Original Research Article

Response Surface Methodology Driven Systematic Development of a Novel RP-UPLC Method for The Quantification of Aliskiren: A Renin Inhibitor

ABSTRACT

ABSTRACT:

Aims: The current study envisages experimental design enabled rapid, sensitive, and stability-indicating RP-UPLC method ~~for the quantification of~~to quantify Aliskiren in its pharmaceutical formulations.

Study design: Box-Benkhen experimental Design using Response surface methodology.

Place and Duration of Study: Department of Analytical Research and DevelopmentBrawn laboratories ltd., Gurugram, ~~india~~India, and Department of Pharmacy KL College Pharmacy, KL Deemed to be University, Vaddesearam, Guntur, Andhra Pradesh, between May 2021 and September 2021.

Methodology: The chromatographic partitioning was achieved on a Waters Acuity H class UPLC system, with BEH 130^{o}A, C18 column (100 x 2.1 mm,1.7 µm) using isocratic elution with PDA detector. The optimum conditions were delineated, selecting three influential factors (CMPs), i.e., mobile phase composition, flow rate, and injection volume. Systematic optimization was accomplished by 3^2 Box-Benkhen design using response surface methodology (RSM).

Results: The selected variables are evaluated for obtained responses (CAAs), i.e., peak area, retention time (Rt), USP Plate count. The optimized method employed, mobile phase composition 0.2 % Glacial acetic acid (pH 3.0) and acetonitrile 50:50 (% v/v) with 0.3 mL min^{-1} flow rate. The injection volume was maintained as 2µL with 2 minutes run time and **λ**max 280 nm.

Conclusion: ~~Linearity was performed~~The method was linear for ~~at~~ 5-300 ppm, ~~and~~ with R^2 ~~was found~~ 0.9995. Forced degradation studies were performed, enduring stability profile of drug as per ICH. The short Rt 1.214, minute implies superior robustness, sensitivity, and cost-effectiveness for routine analysis. The results exhibited RSM approach of QbD could be competently used to optimize the RP-UPLC method with fewer experimental trials and error-free investigation.

Comment [TAW1]: Accronyms need to be defined on their first use

Comment [TAW2]: What does this sentence mean

Comment [TAW3]: Define acronyms, so the authors are not sure since they used the word «could»

Keywords: Chromatography; stability; specificity; renin-inhibitor; Design of experiment; Validation.

1. INTRODUCTION

Aliskiren is recognized as a potent drug of choice known as direct orally active nonpeptide renin inhibitors. The drug is applicable predominantly during high blood pressure. Chemically known as N-Methyl-2-[[3-[(1E)-2-(2-pyridinyl) ethenyl]-1H-indazol-6-yl] thio] benzamide (Fig. 1). Aliskiren is oral, potent, and selective inhibitor of vascular endothelial growth factor receptors [1]. For its clinical use, this can exhibit a novel and advantageous pharmacokinetic and pharmacodynamic profile for the long-term treatment of hypertension so-termed as antihypertensive [2]. In association with selected antihypertensive drugs like calcium channel blockers, the medication of Aliskiren might also be applied with thiazides in product form to provide additional hypertension recoveries. Due to the most acceptable resolution, rapidity, and sensitivity of analysis, UPLC is considered ~~as~~ a budding part of the systematic development of chromatographic science, which holds the sensibleness ~~as~~ compared to conventional HPLC techniques. Unification of the three dynamic factors (speed, resolution, and sensitivity) of advanced UPLC systems and with a configuration of UPLC with PDA detection helps in the isolation with the high-speed scan rates and identification of degradation products by reducing the time required to develop stability-indicating methods [3,4]. The modern application of UPLC with the design of experiments (DoE) paradigm is to improve the analysis of the complex mixture of samples, and hiccups originated during product development and analytical research. UPLC takes full advantage of chromatographic principles to run the separations using columns packed with smaller particles and higher flow rates for improved speed and sensitivity rather than traditional HPLC development, which is pretty tedious.~~4~~ UPLC with QbD served as a proven arena and presiding as an emerging concept based on the robust, rapidity of analysis, regulatory flexibility as well as stability outline of drug products as per ICHQ2R1 guidelines [4],[5] Literature findings revealed for Aliskiren some works with combination dosages have been reported in HPLC, UFLC, etc. ~~But t~~There are limited works that have ~~been~~ reported ~~until now in~~ Response Surface Methodology (RSM) driven Analytical QbD (AQbD) approach, with stability profile analysis. As, QbD based UPLC system ~~has been discovered to~~ produce intense peak capacities with enhanced spectrum quality, separation efficiency, faster elution, and ~~this is found to be~~is quite beneficial in analyzing the complex mixtures ~~rather than depending trial and error basis~~ [4-6].QbD based statistical methodology intensifies analytical design space concept, ~~R~~risk assessments strategy, MFAT (multiple-factors-at-a-time) approach as a contrast to traditional one-factor-at-a-time (OFAT) operations [2]. Hence, an effort ~~has been~~ was made to develop and validate a QbD based precise, ~~cost-effective,~~ sensitive UPLC method [2,3], for the quantification of Aliskiren in its pharmaceutical formulations, which is also stability-signifying as per ICH stability guidelines of ICHQ2R1 and ICHQ8, Q9, Q10 [4,6,7].

Comment [TAW4]: Define UPLC on its first use

Comment [TAW5]: Add reference:
Journal of liquid chromatography & related technologies 34 (20), 2583-2595

Comment [TAW6]: Define PDA

Comment [TAW7]: Some more refernces can be added here
South African Journal of Chemistry 68 (1), 93-98
Current Pharmaceutical Analysis 10 (1), 51-57
Analytical Methods 5 (15), 3693-3699

Comment [TAW8]: What is QbD

Comment [TAW9]: Add refernces
LATIN AMERICAN JOURNAL OF PHARMACY 35 (8), 1768-1775
Journal of Computational and Theoretical Nanoscience 12 (10), 3598-3604
Lat. Am. J. Pharm 34 (2), 351-7

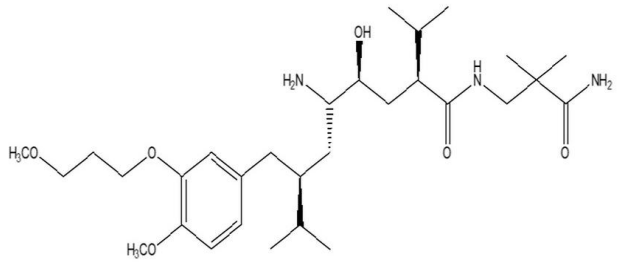


Fig. 1. Chemical structure of Aliskiren

2. METHODOLOGY

2.1.1. Materials and Chemicals

Reference standard or API of Aliskiren (purity 99.4 % w/w) ~~were~~ was obtained from Sun Pharmaceutical Laboratories Pvt. Ltd, (Gujarat), India. The commercial pharmaceutical formulations were procured from the local market. The other foremost solvents used for the research include Acetonitrile UPLC Grade (Merck), Mili-Q-Water (Merck) Methanol and Glacial Acetic acid (Spectro chem). The filtration was performed by the Nylon filter (0.22 µm)–Millipore, Mumbai, India. The pH measurements were made using a Metsar Tech. pH meter.

2.1.2.Instrumentation

The chromatographic development was carried out by a Waters Acquity H class UPLC system with ~~BEH (100 x 2.1 mm), with a particle size of 1.7 µm) C18 Column 130~~ with auto-injector equipped with PDA detector regulated by Empower 2 software. The maximum wavelength was detected with PDA spectra.

Comment [TAW10]: These details are provided in chromatographic conditions

Comment [TAW11]: Update the sentence

2.2. Methods

2.2.1. Statistical Analysis

The advanced statistical software of Design Expert (Ver.12, ~~Stat~~ Stat-Ease, ~~Minepolis~~Minneapolis, USA) was employed for screening with method optimization for assessing CPPs to obtain CAAs through experimental runs [8]. The calculations for the analysis of the regression equation and its ANOVA were premeditated by Microsoft ~~excels~~ Excel 2019 ~~(Microsoft, USA)~~ [1,9].

2.2.2. Preparation of Standard

Comment [TAW12]: Please rewrite the paragraph

75 mg of working standard Aliskiren ~~75 mg of working standard~~ was ~~systematically~~ weighed and ~~allowed to transfer~~transferred ~~into~~ to a 50 mL volumetric flask, ~~, add~~ 30 ml of diluent was added, ultra-sonicated for 10 minutes, the final volume was made up with diluents to obtain a final concentration of Aliskiren 150 µg mL^{-1}. From the stock standard solution, 10 mL was pipetted out in to a 100 ml volumetric flask, and then final volume was made with the diluent. The ensuing chromatogram by injecting blank, standard, and a mixture of excipients (placebo) and formulations are depicted in Fig. 2 (a), (b), (c), and (d), respectively.

Comment [TAW13]: Name the diluent

Formatted: Superscript

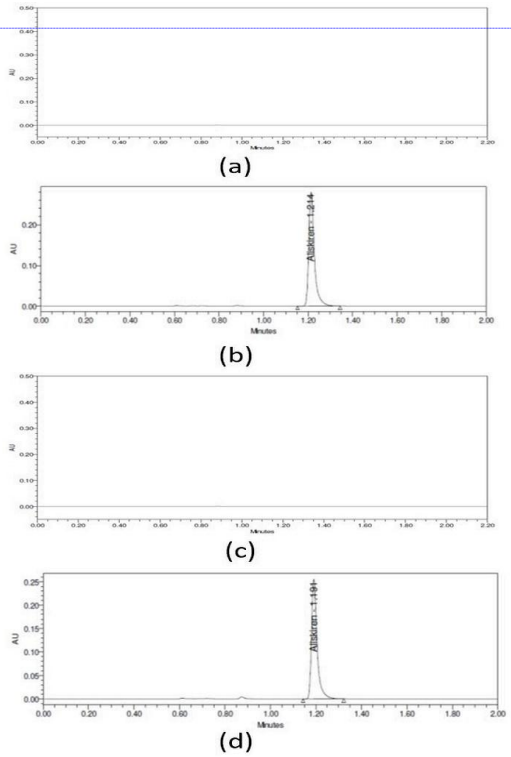


Fig. 2. Optimized Chromatograms of blank (a), standard 150µg/mL (b); mixture of excipients (c) and Formulation (d).

2.2.3. Sample Preparation

Twenty tablets of commercial brands of Aliskiren ~~have~~ were weighed accurately, and each Tablets' average weight was calculated. ~~accurately. After that,~~ the weight equivalent to 300 mg Tablet was transferred into a 100 ml volumetric flask. Diluent of ~~appropriately~~ 50 mL was added and ~~then~~ ultrasonicated for 30 minutes. Further, the volume ~~is~~ was made up ~~of~~ with diluent and filtered. The filtered solution 1ml was pipetted out into a 10 mL volumetric flask and made up to mark with diluent.

2.2.4.Method Development using Box-Benkhen Design (BBD)

AQbD ~~usually delivers assistance by reducing the time, resources, and~~ efforts to develop robust ~~based~~ methods with pertinency in drug substance analysis, degradation products, and other metabolites.~~9~~ For development, Box-Behnken experimental design (BBD) was incorporated [10]~~,~~ [11]~~.~~ to compute independent variables (CMPs) and their capable effects upon~~,~~ the distinct critical analytical or quality attributes (CQAs) like peak area, retention time (Rt), and USP plate count. The focal, interactions, and quadratic effects upon the influential critical variables like mobile phase ratio, flow rate, and injection volume upon the dependent variables like peak area (Y1), retention time (Y2), USP plate count (Y3), are analyzed with

Comment [TAW14]: Figure is not clear. Nothing is visible in figure and also appears that there is some tailing in the peak.

Comment [TAW15]: Rewrite the sentence

Comment [TAW16]: Provide name of diluent

Comment [TAW17]: The authors should provide exact varaiables instead of likes

total 17 experimental runs [12-15]. A method operable design region (MODR) or appropriate design space was earmarked, providing the best method concert via numerical and graphical optimizations over counterplots and by comparing predicted vs. experimental values. The Graphical and statistical chromatographic BBD includes basically the qualitative polynomial equations, 2-D, 3-D counter plot illustrations under the principle of Response surface methodology (RSM) [14],[15].

3. RESULTS AND DISCUSSION

The trail runs aids in the construct of an arithmetical model involving the comprehensive analysis of critical factors~~16~~. Similarly, the 17 experimental runs with three critical factors and ~~its~~ their associated responses 3^2 of BBD experimental design have been elucidated. The design matrix of all the encoded critical factors (independent variables, i.e., % mobile phase and flow rate, injection volume) and its associated observed responses (CAAs) are summarized in Table 1.

Table 1. Design matrix as per Box-Benkhen design for the optimization of chromatographic method

	Low level (−1)	High level (+1)
Independent factors		
***X*1**: Mobile Phase (% v/v)	30	70
***X*2**: Flow rate (ml/min-^{1})	0.2	1
***X*3**: Injection volume (µl)	1	5
Dependent factors (responses)		
***Y1*1**: Peak Area		
***Y2*2**: Retention Time		
Y3: USP Plate count		

Comment [TAW18]: Why the authors used such a huge range?

Were the authors able to get results at both 30 as well as 70 % of organic phase if not then why authors selected 30. As per my understnading the authors wanted to optimise the best condition for analysis. Are there any references for 30% organic phase . please provide representative chromatograms for the 30, 50 and 70 % of organic phase

Similarly, the 17 experimental runs with ~~3~~ three critical factors and ~~its~~ their associated responses (3^2) of BBD experimental design ~~has~~ have been enlisted in Table 2.

Table 2.Optimization of method by 3^2 Box–Behnken design using RSM

Experimental runs	Organic phase (% v/v)	Flow rate (ml min^{-1})	Injection volume (µl)	Peak Area (Cm^2)	Rt (minute)	USP Plate count
1	30	1	3	1020021	1.911	12762
2	50	0.6	3	1290876	1.208	13985
3	30	0.6	1	509876	1.211	10765
4	50	0.2	5	2423217	1.204	12876
5	70	0.6	5	2208761	0.811	12498
6	30	0.6	5	2406541	2.073	10783
7	70	0.2	3	1001245	1.106	15678
8	30	0.2	3	1301456	2.421	10877
9	70	1	3	1100023	0.916	11097
10	70	0.6	1	309871	1.046	11031
11	50	0.2	1	414527	1.215	14608
12	50	0.6	3	965431	0.912	13985
13	50	1	1	122134	0.829	10876
14	50	0.6	3	804321	0.976	13912
15	50	1	5	576843	0.821	11056
16	50	0.6	3	840654	0.917	13985
17	50	0.6	3	840165	0.914	13980

3.1. Optimized Chromatographic Conditions

The chromatographic column used was, Waters Acuity **H** class UPLC BEH 130o A, C18, Column (100 x 2.1 mm, 1.7 µm); mobile phase used was 0.2 % GAA: acetonitrile with 50:50 (% v/v), and the flow rate was maintained as 0.3 mL min-1 during the study. Similarly, the desired pH was monitored 3.0, and the detector employed was PDA with λmax 255 nm. The ultimate temperature was maintained at 30°C during method optimization.

Comment [TAW19]: Are these condition based on results of RSM

Comment [TAW20]: Define GAA

3.1.1 Optimization of chromatographic method using RSM methodology

After selecting optimal chromatographic conditions, the Box-Benkhen Design (BBD) with response surface methodology (RSM), was executed through principles of ANOVA for achieving the enhanced method performance like robustness and leaving scope for continuous enhancement within the specified design space [14],[16],[17]. The multivariate linear regression analysis performed the data optimization analysis to screen out the

tentative responses 2-D counter and 3-D response surface plots. The experimental results or the solutions from the graphical optimization (Experimental run 11) designate that, Organic phase composition (50 % v/v), flow rate (0.2 mL/minute) with injection volume (1 µL) are the utmost influential variables for the method optimizations affecting the Critical Analytical attributes (CAAs), that elucidate final obtained actual responses, i.e., Peak Area (414527 cm^2), retention time (Rt) 1.215, with USP plate count 14608 (Table 3).

Comment [TAW21]: The sentence is hard to read please simplify

Table 3. ANOVA and its significance value with respect to quadratic model post prediction and confirmation data

Comment [TAW22]: Please provide some disscussions as per the table 3 data and what does the table signify

Source	Peak area (Cm^2) F value	Peak area (Cm^2) P- value	Retention time (Minute) F value	Retention time (Minute) P- value	USP plate count F-value	USP plate count P-value
Model	6.12	0.0131*	12.24	*0.0016	13.39	*0.0012
A-Mobile phase	0.3860	0.5541	56.83	0.0001	9.78	0.0167
B-Flow rate	5.45	0.0523	8.78	0.0210	25.40	0.0015
C-injection volume	39.59	0.0004	1.50	0.2597	0.0017	0.9685
AB	0.2922	0.6056	0.8334	0.3916	31.22	0.0008
A^2	3.40	0.1078	26.00	0.0014	18.88	0.0034
B^2	0.8563	0.3856	3.85	0.0906	0.2485	0.6334
C^2	0.3039	0.5986	2.52	0.1561	27.36	0.0012
Lack of fit	5.81	0.0612*	3.09	0.1521*	753.9	<0.0001*

Run 11 Response	Predicted Mean	Predicted Median	Selected values/ Solutions	Observed values	Std Dev.	SE Pred.	95% PI low	95% PI high
Peak Area	3494	3494	12,23561	414527	351676	465223	1096584	1103571
Retention Time	1.12562	1.12562	1.225	1.215	0.175265	0.231853	0.577379	1.67387
USP plate count	13871.4	13871.4	15890	14608	578.595	765.409	12061.5	15681.3

**Significant levels, i.e., less than α value (0.05); *P. I: prediction interval; Std Dev: standard deviation; SE: standard error.*

The graphical optimization solutions elucidate that the predicted values are almost close to obtained experimental values. The counter plots (2D and 3D) responses and their significant interactions of three critical factors upon are depicted in Fig. 3.

Comment [TAW23]: Nothing is visible in graphs

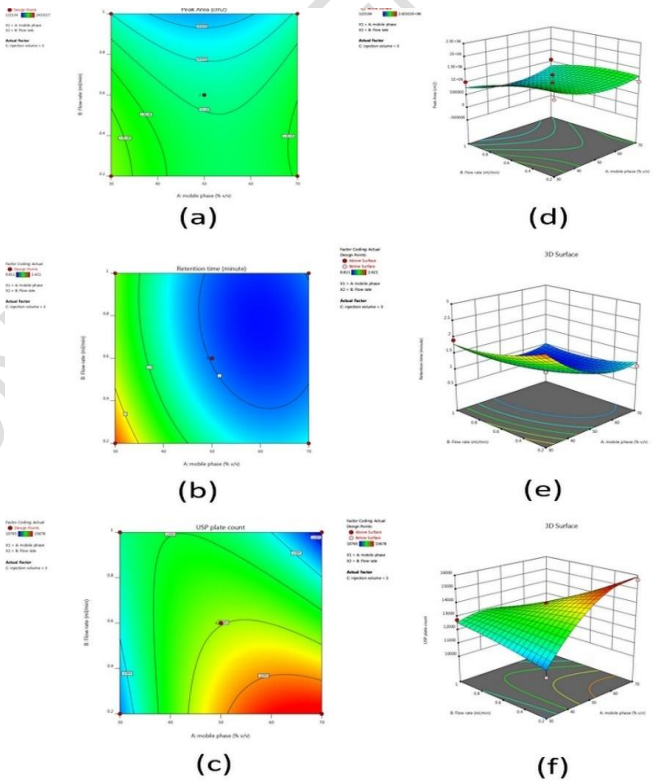
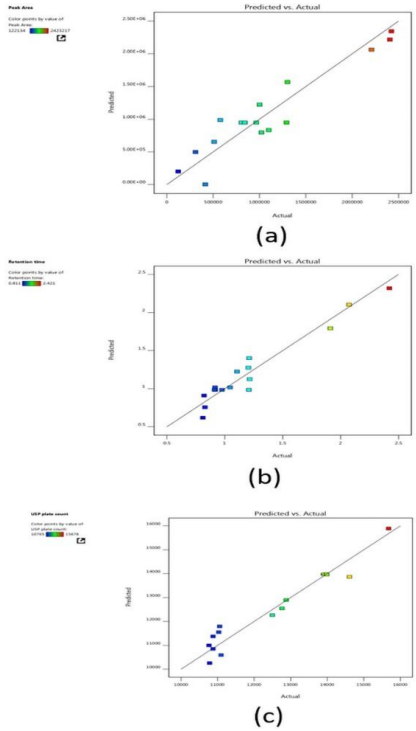


Fig. 3. Schematic diagram indicating 2-D surface contour plot analysis of peak area [Y1] response (a), Retention time [Y2] response (b), USP plate count [Y3] (c); 3-D surface contour plot analysis of peak area [Y1] response (d), Retention time [Y2] response (e), USP plate count [Y3] response.

Similarly, the schematic plot indicating predicted values with and actual experimental values are demonstrated in Fig. 4.



Comment [TAW24]: Figures are unclear

Fig. 4. Predicted vs. Actual value for Peak Area [Y1] (a); Predicted vs. Actual value for Retention Time [Y2]; (b) and Predicted vs. Actual value for USP Plate count [Y3] (c).

3.2. Method Validation

Analytical method validation (AMV) proves that an analytical method that affords analytical results is acceptable for the envisioned practice [8],[12],[14]. As per ICH recommended guidelines, and the drug was subjected to various validation parameters like system suitability test (SST), linearity, accuracy, precision (system, intra and interday), robustness, LOD, and LOQ, etc.

3.2.1. Results of method validation parameters

3.2.1.1. Linearity

~~Linearity~~ The linearity of the method was analyzed for the drug concentrations from 5-300 µg mL-1, employing an injection volume of 10 µL for each concentration. Regression analysis was performed on the obtained data by correlating concentrations and its responses (Peak Area) using an MS-Excel 2019 spreadsheet (M/s Microsoft Inc., Washington, USA), forcing the line through the origin~~,~~ and value of pertinent statistical parameters with Y= 3220X + 32668, and regression co~~-~~efficient (R^2) was obtained 0.9995. The representative linearity plot or calibration curve ~~was~~ is depicted in Fig. 5.

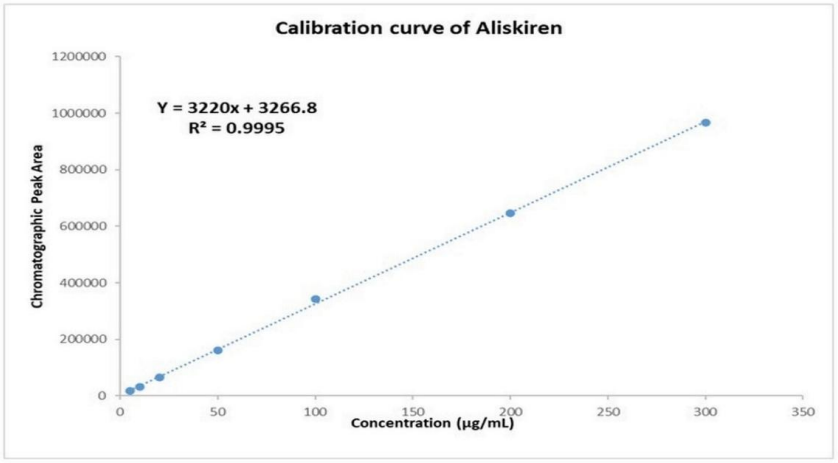


Fig. 5. Schematic diagram of Calibration Plot (a) of Aliskiren

3.2.1.2. Precision

Precision is denoted as the intimacy of preparation (degree of scattering) amongst a series of measurements obtained from multiple samplings of the equal homogeneous sample [8,12]. Precision studies of the drug were carried out by the system, method, and intermediate precision testing. The results of (system, intraday, and interday) ~~P~~precision studies are demonstrated in Table 4.

Table 4. System precision, intraday and interday precision test

Comment [TAW25]: Provide seperate tables for all the three parameters

System Precision				Intra day				Interday			
Conc. (µg/mL)	**Peak Area**	**USP Tailing**	**USP plate count**	**Conc. (µg/mL)**	**Peak Area of at different time intervals (Day 1)**			**Conc. (µg/mL)**	**Peak Area of at different time intervals (Day 2)**		
					10 A.M	**2 P.M**	**5 P.M**		**10 A.M**	**2 P.M**	**5 P.M**
10	48207	1.61	7486	**5**	241190	240123	241987	**5**	241554	241487	241442
10	485124	1.62	7453	**5**	241132	240564	241765	**5**	241431	241541	241643
10	492661	1.51	7359	**5**	241087	240221	241889	**5**	241023	241879	241877
10	483654	1.53	7422	**Average**	241136.3	240302.6	241880.3	**Average**	241336	241635	241654
				SD	51.636	231.565	111.253	**SD**	277.95	212.45	217.70
				% RSD	0.021	0.096	0.045	**% RSD**	0.115	0.087	0.090
10	482854	1.52	7356	10	482392	482454	482776	10	483018	483968	483765
				10	482129	482146	483543	10	485931	484961	484886
10	486059	1.57	7468	10	482736	486263	483456	10	482625	484066	484134
Average	485404			**Average**	485228	483621	483258	**Average**	483858	484331	484261
SD	3842.7			**SD**	304.3	2293.2	419.9	**SD**	1805.99	547.21	571.30
% RS				**% RSD**	0.062	0.474	0.086	**% RSD**	0.062	0.474	0.086

D	0.791						
20	970453	970764	971567	20	971732	971837	971728
20	970452	970771	971569	20	971765	971880	971762
20	970437	971732	971498	20	971754	971878	971769
Average	970447.3	970989	971544.7	**Average**	971750.3	971865	971753
SD	8.9628	383.66	40.426	**SD**	16.802	24.269	21.931
% RSD	0.00092	0.03951	0.00416	**% RSD**	0.00172	0.00249	0.00225

**RSD: relative standard deviation; SD: standard deviation*

3.2.1.3. System suitability Testing (SST)

System suitability parameters were studied by injecting the typical standard solution six times, and results were well under the acceptance criteria. Instrumental performance parameters like peak area, retention time, and USP plate count (> 2000) were evaluated and established, which showed that % RSD was not more than 2%. The % RSD for six replicate injections of the standard was to be 0.791 % The results of the system suitability test are demonstrated in Table 5.

3.2.1.4. Robustness

The robustness of an analytical process is about the degree of its capacity to persevere unaffected by a minor, but deliberate disparities in method performance and its parameters, which indicates its reliability during normal usage [12,19]. The study was performed by altering flow rate, wavelength, and % mobile phase composition. The % RSD less than 0.547 indicates a robust method. The results of robustness studies are demonstrated in Table 5.

Table 5. System suitability and robustness data of Aliskiren

Comment [TAW26]: Provide seperate table for both parameters

**RSD: Relative standard deviation, *SD: Standard deviation*

SYSTEM SUITABILITY TEST (SST)				**ROBUSTNESS STUDY**						
Injection No's	Optimized condition (SST)	USP plate count	Tailing Factor	Parameter	Flow Rate [1+ 0.2ml min^{-1}]	Flow rate [1- 0.2ml min^{-1}]	Wavelength [254 +2 nm]	Wavelength [254 –2 nm]	Amountof [ACN + 2%v/v]	Amountof [ACN - 2%v/v]
Peak Area (Inj.1)	483356	13090	1.6	**Peak Area (Inj.1)**	474365	485595	472465	468476	485267	476253
Peak Area (Inj.2)	472178	13143	1.6	**Peak Area (Inj.2)**	475947	483997	474381	467429	480178	471122
Peak Area (Inj.3)	475965	13069	1.5	**Peak Area (Inj.3)**	472863	481359	474972	464419	482659	474337
Peak Area (Inj.4)	486824	12962	1.5	**Mean**	474391.6	483650.33	473939.33	466774.6	482701.3	473904
Peak Area (Inj.5)	487877	13021	1.5	**Std**	1542.17	2139.17	1310.55	2106.16	2544.76	2592.76
Peak Area (Inj.6)	483257	12964	1.5	**% RSD**	0.325	0.442	0.276	0.451	0.527	0.547
Mean	481576.16			**Difference w.r.t SST (%)**	0.90	-0.43	1.58	3.07	0.233	1.59
Std	2339.13									
% RSD	0.485									

3.2.1.5.Accuracy

~~As per the ICH guidelines about the validation of analytical procedures[19]denotes that~~The ICH guidelines about the validation of analytical procedures [19] denote the accuracy or trueness of experimental observations. Accuracy study was performed at three level (50%, 100 %, and 150%) and the results indicate the mean %recovery studies of all, percentage (%) level data are within acceptance level (98-102 %). The results of robustness studies are demonstrated in Table 6.

Table 6. Accuracy data of Aliskiren

% Level	Amount Spiked (µg/mL)	Amount Recovered (µg/mL)	% Recovery	Mean % Recovery
50%	75	75.02	100.03	100.26 %
	75	75.31	100.42	
	75	75.62	100.83	
100%	150	150.3	100.20	
	150	149.13	99.42	
	150	150.49	100.33	
150%	225	223.74	99.54	
	225	223.80	99.47	
	225	226.86	100.83	

Comment [TAW27]: Provide the standard deviation

3.2.1.6. LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ)~~of the current studies~~ were ~~evaluated~~ calculated from the baseline noise of Aliskiren through findings of calculated signals of samples with known concentrations of analyte with that of the blank by (signal-to-noise) S/N ratio 3:1 (LOD) & 10:1 (LOQ) as per ICHQ2B guidelines [14],[19]. The ~~calculated values of~~ LOD and LOQ were found to be ~~obtained as~~ 0.48 µg mL^{-1} and 1.45 µg mL^{-1}, respectively.

3.2.1.7. Specificity

Specificity is the capability to evaluate the analyte explicitly in the occurrence of components, i.e., degradants, matrix, which may be anticipated to be present. The specificity of the method ~~can be~~was studied by performing stress testing or forced degradation studies. The mixture of excipients was injected to check the interference with the main peak. The results established that~~,~~ there is no interference ~~was detected~~ from the mixture of excipients ~~which are~~and are depicted in Fig. 6 (a-d).

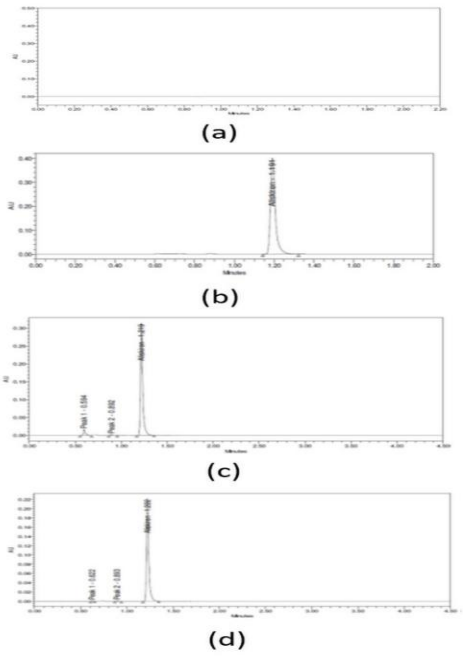


Fig. 6. (a-d). Schematic diagram indicating (a) mixture of excipients, (b) sample acidic degradation, (c) alkali degradation and (d) Peroxide degradation.

Comment [TAW28]: Figure quality needs to be improved

3.3. Forced Degradation Studies

Forced degradation studies ~~was~~ were carried out to patterned the stability of the drug substance and drug product as per recommendations of ICHQ2R1[12],[19]. The drug was subjected to different stress conditions as per ICH. Acidic degradation was performed by taking 1 mL of stock ~~s~~ solution of Aliskiren, and to this 1mL of 2N Hydrochloric acid was added and refluxed for 10 minutes at 60°C. Alkali Degradation studies were carried out by taking 1 mL of stock solution Aliskiren, and to this 1 mL of 2N sodium hydroxide (NaoH) was added and allowed to refluxed for 30 mins at 60°C. Peroxide degradation was carried out by taking 1mL of stock solution of Aliskiren, and to it 1 mL 3% H_2O_2, hydrogen peroxide (H_2O_2) was added separately. Finally, the ensuing solutions of acidic, alkai and peroxide degradations were diluted to obtain 150 µg mL-1 and 2.0 µL were injected into the UPLC system. The chromatograms with results of degradations studies were recorded Fig. 6 (a-d) and Table 7.

Comment [TAW29]: ????

Table 7. Forced degradations and solution stability data of Aliskiren

Stress conditions	Chromatographic Peak Area	*Drug Recovered (%)	*Drug decomposed (%)
Aliskiren standard (Control)	418252	99.8	---
Acidic degradation 1 ml of 2N Hydrochloric acid 60°C, 10 minutes	373215	94.53	5.27
Alkali degradation 1 ml of 2N sodium hydroxideNaOH, 60°C, 30 mins	412367	95.65	4.15
Peroxide degradation 1 ml 3% H_2O_2, room temperature, 10 minutes	486381	96.66	3.14
Thermal degradation 60°C 6 hours	492666	98.22	1.58
Photolytic degradation 365 nm, 3 hours in UV Chamber	495459	99.41	0.39

Solution stability data of Aliskiren

Time in Hrs	Standard Peak Area	% Difference
Initial	485404	---
4	484521	0.18
6	484235	0.24
8	482565	0.58
12	481256	0.85

24	478569	1.41
28	477856	1.55
32	476589	1.82
36	476025	1.93

Likewise, thermal and photolytic degradations of the drug were also premeditated by exposing the 150 µg mL-1 solution to UV light by keeping the beaker in UV Chamber for one day with 200-Watt hours/m^2 in photostability chamber (Fig. 7a & 7b).

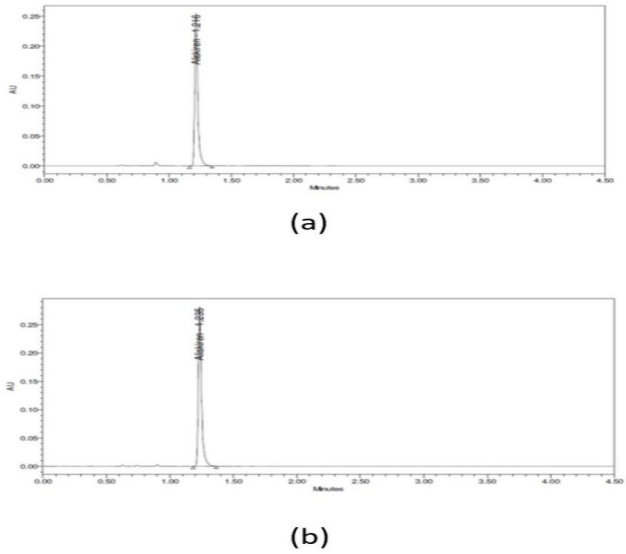


Figure 7 (a-b). Schematic diagram indicating thermal degradation (a), and UV degradations (b)

Finally, the subsequent solution was diluted to obtain 150 µg mL-1 solutions, and 0.2µL were injected into the UPLC system fitted out with PDA detector. The resultant chromatograms were recorded to assess the stability of the sample [12],[18],[19]and the results are enlisted in Table 7.

3.3.1. Stability of Analytical Solutions

~~Stability~~ The stability of the analytical solution was calculated by monitoring the standard and sample solution at 25 ± 2 ºC for the diverse time intervals. Eventually, to assess the stability of the sample, the standard drug and samples were monitored carefully, which signifies those solutions will be stable for up to 36 hours, demonstrated in Table 7 [18-20].

3.4. Assay of Pharmaceutical Formulations

The measured values of % assay of two different marketed formulations of Aliskiren are embodied in Table 8. The results demonstrate that all the values of marketed formulations are within the acceptance limit, i.e., 98-102 % (Table8).

Table 8. Assay of Formulations

Sample No	Brands	Label claims (mg)	% Drug obtained	% Recovery
1	Rasilez, Novartis	300	298.69	99.56
2	Aliskiren Tablets, PAR Formulations Pvt. Ltd.	300	300.08	100.02

4. CONCLUSION

The present article productively reveals the efficiency of the Response surface methodology (RSM) through the AQbD approach~~, and~~. It ~~is to enhance~~enhances the UPLC chromatographic method for the analysis with an improved understanding of the critical factor-response relationship for expanding the method performance. As~~,~~ AQbD is widely being accepted as a scientifically-sound and legitimate paradigm~~,~~ that possesses significant strategies for its execution, ~~mostly~~ primarily when there is not precisely a regulatory need. The overall stress degradation studies practicing the RSM based~~,~~ estimation of the Aliskiren in tablet dosage forms ensured and ~~stability~~ stability-indicating systematic holistic method which is sensitive, precise, and highly robust. ~~These indicate that,~~The quality assurance will be guaranteed in th~~e~~ developed method with regulatory flexibility and can~~. Ultimately, the method also~~the method can find practical application ~~to~~ in the quality control labs for routine analysis.

Comment [TAW30]: Rewrite the sentence

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFRENCES

1. Swarbrick J.Encyclopedia of PharmaceuticalTechnology,2nd ed. Informa Healthcare USA Inc., 270, Madison Avenue New York, 2007;1-648.

2. Moutzouri E, Florentin M, Elisaf MS, *et al*. Aliskiren a direct renin inhibitor, in clinical practice: a new approach in the treatment of hypertension. Curr. Vasc Pharmacol. 2010; 8:344.Available:https://doi:10.2174/157016110791112322.

3. Jain A, Sharma T, Sharma G, Khurana RK, Katare OP, Singh B. QbD-Driven Analytical Method Development and Validation for Raloxifene Hydrochloride in Pure Drug and Solid Oral Dosage Form. Anal Chem. Let. 2019;9:463.Available:https://doi: 10.1080/22297928.2019.1624193.

4. A Otoo, Agarabi DC, Faustino PJ, MJ, Lee HS, Khan MA, Shah RB.Application of quality by design elements for the development and optimization of an analytical method for protamine sulfate. J. Pharm Biomed Anal. 2012; 62.Available:https://doi: 10.1016/j.jpba.2012.01.002.

5. Jena BR, Panda SP, Umasankar K, Swain S, Rao GSN, Dalu D. et al., Applications of QbD-based Software's in Analytical Research and Development. Curr Pharm. Anal. 2021;17 (4). Available:https://doi.org/10.2174/1573412916666200108155853.

6. ICH, Pharmaceutical Quality System Q10, International Conference on Harmonization, IFPMA, Geneva, Switzerland, 2008.

7. ICH Quality Implementation Working Group Points to Consider (R2), ICH-Endorsed Guide for ICH Q8/Q9/Q10 Implementation.Accessed 6 December 2011. Available from: https://database.ich.org/sites/default/files/Q8_Q9_Q10_Q%26As_R4_Points_to_Consider_2.pdf.

8. Stat-ease. (2019). Design-Expert® Software Version 12 - Stat-Ease. Accessed 2 July 2019. Available from: https://www.statease.com/software/design-expert/.

9. Beg S, Jainb A, Kaur R, Panda SS. Katare, OP. Singh B. QbD-driven development and validation of an efficient bioanalytical UPLC method for estimation of olmesartan medoxomil. J Liq Chromatogr Relat. Technol. 2016**;** 39(13): 587–597.Available: http://dx.doi.org/10.1080/10826076.2016.1206023.

10. Jayagopal B, Shivashankar M. Analytical Quality by Design – A Legitimate Paradigm for Pharmaceutical Analytical Method Development and Validation. Mechanics, Mater Sci Eng Magnolithe, 2017; 9. Available:10.2412/mmse.96.97.276. hal-01504765.

11. Patel M, Kothari C. Comprehensive stability-indicating method development of Avanafil Phosphodiesterase type 5 inhibitor using advanced Quality-by-Design approach. J Anal Sci Technol. 2020;11:(29). Available: https://doi.org/10.1186/s40543-020-00228-4).

12. Guidance for Industry:Q8(R2) Pharmaceutical Development. US Food and Drug administration, FDA, 2009.

13. Ramalingam P. Jahnavi, B. QbD Considerations for Analytical Development, In: Beg S, Hasnain MS. (Ed.). Pharmaceutical Quality by Design, Academic Press, 2019, pp. 77-108, ISBN 9780128157992. Availabe:https://doi.org/10.1016/B978-0-12-815799-2.00005-8.

14. Jain A, Beg S, Saini S, Sharma T, Katare OP, Singh B. Application of chemometric approach for QbD-Enabled development and validation of an RP-HPLC method for

estimation of methotrexate. J Liq. Chromatogr Relat Technol. 2019; 42:502. Availabe: http://doi: 10.1080/10826076.2019.1626742.

15. Ghose D, Patro CN, Kumar B, Swain S, Jena BR, Choudhury P & Shre D. QbD-Based Formulation Optimization and Characterization of Polymeric Nanoparticles of Cinacalcet Hydrochloride with Improved Biopharmaceutical Attributes. Turk J of Pharm Sci. 2021, 18:(4):452. Availabe:http://doi:10.4274/tjps.galenos.2020.08522.
16. EMA-FDA pilot program for parallel assessment of Quality-by-Design applications: lessons learnt and Q&A resulting from the first parallel assessment. (Available from:http://www.ema.europa.eu/docs/en_GB/document_library/Other/2013/08/WC500148215.pdf) [Accessed 20 August 2013].
17. Komati S, Swain S, Bhanoji Rao ME, Jena BR. Unnam S, Dasi V. QbD-based design and characterization of mucoadhesive microspheres of quetiapine fumarate with improved oral bioavailability and brain biodistribution potential. Bull. Fac. Pharm. Cairo Univ. 2018; 56: (2),129-145. Availabe:https://doi.org/10.1016/j.bfopcu.2018.09.002.
18. Blessy M, Patel RD, Prajesh N. Prajapati YK. Agrawal, Development of forced degradation and stability indicating studies of drugs- A review, J Pharm Anal2014; 4 (3):159-165.Availabe:https://doi.org/10.1016/j.jpha.2013.09.003.
19. ICH, Pharmaceutical Development Q8 (R2), International Conference on Harmonization, IFPMA, Geneva, Switzerland, 2009.Available:http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.
20. Rao TN. Validation of Analytical Methods, Calibration and Validation of Analytical Methods- A Sampling of Current Approaches, In: Stauffer MT. (Ed.),Intech Open.2018;(https://doi.org/10.5772/intechopen.72087.

LIST OF ABBREVIATIONS

ICH:International Conference on Harmonization
λmax: Maximum Wavelength
RSD: Relative standard deviation
UPLC: Ultra Performance Liquid Chromatography

ANOVA: Analysis of Variance
% RSD: % Relative Standard Deviation
2D: Two dimensional
3D: Three dimensional
BBD: Box-Benkhen Design
DoE: Design of Experiment
FDA: Food and Drug Administration
CMPs: Critical Method Parameters
MODR: Method Operable design region
ATP: Analytical Target Profile
AQbD: Analytical Quality By design