INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

DOI: https://dx.doi.org/10.33289/IJRPC.30.5.2023.13(34)

METHOD DEVELOPMENT AND VALIDATION OF ETORICOXIB BY USING RP-HPLC IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A rapid and sensitive RP-HPLC method with UV detection (207.5 nm) for routine analysis of Etoricoxib in a pharmaceutical formulation was developed. Chromatography was performed with a mobile phase containing a methanol of assay (99.8%) with flow rate of 1 ml/min. Quantitation was accomplished with an internal standard method. The procedure was validated for linearity (correlation coefficient = 0.999), accuracy and limit of detection (LOD) intraday precision. To test validation of the Etoricoxib three factors were considered as linearity, precision, LOD where mobile phase, flowrate and pressure are respectively selected as methanol, 1 ml/min, 1600 pascals. For intraday precision measure the variables considered were: analyst, equipment. The RSD value (0.25%) indicated a good precision of the analytical method. The proposed method was simple; highly sensitive, precise, accurate and retention time less than 3 min indicating that the method is useful for routine quality control

Keywords: Etoricoxib, HPLC, validation, precision and LOD.

INTRODUCTION

Description

Etoricoxib is a synthetic, nonsteroidal anti-inflammatory drug (NSAID) with antipyretic, analgesic, and potential antineoplastic properties. Etoricoxib specifically binds to and inhibits the enzyme cyclooxygenase-2 (COX-2), resulting in inhibition of the conversion of arachidonic acid into prostaglandins.

Drug Name: Etoricoxib
Brand Name: Etozox-90
IUPAC Name: 5-Chloro-6'-methyl-3-[4-(methylsulfonyl)phenyl]-2,3'-bipyridine.
Molecular formula: $C_{18}H_{15}ClN_2O_2S$
Molecular Weight: 358.842g/mol
Boiling Point: 510 °C
Category: Non steroidal anti-inflammatory drug (NSAID).
Solubility: practically insoluble in water.

Mechanism of Action

Like any other selective COX-2 inhibitor ("coxib"), etoricoxib selectively inhibits is form 2 of the enzyme cyclooxygenase(COX-2). It has approximately 106-fold selectivity for COX-2 inhibition overCOX-1. This reduces the generation of prostaglandine (PGs) from arachidonic acid. Among the different functions exerted by PGs, their role in the inflammation cascade should be highlighted. Selective COX-2 inhibitors show less activity on COX-1 compared to traditional non steroidalanti-

inflammatory drugs(NSAID). This reduced activity is the cause of reduced gastrointestinal side effects, as demonstrated in several large clinical trials performed with different coxibs.

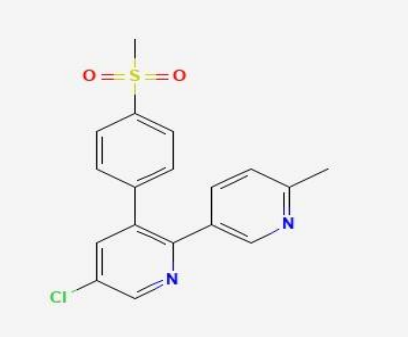

Fig. 1: Etoricoxib structure

MATERIALS AND METHODS

The materials used in this procedure is peak HPLC, UV Detector, C18 Column, weighing machine, Borosil pipettes, burettes, and beakers

The Chemical used are Etoricoxib drug, Methanol, Acetonitrile.

Preparation of solution

1.Preparation of Standard Stock Solution

Accurately weigh and transfer 0.05mg standard drug of Etoricoxib into volumetric flask and add 5 ml of methanol and dissolve by sonication process for 3 minutes and label it as standard stock solution of 1000µg/ml.From the 1000µg/ml prepare 100µg/ml concentration by taking the volumes as 1 ml of stock solution and 9 ml of methanol into a test tube and label it as 100µg/ml.From 100µg/ml prepare 10µg/ml concentration by taking the volumes as 1 ml of stock solution and 9 ml of methanol into an empty test tube and label it as 10µg/ml.

2.Preparation of Sample Solution

To detect the Etoricoxib tablet concentration take any branded tablet like ETOZOX-90 of Etoricoxib drug of powered dosage 10mg of equivalent weight and dissolve it in 10 ml of methanol of equivalent weight and sonicate it for 5 minutes, label it as sample solution.

3.Preparation of Standard Dilutions

Mobile phase(methanol) is used as a diluent.

From the stock solution of concentration 100µg/ml pipette out the required volumes of concentration as 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml,50µg/ml,60µg/ml.

4.Preparation of mobile phase

Methanol:Acetonitrile(20:80).

Preparation of 10µg/ml concentration

1 ml of standard stock solution is taken through pipette into the test tube and 9ml of methanol is added for 10 ml of solution.

Preparation of 20µg/ml concentration

2 ml of standard stock solution is taken through pipette into the test tube and 8ml of methanol is added for 10ml of solution.

Preparation of 30µg/ml concentration

3ml of standard stock solution is taken through pipette into the test tube and 7ml of methanol is added for 10ml of solution.

Preparation of 40µg/ml concentration

4ml of standard stock solution is taken through pipette into the test tube and 6ml of methanol is added for 10 ml of solution.

Preparation of 50µg/ml concentration
5ml of standard stock solution is taken through pipette into the test tube and 5ml of methanol is added for 10 ml of solution.

Preparation of 60µg/ml concentration
6ml of standard stock solution is taken through pipette into the test tube and 4ml of methanol is added for 10 ml of solution.

4.Preparation of Sample Dilutions
From the sample stock solution of 1000µg/ml prepare 100µg/ml by taking volumes as 1 ml of stock solution and 9ml of methanol for 10 ml of solution and label it as sample dilution.

Procedure
Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis peak area) and calculate the correlation co- efficient.

Acceptance criteria
Correlation coefficient should be not less than 0.990 and not more than 0.999.

RESULTS AND DISCUSSION
Method validation: Trail 1

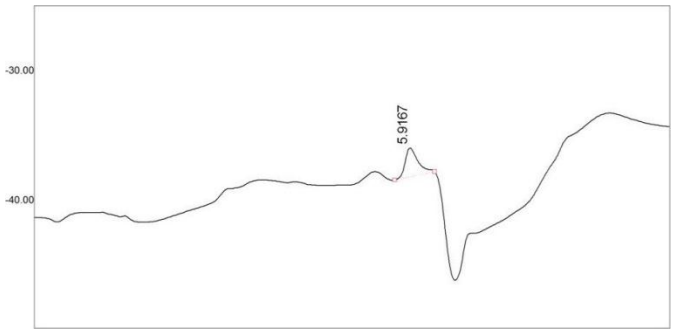

Fig. 2: Trail 1 Chromatogram

Table 1: Result: Trail 1

No.	Name	RT[min]	Area[mV*s]			Resolution
1		5.9167	31.1757	4978.6	1.5215	0.0000
Sum			31.1757			

Observation
Spike of Etoricoxib Peak was observed, but the peak does not have symmetrical shape and does not meet system suitability conditions.

Trail 2

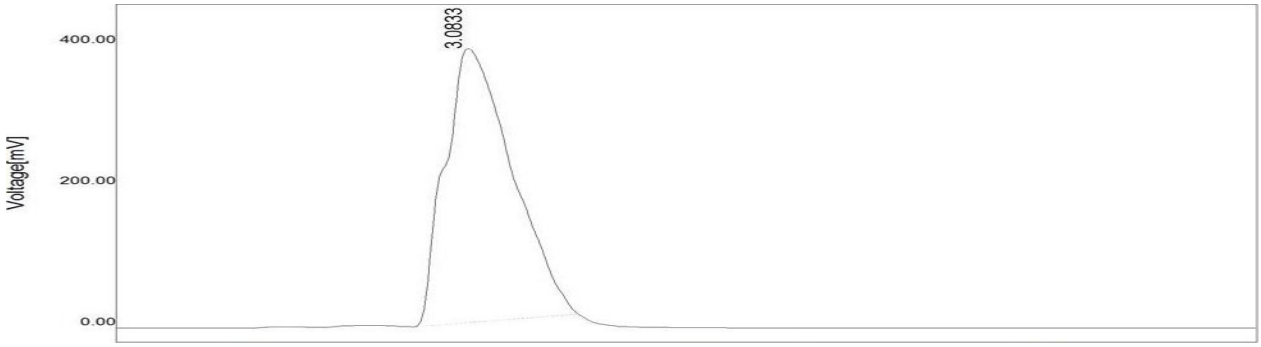

Fig. 3: Trail 2 Chromatogram

Table 2: RESULT: Trail 2

No.	Name	RT[min]	Area[mV*s]	Height[mV]	AmountO		
		3.0833	9507.0833	139.8836	0.0000	476.8	2.5074
Sum			9507.0833	139.8836	0.0000		

Observation

No specific change was observed when compared with trail 1.

Spike of Etoricoxib Peak was observed, but the peak does not have symmetrical shape and does not meet system suitability conditions

Trail 3: Optimised Chromatogram

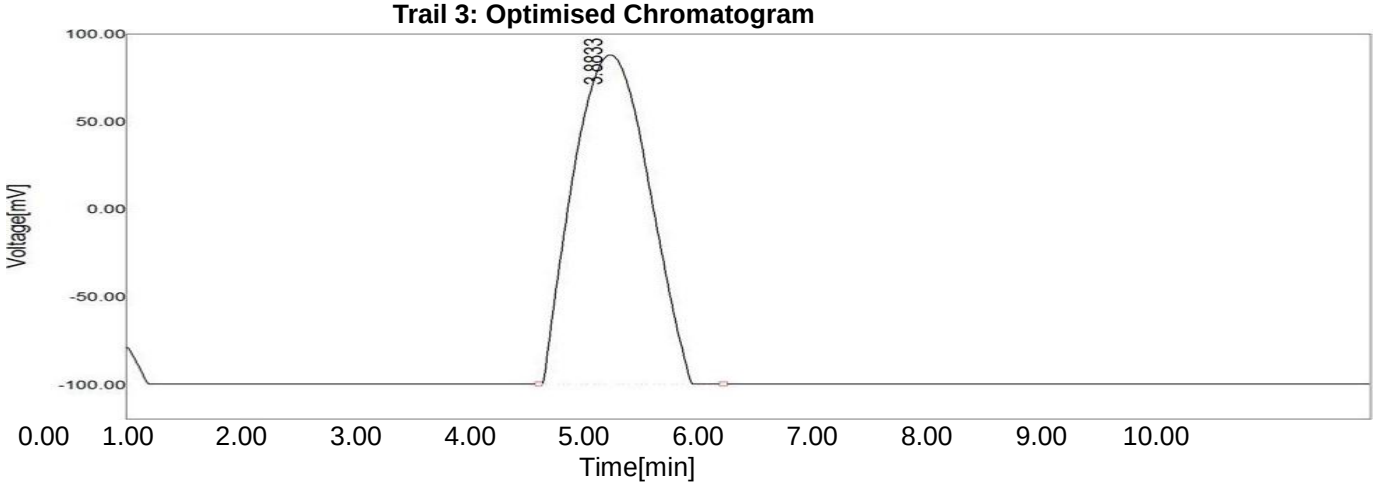

Fig. 4: Optimised Chromatogram

Table 3: Result

No.	**Name**	**RT[min]**	**Area[mV*s]**	**Area%**			**Resolution**
		3.8833	1151.5804	100.00	549.7	0.7892	0.0000
Sum			1151.5804				

Observation

Sharpening of peaks was observed with the previous trails. Spike of a Etoricoxib Peak was observed, the peak have symmetrical shape and meet system suitability condition

Formulation

The sample solution prepared at a concentration of 100µg/ml was analyzed in the developed method conditions. The method can successfully separate and identify the Etoricoxib. Hence the method was found to be suitable for routine analysis of Etoricoxib and formulations.

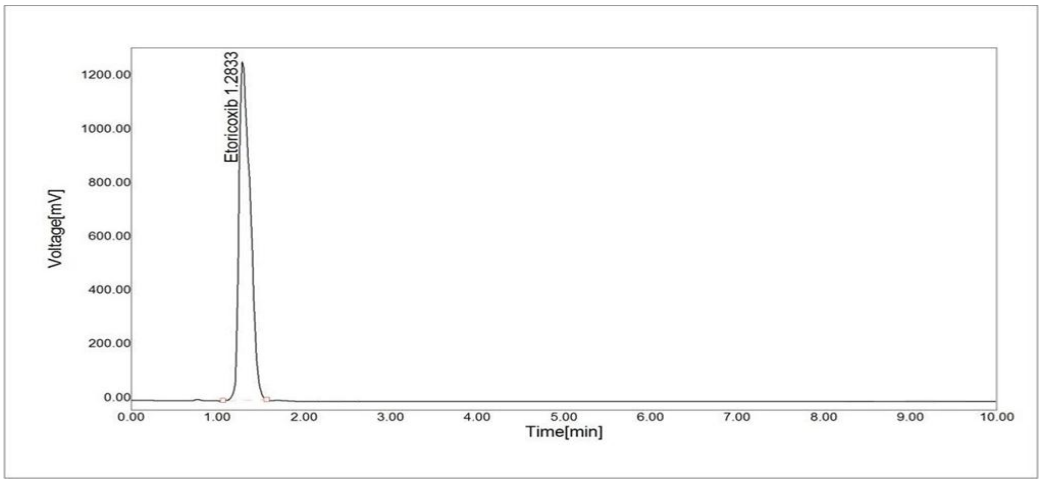

Fig. 5:

Table 4: Result

No.	Name	RT[min]	Area[mV*s]	Area%			Resolution
	Etoricoxib	1.2833	8557.3379	100.00	510.3	1.6784	0.0000
Sum			8557.3379				

Calculation

Table 5: Formulation assay

Formulation	Dosage	Assay%
ETOZOX Tablets	90mg	91.6%

Optimized conditions

Table 6

PARAMETER	CONDITION
Mobile phase	Methanol and Acetonitrile (20:80)
Pump mode	Isocreatic method
PH	4.8
Diluents	Hplc grade Methanol
Column	C_{18} (5 µm pore size)
Column Temp	27^{O}C
Wavelength	207nm
Injection Volume	20 µl
Flow rate	1ml/min
Run time	5 minutes
Retention Time	1.5 minutes

Method validation

Linearity

From the prepared stock solution, a series of calibration standards were prepared at concentrations of 10,20,30,40,50 and 60µg/ml using mobile phase as solvent. Each of these drug solutions (20µl) was injected into the column, the peak area and retention times were recorded. The calibration curve for Etoricoxib was constructed by plotting the mean peak area against the drug concentration. Regression equation was found to be y = 3861x+3844.(r2 = 0.996).

Table 7:

Level	Concentration(µg/ml)	peak area
Level – 1	10µg/ml	458
Level – 2	20µg/ml	1545
Level – 3	30µg/ml	2404
Level – 4	40 µg/ml	3500
Level – 5	50µg/ml	4362
Level - 6	60µg/ml	5319

Calibration curve

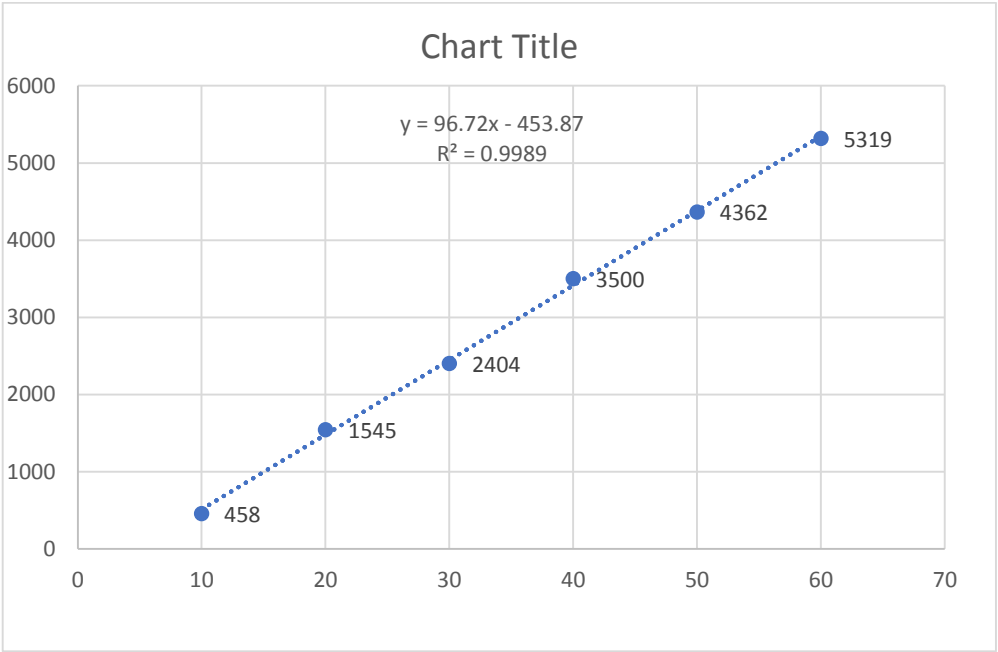

Fig. 6:

Precision

Four replicate analysis of 30µg/ml stock solution of Etoricoxib was analyzed. The % RSD was found to be 0.25 for intraday precision. The % RSD was found to be less than 2 hence the method was found to be précised.

Chromatogram

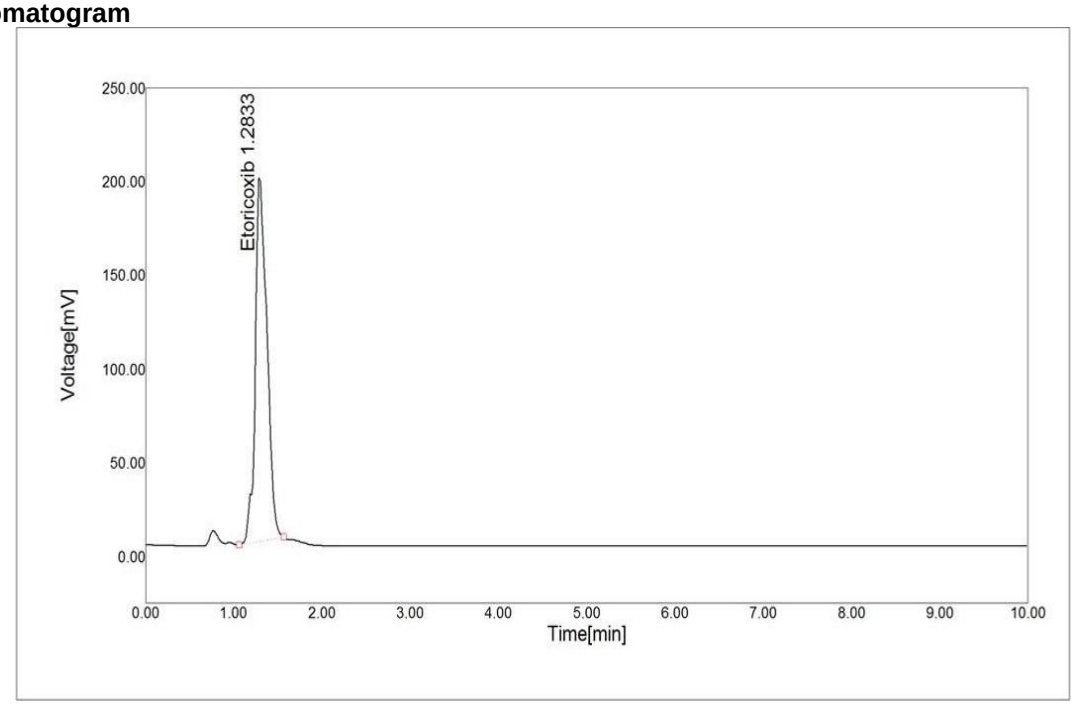

Fig. 7:

Table 8: Precision table

S.NO	Injection	Area value
1	Injection 1	2404
2	Injection 2	2404
3	Injection 3	2404
4	Injection 4	2404
AVERAGE		2404
% RSD		0.25%

LOD Chromatogram

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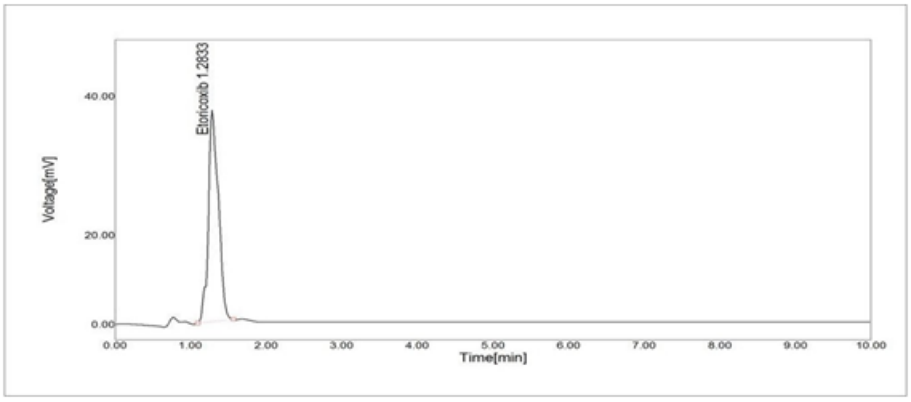

Fig.8:

Table 9: Result

No.	Name	RT[min]	Area[mV*s]	Area%			Resolution
	Etoricoxib	1.2833	163.3701	100.00	3830.5	0.7864	0.0000
Sum			163.3701				

CONCLUSION

The estimation of Etoricoxib was done by RP-HPLC. The assay of Etoricoxib was performed with tablets and the % assay was found to be 91.6% which shows that the method is useful for routine analysis. The linearity of Etoricoxib was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.25 etoricoxib which shows that the method is precise. The accuracy limit is the percentage recovery should be in the range of 90% - 103.0%. The total recovery was found to be 91.6% for etoricoxib. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The acceptance criteria for LOD is 3 .The LOD for etoricoxib was found to be 3.02. The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

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