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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF AMLODIPINE AND LISINOPRIL TABLETS BY RP-HPLC

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ABSTRACT

The present work describes a simple, rapid, and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of amlodipine and lisinopril.columnInertsil 250X 4.6mm, 5µm, C8 and a mobile phase containing KH2PO4 adjusted pH 3.5 using0.1%OPA: methanol (40 : 60 v/v) mixture was used for the separation and quantification. The flow rate was 1.0 mL/min and the eluents were detected by PDA detector at 238 nm. The retention times were found to be 3.411 and 4.605 mins, respectively. The developed method was validated according to ICH guidelines Q2 (R1) and found to be linear within the range of 50–150 µg/mL for both drugs. The developed method was applied successfully for assay of amlodipine and lisinopril.

INTRODUCTION:

Amlodipine, a vasoselective dihydropyridine calcium antagonist, has a pharmacokinetic profile that sets it apart from other calcium antagonists. Differential features include a slow onset of action, a prolonged effect, high bioavailability and relatively minor differences in peak to trough plasma levels.

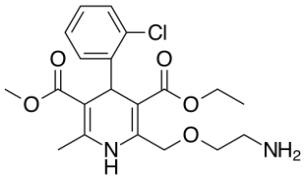

Figure 1: Structure of amlodipine

Lisinopril is a potent, competitive inhibitor of angiotensin-converting enzyme (ACE), The enzyme responsible for the conversion of angiotensin I (ATI) to angiotensin II (ATII). ATII regulates blood pressure and is a key component of the renin-angiotensin-aldosterone system (RAAS). Lisinopril may be used to treat hypertension and symptomatic congestive heart failure, to improve survival in certain individuals following myocardial infarction, and to prevent progression of renal disease in hypertensive patients with diabetes mellitus and micro albuminuria or overt nephropathy. The present study was designed to develop a simple, precise, and rapid analytical RP-HPLC procedure, which can be used for the analysis of assay method for simultaneous estimation of amlodipine and lisinopril.

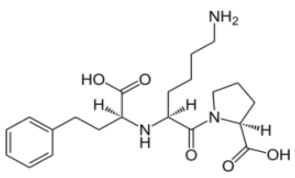

Figure 2: Structure of lisinopril

The developed method was validated as per ICH guidelines and its updated international convention. The linearity of response, precision, ruggedness and robustness of the described method has been checked.

2. EXPERIMENTAL

2.1. Chemicals and Reagents

Amlodipine standard and API were procured by Dr. Reddy's Laboratories, India. lisinopril standard and API were gifted by Dr. Reddy's Laboratories. HPLC grade methanol was purchased from finar, New Delhi, India. All the other reagents used were of analytical grade.

2.2. HPLC Instrumentation and Conditions

The analysis was carried out on a WATERS HPLC, Model: Aglient 2695, Photo diode array detector (PDA), with an automated sample injector. The output signal was monitored and integrated using Empower 2 software.. Inertsil 250X 4.6mm, 5µm, C8 was used for separation. Mobile phase used for separation was mixture containing KH2PO4 adjusted pH 3.5 using 0.1% OPA: methanol (40 : 60 v/v). The flow rate was kept at 1.0 mL/min, column temperature was 38°C, eluents were detected by PDA detector at 238 nm, and the injection volume was 10 µL.

2.2.1. Preparation of Mobile Phase

Transfer 500ml of HPLC water into 500ml of beaker and KH2PO4 adjust pH 3.5 using0.1%OPA. Transfer the above solution 400ml of KH2PO4, 600ml of Methanol is used as mobile phase. They are mixed and sonicated for 20min.

2.2.2Preparation of Amlodipine and Lisinopril standard and Sample Solution:

Preparation of standard solution: Accurately weighed and transferred 50mg Amlodipine and 50mg Lisinopril into 50ml of volumetric flask and add 10ml of Methanol and sonicate 10min (or) shake 5min and make with water. Transferred the above solution into 2.5ml into 25ml volumetric flask dilute to volume with water.

Preparation of sample stock solution:

Commercially available 20 tablets ware weighed and powdered the powdered equivalent to the 768.25mg of Amlodipine and Lisinopril of active ingredients were transfered into a 50ml of volumetric flask anded 10ml of Methanol and sonicated 20min (or) shaked 10min and makeup with water. Transferred above solution 2.5ml into 25ml of the volumetric flask dilute the volume with Methanol. And the solution was filtered through 0.45µm filter before injecting into HPLC system.

3 Assay result for formulation

Label contains: Each film coated tablet contains Amlodipine-5mg. Lisinopril-5 mg. Average weight of each tablet is 768.25mg

Purity of working standards: Amlodipine: % purity - 96.8% and Lisinopril: % purity –98%

Sample preparation: 10 tablets were weighed and crushed, from the powdered tablets, weighed accurately about 768.25mg(5mg Amlodipine and 5mg Lisinopril) into a 50 ml volumetric flask and 50 ml of mobile phase was added.

The mixture was subjected to sonication for 20 min with intermediate shaking for complete extraction of drugs. Filtered and cooled to room temperature and solution was made up to mark with mobile phase. From the above solution 2.5 mL is taken and further diluted in 25 ml volumetric flasks with mobile phase. To acquire a concentration of 5mg Amlodipine and 5mg Lisinopril.

Table 1: Precision

(RSD %)	Amlodipine	Lisinopril	Inference
RT	0.055	0.071	% RSD was found to be < 2
Area	0.070	0.041	
%Assay	0.07	0.04	

Table 2:LOD data for Amlodipine and Lisinopril

S.No	Sample name	RT	Area
1	Amlodipine	3.413	1339210
2	Lisinopril	4.512	5960643

Table 3:LOQ data for Amlodipine and Lisinopril

S.no	Sample name	RT	Area
1	Amlodipine	3.408	2329142
2	Lisinopril	4.512	5960643

Table 4: Recovery data for amlodipine.

S.NO	Accuracy Level	Sample name	Sample weight	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	1	384.13	24.750	24.83	100	99
		2	384.13	24.750	24.83	100	
		3	384.13	24.750	24.82	100	
2	100%	1	768.25	49.500	49.61	100	100
		2	768.25	49.500	49.61	100	
		3	768.25	49.500	49.54	100	
3	150%	1	1152.38	74.250	74.28	100	100
		2	1152.38	74.250	74.33	100	
		3	1152.38	74.250	74.33	100	

Table 5: Recovery data for lisinopril

S.NO	Accuracy Level	Sample name	Sample weight	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	1	384.13	25.000	24.91	100	99
		2	384.13	25.000	24.87	99	
		3	384.13	25.000	24.86	99	
2	100%	1	768.25	50.000	49.81	100	100
		2	768.25	50.000	49.81	100	
		3	768.25	50.000	49.80	100	
3	150%	1	1152.38	75.000	74.76	100	100
		2	1152.38	75.000	74.85	100	
		3	1152.38	75.000	74.68	100	

Table 6: Robustness

Parameter	RT	Theoretical plates	Asymmetry
Decreased flow rate(0.8ml/min)	4.297	3920	1.22
Increased flow rate(1.2ml/min)	2.822	3224	1.37
Decreased temperature(20^0c)	4.297	4083	1.19
Increased temperature(30^0c)	2.828	3332	1.35

Table 7: Results of Robustness for Lisinopril

Parameter	RT	Theoretical plates	Asymmetry
Decreased flow rate (0.8ml/min)	5.680	4158	1.16
Increased flow rate (1.2ml/min)	3.747	3480	1.28
Decreased temperature(20^0c)	5.683	4422	1.14
Increased temperature(30^0c)	3.752	3591	1.27

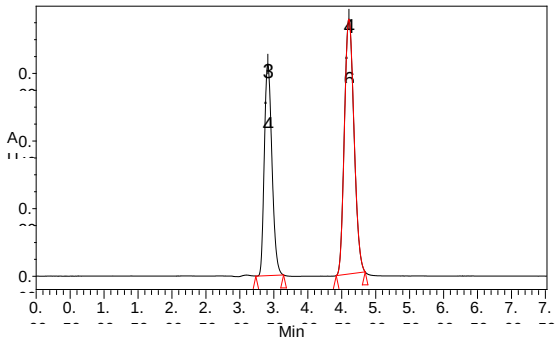

Figure 3: Optimized chromatogram of amlodipine and lisinopril.

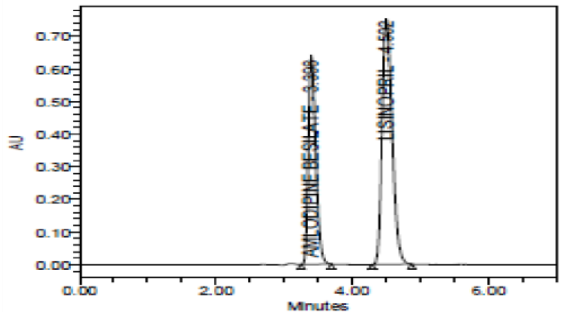

Figure 4: chromatogram representing specificity of standard

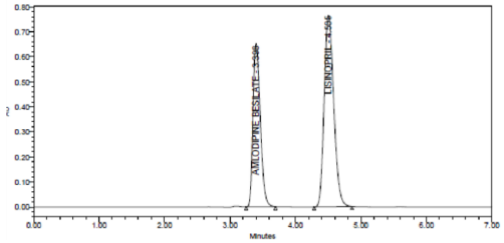

Figure 5: chromatogram representing specificity of sample

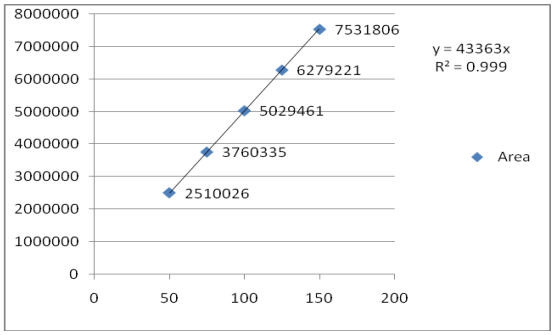

Figure 6: Linearity plot of Amlodipine

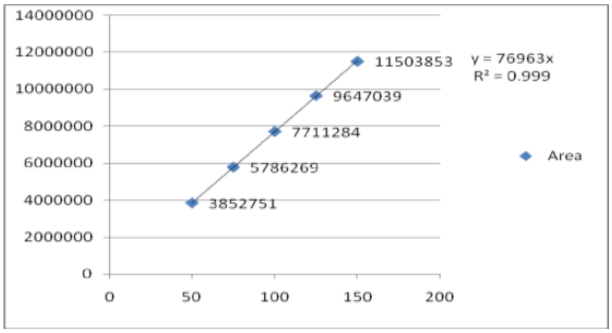

Figure 7: Linearity plot of Lisinopril.

Standard preparation: Accurately weighed quantity of 50mg Amlodipine and 50mg Lisinopril was taken in a 50 ml volumetric flask and 50 ml of mobile phase was added. The mixture was subjected to sonication for 20 min with intermediate shaking for complete extraction of drugs. Filtered and cooled to room temperature and solution was made up to mark with mobile phase. From the above solution 2.5 ml is taken and further diluted in 25 mL volumetric flasks with mobile phase.To acquire a concentration of 50mg Amlodipine and 50mg Lisinopril.

Procedure: Separately injected both the standard (2 injections) and sample preparations (2 injections) into the chromatographic system and recorded the peak area responses.

METHOD VALIDATION

4.1. Specificity: Specificity of the method was determined by comparison between standard drug and sample. Fixed concentrations of 100 µg/mL of standard and working test solutions were injected to the HPLC system for six times and were analyzed. Percentage of RSD was calculated from their peak areas.

4.2. Precision: Repeatability. Precision of the method was studied by making repeated injections of the mixture of drugs on the same day for intraday precision.

The coefficient of variation (CV) after five determinations was determined at 100 µg/mL for both drugs. Intermediate precision was carried out by injecting three replicates of standard concentration (100 µg/mL) by different analysts. The % RSD was calculated.

4.3. Linearity: The linearity of measurement was evaluated by analyzing standard solutions of amlodipine and lisinopril in the range of 50–150 µg/mL for both drugs and calibration plot was constructed.

4.4. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ of amlodipine and lisinopril were determined by calibration curve method. Solutions of amlodipine and lisinopril were prepared in the range of 50–150 µg/mL and injected in triplicate.

4.5. Accuracy: Accuracy of the method was calculated by recovery studies at three levels by standard addition method, that is, spiking about 50% to 150% of the target concentration. The samples were injected in triplicate and their % recovery was determined.

4.6. Robustness: Influence of small changes in chromatographic conditions such as change in flow rate, that is, ±0.2 mL/mins and wavelength of detection ±2 nm, was studied to determine the robustness of the method for the development of RP-HPLC method for the simultaneous estimation.

4.7. System Suitability: The stock solution containing 100 µg/mL was injected and repeated five times and the chromatograms were recorded. The resolution, number of theoretical plates, and peak asymmetry were calculated to determine whether the result complies with the recommended limit

5. RESULTS AND DISCUSSION

5.1. Optimization of Chromatographic Conditions: To develop suitable RP-HPLC method for simultaneous estimation of amlodipine and lisinopril, different chromatographic conditions were applied and optimized chromatographic conditions were developed. Optimized chromatographic conditions are as follows: instrument: WATERS HPLC, Model: Aglient 2695, mobile phase: KH2PO4: Methanol (40:60), column: Inertsil 250X 4.6mm, 5µm, C8, injection volume: 10 µL, flow rate: 1.0 mL/min, detection wavelength: 238 nm, temperature: Ambient (30°C).

5.2.2. Precision: Precision of the method was studied by making repeated injections of the mixture of drugs. Percentage relative standard deviation (%RSD) was found to be less than 2% which proves that method is precise**.**

5.2.3. Linearity: The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. LOD and LOQ of amlodipine and lisinopril were determined by calibration curve method. Solutions of amlodipine and lisinopril were prepared in the range of 50–150 µg/mL and injected in triplicate.

5.2.4. Limit of Detection (LOD) and Limit of Quantitation (LOQ): LOD and LOQ of amlodipine and lisinopril were determined by calibration curve method. Solutions of amlodipine and lisinopril were prepared in the range of 50–150 µg/mL and injected in triplicate.

5.2.5. Accuracy: Accuracy of the method was calculated by recovery studies at three levels by standard addition method. The mean percentage recoveries obtained for amlodipine and lisinopril were 100% and 100%, respectively.

5.2.6. Robustness: The method for the development of RP-HPLC method for the simultaneous estimation of amlodipine and lisinopril was found to be robust as the % RSD was found to be less than 2.

5.2.7. System Suitability: The resolution, number of theoretical plates, and peak asymmetry were calculated for the standard solutions. The stock solution containing 100 µg/mL was injected and repeated five times and the chromatograms were recorded. The resolution, number of theoretical plates, and peak asymmetry were calculated to determine whether the result complies with the recommended limit.

6. CONCLUSION

The proposed RP-HPLC method was used for the simultaneous estimation of amlodipine and lisinopril was found to be sensitive, accurate, precise, simple, and rapid. For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of samples in a short time period. The method shows good reproducibility and good recovery. From the specificity studies, it was found that the developed methods were specific for Amlodipine and Lisinopril.

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