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High Performance Thin Layer Chromatographic Analysis of Paracetamol and Lornoxicam in Combined Tablet Dosage Form

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ABSTRACT

A new simple, accurate, and precise densitometric method for determination of Paracetamol and Lornoxicam in combined tablet dosage form has been developed and validated. Separation of the drugs was carried out using Toluene: Methanolic NaOH: Glacial acetic acid (7: 2: 1.5, v/v/v) as mobile phase on precoated Silica gel 60 F_{254} plates. The retention factors for Paracetamol and Lornoxicam were found to be 0.47 ± 0.02 and 0.67 ± 0.02, respectively. The densitometric evaluation of bands was carried out at 265 nm. The calibration curve was linear in the concentration range 100 to 600 ng/band for both the drugs. The method has been successfully applied for the analysis of drugs in pharmaceutical formulation. The (%) assay (Mean ± S.D.) was found to be 99.93 ± 0.48 for Paracetamol and 100.04 ± 0.68 for Lornoxicam, respectively. The method was validated with respect to linearity, accuracy, precision and robustness as per the International Conference on Harmonisation (ICH) guidelines. The method can be used for routine analysis of these drugs in combined tablet dosage forms in quality-control laboratories.

Key Words: Paracetamol, Lornoxicam, Silica gel, Densitometry, Tablet dosage form.

INTRODUCTION

Paracetamol (PARA), 4-hydroxyacetanilide is a widely-used analgesic and antipyretic drug [1]. Lornoxicam (LORN), chemically, (3*E*)-6-chloro-3-[hydroxy(pyridin-2-ylamino)methylene]-2-methyl-2,3-dihydro-4*H*-thieno[2,3-*e*][1,2]thiazin-4-one 1,1-dioxide is a non-steroidal anti-inflammatory drug (NSAID) of the oxicam class with analgesic, anti-inflammatory and antipyretic properties [2].

Extensive Literature survey reveals High Performance Liquid Chromatographic (HPLC) methods for determination of PARA either in single or in combination with other drugs [3-4]. Spectrophotometric [5-7], High Performance Thin Layer Chromatographic (HPTLC) [8-10] methods for determination of PARA either in single or in combination with other drugs are also

reported. Analytical methods have been reported for the determination of LORN includes HPLC [11-13], Spectrophotometric [14], Polarographic [15] as single component or in combinations with other drugs.

To the best of our knowledge no HPTLC method of analysis has yet been reported for simultaneous analysis of PARA and LORN in combination. This paper describes simple, accurate and precise HPTLC method for simultaneous determination of Paracetamol and Lornoxicam in combined tablet dosage form. The method was optimized and validated as per the International Conference on Harmonization (ICH) guidelines [16].

MATERIALS AND METHODS

Materials

Pharmaceutical grade working standards of PARA and LORN were obtained from Cipla Ltd. (Mumbai) and Ajanta Pharma Ltd.(Mumbai, India), respectively used as such without further purification. The pharmaceutical dosage form used in this study was LOROX- P tablets (Glenmark Generics Ltd., India), labelled to contain 500 mg PARA and 8 mg LORN were procured from the local market. Toluene, Methanol, NaOH, Glacial acetic acid (all AR grade) were obtained from Sisco Research Laboratories (Mumbai, India).

Instrumentation and chromatographic conditions

Silica gel 60 F_{254} TLC plates (E. Merck, Germany) were used as stationary phase. A Camag HPTLC system containing Camag Linomat V semiautomatic sample applicator, Hamilton syringe (100 µl), Camag TLC Scanner-3 with winCATS software version 1.4.2 and Camag twin-trough chamber (10 × 10 cm) were used for the present study.

The plates were prewashed with methanol and activated at 110 ^{0}C for 5 min, prior to chromatography. The slit dimensions 5 mm × 0.45 mm and scanning speed of 20 mm sec^{-1} was employed. The linear ascending development was carried out in 10 cm × 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) using Toluene: Methanolic NaOH: Glacial acetic acid (7: 2: 1.5, v/v/v) as mobile phase, after saturation of the chamber with mobile phase vapor for 20 min. The development distance was 9 cm and the development time approximately 30 min. After chromatography, plates were dried in a current of air with the help of a hair dryer. Densitometric scanning was performed on CAMAG thin layer chromatography scanner 3 at 265 nm for all developments operated by winCATS software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

Preparation of Standard stock solutions

Standard stock solution of PARA and LORN was prepared by dissolving 10 mg of each drug in 10 ml of methanol separately to get concentration of 1 mg/ml from which 1 ml was further diluted to 10 ml to get stock solution of 100 ng/µl of each drug.

Selection of detection wavelength

After chromatographic development bands were scanned over the range of 200-400 nm and the spectra were overlain. It was observed that both drugs showed considerable absorbance at 265 nm. So 265 nm was selected as the wavelength for detection as shown in Figure 1.

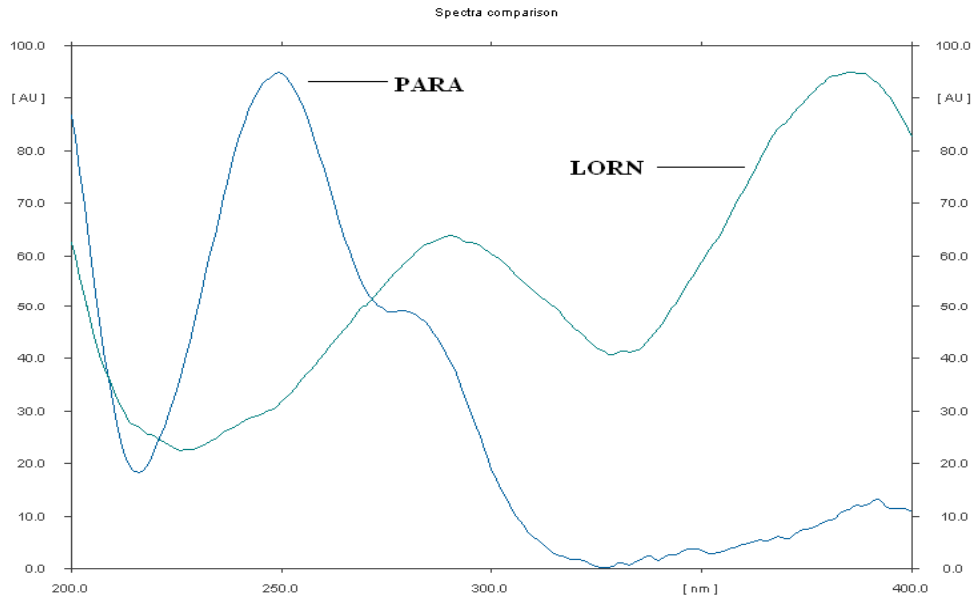

Figure 1: Overlain spectra of PARA and LORN

Analysis of Tablet formulation

Twenty tablets were weighed accurately and finely powdered. A quantity of powder equivalent to 10 mg of PARA was weighed and transferred to a 100 ml volumetric flask containing approximately 60 ml of methanol, sonicated for 10 min, and volume was made up to the mark with the methanol. The solution was filtered through Whatman filter paper No. 41. Two microlitre volume was applied to a TLC plate to furnish final concentration of 200 ng/band for PARA.

For LORN, quantity of powder equivalent to 10 mg was weighed and transferred to a 100 ml volumetric flask containing approximately 60 ml of methanol, sonicated for 10 min, and volume was made up to the mark with the methanol. The solution was filtered through Whatman filter paper No. 41. Two microlitre volume was applied to a TLC plate to furnish final concentration of 200 ng/band for LORN. After chromatographic development the peak areas of the bands were measured at 265 nm and the amount of each drug present in sample was estimated from the respective calibration curves. Procedure was repeated six times for the analysis of homogenous sample.

Method Validation

The method was validated for linearity, accuracy, intra-day and inter-day precision and robustness, in accordance with ICH guidelines [16].

Linearity

The standard stock solutions of PARA and LORN (100 ng/µl each) were applied by overspotting on TLC plate in range of 1 – 6 µl with the help of CAMAG 100 µl sample syringe, using Linomat 5 sample applicator to get concentrations 100, 200, 300, 400, 500 and 600 ng/band. The plate was developed and scanned under above established chromatographic conditions.

Calibration curves of PARA and LORN were plotted separately of peak area vs respective concentration of PARA and LORN.

Recovery studies

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50, 100 and 150 %. Chromatogram was developed and the peak areas were noted. At each level of the amount, three determinations were carried out. The results of recovery studies were expressed as percent recovery and are shown in Table 1.

Table 1: Recovery studies of PARA and LORN

Drug	Amount taken (ng/band)	Amount added (ng/band)	Total amount found (ng/band)	% Recovery*	% RSD
PARA	200	100	301.44	100.48	0.81
	200	200	401.16	100.29	0.46
	200	300	502.85	100.57	0.79
LORN	200	100	302.76	100.92	0.83
	200	200	402.92	100.73	0.89
	200	300	500.3	100.06	0.63

**Average value of three determinations, R.S.D. is relative standard deviation*

Precision

Set of three different concentrations in three replicates of mixed standard solutions of PARA and LORN were prepared. All the solutions were analyzed on the same day in order to record any intra day variations in the results. For Inter day variation study, three different concentrations of the mixed standard solutions in linearity range were analyzed on three consecutive days.

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ for both the drugs were calculated by using the values of slopes and intercepts of the calibration curves.

Robustness studies

In the robustness study, the influence of small, deliberate variations of the analytical parameters on peak area of the drugs were examined. Factors varied were development distance (± 5 %), time from application to development (0, 10, 20, and 30 min) and from development to scanning (0, 30, 60, and 90 min). One factor at a time was changed to study the effect. Robustness of the method was checked at a concentration level of 200 ng/band for both PARA and LORN. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters (% RSD < 2). The results are given in Table 2.

Table 2: Results of Robustness study

Parameters varied	(%) RSD*	
	PARA	LORN
Development distance (± 5%)	0.82	0.46
Time from application to development (min)	0.61	0.39
Time from development to scanning (min)	0.41	0.83

**Average value of three determinations, R. S. D. is Relative standard deviation*

RESULTS AND DISCUSSION

Different mobile phases containing various ratios of Toluene, Methanol, Ethyl acetate, Chloroform, Acetone were examined (data not shown). Finally the mobile phase containing Toluene: Methanolic NaOH: Glacial acetic acid (7: 2: 1.5, v/v/v) was selected as optimum for obtaining well defined and resolved peaks. The optimum wavelength for detection and quantitation used was 265 nm. The retention factors for PARA and LORN were found to be 0.47 ± 0.02 and 0.67 ± 0.02, respectively. Representative densitogram obtained from a mixed standard solution of PARA and LORN is shown in Figure 2.

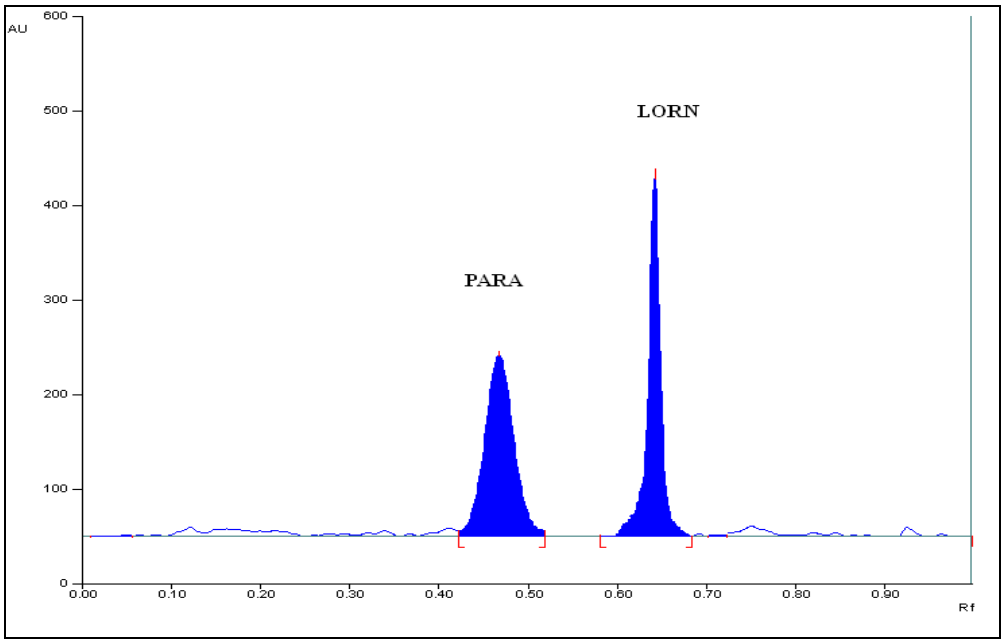

Figure 2: Representative densitogram of mixed standard solution of PARA (R_f = 0.47 ± 0.02) and LORN (R_f = 0.67 ± 0.02)

The results were found to be linear over a range of 100-600 ng/band for both the drugs with correlation coefficient 0.997 for PARA and 0.995 for LORN. The proposed method was also evaluated by the assay of commercially available tablets containing PARA and LORN. The % assay (Mean ± S.D.) was found to be 99.93 ± 0.48 for PARA and 100.04 ± 0.68 for LORN.

For PARA, the recovery study results ranged from 100.29 to 100.57 % with % RSD values ranging from 0.46 to 0.81. For LORN, the recovery results ranged from 100.06 to 100.92 % with % RSD values ranging from 0.63 to 0.89. The method was found to be accurate and precise, as indicated by recovery studies as recoveries were close to 100 % and % RSD not more than 2. Intra-day variation, as RSD (%), was found to be in the range of 0.38–0.70 for PARA and 0.39–0.58 for LORN. Interday variation, as RSD (%) was found to be in the range of 0.47–0.81 for PARA and 0.58–0.68 for LORN. The summary of validation parameters of proposed method are given in Table 3.

Table 3: Summary of validation parameters of proposed method

Parameters	PARA	LORN
Linearity range (ng/band)	100 -600	100 -600
Correlation coefficient (r)	0.997	0.995
LOD[a] (µg/ml)	30.28	32.59
LOQ[b] (µg/ml)	91.75	98.75
Accuracy (% Recovery)	100.29-100.57	100.06-100.92
Precision (% RSD)[c]		
Intra day (n[d] = 3)	0.38-0.70	0.39-0.58
Inter day (n = 3)	0.47-0.81	0.58-0.68

[a]LOD = Limit of detection.; [b]LOQ = Limit of quantitation.; [c]RSD = Relative standard deviation.
[d]n = Number of determinations

CONCLUSION

The validated HPTLC method employed proved to be simple, fast, accurate, precise and robust, thus can be used for routine analysis of PARA and LORN in combined tablet dosage form.

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