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Development and validation of stability indicating RP-HPLC method for the estimation of azilsartan in Bulk

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***Abstract*---**Angiotensin-receptor blockers are often considered adequately efficacious in reducing blood pressure. By accepting the current regulatory requirements for an analytical method

development, a reversed phase high performance liquid chromatographic method for routine analysis of Azilsartan has been developed. The optimized method was achieved using unisol C-18 (3µm, 4.6×150mm) coloumn with mobile phase consisting of mixture acetonitrile and methonal (90:10v/v) with a flow rate of 0.8ml/min at 249nm. The Linear concentration range is 2-10µg/ml. The detection and quantitation limit was found to be 0.45µg/ml. And the detection limit was found to be 0.149µg/ml. There are no interfering peaks under performed degradation conditions. The optimized method was validated according to ICH guidelines.

***Keywords*---**Azilsartan, RP-HPLC, Validation, development

Introduction

Angiotensin-receptor blockers are recommended by the current hypertension guidelines as one of the firstline antihypertensive drug classes. Angiotensin-receptor blockers lower blood pressures through inhibiting the actions of angiotensin II and exhibit good tolerability. Until recently, a number of agents in this class are available for the treatment of hypertension. The more potent blood pressure lowering action was with a problem of more side effects. Recent technological advances have led to the discovery of a structurally and chemically even newer agent, azilsartan drug. In clinical studies, this new angiotensin-receptor blocker showed strong blood pressure lowering efficacy with similar tolerability and side effects [1-6]. This new drug has been compared with several but not all available older agents Azilisartan, trade name Edarbi, is a licensed drug for use in the treatment of hypertension. It is angiotensin receptor antagonist used to decrease the blood pressure. Azilisartan is a angiotensin receptor blocker used to reduce the high blood pressure. 2- ethoxy-3-[[4-[2-(5-oxo-2H-1,2,4-oxadiazol-3- yl]phenyl]methyl]benzimidazole-4-carboxylic acid. Having the empirical formula of C25H20N4O5 having molecular weight-456.4g/mol[7, 8]. Solubility-250c in methanol, dimethyl sulfoxide.

Protein binding-99%. Metabolism at Liver. Azilsartan lowers blood pressure by blocking theaction of angiotensin II at the AT1 receptor, a harmone that contracts blood vessels and reduces water excretion through the kidneys [9-11]. Having Pka value 6.1 (Figure 1).

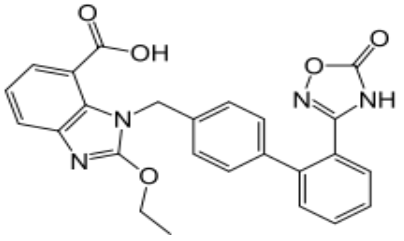

Figure 1: Structure of Azilsartan

Materials and Methods

Materials

Azilsartan drug sample was purchased from the Hetero drugs Limited from Hyderabad, Distilled water, Acetonittrile, Methanol, Hydrochloric acid, sodium hydroxide and Hydrogen peroxide. All the above chemicals and solvents are from Desai chemicals.

Methods

Reason for selection of solvent
Physiochemical properties of a drug molecule plays an important role method development. Physical properties like solubility helps an analyst to decide the solvent and composition of mobile phase. Azilsartan is insoluble in water and soluble in acetonitrile and methanol and showed maximum absorbance in methanol and acetonitrile at 249nm. acetonitrile and methanol are freely available and at low cost, and also retention rate in the previous articles is also less with this mobile phase. Hence acetonitrile and water was used as solvent and 249nm used as maximum wavelength for detection of azilsartan.

Detection of Wavelength
The sensitivity of the HPLC method depends upon the proper selection of wavelength. Drug solution of 100 µg/ml was scanned over the range of 200-400 nm in UV region using different solvents like acetonitrile, hexane and methanol. It was observed that the drug showed maximum absorbance in methanol, acetonitrile at 249 nm and hence methanol and acetonitrile was used as solvent and 249nm was used as maximum wavelength for detection of azilsartan.

Preparation of standard stock solution
Accurately weighed 100mg of Azilsartan, and transferred to 100ml volumetric flask and 3/4th of diluents was added to this flask and sonicated for 10 minutes. Flask was up with diluents and labeled as Standard stock solution.[1000µg/ml azilsartan]

Preparation of Standard working solution[100% solution]
10ml from each stock solution was pipette out and taken into a 100ml volumetric flask and made up with diluents. [100µg/ml azilsartan]

Preparation of Dilutions
Dilute the working standard solution [100µg/ml] by pipetting 2, 4, 6, 8 and 10ml of 100µg/ml solution into 10ml volumetric flask and make up the volume with diluents. The given dilutions of 20, 40, 60, 80, 100µg/ml solution respectively

Mobile Phase selection
Experiments were conducted with mobile phase consisting of acetonitrile and methanol (90:10) trails were conducted taking different combinations of

mobile phase to achieve maximum possible theoretical plates, least possible tailing factor and retention time. Based on this data, the best separation was obtained with acetonitrile and methanol (90:10) mobile phase composition.

How Validation parameters are calculated

Validation parameters are calculated according to international conference on harmonization [ICH] guidelines- validation of analytical procedures: text and methodology Q2 [R1]

System suitability parameters

The system suitability parameters were determined by preparing standard solution of azilsartan and the 10µ/ml solutions were injected four times and the parameters like peak tailing, resolution and USP platecount were determined [12].

Acceptance criteria

The % RSD for area and retension times of four standard injections results should not be more than 2%.

Specificity

Standard solution of 10µg/ml was injected into the system and chromatogram was recorded. Diluent [90:10 acetonitrile : methanol] used as blank and chromatogram was recorded after injection into the system.

Acceptance criteria

Chromatogram of blank should not show any peak at the retention time of analyte peak.

Precision

The precision was determined for azilsartan in terms of intraday precision and inter day precision. Sample solution of 10µg/ml was prepared and injected into the system four times intervals within a day [intraday] and at 4 different days [intra day]. Statistical parameters such as mean, standard deviation and percentage relative standard deviation were calculated.

Acceptance criteria

The % RSD for the area and retention times of six standard injections results should not be more than 2%.

Linearity

Linearity was found by preparing various dilutions from the working standard solution and recording their responses at the optimized set of chromatographic conditions. The calibration plots were constructed between concentrations versus peak areas and the linearity was found in the range from 2µg/ml to 10 µg/ml. The regression equation and correlation coefficient were calculated [13, 14].

Acceptance criteria

The regression coefficient should NMT 1%

Accuracy
To the pre analyzed sample three different amounts of 50%, 100% and 150% of working standard was added, at each level 3 replicate samples were prepared and samples were analyzed to determine percentage recovery from the sample. Percentage recovery is calculated for all nine readings from the ratio of amount of drug found.

Acceptance Criteria
The % Recovery for each level should be between 98.0 to 102.0.

Robustness
Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines. Robustness conditions like Flow minus (0.8ml/min), Flow plus (0.7ml/min), mobile phase minus, mobile phase plus, was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Acceptance Criteria
The % RSD results should not be more than 2%.

Limit of detection (LOD) and Limit of quantitation (LOQ)
Limit of detection (LOD) is the minimum concentration at which the analyte can be detected without actually being quantitated where as Limit of Quantitation (LOQ) is the minimum concentration at which the analyte response can be taken for quantitation i.e., being able to measure various chromatographic parameters like area 0measured with reliable accuracy and precision. These are obtained by comparing the signal to noise ratio (S/N) of blank and drug at different concentrations. The LOD value was found at 0.39 µg/ml concentration where the signal to noise ratio is found to be 3:1 and the LOQ value was found at 1.2 µg/ml with a signal to noise ratio of 10:1 [15, 16].

How degradation studies are done

Oxidation
To 2 ml of stock solution azilsartan, 1 ml of 10% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 600c. For HPLC study, the resultant solution was diluted to obtain 10 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acceptance Criteria
The percentage of drugs degraded should NMT 20%

Acid Degradation Studies
To 2 ml of stock solution, azilsartan 1 ml of 0.1N Hydrochloric acid was added and refluxed for 30mins at 600c. The resultant solution was diluted to

obtain 10µg/ml, 10µl solutions were injected into system and the chromatograms were recorded to assess the stability of samples.

Acceptance Criteria
The percentage of drugs degraded should NMT 20%

Alkali Degradation Studies
To 2 ml of stock solution azilsartan, 1ml of 2N sodium hydroxide was added and refluxed for 30mins at 600c. The resultant solution was diluted to obtain 10 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acceptance Criteria
The percentage of drugs degraded should NMT 20%

Thermal Degradation Studies
The standard drug solution was placed in oven at 80°C for 1 hr to study dry heat degradation. For HPLC study, the resultant solution was diluted to10 µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Acceptance Criteria
The percentage of drugs degraded should NMT 20%

Photo Stability studies
The photochemical stability of the drug was also studied by exposing 100 mg of drug sample in sunlight for 12 hours then the solution was diluted to obtain 10µg/ml solution and 10 µl were injected into the system. and the chromatograms were recorded to assess the stability of sample.

Acceptance Criteria
The percentage of drugs degraded should NMT 20%

Results

Method development
Method development was done by changing various, mobile phase ratios and flow rate.
Trial 1:
Chromatographic conditions:
Mobile phase - Acetonitrile and Methanol taken in the ratio 90:10 Flow rate - 0.9ml/min
Coloumn - Unisol C18 [3µm, 4.6×150mm] Detector wavelength – 249nm
Coloumn temperature – 300c Injection volume - 10µL Run time –10min
Observation – Peak obtained was good but the retention time is more when compared with the retention time
Inference – rate to 0.7ml/min (Figure 2).

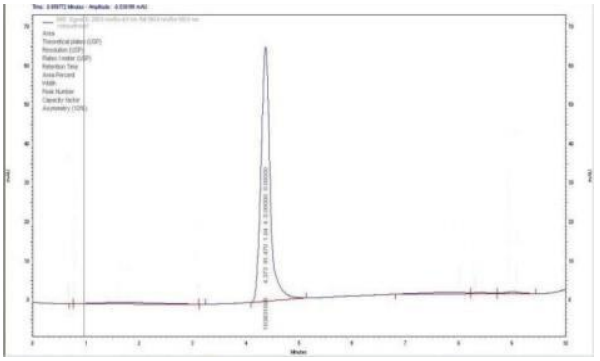

Figure 2: Trial chromatogram 1

Trail: 2
Chromatographic conditions:
Mobile phase - Acetonitrile and Methanol taken in the ratio 90:10 Flow rate - 0.9ml/min
Coloumn - unisol C18 [3µm, 4.6×150mm] Detector wavelength – 249nm
Coloumn temperature – 300c Injection volume - 10µL
Run time– 10min
Observation – Peak obtained was good but the retention time is more when compared with the retention time
Inference – rate to 0.8ml/min (Figure 3).

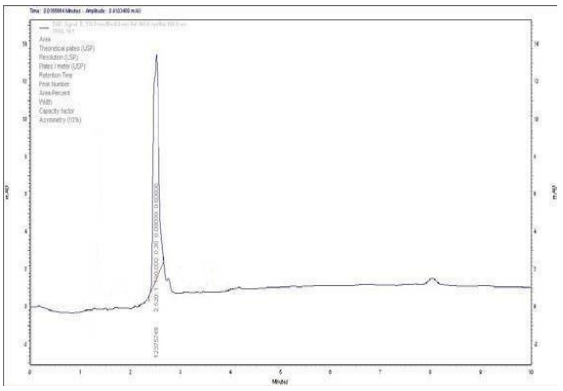

Figure 3: Trial chromatogram 2

Trail: 3
Chromatographic conditions:
Mobile phase - Acetonitrile and Methanol taken in the ratio 90:10 Flow rate - 0.9ml/min
Coloumn - unisol C18 [3µm, 4.6×150mm] Detector wavelength – 249nm
Coloumn temperature – 300c Injection volume - 10µL
Run time – 10min
Observation – Azilsartan peak obtained was good with excellent peak characteristics. Peak was eluted at 2.2 platecount and tailing factor was satisfactory.
Inference – This method was optimized and to validated (Figure 4).

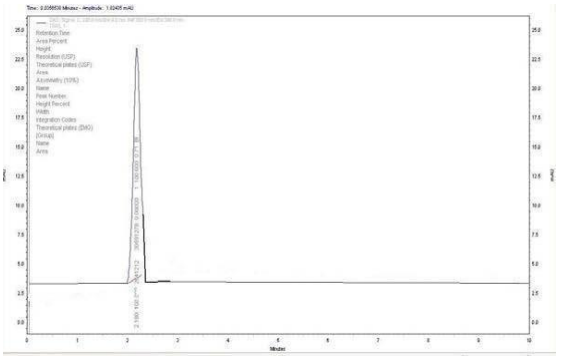

Figure 4: Trail chromatogram 3 [optimized chromatogram]

Trail :4

Chromatographic conditions:

Mobile phase – Acetonitrile and Methanol taken in the ratio 80:20
Flow rate – 0.6ml/min
Coloumn - unisol C18 [3µm, 4.6×150mm] Detector wavelength – 249nm
Coloumn temperature – 300c Injection volume - 10µL Run time – 10min
Observation – Peak obtained was good but the retention time is more when compared with the retention time
Inference – The trail 3 method is selected as optimized method for validation (Figure 5).

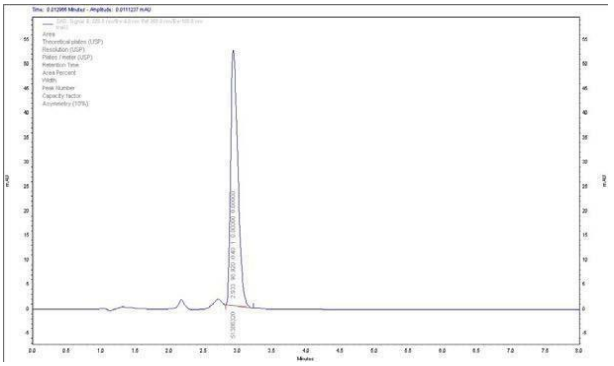

Figure 5: Trail chromatogram 4 [optimized chromatogram]

Method validation

System suitability:

All the system suitability parameters were within the range and satisfactory as per ICH guidelines (Table 1; Figure 6 A-D)

Table 1: System suitability parameters for Azilisartan

S.No	Azilsartan		
S.No	RT(min)	USP Plate Count	Tailing
1	2.248	4457	1.01
2	2.250	4363	1.09
3	2.247	4300	1.08
4	2.249	5910	1.13

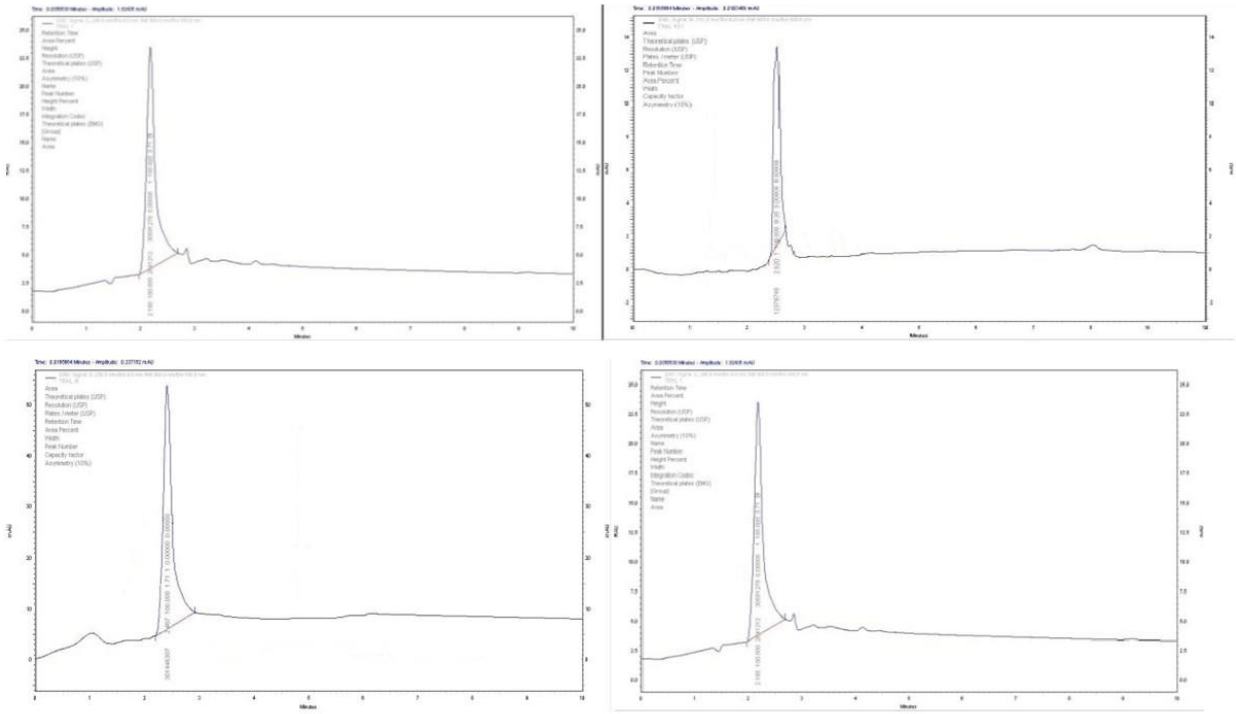

Figure 6: (A) System suitability chromatogram of injection 1. (B) System suitability chromatogram of injection 2. (C) System suitability chromatogram of injection 3. (D) System suitability chromatogram of injection 4

Inference

According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2. All the system suitable parameters were passed and were within the limits (Figure 7 and 8).

Specificity:

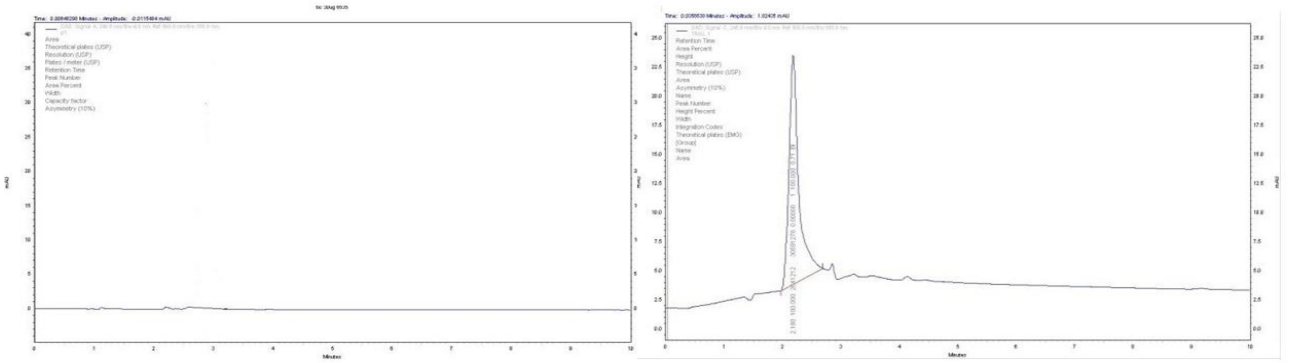

Figure 7: (A). Chromatogram of blank; (B). Typical Chromatogram

Linearity:

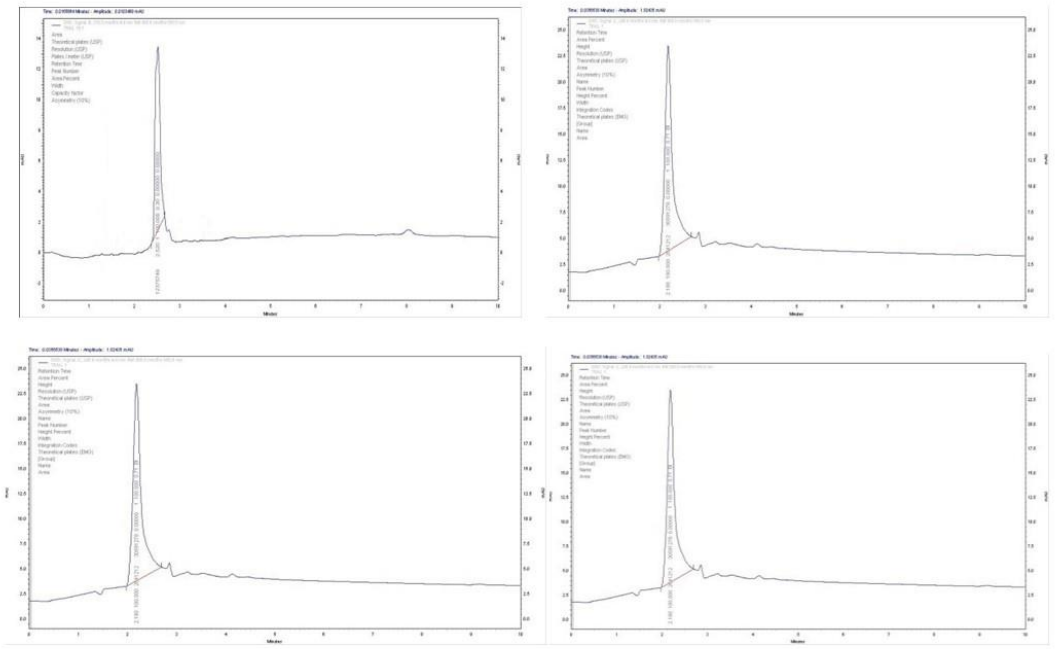

Figure 8: (A). Linearity chromatogram of 2µg/mlAzilsartan; (B). Linearity chromatogram of 4µg/mlAzilsartan; (C). Linearity chromatogram of 6µg/mlAzilsartan; (D). Linearity chromatogram of 8µg/mlAzilsartan

Discussion

A simple, rapid, reliable, robust and optimized reversed phase high performance liquid chromatographic method for estimation of azilsartan was successfully developed and validated as per International Conference on Harmonization guidelines. The optimized method was achieved by using unisol C-18 (150 mm × 4.6 mm × 3 µm) column with mobile phase consisting a mixture of acetonitrile and methanol (90 : 10 v/v) with a flow rate of 0.8 ml/min at 249 nm [17-21]. The developed method was found linear over the concentration range of 2-10 µg/ml for azilisartan the detection and quantitation limit was found to be 0.149 µg/ ml and 0.45µg/ ml .

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