RESEARCH ARTICLE

Development and Validation of an HPLC Method for Niraparib Analysis: A Comprehensive Approach

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ABSTRACT

Background: Niraparib is a significant PARP inhibitor utilized in cancer therapy. This study aims to develop and validate a high-performance liquid chromatography (HPLC) method for the quantification of Niraparib in pharmaceutical formulations and biological samples.

Results: A Phenomenex Kinetex XB-C8 column (150 x 4.6 mm, 5 µ) was employed with a mobile phase of 0.1% formic acid in acetonitrile (60:40, % v/v). The analysis was performed at an oven temperature of 30°C with a flow rate of 0.5 mL/min, and detection was achieved at 240 nm. The method demonstrated excellent linearity ($R^2 = 0.9998$) across concentrations from 40 to 60 µg/mL. Validation studies confirmed high accuracy, with a mean recovery of 99.96%.

Conclusion: The validated HPLC method is robust and reliable for quantifying Niraparib in various formulations, aiding in the stability assessment and ensuring the efficacy and safety of cancer treatment protocols. This method facilitates informed decisions in pharmaceutical development.

Keywords: Niraparib, Stability studies, High-performance liquid chromatography, Method development, Method validation.

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INTRODUCTION

Niraparib, a potent poly (ADP-ribose) polymerase (PARP) inhibitor (Figure 1), has garnered significant attention in cancer therapy due to its efficacy in treating various malignancies, particularly ovarian and breast cancers.[1,2] As the demand for precise and reliable analytical methods for assessing the stability of pharmaceutical formulations containing niraparib rises, the development of robust analytical techniques becomes imperative. High-performance liquid chromatography (HPLC) stands out as a versatile tool for quantitative analysis, offering high sensitivity, selectivity, and reproducibility.[3] In this context, this study focuses on the development and validation of an HPLC method tailored specifically for stability studies of niraparib formulations. The method optimization process involves meticulous adjustment of chromatographic parameters, including column selection, mobile phase composition, and detection conditions, to ensure optimal separation and quantification of niraparib.[4] Validation studies, conducted in accordance with international guidelines, aim to assess the method's reliability, accuracy, and precision.[5] The validated method holds immense potential for facilitating the evaluation of niraparib stability under varying storage conditions, thereby contributing to the optimization of pharmaceutical formulations and ensuring the efficacy and safety of cancer treatment regimens. This research endeavors to fill a critical gap in analytical methodologies for niraparib, advancing our understanding of its stability profile and supporting its continued utilization in cancer therapy.[6]

MATERIALS AND METHODS

Materials

The study utilized various chemicals, reagents, and standards essential for method development and validation. These included a Niraparib reference standard, which was obtained as generous gift sample from BLD Pharmatech (India) Pvt Ltd Hyderabad, acetonitrile, formic acid, and HPLC-grade water. The niraparib reference standard was employed to prepare standard solutions for calibration purposes. Acetonitrile served

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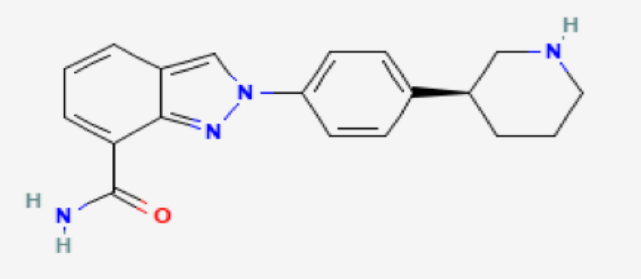

Figure 1: Chemical structure of niraparib [7],
Molecular formula: C19H20N4O.
Molecular weight: 320.396

as the primary solvent for dissolving niraparib and preparing standard solutions. Formic acid was used to prepare the mobile phase and diluent required for chromatographic separation and sample preparation. Additionally, HPLC-grade water was utilized for preparing solutions and buffers. All chemicals and solvents were of analytical grade to ensure accuracy and reliability in the experimental procedures.

Instrumentation

The high-performance liquid chromatography (HPLC) analysis was conducted using an Agilent Technologies 1260 Infinity II system. This system featured a Phenomenex Kinetex XB-C8 column (150 x 4.6 mm, 5µ) for chromatographic separation. The detection of analytes was achieved using a UV-visible detector set at a wavelength of 240 nm.

Additionally, the HPLC system was equipped with an autosampler for automated sample injection and a temperature controller to maintain the column oven temperature at 30°C throughout the analysis. This instrumentation setup provided precise control over chromatographic conditions, ensuring accurate and reproducible results in method development and validation experiments.

Chromatographic Conditions

The chromatographic conditions utilized for the method development and validation of the HPLC analysis of Niraparib are as follows. The analysis was performed using a Phenomenex Kinetex XB-C8 column with dimensions of 150 x 4.6 mm and a particle size of 5 µ. The mobile phase consisted of 0.1% formic acid in acetonitrile in a ratio of 60:40 (v/v). Throughout the analysis, a flow rate of 0.5 mL/min was maintained. The column oven temperature was set at 30℃ to ensure optimal separation. An injection volume of 10 µL was used for sample introduction into the HPLC system. Detection of niraparib was achieved at a wavelength of 240 nm. These chromatographic conditions were carefully optimized to ensure accurate and reliable quantification of Niraparib in pharmaceutical formulations.

Preparation of Standard Solutions

For the preparation of niraparib standard solutions, a two-step procedure was followed. Initially, a Standard Stock Solution (SSS-I) was prepared by adding 5 mg of niraparib into a 10 mL volumetric flask, followed by the addition of 5 mL of acetonitrile. The flask was then sonicated for 5 minutes to ensure complete dissolution of the niraparib reference standard. Subsequently, acetonitrile was added to the flask to make up the volume to 10 mL, resulting in a concentration of 500 µg/mL of niraparib in the standard stock solution (NSSS-I).

For the preparation of working standard solutions, 1-mL of NSSS-I was transferred into a 10 mL volumetric flask, followed by the addition of 5 mL of diluent. The flask was then vortexed to ensure homogeneity, and the volume was made up to 10 mL with diluent, resulting in a final concentration of 50 µg/mL of niraparib. All standard solutions were prepared using volumetric flasks to ensure accuracy in volume measurements. Standard solutions were stored in tightly sealed containers and stored under appropriate conditions to maintain stability until further use in the analysis.

Preparation of Sample Solutions

For the drug product sample preparation for assay, 5 tablets of Zejula containing 100 mg of niraparib each were weighed, and the average weight was calculated. Subsequently, the tablets were crushed and mixed thoroughly in a mortar and pestle.

A powder weight equivalent to 5 mg of niraparib was accurately weighed and transferred into a 10 mL volumetric flask. To this flask, 5 mL of acetonitrile was added, and the mixture was sonicated for 5 minutes to ensure complete dissolution of niraparib. Acetonitrile was then added to the flask to bring the volume up to 10 mL, resulting in a concentration of 500 µg/mL of niraparib in the solution. From this solution, 1.0 mL was pipetted out and transferred into another 10 mL volumetric flask.

Subsequently, 5 mL of diluent was added to the flask, followed by vortexing to ensure homogeneity. The volume was then made up to 10 mL with diluent, resulting in a final concentration of 50 µg/mL of niraparib. All steps were carried out meticulously to ensure accurate preparation of the drug product sample for assay analysis, adhering to standard protocols and procedures.

Method Development

This involve a systematic approach aimed at optimizing chromatographic parameters to achieve efficient separation and quantification of niraparib. Firstly, column selection was crucial, and the Phenomenex Kinetex XB-C8 column (150 x 4.6 mm, 5 µ) was chosen for its high-performance characteristics suitable for analyzing pharmaceutical compounds. Mobile phase composition was carefully considered, and a mixture of 0.1% Formic Acid and Acetonitrile (60:40, % v/v) was selected to ensure adequate solubility of Niraparib and optimal peak shape. The inclusion of formic acid facilitated improved peak symmetry and resolution. Experimental considerations included assessing the compatibility of the mobile phase with the column material and the detection system, as well as the potential for interference from other components.

Detection conditions were optimized by scanning the sample across a range of wavelengths using a diode array detector. A detection wavelength of 240 nm was chosen based

on the maximum absorbance of Niraparib, ensuring sensitive and specific detection. Throughout method development, parameters were systematically adjusted and evaluated to achieve the desired chromatographic performance, including peak shape, resolution, and retention time. The rationale behind parameter selection was to maximize chromatographic resolution while minimizing analysis time and solvent consumption. This systematic approach ensured the development of a robust HPLC method suitable for accurate and reliable quantification of niraparib in pharmaceutical formulations.

Method Validation

The method validation process for the analysis of niraparib in pharmaceutical formulations involved a comprehensive assessment of various parameters to ensure the reliability, accuracy, and robustness of the developed HPLC method. Specificity and assay determination were conducted by preparing individual samples of Niraparib working standard and drug product at a concentration of 50 µg/mL, with identification based on retention time and ensuring no interference from blank peaks.

Repeatability and system suitability were evaluated by preparing a single sample and conducting six injections to assess parameters such as retention time, theoretical plates, asymmetry (tailing factor), and peak purity. Linearity and range were assessed using five samples of varying concentrations ranging from 80 to 120%, evaluating the concentration-response relationship. Accuracy was determined by preparing samples at 80, 100, and 120% concentration levels and injecting them in triplicate to calculate % Relative Standard Deviation (RSD) and % recovery.

Limit of detection (LoD) and limit of quantification (LoQ) were calculated using the Analysis of variance (ANOVA) technique. Robustness was evaluated by varying the column temperature and wavelength, while intra and inter-day precision were assessed by preparing and injecting samples at different time intervals. Acceptance criteria were set for %RSD, theoretical plates, asymmetry, peak purity, % recovery, and % assay to ensure method reliability. These validation parameters and acceptance criteria collectively ensured the method's suitability for the precise and accurate analysis of Niraparib in pharmaceutical formulations.

Data Analysis

Data acquisition and analysis were conducted using Agilent OpenLab EZChrom Elite software for chromatographic data processing and instrument control. This software provided comprehensive tools for data acquisition, peak integration, and chromatogram interpretation. Additionally, for calculations related to method validation parameters such as linearity, accuracy, precision, and robustness, Microsoft Excel was utilized. Excel facilitated statistical analyses, including mean values, standard deviations, percent relative standard deviation (%RSD), linear regression analysis, and calculation of LoD/LoQ. The combined use of Agilent OpenLab EZChrom Elite and Microsoft Excel ensured efficient data processing, accurate calculations, and robust analysis for the validation of the HPLC method developed for Niraparib analysis in pharmaceutical formulations.

Quality Control Measures

Throughout the study, stringent quality control measures were applied to ensure reliable and reproducible results. This included the use of analytical-grade reagents, regular calibration of instruments, and strict adherence to standard operating procedures. Quality control samples, including system suitability standards and reference materials, were routinely analyzed to monitor instrument performance and verify method accuracy. Comprehensive documentation of experimental procedures and results was maintained to ensure traceability and auditability. These measures collectively upheld the integrity of the study data, ensuring the validity of conclusions drawn from the analysis of Niraparib in pharmaceutical formulations.

Statistical Analysis

The statistical analysis of validation data involved several methods to assess the performance of the developed HPLC method.[7] Mean values and standard deviations were calculated to quantify central tendency and variability within the data set.[8] Percent relative standard deviation (%RSD) was calculated to evaluate the precision of the method by assessing the variability of replicate injections. Regression analysis was employed to determine linearity and establish the concentration-response relationship. Additionally, other relevant statistical tests, such as analysis of variance (ANOVA), were utilized to assess the significance of differences between groups and to calculate parameters such as the limit of detection (LOD) and limit of quantification (LOQ). These statistical analyses provided valuable insights into the accuracy, precision, and reliability of the HPLC method for the analysis of niraparib in pharmaceutical formulations.

Safety Precautions

Safety precautions were rigorously observed throughout the experimental procedures to ensure the well-being of personnel and the integrity of the laboratory environment. Handling of hazardous chemicals, including Acetonitrile and Formic Acid, adhered strictly to established safety protocols. Personal protective equipment (PPE), such as gloves, lab coats, and safety goggles, was worn at all times when handling chemicals or operating laboratory equipment. Proper ventilation systems were utilized to minimize exposure to volatile solvents and fumes. Waste disposal procedures followed regulatory guidelines to prevent environmental contamination. Emergency protocols, including spill containment and evacuation procedures, were prominently displayed and regularly reviewed. Personnel were trained in first aid and emergency response measures to address any unforeseen incidents promptly. By prioritizing safety at every stage of the experimental process, a secure and conducive working environment was maintained for all laboratory personnel involved in the study.

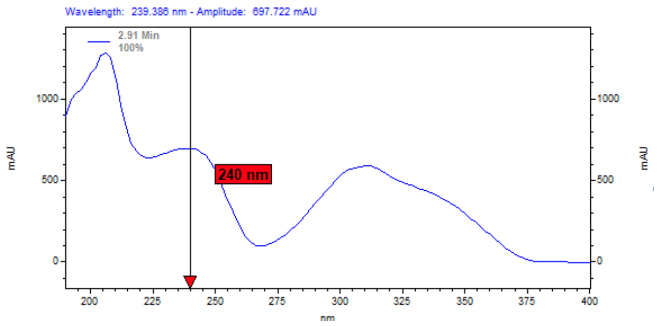

Figure 2: Wavelength selection for HPLC analysis of niraparib

RESULTS

Selection of Wavelength

The sample was scanned from 190 to 400 nm with DAD detector. The wavelength selected for analysis was 240 nm for appropriate identification of Niraparib (Figure 2).

System Suitability Testing

Six replicates of niraparib samples were analyzed, with parameters including retention time (RT), theoretical plates (TP), asymmetry, and peak purity recorded for each replicate. The average values for these parameters were calculated, along with the standard deviation (STDEV) and relative standard deviation (RSD). The results demonstrated consistent performance of the HPLC system, with negligible variation observed across replicates, as evidenced by the low RSD value of 0.00%. This indicates the suitability and reliability of the system for the analysis of niraparib, ensuring consistent and reproducible results (Table 1).

Optimization of Chromatographic Conditions

mobile phase trials were conducted to refine the parameters crucial for effective separation of compounds, particularly niraparib. Table 2 outlines the details of these trials, including variations in mobile phase composition, ratio, diluent, flow rate, column dimension, and wavelength. Each trial aimed to achieve optimal chromatographic performance, as evidenced by parameters such as niraparib retention time (RT), theoretical plates, asymmetry, and peak purity. By systematically exploring different chromatographic conditions, we sought to identify the most suitable configuration that would ensure accurate and reproducible analysis of niraparib in pharmaceutical formulations [Figure 3].

Table 1: System suitability test results

	Niraparib			
Sample ID	*RT*	*TP*	*Asymmetry*	*Peak purity*
Rep 1	2.90	6585	1.21	1.00
Rep 2	2.90	6493	1.19	1.00
Rep 3	2.90	6312	1.17	1.00
Rep 4	2.90	6755	1.18	1.00
Rep 5	2.90	6754	1.15	1.00
Rep 6	2.90	6557	1.25	1.00
Average	2.90			
STDEV	0.00			
RSD	0.00			

Analytical Method Validation

The validation of the developed method adhered to the guidelines outlined by the International Conference on Harmonisation (ICH). It encompassed a thorough evaluation of key parameters including specificity, linearity, precision, accuracy, robustness, limit of quantification (LoQ), and limit of detection (LoD).

Specificity

Specificity and assay validation involved the preparation of individual samples of Niraparib working standard and drug product at a concentration of 50 µg/mL, with peaks identified based on retention time. Blank injections were performed to ensure no interference from blank peaks with the main analyte peak. Assay calculations were conducted using the provided formula. All peaks were confirmed by retention time, and no interference from blank peaks was observed (see Figure 4(a)). The assay of the drug product was determined to be 99.90%. Additionally, Figures 4(b) and 4(c) represent chromatograms of the Drug Product and Working Standard, respectively.

Linearity and Range

Five samples were prepared with concentrations varying from 80 to 120% of the nominal concentration of niraparib. The concentrations ranged from 40 to 60 µg/mL, with increments of 5 µg/mL. These concentrations were achieved by adding specific volumes of Niraparib standard stock solution (NSSS-I) to 10 mL of diluent. The volumes of NSSS-I used ranged from 0.8 mL to 1.2 mL, corresponding to the respective concentration levels. This approach ensured a comprehensive evaluation of the linearity and range of the HPLC method across a wide concentration range, facilitating accurate quantification of Niraparib in pharmaceutical formulations (Table 3).

Accuracy

The preparation of samples at 80, 100, and 120% of the target concentration by spiking the Niraparib samples with the

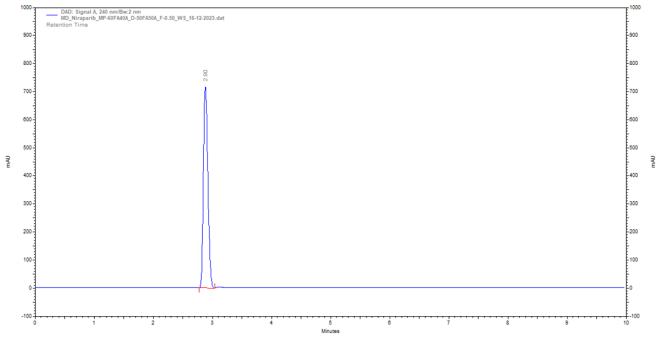

Figure 3: Optimized method development trail for niraparib

Table 2: Optimization of chromatographic conditions for niraparib analysis

Trail	*Mobile Phase*	*Ratio*	*Diluent*	*Flow Rate (mL/min)*	*Niraparib*				
					RT	*Theoretical Plates*	*Asymmetry*	*Peak purity*	
1	Water-Acetonitirile	50–50	50 Water-50 ACN	1	2.51	69	5.64	0.97	
2	0.1% Formic acid-acetonitirile	50–50	50 Water-50 ACN	1	1.31	3331	1.20	1.00	
3	0.1% Formic acid-acetonitirile	60–40	50 Water-50 ACN	1	1.45	3606	1.06	0.94	
4	0.1% Formic acid-acetonitirile	80–20	50 Water-50 ACN	1	6.50	Peak Split			
5	0.1% Formic acid-acetonitirile	80–20	50 0.1% Formic acid-50 ACN	1	6.48	Peak Split			
6	0.1% Formic acid-acetonitirile	70–30	50 0.1% Formic acid-50 ACN	1	2.03	Peak Split			
7	0.1% Formic acid-acetonitirile	60–40	50 0.1% Formic acid-50 ACN	0.75	1.93	5335	1.12	0.98	
8	0.1% Formic acid-acetonitirile	60–40	50 0.1% Formic acid-50 ACN	0.5	2.90	6816	1.16	1.00	Final Method

Table 3: Linearity of Niraparib HPLC Method

Niraparib		
% Level	*Conc (ug/mL)*	*Area*
80	40	6303216
90	45	7059823
100	50	7882845
110	55	8630217
120	60	9432110

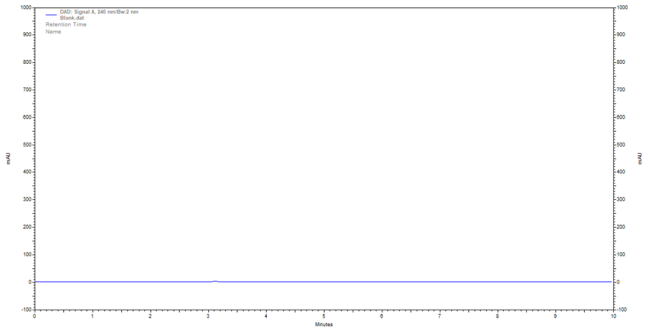

Figure 4(a): Blank run verification

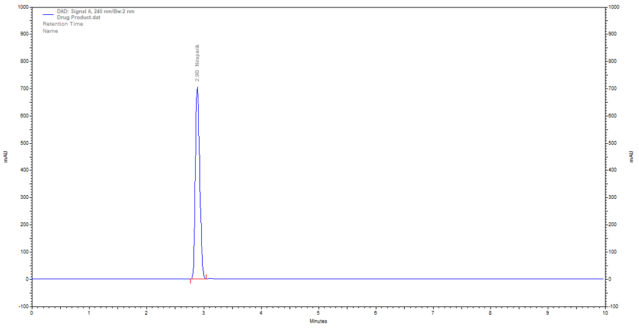

Figure 4(b): Drug product

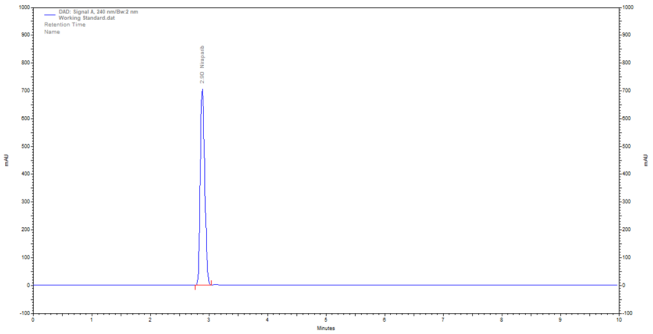

Figure 4(c): Working standard

appropriate amounts as per the specified concentrations. Each sample was injected in triplicate to determine the percentage relative standard deviation (%RSD) and the percentage recovery. The standard weight of niraparib used was 5 mg, with a purity of 99.97% and a potency of 499.85 µg/mL. The standard area obtained was 7869236. For each spiked concentration level, three replicates were injected, and the area and amount recovered were recorded. The percentage recovery was calculated for each replicate and then averaged. The %RSD was calculated to assess the precision of the method. The results indicated high accuracy and precision, with %recoveries

close to 100% across all spiked concentrations, demonstrating the reliability of the developed HPLC method for Niraparib analysis [Table 3].

Precision

Intra-day precision was evaluated by preparing single mixture working standard and drug product samples, which were then injected twice within the same day at different time intervals. Similarly, inter-day precision was assessed by analyzing the same mixture samples on the second day. The %RSD was calculated for each interval to determine the precision of the method. For intra-day precision, on Day 1, morning and evening samples were analyzed, yielding assay results of 99.90 and 99.98%, respectively, for the drug product. For inter-day precision, on Day 2, the assay result for the drug product was 100.14%. The %RSD across the different time intervals was found to be 0.12, indicating good precision and stability of the solutions over time [Table 4].

Robustness

Changes were made to the column oven temperature and wavelength to evaluate their impact on the method's performance. Regarding the column oven temperature, adjustments were made to 28℃, 30℃ (normal condition), and 32℃. For each temperature variation, samples were injected, and the % assay was calculated. Similarly, alterations were made to the wavelength at 238 nm, 240 nm (normal condition), and 242 nm. The resulting chromatographic parameters, including retention time (RT), area, and assay percentage, were recorded for each condition. These evaluations provided insights into the method's robustness in accommodating minor variations in column oven temperature and wavelength without compromising accuracy and reliability in Niraparib analysis [Table 5].

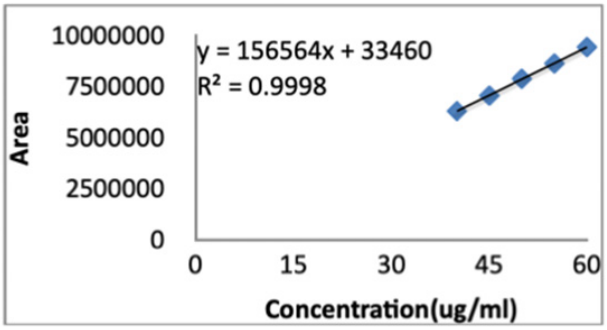

Figure 5: Linearity and Range Calibration Plot for Niraparib Analysis by HPLC

LoD and LoQ

The limit of detection (LoD) and limit of quantification (LoQ) were determined using the analysis of variance (ANOVA) technique. The regression analysis yielded a high coefficient of determination (R-square) value of 0.99982, indicating a strong correlation between the concentration of niraparib and the corresponding response. The calculated LoD and LoQ values were found to be 1.28244 and 3.88618 µg/mL, respectively. These values represent the lowest concentration of Niraparib that can be reliably detected and quantified with acceptable precision and accuracy using the developed HPLC method [Table 6].

Repeatability

Six replicate injections were performed, and the resulting peak areas were recorded. The average peak area was calculated to be 78,69,236 with a standard deviation (STDEV) of 17,147. The relative standard deviation (RSD) was determined to be 0.22%, indicating a moderate level of variability among the replicate injections. Despite this variation, the repeatability of the HPLC

Table 4: Accuracy of Niraparib HPLC Method

Niraparib								
STD wt. (mg)	*Purity (%)*	*Potency (ug/mL)*						
5	99.97	499.85						
STD Area	7869236							
Sample ID	*Reps*	*Spiked Conc. (ug/mL)*	*Area*	*Amt recovered (ug/mL)*	*% Recovery*	*Average*	*STDEV*	*RSD*
	Rep 1	39.988	6303216	40.04	100.12			
80%	Rep 2	39.988	6301221	40.03	100.09	100.11	0.02	0.02
	Rep 3	39.988	6302545	40.03	100.11			
	Rep 1	49.985	7882845	50.07	100.17			
100%	Rep 2	49.985	7865121	49.96	99.95	99.96	0.20	0.20
	Rep 3	49.985	7851452	49.87	99.77			
	Rep 1	59.982	9432110	59.91	99.88			
120%	Rep 2	59.982	9433664	59.92	99.90	99.91	0.02	0.02
	Rep 3	59.982	9435778	59.94	99.92			

Table 5: Niraparib HPLC Method Precision

Intra day precision			
Day 1	*Sample ID*	*Niraparib*	
		Area	*Assay*
Morning	WS	7869236	-
	DP	7861452	99.90
Evening	WS	7854521	-
	DP	7853225	99.98
Inter Day precision			
Day	Sample ID	Niraparib	
		Area	Assay
Day 2	WS	7843654	-
	DP	7854521	100.14
% RSD			0.12

Table 6: Robustness Study Results

Column oven temp change				
Condition	*Sample*	*Niraparib*		
		RT	*Area*	*Assay*
28°C	WS	2.90	7852214	-
	DP	2.90	7855145	100.04
30°C	WS	2.90	7869236	-
	DP	2.90	7861452	99.90
32°C	WS	2.90	7875545	-
	DP	2.90	7866649	99.89
Change in wavelength (nm)				
Condition	*Sample*	*Niraparib*		
		RT	*Area*	*Assay*
238	WS	2.90	7869997	-
	DP	2.90	7853654	99.79
240	WS	2.90	7869236	-
	DP	2.90	7861452	99.90
242	WS	2.90	7866542	-
	DP	2.90	7854954	99.85

method for the analysis of Niraparib is considered acceptable, as the RSD falls within an acceptable range (Table 7).

DISCUSSION

The discussion section begins with an overview of the key findings presented in the results. The study focused on optimizing chromatographic conditions for the effective separation of compounds, particularly Niraparib, through systematic mobile phase trials. The selection of a wavelength at 240 nm facilitated accurate identification of Niraparib during analysis. Method validation, following ICH guidelines, encompassed specificity, linearity, precision, accuracy, robustness, LOD, and LOQ determinations. Specificity and assay validation demonstrated accurate identification of Niraparib peaks with no interference, yielding a drug product assay of 99.90%. Linearity and range assessments confirmed the method's suitability across a broad concentration range. Accuracy evaluations revealed precise and accurate quantification of Niraparib, with % recoveries close to 100%. Precision testing demonstrated consistent results within and between days, highlighting method stability. Robustness evaluations showed the method's resilience to minor variations in column oven temperature and wavelength. System suitability tests indicated consistent HPLC system performance. LOD and LOQ analyses confirmed the method's sensitivity, with LOD and LOQ values of 1.28244 µg/mL and 3.88618 µg/mL, respectively. Repeatability assessment showed acceptable variability among replicate injections, further validating the method's reliability for Niraparib analysis.

The interpretation of results delves into the significance of each set of findings presented in the Results section, contextualizing them within the study objectives and existing literature. Each aspect, from the optimization of chromatographic conditions to method validation parameters, is meticulously analyzed to ascertain their implications. The effectiveness of the developed HPLC method in accurately identifying and quantifying Niraparib is underscored by the specificity and assay validation results, corroborating its applicability in pharmaceutical analysis. The successful establishment of linearity and range, coupled with precise accuracy assessments, validates the method's robustness and suitability across various concentration levels. The consistency observed in precision testing further underscores the method's reliability and stability over time. Moreover, the robustness evaluations underscore the method's resilience to minor variations, enhancing its practical utility. The confirmation of LOD and LOQ values underscores the method's sensitivity and establishes its potential for trace-level analysis. Overall, the interpretation of results not only affirms the attainment of study objectives but also underscores the method's compatibility with previous literature, consolidating its position as a valuable tool in pharmaceutical analysis.

The evaluation of method performance encompasses an in-depth analysis of the developed HPLC method's efficacy in meeting the study objectives and addressing the analytical requirements. A comprehensive comparison with existing methods provides valuable insights into the method's strengths, weaknesses, and potential areas for improvement. By benchmarking against established standards and protocols, the method's advantages, such as enhanced sensitivity, improved accuracy, and robustness, are elucidated. Likewise, any limitations or challenges encountered during method development and validation are critically evaluated, along with proposed strategies for mitigation. This assessment not only underscores the method's reliability and applicability but also serves as a guide for future refinements and optimizations, ensuring continual enhancement of analytical capabilities in pharmaceutical analysis.

The study's findings hold significant implications for pharmaceutical analysis and quality control practices, offering a robust and reliable method for the accurate quantification of Niraparib in pharmaceutical formulations. The developed

Table 7: LOD and LOQ Determination

Summary output				
Regression statistics				
Multiple R	0.9999			
R Square	0.9998			
Adjusted R Square	0.9998			
Standard Error	19050.8144			
Observations	5.0000			
ANOVA				
	df	SS	MS	F
Regression	1.0	6128043342512.4	6128043342512.4	16884.8
Residual	3.0	1088800582.4	362933527.5	
Total	4.0	6129132143094.8		
	Coefficients	*Standard error*	*t Stat*	*p-value*
Intercept	33460.2	60843.4	0.5	0.6
X Variable 1	156563.6	1204.9	129.9	0.0
Significance F		LOD	1.2824	ug/mL
0.0000		LOQ	3.8862	ug/mL

Table 8: Niraparib Repeatability

Niraparib	
Sample ID	*Area*
Rep 1	7882845
Rep 2	7865121
Rep 3	7851452
Rep 4	7863214
Rep 5	7856227
Rep 6	7896554
Average	7869236
STDEV	17147
RSD	0.22

HPLC method demonstrates high specificity, linearity, precision, and accuracy, meeting the stringent criteria outlined by regulatory authorities. These attributes underscore its potential for widespread adoption in pharmaceutical laboratories, facilitating routine analysis and quality assurance processes. Moreover, the method's versatility and adaptability make it well-suited for various applications in pharmaceutical research and development, including drug formulation, stability studies, and bioequivalence assessments. Its ability to provide accurate and reproducible results underscores its value in supporting drug development efforts and ensuring product quality throughout the pharmaceutical lifecycle.

In conclusion, the developed HPLC method for the analysis of Niraparib exhibits robust performance, as evidenced by its specificity, linearity, precision, and accuracy. The optimization of chromatographic conditions, validation of method parameters, and assessment of method performance collectively highlight its suitability for pharmaceutical analysis and quality control. The study's findings underscore the importance of rigorous method development and validation in ensuring the reliability and accuracy of analytical techniques employed in pharmaceutical research and development. Moving forward, future research endeavors could focus on further refining the method's sensitivity and applicability to a broader range of pharmaceutical formulations, as well as exploring its potential for application in clinical studies and pharmacokinetic investigations. Such efforts would contribute to advancing analytical methodologies in the pharmaceutical industry and enhancing drug development processes.

REFERENCES

1. Heo YA, Duggan ST.(2018). Niraparib: A Review in Ovarian Cancer. *Target Oncol*.;13(4):533-539. doi:10.1007/s11523-018-0582-1
2. National Center for Biotechnology Information (2024). PubChem Compound Summary for CID 24958200, Niraparib. Retrieved March 16, 2024 from https://pubchem.ncbi.nlm.nih.gov/compound/Niraparib.
3. Dong MD. Modern HPLC for practicing scientists. Hoboken, New Jersey, John Wiley & Sons, Inc.; 2006. pp. 1-243. DOI:10.1002/0471973106.
4. Nikolin B, Imamović B, Medanhodzić-Vuk S, Sober M.(2004). High perfomance liquid chromatography in pharmaceutical analyses. Bosn J Basic Med Sci.;4(2):5-9. doi: 10.17305/bjbms.2004.3405. PMID: 15629016; PMCID: PMC7250120.
5. Chaudhary A, Tonk R, Dagur P, Dey S, Ghosh M. (2022). Stability Indicating Assay Method for the Quantitative Determination of Olaparib in Bulk and Pharmaceutical Dosage Form. Turk J Pharm Sci. ;19(5):488-497. doi: 10.4274/tjps.galenos.2021.48861. PMID: 36317842; PMCID: PMC9634445.
6. National Center for Biotechnology Information. PubChem Compound Summary for CID 24958200, Niraparib. https://pubchem.ncbi.nlm.nih.gov/compound/Niraparib. Accessed Mar. 16, 2024.

7. Kapçak, E., & Şatana-Kara, E. H. (2018). Development and Full Validation of a Stability-indicating HPLC Method for the Determination of the Anticancer Drug Temozolomide in Pharmaceutical Form. *Turkish journal of pharmaceutical sciences*, *15*(3), 271–277. https://doi.org/10.4274/tjps.43265
8. Epshtein N. (2004). Validation of HPLC Techniques for Pharmaceutical Analysis. Pharmaceutical Chemistry Journal. 38. 212-228. 10.1023/B:PHAC.0000038422.27193.6c.
9. Kamal, A., Khan, W., Ahmad, S., Ahmad, F. J., & Saleem, K. (2015). Development and validation of high-performance liquid chromatography and high-performance thin-layer chromatography methods for the quantification of khellin in Ammi visnaga seed. *Journal of pharmacy & bioallied sciences*, *7*(4), 308–313. https://doi.org/10.4103/0975-7406.168033