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UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION OF LORNOXICAM IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, rapid, and reliable UV–visible spectrophotometric method was developed and validated for the quantitative determination of lornoxicam using 40% (v/v) methanol in phosphate buffer pH 7.4 as the solvent system with detection at 378 nm. The developed method exhibited excellent linearity over the concentration range of 2.5–25 µg/mL with a regression equation of $y = 0.0404x + 0.0042$ and a correlation coefficient (r) of 0.9997 ± 0.006, confirming compliance with Beer–Lambert's law. Precision studies demonstrated high repeatability with intra-day relative standard deviation (RSD) values ranging from 0.222% to 0.480% and inter-day RSD values between 0.904% and 2.41%. Accuracy of the method was confirmed through recovery studies at three concentration levels (15, 20, and 25 µg), yielding mean recoveries in the range of 99.35–99.88% with RSD values below 0.63%. Stability studies indicated that lornoxicam solutions remained stable for up to 72 hours under both refrigerated conditions (8 ± 1°C) and laboratory temperature (25 ± 1°C), with RSD values of 0.987% and 0.865%, respectively. The method was successfully applied to the analysis of marketed tablet formulations, showing no interference from pharmaceutical excipients and yielding drug content of 99.46% with an RSD of 0.94%. The validated method was also effectively employed for the quantification of lornoxicam in solid lipid nanoparticle (SLN) formulations. Owing to its simplicity, accuracy, precision, reproducibility, and cost-effectiveness, the developed UV spectrophotometric method is suitable for routine pharmaceutical analysis and quality control applications.

KEYWORDS: Lornoxicam, UV–Visible spectrophotometry, Validation, Linearity,

INTRODUCTION

Lornoxicam 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 non steroidal anti inflammatory drug[1,2] (NSAID). It belongs to oxicam class with analgesic (pain relieving), anti-inflammatory and antipyretic (fever reducing) properties. It works by blocking the action of Cyclooxygenase, an enzyme involved in the production of chemicals, including some prostaglandins in the body.[3] It is distinguished from established oxicams by a relatively short elimination half-life (3 to 5 hours), which may be advantageous from a tolerability standpoint.[4] It is available in oral and parenteral formulations

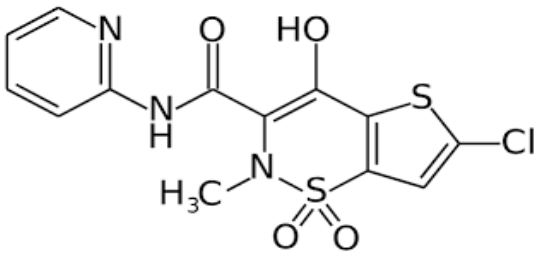

Figure 1: Structure of Lornoxicam.

The UV spectrophotometric method is very simple, rapid, economical, and it allows the determination of pharmaceuticals with enough reliability. For the UV spectrophotometric method, the survey of literature revealed very complex methods, using bands of the visible range using complexometry, derivative or chemometric assistance and interpolation on the calibration curve.[5,6] The aim of this work was the development and validation of a new UV

spectrophotometric method, which can be more economical and simpler than the official methods and with other methods published. Analysis is the most important aspect of any drug development whether in bulk or in combination, a suitable method must be developed so as to ensure that any drug either in dosage form or bulk form can be pointed out. The method development ensures that the amount of a particular drug can be easily determined. The validation parameters confirm that the developed method is precise, accurate and reproducible and can be used for routine evaluation of Lornoxicam in bulk and combined dosage form.[7] Very few analytical methods have been reported for the estimation of Lornoxicam in pharmaceutical formulations.[8-13]

MATERIALS AND METHODS

Instrumentation: Shimadzu UV-1800 spectrophotometer, REMI Laboratory centrifuge, ELICO pH meter, CM101 cyclo mixer was used and all weighing were done on electronic balance (Model Shimadzu AUW-220D).

Method

Selection of wavelength: Good analytical results for analytes will be obtained only by careful selection of the wavelength used for detection. UV spectra can be measured for determining wavelength in case of UV. Standard solution of lornoxicam was scanned in UV spectral range of 200-400nm. The spectrum corresponding to wavelength of maximum absorbance was recorded.

Spectroscopic conditions: The solvent system composed of a mixture of 40 % (V/V) methanol in phosphate buffer pH 7.4. The experiment was performed at 25^{o}C. The absorbance of every calibration standard was estimated atλmax 255nm using fixed wavelength measurement mode.

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 25µg 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

Calibration curve: 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 analysed 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. Thestandard curves were evaluated for intra-day and inter-day reproducibility.

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 (10µg/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) were calculated for each concentration.

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 72h 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 pH 7.4 and suitably diluted with blank solvent medium to get a final concentration of 15µg/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 (strength: 4 mg/tablet) were crushed and triturated well in a mortar. A powder sample, equivalent to 4mg of lornoxicam was accurately weighed and transferred to a25ml 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 micron pore filter after making up the volume, adequately diluted with mobile phase and analyzed by the proposed UV method. The possibility of interference of excipients with the analysis was studied.

RESULTS AND DISCUSSION

Method development

The UV absorption spectrums 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 = 0.9997±0.006in40 % (V/V) methanol in phosphate buffer pH 7.4medium over the concentration range studied. 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 (Table 1).

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, intermediate precision and reproducibility are listed in Table 2&3. Reproducibility was checked by measuring the precision of the proposed method with analysis being performed by another person. The low % RSD values indicate the repeatability and reproducibility of the method.

Table 1: Results of calibration curve at 378 nm.

Concentration (µg/mL)	Absorbance
2.5	0.10
5	0.21
7.5	0.32
10	0.42
12.5	0.51
15	0.62
17.5	0.71
20	0.80
22.5	0.89
25	0.99

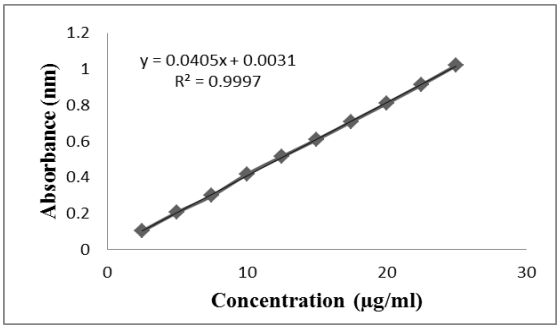

Figure 2: Calibration curve of Lornoxicam (40% V/V) methanol in Phosphate buffer, pH 7.4.

Table 2: Intraday precision of the proposed method.

S. No	Concentration (µg/ml)	n	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

Table 3: Inter day precision of the proposed method.

S. No	Concentration (µg/ml)	n	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 and RSD values of six determinations performed on each day.
X: Mean; SD: Standard deviation; RSD: Relative standard deviation

Table 4: Recovery results of Lornoxicam (40% V/V) methanol in phosphate buffer pH 7.4.

S.No	Amt of sample (µg)	Amt of Std added (µg)	Total amt of lornoxicam (µg)	Total amt of lornoxicam (µg)	Recovery (%)	Mean Recovery (%)	SD	RSD (%)
1	10	5	15	14.92	99.46	99.416	0.466	0.448
2	10	5	15	14.84	98.93			
3	10	5	15	14.98	99.86			
4	10	10	20	19.96	99.8	99.883	0.480	0.480
5	10	10	20	19.89	99.45			
6	10	10	20	20.08	100.4			
7	10	15	25	24.92	98.68	99.346	0.625	0.629
8	10	15	25	24.98	99.92			
9	10	15	25	24.86	99.44			

Recovery: The recovery of the method, determined by spiking a previously analyzed test solution with additional drug standard solution, was found to be in the range of 99.34– 99.88%. The values of recovery (%) and RSD (%) is shown in Table 4.The results clearly indicate the method is accurate.

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 0.987% and 0.865% respectively, suggesting that the drug solution can be stored without any degradation over the time interval studied.

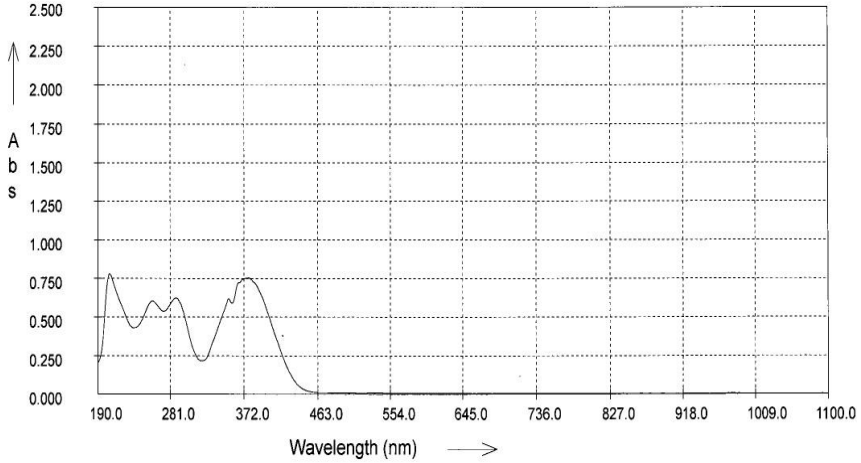

Figure 3: UV spectrum of lornoxicam (20µg/ml) from (40% (V/V) methanol in phosphate buffer, pH 7.4.

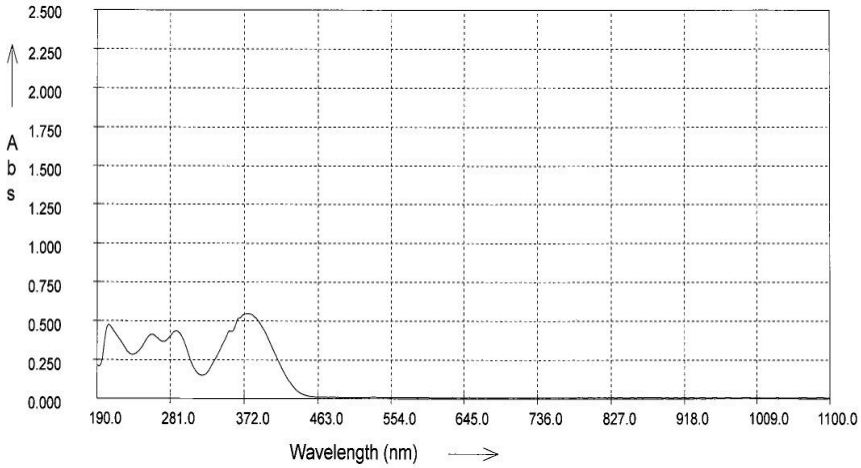

Figure 4: UV spectrum of marketed lornoxicam tablet (15µg/ml) from (40%V/V) methanol in phosphate buffer, pH 7.4.

Analysis of Lornoxicam from marketed tablets

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 99.46% and the RSD was 0.94%. 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 graphs 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.

CONCLUSION

A simple and efficient UV-visible spectrophotometric method was developed and comprehensively validated for the quantification of lornoxicam using 40% (v/v) methanol in phosphate buffer pH 7.4 as the medium, with detection at 378 nm. The method demonstrated excellent linearity with a polynomial regression equation

of y = 0.0404x + 0.0042 and a high correlation coefficient (r = 0.9997 ± 0.006) over the validated concentration range of 2.5–25 µg/mL. Precision studies revealed outstanding repeatability with intra-day relative standard deviation (RSD) values ranging from 0.222% to 0.480%, while inter-day precision showed RSD values between 0.904% and 2.41%, both indicating excellent reproducibility of the method. Recovery studies conducted at three different levels (15, 20, and 25 µg) yielded mean recoveries of 99.35–99.88% with RSD values below 0.63%, confirming the accuracy of the proposed method. Stability studies demonstrated that lornoxicam solutions remained stable over 72 hours at both refrigeration (8 ± 1°C, %RSD = 0.987%) and laboratory temperature (25 ± 1°C, %RSD = 0.865%), indicating no significant degradation under normal storage conditions. Analysis of marketed lornoxicam tablets confirmed the absence of interference from excipients, with lornoxicam content determined at 99.46% and RSD of 0.94%.

The developed UV-visible spectrophotometric method for lornoxicam quantification is validated and suitable for routine pharmaceutical analysis and quality control applications. The method exhibits excellent analytical performance characteristics including high linearity, exceptional precision (RSD < 2.5%), accurate recovery (99.35–99.88%), demonstrated stability of drug solutions under various storage conditions. The absence of interference from pharmaceutical excipients in tablet formulations, coupled with high correlation coefficients and small intercept values, establishes the method's specificity and compliance with Beer's law. The combination of simplicity, economy, precision, accuracy, and reproducibility makes this method particularly valuable for routine analysis of lornoxicam in pharmaceutical dosage forms. Accordingly, this validated UV method has been successfully adopted for the determination of lornoxicam content in lornoxicam-loaded solid lipid nano particle (SLN) formulations, demonstrating its versatility and applicability across diverse pharmaceutical matrices and advanced drug delivery systems.

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