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# Develop a Simple RP-HPLC and UV-Visible Method for Estimation of Etoricoxib from Pharmaceutical Dosage

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### ABSTRACT

**Objective:** The main purpose of present study was to develop a validated and stable isocratic reverse phase high performance liquid chromatography and UV-Visible method for regulate the quality and estimation of Etoricoxib drug content from the marketed pharmaceutical tablets.

**Methods:** The reverse phase high performance liquid chromatography and UV-Visible method validation for Etoricoxib have done followed by assay methodology.

**Results:** The retention time ( $R_t$ ) of Etoricoxib was 10.0 min with the flow rate of 1.0 mL/min at wave length 272 nm. The linearity of method was validated for Etoricoxib drug content in the range of 5-100  $\mu\text{g/mL}$  with correlation coefficient ( $r$ ) values 0.997 and 0.998 for RP-HPLC and UV-Visible, respectively.

**Conclusions:** This method is stable and validated to assay analysis. Thus, the validated method is can be successfully applied to routine analysis for regulate the quality. It also should be used for analytical research purpose.

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## Introduction

The name of Etoricoxib according to IUPAC is (5-chloro-2-[6-methylpyridin-3-yl]-3-[4-methylsulfonyl phenyl] pyridine). It is a second generation drug. This is selective drug and inhibit to cyclo-oxygenase-2. It is applicable as orally as analgesic and anti-inflammatory drug<sup>1,2</sup>. Physically appearance of active ingredients of Etoricoxib is white crystalline powder. It is relatively insoluble in water but freely soluble in alkaline aqueous solutions<sup>3</sup>. It is used for symptomatic management of osteoarthritis<sup>4,5</sup>, rheumatoid arthritis<sup>6-8</sup>, primary dysmenorrhoea, postoperative dental pain, acute gouty arthritis<sup>9</sup>, cancer treatment and prevention and migraine<sup>10-12</sup>. The step of assay evaluation is the most important part of the quality control lab of each and every pharmaceutical industry<sup>13</sup>. The main purposes of assay evaluation are the improvement and maintain the quality of the pharmaceutical products<sup>14,15</sup>. During the literature survey these are notified that the various method validation of Etoricoxib was reported by UV-Spectrophotometry<sup>16</sup>, High Performance Liquid Chromatography (HPLC)<sup>17,18</sup> and High Performance Thin Layer Chromatography (HPTLC)<sup>19</sup>. The main purpose of this study is to develop a cheap and suitable reversed phase high performance liquid chromatography (RP-HPLC) and UV-Visible Spectrophotometer method for the estimation of Etoricoxib from pharmaceutical tablets, accordance International conference harmonized guidelines (ICH)<sup>20</sup>. (See figure 1.)

## Experimental

### Chemicals and reagents

Analytical grade pure drug of Etoricoxib (99.9%) was purchased from Sun Pharma Pvt. Ltd. Dehradun, Uttarakhand, India. The HPLC grade methanol and distilled water were purchased from Molychem. The Nylon filter (pore size

0.45 $\mu$ m) was purchased from Merck India Ltd.

### Instrumentation

A Binary analytical CYBERLAB™ High Performance Liquid Chromatography (HPLC) instrument was used for assay evaluation. The HPLC instrument equipped with a degasser and thermostat column. The drug analyte were detected by UV detector after separation on C<sub>18</sub> column. In 2<sup>nd</sup> method, a double beam Thermo Scientific UV-Visible Spectrophotometer was used for the assay evaluation. An ultrasonic sonicator (company name Tischo) was used for the sonication of mobile phase.

### Preparation of mobile phase

The appropriate mobile was prepared by HPLC grade Methanol and distilled water. According to trial method the solvents in the ratio of 70:30 methanol water (v/v) was selected as mobile phase. Prepared mobile phase was filtered through a 0.45  $\mu$ m nylon membrane and degassed by ultrasonic sonicator.

### Preparation of standard stock solution

A stock solution of 1000 ppm was prepared by transferring 100 mg Etoricoxib in 100 mL volumetric flask and finally diluted up to the meniscus with mobile phase. To remove the air from the prepared stock solution was sonicated for 5.0 min and filtered it with 0.45  $\mu$ m nylon filter membrane.

### Preparation of sample solution

To the preparation of sample solution, 90 mg 25 tablets of Etoricoxib (formulated by Macleods pharmaceuticals Ltd. India) were weighed accurately and crushed it by mortar pistol finely it converted into powder. The equivalent weight (100 mg) of Etoricoxib powder of crushed tablets were mixed well and

transferred into a small conical flask. Separate the Etoricoxib active ingredient by extraction method with mobile phase (methanol/water). The separate active ingredient was transferred into a 100 ml volumetric flask and the volume make up with mobile phase. Prepared sample solution was filtered with 0.45  $\mu\text{m}$  nylon filter paper. This sample solution was covered the working concentration range.

#### Preparation of calibration curve

The sample application volume 25  $\mu\text{L}$  was injected in thrice replication. The different application samples were prepared in the serial concentrations 5,10,25,50 and 100 ppm. Consequently, the calibration curve was prepared by plotting the average peak area and absorbance (OD) against the different known ppm concentrations.

#### Method validation

Precede the procedure of method validation through leading the various parameters such as system suitability, Linearity, limit of detection (LOD), limit of quantification (LOQ) and accuracy. These all parameters were performed according to the ICH guide lines<sup>20</sup>.

#### System suitability test

The system suitability test was performed during the method development. It is the prior need of the method development, so this purpose the system suitability test have been done in the serial concentration of 5,10 and 25  $\mu\text{g/mL}$ . The sample application inject in thrice replication. The system suitability is measured with the different parameters peak area, retention time, resolution factor, theoretical plates, and tailing factor. These all parameters were checked according to international conference harmonization guide line.

#### Linearity

Basically it is the scale of measure the linearity of the method. The linearity ranges in both methods were 5-100 ppm. These both concentrations ranges were covered whole applied experimental work. The linearity ranges were referred to the highest and lowest quantity of the drug analyte. The linearity of the method was calculated by regression analysis.

#### Limit of detection (LOD)

It is the detectable value of the lowest concentration of the sample. It is the 3.3 times multiplication of the ratio of standard deviation of the peak area of the drug and slope of the corresponding calibration curve. The formula for calculation of Limit of detection (LOD) is described below:

$$\text{Limit of Detection} = 3.3 \times \frac{\text{Standard deviation of the Peak Area of the Drug}}{\text{Slope of the Corresponding Calibration Curve}}$$

#### Limit of quantification (LOQ)

It is the detectable value of the lowest concentration of the sample. It is the 10.0 times multiplication of the ratio of standard deviation of the peak area of the drug and slope of the corresponding calibration curve. The formula for calculation of Limit of Quantification (LOQ) is described below:

$$\text{Limit of Quantisation} = 10 \times \frac{\text{Standard deviation of the Peak Area of the Drug}}{\text{Slope of the Corresponding Calibration Curve}}$$

#### Accuracy

To confirm the accuracy of the validated method were applied three concentrations label 5, 10 and 25 ppm standard sample solution. The Performance of the validated method was performed by inter-day and intra-day recovery study. These three concentrations diluted from the stock solution and were added to an extract with a known drug active ingredient of

Etoricoxib. The percentage recovery of the respective constituents was calculated. The formula of percentage recovery described below:

$$\text{Percentage Recovery} = \frac{\text{Peak Area of the Drug in Standard}}{\text{Peak Area of the Drug in Sample Mix}} \times 100$$

## Results

The reverse phase HPLC method is depend basically two basic thinks one is stationary phase and another is mobile phase. According to the nature of the active ingredient the C<sub>18</sub> column was selected as the stationary phase. The mobile phase was select according to dipole moment of the active ingredient but this was achieved by isocratic trial method. According to isocratic trial method which mentioned as above an appropriate suitable mobile phase methanol/water in the ratio of 70:30 was reported at 25 °C<sup>21</sup>. The mobile phase methanol/water in the ratio of 70:30 (v/v) was given suitable retention time and better resolution. There was no interference with excipients. The resolution of Etoricoxib was showed in following chromatogram. (See figure 2.)

For the better resolution the wave length of the Etoricoxib active ingredient was achieved by scanned method. The active ingredient of Etoricoxib was scanned with UV-Visible region 200 to 400 nm. The scanned wave length of active ingredient of Etoricoxib was 270 nm. The obtained results of system suitability test were listed in the table No. 1. The system suitability results were listed with corresponding important HPLC parameters which play an important role in better resolution such as Peak area, Retention time (R<sub>t</sub>), Resolution factor (R<sub>f</sub>), Theoretical plates (T<sub>p</sub>), Tailing factor (T<sub>f</sub>). The system suitability parameters were calculated by statistics formulas such as mean, standard deviation, coefficient variation and standard error. The coefficient

of variation percentages were calculated 0.02, 1.03, 1.67, 0.05 and 1.14 with standard errors 0.74, 0.01, 0.01, 0.43 and 0.01 for the corresponding reverse phase high performance liquid chromatography (RP-HPLC) parameters. (See table 1.)

The linearity for both methods RP-HPLC and UV-VIS Spectrophotometer was measured by regression analysis. The linearity range of Etoricoxib was 5-100 µg/mL for both methods. The results of regression coefficient (R<sup>2</sup>) were calculated 0.997 and 0.998 for both methods. The values of regression coefficient were indicated that the both methods were linear. The linearity results of Etoricoxib were listed in the Table 2.

The results of limit of detection (LOD) and limit of quantification (LOQ) for both methods reversed phase-high performance liquid chromatography (RP-HPLC) and UV-Visible Spectrophotometer were reported 0.03, 0.2, 0.09 and 0.6 µg/mL, respectively. The results were listed in the Table 3.

The results of recovery study were calculated for RP-HPLC 100% for 5µg/mL, 99% for 10µg/mL and 99.64% for 25 µg/mL. The calculated recovery results analyzed by RP-HPLC were listed in Table 4.

The results of recovery study were calculated for UV-Visible method 98% for 5µg/mL, 98% for 10µg/mL and 97% for 25 µg/mL. The calculated recovery results analyzed by RP-HPLC were listed in Table 5.

## Discussion

Wave length selection is the primary need for the chromatographic analysis. The suitable wave length has been find at 272 nm. The selection of mobile phase is an important secondary basic need for chromatographic analysis. The mobile phase has been selected under isocratic reversed

phase-partition chromatographic conditions. The results of linearity have statistically significant ( $R^2=0.997$  and  $0.998$ ) for both technique, hence the applied method is linear. The results of LOD (limit of detection) and LOQ (limit of quantification) have provided suitable condition for the quantitative estimation of drug content. Thus, the instrument working is proper mode. The results of system suitability test have significant with less than 10 CV percent and less than one standard error, hence the proposed methods give good reliability<sup>22-25</sup>. The results of recovery have been given a good accuracy for the both methods. These both methods have been shown minor difference. Meanwhile these both methods can be used for analytical purpose.

### Conclusion

The entire experimental work was developed and validated isocratic RP-HPLC and UV-VIS Spectrophotometer method for the simultaneous determination of Etoricoxib from formulated pharmaceutical marketed tablets. The proceeding conditions in both methods during entire experimental work were optimized to provide high resolution and reproducible absorbance and peak area. The all results were statistically significant. Thus, the both methods are suitable for the quantitative determination of drug content.

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**Table 1.** Summary of system suitability for RP-HPLC analysis

S. No.	Parameters	Mean	SD	CV%	SE
1	Peak area	67864	1.65	0.02	(±) 0.74
2	Retention Time	3.46	0.03	1.03	(±) 0.01
3	Resolution Factor	1.344	0.02	1.67	(±) 0.01
4	Theoretical Plates	2735	0.09	0.05	(±) 0.43
5	Tailing Factor	1.646	0.02	1.14	(±) 0.01

SD=Standard Deviation

CV%=Coefficient of Variation Percentage

SE=Standard Error (±)

**Table 2.** Linearity results for both methods RP-HPLC and UV-Visible

S. No.	Parameters	RP-HPLC	UV-Visible
1	Correlation range	5-100 ppm	5-100 ppm
2	Regression equation	$y=14679x-16229$	$y=0.096x+0.087$
3	Regression coefficient ( $R^2$ )	0.997	0.998

**Table 3.** The calculated value of LOD and LOQ for RP-HPLC and UV-Visible

S. No.	Parameters	RP-HPLC	UV-Visible
1	Limit of detection (LOD)	0.03 µg/mL	0.2 µg/mL
2	Limit of quantification (LOQ)	0.09 µg/mL	0.6 µg/mL

**Table 4.** The calculated recovery results of RP-HPLC method

S. No.	Added in µg/mL	Recovery in µg	Recovery in %
1	5	5.02	100
2	10	9.99	99
3	25	24.91	99.64

**Table 5.** The calculated recovery results of UV-Visible method

S. No.	Added in µg/mL	Recovery in µg	Recovery in %
1	5	4.9	98
2	10	9.8	98
3	25	24.23	97

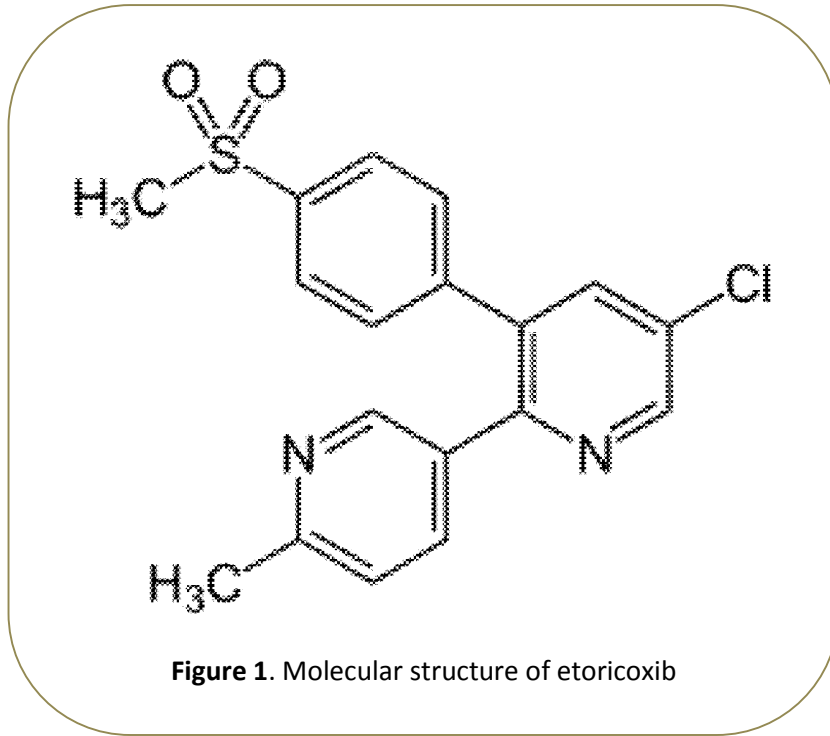


Figure 1. Molecular structure of etoricoxib

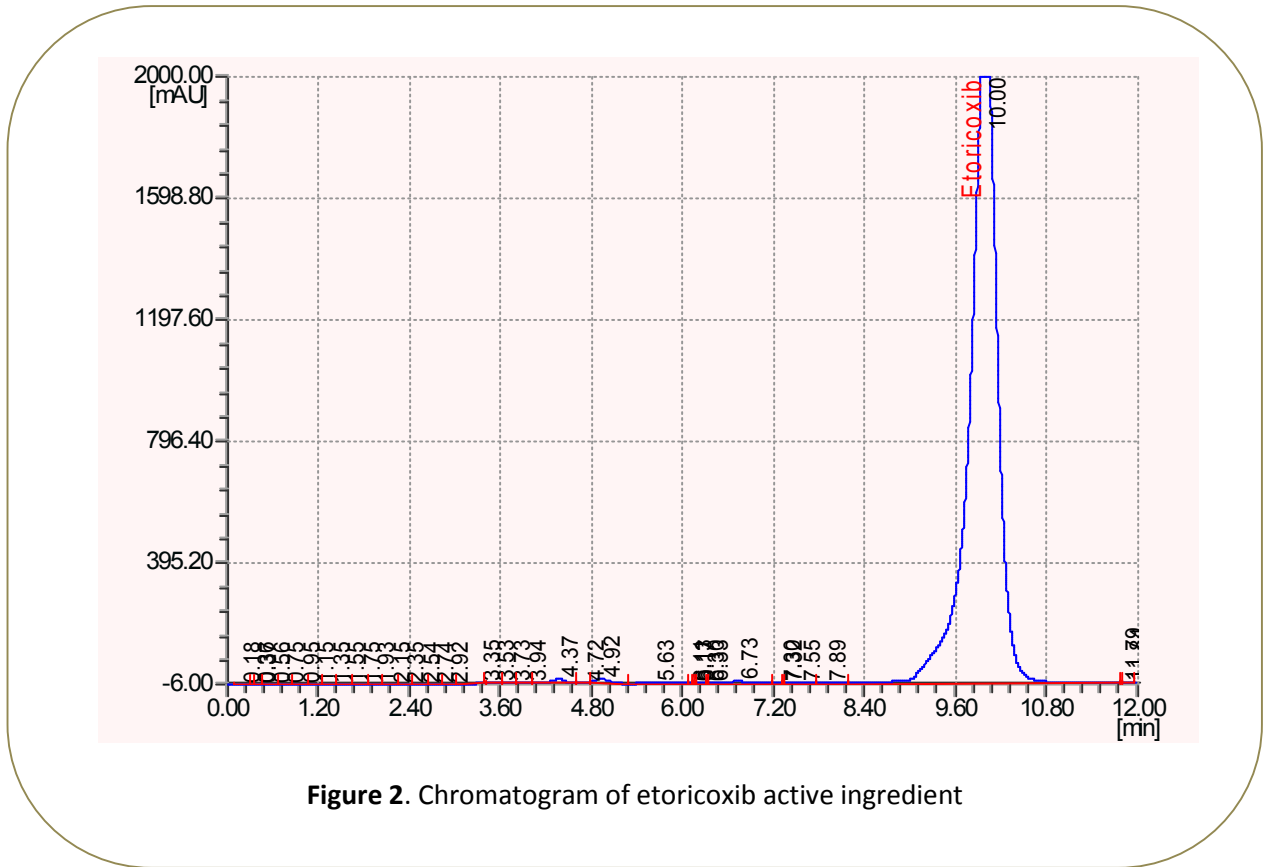


Figure 2. Chromatogram of etoricoxib active ingredient