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DEVELOPMENT AND VALIDATION FOR THE ANALYSIS OF OLAPARIB IN API FORM AND MARKETED PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC

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ABSTRACT

An analytical, new, simple, rapid, robust, precise, accurate and reproducible RP-HPLC method for estimation of Olaparib in bulk form and marketed formulation. Separation of Olaparib was successfully achieved on a Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d. column in an isocratic mode of separation utilizing Methanol : Phosphate buffer (0.02M, pH-3.6) in the ratio of 45:55% v/v at a flow rate of 1.0 mL/min and the detection was carried out at 255nm. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The response was found to be linear in the drug concentration range of 12-28mcg/mL for Olaparib. The correlation coefficient was found to be 0.9995 for Olaparib. The LOD and LOQ for Olaparib were found to be 5.004µg/mL and 15.164µg/mL respectively. The proposed method was found to be good percentage recovery for Olaparib, which indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard solution with the sample solution. Therefore, the proposed method specifically determines the analyte in the sample without interference from excipients of pharmaceutical dosage forms.

KEYWORDS: Olaparib, RP-HPLC, Accuracy, Precision, Robustness, ICH Guidelines.

INTRODUCTION

Olaparib is a small molecule inhibitor of the nuclear enzyme poly (ADP-ribose) polymerase (PARP) with potential chemosensitizing, radiosensitizing, and antineoplastic activities. Olaparib[1] selectively binds to and inhibits PARP, inhibiting PARP-mediated repair of single strand DNA breaks; PARP inhibition may enhance the cytotoxicity of DNA-damaging agents and may reverse tumor cell chemoresistance and radioresistance. PARP catalyzes post-translational ADP-ribosylation of nuclear proteins and can be activated by single-stranded DNA breaks. Olaparib is indicated for the maintenance treatment of adults with deleterious or suspected deleterious germline or somatic BRCA-mutated advanced epithelial ovarian, fallopian tube or primary peritoneal cancer who are in complete or partial response to first-line platinum-based chemotherapy. Olaparib is a cytotoxic and anti-tumour agent. Olaparib[2] inhibits the growth of selective tumour cell lines in vitro and decreases tumour growth in mouse xenograft models of human cancer, both as monotherapy or following platinum-based chemotherapy. The drug exerts anti-tumour effects in cell lines and mouse tumour models with deficiencies in BRCA1/2, ATM, or other genes involved in the homologous recombination repair (HRR) of DNA damage and correlated with platinum response. Olaparib[3] is used as maintenance treatment in patients with advanced ovarian cancer, fallopian tube cancer, or primary peritoneal cancer with a certain type of inherited (germline) or acquired (somatic) abnormal BRCA gene. Your doctor will test for the presence of this gene. The IUPAC name of Olaparib is 4-[[3-[4-(cyclo propane carbonyl) piperazine-1-carbonyl]-4-fluoro phenyl] methyl]-2H-phthalazin-1-one. The Chemical Structure of Olaparib is shown in following page.

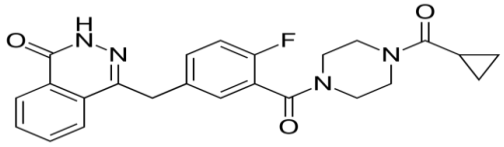

Fig-1: Chemical Structure of Olaparib.

Several analytical methods[31-36] have been devised for the determination of Olaparib. These methods include titrimetric method, HPLC methods, HPTLC methods, LC methods, Uv- visible spectrophotometric methods etc. These methods are required expensive or sophisticated instruments and not simple for routine analysis. High performance liquid chromatography (HPLC) can be used for determination of drug. It has the advantages of being accurate, sensitive, rapid, selective, and reproducible. The present paper reports the development of a new high performance liquid chromatography (HPLC) method for determination of Olaparib in API form and Marketed Pharmaceutical Dosage Form.

MATERIALS AND METHODS

Instruments Used

Table 1: Instruments used.

S.No.	Instruments and Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Chemicals Used

Table 2: Chemicals used.

S.No.	Chemical	Brand names
1	Olaparib	Astra Zeneca
2	Water and Methanol for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for HPLC	Merck
4	Ethanol	Sd fine-Chem ltd; Mumbai
5	DMSO	Sd fine-Chem ltd; Mumbai
6	DMF	Sd fine-Chem ltd; Mumbai
7	Orthophosphoric Acid	Sd fine-Chem ltd; Mumbai

HPLC Method Development

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Olaparib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.1ml of the above Olaparib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Preparation of Sample Solution

Twenty capsules were taken and the average weight was calculated as per the method prescribed in I.P. The weighed tablets were finally powdered and triturated well. A quantity of powder of Olaparib equivalent to 10mg were transferred to clean and dry 10 ml volumetric flask and 7 ml of HPLC grade methanol was added and the resulting solution was sonicated for 15 minutes. Make up the volume up to 10 ml with same solvent.[4] Then 1 ml of the above solution was diluted to 10 ml with HPLC grade methanol. One ml (0.1 ml) of the prepared stock solution diluted to 10 ml and was filtered through membrane filter (0.45µm) and finally sonicated to degas.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.[25,30]

Mobile Phase Optimization

Initially the mobile phase[5] tried was Methanol and Methanol: Water with varying proportions. Finally, the mobile phase was optimized to Methanol and Phosphate buffer (0.02M, pH-3.6) in proportion 45:55% v/v.

Optimization of Column

The method was performed with various C18 columns like, X- bridge column, Xterra, and C18 column. Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d. was found to be ideal as it gave good peak shape and resolution[6] at 1.0ml/min flow.

Preparation of Buffer and Mobile Phase

Preparation of Potassium Dihydrogen Phosphate (KH2PO4) Buffer (0.02M) (pH-3.6)

Dissolve 2.72172g of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 3.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra-sonication.[7]

Preparation of Mobile Phase
Accurately measured 450 ml (45%) of Methanol and 550 ml of Phosphate buffer (55%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation
The Mobile phase was used as the diluent.

Validation Parameters
System Suitability
Accurately weigh and transfer 10 mg of Olaparib working standard into a 10ml of clean dry volumetric flasks[8] add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Olaparib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure
The standard solution was injected for five times and measured the area for all five injections in HPLC.[9] The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity
Preparation of Standard Solution
Accurately weigh and transfer 10 mg of Olaparib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Olaparib stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution
Weight 10 mg equivalent weight of Olaparib sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.1ml of Olaparib above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure
Inject the three replicate injections of standard and sample solutions[10] and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Linearity and Range
Accurately weigh and transfer 10 mg of Olaparib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (12ppm of Olaparib)
Take 0.12ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – II (16ppm of Olaparib)
Take 0.16ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – III (20ppm of Olaparib)
Take 0.2ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – IV (24ppm of Olaparib)
Take 0.24ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – V (28ppm of Olaparib)
Take 0.28ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Procedure
Inject each level into the chromatographic system[11] and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Precision
Repeatability
Preparation of Olaparib Product Solution for Precision
Accurately weigh and transfer 10 mg of Olaparib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Olaparib stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure
The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Intermediate Precision
To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure
Analyst 1
The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Analyst 2
The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy
For preparation of 80% Standard stock solution
Accurately weigh and transfer 10 mg of Olaparib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.08ml of the above Olaparib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 100% Standard stock solution
Accurately weigh and transfer 10 mg of Olaparib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Olaparib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 120% Standard stock solution
Accurately weigh and transfer 10 mg of Olaparib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.12ml of the above Olaparib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure
Inject the Three replicate injections of individual concentrations (80%, 100%, 120%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Olaparib and calculate the individual recovery and mean recovery values.[12]

Limit of Detection and Limit of Quantification (LOD & LOQ)
Preparation of 5.004µg/ml Solution (For LOD)
Accurately weigh and transfer 10 mg of Olaparib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents[13] and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.05004ml of the above Olaparib stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of 15.164µg/ml Solution (For LOQ)
Accurately weigh and transfer 10 mg of Olaparib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15164ml of the above Olaparib stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Robustness
The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

For preparation of Standard Solution
Accurately weigh and transfer 10 mg of Olaparib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Olaparib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation of Flow Conditions
The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of Mobile Phase Organic Composition

The sample was analyzed by variation of mobile phase i.e. Methanol: Phosphate Buffer was taken in the ratio and 50:50, 40:60 instead (45:55), remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT

Wavelength Detection

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The UV spectrum[14] of Olaparib was obtained and the Olaparib showed absorbance's maxima at 255nm. The UV spectra of drug are follows:

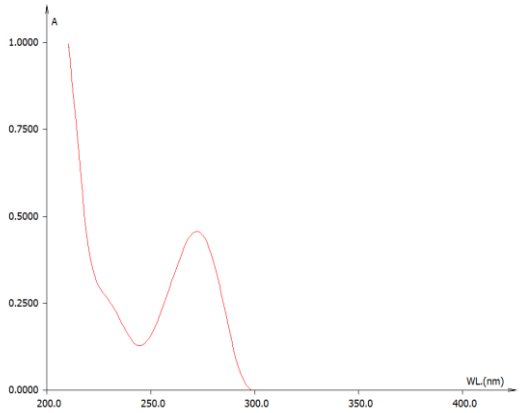

Fig-2: UV Spectrum of Olaparib.

Observation: While scanning the Olaparib solution we observed the maxima at 255nm. The UV spectrum has been recorded on T60-LAB INDIA make UV – Vis spectrophotometer model UV-2450.

Optimized Chromatographic Method

Table 3: Optimized Chromatographic Conditions.

Mobile phase	Methanol : Phosphate buffer (0.02M, pH-3.6) = 45:55 v/v
Column	Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.
Column Temperature	Ambient
Detection Wavelength	255 nm
Flow rate	1.0 ml/ min.
Run time	07 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	20µl
Type of Elution	Isocratic

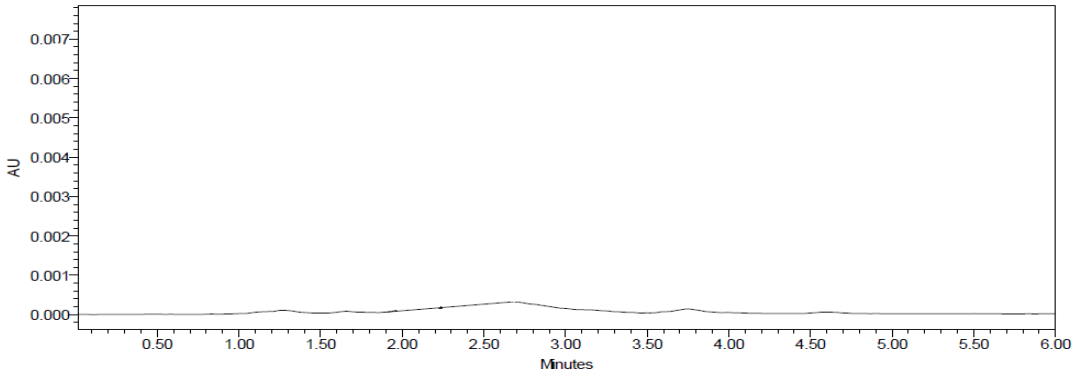

Fig-3: Chromatogram of Blank Solution.

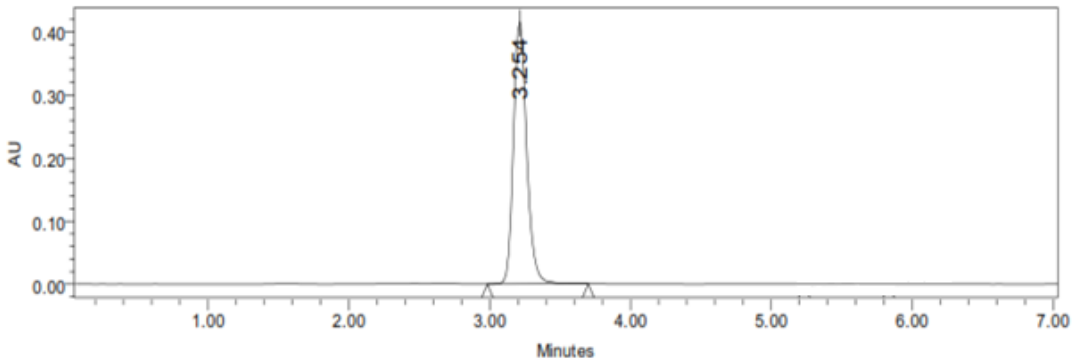

Fig-4: Chromatogram of Olaparib in Optimized Chromatographic Condition.

METHOD VALIDATION

System Suitability: System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters[15-17] were established. The data are shown in Table-4.

Table 4: System Suitability Results for Olaparib (Flow rate)

S.No.	Parameter	Limit	Result
1	Asymmetry	T ≤ 2	Olaparib = 0.12
2	Theoretical plate	N > 2000	Olaparib = 7258
3	Tailing Factor	(Tf) < 2	Olaparib = 1.25

Specificity

Specificity[18] can be determined by comparing the chromatograms obtained from the drugs with the chromatogram obtained from the blank solution. Blank solution was prepared by mixing the excipients in the mobile phase without drug. Drug solutions were prepared individually and the sample containing three drugs was also prepared. Now these mixtures were filtered by passing through 0.45 µ membrane filter before the analysis. In this observation no excipient peaks were obtained near the drug in the study run time. This indicates that the proposed method was specific.

Linearity: To evaluate the linearity, serial dilution of analyte were prepared from the stock solution was diluted with mobile phase to get a series of concentration ranging from 0-28µg/ml for Olaparib. The prepared solutions were filtered through Whatman filter paper (No.41). From these solutions, 20µl injections of each concentration were injected into the HPLC system[19] and chromatographed under the optimized conditions. Calibration curve was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis).

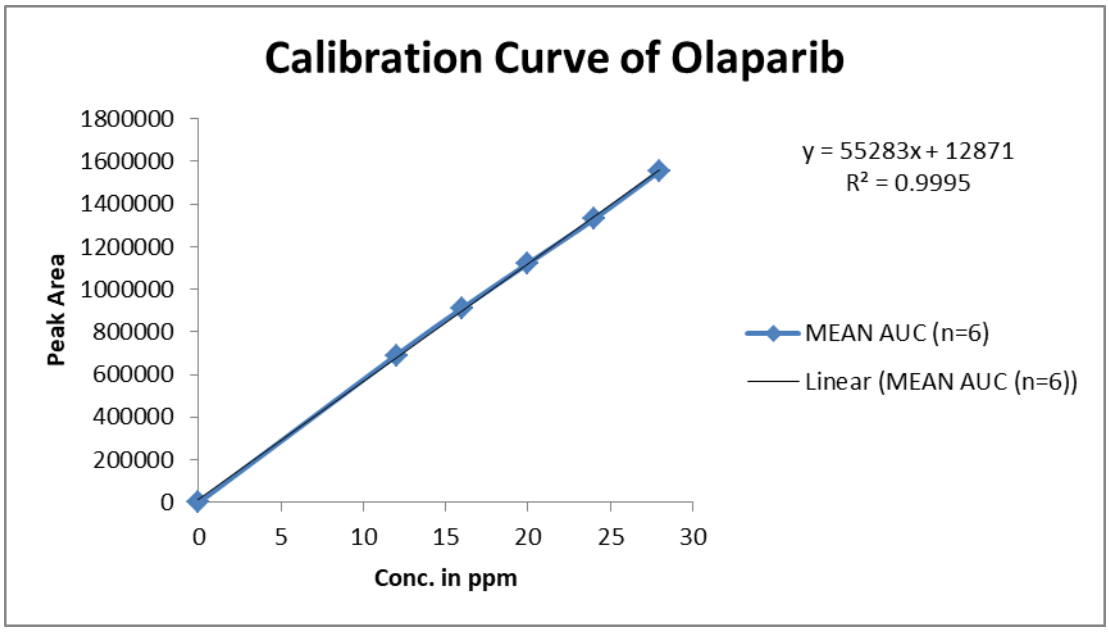

Fig-5: Standard Curve for Olaparib.

Observation: Linearity range[20] was found to be 0-28µg/ml for Olaparib. The correlation coefficient was found to be 0.9995, the slope was found to be 55283 and intercept was found to be 12871 for Olaparib.

Table 5: Linearity Readings for Olaparib.

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
12	690316
16	910621
20	1121057
24	1328903
28	1554666

Accuracy

Inject the three replicate injections of individual concentrations (80%, 100%, 120%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Olaparib and calculate the individual recovery[21] and mean recovery values.

Accuracy at different concentrations (80%, 100%, and 120%) was prepared and the % recovery was calculated.

Table 6: Accuracy results of Olaparib.

Sample ID	Concentration (µg/ml)			%Recovery of Pure drug	Statistical Analysis
	Conc. Found	Conc. Recovered	Peak Area		
S_1 : 80 %	8	8.064107	458679	99.867	Mean= 100.4113% S.D. = 0.473694346 % R.S.D.= 0.471753
S_2 : 80 %	8	7.843532	446485	100.637	
S_3 : 80 %	8	8.19449	465887	100.73	
S_4 : 100 %	10	9.892661	559767	99.41	Mean= 100.6646667% S.D. = 1.166369295 R.S.D.= 1.158667
S_5 : 100 %	10	9.978655	564521	100.868	
S_6 : 100 %	10	10.19623	576549	101.716	
S_7 : 120 %	12	11.85907	668476	99.878	Mean= 100.4637% S.D. = 0.51154309 % R.S.D. = 0.509181
S_8 : 120 %	12	12.16785	685546	100.69	
S_9 : 120 %	12	12.18644	686574	100.823	

Precision: The precision of each method was ascertained separately from the peak areas obtained by actual determination of six replicates of a fixed amount of drug Olaparib. The percent relative standard deviations[22] were calculated for Olaparib are presented in the Table-7.

i) Repeatability: Obtained Six (6) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table 7: Repeatability Results of Olaparib.

HPLC Injection Replicates	AUC for Olaparib
Replicate – 1	285479
Replicate – 2	284571
Replicate – 3	286954
Replicate – 4	283261
Replicate – 5	285964
Replicate – 6	284259
Average	**285081.3**
Standard Deviation	**1318.666**
% RSD	**0.462558**

ii) Intermediate Precision / Ruggedness

To evaluate the intermediate precision[23] (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure

Analyst 1: The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.[24]

Analyst 2

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.[26]

Intra Day (Day-1)/Analyst-1

Table 8: Results of Ruggedness for Olaparib (Analyst-1)

S.No.	Peak Name	RT	Peak Area	Theoretical Plates	Tailing Factor
1	Olaparib	3.253	284568	7368	1.26
2	Olaparib	3.254	285684	7295	1.25
3	Olaparib	3.215	283659	7346	1.27
4	Olaparib	3.204	286598	7457	1.22
5	Olaparib	3.202	287965	7635	1.29
6	Olaparib	3.297	285698	7459	1.28
Mean			**285695.3**		
Std. Dev.			**1508.898**		
% RSD			**0.528149**		

Inter Day (Day -2/Analyst-2)

Table 9: Results of Ruggedness for Olaparib (Analyst-2)

S.No.	Peak Name	RT	Peak Area	Theoretical Plates	Tailing Factor
1	Olaparib	3.297	294754	7394	1.29
2	Olaparib	3.253	293695	7425	1.25
3	Olaparib	3.213	294578	7385	1.27
4	Olaparib	3.297	296534	7584	1.23
5	Olaparib	3.210	296571	7745	1.24
6	Olaparib	3.254	298698	7658	1.25
Mean			**295805**		
Std. Dev.			**1819.334**		
% RSD			**0.615045**		

Robustness: Robustness[27] is defined as the capacity of that method to be unaffected by even small deliberate changes that occur in the method parameters. The evaluation of robustness of a method is done by varying the chromatographic parameters such as pH, temperature, flow rate, mobile phase proportions change, ionic strength etc., and determining any possible effect on the results obtained by that method.

Table 10: Result of Method Robustness Test for Olaparib.

Parameter used for Sample Analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	283261	3.254	7258	1.25
Less Flow rate of 0.9 mL/min	315864	3.297	7569	1.29
More Flow rate of 1.1 mL/min	298542	3.212	7841	1.41
Less organic phase	279856	3.253	7965	1.27
More organic phase	306985	3.215	7458	1.28

LOD: The limit of detection (LOD) is the lowest concentration of analyte in a sample which can be detected, but not quantitated. LOD[28] is a limit test that specifies whether an analyte is above or below a certain value. Signal-to-noise ratio of three-to-one is used to determine LOD.

L.O.D. = 3.3 (SD/S).

Where, SD = Standard deviation of the response
S = Slope of the calibration curve

Table 11: Results of LOD.

	LOD
SD of Intercept	19518.16286
Slope	55283

Observation: The LOD was found to be 1.165µg/ml for Olaparib.

LOQ: The Limit of Quantitation (LOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. Signal-to-noise ratio of ten-to-one is used to determine LOQ.[29]

L.O.Q. = 10 (SD/S)

Where, SD = Standard deviation of the response
S = Slope of the calibration curve

Table 12: Results of LOD.

	LOQ
SD of Intercept	19518.16286
Slope	55283

Observation: The LOQ was found to be 3.53µg/ml for Olaparib.

Assay of Marketed Pharmaceutical Dosage form

Twenty tablets/Capsules were taken and the I.P. method was followed to determine the average weight. Finally the weighed tablets are powdered and triturated well by using mortar and pestle. A quantity of powder which is equivalent to the 100mg of drugs were transferred to a clean and dry 100ml of volumetric flask and add 70 ml of mobile phase and the resulted solution was sonicated for 15 minutes by using ultra sonicator, Then the final volume was make up to the mark with the mobile phase. The final solution was filtered through a selected membrane filter (0.45 µm) and in order to sonicate to degas the mobile phase (Solvent system). From this above stock arrangement (1 ml) was exchanged to five distinctive 10 ml volumetric flagons and volume was made up to 10 ml with same dissolvable framework (Mobile stage).

The readied arrangements were infused in five repeats into the HPLC framework and the perceptions were recorded.

A duplicate injection (Blank Solution) of the standard arrangement likewise infused into the HPLC framework and the chromatograms and peak zones were recorded and figured-13.

ASSAY

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg/tab}$$

Where:

AT = Peak Area of drug obtained with test preparation

AS = Peak Area of drug obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

The assay was performed as explained in the previous chapter. The results which are obtained are following:

Table 13: Recovery Data for estimation Olaparib in Lynparza Capsules.

Brand name of Olaparib	Labelled amount of Drug (mg)	Amount (mg) found by the proposed method (n=3)	Assay %
Lynparza Capsules (Astra Zeneca)	50mg	49.695mg	99.598%

Result & Discussion: The amount of drug in Lynparza Capsule was found to be 49.695 (± 0.789) mg/tab for Olaparib & % Purity was 99.598 (± 0.695) %.

Forced Degradation Studies

Following protocol was strictly adhered to for forced degradation of Olaparib Active Pharmaceutical Ingredient (API). The API (Olaparib) was subjected to keep in some stress conditions in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage and/or after administration to body. It is one type of accelerated stability studies of the drugs that is used to help us to determining the total fate of the drug that is likely to happen after long time storage, within a very short time as compare to the real time or long term stability testing. The different types of forced degradation pathways/studies are studied here are acid hydrolysis, basic hydrolysis, thermal degradation and oxidative degradation.

Results of Degradation Studies: The results of the forced degradation studies[30] indicated the specificity of the developed method that has been developed. Olaparib were stable only in acidic, thermal and basic stress conditions. The results of stability studies are given in the following Table-14.

Table 14: Results of Forced Degradation Studies of Olaparib API.

Stress Condition	Time (hours)	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1N HCl)	24Hrs.	91.326	8.674	100.00
Basic Hydrolysis (0.IN NaOH)	24Hrs.	83.215	16.785	100.00
Thermal Degradation (60 ^{0}C)	24Hrs.	90.311	9.689	100.00
UV (254nm)	24Hrs.	81.322	18.678	100.00
3% Hydrogen Peroxide	24Hrs.	73.514	26.486	100.00

SUMMARY AND CONCLUSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Olaparib, different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat

baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d. column was preferred because using this column peak shape, resolution and absorbance were good. Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Olaparib it is evident that most of the HPLC work can be accomplished in the wavelength range of 255 nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 20µl were found to be the best analysis. The result shows the developed method is yet another suitable method for assay which can help in the analysis of Olaparib in different formulations.

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