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Development and Validation of RP-HPLC Assay Method for Estimation of Rucaparib in active ingredient and Marketed Formulation

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Abstract

Rucaparib is an anti-cancer PARP inhibitor that is marketed under the Nuparp brand. Rucaparib is available in the market as a tablet. A susceptible HPLC method is required to quantify rucaparib as an active ingredient and formulation. The study aimed to develop a novel reverse phase-HPLC stability indicating technique for Rucaparib quantification. Utilizing a Symmetry ODS (C18) column (250 mm x 4.6 mm, 5 µm) and a mobile phase consisting of phosphate buffer (0.02M, pH 2.8) and acetonitrile (48:52% v/v), an isocratic, RP-HPLC technique was developed. This method was developed at a flow rate of 1.0 ml/min, with detection at 248 nm using a UV detector. ICH guideline was followed for validation of this method. This method was highly sensitive and reproducible. Accuracy was in the range of 98-102%. The method was robust and rugged in different conditions. The suggested RP-HPLC approach offers simplicity and robustness for pharmaceutical applications and is effective, economical, and appropriate for regular Rucaparib analysis in quality control under stability circumstances.

Keywords: Rucaparib, RP-HPLC, PARP Inhibitor, Validation, Assay method.

INTRODUCTION

A medication called rucaparib is taken orally to treat cancer. It is a member of the tricyclic indole class of chemicals and functions by blocking particular enzymes termed poly (ADP-ribose) polymerases (PARP1, PARP2, and PARP3). These enzymes are essential for mending cells' damaged DNA. Rucaparib stops cancer cells from fixing their DNA by interfering with their function, which results in cell death. Because of this, the medication works well to intensify the effects of radiation and chemotherapy.

By binding to the PARP enzymes, rucaparib prevents cancer cells' DNA damage from being repaired. The cells become unstable and ultimately perish as a result of the accumulation of DNA strand breakage. As a result, the medication increases the effectiveness of radiation and chemotherapy while also increasing resistance in tumor cells.

PARP enzymes are normally activated when DNA damage occurs. They help repair the damage by attaching ADP-ribose molecules to nuclear proteins, which signals other proteins to start the repair process. However, in many cancers, this repair system becomes faulty, allowing cancer cells to grow uncontrollably. Rucaparib disrupts this process, making it a valuable treatment option.

Rucaparib is primarily used to treat advanced ovarian cancer, especially in patients with BRCA gene mutations. It is prescribed for adults with recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer who have responded (partially or completely) to platinum-based chemotherapy.

While Rucaparib is generally well-tolerated, it may cause temporary increases in liver enzyme levels. However, it has not been linked to serious liver damage.

The chemical name of Rucaparib, according to IUPAC, is **6-fluoro-2-[4-(methylaminomethyl)phenyl]-3,10-diazotricyclo[6.4.1.0^4,13^]trideca-1,4,6,8(13)-tetraen-9-one**.

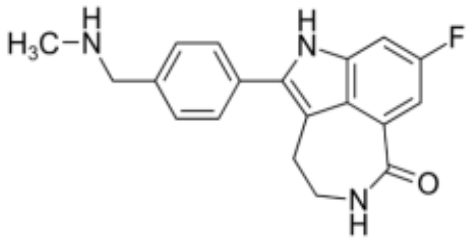

Fig-1: Chemical Structure of Rucaparib

Scientific studies have used various methods to analyze Rucaparib in both bulk and pharmaceutical formulations. Techniques such as reverse-phase high-performance liquid chromatography (RP-HPLC) and liquid chromatography-mass spectrometry (LC-MS) have been successfully applied for its determination in biological fluids.

In this study, we have developed a simple, fast, precise, and reliable stability-indicating liquid chromatography method to determine Rucaparib in its pure form and pharmaceutical products. This method follows the guidelines set by the International Council for Harmonisation (ICH).

MATERIALS AND METHODS

Table-1: List of Instrument used

Instruments/Equipments/Apparatus
Waters HPLC with Empower2 Software with Isocratic with UV-Visible Detector.
ELICO SL-159 UV – Vis spectrophotometer
Electronic Balance **(SHIMADZU ATY224)**
Ultra Sonicator **(Wensar wuc-2L)**
Thermal Oven

Symmetry RP C18, 5µm, 250mm x 4.6mm i.d.
PH Analyzer **(ELICO)**
Vacuum filtration kit **(BOROSIL)**

Table-2: List of Chemicals used

Name	Specifications		Manufacturer/Supplier
	Purity	Grade	
Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
Methanol	99.9%	HPLC	Loba Chem; Mumbai
Dipotassium Hydrogen orthophosphate	96%	L.R.	Sd fine-Chem ltd; Mumbai
Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai
Potassium dihydrogen orthophosphate	99.9%	L.R.	Sd fine-Chem ltd; Mumbai
Sodium hydroxide	99.9%	L.R.	Sd fine-Chem ltd; Mumbai
Hydrochloric acid	96%	A.R.	Sd fine-Chem ltd; Mumbai
3% Hydrogen Peroxide	96%	A.R.	Sd fine-Chem ltd; Mumbai

Selection of Wavelength

The standard and sample stock solutions were prepared separately by dissolving both the standard and sample in a suitable solvent, followed by dilution with the same solvent used in the mobile phase. After optimizing all conditions, the solutions were analyzed using UV spectroscopy.

A UV scan was performed within the wavelength range of 200 to 400 nm to determine the maximum absorbance (λmax) of Rucaparib. Identifying this peak wavelength is essential, as it allows the same wavelength to be used in the HPLC UV detector for accurate measurement of Rucaparib.

Preparation of Standard Solution

1. Accurately weigh **10 mg** of Rucaparib working standard and transfer it into a clean, dry **10 mL volumetric flask**.
2. Add approximately **7 mL of methanol** and sonicate the solution to ensure complete dissolution and remove any air bubbles.
3. Once dissolved, add more methanol to bring the volume up to **10 mL** (marked level on the flask).
4. Take **0.5 mL** of this stock solution and transfer it into another **10 mL volumetric flask**.
5. Dilute it to **10 mL** with methanol to obtain the final working solution.

Procedure

1. Inject the sample into the HPLC system while adjusting the chromatographic conditions.
2. Record the chromatograms and observe the peak elution.
3. Identify the optimal conditions for proper peak separation.
4. Once the ideal conditions are established, proceed with validation as per **ICH guidelines**.

Preparation of Sample Solution

1. Take **twenty tablets** and calculate the average weight as per **Indian Pharmacopoeia (I.P.)** guidelines.
2. Finely powder and **triturate** the tablets to ensure uniform mixing.
3. Weigh a portion of the powdered sample equivalent to **10 mg of Rucaparib** and transfer it into a clean, dry **10 mL volumetric flask**.
4. Add **7 mL of HPLC-grade methanol** and sonicate for **15 minutes** to dissolve the sample completely.
5. After sonication, make up the volume to **10 mL** using the same solvent.
6. Take **1 mL** of this solution and further dilute it to **10 mL** with HPLC-grade methanol.
7. From this diluted solution, take **0.5 mL**, dilute it to **10 mL**, and filter it through a **0.45 µm membrane filter**.
8. Finally, sonicate the filtered solution to remove any dissolved gases before analysis.

Preparation of 0.02M Potassium Dihydrogen Orthophosphate Solution

1. Accurately weigh **2.72172 grams** of **potassium dihydrogen orthophosphate**.
2. Transfer the weighted compound into a **1000 mL beaker**.
3. Add a sufficient amount of **HPLC-grade water** and stir until completely dissolved.
4. Add more **HPLC-grade water** to make the final volume **1000 mL**.
5. Adjust the **pH to 2.80** using **diluted orthophosphoric acid**.

Preparation of Mobile Phase

1. Take **480 mL (48%)** of the prepared phosphate buffer solution.
2. Add **520 mL (52%)** of **HPLC-grade acetonitrile** and mix thoroughly.
3. Degas the solution by placing it in an **ultrasonic water bath** for **15 minutes**.
4. Filter the prepared mobile phase through a **0.45 µm membrane filter** using vacuum filtration.

Optimization of Chromatographic Conditions

The chromatographic conditions were optimized by testing various parameters, including:

- **Different columns** to achieve better separation.
- **Different mobile phase compositions** for improved peak resolution.
- **Varying flow rates** to determine the best efficiency.
- **Different detection wavelengths** to enhance sensitivity.
- **Various diluents for sample preparation** to ensure accuracy and stability.

Each parameter was carefully adjusted to achieve optimal chromatographic performance.

RESULTS AND DISCUSSION

Method Development and its Validation for Rucaparib by RP-HPLC Method Development:

Selection of Wavelength

During the UV scanning of the **Rucaparib solution**, the maximum absorbance (**λmax**) was observed at **248 nm**. The UV spectrum was recorded using an **ELICO SL-159 UV-Vis spectrophotometer**, model **UV-2450**. The scanned UV spectrum is attached in the following page.

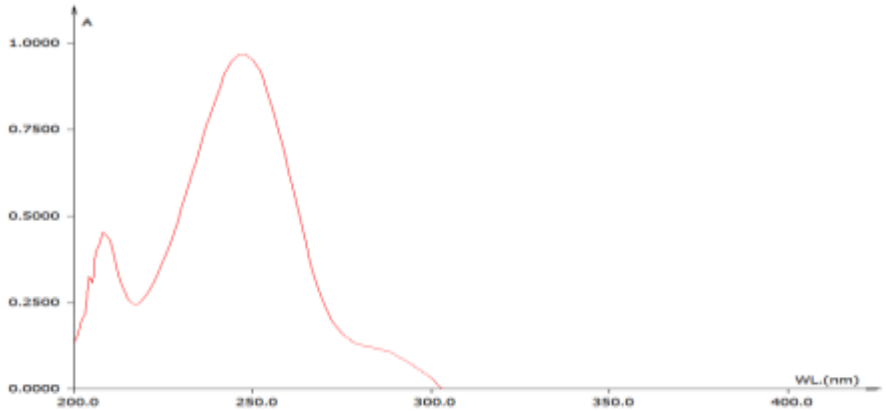

Fig-2: UV Spectrum for Rucaparib

Summary of Optimized Chromatographic Conditions:

The Optimum conditions obtained from experiments can be summarized as below:

Table-3: Summary of Optimised Chromatographic Conditions

Mobile phase	Phosphate Buffer (0.02M): Acetonitrile = 48:52 (pH-2.80)
Column	Symmetry ODS (C18) RP Column, 250 mm x 4.6 mm, 5µm
Column Temperature	Ambient
Detection Wavelength	248 nm
Flow rate	1.0 ml/ min
Run time	08 min
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	20µl
Mode of Elution	Isocratic
Retention time	3.649 minutes

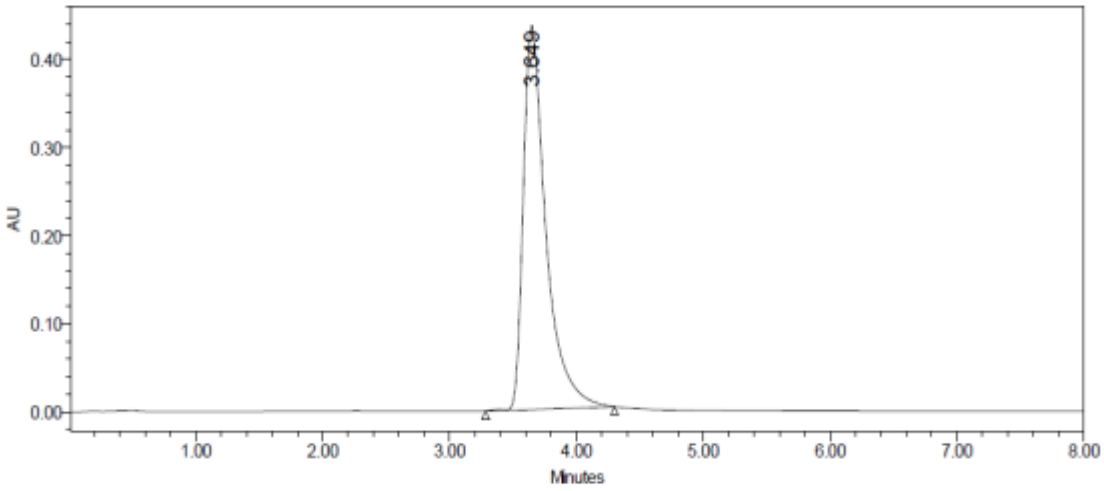

Fig-3: Chromatogram of Rucaparib in Optimized Chromatographic Condition

Validation of Analytical Method

Once the chromatographic and experimental conditions were finalized, the method was validated by assessing key parameters, including:

- **Specificity** – Ensuring the method detects only Rucaparib without interference.
- **System Suitability** – Verifying the system's performance for accurate results.
- **Linearity** – Checking the consistency of response over different concentrations.
- **Precision** – Evaluating repeatability and reproducibility of results.
- **Accuracy** – Measuring how close the obtained values are to the true values.
- **Robustness** – Testing the method's reliability under small variations in conditions.
- **Limit of Detection (LOD)** – Determining the lowest amount of Rucaparib that can be detected.
- **Limit of Quantitation (LOQ)** – Establishing the minimum concentration that can be accurately quantified.

1. Accuracy

Preparation of Standard Solution:

1. Accurately weigh **10 mg** of **Rucaparib working standard** and transfer it into a clean, dry **10 mL volumetric flask**.
2. Add approximately **7 mL of diluent** and sonicate until completely dissolved.
3. After dissolution, add more diluent to bring the final volume to **10 mL** (this forms the stock solution).
4. Take **0.5 mL** of the prepared stock solution and transfer it into another **10 mL volumetric flask**.
5. Dilute it to **10 mL** using **methanol** to obtain the final working solution.

Preparation of 80% Standard Stock Solution

1. Accurately weigh **10 mg** of **Rucaparib working standard** and transfer it into a clean, dry **10 mL volumetric flask**.

2. Add approximately **7 mL of diluent** and sonicate until completely dissolved.
3. After dissolution, add more diluent to bring the final volume to **10 mL** (this forms the stock solution).
4. Take **0.4 mL** of the prepared stock solution and transfer it into a **10 mL volumetric flask**.
5. Add diluent to make up the final volume to **10 mL**.

Preparation of 100% Standard Stock Solution

1. Accurately weigh **10 mg** of **Rucaparib working standard** and transfer it into a clean, dry **10 mL volumetric flask**.
2. Add approximately **7 mL of diluent** and sonicate until fully dissolved.
3. After dissolution, add more diluent to bring the final volume to **10 mL** (this forms the stock solution).
4. Take **0.5 mL** of the prepared stock solution and transfer it into a **10 mL volumetric flask**.
5. Add diluent to make up the final volume to **10 mL**.

Preparation of 120% Standard Stock Solution

1. Accurately weigh **10 mg** of **Rucaparib working standard** and transfer it into a clean, dry **10 mL volumetric flask**.
2. Add approximately **7 mL of diluent** and sonicate until fully dissolved.
3. After dissolution, add more diluent to bring the final volume to **10 mL** (this forms the stock solution).
4. Take **0.6 mL** of the prepared stock solution and transfer it into a **10 mL volumetric flask**.
5. Add diluent to make up the final volume to **10 mL**.

Recovery Study

To evaluate the **accuracy** of the proposed method, a **recovery study** was performed. Different amounts of **pure Rucaparib** (80%, 100%, and 120%) were added to a pre-analyzed formulation with a concentration of **50 µg/mL**. The percentage recovery was then calculated to assess the method's accuracy. The results were shown in table 4.

Table-4: Accuracy Readings

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S1 : 80 %	40	40.141	502647	100.352	Mean= 100.3947% S.D. = 0.071319 %R.S.D.= 0.071038
S2 : 80 %	40	40.191	503214	100.477	
S3 : 80 %	40	40.142	502656	100.355	
S4 : 100 %	50	50.044	614215	100.088	Mean= 99.98533% S.D. = 0.183045 % R.S.D.= 0.183071
S5 : 100 %	50	49.887	612451	99.774	
S6 : 100 %	50	50.047	614254	100.094	
S7 : 120 %	60	60.192	728547	100.32	Mean= 100.311% S.D. = 0.408574 % R.S.D.= 0.407308
S8 : 120 %	60	59.939	725698	99.898	
S9 : 120 %	60	60.429	731211	100.715	

2. Precision

2.1 Repeatability

Preparation of Rucaparib Product Solution for Precision:

1. Accurately weigh **10 mg** of **Rucaparib working standard** and transfer it into a clean, dry **10 mL volumetric flask**.
2. Add approximately **7 mL of diluent** and sonicate until fully dissolved.
3. After dissolution, add more diluent to bring the final volume to **10 mL** (this forms the stock solution).
4. Take **0.5 mL** of the prepared stock solution and transfer it into a **10 mL volumetric flask**.
5. Add diluent to make up the final volume to **10 mL**.

Procedure

1. The **standard solution** was injected **six times** into the **HPLC system**.
2. The **peak area** was recorded for all six injections.
3. The **% Relative Standard Deviation (%RSD)** of the peak area was calculated to ensure it was within the acceptable limits.
4. The **precision** of the method was evaluated by analyzing the **peak areas and retention times** obtained from six replicate injections of **Rucaparib (API)**.

The % relative variance was calculated for Rucaparib square measure bestowed within the table 5.

Table-5: Repeatability Readings

HPLC Injection Replicates of Rucaparib	Retention Time (Minutes)	Peak Area
Replicate – 1	3.649	5674158
Replicate – 2	3.684	5654715
Replicate – 3	3.687	5665841
Replicate – 4	3.688	5654578
Replicate – 5	3.688	5652284
Replicate – 6	3.687	5641487
Average		**5657177**
Standard Deviation		**11369.72**
% RSD		**0.200979**

3. Linearity & Range

Preparation of Drug Solutions for Linearity:

1. **Stock Solution:**
   - Accurately weigh **10 mg** of **Rucaparib working standard** and transfer it into a clean, dry **10 mL volumetric flask**.
   - Add approximately **7 mL of diluent**, sonicate to dissolve completely, and then make up the volume to **10 mL** with the same solvent.
2. **Preparation of Working Solutions at Different Levels:**

- **Level I (30 ppm of Rucaparib):** Take **0.3 mL** of the stock solution into a **10 mL volumetric flask** and dilute to the mark with the **diluent**.
- **Level II (40 ppm of Rucaparib):** Take **0.4 mL** of the stock solution into a **10 mL volumetric flask** and dilute to the mark with the **diluent**.
- **Level III (50 ppm of Rucaparib):** Take **0.5 mL** of the stock solution into a **10 mL volumetric flask** and dilute to the mark with the **diluent**.
- **Level IV (60 ppm of Rucaparib):** Take **0.6 mL** of the stock solution into a **10 mL volumetric flask** and dilute to the mark with the **diluent**.
- **Level V (70 ppm of Rucaparib):** Take **0.7 mL** of the stock solution into a **10 mL volumetric flask** and dilute to the mark with the **diluent**.

Procedure:

1. Inject each concentration level into the **HPLC system** and record the **peak area**.
2. Plot a **calibration curve** with **concentration (X-axis)** and **peak area (Y-axis)**.
3. Calculate the **correlation coefficient (r^2)** to evaluate linearity.

Results:

- The calibration curve exhibited **good linearity** in the range of **0–70 µg/mL** for **Rucaparib (API)**.
- The **correlation coefficient (r^2) was 0.999**, confirming the method's linearity.
- The **regression equation** was found to be **y = 11266x + 50416** for Rucaparib.

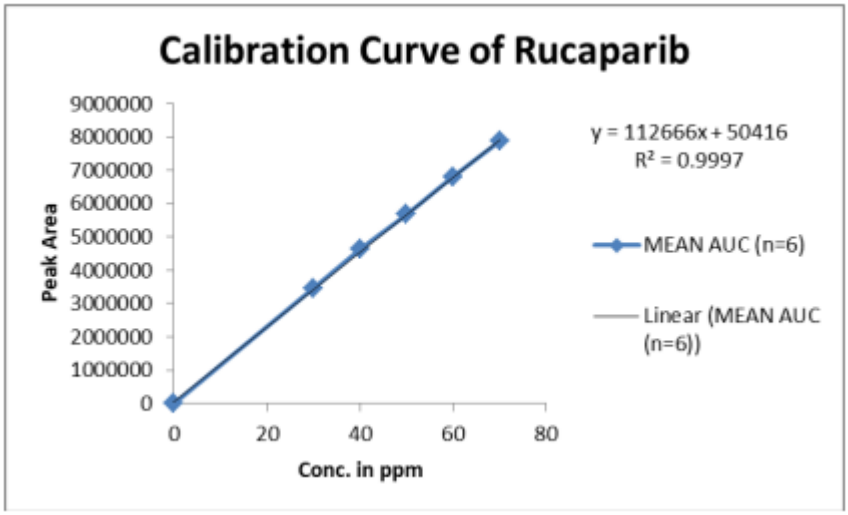

Fig-4: Calibration Curve of Rucaparib (API)

Table-6: Linearity Results

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
30	3465974

40	4626478
50	5682284
60	6815478
70	7878721

Linearity Plot

The linearity of the method was evaluated by plotting **Concentration (X-axis) vs. Average Peak Area (Y-axis)** for Rucaparib. The resulting plot was a straight line, confirming the method's linearity.

- **Equation:** Y = mx + c
- **Slope (m):** 112666
- **Intercept (c):** 50416
- **Correlation Coefficient (r):** 0.99

Validation Criteria:

The method is considered linear if the Correlation Coefficient (r) ≥ 0.99.

Conclusion:

Since the obtained **r-value is 0.99** and the intercept is **50416**, the method meets the validation criteria, confirming the linearity of Rucaparib detection.

4. Method Robustness

Robustness testing was conducted by varying **flow rate** and **mobile phase composition** to assess the method's stability under different conditions.

Effect of Flow Rate Variation:

The method was tested by adjusting the **flow rate** from **0.9 mL/min to 1.1 mL/min** (instead of the standard 1.0 mL/min), while keeping all other conditions unchanged. A **10 µL sample** was injected, and chromatograms were recorded. The method remained robust in **lower flow rate conditions**.

Effect of Mobile Phase Composition Variation:

The method was further tested by modifying the **Acetonitrile:Phosphate Buffer ratio** from the standard **35:65** to **40:60** and **30:70**. A **10 µL sample** was injected, and chromatograms were analyzed. The method remained robust even with **±5% changes** in mobile phase composition.

Preparation of Standard Solution for Robustness Testing:

1. **Stock Solution Preparation:**
   - Weigh **10 mg of Rucaparib working standard** and transfer it into a **clean, dry 10 mL volumetric flask**.

- Add approximately **7 mL of diluent**, sonicate until completely dissolved, and make up the volume to **10 mL** with the same solvent.

2. **Working Solution Preparation:**
   - Take **0.5 mL** of the stock solution into a **10 mL volumetric flask** and dilute to the mark with the **diluent**.

Conclusion:

No significant changes were observed in **resolution, tailing factor, asymmetry factor, or plate count** under varied conditions. This confirms that the method is **robust** and reliable for Rucaparib analysis.

Table-7: Results for Robustness

Parameter Used for Sample Analysis	Peak Area	Retention Time	Theoretical Plates	Tailing Factor
Actual Flow rate of 1.0 mL/min	584624	3.649	1.42	4765
Less Flow rate of 0.9 mL/min	598676	3.687	1.49	4856
More Flow rate of 1.1 mL/min	612543	3.649	1.46	4965
Less organic phase	578642	3.688	1.49	4758
More organic phase	569896	3.684	1.47	4962

5. Limit of Detection (LOD) & Limit of Quantification (LOQ)

Limit of Detection (LOD):

The **LOD** is the **lowest concentration** of Rucaparib that can be **detected** by the analytical method but **not necessarily quantified** with precision. It is calculated using the formula:

$$\mathbf{LOD = 3.3 \times \sigma / s}$$

Where:

- **σ** = Standard deviation of the response
- **S** = Slope of the calibration curve

Limit of Quantification (LOQ):

The **LOQ** is the **minimum concentration** of Rucaparib that can be **accurately quantified** using the method. It is determined using the equation:

$$LOQ = 10 \times \sigma / S$$

Where:

- **σ** = Standard deviation of the response
- **S** = Slope of the calibration curve

These parameters ensure the method's sensitivity and reliability for detecting and quantifying Rucaparib in analytical procedures.

Table-8: Results of LOD & LOQ

SE of Intercept	48846.22527
SD of Intercept	109223.4801
LOD	3.199168
LOQ	9.694449

7. Specificity

Definition:
Specificity is determined by **comparing chromatograms** of the **drug sample** with a **blank solution** to check for any interference from excipients.

Procedure:

1. **Blank Solution Preparation** – A solution was prepared by mixing only the excipients in the **mobile phase**, without adding the drug.
2. **Drug Solution Preparation** – A separate solution containing **only Rucaparib** was prepared.
3. **Filtration** – These solutions were passed through a **0.45 µm membrane filter** before analysis.
4. **Chromatographic Analysis** – The blank solution, drug solution, and sample mixture were injected into the **HPLC system**, and their chromatograms were recorded.

Observation:

- No peaks were observed for excipients near the drug peak in the chromatogram.
- The **active drug** was **well separated** from the blank and excipients.
- This confirms that the **method is specific**, meaning it effectively differentiates the drug from other components in the formulation.

9. Estimation of Rucaparib in Pharmaceutical Dosage Form

Procedure:

1. **Sample Preparation:**
   - **Twenty tablets** were weighed and finely powdered.
   - A quantity **equivalent to 25 mg** of Rucaparib was transferred into a **25 mL volumetric flask**.
   - The sample was **sonicated for 15 minutes** to ensure complete dissolution.
   - The volume was adjusted to **25 mL** with the same solvent.
2. **Dilution:**
   - **10 mL** of the prepared solution was further diluted to **100 mL** with the **mobile phase**.
   - The final solution was **filtered through a 0.45 µm membrane filter** and **sonicated** to remove any air bubbles.
3. **HPLC Analysis:**
   - The prepared sample solution was **injected five times** into the **HPLC system**.
   - A **standard solution** was also injected for comparison.
   - The **peak areas** were recorded, ensuring **accuracy and consistency** in the results.

ASSAY:

Assay % = (AT X WS X DT X P)/ (AS X DS X WT X 100) X Avg. Wt = mg/tab

Where:

AT = Peak space of drug obtained with check preparation

AS= Peak space of drug obtained with normal preparation

WS= Weight of operating normal taken in mg

WT= Weight of sample taken in mg

DS= Dilution of normal resolution

DT= Dilution of sample resolution

P = proportion purity of operating normal

Table-8: Recovery Data for estimation Rucaparib

Brand Name of Rucaparib	Labelled Amount of Drug (mg)	Mean (± SD) Amount (mg) found by the Proposed Method (n=6)	Assay % (± SD)
Nuparp 200 Tab (200mg) (Zydus Oncosciences)	200mg	199.589 (± 0.258)	99.698 (± 0.639)

RESULTS & DISCUSSION

Assay Results:

The **amount of Rucaparib** in the tablet formulation was found to be **199.589 mg per tablet** (± 0.258), with a **% assay of 99.698% (± 0.639)**, confirming the accuracy of the developed method.

Forced Degradation Studies

Objective:
Forced degradation studies were conducted to assess the **stability** of Rucaparib under various **stress conditions**. These studies help predict the **long-term stability** of the drug and simulate the degradation that may occur **during storage or after administration**.

Methodology:
The Rucaparib **Active Pharmaceutical Ingredient (API)** was exposed to different **stress conditions**, including:

1. **Acid Hydrolysis** – Exposure to acidic conditions.
2. **Alkaline Hydrolysis** – Exposure to basic conditions.
3. **Thermal Degradation** – Subjected to high temperatures.
4. **Photolytic Degradation** – Exposure to light.
5. **Oxidative Degradation** – Exposure to oxidative agents.

Results:

- The study confirmed the **specificity** of the developed method.
- Rucaparib remained **stable** under **photolytic** and **peroxide stress conditions**, indicating **no significant degradation** in these environments.

The results of forced degradation studies are given in the following table-14.

SUMMARY AND CONCLUSION

An **analytical method** for Rucaparib was successfully developed and validated by optimizing various parameters:

- **Maximum absorbance** was observed at **248 nm**, confirming excellent **peak purity**.
- An **injection volume** of **20 µL** provided a well-defined **peak area**.
- The analysis was conducted using a **Symmetry ODS (C18) RP Column** (250 mm × 4.6 mm, 5 µm particle size), which produced a **sharp and well-resolved peak**.
- The method was optimized for **ambient temperature**, which was suitable for the drug solution.
- A **flow rate** of **1.0 mL/min** was chosen, ensuring an optimal **peak area** and **retention time**.
- The **mobile phase** consisted of **0.02M Phosphate Buffer and Acetonitrile (48:52% v/v, pH 2.80)**, which provided a **symmetrical peak** and was therefore selected for the study.
- **Methanol** was used for **maximum drug extraction**, and a **sonication time of 10 minutes** was sufficient for complete solubility and **high recovery**.
- A **run time** of **8.0 minutes** was chosen to allow for efficient analysis while ensuring the drug **peak appeared at 3.649 minutes**, reducing total analysis time.
- **Percent recovery** ranged from **98.0% to 102%**, confirming **linearity and precision**.
- The method was found to be **linear within 30-70 ppm** of the target Rucaparib concentration.
- The method passed **robustness and ruggedness** tests, with **relative standard deviation (RSD) values well within the acceptable range**, ensuring accuracy and reproducibility.

Conclusion:
The developed **HPLC method** is **accurate, precise, robust, and linear**. It is suitable for routine **quality control analysis** of Rucaparib in pharmaceutical formulations.

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