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Development of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Amlodipine and Olmesartan in Pure and Pharmaceutical Dosage Form

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ABSTRACT

A precise, accurate, economical and simple stability indicating RP-HPLC method was developed for the estimation of Amlodipine (AML) and Olmesartan (OLM) in bulk and pharmaceutical dosage form. Method was performed on a octadecyl silane column with dimensions 4.6 x 250 mm having particle size 5 micron. The mobile phase used in the method is TEA Buffer (pH 3.0) and acetonitrile in proportion of 25:75 respectively. The flow rate was maintained at 1.0 ml/ min and effluent was monitored at 258 nm. The drug was subjected to acid and alkali hydrolysis, oxidation, photolysis and heat as stress conditions. The method was validated for specificity, linearity, precision, accuracy, robustness and system suitability. The retention times were observed at 2.39 min and 3.33 min for AML and OLM respectively. The standard curve was found linear over a range of 05–35 µg/ml for AML and OLM. Similarly an average correlation coefficient was also obtained at 0.999 for AML and OLM. The limit of quantitation (LOQ) of this method was 2µg/ml for Amlodipine and Olmesartan. The absolute recovery was 100% for Amlodipine and 100.3 for Olmesartan. Degradation products produced as a result of stress studies did not interfere with the detection of AML and OLM and the assay can thus be considered stability-indicating.

Keywords: Amlodipine, Olmesartan, RP-HPLC, TEA Buffer: Acetonitrile, Validation.

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INTRODUCTION

Amlodipine is a long-acting 1,4-dihydropyridine calcium channel blocker. It acts primarily on vascular smooth muscle cells by stabilizing voltage-gated L-type calcium channels in their inactive conformation. By inhibiting the influx of calcium in smooth muscle cells, amlodipine prevents calcium-dependent myocyte contraction and vasoconstriction. Amlodipine

decreases arterial smooth muscle contractility and subsequent vasoconstriction by inhibiting the influx of calcium ions through L-type calcium channels. Calcium ions entering the cell through these channels bind to calmodulin. Calcium-bound calmodulin then binds to and activates myosin light chain kinase (MLCK). Activated MLCK catalyzes the phosphorylation of the regulatory light chain subunit of myosin, a key step in muscle contraction. [1]

Olmesartan is an antihypertensive agent, which belongs to the class of medications called angiotensin II receptor blockers. Olmesartan is an ARB that selectively inhibits the binding of angiotensin II to AT1, which is found in many tissues such as vascular smooth muscle and the adrenal glands. This effectively inhibits the AT1-mediated vasoconstrictive and aldosterone-secreting effects of angiotensin II and results in a decrease in vascular resistance and blood pressure.

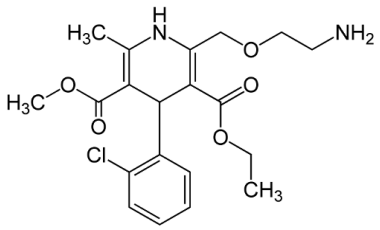

Fig. 1: Amlodipine

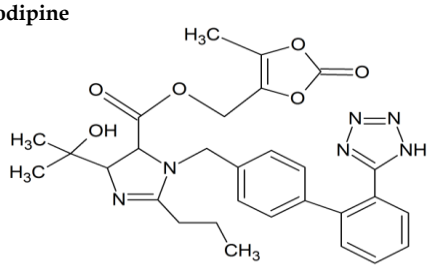

Fig. 2: Olmesartan

Literature survey shows that very few works on stability-indicating RP-HPLC method has been reported so far for the simultaneous estimation of both the drugs using mobile phase TEA buffer and Acetonitrile in proportion of 25:75. Some of the reported methods are for OLM using UV-Spectroscopic method [1-4], RP-HPLC method [5-6], for combination of three drugs AML, OLM and HTZ using RP-HPLC method [7-9], for AML using RP HPLC method. [10] Very few methods have been reported so far for simultaneous estimation of both the drugs AML and OLM. [11-16] The objective of this work was to develop a new rapid, novel, and economical RP-HPLC method which can be used as a stability-indicating assay for combination drug product of AML and OLM.

MATERIALS AND METHODS

Drugs

Pure pharmaceutical sample of AML and OLM was obtained from Yucca Pharma. Olmark tablet (Intas Pharmaceuticals Ltd) containing amlodipine besylate (5 mg) and olmesartan medoxomil (20 mg) were procured from the local drug market.

Chemicals

Sodium dihydrogen phosphate (AR Grade), 85% Orthophosphoric acid (AR Grade), Acetonitrile (HPLC Grade), Hydrochloric Acid (AR Grade), Triethyl-Amine (AR Grade), Sodium Hydroxide (AR Grade) were purchased from Sd fine-Chem limited.

Instrument

Liquid chromatographic system from Waters alliance 2695 with Waters UV detector equipped with Empower software was used.

Preparation of mobile phase

Mobile phase was prepared by dissolving Buffer of pH 3 in Acetonitrile in the ratio of 25:75. The Mobile phase was filtered through 0.45µm membrane filter and degassed by ultrasonic bath.

Preparation of TEA buffer (pH 3)

The buffer solution was prepared by dissolving 1.5 ml of Triethyl amine dissolved in 250 ml of HPLC Water. The pH was adjusted at 3.00 with ortho phosphoric acid.

Diluent preparation

The Mobile phase was used as the diluent.

Stock solutions and standards

A stock solution of drugs were prepared by transferring accurately weighed 25 mg of AML and OLM in two separate 25 ml volumetric flask and dissolved in 15 ml of mobile phase. The solutions were sonicated and the volumes were made up to mark with mobile phase to get concentration of 1000µg/ml of AML and OLM.

Preparation of Sub Stock Solution

1 ml was pippeted from Amlodipine stock solution and 4 ml from Olmesartan stock solution. The solutions were transferred in 100 ml volumetric flask separately. The volume was made up to the mark with mobile phase to get concentration of 10µg/ml and 40µg/ml solution of AML and OLM respectively.

Preparation of sample solution

Accurately weighed ten tablets were taken and crushed in mortar and pestle. 100 mg equivalent weight of powdered drug containing OLM and AML were transferred into a 100 mL volumetric flask and volume was made up to the mark with the solvent. Further 2 ml pipetted out from stock solution and transferred into a 50 ml volumetric flask and diluted up to the mark with diluent.

Stability Study

Tablet powder equivalent to the weight of one tablet was transferred to a 250 ml round bottomed flask and treated under acidic, alkaline, oxidizing, thermal and photolytic stress conditions. When degradation was complete, the solution were left to equilibrate to room temperature and diluted with diluents to furnish solutions of concentration equivalent to 40µg/ml OLM and 10µg/ml AML. The specific conditions are described below. In acidic degradation drug was heated under reflux with 1M hydrochloric acid for 30 min at 80°C and the drug was treated with 0.1N NaOH at room temperature for 2 h in alkaline degradation.

Then resulting solution was neutralized. The drug was treated with 2% v/v H_2O_2 at room temperature for 2 hour in oxidative degradation. Thermal degradation was performed by exposing the solid drug to dry heat in a convection oven at 70°C for 72 h and photolytic degradation was performed by exposing the drug to sunlight for 72 h.

Apparatus and Chromatographic conditions

Quantitative HPLC was performed on Waters HPLC system with UV detector. Empower software is used along with a stainless steel column 4.6 × 150 mm, packed with Octa decyl silane bonded to porous silica (C18) with particle size 5 micron. To develop a suitable and robust HPLC method for the determination of OLM and AML, different mobile phases containing TEA buffer and Acetonitrile were used in different compositions like (30:70, 40:60, 50:50, 70:30, 80:20) at different flow rates (0.5, 0.75, 1.0, 1.2, 1.5 ml/min). The mobile phase TEA buffer and Acetonitrile with a flow rate of 1.0 ml/ min gave peaks of good resolution and were eluted at retention times around 2.39 min, 3.33 min with symmetric peak shape. The detection is performed at the wavelength 258 nm.

Running the standard solution of Amlodipine

1 ml of stock solution (1000 ppm) was pipetted out into a 100 ml volumetric flask. The volume was made up to the mark with mobile phase. The solution was filtered through the 0.45µm membrane filter and degassed under ultrasonic bath prior to use. The solution was injected into the HPLC system. The chromatogram obtained is shown in figure 3.

Running the standard solution of Olmesartan

4 ml of stock solution was pipetted into a 100 ml volumetric flask. The volume was made up to the mark with mobile phase. The solution was filtered through the 0.45µm membrane filter and degassed under ultrasonic bath prior to use. The solution was injected into the HPLC system. The chromatogram obtained is shown in figure 4.

Running the standard solution of Amlodipine and Olmesartan

1 ml of AML stock solution and 4 ml OLM stock solution was pipetted into a 100 ml volumetric flask. The volume was made up to the mark with mobile phase. The solution was filtered through the 0.45µm membrane filter and degassed under ultrasonic bath prior to use. The solution was injected into the HPLC system. The chromatogram obtained is shown in figure 5.

Method development and optimization

The main target of the chromatographic method is to get the separation of closely eluting drugs Amlodipine and Olmesartan, The drugs were co-eluted by using different stationary phases like C18, C8 with varying lengths and different mobile phases containing buffers like phosphate, sulphate and acetate with different pH (2-7) and using organic modifiers like acetonitrile, methanol and ethanol in the mobile phase. pH of the buffer has played a significant role in achieving the separation between drugs.

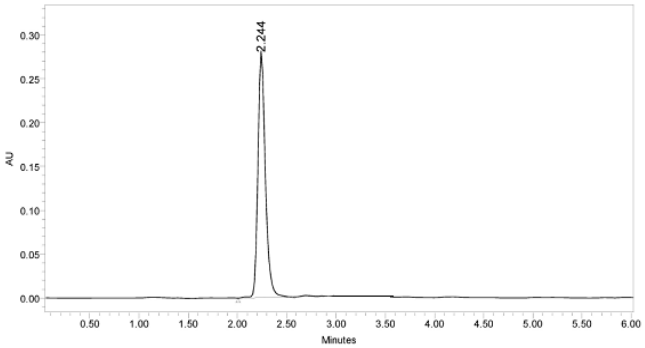

Fig. 3: Chromatogram of Amlodipine (Rt 2.395 min)

S. No.	Peak Name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate
1	Amlodipine	2.316	1232142	194123	3.6	1.2	4651

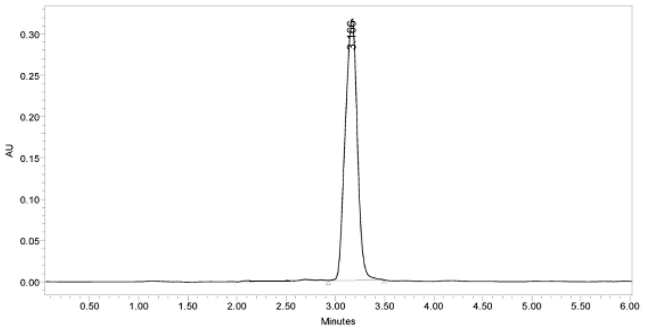

Fig. 4: Chromatogram of Olmesartan (Rt 3.339 min)

S. No	Peak Name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Olmesartan	3.304	1491465	176582	5.6	1.5	3982

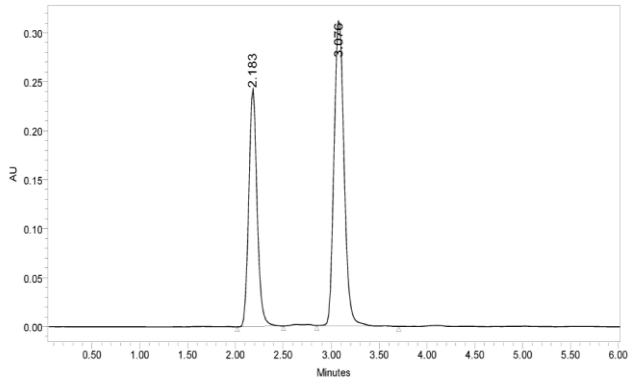

Fig. 5: Chromatogram of Amlodipine (Rt 2.395 min) and Olmesartan (Rt 3.339 min).

S. No	Peak Name	R_t	Area	Height	USP Resolut	USP Taili	USP plate count
1	Amlodipine	2.395	1242388	197332		1.1	4741
2	Olmesartan	3.339	1494848	177825	5.2	1.2	3793

The chromatographic separation was achieved on a stainless steel column (4.6 × 250 mm) column packed with Octa decyl silane bonded to porous silica (C18) with particle size 5 micron, by using solutions TEA Buffer and Acetonitrile in the ratio of (25:75), pH adjusted to 3 using ortho phosphoric acid. The flow rate of the mobile phase was maintained at 1.0 ml/min. At 25°C of column temperature, the peak shape of AML AND OLM was found symmetrical with mobile phase 60:40 ratio. In the optimized conditions AML AND OLM were well separated with a good resolution and the typical retention times of AML AND OLM were about 2.3 min and 3.3 min, respectively. The system suitability results are given in table 1 and the developed LC method was validated. [16]

Table 1: System suitability parameters

Instrument used	Waters HPLC with auto sampler and UV detector
Temperature	Ambient
Column	Symmetry C18 (4.6mm × 150 mm, 5µm, Make: Waters)
Buffer	1.5 ml of Triethyl amine dissolve in 250 ml of HPLC water. Adjust pH 3.00 with orthophosphoric acid.
pH	3
Mobile phase	TEA Buffer (pH-3.00), Acetonitrile in proportion of 25:75
Flow rate	1 ml per min
Wavelength	258 nm
Injection volume	20µl
Run time	6 min

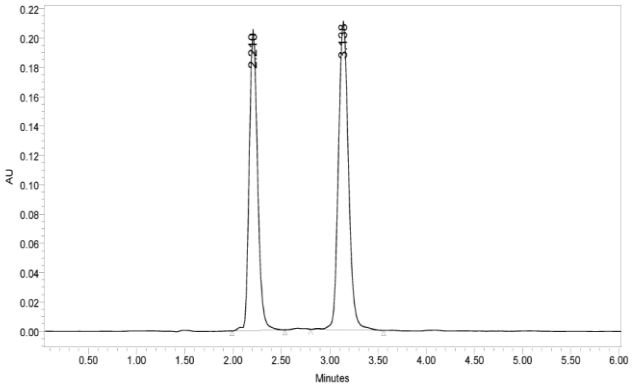

Fig. 6: Chromatogram showing degradation for Amlodipine and Olmesartan in 0.1 N HCl

S. No	Peak Name	R_t	Area	Height	USP Tailing	USP plate count
1	Amlodipine	2.210	1113179	198754	1.2	4854
2	Olmesartan	3.138	1339383	176582	1.3	3872

Stability Studies

Acid Hydrolysis

An accurately weighed 25 mg of pure drugs AML and 100 mg of OLM were transferred to a clean & dry 25 ml volumetric flask separately. To which 0.1 N Hydrochloric acid was added & made up to the mark & kept for 24 hours. From both drug solutions 0.5 ml was taken and transferred in to a 50 ml volumetric flask & made up to the mark with mobile phase and then injected into the HPLC system against a blank of HCl (after all optimized conditions).

Basic Hydrolysis

An accurately weighed 25 mg of pure drugs AML and 100 mg of OLM were transferred to a clean & dry 25 ml volumetric flask separately. To which 0.5N Sodium hydroxide was added & make up to the mark & kept for 24 hours, From both drug solution 0.5 ml was taken in to a 50 ml volumetric flask & make up to the mark with mobile phase, then injected into the HPLC system against a blank of NaOH (after all optimized conditions).

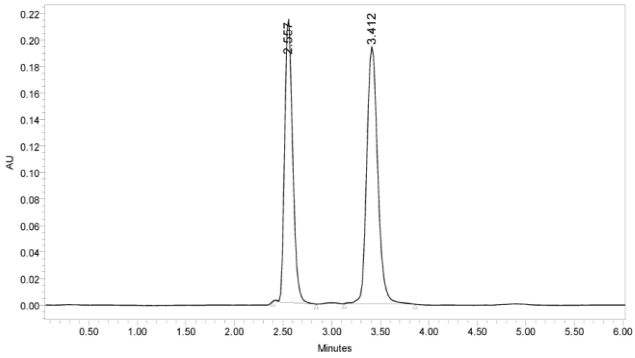

Fig. 7: Chromatogram showing degradation related impurity in 0.1 N NaOH

S. No.	Peak Name	R_t	Area	Height	USP Tailing	USP plate count
1	Amlodipine	2.557	1153184	198574	1.0	4658
2	Olmesartan	3.412	1387517	187452	1.1	3694

Dry Heat Degradation

An accurately weighed 25 mg of pure drugs AML and 100 mg of OLM were transferred in to a 25 ml volumetric flask, volume was made up to the mark with mobile phase & maintained at 50°C for 24 hours. From both drug solutions 0.5 ml was taken in to a 50 ml volumetric flask & make up to the mark with mobile phase. Further it is injected into the HPLC system against a blank of mobile phase.

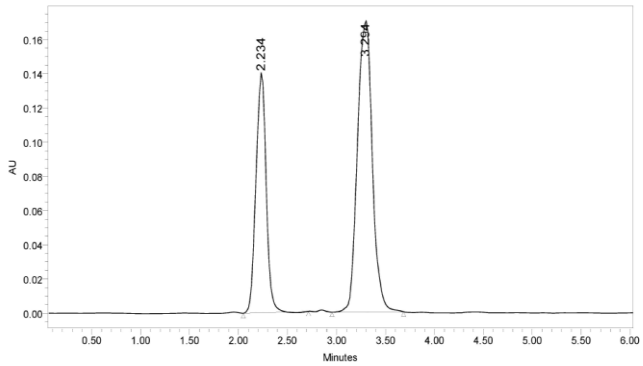

Fig. 8: Chromatogram showing thermal degradation studies

S. No.	Peak Name	R_t	Area	Height	USP Tailing	USP plate count
1	Amlodipine	2.234	1181262	198698	1.2	4821
2	Olmesartan	3.294	1432363	169587	1.4	3365

Photolytic Degradation

Approximately 25 mg of pure drugs AML and 100 mg of OLM were taken in a clean & dry Petri dish. It was kept in a UV cabinet at 254 nm wavelength for 24 hours without interruption. Accurately weighed 1 mg of AML and 4 mg of OLM the UV exposed drug was transferred to a clean & dry 100 ml volumetric flask. First the UV exposed drug was dissolved in methanol & make up to the mark.Then injected into the HPLC system against a blank of mobile phase (after all optimized conditions).

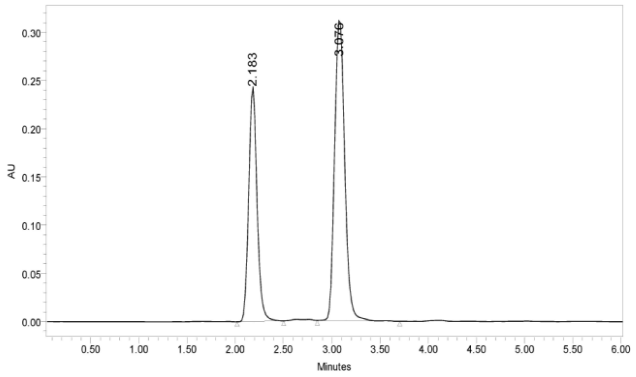

Fig. 9: Chromatogram is showing photolytic degradation.

S. No.	Peak Name	R_t	Area	Height	USP Tailing	USP plate count
1	Amlodipine	2.183	1212073	186954	1.0	4857
2	Olmesartan	3.076	1458373	169587	1.2	3635

Oxidation with 3% H_2O_2

Accurately weighed 1 mg of AML and 4 mg of OLM of pure drugs were taken in a clean & dry 100 ml. volumetric flask. 30 ml. of 3% H_2O_2 and a little methanol was added to it to make it soluble & then kept as such in dark for 24 hours. Final volume was made up to 100 ml. using water to prepare 10 ppm and 40 ppm of AML and OLM solution respectively. The above sample was injected into the HPLC system.

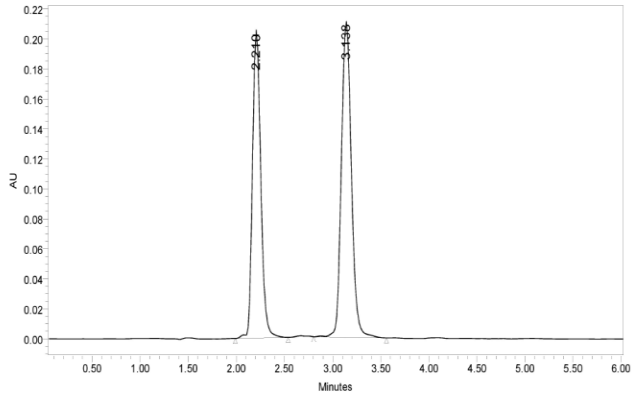

Fig. 10: Chromatogram shows oxidative degradation.

S. No.	Peak Name	R_t	Area	Height	USP Tailing	USP plate count
1	Amlodipine	2.210	1120882	198596	1.2	4635
2	lmesartan	3.138	1348651	177854	1.5	3458

RESULTS AND DISCUSSION

Results of forced degradation studies

The results of the stress studies indicated the specificity of the method that has been developed. Amlodipine and Olmesartan were stable in photolytic, thermal and basic stress conditions. The result of forced degradation studies are given in the following table 2.

Results of method validation

Linearity

Linear calibration plot for assay method was obtained over the calibration ranges tested, i.e. 1-3µg/ml for Amlodipine and 2µg/ml to 30µg/ml for Olmesartan and the correlation coefficient obtained was greater than 0.999. The results show that an excellent correlation existed between the peak area and concentration of the analyte which is given in table 3 and 4.

Table 2: Results of forced degradation studies of Amlodipine and Olmesartan API

Stress condition	Time (h)	Assay of degraded products	Assay of active substance	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24	10.4	89.6	100
Basic Hydrolysis (0.I M NaOH)	24	7.18	92.82	100
Thermal Degradation (50°C)	24	4.92	95.08	100
UV (254 nm)	24	2.44	97.56	100
3% Hydrogen peroxide	24	9.78	90.22	100

Table 3: Linearity results for Amlodipine

Concentration of AML in ppm	Peak area
0	0
5	224748
10	475848
15	692648
20	944621
25	1180741
30	1390935
35	1598929

Table 4: Linearity results for Olmersartan

Concentration of OLM in ppm	Peak area
0	0
5	1234613
10	2472924
15	3570426
20	4853049
25	6053925
30	6990601
35	7817235

Intermediate precision/ruggedness

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions.

Recovery and accuracy

The percentage recovery of AML and OLM in bulk drugs samples was ranged from 99.4-99.6% which

indicates that the method was accurate which is given in table 7.

Table 5: Results of method precession for Amlodipine

S. No.	Peak name	Rt	Area (µV*sec)	USP Plate Count	USP Tailing
1	Amlodipine	2.234	1010585	1.0	3802
2	Amlodipine	2.261	1011075	1.1	3546
3	Amlodipine	2.183	1011924	1.4	4633
4	Amlodipine	2.244	1014299	1.1	4812
5	Amlodipine	2.458	1022159	1.0	3802
	Mean		1014008		
	Std. Dev		4774.567		
	% RSD		0.470861		

Table 6: Results of method precession for Olmesartan

S. No.	Peak name	Rt	Area (µV*sec)	USP Plate Count	USP Tailing
1	Olmesartan	3.294	1513391	1.2	4759
2	Olmesartan	3.191	1513391	1.1	3695
3	Olmesartan	3.076	1526673	1.1	4741
4	Olmesartan	3.166	1560819	1.2	3793
5	Olmesartan	3.319	1560819	1.1	4741
	Mean		1535019		
	Std. Dev.		24168.56		
	% RSD		1.57448		

Table 7: Accuracy studies for Amlodipine

% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% recovery	Mean Recovery
80%	605652.5	4	4.0	100.0%	99.9%
100%	1246314	5	4.94	98.0%	
120%	1869868	6	6.1	101.6%	

Table 8: Accuracy studies for Olmesartan

% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% recovery	Mean Recovery
80%	774787.7	16	15.9	99.37%	99.8%
100%	1537580	20	19.9	99.5%	
120%	2285575	24	24.1	100.4%	

Accuracy results

The accuracy of the method was determined by preparing solutions of different concentrations of AML and OLM that is 80%, 100% and 120% in which the amount of marketed formulation (AML and OLM 5 mg and 20 mg respectively) was kept constant and the amount of pure drug was varied that is 4 mg, 5 mg and 6 mg for AML and 16 mg, 20 mg and 24 mg for OLM i.e. 80%, 100% and 120% respectively. The solutions were prepared in triplicates and the accuracy; similarly was indicated by % recovery in table 7 and 8.

Specificity

5 mg/ml of AML was spiked with 50% (2.5 mg), 100% (5 mg) and 150% (7.5 mg) of excipient mix (Magnesium Stearate), Further 01 ml is pippeted out from the all three samples and diluted to 100 ml in three separate volumetric flask and analysed for % recovery of AML. Similarly 20 mg/ml OLM sample were prepared and analysed.

LOD and LOQ

Detection limit and Quantitation limit of described method were observed as 0.653 mg/ml and 1.959 mg/ml for AML, 0.646 mg/ml and 1.638 mg/ml for OLM.

Robustness

Robustness is a measure of capacity of a method to remain unaffected by small, but deliberate variations in the method conditions, and is indications of the reliability of the method. A method is robust, if it is unaffected by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at three different levels and retention time and chromatographic response were evaluated. One factor at a time was changed to study the effect. Variation of wavelength (235 and 239 nm) and mobile phase flow rate by 0.1 ml/min (0.9 and 1.1 ml/min) had no significant effect on the retention time and chromatographic response of the 50µg/ml solution, indicating that the method was robust.

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1 ml/min), Temperature (± 2°C), Wavelength of detection (± 2 nm) & Acetonitrile content in mobile phase (± 2%) studied to determine the robustness of the method are also in favour of (Table 10 and 11, % RSD < 2%) the developed RP-HPLC method for the analysis of Amlodipine.

The developed method was found to be precise as the %RSD values for intra-day and inter-day were found to be less than 2%. Good recoveries (98.9% to 101.9%) of the drug were obtained at each added concentration, indicating that the method was accurate.

Table 9: Results of specificity studies for Amlodipine and Olmesartan

Specificity data for Amlodipine						
% Concentration (at specification Level)	Area	Drug Added (mg)	Excipient Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	444310	5	2.5	`4.98	99.6%	
100%	885413	5	5	4.97	99.7%	99.6%
150%	1319238	5	7.5	4.96	99.7%	
Specificity data for Olmesartan						
% Concentration (at specification Level)	Area	Drug Added (mg)	Excipient Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	50577	20	10	19.97	99.4%	
100%	104365	20	20	19.96	99.6%	99.5%
150%	156541	20	35	19.95	99.6%	

Table 10: Results of robustness for Amlodipine

Change in parameter	% RSD
Flow (1.1 ml/min)	1.03
Flow (0.9 ml/min)	0.68
Temperature (27°C)	0.42
Temperature (23°C)	0.57
Wavelength of Detection (250 nm)	0.23
Wavelength of detection (266 nm)	0.12

Table 11: Results of robustness for Olmesartan

Change in parameter	% RSD
Flow (1.1 ml/min)	0.03
Flow (0.9 ml/min)	0.08
Temperature (27°C)	0.19
Temperature (23°C)	0.73
Wavelength of Detection (250 nm)	0.82
Wavelength of detection (266 nm)	0.46

The method was also found to be specific indicated by the % recoveries ranging from 99.8% to 99.9%. The LOD and LOQ were found to be in sub-microgram level indicating the sensitivity of the method. The method was also found to be robust and rugged as indicated by the %RSD values which are less than 2%. The results of Assay show that the amount of drug was in good agreement with the label claim of the formulation as indicated by % recovery (99.6% for AML and 99.5% for OLM. The stress degradation studies showed that AML and OLM undergoes degradation in acidic and alkaline conditions whereas it is relatively stable when exposed to Amlodipine and Olmesartan were stable in photolytic, thermal and basic stress conditions. Summary of the results of stress degradation studies of AML and OLM are shown in the table 2.

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