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DEVELOPMENT AND VALIDATION OF RP HPLC METHOD FOR THE ESTIMATION OF ETORICOXIB IN BULK AND TABLETS

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Abstract

Objective: Objective of the present analytical research work was to develop and validate Reverse Phase High Performance Liquid Chromatographic method (RP-HPLC Method) for the Etoricoxib in bulk and tablets dosage form.

Methods: A RP-HPLC method has been developed and validated for estimation of ETOR in pharmaceutical oral dosage form.

Method A (RP-HPLC Method): The RP-HPLC Method for Etoricoxib was developed using Shimadzu HPLC, LC-10, temperature maintained 25 ^{0}C, phenorex Gemini C18 (250 mm × 4.60 mm × 5µm), as stationary particle, isocratic mode. Water: ACN: OPA: TEA (40:60:0.1:0.1, v/vv/v). Mobile phase was maintained at a flow rate of 1.0 ml/min and detection was carried out at 245 nm.

Results: Etoricoxib was found to be linear in the concentration range of 8 - 12 µg/ml for RP-HPLC method. Retention time was found to be 2.7 min for Etoricoxib. The amount of Etoricoxib in marketed formulation was found to be 99.33 %.

Interpretation and Conclusion: Results of assay and validation study were found to be satisfactory. So, the method can be successfully applied for the routine analysis of Etoricoxib.

***Keywords*:** Etoricoxib, RP-HPLC, ICH Guidelines

1. Introduction

Arthritis (from Greek arthro-, joint + -itis, inflammation; plural: arthritides) is a form of joint disorder that involves inflammation of one or more joints. The pain from arthritis is due to inflammation that occurs around the joint, damage to the joint from disease, daily wear and tear of joint, muscle strains caused by forceful movements against stiff painful joints and fatigue. Arthritis is very common and occurs in 30% to 50% of the general population. 10% of the population may suffer from chronic (long-standing) arthritis. Arthritis affects people of all ages, rarely in children, although it is more common in adults and its frequency increases with age. In general, women are affected more frequently than men.

Etoricoxib is a COX-2 selective inhibitor. Cyclooxygenase (COX), also known as prostaglandin endoperoxide synthase, is the key enzyme required for the conversion of arachidonic acid to prostaglandins. Two COX isoforms have been identified, COX-1 and COX-2. In many situations, the COX-1 enzyme is produced constitutively (e.g., in gastric mucosa), whereas COX-2 is highly inducible (e.g., at sites of inflammation and cancer). Traditional nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit both enzymes, and a new class of COX-2 selective inhibitors (COXIBs) preferentially inhibit the COX-2 enzyme.

Literature survey reveals that only a few analytical methods are reported for the estimation of Etoricoxib by RP-HPLC. In this study efforts were made to develop simple, easy and economic HPLC methods for the estimation of Etoricoxib. The developed method was optimized and validated as per the guidelines of International conference on Hormonization (ICH) and demonstrated excellent specificity, linearity, precision and accuracy for Etoricoxib. Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. Method validation is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, quality, purity, and potency of the drug substances and drug products. [1, 2, 3, 4]

2. Materials and methods

2.1 Preliminary analysis of drug: Etoricoxib (ETOR) is official in British Pharmacopoeia (BP). It is in Authorized British Approved Names 2002, in Supplement No. 3 Hence, Preliminary analysis of ETOR was performed according to BP.

2.1.1 Description: The sample of ETOR was observed for its color and texture.

***2.1.2 Solubility*:** The sample of ETOR was taken in test tubes and observed for solubility in various solvents like DMF, DMSO, diluted mineral acids water, alcohol, ethanol and water.

2.1.3 Water Determination: Water content can be determined using Karl Fischer Titrimeter. Karl Fischer reagent is standardized with sodium tartrate, then it is titrated with the known amount of sample i.e. ETOR and then once the color change is observed, it will indicate the end point of the titration

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and this in turn will give the amount of moisture present in the sample.
Moisture/water content can be determine using following formula,

Water content =

$$\frac{\text{Burette reading} \times \text{Karl Fischer Factor} \times 100}{\text{Weight of sample (mg)}}$$

(Limit: Not more than 0.5 %)

2.2 Chemicals and Reagents (HPLC): Analytically pure samples of Etoricoxib were kindly supplied by Sun Pharmaceuticals Ltd, Vapi, Gujarat, India. Arcoxia-60mg (Marketed Formulation), Water (HPLC Grade), ACN (HPLC Grade), OPA, TEA Themis laboratories Pvt.Ltd (Mumbai,India), were used for the method development.
2.2 Instrument Used: Electronic Weighing Balance (Tapson's Analytical Balance), Ultrasonicator (Tapson's TP-101), Cellulose Acetate Filter, 0.45 µm (Nylon 66), HPLC System (Shimadzu)
2.3 Selection of Mobile Phase: The selection was made on the basis of literature survey. After assessing the solubility of drug in different solvents as well on the basis of literature survey, water and acetonitrile were selected as a first choice. OPA (0.1%) and TEA (0.1%) was added to avoid the degradation of the etoricoxib.
2.4 Selection of column (stationary phase): To get well resolved, symmetric peak with highest no. of theoretical plates, the solution of ETOR was analyzed using different column as a stationary phase like Phenyl, C18, Amino column.
2.5 Selection of Analytical Wavelength: To investigate the appropriate wavelength for determination of ETOR, the solution of the same in the mobile phase were scanned separately by UV–Visible spectrophotometer in the range of 200-400 nm and the spectrum was recorded.
2.6 Preparation of Mobile Phase
***Mobile Phase A*:** HPLC grade water.
***Mobile Phase B*:** Acetonotrile was degassed in sonicator for 15 min.
2.7 Preparation of Standard Stock Solution: Standard stock solution was prepared by dissolving 10 mg of ETOR in 100 ml mobile phase which containing OPA (0.1%) and TEA (0.1%) was added for stability of the etoricoxib, that gives concentration of 100 µg/ml of ETOR and labeled as Standard stock ETOR.
2.8 Preparation of Calibration Curve for ETOR
2.9 Analysis of Arcoxia-60 (Sun Pharmaceutical Ltd): To determine the content of ETOR in conventional tablets (Label claim 60 mg ETOR per tablet); the twenty tablets were weighed, their mean weight determined and they were finely powered and powder equivalent to 10.0mg ETOR was transferred into a 100 ml volumetric flask containing ratio of mobile phase, sonicated for 30 min and diluted to 100 ml with mobile phase (10 µg/ml). OPA and TEA was added to avoid the degradation of the etoricoxib. The resulting solution was filtered, using 0.22 µm filter (Millifilter, Milford, MA) and injected into system. The amount of ETOR was determined. The assay procedure was repeated for six times and calculated using following equation.

$$Ct = \frac{Rt \times Cs}{Rs}$$

Where, Ct and Cs = Concentration of Sample and Standard Solution, respectively.
Rt and Rs = Peak Area for Sample and Standard Solution, respectively. [9, 12, 13]

2.10 Validation of RP-HPLC Method
2.10.1 Accuracy: Accuracy of the method was assessed by standard addition method at three different concentration levels i.e. 80%, 100%, 120%. From sample stock solution ETOR (100 µg/ml), pipette out 10 ml to each of four different 100 ml volumetric flask and add to it 0.0 ml, 8 ml, 10 ml and 12 ml of standard stock solution ETOR (100 µg/ml) and make up the volume with mobile phase. The % Recoveries was calculated by applying regression equation.
2.10.2 Precision: The precision of an analytical method was studied by performing repeatability and intermediate precision.
2.10.3 Repeatability: Suitable aliquot from standard stock solution ETOR (100 µg/ml) 1 ml was pipetted in 10 ml volumetric flask and make up the volume to get final concentration of 10 µg/ml and analyzed six times on the same at optimized chromatographic conditions
2.10.4 Intermediate Precision
2.10.4.1 Intra-day Precision: Intra-day precision was determined by analyzing the standard solutions of ETOR (10, 11, 12 µg/ml) and at three different time intervals on same day.
2.10.4.2 Inter-day Precision: Inter-day precision was determined by analyzing the combined standard solutions of ETOR (10, 11, 12 µg/ml) on three consecutive days. The results were reported in terms of % RSD.
2.10.5 Linearity and Range: The linearity of analytical method for ETOR was determined by studying standard calibration curves. The range of analytical method was decided from the interval between upper and lower level of calibration curves by plotting the log curve.
2.10.6 Limit of Detection and Limit of Quantitation: Detection limit and quantitation limit were determined based on the standard deviation of y-intercepts of six calibration curves and average slope of six calibration curves.

$$LOD = 3.3 \times \frac{\text{Standard deviation of intercept}}{\text{Slope}}$$

$$LOD = 10 \times \frac{\text{Standard deviation of intercept}}{\text{Slope}}$$

2.10.7 Robustness: Standard stock solution of ETOR (100 µg/ml)) were used and analyzed at different flow rate (0.9, 1.00, 1.1 ml/min) and at different mobile phase ratio (39:61:0.1:0.1, 40:60:0.1:0.1, 41:59:0.1:0.1) separately.

2.10.8 System Suitability: Standard solution of ETOR (10 µg/ml) was prepared and analyzed. Chromatograms were studied for different parameters such as tailing factor, resolution and theoretical plates to see that whether they comply with the recommended limit or not.

2.10.9 Specificity and Selectivity: The analytes should have no interference from other extraneous components and be well resolved from them. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix. The method is quite selective. There was no other interfering peak around the retention time of ETOR; also the base line did not show any significant noise.

2.10.10 Ruggedness: Ruggedness of the method was checked by two different analysts keeping same experimental and environmental conditions. An appropriate concentration 10 µg/ml of ETOR was subjected to analysis and concentration was determined. This procedure was repeated six times. [4]

3. Results

3.1 Preliminary analysis of drug: Preliminary analysis of Etoricoxib such as description, solubility, identification test and assay were performed and was complied with BP. A white to slightly off-white fine powder was found which was complied with BP. Drug was soluble in isopropyl acetate, ethanol and toluene. Sparingly soluble in 2- propanol. Practically insoluble in water. Water content was found 0.2%.

3.2 High Performance liquid Chromatographic Method

3.2.1. Reverse Phase HPLC method: By the literature survey RP-HPLC method for estimation of Etoricoxib was reported using RP8 column (150mm×4.6mm i.d., 3.5µm particle size) column. Initially, RP8 column was tried to achieve good peak, but it was not giving good resolved peak and also giving a fronting of the peak, thus this prompted to use Phenomenex Gemini C18 column (250 mm x 4.6.0 mm, 5 µ), thus Phenomenex Gemini C18 column was selected. According to the literature survey, mobile phase containing a mixture of water: ACN: OPA: TEA (40: 60: 0.1: 0.1) was used which showed good resolved peak at a flow rate 1ml/min and retention time was 2.7min with UV detection at 245nm (Table 1, Figure 1, 2, 3).

Table 1 Observation and remarks of mobile phase Optimization of Chromatographic Conditions

Mobile Phase Composition	Inference	Conclusion
Water: ACN:OPA:TEA (10 :90 :0.1 : 0.1,v/v/v/v)	Rt of ETOR greater than 10	Mobile phase was not satisfactory
Water: ACN:OPA:TEA (20 :80 :0.1 : 0.1,v/v/v/v)	Rt of ETOR was around 7.10	Mobile phase was not satisfactory
Water: ACN:OPA:TEA (30 :70 :0.1 : 0.1,v/v/v/v)	Rt of ETOR was around 5	Mobile phase was not satisfactory
Water: ACN:OPA:TEA (40 :60 :0.1 : 0.1,v/v/v/v)	Rt of ETOR was Around 2.7 , Assymmetry was good	Mobile phase was Satisfactory (optimized)
Water: ACN:OPA:TEA (50 :50 :0.1 : 0.1,v/v/v/v)	Asymmetry was less	Mobile phase was not suitable

Figure 1 HPLC chromatogram of Blank (diluents) Water: ACN: OPA: TEA (40: 60: 0.1: 0.1, v/v/v/v)

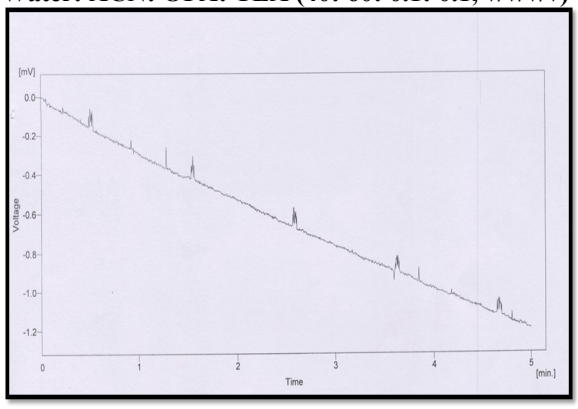


Figure 2 HPLC chromatogram of ETOR standard (10 µg/ml) mobile phase at flow rate of 1 ml/min, at 245nm,

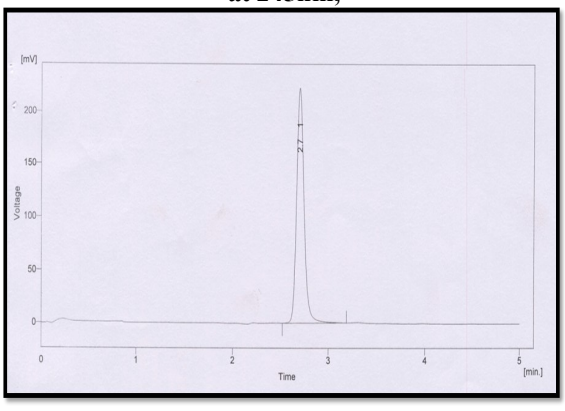
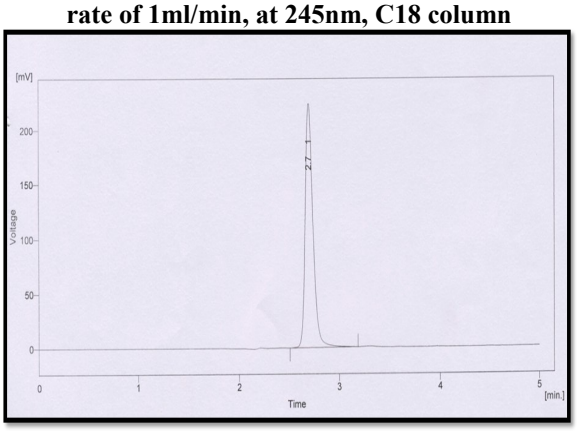


Figure 3 HPLC chromatogram of sample ETOR (10 µg/ml) in the optimized mobile phase at flow rate of 1ml/min, at 245nm, C18 column



3.2.2 Selection of Analytical Wavelength: The standard solutions of ETOR (10 µg/ml) in mobile phase were scanned in the UV region of 200 - 400 nm and the overlain spectra were recorded. It was observed that ETOR drugs showed the absorbance at 245 nm. So, the wavelength of detection used was 245 nm.

3.2.3 Linearity Study: ETOR was found to be linear in the concentration range of 8-12 µg/ml, the results of which are given in Table 2, Table 3 and figure 4.

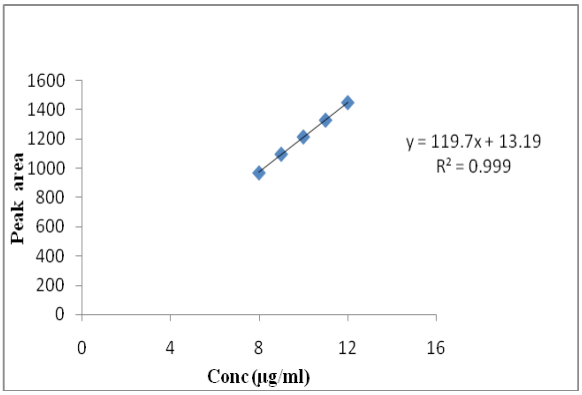
Table 2 Results of Calibration Curve of ETOR

S. No.	Concentration of ETOR [µg/ml]	Area Mean	% RSD
1	8	966.866	0.10
2	9	1094.57	0.11
3	10	1213.383	0.08
4	11	1328.304	0.07
5	12	1448.567	0.08

Table 3 Linear Regression Analysis of Calibration Curves for ETOR

Linearity Range (µg/ml)	8-12
Slope	119.71
Intercept	13.199
Correlation Coefficient (r2)	0.9997
LOD (µg/ml)	0.310
LOQ (µg/ml)	0.572

Figure 4 Calibration Curve of ETOR of RP-HPLC Method



3.2.4 Assay of Marketed Formulation: Amount of ETOR present in the marketed formulation (Arcoxia-60mg, Sun Pharmaceutical Ltd.) was found and the results are presented in Table 4.

Table 4 Assay Results of Arcoxia RP-HPLC Method

Amount present [µg/ml]	Amount found [µg/ml]	[%] of Assay
10	9.94	99.4
10	9.97	99.7
10	9.90	99.0

3.2.5 Validation Parameters

The developed method was validated in accordance to ICH guidelines. Percentage of recoveries of ETOR was found in the range from 99.53 – 99.72%. Precision of the method was determined by % RSD found among intra-day precision, inter-day precision, repeatability. LOD and LOQ of ETOR were found to be 0.310 and 0.572µg/ml, respectively. For robustness study, the effect of change in of mobile phase, mobile phase ratio and flow rate (1.0 ± 0.2 ml/min) on the Mean peak area, % RSD and % Assay were studied. Standard solutions of ETOR (10 µg/ml), was prepared and analyzed at different mobile phase ratio Water: ACN:OPA:TEA (10 :90 :0.1 :0.1, 20: 80: 0.1: 0.1, 30: 70: 0.1: 0.1, 40: 60: 0.1: 0.1, 50: 50: 0.1: 0.1, v/v) and at different flow rate (0.99, 1.00, 1.1 ml/min). Percentage RSD of each peak in all variables was found to be less than 3 % was mentioned in the Table 5, 6, 7, 8, 9, 10.

Table 5 Results of Accuracy for RP-HPLC Method

Initial Amount [µg/ml]	Amount added [µg/ml]	Amt recovered ± S.D [µg/ml, n=3]	% Recovery	% RSD
10	8	17.97 ± 0.06	99.72	0.11
10	10	19.89 ± 0.05	99.53	0.10
10	12	21.87 ± 0.05	99.57	0.18

Table 6 Results of intra-day precision and inter-day precision

Conc. [µg/ml]	Intra-day Precision		Inter-day Precision	
	Mean	% RSD [n= 3]	Mean	% RSD [n= 3]
10	1213.383	0.08	1213.503	0.08
11	1328.304	0.07	1328.937	0.11
12	1448.437	0.08	1449.053	0.04

Table 7 Results of Repeatability Study for ETOR

Mean	1213.558
S.D	0.905
% RSD	0.075

Table 8 Result of Robustness Study: Variation in Mobile phase Ratio

Percentage Methanol in Mobile phase (v/v)	RT	T
81	2.76	1.47
80	2.7	1.40
79	2.65	1.45
Mean ± SD	2.70±0.0550	1.44 ± 0.04

Table 9 Results of Ruggedness

Analysts [n=3]	Amount found of ETOR [%]	% RSD
I	99.51	0.20
II	99.27	0.23

Table 10 Results of System Suitability Parameters

Analyte	Retention time (min)	Tailing Factor	Theoretical plates (N)	Capacity Factor
ETOR	2.7	1.40	4122	0.92

4. Discussion

From the results obtained, chromatographic separation was achieved using a Phenomenex Gemini C18 column (250 mm x 4.6.0 mm, 5 µ) as a stationary phase was finalized; water, ACN, OPA, TEA in the ratio of 40:60:0.1:0.1 (v/v/v/v) as a mobile phase and retention time 2.7 min. This mobile phase is simple to prepare and economical. From the optical characteristics of the proposed method it was found that the drug obeys linearity range within the concentration of 8-12µg/ml. The results of precision study showed that the %RSD was less than 2%, which indicated that the method has good reproducibility. From the results shown in accuracy study, it was found that the % recovery values of pure drug from the preanalysed solutions of formulations were in between 99.53-99.72%, which indicated that the method is accurate. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients with the analysis of drug in the proposed method. The system suitability parameters were within the specified limits. Hence the developed method was found to be simple, precise, accurate, robust and rapid for determination of etoricoxib from pure drug and its dosage forms.

5. Conclusion

RP-HPLC was found to more precise, accurate, rugged and robust for determination of Etoricoxib. The excipients usually present in the pharmaceutical formulation did not interfere with determination of Etoricoxib. Developed method can be successfully used in laboratory to measure the concentration of API in specific dosage form .This method is very beneficial in formulation and development department. This method is useful for identifying of analyte, purity testing, and assay. This is new concept for the validation of method development and method transfer in pharmaceutical companies. These methods are very easy and less time consuming compare to other hectic methods and in these methods very less chemicals are used and also a less cost methods.

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