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Forced Degradation Studies by HPTLC-MS Method for Estimation of Azilsartan Kamedoxomil in Pharmaceutical Dosage Form

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ABSTRACT

A high-performance thin-layer chromatographic method was developed and validated for estimation of AzilsartanKamedoxomil in pharmaceutical dosage form. The proposed method was applied successfully to the pharmaceutical analysis of the recently approved dosage form of AzilsartanKamedoxomil which is available in market as a brand name of Edarbi 40 tablets. The drugs were satisfactorily show peak with RF 0.58 for AzilsartanKamedoxomil. Method was validated according to the ICH guidelines. Accuracy and precision of the proposed method was evaluated by recovery studies (% recovery for Azilsartan Kamedoxomil was 99.49 %) and intra-day and inter-day precision studies. In stability testing, Azilsartan Kamedoxomil were found susceptible to acid hydrolysis and alkaline degradation. Because the method could effectively separate the drugs from their degradation products, it can be used as a stability indicating method. The proposed validated stability indicating assay for the sensitive determination of the mentioned drugs is suitable for Quality control laboratories as a simple fast economic method. Degradation product of AzilsartanKamedoxomil in alkaline condition was carried out and its degradation product is successfully separated and isolated by HPTLC method. degradation product was identified by using MS-MS technique.

Introduction

Azilsartankamedoxomil (AZM) is designated chemically 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}-1H-benzimidazole carboxylate monopotassium salt (Fig.1). The active moiety is revealed by hydrolysis of the medoxomil ester. It has molecular formula C30H23KN4O8, molecular weight 606.62.

AzilsartanKamedoxomil selectively inhibits angiotensin II from binding to the angiotensin II type-1 receptor (AT1). This receptor inhibition provides the antihypertensive activity of azilsartanmedoxomil because it blocks the pressor effects of angiotensin II. Azilsartanmedoxomil is a prodrug, it is hydrolyzed to the active moiety, azilsartan, in the gastrointestinal tract during the absorption phase. It has a superior ability to control systolic blood pressure relative to other widely used ARBs. Greater antihypertensive effects of AZL might be due in part to its unusually potent and persistent ability to inhibit binding of angiotensin II to AT1 receptors. Preclinical studies have indicated that azilsartanmedoxomil may also benefit cellular mechanisms of cardiometabolic disease and insulin sensitizing activity. Because azilsartanmedoxomil is a new product and was recently introduced into the market, it is not yet official in any of the pharmacopeia's.

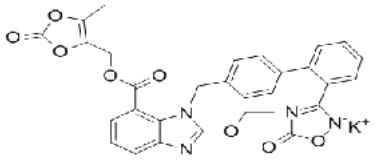

Figure 1: Structure of AzilsartanKamedoxomil.

The current investigation depicts improvement and approval of a basic delicate, explicit, and exact strength demonstrating HPTLC technique for the estimation of AzilsaratnKamedoxomil in mass medications and pharmaceutical plans. The International Conference on

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Harmonization [ICH] [1] rules underline the pressure testing of the medication substance which can assist with recognizing the conceivable debasement items and approve the soundness showing intensity of the investigative strategies utilized. Chromatographic strategies are broadly utilized for the soundness concentrates as it very well may be proficient instruments to isolate and measure the analyte in nearness of decay items. The benefit of HPTLC is that few examples can be run all the while by utilizing a little amount of portable stage not at all like HPLC, therefore bringing down investigation time and cost. The point of the current work was to build up a precise, explicit, and reproducible security showing HPTLC strategy for the assurance of Azilsaratnkamedoxomil within the sight of debasement items under pressure testing in Bulk sedates according to ICH rules [2].

The parent medicates dependability test rules (Q1A R2) gave by International Conference on Harmonization (ICH) necessitates that scientific test strategies for soundness tests ought to be completely approved and the measures ought to be strength demonstrating. Stress testing is a piece of advancement methodology under ICH prerequisites and is done under more serious conditions than quickened examines. The essential objective of the current investigation was to create and approve a HPTLC strategy according to ICH rules [3]. A subsequent target was to build up a soundness demonstrating strategy, which could be utilized for the standard measurement of low degrees of Azilsartankamedoxomil within the sight of debasement items for appraisal of virtue of the mass medication and security of its mass definitions [4]. Constrained corruption contemplates are utilized to encourage the advancement of logical philosophy, to increase a superior comprehension of dynamic pharmaceutical fixing and medication item dependability [5]. Till to day no article identified with the strength demonstrating elite slight layer chromatographic (HPTLC) assurance of Azilsartankamedoxomil in mass medication and pharmaceutical measurements structures was accounted for in writing or in Pharmacopeias. Basic data on sedate degradants and polluting influences can serve to quicken the medication revelation and improvement cycle [6]. The target of steadiness testing is to decide for what timeframe and under what condition the item is agreeable [7]. Pharmaceutical organizations perform constrained debasement concentrates during preformulation to help select mixes and excipients for additional turn of events, to encourage salt determination or detailing improvement, and to create tests for creating strength prosecuting systematic strategies [8-10].

EXPERIMENTAL METHODS

HPTLC Instrumentation

The samples were applied in the form of bands on the plate, width 6 mm, and 10mm from the bottom of the edge using a Camag precoated silica gel aluminium plate 60F254 (10 × 10 cm with 0.2 mm thickness, E.Merck, Germany) with Linomat IV (Switzerland) sample applicator equipped with a 100µL Hamilton (USA) syringe. A constant application rate of 100nL/s was employed and the standard, sample volume was 10µL, the space between two bands were 6 mm and slit dimension was kept 5 mm x 0.45 mm micro and 5 mm sec-1 scanning speed was employed. The eluting solvent was consisted of Toluene: ethyl acetate: methanol solution (5: 3: 2 % v/v). Linear ascending development was carried out in twin trough glass chamber (Camag) saturated with mobile phase. Previously the glass chamber was saturated with help of filter paper and the optimized chamber saturation time was found to be 10 min at room temperature. The length of chromatogram run was approximately 80 mm. After the development the plates were dried in air with the help of an air-dryer. Densitometric scanning was performed on Camag TLC scanner III in the absorbance mode at 260 nm with CATS3 software. The source of radiation utilized was a deuterium lamp. The slit dimensions were 5mm x 0.45 mm micro and the scanning speed was 5 mm sec-1.

Stock Preparation

Preparation of Stock Solution: The stock solution was prepared by weighing 40.0 mg of pure AzilsartanKamedoxomil transferring into 10.0 mL. volumetric flask. Methanol 5.0 mL was added and sonicated for 10 min, and volume made up to the mark by addition of methanol. From this 10.0, 20.0 & 30.0, 40.0, 50.0µg/ml solutions were prepared in methanol.

Method Validation: Method validation was carried out as per the ICH guidelines of analytical method validation (Q2R1) [11,12].

Calibration curve of Azilsartankamedoxomil: The working standard solution each of 10 µl were applied on HPTLC plate to obtain concentration of 10.0, 20.0 & 30.0, 40.0, 50.0µg/ml spot of AzilsartanKamedoxomil. The curve was plotted using peak area against the drug concentrations and the data was treated by the linear least square regression.

Accuracy and Precision:

Precision: To check the degree of repeatability of the method, suitable statistical evaluation was carried out. six samples of the marketed tablets preparation were analysed. the standard deviation (S.D.), % Relative standard deviation (%RSD) was calculated.

Accuracy: The analysed samples were spiked with extra 80, 100, 120 % of the standard Azilsartankamedoxomil and the mixture were analysed by the proposed method. At each level of the above said amount, three determinations were performed. This was done to check the recovery of the drug at different levels in the formulations. Recovery was calculated using the standard formula.

Repeatability:

Repeatability of measurement of peak area: AzilsartanKamedoxomil (40 mg/spot) of 10 µl was spotted on a HPTLC plate, developed, dried and the spot was scanned seven times without changing the plate position and % RSD for measurement of peak areas were estimated.

Repeatability of measurement of sample applications: The drug solution, AzilsartancKamedoxomil, 100 µl (40 mg/spot) was spotted seven times on a HPTLC plate, developed and dried. The spots were scanned and RSD% for measurement of peak areas was estimated.

Robustness: By introducing small changes in the mobile phase composition, the effects on the results were examined. The mobile phase composition, chamber saturation time were tried in the varied range (5 and 15 minutes). Robustness of the method was done at concentration level of 40 mg/ spot.

Limit of detection and limit of quantification:

In order to estimate the limit of detection (LOD) and limit of quantitation (LOQ), blank methanol was spotted six times. The signal-to-noise ratio was determined. LOD was considered as 3:1 and LOQ as 10:1.

Forced degradation of Azilsartankamedoxomil: All stress degradation studies were performed at initial concentration of 100µg/ml. For these studies, 10 mg of AzilsartanKamedoxomil was accurately weighed and transferred to a 100 mL volumetric flask [13,14].

Acid and base induced degradation: To the step one, 3mL of each 0.01N NaOH (for Alkaline degradation), 0.1N HCl (for Acid degradation), were added to separate volumetric flasks and dissolved the content and diluted with methanol to make up the volume and heat the solution for 2 hrs at 80°C.

The mixtures were allowed to cool and 1.0 mL of these solutions were then transferred to 10 mL volumetric flasks neutralized with 1.0 mL of 1N NaOH for acid and neutralized with 1.0 mL of 1N HCl for alkaline degradation and the volumes were made up with methanol. From this solution 50µL and 2 x 50 µL samples were plotted in the form of bands by using Linomat IV, Switzerland applicator on precoated silica gel aluminium plate 60GF-254 to get 500ngspot and 100ng spot respectively for Azilsartan kamedoxomil.

Hydrogen peroxide-induced degradation, Neutral Hydrolysis degradation.

To the step one, 3.0mL of 3% Hydrogen peroxide (for Oxidative degradation) and 3.0 mL of water (for Hydrolytic degradation) were added in two separate volumetric flasks and dissolved the content and diluted with methanol to make up the volume and heat it 2 hrs for 80°C.

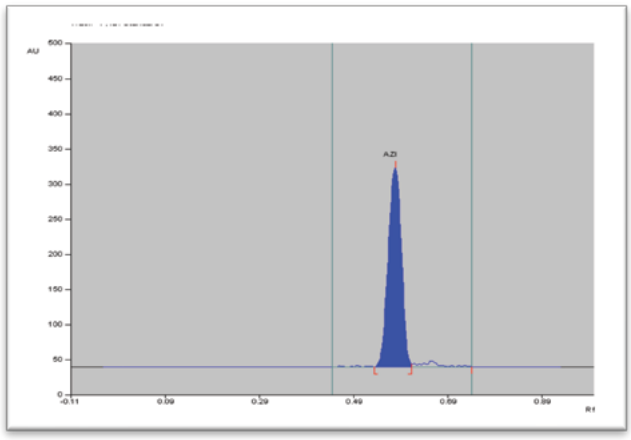


Figure 2: A typical HPTLC chromatogram of AK (Rf = 0.58). HPTLC chromatogram of alkali degraded AK.

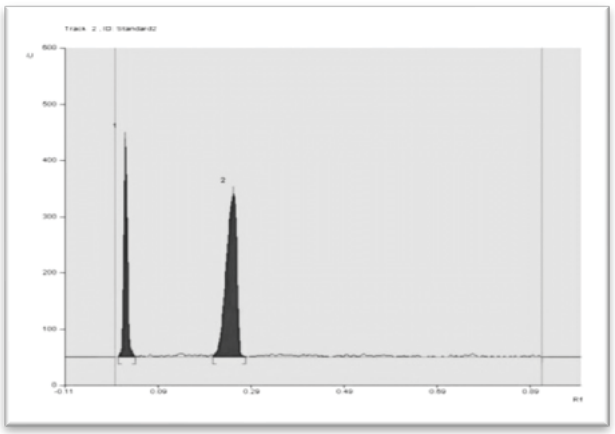


Figure 3

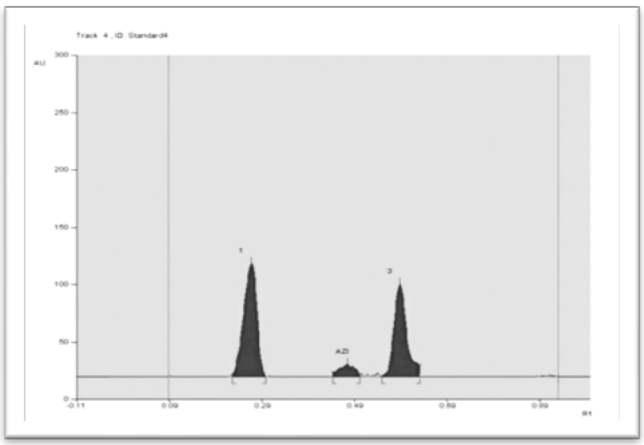


Figure 4

The mixtures were allowed to cool and made up to the mark with the diluent. 1.0 mL of this solution was then transferred to a 10 mL volumetric flask and the volume was made up with methanol. From this solution 50µL and 2 x 50µL samples were plotted in the form of bands by using linomat IV applicator on precoated silica gel aluminium plate 60GF-254 to get 500ng spot1 and 100ng spot-1 respectively for Azilsartan Kamedoxomil.

RESULTS AND DISCUSSION

Method Development:

Development of mobile phase: HPTLC procedure was optimized with a view to developing a stability indicating assay method. Three solvent ratios were selected as to optimize the best among them. The mobile phase consisting of toluene: ethyl acetate: methanol solution (5:3:2 v/v %) gave a sharp and well-defined peak at Rf value of 0.58 (Figure 2). Well-defined spots were obtained when the chamber was saturated with the mobile phase for 10 min at room temperature.

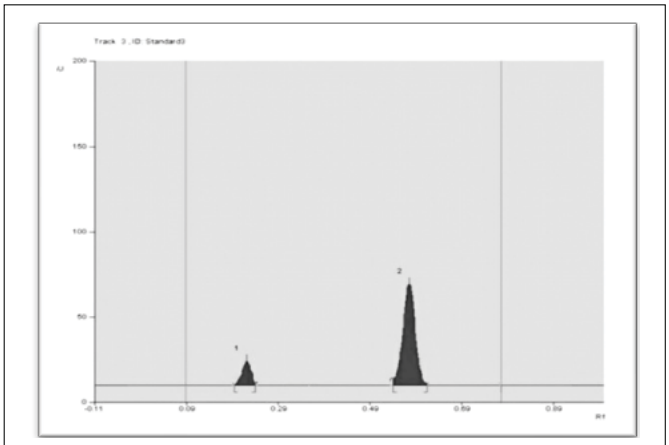


Figure 5: HPTLC chromatogram of hydrogen peroxide degraded AK. HCl degradation.

Table 1: Statistical Evaluation of Precision of Developed Method.

Precision Parameter	% label claim	S.D.	%R.S.D.
Repeatability	101.03	1.2	1.15
Intra-day	101.08	0.12	0.12
Inter-day	100.7	0.22	0.22

Table 2: Robustness of the Method.

Parameter	S.D. of peak area	RSD%
Mobile phase composition: Toluene: ethyl acetate: methanol (4:5:1 v/v)	0.325	0.548
Mobile phase composition: Toluene: ethyl acetate: methanol (6:3:1 v/v)	1.456	0.158
Chamber saturation time (5 min)	0.535	0.176
Chamber saturation time (15 min)	0.278	0.148

Table 3: Recovery Studies.

Level of recovery (%)	Wt. of tablet powder taken equivalent (mg)	Amount of drug added (mg)	Amount of drug recovered (mg)	% Recovery
80	11.7	3.2	8.1	98.64
	11.7	3.2	8.15	99.20
	11.7	3.2	8.16	99.03
100	11.7	4.0	8.61	98.91
	11.7	4.0	8.65	99.78
	11.7	4.0	8.60	99.55
120	11.7	4.8	9.78	99.76
	11.7	4.8	9.77	99.56
	11.7	4.8	9.76	99.67

Degradation of Azilsaratan Kamedoxomil by using Mass spectroscopy:

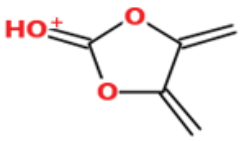
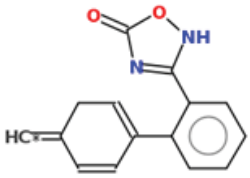
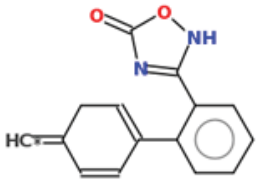
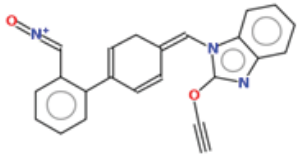
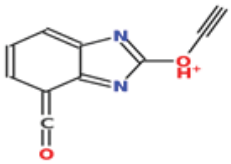
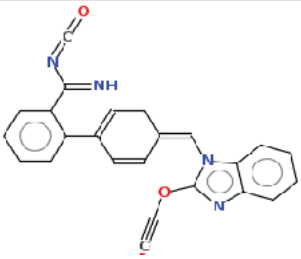
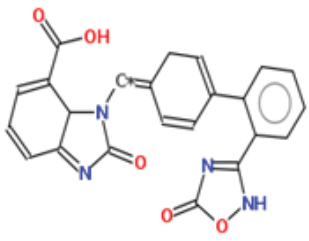
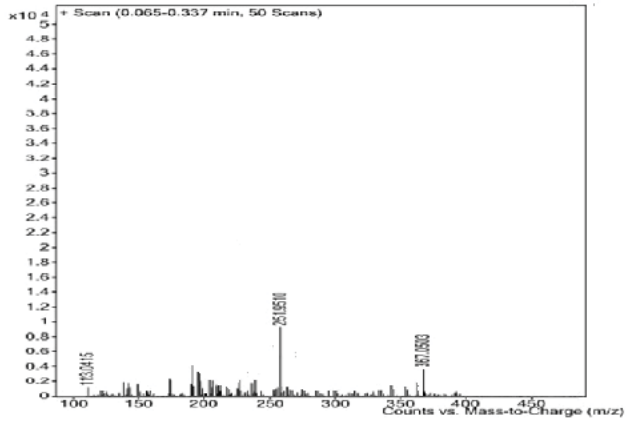
Acid Degradation		
DP1		MW 113.09
DP2		MW 251.09
DP3		MW 251.09
DP4		MW 367.09
DP5		MW 367.09
Alkali Degradation		
DP5		MW.391.28
H_2O_2 Degradation		
DP5		MW.436.10

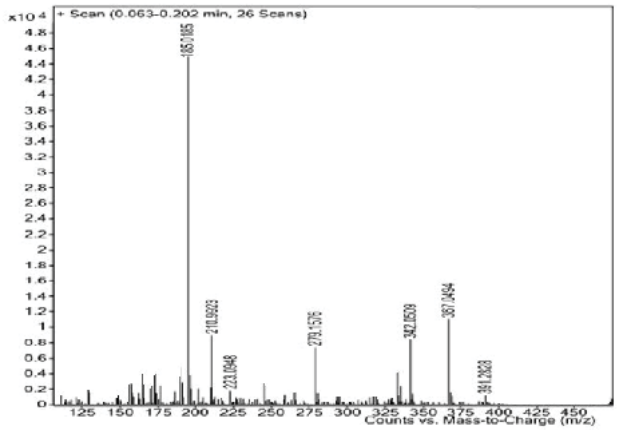
Table 4: Summary of Validation Parameters.

Parameters	Data
Linearity range	10-50 µg/ml
Correlation coefficient	0.9994
Limit of detection (µg/ml)	0.0011
Limit of quantitation (µg/ml)	0.0037
Percent recovery	99.49
Precision (RSD%)	99.49
Repeatability of application (n = 6)	1.15
Repeatability of measurement (n = 6)	0.09
Inter-day (n = 6)	0.12
Intra-day (n = 6)	0.22
Robustness	Robust

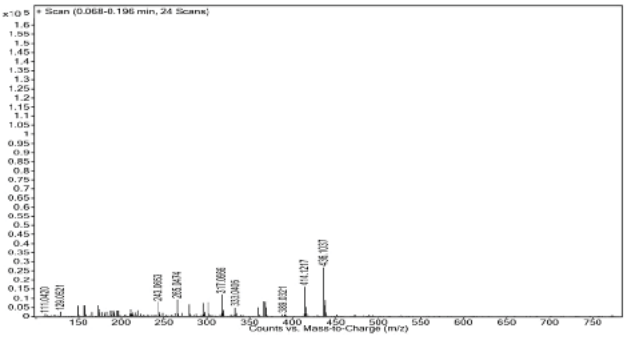
Mass Fragmentation Peak



Acid Degradation



Alkali Degradation



Validation of Method:

Calibration curves: The linear regression data for the calibration curves showed a good linear relationship over the concentration range 40 mg spot with respect to peak area. The regression-coefficient (r2) was found to be 0.9994 which is within the acceptance criteria limit of > 0.99.

Precision: The repeatability of sample application and measurement of peak area were expressed in the terms of % RSD and the results are depicted in Table 1, which revealed intra-day and inter-day variation of AzilsartanKamedoxomil at concentration level of 40 mg spot.

Robustness of the method: The standard deviation of peak areas was calculated for each parameter and %R.S.D. was found to be less than 2%. The low values of %RSD values as shown in Table 2 indicated robustness of the method.

LOD and LOQ: The signal-to-noise ratio 3:1 and 10:1 was considered as LOD and LOQ, respectively.

Recovery studies (Accuracy): The accuracy was assayed by the recovery test. Samples were spiked with three known amounts of AzilsartanKamedoxomil 80, 100 & 120% and then the percentage recovery of the added drug was determined. The data of summary of validation parameters are listed in Tables 3, 4.

Isolation and identification of degradation product of Azilsartankamedoxomil:

A total six degradation products (DP 1 to DP 6) were formed under various stress conditions and their structure were proposed with the help of tandem mass spectrometry (MS/MS) experiments and accurate mass data. In acid hydrolysis total three number of degraded products were found and their molecular weight was mentioned above. In alkali degradation total two degraded products were observed and their molecular weight mentioned above and in hydrogen peroxide one degraded product was observed and molecular weight mentioned above.

Conclusion:

The new HPTLC method is specific, and sensitive for the estimation of Azilsartan kamedoxomil. Statistical analysis proves the method is repeatable and selective for analysis of Azilsartan kamedoxomil. The proposed method is less time consuming and it can be used for routine Quality control test and stability studies of Azilsartan kamedoxomil in Bulk drug. As the method separates the drug from its degradation products, it can be employed as a stability indicating one.

Acknowledgement: None

Conflict Of interest: None

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