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Develop a new, simple, fast, rapid, accurate, efficient and reproducible RP-HPLC method and spectroscopic method for the simultaneous analysis of Azelnidipine and Telmisartan

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A b s t r a c t

Introduction: Quality control in manufacturing industries, the monitoring of clinical and environmental samples, the assaying of geological specimens, and the support of fundamental and applied research are the principal applications. **Aim:** The study aimed to develop a new, simple, fast, rapid, accurate, efficient and reproducible RP-HPLC method and spectroscopic method for the simultaneous analysis of Azelnidipine and Telmisartan. The developed method will be validated according to ICH guidelines. **Methods:** UV spectrum of 10 µg/ml Telmisartan and 10µg/ml Azelnidipine in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 225 nm. At this wavelength both the drugs show good absorbance. **Results and Discussion:** The chromatographic conditions were successfully developed for the separation of Azelnidipine and Telmisartan by using Inertsil ODS C18 column (4.6×250mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) ACN : KH2PO4 ph 3, detection wavelength was 225nm. The instrument used for HPLC , WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.798 mins and 3.587 mins. The % purity of Azelnidipine and Telmisartan was found to be 99.87% and 100.27% respectively. **Conclusion:** The study concluded that RP-HPLC can be used for routine analysis of Azelnidipine and Telmisartan in API and Pharmaceutical dosage form.

Keywords: UV spectrum, Azelnidipine, Telmisartan, RP-HPLC, spectroscopic method.

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C O N T E N T S

1. Introduction

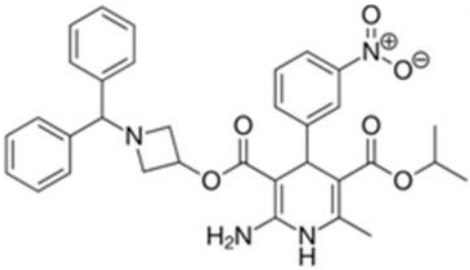

Figure 1: Azelnidipine

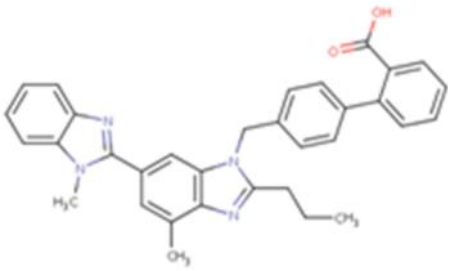

Figure 2: Telmisartan

IUPAC Name: 3-[1-(diphenylmethyl) azetidin-3-yl] 5-propan-2-yl 2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate. It has a gradual onset of action and produces a long-lasting decrease in blood pressure, with only a small increase in heart rate, unlike some other calcium channel blockers [3]. It is currently being studied for post-ischemic stroke management[5-7].

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. Telmisartan can improve carbohydrate and lipid metabolism, as well as control insulin resistance without causing the side effects that are associated with full PPARγ activators.

2. Methodology

Table 1: Instruments Used

S. No	Instrument	Model
1	HPLC	WATERS, software: Empower, 2695 separation module, uv detector.
2	UV/VIS spectrophotometer	LABINDIA UV 3000^{+}
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

Table 2: Chemicals used

SL. No	Chemical	Company Name
1	Telmisartan	Glenmark
2	Azelnidipine	Glenmark
3	KH_2PO_4	FINER chemical LTD
4	Water and Methanol for HPLC	LICHROSOLV (MERCK)
5	Acetonitrile for HPLC	MOLYCHEM
6	Ortho phosphoric Acid	MERCK

HPLC Method Development

Mobile Phase Optimization:

Initially the mobile phase tried was methanol: Ortho phosphoric acid buffer and Methanol: phosphate buffer, Acetonitrile: methanol with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to Phosphate buffer (pH 3.0), Acetonitrile in proportion 70: 30 v/v respectively.

Wave length selection:

UV spectrum of 10µg/ml Telmisartan and 10µg/ml Azelnidipine in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 225 nm. At this wavelength both the drugs show good absorbance.

Optimization of Column:

The method was performed with various columns like C18 column Phenomenex column, YMC, and Inertsil ODS column. Inertsil ODS (4.6 x 250mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1.0 ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used : Waters UPLC with auto sampler and uv detector.
Temperature : Ambient (25°C)
Mode of separation : Isocratic mode
Column : Inertsil ODS (4.6*250mm, 5µ)
Buffer : Phosphate buffer
pH : 3.0
Mobile phase : 70% buffer 30% ACN
Flow rate : 1.2 ml per min

Wavelength : 225 nm
Injection volume : 20 µl
Run time : 8 min.

Preparation of the telmisartan & azelnidipine standard & sample solution:

Standard Solution Preparation: Accurately weigh and transfer 40mg of Telmisartan and 8mg of Azelnidipine working standard into a 10 ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.75 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation: Accurately weigh and transfer the equivalent weight of 40mg of Telmisartan and 8mg of Azelnidipine Tablet powder into a 10 ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 0.75 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of the telmisartan & azelnidipine standard & sample solution:

Standard Solution Preparation: Accurately weigh and transfer 40mg of Telmisartan and 8mg of Azelnidipine working standard into a 10 ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution)**.** Further pipette 0.75 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh and transfer the equivalent weight of 40mg of Telmisartan and 8mg of Azelnidipine Tablet powder into a 10 ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.75 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure: Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Telmisartan and Azelnidipine peaks and calculate the %Assay by using the formulae.

System Suitability: Tailing factor for the peaks due to Telmisartan and Azelnidipine in Standard solution should not be more than 2.0**.** Theoretical plates for the Telmisartan and Azelnidipine peaks in Standard solution should not be less than 2000. Resolution for the Telmisartane and Azelnidipine peaks in standard solution should not be less than 2.

Sample and Standard details

S. No.	Samples
1	Telmisartan &Azelnidipine Tablets 40 mg & 8 mg
2	Telmisartan & Azelnidipine

3. Results and discussion

Table 3: Chromatogram of Telmisartan and Azelnidipine (500 &100µg/ml)

S. No	Linearity Level	Concentration(µg/ml)	Area
1	I	100	65787
2	II	200	131783
3	III	300	194311
4	IV	400	256245
5	V	500	317748
Correlation Coefficient			0.999

Table 4: The accuracy results for Trifluridine

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	95505	10	9.97	99.67	99.59
100%	191399	20	19.97	99.87	
150%	285309	30	29.77	99.25	

Table 5: The accuracy results for Tipracil

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	53846	4.05	4.06	100.23	100.01
100%	107344	8.1	8.09	99.90	
150%	159676	12.04	12.04	99.89	

Table 6: The results are summarized for Trifluridine and Tipracil

Injection	Area for Trifluridine	Area for Tipracil
Injection-1	191345	107339
Injection-2	191232	107232
Injection-3	191671	107131
Injection-4	191999	107399
Injection-5	192898	107018
Injection-6	194679	107089
Average	192304.0	107201.3
Standard Deviation	1308.1	148.4
%RSD	0.7	0.1

Table 7: The results are summarized for Trifluridine and Tipracil

Injection	Area for Trifluridine	Area for Tipracil
Injection-1	192345	104533
Injection-2	192432	104232
Injection-3	192971	104531
Injection-4	192899	104399
Injection-5	192898	104018
Injection-6	192333	104689
Average	192646.3	104400.3
Standard Deviation	305.8	241.9
%RSD	0.2	0.2

Table 8: Robustness studies for Telmisartan and Azelnidipine

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	3828.18	1.21
2	1	3417.62	1.14
3	1.1	3328.18	1.11

Table 9: System suitability results for Azelnidipine

S. No	Flow Rate (ml/min)	System Suitability Results		
		USP Plate Count	USP Tailing	USP Resolution
1	0.9	3213.92	1.23	4.96
2	1	2381.56	1.11	4.42
3	1.1	3415.92	1.21	4.96

Table 10: System suitability results for Telmisartan:

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3726.18	1.21
2	*Actual	3417.62	1.14
3	10% more	3343.64	1.34

Table 11: System suitability results for Azelnidipine

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results		
		USP Plate Count	USP Tailing	USP Resolution
1	10% less	3175.92	1.31	4.96
2	*Actual	2381.56	1.11	4.42
3	10% more	34445.92	1.23	4.96

***** Results for actual Mobile phase composition (50:50 Buffer: ACN) have been considered.

Table 12: Comparison of Telmisartan and Azelnidipine:

Sample Name	Telmisartan	
	Area	**% Degraded**
Standard	191642	
Acid	183252	4.38
Base	183532	4.23
Peroxide	183253	4.38
Thermal	187552	2.13
Photo	186452	2.71
Sample Name	**Azelnidipine**	
	Area	**% Degraded**
Standard	107223	
Acid	98959	7.71
Base	98921	7.74
Peroxide	98978	7.69
Thermal	98851	7.81
Photo	98789	7.87

4. Conclusion

The study has been concluded that a new method was established for simultaneous estimation of Azelnidipine and Telmisartan by RP-HPLC methods. The chromatographic conditions were successfully developed for the separation of Azelnidipine and Telmisartan by using Inertsil ODS C18 column (4.6×250mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) ACN : KH2PO4 ph 3, detection wavelength was 225nm. The instrument used for HPLC , WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.798 mins and 3.587 mins[8-11]. The % purity of Azelnidipine and Telmisartan was found to be 99.87% and 100.27% respectively. The system suitability parameters for Azelnidipine and Telmisartan such as theoretical plates and tailing factor were found to be 4260, 1.2 and 5085 and 1.2, the resolution was found to be 7.67. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Azelnidipine and Telmisartan was found in concentration range of 50µg-250µg and 15µg-55µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 98.56% and 99.96%, %RSD for repeatability was 1.2, % RSD for intermediate precision was 1.9. The precision study was precision, robustness and repeatabilty.LOD value was 3.72 and 0.0242 and LOQ value was 7.40 and 0.0202 respectively.

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