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Response Surface Methodology Driven Systematic Development of a Stability- indicating RP-UPLC Method for the Quantification of Aliskiren: A Renin Inhibitor

Bikash Ranjan Jena [a], G. S. N. Koteswara Rao [a*], Rajasekhar Reddy Alavala [a], Prasanna Kumar Desu [a], G. Chakravarthi [a], Suryakanta Swain [b], Debi Prasad Pradhan [c] and P. Niharika [d]

[a] KL College of Pharmacy, Koneru Lakshmaiah Education Foundation, Green Fields, Vaddeswaram, Guntur-522502, Andhra Pradesh, India.
[b] Department of Pharmacy, School of Health Sciences, The Assam Kaziranga University, Koraikhowa, NH-37, Jorhat-785 006, Assam, India.
[c] Research and Development Center, Endo Pharmaceuticals Inc, Indore- 453001, Madhya Pradesh, India.
[d] Department of Pharmaceutical Chemistry, SIMS College of Pharmacy, Mangaldas Nagar, Guntur-522001, India.

Authors' contributions

This work was carried out in collaboration among all authors. Authors BRJ and GSNKR conceptualized the study design, writing and interpretation of results. Authors RRA, PKD and GC managed the literature collection and analysis of data. Author SS performed the statistical design interpretation. Author DPP and PN reviewed the final draft of manuscript, managed data verification and its available resources. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The current study envisages experimental design enabled rapid, sensitive, and stability-indicating RP-UPLC method to quantify Aliskiren in its pharmaceutical formulations.
Study Design: Box-Benkhen experimental Design using Response surface methodology.

**Corresponding author: E-mail: drgsnkrao@kluniversity.in, drgsnkrao@gmail.com;*

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Methodology: The chromatographic partitioning was achieved on a Waters Acuity H class UPLC system, with BEH 130°A, C18 column (100 x 2.1 mm,1.7 µm) having isocratic elution containing (50:50 %v/v) of 0.2% Glacial acetic acid (GAA) : acetonitrile, at constant flow rate using PDA detection. 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 final optimized method employed, organic phase composition 0.2 % GAA (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 λmax 280 nm.

Conclusion: The method was linear for 5-300 ppm, with regression co-efficient (R^2) 0.9995. As per ICH guidelines, forced degradation studies were carried out to analyse the stability profile of drug. The short Rt 1.214, minute implies superior robustness, sensitivity, and cost-effectiveness for routine analysis. The results exhibited that RSM approach of QbD will be competently used to optimize the RP-UPLC method with fewer experimental trials and error-free investigation.

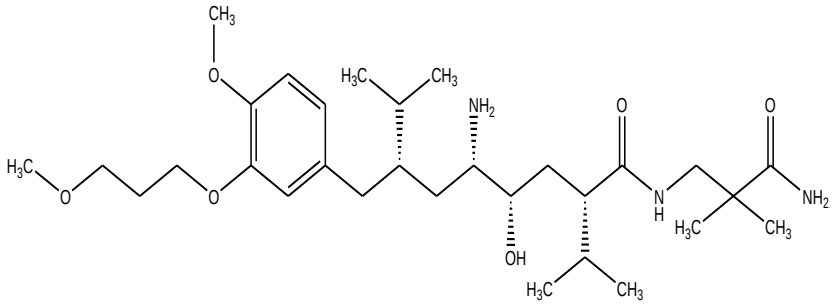
Keywords: Chromatography; stability; specificity; renin-inhibitor; Design of experiment; Validation.

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
Psi : Pound per square inch

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 (2*S*,4*S*,5*S*,7*S*)-5-amino-*N*-(3-amino-2,2-dimethyl-3-oxopropyl)-4-hydroxy-7-[[4-methoxy-3-(3-methoxypropoxy)phenyl]methyl]-8-methyl-2-propan-2-ylnonanamide (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 most acceptable resolution, rapidity, and sensitivity of analysis, UPLC stands for Ultra Performance Liquid Chromatography used for the separation and identification of components under tremendous pressure about 6000-15000 psi and which provides reliable and authenticated data [3]. It is considered a budding part of the systematic development of chromatographic science, which holds the sensibleness compared to conventional HPLC techniques [4]. Unification of the three dynamic factors (speed, resolution, and sensitivity) of advanced UPLC systems.Photodiode array detector (PDA) is powerful detector which has the effective advantage of measutring the spectral profile of components. It is also helpful for detection of purity of analytes, providing wide range of wavelengths in short span of time [5]. The 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 [6,7]. The modern application of UPLC with the design of experiments (DoE)



(2S,4S,5S,7S)-5-Amino-N-(2-carbamoyl)-2-methylpropyl)-4-hydroxy-2-isopropyl-7-(4-methoxy-3-(3-methoxypropoxy)benzyl)-8-methylnonamide

Fig. 1. Chemical structure of Aliskiren

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 [8]. Quality by design is a modern, systematic and holistic approach which includes pre-defined objectives, quality product characteristics relied on product and process understanding, it's control within the required design space [9]. 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 [10,11] Literature findings revealed for Aliskiren some works with combination dosages have been reported in HPLC, UFLC, etc. There are limited works that have reported Response Surface Methodology (RSM) driven Analytical QbD (AQbD) approach, with stability profile analysis. As, QbD based UPLC system produce intense peak capacities with enhanced spectrum quality, separation efficiency, faster elution, and is quite beneficial in analyzing the complex mixtures [12,13]. QbD based statistical methodology intensifies analytical design space concept, 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 was made to develop and validate a QbD based precise, sensitive UPLC method [14-17], 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 [10-12].

2. METHODOLOGY

2.1 Materials and Chemicals

Reference standard or API of Aliskiren (purity 99.4% w/w) 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.

2.1.1 Instrumentation

The chromatographic development was carried out by a Waters Acquity H class UPLC system equipped with auto-injector PDA detector regulated by Empower 2 software. The maximum wavelength was detected with PDA spectrum.(Photo Diode Array detector).

2.2 Methods

2.2.1 Statistical analysis

The advanced statistical software of Design Expert (Ver.12, Stat-Ease, Minneapolis, USA) was employed for screening with method optimization for assessing CPPs to obtain CAAs through experimental runs [18-20]. The calculations for the analysis of the regression equation and its ANOVA were premeditated by Microsoft Excel 2019 [1,21].

2.2.2 Preparation of mobile phase / diluent

2 mL of glacial acetic acid was added to 1000mL, Milli-Q water and mixed well. Mix 500 mL of acetonitrile and 500 mL of prepared buffer, sonicated to degas and filtered through 0.22 µ nylon membrane filter.

2.2.3 Preparation of standard

75 mg of working standard Aliskiren was weighed and transferred to a 50 mL volumetric flask. 30 mL of diluent of 0.2 % glacial acetic acid **:** acetonitrile (50:50 % v/v) 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 into a 100 ml volumetric flask, and then final volume was made with the diluent. The ensuing chromatogram by injecting blank, standard, mixture of excipients (placebo) and formulations are depicted in Fig. 2 (a), (b), (c), and (d), respectively.

2.2.4 Sample preparation

Twenty tablets of Aliskiren commercial brands were accurately weighed, and each tablet's average weight was calculated. The weight equivalent to 300 mg Tablet was transferred into a 100 ml volumetric flask. Diluent of 0.2 % glacial acetic acid **:** acetonitrile (50:50) (prepared mobile phase) of 50 mL was added and ultrasonicated for 30 minutes. Further, the volume was made up with diluent and filtered. The filtered solution 1ml was pipetted out into a 10 mL volumetric flask and made up to mark with diluent.

2.2.5 Method Development using Box-Benkhen Design (BBD)

AQbD efforts to develop robust methods with pertinency in drug substance analysis, degradation products, and other metabolites. For development, Box-Behnken experimental design (BBD) was incorporated [22,23]. To compute the independent variables (CMPs) and their capable effects upon the desired critical quality attributes (CQAs), regarded as independent factors such as peak area, retention time (Rt), and USP plate count. The focal, interactions, and quadratic effects of the influential critical method parameters i.e mobile phase ratio, flow rate, and injection volume upon the dependent factors (responses) peak area (Y1), retention time (Y2), USP plate count (Y3), are analyzed with total 17 experimental runs [24-27]. A method operable design region (MODR) or appropriate design space was earmarked, providing the best method

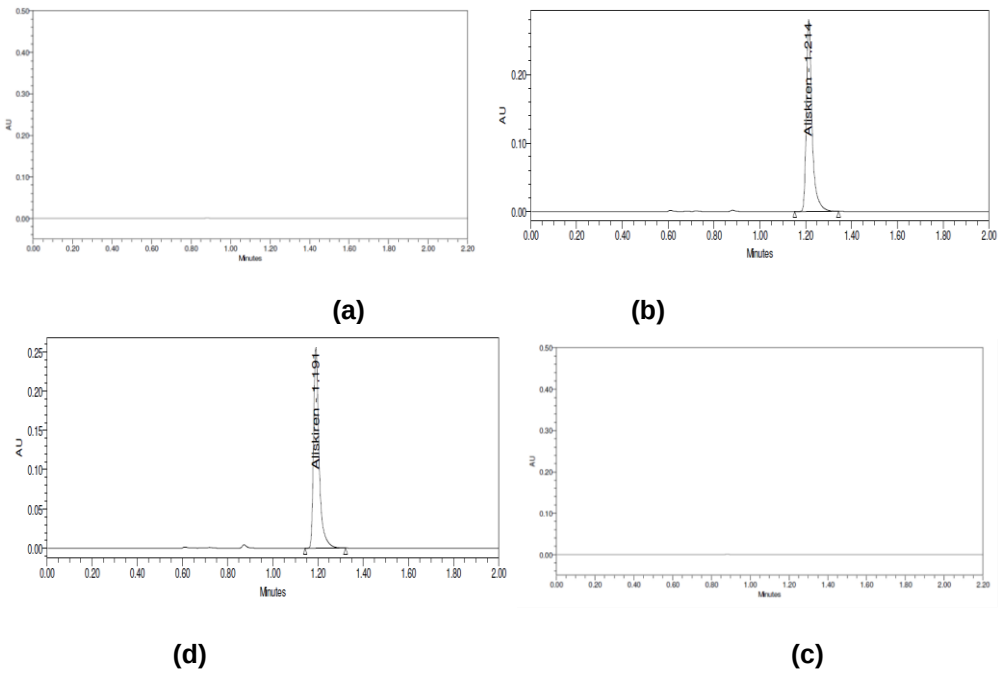


Fig. 2. Optimized Chromatograms of blank (a), standard 150µg/mL (b); mixture of excipients (c) and Formulation (d)

concert via numerical and graphical optimizations over counterplots and by comparing the predicted vs. experimental values. The graphical and statistical chromatographic BBD includes basically the quadratic polynomial equations, 2-D, 3-D counter plot illustrations under the principle of Response surface methodology (RSM) [20], [26],[27].

3. RESULTS AND DISCUSSION

The trail runs aids in the construct of an arithmetical model involving the comprehensive analysis of critical factors. Similarly, the 17 experimental runs with three critical factors and their associated responses 3^2 of BBD experimental design have been elucidated. The design matrix containing encoded values of low, intermediate and high [-1, 0, +1], levels [19],[20]. The selected independent factors for the model such as % mobile phase, flow rate, and injection volume and its dependent factors (observed responses) are represented in Table 1.

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

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

Low level (−1)		Intermediate (0)	High level (+1)
Independent factors			
X1: Mobile Phase (% v/v)	30	50	70
X2: Flow rate (mL/min-^{1})	0.2	0.6	1
X3: Injection volume (µL)	1	3	5
Dependent factors (responses)			
Y1: Peak Area			
Y2: Retention Time			
Y3: USP Plate count			

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

After interpretation of BBD, the calculated responses (Y1), (Y2) & (Y3) of dependent variables (CAAs) are represented as below equations Eq. (1), (2) (3) respectively.

$$Peak\ Area\ (Y1) = +9.483E+05 - 77249.25A - 2.902E+05\,B - 2.902E+05B + 7.824E+05\,C + 95053.25\,AB + 556.25AC - 3.885E+05BC + 3.160E+05\,A^2 - 1.586E+05B^2 - 1.586E+05B^2 \quad (1)$$

$$Retention\ Time\ (Y2) = +0.9854 - 0.4671A - 0.1836B + 0.0760C + 0.0800AB - 0.2743AC + 0.0008BC + 0.4356A^2 + 0.1676B^2 - 0.1357C^2 \quad (2)$$

$$USP\ plate\ count\ (Y3) = +13969.40 + 639.62A - 1031.00B - 8.37C - 1616.50AB + 362.25AC + 478.00BC - 1225.33A^2 - 140.58B^2 - 1474.82C^2 \quad (3)$$

3.1 Optimized Chromatographic Conditions

Using the principles of response surface methodology ultimately, in Waters Acuity **H** class UPLC, BEH 130^{o} A, C18, (100 x 2.1 mm, 1.7 µm) chromatographic column was employed for the developed method; composition of mobile phase 0.2 % glacial acetic acid**:** acetonitrile with 50:50 (% v/v), and 0.3 mL min^{-1} flow rate was maintained 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^{o}C during method optimization.

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 [26],[28],[29]. 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 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 most influential variables for the method optimizations which are closer to predicted values. These critical process parameters have a siginificant impact upon the Critical Analytical attributes (CAAs), or obtained responses, such as Peak Area (414527 cm^2), retention time (Rt) 1.214, with USP plate count 14608. The results of ANOVA of observed responses with *P* value (*P* = .05), *F* value, significant levels, as well as the experimental runs of predicted and actual values of dependent factors (responses) are discussed in Table 3**,** which indicate model is significant.

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

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

3.2 Method Validation

Analytical method validation (AMV) proves that an analytical method that affords analytical results is acceptable for the envisioned practice [20],[22],[24],[26]. As per ICH recommended guidelines [10], 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

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 coefficient (R^2) was obtained 0.9995. The representative linearity plot or calibration curve is depicted in Fig. 5.

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

Source	Peak area (Cm^2)				Retention time (Minute)		USP plate count	
	F value	**P- value**			**F value**	**P- value**	**F value**	**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*

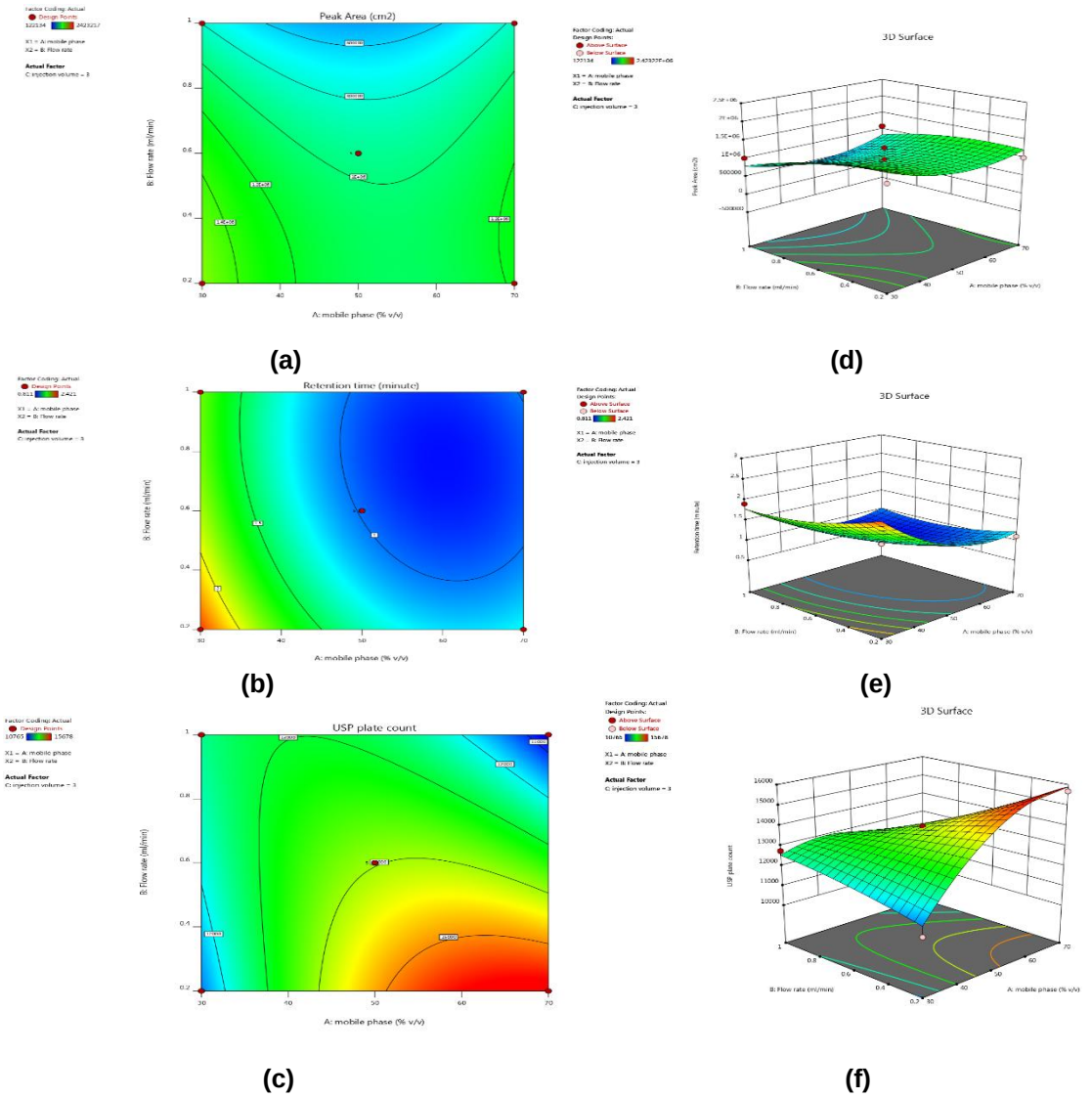
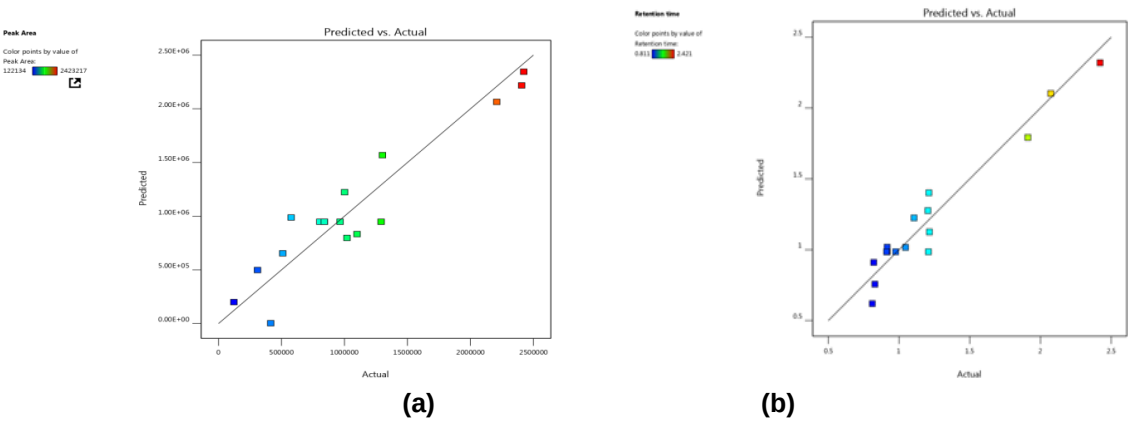
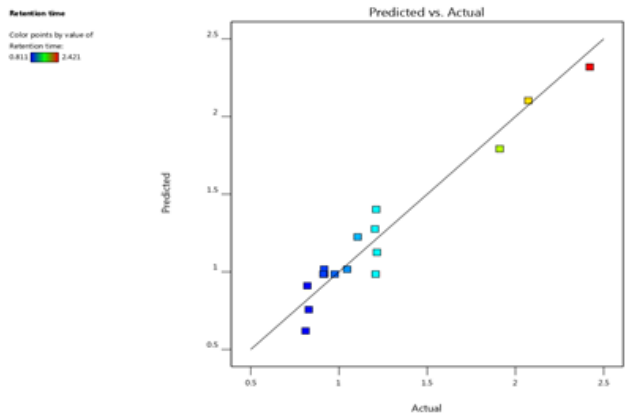


Fig. 3. Schematic diagram indicating 2-D surface contour plot analysis of peak area [Y1] response (a), Retention time [Y2] response (b), USP plate count [Y3] (c); 3-D surface contour plot analysis of peak area [Y1] response (d), Retention time [Y2] response (e), USP plate count [Y3] response





(c)

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)

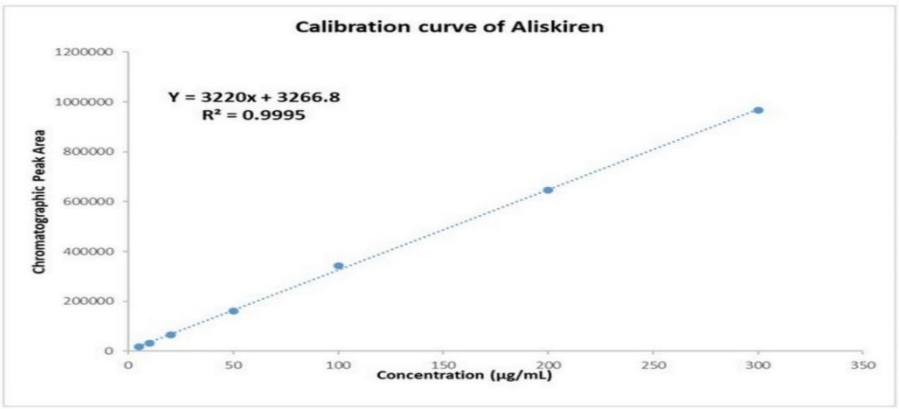


Fig. 5. Schematic diagram of Calibration Plot (a) of Aliskiren

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 [20,24]. Precision studies of the drug were carried out by the system, method, and intermediate precision testing. The results of (system, intraday, and interday) precision studies are demonstrated in Table 4, Table 5 and Table 6 respectively

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 7.

Table 4. System precision data of Aliskiren

System Precision			
Conc. (µg/mL)	**Peak Area**	**USP Tailing**	**USP Plate Count**
10	48207	1.61	7486
10	485124	1.62	7453
10	492661	1.51	7359
10	483654	1.53	7422
10	482854	1.52	7356
10	486059	1.57	7468
Average	485404		
SD	3842.7		
% RSD	0.791		

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

Table 5. Intra day Precision data of Aliskiren

Intra day			
Conc. (µg/mL)	**Peak Area at different time intervals (Day 1)**		
	10 A.M.	**2 P.M.**	**5 P.M.**
5	241190	240123	241987
5	241132	240564	241765
5	241087	240221	241889
Average	241136.6	240302.6	241880.3
SD	51.636	231.565	111.253
% RSD	0.021	0.096	0.045
10	482392	482454	482776
10	482129	482146	483543
10	482736	486263	483456
Average	485228	483621	483258
SD	304.3	2293.2	419.9
% RSD	0.062	0.474	0.086
20	970453	970764	971567
20	970542	970771	971569
20	970437	971732	971498
Average	970447.3	970989	971544.7
SD	8.9628	383.66	40.426
% RSD	0.00092	0.03951	0.00416

Table 6. Inter day precision data of Aliskiren

Inter Day			
Conc. (µg/mL)	**Peak Area of different time intervals (Day 1)**		
	10 A.M.	**2 P.M.**	**5 P.M.**
5	241554	241487	241442
5	241431	241541	241643
5	241023	241879	241877
Average	241336	241635	241654
SD	277.95	212.45	217.70
% RSD	0.115	0.087	0.090
10	483018	483968	483765
10	482625	484961	484886
10	485931	484066	484134
Average	483858	484331	484261
SD	1805.99	547.21	571.30
% RSD	0.062	0.474	0.086
20	971732	971837	971728
20	971765	971880	971762
20	971754	971878	971769
Average	971750.3	971865	971753
SD	16.802	24.269	21.931
% RSD	0.00172	0.00249	0.00225

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

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 [24,30]. 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 8.

Table 7. System suitability data of Aliskiren

System suitability test			
Injection No's	**Optimized condition (SST)**	**USP plate count**	**Tailing Factor**
Peak Area (Inj.1)	483356	13090	1.6
Peak Area (Inj.2)	472178	13143	1.6
Peak Area (Inj.3)	475965	13069	1.5
Peak Area (Inj.4)	486824	12962	1.5
Peak Area (Inj.5)	487877	13021	1.5
Peak Area (Inj.6)	483257	12964	1.5
Mean	481576.16		
SD	2339.13		
% RSD	0.485		

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

Table 8. Robustness data of Aliskiren

Robustness study						
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]**	**Amount of [ACN -2%v/v]**
Peak Area (Inj.1)	474365	485595	472465	468476	485267	476253
Peak Area (Inj.2)	475947	483997	474381	467429	480178	471122
Peak Area (Inj.3)	472863	481359	474972	464419	482659	474337
Mean	474391.6	483650.33	473939.33	466774.6	482701.3	473904
Std	1542.17	2139.17	1310.55	2106.16	2544.76	2592.76
% RSD	0.325	0.442	0.276	0.451	0.527	0.547
Difference w.r.t SST (%)	0.90	-0.43	1.58	3.07	0.233	1.59

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

Table 9. **Accuracy data of Aliskiren**

% Level	Amount spiked (µg/mL)	Amount Recovered (µg/mL)	% Recovery
50%	75	75.02	100.03
	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
		Mean	100.11
		SD	0.548
		% RSD	0.54

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

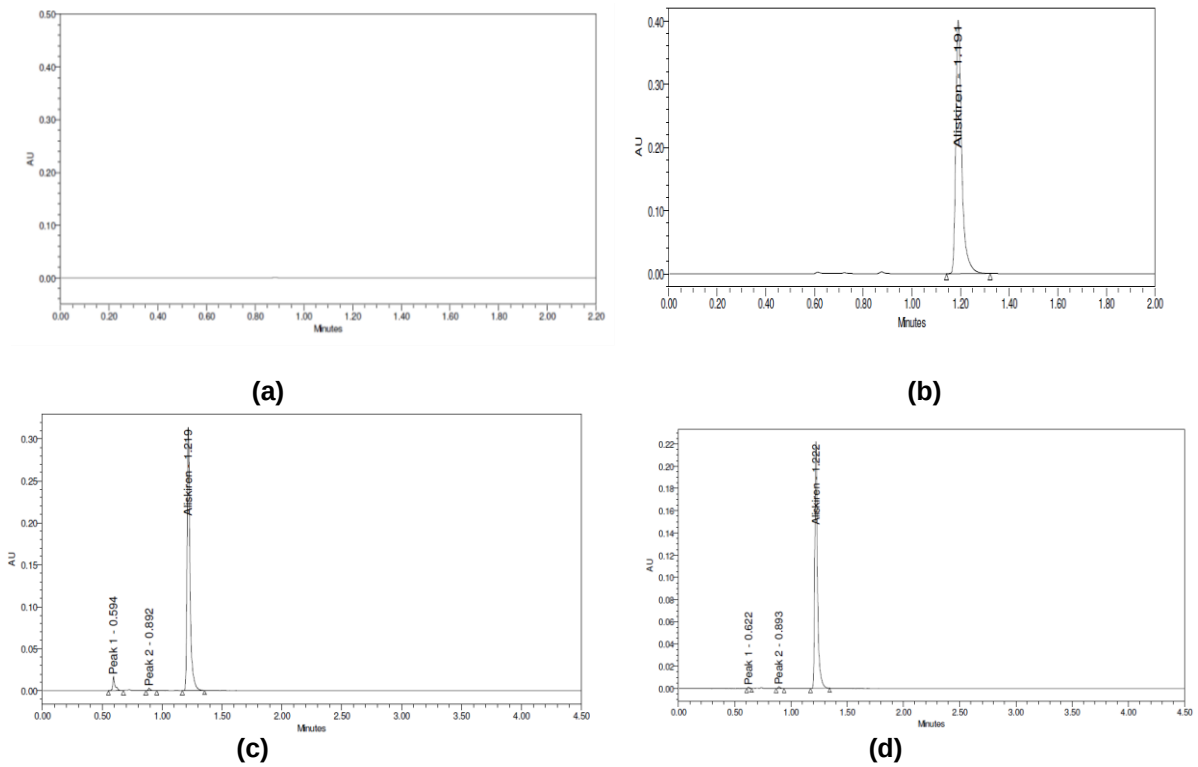


Fig. 6. (a-d). Schematic diagram indicating (a) mixture of excipients, (b) sample acidic degradation, (c) alkali degradation and (d) Peroxide degradation.

3.2.1.5 Accuracy

The ICH guidelines about the validation of analytical procedures [29],[30] 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 9.

3.2.1.6 LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) were 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 [26],[27],[30]. The LOD and LOQ were found to be 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 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 from the mixture of excipients and are depicted in Fig. 6 (a-d).

3.3 Forced Degradation Studies

Forced degradation studies were carried out to characterize the stability of the drug substance and drug product as per recommendations of ICHQ2R1[10],[24] [30]. The drug was subjected to different stress conditions as per ICH. Acidic degradation was performed by taking 1 mL of stock solution of Aliskiren, and to this 1mL of 2N Hydrochloric acid was added and refluxed for 10 minutes at 60^{o}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^{o}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

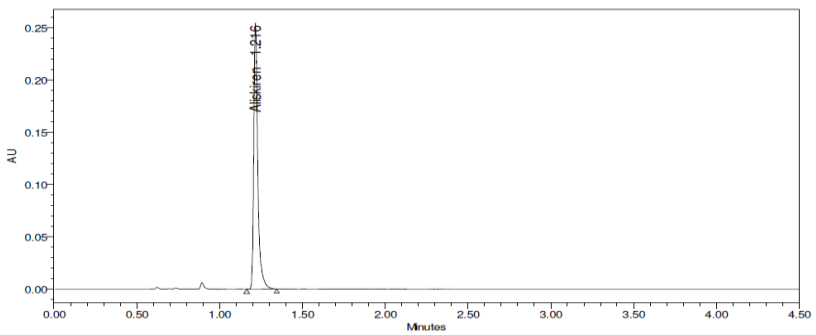
separately. Finally, the ensuing solutions of acidic, alkai and peroxide degradations were diluted to obtain 150 µg mL-1 and 2.0 µL were injected into the UPLC system. The chromatograms with results of degradations studies were recorded Fig. 6 (a-d) and Table 10.

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).

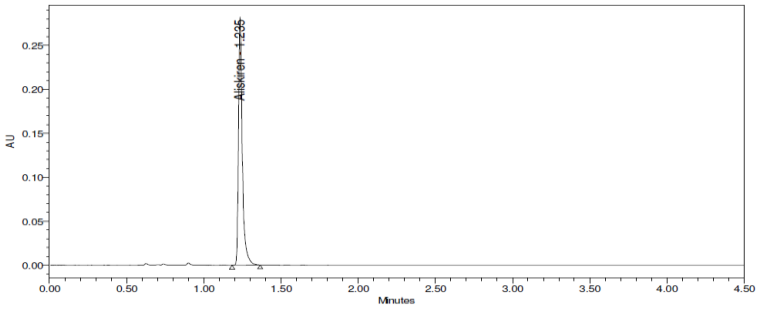
Finally, the subsequent solution was diluted to obtain 150 µg mL^{-1} solutions, and 0.2 µL were injected into the UPLC system fitted out with PDA detector. The resultant chromatograms were recorded to assess the stability of the sample [24],[30] and the results are enlisted in Table 10.

Table 10. 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	



(a)



(b)

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

Table 11. 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

3.3.1 Stability of analytical solutions

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 10 [28-30].

3.4 Assay of Pharmaceutical Formulations

The measured values of % assay of two different marketed formulations of Aliskiren are represented in Table 11. The results demonstrate that all the values of marketed formulations are within the acceptance limit, i.e., 98-102 % (Table 8).

4. CONCLUSION

The present article productively reveals the efficiency of the Response surface methodology (RSM) through the AQbD approach. It 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, primarily when there is not precisely a regulatory need. The results from stress degradation studies using RSM based development confirmed a systematic holistic, stability-indicating method which is also sensitive, precise, and cost effective. The quality assurance will be guaranteed in the developed method with regulatory flexibility and the method can find practical application in the quality control laboratories for routine analysis.

DISCLAIMER

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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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