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Quantitative Analysis of Azelnidipine and Olmesartan Medoxomil by High-Performance Thin-Layer Chromatography with UV Absorption Densitomeric Detection

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Keywords: Azelnidipine, Olmesartan Medoxomil, HPTLC, Method Validation.

ABSTRACT

For the simultaneous quantification of Azelnidipine and Olmesartan Medoxomil in combined tablet dosage form, a high-performance thin-layer chromatographic method was created that is simple, quick, accurate, and selective. The procedure used HPTLC aluminum plates pre-coated with silica gel 60 F_{254} as the stationary phase and Toluene: Chloroform: Ethanol (6:2.5:1.5 v/v/v) as the solvent system. The observed R_f value for Azelnidipine and Olmesartan Medoxomil were 0.627±0.010 and 0.215±0.017 respectively. The technique used exhibited a linear approach in the range of 200-1000 ng/band for Azelnidipine and 500-2500 ng/band for Olmesartan Medoxomil with R^2 value of 0.9963 for both the drugs. The percentage recovery fell within the range of 95.46-95.95%. For Azelnidipine and Olmesartan Medoxomil, the minimum detectable levels were determined to be 64.04 ng/band and 188.30 ng/band respectively, and the limit of quantification was found to be 194.06 ng/band and 570.59 ng/band. The results suggested that this method is poised to become a valuable tool for routine analysis of pharmaceutical dosage forms.

1 INTRODUCTION

Hypertension poses a notable health issue for people globally. Its prevalence is on the rise due to factors such as aging populations, poor dietary habits, and insufficient physical activity. In recent decades, significant advancements in hypertension treatment have been made through the development of numerous combination medications. Most guidelines now advocate for combination therapies to effectively manage hypertension. Among these combinations, angiotensin II receptor blockers and calcium channel blockers are often preferred for achieving target blood pressure in hypertensive patients. One newer combination, Azelnidipine and Olmesartan Medoxomil has demonstrated potent antihypertensive effects, further enriching the treatment options available. [1-3]

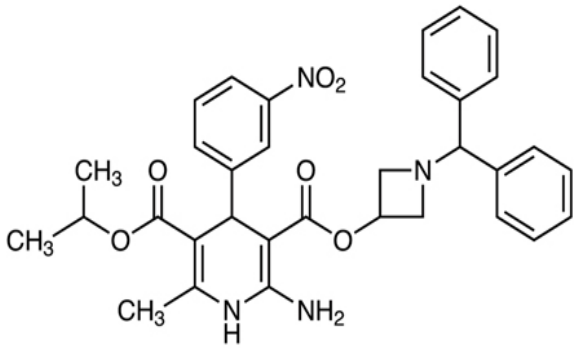

Figure No.1: Structure of Azelnidipine

Azelnidipine (AZEL), 3-(1-diphenyl methyl azetidine-3yl)-5-isopropyl-2-amino-1,4-dihydro-6-methyl-4-(3-nitro phenyl)-3,5-pyridine dicarboxylate (Figure 1), is a new third generation, dihydropyridine, long acting, lipophilic, calcium channel antagonist. Antihypertensive effect is produced by inhibition of trans-membrane Ca^{2+}, particularly blocking L-type calcium channels followed by vasodilation of vascular smooth muscle. AZEL also showed cardio-protective, cerebro-protective, and lipid-lowering effects along with improvement in insulin resistance. [4, 5]

Olmesartan Medoxomil (OLM), (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(Figure 2) is imidazole derivative, pro drug with antihypertensive property. After oral administration, OLM is rapidly hydrolyzed into active metabolite Olmesartan. It reduces blood pressure through peripheral resistance and vasoconstriction by blocking angiotensin II from binding to AT1 receptors (angiotensin II type 1 receptors) in vascular smooth muscles.

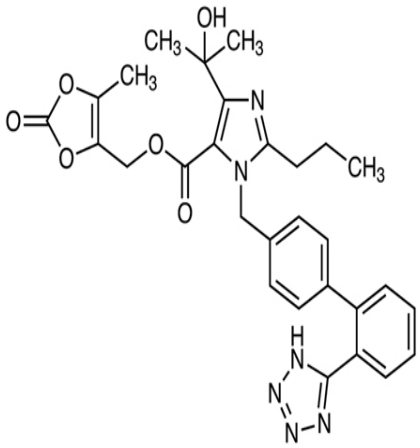

Figure No.2: structure of Olmesartan Medoxomil

The newly introduced AZEL/OLM combination is demonstrating itself as a superior choice among various hypertension treatment combinations. Clinical research has highlighted its efficacy in minimizing potential side effects and maintaining heart rate control, often without significant deviations from the normal heart rate. [7,8]

The results of a comprehensive literature survey revealed that UV [9], HPLC [10-13], methods exist for estimating Azelnidipine and Olmesartan Medoxomil in synthetic mixture/oral dosage forms. This study was initiated since no high-performance thin-layer chromatography (HPTLC) method has been developed for their simultaneous estimation in bulk and pharmaceutical dosage forms. Consequently, the current study presents a newly developed and validated HPTLC method that is both precise and accurate for the simultaneous quantification of these two compounds. This method holds promise for routine analysis of pharmaceutical dosage forms.

2. MATERIALS AND METHODS

Instruments: CAMAG HPTLC, CAMAG LINOMAT 5 sample Applicator with 100µLsyringe (Hamilton, Switzerland), CAMAG TLC Visualizer 2, CAMAG TLC Scanner 4, VisionCATS 3.0(3.0.20196), Twin Trough Chamber (20×10 cm). Chromatography was conducted on aluminum TLC plates measuring 20 × 10 cm that had been pre-coated with silica gel 60 F_{254}.

Chemicals and Reagents: The samples of Azelnidipine and Olmesartan Medoxomil in their chemically pure form were generously provided as gift samples by SYNOKEM PHARMACEUTICALS LTD. Toluene, Chloroform (HPLC), and Ethanol (AR) grade

procured from Merck Chemicals, India. The marketed formulation of Azelnidpine and Olmesartan Medoxomil (Olmezest-Az 20) was purchased from the local market.

3. METHODOLOGY

3.1. Preparation of solutions:

Preparation of Azelnidipine and Olmesartan Medoxomil standard solutions.

Accurately weighed AZEL (8mg) and OLM (20mg) and transferred to two separate 10mL volumetric flasks, each containing 2mL methanol. Flasks were shaken to dissolve the solid content. Volume made up to the mark with methanol, yielding solutions containing 800µg/mL of AZEL and 2000µg/mL of OLM. Using a pipette, 2.5 ml aliquots from each of the aforementioned stock solutions of AZEL and OLM, respectively, were transferred into two separate 10 mL volumetric flasks and solutions were made up.

Preparation of standard mixture solution:

Accurately weighed AZEL (8mg) and OLM (20mg) and transferred to a 10mL volumetric flask, containing 2mL methanol. Flask was shaken to dissolve the solid content. Volume was made up to the mark with methanol, yielding a solution containing a mixture of 800µg/mL of AZEL and 2000µg/mL of OLM. Using a pipette, 2.5 ml aliquot was transferred into a 10mLvolumetric flask and made up the solution.

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 8mg of AZEL and 20 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 5000 rpm for 5 minutes. The supernatant solution was collected for further analysis. Using a pipette, 2.5 mL aliquot was transferred into a 10mL volumetric flask and made up the solution. For the determination of AZEL and OLM, 3µL containing 600 µg/mL of AZEL and 1500 µg/mL of OLM sample solution were applied to the HPTLC plate.

3.2. Chromatographic conditions:

The experiment utilized silica gel 60 F_{254} aluminum sheets (20×10 cm) as the stationary phase. Taking into consideration polarity of the two compounds, trial and error method revealed effective resolution can be attained by using the mobile phase composed of Toluene: Chloroform: Ethanol in a volume ratio of 6: 2.5: 1.5 (v/v/v). The solutions were applied onto the TLC plate as 8 mm wide bands, using a CAMAG LINOMAT 5 semiautomatic sample applicator under a stream of nitrogen gas. The distance between each band was set at 11.4 mm. Ascending development was conducted up to 70 mm in a 20 cm x 10 cm CAMAG twin trough glass chamber saturated with the mobile phase for 20 minutes. Subsequently, the developed TLC plate was air-dried and scanned between 200 to 450 nm using a CAMAG TLC scanner 4 equipped with VisionCATS software.

3.3. Optimization of mobile phase:

Various mobile phase compositions were experimented to optimize the HPTLC parameters. Optimal peak symmetry and satisfactory resolution for AZEL and OLM were achieved using a mobile phase composed of Toluene: Chloroform: Ethanol (6:2.5:1.5v/v/v).

3.4. Preparation of calibration curves:

Volumes ranging from 1-5µL from the stock solution of AZEL and OLM were applied onto pre-coated TLC plates using LINOMAT 5 sample applicator. The method relied on separating the two drugs and then measuring their spots densitometrically at 254 nm. Separation was conducted on Merck TLC aluminum sheets coated with silica gel 60 F_{254}, using Toluene: Chloroform: Ethanol (6:2.5:1.5 v/v/v) as the mobile phase. Linearity was evaluated by visually inspecting the plot of peak area vs concentration.

3.5. Estimation of Azelnidipine and Olmesartan Medoxomil in standard drug mixture and commercial formulation:

The established HPTLC technique was applied to analyze the standard drug mixture and commercial formulation Olmezest-Az 20 tablet.

3.6. Validation of HPTLC method:

The HPTLC method outlined underwent validation, following the standards established by the International Conference on Harmonization (ICH), ensuring compliance with criteria

including linearity, accuracy, precision, as well as determination of limits of detection (LOD) and limit of quantification (LOQ).

4. RESULTS AND DISCUSSION:

From standard solutions containing 200 µg/mL of AZEL and 500 µg/mL of OLM each, 1-5 µL were spotted.3µLeach of standard drug mixture and sample solution with concentration of 600 µg/mL of AZEL and 1500 µg/mL of OLM were also spotted.

4.1. Estimation of Azelnidipine and Olmesartan Medoxomil in standard drug mixture and commercial formulation:

The chromatogram depicted in Figure 3 & 4 illustrates the separation profile of AZEL and OLM in standard drug mixture and sample solution respectively. Both medications were separated at their distinct R_f values that is 0.627±0.010 for AZEL and 0.215±0.017 OLM. No additional peaks from inactive ingredients were detected.

Table.1 presents the estimated drug quantity (mg/tab) and the percentage of the drug found in commercial dosage formulation Olmezest-Az 20. These observations demonstrate the suitability of the proposed for accurately and precisely analyzing AZEL and OLM in their combined commercial formulations.

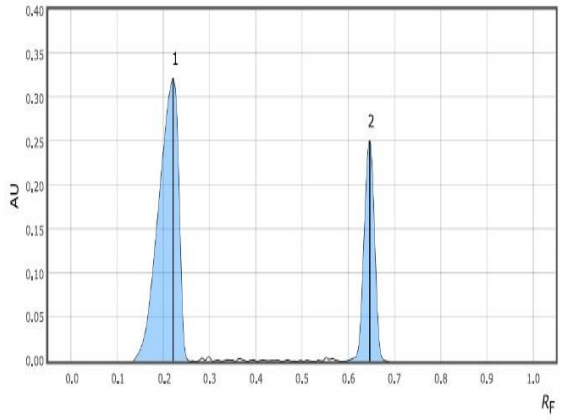

Figure No.3: Chromatogram of AZEL (R_f = 0.637) and OLM (R_f = 0.221) in standard drug mixture solution

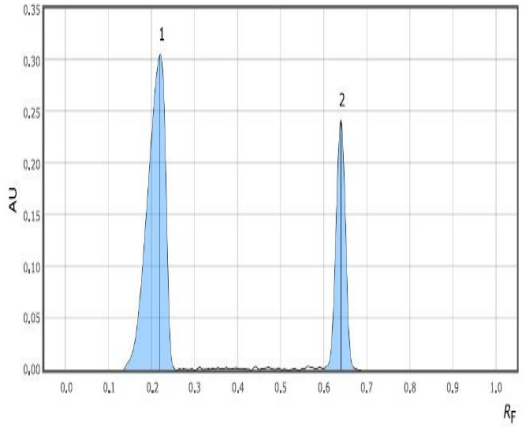

Figure No.4: Chromatogram of AZEL (R_f = 0.637) and OLM (R_f =0.219) in sample solute

Table No: 1.Application of the proposed HPTLC method for assessing the analyzed drugs in a commercially available formulation Olmezest-Az 20 tablet.

Drugs	Label claim(mg/tab)	Estimated drug quantity(mg/tab)	% Amount found
AZEL	8.0	7.62	95.31
OLM	20.0	18.47	92.37

4.2. Validation of the proposed method

Linearity

Linearity assessments were conducted by constructing curves using five different concentration levels spanning a range of 200-1000 ng/band for AZEL and 500-2500 ng/band for OLM. Peak areas were determined and calibration curves plotted as depicted in Figures 5&6.

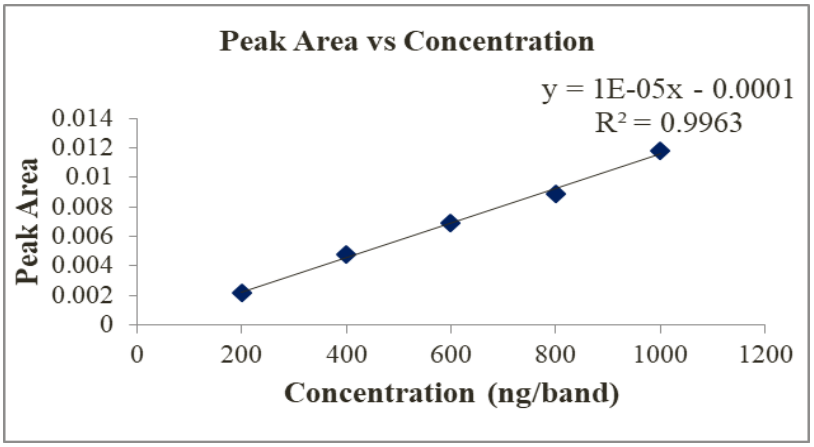

Fig No.5: Calibration curve of Azelnidipine (200-1000ng/band)

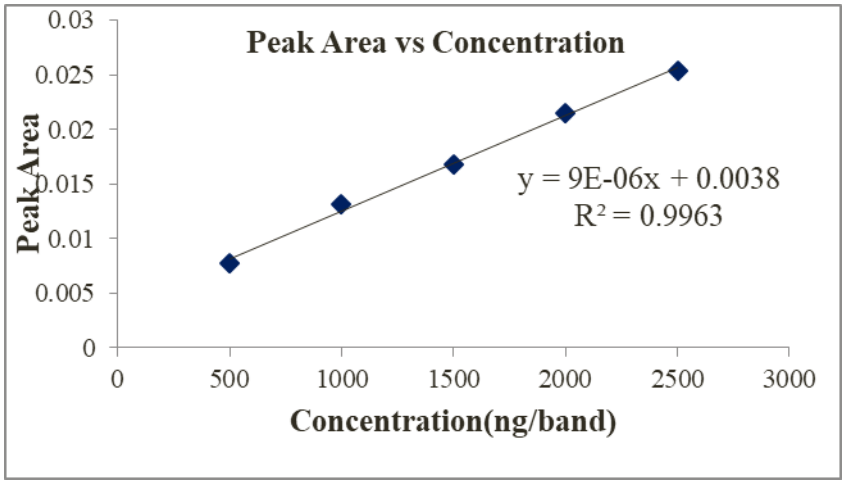

Fig No.6: Calibration curve of Olmesartan Medoxomil(500-2500ng/band)

R^2 value of both AZEL and OLM was found to be 0.9963, and the findings indicate a good correlation between the ratios of peak areas and the concentrations of drugs within the tested range.

Accuracy

The accuracy of the method was evaluated by employing standard additions at three different levels. The reference standards for each drug were introduced into the sample solution at concentrations of 600ng/band for Azelnidipine and 1500 ng/band for Olmesartan Medoxomil corresponding to 80%, 100%, and 120% of the expected concentration levels. Each level was replicated three times; percentage recoveries determined are outlined in Table 2.

Table No.2: Accuracy studies

Recovery level	AZEL			OLM		
	Amount present (ng/band)	Amount added (ng/band)	% Recovery ±RSD	Amount present (ng/band)	Amount added (ng/band)	% Recovery ±RSD
80%	600	480	95.69±0.53	1500	1200	95.56±0.37
100%	600	600	95.95±0.45	1500	1500	95.46±0.61
120%	600	720	95.85±0.50	1500	1800	95.80±0.53

*Average of three observations

Precision

Precision was assessed based on repeatability and intermediate precision in terms of inter-day and intra-day precision.

Repeatability: Repeatability was established by conducting five replicates using a reference standard mixture solution containing 600ng/band of Azelnidipine and 1500ng/band of Olmesartan Medoxomil, where 1µL was drawn from the working standard mixture solution for each analysis. The standard deviation and percentage relative standard deviation were calculated based on the peak areas obtained for each concentration.

Intermediate precision: Intermediate precision was evaluated by analyzing standard solution of AZEL and OLM over three consecutive days. The identical concentrations utilized in the repeatability assessment were applied to the plate. The development process was repeated three times. The same parameters were then calculated for intermediate precision. The results for both repeatability and intermediate precision are detailed in Table 3-5.

Table No.3: Repeatability

Conc. of AZEL (ng/band)	**%RSD***	**Conc. of OLM (ng/band)**	**%RSD***
600	0.73	1500	0.25

*Average of five observations

Table No.4: Inter-day studies

Day	**Conc.of AZEL (ng/band)**	**%RSD***
Day 1	600	0.71
Day 2	600	0.41
Day 3	600	0.54
Day	**Conc.of OLM (ng/band)**	**%RSD***
Day 1	1500	0.28
Day 2	1500	0.35
Day 3	1500	0.69

*Average of five observations

Table No.5: Intra-Day studies

No. of Injection	**Conc. of AZEL (ng/band)**	**% RSD***
6	600	0.86
No. of Injection	**Conc. of OLM (ng/band)**	**% RSD***
6	1500	0.58

*Average of five observations

LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) were determined utilizing equations 1 and 2, respectively. The data is illustrated in the Table No.6.

$$LOD = 3.3\times \sigma/S \text{.....(1)}$$

$$LOQ=10\times\sigma/S \text{.....(2)}$$ where,

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

Table No.6: LOD and LOQ

Parameter	**AZEL (ng/band)**	**OLM(ng/band)**
LOD	64.04	188.30
LOQ	194.06	570.59

*Average of five observations

5. CONCLUSION:

This study introduces a validated HPTLC protocol for quantifying pharmaceutical formulation of Azelnidipine and Olmesartan Medoxomil. The HPTLC method described here is both simple and specific, with high precision, accuracy, reliability, and selectivity, ultimately saving both time and money. Statistical analysis of the result demonstrates that the method accurately quantifies the drug content in tablets without interference from excipients and without the need for any separate extraction step. Thus, this method is suitable for routine

analysis in quality control laboratories for pharmaceutical formulations of Azelnidipine and Olmesartan Medoxomil in tablet dosage forms.

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