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Original Article

Development and Validation of New Analytical RP-HPLC Method for the Estimation of Amlodipine and Perindopril in Tablet Dosage Form.

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ABSTRACT

ABSTRACT : An improved simple, specific, rapid, precise reverse phase HPLC method has been developed and validated for the simultaneous estimation of Perindopril and Amlodipine from combined dosage form. **Method:** The sample was analysed in a Phenomenax (C18) (4.6mm x 100mm, 3.5 m) 0.1% OPA (3.0): Acetonitrile: (70:30) as mobile phase at 230 nm, at a flow rate 0.7 ml/min and the volume of injection was 20 µl. **Results and discussion:** The retention time of Perindopril and Amlodipine were found to be 1.890 & 2.982 minutes respectively. The correlation coefficients of both drugs were found to be 0.998 and 0.999 for Perindopril and Amlodipine respectively. The accuracy of Perindopril was found to be 98.2% - 100.5% whereas for Amlodipine, it was 98.4% - 100.6%. Over all % RSD was found to be less than 2%. The method was validated according to ICH guidelines with respect to linearity, accuracy, precision, robustness, specificity, etc. The developed method can be used for routine analysis of Amlodipine and Perindopril in their pharmaceutical dosage forms.

KEYWORDS: Reversed Phase, ICH guidelines, Amlodipine and Perindopril.

1. INTRODUCTION

Perindopril 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 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 Perindopril is (2S,3aS,7aS)-1-[(2S)-2-[[(2S)-1-ethoxy-1-

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oxopentan-2-yl]amino]propanoyl]-2,3,3a,4,5,6,7,7a-octahydroindole-2- carboxylic acid [2] .The chemical name for Amlodipine is 3-O-ethyl 5-O-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate [3]. The chemical structures of Perindopril and Amlodipine are shown in (Fig. 1 and Fig.2). Literature survey revealed that Methods available for the determination of Perindopril include [HPLC] [4], [LCMS] [5] and [Crystal CE] [6]. Methods available for the determinations of Amlodipine include [HPLC] [7-9], HPTLC [10] simultaneous spectrophotometric determination [11-13]. 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 and Amlodipine in pharmaceutical preparations. The major advantage of the proposed method is that Perindopril and Amlodipine can be determined on a single chromatographic system with the same detection wavelength.

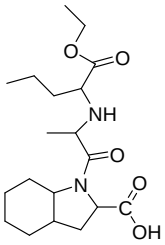

Fig.1 Perindopril

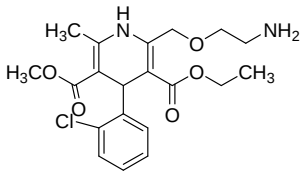

Fig.2 Amlodipine

2. MATERIAL AND METHODS

Chemicals and Materials: Serdia pharmaceuticals supplied Perindopril and Amlodipine. Acetonitrile and Ortho Phosphoric Acid (Merck and Fisher Limited).

Instruments

Waters, 515 pump, equipped with 2487 UV-VIS detector, controlled by N 2000 chromatographic system software. The Phenomenex C_{18} column (4.6X100, 3.5µm) was used as a stationary phase. HPLC conditions are given Table 1.

Preparation of mobile phase:

Taken 1 ml OPA (orthophosphoric acid) into 1000ml of HPLC grade water for preparing the buffer.A mixture of above prepared buffer 700 ml (70%) and 300 ml of HPLC grade Acetonitrile (30%) were mixed and degassed in ultrasonic water bath for 5 minutes. The mobile phase was filtered through 0.45 µ filter under vacuum.

Standard Preparation:

Accurately weighed and transferred 8 mg of Perindopril and 10 mg of Amlodipine working standard into a 10 ml clean dry volumetric flask and added about 7ml of diluent. It was sonicated to dissolve completely and adjusted the volume upto the mark with the same diluent. From the above stock solution 2.5 ml of the solution was pipetted into another 25ml volumetric flask and diluted upto the mark with diluent.

Table 1: Optimized Method Parameters

PARAMETERS	CONDITIONS
Mobile Phase	0.1%OPA (3.0): Acetonitrile: (70:30)
Column (Stationary Phase)	Phenomenax (C18)(4.6mm x 100mm, 3.5µm)
Flow rate (ml/min)	0.7
Column temperature (°C)	Ambient
Volume of injection loop (l)	20
Detection wavelength (nm)	230
Drug RT (min)	1.890 & 2.982

Sample Preparation:

Weigh accurately tablets powdered equivalent to about 8 mg of Perindopril and 10 mg of Amlodipine working standard into a 10 ml clean dry volumetric flask and added about 7ml of diluent. It was sonicated to dissolve completely and adjusted the volume upto the mark with the same diluent and made further dilution 2.5 mL of this solution to 25.0 ml with mobile phase and mix.

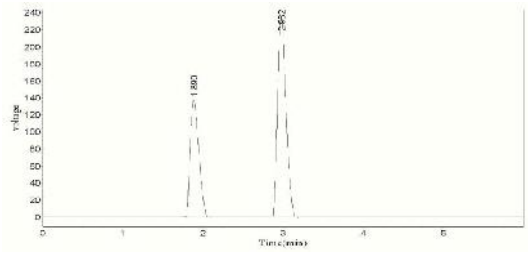

Fig 3: Optimized Chromatogram

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 precision of an analytical method is a measure of the random error and is defined as the agreement between replicate measurements of the same sample. It is expressed as the percentage coefficient of variation (%CV) or relative standard deviation (RSD) of the replicate measurements. The results of system suitability and system precision were presented in Table 2 and Table 3.

Table 2: Observation of System Suitability Parameters

S. No	Parameter	Perindopril	Amlodipine
1	Retention time	1.89	2.99
2	Theoretical plates	2312	3256
3	Tailing factor	1.3	1.1
4	Area	2381448	4665200
5	Resolution		4.4

Table 3: Observation of System Precision

	System Precision		Intermediate system Precision	
INJECTION	Perindopril Area	Amlodipine Area	Perindopril Area	Amlodipine Area
Injection 1	2395835	3972233	2353584	3873160
Injection 2	2406919	3902526	2323586	3875936

Injection 3	2347211	3948577	2343213	3712136
Injection 4	2412101	3996738	2366200	3819600
Injection 5	2352504	3902526	2351153	3875936
Average	2382914	3944520	2347547.2	3831354
Standard Deviation	30799.94	41946.93	15734.48	70841.26
% RSD	1.29	1.06	0.67	1.84

Linearity and Calibration Curve:

The plot of peak area response against concentration is shown in Fig. 4 and Fig. 5. The plot is linear over the concentration range of 8 to 40 mg/mL and 10 to 50 mg / mL for Perindopril and Amlodipine 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 for Perindopril and Amlodipine were presented in Table 4.

Table 4: Linearity for Perindopril and Amlodipine

		Perindopril		Amlodipine	
S.No	Linearity Level	Concentration	Area	Concentration	Area
1	I	8 ppm	988712	10 ppm	1694342
2	II	16 ppm	1750463	20 ppm	2857591
3	III	24ppm	2466919	30ppm	4082787
4	IV	32ppm	3210767	40ppm	5253088
5	V	40ppm	4090603	50ppm	6636476
Correlation Coefficient			0.998		0.999

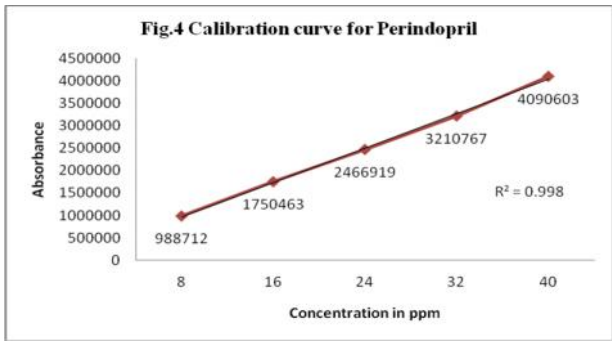

Fig 4: Calibration curve for perindopril

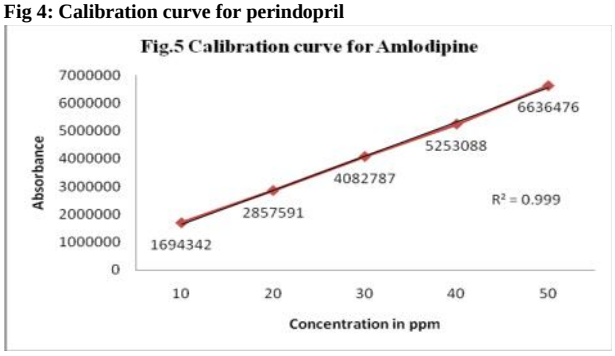

Fig 5: Calibration curve for amlodipine

Accuracy:

The accuracy of the method was determined by calculating percent recovery of Perindopril and Amlodipine by the standard addition method. The recovery experiments were carried out in triplicate (50 %, 100 % and 150 %) by spiking previously analyzed samples of the tablets with three different concentrations of standards. The results (Table 5) are reported in term of percent recovery.

Table 5: Accuracy observation of Perindopril & Amlodipine

	% conc at specification level	Area	Amount added(mg)	Amount found(mg)	% recovery	Mean recovery
Perindopril	50	3606706	12.0	12.07	100.58	99.07
	100	5098608	24.0	23.57	98.20	
	150	6313187	36.0	35.44	98.44	
Amlodipine	50	723798	15.0	14.76	98.40	99.39
	100	1445827	30.0	30.20	100.66	
	150	2858844	45.0	44.60	99.11	

Method robustness:

Robustness of the method was determined by small deliberate changes in flow rate and mobile phase ratio. 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 6.

Table 6: Method robustness

		Perindopril		Amlodipine	
		USP plate count	USP tailing	USP plate count	USP tailing
Flow rate	0.6	2381	1.26	3141	1.06
	0.7	2312	1.32	3256	1.16
	0.8	2232	1.30	2927	1.06
Mobile phase	10% less organic phase	2376	1.27	3300	1.07
	Actual organic phase	2312	1.32	3256	1.16
	10% more organic phase	2083	1.21	2363	0.93

4. CONCLUSION

The detection wavelength of 230 nm was chosen in order to achieve a good sensitivity for quantitative determination of Perindopril and Amlodipine in tablet dosage form. The mobile phase consisting of 0.1% OPA (3.0): Acetonitrile: (70:30) offered a good separation at ambient temperature under these conditions using a flow rate of 0.7 mL/min and a runtime of 6 min, Perindopril elutes at first and then Amlodipine shown in the chromatogram (Fig.3). The correlation coefficient of both drugs was found to be 0.998 and 0.999 for Perindopril and Amlodipine respectively. The accuracy of Perindopril was found to be 98.2% - 100.5% whereas for Amlodipine, it was 98.4% - 100.6%. Over all % RSD was found to be less than 2%. 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.

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