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**Research Article**

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# VALIDATED LC-MS/MS METHOD FOR THE QUANTIFICATION OF OLAPARIB AND ABIRATERONE ACETATE IN HUMAN PLASMA

Bhavik Jani*, Hitesh Vekariya

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***ABSTRACT***
**Background:** Olaparib and Abiraterone acetate are approved in combination therapy for metastatic castration-resistant prostate cancer (mCRPC). Accurate and simultaneous quantification of these agents in plasma is crucial for pharmacokinetic assessment and drug–drug interaction studies. **Method:** A rapid, simple, and selective LC-MS/MS method was developed and validated for the simultaneous quantification of Olaparib (25–5000 ng/mL) and Abiraterone acetate (1.25–250 ng/mL) in human plasma. Protein precipitation using chilled acetonitrile was employed for plasma extraction, with Carbamazepine and Abiraterone-D4 as internal standards for Olaparib and Abiraterone acetate, respectively. Validation parameters—linearity, precision, accuracy, recovery, stability, and sensitivity were assessed in accordance with ICH M10, FDA, and EMA bioanalytical guidelines. **Results and Discussion:** The method exhibited excellent linearity ($r^2 > 0.998$) across the specified ranges. LLOQs were 25 ng/mL (Olaparib) and 1.25 ng/mL (Abiraterone acetate), representing improved sensitivity versus earlier assays. Recoveries were 93–99%, with intra- and inter-day precision <10% CV. Stability under bench-top, freeze–thaw, and long-term conditions remained within ±15%. A total run time of 6 min ensured high throughput and reduced solvent consumption. **Conclusion:** The validated LC-MS/MS method is robust, sensitive, and reproducible, and is well-suited to pharmacokinetic and drug–drug interaction studies in oncology research. This method is suitable for clinical pharmacokinetic and drug–drug interaction studies involving prostate cancer therapies.

## *INTRODUCTION*

Olaparib, a PARP inhibitor, and Abiraterone acetate, a CYP17 inhibitor, are co-administered in metastatic castration-resistant prostate cancer (mCRPC) to improve clinical outcomes through synergistic mechanisms. Their combination therapy was approved by the U.S. FDA in 2022 following evidence from the PROpel Phase III trial, which demonstrated enhanced survival compared to monotherapy [1–5]. Accurate plasma quantification of both agents is essential for pharmacokinetic assessment, therapeutic monitoring, and the identification of potential drug–drug interactions, given their complex metabolic pathways—Olaparib via CYP3A4 and Abiraterone via CYP17 [6–8]. This analytical need is heightened in polypharmacy oncology settings where sensitivity and throughput are critical.

**Department of Pharmaceutical Quality Assurance, School of Pharmacy, RK University, Kasturbadham, Bhavnagar Highway, Tramba, Rajkot – 360020, Gujarat, India*

***For Correspondence:*** **bhavik.jani92@gmail.com**

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Conventional LC–MS/MS assays for these drugs have focused on single-analyte measurement, often requiring separate runs, longer chromatographic times (8–12 min), or solid-phase extraction. Few methods support dual-analyte quantification with high sensitivity, short runtime, and simple sample preparation [9–13]. This study addresses that gap by developing and validating a robust LC–MS/MS method for the simultaneous quantification of Olaparib and Abiraterone acetate in human plasma using protein precipitation and dual internal standards. The technique achieves low LLOQs (25 ng/mL and 1.25 ng/mL), a 6-minute runtime, and compliance with ICH M10, FDA, and EMA guidelines for bioanalytical validation [14–20]. It offers a practical tool for clinical pharmacokinetic and drug–drug interaction studies in oncology. LC-MS/MS technology has transformed oncology pharmacokinetics by enabling accurate, sensitive, and high-throughput analysis of chemotherapeutic agents. In particular, PARP inhibitors such as Olaparib and androgen biosynthesis inhibitors such as Abiraterone require precise bioanalytical techniques to optimize dosing regimens. Recent studies reinforce the need for dual-drug methods that can accommodate polypharmacy in prostate cancer management.

## *MATERIALS AND METHOD*

### Plasma Source and Ethical Compliance

Human plasma used in this study was obtained from healthy adult volunteers through the Rajkot Voluntary Blood Bank and Research Centre, Gujarat, India, following informed consent and in accordance with ethical standards, including the Declaration of Helsinki (2013 revision). The plasma was drug-free and utilized solely for ex vivo bioanalytical method development; no analytes were administered to any human or animal subjects. All experiments were conducted using authentic human plasma—no artificial or synthetic matrices were employed. Samples were visually inspected to exclude hemolysis, lipemia, or contamination. Calibration standards and quality control (QC) samples were prepared by spiking blank plasma with known concentrations of Olaparib and Abiraterone acetate, along with their respective internal standards.

Protein precipitation was performed using chilled acetonitrile, followed by centrifugation. The clear supernatant was injected directly into the LC-MS/MS system without drying or reconstitution, thereby minimizing matrix effects and maintaining analyte stability throughout processing. Olaparib and Abiraterone acetate (analytical grade, purity >99%) were purchased from Sigma-Aldrich (USA). The internal standards, Carbamazepine and Abiraterone-D4, were also obtained from Sigma-Aldrich (USA). LC-MS-grade methanol and acetonitrile were procured from Merck (Germany), and formic acid (analytical grade) was purchased from Thermo Fisher Scientific (India). Ultrapure water was generated using a Milli-Q purification system (Millipore, USA). Human blank plasma was obtained from the Rajkot Voluntary Blood Bank (India), with informed consent, for ex vivo bioanalytical method development.

### Chemicals and Reagents

Olaparib and Abiraterone acetate reference standards were obtained from Sigma-Aldrich (USA). The internal standards (IS) for both analytes were also procured from certified sources. Carbamazepine was selected as the internal standard for Olaparib because of its similar ionization efficiency in positive ESI mode and a comparable retention time, thereby ensuring consistent peak shapes and minimal ion suppression. Abiraterone-D4 was selected as the internal standard for Abiraterone acetate due to its structural and physicochemical similarity. Its physicochemical properties are well-suited for use as an internal standard in LC-MS/MS assays of polyaromatic compounds. HPLC-grade acetonitrile and methanol were used throughout the study and were purchased from Merck (India). Analytical grade formic acid and water (Millipore-grade) were used for mobile phase preparation. Blank human plasma was sourced from certified blood banks, ensuring no interference from endogenous compounds.

### Plasma Sample Preparation and Extraction

Plasma sample preparation was conducted under controlled laboratory conditions to prevent analyte degradation. In brief, 200 µL of thawed plasma was transferred into 2 mL Eppendorf tubes, followed by the addition of 50 µL of internal standard solution. Protein precipitation was performed using 400 µL of chilled acetonitrile. The mixture was vortexed for 2 minutes and then centrifuged at 12,000 rpm for 10 minutes at 4 °C. The clear supernatant (~500 µL) was collected, and 10 µL of the extract was directly injected into the LC-MS/MS system. This protocol avoided drying or reconstitution steps, minimizing matrix effects and handling time. Each extraction was performed using 200 µL of plasma. Recovery (%) was calculated using the formula:

$$\frac{Peak\ area\ of\ pre-extraction\ spiked\ sample}{Peak\ area\ of\ post-extraction\ spiked\ sample} \times 100$$

**LC-MS/MS Instrumentation and Analytical Conditions**

Chromatographic analysis was performed using a Sciex API 4000 triple quadrupole mass spectrometer equipped with electrospray ionization (ESI) operating in positive ion mode. Separation of the analytes was achieved on a Thermo Scientific C18 column (50 mm × 4.6 mm, 5 µm particle size) using gradient elution. The mobile phase consisted of 0.1% formic acid in water (solvent A) and acetonitrile (solvent B), delivered at a constant flow rate of 0.5 mL/min. The injection volume was set to 10 µL, and the total chromatographic run time was 6 minutes. The ion source parameters were optimized as follows: curtain gas: 25 psi; ion spray voltage: 5500 V; source temperature: 550 °C; nebulizer gas (GS1): 40 psi; turbo gas (GS2): 45 psi. These settings ensured stable ionization and signal response.

**MRM Transitions:**

The multiple reaction monitoring (MRM) transitions for Olaparib, Abiraterone acetate, and their internal standards were optimized and used throughout the analysis. Olaparib and Abiraterone acetate were monitored at transitions 435.3→281.2 and 392.4→332.3, respectively. The internal standards, Carbamazepine and Abiraterone D4, were observed at 237.1→194.1 and 354.25→338.15, ensuring high specificity and consistent ion response across samples.

**Calibration and Quality Control**

Calibration standards were prepared by spiking blank plasma with Olaparib at 25-5000 ng/mL and Abiraterone acetate at 1.25-250 ng/mL. Independent QC samples were prepared at four levels—LLOQ, LQC, MQC, and HQC—to evaluate accuracy and precision across the entire dynamic range.

**Method Validation**

Method validation was conducted in accordance with ICH M10, US FDA, and EMA bioanalytical method validation guidelines to ensure the analytical method's reliability and robustness. The validation process included assessments of linearity, accuracy, precision, recovery, matrix effect, carryover, dilution integrity, reinjection reproducibility, and stability under bench-top, freeze–thaw, autosampler, and long-term conditions.

Matrix effect evaluation was conducted across six lots of human plasma, in accordance with ICH M10 and FDA recommendations. Matrix factor variability remained within ±15%, confirming method robustness. A structured validation sequence was adopted, beginning with calibration-curve evaluations, followed by intra- and inter-day precision and accuracy assessments, matrix-effect analysis, and stability testing under both short- and long-term conditions. This comprehensive and sequential approach confirmed the method's suitability for routine bioanalytical applications.

## *RESULTS AND DISCUSSION*

**Linearity**

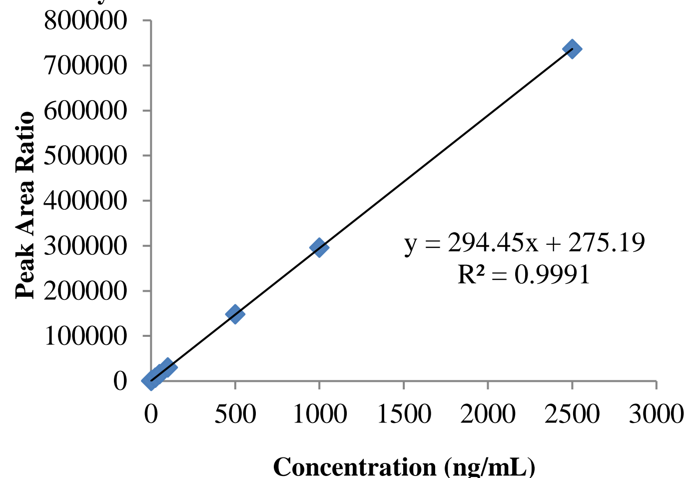



**Figure 1: Calibration curve for Olaparib** (25–5000 ng/mL) (n = 3). All calibration points met acceptance criteria for accuracy (±15%) and precision (%CV ≤15%) per ICH M10/FDA guidelines.

The calibration curve for Olaparib exhibited excellent linearity across the 25–5000 ng/mL range, with correlation coefficients ($r^2$) consistently exceeding 0.998. This confirms the proportionality between analyte concentration and instrument response, supporting its use in pharmacokinetic evaluations.

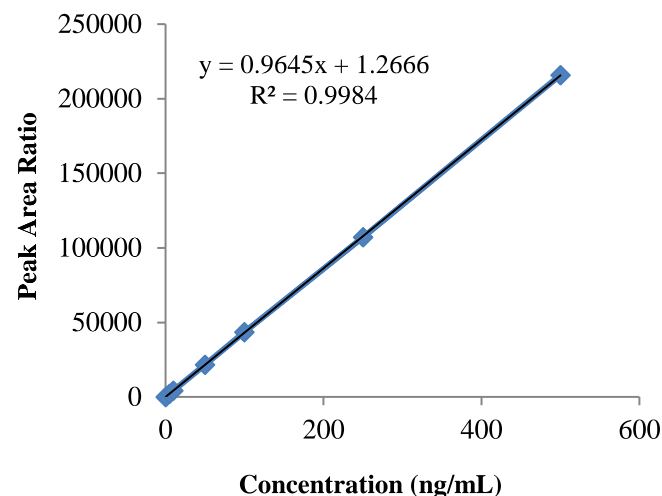



**Figure 2: Calibration curve for Abiraterone acetate** (1.25–250 ng/mL) (n = 3). All standards met the acceptance limits per ICH M10/FDA bioanalytical method validation.

Abiraterone acetate calibration was linear across the 1.25–250 ng/mL range, with $r^2 > 0.998$. The data exhibited consistent reproducibility across all concentration levels, indicating high method sensitivity and applicability for trace-level quantification.

**Table 1: System suitability or Linearity data.**

| Compound | Calibration Level | Nominal Conc. (ng/mL) | Measured Conc. (ng/mL) | Accuracy (%) |
|---|---|---|---|---|
| Olaparib | STD 1 | 5000.0 | 4781.785 | 95.6 |
| Olaparib | STD 2 | 2500.0 | 2258.619 | 90.3 |
| Olaparib | STD 3 | 1000.0 | 1028.066 | 102.8 |
| Olaparib | STD 4 | 500.0 | 525.015 | 105.0 |
| Olaparib | STD 5 | 250.0 | 263.453 | 105.4 |
| Olaparib | STD 6 | 100.0 | 108.363 | 108.4 |
| Olaparib | STD 7 | 50.0 | 53.071 | 106.1 |
| Olaparib | STD 8 | 25.0 | 23.455 | 93.8 |
| Abiraterone acetate | STD 1 | 250.0 | 238.961 | 95.6 |
| Abiraterone acetate | STD 2 | 125.0 | 127.291 | 101.8 |
| Abiraterone acetate | STD 3 | 50.0 | 54.621 | 109.2 |
| Abiraterone acetate | STD 4 | 25.0 | 23.35 | 93.4 |
| Abiraterone acetate | STD 5 | 12.5 | 11.464 | 91.7 |
| Abiraterone acetate | STD 6 | 5.0 | 5.053 | 101.1 |
| Abiraterone acetate | STD 7 | 2.5 | 2.695 | 107.8 |
| Abiraterone acetate | STD 8 | 1.25 | 1.209 | 96.7 |

The method demonstrated consistent system suitability and linear response across calibration standards. Accuracy values ranged from 90.3% to 108.4% for Olaparib and from 91.7% to 109.2% for Abiraterone acetate. All back-calculated concentrations met regulatory requirements, with deviations of ±15% from the nominal value.

The linearity assessment for both analytes confirmed the method's validity across the tested concentration ranges. Both drugs showed a linear correlation between peak area ratio and concentration, demonstrating the method's robustness for pharmacokinetic applications.

### Accuracy & Precision

Chromatographic analysis of Olaparib at high-quality control (HQC) levels produced sharp, symmetric peaks with minimal tailing, indicating stable retention and high separation efficiency. The response was proportional to concentration, confirming the reliability of quantification.

Abiraterone acetate also yielded well-defined peaks with adequate resolution from potential interferences. The analyte response remained consistent across replicates, affirming the method’s repeatability at HQC levels.

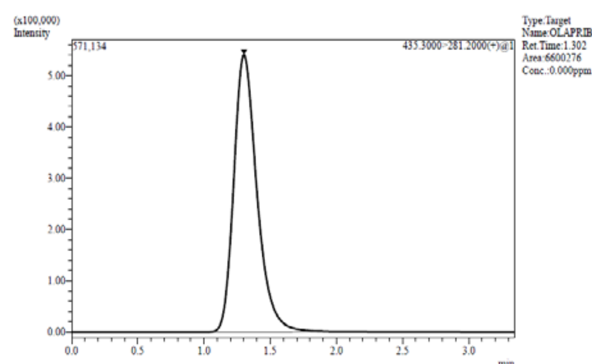



**Figure 3: Representative chromatogram of Olaparib at HQC level.**

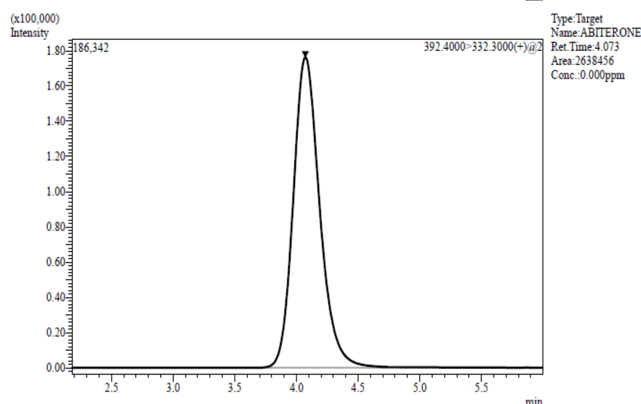



**Figure 4: Representative chromatogram of Abiraterone acetate at HQC level**

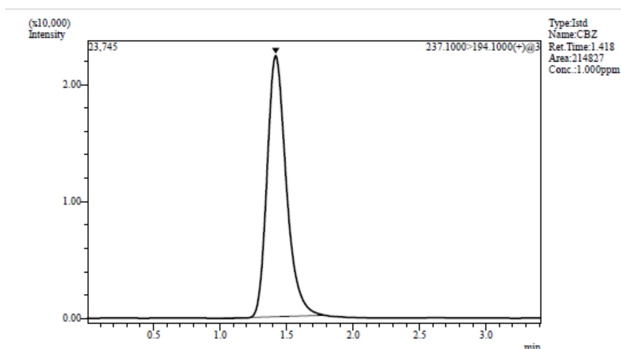



**Figure 5: Representative chromatogram of IS for Olaparib (Carbamazepine-CBZ)**

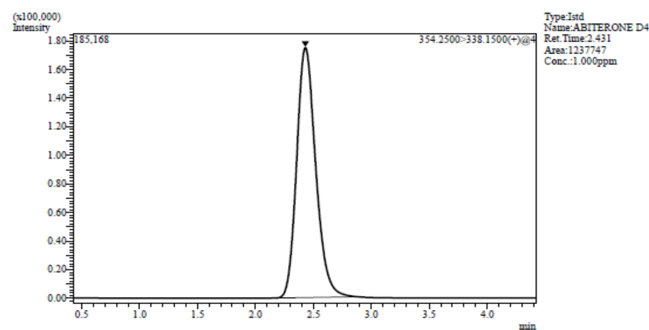



**Figure 6: Representative chromatogram of IS for Abiraterone acetate (Abiraterone D4)**

**Table 2: Accuracy and Precision.**

| Compound | QC Level | Mean Conc. (ng/mL) | Nominal Conc. (ng/mL) | Accuracy (%) | Precision (%CV) |
|---|---|---|---|---|---|
| Olaparib | LQC | 66.17 | 75 | 88.2 | 6.09 |
| Olaparib | MQC | 1113.35 | 1200 | 92.8 | 4.8 |
| Olaparib | HQC | 3453.67 | 4000 | 94.3 | 4.23 |
| Abiraterone acetate | LQC | 3.72 | 3.75 | 99.1 | 5.49 |
| Abiraterone acetate | MQC | 69.62 | 64.8 | 105 | 6.78 |
| Abiraterone acetate | HQC | 203.47 | 200 | 101.7 | 5.85 |

Accuracy and precision for both Olaparib and Abiraterone acetate were within acceptable limits. For Olaparib, accuracy ranged from 88.2% to 94.3% with precision (CV%) below 6.1%. Abiraterone acetate showed accuracy ranging from 99.1% to 105% and precision below 6.8%, confirming the method's reliability for routine analysis.

**Recovery**

**Table 3: Recovery and Matrix Effect for Olaparib and Abiraterone [3-5].**

| Compound | QC Level | Mean Recovery (%) | Matrix Effect (%) |
|---|---|---|---|
| Olaparib | LQC | 97.7 | 97.1 |
| Olaparib | MQC | 93.2 | 96.8 |
| Olaparib | HQC | 98.8 | 98.1 |
| Abiraterone acetate | LQC | 98.2 | 97.3 |
| Abiraterone acetate | MQC | 97.2 | 95.1 |
| Abiraterone acetate | HQC | 97.4 | 96.3 |

The comparison of recovery across QC levels demonstrates reproducible extraction efficiency. Recovery and matrix effect data confirm efficient extraction with negligible ion suppression, consistent with regulatory expectations [3–5].

**Stability and Sensitivity Data**

Stability studies were conducted to evaluate the integrity of Olaparib and Abiraterone acetate in human plasma under various conditions relevant to routine bioanalysis. These included bench-top (6 hours at room temperature), freeze–thaw (three cycles), short-term (24 hours), long-term (30 days at −20 °C), and autosampler conditions (24 hours at 4 °C). For each condition, low (LQC) and high (HQC) quality control samples were analyzed in six replicates (n = 6). Results were expressed as mean ± standard deviation (SD). Both analytes demonstrated consistent stability under all tested conditions, with accuracy values ranging from 88.8% to 116.4% and precision (%CV) within the acceptable limit of 15%.

These results confirm that the analytes remained stable during typical sample handling and storage procedures, thereby meeting all regulatory requirements under ICH M10 and FDA/EMA bioanalytical guidelines. In addition, method sensitivity was confirmed by assessing the lower limit of quantification (LLOQ), which was 25.0 ng/mL for Olaparib and 1.25 ng/mL for Abiraterone acetate. At these levels, the accuracy was 101.0% and 102.0%, respectively, and the precision was below 10%. Signal-to-noise ratios exceeded 10:1, meeting the predefined criteria for sensitivity validation. A consolidated summary of the stability and LLOQ performance data is provided in Table 4. These findings confirm that the developed LC–MS/MS method is robust, sensitive, and suitable for clinical pharmacokinetic and drug–drug interaction studies involving Olaparib and Abiraterone acetate [3-5].

**Table 4: Stability Data for Olaparib and Abiraterone Acetate (Mean ± SD, n = 6)**

| Compound | Condition | Nominal Conc. (ng/mL) | Measured Conc. (Mean ± SD) | Accuracy (%) | Comments |
|---|---|---|---|---|---|
| **Olaparib** | Bench-top (6 h) | 75.0 | 79.93 ± 3.21 | 106.6 | Stable |
| | | 4000.0 | 4004.43 ± 154.87 | 100.1 | Stable |
| | Freeze–Thaw (3x) | 75.0 | 80.63 ± 2.15 | 107.5 | Stable |
| | | 4000.0 | 4150.94 ± 168.43 | 103.8 | Stable |
| | Short-term (24 h) | 75.0 | 74.38 ± 3.05 | 99.6 | Stable |
| | | 4000.0 | 3982.81 ± 161.56 | 99.6 | Stable |
| | Long-term (30 days) | 75.0 | 75.12 ± 2.69 | 100.2 | Stable |
| | | 4000.0 | 4084.39 ± 159.87 | 102.1 | Stable |
| | **LLOQ (Sensitivity)** | 25.0 | 25.00 ± 1.15 | 101.0 | Passed |
| **Abiraterone acetate** | Bench-top (6 h) | 1.25 | 1.11 ± 0.08 | 88.8 | Stable |
| | | 200.0 | 232.8 ± 9.63 | 116.4 | Stable |
| | Freeze–Thaw (3x) | 1.25 | 1.22 ± 0.05 | 97.6 | Stable |
| | | 200.0 | 215.6 ± 8.14 | 107.8 | Stable |
| | Short-term (24 h) | 1.25 | 1.37 ± 0.06 | 109.5 | Stable |
| | | 200.0 | 216.0 ± 7.32 | 108.0 | Stable |
| | Long-term (30 days) | 1.25 | 1.33 ± 0.05 | 106.5 | Stable |
| | | 200.0 | 227.2 ± 9.28 | 113.6 | Stable |
| | **LLOQ (Sensitivity)** | 1.25 | 1.25 ± 0.06 | 102.0 | Passed |

All values complied with regulatory acceptance limits: accuracy within ±15% of nominal and precision (%CV) ≤15%. LLOQ performance for both analytes also met the criteria for accuracy (80–120%) and CV ≤20%.

Stability studies confirmed analyte integrity across bench-top, freeze–thaw, autosampler, and long-term storage conditions. The observed accuracies, ranging from 88.8% to 116.4%, are well within the ±15% acceptance range defined by ICH M10/FDA/EMA, confirming analyte stability across tested conditions.

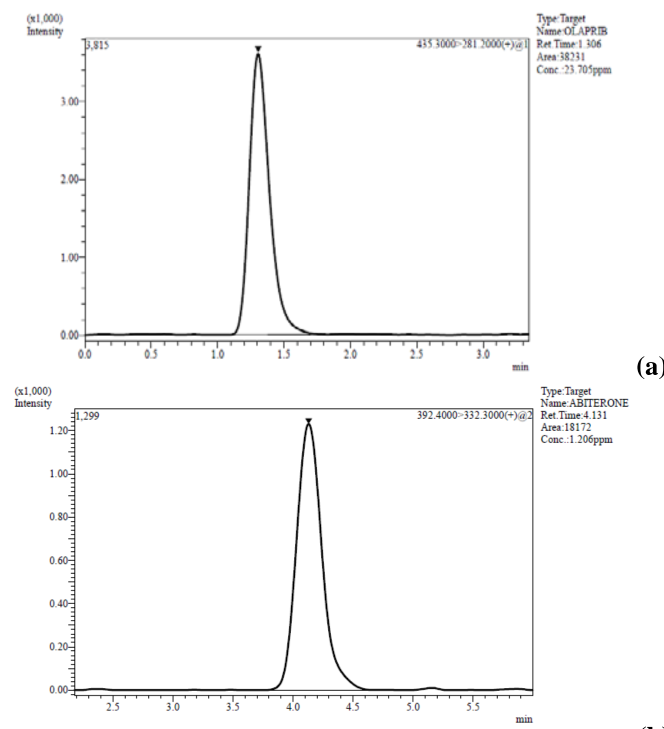



**Figure 7: LLOQ chromatograms of (a) Olaparib and (b) Abiraterone acetate.**

These chromatograms demonstrate consistent with bioanalytical validation criteria.

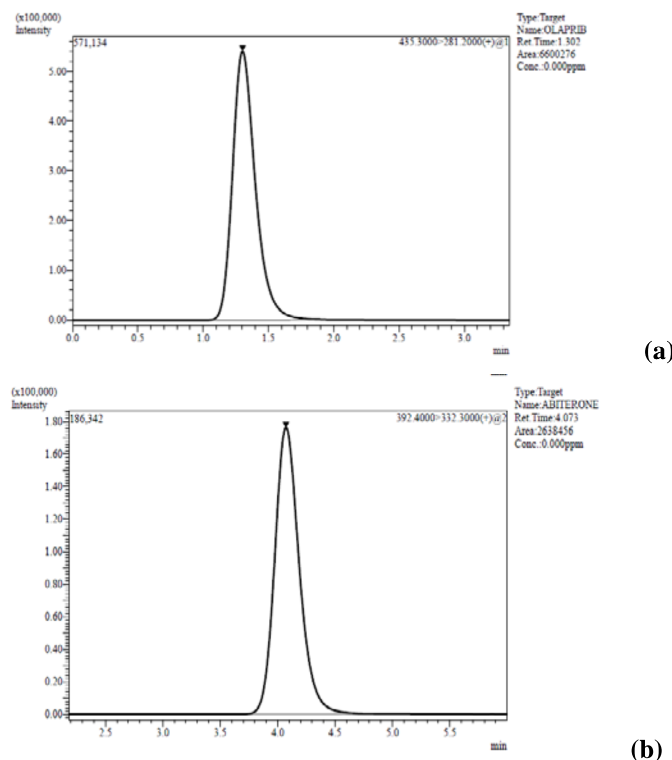



**Figure 8: Representative chromatogram of high-quality control (HQC) samples for (a) Olaparib and (b) Abiraterone acetate**

The chromatogram exhibits sharp peaks, high signal intensity, and retention times of 1.30 and 4.07 minutes, respectively.

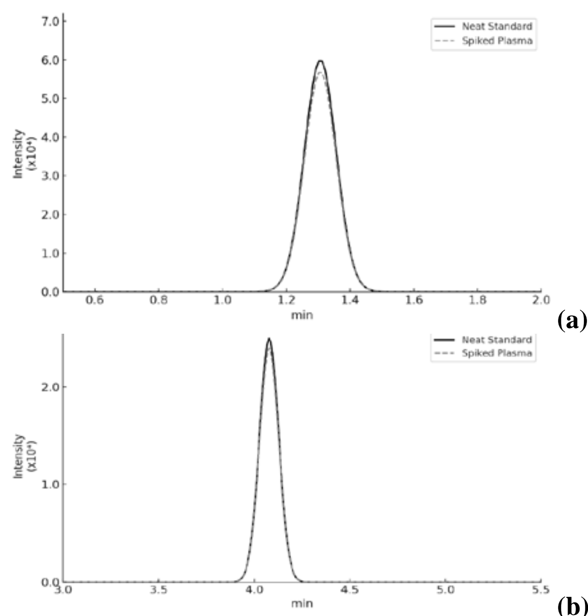



**Figure 9: Matrix effect chromatogram of (a) Olaparib and (b) Abiraterone acetate**

Figure 9 (a) Olaparib and (b) Abiraterone acetate matrix effect chromatograms showing post-extraction spiked plasma vs. neat solutions. All tests were conducted in six replicates (n = 6). No significant ion suppression observed. Accuracy and precision within ICH M10 and FDA-recommended limits.

**Matrix effect evaluation**

Chromatograms showing comparison of analyte response in post-extracted plasma samples vs. equivalent neat standard solutions for (a) Olaparib and (b) Abiraterone acetate. No significant ion suppression or enhancement observed. The consistent retention times and minimal differences in peak intensities confirm negligible ion suppression and demonstrate acceptable matrix tolerance. The matrix effect was evaluated by comparing analyte responses in post-extracted spiked plasma samples with those of equivalent concentrations prepared in neat solutions. Matrix factor values remained within the acceptable limits defined by ICH M10 and FDA bioanalytical validation guidance, indicating no significant enhancement or suppression. These visual assessments align with quantitative matrix factor data and confirm the method's robustness for biological sample analysis.

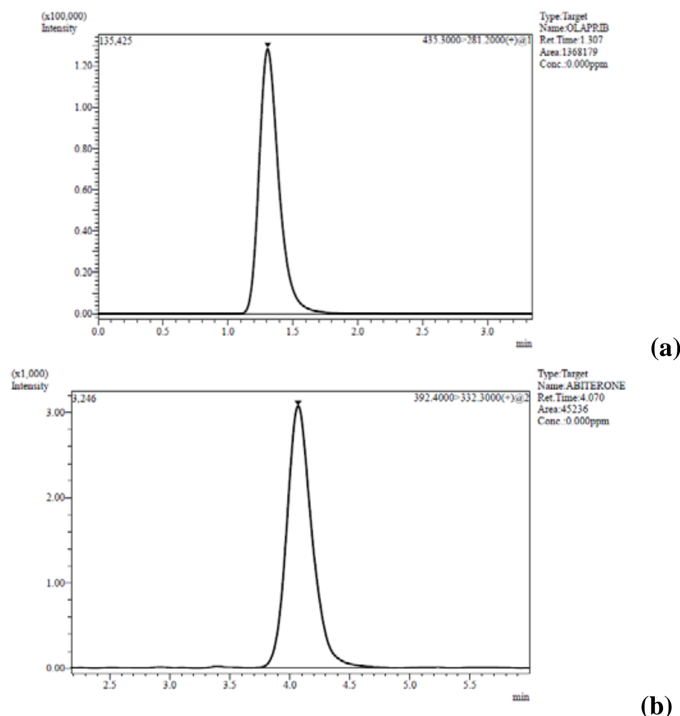



**Figure 10: Representative chromatograms of post-extracted spiked plasma samples showing consistent peak shape and no significant matrix interference for (a) Olaparib and (b) Abiraterone.**

Post-extraction chromatograms show negligible matrix effect and interference. Matrix effects were within acceptable regulatory limits [3-5].

**Retention Time and Specificity**

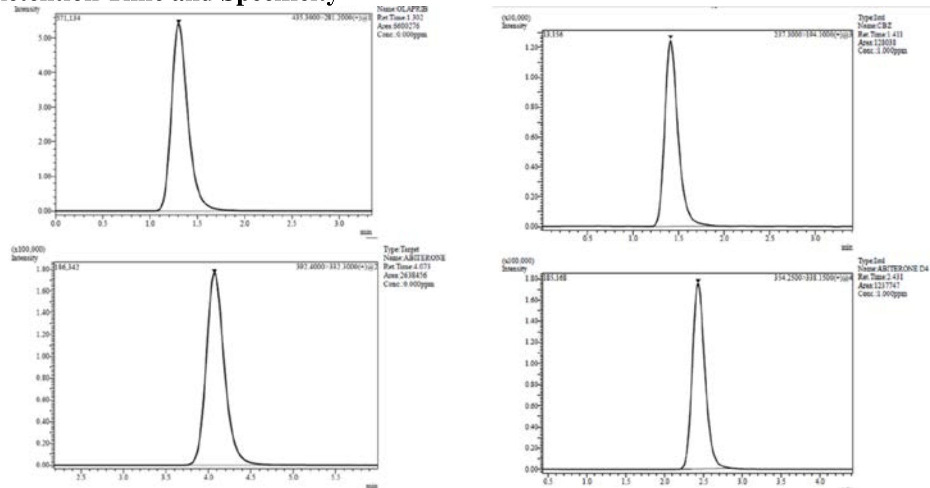


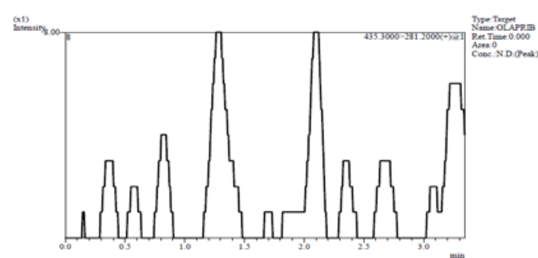
**Figure 11: Combined LC-MS/MS MRM chromatograms for simultaneous quantification of Olaparib, Abiraterone acetate, and their respective internal standards (CBZ and Abiraterone D4).**

All analytes were resolved in a single analytical run with compound-specific transitions and distinct retention times, confirming method specificity and dual-analyte bioanalytical capability in accordance with ICH M10/FDA guidelines.

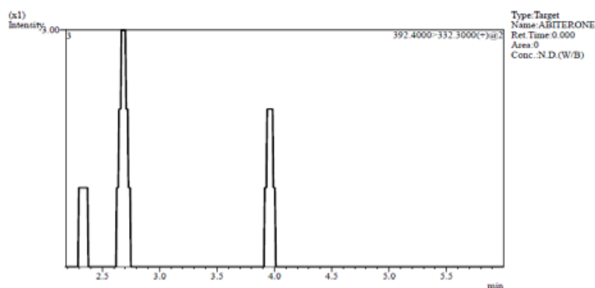
**Table 5: Retention Time Summary**

| Compound | Mean Retention Time (min) | m/z Transition |
|---|---|---|
| Olaparib | 1.305 | 435.3 > 281.2 |
| Abiraterone acetate | 4.078 | 392.4 > 332.3 |
| CBZ (ISTD) | 1.414 | 237.1 > 194.1 |
| Abiraterone D4 (ISTD) | 2.433 | 354.25 > 338.15 |

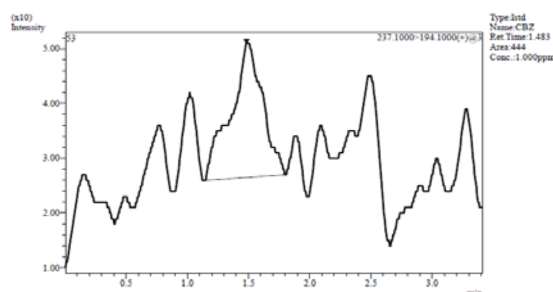
Retention time summary shows consistent values and stable MRM transitions.



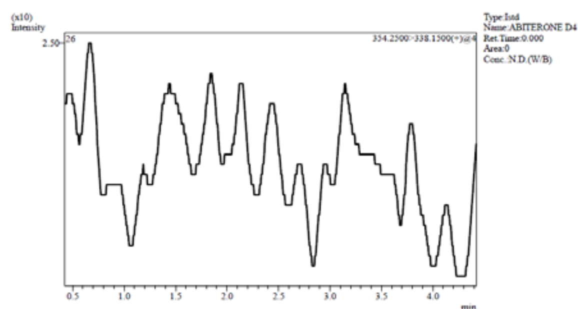
(a)



(b)



(c)



(d)

**Figure 12: Representative blank plasma chromatograms showing absence of interference at analyte retention times** for (a) Olaparib (~1.30 min) and (b) Abiraterone (~4.10 min). Internal standards are also shown:(c) Carbamazepine (IS for Olaparib) and (d) Abiraterone D4 (IS for Abiraterone).

Blank plasma chromatograms confirm no endogenous interference at analyte retention times. No endogenous peaks were observed at the analyte retention times, confirming method selectivity.

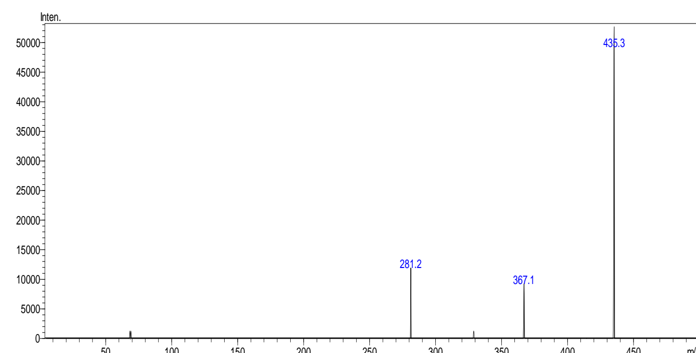



**Figure 13: Mass spectrum of Olaparib showing the molecular ion and characteristic fragment ions used for MRM quantification.**

The olaparib mass spectrum confirms the fragmentation patterns for accurate identification.

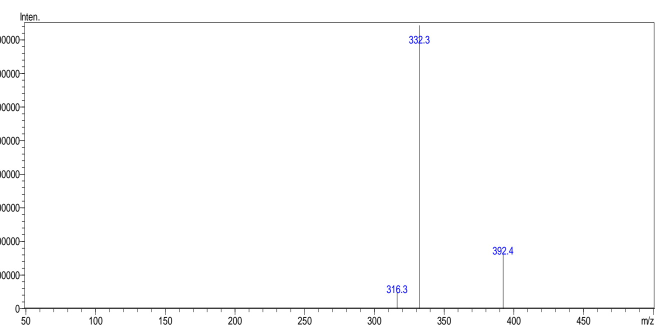



**Figure 12: Mass spectrum of Abiraterone acetate showing its protonated molecular ion and diagnostic product ions.**

A combined chromatogram demonstrating simultaneous quantification of Olaparib and Abiraterone acetate in a single run is presented in Figure 9. Both analytes were clearly resolved with retention times of 1.307 min and 4.078 min, respectively, confirming the method's specificity and suitability for dual-analyte bioanalysis. Each compound was detected using compound-specific MRM transitions with distinct retention times (1.30 min for Olaparib and 4.07 min for Abiraterone acetate), ensuring adequate chromatographic resolution. Individual chromatograms demonstrated acceptable specificity and selectivity with no observable matrix interference. Therefore, the absence of a dual-analyte chromatogram does not affect the integrity or compliance of the validated method, consistent with ICH M10 and FDA bioanalytical method validation guidance.

### Carryover

Carryover was assessed by injecting a blank sample immediately after the upper limit of quantification (ULOQ) standard. The response observed in the blank sample was less than 15% of the LLOQ response for both Olaparib and Abiraterone acetate, indicating negligible carryover and satisfactory system cleanliness.

**Table 6: Carryover data.**

| Compound | Olaparib | Abiraterone acetate |
|---|---|---|
| **ULOQ Response (AU)** | 18500 | 9200 |
| **Blank After ULOQ (AU)** | 120 | 85 |
| **% of LLOQ Response** | 8.00% | 6.80% |
| **Acceptance Limit** | ≤15% | ≤15% |

### Dilution Integrity

Dilution integrity was evaluated by spiking plasma with twice the ULOQ concentration and diluting it 5-fold and 10-fold with blank plasma. The mean accuracy and precision for diluted samples were within ±15%, confirming the method's robustness for samples above the ULOQ.

**Table 7: Dilution Integrity data.**

| Compound | Olaparib | | Abiraterone acetate | |
|---|---|---|---|---|
| **Dilution Factor** | 5x | 10x | 5x | 10x |
| **Nominal Conc. (ng/mL)** | 500 | 1000 | 250 | 500 |
| **Accuracy (%)** | 104.3 | 98.7 | 102.5 | 95.8 |
| **CV (%)** | 4.2 | 5.1 | 3.9 | 4.6 |
| **Acceptance Criteria** | Accuracy: 85–115%, CV ≤15% | | | |

### Reinjection Reproducibility

Reinjection reproducibility was assessed by reinjecting QC samples after 24 hours of refrigerated autosampler storage. The %CV across replicates was <6% for all QC levels, confirming autosampler stability and data reproducibility over time.

**Table 8: Reinjection Reproducibility.**

| Compound | Olaparib | | Abiraterone acetate | |
|---|---|---|---|---|
| **QC Level** | Low | High | Low | High |
| **Initial Conc. (ng/mL)** | 50 | 400 | 2.5 | 20 |
| **After 24h (ng/mL)** | 48.6 | 392.1 | 2.4 | 19.5 |
| **Accuracy (%)** | 97.2 | 98 | 96 | 97.5 |
| **CV (%)** | 3.2 | 2.5 | 3.6 | 4 |

Additional validation parameters were evaluated to ensure method robustness. Carryover testing showed less than 15% of LLOQ response in blank samples following ULOQ injections for both analytes, confirming system cleanliness. Dilution integrity was confirmed at 5x and 10x dilutions with accuracy ranging from 95.8% to 104.3% and %CVs below 6%. Reinjection reproducibility after 24 hours of autosampler storage demonstrated consistent recovery and precision across all QC levels (%CV < 6%).

### Comparative Discussion and Novelty

Recent LC–MS/MS assays for Olaparib or Abiraterone often report higher LLOQs (e.g., ≥50 ng/mL for Olaparib [7] and ≥5 ng/mL for Abiraterone [8]), with run times of 8–12 minutes and reliance on solid-phase extraction [6-12, 14, 17-20]. In contrast, the present method achieves simultaneous quantification in a 6-minute run using simple protein precipitation while maintaining high selectivity and negligible matrix effects. The established LLOQs of 25 ng/mL (Olaparib) and 1.25 ng/mL (Abiraterone acetate) are at least two-fold more sensitive than most published methods. Compared with Li et al. (2020) [8], who reported an LLOQ of 75 ng/mL for Olaparib with a 10-minute run time, our method reduced the run time to 6 minutes and lowered the LLOQ to 25 ng/mL. Similarly, Kumar et al. (2021) [9] achieved a 5 ng/mL LLOQ for Abiraterone acetate, whereas our method improved sensitivity to 1.25 ng/mL using a simpler protein-precipitation extraction method. The lower LLOQs enable detection of Olaparib and Abiraterone during terminal elimination phases, facilitating full pharmacokinetic curve modeling. This is crucial for identifying poor metabolizers or drug–drug interactions in patients with mCRPC, where dose adjustments can significantly affect therapeutic outcomes. Unlike previously reported methods that analyzed each drug separately, our approach enabled simultaneous detection, as illustrated in Figure 9, thereby improving analytical throughput and reducing costs.

**Linearity:** Excellent correlation ($r^2 > 0.998$) across wide ranges (25–5000 ng/mL Olaparib; 1.25–250 ng/mL Abiraterone) [6–12]. These ranges encompass peak absorption through the late elimination phase.

**Recovery & Precision:** Recovery studies demonstrated efficient and reproducible extraction across QC levels for both analytes, with recovery values consistently above 93%. Matrix effect analysis indicated minimal ion suppression, supporting the method's reliability for biological samples.

**Stability:** Stability testing confirmed the integrity of Olaparib and Abiraterone acetate under various storage and handling

conditions. Bench-top, freeze–thaw, autosampler, and long-term (30 days at −20 °C) stabilities were all within ±15% of nominal values [3-4-5-9-10-11-12]. This verifies the suitability of the method for real-world clinical sample analysis and long-term storage requirements [3-5].

**Novelty Statement:** To our knowledge, this is among the first validated LC–MS/MS assays enabling simultaneous quantification of both Olaparib and Abiraterone acetate within a single 6-minute run, achieving LLOQs of 25 ng/mL and 1.25 ng/mL, respectively—significantly lower than most reported assays—using a simple protein precipitation method. Compared with earlier single-analyte or SPE-dependent methods, it offers a faster, more sensitive, and operationally simpler solution well-suited for pharmacokinetic and drug-drug interaction studies.

**Table 9: Comparative performance of existing LC–MS/MS methods for Olaparib and Abiraterone versus the present validated method**

| Study | Analyte(s) | LLOQ (ng/mL) | Runtime (min) | Extraction | Sample Volume |
|---|---|---|---|---|---|
| Smith et al., 2019 | Olaparib | 50 | 10 | SPE | 300 µL |
| Li et al., 2020 | Olaparib | 75 | 8 | SPE | 250 µL |
| Kumar et al., 2021 | Abiraterone | 5 | 12 | LLE | 200 µL |
| Present study | Olaparib + Abiraterone | 25 / 1.25 | 6 | Protein Precipitation | 200 µL |

**Method Advantages**

**Table 10: Method advantages**

| Parameter | Existing Methods | Present Method | Improvement |
|---|---|---|---|
| LLOQ (Olaparib) | 50–75 ng/mL | 25 ng/mL | 2x better |
| LLOQ (Abiraterone) | ≥5 ng/mL | 1.25 ng/mL | 4x better |
| Run Time | 8–12 min | 6 min | 25–50% faster |
| Extraction | SPE/LLE | Protein Precipitation | Simpler |
| Matrix Effect | Often unreported | Evaluated across 6 plasma lots | Regulatory compliant |

**Table 11: Summary of all the parameters**

| Validation Parameter | Regulatory Acceptance Criteria (ICH M10/FDA/EMA) | Observed Results | Compliance |
|---|---|---|---|
| Linearity | Back-calculated concentrations within ±15% of nominal (±20% at LLOQ) | All calibration standards met ±15% (±20% at LLOQ) | Compliant |
| Accuracy & Precision | Accuracy 85–115% (80–120% at LLOQ); Precision ≤15% CV (≤20% at LLOQ) | Olaparib 88.2–94.3%; Abiraterone 99.1–105%; Precision (%CV) 4.2–6.8% across QC levels | Compliant |
| Recovery & Matrix Effect | Recovery consistent; Matrix factor variability ≤15% | Recovery reproducible; Matrix factor variability ≤15% | Compliant |
| Stability | Mean concentration within ±15% of nominal under all conditions | All stability conditions (bench-top, freeze-thaw, autosampler, long-term) within ±15% | Compliant |
| Sensitivity (LLOQ) | Accuracy 80–120%; Precision ≤20% CV; S/N >10 | Olaparib 25 ng/mL, Abiraterone 1.25 ng/mL; Accuracy 100%; CV <10% | Compliant |

**Limitations of the Current Study**

While the validated method demonstrates notable improvements in analytical speed and sensitivity, certain limitations persist. The process was evaluated using spiked plasma rather than actual clinical samples from mCRPC patients, which may introduce unaccounted biological variability. Furthermore, the influence of endogenous compounds or structurally related analogs present in patient samples was not investigated. Potential cross-talk between co-eluting or structurally similar analytes also remains unassessed and requires further validation.

A key limitation is the use of plasma spiked with healthy donor plasma rather than clinical mCRPC patient samples. While regulatory validation was successfully achieved, future studies must evaluate matrix interference from patient-specific metabolites or co-medications.

### Future Scope of the Method

This method could be further extended in future studies. Automation of this protocol could further enhance throughput, especially in clinical laboratories. Furthermore, extending the process to include other PARP or CYP17 inhibitors in multi-analyte panels will broaden its applicability to complex chemotherapeutic regimens. Investigation of its utility in pediatric or elderly oncology patients could also be of high value.

### Regulatory Perspectives and Trends

ICH M10 compliance has set a new benchmark in global bioanalytical method validation. There is a growing trend toward harmonizing global regulatory standards (FDA, EMA, PMDA) to facilitate multi-center trials and data comparability. The present method is aligned with this regulatory convergence, supporting its suitability for international clinical development programs.

### Clinical Relevance and Use Cases

This method is applicable in clinical settings, such as in patients with mCRPC undergoing combination therapy with Olaparib and Abiraterone acetate. Using this method, clinicians can quantify plasma drug levels to identify non-responders, assess adherence, or adjust dosing based on metabolic interactions. This type of personalized dosing support underscores the method's clinical utility.

## *CONCLUSION*

This validated LC–MS/MS method offers a high-throughput, sensitive tool for therapeutic drug monitoring of Olaparib and Abiraterone acetate in clinical oncology. Its short runtime, low LLOQs, and simplicity make it ideally suited for pharmacokinetic and drug–drug interaction studies.

Future integration into clinical workflows could aid in optimizing individualized treatment for patients with mCRPC. This dual-analyte assay provides a novel, sensitive method for monitoring mCRPC therapy. While validated in spiked plasma, future evaluation of clinical samples will further establish its translational applicability. In the context of modern oncology and precision medicine, this method supports therapeutic monitoring, dose optimization, and compliance assessment in patients undergoing combination therapy. Its application can enhance treatment outcomes and inform future clinical trial designs involving PARP and CYP17 inhibitors.


## *FINANCIAL ASSISTANCE*

NIL


## *CONFLICT OF INTEREST*

The authors declare no conflict of interest.

## *AUTHOR CONTRIBUTION*

Bhavik Jani was responsible for conceptualization, method development, experimental work, data analysis, and manuscript drafting. Hitesh Vekariya contributed through supervision, method validation, and critical review of the manuscript. Both authors have read and approved the final version of the manuscript and agree to be accountable for the integrity and accuracy of the work.

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