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UV Method of Development and Validation of Olaparib

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Abstract

An analytical method must be developed and validated in the discovery, development, and production of pharmaceuticals to ensure the identification, purity, potency, and performance of drug commodities. Quality control laboratories use official test protocols that emerge from the processes. A simple ultraviolet spectrophotometric approach was designed and validated using various characteristics, including linearity. Precision, reproducibility, accuracy, robustness, ruggedness, limit of detection, and limit of quantification are among the measuring criteria. The present study devised an accessible, economical, precise, and accurate ultraviolet spectrophotometric approach for assessing Olaparib in bulk and pharmaceutical formulation. The International Conference on Harmonization recommendations were followed in the evaluation of the method validation parameters. It was scanned in the range of 200–400 nm and ultraviolet spectrophotometric detection was carried out at absorption maxima (λ_{max}) at 276 nm using methanol as a solvent. In the concentration range of 0.5–6 µg/ml, the techniques were linear (r^2 =0.999). The precision is reported as 1.6% relative standard deviation. Accuracy, precision, robustness, and ruggedness, as well as specificity investigations, were all used to validate the methods. The Olaparib detector response was linear over the prescribed concentration range of 0.5–6 µg/ml. The validity of the described procedure was investigated. The results were statistically examined, and the precision and accuracy were determined to be extremely high. The process was found to be straightforward, precise, and accurate, and it can be utilized for routine quality control of Olaparib in bulk and solid dosage formulations. There is no interference from common excipients in the above approach.

Keywords: UV-Spectroscopy, Olaparib, method development, validation, methanol

INTRODUCTION

In this method, the analyte is made to quantitatively react with a chromospheres-containing molecule, with the decrease in absorbance correlating with the analyte's concentration, or by reacting with a reagent that forms a chromophoric group [1, 2]. Spectroscopy is the most powerful instrument for studying atomic and molecular structure, and it is used to examine a wide variety of samples. A spectrometer is a device that measures the spectrum of a substance. An instrument called a Ultraviolet-Visible (UV–VIS) Spectrophotometer is used to measure absorbance in the visible (400–800 nm) or UV (200–400 nm) ranges [3]. Spectroscopy is a field of science that studies how electromagnetic radiation (EMR) interacts with materials. It is the measurement of EMR received or emitted by a sample's molecules, ions, or atoms as they transition from one energy state to another [4–6].

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A medicine called Olaparib (OLA) is used to treat some malignancies, especially those of the breast and ovarian tissues. Poly ADP-ribose polymerase (PARP) inhibitors are the family of medications that it is a member of OLA functions by inhibiting PARP—an enzyme involved in cell damage repair. OLA delays the repair of cancer cells' DNA, which ultimately results in the death of the cells, by blocking this process. When someone has a particular mutation in one of the DNA repair genes, such *BRCA1* or *BRCA2*, this medication is frequently administered.

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OLA is typically used in advanced ovarian cancer and metastatic breast cancer cases where other treatments may not be as effective [7].

Chemically, OLA is 2-fluoro-5-[(4-oxo-3H-phthalazin-1-yl) methyl] benzoic acid (Figure 1).

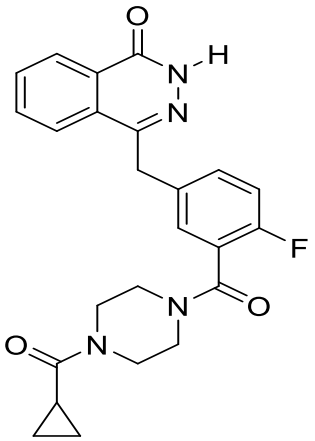

Figure 1. Structure of Olaparib.

MATERIALS AND METHOD

Reagents Used

Methanol (HPLC grade), Olaparib drug.

Equipment

The absorbance of the solutions was measured using a Shimadzu (Kyoto, Japan) UV-1900 double beam UV-Visible spectrophotometer and computer-operated software UV probe 2.0. Two quartz cells that were matched in length by 1 cm were used, and the spectral width was 2 nm with a wavelength precision of 0.5 nm.

UV SPECTROPHOTOMETRIC METHOD

Method Optimization

Selection and Optimization of the Solvent

It is well known that the solvent significantly affects the peak's quality and form. OLA's solubility was assessed using a range of solvents in accordance with Indian Pharmacopoeia guidelines. OLA's solubility was tested in a variety of polar and non-polar solvents. Acetone, Chloroform, and methanol are among the solvents used in the development of UV techniques. At the given wavelength, methanol met all of the criteria for peak quality and non-interference. Based on solubility tests, methanol was chosen as an appropriate solvent for the suggested procedure [8].

Solvent for UV analytical method development was selected by following method:

1. *OLA Solubility in Organic Solvents:* The active pharmaceutical ingredient's (API's) solubility in different inorganic solvents was investigated as shown in Table 1.
2. *Analytical Solvent Selection:* About 10 µg/ml OLA solutions were made in solvents where OLA solubility was discovered, and maximum absorbance was measured with a UV spectrophotometer (Table 2).

Table 1. Specificity.

Volume aliquot (ml)	Diluted up to with methanol (ml)	Concentration (mg/ml)
1	10	0.1
5	10	0.5
5	-	1

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Table 2. Solvent selection.

Solvents	Absorbance (276 nm)	Average absorbance
Methanol	0.372 0.376 0.380	0.376 ± 0.004
Methanol: water (9:1)	0.288 0.289 0.294	0.280 ± 0.0032
Acetonitrile: methanol (9:1)	0.295 0.284 0.291	0.29 ± 0.0055
Acetonitrile	0.327 0.329 0.330	0.328 ± 0.0015

The Choice of Wavelength

To get concentrations of 1 µg/ml, the standard stock solution was further diluted with methanol. With methanol serving as a blank, the solution was scanned in the 200–400 nm range. A λ_{max} of 276 nm was chosen from the UV spectrum to analyze OLA. The stability of OLA in methanol was investigated by monitoring the same solution at this λ_{max} throughout various time periods. It was observed that OLA in methanol was stable for more than 4 h. The wavelength at which maximal absorption occurs in the UV detector is chosen [9].

Preparation of Standard Solution: About 10 mg of the API is accurately weighed and transferred into clean 10 ml volumetric flask and solubilized in 1 ml of methanol and make it up to 10 ml by using distilled water which gives us the concentration of 1000 µg/ml.

Fill a volumetric flask with 10 ml of OLA (about 10 mg). Dilute it with 10 ml pure methanol until it dissolves completely.

Preparation of Standard Stock Solution: Then take aliquot 0.5 ml from standard solution and transfer it into 50 ml of volumetric flask. Then makeup the solution to 50 ml with diluent methanol [10].

Preparation of Calibration

Preparation of Standard Working Solution

From the above solution 0.5, 1, 2, 4, 6 µL transferred into a 10 ml volumetric flask and made up to 10 ml with methanol which gives us the concentration of 0.5 µg/ml, 1 µg/ml, 2 µg/ml, 4 µg/ml, and 6 µg/ml [11].

METHOD VALIDATION

OLA's UV-visible method was developed, and it was validated in accordance with ICH Q2 (R1) recommendations for factors such as specificity, robustness, ruggedness, precision, linearity, accuracy, limit of detection (LOD), and limit of quantification (LOQ).

i. *Precision:* By performing the OLA drug analysis on a different day, the method's (inter-day) accuracy was assessed. In terms of percentage relative standard deviation (percent RSD) for this method, the peak area of the active chemical was identified [12].
   Intra-Day Precision: The average absorbance, standard deviation, and percent RSD for the medication were computed using five working sample solutions of formulation absorbance. The approach was successful in maintaining the system precision because the precision limit was below 2 [13] (Table 3).
   Inter-Day Precision: Five working sample solutions with the same amounts were created from multiple samples obtained from a sample stock solution. Each working sample solution's absorbance was tested, and the findings were documented in a table. The average absorbance, standard deviation, and percent RSD of the medication were determined. In this

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method, the system precision was passed because the precision limit was smaller than 2 [14] (Table 4).

ii. *Linearity:* Based on ICH Q2B recommendations, the procedure was carried out according to the required protocol. Linear regression was used to determine the calibration line's slope and intercept [15] [(Figure 2, Table 5).

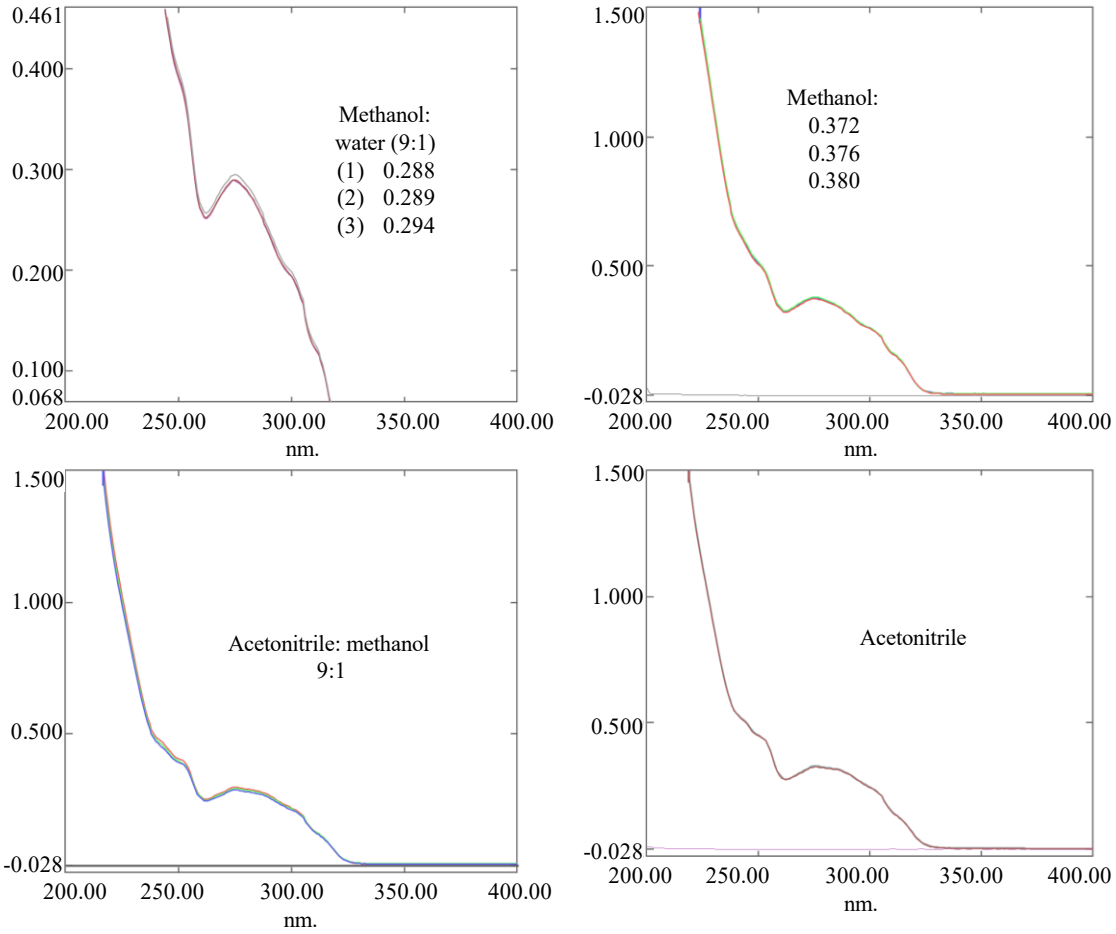

Figure 2. UV spectra of Olaparib in different solvents.

Table 3. Intra-day precision.

S.N.	Working sample solution (µg/ml)	Absorbance (276 nm) n=3	%Relative standard deviation (%RSD)
1	0.5	0.1313 ± 0.0066	5.0697
2	1	0.2213 ± 0.0047	2.1351
3	2	0.3826 ± 0.0056	1.4859
4	4	0.7280 ± 0.0060	0.8241
5	6	1.0666 ± 0.0231	2.1670

Table 4. Inter-day precision.

S.N.	Working sample solution (µg/ml)	Absorbance (276 nm) n=3	%Relative standard deviation (%RSD)
1	0.5	0.1673 ±0.0102	6.1333
2	1	0.2303 ± 0.0066	2.8907
3	2	0.4000 ± 0.0070	1.7500
4	4	0.7516 ± 0.0085	1.1314
5	6	1.0846 ± 0.0065	0.5998

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Table 5. Linearity.

S.N.	Aliquot from flask (µg/ml)	Volume of the flask (ml)	Absorbance (276 nm) n=3
1	0.5	10	0.131
2	1	10	0.221
3	2	10	0.382
4	4	10	0.728
5	6	10	1.066

iii. Accuracy

- *Preparation of 80% Spiked Solution:* A volumetric flask holding 1 mg/ml of sample spiked with 80%, or 0.8 mg of OLA, was filled. The total amount is now 1.8 mg.
- *Preparation of 100% Spiked Solution:* About 1 mg/ml sample spiked with 100%, or 1 mg of OLA, was added to a volumetric flask containing 1 mg/ml sample. The total dosage is now 2 mg.
- *Preparation of 120% Spiked Solution:* A volumetric flask holding a 1 mg/ml sample was spiked with 120%, or 1.2 mg of OLA. The total dosage is now 2.2 mg [16] (Table 6).

iv. *LOD Sample Preparation:* Both the standard deviation (precision) and the slope were used to determine the LOD (linearity graph), using the given equation [17]:

$$LOD = 3.3 \times S.D/Slope$$

v. *LOQ Sample Preparation*: Both the standard deviation (precision) and the slope were used to determine the LOQ (linearity graph), using the given equation [17]:

$$LOQ = 10 \times S.D/Slope$$

vi. *Robustness:* The robustness of the method was tested by varying the concentration. The method's robustness was determined by analyzing it at 276 nm under various concentration conditions [18].

vii. *Ruggedness:* Two distinct analyzers used the same device to deliver different amounts of OLA under optimal circumstances over two days to assess the resilience of the suggested approach. The approach was found to be robust because no discrepancies in the results were noted by different analyzers, indicating that the outcome was reproducible [19] (Figure 3&4, Tables 6&7).

viii. *Specificity:* In methanol, 1 ml of OLA micro emulsion (10 mg) was dissolved (up to 10 ml). About 1 ml of the supernatant was diluted as follows after 1 h of sonicating the mixture and 20 min of centrifugation at 1200 rpm to separate the oily globules [20] (Tables 8&9).

Table 6. Accuracy of Olaparib.

% level	Amount added	Amount spiked	Amount recovered	% Recovery	Mean % recovery
80	0.8 mg	1.8 mg	1.79	99.92	
100	1.0 mg	2.0 mg	1.99	99.98	99.95
120	1.2 mg	2.2 mg	2.19	99.96	

Table 7. Analyst first.

Conc. (µg/ml)	Absorbance (276 nm)			Average (n=3)	%RSD	LOD (mg/ml)	LOQ (mg/ml)
	1	*2*	*3*				
0.5	0.124	0.133	0.137	0.131333 ± 0.00665	5.069793	0.129326	0.391897
1	0.216	0.225	0.223	0.221333 ± 0.004725	2.135158	0.09179	0.278153
2	0.378	0.381	0.389	0.382667 ± 0.005686	1.485951	0.110445	0.334682
4	0.722	0.728	0.734	0.72800 ± 0.00600	0.824176	0.116539	0.353149
6	1.045	1.064	1.091	1.06666 ± 0.02311	2.167092	0.44898	1.360545

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Table 8. Analyte second.

Conc. (µg/ml)	Absorbance (276 nm)			Average absorbance (n=3)	%RSD	LOD (mg/ml)	LOQ (mg/ml)
	1	*2*	*3*				
0.5	0.156	0.170	0.176	0.167 ± 0.0102	5.0697	0.1293	0.3918
1	0.226	0.227	0.238	0.22 ± 0.0066	2.1357	0.0917	0.2781
2	0.393	0.400	0.407	0.400 ± 0.0070	1.4859	0.1104	0.3346
4	0.742	0.755	0.758	0.751 ± 0. 008	0.8241	0.1165	0.3531
6	1.078	1.085	1.091	1.084 ± 0.0065	2.1670	0.4489	1.3605

Table 9. Specificity.

Volume aliquot (ml)	Diluted up to with methanol (ml)	Concentration (µg/ml)	Absorbance (276 nm)			Average absorbance (n=3)	Conc. found (µg/ml)
			1	*2*	*3*		
1	10	0.1	0.024	0.026	0.028	0.026 ±0.002	0.10
5	10	0.5	0.096	0.101	0.102	0.099 ±0.003215	0.53
5	-	1	0.190	0.1912	0.1922	0.191 ±0.001102	1.07

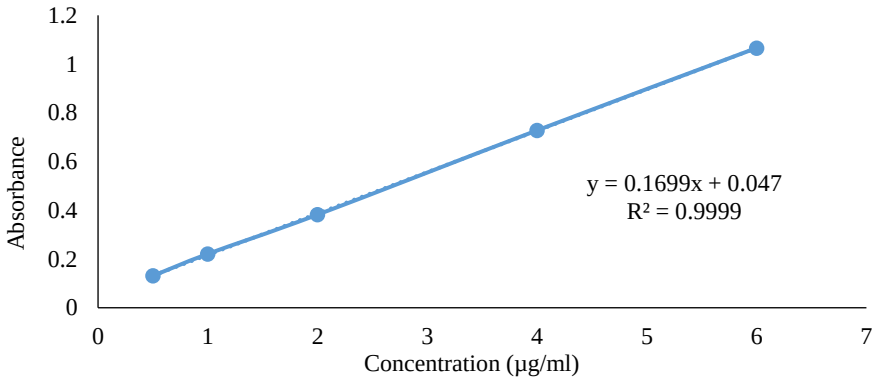
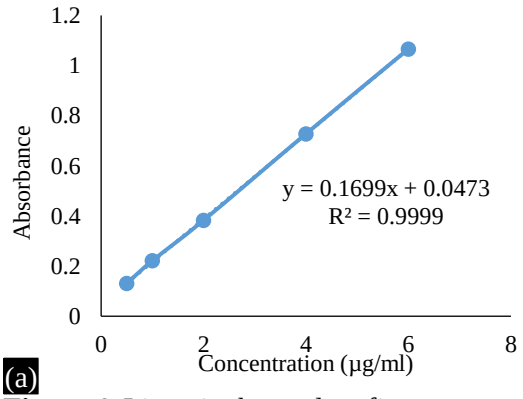
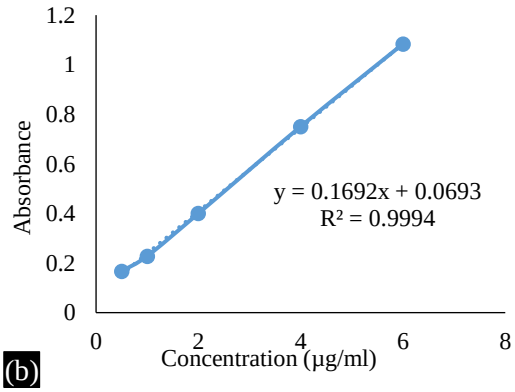


Figure 3. Linearity.



(a)

Figure 4. Linearity by analyte first.



(b)

Figure 4. Linearity by analyte second

ix. *Preparation of Nanostructured Lipid Carrier:* About 50 mg OLA dissolved in Lauric acid and Capmul Lipid mix 1:9 ratios in order to prepare 30 ml with 0.3% of Tween 80: Span 80 (2:1) of surfactant mixture. The emulsion were probe sonicated for 15 min in order to get particle size less than 100 µ.

RESULT

Solubility: Solubility of OLA was found satisfactory in methanol, acetonitrile, dimethyl sulfoxide (DMSO), dimethylformamide (DMF), ethanol, and acetone. All have shown a characteristic peak shape in UV spectra (Figure 2).

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Solvent Selection for Analytical Studies

Methanol is selected as the solvent for UV method development of olaparib due to olaparib is freely soluble in methanol and also methanol has low UV absorbance, which minimizes interference in the detection of olaparib. Moreover, its compatibility with olaparib, leads to its ability to provide a stable and reproducible baseline in the wavelength range of interest. (Table 2 & Figure 2).

Precision

Intra-Day Precision

Intra-day was performed by analysis of OLA drug on the same day.

Inter-Day Precision

Inter-day of the method was checked by repeating analysis of OLA drug on a different day.

Linearity

The linearity of the UV method developed for olaparib using methanol as the solvent was evaluated by preparing a series of standard solutions at different concentrations (0.5, 1, 2, 4, 6 µg/ml). The calibration curve was constructed by plotting the absorbance against the concentration of olaparib. The method demonstrated excellent linearity with a correlation coefficient (R2) of 0.9999, indicating a strong linear relationship between absorbance and concentration over the tested range (Table 5 and Figure 3).

Accuracy

LOD: Detection limit of OLA in this method was found to be 0.29901 µg/ml (Table 7).

LOQ: Quantitation limit of OLA in this method was found to be 0.90613 µg/ml (Table 7).

Specificity

Assay: The percentage drug content of prepared nanostructured lipid carrier (NLC) were determined using above said validation UV-spectroscopy method. Percentage drug content of prepared NLC of OLA was assayed. The absorbance of prepared NLC diluted were found to be 0.8529 and concentration was found to be 4.999 µg/ml. The drug content of prepared NLC was 99.98%.

CONCLUSION

The UV-VIS spectroscopic method for determining OLA content was validated according to ICH requirements, and it passes specified acceptance criteria for linearity, accuracy, intra-day and inter-day precision, detection limit, and quantification limit. The proposed method is straightforward, dependable, and potentially cost-effective, and it can be utilized as a routine quality control method for OLA.

Abbreviations

EMR- Electro Magnetic Radiation
LOD – Limit of Detection
LOQ – Limit of Quantification
NLC – Nanostructured Lipid Carrier
OLA – Olaparib
PARP – Poly Adenosine Diphosphate-Ribose Polymerase
RSD - Relative standard deviation
SD – Standard Deviation
UV-VIS – Ultraviolet – Visible

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