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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP - HPLC METHOD FOR THE ESTIMATION OF LORNOXICAM IN TABLET DOSAGE FORM

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ABSTRACT

A simple reverse phase liquid chromatographic method was developed and validated as per the ICH guidelines for the quantitative determination of Lornoxicam in pharmaceutical dosage forms. The method was simple, precise, specific and accurate. The mobile phase consists of methanol and water (60:40 v/v). The proposed RP-HPLC method utilizes a 3.5µm Symmetry C18 column (150 mm ×4.6 mm) make YMC at ambient temperature. The eluent was monitored at 379 nm and retention time of Lornoxicam was 8 min. The linearity was observed from 10-60µg/ml with r2= 0.9984. The limit of detection and limit of quantitation were found to be 0.01µg/ml and 0.05µg/ml respectively. From the results obtained, it was worthwhile that the provided methods were robust for small and deliberate changes in experimental conditions.

KEYWORDS: Lornoxicam, Anti-pyretic, RP-HPLC, Excipients, Validation.

INTRODUCTION

Lornoxicam: Lornoxicam is a tablet dosage form belonging to NSAIDS which is used as an anti-inflammatory, antipyretic and anti-analgesic I e, for controlling fever, inflammations and pains. These oral administrative dosage forms are always convenient and lead to better compliance. This drug is beneficial and better compliance in terms cost and therapeutic categorization with altogether three therapeutic activities. Foreseeing the need of different analytical methods for the estimation of the active ingredient of NOXFLAM, the ultimate goal of the work was to develop and validate an analytical method better than the existing methods using HPLC technique. This a more or less challenging task due to the formation of drug- excipients interaction.

MATERIALS AND METHODS

Instrumentation

- Waters LC system equipped with 2695 pump and 2996 photodiode array detector was used. The output signals were monitored and integrated using waters Empower 2.0software.
- Analytical balance (Model:AB 204S, Make: Mettle Toledo) and Micro Balance(Model: XP 6, Make: Mettle Toledo) were used for weighing.
- Systronics digital pH meter 361 was used to adjust the pH of the buffer.
- Degassing of the mobile phase was done by sonication using Spinco Biotech Ultra Sonicator)
- Filtration was done by using millipore vaccum filter.

Preparation of solutions

➢ **Preparation of Mobile phase**

• A mixture of HPLC water 400ml (40%) and 600ml of methanol HPLC (60%) were taken and degassed in ultra sonicator for 5 minutes. Filter through 0.45µ filter under vacuum filtration.

➢ **Preparation of diluent**

• The mobile phase itself is used as a diluent.

➢ **Preparation of standard solution**

• Accurately weighed and transferred 10mg of lornoxicam working standard into a 25ml clean dry volumetric flask, added about 15ml of diluent and sonicated to dissolve it completely and made volume up to the mark with the same diluent. Further diluted 1ml of the above solution to 10ml.

➢ **Preparation of placebo solution**

• Weighed accurately 88mg of placebo powder into 25ml volumetric flask, added 15ml of the diluent and sonicated for 20min and diluted to the volume with diluent. Further 1ml of the above solution is diluted to 10ml.

➢ **Test preparation**

• Accurately weighed and finely powdered 10 tablets of **NOXFLAM** and transferred an amount of the powder equivalent to 10mg of lornoxicam into a 25ml of volumetric flask, added 15ml of the diluent and sonicated for 20min and diluted to the volume with diluent. Further 1ml of the above solution is diluted to 10ml.

➢ **Optimized chromatographic conditions**

• After systematic and detailed study of the various parameters involved in the method, the following conditions were employed.

Mobile phase: Water: methanol (40:60 v/v).

Column: Symmetry C18 (4.6 x 150mm, 3.5 µm, Make: YMC) or equivalent.

Flow rate: 1.2 ml per min.

Wavelength: 379 nm.

Injection volume: 20 µL.

Column oven Temperature: Ambient.

Run time: 8min.

Procedure

• Column was equilibrated for at least 60 minutes with the mobile phase flowing through the system at a rate of 1.2ml/min. Detector was set at a wavelength of 379nm. Separately Injected 20µL of diluent, placebo, standard solution, test solutions into the chromatograph and the chromatograms were recorded. The percent assay values of the lornoxicam were calculated by using the following formula.

• **% Assay**

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.\ Wt}{Label\ Claim} \times 100$$

Where:

AT = Peak Area of lornoxicam obtained with test preparation

AS = Peak Area of lornoxicam obtained with standard preparation

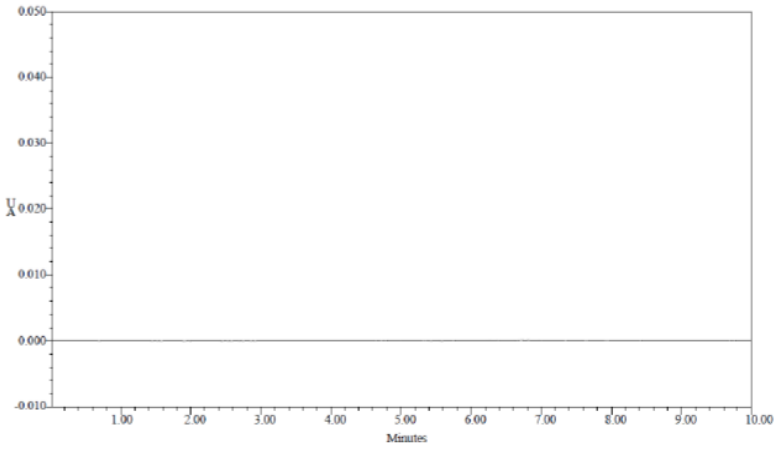
WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

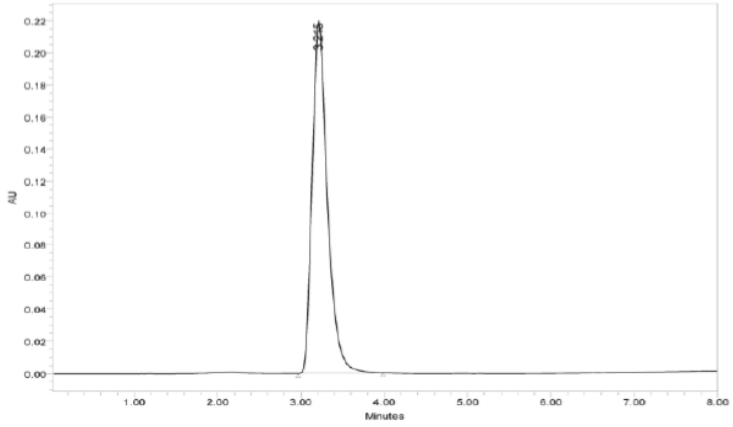
DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard



Representative model chromatogram of Blank solution.



Representative model chromatogram of Standard solution.

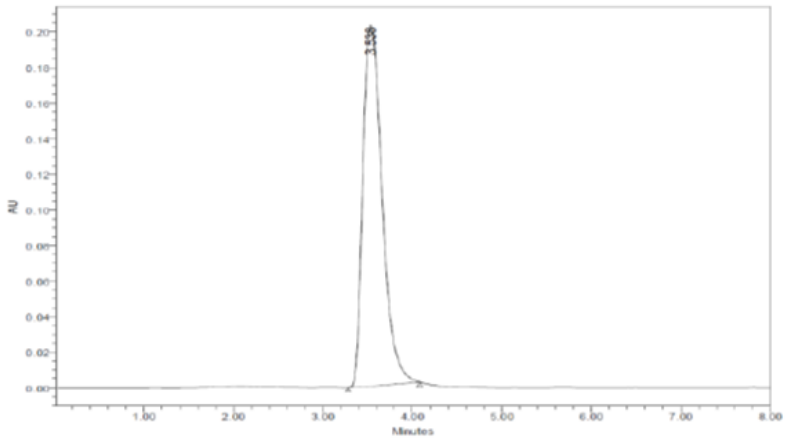
Analytical method Validation

System suitability

To ascertain its effectiveness 20µL of freshly prepared standard solution containing 40µg/ml of lornoxicam was injected 6 times into the HPLC system by using optimized chromatographic conditions and System suitability results were calculated. All the results were tabulated in the below table.

System suitability for Lornoxicam

S. No	Retention time	Peak area	Theoretical plates	Tailing
1	3.123	3113754	7858	1.1
2	3.149	3119256	7589	1.1
3	3.126	3113987	7485	1.2
4	3.128	3119985	7985	1.2
5	3.119	3113585	7854	1.3
6	3.124	3119754	7589	1.1
7	3.116	3113792	7549	1.3
8	3.116	3118978	7598	1.1
9	3.126	3113929	7564	1.1
10	3.106	3119969	7541	1.1
Avarage	3.1233	3116699	7661.2	1.16
SD	0.01	3062.4		
%RSD	0.4	0.1		



Representative model chromatogram of Sample solution

Interference from degradation products

Preparation of degradation samples

Preparation of sample for Acid degradation

NOXFLAM sample was refluxed with the 1M HCl at 60°C for 1hour and then neutralized with 1N NaOH. The sample was prepared as per the test method and then further diluted upto the required concentration with the diluent.

Preparation of sample for Alkaline degradation

NOXFLAM sample was refluxed with the 1M NaOH at 60°C for 1hour and then neutralized with 1M HCl The sample was prepared as per the test method and then further diluted upto the required concentration with the diluents.

Preparation of sample for Oxidative degradation

NOXFLAM sample was refluxed with the 10%H2O2 by heating on water bath at 60°C for 1 hour. The sample was prepared as per the test method and then further diluted upto the required concentration with the diluent.

Preparation of sample for photolytic degradation

NOXFLAM sample was exposed to UV (200watt-hr/m2) and visible (1.2 million lux hrs) The sample was prepared as per the test method and then further diluted upto the required concentration with the diluent.

Preparation of sample for thermal degradation

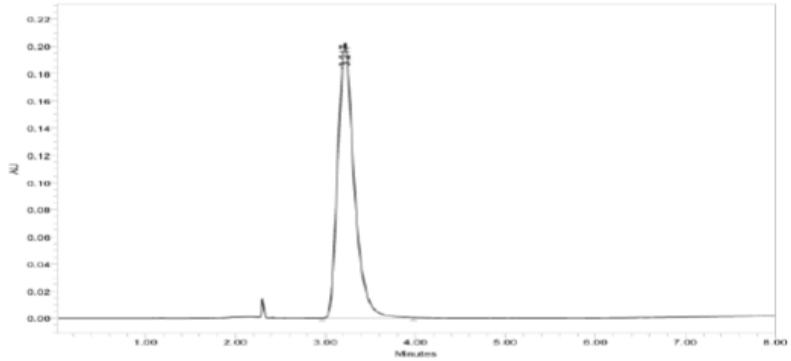
NOXFLAM sample was exposed to temperature at 105°c for 24hrs. The sample was prepared as per the test method and then further diluted upto the required concentration with the diluent.

Preparation of sample for humidity degradation

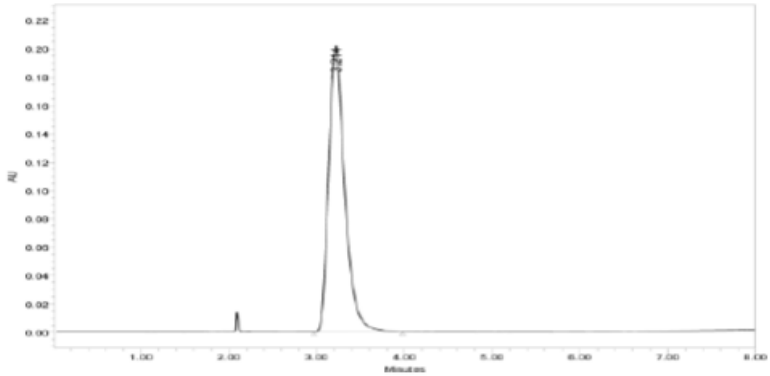
NOXFLAM sample was exposed to 85% humidity for 24hrs. The sample was prepared as per the test method and then further diluted up to the required concentration with the diluent. All the stressed samples were injected into the HPLC system by using optimized chromatographic conditions and the chromatographs were recorded. The chromatograms of the stressed samples were evaluated for peak purity of the drug using PDA detector and Empower software. In all forced degradation samples all the three drugs passed the peak purity (purity angle is less than purity threshold). All the degradant peaks were observed for the drug. Thus the method can be used for estimation of lornoxicam in bulk and pharmaceutical formulations and also the method is stability indicating.

Degradation results of Lornoxicam

Stress Condition	Purity angle	Purity Threshold	% Assay	% Degradation
Acid degradation	0.18	0.23	92.4	7.1
Alkali degradation	0.28	0.32	93.7	5.8
Thermal degradation	0.19	0.41	91.2	8.3
Humidity degradation	0.16	0.20	90.4	9.1
photolytic degradation	0.25	0.29	89.7	9.8
Peroxide degradation	0.36	0.43	92.5	7.0



Representative model chromatogram of acid degradation



Representative model chromatogram of base degradation

Method precision: Precision of the method was conducted by performing the assay of NOXFLAM tablets 6 times. The samples were prepared six times according to the test preparation mentioned earlier and analyzed by using the test method. The % Assay values were calculated for the drug and found to be in between 98.0% - 102.0%. The %RSD values were found to be less than 2.0%.

Method precision for Lornoxicam

S. No.	%Assay
	Lornoxicam
1	100.7
2	99.4
3	98.7
4	98.9
5	99.5
6	99.5
Average	99.5
SD	0.7
% RSD	0.7

Limit of Detection and Limit of Quantification: A study to establish the limit of detection and limit of quantification of lornoxicam was conducted. Limit of detection and limit and quantification were established based on signal to noise ratio. A series of dilutions of the test solution were injected. Limit of detection was established by identifying the concentration which gives signal to noise ratio of about 3. Limit of quantification was established by identifying the concentration which gives signal to noise ratio of about 10.

LOD and LOQ data

Component name	Limit of Detection	Limit of Quantification		
	Concentration (µg/ml)	Concentration (µg/ml)	%Mean recovery	%RSD
Lornoxicam	0.01	0.05	100.8	1.1

Acccuracy: Accuracy for lornoxicam was conducted by spiking the drug to the placebo powder at five different levels of the target concentration (i.e. 50%, 75%, 100% , 125% and 150%) and each level three times. The mean %Recovery and %RSD values were calculated. The %Recovery values for the drug was found to be between 98.0% to 102.0% and %RSD values were found to be less than 2.0%. The accuracy results were tabulated in the table No.7.1.5.

Accuracy for Lornoxicam

S. No.	% Spike level	Amount added (mg)	Amount found (mg)	% Recovery	Statistical parameters
1.	**50%**	19.51	19.49	99.9	**Mean=99.8**
2.		19.79	19.71	99.6	
3.		19.81	19.85	100.2	**SD=0.25**
4.		19.62	19.58	99.8	
5.		19.59	19.49	99.5	**% RSD=0.25**
6.		19.92	19.9	99.9	
7.	**75%**	30.12	30.05	99.8	**Mean=99.8**
8.		30.18	30.1	99.7	**SD=0.09**
9.		29.95	29.92	99.9	**% RSD=0.09**
10.	**100%**	40.15	40.05	99.8	**Mean=99.7**
11.		40.45	40.35	99.8	**SD=0.07**
12.		41.55	41.4	99.6	**% RSD=0.07**
13.	**125%**	50.87	50.79	99.8	**Mean=99.89**
14.		50.12	50.009	99.8	**SD=0.14**
15.		50.18	50.2	100.0	**% RSD=0.14**
16.	**150%**	60.59	60.55	99.9	**Mean=99.9**
17.		60.41	60.35	99.9	
18.		60.48	60.45	100.0	**SD=0.25**

19.		60.73	60.7	99.9	
20.		60.97	60.95	100.0	**% RSD=0.25**
21.		60.81	60.79	100.0	

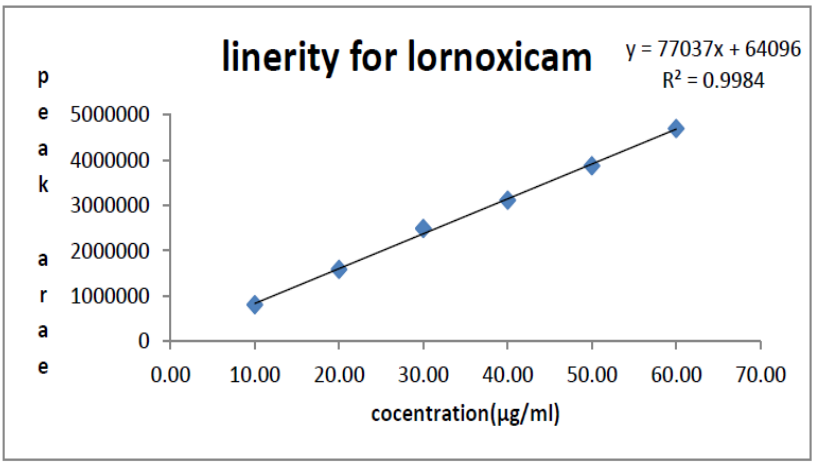
Linearity and range: Linearity of the detector response was established by plotting a graph of concentration versus peak area. A series of solutions of standard were prepared by appropriate dilutions of linearity standard stock solution.

Preparation of Linearity stock solution: Weighed accurately and transferred 25.0 mg lornoxicam WS into 50ml volumetric flask, added 30ml diluent of the diluent and sonicated for 20min and diluted to the volume with diluent, filtered through 0.45µm filter.

Preparation of Linearity solutions: Series of solutions in the range of 25% to 150% of target concentration were prepared by transferring 0.5ml, 1.0ml, 1.5ml, 2.0ml, 2.5ml, 3.0ml of linearity stock solution into separate 25.0mL volumetric flasks and making the volume up to the mark with the diluents. The detector response was found to be linear in the range of 10.0 to 60.0µg/ml for lornoxicam.The correlation coefficient values were found to be with in the limits. The linearity and the regression data was tabulated in Tables.

Linearity for Lornoxicam

S. No.	Linearity level	Concentration (µg/ml)	Peak area
1	25	7.5	438762
2	50	15.0	879523
3	75	22.5	1309285
4	100	30.0	1759146
5	125	37.5	2168808
6	150	45.0	2598569



Graphical Representation of linearity of lornoxicam

Ruggedness: A study to establish ruggedness of the method was conducted by preparing and analyzing the standard and test preparation on two different days by two different analysts on two different columns and two different HPLC systems. The system suitability parameters and the % Assay values of all the three drugs were calculated and the differences between the two analysts were evaluated and the method was found to rugged. The results were tabulated in the below table.

Ruggedness of Lornoxicam

S. No	Lornoxicam		
	Analyst-1	**Analyst-2**	**Overall Results**
1	100.7	98.2	**Mean 100 SD 1.4 %RSD 1.4**
2	99.9	99.2	
3	98.2	98.4	
4	101.7	98.1	
5	100.9	98.5	
Avarage	100.6	98.7	
SD	1.4	0.6	
% RSD	1.4	0.6	

Robustness: A study to establish the effect of variation in flow rate, column temperature, pH of the buffer in the mobile phase was conducted. Standard and test solutions prepared as per the proposed method and were injected into the HPLC system. The system suitability parameters, and the %Assay values were evaluated and the method was found to be robust. All the results were tabulated in the below table.

Robustness for proposed method

Optimum Conditions	**Modifications**	**Retention time**	**Asymmetric factor**	**Theoretical plates**
		LOR	**LOR**	**LOR**
Mobile pHase composition (Water: Methanol) (40:60 v/v)	30:70	3.008	1.10	6896
	50:50	3.261	1.10	7321
Column temperature (25oC)	20	3.512	1.21	7061
	30	3.026	1.28	7161
Flow rate (1.2 mL/min)	1.1	2.798	1.21	7012
	1.3	3.572	0.98	7568
Wave length (379nm)	377	3.123	1.12	7587
	381	3.126	1.11	7558

RESULTS AND DISCUSSIONS

The drug solution was scanned from 200-400 nm, it was observed that the drug show appreciable absorbance at 379nm., hence detection was set at 379nm for method development

purpose. Attempts were made to get good separation of the drug by varying parameters like, flow rate, pH, buffer molarity, buffer components, type of organic modifier, gradient times, and buffer: organic modifier ratio and could get good elution time of lornoxicam in isocratic mode. To achieve this, experiments were conducted by changing the columns and mobile shares but unsuccessful in getting good peaks with less run time. Then method was optimized to separate the main peak.

The satisfactory chromatographic separation, with good peak shapes were achieved on Symmetry C18 (4.6 x 150mm, 3.5 □m, Make: YMC) or equivalent with mobile phase water : methanol (40:60) with a flow rate of 1.2 ml/min. All the System Suitability parameters are within the acceptance limits. The calibration curve for lornoxicam was obtained by plotting the respective peak areas against their concentration. The graph was found to be linear over the range 7.5 -45.0µg/ml for lornoxicam with the correlation coefficient 0.998 respectively the drug which shows that the good correlation exists between peak area and concentration of the drug. This is precise. The high % recovery values obtained for these drugs show that the method is accurate. The LOD value of lornoxicam was found to be 0.01µg/ml, The LOQ was 0.05 µg/ml. The ruggedness was performed and the % RSD was less than 2, hence, method was rugged. The low values of LOD and LOQ show that the method is sensitive and can estimate at micro gram level. The absence of additional peaks indicates the method is specific and the drug was stable in the diluents for 8 hours which is sufficient to complete the work. The stability indicating studies were performed for the above mentioned drug viz…. acid, alkali, thermal, humidity, photolytic, peroxide and the percentage degradation was 7.1%,5.8%,8.3%,9.1%, 9.8%, 7.0%, respectively.

CONCLUSION

The proposed RP-high-performance liquid chromatographic method has been evaluated for the accuracy, precision and linearity. The method was found to be precise, accurate and linear over the linear concentration range. In this method, there was no interference from matrix sources. Moreover, the lower solvent consumption along with the short analytical run time of 8 minutes that allows the analysis of a large number of samples in a short period of time. Therefore, this HPLC method can be used as a routine analysis of the drugs in, pharmaceutical formulations and also for stability studies.

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