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Research Article

The Method Development And Validation Of A High-Performance Liquid Chromatographic Method For Azilsartan Analysis

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ABSTRACT

The goal of the current study is to develop an RP-HPLC-based analytical method for determining the dosage of Azilsartan in bulk and tablet form that is fast, precise, sensitive, selective, and repeatable. Create a novel HPLC technique for Azilsartan estimation, and validate it in compliance with ICH guidelines. In order to make use of the accepted methodology for Azilsartan estimation in pharmaceutical formulations, an RP-HPLC method was utilized to create and validate a stability indicating method. Using an Inertsil-ODS C18 (250×4.6mm, 5µm) column and a 90:10 v/v methanol: acetonitrile mobile phase at a flow rate of 1 ml/min, the estimation was carried out using RP-HPLC. Azilsartan's HPLC linearity range was 20–70 µg/ml, and its R2 value was 0.999. Additionally, the method satisfies the robustness parameter requirement. The method's accuracy, precision, sensitivity, and economy are demonstrated by the findings. HPLC is a faster method. The medicinal dose form was successfully administered using the procedure.

INTRODUCTION

In 2011, the USFDA authorized Azilsartan medoxomil (AZM), a chemically complex monopotassium salt of 5-methyl-2-oxo-1,3-dioxol-4-yl)methyl 2-ethoxy-1-{[2'-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)biphenyl 4yl]methyl}-1Hbenzimidazole-7 carboxylate-7 carboxylate.[1] Fig. 1 shows the structural formula for AZM. AZM is a prodrug that lowers blood pressure. When the medoxomil ester is hydrolyzed, the active component of AZM is exposed. This results in the conversion of azilsartan, an active blocker of the angiotensin II receptor that lowers blood pressure more quickly than valsartan and olmesartan in a 24-hour period.[2, 3]

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There have been reports of several quantification methods for AZM from pure medication, various pharmaceutical formulations, and biological materials. Treatment for hypertension with azilsartan medoxomil and chlorthalidone works well together. Numerous analytical techniques, either by themselves or in conjunction with other medications, are demonstrated in the literature search. These techniques include UV spectrophotometry, high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), and high performance thin-layer chromatography (HPTLC). [4–13]

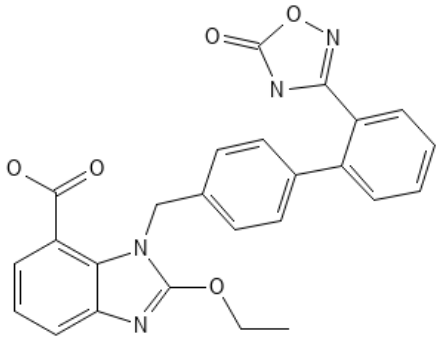

Fig. 1: Chemical structure of Azilsartan

Since RP-HPLC and HPTLC are the most widely used analytical techniques for estimating a drug's specificity and sensitivity, the current study aimed to develop an RP-HPLC method that is straightforward, accurate, sensitive, and precise for the analysis of azilsartan medoxomil in pharmaceutical formulations.

1. Instrumentation:

HPLC System:

Chromatographic separation was accomplished utilizing the RP-HPLC Waters system, which included the Waters Model No. 2690/5 with PDA detector and the Waters Empower-2 software from the Waters Corporation as the data processor. Column for analysis: Inertsil-ODS C18 (250 x 4.6 mm, 5µ).

Sonicator:

Sonication of solvents and various preparation was done by using sonicator of IKON Industries Ultrasonic Bath.

UV System:

The wavelength of Azilsartan was determined using a Systronics-UV Model No. 119 with a pair of 10mm matched quartz cells. The wavelength was calculated by scanning a standard solution in a UV spectrophotometer from 200 nm to 400 nm in spectrum mode.

2. Reagents:

MERCK Limited, Mumbai, contributed HPLC grade methanol and acetonitrile, whereas Bharath Life Science Pvt. Ltd. provided a pure sample of working standard Azilsartan as a gift.

3. Preparation of standard solution:

Take 100mg Azilsartan working standard in 100ml V.F add methanol sonicate it 30 minutes, (That is 1000ppm solution).

4. Preparation of stock solution preparation:

Take 100mg Azilsartan working standard in 100ml V.F add methanol sonicate it 30minutes, (That is 1000ppm solution).

Further Dilution (or) Optimized Method Solutions Preparation:

Take 4ml of above solution in 100ml V.F add Methanol up to mark sonicate it 10minutes (That 40ppm solution).

HPLC METHOD OPTIMIZATION:

For method optimization various mobile phase were tried in different ratio.

Optimized Method Stock Solution Preparation:

Mobile Phase: Methanol:

Acetonitrile (90:10)V/V. Sonicate it 30minets, Filter this mobile phase through 0.45micron filter paper.

Optimized Method Stock Solution Preparation:

Take 100mg Azilsartan working standard in 100ml V.F add methanol sonicate it 30minets,(That is 1000ppm solution).

Further Dilution (or) Optimized Method Solutions Preparation:

Take 4ml of above solution in 100ml V.F add Methanol up to mark sonicate it 10minets(That 40ppm solution).

Chromatographic Conditions:

Table no.1: Chromatographic conditions for optimized trial.

Parameter	Method
Stationary phase (column)	Inertsil-ODS C18(250 x 4.6 mm, 5 µ)
Mobile Phase	Methanol: Acetonitrile (90:10)
Flow rate	1.0 ml/min
Run Time	6 min
Column of Temperature	Ambient
Volume of Injection loop	20
Detection Wavelength	236 nm
Drug RT (min)	3.237 min

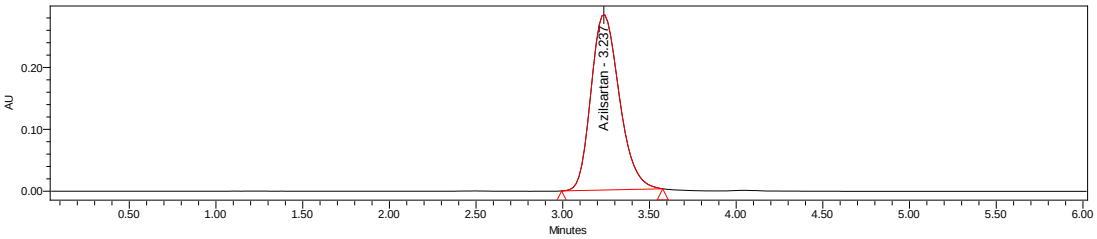

Fig 2. Optimized Method Development Trial Chromatogram of Azilsartan

RESULTS AND DISSCUSSION:

Validation:

1. System suitability:

A Standard solution was prepared by using Azilsartan working standard as per test method and was injected Five times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Azilsartan, retenton times and peak areas.

Table no.2: System suitability data

Injection	RT	Peak Area	USP Plate Count	USP Tailing
1	3.237	1572654	9508	1.111
2	3.236	1573421	9554	1.114
3	3.235	1572901	9599	1.115
4	3.237	1574810	9530	1.112
5	3.232	1573001	9505	1.113
Mean	3.2354	1573357	9539	1.113
SD	0.002074	857.9314	-------	-------
% RSD	0.064092	0.054	-------	-------

2. Precision:

Repeatability:

System precision: Standard solution prepared as per test method and injected five times.

Method precision: Prepared six sample preparations individually using single as per test method and injected each solution.

System Precision:

Table no.3: System precision data

Concentration 40 ppm	Injection	Peak Areas of Azilsartan	% Assay
	1	1572650	100.18
	2	1572935	100.19
	3	1573485	100.23
	4	1575980	100.39
	5	1573805	100.25
Statistical Analysis	Mean	1573771	100.25
	SD	1315.121	0.083
	% RSD	0.083	0.083

Method Precision:

Table no.4: Method precision data

Concentration 40 ppm	Injection	Peak Areas Azilsartan	% Assay
	1	1572550	100.17
	2	1571440	100.10
	3	1570440	100.04
	4	1570015	100.01
	5	1572008	100.14
	6	1572201	100.15
Statistical Analysis	Mean	1571442	100.10
	SD	1016.311	0.064
	% RSD	0.064	0.064

Intermediate precision:

Table no.5: Intermediate precision data

Concentration 40 ppm	Injection	Peak Areas of Azilsartan	% Assay
	1	1578336	100.54
	2	1577003	100.45
	3	1577905	100.51
	4	1578304	100.54
	5	1578005	100.52
	6	1578511	100.55
Statistical Analysis	Mean	1578010	100.52
	SD	541.998	0.034
	% RSD	0.034	0.034

3. Accuracy:

A study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of Azilsartan into each

volumetric flask for each spike level to get the concentration of Azilsartan equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of Azilsartan was calculated.

Table no.6: Accuracy data

Concentration % of Spiked level	Amount Added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery
50 %	20	20.03	100.17	Mean- 100.22 % RSD- 0.046
50%	20	20.04	100.24	
50%	20	20.05	100.26	
100 %	40	40.07	100.17	Mean- 100.27 %RSD-0.083
100%	40	40.12	100.31	
100%	40	40.12	100.32	
150%	60	60.08	100.14	Mean-100.14 %RSD-0.033
150%	60	60.06	100.10	
150%	60	60.10	100.17	

4. Linearity:

A Series of solutions are prepared using Azilsartan working standard at concentration levels from 20ppm to 70 ppm of target concentration.

Table no.7: Linearity data (concentration vs peak area)

Concentration (ppm)	Average Area
0	0
20	786016
30	1179026
40	1572034
50	1950480
60	2358051
70	2751060

Statistical Analysis of Linearity

Table no.8: Statistical analysis of Linearity

Slope	39259
y-Intercept	-554.7
Correlation Coefficient	0.999

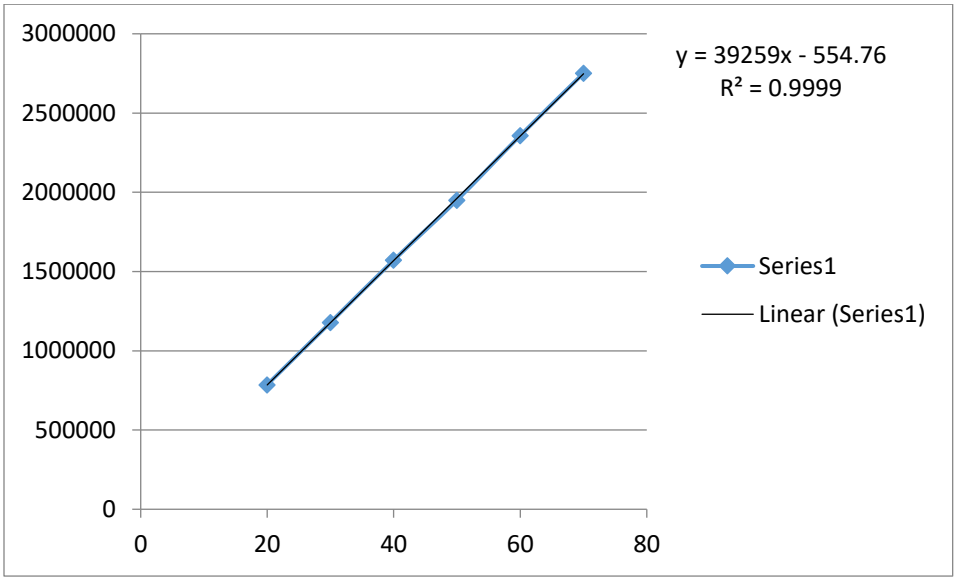

Fig 3: Plot of Linearity (concentration vs peak area)

5. Ruggedness:

System to system variability:

System to system variability study was conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared and each was analyzed as per test metho d. Comparison of both the results obtained on two different HPLC systems, shows that the assay test method were rugged for System-to-system variability. For system 1, refer to system suitability data; Table no.2

Table no.9: Ruggedness data for system 2

Sr no	Peak Area	Assay % of Azilsartan
1	1572006	100.14
2	1572580	100.17
3	1573904	100.26
4	1573308	100.22
5	1572010	100.14
6	1573050	100.20
Mean	1572809	100.19
% RSD	0.047	0.047

6. Robustness:

Effect of variation of flow rate:

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 1.0ml/min and 1.2ml/min. The system suitability parameters were evaluated and found to be within the limits for 1.0ml/min and 1.2ml/min flow.

Table no.10: Data for robustness (Flow rate 0.8ml)

Flow 0.8 ml	Std. Area	Tailing factor
	1551645	1.111
	1552804	1.115
	1552055	1.117
	1552403	1.123

	1553220	1.122
Avg	1552425	1.117
SD	616.7352	0.0049
% RSD	0.039	0.445

Table no.11: Data for robustness (Flow rate 1.0ml)

Flow 1.0 ml	Std. Area	Tailing factor
	1572648	1.115
	1572056	1.117
	1572331	1.115
	1573010	1.116
	1572900	1.117
Mean	1572589	1.116
SD	392.2625	0.001
% RSD	0.025	0.089

Table no.12: Data for robustness (Flow rate 1.2ml)

Flow 1.2	Std. Area	Tailing factor
	1595770	1.128
	1595360	1.130
	1595230	1.129
	1595039	1.129
	1595619	1.128
Mean	1595403	1.128
SD	293.9461	0.0008
% RSD	0.018	0.074

7. LOD and LOQ (limit of detection and limit of quantitation):

Table no.13: Values of LOD AND LOQ

LOD	0.072µg/ml
LOQ	0.218µg/ml

Marketed Sample Analysis:

Table no.14: Information of marketed sample of Azilsartan

Drug name	Brand name	Company
Azilsartan	Abel-40	Lupin

Table no.15: Marketed sample analysis data

Injection	Peak areas of Azilsartan	% Assay
1	1572637	100.18
2	1572091	100.15
3	1572341	100.16
4	1572864	100.19

5	1572065	100.14
Mean	15782400	100.164
SD	347.3655	0.020736
%RSD	0.022091	0.020702

CONCLUSION

Various parameters were examined in order to develop the analytical methodology. To begin with, it was found that Azilsartan had a maximum absorbance of 236 nm. A great peak region was obtained by setting the injection volume at 20µl. In this job, the Inertsil C18 column was used, and ODS selected a good peak form. It was found that the ambient temperature was suitable for the kind of pharmaceutical solution. The flow rate was set at 1.0 ml/min due to the good peak area, sufficient retention length, and good resolution. Several mobile phase ratios were examined; but, due to its symmetrical peaks and good resolution, the mobile phase with a Methanol: Acetonitrile (90:10) ratio was selected. Consequently, this mobile phase was utilized in the intended investigation.

It was found that the system and technique both had precise and well-within-range accuracy. The linearity investigation yielded the correlation coefficient and curve fitting. The analytical method was demonstrated to be linear for both medications throughout a range of 20–70 ppm of the target concentration. The analysis passed the ruggedness and robustness testing. In both cases, the relative standard deviation was very good.

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