

Separation of Etoricoxib and Its Degradation Products in Drug Substance Using UPLC™

Dinesh N. Vora and Arun A. Kadav

Chemistry Dept, Mithibai college of Arts, Chauhan Institute of Science & A.J. College of Commerce and Economics, Vile Parle (W), Mumbai – 400 056, Maharashtra, India.

Abstract

A UPLC™ method was developed and validated for the separation of Etoricoxib and its degradation products in drug substance. The main drug and its seven major and few minor degradation products were well separated within seven minutes on acquity UPLC™ BEH C18 column (1.7 μ , 2.1 x 100 mm) maintained at 25°C, using 0.01M acetate buffer pH 5.0 - acetonitrile (60 : 40, v/v) as mobile phase. The flow rate was 0.3 mL.min⁻¹ and observed backpressure was about 10500 psi. Detection was performed at 235 nm using PDA detector. Linearity was obtained in the concentration range of 0.05 - 120 μ g.mL⁻¹. The method was validated for precision, linearity, LOD and LOQ. Stability indicating capability was established by forced degradation experiments.

Key words: Etoricoxib, degradation products, UPLC, validation.

1. Introduction

Etoricoxib, chemically (5-chloro-2-[6-methyl pyridin-3-yl]-3-[4-methylsulfonylphenyl]pyridine), is a novel highly selective second-generation cyclooxygenase-2 inhibitor administered orally as an analgesic and anti-inflammatory drug. The chemical structure of Etoricoxib is shown in Fig 1. It is an off-white crystalline powder, relatively insoluble in water, and freely soluble in alkaline aqueous solutions. It is used for the treatment of rheumatoid arthritis, osteoarthritis, chronic low back pain, gout, and ankylosing spondylitis.

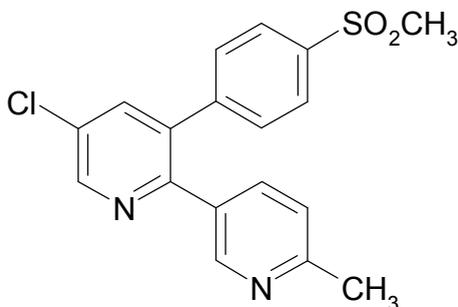


Fig 1. Chemical Structure of Etoricoxib.

Various analytical methods such as LC-Fluorescence [1], LC-UV [2], and LC-MS/MS [3] have been reported for estimation of etoricoxib from biological samples. Also, LC-UV [4, 5] and LC-MS/MS [4] methods are reported for estimation of Etoricoxib from drug and pharmaceutical preparations. A LC-MS/MS method [6] has been reported for determination of impurities and etoricoxib from drug. However, no method is reported which can be used for routine determination of Etoricoxib and its degradation products in drug substance or drug product in a single faster chromatographic run using commonly used detector such as UV/PDA detector.

The conventional isocratic HPLC methods run over approximately 30 minutes when tried to separate all related degradation products. Ultra Performance Liquid Chromatography is a recent technique in liquid chromatography, which enables significant reductions in separation time and solvent consumption. The literature indicates that the UPLCTM system allows about nine fold decrease in analysis time as compared to the conventional HPLC system using 5 μm particle size analytical columns, and about three fold decrease in analysis time in comparison with 3 μm particle size analytical columns without compromise on overall separation. [7, 8]

Stability testing provides the evidence on how the quality of drug substance and drug product is affected with time under influence of environmental conditions such as temperature, humidity and light. Nowadays, the determination of impurities (degradation products) is one of the main and difficult tasks during the development of separation methods for pharmaceuticals, especially if more and more impurities (degradation products) are requested to be determined by drug authorities.

Hence, the purpose of this study was to develop a UPLCTM method for separation of Etoricoxib and its degradation products which are formed by forced degradation under acidic, alkaline, strong oxidizing, thermal and photolytic conditions. Thereafter, this method was validated as per ICH guidelines [9] and successfully applied for separation of Etoricoxib and its degradation products from drug substance.

2. Experimental

2.1. Instrumentation

Analyses were performed on Acquity UPLCTM system (Waters, Milford, USA), consisting of a binary solvent manager, sample manager and PDA detector. The detector was set at sampling rate of 20 points.sec⁻¹ and filter time constant of 0.2 sec. System control, data collection and data processing were accomplished using Waters EmpowerTM chromatography data software. Weighing was performed on Mettler Toledo AG245 balance (Switzerland) and pH was adjusted using pH meter from Thermo Orion (USA)

2.2. Solvents and Chemicals

Standard of Etoricoxib was kindly gifted by a supplier along with declared purity of 99.6% and used as received. Acetonitrile and methanol for HPLC was obtained from J.T. Baker (NJ, USA). Ammonium acetate was obtained from Merck (Mumbai, India) and Excelsior grade glacial acetic acid was supplied by Qualigens fine chemicals (Mumbai, India).

2.3. Chromatography

The buffer solution was prepared by adjusting the pH of 0.01 mol L⁻¹ ammonium acetate solution to 5.0 with glacial acetic acid. This buffer solution was then filtered through 0.2 μ nylon filter. The mobile phase was a mixture of buffer pH 5.0 – acetonitrile, in the ratio of 60: 40 (v/v). The stationary phase was Acquity UPLC™ BEH C18 column (1.7 μ, 2.1 mm x 100 mm) (Waters, Milford, USA). Injection volume was 2 μL, flow rate was 0.3 mL.min⁻¹ and detection was performed at 235 nm. The observed backpressure was about 10500 psi.

2.4. Sample Solution

Sample solution was prepared by dissolving accurately weighed 10 mg of Etoricoxib in 100 mL of methanol. (100 μg.mL⁻¹)

2.5. Degradation Studies

Stress degradation was performed on 100 μg.mL⁻¹ solution of drug in methanol. Acid hydrolysis, base hydrolysis and oxidation was carried out by using 2 mL of 1N hydrochloric acid, 2 mL of 1N sodium hydroxide and 1 mL of 50% hydrogen peroxide solution respectively. Four sets of these solutions were prepared and each set was heated in water bath at 80°C for 1 hr, 2 hr, 3 hr and 4 hr respectively.

Photolytic and thermal degradation was carried out on drug substance by exposing it separately to short wavelength light (254 nm) and heat (105°C) for 4 days. Samples were withdrawn at appropriate time and subjected to analysis.

3. Results and Discussion

3.1. Chromatography

Method development was initiated by optimization of pH of the buffer, which was to be used for mobile phase preparation. Degraded Etoricoxib samples were chromatographed using mobile phases of acetonitrile and buffer solutions of pH 2.5, 5.0 and 6.8. During initial experiments, gradient elution (10% acetonitrile – 70 % acetonitrile in 15 minutes) was used to ensure that all degradation products are eluted, and to determine the total number of major degradation products. The mobile phase of pH 2.5 buffer and acetonitrile was not found

suitable, as some of the degradation products were not resolved from each other or from Etoricoxib peak. Mobile phases prepared with buffer pH 5.0 and 6.8 yielded very much similar chromatograms, wherein Etoricoxib and degradation products were adequately separated. Buffer pH 5.0 was selected for further study. The samples which were degraded for 3 hrs in acidic condition, 2 hrs in oxidative and basic conditions were selected for further study, as reasonable degradation was observed under these conditions.

After confirming the number of major degradation products, development of isocratic method was undertaken. Mobile phases of different proportions of buffer pH 5.0 and acetonitrile were prepared and degraded samples of Etoricoxib were chromatographed using these mobile phases. The mobile phase composition of buffer pH 5.0 and acetonitrile (60:40, v/v) was found most promising for separation of Etoricoxib and all its degradation products, as observed during gradient run. The typical chromatogram of Etoricoxib is given in Fig.2A. To study the effect of temperature, the chromatography was carried out at 30°C column temperature. It was observed that separation between degradation products deteriorates when temperature is increased to 30°C. Hence, 25°C was selected as column temperature. The wavelength of 235 nm was selected for the UV detection because at this wavelength Etoricoxib exhibits maxima.

3.2. Degradation behavior

Studies on Etoricoxib under different stress conditions suggested following degradation behavior.

3.2.1 Oxidative degradation

Etoricoxib degraded under strong oxidative conditions resulting in formation of major degradation products eluting at RRT of 0.38, 0.46 and 1.99, of which degradation product at RRT of 0.46 was highest. All the degradation products were found increasing with time. The chromatogram of oxidation degraded Etoricoxib is presented in Fig.2B.

3.2.2 Acid hydrolysis

Etoricoxib gradually decreased with time on heating at 80°C in 1M hydrochloric acid, and resulted in formation of major degradation products, which eluted at RRT of 0.61, 1.14, 1.62, 1.99 and 2.48. All the degradation products increased during acid hydrolysis upto 3 hrs and after that the peak at RRT of 1.99 was decreased. The chromatogram of acid degraded Etoricoxib is presented in Fig.2c. As seen from this chromatogram the separation between main peak (2.66 min) and its adjacent acid degradation product peak (3.02 min) was critical. This required more aqueous portion in mobile phase and thus resulted in 7 mins total run time.

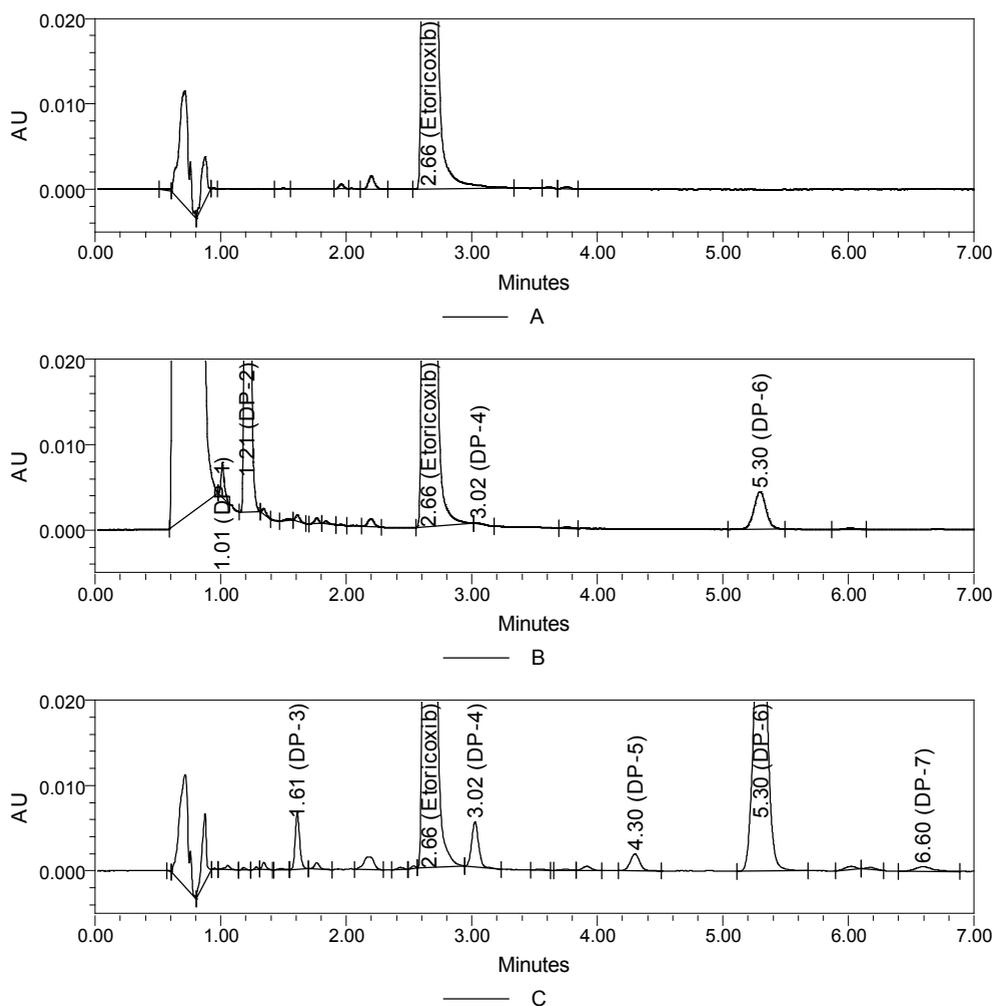


Fig 2. (A) (B) (C): Chromatograms of untreated, oxidation degraded and acid degraded Etoricoxib respectively, chromatographed on acquity UPLC™ BEH C18 column (1.7 μ , 2.1 mm x 100 mm) using 0.01M acetate buffer pH 5.0 - acetonitrile (60 : 40, v/v) as mobile phase and measured at 235 nm. Backpressure = 10500 psi. Major degradation products are labeled as DP-1, DP-2...DP-7.

3.2.3 Alkali hydrolysis

The drug substance degraded to two major degradation products, which eluted at RRT of 1.99 and 2.48. During alkaline hydrolysis, degradation peaks increased upto 3 hrs and after that they were decreased. Etoricoxib undergoes hydrolysis in acidic as well as alkaline conditions, and forms a major degradation product, which elutes at RRT of 1.99. However, the level of this degradation product was higher in acid degradation.

3.2.4 Photolytic and thermal degradation

No major degradation was observed after exposure to photolytic and thermal stress conditions. Nevertheless, a minor degradation product was observed at RRT of 1.99 under photolytic degradation conditions.

3.3. Method validation:

Method validation was performed for following parameters in accordance with ICH guideline [9].

3.3.1 Precision and Intermediate precision

Precision was investigated using sample preparation procedure for six real samples. The developed UPLCTM method was applied to determine the percentage of each impurity and drug by area normalization. Intermediate precision was performed by carrying out analysis on different day, on different column by different analyst. The results are presented in Table 1.

Table 1: Precision and intermediate precision

RRT with respect to Etoricoxib	% ± S.D. (n=6)	
	Precision	Intermediate Precision
0.56	0.01 ± 0.005	0.01 ± 0.005
0.74	0.05 ± 0.0	0.05 ± 0.005
0.83	0.20 ± 0.005	0.20 ± 0.009
1.0 (Etoricoxib)	99.7 ± 0.008	99.7 ± 0.010
1.36	0.02 ± 0.0	0.02 ± 0.004
1.41	0.03 ± 0.0	0.03 ± 0.004

3.3.2 Linearity

The plot of the peak area versus the respective concentration of Etoricoxib was found to be linear in the range of 0.05 µg.mL⁻¹ – 120 µg.mL⁻¹ (n=7). Following linear regression equation could represent the calibration curve.

$$Y = 26916.9 X - 13660.6 \quad (r = 0.9995)$$

Where Y= Area, X = Concentration in µg.mL⁻¹ and r = Correlation coefficient.

3.3.3 Limit of detection and Limit of quantitation

The degradation products separated in this method were unknown and hence their standards were not available. Hence, the limit of detection (LOD) and limit of quantitation (LOQ) were determined using Etoricoxib standard, assuming that degradation products would have equal response as that of Etoricoxib. The LOD and LOQ were determined by using signal to noise (S/N) approach as defined in International Conference on Harmonization (ICH) guideline [9]. The LOD and LOQ for Etoricoxib and thus its unknown degradation products were found to

be $0.02 \mu\text{g.mL}^{-1}$ (S/N = 4.1) and $0.05 \mu\text{g.mL}^{-1}$ (S/N = 10.4) respectively. The RSD of replicate injections at level of LOQ was 1.1%, which shows excellent precision at LOQ level.

3.3.4 Specificity and selectivity

The peak purity of peak due to Etoricoxib and major degradation products was determined in all degradation samples using PDA detector. In all the cases peaks were found to be pure. The resolution between Etoricoxib, major degradation products and respective neighboring peak was greater than 2.0.

To investigate the possibility of overlap of degradation products formed in various conditions, the PDA spectra of individual degradation products were extracted and examined. In all the conditions, peaks eluting at particular retention time showed same spectra, indicating that there was no co elution.

The stability indicating assay was demonstrated by comparing assay of degraded samples with that of undegraded control sample. The assay of degradation samples is presented in Table 2.

Table 2: Assay of degraded Etoricoxib samples

Degradation condition	% Assay with respect to control sample	RRT of major degradation peaks
No degradation (control)	100	-
Oxidation (80°C, 2 hrs, 1mL of 50% hydrogen peroxide)	79.9	0.38, 0.46, 1.99
Acid hydrolysis (80°C, 3 hrs, 2mL of 1N HCl)	72.2	0.61, 1.14, 1.62, 1.99, 2.48
Base hydrolysis (80°C, 2 hrs, 2mL of 1N NaOH)	72.8	1.99, 2.48
Photolytic degradation (105°C, 4 days)	99.5	No major degradation peak observed
Thermal degradation (UV 254 nm, 4 days)	99.4	No major degradation peak observed

4. Conclusion

The proposed method is fast, simple, precise and stability indicating for the separation of Etoricoxib and its degradation products from drug substance. Hence, it can be easily and conveniently adopted for the routine quality control analysis and stability studies.

References

1. Matthews C Z, Subramanian R, Woolf E J, Foster N, Matuszewski B K (2004) Isolation and structural characterization of the photolysis products of Etoricoxib. *Pharmazie* 59: 913.
2. Ramakrishna N V, Vishottam K N, Wishu S, Koteshwara M (2005) Validated liquid chromatography method for the quantitation of etoricoxib in human plasma using liquid liquid extraction. *J. Chromatogr B Analyt Technol Biomed Life Sci.* 816: 215.
3. Liberato B Jr, Danieli C C, Marcio F, Paulo R, Sergio D (2003) Validation of an LC tandem MS/MS method for the determination of etoricoxib in human plasma and pharmaceutical formulations. *J Liq Chromatogr Relat Technol* 29: 123.
4. Liberato B Jr, Danieli C C, Marcio F, Thiago B, Sergio L D (2006) Validation of liquid chromatography and liquid chromatography tandem mass spectrometry methods for the determination of etoricoxib in pharmaceutical formulations. *J AOAC Int* 89: 1268.
5. Ansari T A, Jamil S, Singh R M, Mathur S C, Shivraj, Singh G N (2005) Determination of etoricoxib in bulk drug and its dosage forms by RP-HPLC. *Indian Drugs.* 42: 56.
6. Robert H, Ahmed A, Andrew C, Bing M, Louis S C, Zhihong G (2005) Development and validation of an HPLC method for the impurity and quantitative analysis of etoricoxib. *J Liq Chromatogr Relat Technol* 26: 2551.
7. Wren S, Tchelitcheff P (2006) Use of UPLC in pharmaceutical development. *J Chrom A* 1119: 140.
8. Novakova L, Matysova L, Solich P (2006) Advantages of application of UPLC in pharma analysis. *Talanta* 68:908.
9. International Conference on Harmonization (ICH), Q2 (R1) (2005) Validation of analytical procedures: Text and methodology.

Received: April 14, 2007; Accepted: September 02, 2007

Corresponding author:

Dinesh N. Vora

Address: Chemistry Dept, Mithibai college of Arts, Chauhan Institute of Science & A.J. College of Commerce and Economics, Vile Parle (W), Mumbai – 400 056, Maharashtra, India.

Phone: +91 22 26136059

Fax: +91 22 26130441

E mail: dn.vora@rediffmail.com