RESEARCH ARTICLE

Validated RP-HPLC method for estimation of Etoricoxib & Paracetamol and its applications in analysis of formulations

Prakash Sahu and Shweta Singh *

Shri Satya Sai University of Technology and Medical Science, Bhopal, Madhya Pradesh, India.

Received March 2017; Accepted April 2017

* Corresponding Author

Email: singh.shweta2000@yahoo.com

ABSTRACT

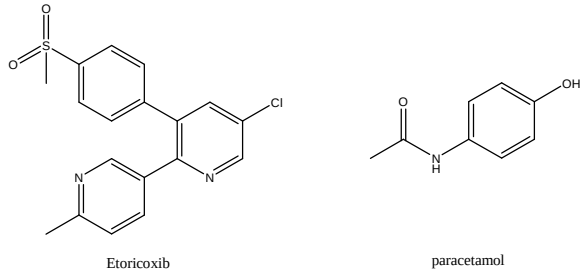
The current paper describes a reversed phase high performance liquid chromatographic method for the simultaneous estimation of Etoricoxib and Paracetamol in formulations. The separation was achieved on the LUNA C_{18} column 5µ (250 x 4.6 mm id), using methanol - water in the ratio 63 - 37 as the mobile phase at 1 ml/min flow rate and 237 nm as detection wavelength. The retention time of Etoricoxib and Paracetamol were 1.6 and 2.9 min respectively. The method was validated in terms of linearity, accuracy, precision, as per ICH Guidelines. The calibration curve was linear in the concentration range from 10-60 µg/ml for etoricoxib and 100-600 µg/ml for paracetamol. Percentage recovery obtained for etoricoxib and paracetamol were 99.60 % and 99.04 % respectively.

Keywords: ICH, HPLC, Validation, Etoricoxib, Paracetamol, Calibration

Introduction

Non-steroidal antiinflammatory drugs (NSAIDS) are widely used for the treatment of pain, inflammation and fever. Etoricoxib (ETO) and Paracetamol (PCM), both are anti-inflammatory, analgesic-antipyretic drugs (Patrignani et al., 2003). Etoricoxib is chemically as 5 chloro-6'-methyl-3-[-4-(methylsulfonyl) phenyl]-2,3'-bipyridine. ETO belongs to the coxib class of NSAIDs. It is highly selective inhibitor of the enzyme cyclooxygenase-2 (COX-2). ETO is used for the treatment of osteoarthritis, rheumatoid, gouty arthritis and chronic low back pain, acute pain, and ankylosing spondylitis (Dallob, 2003).

PCM is chemically as N-acetyl-*para*-aminophenol (Fig.1). It is one of the most commonly used over-the-counter analgesics for headache, musculoskeletal pain, and fever.



Etoricoxib paracetamol

Fig. 1 Structure of Etoricoxib and Paracetamol

A few analytical methods have been reported for the estimation of etoricoxib (Hartman et al., 2003; Brautigam et al., 2003; Werner et al., 2004; Venkata et al., 2005; Mandal et al., 2006; Liberato et al., 2006; Maheshwar et al., 2007; Patel et al., 2007)and paracetamol (Sethi, 2007) the simultaneous estimation of both these drugs in combination dosage form by UV & HPLC (Pattan et al., 2006) but these methods are costly for routine analysis due to the used of expensive chemicals. The focus of present study is to develop and validate a simple, rapid, stable and economic RP-HPLC method for the simultaneous determination of ETO and PCM in their combined tablet dosage form.

Materials and Methods

Chemicals and reagents

All the experiments were performed with pharmaceutical-grade ETO and PCM. HPLC-grade solvents were employed for analysis. Solvents were filtered through 0.45 μm membrane filters. All dilutions were performed in standard volumetric flasks. The pharmaceutical preparations, declaring to contain 60 mg ETO, 500 mg PCM and excipients, were obtained from a local drugstore.

Instrumentation and chromatographic conditions

The separations were performed with a Shimadzu R 1100 series liquid chromatography quaternary gradient, an injector fitted with a 20 μL loop. Compounds were separated on a 250 mm x 4.6 mm C8 column (Luna, Phenomenex, 5 μm particles). The mobile phase constituted of methanol:water (63:37) pumped at a flow rate of 1.0 mL/min. The chromatograms were recorded

employing lab solutions software. The detection wavelength was set at 237 nm.

Mix Standard solution

Mix stock solution containing ETO and PCM containing 60 μg/mL ETO and 500 μg/mL PCM was prepared in methanol. Dilutions were appropriately prepared from the standard solution with mobile phase to obtain concentration of 30 μg/mL ETO and 250 μg/mL PCM.

Test procedure

The mobile phase was allowed to equilibrate with the stationary phase as indicated by steady base line in the chromatogram. The optimized chromatographic conditions were kept constant throughout the experimentation.

A 20 μL solution of above mix standard was injected on to the system. Both ETO and PCM separated properly as indicated by sharp peaks with reasonable retention times. A chromatogram of both the drugs resolved by mobile phase is shown in Fig. 2.

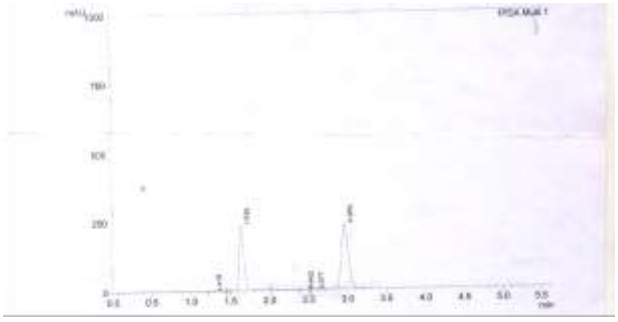


Fig.2 Chromatogram of working standard

System suitability

Seven replicate injections of 20 μL solution of the mix standard were injected on to the injector and the chromatograms were recorded and the system suitability parameters were calculated.

Linearity Range

The mixed standard stock solution was diluted appropriately with mobile phase to obtain different dilutions in range of 10 μg/mL to 100 μg/mL for ETO and 100 μg/mL to 1000 μg/mL for PCM respectively. The linearity curves were obtained by plotting concentration of the drug against the peak area.

Assay of marketed formulation

Twenty tablets were weighed and crushed into fine powder. The quantity of powder equivalent to 60 mg of ETO and 500 mg of PCM was taken in 10 ml volumetric flask, dispersed in methanol and volume was made up to 10 ml. The solution was sonicated for 10 min and filtered through 0.45 μ membrane filter. Accurately measured 0.1 ml aliquot of this solution was diluted up to 10 mL with methanol and filtered through 0.45 μ membrane filter before use.

Results and Discussions

The system suitability studies revealed that the separation of ETO and PCM was achieved properly using a mobile phase comprising of 63:37 ratio of methanol and water. The retention time recorded for ETO was 1.63 min whereas for PCM it was 2.96 min. The chromatogram

obtained exhibited almost Gaussian peaks with asymmetry factor of 1.06 (Table 1).

Table 1 System suitability parameters

Parameter	Peak of ETO	Peak of PCM
Mean peak area	345579	3455793
% RSD	0.78331	0.53388
Retention time	1.639 min	2.965 min
Assymetry	1.062	1.069

The linearity studies indicated that the proposed method was linear up to a range of 10-60 µg/mL for ETO and 100-600 µg/mL for PCM (Fig 3 & 4).

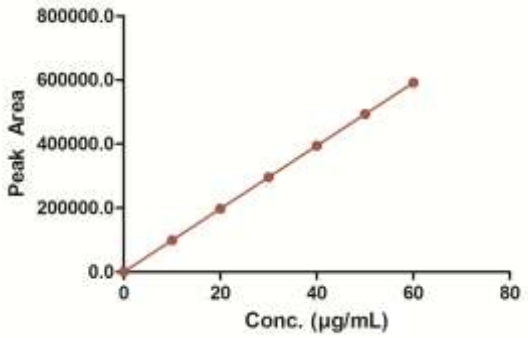


Fig.3 Calibration curve of etoricoxib

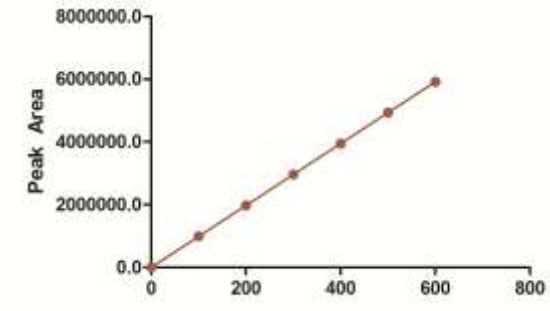


Fig.3 Calibration curve of paracetamol

Validation of method

Validation of the method was carried out in accordance with the ICH guidelines for development and validation of new analytical method (Willard et al, 2001; Kalsi, 2001).

Accuracy

To determine the accuracy of the method, recovery studies were carried out by adding a known quantity of pure drug to a preanalyzed sample and the contents were reanalyzed by the proposed method and the mean percent recovery was calculated. The method was found to be accurate with a % RSD of 0.4892 and 0.6643 for recovery of ETO and PCM respectively (Table 2).

Table 2 Results of recovery studies

S. No.	Wt. taken (mg)	Amount of pure drug added (mg)		Amount recovered (mg)		% Recovery	
		ETO	PCM	ETO	PCM	ETO	PCM
1	150	8.0	110	8.0	108	100	98.18
2	150	20	199	19.8	197	99	98.99
3	150	35	275	34.8	273	99.42	99.27
4	150	56	410	56	409	100	99.75
Mean						99.605	99.04
±SD						0.4873	0.6579
% RSD						0.4892	0.6643

Precision

Precision was studied to find out intra and inter day variations in the proposed method of ETO and PCM. The % RSD was calculated (Table 3).

Table 3 Results of precision studies

Parameter	INTERDAY		INTRADAY	
	ETO	PCM	ETO	PCM
Mean	98.87	98.74	98.91	98.79
SD	0.287	0.291	0.279	0.288

Conclusion

An analytical RP liquid chromatographic method was developed and validated for the estimation of etoricoxib and paracetamol in bulk and tablet dosage form. The developed method was found to be accurate, precise, robust, economic and rapid for its intended use. Hence this method can be used for routine analysis of these two drugs in combination.

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