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

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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.

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**Methodology:** The chromatographic partitioning was achieved on a Waters Acuity H class UPLC system, with BEH 130Å, 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-Benken 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-Benken 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 (2S,4S,5S,7S)-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 measuring 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)
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), Mill-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].

![Chemical structure of Aliskiren](image)

Fig. 1. Chemical structure of Aliskiren
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, ultrasonicated 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-Benken Design (BBD)

AQA 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 (CMRs) 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)](image)
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 3² 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 (3²) of BBD experimental design have been enlisted in Table 2.

Table 1. Design matrix as per Box-Benken 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 3² Box–Behnken design using RSM

| Experimental runs | Organic phase (% v/v) | Flow rate (mL min⁻¹) | Injection volume (μL) | Peak Area (Cm²) | 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                     | 0.1                   | 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.

\[
\text{Peak Area (Y1) } = + 9.483E + 05 - 77249.25A - 2.902E + 05 B - 2.902E + 05 B + 7.824E + 05 C + 95053.25 AB + 556.25AC - 3.885E + 05 BC + 3.160E + 05 A^2 - 1.586E + 05 B^2 - 1.586E + 05 B^2
\]  

(1)
Retrospect Time (Y2)
\[ Y2 = +0.9854 \times 0.4671A - 0.1836B + 0.0760C + 0.0800AB - 0.2743AC + 0.0008BC \\
+ 0.4356A^2 + 0.1676B^2 \\
- 0.1357C^2 \] (2)

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

3.1 Optimized Chromatographic Conditions

Using the principles of response surface methodology ultimately, in Waters Acuity H class UPLC, BEH 130° 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 \( \lambda_{\text{max}} \) 255 nm. The ultimate temperature was maintained at 30°C during method optimization.

3.1.1 Optimization of chromatographic method using RSM methodology

After selecting optimal chromatographic conditions, the Box-Benken 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 significant 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  |
| A²                    | 3.40             | 0.1078  | 26.00   | 0.0014  | 18.88   | 0.0034  |
| B²                    | 0.8563           | 0.3856  | 3.85    | 0.0906  | 0.2485  | 0.6334  |
| C²                    | 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.

(a) 
(b) 
(c) 
(d) 
(e) 
(f)
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

| 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

| Conc. (μg/mL) | 10 A.M. | 2 P.M. | 5 P.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   | 229.3  | 419.9  |        |        |
| % RSD         | 0.062   | 0.474  | 0.086  |        |        |
| 20            | 970453  | 970764 | 971567 |        |        |
| 20            | 970542  | 970771 | 971569 |        |        |
| 20            | 970437  | 971732 | 971498 |        |        |
| Average       | 970447.3| 970968 | 971544.7|        |        |
| SD            | 8.962   | 38.66  | 40.426 |        |        |
| % RSD         | 0.0092  | 0.0395 | 0.00416|        |        |

Table 6. Inter day precision data of Aliskiren

| Conc. (μg/mL) | 10 A.M. | 2 P.M. | 5 P.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

| Injection No’s | System suitability test | USP plate count | Tailing Factor |
|----------------|-------------------------|-----------------|----------------|
| Peak Area (Inj.1) | Optimized condition (SST) | 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 | 2339.13 | 0.485 |
| SD | | 2339.13 | 0.485 |
| % RSD | | 0.485 | 0.485 |

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

### Table 8. Robustness data of Aliskiren

| Parameter | Robustness study | Flow Rate [1+ 0.2ml min⁻¹] | Flow rate [1-0.2ml min⁻¹] | Wavelength [254 +2 nm] | Wavelength [254–2 nm] | Amount of [ACN + 2%v/v] | Amount of [ACN -2%v/v] |
|-----------|------------------|-----------------------------|-----------------------------|-------------------------|-------------------------|--------------------------|--------------------------|
| Peak Area (Inj.1) | | 474365 | 48595 | 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 | 0.548 |
| SD | | 0.548 | 0.548 |
| % RSD | | 0.548 | 0.548 |

*RSD: Relative standard deviation, *SD: Standard deviation
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°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% H₂O₂, hydrogen peroxide (H₂O₂) was added.
separately. Finally, the ensuing solutions of acidic, alkali 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\(_2\)O\(_2\), 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         |
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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REFERENCES

1. Swarbrick J. Encyclopedia of pharmaceutical technology, 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.

3. Nadella PN, Nadh RV, Navuluri S. QbD-Based UPLC Method for Quantification of Brexpiprazole in Presence of Impurities and Application to In Vitro Dissolution. J Chromatogr Sci. 2021;59(3):223-240. PMID: 33333554.

4. Khalil NY, Wani TA, Abunassif MA, Darwish IA sensitive HPLC method with fluorescence detection and on-line wavelength switching for simultaneous determination of valsartan and amlodipine in human plasma, J. Liq. Chromatogr. Relat. Technol., 2011; 34:20:2583-2595, DOI:10.1080/10826076.2011.593069.

5. Haghi ZE. Photodiode array detection in clinical applications; quantitative analyte assay advantages, limitations and disadvantages, photodiodes - communications, bio-sensings, measurements and high-energy physics, Jin-Wei Shi, IntechOpen, September 6th 2011.

6. Wani TA, Khalil NY, Darwish I, Iqbal M and Bakheit AH. Highly Sensitive and Ultra Performance Liquid Chromatography Tandem Mass Spectrometric Method Development and Validation for Determination of Neratinib in Human Plasma. S. Afr. J. 2015;68(1):93-98. DOI: 10.17159/0379-4350/2015/v68a14.

7. Wani TA. Highly sensitive ultra-performance liquid chromatography–tandem mass spectrometry method for the determination of abiraterone in human plasma. Anal. Methods. 2013;(5):3693-3699. DOI:doi.org/10.1039/C3AY26611G.

8. Swetha SR, Bhavya SK, Mounika Ch. A Review on Comparative study of HPLC and UPLC. Research J. Pharm. and Tech. 2020; 13(3):1570-1574.doii. 10.5958/0974-360X.2020.00284.X.

9. Swain S, Parhi R, Jena BR, Babu SM. Quality by Design: Concept to Applications. Curr Drug Discov Technol. 2019;16(3):240-250. DOI:10.2174/15701638156661803081420 16. PMID: 29521238.

10. ICH, Pharmaceutical Quality System Q10, International Conference on Harmonization, IFFPA, Geneva, Switzerland; 2008.

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

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

13. 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). DOI:doi.org/10.2174/15734129166662001 08155853.

14. Ahmad A, Alkharfy KM, Raish M, Alrabiah H, Alanazi AM, Wani TA et al. Response surface methodology-based optimization of robust rp-hplc method for estimation of
sorafenib in pharmaceutical dosage formulation. Lat. Am. J. Pharm. 2016;35(8):1768-1775.

15. Wani TA, Alenazi AM, Zargar S, Ahmad A. Box–Behnken Design Based Statistical Modelling for Optimization of UPLC-MS/MS Method for Analysis of Sorafenib in Bulk and Tablets. J. Comput. Theor. Nanosci. 2015;12(10):3598-3604. DOI:10.1166/jctn.2015.4245.

16. Wani TA, Zargar Seema. Sensitive UPLC-Tandem Mass Spectrometric Method for Determination and Validation of Hydrochlorothiazide in Human Plasma. Lat. Am. J. Pharm. 2015;34(1):161-8.

17. Jain A, Sharma T, Sharma G, Khurana RK, Katore 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. DOI: 10.1080/22297928.2019.1624193.

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

19. 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.

20. 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

21. 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.

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

23. Ramalingam P, Jahnavi, B. QbD considerations for analytical development, In: Beg S, Hasnain MS. (Ed.). Pharmaceutical Quality by Design, Academic Press. 2019;77-108. ISBN 9780128157992. Available:doi.org/10.1016/B978-0-12-815799-2.00005-8.

24. Jain A, Beg S, Saini S, Sharma T, Katarer 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. DOI: 10.1080/10826076.2019.1626742.

25. 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. DOI:10.4274/tjps.galenos.2020.08522.

26. EMA-FDA pilot program for parallel assessment of Quality-By-Design applications: lessons learnt and Q&A resulting from the first parallel assessment. Available:http://www.ema.europa.eu/docs/en_GB/document_library/Other/2013/08/W C500148215.pdf [Accessible 20 August 2013].

27. 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. doi.org/10.1016/j.bfopcu.2018.09.002.

28. Blessy M, Patel RD, Prajesh N. Prajapati YK. Agrawal, Development of forced degradation and stability indicating studies of drugs- A review, J Pharm Anal. 2014;4(3):159-165. DOI:doi.org/10.1016/j.jpha.2013.09.003.

29. ICH, Pharmaceutical Development Q8 (R2), International Conference on Harmonization, IFPMA, Geneva, Switzerland; 2009. Available:http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.
30. Rao TN. Validation of analytical methods, Calibration and Validation of Analytical Methods- A Sampling of Current Approaches, In: Stauffer MT. (Ed.), Intech Open; 2018. Available: https://doi.org/10.5772/intechope72087.

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