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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(9): 461-466

www.thepharmajournal.com
Received: 06-06-2020
Accepted: 04-08-2020

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Method development and validation for simultaneous estimation of amlodipine and Olmesartan in combined tablet dosage form by using RP-HPLC

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DOI: https://doi.org/10.22271/tpi.2020.v9.i9g.5166

Abstract
The objective of the current study was to develop a simple, accurate, precise and rapid RP-HPLC method with subsequently validate as per ICH guidelines for the determination of Amlodipine (AML) and Olmesartan (OLM) using mobile phase [mixture of acetonitrile and methanol in the ratio of 60:40] as the solvent. The proposed method involves the measurement of Retention time at selected analytical wavelength. 260.0 nm was selected as the analytical wavelength. The retention time of AML and OLM was found to be 3.351 and 1.833 respectively. The linearity of the proposed method was investigated in the range of 5-25 µg/ml (r2 = 0.9999) for AML and 10-50 µg/ml (r2 = 0.9998) for OLM respectively. The method was statistically validated for its linearity, accuracy and precision. Both inter-day and intra-day variation was found to be showing less % RSD (Relative Standard Deviation) value indicating high grade of precision of the method.

Keywords: RP-HPLC method, amlodipine, olmesartan, validation

1. Introduction
Amlodipine (AML) 1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5- pyridine carboxylic acid 2- methoxyethyl(2E)-3-phenyl-propenyl ester is a novel and unique dihydropyridine calcium channel blocker that possesses a slow-onset, long-lasting vasodilating effect. It blocks theinflux of calcium ions into both vascular smooth muscle at the level of L-type calcium channels and neuronal cells at the level of N-type calcium channels. [1]
Olmesartan (OLM), chemically 2,3-Dihydroxy-2-butenyl 4(1-hydroxy- 1- methylethyl)-2-propyl-1-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]imidazole5-carboxylate, cyclic 2,3-carbonate is a prodrug used as antihypertensive, which belongs to the class of medications called angiotensin II receptor blockers{ARB}. It is indicated for the treatment of high blood pressure. It 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 vasoconstriction and aldosterone-secreting effects of angiotensin II and results in a decrease in vascular resistance and blood pressure. [2]
Survey of literature revealed that few analytical methods have been developed for the determination of AML and OLM individually [3-9] and in combination with other drugs. [10, 11] Hence an attempt has been made to develop a simple, accurate, precise and reproducible RP-HPLC method for simultaneous estimation of AML and OLM in combined dosage form with validation as per recommendation of ICH guidelines.

2. Experimental
2.1 Chemicals and Reagents
The pure API samples of Amlodipine and Olmesartan were obtained as free gift samples from Unique Pharma Ltd; Mumbai and Micro labs Ltd; Bangalore respectively. The tablet formulation of AML and OLM (Label claim: Amlodipine 10 mg and Olmesartan 20 mg), Nexovas-O tablets (Unique Pharma Ltd. Mumbai) were purchased from local market. Acetonitrile and Methanol (HPLC grade) were obtained from E. Merck Ltd Mumbai, India.

2.2 Instrument used
A Shimadzu class series HPLC unit accomplished with SPD-20AD UV-Visible detector; Enable C18 (250*4.6*5) Column (Shimadzu); LC-20 AD Pump; Quantitative HPLC was performed on a isocratic mode with 20 µl injection of sample loop (manual).

The output signal was monitored and integrated using software class LAB Solutions (Shimadzu).

2.3 Preparation of Mobile Phase

The HPLC grade Acetonitrile and methanol in the ratio of 60:40 was filtered through 0.4 µm membrane filter paper. Mobile phase was prepared by mixing 600 ml of acetonitrile and 400 ml of Methanol and sonicated for 15 min.

2.4 Preparation of Standard Stock Solution

50 mg each of standard AML and OLM was weighed accurately and transferred to two separate 50 ml volumetric flasks. Both the drugs were dissolved in 50 ml of mobile phase with sonication for 15 min and then volume was made up to the mark with mobile phase (solution–A). Further the stock solutions were diluted to get 50 µg/ml of standard stock solution of each drug (solution B). These stock solutions were filtered through 0.4 µ membrane filter paper.

2.5 Preparation of Calibration Curves

Appropriate dilutions were prepared separately and 20 µl of each was injected into the HPLC system and their chromatograms were recorded under the same chromatographic conditions as described below. Peak areas were recorded for all the peaks and a standard calibration curve of AUC against concentration was plotted.

2.6 Chromatographic Condition

The mobile phase containing both Acetonitrile and Methanol in the ratio of 60:40 was selected as the optimum composition of mobile phase, because it was found that this solvent system resolved both the components ideally. The flow rate was set to 1.0 ml/min and UV detection was carried out at 260.0 nm. The mobile phase and samples were degassed by sonication for 15 min and filtered through 0.4 µm membrane filter paper. All determinations were performed at constant column temperature (250C).

2.7 Selection of Analytical Concentration Range

Appropriate aliquots were pipetted out from the standard stock solution (solution B- 50 µg/ml) in to a series of 10 ml volumetric flasks. The volume was made up to the mark with the mobile phase to get a set of solutions having the concentration range, ranging from 5-25µg/ml and 10-50 µg/ml of AML and OLM respectively. Triplicate dilutions of each of the above mentioned concentrations was prepared separately and from these triplicate solutions, 20 µl of each concentration of the drug were injected into the HPLC system two times separately and their chromatograms were recorded under the same chromatographic conditions as described above. Peak areas were recorded for all the peaks and a standard calibration curve of AUC against concentration was plotted.

2.8 Analysis of Tablet Formulation

Twenty tablets of AML and OLM in combination were weighed and their average weight was determined. The tablets were then crushed to fine powder and powder equivalent to 10 mg of AML and 20 mg of OLM was weighed and transferred to 100 ml volumetric flask and dissolved in sufficient quantity of mobile phase. The contents were sonicated for 5 minutes and the final volume was made up to the mark with mobilephase.

The above prepared solution was filtered through 0.4 µ membrane filter paper and was used as standard stock solution. Appropriate aliquot was pipetted out from the standard stock solution and was further diluted with the mobile phase to obtain a mixture containing15

µg/ml of AML and 30 µg/ml of OLM. A replicate mixture containing 15 µg/ml of AML and 30 µg/ml of OLM were prepared as above from the standard stock solution. A 20 µl volume of each sample mixture was injected in to the sample injector of HPLC system and their chromatograms were recorded under the same chromatographic conditions as described above. The area of each peak was determined at 260.0 nm and the amount of drug present in the sample mixture was determined.

2.9 Method Validation

The developed analytical method was subjected to validation with respect to various parameters such as linearity, limit of quantification (LOQ), limit of detection (LOD), accuracy, precision, recovery studies and reproducibility as per the ICH guidelines.

3. Result and Discussion

The present manuscript deals with simultaneous estimation of AML and OLM in combined tablet dosage form by RP-HPLC method using mobile phase as the solvent. The developed method is based upon estimation of both the drugs by determining the area under curve of the chromatogram at selected analytical wavelength. The linearity of the proposed method was established by least square regression analysis of the calibration curve. The constructed calibration curves were linear over the concentration range of 5-25 µg/ml for AML (r^2 = 0.9999) and 10-50 µg/ml OLM (r^2 = 0.9998) respectively as shown in the Table9.

Recovery studies were also performed to determine the accuracy and precision of the proposed method. Recovery experiments were performed at three levels, 80%, 100% and 120% of the labelled amount of both the drugs (10 mg AML and 20 mg OLM) in tablet formulation as shown in Table 1. Three replicate samples of each concentration levels were prepared and the percentage recovery at each level (n = 3), and mean % recovery (n = 3) were determined and summarized in Table 1 and 2. Intra-day precision was estimated by assaying samples of the tablet formulation containing 15µg/ml of AML and 30 µg/ml of OLM, six times and the results were averaged for statistical evaluation. The statistical validation data for intra-day precision is summarized in Table 3 &4.

Inter-day precision was evaluated by analyzing a set of quality control samples of the tablet formulation containing 15µg/ml of AML and 30 µg/ml of OLM, three levels analyzed on three consecutive days. The statistical validation data for inter-day precision is summarized in Table 5. Both intra-day and inter-day variation showed less % RSD value indicating high grade of precision of the method as shown in table6.

The Robustness was evaluated by analyzing the samples by varying few parameters like wavelength and flow rate. The statistical validation data is summarized in table 7 and 8. The validation results obtained confirm the suitability of the proposed RP-HPLC method for simple, accurate and precise analysis of AML and OLM in pharmaceutical preparations. The proposed method does not need prior separation of AML and OLM before analysis. In addition it is suitable for application without interference of excipients and can be applied directly to the commercial preparation without previoustreatment.

Table 1: Recovery of AML and OLM in spiked standard drug solution.

Level of (%) Recovery	Amount present (mg)		Amount added (mg)		Amount found (mg)		Recovery* (%)	
	AML	OLM	AML	OLM	AML	OLM	AML	OLM
80%	10	20	8	16	17.99	35.66	99.94	99.05
	10	20	8	16	17.89	35.98	99.38	99.94
	10	20	8	16	18.10	35.95	100.55	99.86
100%	10	20	10	20	19.98	39.88	99.90	99.70
	10	20	10	20	19.92	39.99	99.60	99.97
	10	20	10	20	19.99	40.10	99.95	100.25
120%	10	20	12	24	21.98	44.10	99.83	100.22
	10	20	12	24	21.96	43.99	99.66	99.97
	10	20	12	24	22.10	43.96	100.83	99.90

Where n *= 3

Table 2: Recovery of AML and OLM in spiked standard drug solution

Level of (%) Recovery	Mean*		Standard deviation*		% Coefficient of Variation*		Standard Error*		%Recovery ± Standard Deviation	
	AML	OLM	AML	OLM	AML	OLM	AML	OLM	AML	OLM
80%	99.95	99.61	0.5851	0.4923	0.5854	0.4942	0.3378	0.2842	99.53	99.89
100%	99.81	99.97	0.1892	0.2750	0.1896	0.2750	0.1092	0.1587	±	±
120%	100.10	100.03	0.6321	0.1682	0.6314	0.1681	0.3649	0.0971	0.4726	0.1305

Where n *= 3

Table 3: Determination of intra-day precision of AML and OLM respectively

Sr. no	Amount present (µg)		Amount found (µg)		Label Claim* %	
	AML	OLM	AML	OLM	AML	OLM
1	15	30	14.99	30.01	99.96	100.01
2	15	30	14.98	29.98	99.9	99.96
3	15	30	15.01	29.97	100.03	99.95
4	15	30	14.98	30.01	99.93	100.03
5	15	30	15.02	29.99	100.13	99.98
6	15	30	15.09	30.02	100.06	100.05

Table 4: Statistical validation data for determination of intra-day precision.

Components	Mean*	Standard deviation*	% Coefficient of Variation*	Standard Error*
AML	100.00	0.0870	0.0870	0.0356
OLM	99.99	0.0398	0.0398	0.0163

Where n *= 6

Table 5: Determination of inter-day precision of AML and OLM respectively

Sr. no	Amount present (mg)		Amount found (mg)		Label Claim* %	
	AML	OLM	AML	OLM	AML	OLM
DAY-1						
1	15	30	15.00	29.99	100.03	99.98
2	15	30	15.09	30.00	100.06	100.03
3	15	30	14.99	30.01	99.96	100.01
4	15	30	15.01	29.98	100.13	99.96
5	15	30	14.98	30.01	99.93	100.05
6	15	30	15.01	30.01	100.10	100.06
DAY- 2						
1	15	30	15.01	29.99	100.03	99.98
2	15	30	14.99	29.98	99.96	99.96
3	15	30	14.98	29.98	99.93	99.95
4	15	30	15.09	29.97	100.06	99.93
5	15	30	14.98	29.97	99.90	99.91
6	15	30	14.97	29.98	99.86	99.95
DAY- 3						
1	15	30	14.99	29.98	99.96	99.95
2	15	30	14.97	29.97	99.86	99.93
3	15	30	14.97	29.97	99.80	99.91
4	15	30	14.96	29.99	99.83	99.88
5	15	30	14.98	29.97	99.90	99.90
6	15	30	14.97	29.98	99.86	99.95

Table 6: Statistical validation data for determination of inter-day precision

Components	Mean*	Standard deviation*	% Coefficient of Variation*	Standard Error*
AML	99.95	0.0967	0.0968	0.0394
OLM	99.96	0.0505	0.0506	0.0206

Where n*= 3

Table 7: Determination of Robustness of AML and OLM respectively.

Levels	Retention time		Tailing factor	
	AML	OLM	AML	OLM
Flow Rate				
-1	3.365	1.728	1.005	0.898
0	3.351	1.833	1.310	0.966
+1	3.201	1.098	1.257	1.159
Wavelength				
-2	3.365	1.823	1.011	1.005
0	3.351	1.833	1.201	0.966
+2	3.366	1.806	1.247	1.157

Table 8: Statistical validation data of determination of Robustness for change in method parameters.

Parameters	Mean		Standard Deviation		(%) Coefficient of variance	
	AML	OLM	AML	OLM	AML	OLM
Flow Rate						
Retention time	3.3086	1.549	0.08573	0.3940	2.5912	25.4357
Tailing factor	1.190	1.008	0.162	0.135	13.6134	13.3928
Wavelength						
Retention time	1.153	1.042	0.125	0.100	10.8412	9.5969
Tailing factor	3.360	1.819	0.0008	0.0008	0.0123	0.0238

Table 9: Summary of validation and System suitability parameters of AML and OLM

Parameters	AML	OLM
Linear range (µg/ml)	5-25	10-50
Slope	58258	39880
Intercept	12486	2938
Regression coefficient (r^2)	0.9999	0.9998
Limit of Detection (µg/ml)	0.02183	0.0312
Limit of Quantification (µg/ml)	0.0661	0.0945
Retention time (min)	3.351	1.833
Tailing factor	1.201	0.966
Resolution factor	11.408	
Theoretical plate	9701.15	3544.80

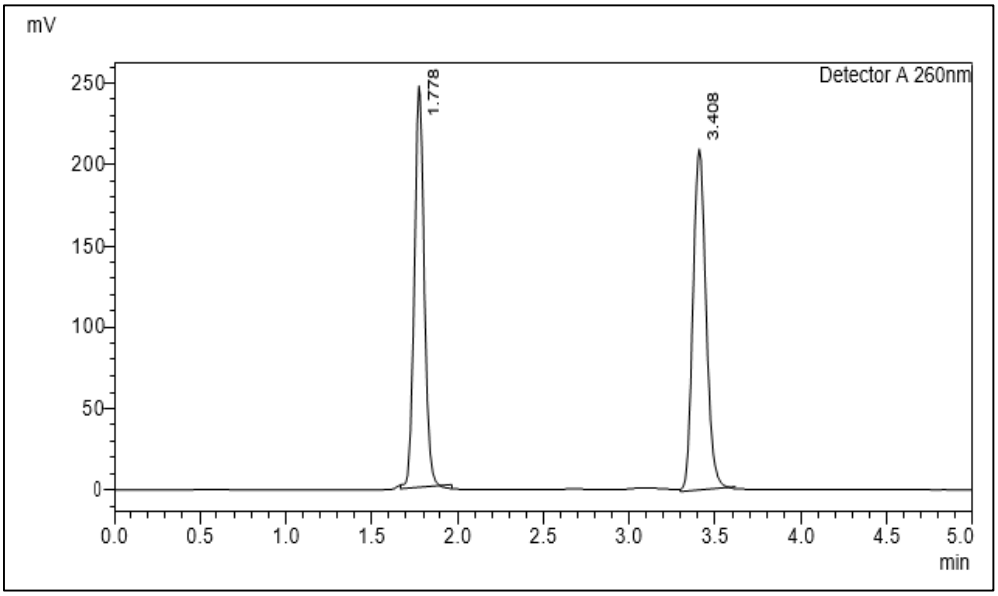

Fig 1: Chromatogram showing retention times of Amlodipine and Olmesartan respectively

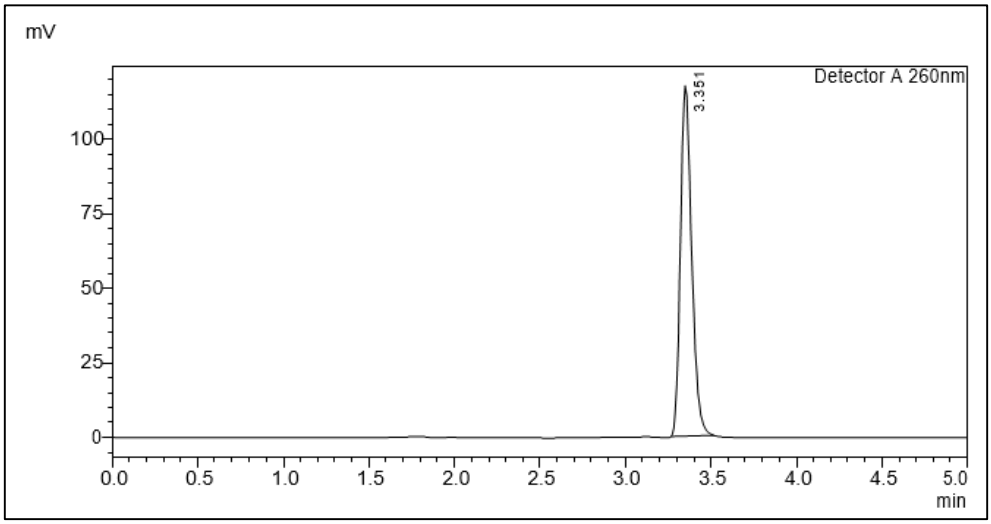

Fig 2: Chromatogram showing retention time of Amlodipine**.**

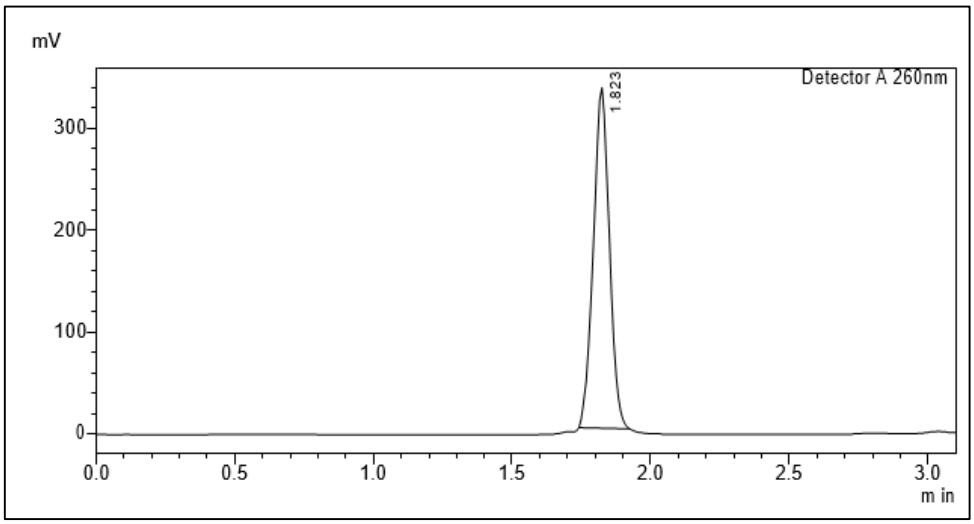

Fig 3: Chromatogram showing retention time of Olmesartan

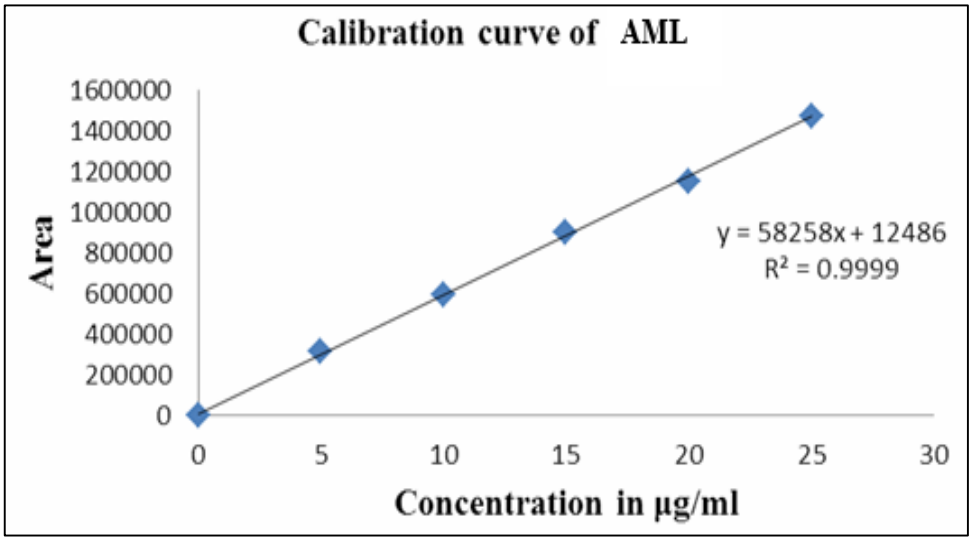

Fig 4: Calibration curve of Amlodipine at 260.0 nm in Acetonitrile and Methanol by RP-HPLC Method.

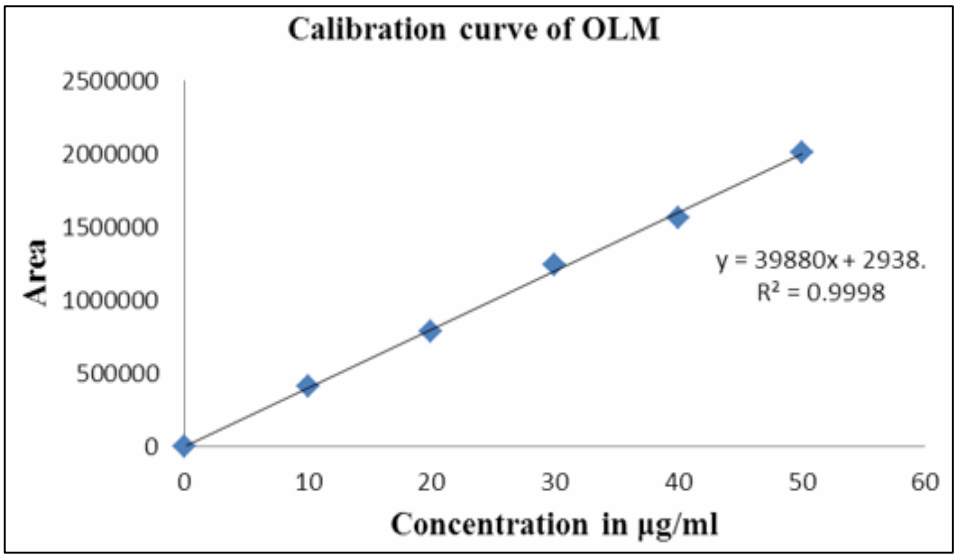

Fig 5: Calibration curve of Olmesartan at 260.0 nm Acetonitrile and Methanol in methanol by RP-HPLC Method

4. Conclusion

Proposed study describes a new RP-HPLC method for the estimation Amlodipine and Olmesartan in combination using simple mobile phase. The method gives good resolution between the compounds with a short analysis time. The method was validated and found to be simple, sensitive, accurate and precise. So the developed method can be used conveniently for analysis of AML and OLM in in combined pharmaceutical dosage form.

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