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RP-HPLC ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ETORICOXIB AND TRAMADOL IN SYNTHETIC MIXTURE

Bhakti Dave, Chainesh Shah*, Komal Chaudhary, Sharma Sheetal, Priyanka Patil, Umesh M. Upadhyay

Sigma institute of Pharmacy, Vadodara, Gujarat.

***Corresponding Author: Dr. Chainesh Shah**

Sigma institute of Pharmacy, Vadodara, Gujarat.

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ABSTRACT

A Specific, precise, accurate, Robust and cost-effective RP-HPLC method was developed for the Simultaneous Estimation of Etoricoxib and Tramadol in Synthetic Mixture. RP-HPLC method was designed using an HPLC system. After optimization of good chromatographic separation was achieved with a mixture of ortho-phosphoric acid (OPA), methanol, and acetonitrile were filled in different mobile phase reservoir after filter and sonicate to degas the mixture. Mobile phase Buffer (pH 5 adjusted with OPA): methanol, and acetonitrile in the volume ratio 30:35:35v/v/v were used as the mobile phase with, Shimpack ODS C18 column 25 cm (4.6 mm x 250mm, 5 um) as stationary phase at flow rate of 1 mL/min and detection wavelength of 220 nm. The retention time of Etoricoxib and Tramadol was found to be 5.092 min and 14.30 min respectively.

KEYWORDS: RP-HPLC, Etoricoxib, Tramadol, orthophosphoric acid, RSD.

INTRODUCTION

The central ring of the dipyridinyl derivative etoricoxib (MK-663) has a phenyl group bonded to it. Etoricoxib has a significant tissue distribution, was highly selective for COX-2, and is 92% bound to plasma. It has an elimination half-life of roughly 22 hours and distributed quickly, reaching its peak concentration in 1 to 2 hours. Etoricoxib was metabolized by cytochrome P-450–dependent oxidation, which causes patients with liver disease to have a prolonged elimination period. The maximum amount of 60 to 90 mg per day that was advised for long-term use is also 120 mg for acute pain.[1]

Tramadol has been thoroughly examined in the management of various chronic pain conditions ranging from moderate to severely severe. Tramadol is effective in treating rheumatoid arthritis, low back pain, osteoarthritis-related pain, and neuropathic pain. Although there is conflicting evidence regarding its effectiveness in treating acute and postoperative pain, it can enhance the analgesic response when used in conjunction with nonopioid analgesics. The majority of research on acute pain has been conducted using parenteral preparations.[2]

The objective of this study to assess patients experiencing back pain, extending from the lowest rib to the gluteal crease, with or without leg radiation. These patients are experiencing either their first episode of back pain or have had a previous episode at least 6 months ago, lasting no more than 6 weeks. As per the physician's judgment, they require treatment with a combination of an anti-inflammatory and an analgesic. This research aims to investigate the effectiveness and safety of a fixed-dose combination comprising 90 mg of etoricoxib and 50 mg of tramadol, for the management of acute low back pain.[3]

EXPERIMENTAL

REAGENTS AND MATERIALS

The drug sample Etoricoxib and Tramadol was procured from Zota health care, Etoricoxib (Zota health care), Tramadol(Zota health care), Methanol (Ranchem Ltd.), Acetonitrile (Ranchem Ltd.), Water, Potassium Dihydrogen Phosphate, Disodium Hydrogen Phosphate(Ranchem Ltd.) Etorocoxib and RANCHEM LTD.)

Instrumentation

Electronic analytical balance(AUW-220D), sonicator(Trans-o sonic), RP-HPLC (Cyber Lab LC 100 model)

Structure

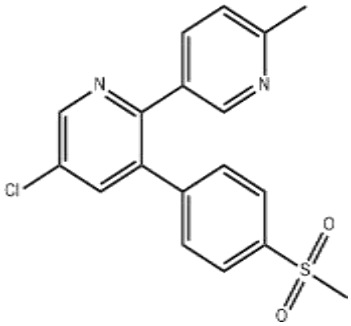

Figure.1 Structure of Etoricoxib

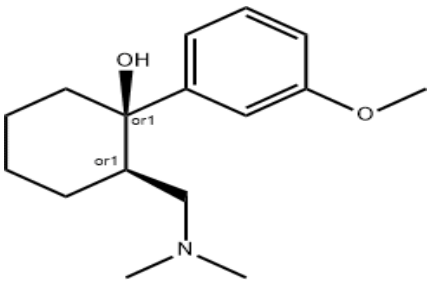

Figure.2 Structure of tramadol.

Chromatographic condition

The mobile phase of etoricoxib (IP-2018) was prepared by mixture of 50 volumes of 0.05 M ammonium acetate and 50 volumes of acetonitrile. Solvent delivery was at a flow rate of 1.0 ml/min. detection of the analytes was carried out at 235nm.The mobile phase of tramadol (IP-2022) was prepared by mixture of 1 volume of trifluoracetic acid, 30 volume of acetonitrile and 69 volume of water. Solvent delivery was at a flow rate of 1.0 ml/min. detection of the analytes was carried out at 271nm.[4]

Sample preparation

Standard solution of Etoricoxib - Accurately weighed 90 mg of Etoricoxib were transferred separately in 10 ml volumetric flasks, dissolved in small volume of methanol and then volume was adjusted to the mark with methanol to obtain concentration of 9000 µg/ml of ETO. These solutions were further diluted to obtain concentration of 900 µg/ml of ETO. Then, five quantities of 0.5, 1.0, 1.5, 2.0, and 2.5 mL were pipetted out to 10-mL flasks and diluted with methanol to prepare a series of standard solutions of ETO with concentrations of 45, 90, 135, 180, and 225 µg/ml.

Standard solution of tramadol - Accurately weighed 50 mg of TRA were transferred separately in 10 ml volumetric flasks, dissolved in small volume of methanol and then volume was adjusted to the mark with methanol to obtain concentration of 5000 µg/ml of TRA. These solutions were further diluted to obtain concentration of 500 µg/ml of TRA. Then, five quantities of 0.5, 1.0, 1.5, 2.0, and 2.5 mL were pipetted out to 10-mL flasks and diluted with methanol to prepare a series of standard solutions of TRA with concentrations of 25, 50, 75, 100, and 125 µg/ml.

Standard solution of preparation of synthetic mixture of etoricoxib and tramadol - The synthetic mixture of Etoricoxib (ETO) and Tramadol (TRA) was made in the ratio of 90:50 (ETOR: TRAM). The same procedure was followed as per above. Except that the same final 10-mL flask was used for both Etoricoxib (ETO) and Tramadol (TRA) with concentrations of 90 µg/mL and 50 µg/mL respectively. Take synthetic mixture equivalent to 90 mg Etoricoxib (ETO) and 50 mg Tramadol (TRA) in 100ml volumetric flask and add methanol up to the mark give solution strength (900, 500µg/ml) and sonicate for 10min and filter. Take 1ml from above solution and transferred in 10ml volumetric flask and make the volume up to mark with methanol give solution strength (90 µg/ml of ETO, 50 µg/ml of TRA).

Method of validation

System suitability of studies - The System suitability was done by analyzing six replicates of Etoricoxib (ETO) and Tramadol (TRA) in the mixture concentration 45 µg/ml and 25 µg/ml. The acceptance criteria of % R.S.D is less than 2% for peak area and retention time, theoretical plates greater than 2000, tailing factor less or equal to 2.0 and resolution greater than 2.0. The results of system suitability analysis were within acceptance criteria.

Table: 1 System suitability analysis.

Drugs	Parameters	volume
Etoricoxib (ETOR)	**Retention Time**	5.09 min
	Theoretical Plate	300 3
	Tailing Factor	1.4 9
	Resolution	-
Tramadol (TRAM)	**Retention Time**	14.30 min
	Theoretical Plate	2165
	Tailing Factor	1.16
	Resolution	11.06

Specificity - The specificity of the method was determined by analyzing standard drugs and sample of Etoricoxib (ETO) and Tramadol (TRA). The excipient present in the synthetic mixture does not interference in the result. So, the results suggested that proposed method is specific**.**

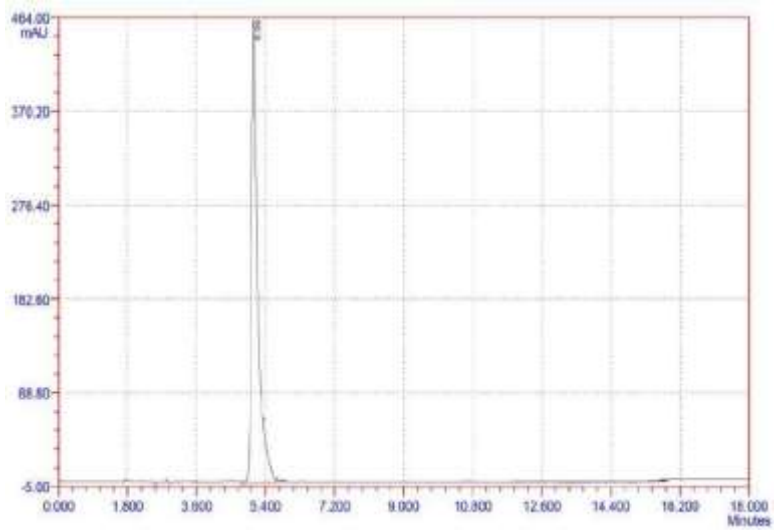

Figure: 3 - Chromatogram of standard Etoricoxib (ETO-45 µg/ml)

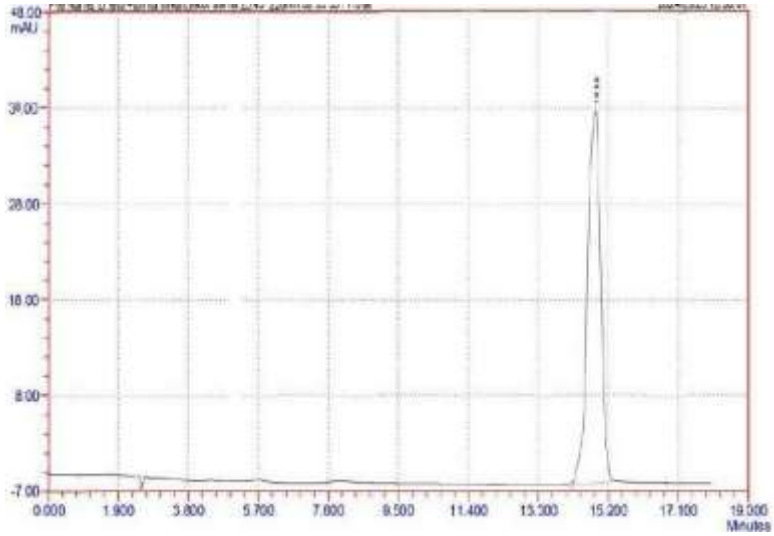

Figure: 4 - Chromatogram of standard Tramadol (TRA- 25 µg/ml)

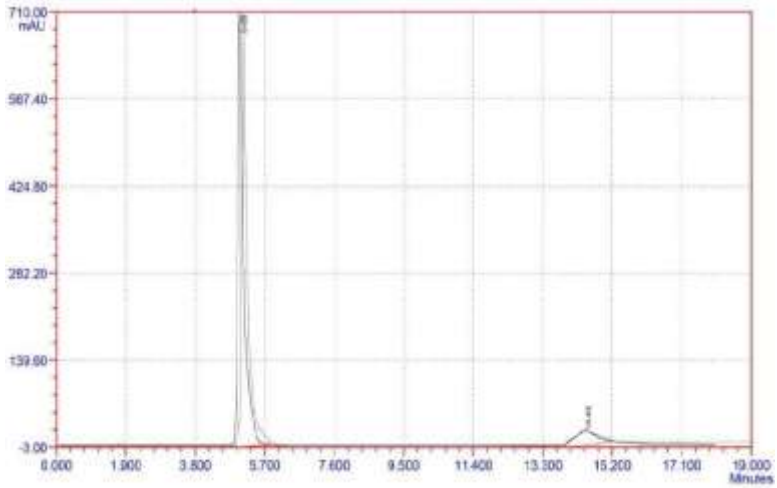

Figure: 5 - Chromatogram of standard and test from synthetic mixtureEtoricoxib (ETO- 45 µg/ml) and Tramadol (TRA- 25 µg/ml).

Linearity and range

The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of ETO with concentrations of 45, 90, 135, 180, and225 µg/mL and TRA with concentrations of 25, 50, 75, 100, and 125 µg/mL, respectively (n=6). The calibration curve of peak area vs. concentration was plotted and determines correlation coefficient. The concentration range of Etoricoxib (ETO) and Tramadol (TRA) was in the range of 45-225 µg/ml and 25-125 µg/ml respectively. The regression coefficient of determination is 0.9999 and 0.9976 for Etoricoxib (ETO) and Tramadol (TRA) respectively.

Table: 2 Linearity data of Etoricoxib (ETO) and Tramadol (TRA).

Sr. no	Etoricoxib (ETO)			Tramadol (TRA)		
	Conc. (µg/ml)	Mean peak area ± S.D. (n=6)	% R.S.D.	Conc. (µg/ml)	Mean peak area ± S.D. (n=6)	% R.S.D.
1	45	287613 ± 5144.98	1.79	25	28267 ± 342.33	1.22
2	90	610045 ± 8108.48	1.32	50	63376 ± 1034.92	1.62
3	13 5	947845 ± 16325.70	1.73	75	113040 ± 2114.78	1.86
4	18 0	1281813 ± 12602.68	0.99	10 0	154950 ± 2950.84	1.91
5	22 5	1621929 ± 26485.99	1.63	12 5	202415 ± 3980.26	1.97

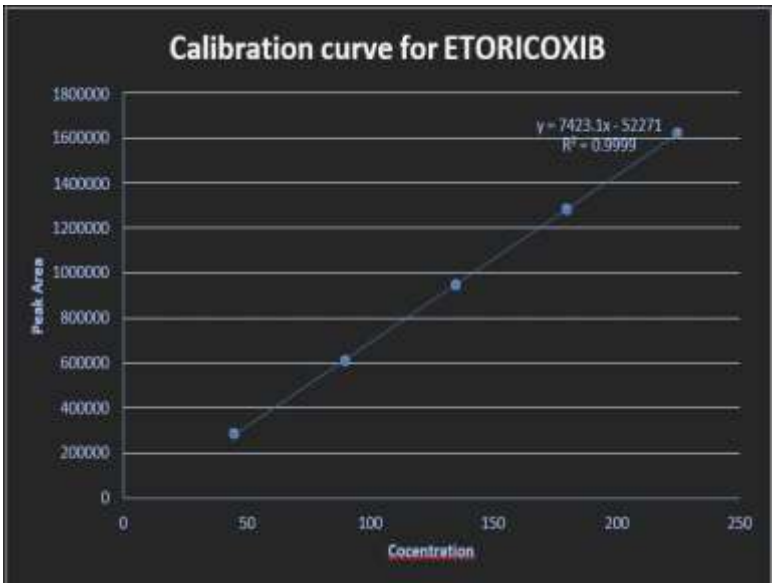

Figure 7: calibration curve of etoricoxib.

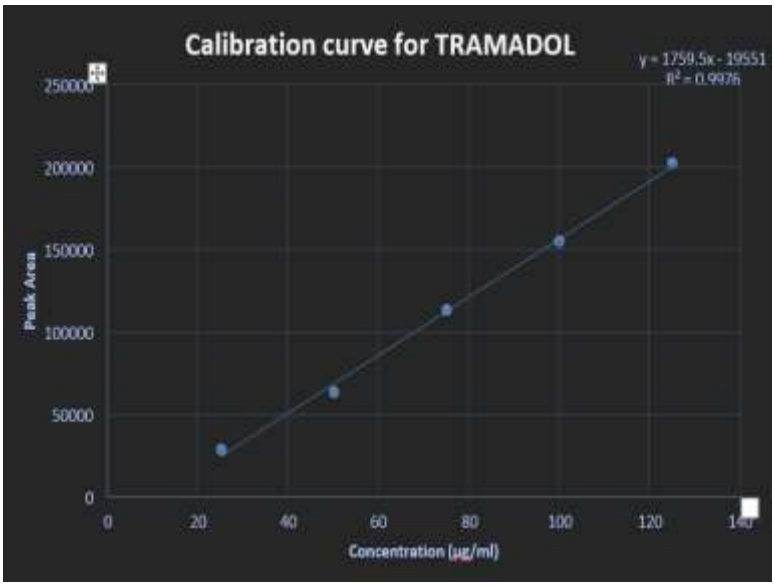

Figure 8: calibration curve of tramadol.

Precision

Repeatability: The repeatability was determined by analyzed solutions containing Etoricoxib (ETO) and Tramadol (TRA) in the mixture concentration 135 µg/ml and 75 µg/ml respectively and same solutions were analyzed six times at 220 nm and % R.S.D was calculated. The Solution of Etoricoxib (ETO) and Tramadol (TRA) containing 135 µg/ml and 75 µg/ml respectively and same solution were analyzed six times. The % RSD was found to be 1.94 % for Etoricoxib (ETO) and 1.90 % for Tramadol (TRA). These %RSD value was found to be less than 2.0% indicated that the method was precise.

Table: 3 repeatability data for Etoricoxib (ETO) and Tramadol (TRA).

Drug	Concentration (µg/ml)	Mean peak area ± S.D. (n=6)	% R.S.D.
Etoricoxib (ETOR)	135	946160 ± 18401.70	1.94
Tramadol (TRAM)	75	113944 ± 2174.36	1.90

Intraday precision: The intraday precision of the developed method was assessed by analyzing solutions containing concentrations 45, 135, 225 µg/ml for Etoricoxib (ETO) and 25, 75, 125 µg/ml for Tramadol (TRA) and three replicate (n=3) each on same day. The Solutions of Etoricoxib (ETO) and Tramadol (TRA) containing 45, 135 and 225 µg/ml and 25, 75 and 125 µg/ml respectively series were analyzed three times on the same day using developed HPLC method and % RSD was calculated. The % RSD was found to be 0.58-1.55 % for Etoricoxib and 0.97- 1.17% for Tramadol. These %RSD value was found to be less than 2.0% indicated that the method is precise. (Table 9)

Table: 4 Intraday precision data for estimation of Etoricoxib (ETO) and Tramadol (TRA) (n=3).

Conc. (µg/ml)		Mean peak area ± SD ETO	% RSD	Mean peak area ± SD TRA	% RSD
Etoricoxib (ETO)	Tramadol (TRA)				
45	25	285695.68 ± 1660.54	0.58	27984.63 ± 282.67	1.01
135	75	950815.32 ± 14728.35	1.55	113002.32 ± 1096.51	0.97
225	125	1638148.24 ± 16216.29	0.99	203703.71 ± 2391.61	1.17

Interday precision[5]- The Interday precision of the developed method was assessed by analyzing samples solutions containing concentrations 45, 135, 225 µg/ml for Etoricoxib (ETO) and 25, 75, 125 µg/ml for Tramadol (TRA) and three replicate (n=3) each on different day. The Solutions of Etoricoxib (ETO) and Tramadol (TRA) containing 45, 135 and 225 µg/ml and 25, 75 and 125 µg/ml series were analyzed three times on the different day using developed HPLC method and % RSD was calculated. The %RSD was found to be 1.00 – 1.88% for ETO and 0.99 - 1.72 % for TRA. The % RSD value was found to be less than 2.0% indicated that the method is precise. (Table 5)

Table: 5 Intraday precision.

Conc. (µg/ml)		Mean peak area ± SD ETO	% RSD	Mean peak area ± SD TRA	% RSD
Etoricoxib (ETO)	Tramadol (TRA)				
45	25	290679.06 ± 5470.00	1.88	28173.08 ± 326.40	1.16
135	75	947845.40 ± 9478.45	1.00	114174.17 ± 1961.18	1.72
225	125	1649069.228 ± 18915.71	1.15	204439.15 ± 2024.15	0.99

data for estimation of Etoricoxib (ETO) and Tramadol (TRA) (n=3).

Accuracy - The developed HPLC method was checked for the accuracy. It was determined by calculating the recovery of Etoricoxib (ETO) and Tramadol (TRA). The spiking was done at three levels 50 %, 100 % and 150 %. The amount of Etoricoxib (ETO) and Tramadol (TRA) was calculated at each level and % recoveries were computed. Percentage recovery for Etoricoxib (ETO) and Tramadol (TRA) by this method was found in the range of 99.26 to 101.56% and 98.14 to 101.55%, respectively.

Table: 6 Solutions for accuracy study.

Concentration of Synthetic mixture (µg/ml)		Concentration of API in spiking solution (µg/ml)		Total concentration of (µg/ml)	
Etoricoxib (ETO)	Tramadol (TRA)	Etoricoxib (ETO)	Tramadol (TRA)	Etoricoxib (ETO)	Tramadol (TRA)
90	50	45	25	135	75
90	50	90	50	180	100
90	50	135	75	225	125

Table: 7 Recovery data of ETO (n=3).[6]

Level	Conc. of ETO from Synthetic mixture (µg/ml)	Amount of Std. ETO added (µg/ml)	Total amount of ETO (µg/ml)	Total amount of ETO Recovered (µg/ml) Mean ± SD	% Recovery
0	90	0	90	91.41 ± 0.46	101.56 %
50%	90	45	135	135.13 ± 1.98	100.10 %
100%	90	90	180	181.41 ± 1.76	100.78 %
150%	90	135	225	223.34 ± 2.19	99.26%

Table: 8 Recovery data of TRAM (n=3).

Level	Conc. of TRA from Synthetic mixture (µg/ml)	Amount of Std. TRA added (µg/ml)	Total amount of TRA (µg/ml)	Total amount of TRA Recovered (µg/ml) Mean ± SD	% Recovery
0	50	0	50	49.07 ± 0.57	98.14 %
50%	50	25	75	73.85 ± 1.82	98.47%
100%	50	50	100	101.55 ± 2.25	101.55 %
150%	50	75	125	125.84 ± 1.86	100.67%

Robustness - The small deliberate change in HPLC conditions were used for determine robustness. In this method two changes were measured for both Etoricoxib (ETO) and Tramadol (TRA) in the mixture concentration 45 µg/ml and 25 µg/ml. Effect of flow rate 0.9 ml/min, 1.1 ml/min and effect of change in detection wavelength 218 nm and 222 nm, change in mobile phase composition on peak area of both drugs was observed. Effect on peak area was observed. Deliberate change in different parameters like Flow rate, Wavelength and buffer ratio showed Relative standard deviation of peak area less than 2 %, indicating that the method was robust. Results, presented in table 7.14 indicate that the selected factors remained unaffected by small variation of these parameters.

Table: 9 Robustness data of Etoricoxib (ETO) and Tramadol (TRA) (n=3).

Parameter	Change in condition	Mean Peak Area ± S.D. (n=3)		% R.S.D.	
		Etoricoxib (ETO)	Tramadol (TRA)	Etoricoxib (ETO)	Tramadol (TRA)
Change in detection wavelength	218nm	988375.84 ± 6674.96	112532.56 ± 1380.00	0.68	1.23
	222nm	955503.99 ± 9772.03	113436.44 ± 1217.83	1.02	1.07
Change inflow rate	0.9 ml/min.	974375.43 ± 14169.19	113899.99 ± 1308.95	1.45	1.15
	1.1 ml/min.	962681.14 ± 14611.91	112574.73 ± 1788.34	1.52	1.59
Mobile Phase Composition pH 5 with ortho-phosphoric acid (OPA), methanol, and acetonitrile in a ratio of 30:35:35	35:30:35	977191.26 ± 10620.58	114232.17 ± 1235.52	1.09	1.08
	25:40:35	976341.74 ± 9815.91	113924.09 ± 1480.96	1.01	1.30

LOD (Limit of detection) AND LOQ (Limit of qualification)[7]

The Limit of etection (LOD) and Limit of Quantification (LOQ) of the developed method was calculated from the five-calibration curve.

The LOD and LOQ were calculated by using this formula.

$$LOD = 3.3 * \frac{\sigma}{Slope}$$

$$LOQ = 10 * \frac{\sigma}{Slope}$$

Where, σ = standard deviation of intercept of 6 calibration curves

Slope = the mean slope of the 6 calibration curves

Table: 10 LOD and LOQ data of Etoricoxib (ETO) and Tramadol (TRA) (n=6).

Parameters	Etoricoxib (ETO) (µg/ml)	Tramadol (TRA) (µg/ml)
LOD	4.26	4.07
LOQ	12.92	12.34

Application of the proposed method for analysis of Etoricoxib (ETO) and Tramadol (TRA) in synthetic mixture. The concentrations of Etoricoxib (ETO) and Tramadol (TRA) in synthetic mixture were determined using the HPLC method. The % assay values are given in Table 15.[8]

Table: 11 Analysis data of formulation (n=3).

Sr. No	Drug	Concentration (µg/ml)	Amount of drug found (µg/ml)	% Assay ± SD
1	Etoricoxib (ETO)	90	91.71 ± 1.67	101.90 ± 1.85
2	Tramadol (TRA)	50	48.82 ± 0.43	97.63 ± 0.85

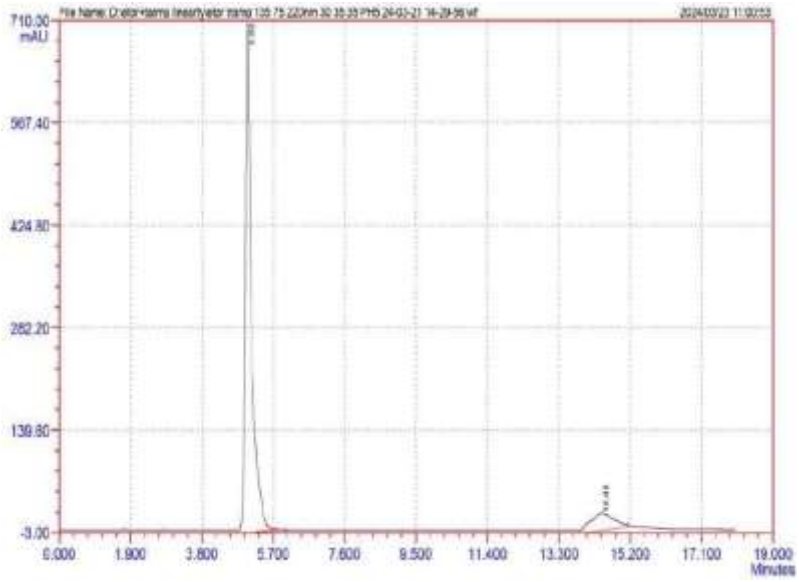

Figure: 14 - Chromatogram of synthetic mixture of Etoricoxib (ETO- 90 µg/ml) and Tramadol (TRA- 50 µg/ml).

Table: 17 Summary of validation parameters.

PARAMETERS	HPLC method	
	Etoricoxib (ETO) (µg/ml)	Tramadol (TRA) (µg/ml)
Concentration range(µg/ml)	45-225	25-125
Regression equation	y = 7423.1x - 52271	y = 1759.5x - 19551
Correlation Coefficient(r2)	0.9999	0.9976
Accuracy(%Recovery) (n=3)	99.26 – 101.56	98.14 – 101.55
Repeatability (%RSD) (n=7)	1.94	1.90
Intra-day Precision (%RSD) (n=3)	0.58 – 1.55	0.97 – 1.17
Inter-day precision (%RSD) (n=3)	1.00- 1.88	0.99 - 1.72
Assay (%Recovery) (n=3)	101.90 ± 1.85	97.63 ± 0.85

Specificity	specific	specific
LOD (µg/ml)	4.26	4.07
LOQ (µg/ml)	12.92	12.34
Robustness	Robust	Robust

All the parameters for two substances met the criteria of the ICH guidelines for the validation and found to be suitable for routine quantitative analysis in synthetic mixture. The result of linearity, accuracy, precision proved to be within limits with lower limits of detection and quantification.

CONCLUSION

In conclusion, RP-HPLC method development and validation for Etoricoxib (ETO) and Tramadol (TRA) in both synthetic mixtures and bulk samples have been successfully achieved. The calibration curves, constructed by plotting the mean peak area against concentration across the ranges of 45-225 µg/ml for ETO and 25-125 µg/ml for TRA, demonstrated excellent linearity. The % Relative Standard Deviation (% RSD) values obtained were 1.94% for ETOR and 1.90% for TRA, indicating high precision as they were both below the acceptable limit of 2.0%. Moreover, the percentage recovery for ETO and TRA ranged from 99.26% to 101.56% and 98.14% to 101.55%, respectively, indicating the accuracy of the method. The robustness of the method was confirmed by the relative standard deviation of peak area being less than 2%. Overall, these results validate the reliability and accuracy of the developed HPLC method for the simultaneous quantification of ETO and TRA in synthetic mixtures and bulk samples.

The developed method holds significant potential for various applications in pharmaceutical analysis, including quality control assessments, batch-to-batch consistency evaluations, and formulation studies. Its capability to quantify ETO accurately and precisely and TRA within the specified concentration ranges makes it suitable for routine analysis in pharmaceutical laboratories. Additionally, the method's robustness ensures consistent performance even in the presence of minor variations in experimental conditions, further enhancing its applicability and reliability in practical.

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