WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 2, 28-35. Research Article ISSN 2277– 7105

SIMPLE, FAST AND RELIABLE REVERSED PHASE HPLC METHOD FOR LORNOXICAM ANALYSIS IN PHARMACEUTICAL FORMULATIONS

Ehab I. Taha, Saleh A. Al-Suwayeh and Gamal M. Mahrous*

Department of Pharmaceutics, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia.

Article Received on
27 Nov. 2018,

Revised on 17 Dec. 2018,
Accepted on 07 Jan. 2019

DOI: 10.20959/wjpr20192-14030

***Corresponding Author**
Dr. Gamal M. Mahrous
Department of Pharmaceutics, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia.

ABSTRACT

Analysis of active ingredients is very important process during drug development and formulation. In this work a simple, fast, economic and reliable analysis method of lornoxicam was developed and validated regarding linearity, specificity, sensitivity, precision and accuracy. limit of detection and limit of quantification were found to be The method was proven to be 0.122 and 0.061 µg/ml. the method consumed very little amount of mobile phase (about 4 ml per each sample). Also the developed analytical method of LOR was very fast which was indicated by a very small retention time (about 3.6 minutes). The analytical method was tested for detection of lornoxicam in commercial Xefo® 8 mg tablets and it was proven to be very effective.

KEYWORDS: Lornoxicam, RP-HPLC, Analysis.

INTRODUCTION

Lornoxicam (LOR) chemically is: (3E)-6-chloro-3-[hydroxyl (pyridin-2-ylamino) methylene]-2-methyl-2,3-dihydro-4H-thieno[2,3-e][1,2]thiazin-4-one 1,1-dioxide. It is a poor water soluble yellow crystalline powder with a pK_a of 4 and has relatively low lipophilicity. It has molecular weight of 371.82 D, Partition coefficient of 1.8 and melting point of 225-230°C with decomposition (Helmy et al 2017, Barr, 2002).

LOR is one of the oxicam class non- steroidal anti-inflammatory drug (NSAID). It has demonstrated clinical efficacy in relieving chronic pain associated with osteoarthritis,

rheumatoid arthritis, dental pain and ankylosing spondylitis (Garg et al 2017, Joseph 2018 Li et al 2018; Yehia et al., 2018). In the treatment of postoperative pain, LOR has been shown to be as effective as morphine (Wertz, 2000).

An in vitro study suggested that LOR is 100 times more potent than tenoxicam as a cox inhibitor and its analgesic potency is 12 and 10 times greater than of piroxicam and tenoxicam, respectively (Cevik et al 2012; Garg et al 2017).

LOR analgesic potency of 16 mg intramuscular is comparable with that of 20 mg morphine (intramuscular) or 50 mg tramadol intravenous (Jain et al., 2017).

Moreover, LOR shows better gastrointestinal tract tolerability compared to other NSAIDs which is extremely advantageous in terms of fewer side effects (Aabakken et al, 1996).

According to the biopharmaceutical classification system LOR is a class II drug, characterized by low solubility–high permeability. Drug dissolution is the rate-controlling step in drug absorption (Williams, 2003).

The objective of this work was to develop and validate a simple, low cost, rapid, accurate, precise and reproducible RP-HPLC method coupled with UV detection for the rapid analysis of LOR utilizing 0.1 M Sodium acetate solution: methanol (in a ratio of 1:1 v/v) as mobile phase taking into consideration a variety of ICH recommended test conditions.

MATERIALS AND METHODS

Chemicals and reagents

LOR was kindly supplied by Al-Jazeera pharmaceutical industries company, Riyadh, Saudi Arabia. Methanol was obtained from BDH chemicals ltd., poole, England. Sodium acetate was obtained from Sigma-Aldrich, St Louis MO., USA. Xefo® 8 mg tablets, Takeda UK. All other chemicals were of reagent grade and all solvents were of HPLC grade.

Mobile phase

The mobile phase composed of isocratic mixture of methanol and 0.1 M Sodium acetate solution in a ratio of 1:1 v/v. The mobile phase was freshly prepared on each day of analysis, filtered through 0.45 µm Millipore filter and degassed by sonication before conducting the experiment.

Stock solution

Stock solution was prepared by dissolving 50 mg of LOR in 50 ml of the mobile phase solution. The obtained stock solution has the concentration of 1000 µg/ml.

Standards solution

Standard calibration curve of LOR was constructed using 12 standard concentrations of LOR ranging from 0.122 to 250 µg/ml. The standard solutions were prepared from the stock solution and diluted by the mobile phase.

Quality control samples

Five quality control samples were prepared form the stock solution (0.65, 1.3, 5.21, 20.83 and 83.33 µg/ml)

Instrumentation and chromatographic conditions

For the analysis of LOR, 10 µl of each standard solution was injected into HPLC Waters system consisting of Autosampler, model no. 717 plus, pump model no. 1525, Dual λ Absorbance UV detector, model no. 2487, was adjusted at 378 λ and for LOR separation Nova-Pak® C_{18}, 4 µm, 3.9 × 250 mm column was used. The column temperature was kept at 29°C during the analysis and the flow rate was adjusted at 1 ml/min. LOR eluted at 3.4 minutes only.

Validation studies

The developed RP-HPLC method was validated according to ICH guidelines for various parameters such as linearity, specificity, sensitivity, precision and accuracy, limit of detection (LOD) and limit of quantification (LOQ) (Haq et. al., 2014).

Linearity

Twelve standard LOR concentrations ranging from 0.122 to 250 µg/ml have been analyzed three times daily on three consecutive days to establish the validation. Standard calibration curve was obtained by plotting LOR concentrations vs. peak areas in order to evaluate the linearity of the assay method.

Selectivity

Selectivity of the developed RV-HPLC method was determined by monitoring and recording the retention time and peak areas of the five quality control samples.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. LOD and LOQ were calculated by using the values of slopes and intercepts of the calibration curves.

Precision and accuracy

Precision of the developed RP-HPLC method was estimated at two different levels i.e. repeatability (intraday precision) and interday (intermediate) precision. Intraday precision of the proposed RV-HPLC method was carried out by quantification of five different concentrations of LOR solution (0.65, 1.3, 5.21, 20.83, and 83.33 µg/ml) three times on the same day while intermediate precision of the proposed RV-HPLC method was determined by reanalyzing the samples on three different days.

Procedure of tablet extraction

Ten tablets of Xefo® 8 mg tablet were crushed into fine powder and added to 50 ml of mobile phase and subjected to shaking at room temperature for one hour to allow complete disintegration of excipients. The mixture was centrifuged for 5 minutes at 5000 rpm. The supernatant was collected and further diluted with mobile phase and analyzed for LOR content using the over mentioned assay method.

Statistical analysis

IBM® SPSS® statistics (version 19.0.0) software was used to analyze the data. One-way ANOVA plus Post Hoc LSD were applied to compare more than two groups or plus Post Hoc Dunnett to compare groups with a control. A value of $p < 0.05$ was denoted significant throughout the analysis of data. Data are expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Chromatography and Method validation

During method development step, by using a mixture of methanol and 0.1 M Sodium acetate solution in a ratio of 1:1 v/v as the mobile phase a good sharp peak was obtained with a short and appropriate retention time of 3.6 ± 0.05 min (Fig. 1).

Linearity

The linearity of detector response to different concentrations of drugs in standard solution, was studied in the range of 0.122–250 µg/.ml at 12 different concentrations (Table 1). The samples were analyzed in triplicates at all concentrations. Calibration curves were constructed and found that correlation coefficient value of the drug was observed to be 1 (Fig. 2). The regression analysis data for calibration curve were calculated using the peak areas and the data are shown in Table 2.

Selectivity, Precision and accuracy

For selectivity, Precision and accuracy study of the developed assay method five different concentrations were used (0.65, 1.3, 5.21, 20.83, and 83.33 µg/ml). The results shown in table 3 clarify that, high selectivity, Precision and accuracy of the method indicated by the low magnitude of % RSD.

Analysis of LOR in commercial products

From the data obtained after analysis of LOR content in Xefo® tables it was found that the developed method is specific for determination of LOR after extraction from Xefo® Tablets as shown in Figure 3.

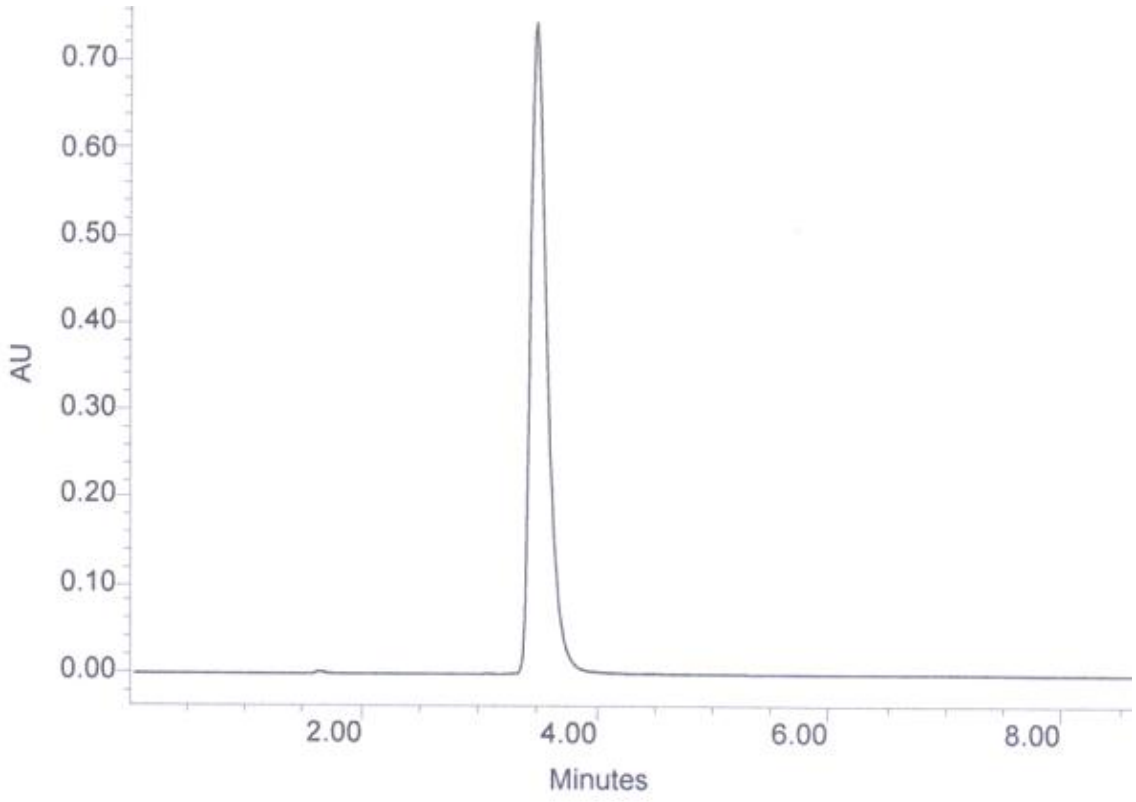

Figure 1: High performance liquid chromatogram of LOR.

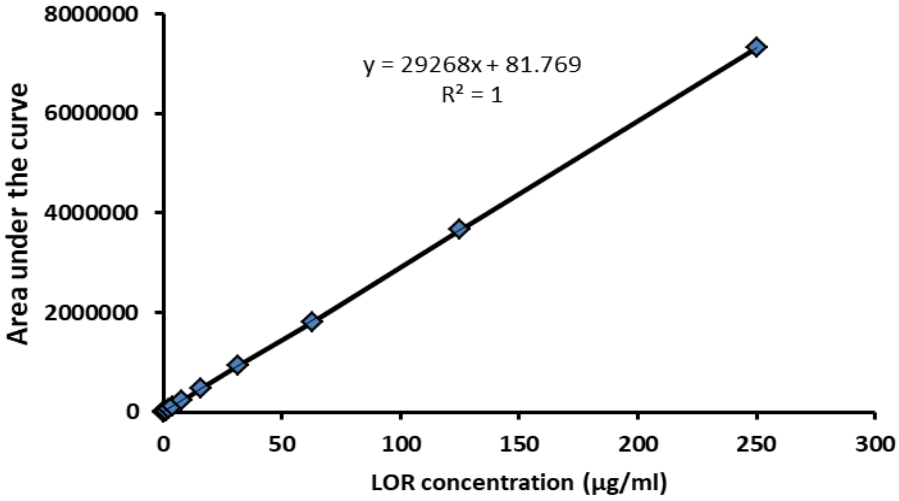

Figure 2: LOR Standard calibration curve.

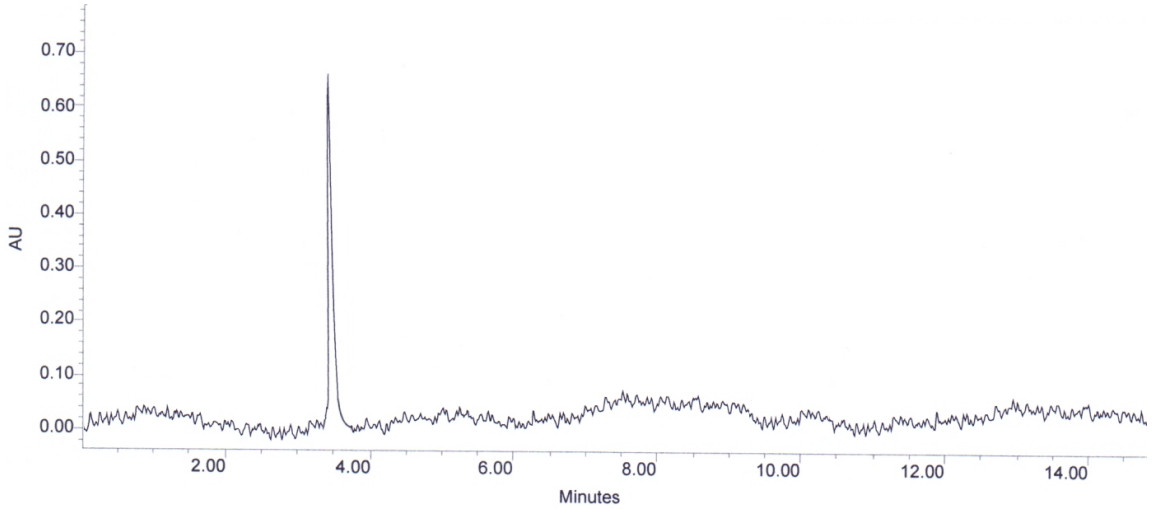

Figure 3: Chromatogram of LOR in commercial tablets (Xefo®).

Table 1: LOR calibration curve.

Conc. (µg/ml)	Average AUC	± SD	RSD %
0.122	2429	84	3.46
0.244	5327	98	1.84
0.488	10734	127	1.18
0.977	24712	347	1.40
1.95	59510	293	0.49
3.91	119359	1111	0.93
7.8125	229623	728	0.32
15.625	466969	932	0.20
31.25	923355	1686	0.18
62.5	1804156	2708	0.15
125	3668670	20256	0.55
250	7316504	25166	0.34

Table 2: Statistical data obtained from the developed assay method.

Parameter	Value
Concentration range	0.122 to 250 µg/ml
Correlation coefficient	1
Slope	29268
Intercept	81.769
LOQ	0.122 µg/ml
LOD	0.061 µg/ml

Table 3: RP-HPLC data for inter-day accuracy and precision of LOR quality control samples.

Assay sample	LOR conc. (µg/ml)		Accuracy (%)	Precision (RSD %)	Retention time (min.)
	Actual	Measured			
Quality control (inter-day)	0.65	0.67 ± 0.05	103.1	7.46	3.6±0.01
	1.3	1.29±0.08	99.2	6.20	3.6±0.02
	5.21	5.27±0.04	101.2	0.76	3.6±0.01
	20.83	21.02±0.03	100.9	0.14	3.6±0.02
	83.33	83.51±0.02	100.2	0.02	3.6±0.02
Quality control (inta-day)	0.65	0.63 ± 0.04	96.9	6.35	3.6±0.01
	1.3	1.24±0.07	95.4	5.65	3.6±0.01
	5.21	5.11±0.05	98.1	0.98	3.6±0.01
	20.83	20.46±0.03	98.2	0.15	3.6±0.01
	83.33	83.24±0.03	99.9	0.04	3.6±0.01
Xefo® 8 mg tablet	8	8.07±0.05	100.9	0.62	3.6±0.03

CONCLUSION

The results in this study showed that the developed HPLC method of analysis provides an acceptable assay which could be used in detection of LOR in pharmaceutical formulations. The described method fast, economic and it is sensitive enough to measure as low as 0.244 µg/ml of LOR.

Conflict of Interest

The authors report no conflict of interest related with this manuscript.

ACKNOWLEDGEMENT

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No. RG-1435-80.

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