Validated RP- HPLC Method for the Assay of Etoricoxib (A Non-Steroidal Anti-Inflammatory Drug) in Pharmaceutical Dosage Forms

SRINIVASU TOPALLI^{*1}, T. G. CHANDRASEKHAR¹ AND M. MATHRUSRI
ANNAPURNA²

¹Ranbaxy Research Laboratories Ltd. Gurgaon, Haryana, India.

²Department of Pharmaceutical Analysis and Quality Assurance, GITAM Institute of
Pharmacy, GITAM University, Visakhapatnam, India

**Corresponding author. E-mail: srinivasu.topalli@gmail.com
srinivasut@yahoo.com*

Abstract: A simple, accurate, sensitive and reproducible reverse phase high performance liquid chromatographic method has been developed for the quantitative determination of Etoricoxib in pharmaceutical dosage forms. The assay was performed on Hypersil ODS C-18 (250 x 4.6 mm., 5µm particle size) column using acetonitrile and potassium dihydrogen phosphate buffer (pH 4.2) (46:54 % v/v) as mobile phase with UV detection at 280 nm (flow rate 1.2 ml/min). Bromhexine was used as an internal standard. Quantization was achieved by measurement of the peak area ratio of the drug to the internal standard. The limit of detection (LOD) and the limit of quantification (LOQ) were 0.0704 µg ml⁻¹ and 0.2134 µg ml⁻¹ respectively. Each analysis required no longer than 10 minutes. The calibration curve was linear over the concentration range from 0.5-85.0 µg ml⁻¹. The retention times of Etoricoxib and Bromhexine were found to be 3.083 and 7.631 minutes respectively. The proposed method was validated according to the ICH guidelines and can be used successfully to analyse marketed formulations.

Key words: Etoricoxib, Bromohexine, Acetonitrile, RP-HPLC, ICH.

Introduction

Etoricoxib¹ (ETC) (Figure 1) is a new non-steroidal anti-inflammatory drug (NSAID) which is chemically 5-chloro-6'-methyl-3-[4-(methylsulphonyl) phenyl]-2,3'-bipyridine. It inhibits the synthesis of prostaglandins by inhibiting the activity²⁻³ of the enzyme, cyclooxygenase-2. It has highest COX-2 selectivity and better safety profile. It is active at low dose and has less gastric toxicity⁴.

Figure 1. Chemical Structure of Etoricoxib (ETC).

Etoricoxib is preferred over conventional NSAIDs as they may lead to serious gastrointestinal complications such as ulcer, severe bleeding and perforation resulting in hospitalization and even death⁵. It is mainly used for the osteoarthritis, rheumatoid arthritis and acute gouty arthritis⁶⁻⁸. ETC administered as a tablet is rapidly and completely absorbed and available; the absolute bioavailability is estimated to be 100% and is metabolized extensively via oxidation 6'-methyl hydroxylation and 1'-N-oxidation and the metabolites are excreted largely in the urine⁹. A high fat meal decreased the rate of absorption without effecting the extent of absorption of ETC; therefore it can be dosed irrespective of food. The dose proportionality of oral ETC in healthy volunteers as well as the Single- and Multiple-Dose Pharmacokinetics of ETC was also studied¹⁰⁻¹¹. ETC was determined in human plasma¹²⁻¹⁵ using HPLC-MS/MS. HPLC¹⁶⁻¹⁷, spectrophotometric¹⁸ and capillary zone electrophoresis¹⁹ were also reported for the determination of ETC. ETC was determined in serum and synovial fluid of patients with inflammatory arthritis by ultra performance liquid chromatography-inductively coupled²⁰ plasma mass spectroscopy and quadrupole time-of-flight mass spectrometry and also by HPTLC²¹ method using chloroform: methanol : toluene (4:2:4 v/v) mobile phase. Matthews et al²² isolated and characterized the photolytic products using HPLC-UV-fluorescence, HPLC-MS/MS, NMR and HPLC-NMR showing that Etoricoxib undergoes a photocyclization reaction when irradiated with UV light (254 nm) leading to the formation of two major isometric photocyclazation products. In the present study the authors proposed a relatively simple, reliable reproducible RP-HPLC method for the determination of Etoricoxib in pharmaceutical dosage forms and validated as per the ICH²³ guidelines.

Experimental

Chemicals and Reagents

Pharmaceutical grade Etoricoxib (ETC) drug was supplied as gift sample from Ranbaxy Research Laboratories Ltd. (Haryana, India). Etoricoxib is available in India as tablets with brand names ETOZOX (Cipla), KINGCOX (Cadila HC) and ETROBAX (Ranbaxy) (60, 90 and 120 mg per tablet) and were purchased from the local market. HPLC grade acetonitrile (Merck) was used and all other chemicals were of analytical grade.

Instrument used

Quantitative HPLC was performed on a binary gradient HPLC with Shimadzu LC-10AT and LC-10AT VP Series HPLC pumps, with a 20 μ L sample injection loop (manual) and SPD 10AT series UV-Visible detector. The output signal was monitored and integrated using Shimadzu Class-VP Version 6.12 SP1 Software. A Hypersil ODS C₁₈ column (250mm $\times$ 4.6mm, 5 μ m) was used for separation. Afcoset analytical electronic balance was used.

Selection of Mobile Phase

The mobile phase acetonitrile: potassium dihydrogen phosphate buffer (0.05 M) was prepared 46:54 % v/v at a flow rate of 1.2 ml/ min produces peaks with good resolution for Etoricoxib and Bromohexine (Internal standard).

Preparation of standard drug solution

Stock solution of Etoricoxib (ETC) (1mg/ml) was prepared by dissolving 25 mg of Etoricoxib in 25 ml of volumetric flask containing 10 ml of mobile phase. The solution was sonicated for about 20 minutes and then made up to volume with mobile phase.

Working standard solutions of Etoricoxib were prepared by suitable dilution of the stock solution with mobile phase. Similarly stock solution of internal standard was prepared by dissolving 25 mg of Bromohexine in 25 ml of volumetric flask containing 10 ml of mobile phase, sonicated for 20 min. then made up to the volume with mobile phase.

Linearity

A series of standard solutions of Etoricoxib were prepared by taking suitable aliquots of drug solution (0.5-85 μ g mL⁻¹) from the stock solution and spiked with internal standard solution (Bromohexine, IS) (10 μ g mL⁻¹) and the volume was made up to 10 ml with mobile phase in a 10 ml volumetric flask.

20 μ L of each of ETC solution spiked with internal standard (Bromohexine, 10 μ g/mL) were injected into the HPLC system thrice and the chromatograms were recorded. A typical chromatogram of ETC was shown in Figure 2. The peak area obtained for ETC and the internal standard solutions were recorded and the peak area ratio of ETC to internal standard was calculated for all the solutions. A calibration curve was constructed by taking concentration of ETC on x-axis and the corresponding peak area ratios on y-axis (Figure 3).

Figure 2. Representative Chromatogram of pure Etoricoxib (20 μ g mL⁻¹) (Rt = 3.083 mins) with Bromohexine (IS) (10 μ g mL⁻¹) (Rt = 7.631 mins).

Figure 3: Calibration curve of Etoricoxib.

Assay procedure for marketed formulations

Twenty tablets were weighed, finely powdered and powder equivalent to 25 mg of ETC was weighed accurately and extracted with mobile phase in a 25ml volumetric flask and sonicated. This solution was filtered through 0.45 μ m filter paper and diluted further with the mobile phase as per the requirement. All determinations were carried out in triplicate. 20 μ l of the above solutions from different brands of formulation spiked with internal standard were injected in to the HPLC system and the chromatograms were recorded (Figure 4 and 5). The assay results are shown in Table 1.

Figure 4. Representative Chromatogram of Etoricoxib Fomulation (ETOZOX - 60 mg) (20 μ g ml⁻¹) (Rt = 3.101 mins) with Bromohexine (IS) (10 μ g ml⁻¹) Rt = 7.652 mins).

Figure 5: Representative Chromatogram of Etoricoxib Fomulation (ETROBAX - 60 mg) (20 μ g ml⁻¹) (Rt = 3.097 mins) with Bromohexine (IS) (10 μ g ml⁻¹) (Rt = 7.645 mins).

Table 1: Analysis of commercial formulation.

S.No.	Tablet brand	Labeled amount (mg)	Amount obtained		
			by proposed method* (mg) $\pm$ SD	% Recovery	% RSD
1	ETZOX	60	59.79 $\pm$ 0.0156	99.65	0.252
	ETROBAX	60	59.84 $\pm$ 0.0231	99.73	0.354
2	KINGCOX	60	59.68 $\pm$ 0.0534	99.47	0.113

*Each value is average of five determinations $\pm$ Standard deviation (SD).

Method validation

Precision

The precision (Inter-day and Intra-day precision) was ascertained by actual determination of three replicates ($n=3$) at three different levels (20, 40 and 60 $\mu\text{g/ml}$) of ETC with 10 $\mu\text{g/ml}$ internal standard. 20 μl of these solutions were injected in to the HPLC system and the peak area ratio of ETC to the internal standard was noted and the percent relative standard deviation was calculated (Table 2).

Table 2: Intra-day and inter-day precision for Etoricoxib ($n = 3$).

Conc. ($\mu\text{g/ mL}^{-1}$)	Intra-day precision		Inter-day precision	
	Mean peak area Ratio		Mean peak area Ratio	
	(Drug / I.S)	RSD (%)	(Drug / I.S) $\pm$ SD	RSD (%)
20	2.01331 $\pm$ 0.00153	0.0759	2.02131 $\pm$ 0.00152	0.0751
40	3.99566 $\pm$ 0.00252	0.0629	3.89923 $\pm$ 0.00252	0.0646
60	6.05766 $\pm$ 00153	0.0252	6.04676 $\pm$ 0.01657	0.274

*Each value is average of three determinations $\pm$ Standard deviation (SD).

Accuracy

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, 120%) of ETC sample solutions to the pre-analyzed ETC formulation solution (within the linearity range) spiked with the internal standard and the percentage recovery values were calculated (Table 3).

Table 3: Accuracy study of Etoricoxib (ETC).

Spike level %	Concentration ($\mu\text{g ml}^{-1}$)		Total conc. ($\mu\text{g ml}^{-1}$)	% Recovery	% RSD
	Pure drug	Formulation			
80	16	20	36	99.48	Mean = 99.733%
80	16	20	36	99.87	SD = 0.219622
80	16	20	36	99.85	% RSD = 0.2202
100	20	20	40	98.94	Mean = 99.523%
100	20	20	40	99.79	SD = 0.505799
100	20	20	40	99.84	% RSD = 0.5082
120	24	20	44	99.97	Mean = 99.840%
120	24	20	44	100.11	SD = 0.353412
120	24	20	44	99.44	% RSD = 0.35398

Results and Discussion

Etoricoxib obeys beer's law in the concentration range of 0.5-85 $\mu\text{g/ml}$ with regression equation $0.101 X - 0.006$ and correlation coefficient 0.999. The LOD and LOQ were found to be 0.0704 and 0.2134 $\mu\text{g/mL}$. The marketed formulations were analyzed and the percentage recovery values were in between 99.47-99.65 % (Table 1). The method is precise (Table 2) as well as accurate (Table 3) as the RSD is less than 2% The theoretical plates are found to be 5384 (>2000) and the tailing factor is 1.1 (<1.5).

Conclusion

The proposed method was found to be simple, precise, accurate, rapid and economical for the determination Etoricoxib in pharmaceutical dosage forms. The sample recoveries in all formulations were in good agreement with their respective label claims suggesting that no interference of excipients from the formulation. Hence this method can be conveniently adopted for routine analysis of Etoricoxib.

Acknowledgements

Authors are grateful to Ranbaxy Research Laboratories Ltd., India for providing the necessary research facilities.

References

1. The Merck Index, An Encyclopedia of Chemical, Drugs and Biologicals, Maryadele J.O. Neil. Eds, 14th Ed., Published by Merck Research Lab, Division of Merck and Co. Inc., Whitehouse Station, NJ, 2006, Monograph No. 3887, 662.
2. Cochrane, D J, Jarvis B and Keating G M, *Drugs*, 2002, **62**, 2637.
3. Leclercq P and Malaise, M G, *Rev. Med. Liege*, 2004, **59**, 345.
4. Lazzaroni M and Bianchi P G, *Pharmacol. Ther.*, 2004, **20**, 48.
5. Rubin B R, Burton R, Navarra S, Antigua J, Londono J, Pryhuber K G, Lund M, Chen E, Najarian D K, Petruschke R A, Ozturk Z E and Geba G P, *Arthritis Rheum.*, 2004, **50**, 598.
6. Ramos-Remus C R, Hunsche E, Mavros P, Querol J, Suarez R, *Curr. Med. Res. Opin.*, 2004, **20**, 691.
7. Duncan M R, and Capell H A, *Int. J. Clin. Pract.*, 2003, **57**, 315.
8. Matsumoto A K, Melian A, Mandel D R, McIlwain H H, Borenstein D, Zhao P L, Lines C R, Gertz B J and Curtis S, *J. Rheum.* 2002, **29**, 1623.
9. Rodrigues A D, Halpin A R, Geer L A, Cui D, Catherine Z Matthews Keith M Gottesdiener, Patrick J Larson, Kenneth C Lasseter and Agrawal N G B, *Drug Metabolism and Disposition*, 2003, **31**, 224.
10. Agrawal N G B, Porras A G, Matthews C Z, Woolf E J, Miller J L, Mukhopadhyay S, *J Clin Pharmacol*, 2001, **41**, 1106.
11. Agarwal N G B, Porras A G, Matthews C Z et al, *J Clin Pharmacol*, 2003, **43**, 268.
12. Braeutigam L, Nefflen J U and Geisslinger G, *J Chromatogr B Analyt Technol Biomed Life Sci.*, 2003, **788**, 309.
13. Rose M J, Agarwal N, Woolf E J and Matuszewski B K, *J. Pharm. Sci.*, 2002, **91**, 405.
14. Werner U, Werner D, Hinz B, Lambrecht C, Brune K, *Biomed Chromatogr.*, 2005, **19**, 113.
15. Sérgio Luiz Dalmora, Liberato Brum Junior, Ricardo Machado Ferretto, Paulo Renato de Oliveira, Thiago Barth and Maximiliano da Silva Sangoi, *Quím. Nova.*, 2008, **31**, 574.
16. Hartman R, Abraham A, Clausen A, Mao B, Crocker L S and Ge Z H, *J. Liq. Chromatogr. Relat. Technol.*, 2003, **26**, 2551.
17. Mandal U, Senthil D, Bose A, Gowda K V, Ghose A, Pal T K, *Ind. J. Pharma. Sci.*, 2006, **68**, 485.
18. Suhagia B N, Patel H M, Shah S A, Rathod I S and Marolia B P, *Ind. J. Pharm. Sci.*, 2005, **67**, 634.
19. Sérgio Luiz Dalmora, Maximiliano da Silva Sangoi, Lucélia Magalhães da Silva, Rui Oliveira Macedo and Thiago Barth, *Journal of Separation Science*, 2008, **31**, 169.
20. Eudoxia Diza, Lucas Settas, Paul Nikolaidis, Christopher Smith, Wilson Ian D, *Journal of Pharmaceutical and Biomedical Analysis*, 2009, **49**, 579.
21. Shah N J, Shah S J, Patel D M, Patel N M, *Ind. J. Pharm. Edu and Res.*, 2006, **68**, 788.
22. Matthews C Z, Subramanian R, Woolf E J, Foster N, Matuszewski B K, *Pharmazie*. 2004, **59**, 913.
23. ICH harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2 (R1) , 2005.