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Research Article

RP HPLC Method Development and Validation on OLAPARIB Tablets

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ABSTRACT

Analytical technique's is a wide-ranging concept covering all matters that individually or collectively influence the quality of the product. Highly specific and sensitive analytical techniques hold the key role to the design, development, standard and quality control of medicinal products. Quality of drug products is very vital, as it involves life. Proper manufacture and quality control of pharmaceuticals is the vital segment of strong primary healthcare program worldwide. Pharmaceutical analysis, a branch of pharmacy, plays a very significant role in quality control of pharmaceuticals through a rigid check on raw materials used in manufacturing of formulation and on finished products. Few analytical methods such as spectroscopic method and chromatographic methods are used for quality control and validation of drugs. In this research article we used RP-HPLC method for development and validation on Olaparib tablets. Olaparib is primarily used for the treatment of ovarian and breast cancer. The purpose of this research is to develop method of a drug and which can be optimized according to different parameter and to validate according to ICH (Q2 R1) guidelines. All the research work was carried out in a systematic, concise, and serial manner which includes a thorough study by literature available for the analysis of Olaparib drug. In this research article we have done optimization of Olaparib tablets using RP-HPLC method with the help of 4 trials for optimization and in validation we have studied about these parameters specificity, linearity, range accuracy, precision, system suitability and robustness.

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Here, we conclude that the developed RP-HPLC validate method is Precise, simple, accurate, sensitive, and reproducible for the quantitative estimation of Olaparib in bulk and its formulation and also concluded that this RP-HPLC method is convenient and effective for research studies, quality control and routine analysis of Olaparib in Pharmaceutical Industries and in assay of Olaparib tablets which can be used in treatment of cancer.

INTRODUCTION

Analytical technique's is a wide-ranging concept covering all matters that individually or collectively influence the quality of the product. It plays a central role in determining the safety and efficiency of medicines. Highly specific and sensitive analytical techniques hold the key role to the design, development, standard and quality control of medicinal products [1]. Quality of drug products is very vital, as it involves life. Proper manufacture and quality control of pharmaceuticals is the vital segment of strong primary healthcare program worldwide. Quality is the total sum of all factors which contribute directly or indirectly to the safety, efficacy and acceptability of the product [2]. The development in analytical sciences has been more significant and prominent in recent years than the past. This has really broaden our vistas and helped to develop new methods of analysis. In pharmacy analytical chemistry is responsible for developing sensitive, reliable and more accurate methods for the estimation of drug in pharmaceutical dosage form [3, 4].

1. **Few Analytical Method:** These analytical methods are classified as classical and instrumental few of them which are routinely used are Spectroscopic methods such as UV-Visible, Infrared, Mass, NMR and chromatography methods such as HPLC, HPTLC, LC-MS, GC-MS [5-8].
2. **High Performance Liquid Chromatography**: - HPLC is an analytical process utilizing special instruments designed to separate, quantify and analyze components of chemical mixture. Samples of interest are introduced to a solvent flow path; carried through a column packed with specialized materials for component separation; and component data is obtained through the combination of a detection mechanism coupled with a data recording system. A typical HPLC separation is based on the selective distribution of analytes between a liquid mobile phase and an immiscible stationary phase [9-14].
3. **Types of High-Performance Liquid Chromatography**
   a) **Normal phase HPLC**
   b) **Reversed phase HPLC** [15]

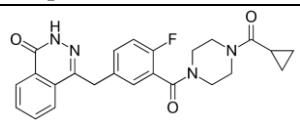
HPLC method development involves several essential steps are HPLC, sample pretreatment, Detection of sample bands, choosing separation conditions, quantitation and method validation and the wide variety of equipment such as column, eluent, operational parameters involved makes high performance liquid chromatography (HPLC) method development seem complex [16-21].

DRUG PROFILE

Olaparib is a pharmaceutical drug that belongs to the class of medications known as poly (ADP-ribose) polymerase (PARP) inhibitors. It is used for the treatment of certain types of cancer, particularly ovarian and breast cancer that have specific genetic mutations. Olaparib is primarily used for the treatment of Ovarian Cancer and Breast Cancer mainly.

Table 1: Brief Overview of Olaparib

Name	OLAPARIB
IUPAC Name	4-[(3-[(4-cyclopropylcarbonyl)piperazin-1-yl]carbonyl)-4-fluorophenyl] methyl(2H)phthalazin-1-one
Appearance	White to off-white crystalline powder

Category	Antineoplastics, PARP Inhibitors.
Chemical Structure	
Mol. Formula	$C_{24}H_{23}FN_4O_3$
Mol. Weight	434.5
Solubility	Soluble in ethanol, water
PKa value	7.4
Storage condition	20-22 ºC
Melting point	198ºC
Route	ORAL
Bioavailability	35% TO 46%
Protein Binding	82% to 87%
Biological Half Life	6.10 Hours
Pharmacological action	Its main pharmacological action is to inhibit the activity of the enzyme PARP, which plays a crucial role in repairing damaged DNA in cells. By inhibiting PARP, Olaparib disrupts the DNA repair process, particularly in cancer cells that already have underlying defects in DNA repair mechanisms, such as those with mutations in the BRCA1 or BRCA2 genes.

RESEARCH ENVISAGED

Plan of Work

The purpose is to develop method of a drug and which can be optimized according to different parameter and to validate according to ICH (Q2 R1) guidelines. All the research work was carried out in a systematic, concise, and serial manner which includes a thorough study by literature available for the analysis of Olaparib drug.

The plan of work includes the following:

Analytical method development and validation of Olaparib by RP-HPLC.

Steps involved in Method development.

- Selection of column
- Selection of detection wavelength
- Selection and optimization of mobile phase
- Preparation of stock standard solution
- Linearity studies
- Estimation of drug in bulk material
- Analysis of marketed formulation.

Validation of Methods: Linearity studies

- Accuracy studies
- Precision studies -System precision and Method precision
- Limit of Detection (LOD) and Limit of Quantification (LOQ)
- Robustness
- Ruggedness studies
- Repeatability
- Recovery studies
- System suitability studies
- Specificity
- Range

MATERIALS AND EQUIPMENTS

Materials and equipments utilized/procured for Analytical Method development and Validation are Olaparib drug supplied by Hetero Pharmaceutical Pvt. Ltd. Hyderabad and its purity assay is 99.53%. The chemicals are used for analysis are HPLC water and ethanol manufactured in Research lab Fine chem. Instruments used are HPLC, UV-spectrophotometer, column, pH meter Ultrasonicator, Glassware, Filters, Water bath and Digital melting point apparatus for analysis and validation of Olaparib tablets.

EXPERIMENTAL WORK

Spectral study of OLAPARIB by using UV spectrophotometer**.** The UV spectrum of

OLAPARIB API was recorded using UV spectrophotometer to determine lambda max.

1 Determination of max wavelength: Standard solutions were scanned separately between 400 to 200 nm using water as a blank and the max wavelength 276nm selected for estimation of drug.

2 Selection of Chromatographic Method: Proper selection of the method depends upon the nature of the sample (ionic/ionizable/neutral molecule, its molecular weight and solubility). The drug selected in the present study is polar in nature so, reverse phase chromatography method can be used. Here, the reverse phase HPLC method was selected for the initial separation owing to its simplicity, suitability, ruggedness, and its wider use

REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD DEVELOPMENT:

1 Optimization of Chromatographic Method: In order to achieve the optimized chromatographic condition to separate elute and quantify OLAPARIB, one or two parameters were modified at each trial and chromatograms were recorded with all specified chromatographic conditions. The standard solution prepared was of strength approximately 20µg/ml with diluent.

Table 2 Optimization of different trial methods of Olaparib

	Trial 1	Trial 2	Trial 3	Trial 4
HPLC system	Agilent infinity 1260 II	Agilent infinity 1260 II	Agilent infinity 1260 II	Agilent infinity 1260 II
Column	Infinity lab Poroshell 120 EC C18 (4.6×150 mm)	Infinity lab Poroshell 120 EC C18 (4.6×150 mm)	Infinity lab Poroshell 120 EC C18 (4.6×150 mm)	TC C18 (250*4.6mm)
Mobile phase	ACETONITRILE and WATER (15:85)	Phosphate Buffer : ACN (80:20) pH 5	Phosphate Buffer : ACN (80:30) pH 5	METHANOL:WATER (70:30)
Flow rate	1.0 ml/min	1.0 ml/min	1.0 ml/min	1.0 ml/min
Pump mode	Isocratic	Isocratic	Isocratic	Isocratic
Injection volume	50µL	50µL	50µL	50µL
Wavelength	270nm	270nm	270nm	276nm
Detector	DAD Detector	DAD Detector	DAD Detector	DAD Detector
Column Temperature	R.T	R.T	R.T	R.T
Run time	10 min	10 min	10 min	10 min
Retention Time	1.09 min	1.09 min	1.09 min	1.8 min

Trial No.1

Observation: When the chromatogram was extracted at 287 nm and integrated with proper integration Retention time was found at 1.9 min.

Trial No.2

Observation: When the chromatogram was extracted at 270 nm and integrated with proper integration Retention time was found at 1.9 min.

Trial No.3

Observation: When the chromatogram was extracted at 270 nm and integrated with proper integration Retention time was found at 1.9 min.

Trial No.4

Observation: When the chromatogram was extracted at 276 nm and integrated with proper integration Retention time was found at 1.85 min.

Conclusion: The eluted peak shape was not good. The next trial was taken by changing the mobile phase proportion in Trial 1-3 and in Trial 4 after changing the proportion of mobile phase the eluted peak shape was good and satisfactory. Retention time was also satisfactory. This trial was finalized and chromatographic condition to finalize.

2 Methodology Followed in Analytical Method Validation

Reagents- HPLC grade water**,** Methanol (HPLC grade)**,** Ethanol**,** distilled water

Preparation of mobile phase: METHANOL AND WATER (70:30) V/V use as mobile phase.

PREPERATION OF DILUENT: Ethanol and Water use as diluent.

PREPERATION OF STANDARD SOLUTION: Weighed accurately about 20 mg of Olaparib and transferred into a 100 ml volumetric flask adds 10 ml ethanol mixed properly and sonicates for 10 min and make up volume with water 100ml.

Preparation of standard I: Pipetted out 10ml from above standard solution and transfer to 100 ml volumetric flask and dilute up to the mark with diluent upto 100 ml.

PREPERATION OF SAMPLE SOLUTION: Weigh accurately Olaparib tablet powder equivalently to 20 mg Olaparib to 100 ml with diluent in 100 ml volumetric flask. Take 10 ml of this solution and further dilution to 100 ml with diluent and sonicate for 10 min.

Assay procedure: Separately injected the equal volume of Blank (diluent), five replicate injection of standard and two injections of sample solution. Chromatogram was recorded of all injections.

Evaluation of system suitability: Standard solution was injected five replicates into chromatogram and the responses of Olaparib peak were measured. The relative standard deviation of five replicate injections should not be more than 2%. The tailing of the Olaparib peak should not be more than 2.0 and the number of theoretical plates should not be less than 2000.

$$\%\ \text{Assay} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{3}{10} \times \frac{100}{WT} \times \frac{10}{0.3} \times \frac{Avg\ wt.}{LC} \times \frac{P}{100} \times 100$$

Where,

AT: Average peak area of sample; AS: Average peak area of standard; WS: Weight of standard Methyldopa API; WT: Weight of sample Methyldopa; Avg wt.: Average weight of tablet formulation; LC: Label claim of injection; P: Potency of Methyldopa standard on as is basis

Estimation of Olaparib tablet dosage form: Standard solution and sample solution were prepared as discussed in the methodology above, peak area was measured and Assay %, label claim was calculated.

Parameters to be evaluated under validation: Test procedure given for the assay of METHYLDOPA in Tablet for testing of Olaparib and method validation studies were performed based on the following acceptance criteria.

Table 3 Parameters to be evaluated under validation

Sr. No.	Validation Parameter	Range of study	Acceptance criteria
1.	Specificity	-	-
1.1	Identification	-	Retention time of standard should be concordant to sample solution.
1.2	Blank interference	-	Blank should not show any peak at the retention time of Olaparib. Peak purity should pass for Olaparib peak in standard and Sample.
2	Linearity and range	25%w/v to 150%w/v	Correlation coefficient should not be less than 0.99
3	Accuracy (%Recovery)	50%, 80%, 100%, 120%, 150% of the specified limit of impurity	Mean recovery should be in the range of 98.0% to 102.0%. The RSD should not be more than 2.0%
4	Precision	-	-
4.1	Method Precision	Six system replicate injection standard solution	RSD should not be more than 2.0%.

		was injected in to thesystem	
4.2	Method Precision	Six replicate preparation of sample solution was injected into the system.	RSD should not be more than 2.0%.
5.	System Suitability	-	Standard solution RSD of five replicate injections should not be more than 2.0%. Tailing factor for Methyldopa peak should not be more than 2.0. Number of theoretical plates should not be less than 2000.
6	Robustness		
6.1	Change in Flow rate 0.8ml/min	276 nm	RSD should not be more than 2.0%.
6.2	Change in flow rate (±1.2ml/min)	276nm	

RESULT AND DISCUSSION

The standard solution was scanned separately between 400nm to 200nm. From the spectrum, 276nm were recorded for the estimation of drug.

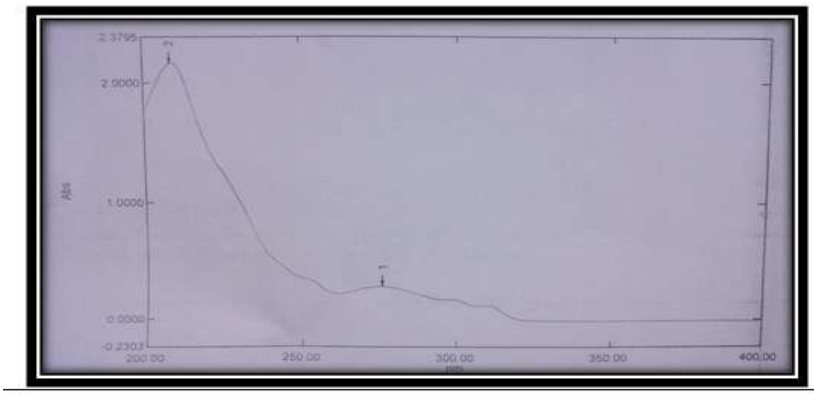


Fig.1 UV Spectra of Olaparib

Determination of Functional group by FTIR

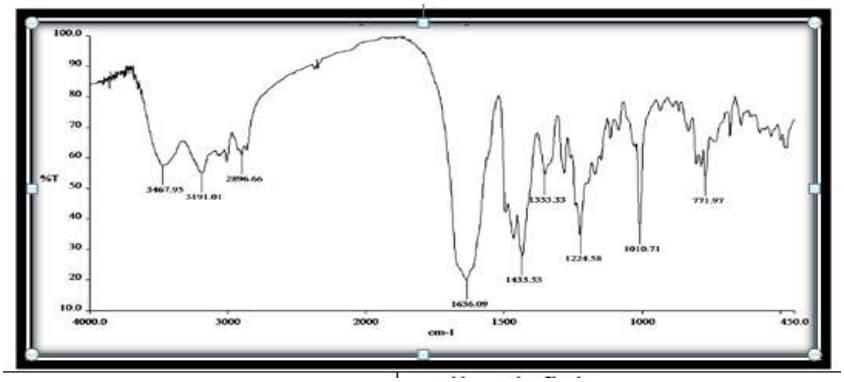


Fig.2 FTIR spectra of Olaparib

Melting point: The melting point was found 198˚ C.

Optimized Method:

Preparation of mobile phase: The Mobile phase was prepared by mixing the Methanol and water in the ratio of 70:30 v/v and mixed well and was

filtered through Whatman filter and degassed by sonication for about 10min**.**

Preparation of diluent: Prepare a mixture of Methanol and Water (70:30v/v).

Preparation of standard solution: An accurately weighed 20 mg of Olaparib dissolve in 10 ml ethanol and sonicate it upto 10 min add water upto the mark 100 ml in 100 ml volumetric flask. Take 10 ml pipette out from above solution and make volume upto 100 ml in 100 ml volumetric flask.

Optimized chromatographic conditions for HPLC method

Table 4 Optimized chromatographic conditions for HPLC method

Parameters	Chromatographic conditions
HPLC system	Agilent infinity 1260 II
Column	TC C18 (250*4.6mm)
Mobile phase	METHANOL:WATER(70:30)
Flow rate	1.0 ml/min
Pump mode	Isocratic
Injection volume	50µL
Wavelength	276nm
Detector	DAD Detector
Column Temperature	R.T
Run time	10 min
Retention Time	1.8 min

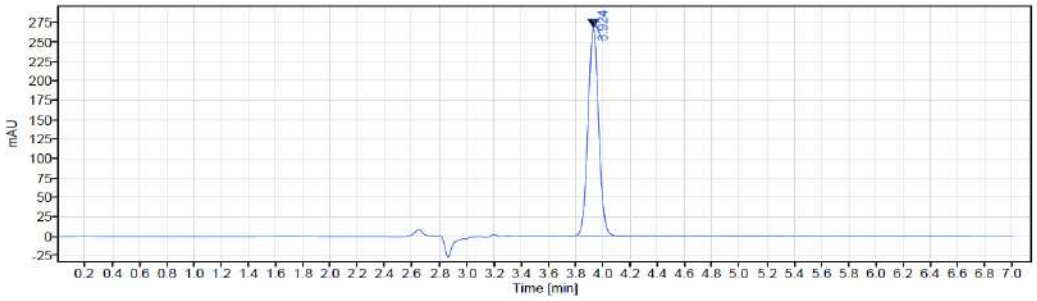


Fig.3 Typical Chromatogram of Final Optimized method: Estimation of Esmolol Hydrochloride in Parentral Dosage Form

Table 5 Estimation of Esmolol Hydrochloride in Parentral Dosage Form

Parameter	Olaparib Area
Curve Area	1447.359
	1463.988
	1460.058
	1441.992
	1462.258
Mean Area	1455.131
%RSD	0.675
Sample Area	1462.258
%Assay	99.78%

Standard solution and sample solution were prepared as discussed in the methodology above, peak area was measured, and Assay %, label claim was calculated.

Melting point: The melting point was found 198℃.

Optimized Method:

Preparation of mobile phase: The Mobile phase was prepared by mixing the Methanol and water in

the ratio of 70:30 v/v and mixed well and was filtered through Whatman filter and degassed by sonication for about 10min**.**

Preparation of diluent: Prepare a mixture of Methanol and Water (70:30v/v).

Preparation of standard solution: An accurately weighed 20 mg of Olaparib dissolve in 10 ml ethanol and sonicate it upto 10 min add water upto the mark 100 ml in 100 ml volumetric flask. Take 10 ml pipette out from above solution and make volume upto 100 ml in 100 ml volumetric flask. Optimized chromatographic conditions **for HPLC method** are similar as mentioned in Table 4.

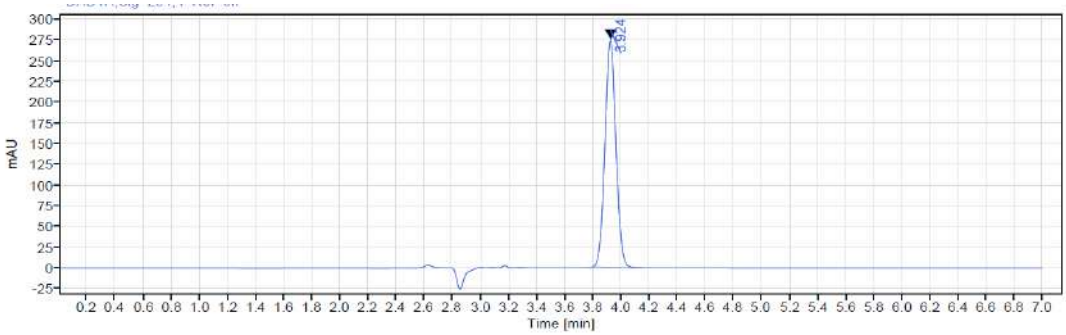


Fig.4 Typical Chromatogram of Final Optimized method: Estimation of Esmolol Hydrochloride in Parentral Dosage Form.

Table 6 Assay of Parentral dosage form

Parameter	Olaparib Area
Standard Area	1447.359
	1463.988
	1460.058
	1441.992
	1462.258
Mean Area	1455.131
%RSD	0.675
Sample Area	1462.258
%Assay	99.78%

Standard solution and sample solution were prepared as discussed in the methodology.
Above, peak area was measured, and Assay %, label claim was calculated.

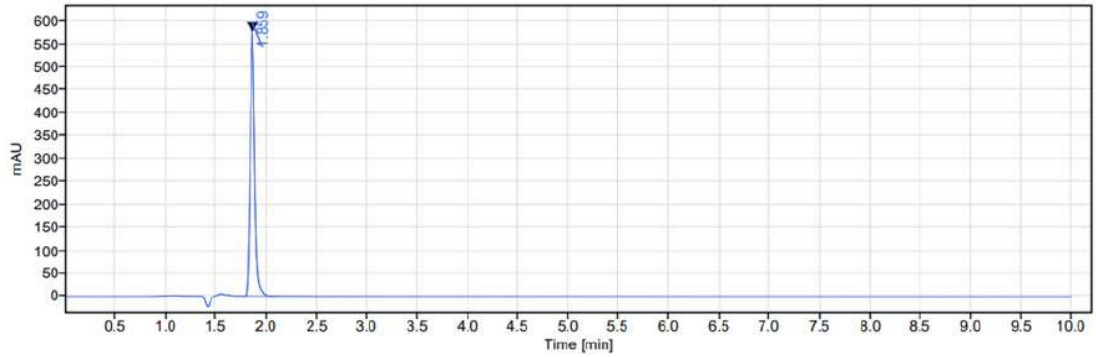


Fig. 5 Typical Chromatogram Obtained from Assay sample

System Suitability: Single injection of Blank solution, six replicates of standard solution was injected in to the HPLC system. The results obtained are summarized in Table 7.

Table 7 System suitability for Olaparib

Sr. No.	Standard Area
1	1447.359
2	1463.988
3	1460.058
4	1441.992
5	1462.258
Mean	1455.131
%RSD	0.6755

Acceptance criteria:

The %RSD of six replicate injections of standard solution for peak area response of Olaparib should not be more than 2.0%.The tailing factor for peak of Olaparib in standard solution should not be more than 2.0. The Theoretical plates for peak of Olaparib in standard solution should not be less than 2000.

Conclusion: The results demonstrate that all the system suitability parameter meets the acceptance criteria. Hence, the system is suitable.

Specificity: The retention time of the Olaparib peak in the chromatogram of the Sample corresponds to that of the Olaparib peak in the chromatogram of the standard preparation. Retention time of the Olaparib in standard solution is 3.93 min. Retention time of Olaparib peak in sample solution is 3.92 min.

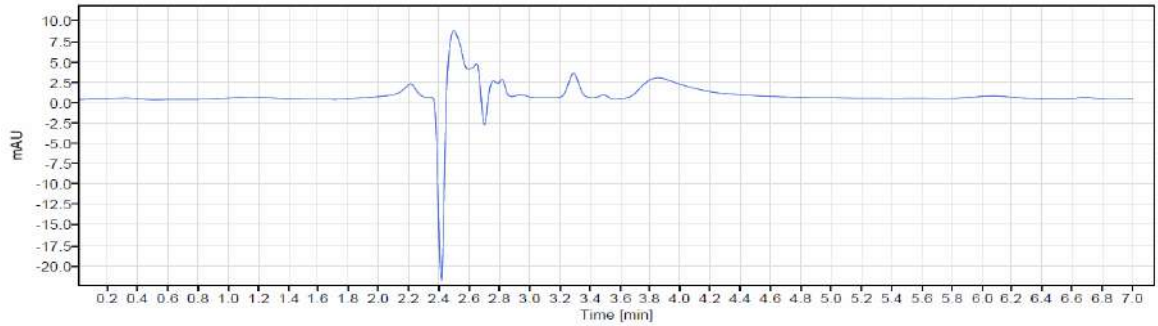


Fig. 6 Specificity Blank chroma

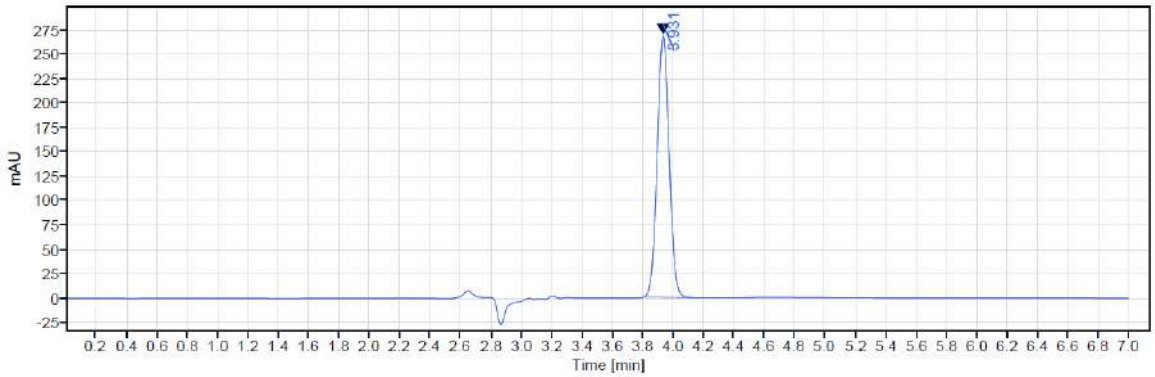


Fig. 7 Specificity Standard solution chromatogram

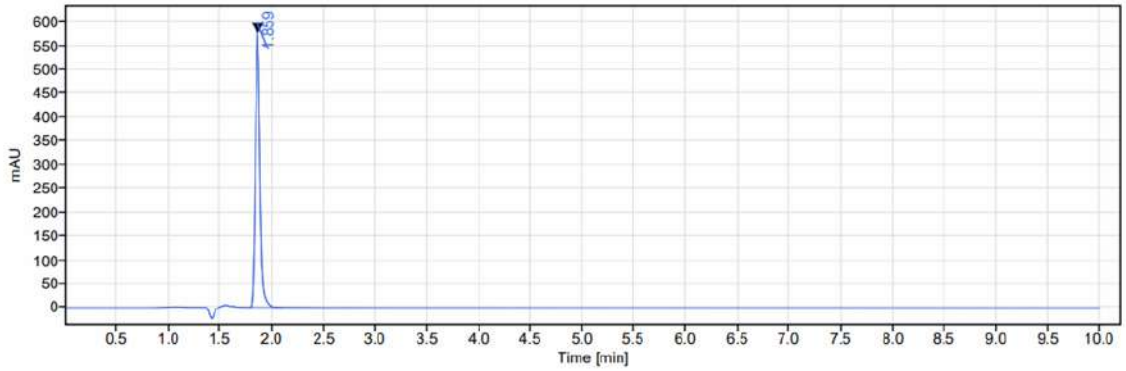


Fig. 8: Specificity Sample solution chromatogram

Table 8: Data sheet for Specificity

Sr. No	Standard Area	Standard R. T	Sample Area	Sample R T	% Assay
1	1447.359	3.92	1487.306	3.92	99.78
2	1463.988	3.92			
3	1460.058	3.93			
4	1441.992	3.93			
5	1462.258	3.93			
Mean	1455.131	-	-	-	-
SD	9.83				
%RSD	0.6755				

Acceptance criteria: Blank should not show any peaks at the Retention time of Olaparib peak Standard and sample peak should be pure for working concentration level.

Conclusion: Chromatogram obtained from sample solution of Olaparib is similar to Olaparib standard solution and there is no interference of blank, known impurities at the Retention time of main peak.

Linearity and Range

A series of standard preparation of Olaparib standard was prepared over a range of 5, 10,16,20,24 and 30 ppm of working concentration level of Olaparib i.e 20 ppm. The above-mentioned range covers the working concentration of Assay and content uniformity.

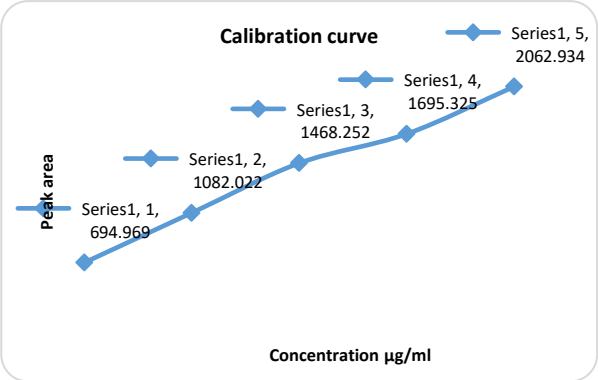


Fig. 9 Linearity graph of OLAPARIB

Table 9 Data sheet for Linearity

Linearity Level	Standard Conc.(ppm)	Mean Peak Area	Statistical Analysis
Level -1	5ppm	368.321	-
Level-2	10 ppm	694.969	-
Level-3	16 ppm	1082.022	
Level-4	20 ppm	1468.252	Slope of Regression line = 3095.4
Level-5	24 ppm	1695.325	Y-Intercept = 633.2
Level-6	30 ppm	2062.934	Correlation Coefficient = 0.9999

Acceptance criteria: System suitability criteria should be fulfilled. Response should be linear. Correlation coefficient (r2) should not be less than 0.99

Conclusion: The results show that all the system suitability parameters meet the acceptance criteria. The results show that the response is found to be linear. Refer linearity graph attached as figure 9. Correlation coefficient is more than 0.999. Hence, the method is linear in the given tested range.

Accuracy (Recovery): The accuracy was evaluated by spiking at five levels from 50%, 80%, 100%, 120% and 150% levels of the corresponding stock concentration level for sample in five and prepared as described under methodology. Each of the sample preparation was injected in triplicate and the average area count to be taken for calculation.

Acceptance criteria: System Suitability criteria should be fulfilled. Mean recovery for 25% to 150% levels should be in the range of 98.0%-102.0%.The RSD for % Recovery of all obtained results should not be more than 2.0%.

Conclusion: Mean recovery is 99.70 % and %RSD is 0.063 % therefore, the HPLC method for the determination of Assay of Olaparib Injection is accurate.

Method precision: Six sample solutions of Olaparib Injection were prepared and injected in to the HPLC. The data obtained is summarized in Table 10.

Table 10 Data sheet for Method precision

Sample	%Assay
1	101.0
2	99.52
3	99.69
4	100.38
5	99.68
6	100.96
Mean	100.205
SD	0.669
%RSD	0.668

Acceptance criteria: RSD should not be more than 2.0%

Conclusion: The RSD of the method precision is 0.668% therefore; the HPLC method for the determination of Olaparib is reproducible.

Robustness: This parameter was studied by making small but deliberate changes in the chromatographic conditions and observing the effect of these changes on the system suitability and the results obtained by injecting the standard solutions. The results are summarized in Table 11.

Table 11: Data sheet for Robustness

Change in Parameter		Area of Standard	Mean	SD	% RSD
Changes in Flow Rate (± 0.2ml/min) 1.0ml/min	0.8ml/min	1889.269	1899.988	3.15	0.166
		1900.626			
		1904.833			
		1896.163			
		1899.05			
	1.2ml/min	1277.789	1270.837		
		1267.625		7.684	0.60
		1259.021			
		1273.240			
		1276.511			

Acceptance criteria: The difference for each modified condition and original condition should not be more than ±2.0%.

Conclusion: The difference for each modified condition and original condition is within the limit. Hence, the method is robust**.**

Stability of Analytical Solutions

Table 12 Data sheet for Stability of Analytical solutions at Room Temperature

Sr. No.	Name	% Content	%Correlation
1.	Standard solution-0hrs	100.0	-
2.	Standard solution-24hrs	100.1	100.1
3.	Sample solution-0hrs	100.8	-
4.	Sample solution-24hrs	100.2	100.5

Acceptance criteria: Standard solution: Assay of old standard against freshly prepared is between 98.0% and 102.0%

Sample solution: Correlation of old sample solution against initial assay is between 98.0% and 102.0%

Conclusion: Standard and sample solutions are stable for 24hrs at room temperature**.**

CONCLUSION

Present study was undertaken with an objective of developing suitable, sensitive and simple analytical method for estimation of olaparib in Bulk and pharmaceutical dosage form. The developed method is validated for specificity, linearity &, Precision, Accuracy, Robustness and found to be meeting the predetermined acceptance criteria. The validated method is specific, linear, precise, accurate, and robust for estimation of olaparib in pharmaceutical jjdosage form. Hence, this method can be introduced into routine use for the assay of Olaparib. The parameters considered for the validation and results obtained are summarized in Table 13.

Table 13: Parameters considered for the validation and results obtained

Sr. No.	Validation parameter	Acceptance criteria	Results obtained
1.	**SPECIFICITY**		
1.1	Identification	Results should be comparable with respect to Retention time	R.T. of standard solution is 3.1 min. R.T. of sample solution is 3.07 min.
1.2	Blank interference	Blank should not show any peak at the retention time of Esmolol hydro-chloride peak	No interference is observed. Esmolol Hydro-chloride peak is pure in standard and sample solution
2.	**LINEARITY AND RANGE**	Correlation coefficient should not be less than 0.999	Correlation coefficient Is 0.9999.
3.	**ACCURACY (%Recovery)**	Mean recovery should be in the range of 98.0% to 102.0%. The RSD should not be more than 2.0%	Mean recovery is 99.27 % and % RSD is 1.18 %
4.	**PRECISION**		
4.1	System Precision	RSD should not be more than 2.0%.	The RSD is 1.18%
4.2	Method Precision	RSD should not be more than 2.0%.	The RSD is 0.90%
4.3	Intermediate Precision	RSD should not be more than 2.0%.	The RSD is 1.03%
5.	**SYSTEM SUITABILITY**	Standard solution RSD of five replicate injections should not be more than 2.0%. Tailing factor for Esmolol Hydrochloride peak should not be more than 2.0. Number of theoretical plates should not be less than 2000.	RSD NMT 2.0% NMT 2.0 NLT 2000
5.1	Change in Wavelength (±5nm)	RSD should not be more than 2.0%	Complies.

5.2	Change in flow rate (±0.2ml/min)	RSD should not be more than 2.0%	
5.3	Change in mobile phase composition (±5%)	RSD should not be more than 2.0%	
6.	**ROBUSTNESS**		
6.1	Change in Wavelength (±5nm)	RSD should not be more than 2.0%	
6.2	Change in Flowrate (±0.2ml/min)	RSD should not be more than 2.0%	
6.3	Change in mobile phase composition (±5%)	RSD should not be more than 2.0%	

HPLC has gained the valuable position in the field of analysis due to ease of performance, specificity, sensitivity, and the analysis of sample of complex nature. This technique was employed in the present investigation for estimation of Olaparib in oral dosage form. Agilent HPLC with OPEN lab software, TC C18 (250*4.6mm) column and UV-Visible detector was used for the study. The standard and sample solution of Olaparib was prepared in diluent. Different pure solvents of varying polarity in different proportions were tried as mobile phase for development of the chromatogram. The mobile phase that was found to be most suitable was METHANOL AND WATER in the ratio of (70:30 v/v). The wavelength selected was 276nm. The selection of wavelength was based on the λ max obtained by scanning of standard. This system gave good resolution and optimum retention time with appropriate tailing factor (<2). After establishing the chromatographic conditions, standard laboratory mixture was prepared and analyzed by procedure described under Materials and methods. It gave accurate, reliable results and was extended for estimation of drugs in parentral formulation. The measured signal was shown to be precise, accurate, and linear over the concentration range tested (10-50 ug/mL) with a correlation coefficient better than 0.9999. Moreover, the lower solvent consumption leads to a cost effective and environmentally friendly chromatographic procedure. The Accuracy mean recovery is 99.70 % and % RSD is 0.063 % is in the limit. The precision RSD should not be more than 2.0%. It is found to be within the range.The results from above clearly indicated that the RP-HPLC technique can be successfully applied for the estimation of Olaparib in their formulation.

Here, we conclude that the developed RP-HPLC validate method is Precise, simple, accurate, sensitive, and reproducible for the quantitative estimation of Olaparib in bulk and its formulation and also concluded that this RP-HPLC method is convenient and effective for research studies, quality control and routine analysis of Olaparib in Pharmaceutical Industries.

CONFLICT OF INTEREST

There is no conflict of interest between authors.

REFERENCES

1. Skoog, West and Holler: Fundamental of Analytical Chemistry, 7th Edition, Saunders college Publishing 1992(1)
2. Sharma BK: Instrumental Methods of Chemical Analysis, 16thEdn.Goel Publishing House, Krishna Prakashan Ltd. Meerut. 2004(3)
3. Beckett AH. and Stanlake JB: Practical Pharmaceutical Chemistry, 4thEdn. Part 2, CBS Publishers and Distributors. New Delhi,2005(1)
4. Khopkar SM: Basic concepts of analytical chemistry, New age International Ltd. Publishers, New Delhi, 1998(2)178-179.

5. Settle F: Handbook of Instrumental techniques for analytical chemistry, Prentice Hall PTR, NJ, 1997 17-19, 56-57.
6. Skoog DA, Holler FJ, Crouch SR, Principle of Instrumental Analysis, Thomson Publications, India, 2007(6)1-3,145-147,180.
7. Mendham J, Denney RC, Barnes JD, Thomas M: Vogel's Textbook of Quantitative Analysis. Pearson Education, Singapore, 2003 8-9
8. Christian GD: Analytical Chemistry, John Wiley and Sons, 2003(5)35-42,131-132.
9. Gennaro AR, Karen BM, Medwick T, Remington: The Science and Practice of Pharmacy, 19th, the Mack Publishing Company, Pennsylvania, 1995(1)437-490.
10. Willard HH, Merritt LL, Dean JA, Settle FA: Instrumental Methods of Analysis, 7th Edn, CBS Publisher and Distributors, New Delhi. 2002. 617.
11. Sethi PD: High Performance Liquid Chromatography, Quantitative Analysisof Pharmaceutical Formulations, CBS Publishers and Distributors, New Delhi, 2001(1) 3-11, 116-120.
12. Christen GD, Analytical Chemistry, 5 edi John Wiley & Sons, Inc.2001.505
13. Weston A, Brown PR: High Performance Liquid Chromatography, Separations in High Performance Liquid Chromatography, Instrumentation for HPLC. In: HPLC and CE – Principles and practice. Academic Press, USA, 1997. 1-4, 8-11, 24-26, 29-32, 71.
14. Kasture AV, Mahadik KR, Wadodkar SG, More HN, Pharmaceutical Analysis-Instrumental Methods. 2008(l) 6-7, 28-30, 49, 64, 67.
15. United State Pharmacopeia, USP 29/NF 24, US Pharmacopeial Convention, Inc, Rockville MD, 2006, 555-556, 1777, 1861, 3050- 3052.
16. Andrew F: - Studied simultaneous analysis of Escitalopram and buproprion-SR by High Performance Liquid Spectroscopy.
17. Hanretta D. - Studied determination of Escitalopram, with Duloxetine and Olanzapine by reversed phase High Performance Liquid Chromatography.
18. Elham A. - Studied Micelle Enhanced Fluorimetric and Thin Layer Chromatography Densitometric Methods for the Determination of (±) Citalopram and its S – Enantiomer Escitalopram.
19. Christine Greiner: - Studied Determination of citalopram and escitalopram together with their active main metabolites desmethyl(es)citalopram in human serum by column- switching high performance liquid chromatography (HPLC) and spectrophotometric detection.
20. ICH guidelines, analytical method validation (Q3). Geneva, July 2000.
21. Guidance for industry, Analytical Procedure and Method Validation, U. S. Department of Health and Human Services FDA, (Aug2000).

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