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DETERMINATION OF LORNOXICAM IN PLASMA BY UV SPECTROSCOPY

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ABSTRACT

Simple, precise and cost effective UV spectrophotometric method has been developed for the estimation of lornoxicam in plasma. Lornoxicam shows λ_{max} at 289.7 nm in plasma. The drug follows Beer-Lambert law in the concentration range of 2.0-26.0 µg/ml with correlation coefficient of 0.999. The method was validated by following analytical performance parameters suggested by the international conference on harmonization. All validation parameters were within the acceptable range. The developed method was successfully applied to estimate the amount of lornoxicam in plasma.

Keywords: Lornoxicam, UV spectrophotometry.

INTRODUCTION

Lornoxicam is chemically, 6-chloro-4-hydroxy-2-methyl-N-2-pyridinyl-2H-thieno-[2, 3-e]-1, 2-thiazine-3-carboxamide 1, 1-dioxide. It is a novel non-steroidal anti-inflammatory drug (NSAID) with marked analgesic activity. It works by blocking the action of cyclooxygenase, an enzyme involved in the production of prostaglandins from arachidonic acid in the body.[1]

Lornoxicam is an official drug in Merck Index. On detailed literature survey, it was found that lornoxicam can be estimated by spectrophotometry[2], polarographic[3], HPLC[4,5], liquid chromatography[6] methods individually or in combination with other drugs. The aim of the present work is to develop and validate UV spectrophotometric method for the estimation of lornoxicam in plasma.

MATERIALS AND METHODS

Chemicals and Reagents

Lornoxicam working standard was kindly provided by Glenmark Generics Ltd., (Pune) and was used as received. A commercial tablet formulation was purchased from the local market. Methanol of analytical grade was used.

Instrument

A double beam UV-VIS spectrophotometer (UV CE7400, Cecil, UK) connected to computer loaded with spectra manager software UV probe was employed with spectral bandwidth of 1 nm and wavelength accuracy of ±0.5 nm with a pair of 10 mm matched quartz cells. All weights were taken on electronic balance (XB120A, Precisa, Switzerland).

Collection of Plasma

The blood was collected and poured into the centrifuge tubes. These centrifuge tubes were then placed into the centrifuge apparatus & allowed to centrifuge for 20 minutes at 8000 rpm

and the plasma was separated and stored in freezer.

Preparation of Standard Stock Solution

10 mg of lornoxicam was accurately weighed and transferred to 100 ml volumetric flask. The drug was dissolved by adding 10 ml of plasma and 10 ml of methanol. The volume was then made up to 100 ml with water to yield the final standard solution of 100 µg/ml concentration.

Determination of Wavelength of Maximum Absorbance of Lornoxicam in Plasma

1.0 ml of above solution was diluted to 10 ml with the same solvent to get the concentration of 10 µg/ml. The UV spectrum of final solution obtained was scanned in the range of 200 to 400 nm against a mixture of plasma, methanol and water (1:1:8) as a blank.

The λ_{max} was found 289.7 nm. The UV spectrum of lornoxicam is shown in figure1.

Preparation of Calibration Curve for Lornoxicam

0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml, 1.2 ml, 1.4 ml, 1.6 ml, 1.8 ml, 2.0 ml, 2.2 ml, 2.4 ml, and 2.6 ml. solutions were pipetted out individually from the stock solution in a series of thirteen, 10 ml volumetric flasks. The volume in each flask was made up to 10 ml with plasma, methanol and water (1:1:8) to yield final solution in the concentration range of 2.0 to 26 µg/ml. Then the absorbances of all the solutions were measured at λ_{max} of drug, i.e. 289.7 nm, against the mixture of plasma, methanol and water (1:1:8) as a blank. The results of calibration curve data for lornoxicam are shown in table1 and the calibration curve is depicted in figure2.

Estimation of Lornoxicam in Tablets

Twenty tablets of lornoxicam were weighed and finely powdered. A quantity of powder equivalent to 8 mg of the drug was transferred to a 100 ml volumetric flask containing plasma, methanol and water in the ratio of 1:1:8 and made up to mark with plasma, methanol and water (1:1:8). The solution was kept in ultrasonic water bath for 15 min to complete dissolution. The solution was filtered through whatmann filter paper no.40. After suitable dilution, the spectrum of the final sample corresponding to 8.0 µg/ml was recorded against blank. The results are shown in table 2.

Method Validation

Linearity

A calibration curve was constructed at optimum experimental condition using absobance values versus concentration in the range of 2.0-26 µg/ml. It has shown linear relationship with the regression equation y= 0.026c + 0.002, where „y" is absorbance at 289.7 nm and „c" is the concentration of the sample in µg/ml. High value of correlation coefficient (0.999) indicates good linearity and adherence of the method to Beer"s law.

Precision

The intraday and interday precisions of developed method were determined by estimating the corresponding response three times on the same day and on three different days over a period of week for three different concentrations of Lornoxicam (11.2 µg/ml, 14.00 µg/ml, 16.80 µg/ml) and the results are reported in terms of relative standard deviation in table 3.

Accuracy

This parameter was evaluated by the percent recovery studies at concentration levels of 80, 100 and 120%, which consisted of adding known amounts of lornoxicam reference materials to a prequantified sample solution. Aliquots of sample solutions containing lornoxicam at 10.0 µg/ml were transferred to three 10 ml volumetric flasks containing, respectively, 0.8, 1.0, and 1.2 ml lornoxicam reference solution (100 µg/ml). The contents were mixed and diluted to volume in order to obtain final concentrations of 18.0, 20.0, and 22.0 µg/ml respectively. The recovery was verified by estimation of drugs in triplicate

preparations at each specified concentration level. The spectrums were recorded in the UV range and then analyzed. The results are reported in terms of % recovery in table 3.

Specificity

Commonly used excipients present in selected tablet formulation were spiked into a preweighed quantity of drugs. Absorbance was measured.

RESULTS AND DISCUSSION

According to the International Conference on Harmonization[7], the main objective of the validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose, and the parameters that need to be selected are the responsibility of the analyst. Methanol was used as solvent for lornoxicam. Lornoxicam in plasma, methanol and water (1:1:8) showed absorption maxima at 289.7 nm in UV spectrum. The response for lornoxicam was found to be linear in the concentration range of 2.0–26.0 µg/ml. The optical characteristics of the method and regression analysis of the calibration curve are shown in table 3. The recovery of Lornoxicam was found to be satisfactory. Excipients used in the specificity study did not interfere with response of the drug at its analytical wavelength. Also, no significant change in response of lornoxicam was observed after 24 hrs. Hence, the method is specific and robust for estimation of lornoxicam.

Table 1: Calibration curve data for Lornoxicam

Conc. (g/ml)	Absorbance
2.0	0.059
4.0	0.110
6.0	0.163
8.0	0.212
10.0	0.261
12.0	0.317
14.0	0.375
16.0	0.417
18.0	0.466
20.0	0.522
22.0	0.579
24.0	0.649
26.0	0.690

CONCLUSION

The method was validated and found to be simple, sensitive, accurate, and precise. Hence, the method can be used successfully for routine analysis of pharmaceutical dosage form of lornoxicam.

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Table 2: Assay result of Lornoxicam in tablets

Label claim (mg/tab)	Amount found (mg/tab)	Standard deviation	% Mean recovery
8.0	7.98	0.899	99.76

Table 3: Optical characteristics and validation parameters of Lornoxicam

Parameter		Values
Beer‟s law limit (g/ml)		2.0-26
λ max (nm)		289.7
Molar absorptivity (mole-1 cm-1)		96670.6
Regression equation (Y=a + bc)		Y=0.026c + 0.002
Correlation coefficient (r2)		0.999
Slope (b)		0.026
Intercept (a)		0.002
Limit of detection (g/ml)		0.23834
Limit of quantitation (g/ml)		0.79446
Precision (RSD, %)	Repeatability	0.96957
	Intraday	0.5028
	Interday	0.7121
Accuracy (% recovery)		100.58

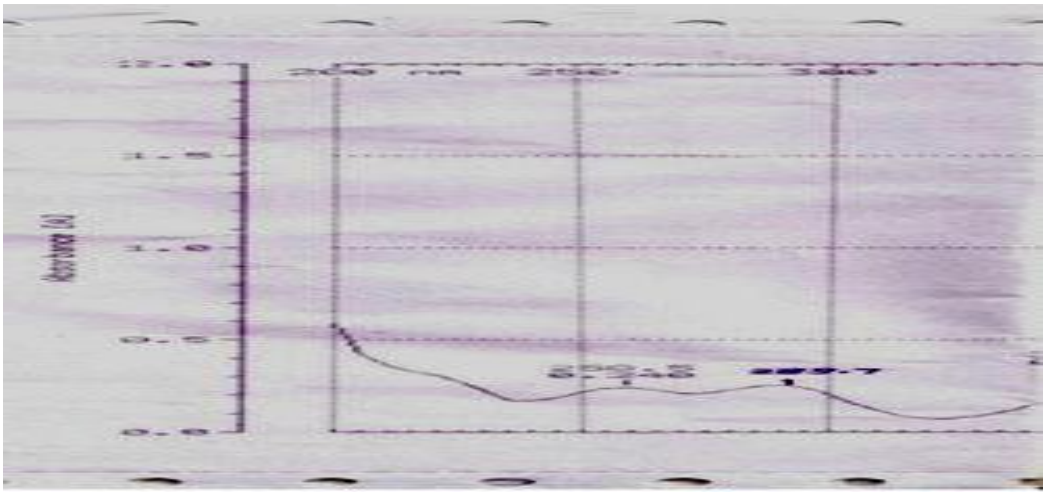

Figure1: UV spectrum of Lornoxicam

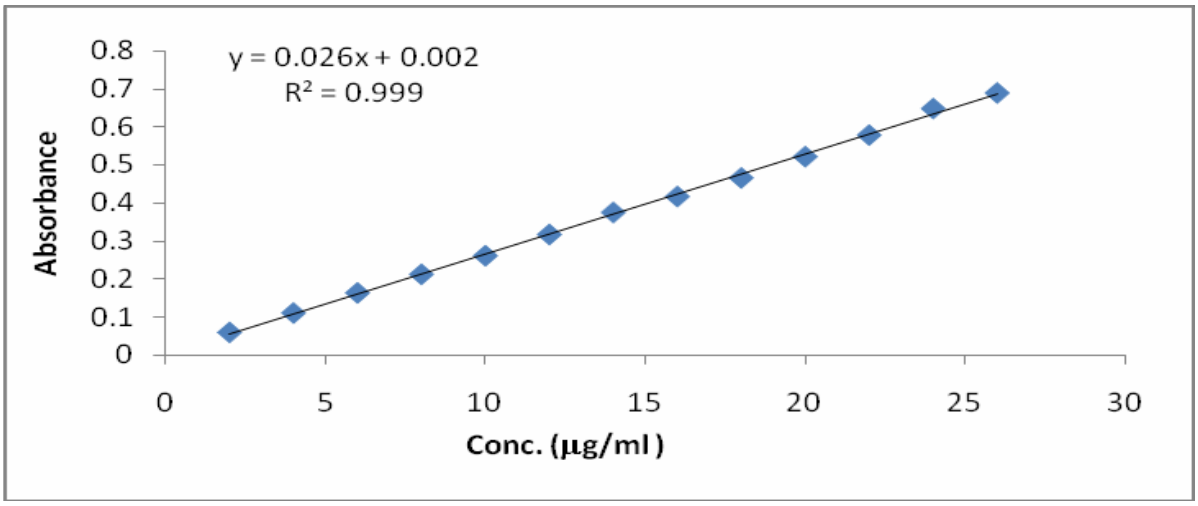

Figure2: Calibration curve of lornoxicam at 289.7 nm

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