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METHOD DEVELOPMENT AND VALIDATION OF KETOROLAC TROMETHAMINE IN TABLET FORMULATION BY RP-HPLC METHOD

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Keywords:

Ketorolac Tromethamine, RP-HPLC method, Method development, Method validation, UV detector

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ABSTRACT: In the present study, a simple, precise, and accurate method was developed and validated for analysis of Ketorolac Tromethamine in Tablet formulation. A gradient HPLC analysis was performed on Grace C18 column (250 cm × 4.6 mm × 5 µ). The compound was separated with a solvent mixture of Methanol and water in a ratio of 65:35 v/v with 0.1% O-phosphoric acid as the mobile phase at a flow rate of 1ml/min. The UV detection was performed at the ʎ max 245 nm. The retention time was found to be 7.70 min. The system suitability parameters such as theoretical plate count, tailing factor, and percentage relative standard deviation (RSD) between six standard injections was within the limit. The method was validated according to the International conference of harmonization (ICH) guidelines. The linearity was found to be in the concentration range of 5-25 µgm/ml as indicated by correlation coefficient (r^2) of 0.999. The robustness of the method was evaluated by deliberately altering the chromatographic condition. The developed method can be applicable for routine quantitative analysis.

INTRODUCTION: Ketorolac tromethamine is a non-steroidal anti-inflammatory drug (NSAID). Chemically 2-Amino-2-(hydroxymethyl) propane-1, 3-diol (1RS) -5- benzoyl-2, 3-dihydro-1H-pyrrolizine-1-carboxylate (API) (European Pharmacopoeia 2008) as shown in **Fig 1.** It is a member of the heterocyclic acetic acid derivative family and is used as an analgesic with an efficacy close to that of the opioid family. It is also a potent antipyretic and anti-inflammatory. It is mainly used for the short term treatment of post-operative pain as it is highly selective for the COX-1 enzyme [5].

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The USA Food and Drug Administration approves this Analgesic, and it is non-narcotic, fast acting, and non-addictive. It can be administered orally or by injection [12].

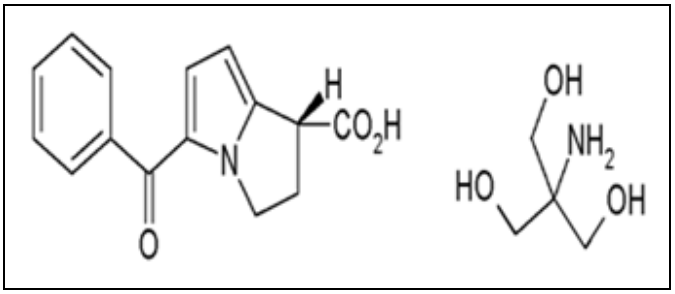

FIG. 1: STRUCTURE OF KETOROLAC TROMETHAMINE (MW=376.4)

Several studies for the estimation of the Ketorolac Tromethamine drug using various techniques have been carried out, some of them being; Development and Validation of Ketorolac Tromethamine in Eye Drop Formulation by RP-HPLC Method [1].

RP- HPLC method development and validation of acuvail drug [2]. New simultaneous UV-Visible spectrophotometric methods for estimation of ofloxacin and ketorolac tromethamine in ophthalmic dosage form [3]. Analytical method development and validation for the simultaneous estimation of febuxostat and ketorolac in tablet dosage forms by RP-HPLC [4]. Development and validation of a rapid liquid chromatographic method for the analysis of Ketorolac Tromethamine and its related production impurities [5].

Simultaneous RP-HPLC estimation of moxifloxacin hydrochloride and ketorolac tromethamine in ophthalmic dosage forms [8]. New spectrophotometric determination of ketorolac tromethamine bulk and pharmaceutical dosage form [9]. Two-dimensional liquid chromatography-ion trap mass spectrometry for the simultaneous determination of ketorolac enantiomers and paracetamol in human plasma application to a pharmacokinetic study [11]. Reversed-phase high–performance liquid chromatography of ketorolac and its application to bioequivalence studies in human serum [12]. A simple and sensitive method for the analysis of ketorolac in human plasma using high-performance liquid chromatography [13]. An indirect (derivatization) and a direct HPLC method for the determination of the enantiomers of ketorolac in plasma [14].

Experimental Procedures:

Instrument: Younglin (S.K 9000) gradient System with UV Detector (Autochrome -3000 software), Sartorius Electronic Analytical balance, Crest sonicator, and Grace C_{18} column (4.6 mm x 250 cm×5µm) was used.

Chemical and Reagents: Gift sample of Ketorolac Tromethamine was obtained from FDC Limited, Mumbai. A Pharmaceutical product (Ketrol- DT) containing the same amount of drug formulation was used in the experiment HPLC grade methanol was procured from Modern lab, Nasik. HPLC grade deionized water was used throughout the experiment.

Mobile Phase: Methanol and Water with 0.1% O-Phosphoric acid in a ratio of 65:35 v/v used as mobile phase. It was used as diluents for the preparation of sample and standard.

METHOD DEVELOPMENT:

Wavelength Detection: Accurately weighed Ketorolac Tromethamine equivalent to 0.1 gm in 100 ml volumetric flask, 100ml methanol was added, sonicate for 5min and filtered through 0.45 nylon membrane filter. Pipette out 1 ml of the above solution and dilute to 10 ml with methanol in 10 ml volumetric flask and scanned between 200-400 nm by UV spectroscopy. The λ_{max} found was 245 nm.

Chromatographic Conditions: Chromatographic separation was achieved at ambient temperature, the detection was carried at 245 nm at a flow rate of 1 ml/min, and run time was kept 16 min. Before the injection of drug solution column was equilibrated for 60 min with the mobile phase flowing through the system. The injection volume was 20 µl throughout the experiment. Blank containing mobile phase was injected to check the solvent interference.

Standard Preparation: The 10 mg of Ketorolac Tromethamine was weighed and transferred into a 10 ml volumetric flask and make up to the volume with methanol. From this take 0.05, 0.10, 0.15, 0.20 and 0.25 ml and dilute with mobile phase up to 10 ml for preparation of 5, 10, 15, 20 and 25 µgm/ml respectively. A representative chromatogram of the standard was shown in **Fig 2.**

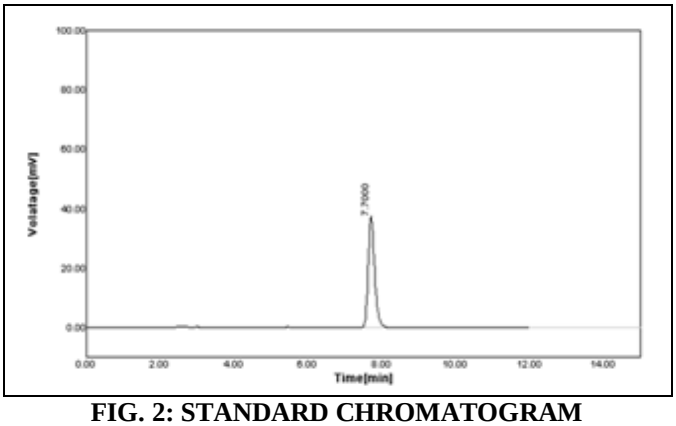

FIG. 2: STANDARD CHROMATOGRAM

Sample Preparation: The 220 mg powder of Ketrol - DT contain 10 mg Ketorolac tromethamine take in 10 ml volumetric flask and make up the volume with methanol. Sonicated for 15 min and filtered through a 0.45 µm nylon membrane filter. A representative chromatogram of the sample was shown in **Fig. 3** and **Table 1.**

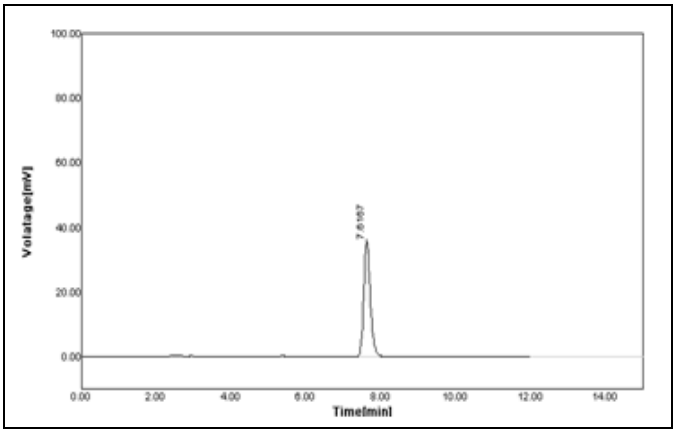

FIG. 3: SAMPLE CHROMATOGRAM

TABLE 1: ANALYSIS OF MARKETED FORMULATION

Commercial formulation	Ingredients	Labeled amount (mg)	Area	Amount found (mg)	% found
Ketrol – DT	Ketorolac Tromethamine	10 mg	365.8	10.14	100.70
Ketrol – DT	Ketorolac Tromethamine	10 mg	368.75	10.32	101.60

Evaluation of System Suitability Evaluation of System Suitability: The 20 µl of the standard solution was injected in six duplicate before and after the analysis, and the chromatogram was recorded. System suitability parameter like column efficiency, plate count, and tailing factor were also recorded. The column efficiency was determined was found to be more than 2000 USP plate count, USP Tailing for the same peak is not more than 2.0 and % RSD of six injections of the standard solution is not more than 2.0% the chromatogram was shown in **Fig. 4** and **Table 2.**

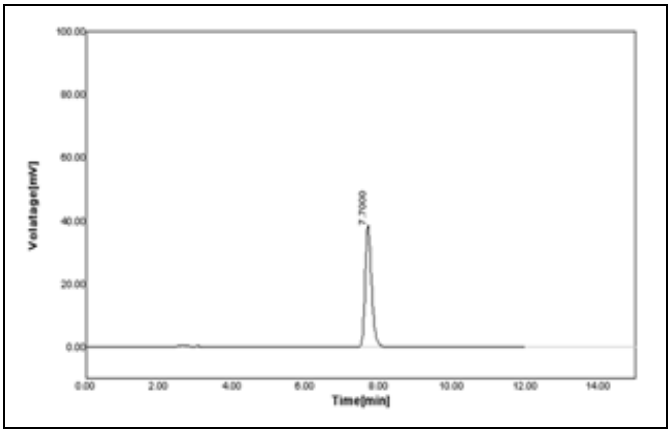

FIG. 4: SYSTEM SUITABILITY CHROMATOGRAM

TABLE 2: SYSTEM SUITABILITY STUDY

Injections	USP Plate count	USP Tailing Factor	RT	Peak Area (min)
1	8219	1.22	7.700	450.25
2	8362	1.35	7.766	453.17
3	8398	1.22	7.783	462.70
4	8219	1.22	7.700	460.36
5	6943	1.22	7.666	457.58
6	8324	1.25	7.712	452.77
		Mean	7.721	456.13
		SD	0.0444	4.8535
		%RSD	0.5750	1.064

Analytical Method Validation:

Linearity: The linearity of Ketorolac Tromethamine was determined by preparing and injecting solution with a concentration of about 5-25 µgm/ml. The calibration curve indicates the response is linear over the concentration range studied for Ketorolac Tromethamine with correlation coefficient (r) of 0.999. Calibration curve for linearity shown in **Fig. 5** and their values in **Table 3.**

TABLE 3: LINEARITY

S. no.	Conc. (µgm/ml)	Area I	Area II	Mean	SD	% RSD
1	5	103.12	106.00	104.56	2.04	1.95
2	10	195.70	192.72	194.21	2.11	1.08
3	15	285.38	283.32	284.35	1.46	0.81
4	20	357.56	362.75	360.16	3.67	1.02
5	25	450.39	444.36	447.38	4.26	0.85

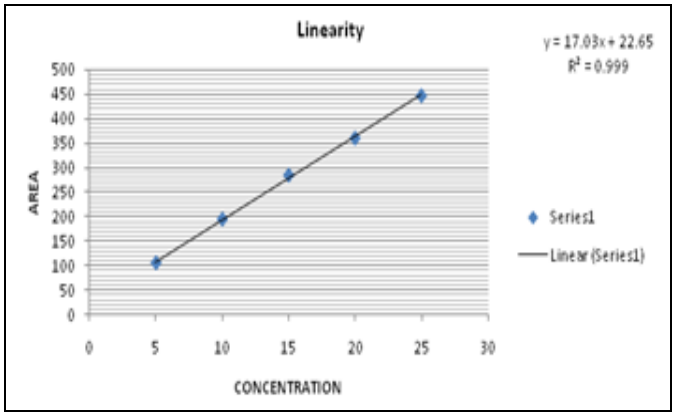

FIG. 5: CALIBRATION CURVE FOR KETOROLAC TROMETHAMINE

Precision: Precision was measured in terms of repeatability of application and measurement. Repeatability of standard application (system precision) was carried out using six replicate of the sample injection (25 µgm/ml) repeatability of sample measurement (method precision) was carried out in six replicate of sample preparation from the same homogenous blend of the marketed sample (25 µgm/ml). The percentage RSD for repeatability of standard preparation was 1.18% whereas the % RSD for repeatability of the sample 0.79%. This shows that the precision of the method is satisfactory as percentage RSD is not more than 2% the chromatogram was shown in **Table 4** and **5.**

TABLE 4: PRECISION STUDY OF THE SYSTEM

S. no.	Sample Area	% Assay Present (µgm)	Amount
1	450.25	100.40	25.10
2	453.17	101.08	25.27
3	462.70	103.32	25.83
4	460.36	102.80	25.70
5	457.58	102.12	25.53
6	450.25	102.70	25.60
	Mean	101.94	25.49
	SD	1.20	0.30
	% RSD	1.18	1.18

TABLE 5: PRECISION STUDY OF THE METHOD

S. no.	Sample area	% Assay	Amount present (µgm)
1	451.37	100.48	25.12
2	456.21	101.36	25.34
3	463.67	101.80	25.45
4	459.36	102.60	25.65
5	456.48	101.72	25.43
6	454.23	100.56	25.14
	Mean	101.42	25.35
	SD	0.8066	0.2016
	% RSD	0.795	0.795

The inter-day precision also carries out using 10, 15, and 25 µgm/ml standard solution % RSD found is not more than 2% shown in **Fig. 6** and **Table 6.**

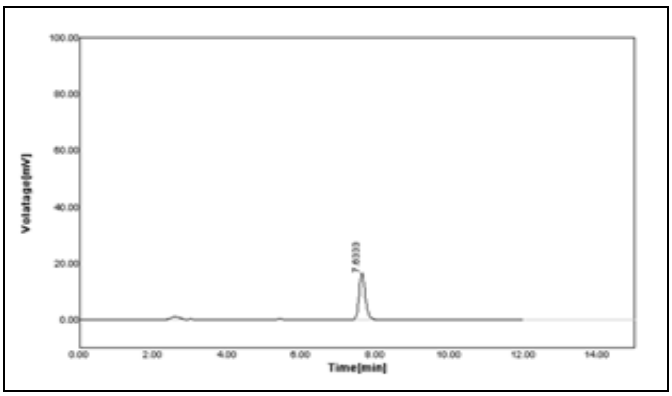

FIG. 6: PRECISION CHROMATOGRAM

TABLE 6: PRECISION (INTERDAY)

S. no.	Conc.	Area I	Area II	Mean	SD	RSD
1	10	194.16	192.93	193.55	0.87	0.45
2	15	277.23	283.71	280.47	4.58	1.63
3	20	441.46	445.94	443.70	3.17	0.71

Accuracy: The percentage of recovery experiments were performed by adding a known quantity of pure standard drug into the pre-analysed sample. The solution equivalent to 100mg of Ketorolac tromethamine was accurately weighed into a 100 ml volumetric flask. The sample was then spiked with the standard at level 80 %, 100 % and 120 % of test concentration. The resulting spiked sample solutions were assayed in triplicate, and the results were compared and expressed as a percentage. The mean percentage recovery of Ketorolac Tromethamine was found to be in the range between 101.7 and 103.6, which are within the acceptance limit was shown in **Table 7** and **Fig 7.**

TABLE 7: ACCURACY STUDY

Spiked level	Amount added µgm/ml	Peak area	Amount found	Percent recovery	Percent mean recovery
80%		341.98	18.75	104.1	
80%	8	344.55	18.90	105.0	103.6
80%		343.25	18.35	101.9	
100%		372.86	20.56	102.8	
100%	10	365.37	20.12	100.6	101.7
100%		368.42	20.39	101.9	
120%		409.56	22.71	103.2	
120%	12	405.88	22.50	102.2	102.8
120%		407.29	22.67	103.0	

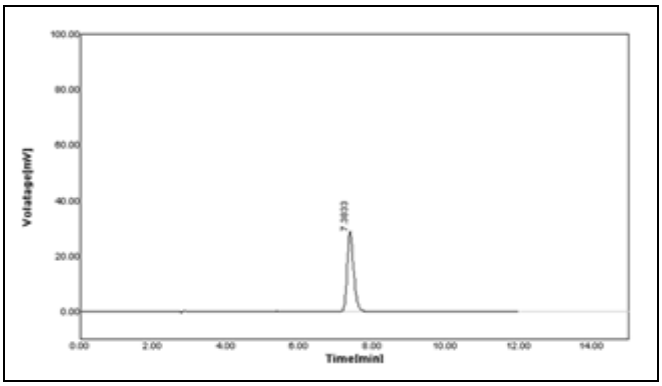

FIG. 7: ACCURACY CHROMATOGRAM

Robustness: Robustness of the method was determined by analyzing the standard solution at normal operating condition by changing some operating analytical conditions such as flow rate, mobile phase, and detection wavelength. The condition with variation and their result was shown in **Table 8.** The tailing factor is around indicative of peak symmetry and theoretical plate count also above 2000. Hence robustness of the extent of variations applied to the analytical condition was shown in **Fig. 8.**

TABLE 8: ROBUSTNESS STUDIES

System suitability	Parameter (variation)	% RSD of peak area response (n=3)	Mean tailing factor (n=3)	Mean retention time (n=3)
Flow change	0.9 ml/min	1.61	1.27	8.23
	1.1 ml/min	1.29	1.25	6.68
Mobile phase volume	64:36	1.81	1.18	7.73
	66:34	1.56	1.15	7.15
Wavelength change	244 nm	1.18	1.20	7.67
	246 nm	1.87	1.18	7.33

Limit of Detection (LOD) and Limit of Quantification (LOQ): The limit of detection (LOD) is the the lowest amount of analyte in a sample that can be detected but not necessarily quantified, under the stated experimental conditions. LOD and LOQ were calculated by using standard deviation and slope value obtained from the calibration curve by using formula LOD = 3.3(SD/S) and LOQ = 10(SD/D). The LOD and LOQ value for Ketorolac Tromethamine was found to be 0.825 µgm/ml and 2.501 µgm/ml, respectively.

Force Degradation Study:

Thermal Degradation: Heat about 1000 mg of tablet powder at 105 ºC for 24 h weigh accurately

this powder equivalent to 100 mg of Ketorolac Tromethamine into a 100 ml volumetric flask added 60 ml of diluent and sonicate 15 min with intermittent shaking and makeup to the mark with diluent. Thermal degradation study was carried out after 1 day, 3 day, and 6day shown in **Fig. 9.**

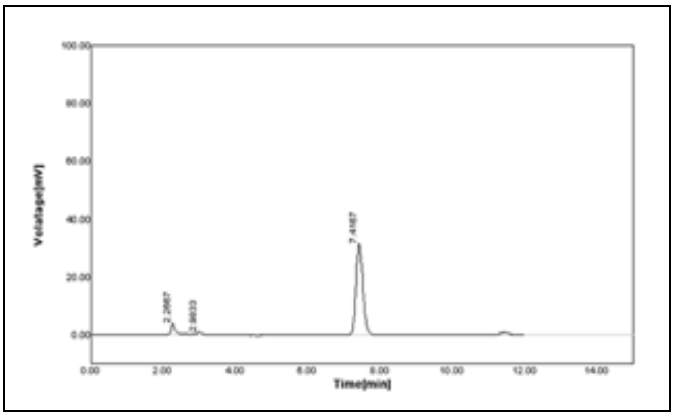

FIG. 9: THERMAL STRESS CONDITION

Photostability: Expose about 1000 mg of tablet powder in photostability for 1.2 million Lux h weigh accurately this powder equivalent to 100 mg of Ketorolac tromethamine into a 100 ml volumetric flask add 60 ml of diluent and sonicate for 15 min with intermittent shaking and makeup to the mark with diluent. Filter the solution through a 0.45µ nylon filter. Photostability was carried out after 1 day, 3 day and 6 days shown in **Fig. 10**.

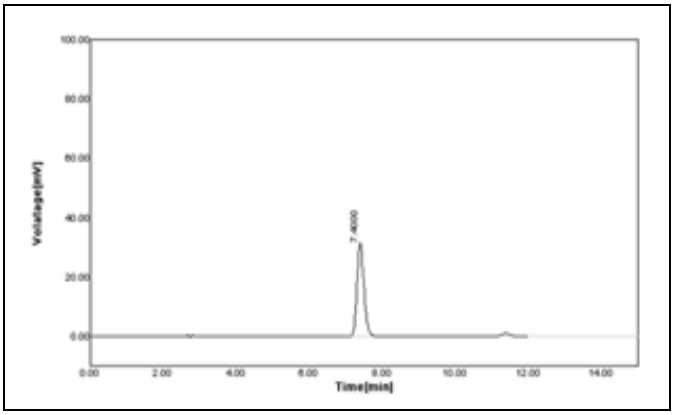

FIG. 10: PHOTODEGRADATION STUDY CHROMATOGRAM

Acid Degradation: Weight accurately tablets powder equivalent to 100 mg of Ketorolac tromethamine into 100 ml volumetric flask add 10 ml of 5 N Hydrochloric acid heat it on water bath at 80 ºC for 8 h, cool it add 10 ml of 5N Sodium Hydroxide and add 60 ml of diluent and sonicate, dissolved the substances, makeup to the mark with diluent and mix well. Filter the solution through 0.45 µ nylon filter. The Chromatogram is shown in **Fig. 11**.

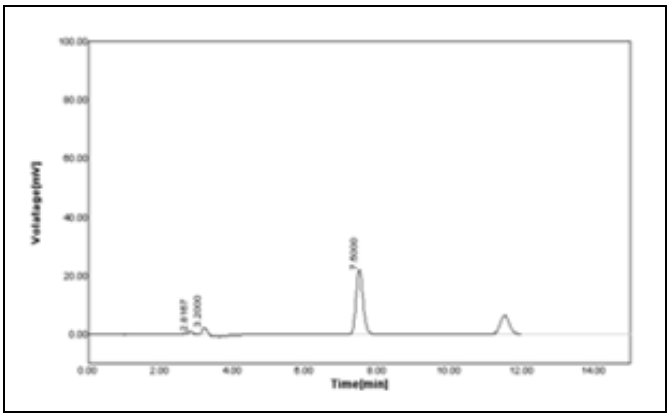

FIG. 11: ACID STRESS CONDITION

Base Degradation: Weight accurately tablets powder equivalent to 100 mg of Ketorolac tromethamine into 100 ml volumetric flask add 10 ml of 5 N Sodium Hydroxide heat it on water bath at 80 ºC for 8 h, cool it add 10 ml of 5N Hydrochloric acid and add 60 ml of diluent and sonicate, dissolved the substances, makeup to the mark with diluent and mix well. Filter the solution through 0.45 µ nylon filter. Chromatogram showed in **Fig. 12.**

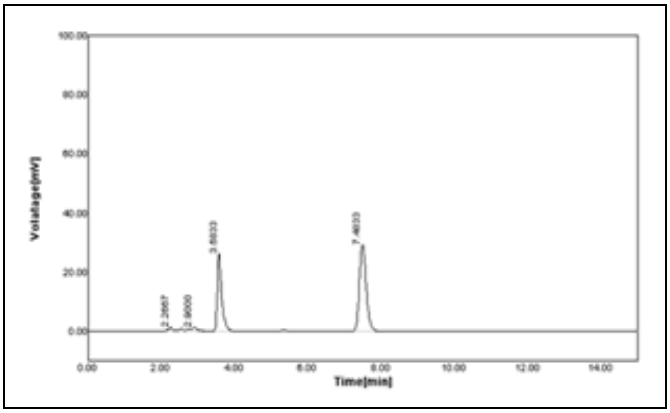

FIG. 12: BASE STRESS CONDITION CHROMATOGRAM

Peroxide Degradation: Weight accurately tablets powder equivalent to 100 mg of Ketorolac tromethamine into 100 ml volumetric flask add 10 ml 30% hydrogen peroxide heat it on a water bath at 80 ºC for 8 h, add 60 ml of diluent and sonicate, dissolved the substances, makeup to the mark with diluent and mix well. Filter the solution through 0.45 µ nylon filter. Chromatogram and force degradation data, as has shown in **Fig. 13** and **Table 9,** respectively.

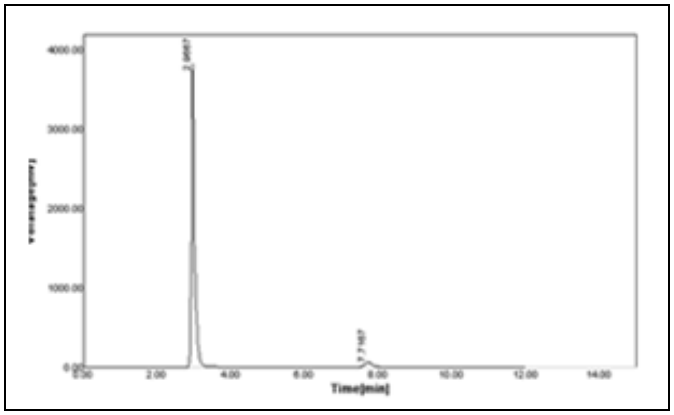

FIG. 13: PEROXIDE STRESS CONDITION

TABLE 9: FORCE DEGRADATION DATA

Treated Parameter	RT	Theoretical plate	Tailing factor
As such Ketorolac	7.88	8615.4	1.27
Thermal treatment			
After 1day	7.41	6497.6	1.12
3 day	7.40	6468.4	1.16
6 day	7.33	6352.4	1.12
Photo Stability			
After 1day	7.40	6468.4	1.12
3 day	7.43	6526.8	1.12
6 day	7.36	6410.4	1.05
Acid heat treatment	7.50	6644.4	1.12
Base after heating	7.45	7694.3	1.12
Oxidation treatment after heat	7.71	6064.9	1.11

RESULTS AND DISCUSSION: A different combination of mobile phases and chromatographic conditions were tried and a mobile phase containing methanol and water with 0.1% O-phosphoric acid (65:35 v/v), Grace C18 (250 cm × 4.6 mm × 5 µ) column, 1.0 mLmin-1 flow rate, 20 µL injection volume, 30 ºC column oven temperature, 245 nm wavelength and 16 min run time was found to be suitable for all combinations. These chromatographic conditions gave a retention time of 7.70 min. The force degradation study of the sample solution was evaluated by preparing a sample solution as per the proposed method and analyzed after 1 day, 3day and 6 days for thermal and photostability study retention time found same as per standard chromatogram. System precision and method precision results showed the % RSD of 1.18 and 0.79, respectively. A good linearity relationship indicated by correlation coefficient (r) value 0.999 was observed between the concentrations of 5 μgmL^{-1} to 25 μgmL^{-1} of Ketorolac Tromethamine. Inter-day Precision was done by changing the analyst, column, with the same chromatographic conditions, and the obtained results were within limits. The Robustness method was evaluated by deliberately varying the chromatographic conditions of the method, such as mobile phase methanol content, flow rate, and wavelength. The parameter like tailing factor and retention time showed adherence to the limits. The accuracy of the method was determined, and the percentage of recovery was calculated. The data indicates an average of 103.6 % recovery of the standard sample.

CONCLUSION: The method developed for Ketorolac Tromethamine was found to be a simple process and the procedure does not involve any experimental conditions. The validation results indicated that the method was specific, accurate, linear, precise, rugged and robust. The runtime was relatively 20 min which enabled rapid quantification of many samples in routine and quality control analysis of tablet formulation.

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CONFLICT OF INTEREST: Nil

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