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Stability Indicating Assay Method For The Quantitative Determination Of Olaparib In Bulk And Pharmaceutical Dosage Form

Short title: Stability Indicating Assay Method for Olaparib

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ABSTRACT

Background: Olaparib is an orally active poly (ADP-ribose) PARP (polymerases) inhibitor known to destroy cancer cells with BRCA1 or BRCA2- deficiency. **Objectives:** An authentic, fast, distinct, and reliable Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) method was developed and promptly validated in tablet formulation for olaparib estimation.
Materials and Methods: The proposed method is focusedon the separation of olaparib in reverse phase mode using a WATERS symmetry C18 (150 × 4.6 mm, 5µm) analytical column with a flow rate of 1.0 mL/min and the injection volume was keptat 20µL. The optimized mobile phase consists of ammonium acetate buffer (pH adjusted to 3.5 by glacial acetic acid): methanol in the ratio of 50:50 v/v.
Results: The eluents were measured at 254 nm and the retention time for the drug encircled was about 4.32 min. The stress degradation studies of Olaparib were carried out under acidic, alkaline, oxidative, photolytic and thermal conditions in order to demonstrate the stability of the drug. The regression value of 0.998 showed that the developed method was linear over the range of 80µg/mL to 120µg/mL. The developed RP- HPLC method is accurate and precise. The method was statistically validated as per ICH guidelines.
Conclusion: The proposed method is suitable and can be applied practically for the quantitative estimation of olaparib without any interference of the excipients used in the drug formulations.
KEYWORDS: Olaparib, Poly ADP-ribose polymerase (PARP) inhibitor, RP-HPLC, Waters, ICH and Validation

INTRODUCTION

During the last decade, inactivation of poly (ADP-ribose) polymerase (PARP), a nuclear enzyme associated with a number of operations including DNA repair and cell death, has emerged as a possible individualised cancer therapeutic approach.[1,2,3,4] In cancer cells with a defective DNA damage repair system, such as those produced by BRCA gene mutations, PARP inhibitors, a new class of anticancer drugs, can cause tumor-specific synthetic lethality.[5,6,7,8] Olaparib (Fig. 1), veliparib, niraparib, and rucaparib are potent PARP inhibitors that have recently moved through advanced clinical studies as combination and/or solo targeted therapies, especially in breast and ovarian malignancies. Olaparib

(Lynparza) was the first medication to be approved for use in individuals with BRCA-mutated ovarian cancer by the European Commission (2014) and the US FDA (2015).[5,9,10]
PARP inhibitors hold a lot of therapeutic potential and will likely be employed in many cancer therapies in the future.[10] However, preclinical and clinical studies have revealed that tumour cell sensitivity to PARP inhibitors varies significantly, indicating that treatment efficacy must be enhanced[11]. Because PARP is an intracellular target, a crucial element influencing tumour cell sensitivity and the efficacy of a PARP targeted treatment is the quantity of PARP inhibitors reaching the intracellular compartment.[12] PARP inhibitors, like any other intracellular target medicine, are affected by processes such as excretion, metabolism,[13] drug absorption, and expression/upregulation of transmembrane drug efflux transporters.[14,15] The latter, which is particularly significant for PARP inhibitors, was discovered as a key resistance mechanism during early preclinical trials.[16,17,18]
Analytical method validation assures that diverse HPLC analytical procedures provide consistent and reproducible results; it is an important stage in the development of novel dosage forms since it gives information on accuracy, linearity, precision, detection, and quantitation limits. "The goal of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose," according to the ICH guideline. Validation data must now be sent to the appropriate authorities during the medication development process. The validation of analytical methods is governed by a set of guidelines. Guidelines from the International Conference on Harmonization (ICH) and the United States Pharmacopeia

Olaparib has not yet been formally included in any of the official pharmacopoeias, and there is no documented RP-HPLC technique for quantifying olaparib in pharmaceutical formulations, according to a comprehensive literature assessment.[19,20] However, only a few techniques for estimating olaparib concentrations in human plasma using UHPLC10 and LC–MS/MS have been reported.[21,22] The goal of this work was to create a genuine, quick, distinct, and reliable analytical technique for quantifying olaparib in pharmaceutical formulations using reverse phase high performance liquid chromatography, which was validated according to ICH guidelines. The proven method for quantifying olaparib in bulk and pharmaceutical formulation was successfully implemented.

MATERIALS AND METHODS

Chemical and Reagents

The various laboratory batch samples and reference standard (99.92%) of Olaparib were provided by AstraZeneca Pharma. HPLC grade Methanol was procured from Merck Sigma Aldrich. Milli-Q purified water by Milli Q plus purification system from Millipore were utilised in the course of experimental studies. HPLC grade Ammonium acetate was obtained from Rankem.

Instrument

In the study, a Waters HPLC 2695 sequence with a pump, auto sampler, auto injector, variable wavelength detector, and 2690 PDA detector, thermostatic column compartment was used. Operation control of the instrument and data collection was done by Empower 3 software.

Optimization of Chromatographic Conditions

The HPLC method was optimised with an objective to estimate olaparib in tablet formulation. Several mobile phases in isocratic mode, along with various columns, were taken into consideration to achieve a sharp peak with the base line. The tailing factor, sharpness of the peak, and symmetry were taken into consideration for selectivity, sensitivity, and appropriate chromatographic conditions suitable for the column and the mobile phase. Different flow rates were also tried and fixed as 1ml/min for the optimised method. The eluents were also checked for their maximum absorbance in the PDA detector and fixed at 254 nm as a detection wavelength. The temperature of the column was maintained at 25°C.

Preparation of Mobile Phase

The mobile phase was prepared in a ratio of 50:50 v/v of buffer and methanol. Ammonium acetate buffer

was created by dissolving 1.0 gm of ammonium acetate in a sufficient volume of Milli Q water (1000 mL).The pH of the solution was adjusted to 3.5 using glacial acetic acid. The mobile phase was degassed by sonication and it was filtered using a 0.45-microne membrane filter. Methanol was used as a diluent. The UV detector was set at a wavelength of 254 nm.

Preparation of standard solution

The standard stock solution of olaparib was prepared by weighing 25 mg of olaparib into a 25 mL volumetric flask, sonicating until dissolved, and finally, the volume was made up to 25 mL with methanol. Appropriate dilutions were made from the above 1.0 mg/mL solution and transferred to a 100 mL volumetric flask, where the final volume was made by the mobile phase. Prior to the filling of the vials for chromatographic analysis, the solution was passed through a membrane filter of diameter 0.45µ.

Sample Solution Preparation for the estimation of Marketed Tablet Formulation

20 tablets were accurately weighed and powdered. Then, a volumetric flask was added with 25 mL of diluent and sonicated till it was completely dissolved. Finally, the volume was made up to 50 mL. Appropriate dilution was made to obtain a concentration of 100µg/mL as a stock solution. Different dilutions were made from the stock solution and chromatographic analysis was carried out. Prior to filling the vial for chromatographic analysis, the solution was filtered via a 0.45µ membrane filter.

Method Validation

The optimized method developed for olaparib was validated in accordance with the International Conference of Harmonization (ICH) guideline Q2 (R1) (ICH) for evaluating linearity, precision, accuracy, specificity, robustness, ruggedness, system suitability, analytical solution stability and force degradation.

Linearity

The linearity range of an analytical method has been assessed by injecting the standard dilutionin duplicates over five different concentrations made in the range of 80 µg/mL to 120 µg/mL. The calibration curve was plotted with the analyte peak area against the analyte concentration to ensure the linearity of the analytical method being developed.

Precision and intermediate precision

The intra-and inter-day precision was determined in terms of the peak area difference of drug solutions for three consecutive days. A relative standard deviation (RSD) was calculated from the alteration of peak area to represent the intra- and inter-day precision.

Intra and inter-day precision were performed at three different concentration levels of 80, 100, and 120 µg/mL. The repeatability study was performed by injecting six replicates of standard preparations of concentration 100 µg/mL.

Accuracy

The accuracy of the developed method was verified by spiking olaparib, which was performed by spiking olaparib with a standard at three different concentrations: 90%, 100%, and 110%. Triplicate analysis of these samples was performed and the results were in the form of % RSD and recovery percentage.

Specificity

The Specificity of the method was established by analyzing standard substances against potent interferences. Specificity was assessed by injecting standard, sample, placebo, and blank preparations into HPLC. The recovery was measured.

Robustness

Alterations were made deliberately in chromatographic parameters like the composition of the mobile phase composition, mobile phase pH, and flow rate. These variations were evaluated for column

efficiency, asymmetry factor, along with their %RSD.

Ruggedness
Different analysts were taken into consideration for the ruggedness study. Solutions of 100 µg/mL were prepared and injected by two different analyst, and the result was given in the form of %RSD.

Assay of Standard formulation of Lynparza (olaparib)
Twenty tablets were weighed and crushed to powder. A quantity of this powder equivalent to 50 mg was taken in a 50 mL volumetric flask to which 25 mL of diluent was added. The solution was sonicated for 30 min, and the volume was adjusted up to the mark with diluent. The solution was further diluted to obtain a concentration of 100µg/mL of olaparib. Prior to the filling of the vials for chromatographic analysis, the solution was passed through a 0.45µ membrane filter.
System Suitability
System suitability parameters such as tailing factor, resolution, theoretical plates, and percent relative standard deviation have been assessed through injecting a blank observed by six replicates of the olaparib standard as well as sample solutions at a concentration of 100µg/mL.

Limit of Detection and Limit of Quantification
The detection limit (LOD) and quantification limit (LOQ) were calculated from the calibration according to the formulas mentioned:

$$LOD = 3.3SD/Slope$$
$$LOQ = 10SD/Slope$$

or detection limit=3.3σ/s, quantification limit=10σ/s, where σ is the standard deviation of y- intercept of regression line, and s is the slope of the calibration curve.

Solution Stability
The stability of the analytical solution was set up by injecting the standard solution at a periodic interval of 48 hours by maintaining the temperature of the auto sampler at room temperature. The solution response was measured and the percentage differences of the peak area have been calculated.
Force degradation Study
According to the ICH guideline Q1A (R2), a force degradation study of olaparib was performed under stress conditions. The olaparib API (100µg/mL) was subjected to hydrolysis (acid and alkali), peroxide, thermal, and photolytic degradation for the stability study.
Acid Degradation
A standard solution of 5 mL olaparib was taken in a 50 mL clean and dry volumetric flask. To the volumetric flask, 2.5 mL of 5M HCl was added and kept for 30 min. After the completion of 30 min., neutralise the solution with 2.5 mL of 5M NaOH and the 50 mL volume was made by the mobile phase. Finally, filter the solution with a 0.45µm filter. The filtered solution was introduced in HPLC and the peak area was compared with the standard chromatogram.
Alkali Degradation
A standard solution of 5 mL olaparib was taken in a 50 mL clean and dry volumetric flask. To the volumetric flask, 2.5 mL of 5M NaOH was added and kept for 30 min. After 30 minutes, neutralise the solution with 2.5 mL of 5M HCl, and the mobile phase was responsible for the 50 mL volume. Finally, filter the prepared solution with a 0.45µm filter. The filtered solution was introduced in HPLC and the peak area was compared with the standard chromatogram.
Peroxide Degradation
A standard solution of 1.0 mL of olaparib was taken in a 10 mL clean and dry volumetric flask. To the volumetric flask, 1ml of 30% H_2O_2 was added and kept in the flask for 30 min. After the completion of the 30 min., a volume of 10 mL was made by the mobile phase. Finally, filter the prepared solution with a 0.45µm filter. The filtered solution was then introduced into HPLC and the peak area was compared with the standard chromatogram.

Thermal Degradation
The powdered sample of olaparib was spread on a petri dish with 1.0 mm thickness and kept at 70 ºC in a hot air oven for 3 hrs. 25 mg of the sample was taken in a 25 mL clean and dry volumetric flask. 10 mL solution was pipette out and the volume of 100 mL was made by the mobile phase. Finally, filter the prepared solution with a 0.45µm filter. The filtered solution was introduced in HPLC and the peak area was compared with the standard chromatogram.
Photolytic Degradation
The powdered sample of Olaparib was spread on a petri dish with 1.0 mm of thickness and kept in direct sunlight for 3 hours. 25 mg of the sample was taken in a 25 mL clean and dry volumetric flask. 10 mL solution was pipette out and the volume of 100 mL was made by the mobile phase. Finally, filter the prepared solution with a 0.45µm filter. The filtered solution was introduced in HPLC and the peak area was compared with the standard chromatogram.

RESULTS
Method Development
Chromatographic separation
Various chromatographic systems (RP-HPLC) were taken into consideration to optimise the separation of olaparib. Olaparib separation was performed on the column C18 (150mm × 4.6mm, 5µm). The mobile phase was a combination that included 500 mL of methanol, 500 mL of buffer, and 1.0 mL of glacial acetic acid, pH adjusted to 3.5 ± 0.05 with ammonium acetate. The flow rate was set to 1.0 mL/min and the detector was set to 254 nm. The injection volume was kept to 20µL. The retention time for olaparib was found to be 4.32 min, as shown in figure 2.

Calibration curve
The calibration curve was prepared and evaluated using the least square method within the Microsoft Excel® program. The coefficient of determination (R^2), slope and intercept for olaparib were 0.998, 23599 and 66731 respectively. The linear equation was found to be y = 23599x + 66731 and the calibration curve is shown in Figure 2.

Validation of the method
Linearity
The analytical calibration curve was plotted for olaparib and was found to be linear in the specified ranges (80-120µg/mL) indicating the correlation coefficient R2 of 0.99 (acceptance limit > 0.98). The slope of the straight line was found to be 23599 and the intercept was found to be 66731. The results are reported in Table 1 and the calibration curve is shown in Figure 2.

Precision and Intermediate precision
The precision value was reported in terms of relative standard deviation (%RSD). The %RSD for olaparib was found to be <2.0% for both inter-and intra-day precision, indicating satisfactory precision (Table 2). The inter-day precision was found to be within 0.05-0.98, whereas intra-day precision was found to be within 0.06-0.43.

Accuracy
The accuracy of this method is determined by the recovery study carried out using standard addition methods at six concentration levels, first 90%, 100%, & 110%. The spiked sample solutions were assayed in triplicate and the results obtained were compared with the expected results and expressed as the percentage of recovery reported in Table 3. The recovery was found to be within the limit.

Specificity
The developed analytical method should reflect that there was no interference due to the presence of excipients in the formulation. The recovery and %RSD of olaparib were measured and were within the limits summarised in Table 4. The recovery was found to be 100.81-101.71 and %RSD was within 0.10-0.80.

Robustness
The method was found to be robust, ensuring that upon applying small variations to the chromatographic conditions in terms of flow rate, mobile phase composition, and pH variation in the mobile phase, no significant changes are detected. The robustness data was expressed in terms of % RSD was found to be 0.68 and given in Table 5.

Ruggedness
Analyst 1 and Analyst 2 performed the ruggedness test and the results are summarised in Table 6 as %RSD was found to be 1.48.
System Suitability
The system suitability test is an important element of chromatographic analysis since it ensures that the chromatographic system's accuracy and repeatability are sufficient for analysis. It was performed by six replicate injections of the standard solution of olaparib. The retaining of olaparib was found to be 4.32 min., having a tailing factor of not more than 1.17 in all the peaks, indicating good peak symmetry. Theoretical plates were found to be 3160. The results are reported in Table 7.

Detection limit and quantification limit
The LOD and LOQ of olaparib were found to be 0.49 µg/mL and 1.49 µg/mL, respectively.

Assay of standard formulation of Lynparza (olaparib)
Assay validation provides reliability assurance during normal use, and is sometime referred to as "the process of providing documented evidence that the method is doing what it intends to do." The purity by HPLC is determined by the percentage recovery of olaparib. The developed method was very accurate, precise and robust as the recovery % was within 100+/-2 given in Figure 3a and Table 8.

Analytical Solution Stability
The Olaparib sample solution was stable for 24 hours at room temperature. The stability results have been analysed for the percentage difference from zero time injection, where there is no decrease in the peak areas of the drug nor there is a sifting of retention time was detected. The observations obtained from the stability phenomenon are reported in Table 9.

Force Degradation Study
The drug degradation study was performed in acid, alkaline and oxidation solutions to determine the stability of the drug in different conditions.

Acid Degradation
The acid degradation of olaparib was carried out at different concentrations of (1-5M) HCl till it was degraded for a time period of 30 min. The degraded chromatogram and the non-degraded chromatogram were compared and the percentage of degradation was calculated. The chromatogram is given in (Figure 3b) and the results are summarised in Table 10. Acid degradation was found to be 12.69% at 5M HCl.
Alkali Degradation
The alkali degradation of olaparib was carried out at different concentrations of (1-5M) NaOH till it was found to be degraded. The degraded chromatogram and the non degraded chromatogram were compared and the percentage of degradation was calculated. The chromatogram is given in Figure 4a and the results are summarised in Table 9. Alkali degradation was found to be 2.60% at 5M NaOH.

Peroxide Degradation
Peroxide degradation of olaparib was carried out at a concentration of 30%. A comparative study of the peroxide degraded olaparib chromatogram and the non degraded chromatogram was conducted to calculate the 2.55% degradation. The chromatogram is given in Figure 4b and the results are summarised in Table 10.
Thermal Degradation
Thermal degradation of the drug was found to be negligible. The olaparib drug was found to be thermal stable as there was no degradation when exposed to thermal conditions. The chromatogram is given in Figure 5a, and the results are summarised in Table 10.
Photolytic Degradation
Degradation by photolysis of olaparib was found to be negligible. The olaparib drug was found to be light stable as there was no degradation when exposed to light. The chromatogram is given in (Figure 5b) and the results are summarised in Table 10.

DISCUSSION

Olaparib is a new drug, so nearly no method is available to estimate olaparib in bulk and pharmaceutical dosage form. So our present aim is to develop a new, compatible, stable, robust method for the determination of olaparib in bulk and formulations by RP-HPLC.
According to the ICH guidelines, the developed method was validated for the following parameters: system suitability, linearity, accuracy, precision, robustness, analytical solution stability. The % RSD value was well below 2 and the % recovery was within the limit of 100+/-2. Stability of the drugs is a very big issue during formulation and still no stability data has been reported. To know the stability of olaparib, we have performed a force degradation study.
The drug was found to be very stable when exposed to heat and light. It was also found to be quite stable in both acidic and basic conditions. Higher concentrations of acids and bases (5M) were able to degrade the drug, too, by 12.69% in acidic conditions, where as in the case of basic and oxidation conditions, degradation was found to be 2.60% and 2.55%. In thermal and photolytic conditions, they were found to be stable.

CONCLUSION

The developed method in RP-HPLC was established to be simple, cost-effective, accurate, and robust so that it can be extensively applied for the estimation of any formulations of olaparib. The degradation study was performed on HPLC for the first time. As the HPLC method is less cost-effective as compared to the LC-MS method, this will prove an effective method for the estimation of olaparib.
Degradation studies were performed and from the study we can conclude that the degradation of olaparib was very low in the case of basic and oxidation conditions, whereas in the case of acidic degradation by HCl, the highest degradation was observed. The degradation was found to be 12.69%. No degradations were found in the case of thermal and photolytic conditions. The drug olaparib can be considered as a very stable drug in all conditions except the acidic condition.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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REFERENCES

1. O. Dizdar, C. Arslan, K. Altundag, Advances in PARP inhibitors for the treatment of breast

cancer, Expert Opin. Pharmacother. 16 (2015) 2751–2758.
2. D.V. Ferraris, Evolution of poly(ADP-ribose) polymerase-1 (PARP-1) inhibitors From concept to clinic, J. Med. Chem. 53 (2010) 4561–4584.
3. B. Lupo, L. Trusolino, Inhibition of poly(ADP-ribosyl)ation in cancer: old and new paradigms revisited, Biochim. Biophys. Acta 1846 (2014) 201–215.
4. V. Schreiber, F. Dantzer, J.C. Ame, G. de Murcia, Poly(ADP-ribose): novel functions for an old molecule, Nat. Rev. Mol. Cell Biol. 7 (2006) 517–528.
5. J.S. Brown, S.B. Kaye, T.A. Yap, PARP inhibitors: the race is on, Br. J. Cancer 114 (2016) 713–715.
6. J. Murai, S.Y. Huang, B.B. Das, A. Renaud, Y. Zhang, J.H. Doroshow, Y. Pommier, Trapping of PARP1 and PARP2 by clinical PARP inhibitors, Cancer Res. 72 (2012) 5588–5599.
7. S. Tangutoori, P. Baldwin, S. Sridhar, PARP inhibitors: a new era of targeted therapy, Maturitas 81 (2015) 5–9.
8. S. Murata, C. Zhang, N. Finch, K. Zhang, L. Campo, E.K. Breuer, Predictors and modulators of synthetic lethality: an update on PARP inhibitors and personalized medicine, Biomed. Res. Int. (2016) 2346585.
9. E.D. Deeks, Olaparib: first global approval, Drugs 75 (2015) 231–240.
10. M.J. O'Connor, Targeting the DNA damage response in cancer, Mol. Cell 60 (2015) 547–560.
11. C.T. Dollery, Intracellular drug concentrations, Clin. Pharmacol. Ther. 93 (2013) 263–266.
12. T. Fojo, S. Bates, Mechanisms of resistance to PARP inhibitors-three and counting, Cancer Discov. 3 (2013) 20–23.
13. C.J. Lord, A. Ashworth, Mechanisms of resistance to therapies targeting BRCA-mutant cancers, Nat. Med. 19 (2013) 1381–1388.
14. R. Dufour, P. Daumar, E. Mounetou, C. Aubel, F. Kwiatkowski, C. Abrial, C. Vatoux, F. Penault-Llorca, M. Bamdad, BCRP and P-gp relay overexpression in triple negative basal-like breast cancer cell line: a prospective role in resistance to Olaparib, Sci. Rep. 5 (2015) 12670.
15. Aoki D and Chiyoda T. PARP inhibitors and quality of life in ovarian cancer. The Lancet Oncol. 2018;19:10-12.
16. Weil MK and Chen A. PARP inhibitor treatment in ovarian and breast cancer. Curr.Probl. Cancer. 2011; 35:7.
17. Faraoni I, Aloisio F, De Gabrieli A, Consalvo MI, Lavorgna S, Voso MT, Lo-Coco F and Graziani G. The poly (ADP-ribose) polymerase inhibitor olaparib induces up-regulation of death receptors in primary acute myeloid leukemia blasts by NF-κB activation. Cancer Lett. 2018;423:127-38.
18. Liu CH, Chang Y and Wang PH. Poly (ADP-ribose) polymerase (PARP) inhibitors and ovarian cancer. Taiwan J. Obstet. Gynecol. 2017; 56:7-13.
19. Nijenhuis CM, Lucas L, Rosing H, Schellens JH and Beijnen JH. Development and validation of a high-performance liquid chromatography–tandem mass spectrometry assay quantifying olaparib in human plasma. J. Chrom. B. 2013; 940:121-5.
20. Daumar P, Dufour R, Dubois C, Penault-Llorca F, Bamdad M and Mounetou E. Development and validation of a high-performance liquid chromatography method for the quantitation of intracellular PARP inhibitor Olaparib in cancer cells. J. pharm. Biomed. anal. 2018; 152:74-80
21. Roth J, Peer CJ, Mannargudi B, Swaisland H, Lee JM, Kohn EC and Figg WD. A sensitive and robust ultra HPLC assay with tandem mass spectrometric detection for the quantitation of the PARP inhibitor olaparib (AZD2281) in human plasma for pharmacokinetic application. Chrom. 2014; 1:82-95.
22. Nijenhuis CM, Rosing H, Schellens JH and Beijnen JH. Development and validation of a high-performance liquid chromatography–tandem mass spectrometry assay quantifying vemurafenib in human plasma. J. Pharm. Biomed. Anal. 2014; 88:630-5.

Table 1. Linear regression equation generated from validation of Olaparib: Slope,Intercept and Coefficient of determination

Conc. (mg/mL)	Conc. (µg/mL)	Peak area1	Peak area 2	Peak area 3	Average area
0.080	80	1938729	1945764	1942428	1942307
0.090	90	2215489	2215608	2210027	2213708
0.100	100	2413316	2411985	2415423	2413575
0.110	110	2671899	2672202	2663858	2669319.600
0.120	120	2894206	2891855	2897362	2894474.300
Slope					23599
Intercept					66731
R^2(Correlation	Coefficient)				0.998

Table 2a. Intra-day (n = 3) precision

Conc. (µg/mL)	PeakArea	Conc. found (µg/mL)	Avg.	S.D	% RSD
80	1942307	79.477	79.475	0.149	0.187
80	1945764	79.623			
80	1938729	79.325			
100	2475997	102.092	101.585	0.440	0.433
100	2458935	101.369			
100	2457207	101.296			
120	2894210	119.813	119.862	0.073	0.061
120	2894544	119.828			
120	2897362	119.947			

Table 2b. Inter-day (n = 3) precision.

Conc. (µg/mL)	PeakArea	Conc. found (µg/mL)	Avg.	S.D	% RSD
80	1939789	79.370	79.417	0.043	0.054
80	1941781	79.455			
80	1941161	79.428			
100	2442327	100.665	101.802	1.005	0.986
100	2487270	102.569			
100	2477898	102.172			
120	2894277	119.816	119.868	0.157	0.131
120	2892563	119.743			
120	2899665	120.044			

Table 2c. Repeatability

Conc. (µg/mL)	PeakArea	Conc. found (µg/mL)	Avg.	S.D	% RSD
100	2443258	100.704	100.959	0.250	0.248
100	2448178	100.913			
100	2446070	100.824			
100	2444889	100.774			
100	2457432	101.305			
100	2455724	101.232			

Table 3. Accuracy Observation Table

Recovery Level	API Added (mg)	API Recovered (mg)	%Recovery	Average % Recovery	%RSD
90%		22.110	98.680		
90%	24.900	22.100	98.610	98.650	0.230
90%		22.500	100.390		
100%		24.720	99.290		
100%	24.900	24.920	100.070	99.680	0.140
100%		25.060	100.000		
110%		26.920	98.300		
110%	24.900	26.900	98.220	98.260	0.050
110%		26.870	98.110		

Table 4. Specificity table of Olaparib

Analyte	% Added	Excipient amount added (mg)	Conc. Found (µg/mL)	% Recovery	Avg. % recovery	S.D	% RSD
Olaparib	50	5	100.705	100.705	100.814	0.105	0.104
(10 mg)	50	5	100.913	100.913			
	50	5	100.824	100.824			
	100	10	100.774	100.774	100.915	0.342	0.340
	100	10	101.305	101.305			
	100	10	100.665	100.665			
	150	15	102.569	102.57	101.710	0.892	0.877
	150	15	100.789	100.789			
	150	15	101.773	101.774			

Table 5. Robustness study with flow rate, pH and mobile phase composition

Sample I.D.	Analytical condition	Olaparib input (mg)	Olaparib Rec. (mg)	Olaparib Rec. (%)	Mean Rec. Olaparib (%)	S.D.	% RSD
1	Flow rate: 1.1 mL/min Mobile phase pH: 3.5 Mobile phase ratio: 50:50 Column: C18 (150mm × 4.6 mm, 5µm)	25	24.860	99.500	100.060	0.680	0.680
2	Flow rate: 0.9 mL/min Mobile phase pH: 3.5 Mobile phase ratio: 50:50 Column: C18 (150mm × 4.6 mm, 5µm)	25	25.090	100.300			
3	Flow rate: 1 mL/min Mobile phase pH: 3.6 Mobile phase ratio: 50:50 Column: C18 (150mm × 4.6 mm, 5µm)	25	24.820	99.200			
4	Flow rate: 1 mL/min Mobile phase pH: 3.4 Mobile phase ratio: 50:50 Column: C18 (150mm × 4.6 mm, 5µm)	25	25.110	100.400			
5	Flow rate: 1 mL/min Mobile phase pH: 3.5 Mobile phase ratio: 55:45 Column: C18 (150mm × 4.6 mm, 5µm)	25	24.980	99.900			
6	Flow rate: 1 mL/min Mobile phase pH: 3.5 Mobile phase ratio: 45:55 Column: C18 (150mm × 4.6 mm, 5µm)	25	25.290	101.100			

Table 6. Ruggedness of Olaparib

S.No.	Assay % Olaparib	
	Analyst 1 (Assay %)	**Analyst 2 (Assay %)**
1	99.390	100.560
2	100.210	99.640
3	98.040	99.800
4	102.690	100.410
5	99.980	100.200
6	101.560	97.100
Mean	100.480	99.620
% RSD	1.660	1.290
Overall % RSD	1.480	

Table 7. System Suitability parameters and achieved values Parameters

Values achieved in the validation phenomenon	
Theoretical Plates	3160
Retention Time	4.32 Minutes
Asymmetry	1.170
RSD	0.240 %

Table 8. Summery of validation

Parameter	**Acceptable limit**	**Remark**
Accuracy	98.260-99.680	Passed
Specificity	0.100-0.800	Passed
Precision	0.056-0.980	Passed
Linearity	0.998	Passed
Ruggedness and Robustness	1.480 and 0.680	Passed
Assay	98.680%	Passed

Table 9. Solution Stability data

Time	Inject	Time	Difference (sec)	Area	Calculation
Initial	9	2	0	2442536	0.00
After 1 hr	10	9	67	2450664	0.00
After 24 hr	10	5	1436	2473824	-0.01

Table 10. Force Degradation data

Type of Degradation	Area	% Degradation	Peak Purity
No degradation (standard chromatogram 100µg/mL)	2443258	-	Passed
2.5 mL of 5M HCl (acidic)	2133134.666	12.690	Passed
2.5 mL of 5M NaOH (alkaline)	2507018.666	2.600	Passed
1 mL of 30% H_2O_2 (peroxide)	2380875	2.550	Passed
Thermal degradation	2443258	0	passed
Photolytic degradation	2443258	0	passed

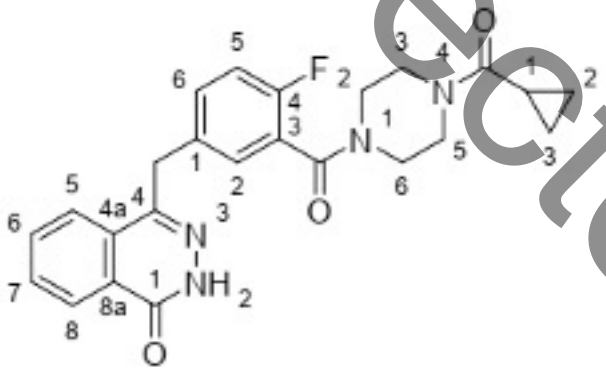

Figure 1. Chemical structure of olaparib

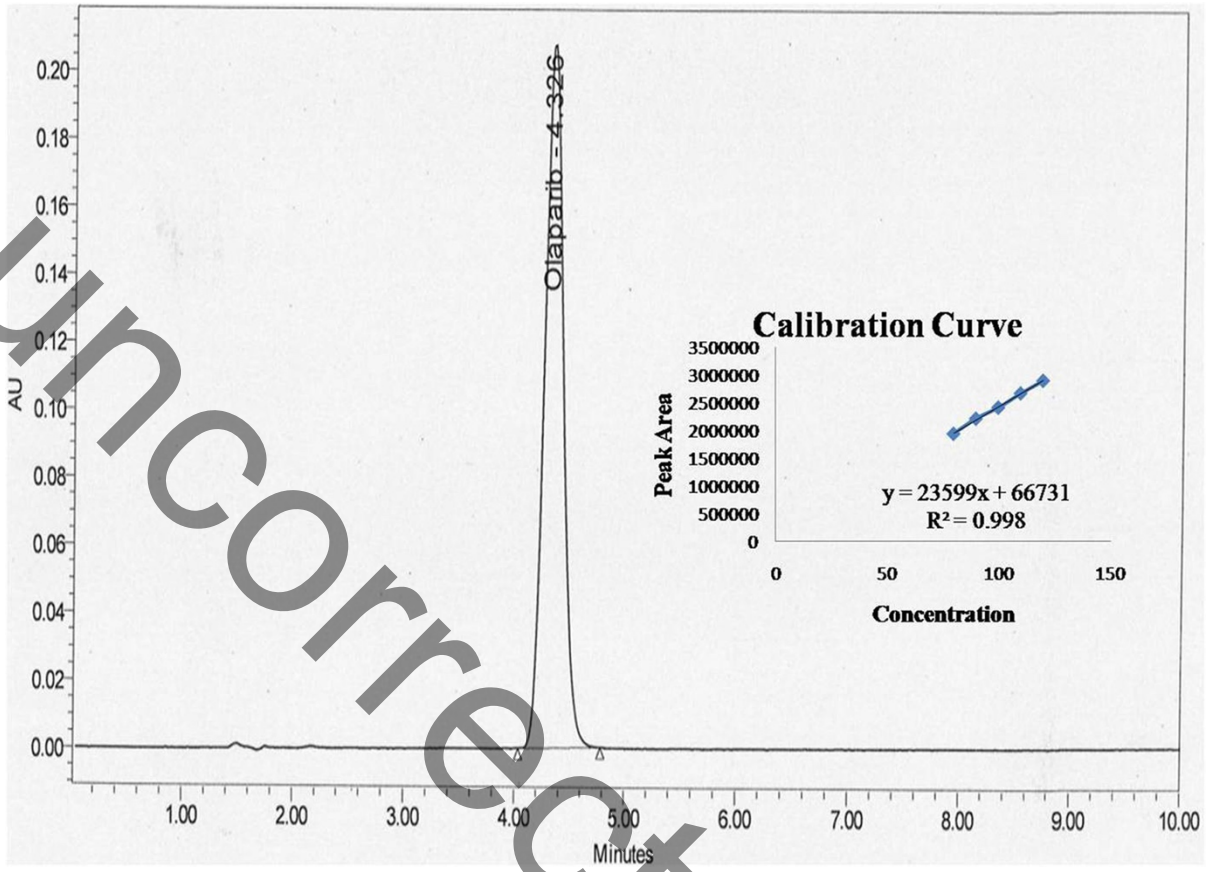

Figure 2. HPLC chromatogram of olaparib at 254 nm. Inset: Calibration curve of olaparib

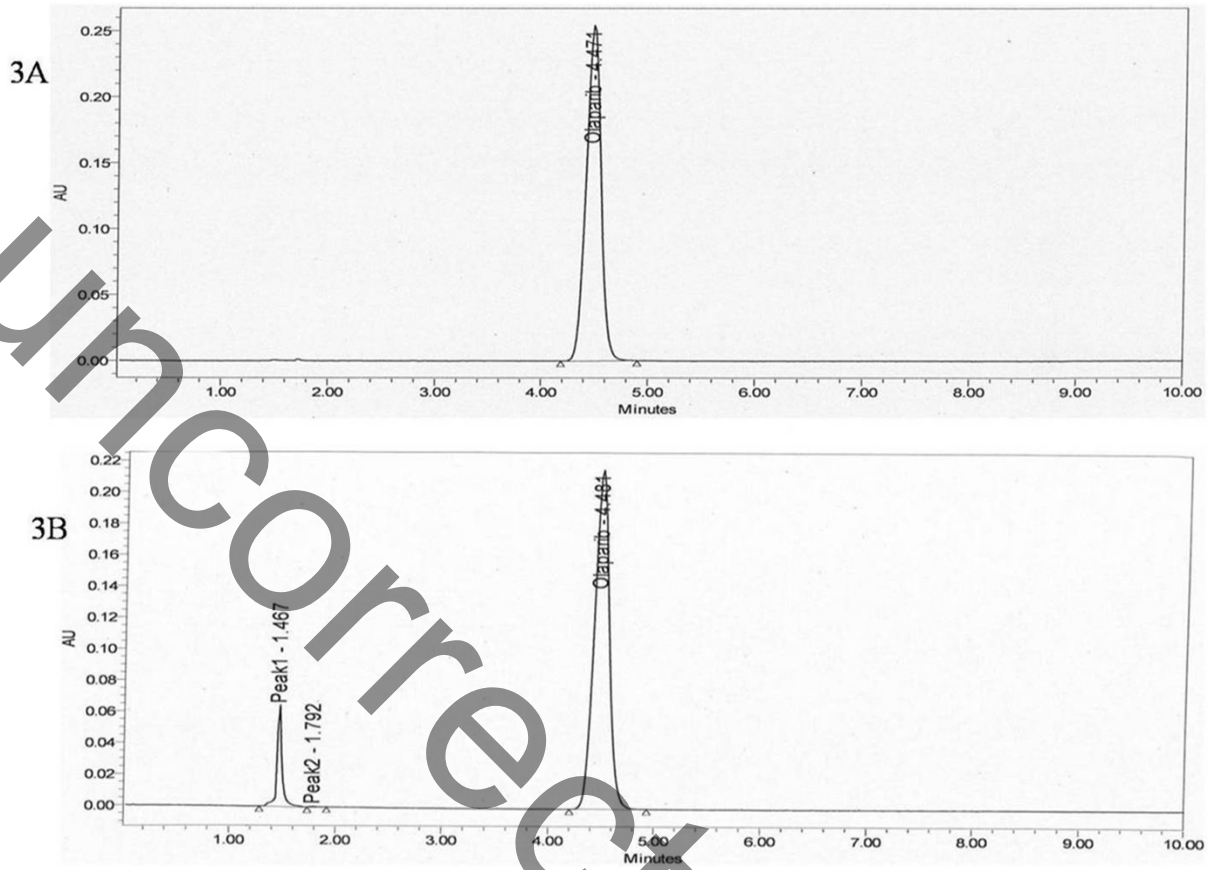

Figure 3a. Chromatogram showing assay of standard formulation, b) Chromatogram of acid degraded olaparib

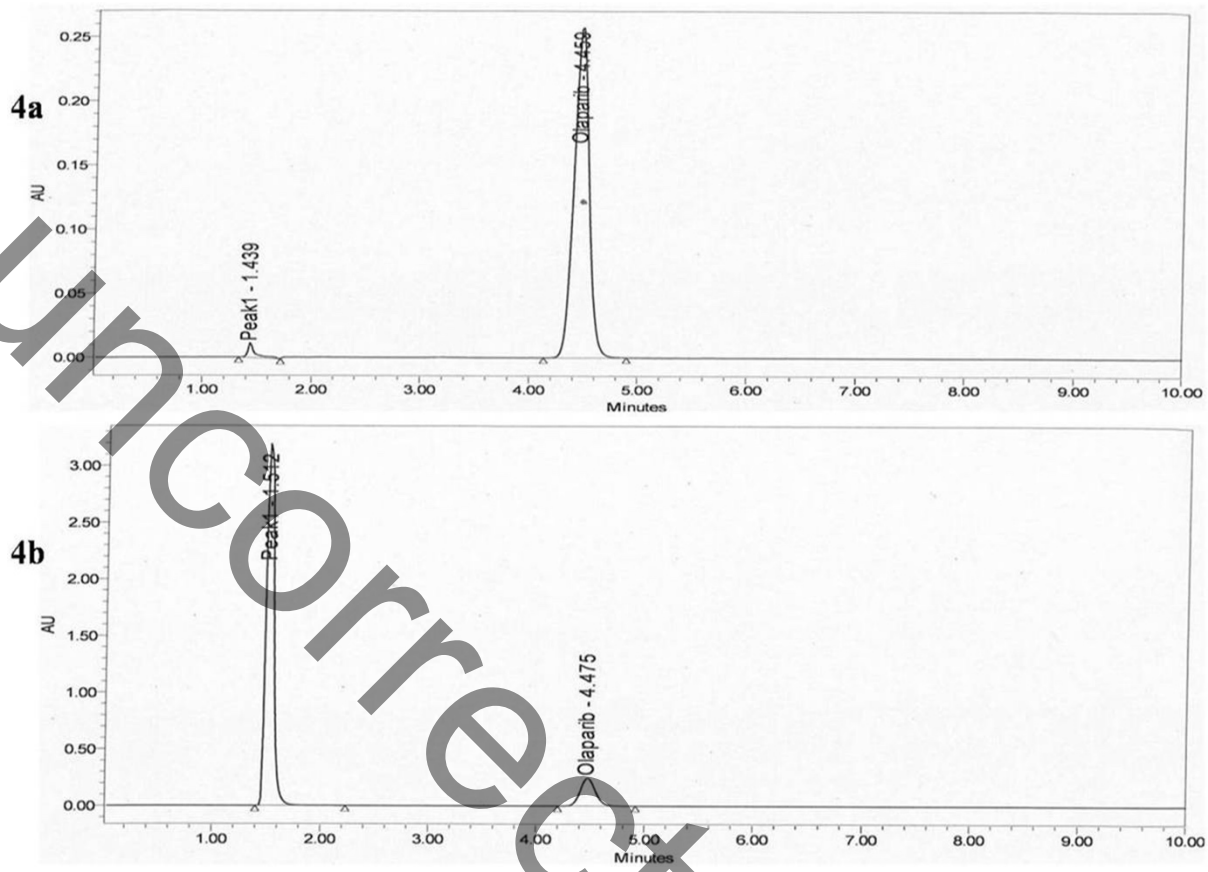

Figure 4a. Chromatogram of base degraded olaparib, b) Chromatogram of hydrogen peroxide degraded olaparib

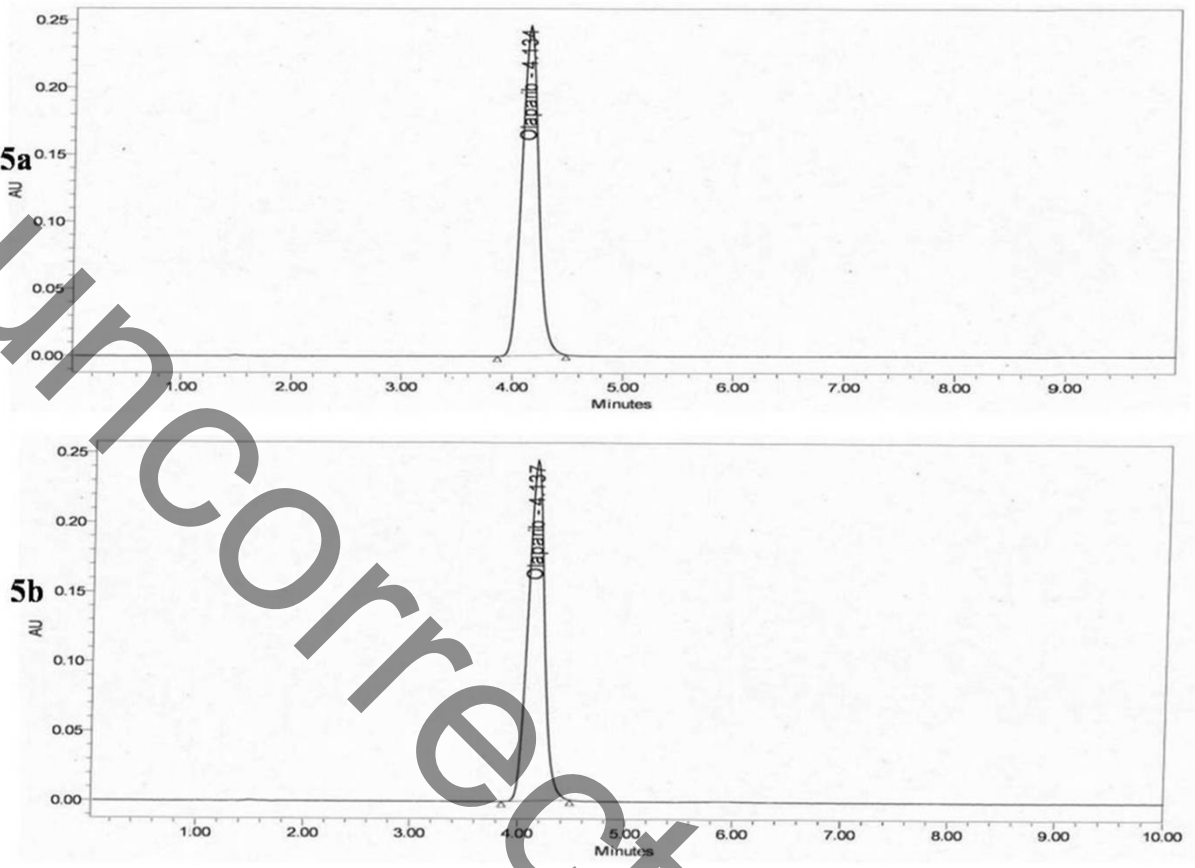

Figure 5a. Chromatogram of thermal degraded olaparib, b) Chromatogram of photolytic degraded olaparib