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DEVELOPMENT AND VALIDATION OF A RP - HPLC METHOD FOR DETERMINATION OF ETORICOXIB IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

To develop a simple, cheap, accurate, precise, linear and rapid Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method and validate as per ICH & USP guidelines for the quantitative estimation of Etoricoxib in pharmaceutical dosage forms. The separation was conducted by using mobile phase consisting of methanol: ammonium acetate buffer:acetonitrile in the ratio (70:20:10). The wavelength was found at 235nm. Chromatographic determination was performed on Agilent 1220 Infinity LC with ezchrome software with variable wavelength detector. The separation was conducted by using Zebra Eclipse XDB-C-18 (4.6×150×5µm) at the flow rate of 1.0 ml/min using variable wavelength detector. The developed method resulted in etoricoxib eluting at 2.083 min. The method was found to be linear over the concentration range 2-12µg/ml with coefficient regression R^2-0.9988. The precision is exemplified by relative standard deviation of 1.15 to 1.8 %. Percentage Mean recovery was found to be in the range of 97to99%, during accuracy studies. The limit of detection (LOD) and limit of quantitiation (LOQ) was found to be 5 ng/ml and 15 ng/ml respectively. A cheap, accurate, precise, linear and rapid RP-HPLC method was developed and validated for the quantitative estimation of etoricoxib tablets as per ICH guidelines and hence it can be used for the routine analysis in various pharmaceutical industries.

KEY WORDS: RP-HPLC, Etoricoxib, Method Validation

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INTRODUCTION

Nonsteroidal anti-inflammatory (NSAIDs) drugs are widely used for the treatment of pain, inflammation and fever. Their mechanism of action involves the inhibition of COX1, a hemeprotein that exists in two forms (COX-1 and COX-2) and converts arachidonic acid to proinflammatory prostaglandins and their subsequent metabolic products. They are among the most widely used medications in the world and are often taken long term by patients with osteoarthritis and rheumatoid arthritis. A major factor limiting the use of NSAIDs is concern for the development of gastrointestinal complications such as bleeding. Cyclo-oxygenase-2 (COX-2) selective inhibitors were developed to decrease the risk of gastrointestinal tract injury and avoid the anti-platelet effect of traditional NSAIDs, and large outcome trials have shown a decrease in upper gastrointestinal complications with COX-2 selective inhibitors as compared with traditional NSAIDs. Etoricoxib (Fig. 1) is chemically, 5-chloro-3-(4- methanesulfonylphenyl)-2-(6-methylpyridin- 3-yl) pyridine. It is a new COX-2 selective inhibitor and used in the treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, chronic low back pain, acute pain and gout. Etoricoxib is chemically, 5-chloro-2-(6-methylpyridin-3-yl)-3-(4-methylsulfonylphenyl) pyridine and it is used as a muscle relaxant with anti inflammatory and analgesic effects[1-3]. Analytical method development and validation plays an important role in discovery, development and manufacture of pharmaceuticals. Validation is the process of providing documented evidence that the method does what it is intended to do. In other word the process of method validation ensures that the proposed analytical methodology is accurate, specific, reproducible and rugged for its intended use. Analytical techniques have different degrees of sophistication, sensitivity and selectivity, as well as, different cost and time requirements. First stage in the selection or development of method is to establish what is to be measured and how accurately it should measure.Effective method development ensures that laboratory resources are optimized, while methods meet the objectives required at each stage of drug development. Method validation, required by regulatory agencies at certain stages of the drug approval process, is defined as the “process of demonstrating that analytical procedures are suitable for their intended use[4-6].

MATERIALS AND METHODS

Chemicals and Reagents

Water, Methanol, Acetonitrile of Analytical and HPLC grade purchased from HiMedia (Mumbai). Ammonium acetate buffer of AR grade purchased from S.D. Fine Chemicals (Hyderabad). 0.45µm Nylon membrane filters were obtained from Spincotech Private Limited, Hyderabad, India.

Instrument

HPLC analysis was performed on Agilent 1220 Infinity LC with *EZ*chrome software with variable wavelength detector. With made of Agilent technologies, A manually operating Rheodyne injector with 20 µL sample loop was equipped with the HPLC system., Zobrax Eclipse XDB-C18 column (4.6×150×5µm), Electronic weighing balance BL- 220 H made of Shimatzu Corporation, Hot air oven made of Nisco Company, Sonicator made of the Ultrasonic’s PCi Analytics sonicator.

METHODS

Selection of Wavelength

Suitable wavelength for the HPLC analysis was determined by recording UV spectrum in the range of 200-400 nm for etoricoxib. Suitable wavelength selected was 235 nm (Figure 2).

Figure 1
Chemical structure of Etoricoxib

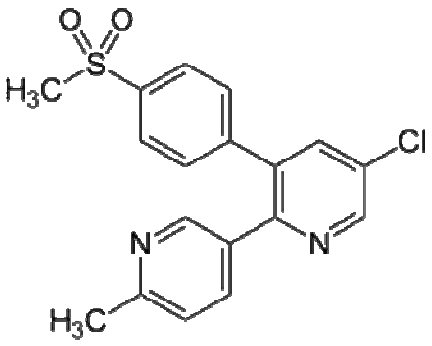


Figure 2
UV spectrum of Etoricoxib

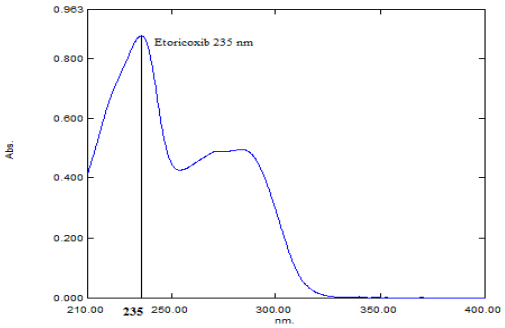


Figure 3
Typical Chromatogram of Blank solution

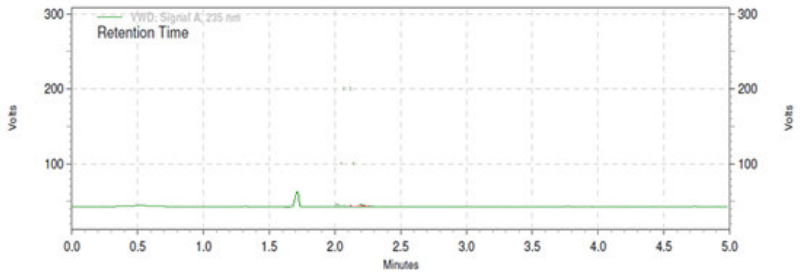


Figure 4
Typical chromatogram of the standard solution

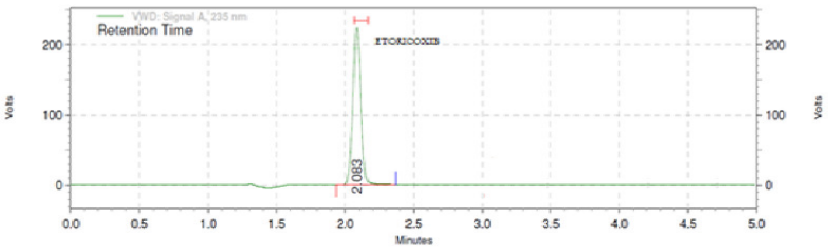


Figure5
Typical chromatogram for the tablet formulation

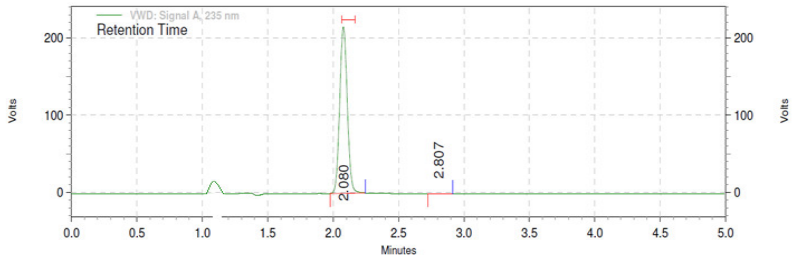
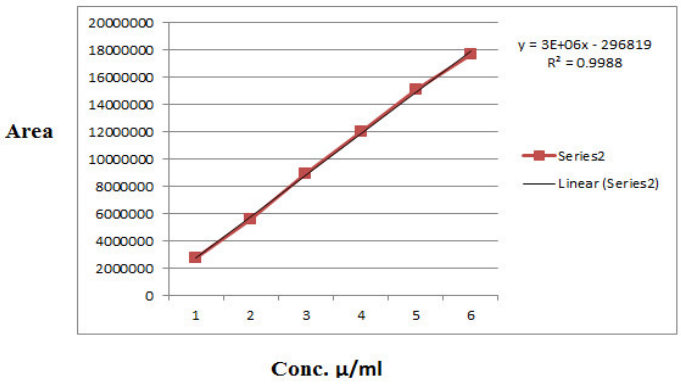


Figure 5
Calibration Curve



Chromatographic conditions
The developed method uses a reverse phase C18 column, Zobrax Eclipse XDB-C18 column (4.6×150×5µm), mobile phase consisting of a mixture of methanol: ammonium acetate buffer:acetonitrile in the ratio (70:20:10). The mobile phase was set at a flow rate of 1 ml/min and the volume injected was 20 µl for every injection. The detection wavelength was set at 235 nm.

Preparation of Ammonium acetate buffer
2.7gm of ammonium acetate was weighed and dissolved in 1000ml volumetric flask and volume was made up to 1000ml with water.

Preparation Mobile Phase
A mixture of 70 volumes of Methanol, 20 volumes of Ammonium acetate buffer and 20 volumes of Acetonitrile was prepared (70:20:10). The mobile phase was sonicated for 10min to remove gases.

Preparation of standard stock solution
Weigh accurately 10 mg of Etoricoxib drug in 100 ml volumetric flask and dissolve in 100 ml methanol.

Preparation of sample solution
20 Tablets (each tablet contains 10 mg of Etoricoxib) were weighed and taken into a mortar uniformly mixed. Test stock solutions of Etoricoxib (10µg/ml) and was prepared by dissolving weight equivalent to 10 mg of Etoricoxib and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 10 µg/ml of Etoricoxib was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Calibration Curve
Appropriate aliquots of standard stock solution (1000 µg/ml) was diluted to 100 mg/ml in 10ml volumetric flask and resultant solution was diluted up to the mark with mobile phase to obtain a final concentration of 2 4, 6, 8, 10, and 12 µg/ml. These solutions were injected into chromatographic system and chromatograms were obtained and peak area ratio was determined for each concentration of drug solution. Calibration curve of Etoricoxib were constructed by plotting peak area ratio vs applied concentration of Etoricoxib and regression equation was computed. The sample solution was chromatographed and concentration of montelukast sodium in tablet samples was calculated using regression equation

RESULTS AND DISCUSSION

METHOD DEVELOPMENT
A Reverse phase HPLC method was developed keeping in mind the system suitability parameters i.e. tailing factor (T), the number of theoretical plates (N), run time and the cost effectiveness. The optimized method developed resulted in the elution of etoricoxib at 2.08 min. Figures 3 & 4 represent chromatograms of blank and standard solution (10µg/ml) respectively. The total run time is 5 minutes. System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (Rt), number of theoretical plates (*N*) and peak Tailing factor (T) were evaluated for six replicate injections of the standard at working concentration. The results are given in Table 1.

Table 1
System suitability studies results.

Parameters*	Etoricoxib
Retention time (min)	2.08
Number Of Theoretical plates (N)	6245
Tailing factor (T)	1.8
% RSD	1.35

**** Mean of six injections***

Table 2
Calibration data for Etoricoxib.

Sr. No	Concentration(µg/ml)	Area
1	2	2774649
2	4	5564328
3	6	8897861
4	8	11997659
5	10	15139340
6	12	17694099
SD		5691845
Slope	3E+06X	

Table 3
Results of Accuracy studies for Etoricoxib

Recovery levels	Accuracy Etoricoxib					Average % Recovery
	Amount taken (mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	% Recovery	
80	2	26953657				
	2	26705613			97%	
	2	26557801	26739023	1.94		
100	6	29675784				
	6	29769547			99%	
	6	29593579	29679637	5.94		98
120	10	32753478				
	10	32578473	32739139	9.8	98%	
	10	32885467				

Table 4
System precision results

Etoricoxib		
Injection No.	Rt	Area
1	2.084	15253952
2	2.083	15378236
3	2.082	15493825
4	2.084	15638931
5	2.086	15849584
6	2.084	15428495
Mean	2.083	15507171
SD	0.001	210579.9
%RSD	0.063	1.35

Table 5
Intra day precision results

Injection No.	% Assay of Etoricoxib
1	97.58
2	98.36
3	97.23
4	100.12
5	98.23
6	97.01
Mean	**98.09**
SD	**1.130**
%RSD	**1.152**

Table 6
Inter day precision results

% Assay of Etoricoxib			
Injection No.	**Day 1**	**Day 2**	**Day 3**
1	98.65	96.96	100.25
2	97.28	97.28	96.33
3	98.54	100.97	98.69
4	100.23	97.14	98.33
5	99.23	99.87	99.95
6	100.98	96.45	96.01
Mean	**99.15**	**98.11**	**98.26**
SD	**1.315**	**1.483**	**1.777**
%RSD	**1.326**	**1.879**	**1.809**

Table 7
Robustness results of Etoricoxib

Parameter	Etoricoxib	
	Retention time (min)	**Tailing factor**
Flow		
0.8ml/min	2.214	1.384
1.2ml/min	1.853	1.206
Wavelength		
233nm	2.045	1.339
237nm	2.104	1.104

METHOD VALIDATION[6-7]

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. RP-HPLC method developed was validated according to International Conference on Harmonization (ICH) and USP guidelines for validation of analytical procedures. The method was validated for the parameters like system suitability, specificity, linearity, accuracy, precision, ruggedness, limit of detection (LOD) and limit of quantitiation (LOQ).

System suitability parameters

The system suitability parameters were determined by preparing standard solutions of Etoricoxib and the solutions were injected six times and the parameters like % RSD, peak tailing, resolution and USP plate count were determined. The results are mentioned in Table 1. The standard chromatogram is shown in Fig. 4.

Specificity

Figures 3-5 for blank, standard drug solution and sample chromatogram reveal that the peaks obtained in the standard solution and sample solution at working concentrations are only because of the drugs as blank has no peak at the retention time of etoricoxib. Accordingly it can be concluded that, the method developed is said to be specific.

Linearity

Linearity the method was tested from 80-120 % of the targeted level of the assay concentration for analyte. Standard solutions contained 2-12 µg/mL of etoricoxib. Linearity solutions were injected in triplicate. The calibration graphs were obtained by plotting peak area against the concentration of the drugs. The equations of the calibration curves for Montelukast sodium obtained were y = 02E+06X in the etoricoxib determination, the calibration graphs were found to be linear in the aforementioned concentrations with correlation coefficients 0.9988. The results are mentioned in Table 2 & calibration curve Fig 5.

Accuracy
Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample at three different levels (80-120%). 20 blank tablets were powdered and mixed. This powder was then spiked with a quantity of etoricoxib corresponding to 80%, 100% and 120% of the labeled claim. Each of these powder mixtures was analyzed in triplicate and the quantity of Etoricoxib was determined using calibration equation. Accuracy was reported as 98 % of etoricoxib recovered. The results are mentioned in Table 3.

Precision
System precision
Six replicate injections of the standard solution at working concentration showed % RSD (Relative Standard Deviation) less than 2 concerning the peak area for the drug, which indicates the acceptable reproducibility and thereby the precision of the system. System precision results are tabulated in Table 4.

Method precision
Method precision was determined by performing assay of sample under the tests of (i) repeatability (Intraday precision) and (ii) Intermediate precision/ Ruggedness/ Inter day precision) performed during 3 consecutive days by three different analysts, at working concentration.

Repeatability (Intraday precision)
Six consecutive injections of the sample from the same homogeneous mixture at working concentration showed % RSD lessthan 2 concerning % assay for the drug which indicate that the method developed is method precise by the test of repeatability and hence can be understood that the method gives consistently reproducible results (Table 5).

Intermediate Precision (Ruggedness / Inter day precision)
Six consecutive injections of the sample solution from the same homogeneous mixture at working concentration on three consecutive days by three different analysts, showed % RSD less than 2 for % assay for the drug within and between days, which indicate the method developed is inter day precise / rugged (Table 6).

Robustness
The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variation in the analytical procedure parameters. To evaluate HPLC method robustness a few parameters were deliberately varied. The parameters included variation of columns C8 (old & new), percentage of acetonitrile in the mobile phase and acetonitrile of different lots. Change in wavelength ± 2nm Change in flow rate ±0.2 ml/min. The results are mentioned in Table 7.

Limit of Detection (LOD)
The limit of detection (LOD) of an analytical method may be defined as the concentration, which gives rise to an instrument signal that is significantly different from the blank. For spectroscopic techniques or other methods that rely upon a calibration curve for quantitative measurements, the IUPAC approach employs the standard deviation of the intercept (Sa), which may be related to LOD and the slope of the calibration curve, b. The limit of detection was found to be 5ng/ml

Limit of Quantification (LOQ)
The LOQ is the concentration that can be quantitate reliably with a specified level of accuracy and precision. The LOQ represent the concentration of analyte that would yield a signal-to-noise ratio of 10.The limit of quantification was found to be 15 ng/ml

CONCLUSION

A reverse phase HPLC isocratic method developed has been validated as per ICH & USP guidelines in terms of specificity, accuracy, precision, linearity, ruggedness, limit of detection and limit of quantitation, for the quantitative estimation of etoricoxib in pharmaceutical dosage form. A good linear relationship was observed for the drug between concentration ranges of 2-12µg/ml. The inter day and intraday precision results were good enough to say that the method developed is precise and reproducible. Accuracy studies revealed that mean recoveries were between 97 and 99%, an indicative of accurate method. Accordingly it can be concluded that the developed reverse phase isocratic HPLC method is accurate, precise, linear and rugged and therefore the method can be used for the routine analysis of etoricoxib in pharmaceutical dosage form.

CONFLICT OF INTEREST
I certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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