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RP-HPLC Method for the Simultaneous Estimation of Perindopril and Losartan in Pure Form and Tablet Formulations

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ABSTRACT
The combination of Perindopril (PRN) and Losartan (LRN) is used in the management of hypertension. The present work was focused on the development of RP-HPLC method for the estimation of perindopril and losartan in binary mixtures. The separation was performed on LUNA C_{18} column at 210 nm by isocratic elution. Methanol and phosphate buffer (pH 6.8) in the ratio of 85:15 with a flow rate of 0.8 ml/min at ambient temperature was found to be suitable for chromatographic separation. Perindopril and losartan exhibits linearity in the ranges of 200-500 and 30-80 µg/mL respectively. The proposed method evidenced the absence of chromatographic interference by pharmaceutical excipients. Separation of the mixtures was proved to be good from the resolution of the peaks. Validation and recovery studies were performed and the results proved the method to be suitable for routine analysis.

Keywords: Perindopril, Losartan, RP-HPLC, Simultaneous estimation.

INTRODUCTION

Hypertension is the most prevalent cardiovascular disease in people above 50 years of age. [1] The pathological changes in the vasculature and hypertrophy of the left ventricle are caused by increased arterial pressure. As a consequence hypertension is the major cause of several complications of cardiovascular system like myocardial infarction, coronary artery disease, renal insufficiency etc. In past five decades antihypertensive therapy has been remarkably improved. By the combination therapy, hypertension can be easily controlled with minimum discomfort. Angiotensin converting enzyme inhibitors in combination with angiotensin II antagonists proved to be effective for the management of hypertension.

Perindopril is an ACE inhibitor used in the treatment of heart failure and hypertension and chemically known as (2S)-2-{(1S)-2-carbethoxy butyl amino}-1-oxo propyl-(2s, 3as, 7as)-1-perhydro indole-2-caboxylic acid. [2] Losartan, an angiotensin II receptor antagonist, chemically known as I,2-n-butyl-4-chloro-1-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]-imidazole-5-methanol mono potassium salt. [3] It is indicated for the management of hypertension.

Literature survey reveals few LC [4-5] and spectrophotometric [6-7] methods for the determination of PRN or LRN alone, which indicates the need for method development for the quantitative estimation of PRN in combination with LRN. Hence, an attempt has been made to develop and validate a sensitive, reproducible and specific method for the quantitation of PRN and LRN in mixtures.

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MATERIALS AND METHODS

Apparatus

The Schimadzu chromatographic system consisted of prominence LC20AD binary pump, rheodyne injector, with 20µL loop, UV/Visible detector and sphinchrom software. LUNA C_{18}, (250 mm × 4.6 mm id) was used for the separation and quantification of mixtures. An ELICO (LI 610) pH meter was used for the adjustment of pH in the preparation of buffer.

Reagents and Standards

HPLC grade methanol (99.9% purity) and water from Merck, Mumbai were used in the analysis. Potassium dihydrogen orthophosphate and sodium hydroxide were of analytical grade (S D Fine Chem ltd, Mumbai). Phosphate buffer pH (6.8) was prepared by adding 22.4 ml of 0.1M sodium hydroxide to 50 ml of 0.02 M potassium dihydrogen ortho phosphate solution and diluting to 200 ml with water. The prepared buffer was then filtered through 0.42µM membrane filter. The mobile phase was degassed using bath sonicator.

Chromatographic conditions

Chromatographic separation was achieved at ambient temperature on C_{18} column using the mobile phase,

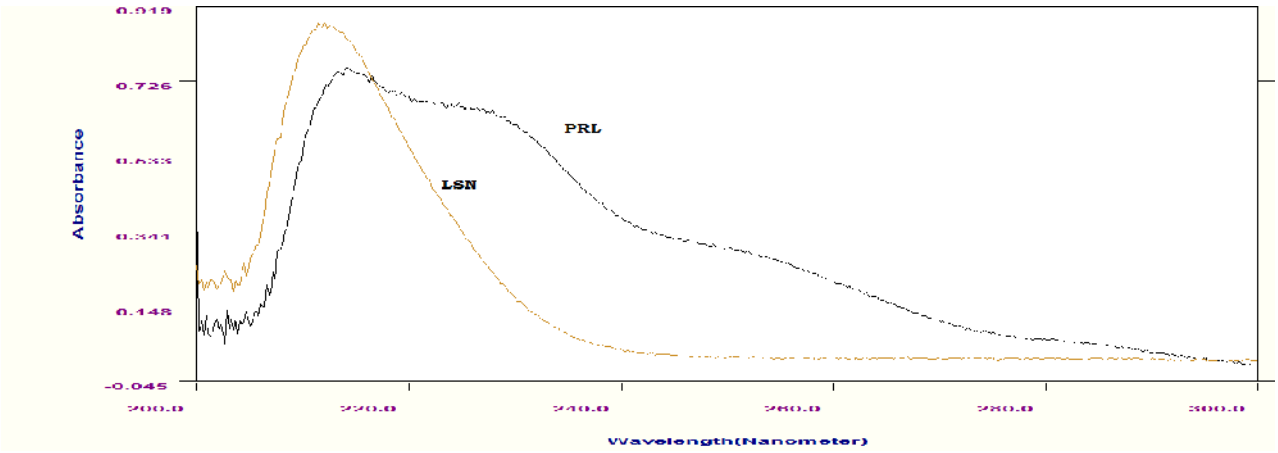

Fig. 1: UV overlain absorption plot of PRN and LRN

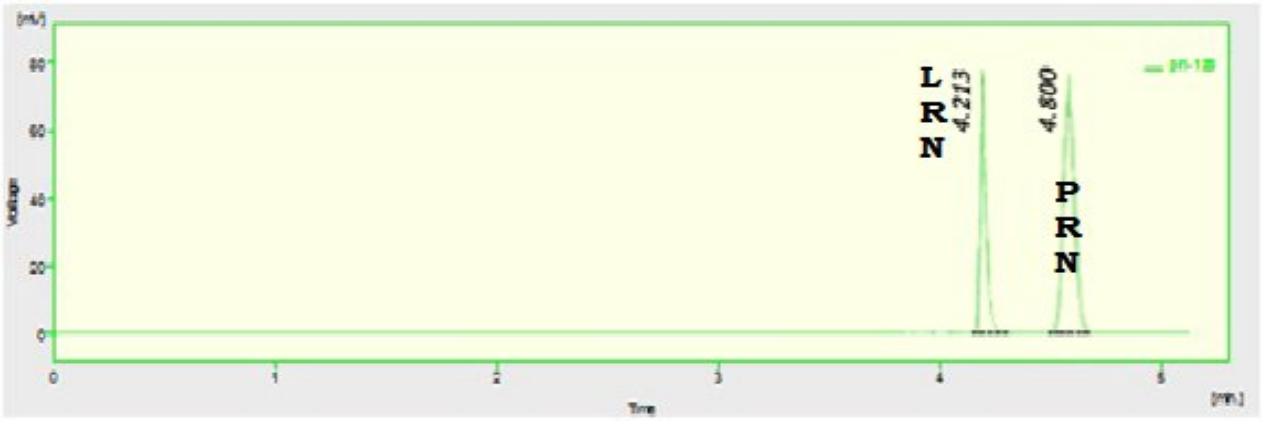

Fig. 2: Chromatogram of PRN and LRN

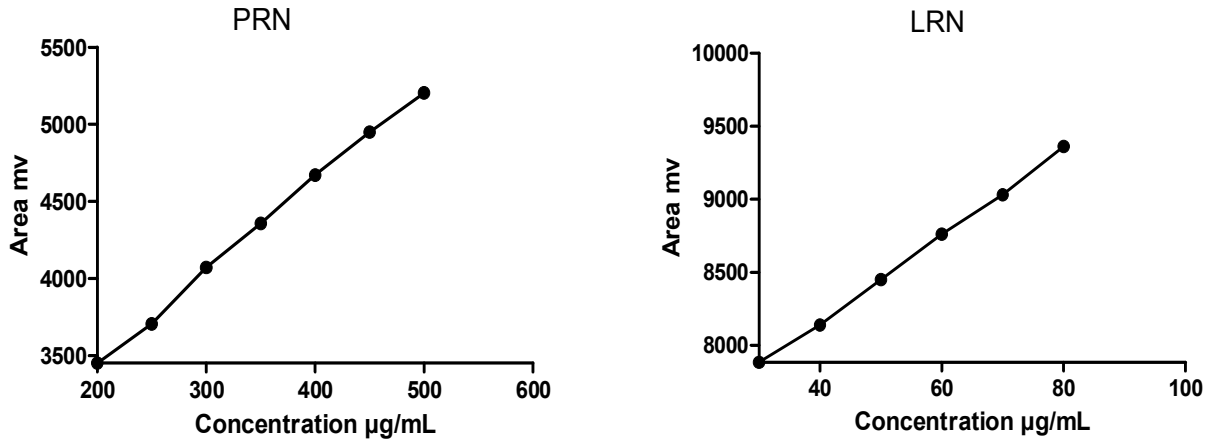

Fig. 3: Linearity plot of PRN and LRN

methanol: buffer (85:15 v/v) at the flow rate of 0.8 ml/min and at 210 nm.

Calibration graph

Standard solution of PRN/LRN was prepared by dissolving 50 mg of PRN/LRN in 25 ml of methanol and diluted to 50 ml with the same (1mg/mL). Working standard solution of PRN was prepared by diluting 25 mL of the above solution to 50 mL with methanol (500µg/mL). Further dilutions of the working standard solution of PRN were made using methanol to meet the concentration range of 200-500 µg/mL. Working standard solution of LRN was prepared by diluting 5mL of the standard stock solution to 50 mL with methanol to produce 100µg/mL. Triplicate 20 µL injections were made for each concentration and chromatographed under the specified conditions. Linear relationship was found when the graph was plotted between peak area values and its corresponding concentration.

Pharmaceutical sample preparation

Twenty tablets were weighed and powdered. The quantity of powder equivalent to 10 mg of PRN was taken and 5 mL of methanol was added, sonicated for 10min, filtered and diluted to 10 mL with methanol. 5 mL of the above said solution was diluted to 10mL with methanol, degassed, filtered. General procedure for HPLC method described under calibration was followed and the concentration of PRN/LRN was estimated.

Table 1: Results of optical and regression parameters

Parameters	PRN	LRN
Calibration range (µg/mL)	200-500	30-80
Retention time	4.62	4.09
Detection limit (µg/mL)	8.949	1.618
Quantitation limit (µg/mL)	27.065	4.9046
Slope (b)	15.051	119.862
SD of Slope (S_b)	0.23	0.45
RSD of Slope (%)	1.2	1.41
Intercept (a)	-2388.57	-2602.7904
LOD	0.178	0.03
LOQ	0.542	0.098
SD of Intercept (S_a)	0.34	0.46
Correlation coefficient	0.9997	0.9997
Theoretical plates	4356	8760
Symmetry factor	0.97	0.96
Resolution	1.7695	

Table 2: Recovery studies and assay results of PRN and LRN

Drug	Added	Measured	Mean % recovery ± S.D	% label claim
PRN	200	202.41	100.58	98.36
	250	249.32		
	300	302.54		
LRN	30	31.21	102.8	101.36
	40	40.9		
	50	52.1		

Table 3: Intra-day and inter-day precision (n=3)

Drug	Added, µg/ml	Intraday- precision Found ± S.D	Interday precision Found ± S.D
PRN	200	202±0.34	203±0.42
	300	298±0.26	302±0.36
	400	401±0.28	398±0.43
LRN	40	40.2±0.36	41.98±0.21
	50	51.2±0.42	52.3±0.43
	60	61.5±0.37	62.1±0.54

RESULTS AND DISCUSSION

Method development

The selection of the buffer and the composition of mobile phase were studied and optimized. Separation was found to be satisfactory with methanol and buffer (pH 6.8) in the ratio of 85:15 v/v. Increasing the buffer concentration led to very short retention time and distortion of the peak. UV detection was carried out at 210 nm where both the drugs exhibit maximum absorption and is shown in fig. 1.

Method Validation

The developed method was validated for several parameters including linearity, precision, accuracy, sensitivity, robustness and system suitability. The optical and regression parameters were given in table 1.

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of interfering substances. This was examined by the validation parameters obtained from the chromatogram. The theoretical plates and tailing factor was found to be 5696 and 0.407 for PRN; 5212 and 0.343 for LRN, respectively. The responses were found to be satisfactory which indicates the system suitability of the method.

Linearity

Calibration plot was established by analyzing a series of different concentrations of each compound. In the present study eight concentrations were chosen ranging between 200-500 µg/mL for PRN, and 30-80 µg/mL for LRN. Each concentration was repeated three times. The regression equation and correlation coefficient for PRN and LRN were found to be Y=752.57 X-2388.57; 0.9997 and Y= 5993.14 X -2602.79; 0.9997, respectively and results were shown in table 1.

Accuracy and precision

The accuracy and intraday precision of pure PRN and LRN at three different concentrations were analysed in six replicates. The mean percentage recoveries and their standard deviations for the proposed method for six replicates of PRN and LRN were calculated and found to be satisfactory. Consequently, the interference by the excipients in pharmaceutical formulations was not observed and the results were presented in Table 2 and 3.

Ruggedness

Intermediate precision of six replicate determinations of PRN/LRN was analysed by three analysts on different days. The percentage RSD of assay was found to be less than 2.0%.

Robustness

The chromatographic separation was not influenced by minor variations on pH of the buffer by ±0.2 pH units as well as flow rate ± 0.1 mL/min.

Limit of quantitation and limit of detection

LOD and LOQ were established as per ICH recommendations, based on the approach of S.D of the response and the slope. The detection limits were found to be 0.053 and 0.028 for PRN and LRN, respectively. The quantitation limits were found to be 0.162 and 0.085 for PRN and LRN, respectively. The results were given in table 1.

Analysis of tablets

The proposed HPLC method was applied to the simultaneous estimation of PRN and LRN in commercial tablets. Satisfactory results were obtained for each compound in good agreement with label claim.

The proposed HPLC method is simple, rapid and specific. A clear resolution was achieved even when applied to the formulations. The method is characterized by short retention time and is without interference from excipients. The statistical measures and recovery studies indicated that the proposed method could be extended to effective quantitation of PRN and LRN in tablet formulation.

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