WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 17, 776-787. Research Article ISSN 2277–7105

A NOVEL UV SPECTROPHOTOMETRIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF AZELNIDIPINE AND OLMESARTAN MEDOXOMIL IN TABLET DOSAGE FORM

Mary Jiffy[1*] and Dr. Haripriya M.[2]

[1]Department of Pharmaceutical Analysis, College of Pharmaceutical Sciences, Govt. Medical College, Thiruvananthapuram, Kerala, India. 695011.

[2]Professor, Department of Pharmaceutical Analysis, College of Pharmaceutical Sciences, Govt. Medical College, Thiruvananthapuram, Kerala, India. 695011.

Article Received on
15 July 2024,

Revised on 05 August 2024,
Accepted on 26 August 2024

DOI: 10.20959/wjpr202417-33752

***Corresponding Author**
Mary Jiffy
Department of Pharmaceutical Analysis, College of Pharmaceutical Sciences, Govt. Medical College, Thiruvananthapuram, Kerala, India.695011.

ABSTRACT

An efficient, sensitive, precise, accurate, and economical Q-absorption ratio method for the quantitative analysis of Azelnidipine and Olmesartan Medoxomil in both bulk and combined dosage forms has been developed and validated. This method utilizes the ratio of absorbances at two distinct wavelengths: one at the iso-absorptive point (286 nm in methanol) and the other at the λ_{max} of Azelnidipine (256nm in methanol). The method demonstrates linearity within the concentration range of 2-18µg/mL for both drugs in methanol. It is ideal for routine quality control and has been validated through rigorous statistical analysis and recovery studies.

KEYWORDS: Azelnidipine, Olmesartan Medoxomil, Simultaneous estimation, Q-absorption ratio method, Validation.

1. INTRODUCTION

Hypertension is a pressing global health challenge, exacerbated by aging populations, poor dietary habits, and sedentary lifestyles. Significant strides in hypertension management have been made with the advent of combination therapies, now widely endorsed in treatment guidelines. Among these, combinations involving angiotensin II receptor blockers (ARBs) and calcium channel blockers (CCBs) are favored for achieving optimal blood pressure control. Azelnidipine and Olmesartan Medoxomil, a newer combination, has emerged as a potent option in effectively

managing hypertension, contributing to the expanding array of treatment choices available.[1-3]

Azelnidipine (AZEL), illustrated in Figure 1 as 3-(1-diphenylmethylazetidine-3yl)-5-isopropyl-2-amino-1,4-dihydro-6-methyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate, represents a new generation of dihydropyridine calcium channel antagonists. Known for its long-acting and lipophilic properties, AZEL achieves its antihypertensive effects by inhibiting transmembrane calcium influx, particularly targeting L-type calcium channels to induce vasodilation of vascular smooth muscle. Furthermore, AZEL has exhibited cardioprotective, neuroprotective, and lipid-lowering effects, alongside improvements in insulin resistance.[4, 5]

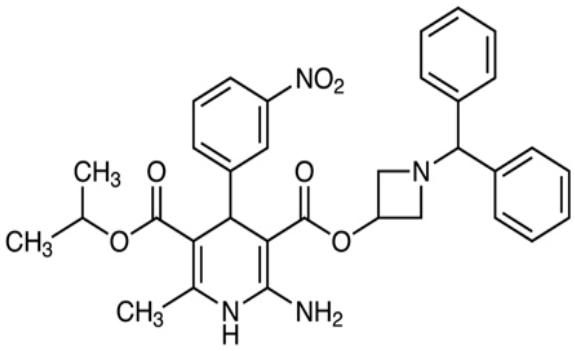

Figure 1: Structure of Azelnidipine.

Olmesartan Medoxomil (OLM), depicted in Figure 2 as (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl 4-(2-hydroxypropan-2-yl)-2-propyl-1-{[2'-(2H-tetrazol-5-yl)biphenyl-4-yl]methyl} -1H-imidazole-carboxylate, is an imidazole derivative and prodrug with potent antihypertensive properties. Upon oral administration, OLM undergoes rapid hydrolysis to its active metabolite, Olmesartan. This metabolite exerts its antihypertensive effects by reducing blood pressure through peripheral resistance and vasoconstriction. This is achieved by blocking the binding of angiotensin II to AT1 receptors (angiotensin II type 1 receptors) in vascular smooth muscle cells.[6]

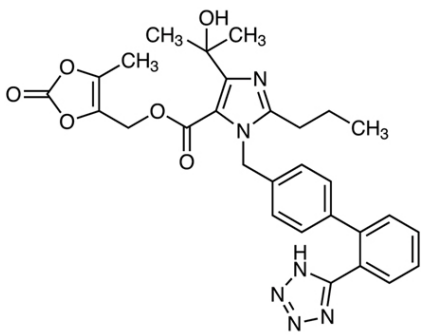

Figure 2: Olmesartan Medoxomil.

The AZEL/OLM combination, newly introduced for hypertension treatment, is proving to be a standout choice. Clinical research has emphasized its efficacy in reducing potential side effects and maintaining stable heart rates, with minimal deviation from normal values.[7,8] Thorough literature survey revealed that existing methods such as UV[9] and HPLC[10-13] are available for assessing Azelnidipine and Olmesartan Medoxomil in synthetic mixtures and oral dosage forms. Notably, there exists unique UV spectrophotometric technique, specifically the first derivative spectrophotometric method, for the determination of AZEL and OLM for synthetic mixture. The present study was prompted by the lack of a simple UV spectrophotometric method for the simultaneous analysis of these compounds in bulk and pharmaceutical formulations, other than synthetic mixture. The study resulted in the introduction of validated UV method that is both precise and accurate for the concurrent quantification of these compounds. This innovative method is poised to enhance routine analysis in pharmaceutical quality control.

2. MATERIALS AND METHODS

Instruments: Double beam spectrophotometer Hitachi UH 5300, Shimadzu ATX 224 weighing balance, Power sonic 405 sonicator was used for the spectrophotometric analysis.

Chemicals and Reagents: Chemically pure samples of Azelnidipine and Olmesartan Medoxomil were graciously provided as gift samples by SYNOKEM PHARMACEUTICALS LTD. Methanol of HPLC grade were sourced from Merck Chemicals, India. The commercial formulation containing Azelnidipine and Olmesartan Medoxomil (Olmezest-Az 20) was procured from the local market.

Method: Q-ABSORPTION RATIO METHOD

Q-Absorption ratio method uses the ratio of absorption of two drugs X and Y at two selected wavelengths; one is at iso-absorptive point and other being the λ_{max} of one of the two components. The individual concentration of X and Y was determined using the following equations.

$C_x = \{(Q_M-Q_y)/(Q_x-Q_y)\}\ (A_1/ax_1)$

$C_y = \{(Q_M-Q_x)/(Q_y-Q_x)\}(A_1/ay_1)$

$Q_M = A_2/A_1$, $Q_X = ax_2/ ax_1$ and $Q_Y = ay_2/ ay_1$; A_1 & A_2: Absorbance of mixture at λ_1 & λ_2 respectively, ax_1 & ay_1 are the absorptivities of X & Y at λ_1; ax_2& ay_2 are the absorptivities of X & Y at λ_2.[14]

3. METHODOLOGY

3.1. Preparation of solutions

3.1.1. Preparation of standard solution of Azelnidipine

Accurately weighed AZEL (10mg) and transferred to 10mL volumetric flask, containing 2mL methanol. Flask was shaken to dissolve the solid content. Volume made up to the mark with methanol, yielding solution containing 1000µg/mL of AZEL. (Stock solution A).

Using a pipette, 1mL aliquot from the solution A, was transferred into a 10 mL volumetric flask and solution was made up to the mark with methanol, yielding solution containing 100µg/mL of AZEL (working standard solution A).

3.1.2. Preparation of standard solution of Olmesartan Medoxomil

Accurately weighed OLM (10mg) and transferred to 10mL volumetric flask, containing 2mL methanol. Flask was shaken to dissolve the solid content. Volume made up to the mark with methanol, yielding solution containing 1000µg/mL of OLM.(Stock solution B).

Using a pipette, 1mL aliquots from the aforementioned stock solutions of OLM, was transferred into a 10 mL volumetric flasks and solution was made up to the mark with methanol, yielding solution containing 100µg/mL of OLM.(working standard solution B).

3.2. Determination of Iso-absorptive point and wavelength of maximum absorbance (λ_{max})

Solutions of 4µg/ mL of AZEL and 10µg/ mL of OLM and were prepared from their working standard solutions and scanned in the 230-350nm region against methanol as blank. The individual absorption spectra (Figure: 3& 4) as well as the overlain spectrum (Figure:5) were also obtained to determine wavelength of maximum absorbance and iso-absorptive point respectively.

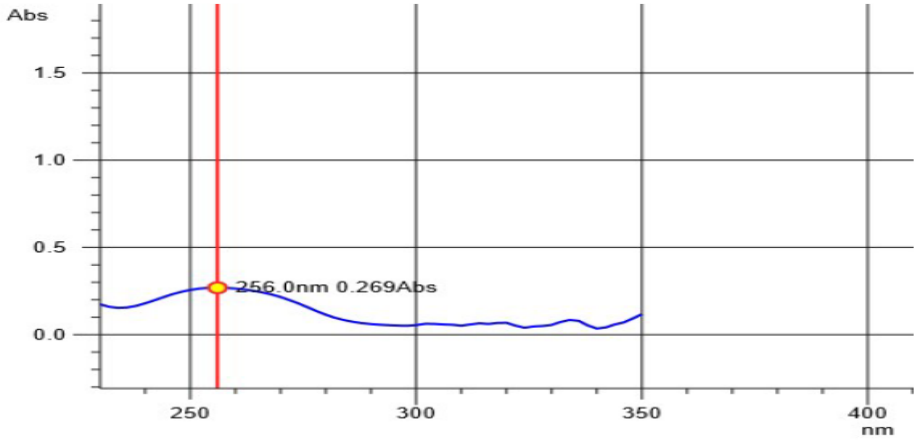

Figure 3: Absorption spectra of Azelnidipine(4µg/ mL).

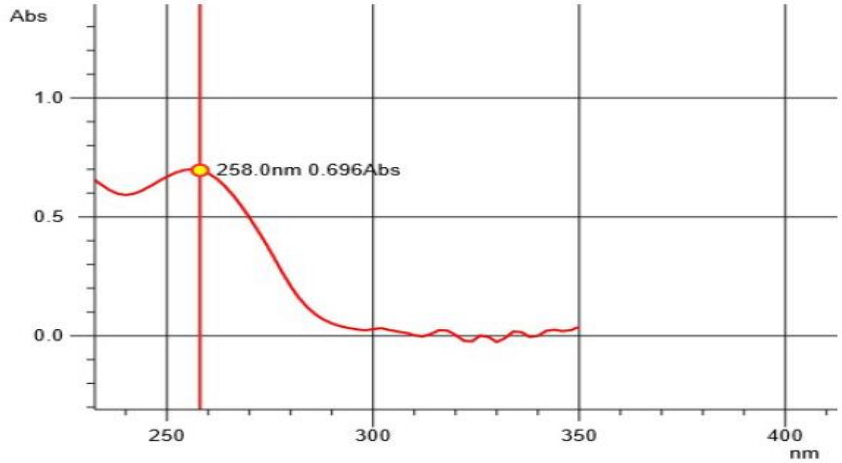

Figure 4: Absorption spectra of Olmesartan medoxomil(10µg/ mL).

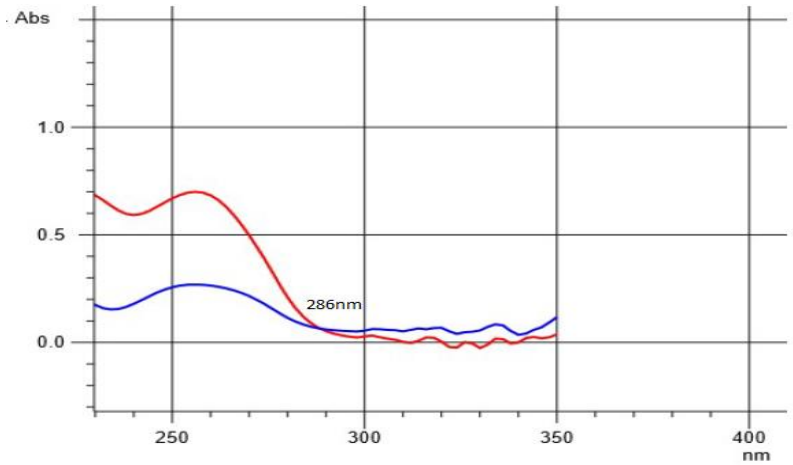

Figure 5: Overlain spectra of Azelnidipine & Olmesartan medoxomil.

3.3. Preparation of sample solution

Twenty tablets of Olmezest-Az 20, each containing 8mg of AZEL and 20mg of OLM, were accurately weighed to determine their average weight. They were finely powdered. An

amount of powder equivalent to 4mg of AZEL and 10 mg of OLM was transferred to a 10 ml volumetric flask, and 5 ml methanol was added to dissolve the substance. Sonicated for 15 minutes. The volume was then made up to 10 mL with methanol. The resulting solution was centrifuged at 800 rpm for 10 minutes and filtered through Whatman No.1. The required dilutions of filtrate were made with methanol to get final concentration 4µg/ mL of AZEL and 10µg/ mL of OLM.

3.4. Preparation of calibration curves

For the purpose of calibration, a series of standard solutions of drugs were prepared containing AZEL 2.0, 6.0, 10.0, 14.0, 18.0µg/ mL and OLM 2.0, 6.0, 10.0, 14.0, 18.0µg/ mL through the dilution of the working standard solution with methanol in standard volumetric flasks of 10mL capacity.

3.5. Determination of Azelnidipine and Olmesartan Medoxomil in a a commercial dosage form

The UV spectrophotometric method was employed to analyze the commercial tablet formulation Olmezest-Az 20.

3.6. Validation of the proposed method

The UV spectrophotometric method described was validated according to the guidelines set by the International Conference on Harmonization (ICH), ensuring conformity with criteria such as linearity, accuracy, precision, and determination of limits of detection (LOD) and quantification (LOQ).

4. RESULTS AND DISCUSSIONS

The absorption ratio method uses the ratio of absorbances at two selected wavelengths: one at the iso-absorptive point and the other at the λmax of one of the two components. According to the overlaid spectra of Azelnidipine and Olmesartan Medoxomil (shown in figure 5), they share an iso-absorptive point at 286 nm (λ_1). The second wavelength used is 256 nm(λ_2), which corresponds to the λ_{max} of Azelnidipine.

4.1. Determination of Azelnidipine and Olmesartan Medoxomil in a commercial formulation

The absorbance of the tablet mixture solution, which contained 4µg/mL of AZEL and 10µg/mL of OLM, was measured at 286 nm (iso-absorptive point) and 256 nm (λ_{max} of

AZEL). These values were substituted into the respective formula of the absorption ratio method to determine the concentrations of AZEL and OLM. The results are depicted in the table.1.

Table no. 1: compilation of outcomes from the commercial product.

Drugs	Label claim (mg/tab)	Estimated amount of drug(mg/tab)	%Amount found
AZEL	8.0	7.745	97.09
OLM	20.0	19.49	97.17

4.2. Validation of the suggested method

Linearity

Calibration curves were generated by plotting absorbance against the concentration of AZEL and OLM at 286nm((λ_1) and256nm (λ_2), and regression equations were derived. These curves encompassed five concentrations ranging from 2-18µg/mL for both AZEL and OLM, respectively (Figures 6, 7, 8 & 9.

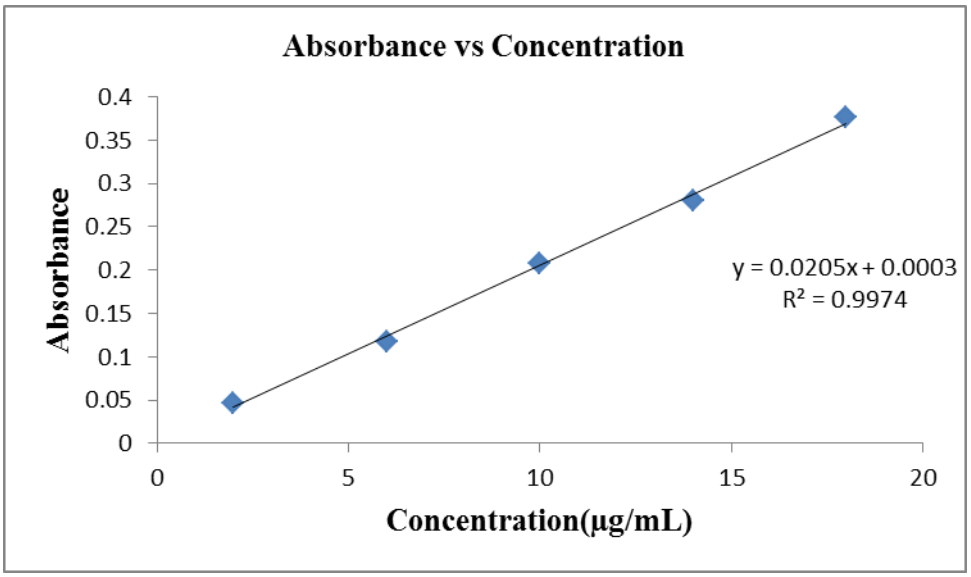

Figure No. 6: Calibration curve of AZEL at λ_1=286nm.

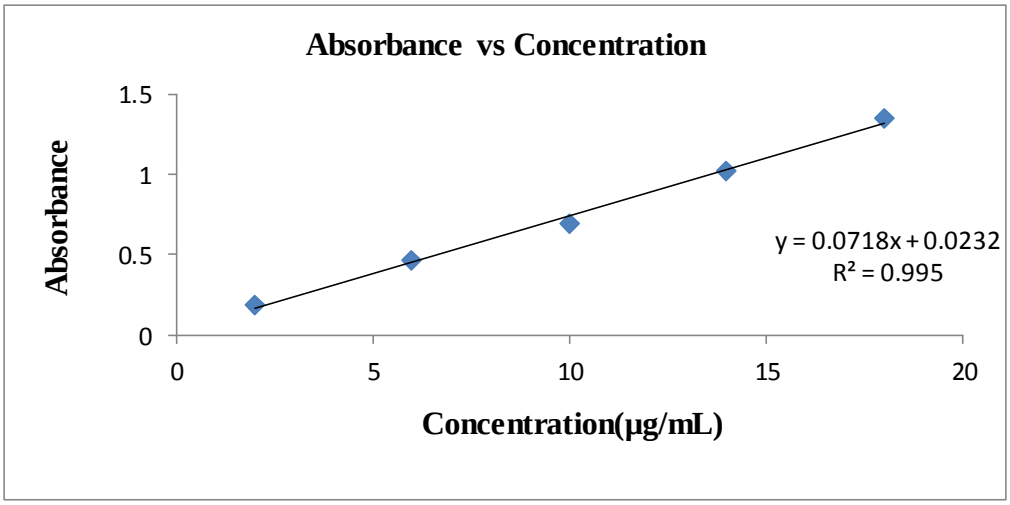

Figure No. 7: Calibration curve of AZEL at λ_2=256nm.

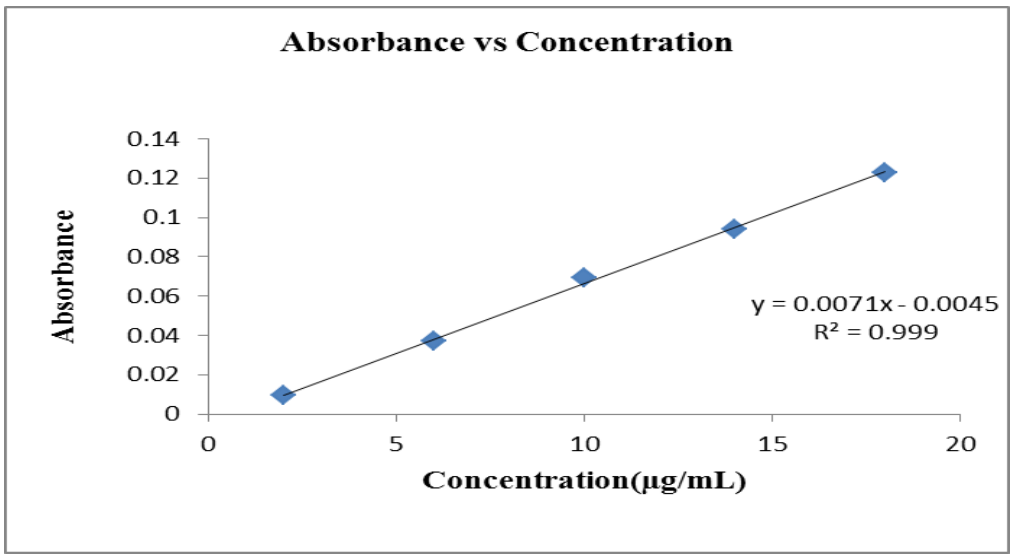

Figure No. 8: Calibration curve of OLM at λ_1=286nm.

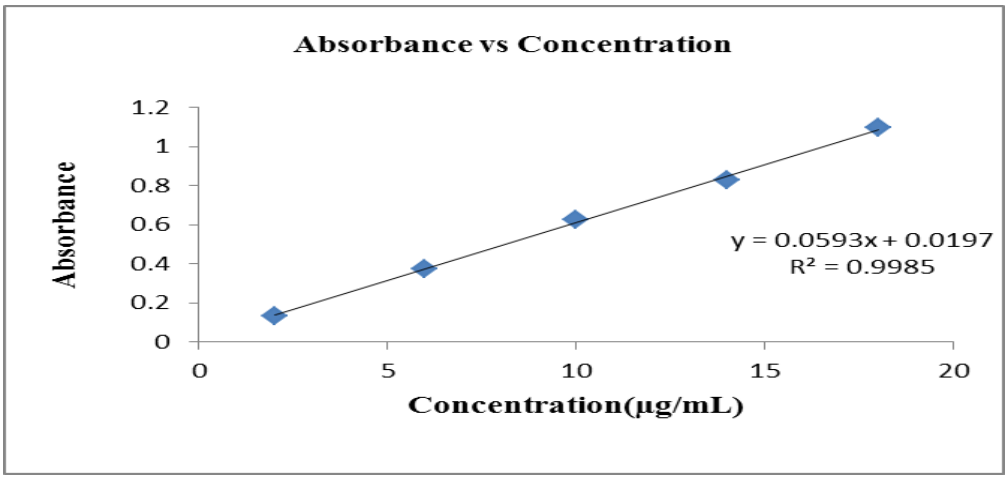

Figure No. 9: Calibration curve of OLM at λ_2=256nm.

R^2 value of AZEL at λ_1 & λ_2 was found to be 0.9974 & 0.995 respectively, and for OLM, R^2 at λ_1 and λ_2 were determined to be 0.999 and 0.9985, respectively. The results suggest a strong correlation between absorbance and the concentrations of drugs across the tested range.

ACCURACY

The accuracy of the method was assessed using standard additions at three levels. Reference standards of each drug were introduced into the sample solution at concentrations of 4µg/mL for Azelnidipine and 10µg/mL for Olmesartan Medoxomil, representing 80%, 100%, and 120% of the expected concentration levels. Each level was replicated three times, and the resulting percentage recoveries are detailed in Table 2.

Table No. 2: Accuracy studies.

Recovery level	AZEL			OLM		
	Amount present (µg/mL)	Amount added (µg/mL)	% Recovery ±RSD	Amount present (µg/mL)	Amount added (µg/mL)	% Recovery ±RSD
80%	4	3.2	98.91±0.84	10	8	99.04±0.50
100%	4	4	98.58±0.73	10	10	98.39±81
120%	4	4.8	96.87±0.93	10	12	99.50±0.35

*Average of three observations

Precision

The precision of the proposed method was evaluated through repeatability, intra-day precision, and inter-day precision. Repeatability was determined by conducting six replicates of the reference standard drug mixture solution. For assessing intermediate precision, both intra-day and inter-day precision tests were conducted by analyzing the responses of six replicates of a standard drug mixture solution containing 4 µg/mL of AZEL and 10 µg/mL of OLM. The variability in the results was expressed as the relative standard deviation (RSD). The outcomes of both repeatability and intermediate precision are delineated in the table 3,4 & 5.

Table No. 3: Repeatability.

Conc. of AZEL (µg/mL)	%RSD*	Conc. of OLM (µg/mL)	%RSD*
4	1.03	10	0.58

*Average of six observations

Table No. 4: Inter-day studies.

Day	Conc.of AZEL (µg/mL)	%RSD*
Day 1	4	0.79
Day 2	4	0.82
Day 3	4	0.79
Day	**Conc.of OLM (µg/mL)**	**%RSD***
Day 1	10	0.65
Day 2	10	0.35
Day 3	10	0.98

*Average of six observations

Table No. 5: Intra-day studies.

Drug	Concentration (µg/mL)	% RSD*
AZEL	4	1.03
Drug	**Concentration (µg/mL)**	**% RSD***
OLM	10	0.58

*Average of six observations

LOD and LOQ

Equations 1 and 2 were employed to establish the limits of detection (LOD) and quantification (LOQ) respectively.

LOD = 3.3× σ/S……..(1)

LOQ=10×σ/S…….(2) where

σ : Standard deviation of intercept S: Slope of the calibration curve.

Table No. 6: LOD & LOQ.

Parameter	AZEL (µg/mL)		OLM(µg/mL)	
	286nm	256nm	286nm	256nm
LOD	1.071	1.480	0.674	0.799
LOQ	3.247	4.484	2.04	2.422

*Average of five observations

5. CONCLUSION

The UV spectrophotometric Q absorption ratio method was created and validated for the concurrent estimation of AZEL and OLM. The findings collectively confirmed that the method is simple, accurate, precise, reproducible, and sensitive. Statistical analysis indicates

that the method effectively quantifies the drug content in tablets without interference from excipients or the need for separate extraction. Consequently, it is ideal for routine quality control in laboratories for Azelnidipine and Olmesartan Medoxomil tablet formulations.

6. REFERENCES

1. Nozari F, Hamidizadeh N. The effects of different classes of antihypertensive drugs on patients with COVID-19 and hypertension: A mini-review. Int J Hyperten, 2022; 2022: 1-8.
2. E1-Hanboushy S, Marzouk HM, Fayez YM, Abdelkawy M, Lotfy HM. Eco-friendly spectrophotometric evaluation of triple-combination therapies in the treatment strategy of patients suffering from hypertension during coronavirus pandemic-Spectralprint recognition study. Spectrochim Acta A Mol Biomol Spectrosc, 2022; 280(121523).
3. Bakris G, Ali W, P arati G. ACC/AHA versus ESC/ESH on hypertension guidelines: JACC Guideline Comparison. J Am Coll Cardiol, 2019; 73(23): 3018-3026.
4. Zhang W, Chem B, Zhang Y, Luo J. Clinical use of azelnidipine in the treatment of hyprtesion in Chinese patients. Ther Clin Risk Manag, 2015; 11: 309-318.
5. Ram CVS. Therapeutic usefulness of a novel calcium channel blocker azelnidipine in the treatment of hypertension: A narrative review. Cardiol Ther, 2022; 11(4): 473-489.
6. Brunner HR. The new oral angiotensin II antagonist olmesartan medoxomil: a concise overview.J Hum Hypertens, 2002; 16(S2): S13-S16.
7. Shimada K, Ogihara T, Saruta T, Kuramoto K. Effects of combination olmesartan meoxomil 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. Clin Ther, 2010; 32(5): 861-881.
8. Ogihara T, Saruta T, Shimada K, Kuramoto K. A randomized, double-blind, four-arm parallel-group study of the efficacy and safety of azelnidipine and olmesartan medoxomil combination therapy compared with each monotherapy in Japanese patients with essential hypertension: the REZALT study. Hypertens Res, 2009; 32(12): 1148–1154.
9. Patel JK, Patel NK. Validated stability-indicating RP-HPLC method for the simultaneous determination of azelnidipine and olmesartan in their combined dosage form. Sci Pharm, 2014; 82(3): 541-554.
10. Ganduri RB, Peddapapireddigari JR, Vurimindi H, Ramprakash. Stability indicating liquid chromatographic method for the simultaneous determination of olmesartan

medoxomil and azelnidipine in combined tablet dosage form. Int. J. Pharma Sci. Res, 2014; 5(6): 275-282.

11. Dharuman N, Karunanidhi Santhana L, Krishnan M. Eco-friendly design of experiment based approach for estimation of azelnidipine and olmesartan medoxomil in RP-HPLC: Greenness appraisal. J Liq Chromatogr Relat Technol, 2023; 46(16–20): 302-315.
12. Bhosale AP. Bioanalytical RP-HPLC method development and validation for estimation of azelnidipine and Olmesartan medoxomil in human plasma. J Med Pharm Allied Sci, 2022; 11(5): 5232-5239.
13. Prasanthi T, Atmakuri LR, Vasavi T. Rp-hplc method development and validation for the simultaneous estimation of olmesartan and azelnidipine in bulk and pharmaceutical dosage form. IJRPC, 2023; 13(1): 87-92.
14. Beckett AH, Stenlake JB.Practical pharmaceutical chemistry: Pt. 2. 4th ed. New Delhi:CBS publishers and distributers, 2002; 275-288.
15. M.Haripriya, Neethu Antony, P. Jayasekhar. Development and validation of uv spectrophotometric method for the simultaneous estimation of cilnidipine and telmisartan in tablet dosage form utilising simultaneous equation and absorbance ratio method. ijpbs, 2013; 3(1): 343-348.
16. Pandey G, Mishra B. A new analytical *Q*-absorbance ratio method development and validation for simultaneous estimation of lamivudine and isoniazid. ISRN Spectrosc, 2013; 2013: 1–5.
17. ICH Harmonized Tripartite Guidelines, Validation analytical procedures: Text and Methodology, 2005; Q2(R1). Geneva.