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Research Article

A VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF PARACETAMOL AND LORNOXICAM IN COMBINED TABLET DOSAGE FORM

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ABSTRACT

A simple, accurate and precise reverse phase high performance liquid chromatographic method for the simultaneous estimation of Paracetamol and Lornoxicam in combined tablet dosage form has been developed and validated. Chromatographic separation was carried out on Jasco HPLC system equipped with Grace C_{18} column (150 mm×4.6 mm i.d.) and UV/VIS detector using Acetonitrile: 0.04 mM Potassium hydrogen phosphate buffer in the ratio of (60:40, v/v) as mobile phase at a flow rate of 1.0 mL/min and detection was carried out at 270 nm. The retention time for Paracetamol and Lornoxicam was found to be 1.956 ± 0.002 and 3.171 ± 0.018 min, respectively. Results were linear in the range of 2-12 µg/mL for both the drugs. The method has been successfully applied for the analysis of drugs in pharmaceutical formulation. Results of analysis were validated statistically and by recovery studies.

Keywords: Paracetamol, Lornoxicam, RP-HPLC, 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)[3,4], Spectrophotometric[5-8], High Performance Thin Layer Chromatographic (HPTLC)[9-11] methods for determination of PARA either in single or in combination with other drugs. Analytical methods have been reported for the determination of LORN includes HPLC[12-14], Spectrophotometric[15], Polarographic[16] as single component or in combination with other drugs.

To the best of our knowledge no RP-HPLC method of analysis has yet been reported for simultaneous analysis of PARA and LORN in combination. This paper describes simple, accurate and precise RP-HPLC method for simultaneous determination of PARA and LORN in combined tablet dosage form. The method was optimized and validated as per the International Conference on Harmonization (ICH) guidelines[17].

EXPERIMENTAL

Chemicals and reagents

Pharmaceutical grade working standards of PARA and LORN were obtained from Cipla Ltd. (Mumbai, India) and Ajanta Pharmaceuticals 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. Acetonitrile (HPLC grade), Potassium dihydrogen phosphate (AR grade) purchased from Merck specialties Pvt. Ltd. (Mumbai, India) and double distilled water were used in analysis.

Instrumentation and chromatographic conditions

Jasco HPLC system consisting of Jasco PU-2080 plus HPLC pump and UV-2075 plus UV/VIS detector and JASCO Borwin 1.50.8.0 version software was used for analysis. Separation was carried out on Grace C_{18} column (150 x 4.6 mm i.d.) using Acetonitrile: 0.04 mM Potassium dihydrogen phosphate buffer in ratio of (60:40, v/v) as mobile phase at flow rate of 1.0 mL/min. Samples were injected using Rheodyne injector with 20 µL loop and detection was carried out at 270 nm. All weighing were done on Shimadzu balance (Model AY-120).

Preparation of standard stock solutions

Standard stock solutions of pure drugs were prepared separately by dissolving 10 mg of each drug in 10 mL of mobile phase to get concentration of 1000 µg/mL from which one mililitre of solution was further diluted to 10 mL with mobile phase to get a solution having concentration 100 µg/mL. One mililitre of this stock solution was further diluted to 10 mL with mobile phase to get a working standard solution having concentration 10 µg/mL for both drugs.

Preparation of sample solution

Twenty tablets were weighed accurately and powdered. A quantity of tablet powder equivalent to 10 mg of PARA was transferred to 100 mL volumetric flask containing 70 mL of mobile phase and ultrasonicated for 5 min. The volume was made upto the mark with the mobile phase and filtered through Whatman paper No. 41. 0.4 mL of filtrate was further diluted to 10 mL of mobile phase to get final solution of concentration 4 µg/mL. For LORN, powder equivalent to 10 mg was weighed and transferred to 100 mL volumetric flask containing 70 mL of mobile phase and ultrasonicated for 5 min. The volume was made upto the mark with the mobile phase and filtered through Whatman paper No. 41. 0.4 mL of filtrate was further diluted to 10 mL of mobile phase to get final solution of concentration 4 µg/mL. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the tablet sample solution was injected, chromatogram was obtained and the peak areas were recorded. The injections were repeated six times and the amount of each drug present per tablet was estimated from the respective calibration curves.

System suitability

The system suitability was assessed by six replicate injections of the mixture containing 10 µg/ml of both the drugs. The resolution, peak asymmetry, number of theoretical plates and HETP were calculated as represented in Table 1. The values obtained demonstrated the suitability of the system for the analysis of these drugs in combination.

Table 1: System suitability parameters for RPHPLC method

Parameters	PARA	LORN
Theoretical plates	1239.58	2313.46
HETP (cm)	0.121	0.06
Resolution*	1.14	2.9
Asymmetry factor	2.02	1.85

*With respect to previous peak

Method validation

The method was validated for linearity, accuracy, intra-day and inter-day precision and robustness, in accordance with ICH guidelines[17].

Linearity

Aliquots 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 mL of working standard solutions of PARA and LORN were transferred in a series of 10 ml volumetric flasks and the volume was made up to the mark with the mobile phase. Five replicates per concentration were injected and chromatograms were recorded. The peak areas were recorded and calibration curve was plotted of peak area against concentration of drug.

Accuracy

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 %. The percentage of recoveries were calculated, results of which are represented in Table 2.

Table 2: Recovery studies of PARA and LORN

Drug	Amount taken (µg/mL)	Amount added (µg/mL)	Total amount found (µg/mL)	% Recovery*	% RSD
PARA	4	2	6.00	100.00	0.46
	4	4	7.96	99.62	0.43
	4	6	9.99	99.90	0.22
LORN	4	2	5.96	99.48	0.23
	4	4	7.99	99.89	0.31
	4	6	10.02	100.21	0.80

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

Precision

One set of three different concentrations of mixed standard solutions of PARA and LORN were prepared. All the solutions were analyzed thrice, in order to record any intra day variations in the results. For Inter day variations study three different concentrations of the mixed standard solutions in linearity range were analyzed on three consecutive days. The peak areas were recorded and Relative standard deviation (RSD) was calculated for both series of analyses.

LOD and LOQ

LOD and LOQ were calculated as 3.3 σ /S and 10 σ /S respectively, where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

Robustness studies

In the robustness study, the influence of small, deliberate variations of the analytical parameters on retention time of the drugs was examined. The following three factors were selected for change: Flow rate of the mobile phase (0.8 ± 0.05 mL/min), a wavelength at which the drugs were recorded (270 ± 1 nm) .One factor at the time was changed to estimate the effect. The solutions containing 4 µg/mL of both the drugs were applied onto the column. A number of replicate analyses (n = 3) were conducted at 3 levels of the factor (-, 0, +). It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

RESULTS AND DISCUSSION

For RP-HPLC method different mobile phases were tried and the mobile phase containing Acetonitrile: 0.04 mM Potassium dihydrogen phosphate buffer in ratio of (60:40 v/v) was found to be optimal for obtaining well defined and resolved peaks with mean retention times 1.956 **±** 0.002 and 3.171 **±** 0.018 min (Mean ± S.D.) for PARA and LORN respectively. The representative chromatogram of the standard solution of mixture is shown in Fig. 1.

Results were found to be linear in the concentration range of 2-12 µg/mL for both PARA and LORN. The correlation coefficients for the plots were 0.997 for PARA and 0.999 for LORN. The proposed method was also evaluated by the assay of commercially available tablets containing PARA and LORN. The % assay was found to be 99.99 ± 0.65 for PARA and 100.16 ± 0.799 for LORN (mean ± S.D., n = 6). The method was found to be accurate and precise, as indicated by recovery studies and % RSD not more than 2. Robustness of the method (data not shown), checked after deliberate alterations of the analytical parameters shown no marked changes in the chromatograms (RSD < 2), which demonstrated that the RP-HPLC method developed is robust.

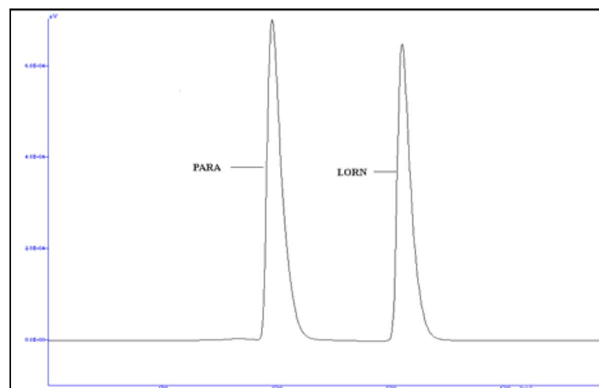

Fig. 1: Representative chromatogram obtained for standard mixture of PARA (10 µg /mL, 1.956 ± 0.002 min), LORN (10 µg/mL, 3.171 ± 0.018 min)

The summary of validation parameters of proposed RP-HPLC method is given in Table 3.

Table 3: Summary of validation parameters of proposed RP-HPLC method

Parameters	PARA	LORN
Linearity range (µg/mL)	2-12	2-12
Correlation co-efficient	0.997	0.999
Slope (m)	59122	65977
Intercept (c)	22158	14748
LOD[a] (µg/mL)	0.3	0.09
LOQ[b] (µg/mL)	0.91	0.29
Accuracy (% Recovery)	99.62 - 100.04	99.48 -100.21
Precision (% RSD) [c]		
Intra day ([d] n= 3)	0.28- 0.68	0.21- 0.73
Inter day (n = 3)	0.29 -0.69	0.24-0.39

[a]LOD = Limit of detection

[b]LOQ= Limit of quantitation

[c]RSD = Relative standard deviation

[d]n = Number of determination

CONCLUSION

The validated RP-HPLC method employed here 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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