Advances in Bioresearch
Adv. Biores., Vol 15 (5) September 2024: 143-149

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.15.5.143149

Advances in Bioresearch

ORIGINAL ARTICLE

Advanced Analytical Method Development and Validation for the Combined Formulation of Lipid-Regulating Agent and Cholesterol Transport Inhibitor

J. Sumalatha[1], V. Viswanath[2], Divya[3], P.Anitha[2] D.Vasavi devi[4*]
[1]Department of Pharmaceutical Chemistry, P. Rami Reddy Memorial College of Pharmacy, Kadapa, Andhra Pradesh, – 516003.
[2]Department of Pharmaceutics, Annamacharya College of Pharmacy, New Boyanapalli, Rajampet, Annamayya Dt., Andhra Pradesh -516 126.
[3]Department of Pharmaceutics, P. Rami Reddy Memorial College of Pharmacy, Kadapa, Andhra Pradesh, – 516003.
[4]Department of Pharmaceutical Analysis, Annamacharya College of Pharmacy, New Boyanapalli, Rajampet, Annamayya Dt., Andhra Pradesh -516 126.
*Corresponding author email: drdvas.reddy@gmail.com

ABSTRACT
The primary goal of this investigation was to develop and validate a high-performance liquid chromatograophy (HPLC) method that is simple, reliable, sensitive, and accurate in quantifying the concentrations of Olaparib and Bevacizumab in pharmaceutical dosage form. Waters Alliance-e2695 system with an autosampler and a PDA detector was used to create the HPLC technique. With a particle size of 5 µ and dimensions of 250 x 4.6 mm, an Agilent Eclipse XDB column was used for the separation. A combination of acetonitrile and Ammonium formate of pH-3.0 with a Formic acid ratio of 40:60 v/v makes up the mobile phase. The flow rate was 1.0 ml/min; detection was carried out by absorption at 265nm using a photodiode array detector at ambient temperature. The findings showed that 2.245 and 4.232 minutes were optimal for separating Olaparib and Bevacizumab, correspondingly. The number of theoretical plates and tailing factor for ACN: Ammonium formate of pH-3.0 with Formic acid ratio of 40:60 v/v Olaparib and Bevacizumab were NLT 2000 and should not more than 2 respectively. The current procedure has been verified in accordance with ICH standards Q2 R1, and stability-indicating tests have been performed in accordance with ICH standards Q1A R2. % Relative standard deviation of peak areas of all measurements always less than 2.0. The method was found to be simple, economical, suitable, precise, accurate & robust method for quantitative analysis of Olaparib and Bevacizumab study of its stability.
Keywords: *RP-HPLC, Olaparib and Bevacizumab, stability indicating method*

Received 04.06.2024 Revised 21.07.2024 Accepted 21.09.2024

How to cite this article:
J. Sumalatha, V. Viswanath, Divya, P.Anitha D.Vasavi D. Advanced Analytical Method Development and Validation for the Combined Formulation of Lipid-Regulating Agent and Cholesterol Transport Inhibitor. Adv. Biores. Vol 15 [5] September 2024. 143-149

INTRODUCTION

During the last decade, the inactivation of poly (ADP-ribose) polymerase (PARP), a nuclear enzyme associated with many operations including DNA repair and cell death, has emerged as a possible individualized cancer therapeutic approach[1-3]. 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[4,5]. Olaparib and Bevacizumab are two significant agents in cancer therapy, each targeting different mechanisms to inhibit tumor growth. Olaparib is a poly (ADP-ribose) polymerase (PARP) inhibitor primarily used in treating ovarian and breast cancers with BRCA mutations. By disrupting the DNA damage repair mechanism, Olaparib enhances the sensitivity of cancer cells to chemotherapy and radiation. On the other hand, Bevacizumab is a monoclonal antibody that inhibits vascular endothelial growth factor (VEGF), thereby preventing angiogenesis—the formation of new blood vessels that tumors need to grow[6,7]. Due to their clinical importance, the accurate

determination of these drugs' concentrations in biological fluids and pharmaceutical formulations is crucial. High-Performance Liquid Chromatography (HPLC) is one of the most widely employed analytical techniques for this purpose, owing to its high sensitivity, specificity, and efficiency in separating complex mixtures. HPLC enables the quantification of these drugs, supporting both pharmacokinetic studies and therapeutic drug monitoring.

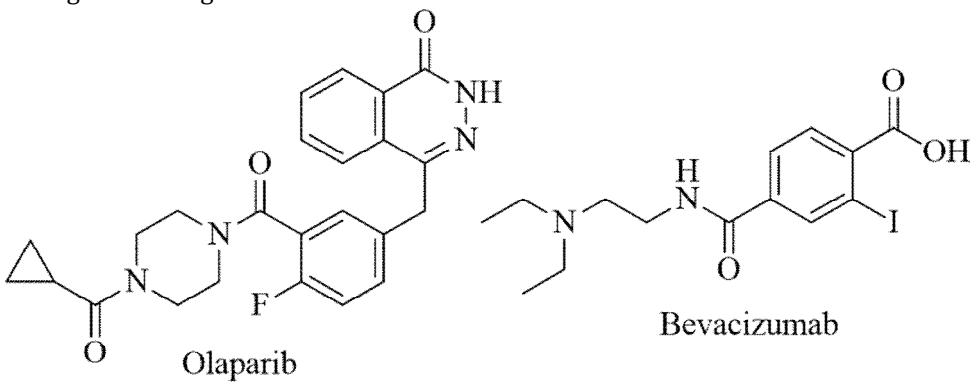

Figure 1: Structure of Olaparib and Bevacizumab

A literature survey reported that limited methods are available for simultaneous estimation of Olaparib and Bevacizumab, and a few articles reported spectrophotometric techniques for estimation of Olaparib and Bevacizumab alone and with other drugs, such as UV-spectrophotometer methods, UPLC, and RP-HPLC [8-18]. This study aims to create a simple, precise, accurate, relatively sensitive, and fast RP-HPLC technique for estimating Olaparib and Bevacizumab in bulk and formulations. The developed method was validated per ICH guidelines [19] and can be applied successfully to quality control determinations.

MATERIAL AND METHODS

Chemicals and Reagents

Olaparib and Bevacizumab pure drugs (API) were received from Supriya Life Sciences, Mumbai. A combination of Olaparib and Bevacizumab LYNPARZA was received from the local market. Acetonitrile HPLC grade received from Rankem, Water (Milli Q) produced in-house, Phosphate buffer, Methanol, Potassium dehydrogenate ortho phosphate buffer, Ammonium Formate, orthophosphoric acid, and Formic Acid of HPLC grade received from analytical reagents.

Preparation of Standard stock solutions

To accurately measure and transfer 150 mg of Olaparib and 25 mg of Bevacizumab working standard, use a clean and dry volumetric flask with a capacity of 100 ml. Add the appropriate amount of Diluent to the flask and then sonicate the mixture until completely dissolved. Finally, fill the flask up to the mark with the same solvent (Stock solution). To proceed, carefully transfer 5 ml of the previously mentioned stock solutions into a 50 ml volumetric flask. Then, add diluent to the flask until it reaches the mark on the flask, ensuring proper dilution. The concentration of Olaparib is 150 parts per million (ppm), while the concentration of Bevacizumab is 25 ppm.

Preparation of Sample stock solutions

Transfer 279mg of Olaparib sample and 1mL of Bevacizumab sample into a 100mL clean dry volumetric flask and weigh accurately. Diluent and sonicate it for 30 minutes to dissolve, then centrifuge for 30 minutes to thoroughly dissolve and bring the volume up to the mark using the same solvent. The solution is then passed through a 0.45-micron Injection filter (stock solution). Pipette 5 mL of the following stock solutions into a 50 mL volumetric flask and dilute with diluents to the desired concentration (Olaparib 150 ppm, Bevacizumab 25 ppm).

Method Validation

Specificity/Selectivity

The method's ability to distinguish and quantify the analyte in the presence of other components like impurities, degradation products, or matrix components. To ensure no interference at the analyte retention time, analyze blank samples, spiked samples with known analyte concentrations, and samples containing potential interferences.

Linearity

The method’s ability to elicit results directly proportional to the concentration of the analyte within a given range. Prepare calibration curves by analyzing samples of known concentrations (typically 5–7

levels) and plot the response vs. concentration. Calculate the correlation coefficient (R^2), which should be ≥ 0.99 for most applications.

Accuracy

The closeness of the test results to the actual value. Analyze known concentrations of the analyte and compare the measured value with the actual value. Express results as a percentage recovery, typically within 98-102% for pharmaceutical assays.

Precision

Perform replicate analyses (typically six) on homogeneous samples and calculate %RSD (Relative Standard Deviation), with the acceptable limit generally being ≤ 2%.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The lowest amount of analytes can be detected but not necessarily quantified. The lowest amount of analyte can be quantitatively determined with acceptable precision and accuracy. Based on the signal-to-noise ratio, 3:1 for LOD and 10:1 for LOQ is typically acceptable. LOD and LOQ can also be estimated from the response's calibration curve slope and standard deviation.

Robustness

The method's capacity to remain unaffected by minor but deliberate variations in method parameters (e.g., changes in pH, temperature, flow rate). Intentionally vary method parameters and assess if the changes affect the results significantly. The method should maintain acceptable performance under varying conditions**.**

Degradation studies

Degradation studies for Olaparib and Bevacizumab were conducted under various stress conditions to assess their stability. Oxidative degradation involved adding 1 ml of 20% hydrogen peroxide to 1 ml of stock solution, heating at 60°C for 30 minutes and analyzing by HPLC after dilution to 150 µg/ml and 25 µg/ml. Acid degradation was performed by refluxing 1 ml of stock solution with 1 ml of 2N hydrochloric acid at 60°C for 30 minutes, followed by HPLC analysis at the same concentrations. Similarly, alkali degradation was conducted by refluxing the stock solution with 2N sodium hydroxide at 60°C for 30 minutes. Dry heat degradation was tested by placing the drug at 105°C for 6 hours, and Photostability was assessed by exposing the drug solution to UV light for seven days or 200 Watt hours/m^2. In neutral degradation studies, the drug was refluxed in water for 6 hours at 60°C. Reduction degradation was executed by treating the sample with 1ml of 10% Sodium bisulfate at 60 °C for 1 hour. For each condition, the resulting solution was diluted to 150 µg/ml and 25 µg/ml, and 10 µl was injected into the HPLC system to record chromatograms and evaluate sample stability.

RESULTS AND DISCUSSION

The chromatographic analysis was performed using a Symmetry C-18 (150 x 4.6mm, 3.5µm) with a mobile phase consisting of acetonitrile (ACN) and ammonium formate of pH-3.0 with Formic acid (40:60). The flow rate was set at 1 ml/min, and the detector wavelength was 265 nm. The column temperature was maintained at 25°C, and an injection volume of 10 µL was used. The total run time for each analysis was 6.0 minutes. Both peaks have good resolution, tailing factor, Theoretical plate count, and resolution. The total runtime for each validation parameter was set to 6 minutes.

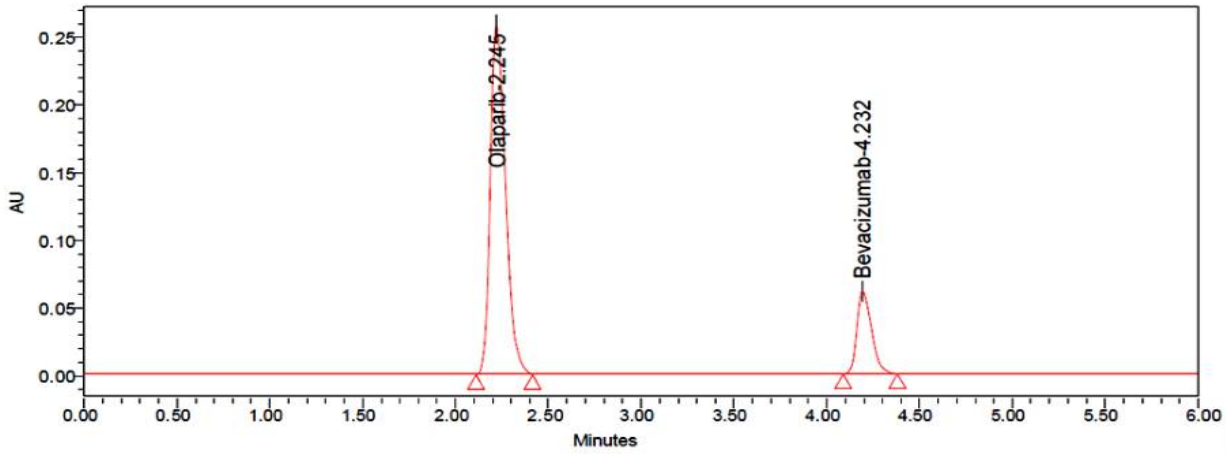

Figure 2: Optimized chromatogram of Olaparib and Bevacizumab

Method Validation

The following parameters were studied to validate the HPLC method for the determination of Olaparib and Bevacizumab as per the protocol and demonstrate that the method is appropriate for its intended use. All the validation parameters were carried out according to ICH.

Specificity

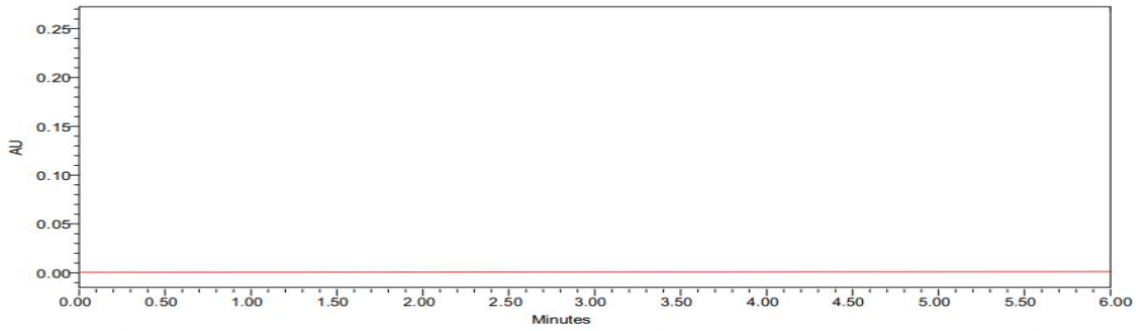

Figure 3: Chromatogram of blank

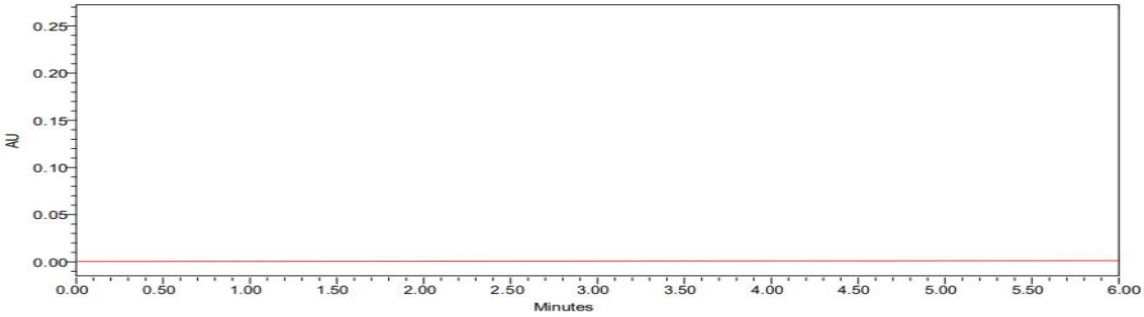

Figure 4: Chromatogram of placebo

Linearity

Six linear concentrations of Olaparib (25-150µg/ml) and Bevacizumab (6.25-37.5µg/ml) were injected and duplicated. Average areas were mentioned in table No. 1, and the linearity equations obtained for Olaparib was y = 27402.84x+9816.79, and for Bevacizumab, was y = 27613.65x +1566.14. The correlation coefficient obtained was 0.999 for the two drugs.

Table 1: Linearity table for Olaparib and Bevacizumab

S.No.	Olaparib		Bevacizumab	
	Conc.(µg/ml)	Peak area	Conc.(µg/ml)	Peak area
1	25.00	689742	6.25	175620
2	50.00	1383465	12.50	348596
3	75.00	2078312	18.75	524670
4	100.00	2750435	25.00	683767
5	125.00	3465264	31.25	859346
6	150.00	4087992	37.50	1043255
Regression equation	y = 27324x + 18325		y = 27564x + 2923	
Slope	27324		27564	
Intercept	18325		2923	
R^2	0.999		0.999	

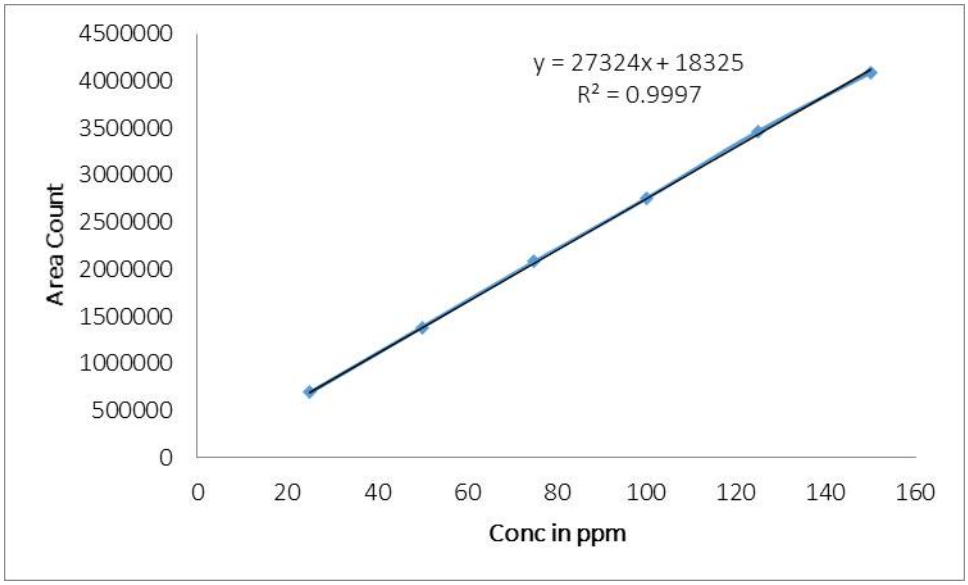

Figure 5: Calibration curve of Olaparib.

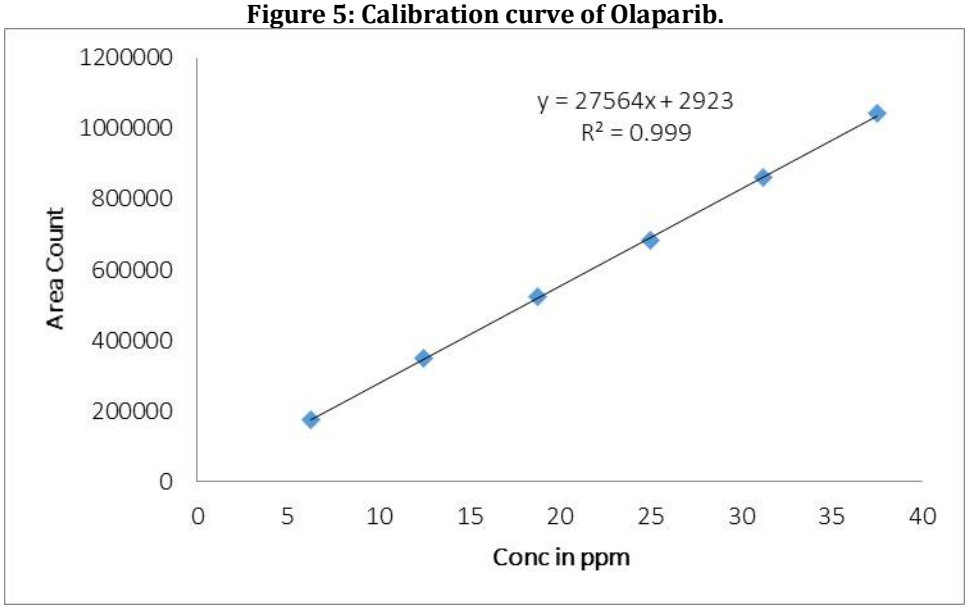

Figure 6: Calibration curve of Bevacizumab

Precision

Method precision

The %RSD (Relative Standard Deviation) for Olaparib and Bevacizumab was calculated using six replicate injections. The mean area for Olaparib was 2765171 with a standard deviation (S.D) of 29074.03, resulting in a %RSD of 1.051%. Similarly, the mean area for Bevacizumab was 686120, with a standard deviation of 1985.35, giving a %RSD of 0.289%. These low %RSD values indicate high precision and reproducibility of the method for both compounds.

Intermediate precision

Intermediate precision of Olaparib and Bevacizumab based on six replicate injections. The mean area for Olaparib was 2760609, with a standard deviation (S.D) of 30009.56, resulting in a %RSD of 1.087%. Bevacizumab mean area was 686119, with a standard deviation of 2219.69, yielding a %RSD of 0.324%. These results indicate good intermediate precision for both analytes, demonstrating the method's consistency when tested under different conditions or over different days.

Accuracy

Three levels of Accuracy samples were prepared by the standard addition method. Triplicate injections were given for each level of accuracy, and the mean Recovery was 100.17% and 100.23% for Olaparib and Bevacizumab, respectively.

Sensitivity

Olaparib Limit of Detection (LOD) was 0.6 µg/ml, and the Limit of Quantitation (LOQ) was 2.0 µg/ml. The LOD for Bevacizumab was 0.15 µg/ml, and the LOQ was 0.5 µg/ml. These values indicate the method's ability to detect and quantify deficient concentrations of both analytes with high sensitivity.

Robustness

The method's robustness for Olaparib and Bevacizumab was evaluated under various conditions by altering the flow rate, mobile phase composition, and temperature. When the flow rate was decreased to 0.9 ml/min, the %RSD for Olaparib was 0.21%, and for Bevacizumab, was 0.15%. When the flow rate was increased to 1.1 ml/min, the %RSD values were 0.4% and 0.75%, respectively. For mobile phase composition changes, a ratio of 27A:73B resulted in %RSD values of 0.25% for Olaparib and 0.21% for Bevacizumab, while a 33A:67B ratio yielded %RSDs of 0.35% and 0.63%. Lastly, temperature variations at 27°C showed %RSDs of 0.6% and 1.0%, indicating that the method is robust under these conditions.

Table 2: Robustness data for Olaparib and Bevacizumab

S.No.	Condition	%RSD of Olaparib	%RSD of Bevacizumab
1	Flow rate (-) 0.9ml/min	1.05	0.59
2	Flow rate (+) 1.1ml/min	0.45	0.44
3	Mobile phase (-) 37A:63B	0.56	0.46
4	Mobile phase (+) 43A:57B	0.44	0.34
5	Temperature 27°C	0.7	1.1

Assay

A LYNPARZA assay was performed using the above formulation. The average % Assay for Olaparib and Bevacizumab obtained was 99.4% and 99.3%, respectively.

Degradation Studies:

The method's specificity was demonstrated through forced degradation studies conducted on the sample using acid, alkaline, oxidative, reductive, and thermal degradation. The sample was exposed to these conditions, and the main peak was studied for peak purity, thus indicating that the method effectively separated the degradation products from the pure active ingredient. Regulatory guidance in ICH Q2A, Q2B, Q3B, and FDA 21 CFR section 211 requires developing and validating stability-indicating potency assays.

Table 3: Forced Degradation results for Olaparib and Bevacizumab

Degradation	Area	% Assay	% Deg	Area	% Assay	% Deg
Control	2760140	100	0	686011	100	0
Acid	2403763	87.1	12.9	593426	86.5	13.5
Alkali	2372274	85.9	14.1	601349	87.6	12.4
Peroxide	2394211	86.7	13.3	585460	85.3	14.7
Reduction	2629211	95.2	4.8	661437	96.4	3.6
Thermal	2721455	98.6	1.4	680533	99.2	0.8
Photolytic	2665439	96.6	3.4	671956	97.9	2.1
Hydrolysis	2698172	97.7	2.3	678021	98.8	1.2

CONCLUSION

The study concluded that the HPLC method developed and validated for simultaneous quantifying Olaparib and Bevacizumab in drug products proved simple, precise, accurate, and sensitive. The technique demonstrated excellent separation of both analytes with well-defined retention times and consistent results across different levels of accuracy. Validation parameters, including % recovery, LOD, and LOQ, confirmed the method's reliability and robustness for routine analysis. This method can be effectively applied for quality control and routine analysis of Olaparib and Bevacizumab in pharmaceutical formulations.

REFERENCES

1. Dizdar O, Arslan C, Altundag K.(2015). Advances in PARP inhibitors for the treatment of breast cancer. Expert Opin Pharmacother.16(2):2751–2758.
2. Ferraris DV. (2010) Evolution of poly(ADP-ribose) polymerase-1 (PARP-1) inhibitors. From concept to clinic. J Med Chem. 53(3):4561–4584.
3. Brown JS, Kaye SB, Yap TA. (2016).PARP inhibitors: the race is on. Br J Cancer.114(8):713–715.
4. Murai J, Huang SY, Das BB, Renaud A, Zhang Y, Doroshow JH, Ji J, Takeda S, Pommier Y.(2012). Trapping of PARP1 and PARP2 by clinical PARP inhibitors. Cancer Res. 72(2):5588–5599.

5. Tangutoori S, Baldwin P, Sridhar S.(2015). PARP inhibitors: a new era of targeted therapy. Maturitas. 81(2):5–9.
6. Patel, M., et al. (2019). "Pharmacokinetics and safety of Olaparib in patients with advanced solid tumors." Cancer Chemotherapy and Pharmacology, 83(5), 877-885.
7. Zeng, H., et al. (2020). "Analytical methods for the characterization of Bevacizumab: A review." Journal of Pharmaceutical Sciences, 109(4), 1344-1355.
8. Nijenhuis CM, Lucas L, Rosing H, Schellens JH, Beijnen JH. (2013).Development and validation of a high-performance liquid chromatography-tandem mass spectrometry assay quantifying olaparib in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci. ;940(2):121–125.
9. Roth J, Peer CJ, Mannargudi B, Swaisland H, Lee JM, Kohn EC, Figg WD(2014). 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. Chromatography. 1(1):82–95.
10. Kavitapu et,al (2019). New Rapid Stability indicating RP-UPLC Method for the Determination of Olaparib, its Related Substances and Degradation Products in Bulk drug and Dosage Form ,Materials Today: Proceedings, 14,(2) , 492- 503.
11. Ottria R, Ravelli A, Miceli M, Casati S, Orioli M, Ciuffreda P(2019). Quantitative Characterization of Olaparib in Nanodelivery System and Target Cell Compartments by LC-MS/MS. Molecules. 24(5):989.
12. Oliva, A.; Llabrés.,M (2019). Validation of a Size-Exclusion Chromatography Method for Bevacizumab Quantitation in Pharmaceutical Preparations: Application in a Biosimilar Study. Separations . 6(1), 43.
13. Pierre Daumar et al (2018) 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. 15(4); 152:7480.
14. Martínez-Ortega A, Herrera A, Salmerón-García A, Cabeza J, CuadrosRodríguez L, Navas N(2018). Validated reverse phase HPLC diode array method for the quantification of intact bevacizumab, infliximab, and trastuzumab for the long-term stability study. Int J Biol Macromol. Sep;116(9):993-1003.
15. Sousa F, Gonçalves VMF, Sarmento B. (2017).Development and validation of a rapid reversed-phase HPLC method for the quantification of monoclonal antibody bevacizumab from polyester-based nanoparticles. J Pharm Biomed Anal. 5(8);142:171-177.
16. Antima Chaudhary, Rajiv Tonk, Pankaj Dagur, Suddhasattya Dey, Manik Ghosh(2022). Stability Indicating Assay Method for the Quantitative Determination of Olaparib in Bulk and Pharmaceutical Dosage Form. Turk J Pharm Sci .19(5):488-497.
17. Bansal, S., & Reddy, A. (2015). "High-Performance Liquid Chromatography: Principles and Applications." International Journal of Pharma Sciences and Research, 6(2), 143-150.
18. ICH Harmonised Tripartite Guideline (2003), Validation of analytical procedures: Text and methodology, Q2 (R1), International Conference on Harmonization, Geneva (2005): 1-13.
19. International Conference on the Harmonization, (2003) ICH Harmonized Tripartite Guideline. Stability Testing of New Drug Substances and Products Q1A (R2), 32-35.