Development of Rp-Hplc Method for Simultaneous Determination of Azelnidipine and Olmesartan Medoxomil in Bulk and Marketed Tablet Dosage Form

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ABSTRACT :
Objective: To develop a simple, selective and rapid reverse phase high performance liquid chromatography (RP-HPLC) method and validate as per ICH guidelines for simultaneous determination of Azelnidipine and Olmesartan Medoxomil in a combined dosage form.
Methods: The chromatographic separation of the two anti-Diabetic drugs were achieved using Develosil C_{18} (4.6mm×250mm, 5µm) Particle size, maintained at 34 °C throughout the analysis. The drugs were separated in isocratic elution mode with a mobile phase of Methanol: Phosphate Buffer (45:55% v/v) at a flow rate of 1.0 mL/min and a detection wavelength of 261 nm using a PDA detector.
Results: The linearity and range for Azelnidipine and Olmesartan Medoxomil were 60 to 140 mg/mL (R2> 0.9997) and 100 to 500 mg/mL (R2> 0.9997), respectively. Mean recoveries observed for Azelnidipine and Olmesartan Medoxomil were 100.36% and 100.15%, respectively. The precision of the method obtained was for Azelnidipine and for Olmesartan Medoxomil with a relative standard deviation less than 2%. The lower degree of % RSD that was obtained for intermediate precision has proved that the method is robust and rugged.
Conclusion: A simple and rapid RP-HPLC method was developed and validated for simultaneous determination of Azelnidipine and Olmesartan Medoxomil in a combined dosage form and hence, it can be used in the quality control analysis of an active pharmaceutical ingredient and pharmaceutical dosage form.
Key Words: Azelnidipine and Olmesartan Medoxomil, RP-HPLC, ICH Guidelines.

I. INTRODUCTION

Azelnidipine is a third-generation dihydropyridine calcium channel blocker (CCB) used to treat high blood pressure (hypertension). It works by relaxing blood vessels, which reduces the workload on the heart and lowers blood pressure. Azelnidipine is a dihydropyridine calcium channel blocker[1]. It is marketed by Daiichi-Sankyo pharmaceuticals, Inc. in Japan. It has a gradual onset of action and produces a long-lasting decrease in blood pressure, with only a small increase in heart rate, unlike some other calcium channel blockers. It is currently being studied for post-ischemic stroke management[2].Azelnidipine belongs to a class of anti-hypertensive drugs primarily used to treat hypertension (high blood pressure). Hypertension (high blood pressure) is a lifelong or chronic condition in which the force exerted by the blood against the artery walls becomes high[3]. The IUPAC name of Azelnidipine is 3-O-(1-benzhydrylazetidin-3-yl) 5-O-propan-2-yl 2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate. The Chemical Structure of Azelnidipine is shown in following figure-1.

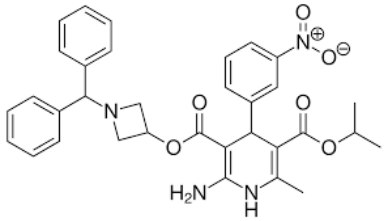

Fig-1: Chemical Structure of Azelnidipine

Olmesartan belongs to the angiotensin II receptor blocker (ARB) family of drugs, which also includes Telmisartan, candesartan, losartan, valsartan, and Irbesartan. ARBs selectively bind to angiotensin receptor 1

(AT1) and prevent the protein angiotensin II from binding and exerting its hypertensive effects, which include vasoconstriction, stimulation and synthesis of aldosterone and ADH, cardiac stimulation, and renal reabsorption of sodium, among others[4]. Overall, Olmesartan's physiologic effects lead to reduced blood pressure, lower aldosterone levels, reduced cardiac activity, and increased excretion of sodium[5].Olmesartan is indicated for the treatment of hypertension either alone or in combination with other antihypertensive agents. The IUPAC Name of OlmesartanMedoxomil is (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl 5-(2-hydroxypropan-2-yl)-2-propyl-3-[[4-[2-(2H-tetrazol-5-yl)phenyl]phenyl]methyl]imidazole-4-carboxylate[6]. The Chemical Structure of OlmesartanMedoxomilis shown in following figure-2.

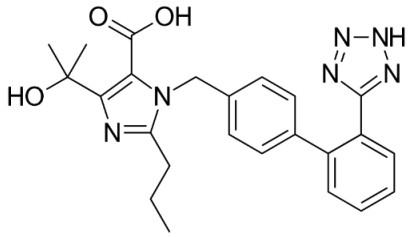

Fig-2: Chemical Structure of Olmesartan Medoxomil

II. EXPERIMENTAL WORK

Instruments Used:

Table-1: Instruments Used

S.No.	Instruments and Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module. 996 PDA detector, software: Empower 2
2	pH meter	LabIndia
3	Weighing machine	Sartorius
4	Digital Ultra Sonicator	Labman

Chemicals Used:

Table-2: Chemicals used

S.No.	Chemical	Brand Names
1	Azelnidipine	Synpharma Research Lab, Hyderabad
2	Olmesartan Medoxomil	Synpharma Research Lab, Hyderabad
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

HPLC Method Development:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Azelnidipine and Olmesartan Medoxomil working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 1ml of the above Azelnidipine and 3ml of Olmesartan Medoxomil stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol[7].

Preparation of Sample Solution:

Take average weight of the Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight ofAzelnidipine and Olmesartan Medoxomil sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 1ml of Azelnidipine and 3ml Olmesartan Medoxomil above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent[8].

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines[9-15].

Preparation of Mobile Phase:
Accurately measured 450ml (45%) of Methanol, 550ml of Phosphate Buffer (55%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration[16].

Diluent Preparation:
The Mobile phase was used as the diluent.

Method Validation Parameters
System Suitability
Accurately weigh and transfer 10 mg of Azelnidipineand 10mg of Olmesartan Medoxomil working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).Further pipette 1ml of Azelnidipine and 3ml of Olmesartan Medoxomil from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:
The sample solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits[17].

Specificity:
Preparation of Standard Solution:
Accurately weigh and transfer 10 mg of Azelnidipineand 10mg of Olmesartan Medoxomil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 1ml of Azelnidipine and 3ml of Olmesartan Medoxomil from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution:
Take average weight of the Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight ofAzelnidipine and Olmesartan Medoxomil sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 1ml of Azelnidipine and 3ml Olmesartan Medoxomil above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:
Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula[18]:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Linearity:
Accurately weigh and transfer 10 mg of Azelnidipineand 10mg of Olmesartan Medoxomil working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Preparation of Level – I (60ppm of Azelnidipine&100ppm of Olmesartan Medoxomil):
Pipette out 0.6ml of Azelnidipine and 1ml of Olmesartan Medoxomil stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (80ppm of Azelnidipine & 200ppm of Olmesartan Medoxomil):
Pipette out 0.8ml of Azelnidipine and 2ml of Olmesartan Medoxomil stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (100ppm of Azelnidipine & 300ppm of Olmesartan Medoxomil):
Pipette out 1ml of Azelnidipine and 3ml of Olmesartan Medoxomil stock solutions was take in a 10ml of

volumetric flask dilute up to the mark with diluent[19].

Preparation of Level – IV (120ppm of Azelnidipine & 400ppm of Olmesartan Medoxomil):
Pipette out 1.2ml of Azelnidipine and 4ml of Olmesartan Medoxomil stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (140ppm of Azelnidipine & 500ppm of Olmesartan Medoxomil):
Pipette out 1.4ml of Azelnidipine and 5ml of Olmesartan Medoxomil stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure:
Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient[20-22].

Precision
Repeatability
Preparation of Azelnidipine and Olmesartan MedoxomilSolution for Precision:
Accurately weigh and transfer 10 mg of Azelnidipineand 10mg of Olmesartan Medoxomil working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).Further pipette 1ml of Azelnidipine and 3ml of Olmesartan Medoxomil from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.
The sample solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits[23].

Intermediate Precision:
To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:
Day 1: The sample solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.
Day 2: The sample solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits[24].

Accuracy:
For preparation of 50% SampleStock solution:
Accurately weigh and transfer 10 mg of Azelnidipineand 10mg of Olmesartan Medoxomil working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).
Further pipette 0.5ml of Azelnidipine and 1.5ml of Olmesartan Medoxomil from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 100% SampleStock solution:
Accurately weigh and transfer 10 mg of Azelnidipineand 10mg of Olmesartan Medoxomil working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).
Further pipette 1ml of Azelnidipine and 3ml of Olmesartan Medoxomil from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents[25].

For preparation of 150% SampleStock solution:
Accurately weigh and transfer 10 mg of Azelnidipineand 10mg of Olmesartan Medoxomil working Sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).
Further pipette 1.5ml of Azelnidipine and 4.5ml of Olmesartan Medoxomil from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:
Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Azelnidipineand Olmesartan Medoxomil and calculate the individual recovery and mean recovery values[26].

Robustness:
The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

For preparation of Standard solution:
Accurately weigh and transfer 10 mg of Azelnidipineand 10mg of Olmesartan Medoxomil working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).
Further pipette 1ml of Azelnidipine and 3ml of Olmesartan Medoxomil from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation of Flow Conditions:
The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded[27].

Effect of Variation of Mobile Phase Organic Composition:
The sample was analyzed by variation of mobile phase i.e. Methanol: PhosphateBuffer (45:55% v/v)was taken in the ratio and 50:50, 40:60 instead (45:55% v/v) remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

III. RESULTS AND DISCUSSION

Development of an Analytical Method:
Optimized Chromatogram (Standard)

Mobile phase : Methanol: Phosphate Buffer (45:55% v/v)
Column : Develosil C_{18} (4.6mm×250mm, 5µm) Particle size
Flow rate : 1.0 ml/min
Wavelength : 261 nm
Column temp : 34ºC
Injection Volume : 20 µl
Run time : 10 minutes

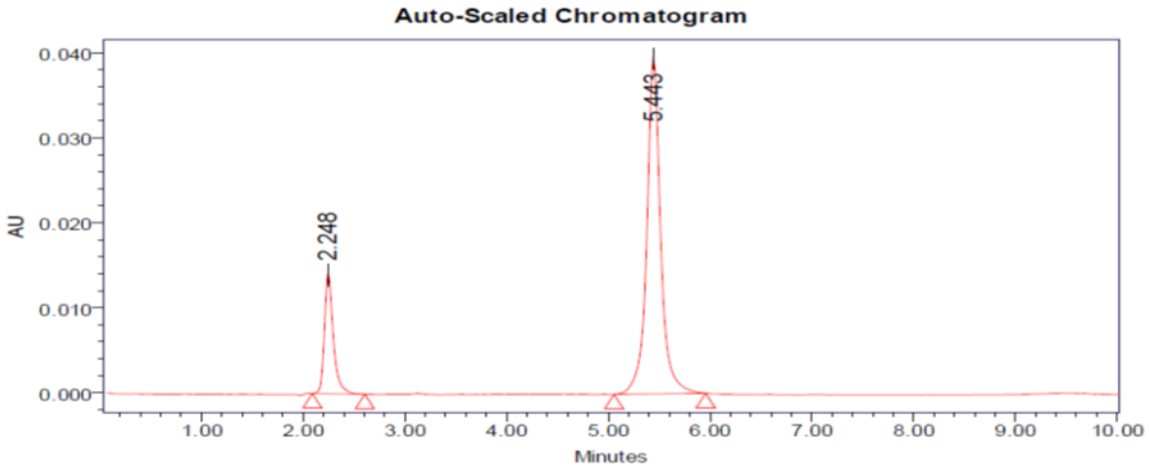

Fig-3: Optimized Chromatogram

Analytical Method Validation:
System Suitability:

Table-4: Results of System Suitability for Azelnidipine

S.No.	Name	Rt	Peak Area	Height	USP plate Count	USP Tailing
1	Azelnidipine	2.247	105698	18652	7592	1.08
2	Azelnidipine	2.246	105874	18754	7584	1.09
3	Azelnidipine	2.248	105698	18698	7562	1.08
4	Azelnidipine	2.252	105465	18689	7549	1.08
5	Azelnidipine	2.248	105236	18695	7591	1.09
Mean			**105594.2**			
Std. Dev			**247.4049**			
% RSD			**0.234298**			

Table-5: Results of System Suitability for Olmesartan Medoxomil

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Olmesartan Medoxomil	5.452	1856985	63659	6359	1.05	5.86
2	Olmesartan Medoxomil	5.484	1856754	63598	6384	1.04	5.85
3	Olmesartan Medoxomil	5.491	1856985	63845	6395	1.05	5.86
4	Olmesartan Medoxomil	5.482	1856574	63989	6345	1.04	5.86
5	Olmesartan Medoxomil	5.491	1854735	63895	6395	1.05	5.85
Mean			**1856407**				
Std. Dev			**950.2696**				
% RSD			**0.051189**				

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitates Azelnidipine and Olmesartan Medoxomil in marketed formulation[28].

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Azelnidipine and Olmesartan Medoxomil in pharmaceutical dosage form (marketed formulation) was found to be 99.72%.

Linearity:

Table-6: Chromatographic Data for Linearity Study of Azelnidipine

Concentration µg/ml	Average Peak Area
60	648743
80	856982
100	1068542
120	1268984
140	1469853

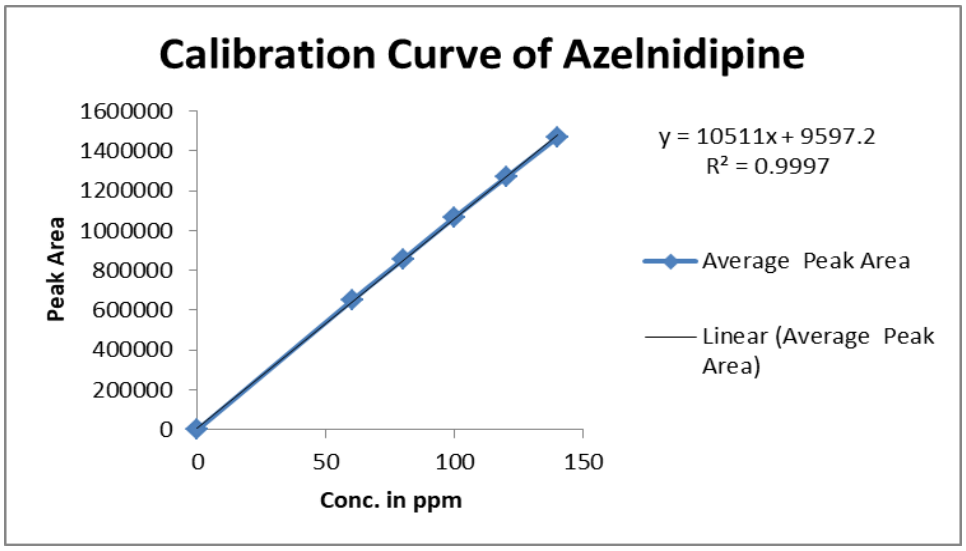

Fig-4: Calibration Graph for Azelnidipine

Linearity Plot: The plot of Concentration (x) versus the Average Peak Area (y) data of Azelnidipine is a straight line.

Y = mx + c
Slope (m) = 10511
Intercept (c) = 9597
Correlation Coefficient (r) = 0.999

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.
Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 9597. These values meet the validation criteria[29].

Table-7: Chromatographic Data for Linearity Study of Olmesartan Medoxomil

Concentration µg/ml	Average Peak Area
100	667564
200	1268547
300	1868598
400	2465487
500	3085864

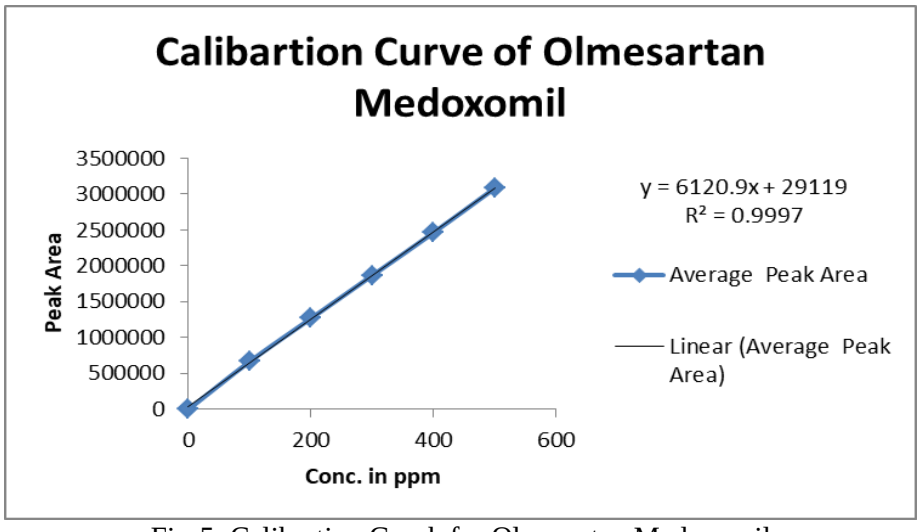

Fig-5: Calibration Graph for Olmesartan Medoxomil

Linearity Plot: The plot of Concentration (x) versus the Average Peak Area (y) data of Olmesartan Medoxomil is

a straight line.

Y = mx + c
Slope (m) = 6120
Intercept (c) = 29119
Correlation Coefficient (r) = 0.999

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.
Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 29119. These values meet the validation criteria.
Precision:
The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions[30].
Repeatability: Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table-8: Results of Repeatability for Azelnidipine

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Azelnidipine	2.269	105698	18569	7598	1.08
2	Azelnidipine	2.255	105684	18547	7546	1.09
3	Azelnidipine	2.252	105421	18594	7549	1.09
4	Azelnidipine	2.267	105879	18574	7538	1.08
5	Azelnidipine	2.260	105326	18563	7582	1.08
Mean			105601.6			
Std. Dev			224.5023			
% RSD			0.212594			

Table-9: Results of Method Precision for Olmesartan Medoxomil

S. No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Olmesartan Medoxomil	5.274	1856985	63598	6359	1.05	5.86
2	Olmesartan Medoxomil	5.266	1857458	63579	6357	1.04	5.85
3	Olmesartan Medoxomil	5.265	1854795	63547	6358	1.04	5.86
4	Olmesartan Medoxomil	5.278	1857469	63592	6357	1.05	5.86
5	Olmesartan Medoxomil	5.305	1857685	63569	6345	1.04	5.85
Avg			**1856878**				
Std. Dev			**1192.4**				
% RSD			**0.064215**				

Intermediate Precision:
Day 1:

Table-10: Results of Intermediate Precision for Azelnidipine

S. No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Azelnidipine	2.248	115246	19685	7698	1.09
2	Azelnidipine	2.245	116985	19654	7685	1.09
3	Azelnidipine	2.242	115847	19675	7645	1.09
4	Azelnidipine	2.239	116985	19682	7682	1.09

5	Azelnidipine	2.243	115848	19654	7691	1.09
6	Azelnidipine	2.246	116582	19647	7642	1.10
Mean			**116248.8**			
Std. Dev			**710.3091**			
% RSD			**0.611025**			

Table-11: Results of Intermediate Precision for Olmesartan Medoxomil

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Olmesartan Medoxomil	5.284	1948592	64582	6459	1.05	5.96
2	Olmesartan Medoxomil	5.293	1958245	64256	6475	1.06	5.95
3	Olmesartan Medoxomil	5.306	1947584	64598	6498	1.05	5.96
4	Olmesartan Medoxomil	5.319	1948675	64785	6472	1.06	5.95
5	Olmesartan Medoxomil	5.346	1959854	64585	6493	1.05	5.96
6	Olmesartan Medoxomil	5.352	1958246	64924	6438	1.06	5.96
Mean			**1953533**				
Std. Dev			**5792.661**				
% RSD			**0.296522**				

Day 2:

Table-12: Results of Intermediate Precision Day 2 for Azelnidipine

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Azelnidipine	2.255	102658	62584	6259	1.03
2	Azelnidipine	2.260	102856	62359	6276	1.02
3	Azelnidipine	2.242	102658	62451	6215	1.03
4	Azelnidipine	2.245	102698	62584	6285	1.02
5	Azelnidipine	2.260	102451	62758	6235	1.03
6	Azelnidipine	2.255	102368	62154	6298	1.02
Mean			**102614.8**			
Std. Dev			**176.9592**			
% RSD			**0.17245**			

Table-13: Results of Intermediate Precision for Olmesartan Medoxomil

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Olmesartan Medoxomil	5.266	1798952	62859	6265	1.03	5.42
2	Olmesartan Medoxomil	5.265	1789854	62985	6289	1.02	5.43
3	Olmesartan Medoxomil	5.306	1798659	62895	6279	1.03	5.42
4	Olmesartan Medoxomil	5.293	1789898	62785	6285	1.02	5.43
5	Olmesartan Medoxomil	5.265	1796856	62354	6249	1.03	5.42
6	Olmesartan Medoxomil	5.266	1798568	62589	6245	1.02	5.43

Mean			**1795465**				
Std. Dev			**4390.879**				
% RSD			**0.244554**				

Accuracy: Accuracy at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated[31].

Table-14: The Accuracy Results for Azelnidipine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	539070	50	50.373	100.746%	100.36%
100%	1063578	100	100.274	100.274%	
150%	1587149	150	150.085	100.056%	

Table-15: The Accuracy Results for Olmesartan Medoxomil

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	949127	150	150.328	100.218%	100.15%
100%	1867824	300	300.441	100.147%	
150%	2785321	450	450.359	100.079%	

Limit of Detection : The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value[32].

$$\mathbf{LOD = 3.3 \times \sigma / s}$$

Where
σ = Standard deviation of the response
S = Slope of the calibration curve
Result:
Azelnidipine: =2.63µg/ml
Olmesartan Medoxomil:=3.84µg/ml
Limit of Quantitation: The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined[33].

$$\mathbf{LOQ = 10 \times \sigma / S}$$

Where
σ = Standard deviation of the response
S = Slope of the calibration curve
Result:
Azelnidipine: = 7.92µg/ml
Olmesartan Medoxomil:= 11.54µg/ml
Robustness: The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Azelnidipine and Olmesartan Medoxomil. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The samples (marketed formulation) of Azelnidipine and Olmesartan Medoxomil were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count[34].

Table-16: Results for Robustness of Azelnidipine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	105265	2.256	7589	1.08
Less Flow rate of 0.9 mL/min	109898	2.505	7256	1.05
More Flow rate of 1.1 mL/min	102365	2.046	7469	1.07
Less organic phase	101548	2.505	7358	1.06

More organic phase	104645	2.046	7659	1.02

Table-17: Results for Robustness of Olmesartan Medoxomil

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1858475	5.427	6354	1.04
Less Flow rate of 0.9 mL/min	1925684	5.599	6253	1.05
More Flow rate of 1.1 mL/min	1863525	4.576	6248	1.03
Less organic phase	1825471	5.599	6415	1.02
More organic phase	1836594	4.576	6529	1.06

STABILITY STUDIES

The specificity of the method can be demonstrated by applying stress conditions using acid, alkaline, peroxide, thermal, UV, water degradations. The sample was exposed to these conditions the main peak of the drug was studied for peak purity that indicating the method effectively separated the degradation products from the pure active ingredient[35-39].

Table-18: Results of Forced Degradation Studies of Azelnidipine

S.No.	Stress Condition	Peak Area	% of Degraded Amount	% of Active Amount	Total % of Amount
1	Standard	105265	0	100%	100%
2	Acidic	101557.566	3.522	96.478	100%
3	Basic	104200.770	1.011	98.989	100%
4	Oxidative	102830.220	2.313	97.687	100%
5	Thermal	103990.240	1.211	98.789	100%
6	Photolytic	105154.471	0.105	99.895	100%

Table-19: Results of Forced Degradation Studies of Olmesartan Medoxomil

S.No.	Stress Condition	Peak Area	% of Degraded Amount	% of Active Amount	Total % of Amount
1	Standard	1858475	0	100%	100%
2	Acidic	1795045.248	3.413	96.587	100%
3	Basic	1832214.748	1.251	98.749	100%
4	Oxidative	1800100.300	3.141	96.859	100%
5	Thermal	1829910.239	1.537	98.463	100%
6	Photolytic	1839276.953	1.033	98.967	100%

CONCLUSION

The analytical method was developed by studying different parameters.First of all, maximum absorbance was found to be at 261 nm and the peak purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Develosil C_{18} (4.6mm×250mm, 5µm) Particle size because it was giving good peak.Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Methanol: Phosphate Buffer (45:55% v/v)was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Run time was selected to be 10 min because analyze gave peak around 2.248, 5.443 ±0.02min respectively and also to reduce the total run time.The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. The analytical method was found linearity over the range 60-140µg/ml of Azelnidipineand 100-500µg/ml of Olmesartan Medoxomil of the target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

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