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R E S E A R C H A R T I C L E

Simultaneous Estimation of Amlodipine Besylate and Perindopril Erbuminein combined Dosage form by RP-HPLC

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A B S T R A C T

The present work was developed and validated an 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), 5mm. 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. The retention time for Perindopril Erbumine and Amlodipine Besylate 3.168 & 5.504 min respectively. Calibration curves were linear with correlation coefficient between 0.99 to 1.0 over a concentration range of 8 to 60 mg/mL of Perindopril Erbumine and 10 to 75 mg/mL of Amlodipine Besylate. The relative standard deviation (RSD) was found< 2.0%.

Keywords: Perindopril Erbumine; Amlodipine Besylate; Reversed-phase HPLC.

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C O N T E N T S

1. 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.

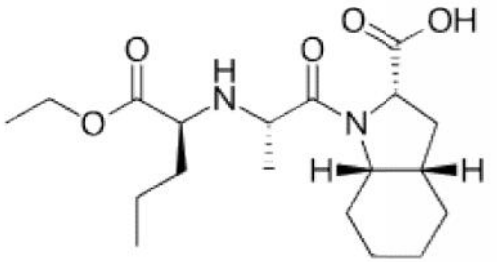


Fig 1: Structure of Perindopril

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].

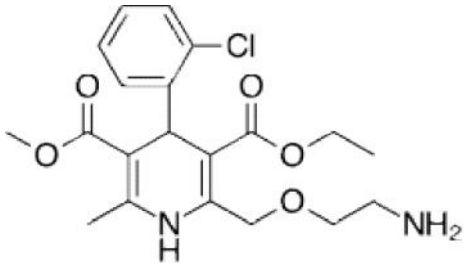


Fig 2: Structure of Amlodipine

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.

2. Materials and Methods

Chemicals and Materials:

Cadila Healthcare Limited. Supplied Perindopril Erbumine and Vent Unimark remedies supplied Amlodipine Besylate. Acetonitrile and Ortho Phosphoric Acid was supplied by Spectrochem and EMerck 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, 5mm) was used as a stationary phase. HPLC conditions are given Table 1.

Table 1: Chromatographic conditions

Column	Eclipse XDB C8 column (150 X 4.6, 5mm).
Detector	210 nm
Injection volume	20 µL
Temperature	30˚ C
Run time	8 min
Flow rate	1.0 mL/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 PerindoprilErbumine 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.

3. Results and Discussion

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 below.

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 mg/mL and 10 to 75 mg / 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 below.

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.

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 below.

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 below.

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 below.

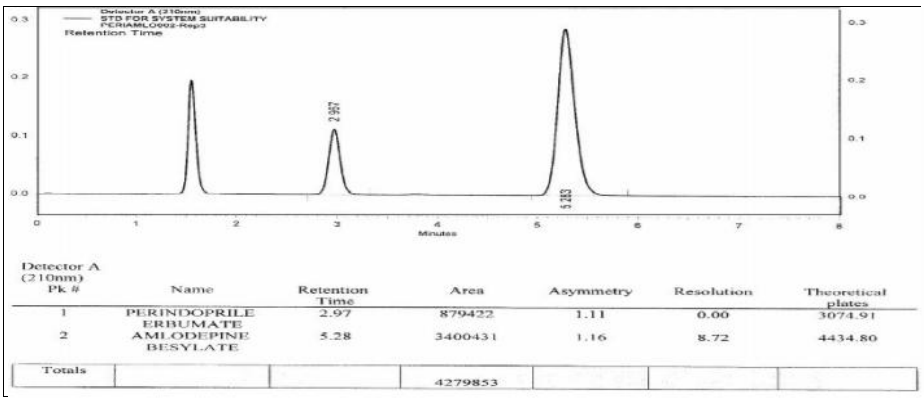
Discussion

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 peak(at RT 1.5 min) due to Besylate Salt of Amlodipine is detected due to lower common wavelength selected, 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.

Table 2: System Suitability and System Precision

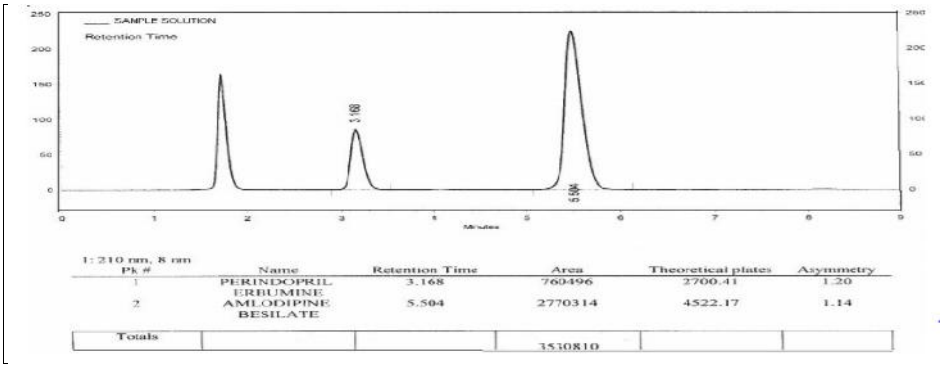
Compound	Retention time	N	k'	R	T	α
Perindopril Erbumine	3.172 ± 0.004	2694.43	30.79	--	1.11	--
Amlodipine Besylate	5.504 ± 0.000	4457.92	54.04	8.10	1.05	1.755

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



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 3: Chromatogram of Standard 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 4: Chromatogram of Sample Solution

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 regression µg/mL	Residual std. 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

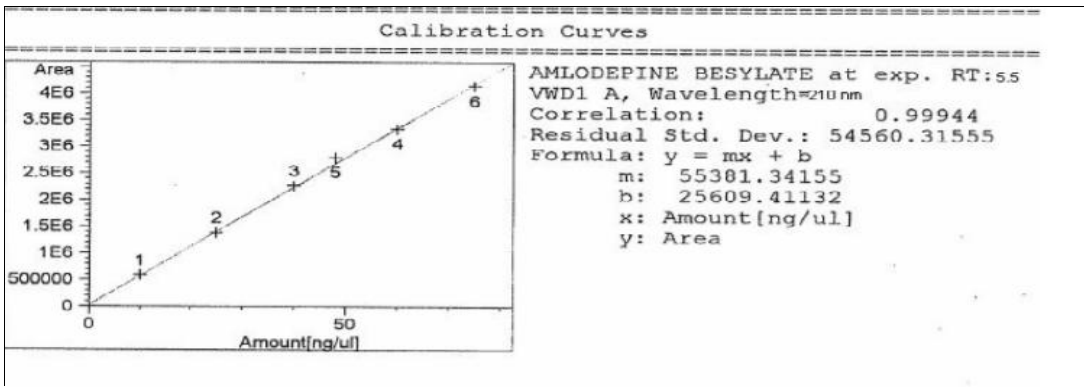


Fig 5: Calibration curve of Amlodipine Besylate

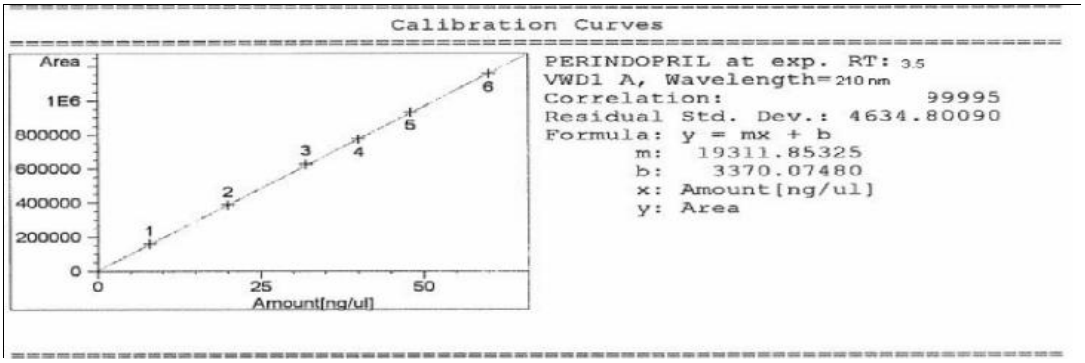


Fig 6: Calibration Curve of Perindopril Erbumine

Table 4: Precision results

Compound	Concentration of µg/mL (n=6)	Retention time Mean ± SEM (n=6)	% Assay Mean ± SEM (n=6)	% RSDAssay
PerindoprilErbumine	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: Accuracy results

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: Ruggedness results

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: Robustness results

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

4. Conclusion

A simple and elective LC method is described for the determination of Amlodipine and Perindopril in tablet dosage forms. Chromatographic separation was achieved on a C-18 column using mobile phase consisting of a mixture of 80 volumes of methanol and 20 volumesof water with detection of 240 nm. Linearity was observed in the range 6-14 µg /ml forAmlodipine(r 2 =0.998) and 6-14 µg /ml for Perindopril(r 2 =0.996) for the amount of drugsestimated by the proposed methods was in good agreement with the label claim.The proposed methods were validated. The accuracy of the methods was assessed byrecovery studies at three different levels. Recovery experiments shows that there is completeabsence in the interference from commonly encountered pharmaceutical additives. The methodwas foundto be precise as indicated by the repeatability analysis, showing %RSD less than 2.All statistical data proves validity of the methods and can be used for routine analysis ofpharmaceutical dosage form.V.

Conflict of Interest: We declare no conflict of interest.

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