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Stability-Indicating RP-HPLC Assay Method for Estimation of Ketoprofen in Bulk and Tablets

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Abstract

Objective

To develop and validate a simple, precise, accurate and stability-indicating reverse phase-high performance liquid chromatographic (RP-HPLC) assay method for the estimation of Ketoprofen in bulk and pharmaceutical tablet dosage forms.

Methods

Chromatographic separation was achieved using an Enable C_{18} column (250 mm × 4.6 mm, 5 µm) and a mobile phase comprising aqueous phosphate buffer (pH 3.0): acetonitrile (40:60 v/v) at a flow rate of 1.0 ml/min. UV detection was performed at 260 nm. The method was validated according to ICH Q2(R1) guidelines for linearity, precision, accuracy, robustness, LOD and LOQ. Forced degradation studies were performed under acidic, basic, oxidative, thermal and photolytic conditions as per ICH Q1A(R2).

Results

The retention time of Ketoprofen was found to be 4.53 min. The method was linear in the range of 10–100 µg/ml with a correlation coefficient (r^2) of 0.9999. The mean % recovery was 99.13 and %RSD for repeatability was below 0.5%. LOD and LOQ were found to be 0.35 µg/ml and 1.06 µg/ml, respectively. The drug was found stable under acidic, hydrolytic and thermal conditions but degraded in basic, photolytic and oxidative environments, indicating the stability-indicating capability of the method.

Conclusion

The developed RP-HPLC method is simple, rapid, specific, sensitive and validated as per ICH guidelines. It can be effectively employed for routine quality control and stability testing of Ketoprofen in bulk and tablet dosage forms.

Keywords: Ketoprofen, RP-HPLC, Method Validation, Stability Indicating Assay, ICH Q2(R1).

1. Introduction

Ketoprofen is a propionic acid derivative nonsteroidal anti-inflammatory drug (NSAID) chemically known as (±)-2-(3-benzoylphenyl)propionic acid. It is widely used for its analgesic, anti-inflammatory and antipyretic properties in the treatment of arthritis, musculoskeletal disorders, and postoperative pain.[1-2] Ketoprofen acts by inhibiting cyclooxygenase (COX-1 and COX-2) enzymes, thereby reducing prostaglandin synthesis and inflammation.[3] Despite its extensive therapeutic application, there is a need for a reliable and stability-indicating analytical method for Ketoprofen in bulk and dosage forms. Literature surveys report several analytical approaches, including spectrophotometric methods (UV and derivative spectroscopy), HPTLC and non-stability-indicating HPLC methods. However, few reports include forced degradation studies that establish the stability-indicating nature of the method.[4-5] Hence, the present study aims to develop a validated, stability-indicating RP-HPLC method for the estimation of Ketoprofen in bulk and tablet formulations, following ICH Q1A(R2) and Q2(R1) guidelines.[6-7]

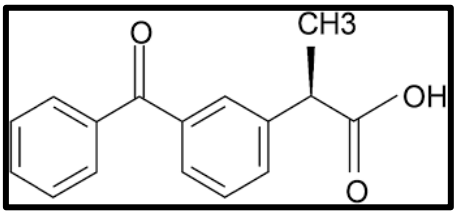

Fig. 1: Chemical Structure of Ketoprofen

2. Materials and Methods

2.1 Chemicals and Reagents

Ketoprofen reference standard was used for the study. Marketed tablets (Ketafen® 100 mg) were purchased locally. HPLC-grade acetonitrile and methanol were used. Analytical grade orthophosphoric acid and potassium dihydrogen phosphate was used for buffer preparation. Distilled water was prepared using a Millipore system.

2.2 Instrumentation

The analysis was performed on a Waters 2695 HPLC 2695 Alliance system equipped with a UV-visible detector. The data were processed using LC Solution software. A C_{18} column (250 mm × 4.6 mm, 5 µm) was used for chromatographic separation.

2.3 Preparation of Solutions

2.3.1 Standard Stock Solution

An accurately weighed 100 mg of Ketoprofen was dissolved in methanol and diluted to 100 ml with the same solvent to obtain a stock solution of 1000 µg/ml.

2.3.2 Working Standard Solution

Aliquots of the stock solution were diluted with the mobile phase to obtain concentrations in the range of 10–100 µg/ml.

2.3.3 Sample Solution (Tablet Assay)

Twenty tablets were weighed and powdered. A portion equivalent to 100 mg of Ketoprofen was transferred to a 100 ml volumetric flask, dissolved in methanol, and sonicated for 10 min. The solution was filtered and diluted appropriately to obtain 50 µg/ml of Ketoprofen. This procedure was repeated in triplicate. The results of the assay of tablets are shown in Table 2.

3. Method Validation[8]

The developed chromatographic method was validated in accordance with ICH guidelines for system suitability, linearity, range, accuracy, precision and robustness parameters.

3.1 Linearity and Range

Working standard solutions of Ketoprofen were injected under the optimized chromatographic conditions and the corresponding peak areas were recorded at 260 nm. A calibration curve was constructed by plotting peak area versus concentration. The linear regression data for the calibration curve is presented in Fig. 3. The concentration range for the standard solutions was selected based on the correlation coefficient obtained from the regression equation.

3.2 Precision

Repeatability study was carried out with six replicates, and intermediate precision studies were carried out with three concentrations of Ketoprofen with three replicates. The values of % relative standard deviation (% RSD) of precision study are shown in table 3.

3.3 Robustness

Robustness of the optimized method was studied by changing flow rate (±0.1 ml/min), change in wavelength (±2 nm) and change in mobile phase composition (±5%) during analysis. The sample was injected in triplicate for every condition and % RSD was calculated for each condition is shown in table 5.

3.4 Limit of detection (LOD) and Limit of quantification (LOQ)

Five sets of Ketoprofen standard solutions were prepared in the concentration range of 10–100 µg/ml, and the corresponding peak areas were recorded. Calibration curves were constructed for each set. The standard deviation of the y-intercept and the average slope of the calibration curves were used to determine the LOD and LOQ of Ketoprofen using the following equations.

LOD = 3.3 (SD/S) and

LOQ = 10 (SD/S).

Where SD is the standard deviation of y-intercepts of the calibration curves; S is the mean slope of six calibration curves.

3.5 Accuracy

The accuracy of the method was evaluated by determining the percent recovery of Ketoprofen using the standard addition technique. Percent recovery was assessed at three concentration levels - 80%, 100%, and 120% of the target concentration, each analyzed in triplicate. The results of the accuracy study are presented in Table 4.

4. Forced Degradation Studies[9]

To evaluate the stability of Ketoprofen, the drug was subjected to forced degradation studies under acidic, basic, neutral hydrolysis, oxidative, thermal and photolytic conditions as per the International Conference on Harmonisation (ICH) guidelines.

4.1 Alkaline Hydrolysis

An accurately weighed quantity of 100 mg of Ketoprofen was transferred into a 250 mL round-bottom flask containing 100 mL of 0.1 N sodium hydroxide (NaOH) solution. The mixture was refluxed at 80 °C to induce alkaline degradation. At specific time intervals of 30 minutes, 5 mL aliquots of the refluxed solution were withdrawn and neutralized immediately with an equal volume (5 mL) of 0.1 N hydrochloric acid to prevent further degradation. The neutralized samples were then diluted tenfold with the mobile phase to obtain a final concentration of 100 µg/mL of Ketoprofen and 20 µL of each was injected into the HPLC system. The chromatogram obtained after 2 hours of alkaline hydrolysis is shown in Fig. 4A.

4.2 Acid Hydrolysis

A weighed quantity of 100 mg of Ketoprofen was transferred into a 250 mL round-bottom flask containing 100 mL of 0.1 N hydrochloric acid (HCl) and refluxed at 80 °C. At regular intervals of 30 minutes, 5 mL aliquots were withdrawn and neutralized with an equal volume of 0.1 N sodium hydroxide (NaOH). The samples were further diluted tenfold with the mobile phase to achieve 100 µg/mL concentration. The chromatogram obtained after 2 hours of alkaline hydrolysis is shown in Fig. 4B.

4.3 Oxidative Degradation

For oxidative stress, 100 mg of Ketoprofen was accurately weighed and transferred into a 250 mL round-bottom flask containing 100 mL of 3% hydrogen peroxide (H_2O_2) solution. The mixture was kept at room temperature (25 °C) for 24 hours to induce oxidation. After exposure, 5 mL of the sample was withdrawn and diluted tenfold with the mobile phase to obtain 100 µg/mL solution. The chromatogram obtained after oxidative degradation is shown in Fig. 4C.

4.4 Hydrolytic (Neutral) Degradation

An accurately weighed 100 mg of Ketoprofen was dissolved in 100 mL of distilled water in a 250 mL round-bottom flask and refluxed at 80 °C for 2 hours. Samples (5 mL each) were collected every 30 minutes and diluted tenfold with the mobile phase to obtain 100 µg/mL concentration. The chromatogram obtained after 2 hours of hydrolytic degradation is shown in Fig. 4D.

4.5 Thermal Degradation

To study thermal stability, Ketoprofen powder (100 mg) was placed in a hot air oven at 80 °C for 48 hours. After cooling to room temperature, an amount equivalent to 100 µg/mL was dissolved in mobile phase and injected into the HPLC system. The chromatogram obtained after thermal degradation is shown in Fig. 4E.

4.6 Photolytic Degradation

For photolytic degradation, Ketoprofen powder and aqueous solution (100 µg/mL) were exposed to UV light (254 nm) and visible light in a photostability chamber for 48 hours. After

exposure, samples were injected into the HPLC system. The chromatogram obtained after photolytic degradation is shown in Fig. 4F.

5. Results and Discussion

5.1 Selection of detection wavelength

Detection wavelength of Ketoprofen was selected as the wavelength maxima (λmax) from the UV absorption spectrum of the drug in the range of 200–400 nm. The value of λmax was found to be 260 nm.

5.2 Optimization of chromatographic conditions

Initially, various chromatographic conditions were tried in order to obtain better separation characteristics by altering the mobile phase composition, pH and flow rate. Finally, a mobile phase consisting of aqueous phosphate buffer (pH 3.0): acetonitrile (40:60 v/v) was selected, delivered at a flow rate of 1.0 ml/min, with UV detection at 260 nm. The retention time of Ketoprofen was found to be 4.53 min, indicating that the method is rapid and efficient. The chromatogram of Ketoprofen is shown in Fig. 2, and the optimized chromatographic conditions are summarized in Table 1.

Table 1: Optimized chromatographic conditions

Parameter	Specification
Column	Enable C_{18} (250 × 4.6 mm, 5 µm)
Mobile Phase	Aqueous phosphate buffer (pH 3.0): Acetonitrile (40:60 v/v)
Flow Rate	1.0 ml/min
Detection Wavelength	260 nm
Injection Volume	20 µl
Run Time	10 min
Retention Time	4.53 ± 0.02 min
Temperature	Ambient (25°C)

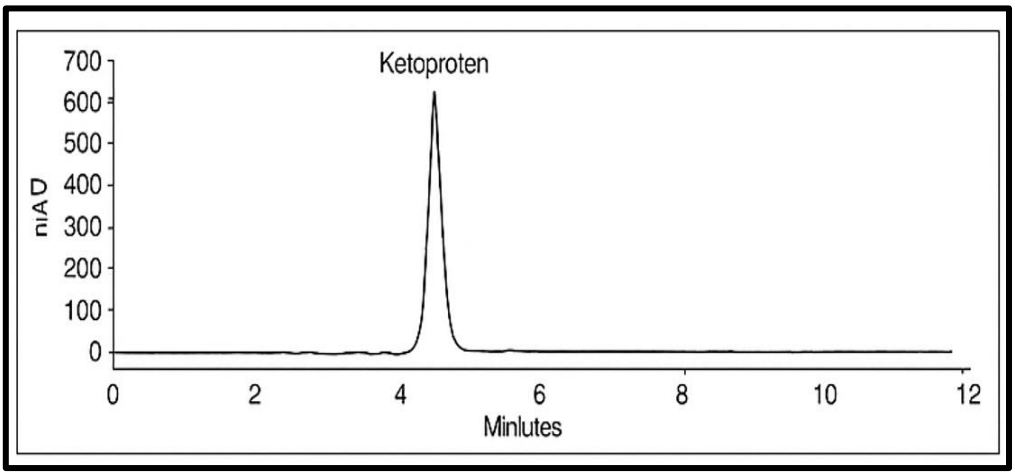

Fig 2: Chromatogram of Ketoprofen

5.3 Assay of tablet formulation

The value of mean % drug found to be 99.30% which was within acceptance criteria.

Table 2: Results of Assay of Ketoprofen Tablets

Sr. No.	Sample Solution Concentration (µg/ml)	Actual Concentration Found (µg/ml)	Amount of Drug Estimated (mean ± SD)
1	50	49.43	99.30 ± 0.214
2	50	49.65	
3	50	49.51	

* The value is represented as mean ± SD of 3 observations.

5.4 Linearity and Range

Linearity was evaluated using working standard solutions (10–100 µg/ml). A calibration curve was plotted between peak area and concentration. Linear regression data for calibration curve was shown in Fig. 3. The correlation coefficient ($r^2 = 0.9999$) confirmed excellent linearity.

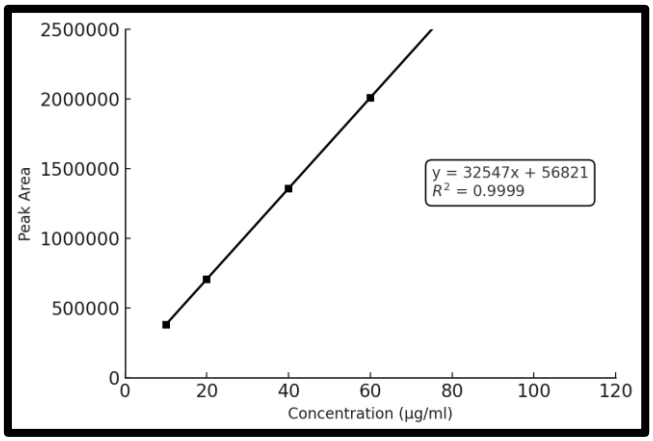

Fig. 3: Calibration curve of Ketoprofen

5.5 Precision:

Precision was evaluated by repeatability and intermediate precision studies. % RSD values were <1% in all cases, confirming high reproducibility. The values of % RSD of precision study are shown in table 3.

Table 3: Repeatability and Intermediate Precision for Ketoprofen

Precision	Concentration of drug (µg/ml)	Mean Area ± SD*	%RSD
Repeatability (n=6)	60	1,950,865 ± 8,220.41	0.421
Intra-day (n=3)	40	1,289,742 ± 5,981.23	0.464
	60	1,950,312 ± 6,124.58	0.314
	80	2,610,473 ± 4,981.05	0.190
Inter-day (n=3)	40	1,295,104 ± 6,022.11	0.465
	60	1,953,687 ± 9,028.74	0.462
	80	2,609,518 ± 7,652.47	0.293

*Each value is represented as mean ± SD of *n* observations. The value of *n* is 6 for repeatability and 3 for intra-day and inter-day precision.

SD: Standard deviation; %RSD: Percent relative standard deviation.

5.6 Accuracy

Accuracy was determined using standard addition (80%, 100%, 120% levels). Recovery values were between 98.70% and 99.50%, indicating accuracy. The results of accuracy study are shown in table 4.

Table 4: Recovery Study for Ketoprofen

Level	Amount taken (µg/ml)	Amount found (µg/ml)	% Recovery *	Mean % Recovery ± %RSD
80 %	48	47.42	98.80	98.82 ± 0.21
		47.38	98.70	
		47.50	99.00	
100 %	60	59.49	99.15	99.27 ± 0.18
		59.56	99.26	
		59.68	99.40	
120 %	72	71.45	99.24	99.31 ± 0.26
		71.42	99.20	
		71.56	99.50	

*Percent recovery was done in triplicate.

% RSD: Percent relative standard deviation.

5.7 Robustness

Small deliberate variations in flow rate (±0.1 ml/min), wavelength (±2 nm), and mobile phase ratio (±5%) did not significantly affect assay performance (%RSD < 1%), demonstrating robustness. The sample was injected in triplicate for every condition and % RSD was calculated for each condition is shown in table 5.

Table 5: Robustness Study for Ketoprofen

Factor	%RSD*
A: Change in flow rate	
0.9 ml/min	0.212
1.0 ml/min	0.185
1.1 ml/min	0.243
B: Change in wavelength	
258 nm	0.314
260 nm	0.281
262 nm	0.297
C: Change in mobile phase composition	
Buffer: Acetonitrile (38:62 v/v)	0.256
Buffer: Acetonitrile (40:60 v/v)	0.219
Buffer: Acetonitrile (42:58 v/v)	0.244

*Each value is represented as % RSD of *n* observations.

The value of *n* is 3 for each condition.

%RSD: Percent relative standard deviation.

5.8 Limit of detection (LOD) and Limit of quantitation (LOQ)

They were found to be 0.35 µg/ml and 1.06 µg/ml respectively, indicating method sensitivity.

5.9 Forced degradation studies

Forced degradation studies were performed to establish the stability-indicating nature of the method, following ICH Q1A(R2) guidelines. The results of stress testing indicate that Ketoprofen is stable in acidic, neutral, and thermal conditions, but degrades extensively under basic, oxidative, and photolytic conditions. These findings confirm that the developed RP-HPLC method is stability-indicating, as it successfully resolved the drug from its degradation products.

The chromatogram obtained after 2 hours of alkaline hydrolysis showed a significant decrease in the peak area of the parent drug and the appearance of additional peaks corresponding to degradation products, indicating extensive alkaline degradation of Ketoprofen due to base-catalyzed hydrolysis and decarboxylation reactions. (Figure 4A).

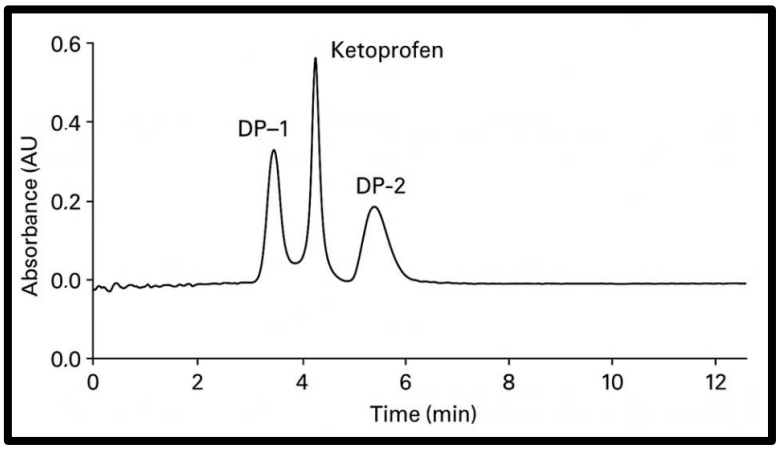

Fig 4A: Alkaline degradation of Ketoprofen

The chromatogram obtained after 2 hours of acid exposure showed minimal change in the drug peak and no significant secondary peaks, indicating that Ketoprofen is relatively stable in acidic medium. (Figure 4B).

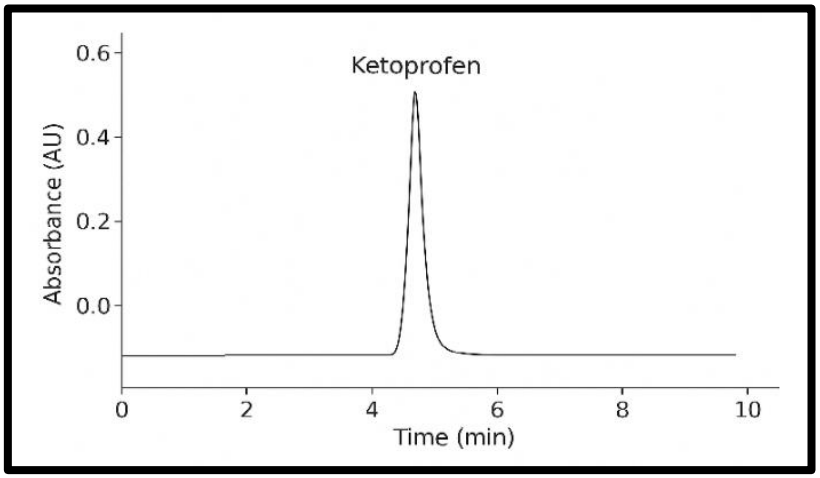

Fig 4B: Acidic degradation of Ketoprofen in 0.1 N HCl at 80 ◦C after 2 hr.

The chromatogram obtained after oxidative degradation showed formation of additional peaks at lower retention times, confirming the oxidative degradation of Ketoprofen. This degradation likely occurs through oxidation of the aromatic ring and formation of quinone-like structures (Figure 4C).

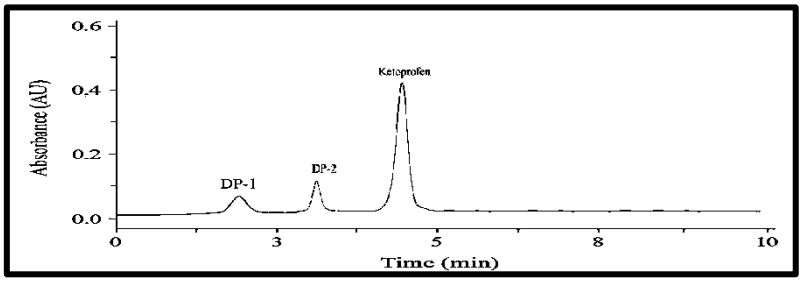

Fig 4C: Oxidative degradation of Ketoprofen

The chromatogram obtained after 2 hours hydrolytic degradation showed a slight decrease in the peak area with no prominent degradant peaks, indicating that Ketoprofen is moderately stable in aqueous (neutral) medium (Figure 4D).

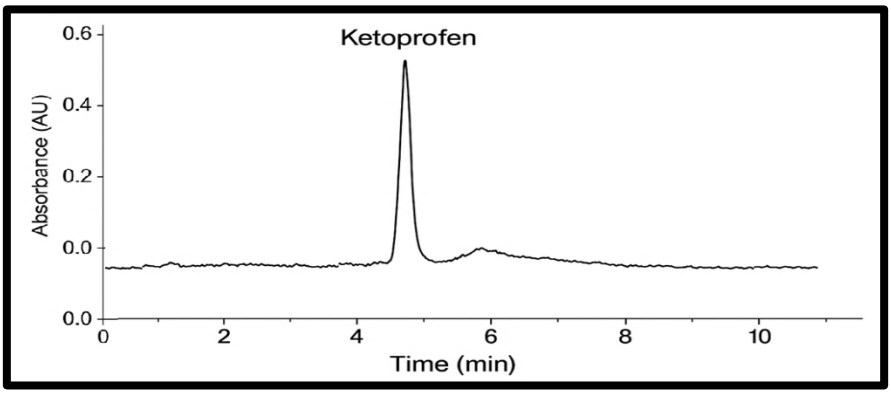

Fig 4D: Hydrolytic degradation of Ketoprofen

The chromatogram obtained after thermal degradation showed no significant additional peaks and only a minor decrease in drug peak area, indicating that Ketoprofen is thermally stable under dry heat conditions (Figure 4E).

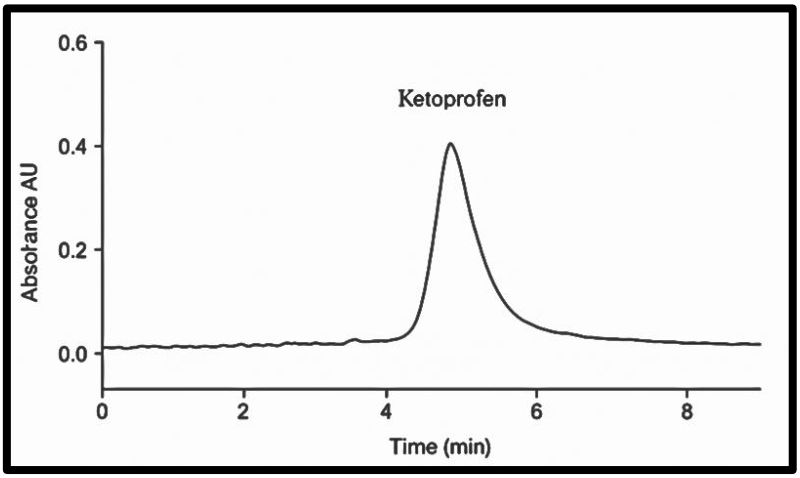

Fig 4E: Thermal degradation of Ketoprofen

The chromatogram obtained after photolytic degradation revealed extensive degradation, with multiple new peaks and a significant decrease in the main drug peak, confirming that Ketoprofen is highly photosensitive and undergoes photo-oxidative decarboxylation, producing benzophenone-type photoproducts (Figure 4F).

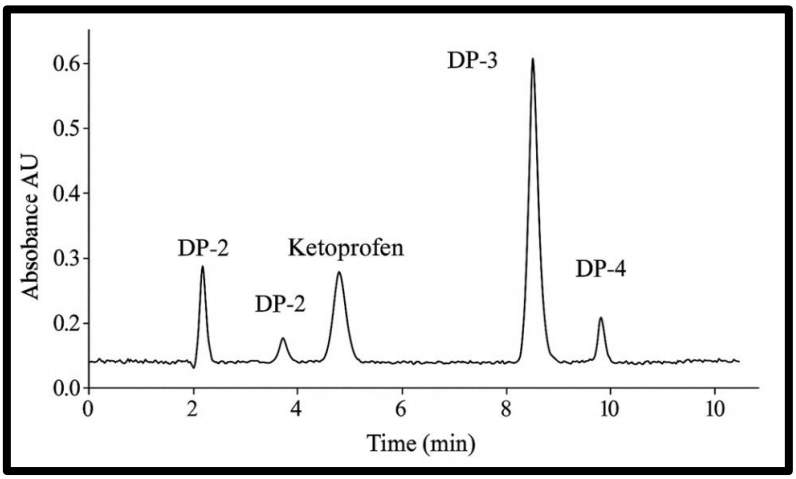

Fig 4F: Photolytic degradation of Ketoprofen

5.10 Stability testing on commercial Tablets:

The assay content of Ketoprofen, commercially available marketed formulation was analysed by the proposed method after exposure to accelerated storage condition. The peak at retention time 4.53 min for the drug was observed in the chromatogram of the drug samples extracted from tablets and no additional peak was found (Fig. 5). Experimental results of the amount of Ketoprofen in tablets, expressed as percentage of label claim were in good agreement with the label claims thereby suggesting that there is no interference from any excipients, which are normally present in tablets and packaging material is of good quality (Table 2).

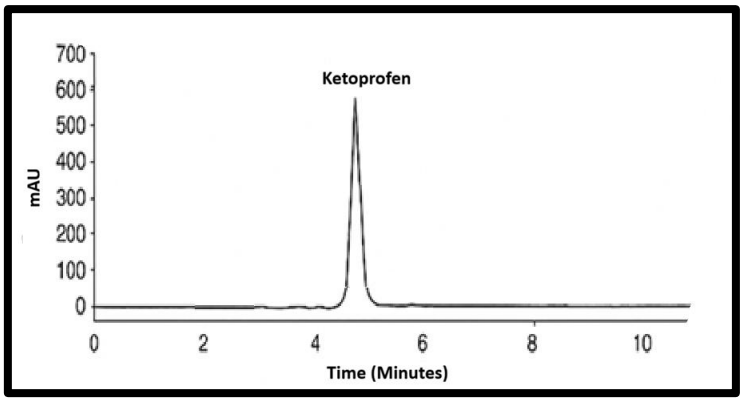

Fig 5: Chromatogram of marketed formulation

6. Conclusion:

A new, simple and accurate RP-HPLC method was successfully developed and validated for the estimation of Ketoprofen in bulk and tablet formulations. The method proved to be specific, precise and stability-indicating as per ICH guidelines. It can be effectively used for quality control and stability analysis in routine pharmaceutical applications.

The forced degradation results demonstrated that Ketoprofen undergoes significant degradation under alkaline, oxidative, and photolytic conditions, while it remains relatively stable in acidic, hydrolytic (neutral), and thermal environments. Hence, light protection and pH control (pH 4–6) are essential for formulation and storage of Ketoprofen products.

7. Acknowledgment:

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8. Conflict of Interest:

The authors declare no conflict of interest.

9. References:

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