Journal of PharmaSciTech
ISSN: 2231 3788 (Print)
2321 4376 (Online)
Research Article

Stability Indicating Ultra-Fast Liquid Chromatography Coupled with Photodiode Array Method for Simultaneous Estimation of Azelnidipine and Olmesartan Medoxomil in Pharmaceutical Formulations

Mohamed A. Amin [1,*], Alaa S. Amin [2], Mounir Z. Saad [3]

[1] *Mepaco Pharmaceutical Company, Sheraton Heliopolis, Cairo 11361, Egypt*
[2] *Department of Chemistry, Benha University, Benha 13511, Egypt*
[3] *Department of Chemistry, Zagazig University, Zagazig 44519, Egypt*

Correspondence: alichem2005@yahoo.com (Tel.: 002/01009560368)

Article Info:
Received: 31.03.2019
Accepted: 07.09.2019
Available online: 01.9.2020

https://doi.org/10.33981/JPST.2020.v09i01.002

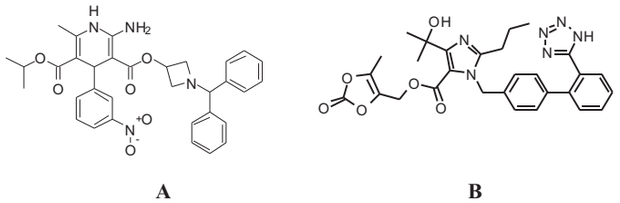
Abstract

A simple, rapid, sensitive, specific, precise and accurate stability indicating Ultra-Fast liquid chromatography (UFLC) method was developed for simultaneous determination of Azelnidipine and Olmesartan medoxomil in pharmaceutical formulations. The chromatographic separation was conducted on Shimadzu (Prominence LC 20 UFLC XR) connected with photodiode array (PDA) detector; using column ACE, C18/CN (100 x 4.6 mm, 5 μm) as stationary phase with mobile phase was isocratic consisted of methanol and 0.1% Phosphoric acid 85% in the ratio of (60:40 v/v) at flow rate of 1.5 mL min^{-1}. An injection volume of 20 μL was used for Azelnidipine and Olmesartan medoxomil. The detection wavelength (λ max) was 255 nm using a diode array detector. Linearity of the method was established over the concentration ranges of 2.0 – 60.0 μg mL-1 for Azelnidipine with a retention time of 4.30 minutes and 2.0 – 60.0 μg mL^{-1} for Olmesartan medoxomil with a retention time of 1.21 minutes (correlation coefficients greater than 0.999). The recovery level of Azelnidipine and Olmesartan medoxomil were 99.48% and 99.40%; respectively. The relative standard deviation (RSD) was found to be < 2. The method can therefore be considered as stability-indicating and can be used successfully for simultaneous determination of Azelnidipine and Olmesartan medoxomil in pharmaceutical formulations.

Keywords: UFLC, Azelnidipine, Olmesartan medoxomil, PDA detector, Method validation.

Introduction

Azelnidipine is (±)-3-[1-(diphenylmethyl) azetidin-3-yl] 5-propan-2-yl 2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (Figure 1). Antihypertensive effects of CS-905, a novel dihydropyridine Ca2+ channel blocker [1]. 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 [2-4], LC-MS method [5-6], LC-ESI-MS [7-[8], HPLC-MS-MS [9], in which two methods for formulation and remaining for human plasma.



A B

Figure 1: Chemical structures of azelnidipine (A) and olmesartan medoxomil (B).

Olmesartan medoxomil (Figure 1) 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-carb-oxylate. 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; [10], MS spectroscopy [11], degradation product HPLC [12], HPTLC [13], HPLC with dissolution study [14] and few UV-Visible methods [15-17] alone or in combination [18] with other dosage form.

Several clinical trials prove that Olmesartan medoxomil and Azelnidipine gives better therapeutic effect in essential hypertension rather than in single dosage form [19]. There was only one first derivative spectrophotometric method reported for simultaneous analysis [20], HPLC simultaneous analysis [21] and UFLC simultaneous analysis [22]. Low chromatographic method published for the combination dosage form. So, the present work was to develop a simple, rapid, sensitive, and cost-effective UFLC method for routine analysis. The proposed method was validated according to ICH guidelines [23].

Materials and Methods

Chemicals and reagents

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 Fisher Chemical (UK). Ortho-Phosphoric acid 85% was HPLC grade from Fluka chemicals (Germany). HPLC grade Acetonitrile from Romil (England). Water for chromatography was purchased from Merck (Germany). Mobile phase was filtered using 0.45 μm nylon membrane filter (UK).

Equipment and chromatographic conditions

The analysis of drugs was carried out on a Shimadzu LC-20 XR, prominence (Kyoto, Japan) is equipped with an auto sampler (SIL-20AC XR, Shimadzu, Japan) and PDA detector (SPD- M20A, Japan) was used for the analysis. Peak areas were integrated using a Shimadzu LC solution (version 5.41.240) software program. The data was recorded using LC-solution software. A NSXX sonics ultrasonic bath (NS-A-12-7H, Germany) was used for degassing of the mobile phase.

Experimental conditions were optimized on a ACE C18/CN column (100 x 4.6 mm, 5 μm) at a temperature of 25ºC with column oven (CTO-20AC) and the flow rate of the mobile phase was 1.5 ml min-1.

The mobile phase was consisting of HPLC grade methanol and 0.1% phosphoric acid 85% in the ratio of 60:40 v/v, respectively. Analysis was performed with injection volume of 20 µL using PDA detection at 255 nm. The run time was set for 6.0 min. The optimized chromatographic condition is shown in Table 1.

Table 1: Optimized chromatographic conditions

Parameters	Conditions
Stationary phase	ACE, C18/CN, 100 x 4.6 mm, 5 μm
Mobile phase	Methanol and 0.1% H_3PO_4 (60:40 v/v)
Flow rate (mL min-1)	1.5
Run time (min)	6.0
Column temperature (^{0}C)	Ambient (25 ^{0}C)
Injection volume (μL)	20
Detection wavelength (nm)	255nm
Retention time of azelnidipine (min)	4.30
Retention time of olmesartan (min)	1.21

Preparation of standard stock and standard solution

Standard stock solutions of azelnidipine and olmesartan medoxomil were prepared by accurately weighing 10 mg azelnidipine and 10 mg olmesartan medoxomil working standard were weighed and transferred into a 100 ml volumetric flask. 70 ml of the acetonitrile and water at the ratio of (1:1% v/v) was added and shake on vortex for 2 min; then was sonicated for 5 minutes. Working standard solutions were prepared and further diluted in acetonitrile and water at the ratio of (1:1% v/v) to contain a mixture of azelnidipine and olmesartan medoxomil in over the linearity range from 2.0 – 60.0 μg mL-1 and 2.0 – 60.0 μg mL-1 respectively. The solution was filtered through a 0.45 μm nylon filter before analysis.

Linearity

Linear calibration plots of the proposed method were obtained over concentration ranges of 2.0-60.0 μg mL-1 (2.0, 10.0, 20.0, 40.0, 50.0 and 60.0 μg mL-1) for azelnidipine and 2.0-60.0 μg mL-1 olmesartan medoxomil (2.0, 10.0, 20.0, 40.0, 50.0 and 60.0 μg mL-1). Each solution was prepared in triplicate.

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.

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	1.03	1.16
2	Retention time	4.30	1.21
3	Theoretical plates	1042	722

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

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.

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 (25 ^{o}C). 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.

Table 3: Stability of standard and sample solution of azelnidipine and olmesartan medoxomil

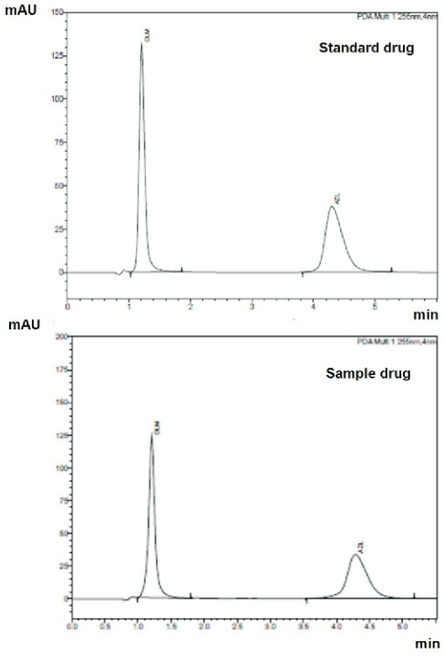
Time interval (h)	Azelnidipine				Olmesartan			
	Standard		Sample		Standard		Sample	
	Peak area	% Difference	Peak area	% Difference	Peak area	% Difference	Peak area	% Difference
0	1588659	-	1587895	-	1750236	-	1751256	-
12	1587993	0.04	1587456	0.03	1750026	0.01	1750598	0.04
24	1587723	0.06	1587036	0.05	1749963	0.02	1750922	0.02

Limit of detection (LOD) and limit of quantitation (LOQ)

Detection and quantification limits were determined by the signal-to-noise (S/N) approach. In order to examine the limit of quantitation and limit of detection solutions of different concentrations were prepared by spiking known amounts of azelnidipine and olmesartan medoxomil. Each solution was prepared according to the defined protocol and analysed repeatedly to determine the S/N ratio. The average S/N ratio from all the analyses at each concentration level was used to calculate the limit of quantitation and limit of detection. The concentration level that gives an S/N ratio of 10:1 at which analytes can be readily quantified with accuracy and precision was reported as the limit of quantitation. The concentration level that gives an S/N ratio of 3:1 at which analytes can be readily detected was reported as the limit of detection.

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.



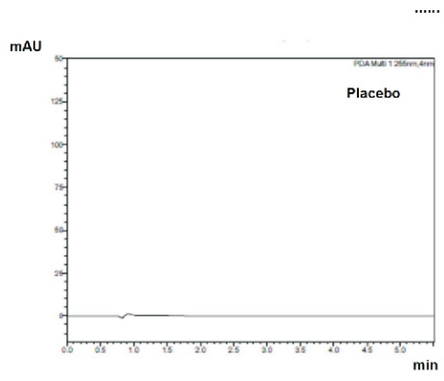


Figure 2: A typical chromatogram for azelnidipine and olmesartan medoxomil (standard drug, sample drug and placebo).

There was clear resolution between azelnidipine and olmesartan medoxomil with retention time of 4.30 and 1.21 minutes; respectively. The developed chromatographic method was validated using ICH guidelines [23]. Validation parameters include linearity, accuracy, precision, robustness, specificity, limit of detection and quantitation.

Linear calibration plots for the proposed method were obtained in concentration ranges of 2.0-60.0 μg mL^{-1} (2.0, 10.0, 20.0, 40.0, 50.0 and 60.0 μg mL^{-1}) for azelnidipine as shown in Figure 3 and data are shown in Table 4 and 2.0-60.0 μg mL^{-1} olmesartan

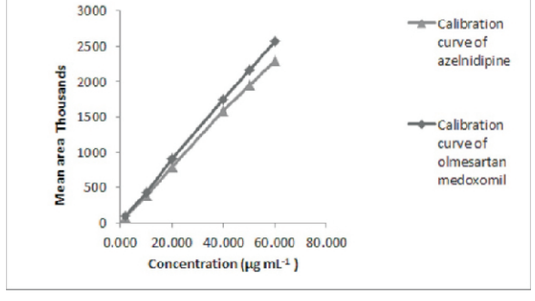


Figure 3: Calibration curve of azelnidipine and olmesartan medoxomil.

Table 4: Statistical data of calibration curves of azelnidipine and olmesartan medoxomil

S. No	% test Concentration		Concentration (μg mL-1)		Average peak area	
	Azelnidipine	Olmesartan	Azelnidipine	Olmesartan	Azelnidipine	Olmesartan
1	5	5	2	2	76705	90807
2	25	25	10	10	390321	435428
3	50	50	20	20	785694	908160
4	100	100	40	40	1585150	1750145
5	125	125	50	50	1944125	2157840
6	150	150	60	60	2290374	2567566
Regression co-efficient (azelnidipine) = 0.9994						
Regression co-efficient (olmesartan) = 0.9996						

medoxomil (2.0, 10.0, 20.0, 40.0, 50.0 and 60.0 μg mL-1) as shown in Figure 3 and data are shown in Table 4.

Each of the concentrations was injected in triplicate to get reproducible response. Calibration curves were constructed by plotting peak area versus concentration. Each reading was average of three determinations. They were represented by the linear regression equation.

$$Y_{\text{Azelnidipine}} = 38488.2012x + 11252.6759,\ r^2 = 0.9994$$

$$Y_{\text{Olmesartan medoxomil}} = 42751.2322x + 21536.7339,\ r^2 = 0.9996$$

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.

The limit of detection (LOD) and quantitation (LOQ) were determined by making serial dilutions. LOD was found to be 0.334 μg mL^{-1} and 0.330 μg mL^{-1} for azelnidipine and olmesartan medoxomil, respectively (signal to noise ratio of 3:1). LOQ was found to be 1.0 μg mL^{-1} and 0.99 μg mL^{-1} for azelnidipine and olmesartan medoxomil, respectively (signal to noise ratio of 10:1).

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 5 and % RSD is 0.110 and 0.373 of azelnidipine and olmesartan medoxomil respectively which is within the acceptable limit (less than 2.0). Recovery studies showed the method to be highly accurate and suitable for intended use.

Table 5: 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	3.12	3.10	99.36	3.18	3.16	99.37
100	6.25	6.22	99.52	6.28	6.22	99.04
150	9.33	9.29	99.57	9.30	9.28	99.78
	Average recovery		99.48	Average recovery		99.40
	SD		0.110	SD		0.371
	% RSD		0.110	% RSD		0.373

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. Figure 2 showed that there were no interferences at the retention times for azelnidipine and olmesartan medoxomil due to the placebo. Therefore, the proposed method is suitable for the quantification of the active ingredients in tablet formulation.

Inter-day Precision of the solution containing 40 μg mL^{-1} and 40 μg mL^{-1} 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 6.

Table 6: 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	99.88	99.25	99.43	98.24	99.42	98.77
Sample-2	99.75	99.14	99.99	99.52	99.36	99.62
Sample-3	99.62	100.09	99.47	98.88	99.96	99.55
Sample-4	98.79	99.55	98.55	99.87	99.24	100.13
Sample-5	100.09	99.89	98.65	100.11	98.99	98.98
Sample-6	99.39	98.44	99.92	100.21	99.25	99.24
Average	99.59	99.39	99.34	99.47	99.37	99.38
SD	0.456	0.592	0.614	0.772	0.324	0.490
% RSD	0.458	0.595	0.618	0.776	0.327	0.493

Inter-day Reproducibility 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 7.

Table 7: 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	100.15	99.47	98.81	99.78	98.77	98.73
Sample-2	99.88	99.65	99.11	99.82	99.86	99.21
Sample-3	99.74	99.85	99.14	99.24	99.69	98.50
Average	99.92	99.66	99.02	99.61	99.44	98.81
SD	0.208	0.190	0.182	0.324	0.586	0.362
% RSD	0.209	0.191	0.184	0.325	0.590	0.367

Stability of analytical solution 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 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 ±2% standard deviation range during routine performance of the methods.

Conclusion

A simple, sensitive, fast, isocratic and accurate UFLC method is described for simultaneous determination of azelnidipine and olmesartan medoxomil in pharmaceutical formulations and 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. All these convince us to conclude that the method can be successfully used for any sort of stability and validation studies.

Acknowledgement

The authors are grateful to Mepaco-Medifood Pharmaceutical Company (El Sharkia, Egypt) for its ultimate support for research and to the development team.

Conflict of Interest

There is no competing interest in connection to the work discussed above.

References

[1] Oizumi K, Nishino H, Koike H, Sada T, Miyamoto M, Kimura T. Antihypertensive effects of CS-905, a novel dihydropyridine Ca2+ channel blocker. Jpn J Pharm 1989; 51:57-64.

[2] An H-M, Wang J-C. Determination of content and related substances of azelnidipine by HPLC. West China J Pharm Sci 2006; 06:581–582.

[3] Pan YF, Zhang JB, Ding J, Wang TM. Determination of azelnidipine tablets by HPLC. Qilu Pharm Affairs 2008; 07:398–399.

[4] Megha GG, Pratap SD. RP-HPLC method development and validation of azelnidipine. Int J Pharm Sci Res 2016; 07:5111–5114.

[5] Kawabata K, Urasaki Y. Simultaneous determination of azelnidipine and two metabolites in human plasma using liquid chromatography-tandem mass spectrometry. J Chromatogr B 2006; 844:45–52.

[6] Kawabata K, Samata N, Urasaki Y, Fukazawa I, Uchida N, Uchida E, Yasuhara Y. Enantioselective determination of azelnidipine in human plasma using liquid chromatography-tandem mass spectrometry. J Chromatogr B 2007; 852:389-97.

[7] Zou J-J, Ji H-J, Zhou X-H, Zhu Y-B, Fan H-W, Xiao D-W, Hu Q. Determination of azelnidipine by LC-ESI-MS and its application to a pharmacokinetic study in healthy Chinese volunteers. Pharmazie 2008; 63:568-70.

[8] Ding L, Li L, Ma P. Determination of azelnidipine in human plasma by liquid chromatography–electrospray ionization-mass spectrometry. J Pharm Biomed Anal 2007; 43:575-79.

[9] Jia J, Nan F, Liang M-Z, Yu Q, Qin Y-P, Xiang J. Determination and pharmacokinetics of azelnidipine in human plasma by HPLC-MS-MS. Chin J Hosp Pharm 2010; 24:2088-91.

[10] Vaidya VV, Shikha MN. LC - MS - MS Determination of Olmesartan in Human Plasma. Chromatographia 2008; 67:147-50.

[11] Liu D, Hu P, Matsushima N, Li X, Li L, Jiang J. Quantitative determination of olmesartan in human plasma and urine by liquid chromatography coupled to tandem mass spectrometry. J Chromatogr B 2007; 856:190-97.

12] Tomonori M, Hidetoshi K, Naoto F, Michinobu O, Takao K, Fumiyo K. Identification of a degradation product in stressed tablets of olmesartan medoxomil by the complementary use of HPLC hyphenated techniques. J Pharm Biomed Anal 2008; 47:553-59.

[13] Shah NJ, Suhagia BN, Shah RR, Patel NM. Development and validation of a simultaneous HPTLC method for the estimation of olmesartan medoxomil and hydrochlorothiazide in tablet dosage form. Indian J Pharm Sci 2007; 69:834-36.

[14] Sagirli O, Nall AO, Toker SE, Sensoy D. Simultaneous HPLC analysis of olmesartan and hydrochlorothiazide in combined tablets and in vitro dissolution studies. Chromatographia 2007; 66:213-18.

[15] Bindi NV, Rajesh RP, Pratik PN, Dushyant AS. Development and validation of the simultaneous UV. Spectrophotometric method for estimation of metaprolol succinate and olmesartan medoxomil in tablet dosage form. Pharmaceutical Methods 2010; 03:36-41.

[16] Tripti S, Si SC, Gowrishankar D. Difference spectrophotometric method for the determination of olmesartan medoxomil in tablets. J Pharmacy Res 2010; 03:1553-55.

[17] Anuradha V, Rashmita G, Rameezuddin MD, Gayatri P, Ramesh T, Vasanth PM. Development and validation of a sensitive spectrophotometric method for the estimation of olmesartan medoxomil in pharmaceutical dosage form. Int J Biolog Pharm Res 2012; 03:796-99.

[18] Celebier M, Altinoz S. Determination of Olmesartan medoxomil in tablets by UV - Visspectrophotometry. Pharmazie 2007; 64:419-22.

[19] Shimada K, Ogihara T, Saruta T, Kuramoto K, Rezalt Study Group. Effects of combination olmesartan medoxomil plus azelnidipine versus monotherapy with either agent on 24-hour ambulatory blood pressure and pulse rate in Japanese patients with essential hypertension: Additional results from the Rezalt study group. Clin Therap 2010; 32:861-81.

[20] Patel N, Patel J. Simultaneous determination of azelnidipine and olmesartan medoxomil by first derivative spectrophotometric method. Der Pharm Lett 2012; 4:1080-84.

[21] Patel N, Patel J. Validated stability-indicating RP-HPLC method for the simultaneous determination of azelnidipine and olmesartan in their combined dosage form. Sci Pharm 2014; 82:541-54.

[22] Alaa EAA, Mounir ZS, Mohamed AAA. Simultaneous determination of azelnidipine and olmesartan medoxomil in pharmaceutical dosage forms by UFLC method. J PharmaSciTech 2016; 06:69-74.

[23] I.C.H, (Q2B). Note for guidance on validation of analytical procedures: methodology. In: International Conference on Harmonization, IFPMA, Geneva 1996.