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Determination of PARPi in Human Plasma and its Application in Therapeutic Drug Monitoring in Indian Cancer Patients

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Abstract

A rapid, sensitive, and reliable LC-MS/MS method was developed for the quantification of Olaparib in human plasma using Rucaparib as the internal standard. Chromatographic separation was achieved on a C18 column with positive electrospray ionization (ESI+) and multiple reaction monitoring (MRM), providing a total run time of 7 minutes. The method demonstrated good selectivity and linearity over a calibration range of 1.25 - 1000 ng/mL, enabling accurate determination of Olaparib in plasma. The optimized method offers simplified sample preparation, high sensitivity, and suitability for pharmacokinetic studies, therapeutic drug monitoring, bioequivalence studies, and clinical research. Its rapid analysis and robust performance make it a valuable bioanalytical tool for supporting precision oncology and the clinical use of Olaparib.

Keywords: PARP Inhibitor, Human Plasma, Therapeutic Drug Monitoring, Cancer Patients

1. Introduction

Cancer continues to be a major global health challenge, prompting the development of targeted therapies with improved efficacy and safety[1,2]. Olaparib, the first approved poly (ADP-ribose) polymerase (PARP) inhibitor, is widely used for the treatment of BRCA1/BRCA2-mutated ovarian, breast, pancreatic, and prostate cancers[3,4]. By inhibiting PARP-mediated DNA repair, Olaparib induces synthetic lethality in cancer cells with homologous recombination deficiencies, making it an effective targeted anticancer agent[5,6].

Accurate quantification of Olaparib in human plasma is essential for pharmacokinetic studies, therapeutic drug monitoring, bioavailability and bioequivalence studies, and clinical research[7,8]. However, the complexity of plasma and the low drug concentrations encountered during pharmacokinetic studies require highly sensitive and selective analytical techniques[9].

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is the preferred bioanalytical method due to its high sensitivity, specificity, and rapid analysis[10,11]. Coupled with appropriate sample preparation and multiple reaction monitoring (MRM), LC-MS/MS enables reliable quantification of Olaparib with minimal matrix interference[10,12].

A robust bioanalytical method must comply with ICH M10, USFDA and EMA validation guidelines, including assessment of selectivity, linearity, accuracy, precision, recovery, matrix effects, and stability[13-15]. Therefore, the present study aims to develop and validate a simple, rapid, and sensitive LC-MS/MS method for the determination of Olaparib in human plasma, supporting pharmacokinetic, therapeutic monitoring, and bioequivalence studies.

2. Objective

a) To develop and optimize a rapid, sensitive, and selective LC-MS/MS method for the quantification of Olaparib in human plasma using Rucaparib as an internal standard.
b) To establish a reliable calibration curve and optimize chromatographic and mass spectrometric conditions for accurate determination of Olaparib in human plasma.
c) To evaluate the suitability of the developed method for pharmacokinetic studies, therapeutic drug monitoring, bioavailability, and bioequivalence studies.

d) To develop a robust bioanalytical method that complies with USFDA and EMA bioanalytical method validation guidelines.

3. Materials and Method

3.1 Materials:

The study utilized the following materials and laboratory equipment: a Mettler Toledo analytical balance; a centrifuge operated at 13,500 rpm for 10 minutes at 6°C; Tarson automatic micropipettes of 200 µL (Code No. SUR/CEN/LAB/SE/200) and 1000 µL (Code No. SUR/CEN/LAB/SE/321); disposable pipette tips (P100, P200, and P1000); a solvent filtration apparatus fitted with 0.45 µm cellulose nitrate membrane filters (47 mm diameter); an ultrasonic sonicator (Code No. SUR/CEN/LAB/SE/310); A 100 mL measuring cylinder; a 1000 mL conical flask; a 500 mL Duran bottle; a C18 chromatographic column (50 x 4.6 mm, 5 µm particle size; Agilent Technologies) equipped with a guard column; Parafilm; 5 mL disposable syringe injections; syringe filters; a vortex mixer (Code No. SUR/CEN/LAB/LCMS/SE/117); refrigerators maintained at -20°C and -80°C; 15 mL Abdos centrifuge tubes and 50 mL Falcon tubes; and 1.5 mL Tarsons microcentrifuge tubes.

3.2 Chemicals

The chemicals and reference standards used in this study included Olaparib reference standard (Batch No. OPOZ230004; Working standard No. DS/PS/23/022) and Rucaparib reference standard (Batch No. RCCOZ240001; Working Standard No. BDS/PS/24/008). LC-MS grade Acetonitrile [Batch No. KOLGO-TR-RC-(MeCN)-060924-01], LC-MS grade Water (Batch No. KOLGO-TR-RC-(H_2O)-060924-01), and LC-MS grade Formic Acid (Batch No. ZJ836436) were used throughout the study for the preparation of mobile phases, standard solutions, and sample analysis.

(A)

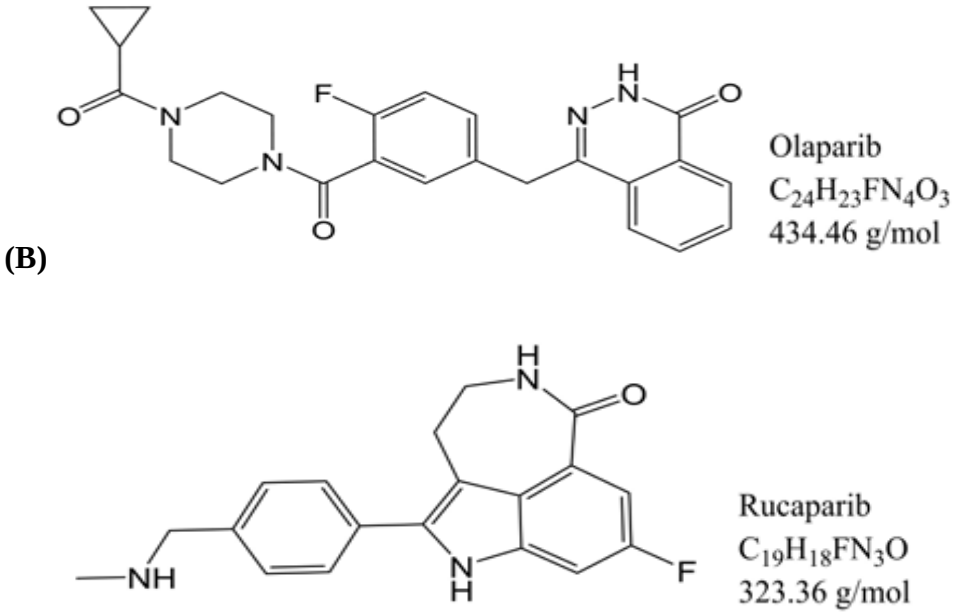

Figure 1. Chemical structures, molecular formula, and molecular weight of **(A)** Olaparib and **(B)** Rucaparib.

3.3 Plasma Source and Ethical Consideration

Human plasma used for this study was collected from cancer patients through the Dum Dum Medical Centre, Kolkata, India, after obtaining written informed consent. Plasma collection was carried out in compliance with applicable ethical guidelines and the principles outlined in the Declaration of Helsinki (2013 revision). The collected plasma was confirmed to be free from drugs and was used exclusively for ex-vivo bioanalytical method development. No investigational analyte is administered to any human subjects during the study. Authentic human plasma was employed throughout the experimental work, and no artificial or surrogate matrices were used. Prior to use, all plasma samples were visually examined and samples exhibiting signs of hemolysis, lipemia, or contamination were excluded. Calibration standards and quality control (QC) samples were prepared by spiking blank human plasma with predetermined concentrations of Olaparib with its corresponding internal standard.

3.4 Plasma Sample Preparation and Extraction

Plasma sample were processed under controlled laboratory conditions to maintain analyte stability throughout the extraction procedure. Briefly, 200 µL of thawed plasma was transferred into a 2 mL Eppendorf tube, followed by the addition of 50 µL of the internal standard solution. Protein precipitation was carried out by adding 400 µL of ice-cold acetonitrile. The mixture was vortex-mixed for 2 min and centrifuged at 12,000 rpm for 10 min at 4°C. After centrifugation, approximately 500 µL of the clear supernatant was carefully collected, and a 10 µL aliquot was directly injected into the LC-MS/MS system for analysis. The sample preparation procedure eliminated the need for evaporation and reconstitution steps, thereby reducing sample handling time and minimizing potential matrix effects. Each plasma sample was extracted using a fixed volume of 200 µL. Extraction recovery (%) was determined by comparing the peak area of analytes extracted from spiked plasma samples with that of post-extraction spiked sample, according to the following equation:

Recovery (%) = (Peak area of pre-extraction spiked sample)/ (Peak area of post-extraction spiked sample) x 100

3.5 LC-MS/MS Instrumentation and Analytical Conditions

Chromatographic analysis was carried out using Shimadzu LCMS-8045 coupled with an electrospray ionization (ESI) source operated in the positive ionization mode. Separation of Olaparib and Rucaparib (IS) was archived on an Agilent Technologies C18 column (50 mm X 4.6 mm, 5 µ Particle size) with guard column employing a gradient elution programme. The mobile phase comprised acetonitrile : water :: 50:50 (v/v) as solvent A and acetonitrile : water containing 0.1% formic acid :: 9:1 (v/v) with a gradient elution at Pump A : Pump B :: 7:3, delivered at a flow rate of 0.35 mL/min. A sample injection volume of 10 µL was used, with a total run time of 7.0 minutes for each analysis. The mass spectrometer was operated under optimized source conditions, including a curtain gas (CUR) pressure of 30 psi, interface voltage of 4.0 kV, interface current 1.1 µA, interface/source temperature 298°C, nebulizing gas F1 3.0 L/min., drying gas flow 10.0 L/min., conversion dyno of 10.0 kV, detector voltage of 1.86 kV, DL temperature was 250°C and heat block temperature 400°C. These optimized operating parameters operating parameters provided consistent ionization efficiency and reliable analytical response throughout the study.

3.6 MRM Transitions

Multiple reaction monitoring (MRM) conditions were optimized for Olaparib and its respective internal standard i.e., Rucaparib to achieve selective and sensitive detection. The optimized precursor-to-product ion transitions were *m/z* 435.3 → 281.2 for Olaparib. Rucaparib employed as the internal standard, was monitored using the transitions *m/z* 324.2 → 293.2. These optimized MRM transitions provided excellent analyte specificity, minimal interference, and reproducible ion responses throughout the analytical sequence.

3.7 Calibration and Quality Control

Calibration standards were generated by spiking blank plasma with Olaparib at concentrations ranging from 1.25 to 1000 ng/mL. Separate quality control (QC) samples were independently prepared at four concentration levels – lower limit of quantification (LLOQ), low QC (LQC), medium QC (MQC), and high QC (HQC) to assess the method's accuracy and precision throughout the entire calibration range.

3.8 Method Validation

The analytical method was validated in compliance with the ICH M10, USFDA, and EMA bioanalytical method validation guidelines to demonstrate its reliability and robustness. Validation parameters included linearity, accuracy, precision, recovery, matrix effect, carryover, dilution integrity, reinjection reproducibility, and stability.

The matrix effect was assessed using six independent lots of human plasma in accordance with the recommendations outlined in the ICH M10 and USFDA bioanalytical method validation guidelines. The variability of the matrix factor remained within the acceptable limit of ± 15%, demonstrating the robustness of the analytical method. Validation was performed using a systematic approach that included calibration curve evaluation, intra- and inter-day accuracy and precision studies, matrix effect assessment, and stability investigations under both short-term and long-term storage conditions. The successful completion of these validation experiments established the method as reliable and suitable for routine bioanalytical analysis.

4. Results

4.1 Linearity

The calibration curve for Olaparib demonstrated excellent linearity over the concentration range of 1.25 to 1000 ng/mL, with correlation coefficients (r^2) consistently greater than 0.996. The strong linear relationship between analyte concentration and detector response indicates the reliability of the method for accurate quantification and supports its application in pharmacokinetic studies.

The linearity study demonstrated that the developed method provided reliable quantification of analyte throughout its respective calibration range. A strong linear relationship was observed between the analyte concentration and the corresponding peak area ratio, confirming the method's accuracy, consistency, and suitability for pharmacokinetic and bioanalytical applications.

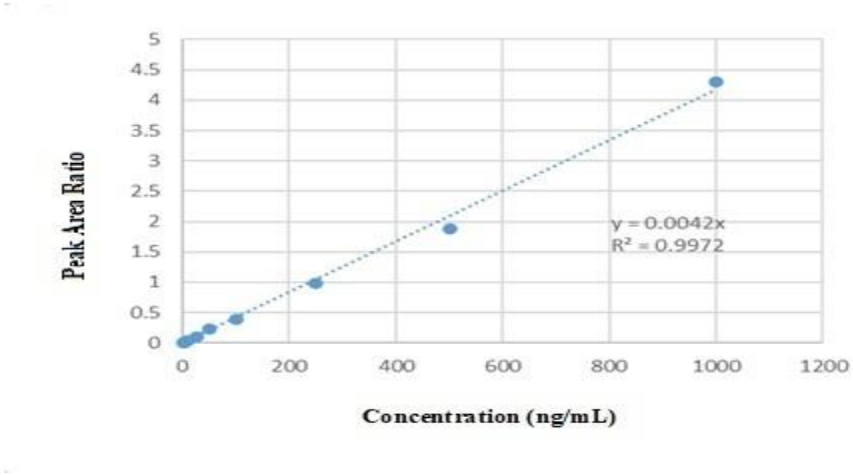

Figure 2. Calibration curve for Olaparib (1.25-1000 ng/mL) (n = 3). All calibration points met acceptance criteria for accuracy (±15%) and precision (%CV ≤15%) per ICH M10/ FDA guidelines.

Table 1: System suitability or Linearity data of Olaparib

Calibration Level	Nominal Conc.	Measured Conc.	Accuracy (%)

	(ng/mL)	(ng/mL)	
STD 1	1.25	1.30	104.00
STD 2	2.5	2.36	94.40
STD 3	5	4.75	95.00
STD 4	10	10.58	105.80
STD 5	25	24.61	98.44
STD 6	50	48.33	96.66
STD 7	100	97.54	97.54
STD 8	250	233.18	93.27
STD 9	500	479.64	95.92
STD 10	1000	978.11	97.81

The developed method exhibited satisfactory system suitability and maintained a consistent linear response across the calibration range. The accuracy of Olaparib was observed between 93.27% and 105.80%. All back-calculated calibration standard concentrations complied with the established regulatory acceptance criteria, with deviations remaining within ±15% of its respective normal concentrations.

4.2 Accuracy and Precision

Chromatographic evaluation of Olaparib at the high-quality control (HQC) concentration produced well-defined, symmetrical peaks with negligible tailing, demonstrating excellent chromatographic performance and consistent retention behavior. The detector response increased proportionally with analyte concentration, confirming the accuracy and reliability of the quantification method.

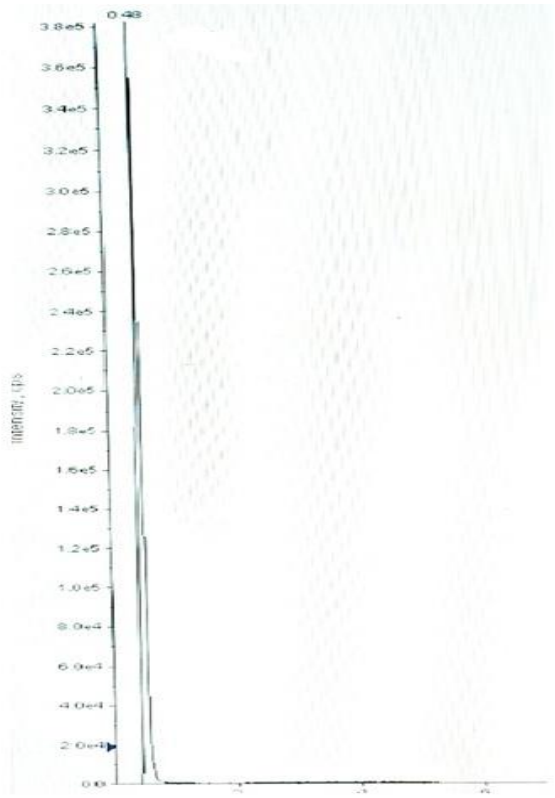

Figure 3. Representative chromatographic of Olaparib at HQC level.

Table 2: Accuracy & Precision of Olaparib

QC Level	Mean Conc. (ng/mL)	Nominal Conc. (ng/mL)	Accuracy (%)	Precision (%CV)
LLOQ	1.13	1.25	90.03	7.11
LQC	3.40	3.75	90.76	8.44
MQC	344.36	375.00	91.83	9.16
HQC	746.53	750.00	99.54	5.00

The developed method demonstrated satisfactory accuracy and precision for the quantification of Olaparib, accuracy values ranged from 90.03% to 99.54% with coefficient of variation (CV%) remaining below 9.16%. These findings confirm that the method provides reliable and reproducible quantification, making it suitable for routine bioanalytical applications.

4.3 Recovery

The recovery results obtained across all QC levels of both Olaparib and IS indicate consistent and reproducible extraction efficiency [see Table 3]. The recovery and matrix effect assessments demonstrate effective analyte extraction with minimal ion suppression, meeting established regulatory expectations for bioanalytical method performance[13-15].

Table 3. Recovery & Matrix Effect of Olaparib & IS

Compound	QC Level	Mean Recovery (%)	Matrix Effect (%)
Olaparib	LQC	101.85	95.08
Olaparib	MQC	93.43	95.13
Olaparib	HQC	94.73	102.39
IS	LQC	103.83	96.24
IS	MQC	95.24	87.00
IS	HQC	92.94	94.89

4.4 Stability and Sensitivity

Stability assessments were performed to investigate the stability of Olaparib in human plasma under conditions representative of routine bioanalytical sample handling and storage. The evaluated conditions included bench-top stability (8 hours at room temperature), freeze-thaw stability (three cycles), autosampler stability (24 hours at 15°C), short-term stability (24 hours), and long-term stability (30 days at -20°C). Low-quality control (LQC), medium-quality control (MQC), and high-quality control (HQC) samples were analyzed in five replicates (n = 5) foe each stability condition, and the results were reported as mean ± standard deviation (SD). Olaparib remained stable throughout the study, with measured accuracies ranging from 90.58% to 109.96% and precision (%CV) within the acceptable limit of ±15%.

Table 4. Stability Data of Olaparib (Mean ± SD, n = 5)

Condition	Nominal Conc. (ng/mL)	Measured Conc. (Mean ± SD)	Accuracy (%)	Comments
Bench-top stability (8 hrs.)	3.75	3.72 ± 0.40	104.31	Stable
	375	353.59 ± 38.51	98.56	Stable
	750	766.86 ± 30.37	96.99	Stable
Freeze-thaw stability (3 cycles)	3.75	3.48 ± 0.29	97.37	Stable
	375	394.50 ± 25.36	109.96	Stable
	750	787.99 ± 20.50	101.82	Stable
Autosampler stability (24 hours at 15°C)	3.75	3.49 ± 0.42	94.07	Stable
	375	376.17 ± 31.20	104.85	Stable
	750	756.48 ± 26.04	96.46	Stable
Short-term stability (24 hours)	3.75	3.90 ± 0.27	104.05	Stable
	375	397.45 ± 29.07	90.58	Stable
	750	764.90 ± 27.95	96.35	Stable
Long-term stability (30 days at -20°C)	3.75	3.61 ± 0.45	93.77	Stable
	375	387.09 ± 54.91	107.89	Stable
	750	770.13 ± 45.17	97.01	Stable
LLOQ (Sensitivity)	1.25	1.09 ± 0.13	87.20	Passed

All measured values satisfied the predefined regulatory acceptance criteria, with accuracy remaining within ± 15% of the nominal concentration and precision (%CV) not exceeding 15% i.e., ≤15%. At the LLOQ, Olaparib also met the required acceptance limits, demonstrating accuracy within 80-120% and precision (%CV) of ≤ 20%.

The stability results demonstrated that the analyte remained stable under routine sample handling and storage conditions, fulfilling the regulatory acceptance criteria outlined in the ICH M10 and FDA/EMA bioanalytical method validation guidelines. The sensitivity of the developed LC-MS/MS method was further established through evaluation of the lower limit of quantification (LLOQ), which was determined to be 1.25 ng/mL. At this level, the accuracy was 87.20%, and the precision was below 12%. Furthermore, the signal-to-noise ratio exceeded 10:1

for the analyte, satisfying the predefined acceptance criteria for sensitivity. A comprehensive summary of the stability and LLOQ validation results is presented in Table 4. Collectively, these findings demonstrate that the validated LC-MS/MS method is robust, sensitive, and well suited for clinical pharmacokinetic investigations and drug-drug interaction studies involving Olaparib[13-15].

Stability evaluations demonstrated that the analyte maintained its integrity under bench-top, freeze-thaw, autosampler, and long-term storage conditions. The measured accuracies ranged from 90.58% to 109.96%, remaining within the ± 15% acceptance limits specified by the ICH M10 and FDA/EMA bioanalytical validation guidelines, thereby confirming the stability of the analyte throughout the evaluated storage and handling conditions.

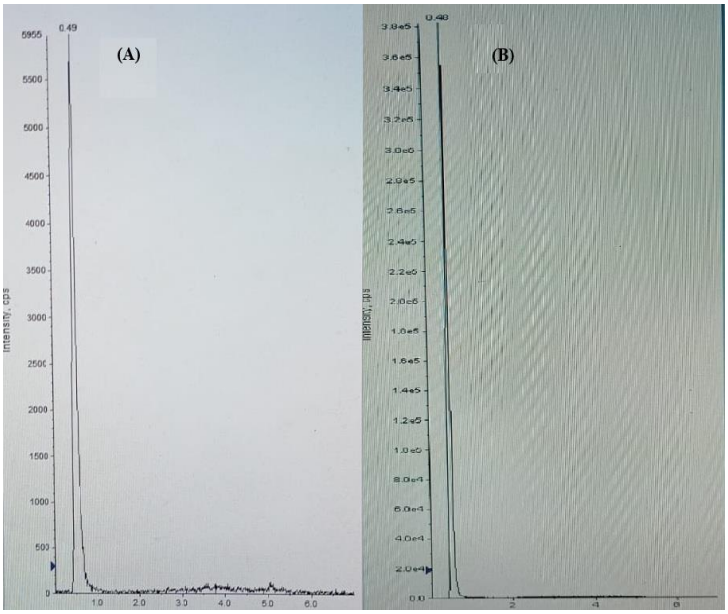

Figure 4. Representative chromatographic of Olaparib at (A) LLOQ & (B) HQC level.

The representative chromatograms demonstrated performance consistent with established bioanalytical validation criteria. Well-defined, symmetrical peaks with high signal intensity were observed for the analyte with retention time 0.49 min, indicating reliable chromatographic separation and detection.

4.5 Matrix Effect Evaluation

Representative chromatograms comparing analyte and IS responses in post-extracted plasma samples with those of equivalent neat standard solutions are presented for (A) Olaparib and (B) IS (Rucaparib). No evidence of significant ion suppression or enhancement was observed. The comparable peak intensities and consistent retention times indicate minimal matrix interference and confirm satisfactory chromatographic performance. Matrix effect was further assessed by comparing the responses of post-extraction spiked plasma samples with the analyte concentrations prepared in neat solutions. The calculated matrix factor values complied with the acceptance criteria specified in the ICH M10 and FDA bioanalytical method validation guidelines, demonstrating the absence of significant matrix-induced signal enhancement or suppression. These qualitative chromatographic observations were consistent with the quantitative matrix factor results, confirming the robustness and reliability of the developed LC-MS/MS method for the analysis of biological plasma samples.

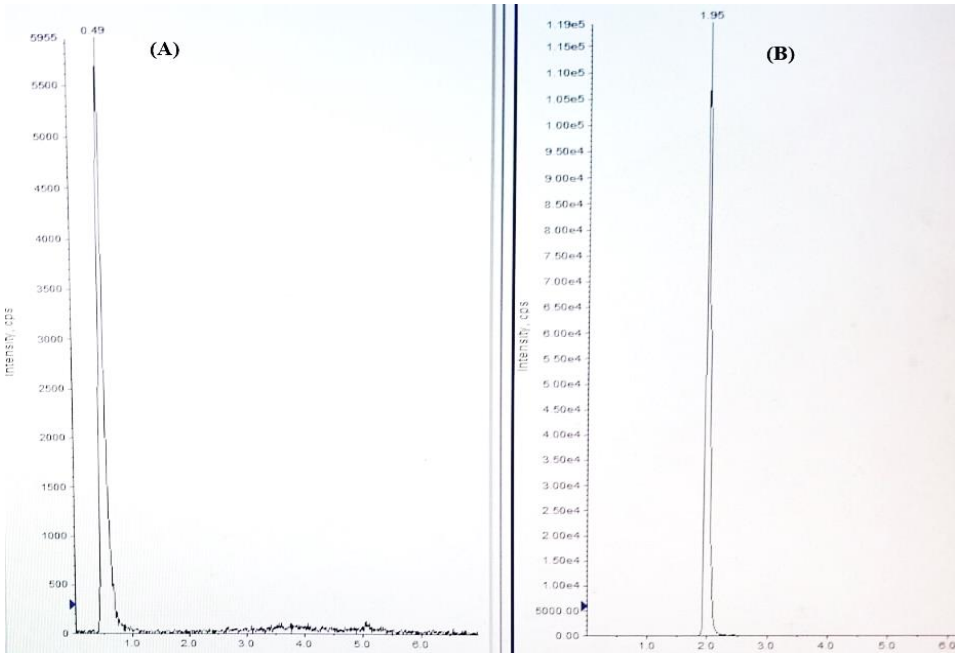

Figure 5. Matrix effect chromatogram of (A) Olaparib and (B) IS (Rucaparib).

Post-extraction chromatograms demonstrated negligible matrix effects and no significant interference[13-15].

4.6 Selectivity and Specificity

The developed LC-MS/MS method enabled the simultaneous quantification of Olaparib in a single analytical run using compound-specific MRM transitions. The analyte and IS were well resolved with distinct retention times of 0.49 min and 1.95 min, demonstrating excellent chromatographic resolution and method specificity. Blank plasma chromatograms showed no endogenous interference at the respective retention times, confirming method selectivity. The Olaparib and Rucaparib (IS) mass spectrum verified the characteristic fragmentation pattern, ensuring accurate analyte and IS identification. Individual chromatograms exhibited acceptable specificity and negligible matrix interference, supporting reliable analyte detection. Collectively, these findings confirm the suitability of the method for dual-analyte bioanalysis and its compliance with ICH M10 and FDA bioanalytical method validation guidelines.

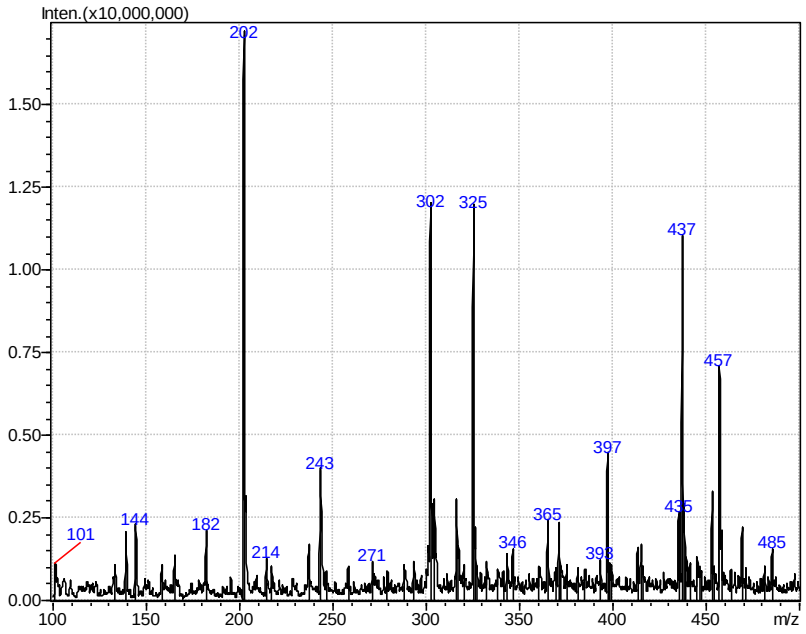

Figure 6. Mass spectrum of Olaparib illustrating the precursor ion and characteristic product ions employed for MRM quantification.

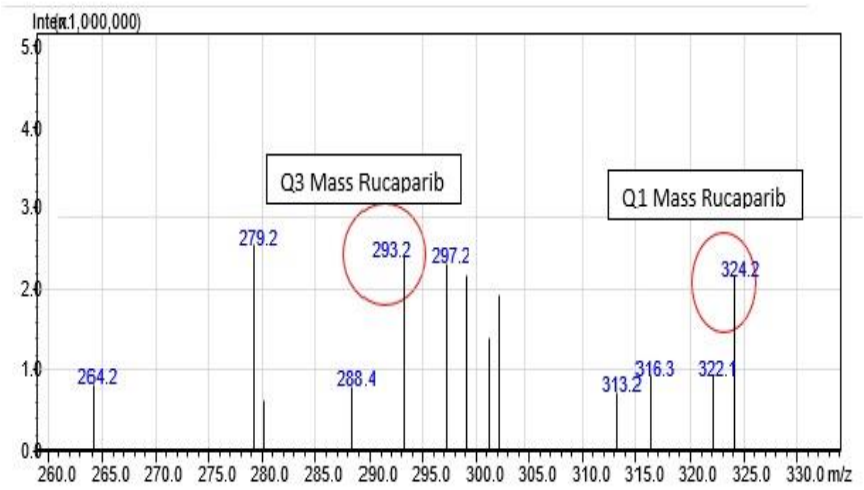

Figure 7. Mass spectrum of Rucaparib (IS) illustrating the precursor ion and characteristic product ions employed for MRM quantification.

4.7 Application in Therapeutic Drug Monitoring

The validated LC-MS/MS method was successfully applied to the therapeutic drug monitoring (TDM) of Olaparib in plasma samples collected from Indian cancer patient. The method demonstrated reliable quantification of Olaparib in all clinical samples with satisfactory sensitivity, selectivity, and reproducibility. Measured plasma concentrations were within the validated calibration range, confirming the method's suitability for routine clinical pharmacokinetic studies and TDM applications.

Table 5. Trough concentration of Olaparib

Parameter	Tablet
Dose	300 mg
No. of Patient	1
Drug level sample	2

C_{trough} [ng/mL]	875

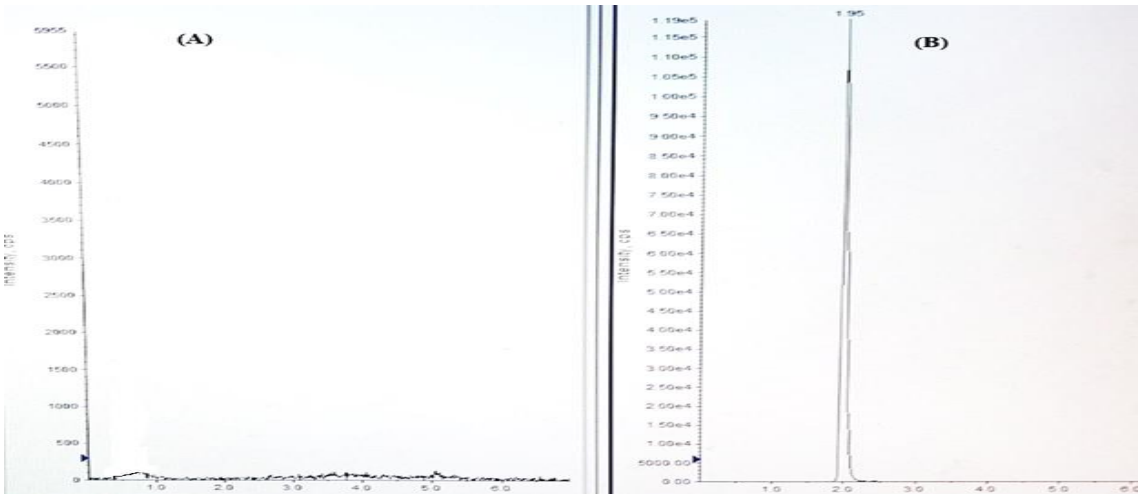

Figure 8. Representative Chromatograms of (A) Olaparib and (B) IS (Rucaparib) in a Drug-Free Patient's Plasma Sample

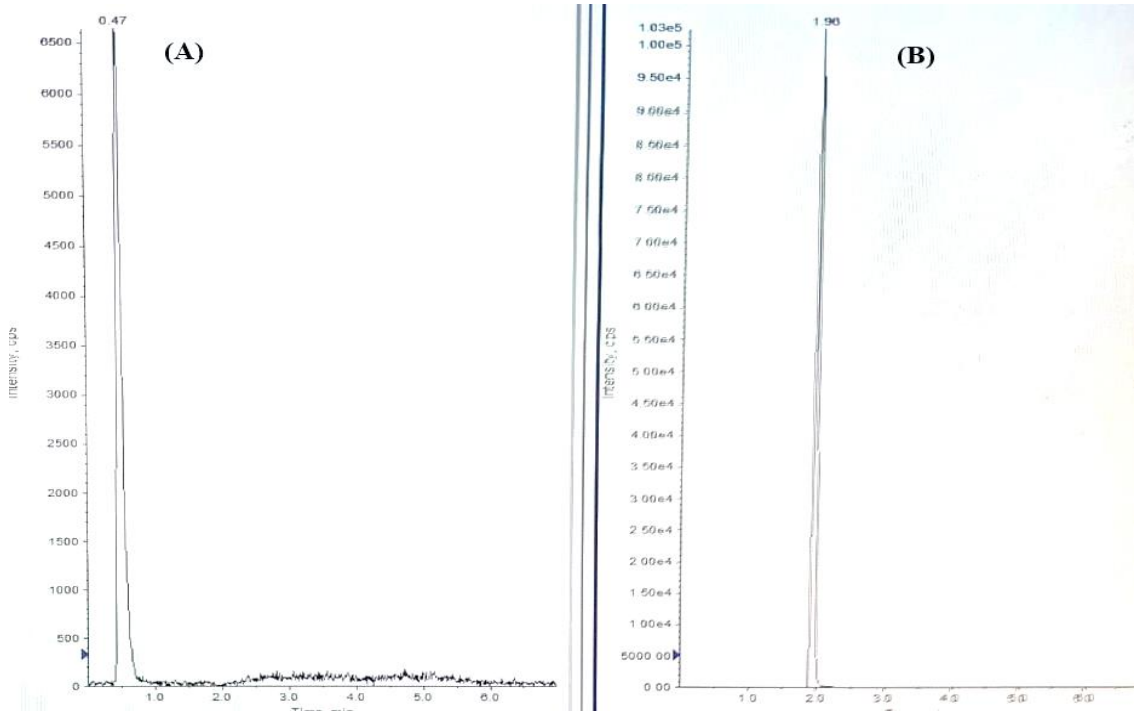

Figure 9. Representative Chromatograms of (A) Olaparib and (B) IS (Rucaparib) in a Patient's Plasma Sample after Dosing 300 mg Tablet of Olaparib

5. Discussion

Several published LC-MS/MS methods for the determination of Olaparib has reported relatively higher lower limits of quantification (LLOQs), such as ≥ 50 ng/mL[16]. These methods also typically require longer chromatographic run times (8-12 minutes) and rely on solid-phase extraction for sample preparation[16-25]. In comparison, the present method enables quantification of the analyte within 7 minutes chromatographic run using a simple protein precipitation procedure, while maintaining excellent selectivity and minimal matrix interference. The validated LLOQ of 1.25 ng/mL for Olaparib at least a five-fold improvement in sensitivity over most previously reported methods. For example, Takeo Yasu et al. (2023) reported an LLOQ of 100 ng/mL for Olaparib with a 15 minutes analytical run time to 7 minutes whereas the present method further enhanced sensitivity to 1.25 ng/mL using a simpler protein precipitation extraction approach instead of more complex sample preparation techniques. The improved sensitivity of the method allows reliable quantification of both analytes during the terminal elimination phase, thereby supporting complete pharmacokinetic profile characterization. This enhanced analytical performance is particularly valuable in patients with metastatic castration-resistant prostate cancer (mCRPC), where accurate assessment of drug exposure can aid in detecting altered metabolism, identifying potential drug-drug interactions, and optimizing dose adjustments to improve therapeutic outcomes.

6. Limitations of the Present Study

Although the validated method showed improved analytical speed and sensitivity, it was evaluated only using spiked plasma samples and not clinical samples from patients with metastatic castration-resistant prostate cancer (mCRPC). Consequently, biological variability and potential interference from endogenous compounds or structurally related analogs present in patient samples were not assessed. On the other hand, we used just one patient plasma sample after the dosing of Olaparib.

7. Future Scope of the Method

Future studies should validate the method using clinical samples and explore its automation to improve laboratory throughput. The method can also be expanded to simultaneously quantify other

PARP and CYP17 inhibitors in multi-analyte panels and evaluated in diverse patient populations, including pediatric and elderly oncology patients.

8. Conclusion

The validated LC-MS/MS method provides a rapid, sensitive, and reliable approach for the simultaneous quantification of Olaparib in plasma. Its short analysis time and low LLOQ make it suitable for therapeutic drug monitoring, pharmacokinetic, and drug-drug interaction studies. Although validated in spiked plasma, further evaluation using clinical samples is required to confirm its clinical applicability. This method has the potential to support dose optimization, treatment monitoring, and precision oncology in patients with metastatic castration-resistant prostate cancer (mCRPC).

9. Confidentiality Statement

Patient confidentially was preserved at all stages of the study, and the collected data were used solely for scientific and research purposes.

10. Conflict of Interests

Authors have no conflict of interests.

11. Authors' Contributions

Kajal Baidhar Ghadai conceived the study, developed the analytical method, performed the experimental work, analyzed the data, and prepared the manuscript. Dr. Amlan Kanti Sarkar provided overall supervision, Dr. Dhiman Halder and Dr. Pushpanjali Das contributed to method validation, and critically reviewed the manuscript. Dr. Amlan Kanti Sarkar reviewed and approved the final manuscript and accept responsibility for the accuracy, integrity, and authenticity of the work.

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