ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2012; 3(4):1012-1016

ORIGINAL RESEARCH ARTICLE

Method Development and Validation of Lornoxicam in Pharmaceutical Dosage Form by Using UV-Visible Spectrophotometry

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Received 28 May 2012; Revised 12 Aug 2012; Accepted 19 Aug 2012

ABSTRACT

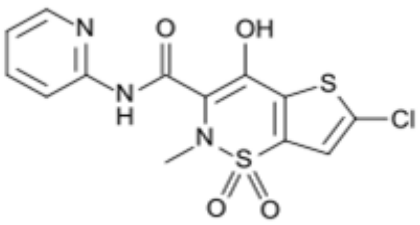
Lornoxicam is a Non steroidal anti inflammatory drug (NSAID) of the oxicam class with analgesic, anti–inflammatory and antipyretic properties. Three simple UV-Visible spectrophotometric methods were developed for the determination of lornoxicam in pharmaceutical dosage form. Method A was UV method, Lornoxicam exhibiting λ max at 377nm in 0.02 N NaOH and obeyed linearity in the concentration range of 5-30µg. The proposed method was statistically validated. The method (B) is based on the formation of chloroform extractable ion-association complexes of lornoxicam with Alizarin red S (Method B) exhibiting absorption maximum at 440nm. Method C is diazotization of Para nitro Aniline (PNA) with sodium nitrate followed by coupling with drug in alkaline medium (PNA Method) exhibiting absorption maximum at 420nm. Linearity ranges and RSD will be 5-30ppm and 0.2106 for Method A and 50-250ppm and 0.495 for Method B and 5-30ppm and 1.023 for method C. All these methods are Accurate, precise and very effective even at low concentrations and used for the quantitative estimation of Lornoxicam in commercial formulations.

Key words: Lornoxicam, uv-visible spectrophotometry methods, 0.02N NaOH, ARS, PNA.

INTRODUCTION

Lornoxicam is a non steroidal anti inflammatory drug (NSAID) .It belongs to oxicam class with analgesic (pain relieving), anti-inflammatory and antipyretic (fever reducing) properties. It works by blocking the action of Cyclooxygenase, an enzyme involved in the production of chemicals, including some prostaglandins in the body [1]. It is available in oral and parentral formulations. It is distinguished from established oxicams by a relatively short elimination half-life (3 to 5 hours), which may be advantageous from a tolerability standpoint [2].

Figure 1: Structure of Lornoxicam



Data from preliminary clinical trials suggest that lornoxicam is as effective as the opioid analgesics morphine, pethidine (meperidine) and tramadol in relieving postoperative pain following gynaecological or orthopaedic surgery, and as effective as other NSAIDs after oral surgery. Lornoxicam was also as effective as other NSAIDs in relieving symptoms of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, acute sciatica and low back pain [3]. Lornoxicam has a tolerability profile characteristic of an NSAID, with gastrointestinal disturbances being the most common adverse events.

The severe or irreversible adverse effects of Lornoxicam, which give rise to further complications include Anemia, Thrombocytopenia, Leucopenia, Hypertension, Hypersensitivity ,Skin reactions, Palpitation [4]. Lornoxicam produces potentially life-threatening effects which include discontinuation of Lornoxicam therapy. The signs and symptoms that are produced after the acute overdosage of Lornoxicam include Nausea, Vomiting, Coma, Ataxia, Dizziness, Coagulopathy, Renal damage.

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Very few analytical methods have been reported for the estimation of Lornoxicam in pharmaceutical formulations [5-11].

MATERIALS AND METHODS

Instrument:

Elico SL-150 Single beam uv-visible spectrophotometer, Lab India UV-3200 double beam uv-visible spectrophotometer was used.

Preparation of reagents:

0.02N NaOH: Dissolve 0.08gm of NaOH in 100ml of distilled water to make 0.02 N NaOH.

ARS solution: 200mg of ARS dissolved in 100ml of distilled water.

0.1M Hcl solution: 8.6 ml concentrated Hcl in 100ml of distilled water.

PNA reagent: Prepared by dissolving 100mg of para nitro aniline in 100ml of 0.2M Hcl.

$NaNO_2$ Solution: Prepared by dissolving 100mg of sodium in (0.4%, 5.796×10^{-2}M) nitrite in 100ml of distilled water.

NaOH 4% 1M: Prepared by dissolving 4g of NaOH in 100ml of distilled water.

Preparation of Standard drug solution:

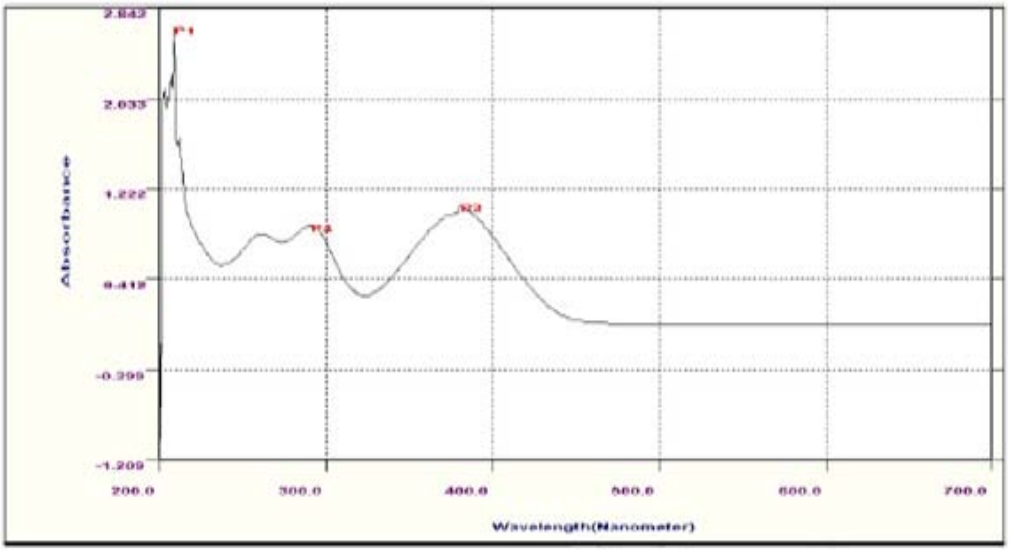
Standard stock solution was prepared by dissolving 100mg of lornoxicam in 100ml of 0.02N NaOH to get concentration of 1000µg/ml solution (stock solution-1). From this required concentrations were prepared by proper dilution from the stock solution.

Method A: UV Method:

Determination of Wavelength maximum:

Standard drug solution was scanned between 400-200nm against 0.02N NaOH solution as blank. The absorption spectra maximum shows at 377nm. The absorption spectrum was shown in (**Fig 2**).

Figure 2: Determination of maximum wavelength of Lornoxicam



Preparation of working standard solution and Construction of calibration curve: The prepared stock solution was further diluted with mobile phase to get working standard solution of 5, 10, 15, 20, 25, and30µg/ml of lornoxicam to construct beer's law plot for the pure drug, the absorbance was measured at λ max at 377nm, against mobile phase as a blank. The standard graph was plotted by taking concentration of drug on X-axis and absorbance on Y-axis in the concentration range of 30mcg. The Results were shown in (**Table 1).**

Table 1: calibration results of Lornoxicam in UV Method (Method A)

S. No	Concentration(µg/ml)	Absorbance
1	5	0.298
2	10	0.499
3	15	0.723
4	20	0.998
5	25	1.248
6	30	1.457

Regression: 0.9991; Intercept: 0.0130; Slope: 0.048352

Method B: ARS Method [12]:

Into a series of 125ml separating funnels, containing aliquots (0.5-3ml) of standard drug solution.6ml of 0.1M hydrochloric acid solution & 2ml 0.2% dye solution were added successively. The total volume of aqueous phase in each separating funnel was adjusted to 15ml with distilled water. To each separating funnel 10ml of chloroform was added & the contents were shaken for 2 minutes. The two phases were allowed to support & absorbance of the separated chloroform layer was measured at λ max 440nm against a similar reagent blank.

Method C: PNA Method [13]:

Into a series of 10ml graduated test tubes 1ml of PNA solution & 1ml of $NaNO_2$ solution were successively added & allowed stand for 2 minutes. Later aliquots of the standard drug were delivered into the test tubes. The 1.5ml of NaOH solution

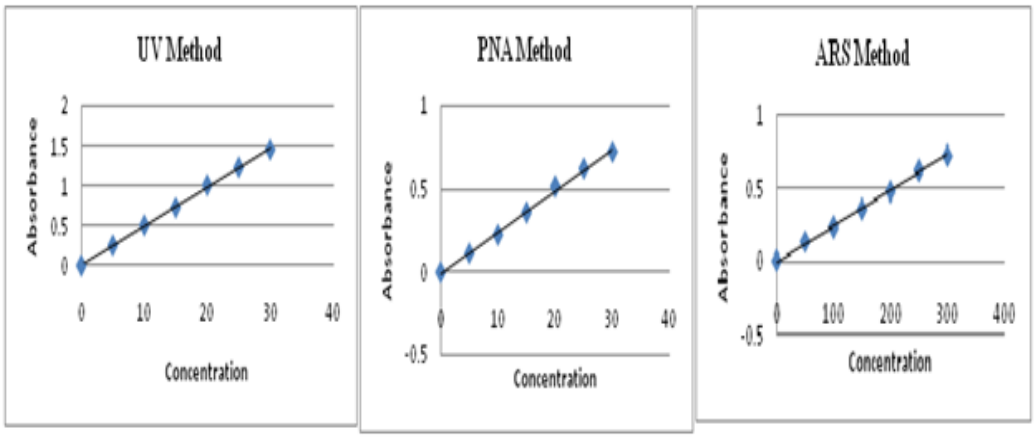
was added & the volume in each tube was made up to 10ml with distilled water. The absorbance was measured at 480nm against a reagent blank.

Validation for the proposed methods:

Linearity:

To establish the linearity of proposed methods, six separate series of solutions of lornoxicam(5-30µg) in diluent were prepared from the stock solutions and analyzed. To construct beer's law plot for the pure drug, the absorbance was measured at λ max at 377nm, 440nm and 480nm for methods A, B, C respectively against blank.

Figure 3: linearity curve of Lornoxicam



Precision:

The precision of the proposed was ascertained by determination of six replicates of same concentration of sample and standard for method precision and system precision. RSD of the precision was calculated and was found to be less than 2, which proves the precision for the proposed methods.

Table 2: System precision results of Lornoxicam

S. No	Absorbance Method A 15(µg/ml)	Absorbance Method B 150(µg/ml)	Absorbance Method C 15(µg/ml)
1	0.723	0.354	0.366
2	0.721	0.352	0.361
3	0.721	0.354	0.355
4	0.722	0.353	0.365
5	0.723	0.356	0.359
6	0.719	0.351	0.365
SD	0.001516	0.001751	0.004308
%RSD	**0.21**	**0.50**	**1.19**

Table 3: Method precision results of Lornoxicam

S. No	Absorbance Method A 15(µg/ml)	Absorbance Method B 150(µg/ml)	Absorbance Method C 15(µg/ml)
1	0.719	0.356	0.359
2	0.719	0.354	0.356
3	0.720	0.351	0.363
4	0.721	0.352	0.361
5	0.722	0.351	0.356
6	0.720	0.353	0.365
SD	0.001169	0.0019407	0.0036878
%RSD	**0.16**	**0.55**	**1.02**

ACCURACY:

To determine the accuracy of proposed method, recovery studies were carried out by adding different amounts (50%, 100%, 150%) of standard bulk sample of lornoxicam within the linearity range were taken and added to pre analysed formulation of concentration 10mcg and percentage recovery values are calculated. Test solutions should be prepared in triplicates at each spike level and assay should be done as per the test method.

Table 4: Recovery results of Lornoxicam in method A

S. No	Amount of sample (µg)	Amt of Standard added(µg)	Total amt of lornoxicam (µg)	Amt of Lornoxicam found	% Recovery	% Mean Recovery	SD	%RSD
50%	10	5	15	14.896	99.308	99.442	0.014	0.139084
50%	10	5	15	14.917	99.58			
50%	10	5	15	14.9170	99.44			
100%	10	10	20	20.099	100.4	100.2	0.00152	0.1520
100%	10	10	20	20.05	100.2			
100%	10	10	20	20.06	100.1			
150%	10	15	25	25.0408	100.1	99.924	0.00152	0.1249
150%	10	15	25	25.00	100			
150%	10	15	25	24.918	99.672			

Table 5: Recovery results of Lornoxicam in method B

S. No	Amt of sample µg)	Amt of std added (µg)	Total amount of lornoxicam(µg)	Amount Lornoxicam found	% Recovery	% Mean Recovery	SD	%RSD
50%	100	50	150	150.84	100.56	99.72	0.00264	0.74929
50%	100	50	150	149.15	99.45			
50%	100	50	150	148.72	99.15			

100%	100	100	200	196.65	98.32	99.37	0.008736	1.82646
100%	100	100	200	200.4	100.2			
100%	100	100	200	198.7	99.37			
150%	100	150	250	249.19	99.67	99.40	0.001527	0.24656
150%	100	150	250	248.3	99.35			
150%	100	150	250	247.998	99.19			

Table 6: Recovery results of Lornoxicam in method C

S. No	Amount of sample (µg)	Amt of std added (µg)	Total amount of lornoxicam (µg)	Amount Lornoxicam found	% Recovery	% Mean Recovery	SD	%RSD
50%	10	5	15	15.08	100.54	99.99	0.005291	1.4495
50%	10	5	15	15.16	101.09			
50%	10	5	15	14.75	98.35			
100%	10	10	20	19.88	99.41	9.863	0.003214	0.6286
100%	10	10	20	19.92	99.60			
100%	10	10	20	20.117	100.58			
150%	10	15	25	24.87	99.51	99.5566	0.003511	0.56969
150%	10	15	25	25.046	100.16			
150%	10	15	25	24.757	99			

Stability studies:

The sample was subjected for stability studies under room temperature and at refrigerator at 25^0C. Stabilities were studied by performing experiment to check the changes the absorbance with the freshly prepared standard solution. Stability studies were conducted for uv method only.

Table 7: Stability studies results of lornoxicam in UV method

S. No	Time (in min)	Concentratio	Absorbance	% difference w.r.t initial
	0	15	0.723	-
1	12hrs(Room Tem)	15	0.718	0.69
2	12hrs (at 25^0C)	15	0.719	0.55
3	24hrs(Room Tem)	15	0.709	1.93
4	24hrs(at 25^0C)	15	0.716	0.96
5	48hrs(Room Tem)	15	0.702	2.90
6	48hrs(at 25^0C)	15	0.715	1.10

Assay procedure:

Ten tablets (Lornoxi-4) were accurately weighed and average was calculated. The tablets were then crushed to obtain fine powder equivalent to about 4mg of lornoxicam was transferred to 100ml volumetric flask, few ml of solvent was added and shaken for a while and it gives 1000µg/ml. From this 10ml was taken into 100ml volumetric flask make up with 0.02N NaOH it gives 100µg/ml.

From this solution required concentrations was selected and apply same procedure as the method proposed and values are recorded. From the results of the determination it is clear that there is close agreement between the results obtained by the proposed methods and the label claim. These results indicating that there was no significant difference between the proposed methods and the reference methods in respect to accuracy and precision.

Table 8: assay of Lornoxicam tablet

Method	Brand Name	Lable Claim	Amount Prepared	Concen. Obtained	% Purity
Method A	Lornoxi 4	4mg	15µg/ml	14.78	98.53
Method B	Lornoxi 4	4mg	150 µg/ml	148.44	98.96
Method C	Lornoxi 4	4mg	15µg/ml	14.87	99.13

Robustness was carried out by typical variations in analytical conditions at standard concentration and results were found to be under the acceptance criteria. Ruggedness was performed by using six replicate analysis of standard concentration and RSD was calculated and was found to be very low indicating that the proposed methods are valid. LOD and LOQ value are found to be very low indicating that the proposed methods are highly sensitive.

Discussion:

Method A is based on the UV absorption of the drug in 0.02N NaOH solution. The drug shows maximum absorbance at 377nm. In method B, drug being a base, form an ion association complex with acid dye ARS [12]. The formed complex is extractable in to Chloroform from the aqueous phase. The protonated nitrogen positive charge of the drug molecule in acid medium is expected to attack the positive charge of the dye. Hence form a colored complex which is extracted with Chloroform. In method C diazotization of PNA with sodium nitrate fallowed by coupling with drug in alkaline medium. The formed PNA-DRUG complex develop brown color, the developed color can be estimated by using spectrophotometer at a wavelength 440nm [13].

Lornoxicam follow linearity within the concentration range of 5-30 µg/ml for method A and c, 50-300 µg/ml for method B. The observed linearity range fitted well Beer-Lambert's law and corresponding regression coefficient(r=0.999) is an indicating of a high degree of method sensitivity.

The percentage of drug found in formulations and results of analysis shows that the amount of drug was in good agreement with the label claim of the formulation. The %RSD is less than 2 which show that the system and method has good

reproducibility were tabulated in table. The percentage recovery values of pure drug from the analyzed solution of formulation were in between 98 to 102 which indicated the proposed method was accurate. And also reveals that the commonly use excepients and additives in the pharmaceutical formulation were not interfering in the proposed method.

CONCLUSION

The three proposed methods were found to be rapid, economical, accurate and precise for the determination of lornoxicam in bulk drug and in tablets by UV spectrophotometer and visible methods produce comparable results and can be used for precise and accurate analysis of Lornoxicam in its pure and tablet dosage form. Interference studies revealed that the common excipients and other activities usually present in the dosage form did not interfere in three methods. The values of % recovery was close to 100% indicating reproducibility and accuracy of the proposed methods can be successfully employed as a quality control tool for the analysis of lornoxicam in its tablet dosage form and in bulk drug.

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