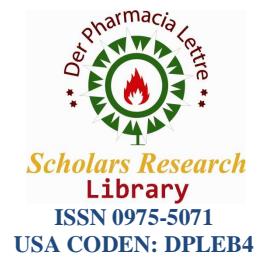




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## RP-HPLC method for degradation study of Lornoxicam

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### ABSTRACT

The main objective of this paper is to develop simple, precise, accurate stress degradation assay method for lornoxicam. For present work ACN: Water (70:30) was used as mobile phase. It showed sharp peaks and good resolution (column-Grace smart RP 80 (250×4.5Mm) 5µm), (HPLC PU – 2080 Plus, Jasco). The retention time was found to be 2.3 min. The drug showed λ<sub>max</sub> at 382 nm. The method obeys Beers-Lamberts law in the concentration range of 5-30 µg/ml for HPLC analysis. From the degradation studies Lornoxicam showed sufficient degradation in acidic and alkaline degradation. Lornoxicam was found to be less susceptible to oxidative degradation. No degradation was observed in photolytic and thermal degradation. There was no significant difference observed in degradation pattern of bulk drug and pharmaceutical formulation. ICH guidelines were followed throughout the degradation studies and the proposed method found accurate stress degradation assay method. The proposed method is precise, accurate and stability indicating, resolving the entire degradation product from the drug. Thus the proposed method can have its application in the determination, of Lornoxicam in bulk drug, as well as in presence of its degradation products.

**Keywords:** HPLC column, Lornoxicam, Stress degradation.

### INTRODUCTION

Lornoxicam is a non-steroidal anti-inflammatory drug (NSAID) that belongs to the oxicam class. As with other NSAIDs, Lornoxicam is a potent inhibitor of the cyclooxygenase enzymes, which are responsible for catalyzing the formation of prostaglandins (act as messenger molecules in the process of inflammation) and thromboxane from arachidonic acid<sup>(1-3)</sup>. A survey of literature revealed some spectrophotometric and HPLC method for analysis of Lornoxicam alone and in combination with other drugs. A few reports on LC and LC-MS methods for the detection of Lornoxicam and its metabolites in biological matrices are available<sup>(4-10)</sup>. No far work has been done for analysis of lornoxicam with its degradants hence it is selected for the study. Study of extent of drug degradation in the different degradation conditions (acidic, alkaline, oxidative, thermal and photolytic) using suitable analytical method is the part of work. Present study concludes that Lornoxicam is most labile to alkaline hydrolysis followed by acidic degradation and oxidative degradation. It is stable to thermal and photochemical stress conditions. The proposed method is precise, accurate and resolving the entire degradation product from the drug. The ICH guidelines are followed throughout the study for method validation and stress testing, and thus proposed method has wide industrial applicability.

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**MATERIALS AND METHODS****Reagents and chemicals:**

Lornoxicam from Glenmark Pharmaceuticals Ltd. Mumbai, Acetonitrile (HPLC grade) Methanol (HPLC grade), Sodium Hydroxide pellets, Hydrochloric acid, Hydrogen peroxide. All chemicals from Loba Chemie. Tab. Lorcarn (Sun Pharma).

**Instruments:**

The equipment used for method was UV Visible Spectrophotometer (Shimadzu 1800), HPLC PU-2080 Plus (Jasco), HPLC Detector (UV-2075 plus Intelligent UV/VIS), Sonicator (PCI), column (Grace smart RP 80(250×4.5Mm) 5µm), PH Meter (Eutech), Micro syringe (Hamilton), Hot Air Oven (S. M. Scientific, Delhi), UV Cabinet (Neolab Bombay). Flow rate was monitored at 0.8 mL/min. The wavelength selected for the method was 382 nm and the injection volume was 20µL.

**Method Development:**

For selection of mobile phase, various mobile phases containing ACN with Water in different ratios were tried but the best resolution was found to be in ACN and Water (70:30v/v). Finally, mobile phase containing ACN and Water in 70:30v/v proportions were found to give best resolution for drug and degradant.

**Optimization of Detection Wavelength:**

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is one that gives good response for the drugs that are to be detected. For good response, optimization of wavelength was done at different wavelengths by UV detector. In the present study, drug solutions of 10 µg/ml were prepared in mobile phase. After observing UV spectra of the drug, wavelength of 382nm was selected for further study.

**Preparation of Standard Drug Solution:**

Standard stock solution containing Lornoxicam was prepared by dissolving 10 mg of drug in few ml of mobile phase in 100ml of volumetric flask by sonication. Final volume was then made up to 100ml with mobile phase. (100 µg/ml).

**Preparation of sample from formulation:**

Twenty tablets were triturated Lornoxicam equivalent to 10 mg was weighed accurately and dissolved using ACN: Water (70:30v/v) by sonication. Final volume is made with the mobile phase up to 100 ml.

**Forced Degradation Study:****Alkaline Degradation Study:**

The alkaline degradation was done against 1N sodium hydroxide.

**Procedure:**

To 5ml of above stock solution (100µg/ml) 5ml of 1N sodium hydroxide was added. The solution was refluxed for 2hr cooled and then it was neutralized with 1N HCl and volume is made up to 100 ml with mobile phase. 1ml of resultant solution was taken and volume made up to 10ml with ACN: Water (70:30v/v) It was filtered through 0.45µ filter paper and sonicated for 15 min and used for study. The chromatogram of Lornoxicam and degraded product in 1N sodium hydroxide is shown in Fig No.1

**Acidic Degradation Study:**

The acidic degradation was done against 2N hydrochloric acid.

**Procedure:**

To 5ml of above stock solution (100µg/ml) 5ml of 2N hydrochloric acid was added. The solution was refluxed for 2hr cooled and then it was neutralized with 2N NaOH and volume is made up to 100 ml with mobile phase. 1ml of resultant solution was taken and volume was made up to 10ml with ACN: Water (70:30v/v) The chromatogram of Lornoxicam and degraded product in 2N Hydrochloric acid is shown in Fig. No. 2

**Oxidative Degradation Study:**

The oxidative degradation was done against 6% Hydrogen peroxide.

**Procedure:**

To 5ml of above stock solution (100µg/ml) 5ml of 6% Hydrogen peroxide was added. The solution was refluxed for 1hr cooled. 1ml of resultant solution was taken and volume was made up to 10ml with ACN: Water (70:30v/v). It was filtered through 0.45µ filter paper and sonicated for 15 min and used for study. It was filtered through 0.45µ filter paper and sonicated for 15 min and used for study. The chromatogram of Lornoxicam and degraded product is shown in Fig. No. 3

**Thermal Degradation Study:**

The thermal degradation was done by heating the Lornoxicam at 80 °C.

**Procedure:**

Under Dry Heat condition 50 mg of Lornoxicam was heated at 80 °C in hot air oven for 2 hrs 10 mg of this drug was dissolved in 100 ml of mobile phase .1 ml of resultant solution was diluted by mobile phase upto 10 ml. It was filtered through 0.45µ filter paper and sonicated for 15 min. and used for study. The chromatogram of Lornoxicam and degraded product is shown in Fig.No. 4

**Photo stability studies:**

The photochemical stability of the drug was studied by exposing the 50mg of Lornoxicam to UV light in UV cabinet for 24 hrs. 10 mg of this drug was dissolved in 100 ml of mobile phase. 1 ml of resultant solution was diluted up to 10 ml with mobile phase to get 10 µg/ml solution , filtered through 0.45µ filter paper and sonicated for 15 min. and used for study. The chromatogram of Lornoxicam and degraded product is shown in Fig. No. 5

**METHOD VALIDATION:****Linearity Study of Drug at Selected Wavelength:**

In a series of 10 ml volumetric flasks, 0.5 ml to 3.0 ml of drug stock solution (100µg/ml) was pipetted and final volume of the solutions was made up to 10 ml with mobile phase. A 20 µl of sample solution was injected into the chromatographic system using fixed volume loop injector. Chromatograms were recorded. Retention time for Lornoxicam was found to be 2.3 min. The peak area was plotted against concentration to get the calibration curve. The data for calibration curve is given in Chromatograms seen in Fig no.6 and 7 parameters are shown in Table no. 2, 3, 4.

**Precision Studies:**

The precision studies were carried out by performing repeatability and intermediate precision. The Repeatability (Intra-Day) was performed by taking three concentration on the same day (n=6). Intermediate precision (Inter-Day) of the method was carried by repeating the analysis of given concentrations for three different days. The results for precision studies were given in Table No.5

**Accuracy (Recovery Studies):**

Recovery studies was carried out by applying the method to drug sample to which the known amount of pure Lornoxicam was added corresponding to 50,100 and 150% of the label claim (standard addition method). At each level six absorptions were taken and % drug recovery was calculated, shown in Table No. 6

**Selectivity :**

The selectivity of method was determined by complete separation of Lornoxicam along with its degradation products by checking various parameters such as retention time, asymmetry factor etc. The selectivity by retention time was illustrated in Table No. 7

**Limit of Detection & Limit of Quantitation:**

The limit of detection (LOD) & limit of quantitation (LOQ) for the developed method was calculated by using following formulae.

$$\text{LOD} = 3.3 \sigma / S$$

$$\text{LOQ} = 10 \sigma / S$$

Where,

$\sigma$  =Standard deviation of response.

S = slope of calibration curve.

The results of LOD and LOQ were given inTable No. 8

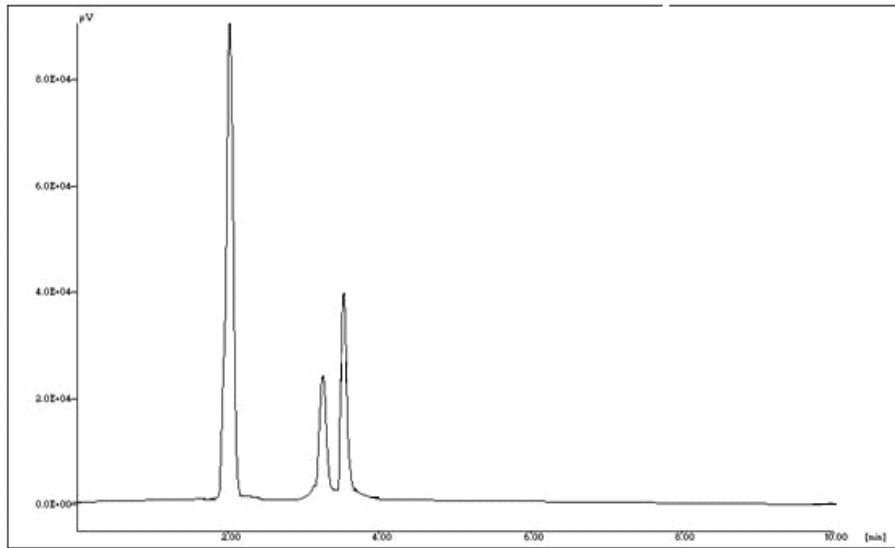


Fig.No.1chromatogramof alkali degradation of Lornoxicam (API) in by NaOH

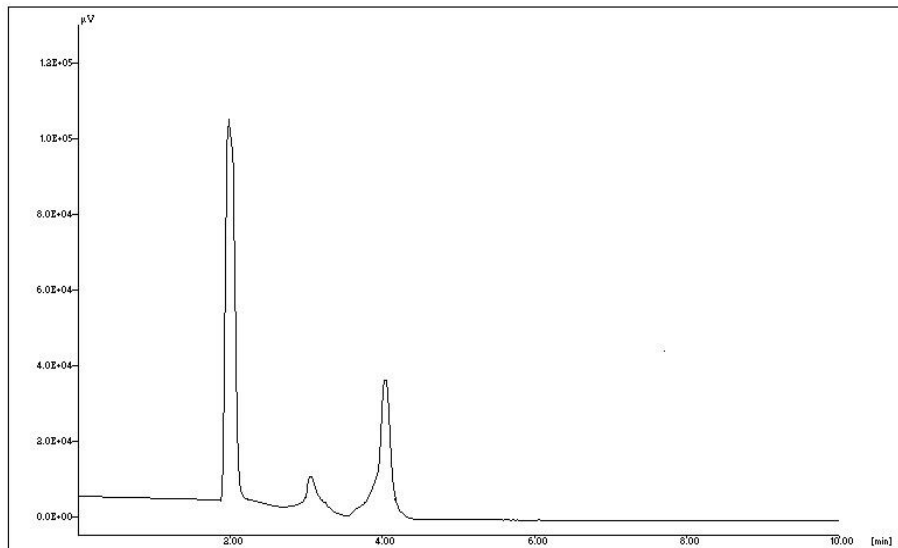


fig. no.2 Chromatogram of acid degradation of Lornoxicam (API) by 2N HCl

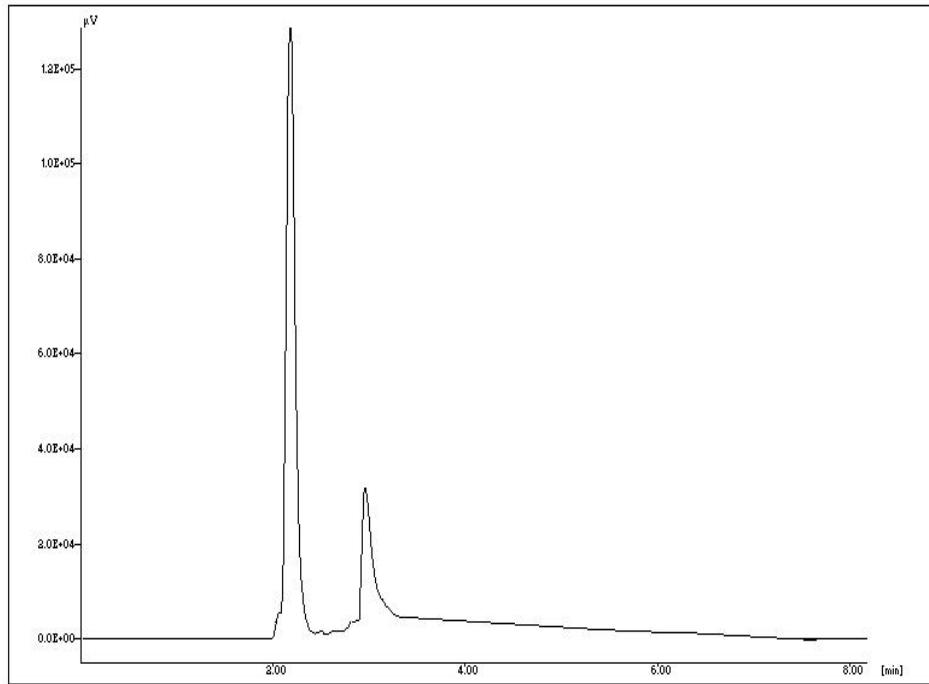


Fig.No.3 Chromatogram of oxidative degradation of Lornoxicam (API) by 6% H<sub>2</sub>O<sub>2</sub>.

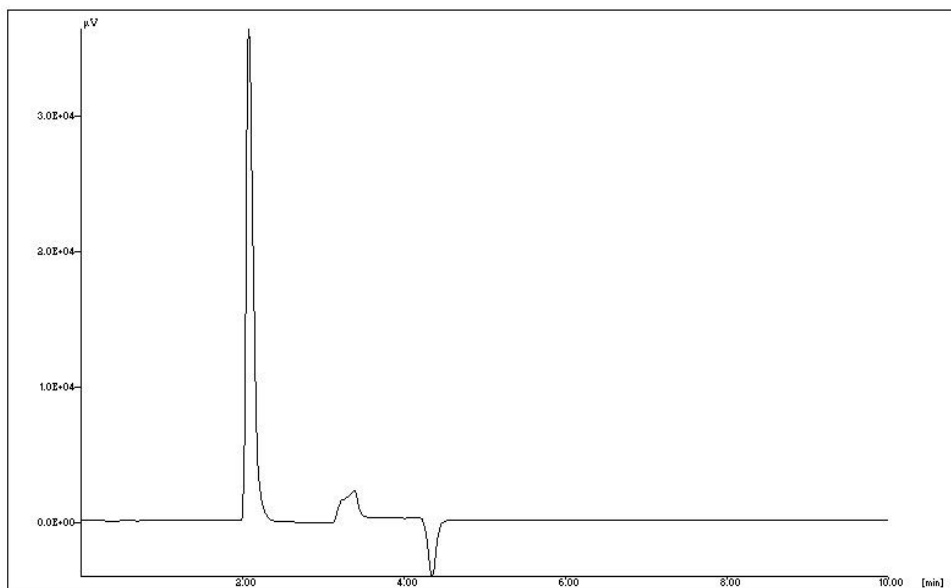
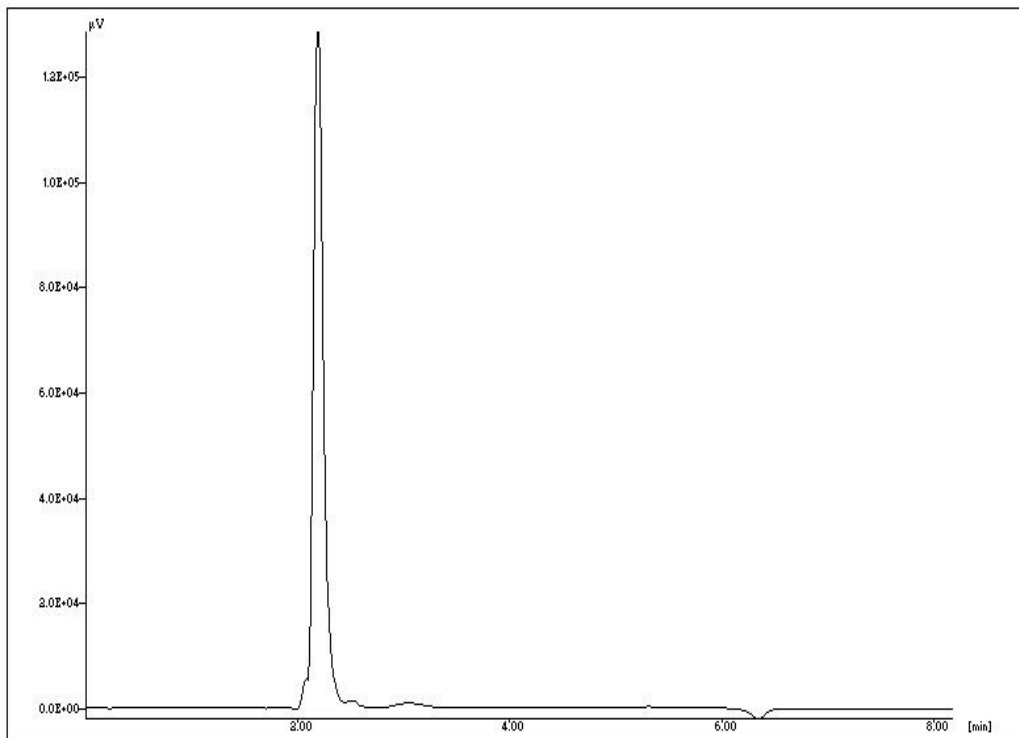


Fig.No.4 Chromatogram of thermal degradation of Lornoxicam (API)



FigNo.5 Chromatogram of photolytic degradation of Lornoxicam (API)

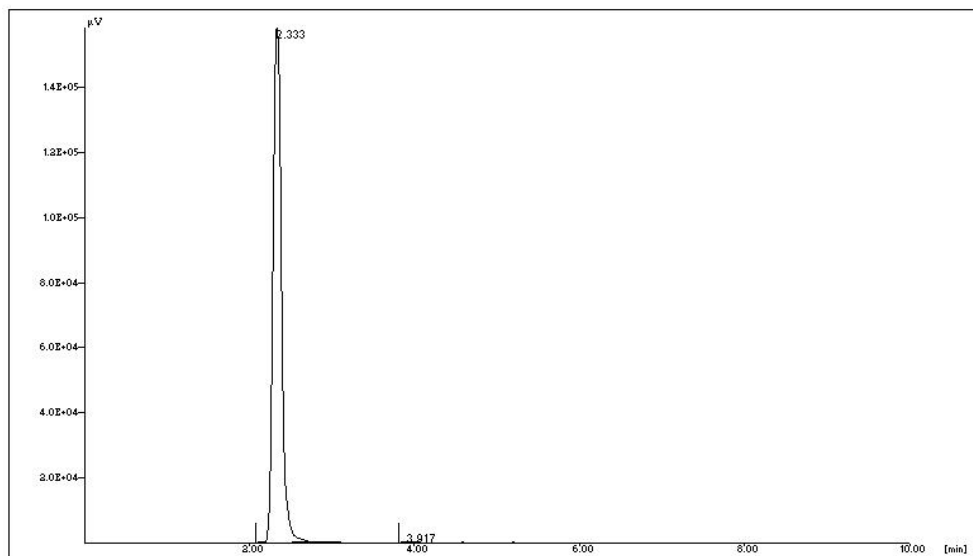


Fig.No. 6.Chromatogram of Lornoxicam (API)

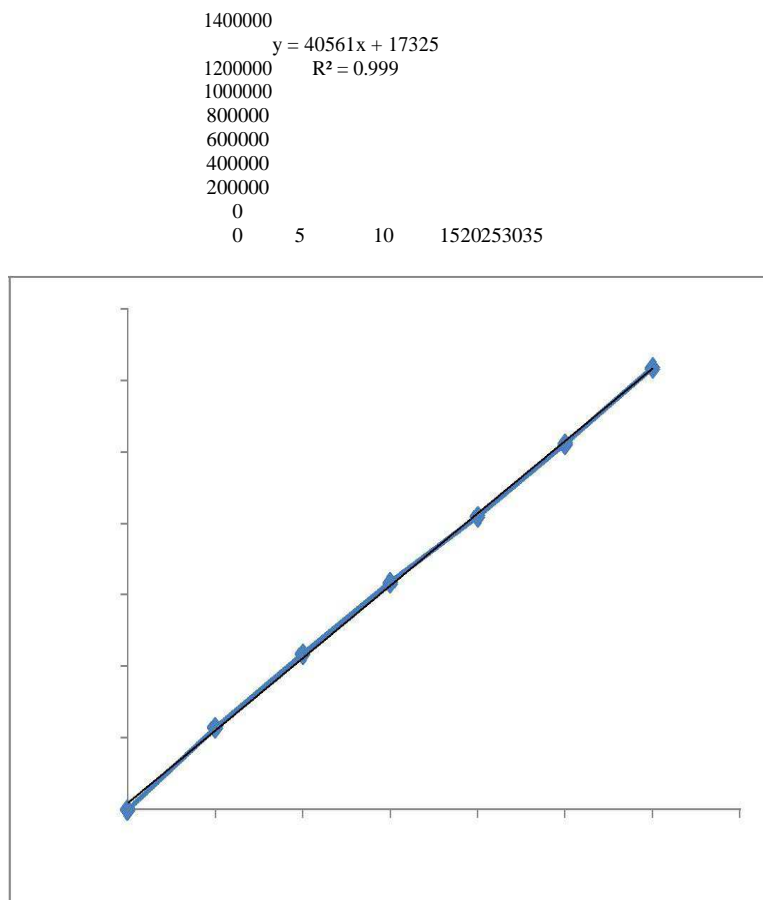


Fig. No. 7. Calibration plot of Lornoxicam at 382 nm

Table No.1 Results of Degradation of Lornoxicam by Rp-HPLC

CONC. (µg/ml)	1N NaOH		2N HCL		6% H <sub>2</sub> O <sub>2</sub>		Heat		UV	
	%*	R.S. D.	%*	R.S.D.	%*	R.S.D.	%*	R.S.D.	%*	R.S.D.
10	33.79	0.118	24.46	1.221	19.07	0.144	5.17	0.025	4.25	0.047

\*Average of three determinations S.D., standard deviation; R.S.D.relative Standard deviation

Table No.2. Linearity of Lornoxicam

Sr. No.	Concentration (µg ml <sup>-1</sup> )	Peak area
1	5	229843
2	10	435698
3	15	635536
4	20	819869
5	25	1023563
6	30	1235623

Table No.3. Regression Equation Data

Regression Equation Data For Lornoxicam, Y = A + B*C	
Slope (B)	40561
Intercept (A)	17325
Correlation coefficient	0.999

Where C is the concentration in µg ml<sup>-1</sup> and Y is the unit of response (area).

**Table No.4 Chromatographic Data for Lornoxicam (10 µg/ml)**

Drug	Retention time	Peak area	Peak height
Lornoxicam	2.333	435698.17	97352

\*Average of six determinations; S.D., standard deviation;  
R.S.D., relative standard deviation.

**Table No.5 Precision of the method:**

Analyte	Precision	%Concentration estimated*(Mean ± S.D.)	R.S.D
Lornoxicam	Intra day	100.79 ± 0.7162	
	Inter day	100.46 ± 0.73	

\*Average of six determinations; S.D., standard deviation;  
R.S.D., relative standard deviation.

**Table No.6 Results of recovery study of Lornoxicam**

Level of % Recovery	% Mean Recovery	S.D.	R.S.D
50	100.4	1.2263	1.2214
100	101.1	0.5819	0.5752
150	100.2	0.3634	0.3626

\*Average of six determinations; S.D., standard deviation;  
R.S.D., relative standard deviation.

**Table No.7 Results of Assay of Lornoxicam**

Analyte	Label claim(mg/tab)	%Recovery estimated* (Mean ± S. D.)	R.S.D.
LORCAM (Lornoxicam)	8 mg	99.235 ± 0.042	0.0441

\*Average of six determinations S.D., standard deviation;  
R.S.D., relative Standard deviation.

**Table No.8: Sensitivity of method**

Parameter	At 382nm
LOD µg ml <sup>-1</sup>	0.0254
LOQ µg ml <sup>-1</sup>	0.0769

**Table No.9: Selectivity of method**

Analyte	Retention time* (Mean ± S.D.)	%R.S.D.
Lornoxicam	2.192 ± 0.0534	0.910

\*Average of six determinations S.D., standard deviation;  
R.S.D., relative standard deviation.

**Table No.10 System suitability parameters**

Sr.No.	Parameters	Lornoxicam
1	Retention Time in minutes	2.192
2	Theoretical plates (N)	3451
3	Asymmetry	1.20
4	Calibration Curve (µg/ml)	5-30
5	Resolution (RS)	3.30

## RESULTS AND DISCUSSION

HPLC studies of samples obtained on stress testing of Lornoxicam under different conditions using Acetonitrile: Water (70:30v/v) as a mobile phase suggested the following degradation behavior:

### Hydrolysis:

#### Acidic Hydrolysis:

Studies were performed in 2 N hydrochloric acid which was refluxed at 80 °C for 2 hrs, degradation was found to be 24.46 %. Hence Lornoxicam was found to be sensitive to acid Degradation.

**Basic Hydrolysis:**

Studies were performed in 1 N sodium hydroxide at 80 °C for 2 hrs. The drug showed 33.79 % degradation. Hence Lornoxicam was found to be sensitive to base degradation.

**Oxidation:**

The drug was found to be less labile to oxidative degradation. The reaction in 6 % H<sub>2</sub>O<sub>2</sub> was carried out at 80 °C for 1 hr. The degradation was slow and around 19.07 % of the drug was degraded. The drug was found to be less susceptible to oxidative degradation as compared to hydrolytic degradation.

**Photochemical degradation**

Lornoxicam was found to be stable to photochemical degradation as around 4.25 % of the degradation was seen after exposing drug to uv radiation for 48 hrs.

**Thermal Degradation**

Lornoxicam was found to be stable to thermal degradation as around 5.17 % of the degradation was seen after exposing drug to dry heat in an oven for 4 hrs.

**Validation of the stability indicating method**

The results of validation studies on the stability indicating method developed for Lornoxicam in the current study involving Acetonitrile : Water (70:30v/v) as a mobile phase are given below.

**Linearity**

The response for the drug was linear ( $r^2 = 0.999$ ) in the concentration range between 5-30 µg/mL. The values of slope, intercept and correlation coefficient were 47874, 17325 and 0.999, respectively

**Precision**

The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were < 2%, respectively as recommended by ICH guideline. Separation of the drug and different degradation products in stressed samples was found to be similar when analysis was performed on different chromatographic system on different days

**LOD and LOQ**

The LOD and LOQ were found to be 0.0254 µg/mL and 0.0769 µg/mL respectively

**Specificity**

The specificity of the HPLC method is illustrated where complete separation of Lornoxicam in presence of its degradation products was noticed. The peaks obtained were sharp and have clear baseline separation. The resolution factor for drug from nearest resolving was > 3.

**Recovery studies**

Good recoveries of the drug in the range from 100.2±0.3634 to 101.16 % ± 0.5819 were obtained at various added concentrations, despite the fact that the drug was fortified to a mixture that contained drug as well as degradation product formed at various reaction conditions

**CONCLUSION**

Present study concludes that Lornoxicam is most labile to alkaline hydrolysis followed by acidic degradation and oxidative degradation. It is stable to thermal and photochemical stress conditions. The proposed method is precise, accurate and stability indicating, resolving all the degradation product from the drug. Thus the proposed method can have its application in the determination, of Lornoxicam in bulk drug, as well as in presence of its degradation products. The ICH guidelines are followed throughout the study for method validation and stress testing, and thus proposed method has wide industrial applicability.

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