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DEVELOPMENT AND VALIDATION OF RP-HPLC CHROMATOGRAPHIC ASSAY METHOD FOR THE SIMULTANEOUS ESTIMATION OF PERINDOPRIL ERBUMINE AND AMLODIPINE BESYLATE IN FORMULATION

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ABSTRACT

Background: The present paper reports are the simple, accurate, rapid & precise RP-HPLC method for the simultaneous estimation of Perindopril erbumine & Amlodipine besylate in the bulk & in the formulated drug substance.

Methodology: The analytical method of Reverse Phase Liquid chromatographic has been performed on a Kromasil C8 (4.6mm x 250mm, 5µ particle size) column with the Buffer (6.8g Potassium dihydrogen orthophosphate) & Acetonitrile in the ratio 59:41 with adjusted pH 2.6 with orthophosphoric acid as a mobile phase & column oven temperature 40°C. The mobile phase flow rate was adjusted to 1.0 ml/min. & the injection volume should be 10 µl. 210 nmwas used as the wavelength for the detection of the sample**.**

Result: The retention time of Perindopril erbumine and Amlodipine Besylate were found to be 4.483 min. and 6.767 min respectively. The concentration range of linearity was observed from 20 % to 160 % of the nominal concentration of Perindopril erbumine & Amlodipine Besylate

correlation coefficient was 0.999 for both drugs. The percent recovery was found within the limits of the acceptance criteria with an average recovery of 99.4 % for perindopril erbumine & 99.6 % for Amlodipine besylate.

Conclusion: The % RSD below 2.0 shows the high precision of the proposed method. The method can be adopted for the routine analysis of simultaneous estimation of Perindopril erbumine & Amlodipine Besylate in pharmaceutical solid dosage form

Keywords: RP-HPLC; UV detector; Amlodipine Besylate; Perindopril Erbumine; Solid oral dosage form (tablets)

INTRODUCTION

Nowadays Chromatography is the most powerful analytical method available for modern chemists. It is most commonly used because of its capacity to determine many individual components quantitatively present in the mixture by a single analytical procedure. HPLC i.e. High-performance liquid chromatography is a chromatographic technique that can be used to separate a mixture of compounds in analytical chemistry and biochemistry to identify, quantify & purify the individual components of the mixture. Among the chromatographic method, the Reversed-phase chromatography has found both analytical and preparative applications in the area of biochemical separation and their purification. Molecules that show some degree of hydrophobic characters, such as proteins, peptides & nucleic acids, can be separated by the Reversed-phase chromatography method with excellent recovery & resolution.

Recently the reversed-phase chromatography is the most commonly used separation technique in HPLC methods, due to its broad range of applications. It is estimated that over 65% (possibly up to 90%) of all separation of HPLC are carried out by using the reversed-phase mode of chromatography. The reasons behind that are its simplicity, versatility, and its ability to handle compounds of a diverse polarity and molecular mass.

Perindopril erbumine: Chemically it is 2-Methylpropan-2-amine (2*S*, 3aS, 7aS)-1-[(2*S*)-2-[[(1*S*)-1-(ethoxy carbonyl) butyl] amino] Propionyl] octahedron-1*H*-indole-2-carboxylate. It acts as an angiotensin-converting enzyme inhibitor. Mostly it is used in patients with hypertension and heart failure. Its Molecular formula-$C_{23}H_{43}N_3O_5$ & molecular weight-441.613 g/mol. Solubility-It's freely soluble in water and ethanol (96 %), and soluble or sparingly soluble in methylene chloride **[1]**.

Amlodipine Besylate: - It is chemically 3-Ethyl 5-methyl -2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzenesulfonate. It belongs to the class of calcium channel blocker which acts by widening blood vessels. Its Molecular formula-$C_{26}H_{31}CIN_2O_8S$ and molecular weight-567.05 g/mol. Solubility- it is slightlysoluble in water, freely soluble in methanol, sparingly soluble in anhydrous ethanol, and slightly soluble in 2-propanol **[1, 2]**.

The perindopril erbumine & Amlodipine besylate tablets have involved a combination of two active ingredients i.e. perindopril & Amlodipine. In that, the drug Amlodipine is acting as a calcium channel blocker that dilates blood vessels, and the second drug Perindopril is an angiotensin-converting enzyme inhibitor agent. In combined form, they work to widen and relax the blood vessels, which results in the reduction of blood pressure, and because of that blood can flow through the whole body very easily.

In the literature survey, we conclude that individual & combination of these drugs have been analyzed by many spectroscopic methods. The Amlodipine besylate API is official in British pharmacopoeia and Indian pharmacopeia **[2]**. And the Perindopril erbumine is official in the British Pharmacopoeia. The combination of Perindopril erbumine & Amlodipine besylate is not included in any pharmacopeias. So, the objective of this work describes simple, rapid, economical, selective, precise & reproducible HPLC method for pharmaceutical importance. This method was validated as per the ICH guidelines **[3]**.

MATERIALS AND METHODS:

Reagents and chemicals

Acetonitrile (HPLC Grade), Potassium dihydrogen orthophosphate (Merck, AR Grade), Orthophosphoric acid (AR Grade), water (HPLC Grade), The standard drug samples of perindopril erbumine and amlodipine besylate, as well as tablet available in the ratio of 1:1 containing perindopril erbumine 5mg and Amlodipine 5mg, perindopril erbumine 10mg and Amlodipine 10mg gifted from Generic Healthcare Pvt. Ltd., Pune.

For Assay and content uniformity:

Chromatographic condition:

Analysis was performed on a chromatographic system consisted of Shimadzu, series LC 2010 A (pump Quaternary system). Separation was carried out with a Kromasil C8 (4.6mm x 250mm, 5µ particle size) column at a temperature of 40°C, with a flow rate of 1.00mL per min. with an isocratic mobile phase constituting

Buffer & Acetonitrile having the ratio 59:41. pH was adjusted up to 2.6 with Orthophosphoric acid. Perindopril & Amlodipine was determined by using the UV detection method at 210nm where the injection volume was 10µL and the run time was 10 min **[4, 5]**.

Preparation of buffer solution for mobile phase:

Weigh accurately about 6.8gm of Potassium dihydrogen orthophosphate and transfer it to 500 ml of HPLC grade water then shake and sonicate to dissolve completely. Finally, make the solution up to 1000 ml with HPLC grade water.

Preparation of standard solution:

Weigh accurately 50 mg of Perindopril erbumine & 69 mg of Amlodipine besylate, transferred to 100 mL volumetric flask and dissolved it in 70 ml of mobile phase and make volume up to the mark with mobile phase to get 500µg/ml of Perindopril erbumine & 690µg/ml of Amlodipine besylate stock solution. The final solution prepared by 5 ml of this solution into 100 ml volumetric flask then made volume up to the mark with mobile phase to get 50µg/mL of Perindopril erbumine and 69µg/mL of Amlodipine besylate respectively. **Figure 1** represents the typical chromatogram of standard Perindopril and Amlodipine respectively **[6, 7]**.

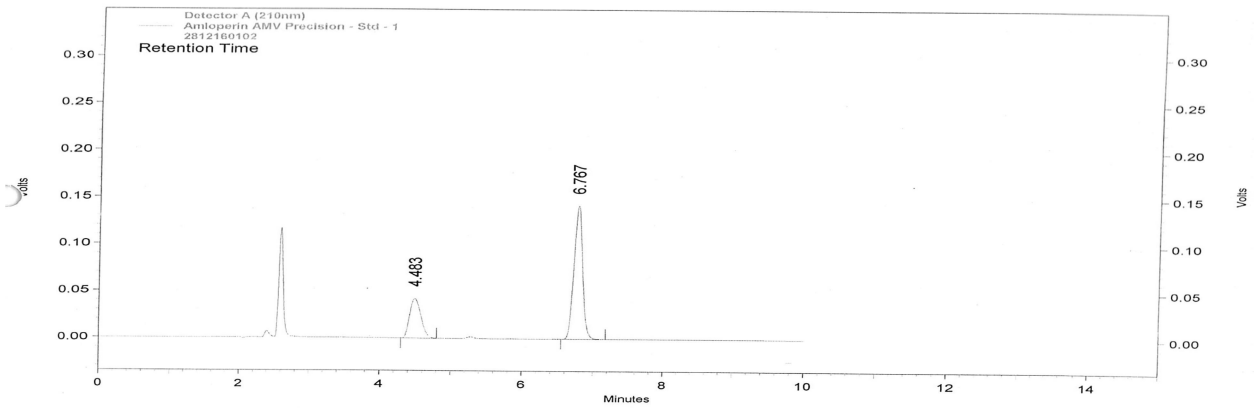
Preparation of sample solution:

For Assay, 20 tablets of Perindopril erbumine labeled as containing 5mg and 5 mg of Amlodipine besylate together with excipients were weigh accurately and made a fine powder. Take the accurate weight of powder equivalent to 5 mg of Perindopril erbumine and 5 mg of Amlodipine and transferred it into 100 ml volumetric flask and 50 ml of mobile phase was added, sonicated for 10 min. Cool it and make volume up to the mark with the mobile phase. Filter a sufficient amount of this solution through a 0.45µm membrane syringe filter. The final solution was prepared by transferring 5 ml of this filtered solution into a 100 ml volumetric flask then make the volume up to the mark by adding a mobile phase to get 50µg/ml of Perindopril erbumine & 69µg/ml of Amlodipine besylate respectively. **Figure 2** represents the typical chromatogram of the sample Perindopril & Amlodipine respectively.

For Content uniformity, one tablet was placed into each of ten 100 ml volumetric flask. Approximately 70 ml of mobile phase was added to each volumetric flask & sonicate till tablets were dispersed in the solution. Cool the resultant solutions and

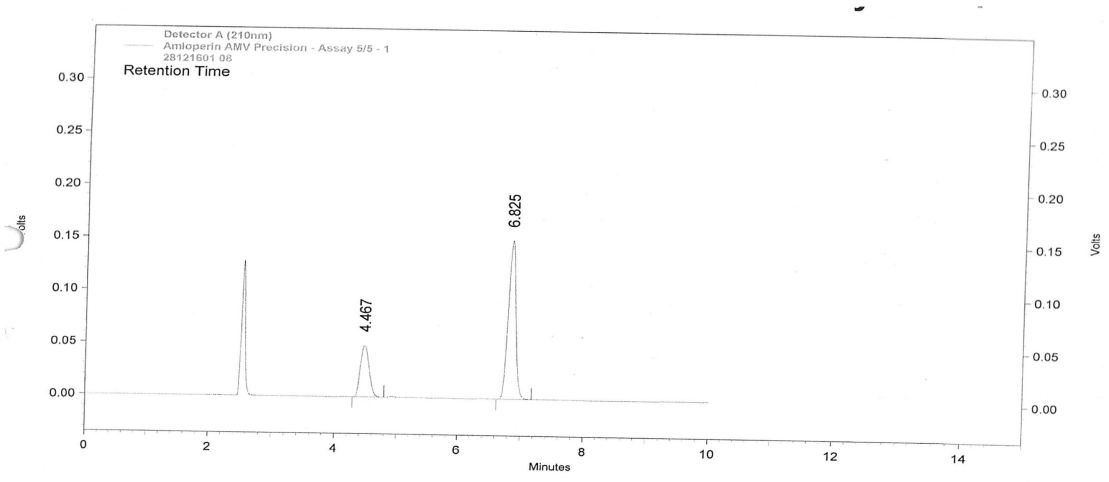
make volume up to the mark with the mobile phase. Shake the solution well for uniform distribution. Filtered a portion of the solution by using a 0.45µm membrane syringe filter & then the filtrate was injected for analysis.

Figure 3 represents the typical sample chromatogram of Perindopril and Amlodipine respectively **[8, 9]**.



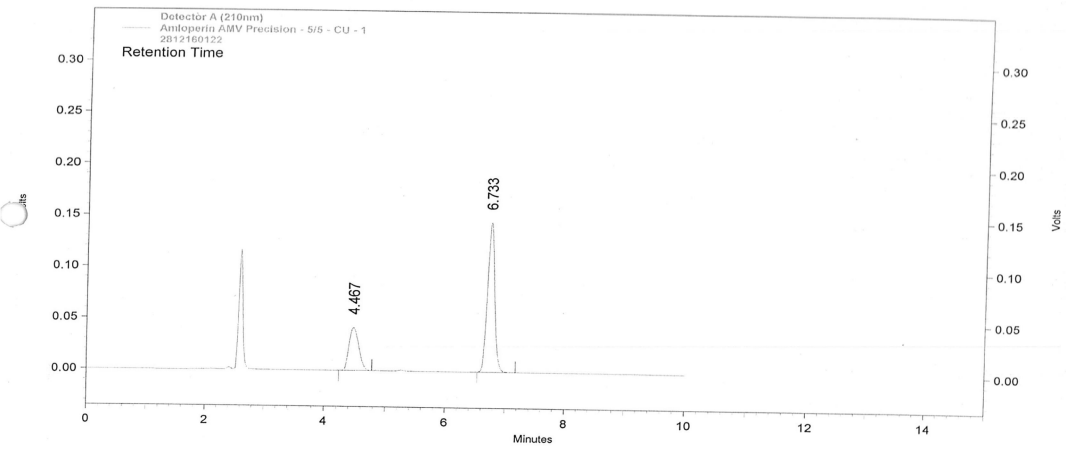
Detector A (210nm) Name	Retention Time	Area	Area %	Theoretical plates	Resolution	Asymmetry
Perindropil	4.483	463562	27.74	3669	0.00	1.28
Amlodipine	6.767	1207611	72.26	14098	8.71	1.15

Figure 1: Chromatogram of the standard preparation



Detector A (210nm) Name	Retention Time	Area	Area %	Theoretical plates	Resolution	Asymmetry
Perindropil	4.467	472053	27.69	4750	0.00	1.26
Amlodipine	6.825	1232844	72.31	15146	9.80	1.24

Figure 2: Chromatogram of sample Preparation (Assay)



Detector A (210nm)

Name	Retention Time	Area	Area %	Theoretical plates	Resolution	Asymmetry
Perindropil	4.467	459037	27.11	3621	0.00	1.27
Amlodipine	6.733	1234034	72.89	13893	8.63	1.20

Figure 3: Chromatogram of sample preparation (CU)

RESULTS AND DISCUSSION

Specificity and system suitability

Specificity test used to determine the effect of excipients on the result of the assay. To determine the specificity of the method, filtered as well as unfiltered solutions of blank, placebo, diluent and standard of Perindopril erbumine and Amlodipine Besylate injected.

The system suitability study of the above method was carried out by five repeated analyses of the solution containing 100 % target concentration of Perindopril erbumine& Amlodipine besylate. Various parameters of chromatographic techniques such as retention time, column efficiency, peak area, tailing factor & resolution between the peaks were determined and the method was evaluated by analyzing these parameters **[10, 11]**.

Linearity and range

In the above method, linearity was determined by constructing the calibration curves. For this purpose, different standard solution of Perindopril erbumine & Amlodipine besylate of different concentration level (20%, 40%, 60%, 100%, 120%, 140% and 160%) were used. Measurement of each concentration was carried out & the peak areas of the chromatograms were plotted against the concentrations to obtain the calibration curves & correlation coefficients. **Table no.1a & 1b** represents the results that were directly proportional to the concentration of analyte in the given sample.

Precision

System precision & method precision

The method precision is performed by carrying out standard replicate & six independent sample preparations of a single lot of formulation. For this method, the sample solution was prepared the same as described in sample preparation. The percentage relative standard deviation was found less than 2.0% for both the analyte.

Intermediate precision

The Intermediate precision was performed by carried with standard replicate & six independent samples from two different analysts by using the different chromatographic system on different days. The chromatographic sample results are summarized in the following **Table 2a and 2b**, the percentage relative standard deviation was found less than 3.0% for both analytes.

Effect of variation in the mobile phase

This study was performed to determine the effect of variations in the composition of the mobile phase. The standard solution and test solution were prepared & injected into the HPLC system by changing the composition of the mobile phase by ± 5 and system suitability parameters were evaluated. The values were given in the following **Table no. 3, 4a, 4b and 4c**.

Solution Stability

For the demonstration of the stability of the standard solution during its analysis, the standard solution was analyzed throughout the 24^{th} at room temperature. The results obtained for all the solutions, state that the retention times and peak areas of Perindopril and Amlodipine almost remained unchanged (RSD%) which indicates that no significant degradation occurred within this period. i.e., solutions were stable for at least the 24^{th}hour which was sufficient for completion of the whole analytical process. The results were displayed in the following **Table no.5 [8, 11]**.

Table 1a: Linearity, and range (For Perindopril peak)

Linearity Level	Level 1. (20%)	Level 2. (40%)	Level 3. (60%)	Level 4. (100%).	Level 5. (120%).	Level 6. (140%).	Level 7. (160%)	Regression coefficient	% Y Intercept
Conc. Of Perindoprilw.r.t. working level conc. (i.e. 0.5mg/ml)	0.01	0.02	0.03	0.05	0.06	0.07	0.08		
Peak area injection 1.	98762	190813	288351	480470	576022	672996	765324		
Peak area injection 2.	98817	190906	288256	479660	576169	672772	765810		
Peak area injection 3.	98903	190716	288507	480000	576429	673135	768005	0.99999	0.3
Peak area injection 4.	98907	……	……	479778	……	……	766996		

Peak area injection 5.	**98975**			**479484**			**766358**		
Average peak area.	**98873**	**190812**	**288371**	**479878**	**576207**	**672968**	**766499**		
Response factor.	**9887300**	**9540600**	**9612366.7**	**9597560**	**9603450**	**9613829**	**9581238**		
Relative response factor.	**1.0302**	**0.9941**	**1.0015**	**1.0000**	**1.0006**	**1.0017**	**0.9983**		
Average.	**1.0038**								
% RSD.	**1.19**								

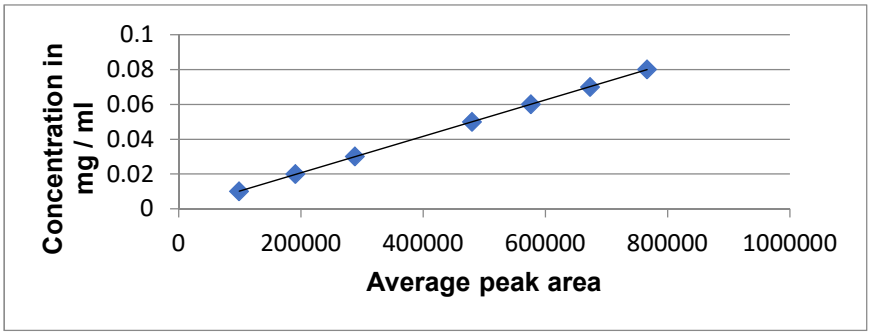


Table 1b: Linearity, and range (For Amlodipine peak)

Linearity Level	**Level 1. (20%)**	**Level 2. (40%)**	**Level 3. (60%)**	**Level 4. (100%)**	**Level 5. (120%)**	**Level 6. (140%)**	**Level 7. (160%)**	**Regression coefficient**	**% Y Intercept**
Conc. Of Amlodipine w.r.t. working level conc. (i.e. 0.5mg/ml)	**0.01**	**0.02**	**0.03**	**0.05**	**0.06**	**0.07**	**0.08**		
Peak area injection 1	**252727**	**485684**	**733158**	**1219653**	**1460105**	**1704408**	**1936794**		
Peak area injection 2	**252303**	**485804**	**732671**	**1216435**	**1459867**	**1702293**	**1935237**		
Peak area injection 3	**252403**	**485529**	**732670**	**1218202**	**1459349**	**1703700**	**1941934**		
Peak area injection 4	**252280**			**1216642**			**1938703**	**0.99999**	**0.6**
Peak area injection 5	**252394**			**1216177**			**1937040**		
Average peak area	**252421**	**485672**	**732833**	**1217422**	**1459774**	**1703467**	**1937942**		
Response factor	**25242100**	**24283600**	**24427767**	**24348440**	**24329567**	**24335243**	**24224275**		
Relative response factor	**2.6301**	**2.5302**	**2.5452**	**2.5369**	**2.5350**	**2.5356**	**2.5240**		
Average	**2.5481**								
% RSD	**1.44**								

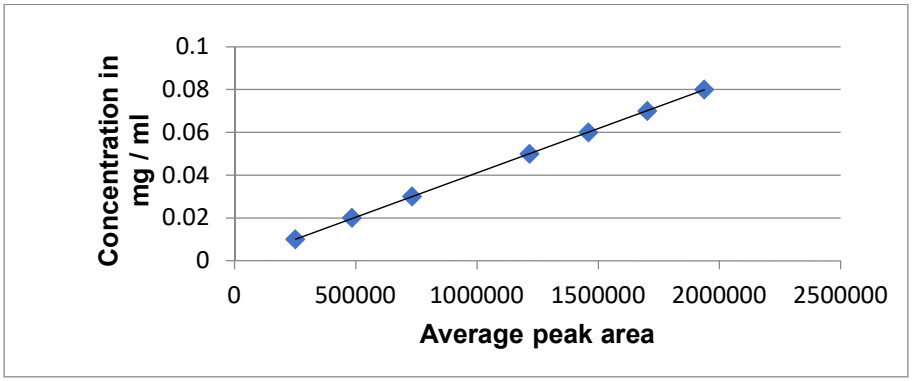


Table 2a: Precision Study for Assay

Sr. No.	Method Precision		Intermediate Precision (Analyst 2, Day 2)			
	Content in %		Content in %			
	Perindopril	Amlodipine	Perindopril (MP)	Perindopril (IP)	Amlodipine (MP)	Amlodipine (IP)
1	99.7	100.0	99.7	98.5	100.0	97.0
2	99.9	100.2	99.9	98.3	100.2	96.8
3	100.3	100.6	100.3	98.0	100.6	96.5
4	100.1	100.4	100.1	98.3	100.4	96.8
5	100.2	100.5	100.2	97.9	100.5	96.4
6	99.6	99.9	99.6	98.8	99.9	97.4
Mean	100.0	100.3	99.1		98.5	
% RSD	0.3	0.3	0.9		0.9	

SD = Standard deviation/ RSD = Relative standard deviation/ MP = Method Precision/ IP = Intermediate Precision.

Table 2b: Precision Study for CU

Sr. No.	Method Precision		Intermediate Precision (Analyst 2, Day 2)			
	Content in %		Content in %			
	Perindopril	Amlodipine	Perindopril (MP)	Perindopril (IP)	Amlodipine (MP)	Amlodipine (IP)
1	97.7	100.9	97.7	101.8	100.9	99.4
2	98.1	101.4	98.1	97.1	101.4	100.8
3	97.9	101.2	97.9	102.1	101.2	99.7
4	102.1	103.9	102.1	97.2	103.9	100.9
5	102.3	104.1	102.3	97.4	104.1	101.1
6	97.8	100.8	97.8	102.2	100.8	99.7
7	98.2	101.4	98.2	97.4	101.4	101.2
8	97.9	101.0	97.9	102.4	101.0	99.8
9	102.1	103.8	102.1	97.4	103.8	101.1
10	102.5	104.1	102.5	97.3	104.1	101.0
Mean	99.7	102.3	99.4		101.4	
% RSD			2.3		1.4	

SD = Standard deviation/ RSD = Relative standard deviation/ MP = Method Precision/ IP = Intermediate Precision.

Table 3: % Recovery

Levels	Perindopril	Amlodipine
1 (50%)	99.4	99.0
2 (100%)	99.7	100.8
3 (150%)	99.0	98.9
Average	99.4	99.6
% RSD	0.4	1.1

Table 4a: Flow Rate (Robustness)

Flow Rate: - 0.9 ml/Min.			Flow Rate: - 1.1 ml/Min.		
Sr. No.	**Perindopril in %**	**Amlodipine in %**	**Sr. No.**	**Perindopril in %**	**Amlodipine in %**
Sample- A	**97.5**	**100.3**	**Sample- A**	**97.6**	**100.1**
Sample- B	**98.0**	**100.9**	**Sample- B**	**98.1**	**100.5**
Average	**97.8**	**100.6**	**Average**	**97.9**	**100.3**

Table 4b: Column oven temperature (Robustness)

Column oven temperature: - 38°C.			Column oven temperature: - 42°C.		
Sr. No.	**Perindopril in %**	**Amlodipine in %**	**Sr. No.**	**Perindopril in %**	**Amlodipine in %**
Sample- A	**98.1**	**100.9**	**Sample- A**	**97.6**	**100.7**
Sample- B	**97.9**	**100.7**	**Sample- B**	**97.6**	**100.6**
Average	**98.0**	**100.8**	**Average**	**97.6**	**100.7**

Table 4C: Mobile phase (Robustness)

Mobile phase - 5%			Mobile phase + 5%		
Sr. No.	**Perindopril in %**	**Amlodipine in %**	**Sr. No.**	**Perindopril in %**	**Amlodipine in %**
Sample- A	**99.3**	**99.1**	**Sample- A**	**99.2**	**98.9**
Sample- B	**99.1**	**98.9**	**Sample- B**	**98.6**	**98.5**
Average	**99.2**	**99.0**	**Average**	**98.9**	**98.7**

Table 5: Solution Stability

Sr. No.	Time interval in Hour	Content of perindopril in %	% Relative difference with time interval	Content of Amlodipine in %	% Relative difference with time interval
1	**2 Hour**	**96.4**	**0.00**	**97.2**	**0.10**
2	**4 Hour**	**96.4**	**0.00**	**97.2**	**0.10**
3	**8 Hour**	**96.3**	**0.10**	**97.0**	**0.31**
4	**12 Hour**	**96.4**	**0.00**	**97.1**	**0.21**
5	**16 Hour**	**96.3**	**0.10**	**96.9**	**0.41**
6	**20 Hour**	**96.3**	**0.10**	**97.0**	**0.31**
7	**24 Hour**	**96.3**	**0.10**	**96.9**	**0.41**

CONCLUSION

Results obtained from all validation parameters, it is concluded that the developed RP-HPLC method is sensitive, linear, accurate, precise, robust, and can be adopted for the routine analysis of simultaneous estimation of Perindopril erbumine & Amlodipine Besylate.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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