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Research Article

STABILITY-INDICATING LC METHOD FOR ANALYSIS OF LORNOXICAM IN THE FINISHED DOSAGE FORM

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ABSTRACT

A selective isocratic liquid chromatography (LC) method for the determination of Lornoxicam (LX) and its related substances in pharmaceutical drugs has been developed. The method uses a reversed-phase C_{18} column (25 cm x 4.6mm i.d), 5µm maintaining the temperature at 30°C. The mobile phase consists of methanol, triethylamine buffer pH 7.0 with H_3PO_4 (55:45). The flow rate was 1.0mL/min and effluent was monitored at 380nm.The separation towards LX component was investigated on different C18 columns. The developed method was further validated with respect to robustness, precision, sensitivity, recovery and linearity. A central composite design was applied to examine the robustness of the method. The method shows good preision, sensitivity, linearity, recovery and robustness. Two commercial LX samples were examined using this method. Furthermore, the method proved to be successful when applied to analyze a marketed LX formulation for dosage forms.

Keywords: Lornoxicam, HPLC, Validation, Isocratic, C_{18} column.

INTRODUCTION

Lornoxicam (LX) is chemically described as, 6-chloro-4-hydroxy-2-methyl-N-2-pyridinyl-2H-thieno [2, 3-e]-1, 2-thiazine-3-carboxamide1, 1-dioxide is a new non steroidal anti inflammatory drug (NSAID) of oxicam class. This is used in the treatment of rheumatoid arthritis, osteoarthritis and related conditions. The pharmacological action of this oxicam is related to inhibition of cyclo oxygenase, a key enzyme of prostaglandin biosynthesis at the site of inflammation .Lornoxicam is not official in any pharmacopoeia and a literature survey revealed that a few attempts have been made as stability indicating methods like spectrophotometric[1], electro chemical[2] and few HPLC[3] methods were reported for its determination. A successful attempt was made to estimate the Lornoxicam. Therefore it was thought worthwhile to develop and validate an accurate and rapid RP-HPLC method for the estimation of Lornoxicam (LX) from tablet formulations. The chemical structure of Lornoxicam is as shown in the figure number 1.

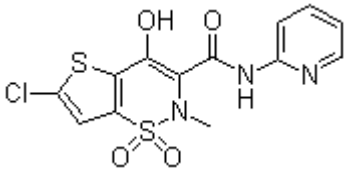

Fig. 1: Chemical Structure of Lornoxicam

MATERIALS AND METHODS

Instrumentation

For HPLC method development, an Agilent 1200Series HPLC system equipped with a quaternary pump, an auto sampler, a thermostated column heater and Diode Array Detector (DAD) was used (Agilent, CA, USA) Diode array data analysis was performed using Agilent Chemstation software version A.08.04.

Materials

Lornoxicam (99.0% purity, fig.1) used as a standard was purchased from sigma chemical (St.Louis, Missouri, USA).The water, methanol, Tri ethyl amine and Phosphoric acid were purchased from Rankem chemicals India.5M NaOH, 5M HCl, H_3PO_4 and 30% H_2O_2 used were purchased from Merck Chemicals with analytical reagent grade. Sample preparation solvent was made by combining 500mL of methanol and 500mL of 0.1N NaOH. Tri ethyl amine buffer was made by adding 5mL of TEA in 1lit of water and adjusting the pH 4.0 ± 0.05 with H_3PO_4.An in house formulation (tablet) and placebo were used for the validation of method accuracy, precision and specificity. Each tablet contains a declared amount of 4mg and 8mg of Lornoxicam. In addition, two commercially available solid dosage forms containing Lornoxicam analysed using this method.

Liquid Chromatographic Conditions and Mobile phase

The column used was a Hypersil BDS C_{18} (250 x 4.6 mm) 5µm column. The mobile phase was 45:55 (v/v) 0.5% of triethyl amine buffer pH 4.0 with H_3PO_4/methanol. The flow rate was 1.0mL/min,the wavelength was 380nm, the injection volume was 20µL,the column temperature was thermostated at 30°C,the run time was 15min,the diluent was 1:1 ratio of 0.1M sodium hydroxide and methanol and quantitation was performed using peak area counts.

Standard Preparation

An accurately weighed amount approximately 50mg of LX standard was transferred into a 50mL volumetric flask, approximately 30mL of diluent was added, sonicate for 5min and make up to volume with the sample preparation solvent, and mix well. Further dilute 5mL of this solution to 50mL with the same. This is the working standard with a concentration of approximately 0.1mg/mL

Assay Sample Preparation

Twenty tablets were weighed, and the average tablet weight was determined. The tablets were crushed to homogeneous powder. A portion of the powder corresponding to 10mg of Lornoxicam was weighed and transferred into a 100mL volumetric flask. Add about 60mL of sample preparation solvent and sonicate for a minimum of 15minutes to dissolve and make up to volume and mix well. The sample was filtered into a HPLC vial using a 25mm, 0.45µ PVDF syringe filter.

RESULTS AND DISCUSSION

Method Development

As the product is not official in any pharmacopoeia, the experiment was started using different buffers and mobile phases. A longer column Zorbax Eclipse XDB C18 (250 x 4.6mm ID) 5µm was used in order to improve the overall separation. UV detection was performed at 380nm.The flow rate was 1.0mL/min and the column was used to evaluate temperature 30°C, 35°C and 40°C. Increasing the temperature shortened retention times for Lornoxicam and its degradants, with minor selectivity changes, and 30°C was chosen because of improvements in column efficiency compared to 35°C and 40°C. Increasing the buffer strength increased Lornoxicam

retention relative to the degradants, with 0.5% TEA buffer being chosen as optimal. The UV absorbance for Lornoxicam was highest at 380 nm, and was chosen. The pH 7.0 was selected as the pKa value of Lornoxicam was 4.7 and ratio of methanol to buffer were optimized with the set conditions at 30°C temperature,380 nm wavelength, 0.5% TEA (aqueous) buffer strength, and 1.0 ml min−1 flow rate. The pH (6.5, 7.0, and 7.5) and percent methanol (50, 55, and 60) were varied, and retention time data on Lornoxicam and its degradants were entered into Chemstation for analysis. The optimal conditions were finalized to those listed in the Section 2. Fig. 2 shows typical HPLC chromatograms of Lornoxicam standard and Lornoxicam in a pharmaceutical formulation.

System Suitability

A system suitability test of the chromatography system was performed before each validation run. Five replicate injections of a system suitability/calibration standard and one injection of a check standard were made. Area relative standard deviation, tailing factor, and efficiency for the five suitability injections were determined. The check standard was quantified against the average of the five suitability injections. For all sample analyses, the tailing factor was ≤2.0; efficiency ≥5000, %R.S.D. ≤2.0%, and 100±2.0% check standard recovery.

Linearity

A set of six standards at the following concentrations were prepared: 0.02, 0.04, 0.06, 0.08, and 0.10, 0.12 and 0.15 mg mL^{-1} of Lornoxicam. This set ranges from 20% to 150% of the nominal assay concentration of 0.1 mg/ml of Lornoxicam. Each of the six standards was analyzed. Table 1 shows the results. The calibration curve was constructed by plotting the peak area against the concentration using linear regression analysis. It showed that the slope was 6806 with a *y*-intercept of -2.378 and a correlation coefficient or regression coefficient of 0.9998, indicates an excellent linearity. At the range, the percent relative standard deviations of the peak areas of three replicate injections were found to be between 0.3 and 1.2%.

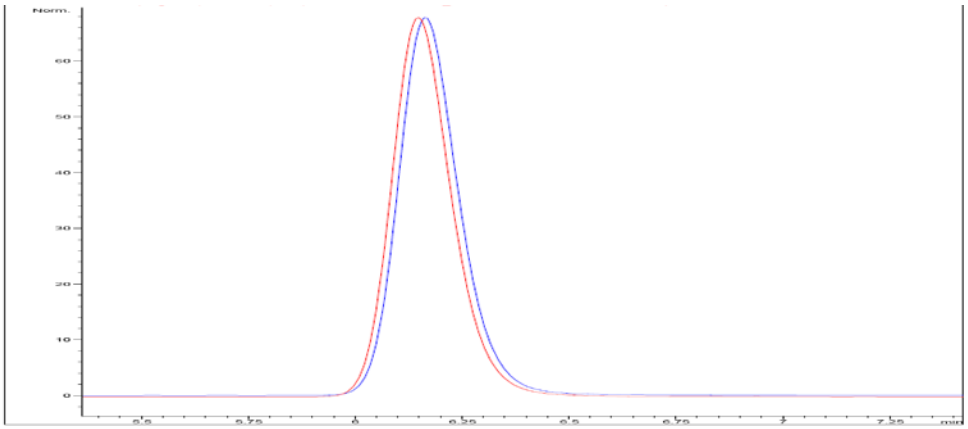

Fig. 2: Lornoxicam standard and sample chromatogram

Table 1: Linearity Results

%Nominal(Assay)	Concentration mg/mL	Peak Area	
20	0.02	134.608	
40	0.04	270.346	Y=6806x-2.378
60	0.06	403.810	
80	0.08	541.724	R^2 = 0.9998
100	0.10	674.250	
120	0.12	822.800	Slope=6.806
150	0.15	1015.215	

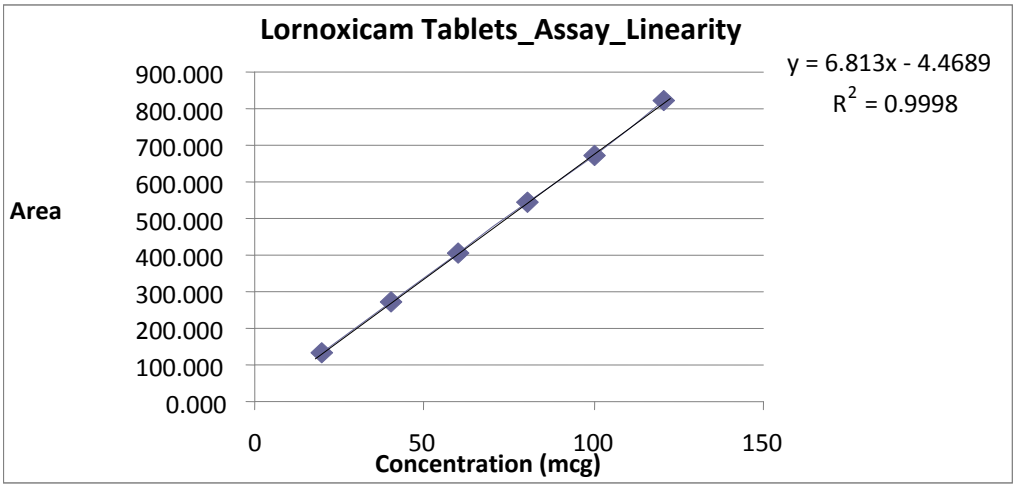

Fig. 3: Lornoxicam Assay Linearity graph

Specificity/Forced Degradation Studies

The forced degradation study was conducted by subjecting standard, placebo, and tablet formulation samples to heat, oxidation, acid, and alkali degradation. The samples were appropriately neutralized and analysed using the method. Single wavelength data at 380 nm was collected by following the method. Additional photodiode array data was collected for the purposes of the peak purity evaluation. Thermal degradation was induced by storing the samples at 105±2◦ C for a period of 24 hours. Oxidative degradation was induced by storing the samples at 50°C and 100°C temperature in 30% hydrogen peroxide for a period of 1hour. The specificity experiments showed that, in all the conditions Lornoxicam degraded was less than 5%, the degradation conditions Fig. 4, 5, 6&7 shows chromatograms of acid, base, peroxide and thermal stressed placebo, standard, and an in-house formulation.

Baseline resolution between Lornoxicam and degradation product peaks was achieved. Diode array detection peak homogeneity tests showed that no peak interfered with the lornoxicam peak. Peak purity for Lornoxicam was established with the help of chemstation software fig 8.The purity factor is within the threshold limit.

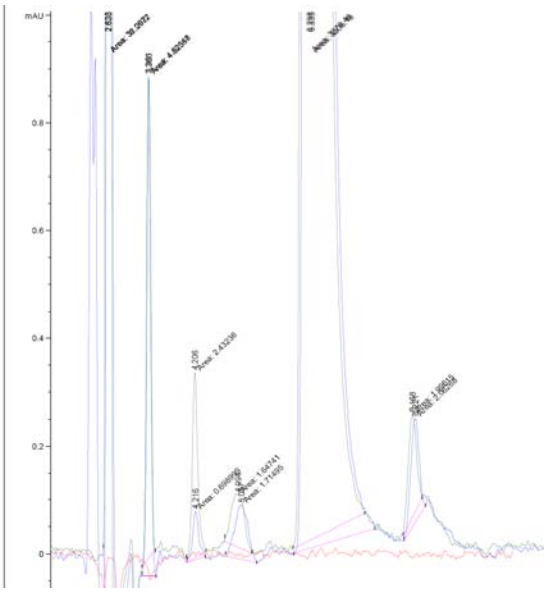

Fig. 4: Alkali degradation

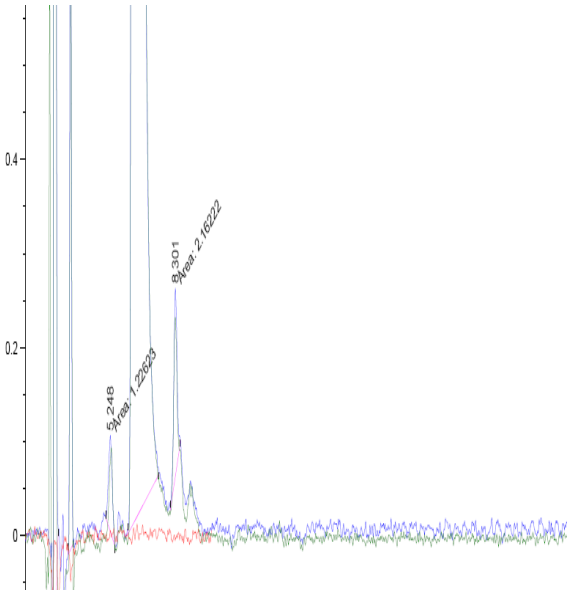

Fig. 5: Acid degradation

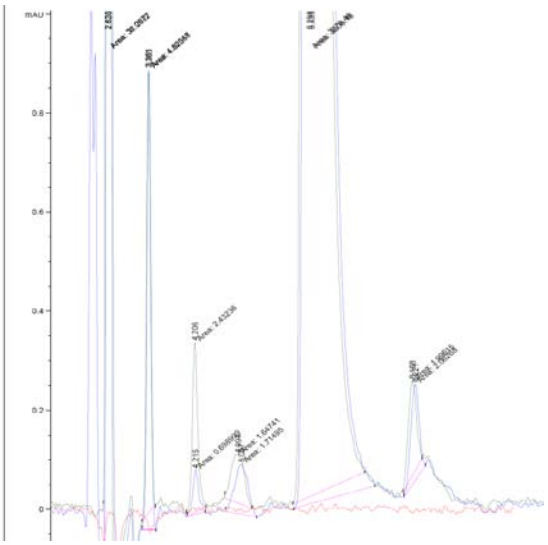

Fig. 6: Peroxide degradation

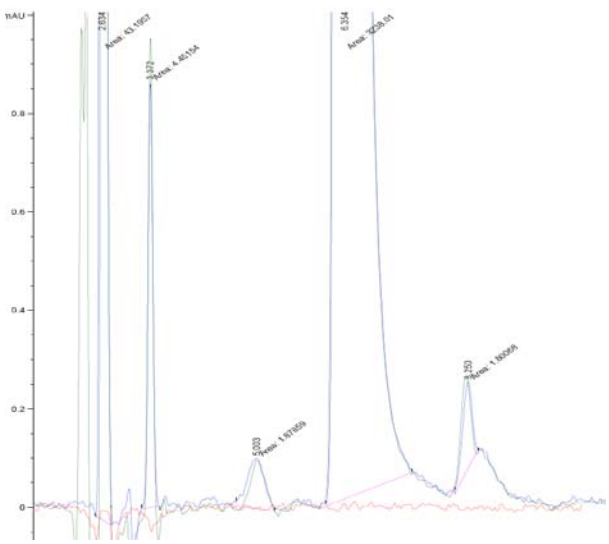

Fig. 7: Thermal degradation

Accuracy

The accuracy was demonstrated by preparing placebo samples that were additionally spiked to approximately 50%,80%,100%,120% and 150% of the theoretical lornoxicam concentration level in sample .The spiked placebo samples at the 50%,80%,100%, and 120% level were prepared in triplicate, using lornoxicam in the solid form for spiking the placebo. The results in Table 2 shows that the average recovery at each level was within 100±2.0% and the %RSD at each level was ≤2.0%.

Method Precision

The repeatability of the method was demonstrated by preparing and analyzing six sample replicates from a homogenous composite blend of 20 tablets. The result in Table 3 shows that the average recovery

was within 100.0 ±2.0% of label claim, and the %R.S.D. was 1.0%, indicating excellent precision.

Solution Stability

The stability of lornoxicam in standard and sample preparation was evaluated. The solutions were stored at ambient temperature and tested at intervals of 0, 2, 4, and 48 hours. The responses for the aged solutions were evaluated using a freshly prepared standard. The results in Table 4 show that sample and standard solutions retained a potency of 100.0±2.0% as compared with the fresh solution over a time of 1 week.

Application to solid dosage forms

The developed method was applied for the determination of lornoxicam content in two marketed products .Products contain single active, lornoxicam . Products are in tablet forms. The method was also used for analyzing the stability samples of formulation. The samples were stored at 40°C/75%RH, pulled periodically and analyzed for the active content. The chromatograms from stability samples and commercial products showed the well separation of lornoxicam peak with other degradants or components. The purity of the lornoxicam peak was checked by PDA and found to be 100%, demonstrating that no interference existed between lornoxicam and degradants or other components. The results showed the method was suitable for stability-indicating analysis and assay for commercial products.

Robustness Study

The influence of chromatographic parameters on the separation was investigated. The parameters examined were the amount of methanol in mobile phase, the amount of triethylamine buffer in the mobile phase and the column temperature (°C).Their effects on the resolution of different peaks were evaluated by means of an experimental design .The chromatographic parameter settings in the experimental design were showed in the Table 5 and results were tabulated in table 6.

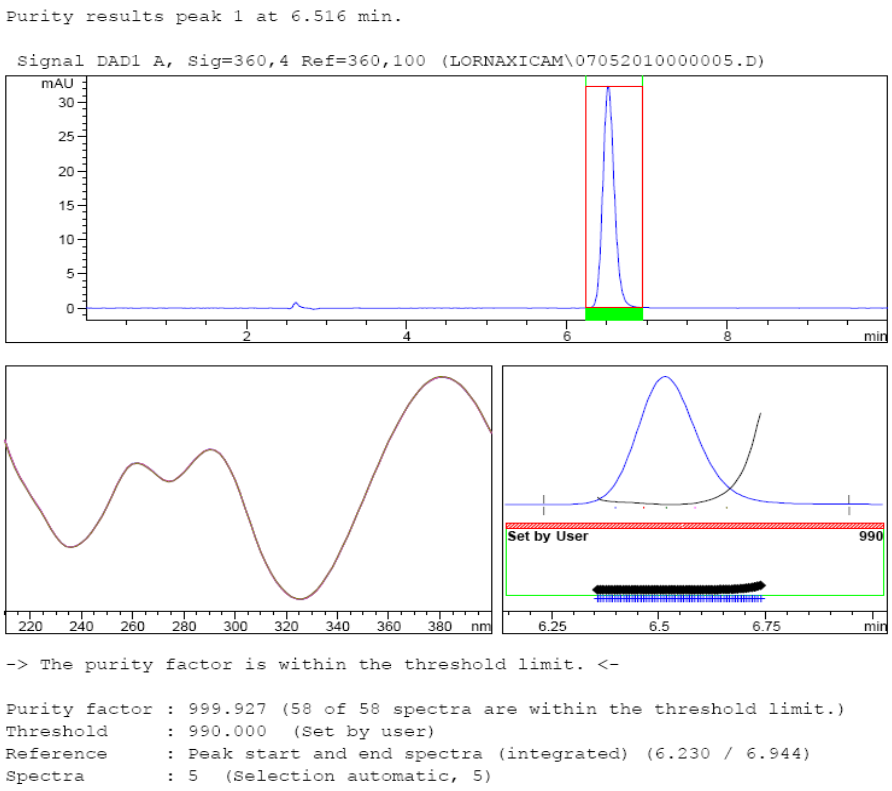

Fig. 8: Peak purity spectrum

Table 2: Accuracy Results

%Level	Sample	% Recovery	Mean	% R.S.D.
50	1	100.12	99.54	0.58
	2	99.54		
	3	98.96		
80	1	99.94	100.55	0.81
	2	100.23		
	3	101.47		
100	1	100.70	100.49	0.30
	2	100.15		
	3	100.61		
120	1	100.32	100.63	0.31
	2	100.93		
	3	100.65		
150	1	99.58	99.54	0.21
	2	99.31		
	3	99.72		

Table 3: Precision Results

Sample	Lornoxicam for 4mg	%Assay	Lornoxicam for 8mg	%Assay
1	3.96	99.1	7.92	99.0
2	3.99	99.7	7.95	99.4
3	4.01	100.3	7.99	99.9
4	4.05	101.3	8.01	100.1
5	3.99	99.9	8.00	100.0
6	3.96	99.0	8.05	100.6
Mean	3.99	99.85	7.99	99.83
%RSD	0.85	NA	0.57	NA

Table 4: Solution stability results

Interval	Standard Solution Stability	Sample Solution Stability
Initial	100.0	99.0
2 hours	100.2	99.2
8 hours	100.0	99.8
15 hours	99.9	99.5
24 hours	99.5	98.9
48 hours	99.2	99.0

Table 5: Robustness parameters

Parameter	Lower Value(-)	Central Value	High Value(+)
Methanol	50	55	60
Flow	0.8	1.0	1.2
pH	6.8	7.0	7.2
Temp(°C)	25	30	35

Table 6: Robustness Results

Parameter	Variation	% Assay
Methanol	40	99.02
	60	99.72
Flow	0.8	98.96
	1.2	99.44
pH	6.8	98.81
	7.2	98.80
Temp	25°C	99.35
	35°C	99.57

CONCLUSIONS

A simple HPLC method using a thermo column was developed for the analysis of lornoxicam in solid dosage formulations. This method was also successfully used for the analysis of lornoxicam in various different marketed formulations. Since the forced degradation and stability studies of the in-house formulation showed no interference with the lornoxicam peak, the method is specific and stability-indicating. The method is also accurate and precise. Hence, the method is recommended for routine quality control analysis.

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