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ORIGINAL ARTICLE

AQbD Driven Development of an RP-HPLC Method for the Quantitation of Abiraterone Acetate for its Pharmaceutical Formulations in the Presence of Degradants

Degradantların Varlığında Farmasötik Formülasyonları için Abirateron Asetat Miktarının Belirlenmesi için AQbD Güdümlü Bir RP-HPLC Yönteminin Geliştirilmesi

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ABSTRACT

Objectives: Abiraterone acetate is a well-known anticancer drug and a steroidal derivative of progesterone for treatment of patients with hormone-refractory prostate cancer. Chemometrics-assisted reverse phase high performance liquid chromatography (RP-HPLC) development of the drug abiraterone acetate has been employed in this study using an analytical quality by design (AQbD) approach.

Materials and Methods: Drug separation was performed using a Princeton Merck-Hibar Purospher STAR (C18, 250 mm × 4.6 mm) i.d., 5 µm particle size) with ultraviolet 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. The impact of three independent variables, namely, composition of the mobile phase, pH, and flow rate, on response retention time and peak area was studied by constructing an arithmetic model from these variables.

Results: Optimized experimental conditions for the proposed work include the mobile phase acetonitrile and phosphate buffer (10 mM KH_2PO_4) (20:80 %v/v). At the concentration range of 2-100 µg/mL, a linear calibration curve was found. Recovery was performed at three concentrations and was foun to be between 98% and 102%. The 3D response surface curves revealed that mobile phase composition and flow rate were the most substantial critical factors affecting desired responses.

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

Key words: Precision, accuracy, ICH guidelines, method validation, experimental design

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ÖZ

Amaç: Abirateron asetat, iyi bilinen bir antikanser ilacıdır ve hormona dirençli prostat kanseri olan hastaların tedavisi için progesteronun steroidal bir türevidir. Bu çalışmada abirateron asetat ilacının kemometri destekli ters fazlı yüksek performanslı sıvı kromatografisi (RP-HPLC) ile geliştirmesi, analitik kalite tasarım (AQbD) yaklaşımı kullanılarak gerçekleştirilmiştir.

Gereç ve Yöntemler: İlaç ayrımı, 235 nm'de ultraviyole saptamalı bir Princeton Merck-Hibar Purospher STAR (C18, 250 mm x 4,6 mm) i.d., 5 um partikül boyutu) kullanılarak yapılmıştır. Tepki yüzeyi metodolojisine sahip bir Box-Behnken istatistiksel deney tasarımı, yöntem optimizasyonu ve birkaç deneysel deneme ile formülasyonundan istenen kromatografik ayırma için uygulanmıştır. Üç bağımsız değişkenin, yani mobil fazın bileşimi, pH ve akış hızının, yanıt tutma süresi ve tepe alanı üzerindeki etkisi, bu değişkenlerden bir aritmetik model oluşturularak incelenmiştir.

Bulgular: Önerilen çalışma için optimize edilmiş deneysel koşullar, mobil faz asetonitril ve fosfat tamponunu (10 mM KH_2PO_4) (20:80 %h/h) içerir. 2-100 µg/mL konsantrasyon aralığında doğrusal bir kalibrasyon eğrisi bulunmuştur. Geri kazanım, üç konsantrasyonda gerçekleştirilmiş ve %98 ile %102 arasında bulunmuştur. 3B yanıt yüzey eğrileri, mobil faz bileşimi ve akış hızının, istenen yanıtları etkileyen en önemli kritik faktörler olduğunu ortaya çıkarmıştır.

Sonuç: Kalite kontrol laboratuvarlarında abirateron asetatın rutin analizi için başarıyla kullanılabilecek ekonomik, kesin, sağlam, stabiliteyi gösteren AQbD tabanlı RP-HPLC yöntemini geliştirmek ve doğrulamak için bir girişimde bulunulmuştur.

Anahtar kelimeler: Kesinlik, doğruluk, ICH yönergeleri, yöntem validasyonu, deneysel tasarım

INTRODUCTION

The potent anticancer drug abiraterone acetate {[(3*S*,8*R*,9*S*,10*R*,13*S*,14*S*)-10,13-dimethyl-17-pyridin-3-yl-2,3,4,7,8,9,11,12,14,15-decahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl]acetate} is an acetyl ester and a significant prodrug of its active metabolite abiraterone.[1-3] It is a well-built receptor blocker of androgen with potent bioavailability, especially in oral administration.[4] Abiraterone acetate is found to be more intense and effective than ketoconazole and liarozole in CYP17A1 inhibition; which is a rate-limiting enzyme for the biosynthesis of androgens.[4,5] Principally, abiraterone acetate is specified for use in combination with prednisone for treatment of men with metastatic castration-resistant prostate cancer who have already received prior chemotherapy comprising docetaxel.[5] Abiraterone acetate is poorly soluble over an extensive pH range. However, it is faintly soluble in HCl as well as in organic solvents (Figure 1). Abiraterone acetate can be detected by liquid chromatography-tandem mass spectrometry,[6-8] spectrofluorimetric for measuring fluorescence emission, and absorption spectroscopy in cancer patients.[9-11] Furthermore, the drug has been thoroughly recognized by other chromatographic techniques for the quantification of metabolites in biological samples bioanalytically.[8,9,12] However, there is scant research using QbD Paradigm, MODR Concepts, design space, and systematic development by chemometrics-assisted statistical optimization of critical method parameters (CMPs) and critical method attributes.[13] These optimization techniques with analytical quality by design (AQbD) are efficient, cost-effective, and innovative methodologies, which signify the "finest solution" to a meticulous "complexity" or problems raised in method development but deliver consistent quality output where other general High-performance liquid chromatography (HPLC) methods have been unable to achieve.[13-16] Notably, more relatively efficient optimization techniques such as Box-Behnken design (BBD) than other designs (central composite, d-optimal, and mixture design) have been applied for analytical method development and optimization of pharmaceutical drugs. BBD is a second-order three-level factorial design with advantages of user-friendly, economical, simple, and limited experimental runs.[17,18] Apart from that, such BBD optimization is a sequential design empowered with a feasible design matrix showing the quadratic model with a straightforward assessment of CMPs or critical material attributes. It also requires limited variables or responses, mainly three coded levels such as [low (-1), middle (0), and high (+1)], for the experimental design to fit the statistics or lack of fit values of the distinctive quadratic model.[17-19] Therefore, the developed method of validation, optimization, and stability-indicating reversed-phase (RP)-HPLC by employing the AQbD approach for the quantification of the bulk drug and its pharmaceutical formulations of abiraterone acetate is more economical, robust, and less expensive and reduces the heat and trial methods of optimization as well as the revalidation process as compared to one factor-at-a-time approach.

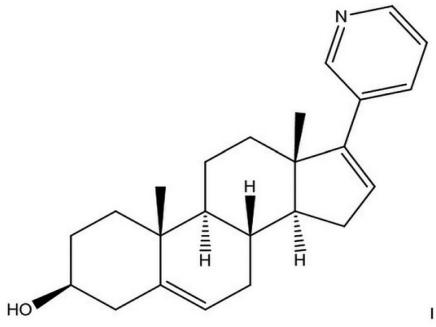

Figure 1. Chemical structure of abiraterone acetate

MATERIALS AND METHODS

A pure standard of the drug abiraterone acetate was obtained from Sun Pharmaceutical Industries Ltd., Halol, Gujarat, India. Abiraterone acetate was obtained commercially [XIBRA (Cipla Ltd, India), ZYTIGA (Janssen-Cilag Ltd., India), and ABIRAPRO (Glenmark Pharmaceuticals, India)] with a labeled claim of 250 mg. Phosphate buffer and acetonitrile (HPLC grade) were procured from Merck Laboratories, Mumbai.

Instrumentation

A Shimadzu HPLC system (LC-2010C HT) with a ultraviolet (UV)-visible detector, ultra-sonicator from Remi Instruments, Mumbai, and nylon filter (0.45 µm) from Millipore, Mumbai, India, was used.

Chemicals and reagents

Orthophosphoric acid (OPA) (AR grade) and acetonitrile (HPLC grade) were obtained from Merck Laboratories Pvt. Ltd., Mumbai. Potassium dihydrogen phosphate (KH_2PO_4) was obtained from Fischer Scientific, Mumbai, India.

Methods

Method optimization was performed with Box-Behnken statistical design comprising the CMPs, which include three significant factors (the composition of the determined mobile phase, flow rate, and pH of buffer), encompassing three levels. Seventeen experimental runs were established with five center points. The flow rate was tested at 1.0, 1.2, and 1.5 mL/min; the pH was measured at 4, 5, and 6; and the concentration of the mobile phase was monitored at 20%, 50%, and 80%. The responses considered were retention time (Rt) and peak area, which were 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 executed for design space navigation. The 2D & 3D response surface techniques and perturbation sequential plots were scrutinized to assess the indicative 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 was acquired within the design space of the optimized results.[19,20]

Statistical analysis

Design-Expert (Version 12), Stat-Ease Inc., Minneapolis, MN, USA, was utilized for method optimization and estimation of its CMPs and randomization of the runs.[14] Microsoft Excel 2007 (Microsoft, USA) was used for the remaining calculations.

Preparation of solvent

Phosphate buffer (pH 4): Disodium hydrogen phosphate (5.04 g) and KH_2PO_4 (3.01 g) were dissolved in water to a volume of 1000 mL. The pH was adjusted to 4.0 with OPA. The resulting solution was passed through (0.45 µm) filter paper, filtered by vacuum filtration, and sonicated for about 15 min.[15] While preparing the mobile phase, only the phosphate buffer was filtered using (0.45 µm) nylon membrane filters. Acetonitrile was not filtered and was used as provided by the supplier.

Procedure for the stock standard solution

Standard stock solution of the drug was made by accurately weighing 10 mg of the drug and mixed with 10 mL of acetonitrile to acquire a concentration of 1000 µg mL^{-1}. Serial dilutions of the stock solution were prepared by diluting with the mobile phase to obtain concentrations of 1 to 100 µg mL^{-1} and filtered through a 0.45 µm syringe filter before chromatography. Calibration curves were constructed as depicted in Figures 2a-c. Here, a mixture of acetonitrile and phosphate buffer (10 mM KH_2PO_4) (20:80, %v/v) was selected as a mobile phase with a flow rate of 1 mL min^{-1} and UV detection at 235 nm. The limit of quantification (LOQ) and limit of detection (LOD) were estimated based on the linearity plot (concentration vs. peak area).

Chromatographic conditions

A Merck-Hibar Purospher STAR Analytical column (C18, 250 mm × 4.6 mm × 5 µm) was used for chromatographic separation due to its advantage of having the highest carbon loading for better separation of the desired analyte, minimizing the variabilities of the mode of selection. A mobile phase of acetonitrile and phosphate buffer (10 mM KH_2PO_4) (20:80 %v/v) was used. The flow rate was 1 mL min^{-1} with an injection volume of 10 µL. The oven temperature of the chromatographic column was 30°C, and the sample temperature was ambient.

Optimization of the chromatographic method using the analytical QbD approach

Initially, trial and error practices were executed to obtain rigorous data for 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 BBD principles. The statistical design intensifies ANOVA principles for establishing the optimized experimental conditions of the method.[16,21,22] A simple, accurate, and AQbD-based RP-HPLC method was developed, which is also stability indicating. This was further subsequently validated as per the International Conference on Harmonization (ICH) recommended stability guidelines for quantification of abiraterone acetate in its various pharmaceutical formulations (tablets). A mixture of phosphate buffer (10 mM KH_2PO_4 and acetonitrile) (20:80, %v/v) was used as the mobile phase with a 1 mL min^{-1} flow rate.

Optimized chromatographic conditions

The optimized conditions are as follows: Optimized trail concentration: 10 µg mL^{-1}; flow rate: 1mL min^{-1}; mobile phase: Acetonitrile/phosphate buffer [10 mM KH_2PO_4, (20:80) %v/v]. The optimized chromatograms of the blank, standard, and mixture of excipients (10 µg mL^{-1}) of the developed analytical method are shown in Figure 2a-c, respectively.

Method validation

Method validation was performed to substantiate that the analytical procedure employed for a definite experiment is appropriate for use.[19] The outcomes of method validation parameters can be highly requisite to judge the reliability, quality, and steadiness of analytical results. Validation of a method for parameters linearity, accuracy, precision, and robustness was done according to recommendations of ICH guidelines (ICH Q2) (R1).[23-25]

Linearity

The linearity plot of the proposed method was constructed as per the stated ICH guidelines.[26-28] The linearity chart of abiraterone acetate was found to be within the concentration range of 2-100 µg mL^{-1}; further, the calibration plot was constructed using the peak area versus the concentration.

Accuracy

A series of solutions were spiked with known standard concentrations of abiraterone acetate of 50%, 100%, and 150% (5, 10, and 15 µg/mL) in triplicate, performed as per recommendations of ICH guidelines.[26-28]

Precision

The precision of the developed technique, expressed in % relative standard deviation (RSD), was calculated by performing repeatability and intermediate precision studies.[21] The developed analytical QbD-based method was validated by the precision studies (both intraday and interday).

LOD and LOQ

The LOD and 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 ratios of 3:1 (LOD) & 10:1 (LOQ) as per guidance and protocol recommended by ICHQ2B.[29]

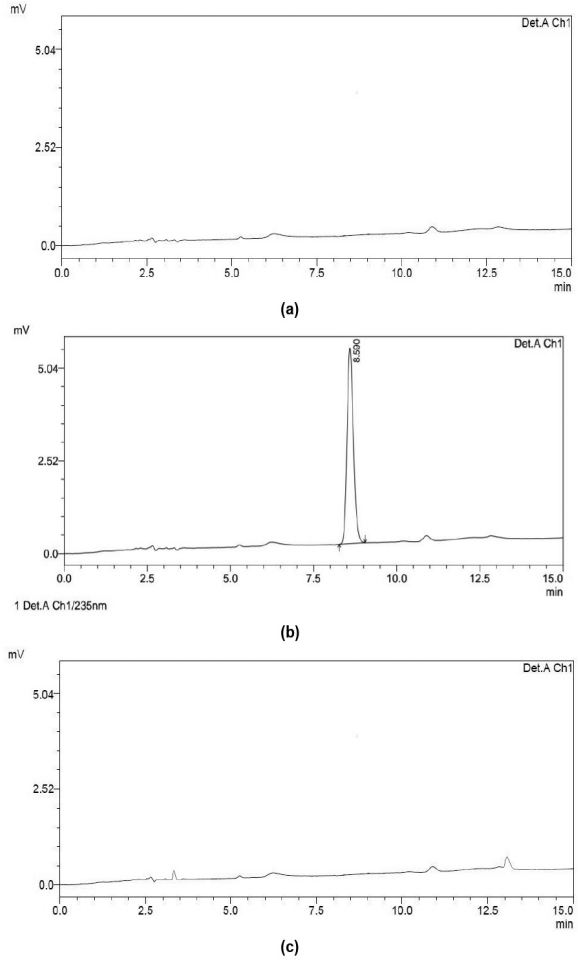

Figure 2. Optimized chromatograms of blank (a), standard 10 µg mL^{-1}, and the mixture of excipients (c)

Robustness

A robustness study was performed to recognize and evaluate the toughness of an analytical method.[30] To check the ability of the projected method, different factors were deliberately altered such as alternation in mobile phase composition, change in flow rate, etc.

Specificity

Specificity was carried out to evaluate the analyte noticeably in the occurrence of components that may be expected to be present during development. Specificity was established by representing no interference from the excipients and the degradation products.[28,29]

Forced degradation studies

Forced degradation generally includes exposure of drug substances to a range of stress conditions to demonstrate the stability profile and possible degradants of developed analytical methods.[26-28] Acid degradation was performed by adding 1 mL of 0.1 N HCl, then heating at 60˚C for 30 min, cooling to room temperature, and neutralizing. Alkali degradation was performed by adding 1 mL of 0.1 N NaOH, heating at 60˚C for 30 min, cooling to room temperature, and neutralizing. The degradative process through oxidation was performed by exposing the drug to 1 mL of 3% H_2O_2 and heating at 60˚C for 30 min. The degradation study was achieved by heating the drug content solutions at 105˚C on a thermostatically regulated water bath for half an hour. Photolytic degradation was done by subjecting the drug solutions to UV light in a UV chamber at 365 nm for 3 h. 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.[26,29] 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 the chemical stability of the drug and sample solution whether any significant changes occurred at varied time intervals.[29,31,32]

Assay of formulations

Twenty tablets of commercial brands of abiraterone acetate were chosen arbitrarily, and their average weight was determined.[31] The tablets were crushed to fine powder, 250 mg was weighed, and the powder was dissolved in 200 mL of acetonitrile (in a 250 mL volumetric flask). Then, it was shaken for 20 min and ultrasonicated for 20 min. After that, it was allowed to cool at room temperature, and the solution was diluted up to mark with diluents (1000 mcg mL^{-1}). The final obtained solution was

then diluted to 10 mcg mL^{-1} with acetonitrile/phosphate buffer (20:80 %v/v) and subsequently injected to an HPLC system for estimation in triplicate.

RESULTS AND DISCUSSION

Method development and optimization Using Box-Behnken experimental design

In the current investigation, trials were proposed and conducted using Box-Behnken experimental design.[21] The proposed BBD desires 17 experimental runs of examination to acquire data and model the response surface for a reliable analysis. A 3^2 level with 17 experimental runs was executed to identify the optimized design space for detection of the predicted response. The 3^2 BBD with RSM was executed.[16,21] The statistical design with optimization methodology and its data analysis through BBD were statistically evaluated using Design-Expert-Ver.12 software.[21,22] The three most influential factors (CMPs), the composition of the mobile phase (X1), flow rate (X2), and pH (X3), were selected; the peak area (Y1) and Rt (Y2) were used as observed responses. The design matrix of the statistical BBD and experimental runs are shown in Table 1.[21] 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) is found to produce a nullified effect upon the obtained responses. The outline of 3^2 factors and ANOVA results, through its calculated mean and standard deviation values, are summarized in Table 2.[22] The statistically constructed model was suitably validated by interaction studies using the effect of various factors upon the found responses. The 2D counter plot analysis of peak area and Rt with the observed responses (R1) & (R2) are depicted in Figure 3a, b, respectively. Similarly, the 3D counter plot analysis of peak area (R1) response and Rt (R2) response is depicted in Figure 3c, d, respectively. The statistical model signifies that predicted values for both the responses (Rt and peak area) are a bit closer to the actual values representing superior accuracy and precision values for the obtained responses. The 2D and 3D counter plots of predicted versus actual values for peak area

Table 1. Box-Behnken 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. ANOVA by selecting 3^2 factors

Factors	Name	Units	Type	Minimum	Maximum	Coded low	Coded high	Mean	Standard deviation
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

(R1) and Rt (R2) are depicted in Figure 4a, 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 peak area and Rt. The representative plots of perturbation analysis for the observed responses peak area (R1) and Rt (R2) are depicted in Figure 4c, d, respectively. Here, for 2D and 3D surface numerical optimization, the Rt (R2) and peak area (R1) are depicted in Figure 5a-d, respectively. Figure 6 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).

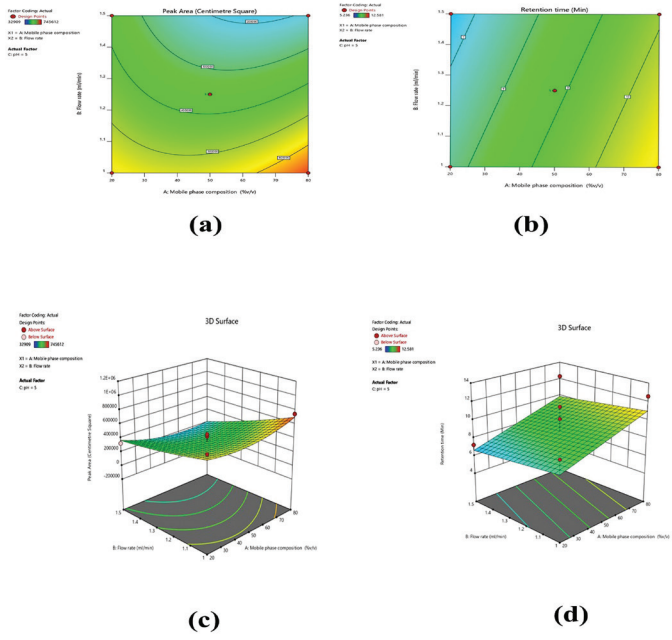

Figure 3. 2D surface contour plots of peak area (R1) response (a) and retention time (R2) response (b); 3D surface contour plots of peak area (R1) response (c) and retention time (R2) response (d)

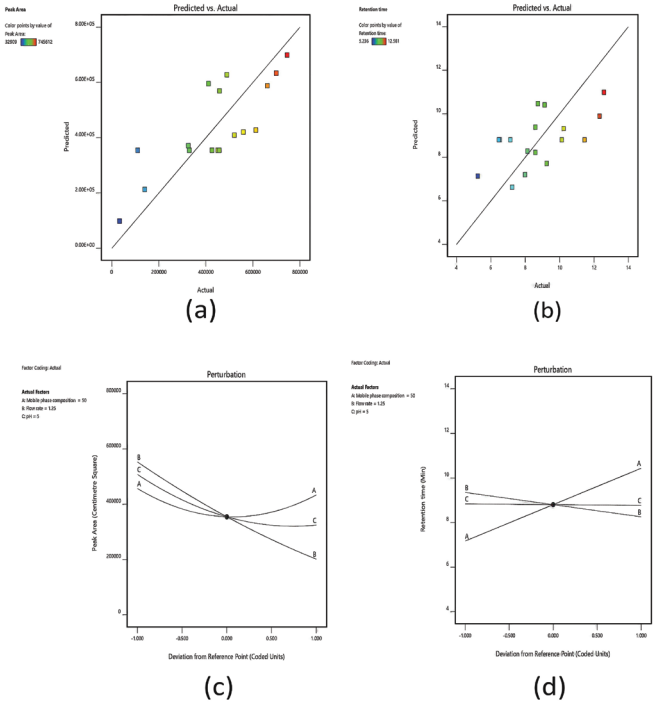

Figure 4. Predicted versus actual values for peak area (R1) (a) and predicted versus actual values for retention time (R2) (b). Representative plots of perturbation for (c) response peak area (R1) and (d) retention time (R2)

Results of validation studies

The optimized feasible chromatographic conditions were aggrandized and effectively implemented to validate the QbD-based method by a range of validation parameters, linearity, accuracy, precision, LOD, LOQ, robustness, etc., as per recommendations of ICH guidelines.

Linearity

Six concentrations of the abiraterone acetate working standard ranging from 2 to 100 ppm were prepared and analyzed for the linearity study. The calibration plot was constructed by plotting the chromatographic peak areas versus the known predicted concentrations in µg mL^{-1}, and values of observed concentrations were determined. The regression equation was found to be Y= 73399x - 71154, and the regression coefficient r^2 is 0.998. The detail of the calibration plot and its residual plot data analysis are depicted in Figure 7a, b, respectively.

Accuracy

The accuracy of the developed method was studied to make sure the agreement between the true and reference values in

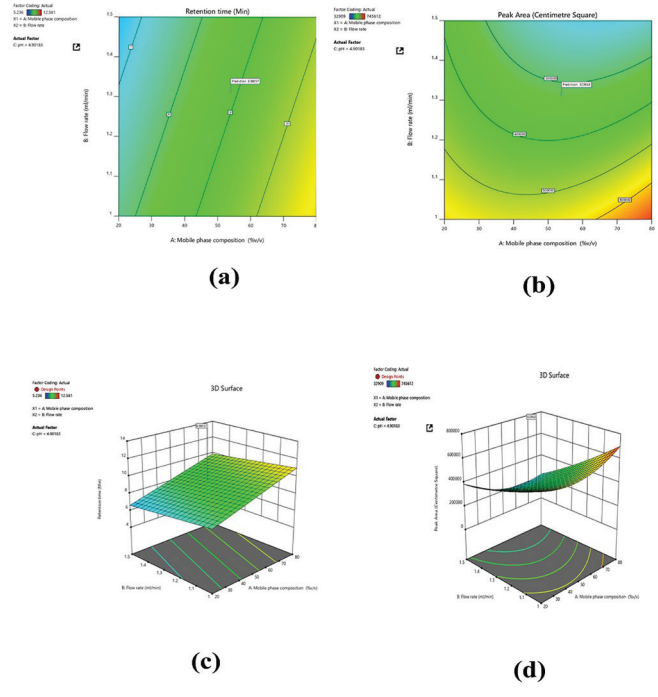

Figure 5. 2D surface numerical optimization for retention time (R2) (a) and peak area (R1) (b); 3D surface numerical optimization for retention time (R2) (c) and peak area (R1) (d)

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 was obtained within the acceptance limit, that is, NMT 2.0%. The data of all the recovery studies are shown in Table 4.

Precision

Precision studies of the developed method were carried out by the system, method, and intermediate precision studies. Precision of the system suitability test for six replicate standard injections was calculated, within the acceptance criteria, that is, NMT 2%, and both intraday and interday precisions through three replicate injections of standards were calculated. Data of % RSD are reported in Table 5.

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) ratio of 3:1, whereas the quantification limit was indicated as a S/N ratio of 10:1, as an effect of the response by the detector. The estimated value for LOD was found to be 0.45 ppm, and the estimated value for LOQ was 1.35 ppm. The calculated values are shown in Table 3.[23]

Robustness

Robustness is 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 were taken, and changes in flow rate and mobile phase data are summarized in Table 6. The results show the calculated % RSD within the acceptance limit, that is, less than 2%.

Specificity

The specificity of the method has been studied to determine the interference from the degradation products as per ICH through a forced degradation study. The results denote no sign of peak formation at the Rt of abiraterone acetate and the case of degradation products since the peak purity passed. The data reveal that the purity angle was less than the purity threshold, presenting no specific interference (Table 7).

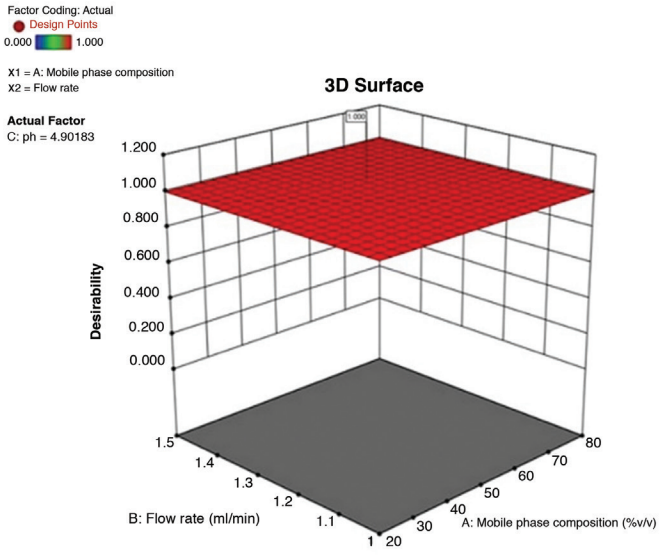

Figure 6. Numerical optimization for desirability data

Table 3. Optimum chromatographic conditions of abiraterone acetate

Optimum chromatographic conditions	
Run time	15. 0 minute
Retention time	8.59 minutes
Flow rate	1 mL/min
Linearity range	(2-100) µg/mL (r^2=0.998)
Accuracy	% recovery: Within (98-102%) (RSD <2%)
Precision	(RSD <2%)
LOD	0.45 µg/mL
LOQ	1.35 µg/mL

RSD: Relative standard deviation, LOD: Limit of detection, LOQ: Limit of quantification

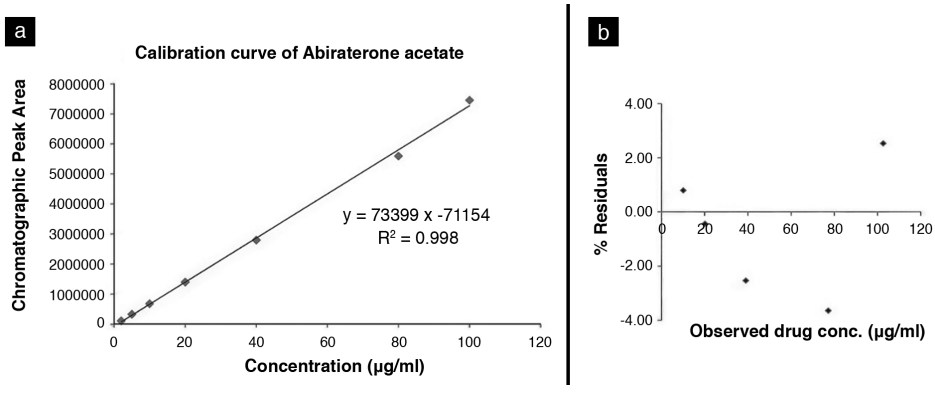

Figure 7. Schematic diagram of calibration plot (a) and residual plot (b) of abiraterone acetate

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 tests

System precision				Intra-day			Inter-day			
Conc. (µg/mL)	Peak area	USP tailing	USP plate count	Conc. (µg/mL)	Peak area of at different time intervals (day 1)			Conc. (µg/mL)	Peak area of at different time intervals (day 2)		
					10 a.m	2 p.m	5 p.m		10 a.m	2 p.m	5 p.m
10	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
10	695971	1.02	7356	**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	695272	698777	697651	10	696565	694872	697981
10	695332	1.03	7287	10	695773	698874	697236	10	696834	698671	697123
				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

RSD: Relative standard deviation, SD: Standard deviation

Table 6. Robustness data of abiraterone acetate

Robustness	Peak area	Average	SD	% RSD
Flow rate (1+0.2 mL/min) 10 (µg/mL)	689234	689558	384.448	0.05575
	689458	-	-	-
	689983	-	-	-
Flow rate (1-0.2 mL/min) 10 (µg/mL)	699764	699770	85.675	0.01224
	699859	-	-	-
	699688	-	-	-
Amount of (ACN +2% v/v) 10 (µg/mL)	699272	699641	323.013	0.04617
	699874	-	-	-
	699776	-	-	-
Amount of (ACN -2 %v/v) 10 (µg/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	-	-	-

RSD: Relative standard deviation, SD: Standard deviation

Table 7. Forced degradation and solution stability data 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_2O_2, 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

*With respect to assay value of control

Forced degradation studies

Forced degradation studies provide knowledge on practical degradation pathways and degradation products of the active ingredients and help expound the configuration of the degradants as per ICH recommendations.[27] The following conditions were applied for degradation: Acid (1 mL of 0.1 N HCl, 60°C for 30 min), alkali (1 mL of 0.1 N NaOH, 60°C for 30 min), peroxide (1 mL of 3% v/v H_2O_2), thermal (105°C), and photolytic degradations at 231 nm, for determining the steady nature of drugs. Percent drug degradation values during acidic and alkaline degradation were observed to be 26.5% and 12.3%, respectively. In contrast, less than 2% degradation was reported in the case of oxidative, thermal, and photolytic degradations, which indicate the drug's completely resistant behaviors to the above stress conditions. The detailed descriptions of forced degradation activities are listed in Table 7. The representative chromatograms of the sample in various stress conditions by incorporating a mixture of excipients (a), acid (b), alkali (c), oxidative (d), photolytic (e), and thermal degradation (f) studies are depicted in Figure 8a-f, respectively.

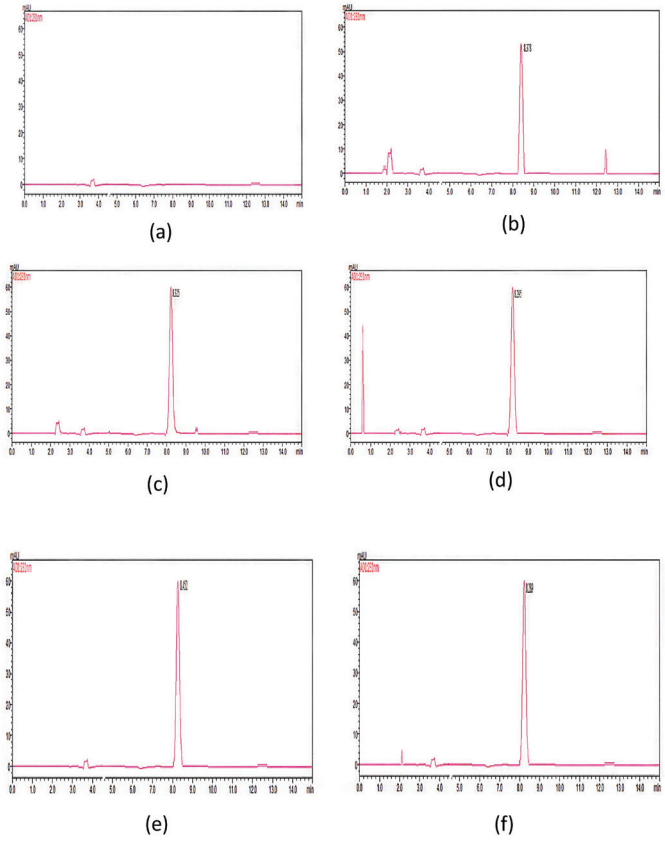

Figure 8. Schematic diagram indicating (a) the mixture of excipients, (b) sample acidic degradation, (c) alkali degradation, (d) peroxide degradation, (e) photolytic degradation, and (f) thermal degradation studies

Stability of analytical solution

The solution stability study was carried out by observance of sample and standard solutions at 25±2°C for 40 h. After analysis, it has been concluded that the drug standard and sample were stable for up to 40 h (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 the assay of various marketed formulations are exhibited in Table 8. The results show that all the values of the formulations are within the acceptance limit, that is, 98-102%.

CONCLUSION

Chemometrics-assisted method development affords regulatory flexibility, the formation of homogeneous or robust finished products of assured quality characteristics as per Food and Drug Administration (FDA) concerns, and ICH stability stressed conditions. In addition to this, the AQbD method minimizes the process of revalidation, lessens solvent consumption and development time, and enhances optimum robust analytics. By implementing the DoE, the Box-Behnken statistical design can evaluate the independent variables (CMPs) concurrently with adding common interactions among the critical factors to optimize the tentative conditions. It is explicit that the application of this BBD with RSM is an adaptable practice to reduce the

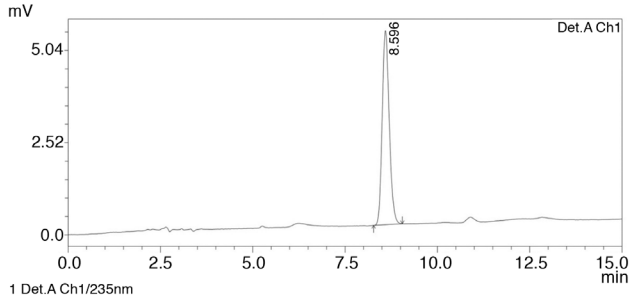

Figure 9. Chromatogram of the formulation (10 µg mL^{-1})

Table 8. Assay data 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

total experimental runs to obtain sustainable, robust analytics, reducing the chance of revalidation, requisite for analytical development. The optimization of the RP-HPLC method for abiraterone acetate can produce the highest intense data and boost efficiency within a short period as per the ICH Q8 (R2) and FDA perspectives. The proposed method was found to be linear, with concentrations of 2-100 ppm having r^2: 0.998. The remarkable % recovery (within 98-102%) of the drug reflects that the excipients existing 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 stability indicating. Therefore, the developed stability-indicating method was economical, accurate, and precise. It can be successfully implemented for the routine analysis of abiraterone acetate in its bulk and pharmaceutical formulations.

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