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QUALITY BY DESIGN (QbD) APPROACH FOR METHOD DEVELOPMENT FOR AZILSARTAN MEDOXOMIL USING UPLC: APPLICATION TO HYDROLYTIC, THERMAL AND OXIDATIVE DEGRADATION KINETICS

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Keywords:

Azilsartan medoxomil, Angiotensin II receptor antagonist, QbD, UPLC, Kinetic study

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ABSTRACT: Quality by Design (QbD) is a systematic approach for the development of products and processes. Azilsartan Medoxomil is a competitive antagonist of the Angiotensin II Type 1 receptor. A precise and reproducible ultra-performance liquid chromatographic (UPLC) method was developed and validated for the estimation of Azilsartan Medoxomil from its marketed tablet dosage form applying Quality by Design approach. Eight experiments were conducted using the full factorial design in order to rationally examine the effects of stationary phase, buffer pH and organic phase concentration in primary screening followed by method optimization with variation in Column temperature and flow rate. Chromatographic separation of AZIL was achieved at 40 °C temperature using an Acquity UPLC BEH C18 (4.6mm × 2.1mm, 1.7µ) analytical column; the mobile phase consisted of Acetonitrile: phosphate buffer (pH 5.5, 0.02mM) (40:60, v/v) at a flow rate of 0.3 ml min^{-1}. Azilsartan eluted with retention time (Rt value) 2.41 min. The linear regression analysis showed good linear relationship with a correlation coefficient of 0.999. The limit of detection and quantitation were found to be 0.171µg/ml and 0.57µg/ml, respectively. The method was validated as per ICH guidelines. Exposure of analyte to different stress conditions showed that AZIL follows second order reaction in both acidic and basic medium and first-order reaction in peroxide medium with rate constant 7.351×10^{-3} mol L^{-1} s^{-1}, 7.427×10^{-3} mol L^{-1} s^{-1} and 1.277×10^{-2} mol L^{-1} s^{-1} respectively. The method developed is a stability-indicating, robust, simple, accurate, and reproducible for the determination of AZIL in bulk and formulation.

INTRODUCTION: Azilsartan Medoxomil (AZIL) is an Angiotensin II receptor antagonist, used to treat high blood pressure (hypertension). Chemically, it is Benzimidazole derivative (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}-1H-benzimidazole-7-carboxylate).

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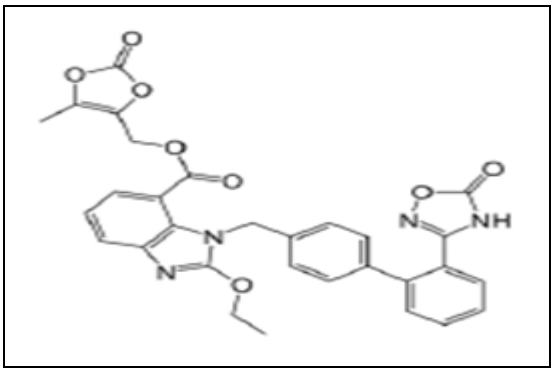

CHEMICAL STRUCTURE OF AZILSARTAN MEDOXOMIL (AZIL)

It is practically insoluble in water and freely soluble in methanol, dimethylformamide, dimethyl

sulfoxide, soluble in acetic acid, slightly soluble in acetone [1]. Literature review reveals RP-HPLC method development and validation for azilsartan medoxomil potassium for quantitation in human plasma [2], HPLC methods reported for the estimation of Azilsartan medoxomil as a single drug in pharmaceutical dosage form and combined dosage form [3-6]. LC-MS compatible stability-indicating assay method [7,] and HPTLC methods [8] and UV spectroscopic method [9], UPLC –MS/MS method for pharmacokinetic studies [10]. Stability indicating method for azilsartan medoxomil/ chlorthalidone using QbD approach [11]. Further UPLC method for combination of Azilsartan Medoxomil and Chlorthalidone to identify degadants and *in-silico* Toxicity Prediction of Degradation Products was reported by Samanthula G *et al*. [12] Another stability-indicating RP-UPLC method was developed for simultaneous determination of azilsartan medoxomil and chlorthalidone in tablets in the presence of its degradation products [13].

Presently stability-indicating methods (SIMs) of analysis are in great demand as Environmental factors, such as temperature, pH, buffer species, ionic strength, light, oxygen, moisture, additives, and excipients, affect the stability of drug substances to a large extent. Stress testing can help in identifying degradation products and provide important information about the intrinsic stability of drug substances [14]. With the advent of the International Conference on Harmonization (ICH) guidelines [15, 16] requirements for the establishment of SIMs have become more clearly mandated. The guidelines explicitly require the conduct of forced decomposition studies under a variety of conditions, like pH, light, oxidation, dry heat, *etc*. and separation of drugs from degradation products. Moreover, kinetic studies on the decomposition of drugs using stability testing techniques are essential for their quality control and to predict the expiry date of pharmaceutical products [17]. The first stability indicating UPLC method for the analysis of AZIL reported by Peraman R *et al*., [18] and in combination with chlorthalidone by Hussien LA and coworkers [19].

QbD has become an important concept for the pharmaceutical industry, which suggests looking into the quality of the analytical process during the development stage itself as discussed by Reddy MC *et al*. [20] QbD approach helps to depict the combined effect of all the factors involved in a study. A response surface methodology (RSM) approach was used to identify the optimum conditions for analysis during method development. The process involves performing experiments with factors (variables) to understanding their effect on responses within the predefined limits. Compared with the traditional optimization method of implementing single factor study at a time leading to poor optimization as other factors are constant, RSM uses a minimum number of experiments, short operating time span and analyzing the generated data statistically to obtain valuable information on the interactions among experimental parameters.

Implementation of Quality by Design approach for the development of a stability-indicating method for furosemide [21,] Quality by Design (QbD) approach was reported for developing the HPLC method for Eberconazole nitrate with its application to hydrolytic, thermal, oxidative and photolytic degradation kinetics [22]. Analytical methods based on QbD, such as an efficient bioanalytical UPLC method for estimation of olmesartan medoxomil was reported by Beg *et al.* [23] QbD based development of a simple, ultra-fast, robust, sensitive, effective and economical RP-UPLC method for estimation of docetaxel trihydrate reported by Khurana *et al*. [24]

Jain A *et al*., reported QbD based analytical method development and validation for raloxifene hydrochloride solid oral dosage form [25]. The objective of the present work was to develop the RP-UPLC method for the estimation of Azilsartan medoxomil in bulk dosage form by implementing the QBD approach and further application of the same for degradation kinetic study.

MATERIALS AND METHODS:

UPLC Instrumentation and Chromatographic Conditions: The UPLC system consisted of two pumps, a manual injector with 10 µl capacity per injection, a temperature-controlled column oven, and the PDA detector. The software used was to Empower 2 software. The experimental data generated from experimental design was statistically evaluated using MINITAB 17.

Chemicals, Reagents and Solutions: Pharmaceutical grade Azilsartan Medoxomil was gifted by Lupin Laboratories Ltd. (Pune, India). Methanol (UPLC grade) was purchased from Merck Chemical Company (India). Potassium dihydrogen orthophosphate, tetra butyl ammonium hydroxide (TBAH) and o-phosphoric acid, hydrochloric acid, sodium hydroxide, and 30% hydrogen peroxide used were of analytical grade. Buffer was prepared by dissolving 2.72 mg (0.02 mM) of potassium dihydrogen orthophosphate in 1 L of HPLC grade water, adding 1ml of TEA, and then adjusting pH with OPA.

Chromatographic Conditions: Full factorial design (3 factors, 2 levels, 8 runs) was used to rationally examine the effects of stationary phase, buffer pH, and organic phase concentration in primary screening. Further method optimization included eight experiments using the full factorial design (3 factors, 2 levels, 8 runs), in order to examine the effects of flow rate, column temperature, and the final concentration of the organic phase. Experimental factors and levels used in the experimental design are shown in **Table 1**.

Chromatographic separation of AZIL was achieved at 40 °C temperature using an Acquity UPLC BEH C18 (4.6mm × 2.1mm, 1.7µ) analytical column; the mobile phase consisted of Acetonitrile: phosphate buffer (pH5.5, 0.02mM) (40:60, v/v) at a flow rate of 0.3 ml min^{-1}. The mobile phase was filtered through a 0.22µ nylon membrane filter and sonicated for 15 min. The injection volume was 2µL, and the optimum wavelength selected for quantification was 249 nm.

Preparation of Standard Stock Solution: Standard stock solution of AZIL was prepared in the mobile phase (50:50, v/v) at a concentration of 100 µg mL^{-1} and further diluted with the mobile phase to furnish the working standard stock solution of 20µg mL^{-1}. The working standard stock solution was diluted with the mobile phase to prepare calibration samples in the concentration range of 10 to 30µg mL^{-1}. Triplicate injections of 2µL were made for each calibration sample and chromato-graphed under the specified UPLC conditions described previously. Peak areas were plotted against the corresponding concentration to obtain the calibration curve.

Forced Degradation Studies of AZIL: [26]

Hydrolytic Conditions: Acid, Alkali and Water Induced Degradation: Standard stock solution (1 mL) was transferred to each of three 10 mL volumetric flasks. 0.1 M HCl (0.5 ml) and 0.1 M NaOH (0.5 ml) and water are added in each separate flask, and the volume was made up to the mark with the mobile phase. For Hydrogen peroxide-induced degradation also a standard stock solution (1 mL) was transferred to 10 mL volumetric flasks. 10% H_2O_2 (1ml) then added in the flask, and the volume was made up to the mark with the mobile phase. Then subjected to the conditions specified in **Table 7**.

Thermal Conditions: For Moist heat-induced degradation, standard stock solution (1 mL) was transferred to each of two 10 mL volumetric flasks, and the volume was made up to the mark with the mobile phase. For dry heat-induced degradation, the analyte is exposed to dry heat 60 °C for 24 h. weighed accurately to prepare a stock solution of 100 µg/ml from which 1 mL was transferred to a 10 mL volumetric flask, and the volume was made up to the mark with the mobile phase. These were subjected to the conditions indicated in **Table 7**.

Photolytic Degradation: Azilsartan medoxomil drug sample is exposed to UV-light of 249nm for 24 h weighed accurately to prepare a stock solution of 100 µg/ml from which 1 mL was transferred to a 10 mL volumetric flask and the volume was made up to the mark with the mobile phase. These were subjected to the conditions indicated in **Table 7**.

The samples from acid and base induced degradation were neutralized by adding 0.5ml of appropriate strength of sodium hydroxide and hydrochloric acid. All samples were stored at 2-8 °C in the refrigerator, filtered with a 0.22 µm membrane syringe filter and injected three times for each sample into UPLC.

Marketed Formulation Analysis: [27] Twenty tablets (EDARBI containing 40 mg of Azilsartan Medoxomil and was weighed, their mean weight determined, and crushed to a fine powder. Tablet powder equivalent to 20 mg of AZIL was transferred to 100.0 ml volumetric flask; 50ml of ACN was added and mixed well. The solution was ultrasonicated for 40 min. and then diluted up to the

mark with phosphate buffer pH 5.5. The solution was mixed and filtered through Whatman filter paper no. 42. The filtrate was further diluted with mobile phase to obtain a final concentration of 20 µg/ml of AZIL. The diluted solution was filtered through NNSY 25, 0.22 µ NYLON filter. From the dilution, 2 µl was injected into the sample injector under the optimized chromatographic conditions. The area of each peak was measured at the selected wavelength. The results of the analysis of tablet formulation and its statistical evaluation are given in **Table 8**.

RESULTS AND DISCUSSION:

Method Development and Optimization: Optimum wavelength of 249 nm was selected to reduce the baseline noise at the absorption maximum of AZIL. Based on AZIL solubility, Acetonitrile was selected as the organic phase. Initially, reversed-phase C18 analytical columns (Acquity UPLC BEH C18 (4.6mm × 2.1mm, 1.7µ) / Phenomax kinetex XB C18 (50mm × 4.6mm, 2.6 µ) were tested with mobile phase composed of variable composition of acetonitrile (40-30% v/v) and phosphate buffer 0.02 mM potassium dihydrogen orthophosphate) at different pH levels ranging from 4.5 to 5.5 with a flow rate from 0.3 to 0.5 mL min^{-1}. Azilsartan is a basic drug having pKa of 6.2, so pH of the buffer (0.02 mM potassium dihydrogen orthophosphate) was adjusted to 5.5 with o-phosphoric acid, *i.e.*, more than two units below the pKa (6.2) to ionize AZIL by 100%.

Eight experiments each were conducted using the full factorial design (3 factors, 2 levels, 8 runs), in order to rationally examine the effects of stationary phase, buffer pH and organic phase concentration in primary screening followed by optimization of an analytical method. It was observed that responses like theoretical plates, retention time, and tailing factor were optimum with a mobile phase composed of Acetonitrile: buffer at the 40:60 ratio eluted AZIL through the C18 stationary phase (Acquity UPLC BEH C18 (4.6mm × 2.1mm, 1.7µ). In method optimization again, eight experiments were conducted using the full factorial design (3 factors, 2 levels, 8 runs), in order to examine the effects of flow rate, column temperature, and the final concentration of the organic phase. Experimental factors and levels used in the experimental design are shown in **Table 1**.

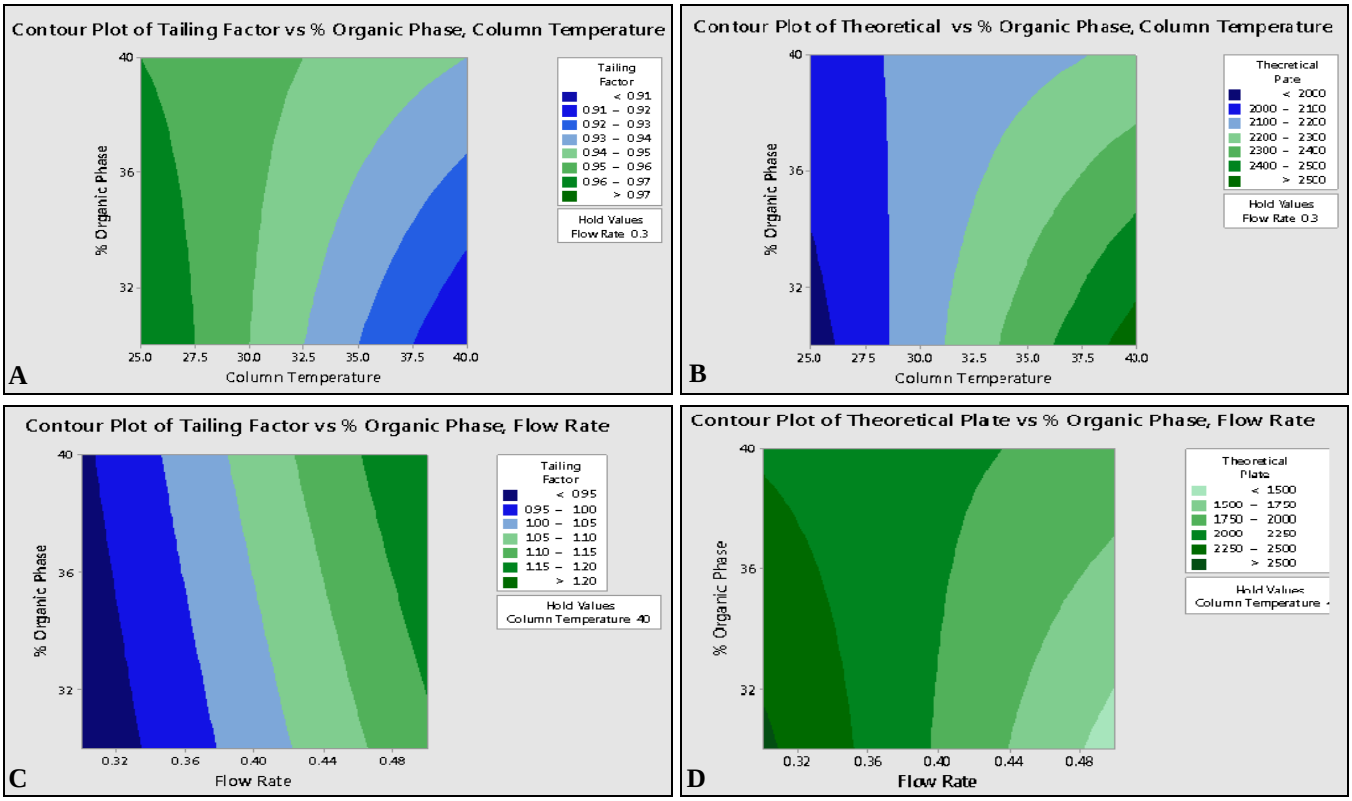

FIG. 1: CONTOUR PLOTS FOR EFFECT OF ORGANIC PHASE AND COLUMN TEMP ON A] TAILING FACTOR B.THEORETICAL PLATES AND ORGANIC PHASE AND FLOW RATE ON C] TAILING FACTOR, D] THEORETICAL PLATES

TABLE 1: FACTORS AND LEVELS USED IN THE EXPERIMENTAL DESIGN

Phase I: Preliminary Screening		Phase II: Method optimization	
Column type	Acquity UPLC BEH C18 (4.6mm×2.1mm,1.7µ) Phenomax kinetex XB C18 (50mm×4.6mm,2.6 µ)	Flow rate	0.3ml/min-0.5ml/min
Buffer pH	4.5-5.5	Final % organic phase	30% to 40%
Organic Phase	Acetonitrile or Methanol	Column temperature	25 °C to 40 °C

The factors and ranges selected for consideration were based on previous univariate studies and chromatographic intuition. The data generated were analyzed using MINITAB 17. The responses obtained after carrying out trial runs were entered back to DOE software, and the Contour Plots of Retention Time (RT), Tailing Factor (TF), and Theoretical Plates (TP) were plotted. Three dimensional overlaid contour plots are presented in **Fig. 1** and **2** are very useful for studying the design space available. The unshaded region in the above plots indicates the design space where all the responses are feasible. QBD Design Space is exhibited as an unshaded area where the method meets the mean performance goals and robustness criteria. The final method conditions are listed along with predicted response results. Optimization plot **Fig. 5** reflects the appropriate condition to reach the goal. The optimized chromatographic conditions obtained from the design were Acquity UPLC BEH C18 (4.6mm × 2.1mm, 1.7µ) column, mixture of 10 mM potassium dihydrogen orthophosphate (pH 5.5) containing 0.02 mM phosphate buffer and Acetonitrile (60:40, v/v), at a flow rate of 0.3 mL min^{-1} with column oven temperature 40 °C. These chromatographic conditions achieved reasonable retention of 2.41 min.and symmetric peak shape for AZIL **Fig. 3**. No interference from the blank and tablet formulation excipients was observed at the retention time of AZIL.

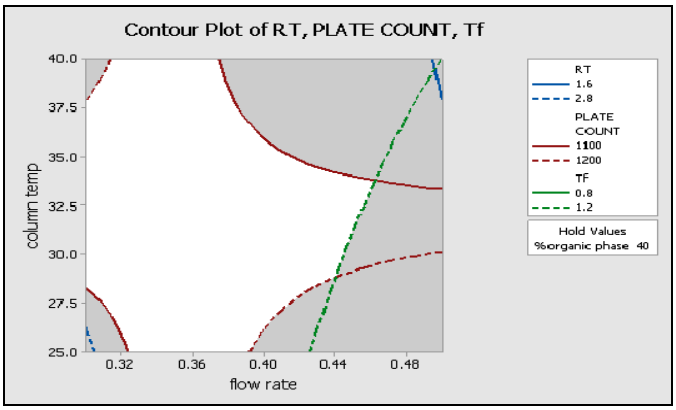

FIG. 2: OVERLAID CONTOUR PLOTS FOR RETENTION TIME, THEORETICAL PLATES AND TAILING FACTOR AGAINST COLUMN TEMPERATURE AND FLOW RATE

Solution Stability: The stability of AZIL in the mobile phase was investigated by analyzing the standard of AZIL (20 µg mL^{-1}) at 0, 6, 12, 24 and 48 h. No significant variation in the peak area of standard solution was observed **Table 2** and also no additional peaks were found in the chromatogram, indicating that AZIL was stable in the mobile phase.

TABLE 2: SYSTEM SUITABILITY DATA

Property/Limit	Observations (n=3)
Retention time (Rt) RSD < 2%	2.41±0.12
Capacity factor (k)	1.41±0.36
Theoretical plates (N) N >2000	9470.58
Tailing factor (T) T < 2	1.1
Stability of solution Peak area ±S.D (0-24 hr)	279008±709.37

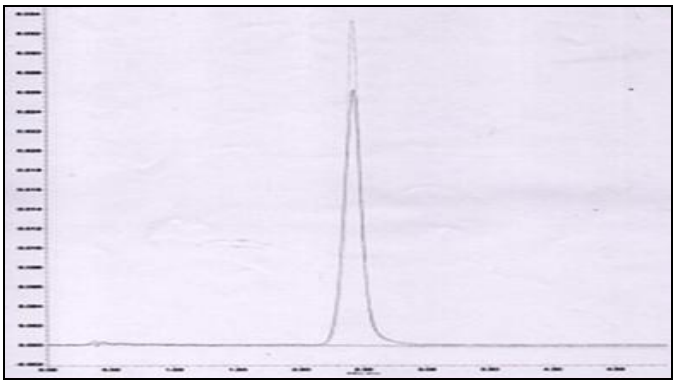

FIG. 3: REPRESENTATIVE CHROMATOGRAMS OF AZIL STANDARD

Method Validation: To confirm the suitability of the method for its intended purpose, the method was validated in accordance with the ICH guidelines [28] for system suitability, linearity, limits of detection and quantification, accuracy, method, and system precision, specificity, and robustness.

System Suitability Parameters: System-suitability test is an integral part of method development and has been used to ensure the adequate performance of the chromatographic system. Retention time (Rt), capacity factor (k), number of theoretical plates (N) and tailing factor (T), were evaluated for six replicate injections of the drug at a concentration of 20 µg mL^{-1}. The results presented in **Table 2** are within the acceptable limits.

Linearity: Linearity of the proposed method was evaluated according to the ICH guidelines. AZIL showed linearity in the concentration range of 10–30µg ml^{-1}, (r^2 = 0.999). The regression equation obtained was Y = 13836x + 2007, where Y is peak area, and X is the concentration of AZIL (µg ml^{-1}). This equation was used to determine the amount of AZIL present in the stability samples.

Limits of Detection and Quantification: The limit of detection (LOD) was defined as the lowest concentration of AZIL, resulting in a signal-to-noise ratio of 3:1 and limit of quantification (LOQ) was expressed as a signal-to-noise ratio of 10:1. The LOD and LOQ obtained were 0.17 and 0.57µg mL^{-1}, respectively.

TABLE 3: RECOVERY STUDIES

Level of % Recovery	Label Claim (mg/tablet)	Total Amount Added (mg)	Amount Recovered[#] (mg)	% Mean Recovery
50	40	20	60.03	100.05
100	40	40	79.92	99.65
150	40	60	100.06	100.06

TABLE 4: RESULTS OF METHOD AND SYSTEM PRECISION

	Method precision		System precision	
Sample no.	Mean Area	% Assay	Mean Area	% Assay
Sample -1	277168	99.8	277078	99.8
Sample -2	276102	99.3	276149	99.3
Sample -3	277959	100.1	275847	99.4
Sample -4	276082	99.5	275888	99.4
Sample -5	276557	99.6	275942	99.4
Sample -6	276537	99.4	275937	99.2
Mean		99.6		99.4
SD		0.293		0.204
%RSD		0.29		0.21

Accuracy: Accuracy of the method was determined by performing the recovery experiments. A known amount of the standard at 50%, 100%, and 150% levels was fortified to the tablet sample. Three replicate samples of each concentration level were prepared, and the percentage recovery at each level (n = 3) was determined **Table 3**. For AZIL, the results obtained are in good agreement with the added amounts.

Method and System Precision: System precision was evaluated by injecting 6 replicate samples of AZIL from different stock on the same system. For method variation, sets of six replicates of the sample from the same stock were analyzed on the different systems. The system and method precision (% RSD) was found to be less than 2% **Table 4**, indicating that the method was precise.

Specificity: Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to be present in the sample matrix. The specificity of the UPLC method was illustrated in **Fig. 4**, where the complete separation of AZIL was noticed in the presence of degradants.

The average Rt ± standard deviation for AZIL was found to be 2.41 ± 0.04 min, for six replicates. The peaks obtained were sharp and had clear baseline separation.

Robustness: A method is robust if it is unaffected by small changes in operating conditions. To evaluate the UPLC method's robustness, few parameters were deliberately varied. The parameters included were a variation of flow rate, wavelength, and percentage of acetonitrile in the mobile phase. Each of the three examined factors (wavelength, flow rate, and acetonitrile percentage) selected was changed one at a time to estimate the effect. Replicate injections (n = 6) of standard solution (20 ug mL^{-1}) were performed under small changes of chromatographic parameters (factors). Flow rate was varied by 0.3± 0.2 mL min^{-1}; the level of acetonitrile in the mobile phase was varied by 40 ±20% (v/v), while wavelength was varied by 249 nm ± 20 nm. Results obtained are presented in **Table 5**, indicating that the results remained unaffected by small variations of these parameters. These results indicate that the applied design of the experiment is useful and meets with set goals.

TABLE 5: RESULTS FOR THE ANALYSIS OF ROBUSTNESS

Chromatographic changes		
Parameter	**Area (Mean ± S.D.)**	**Retention time (Mean ± S.D.)**
Flow Rate (0.3ml/min)±0.2	275879 ± 1707.388	2.813 ± 1.4017
% Organic Phase (40% ACN ± 20v/v)	276541.4 ± 1228.223	2.651 ± 1.9504
Absorbance maxima (249 ± 20nm)	278437.8 ± 944.2689	1.401 ± 0.0088

Stability-Indicating Property: An analytical method is stability-indicating if this method can separate all the process-related impurities and all the degradation products from the major peak of the sample. The model chromatograms of AZIL under acidic, basic, and oxidative stress conditions are presented in **Fig. 4**. AZIL under acidic stress conditions showed degradant peaks at the retention time of 0.472 and 0.988 min and in basic stress conditions showed degradant peaks at the retention time of 0.438 min. Stress samples under dry heat, moist heat, and water hydrolysis showed degradant peaks at 0.44 min, 0.483 min, and 0.482 min. Under oxidative stress conditions, AZIL showed degradant the peak at 0.39 min. This indicates that the drug is susceptible to hydrolytic (acid, base, and water), oxidative, and thermal degradation. In all the above cases, the degradant peaks did not interfere with the AZIL peak, suggesting that the method enabled a specific analysis of AZIL in the presence of its degradation products in **Table 6**.

TABLE 6: FORCED DEGRADATION STUDIES OF AZIL

S. no.	Stress Parameter	Experimental Details	% assay of active substance
1	Acid (0.1 M HCl)	0.5 ml for 10 min at 80 °C	97.48
2	Alkali (0.1 M NaOH)	0.5 ml for 10 min at 80 °C	67.11
3	H_2O_2 (10%)	1 ml for 10 min at 80 °C	65.28
4	Heat	1 hr at 80 °C	99.13
5	Wet	1 hr at 80 °C	55.05
6	UV light	24 hr at 249nm	88.25

TABLE 7: KINETIC PARAMETERS OF DEGRADATION STUDIES

Kinetic parameters of degradation	Parameter	Orders		
		Zero (C × time)	**First (log C × time)**	**Second (1/log C × time)**
Basic medium	Linear correlation coefficient (R^2)	0.723	0.828	0.901
	K (Rate constant)	19.67092×10^{-2}	1.267882×10^{-2}	0.735102×10^{-3}
Acid medium	Linear correlation coefficient (R^2)	0.907	0.624	0.971
	K (Rate constant)	19.35122×10^{-2}	1.258056×10^{-2}	0.742766×10^{-3}
Peroxide medium	Linear correlation coefficient (R^2)	0.882	0.99	0.731
	K (Rate constant)	19.82967×10^{-2}	1.277416×10^{-2}	0.721568×10^{-3}

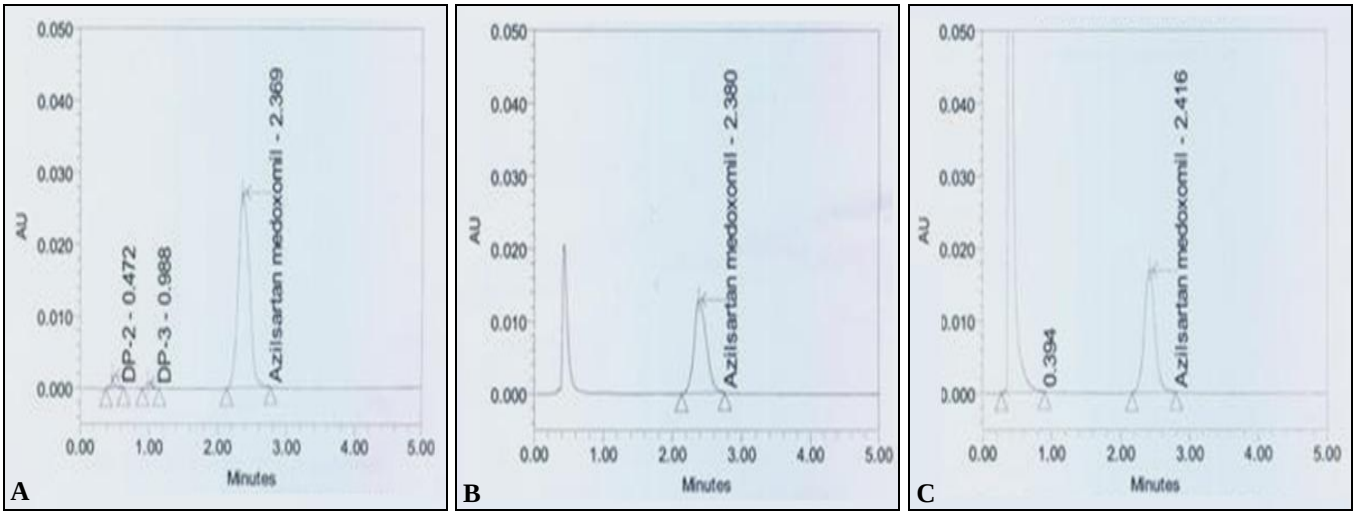

FIG. 4: FORCED DEGRADATION STUDIES WHEN SUBJECTED TO A) ACID B) BASE C) OXIDATIVE

Stress Kinetic Investigation: Treatment of AZIL under specified stress conditions resulted in a gradual decomposition of AZIL in all conditions. The degradation profile was studied, for first-order kinetics linear relationship between log C (concentration of AZIL) percentage of AZIL remaining and time was established while for second-orderr kinetics a linear relationship between 1/log C (concentration of AZIL) remaining and time was plotted. First-order is the term used when the reaction rate of change is proportional to drug concentration. And second-order is the term used when you have two components reacting with each other. The kinetic parameters are presented in **Table 7**. The rate constant (K), for each stress condition, were calculated using Eqs. (1 and 2), respectively

First-order reaction: K= - (log Co+ log C/t) ×2.303 = - slope × 2.303

Second-order reaction: K = (1/C - 1/Co) / t = slope

Where K is the rate constant, $[C_0]$ is the concentration of AZIL at time t= 0, and [Ct] is its concentration at time t. The K values per day were found to be 7.42766×10^{-3}, 7.35102×10^{-3}, and 1.277416×10^{-2} mol L^{-1} s^{-1} for 0.1 M HCl, 0.1 M NaOH, 10% H_2O_2 conditions, respectively. K values indicate more susceptibility of AZIL under peroxide media compared to acidic and basic.

The K value for 0.1 M HCl induced degradation was found to be similar to the degradation by 0.1 M NaOH. Extensive degradation was observed in oxidative conditions, where K value was found to be highest among all the tested conditions. Hence the effect of oxygen needs to be considered for the formulation of AZIL. Suitable antioxidants need to be a part of the formulation of AZIL.

Marketed Formulation Assay: Azilsartan medoxomil (as azilsartan medoxomil potassium) available as, 40 mg and 80 mg, Angiotensin II AT1 Receptor Blocker, Using the proposed chromatographic method, assay of the formulation was carried out. The peak at R_f for AZIL was observed in the densitogram of the drug samples extracted from Tablets. There was no interference observed from the excipients used in the formulation of DFZ tablets. The drug content was found to be 99.102 ± 0.65 **Table 8**.

TABLE 8: RESULTS OF ANALYSIS OF TABLET FORMULATION BY UPLC METHOD

S. no.	Label Claim (mg/tab)	Area	Amount Found (mg/tab)	% of Label Claim
1	40	277794	39.98	99.65
2	40	278945	40.14	100.07
3	40	273156	39.31	97.98
4	40	276599	39.80	99.22
5	40	276097	39.73	99.04
6	40	275750	39.68	98.92
Mean content (n=6) (%)				99.10
S.D.				0.6580
% R.S.D.				0.0066

CONCLUSION: The proposed UPLC method was developed based on Quality by design approach. The optimization plot indicated that the optimum chromatographic conditions could be achieved by using a flow rate of 0.3 ml/min, Column Temperature of 40 °C and % Organic phase of 40% to obtain the required response. The method developed provides simple, accurate, and reproducible quantitative analysis for the determination of AZIL in the presence of its degradants. It was found that AZIL was rapidly degraded under oxidative, hydrolytic (acid and alkali), and photolytic conditions. The degradation of AZIL was found to be of first-order kinetics in oxidation and second-order in acid and base condition.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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