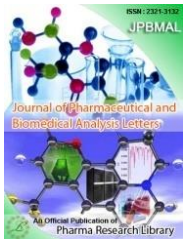

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Analytical Method Development and Validation for Simultaneous Estimaton of Azelnidipine and Telmisartan by RP-HPLC in Bulk and Tablet Dosage Forms

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A B S T R A C T

A new, simple, rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validation of Azelnidipine and Telmisartan in its pure form as well as in combined marketed formulation. Chromatography was carried out on a Phenomenex Luna C18 (4.6mm×250mm) 5µm particle size column using a mixture of Methanol: Phosphate Buffer (pH-4.2) (37:63% v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 260 nm. The retention time of the Azelnidipine and Telmisartan was found to be was 2.133, 3.692±0.02 min respectively. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The method produce linear responses in the concentration range of 20-60mg/ml of Azelnidipine and 10-30mg/ml of Telmisartan. The inter-day and intra-day precisions were found to be within limits. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Azelnidipine and Telmisartan, RP-HPLC, Validation, Accuracy, Precision.

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

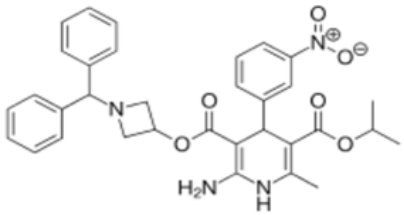

Fig.1. Azelnidipine

Drug category : Calcium channel blocker
Chemical name/ Nomenclature / IUPAC Name: 3-[1-(diphenyl methyl) azetidin-3-yl] 5-propan-2-yl 2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3, 5-dicarboxylate
Molecular Formula : $C_{33}H_{34}N_4O_6$
Molecular Weight : 582.657gm/mole.
Official Pharmacopoeia : JP

Physicochemical properties

Description (Physical State): Solid
Solubility: Water Solubility 0.00082 mg/ml (Insoluble in water)
Melting point : 193-195°C

pKa(strongest acidic) : 19.88
Log P : 5.12
Pharmacokinetic properties:
Half-life:16-28 hr
Absorption : Oral ingestion of azelnidipine demonstrates rapid and dose dependent absorption.
Volume of Distribution : In a Chinese study examining the pharmacokinetics of the drug, the volume of distribution was found to be 1749 +/- 964
Protein binding : 90-91 %
Metabolism : First-Pass Metabolism
Excretion : In one study, following a single 4mg oral dose of 14C-labeled azelnidipine in humans, about 26% of the drug was excreted in the urine and 63% in the faeces during the 1 week period post administration.

Pharmacodynamics:

Azelnidipine is a vasodilator that induces a gradual decrease in blood pressure in hypertensive patients. Unlike other members of its drug class, azelnidipine does not induce reflex tachycardia due to vasodilation. This is likely due to the fact that it elicits a gradual fall in blood pressure. It also exhibits a prolonged hypotensive effect and has been shown to have a strong anti-arteriosclerotic action in vessels due to its high affinity for vascular tissue and antioxidative activity. Clinical studies have demonstrated that azelnidipine markedly reduced heart rate and proteinuria in hypertensive patients by inhibiting sympathetic nerve activity. Azelnidipine has also been confirmed to have cardio-protective, neuroprotective, and anti-atherosclerotic properties, and has also been found to prevent insulin resistance.

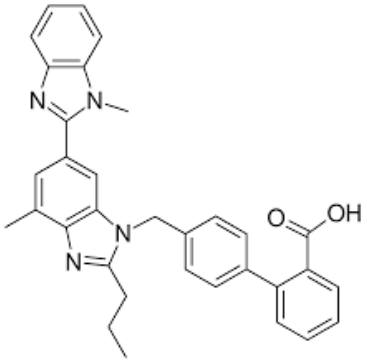

Fig.2. Telmisartan

Drug category : Angiotensin 2 Receptor Blocker
Chemical name/ Nomenclature / IUPAC Name: 2-(4-{[4-methyl-6-(1-methyl-1H-1,3-benzodiazol-2-yl)-2-propyl-1H-1,3-benzodiazol-1-yl]methyl}phenyl)benzoic acid
Molecular Formula : C33H30N4O2
Molecular Weight : 514.6169 gm/mole.
PHYSICOCHEMICAL PROPERTIES:
Description (Physical State): Solid
Solubility: water solubility
Storage Conditions: Store at 25°C (77°F)
Melting point: 261-263 °C
pKa (strongest acidic): 3.65
Log P: 7.7

Pharmacokinetic properties:

Bioavailability : 42–100%
Half-life : 24 hours
Absorption : Absolute bioavailability depends on dosage. Food slightly decreases the bioavailability (a decrease of about 6% is seen when the 40-mg dose is administered with food).
Volume of Distribution : 500 L
Protein binding : >99.5 %
Metabolism : Minimal hepatic Metabolism
Time of peak action : Plasma: 0.5 to 1 hours
Excretion: Faecal 97%

Adverse effects/Side effects:

Side effects are similar to other angiotensin II receptor antagonists and include tachycardia and bradycardia (fast or slow heartbeat), hypotension (low blood pressure), oedema (swelling of arms, legs, lips, tongue, or throat, the latter leading to breathing problems), and allergic reactions.

2. Materials and Methods

Table 1: Instruments used

S.No	Instruments	Model
1	HPLC	WATERS, software: Empower 2, Alliance 2695 separation module. 996 PDA detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Lab man

Table 2: Chemicals used

S.No	Chemical	Brand names
1	Azelnidipine	AZ
2	Telmisartan	TELMA
3	Water and Methanol for HPLC	Lichrosolv (MERCK)
4	Acetonitrile for HPLC	Merck
5	Potassium Dihydrogen Phosphate	Merck

Mobile Phase Optimization:

Initially the mobile phase tried was methanol: Water, Methanol: Phosphate buffer and ACN: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer (pH-4.2) (37:63v/v) in proportion 37:63 v/v respectively.

Optimization of Column: The method was performed with various C18columns like Symmetry, X terra and ODS column. Phenomenex Luna C18 (4.6mm×250mm) 5µm particle size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions:

Instrument used : Waters Alliance 2695 HPLC with PDA Detector 996 model.
Temperature : 35ºC
Column : Phenomenex Luna C18 (4.6mm×250mm) 5µm particle size
Mobile phase : Methanol: Phosphate Buffer (pH-4.2) (37:63v/v)
Flow rate : 1ml/min
Wavelength : 260 nm
Injection volume : 10µl
Run time : 6minutes

Method Validation

Preparation of buffer and mobile phase:

Preparation of Potassium dihydrogen Phosphate (KH2PO4) buffer (pH-4.2): Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 4.2 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultrasonication.

Preparation of Mobile Phase:

Accurately measured 350 ml (35%) of TEA buffer and 650 ml of HPLC Methanol (65%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

3. Results and Discussion

Optimized Chromatogram (Standard)

Mobile phase ratio: Methanol: Phosphate Buffer (pH-4.2) (37:63v/v)
Column : Phenomenex Luna C18 (4.6mm×250mm) 5µm particle size
Column temperature : 35ºC
Wavelength : 260 nm
Flow rate : 1ml/min
Injection volume : 10µl
Run time : 6minutes

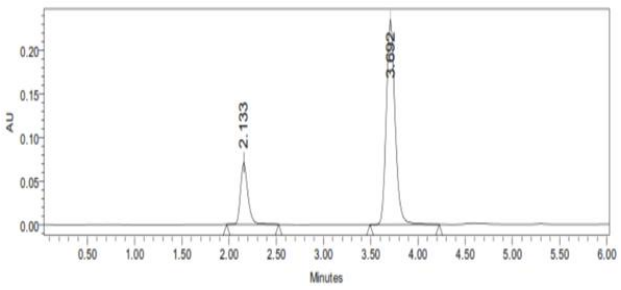

Fig.3: Optimized Chromatogram (Standard)

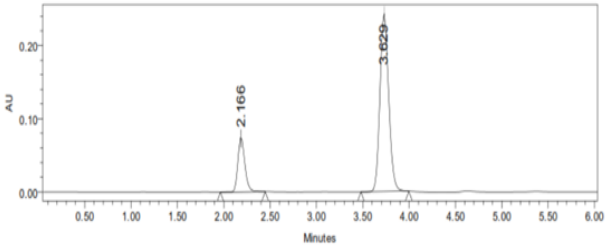

Fig.4: Optimized Chromatogram (Sample)

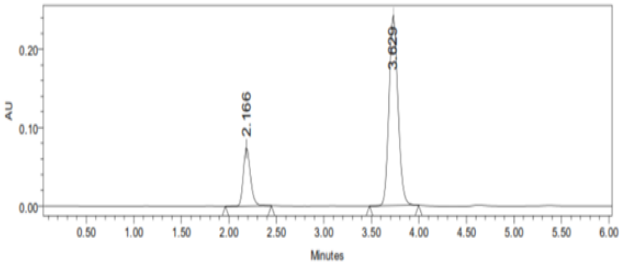

Fig.5: System Suitability

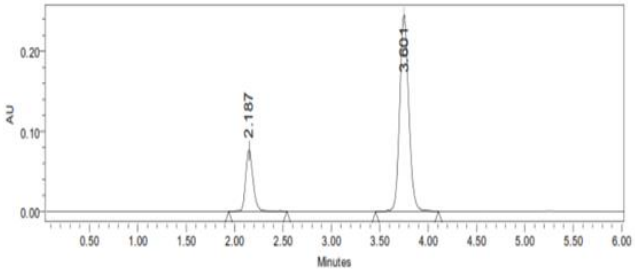

Fig.6: Assay of sample injection

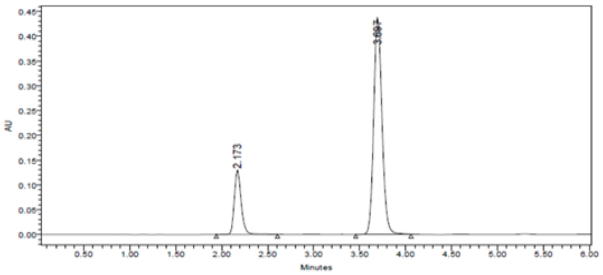

Fig.7: Chromatogram showing linearity

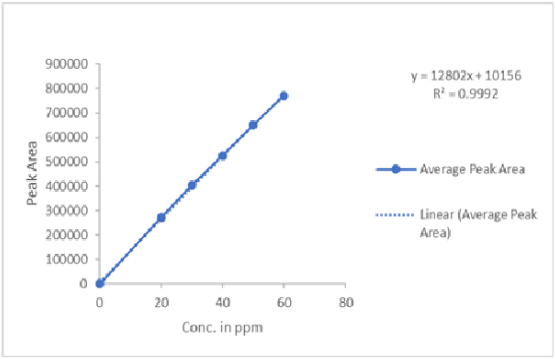

Fig.8: Calibration Curve of Azelnidipine

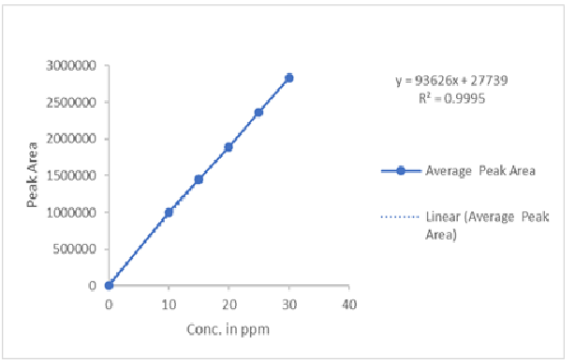

Fig.9: Calibration Curve of Telmisartan

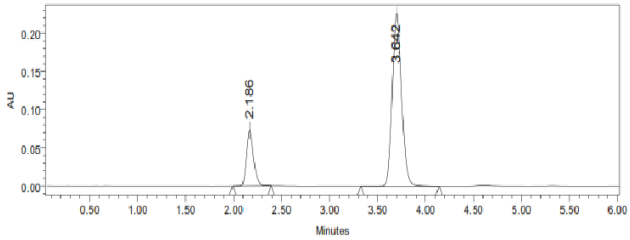

Fig.10: Intermediate precision Chromatogram

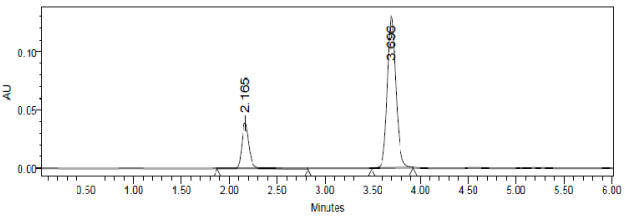

Fig.11: Accuracy-50% injection

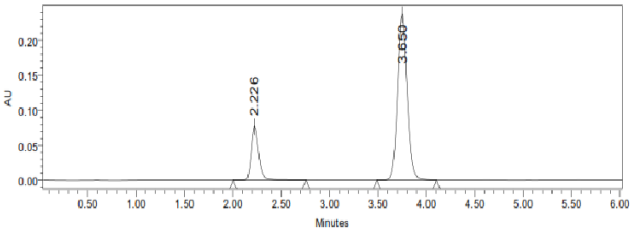

Fig.12: Accuracy-100% injection

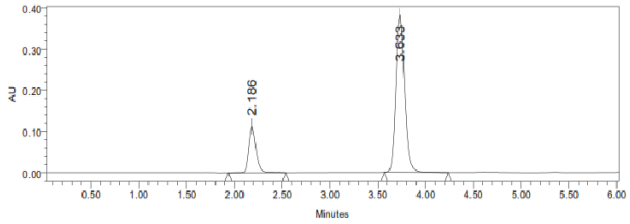

Fig.13: Accuracy-150% injection

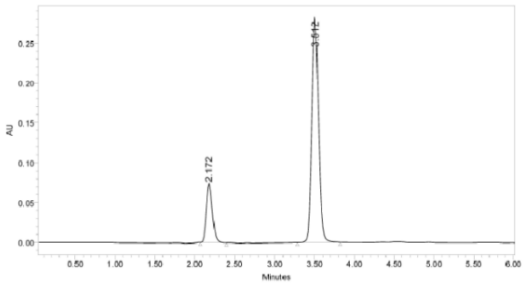

Fig.14: Chromatogram showing less organic composition

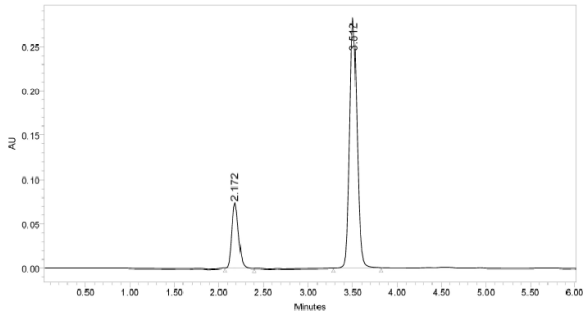

Fig.15: Chromatogram showing more organic composition

Table 3: Accuracy results for Azelnidipine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	267011.3	20	20.063	100.315%	100.28%
100%	523752.3	40	40.118	100.295%	
150%	778457.3	60	60.133	100.221%	

Table 4: Accuracy results for Telmisartan

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	972876.3	10	10.094	100.94%	100.48%
100%	1900122	20	19.998	99.99%	
150%	2851152	30	30.156	100.52%	

Table 5: Results for Robustness of Azelnidipine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	526389	2.133	5679	1.56
Less Flow rate of 0.9 mL/min	542685	2.210	5264	1.54
More Flow rate of 1.1 mL/min	526483	2.184	5426	1.52
Less organic phase	516854	2.200	5163	1.57
More Organic phase	506898	2.172	5098	1.51

Table 6: Results for Robustness of Telmisartan

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1687285	3.692	8685	1.79
Less Flow rate of 0.9 mL/min	1725468	4.498	8265	1.68
More Flow rate of 1.1 mL/min	1652847	3.505	8415	1.59
Less organic phase	1687485	4.504	8326	1.62
More organic phase	1674524	3.512	8415	1.63

4. Conclusion

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Azelnidipine and Telmisartanin bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Azelnidipine was found to be freely soluble in chloroform, soluble in water and in glacial acetic acid, slightly soluble in ethanol and in acetonitrile and practically insoluble in ethyl acetate and in n-hexane. Telmisartan was found to be soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide, soluble in water. Methanol: Phosphate Buffer (pH-4.2) (37:63v/v was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising.

The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Azelnidipine and Telmisartan bulk drug and in Pharmaceutical dosage forms

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