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Stress Degradation Studies on Azilsartan Medoxomil and Development of a Validated Method by UV Spectrophotometry in Bulk Drug

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Abstract

The present research work discusses the development and validation Azilsartan medoxomil in bulk drug by stress degradation studies using UV Spectrophotometry method. Simple, specific, accurate and cost effective spectroscopic method has been developed for the estimation of Azilsartan medoxomil. The maximum wavelength (λmax) was found to be 251nm. The validation was performed as per ICH guidelines for linearity, accuracy, precision, LOD and LOQ. Methanol used as a solvent. The Beer's Law obey the linearity in the range of 2-10 µg/ml and shows a linear relationship between the absorbance and concentration with coefficient of correlation 0.998. The % recovery of Azilsartan medoxomil is 99.13%.the recovery of the drug was found to be within the limit. The precision study the relative standard deviation was found to be less than 2.0%. The LOD and LOQ were found to be 12.35µg/ml and 37.43µg/ml respectively. Azilsartan medoxomil was subjected to various stress degradation conditions recommended by I.C.H. Stress degradation studies were carried out to provide indications of stability indicating property of proposed method. Hence it could be concluded that the proposed methods would be suitable for the analysis of Azilsartan medoxomil in bulk and pharmaceutical preparations.

Keywords: Azilsartan medoxomil, ICH guidelines, Stress degradation method, UV Spectroscopic method.

INTRODUCTION

Azilsartan medoxomil is chemically known as 2-ethoxy-1-{[2'-(5-oxo-2, 5-dihydro-1, 2, 4-oxadiazol-3-yl)-4-biphenylyl] methyl}-1*H*-benzimidazole-7-carboxylic acid. It is freely soluble in methanol, acetone, acetic acid, n-propanalol, diethyl ether, benzene, sodium hydroxide, very slightly soluble in water. It shows molecular formula as $C_{25}H_{20}N_4O_5$ with molecular weight 456.46 g/mol. Belongs to a class of Anti-Hypertensive drug known as Angiotensin II receptor antagonists. Azilsartan medoxomil lowers blood pressure by blocking the action of Angiotensin II at AT1 receptor, a hormone that contracts blood vessels and reduces water excretion through the kidneys[1-2]. Fewer methods have been reported for the quantitative determination of Azilsartan medoxomil, which includes UV [3], HPLC [4, 5], HPTLC [6, 7], Spectroflourimetry [8], UPLC [9].UPLC–MS/MS [10]. Liquid chromatography-mass spectrometry [11].

The present study, we are planning to develop simple, sensitive, precise, accurate and cost effective stability and its methods by using UV Spectrophotometry for Azilsartan medoxomil.

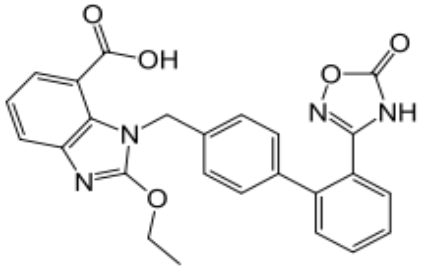

Fig no: 1 Structure of Azilsartan medoxomil

MATERIALS AND METHODS

Instrumentation

UV/VIS spectrophotometer (Perkin Elmer lambda 25) used with 1cm path length quartz cell, analytical Balance (shimadzu) were used.

Chemicals

Working standards of pharmaceutical grade Azilsartan medoxomil was obtained reference sample, Methanol, ethanol, 0.1M Hcl, 0.1M NaOH were purchased from Merck chemicals, Mumbai, India.

METHOD DEVELOPMENT

Selection of wavelength

Weigh accurately 100mg of Azilsartan medoxomil dissolved in 100ml of methanol in a 100ml of standard volumetric flask to obtain the concentration 1000µg/ml. From the above stock solution 10ml was pipette out and transferred into 100ml standard volumetric to obtain the concentration 100µg/ml. Further diluted with methanol to obtain a 50µg/ml. The above solution was scanned between 200 – 400nm against the reagent blank.

Assay of Azilsartan medoxomil

Standard preparation

Weigh accurately 100mg of standard Azilsartan medoxomil (Figure 1) dissolved in 100ml of methanol in a 100ml of standard volumetric flask to obtain the concentration 1000µg/ml. From the above stock solution 10ml was pipette out and transferred into 100ml standard volumetric flask using with methanol to obtain the concentration 100µg/ml. Further diluted with methanol to obtain a 6µg/ml. Measure the absorbance of prepared standard solution at 251nm using reagent as blank.

METHOD VALIDATION

This method validated by evaluating linearity, accuracy method and limit of detection (LOD) and limit of quantification (LOQ) were performed according with ICH guidelines.

Linearity

Linear regression data from the calibration plots revealed good linear relationships between area and concentration over the range of 2-10µg/ml respectively. The correlation coefficient was found to be 0.998 with a straight line equation Y=0.023x + 0.000. It is indicates that the method is linear.

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels 80%, 100%, 120%. The values of recovery (%), RSD (%) indicate the method is accurate.

Precision

The reproducibility of the proposed method was determined by performing at different intervals on same day (intraday precision) and on three different days (inter day precision) In the inter-day variation study, 6 µg/ml of solution was prepared and analyzed thrice, for three consecutive days, and the absorbance was recorded. In the intra-day variation study, three different solutions of the same concentration (6µg/ml) were prepared and analyzed thrice a day (morning, afternoon, and evening). The results were indicated by % RSD.

Ruggedness

In the present study, ruggedness was carried out through different nanometer 241 and 261nm.The ruggedness of method was verified by conducting the precision study by using different nanometre. The mean standard deviation, and %RSD for the two set of data are ruggedness of the method is indicated by the overall RSD between two set of data.

Limit of Detection (LOD) and Limit of Quantization (LOQ)

LOD and LOQ of the drug were calculated using the following equations according to International Conference on Harmonization (ICH) guidelines

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where,

σ = the standard deviation of the response and

S = the slope of the regression equation.

DEGRADATION STUDIES

Stress degradation by hydrolysis under acidic condition

To 3ml of stock solution (1000µg/ml) of azilsartan medoxomil, 1ml of 3N HCl was added in 10ml volumetric flask using with methanol. Then kept at normal condition for 90mins. After 60mins, time interval, 1ml of solution was pipette out from this flask, to obtain (30µg/ml) concentration by using a methanol. Used as a blank, 0.5ml solution of 3N HCl and 0.5ml solution of 3N NaOH were diluted with methanol in 10ml of volumetric flask. After 90mins, again 1ml of the solution was pipette out from the flask and the above procedure was repeated.

Stress degradation by hydrolysis under alkaline condition

To 3ml of stock solution (1000µg/ml) of azilsartan medoxomil, 1ml of 0.1N NaOH was added in 10ml volumetric flask using with methanol. Then, kept at normal condition for 90mins. After 60mins, time interval, 1ml of solution was pipette out from this flask, to obtain (30µg/ml) concentration by using a methanol. For the blank, 0.5ml solution of 0.1N HCl and 0.5ml solution of 0.1N NaOH were diluted with methanol in 10ml of volumetric flask. After 90mins, again 1ml of the solution was pipette out from the flask and the above procedure was repeated.

Dry heat induced degradation

Azilsartan medoxomil sample was taken in a petriplate and exposed to a temperature of 70°C for 48hours in an oven. After 48hours, 10mg of the sample was diluted methanol in order to make the volume up to 10ml. from this solution, dilutions were carried out to achieve the concentration (30µg/ml) for the UV-VIS analysis.

Oxidative degradation

To 1.5ml of the stock solution of Azilsartan medoxomil (1000µg/ml), 1ml of 30%w/v of hydrogen peroxide added in 10ml of volumetric flask with methanol. then kept at room temperature for 15min. For the blank, 1ml of the 30%w/v of hydrogen peroxide was kept at normal condition for overnight in 10ml of volumetric flask. Both solutions were heated on boiling water bath to remove excess of hydrogen peroxide. Finally, after 15mins dilutions were made from the stock solution to achieve the required concentration (30µg/ml) analysed in UV.

Photolytic degradation

Sample of Azilsartan medoxomil was exposed to near ultraviolet lamp in photostability chamber providing illumination of not less than 1.2 million lux hours. 10mg sample was dissolved in methanol and volume made up to 10ml. From this solution appropriate dilution (30µg/ml) was made using methanol and taken for the UV analysis.

RESULTS AND DISCUSSION

Azilsartan medoxomil shows the absorbance maxima at 251 nm it shown in the (Figure no: 2). the percentage assay of Azilsartan medoxomil was found to be 99.62 %w/v. the calibration plots showed good linear relationship in the concentration range of 2-10 µg/ml and given in (Figure no: 3, Table no: 1). The % recovery of Azilsartan medoxomil is 99.13%. As per ICH guidelines, percentage recovery limit should be more than 99-102%.The recovery of the drug was found to be within the limit result are shown in (table no:2). The precision of the method expressed as % RSD of intraday and interday validation is given in the (table no: 3). ruggedness of the method and the % RSD values listed in (table no: 4). The Limit of detection and quantification of Azilsartan medoxomil was found to be 12.35µg/ml and 37.43µg/ml. As per ICH guidelines. The percentage degradation limit should be 5-20%. The drug Azilsartan medoxomil was found to be within the limit As per ICH guidelines. The percentage degradation limit should be 5-20%. The drug

Azilsartan medoxomil was found to be within the limit. Results are shown in (table no: 5).

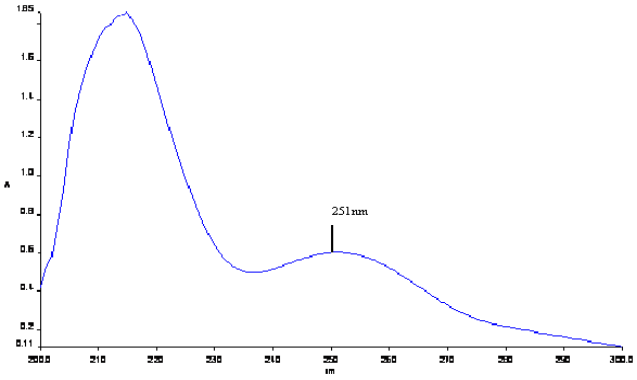

Fig no: 2 UV absorption spectrum of Azilsartan medoxomil

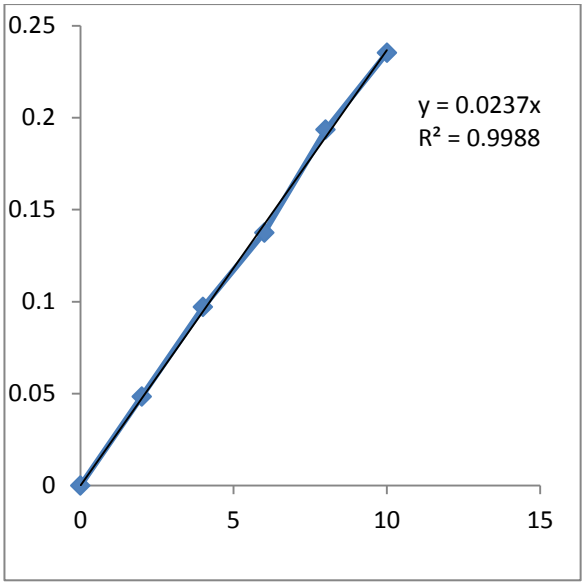

Fig no: 3 Calibration curve of Azilsartan medoxomil

Table no: 1 Calibration Parameters for Azilsartan medoxomil

PARAMETERS	AZILSARTAN MEDOXOMIL
Wavelengths(nm)	**251nm**
Concentration range (μgmL^{-1})	**2-10μgmL^{-1}**
Intercept (a)	**0.0006**
Slope (b)	**0.0235**
Correlation coefficient (r)	**0.998**
LOD(μgmL^{-1})	**12.35**
LOQ(μgmL^{-1})	**37.43**

Table no: 2 Results of Accuracy Studies

Drug	Absorbance	Level	Amount Added (mg)	Amount Recovered (gm)	% recovery	Average % recovery
Azilsartan medoxomil	0.1286	80%	4.8	4.77	99.37%	99.13%
	0.1037	100%	6	5.90	98.33%	
	0.0852	120%	7.2	7.18	99.70%	

Table no: 3 Results of precision Studies

S.No	AZILSARTAN MEDOXOMIL					
	INTERDAY PRECISION			INTRADAY PRECISION		
	DAY 1	DAY 2	DAY 3	At 10.30am	At 11.30am	At 12.30pm
Avg (n=3)	0.1528	0.1545	0.1486	0.1524	0.1657	0.1582
S.D	0.000208	0.000216	0.000163	0.00020	0.000251	0.000163
%R.S.D	0.1362	0.1398	0.1098	0.1312	0.1518	0.1030

Table no: 4 Results for Ruggedness

S.NO	AZILSARTAN MEDOXOMIL	
	241nm	261nm
1	1.4706	1.0678
2	1.4712	1.0681
3	1.4720	1.0676
Avg.	1.4712	1.0678
S.D	0.000577	0.000200
%R.S.D	0.0392	0.0119

Table no: 5 Stress degradation studies for the determination of Azilsartan medoxomil

CONDITION	TIME	% DEGRADATION
0.1N NaOH (1ml)	60min	4.97
	90min	7.25
3N HCl (1ml)	60min	6.41
	90min	13.55
Dry heat 70°c	48 hr	11.86
Photolytic	3hr	15.44
	6hr	16.86

CONCLUSION

The proposed method was found to be simple, robust, selective and sensitive. The validation parameters were also found to be within the limits. The method showed acceptable linearity and accuracy and is highly sensitive. All the analytical reagents used have excellent shelf life, inexpensive, and are available easily in any analytical laboratory. Developed method can be applied successfully for the estimation of Azilsartan medoxomil in bulk and pharmaceutical formulation. Therefore, it could be used easily for the routine analysis of pure drugs in quality control and clinical laboratories.

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