Research Article

RP-HPLC Method Development and Validation for the Estimation of Lornoxicam in Bulk and it's Solid Dosage Form

Pavan Kumar K*, Kiran Kumar DP, Mohan Kandhasamy R, Jagadeeswaran M and Siva Kumar T

Department of Pharmaceutical Analysis, Nandha College of Pharmacy and Research Institute, Erode, TamilNadu, India.

ABSTRACT

The present paper describes simple and sensitive, reversed phase high performance liquid chromatography (RP-HPLC) method has been developed for the determination of lornoxicam in bulk and its tablet dosage form. Chromatographic separation was achieved by using phenomenex C_{18} column, 250×4.6 mm, 5 µm as stationary phase. Mixture of acetonitrile: phosphate buffer (60:40 v/v) was used as a mobile phase and the pH was adjusted into 7.0 using o-phosphoric acid, at a flow rate of 1.2 ml/min. Lornoxicam standard shows maximum absorption in UV at 390 nm. The method was validated statistically and recovery study was performed as per ICH guidelines. The result of percentage recovery and placebo interference shows that the method was not affected by the presence of excipients which proves suitability of the method and also it was found to be rapid, accurate and precise.

Keywords: Lornoxicam, RP-HPLC, Validation, Tablet dosage form.

INTRODUCTION

Lornoxicam (LOR)[1], (2H-Thieno(2,3-e)-1,2-thiazine-3-carboxamide), (6-chloro-4-hydroxy-2-methyl-N-2-pyridinyl-2H-thieno[2,3-e]-1,2-thiazine-3-carboxamide1,dioxide), (Chlortenoxicam) $C_{13}H_{10}C_lN_3O_4S_2$, Fig. 1) is a novel non-steroidal anti-inflammatory drug in the enolic acid class of compound with analgesic, anti-inflammatory and antipyretic properties. It acts by non-selective inhibition of cyclo-oxygenase-1 and -2 an enzyme involved in the production of chemicals, including some prostaglandins in the body.[2, 3, 4]

In the literatures, a voltametric[5], polarographic[6], UV spectrophotometric[7], LC-MS/MS[8] and high performance liquid chromatographic methods[9] have been proposed for the analysis of lornoxicam. The aim of present work is to develop a simple, rapid, precise, accurate and selective reversed phase chromatographic method and to estimate the lornoxicam in bulk and its solid dosage forms. The proposed method was optimized and validated in accordance with International Conference on Harmonization (ICH) guidelines.[10]

EXPERIMENTAL PART

Chemicals and reagents

Bulk sample of lornoxicam was obtained from Hetero drugs, Hyderabad, India. The commercial samples of tablets containing 8 mg of lornoxicam were purchased from local market. Milli-Q-Water and acetonitrile (HPLC grade) were procured from Sigma Aldrich (Switzerland).

Equipment

A shimadzu LC system consisted of a solvent delivery system, an auto-injector fitted with 100 µl syringe, an online degasification system, and an SPD-10A UV-Vis detector. The output signal was monitored and integrated using chemistry station software (LC Solution).

Chromatographic equipment and conditions

The shimadzu LC10ATvp series, (Kyoto, Japan) HPLC instrument with a Phenomenex C_{18} analytical column, (250 mm×4.6 mm, 5 µm) was used for the study. UV detection was made at 390 nm. The volume of injection was 20 µl. All analyses were done at ambient temperature. The mobile phase was prepared freshly and vacuum-filtered through a 0.45 µm Millipore nylon filters in the beginning of the experiment.

Mobile phase

For the analysis, the mobile phase consisted of acetonitrile and buffer in the ratio (60:40 v/v), adjusted the pH into 7.0 with ortho-phosphoric acid and flow rate of 1.2 ml/min.

Preparation of standard stock solutions

The stock solution of lornoxicam (1000 µg/ml) was prepared by dissolving 10 mg of lornoxicam (99.8%) is dissolved in 10 ml volumetric flask using mobile phase. Aliquots of 10-50 µg/ml were prepared from the stock solution.

Sample preparation

Twenty tablets were weighed and their average weight was calculated. The tablets were crushed into a homogeneous powder and a quantity equivalent to one tablet (8 mg) was transferred to a 10 ml volumetric flask, dissolved in acetonitrile: buffer (60:40 v/v) and filtered through a 0.45 µm Millipore nylon filters.

Recommended procedure for standard graph

After a systematic and detailed study of various parameters involved, the following procedure and conditions recommended for the determination of lornoxicam in pure samples and in dosage forms.

The calibration curve for lornoxicam was constructed by analyzing lornoxicam solutions containing 10-50 µg/ml in triplicate. The standard solutions were prepared by diluting the stock solution in mobile phase. Each of samples (20 µl) was injected six times into the column and the peak area of absorption was determined. Standard graph was plotted by taking concentration of drug on x-axis and peak area of absorption on y-axis.

RESULTS AND DISCUSSION

Chromatograms of lornoxicam were recorded and shown in Fig. 2. The retention time was recorded at 5.260 min respectively. The results from development activity are that a suitable, easy, less time consuming validated method has been developed for lornoxicam.

Linearity

Calibration curves were obtained from the peak area and concentration of the drug were subjected to regression analysis and correlation coefficients. Table 1 represents the mean RP-HPLC area responses for Lornoxicam at different concentrations. As shown, the responses for the drug was strictly linear (r^2>0.999) in the concentration range of 10-50 µg/ml. The slope and intercept was found to be 47.06 and 13.4 respectively.

Precision

Table 2 gives the intra-day precision and inter-day precision values of measured concentrations of the lornoxicam, as calculated from the linearity plots. In both situations the RSD values were found to be 0.176% for intra-day assay and 0.705% for inter-day assay respectively, demonstrating that the method was precise.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were determined from the calculated standard deviations of each calibration standard and it was found to be 0.013 µg/ml and 0.465 µg/ml respectively. It reveals that the method can be effectively used for the estimation of lornoxicam.

Accuracy

The respective HPLC area responses from the accuracy determination study are shown in Table 3. Recovery experiment was carried out by applying the standard addition method. Drug assay was performed in triplicate by spiking with equivalent amount of raw material into each volumetric flask for each spike level to get the concentrations of lornoxicam equivalent to 80%, 100%, and 120% of the standard concentrations of lornoxicam. The average percentage recovery of lornoxicam was found to be within the limits and it was highly accurate.

Specificity

Specificity is the ability of the method to accurately measure the analyte response in the presence of all potential sample components (excipients).The method was found to be specific to the lornoxicam, as the peaks of the degradation products did not interfere with the drug peaks.

CONCLUSION

The proposed method is validated as per ICH recommended conditions and it was found to be simple, rapid, precise, accurate and sensitive for the determination of lornoxicam. It can be used for the routine quality control analysis of lornoxicam in bulk drug and its solid dosage form.

Table 1: Linearity of RP-HPLC method

Drug	Concentration (µg/ml)	Mean Peak Area ± S.D*	RSD (%) **
Lornoxicam	10	1960747±14142.14	0.721
	20	2125202±14181.54	0.697
	30	4138503±38876.02	0.939
	40	10384361±58041.45	0.559
	50	11212788±94752.31	0.845

*Average ± standard deviation of 3 observations.
**Average of 3 observations.

Table 2: Precision of RP-HPLC method

Drug	Concentration (µg/ml)	Intra-day precision		Inter-day precision	
		Peak area	RSD (%)*	Peak area	RSD (%)*
Lornoxicam	10	11.746311	1.31	11.982756	1.78
	20	15.468241	1.48	16.789541	1.15
	30	16.606869	1.09	19.012542	1.22
	40	19.363271	0.93	20.541478	1.05
	50	20.497361	0.79	25.124571	0.88

*Average of 6 observations.

Table 3: Analysis of formulations and recovery studies by RP-HPLC

Drug	Spike level (%)	Amount of drug added (µg)	Amount of drug recovered (µg)	Recovery (%)	RSD (%)*
Lornoxicam	80	10	10.21	102.1±0.4578	0.153
	100	30	30.51	101.7±0.2148	0.565
	120	50	50.06	100.1±0.1025	0.550

*Mean value of three determinations

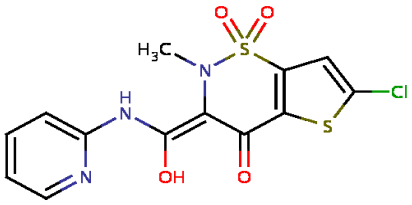

Fig. 1: Structure of Lornoxicam

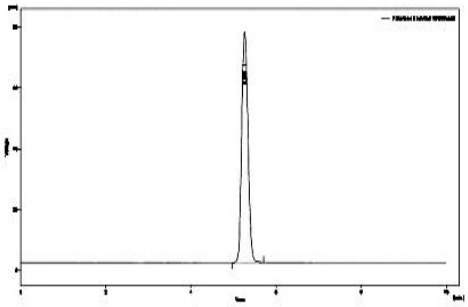

Fig. 2: Chromatogram of Lornoxicam

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