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Development and validation of HPTLC stability indicating method for estimation of Azilsartan Medoxomil using Fluorescence mode

Santosh V. Gandhi*, Vrushpriya H. Habde

AISSMS College of Pharmacy, Kennedy Road, Pune- 411001, Maharashtra, India.

***Corresponding Author: Santosh V. Gandhi**
Email: santoshvgandhi@rediffmail.com

ABSTRACT

Azilsartan medoxomil is an angiotensin II receptor antagonist used in the management of hypertension. A simple, selective, and sensitive High Performance Thin layer Liquid Chromatographic (HPTLC) method for the determination of Azilsartan medoxomil using fluorescence mode has been developed. The HPTLC separation was achieved on the aluminum backed layer of silica gel $60F_{254}$ using Toluene: Methanol (8:2 v/v) as mobile phase. Quantification was achieved using fluorescence mode at 312 nm over the concentration range of 200 to 1000 ng/band with correlation coefficient of 0.991.The recovery study results ranged from 99.33- 99.59%for Azilsartan medoxomil. Retention factor (Rf) was found to be 0.38 ± 0.04respectively. The method is simple and was successfully validated according to ICH guidelines Q2 (R1). The method showed acceptable values for assay, accuracy, precision, robustness, sensitivity and stability.

Keywords: Azilsartan medoxomil, High Performance Thin Layer Chromatography (HPTLC), Fluorescence mode.

INTRODUCTION

Azilsartan medoxomil (AZL-M) (5-methyl-2-oxo-1,3-dioxol-4yl) methyl 2-ethoxy-1-{[2′-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]methyl}-1H-benzimidazole-7 carboxylate (Fig.1)is practically insoluble in water, freely soluble in methanol and dimethylsulfoxide. Azilsartan, the activemetabolite of AZL-M, is a novel non-peptide angiotensin II type 1 (AT1) receptor blocker (ARB) that was approved for treatment of hypertension. It has a superior ability to control systolic blood pressure (BP) relative to other widely used ARBs [1]. The drug substance belongs to BCS Class 4, i.e. low solubility and low permeability and is unstable in aqueous solution of pH 1 and pH 7 and relatively stable from pH 3 to pH 5 [2].

Literature Survey reveals that there are few reported methods to determine the Azilsartan medoxomil alone and in combination with other drugs by RP-HPLC [3-8], HPTLC [9-10], UPLC MS-MS [11] and UV spectrophotometric methods [12]. No HPTLC method has been reported for estimation of Azilsartan medoxomil using fluorescence mode. The present method describes a simple, selective, and sensitive HPTLC method in fluorescence mode. The method has been validated as per the ICHQ2 (R1) Guidelines [13].

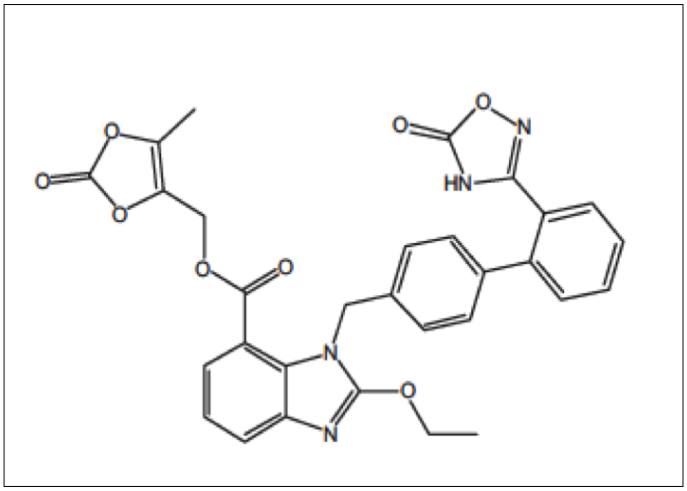

Fig 1: Structure of Azilsartan medoxomil (AZL-M)

MATERIALS AND METHODS

Reagents and Chemicals

Azilsartan medoxomil (AZL-M) was provided as a gift sample by Lupin Pharmaceuticals, Aurangabad and it was used as such without further purification. All weighing was done on Shimadzu balance (Model AY 120).Methanol (AR grade), Toluene (AR grade) were purchased from S. D. fine chemical Laboratories, Mumbai.

Preparation of mobile phase and stock solutions

AR grade Toluene and Methanol were mixed in 8:2v/v proportions. Stock solution was prepared by dissolving 10 mg AZL-M in 10 mL of acetonitrile to get concentration of 1000 µg/mL. Working stock solution for AZL-M was prepared by diluting appropriately stock solution to get the final concentration of 100µg/mL.

Chromatographic conditions and Instrumentation

The HPTLC analysis was performed on aluminium plates pre-coated with silica gel 60 F_{254}, purchased from E-Merck, Germany. Samples were applied on the plate as a band with 4 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland). Linear ascending development was carried out in a twin trough glass chamber (10 x 10 cm) at room temperature and a densitometric scanning was performed using Camag TLC scanner 3 using fluorescence mode at 312 nm, operated by WinCATS software (Version 1.4.3, Camag). Mercury lamp was used as a radiation source. Representative Densitogram obtained from the standard solution of Azilsartan medoxomil is shown in Fig. 2.

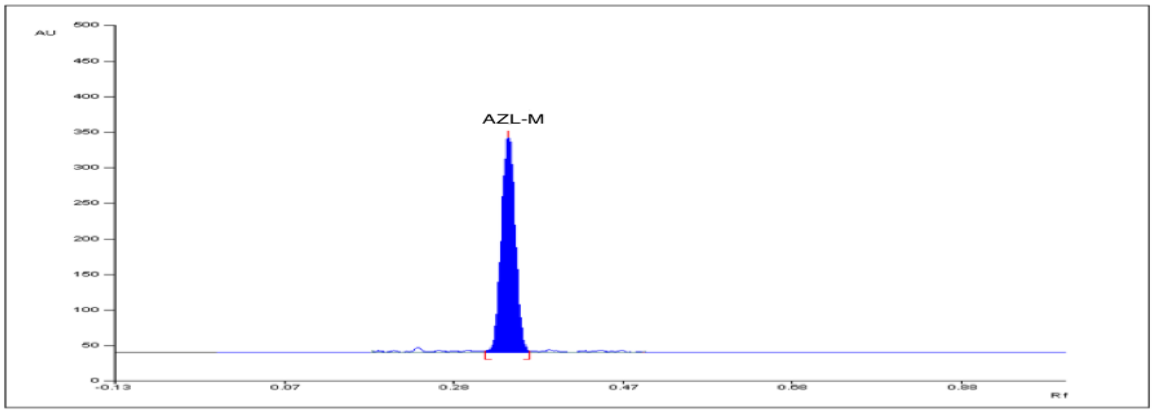

Fig 2: Representative chromatogram of Azilsartan medoxomil (400 ng/band) Rf - 0.38 ± 0.04.

Method Validation

Developed method was validated as per ICH Q2 (R1) guidelines in terms of linearity, assay, accuracy, precision and limit of detection and quantitation [13].

Linearity

The linearity was determined by analyzing six solutions over the concentration range of 200-1000 ng/band for AZL-M. Five replicates per concentrations were analyzed respectively.

Assay

Preparation of sample solution (blend)

Preparation of blend [10]

Blend equivalent to 40 mg of AZL-M was prepared to satisfy dose/tablet of marketed formulation. Blend containing 40 mg AZL-M was prepared by spiking drug into excipients (20 mg starch, 4 mg Magnesium Stearate and lactose (q.s upto 200 mg)). Mixing was done by geometric addition method.

Preparation of test solutions

Blend equivalent to 10 mg of AZL-M was weighed and dissolved in 8 mL acetonitrile. This solution was centrifuged, filtered and volume was made up to 10 mL with acetonitrile, which contained 1 mg/mL Azilsartan medoxomil (A). From solution A, further dilution was made with acetonitrile to get a concentration of 100 µg/mL of Azilsartan medoxomil (B). 4µl of the resultant solution was then applied at TLC plate and densitogram was developed. The retention factor for Azilsartan medoxomil was
found to be 0.38 ± 0.04.

Accuracy

Accuracy was carried out at 3 different levels of 50, 100 and 150 % of assay concentration by adding standard solution to sample solution. Percentage recovery was determined from linearity equation.

Precision

The precision of the method was demonstrated by Intra-day and Inter-day variation studies. In the Intra-day studies 3 replicates of 3 concentrations were analyzed on the same day. For the inter day variation studies, 3 concentrations were analyzed on 3 consecutive days and % RSD were calculated.

Limit of detection and quantification (LOD and LOQ)

From the linearity data the limit of detection and quantitation was calculated, using the formula LOD = 3.3 σ/ S and LOQ = 10 σ/ S where σ is standard deviation of the y intercept of linearity equation and S is slope of the calibration curve of the analyte.

Stress Degradation

Stability studies were carried out to provide evidence on how the quality of drug varies under the influence of variety of environmental conditions like hydrolysis, oxidation, temperature, etc. (Table No 1). For each stress study 4µL of the resultant solution was applied on TLC plate and densitogram was developed (Fig. 3).

Degradation under acid catalyzed hydrolytic condition

To 1 mL of 1000 µg/mL solution of Azilsartan medoxomil, 1 mL of 0.1 N HCl was added. The volume was made up to 10 mL with acetonitrile. The above solution was kept for 2 hours at room temperature.

Degradation under alkali catalyzed hydrolytic condition

To 1 mL of 1000 µg/mL solution of Azilsartan medoxomil, 1 mL of 0.1 N NaOH was added. The volume was made up to 10 mL with acetonitrile. The above solution was kept for 0.5 hours at room temperature.

Degradation under oxidative condition

To 1 mL of 1000 µg/mL solution of Azilsartan medoxomil, 1 mL of 30 % H_2O_2 was added. The volume was made up to 10 mL with acetonitrile. The above solution was kept for 2 hours at room temperature.

Degradation under neutral hydrolytic condition

To 1 mL of 1000 µg/mL solution of Azilsartan medoxomil, 1 mL of distilled water was added. The volume was made up to 10 mL with acetonitrile. The above solution was kept for 2 hours at room temperature.

Degradation under dry heat

Dry heat studies were performed by keeping drug sample in oven (80^0C) for a period of 72 hours. After exposure 100 µg/mL solution of Azilsartan medoxomil was prepared in acetonitrile.

Photo-degradation studies

The photo degradation study of the drug was carried out by exposing the drug to UV light providing illumination of NLT 200 watt hr/m^2 and was subsequently exposed to cool white fluorescence light of NLT 1.2 million Lux-Hr. After exposure 100 µg/mL solution of Azilsartan medoxomil was prepared in acetonitrile.

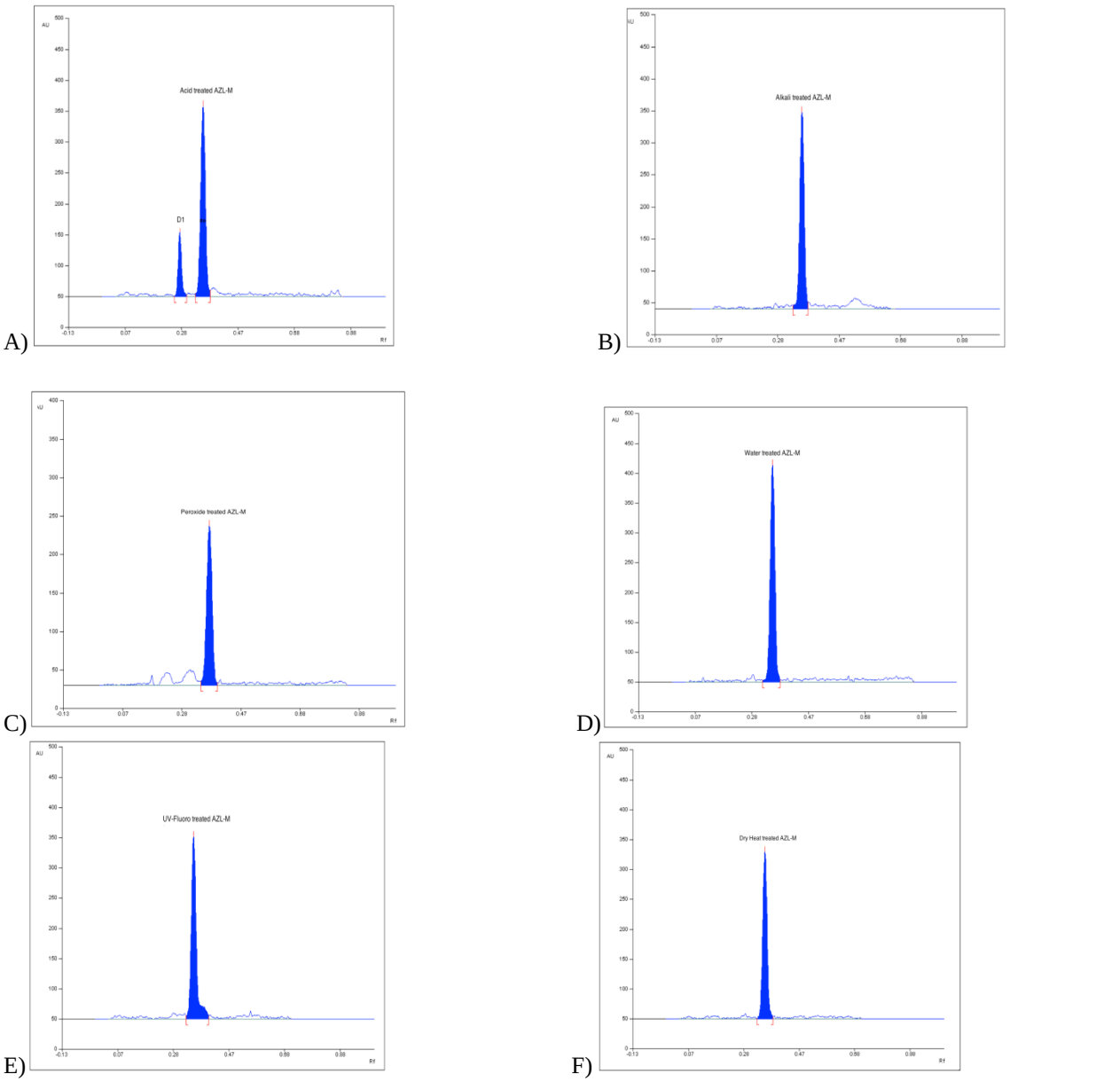

Fig.3: Representative densitogram of Acid (A), Alkali (B), Oxidative (C), Neutral (D), Dry Heat, (E), Photolytic degradation (F) of Azilsartan medoxomil.

Table No 1: Summary of Stress Degradation Study of Azilsartan Medoxomil

Sr.No	Stress Degradation Condition	% Recovery AZL-M	% Degraded
1.	Acid 0.1 N HCl (Kept for 2 hours)	80.81	19.19
2.	Base 0.1 N NaOH (Kept for 0.5 hour)	81.87	18.13
3.	H2O2, 30% (Kept for 2 hours)	77.31	22.69
4.	Neutral (Kept for 2 hours)	78.85	21.15
5.	Dry heat (80^0C for 72 hours)	80.44	19.56
6.	Photo stability UV(200 watt hours/square meter) and Fluorescence, (1.2 million Lux. hours)	76.53	23.47

RESULTS AND DISCUSSION

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 0.996, indicating the non-interference of any other peak of degradation product or impurity at the retention time of the drug.

Linearity of Azilsartan medoxomil was carried out on TLC plate to get final concentration of 200-1000 ng/band. Five replicates per concentration were spotted. The equation of the calibration curve found to be y=4.8584x+1354.1with coefficient of correlation 0.991.

Accuracy results showed almost 100 % recovery at various levels with % RSD of less than 2. The results obtained are shown in Table No. 2. Assay results also showed 100 % recovery. % Recovery was determined from linearity equation. Percent assay was found to be 99.20 ± 0.225.

The %RSD for both Interday and Intraday precision was within the acceptance limit. The results are tabulated in Table No. 3. The LOD and LOQ were found to be 56.06 ng/b and and 169.89 ng/band, respectively.

Robustness

Robustness of the method was determined by varying mobile phase ratio, chamber saturation time, detection wavelength, time from application to development and time from development to scanning. The effects on the area were noted. It was found that method is robust as % RSD found to less than 2.The developed method was found to be simple, sensitive and accurate and can be used for routine analysis.

Table No.2: Intra-Day and Inter-Day Precision results for Azilsartan medoxomil

Intraday precision				Interday Precision			
Replicate No.	**400 ng/band**	**600 ng/band**	**800 ng/band**	**No of Days**	**400 ng/band**	**600 ng/band**	**800 ng/band**
1.	3326.7	4224.5	5168.4	1.	3286.3	4264.1	5274.5
2.	3264.4	4268.2	5256.8	2.	3258.8	4224.6	5170.6
3.	3259.5	4214.7	5269.3	3.	3317.6	4213.2	5186.7

Mean Area	3283.5	4269.1	5231.5	**Mean Area**	3290.9	4241.6	5210.6
SD	37.463	45.107	55.002	**SD**	34.629	44.251	55.921
%RSD	1.140	1.056	1.051	**%RSD**	1.052	1.043	1.073

Table No 3: Results of recovery for Azilsartan medoxomil

Level (%)	Sample (ng/band)	Std. (ng/band)	Area of AZL-M	Average Area of AZL-M	Recovered Conc. (ng/band)	% Recovery
50	400	200	4265.9 4247.6 4258.6	4257.36	597.57	99.59
100	400	400	5233.7 5199.4 5231.6	5221.56	796.03	99.50
150	400	600	6177.3 6153.7 6210.1	6180.36	993.38	99.33

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