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METHOD DEVELOPMENT AND VALIDATION OF LORNAXICAM FORMULATED DOSAGE FORMS

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ABSTRACT

Lornoxicam is a non- steroidal anti-inflammatory drug (NSAID) of the oxicam class having analgesic, anti–inflammatory and antipyretic properties. A rapid, specific and economic UV spectrophotometric method has been developed using a solvent composed of methanol in phosphate buffer PH 7.4. In the proposed study, the lornoxicam content in bulk and pharmaceutical dosage formulations was determined. However at a pre determined λmax of 292 nm, it was proved to be linear in the range of 2.5-25 μg/mL. This method exhibited a good correlation coefficient (R^2 = 0.9997) and excellent mean recovery (99.36–99.88%). Hence this process was successfully applied to determine the lornoxicam content in six marketed brands from andhra pradesh. The results were in good agreement with the label claims and within the limits. The method was validated statistically as per the International conference of harmonization(ICH) guidel ines. The recovery studies for linearity, precision, repeatability, and reproducibility were found to be in line with the acceptance criteria. The method was found to be rapid, specific, precise & accurate and can be successfully applied for routine analysis of lornoxicam.

Key Words: Lornoxicam, UV-spectroscopic method, validation.

INTRODUCTION

Lornoxicam (LOX) is 6-chloro-4-hydroxy-2-methyl-N-2-pyridinyl-2H-thieno-[2,3-e]-1,2-thiazine-3-carboxamide 1,1-dioxide; is a novel non-steroidal anti-inflammatory drug (NSAID) with marked analgesic properties. LOX belongs to the chemical class oxicams, which includes piroxicam

tenoxicam and meloxicam. LOX, which is commercially available as an 8-mg tablet, is used to treat inflammatory diseases of the joints, osteoarthritis, and pain after surgery. It works by blocking the action of cyclooxygenase, an enzyme involved in the production of chemicals, including some prostaglandins in the body [1]. The present work was undertaken to develop such method of analysis, which can estimate drug in formulation which is a precise, accurate, simple, reliable and less time consuming method for estimation of drugs in tablet.

MATERIALS AND METHODS

Materials

Lornoxicam was a gift from Glenmark pharmaceutical (Nasik, India). Methanol (HPLC grade) was procured from Merck. All chemicals used were analytical grade and glasswares used were Class A grade.

Methods

Preparation of standard solution

Stock solution of lornoxicam for UV determination was prepared at concentration of 50 µg ml^{-1} in 40 % (V/V) methanol in phosphate buffer, pH 7.4. The working standard solutions were prepared by diluting the stock solution in the concentration range from 2.5 to 25 µg ml^{-1}. Ten different concentrations of lornoxicam as the working standard solutions, chosen for the calibration curve were 2.5, 5, 7.5, 10, 12.5, 15, 17.5,20, 22.5 and 25 µg ml^{-1} (n = 6). The standard solutions were prepared by dilution of different volumes of the stock solution to a constant volume with 40 %(V/V) methanol in phosphate buffer, pH 7.4. UV spectra were recorded against 40 %(V/V) methanol in phosphate buffer pH, 7.4 as reference substance.

Method validation

The developed method was validated as per ICH guidelines (ICH Q2B, 1996) for following parameters.

Linearity of calibration curves

Ten level calibration series with six analyses at each concentration level were measured for UV determination. The standard calibration curves of lornoxicam were constructed by plotting absorbance vs. concentration for 40% (V/V) methanol in phosphate buffer, pH 7.4. The results were averaged and analyzed by linear simple regression model of y=mx + c method. A series of standard curves were prepared over a concentration range of 2.5 - 25 µg/ml from a stock solution of lornoxicam (50µg/ml) in 40 %(V/V) methanol in phosphate buffer, pH7.4.The standard curves were evaluated for intra-day and inter-day reproducibility. Each experiment was repeated in triplicate.

Precision

Intra-day variation

Measurement of intra-day variation of Lornoxicam solutions at three different concentrations (5, 10 and 15 µg/ml) was carried out by UV on the same day at different time intervals.

Inter-day variation

Measurement of inter-day variation of lornoxicam solutions at three different concentrations (5, 10 and 15 µg/ml) in triplicate on three consecutive days determined the intermediate precision.

Accuracy

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analyzed samples of lornoxicam

(10 µg/ml) were spiked with 50, 100, and 150% extra lornoxicam standard and the mixtures were analyzed by the proposed method. The experiment was performed in triplicate. Recovery (%), RSD (%) was calculated for each concentration.

Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of quantification (LOQ) was determined as the lowest concentration on the standard calibration curve that was measured with a precision of 20% and accuracy of 80% or 120%.The limit of detection (LOD), expressed as the lowest amount of analyte that was detected but not quantified, was calculated[1,3].The LOD and LOQ for lornoxicam by the proposed method were determined using calibration standards. LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation (n = 6) [4].

Sample solution stability

The stability of the drug in solution during analysis was determined by repeated analysis of samples during the course of experimentation on the same day and also after storage of the drug solution for 72 h under laboratory bench conditions (25 ± 1°C) and under refrigeration (8 ± 1°C). An accurately weighed quantity of the pure drug was dissolved in 40 % (V/V) methanol in phosphate buffer, pH7.4 and suitably diluted with blank solvent medium to get a final concentration of 15 µg/ml. The solution was subjected to UV analysis immediately and after a period of 24, 48 and 72 h.

Analysis of lornoxicam in marketed tablets

Ten tablets (Flexilor tablet; strength: 4 mg/tablet) were crushed and triturated well in a mortar. A powder sample, equivalent to 4 mg of lornoxicam, was accurately weighed and transferred to a 50 ml volumetric flask. The drug was extracted into 40 % (V/V) methanol in phosphate buffer, pH 7.4 and mixed thoroughly for 30 min using a sonicator. The solution was filtered through 0.45 µm pore size cellulose membrane filter after making up the volume, adequately diluted with solvent and analyzed by the proposed UV method. The possibility of interference of excipients with the analysis was studied.

Data analysis

The SD and RSD were determined using Microsoft Excel 2007 application.

Results and Discussion

Method development

The UV absorption spectra of lornoxicam were monitored a single well-defined maximum peak for 40 % (V/V) methanol in phosphate buffer pH 7.4 medium at 378 nm in the measuring wavelength range of 200–1100 nm. No difference was observed in the maximum wavelengths of all spectra (n = 6).

Linearity

Absorbance versus drug concentration was plotted to construct a standard curve for lornoxicam. The polynomial regression for the calibration plots showed good linear relationship with coefficient of correlation, $y = 0.0404x + 0.0042$, $R^2 = 0.9997$ in 40 % (V/V) methanol in phosphate buffer, pH 7.4 medium over the concentration range studied (Figure 4.1). The range of reliable quantification was set at 2.5 – 25 µg/ml as no significant difference was observed in the slopes of the standard curves in this range. The linear regression data for the calibration

plot is indicative of a good linear relationship between absorbance and concentration over a wide range. The correlation coefficient was indicative of high significance. The low values of the standard deviation of slope, and the intercept of the ordinate showed the calibration plot did not deviate from linearity.

Precision

Repeatability of sample injection was determined as intra-day variation while intermediate precision was determined by measuring inter-day variation for triplicate determination of lornoxicam at three different concentrations. The results of the determination of repeatability and intermediate precision are shown in Table 4.1 (A) and Table 4.1 (B). The RSD value of evaluated intra-day precision in 40% (V/V) methanol in phosphate buffer, pH 7.4 was found to be 0.222–0.480%. The RSD value of the evaluated inter-day precision was found to be 0.904 – 2.41%. The low % RSD values indicated that the developed methods have a good repeatability.

Figure No.1. Calibration curve of lornoxicam in 40 %(V/V) methanol in Phosphate buffer, pH 7.4.

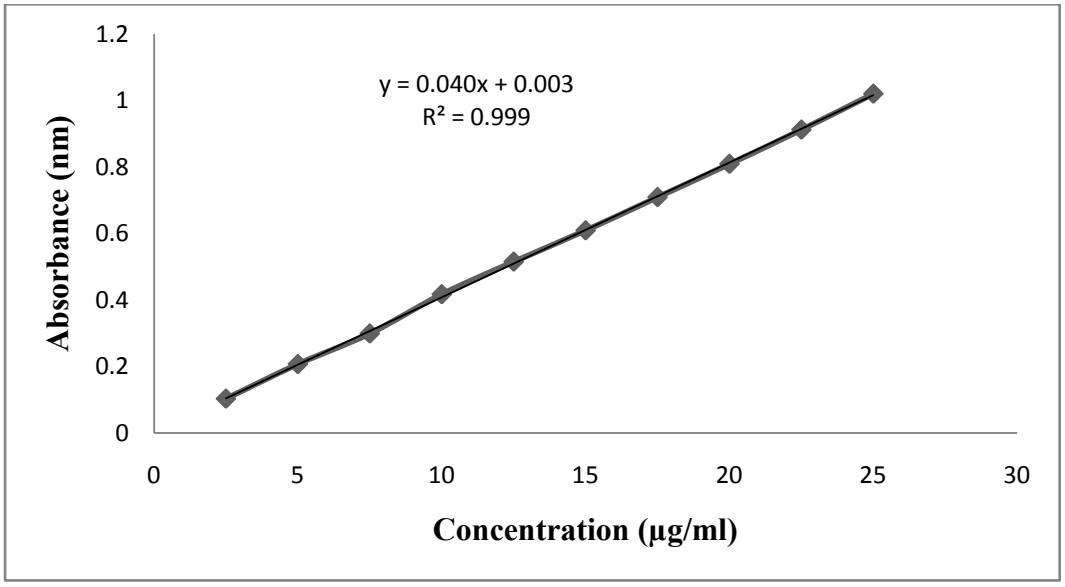


Table No.1. Intra-day precision of the proposed method

Sl. No.	Concentration (µg/ml)	n	$\bar{x}$	SD	RSD (%)
1	5	6	0.208	0.0015	0.480
2	10	6	0.420	0.0025	0.476
3	15	6	0.611	0.0020	0.328
4	20	6	0.810	0.0018	0.222

Means and RSD values of six determinations performed on each day.

$\bar{x}$: Mean; SD:Standard deviation; RSD: Relative standard deviation.

Table No.2. Inter-day precision of the proposed method.

Sl. No.	Concentration (µg/ml)	n	$\bar{x}$	SD	RSD (%)
1	5	6	0.198	0.0047	2.37
2	10	6	0.402	0.0097	2.41
3	15	6	0.597	0.0066	1.10
4	20	6	0.796	0.0072	0.904

Means RSD values of four determinations performed on 3 different days.

$\bar{x}$: Mean; SD: Standard deviation; RSD: Relative standard deviation.

Recovery

The recovery of the method was determined by spiking a previously analyzed test solution with additional drug standard solution. It was found to be in the range of 99.36 – 99.88%. The values of recovery (%) and RSD (%) is shown in in Table 4.2. The results clearly indicate the method was accurate.

Table No.3. Recovery results of Lornoxicam in 40% (V/V) methanol in phosphate buffer, pH7.4.

Amount (%) of drug added to Analyte	Theoretical content (µg/ml)	Conc. found (µg/ml)	Recovery (%)	Mean Recovery (%)	SD	RSD (%)
0	10	9.93	99.30	99.366	0.208	0.209
0	10	9.96	99.60			
0	10	9.92	99.20			
50	15	14.92	99.46	99.416	0.466	0.448
50	15	14.84	98.93			
50	15	14.98	99.86			
100	20	19.96	99.8	99.883	0.480	0.480
100	20	19.89	99.45			
100	20	20.08	100.4			
150	25	24.92	99.68	99.680	0.625	0.629
150	25	24.98	99.92			
150	25	24.86	99.44			

Sensitivity

The LOD for lornoxicam was found to be 0.49 µg/ml while the value of LOQ was found to be 1.48 µg/ml. This indicated the method can be used for detection and quantification of lornoxicam over a very wide range of concentrations.

Stability of lornoxicam solutions

There was no significant change in analyte composition (concentration = 15 µg/ml) over a period of 72 h. The % RSD for the samples stored under refrigeration (8 ± 1°C) and at laboratory temperature (25 ± 1°C) was found to be 0.926 % and 1.10%, respectively, suggesting that the drug solution can be stored without any degradation over the time interval studied.

Figure No.2. UV spectrum of lornoxicam contained marketed lornoxicam tablet (15µg/ml) from 40%(V/V) methanol in phosphate buffer, pH 7.4.

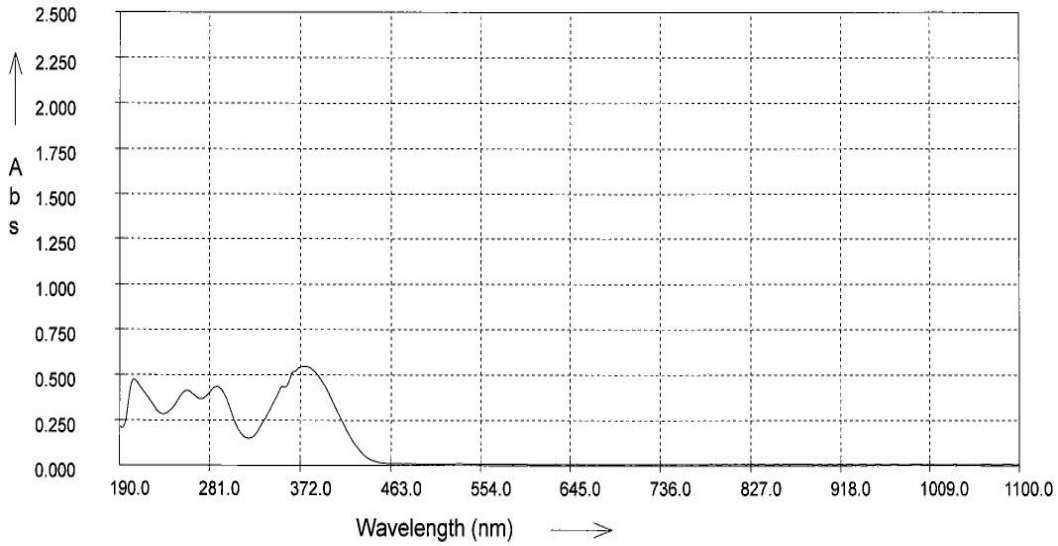
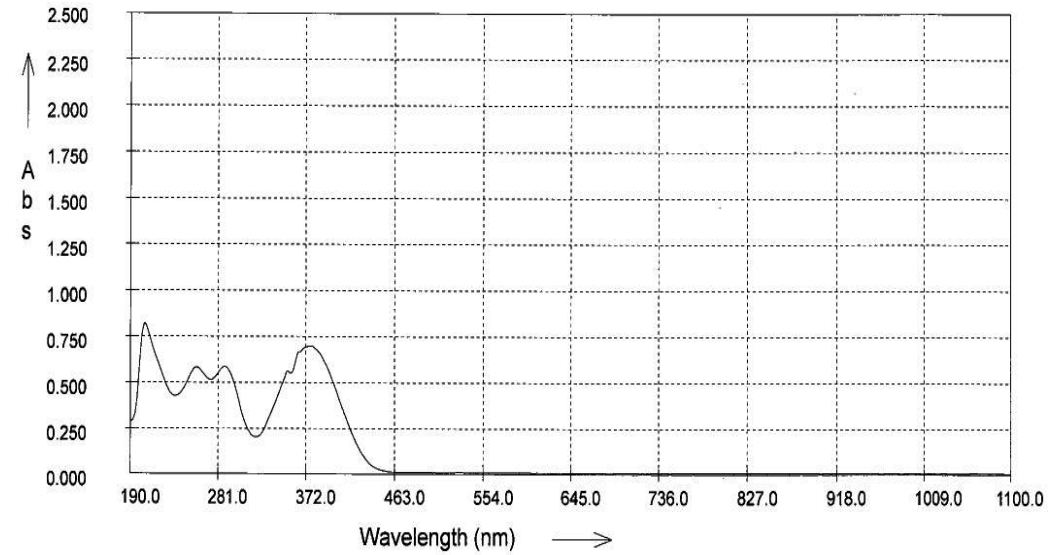


Figure No.3.UV spectrum of lornoxicam contained marketed lornoxicam tablet (20µg/ml) from 40% (V/V) methanol in phosphate buffer, pH 7.4.



Analysis of lornoxicam from marketed tablets

Lornoxicam was analyzed using the same method described in section 4.1.2.6. This method has been applied successfully to commercial tablet. The UV spectra of lornoxicam in the concentration of 15µg/ml and 20µg/ml prepared from commercial tablet in 40 %(V/V) methanol in phosphate buffer, pH 7.4 are shown in Figure 4.2(A) and Figure 4.2(B), respectively. A single well defined maximum peak was observed of lornoxicam. No interaction was observed between lornoxicam and excipients present in the tablets. The lornoxicam content was found to be 99.62% and the RSD was 0.91%. The low RSD indicated the suitability of this method for routine analysis of lornoxicam in pharmaceutical dosage forms. The proposed UV method of analysis was also found to be precise and accurate, as depicted by the statistical data of analysis. High values of correlation coefficients and small values of intercepts validated the linearity of the calibration plots and obedience to Beer's laws. The RSD values and the slopes and intercepts of the calibration curve indicate the high reproducibility of the proposed method. Furthermore, the low values of LOD and LOQ indicate that the method can be employed over a wide concentration range for linearity. Thus this method was adopted for the analysis of lornoxicam from prepared lornoxicam loaded SLNs formulations.

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