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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF LORNOXICAM USING RP-HPLC IN BULK DRUG AND FORMULATION

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ABSTRACT

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the quantitative determination of Lornoxicam in bulk drug and formulation. The developed method consists of mobile phase, ammonium dihydrogen phosphate Buffer: acetonitrile (50:50) with isocratic programming, Hypersil BDS C_{18}, 250×4.6mm, 5µm column as stationary phase with a flow rate of 1.0 mL/min. Proposed method was found to be linear in the concentration range of 5 – 30 µg/mL, the correlation coefficient was found to be 0.999. System suitability parameters were studied by injecting the standard solution five times and results were well under the acceptance criteria, the proposed method is found to be sensitive, rapid, reproducible, and accurate.

Keywords: Lornoxicam, RP-HPLC, Stationary phase.

INTRODUCTION

Lornoxicam (LOM), 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 nonsteroidal anti-inflammatory drug (NSAID) in the enolic acid class of compound with analgesic, anti-inflammatory and antipyretic properties. LOM, which is commercially available as an 8-mg tablet, is used to treat inflammatory diseases of the joints, osteoarthritis, and pain after surgery, and sciatica. It works by blocking the action of cyclooxygenase, an enzyme involved in the production of chemicals, including some prostaglandins, in the body [1, 2].

Methods for analysis of some oxicams by reversed-phase high performance liquid chromatography [3-8], spectrofluorimetric and spectrophotometric methods using 7-chloro-4-nitrobenz- 2-oxa-1, 3-diazole [9] and a voltammetric [10, 11] have been reported in the literature. A literature survey reveals that a spectrophotometric method has been used for analysis of LOM [12]; an LC method has been used for analysis of LOM and its metabolite in plasma and synovial fluid [13], and a liquid chromatographic–electrospray ionization tandem mass spectrometric method [14] has also been used for analysis of LOM.

The objective of the present study was to develop and validate RP-HPLC method for the assay of lornoxicam in pharmaceutical dosage forms.

MATERIALS AND METHODS

Instrumentation: A Waters HPLC system consisting of a Water 2695 binary gradient pump, an inbuilt auto sampler, a column oven and Water 2487 dual wavelength absorbance detector (DAD) was employed throughout the analysis. The data was acquired using Empower 2 software. The column used was Hypersil BDS C_{18} (250×4.6 mm, 5µm). A Bandline sonerex sonicator was used for enhancing dissolution of the compounds. A Digisum DI 707 digital pH meter was used for pH adjustment.

Reagents and chemicals: Lornoxicam was generous gift from Suven Life Sciences Limited, Hyderabad (INDIA). HPLC grade acetonitrile, methanol, ortho phosphoric acid, Ammonium Dihydrogen Phosphate and potassium dihydrogen phosphate were obtained from Rankem, New Delhi, India. High purity deionized water was obtained from a Millipore, Milli-Q (Bedford) purification system.

Chromatographic conditions: The chromatographic elution was carried out in isocratic mode using a mobile phase consisting of acetonitrile and ammonium dihydrogen phosphate buffer (pH 5.5, pH adjusted with ortho phosphoric acid) in a ratio of 50:50 v/v. The analysis was performed at ambient temperature using a flow rate of 1.0 mL/min with a run time of 5 min. The eluent was monitored using DAD at wavelength of 380 nm. The mobile phase was filtered through 0.45 µm micron filter prior to use.

Preparation of stock and standard solutions: A stock solution of LOM (1000 µg/mL) was prepared by accurately weighed 100 mg of LOM reference standard into 100 mL volumetric flask and dissolved in 50 mL deionized water and volume was made up to the mark with deionized water. The stock solution is protected from light using aluminum foil. Aliquots of the standard stock solutions of LOM ware transferred using A-grade bulb pipettes into 100 mL volumetric flasks and solutions were made up to the mark with mobile phase to give the final concentrations of 5-30 µg/mL.

Estimation of lornoxicam from tablet dosage form: To determine the content of LOM in tablets (label claim: 5 mg), 20 tablets were taken and contents were weighed and mixed. An aliquot of powder equivalent to the weight of one tablet was accurately weighed and transferred to 50 mL volumetric flask and was dissolved in 25 mL of deionized water and volume was made up to the mark with deionized water. The flask was sonicated for 25 min to affect complete dissolution.

The solution was filtered through a 0.45 µm micro filter. Suitable aliquot of the filtered solution was transferred into a 100 mL volumetric flask and made up to the volume with mobile phase to yield the concentration of 20µg/mL. The experiments were performed six times under the chromatographic conditions described above. The peak areas were measured at 229 nm and concentration in the sample was determined by comparing the area of sample with that of the standard.

Method validation

Linearity By appropriate aliquots of the standard LOM solution with mobile phase, five working solutions ranging between 5-30 µg/mL were prepared. Each experiment was performed in triplicate according to optimized chromatographic conditions. The peak areas of the chromatograms were plotted against the concentration of LOM to obtain the calibration curve.

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 LOM to which known amounts of standard LOM corresponding to 50, 100 and 150% of label claim were added. The accuracy was expressed as the percentage of analyte recovered by the proposed method.

Precision: Precision was determined as repeatability and intermediate precision, in accordance with ICH guidelines [15, 16]. The intra-day and inter-day precision were determined by analyzing the samples of LOM at concentration of 10, 20 and 30 µg/mL. Determinations were performed with three replicates on the same day as well as on three consequent days.

Reproducibility: The reproducibility of the method was checked by determining precision on a same instrument, analysis being performed by another person in same laboratory. It was analyzing the samples of LOM at different concentration (10, 20 and 30 µg/mL) were determined in triplicate and calculate the amount of drug present in the sample.

Limit of detection and limit of quantification: Limit of detection (LOD) and limit of quantification (LOD) were calculated based on the ICH guidelines [15, 16].

Robustness: The robustness of the method was performed by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from1.0 to 0.9 mL/min and 1.1 mL/min. The organic strength of the mobile phase was varied by ±2%.

System suitability tests: To ensure the validity of the analytical procedure, a system suitability test was established. Data from ten injections of 20 µL of the working standard solution containing 20 µg/mL were used for the evaluation of the system suitability parameters like tailing factor, number of theoretical plates and retention time.

RESULTS AND DISCUSSIONS

A RP-HPLC was proposed as a suitable method for the quantification of LOM in tablet dosage forms. The best chromatographic conditions were adequately selected. The selection of mobile phase and flow rate were made on the basis of peak shape, baseline drift, time required for analysis, economical and the mobile phase consisted of acetonitrile and ammonium dihydrogen phosphate buffer (pH 5.5, adjusted pH with ortho phosphoric acid) in the ratio of 50:50 v/v at flow rate of 1.0 mL/min and analyzed at 380 nm. The retention time observed (2.643) allows a rapid determination of the drug. In Figure 1, a typical chromatogram obtained under these conditions is shown.

The calibration plot of peak area against concentration was linear in the range of 10-50 µg/mL. The calibration data, with their linear regression equation was shown in figure 2. The range of reliable quantification was set at 10-50 µg/mL as no significant difference was observed in the slope of the standard curve in this range. The linear regression data for the calibration curve is indicative of a good linear relationship between peak area and concentration over a wide range. The correlation coefficient was indicative of high significance.

The LOD and LOQ were determined based on a signal-to-noise ratios and were based on analytical responses of 3 and 10 times the background noise, respectively. The LOD was found to be 0.08µg/mL. The LOQ was found to be 0.26µg/mL. The system suitability tests were also carried out to evaluate the reproducibility of the system for the analysis to be performed. The results of system suitability tests are given in Table 1, showing that the parameters are within the suitable range.

The accuracy was assessed from three replicates containing concentration of 15, 20 and 25µg/mL. The recovery of the method, determined by spiking a previously analyzed test solution with addition of standard LOM solution, was found to be in the range of 99.28-100.6%. The values of % recovery and %RSD are listed in Table 2, indicates that the method is accurate.

Precision of the method was measured in accordance with ICH guidelines. Repeatability of the method was determined as intra-day variation while intermediate precision was determined by measuring inter-day variation for triplicate determination of LOM at three different concentrations. The low %RSD values indicate that the method is precise. Reproducibility of the method was performed in the same laboratory on a same instrument which was performed by another analyst. The assay values and low %RSD values indicate that the method is reproducible.

The proposed method was applied to the analysis of marketed formulations and the results obtained are given in Table 3. The blank solution was prepared containing the components indicated in tablet dosage form except the active ingredient. No interference was observed from the tablet excipients. The percentage drug found and low %RSD indicated the suitability of this method for routine analysis of LOM in pharmaceutical dosage forms.

CONCLUSION

The proposed RP-HPLC method is rapid, specific, accurate and precise for the quantification of LOM from its tablet dosage form. The method has been found to be better than previously reported methods, because of its wide range of linearity, use of readily available mobile phase, lack of extraction procedures and low t_R. All these factors make this method suitable for quantification of LOM in tablet dosage forms. The method can be successfully used for routine analysis of LOM in bulk drugs and pharmaceutical dosage forms without interference.

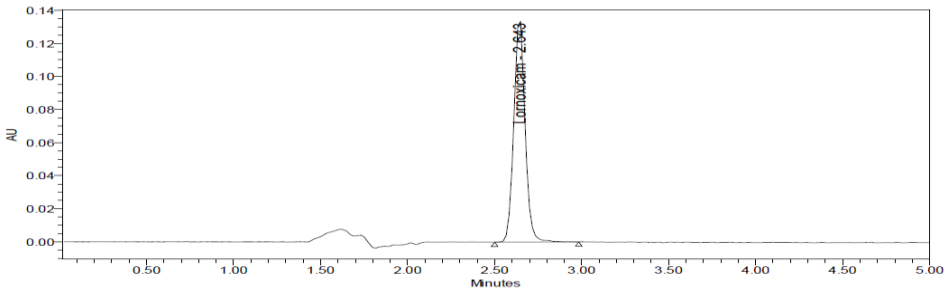

Figure 1: A typical Chromatogram of Lornoxicam

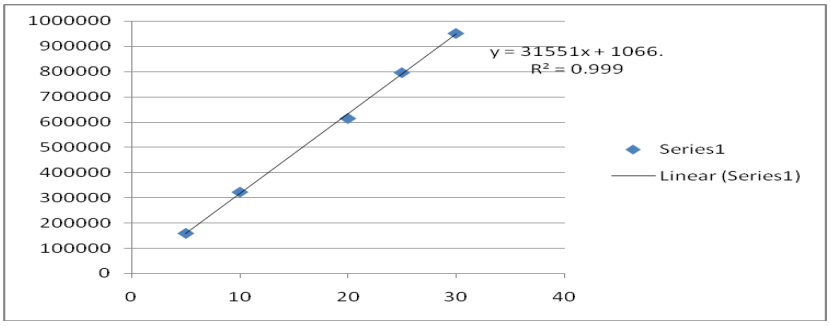

Figure 2: Graph Showing Linearity of Lornoxicam

Table 1: Results of system suitability tests

Parameters	Results
Retention time (min)	2.637
Tailing factor	1.07
Theoretical plates (N)	8186

Table 2: Results of recovery studies

Analyte	Amount (%) of drug added to analyte	Theoretical content (µg/mL)	Conc. found (µg/mL) ± SD (n=3)	RSD (%)	Recovery (%)
LOM	50	15	15.06±0.02	0.134	100.39
	100	20	20.12±0.01	0.05	100.6
	150	25	24.82±0.11	0.442	99.28

Table 3: Analysis of lornoxicam in tablets

Tablet Formulation	Label Claim per Tablet (mg)	% Drug found ± SD (n=6)	RSD (%)	SEM
Brand 1	5	99.58±0.1999	0.201	0.0816
Brand 2	5	100.08±0.2038	0.204	0.0832

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