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Research Article

A NOVEL STABILITY INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF KETOROLAC TROMETHAMINE IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

Objective: The main objective of the present investigation was to develop a simple isocratic stability indicating reversed phase liquid chromatographic method and validate the proposed method and to apply it for the estimation of ketorolac tromethamine, a non-steroidal anti-inflammatory drug in pure and pharmaceutical formulations.

Methods: Waters high performance liquid chromatographic 2695 series system, inertsil ODS, C18 (150 mm × 4.6 mm × 5.0 µ) column and UV-visible detector with photo diode array detection was adopted for the method development. The components were separated by injecting about 20 µL working standard solution of concentration 5 µg/mL, using mobile phase prepared by mixing buffer of 100 mM potassium dihydrogen orthophosphate solution of pH 4.5 and acetonitrile in the ratio 60:40 v/v at a flow rate of 0.8 mL/minute, and the components were detected at a wavelength of 316 nm.

Results: The proposed method was validated as per ICH guidelines. The developed method was found to be precise, accurate, linear, robust and rugged. A study of forced degradation was carried out and found that the drug was stable under variety of degradation conditions.

Conclusion: The proposed method was adopted for assay of pharmaceutical formulations and recommended for routine analysis in any quality control laboratory.

Keywords: Ketorolac tromethamine, Validation, Assay, Forced degradation, Pharmaceutical formulations.

INTRODUCTION

Ketorolac tromethamine (KTR) is a member of the pyrrolo-pyrrole group of non-steroidal anti-inflammatory drugs (NSAIDs) which acts by inhibiting the bodily synthesis of prostaglandins. An ophthalmic solution of KTR is available and is used to treat eye pain and to relieve the itchiness and burning of seasonal allergies. KTR in its oral (tablet or capsule) and intramuscular (injected) preparations is a racemic mixture of both (*S*)-(−)-ketorolac, the active isomer, and (*R*)-(+)-ketorolac. The chemical name for KTR is (±)-5-benzoyl-2,3-dihydro-1 H-pyrrolizine-1-carboxylic acid, compound with 2-amino-2-(hydroxymethyl)-1,3-propanediol (1:1). The molecular formula and molecular weight of the selected drug are $C_{15}H_{13}O_3$ $C_4H_{11}NO_3$ ($C_{19}H_{24}N_2O_6$) and 376.41 g per mole respectively. KTR may exist in three crystal forms soluble in water and is available as toradol, acular and sprix. Toradol is available as round, white, film-coated, red-printed tablets. Each tablet contains 10 mg KTR, the active ingredient, with added lactose, magnesium stearate and microcrystalline cellulose. The white film-coating contains hydroxypropyl methylcellulose, polyethylene glycol and titanium dioxide. Its chemical structure of KTR is given in Fig. 1.

An extensive literature survey was carried out and found a few high performance liquid chromatographic (HPLC) methods for the determination of KTR in different biological fluids [1-5] and formulations [6-9] were reported for the impurity profile study of KTR. Stability indicating and simultaneous determination methods [10-12] in human plasma and formulations were also reported. One HPTLC method [13] and an automatic fluorescence method [14] were also developed for the determination of KTR. The main objective of the proposed method is to develop a novel, rapid and economic liquid chromatographic procedure for the determination of KTR in pure and formulations, and also to study the stability of the drug under the forced degradation studies. Based on the nature of the drug and available chromatographic procedures the authors made some trails to reduce the retention time and run time of the analysis by changing polarity of the mobile phase, choosing chromatographic column having long carbon chain length in the column material and flow rate of the mobile phase, and succeeded in their attempts.

METHODS

Instrumentation

Waters HPLC 2695 series consisting 4 pumps, auto sampler, having capacity of volume of injection 5-500 µL, with 5 racks and each has 24 vials holding capacity with temperature control, UV-visible detector with photo diode array detection equipment was used in the present investigation. Thermostat column compartment connected it has a capacity to maintain 5-60°C column temperature. Empower software-2 software was used for acquisition of chromatographic data.

Chemicals and reagents

Analytical grade potassium dihydrogen orthophosphate, ortho phosphoric acid hydrochloric acid, sodium hydroxide and hydrogen peroxide were purchased from Qualigens Fine Chemicals Ltd., Mumbai. Acetonitrile of HPLC grade is obtained from E. Merck. (India) Ltd., Mumbai. Kerorolac tromethamine is gifted by Hetro Drugs Limited, Hyderabad. The commercially available KTR tablets were purchased from the local pharmacy.

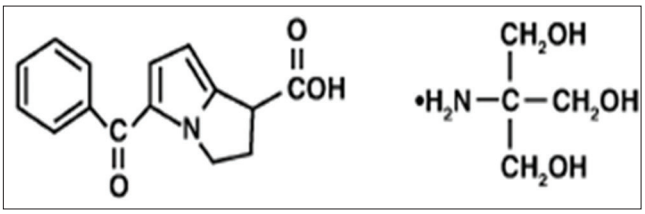

Fig. 1: Chemical structure of Ketorolac tromethamine

Buffer preparation: Prepared 100 mM potassium dihydrogen orthophosphate solution, and its pH was adjusted to 4.5 with dilute orthophosphoric acid solution.

Mobile phase preparation: Mobile phase was prepared by mixing buffer and acetonitrile at 60:40 ratio and sonicated the resulting solution and degassed it using vacuum filtration through 0.4 µ membrane filter.

Preparation of standard solution: Weighed and transferred 50 mg of KTR working standard into 100 mL volumetric flask, added 50 mL of diluent and sonicated to dissolve and finally diluted to volume with diluent. Then about 1.0 mL of above solution was transferred into 100 mL volumetric flask and diluted to volume with diluent.

Preparation of sample solution: An amount of fine powder of Toradol equivalent to 50 mg was weighed and transferred into a cleaned 100 mL volumetric flask and dissolved in 50 mL of diluent, sonicated for 10 minutes and diluted up to the mark. Further filtered the solution through 0.4 µ membrane filter and then 1.0 mL of the solution was diluted to 100 mL to obtain the final concentration of 5 µg/mL.

Chromatographic method development

To optimize the HPLC method, one of the chromatographic conditions such as column, mobile phase, injection volume, flow rates, column temperature and detection wavelength was varied keeping other constant, chromatograms were recorded for each variation, and chromatographic parameters such as retention time, number of theoretical plates, tailing factor, area of the peak and peak height were obtained. Finally, the following procedure was found to be optimized chromatographic procedure.

About 20 µL of blank solution or placebo solution or standard solution was injected into an Inertsil ODS, C18 (150 mm × 4.6 mm × 5.0 µ) column maintained at a temperature of 30°C. The component were separated by using mobile phase of buffer and acetonitrile in the ratio 60:40 (v/v) at a flow rate 0.8 mL/minute under an isocratic mode, and the components were detected at a wavelength 316 nm using photodiode array (PDA) detector.

Method validation

The developed method was validated to demonstrate the proposed method is scientifically sound and that it serves the intended analytical purpose. Validation of the assay procedure was carried out using the following parameters.

Specificity

To find out the specificity of the proposed method, about 20 µL of blank or working standard or sample solution was injected into the HPLC system, and chromatograms were recorded under the optimized chromatographic conditions. In blank chromatogram, no peaks were found where as in standard and sample chromatograms a sharp symmetrical peak with tailing <2.0 and plate count more than 2000 was found.

Precision

System precision: To find the system precision, about 20 µL of working standard solution of concentration 5 µg/mL was injected into the HPLC system 6 times, chromatograms were recorded under the optimized chromatographic conditions, and the chromatographic parameters such as retention time, peak area, peak height, tailing factor and number of theoretical plates were obtained.

Method precision: To obtain method precision, a standard solution of concentration 5 µg/ml was prepared 6 times and chromatographic parameters were obtained under the normal conditions.

Linearity

In the present investigation, a study of linearity was established by preparing a series of six standard solutions over the range of concentration 1.25-7.50 µg/mL from standard stock solution, and chromatograms were obtained under the optimized conditions by injecting each of the solution twice into the system and chromatographic parameters were evaluated.

Sensitivity

The sensitivity is expressed in terms of limit of detection (LOD) and limit of quantitation (LOQ). The LOD of an individual analytical procedure is the lowest concentration of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The LOQ is a parameter of the quantitative assay for low levels of compounds in sample matrices and is used particularly for the determination of impurities and/or degradation products. The values of LOD and LOQ were calculated from the slope and standard deviation of the intercept.

Accuracy

In the proposed method accuracy was evaluated by using standard addition method, in which three concentration levels (80%, 100% and 120% with respect to precision concentration) were prepared by adding different amounts of standard to a constant amount of pre analyzed sample, and each solution was injected into column in triplicate, chromatograms were recorded and chromatographic parameters were evaluated. The percent of recovery was calculated by from mean peak area of standard and spike level.

Ruggedness

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions. In the proposed method, the study of ruggedness was carried out by preparing working standard solutions of 5.0 µg/ml and analyzed in 2 different days.

Intraday precision: Working standard solution of concentration 5 µg/mL was prepared 6 times from the standard stock solution on the same day (day-1), about 20 µL of these solutions was injected into the column and the chromatograms were obtained under the same optimized conditions, and the relative standard deviation (%RSD) was calculated on area of the six peaks and found to be <2.0.

Inter day precision: About 5 µg/mL working standard solution was prepared 6 times from the standard stock solution on day-2, and about 20 µL of these solutions was injected into the column and the chromatograms were obtained under the same optimized conditions, and the %RSD was calculated on area of the six peaks and found to be <2.0.

Robustness

The robustness of the proposed method was demonstrated by carrying out intentional method variations like mobile phase flow changes and column oven temperature variations. In the present investigation, working standard solution of concentration 5 µg/ml was prepared, and chromatogram was obtained for deliberate variations in method parameters like flow rate and temperature.

Degradation Studies

Acid hydrolysis

Transferred quantitatively 100 mg of ketorolac API in to 200 mL RB flask, added 100 mL of freshly prepared 0.1 N HCl and left for 12 hrs. After that filtered the solution through filter paper and neutralized the solution with 0.1 N NaOH, then about 0.5 mL of filtrate was diluted to 100 mL with mobile phase.

Base hydrolysis

About 100 mg of ketorolac API was accurately weighed and transferred into a 200 mL RB flask and dissolved in 100 mL of freshly prepared 0.1 N NaOH, and left the solution for 12 hrs. After 12 hrs, the solution

was filtered and neutralized the solution with 0.1 N HCl, then about 0.5 mL of filtrate was diluted to 100 mL with mobile phase.

Oxidation

An amount of 100 mg of ketorolac was added to 100 ml of freshly prepared 1.0% hydrogen peroxide taken in a 200 mL RB flask and allowed to react it for 12 hrs and then 0.5 ml of above filtered solution was to 100 ml with mobile phase.

Heat exposure

Transferred quantitatively 100 mg of ketorolac API on to clean and dry petri dish, spread it throughout the plate and placed the petri dish in an oven, which is maintaining at 100°C for 12 hrs. After 12 hrs, transferred the contents into 100 mL volumetric flask and added 100 mL of diluents, sonicated it for 10 minutes and dilute volume with diluent. Further filtered the solution and about 0.5 ml of this filtrate was diluted to 100 ml with mobile phase.

UV exposure

About 100 mg of ketorolac was taken on to clean and dry petri dish, placed in UV Chamber for 12 hrs. After 12 hrs transferred the contents into 100 mL volumetric flask added 100 mL of diluent and sonicated it for 10 minutes and diluted to volume with diluent. Further filtered the solution through filter paper and 0.5 mL of the solution was diluted to 100 mL with mobile phase.

RESULTS AND DISCUSSION

System suitability

About 20 µL of standard solution of concentration 5 µg/mL was injected into an Inertsil ODS, C18 (150 mm × 4.6 mm × 5.0 µ) column and the separation was achieved by using mobile phase of buffer and acetonitrile in the ratio 60:40 (v/v) at a flow rate 0.8 mL/minute under an isocratic mode, and the components were detected at a wavelength 316 nm using PDA detector. The system suitable parameters were given in Table 1.

Specificity

Specificity of the proposed method was determined by injecting about 20 µL of blank or working standard or sample solution into the HPLC system and chromatograms (Figs. 2 and 3) were recorded under the optimized chromatographic conditions. The chromatograms of standard and sample showed that only one peak with 100% peak area at retention time 2.927 and 2.922 minutes respectively.

Table 1: System suitable parameters

Serial no	RT	Area	Height	% area	Tailing	Plate count
Standard	2.927	3265045	310226	100.00	1.13	3795
Sample	2.922	3267545	318116	100.00	1.15	3805
Blank						

RT: Reverse transcription

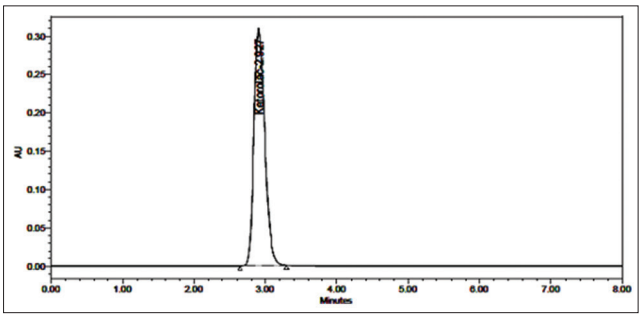

Fig. 2: Reversed phase-high performance liquid chromatographic chromatogram of ketorolac standard

Precision

Precision, repeatability of a method was expressed as a percent of %RSD of finite replicate measurements. In the present investigation, the system precision was evaluated by injecting about 20 µL of working standard solution into the HPLC system 6 times, chromatograms were recorded under the optimized chromatographic conditions and %RSD was evaluated on retention time and peak area, and found to be within the acceptable limits. Method precision was determined for six chromatograms of six working standard solutions of concentration 5 µg/mL, the %RSD was determined on retention time and peak area of six chromatograms and found to be <2.0. The results of precision were presented in Table 2.

Linearity

Linearity, a study of dependence of instrument response on concentration of the analyte was determined by getting chromatograms twice and their peak area of a series of six standard solutions of concentration from 1.25 µg/mL to 7.50 µg/mL. A calibration curve (Fig. 4) was plotted peak area as a function of concentration of the drug

Table 2: Results of precision studies

System precision			Method precision		
Serial no	Ketorolac		Serial no	Ketorolac	
	RT	Area		RT	Area
1	2.924	3305808	1	2.921	3285212
2	2.927	3265045	2	2.922	3265244
3	2.923	3282897	3	2.921	3285612
4	2.928	3282585	4	2.925	3281866
5	2.926	3265864	5	2.926	3284688
6	2.931	3283162	6	2.928	3285275
Mean	2.927	3280894	Mean	2.924	3281316.167
SD	0.0029	14898.4	SD	0.0029	7991.122
RSD	0.098	0.454	RSD	0.100	0.244

RT: Reverse transcription, %RSD: Relative standard deviation, SD: Standard deviation

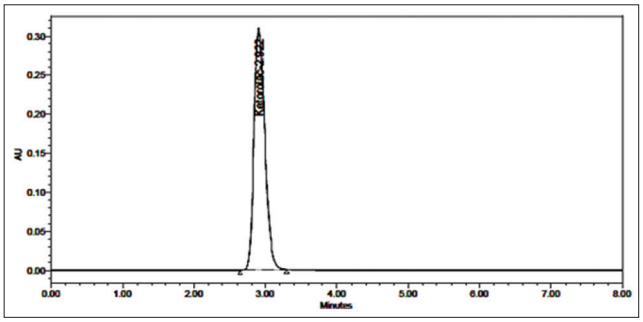

Fig. 3: Reversed phase-high performance liquid chromatographic chromatogram of ketorolac sample

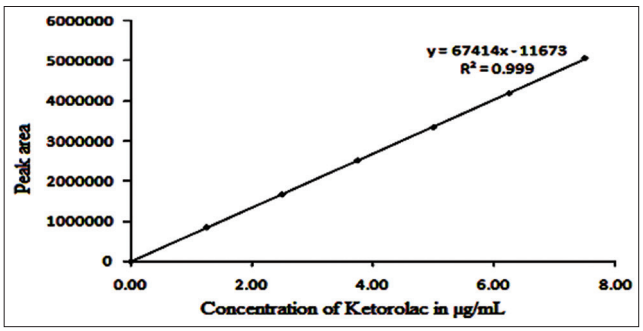

Fig. 4: Calibration plot drawn mean peak area against concentration of the drug

and found to be linear with positive slope. The slope, intercept and correlation coefficient were determined and presented in Table 3.

Sensitivity

The sensitivity of the proposed method was presented in terms of LOD and LOQ and these were calculated from standard deviation of response and slope of the calibration curve by using the formulae LOD=$3S_a/b$ and LOQ=$10S_a/b$ respectively and found to be 0.0261 and 0.0869 µg/mL.

Accuracy

Accuracy, percent of recovery was evaluated by using standard addition method at 80%, 100% and 120% concentration levels with respect to target concentration, and each solution was injected into column in triplicate, chromatograms were recorded and percent of recovery was calculated by from mean peak area of standard and spiked level. The results of accuracy were presented in Table 4.

Ruggedness

The ruggedness of an analytical method was demonstrated in terms of intraday precision and inter day precision. Chromatograms were obtained for six working standard solutions of concentration 5 µg/mL under the optimized conditions on day-1, and the %RSD was calculated on the area of the six peaks and found to be <2.0. Similarly, about 5 µg/mL working standard solution was prepared 6 times from the standard stock solution on day-2, and the chromatograms were obtained under the optimized conditions, and the %RSD was calculated on area of the six peaks and found to be <2.0 and results were shown in Table 5.

Table 3: Linearity studies and regression analysis

Serial no	Concentration	Area of peak
1	0.00	0
2	1.25	841105
3	2.50	1662993
4	3.75	2506960
5	5.00	3334447
6	6.25	4201197
7	7.5	5067749
Slope		674139.57
Intercept		−11673.25
Correlation coefficient		0.9999

Table 4: Results of accuracy of the proposed method

Spike level %	Taken	Added	Found	Mean peak area*	% recovery
80	50	40	89.541	2633796	99.49
100	50	50	99.56	3294504	99.56
120	50	60	110.649	3994385	100.59

*Mean of three determinations

Table 5: Intraday and inter day precision

Serial no	Day-1		Day-2	
	RT	Area	RT	Area
1	2.924	3305808	2.925	3285556
2	2.927	3265045	2.922	3285345
3	2.923	3282897	2.926	3283325
4	2.928	3282585	2.923	3284478
5	2.926	3265864	2.921	3291245
6	2.931	3283162	2.927	3287542
Mean	2.927	3280894	2.924	3286249
SD	0.0029	14898.419	0.0024	2814.802
%RSD	0.098	0.454	0.081	0.086

RT: Reverse transcription, RSD: Relative standard deviation, SD: Standard deviation

Robustness

In the present investigation, the study of robustness was carried out by evaluating system suitable parameters under the different flow rates (0.6 ml/minute and 1.0 ml/minute) and temperatures at 30±2°C, and the results were presented in Table 6.

Degradation studies

A study of forced degradation was carried out to examine the stability of the drug under different degradation conditions such as 0.1 N HCl, 0.1 N NaOH, 1.0% H_2O_2, exposure to heat and photo light. Chromatograms were recorded for each degradation condition, peak area of each of the chromatogram was determined, and percent of degradation was calculated. From the studies, it was evident that the

Table 6: Results of study of robustness

Serial no	Peak	RT	Area	Height	% area	Tailing	Plate count
			Flow rate-1				
1	Ketorolac	3.006	3412169	330848	100.00	1.14	3805
			Flow rate-2				
2	Ketorolac	2.511	2923251	312909	100.00	1.12	3645
			Temp-1				
3	Ketorolac	3.304	3444583	316010	100.00	1.15	3873
			Temp-2				
4	Ketorolac	2.956	3155612	313422	100.00	1.15	3856

RT: Reverse transcription

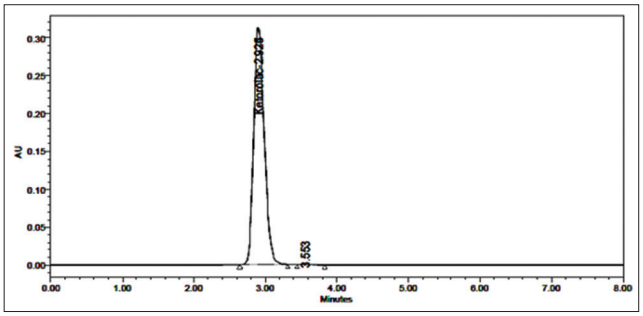

Fig. 5: Chromatogram of ketorolac in acid degradation studies

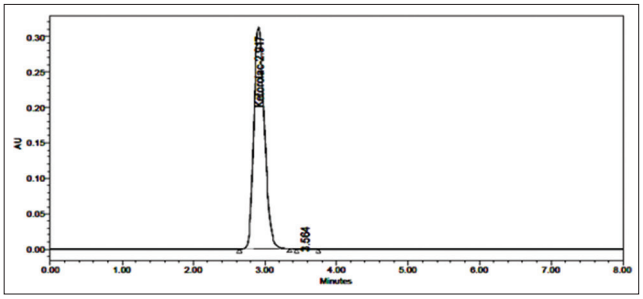

Fig. 6: Chromatogram of ketorolac in base degradation studies

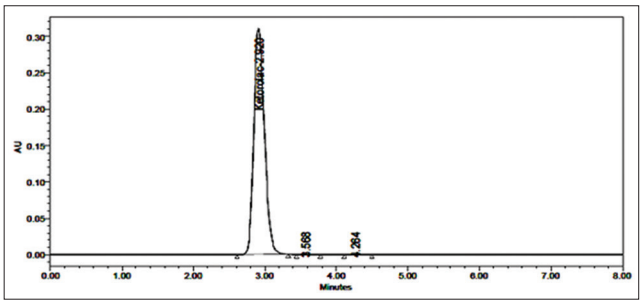

Fig. 7: Chromatogram of ketorolac in peroxide degradation studies

Table 7: Study of degradation of KTR under variuos degradation conditions

Serial no	Peak	Retention time	Peak area	Height	% area	Resolution	% degradation
		Degradation standard					
1	Ketorolac	2.925	3323452	326465	100.00		
		Acid degradation					
1	Ketorolac	2.925	3125910	314056	99.941	2.41	6.62
2	Ketorolac	3.553	1857	194			
		Base degradation					
1	Ketorolac	2.917	3185830	312040	99.938	2.40	4.35
2	Ketorolac	3.564	1978	208	0.062		
		Peroxide degradation					
1	Ketorolac	2.920	3115909	309733	99.888	2.38	6.36
2	Ketorolac	3.568	2054	209	0.064	2.24	
3	Ketorolac	4.264	1556	131	0.0148		
		Thermal degradation					
1	Ketorolac	2.914	3201388	311768	99.932	2.34	3.89
2	Ketorolac	3.565	2178	218	0.068		
		UV-degradation					
1	Ketorolac	2.925	3221415	312459	99.942	2.31	3.58
2	Ketorolac	3.604	2384	2157	0.058		

KTR: Ketorolac tromethamine, UV: Ultraviolet

Table 8: Assay of ketorolac in toradol tablets

	Mean peak area	% of assay
Standard	3272851	
Sample	3285089	99.83

*Mean of three determinations

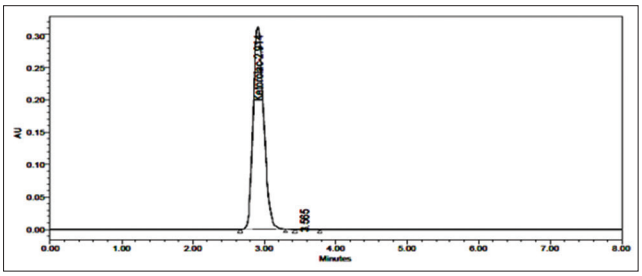

Fig. 8: Chromatogram of ketorolac in thermal degradation studies

drug was found to be stable, and the percent of degradation was found to be 3.58-6.62%. The results were presented in Table 7 and respective chromatograms were presented from Figs. 5-9.

Analysis of formulations

Preparation of sample solution: An average weight of ten tablets (toradol 10 mg) was calculated and made them into a fine powder. An amount of fine powder equivalent to 50 mg of standard ketorolac was weighed and transferred into a cleaned 100 mL volumetric flask and dissolved in 50 mL of diluent, sonicated for 10 minutes and diluted up to the mark and filtered the solution. Then about 1.0 mL of the above solution was transferred into a 100 mL volumetric flask and diluted up to the mark. The triplicate chromatograms of standard and sample were taken under the similar chromatographic conditions, and the percent of assay was determined, results were reported in Table 8.

CONCLUSIONS

A simple isocratic RP-HPLC method was developed for the determination of ketorolac in pure and pharmaceutical formulations. The proposed method was found to be simple, precise, accurate, robust and rugged. Therefore, the method can be used for routine analysis in quality control.

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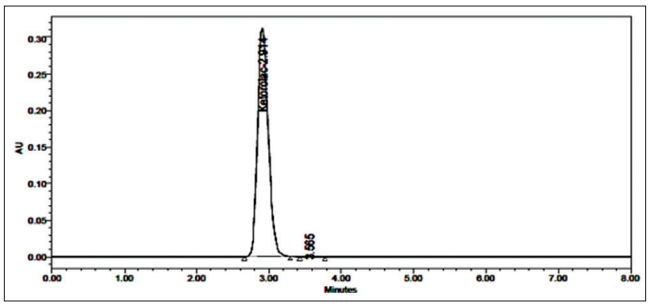

Fig. 9: Chromatogram of ketorolac in UV degradation studies

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