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HPLC validation and stress degradation behavior of Etoricoxib in tablets dosage form

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Abstract

Etoricoxib is a new COX-2 selective inhibitor. Current therapeutic indications are the treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, chronic low back pain, acute pain, and gout. Like any other COX-2 selective inhibitor, Etoricoxib selectively inhibits isoform 2 of cyclo-oxygenase enzyme (COX-2) to reduce the generation of prostaglandins (PGs) from arachidonic acid. Effective chromatographic separation method for etoricoxib was achieved using HPLC instrument by Luna C18 – (150X4.6mm, 5µm) column with isocratic elution of the mobile phase consisting of buffer pH 3.5 of 0.01M potassium dihydrogen phosphate (1.36g/L): acetonitrile: methanol (40:15:45 v/v/v). The wavelength of detection was set to be 235 nm (UV detector), and a flow rate of 1.0 ml/min was employed, 10 µl was used as injection volume and the column temperature was maintained at 30°C. A rapid, simple, and selective reversed-phase high-performance liquid chromatographic method was developed and validated for the assay of Etoricoxib in its pharmaceutical formulation, under these chromatographic conditions, the peak of Etoricoxib was obtained at a retention time about 5.1 min. and run time of about 7.0 minutes. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, and limit of quantitation. The proposed method can be used for the assay of these drugs in dosage form for routine analysis, this has already been applied to different samples, and this is explained in the applications section.

Keywords: Etoricoxib; HPLC; Validation; ANOVA (Analysis of variances); Forced degradation.

1. Introduction

Etoricoxib {5-Chloro-6'-methyl-3- [4-(methylsulfonyl) phenyl]-2,3'-bipyridine} (**Figure 1**) is the newest addition to the group of nonsteroidal anti-inflammatory drugs (NSAIDs) known as selective cyclooxygenase-2 (Cox-2) inhibitors, which are indicated for the treatment of rheumatoid arthritis, psoriatic arthritis, osteoarthritis, ankylosing spondylitis, chronic low back pain, acute pain, and gout. Approved indications differ by country. It is also used for the short-term treatment of moderate pain after dental surgery in adults [1]. A tablet formulation containing 90 mg of Etoricoxib has been introduced into clinical practice. A survey of literature revealed that few HPLC and spectrophotometric methods are reported for the determination of Etoricoxib.

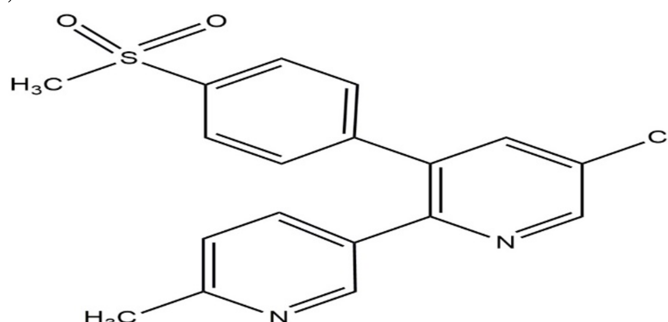


Figure 1. Chemical structure of Etoricoxib.

The drug alone was determined by ultraviolet spectrophotometric[2],Spectrofluorophotometric [3], and reverse phase (RP)-HPLC for Coxibs (NSAIDs) like Celecoxib, Rofecoxib, and Etoricoxib [4]–[9] methods. Thin-Layer Chromatographic Determination of Etoricoxib [10]. LC/MS/MS was applied for the quantification of Etoricoxib using a stable isotope as an internal standard which was validated over the concentration range of 0.5–250 ng/ml [11]. The determination and pharmacokinetics of the drug in thoroughbred horses by LC/MS/MS has been performed and published [12]. Liquid chromatography-tandem mass spectrometry was applied to the determination of Etoricoxib [13].

Liquid chromatography has been also applied to determine Etoricoxib in human plasma [14], [15]. An HPLC-MS/MS method for the simultaneous determination of Etoricoxib and its carbon-13 analog from human plasma was also developed [16]. A rapid reversed-phase HPLC-UV method for the separation and analysis of etoricoxib with common suspension additives in drug suspensions in a single run has been done [17].

This work has been performed to develop and validate an HPLC method for the quantification of etoricoxib and evaluate its stability. The proposed method was successfully applied to the assay of commercial tablets.

This method has been successfully used in either the quality control testing or the stability testing under various conditions according to ICH guidelines [18] of the selected pharmaceutical formulations, brand product (ARCOXIA FC tablets) [19], and pure raw material.

Analytical method objectives are often defined as method acceptance criteria for peak resolution, precision, specificity, and sensitivity. The focus in reversed-phase liquid chromatography (RPLC), The separation is based on analytes partition coefficients between a polar mobile phase and a hydrophobic (nonpolar) stationary phase. The stationary phases were solid particles coated with nonpolar liquids. These were quickly replaced by more permanently bonding hydrophobic groups, such as octadecyl (C18) bonded groups, on a silica support [20], [21].

2. Experiment

2.1. Reagents and Material

Potassium dihydrogen phosphate, acetonitrile (HPLC grade), methanol (HPLC grade), Ortho phosphoric acid and Hydrochloric acid from Scharlau were used in this study. Sodium hydroxide and 30% Hydrogen peroxide were purchased from Merck KGaA. Ultra-pure water and the working standard of Etoricoxib and Pharmaceutical formulation (Ricoxitib 90 mg tablets are white round film coated tablets with average weight, 309.0 mg/tablet) were provided by Alandalous Pharmaceutical Company, Egypt.

2.2. Instruments

The integrated high-performance liquid chromatography system (Agilent 1260 infinity II) was equipped with a quaternary pump, a degasser, an autosampler, an injector with a 100 μl loop, a column oven, a UV detector with diode array and Empower3 software. The separation of compounds was made on Phenomenex Luna C18(2)-150X4.6 mm, 5μ with isocratic elution of the mobile phase consisting of 0.01 M potassium dihydrogen phosphate solution in water (1.36 g/L, adjusted to pH 3.5 using orthophosphoric acid): Acetonitrile: Methanol (40:15:45 v/v/v). The wavelength of detection was set to be 235 nm (Uv detector), and a flow rate of 1.0 ml/min. was employed, 10 µl was used as injection volume and the column temperature was maintained at 30°C, peak of etoricoxib was obtained at retention time about 5.1 minutes (**Figure 2**) and run time of about 7.0 minutes. A detector with a diode array explains peak purity, it is useful to show that the analyte chromatographic peak is not attributable to more than one component as shown in **Figure 3**.

2.3. Method development

We experimented with various mobile phases that contained buffer, methanol, acetonitrile, and numerous C18 columns from various manufacturers. In relation to the etoricoxib peak, peak separation was symmetrical. Acceptable theoretical plates and the tailing factor are also included.

2.3.1. Preparation of standard solution

Etoricoxib working standard of 45 mg is carefully weighed into a 50 ml volumetric flask, then is dissolved in 30 ml mobile phase as diluent, sonicated for five minutes, and then diluted to volume using the same solvent (Standard stock 1). Transfer 5 ml from (Standard stock 1) into a 50 ml volumetric flask, and then add the mobile phase to fill the flask to volume (Standard solution), which contains 90 µg /ml of etoricoxib.

2.3.2. Preparation of sample test

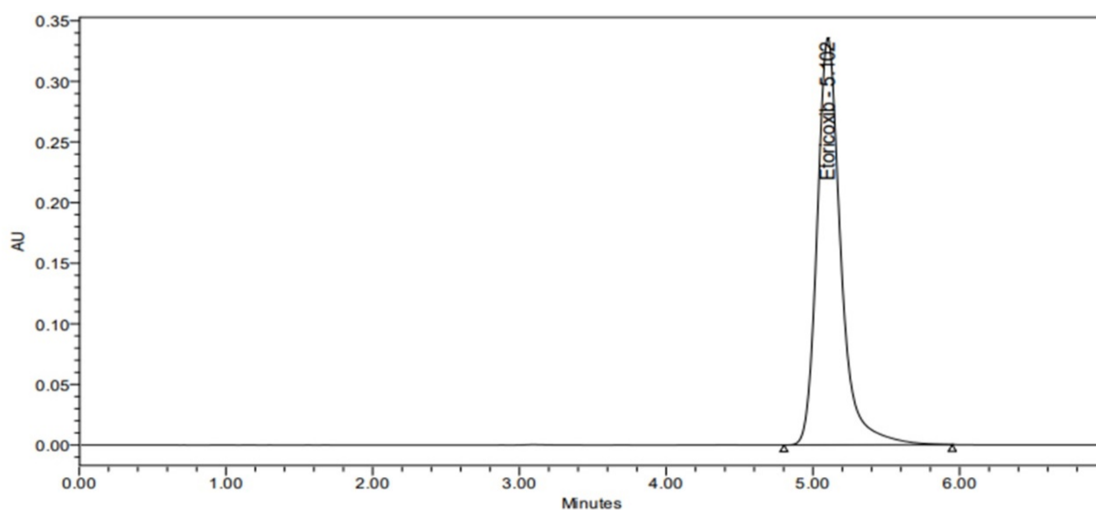
Ten tablets were weighed and finely powdered. The exact weight of the powdered tablets about 309 mg, was put into a volumetric flask of 100 ml, dissolved in 80 ml mobile phase as diluent, sonicated for 20 minutes, cooled, and completed to volume with the same solvent (sample stock 1). Transfer 5 ml from (Sample stock 1) into a 50 ml volumetric flask, and then add the mobile phase to fill the flask to volume (Sample test solution), which contains 90 µg /ml of etoricoxib. Note: Filter all solutions through a 0.45 µ Nylon filter, discard the first 5 ml of the filtrate.

2.4. HPLC conditions for Organic impurities

Apply the assay procedure with the exception that the run time is 30 minutes, considering the chromatographic conditions, mobile phase, diluent, and test solution preparations.

2.4.1. Preparations

Inject stock solution of assay as a sample test for organic impurities, inject mobile phase as Blank, and Prepare standard solution for related by transfer 10 ml of sample test Solution into 100 ml volumetric flask, and complete to the volume with mobile phase, then dilute 2 ml into 100 ml volumetric flask, and complete to the volume with mobile phase.



	Peak Name	RT	Area	% Area	Height	Amount	USP Plate Count	USP Resolution	USP Tailing	s/n
1	Etoricoxib	5.102	3759470	100.00	335916		5401		1.29	

Figure 2. Chromatogram of Etoricoxib peak.

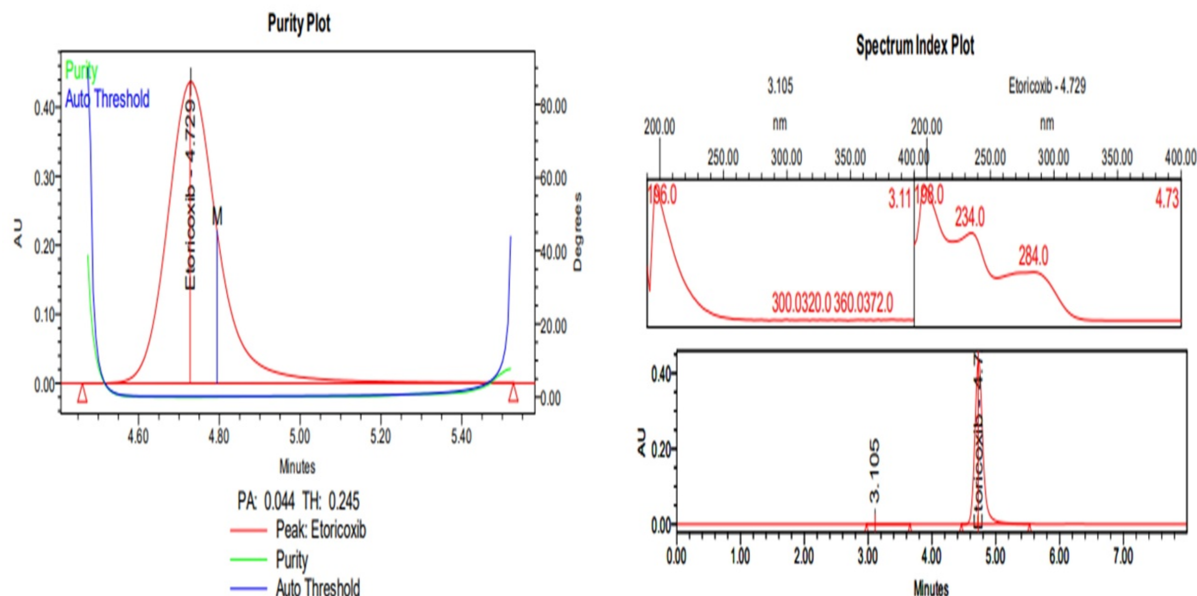


Figure 3. Peak purity and spectrum index plot.

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3. Results and discussion

3.1. Optimization of the method conditions

Any method's goal throughout development is to obtain peaks with acceptable peak symmetry, good resolution between analytes if there are many of them, and in a fair amount of time.

3.2. Stationary phase

The stationary phase was optimized using a variety of C18 columns from various manufacturers. It was determined that Phenomenex-Luna C18 - (150X4.6mm, 5 µm) was the finest of them all. In relation to the etoricoxib peak, peak separation was symmetrical. Acceptable theoretical plates (approximately 5390.69) and a tailing factor of no more than 2.0 are also required (about 1.29).

3.3. Mobile phase

Potassium dihydrogen phosphate was utilized as a buffer in a mobile phase with a moderate pH (3.5) to extend column lifespan and make the procedure more appropriate for frequent analysis. The mobile phase's organic content was optimized. The optimal ratio for providing satisfactory peak separation and an appropriate retention duration was discovered to be acetonitrile to methanol and buffer.

3.4. Wavelength of detection

Wavelengths from 230 nm to 270 nm were tried. The analyte's response was discovered to be marginally low at 230 nm and too low at 254 nm till 270 nm, but the best peak response and signal-to-noise ratio were obtained while utilizing 235 nm as a wavelength for detection.

3.5. Concentration of standard and test solutions

Samples were prepared by weighing a specific amount of the sample to equal the same concentration so that there would be an equal response in the same amount of the standard substance and samples. An appropriate concentration of the standard substance, around 0.09, was used to give an appropriate response.

4. Validation of proposed method

According to the International Conference on Harmonization (ICH) validation guidelines, the suggested HPLC-UV technique was

verified. Validation of analytical procedures: text and methodology [22], [23].

4.1. Precision &Repeatability

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at two levels: repeatability, ruggedness (intermediate precision). Repeatability expresses the precision under the same operating conditions

over a short interval of time. Repeatability is also termed intra-assay precision. A minimum of 6 determinations of the same homogenous sample at 100% of the test concentration showed in **Table 1**. Relative standard deviation (%RSD) of repeatability 0.105% for Etoricoxib standard and 0.466 % for test assay.

Table 1. Data for standard and sample test repeatability of Etoricoxib.

No.	Standard Repeatability	Sample test Repeatability	
	Standard response (PA)	Average test response	% Assay
1	3768483	3665431	95.66
2	3759618	3726112	95.00
3	3761925	3771425	96.19
4	3759157	3700417	95.58
5	3759470	3677084	95.21
6	3757289	3730827	95.10
Average	**3760990**	**3711882**	**95.46**
Standard deviation (STDEV)	**3956.497**	**39004.741**	**0.445**
% RSD	**0.105**	**1.05**	**0.466**
		Acceptance criteria: RSD ≤ 2 % for Assay	

4.2. System suitability

Analyzing replicate injections of the standard solution allowed for the evaluation of system suitability tests [24]. Using the previously indicated parameters, the HPLC-UV method was used to examine the system suitability solution. The following are the standards for system appropriateness that were established: The number of theoretical plates (N) should be greater than 2000. (Found to be 5390.69). Should be less than 2.0 for the tailing factor (Found to be 1.29). Replicate injections' percentage RSD of the analyte peak should be less than 2.0%. (Found to be 0.105).

4.3. Linearity and concentration ranges

The capacity of an analytical technique to produce test results that are directly proportional to the concentration of analyte in the sample, within a certain range. A minimum of 5 distinct concentrations were prepared, and 3 replicates of each concentration were then made to test for linearity. Assessment using the least-squares approach. Linearity is determined by the correlation coefficient, which, when utilizing peak area responses, should be found to be not less than 0.998. It is necessary to provide the correlation coefficient, y-intercept, slope of the regression line, and residual sum of squares.

In this experiment, nine percentages of different concentrations were prepared from Etoricoxib standard represented in **Table 2**.

The linearity study was evaluated based on correlation coefficient (r) and r-squared should have values higher than 0.998 (**Figure 4**), significance of intercept (the 95 % confidence interval of intercept should include the zero value) this indicates statistically insignificant intercept, and ANOVA test of linearity data that indicates

the probability that the regression was not obtained by chance, small significance of F confirms the validity of the regression output **Table 3**.

Table 2. Data for linearity of Etoricoxib**.**

Level (%)	Concentration (µg/ml)	Mean peak area	*SD (standard deviation)*	*%RSD*
20	18.2	751908	870.39	0.12
40	36.3	1487099	801.18	0.05
60	54.5	2260325	1407.96	0.06
80	72.6	2991974	783.58	0.03
100	90.8	3760794	2899.94	0.08
120	109.0	4488600	4612.66	0.10
140	127.1	5267269	3605.61	0.07
160	145.3	6004314	4310.55	0.07
200	181.6	7472369	5083.88	0.07
Slope	**41234.0744**			
Intercept	**4372.7699**			
R^2	**0.99996**			

Table 3. Data from ANOVA and Statistical analysis for linearity of Etoricoxib.

Multiple R	0.99998
R Square	0.99996
Adjusted R Square	0.99995
Standard Error	14852.84
Observations	9
F	175095.14
Significance F	1.17561E-16
P-Value	0.68676
SD intercept	10400.4128

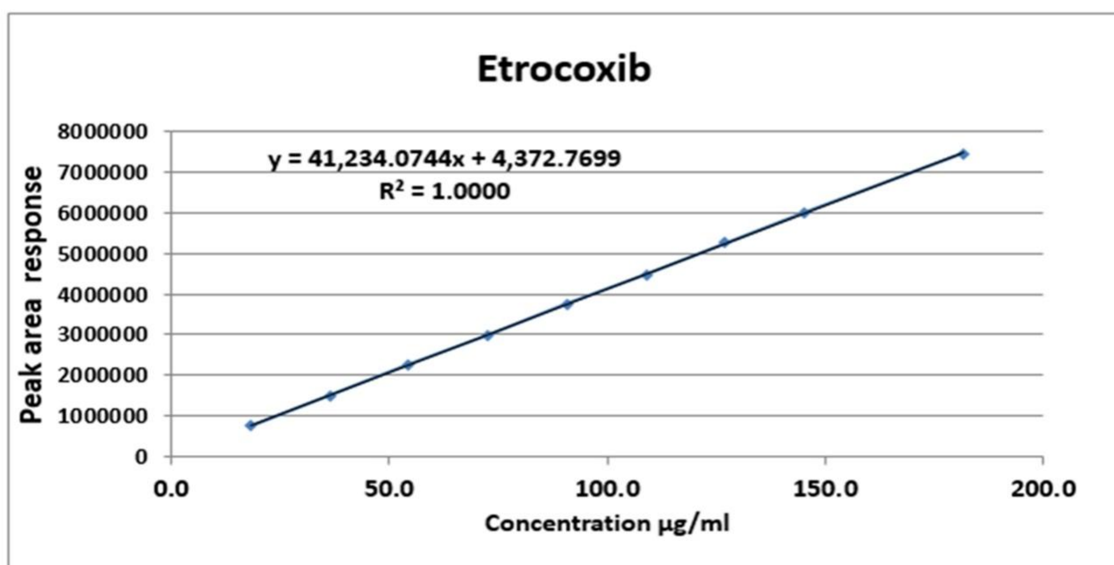


Figure 4. Linearity plot of Etoricoxib by the proposed method.

4.4. Sensitivity

4.4.1. Limit of Detection (LOD)

It is the concentration of analyte can be detected but not necessarily quantified.

$$LOD = \frac{3.3 \; x \; SD \; intercept}{Slope} \quad (1)$$

LOD result is 0.832 µg/ml.

4.4.2. Limit of Quantitation (LOQ)

It is the concentration at which the peak of analyte quantified.

$$LOQ = \frac{10 \; x \; SD \; intercept}{Slope} \quad (2)$$

LOQ result is 2.522 µg/ml.

Where: SD stands for standard deviation.

4.5. Accuracy and Recovery

Nine determinations at three concentration levels covering the prescribed range were used to evaluate accuracy (i.e., three concentrations and three replicates). The method was determined to be accurate within (98% - 102%) at the range of around 80% to 120% of the working concentration. The measurements were made at a concentration, which is to be the (100%) specification, and at reasonable concentration intervals around this concentration.
Three percentages of various concentrations were created from the etoricoxib sample used in this experiment, which is shown in **Table 4**.

Table 4. Data for Accuracy & Recovery of Etoricoxib.

%Conc.	Mean Peak area	SD (standard deviation)	%RSD	Actual conc. (µg/ml)	Theoretical conc. (µg/ml)	Recovery %
80	2949532	2102.507	0.071	70.68	71.56	98.76
100	3677358	2778.680	0.076	88.12	89.46	98.50
120	4388671	4979.088	0.113	105.16	107.35	97.96
Average Recovery						**98.41**

4.6. Robustness

Determined by examining how a technique holds up to minor changes in its usual operating conditions. With HPLC, for example, this could be a change in flow rate, pH, or a tiny modification in the mobile phase's composition. The robustness of an analytical technique is a measurement of its ability to be unaffected by minute but intentional changes to the procedure parameters mentioned in the procedure documentation and gives a clue as to its applicability under typical conditions.
For instance, a little modification in the standard operational parameters (Flow rate, Wavelength, and/or Temperature, etc.) could be used.

Acceptance standards: The results' pooled RSD was less than 3%, and at a 5% level of significance, Use ANOVA statistics; the P value should be greater than 0.05 to indicate that the results from the two sets are similar.
The robustness of the analytical procedure was examined by studying the effect of changes on the suitability of the analytical method as, changing the flow rate by ± 5%, change the wavelength by plus 3nm and minus 2 nm, and changing the Temperature by ± 5°C.
ANOVA statistical analysis was used to examine the system suitability characteristics for each change. Results obtained demonstrated that these adjustments had no appreciable impact on the system suitability characteristics assessed for the suggested shown in **Table 5**.

Table 5. Data and Results ANOVA Statistical analysis for Robustness.

Effect of changes	Pooled RSD	F	P-value
Flow rate	0.491	0.00900	0.99105
Wavelength	0.487	0.05200	0.94975
Temperature	0.485	0.02653	0.97393

4.7. Ruggedness (Intermediate precision)

Variations within laboratories are expressed as different days, different analysts, and various columns. For each change, a minimum of six sample determinations at 100% of the test concentration are made, i.e., two analysts will make six sample determinations. Each set's RSD needs to be recorded. Acceptance criteria: The results' pooled RSD was less than 3%, and at a 5% level of significance, Use ANOVA statistics; the P value should be greater than 0.05 to indicate that the results from the two sets are similar.

Results: Pooled RSD = 0.459 %, P Value = 0.20744

4.8. Selectivity and Specificity

In order to determine the stability indicating characteristics, selectivity, and specificity of the technique, forced degradation studies were carried out. The use of heat, light, oxidation, acid and base hydrolysis, as well as the injection of well-known degradation products if present and identified, were all used to try and accelerate degradation. Acceptance criteria: The peaks of the analyte should be resolute from any other peaks that may occur as a result of degradation for the approach to be selective and stability suggesting.

4.8.1. Procedure of forced degradation for Etoricoxib

Acid hydrolysis (by using 1N Hydrochloric acid) chromatogram as shown in **Figure 5**, base hydrolysis (by using 1N Sodium hydroxide) chromatogram as shown in **Figure 6**, heat hydrolysis in water bath at 70°C for 4 hours chromatogram as shown in **Figure 7**, light hydrolysis sample was exposed to direct sun light for 1 hour chromatogram as shown in **Figure 8**, and oxidation hydrolysis (by using 30% Hydrogen per oxide) chromatogram as shown in **Figure 9**.

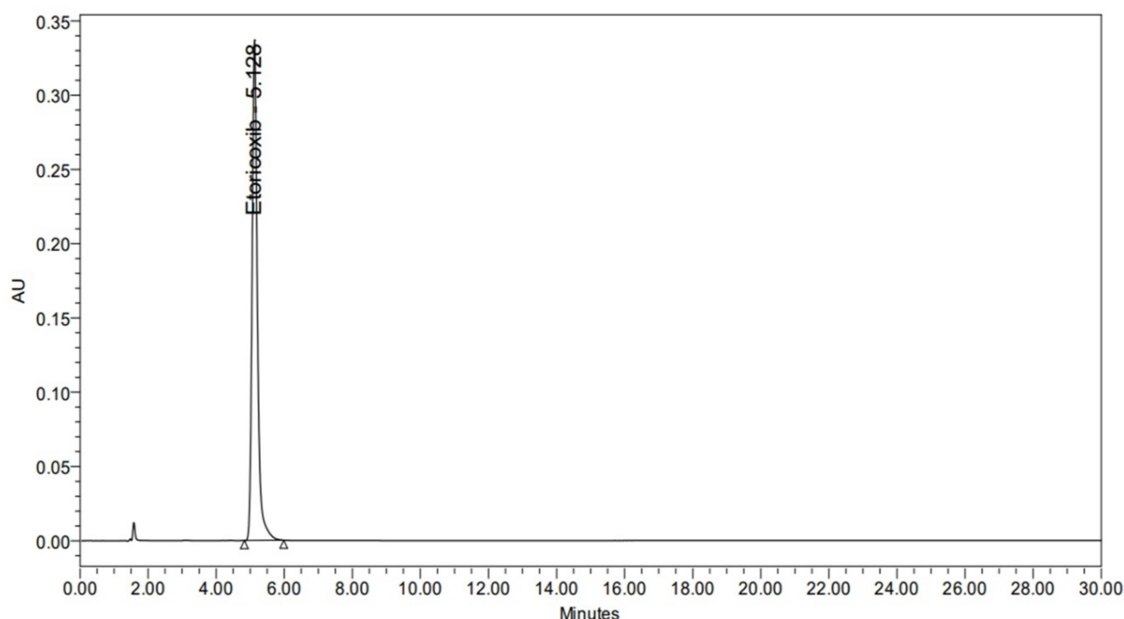


Figure 5. Acid-forced degradation chromatograms for Etoricoxib.

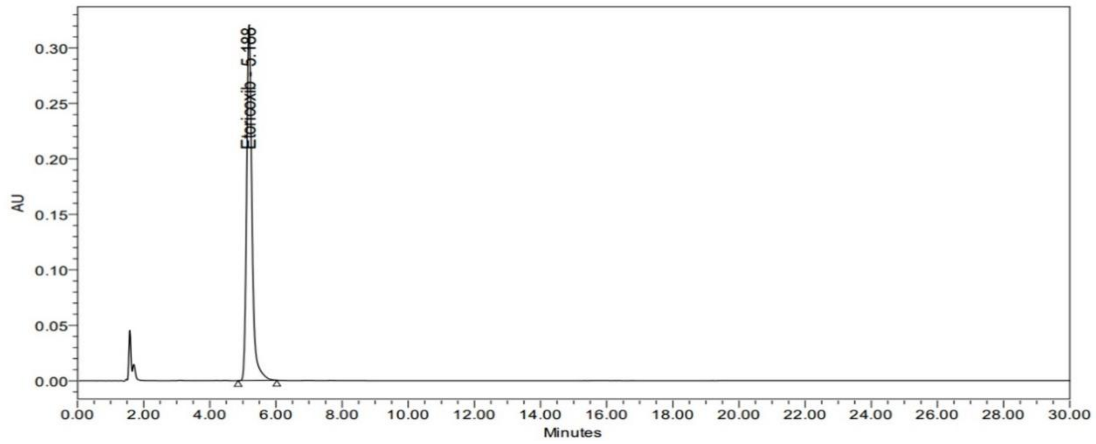


Figure 6. Base-forced degradation chromatograms for Etoricoxib.

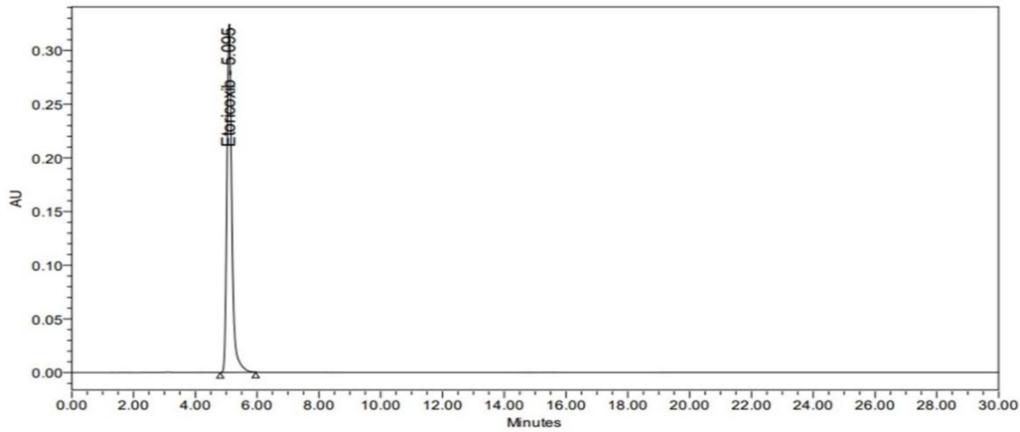
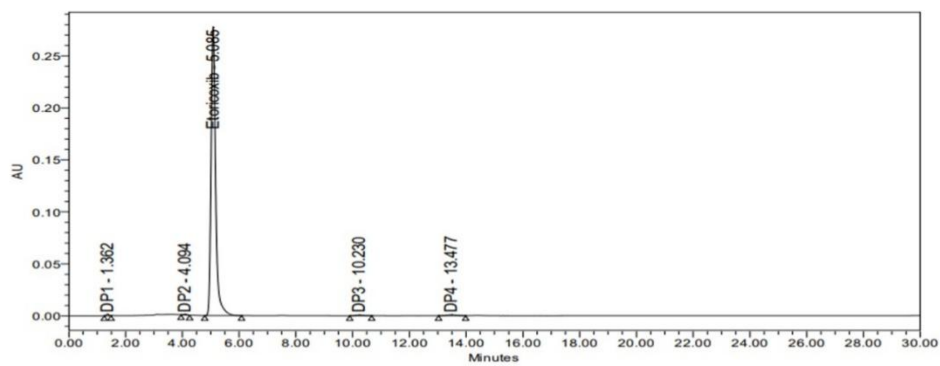
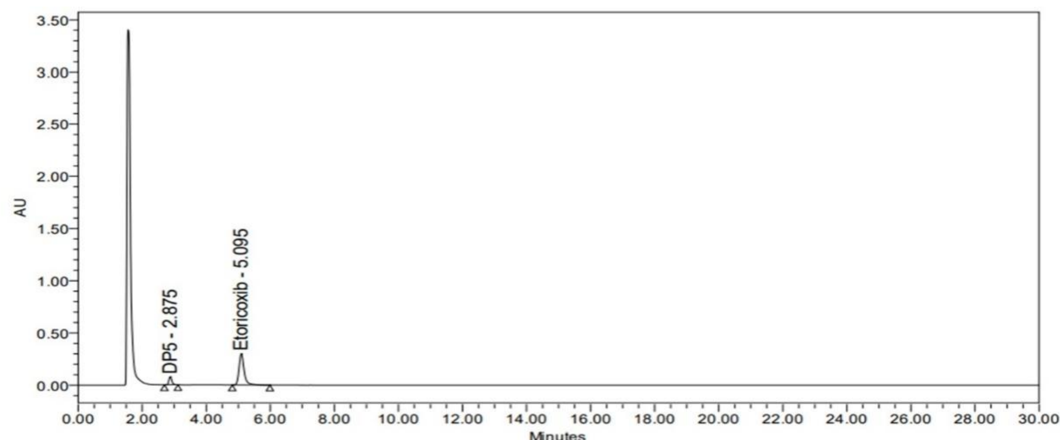


Figure 7. Heat-forced degradation chromatograms for Etoricoxib.



	Peak Name	RT	Area	% Area	Height	Amount	USP Plate Count	USP Resolution	USP Tailing	s/n
1	DP1	1.362	7751	0.25	1536		1673		1.10	
2	DP2	4.094	6073	0.20	742		5505	15.18	1.19	
3	Etoricoxib	5.085	3067967	98.52	278038		5566	3.93	1.32	
4	DP3	10.230	12564	0.40	671		6420	13.09	1.15	
5	DP4	13.477	19742	0.63	811		6859	5.54	1.07	

Figure 8. Light-forced degradation chromatograms for Etoricoxib.



	Peak Name	RT	Area	% Area	Height	Amount	USP Plate Count	USP Resolution	USP Tailing
1	DP5	2.875	491589	12.93	76947		4894		1.21
2	Etoricoxib	5.095	3309121	87.07	300087		5584	9.90	1.34

Figure 9. Oxidation-forced degradation chromatograms for Etoricoxib.

4.8.2. The results of forced degradation and API percent remaining for etoricoxib are represented in **Table 6**. For etoricoxib, several degradation products were detected with forced degradation. The ICH Q3A guidance on the nature and classification of impurities [25].

Table 6. Results of forced degradation for Etoricoxib.

Conditions	% Remaining of API (Standard)	% Remaining of API (Sample)
Acid	97.91	98.02
Base	98.56	98.21
Heat	98.04	97.53
Light	81.48	93.91
Oxidation	88.03	76.86

4.9. Stability of solution

The stability of the drug substance-containing solution is examined over a predetermined duration, with a freshly generated solution being used as a baseline for comparison. The drug substance-containing solution is stored in a stable environment. Between 98.0% and 102.0% of the anticipated final concentration is the acceptable range. At room temperature, the stability of the standard solution was tested over a 24-hour period, and the result was 100.82%. Throughout the day of preparation, the solutions showed no signs of degradation.

5. Practical Application

This section demonstrates the use of the analytical method in practice on a variety of samples that contain the active ingredient in etoricoxib in pure form as well as pharmaceutical formulations with varying concentrations and manufacturing locations. This technique has been utilized successfully for verifying the stability or the quality control of certain pharmaceutical formulations, brand products, and pure raw materials.

Table 7 contains all the information and findings regarding the samples utilized in the analysis of etoricoxib.

Table 7. Raw data and Results for Etoricoxib analysis.

No.	Sample name and Conc.	B.No.	Conditions	Av.wt. mg/tablet	%Assay	%Large impurities	%Total impurities
1	Etoricoxib (Raw material)	EX20090031 (Hetero Labs Limited)	Ambient temperature	Powder	100.7	0.03	0.09
2	Ricoxitib 60mg FCT	210182	Ambient temperature	207.19	98.1	0.03	0.09
3	Arcoxia 60mg FCT	T030417 (Merk sharp& Dohme B.V.)	Ambient temperature	210.41	98.5	0.04	0.07
4	Ricoxitib 90mg FCT	210160	06m Accelerated stability (40°C,75% RH)	313.07	98.3	0.03	0.09
5	Ricoxitib 90mg FCT	210160	06m Lon term stability (30°C,65% RH)	313.59	99.4	0.03	0.09

6. Conclusion

Shorter retention time makes this method more acceptable and cost-effective. The HPLC-UV method was validated in accordance with ICH requirements for the validation of analytical methods. It is highly sensitive, accurate, precise, fast, and easy to use. The suggested technique was effectively used. The technique can be applied to routine analyses of formulations and raw ingredients. The validity of the technique used to show the by-products of decomposition or degradation during stability studies in various temperature and humidity conditions was also shown in the case of forced degradation, where its by-products were discernible with good resolution between the peaks in the types used in the process of degradation for the active substance.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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