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A RP-HPLC Method For The Estimation Of Etoricoxib In Pharmaceutical Formulation

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ABSTRACT

A simple, selective, rapid, precise and economical reverse phase HPLC method has been developed for the estimation of etoricoxib from pharmaceutical formulation. The method was carried out on a Hichrom C_{18} (25 cm × 4.6 mm i.d., 5µ) column with a mobile phase consisting of acetonitrile: 0.5% triethylamine buffer (adjusted to pH 3.5 using orthophosphoric acid) (60:40 v/v) at a flow rate of 1.0 ml/min. Detection was carried out at 245 nm. Cetrizine hydrochloride was used as an internal standard. The retention time of etoricoxib and cetrizine hydrochloride was 11.6 and 7.0 min, respectively. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation and solution stability. The proposed method can be used for the estimation of these drugs in combined pharmaceutical formulation.

KEYWORDS

RP-HPLC;
Etoricoxib;
Cetrizine hydrochloride.

INTRODUCTION

Etoricoxib is chemically {5-chloro-3-(4-methanesulfonyl-phenyl)-69-methyl-[2,3]-bipyridinyl} used for the treatment of osteoarthritis. Detailed survey of literature for etoricoxib revealed only limited methods are available by LCMS[1] and HPLC for its determination in human plasma. The present RP-HPLC method was validated following the ICH guidelines[3-10]. The method was used to quantify the drug in tablet formulation.

Reagents and instrument

Acetonitrile HPLC grade was procured from E Merck (India) Ltd, Mumbai. Triethylamine and orthophosphoric acid AR grade were procured from Qualigens fine chemicals, Mumbai. Water HPLC grade was obtained from a Milli-Q R water purification system. Reference standard of etoricoxib was procured from Cadila Pharmaceuticals Ltd,

Ahmedabad and cetrizine hydrochloride was procured from Aristo Pharmaceuticals, Mumbai.

Chromatographic separation was performed on a Shimadzu® liquid chromatographic system equipped with a LC-10AT-vp solvent delivery system (pump), SPD M-10AVP photo diode array detector, Rheodyne 7725i injector with 50 µl loop volume. Class-VP 6.01 data station was applied for data collecting and processing (Shimadzu, Japan). A Hichrom C_{18} column (25cm × 4.6mm i.d., 5µ) was used for the separation.

Mobile phase and preparation of standard solution

The mobile phase prepared is a mixture of acetonitrile and 0.5% triethylamine buffer (pH 3.5 adjusted with orthophosphoric acid) (60:40 v/v). It was filtered through a 0.2 µ membrane filter and degassed. Standard stock solutions of 1 mg/ml of etoricoxib and cetrizine hydrochloride were prepared separately using a mixture of water and acetonitrile in the ratio 1:1 v/v. From the standard stock solution, mixed standard solution was prepared to contain 50 µg/ml of etoricoxib and cetrizine hydrochloride (100 µg/ml) of internal standard. The mobile phase was delivered at a flow rate of 1.0 ml/min with detection at 245 nm. The injection volume was 50 µl and analysis was performed at ambient temperature.

Assay

Twenty tablets, each containing 90 mg of etoricoxib (ETROBAX-Ranbaxy pharmaceuticals, Mumbai) were weighed and finely powdered; a quantity of powder equivalent to 50 mg of etoricoxib was weighed and transferred to a sintered glass crucible. To this 10 ml of 1 mg/ml solution of cetrizine hydrochloride was added and the drugs were extracted with three quantities, each of 20 ml of mixture of acetonitrile and water (1:1 v/v). The combined extracts were made up to 100 ml with mobile phase and further dilutions were made to get a concentration of 50 µg/ml of etoricoxib, (theoretical value) and 100 µg/ml of cetrizine hydrochloride as internal standard and this solution was used for the estimation.

With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solution was injected and the chromatogram was recorded. The retention time of etoricoxib and cetrizine hydrochloride was found to be 11.6 and 7.0 min, respectively. This procedure was repeated for the sample solution obtained from the formulation. The response factor (peak area ratio of standard peak area and internal standard peak area) of the standard solution and sample solution were calculated. The concentration of the drugs were calculated (TABLE 1) using following formula,

TABLE 1: Results of analysis of formulation and recovery studies

Drug	Amount mg/ tab		% Label claim**	% Recovery**
	Labelled	Found*		
Etoricoxib	90	89.56	99.51±1.56	99.85

*Average of six determinations, mean ± S.D
**ETROBAX (Ranbaxy pharmaceuticals) tablet

$$\text{Concentration of drugs} = \frac{\text{Response factor of the sample}}{\text{Response factor of the standard}} \times \text{Concentration of standard}$$

The typical chromatogram of sample solution is given in figure 1. Detection was done at 245 nm. The peak area ratio of standard and sample solutions was calculated. The assay procedure was repeated for six times and mean peak area ratio and mean weight of standard drugs were calculated. The percentage of individual drugs found in formulation, mean, standard deviation in formulation were calculated and presented in TABLE 1. The results of analysis show that the amount of drugs was in good agreement with the label claim of the formulation.

The method was validated as per ICH guidelines. The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in TABLE 1. From the data obtained, added recoveries of standard drugs were found to be accurate.

Validation

The precision of the method was demonstrated by inter day and intra day variation studies. In the intra day studies, six repeated injections of standard

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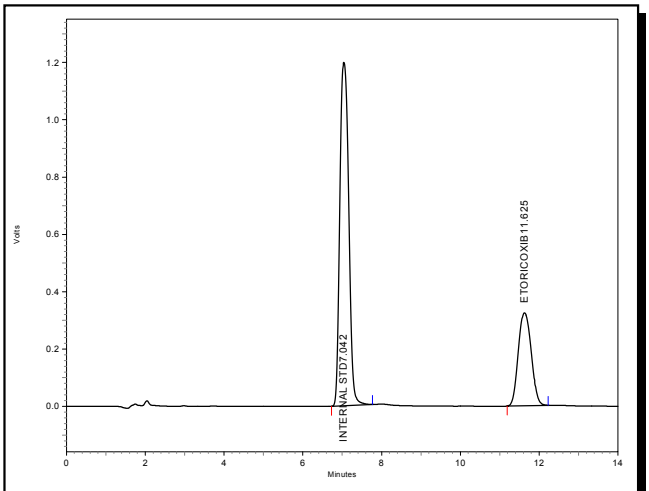

Figure 1: Typical chromatogram of sample solution

and sample solutions were made and the response factor of drug peaks and percentage relative standard deviation (RSD) were calculated**.** In the inter day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks and percentage RSD were calculated. From the data obtained, the developed HPLC method was found to be precise.

The linearity of the method was determined at seven concentration levels ranging from 25 to 125 µg/ml for etoricoxib**.** The calibration curve was constructed by plotting response factor against concentration of drugs (Figure 2). The slope and intercept value for calibration curve was y = 0.0046 × -0.0012 (R^2=0.9994) for etoricoxib. The results show that an excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above.

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for etoricoxib was found to be 5 ng/ml respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ was found to be 15 ng/ml for etoricoxib (TABLE 2).

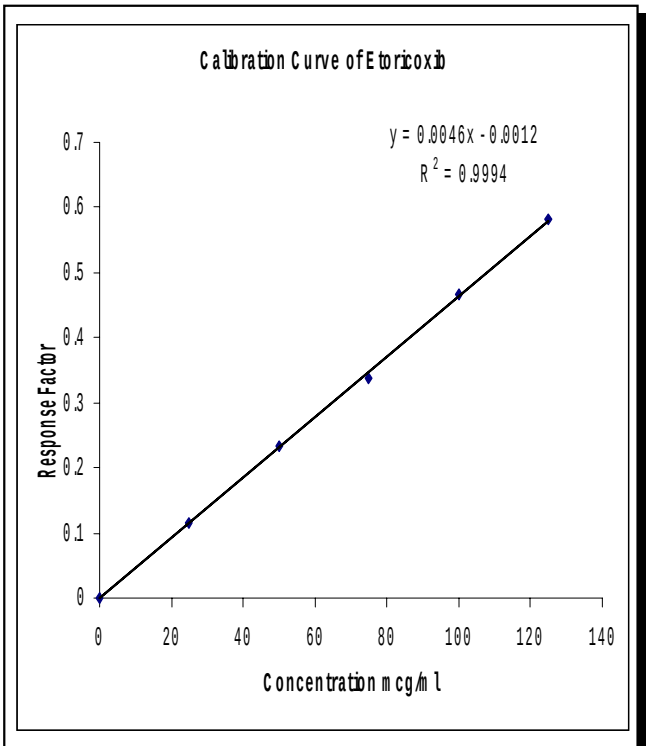

Figure 2: Calibration curve of etoricoxib

TABLE 2: Validation and system suitability studies

S. No.	Parameters	Etoricoxib
1	Linearity range	25 - 125 mcg
2	Theoretical plate/meter	4159
3	Resolution factor	1.3
4	Asymmetric factor	0.56
5	Tailing Factor	0.25
6	LOD (ng/ml)	5
7	LOQ (ng/ml)	15

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC-10AT), Agilent HPLC and Water's Breeze HPLC by different operators using different columns of similar type like Hypersil C_{18}, Phenomenex LUNA C_{18} and Hichrom C_{18}. Robustness of the method was determined by making slight changes in the chromatographic conditions. No marked changes in the chromatograms demonstrated that the HPLC method developed are rugged and robust.

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 5 h at room temperature. The results show that for both solu-

tions, the retention time and peak area of etoricoxib remained almost unchanged (% RSD less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 5 h, which was sufficient to complete the whole analytical process.

The system suitability studies were carried out to determine theoretical plate/meter, resolution factor, asymmetric factor and tailing factor. The results were given in the TABLE 2**.** The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 % standard deviation range during routine performance of the method.

Thus the proposed RP-HPLC method for the estimation of etoricoxib in dosage forms is accurate, precise, linear, rugged, robust, simple and rapid. Hence the present RP-HPLC method is suitable for the quality control of the raw materials, formulations and dissolution studies.

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