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Development and Validation of RP-HPLC Method for Simultaneous Estimation of Losartan Potassium and Perindopril In Tablet Dosage Form

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ABSTRACT

The present work describes a new simple, sensitive and precise reverse phase high performance liquid chromatographic method (RP-HPLC) for the simultaneous estimation of Losartan potassium (LP) and Perindopril erbumine (PE) in bulk and in pharmaceutical dosage forms. Chromatographic separation was performed on KNAUER High Performance Liquid Chromatographic System with C18 column of Make: Thermo Hypersil – ODS of dimensions 250 x 4.6mmwith a mobile phase comprising of 0.01M potassium phosphate buffer (pH 3.5): Acetonitrile: Methanol in the ratio of 5:55:40v/v. the pH of buffer was adjusted with ortho phosphoric acid. The flow rate was 1.0 ml/min with detection with detection at 210nm.As per International Conference on Harmonization (ICH) guidelines the method was validated for linearity, precision, limit of quantitation, limit if detection and robustness. Linearity of LP was found to be in the range of 350µg/ml-650µg/ml and 28µg/ml-52µg/ml for PE. The correlation coefficient for LP and PE was found to be 0.997 and 0.998 respectively. The mean recoveries obtained for LP and PE was found to be 100.222% and 99.844% respectively. The developed analytical method was found to be accurate, linear, specific, and precise which is evident from the statistical data.

Keywords: Losartan Potassium, Perindopril Erbumine, RP-HPLC, simultaneous estimation, validation.

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INTRODUCTION

Losartan potassium (LP): LP (Figure 1) is chemically potassium salt of 2Butyl- 4-chloro-1-[[2-(1H-tetrazol-5-yl) [1,1-biphenyl]-4yl]methyl]-1H-imidazole-5-methanol, represents the first of a new class of orally active non-peptide angiotensin II (Type AT1) receptor antagonists employed in the management of essential hypertension.

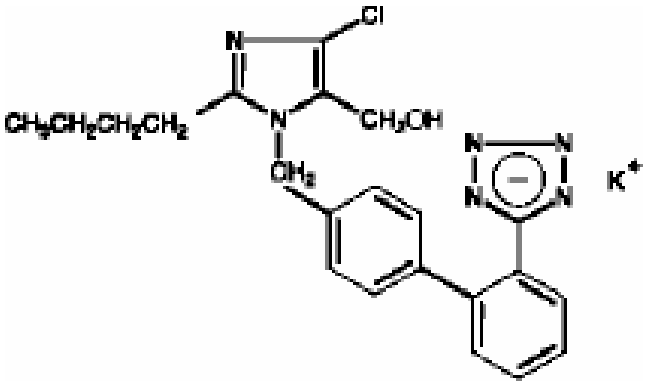

Figure 1: Structure of Losartan Potassium

Perindopril erbumine (PE): PE (Figure 2) BP, 2007 is chemically (2S, 3αS, 7αS)-1-[(S)-N-[(S)-1-Carboxy-butyl] alanyl] hexahydro-2-indoline carboxylic acid,1-ethyl ester, compound with tert-butylamine. Perindopril erbumine is an anti-hypertensive agent and prodrug for perindoprilat, which inhibits ACE in human subjects and animals. Perindopril Erbumine is one of the non-peptide Angiotensin II receptor antagonists, and is used for the treatment of patients with hypertension and symptomatic heart failure.

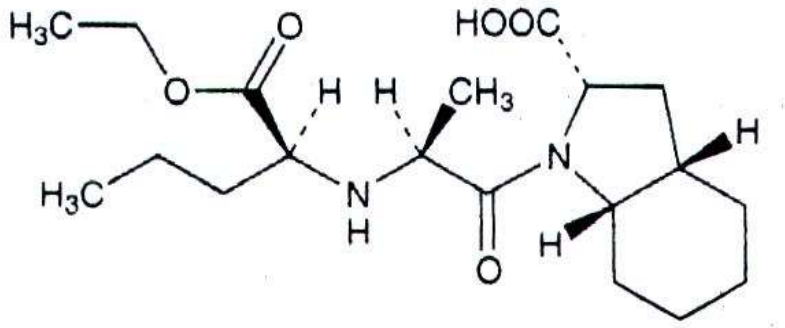

Figure 2: Structure of Perindopril Erbumine

The combined oral administration of perindopril with losartan has been found to be more effective than either of the drugs alone in the treatment of hypertension. Their combination is not official in any pharmacopoeia, so no official method is available for the estimation of these two drugs in combination.

Literature reveals one Spectrophotometric with LC method[3],one stability indicating RP-HPLC method, Nine RP-HPLC methods has been reported with other drugs combination[5-13]. To the best of our knowledge, only one RPHPLC method has been described for simultaneous estimation of both the drugs in tablets[14]. The present work describes the simple, accurate, precise, sensitive RP-HPLC method for the determination of these drugs in combined tablet dosage form. The method was validated as per the ICH guidelines.[10-15].

MATERIALS AND METHOD

Instrumentation:

KNAUER HPLC System comprising of SMARTLINE Pump 1000, SMARTLINE UV Detector, SMARTLINE Manager 5000, RHEODYNE injector fitted with 20µl capacity loop along with EUROCHROM Software was used in the study. Separation and quantitation were done using Thermo Hypersil – ODS C18 (250x4.6 mm) column. Standard and sample solution was filtered using Filtration Assembly comprising of Nylon 66 membrane filter of 13mm diameter, having pore size of 0.45 micron using syringe and needle. Wavelength of detection was obtained from the PDA detector where the two drugs showed the absorption maxima at 210nm. UV-Visible spectrophotometer was used to record the UV spectra of Losartan potassium (LP) and Perindopril erbumine (PE). A Digital Weighing Balance (Make: Precisa Instruments AG, Precisa 310M) was used for preparations of all samples and buffer solutions required.

Chemicals and reagents:

The reference samples of Losartan Potassium and Perindopril were obtained from Zydus Cadila Pvt. Ltd., Goa, India and GlenpharmaPvt. Ltd., Goa, India respectively. Acetonitrile (HPLC grade), Methanol (HPLC grade) and Water (HPLC grade) was purchased from S. D. Fine Chem. Pvt. Ltd., Mumbai India. Potassium dihydrogen orthophosphate (AR) and Orthophosphoric acid (AR) were purchased from LobaChemie Pvt. Ltd., Mumbai India. ADPACE-4, a commercial tablet containing Losartan Potassium(50mg) and Perindopril Erbumine (4mg) manufactured by Sun Pharmaceuticals Ltd. was procured from local firms.

Mobile Phase:

The mobile phase was prepared by mixing Acetonitrile, Methanol and 0.01M potassium phosphate buffer (pH 3.5) in the ratio of 55:40:5v/v/v. The pH of the buffer solution was adjusted by 0.1%v/v ortho phosphoric acid. The mobile phase was filtered using 0.22 µm membrane filter after sonication of each solvent for 15 min.

METHOD DEVELOPMENT

Selection of Wavelength

Wavelength of detection based on the density plot of the standard solution of the drugs was obtained from the UV detector, wherein the two drugs were scanned in the range of 190 nm to 400 nm. Both the components show reasonably good absorption maxima at 210nm.

Optimization of Mobile Phase:

A number of eluting experiments were conducted for the optimization of separation of the drugs using mobile phase. A mixture of Acetonitrile, Methanol and 0.01M potassium phosphate buffer were screened as possible eluting systems in different proportions like 30:60:10, 35:55:10 and 40:50:10 v/v. A suitable optimized condition with a mixture of Acetonitrile, Methanol and 0.01M potassium phosphate buffer (pH adjusted to 3.4 with OPA), in a ratio of 55:40:5v/v provided an efficient separation of the drugs with good peak symmetry as well as retention times. A flow rate of 1 mL/min was found to be optimum in which the retention time was 2.067 min for LP and 3.150 min for PE with baseline stability.

Preparation of Standard Stock Solution:

The stock solutions were prepared by dissolving a suitable quantity of LP and PE to get the final concentration of 0.5mg/mL (500 µg /mL) and 0.04 mg/mL (40 µg/mL) in standard volumetric flask and volume was made up with methanol. Further dilutions were made from the stock solution with acetonitrile in the required concentration range in 10mL volumetric flasks for the calibration curve.

Preparation of Sample Solution:

Twenty tablets (ADPACE-4 tabs) each containing 50mg of losartan potassium and 4mg of perindopril were weighed and powdered. Weight of tablet powder equivalent to 500mg of LP and 40mg of PE was weighed and transferred to 100ml volumetric flask. The solutions were shaken for 20mins for complete solubility of the drugs. After 20 minutes volume was made up to 100ml with methanol. The solution was then filtered through What man filter paper No. 41. From the filtrate, dilution was made in a 10ml volumetric flask with acetonitrile. Sample solution was then filtered using Sample Filtration Assembly.

METHOD VALIDATION

As per the International Conference on Harmonization (ICH) guidelines[17-19], the method validation parameters like linearity, precision, accuracy, limit of detection, limit of quantitation, robustness and specificity were experimentally determined and the method validated.

Selectivity/Specificity of the proposed method

Selectivity was demonstrated by the resolution of the two compounds, Losartan Potassium and Perindopril. Sample matrix did not show any interference with the analyte peaks. Retention times for Losartan Potassium and Perindopril were 2.067 and 3.150 respectively.

Linearity and range

Series of mixed standard solutions of Losartan Potassium and Perindopril were prepared. Linearity of the method was studied by injecting five concentrations of the standard solution in the range of 350-650 µg /ml and 28-52 µg /ml for Losartan Potassium and Perindopril respectively, in the HPLC system noting the peak areas.

Accuracy

The accuracy of the method was determined by recovery studies. The recovery studies were performed by standard addition method; at 70%, 100%, 130% level for both the drugs i.e; three different levels. Accuracy of the method was studied by calculating recovery of the spiked samples.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ of LP and PE by the proposed methods were determined on the basis of response and slope of the regression equation. LOD and LOQ values were calculated using the formula 3.3×s/S and 10×s/S, respectively, where Sis the slope of calibration curve and s is the standard deviation of y-intercept of regression equation.

Robustness

The robustness of the developed method was determined according to ICH guidelines. Experimental conditions were deliberately altered. Variations of the analytical parameters on retention time of the drugs was examined wherein the method was changed in pH by unit of 0.1 above and below the normal pH of 0.01M potassium phosphate buffer was done.

System suitability

For system suitability, six replicates of the working standard sample were injected and the parameters like plate number(N), retention time, resolution and peak asymmetry of samples were calculated.

RESULTS AND DISCUSSION

The goal of this present study was aimed at developing a sensitive, precise and accurate HPLC method for the analysis of Losartan Potassium and Perindopril Erbumine in its bulk and pharmaceutical combined dosage form. In order to achieve optimum separation of the components peaks, various proportions of buffer with acetonitrile and methanol were tested as mobile phase on

the KNAUER HPLC C18 column. Mobile phase containing a mixture of Acetonitrile, Methanol and 0.01M potassium phosphate buffer (pH adjusted to 3.4 with OPA), in a ratio of 55:40:5v/v was selected as it resulted in peaks with good symmetry and resolution. A flow rate of 1.0mL/min was found to be optimum in the 0.5 to 1.0 mL/min range resulting in the short retention time, baseline stability and minimum noise. With the above optimized conditions, the retention times of LP and PE were found to be 2.067 min and 3.150 min respectively showing the proposed method is time saving. (Figure 3) along with the 3D density plot of combined dosage form (Figure 5). The calibration curve showed linearity in the concentration range of 350-650 µg /ml and 28-52 µg /ml for Losartan Potassium and Perindopril respectively (Figures 6 & 7). The regression coefficients of concentration over their peak areas were found to be 0.997 for LP and 0.998 for PE (Table 1). The results of intraday and interday precision values are represented in (Table 2). The RSD% for assay of drugs during intra-day and inter-day were 0.275 and 0.301 for LP and 0.625 and 0.794 for PE. Assay of two drugs using the developed method showed acceptable relative error values that are less than 2 indicating that the method is highly precise. The percentage mean recovery of individual analyte was high, satisfactory and indicates that the proposed method is accurate (Table3). The number of theoretical plates was determined to be 2673 and 2324 for LP and PE respectively, which indicates high sensitivity of the method (Table 4). The excipients used in formulation did not interfere with the drug peaks and that the method is specific. The HPLC chromatograms recorded for the drug matrix (mixture of the drug and excipients) showed almost no interfering peaks within retention time ranges. Figures 3 and 4 show the representative chromatograms for the standard and the formulation. The chromatograms show that the selected drugs were clearly separated and thus the proposed HPLC method is selective. In robustness study, pH of buffer was deliberately altered. Under all the above conditions specified above, asymmetric factor was less than 2.0 theoretical plates were more than 2000 for LP and PE peaks, which illustrates good robustness of the developed method. (Table 5). The amount of LP and PE present in the sample solutions were determined by fitting the responses into the regression equations (Figures 6 and 7) of the calibration curve for LP and PE respectively and the results obtained were comparable with the corresponding label claim (Table 6 and 7).

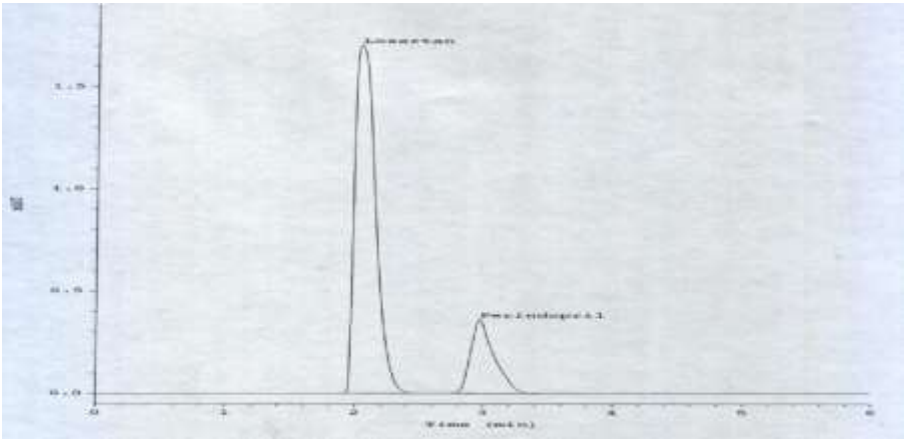

Figure 3: Chromatogram of standard solution of Losartan Potassium and Perindopril Erbumine

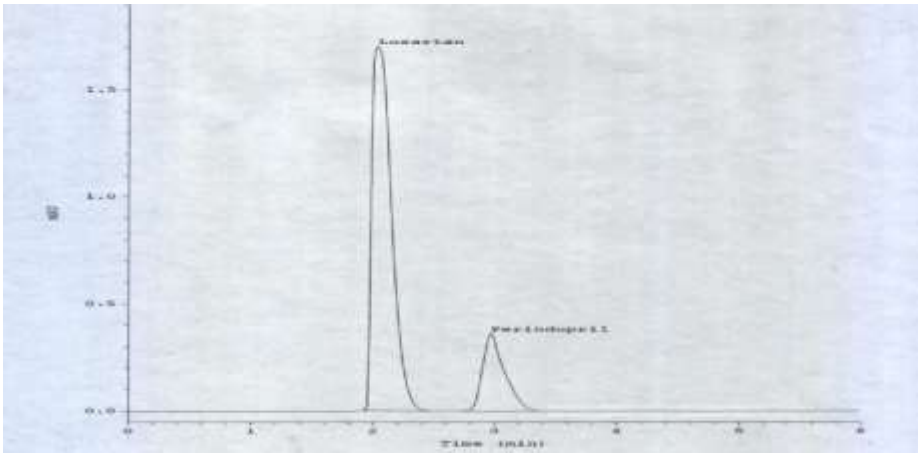

Figure 4: Typical chromatogram of ADPACE-4 tablet formulation

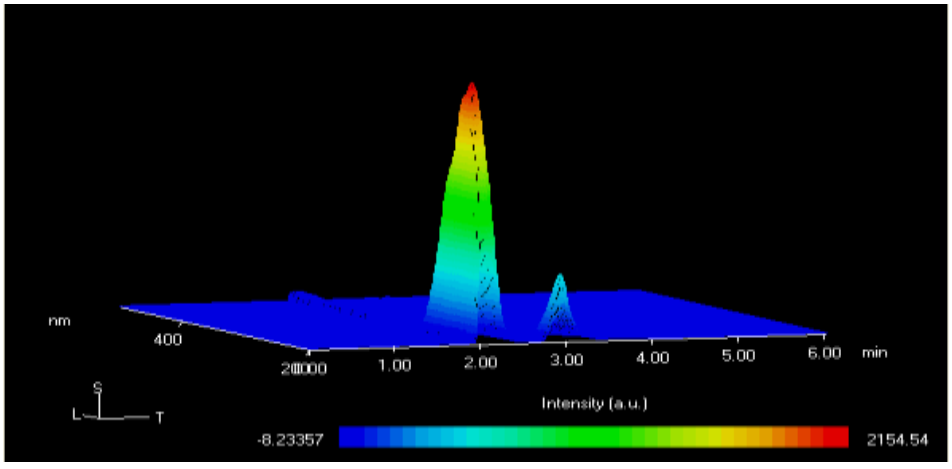

Figure 5: 3D Density Plot of Losartan Potassium and Perindopril Erbumine

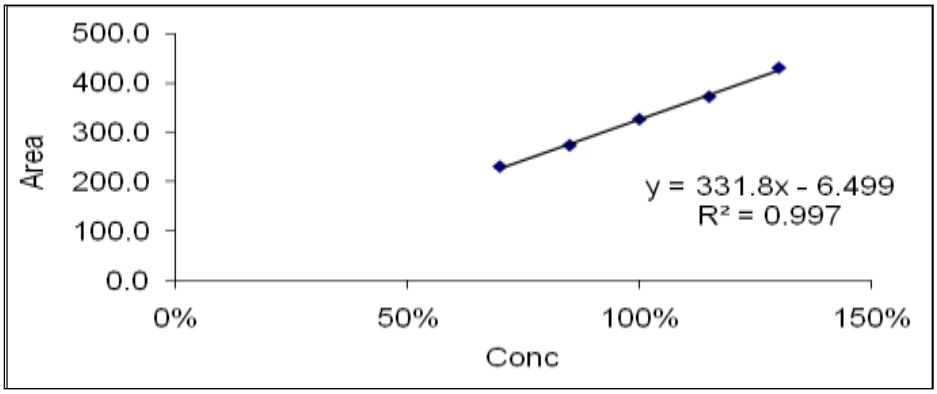

Figure 6: Linearity Curve of Losartan Potassium

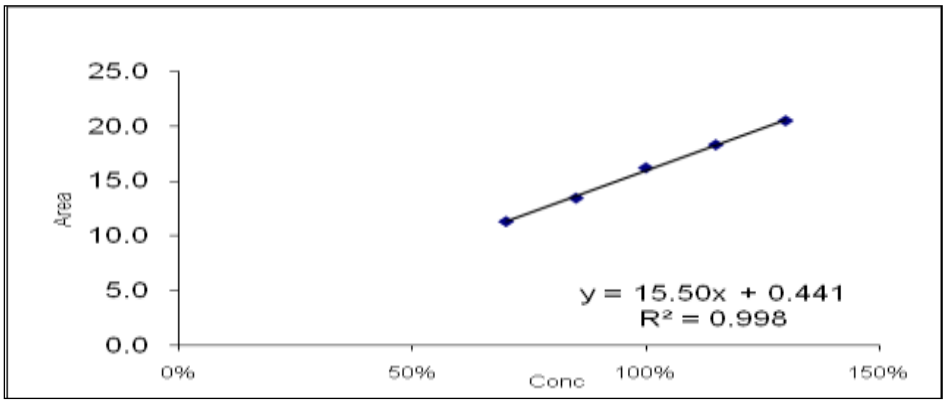

Figure 7: Linearity Curve of Perindopril Erbumine

Table 1: Results of Linearity Study from Calibration Curve

Drug	Conc.(µg/ml)	R^2
LP	350-650	0.997
PE	28-52	0.998

Table 2: Results of Precision Study (n=6)

Drug	%RSD(Intraday)	%RSD(Interday)
Losartan Potassium	0.275	0.301
Perindopril Erbumine	0.625	0.794

Table 3: Results of Accuracy Study (n=3)

Analyte	Concentration of solution in percentage	Amount Spiked (µg/ml)	Mean Peak area of Standard	Mean Peak area of Sample	Amount Recovered (µg/ml)	% Recovery
Losartan	70	350	326.867	228.618	35.013	100.037
Potassium	100	500		327.527	50.161	100.322
	130	650		425.720	65.200	100.307
Mean % recovery						100.222
	70	28		11.385	2.789	99.607
Perindopril	100	40	16.289	16.358	4.007	100.175
Erbumine	130	52		21.174	5.187	99.750
Mean % recovery						99.844

Table 4: System Suitability Parameters

Parameters	Losartan Potassium	Perindopril Erbumine
Theoretical plates	2673	2321
Tailing factor	1.346	1.368
Resolution factor	-	3.769
RSD of Area	0.151	0.348
RSD of Rt	0.423	0.646

Table 5: Evaluation of Robustness For Losartan Potassium and Perindopril Erbumine

Parameter	Content in mg/ tablet	
	Losartan Potassium	Perindopril Erbumine
Set-I (OPA buffer pH 3.4)	49.988	4.012
Set-II (OPA buffer pH 3.6)	50.015	3.918

Table 6: LOD and LOQ for losartan potassium and perindopril erbumine

Parameter	Losartan Potassium	Perindopril Erbumine
LOD	0.5 µg/ml	2 µg/ml
LOQ	1.5 µg/ml	3 µg/ml

Table 7: Results of Assay from Tablet Dosage Form

Drug	Content (mg)	% Purity
Losartan Potassium	50.053	100.106
Perindopril Erbumine	4.015	100.370

CONCLUSION

Proposed study describes a new isocratic RP-HPLC method for the estimation of Losartan Potassium and Perindopril Erbumine in combination using simple mobile phase. The method gives good resolution between the compounds with a short analysis time. The method was validated and found to be simple, sensitive, accurate, precise and can be used for analysis of regular quality control samples.

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