

Original Article

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## AQbD Driven Development of a RP-HPLC Method for the Quantitation of Abiraterone acetate for Its Pharmaceutical Formulations in presence of Degradants

### Ayrıştırıcıların varlığında farmasötik formülasyonları için abirateron asetatın miktar tayini için bir RP-HPLC yönteminin optimize edilmiş AQbD güdümlü bir gelişimi

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#### Abstract

**Objective:** Abiraterone acetate is well known as an anticancer drug, a steroidal derivative of progesterone that affords clinical assistance to hormone-refractory prostate cancer patients. Chemometrics-assisted RP-HPLC development of the drug abiraterone acetate has been employed in this investigation using an analytical QbD (AQbD) approach.

**Materials and Methods:** The separation of the drug was performed on Princeton Merck-Hibar ®Purospher ® STAR (C18, 250 mm×4.6 mm) i.d., 5µm particle size) with UV-detection at 235 nm. A Box-Behnken statistical experimental design with response surface methodology was executed for method optimization and desired chromatographic separation from its formulation with a few numbers of experimental trials. Three independent variables, namely composition of the mobile phase, pH, and flow rate, were used to construct an arithmetical model to study the impact of these independent variables upon the responses retention time and peak area.

**Results:** Optimized experimental conditions for proposed work include the mobile phase acetonitrile and phosphate buffer (10mM KH<sub>2</sub>PO<sub>4</sub>) (20:80 %v/v). The Calibration curve was found to be linear over the concentration range 2–100 µg/mL, and recovery was performed at three levels, within 98–102%. The 3D response surface curves revealed that mobile phase composition and flow rate were the most substantial critical factors affecting desired responses.

**Conclusion:** Hence, an attempt has been made to develop and validate an economical, precise, robust, stability-indicating AQbD based RP-HPLC method, which can be employed successfully for the routine analysis of abiraterone acetate in quality control labs.

Keywords: Precision, Accuracy, ICH guidelines, Method Validation, Experimental Design

## Öz

**Amaç:** Abiraterone asetat, hormona dirençli prostat kanseri hastalarına klinik yardım sağlayan progesteronun steroidal bir türevi olan bir antikanser ilaç olarak bilinir. Bu çalışmada, analitik bir QbD (AQbD) yaklaşımı kullanılarak ilaç abirateron asetatın kemometri destekli RP-HPLC geliştirmesi kullanılmıştır.

**Gereç ve Yöntemler:** İlacın ayrılması, 235 nm'de UV-saptama ile Princeton Merck-Hibar®Purospher® STAR (C18, 250 mm × 4.6 mm) i.d., 5µm partikül boyutu) üzerinde gerçekleştirildi. Metot optimizasyonu ve birkaç deneysel denemeyle formülasyonundan istenen kromatografik ayırma için yanıt yüzey metodolojisine sahip bir Box-Behnken istatistiksel deney tasarımı yürütüldü. Bu bağımsız değişkenlerin yanıt tutma süresi ve tepe alanı üzerindeki etkisini incelemek için aritmetik bir model oluşturmak için mobil faz, pH ve akış oranının bileşimi olmak üzere üç bağımsız değişken kullanıldı.

**Sonuçlar:** Önerilen çalışma için optimize edilmiş deneysel koşullar arasında mobil faz asetonitril ve fosfat tamponu (10 mM KH<sub>2</sub>PO<sub>4</sub>) (% 20:80 h / h) yer alır. Kalibrasyon eğrisinin 2–100 µg / mL konsantrasyon aralığında doğrusal olduğu bulundu ve geri kazanım% 98–102 arasında üç seviyede gerçekleştirildi. 3D yanıt yüzey eğrileri, mobil faz bileşiminin ve akış hızının, istenen yanıtları etkileyen en önemli kritik faktörler olduğunu ortaya koydu.

**Sonuç:** 7Bu nedenle, kalite kontrol laboratuvarlarında abiraterone asetatın rutin analizi için başarıyla kullanılabilen ekonomik, hassas, sağlam, stabilite gösteren AQbD bazlı RP-HPLC yöntemini geliştirmek ve doğrulamak için bir girişimde bulunulmuştur.

**Anahtar Kelimeler:** Kesinlik, Doğruluk, ICH kılavuzları, Yöntem Doğrulama, Deneysel Tasarım

## INTRODUCTION

The potent anticancer drug Abiraterone acetate  $\{[(3S, 8R, 9S, 10R, 13S, 14S)- 10,13\text{-dimethyl-17-pyridin-3-yl-2,3,4,7,8,9,11,12,14,15- decahydro-1H-cyclopenta [a] phenanthren-3-yl}\}$  acetate} is widely known as an acetyl ester and a significant prodrug of its active metabolite Abiraterone.<sup>1-3</sup> It is a well-built receptor blocker of the androgen that is having potent bioavailability, especially in the oral administration.<sup>4</sup> The drug Abiraterone acetate is obtained to be intense and extra effective than ketoconazole and liarozole in the inhibition of CYP17A1; which is a rate-limiting enzyme for the biosynthesis of androgens.<sup>4,5</sup> Principally, the drug abiraterone acetate is specified for the use in grouping with the prednisone for curing of men with metastatic castration-resistant prostate cancer who have already received prior chemotherapy comprising docetaxel.<sup>5</sup> Abiraterone acetate is nearly not soluble in the reagents

over an extensive pH range. However, it is faintly soluble in HCl as well as in the organic solvents (Figure 1). Abiraterone acetate was detected by few hyphenated techniques like LC-MS/MS techniques 6-8, spectrofluorimetric for measuring fluorescence emission and absorption spectra in cancer patients.9-11 In addition to this, the drug has been thoroughly recognized by other chromatographic techniques for the quantification of metabolites in biological samples bio-analytically.8,9,12 However, there are insufficient works reported using QbD Paradigm, MODR Concepts, design space, as well as systematic development by chemometric assisted statistical optimization of critical method parameters (CMPs), critical method attributes (CMAs).13 As these optimization techniques with analytical quality by design (AQbD) approach denotes an efficient and cost-effective analysis and production of innovative analytical methodologies which signify the "finest solution" to a meticulous "complexity" or problems raised in method development, but finally, deliver consistent quality output; where other general HPLC methods unable to achieve that.13-16 The Box-Behnken design (BBD) is a rotatable, second-order three-level factorial design. This design is a notable, quite efficient, and cost-effective tool with a fewer number of experimental runs for analytical development & method optimization.17,18 The BBD is also regarded as a glowing viable methodology from other available designs like Central composite, d-optimal, and mixture designs.17 The design sketch ensemble to obtain a quadratic process model and recommend each factor, or independent variable, that is located at one of the noted three equally spaced values, that are encoded as low, intermediate, and high [-1, 0, +1] levels. Due to feasible design matrix, BBD is recognized as a superfluous economic tool as it aids in clear-assessment of the critical method parameters (CMPs) of the model; creation of sequential designs; finding of fit statistics or lack of fit values of the distinctive quadratic model; as well as the routine implementation of blocks.17-19 Hence, an effort was undertaken to develop and validate an economical, highly robust, stability-indicating RP-HPLC method of abiraterone acetate by Box-Benkhen Design of QbD paradigm for quantification of the bulk drug and its pharmaceutical formulations which minimize the time taken for trial-and-error methodologies, minimize the re-validation process, reagents rather than depending on One factor-at-a-time (OFAT) approach.

## **MATERIALS AND METHODS**

The pure standard of the drug abiraterone acetate was obtained from Sun Pharmaceutical Industries Ltd., Halol, Gujarat, India. Abiraterone acetate is obtainable as a tablet dosage form with brand names XIBRA (Cipla Ltd, India), ZYTIGA (Janssen-Cilag Ltd, India), and ABIRAPRO (Glenmark Pharmaceuticals, India) with a suitably labeled claim as 250 mg. The solvents selected for the research are phosphate buffer and acetonitrile (HPLC grade) were procured from Merck Laboratories, Mumbai.

### **Instrumentation**

An HPLC Shimadzu HPLC system (LC-2010C HT) with UV-Visible detector, Ultra-Sonicator from Remi Instruments, Mumbai, Nylon filter (0.45 µm) from Millipore, Mumbai, India are used for the research work.

### **Chemicals and Reagents**

Significant chemicals like orthophosphoric acid (AR) grade, acetonitrile (HPLC grade) were obtained from Merck Laboratories Pvt. Ltd., Mumbai. Potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>) was obtained from Fischer Scientific, Mumbai, India.

### **Methods**

The method optimization was performed with a Box-Behnken statistical design comprising the critical method parameters (CMPs), which include three significant factors (Composition of the

intent mobile phase, flow rate, and pH of buffer) encompass three levels. Seventeen experimental runs are established with five center points. The flow rate was tested at 1.0, 1.2, and 1.5 mL/min, pH was measured at 4, 5, and 6, and concentration of mobile phase was monitored at 20%, 50%, and 80% accordingly. The responses taken into account were the retention times (Rt), Peak Area for abiraterone acetate, which was designated as critical analytical attributes (CAAs). The data were analyzed, and the model was validated with Design-Expert software. The quadratic model revealed a virtuous correlation with the experimental data and is employed to navigate the design space. The 2D & 3D Response-surface and perturbation plots were also analyzed to assess the critical factors' impact upon the observed responses, or CAAs found within the predicted range. The predicted values from the practical responses were found to be satisfactory, and it was confirmed that it had been acquired within the design space of the optimized results.<sup>19,20</sup>

### **Statistical Analysis**

Design-Expert® (Version 12), Stat-Ease Inc., Minneapolis, MN, advanced statistical software of USA, was utilized for method optimization and the estimation of its critical method parameters and randomized the runs.<sup>14</sup> The remaining calculations for the analysis were performed by Microsoft excels 2007 (Microsoft, USA).

### **Preparation of Solvent**

Phosphate buffer (pH 4): Dissolve accurately 5.04 gram of disodium hydrogen phosphate and 3.01 gm of potassium dihydrogen phosphate in adequate water to make 1000 mL, and pH was adjusted to 4.0 with orthophosphoric acid (OPA). The resulting solution was passed through (0.45 $\mu$ ) filter paper and filtered using vacuum filtration and allowed to sonicate for about 15 minutes.<sup>15</sup> While preparing the mobile phase, only phosphate buffer was filtered using (0.45  $\mu$ m) nylon membrane filters. However, acetonitrile was not filtered and is used as provided by the supplier.

### **Procedure for the stock standard solution**

The pure drug's standard stock solution was made by accurately weighing 10mg of the drug and mixed with 10mL of acetonitrile to obtain the concentration (1000  $\mu$ g.mL<sup>-1</sup>). Applying this stock solution, diverse serial dilutions were prepared by diluting with the mobile phase and are used as diluents to obtain the concentrations ranging between 1 and 100  $\mu$ g.mL<sup>-1</sup>. The made serial dilutions of the drug were filtered through a 0.45  $\mu$ m syringe filter and subjected to chromatography for preparing the calibration curve by injecting blank, standard, and the mixture of excipients (placebo) depicted in Figures 2 (a), (b), & (c) respectively. Here acetonitrile and phosphate buffer (10mM Potassium dihydrogen phosphate (20:80, %v/v) is selected as a mobile phase with flow rate 1mL/min and UV detection 235 nm. The chromatogram's peak area was noted down, and a linearity graph was plotted (Concentration vs. Peak area). The values of the limit of quantification (LOQ) and limit of detection (LOD) were estimated based on the linearity plot response.

### **Chromatographic Conditions**

Chromatographic separation was carried out by using Merck-Hibar® Purospher® STAR Analytical column (C18, 250 mm  $\times$  4.6 mm  $\times$  5 $\mu$ ). The advantage of such a column is having the highest carbon loading for better separation of the desired analyte, which minimizes the

variabilities of the mode of selection. The mobile phase contains principally the acetonitrile and phosphate buffer (10 mM KH<sub>2</sub>PO<sub>4</sub>) (20:80 %v/v) was used. The flow rate was maintained as 1 mLmin<sup>-1</sup> with an injection volume of 10µL. The oven temperature of the chromatographic column was maintained as 30° C. The sample temperature has been maintained as ambient.

### **Optimization of the Chromatographic Method using Analytical QbD approach**

Initially, trial and error practices were executed to obtain rigorous data about the selected chromatographic method's performance and its finding of vital independent variables with its considerable effect upon the dependent variables. The utmost significance of developing the RP-HPLC method mostly separates the drug from its excipients and the degradants. Statistical analysis was accomplished by implementing a suitable experimental design by response surface methodology (RSM) through Box-Benken design principles. The statistical design intensifies ANOVA principles for establishing the optimized experimental conditions of the method.<sup>16,21,22</sup>

A simple, accurate, AQbD based stability-indicating new reverse phase HPLC method has been developed and subsequently validated as per ICH recommended stability guidelines for quantification of abiraterone acetate in its various pharmaceutical formulations (tablets). A mixture of phosphate buffer (10mM Potassium dihydrogen phosphate and acetonitrile (20:80, %v/v) was used as the mobile phase with a 1 mL/min flow rate.

### **Optimized Chromatographic Conditions**

Optimized Trail: Concentration (10 µg/mL)

Flow rate: 1mL /min

Mobile Phase used: Acetonitrile : phosphate Buffer [10mM KH<sub>2</sub>PO<sub>4</sub>, (20:80%) v/v]. The optimized chromatograms of the blank, standard, and mixture of excipients (10 µg/mL) of the developed analytical method were depicted in Figure 2 (a), (b) and, (c), respectively.

### **Method Validation**

Method validation is carried out in order to substantiate that the analytical procedure is employed for a definite experiment is appropriate for its anticipated usage.<sup>19</sup> The outcomes of method validation parameters can be highly requisite to judge the reliability, quality, and steadiness of analytical results. The validation of a method for parameters like linearity, accuracy, precision, robustness, etc., is carried out according to recommendations of ICH guidelines [ICH Q2 [R1]].<sup>23-25</sup>

### **Linearity**

The linearity plot of the proposed method was carried out as per the stated ICH guidelines.<sup>26-28</sup> The linearity chart of abiraterone acetate was found within the concentration range of (2–100) µg/ml; further, the calibration plot was constructed within the peak area in opposition to the concentration.

### **Accuracy**

A sequence of solutions was arranged in triplicates by spiking the known standard concentrations of abiraterone acetate in the range of 50%, 100%, and 150% on the tablet solution as analyzed. The accuracy of the method was provided at three diverse concentration levels at 5, 10, 15µg/ml

of Abiraterone acetate standard, and it is performed as per recommendations of ICH guidelines.  
26-28

### **Precision**

The precision of the developed technique was calculated by performing the repeatability and intermediate precision studies, and it was expressed in % RSD.<sup>21</sup> The developed analytical QbD based method was validated by the precision studies (both intraday and interday).

### **LOD and LOQ**

The limit of detection (LOD) and limit of quantification (LOQ) of the current investigation were evaluated from the baseline noise of abiraterone acetate through comparisons of measured signals of samples with known analyte concentrations with that of the blank by (signal-to-noise) S/N ratio 3:1 (LOD) & 10:1 (LOQ) as per guidance and protocol recommended by ICHQ2B.<sup>29</sup>

### **Robustness**

A Robustness study is an experimental preparation or set-up which is performed to recognize and evaluate the toughness of an analytical method.<sup>30</sup> To check the ability of the projected method, different factors were deliberately altered like an alternation in mobile phase composition, change in flow rate, etc.

### **Specificity**

Specificity is carried out in order to evaluate the analyte noticeably in the occurrence of components that may be expected to be present during development. Specificity is established by representing no interference from the excipients and the degradation products.<sup>28,29</sup>

### **Forced degradation studies**

Forced degradation generally includes exposure of drug substances to a range of stress conditions to demonstrate the stability profile, possible degradants of developed analytical methods.<sup>26-28</sup> Acidic degradation was performed by adding up 1 ml of 0.1N HCl and then heated at 60°C for 30 minutes cool to room temperatures and neutralized. Alkali degradation was performed by adding up 1 ml of 0.1N NaOH, heated at 60°C for 30 minutes, cool to room temperatures, and neutralized. Oxidative degradation was executed by exposing the drug to 1ml of 3% H<sub>2</sub>O<sub>2</sub>, heated at 60°C for 30 minutes. Thermal degradation was achieved by heating the solutions of the drug at 105°C on a thermostatically regulated water bath for half an hour. Photolytic degradation was carried out by exposing the drug solutions to ultraviolet light within a UV chamber at 365nm for 3 hours. The degradation samples were accurately prepared through appropriate aliquots of the drug and in the solution form of their drug products, instructed by the stress testing protocol.<sup>26,29</sup> After a definite time, the treated solutions were adjusted with the mobile phase.

### **Stability of analytical solution**

The solution stability study was effectively performed by observing the standard and sample solutions to determine the stability potential of the drug substance. This factor was analyzed by injecting the drug sample and standard solutions at distinctive intervals. This evaluation parameter is intended for the evaluation of chemical stability of drug and sample solution whether any significant changes occurred at varied time intervals.<sup>29, 31,32</sup>

## Assay of Formulations

Twenty tablets of the commercial brands were chosen arbitrarily, and their average weight was found out 31. The tablets are crushed uniformly to get the fine powder form. From this powder, the weight equivalent to 250 mg of abiraterone acetate was taken and allowed to dissolve in 200 ml of acetonitrile (in 250 ml volumetric flask. Then it was shaken for 20 minutes and ultrasonicated for 20 minutes. After that, it was allowed to cool with optimum room temperature, and the volume was made up to the mark with diluents (1000 mcg/ml). The final obtained solution was then diluted to 10 mcg/ml with mobile phase acetonitrile: phosphate Buffer (20:80 %v/v) and subsequently injected in triplicates to the HPLC system for estimation.

## RESULTS AND DISCUSSION

Method Development and Optimization using Box-Benken experimental design

In the current investigation, trials were proposed and conducted ensuing Box-Benken experimental design.<sup>21</sup> The proposed Box-Benken design desires 17 experimental runs of examination to acquire data and model the response surface for reliable analysis. A 32 level with 17 experimental runs were executed to identify the optimized design space for detection of predicted response. The 32-factorial design was employed utilizing a Box-Benken experimental design with response surface methodology.<sup>16,21</sup> The experimental design with optimization methodology and its data analysis through BBD were statistically evaluated by “Design-Expert-Ver. 12” software. <sup>21,22</sup> Selection of the three most influential factors (CMPs) like the composition of mobile phases (X1), flow rate (X2), pH (X3); whereas, the peak area (Y1) and retention time (Y2) were used as observed responses. The design matrix of the statistical BBD; experimental runs have demonstrated in Table 1. The contour plots illustrate that the effect of both the responses stand in need about factors X1 (acetonitrile and phosphate buffer %) and X2 (flow rate), while the X3 (pH) has no significant effect upon the obtained responses. The outline of (3)2 factors, ANOVA results, through its calculated mean and standard deviation values, are summarized in Table 2. The model was effectively validated by the interaction studies using the effect of various factors on the obtained responses. The 2D counter plot analysis of peak area and retention time have the observed responses [R1] & [R2] is depicted in Figure 3 (a) & (b), respectively. Similarly, the 3D counter plot analysis of peak area [R1] response and retention time [R2] response is depicted in Figure 3 (c) & (d), respectively. The statistical model signifies that predicted values for both the response (Retention Time and Peak Area) are a bit closer to the actual values representing higher accuracy as well as precision for the obtained responses. The 2D and 3D counter plot analysis of Predicted vs. Actual value for Peak Area [R1] and Retention Time [R2] are depicted in Figure 4 (a) & (b), respectively. The Perturbation plot displays the impact of all the influential factors (mobile phase and flow rate) at a particular point within the design space for the selected responses like Peak Area and Retention time. The representative plot of Perturbation analysis for the observed Responses Peak Area [R1] and Retention Time [R2] are depicted in Figure 4 (c) & (d), respectively.

Here for 2D and 3D Surface numerical optimization, the retention time [R2] and peak area [R1] are depicted in Figure 5 (a) (b) and (c) & (d), respectively. Figure 6 eventually elucidates the parameters intended for numerical optimization for desirability and optimized data for factors indorsed by design. Finally, the optimized apparent chromatographic conditions can be well predicted from the arithmetical model, and it has strongly been recommended for the developed analytical method (Table 3).

## Results of Validation Studies

The optimized feasible chromatographic conditions were aggrandized and effectively implemented to validate the QbD avenue method by a range of validation parameters such as Linearity, Accuracy, Precision, LOD, LOQ, Robustness, etc., as per recommendations of ICH guidelines.

### Linearity

Six diverse concentrations of drug abiraterone acetate working standard ranging from 2-100 ppm were prepared and analyzed for linearity study. The calibration plot was achieved by plotting the chromatographic peak areas versus known predicted concentrations in  $\mu\text{g/mL}$ , and values of observed concentrations are found out. The regression equation was found to be  $Y = 73399x - 71154$  and regression coefficient  $r^2 = 0.998$ . The detail of the calibration plot and its residual plot data analysis are depicted in Figure 7(a) and (b), respectively.

### Accuracy

Accuracy of the developed method was executed to make sure the convenience of agreement among true and reference value in three significant levels of 50%, 100%, and 150%. The percentage recoveries of three various concentrations were found to be within the range of 98 to 102%, and % RSD is obtained within the acceptance limit, i.e., NMT 2.0%. The data of all the recovery studies are represented in Table 5.

### Precision

Precision studies of the developed method were carried out by the system, method, and intermediate precision studies. The outcomes of system suitability by six replicate standard injections of system Precision (USP plate count and tailing factor values), intraday, and interday precision through three replicate injections of standards are performed, and data's of % RSD are reported Table 5 respectively. The calculated values are obtained within the acceptance criteria, i.e., NMT 2%.

### LOD and LOQ

The detection and quantification limits (LOD & LOQ) of the current investigation were actively quantified as per the recommendation of ICHQ2B guidelines for validation of analytical methodology. The detection limit was derived as a signal-to-noise (S/N) approach of ratio 3:1, whereas the quantification limit was indicated as S/N ratio 10:1; as an effect of the response by the detector. The estimated value for LOD was found to be 0.45 ppm & the estimated value for LOQ was 1.35 ppm. The calculated values are reflected in Table 3.

### Robustness

Robustness can be illuminated as the capacity to replicate the analytical method in diverse labs or under varied conditions without the manifestation of unanticipated differences in the obtained results. The estimated values of mobile phase composition and flow rates are taken, and changes in flow rate and mobile phase data are summarized in Table 6. The results show that the calculated % RSD is established within the acceptance limit, i.e., less than 2%.

### Specificity

The specificity of the method has been performed to determine the interference from the degradation products as per ICH through forced degradation study. The results denote that there is no sign of peak formation at the retention time (Rt) of Abiraterone acetate and the case of degradation products since the peak purity passed. The data are revealed that purity angle was less than purity threshold; elucidate no specific interference (Table 7).

### **Forced Degradation Studies**

As specified, the specificity has performed by stress degradation studies that afford the understanding of practical degradation pathways and degradation samples of the active ingredients and help out expound the configuration of the degradants as per ICH recommendations.<sup>27</sup> The stress degradation studies were efficiently performed with the addition of acid (1mL of 0.1 N HCl, 60°C for 30 mins), alkali (1mL of 0.1 N NaOH, 60°C for 30 mins); peroxide (1ml of 3% v/v H<sub>2</sub>O<sub>2</sub>); thermal (105 °C) and photolytic degradations at 231nm consequently, for determining the steady nature of drugs. The % drug degradation during acidic and alkaline conditions was observed as 26.5% & 12.3%, respectively. In contrast, less than 2% degradation is reported in the case of oxidative, thermal, and photolytic degradations, which indicate the drug's completely resistant behaviors to above stress conditions. The detailed descriptions of forced degradation activities have been demonstrated in Table 7. The representative chromatograms of the sample in various stress conditions by incorporating the mixture of excipients (a), acid (b), alkali (c), oxidative (d), photolytic (e), and thermal degradation (f) studies are depicted in Figure 8 (a-f) respectively.

### **Stability of analytical solution**

The solution stability study was carried out by observance of sample and standard solutions at 25±2 °C for 40 hours. After analysis, it has concluded that the drug standard and sample were observed to be stable for up to 40 hours (Table 7). The anticipated method was effectively validated and met the necessities as per the recommended stability guidelines of ICH.

### **Assay of formulations**

The chromatogram of the marketed sample is depicted in (Figure 9). The calculated values of the percentage of assay of various marketed formulations are represented in Table 8. The results illustrate that all the values formulations are within the acceptance limit, i.e., 98-102%.

## **CONCLUSION**

Chemometrics-assisted method development affords regulatory flexibility, the formation of homogenous or robust finished products of assured quality characteristics as per FDA concerns, and ICH stability stressed conditions. In addition to this AQBd method minimizes the process of revalidation, lessens solvent consumption, development time, and enhances optimum robust analytics. By implementing the DoE, the Box–Behnken statistical design is allowed to evaluate the independent variables (CMPs) concurrently with adding common interactions between the critical factors or CMPs to optimize experimental conditions. It is explicit that the application of this Box-Behnken design with RSM is an adaptable practice to reduce the total experimental runs to obtain sustainable, robust analytics, reducing the chance of revalidation, requisite for the analytical development. The optimization of the RP-HPLC method for abiraterone acetate can produce the highest intense data, boost efficiency within a short period as per the ICH Q8 (R2)

and FDA perspectives. The proposed method was found to be linear, with concentration 2-100 ppm having  $r^2=0.998$ . The remarkable % recovery (within 98-102%) of the drug reflects that the excipients present in the tablet formulation have no impediment in the quantitation of the drug. The optimized conditions by AQbD of the anticipated method established that the proposed study was cost-effective, extremely robust, and stable signifying. Therefore, the developed stability-indicating method was an economical, accurate, and precise. It can be successfully implemented for the routine analysis of abiraterone acetate in its bulk and pharmaceutical formulations.

#### Conflict of Interest

The authors have no conflict of interest

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Table 1. Box-Benkhjen design experimental runs by Selecting ( $3^2$ ) factors

Serial No	Factor 1: Mobile Phase composition (%v/v)	Factor 2: Flow rate (mL/min)	Factor 3: pH	Response 1 Peak Area	Response 2 Retention Time
1	50	1.5	6	32909	8.59
2	50	1.25	5	109912	10.124
3	50	1.25	5	329581	11.457
4	50	1	4	699272	8.59
5	80	1.5	5	139884	12.321
6	80	1.25	6	559463	9.125
7	80	1	5	745612	12.581
8	50	1	6	411153	10.235
9	20	1.5	5	325114	7.235
10	50	1.25	5	451231	6.512
11	20	1.25	6	521231	5.236
12	50	1.25	5	425851	6.458
13	50	1.25	5	456875	7.126
14	50	1.5	4	612578	8.127
15	20	1	5	661257	9.245
16	80	1.25	4	457812	8.736
17	20	1.25	4	489254	7.984

Table 2. Analysis of Variance results (ANOVA) by Selecting ( $3^2$ ) factors

Factors	Name	Units	Type	Minimum	Maximum	Coded low	Coded High	Mean	Std. dev
A	Mobile Phase Composition	% v/v	Numeric	20.00	80.00	1— 20.00	1— 80.00	50.00	21.21
B	Flow rate	mL/ min	Numeric	1.0000	1.50	1— 1.00	1— 80.00	1.25	0.1768
C	pH	Moles/ltr	Numeric	4.00	6.00	1— 4.00	1— 80.00	5.00	0.7071

Table 3. Optimum chromatographic conditions of abiraterone acetate

Optimum Chromatographic Conditions	
Run time	15.0 minute
Retention time	8.590 minutes
Flow rate	1 mL/min
Linearity range	(2–100) µg/mL ( $r^2=0.998$ )
Accuracy	% Recovery: Within [98-102%] Relative Standard Deviation (RSD < 2%)
Precision	Relative Standard Deviation (RSD < 2%)
LOD	0.45 µg/mL
LOQ	1.35 µg/mL

Table 4. Accuracy data of abiraterone acetate

Amount added (µg/mL)	Levels	Sl. No.	Chromatographic Area	Mean Area	SD	Amount recovered (µg/mL)	%Recovery	% RSD
5	50%	1	337854	339294	1239.41	4.983	99.66 %	0.365
		2	339816					
		3	340147					
10	100%	1	697482	697500	341.86	9.971	99.71%	0.049
		2	697168					
		3	697851					
15	150%	1	1050226	1049216	1415.78	14.968	99.78%	0.134
		2	1047598					
		3	1049825					

• RSD: Relative standard deviation

• SD: Standard deviation

Table 5. Standard injection of abiraterone acetate peak response by system, intra and interday precision test.

System Precision				Intra-day				Inter-day			
Conc. ( $\mu\text{g/mL}$ )	Peak Area	USP Tailing	USP plate count	Conc. ( $\mu\text{g/mL}$ )	Peak Area of at different time intervals (Day 1)			Conc. ( $\mu\text{g/mL}$ )	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	695445	1.01	7486	5	329851	326885	327519	5	326511	326578	326899
10	695872	1.02	7453	5	329557	326699	327157	5	326789	326576	326858
10	695234	1.01	7359	5	329259	326544	327556	5	326724	326544	326891
10	695982	1.03	7422	Average	329556	326709.3	327411	Average	326675	326566	326883
				SD	296.00	170.73	220.46	SD	145.418	19.079	21.733
				% RSD	0.09	0.05	0.07	% RSD	0.045	0.006	0.007
10	695971	1.02	7356	10	695272	698777	697651	10	696565	694872	697981
				10	695773	698874	697236	10	696834	698671	697123
10	695332	1.03	7287	10	695859	698349	697987	10	696785	698956	697855
Average	695639			Average	695635	698667	697625	Average	696728	697500	697653
SD	340.02			SD	317.01	279.35	376.19	SD	143.27	2280.08	463.30
% RSD	0.0489			% RSD	0.05	0.04	0.05	% RSD	0.02	0.33	0.07
				20	1373567	1398971	1385569	20	1398171	1373267	1385669
				20	1373776	1398885	1386981	20	1398985	1373976	1385781
				20	1373962	1398934	1385856	20	1398734	1373562	1385113
				Average	1373768	1398930	1386135	Average	1398630	1373602	1385521
				SD	197.61	43.14	746.30	SD	416.85	356.16	357.75
				% RSD	0.01	0.03	0.05	% RSD	0.03	0.03	0.03

Table 6. Robustness data's of abiraterone acetate

Robustness				
	Peak Area	Average	SD	% RSD
Flow Rate [1 + 0.2 mL/min] 10 ( $\mu\text{g/mL}$ )	689234	689558	384.448	0.05575
	689458			
	689983			
Flow Rate [1 - 0.2 mL/min] 10 ( $\mu\text{g/mL}$ )	699764	699770	85.675	0.01224
	699859			
	699688			
Amount of [ACN + 2% v/v] 10 ( $\mu\text{g/mL}$ )	699272	699641	323.013	0.04617
	699874			
	699776			
Amount of [ACN - 2% v/v] 10 ( $\mu\text{g/mL}$ )	685761	686455	748.890	0.1090
	686357			
	687249			
Detector wavelength 235 nm [+2 nm]	689071	689068	301.506	0.0437
	689369			
	688766			
Detector wavelength 235 nm [-2 nm]	683866	684357	687.714	0.10049
	684062			
	685143			

Table 7. Forced Degradation & Solution stability data's of abiraterone acetate

Stress conditions	Peak Area	*Drug Recovered (%)	*Drug decomposed (%)	Theoretical plates	Tailing factor	Purity Angle	Purity Threshold
Abiraterone acetate standard (Control)	698252	100	---	7526	1.08	0.301	0.425
Acidic degradation 1 ml 0.1N HCl, 60°C, 30 minutes	513215	73.5	26.5	6326	1.29	0.118	0.192
Alkaline degradation 0.1 ml 0.1N NaOH, 60°C, 30 mins	612367	87.7	12.3	6823	1.24	0.356	0.526
Oxidative degradation 1 ml 3% H <sub>2</sub> O <sub>2</sub> , 60°C, 30 minutes	686381	98.3	1.7	7067	1.21	0.319	0.423
Thermal degradation 105°C, 30 minutes	692666	99.2	0.8	7236	1.12	0.238	0.469
Photolytic degradation 365 nm, 3 hours	695459	99.6	0.4	7468	1.17	0.278	0.568

**Solution stability data**

Area Counts			% Deviation from Mean: ±3.0%	
Time (Hrs)	Standard	Sample	Standard	Sample
Initial	695773	686747	0.0	0.0
7 hrs	694105	686875	0.23	0.01
16 hrs	697297	689303	0.21	0.37
25 hrs	698567	689473	0.40	0.39
32 hrs	699875	692615	0.58	0.85
40 hrs	699948	698543	0.60	1.71

Table 8. Assay data's of abiraterone acetate

Assay of marketed formulations			
Brand Name	Label Claim (mg)	Drug obtained	% Recovery
Brand I (XIBRA)	250	247.96	99.18
Brand II (ZYTIGA)	250	251.02	100.41
Brand III (ABIRAPRO)	250	249.47	99.79

- RSD: Relative standard deviation
- SD: Standard deviation

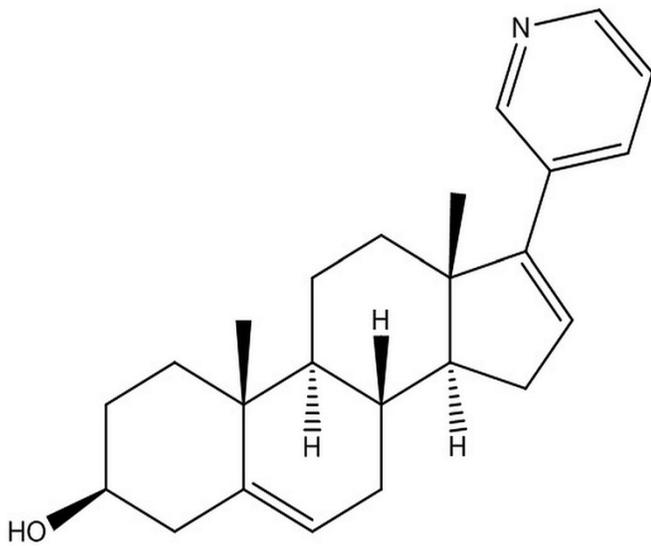
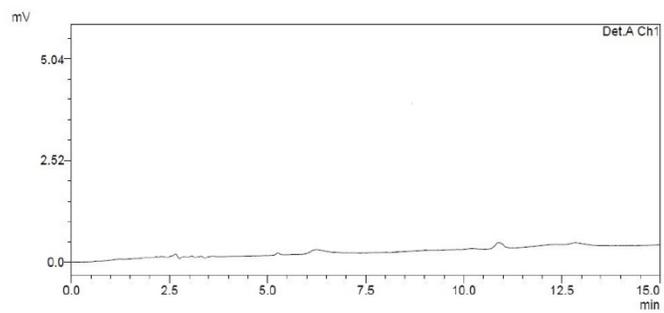
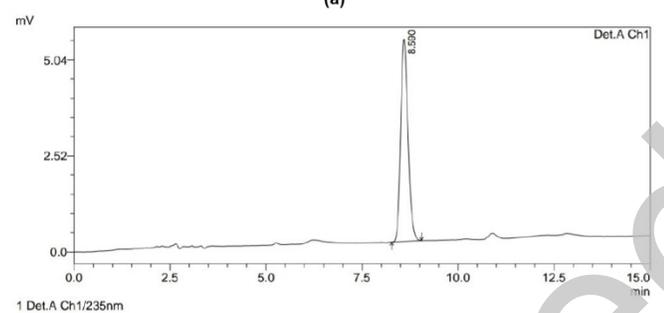


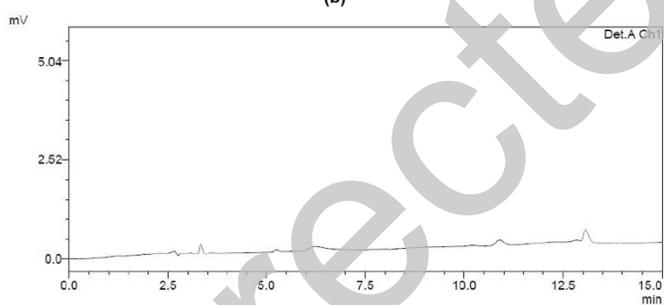
Figure 1. Chemical structure of abiraterone acetate



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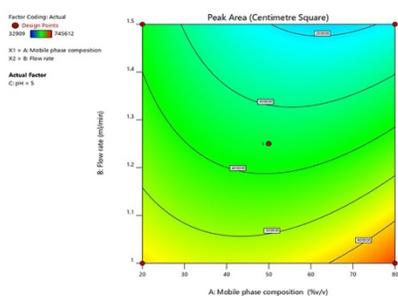


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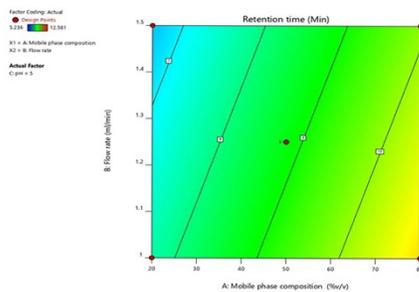


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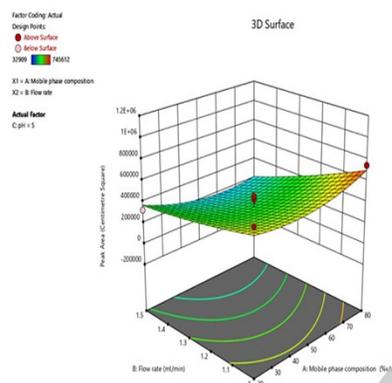
Figure 2. Optimized Chromatograms of blank (a), standard 10 $\mu$ g/mL and mixture of excipients (c)



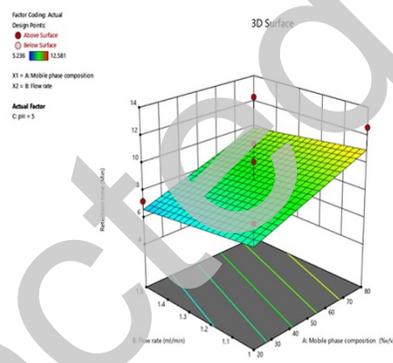
(a)



(b)



(c)



(d)

Figure 3. 2-D surface contour plot analysis of peak area [R1] response (a), Retention time [R2] response (b), 3-D surface contour plot analysis of peak area [R1] response (c), Retention time [R2] response (d)

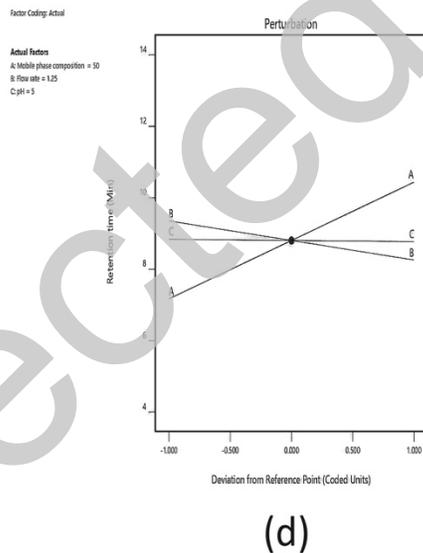
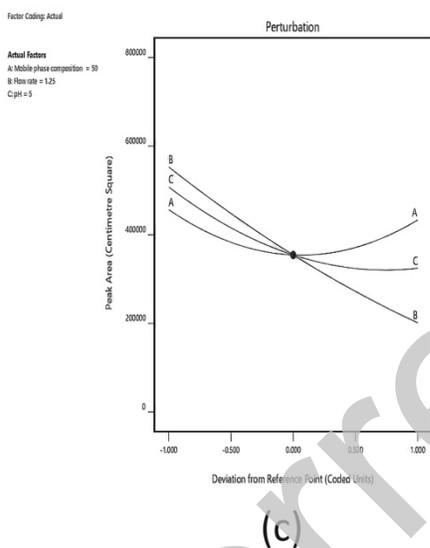
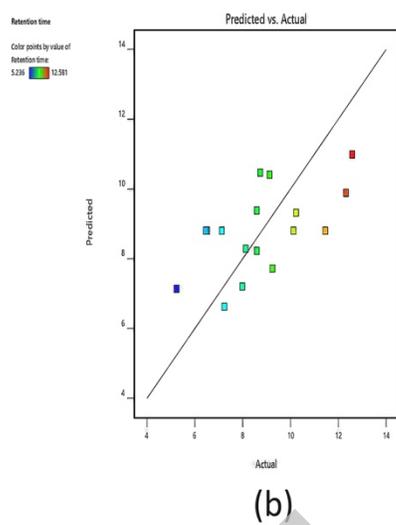
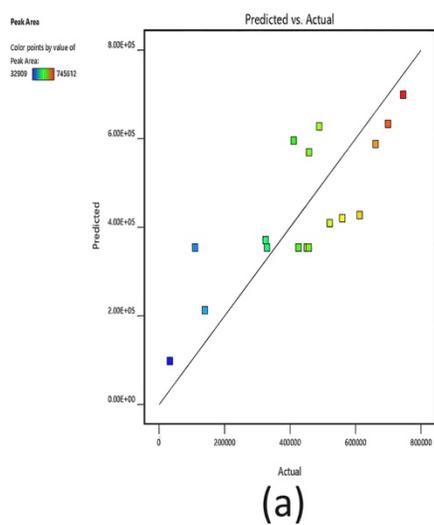
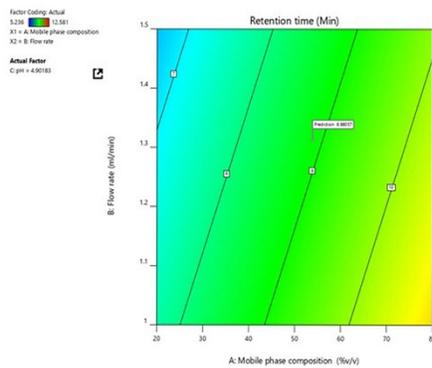
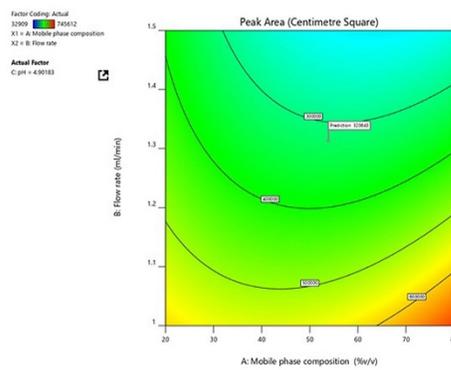


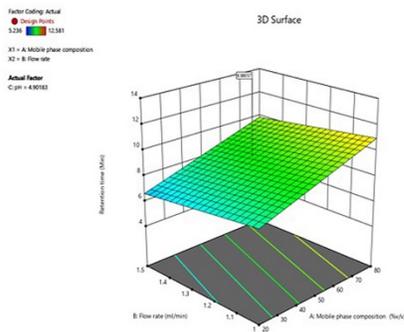
Figure 4. Predicted vs. Actual value for Peak Area [R1] (a), and Predicted vs. Actual value for Retention Time [R2]; (c) Representative plot of Perturbation for Response Peak Area [R1], and (d) Retention Time [R2]



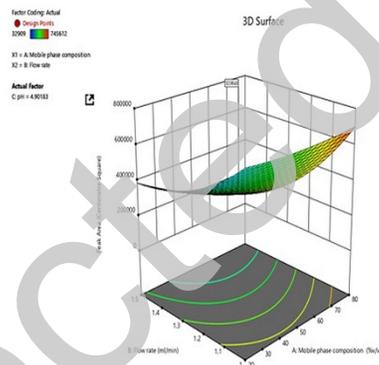
(a)



(b)



(c)



(d)

Figure 5. 2D surface numerical optimization for retention time [R2] (a), peak area [R1] (b), 3-D Surface numerical optimization for retention time [R2] (c), Peak area [R1] (d).

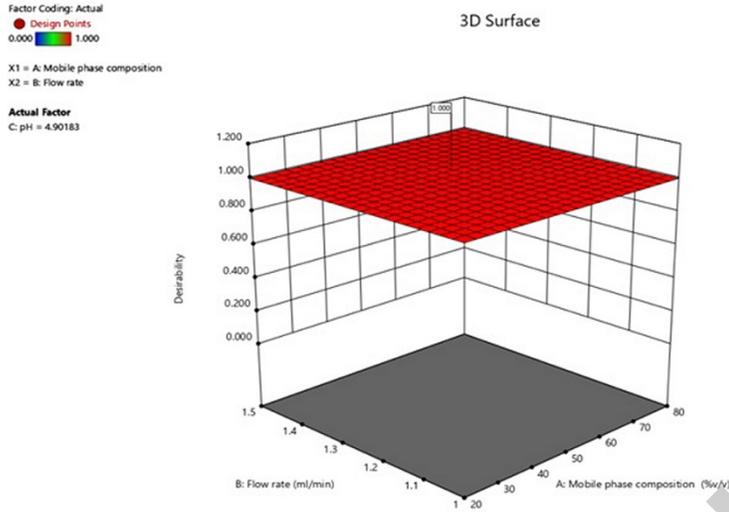


Figure 6. Numerical Optimization for Desirability data

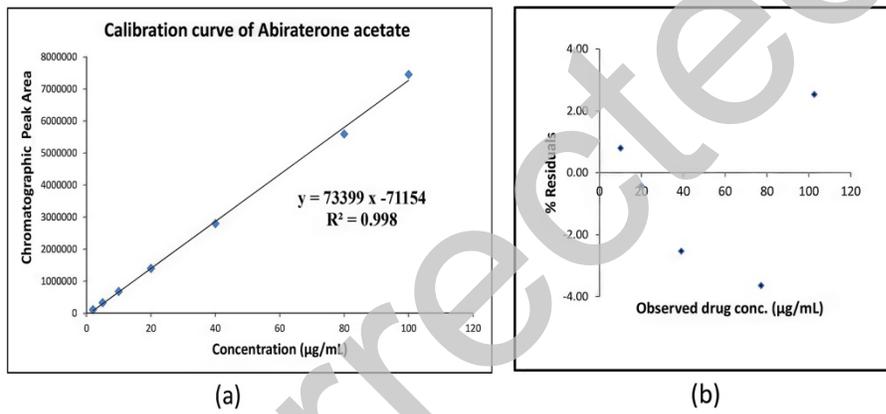


Figure 7. Schematic diagram of Calibration Plot (a) and Residual Plot (b) of abiraterone acetate

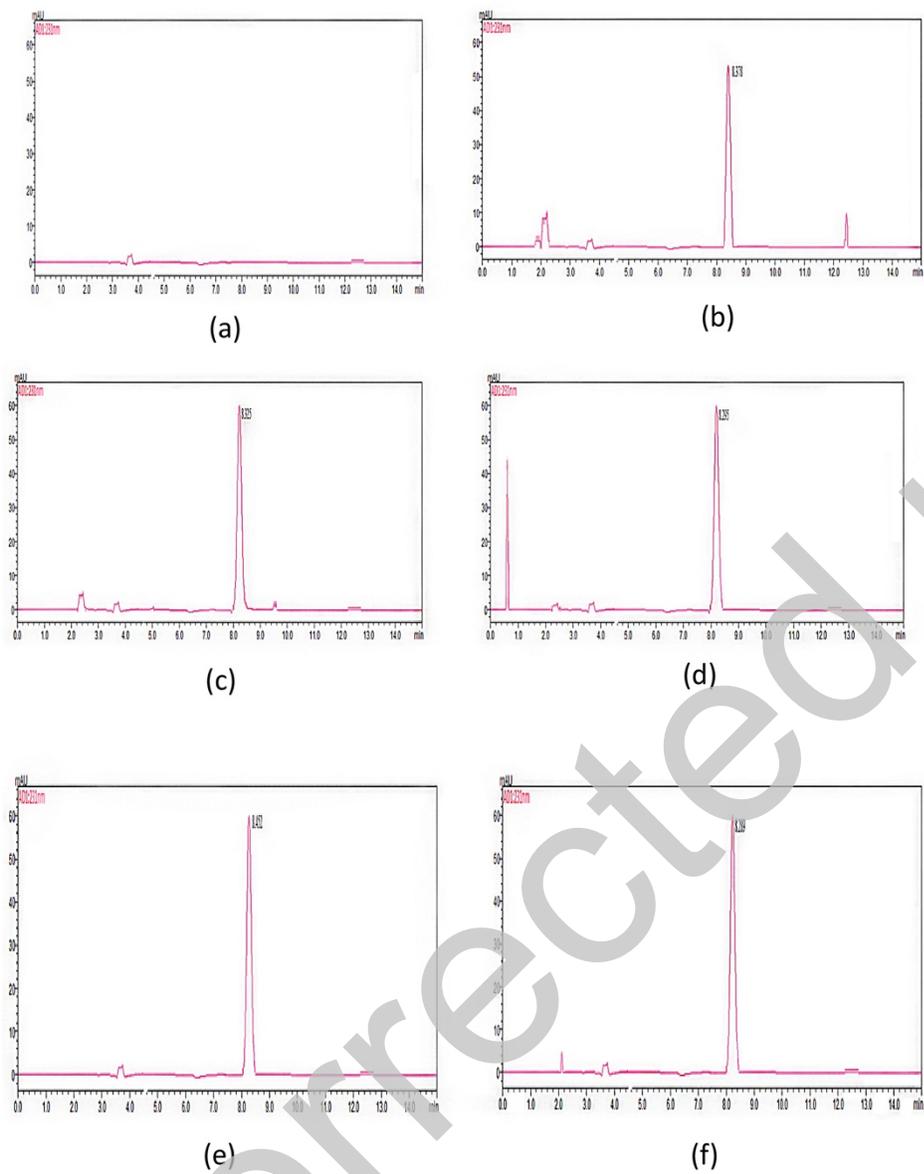


Figure 8. Schematic diagram indicating (a) mixture of excipients, (b) sample acidic degradation (c) alkali degradation (d) Peroxide degradation (e) Photolytic degradation (f) thermal degradation studies

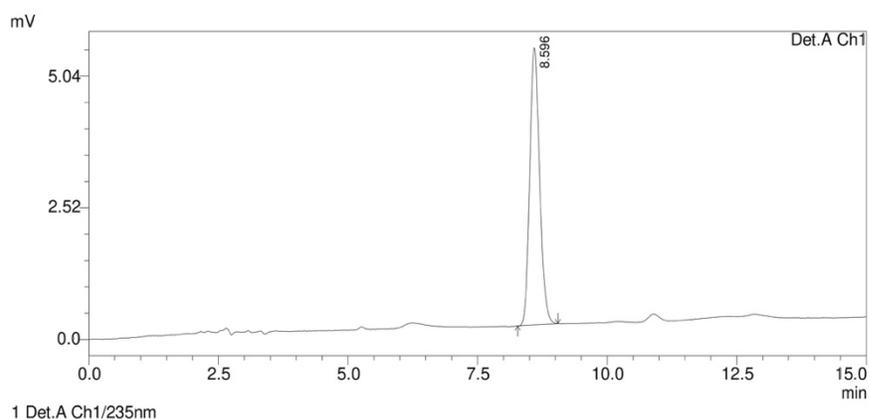


Figure 9. Chromatogram of formulation (10 µg/mL).

**List of abbreviations:**

ICH: International Conference on Harmonization  
 $\lambda_{\max}$ : Maximum Wavelength  
 RSD: Relative standard deviation  
 HPLC: High Performance Liquid Chromatography  
 QbD: Quality by Design  
 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  
 RSM: Response Surface Methodology  
 AQbD: Analytical Quality By design  
 MODR: Method Operable design region  
 ATP: Analytical Target Profile