ORIGINAL ARTICLE

Development and Validation of a New Stability-indicating RP-HPLC Method for the Quantification of Etoricoxib in Tablets

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Abstract

Introduction: Etoricoxib is also a bipyridine derivative used as a non-steroidal anti-inflammatory drug. A new stability-indicating reverse phase high-performance liquid chromatographic (HPLC) method has been developed for the quantification of Etoricoxib in tablet dosage forms. **Materials and Methods:** A Shimadzu HPLC system equipped Agilent C18 column was chosen for the chromatographic study with mobile phase formic acid:acetonitrile (52:48) and a flow rate of 0.8 mL/min (UV detection at 247 nm). **Results and Discussion**: Linearity was obeyed over the concentration range of 0.5–100 µg/mL with a linear regression equation y = 95013× – 11970 and a correlation coefficient of 0.9998. The limit of detection and limit of quantification were found to be 0.1557 and 0.4791 µg/mL, respectively. Stress degradation studies were performed, and the method was validated as per the International Council for Harmonisation guidelines. **Conclusion:** The RP-HPLC method so developed was found to be selective, specific, precise, accurate, robust, and useful for the estimation of Etoricoxib tablets.

Key words: Etoricoxib, International Council for Harmonisation guidelines, reverse phase high-performance liquid chromatography, stability indicating, validation

INTRODUCTION

Etoricoxib[1] [Figure 1] (molecular weight: 358.8419 g/mol) is used for the treatment of osteoarthritis, chronic low back pain, rheumatoid arthritis, gout, and acute pain. Etoricoxib[2] (CAS no. 202409-33-4) was patented in 1996 and approved in 2002 for medical use. Chemically, Etoricoxib ($C_{18}H_{15}ClN_2O_2S$) is 5-chloro-2-(6-methylpyridin-3-yl)-3-(4-methylsulfonylphenyl) pyridine, which is a methyl sulfone derivative with pKa 3.9 and 4.6. It is a COX-2 inhibitor[3] and administered through the oral route.[3,4]

A literature survey reveals that various analytical techniques such as capillary zone electrophoresis, spectrophotometry, UPLC-MS/MS, LC-ESI-MS/MS, LC-APCI/MS/MS, HPTLC, and high-performance liquid chromatography (HPLC) were developed for the estimation of Etoricoxib in pharmaceutical formulations as well as biological fluids.

Dalmora *et al*.[5] developed a capillary zone electrophoresis method for the determination of Etoricoxib in pharmaceutical formulations.

Different spectrophotometric methods[6-10] were developed using methanol, 0.1 N HCl, chloroform, etc., for the assay of Etoricoxib.

Zhang *et al*. developed UPLC-MS/MS method[11] for the quantitative analysis of Etoricoxib in human plasma using acetonitrile:2 mM ammonium acetate (gradient mode), and the linearity was observed as 0.005–5.0 µg/mL.

Junior *et al*. developed LC-ESI-MS/MS method[12] for the quantitative analysis of Etoricoxib in human plasma using a mobile phase mixture consisting of acetonitrile:water (95:5)/0.1% acetic acid (90:10) in the presence of an internal standard, piroxicam, and another HPLC method using 0.01M phosphoric acid (pH 3.0) adjusted with sodium hydroxide:acetonitrile (62:38).

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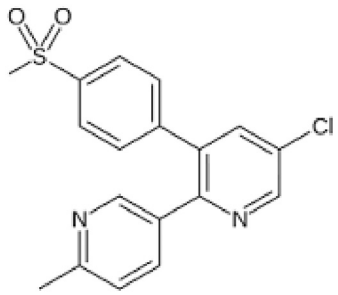

Figure 1: Chemical structure of Etoricoxib

Dalmora *et al*. developed LC-APCI/MS/MS method[13] for the quantitative analysis of Etoricoxib in human plasma using an automated online solid-phase extraction with a mobile phase mixture consisting of acetonitrile:water (95:5)/10 mM ammonium acetate (pH 4.0), and the linearity was observed as 0.001–5.0 µg/mL.

Maheshwari *et al*. developed HPTLC method[14] for the determination of Etoricoxib in pharmaceutical dosage forms using toluene:1,4-dioxane:methanol (8.5:1.0:0.5) as mobile phase in the presence of internal standard rofecoxib (UV detection 235 nm).

Patel *et al*. developed reverse phase (RP)-HPLC method[15] for the determination of Etoricoxib using a mobile phase mixture consisting of acetonitrile:methanol:10 mM potassium dihydrogen phosphate (pH 3.0 adjusted with orthophosphoric acid) (UV detection at 234 nm) and linearity was shown as 0.025–0.4 µg/mL.

Topalli *et al*. developed RP-HPLC method[16] for the determination of Etoricoxib using a mobile phase mixture consisting of acetonitrile:potassium dihydrogen phosphate buffer (pH 4.2) (46:54) (UV detection at 280 nm), and linearity was shown as 0.5–85 µg/mL.

Thimmaraju *et al*. developed the RP-HPLC method[17] for the determination of Etoricoxib in pharmaceutical formulations using a mobile phase mixture of acetonitrile:(0.05M) KH_2PO_4 buffer (50:50) with UV detection at 283 nm, and linearity was shown as 0.5–85 µg/mL.

Gangane *et al*. developed the RP-HPLC method[18] for the determination of Etoricoxib in tablet formulations using methanol (UV detection at 233 nm), and linearity was shown as 20–55 µg/mL.

Shakya *et al*. developed the RP-HPLC method[19] for the determination of Etoricoxib in human plasma using a mobile phase mixture consisting of aqueous buffer containing triethylamine and orthophosphoric acid with acetonitrile (62:38) (UV detection at 284 nm), and linearity was shown as 0.015–3.2µg/mL.

Aluri *et al*. developed a RP-UFLC method[20] for the determination of Etoricoxib using a mobile phase mixture consisting of acetonitrile:water (50:50) (UV detection at 230 nm), and linearity was shown as 0.1–100 µg/mL.

Alzweiri *et al*. developed RP-HPLC method[21] for the determination of Etoricoxib using a mobile phase mixture consisting of methanol:phosphate buffer (pH 6) (70:30) in the presence of the internal standard, celecoxib (UV detection at 215 nm), and linearity was shown as 1.0–8.0 µg/mL.

Srikar *et al*. have published a review article[22] on the analytical techniques reported so far the determination of Etoricoxib.

MATERIALS AND METHODS

Procedure

25 mg of Etoricoxib was accurately weighed and transferred into a 25 mL volumetric flask (1000 µg/mL) and dissolved in HPLC-grade acetonitrile. The stock solution (1000 µg/mL) was further diluted with mobile phase as per the requirement for linearity, precision, accuracy, robustness, and other studies, and all the solutions were filtered through a 0.45 µm membrane filter before injection.

Instrumentation and chromatographic conditions

A Shimadzu Model HPLC system with PDA detector and Agilent C18 column was used for the chromatographic study. A mobile phase mixture consisting of formic acid:acetonitrile (52:48) was used for the present chromatographic study with a flow rate of 0.8 mL/min (UV detection at 247 nm).

Method validation[23]

Linearity study

Etoricoxib solutions (0.5–100 µg/mL) were prepared from the stock solution and diluted with the mobile phase, formic acid:acetonitrile (52:48) and injected (n = 3) into the HPLC system, and the peak area was noted from the respective chromatograms. Finally, the mean peak area (n = 3) was calculated, and a calibration curve was drawn by plotting the Etoricoxib concentration on the X-axis and the corresponding mean peak area on the Y-axis. The limit of detection (LOD) and limit of quantification (LOQ) were calculated from the S/N ratio.

Precision study

Intraday and interday precision studies were performed on the same day and on three different days (20, 40, and 60 µg/mL), and the peak area of the chromatograms was recorded during the study from which the mean peak area (n = 3) was calculated. The percentage relative standard deviation was also calculated from the mean peak area and the standard deviation.

Accuracy study

The accuracy study was performed by spiking the Etoricoxib formulation (36 µg/mL) solution (50, 100, and 150%) with a known concentration of 18, 36, and 54 µg/mL API. These solutions were also injected (n = 3) into the HPLC system, and the chromatograms were recorded followed by the peak areas from which the mean peak area and the % RSD were calculated from the linear regression equation.

Robustness study

The robustness of the method was proved by incorporating very small changes in the optimized chromatographic conditions, such as flow rate, mobile phase composition, and detection wavelength.

Stress degradation studies[24]

Stress degradation studies were performed to determine the stability of Etoricoxib (20 µg/mL) toward acidic hydrolysis, basic hydrolysis, oxidation, and thermal degradation. The specificity of the method was determined from the stability studies; therefore, Etoricoxib was exposed to different stress conditions as explained below.

Acidic hydrolysis was performed by heating Etoricoxib (20 µg/mL) solution with 1 mL of 0.1 N HCl solution at 80°C for 30 min on a water bath. The stressed sample was then cooled, neutralized with 1.0 mL 0.1 N sodium hydroxide solution, and diluted with diluent, and then, 20 µL of the solution was injected into the HPLC system.

Alkaline hydrolysis was performed by heating Etoricoxib (20 µg/mL) solution with 1.0 mL 0.1 N sodium hydroxide solution at 80°C for 30 min on a water bath. The stressed sample was then cooled, neutralized with 1.0 mL of 0.1 N HCl solution, and diluted with mobile phase, and then, 20 µL of the resulting solution was injected into the HPLC system.

Thermal degradation was performed by heating the Etoricoxib (20 µg/mL) solution at 80°C for 30 min on a water bath and then cooled, diluted with mobile phase, and 20 µL of the resulting solution was injected into the HPLC system.

Table 1: Literature survey of Etoricoxib

Mobile phase (v/v)	Detection wavelength (nm)	Linearity (µg/mL)	Comment	References
Methanol	284	2–24	Spectrophotometry	[6]
0.1 N HCl	233	2–24	Spectrophotometry	[7]
Chloroform	247	1–40	Spectrophotometry	[8]
0.1M HCl	233	0.1–0.5	Spectrophotometry	[9]
Methanol	234	1–11	Spectrophotometry	[10]
Acetonitrile: 2 mM Ammonium acetate (Gradient mode)	-	0.005–5	UPLC-MS/MS (Human plasma)	[11]
a) Acetonitrile: water (95:5)/0.1% acetic acid (90:10) and piroxicam (Internal standard) 0.01M phosphoric acid (pH 3.0 adjusted with sodium hydroxide 3 M: acetonitrile (62:38)	-234	0.001–5	LC-ESI-MS/MS (Human plasma) RP-HPLC	[12]
Acetonitrile: water (95:5)/10 mM Ammonium acetate (pH 4.0)	-	0.001–5	LC-APCI/MS/MS (Human plasma)	[13]
Toluene: 1,4-dioxane: methanol (8.5:1.0:0.5) rofecoxib (Internal standard)	235	0.1–1.5/spot	HPTLC	[14]
Acetonitrile: methanol: 10 mM potassium dihydrogen phosphate (pH 3.0 adjusted with orthophosphoric acid)	234	0.025–0.4	RP-HPLC	[15]
Acetonitrile: potassium dihydrogen phosphate buffer (pH 4.2) (46:54)	280	0.5–85	RP-HPLC	[16]
Acetonitrile:(0.05M) KH_2PO_4 buffer (50:50)	283	0.5–85	RP-HPLC	[17]
Methanol	233	20–55	RP-HPLC	[18]
Aq. buffer containing triethylamine and orthophosphoric acid):acetonitrile (62:38)	284	0.015–3.2	RP-HPLC (Human plasma)	[19]
Acetonitrile: water (50:50)	230	0.1–100	RP-UFLC	[20]
Methanol: phosphate buffer (pH 6) (70:30) celecoxib (Internal standard)	215	1–8	RP-HPLC	[21]
Formic acid: acetonitrile (52:48)	247	0.5–100	RP-HPLC	Present method

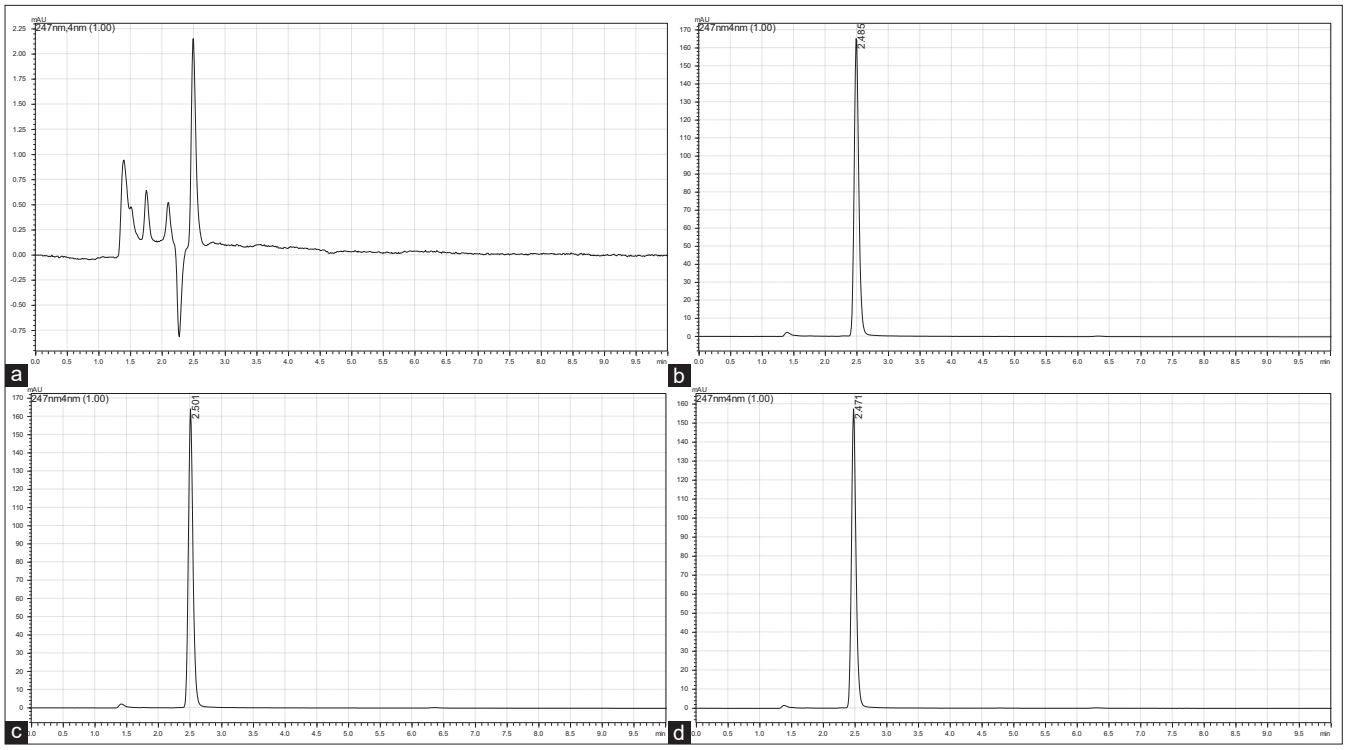

Figure 2: Representative chromatograms of (a) Blank (b) Etoricoxib (10 µg/mL) (API) (Rt: 2.485 min) (c) Etoricoxib formulation (10 µg/mL) (Brand I) (Rt: 2.501 min) (d) Etoricoxib formulation (10 µg/mL) (Brand II) (Rt: 2.471 min)

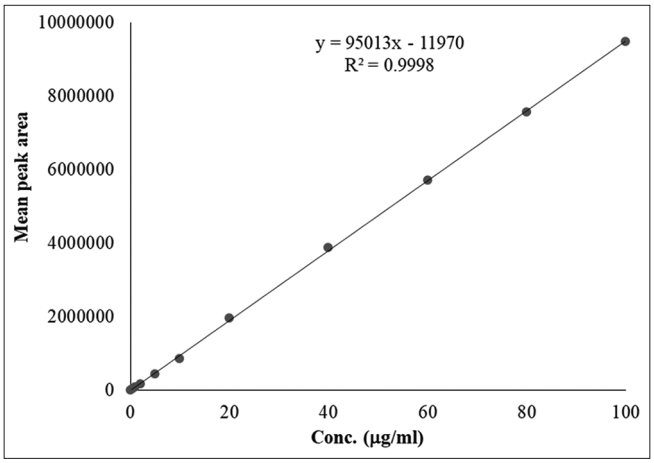

Figure 3: Calibration curve of Etoricoxib

Oxidative degradation was performed by heating Etoricoxib (20 µg/mL) solution with 1.0 mL of 30% hydrogen peroxide solution at 80°C for 30 min in a water bath. The stressed sample was then cooled and diluted with mobile phase, and then, 20 µL of the resulting solution was injected into the HPLC system.

Assay of Etoricoxib formulations

Etoricoxib was obtained as a gift sample from Glenmark Pharmaceuticals Ltd. (India). Etoricoxib is available in India with brand names KRETOS (label claim: 90 mg; 120 mg) (Glenmark Pharmaceuticals Ltd.,) and NUCOXIA (label claim: 60 mg; 90 mg) (Zydus Cadila) as tablets. Two different Indian brands of Etoricoxib were selected and 20 tablets of each were procured from a local pharmacy store, and then, the contents were accurately weighed, powdered, and transferred into two different volumetric flasks and extracted with HPLC-grade acetonitrile. The mixture was sonicated for 30 min and then filtered. These solutions were diluted according to the requirement with the mobile phase, and 20 µL of each solution was injected (n = 3) into the HPLC system, and the peak area was noted from the resultant chromatogram. The mean peak area was calculated and the assay of the tablets was calculated from the linear regression equation.

Table 2: Linearity of Etoricoxib

Conc. (µg/mL)	*Mean peak area	% RSD
0	0	-
0.5	43018	0.27
1	85219	0.31
2	168764	0.58
5	423927	0.84
10	856142	0.51
20	1958924	0.29
40	3860192	0.59
60	5701390	0.72
80	7559258	0.46
100	9473125	0.62

*Mean of three replicates

Table 3: Precision studies of Etoricoxib

Intraday precision study		
Conc. (µg/mL)	**Mean peak area**	**Statistical analysis *Mean peak area±SD (% RSD)**
20	1958924	1957931.67±1005.24 (0.0513)
20	1957957	
20	1956914	
40	3860192	3860066±218.24 (0.0057)
40	3859814	
40	3860192	
60	5701390	2158729.2±111.16 (0.0052)
60	5701426	
60	5701598	

Interday precision study				
Conc. (µg/mL)	**Day 1**	**Day 2**	**Day 3**	**Statistical analysis *Mean peak area±SD (% RSD)**
20	1958924	1946549	1953841	1953104.67±6220.27 (0.3185)
40	3860192	3799684	3859612	3839829.33±34768.09 (0.9055)
60	5701390	5695847	5715875	5704370.67±10341.35 (0.1813)

*Mean of three replicates

Table 4: Accuracy study

Conc. (µg/mL)	Formulation (µg/mL)	Total Conc. (µg/mL)	*Conc. obtained (µg/mL)±SD (%RSD)	% recovery
18 (50%)	36	54	53.91±0.4959 (0.92)	99.83
	36	54		
	36	54		
36 (100%)	36	72	71.81±0.5960 (0.83)	99.74
	36	72		
	36	72		
54 (150%)	36	90	89.88±0.7101 (0.79)	99.87
	36	90		
	36	90		

*Mean of three replicates

RESULTS AND DISCUSSION

Etoricoxib is a non-steroidal anti-inflammatory drug, and a new stability-indicating RP-HPLC method was developed and validated for the quantification of Etoricoxib in tablets. Previously published literature on analytical techniques so far developed is briefly shown in Table 1.

Method optimization

Etoricoxib drug solution (10 µg/mL) was injected into the HPLC system initially using various mobile phase mixtures such as acetonitrile:water, acetonitrile:phosphate buffer, methanol:water, methanol:formic acid, and finally, a mobile phase consisting of formic acid:acetonitrile (52:48) (flow rate: 0.8 mL/min) with an Agilent C18 column where Etoricoxib and the system suitability parameters were within the acceptable criteria. The optimized chromatogram of Etoricoxib (RT 2.485 min) along with the blank were shown in Figure 2.

Method validation

Etoricoxib shows linearity over the concentration range 0.5–100 µg/mL [Table 2] (0.27–0.84), and the linear regression equation was found to be $y = 95013\times - 11970$ with a correlation coefficient of 0.9998 [Figure 3]. The LOD and LOQ were found to be 0.1557 and 0.4791 µg/mL, respectively. The % RSD was found to be 0.0052–0.0513 (intraday) [Table 3] and 0.1813–0.9055 (inter-day) [Table 3] in precision studies, which is <2.0, indicating that the method is precise. The % recovery in accuracy studies was found to be 99.74–99.87% [Table 4], and the % RSD was 0.79–0.92 which was <2%, indicating that the method is accurate. The % RSD in the

Table 5: Robustness study (40 µg/mL)

Parameter	Condition	*Mean peak area	Statistical analysis *Mean±SD (% RSD)
Flow rate (±0.1 mL/min)	0.9	3863212	3861650.67±1512.62 (0.0392)
	0.8	3860192	
	0.7	3861548	
Detection wavelength (±2 nm)	245	3859862	3860436±727.37 (0.0188)
	247	3860192	
	249	3861254	
Mobile phase composition Formic acid: acetonitrile (v/v) (±5%)	47:53	3859651	3859943.33±273.13 (0.0071)
	52:48	3860192	
	57:43	3859987	

*Mean of three replicates

Table 6: Assay of Etoricoxib formulations

S. No.	Brand name	Label claim (mg)	*Observed amount (%w/w)	% Recovery*
1	Brand I	90	89.37	99.30
2	Brand II	90	89.52	99.47

*Mean of three replicates

Table 7: Stress degradation studies of Etoricoxib

Stress condition	R_t (min)	Mean peak area	% recovery	% drug Degradation	Theoretical plates (>2000)	Tailing factor (<1.5)	Resolution (>2)
Standard drug	2.514	1958924	100	0	4370.04	1.117	-
Alkaline hydrolysis 0.1 NaOH/75°C/30 min	2.532	1864422	95.18	4.82	4950.24	1.086	-
Acidic hydrolysis 0.1 N HCl/75°C/30 min	2.547	1938506	98.96	1.04	5592.76	1.070	-
Oxidation degradation H_2O_2/75°C/30 min	2.494 2.122	1950106	99.55	0.45	4047.29	1.417	2.101
Thermal degradation Water/75°C/30 min	2.498	1901943	97.09	2.91	4681.48	1.093	-

*Mean of three replicates

robustness study was found to be 0.0071–0.0392, which was <2%, indicating that the method is robust [Table 5].

Assay of Etoricoxib formulations

Etoricoxib tablets (2 different brands) were chosen, and the proposed RP-HPLC method was applied. The percentage of purity of Etoricoxib was found to be 99.30–99.47 [Table 6]. The representative chromatograms of Etoricoxib obtained from the two brands are shown in Figures 2c and d.

Stress degradation studies

Etoricoxib was eluted at 2.514 min with theoretical plates 4370.04 and tailing factor 1.117. During the alkaline degradation, Etoricoxib showed 4.82% degradation and was eluted at 2.532 min with theoretical plates 4950.24 and tailing factor 1.086. During the acidic degradation, Etoricoxib has shown 1.04% degradation. Etoricoxib was eluted at 2.547 min with theoretical plates 5592.76 and tailing factor 1.070. During oxidative degradation, Etoricoxib has shown 0.45% degradation. Etoricoxib was eluted at 2.494 min with theoretical plates 4047.29 and tailing factor 1.417, and a degradant was also eluted at 2.122 min with resolution 2.101. During the thermal degradation, Etoricoxib has shown 2.91%; degradation was eluted at 2.498 min with theoretical plates 4681.48 and tailing factor 1.093. Less than 5% degradation of Etoricoxib was observed during all the degradation studies indicating that Etoricoxib is highly resistant toward all stress conditions. The system suitability parameters were within the acceptable criteria, and the method is selective and specific as there is no interference of degradant peaks. The results observed during the stress degradation studies are shown in

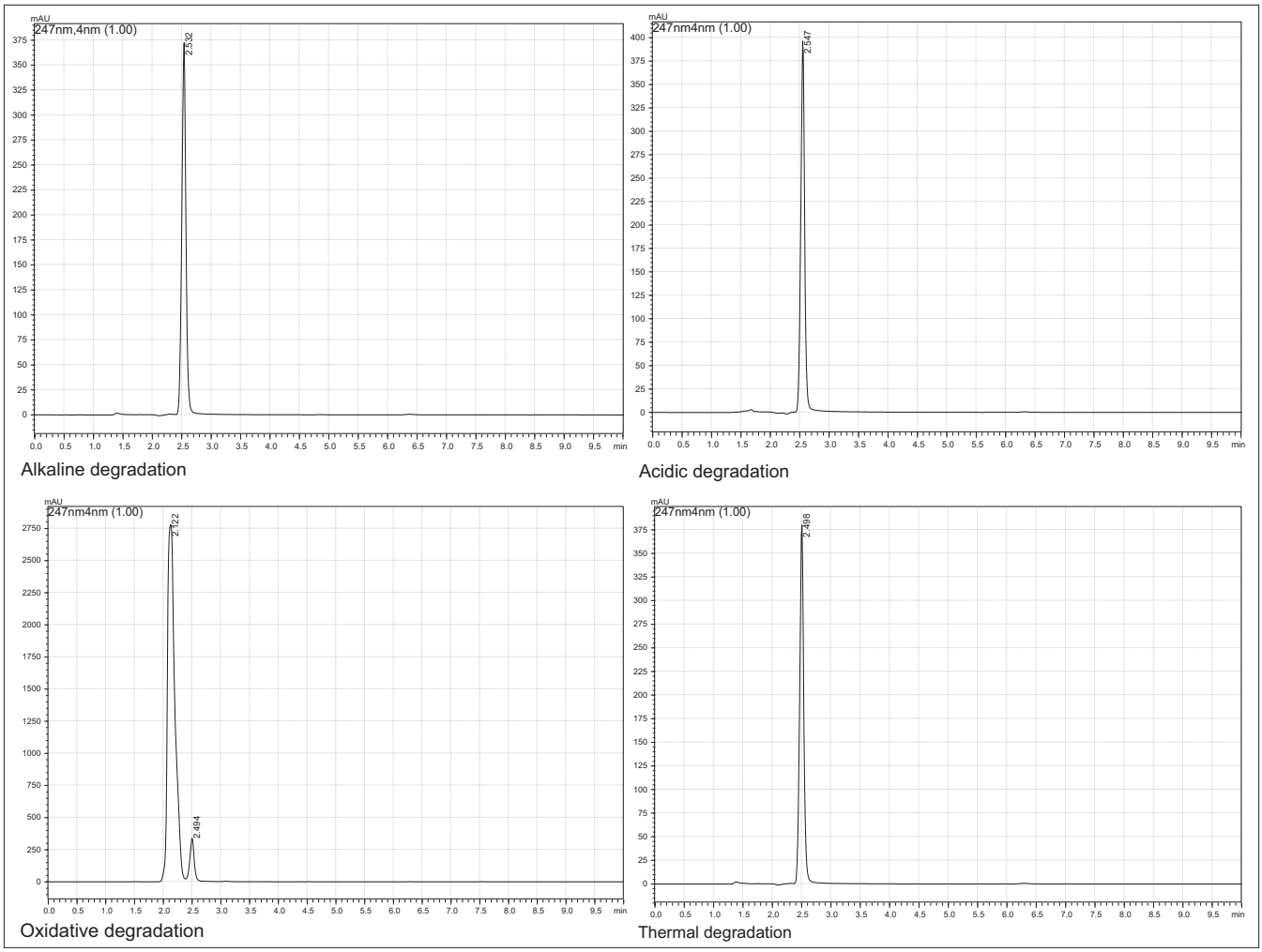

Figure 4: Representative chromatograms of Etoricoxib (20 µg/mL) during stress degradation studies

Table 7, and the corresponding chromatograms are shown in Figure 4.

CONCLUSION

Etoricoxib is an anti-inflammatory drug. A validated stability-indicating RP-HPLC method was proposed for the quantification of Etoricoxib in tablets. During the degradation studies, it was observed that Etoricoxib has shown less than 5% degradation, i.e., it is highly resistant toward all the stress conditions, and there is no interference of degradant peaks, indicating that the method is selective and specific. The system suitability parameters are within acceptable criteria, and the method is simple, economical, accurate, precise, robust, and useful for the quantification of Etoricoxib in pharmaceutical formulations.

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