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Research Article

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Development and Validation of Rp-Hplc Chromatographic 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 and precise RP-HPLC method for the simultaneous estimation of Perindopril erbumine and Amlodipine besylate in the bulk and in the formulated drug substance.*

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

Results: *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 nominal concentration of Perindopril erbumine and Amlodipine Besylate correlation coefficient was 0.999 for the both drugs. The percent recovery was found within the limits of the acceptance criteria with an average recovery 99.4% for perindopril erbumine and 99.6% for Amlodipine besylate.*

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

Keywords: RP-HPLC; UV detector; Amlodipine besylate; Perindopril erbumine

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INTRODUCTION

Now days Chromatography is the most powerful analytical method available for the modern chemist. It is most commonly used because of its capacity to determine many individual components quantitatively present in the mixture by 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 and 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 which shows some degree of hydrophobic character, such as proteins, peptides and

nucleic acids, can be separated by the Reversed phase chromatography method with excellent recovery and resolution.

Recently the reversed-phase chromatography is most commonly used separation technique in HPLC methods, due to its broad range of application. It is estimated that over 65% (possibly up to 90%) of all separation of HPLC are carried out by using reversed-phase mode of chromatography. The reasons behind that is 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 (2S, 3aS, 7aS)-1-[(2S)-2-[[(1S)-1-(ethoxy carbonyl) butyl] amino] Propanoyl] octahydro-1H-indole-2-carboxylate. It acts as an angiotensin-converting enzyme inhibitor. Mostly it is used in the patients with hypertension and heart failure. Its Molecular formula-$C_{23}H_{43}N_3O_5$ and molecular weight-441.613 g/mol. Solubility-Its freely soluble in water and in 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 is belongs to the class of calcium channel blocker which act by widening of blood vessels. Its Molecular formula-$C_{26}H_{31}ClN_2O_8S$ and molecular weight-567.05 g/mol. Solubility-it is slightly soluble in water, freely soluble in methanol, sparingly soluble in anhydrous ethanol, and slightly soluble in 2-propanol [1,2].

The perindopril erbumine and Amlodipine besylate tablets are involves combination of two active ingredients i.e. perindopril and Amlodipine. In that the drug Amlodipine is act as a calcium channel blocker which dilates blood vessels and the second drug Perindopril is an angiotensin converting enzyme inhibitor agent. In combine 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 literature survey, we conclude that individual and combination these drugs has been analyzed by many spectroscopic methods. The Amlodipine besylate API is official in British pharmacopoeia and Indian pharmacopoeia [2]. And the Perindopril erbumine is official in the British Pharmacopoeia. The combination of Perindopril erbumineand Amlodipine besylate is not included in any pharmacopoeias. So, objective of this work describes simple, rapid, economical, selective, precise and reproducible HPLC method for pharmaceutical importance. This method was validated as per the ICH guidelines [3].

MATERIALS AND METHODS

Reagents and Chemicals

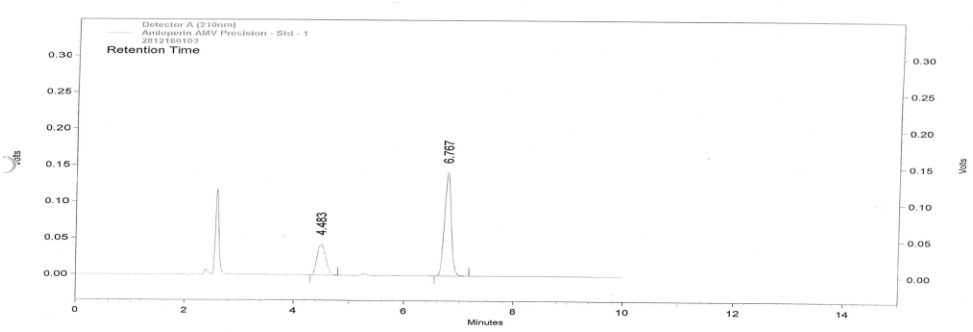
Acetonitrile (HPLC Grade), Potassium dihydrogen orthophosphate (Merck, AR Grade), Orthophosphoric acid (AR Grade), water (HPLC Grade), The standard drug samples of perindopril erbumine and amlodipine besylate, as well as tablet available in the ratio of 1:1 containing perindopril erbumine 5 mg and Amlodipine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

For Assay and Content Uniformity

Chromatographic condition: Analysis was performed on chromatographic system consisted of Shimadzu, series LC 2010 A (pump Quaternary system). Separation was carried out with a Kromasil C8 (4.6 mm × 250 mm, 5 µ particle size) column at a temperature 40°C, with flow rate 1.00 mL per min. with an isocratic mobile phase constituting Buffer and Acetonitrile having the ratio 59:41. pH was adjusted up to 2.6 with Orthophosphoric acid. Perindopril and Amlodipine was determined by using UV detection method at 210 nm where the injection volume was 10 µL and the run time was 10 min [4,5].

Preparation of buffer solution for mobile phase: Weigh accurately about 6.8 fgm of Potassium dihydrogen orthophosphate and transfer in 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.

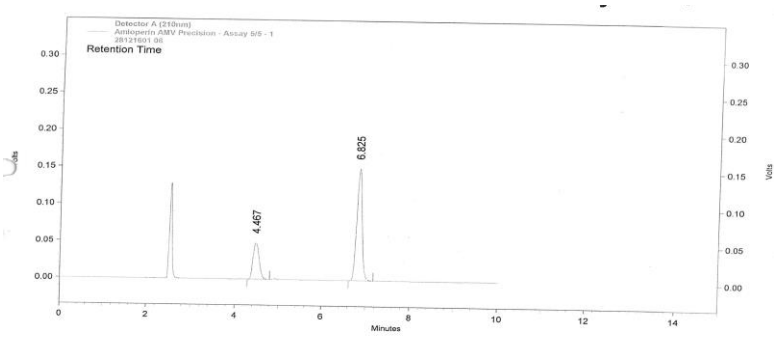
Preparation of standard solution: Weigh accurately 50 mg of Perindopril erbumine and 69 mg of Amlodipine besylate, transferred to 100 mL volumetric flask and dissolved it in 70 ml of mobile phase and make volume up to the mark with mobile phase to get 500 µg/ml of Perindopril erbumine and 690 µg/ml of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



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: C**hromatogram of standard preparation.**

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

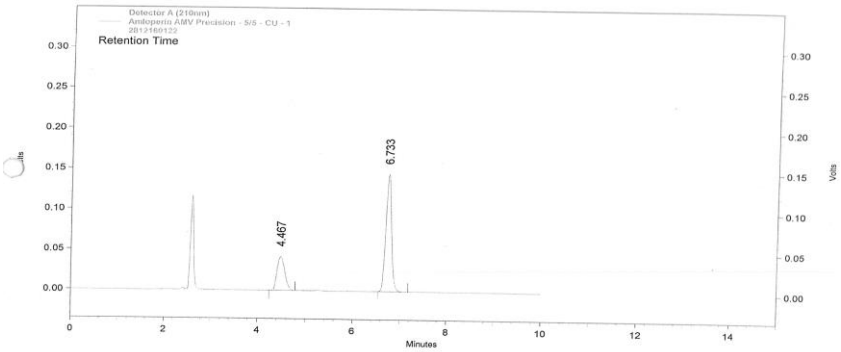


Detector A (210nm) Name	Retention Time	Area	Area %	Theoretical plates	Resolution	Asymmetry
Perindropil	4.467	472053	27.69	4750	0.00	1.26
Amlodipine	6.825	1232844	72.31	15146	9.80	1.24

Figure 2: C**hromatogram of sample preparation (Assay).**

For Content uniformity, one tablet was placed in to each of ten 100 ml volumetric flask. Approximately 70 ml of mobile phase was added to each volumetric flask and 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 solution by using 0.45 µm membrane syringe filter and then filtrate was injected for analysis.

A 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	4.467	459037	27.11	3621	0.00	1.27
Amlodipine	6.733	1234034	72.89	13893	8.63	1.20

Figure 3: C**hromatogram of sample preparation (CU).**

RESULTS AND DISCUSSION

The Specificity and System Suitability

Specificity test used to determines the effect of excipients on the result of assay. To determine the specificity of 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 above method was carried out by five repeated analysis of solution containing 100% target concentration of Perindopril erbumine and Amlodipine besylate. Various parameters of chromatographic techniques such as retention time, column efficiency, peak area, tailing factor and resolution between the peaks were determined and the method was evaluated by analyzing these parameters in Figure 4,5 [10,11].

Linearity and Range

In above method linearity was determined by constructing the calibration curves. For this purpose different standard solution of Perindopril erbumine and Amlodipine besylate of different concentration level (20%, 40%, 60%, 100%, 120%, 140% and 160%) were used. Measurement of each concentration was carried out and the peak areas of the chromatograms were plotted against the concentrations to obtain the calibration curves and correlation coefficients. Tables 1 and 2 represents the results were directly proportional to the concentration of analyse in the given sample.

Table 1: 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 Perindopril w.r.t. working level conc. (i.e. 0.5 mg/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	0.99999	0.3
Peak area injection 2.	98817	190906	288256	479660	576169	672772	765810		
Peak area injection 3.	98903	190716	288507	480000	576429	673135	768005		
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	9612367	9597560	9603450	9613829	9581238		
Relative response factor.	1.0302	0.9941	1.0015	1	1.0006	1.0017	0.9983		
Average.	1.0038								
% RSD.	1.19								

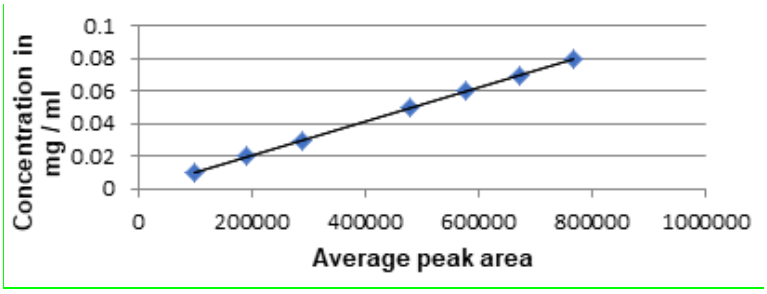


Figure 4: Linearity and range.

Table 2: 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., 0.5 mg/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	0.99999	0.6
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		
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	2.535	2.5356	2.524		
Average	2.5481								
% RSD	1.44								

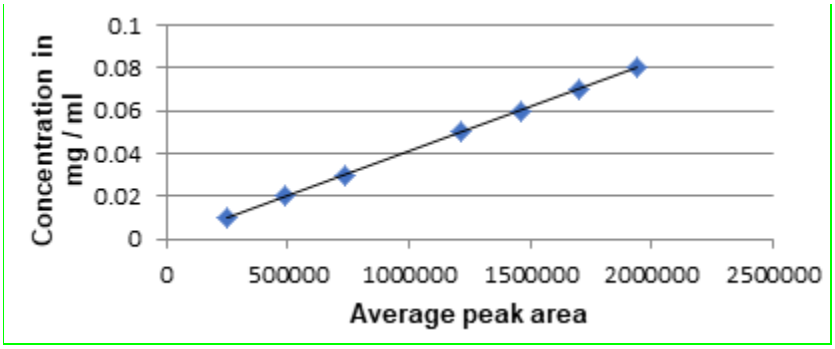


Figure 5: Linearity and range.

Precision

System precision and method precision: The method precision is performed by carry out standard replicate and six independent sample preparations of a single lot of formulation. For this method the sample solution was prepared

same as described in sample preparation. The percentage relative standard deviation was found less than 2.0% for the both the analyse.

Intermediate precision: The Intermediate precision performed by carried with standard replicate and six independent samples from two different analysts by using different chromatographic system on different days. The chromatographic sample results are summarized in following Table 3 and 4, the percentage relative standard deviation was found less than 3.0% for both analysts in Table 5.

Table 3: 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	99.7	98.5	100	97
2	99.9	100.2	99.9	98.3	100.2	96.8
3	100.3	100.6	100.3	98	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	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 4: 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	97.9	102.4	101	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

Mean	99.7	102.3	99.4	101.4
% RSD			2.3	1.4

Table 5: % Recovery.

Levels	Perindopril	Amlodipine
1 (50%)	99.4	99
2 (100%)	99.7	100.8
3 (150%)	99	98.9
Average	99.4	99.6
% RSD	0.4	1.1

Effect of variation in mobile phase: This study was performed to determine the effect of variations in composition of mobile phase. The standard solution and test solution was prepared and injected in to HPLC system by changing the composition of mobile phase by ± 5 and system suitability parameters were evaluated. The values were given in the following Table 6-8.

Table 6: 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	100.9	Sample-B	98.1	100.5
Average	97.8	100.6	Average	97.9	100.3

Table 7: 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	100.8	Average	97.6	100.7

Table 8: 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	Average	98.9	98.7

Solution Stability

For the demonstration of the stability of standard solution during its analysis, the standard solution was analyzed over a period of 24th 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 indicate that no any significant degradation occurred within this period. i.e. solutions were stable for at least 24th hour which was sufficient for completion of whole analytical process. The results were displayed in the following Table 9 [8,11].

Table 9: 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	97.2	0.1
2	4 Hour	96.4	0	97.2	0.1
3	8 Hour	96.3	0.1	97	0.31
4	12 Hour	96.4	0	97.1	0.21
5	16 Hour	96.3	0.1	96.9	0.41
6	20 Hour	96.3	0.1	97	0.31
7	24 Hour	96.3	0.1	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 and Amlodipine Besylate.

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