Research Article

Development and Validation of A Stability Indicating Assay Method of Lornoxicam By Using UV-Visible Spectrophotometer

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ABSTRACT

Our objective was to study and develop a simple accurate, precise and cost effective UV-Vis spectrophotometric method for the estimation of Lornoxicam in bulk and pharmaceutical dosage form. The solvent used was methanol and the λmax or the absorption maxima of the drug was found to be 381nm. A linear response was observed in the range of 2-20 μg/ml with a regression coefficient of 0.999. The method was then validated for different parameters as per the International Conference for Harmonization guidelines (ICH). This method can be used for the determination of Lornoxicam in quality control of formulation without interference of the excipients. Lornoxicam was subjected to stress degradation under different conditions recommended by ICH. The samples generated were used for degradation studies using the developed method.

Keywords: Lornoxicam, stress conditions studies, validation, and UV-Vis spectroscopy.

INTRODUCTION

Lornoxicam is chemically known as (3*E*)-6-chloro-3-[hydroxy (pyridin-ylamino)methylene]-2-methyl-2,3-dihydro-4*H*-thieno[2,3-*e*][1,2]thiazin-4-one 1,1-dioxide,belonging to the class of cyclooxygenase inhibitor of non-steroidal antiimmflamtory drug (NSAID) of the Lornoxicam class with analgesic, antiimmflamtory and antipyretic properties. Lornoxicam wavelength corresponding to maximum absorbance was noted which is its □max i.e. at 381 nm as shown in fig no. 2 and the chemical structure of Lornoxicam as shown in fig. no. 1

Assay of Lornoxicam[1, 3]

A quantity of powder equivalent to 10 mg of Lornoxicam was taken in a 100 ml volumetric flask and it was dissolved in 20 ml of methanol. The resultant solution was ultrasonicated for 5 minutes. The solution was then filtered using whatmann filter paper No. 40. The given solution was diluted up to 100 ml with methanol. This gives 100 µg/ml solution.

EXPERIMENTAL

Materials and methods

The drug, Lornoxicam was procured as a gift sample from Neha Pharma Pvt. Ltd. Mumbai. The instrument used for the present study was a Jasco V-530 UV-Vis Double Beam Spectrophotometer. The solvent used was Methanol which was of AR grade. The chemicals NaOH, HCl, and H_2O_2 used for present study are of AR grade and these chemicals are purchased from Merck Chemicals (Mumbai, India).

UV method development[3]

Preparation of stock solution

Standard stock solution of Lornoxicam was prepared by dissolving 10 mg Lornoxicam of in 100 ml of methanol which gives 100 µg/ml solution.

Preparation of series of dilution

From the above stock solution 2 ml was transferred into 10 ml volumetric flask and volume was make with methanol up to which to give 20 µg/ml. Lornoxicam was scanned with UV-Visible Spectrophotometer in the range 200-400 nm against methanol as blank and the wavelength corresponding to maximum

absorbance was noted which is its □max i.e. at 381 nm and 279 nm. But for present study λmax at 381 nm is to be considered (fig.no.2).

Preparation of calibration curve

0.2 ml-2 ml of 100 µg/ml solution were diluted and the volume was made up to 10 ml using methanol to produce 2-20 µg/ml sub stock solutions respectively. Then the absorbance of solution was taken. The calibration curve of Lornoxicam was plotted by taking concentration on X-axis and absorbance on Y-axis (fig.no.3). This drug shown line in concentration range of 2-20 µg/ml and correlation coefficient was found to be 0.999.

Acid Degradation [4,5]

2 ml of stock solution of Lornoxicam and 5 ml of 3N HCl was added in 10 ml of volumetric flask and the volumetric flask was kept at normal condition for 3 hours. After 3 hours time interval, solution was neutralized and diluted with methanol in order to make the volume up to 10 ml which gives 20 µg/ml solutions (table no.8 and fig.no.4).

Alkali Degradation [4,5]

2 ml of stock solution of Lornoxicam and 5 ml of 3N NaOH was added in 10 ml of volumetric flask and the volumetric flask was kept at normal condition for 4 hours. After 4 hours time interval, solution was neutralized and diluted with methanol in order to make the volume up to 10 ml which gives 20 µg/ml solution (table no.8 and fig.no.5).

Oxidative degradation [4,5]

2 ml of the stock solution of Lornoxicam and 5 ml of 6 % w/v of hydrogen peroxide was added in 10 ml of volumetric flask and volumetric flask was kept at normal condition for 3 hours. After 3 hours time interval, solution was diluted with methanol in order to make the volume up to 10 ml which gives 20 µg/ml solution (table no.8 and fig.no.6).

Dry heat induced degradation [4,5]

Lornoxicam sample was taken in a petriplate and exposed to a temperature of 50°C for 3 hours in an oven. After 3 hours, 10 mg of the sample was diluted with methanol in order to make the volume up to 10 ml which gives 20 µg/ml solution and the solution was scanned for the UV-Vis analysis (table no.8 and fig. no.7).

UV-Degradation at 254 nm [4, 5]

Sample of Lornoxicam was exposed to UV short (254 nm) light for 3 hours , 10 mg sample was dissolved in methanol and volume was made up to 10 ml which gives 20 µg/ml solution and scanned for the UV analysis. (table no.8 and fig.no.6).

Method Validation [4,5]

Linearity

Various aliquots were prepared from the stock solution (100 µg/ml) ranging from 2-20 µg/ml. The samples were scanned in UV-Vis Spectrophotometer against methanol as blank. It was found that the selected drug shows linearity between the ranges of 2-20 µg/ml (table no.2 and fig. no.3).

Accuracy [4,6]

Solutions were prepared in triplicate at levels 80%, 100%, and 120% of test concentration using Lornoxicam working standard as per the method and taken absorbance of each solution in triplicate. The recovery result showed that the proposed method has an acceptable level of accuracy for level for Lornoxicam which is from 80% - 120% of test concentration is from 98.38 % - 102.50% (table no.2).

Precision

Precision of the method was demonstrated by intraday and interday variation studies. In intraday variation study six different solutions of same concentration 20 µg/ml were analyzed three times in a day i.e. from morning, afternoon and evening. In the interday variation studies, solution of same concentration i.e. 20 µg/ml were analyzed three times for the three consecutive days and the absorbance result, mean, standard deviation and % RSD was calculated (table no.3 and no.4).

Robustness

Robustness of the method was determined by carrying out the analysis

under different temperature condition i.e. at 21°C, 25°C and at 30°C. The respective absorbances of 20 µg/ml were noted and the result was indicated as % RSD (table no.5).

Ruggedness

Ruggedness of the method was determined by carrying out the analysis by different analyst and the respective absorbance of 20 µg/ml was noted. The result was indicated as % RSD (table no.6).

Limit of Detection (LOD) [4, 5]

The limit of detection (LOD) was determined by preparing solutions of different concentrations ranging from 0.1-0.8 µg/ml. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantification as an exact value (table no.7).

Limit of Quantification (LOQ)[5,6]

The LOQ is the concentration that can be quantification reliably with a specified level of accuracy and precision. The LOQ was calculated using the formula involving standard deviation of response and slope of calibration curve (table no.7).

RESULTS AND DISCUSSION

The developed method was found to be precise as the % RSD values for intraday and inter-day were found to be less than 2%. Good recoveries (99% to 100.42%) of the drug were obtained at each added concentration, indicating that the method was accurate. The method was also found to be specific indicated by the % recoveries ranging from 98.65%-100.42%. The LOD and LOQ were found to be in sub-microgram level indicating the sensitivity of the method. The method was also found to be robust and rugged as indicated by the % RSD values which are less than 2%. The results of assay show that the amount of drug was in good agreement with the label claim of the formulation as indicated by % recovery (100.42%). The summary of validation parameters of proposed spectrophotometric method is shown in table no.1& 7. The stress degradation studies showed that Lornoxicam undergoes degradation in acidic, oxidative, alkaline and dry heat condition (23.69%, 33.34%, 21.06%, 8.78%, 27.20%) respectively.

ACKNOWLEDGEMENTS

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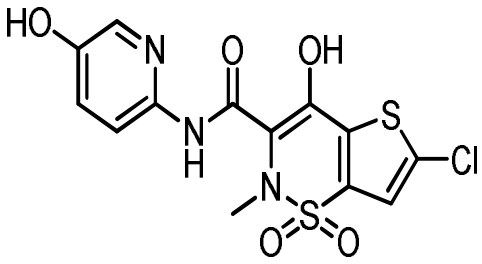

Fig. 1: Structure of Lornoxicam

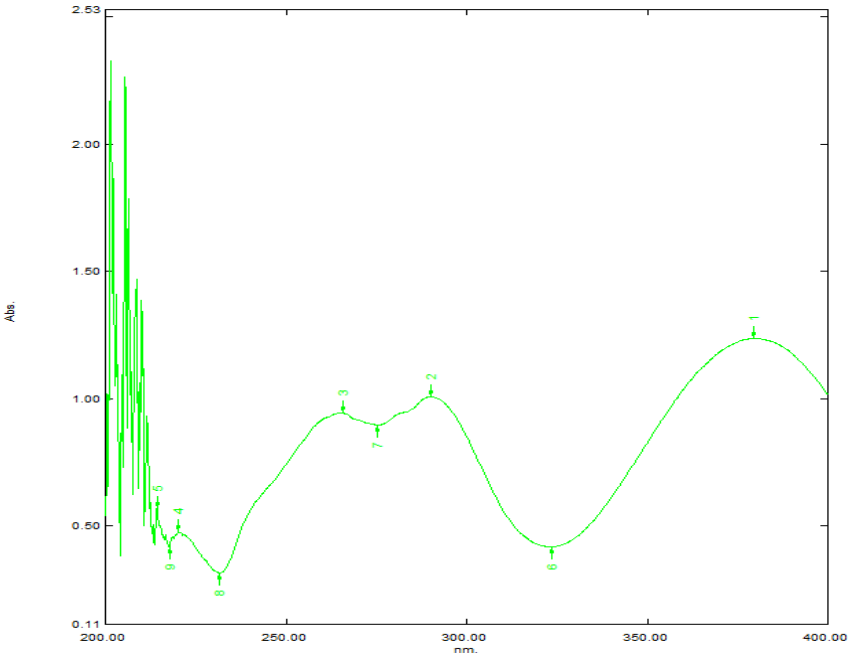

Fig. 2: λmax of Lornoxicam showing at 381nm

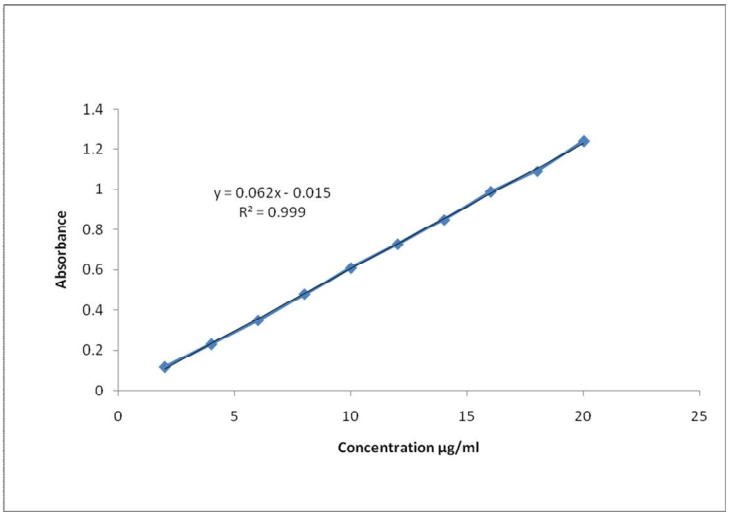

Fig. 3: Calibration curve for Lornoxicam

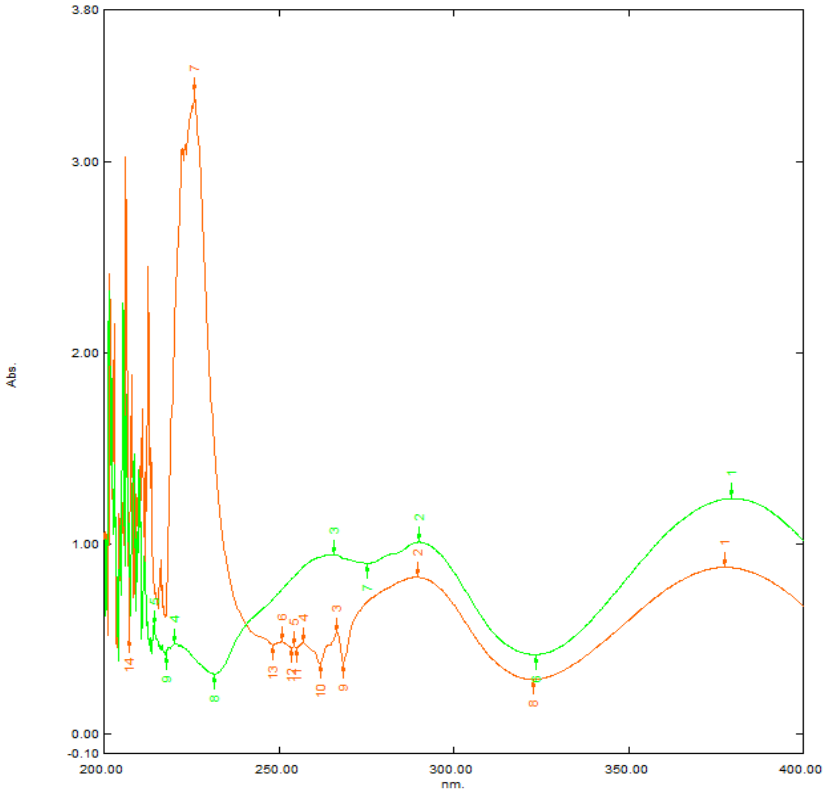

Fig. 4: Comparison between standard Lornoxicam (20 µg/ml) & acid degraded sample of Lornoxicam (20 µg/ml) after 3 hours. 3N HCl by drug got degraded and its ƛmax shifted

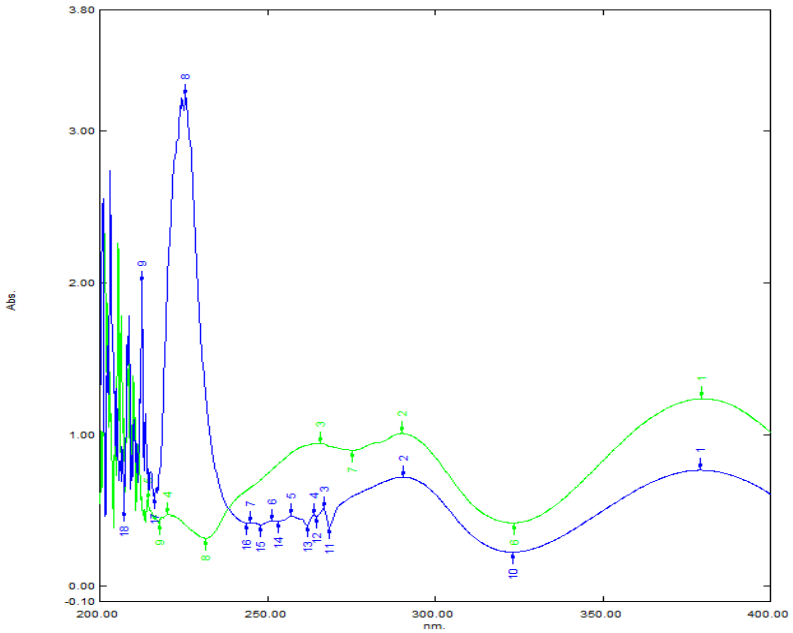

Fig. 5: Comparison between standard Lornoxicam (20 µg/ml) & alkali degraded sample of Lornoxicam (20 µg/ml) after 4hours by 3N NaOH, drug got degraded and its ƛmax shifted

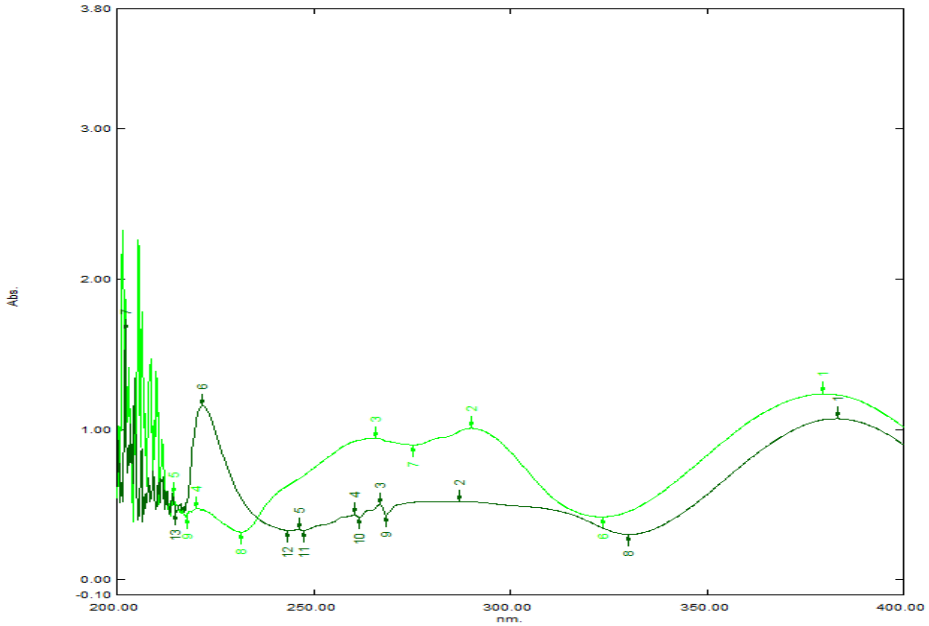

Fig. 6: Comparison between standard Lornoxicam (20 µg/ml) & Oxidised sample of Lornoxicam (20 µg/ml). Drug got degraded and its λmax shifted

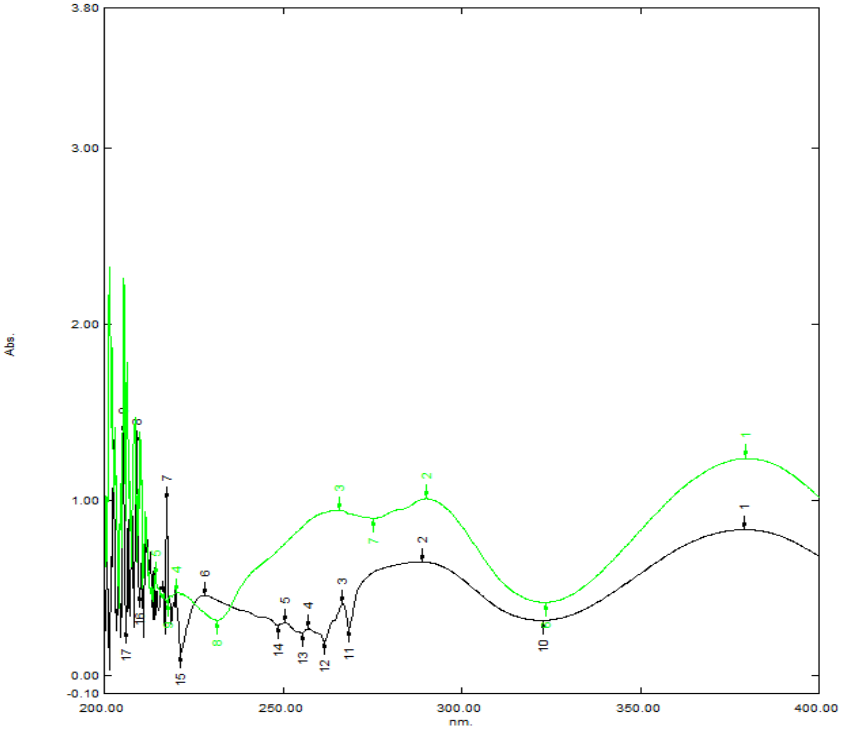

Fig. 7: Comparison between standard Lornoxicam (20 µg/ml) & temperature degraded sample of Lornoxicam (20 µg/ml), after 3 hours drug got degraded

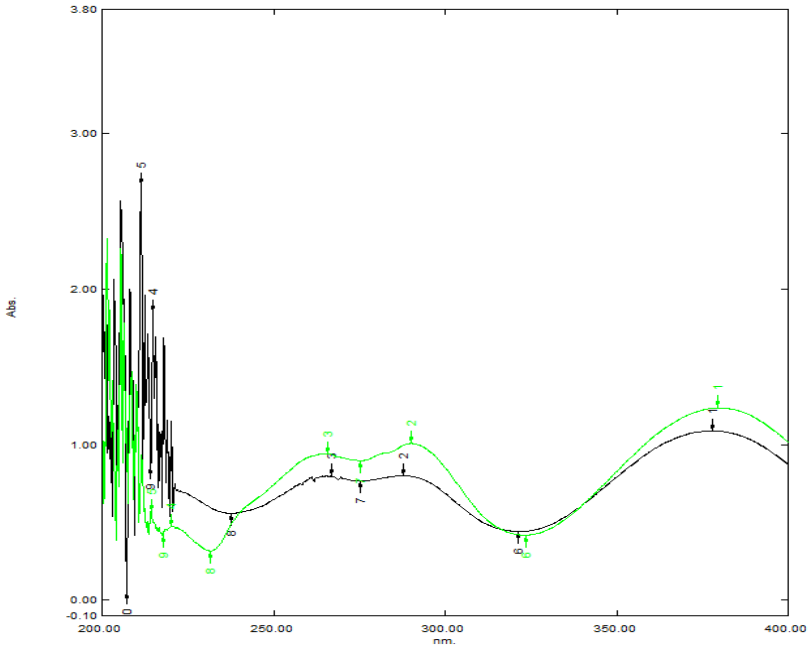

Fig. 8: Comparison between standard Lornoxicam (20 µg/ml) & UV degraded sample of Lornoxicam (20 µg/ml), after 3 hours drug got degraded

Table 1: Optical characteristics

Parameters	Results
Beer's law limit (µg/ml)	2-20 (µg/ml
Correlation coefficient	0.999
Regression equation (Y*)	0.622x-0.0153
Slope (a)	0.622x
Intercept (b)	0.0153

Table 2: Accuracy reading of Lornoxicam

No. of Preparation	Concentration (µg/ml)		%Recovery	Statistical results		
	Formulation	Pure drug		Mean	SD	%RSD
S_1: 80%	10	8	97.58	98.38	1.11	1.11%
S_2: 80%	10	8	98.79			
S_3: 80%	10	8	98.79			
S_4:100%	10	10	102	100.39	0.0324	0.032%
S_5:100%	10	10	99.19			
S_6:100%	10	10	100			
S_7:120%	10	12	104.8	102.5	0.036	0.036%
S_8:120%	10	12	100			
S_9:120%	10	12	102.8			

Table 3: Interday precision of Lornoxicam

Concentration (µg/ml)	Absorbance 1	Absorbance 2	Absorbance 3	Stastistical analysis
20	1.14	1.14	1.10	Mean=1.127
20	1.13	1.13	1.10	
20	1.12	1.16	1.12	SD=0.0153
20	1.12	1.14	1.13	
20	1.12	1.14	1.10	%RSD=1.35%
20	1.15	1.15	1.12	

Table 4: Intraday precision of Lornoxicam

Concentration (µg/ml)	Absorbance 1	Absorbance 2	Absorbance 3	Statistical analysis
20	1.14	1.14	1.13	Mean=1.124
20	1.12	1.13	1.13	
20	1.13	1.16	1.13	SD=0.0158
20	1.10	1.10	1.13	
20	1.10	1.14	1.13	%RSD=1.02%
20	1.11	1.15	1.13	

Table 5: Robustness study of Lornoxicam

Concentration (µg/ml)	Temperature 21^{0}C	Temperature 25^{0}C	Temperature 30^{0}C	Statistical analysis
20	1.15	1.13	1.14	Mean=1.13
20	1.16	1.15	1.13	
20	1.15	1.12	1.14	SD=0.011
20	1.15	1.14	1.14	
20	1.15	1.13	1.16	%RSD=1.04%
20	1.15	1.14	1.14	

Table 6: Ruggedness study of Lornoxicam

Concentration (µg/ml)	Analyst 1	Analyst 2	Analyst 3	Statistical analysis
20	14	13	13	Mean=1.13
20	13	15	12	
20	12	12	13	SD=0.0077
20	12	14	14	
20	13	13	13	%RSD=0.68%
20	15	14	14	

Table 7: Summary of validation

Parameters	Result
Linearity indicated by correlation coefficient	0.999
Precision indicated by % RSD	1.35%
Accuracy indicated by %Recovery	100.42%
Limit of Detection	0.8µg/ml
Limit of Quantification	2.45µg/ml
Range	2-20µg/ml
Linear Regression Equation	0.0622x-0.0153
Robustness indicated by %RSD	0.76%

Table 8: Summary of results of stress degradation studies

Stress condition	Time	Observation	%Degradation
Acidic degradation	3 hours	λ max shifted	23.69%
Alkali degradation	4 hours	λ max shifted	33.34%
6% Hydrogen peroxide	3 hours	λ max shifted	21.06
Dry heat 50 degree	3 hours	λ max shifted	8.78%
Photolytic	3 hours	λ max shifted	27.20%

REFERENCES

1. Vitthal VC, Navneet BT, Shailaja BJ and Pravin DC. Development and Validation of A Stability Indicating Assay Method of Mefloquine HCl by Using Different Stress Degradation Conditions. Journal of Pharmacy Research. 2012;5(5):2631-2635
2. Sagar BW and Sohan SC. Estimation of Thiocolchicoside and Ketoprofen in Pharmaceutical Dosage Form by Spectrophotometric Methods. Journal of Pharmaceutical Resarch 2010;3(4):707-710.
3. Beckett AH and Stenlake JB. Practical Pharmaceutical

Chemistry. Fourth edition- Part two. CBS Publisher and Distributors Pvt. Ltd; New Delhi, 2001.

4. Walfish S. Analytical methods: A Statistical Prespective on the ICH Q2A Guidelines for Validation of Methods, Biopharm International: 1-6.
5. Monika B and Saranjeet S: Development of Validate Stability Indicating Assay Methods- Critical Review, Journal of Pharmaceutical and Biomedical Analysis 2002;(28): 1011-1040.
6. Ravichandran V, Shalini S, Sundaram KM and Harish R. Validation of Analytical Methods-Strategies and Importance Review, International Journal of Pharmacy and Pharmaceutical Sciences 2010; 2(3):18-22.