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Rapid RP-HPLC Method for Quantitative Determination of Lornoxicam in Bulk and Pharmaceutical Formulations

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Abstract: A simple, rapid, and precise method has been developed for quantitative analysis of Lornoxicam (LXM) in pharmaceutical dosage forms. Chromatographic separation of LXM was achieved on a C_{18} analytical column with potassium dihydrogen phosphate buffer - acetonitrile, 70:30 (v/v), as mobile phase at ambient temperature. The flow rate was 1.0 ml/min and detection was by absorption at 291 nm using a photodiode-array detector. The number of theoretical plates and tailing factor for LXM were 6,577 and 1.03, respectively. The linearity of the method was excellent over the range 10–100 µg/ ml LXM. The correlation coefficient was 0.9999. Relative standard deviations of peak areas from six measurements were always less than 2%. The proposed method was found to be suitable and accurate for quantitative analysis of LXM.
Keywords: Lornoxicam, RP-HPLC, method validation.

INTRODUCTION

Lornoxicam (LXM, 6-chloro-4-hydroxy- 2-methyl-N-2-pyridinyl-2H-thieno-[2,3-e]-1,2-thiazine-3-carboxamide 1,1-dioxide; Figure 1) is a novel non-steroidal anti-inflammatory drug (NSAID) in the enolic acid class of compound with analgesic, anti-inflammatory and antipyretic properties [1, 2]. LXM, which is commercially available as an 8-mg tablet, is used to treat inflammatory diseases of the joints, osteoarthritis, 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. All NSAIDs reduce inflammation caused by the body's own immune system and are effective pain killers [3]. Methods for analysis of some oxicams by reversed-phase high-performance liquid chromatography (LC) [4–10], spectrofluorimetric and spectrophotometric methods using 7-chloro-4-nitrobenz- 2-oxa-1,3-diazole [11] and a voltammetric [12, 13] have been reported in the literature. A literature survey reveals that a spectrophotometric method has been used for analysis of LXM [14]; an LC method has been used for analysis of LXM and its metabolite in plasma and synovial fluid [15], and a liquid chromatographic–electrospray ionization tandem mass spectrometric method [16] has also been used for analysis of LXM.

The main purpose of the work presented was to develop simple, rapid, accurate, precise, linear, sensitive, robust and rugged HPLC method for the determination of LXM in bulk drug and pharmaceutical formulations.

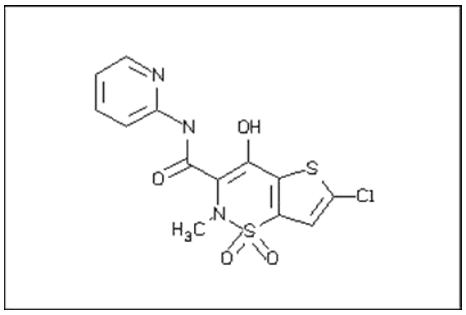


Figure 1: Structure of LXM

EXPERIMENTAL

Materials and reagents

LXM reference standard was obtained from Themis Lab (Mumbai, India), acetonitrile (HPLC grade) from Merck, Potassium dihydrogen orthophosphate (KH_2PO_4) from Qualigens Fine Chemicals (Glaxo,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 product of LXM, i.e. Zion tablet (Unichem Laboratories, Mumbai, India) with a label claim of 8 mg drug was purchased commercially. Milli-Q water was used throughout the work. Other chemicals used were analytical or HPLC-grade and glasswares used were Class A grade.

Equipment

A gradient high-performance liquid chromatograph of an Agilent 1100 series instrument comprising of degasser, quaternary pump, auto injector, column compartment, and variable wavelength programmable UV–Vis detector was used. The system was controlled by Chemstation software. Analytical balance used for weighing was Make-Mettler Toledo, Model-XP 105. Sonarex Super RK 102 (35 KMZ) Bandelin (Berlin, Germany) equipment with thermostatically controlled heating (30–80 ◦C) was used for ultrasonication.

Chromatographic conditions

Chromatography was performed on Hypersil BDS C_{18} (200 X 4.6 mm, 5µm particle) column (LGC Promochem, Banglore, India) at ambient temperature. The isocratic mobile phase was a 70:30(v/v) mixture of potassium dihydrogen phosphate buffer and acetonitrile at a flow rate of 1mL/min. the variable wavelength programmable UV–Vis detector was set at 291 nm.

Preparation of Potassium Dihydrogen Phosphate Buffer

Accurately weighed 6.8 g of potassium dihydrogen phosphate was transferred to a 1000 mL volumetric flask, dissolved in Milli-Q water and volume adjusted up to the mark with the same solvent.

Diluent preparation

Mobile phase and acetonitrile were mixed in the ratio of 1:1

Preparation of standard stock solution and calibration curve

Standard stock solution of concentration 100µg/mL was prepared by dissolving 10 mg of LXM reference standard in diluent and diluting to 100 ml with the same solvent. Aliquots from the stock solution were diluted with the diluent to give the solutions in the concentration range 10-100 µg/mL. The solutions were injected in HPLC and area was measured for each solution. The calibration curve was obtained by plotting peak area on ordinate against drug concentration on abscissa. Linear regression data is shown in table 1.

Preparation of Sample solution for Assay

Twenty tablets were weighed accurately and finely powdered. A powder equivalent to 10mg of LXM 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. Results obtained are summarized in table 2.

Table 1: Linear regression data of calibration graph

Linearity range (µg/mL)	10-100
Slope	37.16
Y-intercept	0.934
Correlation coefficient (r)	0.9999

Table 2: Assay of LXM in tablets

Label claim (mg)	8
Amount found (mg) ± SD	7.95 ± 0.018
% label claim	99.39 %
% RSD (n=6)	0.229

Method validation

The developed method was validated as per ICH guidelines [17,18].

Linearity

Linearity was studied in the concentration range of 10-100µg/mL. All measurements were repeated three times for each concentration. Correlation coefficient (r) of the line, constructed by plotting mean of peak areas against corresponding concentration, was found to be 0.9999.

Specificity

The optimized solvent system yielded a symmetric peak for the drug with Rt 8.184 min (Figure 2). The peak for the drug from tablets was identified by comparing the Rt and also comparing its absorbance spectrum with that obtained with the standard drug. Peak purity values were >999 for the drug product, which shows that the analyte peaks were pure and there were no interferences from formulation excipients in the analyte peak.

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 3.

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 4.

Intra – day and Inter – day Precision: Intra-day precision was determined by analyzing the drugs at three different concentrations and each concentration for three times, on the same day. Inter-day precision was determined similarly, but the analysis being carried out daily, for three consecutive days. The results are summarized in table 5.

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 6.

Table 3: Results of recovery studies

Initial Concentration (µg/mL)	Excess drug added (µg/mL)	Amount recovered (µg/mL ± SD)	% Recovery	% RSD (n=3)
10	8	7.95± 0.081	99.37	1.021
10	10	10.02± 0.015	100.18	0.154
10	12	12.09± 0.035	100.79	0.287

Table 4: Repeatability of the method

Concentration taken (µg/mL)	10
Concentration found (µg/mL±SD)	9.97± 0.025
% RSD (n=6)	0.255

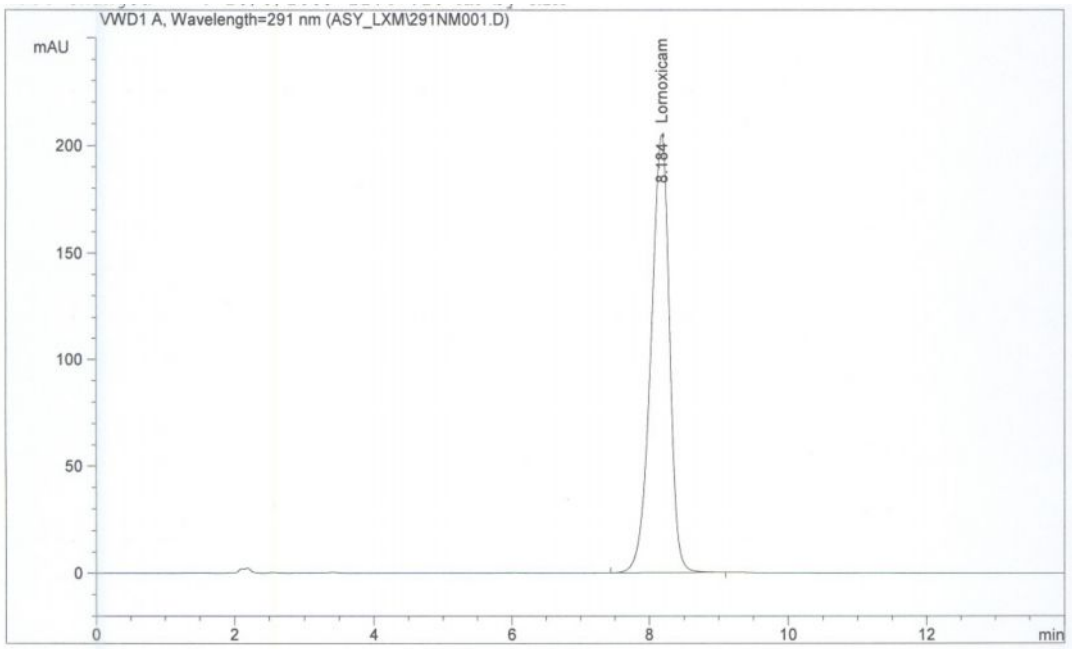
Table 5: Results of intra-day and inter-day precision

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
10	9.94± 0.026	0.263	9.97±0.022	0.217
40	39.82± 0.084	0.212	39.94±0.089	0.222
90	89.93± 0.341	0.380	89.69±0.373	0.416

Table 6: Results of ruggedness studies

Parameter	Analyst I	Analyst II
Label claim (mg)	8	8
Amount found (mg)	7.97	7.98
% Label claim	99.57	99.76
% RSD	0.264	0.224

Figure 2: Chromatogram of standard LXM



RESULTS AND DISCUSSION

Several mobile phases were tried for the analysis. The mobile phase 70:30 (v/v) mixture of potassium dihydrogen phosphate buffer and Acetonitrile showed good resolution and good peak symmetry (Figure 2). *R*t of LXM was found to be 8.184 min.

Variable columns were used such as C18 (YMC, Alltima, and ACE) and CN (YMC and Alltima), a u Bondapack 5um (300 X4.6 mm), Sherisorb ODS 5um (200 X4.6). Hypersil 5um BDS C18 (200 X4.6 mm) was selected for the analysis as it showed minimum elution time with good resolution.

Linearity was observed in the concentration range of 10-100 µg/mL, correlation coefficient (r) being 0.9999. The %RSD for intraday and inter-day precision was ranged between 0.212-0.380% and 0.217-0.416% respectively. The recoveries were ranged between 99.37-100.79%.

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