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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF OLMESARTAN AND AZELNIDIPINE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, rapid, precise, accurate, and sensitive and stability indicating RP-HPLC method for the simultaneous quantification of Olmesartan and Azelnidipine in pure and tablet dosage form. Chromatographic separation was achieved using Agilent C18 (150 mm x 4.6 mm, 5µ)and with the mobile phase of 50:45 v/v of 0.1% Na_2HPO_4 and acetonitrile pumped at a flow rate of 1 mL/min. Linear response was observed in the range of 5-30 µg/mL for Olmesartan and 2-12µg/mL for Azelnidipineand also the correlation coefficient was found to be0.999 for both drugs . Olmesartan and Azelnidipine were eluted at 2.399 min and 2.984 min respectively with good resolution.The mean percentage recoveries of Olmesartan and Azelnidipine were found to be 101.53% and 100.84% respectively.ICH guidelines were adhered for validation of proposed method regarding specificity, precision, linearity, accuracy, system suitability and robustness.. The stress testing of both the drugs individually and their mixture is carried out under acidic, alkaline, oxidation, photo-stability and thermal degradation conditions and its degradation products are well resolved from the analyte peaks..The developed method was successfully employed for routine quality control analysis.

Keywords: Olmesartan, Azelnidipine, Degradation, Validation and RP-HPLC.

INTRODUCTION

Albuminaria is an associated condition with Type-II diabetic and Hypertensive patients which leads to cardiovascular diseases. Olmesartan and Azlendipine combined dosage form is an effective medicine used to treat albuminaria.

Olmesartan(Figure 1)is a selective blocker of Angiotensin-II receptor and emerged as an effective agent in lowering blood pressure[1]. Chemically it is (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl5-(2-hydroxypropan-2-yl)-2-propyl-3-[[4-[2-(2*H*-tetrazol-5yl) phenyl] phenyl] methyl]imidazole-4-carboxylate. Olmesartan mainly regulates the hemostasis, kidney, vascular and cardiac functions.

Azelnidipine(Figure 2) is a third generation long lasting dihydro pyridine calcium channel blocker shows anti hypertensive effect via inhibition of trans-membrane Ca2+ influx through the voltage-dependent channels of smooth muscles in vascular walls[2]. It is highly lipid soluble and expected to have an increasing effect of cerebral blood flow. It is chemically known as 3-*O*-(1-benzhydrylazetidin-3-yl) 5-*O*-propan-2-yl (4*R*)-2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate.

Literature review disclosed few HPLC methods[3-7] were reported for simultaneous estimation of Olmesartan and Azelnidipine. Hence an attempt was made to develop a simple, rapid and accurate RP-HPLC method for estimation of Olmesartan and Azelnidipine in pure and tablet dosage form.

MATERIALS AND METHODS

Instrument

Agilent HPLC 1260 affinity model equipped with binary pump operated using open lab software was utilized for chromatographic studies.

Mobile phase

Mobile phase was prepared by mixing 0.01 N Na_2HPO_4 and acetonitrile taken in ratio 55:45

%v/v. It was filtered through 0.45 µ membrane filter to remove the impurities which may interfere in the final chromatogram.

Diluent
Based up on the solubility of the drugs, diluent was selected, water and acetonitriletaken in the ratio of 55:45 %v/v

Preparation of standard stock solution
Standard stock solution was made by accurately weighing10 mg of Olmesartan and4 mg of Azelnidipine and shifted to 50 mL volumetric flasks. Add some diluent to dissolve the drugs, sonicate if required, finally volume made upto mark with diluent. Working standard solutionswere prepared by further dilution with diluent.

Preparation of Sample solution
10 tablets were accurately weighed and average weight equivalent to 1 tablet was transferred into a 100mL volumetric flask, 50mL of diluent was added and sonicated for 25 min, further the volume was made up with diluent. The resulting solution was filtered through a 0.22 µm membrane filter. The filtrate was diluted further with mobile phase to get the working sample solution.

Detection of wave length
Standard stock solutions were further diluted and scanned in the wavelength range of 200-400 nm and from the spectral results 260 nm was selected for measuring the response of drugs.

RESULTS

Method development
A new method was proposed for simultaneous determination of Olmesartan and Azlendipine after performing various trails with different mobile phases and different columns. Optimised chromatographic conditions include the mobile phase composed of 0.01N Na_2HPO_4: Acetonitrile(55:45 %v/v) pumping in the column agilent C18 (150 mm x 4.6 mm, 5µm) at a flow rate 1 mL/min. The response of drug substances were recorded at 260 nm. The peaks for Olmesartan and Azelnidipine were detected at 2.39 min and 2.984 min.

Method Validation
System suitability
System suitability is a testing parameter for checking the performance of HPLC system by injecting standard solution for 6-10 times and %RSD was calculated from peak areas. The data was presented in Table 1.

Specificity
The method was so specific as it detects only standard and sample peaks and no response was observed in blank and placebo peaks.

Linearity
Linearity is a performance characteristic for measuring the relationship between concentration and response. Calibration curve was constructed with 5-10µg/mL dilutions of Olmesartan and 2-12µg/mL dilutions of Azelnidipine. Linearity results were furnished in Table 2 and calibrations graphs were represented in Figure 4& 5.

Precision
Precision was studied to find out intra-day and inter-day variation in the test methods for 6 times on the same day and different day. The intra-day and inter-day precision obtained was %RSD (<2.0) indicates that the proposed method is quite precise and reproducible and results are shown in Table 3.

Accuracy
The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed drug sample solution. Percent recovery was calculated by comparing the peak area before and after the addition of the standard drug. The standard addition method was performed at three concentration levels in triplicate at 50%, 100% and 150%. The results were shown in Table 4.

Robustness
To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and wavelength. System suitability parameters (Table 5) were compared with that of method precision.

Limit of detection and Limit of quantification
Limit of detection (LOD) is defined as the lowest concentration of analyte that gives a detectable response. Limit of quantification (LOQ) is defined as the lowest concentration that can be quantified reliably with a specified level of accuracy and precision. The results were furnished in Table 6.

Assay of tablet dosage form
The assay results of tablet dosage form were comparable with the value claimed on the label. The obtained results, indicated the suitability of the method for routine analysis of Olmesartan and Azelnidipine.

Degradation studies

Degradation studies were performed in presence of acid, alkali, peroxide, temperature, light and water. The percentage degradation of Olmesartan and Azelnidipine were calculated by comparing the peak area of Olmesartan and Azelnidipine before and after treatment. The degradation peaks were well resolved from that of drugs in the absence of impurity. The results were furnished in Table 7.

CONCLUSION

The present investigation described a simple, rapid, accurate, precise, robust and stability indicating HPLC method for the simultaneous estimation of Olmesartan and Azelnidipine in pure and tablet dosage form. The mobile phase and solvents are simple to prepare and economical, reliable, sensitive and less time consuming. The sample recoveries were in good agreement with their respective label claims and they suggested no interference of formulation recipients in the estimation and can be used in laboratories for the routine analysis of selected drugs. Since the system validation parameters of HPLC method used for estimation of selected drugs in pure and have shown satisfactory, accurate and reproducible results (without any interference of recipients) as well, it is deduced that the simple and short proposed methods be most useful for analysis purpose.

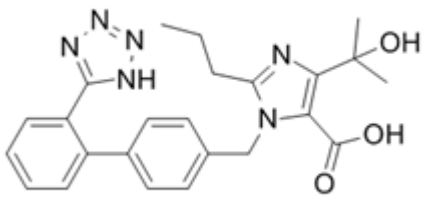

Fig. 1: Structure of Olmesartan

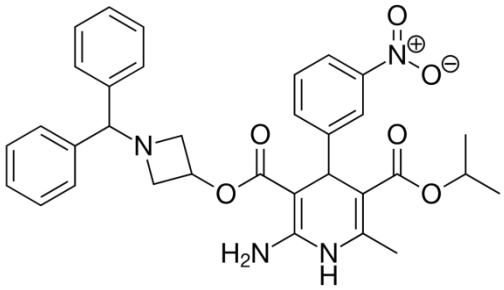

Fig. 2: Structure of Azelnidipine

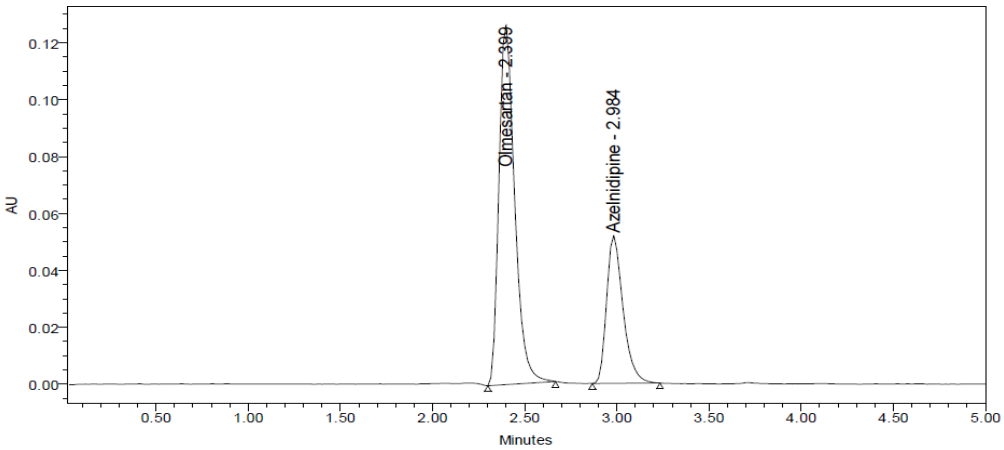

Fig. 3: Optimized Chromatogram of Olmesartan and Azelnidipine

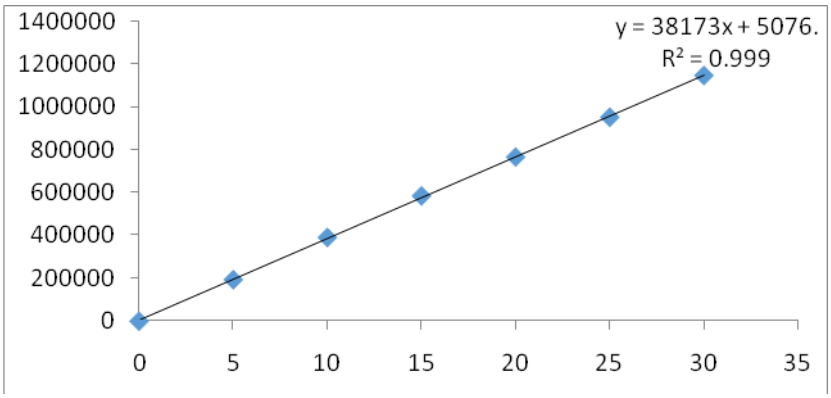

Fig. 4: Calibration curve of Olmesartan

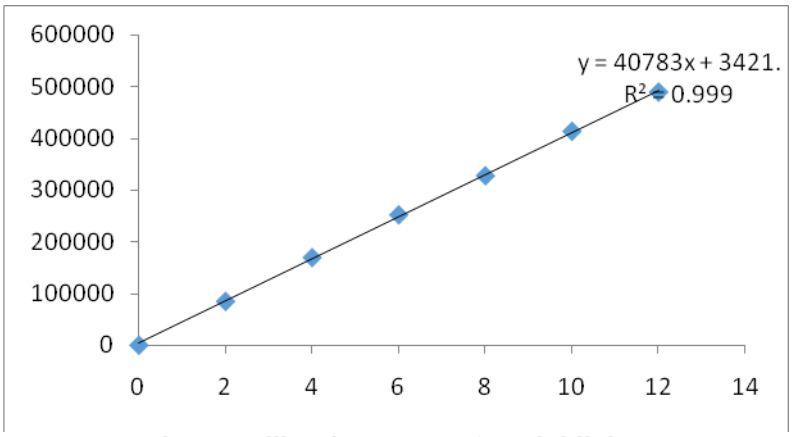

Fig. 5: Calibration curve of Azelnidipine

Table 1: Results for System suitability

S. No.	Olmesartan			Azelnidipine			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	RS
1	2.364	3490	1.33	2.953	4985	1.35	3.5
2	2.380	3544	1.35	2.976	4870	1.36	3.5
3	2.389	3444	1.36	2.994	4874	1.34	3.5
4	2.394	3457	1.36	2.999	5035	1.34	3.6
5	2.398	3571	1.34	3.000	5069	1.36	3.5
6	2.405	3577	1.33	3.009	4908	1.35	3.5

Table 2: Results for Linearity

S. No.	Olmesartan		Azelnidipine	
	Conc. (µg/mL)	Peak area	Conc. (µg/mL)	Peak area
1	0	0	0	0
2	5	194360	2	84748
3	10	391770	4	169442
4	15	585953	6	252059
5	20	767884	8	327739
6	25	954354	10	413524
7	30	1149367	12	489342
Slope	38173		40783	
Intercept	5076		3421.2	
R2	0.999		0.999	

Table 3: Intra-day and Inter-day Precision results

S. No	Intra-day		Inter-day	
	Area of Olmesartan	Area of Azelnidipine	Area of Olmesartan	Area of Azelnidipine
1	751061	329028	754022	317917
2	751074	327696	733093	315264
3	759533	326706	748350	315504
4	759448	331505	756199	319022
5	763108	327461	749869	314518
6	762888	331303	741313	319823
Mean	757852	328950	747141	317008
S.D	5484.9	2044.3	8597.8	2205
%RSD	0.7	0.6	1.2	0.7

Table 4: Results for Accuracy

% Level	Olmesartan			Azelnidipine		
	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Amount Spiked	Amount recovered	% Recovery
50%	10	10.2	101.5	4	4.05	101.27
	10	10.3	103	4	4.02	100.38
	10	10.2	102.4	4	3.98	99.4
100%	20	20.4	101.9	8	8.14	101.71
	20	20	100.2	8	8.06	100.8
	20	20.3	101.6	8	8.15	101.87
150%	30	30	100.1	12	12	99.97
	30	30.4	101.3	12	12.15	101.29
	30	30.6	101.8	12	12.1	100.87

Table 5: Results for Robustness

S.No.	Condition	%RSD of Olmesartan	%RSD of Azelnidipine
1	Flow rate (-) 0.9mL/min	0.5	0.2
2	Flow rate (+) 1.1mL/min	0.8	1.3
3	Mobile phase (-) 60B:40A	0.4	0.2
4	Mobile phase (+) 50B:50A	0.2	0.5
5	Temperature (-) 25°C	0.6	0.8
6	Temperature (+) 35°C	1.2	0.2

Table 6: LOD and LOQ values

Molecule	LOD	LOQ
Olmesartan	0.18	0.56
Azelnidipine	0.08	0.25

Table 7: Degradation data

S.No.	Degradation Condition	% Degradation of Olmesartan	% Degradation of Azelnidipine
1	Acid	5.41	4.49
2	Alkali	4.04	3.38
3	Oxidation	4.76	3.59
4	Thermal	3.01	2.98
5	UV	1.69	1.83
6	Water	0.27	0.32

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