INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN: 2320-2831

IJPAR |Vol.3 | Issue 4 | Oct-Dec-2014
Journal Home page: www.ijpar.com

Research article *Open Access*

Stability indicating RP-HPLC method for determination of azilsartan medoxomil in bulk and its dosage form

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ABSTRACT

A simple, accurate, rapid, precise and novel Reverse phase High Pressure liquid chromatographic method (RP-HPLC) has been developed and validated for determination of Azilsartan Medoxomil in bulk and its dosage form. The selected and optimized mobile phase was 0.05M potassium dihydrogen phosphate pH 4.0: Acetonitrile in ratio of 60:40 and conditions were flow rate (1.0 ml/minute), wavelength (248 nm), Run time was 10 min. The retention time was found to be 3.8.Linearity and range was found to be 10-60 µg/ml. The proposed chromatographic conditions were found appropriate for the quantitative determination of the drugs. The method was validated for accuracy, precision, specificity, linearity, robustness, sensitivity, LOD and LOQ.it was also determined by UV-spectroscopy method. The proposed methods were successfully used for quantitative analysis of tablets. No interference from any component of pharmaceutical dosage form was observed. Validation studies revealed that methods were specific, rapid, reliable, and reproducible.

Keywords: RP-HPLC, Azilsartan Medoxomil, Acetonitrile and linearity

INTRODUCTION

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, liner, precise and accurate and the limit of detection is low and also it offers the following advantages[1].

1. Speed (many analysis can be accomplished in 20 min or less)
2. Greater sensitivity (various detectors can be employed)
3. Improved resolution (wide variety of stationary phases)
4. Reusable columns (expensive columns but can be used for many analysis)
5. Ideal for the substances of low viscosity.
6. Easy sample recovery, handling and maintenance.
7. Instrumentation leads itself to automation and quantification (less time and less labour)
8. Precise and reproducible.
9. Integrator itself dose calculations[2,3].

NORMAL PHASE MODE

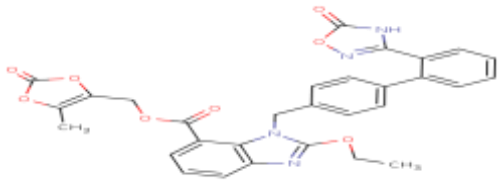
In normal phase mode stationary phase is polar in nature and the mobile phase is non polar. In these techniques non polar compounds travel faster and eluted first.

REVERSE PHASE MODE

In this mode a non polar stationary phase is used and the mobile phase is polar in nature. Hence polar compounds get eluted first and non polar compounds are retained for a long time[2, 3].

DRUG PROFILE OF AZILSARTAN MEDOXOMIL

Structure of Azilsartan Medoxomil



Chemical name: (5-methyl-2-oxo-4-yl) methyl2-ethoxyl-1([2'-(5-oxo-4, 5-dihydro-1,2,4-oxadiazol-3-yl) biphenyl-4-yl] methyl)-1-H-benzimidazole-7-carboxylate

Molecular formula: $C_{25}H_{20}N_4O_5$

Molecular mass: 456.48g/mol

Description: white to nearly white powder

Category: anti –hypertensive (angiotensin2 receptor antagonist)

Dose: it is given in an initial dose of 40mg once daily. This may be increased ,if necessary to a maximum dose of 80mg once daily

Solubility: It is practically insoluble in water and freely soluble in methanol[4,5].

MATERIALS & METHODS

ANALYTICAL METHOD DEVELOPMENT

PREPARATION OF MOBILE PHASE

6.8 gms Potassium dihydrogen phosphate was accurately weighed and taken in a clean 1000ml volumetric flask containing 1 litre milliQ water ,pH 4.0 was adjusted with dilute phosphoric acid The PH was checked by using pH meter and it was recorded as 4.0 and Acetonitrile was taken in the ratio 60 : 40

PREPARATION OF ANALYTICAL CONCENTRATIONS

Accurately weighed and transferred 10 mg of AZL working standard into 10 mL volumetric flask, add 3 mL of diluent and sonicated for 5 min to dissolve and dilute to volume with diluent to get final conc of 1000µg/mL.

Transfer 1 mL of standard stock solution into 10 mL volumetric flask and dilute to volume with diluent to get the final conc of 100µg/mL. Azilsartan medoxomil was prepared 10mcg/mL in mobile phase.

SELECTION OF ANALYTICAL WAVELENGTH

A dilution of 10µg/ml dilution was prepared from standard stock solution .these drug solution was then scanned in the UV region of 200-400 nm and the spectrum was recorded. Azilsartan medoxomil showed good absorbance at 248nm which was selected as wavelength for analysis.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

The best peak shape and maximum separation was achieved with mobile phase composition consisting 0.05M potassium dihydrogen phosphate buffer ph4and acetonitrile in the ratio of 60:40 v/v. the best separation ,peak symmetry and reproducibility were obtained on HYPERSIL BDS c18 250 X 4.6 mm, 5µ, particle size

column. The optimum wavelength for detecting the analyte was found to be 248nm. A flow rate of 1mL/min yielded optimum separation and peak symmetry.

Column	Hypersil BDS C_{18}, 250 X 4.6 mm, 5µ,
Flow rate	1.0 mL /min
Wavelength	248nm
Column temperature	25°C
Injection volume	20 µL
Run time	10 minutes
Diluent	Mobile phase
Elution	Isocratic
Mobile phase	0.05M potassium dihydrogen phosphate pH 4.0 : Acetonitrile 60:40
Remarks	good peak

SPECTROSCOPY METHOD

Four methods were used
Method A: zero order spectroscopic method
Method B: Area under Method
Method C: first order derivative spectroscopy
Method D: Second Order Derivative Spectroscopic method

PREPARATION OF STANDARD STOCK SOLUTION

Accurately weighed 10mg of AZL working std in 10mL volumetric flask containing 5ml of methanol shaken for 5min then remaining volume made up with methanol. The final concentration obtained was 1000µg/mL . It was further diluted to get concentration 100µg/ml was prepared with distilled water .From this a series of aliquots were prepared to get concentration ranging from 5-30µg/mL in 10ml vol. flask using distilled water.

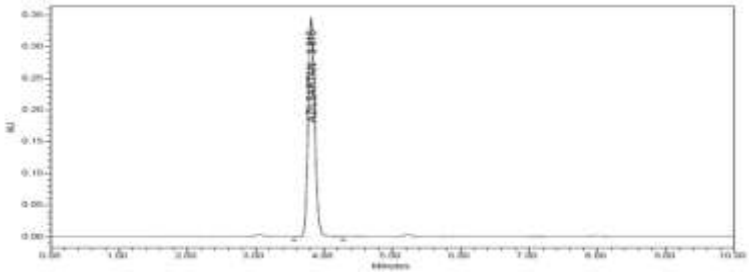
PREPARATION OF SAMPLE SOLUTION

For the estimation of Azilsartan in the commercial formulations, 5 tablets each containing 40 mg of Azilsartan were weighed and the average weight was calculated. The tablets were crushed and powdered in glass mortar. For the analysis of drug, quantity of powder equivalent 10 mg of Azilsartan was transferred to 10 mL volumetric flask and dissolved in sufficient quantity of methanol and volume made upto the mark with methanol to obtain conc. of 1000 µg/mL of Azilsartan. Then the solution was filtered through Whatman filter paper no.41.Further dilutions of the solution were made in water to get required concentration of 10 µg/mL. The concentration of Azilsartan in formulation was determined by above developed methods. The assay procedure was repeated six times (n= 6) for each method.

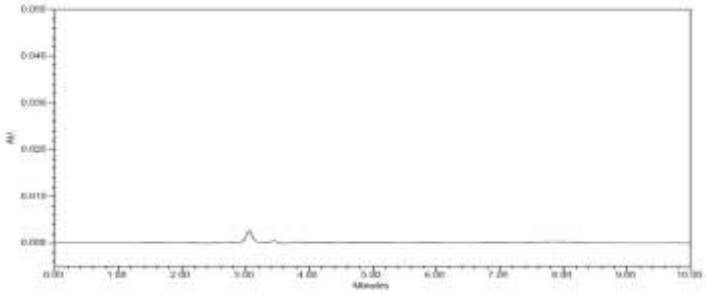
RESULTS & DISCUSSION

ESTIMATION OF AZILSARTAN MEDOXOMIL IN TABLET DOSAGE FORM

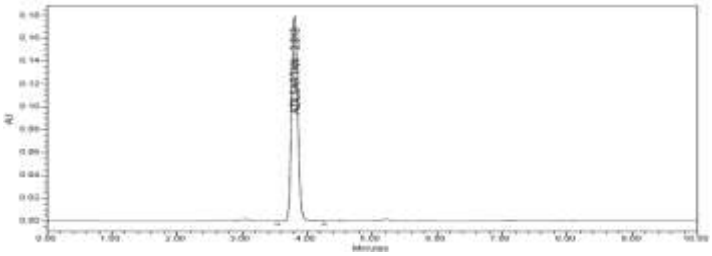
Accurately twenty tablets were weighed and triturated into fine powdered in a glass mortar. A portion of powder equivalent to 10mg was accurately weighed into 10ml volumetric flask and 5ml of mobile phase was added. The volumetric flask was sonicated for 15min for complete dissolution of Azilsartan medoxomil the solution was then made up to volume with mobile phase to get concentration of 1000µg/mL solution. The solution was then passed through 0.45µ membrane filter. From this, working solution concentration of 40µg/mL AZL was prepared .20µL of the test solution was injected (n=6) and chromatogram was recorded under same optimized chromatographic conditions. The drug content was quantified using the regression equation obtained for the pure sample.



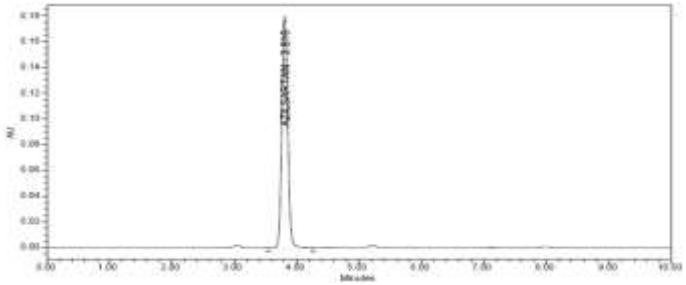
Typical chromatogram of AZL



Chromatogram of Blank



Typical standard chromatogram of AZL (API)



Typical chromatogram of AZL (formulation)

Tablet analysis results of AZL by RP-HPLC method

Brand Name	Labeled amount of drug (mg)	Mean amount found by the proposed method (n=6)	% Assay
Edarbi	40	39.923	99.807

System suitability parameters of AZL

Parameter	Result
Retention Time (mins)	3.8
Theoretical Plates (N)	7445
Tailing Factor	1.1
Limit of Detection (µg/ml)	0.1575
Limit of Quantification (µg/ml)	0.4775

Linearity characteristics of AZL

S.no	Parameter	Result
1.	Concentration range (µg/ml)	10-60µg/mL
2.	Slope (m)	56665
3.	Intercept (b)	0.001
4.	Correlation coefficient	0.999
5.	% RSD	0.77

Recovery studies of proposed method

Spiking levels	Conc taken	Amount added(µg)	Area(n=6)	Amount recovered(µg)	% Recovery	% RSD
50 %	20µg	10µg	1728826	29.89	99.78	0.65
100 %	20µg	20µg	2291755	39.62	98.62	0.78
150 %	20µg	30µg	2868989	49.54	99.03	0.57

Intra-day and inter-day precision studies of the proposed method

Concentration of Azilsartan medoxomil (µg/mL)	Observed concentration of Azilsartan medoxomil (µg/mL)			
	Intra-day		Inter-day	
	Mean (n=3)	RSD%	Mean(n=6)	RSD%
10	580270	0.91	581148	0.89
20	1155381	0.36	1154520	0.32
30	1752615	0.15	1752444	0.13

Robustness results of AZL by RP-HPLC

S.No.	Parameter		RT	AREA	avg	Std dev	%RSD
1.	Flow rate(f1)	0.9mL/m	3.407	2025785	3.877	233649	1.01
2.	Flow rate(f2)	1.1mL/m	4.353	2596596			
3.	Temperature(t1)	27ºc	3.801	2276525	3.823	2297358	1.04
4.	Temperature(t2)	23ºc	3.846	2290527			

Stability studies of AZL

S.no		RT	AREA
1.	STD		2314385
2.	ACID	3.824	1693947
3.	BASE	3.831	1997678
4.	h2o2	3.824	1693170
5.	HEAT	3.831	2099994

Summary of validation parameters

Parameter	Result
Λmax	248nm
Linearity indicated by correlation coefficient	0.999
Beer's limit	10-60µg/mL
Slope	56665
Intercept	18966
Accuracy	98-102%Recovery
Precision	0.24%RSD
Robustness	0.683%RSD
LOD	0.1575µg/mL
LOQ	0.4775µg/mL

SPECTROSCOPY METHOD

Analysis of AZL (formulation)

method	Label claim (mg/tablet)	Amount found (mg) (n= 6)	% assay	% RSD
A	40 mg	9.94	98.16	0.094
B	40 mg	10.05	101.6	0.064
C	40 mg	10.02	100.7	0.038
D	40 mg	10.01	100.3	0.011

VALIDATION OF PROPOSED METHODS

Validation is a process of establishing documented evidence ,which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics.

The method was validated as per ICH guide lines for different parameters like linearity, accuracy, precision.

LINEARITY

The linearity of the proposed UV spectroscopic methods were evaluated by analysing different concentrations of standard solutions of Azilsartan and by plotting absorbencies of analyte against concentrations of the analyte. Beer's law was obeyed for all four methods in the concentration range of 5 – 30 µg/mL. A good linear relationship (R^2 =0.99) was observed between the concentrations of Azilsartan medoxomil and the corresponding absorbance.

ACCURACY

Accuracy is express as degree of closeness of experimental value to the true value. To study the accuracy of the proposed method and to check the interferences from excipients used in the dosage forms, recovery experiments were carried out by standard addition method. This parameter is evaluated by percent recovery studies at concentration levels of 50,100 and 150 % which includes addition of known amounts of Azilsartan working standard to a prequantified sample solution. Each of the dilution was observed six times. The samples were reanalyzed by proposed methods. The amount of Azilsartan was estimated by applying obtained values to regression equation. The percentage recovery of the drug was calculated.

PRECISION

Precision is the level of repeatability of results as reported between samples analyzed on the same day (Intra – day) and samples run on three different days (Inter – day). To check the intra – day and inter – day variation of the methods, solutions containing 5, 10 and 15 µg/mL concentrations of Azilsartan were subjected to the proposed spectrophotometric methods of analysis and the recoveries obtained were noted. The precision of the proposed method i.e. the intra and inter – day variations in the absorbance of the drug solutions was calculated in terms of % RSD . Statistical evaluation revealed that relative standard deviation of drugs at different concentration levels for three times was less than 2.0. (Intra – day – 0.48, inter – day – 0.473).

Linearity characteristics of AZL

Parameter	Method A	Method B	Method C	Method D
λmax	243	238-248	247	257
Beer's limit (µg/mL)	5 – 30	5 – 30	5 – 30	5 – 30
Molar extinction coefficient (L/Mol.cm)	0.026	0.028	0.004	0.0002
Correlation coefficient (r^2)	0.991	0.997	0.994	0.998
Slope (a)	0.013	0.335	0.252	0.036
Intercept (b)	0.030	0.710	0.278	0.093

Recovery studies of AZL

Concentration taken (µg/mL)	Spiked level(%)	Amount added(mg)	Amount found (mg) (n = 6)				% Recovery			
10	0	5	5.34	5.48	5.45	5.42	8.8	01.4	00.9	00.3
10	00	10	9.96	0.07	0.04	0.02	9.3	01.6	00.6	00.1
10	150	15	4.55	4.69	4.69	4.63	9.2	01.3	00.5	00.4

Intraday and inter day precision studies of AZL

Concentration taken (µg/mL)	Intra – day precision *mean ±SD	% RSD	Inter – day precision *mean ± SD	% RSD
5	0.134 ± 0.003	0.96	0.12 ± 0.004	1.24
10	0.296 ± 0.005	0.62	0.279 ± 0.004	0.96
15	0.504 ± 0.004	0.58	0.526 ± 0.005	0.74

Summary of validation parameters of azilsartan medoxomil

Parameter	Method A	Method B	Method C	Method D
λ_{max}	243 nm	238 – 248 nm	254 nm	247 nm
Beer's limit (µg/mL)	5-30	5-30	5-30	5-30
Linearity indicated by correlation coefficient	0.992	0.993	0.994	0.996
Precision indicated by % RSD	1.1	0.79	0.66	0.72
Accuracy indicated by % recovery	99.2	101.3	100.5	100.4

The calibration curve was linear in the concentration range 5-30 µg/mL. The % assay by the four methods was found to be in the range 98.16 – 100.3% for Azilsartan medoxomil. No interference was observed from the pharmaceutical excipients. The recovery studies showed that there methods were accurate and reproducible. The results revealed that any change in the drug concentration could be accurately determined by the proposed method. Accuracy and reproducibility of the proposed methods were further confirmed by percent recovery values, Intermediate precision study for the method % RSD is not more than 2.0 indicate good intermediate precision. Hence, the proposed methods were validated in terms of linearity, precision and accuracy. Characteristic parameters and summary of validation parameters for all the four methods were given in table 8. By observing the validation parameters, the methods were found to be simple, accuracy and precise. Hence these methods can be employed for the routine analysis of Azilsartan medoxomil in tablet formulations.

CONCLUSION

The developed HPLC, UV and Visible methods for the determination Assay of selected drugs are simple, rapid, accurate, precise, robust and economical. The mobile phase and solvents are simple to prepare and economical reliable, sensitive and less time consuming.

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