Research Article

Current Pharma Research

ISSN: 2230-7842

CPR 3(3), 2013, 879-883.

Simultaneous HPLC Estimation of Paracetamol and Lornoxicam from Tablets.

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Abstract

The present work describes a simple reverse phase HPLC method for the determination of Paracetamol and Lornoxicam from tablet formulations. The determination was carried out on a Phenomenex ODS, C-18 (150 × 4.6 mm, 5 micron) column using mobile phase of acetonitrile: methanol: 0.1M sodium dihydrogen phosphate (pH 3.6) in the ratio of 80:10:10 v/v/v. The flow rate and the run time were 1 ml/min and 10 min, respectively. The eluent was monitored at 260 nm. The method was reproducible, with good resolution between paracetamol and lornoxicam. The detector response was found to be linear in the concentration range of 100 – 500 µg/ml for Paracetamol and 10 – 50 µg/ml for Lornoxicam.

Key Words

RP-HPLC, Paracetamol, Lornoxicam, Estimation.

Introduction

Paracetamol and Lornoxicam are available in tablet dosage form. Chemically Paracetamol (PAR) is N acetyl P-aminophenol. It has antipyretic and analgesic activity. Lornoxicam is (3E)-6-chloro-3-[hydroxy (pyridine-2-ylamino) methylene] 2-methyl-2, 3-dihydro-4H-thieno [2, 3-e][1,2] thiazin-4-one 1, 1-dioxide. It has non-steriodal anti-inflammatory activity. Paracetamol is official in IP[1], BP[2] and USP[3], while Lornoxicam is not official in any Pharmacopoeia, but listed in the Merck Index[4]. Literature survey reveals many analytical methods for determination of Paracetamol such as UV Spectrophotometry[5], HPLC[6-11], and capillary electrophoresis[12] methods from pharmaceutical preparations. Few analytical methods for determination of Lornoxicam using UV Spectroscopy[13,14] HPLC[15,16] and polarography[17] in plasma and pharmaceutical formulation have been reported. The present work describes the development of RP-HPLC method using isocratic mobile phase that offers certain advantages in its simplicity and time saving.

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Materials and Methods

Standard samples of Paracetamol and Lornoxicam, were prepared from reference standard procured from a pharmaceutical company (Burgeon Pharma Ltd, Chennai). HPLC grade

methanol manufactured by E.Merck was procured from commercial sources. Double distilled water was prepared in the laboratory. Tablet formulation, Lorsum Forte (Glenmark Pharmaceuticals, Ahmedabad) containing both Paracetamol and Lornoxicam were obtained from local market. A Shimadzu HPLC (Kyoto, Japan) system coupled with SPD 10A UV detector was used. Separations were carried out on a Phenomenex BDS C18 column (150 × 4.6 mm ID) packed with 5µ particle size as the stationary phase. The mobile phase consisting of acetonitrile, methanol and 0.1 M sodium dihydrogen phosphate (80:10:10) was pumped at a flow rate 1ml/min, the detection was monitored at 260 nm and the run time was 10 min. Paracetamol and Lornoxicam (100 mg each) were weighed accurately in two 100 ml volumetric flasks separately and both standards were dissolved in 30 ml of 0.1M NaOH solution. The volume was made up to 100 ml with water (stock solution). Six different aliquots of solutions ranging from 1-5 ml stock solutions of Paracetamol and 0.1-0.5 ml of Lornoxicam were diluted to 10 ml with distilled water in separate volumetric flasks to get the concentrations ranging from 100 – 500 µg/ml. An aliquot of 20 µl of the solution from each flask was injected two times. Calibration curves were constructed by plotting mean peak areas against corresponding drug concentrations. The detector response was found to be linear in the concentration range of 100-500 µg/ml for Paracetamol and 10-50 µg/ml for Lornoxicam. Twenty tablets were powdered finely. A quantity equivalent to one tablet was transferred to a 100 ml volumetric flask and 30 ml of 0.1M NaOH solution was added. The flask was shaken for 15 min and then contents were diluted to 100 ml and filtered through Whatman No.1 filter paper. Ten ml of this solution was further diluted to 100 ml with distilled water to get a concentration of 1000 µg/ml of Paracetamol and 16 µg/ml of Lornoxicam. This solution was used for further analysis. Results of triplicate analysis are given in Table I.

Results and Discussion

This method was validated for statistical parameters i.e, precision, accuracy, specificity, linearity and range, stability of analytical solutions and ruggedness criteria. Results of the method validation experiments are given in Table 2. The precision of the method was determined by knowing percentage RSD of means of three replicate solutions of all the three independent samples. The accuracy of the method is determined by adding known amount of standard to that of sample (above and below the normal level) at 3 different levels to cover both above and below (75 to 125%) the normal levels expected in the sample. The normal expected level for the assay of Paracetamol and Lornoxicam is about 400 µg/ml and 4.8 µg/ml respectively. So the study range was 300, 400 and 500 µg/ml for Paracetamol and 4.8, 6.4 and 8.0 µg/ml for Lornoxicam. The linearity of analytical method was studied by analyzing response of standard with predetermined concentration range,

linearity curve was plotted for response areas against the concentration of the solution. Regression coefficient was calculated using above plot. For paracetamol the prepared solutions were within concentration range of 100-500 µg/ml at 5 constant consecutive concentration levels i.e. 100, 200, 300, 400 and 500 µg/ml. For Lornoxicam prepared solutions were within concentration range of 10-50 µg/ml at constant consecutive concentration levels i.e. 10, 20, 30, 40 and 50 µg/ml. The regression coefficient of area of above consecutive concentrations was calculated. The stability of analytical solutions was studied by preparing a series of standards and samples and analysed immediately. They were stored at normal lab conditions and in a dark refrigerator, then reanalysed 120 h later against freshly prepared solutions. The ruggedness of analytical method for Paracetamol and Lornoxicam in assay determination was studied by analyzing the samples by two sets. (i.e. different analysts, different reagents and solutions and different days). A typical chromatogram obtained in the present investigation is shown in Figure 1. The results obtained are summarized in Table 1. Prior to the analysis, the method was subjected to system suitability tests. The resolution factor was found to be 3.93, which indicated that there is good resolution between Paracetamol and Lornoxicam. This method is highly sensitive to estimate Paracetamol and Lornoxicam in tablet formulations.

Conclusion

The statistical parameters in method validation studies for precision, accuracy specificity, stability of analytical solutions and ruggedness were justified the validity of the proposed method. The results of assay and method validation studies given in Tables 1 and 2 have shown that the method is simple, accurate, precise and non-interference from tablet excipients.

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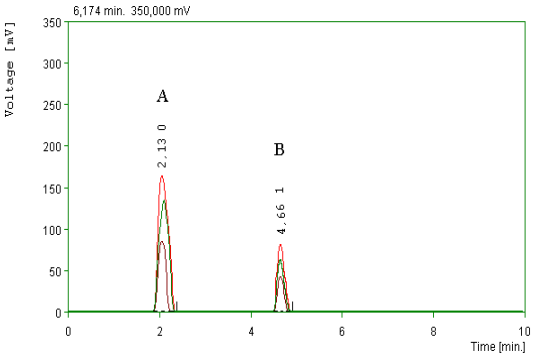

Fig 1: Chromatogram showing Paracetamol (R_t 2.10) and Lornoxicam (R_t 4.63).

Table 1: Analysis of Tablets Containing Paracetamol and Lornoxicam.

Formulation*	Label Content (mg/tab)	Mean amount found** (mg/tab)	Mean % drug**	Standard deviation found (±)
Paracetamol	500	498.2	99.64	1.12
Lornoxicam	8	7.85	98.12	1.23

*Lorsum Forte, Glenmark Pharmaceuticals Ltd, **Mean of three estimations.

Table 2: Results of Method Validation Experiments of Paracetamol and Lornoxicam.

Performance Parameters	Drug	Results	Acceptance Limit
Precision	Paracetamol	1.22	NMT 2.0% RSD
	Lornoxicam	1.73	
Accuracy	Paracetamol	99.30	% Bias NMT 5%
	Lornoxicam	100.34	
Linearity (Regression Coefficient – r)	Paracetamol	0.9998	Linear NLT 0.995
	Lornoxicam	0.9998	
Stability of analytical solutions (normal conditions)	Paracetamol	1.33	NMT 2.0 % RSD
	Lornoxicam	1.41	
Stability of analytical solutions (in a dark refrigerator)	Paracetamol	1.42	NMT 2.0 % RSD
	Lornoxicam	1.44	
Ruggedness	Paracetamol	1.26	NMT 2.0 % RSD
	Lornoxicam	1.36	

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