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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF AZILSARTAN MEDOXOMIL IN TABLET DOSAGE FORM

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ABSTRACT

Present study aims to develop a simple, precise and accurate method for estimation of Azilsartan Medoxomil in tablet dosage form. The wavelength and solvent system were optimized in order to maximize the sensitivity of the proposed method. Azilsartan shows the maximum absorbance at 248 nm. The separation was achieved on RP-HPLC Isocratic system equipped with HPLC Agilent 1100 series. The mobile phase was prepared with buffer pH 3.0 and ACN in the ratio of 55:45, pH was adjusted with o – phosphoric acid (pH 3.0). Run through Octadecylsilane Column 150mm X 4.6mm, 5µ (Hypersil BDS 18C) with a flow rate of 1.5 ml/min for 10 min run time. System suitability parameters like plate count and tailing factor were passed according to ICH guidelines. Percentage recovery of Azilsartan was 99.03%. Linearity was observed in the concentration range of 70-130% and correlation coefficient of Azilsartan was obtained 0.99962. Robustness was found to be within the limits i.e. not 2. Stability studies were done and % degraded was within limits. A simple method was developed for estimation of Azilsartan. The RP-HPLC method was developed and validated for estimation of Azilsartan Medoxomil in tablet dosage form.

KEYWORDS: Azilsartan Medoxomil, RP-HPLC, Stability indicating, Method development, Validation.

1. INTRODUCTION

RP-HPLC includes the partition of atoms based on hydrophobicity. Excellent resolution can be achieved under a wide range of chromatographic conditions. Chromatographic selectivity can be manipulated through changes in mobile phase characteristics.

Azilsartan medoxomil chemically (AZM) known as 5-methyl-2-oxo-1,3-dioxol-4-yl) methyl 2-ethoxy-3-[[4-[2-(5-oxo-2~{H}-1,2,4-oxadiazol-3-yl)phenyl]phenyl]methyl] benzimidazole-4-carboxylate and molecular formula is $C_{25}H_{20}N_4O_5$. Azilsartan was practically insoluble in water but freely soluble in methanol.

Azilsartan Medoxomil is an anti-hypertensive. Azilsartan medoxomil hinders the angiotensin ii type 1 receptor counteracting angiotensin ii from authoritative and causing vasoconstriction. Azilsartan's capacity to remain firmly bound to AT1 receptors for extensive stretches after medication washout is among its most abnormal highlights. Azilsartan medoxomil decreases the pressure effect of angiotensin II. Accordingly, angiotensin I, angiotensin II, and renin are expanded while aldosterone is diminished. After oral administration Azilsartan medoxomil blocks the angiotensin II type 1 receptor preventing angiotensin II from binding and causing vasoconstriction. In this paper we portray a straightforward yet quick, particular and exceedingly touchy HPLC strategy, not requiring test treatment, for assurance of Azilsartan Medoxomil. We also have laid emphasis on the stability indicating assay method of sample by HPLC.

Chemical Structure of Azilsartan Medoxomil

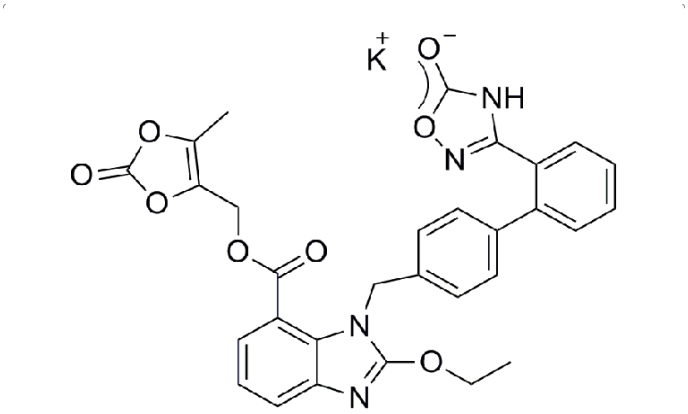


Figure 1: Azilsartan Medoxomil.

2. MATERIALS AND METHOD

2.1 Materials

Azilsartan Medoxomil, Active Pharmaceutical ingredient (API) and working standard was supplied by Alkem Laboratories (Taloja, Navi Mumbai). Tablet of Azilsartan Medoxomil as a sample for respected study were provided by the same firm.

2.2 Chemicals and Reagents

SR NO.	Chemicals/Solvent		Grade
1.	Potassium Dihydrogen Phosphate	:	AR Grade or Equivalent
2.	Water	:	HPLC Grade or Equivalent
3.	Methanol	:	AR Grade
4.	Acetonitrile	:	HPLC Grade
5.	Orth phosphoric Acid	:	AR Grade

2.3 Apparatus and Equipment

A. HPLC Equipped with Pump, UV Detector, Make: Agilent 1100 Series.

B. HPLC Equipped with Pump, Injector and PDA Detector, Make: Agilent 1100series.

C. Analytical Balance, Make: Mettler Toledo.

D. Hot Air Oven, Make: Thermolab.

E. Photo stability Chamber, Make: Newtronic

F. Sonicator, Make: Spectra Lab UCB 300D.

2.4 Chromatographic Conditions

Chromatographic separation was performed on a reverse phase Thermo Fisher Sceintifc C18 Shield. The mobile phase was a mixture of buffer pH 3.0: ACN (55:45) v/v.

Column	**:**	**Octadecylsilane Column 150 x 4.6 mm, 5µ (Hypertsil BDS C18, or equivalent)**
Flow Rate	**:**	1.5 ml/min
Wavelength	**:**	248 nm
Injection Volume	**:**	10 µL
Column Temperature	**:**	40°C
Sample Compartment Temperature	**:**	10°C
Run Time	**:**	10 min
Retention Time	**:**	6.5 min

2.5 Preparation of buffer pH 3.0

Dissolve 6.8g of Potassium Dihydrogen Orthophosphate in 1000ml of water. Adjust pH to 3.0 with Orthophosphoric acid.

2.6 Preparation of diluent

ACN: Water (75:25).

2.7 Preparation of standard solution

Weigh accurately about 43mg of Azilsartan Medoxomil working standard into 50ml volumetric flask. Add 40ml of diluent, sonicate to dissolve and dilute to the mark with

diluent. Further dilute 5ml of this solution with 50 ml of mobile phase. (maintain cold condition during processing).

2.8 Analysis of Marketed Formulation

Assay was performed by using tablets of Azilsartan Medoxomil for the preparation of sample solution 5 tablets were used. Lightly crush 5 tablets into 2-3 pieces. Weigh and transfer five lightly crush tablets (equivalent about 400mg of Azilsartan Medoxomil) into 50ml volumetric flask, and add about 20ml of water then sonicate fir 5min with intermittent shaking and add 350ml of diluent and sonicate for about 30min with intermittent shaking and make up the volume with diluent. Filter through 0.45µ PVDF filter. Further dilute 5ml of this solution to 50ml of mobile phase.

(Maintain cold condition during processing).

3. METHOD VALIDATION

The method was validated for linearity, accuracy, precision, specificity, robustness, filter paper study, solution stability and forced degradation study.

3.1 Accuracy

Placebo was spiked with the known amount of Azilsartan Medoxomil at 70%. 100% and 130% of test concentration as Azilsartan Medoxomil Tablet (80mg Azilsartan Medoxomil per tablet). The amount of Azilsartan Medoxomil was quantified as per the test method. The percentage recovery was calculated from the amount found and the actual amount added.

3.2 Precision

The instrument precision was evaluated by determining the absorbance of the standard solution six times repeatedly. The outcomes are accounted for as far as relative standard deviation. The intra-day and inter-day variation for the determination was carried out in triplicate for the standard solution.

3.3 Linearity

Linearity for AZM performed over range of 5.659 mcg/ml to 10.510mcg/ml (about 70% to 130%) test concentration. A graph was plotted with concentration (in mcg/ml) on X- axis and peak areas on Y-axis. Slope, Y-intercept, correlation coefficient (r-value) and residual sum of sequences (RSS) were determined.

3.4 Specificity

Specificity is the ability of a method to discriminate between the analyte(s) and other components in sample. Blank (mobile phase), Placebo, standard and sample solution were injected into the HPLC system (in triplicate preparation). Volume of 10µl working placebo sample solution was injected into the chromatogram and chromatogram was recorded.

3.5 Stability in analytical solution

Prepared standard and one sample solution as per test method and injected into HPLC at initial and different time intervals up to 24 hours and further at 25°C. Determined % assay at different intervals. The difference in the results shall not be more than 2.0

3.6 Filter paper selection study

Selection of filter paper was evaluated by preparing the assay preparation in triplicate as per test method. Filtered the test solution through 0.45µm PVDF filter and 0.45µm Nylone filter analysed the samples against centrifuged a portion of sample solution.

3.7 Robustness

Robustness of proposed method for Azilsartan Medoxomil was carried out by changing the optimum HPLC conditions set for this method. The small changes include,

a) Flow rate: ± 10%.
b) pH mobile phase: ± 2.
c) Temperature: ± 5°C.
d) Organic content: ± 2 %.

3.8 Force degradation

Force degradation studies were carried out as per ICH guideline. The study was performed by subjecting the drug substance to acid, alkaline, oxidative, thermal, photolytic and ultraviolet degradations.

Degradation Condition	Condition details
Acid	1 mL of 0.1N HCL at RT
Alkali	1 mL of 0.01N NaOH at RT
Oxidation	1 mL of 3.0% H_2O_2 at RT
Humidity	25°C /90% RH for 24 Hrs
Thermal	105°C for 6 Hrs
U.V.	248 nm for 48 Hrs

3.9 System suitability

System suitability for this method was tested by five replicates injections of standard preparation. Plate tally, following variable, goals and % RSD were accounted for.

3.10 Solution Stability

Solution stability of standard and sample solutions were prepared at room temperature. The solutions were analysed after 4, 6, 8, 10, 12, 14 hours. The relative standard deviation was found to be below 2.0% in this method.

3.11 Assay

Percentage labeled amount was found by performing assay. Sample and standard solution of same concentration were prepared and sample peak area were compared to the standard peak area.

4. RESULT AND DISCUSSION

An Isocratic reverse - phase HPLC procedure was suggested as a suitable method for the assay of Azilsartan Medoxomil. In this method development process, many trials were done with different columns, mobile phase compositions, by changing the buffer and its pH. The peaks of active ingredient and other additives as well as the degradation product were separated out by the RP-HPLC method. Complete validation studies for this method proved them to be specific, linear, robust and reproducible.

4.1 System Suitability

Framework appropriateness was assessed by infusing standard arrangement amid different long stretches of approval. The tailing factor for the first standard injection and % relative standard deviation for the peak areas of Azilsartan Medoxomil from five replicate injections of standard solution were verified at every stage.

The results are tabulated in table -

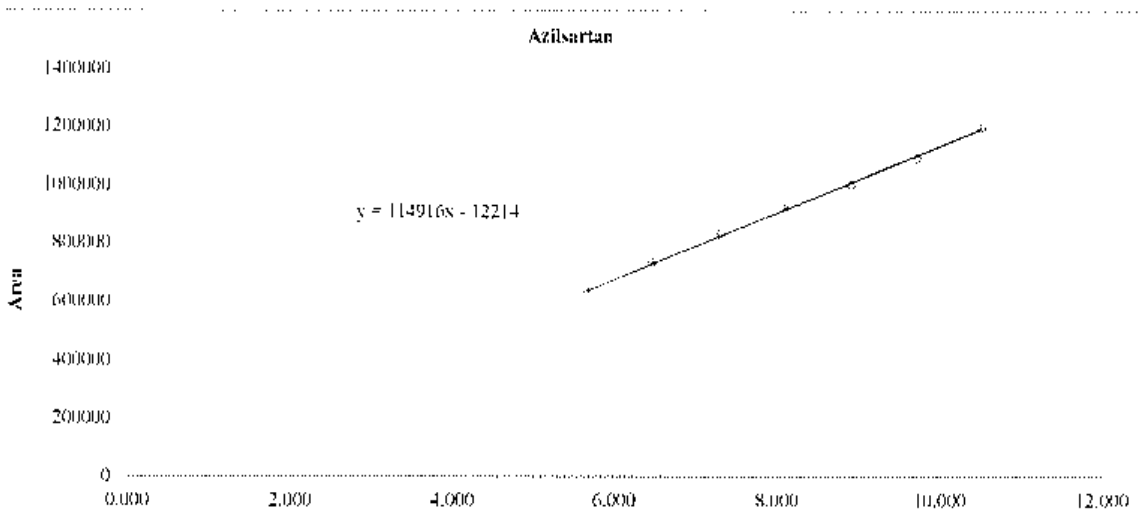
Retention Time (Mean ± SEM)	Theoretical plates (n)	Tailing (T)
6.580	7717	1.2

4.2 Linearity

Linearity was established by plotting a graph between concentration verses peak area and the correlation coefficient was determined.

Table- Linearity data of AZM.

Spike level in %	Concentration mcg/ml	Area
70	5.659	634352
80	6.468	730908
90	7.276	831166
100	8.085	921123
110	8.893	1003584
120	9.702	1096455
130	10.510	1200335
Slope	114916	
y-intercept	-12214	
r-value	0.99962	
RSS	184307183	



Linearity plot for Azilsartan.

4.3 Specificity

Blank (mobile phase), Placebo, standard and sample solution were injected into the HPLC system. There was no interference from the blank and placebo at the retention time of Azilsartan Medoxomil peak. Peak purity data reveals that Azilsartan Medoxomil peak was homogeneous and there were no co-eluting peaks at the retention time of Azilsartan Medoxomil peak.

Sample type	Retention Time (min)	Peak purity
Standard	6.307	999.995
Control Sample	6.327	999.997

4.4 Method Precision

The precision of method was evaluated by calculating the % RSD of peak areas of six replicate injections of standard concentration.

Method Precision

Sr. No.	% Assay
1	102.33
2	102.64
3	103.54
4	102.58
5	102.54
6	103.37
Mean	102.83
SD	0.496
% RSD	0.48

4.5 Method Recovery

The accuracy was carried out using various set of different standard addition method of different concentration levels, 70%, 100% and 130%, and then comparing the difference between the spiked value and actual found value.

Table – Method Precision.

Level no/Spike level in %	Actual Amount of Azilsartan Medoxomil added in mg	Amount of Azilsartan Medoxomil found in mg	%Recovery	Mean	SD	% RSD
Level – 1 (70%)	279.61	299.85	100.70	100.81	0.297	0.29
	279.70	299.94	100.59			
	279.93	300.19	101.15			
Level – 2 (100%)	400.26	429.23	98.63	98.72	0.090	0.09
	400.03	428.98	98.81			
	400.46	429.44	98.72			
Level – 3 (130%)	519.47	557.06	99.08	99.03	0.146	0.15
	519.79	557.41	98087			
	519.36	556.95	99.15			
Over all mean			99.52			
Over all SD			0.993			
Over all % RSD			1.00			

4.6 Method Robustnes

Robustness of proposed method for Azilsartan Medoxomil was carried out by changing the optimum HPLC conditions set for this method. The percentage recovery and RSD were noted for Azilsartan Medoxomil.

Sr. No.	I	II	III	IV	V	VI	VII
1	102.33	100.66	100.73	100.66	101.04	103.74	103.69
2	102.64	100.75	100.82	100.59	101.06	103.75	103.85
3	103.54	100.63	100.80	100.82	101.20	103.82	104.35
4	102.58						

5	102.54						
6	103.37						
Over all Mean		102.12	102.15	102.12	102.26	103.15	103.21
Over all SD		1.146	1.098	1.143	0.952	0.611	0.709
Overall % RSD		1.12	1.07	1.12	0.93	0.59	0.69

Sr. No	Experiment (Actual Value)
I	Method Precision data
II	Plus Temperature (35°C)
III	Minus Temperature (25°C)
IV	Plus flow (1.65ml/min)
V	Minus flow (1.35ml/min)
VI	Plus organic content
VII	Minus organic content

The method is robust for change in flow rate, change in column oven temperature and change in organic content in mobile phase.

4.7 Solution Stability

Stability of Azilsartan Medoxomil peak in analytical solution was verified by analyzing the standard solutions and sample solutions, initially and also at different time intervals as mentioned below by storing in sample compartment of HPLC instrument at 10°C. Calculated the % assay for standard solution and sample solutions.

Table of Solution Stability for standard solution.

Time in hours	% Assay	Difference
Initial	100.05	-
2	100.17	0.12
4	100.51	0.46
6	100.73	0.68
8	100.48	0.43
10	100.9	0.85
12	100.97	0.92
14	100.85	0.80

Table of Solution Stability for sample solution.

Time in hours	% Assay	Difference
Initial	3.18	-
2	3.19	0.01
4	3.19	0.01
6	3.19	0.01
8	3.21	0.03
10	3.21	0.03
12	3.21	0.03
14	3.21	0.03

The difference in assay in both the standard and sample solutions were within the acceptance criteria, hence it was concluded that the standard solution and sample solution is stable up to 14 hours at 10°C.

4.8 Force Degradation Studies

Prepared Azilsartan Medoxomil Placebo, AZM API samples were exposed to different stress conditions to determine peak purity as that of the normal condition. The method was found to be stability indicating for assay of Azilsartan Medoxomil in Azilsartan Tablet (80mg Azilsartan Medoxomil per tablet).

Condition	% Assay	% Degradation	Peak Purity
Untreated Sample	100.49		999.997
Acid Treated sample	89.20	11.23	999.941
Base Treated sample	94.71	5.57	999.933
Peroxide Treated sample	87.69	12.74	999.993
Water Treated sample	76.36	23.58	999.996
Heat Treated sample	100.91	-0.42	999.996
UV Treated sample	97.90	1.97	999.998
Humidity Treated Sample	112.89	-12.34	999.965

4.9 Assay

The assay results were about 998-100%. The mean retention time was found 6.5. The results of assay by using this method indicate that the method is specific for the analysis of Azilsartan Medoxomil without interference from the excipients used to prepare and formulate these tablets.

5. CONCLUSION

RP- HPLC method was found to be stability indicating for estimation of Azilsartan Medoxomil in presence of other degradation products and various excipients used in sold dosage form. Technique approval results have ended up being particular, exact, precise and vigorous just as soundness demonstrating. Under FD experiment samples were stressed various stress conditions and analyzed along with unstressed samples. Therefore, this method can successfully apply for routine analysis.

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