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DEVELOPMENT OF SPECTROPHOTOMETRIC METHOD FOR DEGRADATION STUDY OF LORNOXICAM

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ABSTACT

The main objective of this paper is to develop simple, precise, accurate stress degradation assay method for Lornoxicam. Lornoxicam, a non-steroidal anti-inflammatory drug (NSAID).For present work ACN: Water (70:30) was used as mobile phase. The drug showed λmax at 382 nm. The method obeys Beers-Lamberts law in the concentration range of 5-30 µg/ml for UV 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. 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: Lornoxicam, Stress degradation, UV analysis, Beers-Lamberts law NSAID.

INTRODUCTION

Lornoxicam, a non-steroidal anti-inflammatory drug (6-chloro-4-hydroxy-2-methyl-N-pyridin-2-yl-2H-thieno [2,3- e].[1,2] thiazine-3-carboxamide-1, 1-dioxid.It acts by balanced COX-1/COX-2 enzyme inhibition.[1] The literature survey has been done for analysis of various NSAID drugs for its impurity and degradation products. There found very few reported methods for analysis of degradation products and impurities of Lornoxicam by UV analysis and hence it is selected for the analysis.[2-8] 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. A few reports on LC and LC–MS methods for the detection of Lornoxicam and its metabolites in biological matrices are available.[9-18]

MATERIALS AND METHODS

Reagents and chemicals

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

All the chemicals used are of AR grade.

Diluents: Diluent used was Acetonitrile and Water. ACN was used to dissolve the drug completely and then water was used to make up the volume.

Preparation of Standard Drug Solution

Standard stock solution containing Lornoxicam was prepared by dissolving 10 mg of Lornoxicam in few ml of diluents sonicated for 10 minutes and then final volume of the solutions was made up to 100 ml with the solvent (100µg/ml). 1ml of this solution was further diluted up to 10 ml to get stock solution of 10 µg ml^{-1} and was used to run spectrum. Lornoxicam showed maximum absorbance at 382 nm.

FORCED DEGRADATION STUDY BY UV METHOD

1) Alkaline Degradation Study: The alkaline degradation was done against 1N sodium hydroxide.

Procedure: To 5ml of above stock solution (100µg/ml) 5 ml of 1N sodium hydroxide was added. The solution was refluxed for 2 hr cooled and then it was neutralized with 1N HCl and volume is made up to100 ml with mobile phase.1ml of resultant solution was taken and volume made up to 10 ml with diluents. It was filtered through 0.45µ filter paper and sonicated for 15 min and used for study.

2) 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 to100 ml with mobile phase.1ml of resultant solution was taken and volume made up to 10ml with diluents. It was filtered through 0.45µ filter paper and sonicated for 15 min and used for study.

3) 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 made up to 10ml with diluent It was filtered through 0.45µ filter paper and sonicated for 15 min and used for study.

4) Thermal Degradation Study: The thermal degradation was done by heating the Lornoxicam at 80 ºC.

Procedure: Under Dry Heat condition 50 mg of Lornoxicam was heated at 80 ºC 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 diluent to get 10 µg/ml solutions. It was filtered through 0.45µ filter paper and sonicated for 15 min. and used for study.

5) Photolytic Degradation Study
The UV degradation was done by placing the Lornoxicam under UV radiation.

Procedure: 50 mg of Lornoxicam was placed under UV radiation in UV cabinet for 24 hrs ,10 mg of this drug was dissolved in 100 ml of diluent.1 ml of resultant solution was diluted by mobile phase to get 10 µg/ml solution . It was filtered through 0.45µ filter paper and sonicated for 15 min. and used for study.

METHOD VALIDATION

Linearity Study of Drug at Selected Wavelength
In to a series of 10 ml volumetric flasks, 0.3 to 1.8 ml of drug stock solution (100µg/ml) was pipette and final volume of the solutions was made up to 10 ml with diluent .These solutions were scanned at 200-400nm and the absorbance at 382nm was measured and plotted against concentration which is shown in Table No.2.

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 are given in Table No.5.

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.
LOD = 3.3 / S
LOQ = 10 / S
Where,
σ =Standard deviation of response.
S = slope of calibration curve.

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.

Assay: For assay calculation marketed formulation of Lornoxicam (Tab. Lorcam 4 mg- Sun Pharma.) was taken and triturated. Powder equivalent to 4mg was taken and absorbances were noted and mean % recovery was calculated.

RESULT AND DISCUSSION

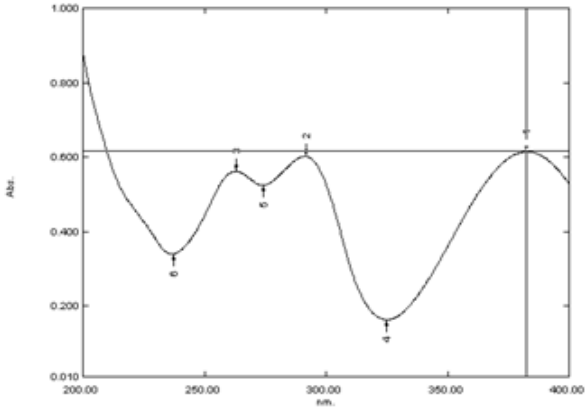

Fig. No. 1. UV spectra of lornoxicam.

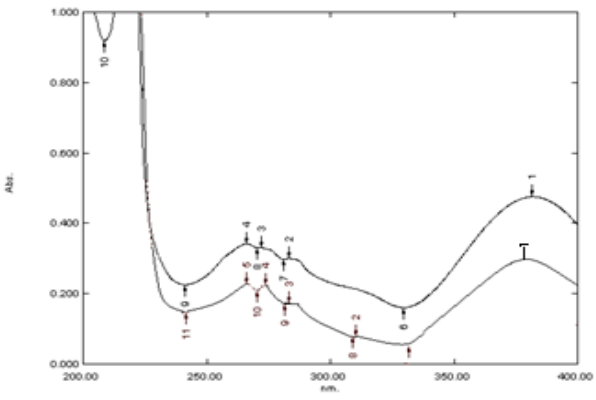

Fig No. 2. UV spectra of alkaline degradation.

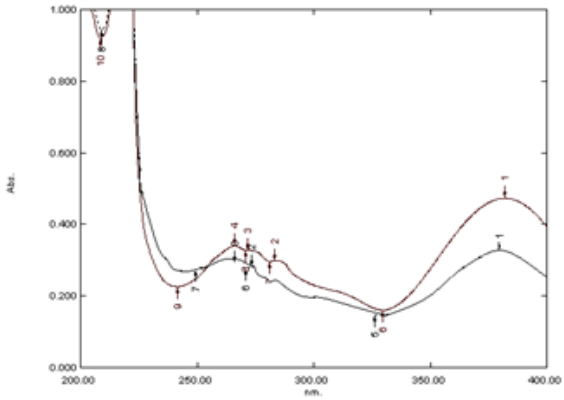

Fig. No. 3. UV spectra of acid degradation.

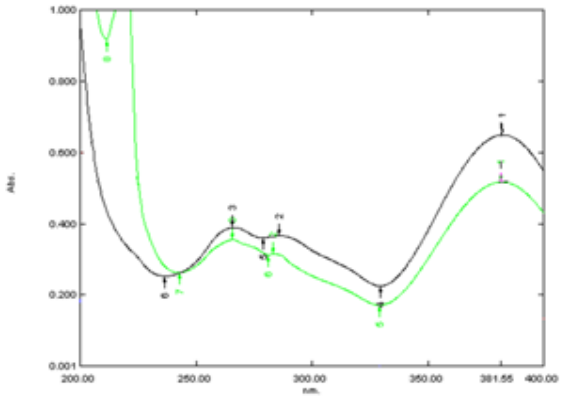

Fig. No. 5. UV spectra of thermal degradation.

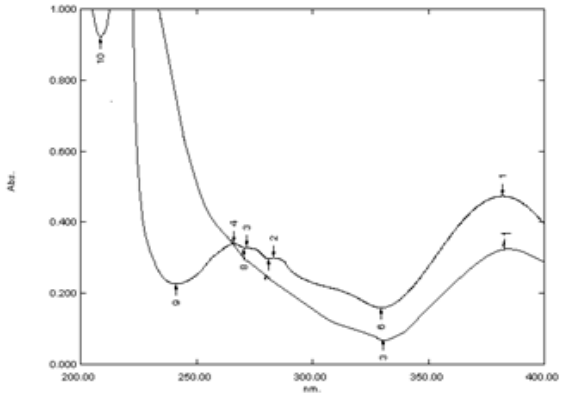

Fig. No. 4. UV spectra of oxidative degradation.

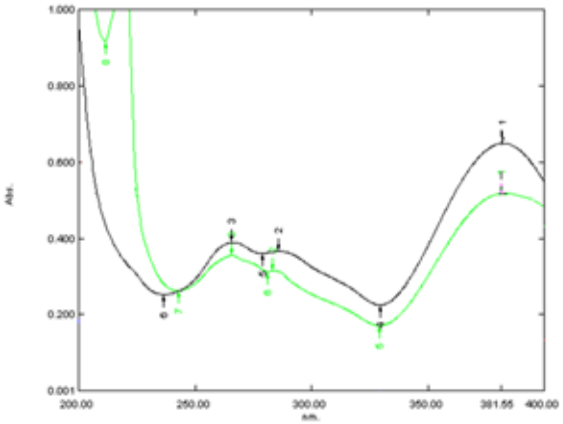

Fig. No. 6. UV spectra of UV degradation.

Table No. 1: Degradation of Lornoxicam by UV method.

Conc. (µgml^{1})	DGRN. by 1N NaOH		DGRN. By 2N HCL		DGRN. By 6% H_2O_2		DGRN. by heat		DGRN. by UV	
10	%*	R.S.D.	%*	R.S.D.	%*	R.S.D.	%*	R.S.D.	%*	R.S.D.
	32.96	0.134	23.51	0.068	19.56	0.215	4.70	0.450	4.38	0.214

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

Table. No. 2: Linearity of Lornoxicam at 382 nm.

Sr. No.	Concentration (µg ml^{-1})	Absorbance
1	3	0.12
2	6	0.242
3	9	0.369
4	12	0.49
5	15	0.601
6	18	0.722
7	21	0.843
8	24	0.97
9	27	1.08

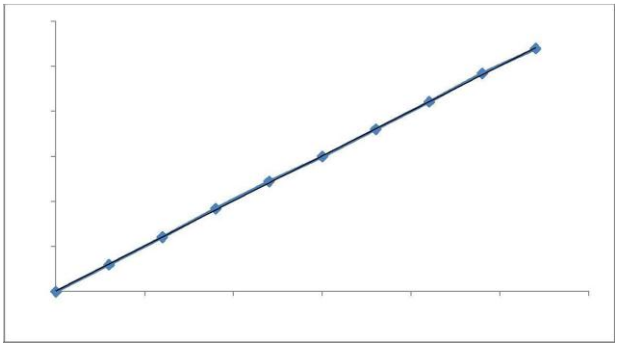

Fig. No. 12. Calibration plot of Lornoxicam at 382nm.

Table No. 3. Regression Equation Data Regression Equation Data For Lornoxicam, Y = mx+C.

Slope (m)	0.040
Intercept (C)	0.002
Correlation coefficient	0.999

Where x is the concentration in µg ml^{-1} and Y is the absorbance.

Table. No. 4: Sensitivity of method.

Parameter	At 382nm
LOD µg ml^{-1}	0.1195
LOQ µg ml^{-1}	0.3623

Table No. 8: Precision of the method.

Analyte	precision	%Concentration estimated*(Mean±S.D.)	% R.S.D.
Lornoxicam	Intra day	98.94 ± 0.4655	0.4701
	Inter day	98.70±0.4359	0.4419

*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	98.94 ± 0.4655	0.4701
	Inter day	98.70±0.4359	0.4419

Table No. 6: Recovery study of Lornoxicam.

Level of % Recovery	% Mean recovery	S.D.	%R.S.D.
50	100.08	0.6968	0.6962
100	100.99	0.4281	0.4239
150	101.07	0.6933	0.6859

*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)	4 mg	99.33 ± 0.7651	0.7702

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

Stability indicating property by UV method

UV studies of samples obtained on stress testing of Lornoxicam under different conditions using Acetonitrile Water as a diluent suggested the following degradation behavior Table No.1.

1. Hydrolysis

Acidic Hydrolysis

Studies were performed in 2 N hydrochloric acid which was refluxed at 80°C for 2 hrs, degradation was found tobe 23.51% 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 show32.96% degradation. Hence Lornoxicam was found to be sensitive to base degradation.

2. Oxidation

The drug was found to be less labile to oxidative degradation. The reaction in 6% H_2O_2 was carried out at 80°C for 1 hr .The degradation was slow and around 19.56% of the drug was degraded. The drug was less sensitive to oxidative degradation as compaired to hydrolytic degradation.

3. Photochemical degradation

Lornoxicam was found to be stable to photochemical degradation as around 4.38% of the degradation was seen after exposing drug to uv radiation for 24 hrs.

4. Thermal Degradation

Lornoxicam was found to be stable to thermal degradation as around 4.70% 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 UV method developed for Lornoxicam in the current study involving Acetonitrile: Water (70:30v/v) as a mobile phase are given below.

1. Linearity

The response for the drug was linear (r^2= 0.999) in the concentration range between 3-27 µg/mL. The values of slope, intercept and correlation coefficient were 0.040, 0.002 and 0.999, respectively Table No.2.

2. 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 Table No.5.

3. LOD and LOQ

The LOD and LOQ were found to be 0.1195 µg/mL and 0.3623 µg/mL respectively Table No.4.

4. Recovery studies

Good recoveries of the drug in the range from 100.08±0.6968 to 101.07% ± 0.6933 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 Table No.6.

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, pharmaceutical formulation 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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