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Analytical Method Development and Validation by RP-HPLC for Simultaneous Estimation of Losartan Potssium and Perindopril Erbumine in Combined Tablet Dosage Form

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A B S T R A C T

A simple, sensitive and precise reverse phase high performance liquid chromatographic method has been developed for the simultaneous estimation of Losartan Potassium and Perindopril erbumine in pharmaceutical dosage form. The mobile phase consisted of ortho phosphoric acid: HPLC water: 55:45 0.1ml /10 min and wavelength of detection at 218 nm. The retention times of were Losartan Potassium and Perindopril erbumine 3.016 min and 3.43 min respectively. The method was validated in terms of linearity, precision, accuracy, limit of detection, limit of quantification. The method was found to be linear in the range of inertsil-ODS C18 (250 x 4.6mm, 5µ) g/ml for Losartan potassium and Perindopril erbumine respectively. The coefficient of variance for both the drug was more than 0.999.The proposed method can be used for determination of these drugs in combined dosage forms.

Keywords: Losartan potassium, perindopril erbumine, RP-HPLC

A R T I C L E I N F O

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1. Introduction

Losartan Potassium is a chemically named as [2-butyl-4-chloro-1-({4-[2-(2H-1, 2, 3, 4-tetrazol-5-yl)phenyl] phenyl} methyl)-1H-imidazol-5-yl] methanol. Losartan is an angiotensin-receptor blocker (ARB) that may be used alone or with other agents to treat hypertension [1,2]. Losartan and its longer acting metabolite, E-3174, lower blood pressure by antagonizing the renin-angiotensin-aldosterone system (RAAS); they compete with angiotensin II for binding to the type-1 angiotensin II receptor (AT1) subtype and prevents the blood pressure increasing effects of angiotensin II[3]. Unlike angiotensin-converting enzyme (ACE) inhibitors, ARBs do not have the adverse effect of dry cough [4]. Losartan may be used to treat hypertension, isolated systolic hypertension, left ventricular hypertrophy and diabetic nephropathy. It may also be used as an alternative agent for the treatment of systolic dysfunction, myocardial infarction, coronary artery disease, and heart failure. Perindopril erbumine is a chemically named as (2S, 3aS, 7aS)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxopentan-2-yl] amino} profanely]-octahydro-1H-indole-2-carboxylicacid [8,9]. Perindopril is a nonsulfhydryl prodrug that belongs to the angiotensin-converting enzyme (ACE) inhibitor class of medications. It is rapidly metabolized in the liver to perindoprilat, its active metabolite, following oral administration [5].

Perindoprilat is a potent, competitive inhibitor of ACE, the enzyme responsible for the conversion of angiotensin I (ATI) to angiotensin II (ATII). ATII regulates blood pressure and is a key component of the renin-angiotensin-aldosterone system (RAAS)[6]. Perindopril may be used to treat mild to moderate essential hypertension, mild to moderate congestive heart failure, and to reduce the cardiovascular risk of individuals with hypertension or post-myocardial infarction and stable coronary disease.

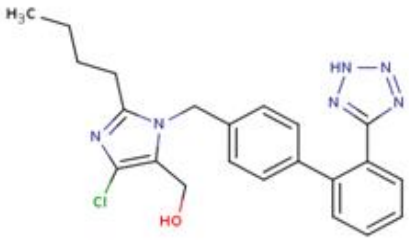

Figure 1: Structure of Losartan Potassium

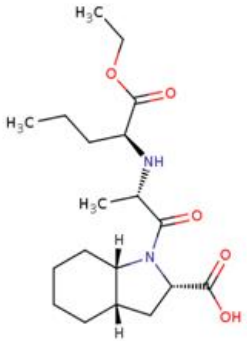

Figure 2: Structure of Prednisolone

2. Materials and Methods

Instruments:

HPLC –Waters Model NO.2690/5 series Compact System Consisting of

- Inertsil-C18 ODS column.
- Electronic balance (SARTORIOUS)
- Sonicator (FAST CLEAN)

Chemicals:

- Methanol HPLC Grade.
- Water HPLC Grade.

Raw Material:

Metformin and Vildagliptin Working Standards.

Method Development for HPLC:

The objective of this experiment was to optimize the assay method for simultaneous estimation of Perindopril erbumine and Losartan potassium on the literature survey made. So here the trials mentioned describes how the optimization was done [8].

Trial: 1

Mobile Phase: Degassed Methanol 100%.

Preparation of Standard Solution:

Weigh down 10mg's of Perindopril erbumine and Losartan potassium drugs and dissolved in 10ml of Mobile phase taken in two 10ml of volumetric flasks seperately and sonicated for 20 minutes to get 1000ppms and 1 ml was taken from each solution into a 10ml volumetric flask and diluted to 10 ml with mobile phase.

Chromatographic Conditions:

Flow rate	: 1.0ml/min
Column	: Inertsil - C18, BDS column
Detector wavelength	: 218nm
Columntemp	: Ambient
Injection volume	: 20µl
Run time	: 8min
Retention time	:2.6min for Perindopril erbumine and 2.7 for Losartan potassium

Observation:

Two peaks are merged and not separated completely. The trial 1 chromatogram result was shown in Fig:3.

Trail: 2

Mobile Phase: Degassed Methanol and orthophosphoric acid in the ratio of 60:40 V/V.

Preparation of Standard Solution: Weigh down 10mg's of Perindopril erbumine and Losartan potassium drugs and dissolved in 10ml of Mobile phase taken in two 10ml of volumetric flasks seperately and sonicated for 20 minutes to get 1000ppms and 1 ml was taken from each solution and diluted to 10 ml with mobile phase[9].

Chromatographic Conditions:

Flow rate	: 1ml/min
Column	: inertsil-C18, BDS column
Detector wavelength	: 245
Columntemp	: Ambient
Injection volume	: 20µl
Run time	: 10 min
Retention time	: 2.7min for Hepatic metabolism by hydroxylation, oxidation, glucuronidation and 4.0min for Losartan potassium

Observation:
The two peaks are separated completely but peak shapes are not good. The trial 2 chromatogram result was shown in Fig:4.

Trail: 3

Mobile Phase: Degassed Methanol and Orthophosphoric acid in the ratio of (50:50)

Preparation of Standard Solution: Weigh down 10mg's of Perindopril erbumine and Losartan potassium drugs and dissolved in 10ml of Mobile phase taken in two 10ml of volumetric flasks separately and sonicated for 20 minutes to get 1000ppms and 1 ml was taken from each solution and diluted to 10 ml with mobile phase.

Chromatographic Conditions:

Flow rate : 1.0ml/min
Column : inertsil- C18, BDS column
Detector wavelength : 218nm
Column temp : Ambient
Injection volume : 20µl
Run time : 10 min
Retention time : 2.7min for Hepatic metabolism by hydroxylation, oxidation, and glucuronidation and 3.1min for Losartan potassium

Observation: Tinidazol got peak fronting and base line between two peaks is not straight. The trial 3chromatogram result was shown in Fig:5

Optimized Method

Mobile Phase: Orthophosphoric acid and methanol in the ratio of (45:55)

Preparation of stock solution:

Reference solution: The solution was prepared by dissolving 20.0 mg of accurately weighed Perindopril erbumine 25.0 mg Losartan potassium l in Mobile phase, in two 100.0 mL volumetric flasks separately and sonicate for 20min. From the above solutions take 10.0 ml from each solution into a 50.0 mL volumetric flask and then makeup with mobile phase and sonicate for 10min [10].

Preparation of working standard solution: The stock solutions equivalent to 20ppm to 80ppm with respect to both drugs were prepared in combination of Perindopril erbumine and Losartan potassium above, sonicated and filtered through 0.45µ membrane.

Table 1: Optimized chromatographic conditions

Parameters	Method
Stationary phase (column)	Inertsil -BDS C18(250 x 4.6 mm, 5 µ)
Mobile Phase	Ortho phosphoric acid : water (55:45)
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	10 min
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	218nm
Drug RT (min)	3.016min for Perindopril erbumine and 3.434for Losartan potassium

Observation: Got chromatogram at RT's pf 3.016min to perindopril erbumine and 3.434min of Losartan potassium for standard. As shown in Table no:5

Observation: Got chromatograms perindopril erbumine and Losartan potassium for sample. As shown in Table no:6

Method Validation

(a) System Suitability:

A Standard solution was prepared by using Perindopril erbumine and Losartan potassium. Potassium working standards as per test method and was injected Five times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Perindopril erbumine and Losartan potassium, retention times and peak areas [11].

Acceptance Criteria:

1. The % RSD for the retention times of principal peak from 5 replicate injections of each Standard solution should be not more than 2.0 %
2. The % RSD for the peak area responses of principal peak from 5 replicate injections of each standard Solution should be not more than 2.0%.
3. The number of theoretical plates (N) for the Perindopril erbumine and Losartan potassium peaks is NLT 3000.
4. The Tailing factor (T) for the Perindopril erbumine and Losartan potassium peaks is NMT 2.0

Observation:

The %RSD for retention times and peak areas were found to be within the limit.

(b) Specificity:

Perindopril erbumine and Losartan potassium:

Solutions of standard and sample were prepared as per the test method are injected into chromatographic system.

Acceptence Criteria:

Chromatograms of standard and sample should be identical with near Retention time.

Observation:

The chromatograms of Standard and Sample were same identical with same retention time.

(c) Precision:

Repeatability:

System precision: Standard solution prepared as per test method and injected five times.

Method precision: Prepared six sample preparations individually using single as per test method and injected each solution.

Acceptance Criteria: The % relative standard deviation of individual Perindopril erbumine and Losartan potassium, from the six units should be not more than 2.0%. The individual assays of Perindopril erbumine and Losartan potassium shouldbe not less than 98% and not more than 102.0%.

Observation: Test results are showing that the test method is precise. Refer tables 7 and 8 for system precision. Refer tables 9 and 10 for method precision.

Intermediate precision (analyst to analyst variability):

A study was conducted by two analysts as per test method

Acceptence Criteria:

The individual assays of Perindopril erbumine and Losartan potassium should be not less than 98% and not more than

102% and %RSD of assays should be NMT2.0% by both analysts.

Observation:

Individual %assays and %RSD of Assay are within limit and passes the intermediate precision, Refer table: 11 and 12.

(d) Accuracy (Recovery):

a study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of Perindopril erbumine and Losartan potassium into each volumetric flask for each spike level to get the concentration of Perindopril erbumine and Losartan potassium equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of Perindopril erbumine and Losartan potassium were calculated [12].

Acceptance Criteria:

The mean % recovery of the Perindopril erbumine and Losartan potassium at each spike level should be not less than 98.0% and not more than 102.0% for both the drugs separately.

Observation: Refer tables: 13 and 14.Observation

$$\% \text{ Recovery} = \frac{\text{Amount found}}{\text{Amount added}} \times 100$$

The recovery results indicating that the test method has anacceptable level of accuracy.

(e) Ruggedness of Test Method:

a) System to system variability:

System to system variability study was conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared and each was analyzed as per test method. Comparison of both the results obtained on two different HPLC systems, shows that the assay test method are rugged for System to system variability.

Acceptance Criteria:

The % relative standard deviation of Perindopril erbumine and Losartan potassium from the six sample preparations should be not more than 2.0%**.** The % assay of Perindopril erbumine and Losartan potassium should be between 98.0%-102.0%.

Observation: The % RSD was found within the limit.

b) Column to column variability:

Column to column variability study was conducted by using different columns. Six samples were prepared and each was analyzed as per test method

Acceptance Criteria:

The %RSD of Perindopril erbumine and Losartan potassium tablets should be NMT2.0%. The %assay of Perindopril erbumine and Losartan potassium should be between 98.0% and 102.0% for individual drugs.

Observation: The results obtained by comparing with both two types were within limit.

(f) Robustness:

a) Effect of variation of flow rate:

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 1.0ml/min and 1.2ml/min. The system suitability parameters were evaluated and found to be within the limits for 1.0ml/min and 1.2ml/min flow.

Perindopril erbumine and Losartan potassium and was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates 1.0ml/min.

Acceptance Criteria:

The Tailing Factor of Perindopril erbumine and Losartan potassium standards should be NMT 2.0 for Variation in Flow.

Observation:

The tailing factor for Perindopril erbumine and Losartan potassium was found to be within the limits.

b) Effect of variation of temperature:

A study was conducted to determine the effect of variation in temperature. Standard solution prepared as per the test method was injected into the HPLC system at 20ºC temperature. The system suitability parameters were evaluated and found to be within the limits for a temperature change of 20ºc. Similarly sample solution was chromatographed at 25ºC temperature. Perindopril erbumine and Losartan potassium were resolved from all other peaks and the retention times were comparable with those.

Acceptance Criteria:

The Tailing Factor of Perindopril erbumine and Losartan potassium standard and sample solutions should be NMT 2.0 for Variation in temperature.

Observation:

The tailing factor for Perindopril erbumine and Losartan potassium is found to be within the limits.

(g) Linearity of Test Method:

A Series of solutions are prepared using Perindopril erbumine and Losartan potassium working standards at concentration levels from 25ppm to 150 ppm of target concentration. Measure the peak area response of solution at Level 1 and Level 6 six times and Level 2 to Level 5 two times.

Acceptance Criteria:

Correlation Coefficient should be not less than 0.9990.

% of y- Intercept should be ±2.0.

% of RSD for level 1 and Level 6 should be not more than 2.0%.

Observation:

The linear fit of the system was illustrated graphically. The results are presented in table 15,16..

(h) Limit of Detection and Quantitation (LOD and LOQ):

From the linearity data calculate the limit of detection and quantitation, using the following formula.

$$\text{LOD} = \frac{3.3\,\sigma}{S}$$

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

$$\text{LOQ} = \frac{10\,\sigma}{S}$$

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

3. Results and Discussion

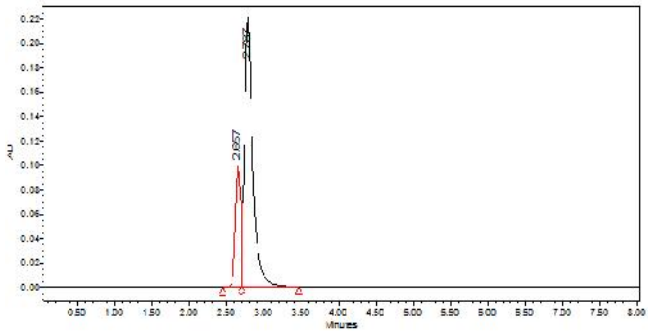

Figure 3: Chromatogram of Trial I

Table 2: Retention time of Losartan potassium and perindopril erbumine

S.NO	Name of the peak	Retention time (min)
1	Perindopril erbumine	2.6
2	Losartan potassium	2.7

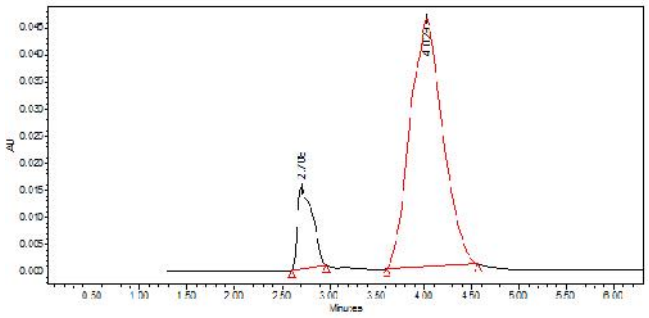

Figure 4: Chromatogram of Trial II

Table 3: Retention Time of Losartan potassium and perindopril erbumine

S.NO	Name of the peak	Retention time (min)
1	Perindopril erbumine	2.7
2	Losartan potassium	4.0

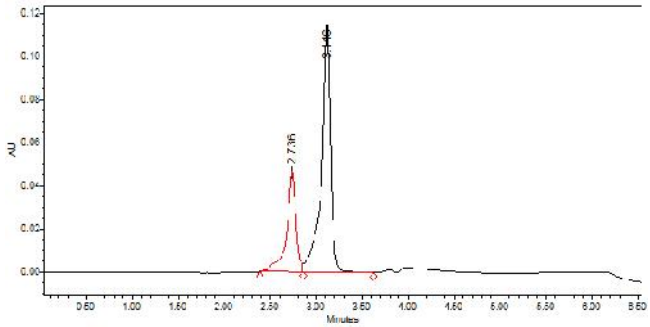

Figure 5: Chromatogram of Trial III

Table 4: Retention Time of Losartan potassium and perindopril erbumine

S.NO	Name of the peak	Retention time (min)
1	Perindopril erbumine	2.7
2	Losartan potassium	3.1

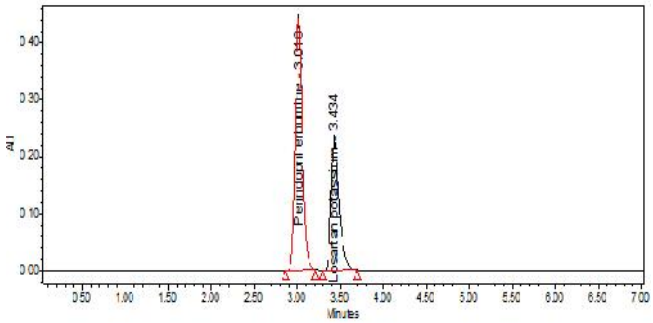

Figure 6: Chromatogram of standard

Table 5: Retention Time of Losartan potassium and perindopril erbumine

S.NO	Name of the peak	Retention time (min)
1	Perindopril erbumine	3.016
2	Losartan potassium	3.434

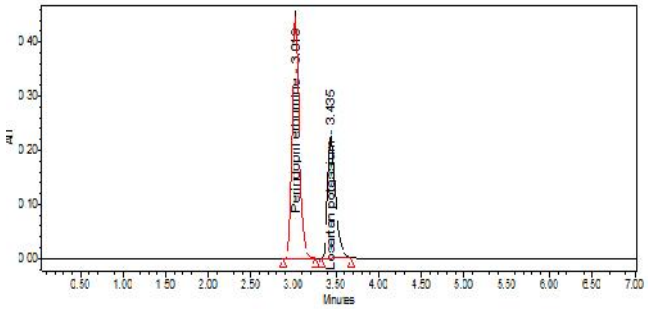

Figure 7: Chromatogram of sample

Table 6: Retention Time of Losartan potassium and perindopril erbumine

S.NO	Name of the peak	Retention time (min)
1	Perindopril erbumine	3.019
2	Losartan potassium	3.435

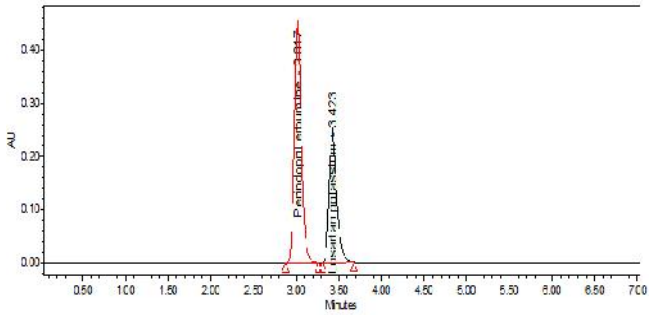

Figure 8: Chromatograms of system precision

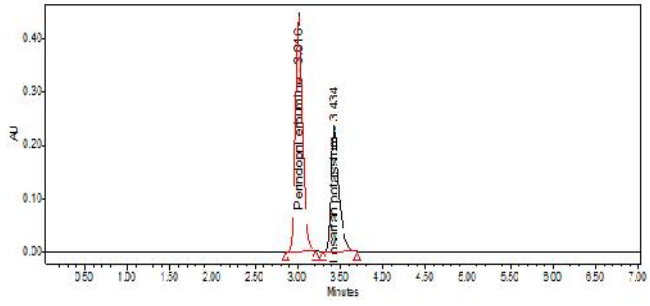

Figure 9: Chromatograms of Repeatability STD–1

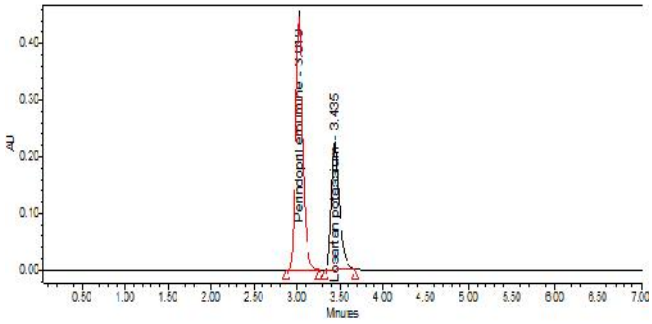

Figure 10: Chromatograms of Repeatability STD–2

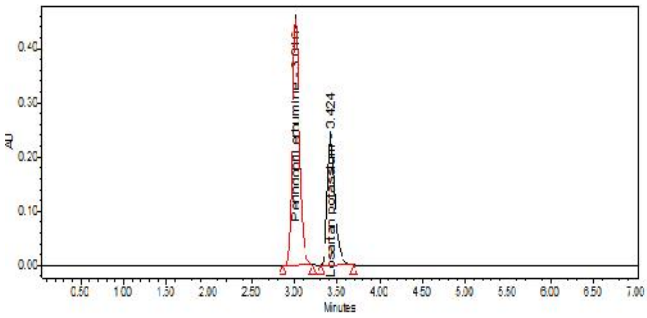

Figure 11: Chromatograms of Intermediate precision

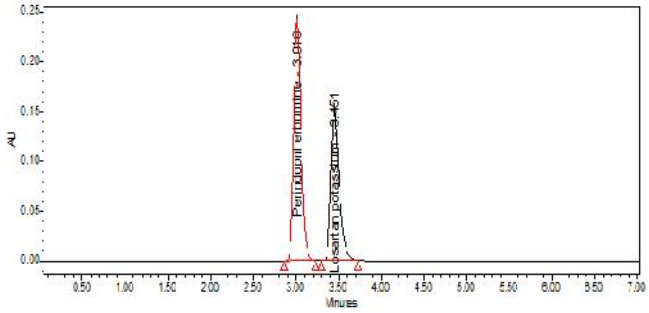

Figure 12: Chromatogram for accuracy (50%) STD-1

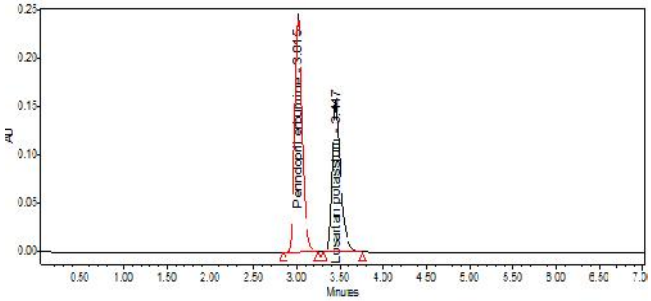

Figure 13: Chromatogram for accuracy (50%) STD-2

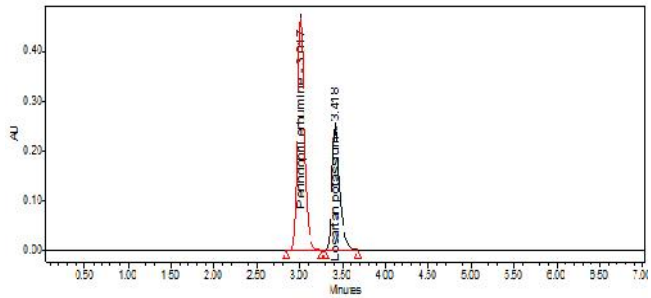

Figure 14: Chromatogram for accuracy (100%) STD-1

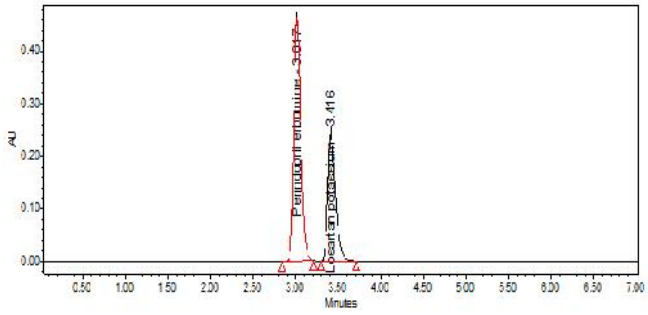

Figure 14: Chromatograms for accuracy (100%) STD-2

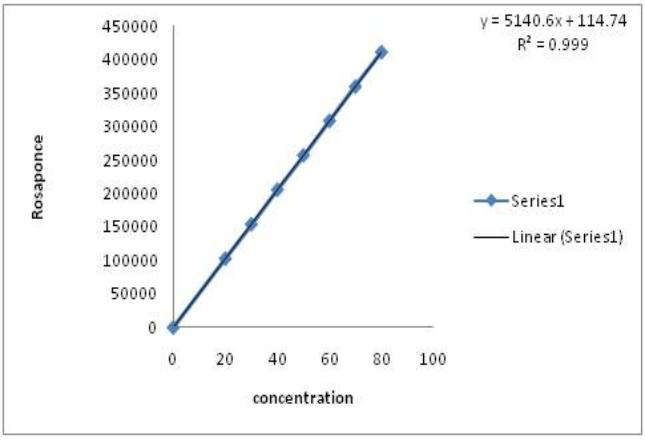

Figure 16: Linearity Plot (Concentration Vs Response) of Perindopril erbumin

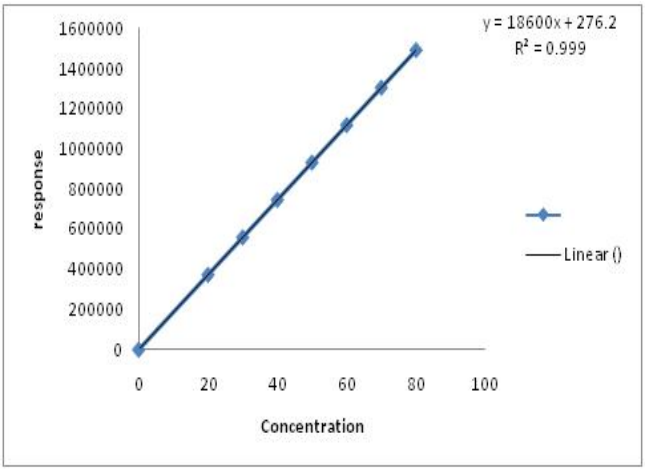

Figure 17: Linearity Plot (Concentration Vs Response) of Losartan potassium

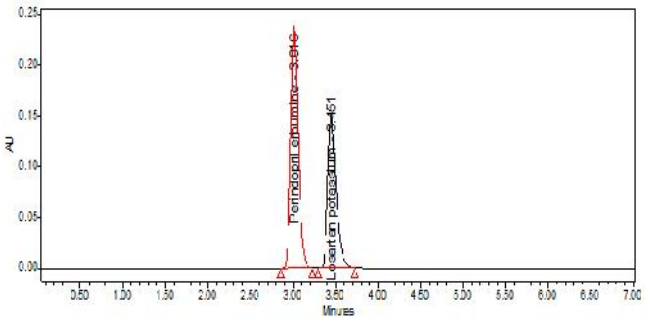

Figure 18: Chromatograms for 20 ppm

Table 7: Data of Repeatability (System precision) for Perindopril erbumine

	Injection	Peak Areas of Perindopril erbumine	% Assay
Concentration 40ppm	1	205625	99.95
	2	206225	100.24
	3	205840	100.06
	4	204283	99.30
	5	205735	100.00
Statistical Analysis	**Mean**	205541.6	99.91
	SD	739.0046	0.35819
	% RSD	0.35	0.35

Table 8: Data of Repeatability (System precision) for Losartan potassium

	Injection	Peak Areas of Losartan potassium	% Assay
Concentration 40ppm	1	734360	98.66
	2	739098	99.30
	3	755696	101.53
	4	748289	100.53
	5	744147	99.98
Statistical Analysis	**Mean**	744318	100.00
	SD	8241.164	1.107678
	% RSD	1.1	1.10

Table 9: Data of Repeatability (Method precision) for Perindopril erbumine

	Injection	Peak Areas of Perindopril erbumine	% Assay
Concentration 40ppm	1	202110	98.6
	2	203700	99.02
	3	201851	98.12
	4	202255	98.31
	5	203283	98.81
Statistical Analysis	**Mean**	202349	98.36
	SD	202687.6	98.48
	% RSD	771.5483	0.352647

Table 10: Data of Repeatability (Method precision) for Losartan potassium

	Injection	Peak Areas of Perindopril erbumine	% Assay
Concentration 40ppm	1	733495	98.55
	2	735992	98.88
	3	739828	99.40
	4	739098	99.30
	5	748289	100.53
Statistical Analysis	**Mean**	731322	98.28
	SD	738004	99.278
	% RSD	5988.879	0.827236

Table 11: Data of Intermediate precision for Perindopril erbumine

	Injection	Peak Areas of Perindopril erbumine	% Assay
Concentration 40ppm	1	202110	98.6
	2	203700	99.02
	3	201851	98.12
	4	202255	98.31
	5	203283	98.81
Statistical Analysis	**Mean**	202349	98.36
	SD	202687.6	98.48
	% RSD	771.5483	0.352647

Table 12: Data of Intermediate precision (Analyst 2) for Losartan potassium

	Injection	Peak Areas of Perindopril erbumine	% Assay
Concentration 40ppm	1	736792	99.99
	2	734360	99.66
	3	755696	101.53
	4	744147	99.98
	5	744127	99.97
Statistical Analysis	**Mean**	752525	101.10
	SD	744607.8	100.37
	% RSD	8392.59	0.753536

Table 13: Data of Accuracy for Perindopril erbumine

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50%Injection 1	20	20.15	100.75	**MEAN**	99.69333
50%Injection 3	20	19.86	99.31	**%RSD**	0.92
100 % Injection 1	40	19.80	99.02	**MEAN**	99.83333
100 %Injection 2	40	39.88	99.70		
100%Injection 3	40	40.12	100.30	**%RSD**	0.41
150%Injection 1	60	39.80	99.50	**MEAN**	99.97333
150%Injection 2	60	60.12	100.21		
150%Injection 3	60	59.76	99.61	**%RSD**	0.31

Table 14: Data of Accuracy for Losartan potassium

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50%Injection 1	20	20.04	100.22	**MEAN**	100.06
50%-Injection3	20	19.97	99.85	**% RSD**	0.18
100 %Injection 1	40	20.02	100.11	**MEAN**	100.04
100 %Injection 2	40	40.01	100.02		
100%Injection 3	40	40.05	100.14	**%RSD**	0.091
150%Injection 1	60	39.98	99.96	**MEAN**	100.02
150%Injection 2	60	60.08	100.14		
150%Injection 3	60	59.97	99.96	**%RSD**	0.09

Table 15: Data of Linearity (Perindopril erbumine)

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	5140
20	102965	y-Intercept	114.7
30	154371	Correlation Coefficient	0.999

Table 16: Data of Linearity (Losartan potassium)

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	18600
20	372546	y-Intercept	276.2
30	558296	Correlation Coefficient	0.999

4. Conclusion

The maximum absorbance was found to be at 215nm for Perindopril erbumine and 256nm for Losartan potassium. Common wavelength is 218nm and the peaks purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Inertrsil C_{18}, ODS chosen good peak shape. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area, satisfactory retention time and good resolution. Different ratios of mobile phase were studied with ratio of 45:55 orthophosphoric acid: water was fixed due to good symmetrical peaks and for good resolution. So this mobile phase was used for the proposed study. The present recovery was found to be 98.0-101.50 was linear

and precise over the same range. Both system and method precision was found to be accurate and well within range. Detection limit was found to be 0.56 for Perindopril erbumine and 0.57 for Losartan Losartan potassium. Linearity study was, correlation coefficient and curve fitting was found to be 0.999. The analytical method was found linearity over the range of 20-80ppm of the target concentration for both the drugs. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

5. References

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