Analytical Quality by design method development and validation of Niraparib and Abiraterone using RP-HPLC method

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Abstract:

A robust RP-HPLC technique was set up for the estimation of Abiraterone & Niraparib. SST parameters, including resolution, theoretical plates, & tailing factor, were within acceptable boundaries, ensuring the technique's reliability. Technique verification followed ICH rules, demonstrating high assay accuracy with recoveries within 98-102%. The linearity scale was 10-50 µg per ml for Abiraterone & 2-10 µg per ml for Niraparib, with R^2 of 0.999. Precision, ID precision, & robustness tests confirmed the technique's consistency across different conditions. LOD & LOQ were established, showcasing the technique's sensitivity. This verified RP-HPLC technique is suitable for regular analysis of Abiraterone & Niraparib in l formulations.

Key Words: Niraparib, Abiraterone, QbD, RP-HPLC

INTRODUCTION

Niraparib is a small molecule poly ADP ribose polymerase 1 and 2 inhibitors prescribed for treating ovarian cancer in adult patients. It is also used to treat primary peritoneal cancer and fallopian tube cancer for patients who are showing partial or complete response to platinum-based chemotherapy. Niraparib works by irreversible multiple breaks in the double strand with Breast cancer susceptibility protein (BRCA) type 1 and 2 mutations leading to the death of the cells. [1,2]

According to Global Cancer Statistics 2020, prostate cancer is the second most commonly occurring cancer in men and the fifth most common cause of cancer-related death. Abiraterone (ART) acetate is used to treat metastatic castration-resistant prostate cancer and metastatic high-risk castration sensitive prostate cancer. ART acetate is recommended in combination with prednisone or prednisolone. In vivo, ART is an active metabolite of ART acetate. Both ART acetate and ART are potent, selective, and irreversible inhibitors of 17α-hydroxylase/C17, 20-lyase (CYP17), a key enzyme used in testosterone biosynthesis. By blocking CYP17, ART leads to the termination of androgen biosynthesis in testicular, adrenal, and prostatic tumour tissues. ART chemically known as (3b)-17-(pyridin-3-yl) androsta-5, 16-dien-3-ol. [3; 4]

Several analytical techniques, including high-performance liquid chromatography (HPLC)-fluorescence [11], HPTC [8], ultraviolet (UV)[5,6], UPLC[7] and liquid chromatography-mass spectrometry (LC-MS/MS)[9] have been reported to quantify Abiraterone and Niraparib. In the extensive literature review, it was confirmed that no analytical Liquid Chromatographic Method was reported for estimation of Abiraterone and Niraparib.

Hence the present work intended to establish an analytical Liquid Chromatographic Method for the estimation of Niraparib and Abiraterone.

MATERIAL AND METHODS

A) Chemicals and Reagents

Niraparib, Abiraterone (ART) acetate, Sodium hydroxide (NaOH), water, Phosphate buffer, Orthophosphoric acid (OPA) and Potassium dihydrogen phosphate (KH_2PO_4) acetonitrile (ANC) and trifluoroacetic acid chemicals are used in this research.

B) INSTRUMENTS

HPLC System: Shimadzu (LC-2010C HT) with a ultraviolet (UV)-visible detector, ultra-sonicator and nylon filter (0.45 μm) was used

Detector: photodiode array, Software Empower 3 ,2695 separation modules 996 PDA

C) METHODS

Preparation of mobile phase

Mix a 300ml TFA PH: 3 (30%) & 700 ml ACN (70%) & remove gases in ultra sonication water bath for 5 mins. Filter using 0.45micron vacuum filtration.

Standard Solution Preparation:
Accurately weigh and transfer 50 mg of Abiraterone and 10 mg of Niraparib working standards add into a separate 25 ml clean dry volumetric flasks add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.3ml & 0.3 ml of the above stock solutions into a 20ml volumetric flask and dilute up to the mark with Diluents

Results and Discussion:

Method development and Optimization:

Design Space

Method optimization was performed with Box-Behnken statistical design comprising the CMPs, which include five significant factors (the composition of the determined flow rate, organic phase ratio, column, detector wavelength and column temperature), encompassing five levels and include five significant responses (tailing factor of 1st and 2nd drug, retention time of 1st and 2nd drug and resolution. Seventeen experimental runs were established. Design-Expert software was used to validate the model after the data was examined. A positive correlation was found between the quadratic model and the experimental data used for design

space navigation. It was verified that the predicted values from the practical responses were obtained within the design space of the optimized results, and they were found to be satisfactory.

Method development and optimization Using Box-Behnken experimental design

Table 1: Experimental runs and responds values

Std	Run	Block	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4	Response 5
15	1	Block 1	0.90	50.00	35.00	1.06	1.01	2.122	2.76	5.09
2	2	Block 1	1.00	40.00	35.00	1.07	1.02	2.118	2.72	5.05
8	3	Block 1	1.00	50.00	45.00	1.08	1.03	2.119	2.73	5.06
1	4	Block 1	0.80	40.00	35.00	1.02	1	2.06	2.4	5
12	5	Block 1	0.90	60.00	45.00	1.15	1.19	2.13	2.64	4.9
4	6	Block 1	1.00	60.00	35.00	1.09	1.04	2.12	2.74	5.07
7	7	Block 1	0.80	50.00	45.00	1.04	1.01	2.08	2.6	5.01
5	8	Block 1	0.80	50.00	25.00	1.04	1.01	2.08	2.6	5.01
6	9	Block 1	1.00	50.00	25.00	1.08	1.03	2.119	2.73	5.06
13	10	Block 1	0.90	50.00	35.00	1.06	1.01	2.122	2.76	5.09
9	11	Block 1	0.90	40.00	25.00	1.16	1.21	2.132	2.65	5.01
14	12	Block 1	0.90	50.00	35.00	1.06	1.01	2.122	2.76	5.09
3	13	Block 1	0.80	60.00	35.00	1.05	1.02	2.09	2.7	5.02
17	14	Block 1	0.90	50.00	35.00	1.06	1.01	2.122	2.76	5.09
16	15	Block 1	0.90	50.00	35.00	1.06	1.01	2.122	2.76	5.09
10	16	Block 1	0.90	60.00	25.00	1.09	1.04	2.12	2.74	5.07
11	17	Block 1	0.90	40.00	45.00	1.16	1.21	2.132	2.65	5.01

Table 2: ANNOVA of quadratic model

Ource	Sum of Squares	df	Mean Square	F Value	p-value
Model	0.025	9	2.786E-003	7.57	0.0071
Flow rate	3.613E-003	1	3.613E-003	9.82	0.0165
Organic mp ratio	1.125E-004	1	1.125E-004	0.31	0.5975
Column Temp	4.500E-004	1	4.500E-004	1.22	0.3053

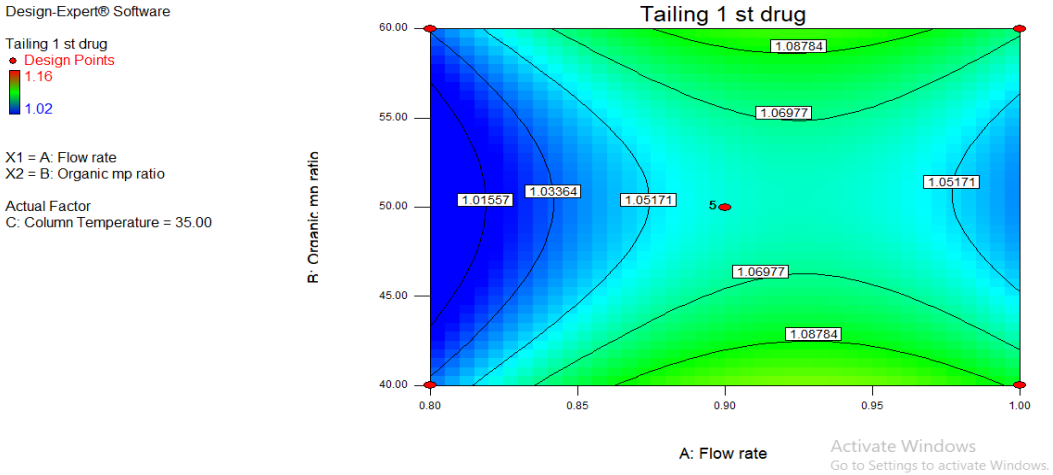

Figure 1: Tailing factor of Niraparib

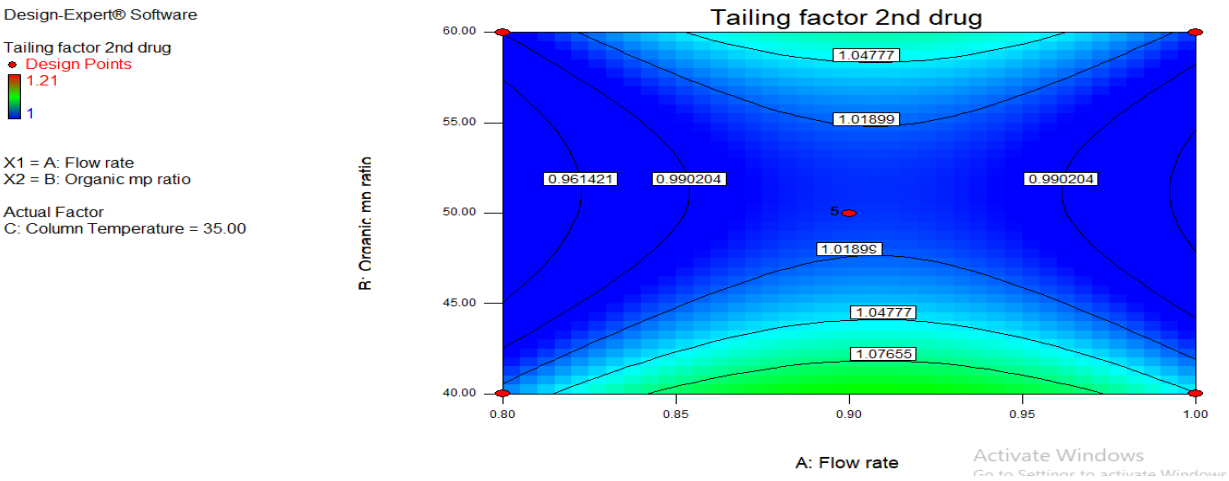

Figure 2: Tailing factor of Abiraterone

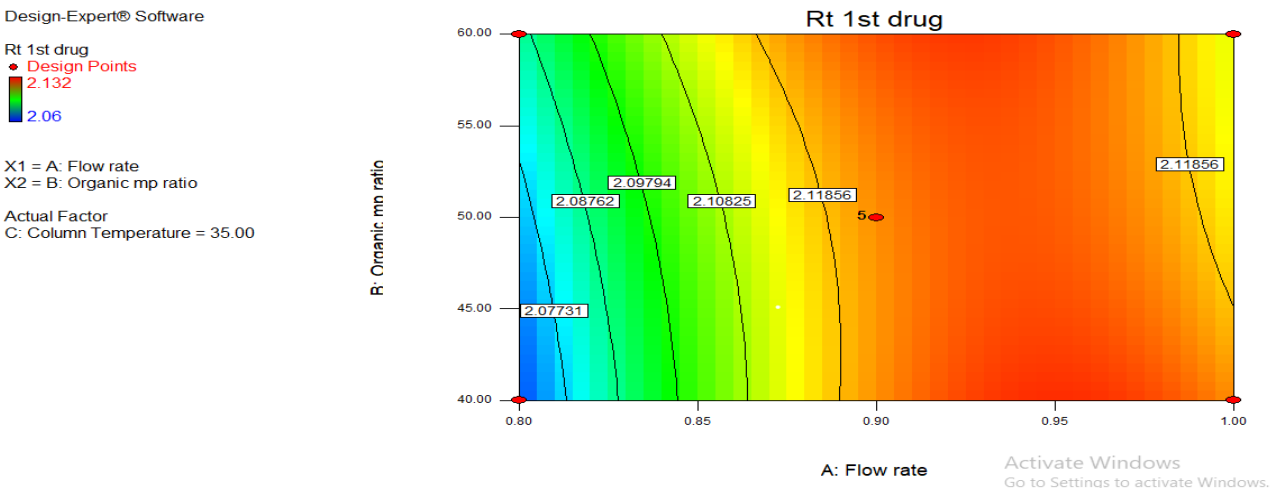

Figure 3: Retention of Niraparib

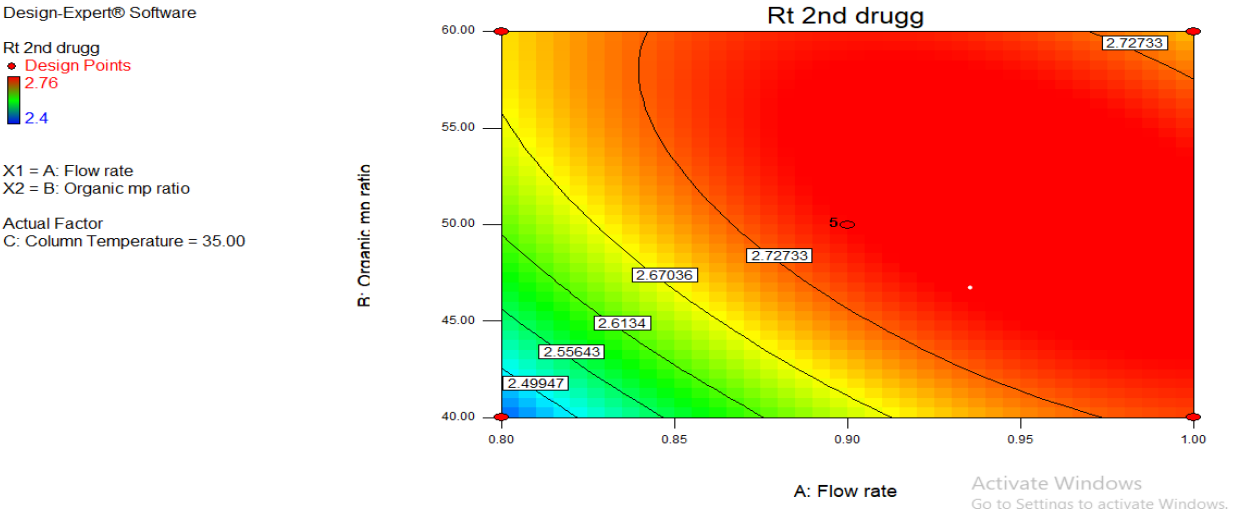

Figure 4: Retention of Abiraterone

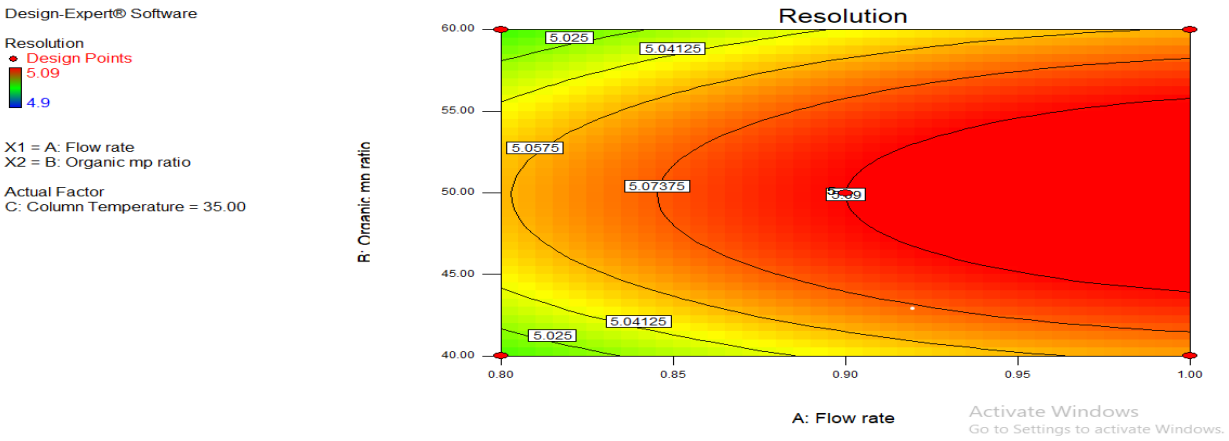

Figure 5: Resolution of Niraparib and Abiraterone

METHOD VALIDATION

A) System Suitability:

All system suitability parameters are within the range and in accordance with ICH guidelines as shown in below table

Table 3: Outcomes of System Suitability parameters

S.No	Name's	RT(min)	Area (µV sec)	Height (µV)	USP tailing	USP plate count
1	Abiraterone	2.726	3258850	201231	1.16	4315
2	Niraparib	3.568	651770	40246	1.05	2543

B) Specificity:

The selectivity was established by comparing the chromatograms obtained with the standard solution, sample solution and mobile phase blank. The chromatograms of the same are shown in Figure. 6. No interference was observed by co elution of the mobile phase blank at the same retention times of Analytes.

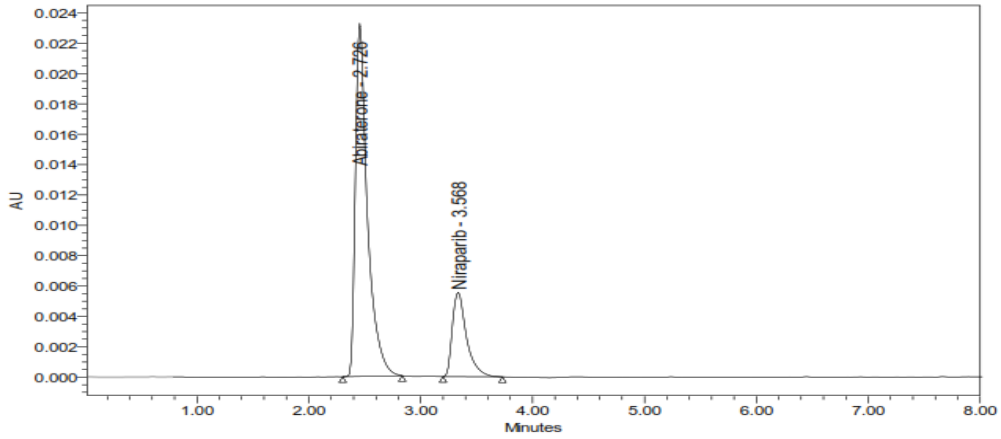

Figure 6: Chromatogram for Specificity

C) Linearity

Six concentrations of the niraparib and abiraterone acetate working standard ranging from 10 to 50 ppm were prepared and analysed. The calibration plot was constructed by plotting the chromatographic peak areas versus concentrations and values of observed concentrations were determined. The correlation coefficient obtained was 0.999 which is in the acceptance limit. The linearity range was found to lie from 10µg/ml to 50µg/ml of Abiraterone and 2µg/ml to 10µg/ml Niraparib and chromatograms are shown below.

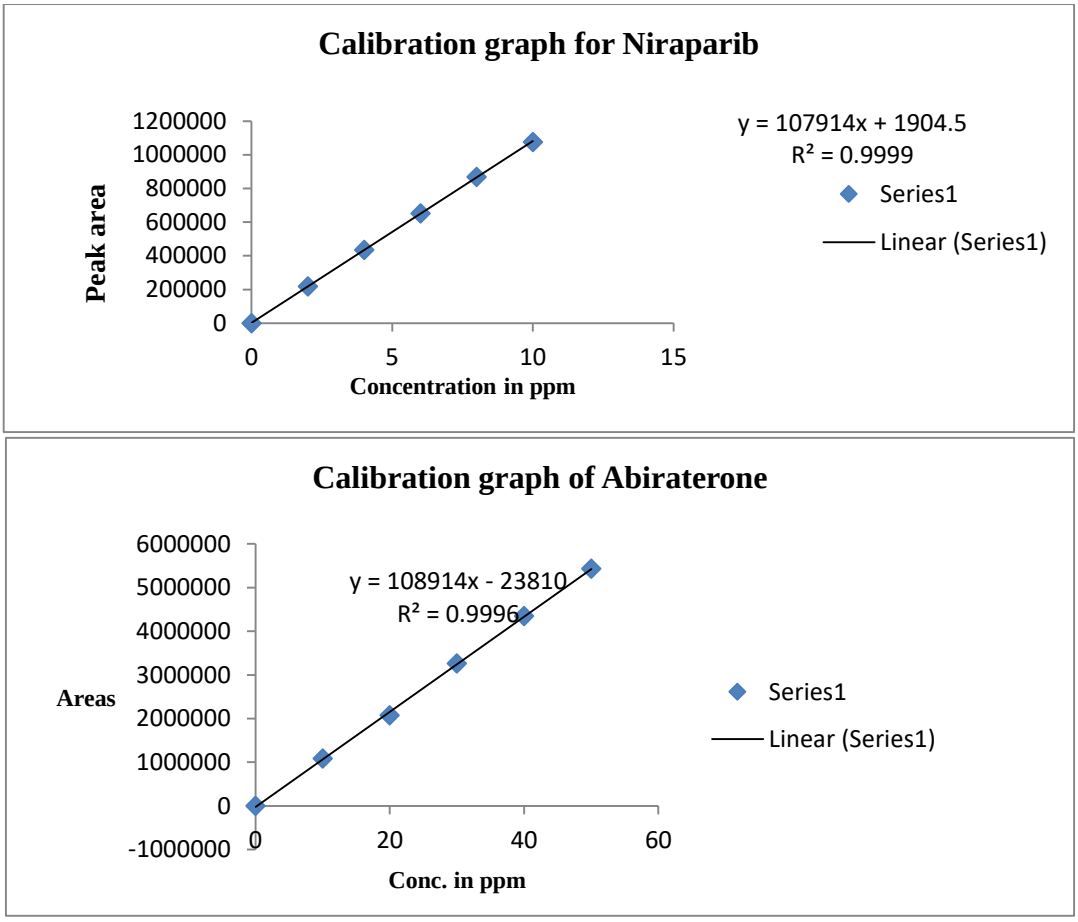

Figure 7: Calibration graphs of Niraparib and Abiraterone

D) Accuracy

A series of solutions were prepared with known standard concentrations of niraparib and abiraterone acetate of 50%, 100%, and 150% (5, 10, and 15 µg/mL) in triplicate and the % recovery was calculated.

Table 4: Accuracy (recovery) data for Abiraterone

%Concentration Abiraterone	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1629425	25	24.95	99.81	99.8
100%	3258850	50	49.90	99.81	
150%	48883005	75	74.77	99.70	

Table 5: Accuracy (recovery) data for Niraparib

%Concentration Niraparib	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	325885	5	4.95	100.09	100.03
100%	651070	10	9.84	99.98	
150%	977255	15	14.92	100.05	

E) Precision:

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

Table 6: Results of Precision for Abiraterone and Niraparib

Injection	Areas of Abirateron	Areas of Niraparib
Injection-1	3258850	651770
Injection-2	3248850	641770
Injection-3	3158850	650770
Injection-4	3248850	651770
Injection-5	3238850	651770
Injection-6	3258850	651770
Average	3235517	649936.7
Standard Deviation	38297.08	4020.779
%RSD	1.18	0.61

F) LOD and LOQ:

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio. The estimated value for LOD was found to be 0.08 µg/ml, 0.024 µg/ml for Abiraterone and Niraparib and the estimated LOQ value was found to be 0.264 µg/ml and 0.079 µg/ml.

Assay of formulations

Twenty tablets of commercial brands of niraparib and abiraterone acetate were chosen and their average weight was determined. 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.

Table 7: Assay of niraparib and abiraterone

Drugs	Label Claim (mg)	% Assay
Abiraterone and Niraparib	500mg/100mg	99.95

CONCLUSION

The AQbD approach proved effective for holistic process understanding and analytical method development. By identifying CMPs and optimizing key parameters, the developed method achieved reliable results for niraparib and abiraterone. This approach can be extended to other drug analyses, promoting quality and efficiency in pharmaceutical research.

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 niraparib abiraterone acetate in bulk and pharmaceutical formulations.

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