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DEVELOPING A REVERSE PHASE-HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR ESTIMATING TELMISARTAN AND AZELNIDIPINE SIMULTANEOUSLY IN BOTH TABLET PRODUCT AND BULK

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ABSTRACT

A new, accurate, precise, and durable RP-HPLC method with sensitive features has been developed for the simultaneous assessment of Telmisartan (TEL) and Azelnidipine (AZEL) in both bulk and tablet format. An Agilent C_{18} column with a size of 100 mm × 4.6 mm, 5 µm was used to estimate the solutes. TEL and AZEL were eluted in a 10-minute gradient trial at a flow rate of 0.7 ml/min with an ambient column temperature of 25°C and monitored at a wavelength of 257 nm using orthophosphoric acid (0.05%) buffer: acetonitrile in a 70:30 v/v ratio. The retention times of TEL and AZEL were found to be 3.587 minutes and 5.633 minutes, respectively. The Q2A and Q2B validations of the analytical method demonstrated good linearity throughout the concentration ranges of 20-100 µg/mL for TEL and 4-20 µg/mL for AZEL, with r^2 of 0.999 in both cases. High accuracy, excellent precision (inter-day and intra-day), and remarkable resilience values were also shown by the technique. The suggested analytical method proved precise, accurate, and robust for frequent analysis of the drug combination in bulk and tablet forms.

Keywords: Telmisartan, Azelnidipine, RP-HPLC, Simultaneous, Estimation, Validation

INTRODUCTION

Angiotensin-II receptor antagonist (ARB) telmisartan (TEL) (**Figure 1A**) is used to treat hypertension. Angiotensin-II receptor blockers (ARBs) like telmisartan bind to the angiotensin-II type-1 (AT_1) receptors with high affinity, inhibiting angiotensin-effect II's on vascular smooth muscle and, as a result, lowering arterial blood pressure. According to recent research, telmisartan may also exhibit PPAR-γ agonistic characteristics, which may result in favorable metabolic benefits. It may be used alone or in conjunction with other anti-hypertensive medications to treat hypertension. Also used to treat diabetic nephropathy in individuals with type 2 diabetes mellitus who are hypertensive, as well as congestive heart failure (only in patients who cannot tolerate ACE inhibitors). It binds reversibly and selectively to the receptors in vascular smooth muscle and the adrenal gland, interfering with the binding of angiotensin-II to the angiotensin-II AT1-receptor. Blocking the effects of angiotensin-II, a vasoconstrictor that simultaneously promotes the production and release of aldosterone, lowers systemic vascular resistance. The angiotensin converting enzyme, other hormone receptors, and ion channels are not inhibited by TEL. It is also thought to be a partial agonist of PPAR-γ, which is a well-known target for anti-diabetic medicines. This indicates that TEL may enhance carbohydrate and lipid metabolism as well as insulin resistance management without the negative side effects associated with full PPAR-γ activators **[1]**.

Azelnidipine (AZEL) is a calcium channel blocker that is a dihydropyridine. Daiichi-Sankyo Pharmaceuticals Inc. in Japan markets it. Unlike some other calcium channel blockers, it has a delayed start of action and results in a long-lasting reduction in blood pressure with just a little rise in heart rate. It's presently being researched as a treatment for post-ischemic stroke. AZEL is a vasodilator that lowers blood pressure gradually in hypertensive individuals. AZEL, unlike other members of its medication family, does not cause reflex tachycardia as a result of vasodilation. This is most likely because it causes a steady drop in blood pressure. It also has a long-acting hypotensive impact and, owing to its affinity for vascular tissue and anti-oxidative activity, has been found to have a potent anti-arteriosclerotic effect in vessels. It has been shown in clinical trials to decrease heart rate and proteinuria in hypertension individuals by decreasing sympathetic nerve activity. It

has also been shown to have cardioprotective, neuroprotective, and anti-atherosclerotic effects, as well as the ability to prevent insulin resistant ischemic stroke. It inhibits trans-membrane Ca^{2+} influx via vascular smooth muscle voltage-dependent channels. L-type, T-type, N-type, P/Q-type, and R-type Ca^{2+} channels are among the several types of Ca^{2+} channels. Calcium normally causes smooth muscle contraction, which contributes to hypertension. The vascular smooth muscle does not contract when calcium channels are closed, resulting in vascular smooth muscle wall relaxation and lower blood pressure **[2]**.

Several studies have used validated analytical methods such as reverse phase-high performance liquid chromatography (RP-HPLC), ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), fluorescence, and spectrophotometric methods to estimate TEL and AZEL, as well as their metabolic products, in plasma, bulk formulation, and pharmaceutical formulation (capsule, controlled-release). There have, however, been no studies published that estimate the medication in both bulk and tablet form at the same time **[3, 4]**.

There was no single article found when scanning standard global databases for literature on any analytical RP-HPLC method for the routine simultaneous determination of TEL and AZEL drug combinations in bulk and pharmaceutical formulations. To solve the issue, a simple, long-lasting, precise, low-cost, and accurate solution was developed. The purpose of this research is to develop a reliable RP-HPLC method for quantifying TEL and AZEL in bulk and tablet formulations.

MATERIAL AND METHODS

Materials

Ankaleshwar, Gujarat-based Purechem Pvt. Ltd. provided a big sample of TEL and AZEL as a present. The Telma AZ® 40/8 Tablet (CONTAINING40 mg of TEL and 8 mg of AZEL) was supplied by Glenmark Pharmaceuticals Ltd., Mumbai. HiMedia Ltd., Mumbai provided analytical quality chemicals and HPLC grade solvents for the study.

Instruments

A Shimadzu® AUW220D balance was used for the weighing (Kyoto, Japan). The pH was measured using a VSI® VSI-1B digital pH meter (Mohali, India). The sonication was done using a Transonic Digital S sonicator (Mumbai, India). The method was developed using a reverse-phase Denali C_{18} column with a particle size of 5 µm and a dimension of 100 mm × 4.6 mm, which was connected

to an Agilent® 1100 Gradient HPLC system with a PDA detector 2996 and a manual rheodyne injector (20 L loop), all of which were controlled by Chemstation v.2 software.

Selection of the mobile phase

The mobile phase must be carefully selected for the elution of the solutes. The mobile phase was selected based on theoretical plates, peak purity index, and peak symmetry. The experiment started with buffer systems and an eluant like methanol, acetonitrile, or other solvents. Low-intensity peaks with a lot of tailing were produced by elution with an equal combination of buffer KH_2PO_4 and methanol. Although this was an improvement over the previous experiment, the combination of KH_2PO_4 buffer (pH 4.8) with acetonitrile resulted in the formation of a broad peak with tailing. When employed in an equal ratio with methanol, the peak symmetry improved considerably and tailing was reduced when the buffer was replaced with orthophosphoric acid (OPA) (0.05%), but it was still inadequate to elute the solutes. Methanol was combined with OPA to produce a crisp peak with a good Gaussian peak. The 70:30 v/v ratio generated the most theoretical plates as well as the greatest peak purity index. The mobile phase was degassed under vacuum before being filtered using a 0.45 µm membrane filter. Allowing the mobile phase to equilibrate until it achieved a stable baseline was permitted.

Chromatographic conditions

TEL and AZEL were eluted at a flow rate of 0.7 ml/min with an ambient column temperature of 25°C in a 10-minute gradient trial and monitored at a wavelength of 257 nm using a 70:30 v/v Methanol: OPA (0.05%) buffer.

Preparation of analytical solutions

Preparation orthophosphoric acid (0.05%) buffer

Before being sonicated to remove gas, 0.5 mL orthophosphoric acid was accurately weighed and diluted with 1000 mL HPLC grade water.

Preparation of mobile phase

The above-prepared buffer was thoroughly mixed with methanol in a 70:30 v/v ratio. After that, the solution was degassed for 5 minutes with sonication before being filtered under vacuum through a 0.45 µm membrane filter.

Diluent preparation

Methanol and water were employed as diluents in the preparation of the standard and sample solutions in a 70:30 v/v ratio.

Standard preparation

In a 10 mL dry volumetric flask, a precise quantity of 40 mg TEL and 8 mg AZEL were introduced. Sufficient amount of mobile

phase was added to dissolve the drug to get a standard stock solution of 400 µg/mL and 80 µg/mL concentrations. The aforementioned content was sonicated for 10 minutes and the volume was made up to 10 mL.

Sample preparation

The average weight of 20 tablets was determined (6.12 g) after they were properly weighed. A weight equal to a tablet (306 mg) was transferred to a 100 mL volumetric flask and half-filled with the diluent. The contents were sonicated for 20 minutes and then filtered to produce 400 mg/mL of TEL and 80 mg/mL of AZEL.

Method validation

The technique was verified using the Q2A and Q2B guidelines from the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), as well as guidance from the USFDA.

Linearity and Range

The linearity of the technique was tested using five different solute concentrations, ranging from 20 to 100 µg/mL for TEL and 4 to 20 µg/mL for AZEL. The solutions were prepared with the diluent and an equal quantity was injected into the HPLC equipment to determine the peak area. On a linearity graph, the concentration and average area of each solute were plotted. The r^2 value of the regression coefficient was computed as well **[5]**.

Accuracy

The accuracy of the HPLC system was tested by spiking the reference drug solutions at concentrations of 80%, 100%, and 120% (recovery). The experiment was repeated three times, with the results given as % recovery % relative error based on the concentrations used **[6]**.

Precision

The precision of the suggested method was tested in terms of inter-day and intra-day variability by spiking concentrations of 40%, 60%, and 80% six times in a single day (intra-day) and on three different days (inter-day). % relative error precision was used to describe the data **[7]**.

Robustness

The method's robustness was evaluated by varying the mobile phase composition by 1% v/v (i.e. 71:29 % v/v and 69.31 % v/v), flow rate by 0.1 mL/min (i.e. 0.6 mL/min and 0.7 mL/min), and wavelength by 1 nm (i.e. 256 nm and 258 nm), while keeping all the other chromatographic parameters fixed **[8]**.

Systems suitability parameters

The analytical method's repeatability profile was determined by injecting five times the standard solution and monitoring data such

as retention length, peak area, theoretical plates, and tailing factor **[9]**.

Limit of detection

Although it is not necessary to define the exact amount, the limit of detection (LOD) is the lowest concentration that any analytical method can detect **[10]**.

The limit of detection (LOD) was determined by the formula:

$$\mathbf{LOD = 3.3\ (\sigma / S)}$$

Where, **σ =** standard deviation of response; **S** = slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte.

Limit of quantification

The limit of quantification is the smallest amount that can be measured with a given degree of accuracy and precision using any analytical method (LOQ) **[11]**.

The limit of quantification (LOQ) is determined by the formula:

$$\mathbf{LOQ = 10\ (\sigma / S)}$$

Where, **σ =** standard deviation of response; **S** = slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte.

RESULTS AND DISCUSSION

Method development and optimization of chromatographic conditions

Because there were no previous similar methods, the new methodology was entirely based on trial and error. However, considerable influence was drawn from earlier reports when deciding on the stationary phase. The reverse phase C_{18} stationary phase from Agilent® was utilized, with a particle size of 5 µm and a diameter of 100 mm × 4.6 mm i.d. The mobile phase Methanol: OPA (0.05 %) in the ratio 70:30 v/v was utilized for the elution after several continuous trials. Peak tailing was minimized and the analytical method's robustness was significantly enhanced by keeping the mobile phase at a low pH. The use of acidic pH was justified to a greater extent because high basic pH caused dissolution in silica-based reverse-phase columns. The pH of the mobile phase and the pKa of the solute were also found to be in close agreement, enabling them to remain in the unionized state. As a consequence, the pH value was chosen based on two units. The elution was place on an Agilent® C_{18} column in gradient mode for 10 minutes with a mobile phase of 70:30 v/v Methanol: OPA (0.05%). The flow rate was maintained at 0.7 mL/min, the column temperature at 25°C, and the detection wavelength at 257 nm. The retention times for TEL and AZEL were 3.587 minutes and 5.633 minutes, respectively (**Figure 2A**). In the tablet sample solution, TEL had a retention time of 3.566 minutes while AZEL

had a retention time of 5.954 minutes (**Figure 2B**). This clearly showed that the suggested analytical method for routine medicine combination analysis in bulk and tablet forms was exact, accurate, and robust.

Method validation

Linearity and range

Throughout the dose and peak area ranges of 20-100 g/mL for TEL and 4-20 g/mL for AZEL, there was very high linearity, with linear regression equations of y = *110.4x + 350.3* and y = *75.45x + 26.08*, respectively (**Table 1**). The regression coefficient values were 0.999 in both cases, suggesting that there was a high level of linearity (**Figure 3**).

Accuracy

The % recovery characteristic of the proposed method for simultaneous estimation by utilizing the calibration curve was determined in part by the Y-intercept and slope of the graph. TEL's % RSD values were 0.80, 0.11, and 0.42, respectively, while AZEL's were 0.33, 0.17, and 0.18, all of which were less than the US Pharmacopeia's acceptance threshold of 2% (**Table 2**). Overall, the method revealed that the data retrieved was correct.

Precision

In both intra-day and inter-day variability testing for precision data, the method was proven to be highly accurate across the tested ranges of 20-100 µg/mL for TEL and 4-20 µg/mL for AZEL. The peak area of the sample solution matched that of the standard solution in both cases, with a % RSD of less than 2%. TEL and AZEL had % RSDs of 0.02 % - 1.22 % and 0.04 % - 0.48 % in intra-day studies (**Table 3**), respectively, whereas TEL and AZEL had % RSDs of 0.02 % - 0.76 % and 0.07 % - 1.42 % in inter-day studies, indicating high precision and minimal variation (**Table 4**).

Robustness

The intentional change of several critical chromatographic parameters such as mobile phase composition, flow rate, and wavelength by 1%, 0.1 mL/min, and 1 nm, respectively, resulted in a substantial shift in the chromatogram for both medicines. When the mobile phase combination was adjusted to 71:29 v/v, the % RSD was determined to be 2%. (0.11 for TEL and 0.23 for AZEL). Similarly, the % RSD was found to be less than 2% when the composition was altered by 69:31 v/v where TEL has a value of 0.15, whereas AZEL has a value of 0.38. When the flow rate was raised by 0.1 ml/min, the % RSD was determined to be 2%. (0.01 for TEL and 0.20 for AZEL). A similar reduction in flow rate, on the other hand, resulted in a % RSD of < 2 (specifically, TEL showed 0.04 while AZEL showed 0.26).

A variation of 1 nm in wavelength resulted in a RSD value of less than 2% where TEL demonstrated 0.10 and 0.09, respectively and AZEL demonstrated 0.15 and 0.17, respectively. All of the tests indicated that the suggested method has robust characteristics due to the deliberate change of the parameters.

Systems suitability parameters

The system suitability features of the suggested approach demonstrated a high degree of repeatability and may be utilized for routine drug combination analyses. The suggested TEL method yielded an average retention time (Rt) of 3.585 minutes and a mean theoretical plate (TP) of 6033. The Rt and TP for AZEL were 5.635 minutes and 5755, respectively (Table 5). A tailing value of less than 2% showed no specific tailing in any cases. Both symmetric and asymmetric components are of similar magnitude in an ideal Gaussian peak with excellent peak symmetry (asymmetric factor = 1). Because the suggested method met the minimum requirements of US Pharmacopoeia monographs (minimum theoretical plates of 2000 and tailing factor of less than 2%), it has a high resolution, significant separation, high column effectiveness, and enhanced repeatability. The separation factor (α) and resolution factor (Rs) were found to be significantly higher than the ICH limits and required recommendations of 1 and 1.5, respectively, indicating that the suggested analytical technique produces a greater separation of both peaks with less tailing and greater resolution. The method may be utilized for routine analysis because of its high precision, reproducibility, and accuracy.

Limit of detection and Limit of quantification

TEL had a LOD of 0.201766 µg/mL and a LOQ of 0.611413 µg/mL, while AZEL had a LOD of 0.103658 µg/mL and a LOQ of 1.7045 µg/mL, showing the method's remarkable detection capacity for the lowest possible concentration of the solute concurrently from the combination or formulation.

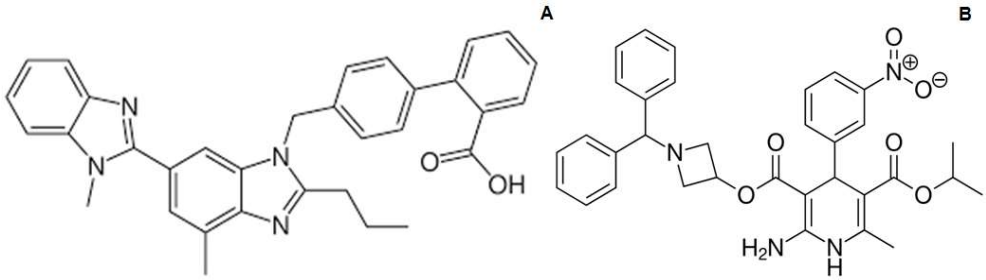

Figure 1: Structure of (a) Telmisartan and (b) Azelnidipine.

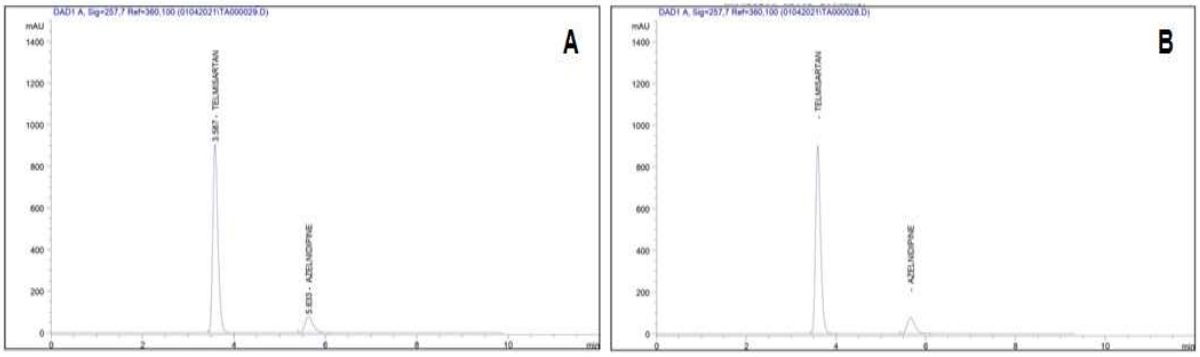

Figure 2: Chromatogram for Telmisartanand Azelnidipine (A) after method optimization and (B) after tablet sample solution

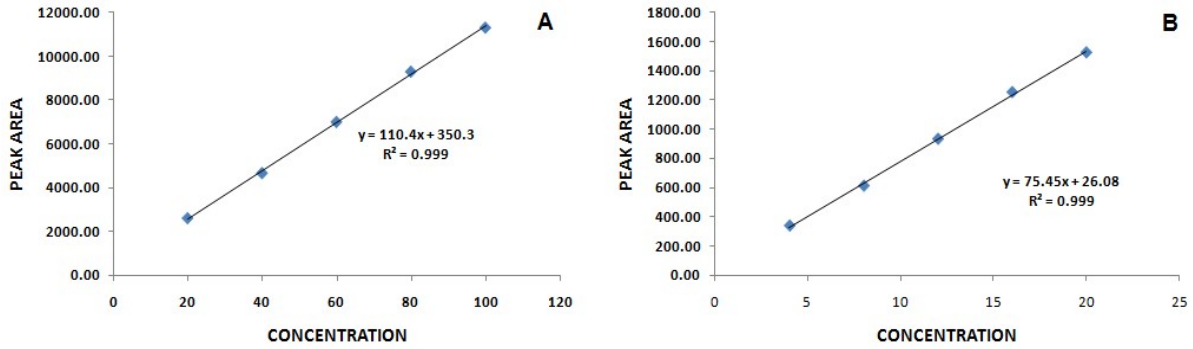

Figure 3: Linearity plot of (a) Telmisartanand (b) Azelnidipine.

Table 1: Linearity study of Telmisartan and Azelnidipine

TELMISARTAN		AZELNIDIPINE	
Concentration (µg/mL)	Peak Area (mV)	Concentration (µg/mL)	Peak Area (mV)
20	2596.37	4	337.00
40	4661.01	8	610.75
60	7002.64	12	932.18
80	9304.95	16	1252.58
100	11318.10	20	1525.16

Table 2: Recovery for accuracy studies for the combination.

Spiked level %	Conc. of drug added (µg/mL)	Conc. of drug found (µg/mL)	Recovery %	Mean %	% RSD
		TELMISARTAN			
80	32	16.24386	100.20	100.77	0.80
	32	16.29407	101.34		
100	40	20.24659	99.17	99.24	0.11
	40	20.0717	99.32		
120	48	24.3347	99.71	100.88	0.42
	48	24.31274	99.97		
		AZELNIDIPINE			
80	6.4	6.517429	101.83	102.08	0.33
	6.4	6.548364	102.32		
100	8	7.960449	99.51	99.62	0.17
	8	7.979085	99.74		
120	9.6	9.572432	99.71	99.84	0.18
	9.6	9.597307	99.97		

Conc., Concentration; RSD, relative standard deviation

Table 3: Precision data of intra-day variability

Drug	Conc. (µg/mL)	Peak area of standard (mV)	Peak area of sample (mV)	% label claim	%RSD
TEL	40	4719.767	4691.41	98.30	0.85
	60	7001.133	7000.07	100.39	0.02
	80	9467.191	9386.05	102.31	1.22
AZEL	8	622.0565	621.86	98.70	0.04
	12	931.2762	931.50	100.00	0.03
	16	1240.078	1244.28	100.91	0.48

Conc., Concentration; RSD, relative standard deviation

Table 4: Precision data of inter-day variability

Drug	Conc. (µg/mL)	Peak area of standard (mV)	Peak area of sample (mV)	% label claim	%RSD
TEL	40	4687.125	4688.33	98.23	0.04
	60	7012.456	6975.04	100.01	0.76
	80	9321.254	9320.11	101.56	0.02
AZEL	8	626.1541	619.92	98.38	1.42
	12	929.3145	928.88	99.71	0.07
	16	1266.452	1267.52	102.84	0.12

Conc., Concentration; RSD, relative standard deviation

Table 5: Systems suitability parameters

TELMISARTAN						AZELNIDIPINE					
Rt (min)	Area (mV)	Theoretical Plates (TP)	Separation Factor	Resolution Factor	Tailing Factor	Rt (min)	Area (mV)	Theoretical Plates (TP)	Separation Factor	Resolution Factor	Tailing Factor
3.587	368856	6019	1.642	1.847	1.22	5.633	2432754	5760	1.645	1.989	1.60
3.589	364903	6049	1.648	1.843	1.41	5.632	2428072	5762	1.638	1.998	1.69
3.583	367942	6055	1.647	1.832	1.36	5.637	2427628	5759	1.648	1.981	1.76
3.581	367493	6021	1.643	1.841	1.29	5.638	2428903	5753	1.643	1.987	1.56
3.582	366224	6027	1.652	1.837	1.33	5.630	2423582	5776	1.657	1.992	1.64
3.585	366288	6033	1.648	1.834	1.34	5.635	2628386	5755	1.642	1.994	1.68
% RSD		0.57							0.88		

CONCLUSION

The suggested analytical method may be utilized to estimate TEL and AZEL in bulk and tablet formulations at the same time. According to the ICH validation criteria. The validated stress degradation tests under thermal, oxidative, alkali, and acid conditions showed the possibly damaged components, which chemists would find very helpful for quality control and assurance. The method may be utilized for routine analysis because of its high precision, reproducibility, and accuracy.

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