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Original Article

Development and Validation of a HPTLC Method for the Estimation of Lornoxicam in Bulk Drug and in Tablet Dosage Form

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ABSTRACT

Introduction: Lornoxicam is a selective cyclooxygenase-1 and 2 inhibitor that exhibit anti inflammatory, analgesic and anti pyretic activities. It is used in the management of osteoarthritis, rheumatoid arthritis, postoperative pain and primary dysmenorrhoea. **Aim:** The present work describes a simple, precise and accurate HPTLC method for its estimation as bulk and in tablet dosage form. **Methods:** The chromatographic separation was carried out on precoated silica gel 60 GF254 aluminium plates using mixture of Tetrahydrofuran: Methanol:Ethyl acetate:Ammonia (2:1.5:5.5:0.6 v/v/v/v) as mobile phase and densitometric evaluation of spots was carried out at 377 nm using CAMAG TLC Scanner-3 with win CATS 1.4.1 version software. The experimental factors like band width of spot, chamber saturation time, slit width, solvent front migration etc. were critically studied and optimum conditions were developed. **Results:** The drug was satisfactorily resolved with R_f value 0.34 ± 0.01. The accuracy and reliability of the proposed method was determined by evaluating various validation parameters like linearity (100-1000 ng/band), precision (intra-day RSD 0.05-0.10 %, inter-day RSD 0.04-0.13%), accuracy (98.26–100.60%) and specificity according to ICH guidelines. The drug was also subjected to acid, alkali, oxidation, thermal and photochemical degradation studies and the method was found to be effective in separating the drugs from their degradation products. **Conclusion:** The proposed method is simple, accurate, precise and may be used as a cost-effective quality control tool for routine analysis of Lornoxicam as bulk drug and in tablet formulation.

Key words: Densitometric estimation, HPTLC, Lornoxicam, Precision, Stability, Validation.

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INTRODUCTION

Lornoxicam (LNX) is a potent inhibitor of both COX-1 and COX-2 enzymes that exhibit anti inflammatory, analgesic and anti pyretic activities. The mechanism of the analgesic action is mainly the inhibition of cyclo-oxygenase, which suppresses the synthesis of prostaglandins and thromboxanes thereby reducing pain and inflammation. It is used in osteoarthritis and rheumatoid arthritis; in treatment of postoperative pain and primary dysmenorrhoea. LNX is sparingly soluble in methanol and acetonitrile and insoluble in water. It is highly unstable in solution undergoing rapid oxidation and hydrolytic cleavage, leading to the formation of many degradation products, even on standing at room temperature. Lornoxicam is absorbed rapidly and completely from the gastro intestinal tract. Its bioavailability after oral administration is more than 90%. Maximum plasma concentrations are achieved after about 2 hours and the plasma half life is about 4 hours.[1-3]

Extensive literature survey has revealed that many HPTLC methods have been reported for the estimation of Lornoxicam in combination with other drugs from formulations or from biological fluids, but no method has been reported for the estimation of Lornoxicam alone.[4-7]

MATERIALS AND METHODS

Materials

Lornoxicam working standard was a generous gift sample from Life Care Formulations Pvt. Ltd., Pondicherry. Fixed dose tablets (LORNOXI 4, Hetero Healthcare Ltd.) containing 4 mg of Lornoxicam were procured from the local pharmacy. Silica gel 60 F254 TLC plates (20×10 cm, 200 µm layer thickness; E. Merck, Germany) were used as stationary phase. All chemicals and reagents used were of grade and were purchased from Merck Chemicals Corporation Ltd. Mumbai, India. A CAMAG HPTLC system containing CAMAG Linomat V automatic sample applicator, CAMAG microlitre syringe (100 µL), CAMAG TLC Scanner-3 with winCATS software version 1.4.1 and CAMAG twin-trough chamber (20×10 cm) were used for the present study.

Instrumentation and chromatographic conditions

Chromatography was performed on precoated silica gel 60 F_{254} aluminium TLC plates (10×10 cm, 200 µm layer thickness) as stationary phase. Before use the plates were prewashed with methanol, dried in a current of dry air and activated at 105°C for 20 min. Samples were spotted in the form of bands 6 mm wide, 10 mm apart with a CAMAG microlitre syringe using CAMAG Linomat V automatic sample applicator. The parameters such as Slit dimension 6.00 x 0.45 mm and scanning speed of 20 mm/s were optimized for smooth working of the equipment. A constant application rate of 150 nLs^{-1} was used. Trials were carried out on mobile phases containing water, chloroform, methanol, acetonitrile, ethyl acetate, ammonia in various combinations. Of these, the mixture containing Tetrahydrofuran: Methanol: Ethyl acetate: Ammonia (2:1.5:5.5:0.6 v/v/v/v) were found to be most suitable as it gave sharp distinct peaks. Linear ascending development was performed in a CAMAG twin- trough chamber (20×10 cm) previously saturated with the mobile phase for 30 min. The plates were developed to a distance of 90mm and dried. The densitometric scanning was performed at 377 nm in absorbance mode with CAMAG TLC Scanner-3 and winCATS software version 1.4.1.

Preparation of standard stock solution of Lornoxicam

A stock solution containing 1 mg/mL of Lornoxicam was prepared by dissolving an accurately weighed 10mg of the drug in methanol in a 10 mL volumetric flask. For analysis, 1 mL of the stock solution was further diluted to 10 mL with methanol to obtain a concentration of 0.1 µg/uL. This solution was used for the subsequent trials.

Method Validation

The method was validated in accordance with ICH guidelines. The parameters assessed were linearity, specificity, sensitivity, accuracy, precision, robustness and ruggedness.

Calibration curve and Linearity

A stock solution containing 0.1µg/µL of LNX was prepared using methanol as solvent. Different volumes of stock solution (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 µL) were spotted on a TLC plate to obtain concentrations of 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 ng/band respectively. Peak areas were plotted against the corresponding concentrations to generate the calibration plot. Each response was an average of six determinations.

Three quality control levels, Lower quality control samples (LQC) = 200 ng/band; medium quality control (MQC) = 500 ng/band and higher quality control (HQC) = 900 ng/band samples were determined for various validation studies.

Specificity

Specificity is the ability of the analytical method to measure the analyte response in the presence of interferences including degradation products and related substances. In the present work, the densitograms of the samples were checked for the appearance of any extra peaks due to the presence of excipients. The specificity of the developed method was established by analyzing the solutions containing Lornoxicam pure drug (standard) and Lornoxicam extracted from marketed tablet formulation (LORNOXI- 4). Twenty tablets were accurately weighed and crushed into a fine powder in a mortar. An amount of powder equivalent to 10mg of LNX was extracted with methanol, sonicated for 20 min and diluted to 10 mL with the same solvent. The resulting solution was filtered and 1mL of the filtrate was again made upto 10 mL with methanol. 4µL of Lornoxicam pure drug solution and 4µL of the tablet extract were spotted on the same HPTLC plate under optimized chromatographic conditions. The peak areas were measured at 377 nm. The Rf value and peak area of the Lornoxicam sample was compared with that of Lornoxicam standard.

Sensitivity

The sensitivity of measurement was estimated in terms of Limit of detection (LOD) and Limit of Quantitation (LOQ). LOD and LOQ were calculated by the use of equations LOD= 3.3σ /S and LOQ = 10σ/S, where S is the slope of the corresponding calibration plot and σ is the standard deviation of response.[8]

Accuracy

Recovery studies were performed to check the accuracy of the method, by the addition of a standard drug solution at three different levels (50%, 100% and 150%) to a pre-analyzed tablet sample solution (400ng/ band). The peak areas obtained after extraction were compared with peak areas resulting from standard solutions at the same concentrations. Three determinations were carried out for each concentration level.

Precision

The precision of analytical method is the degree of agreement among the individual test results, when the method is applied repeatedly to multiple sampling of homologous samples. The precision of the method was checked by repeatability of sample application, intermediate precision (intraday, interday) and reproducibility. Sample application repeatability was studied by calculating the percentage relative standard deviation (% RSD) for three determination of peak areas of LNX (400ng per band), performed on the same day.

The intraday and interday precision studies were carried out by estimating the responses of three quality standards of LNX (200, 500, 900 ng per band) in triplicates under the same experimental conditions three times on the same day and on three different days. From the results obtained, the precision was expressed as percentage relative standard deviation (% RSD) from mean intraday and interday assays.

Robustness

Robustness studies were done in triplicate at a concentration level of 400 ng/ spot, by making small changes in chromatographic parameters such as mobile phase composition, mobile phase volume and chamber saturation time.

Forced degradation studies

A stock solution containing 1 mg/mL of Lornoxicam in methanol was used for forced degradation to provide an indication of the stability indicating nature and specificity of the proposed HPTLC method. In all degradation studies the average peak area of Lornoxicam after application (1000 ng/spot) of three replicates was obtained after development and scanning of the plate.[9-11]

Acid degradation

10 mL of the drug stock solution was mixed with 10 mL of 0.1 M HCl and refluxed at 80°C for 2 h in the dark in order to rule out any possible degradation by light. 1 mL of treated solution was then applied to a TLC plate in triplicate (1000 ng/spot) and chromatography was performed as described above.

Alkali degradation

10 mL of the drug stock solution was mixed with 10 mL of 0.1 M NaOH and refluxed at 80°C for 2 h in the dark in order to rule out any possible degradation by light. 1 mL of treated solution was then applied to a TLC plate in triplicate (1000 ng/spot) and chromatography was performed.

Oxidative degradation

10 mg of Lornoxicam was dissolved in 10 mL of methanolic solution of hydrogen peroxide (10%, *v/v*). The solution was kept for 8 h at room temperature in the dark in order to exclude the possible degradative effect of light. 1 mL of the above solution was applied on TLC plate in triplicate (1000 ng/ spot) and chromatography was performed.

Photo degradation

The 10 mg Lornoxicam was dissolved in 10 mL of methanol. The solution was kept exposed to sun light for 8 h. The resultant solution was applied on TLC plate in triplicate (1000 ng/ spot) and chromatography was performed.

Thermal degradation

The powdered drug was stored in an oven at 55^0 C for 3 h. The samples were then dissolved in methanol, diluted suitably and appropriate volumes of resultant solution were applied on TLC plate and densitograms were developed.

RESULTS AND DISCUSSION

For HPTLC method development, initial trials were performed with the objective of selecting adequate and optimum chromatographic conditions. Parameters, such as ideal mobile phase and their proportions, detection wave length and concentrations of the standard solutions were carefully studied. Several solvents were tested in varying proportions. Finally, a mixture of Tetrahydrofuran: Methanol: Ethyl acetate: Ammonia (2:1.5:5.5:0.6 v/v/v/v) was selected as the optimum mobile phase. The retardation factor (R_f) value of LNX was found to 0.34 ± 0.01 in the optimized mobile phase. The densitogram obtained from the standard LNX solution is shown in Figure 1. The developed HPTLC method was validated in terms of linearity, sensitivity, specificity, accuracy, precision, robustness and stability as per ICH guidelines

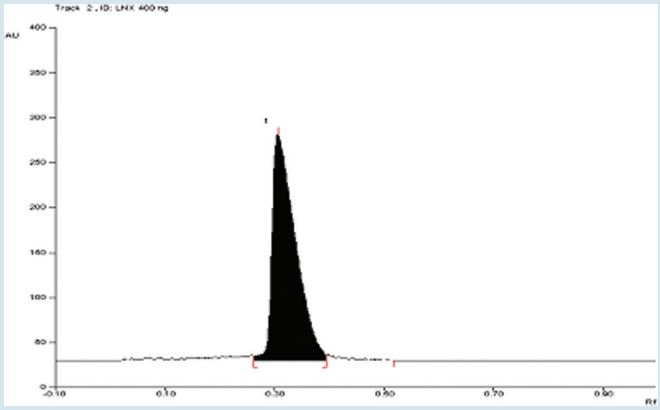

Figure 1: Chromatogram of Standard Lornoxicam-peak of 400 ng (R_f =0.34).

Table 1: Summary of linearity data and sensitivity of Lornoxicam

Parameters	Values for lornoxicam
Linearity range	100-1000 ng/band
Wavelength	377 nm
R_f	0.34
Correlation coefficient (r^2)*	0.995
Slope*	18.619
Intercept*	520.52
LOD*	29.94 ng/band
LOQ*	90.71 ng/band

*Average of 3 determinations

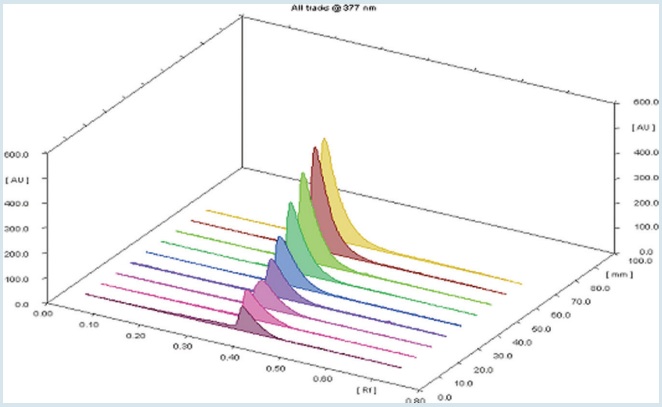

Figure 2: 3 D chromatogram of Calibration linearity.

Calibration curve and Linearity

A calibration curve was constructed by plotting peak area against concentration of Lornoxicam (ng/spot). LNX was well resolved at Rf 0.34. The peak area vs. concentration fitted well to a straight line, with the following equation y = (18.619) x + 520.52. The results of the regression analysis are shown in Table 1. The data confirms the linearity of the standard curve over the range studied (100 -1000ng/band). Linear regression of concentration versus peak area plots resulted in an average correlation coefficient (r^2) of 0.995. The 3-D chromatograms of the calibration concentrations and QC samples are shown in Figure 2 and 3 respectively.

Specificity

The specificity of the method was determined by comparing the spots and Rf value of the marketed formulation with that of the standard drug

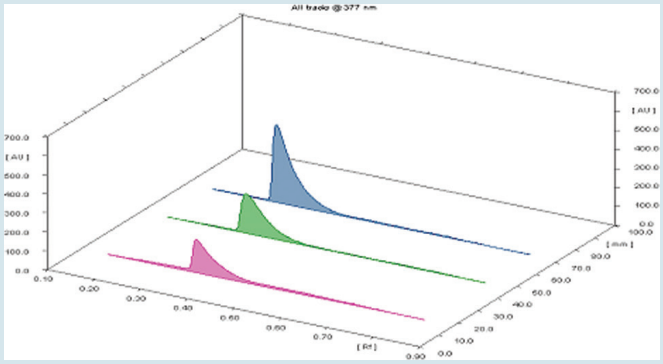

Figure 3: 3 D chromatogram of QC concentrations.

Table 2: Results of specificity study of Lornoxicam

Sample	Conc. (µg/µl)	Vol. spotted (µl)	Conc. (ng/band)	R_f	Area
Lornoxicam Pure drug	0.1	4	400	0.34	10322.8
LORNOXI-4 tablet	0.1	4	400	0.34	9847.7

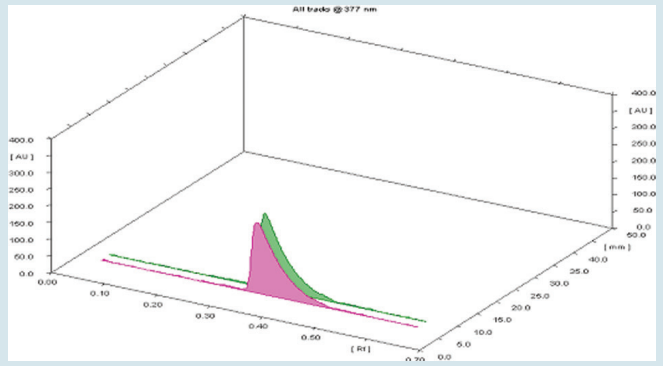

Figure 4: 3D graph of Lornoxicam peaks in standard and tablet samples.

solution. The Rf of formulation and standard drug was found to be 0.34 (Table 2). The excipients present in the formulation did not show any interaction with the peak of Lornoxicam. In addition, the densitogram of drug in the sample solution was found identical to the densitogram of the standard drug solution at the wavelengths applied. These results confirm the specificity of the proposed method. The 3-D graph and the overlay spectra are shown in Figures 4 and Figure 5 respectively.

Sensitivity

Limit of Detection and Limit of Quantification were found to be 29.94 ng/band and 90.71 ng/band respectively. Hence the method was found to be highly sensitive for the determination of LNX. The data for linearity and sensitivity is summarized in Table 1.

Accuracy

Accuracy of the analysis was carried out by carrying out recovery studies at three different levels namely 50, 100 and 150%. The developed method showed high and consistent recoveries at all studied levels. The mean % recovery ranged from 98.26-100.60% (Table 3). The results indicated that the proposed method is accurate of the estimation of drug in tablet dosage form.

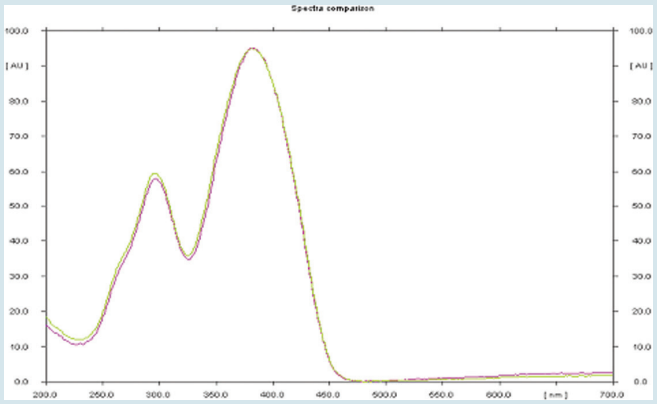

Figure 5: Overlay spectra of standard drug and tablet samples.

Table 3: Results of recovery studies of Lornoxicam

Label claim (mg/tablet)	Standard (LNX) added (%)	Total standard (LNX) added (mg)	Amount recovered (mg)	% Recovery ± SD	% RSD
LORNOXI-4 (4 mg)	50	2	1.965	98.26 ± 0.51	0.51
	100	4	3.95	98.82 ± 0.58	0.59
	150	6	6.03	100.60 ± 0.50	0.50

Table 4: Results of precision studies of Lornoxicam

Concentration (ng/band)	Intraday		Interday	
	Mean peak area* ± SD	% RSD	Mean peak area* ± SD	% RSD
200	5627.93 ± 5.90	0.10	5628.8 ± 7.83	0.13
500	9715.66 ± 6.21	0.06	9609.33 ± 5.10	0.05
900	17554 ± 9.64	0.05	17524.7 ± 7.27	0.04

* Average of 3 determinations

Table 5: Results of robustness studies of Lornoxicam

Parameters	R_f	Mean peak area*± SD	% RSD
Mobile phase composition Tetrahydrofuran: Methanol: Ethyl acetate: Ammonia (1.5:1.5:5.5:0.5 v/v/v/v)	0.29	3653.7 ±7.39	0.20
Mobile phase volume 20 mL	0.34	7723.97 ±5.15	0.06
Chamber saturation time 20 minutes	0.34	7689 ± 5.44	0.07

* Average of 3 determinations

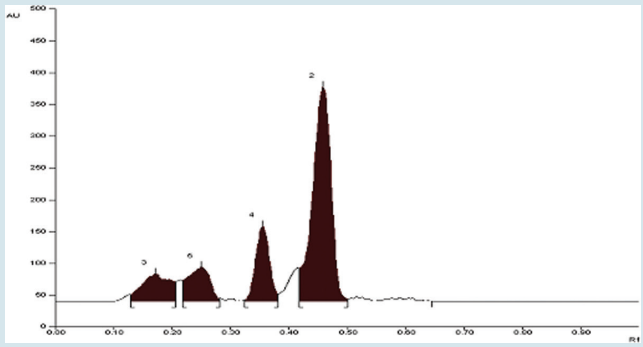

Figure 6: Peaks obtained after acid degradation of Lornoxicam.

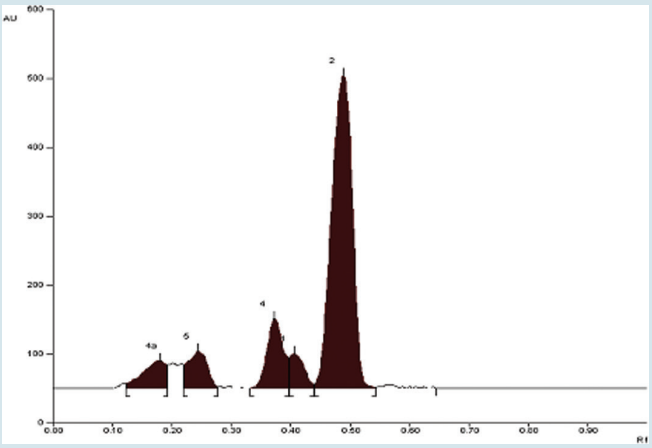

Figure 7: Peaks obtained after alkali degradation of Lornoxicam.

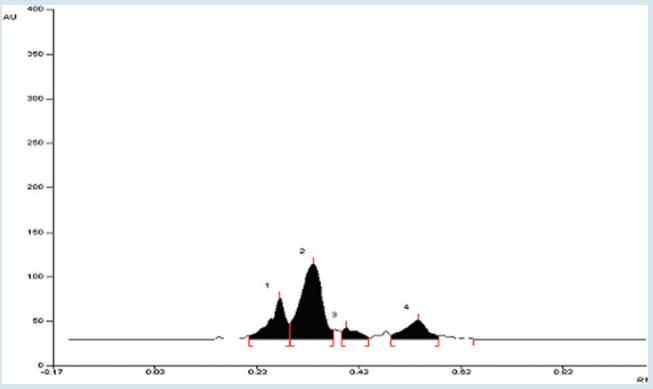

Figure 8: Peaks obtained after oxidative degradation of Lornoxicam.

Table 6: Forced degradation studies

Stress conditions	Number of degradation products (Rf values)	Lornoxicam (R_f)
1 M HCl, 2h, 80₀C, Reflux	3 (0.17,0.25,0.46)	0.35
1 M NaOH, , 2h, 80₀C, Reflux	4 (0.18,0.24,0.41,0.49)	0.37
10 % H_2O_2, 8h, RT	3 (0.28, 0.41,0.55)	0.35
Heat, 3h, 55₀C	1 (0.06)	0.35
Sunlight, 8 h	2 (0.06)	0.37

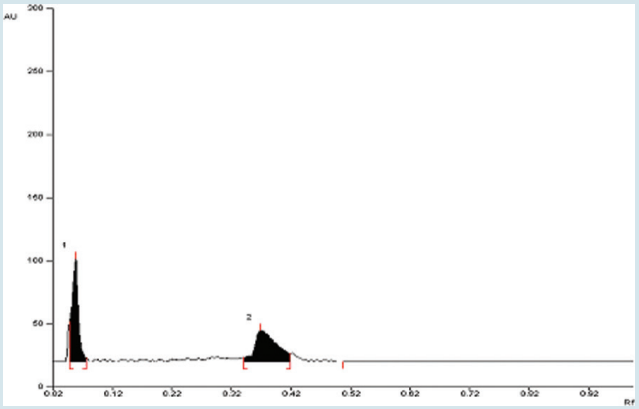

Figure 9: Peaks obtained after photo degradation of Lornoxicam.

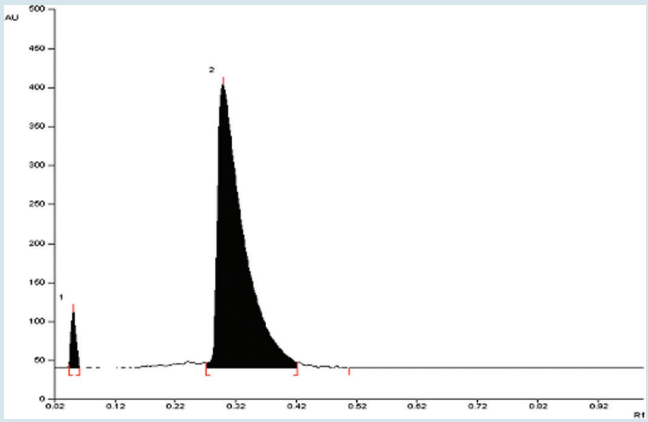

Figure 10: Peaks obtained after thermal degradation of Lornoxicam.

Precision

The precision of the method was checked by repeatability of sample application and intermediate precision (intraday, interday). The precision of the developed HPTLC method was expressed in terms of % RSD. The RSD value for repeatability was found to be 0.05%. The low values of % RSD are indicative of the high repeatability of the method. The results depicted in Tables 4 revealed good accuracy and high precision of the assay method.

Robustness

The results obtained in the new conditions showed slight variation in the Rf values in comparison with the original results. The % RSD values for peak area was less than 1 indicating the highly robust nature of the developed method (Table 5).

Forced degradation studies

The chromatograms obtained from samples degraded by treatment with acid, alkali, hydrogen peroxide, sunlight, and heat contained well separated spots of the pure drugs and some additional peaks at different Rf values. It is apparent from Figures 6–10 that the spots of the degradation products were well resolved from those of the drug. The peaks of lornoxicam were not significantly shifted in the presence of the degradation peaks, which indicated the stability-indicating nature of the method. The Rf values of the degradation products are listed in Table 6.

CONCLUSION

Based on the studies conducted and the results obtained, it can be concluded that the developed HPTLC method is simple, selective, precise, sensitive and accurate, duly developed and validated for the determination of Lornoxicam in both bulk drug and tablet dosage forms. The peaks of the degradation product did not interfere with the peak of drugs and could be resolved easily. Hence this method can be used as a stability indicating method. The method can also minimize the time and cost of analysis and several samples can be analyzed simultaneously. The method does not require expensive chemical and solvents and can be used as an alternative for existing HPLC methods.

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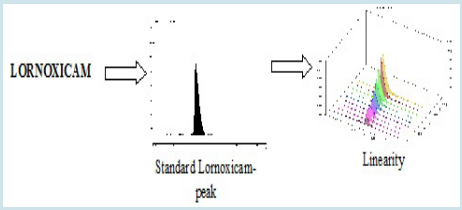
ABBREVIATION USED

COX: Cyclooxygenase; **HPTLC:** High Performance Thin Layer Chromatography; **LNX:** Lornoxicam; **LOD:** Limit of detection; **LOQ:** Limit of Quantitation; **LQC:** Lower quality control; **MQC:** Medium Quality Control; **HQC:** Higher quality control; **ICH:** International Conference on harmonization; **R_f:** Retardation factor; **RSD:** Relative Standard Deviation; **RT:** Room Temperature.

REFERENCES

1. Prasad Byrav DS, Medhi B, Prakash A, Patyar S, Wadhwa S. Lornoxicam: a Newer NSAID. Int J Pharm Med Res. 2009; 20: 27-31.
2. Neil SM, Neal Davies M. Clinical Pharmacokinetics of Lornoxicam- A Short Half-life Oxicam. Clin Pharmacokinet. 1998; 34: 421-28.
3. Mahrous OA, Abdullah AA. Profiles of drug substances, excipients, and related methodology: Lornoxicam. Oxford: Elsevier Inc; 2011. p. 206-237.
4. Dinesh KJ, Nitin D, Yash S. Development and validation of a stability-indicating HPTLC method and HPLC method for simultaneous estimation of lornoxicam and paracetamol in combined tablet dosage forms. Int J Res Pharm Biol Sci. 2011; 2: 55-60.
5. Raja T, Lakshmana Rao A. Validated HPTLC method for simultaneous quantitation of Paracetamol and Lornoxicam in bulk drug and pharmaceutical formulation. Int J Pharm Biomed Res. 2012; 3: 162-66.
6. Shital SB, Santosh VG, Padmanabh BD, Navjot G. High performance thin layer chromatographic determination of lornoxicam in human plasma. J Chem Bio Phy Sci Sec. B 2011; 2: 279-83.
7. Madhusmita S, Pratima S, Asawaree AH, Rahul PR, Vishnu PC, Bhanudas SK. Development and validation of a HPTLC method for simultaneous estimation of lornoxicam and thiocolchicoside in combined dosage form. Pharm Methods. 2011; 2: 178–83.
8. Baghel US, Dhiman V. (2012) Application of hydrotropic solubilization phenomenon for estimating Diacerein in capsule dosage forms by spectrophotometric methods. *Iraqi J Appl Phys.* 2012; 8: 17–23.
9. Monika B, Saranjit S. Development of validated stability-indicating assay methods-critical review. J Pharm Biomed Anal. 2002; 28: 1011–40.
10. Keyur BA, Emanuel MP, Falgun AM. Development of a Validated Stability- Indicating HPTLC Method for Nitrazoxanide in Pharmaceutical Formulation. J Chromatogr Sep Tech. 2012; 3: 1-5.
11. Gaurang PP, Hitendra SJ. Stability Indicating HPTLC Method for Estimation of Modafinil in the Bulk and Tablet Formulation. *IOSR J Pharm Biol Sci.* 2013; 5: 22-28.

PICTORIAL ABSTRACT



SUMMARY

- A simple, precise and accurate HPTLC method was developed for the estimation of Lornoxicam as bulk and in tablet dosage form.
- The drug was satisfactorily resolved with R_f value 0.34 ± 0.01. The accuracy and reliability of the proposed method was determined by evaluating various validation parameters like linearity, precision, accuracy and specificity according to ICH guidelines.
- The method was also found to be effective in separating the drugs from their degradation products.
- The proposed method could be used as a cost-effective quality control tool for routine analysis of Lornoxicam as bulk drug and in tablet formulation.

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