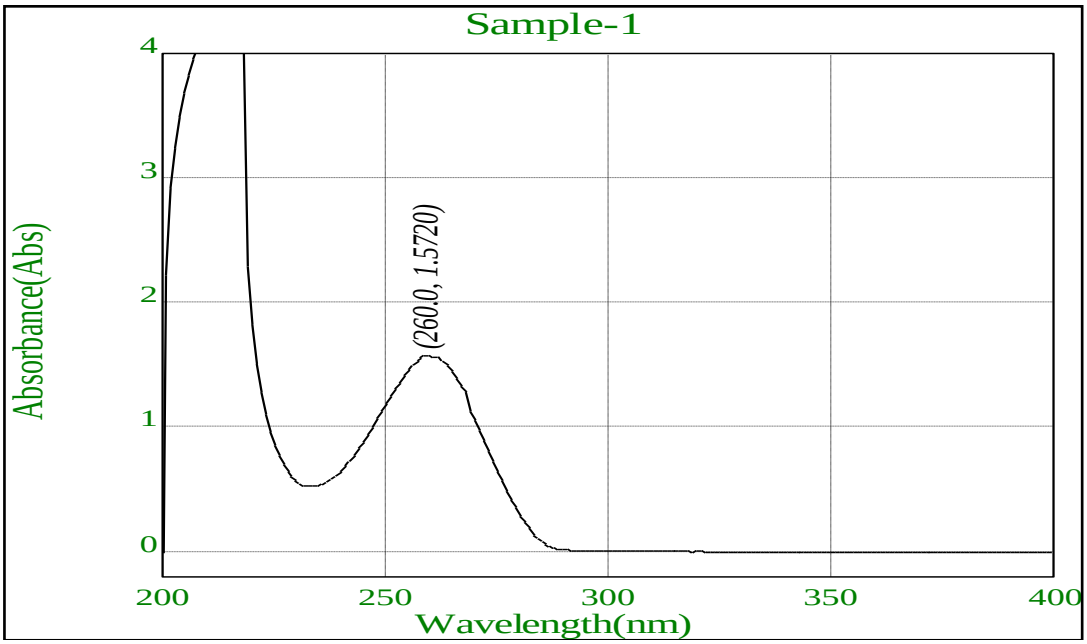

Fig.1: UV Spectrum of Paracetamol

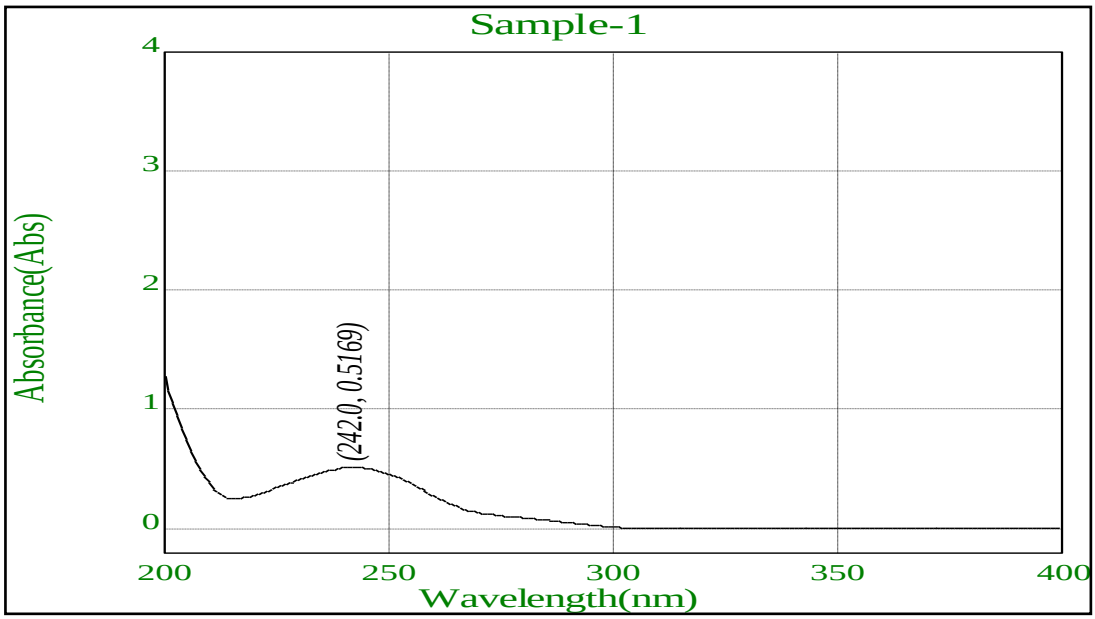

Fig.2: UV Spectrum of Etoricoxib

Table 2: Studies on the chromatographic behavior of Paracetamol and Etoricoxib

Sr No.	Mobile Phase	Retention Time (min)		Remark
		PARA	ECXB	
1.	[80% MEOH +20% Water (pH 3.0 adjust with OPA) Flow 0.7 ml/min abs at 254 nm (column 250mm X 4.6, 5.0 µm)	3.395	6.103	No peak

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2	[80% MEOH +20% buffer (pH 3.0 adjust with 0.05 % OPA) Flow 0.7 ml/min abs at 254 nm (column 250mm X 4.6, 5.0 µm)	6.187	9.226	No peak
3	[70% MEOH +30% Water (pH 3.0 adjust with OPA) Flow 0.7 ml/min abs at 254 nm (column 250mm X 4.6, 5.0 µm)	10.053	19.962	No peak
4	[75 % MEOH +25 % Water (pH 3.0 adjust with OPA) Flow 0.7 ml/min abs at 254 nm (column 250mm X 4.6, 5.0 µm)	5.406	9.271	No peak
5	[75 % MEOH +25 % Water (pH 3.0 adjust with OPA) Flow 1.0 ml/min abs at 275, 230 and 276 nm (column 250mm X 4.6, 5.0 µm)	5.407	9.271	No peak
		5.406	9.272	
		5.407	9.271	
6	[75 % MEOH +25 % Water (pH 3.0 adjust with OPA) Flow 1.0 ml/min abs at 276 nm (column 250mm X 4.6, 5.0 µm)	5.380	9.191	RESOLVE PEAK AND SHARP

It was observed thatusing a mobile phase of MEOH + Water (0.05 % OPA) 10 mm (75+25% v/v) 275 nm, 1.0 ml, observed sufficient retention time at 5.380 min and 9.191 min. having the excellent shape of peak (Theoretical plates of 10834 of Paracetamol &10501 of Etoricoxib).

Table 3: Optimized chromatographic conditions for Paracetamol.

Parameter	**Paracetamol**	**Etoricoxib**
Analytical column	C18 column (4.6mm x100mm)	C18 column (4.6mm x 100mm)
Injection volume	20µl	20µl
Flow rate	1.0 ml/min	0.7 ml/min
Mobile phase	MEOH+0.1OPA(75+25 % v/v)	MEOH +0.1OPA(25: 75 % v/v)
Detection	275 nm	239 nm
Run Time	15 min	15 min

3.2 Calibration experiment

3.2.1 Linearity:

When the data from the calibration trials were analyzed using linear regression, it revealed a linear association between peak areas and concentrations in the ranges of 10-50 g/mL for Paracetamol and 0.4-2.0 g/mL for Etoricoxib. The linear equation for Paracetamol was y = 177.82 x + 242.49 and Etoricoxib equation y = 400.03 x + 28.33 where x represents the concentration of the drug and y represents the peak area. The correlation coefficient was 0.999 and the calibration curve of Paracetamol and Etoricoxib is depicted in figure 3 and figure 4. Linearity data for the Paracetamol and

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etoricoxib is represented in tables no 4 and 5 respectively.

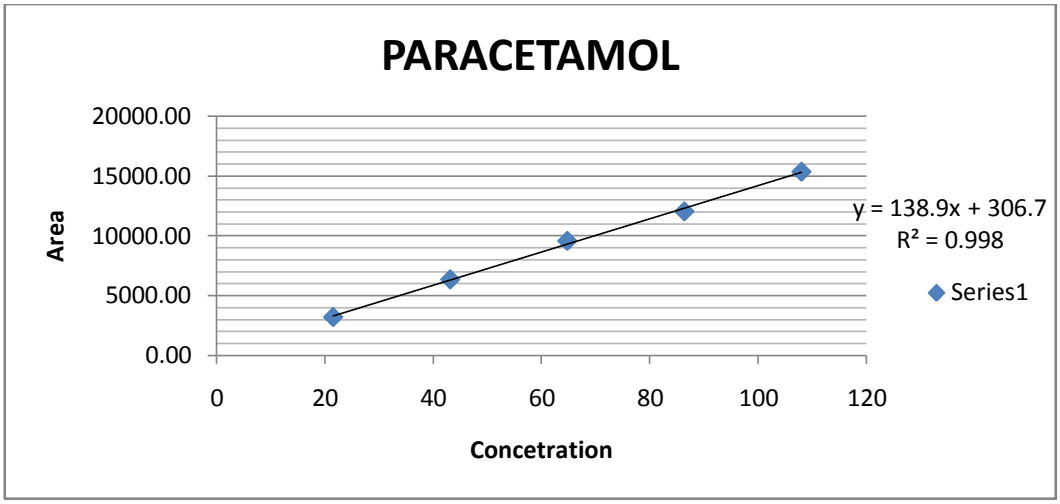

Figure 3: Calibration curve of Paracetamol

Table 4: Linearity data of Paracetamol:

Method	Conc µg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
HPLC Method	21.6	3205.37	3204.45	3204.91	0.65	0.02
	43.2	6365.84	6339.27	6352.56	18.79	0.30
	64.8	9543.59	9610.12	9576.86	47.04	0.49
	86.4	11997.4	12087.5	12042.45	63.71	0.53
	108	15416.2	15306.8	15361.50	77.36	0.50
	Equation		y =138.9 x +306.73			
	R^2		0.999			

Table 5: Linearity data of Etoricoxib

Method	Conc µg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
HPLC Method	4	368.98	377.77	373.38	6.22	1.66
	8	766.54	765.55	766.05	0.70	0.09
	12	1219.87	1223.17	1221.52	2.33	0.19
	16	1631.26	1656.30	1643.78	17.71	1.08

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	20	1964.65	1971.31	1967.98	4.71	0.24
	Equation		y = 106.42 X – 60.537			
	R^2		0.9998			

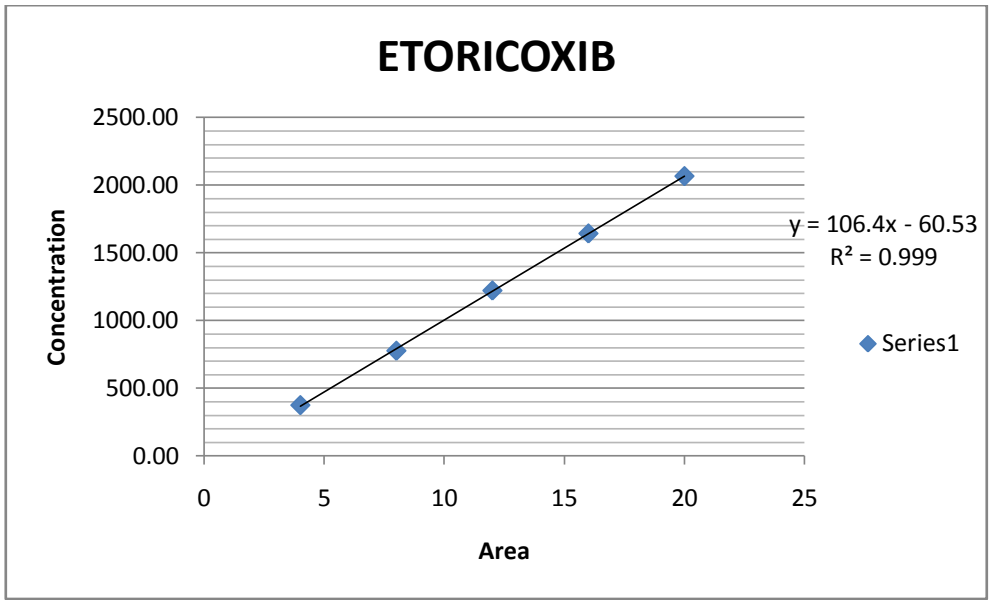

Figure 4: Calibration curve of Etoricoxib

The RP-HPLC method for the respective linear equation for Etoricoxib was y =400.03 X + 28.33 in which X represents the concentration whereas y represents the peak area. The correlation coefficient was 0.999. The calibration curve of Etoricoxib is depicted in Fig. 4.

3.2.2 Accuracy:

Validation of Recovery Studies which is done statistically is represented in Table 6 which shows the effects of Paracetamol and etoricoxib.Recovery studies were carried out to ensure that the developed approach was accurate.The solution which is previously analyzed means the specific standard drug concentration 80, 100, and 120 percentageswere mixed and then allowed for the recovery analysis. Recovery experiments at various concentration levels are used to verify the accuracy of the RP-HPLC and UV Spectrophotometric methods. The recovery rate was determined to be between 98 and 101%.

Table 6: Statistical Validation of Recovery Studies Paracetamol and Etoricoxib

METHOD	Level of Recovery (%)	Drug	% RSD	Standard Deviation*	Mean % Recovery
Rp-HPLC Method	80%	PARA	1.01	1.01	99.99
		ECXB	0.75	0.75	100.47
	100%	PARA	0.61	0.61	100.15
		ECXB	0.02	0.02	99.22
		PARA	0.21	0.21	99.81

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	120%	ECXB	1.14	1.14	101.58

*For the RP-HPLC and UV methods, this is the average of three determinations.

3.2.3 Repeatability:

Repeatability studies on RP-HPLC for Paracetamol and Etoricoxib are shown in table 7. Repeatability studies on RP-HPLC and Ultraviolet method for Paracetamol and Etoricoxib wereobserved as %RSD was ≥2%, which meansa higher amount was observed in between 100% to 102% which is the indication that analytical method concluded.

Table No.7: Repeatability analysis on RP-HPLC for Paracetamol and Etoricoxib

Method	Concentration of Paracetamol & Etoricoxib (mg/ml)	Peak area	Amount found (mg)	% Amount found
HPLC EMPA METHOD	20	945.06	19.99	99.99
	20	944.25		
		Mean	944.66	
		SD	0.57	
		%RSD	0.06	
	10	730.14	10.01	100.17
HPLC LINA METHOD	10	728.29		
		Mean	729.22	
		SD	1.31	
		%RSD	0.18	

3.2.4 Precision:

Intraday and inter-day precision investigations on the RP-HPLC method for paracetamol and etoricoxib demonstrate high precision percent amounts ranging from 97 to 101 percent, indicating an analytical procedure that was concluded. Table 8 shows the results of intraday and inter-day precision experiments on the RP-HPLC technique for paracetamol and etoricoxib.

Table 8: Result of Intraday and Inter day Precision analysis on RP-HPLC method for Paracetamol and Etoricoxib

METHOD	Drug	Conc (µg/ml)	Interday Precision		Intraday Precision	
			Mean± SD	%Amt Found	Mean± SD	%Amt Found
Rp-HPLC METHOD	PARA	10	462.24±1.43	101.76	460.97±0.78	101.50
		30	1431.44±1.62	99.70	1430.78±0.67	99.65
		50	2427.94±1.48	100.44	2429.47±1.56	100.46
	ECXB	5	356.57±0.93	100.83	355.93±0.52	100.87
		15	1092.03±0.22	99.00	1090.18±1.39	98.84
		30	1848.22±2.98	99.24	1851.03±0.75	94.96

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3.2.5 Robustness

The ability of a procedure to remain unaffected by tiny deliberate changes in parameters is known as robustness. For evaluation of this, there is one proposed method which is small However, purposeful changes to the optimum procedure parameters were made. The impact of modifications in mobile phase composition and flow rate, as well as wavelength, on time of retention of drug and tailing factor was investigated. The mobile phase composition was adjusted in a percentage of (1 ml/min-1), the rate of flow was changed by (1 ml/min-1), and the wavelength of the optimum chromatographic condition was altered in a proportion of (1 ml/min-1).Robustness as a result Table 9 shows the results of a study with paracetamol and etoricoxib. The robustness parameters were also found to be satisfactory, so the analytical technique was completed.

Table 9: Robustness Study of Paracetamol and Etoricoxib

Parameters	**Conc.(µg/ml)**	**Detected amount (mean ±SD)**	**%RSD**	**Detected amount(mean ±SD)**	**%RSD**
		For Paramcetamol		**For Etoricoxib**	
Chromatogram of flow change 0.6 ml	40+1.6	1296.26±1.61	0.12	1689.44±1.16	0.07
Chromatogram of flow change 0.8 ml	40+1.6	969.58±1.39	0.14	1260.09±2.11	0.17
Chromatogram of comp change wavelength change 238 nm	40+1.6	1247.9±1.50	0.12	1674.5±1.24	0.07
Chromatogram of comp change wavelength change 240 nm	40+1.6	975.56±1.45	0.15	1233.80±2.49	0.20
Chromatogram of mobile phase change 24+76 ml	40+1.6	1673.9±0.64	0.47	1447.2±0.41	0.03
Chromatogram of mobile phase change 26+74 ml	40+1.6	1108.46±2.51	0.23	1448.21±2.84	00.20

3.2.6 Limit Detection

Depending on the standard deviation of response and slope, the limit of detection means LOD is detected.The LOD is the lowest limit that can be detected. The LOD and LOQ of Etoricoxib wereobserved as 0.2375 (ug/mL) and 0.7199 (ug/mL), the analytical method that concluded.

3.2.7 Limit Quantification:

Limit of detection = 3.3X12.8/177.8=0.2375 (ug/mL)

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Limit of Quantitation = 10 X12.80/ 177.8=0.7199 (µg/mL). The values of LOD and LOQ for Paracetamol was observed as 0.2375 (ug/mL) and 0.7199 (ug/mL).

3.3 Analysis of tablet formulation

The quantity of Paracetamol and Etoricoxib in each tablet was estimated by the extrapolation method by taking the value of area from the calibration curve. The process of Analysis procedure was done repeatedly about five times by using tablet formulation. For calculation of %Label claim and %RSD, tablet assay was estimated.In Table 10 and figure 5, results are represented. Brand Name Arthopen (crescent therapeutics) was used for the analysis. The average weight of the tablets was 0.838 gms. /Tab. Its equivalent weight for 10 mg would be 100X 838 / 500 =167.6 mg. Hence, 167.6 mg in 10 ml water was taken and sonicated 10 min. Analysis of marketed formulation was analyzed and % Label Claim was observed as 100-101% which was concluded satisfactorily.

Table 10: Analysis of marketed formulation.

Assay	Drug	Amt. Found	%Label Claim	SD	%RSD
Rp-HPLC Method	**PARA**	50.14	99.89	0.06	0.06
	ECXB	50.14	99.33	0.03	0.03

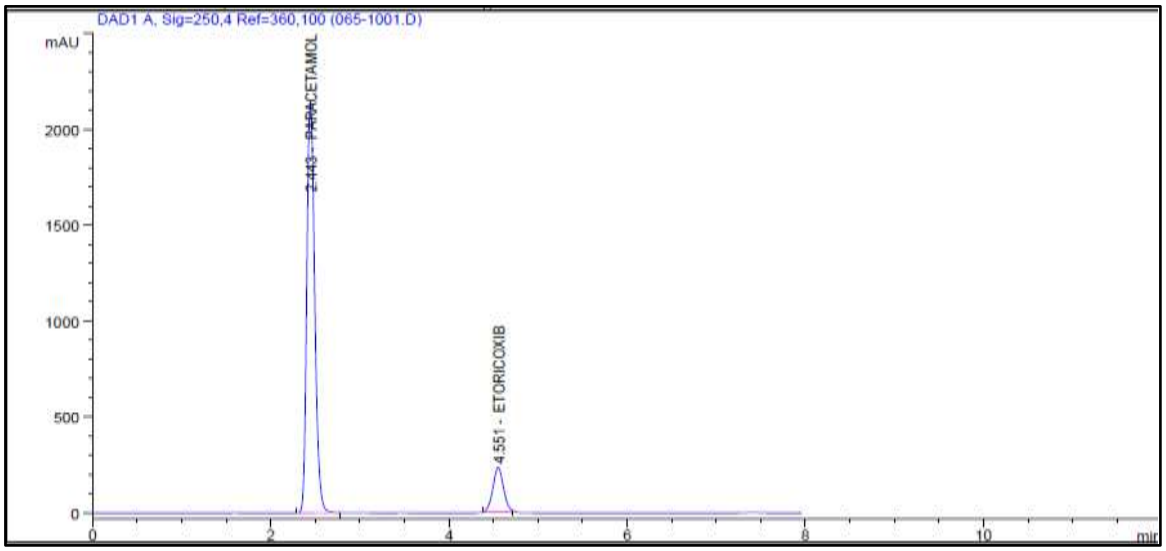

Figure 5: Chromatogram for Marketed Formulation

IV. CONCLUSION

For the analysis of Paracetamol and Etoricoxib in API as well as in tablet, simple, rapid, accurate, and precise RP-HPLC and spectrophotometric methods have been developed and validated. Both procedures are suitable for determining paracetamol and etoricoxib simultaneously in multi-component formulations without interfering with one another.The methodologies developed are suggested for quality control examination of medicines in a combination of two medicament formulations. The quantity discovered using the recommended procedure was consistent with the formulation's label claim. The calculated standard deviation and coefficient of variation were also acceptable, showing that the proposed approaches are suitable for routine assessment of tablet dosage forms.

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Conflict of interest
The authors declared no conflict of interest .

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