RESEARCH PAPER

"ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF OLAPARIB USING RP-HPLC, LC-MS AND UV–VISIBLE SPECTROPHOTOMETRY"

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Graphical Abstract:

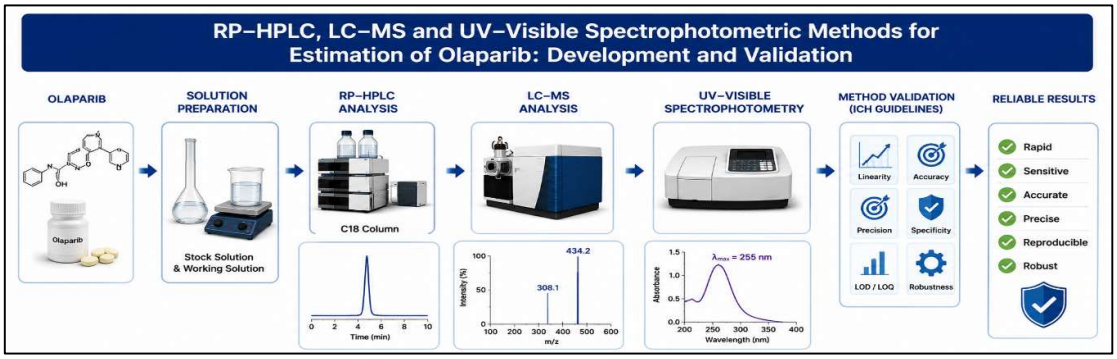

Figure No.1: Graphical Abstract of Analytical Method Development and Validation of Olaparib

Abstract

The present study focuses on the development and validation of RP–HPLC, LC–MS, and UV–Visible spectrophotometric methods for the estimation of Olaparib, an anticancer drug used in ovarian and breast cancer therapy. RP–HPLC analysis was performed using a C18 column with optimized chromatographic conditions to obtain sharp and well-resolved peaks. LC–MS was developed for sensitive identification and characterization of Olaparib through mass spectral analysis, while the UV–Visible method was based on determination of λmax in the range of 200–400 nm. All methods were validated according to ICH guidelines for parameters including accuracy, precision, linearity, robustness, specificity, LOD, and LOQ. The developed methods were found to be simple, accurate, precise, sensitive, and suitable for routine quality control analysis of Olaparib in bulk and pharmaceutical dosage forms.

Keywords

Olaparib, RP–HPLC, LC–MS, UV–Visible Spectrophotometry, Method Development, Method Validation, ICH Guidelines, Pharmaceutical Analysis

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1. Introduction:

Poly (ADP-ribose) polymer (PARP) inhibitors are a class of specific therapeutic medicines that includes the powerful oral anticancer medication olaparib[1-2]. Because they can target cancer cells specifically while causing the least amount of harm to healthy, normal tissues, targeted treatments for cancer have received a lot of interest in recent years [3]. Olaparib is a significant breakthrough in this area, especially when it comes to treating malignancies linked to flaws in DNA repair processes [4-5].

By helping to repair single-strand DNA breaks via the base excision repair pathway, PARP enzymes are essential for preserving genomic integrity [6]. PARP enzymes recognize breaks in DNA under normal physiological settings and start repair activities to restore DNA integrity [7-8]. However,

this repair mechanism is disrupted by PARP enzyme suppression, which causes DNA damage to accumulate. Double-strand DNA breaks, which are more deadly to the cell, are the result of this accumulating over time [9-10].

PARP inhibition is especially dangerous for cancer cells with mutations in the BRCA1 or BRCA2 genes [11]. These genes are in charge of the high-fidelity DNA repair process known as homologous recombination repair. Cancer cells cannot successfully repair DNA damage when both PARP-mediated cellular repair and BRCA-mediated repair pathways are impaired, which eventually culminates in cell death [12–13]. Olaparib's therapeutic activity is based on a phenomenon known as synthetic lethality.

Olaparib has been a successful therapy choice for a number of cancers, including ovarian cancer, breast cancer, pancreatic cancer, and prostate cancer, particularly in patients with BRCA mutations, because of this distinct mechanism [14–15]. Olaparib provides a more targeted approach than traditional chemotherapy, which frequently affects both malignant and normal cells. This reduces systemic toxicity and improves patient outcomes [16].

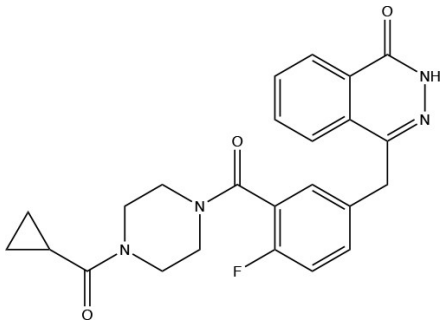

Figure 2: Chemical structure of olaparib

Olaparib is a complicated small-molecule compound that has the IUPAC designation. 4-[[3-[4-(cyclopropane carbonyl) piperazine-1-carbonyl]-4-fluorophenyl] methyl]-2H-phthalazin-1-one [17–18].
Its pharmacological activity is influenced by a variety of functional groups found in its molecular structure, such as heterocyclic elements, amide bonds, and aromatic rings. Its identification by spectroscopic methods like UV-visible spectrophotometry is also made possible by the existence of these chromophoric groups.

The creation of precise, accurate, and trustworthy analytical techniques is necessary to guarantee the efficacy, safety, and quality of pharmaceutical substances containing olaparib [19]. The development of analytical methods is essential to pharmaceutical research and quality control because it offers the instruments required to detect, measure, and track the medicinal material in both bulk and formulated dose forms [20–21]. Additionally, stability studies, drug development, regulatory submissions, and regular quality assurance all depend on these techniques.

Drug analysis can be done using a variety of analytical techniques, but choosing the right one depends on a number of parameters, including cost, convenience of use, sensitivity, specificity, and accuracy[22]. Reverse-phase high-performance liquid chromatography (RP-HPLC), liquid chromatography–mass spectrometry (LC-MS), and UV–visible spectrophotometry are among the many methods that are frequently employed because of their complementing benefits[23–24].

One of the most straightforward and affordable analytical methods is UV-visible spectrophotometry [25]. Its foundation is the evaluation of a substance's absorption of visible or ultraviolet light [26]. This approach's simplicity, speed, and low sample preparation requirements make it very helpful for routine examination in quality control labs. However, in the presence of interfering compounds, it might not be as specific [27].

Even in complicated matrices, RP-HPLC is a potent chromatographic method that offers precise drug quantification and excellent resolution [28]. It works on the basis of hydrophobic relationships between the analyte with the stationary phase [29]. Olaparib and other moderately non-polar chemicals can be effectively separated using a C18 column. Because of its accuracy, consistency, and durability, RP-HPLC is considered the gold standard for pharmaceutical analysis [30].
Liquid chromatography's separation capacity and mass spectrometry's detection power are combined in LC-MS [31]. With remarkable sensitivity and selectivity, it is regarded as one of the most

sophisticated analytical methods [32]. In addition to enabling precise drug measurement at trace levels, LC-MS offers structural information by mass spectral analysis [33]. Multiple reaction monitoring (MRM) and electrospray ionization (ESI) increase the specificity of detection, which makes it ideal for confirmatory analysis [34].

Combining these analytical methods offers a thorough method for analyzing drugs. RP-HPLC guarantees accurate quantification, LC-MS delivers excellent sensitivity and confirmation identification, and UV spectrophotometry is straightforward and economical. When combined, these techniques meet the criteria for a comprehensive assessment of Olaparib in pharmaceutical dosage forms as well as bulk drugs.

Even though there are many analytical techniques available in the literature, Olaparib specific proven, dependable, and effective techniques still need to be developed [35]. The normal use of many documented procedures in quality control laboratories is limited because they are either complicated, time-consuming, or require costly equipment. Additionally, some methods lack adequate specificity, particularly in the presence of excipients or degradation products [36].
As a result, it is crucial to create analytical techniques that are easy to use, affordable, accurate, and exact. The International Council for Harmonization's (ICH) guidelines, which guarantee the dependability, repeatability, and regulatory acceptability of analytical processes, must also be followed by all new methods. Validation characteristics like linearity, accuracy, precision, specificity, robustness, and sensitivity are highlighted in these guidelines [37].

The current work focuses on the methodical creation and verification of analytical procedures for Olaparib estimation employing RP-HPLC, LC-MS, and UV-visible spectrophotometry. The objective is to develop a highly sensitive LC-MS approach for confirmatory identification and trace-level analysis, a reliable and accurate RP-HPLC method for quantitative determination, and a straightforward and affordable UV method for regular analysis. To guarantee that the proposed procedures are appropriate for pharmaceutical applications, the study also involves validation of the created methods in compliance with ICH guidelines. Olaparib is then analyzed in pharmaceutical dosage forms and bulk drugs using the verified techniques.
The ultimate objective of this study is to create an all-encompassing analytical method that blends simplicity, precision, sensitivity, and dependability. Regular quality control, regulatory compliance, and guaranteeing consistent pharmaceutical product quality will all greatly benefit from this strategy.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

A certified pharmaceutical source provided the reference norm for Olaparib (purity ~99.9%) and the different laboratory batch samples. Throughout the investigation, Milli-Q purified water from a Millipore filtration system was utilized. The mobile phase and buffers were prepared using the analytical variety formic acids and ammonium acetate. To guarantee accuracy and repeatability of the outcomes, all reagents selected of analytical or HPLC grade.

2.2. Instruments Used

Sr. No.	Instrument	Manufacturer
1	HPLC System	Shimadzu/Waters
2	UV–Visible Spectrophotometer	Shimadzu UV-1800
3	LC-MS System	Agilent/Waters
4	Analytical Balance	Shimadzu
5	Ultrasonic Bath	Remi
6	pH Meter	Elico

2.3. RP-HPLC METHOD

2.3.1. Instrumentation

Chromatographic analysis was carried out using a Waters HPLC 2695 system equipped with a quaternary pump, autosampler, auto injector, and UV detector. The chromatographic data were processed using Empower software. Separation of Olaparib was achieved using a C18 analytical column having dimensions of 250 mm × 4.6 mm and particle size of 5 µm.

2.3.2. Preparation of Mobile Phase

The mobile phase was prepared by mixing acetonitrile and 0.1% formic acid in water in the ratio of 60:40 v/v. The prepared mobile phase was filtered using a 0.45 µm membrane filter followed by sonication to eliminate dissolved gases.

2.3.3. Preparation of Standard Solution

An accurately weighed quantity of 25 mg of Olaparib was transferred into a 25 mL volumetric

flask and dissolved in methanol with the help of sonication. The final volume was adjusted with methanol to obtain a stock solution containing 1 mg/mL concentration. Further dilutions were prepared using mobile phase to obtain the desired working concentrations.

2.3.4. Preparation of Sample Solution

Twenty tablets of Olaparib formulation were weighed accurately and powdered finely. Powder equivalent to 50 mg of Olaparib was transferred into a volumetric flask containing suitable diluent. The mixture was sonicated for approximately 30 minutes to ensure complete extraction of the drug. The solution was filtered through
a 0.45 µm membrane filter and diluted appropriately before chromatographic analysis.

2.3.5. Optimized Chromatographic Conditions Table No.1: Optimized RP-HPLC Conditions

Parameter	Optimized Condition
Column	C18 Column (250 × 4.6 mm, 5 µm)
Mobile Phase	Acetonitrile: 0.1% Formic Acid (60:40 v/v)
Flow Rate	1.0 mL/min
Detection Wavelength	268 nm
Injection Volume	10–20 µL
Run Time	10 min
Retention Time	4.8–5.1 min

2.4. LC-MS METHOD

2.4.1. Instrumentation

LC-MS analysis was performed using a liquid chromatography system coupled with a mass spectrometer equipped with an electrospray ionization (ESI) source operating in positive ionization mode.

2.4.2. Chromatographic and Mass Conditions

A suitable C18 column was used for chromatographic separation. The mobile phase consisted of acetonitrile and aqueous formic acid solution. The flow rate was maintained at 0.5 mL/min throughout the analysis.

Table No.2: LC-MS Operating Conditions

2.4.3. Preparation of Standard Solution

A standard stock solution containing 1 mg/mL of Olaparib was prepared using methanol. Appropriate serial dilutions were prepared with mobile phase to obtain solutions in nanogram concentration range

Parameter	Condition
Ionization Mode	Positive ESI
Mobile Phase	Acetonitrile : Formic Acid Solution
Flow Rate	0.5 mL/min
Scan Range	100–1000 m/z
Molecular Ion Peak	m/z 435

suitable for LC-MS analysis.

2.4.4. Preparation of Sample Solution

The sample solution was prepared similarly to the RP-HPLC procedure. After filtration through a 0.45 µm membrane filter, the sample was diluted suitably and injected into the LC-MS system for analysis.

2.5. UV–VISIBLE SPECTROPHOTOMETRIC METHOD

2.5.1. Instrumentation

UV spectrophotometric analysis was carried out using a double beam UV–Visible spectrophotometer equipped with matched quartz cells of 1 cm path length.

2.5.2. Determination of λmax

A diluted standard solution of Olaparib was scanned in the wavelength range of 200–400 nm using methanol as blank solution. The maximum absorbance wavelength (λmax) was observed at 268 nm.

2.5.3. Preparation of Standard Stock Solution

Accurately weighed 25 mg of Olaparib was dissolved in methanol and diluted up to 25 mL in a volumetric flask to obtain a stock solution having concentration of 1 mg/mL.

2.5.4. Preparation of Calibration Curve

Working standard solutions in the concentration range of 5–25 µg/mL were prepared by suitable dilution of the stock solution. The absorbance of each solution was measured at 268 nm and a calibration curve was constructed by plotting absorbance against concentration.

Table No.3 : UV Spectrophotometric Conditions

Parameter	Condition
Solvent	Methanol
Detection Wavelength	268 nm

Concentration Range	5–25 µg/mL
Cell Path Length	1 cm

2.5.5. Preparation of Sample Solution

Tablet powder equivalent to 50 mg of Olaparib was transferred into a volumetric flask and dissolved in methanol using sonication. The prepared solution was filtered and diluted suitably within the Beer-Lambert's concentration range before analysis.

2.6. METHOD VALIDATION

The developed RP-HPLC, LC-MS, and UV spectrophotometric methods were validated according to ICH Q2(R1) guidelines. Validation parameters such as linearity, accuracy, precision, specificity, robustness, ruggedness, limit of detection (LOD), limit of quantification (LOQ), and system suitability were evaluated. The obtained res
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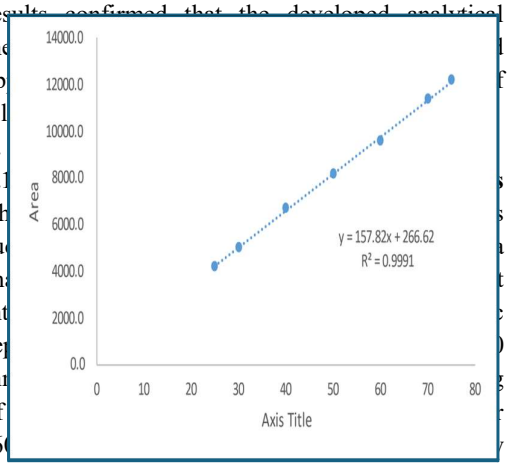

rate of 1.0 mL/min with detection at 268 nm. Under optimized conditions, Olaparib showed a distinct peak with a retention time of approximately 5.1 min, indicating good specificity and chromatographic performance.

Table No.4 : System Suitability Parameters

Parameter	Value
Retention Time (min)	5.14
Tailing Factor	1.17
Theoretical Plates	3200–4500
%RSD (Peak Area)	< 2%

The system suitability results indicate that the chromatographic system is performing adequately and is suitable for analysis. The low %RSD value confirms the repeatability of the system

3.1.1. Linearity Study of RP-HPLC Method

The linearity of the developed RP-HPLC method was evaluated using standard solutions in the concentration range of 25–75 µg/mL. A calibration curve was plotted between concentration and corresponding peak area.

Table No.5: Calibration Data for RP-HPLC Method

Sr. No.	Concentration (µg/mL)	Peak Area
1	25	4200.8
2	30	4976.3
3	40	6705.8
4	50	8144.3
5	60	9560.6
6	70	11357.7
7	75	12157.4

Figure No.3: The calibration graph exhibited excellent linearity with correlation coefficient (R^2) value of 0.9991, indicating a direct proportional relationship between concentration and peak area.

3.1.2. Precision Study

Precision of the RP-HPLC method was assessed by analyzing six replicate injections of standard solution under the same experimental conditions.

Table 8: Precision Data for RP-HPLC Method

Injection No.	Peak Area
1	8142.5
2	8145.2
3	8143.8
4	8146.1
5	8144.6
6	8143.9

The %RSD value was found to be less than 2%, demonstrating excellent repeatability and precision of the developed method.

3.1.3. Accuracy Study

Accuracy of the method was evaluated by recovery studies using standard addition technique at three concentration levels.

Table No.6: Accuracy (Recovery Study)

Level	Amount Added (µg/mL)	% Recovery
80%	40	99.2
100%	50	100.4
120%	60	99.6

The percentage recovery values were found within acceptable range (98–102%), confirming the accuracy of the method.

3.1.4. LOD and LOQ Data

Table No.7: LOD and LOQ data

Parameter	Value
LOD (µg/mL)	0.42–0.49
LOQ (µg/mL)	1.28–1.49

By calculating the limit of detection, or LOD, and limit of quantitation (LOQ), the sensitivity of the RP-HPLC method was evaluated. The LOQ varied from 1.28 to 1.49 µg/mL, and the LOD was determined to be between 0.42 and 0.49 µg/mL. The low values show that the technique is very sensitive and can successfully identify and measure minute levels of olaparib.

Observations

- Retention time ≈ **5.14 min**
- Peak shape: Sharp and symmetrical
- No interference observed
- Excellent reproducibility

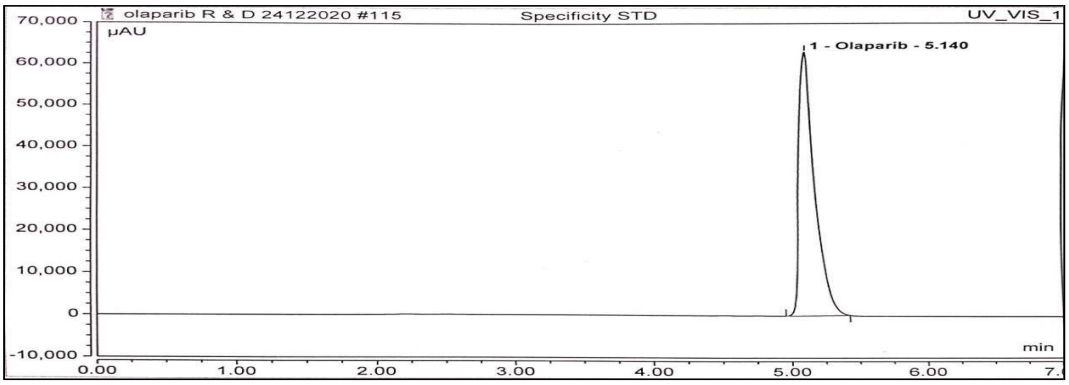

Figure No.4: Chromatogram of Standard Olaparib

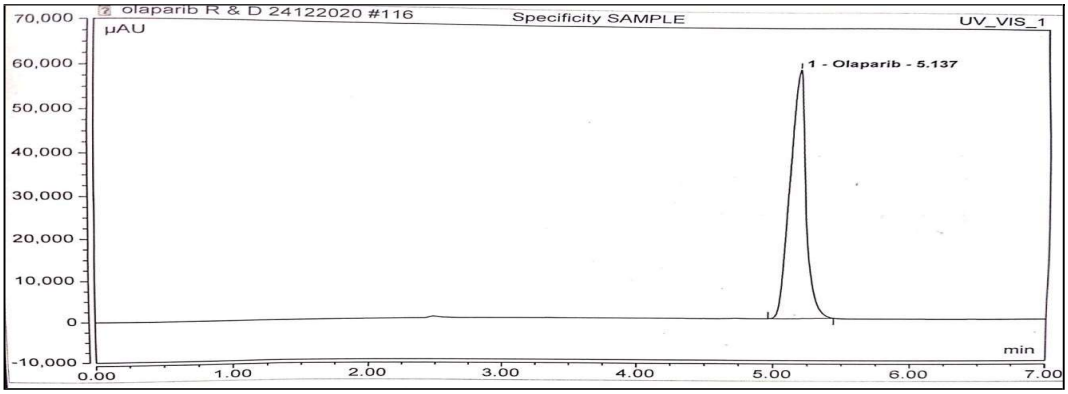

Figure No.5: Chromatogram of Sample Olaparib

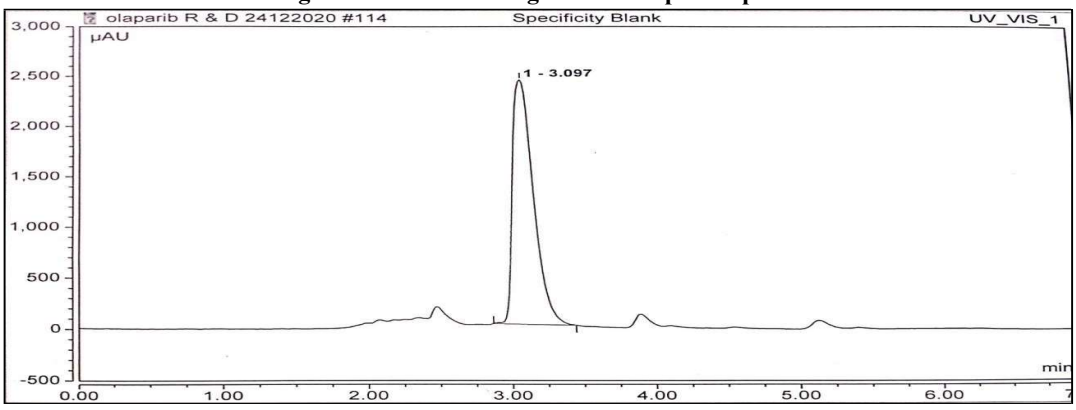

Figure No.6 : Blank Chromatogram of Olaparib

3.1.5. Robustness Study

Robustness of the developed RP-HPLC method was studied by making small deliberate changes in chromatographic conditions such as flow rate and mobile phase composition. No significant variation in chromatographic response or retention behavior was observed, indicating that the method is robust and reliable under slightly varied experimental conditions.

Table No.8: Robustness Study Data

Parameter Changed	Condition	Retention Time (min)	Peak Area	Tailing Factor
Flow rate	0.9 mL/min	5.32	8125.6	1.19
	1.0 mL/min	5.14	8144.3	1.17
	1.1 mL/min	4.98	8138.2	1.18
Mobile Phase	58:42	5.21	8130.5	1.20
	60:40	5.14	8144.3	1.17
	62:38	5.07	8150.1	1.16
Wavelength	266 nm	5.14	8105.4	1.18
	268 nm	5.14	8144.3	1.17
	270 nm	5.15	8128.7	1.19

3.1.6. Ruggedness Study (RP-HPLC)

To ascertain the reproducibility of findings under various circumstances, such as analysis carried out by different analysts, the robustness of the RP-HPLC method was assessed. Two distinct analysts conducted the study under comparable experimental conditions using the same tool. Both analyzers generated and independently examined a standard solution of Olaparib at a concentration of 100 µg/mL, and the findings were compared.

Table No.9: Ruggedness Study Data

Analyst	Injection No.	Peak Area
Analyst 1	1	8142.5
	2	8145.2
	3	8143.8
Analyst 2	1	8146.1
	2	8144.6
	3	8143.9

The toughness investigation showed that there was very little variance in peak area values and that the results from several analysts were quite consistent. It was discovered that the computed %RSD was less than 0.5%, which is well within allowable bounds. This suggests that regardless of the operator conducting the analysis, the approach yields repeatable results.

The new RP-HPLC method is robust and dependable, which makes it appropriate for routine quality control analysis in various laboratory settings, as seen by the minimal variability observed.

3.2. LC–MS Method Development and Validation for Olaparib

LC–MS Method Development

An LC–MS method was developed and optimized for the sensitive, selective, and confirmatory analysis of Olaparib. Chromatographic separation was achieved using a reverse-phase C18 analytical column with a mobile phase consisting of 0.1% formic acid in water and acetonitrile. The flow rate was maintained at 0.5 mL/min to ensure efficient chromatographic separation and compatibility with mass spectrometric detection.

Mass spectrometric detection was carried out using an electrospray ionization (ESI) source operating in positive ion mode, which provided efficient ionization of Olaparib. Under optimized conditions, Olaparib exhibited a prominent protonated molecular ion peak [M+H]+ at m/z 435, confirming the molecular identity of the drug. The chromatographic peak obtained was sharp, symmetrical, and free from interfering peaks, indicating good specificity and suitable chromatographic performance.

3.2.1. System Suitability Studies

Prior to sample analysis, system suitability studies were performed to evaluate the performance and reliability of the LC–MS system. Important

chromatographic and mass spectrometric parameters including retention time, signal-to-noise ratio, molecular ion detection, and peak symmetry were assessed.

Table No.10: LC–MS System Suitability Parameters

Parameter	Value
Retention Time (min)	3.2–3.8
Molecular Ion (m/z)	435
Signal-to-Noise Ratio	>10
Peak Shape	Symmetrical

The retention time of Olaparib was observed between 3.2 and 3.8 min, indicating rapid and efficient chromatographic separation. The protonated molecular ion peak [M+H]+ at m/z 435 confirmed the specificity of the developed method.

The signal-to-noise ratio greater than 10 demonstrated adequate method sensitivity for trace-level analysis. Furthermore, the chromatographic peak exhibited excellent symmetry without significant tailing or fronting, indicating appropriate method optimization and reliable peak integration.

Overall, the system suitability results confirmed that the developed LC–MS method was stable, reproducible, and suitable for routine quantitative analysis of Olaparib.

3.2.2. Calibration Curve and Linearity

Calibration standards of Olaparib were prepared over a concentration range of 25–5000 ng/mL and analyzed under optimized LC–MS conditions. The corresponding peak areas were recorded to evaluate the linearity of the developed method.

Table No.11: Calibration Data for LC–MS Method

Sr. No.	Concentration (ng/mL)	Peak Area
1	25	15230
2	50	30125
3	100	60280
4	500	301540
5	1000	602315
6	2500	1504780
7	5000	3012650

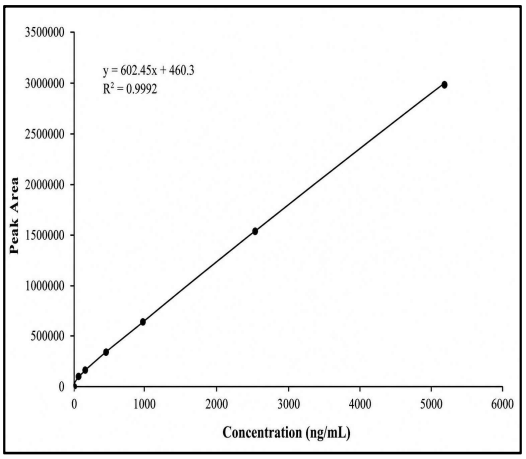

Figure No.7: Calibration curve of Olaparib showing linear relationship between concentration (ng/mL) and peak area using LC–MS method.

A proportional increase in peak area was observed with increasing concentration of Olaparib, demonstrating a direct relationship between analyte concentration and detector response. The calibration curve constructed by plotting concentration versus peak area exhibited excellent linearity with a correlation coefficient (R^2) greater than 0.998.

The wide linearity range of 25–5000 ng/mL demonstrated the applicability of the method for both trace-level detection and higher concentration analysis. The developed LC–MS method was therefore found suitable for quantitative estimation of Olaparib in pharmaceutical and biological samples.

3.2.3. Precision Studies (Repeatability)

Method precision was evaluated by performing six replicate injections of Olaparib under identical analytical conditions.

Table No.12: Precision Data (Repeatability)

Injection No.	Peak Area
1	602280
2	602350
3	602310
4	602295
5	602340
6	602300

The obtained peak areas were highly consistent, with a mean peak area of approximately 602312 and a percentage coefficient of variation (%CV) below

1%. The low variability among replicate injections indicated excellent repeatability and precision of the developed method.

According to International Council for Harmonisation (ICH) guidelines, a %CV value below 2% is considered acceptable for precision studies. Therefore, the developed LC–MS method demonstrated excellent reproducibility and was considered reliable for routine quantitative analysis.

3.2.4. Mass Spectral Analysis of Olaparib

Mass spectral analysis of Olaparib was carried out using LC–MS/MS in Multiple Reaction Monitoring (MRM) mode to achieve highly selective and sensitive detection.

A significant protonated molecular ion peak [M+H]+ was observed at m/z 435, confirming the molecular weight and identity of Olaparib. In addition, characteristic fragment ions generated from collision-induced dissociation were detected and utilized for MRM transitions.

The use of MRM mode enhanced method selectivity by monitoring specific precursor-to-product ion transitions while minimizing interference from matrix components. The observed fragmentation pattern confirmed the identity of Olaparib and validated the suitability of the LC–MS/MS method for quantitative analysis.

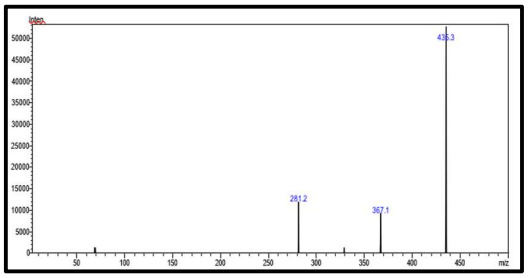

Figure No. 8: Mass spectrum of Olaparib showing molecular ion and characteristic fragment ions used for MRM quantification.

3.2.5. Accuracy Studies (Recovery)

The accuracy of the developed method was evaluated through recovery studies at three concentration levels corresponding to 80%, 100%, and 120% of the target concentration.

Table No.13: Accuracy (Recovery Studies)

Level (%)	Concentration (ng/mL)	% Recovery
80	800	98.9
100	1000	100.2
120	1200	99.5

The percentage recovery values ranged from 98.9% to 100.2%, which falls within the acceptable recovery limit of 98–102%. These results confirmed the accuracy of the method and indicated the absence of interference from excipients or matrix components.

The developed method was therefore found suitable for accurate quantitative estimation of Olaparib.

3.2.6. Sensitivity Studies (LLOQ and LOD)

The sensitivity of the LC–MS method was determined by evaluating the lower limit of quantification (LLOQ) and limit of detection (LOD).

Table No.14: Sensitivity (LLOQ and LOD)

Parameter	Value
LLOQ (ng/mL)	25
LOD (ng/mL)	8–10

The LLOQ value of 25 ng/mL demonstrated the capability of the method to accurately quantify very low concentrations of Olaparib, while the LOD value of approximately 8–10 ng/mL indicated excellent sensitivity.

These results confirmed the suitability of the developed method for trace-level analysis and routine quality control applications.

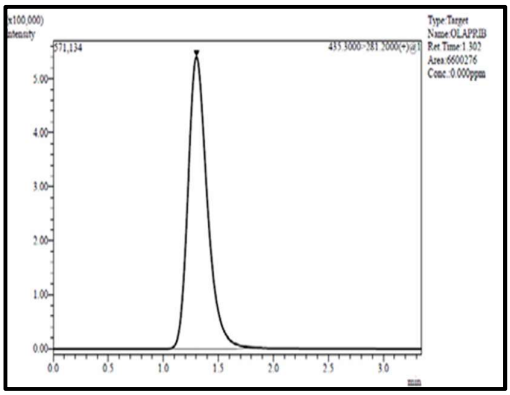

Figure No.9: LLOQ chromatogram of Olaparib.

3.2.7. Stability Studies

The stability of Olaparib was evaluated under various experimental and storage conditions to ensure analyte integrity during sample handling and analysis.

Table No.15: Stability Studies data

Condition	% Stability
Bench-top (6 h)	99.1
Freeze–thaw (3 cycles)	98.7
Short-term	99.3
Long-term	98.9

The bench-top stability result (99.1%) indicated that Olaparib remained stable during routine laboratory handling at room temperature. Freeze–thaw stability (98.7%) demonstrated that repeated freezing and thawing cycles did not significantly affect analyte integrity.

Similarly, short-term (99.3%) and long-term (98.9%) stability studies confirmed that Olaparib remained chemically stable during extended storage periods. All stability values were within acceptable limits, indicating negligible degradation under the tested conditions.

The developed LC–MS method effectively quantified Olaparib without interference from degradation products, demonstrating excellent specificity and robustness.

Overall, the results confirmed that the developed method is reliable and suitable for routine stability studies and quantitative analysis.

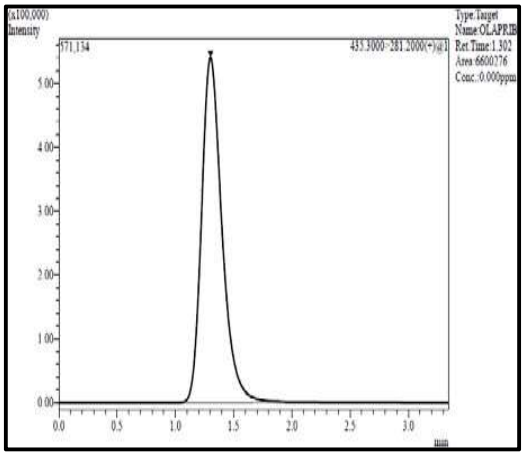

Figure No.9: Representative chromatogram of Olaparib at HQC level.

3.2.8. High Quality Control (HQC) Analysis

High Quality Control (HQC) samples representing the upper concentration range were analyzed to evaluate method performance at higher analyte concentrations. The obtained chromatograms demonstrated excellent peak symmetry, reproducibility, and consistent detector response.

The HQC results confirmed the accuracy and precision of the developed method across the entire calibration range, including concentrations near the upper limit of quantification.

Key Observations

- Molecular ion peak observed at m/z 435
- High sensitivity and selectivity of the developed LC–MS method
- Wide linearity range of 25–5000 ng/mL
- No significant matrix interference observed
- Excellent reproducibility and repeatability
- Stable analyte response under various storage conditions

Conclusion

A sensitive, precise, accurate, and selective LC–MS method was successfully developed and validated for the analysis of Olaparib. The method demonstrated excellent chromatographic performance, strong linearity over a wide concentration range, high sensitivity, and reliable reproducibility.

The developed method also exhibited acceptable accuracy, precision, and analyte stability under various experimental conditions. The absence of matrix interference and excellent system suitability parameters further confirmed the robustness of the analytical procedure.

Therefore, the validated LC–MS method can be effectively applied for routine quantitative estimation, trace-level detection, confirmatory analysis, and stability studies of Olaparib in pharmaceutical formulations and biological samples.

3.3. UV–Visible Spectrophotometric Analysis

3.3.1. Linearity Study:

The development of a UV–Visible spectrophotometric method for quantitative estimation of Olaparib was based on its characteristic ultraviolet absorption properties. A standard stock solution of Olaparib was prepared using methanol as the solvent, followed by suitable serial dilutions to obtain working standard solutions of desired concentrations. The prepared solution was scanned in the wavelength range of 200–400 nm against methanol as blank in order to determine the wavelength of maximum absorbance (λmax).

A distinct absorption peak was observed at 268 nm, indicating the presence of a suitable chromophoric group in the drug molecule. The wavelength of 268 nm was selected as the analytical wavelength

because it exhibited maximum absorbance with good sensitivity and reproducibility.

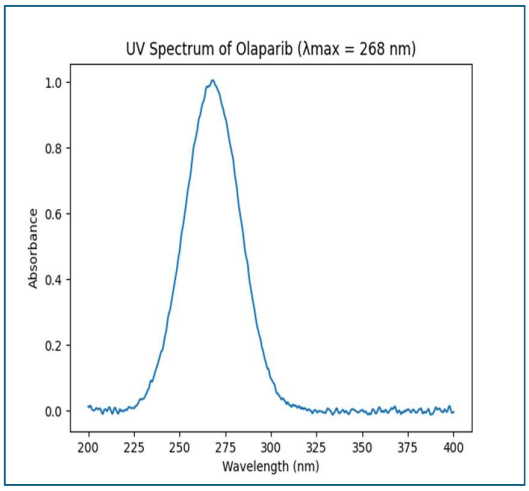

Figure No. 10: UV absorption spectrum of Olaparib showing maximum absorbance (λmax) at 268 nm.

To evaluate the linearity of the developed method, standard solutions were prepared in the concentration range of 5–25 µg/mL. The absorbance of each solution was measured at 268 nm and a calibration curve was constructed by plotting concentration versus absorbance. The results demonstrated a direct proportional relationship between concentration and absorbance, confirming compliance with Beer–Lambert's law within the selected range.

Table No. 16: Calibration Data for UV Spectrophotometric Method

Sr. No.	Concentration (µg/mL)	Absorbance at 268 nm
1	5	0.182
2	10	0.365
3	15	0.548
4	20	0.732
5	25	0.915

The calibration curve obtained from the above data exhibited excellent linearity with a correlation coefficient (R^2) of approximately 0.999, indicating the reliability and suitability of the method for quantitative estimation of Olaparib.

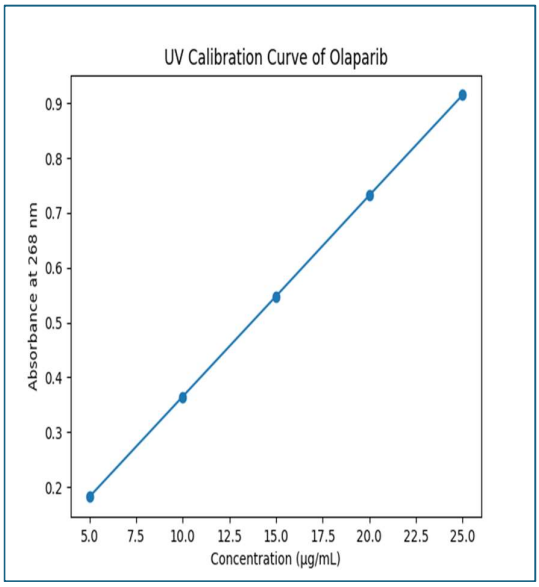

Figure No.11: Calibration curve of Olaparib by UV spectrophotometry (Concentration vs. Absorbance at 268 nm).

Overall, the developed UV spectrophotometric method was found to be simple, rapid, sensitive, accurate, and economical for routine analysis of Olaparib. The method requires minimal sample preparation and provides reliable analytical performance, making it suitable for quality control and research applications.

3.3.2. Precision Study

Table No. 17: Precision Data for UV Spectrophotometric Method

Trial	Absorbance
1	0.548
2	0.550
3	0.547

The precision of the developed UV spectrophotometric method was evaluated by measuring the absorbance of replicate samples under the same experimental conditions. The absorbance values obtained were found to be highly consistent, indicating excellent repeatability of the method. The percentage relative standard deviation (%RSD) was found to be less than 2%, confirming the precision and reproducibility of the analytical procedure.

3.3.3. Accuracy Study

Table No. 18: Accuracy Data (Recovery Studies) for UV Spectrophotometric Method

Level	% Recovery
80%	99.2
100%	100.1
120%	99.8

Accuracy of the method was determined by recovery studies performed at three different concentration levels: 80%, 100%, and 120%. The percentage recovery values were found within the acceptable range of 98–102%, indicating good accuracy of the method and absence of interference from excipients or solvent components.

3.3.5. Limit of Detection (LOD) and Limit of Quantification (LOQ)

Table No. 19: Limit of Detection (LOD) and Limit of Quantification (LOQ) for UV Spectrophotometric Method

Parameter	Value
LOD	~0.4 µg/mL
LOQ	~1.2 µg/mL

The sensitivity of the developed UV spectrophotometric method was evaluated by determining the limit of detection (LOD) and limit of quantification (LOQ). The low values obtained for LOD and LOQ indicate that the method is sufficiently sensitive for detection and quantification of very small concentrations of Olaparib.

4. Conclusion

In the present study, analytical methods using UV–Visible spectrophotometry, RP–HPLC, and LC–MS/MS were successfully developed and validated for the estimation of Olaparib in bulk drug and pharmaceutical dosage forms. The UV spectrophotometric method provided a simple and cost-effective approach for routine analysis, whereas the RP–HPLC method offered excellent chromatographic separation with sharp peak symmetry, satisfactory retention time, and high reproducibility. The LC–MS/MS method demonstrated superior sensitivity and specificity through selective ion monitoring in MRM mode, enabling accurate identification and quantification of Olaparib.

All developed methods were validated according to ICH guidelines for parameters such as linearity, accuracy, precision, specificity, robustness, LOD, and LOQ. The validation results confirmed that the methods were reliable, accurate, precise, sensitive, and reproducible within acceptable limits.

Furthermore, successful application of these methods to pharmaceutical formulations established their suitability for routine quality control and analytical purposes. Overall, the developed UV–Visible spectrophotometric, RP–HPLC, and LC–MS/MS methods can be effectively utilized for regular estimation and quality assessment of Olaparib in pharmaceutical industries and research laboratories.

Discussion

The developed RP-HPLC, LC-MS, and UV spectrophotometric methods demonstrated excellent analytical performance for the estimation of Olaparib. RP-HPLC method provided good peak symmetry, acceptable retention time, and high reproducibility. LC-MS analysis confirmed the molecular identity of Olaparib and exhibited excellent sensitivity for trace-level quantification. The UV spectrophotometric method offered a simple, rapid, and economical approach for routine analysis.

All validation parameters complied with ICH guidelines, confirming the reliability and applicability of the developed methods for pharmaceutical quality control and routine analytical purposes.

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