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Analytical Method Development and Validation for the Simultaneous Estimation of Azelnidipine and Telmisartan

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ABSTRACT

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, thedetection 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.

INTRODUCTION

Chromatography

The chromatographywas discovered by Russian Chemist and botanist *Micheal Tswett* (1872-1919) who first used the term chromatography (colour writing derived from Greek for colour – Chroma , and write – graphein) to describe his work on the separation of coloured plant pigments into bands on a column of chalk and other material such as polysaccharides, sucrose and insulin. " *Chromatography is a method in which the components of a mixture are separated on an adsorbent column in a flowing system*".The adsorbent material, or stationary phase, first described by Russian scientist named Tswett in 1906, has taken many forms over the years, including paper, thin layers of solids attached to glass plates, immobilized liquids, gels, and solid particles packed in columns. "Chromatography is a physical method of separation in which the component to be separated are distributed between two phases of which in stationary while other moves in a definite direction (IUPAC)"

Types of Chromatography

The mobile phase could be either a liquid or a gas, and accordingly we can subdivide chromatography into Liquid Chromatography (LC) or Gas Chromatography (GC). Apart from these methods, there are two other modes that use a liquid mobile phase, but the nature of its transport through the porous stationary phase is in the form of either (a) capillary forces, as in planar chromatography (also called Thin-Layer Chromatography, TLC), or (b) electro osmotic flow, as in the case of Capillary Electro Chromatography (CEC).

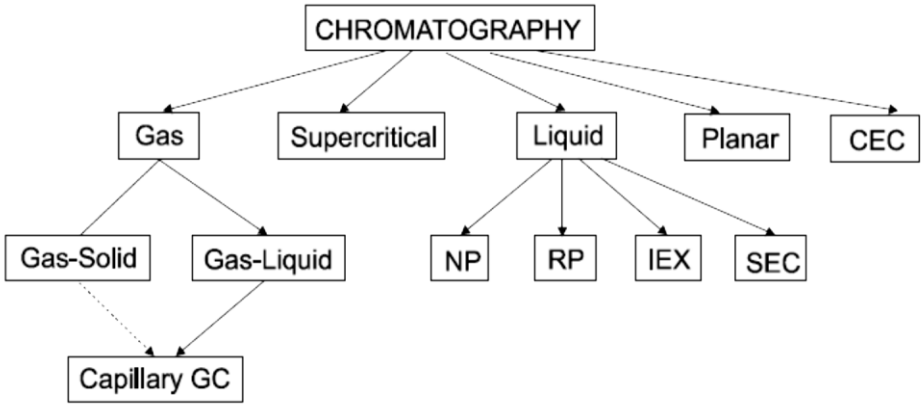

Fig 1: Classification of chromatography[4]

Objective

- The objective of the present work is to development and validates a HPLC method with PDA detector for the development and validation Azelnidipine and Telmisartan of tablets.
- To be employed in routine and stability tests.In the method development of Azelnidipine and Telmisartan we have decided to carry out our project work by incorporating the reverse phase high performance liquid chromatography (HPLC).
- Then the developed method will be validated according to ICH guidelines for its various parameters.

MATERIALS AND METHODS

Table 1: Instruments used

S.No	Instruments And Glass wares	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	Labman

Table 2: Chemicals used

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

HPLC METHOD DEVELOPMENT

TRAILS

Preparation of standard solution: Accurately weigh and transfer 10 mg of Azelnidipine and Telmisartan working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.4ml of Azelnidipine and 0.2ml of Telmisartan from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

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) (37:63 v/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) (37:63 v/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 ultra sonication.

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.

RESULTS AND DISCUSSION

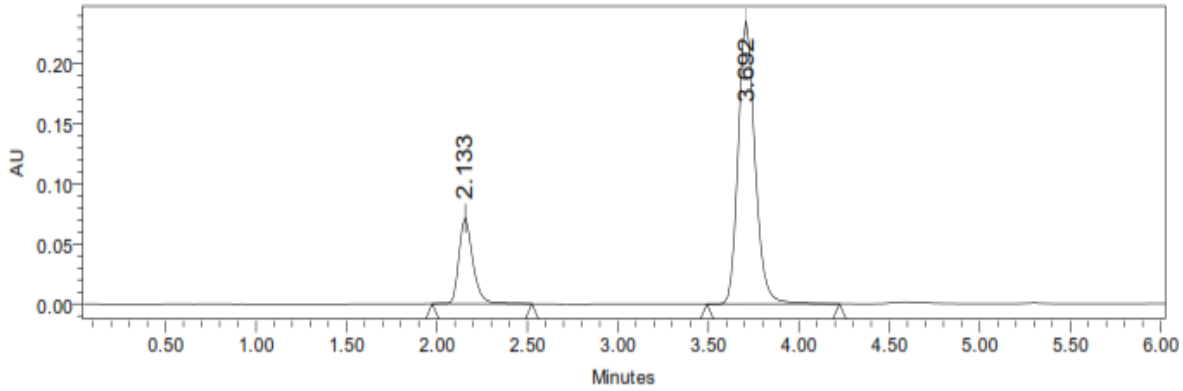

Fig 2: Optimized Chromatogram (Standard)

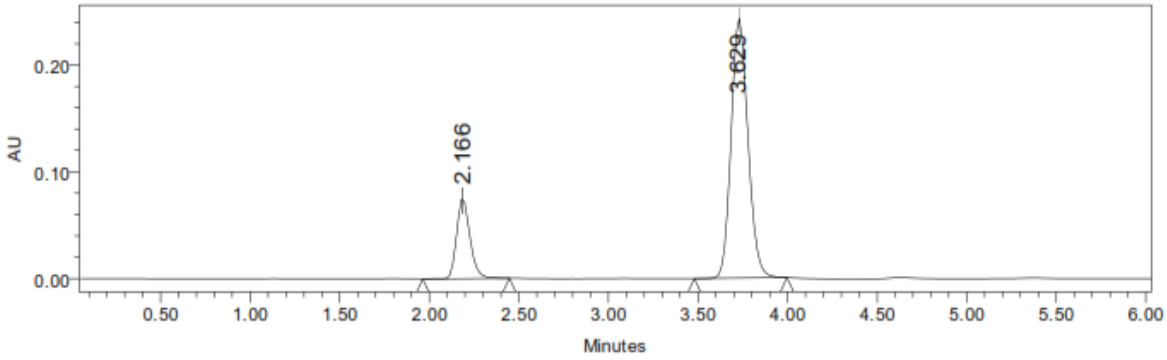

Fig 3: Optimized Chromatogram (Sample)

VALIDATION

System Suitability

Table 3: Results of system suitability for Azelnidipine

S.No.			Area	Height		
1	Azelnidipine	2.152	526358	86598	5695	1.56
2	Azelnidipine	2.157	526548	86254	5652	1.57
3	Azelnidipine	2.141	526854	86598	5627	1.56
4	Azelnidipine	2.133	526598	86245	5692	1.57
5	Azelnidipine	2.166	524874	86521	5641	1.56
Mean			526246.4			
Std. Dev.			787.353			
% RSD			0.149617			

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

Table 4: Results of system suitability for Telmisartan

S.No.			Area	Height			Resolution
1	Telmisartan	3.674	1682821	1686958	8659	1.56	9.8
2	Telmisartan	3.631	1682726	1685745	8675	1.57	9.9
3	Telmisartan	3.625	1687361	1685421	8692	1.56	9.8
4	Telmisartan	3.692	1682811	1685242	8642	1.57	9.8
5	Telmisartan	3.629	1683816	1685364	8635	1.58	9.8

Mean			1683907				
Std. Dev.			1982.03				
% RSD			0.117704				

Specificity

Table 5: Peak results for assay standard of Azelnidipine

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Azelnidipine	2.152	526358	86598	1.56	5698	1
2	Azelnidipine	2.198	526584	86784	1.57	5687	2
3	Azelnidipine	2.179	529658	86253	1.56	5639	3

Table 6: Peak results for assay standard of Telmisartan

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Telmisartan	3.646	1687589	365879	1.80	8659	1
2	Telmisartan	3.604	1685987	365854	1.79	8697	2
3	Telmisartan	3.610	1685974	369854	1.80	8675	3

Table 7: Peak results for Assay sample of Azelnidipine

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Azelnidipine	2.152	536859	87584	1.58	5789	1
2	Azelnidipine	2.150	532654	87965	1.59	5784	2
3	Azelnidipine	2.187	532685	87465	1.58	5769	3

Table 8: Peak results for Assay sample of Telmisartan

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Telmisartan	3.646	1698568	378562	1.81	8759	1
2	Telmisartan	3.651	1698574	375847	1.80	8795	2
3	Telmisartan	3.601	1698547	376584	1.81	8745	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

= 99.89%

The % purity of Azelnidipine and Telmisartan in pharmaceutical dosage form was found to be 99.89%

Linearity

Chromatographic Data For Linearity Study Of Azelnidipine

Table 9: Chromatographic Data for Linearity Study of Azelnidipine

Concentration µg/ml	Average Peak Area
20	272897
30	402986
40	526389
50	649785
60	769287

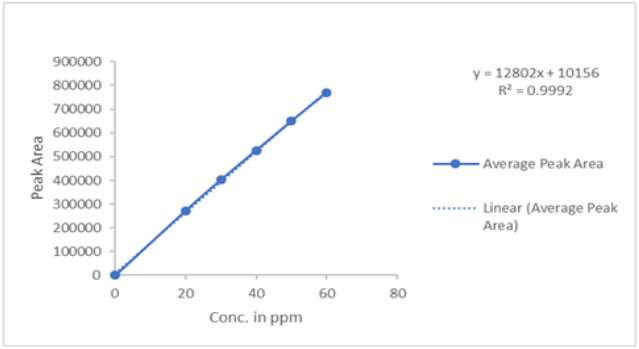

Fig 4: Calibration Curve of Azelnidipine

Correlation Coefficient (r) is 0.99, and the intercept is 10156. These values meet the validation criteria.

Chromatographic Data For Linearity Study Of Telmisartan

Table 10: Chromatographic Data for Linearity Study of Telmisartan

Concentration µg/ml	Average Peak Area
10	1000237
15	1448768
20	1887285
25	2365897
30	2826845

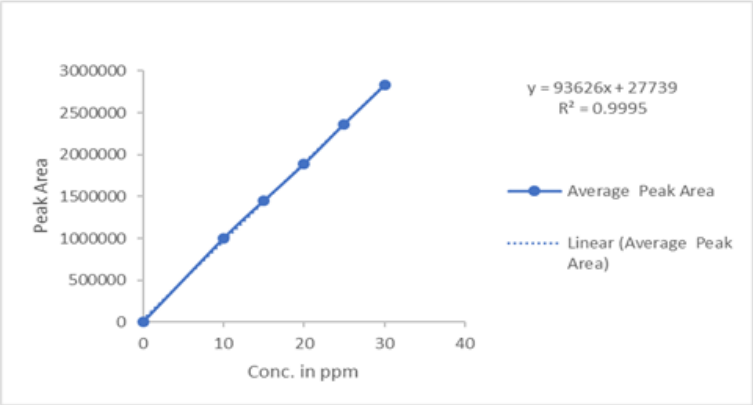

Fig 5: Calibration Curve of Telmisartan

Correlation Coefficient (r) is 0.99, and the intercept is 27739. These values meet the validation criteria.

Precision

Repeatability

Table 11: Results of repeatability for Azelnidipine

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Azelnidipine	2.157	526358	86598	5689	1.56
2	Azelnidipine	2.159	524856	86542	5687	1.57
3	Azelnidipine	2.186	526985	86578	5684	1.56
4	Azelnidipine	2.160	528654	86354	5689	1.56
5	Azelnidipine	2.170	528457	86958	5639	1.56
Mean			527062			
Std.dev			1569.114			
%RSD			0.297709			

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table 12: Results of Repeatability for Telmisartan:

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Telmisartan	3.603	1687589	367859	8659	1.79
2	Telmisartan	3.608	1685987	368547	8679	1.80
3	Telmisartan	3.600	1685987	367985	8645	1.80
4	Telmisartan	3.696	1685754	365874	8695	1.79
5	Telmisartan	3.629	1685985	364589	8625	1.79
Mean			1686260			
Std.Dev			749.493			
%RSD			0.044447			

Accuracy

Table 13: The 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%	

- The percentage recovery was found to be within the limit (98-102%).

Table 14: The 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%	

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

LIMIT OF DETECTION

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

AZELNIDIPINE

Result:

= 1.04µg/ml

TELMISARTAN

Result: = 3.12µg/ml

QUANTITATION LIMIT

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

AZELNIDIPINE

Result: =2.1µg/ml

TELMISARTAN

Result: =6.3µg/ml

Robustness

Table 19: Results for Robustness 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

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Table 20: Results for Robustness 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

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Azelnidipine and Telmisartan in 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) (37:63 v/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 Telmisartanin bulk drug and in Pharmaceutical dosage forms.

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