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Ketorolac Tromethamine Force Degradation And Stability Indicating Study By Using RP-HPLC

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Abstract: The present work aims to perform force degradation study using RP-HPLC method and to study impurity profiling of Ketorolac to know interference of impurities with the main analyte peaks.The Chromatographic separation was performed for Ketorolac Tromethamine drug using Phenomenex C18 150 x 4.6 mm using (60:40% v/v) Acetonitrile and pH 3 Phosphate buffer , 5µ column, detection wavelength was 324 nm and run time is 5.9 min. It isindicated that the drug was degraded by 3.65%, 2.45% and 19.83% when subjected to acid, base hydrolysis and degradation by oxidation respectively. It shows that there were not found any impurity. Drug was stable in different conditions of degradation except oxidative condition.

Keywords: Force degradation, RP-HPLC, ICH, degradation product, Ketorolac

INTRODUCTION

The aim of forced degradation studies is to learn more about the breakdown products and pathways while also looking at stability-related properties of an API. [1]

Forced degradation is a procedure in which drug products and drug substances aredegraded under conditions that are more severe than accelerated settings, resulting in degradation products that may be analyzed to determine the molecule's stability. The following ICH recommendations apply to investigations involving forced degradation:

a. New Drug Substances and Products: Stability Testing-ICH Q1A
b. Photostabilization Testing of Novel Drug Substances and Products (ICH Q1B)
c. ICH Q2B: Methodology for Analytical Procedure Validation. [2]

According to the ICH recommendation, stress testing is meant to confirm the stability indicating techniques that have been employed and to identify the expected degradation products, which aid in determining the molecule's inherent stability and identifying degradation pathways. [3,4,5]

Objective of forced degradation studies:-

The following goals are pursued through forced deterioration studies:

1. To identify drug substance and drug product breakdown pathways.

2. To distinguish between degradation products derived from drug products andthose derived from non-drug compounds in a formulation.
3. To figure out how degradation products are made.
4. To determine a pharmacological substance's intrinsic stability in a formulation.
5. To determine the drug substance and drug product's degradation mechanisms,such as hydrolysis, oxidation, thermolysis, or photolysis .
6. To establish the nature of a created method's stability.
7. To gain a better understanding of medicinal compounds' chemical characteristics.
8. To create formulas that is more stable.
9. To create a degradation profile that is similar to what would be seen in a formalstability study conducted under ICH conditions.
10. To establish the nature of a created method's stability.
11. To address issues relating to stability.[1,6,7,]

MATERIALS AND METHODS

Ketorolac working standard was received as gift samples from Pratima Pharmaceuticals, Shikrapur, Pune, All chemicals and reagents used were either analytical or HPLC grade. The tablet Ketonic was procured from the local market.

Thermo 2080 system,P4000 Quaternary pump, UV 6000 PDA detector.The Chromatographic separation was performed using Phenomenex C18 150 x 4.6 mm, 5µ column, detection wavelength is 324 nm and run time is 5.9 min.

Chromatographic conditions

During these investigations a mobile phase was composed Acetonitrile and Phosphate buffer pH 3 in the proportion of 60:40 % v/v gave the best outcomes. During the courseof these studies the injection volume, mobile phase flow rate and analytical wavelength was kept constant (20µL, 1.0 ml/min and 324 nm) for the above selected respectively.

Preparation of standard stock solution:

10mg of Ketorolac was moved in 100 ml volumetric flask and dissolved in Acetonitrile and required volume adjusted with the Acetonitrile.This was further diluted to get 100µg/ml of Ketorolac and chromatogram was recorded shown in fig. no. 1.

Preparation of sample solution:

Tablet Powder comparable to Ketorolac 10mg was dissolved in 75 ml acetonitrile in 100 ml volumetric flask, sonicated and filtered through 0.2µm membrane filter, then volume adjusted up to the mark with acetonitrile.

Force degradation study of drug[3,4,5]

Procedure for acid degradation

Ketorolac10µg/ml was used as standard stock solution in procedure. In 10 ml ofketorolac stock solution, 10ml of 2N HCl was added and refluxed for 45min at 60˚C, then cooled to room temperature and neutralized with 10ml of 2N NaOH solution. Then volume adjusted up to the mark with diluent. About 10µl of this solution was injected in to RP-HPLC system and chromatogram was recorded as shown in the figure no.2.

Procedure for base degradation

Ketorolac stock solution 10ml and 10ml of 2N NaOH were transferred in a 100ml volumetric flask,refluxed for 45min at 60˚C temperature, cooled to room temperature and neutralized With 10ml of 2NHCl solution. Then volume adjusted up to the mark with diluent.
About 10µl of this solution was injected in to RP-HPLC system and the chromatogram was recorded shown in the figure no.3.

Procedure for heat degradation

The Ketorolac powder equivalent to 10mg of Ketorolac and 5 ml diluent sonicated for 25min in 10 ml volumetric flask. Then heated at 80˚C for 45min in a thermostatically controlled water bath and filtered through 0.2µm filter to and 1 ml of the filtrate was pipette out into a 10ml volumetric flask and finally adjusted volume with diluent. About 10µl of this solution was injected in to RP-HPLC system and the chromatogram was recorded shown in the figure no.4.

Procedure of oxidative degradation

The Ketorolac powder equivalent to 10mg of Ketorolac and 5 ml diluent sonicated for 25min in 10 ml volumetric flask. 5ml of 20% v/v H_2O_2 was added to the solution and heated for 45min in a thermostatically controlled water bath, Then filtered through 0.2µm filter and 1 ml of the filtrate was pipette into a 10ml volumetric flask and made up to the mark with diluent. About 10µl of this solution was injected in to RP-HPLC system and the chromatogram was recorded shown in the figure no.5.

Procedure of Photolytic degradation

he Ketorolac powder equivalent to 10mg of Ketorolac and 5 ml diluent sonicated for 25min in 10 ml volumetric flask. 5ml of diluent was added to the solution and exposed for UV light in photo stability chamber for 180min. Then filtered through 0.2µm filtrate and 1ml of the filtrate was pipetteinto a 10ml volumetric flask and made up to the mark with diluent. About 10µl of the solution was injected in to RP-HPLC system and the chromatogram was recorded shown in the figure no.5.

Analysis of marketed formulation

10 tablets contains Ketorolac (10mg) were weighed and triturated into mortar for fine powder. Accurately weighed fine Ketorolac and transferred into a 10 ml volumetric flask and sonicated for 20 min with 7 ml of Distilled water (diluent). The resulting solution was filtered by using whatman 1 filter paper, final solution diluted up to 10 ml with diluent to obtain a final concentration of 10µg/ml. Plotted chromatogram for analysis of formulation. Graphs of peak purity are shown in Figure no. 6.

RESULT AND DISCUSSION

Method Optimization

Many trials were conducted for the optimization of the method for the simultaneous estimation of Ketorolac Tromethamine tablet by changing ratio of buffer and solvents. The chromatographic conditions optimized were shown in table no. 1.

Table No. 1: Optimized chromatographic conditions for Ketorolac

Sr. No	Parameters	Conditions
1.	Column	C18 column 150 x 4.6 mm x 5µ
2.	Flow Rate	1.0ml/min
3.	Mobile Phase Ratio	Acetonitrile and Phosphate buffer pH 3 in the ratio (60:40 % v/v)

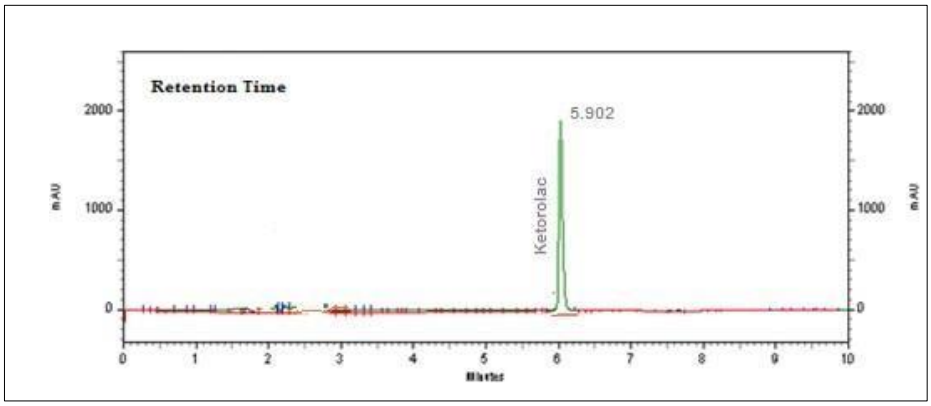

Figure No. 1: Optimized RP-HPLC chromatogram of Ketorolac Tromethamine

Forced degradation study of for Ketorolac Tromethamine in tablet form

Forced degradation studies were carried out to know interference of impurities with the main analyte peaks. The results of acid, base, peroxide, heat, and UV and water degradation were presented in table no. 8 The chromatograms of acid, base, photolytic, peroxide, heat of Ketorolac Tromethamine in tablet sample solution were presented in figure no.2, figure no.3, figure no. 4, figure no. 5, and figure no. 6.

Table No. 2 Peak purity results of acid degraded Ketorolac Tromethamine sample solution

Sr. No.	Peak name	Purity angle	Purity threshold	Purity Flag	Peak purity
1.	Ketorolac Tromethamine	0.247	0.430	No	Pass
2.	Peak 1	0.420	0.552	No	Pass

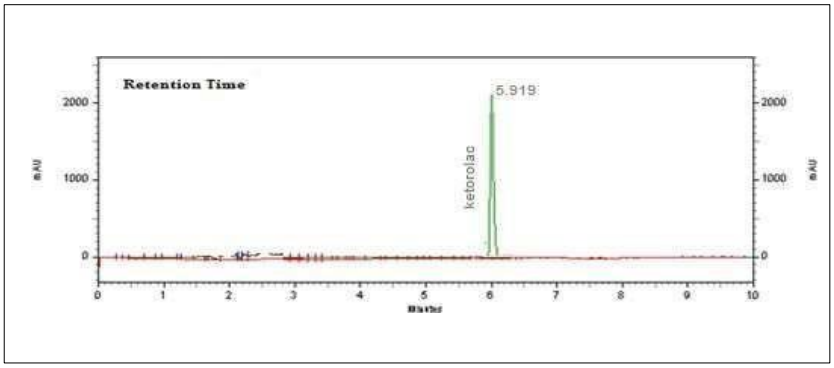

Figure No. 2: The chromatogram of Acidic condition

Table No. 3: Peak purity results of base degraded Ketorolac Tromethaminesample solution

Sr.No.	Peak name	Purity angle	Purity threshold	Purity Flag	Peak purity
1.	Ketorolac Tromethamine	0.2135	0.341	No	Pass

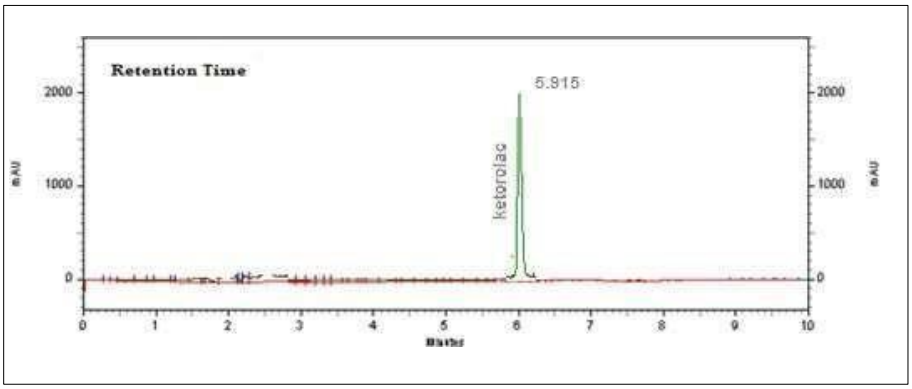

Figure no. 3: The chromatogram of Basic condition

Table No.4: Peak purity results of photolytic degraded Ketorolac Tromethaminesample solution

Sr.No.	Peak name	Purity angle	Purity threshold	Purity Flag	Peak purity
1.	Ketorolac Tromethamine	0.347	0.441	No	Pass

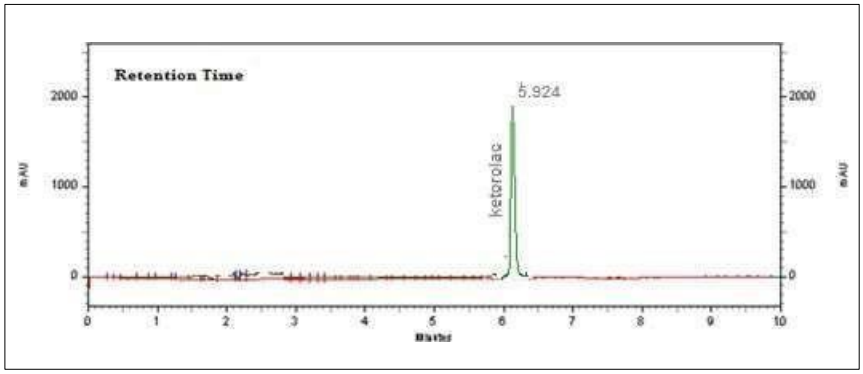

Figure no. 4: The chromatogram of Photolytic condition

Table No. 5: Peak purity results of oxidative degraded Ketorolac Tromethaminesample solution

Sr. No.	Peak name	Purity angle	Purity threshold	Purity Flag	Peak purity
1.	Ketorolac Tromethamine	0.857	0.640	No	Pass
2.	Peak 1	0.322	0.423	No	Pass

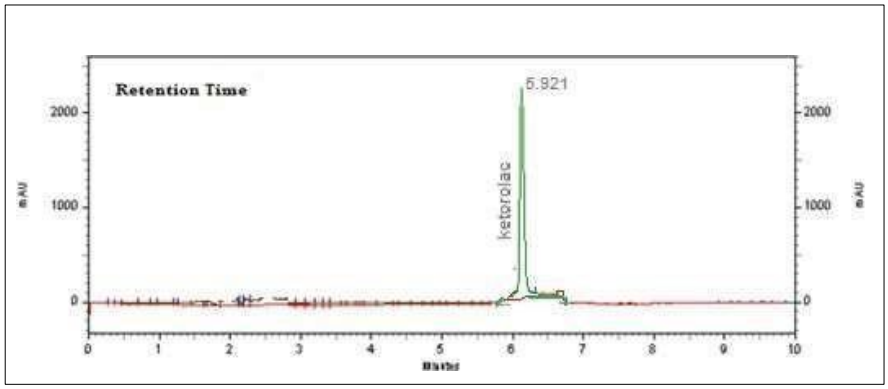

Figure no. 5: The chromatogram of oxidative condition

Table No. 6: Peak purity results of heat degraded Ketorolac Tromethamine samplesolution

Sr.No.	Peak name	Purity angle	Purity threshold	Purity Flag	Peak purity
1.	Ketorolac Tromethamine	0.314	0.342	No	Pass

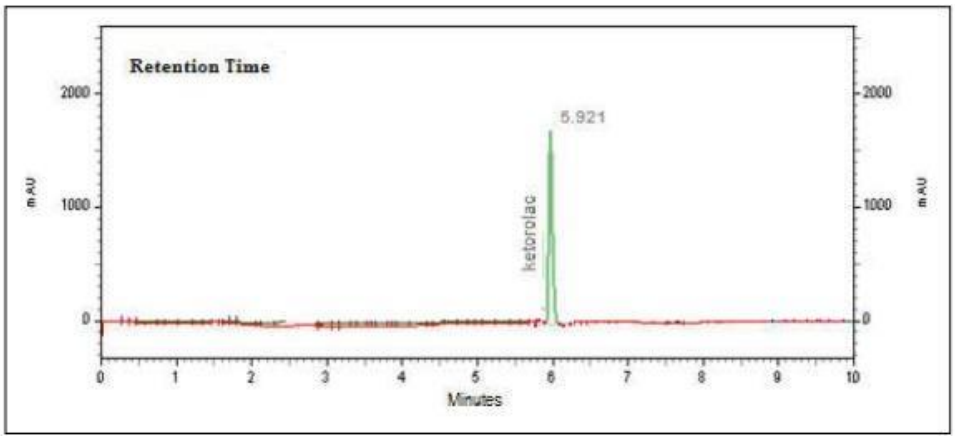

Figure no. 6: The chromatogram of heat condition

Table No. 7: Peak purity results of UV degraded Ketorolac Tromethamine sample solution

Sr.No.	Peak name	Purity angle	Purity threshold	Purity Flag	Peak purity
1.	Ketorolac Tromethamine	0.289	0.312	No	Pass

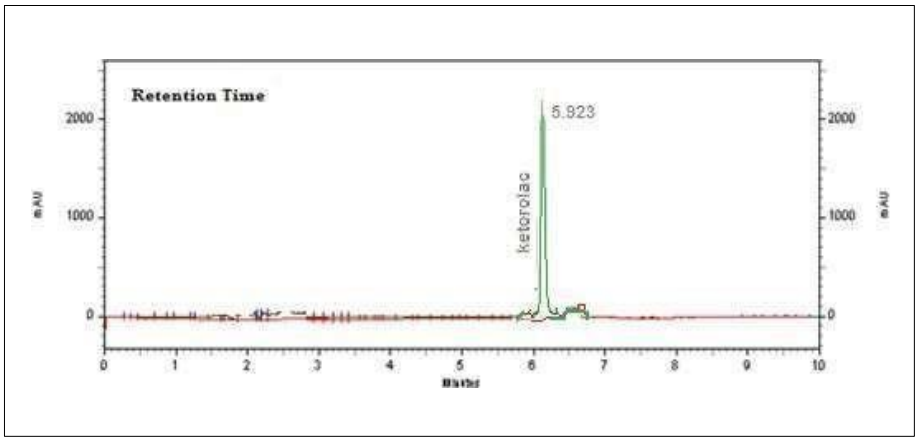

Figure no.7: The chromatogram of UV condition

Table No. 8: Forced degradation study of Ketorolac Tromethamine

	% Assay of active ingredients	
Stress conditions	**Ketorolac Tromethamine**	**% Degradation**
Acidic	96.35	3.65
Basic	97.55	2.45
Oxidative	80.17	19.83
Photolytic UV light Visible light	99.45	0.55
Heat	99.17	0.83

Stability Indicating Assay of Ketorolac Tromethamine

The force degradation study was performed for stability of Ketorolac Tromethamine. It is indicated that the drug was degraded by 3.65%, 2.45% and 19.83% when subjected to acid, base hydrolysis and degradation by oxidation respectively. This shows that there were not found any impurity. Drug was found to be stable in different degradation conditions like acid, base hydrolysis, heat, photolytic degradation except oxidation degradation.

Conclusion:

In force degradation study of Ketorolac Tromethamine negligible degradation obtained. There were not found any impurity other than reported. Peak purity test was passed. In force degradation study oxidative condition shown marked degradation.

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