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Research Article

Simultaneous Determination of Azelnidipine and Olmesartan Medoxomil in Pharmaceutical Dosage Forms by UFLC Method

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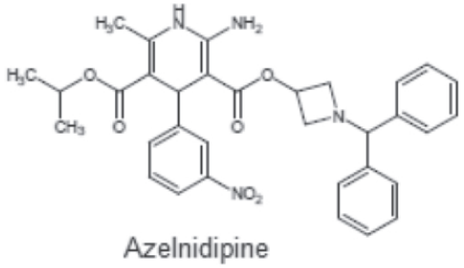
Abstract

A simple, precise, sensitive and rapid reversed phase Ultra-Fast liquid chromatography method was developed for simultaneous determination of azelnidipine and olmesartan medoxomil in Pharmaceutical Dosage form with greater precision and accuracy has been developed and validated. The chromatographic separation was achieved by using Phenomenex, Prodigy, ODS3, 5 μm, 100 Å, (250 x 4.6 mm) analytical column with a mobile phase consisting of methanol and water at the ratio of (85:15% v/v). The chromatographic condition was set at a flow rate of 1.5 ml/min, column oven temperature 25°C and detector wavelength of 255 nm using a photodiode array detector. An injection volume of 10 μl was used for azelnidipine and olmesartan medoxomil. The calibration curve of azelnidipine was linear with correlation coefficient (r^2) = 0.9999; over a concentration range of 1.0 - 60.0 μg/ml for; with a retention time of 6.80 min. While the calibration curve of olmesartan medoxomil was linear with correlation coefficient (r^2) = 0.9998; over a concentration range of 1.0 - 60.0 μg/ml for; with a retention time of 1.72 min. The recovery level of azelnidipine and olmesartan medoxomil was 99.62% and 100.12%; respectively. The validated UFLC method was successfully applied to the analysis of azelnidipine and olmesartan medoxomil in pharmaceutical dosage form.

Keywords: UFLC, azelnidipine, olmesartan medoxomil, method validation, pharmaceutical dosage forms.

Introduction

Azelnidipine (AZL), (±)-3-[1-(diphenylmethyl) azetidin-3-yl] 5-propan-2-yl 2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate as shown in Figure 1, is a new dihydropyridine derivative with calcium antagonistic activity [1].



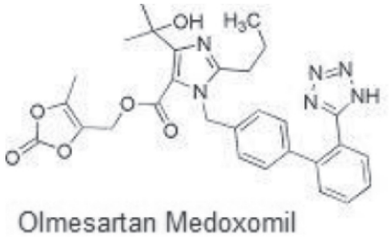


Figure 1: Chemical structure for azelnidipine and olmesartan medoxomil

The recommended dosing of Azelnidipine is 16 mg per day. A literature survey revealed that Azelnidipine is not yet official in any pharmacopoeia. Very few analytical methods have been reported for the determination of Azelnidipine includes HPLC [2-3], LC-MS method [4-5], LC-ESI-MS [6-7], HPLC-MS-MS [8], in which two methods for formulation and remaining for human plasma. Olmesartan medoxomil (OLM), is (5-methyl-2-oxo-1,3-dioxol-4-yl) methyl 4-(2-hydroxy-propan-2-yl)-2-propyl-1-{[2'-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl}-1H-imidazole-5-carboxylate as shown in Figure 1. A literature survey revealed that Olmesartan is not yet official in any pharmacopoeia. Several analytical methods have been reported for the determination of Olmesartan medoxomil in biological fluids, which includes LC-MS-MS [9], degradation product HPLC [10], HPTLC [11] and HPLC with dissolution study [12].

Several clinical trials prove that Olmesartan medoxomil and Azelnidipine gives better therapeutic effect in essential hypertension rather than in single dosage form [13]. There was only one first derivative spectrophotometric method reported for simultaneous analysis [14] and HPLC simultaneous analysis [15].

Materials and Methods

Materials

All chemicals and reagents used were HPLC grade. Pure standards of azelnidipine, Zhejiang Gaobang Pharmaceutical Co. Ltd., and olmesartan medoxomil Qilu Tianhe Pharmaceutical Co. Ltd., were obtained from Chinese. HPLC grade Methanol was purchased from Romil. Water for chromatography was purchased from Merck, Germany.

Chromatographic conditions

The analysis of drugs was carried out on a Shimadzu LC-20 XR, prominence, equipped with an auto sampler (SIL-20AC XR, Shimadzu, Japan) and PDA detector (SPD- M20A, Japan) was used for the analysis. The data was recorded using LC-solution software. A Phenomenex, Prodigy, ODS3, (250mm x 4.6mm, 5µm) column was used for the analysis. A NSXX sonics ultrasonic bath (NS-A-12-7H, Germany) was used for degassing of the mobile phase.

In this UFLC method separation was carried out using a mobile phase

consisting of HPLC grade methanol and water at the ratio of (85:15% v/v). The mobile phase was filtered by using a 0.45 μm nylon membrane filter. The column was maintained at a temperature of 25ºC with column oven (CTO-20AC) and the flow rate was 1.5 ml/min. Analysis was performed with injection volume of 10 µl using PDA detection at 255 nm. The run time was set for 8.0 min. The optimized chromatographic condition is shown in Table 1.

Table 1: Optimized chromatographic conditions

Parameters	Conditions
Stationary Phase	Prodigy, ODS3, 250 x 4.6 mm, 5 μm
Mobile Phase	Methanol and Water (85:15 v/v)
Flow Rate (ml/min)	1.5
Run Time (min)	8.0
Column Temperature (ºC)	Ambient (25ºC)
Injection Volume (μl)	10
Detection Wavelength (nm)	255nm
Retention Time of Azelnidipine (min)	6.80
Retention Time of Olmesartan (min)	1.72

Preparation of stock and working standard solution

A 10 mg of azelnidipine and A 10 mg of olmesartan medoxomil working standard were weighed and transferred into a 100 ml volumetric flask. 85 ml of the methanol was added and shake on vortex for 2 min; then was sonicated for 10 minutes. Working standard solutions were prepared and further diluted in methanol to contain a mixture of azelnidipine and olmesartan medoxomil in over the linearity range from 1.0 - 60.0 μg/ml and 1.0 - 60.0 μg/ml respectively.

Method validation

The present method of analysis was validated according to the recommendations of ICH- 1996 and USP-30 for the parameters like specificity, system suitability, accuracy, linearity and precision.

Specificity

It provides an indication of the selectivity and specificity of the procedure. The method is to be selective, if the main peak is well resoluted from any other peak by resolution of minimum 2. This could be done injecting placebo and compare it with that of standard and placebo spiked with standard and sample, then peak purity was ascertained by use of PDA.

System suitability

System suitability was performed by injecting six replicates of standard solution at 100% of the test condition at a 100% level to verify the precision of the chromatographic system. The purposed UFLC method permits the determination of azelnidipine and olmesartan medoxomil in sample drug have different retention times. System suitability data are given in Table 2.

Table 2: System suitability parameters for azelnidipine and olmesartan medoxomil

S. No.	Parameters	Azelnidipine	Olmesartan
1	Tailing factor	1.08	0.86
2	Retention Time	6.80	1.72
3	Theoretical plates	7044	839

Linearity

Is defined by the correlation coefficient, which should be found NLT 0.99, using peak area responses, Linearity for single point standardization should extend to at least 20% beyond the specification range and include the target Conc. This was performed by preparing 7 different concentrations (2.5%, 5%, 25%, 50%, 100%, 125% and 150%), and then making 3 replicates of each concentration. The linear working range was determined from the constructed standard calibration curve.

Intraday Precision

This study was conducted by performing multiple analyses on a suitable number of portions of a homogeneous sample. This was performed by assaying multiple aliquots with the same concentration. The analytical precision of the method was determined by the relative standard deviation.

Inter-day Reproducibility (Method Ruggedness)

The degree of reproducibility determined by analysis of samples from homogeneous lot of materials, under different but typical test conditions The method is to be rugged, at any item if the pooled %RSD of the total number of replicates that have been made in this item is within the acceptance criteria, 3 replicates of a single sample of powder material are used for each determination. First day: 3 replicates, on a second day: 3 replicates, then on third day: 3 replicates of freshly prepared test from the same sample are analyzed, under the same conditions.

Accuracy

Accuracy was evaluated by spiking standard with sample solution. The measurements are made at a concentration of standard mix, which is found to be the target concentration, and at suitable intervals around this point. The test samples was spiked with known quantities of standard azelnidipine and olmesartan medoxomil using three determinations over three concentrations level covering the specified range. Relative recoveries of standard azelnidipine and olmesartan medoxomil used in the standards were evaluated by comparing their peak area with those obtained from the calibration curve equation.

Stability of Analytical solution

The stability of analytical solutions was established by injecting the

standard solution and sample solution at different time intervals up to 24 h (0, 12, and 24 h) by keeping the auto sampler temperature at room temperature (25ºC). The % differences of peak area of standard solution and sample solution that were injected at periodic intervals found to be the specified limit. The values are presented in the Table 3 and Table 4.

Table 3: Stability of standard and sample solution of azelnidipine

Time Interval (h)	Standard		Sample	
	Standard Peak area	% Difference	Sample Peak area	% Difference
0	841726	-	841423	-
12	841683	0.01	841375	0.01
24	841662	0.01	841303	0.01

Table 4: Stability of standard and sample solution of olmesartan medoxomil

Time Interval (h)	Standard		Sample	
	Standard Peak area	% Difference	Sample Peak area	% Difference
0	1277723	-	1277136	-
12	1277526	0.02	1276921	0.02
24	1277397	0.03	1276858	0.02

Results and Discussion

The proposed UFLC method required fewer reagents and materials, and it is simple and less time consuming. This method could be used in quality control test in pharmaceutical industries. The chromatogram of azelnidipine and olmesartan medoxomil was shown in Figure 2. There was clear resolution between azelnidipine and olmesartan medoxomil with retention time of 6.80 and 1.72 minutes; respectively.

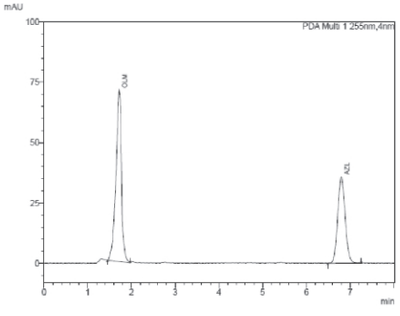


Figure 2: A typical chromatogram for azelnidipine and olmesartan medoxomil standard drug

Specificity

Generally, the specificity of a method is its suitability for the analysis of a compound in the presence of potential impurities. Placebo, standards, and sample test solutions were all injected at the same wavelength of 255 nm to demonstrate the specificity of the optimized method. A comparison of the retention times of azelnidipine and olmesartan medoxomil in sample solutions and in the standard solutions were exactly the same. Figures 2, 3 and 4 showed that there were no interferences at the retention times for azelnidipine and olmesartan medoxomil due to the placebo.

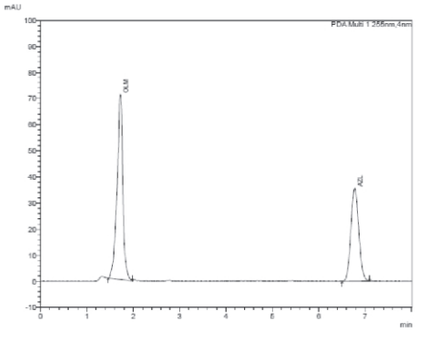


Figure 3: A typical chromatogram for azelnidipine and olmesartan medoxomil sample drug

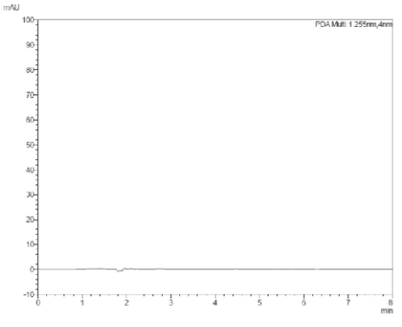


Figure 4: UFLC chromatogram of placebo

Therefore, the proposed method is suitable for the quantification of the active ingredients in tablet formulation.

Linearity

The response for the detector was determined to be linear over the range of 1.0-60.0 μg/ml (1.0, 2.0, 10.0, 20.0, 40.0, 50.0 and 60.0) for azelnidipine as shown in Figure 5 and data are shown in Table 5. The response for the detector was determined to be linear over the range

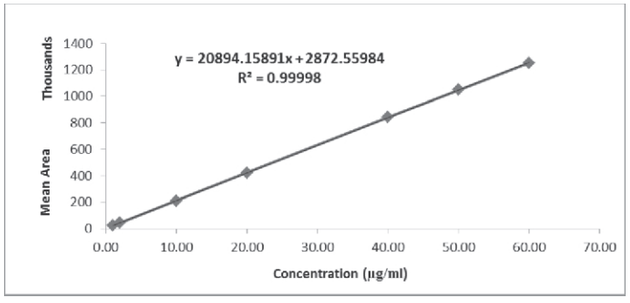


Figure 5: Calibration curve of azelnidipine

Table 5: Statistical data of calibration curves of azelnidipine

S. No.	% test Concentration	Concentration (μg/ml)	Average Peak area
1	2.5	1	23094
2	5	2	44592
3	25	10	210639
4	50	20	421299
5	100	40	841858
6	125	50	1049211
7	150	60	1253045
Regression co-efficient = 0.9999			

of 1.0-60.0 μg/ml (1.0, 2.0, 10.0, 20.0, 40.0, 50.0 and 60.0) for olmesartan medoxomil as shown in Figure 6 and data are shown in Table 6.

Accuracy

Accuracy was calculated by addition of standard drugs to preanalyzed sample at 3 different concentration levels (50%, 100% and 150%) and computing percentage recoveries. Standard limit of % recovery study is 98 - 102 % as per ICH guideline. From the studies it was concluded that % recovery study of azelnidipine and olmesartan medoxomil complies with standard limit of ICH guideline. Results of accuracy were proven by the Table 7 and % RSD is 0.313 and 0.253 of

Each of the concentrations was injected in triplicate to get reproducible response. Calibration curves were constructed by plotting peak area versus concentration.

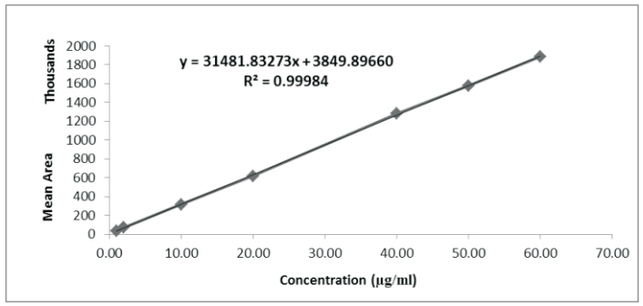


Figure 6: Calibration curve of olmesartan medoxomil.

Each reading was average of three determinations. They were represented by the linear regression equation.

$Y_{Azelnidipine} = 20894.15891x + 2872.55984, r^2 = 0.9999$

$Y_{Olmesartan\ medoxomil} = 31481.83273x + 3849.89660, r^2 = 0.9998$

Slopes and intercepts were obtained by using regression equation ($Y = mx + c$) and least square treatment of the results used to confirm linearity of the method developed.

Table 6: Statistical data of calibration curves of olmesartan medoxomil

S. No.	% test Concentration	Concentration (μg/ml)	Average Peak area
1	2.5	1	37007
2	5	2	71212
3	25	10	317174
4	50	20	618424
5	100	40	1278919
6	125	50	1578673
7	150	60	1886715
Regression co-efficient = 0.9998			

Table 7: Results of accuracy for azelnidipine and olmesartan medoxomil

Level (%)	Azelnidipine			Olmesartan		
	Amount of drug spiked (mg)	Found (mg)	Recovery (%) (n=3)	Amount of drug spiked (mg)	Found (mg)	Recovery (%) (n=3)
50	2.96	2.94	99.29	2.88	2.89	100.35
100	5.92	5.90	99.66	5.76	5.77	100.17
150	8.88	8.87	99.91	8.64	8.63	99.85
	Average Recovery		99.62	Average Recovery		100.12
	SD		0.312	SD		0.253
	% RSD		0.313	% RSD		0.253

azelnidipine and olmesartan medoxomil respectively which is within the acceptable limit (less than 2.0).

Inter-day Precision

Solution containing 40 µg/ml and 40 µg/ml of azelnidipine and olmesartan medoxomil was prepared from their respective standard stock solution. Analysis was replicated for 3 different days. The result of inter-day precision studies was shown in Table 8.

Table 8: Inter -day precision data of azelnidipine and olmesartan medoxomil

Sample ID	Assay (% labeled amount)					
	Azelnidipine			Olmesartan		
	(Day 1)	(Day 2)	(Day 3)	(Day 1)	(Day 2)	(Day 3)
Sample-1	99.23	99.44	98.88	99.12	99.62	98.98
Sample-2	99.22	99.36	99.14	99.18	99.23	99.13
Sample-3	98.62	100.11	99.61	99.88	100.06	99.53
Sample-4	100.02	100.15	99.55	98.45	99.65	99.41
Sample-5	98.99	99.97	98.77	98.96	98.98	98.74
Sample-6	99.22	99.45	99.31	99.33	99.45	99.42
Average	99.22	99.75	99.21	99.15	99.50	99.20
SD	0.459	0.368	0.344	0.468	0.373	0.305
% RSD	0.463	0.369	0.347	0.472	0.375	0.308

Inter-day Reproducibility (Method Ruggedness)

Three replicates of a single sample of powder material are used for each determination. First day: 3 replicates, on a second day: 3 replicates, then on third day: 3 replicates of freshly prepared test from the same sample are analyzed, under the same conditions. The result of inter-day reproducibility studies was shown in Table 9.

Table 9: Inter-day reproducibility data of azelnidipine and olmesartan medoxomil

Sample ID	Assay (% labeled amount)					
	Azelnidipine			Olmesartan		
	(Day 1)	(Day 2)	(Day 3)	(Day 1)	(Day 2)	(Day 3)
Sample-1	99.44	99.56	100.12	99.56	98.86	98.89
Sample-2	99.26	98.36	99.56	99.49	99.46	98.78
Sample-3	99.58	99.26	99.72	98.66	98.60	98.58
Average	99.43	99.06	99.80	99.24	98.97	98.75
SD	0.160	0.624	0.288	0.501	0.441	0.157
% RSD	0.161	0.630	0.289	0.504	0.446	0.159

Quantification limit

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method was determined by injecting progressively low concentrations of the standard solutions using the developed methods. The LOD is the lowest concentration of the analyte that can be detected with signal to noise ratio (3:1) and LOQ is the lowest concentration that can be quantified with acceptable precision and accuracy with signal to noise ratio (10:1). The LOD of azelnidipine and olmesartan medoxomil found to be 0.167 μg/ml and 0.170 μg/ml respectively. The LOQ of azelnidipine and olmesartan medoxomil found to be 0.50 μg/ml and 0.51 μg/ml respectively.

Stability of analytical solution

In this study, the mobile phases, the standard solutions, and the sample solution were subjected to long term (24 h) stability studies. The stability of these solutions was studied by performing the experiment and looking for changes in separation, retention, and asymmetry of the peaks which were then compared with the pattern of the chromatogram of freshly prepared solutions

System suitability

The system suitability was determined by injecting six replicates of the standard solutions and analyzing each active ingredient for its peak area, peak tailing factor, resolution, number of theoretical plates, and capacity factor. The values obtained demonstrated the suitability of the system for the analysis of the above drug combinations System suitability parameters might be fall within $\pm$2% standard deviation range during routine performance of the methods.

Conclusion

The validated UFLC method developed for the quantitative quality control determination of azelnidipine and olmesartan medoxomil in combination was evaluated for system suitability, specificity,

linearity, range, accuracy (recovery), precision (repeatability and intermediate precision). This method enables simultaneous determination of azelnidipine and olmesartan medoxomil because of good separation and resolution of the chromatographic peaks. As a result, the proposed UFLC method could be adopted for the quantitative quality control and routine analysis of tablet dosage form.

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Conflict of Interest: The authors confirm that this article content has no conflict of interest.

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