ORIGINAL ARTICLE

A Novel Reverse Phase High-Performance Liquid Chromatography Method Development and Validation for Determination and Estimation of Niraparib and Abiraterone Drug with its Bulk Form and Tablet Formulation

B. Sravan Kumar[1], Roopa Redamala[2]

[1]Department of Chemistry, Telangana State Model School, Nalgonda, Telangana, India, [2]Department of Chemistry, Mahatma Gandhi University, Nalgonda, Telangana, India

Abstract

Aim: Measurement which is simultaneous of Niraparib and Abiraterone in/to bulk and pharmaceutical dosage form with forced degradation investigations, a straightforward, stability-indicating, and reliable reverse liquid reverse phase high-performance liquid chromatography approach has been devised and validated. **Materials and Methods:** The Sunfire C18 Column, measuring 100Å, 5 µm, 3 mm, and 250 mm, was employed as the stationary liquid for the separation. The mobile phase consisted of CH_3OH and 0.1% trifluoroacetic acid in a 60:40 ratio, maintained at a flow velocity of 0.9 mL/min, with a maximum wavelength of 220 nm at a temp of 30°C. Niraparib and Abiraterone were shown to have average retention durations of 4.378 and 3.350 min, respectively. **Results:** Six injections of the Standard were used to study the system suitability characteristics, and the findings fell considerably below the accepted table threshold (limit of <2). A sequentiality analysis was conducted at 25–150% levels, and the R^2 value was 0.999. Numerous validation measures, including robustness, precision, accuracy, limit of quantification,and limit of detection, were determined to be within the accepted table ranges. For Niraparib and Abiraterone, the recovery percentages were 100.29% and 100.13%, respectively. **Conclusion:** This method found very simple, accurately, responsive, fast, and cheap with a runtime within 8 min. Practically this method can be applied for the determination of assay in Tablet formulation as well.

Key words: Abiraterone, CH_3OH, niraparib, reverse phase high-performance liquid chromatography, trifluoroacetic acid

INTRODUCTION

A tumor is a condition characterized by uncontrolled growth of the body's cells. A prostate tumor specifically originates in the prostate gland. Many men diagnosed with prostate tumors, especially those whose tumors have not advanced beyond the prostate, often succumb to other causes without ever showing any symptoms related to the tumor. Approximately 96% of men diagnosed with prostate tumors survive for 5 years. Tumors arise from abnormal cell growth that disrupts normal cellular functions, hindering the body's ability to function effectively. Prostate tumors develop when abnormal cells proliferate within the prostate gland. It is important to note that not all abnormal growths are malignant; some are benign and do not pose a threat to life, such as benign prostatic hyperplasia. These benign growths do not invade surrounding tissues or spread to other parts of the body. While they can be surgically removed and may regrow slowly, this is rare. In contrast, prostate tumors can metastasize to other areas of the body or adjacent organs, such as the bladder or rectum.

Address for correspondence:
Roopa Redamala, Department of Chemistry, Mahatma Gandhi University, Nalgonda, Telangana, India.
E-mail: rooparedamala28@gmail.com

Received: 03-05-2025
Revised: 20-06-2025
Accepted: 29-06-2025

Even after removal, there is a possibility of recurrence. If a prostate tumor spreads beyond the prostate, it can become life-threatening. Prostate tumors represent a serious health concern, as the prostate gland produces a fluid that is essential for maintaining sperm health during conception and pregnancy. Fortunately, most individuals with prostate tumors are diagnosed before the disease progresses beyond the prostate, allowing for effective treatment that typically involves the removal of the cancer. Niraparib and Abiraterone, in combination with prednisone, are utilized to treat a specific type of prostate tumor that has previously undergone treatment and has metastasized.[1-4]

Analyte background

Niraparib [Figure 1] is an orally administered PARP inhibitor that induces cytotoxic effects in tumor cells by obstructing the enzymes responsible for DNA repair. It specifically targets PARP-1 and PARP-2. Food and Drug Administration (FDA) Approved on March 27, 2017, Niraparib is indicated for the treatment of tumors which are primary peritoneal, tumors of fallopian, and epithelial ovarian tumors.[5-7] The Commission of Europe granted approval on November 16, 2017, followed by Health Canada on June 27, 2019. In contrast, Abiraterone functions as an effective, permanent, and carefully selected inhibitor of 17 α-hydroxylase/C17, 20-lyase (CYP17), the enzyme was found in adrenal, prostatic and testicular tumor tissues, to androgen regulation production. It received its initial approvals from the FDA and European Medicines Agency on April 9, July 16, and September 2011, respectively, and is utilized for treating metastatic castration-resistant prostate tumors and hormone-sensitive high-risk metastatic prostate tumors. Due to its low oral bioavailability and susceptibility to hydrolysis by esterases, Abiraterone [Figure 1] acetate has been developed as a more stable and absorbable orally bioavailable prodrug. Common side effects associated with both Niraparib and Abiraterone include muscle or bone discomfort, sleep disturbances, nausea, vomiting, constipation, loss of appetite, and rashes. Overdose symptoms may manifest as rapid or irregular heartbeats, difficulty in breathing, and swelling of hands, lower legs, feet and ankles.[8-10]

Niraparib and Abiraterone combination drug has been manufactured under the brand name as Akeega.

One of the efficient separation analytical techniques for estimating drug content is high-performance liquid chromatography.[11-15] The literature does not disclose reverse phase high-performance liquid chromatography (RP-H-P-L-C) methods for estimating the dosage forms of Niraparib, Abiraterone, and a few additional medicines either alone or in combination. More cost-effective techniques were found in the literature review; therefore, in accordance with International Council for Harmonisation (I-C-H) (Q^2 specification) criteria, a straightforward, economical stability-indicating simultaneous estimate of Niraparib and Abiraterone by RP-H-P-L-C in pharmaceutical dose form needs to be developed and validated.[16-20]

MATERIALS AND METHODS

Pure medications of Niraparib and Abiraterone were acquired from Spectrum Pharma research solutions. Acetonitrile and CH_3OH of H-P-L-C quality were purchased from Rankem Chemical Division in India. (Rankem, India) use of (0.45 µ) Millipore filters pure milli-Q H_2O and sodium hydrogen phosphate are utilized.

Instrumentation and chromatographic conditions

The method was developed and validated using an automated case injector and an H_2O H-P-L-C, model SYSTEM (2695) with a detector photodiode array. For the separation, a Sunfire C18 column (250 mm × 3.0 mm × 5 µm) was utilized. MP A is CH_3OH, and MP B is 0.1 percentage trifluoroacetic acid (TFA) (60:40 ratio). With an injection, vol of 10 µL and a stream velocity of 0.9 mL/min, the analysis was performed in isocratic mode. 8 min of run time was, and the column temp was 30°C. The software Empower 2 was used to collect the data at a detection wavelength of 220 nm.

Arrangement of solutions

Filler

Mixed H_2O and CH_3OH (50:50v/v) ratio.

Arrangement of buffer

Buffer (0.1% TFA): Accurately take 1 mL of TFA in a 1,000 mL of Flask measured volumetrically add about 900 mL of milli-Q H_2O added and degas to sonicate and finally make up the vol with H_2O, adjusted pH to 2.6.

Arrangement of a standard solution

Accurately Weighed and transferred 10 mg of Niraparib and 50 mg of Abiraterone working Standards into 50 mL clean dry flask measured volumetrically, add 10 mL of Filler, sonicated for 10 min, and make up to the final vol with Fillers. (200 µg/mL Niraparib and 1000 µg/mL of Abiraterone). 1 mL from the above two working solutions was taken into a 10 mL flask measured volumetrically and made up to 10 mL. (20 µg/mL Niraparib and 100 µg/mL of Abiraterone).

Arrangement of Case working solution

Accurately weighed equivalent bulk density of the combination powder (Tablet) case transfer into a 100 mL flask measured volumetrically, 50 mL of Fillers was added and

sonicated for 25 min, further the vol was made up with Filler and filtered by 0.45 µ milli-Q filters (1,000 µg/mL Niraparib and 5000 µg/mL of Abiraterone), 0.2 mL of filtered case working solution was transferred to 10 mL flask measured volumetrically and made up with Filler. (20 µg/mL Niraparib and 100 µg/mL of Abiraterone).

METHODS VALIDATION

To show that the H-P-L-C method is suggested for routine analysis, it was validated for the simultaneous quantification of Niraparib and and Abiraterone drug material in accordance with the I-C-H criteria.

System suitability

By injecting a system suitability solution containing 100 µg/mL of Niraparib and 100 µg/mL of Abiraterone, the system appropriateness was checked for each validation parameter. Figure 2 the system suitability Column, and Table 1 lists the values.

Specificity (selectivity)

Testing the optimized approach for interference. When these medications are retained using this approach, we shouldn't see interfering peaks in the blank and placebo. Thus, it was claimed that this approach was specific. Figure 3 table a representative Column, and Table 2 provides experimental data.

According to the Column above, no interference was seen in the blank or placebo solutions during the Niraparib and Abiraterone retention periods. Good resolution is achieved in the separation of all compounds.

Cases were stressed by acid, base, oxidation, heat, light, and H_2O to evaluate the stability indicating the nature of the H-P-L-C technique, Niraparib, and Abiraterone. A photodiode-array detector was used to analyze the deteriorated cases. Niraparib and Abiraterone passed the peak purity test. Table 3 lists the requirements for forced degradation, while Table 4 lists the outcomes.

The results indicated that there was no degradation observed when the samples were subjected to acid, base, hydrolysis, thermal conditions, light, and water. Furthermore, the stress study revealed that none of the degradants co-eluted with the peaks of the active drug that were formed.

The limit of detection (L-O-D) and limit of quantification (L-O-Q) are defined as follows: The detection limit refers to the minimal concentration of an analyte that can be identified, although it may not be quantifiable. Conversely, the L-O-Q represents the lowest concentration. of an analyte that can be measured with an acceptable degree of exactness and

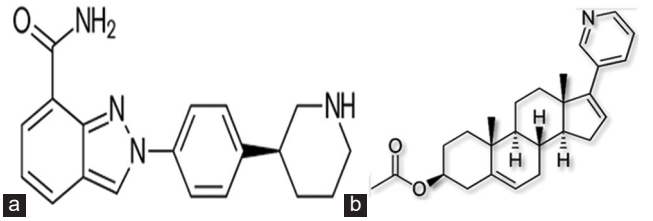

Figure 1: Structures of (a) niraparib and (b) abiraterone

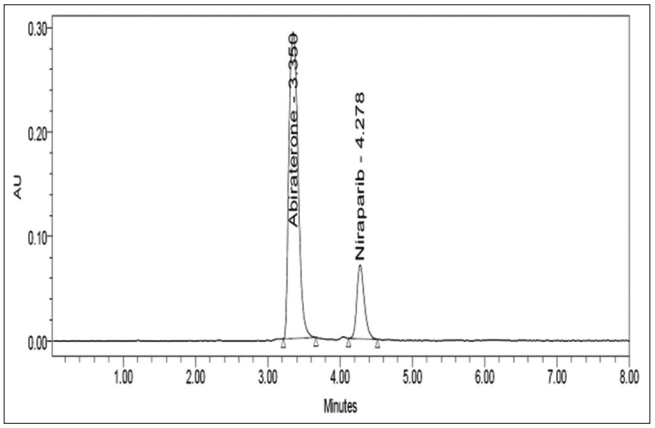

Figure 2: System suitability column of niraparib and abiraterone

Table 1: System suitability

Abiraterone			Niraparib			
(min) RT	TP	Tailing	(min) RT	TP	Tailing	RS
3.334	3485	1.16	4.199	8592	1.32	4.1
3.341	3495	1.16	4.207	8579	1.31	4.1
3.343	3493	1.14	4.218	8558	1.31	4.2
3.343	3499	1.15	4.239	8517	1.32	4.3
3.346	3453	1.15	4.254	8521	1.31	4.2
3.350	3507	1.15	4.278	8577	1.31	4.2

RT: Retention time, TP: Time to positivity

Table 2: Specificity data

Case name	Retention time(min)
Niraparib	3.350
Abiraterone	4.378

correctness according to the method used. The L-O-D values for Niraparib and Abiraterone are presented in Table 5, with the relevant representative column illustrated in Figure 4.

The L-O-Q values obtained for niraparib and abiraterone are listed in Table 5 and the corresponding representative Column is shown in Figure 4.

Sequentiality

Analyzing solutions ranging from 25% to 150% of the specified limit allowed for the demonstration of the

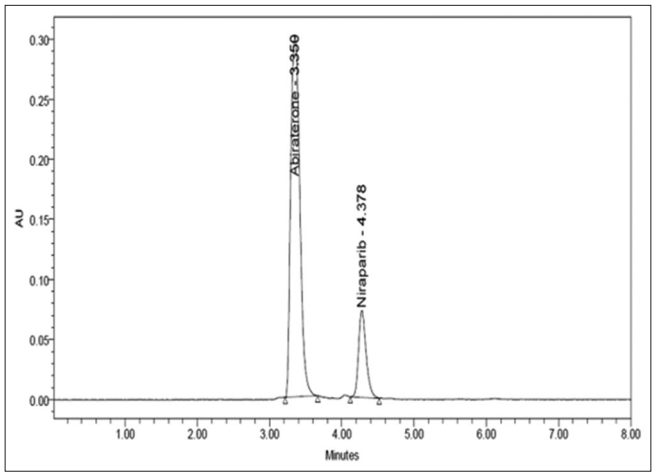

Figure 3: Typical and overlay representation of high-performance liquid chromatography column of niraparib and abiraterone

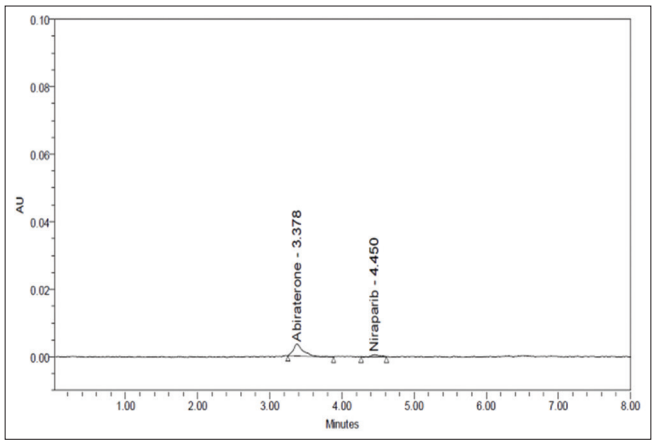

Figure 4: Typical representation of high-performance liquid chromatography column of limit of detection solution. Based on above results for the limit of detection solution, S/N ratio of each component was within the limit

method's sequentiality for Niraparib and Abiraterone [Table 6]. Niraparib and Abiraterone had a correlation value of 0.999. Good sequentiality is indicated by this [Figures 5-8].

Accuracy

Solutions comprising spiked cases of niraparib and abiraterone at 50%, 100%, and 150% of the working strength were used to assess the method's accuracy. Every solution was made 3 times and examined. Table 7 shows the percentage recovery outcomes for each contaminant.

System precision

Six replicate injections of working solution at 100% of the designated limit were analyzed to determine the system's precision in relation to the working strengths of Niraparib and Abiraterone. Table 8 provides a Synopsis of the peak area results.

The percentage relative standard deviation (RSD) for the peak areas of niraparib and abiraterone, derived from six replicate injections of the standard solution, fell within the acceptable limit.

Method precision

A frequently used statistical term is the standard deviation (SD) of a population of observations. The SD is calculated as the square root of the sum of the squared deviations of each individual result from the mean, divided by the total number of results minus one. The SD, denoted as S, is expressed as follows:

Table 3: Forced degradation conditions for niraparib and abiraterone

Stress condition	Solvent	Temp (°C)	Exposed time in min
Acid	2N HCL	60	30
Base	2N NAOH	60	30
Oxidation	20% H_2O_2	60	30
Thermal	Filler	105	360
Photolytic	Filler	-	-
Hydrolytic	H_2O	60	

Table 4: Degradation profile results

Degradation condition	Niraparib percentage undegraded	Abiraterone percentage undegraded
Acid	97.71	98.78
Base	94.89	93.59
Oxidation	98.82	98.48
Thermal	98.97	98.73
Photolytic	99.16	99.87
Hydrolytic	99.78	99.79

Table 5: Synopsis of limit of detection

Case (µg/mL)	Conc Concentration	Peak area	S/N ratio
Niraparib	0.06	4192	5.7
Abiraterone	2.06	34786	4.9

Table 6: Synopsis of the limit of quantification

Case (µg/mL)	Conc	Peak area	S/N ratio
Niraparib	0.19	9567	13.1
Abiraterone	6.24	87640	9.1

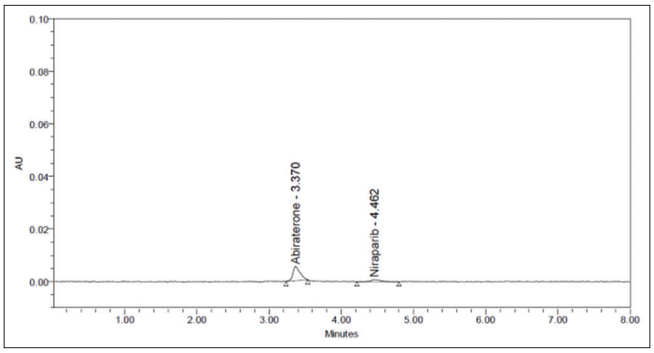

Figure 5: Typical representation of high-performance liquid chromatography column of limit of quantification solution. Based on above results for the limit of detection, S/N ratio of each component was within the limit

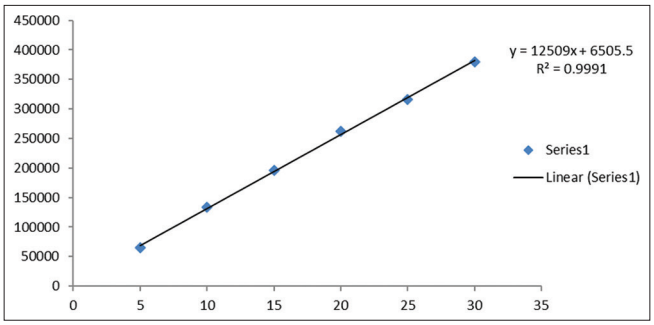

Figure 6: Sequentiality plot of niraparib

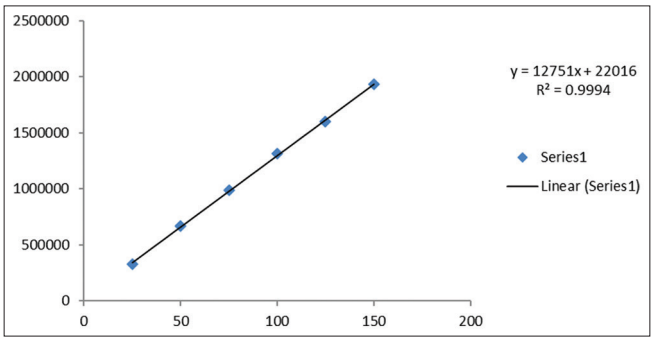

Figure 7: Sequentiality plot of abiraterone

$$S = \sqrt{\frac{\sum_{i=1}^{n}(x - x')^2}{(n-1)}}$$

The SD shares the same units as the measured property. The square of the SD is referred to as variance (S^2). The c is defined as the SD expressed as a fraction of the mean, represented as S/x. Occasionally, it is multiplied by 100 and presented as a percentage of the RSD, which provides a more dependable indication of precision.

$$\text{Percentage RSD} = \frac{SD}{Mean} \times 100$$

A case of niraparib and abiraterone was analyzed to establish the method's precision. (Six separate arrangements of cases). Tables 9,10 provides a Synopsis of the collected data.

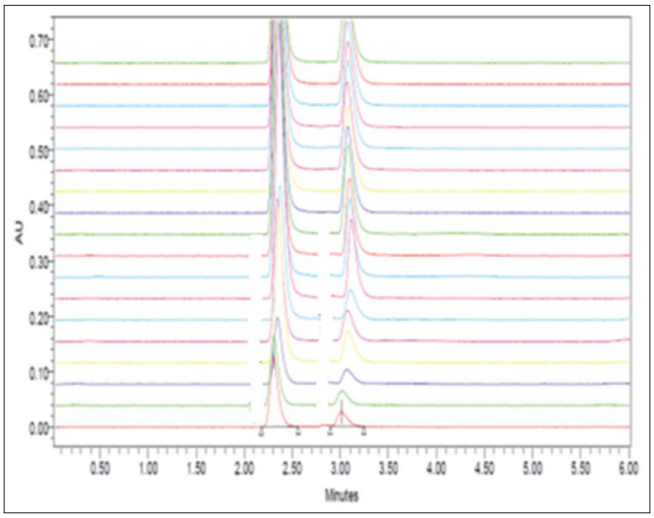

Figure 8: Overlay of sequentiality abiraterone and niraparib

Table 7: Sequentiality data

Percentage level	Niraparib		Abiraterone	
	Conc (µg/mL)	Area	Conc (µg/mL)	Area
25	5	65,047	25	325,900
50	10	133,170	50	665,146
75	15	196,170	75	989,700
100	20	261,912	100	1,315,650
125	25	316,440	125	1,597,239
150	30	379,757	150	1,932,951

Table 8: Percentage recovery data

Percentage level	Percentage recovery	
	Niraparib	Abiraterone
Level 50	99.65	99.83
	100.69	99.80
	100.68	100.34
Level 100	100.63	100.88
	100.72	100.54
	100.36	99.17
Level 150	99.85	100.51
	99.88	99.84
	100.15	100.24
Mean percentage	100.29	100.13

From the above results, the Percentage RSD of the method precision study was within the limit for Niraparib and Abiraterone.

Robustness

To assess the robustness of the present approach, the chromatographic conditions were purposefully altered.

Table 9: System precision data

Injection	Niraparib	Abiraterone
1	259,772	1,362,617
2	260,544	1,343,711
3	260,265	1,339,613
4	264,141	1,333,490
5	267,634	1,333,055
6	266,414	1,341,551
Average	263,128	1,338,284
Standard deviation	3413.6	10822.5
Percentage RSD	1.3	0.8

RSD: Relative standard deviation

Table 10: Method precision data

Injection	Niraparib	Abiraterone
1	261,300	1,347,853
2	262,547	1,337,198
3	265,742	1,353,771
4	261,001	1,345,411
5	262,954	1,330,908
6	262,256	1,347,673
Average	262,633	1,343,802
Standard deviation	1694.6	8285.3
Percentage RSD	0.6	0.6

RSD: Relative standard deviation

Table 11: Robustness results

Chromatographic condition	Niraparib (RSD)	Abiraterone (RSD)
Flow (−)	1.2	1.3
Flow (+)	1.5	0.7
Temp (−)	0.9	1.2
Temp (+)	1.1	0.6
MP (−)	1.3	1.0
MP (+)	1.2	0.8

RSD: Relative standard deviation

System suitability solution is prepared according to the methodology and injected into H-P-L-C at various altered conditions to check the method's ability, such as flow velocity (10%), column oven temp (5°C), and MP (10%) from actual method conditions. This is done to determine the robustness of the method. Changing the flow, temp, MP, and system appropriateness all showed no discernible changes in accordance with the methodology. Table 11 provides a Synopsis of the robustness results.

Intermediate precision is differently from repeatability; the precision obtained within a single laboratory over a longer period of time (generally at least several months) and takes into account more changes than repeatability. In particular: different analysts, calibrants, batches of reagents, columns, spray needles, etc. These factors are constant within a day (i.e. behave systematically within a day timescale) but are not constant over a longer time period and thus behave as random in the context of intermediate precision [Table12]. Because more effects are accounted for by the intermediate precision, its value, expressed as SD (see the next section), is larger than the repeatability SD.

Table 12: Intermediate precision data

Injection	Niraparib	Abiraterone
1	260,179	1,367,301
2	266,587	1,345,728
3	268,562	1,372,425
4	265,616	1,349,532
5	267,653	1,382,972
6	267,317	1,359,080
Avg	265,986	1,362,840
Std dev	3013.5	14150.1
Percentage RSD	1.1	1.0

RSD: Relative standard deviation

CONCLUSION

Based on the aforementioned experimental findings, it was determined that the recently created technique for the simultaneous estimate of Niraparib and Abiraterone was straightforward, accurately, and exact. It also had a shorter retention period, high resolution, and separated degradants. The present suggested methodology is cost-effective and suitable for typical pharmaceutical industry evaluations.

ACKNOWLEDGMENT

We would like to express my sincere gratitude to Spectrum Pharma Research Solutions for the opportunity to conduct part of the experiment.

REFERENCES

1. Young AH, Sean TD. Niraparib: A review in ovarian cancer. Target Oncol 2018;13:533-9.
2. Ison G, Howie LJ, Amiri-Kordestani L, Zhang L, Tang S, Sridhara R, *et al*. FDA approval summary: Niraparib for the maintenance treatment of patients with recurrent ovarian cancer in response to platinum-based chemotherapy. Clin Tumor Res 2018;24:4066-71.
3. Ryan CJ, Cheng ML. Abiraterone acetate for the treatment of prostate cancer. Expert Opin Pharmacother

2013;14:91-6.
4. James A, Vincent B, Sivadas A, Pavithran K. A study on the clinical outcome of abiraterone acetate in castration resistant prostate cancer patients. Int J Hematol Oncol Stem Cell Res 2018;12:4-7.
5. Stappaerts J, Geboers S, Snoeys J, Brouwers J, Tack J, Annaert P, *et al*. Rapid conversion of the ester prodrug Abiraterone acetate results in intestinal supersaturation and enhanced absorption of Abiraterone: *In vitro*, rat *in situ* and human *in vivo* studies. Eur J Pharm Biopharm 2015;90:1-7.
6. Roberts HN, Maurice-Dror C, Chi KN. Combination niraparib and abiraterone for HRR-altered metastatic castration-resistant prostate cancer. Future Oncol 2025;21:201-11.
7. Kim NC, Dana R, Matthew RS, Eleni E, Gerhardt A, David O, *et al*. Niraparib and abiraterone acetate for metastatic castration-resistant prostate cancer. J Clin Oncol 2023;41:3339-51.
8. Maria DS, Sara MB, Paul R, Camille C, Katie P, Suzy VS, *et al*. Feasibility of indirect treatment comparisons between niraparib plus abiraterone acetate and other first-line poly ADP-ribose polymerase inhibitor treatment regimens for patients with BRCA1/2 mutation-positive metastatic castration-resistant prostate cancer. Adv Ther 024;41:3039-58.
9. Shanthi Priya DK, Annapurna MM. Stability indicating RP-UPLC method for the simultaneous estimation of Niraparib and Abiraterone in pharmaceutical dosage forms. Res J Pharm Technol 2024;17:5885-9.
10. Chi KN, Sandhu S, Smith MR, Attard G, Saad M, Olmos D, *et al*. Niraparib plus abiraterone acetate with prednisone in patients with metastatic castration-resistant prostate cancer and homologous recombination repair gene alterations: Second interim analysis of the randomized phase III magnitude trial. Ann Oncol 2023;35:772-82.
11. Ryan CJ, Smith MR, Fizazi K, Saad F, Mulders PF, Sternberg CN, *et al*. Abiraterone acetate plus prednisone versus placebo plus prednisone in chemotherapy-naive men with metastatic castration-resistant prostate cancer (COU-AA-302): Final overall survival analysis of a randomised, double-blind, placebo-controlled phase 3 study. Lancet Oncol 2015;16:152-60.
12. Thakur A, Roy A, Ghosh A, Chhabra M, Banerjee S. Abiraterone acetate in the treatment of prostate cancer. Biomed Pharmacother 2018;101:211-8.
13. Martín AG, Pothuri B, Vergote I, DePont Christensen R, Graybill W, Mirza MR, *et al*. Niraparib in patients with newly diagnosed advanced ovarian cancer. N Engl J Med 2019;381:2391-402.
14. Moore N, Secord AA, Geller MA, Miller DS, Cloven N, Fleming GF, *et al*. Niraparib monotherapy for late-line treatment of ovarian cancer (QUADRA): A multicentre, open-label, single-arm, phase 2 trial. Lancet Oncol 2019;20:636-48.
15. Williams SM, Kuznicki AM, Andrade P, Dolinski BM, Elbi C, O'Hagan RC, *et al*. Treatment with the PARP inhibitor, niraparib, sensitizes colorectal cancer cell lines to irinotecan regardless of MSI/MSS status. Cancer Cell Int 2015;15:14.
16. Wang Z, Sun K, Xiao Y, Feng B, Mikule K, Ma XY, *et al*. Niraparib activates interferon signaling and potentiates anti-PD-1 antibody efficacy in tumor models. Sci Rep 2019;9:1853.
17. Sun K, Mikule K, Wang Z, Poon G, Vaidyanathan A, Smith G, *et al*. A comparative pharmacokinetic study of PARP inhibitors demonstrates favorable properties for niraparib efficacy in preclinical tumor models. Oncotarget 2018;9:37080-96.
18. Luo Y, Xia Y, Liu D, Li X, Li H, Liu J, *et al*. Neoadjuvant PARPi or chemotherapy in ovarian cancer informs targeting effector Treg cells for homologous-recombination-deficient tumors. Cell 2024;187:4905-25.
19. Wang L, Evans JC, Ahmed L, Allen C. Folate receptor targeted nanoparticles containing niraparib and doxorubicin as a potential candidate for the treatment of high grade serous ovarian cancer. Sci Rep 2023;13:3226.
20. Hussain M, Kocherginsky M, Agarwal N, Adra N, Zhang J, Paller CJ, *et al*. Abiraterone, olaparib, or abiraterone + olaparib in first-line metastatic castration-resistant prostate cancer with DNA repair defects (BRCAAway). Clin Cancer Res 2024;30:4318-28.

Source of Support: Nil. **Conflicts of Interest:** None declared.