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"STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF CELECOXIB AND TRAMADOL HCL IN SYNTHETIC MIXTURE"

P Kunjan[1],P Bhumi[2],P Ankita[3], P Divyakant[4], P Jaymin[5]

student[1], Assistant professor[2], Assistant professor[3], Assistant professor[4], Assistant professor[5]

Department of Quality Assurance

Address: Sharda School of pharmacy, Pethapur, Gandhinagar, Gujarat 382610

ABSTRACT: Simple, rapid, economical, precise and accurate Stability indicating RP- HPLC method for the estimation of Celecoxib and Tramadol HCL in synthetic mixture has been developed. A reverse phase high performance liquid chromatographic method was developed for the estimation of Celecoxib and Tramadol HCL in synthetic mixture has been developed. The separation was achieved Column Agilent eclipse XDB-C18 (150 x 4.6 x 5), Gradient program 0.1%TFA: Methanol, as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 225 nm retention time of Celecoxib and Tramadol was found to be 4.138 and 8.870 min. The method has been validated for linearity, accuracy and precision. Linearity observed for Celecoxib and Tramadol HCL in synthetic mixture 27.72-65.67 µg/ml. Developed method was found to be accurate, precise and rapid for estimation of Celecoxib and Tramadol HCL in synthetic mixture. The drug was subjected to stress condition of hydrolysis, oxidation, photolysis and Thermal degradation, under same chromatographic condition. The stress samples were assayed on RP-HPLC system.

KEYWORDS:

Celecoxib, Tramadol Stability indicating RP- HPLC Method, Validation.

I . INTRODUCTION:

Tramadol is a strong painkiller from a group of medicines called opiates and Celecoxib is a non-steroidal anti-inflammatory drug (NSAID) used to treat mild to moderate pain and help relieve symptoms of arthritis (eg, osteoarthritis, rheumatoid arthritis, or juvenile rheumatoid arthritis), such as inflammation, swelling, stiffness, and joint pain.[1] Despite the establishment of new guidelines for pain management and the availability of hundreds of proprietary analgesics on the market with manufacturer's claims of efficacy, postoperative pain management remain often subpar. However, the sensible strategy for treating acute pain, which this study aimed to investigate, is to rely on the best available data from systematic reviews of reliable randomized trials[2-3] Tramadol and celecoxib are NSAIDS that primarily work by inhibiting the cyclooxygenase, an enzyme that promotes inflammation (COX). While celecoxib, a more recent medication, is a selective COX-2 inhibitor, Tramadol is a non-selective traditional NSAID (tNSAID) that inhibits both COX-1 and COX-2 in an unspecific manner. The body's COX-1 constitutive enzyme is found all over and contributes to the production of protective prostaglandins in the kidneys, platelets, and the stomach mucosa. On the other hand, COX-2 is only activated during inflammation and is only expressed in a small number of specialized tissues. Therefore, prostaglandin production is prevented when COX-2 is suppressed. After third molar surgery, this prevents inflammation and sensitization of peripheral nociceptors, which cause discomfort. tNSAIDs, despite being helpful at reducing pain and inflammation by inhibiting COX-2, carry a high risk of major gastrointestinal side effects, especially when used long-term. Traditional NSAIDs' ability to suppress prostaglandin (PGE2) and prostacyclin (PGI2) through the stomach mucosa's COX-1 is mostly responsible for

their ulcerogenic effects. A synthetic analgesic with a central action, tramadol shares structural similarities with morphine and codeine. It is a racemic combination of two pharmacologically active enatiomers, and studies on people have shown that it exerts analgesic effects from both opioid and non-opioid analgesic mechanisms. [4-6]

Because there are some clinically significant differences across the pain models, medications for treating acute postoperative pain that come from distinct pain models have limitations and downsides. To the best of our knowledge, there hasn't been a randomized controlled study on celecoxib, tramadol, or ibuprofen among Nigerians.[7, 8]

individuals undergoing surgery on their mandibular third molars will be given numerous doses of the painkillers ibuprofen, a nonselective COX inhibitor, celecoxib, a selective COX-2 inhibitor, and tramadol, a synthetic opioid[9].

High performance liquid chromatography (HPLC) is fastest growing analytical technique for analysis of drugs[10]

components move at different speed over the stationary phase and there by separated from each other.[11]

II . MATERIALS AND METHODS

Shimadzu HPLC, LC 2010 CHT model and LC Solution software was used. Acetonitrile, methanol, Diammonium hydrogen phosphate, Mili-Q water and ortho phosphoric acid of AR grade from Merck Life Science Pvt. Ltd, was used. A commercial dosage form Zita-D was purchased from local market

IR Identification and wavelength selection

Potassium Bromide IR disc was prepared using 1mg of Celecoxib and Tramadol on Hydraulic Pellet Press. This disc was scanned in the region of 4000–400cm^{-1} in FTIR and obtained IR spectrum was compared with the reference spectrum of celecoxib and tramadol

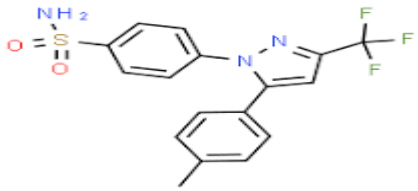


Figure 1. Structure of Celecoxib

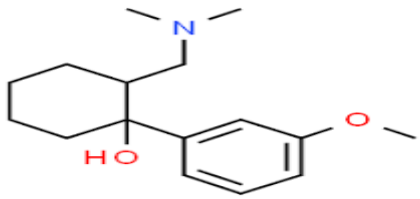


Figure 2. Structure of Tramadol

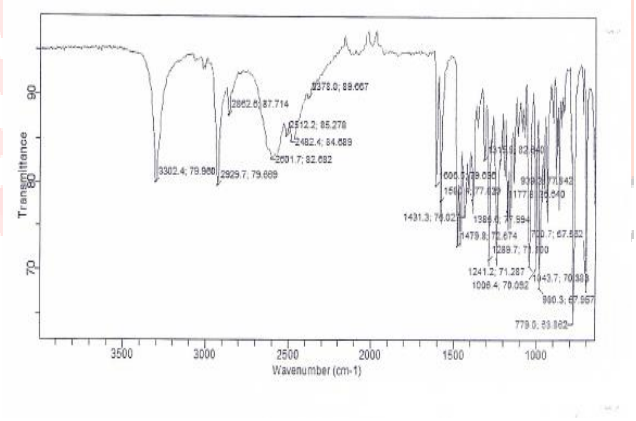


Figure 3: IR Identification of Celecoxib std

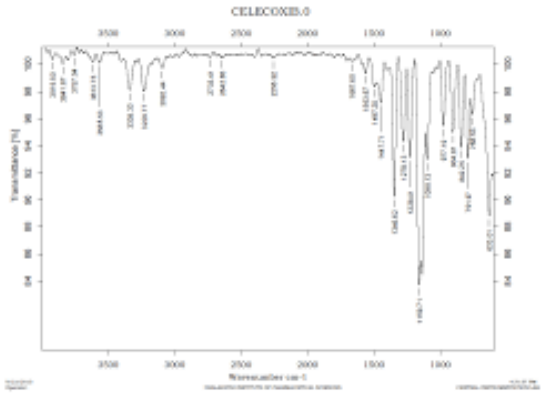


Figure 4: IR Spectrum of celecoxib std

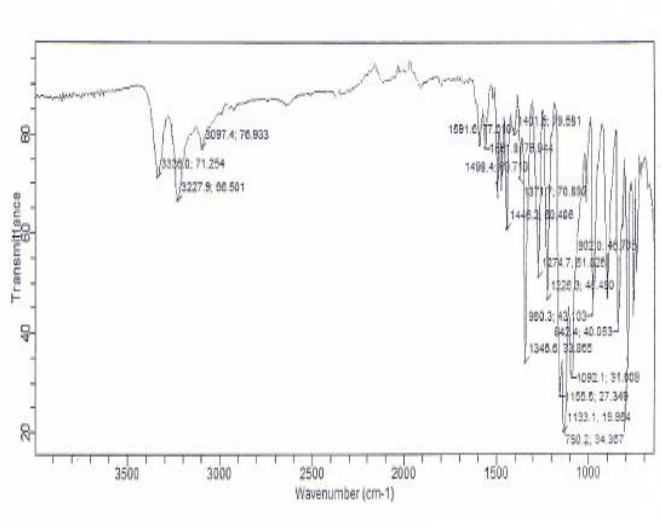


Fig -5 IR Identification of Tramadol

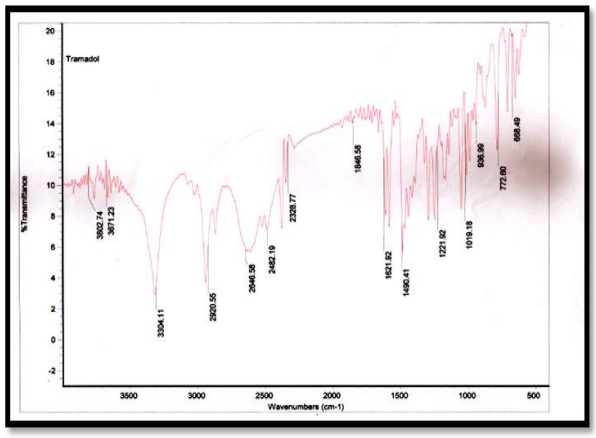


Fig -6 IR Identification of Tramadol

Sr. No.	Functional group	Observed value
1	N-H stretching	3330
2	C-N stretching	1274.7
3	C-H bending	1446.2
4	S=O stretching	1345
5	C-F stretching	1155.5

Table: 1 Interpretation of Celecoxib

sr. No.	Functional group	Observed value
1	O-H stretching	3302.4
2	N-H stretching	2929.7
3	C-H stretching	2852.5
4	C-H bending	1431.3
5	C-O stretching	1289.7
6	C-N stretching	1241.2

Table :2 IR Interpretation of Tramadol

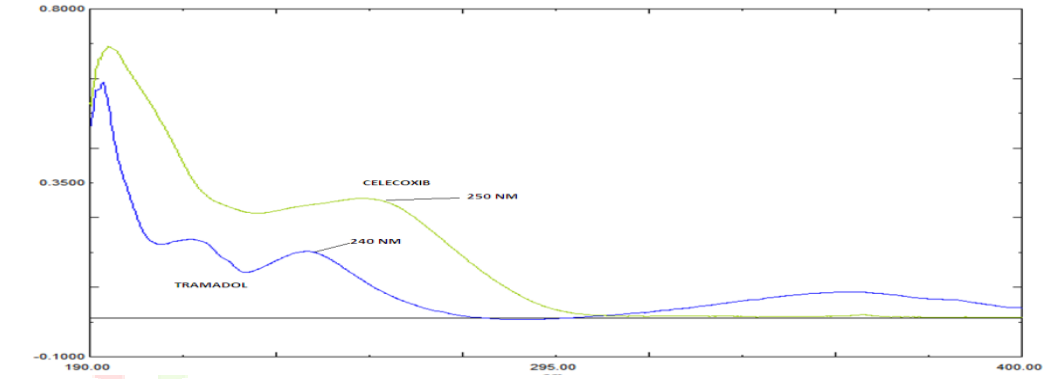


Figure: 7:Determination of wavelength maximum

Selection of Mobile Phase

Trial contains various mobile phase which are considered of Methanol and Water in different proportions and differen t volumes at different flow rate were tried. On the basis of various trial the Mobile phase A (0.1%TFA Buffer) 1 ml trifluoroacetic acid was added into 1000 ml miliQ water. Solution was degassed into sonicator. Mobile phase B: Methanol. at 1 mL/min flow rate, proved to be better than the other mixture in terms of peak shape, theoretical plate and a symmetry

Preparation of sample solution

Sample solution was prepared by taking a weight of granules equivalent to 56 mg of celecoxib and 44 mg of tramadol into 100 ml volumetric flask. To this, 30 ml of methanol was added and dissolved by sonication. The solution was diluted up to the mark with methanol. Further 2 ml aliquot was taken and diluted upto 20 ml with diluent.

METHOD DEVELOPMENT

Trial-1

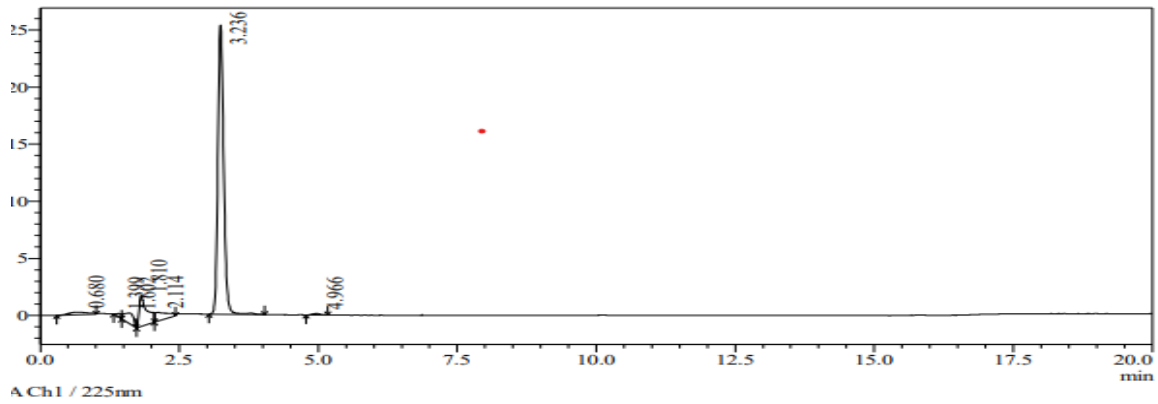


Fig: 8 Trial 1 with mobile phase 0.1% TFA:Methanol (50:50) %v/v

Trial -2

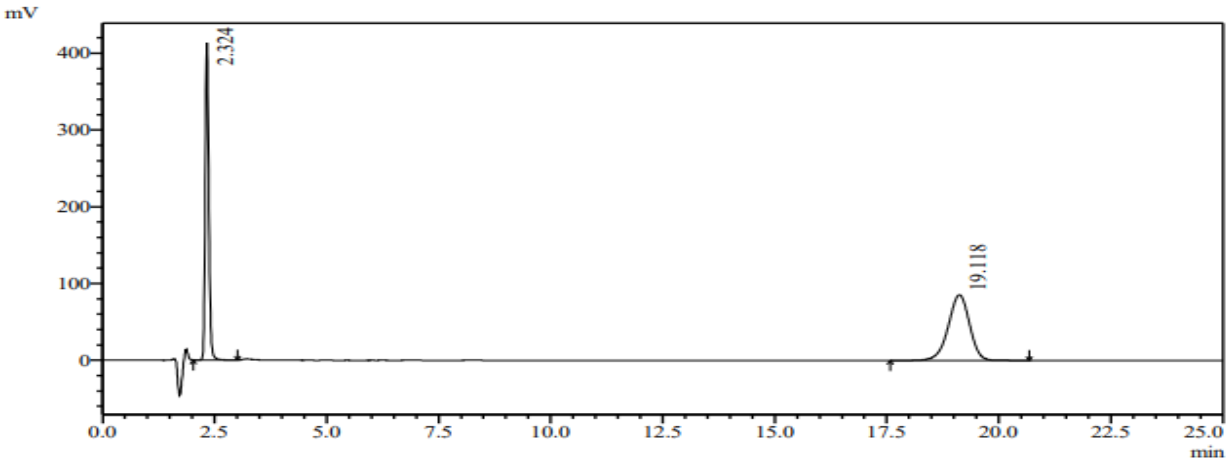
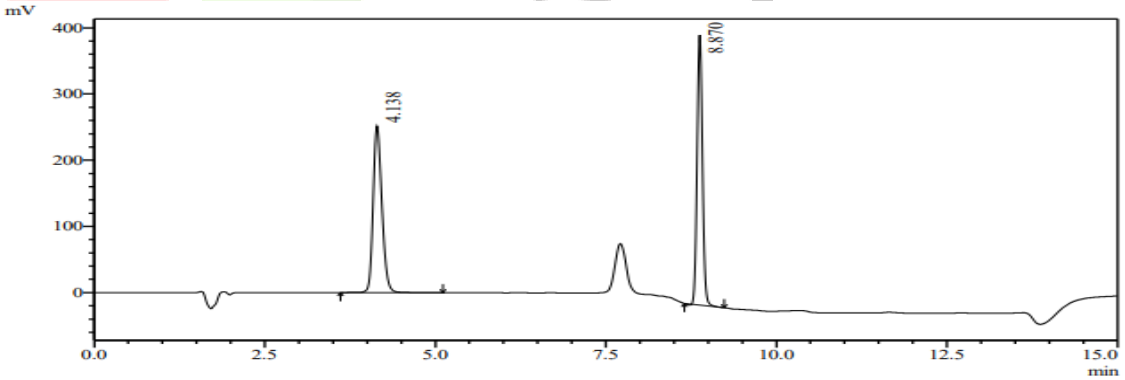


Fig :9 Trial 2 with mobile phase 0.1% TFA:Methanol (40:60) %v/v

Trial-3

Fig: 10 Trial 3 with mobile phase 0.1% TFA:Methanol (30:70) %v/v



Trial 4

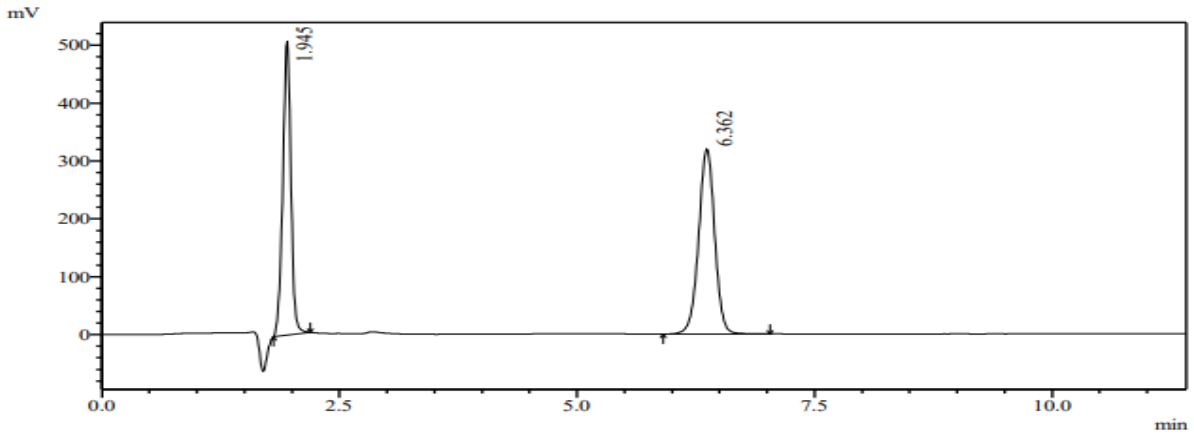


Fig :11 Trial with gradient flow summarized in table 8.1

Trial 5

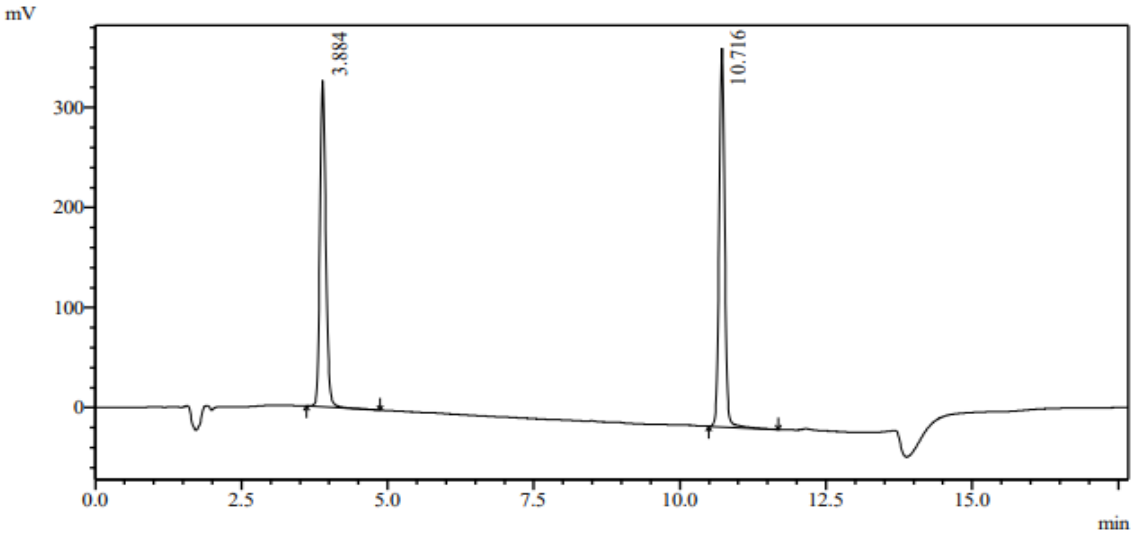


Fig: 12 Trial with gradient flow summarized in table 8.1

Table: 3 Mobile phase selection

Sr. no	Mobile phase composition	Inference
1	0.1% TFA: Methanol (50:50) %v/v	peak did not elute
2	0.1% TFA: Methanol (40:60) %v/v	peak elutes very late
3	0.1% TFA: Methanol (30:70) %v/v	peak elutes in void volume with higher organic concentration
4	0.1%TFA:Methanol (Gradient)	peaks are separated and proper peak shapes.
5	0.1%TFA:Methanol (Gradient)	peaks Baseline noise reduced, Tailing factor less than 2 for tramadol and celecoxib peaks.

IV. METHOD VALIDATION

Specificity

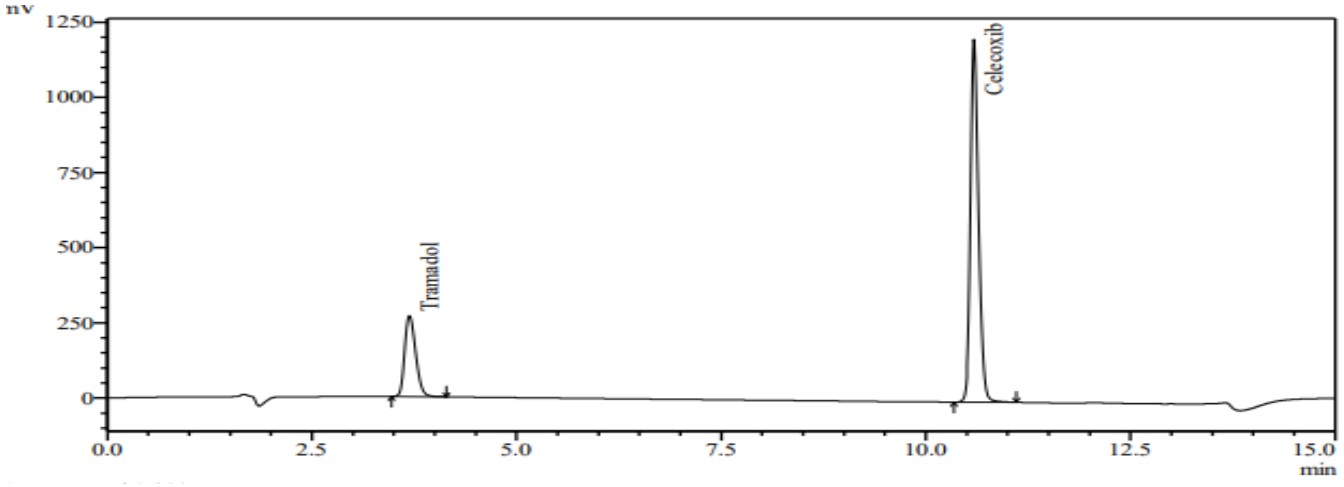


Fig 13 Chromatogram of Standard

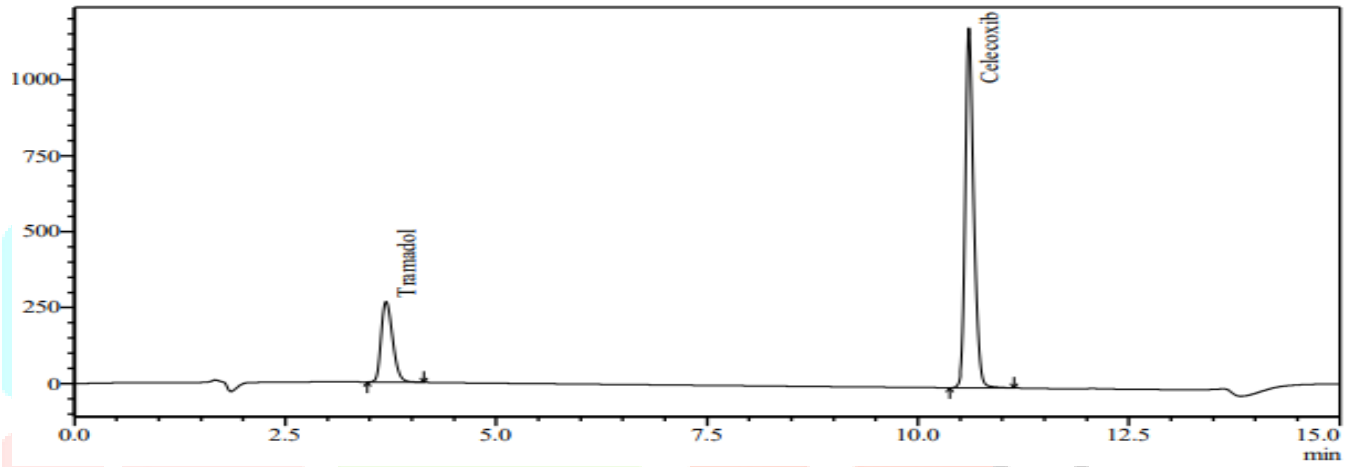


Fig 14 Chromatogram of Sample

Linearity

The linearity for Celecoxib was assessed by analysis of combined standard solution in range of 2.5-7.5µg/ml for Celecoxib

The linearity for tramadol was assessed by analysis of combined standard solution in range of 2.5-7.5µg/ml for tramadol

Regression curve overlay spectra and graph shown in figure no.

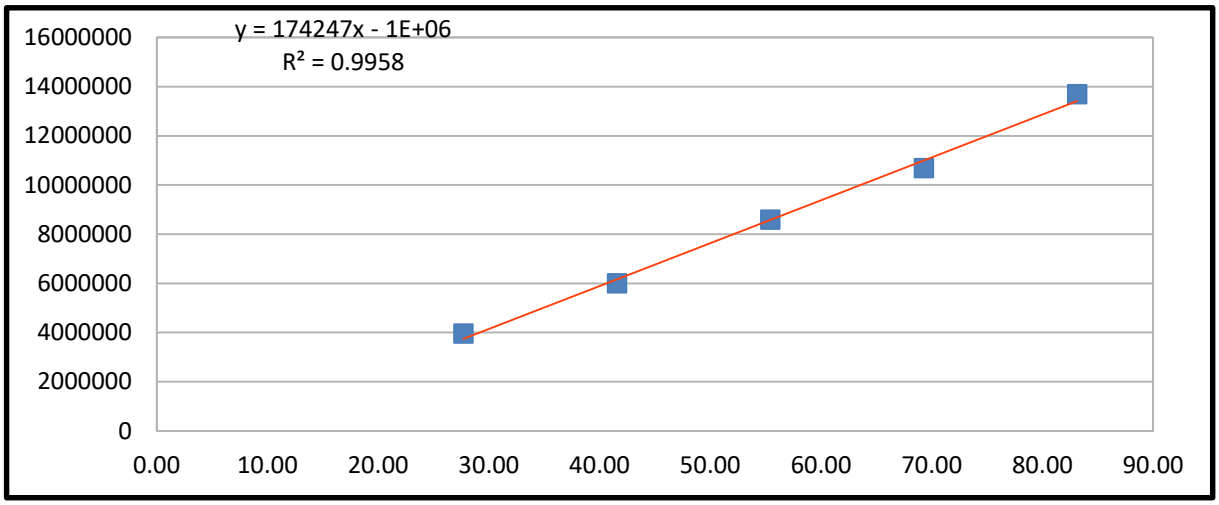


Fig 15 Linearity graph of Celecoxib

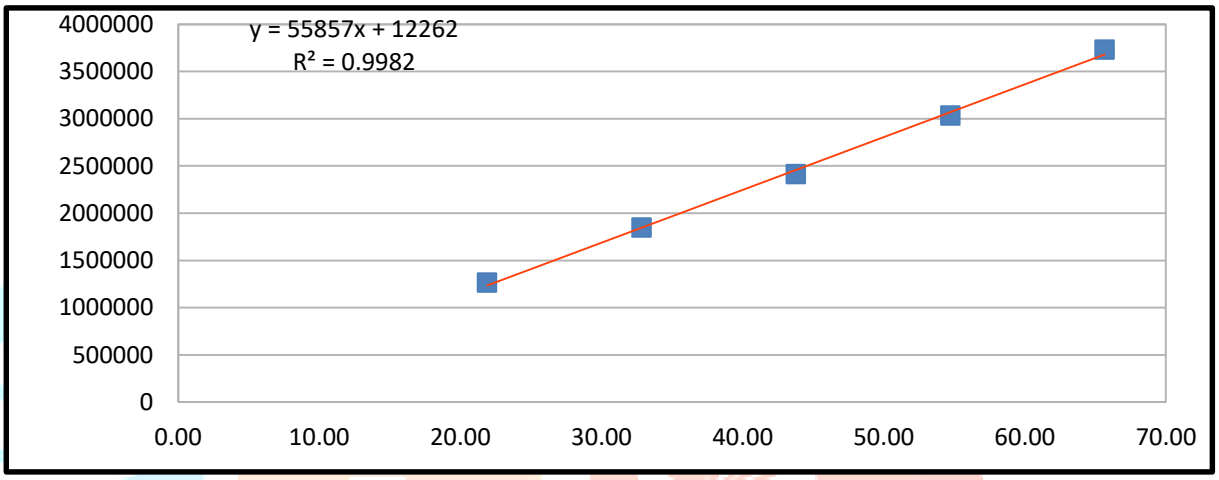


Fig 16 linearity graph of tramadol

Table 4: Linearity study of Celecoxib and tramadol

Celecoxib		Tramadol	
Concentration (µg/ml)	Peak Area	Concentration (µg/ml)	Peak Area
27.72	3951145	21.89	1265871
41.58	5998196	32.84	1846201
55.44	8589330	43.78	2414446
69.30	10678151	54.73	3032227
83.16	13686479	65.67	3729623

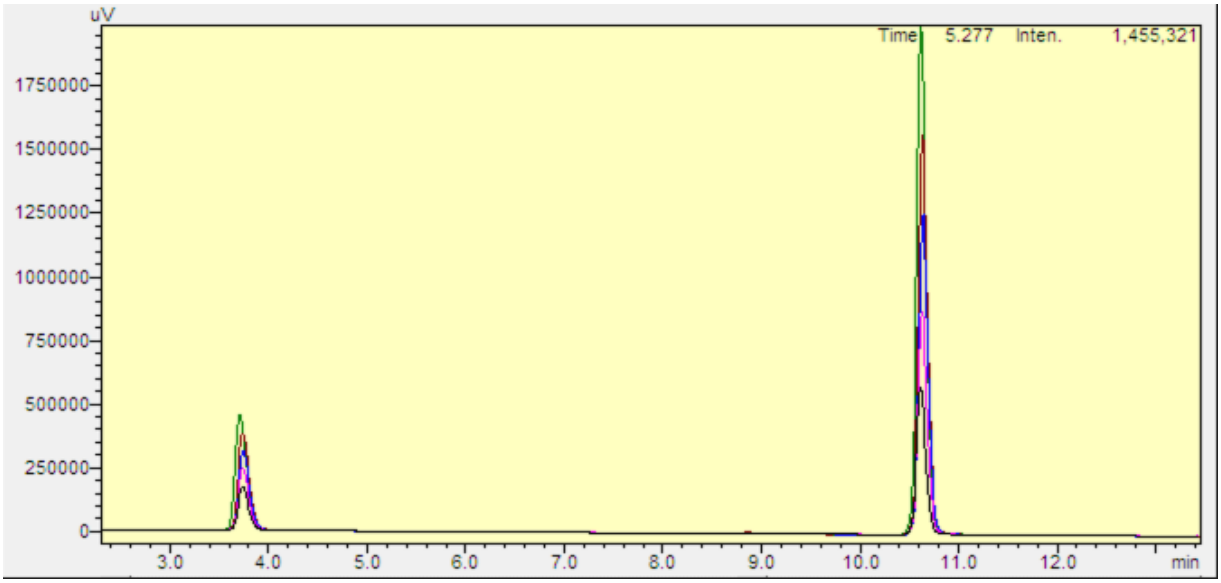


Figure 17: Overlain linearity chromatogram of Celecoxib and tramadol

Repeatability

The data for repeatability of peak area measurement for Celecoxib and Tramadol is based on six measurements of same solution of Celecoxib and Tramadol are depicted in below table. Procedure should be done within a laboratory over a short period of time using the same analyst with the same equipment and was expressed by %RSD. The %RSD for Celecoxib and Tramadol was found within the range

Table 5: Repeatability study

Concentrationof DAPA(µg/ml)	celecoxib		Concentration ofTENE(µg/ml)	Tramadol	
	Mean ± SD(n=6)	%RSD		Mean ± SD(n=6)	%RSD
27.72	3951147.667±1.75	0.0044	27.2	1265875±3.311	0.00026

Table 6: Intraday & Inter-day precision study of Celecoxib

Drug	Conc.(µg/ml)	Intra-dayprecision		Inter-dayprecision	
		Mean ± SD(n=3)	%RSD	Mean ± SD(n=3)	%RSD
Celecoxib	27.72	3951148.3 ±3.51	0.0088	3951123.3±2.51	0.0005

Table 7: Intraday & Inter-day precision study of Tramadol

Drug	Conc.(µg/ml)	Intra-dayprecision		Inter-dayprecision	
		Mean ± SD (n=3)	% RSD	Mean ± SD (n=3)	% RSD
Tramadol	21.89	1265875.7±5.03	0.0003	1265885.3±6.41	0.0005

Accuracy:

Table 8: Recovery study for Celecoxib and Tramadol

Sr. No.	Conc .Level(%)	Sample Amount	Amount Added	Amountrecovered(µg/ml)	Recovery	MeanRecovery ±SD
1	50 %	2.50	1.44	1.46	100.9	101.0± 0.2
2	100 %	5.0	2.81	2.80	99.5	98.02 ± 0.4
3	150 %	7.50	4.22	4.27	101.3	101.3 ± 0.1

Robustness

The Robustness was evaluated by Temperature, Flow rate and Org..During studies of Robustness there was not much change retention time, and symmetry of peak. The effect of changes was found to be with in the acceptance criteria which are show nin below table. The %RSD should be less than 2%.

Intra-day and inter-day Precision

Standard solution containing (27.72, 55.44, 83.16 µg/ml) for celecoxib and (21.89,43.78 and 65.67 µg/ml) was analyzed three times onsame day and %RSD was calculated. The date for intraday precision for celecoxib is shown in below table

Table:9 Robustness data for Celecoxib and Tramadol

Drug	Area at Temp. (-0.2°C)	Area at Temp. (+0.2°C)	Area at Flow (-0.2% ml/min)	Area at Flow (+0.2% ml/min)	Area at Mobile Phase (-0.2%)	Area at Mobile Phase (+0.2%)
Celecoxib	8307579	8316347	9237645	7499588	8299777	8326591
	8306283	8311165	9252417	7656983	8298337	8322950
	8315948	8312284	9263078	7666921	8293865	8319922
% R.S.D	0.1	0.0	0.1	1.2	0.0	0.0
Tramadol	2506344	2498780	2817238	2302335	2463266	2515462
	2508812	2497626	2819882	2243713	2463581	2510502
	2508759	2493323	2824508	2240831	2470573	2511624
% R.S.D	0.1	0.1	0.1	1.5	0.2	0.1

LOD and LOQ

LOD and LOQ was determined by following equation

LOD = 3.3 * SD/ Slope , LOQ = 10*SD/Slope

Where, SD = standard deviation

Table 10: Limit of Detection and Limit of Quantitation Data of Celecoxib and Tramadol

Celeoxib	Tramadol
LOD = 3.3 x (SD / Slope) = 0.921 µg/ml	LOD = 3.3 x (SD / Slope) =0.963 µg/ml
LOQ = 10 x (SD / Slope) = 3.125 µg/ml	LOQ = 10 x (SD / Slope) = 4.125µg/ml

V. Forced Degradation Condition

1. Acid degradation

- Acid decomposition studies were performed by transferring one ml of stock solution in to 50ml of volumetric flask. One ml of 5 N HCl solutions was added and mixed well and put for 4 hrs at Room temperature. After time period one ml of 5 N NaOH Added to neutralize the solution and make up the volume with diluents to get Celecoxib (56 **µg/mL**)and **Tramadol** (44 **µg/mL**)

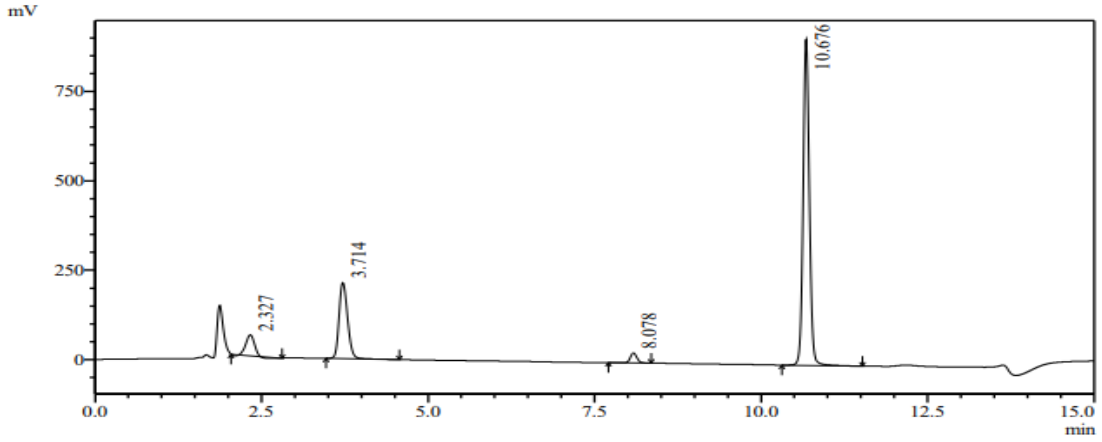


Fig 18 Standard chromatogram of acid degradation

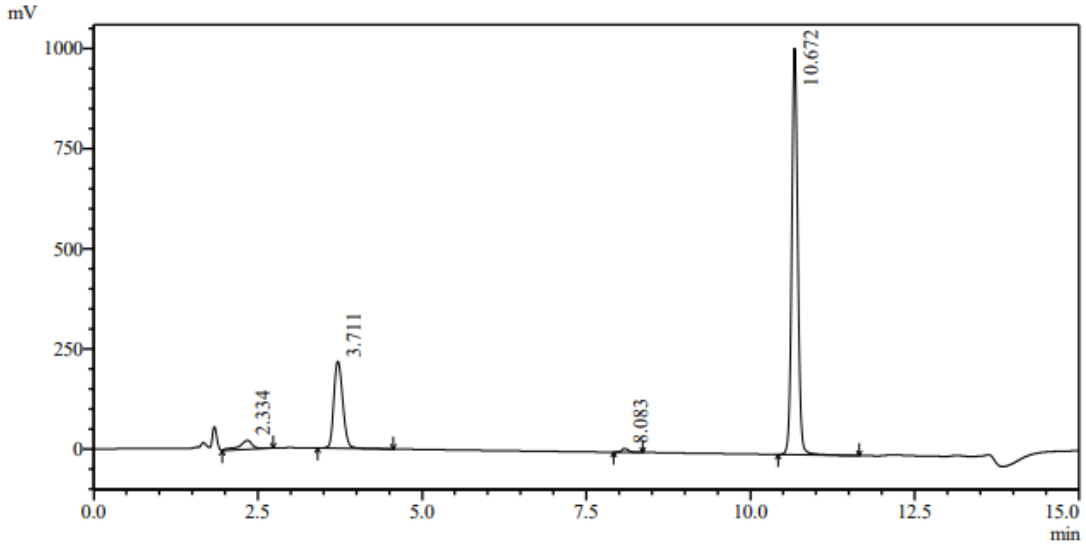


Fig 19 Sample chromatogram of acid degradation

2. Base degradation

- Base decomposition studies were performed by transferring one ml of stock solution in to 50 ml of volumetric flask. one ml of 5 N NaOH solutions was added and mixed well and put for 4 hrs at Room temperature. After time period one ml of 5 N HCl Added to neutralize the solution and make up the volume with diluent to get Celecoxib (56 **µg/mL**)and **Tramadol** (44 **µg/mL)**

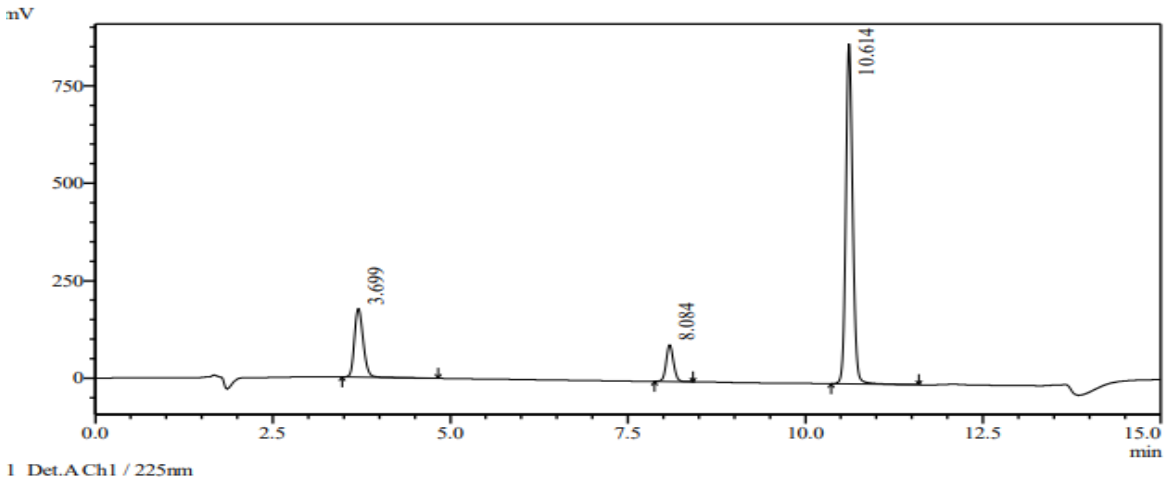


Fig :20 standard chromatogram of base degradation

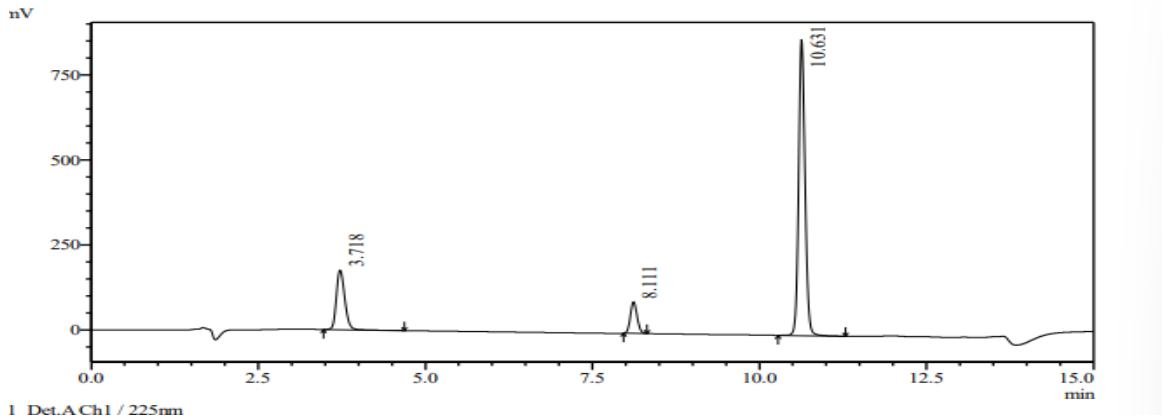


Fig : 21 Sample chromatogram of base degradation

3 . Oxidation degradation

- Oxidation decomposition studies were performed by transferring one ml of stocksolution in to 50 ml of volumetric flask. one ml of 3% H_2O_2 solutions was addedand mixed well and put for 4 hrs at Room temperature. After time period make up the volume with diluents to get Celecoxib (56 **µg/mL**) and **Tramadol** (44 **µg/mL)**

Fig: 22 Standard chromatogram of oxidative degradation

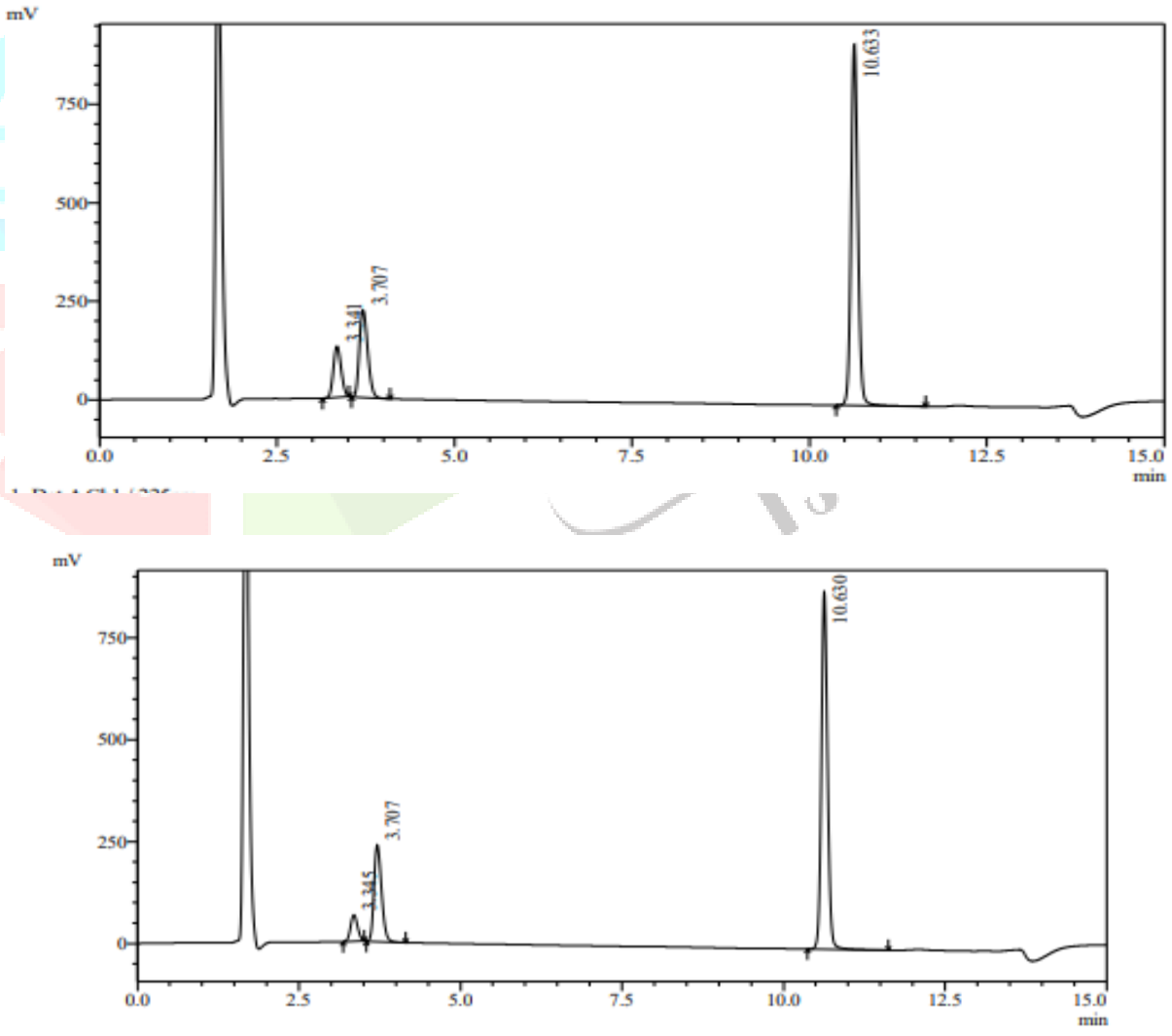


Fig: 23 sample chromatogram of oxidative degradation

4. Thermal Degradation

- Celecoxib (56 **µg/mL**) and **Tramadol** (44 **µg/mL)** was taken in 100ml Volumetric flask and put in oven for 4 hrs at 80^0C temperature, then after Volumetric flask was removed and cool atroom temperature, volume was made up with mobile phase, 1ml of this solutionwas transferred in 10ml volumetric and volume was made up with Diluent to getCelecoxib (56 **µg/mL**) and **Tramadol** (44 **µg/mL)**

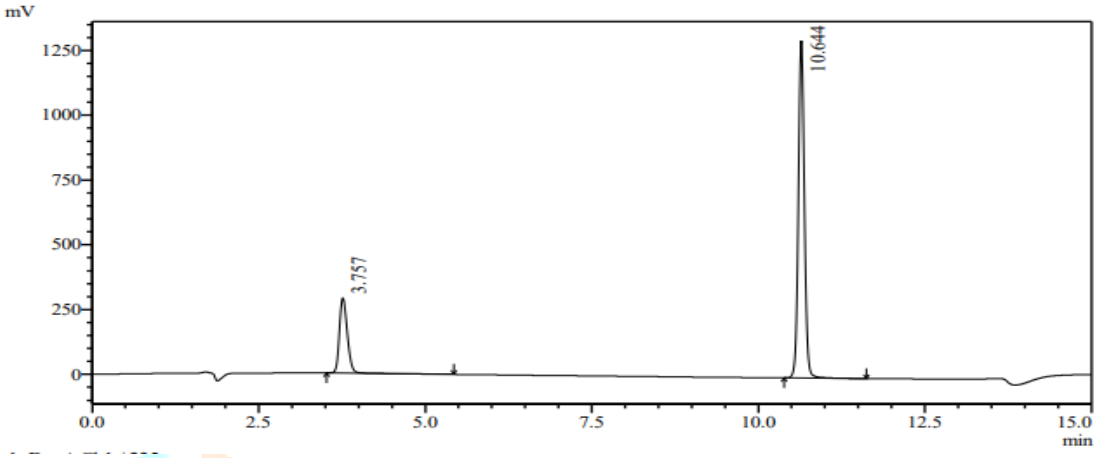


Fig 24 Standard chromatogram of Thermal degradation

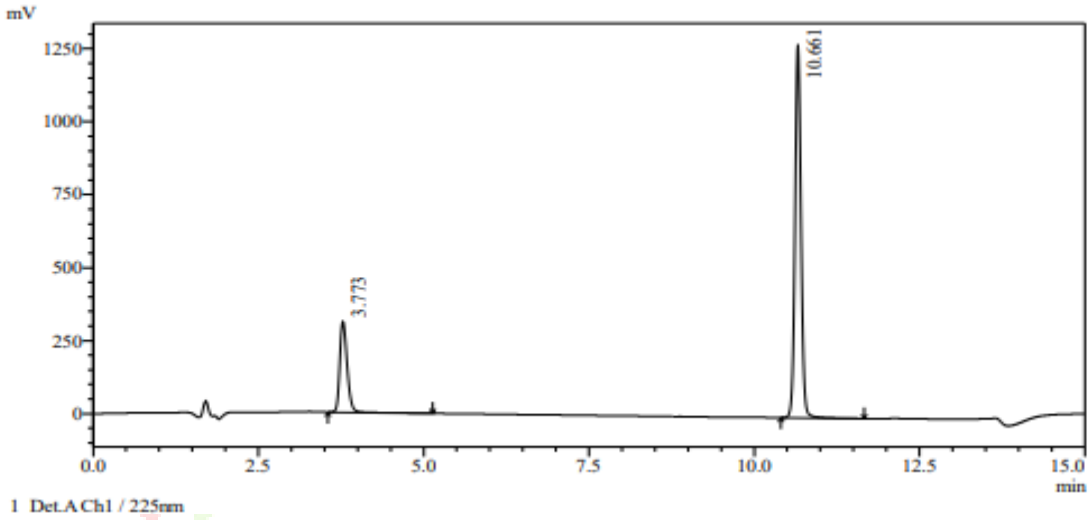


Fig 25 Sample chromate gram of Thermal degradatiom

5. Photo degradation

- Photo decomposition studies were performed by transferring one ml of stock solution in to 50ml of volumetric flask. Volumetric flask was kept in UV Chamber for 4hrs. After time period make up the volume with diluents to get Celecoxib (56 **µg/mL**) and **Tramadol** (44 **µg/mL)**

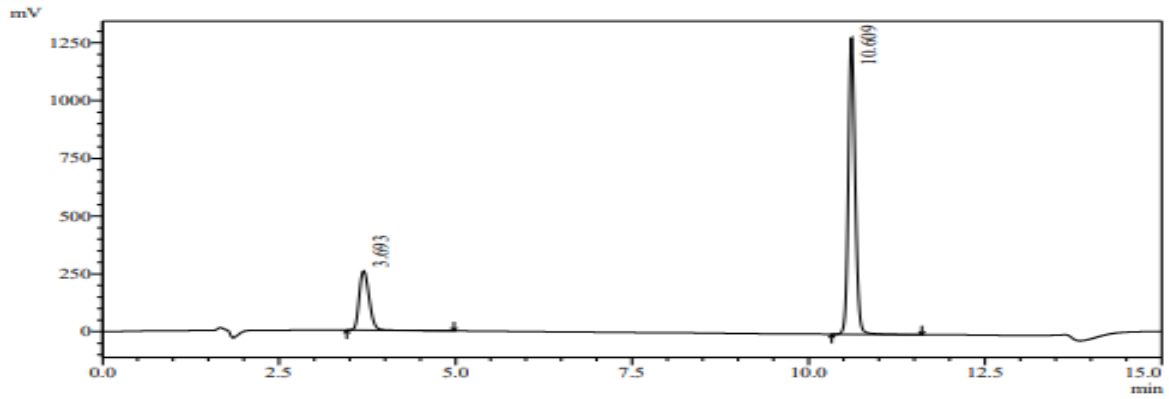


Fig 26 Standard chromatogram of photo degradation

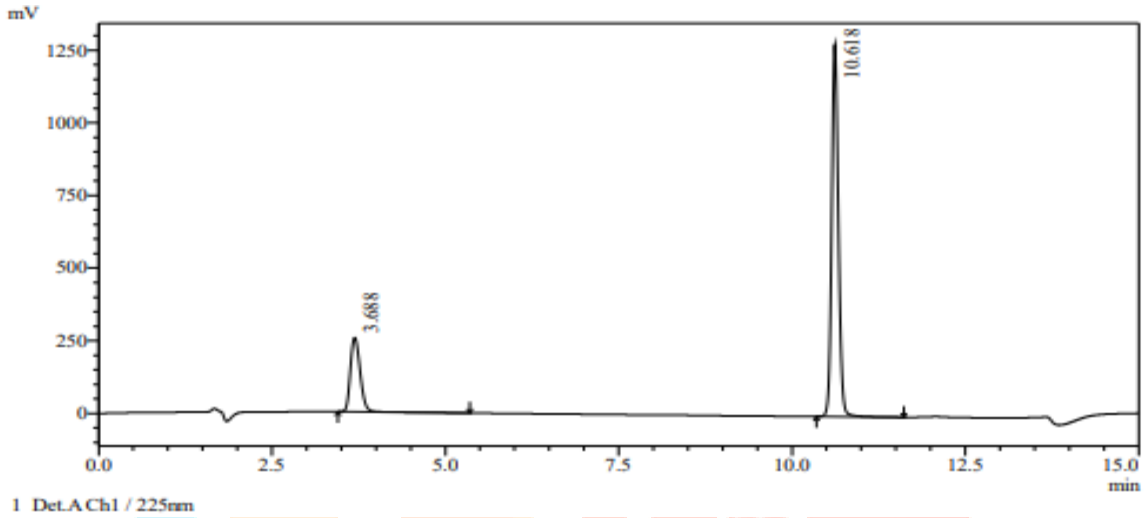


Fig 27 Sample chromatogram of photo degradation

Table 11: Result of stability study of DAPA and TENE

Condition	% Degradation celecoxib		% Degradation tramadol	
	Sample	Standard	Sample	Standard
Acid	21.3	27.3	18.1	24.5
Base	29.25	28.8	39.2	39.2
Oxidation	28.87	25.6	23.5	27.5
Thermal	1.11	0.31	4.29	4.22
Photo	-0.27	0.49	0.177	0.729

RESULT AND DISCUSSION

The present work aimed development and validation of stability indicating RP-HPLC method for simultaneous estimation of Celecoxib and Tramadol. The melting point of Celecoxib(157-159 °C) and Tramadol (178-181°C) was found in the range. Method was developed in mobile phase containing Gradient program0.1%TFA: Methanol. Detection was carried out at 225 nm. Method was validated as per ICH guidelines. Linearity and regression data were shown in table and Figure. % Recovery was within the range (99% - 102%). Results were shown in table. Hence it is found that the developed method is accurate. %RSD values were <2 for repeatability, intra-day and inter-day precision. Results were shown in table. So, the developed method was found to be precise. LOD and LOQ values were shown in table. LOD & LOQ confirms the method to be sensitive. Small changes were carried out in mobile phase and flow rate for robustness study, in that % RSD of area was found to be <2. So, the developed method was found to be robust. Various forced degradation conditions were performed in proposed method and it can efficiently separate all the degradation products from the drugs. % degradation values are 1% to 31% degradation of the drug substance, have been considered as reasonable and acceptable for validation of chromatographic assays. So, the developed method is stability indicating.

CONCLUSION

Tramadol and celecoxib are NSAIDS that primarily work by inhibiting the cyclooxygenase, an enzyme that promotes inflammation (COX). While celecoxib, a more recent medication, is a selective COX-2 inhibitor, Tramadol is a non-selective traditional NSAID (tNSAID) that inhibits both COX-1 and COX-2 in an unspecific manner. The body's COX-1 constitutive enzyme is found all over and contributes to the production of protective prostaglandins in the kidneys, platelets, and the stomach mucosa. On the other hand, COX-2 is only activated during inflammation and is only expressed in a small number of specialized tissues.

RP-HPLC method was developed for simultaneous estimation Celecoxib and Tramadol. In RP-HPLC method, good resolution and separation of two drugs was achieved. Gradient program 0.1%TFA: Methanol, mobile phase. Retention time of Celecoxib and Tramadol were found to be 4.138 and 8.870 min respectively with a flow rate of 1 ml/min. The proposed method was accurate and precise. Therefore, proposed method can be used for routine analysis of Celecoxib and Tramadol synthetic mixture

Forced degradation study of Celecoxib and Tramadol was performed by RP-HPLC method which includes Acid, Base, Oxidation, Photo and Thermal degradation .Results of degradation were found with in limit

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