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QUANTITATIVE METHOD DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR THE ESTIMATION OF PERINDOPRIL ERBUMINE IN PHARMACEUTICAL DOSAGE FORM

Kalyani Bhongade*, Shailesh Jawarkar and Jagruti Dolas

India.

***Corresponding Author: Kalyani Bhongade**
India.

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ABSTRACT
A new rapid, precise and sensitive reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed and validated for the estimation of Perindopril erbumine in bulk and pharmaceutical dosage form. The Perindopril erbumine was estimated on an isocratic method, C18 column, utilizing a mobile phase composition of acetonitrile: water: 70% perchloric acid (45:55:2), v/v, pH 5.0) at a flow rate of 1.0 mL/min with UV detection at 216 nm. The retention time of Perindopril erbumine was 5.08 min respectively. The developed method was validated for specificity, linearity, precision, accuracy, robustness as per ICH guidelines. Linearity for Perindopril erbumine was found in the range of 30.6854 µg/ml. The percentage recoveries for Perindopril erbumine ranged from 98.0-102.0 %. All the parameters were found to be within limits, which indicate that the above method was accurate and precise. Hence, it was concluded that the developed method is suitable for routine analysis of perindopril erbumine due to its less analysis time in the bulk and pharmaceutical dosage forms.

KEYWORDS: Perindopril erbumine, Estimation, RP-HPLC, Validation, Method development.

INTRODUCTION
Perindopril erbumine (PDE) is a tert-butylamine ester of 1-[2S, 3(a)S, 7(a)S]-1-[(S)-N-[(S)-1-Carboxybutyl]alanyl]hexahydro-2-indolinecarboxylic acid, as shown in Figure 1A. Clinically, it is used as a long-acting angiotensin-converting enzyme (ACE) inhibitor in the treatment of hypertension, heart failure and stable coronary artery disease.[1] Inhibition of ACE results in decreased plasma angiotensin II, leading to decreased vasoconstriction, increased plasma rennin activity and decreased aldosterone secretion. The overall effect of this is a drop in blood pressure and a decrease in the workload of the heart.[2] It is rapidly metabolized in liver to perindoprilat, which is an active metabolite that is excreted in urine.[3] 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).[4] The purpose of the present study was to develop a simple, sensitive, accurate and precise HPLC method for the determination of perindopril erbumine in pharmaceutical formulations. The developed method has been validated by evaluation of the system suitability, linearity, specificity, robustness, assay[5], precision and accuracy. Also, the present work is designed to develop a sensitive, precise and selective stability-indicating RP-HPLC method for the estimation of PDE (perindopril erbumine) in bulk drug and dosage form under various stress conditions. A search for stability studies on PDE revealed that a reversed-phase (RP)-HPLC method was reported based on pH-dependent degradation kinetics.[6] The developed method is validated as per International Conference on Harmonization (ICH) Q2 (R2) guidelines.[7-8]

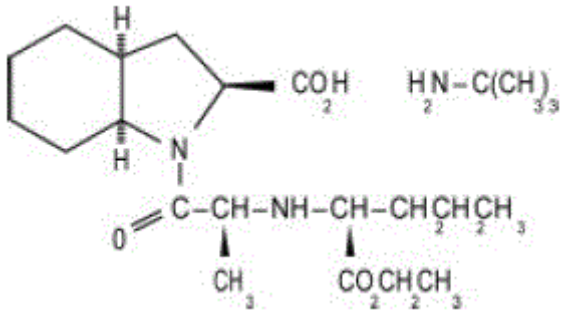

Fig.no.1: Structure of Perindopril Erbumine.

EXPERIMENTAL
MATERIAL AND REAGENTS
The chemicals used were of analytical reagent grade and HPLC grade. HPLC grade acetonitrile, water (HPLC grade), perchloric acid (70%) were used for preparation

of mobile phase. Hydrochloric acid, sodium hydroxide pellets, hydrogen peroxide (30%) were used for force degradation studies. Pharmaceutical grade perindopril erbumine was used as received. Other reagents potassium dihydrogen orthophosphate anhydrous (99.5%) as buffer solution, HPLC grade methanol were used for trials of method development.

INSTRUMENTATION

A High pressure liquid chromatograph (HPLC Gradient System) with two P-3000-M Reciprocating(40MPa) pumps, variable wavelength programmable UV-Visible detector UV-3000-M, and C18 (Hypersil BDS C_{18} (250 mm x 4.6 mm 5 µ) column was used. The HPLC system was equipped with the software Autochro -3000 series version 5.03.

PROCEDURES

Chromatographic conditions

The separation was achieved on the column Hypersil BDS C18 column (4.6mm x 250mm; 5.0 µm) using the mobile phase Acetonitrile (ACN): Water: 70% Perchloric acid at a flow rate of 1 ml/min. The detector wavelength was set at 216 nm.

CALIBRATION GRAPH

Working standard solutions of perindopril erbumine were prepared by appropriate dilution of stock standard solution with the diluent solution. 20 µl aliquot of each solution was injected automatically on to the column for several times of attempt and the chromatograms were recorded. Calibration graph was prepared by plotting the mean peak area versus concentration of PE. The concentration of the unknown was read from the calibration graph or computed from the regression equation derived using the mean peak area-concentration data.

METHOD DEVELOPMENT AND OPTIMIZATION

To optimize the chromatographic conditions, the effect of chromatographic variables such as mobile phase, pH, flow rate and solvent ratio were studied. Various solvent systems were tried for the development of a suitable HPLC method for determination of perindopril erbumine in bulk drug and tablet dosage form. Different mobile phases and the diluent used for the method development were different. Diluent used was 0.2% perchloric acid. Mobile phase tried for this purpose were methanol : water (80 : 20), 0.1 M monopotassium phosphate (buffer) : methanol (20 :80), 0.1% OPA as buffer : acetonitrile acid (50 : 50), acetonitrile : water : 70% perchloric acid (450 : 550 : 2). The peak shape was improved with the mobile phase acetonitrile: water: 70% perchloric acid (450: 550: 2) and diluent 0.2% perchloric acid. The condition that gave best the best peak shape and which are according to the acceptance criteria was selected. HPLC conditions are given in Table no.4.

Preparation of mobile phase

450 ml of acetonitrile acid was mixed with 550 ml of water and 2ml of 70% perchloric acid. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µ filter under vacuum.

Preparation of diluent

2ml of 0.2% perchloric acid is added to the volumetric flask of 1000 ml and volume is adjusted up to the mark of 1000 ml (1 liter).

Preparation of standard solution

Standard stock solution of 1000 µg/ml was prepared by dissolving 10 mg of perindopril erbumine in 10 ml of diluent. From this stock solution, 0.5 ml was pipetted out and the volume was made up to 10 ml with the diluent to prepare working standard solution of 50µg/ml.

Preparation of sample solution

Weigh accurately tablets, powdered equivalent to about 10 mg of perindopril erbumine and transfer it into a 10 ml volumetric flask. Add about 7 ml of diluent and sonicate to dissolve. The volume was made up to the mark and the solution was filtered through 0.45 µ filter under vacuum. From this stock solution, 0.5 ml was pipetted out and the volume was made up to 10 ml with the diluent to prepare sample solution of 50µg/ml.

Assay[5]

Inject 20 µl of the standard and sample solution into the chromatographic system and measure the areas for the perindopril erbumine and calculate the % assay by using the formula. The standard and sample chromatograms were shown in fig. 3.

Formula:

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.wt}{\text{Label claim}} \times 100$$

Where:
AT = average area counts of sample preparation.
AS = average area counts of standard preparation.
WS = Weight of working standard taken in mg.
P = Percentage purity of working standard LC = Label Claim of drug mg/ml.

Method validation

The method was validated as per ICH (Q2) guidelines with respect to assay, linearity, accuracy, precision, specificity, robustness.[9-11]

System Suitability

Injected the replicate five standard solution into the chromatograph, recorded the chromatograms and measured the area counts, retention time, % RSD for area and retention time of standard solution, also theoretical plates, tailing factor for perindopril erbumine chromatographic peak.

Acceptance criteria

The % RSD for area response obtained from five replicate injections of Standard solution should be ≤ 2.0
Tailing factor for perindopril erbumine peak should be ≤ 2.0 in Standard solution.
Theoretical plates for perindopril erbumine peak should ≥ 2000 in Standard solution.

Table no.1: system suitability parameter.

Sr.no.	Parameter	Perindopril Erbumine
1	Retention time	5.08 min
2	Theoretical plate	14251
3	Tailing factor	1.13
4	Area	1552.3090

Linearity and range

Calibration curve was constructed by plotting the mean peak area versus concentration which was linear over the concentration range 25-75 µg/ml. Using the regression analysis, the linear equation, Y = 30.685x - 7.5196, was obtained, where Y is the mean peak area and X concentration in µg/ml. The Linearity co-efficient of mean res ponse of replicate determination plotted against respective concentration was found to be 0.9994.

Accuracy

The accuracy of an analytical method expresses the closeness of agreement between the value, which is accepted reference value, and the value found. Accuracy studies were done by the standard addition method. Accuracy is expressed as % recovery of the standard spiked to previously analyzed test sample of tablet.[6] The active ingredients were spiked in previously analyzed tablet powder sample at different concentration levels viz. 50%, 100%, and 150% each of the labeled claim and injected in developed chromatographic conditions in triplicate. The percentage recoveries were then calculated. The recovery data for accuracy studies were shown in Table no. 11. The accuracy chromatograms were shown in fig no.16, 17, and 18.

Precision

The precision of the method was evaluated in terms of intermediate precision (intra-day and inter-day). Three different concentrations of PE were analyzed in five replicates during the same day (intra-day precision) and three consecutive days (inter-day precision). Within each series, every solution was injected in triplicate. The RSD values of intra-day and intra-day studies (<1%) showed that the precision of the method was satisfactory. The results of this study are given in Table-12A and 12B.

Robustness

Robustness of the method was determined by small deliberate changes in flow rate, change in wavelength and column oven temperature. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was robust. The results of robustness were presented in table 16 and 17.

RESULT AND DISCUSSION

Preliminary studies on perindopril Erbumine:

I. Melting point

The procured reference standard of perindopril erbumine was found to melt in the range of 126-128^{0}C.

II. Solubility

The drug was found to be

- Freely soluble in methanol and Perchloric acid solution, water.

III. UV spectroscopy

Determination of λ_{max} and selection of Analytical Wavelength

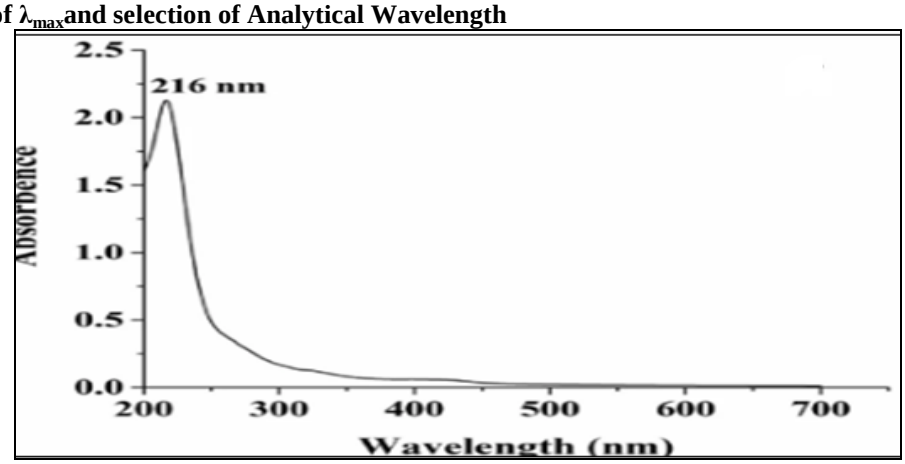

Fig no.2 Determination of λ_{max} of perindopril Erbumine and Selection of Analytical Wavelength.

IV. Optimization of chromatographic condition.

Table no.2: Optimization of chromatographic condition.

Sr.no	Parameters	Specification
1	HPLC	Youngling Acme 9000
2	Software	Autochro-3000
3	Column	Hypersil BDS C18 column (4.6mm x 250mm; 5.0 µm)
4	Particle size packing	5µm
5	Stationary phase	Hypersil BDS C18
6	Detected wavelength	216nm
7	Flow rate	1ml/min
8	Temperature	Ambient
9	Run time	15 min.
10	Filter paper	Nylon 6,6 membrane filter 0.45 µm.

V. Development of Method Validation for Estimation of Drug

Method development: Various solvent systems were tried for the development of a suitable HPLC method for determination of perindopril erbumine in bulk drug and tablet dosage form. Different mobile phases and the diluents were used for the purpose of method development were different.

Table no.3 Showing different mobile phase and diluent for method development.

Sr.no	Mobile phase	Diluent	Observation
1	methanol : water (80 : 20)	Same as mobile phase	Peak shape at retention time was poor.
2	0.1 M monopotassium phosphate (buffer) : methanol (20 :80)	Same as mobile phase	Splitting was observed at retention time.
3	0.1% OPA as buffer : acetonitrile acid (50 : 50)	Same as mobile phase	Splitting was observed at the base.
4	acetonitrile : water : 70% perchloric acid (450 : 550 : 2)	Same as mobile phase	Peak shape was improved but theoretical plate and tailing factor was not in acceptance criteria.
5	acetonitrile : water : 70% perchloric acid (450 : 550 : 2)	0.2% perchloric acid	Peak shape was good with theoretical plate value and tailing factor was found to be with in acceptance criteria.

Table no. 4: The condition that gave best peak shape was selected.

Sr. no.	Parameter	Specification
1	Column	Hypersil BDS C18 column (4.6mm x 250mm; 5.0 µm)
2	Flow rate	1.0 ml per minute.
3	Injection volume	20 µL.
4	Oven Temperature	Ambient
5	Wavelength	216 nm
6	Mobile phase	Acitonitril: water: 70% perchloric acid. (45: 55: 0.2)
7	Diluent	0.2% perchloric acid

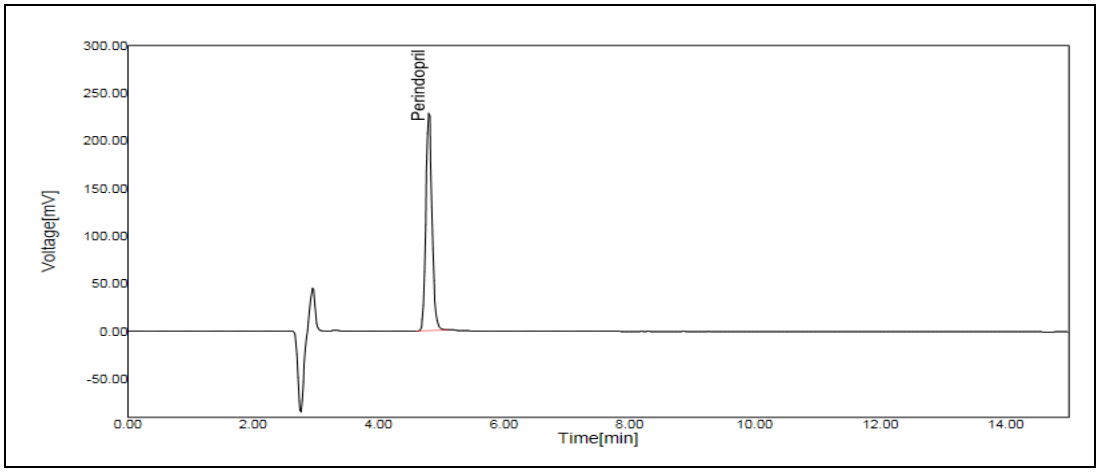

Fig.no.3: Chromatogram of the condition that gave the best peak shape (developed method).

Observation
Peak shape was found improved also theoretical plate and tailing factor was found according to the acceptance criteria, i.e. the theoretical plate value of peak was found greater than 2000 and tailing factor was observed below 2%. Hence this method was suitable.

The Method was developed with satisfactory improved peak shape and with all values according to the acceptance criteria for the perindopril erbumine.

VI. Method Validation Parameter of HPLC.

A) System suitability

The RSD value of peak area and retention time for drugs were within 2% indicating the suitability of the system the values are summarized in (Table no.5).

Table No.5: system suitability Test for perindopril erbumine.

Sr. No.	Std.inj.no.	Peak area	Retention time (min)	Theoretical plate	Tailing factor
1	Std.inj.no.1	1563.7798	5.03	1.15	21262
2	Std.inj.no.-2	1560.2791	4.98	1.13	20943
3	Std.inj.no.-3	1567.1275	4.99	1.15	20817
Mean		1563.729	5	-	-
SD		3.424485	0.026458	-	-
%RSD		0.218995	0.52915	-	-

Observation
All the parameters of system suitability were observed and all the values were within the limits for perindopril erbumine.

B) Specificity

To ensure that there is no interference from blank at the retention of peak of interest, also to be compared the retention times obtained for perindopril erbumine peaks in Standard Solution and Sample Solution.

Table No.6: specificity Stuidies for test and blank solution.

Name of solution	Peak area	Retention time
Blank	0.000	No peak
Test solution	1529.0985	4.85

Table No.7: specificity Stuidies for standard perindopril erbumine solution.

Name of solution	Peak area	Retention time
Standard solution inj-1	1563.7798	4.80
Standard solution inj-2	1563.6798	4.88
Standard solution inj-3	1560.2791	4.90
Standard solution inj-4	1567.1275	4.79
Standard solution inj-5	1560.1285	4.83
Mean	1562.9989	4.84
SD	2.9049645	0.05
%RSD	0.19	1.00

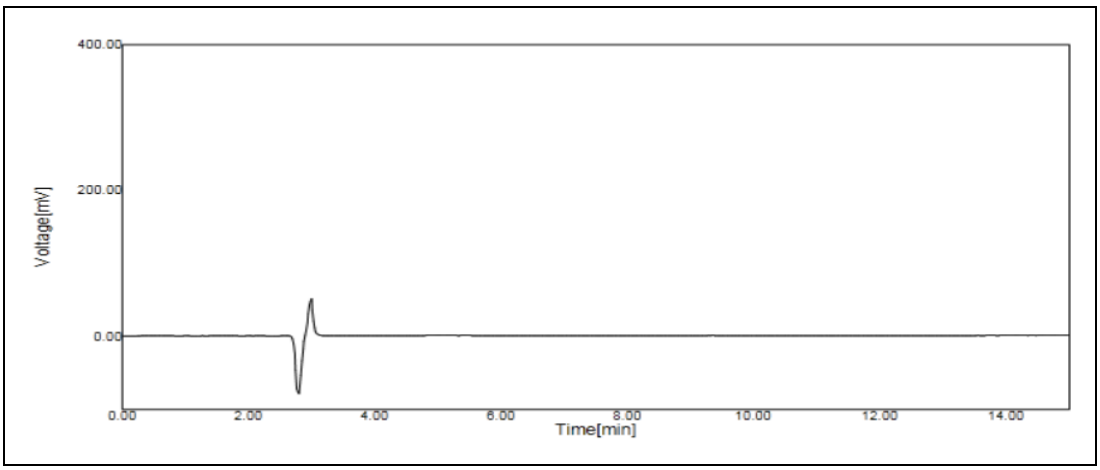

Fig.no.4: Chromatogram showing no interference of blank at the retention time of perindopril erbumine.

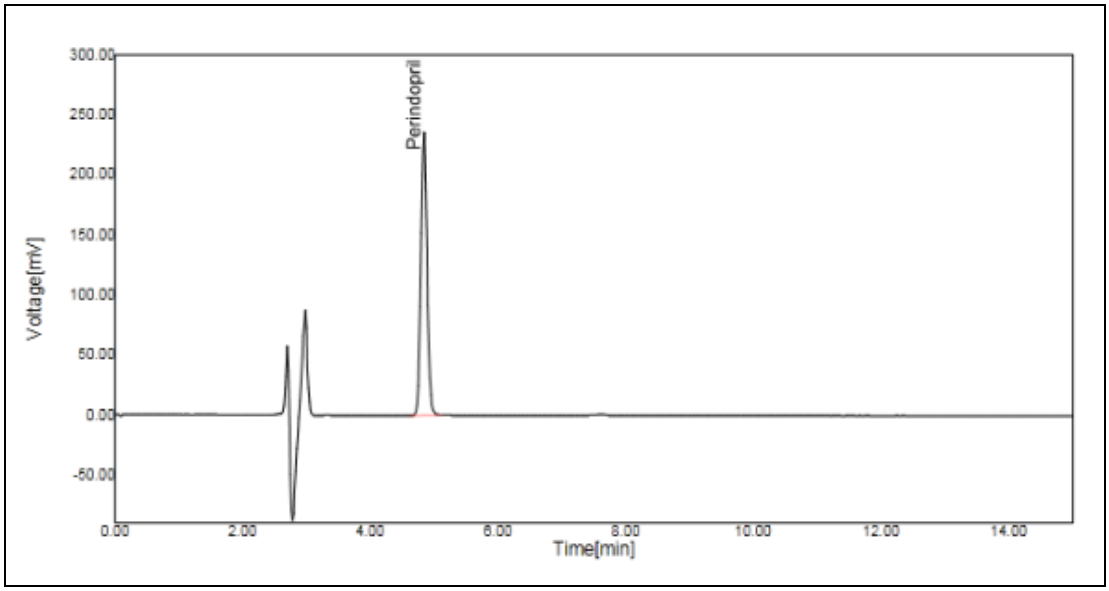

Fig.no.5: Chromatogram showing test of perindopril erbumine.

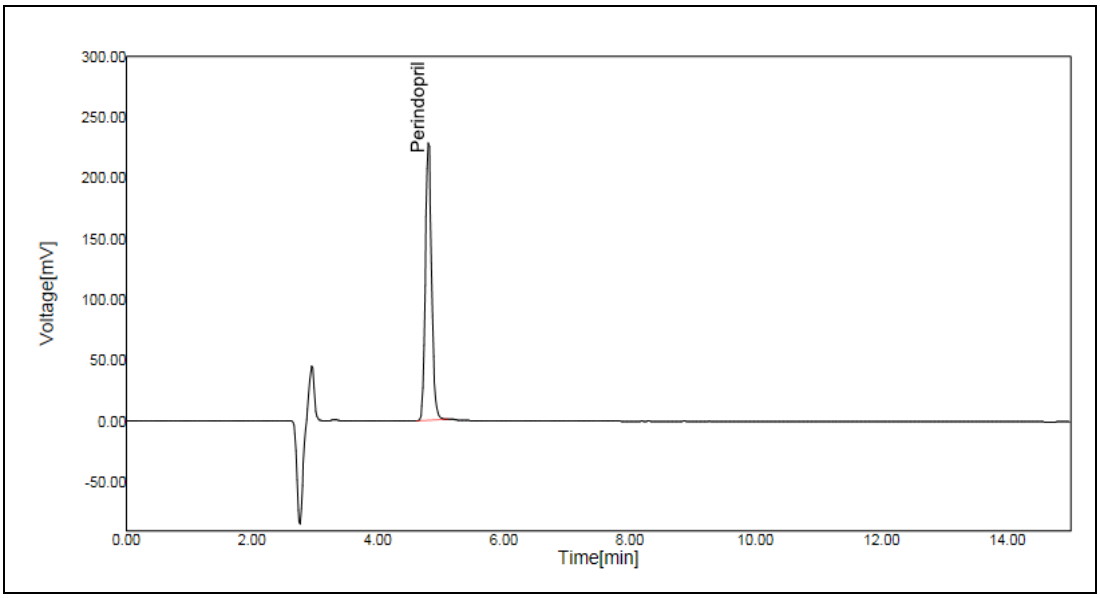

Fig.no.6: Chromatogram showing standard solution of perindopril erbumine.

Observation

The specificity was performed for the developed method and it was observed that there is no interference from blank at the retention of peak of interest.

C) **Linearity and Range**

In these parameter range and linearity of perindopril erbumine concentration range was (r>0.9987), so that these parameter properly performed. Also concentration 25.05, 35.07, 50.10, 65.13, 75.15 ppm or ug/ml properly plot calibration graph on HPLC.

Table No.8: Standard Calibration curves of perindopril erbumine

Sr. No.	Con. (ppm or ug/ml)	Area
1	25.05	739.7516
2	35.07	1074.8964
3	50.10	1557.2593
4	65.13	2004.3132
5	75.15	2272.8848
Slope	30.6854	
Intercept	-7.5196	
Correlation coefficient	0.9994	

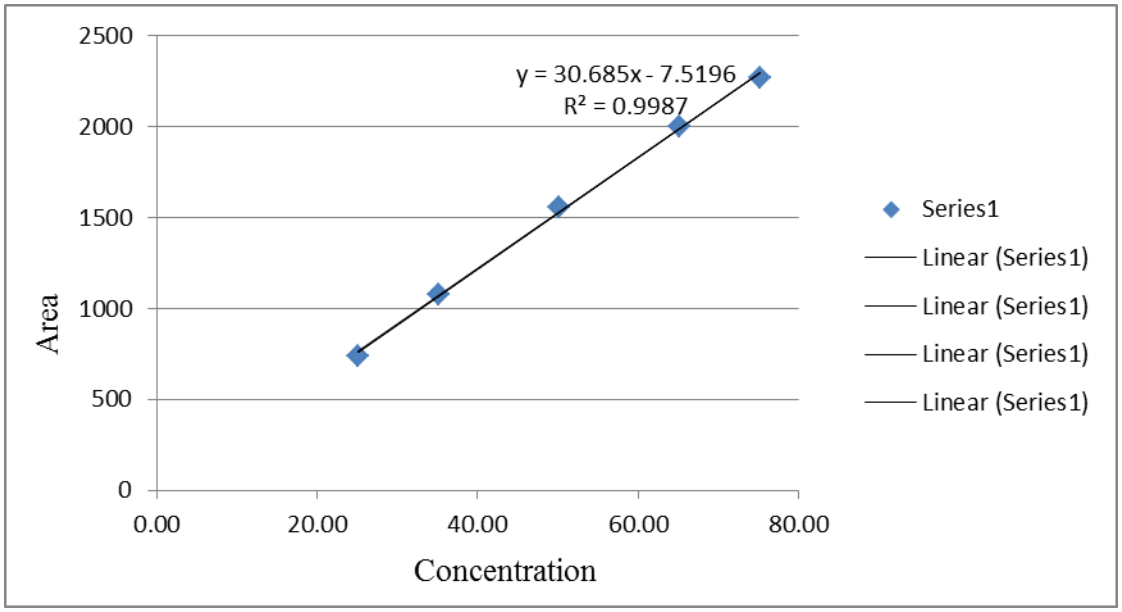

Fig.no.7: Standard calibration Curve of perindopril erbumine.

In calibration curves the Correlation coefficient (r) & the regression equation (**y**) for perindopril erbumine were calculated and shown in above Figure. It indicates the capability of developed method to estimate the drugs over the desired concentration range.

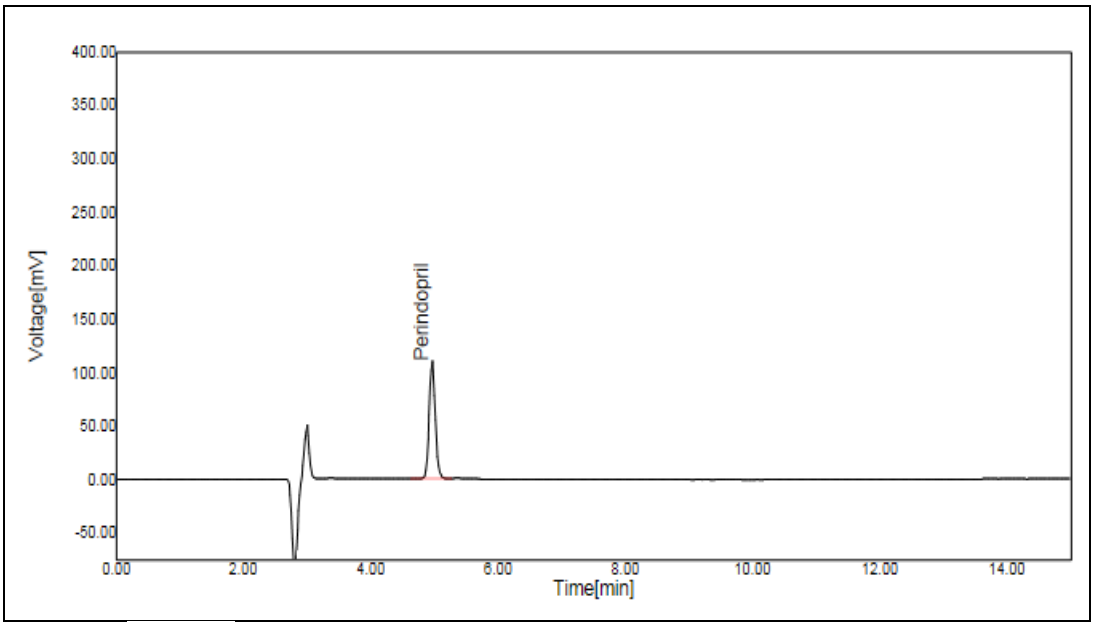

Fig.no.8: Chromatogram of linearity for perindopril erbumine in 25 ppm.

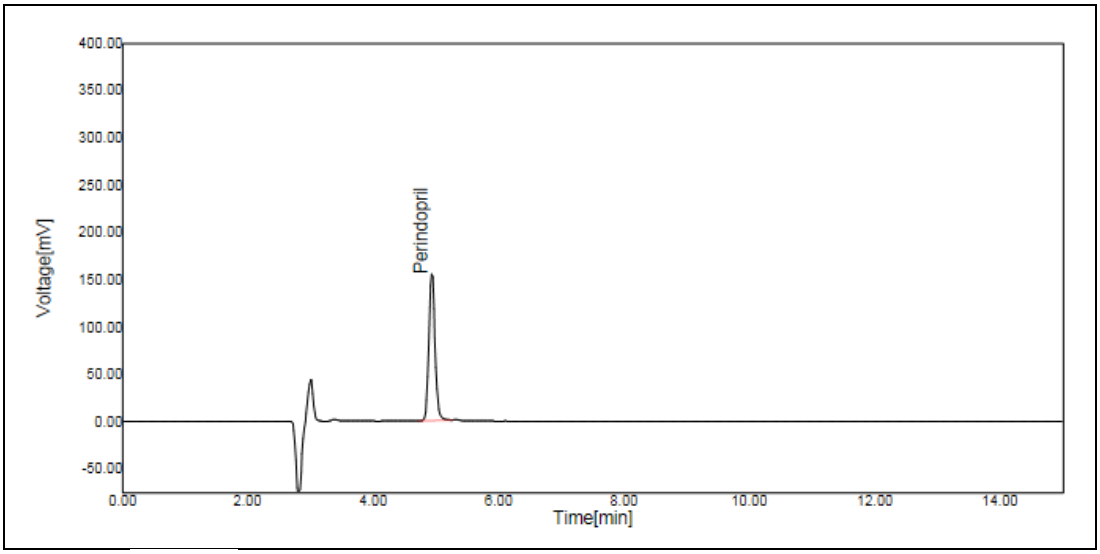

Fig.no.9: Chromatogram of linearity for perindopril erbumine in 35 ppm.

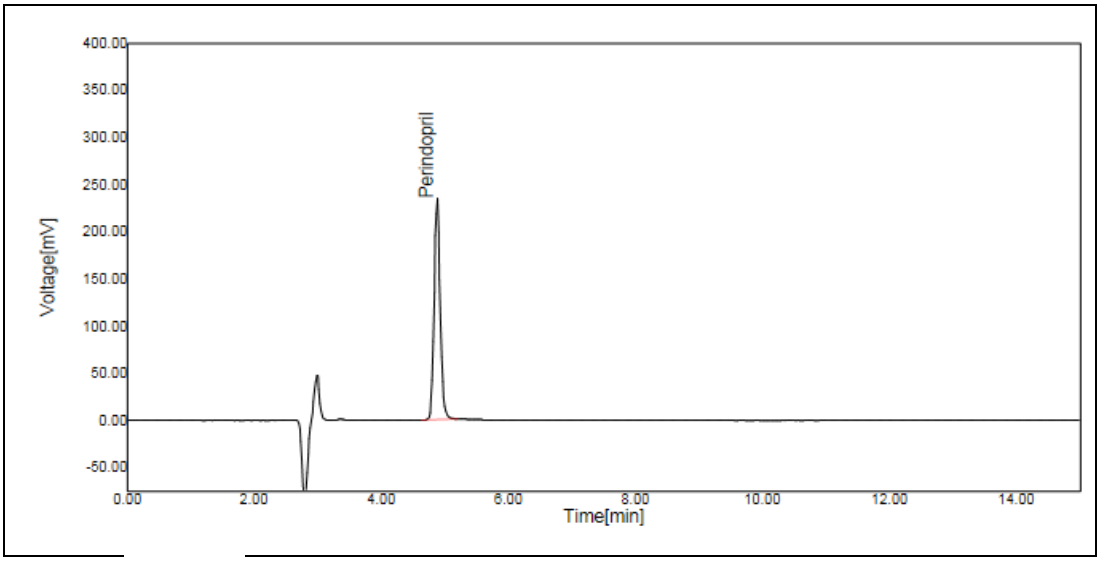

Fig.no.10: Chromatogram of linearity for perindopril erbumine in 50 ppm.

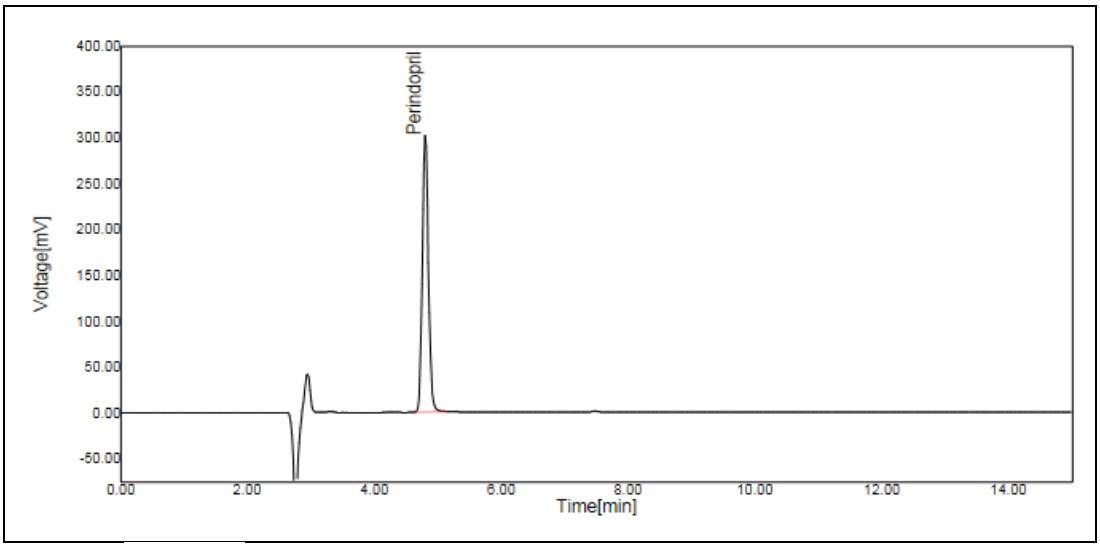

Fig.no.11: Chromatogram of linearity for perindopril erbumine in 65 ppm.

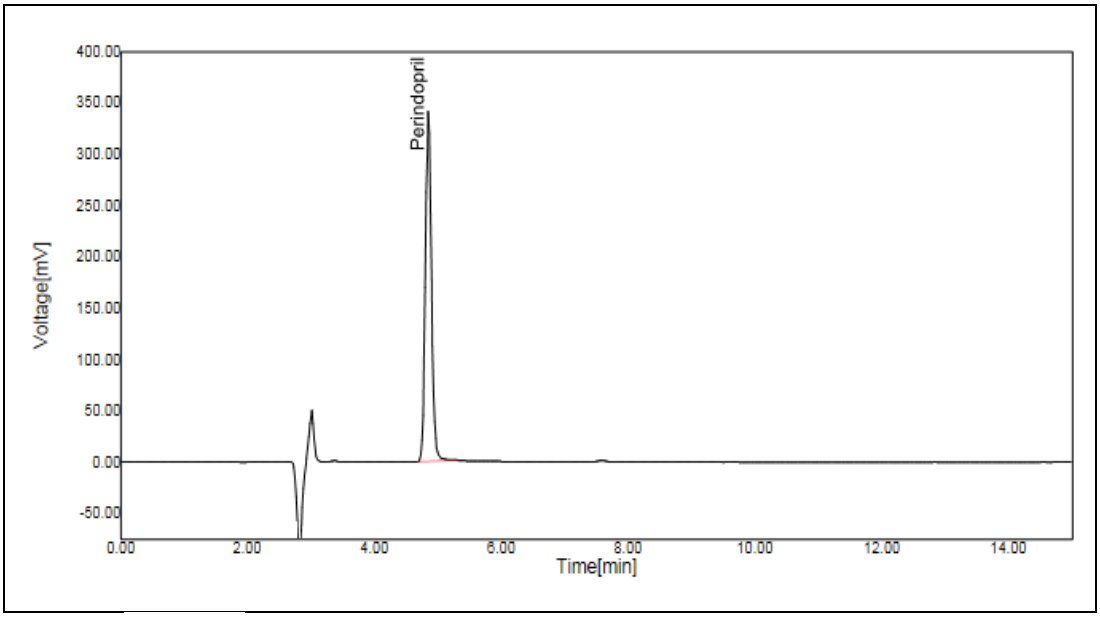

Fig.no.12: Chromatogram of linearity for perindopril erbumine in 75 ppm.

For perindopril erbumine
y=30.685x-7.5196
r = 0.9994

Observation
The linearity parameter was calculated and plotted for the developed method of PE on various concentrations and it was observed that the developed method was linear.

Assay
Initially content Perindopril erbumine in mg was calculated using area observed in standard and test solutions, the assay of perindopril erbumine was calculated by using observed PE content in mg in test preparation against the label claim of formulation.

Table No.9: Assay of standard solution of perindopril erbumine

Name	Area of std.	RT(min)	TP	TF
Std. Inj-1	1552.3090	4.93	14251	1.13
Std. Inj-2	1569.2781	4.93	15641	1.15
Std. Inj-3	1575.6729	4.92	15927	1.15
Mean	1565.7533	4.93		
SD	12.07418489	0.0058		
%RSD	0.77	0.12		

Table no.10: Assay of test solution of perindopril erbumine.

Name	Area of std.	RT(min)	Perindopril erbumine observed in mg	Perindopril erbumine claim in mg	% Assay
Test Preparation-1	1587.2704	4.88	3.9773	4.0000	**99.43**
Test Preparation-2	1568.8246	4.92	3.9681	4.0000	**99.20**

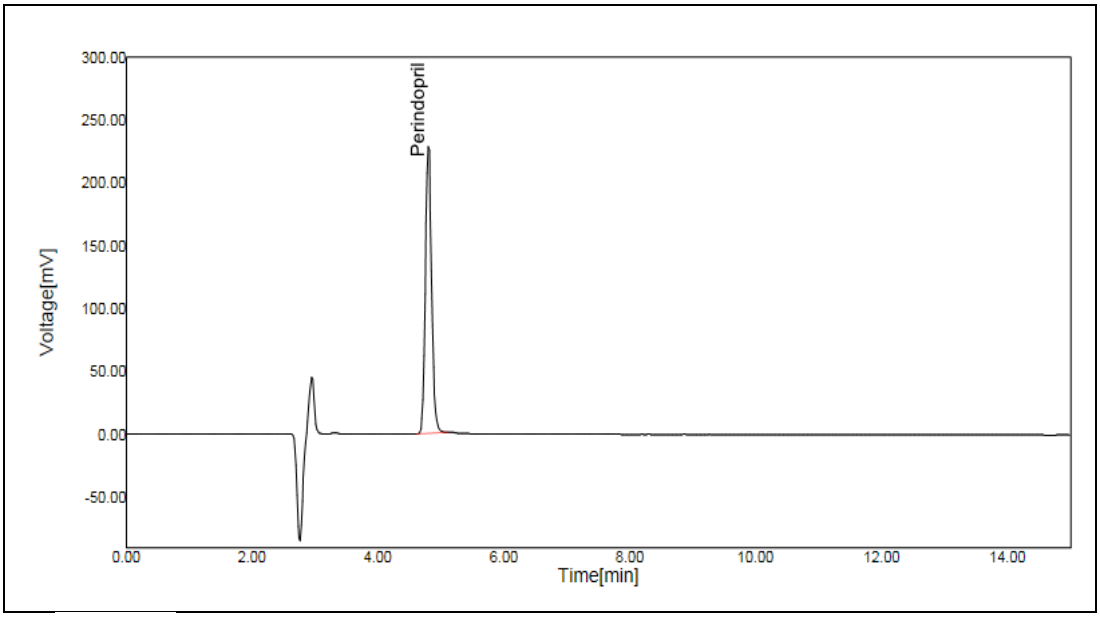

Fig.no.13: Chromatogram showing assay of standard solution of perindopril erbumine.

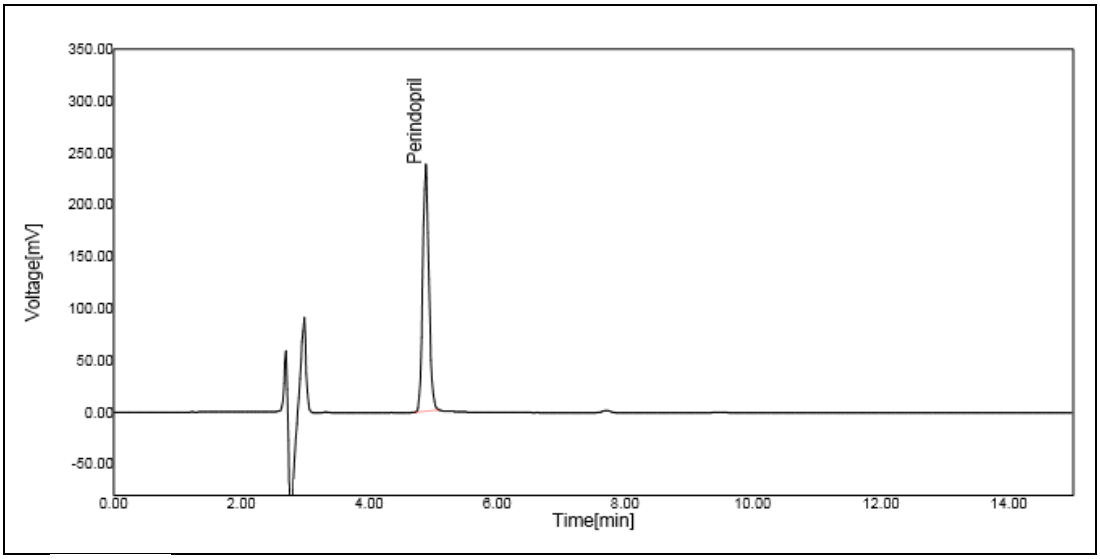

Fig.no.14: Chromatogram showing assay of Test Preparation-1of perindopril erbumine.

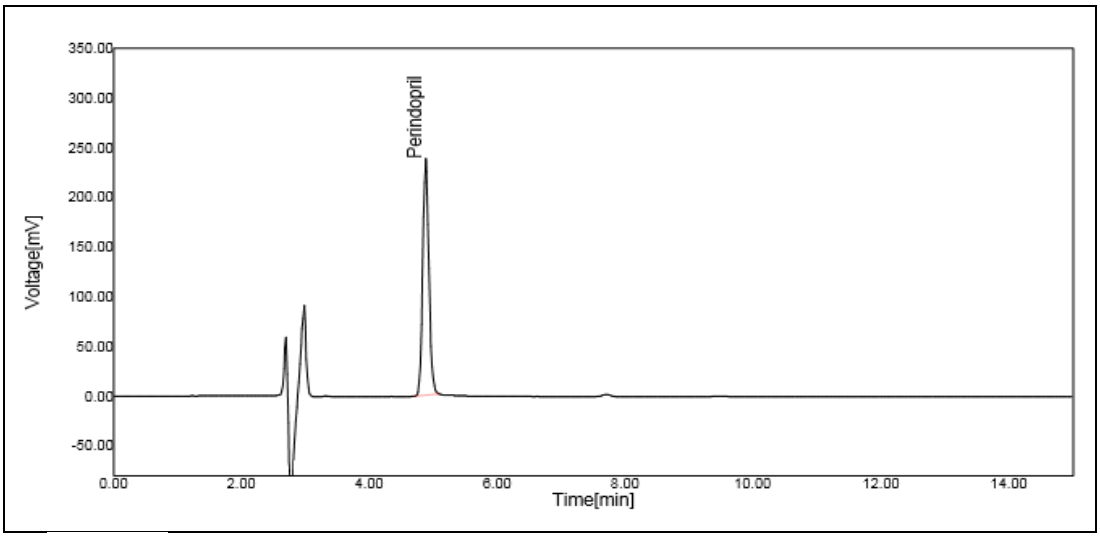

Fig.no.15: Chromatogram showing assay of Test Preparation-2 of perindopril erbumine.

Observation

The assay was performed to observed the PE content in mg in test preparation and the %assay was calculated and the value obtained as 99.43 and 99.20.

Accuracy: (% Recovery)

Recovery studies were performed to validate the accuracy of developed method. To pre analyzed solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Table No 11: Determination of Accuracy (% Recovery).

Recovery levels	Wt. of test in mg	Area	Amount added in mg (w.r.t)	Amount recovered in mg (w.r.t)	% Recovery	Statistical analysis		
						Mean	SD	%RSD
80%	201.2	2809.3855	3.1841	3.1681	99.50	99.71	0.85	0.85
	200.8	2799.9872	3.1904	3.1583	98.99			
	200.1	2815.2739	3.2016	3.2224	100.65			
100%	199.5	3096.7261	4.0140	3.9666	98.82	98.46	0.40	0.41
	200.4	3099.3452	3.9960	3.9376	98.54			
	200.2	3089.8537	4.0000	3.9212	98.03			
120%	199.6	3450.6992	4.8144	4.8708	101.17	100.58	0.67	0.56
	202.5	3448.6579	4.7455	4.7388	99.86			
	201.3	3455.3673	4.7738	4.8079	100.71			

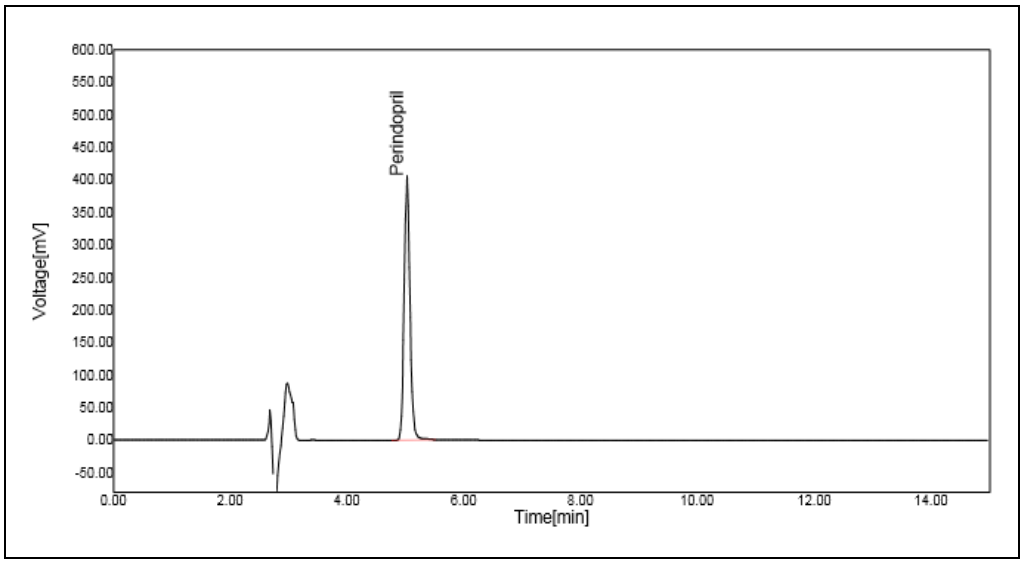

Fig.no.16: Chromatogram of % Recovery for perindopril erbumine at 80%'.

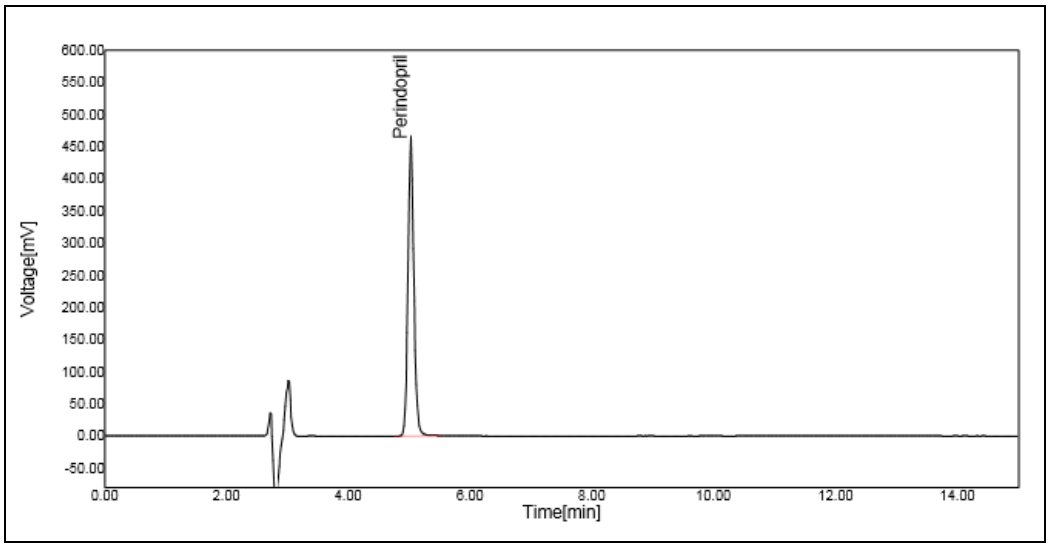

Fig.no.17: Chromatogram of % Recovery for perindopril erbumine at 100%.

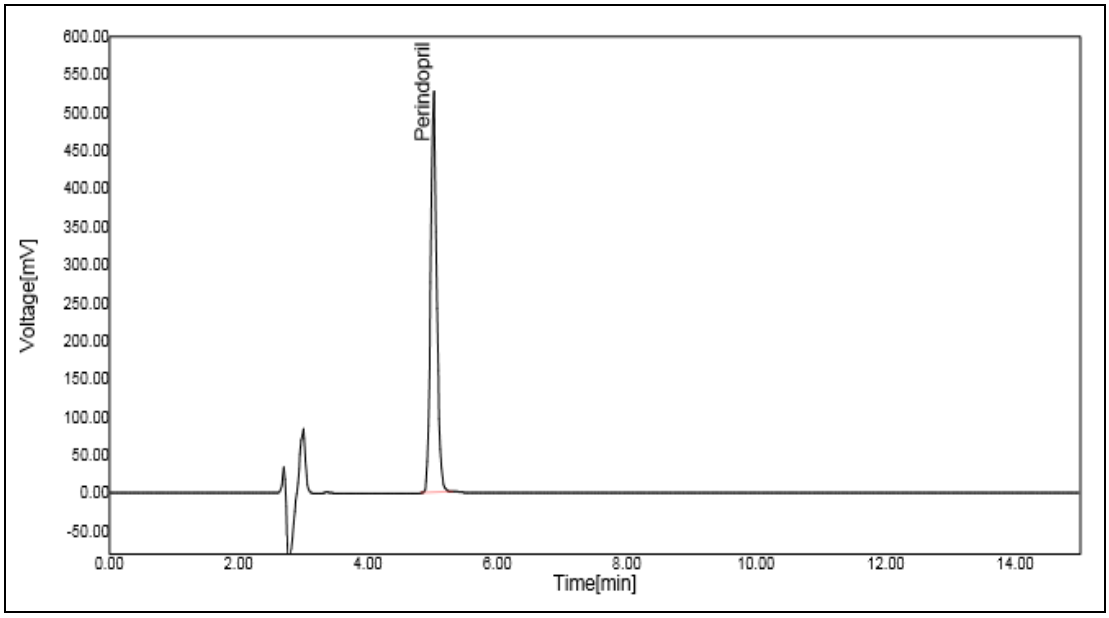

Fig.no.18: Chromatogram of % Recovery for perindopril erbumine at 120%.

Observation

% recoveries was calculated by calculating %RSD or 80%, 100%, 120% and the values obtained are 0.85%, 0.41%, 0.56%.

Precision

In the precision parameter i.e intraday and interday, % RSD was calculated with different interval of time within day and also with two different days results by using proposed method of perindopril erbumine.

Table No 12: A. Studies of intraday precision(std. preparation).

Wt. of standard	Name of preparation	Peak area	Mean	Std. deviation	%RSD
100.1	Std. inj-1	1534.2466	1544.0930	12.8832	0.83
	Std. inj-2	1558.6735			
	Std. inj-3	1539.3589			

Table No 12: B. Studies of intraday precision (test preparation).

Wt. of standard (mg)	Name of preparation	Peak area	Perindopril erbumine observed in mg	% Assay
First analysis				
203.7	Test Preparation-1	1587.2704	3.9773	99.43
201.8	Test Preparation-2	1568.8246	3.9681	99.20
Second analysis				
202.9	Test Preparation-1	1565.0105	4.0002	100.01
202.5	Test Preparation-2	1558.4538	3.9913	99.78
	Mean			**99.61**
	SD			**0.3602**
	%RSD			**0.36**

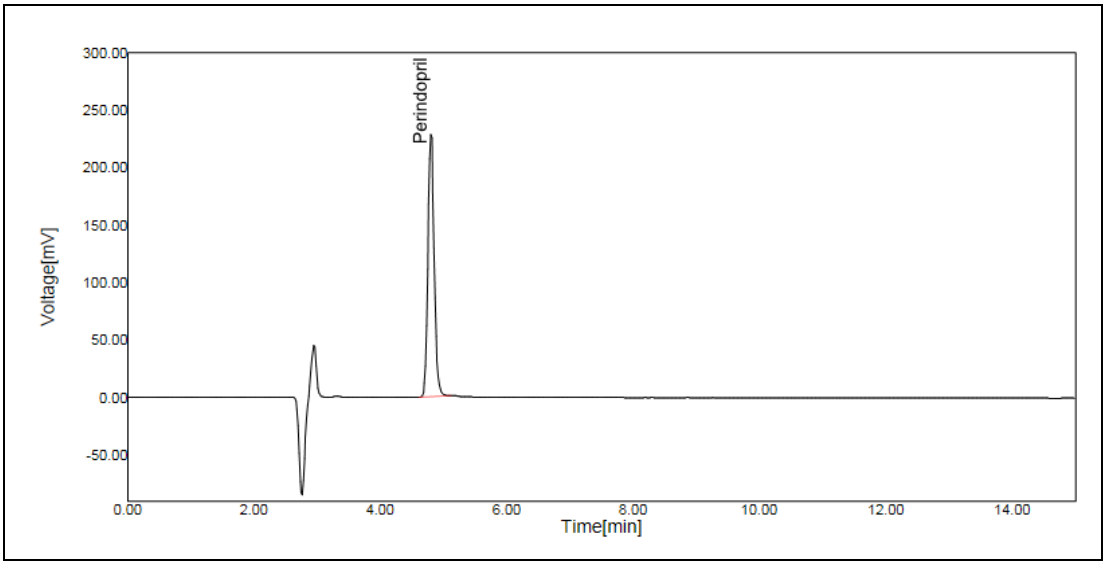

Fig.no 19: Chromatogram showing standard solution of perindopril erbumine of intraday precision.

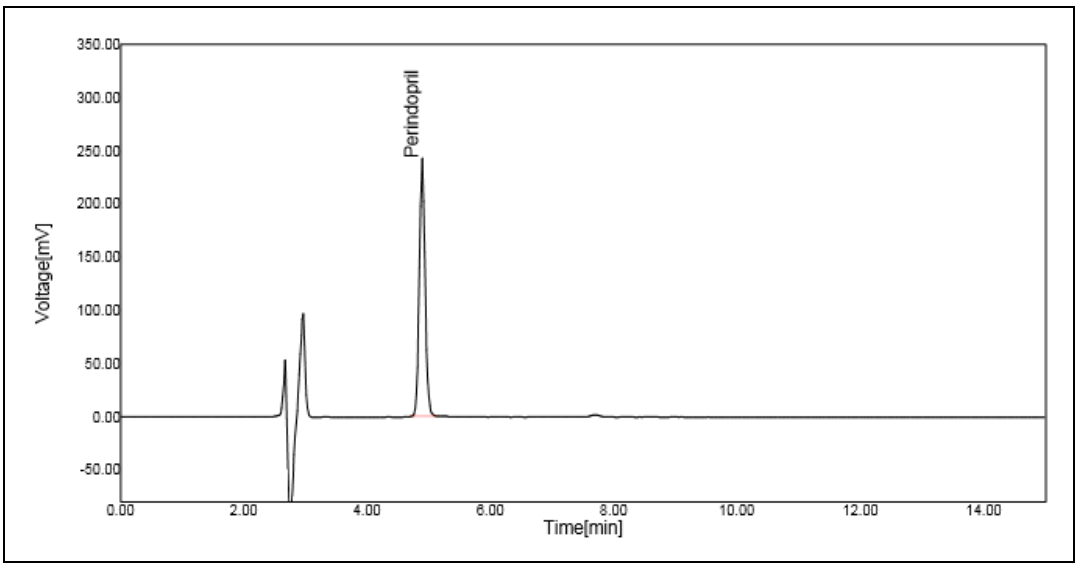

Fig.no 20: Chromatogram showing intraday precision of first analysis.

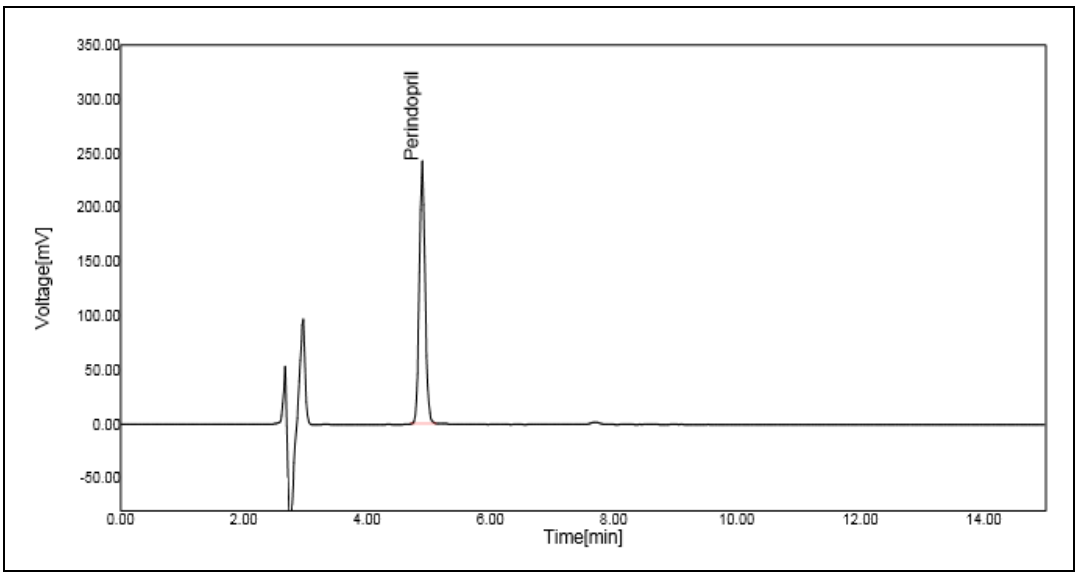

Fig.no 21: Chromatogram showing intraday precision of second analysis.

Observation

In determination of accuracy and precision, the %RSD was in limit, which indicate the method is precise, as %RSD was no more than 2 % indicate the method is precise. The % RSD was found to be 0.36.

Interday Precision Study

The interday study was performed by applying the proposed method on same sample of perindopril erbumine on different days.

Table No: 13: A. Studies of interday precision (std. preparation).

Wt. of standard	Name of preparation	Peak area	Mean	Std. deviation	%RSD
100.5	Std. inj-1	1586.3693	1560.2184	23.3744	1.50
	Std. inj-2	1541.3584			
	Std. inj-3	1552.9274			

Table No: 13.B. Studies of interday precision (test preparation).

Wt. of standard (mg)	Name of preparation	Peak area	Perindopril erbumine observed in mg	% Assay
Day 1				
203.7	Test Preparation-1	1587.2704	3.9773	99.43
201.8	Test Preparation-2	1568.8246	3.9681	99.20
Day2				
200.9	Test Preparation-1	1531.4850	3.9283	98.21
201.4	Test Preparation-2	1545.8067	3.9552	98.88
	Mean			**98.93**
	SD			**0.5303**
	%RSD			**0.54**

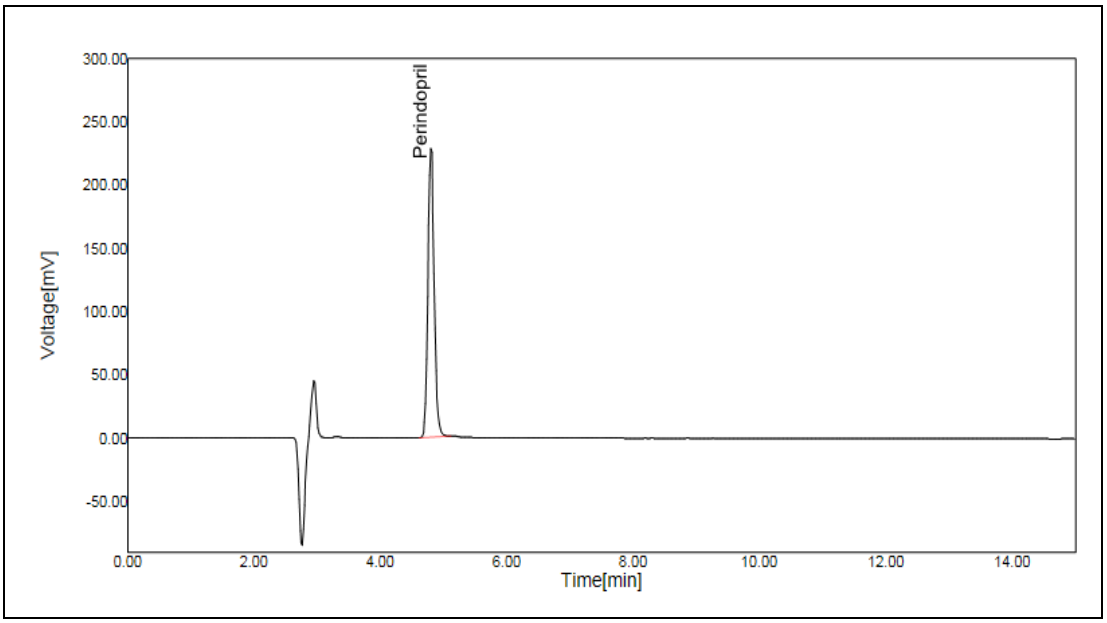

Fig.no.22: Chromatogram showing standard solution of perindopril erbumine of interday precision.

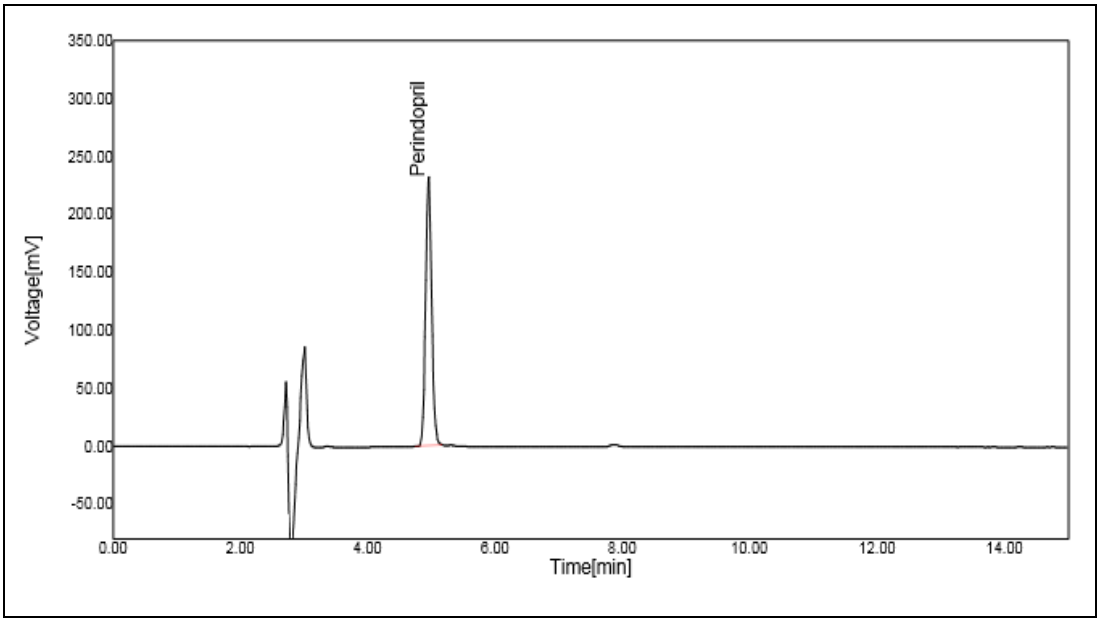

Fig.no.23: Chromatogram showing test solution of perindopril erbumine (intraday precision of day 1).

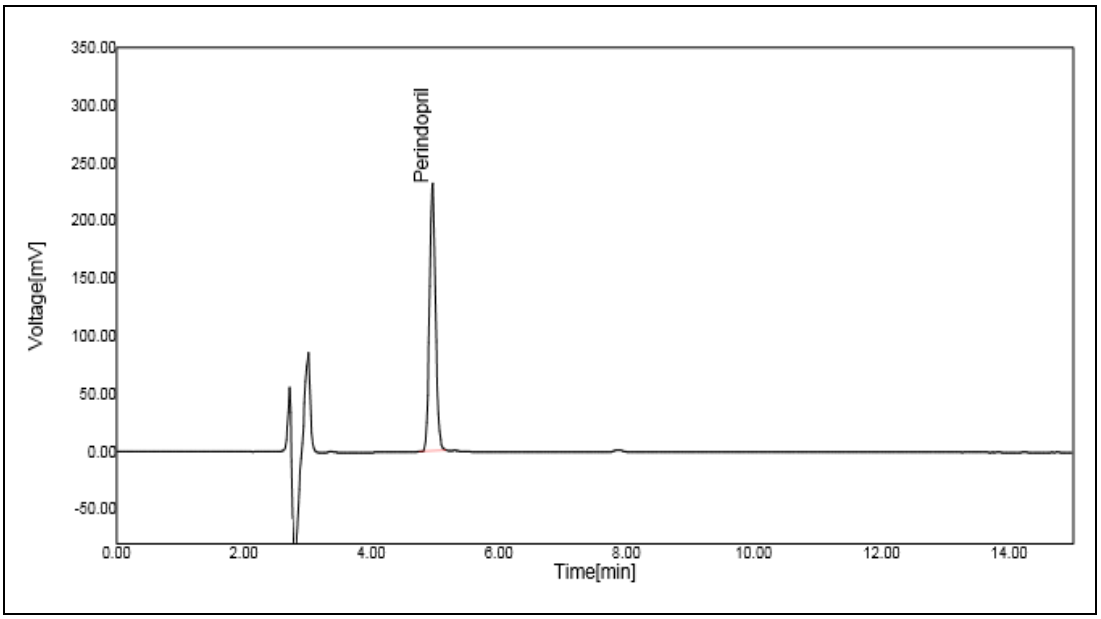

Fig.no.24: Chromatogram showing test solution of perindopril erbumine (intraday precision of day 2).

Observation

In determination of accuracy and precision, the %RSD was in limit, which indicate the method is precise, as %RSD was no more than 2 % indicate the method is precise. The % RSD was found to be 0.54.

Robustness

The minor changeous in the experimental condition is important to demonstrate robustness of the method. To ensure the insensitivity of the HPLC method. These modification does not cause significant change resolution between drugs and peak area %RSD, tailing factor, peak width theoretical plates.

Condition: 1. Change in flow rate.

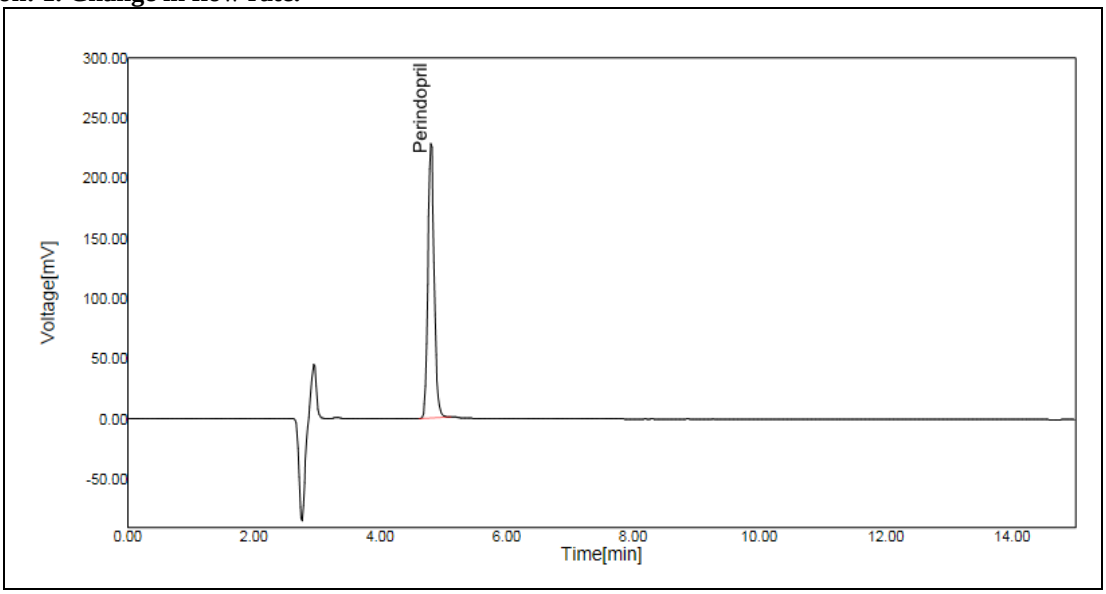

Fig.no.25: Chromatogram showing standard solution of perindopril erbumine.

Table No. 14: Results of robustness (For 0.9 mL).

Robustness (For 0.9 mL)					Change in flow(0.9ml)	Original flow(1.0ml)
Wt. of std (mg)	**Std peak area**	**Wt. of sample (mg)**	**Peak area**	**Perindopril erbumine observed in mg**	**% Assay**	**Initial % assay**
100.2	1690.3169	201.5	1693.9896	3.9879	**99.70**	**99.43**
	1682.6573	201.6	1699.021	3.9978	**99.94**	**99.20**
	1696.557					
Mean	**1689.8437**			**Mean**	**99.57**	
SD	**6.961919997**			**SD**	**0.3216**	
%RSD	**0.41**			**%RSD**	**0.32**	

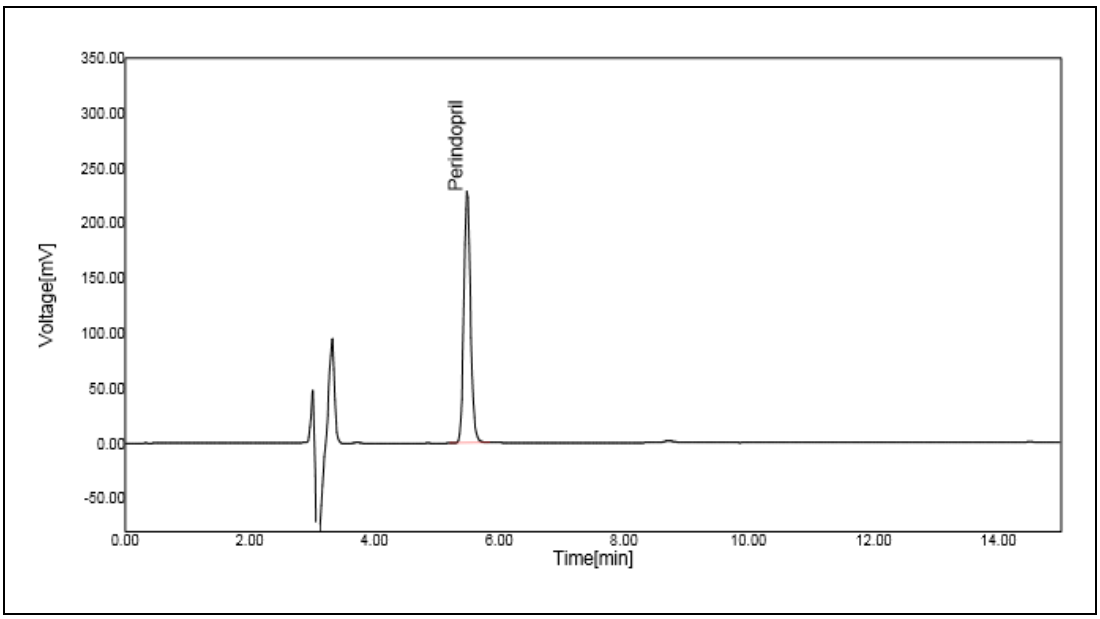

Fig.no.26: Chromatogram of Robustness flow change 0.9ml for Perindopril erbumine.

Table No.15: Results of robustness (For 1.1 mL).

Robustness (For 1.1 mL)					Change in flow(0.9ml)	Original flow(1.0ml)
Wt. of std (mg)	**Std peak area**	**Wt. of sample (mg)**	**Peak area**	**Perindopril erbumine observed in mg**	**% Assay**	**Initial assay**
100.2	1276.1145	203.9	1328.1915	4.0484	101.21	99.43
	1282.6276	201.2	1300.6102	4.0175	100.44	99.20
	1283.5794					
Mean	1280.7738			Mean	100.07	
SD	4.063067947			SD	0.9315	
%RSD	0.32			%RSD	0.93	

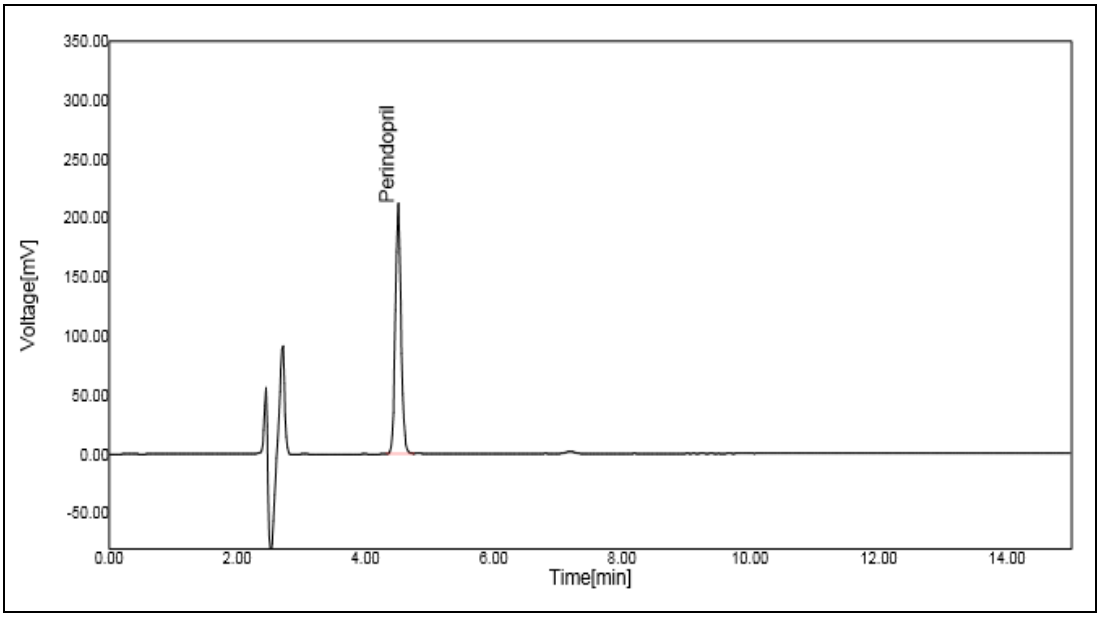

Fig.no 27: Chromatogram of Robustness flow change 1.1 ml for Perindopril erbumine.

Change in wavelength

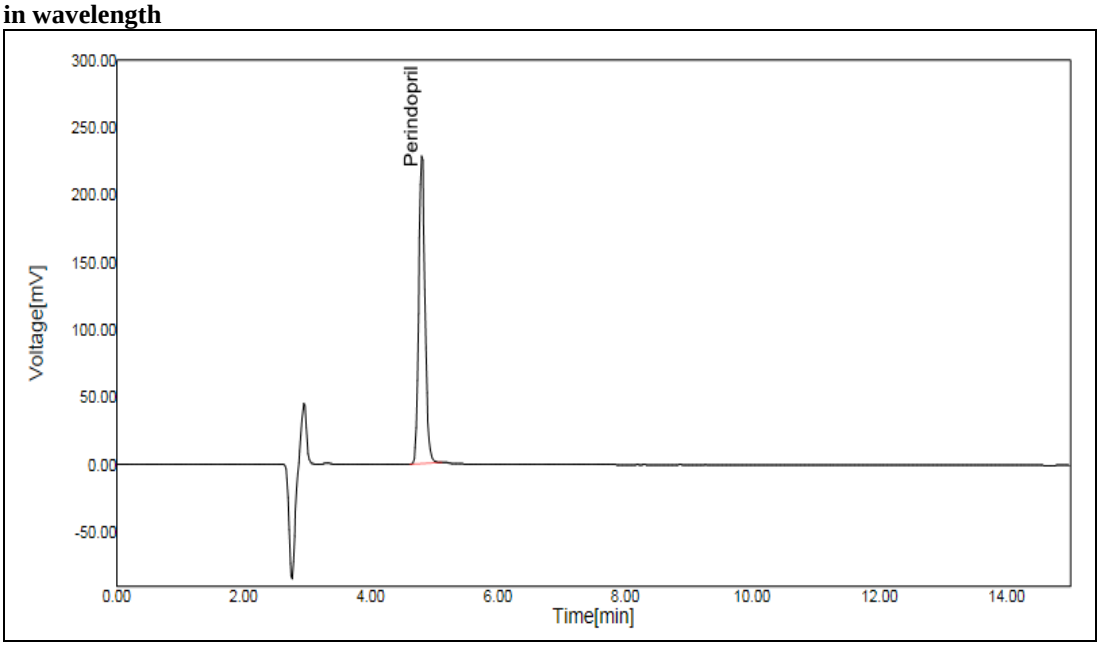

Fig.no 28: Chromatogram showing standard solution of perindopril erbumine.

Table No. 16 Results of robustness(214nm)

Robustness (214nm)					Change in wavelength (214)	Original wavelength (214)
Wt. of std (mg)	**Std peak area**	**Wt. of sample (mg)**	**Peak area**	**Perindopril erbumine observed in mg**	**% Assay**	**Initial assay**
101.1	1244.0122	199.2	1188.1505	3.9364	**98.41**	**99.43**
	1224.5678	200.2	1198.3676	3.9504	**98.76**	**99.20**
	1207.9842					
Mean	**1225.5214**			**Mean**	**98.95**	
SD	**18.03292019**			**SD**	**0.4548**	
%RSD	**1.47**			**%RSD**	**0.46**	

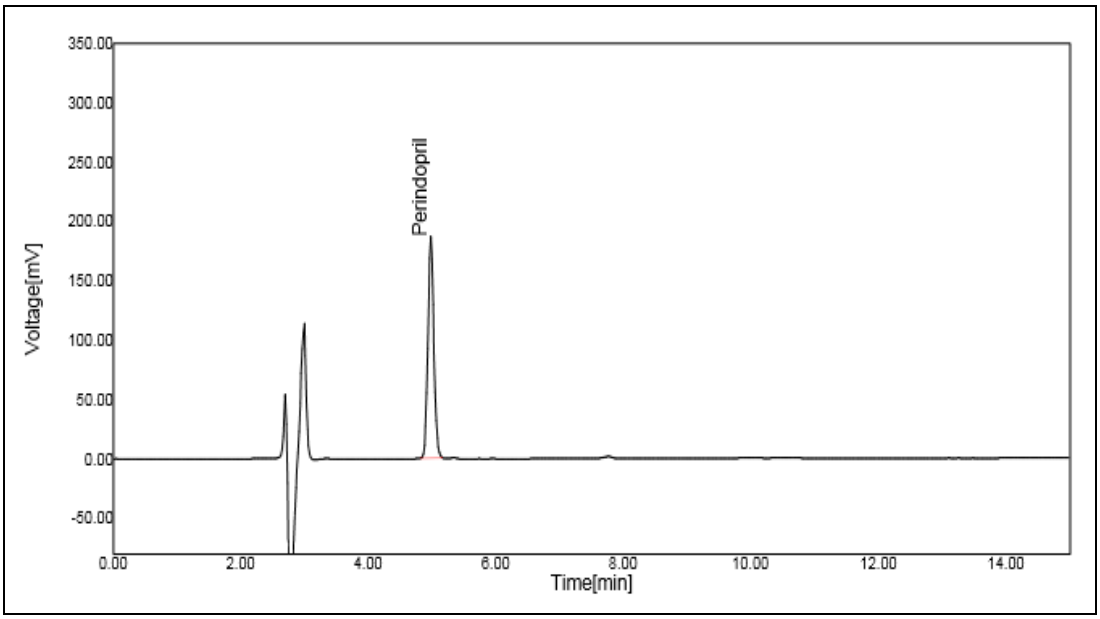

Fig.no 29: Chromatogram of Robustness change in wavelength (214nm) for Perindopril erbumine.

Table no.17: Results of robustness (218 nm).

Robustness (218nm)					Change in wavelength (218)	Original wavelength(218nm)
Wt. of std (mg)	**Std peak area**	**Wt. of sample (mg)**	**Peak area**	**Perindopril erbumine observed in mg**	**% Assay**	**Initial assay**
100.3	1786.3993	200.9	1790.2892	4.0088	**100.22**	**99.43**
	1784.6579	201.5	1783.6487	3.9821	**99.55**	**99.20**
	1779.9927					
Mean	**1783.6833**			**Mean**	**99.60**	
SD	**3.312629584**			**SD**	**0.4381**	
%RSD	**0.19**			**%RSD**	**0.44**	

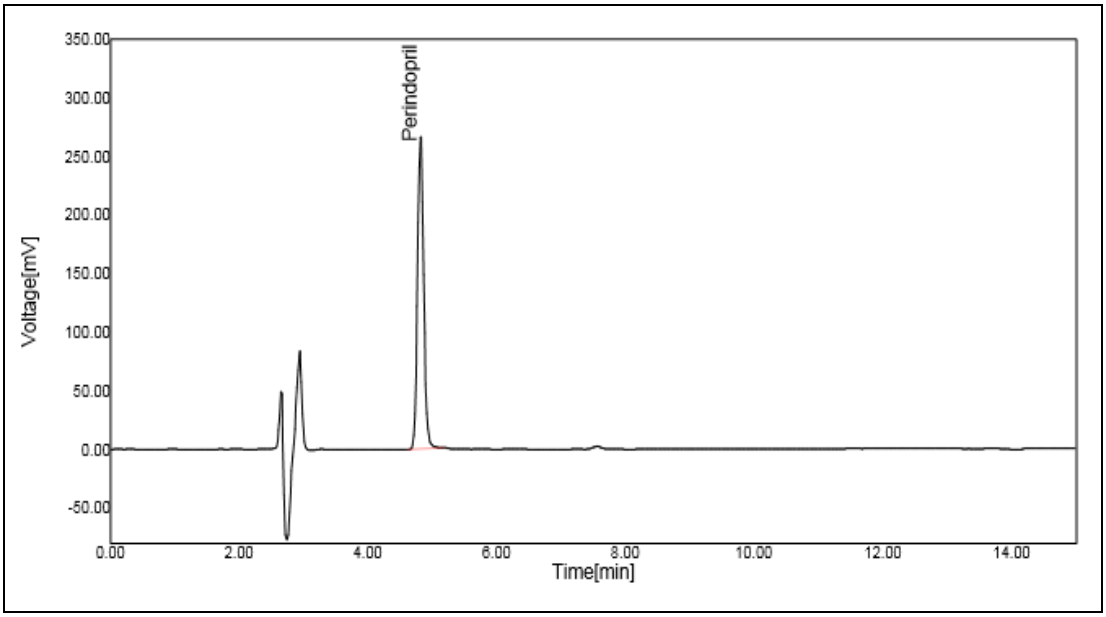

Fig.no.30: Chromatogram of Robustness change inwavelength (218nm) for Perindopril erbumine.

Observation

The %RSD for (Retention time, peak area and % amount found) was not more than 2% for perindopril erbumine which is agree with system suitability. Hence the proposed HPLC method for the determination of perindopril erbumine was found to be robust.

CONCLUSION

The developed methods reported are simple, rapid and stability indicating. These developed Stability indicating HPLC methods can be used to monitor stability and the determination of perindopril erbumine in quality control laboratories.

The developed HPLC method was found to be linear over concentration range. Therefore, the developed HPLC method can be applied for routine quantitative and qualitative analysis of perindopril erbumine in bulk. The developed HPLC method was validated as per the ICH guidelines. The proposed method was validated by performing the parameters as per ICH guidelines and under the acceptance criteria. From the performed parameters the linearity, and the % assay, the % RSD was calculated.

These methods complies the requirement of ICH Q2 guidelines. Thus could act as an indicator to contents of active ingredients in dosage forms, thus indirectly contribute the patient in efficacious treatment and assure quality of medications.

REFERENCE

1. Bounhoure, J.P., Bottineau, G., Lechat, P.; Value of perindopril in the treatment of chronic congestive heart failure: Multicentre doubleblind placebo-controlled study; Clinical Experimental Hypertension, 1989; A11: 575–586.
2. Van den Berg et al., A New Radioimmunoassay for the Determination of the Angiotensinconverting Enzyme Inhibitor Perindopril and its Active Metabolite in Plasma and Urine: Advantages of a Lysine Derivative as Immunogen to Improve the Assay Specificity, J. Pharm. Biomed. Anal, 1991; 9: 517-24.
3. Hisham, E.; Journal of Pharmaceutical and Biomedical Analysis, 1998; 17: 1267.
4. http://www.drugbank.ca/drugs/DB00790.
5. Van Staden J. F., Stefan R. I., Aboul-Enein H. Y., Amperometric biosensor based on Daminoacid oxidase for the R-perindopril assay, Fresenius Journal of Analytical Chemistry, 2000; 367: 178-180.
6. Lin, S.J., Wu, H.L., Chen, S.H., Y.H., Wen, Y.H.; Derivatization-GAS chromatographic determination of perindopril; Analytical Letters, 1996; 29: 1751–1762.
7. Jain, P.S., Badreshiya, P.R., Chalikwar, S.S., Todarwal, A.A., Surana, S.J.; Validation of a dissolution method with RP-HPLC analysis for Perindopril erbumine and indapamide combination tablet; Chemical Industry and Chemical Engineering Quarterly, 2012; 18: 19–25.
8. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guideline, "validation of analytical Procedures": Text and Methodology Q2(R 1), Complementary Guideline on Methodology dated 06 November 1996, incorporated in November 2005, London.
9. The United States Pharmacopeial Convention, Inc.; Validation of compendial methods, United States Pharmacopeia 30, General Chapter 1225, National Formulary 25. Rockville, MD, 2007.
10. Stability testing of new drug substances and products [Q1A(R2)], ICH Harmonized Tripartite

Guideline. International Conference on Harmonization, Geneva, Switzerland, 2003.
11. Nash, R.A., Wachter, A.H.; Pharmaceutical process validation, Marcel Dekkar, 3rd, 129: 507–523.