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METHOD DEVELOPMENT AND VALIDATION FOR QUANTITATIVE ESTIMATION OF OLAPARIB IN TABLET DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT

A simple and new isocratic high-performance liquid chromatography (HPLC) method was developed for quantitative determination of Olaparib in its tablet dosage form. The chromatographic separation was achieved on Thermo Scientific C18 column (250mm x 4.6mm i.d.5µ). The mobile phase selected was 0.1% Trifluoroacetic buffer: Acetonitrile in the ratio of 60: 40 v/v at flow rate 1.0ml/min with column temperature maintained at 35°C and 10µl injection volume. The detection was carried out at 276nm. The retention time of Olaparib was found to be at 5.14 min. The developed HPLC method was validated as per ICH (Q2R1) guideline. The HPLC method was linear over range of 25-75 µg/ml with regression coefficient 0.9991. The results of validation parameters indicate that developed HPLC method was specific, accurate, precise, rapid, reliable and reproducible therefore, it can be applied for routine quality control analysis of Olaparib in its tablet dosage form.

KEYWORDS: RP-HPLC, Olaparib, Method Development, Validation, ICH guidelines.

ABBREVIATIONS

RP-HPLC - Reverse Phase High Performance Liquid Chromatography

LC-MS - Liquid Chromatography – Mass Spectroscopy

PARP-1inhibitor – Poly (ADP-ribose) polymerase inhibitor

BRCA1/2mutations – Breast Cancer gene mutations 1 and 2

1. INTRODUCTION

Olaparib (Lynparza [AstraZeneca, Cambridge, UK], earlier mentioned to as AZD2281 or KU0059436) is an oral poly (ADP-ribose) polymerase (PARP) inhibitor.[1] PARP is tangled in numerous aspects of DNA repair, and the PARP inhibitor Olaparib (Lynparza, AstraZeneca) has newly been approved used for treating ovarian cancers with *BRCA1/2* mutations.[2] It is currently in Phase III progress and has earlier been examined in several clinical trials, both as a single agent and in combination with directed an international, randomized, double-blind, phase 3 trial to estimate the effectiveness of Olaparib as maintenance therapy in patients with recently detected advanced (International Federation of Gynaecology and Obstetrics stage III or IV) high-grade serous or endometrioid ovarian cancer, primary peritoneal cancer, or fallopian-tube cancer (or a combination thereof) with a mutation in *BRCA1*, *BRCA2*, or both (*BRCA1/2*) who had a broad or part clinical response after platinum-based chemotherapy.[3] The chemically it is known as 4-[[3-[4-(cyclopropane carbonyl) piperazine-1-carbonyl]-4-fluorophenyl]methyl]-2H-phthalazin-1-one.

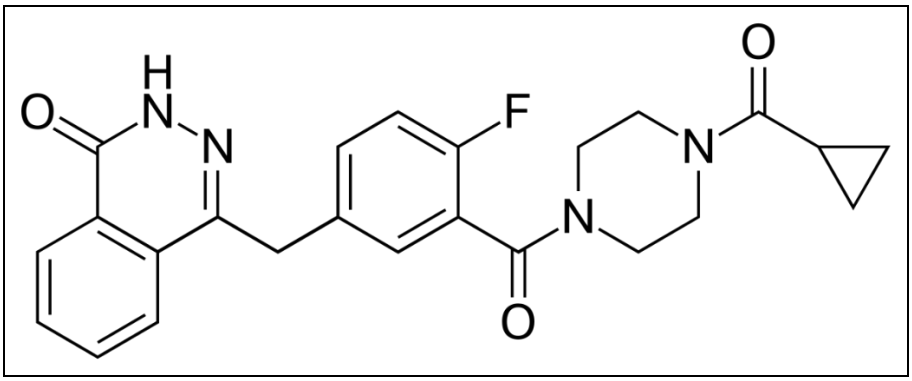

Fig. 1: Structure of Olaparib.

On December 19, 2014, the FDA approved Olaparib Tablets (Lynparza; AstraZeneca) intended for the treatment of patients with toxic or suspected toxic germline *BRCA*-mutated advanced ovarian cancer who have been treated with three or more previous lines of chemotherapy.[4] In Literature survey reveals that very few hyphenated methods such as LC-MS, LC-MS/MS which are reported but, HPLC method is not reported for the determination

of Olaparib. Therefore, an attempt was made to develop a new HPLC method which is simple, rapid, reproducible and economical for the estimation of Olaparib in tablet dosage form. The proposed method was optimized and validated according to International Council on Harmonization (ICH guidelines).[5]

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

An analytically pure Olaparib working standard was procured from Central Drug Testing Laboratory, Mumbai with defined potency 99.6 % (as is basis). LYNPARZA® (Olaparib tablet 100 mg) tablets were received as gift sample from Assistant Drugs Controller Office, Mumbai. Acetonitrile of HPLC grade, Trifluoroacetic acid of analytical grade were from Rankem manufacturers. Water used during the analysis was of Mill Q grade and filter papers used for filtration were of 0.45µm nylon-66 filters.

2.2 Instrumentation

Perkin Elmer UV/ Vis Spectrophotometer Lambda 25 connected to a computer with software Perkin Elmer UV Win lab was used for all the spectrophotometric measurements and HPLC method was developed on Thermo Scientific Dionex Ultimate 3000 HPLC using software Thermo Scientific Dionex Chromeleon Chromatography Data System Version 7.2.6 with LC instrument control.

2.3 Solubility profile of Olaparib

Olaparib is essentially neutral across the physiological pH range and belongs to class 4 within the Biopharmaceutics Classification System (BCS) because of its poor solubility and moderate permeability. Due to low solubility in water, it has a low dissolution rate and as a result exhibit poor bioavailability.[6]

2.4 Selection of Diluents

On the basis of molecular structure and chemical nature and solubility of Olaparib, mixture of water and acetonitrile (1:1) was selected as diluents for preparation of standard and sample solutions.

2.5 Selection of detection wavelength of Olaparib

Olaparib standard solution of 50µg/ml was prepared by weighing about 10mg of Olaparib to volumetric flask and volume was made up to 20ml by diluents. Further, 1ml of the above

stock solution was diluted to 10ml volumetric flask by diluents. The solution was scanned in the range of 200-400 nm.

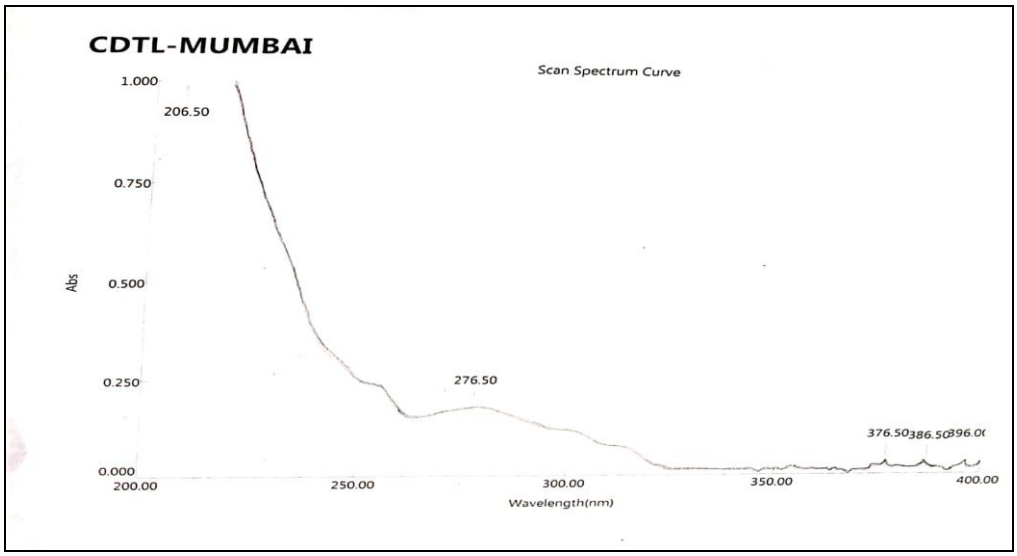

Fig .2 Olaparib UV Spectrum.

2.6 Preparation of standard drug solution

Around 10 mg Olaparib was weighed accurately on analytical balance and added to 20ml volumetric flask, it was dissolved by sonicating in sufficient diluents for 5 minutes and then volume was made up to 20ml with diluents(500µg/ml). From above stock solution 1ml was pipetted and diluted up to 10ml with diluents. Final concentration of Olaparib was 50 µg/ml, which was treated as final working standard solution.

2.7 Preparation of sample solution

Average weight of twenty tablets of LYNPARZA® (100mg) was calculated and then tablets were crushed to fine powder. An accurately weighed quantity of tablet powder equivalent to 100mg of Olaparib was dissolved in diluents and sonicated for 15minutes and then dilution was made up to 200ml with diluents (500µg/ml) and then filtered using syringe filter (0.45µm). Final concentration of test solution (50µg/ml) was prepared by pipetting 1ml from above stock solution of test and diluted to 10ml with diluents.

2.8 Method optimization

Chemical structure of Olaparib reveals that it has poor solubility and moderate permeability in nature. Therefore, the selection of column and mobile phase was done according to the nature of the molecule. Diluents were selected based on solubility of the drug. Initial trials were done with water and acetonitrile by changing ratio of aqueous portion of the mobile

phase with flow rate of 1ml/min on Thermo Scientific C18 column. Further optimization of mobile phase was done by changing the ratio of the mobile phase, changing column to Thermo Scientific C18 column (250mm x 4.6mm i.d.5µ). Further studies were carried out by using 0.1% Trifluoroacetic buffer of different concentrations until peak shape was improved (sharp and narrow) with acceptable system suitability parameters were obtained. Good peak shape with acceptable system suitability parameter were obtained with mobile phase consisting of mixture of 0.1% Trifluoroacetic buffer and acetonitrile in the ratio of 60:40 v/v. Flow rate was kept at 1 ml/min and UV detection wavelength of 276 nm and column oven temperature maintained at 35˚C.

2.9 Solubility profile of Olaparib

Solubility of Olaparib drug was carried according to Indian Pharmacopeia 2018 by dissolving one part by weight (mg) of Olaparib to number of parts by volume (ml) of solvent. Various solvents were used to establish the solubility of drug mentioned in Table no.3.

Table no. 3: Result of solubility studies of Olaparib.

Solvent	Amount of solvent required to dissolve 10mg of Olaparib	Remark
Acetonitrile	50 ml	Soluble
Water	50 ml	Very slightly soluble

3. VALIDATION OF METHOD

The developed RP-HPLC method of Olaparib was validated as per ICH Q2 (R1) guidelines for parameters such as specificity, linearity, accuracy, limit of detection, limit of quantification, robustness.

4.1 Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Specificity study is performed by injecting Blank, standard drug (50µg/ml) and test solution (50µg/ml) of Olaparib into HPLC system. Chromatograms shown in Figure no. 4.1(a), (b), (c) shows that there is no interference of excipient peak at the retention time of Olaparib peak.

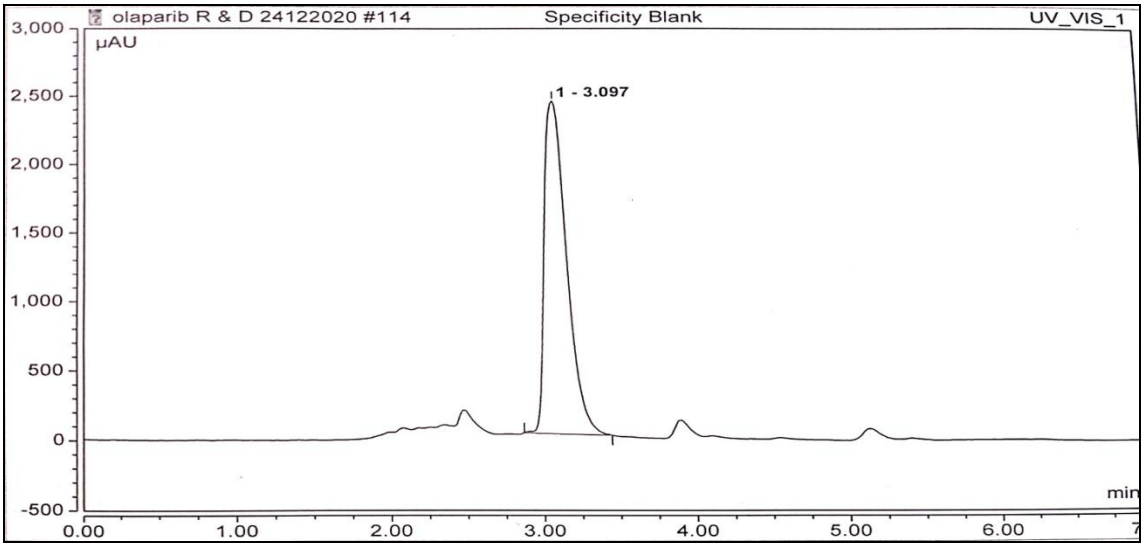

Fig.4.1(a)–Chromatogram of specificity of Olaparib blank solution.

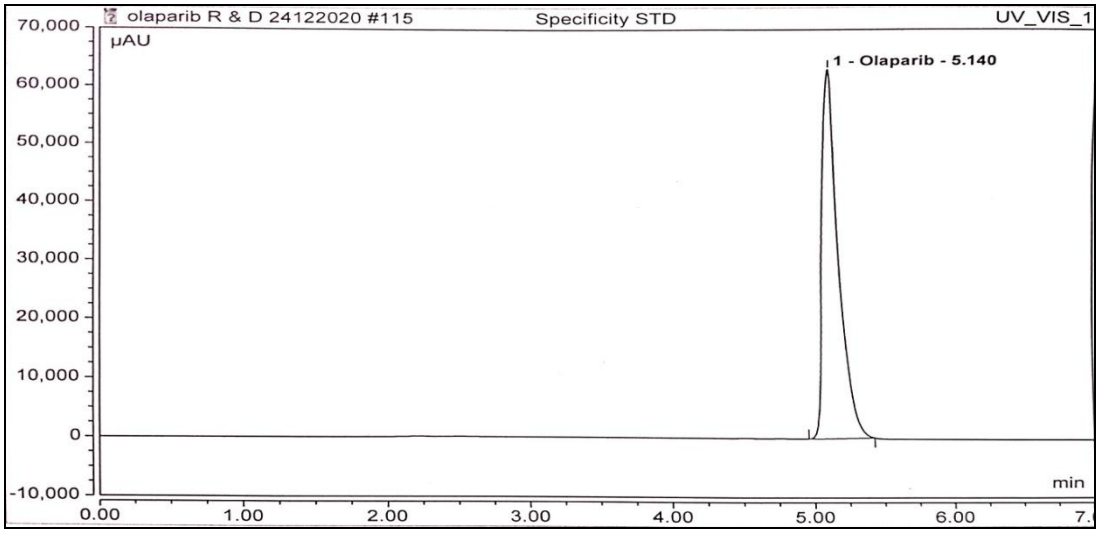

Fig. 4.1(b)–Chromatogram of specificity of Olaparib standard solution.

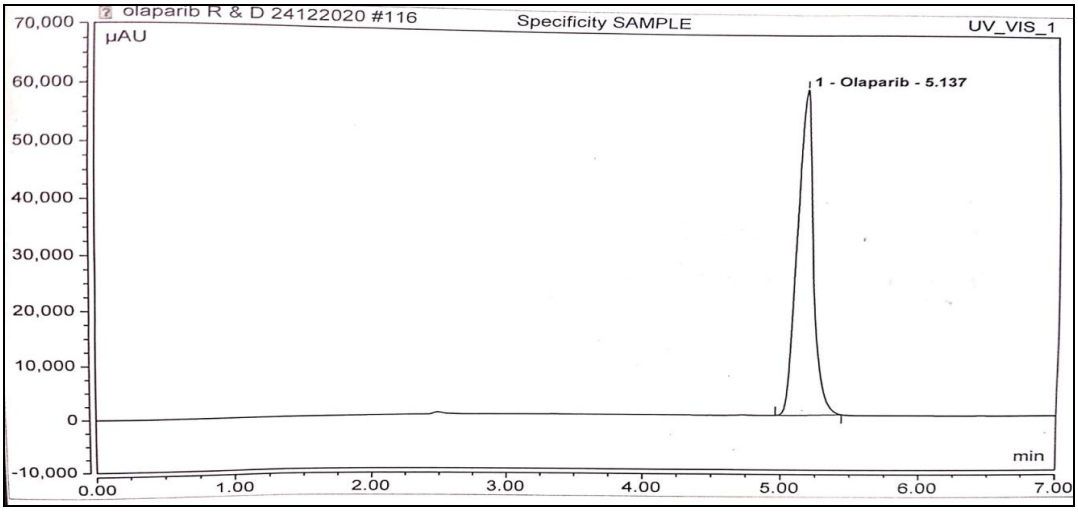

Fig. 4.1(c)-Chromatogram of specificity of Olaparib sample solution.

4.2 Linearity and Range

The study of linearity and range was carried out by preparing appropriate aliquots from standard stock solution of Olaparib (500µg/ml) to obtain concentration in the range of 25-75µg/ml. The linear calibration plot was constructed by analysing the concentration over the selected range versus peak area of standard solution and their results are mentioned in Table no. 4.2. Regression coefficient was found to be 0.9991 from calibration curve shown in Fig.4.2(a).

Table no. 4.2-Result of linearity data of Olaparib.

Linearity level	Concentration (µg/ml)	Area
1	25	4200.8
2	30	4976.3
3	40	6705.8
4	50	8144.3
5	60	9560.6
6	70	11357.7
7	75	12157.4

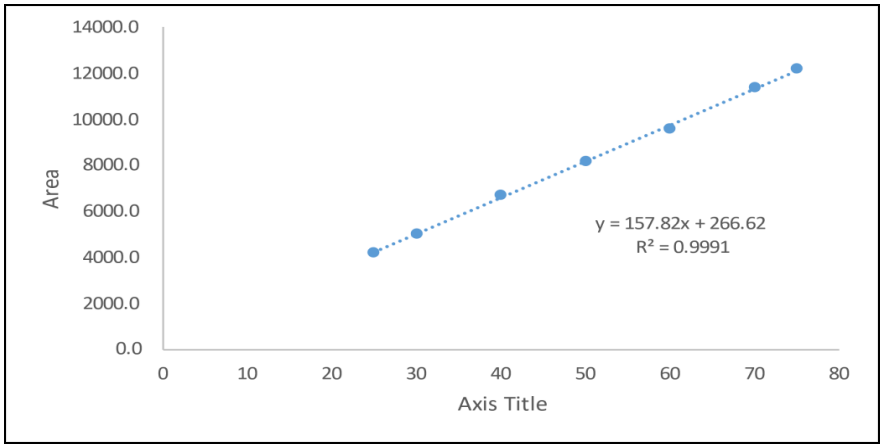

Fig.4.2(a)-Calibration curve of Olaparib.

4.3 Accuracy (Standard Addition method)

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the estimated value. The accuracy method was established by standard addition method at three different level 110%, 120% and 130%. It was determined by recovery of Olaparib (known quantity) added to test solution. The average of % mean recovery at all three levels was found to 100.69% and mean %RSD value was found to be 0.0825% mentioned in Table no. 4.3.

Table no.4.3-Result of accuracy studies of Olaparib.

% Level	Amount STD spiked (mg)	Amount recovered (mg)	Recovery	% Recovery	% Mean recovery	SD	%RSD
110	0.5	5.49	108.16	98.33	98.49	0.15	0.15
	0.5	5.47	108.51	98.64			
	0.5	5.45	108.36	98.51			
120	1	6.01	121.99	101.66	101.67	0.02	0.02
	1	5.98	122.05	101.71			
	1	6.01	121.99	101.66			
130	1.5	6.53	132.57	101.97	101.9	0.06	0.06
	1.5	6.54	132.44	101.88			
	1.5	6.57	132.4	101.85			
				Mean	**100.69**	**0.08**	**0.08**

4.4 Precision

4.4.1. Repeatability: Repeatability expresses the precision under the same operating conditions over a short interval of time.

4.4.2. System Precision: Six replicate injections of working standard solution of Olaparib were injected. The % RSD calculated was found to be 0.03%mentioned in Table no. 4.4.2.

Table no. 4.4.2 -Result of system precision studies of Olaparib.

Injection No.	Area at 276nm	Limit
1	8024.1	NMT 2%
2	8023.6	
3	8019.4	
4	8024.4	
5	8018.3	
6	8021.9	
Mean	8021.95	
± S.D.	2.58	
% RS.D.	0.03	

3.4.3. Method Precision: Six test solution of Olaparib were injected. The %RSD calculated was found to be 0.23%mentioned in Table no. 4.4.3.

Table no. 4.4.3-Result of method precision studies of Olaparib.

Injection No.	Area at 276 nm	Limit
1	8120.8	NMT 2%
2	8099.1	
3	8138.6	
4	8105.9	
5	8105.8	
6	8083.6	
Mean	8108.97	
± S.D.	18.86	
% RS.D.	0.23	

4.4.4 Intermediate Precision: Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

4.4.5. Intraday Precision: Study was carried out by injecting freshly prepared standard and test solution at three different time intervals i.e. at 10am, 1pm and 4pm. The average of %mean recovery and average of % RSD calculated was found to be 100.31% and 0.62% respectively mentioned in Table no.4.4.5.

Table no. 4.4.5-Result of intraday precision studies of Olaparib.

Sr. no	10:00 AM	1:00 PM	4:00 PM	
1	100.46	99.65	100.48	
2	99.45	100.29	100.09	
3	100.69	99.12	100.81	
4	101.52	100.27	101.37	
5	99.54	100.38	100.59	**Mean**
Mean %	100.33	99.94	100.67	**100.31**
SD	0.86	0.54	0.47	**0.62**
% RSD	0.86	0.54	0.47	**0.62**

4.4.6 Interday Precision: Study was carried out by injecting freshly prepared standard and test solution on three different days and by three different analysts. The average % mean recovery and average % RSD calculated was found to be 100.62% and 0.66% respectively mentioned in Table no. 4.4.6.

Table no.4.4.6-Result of interday precision studies of Olaparib.

Sr. No.	Analyst A (Day 1)	Analyst B (Day 2)	Analyst C (Day 3)
1	101.78	99.57	100.28
2	100.38	100.61	101.22
3	100.72	100.82	101.62
4	100.04	100.56	100.02

5	100.82	99.97	101.26
6	99.98	101.63	100.65
Mean %	100.62	100.53	100.84
S.D.	0.66	0.71	0.62
%R.S.D.	0.66	0.71	0.62

4.5 Robustness

The Robustness of the method was established by making deliberate changes in detection wavelength, temperature, flow rate and ratio of mobile phase in the estimation of Olaparib test solution. It gives indication about the reliability of developed method. The mean, standard deviation and %RSD were calculated for each change in method and results mentioned in Table no 4.5.

Table no. 4.5-Result of robustness studies of Olaparib.

Parameter	Change in Parameters		% Estimation	Mean	SD	% RSD	Limit
Wavelength (nm)	274		100.64	100.62	0.05	0.05	NMT 2.0%
	276		100.56				
	278		100.67				
Flow (ml/min)	0.8		100.53	100.58	0.07	0.07	
	1		100.56				
	1.2		100.67				
Temperature (ºC)	38		101.82	101.34	0.68	0.67	
	40		100.56				
	42		101.64				
Mobile Phase Ratio [0.1% TFA buffer (A): Acetonitrile (B)]	A	B					
	55	45	101.32	101.19	0.58	0.57	
	60	40	100.56				
	65	35	101.71				

4.6 System suitability test

System suitability testing is an integral part of method development and it was carried out as per ICH Q2(R1) guidelines. The HPLC system used for analysis must pass the system suitability limits before sample analysis can commence. A blank preparation (single injection) and standard preparation (six replicate injections) at the working concentration (50µg/ml) were injected into the HPLC and the chromatograms were recorded to evaluate the system suitability parameters like peak area, retention time (RT), number of theoretical plates (N) and tailing factor and % RSD of the replicate injections of standard was determined and mentioned in Table no. 4.6.

Table no. 4.6 - Results of system suitability parameters of Olaparib.

Sr. No.	Peak Area	Retention Time	Theoretical Plates	Tailing Factor
1	8296.4	5.140	10487	1.39
2	8312.6	5.143	10429	1.38
3	8318.6	5.143	10443	1.35
4	8295.7	5.143	10420	1.35
5	8313.4	5.143	10413	1.36
6	8333.1	5.143	10466	1.37
Average	8311.63	5.143	10443	1.37
S.D.	14.14	0.001		0.02
%R.S.D.	0.17	0.02		1.19
Limits	NMT 2.0%	NMT 1.0%	NLT 2000	NMT 2.0%

4. RESULTS AND DISCUSSION

High-pressure liquid chromatography (HPLC) is one of the most reliable and essential analytical tool in assessing the drug product. It has the ability to separate, identify, and quantify the compounds that are present in any sample. Therefore, a simple, accurate, precise, sensitive and novel isocratic reverse phase high performance liquid chromatography (RP-HPLC) method was developed for the estimation of Olaparib in its tablet formulation. It can be used both for qualitative as well as quantitative analysis. After optimizing the method, with consideration of system suitability parameters, the method finalized was given below Table no. 5.

Table no. 5 - Optimized RP-HPLC method for Olaparib.

COLUMN	**Thermo Scientific C18 column (250mm x 4.6mm x 5µ)**
WAVELENGHT	276 nm
FLOW RATE	1 ml/min
INJECTION VOLUME	10µl
COLUMN OVEN	35
RUN TIME	10
SOLVENT MIXTURE	Acetonitrile and water (1:1)
MOBILE PHASE	0.1% Trifluoro acetic buffer: Acetonitrile [60:40]
DETECTOR	UV-VIS Variable wavelength

In case of specificity, no interference was observed from blank (diluents) and at the retention time of Olaparib peak. Therefore, the developed HPLC method for the determination of Olaparib is specific.

The results of linearity study of Olaparib standard solution over the concentration range of 25-75µg/ml showed the linear response with correlation coefficient 0.9991 and regression equation y = 157.82x + 266.62.

Accuracy was determined in terms of percent recovery studies at three different levels i.e., 110%, 120% and 130%. The % mean recovery at all three levels was found to between limits 98.0-102.0% and average %RSD values was found to be 0.082.

In repeatability method for system precision, replicate injections (n=6) of Olaparib standard solution were injected and % RSD was found to be 0.03 and for method precision, six replicate injections were injected %RSD was found to be 0.23 indicating substantially high precision of method.

The intermediate precision study (ruggedness) was ascertained on the basis of intraday and interday data obtained by analysing Olaparib in tablet dosage forms by proposed method and it is found to be very much reproducible with mean value 100.31% and 100.66% respectively and has average % RSD less than 2 i.e., 0.62 and 0.67 for intraday and interday precision respectively. This indicates the ruggedness of the method.

In case of robustness, it was performed by changing various parameters such wavelength, flow, temperature and ratio of mobile phase and it was observed that the method was robust and through the validation report, it can also be concluded that system is suitable for analysis.

5. CONCLUSION

The proposed RP-HPLC method developed for the qualitative and quantitative determination of Olaparib in tablet dosage form was simple, selective, sensitive, accurate, precise and rapid. Acceptable regression values, RSD (%) and standard deviations make it versatile and valuable for estimation of Olaparib in tablet formulation. The method was validated as per ICH guidelines and validation acceptance criteria were met in all cases. Thus, the described method is suitable for routine analysis and quality control of pharmaceutical formulation i.e., tablets dosage form.

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