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Simultaneous estimation of paracetamol and lornoxicam by RP-HPLC method from combined dosage forms

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ABSTRACT

A simple, rapid and precise reverse phase high performance liquid chromatographic method was developed for simultaneous estimation of Paracetamol (PL) and Lornoxicam (LO) in combined tablet dosage form. Formulation containing PL and Chromatography was performed on a 150 mm x 4.6 mm, 5-µm particle size, C_8 HiQSil column using methanol: acetate buffer (60:40 v/v) mixture as a mobile phase of pH 6.5 adjusted with Glacial acetic acid. The detection of the combined dosage form was carried out at 265 nm with a flow rate of 1 ml min^{-1}. The retention times were 1.725 & 2.725 minutes for PL and LO respectively. Linearity of the method was excellent over a concentration range 30 to 80 µg mL^{-1} for PL and 0.5 to 1.0 µg mL^{-1} for LO. The correlation coefficient was 0.9998 and 0.9994 for PL and LO respectively.The relative standard deviation values for repeatability and intermediate precision studies were less than 2 %, and % recovery was greater than 98% for both drugs. The proposed method was found to be suitable for the routine estimation of PL and LO in tablet dosage form.

Keywords: Paracetamol, Lornoxicam, RP-HPLC, Simultaneous estimation, Validation.

INTRODUCTION

Paracetamol (PL) is widely used as analgesic and antipyretic and chemically it is *N*-(4-hydroxyphenyl) acetamide [1, 2]. It is official in IP [3], BP [4] and USP [5]. Lornoxicam (LO), 6-chloro-4-hydroxy-2-methyl-N-2-pyridinyl-2*H*-thieno-[2,3-e]1,2-thiazine-3-carboxamide 1,1-dioxide **(Fig.1)** is a novel non-steroidal anti-inflammatory drug (NSAID) with marked analgesic properties [6] . LO belongs to the chemical class oxicams, which includes Piroxicam, Tenoxicam and Meloxicam. A literature survey reveals that HPLC and UV methods are available for analysis of PL as a single component [3-5]. Also Spectrophotometric method has been reported for analysis of LO [7]. Besides LC [8] and LC-MS [9] has also been reported for analysis of LO.

A combination of 8 mg of Lornoxicam and 500 mg of Paracetamol is available commercially as tablets. From the literature it is observed that there is no RP-HPLC method reported for simultaneous estimation of PL and LO from combined dosage form. Hence an attempt has been made to develop a simple, precise, accurate and economical RP-HPLC method for the simultaneous estimation of PL and LO in tablets. The chemical structures of PL and LO are shown in Figure 1.

MATERIALS AND METHODS

Chemicals and Reagents

HPLC-grade methanol and analytical grade sodium acetate were procured from Fin-kem (Mumbai, India) and bulk standards of PL (99.80%) and LO (99.75%) were obtained as gift samples from Whockhardt Ltd (Aurangabad, India). Glacial acetic acid was procured from Merck Specialities (Mumbai, India). HPLC grade water was used throughout the study.

Instrumentation and Chromatographic Conditions

Chromatography was performed with a Jasco HPLC LC-2000 Plus series (Japan), isocratic pump PU-2080, an UV detector PU-2075 Plus, injector with 20-µL loop, C_8 HiQSil column (250 x 4.6 nm, 5-µm particles) was used for chromatographic separation under suitable condition. Detection was carried out at 265 nm and the software used was Borwin.

The mobile phase was a mixture of methanol: sodium acetate buffer (60:40) (*v/v*) of pH 6.5 agjusted with Glacial acetic acid. The mobile phase was filtered through 0.45 µ membrane filter and sonicated before use. The flow rate of mobile phase was maintained at 1 mL min^{-1}.The injection volume was 20 µL and total run time was 8 min. The peaks were identified by retention time. A typical chromatogram is shown in Fig. 2.

Preparation of standard solution for calibration Plots

Weigh accurately about 500 mg of PL and 8 mg of LO and transfer it to a 100 ml volumetric flask separately. Add 60 ml of mobile phase, sonicate it to dissolve the content and make up the volume with mobile phase. Filter through 0.45 µ membrane filter paper. Dilute 1 ml of resulting solution to 10 ml with mobile phase to give a concentration of 500 µg mL^{-1} of PL & 8.0 µg mL^{-1} of LO. Stock solution was diluted with mobile phase to give working standard solution containing 30 to 80 µg mL^{-1} of PL and 0.5 to 1.0 µg mL^{-1} LO respectively. These standard solutions were injected for construction of calibration plots by plotting drug peak-area ratio (*y*) for each of the drug against concentration (*x*). Analysis was performed at ambient temperature. The retention times of PL and LO under these conditions were 1.725 and 2.725 minutes respectively. The values of coefficient of correlation r were 0.9998 and 0.9994 for PL and LO respectively. The linear regression data for the calibration curves indicate that the response is linear over the concentration range studied for both drugs.

Analysis of marketed formulation

Twenty tablets, each containing PL (500 mg) and LO (8.0 mg) were weighed and finely powdered. A quantity of powder sample equivalent to 500 mg of PL and 8.0 mg of LO was taken in 100 ml volumetric flask and dissolved in 60 ml mobile phase. The solution was sonicated for

15 minutes. The volume was made up with mobile phase and filtered through a 0.45 µ membrane filter. One ml of filtrate was transfered to 10 ml volumetric flask and make up the volume with mobile phase. Again pipette out 1 ml of this solution and transfer to 10 ml volumetric flask and make up the volume with mobile phase. The concentrations for PL and LO was 50 µg mL^{-1} and 0.8 µg mL^{-1} respectively. The solutions were then injected. From the peak area, the drug content in the tablets was determined.

RESULTS AND DISCUSSION

Method development

The objective of this study was to develop simultaneous estimation of two components under isocratic conditions. Method development for the simultaneous estimation of PL and LO was a challenging task because of their combination ratio of approximately 62.5:1. Several mobile phase combinations at different flow rate were tried to resolve the peaks of PL and LO and wherein the response could be measured simultaneously.

Finally a mixture of methanol- buffer (pH 6.5) in the ratio of 60:40 (*v/v*) at a flow rate of 1.0 ml was proved to be effective for better resolution of the peaks.The mentioned chromatographic conditions revealed to provide better resolution between PL and LO in 1.725 and 2.725 minutes respectively.The optimum wavelength for detection was 265 nm.

Validation of the method

Specificity

Specificity was tested against standard solution and against potential interferences in the presence of placebo. No interference was detected at the retention time of PL and LO in sample solution. Hence the excipients do not interfere with the estimation of drugs.

Precision

The precision of the method was done in terms of repeatability of measurement, performed by injecting the standard solution six times and measuring the peak areas. In method precision the % RSD was 0.21 and 0.18 for PL and LO respectively. In Intra-day precision % RSD was 0.09 and 0.12 for PL and LO respectively. In Inter-day precision % RSD was 0.17 and 0.19 for PL and LO respectively. This shows that precision of method is satisfactory as % RSD is not more than ±2.0%.

Linearity

Linearity was assessed with the aid of serially diluted calibration solutions as mentioned above. The standards were injected separately. Calibration graphs were plotted on the basis of triplicate analysis of each calibration solutions. Linear correlations were ob-tainted over the range of 30 to 80 µg mL^{-1} for PL and 0.5 to 1.0 µg mL^{-1} for LO, with correlation coefficients ≥ 0. 99. In case of tablets, the regression equation was y = 6422.9x (R2=0.9998) for PL and y = 51150x (R2=0.9994) for LO. The results of linearity and other system suitability parameters are given in Table 1.

Accuracy

The accuracy of the method was determined by recovery study carried out using standard addition method at three different levels. The resulting spiked sample solutions were assayed in triplicate and the results obtained were compared with the expected results and expressed as percentage. The results indicate that the individual recovery of PL ranges from 99.38 to 99.89% and for LO 99.16 to 99.75%. The recovery by proposed method is satisfactory as % RSD is less than ±2% .The results of the recovery study are summerised in Table 2.

Robustness

Robustness of the method was determined by analyzing standard solutions at normal operating conditions and also by changing some operating conditions such as flow rate, wavelength, pH of mobile phase.The results of the robustness study was given in Table 3.

The deliberate aforementioned changes in parameters not have the significant change. The robustness of the method is established as the % deviation from mean assay value. The assay values were within ±2.0%. after 72 h.

Analysis of marketed formulation

The developed method was applied for the simultaneous estimation of PL and LO in tablet dosage form. The results of analysis are given in Table 4**.**

The % RSD for assay in tablet formulation was found to be to be 0.21 and 0.18% for PL and LO respectively. This shows that the method is satisfactory as % RSD is not more than ±2.0%. indicating the suitability of the method for routine analysis of both the drugs in pharmaceutical dosage forms.

Table 1: System suitability parameters

Parameters	**PL**	**LO**
Retention time	1,725	2.725
Resolution factor	-----	1.538
Theoretical plates	3306	11881
Tailing factor	0.583	0.6
Linearity range (µg mL-1)	30-80	0.5-1.0
Intra-day precision RSD (%))*	0.09	0.12
Inter-day precision (RSD (%)*	0.17	0.09

Table 2: Results of the Recovery Tests for the Drugs

Level of % Recovery	**% Recovery**		**Mean S.D**		**% R.S.D**	
	PL	**LO**	**PL**	**LO**	**PL**	**LO**
80	99.62	99.35	0.257	0.176	0.26	0.18
100	99.73	99.64	0.101	0.097	0.1	0.1
120	99.78	99.46	0.075	0.146	0.08	0.15

Table 3: Robustness of the method

Chomatographic changes					
Factor	**Level**	**% Assay**		**% Deviation**	
Flow Rate(ml/min)		PL	LO	PL	LO
0.8	-0.2	99.77	99.76	0.06	0.03
1.0	0	99.71	99.73	0.0	0.0
1.2	+0.2	99.74	99.69	0.03	0.04
pH		**PL**	**LO**	**PL**	**LO**
6.0	-0.5	99.68	99.75	0.03	0.02
6.5	0	99.71	99.73	0.0	0.0
7.0	+0.5	99.73	99.72	0.02	0.01
Wavelength(nm)		**PL**	**LO**	**PL**	**LO**
260	-5	99.75	99.67	0.04	0.06
265	0	99.71	99.73	0.0	0.0
270	+5	99.69	99.77	0.02	0.04

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Table 4: Analysis of Tablet Formulation

Sr.	**Label claim(mg/tab)**		**Amount Found mg/tab**		**% Label Claim**	
No.	**PL**	**LO**	**PL**	**LO**	**PL**	**LO**
1	500	8	499.64	7.95	99.93	99.41
2	500	8	497.90	7.98	99.40	99.85
3	500	8	497.50	7.97	99.50	99.74
4	500	8	499.00	7.99	99.80	99.91
5	500	8	499.35	7.97	99.87	99.69
6	500	8	498.90	7.98	99.78	99.80
Mean					99.71	99.73
S.D					0.213	0.176
%RSD					0.21	0.18

Figure 1:The chemical structure of PL and LO

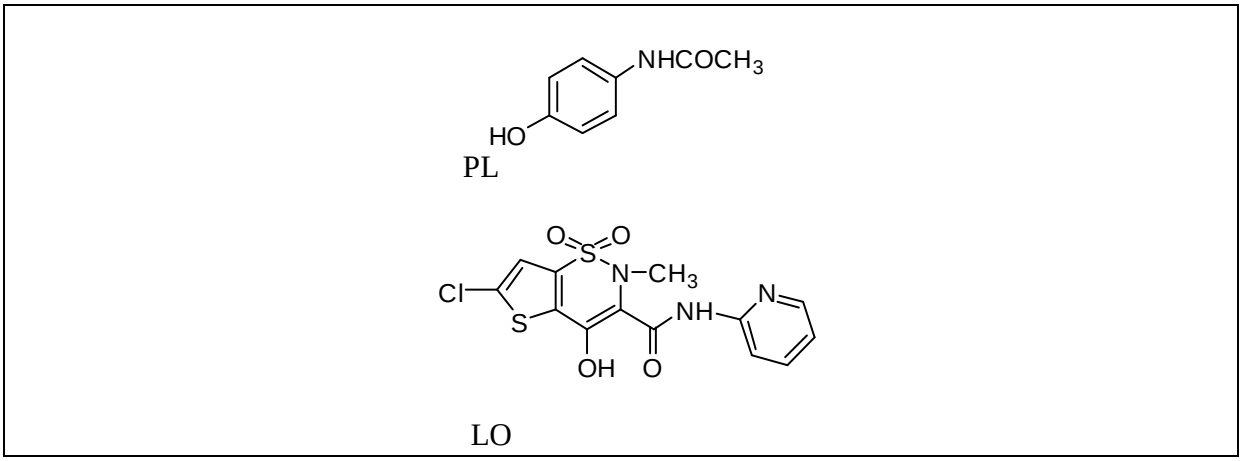
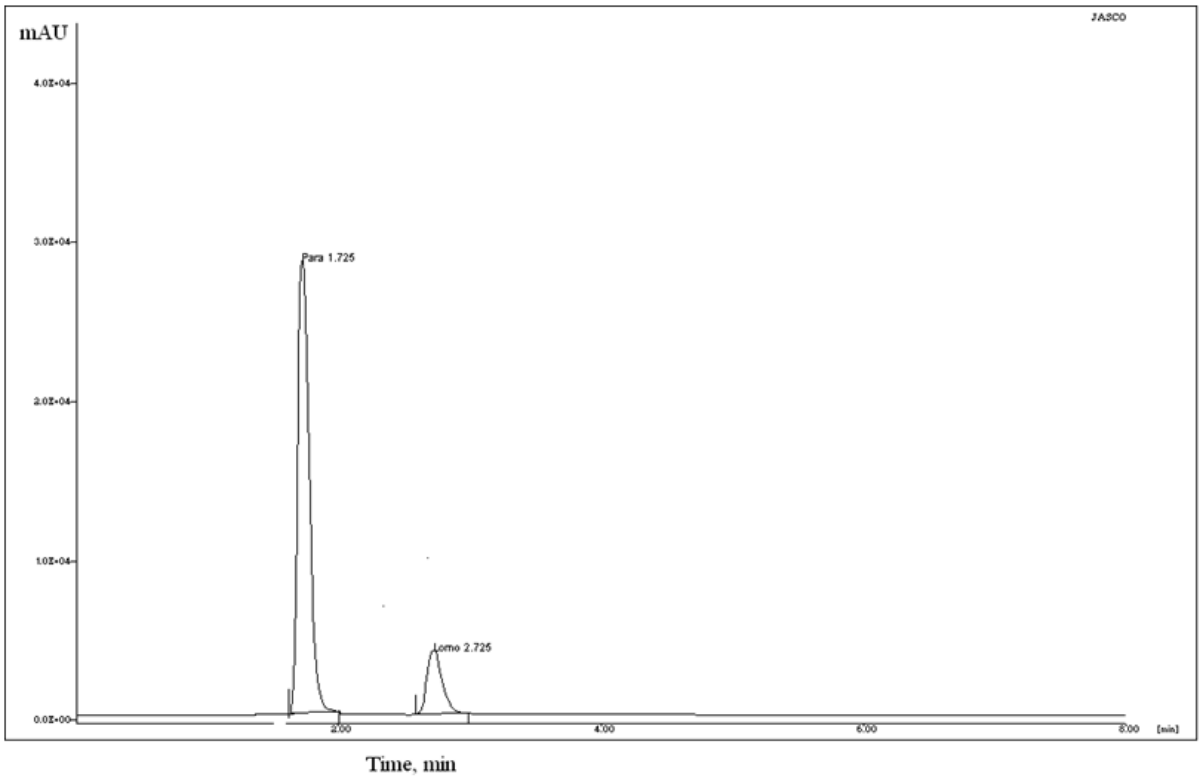


Figure 2:Typical chromatogram obtained from PL and LO



CONCLUSION

The developed liquid chromatographic method is specific, precise, accurate and robust. It can be used as routine method for simultaneous determination of Paracetamol and Lornoxicam.

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