

DEVELOPMENT AND VALIDATION OF A RP-HPLC-PDA METHOD FOR THE ANALYSIS OF LORNOXICAM IN BULK, TABLETS AND POWDER FOR INJECTION

RAVISHANKER DASHAMUKHI, VIJAYA SRI KANAGALA, AJAYKUMAR CHITTIMALLA

Department of Pharmaceutics, Malla Reddy College of Pharmacy, Maisammaguda, Secunderabad -500 014, Andhra Pradesh, India.
Email: vijayasree 2002@yahoo.co.in

Received: 23 Feb 2013, Revised and Accepted: 05 Apr 2013

ABSTRACT

A simple, precise, accurate and robust RP-HPLC-PDA method was developed and validated for the determination of lornoxicam in tablet dosage forms. Reversed-phase chromatography was performed on thermo scientific C₁₈ (250 mm × 3.4 mm, 3.5 μm) column of Waters HPLC with Empower software with photodiode array detector. Acetonitrile-Water containing phosphoric acid 0.1%, 70:30 (v/v) was used as mobile phase at a flow rate of 1 mL min⁻¹ with PDA detection at 254 nm. Linearity was observed in the concentration range of 1–100 μg mL⁻¹ with regression equation $y = 28492x + 69340$ ($R^2 = 0.999$). The limit of quantitation (LOQ) and limit of detection (LOD) were found to be 0.064 and 0.019 μg mL⁻¹ respectively. The method was validated as per ICH guidelines. The RSD for intra-day (0.22-0.36) and inter-day (0.52-0.79) precision were found to be less than 1%. The percentage recovery was in good agreement with the labelled amount in the pharmaceutical formulations and the method is simple, precise, accurate and robust for the determination of lornoxicam in tablets and powder for injection dosage forms.

Keywords: Lornoxicam; RP-HPLC-PDA; Method validation; Tablets; Powder for injection.

INTRODUCTION

Lornoxicam [6-chloro-4-hydroxy-2-methyl-N-2-pyridyl-5H-thieno [2,3-e]-thiazine-3 carboxamide -1,1-dioxide] is a nonsteroidal anti-inflammatory drug of the oxamic class, with analgesic and anti-inflammatory activities [1]. Due to its short half-life compared to other oxicams, lornoxicam has both high therapeutic potency of oxicams and improved gastrointestinal profile [2]. Lornoxicam is also as effective as other NSAIDs in relieving symptoms of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, acute sciatica and low back pain. It inhibits prostaglandin biosynthesis by blocking the enzyme cyclooxygenase (both COX-1 and COX-2).

Methods for analysis of some oxicams by reversed-phase high performance liquid chromatography (LC) [3-9] 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 lornoxicam [12]; an LC method has been used for analysis of lornoxicam 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 lornoxicam.

The aim of the study was to develop a sensitive, precise, accurate and specific RP-HPLC-PDA method for the determination of lornoxicam in tablets and powder for injection dosage forms. The detector responds were linear in the concentration range of 1-100 μg mL⁻¹ of the drug. The method was validated according to ICH [15, 16] guidelines.

MATERIALS AND METHODS

Materials and Reagents

Pure drug sample of lornoxicam was obtained as a gift sample from Macleod Pharmaceutical Ltd (Mumbai, India). HPLC grade Acetonitrile and water were procured from Merck fine Chemicals (Mumbai, India). Phosphoric acid was purchased from Fine Chem (Mumbai, India). The 0.45 μm pump Nylon filter was obtained from Advanced Micro Devices (Ambala Cantt, India) & whatman no 5 filter paper was obtained from Modern Science lab, (Nashik, India). The drug products of lornoxicam, i.e. LOFECAM tablet (SUN PHARMA, Mumbai, India) and FULACTIVE injection (RANBAXY, Haryana, India) with a label claim of 8 mg drug was purchased commercially. Other chemicals used were analytical or HPLC-grade and glasswares used were Class A grade.

HPLC instrumentation and conditions

Chromatographic separation was achieved by using thermo scientific C₁₈ (250 mm × 3.4 mm) column of Waters HPLC with

Empower software with photodiode array detector, maintained at 25 °C. Isocratic elution was performed using Acetonitrile-Water containing phosphoric acid 0.1%, 70:30 (v/v) at a flow rate 1 mL min⁻¹ with PDA detection at 254 nm. The overall run time was 10 min and 20 μL of sample was injected into the HPLC system.

Preparation of stock solution

Lornoxicam stock solution (1000 μg mL⁻¹) was prepared by accurately weighing 25 mg of lornoxicam in a 25 mL amber volumetric flask and making up to volume with mobile phase. Working solutions for HPLC injections were prepared on a daily basis from the stock solution in a solvent mixture of Acetonitrile-Water containing phosphoric acid 0.1%, 70:30 (v/v) (mobile phase). Solutions were filtered through a 0.45 μm membrane filter prior to injection.

Development of calibration curve

Appropriate aliquots of standard lornoxicam stock solution were taken in different volumetric flasks and the resultant solution was diluted up to the mark with the mobile phase to obtain a final concentration of 1, 5, 10, 20, 30, 50 and 100 μg/mL. Each of the solutions was injected using isocratic mode with PDA detection at 254 nm and flow rate of 1 mL/min⁻¹. From the resultant chromatogram obtained, peak area was used to plot the calibration curve.

Method Validation

The method was validated for the following parameters: linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, system suitability and robustness.

Linearity

Linearity test solutions for the assay method were prepared from a stock solution at different concentration levels of the assay analyte concentration (1-100 μg mL⁻¹). 5 μL of each solution was injected in to the HPLC system and the peak area of the chromatogram obtained was noted. Correlation coefficient (r) of the line, constructed by plotting mean of peak areas against corresponding concentration, was found to be 0.999.

Method validation

Accuracy

To ensure accuracy of the method, recovery studies were performed by standard addition method at 80%, 100% and 120% level to pre-

analyzed samples and subsequent solutions were re-analyzed. At each level three determinations were performed and the results obtained are shown in table 1.

Precision

Precision of the method was determined in terms of repeatability and intra-day and inter-day precisions. Repeatability of the method was determined by analyzing six samples of same concentrations of drug. Chromatographs were recorded and area of each chromatograph was measured. Results of this determination are reported in table II. The intra-day precision of the assay method was evaluated by carrying out 9 independent assays of a test sample of lornoxicam at three concentration levels (1, 20 and 50 $\mu\text{g mL}^{-1}$) (n=3) against a qualified reference standard. The % RSD of three obtained assay values at three different concentration levels was calculated. The inter-day precision study was performed on three different days i.e. day 1, day 2 and day 3 at three different concentration levels (10, 20 and 50 $\mu\text{g mL}^{-1}$) and each value is the average of three determinations (n=3). The % RSD of three obtained assay values on three different days was calculated.

System Suitability parameters

To ensure the validity of the analytical procedure, a system suitability test was established. System suitability parameters for the developed HPLC method were determined by injecting six replicates of the standard solution (30 $\mu\text{g/mL}$). Parameters such as a number of theoretical plates (N), tailing factor and retention time were calculated.

Robustness

The drug solution was subjected to small, deliberate changes like flow rate, wavelength and pH. The results obtained were not affected by varying the conditions and were in accordance with the results in original conditions.

Ruggedness

To determine ruggedness, two different analysts performed assay on marketed tablets of the drug in similar operational and environmental conditions using developed method. The results are summarized in table 2.

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

The limit of quantification (LOQ) and limit of detection (LOD) were based on the standard deviation of the response and the slope of the constructed calibration curve (n=3), as described in International Conference on Harmonization guidelines Q2 (R1).

Solution stability and mobile phase stability

The solution stability of lornoxicam in the assay method was carried out by leaving both the sample and reference standard solutions in tightly capped volumetric flasks at room temperature for 48 h. The same sample solutions were assayed at 12 h intervals over the study period. The mobile phase stability was also assessed by assaying the freshly prepared sample solutions against freshly prepared reference standard solutions at 12 h intervals up to 48 h. The prepared mobile phase remained constant during the study period. The %RSD of the lornoxicam assay was calculated for the mobile phase and solution stability experiments. An additional study was carried out using the stock solution by storing it in a tightly capped volumetric flask at 4°C.

Assay procedure for commercial formulations (Tablets and Powder for injection)

Twenty tablets were weighed accurately and finely powdered. A powder equivalent to 10mg of lornoxicam was transferred carefully

to 100mL volumetric flask and about 30mL diluent was added. The mixture was sonicated for 10 minutes. The volume was made up to 100mL with diluent, filtered through whatman no. 5 filter paper. From the filtrate 10mL was pipetted out and diluted to 100mL with diluent. The final solution was injected in HPLC, chromatogram was recorded and area was measured.

To determine the content of lornoxicam in three batches of injectable powder for reconstitution, solid powders existed in 10 vials of each batch were taken out, powdered d in HPLC, chromatogram was recorded and area was measured and mixed thoroughly. An accurately weighed amount of yellow powder (equivalent to 8 mg of lornoxicam) were then put into 100 ml volumetric flask and completely dissolved in mobile phase. Aliquots of 2.0 ml of this solution were transferred into 10 ml measuring flask and mobile phase was then added to make up the volume. The final solution was injected in HPLC, chromatogram was recorded and area was measured.

RESULTS AND DISCUSSION

Several mobile phases were tried for the analysis. The mobile phase 70:30 (v/v) mixture of Acetonitrile and Water containing phosphoric acid 0.1% showed good resolution and good peak symmetry. By the proposed method, the retention time of lornoxicam was found to be 3.6 minutes. The resulting chromatogram obtained is shown in figure 1.

Linearity was obeyed in the concentration range of 1-100 $\mu\text{g/mL}$ and the correlation 0.999. The regression equation of lornoxicam concentration over its peak area ratio was found to be $y = 28492x + 69340$, where y is the mean peak area and x is the concentration of lornoxicam ($\mu\text{g/ml}$). The calibration curve obtained is shown in figure 2.

The intraday and interday precision studies were carried out and the percent relative standard deviation (% RSD) was calculated and it was found to be 0.45 and 0.98 respectively, which is within the acceptable criteria of not more than 2.0. The results are shown in Table 3.

The System suitability parameters like number of theoretical plates (N) was found to be 3600, tailing factor 0.9 and capacity factor 0.95, which indicates efficient performance of column. The results are shown in Table 4.

The drug solution was subjected to small, deliberate changes like flow rate, wavelength and pH. The results obtained were not affected by varying the conditions and were in accordance with the results in original conditions. This shows the method was robust. Using developed method two different analysts performed assay on marketed tablets of the drug in similar operational and environmental conditions. The results obtained were not affected and were in accordance with the results in original conditions. This shows the method was rugged.

The limit of detection and limit of quantification was found to be 0.103 $\mu\text{g/ml}$ and 0.782 $\mu\text{g/ml}$ respectively, indicates the sensitivity of the method. Satisfactory recovery in the range of 98.5-99.6% is obtained by the proposed method. A good percentage of recovery indicates that the proposed method is accurate.

The percentage of purity of lornoxicam in (tablets and injection) commercial formulations was found to be 99.48 and 99.85. The formulations results are summarized in table 5. The result of assay obtained was found to be in good agreement with the labeled claim, indicating the absence of interference of the excipients.

Table 2: Results of ruggedness studies

	Label claim (mg)	Amount found (mg)	% Label claim	% RSD
Analyst I	8	7.89	98.7	0.022
Analyst II	8	7.94	99.3	0.033

Table 1: Results of Accuracy studies

Spike Level %	Pure drug concentration µg/ml	Formulation concentration µg/ml	Total concentration µg/ml	% Recovered	% RSD (n=3)
80	10	8	17.8	99.0	0.08
80	10	8	17.9	99.5	0.54
80	10	8	17.7	98.4	0.21
100	10	10	19.6	99.0	0.08
100	10	10	19.9	99.8	0.54
100	10	10	19.7	98.4	0.21
120	10	12	21.9	99.8	0.08
120	10	12	21.6	99.0	0.54
120	10	12	22.0	100	0.21

Table 3: Results of intra-day and inter-day precision

Concentration taken (µg/mL)	Intra-day precision		Inter-day precision	
	Concentration found (µg/mL±SD)	%RSD n=3	Concentration found (µg/mL±SD)	%RSD n=3
1	0.99±0.32	0.19	0.98±0.81	0.43
20	20.0±0.11	0.23	19.9±0.43	0.65
50	49.3±0.09	0.32	50.0±0.27	0.98

Table 4: System suitability parameters

Parameter	Results
Relative retention	2.2
Capacity factor	2.4
Number of Theoretical plates	3600
Tailing factor	0.9

Table 5: Assay of lornoxicam in tablets and powder for injection

Formulation	Label claim (mg)	Amount found (mg)	% Label claim	% RSD (n=3)
LOFECAM tablet	8	7.98±0.12	99.7	0.132
FULACTIVE Powder for injection	8	7.91±0.15	99.1	0.117

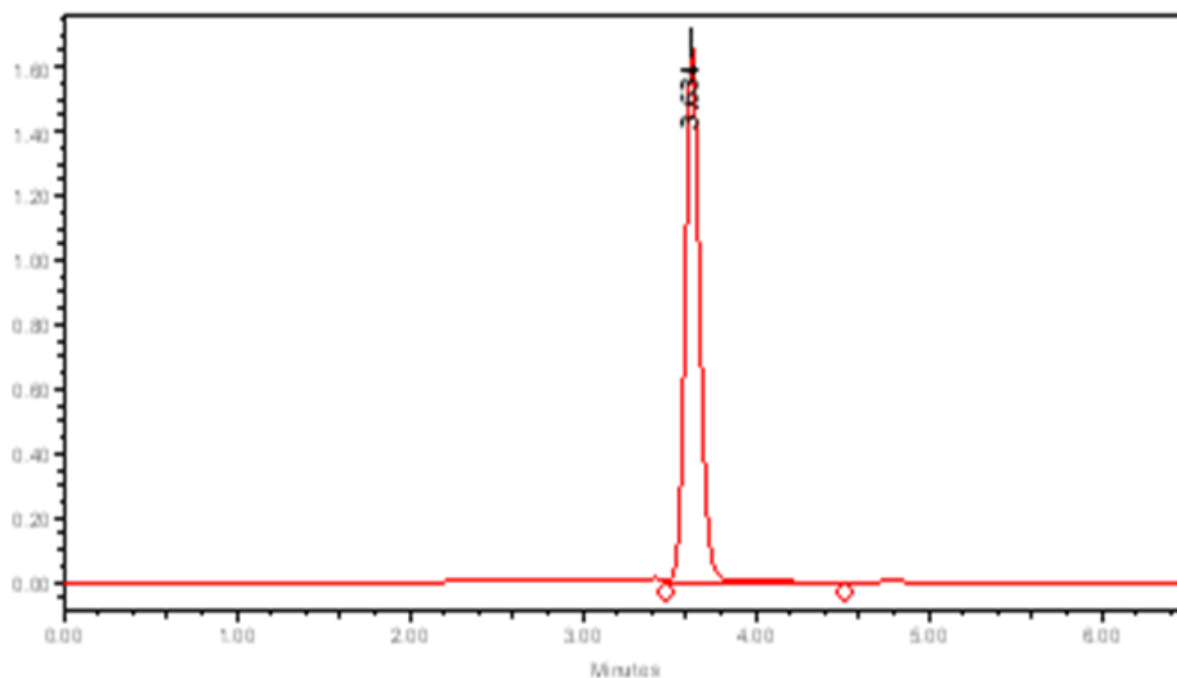


Fig. 1: Chromatogram of lornoxicam

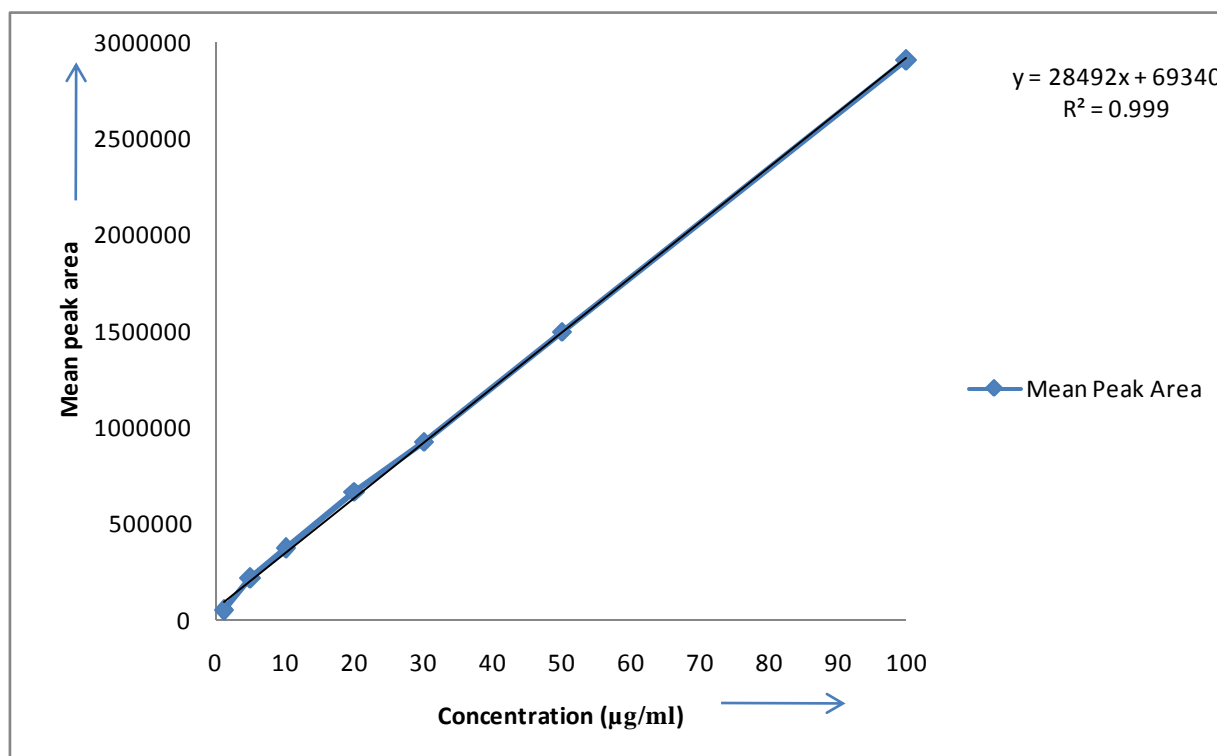


Fig. 2: Calibration curve of lornoxicam

CONCLUSION

The developed HPLC method was found to be simple, precise, accurate and reproducible for the estimation of lornoxicam in bulk and pharmaceutical dosage forms. The proposed method is specific while estimating the commercial formulations without interference of the excipients and other additives. Hence, this method can be used for the routine determination of lornoxicam in pure and pharmaceutical formulations.

ACKNOWLEDGMENT

The authors are thankful to CH. Malla Reddy, Prof. M. Sudhakar, Chairman and Principal of Malla Reddy college of Pharmacy, Hyderabad for providing the facilities to carry out the research work.

REFERENCES

- Balfour JA, Fitton A, Barradell LB. Lornoxicam: A review of its pharmacology and therapeutic potential in the management of painful and inflammatory conditions. *Drugs* 1996; 51:639-657.
- Radhofer-Welte S, Rabasseda X. Lornoxicam, a new potent NSAID with an improved tolerability profile. *Drugs Today* 2000; 36:55-76.
- Wanwimolruk S, Wanwimolruk SZ, Zoset AR. A simple and sensitive HPLC assay for Piroxicam in plasma and its application to bioavailability study. *J Liq Chrom & Rel Technol* 1991; 14(12):2373-2381.
- Owen SG, Roberts MS, Frisen WT. Rapid high-performance liquid chromatographic assay for the simultaneous analysis of non-steroidal anti-inflammatory drugs in plasma. *J Chromatogr* 1987; 416(2):293-302.
- Streete PJ. Rapid high-performance liquid chromatographic methods for the determination of overdose concentrations of some non-steroidal anti-inflammatory drugs in plasma or serum. *J Chromatogr* 1989; 495:179-193.
- Joseph-Charles J, Bertucat M. Simultaneous high performance liquid chromatographic analysis of non-steroidal anti-inflammatory oxicams in pharmaceutical preparations. *J Liq Chrom & Rel Technol* 2005; 22(13):2009-2021.
- Cerretari D, Micheli L, Fiaschi AI, Giorgi G. Rapid and sensitive determination of Piroxicam in rat plasma, muscle and skin by high-performance liquid chromatography. *J Chromatogr* 1993; 614:103-108.
- Mason JL, Hobbs GJ. Simple method for the analysis of tenoxicam in human plasma using high-performance liquid chromatography. *J Chromatogr Analyst Technol Biomed Life Sci* 1995; 665(2):410-415.
- Taha EA, Salama NN, El-S Abdel Fattah. Stability-indicating chromatographic methods for the determination of some oxicams. *JAOAC Int* 2004; 87(2):366-373.
- Bozal B, Uslu B. Applications of carbon based electrodes for voltammetric determination of Lornoxicam in pharmaceutical dosage form and human serum. *Combinatorial Chemistry & High Throughput Screening* 2010; 13:599-609.
- Ghoneim MM, Beltagi AM, Radi A. Wave adsorptive stripping voltammetric determination of the anti-inflammatory drug Lornoxicam. *Anal Sci* 2002; 18(2):183-186.
- Nemutlu E, Demircan S, Kir S. Determination of Lornoxicam in pharmaceutical preparations by zero and first order derivative UV spectrophotometric methods. *Pharmazie* 2005; 60(6):421-425.
- Radhofer-Welte S, Dittrich P. Determination of the novel nonsteroidal anti-inflammatory drug Lornoxicam and its main metabolite in plasma and synovial fluid. *J Chromatogr Analyst Technol Biomed Life Sci* 1998; 707:151-159.
- Kim YH, Ji HY, Park ES, Chae SW, Lee HS. Liquid chromatography electrospray ionization tandem mass spectrometric determination of Lornoxicam in human plasma. *Arch Pharm Res* 2007; 30(7): 905-910.
- International Conference on Harmonization. Guidelines for the photostability testing of new drug substances and products. 1996; step 4, Q1B.
- International Conference on Harmonization. Validation of analytical procedures: text and methodology. In ICH Harmonized Tripartite Guidelines 2005; Q2 (R1).