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A NOVEL VALIDATED STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT FOR DETERMINATION OF AZILSARTAN MEDOXOMIL IN ITS DOSAGE FORM

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Abstract:

A simple, specific, accurate and novel stability indicating reverse phase High performance liquid chromatographic method was developed and validated for the estimation of Azilsartan Medoxil in its Dosage form. Azilsartan medoxil is an angiotensin-II receptor antagonist used in the treatment of hypertension. Chromatography was performed using C18 column Qualisil Gold (250 X 4.6 mm, 5µm) with mobile phase consisting 0.2% trifluroacetic acid in acetonitrile and 0.2% trifluroacetic acid in MilliQ water in the ratio of 62:38.The pH was adjusted to 3 with orthophosphoric acid. The detection was carried out at 248nm and retention time (RT) of Azilsartan Medoxil was found to be 7.353min.The method was validated in terms of linearity(20-120 µg/ml), precision, accuracy, specificity, LOD(0.0186µg/ml), and LOQ(0.0613 µg/ml) were well within limits. Azilsartan Medoxil was subjected to stress conditions including acidic, alkaline, oxidative, photolysis and thermal degradation and the results showed that it was highly sensitive to alkaline conditions followed by liable to photolytic, oxidative, thermal, acidic and neutral stress conditions. The degraded products were well resolved from the analyte peak with significant difference in their RT values.

Keywords: *Azilsartan medoxil, Reverse phase, Stability indicating, Chromatography.*

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INRODUCTION:

Azilsartan medoxomil chemically known as (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 2-ethoxy-1-{[2'-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]methyl}-1*H*-benzimidazole-7-carboxylate,is a white powder, practically insoluble in water ,freely soluble in methanol. Azilsartan medoxomil is an antihypertensive drug used in treatment of hypertension. it is a selective AT_1 subtype angiotensin-II receptor antagonist [1-3].An literature survey reveals that azilsartan medoxomil potassium is quantitatively estimated in human plasma by liquid chromatography [4]. However some UV-Spectrophotometric method and HPLC methods were proposed for the estimation of Azilsartan medoxomil individually or in combined with chlorthalidone [5-7]. In the Present an attempt was made to develop simple, specific, accurate and novel validated stability indicating RP-HPLC method of Azilsartan Medoxomil in its dosage form.

Stress studies were carried out under the conditions mentioned in ICH Q1A (R2) viz dry heat, hydrolysis, oxidation and photolysis. Regulatory guidance in ICH Q2A, Q2B, Q3B and FDA 21 CFR section 211 all requires the development and validation of stability indicating assays [8-11] .

MATERIALS AND METHOD:

Chemicals:

Azilsartan Medoxomil (Aurobindo Pharma Limited, Hyderabad, India), Edarbi (40 mg, Takeda Pharmaceutical Company limited, U.S), HPLC solvents: Acetonitrile, water (Merck, Mumbai (India), Orthophosphoric acid (Qualigens, Mumbai), Triethyl amine (Qualigens, Mumbai), pH meter (Systronics, Digital pH meter 802).

Chromatographic conditions:

HPLC system was composed of a manual rheodyne injector with a 20-µl fixed loop and PDA UV-visible detector. Separation was performed on a Qualisil Gold C_{18} column (250×4.6 mm i.d., 5µm) at ambient temperature. The mobile phase consisted of 0.2% trifluroacetic acid in acetonitrile and 0.2% trifluroacetic acid in MilliQ water in the ratio of 62:38, with the pH adjusted to 3 with orthophosphoric acid. The mobile phase was sonicated for 10 min and filtered through a 0.45µ membrane filter. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1.0 ml/min and the injection volume was 20 µl. The eluents were monitored at 248 nm. All determinations were performed at ambient temperature for a run time of 10 min.

Method Development:

Selection and preparation of mobile phase:

Various mobile phase containing methanol, water, acetonitrile and glacial acetic acid in different ratios were tried with different flow rates. Good symmetrical peak was found with the mobile phase comprising acetonitrile, water (0.2% trifluroaceticacid) in the ratio 62:38(v/v) (pH adjusted to 3 with orthophosphoric acid).

Mobile phase was prepared by mixing 620 ml of acetonitrile in 0.2% trifluroacetic acid with 380 ml of HPLC grade water in 0.2% trifluroacetic acid and the pH was adjusted to 3 with orthophosphoric acid. The mobile phase was sonicated for 10 min and filtered through the 0.45 µm membrane filter.

preparation of standard stock solution:

The standard stock solution of 100µg/ml of the drug were prepared by dissolving 10 mg of pure drug in acetonitrile in a 10 ml volumetric flask and the volume was made up to the mark. Resulting solutions were further diluted with mobile phase to obtain a final concentration of 100µg/ml and stored under refrigeration.

Preparation of sample solution:

For the estimation of Azilsartan medoxomil in its dosage form, 5 tablets each containing 40 mg of azilsartan were weighed and the average weight was calculated. The tablets were crushed and powdered, a quantity of powder equivalent to 10 mg of azilsartan medoxil was transferred to 10 ml volumetric flask and the volume was made up to the mark with acetonitrile. The solution was further diluted with mobile phase to get a final concentration of 100µg/ml.

Method Validation:

The developed method was validated for specificity and selectivity, linearity, accuracy, precision, robustness, ruggedness, detection limit, quantification limit and stability.

Selectivity and specificity:

Specificity is the ability of a method to discriminate between the analyte(s) and other components in the sample. Selectivity of the HPLC method is demonstrated by the separation of the analyte from other potential components such as impurities, degradants, or excipients. Volume of 20 µl of working placebo sample solution was injected into the chromatogram and the chromatogram was recorded.

Linearity:

A stock solution of azilsartan medoxil of 1000µg/ml was prepared with mobile phase. From it , various working standard solutions were prepared in the range of 20-120µg/ml and injected into HPLC. It was shown that the selected drug had linearity in the range of 20-120µg/ml.

Accuracy:

The accuracy of the method was carried out using various set of different standard addition method of different concentration levels, 80%, 100% and 120%, and then comparing the difference between the spiked value and actual found value.

Precision:

The precision of the method was evaluated by calculating the %RSD of peak areas of six replicate injections of standard concentrations. The average RSD of Azilsartan medoxil was found to be 0.12%. The precision of the assay was also determined in terms of intra-day and inter-day variation in the peak areas of a set of drug solutions on three different days.

Robustness:

Robustness of the proposed method for Azilsartan medoxil was carried out by changing the optimum HPLC conditions set for this method. The small changes include, flow rate (± 2 ml), pH of the mobile phase (± 0.1 units) and detection wavelength (± 2 nm). The percentage recovery and RSD were noted for Azilsartan medoxil.

Detection limit and Quantification limit:

The limit of detection and limit of quantification were performed on samples containing very low concentrations of analyte under the ICH guidelines. The LOD and LOQ were established according to the following formulas:

LOD=3.3SD/slope

LOQ=10SD/slope

Forced Degradation Studies:

Forced degradation studies were carried out as per ICH guidelines. The specificity and selectivity of the method can be demonstrated by performing forced degradation studies conducted on the sample using acid, alkaline, oxidative, thermal, photolytic and ultraviolet degradations [12-15].

Acid degradation:

Accurately 2.5 mg of Azilsartan medoxil pure drug was weighed, transferred into 25 ml volumetric flask and dissolved in acetonitrile. The volume was made up to the mark with 0.1N HCl (100µg/ml). The flask was kept aside for 8 days at room temperature. Periodically (0, 1,24, 48 hrs and for up to 8 days) 2 ml was taken in a 10 ml volumetric flask, add 5 ml of mobile phase and adjust the pH between 3-4 by adding 0.1N NaOH, dilute with mobile phase up to the mark (20µg/ml). The solutions were injected under the chromatographic conditions and peak areas were measured.

Alkaline degradation:

Accurately 2.5 mg of Azilsartan medoxil pure drug was weighed, transferred into 25 ml volumetric flask and the volume made up to the mark with 0.05N NaOH(100 µg/ml). The flask was kept aside for 10 min at room temperature. Periodically (0, 10 min) 2 ml was taken in a 10 ml volumetric flask, add 5 ml of mobile phase and adjust the pH between 3-4 by adding 0.05N HCl, dilute with mobile phase up to the mark (20 µg/ml). This solution was injected under chromatographic conditions and peak area was measured.

Oxidative degradation:

Accurately 2.5 mg of the Azilsartan medoxil pure drug was weighed, transferred into 25 ml volumetric flask and the volume was made up to the mark with 0.3% H_2O_2 (100µg/ml). The flask was kept aside for 2 hrs at room temperature. Periodically (0, 30, 60, 90,120 min) 2 ml was taken in 10 ml volumetric flask and made up to the mark with mobile phase (20µg/ml). These solutions were injected into HPLC and peak area was measured.

Photolytic degradation:

Accurately 2.5 mg of the Azilsartan medoxil pure drug was weighed, transferred into 25 ml volumetric flask. The drug was dissolved in small quantity of acetonitrile and volume was made up to the mark with HPLC water (100µg/ml). The flask was exposed to sunlight for 30 min. Periodically (0, 10, 20, 30 min) 2 ml was taken in 10 ml volumetric flask and made up to the mark with mobile phase (20 µg/ml). The solution was injected into HPLC and peak area was measured.

Thermal degradation:

Accurately 300 mg of the Azilsartan medoxil pure drug was weighed, kept in Petridis and maintained at a temperature of 105^0C in an controlled temperature oven . Periodically (0, 1, 3, 6 hrs) 2.5 mg of sample was weighed, transferred into a 25 ml volumetric and dissolved in small quantity of acetonitrile, volume was made up to the mark with HPLC water (100 µg/ml). From this solution 2 ml was taken in 10 ml volumetric flask and made up to the mark with mobile phase (20 µg/ml) and injected into HPLC, peak area was measured.

RESULTS AND DISCUSSION:

Method development and Optimization:

Wavelength for detection was selected by obtaining absorption spectra of Azilsartan Medoxomil in water by using double beam UV-VIS spectrophotometer (Lab India). It shows that Azilsartan Medoxomil has λ_{max} at 248 nm. The same wavelength was used in HPLC method development where the impurities can also be detected.

Several solvent systems were tried to get good optimized conditions for Azilsartan Medoxomil.

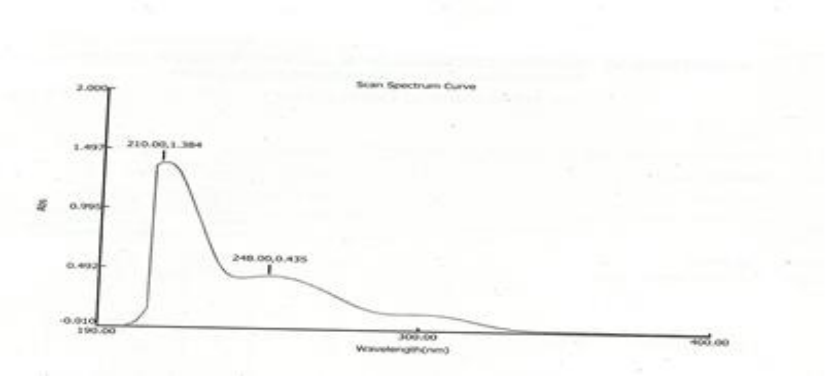

Fig 1: Spectra of Standard APIs:

Table 1: Optimized chromatographic conditions

S.No	Parameter	Optimized Condition
1	Mobile Phase composition	Acetonitrile and water (0.2% TEA, pH 3 with OPA) in the ratio of 62:38
2	Stationary phase	LC-GC Qualisil Gold C_{18} (250 X 4.6 mm i.d., 5µ)
3	Flow Rate	1 ml/min
4	Run time	15 min
5	Column temperature	Ambient
6	Volume of injection	20 µl
7	Detection wavelength	248 nm
8	Retention time of the drug	7.353 min

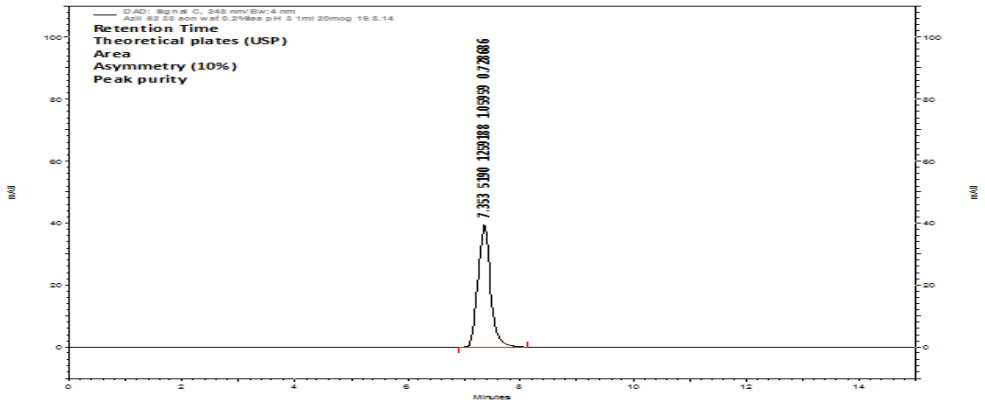

Fig 2: Optimized chromatogram of Azilsartan Medoxomil

Method Validation:

The method was validated for all validation parameters as per ICH guidelines. The linearity range of Azilsartan Medoxomil was 20-120 µg/ml. The value of correlation coefficient (r^2) was 0.9997. The % RSD values in the precision studies were <2%. This confirmed that the method was sufficiently precise. The accuracy of the method was validated by recovery studies and was found to significant and under specification limits, with % recovery 99.92-100.29 (within acceptable range 98-102%). The assay result was found to be 99.64% (i.e. within 95-105%). The method also passes the specifications for robustness parameters. LOD of Azilsartan Medoxomil was found to be 0.0186 µg/ml and LOQ of Azilsartan Medoxomil was found to be 0.0613 µg/ml.

Forced Degradation:

Forced degradation studies of azilsartan in tablet dosage form were carried out in acid/alkaline hydrolysis, oxidative, thermal, photolytic and neutral stress. The peaks of the degraded components were well resolved from the peaks of main component and drug component passed the purity test.

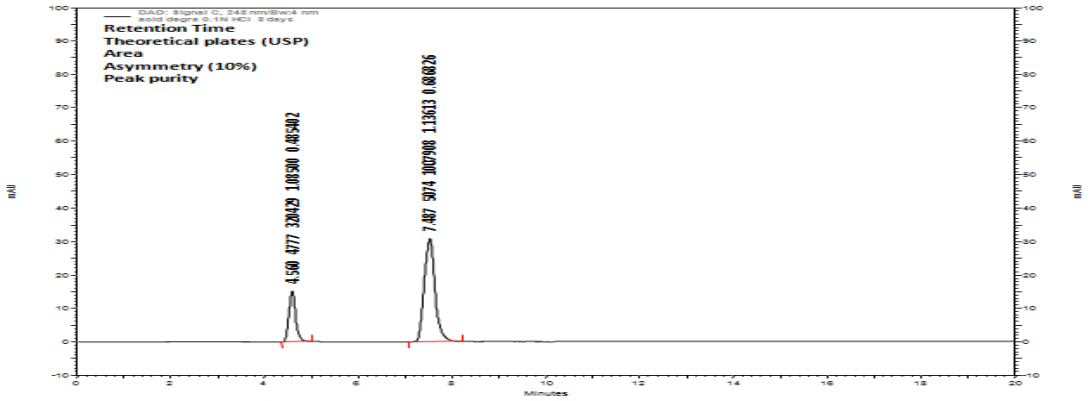

Fig 3: Chromatogram of acid degradation in 0.1N HCl at 8 days

Table 2: Degradants formed during acid degradation in 0.1N HCl

S.No	Degradants	Retention time (min)	Peak area
1	D_1	4.560	320429

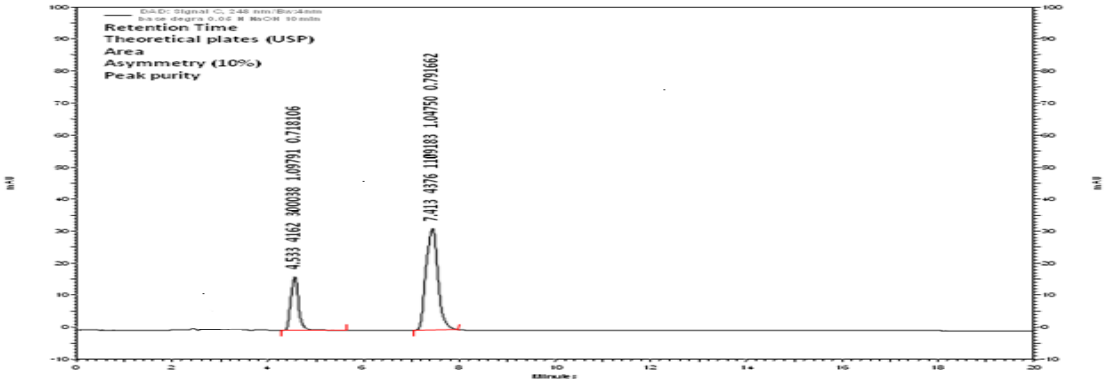

Fig 4: Chromatogram of alkaline degradation in 0.05N NaOH at 10 min

Table 3: Degradants formed during alkaline degradation in 0.05N NaOH

S.No	Degradants	Retention time (min)	Peak area
1	D_1	4.533	300038

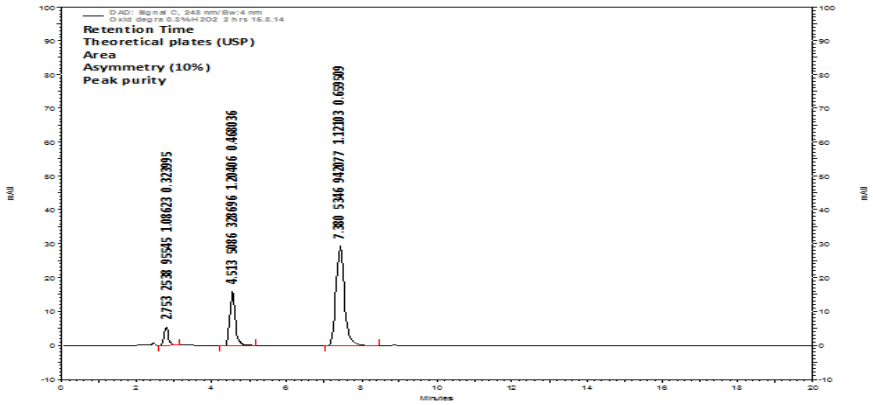

Fig 5: Chromatogram of oxidative degradation in 0.3% H_2O_2 at 120 min

Table 4: Degradants formed during oxidative degradation in 0.3% H_2O_2

S.No	Degradants	Retention time (min)	Peak area
1	D_1	4.513	328696

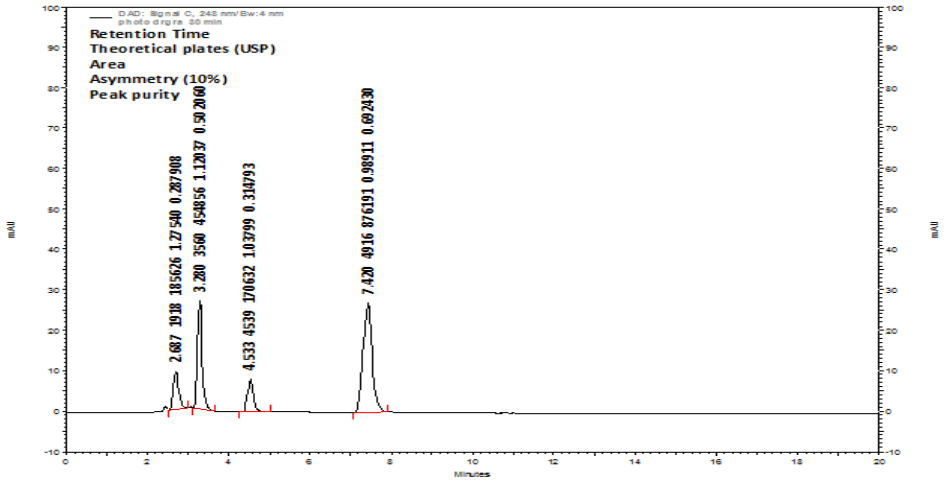

Fig 6: Chromatogram of photo degradation at 30 min

Table 5: Degradants formed during photo degradation

S.No	Degradants	Retention time (min)	Peak area
1	D_1	2.687	185626
2	D_2	3.280	454856
3	D_3	4.533	170632

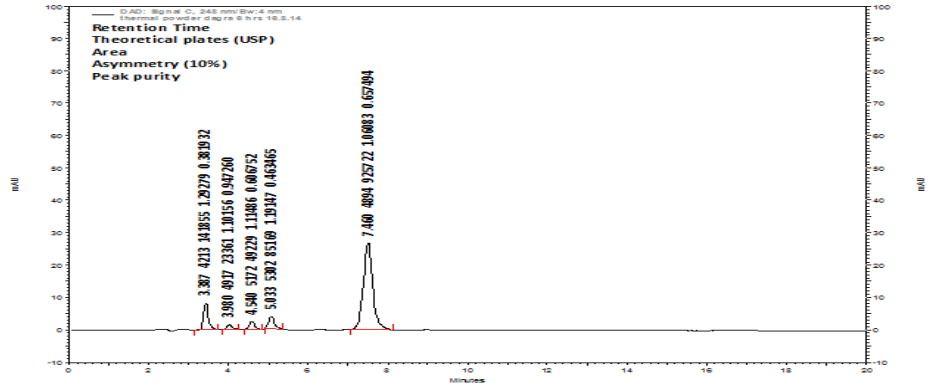

Fig 7: Chromatogram of thermal degradation (105^{0}C) at 6 hrs

Table 6: Degradants formed during thermal degradation at 105ºC

S.No	Degradants	Retention time (min)	Peak area
1	D_1	3.387	141855
2	D_2	3.980	23361
3	D_3	4.540	49229
4	D_4	5.033	85169

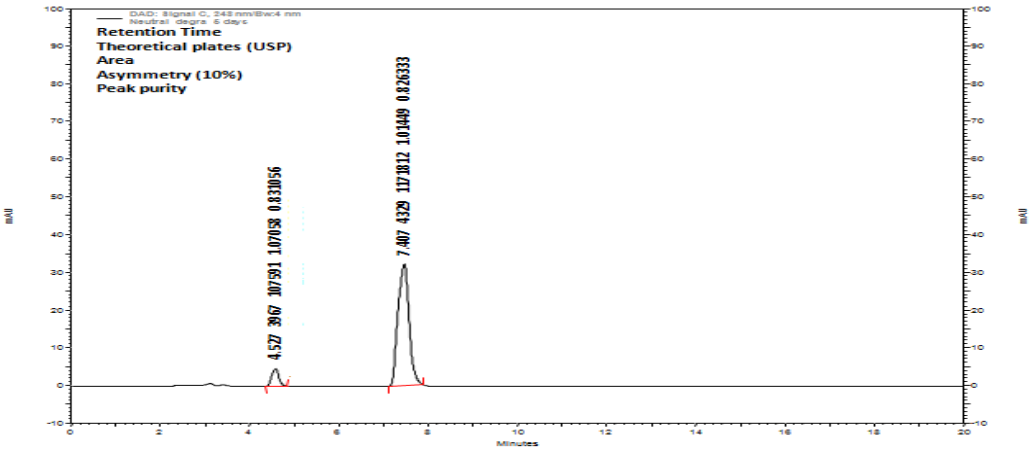

Fig 8: Chromatogram of neutral degradation in pH 7 water at 120 hrs

Table 7: Degradants formed during neutral degradation in pH 7 water

S.No	Degradants	Retention time (min)	Peak area
1	D_1	4.527	107591

CONCLUSION:

The developed RP-HPLC method was found to be suitable for the estimation of Azilsartan Medoxomil in tablet dosage form and was found to be simple, accurate, precise and reliable. The drug azilsartan medoxomil was found to be more degraded when exposed to thermal, photolytic degradation and least degraded when exposed to acid/alkaline hydrolysis, oxidative and neutral degradation.

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