DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD OF AZILSARTAN MEDOXOMIL

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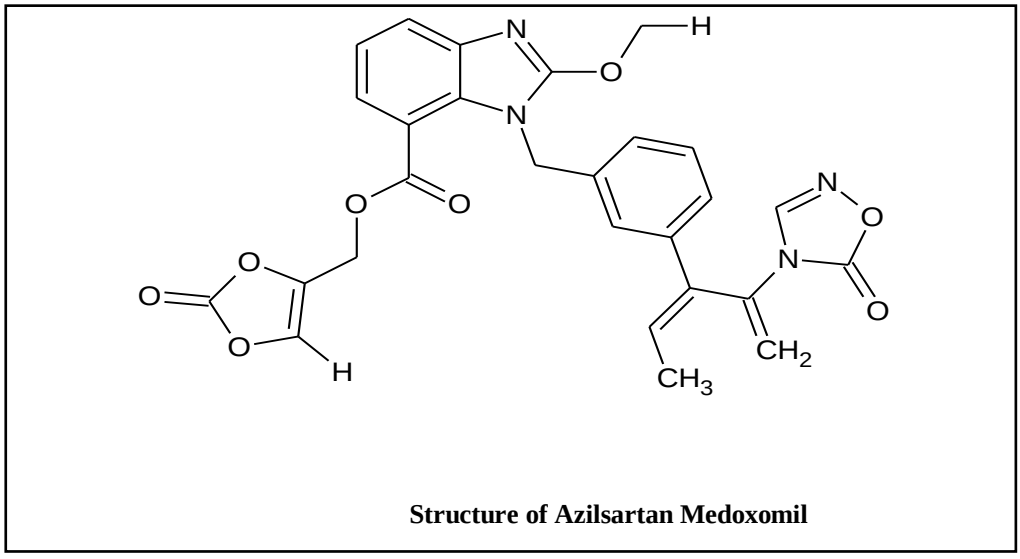
ABSTRACT

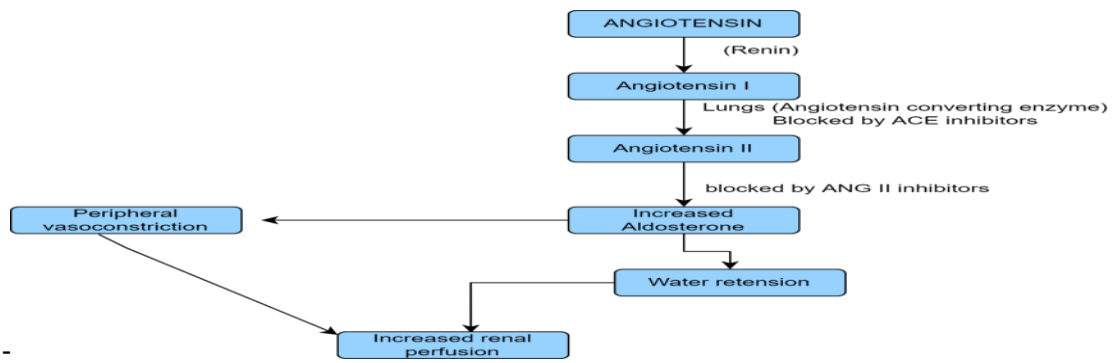
Azilsartan is used for the treatment hypertension and has reliable results in blood pressure reduction and tolerability. It lowers blood pressure by blocking the action of angiotensin II at the AT1 receptor, a hormone that contracts blood vessels and reduces water excretion through the kidneys. There have been numerous methods developed so far for quantitative estimation of azilsartan in bulk and pharmaceutical dosage form. Pharmaceutical analytical methods include UV Visible Spectrophotometry. Azilsartan Medoxomil is rapidly hydrolysed to the active moiety azilsartan by esterases in the gastrointestinal tract and during drug absorption. In this study, a simple, sensitive and highly accurate ultraviolet spectrophotometry method has been developed and validated for determination of Azilsartan medoxomil. Results of analysis were validated for accuracy, precision, LOD, LOQ etc. parameters were found to be satisfactory. The proposed method in simple, rapid and suitable for the routine analysis.

Keywords: Azilsartan medoxomil, Mechanism, Methanol, Spectrophotometry and Validation.

Introduction: - Azilsartan is an Angiotensin 2 Receptor Blocker. Azilsartan Medoxomil is in a class of medications called angiotensin II receptor antagonists. It is used alone or in combination with other medications to treat high blood pressure. Azilsartan Medoxomil is obtained as prodrug. It is associated with a low rate of transient serum aminotransferase elevations and in lowering blood pressure reduces the risk of fatal and nonfatal cardiovascular events, primarily strokes and myocardial infarctions. These benefits have been seen in controlled trials of antihypertensive drugs from a wide variety of pharmacologic classes. Azilsartan medoxomil is (5-methyl-2-oxo-1,3-dioxol-4-yl) methyl 2-ethoxy-1-([2'-(5-oxo-4,5-oldihydro-1,2,4-oxadiazol-3-yl) biphenyl-4-yl] methyl)-1H-benzimidazole-7-carboxylate and chemical formula is $C_{30}H_{24}N_4O_8$ with molecular weight of 456.6g.



Structure of Azilsartan Medoxomil



Mechanism of action: -

MATERIAL AND METHODS

Material

The reference standard of Azilsartan Medoxomil API was provided as a gift sample by Laureate Institute Pharmacy, Kathog, Jawalamukhi, and Himachal Pradesh, India.

Instruments

A double beam UV Visible Spectrophotometer (LABINDIA UV3000$^+$), Electronic Weighing Balance (WENSAR), and Sonicator (Oscar Ultrasonicator micro clean-103) were used to perform this experiment.

Methods Preparation of standard stock solution

An accurately weighed 100 mg of Azilsartan Medoxomil pure drug was dissolved in 100 ml of Methanol using a 100 ml volumetric flask. The solution was then sonicated for 10 min and the final volume was adjusted up to the mark with the same solvent, to give the final concentration of 1000µg/ml. Out of this stock, 10ml was pipetted and diluted up to 100ml (100µg/ml) by Methanol and examined between 200-400 nm. The maximum absorbance was determined using UV Vis Spectrophotometer (LABINDIA UV3000$^+$) to confirm the λmax of the drugs

Selection of wavelength

Azilsartan Medoxomil standard solution (100µg/ml) was prepared by appropriate dilution of standard stock solution and then scanned in the UV range (200-400 nm). Azilsartan Medoxomil showed the absorption maxima at 247 nm.

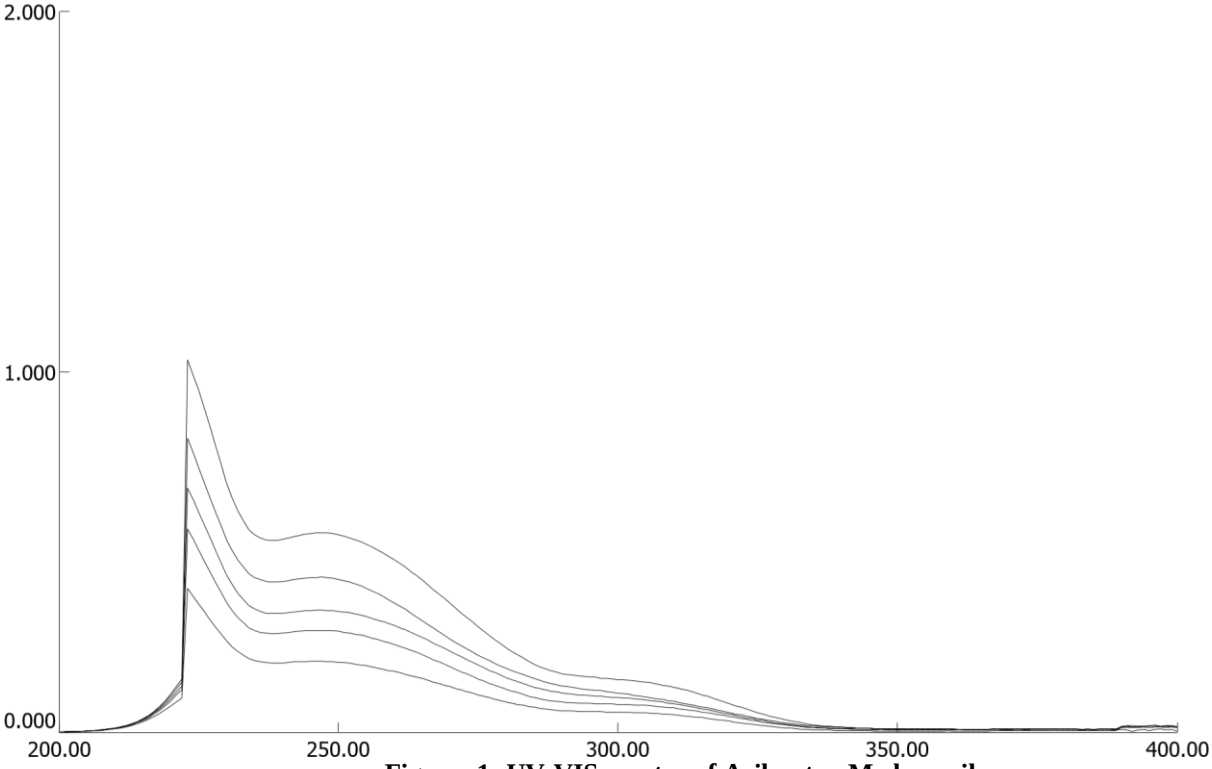


Fig. no. 1: UV-VIS spectra of Azilsartan Medoxomil

Calibration curve

Five concentrations of Azilsartan Medoxomil standard solution (2, 4, 6, 8, 10µg/ml) were prepared by pipetting out 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 10 ml of standard stock solution and transferring into a series of 10 ml volumetric flask. The volume was then adjusted up to the mark with Methanol. The absorbance of each solution was recorded at 247nm using Methanol as a blank. A calibration graph was prepared by plotting absorbance vs respective concentration.

Validation of method

The method was validated as per ICH guidelines for different parameters like Linearity, Precision, Accuracy, LOD, LOQ, Robustness and Ruggedness.

Linearity and Range

Various aliquots of Azilsartan Medoxomil were prepared from a stock solution in the range of 2-10 µg/ml. The samples were scanned in UV-VIS Spectrophotometer and the calibration graph plotted absorbance versus respective concentration. Beer's law was obeyed over the concentration range of 2-10 µg/ml. (Table 1).

Precision

Precision study of the developed method was performed as inter-day precision and intraday precision. Intraday precision (Table 2) was performed by analysing the solution of known concentration i.e., three times a day. Inter-day precision (Table 3) was performed by analysing the solution of the same concentration for 3 days. The % RSD was calculated.

Accuracy

The accuracy study of the developed method was carried out by calculating the recovery of the drug by the standard addition method. In this, a known amount of Azilsartan Medoxomil standard solution was added to the sample solution. The recovery study was performed at three, i.e., 80%, 100%, and 120% of the working different concentration levels of the working concentration of the sample. The percentage recoveries were calculated (Table 4).

Limit of detection (LOD) and Limit of quantification (LOQ)

Limit of detection (LOD) is the lowest concentration of analyte in the sample that can be detected but not necessarily be quantified, under a stated experimental condition, and Limit of Quantification (LOQ) is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental condition. The limit of detection (LOD) and Limit of Quantification (LOQ). The LOD & LOQ were calculated using the following Formula:

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

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

Where 'σ' is the standard deviation of response and 'S' is the slope of the corresponding calibration curve.

Robustness

The robustness of the developed method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters. Robustness was determined by recording the absorbance of the solution at two different wavelengths at Table 5.

Ruggedness

The solution was prepared and analysed with change in the analytical condition like different laboratory conditions and different analyst. (Table 6)

RESULTS

Summary of validation parameters

Sr. No.	Parameter	Azilsartan Medoxomil
1.	**Linear Range (µg/ml)**	2-10 µg/ml
2.	**Slope**	0.7759
3.	**LOD**	0.637
4.	**LOQ**	1.931
5.	**Precision(%RSD)** **At 247nm** **Inter day (n = 3)** **Intraday (n= 3)**	 0.284 - 0.433 0.190 - 0.716
6.	**% Recovery**	99% - 102%
7.	**Robustness**	0.179 - 0.276
8.	**Ruggedness**	0.336 & 0.337

Determination of wavelength of maximum absorption

The wavelength of maximum absorption (λmax) of Azilsartan Medoxomil was found to be 247nm.

Calibration curve of Azilsartan Medoxomil

The calibration curve showed the linearity in the range of 2-10µg/ml with a correlation coefficient of 0.9988 and regression equation as

$$y = 0.0497x + 0.0759 \text{ (Fig. no.2).}$$

Validation parameters Linearity

The linearity was found to be in the range of 2-10µg/ml. $R^2 = 0.9988$

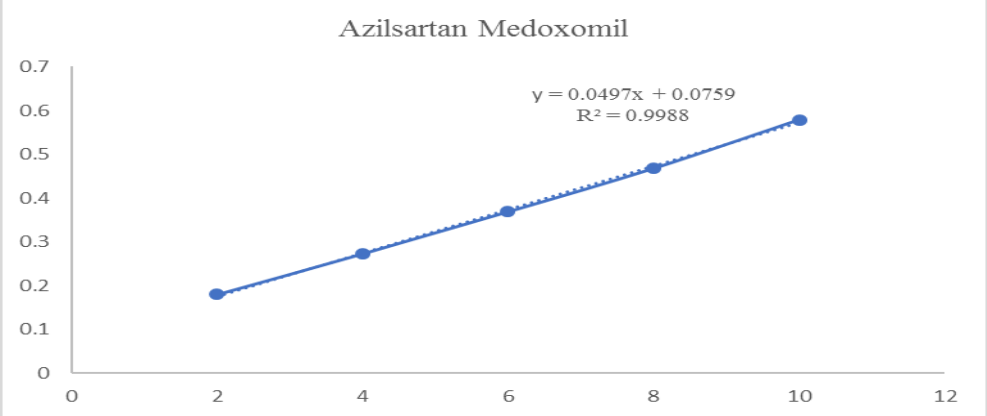


Fig no. 2 Calibration curve for Azilsartan Medoxomil

Table 1: Linearity of Azilsartan Medoxomil

Sr. No.	Conc.(µg/ml)	Abs. Mean ± Std. deviation	%RSD
1	2	0.180 ± 0.001	0.639
2	4	0.273 ± 0.005	0.210
3	6	0.369 ± 0.001	0.271
4	8	0.468 ± 0.001	0.213
5	10	0.579 ± 0.005	0.099

Precision

The % RSD values for inter-day and intraday precision were found to be less than 2 %.

Table 2: Intraday precision

Wavelength	4µg/ml	6µg/ml	8µg/ml
Absorbance	0.276	0.351	0.536
	0.275	0.353	0.538
	0.274	0.354	0.539
MEAN	0.275	0.352	0.537
SD	0.001	0.0015	0.0015
%RSD	0.363	0.433	0.284

Table 3: Intra-day precision

Wavelength	4µg/ml	6µg/ml	8µg/ml
Absorbance	0.212	0.349	0.525
	0.213	0.348	0.526
	0.215	0.347	0.524
MEAN	0.213	0.348	0.525
SD	0.0015	0.001	0.001
%RSD	0.716	0.287	0.190

Accuracy

The % recoveries were found to be in the range of 98 – 101 % indicating that the method was accurate.

Table 4: Accuracy of Azilsartan Medoxomil

Sr. No	Unfortified Sample			Fortified Sample			% Recovery
	Conc. (µg/ml)	Abs.	Mean	Conc. (µg/ml)	Abs.	Mean	
1	2	0.181	0.181	2+6	0.528	0.529	99%
		0.180			0.527		
		0.182			0.529		
2	6	0.348	0.349	6+6	0.701	0.702	101%
		0.349			0.703		
		0.348			0.702		
3	10	0.558	0.559	10+6	0.904	0.905	99%
		0.557			0.903		
		0.559			0.906		

Limit of Detection & Limit of quantification

$LOD = 3.3 \times 0.01466 / \sqrt{5}$

$= 0.63748$

$LOQ = 10 \times 0.01466 / \sqrt{5}$

= 1.93177

Robustness

The robustness was calculated at two different wavelengths 245 nm and 249 nm and it gave reliable results as the % RSD was found to be less than 2 %.

Table 5: Robustness studies.

Wavelength	245nm	247nm	249nm
Absorbance	0.553	0.558	0.551
	0.550	0.557	0.553
	0.552	0.559	0.552
MEAN	0.551	0.558	0.552
SD	0.001	0.001	0.001
%RSD	0.276	0.179	0.181

Ruggedness

At 247nm by Q-Analysis

	Analyst 1	Analyst 2	Inference
Concentration(µg/ml)	**4 µg/ml**		No Significant Difference
Absorbance	0.296	0.295	
	0.298	0.296	
	0.297	0.295	
MEAN	0.297	0.296	
SD	0.001	0.001	
%RSD	0.336	0.337	

CONCLUSION

A simple, sensitive, accurate, and precise UV-Visible spectrophotometric method was developed and validated as per ICH guidelines. All the parameters were found to be within the standard range. The method can be applied for routine analysis of Azilsartan Medoxomil in bulk and pharmaceutical dosage form.

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