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A VALIDATED HPLC METHOD FOR ESTIMATION OF LORN AND PCM IN THEIR COMBINED TABLET DOSAGE FORM

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ABSTRACT

The present work describes a reverse phase high performance liquid chromatographic method without use of buffer in mobile phase for the simultaneous estimation of Lornoxicam and Paracetamol in combined tablet dosage form. Chromatography was performed on Chromasil C18 (250 × 4.6 mm i.d. and particle size 5 µm) column in isocratic mode with mobile phase containing acetonitrile: 0.2% triethylamine aqueous solution (adjusted to pH 6.54 using 1 % ortho phosphoric acid) in the ratio of 62:38 v/v. The flow rate was 0.8 ml/min and effluents were monitored at 310 nm. The selected chromatographic conditions were found to be useful in separating Lornoxicam (run time 4.00 min) and Paracetamol (run time 3.05 min). Linearity for Lornoxicam and Paracetamol was found to be in the range of 2-14 µg/ml and 250-875 µg/ml with correlation coefficient of 0.9995 and 0.9991 respectively. Percent recovery of the drugs was found to lie between 99.06-99.64 % and 99.58-100.65 % for Lornoxicam and Paracetamol respectively. The proposed method was validated by different parameters. It was found to be accurate, precise, reproducible and specific and hence can be used for simultaneous analysis of these drugs in combined tablet dosage forms.

INTRODUCTION: Lornoxicam (LORN) is a non steroidal anti inflammatory drug of the oxicam class with the analgesic, anti inflammatory and anti pyretic properties having chemical name (3E)-6-chloro-3-[hydroxy(pyridin-2-ylamino)methylene]-2-methyl-2, 3-dihydro- 4H- thieno [2, 3-e][1, 2]thiazin-4-one 1, 1-dioxide. It has been found to be effective in inflammatory diseases of the joints, osteoarthritis, pain following surgery, sciatica [1]. Unlike other oxicam it has shorter elimination half life of 3-5 hrs [2]. Paracetamol (PCM) common analgesic having chemical name N-(4-hydroxyphenyl) acetamide. PCM/ acetaminophen is used for the relief of fevers, aches, and pains associated with many parts of the body. It has weak anti-inflammatory properties [3-6]. It is combined with LORN in tablet dosage form for the treatment of rheumatism and pain after surgery. Analytical techniques such as Spectrophotometry [7-8], HPLC [9-11], HPTLC [12], LC/MS\MS [13] etc. are reported for the determination of LORN and PCM individually in plasma and bulk pharmaceutical formulations and also some Spectrophotometry [14-15] and HPTLC [16] methods for estimation of LORN and PCM in their combination have been reported. But there is not any HPLC method has been developed for the estimation of LORN and PCM in their combination.

Hence, our object was to develop a simple HPLC method for the estimation of these drugs in combined tablet.

MATERIALS AND METHODS:

Instrumentation: Chromatographic separation was performed on HPLC2010 CHT system, UV detector and Rheodyne injector with 20 µl loop volume.

Materials: LORN and PCM working standards were obtained as a gift sample from Cirex Pharmaceutical Ltd (Mandal, A.P. India) and Cadila Pharmaceutical Ltd(Ahmedabad,Gujarat, India) respectively. LORN and PCM combined tablets (Claiming 8 mg of LORN and 500 mg of PCM per tablet) of two different brands [Lornasafe, mankind Pharma Ltd; Lorsaid-P, Pyramal Healthcare] were collected from market and analyzed for the LORN and PCM content by the proposed method. All the other chemicals and reagents were of analytical grade.

Selection of Detection Wavelength: PCM and LORN were found in the ratio of 500:8 mg in tablet dosage form. As LORN has relatively less concentration in tablet, 310 nm wavelength was selected where it has reasonable absorbance.

Preparation of solution: Standard stock solution of both drugs were prepared by dissolving 1000 mg of PCM and 16 mg of LORN in acetonitrile to obtain 100 ml stock solution (10000:160 µg/ml) and further serially diluted with mobile phase to get final linear concentrations.

Chromatographic Condition: A mobile phase consisting of acetonitrile: 0.2% triethylamine aqueous solution (adjusted to pH 6.54 using 1 % ortho phosphoric acid) in the ratio of 62:38v/v at a flow rate of 0.8 ml/min was used. The mobile phase was then filtered through membrane filter and sonicated for 5 min in ultrasonic bath. Chromasil C18 (250 × 4.6 mm i.d. and particle size 5 µm) column was used.

Method Validation: This optimized HPTLC method was then validated for the parameters listed below as per ICH guidelines [17].

Linearity: Different concentrations of LORN (4-14 µg/ml) and PCM (250-850 µg/mL) were injected and peak area was measured and calibration curve was plotted against drug concentrations.

Precision: Interday and Intraday precision were evaluated by analyzing three concentration (6 µg/ml, 8 µg/ml and 10 µg/ml), three times and % RSD values obtained were calculated to determine any intraday and interday variation.

Accuracy: To check 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 %. Mean percentage recovery was determined.

Specificity and Selectivity: The absence of any secondary spectra different from LORN and PCM in the typical constituted placebo of the tablet preparation, which may interfere with LORN and PCM peak, indicates the specificity of the analytical method.

Limit of Detection and Limit of Quantitation: The limit of detection (LOD) and limit of quantitation (LOQ) were obtained by calculating using the standard formula as per the ICH guidelines,

$$LOD = 3.3(\sigma/S),\ LOQ = 3.3(\sigma/S)$$

Where σ is Standard deviation of the response and S is slope of the calibration curve.

Formulation Analysis: Ten combined tablets of LORN and PCM were finely powdered and powder equivalent to 1000 mg of PCM and 16 mg of LORN was dissolved in acetonitrile to obtain 100 ml stock solution (10000:160 µg/ml). It was sonicated and filtered through 0.45 µm milipore filter and further serially diluted with mobile phase to get final linear concentrations.

RESULTS AND DISCUSSION:

Development of the optimum mobile phase: Different mobile phases were tried to resolve LORN and PCM. The optimum results were obtained with mobile phase consisting of acetonitrile: 0.2% triethylamine aqueous solution (adjusted to pH 6.54 using 1 % ortho phosphoric acid) (62:38 v/v). The Rt value of LORN and PCM peak were observed about 4.00 and 3.05 min respectively. The representative chromatogram is given in (Figure 1).

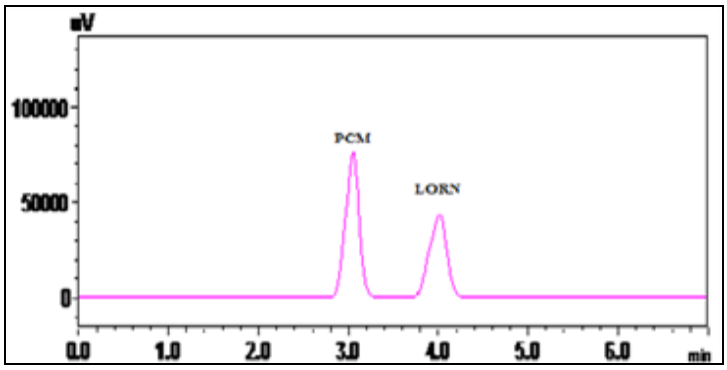

FIG. 1: REPRESENTATIVE CHROMATOGRAM OF LORN AND PCM

Linearity And Range: The responses for the drugs were found to be linear in the concentration range 4-14 µg/ml for LORN and 250-850 µg/ml for PCM. The regression equations were found to be y = 31344x + 3504 and y = 888.5x + 38525 and correlation coefficient were 0.9995 and 0.9991 for LORN and PCM respectively.

Precision: Intraday and interday precision data are listed in table. %RSD value below 2% indicates that method is precise (**Table 1**).

Validation of the method:

TABLE 1: METHOD PRECISION OF THE ANALYTICAL METHOD

Amount in µg/ml		Peak area ± %RSD (n=3) Intraday		Peak area ± %RSD (n=3) Interday	
LORN	PCM	LORN	PCM	LORN	PCM
6	375	193887 ± 0.57	382969 ± 0.62	195537 ± 0.68	383649 ± 0.79
8	500	256319 ± 0.45	490638 ± 0.59	256886 ± 0.63	492005 ± 0.69
10	625	319160 ± 0.49	595479 ± 0.60	319986 ± 0.71	596575 ± 0.73

n= No. of three replicates

Accuracy: Excellent recoveries were obtained at each level of added concentration. Recovery was found to be 99.06-99.64% and 99.58-100.65% for LORN and PCM respectively (**Table 2**).

TABLE 2: RECOVERY STUDY OF THE ANALYTICAL METHOD

Test Concentration		Amount spiked		%Recovery ± %RSD	
LORN	PCM	LORN	PCM	LORN	PCM
4	250	2	125	99.06 ± 0.62	99.58 ± 0.63
4	250	4	250	99.10 ± 0.50	100.65 ± 0.59
4	250	6	375	99.64 ± 0.51	99.81 ± 0.58

Limit of detection: The LOD as calculated by standard formula as given in ICH guidelines was found to be 0.04 µg/ml and 3.48 µg/ml for LORN and PCM, respectively.

Limit of quantitation: The LOQ as calculated by standard formula as given in ICH guidelines was found to be 0.11 µg/ml and 10.5 µg/ml for LORN and PCM, respectively.

Formulation analysis: The % Assay of two different LORN and PCM tablet samples Lornasafe and Lorsaid-P were found to be in the range of 98-102 (**Table 3**).

Specificity: The specificity of the method was ascertained by absence of any other peak of placebo.

Robustness: Robustness of the method was determined by making slight deliberate changes in chromatographic conditions like 1% change in ratio of mobile phase constituents, ± 1nm change in detection wavelength and 10% change in flow rate. It was observed that there were no marked changes in the chromatogram. It suggests that the developed method is robust.

The validation summary is given in **Table 3** and system suitability parameters studied are given in **Table 4**.

TABLE 3: SUMMARY OF VALIDATION

Validation parameter	LORN	PCM
Regression equation	y = 31344x + 3504	y = 888.5x + 38525
Linearity	0.9995	0.9991
Presicion	NMT 2%	NMT 2%
Recovery	98-102%	98-102%
LOD	0.04	3.48
LOQ	0.11	10.5
Specificity	Specific	Specific
%Assaay (Lorsaid-P)	98.83%	100.18%
(Lornasafe-plus)	98.23%	99.45%

TABLE 4: SYSTEM SUITABILITY PARAMETERS

Parameter	LORN	PCM
Rt	4.00 min	3.05 min
Tailing factor	0.90	0.94
HETP	1832	1629
Resolution	2.81	--

Rt- Retension time, HETP- Height equivalent to theoratical plate

CONCLUSION: The proposed method for quantitative determination of LORN and PCM in combined tablet dosage form is efficient and sensitive. The excipients of the commercial sample analyzed did not interfere in the analysis, which proved the specificity of the method for these formulations. The HPLC method was found to be simple (non usage of buffer), rapid, precise, accurate and sensitive. This method can be used for routine quality control of LORN and PCM in tablets samples.

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