METHOD DEVELOPMENT AND VALIDATION OF CARDIOVASCULAR DRUGS BY RPHPLC

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Abstract: The objective of this current work was to design and validate analytical technique for quantitative measurement of Perindopril Erbumine and Amlodipine Besylate in a tablet formulation. The two medications were separated by chromatography using Hypersil ODS C18 (250x4.6mm ID) 5 µm. At a flow rate of 1.0 mL/min, the mobile phase, which consisted of Phosphate buffer pH 5.0: ACN:Methanol (40:40:20) At 230nm, detection was carried out. The separation took place in 5 minutes. Over the concentration range of 8 to 80 mg/mL of perindopril erbumine and 10 to 80 mg/mL of amlodipine besylate, calibration curves were linear with correlation coefficients between 0.99 and 1.0. The result was the relative standard deviation, or R.S.D.

Keywords: Perindopril Erbumine, Amlodipine Besylate, Reverse-Phase HPLC, Isocratic elution.

INTRODUCTION

Amlodipine Besylate and Perindopril Erbumine are antihypertensive medications. The enzyme inhibitor that converts angiotensin is perindopril erbumine. The medication is used to treat hypertension. It can be used both on its own and in conjunction with other antihypertensive medications. Calcium channel blocker amlodipine besylate [1] is the drug. It is applied as an angina therapy and an anti-hypertensive. It prevents spasm by lowering blood pressure, relaxing heart muscles, and dilating heart blood channels. Perindopri Erbumine's chemical name is 2-Methyl Propane.2. Amine (2S, 3As, 7As)-1-[(2S)-2- 2[[(1S)-1- (ethoxycarbonyl) butyl] amine] propanoyl] octahydro-1H-indol-2-carboxylate. Amlodipine

A review of the literature indicated that the following methods are available for determining the presence of perindopri erbumine: [HPLC] [4], [LCMS] [5], and [Crystal CE] [6]. Similarly, the following methods are available for determining the presence of amlodipine besylate: [HPLC] [7] [8] [9], HPTLC [10], simultaneous spectrophotometric determination [11] [12] [13], Spectrofluorometric [14], [LCMS] [15], and stability indicating assay method [16]. The current study presents a validated reverse phase HPLC technique for these medications' simultaneous determination in tablet dose form. Nevertheless, there are no references available for the quantitative assessment of amlodipine besylate and pridopril erbumine in pharmaceutical formulations. The ability to measure both amlodipine besylate and phendiopril erbumine on a single chromatographic system using the same detection wavelength is the main benefit of the suggested approach

MATERIALS AND METHODS

Table 1 Instruments

UV-Visible Spectrophotometer	Analytical Technologies Ltd
HPLC	Cyberlab (Salo Terrace, Millbury, USA)
Ultra sonicator	Citizen, Digital Ultrasonic Cleaner
pH meter	Elico
Electronic balance	Shimadzu
Syringe	Hamilton
HPLC Column	Waters symmetry C18 (150x4.6 ID) 3.5µm

Table 2 Chemicals

Potassium Dihydrogen ortho phosphate	Rankem/ AR Grade
Acetonitrile	Merck/ HPLC Grade
Water	Merck/ HPLC Grade
Methanol	Merck/ HPLC Grade
Triehylamine	Rankem/ AR Grade

Table 3 Drugs Used

LP and AB bulk drugs	Gift samples obtained from Madras pharmaceuticals, Chennai
AB and LP tablets	Obtained from local pharmacy

Preparation of 0.05 M potassium Dihydrogen Orthophosphate buffer pH 6.0:
3.402 gm of potassium dihydrogen phosphate was weighed and dissolved in 100 mL of water and volume was made up to 500 mL with water. Adjust the pH to 6 ± 0.02 using triethylamine. The buffer was filtered through 0.45µm filters to remove all fine particles and gases.

Determination of Working Wavelength (λ_{max})
In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

Preparation of Standard solution
About 50 mg of AB and 40 mg of PE were weighed into a 50 mL volumetric flask, to this 25 mL of mobile phase was added, sonicated and the volume was made up to mark with the mobile phase.

Dilutions
Necessary dilutions are made from standard stock solutions to get the concentration range of 100 µg/mL of AB and 80 µg/mL PE.
The wavelength of maximum absorption (λ_{max}) of the solution of the drugs in mobile phase were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against mobile phase as blank. The absorption curve shows characteristic absorption maxima at 210 nm for AB236 nm for PE and at 230 nm same absorbance for both the drugs i.e., isobestic point. Thus 230 nm was selected as detector wavelength for the HPLC chromatographic method.
Preparation of Sample solution

Sample name   : Coversyl-AM

Manufacture name   : Serdia

Weigh A quantity of powder equivalent to 50 mg of AB and 40 mg of PE in 50 mL volumetric flask and make up mark with mobile phase. From above solution pipette 1 mL of the clear solution in to 10 mL volumetric flask and make up volume with mobile phase. The resulting solution is used to record the chromatogram

METHOD VALIDATION
System Suitability

To verify that the analytical system is working properly and can give accurate and precise results were evaluated by 100 µg/mL of AB and 82 µg/mL of PE were injected six times and the chromatograms were recorded for the same.

Precision:
System precision
The system precision was determined by analysing standard preparation of AB (100 µg/mL) and PE (80 µg/mL) for six times

Method precision

Method precision was determined by injecting sample solutions of concentration AB (100 μg/mL) and PE (80 μg/mL) for six times are prepared separately

Limit of Detection and Limit of Quantitation

LOD and LOQ is calculated from standard deviation of response from precision and slope from linearity

$$LOQ = 10\ \sigma / S$$
$$LOD = 3.3\ \sigma / S$$

Where

σ is standard deviation from response S is slope from calibration curve

Specificity

The standard solution 100μg/mL of AB and 80 μg/mL of PE was injected and the chromatogram was recorded
The tablet sample solution 100 μg/mL of AB and 80 μg/mL of PE was injected and the chromatogram was recorded

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre analysed sample), the reference standards of the drugs were added at the level of 80%, 100%, 120%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug

Robustness

The Robustness of the method was determined. The results obtained by deliberate variation in method parameters
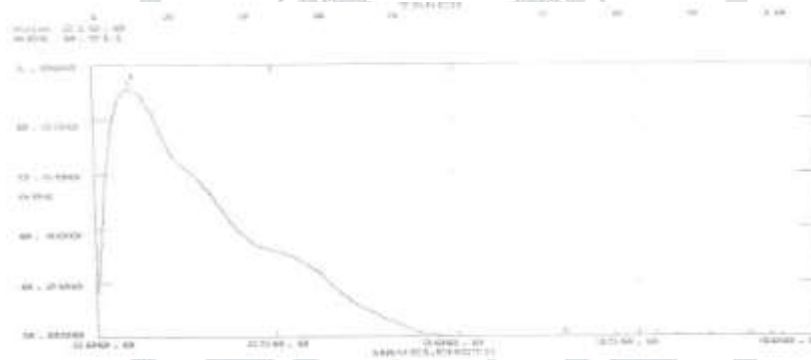
RESULTS AND DISCUSSION:
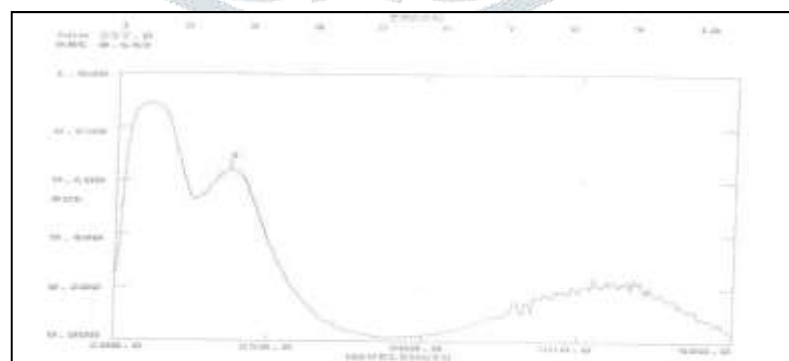

Fig 1 UV-VIS Spectrum of AB
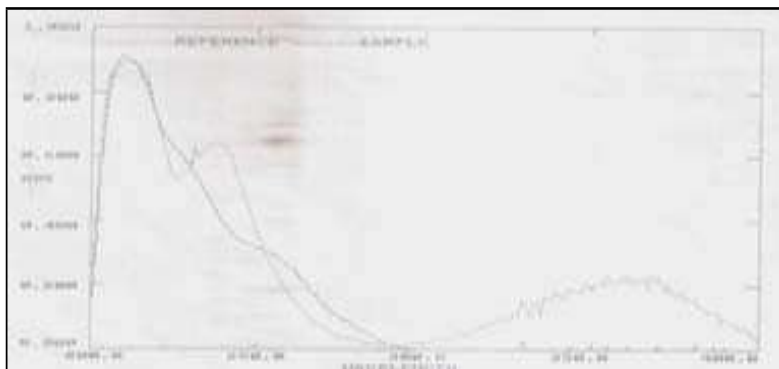

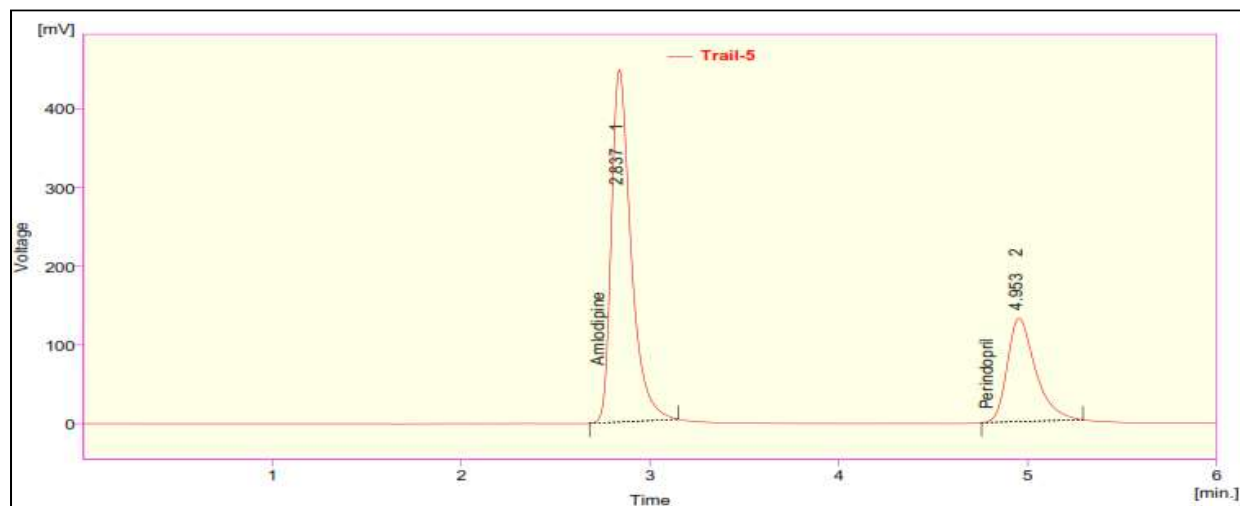Fig 2 UV-VIS Spectrum of PE

Fig 3 UV-VIS overlay Spectrum of AB and PE

Fig 4 Chromatogram of Standard solution

Table 4 Observation values

S.NO	Name	RT	Area	TP	TF	R_S
1	AB	2.836	3234.124	3684	1.860	-
2	PE	4.953	1363.830	5538	1.643	9.341

The AB peak was observed at 2.293 min with peak area 3234.124, theoretical plates 3684 and tailing factor 1.860. The PE peak was observed at 4.953 min with peak area 1363.830, theoretical plates 5538 and tailing factor 1.643 (Table 6.6). The Theoretical plate, tailing factor and resolution was found to be within limits. So, this trail was considered and validated according to ICH guidelines.

Conclusion: Hence this method was finalized for the simultaneous estimation of AB and P

Chromatogram of sample solution.

Table 5 Observation values

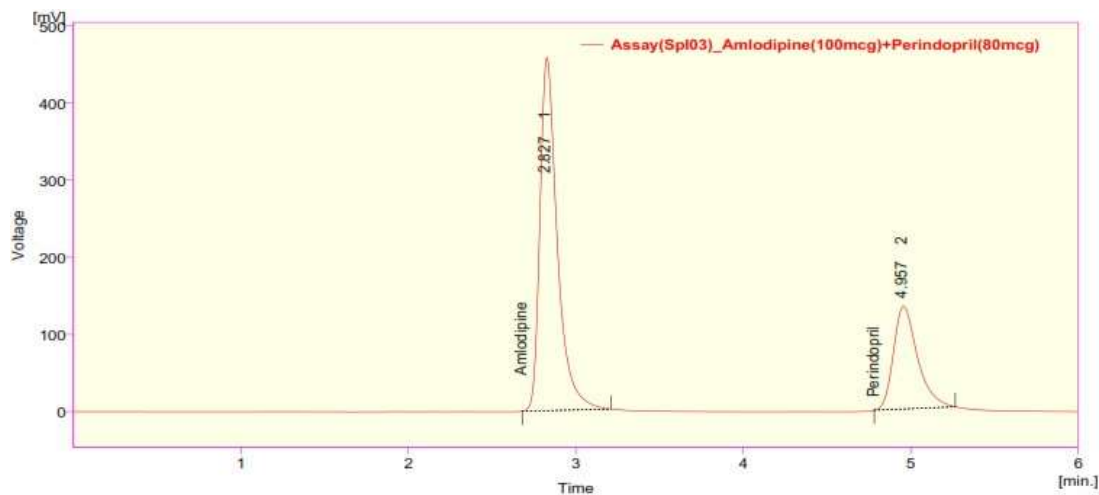| S.NO	Name	RT	Area	TP	TF	R_S
1	AB	2.826	3292.484	3658	36583	-
2	PE	4.956	1330.491	5689	56892	9.519

Fig 5 Chromatogram of Sample solution

Observation

So, the both drugs % assay found to be within the limits. The percentage purity of both AB and AB were found to be within the limits that is 98-102 %.

System suitability

Tables 6 Results for system suitability of AB.

Injection	RT	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	2.84	3226.960	3693	1.692
2	2.836	3234.124	3684	1.86
3	2.84	3245.109	3693	1.692
4	2.83	3253.024	3666	1.692
5	2.84	3146.809	3693	1.692
6	2.83	3255.560	3666	1.692
Mean	2.8362	3226.931	3156	1.805
SD	0.0049	40.648	-	-
%RSD	**0.16**	**1.26**	-	-

Tables 7 Results for system suitability of PE

Injection	Retention time	Peak area	Theoretical plates	Tailing factor	Resolution
1	4.960	1314.66	5565	1.639	9.4
2	4.953	1363.83	5538	1.643	9.341
3	4.963	1335.391	5353	1.666	9.299
4	4.946	1366.214	5295	1.694	9.226
5	4.960	1305.008	5565	1.666	9.4
6	4.946	1335.081	5295	1.694	9.226
Mean	4.960	1336.614	5438	1.684	9.31
SD	0.012	24.889	-	-	-
%RSD	**0.25**	**1.86**	-	-	-

Result

The plate count and tailing factor results were found to be satisfactory and are found to be within the limit. The % RSD was found to be less than 2.

System precision

Table 8 System precision results for AB and PE.

Injection	AB		PE	
	Retention times	Area	Retention time	Area
1	2.84	3226.960	4.960	1314.66
2	2.836	3234.124	4.953	1363.83
3	2.84	3245.109	4.963	1335.391
4	2.83	3253.024	4.946	1366.214
5	2.84	3146.809	4.960	1305.008
6	2.83	3255.560	4.946	1335.081
Average	2.8362	3226.931	4.960	1336.614
SD	0.0049	40.648	0.012	24.889
%RSD	**0.16**	**1.26**	**0.25**	**1.86**

Result

% RSD of 6 determinations of AB and PE for System precision found to be within the acceptance criteria of less than 2.0%.

Table 9 Method precision

Injection	AB		PE	
	Retention times	Area	Retention times	Area
1	2.841	3226.960	4.930	1315.66
2	2.838	3235.124	4.933	1353.83
3	2.842	3246.109	4.963	1335.391
4	2.838	3253.024	4.956	1366.254
5	2.841	3146.629	4.960	1345.008
6	2.833	3255.550	4.936	1345.001
Average	2.8388	3226.233	4.948	1343.541
SD	0.0033	40.946	0.016	16.113
%RSD	**0.12**	**1.26**	**0.35**	**1.26**

Result
The %RSD of 6 determinations of AB and PE for System precision found to be within the acceptance criteria of less than 2.0%.

Linearity and range

Table 10 Linearity data of AB

S. No	Concentration (µg/mL)	Area
1	60	1849.349
2	80	2553.316
3	100	3309.843
4	120	3888.959
5	140	4512.565
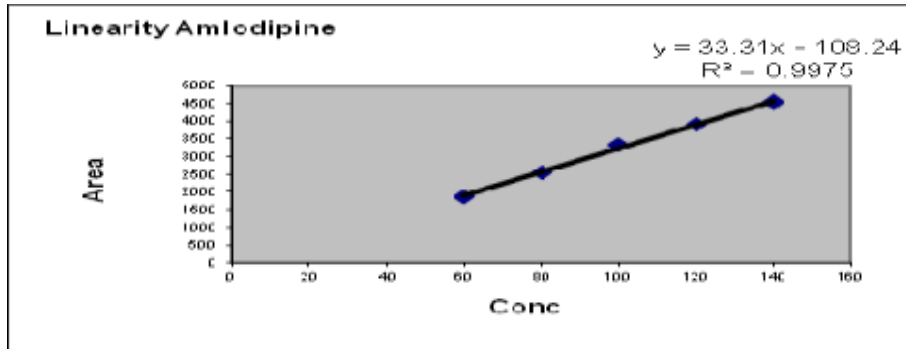
Fig 6 Linearity of AB

Table 11 Linearity data of PE

S. No	Concentration (µg/mL)	Area
1	48	666.584
2	64	1086.620
3	80	1355.935
4	96	1598.886
5	112	1860.699
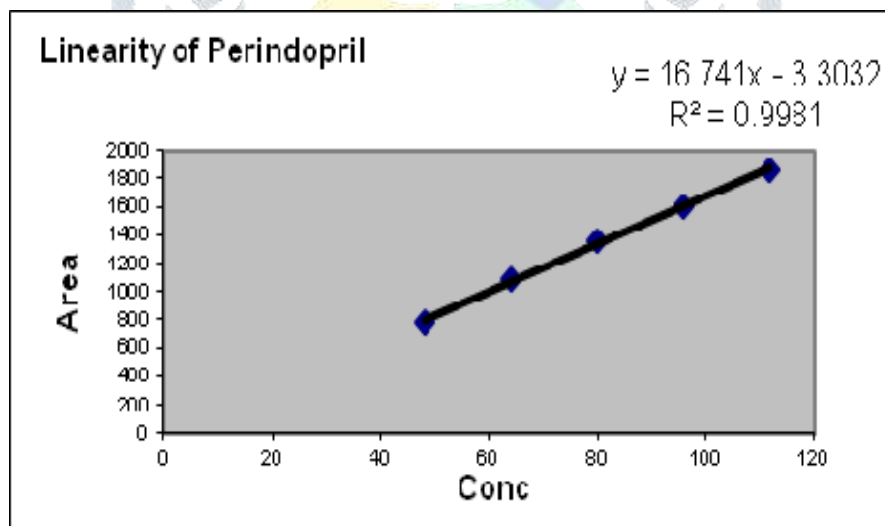
Graph for Linearity data of PE

Table 12 Linearity data of PE

S. No	Parameter	AB	PE
1	Correlation coefficient	0.996	0.998
2	Slope	33.31	16.64
3	Intercept	108.2	3.303

Result

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of AB and PE is 0.996 and 0.998 respectively

Table 13 Results for Robustness of AB and PE.

Chromatographic changes		Retention time(min)		Tailing factor	
		AB	PE	AB	PE
Flow rate (mL/min)	0.8	3.510	6.136	1.842	1.843
	1.0	2.830	4.943	1.848	1.634
	1.2	2.363	4.166	1.842	1.843
Wavelength (nm)	228	2.826	4.953	1.830	1.800
	230	2.830	4.943	1.848	1.634
	232	2.830	4.960	1.842	1.844

LOD &LOQ:

The LOD for this method was found to be 0.125 μg/mL for AB and 0.366 μg/mL for PE respectively. The LOQ for this method was found to be 0.368 μg/mL for AB and 1.11 μg/mL for PE respectively.

Result

The tailing factor was found to be within the limits on small variation of flow rate and wavelength.

CONCLUSION

A new precise, accurate, rapid method has been developed for the simultaneous estimation of Amlodipine Besilate and Perindopril Erbumine in pharmaceutical dosage form by RP-HPLC.

The different analytical performance parameters such as linearity, precision, accuracy, and specificity, LOD, LOQ were determined according to International Conference on Harmonization ICH Q2B guidelines. The calibration curve for PE was obtained by plotting peak area versus the concentration over the range of 48-112 μg/mL For PE and 60-140 μg/mL for AB. From linearity the correlation coefficient R^2 value was found to be 0.998 for PE and 0.997 for AB. The proposed HPLC method was also validated for system suitability, system precision and method precision. The % RSD in the peak area of drug was found to be less than 2%. The number of theoretical plates was found to be more than 4000, which indicates efficient performance of the column. The limit of detection of PE and AB were found to be 0.0.366 μg/mL and 0.125 μg/mL and limit of quantitation were 1.11 μg/mL and0.378 μg/mL respectively, indicates the sensitivity of the method. The percentage of recovery of PE and AB were found to be 100.22 and 99.07 respectively shows that the proposed method is highly accurate

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