Analytical method development and validation of Amlodipine besylate and Perindopril erbumine in combine dosage form by RP-HPLC

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Abstract: The objective of this present work was to develop and validate analytical method for quantitative determination of Perindopril Erbumine and Amlodipine Besylate in a tablet formulation. Chromatographic separation of the two drugs was achieved on an Eclipse XDB C-8 (150 mm X 4.6 mm), 5µm. The mobile phase constituted of Buffer: Acetonitrile (65:35) and pH adjusted to 2.6 with dilute Ortho- Phosphoric Acid was delivered at the flow rate 1.0 mL/min. Detection was performed at 210 nm. Separation was completed within 8 min. Calibration curves were linear with correlation coefficient between 0.99 to 1.0 over a concentration range of 8 to 60 µg/mL of Perindopril Erbumine and 10 to 75 µg/mL of Amlodipine Besylate. The relative standard deviation (R.S.D) was found <2.0%.
Keywords: Perindopril Erbumine; Amlodipine Besylate; Reversed-phase HPLC.

Introduction

Perindopril Erbumine and Amlodipine Besylate are Antihypertensive drugs. Perindopril Erbumine is Angiotensin Converting Enzyme Inhibitor. It is used for the treatment of hypertension. It may be used alone or   in combination with other antihypertensive agents. Amlodipine besylate is the Calcium channel blocker [1]. It is used as an anti-hypertensive and in the treatment of angina. It lowers the blood pressure, relaxes heart muscles and dilates the heart blood vessels to prevent spasm.
The chemical name for Perindopri Erbumine is 2-Methyl Propane-2-amine   (2S,3As,7As)-1-[(2S)-2-2[[(1S)-1-(ethoxycarbonyl)   butyl]amine]propanoyl]octahydro-1H-indol-2-carboxylate… [2]

The chemical name for Amlodipine besylate is 3-ethyl 5-methyl   4RS-2-[(2-aminoethoxy)   methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzene sulphonate...[3]. Literature survey revealed that Methods available for the determination of Perindopri Erbumine include [HPLC] [4], [LCMS] [5] and [Crystal CE] [6].Methods available for the determinations of Amlodipine besylate include [HPLC] [7] [8] [9], HPTLC [10] simultaneous spectrophotometric determination [11] [12] [13], Spectrofluorometric [14], [LCMS] [15] and stability indicating assay method [16]. The present work describes a validated reverse phase HPLC method for simultaneous determination of these drugs in tablet dosage form. However no

references have been found for quantitative determination of Perindopril Erbumine and Amlodipine Besylate in pharmaceutical preparations. The major advantage of the proposed method is that Perindopril Erbumine and Amlodipine Besylate can be determined on a single chromatographic system with the same detection wavelength.

Material and Methods

Chemicals and Materials: Cadila Healthcare Limited. supplied Perindopril Erbumine and Vent Unimark remedies supplied Amlodipine Besylate. Acetonitrile and Ortho Phosphoric Acid (Spectrochem and E-Merck Limited).

Instrumentation

Shimadzu 2010C integrated high performance liquid chromatographic system was used for this experiment. Shimadzu 2010C system equipped with quaternary gradient pump, 2010C UV-VIS detector, 2010C Column Oven and 2010C programmable auto sampler controlled by CLASS-VP software. The Eclipse XDB C8 column (150 X 4.6, 5μm) was used as a stationary phase. HPLC conditions are given **Table 1.**

Table1. HPLC conditions

Column	Eclipse XDB C8 column (150 X 4.6, 5μm).
Detector	210 nm
Injection volume	20 μL
Flow rate	1.0 mL/min
Temperature	30˚ C
Run time	8 min
Mobile phase	Buffer **:** Acetonitrile (65**:**35)

Buffer Preparation

Weigh 6.8 Pottasium Dihydrogen Phosphate in to 1000 ml of Milli Q water and mix.

Mobile Phase

Mixture of Buffer **:** Acetonitrile in the ratio of (65:35), Adjust PH 2.6 with Ortho Phosphoric Acid.

Diluent

Mixture of Buffer **:** Acetonitrile in the ratio of (65:35), Adjust PH 2.6 with orthophosphoric acid.

Standard Preparation

Standard stock solutions of 400μg.mL^{-1} and 700μg.mL^{-1} were prepared by dissolving 40 mg Perindopril

Erbumine and 70 mg Amlodipine Besylate in 100 ml diluent respectively. From this stock solution, working standard solution having concentration 40 μg.mL^{-1} and 70 μg.mL^{-1} were prepared by appropriate dilution for Perindopril Erbumine and Amlodipine Besylate respectively.

Sample Preparation:

Weigh accurately tablets powdered equivalent to about 40 mg of Perindopril Erbumine and 50 mg of Amlodipine besylate in to 200 mL volumetric flask. Add about 150 mL Mobile phase and sonicate to dissolve. Now make volume up to the mark with Mobile phase.Filtered it through 0.45 μ HVLP nylon filter and made further dilution 5.0 mL of this solution to 25.0 ml with mobile phase and mix.

Method Validation

System Suitability and System Precision:

System suitability and system precision was daily performed during entire validation of this method. The results of system suitability and system precision were presented in **Table 2.**

Linearity and Calibration Curve:

The plot of peak area response against concentration is shown in fig. The plot is linear over the concentration range of 8 to 60 μg/mL and 10 to 75 μg / mL for Perindopril Erbumine and Amlodipine Besylate respectively. Linearity of the calibration curve was determined by weighed (1/c) least square regression analysis. The correlation coefficient was found to be 0.99 to 1.00. A linear relationship was found for all components. The results of linearity, limit of detection and limit of quantification were presented in **Table 3.**

Specificity:

There was no interference from sample placebo and peak purity of Perindopril Erbumine and Amlodipine Besylate were 0.99999 and 1.00000 respectively. It showed that developed analytical method was specific for the analysis of Perindopril Erbumine and Amlodipine Besylate in tablet dosage form.

Standard and Sample Solution Stability:

Standard and sample solution stability was evaluated at room temperature for 24 h. The relative standard deviation was found below 2.0%. It showed that both standard and sample solution was stable up to 24 h at room temperature.

Table 2. System Suitability and System Precision

Compound	Retention time (Mean ± SEM)	n	k'	R	T	α
Perindopril Erbumine	3.172 ± 0.004	2694.43	30.79	--	1.11	--
Amlodipine Besylate	5.504 ± 0.000	4457.92	54.04	8.10	1.05	1.755

n= Theoretical plates
k'= Capacity Factor
R= Resolution
T= Asymmetry
α = Selectivity

Table 3. Characteristics of the Analytical Method derived from the Standard Calibration Curve

Compound	LOD µg/mL	LOQ µg/mL	Linearity range n=(5)	Correlation co-efficient µg/mL	Residual std. regression σ	Slope of regression S
Perindopril Erbumine	0.4	1.2	8.0 to 60.0	0.99995	4634.801	19311.853
Amlodipine Besylate	0.28	0.84	10.0 to 75.0	0.99994	54560.316	55381.342

LOD= Limit of detection
LOQ= Limit of quantification

Method Precision:
The precision of the method was established by carrying out the analysis of the analyte (n=6) using the proposed method. The low value of standard deviation showed that the method was precise. The results obtained were presented in table 4.

Method robustness:
Robustness of the method was determined by small deliberate changes in flow rate, mobile phase ratio 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 7.

Method Ruggedness:
Ruggedness test was determined between two different analysts, instruments and columns. The value of percentage RSD was below 2.0%, showed ruggedness of developed analytical method. The results of ruggedness were presented in table 6.

Table 4. Method Precision

Compound	Concentration µg/mL (n=6)	Retention time Mean ± SEM (n=6)	% Assay Mean ± SEM (n=6)	% RSD of Assay
Perindopril Erbumine	40.4	3.170 ± 0.0000	98.4 ± 0.172	0.4
Amlodipine Besylate	70.1	5.498 ± 0.0000	100.6 ± 0.107	0.3

Table 5. Method Accuracy

Level	Drug Added (mg)	Drug recovered (mg)	% Assay (Mean ± SEM) (n=3)	% RSD of Assay (n=3)
For Perindopril Erbumine				
50%	20.36	20.60	101.7 ± 0.61	1.0
100%	40.53	40.26	99.8 ± 0.43	0.8
150%	60.37	59.89	99.7 ± 0.20	0.3
For Amlodipine Besylate				
50%	25.46	18.59	101.4 ± 0.954	1.6
100%	50.27	36.46	100.7 ± 0.241	0.4
150%	75.23	54.28	100.1 ± 0.261	0.4

Table 6. Method Ruggedness

Compound	% Assay Mean ± SEM (n=6)	% RSD of Assay (n=6)
Day 1	**Analyst-1, Instrument-1 & Column-1**	
Perindopril Erbumine	98.4 ± 0.17	0.4
Amlodipine Besylate	100.6 ± 0.11	0.3
Day 2	**Analyst-2, Instrument-2 & Column-2**	
Perindopril Erbumine	99.0 ± 0.34	0.8
Amlodipine Besylate	100.0 ± 0.11	0.3

Table 7. Method Robustness

Compound	% RSD in Normal and Changed condition (n=5)		
Temperature	**% RSD Normal**	**% RSD (-5°C)**	**% RSD (+5°C)**
Perindopril Erbumine	0.07	0.12	0.06
Amlodipine Besylate	0.04	0.06	0.05
pH	**% RSD Normal**	**% RSD (-0.2 unit)**	**% RSD (+0.2 unit)**
Perindopril Erbumine	0.07	1.04	0.06
Amlodipine Besylate	0.04	0.11	0.03
Flow Rate	**% RSD Normal**	**% RSD (-10%)**	**% RSD (+10%)**
Perindopril Erbumine	0.07	0.03	0.37
Amlodipine Besylate	0.04	0.45	0.10
Mobile phase ratio	**% RSD Normal**	**% RSD (-2%)**	**% RSD (+2%)**
Perindopril Erbumine	0.07	0.1	0.08
Amlodipine Besylate	0.04	0.2	0.15

Fig. 1   Chromatogram of Standard Solution.
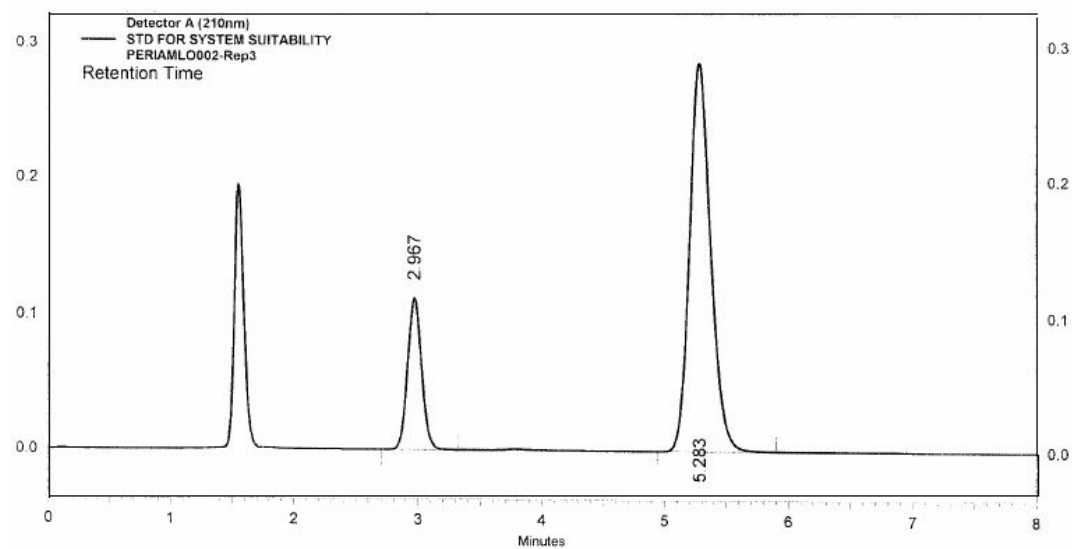
Detector A (210nm) Pk #	Name	Retention Time	Area	Asymmetry	Resolution	Theoretical plates
1	PERINDOPRILE ERBUMATE	2.97	879422	1.11	0.00	3074.91
2	AMLODEPINE BESYLATE	5.28	3400431	1.16	8.72	4434.80
Totals			4279853			

Fig.2 Chromatogram of sample solution.
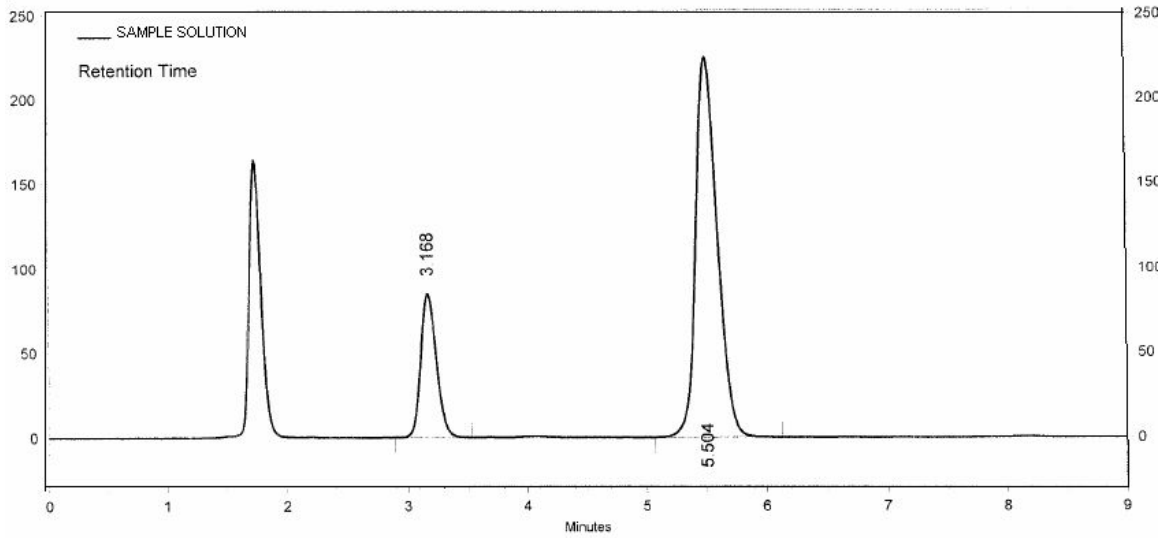
1: 210 nm, 8 nm

Pk #	Name	Retention Time	Area	Theoretical plates	Asymmetry
1	PERINDOPRIL ERBUMINE	3.168	760496	2700.41	1.20
2	AMLODIPINE BESILATE	5.504	2770314	4522.17	1.14

Totals					
			3530810		

Fig.3 Calibration curve of Amlodipine Besylate.

Calibration Curves

AMLODEPINE BESYLATE at exp. RT:5.5
VWD1 A, Wavelength=210 nm
Correlation: 0.99944
Residual Std. Dev.: 54560.31555
Formula: y = mx + b
        m: 55381.34155
        b: 25609.41132
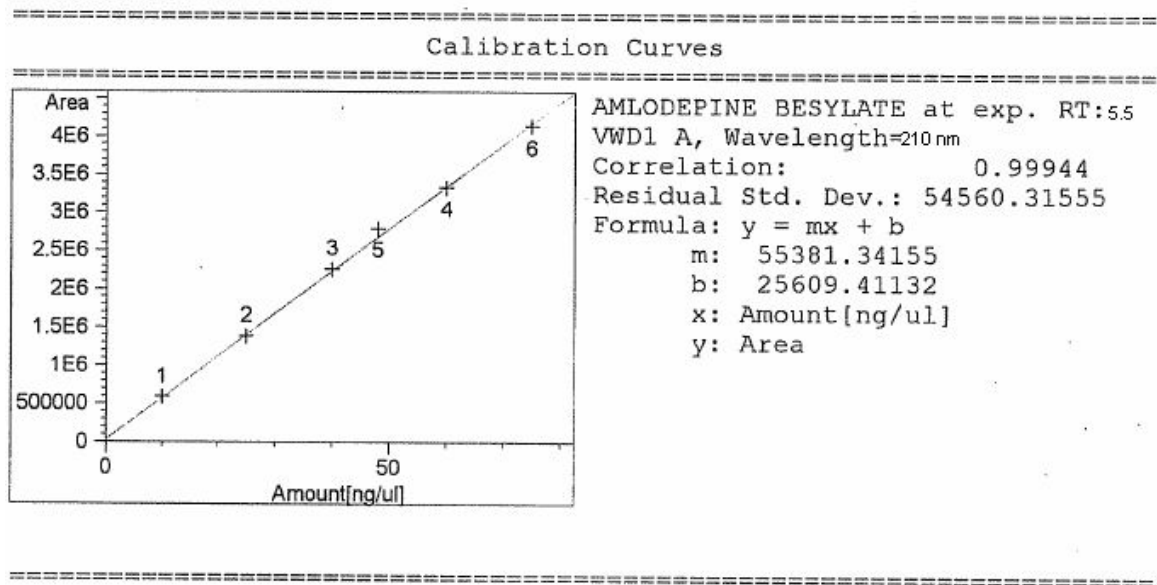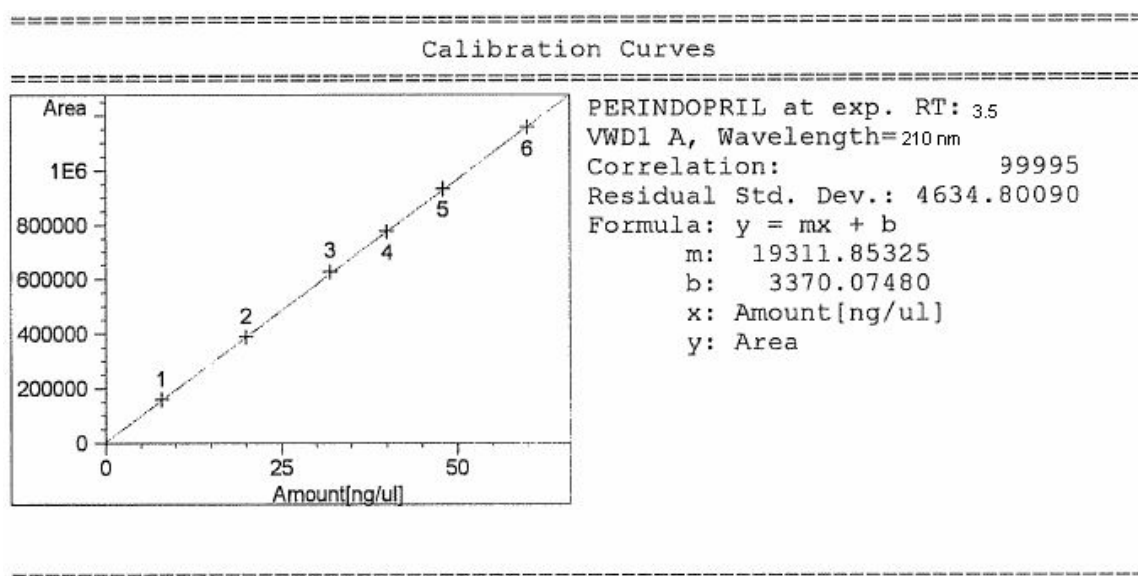   x: Amount[ng/ul]
        y: Area

Fig. 4 Calibration Curve of Perindopril Erbumine.

Results

The detection wavelength of 210 nm was chosen in order to achieve a good sensitivity for quantitative determination of Perindopril Erbumine and Amlodipine Besylate in tablet dosage form. The mobile phase consisting of Buffer **:** Acetonitrile (65:35) with (pH 2.6) with Ortho Phosphoric Acid offered a good separation at ambient temperature under these conditions using a flow rate of 1.0 mL/min and a runtime of 8 min, Perindopril Erbumine elutes at first and then Amlodipine Besylate shown in the chromatogram, In chromatogram first pick(at RT 1.5) due to Besylate Salt of Amlodipine Besylate is detected due to lower common wavelength selected, Fig. 1 and 2 which illustrate the separation of both active ingredients in this system. The isocratic program throughout HPLC method was adopted to analyze both components in a single run. The proposed method is simple and do not involve laborious time-consuming sample preparation.

Acknowledgement

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