Journal of Pharma Research
Available online through
www.jprinfo.com

Research Article
ISSN: 2319-5622

Development and Validation of an Analytical Method for the Estimation of Etoricoxib Tablets by Reverse Phase HPLC

Aayushi Agarwal Bansal
Department of Pharmacy, Rohilkhand University, Bareilly, Uttar Pradesh, India.

Received on: 16-01-2014; Revised and Accepted on: 21-01-2014

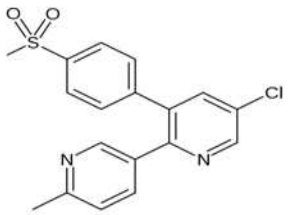
ABSTRACT

A simple, precise and rapid RP-HPLC method was developed for the estimation of Etoricoxib in pharmaceutical dosage forms. Method development incorporated the optimization of stationary phase (column), mobile phase composition and other chromatographic conditions. The method was carried out on Symmetry (Waters) RP-C_{18} (250 x 4.6 mm), 5 µm column using a mixture of Phosphate buffer: Acetonitrile in the ratio/ composition 30:70 v/v. The mobile phase was pumped at a flow rate of 1ml/min and the detection was carried out at 220 nm. The retention time of the drug was 9.585 min. Method validation produced excellent results for specificity, linearity, precision, intermediate precision, accuracy, limit of detection and limit of quantitation and robustness. The linearity was found within concentration range of 70 ppm to 130 ppm with correlation coefficient of 0.998145. The percentage recovery was found to be 99.88 % to 100.44 %. The proposed method was optimized and validated as per the ICH guidelines and was successfully applied for the estimation of Etoricoxib in tablet formulation. Hence the developed and validated HPLC method can be used for routine analysis of Etoricoxib in tablets.

Keywords: *Etoricoxib, RP-HPLC, Method validation.*

INTRODUCTION

Etoricoxib **(Fig. 1)**, a newer Non Steroidal Anti-Inflammatory drug **(NSAID)** and is a selective cyclo-oxygenase-2 inhibitor, is mainly used in the management of osteoarthritis, rheumatoid arthritis and acute gouty arthritis. Chemically, Etoricoxib is a 5-chloro-6'-methyl-3-[4-(methylsulfonyl) phenyl]-2, 3'-bipyridine, and is not yet official in any Pharmacopoeia [1].



ETORICOXIB

Fig. 1: Chemical structure of Etoricoxib

Literature survey reveals that several analytical methods have been reported for the analysis of Etoricoxib in bulk drugs, formulations and biological fluids, *i.e.* spectrophotometric methods including UV and visible [2-8], LC/Mass spectrophotometry [9-11] and chromatographic HPLC methods in combination with other drugs [12-14], but no individual RP-HPLC method has been developed for the estimation of the drug in pharmaceutical dosage form with the chromatographic conditions which have been used in this method. Hence there was the need to develop a new column chromatographic method for the analysis of the drug. Therefore the aim of the present study was to develop a sensitive, precise, accurate and specific HPLC method for the determination of Etoricoxib in tablet formulation.

***Corresponding author:**
Aayushi Agarwal Bansal
Department of Pharmacy, Rohilkhand University,
Bareilly, Uttar Pradesh, India., Ph. No. 08909465863, 09458513931.
**E-Mail: aayushi0709@gmail.com*

MATERIALS AND METHODS

Instrumentation:

Shimadzu LC-2010A-HT high performance liquid chromatographic system was used for this experiment with 2010A UV-VIS detector, 2010A Column Oven and 2010A programmable auto sampler. The Symmetry (Waters) RP-C_{18} (250 x 4.6 mm), 5 µm column was used as a stationary phase. Mettler analytical weighing balance - Model AB 204-S was used for study.

Materials and Reagents:

Sample drug i.e. ETORICOXIB Tablets (Batch no. FLT-02A and 90 mg strength) and working standard were gifted by Akums Drugs and Pharmaceuticals Ltd., Haridwar. HPLC grade Acetonitrile, Methanol and Potassium dihydrogen phosphate (AR grade) were supplied by Merck Specialties Pvt. Ltd. Millipore water was used throughout the study. Mobile phase was degassed by filtration (0.45µm) and sonication.

Method Development:

Different mobile phases containing Methanol, Water, Acetonitrile, and different buffers in different proportion were tried and finally a mixture of Phosphate buffer: Acetonitrile in the ratio/ composition 30:70 v/v was selected as mobile phase which gave acceptable peak parameters for Etoricoxib.

Chromatographic Conditions:

Waters Symmetry RP-C_{18} (250 x 4.6 mm), 5 µm column was used as a stationary phase. The isocratic elution with Phosphate buffer: Acetonitrile (30:70 v/v) mobile phase at the flow rate of 1.0 ml/min was carried out. The run time was set at 10 min and ambient temperature was maintained. The volume of injection was 20 µl, prior to injection of analyte, the column was equilibrated for 30-40 min with mobile phase. Detector signal was monitored at a wavelength of 220 nm.

Preparation of Phosphate Buffer:

3.4gm of Potassium dihydrogen phosphate was dissolved in water to make 1000 ml of solution (pH 4.7).

Preparation of Standard Solution:

50mg of Etoricoxib working standard was accurately weighed and transferred into 100ml volumetric flask. 50ml mobile phase was added and sonicated for 10 min. Volume was made

upto100ml with mobile phase. 5ml of this solution was diluted to 25ml with mobile phase and filtered through 0.45µ nylon membrane filter. (Conc. 100 ppm)

Preparation of Sample Solution:

Ten tablets were weighed and finely powdered, powdered tablets were accurately weighed equivalent to 50mg of Etoricoxib and transferred into 100ml volumetric flask. 50ml mobile phase was added, sonicated for 10 min and cooled to room temperature**.** Volume was made upto100ml with mobile phase. 5ml of this solution was diluted to 25ml with mobile phase and filtered through 0.45µ nylon membrane filter. (Conc. 100 ppm)

Method Validation:

As per the ICH guidelines, the method validation parameters checked were specificity, precision (system, method and intermediate precision), linearity, accuracy, LOD, LOQ, robustness and system suitability tests. Acceptance range is that the % RSD should NMT 2 %.

Specificity:

The specificity of the HPLC method was illustrated, where complete separation of Etoricoxib was noticed in presence of tablet placebo. In addition there was no interference at the retention time of Etoricoxib in the chromatogram of tablet solution. This shows that the peak of analyte was pure and excipients in the formulation did not interfere with the analyte.

Precision:

The precision of the system and method was demonstrated by intraday variation studies, in which five replicate injections of standard solution and six successive injections (in duplicate) were injected and chromatograms were recorded. The % assay and % RSD were calculated for Etoricoxib and presented in **Table 1.**

Inter day variation study was performed by two different analysts (A-I and A-II). Six successive injections (in duplicate) of sample solutions were injected by each analyst and chromatograms were recorded. The % RSD was calculated from the peak areas of replicates and presented in **Table 2.**

Linearity:

It was determined at seven concentration levels ranging from 70 to 130 ppm for Etoricoxib. The Correlation coefficient was 0.998145. The result show that an excellent correlation exists between response factor and concentration of drug within the concentration range. The results obtained are summarized in **Table 3** and calibration curve is presented in **Fig. 2.**

Accuracy:

It was determined by spiking known amount of Etoricoxib raw material (API) in the placebo in triplicate at 70 %, 100 % and 130 % of labeled amount. The amount of Etoricoxib was quantified as per the test method. The % recovery was calculated and presented in **Table 4.**

Limit of Detection and Limit of Quantitation:

According to the determined signal-to-noise ratio, Etoricoxib presented limits of detection of 0.0452 ppm and limits of quantitation of 0.151 ppm, where the compounds proportion found in the sample solutions injected on to the chromatograph. However, the objective of the method is the quantitation of Etoricoxib so that the values obtained should be considered as the limit of method sensitivity.

***Robustness*:**

To determine the robustness of the method, two parameters (flow rate, ratio of mobile phase) from the optimized chromatographic conditions were varied. Statistical analysis showed no significant difference between results obtained employing the analytical conditions established for the method and those obtained in the experiments in which variations of parameters were introduced. Thus the method showed to be robust which is shown in **Table 5.**

System Suitability Tests:

It was evaluated by injecting standard solution during various stages of validation. The tailing factor and theoretical plates for Etoricoxib peak was determined from the five replicate injections of standard solution at every stage of validation. The results are summarized in the **Table 6.**

Assay of Etoricoxib Tablet:

Equal volumes (about 20 µl) of the Blank solution, Standard solution and the Test solution were separately injected into the chromatograph and chromatograms were recorded and areas were measured for the major peak. The quantity of Etoricoxib was calculated in comparison with the standard solution and the results are presented in **Table 7.**

RESULTS AND DISCUSSION

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drug Etoricoxib was preferably analyzed by reverse phase chromatography and accordingly C_{18} column was selected. The elution of the compound from the column was influenced by polar mobile phase. The ratio of acetonitrile to phosphate buffer was optimized to give symmetric peak with short run time. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase of acetonitrile: phosphate buffer at the ratio of 70:30 (v/v). The retention time of Etoricoxib was found to be 9.58 min, which indicates a good base line. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The system suitability parameters are given in **Table 6.** Developed chromatographic method was applied for the determination of Etoricoxib in tablet formulation, given in **Table 8.** A typical chromatogram showing the separation of Etoricoxib is shown in **Fig. 3.**

Table No. 1: Intraday Precision (Method Precision) of Etoricoxib

S. No.	Label Claim	Amount of Drug*	% Assay of Etoricoxib*
1	90 mg	91.93 mg	102.14
2	90 mg	89.85 mg	99.83
3	90 mg	88.71 mg	98.57
4	90 mg	90.86 mg	100.96
5	90 mg	88.39 mg	98.21
6	90 mg	89.64 mg	99.6
Mean		**89.89 mg**	**99.89**
Standard Deviation (SD)			1.47
Relative Standard Deviation (% RSD)			1.47

***Average of two readings**

Acceptance Criteria: The % RSD should not be more than 2 %.

Table No. 2: Interday Precision (Intermediate Precision) of Etoricoxib

S. No.	Amount of drug present*		% Assay of Etoricoxib*	
	Analyst - I	Analyst - II	Analyst - I	Analyst - II
1	91.93 mg	91.47 mg	102.14	101.63
2	89.85 mg	91.27 mg	99.83	101.41
3	88.71 mg	88.57 mg	98.57	98.41

4	90.86 mg	89.70 mg	100.96	99.67
5	88.39 mg	89.34 mg	98.21	99.27
6	89.64 mg	87.61 mg	99.6	97.41
Mean	**89.89 mg**	**89.66 mg**	**99.89**	**99.63**
SD			1.47	1.66
% RSD			1.47	1.67
Overall Mean				99.76
Overall SD				1.57
Overall % RSD				1.57

***Average of two readings**

Acceptance Criteria: The % RSD should not be more than 2%.

Table No. 3: Linearity of Etoricoxib

S. No.	Concentration (in ppm)	Area
1	70	4365120
2	80	4925172
3	90	5450437
4	100	5943270
5	110	6409698
6	120	6785603
7	130	7236921
	Correlation coefficient	**0.998145**

Acceptance Criteria: The Correlation coefficient and Regression coefficient (R2) value should not less than 0.997.

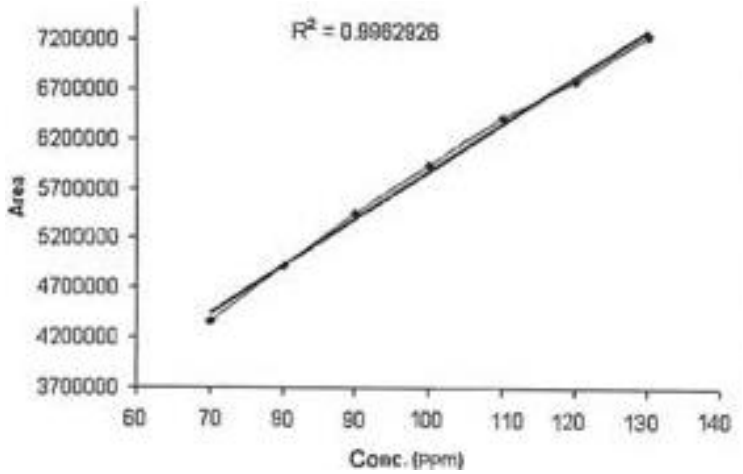


Fig. 2: Standard calibration curve of Etoricoxib

Table No. 4: Accuracy of Etoricoxib

S. No.	Known amount added in Placebo (in mg)		Recovery in mg	% of Recovery
1	70%	69.21	68.38	98.88
2	100%	99.65	97.97	100.44
3	130%	129.09	127.55	99.23

Acceptance Criteria: The % Recovery of Etoricoxib at each spike level should be between 98 and 102 for triplicate determination.

Table No. 5: Robustness of Etoricoxib

S. No.	Parameters	Adjusted to	Amount of drug present*	% assay of Etoricoxib*
1	**Flow rate (1ml/min)**	0.9 ml/min	88.39 mg	98.21%
		1.1 ml/min	88.71 mg	98.57%
2	**Ratio of mobile phase Acetonitrile: phosphate buffer 70: 30**	65:35	87.97 mg	97.74%
		75: 25	88.47 mg	98.30%

*** Average of two readings**

Acceptance Criteria: The % assay of Etoricoxib should be between 90% - 110 %

Table No. 6: System Suitability Test

S. No.	Name of the Experiment	Theoretical Plates	Tailing Factor
1	**ASSAY**	11990.917	1.095
2	**SPECIFICITY**	12009.188	1.092
3	**PRECISION**		
	2.1 System Precision	12014.984	1.095
	2.2 Method Precision	12024.5	1.094
	2.3 Intermediate Precision	11950.75	1.095
4	**LINEARITY**	12105.544	1.092

5	**ACCURACY**	12257.53	1.09
6	**6.1 LOD**	11929.349	1.096
	6.2 LOQ	12924.544	1.092
7	**ROBUSTNESS**		
	7.1 Change in Flow Rate of Mobile Phase		
	7.1.1 Minus Flow (0.9 ml/min)	11359.933	1.092
	7.1.1 Plus Flow (1.1ml/min)	11479.434	1.097
	7.2 Change in Ratio of Mobile Phase		
	7.2.1 ACN : Phosphate buffer (65 : 35)	11059.901	1.09
	7.2.2 ACN : Phosphate buffer (75 : 25)	12459.984	1.095

Acceptance Criteria: The Tailing Factor should not be more than 2 and Theoretical Plates should not be less than 2000.

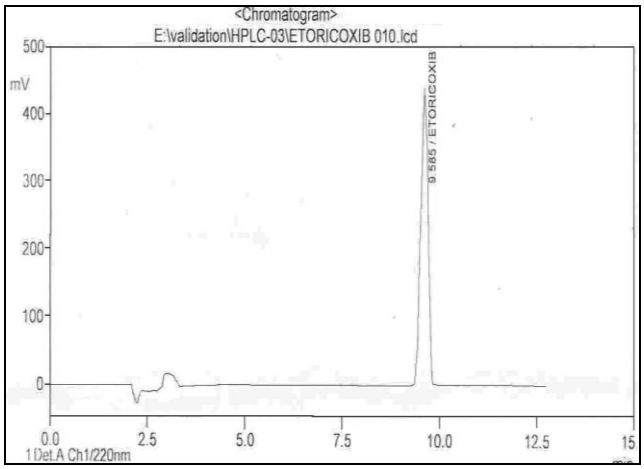
Table No. 7: Assay of Etoricoxib Tablet

S. No.	Name of solution of Etoricoxib	Average area	Average Retention time	Labeled amount (mg) per tablet	Amount found (mg) per tablet	% amount found (% assay)
1	Standard solution	5674077*	9.588*	-	-	-
2	Sample Solution	5725602**	9.586**	90 mg	89.64 mg	99.60%

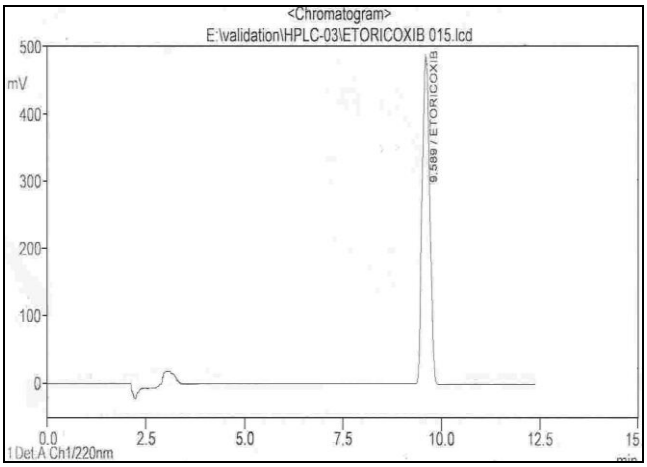
*** Average area and retention time of five replicate injections of standard solution of Etoricoxib**
**** Average area and retention time of duplicate injection of sample solution of Etoricoxib**

Table No. 8: Developed Chromatographic Conditions

Instrument	HPLC (auto sampler) LC-2010A – HT, Shimadzu
Column	Symmetry (Waters) RPC_{18} column (250 x 4.6 mm, 5 µm)
Mobile Phase	Phosphate buffer : Acetonitrile in the ratio 30:70 v/v
Detector	UV detector
Wavelength	220 nm
Flow Rate	1ml/min.
Injection volume	20 µl
Temperature	Ambient
Run Time	10 min.
Retention Time	9.585 min.



Chromatogram of Standard solution of Etoricoxib



Chromatogram of Sample solution of Etoricoxib

Fig. 3: Chromatograms of Standard solution & Test solution of Etoricoxib

CONCLUSION

A validated RP-HPLC method has been developed for the determination of Etoricoxib in tablet dosage form. The proposed method is simple, rapid, accurate, precise, linear, robust and specific and the method was successfully applied for the estimation of Etoricoxib in marketed tablet formulation and assay values confirmed to the label claim of the marketed tablet formulation.

Hence the developed and validated HPLC method can be used for the routine analysis of Etoricoxib in tablets in Pharmaceutical Industries and in Research Laboratories.

ACKNOWLEDGEMENT

The author is thankful to Akums Drugs and Pharmaceuticals Ltd., Haridwar for providing working standard and gift samples of Etoricoxib and necessary facilities to carry out the research work.

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Conflict of interest: The authors have declared that no conflict of interest exists.

Source of support: Nil