A Rapid Stability indicating method Development and validation for the quantification of Talazoparib Tosylate in bulk drug and Pharmaceutical formulation by HPLC

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ABSTRACT:

Analytical method development and validation are the continuous and inter-dependent task associated with the research and development, quality control and quality assurance departments. Analytical procedures play a critical role in equivalence and risk assessment, management. It helps in establishment of product-specific acceptance criteria and stability of results. In view of this the present work is intended to develop an efficient and simple HPLC method for the determination of Talazoparib in bulk and was applied on marketed Talazoparib products. The pharmaceutical drug containing Talazoparibis an orally available poly ADP ribose polymerase (PARP) inhibitor developed for the treatment of advanced breast cancer with germline BRCA mutations. In the HPLC analysis, the mobile phase used for the chromatographic runs consisted of Methanol: acetonitrile: 0.1 % sodium perchlorate in the ratio of 25:75:05 (v/v). The separation was achieved on a Spherisorb ODS2C18 column (250mm × 4.5 mm; 5µm) column using isocratic mode. Drug peak was well separated and were detected by a UV detector at 269 nm.The method was linear at the concentration range of15–90 µg/ml for Talazoparib. The method has been validated according to ICH guidelines with respect to system suitability, specificity, precision, accuracy and robustness. LOD and LOQ were found to be 0.03µg/mL and 0.10µg/mL for Talazoparib. Results confirmed that the method was sensitive and can be useful for the detection and analysis of drugs at very lowest concentrations. The method can effectively separate the forced degradation products formed during the stress study confirms the stability indicating nature of the method. Hence it the method reported in the present study was used for the separation and quantification of Talazoparib in bulk drug, formulations as well as stability testing.

Key words: Talazoparib, Ultraviolet, Analytical method development & High Performance Liquid Chromatography.

INTRODUCTION:

Analytical techniques both qualitative analysis (the presence or absence of one or more elements) and quantitative analysis (how much amount is present) can be done [1]. Analytical chemistry involves the application of range of techniques and methodologies to obtain and access quantitative and structural information on the nature of matter [2, 3]. Analytical information and level of accuracy required. Costs, timing, availability of laboratory instruments and facilities [4, 5]. The most important aspect of analysis is quantitative chemical analysis. In the present age, the physical, chemical and biological analysis, Involve computerized techniques to facilitate better result [6-9]. The process of analytical method validation should demonstrate that the method is fit for its purpose[10-12]. The validation should follow a plan that includes the scope of the method, the method performance characteristics and acceptance limits [13-16].

Talazoparib is an orally available small molecule inhibitor of the DNA repair enzyme poly ADP-ribose polymerase (PARP) which is used as an antineoplastic agent in the treatment of selected cases of breast cancer. Talazoparib is associated with a moderate rate of serum aminotransferase elevations during therapy and is suspected to cause rare instances of clinically apparent acute liver injury [17, 18].

Figure 1: Molecular structure of Talazoparib

Talazoparib was approved by the FDA for use in germline BRCA mutated, HER2 negative, locally advanced or metastatic breast cancer on October 16, 2018 under the trade name Talzenna [19]. Talzenna was granted approval based on the results of the EMBRACA trial in which talazoparib resulted in a mean 8.6 months progression-free survival time versus physician's choice chemotherapy which resulted in 5.6 months progression-free survival [20, 21].

Talazoparib acts as an inhibitor of poly ADP ribose polymerase (PARP) which aids in single strand DNA repair. Cells that have BRCA1/2 mutations are susceptible to the cytotoxic effects of PARP inhibitors because of an accumulation of DNA damage [22]. PARP trapping is the mechanism of action where the PARP molecule is trapped on the DNA, which interferes with the cells ability to replicate. Talazoparib is found to be ~100 fold more efficient in PARP trapping than Olaparib [23].

The literature survey was conducted for the available analytical methods for the estimation of Talazoparib in bulk drug as formulations using various analytical techniques such as HPLC, spectrophotometer, LCMS, UPLC etc techniques. Based on the literature, it can be confirmedthat there is no analytical method reported for the estimation of talazoparib in bulk and pharmaceutical formulations [24-26].

Aim and Objectives:

The aim of the research work to develop and validated analytical method for the determination Talazoparib in single dosage form and estimation of degradants generated during storage of finished products using techniques such as High-performance liquid chromatography.

MATERIALS AND METHODS

Instrumentation:

HPLC System, Deep Freezer, Microbalance,Vibramax,Vacuum pump, PH meter, Micropipettes, Vortexer, Water Purification (Elix 10 / Milli-Q gradient) and Ultra Sonicator(Power Sonic510)Data acquisition was done using LC Solutions version 1.23SP 1 software.

Chemicals and solvents:

The working standard drug TalazoparibTosylate(98.68% purity) along with the formulation dosage form (Talzenna®- 1.0mg) were obtained fromNovartis India Limited, Mumbai. HPLC grade Methanol, Water and Acetonitrile were purchased form Merk chemicals private limited, Mumbai.The buffer solutions used for the study were AR Grade and purchased from Merck Specialties Private Limited, Mumbai, India.

Preparation of standard drug solution:

Preparation of standard stock solutionwas the primary step prior to experimental work. A standard stock solution of 1000µg/mL was prepared by weighing accurately 10mg of the standard drug Talazopariband was taken in a 10mL volumetric flask having little amount of Methanol.Dissolve the drug in the solvent and make up to the mark.Then it was filtered through 0.45µ filter paper to remove un-dissolved particles or any solid substances.The solution was preserved safely and used when required. The standard concentration of Talazoparib used at different concentrations (15, 30, 45, 60, 75&90µg/mL) to compare with test formulation.

Preparation of formulation solution:

Tablets of Talzenna®brand containing 1.0 mg of Talazoparibtosylatewas powdered using a sterile mortar and pestle. Then an amount of tablet powder equivalent to 50 mg of Talazoparibtosylate was accurately weighed and dissolved in 50 mL solvent using sonicator and filtered through 0.45 µ membrane filter. Then it was diluted while doing the formulation analysis.

HPLC Method Development:

***Selection of wavelength*:**

To select an appropriate monitoring wavelength, the standard solutions of 10μg /mL was prepared and scanned by the UV-Vis spectrophotometer. The obtained wavelength maxima was selected as suitable wavelength for the detection.

***Selection of stationary phase*:**

Since the Talazoparibis a Polar drug, a non-polar C18 column was selected for the separation of the drug. Different columns of different companies, manufactures and configurations were tested.

Analytical Method Validation:

The method was validated with respect to linearity, accuracy, precision, repeatability, selectivity, and specificity, according to the ICH guidelines.

***Specificity*:**

Specificity of the method was checked by injecting the solution into the chromatograph. Specificity of the method was assessed by comparing the chromatogram of Talazoparib (standard), blank and sample solutions to those obtained for tablet solutions. Retention time of the Talazoparib in standard solution, and in the sample solution was compared to determine the specificity of the method.

***System suitability*:**

The system suitability was determined by making six replicate injections of the standard solution and analysingTalazoparibfor its peak area, peak USP tailing factor, and number of theoretical plates. The proposed accepted criteria are not more than 2% for RSD%, not less than 2 for resolution, not more than 2 for USP tailing factor, and not less than 2000 for the number of theoretical plates.

***Sensitivity of the method*:**

The limit of detection (LOD) and limit of quantitation (LOQ) were defined as the lowest concentration of analyte in a sample that can be detected and quantified. The standard solutions of Talazoparibfor LOD and LOQ were prepared by diluting them with suitable solvent. The LOD and LOQ were determined by the signal-to-noise (S/N) ratio for each compound through analyzing a series of diluted solutions until the S/N ratio yield 3 for LOD and 10 for LOQ, respectively.

***Linearity and Range*:**

The calibration curve in the developed method was constructed from LOQ concentration. Talazoparibstandard stock solution of 1 mg/mL was used for preparation of subsequent aliquots. Sample solution was loaded and 20µL was injected into column. All measurements were repeated for each concentration. The calibration curve of the area under curve versus concentration was recorded. Form the calibration curve, correlation and regression values were calculated for Talazoparib.

***Precision*:**

The precision studies were carried out by estimating response of Talazoparib six times at a standard concentration of 60 µg/mL and results are reported in terms of %RSD. The intra-day and inter-day precision studies were carried out by estimating the corresponding responses six times on same day for intraday and interday for three different days and it was expressed as the percentage relative standard deviation (%RSD) which was calculated as per the following expression

$$\%RSD = (\text{standard deviation} / \text{mean}) \times 100.$$

Table: 01 Method Development trails HPLC method development

Trail .NO	Parameter	Condition	Trail .NO	Parameter	Condition
I	MP	Water :acetonitrile in 20:80 (v/v)	**IV**	MP	methanol: acetonitrile 80:20 (v/v)
	Wavelength	269nm		Wavelength	269 nm
	Stationary Phase	Spherisorb ODS2C18 column (250mm × 4.5 mm; 5µm)		Stationary Phase	Spherisorb ODS2C18 column (250mm × 4.5 mm; 5µm)
	Flow Rate	1.0 mL/min		pH of MP	5.5
				Flow Rate	1.0mL/min
II	MP	Water : acetonitrile in 80:20 (v/v)	**V**	MP	Methanol: acetonitrile: 0.1 % sodium perchlorate in 25:73:02 (v/v)
	Wavelength	269 nm		Wavelength	269 nm
	Stationary Phase	Spherisorb ODS2C18 column (250mm × 4.5 mm; 5µm)		Stationary Phase	Spherisorb ODS2C18 column (250mm × 4.5 mm; 5µm)
	Flow Rate	1.0 mL/min		pH of MP	5.1
				Flow Rate	1.0mL/min
III	MP	Water: Methanol in 60:40 (v/v)	**VI**	MP	Methanol: acetonitrile: 0.1 % sodium perchlorate in 25:75:05 (v/v)
	Wavelength	269 nm		Wavelength	269 nm
	Stationary Phase	Spherisorb ODS2C18 column (250mm × 4.5 mm; 5µm)		Stationary Phase	Spherisorb ODS2C18 column (250mm × 4.5 mm; 5µm)
	pH of MP	5.2		pH of MP	4.9
	Flow Rate	1.0mL/min			

***Accuracy/ Recovery*:**

Accuracy of method was observed by recovery result from two placebos preparations accurately spiked with different concentration of Talazoparib. Recovery assessment was obtained by using standard addition technique which was by adding known quantities of pure standards at three different levels in 50%, 100% and 150% to the pre analyzed sample formulation. From the amount of drug found, amount of drug recovered and percentage recovery were calculated by using the formula.

%RSD = (standard deviation / mean) x 100.

***Ruggedness*:**

Two laboratory analysts carried out the precision of Talazoparib at a standard concentration of 60 µg/ml was prepared by different analysts in the laboratory conditions, the prepared solution were analyzed in the optimized conditions. Peak area that obtained was used for the determination of ruggedness of the method. Ruggedness was expressed in terms of %RSD which must be less than 2.

***Robustness*:**

Robustness of the proposed method included six deliberate variations to some chromatographic parameters. The modifications include different mobile phase ratios and different detector wavelengths and different percentage in the mobile phase (in the range of ± 5 of the nominal value and the normal %). The % change in each of the changed condition was calculated.

Methodology for Forced degradation study:

Acid Hydrolysis:

50 mg of drugs were mixed with 50ml of 0.1N HCl solution. The solution was neutralized and diluted up to standard concentration (60 µg/ml) and was analyzed in the developed method condition

Base Hydrolysis: 50 mg of drugs were mixed with 50ml of 0.1N NaOH solution. The solution was neutralized and diluted up to standard concentration i.e60 µg/ml and was analyzed in the developed method condition

Oxidative Degradation:

50 mg of drugs were with 50ml of 3% Peroxide solution. The solution was neutralized and diluted up to standard concentration (60 µg/ml) and was analyzed in the developed method condition

Photolytic Degradation:

50 mg of drug sample was kept in UV light [254 nm]. After the selected time of light expose, the drug solution was prepared and was analyzed

Thermal Degradation:

50 mg of drug sample was kept in oven at 60 0C. After the selected time of light expose, the drug solution was prepared and was analysed

Formulation analysis:

This proposed method was applied to the determination of Talazoparibin commercially combined tablets. The sample solution at a concentration of 60 µg/ml of Talazoparib was analysed in the optimized conditions. Peak area of the resultant chromatogram was used for the estimation of assay using label clime recovery method. The % assay was calculated for Talazoparib using the standard calibration values.

RESULTS AND DISCUSSION

HPLC Method Development:

The spectrum of diluted solutions of the Talazoparib in methanol was recorded separately on UV spectrophotometer.The ultraviolet absorption spectra of the Talazoparibdemonstrated that the maximum absorption at a wavelength near 269 nm, and it was therefore chosen during the entire study.

Method development consists of selecting the appropriate wavelength by spectrophotometer and choice of stationary and mobile phases. The following studies were conducted for this purpose.

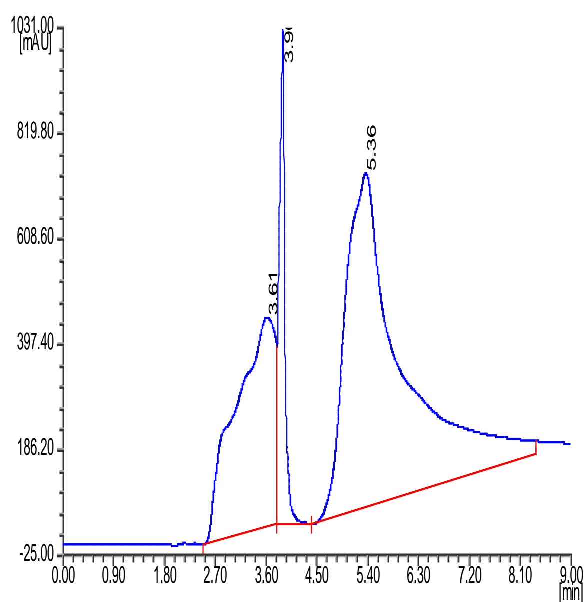


Figure 2: Chromatogram observed for Talazoparibin trail 1

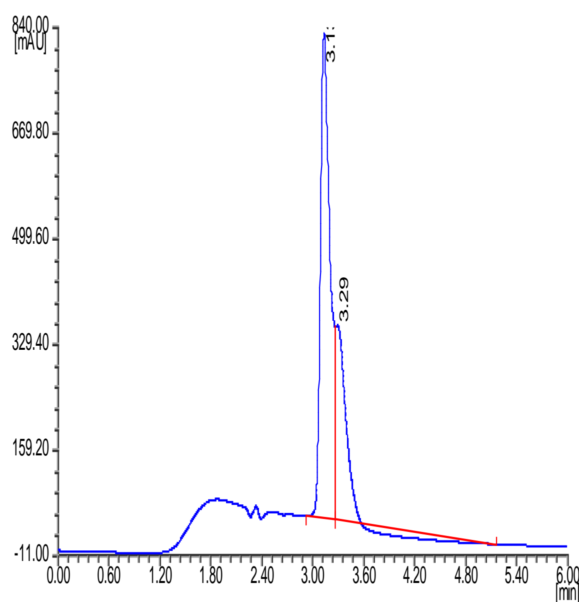


Figure 3: Chromatogram observed for Talazoparib in trail 2

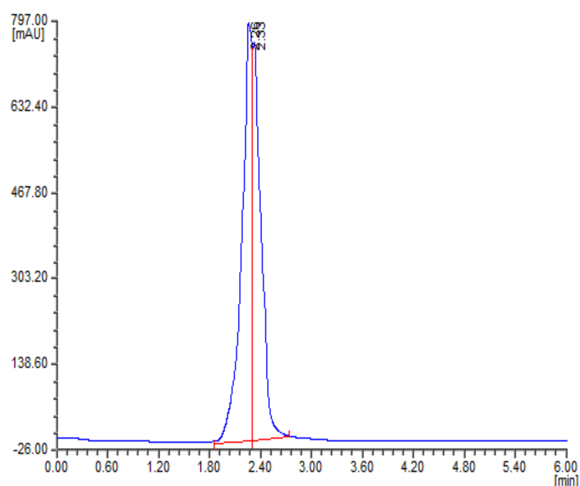


Figure 4: Chromatogram observed for Talazoparib in trail 3

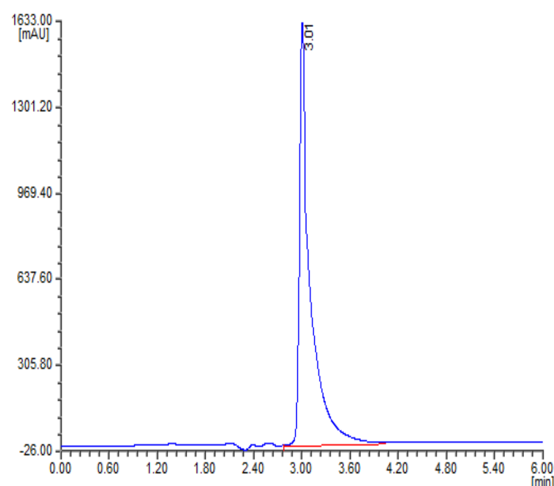


Figure 5: Chromatogram observed for Talazoparib in trail 4

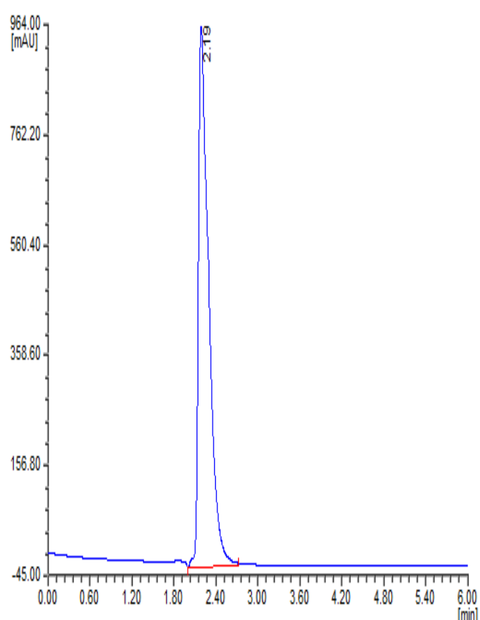


Figure 6: Chromatogram observed for Talazoparib in trail 5

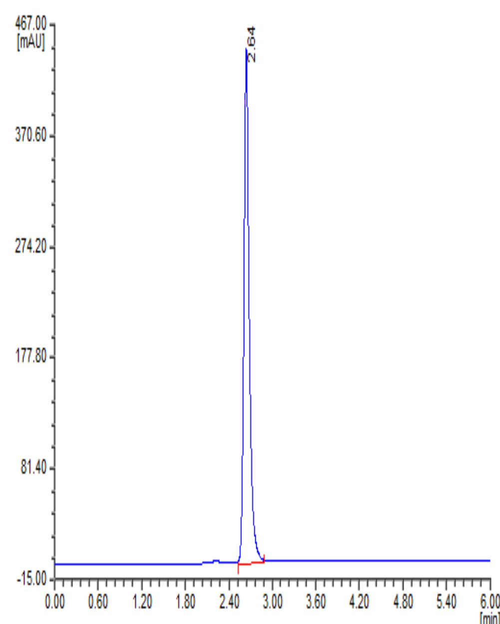


Figure 7: Optimized Chromatogram observed for Talazoparib in trail 6

In the trail 6, single sharp symmetric peak with acceptable system suitability was observed (Figure 7).
Hence these conditions were found to be suitable and further valuation was carryout using these conditions.

Method Validation:

The proposed method was validated as per ICH guidelines. The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification, and stress degradation studies.

Specificity:

The specificity of method was performed by comparing the chromatograms of standard (Figure 8) and sample solutions (Figure 9). It was found that there is no interference due to excipients in the tablet formulation and also found good correlation between the retention times of standard and sample. Sharp peak was obtained for Talazoparib retention times of 2.74min. This result indicated specificity of the method. Furthermore, there was no interference from the excipients present in the tablets; thus, the method was considered specific and selectivity.

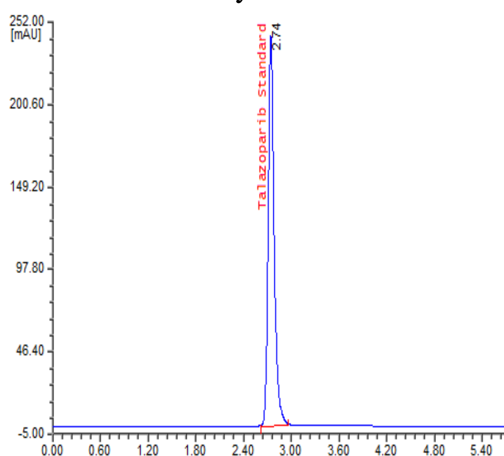


Figure 8: Chromatogram of Standard in the optimized conditions

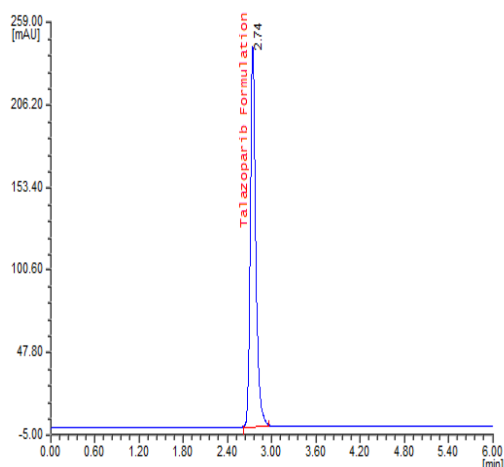


Figure 9: Chromatogram of Sample in theoptimized conditions

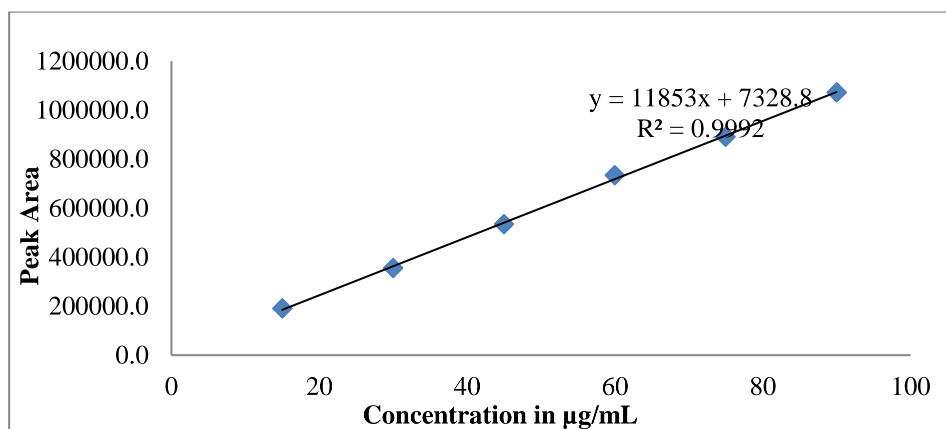


Figure 10: Linearity graph

Precision&Ruggedness

The %RSD was found to be 0.22 and 0.50for Talazoparibin intraday precision and interday precisionrespectively. Ruggedness was expressed in terms of %RSD which must be less than 2. The %RSD was found to be 0.80 in the developed method.

S. No	Intraday Precision	Interday Precision	S. No	Peak area observed
1	733305.3	730372.1	1	727450.6
2	733416.4	731949.6	2	730485.7
3	733340.2	728940.2	3	724566.5

4	732767.3	730569.0	4	728377.3
5	731155.1	724574.7	5	718053.5
6	729351.5	722787.4	6	716282.3
% RSD	**0.22**	**0.50**	**RSD**	**0.80**

Table 02: Precision results

Accuracy/ Recovery:

Accuracy was confirmed by carrying out recovery study as per ICH guidelines, where for a pre analyzed sample solution, known concentration of standard solution was added equivalent to 50%, 100%, 150% of labile claimed.The %recovery was fond to be within the range of 98.13 to 99.67 %. The %RSD in each spiked level was found to be 0.63, 0.28 and 0.76 % for Talazoparib in 50 %, 100 % and 150 % spiked level respectively. The results found to be with in the acceptance limit of 98-102 and % RSD of <2 which sense to conformation that the proposed method was accurate. Table 3 gives the accuracy results.

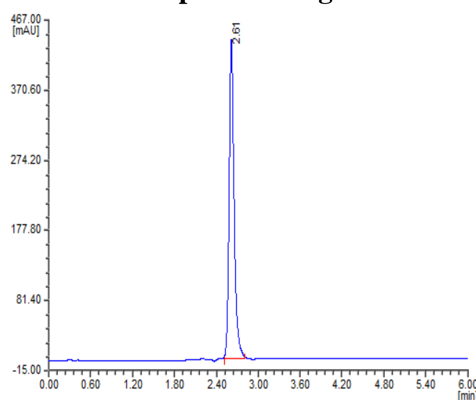
% Recovery	**Concentration in µg/ml**			**Amount Found**	**% Recovery**	**% RSD**
	Target	**Spiked**	**Total**			
50%	30	15	45	44.708	99.35	0.63
	30	15	45	44.159	98.13	
	30	15	45	44.361	98.58	
100%	30	30	60	59.178	98.63	0.28
	30	30	60	59.448	99.08	
	30	30	60	59.478	99.13	
150%	30	45	75	74.753	99.67	0.76
	30	45	75	73.838	98.45	
	30	45	75	73.718	98.29	

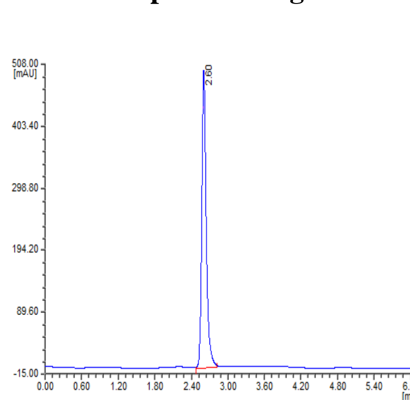
Table 03: Accuracy results

Robustness:

The robustness study was performed by slight modification in flow rate of the mobile phase, pH of the buffer and composition of the mobile phase. Talazoparib at 15 µg/ml concentration was analyzed under these changed experimental conditions.

Mobile phase change 1 **Mobile phase change 2**





Mobile phase pH change 1 **Mobile phase pH change 2**

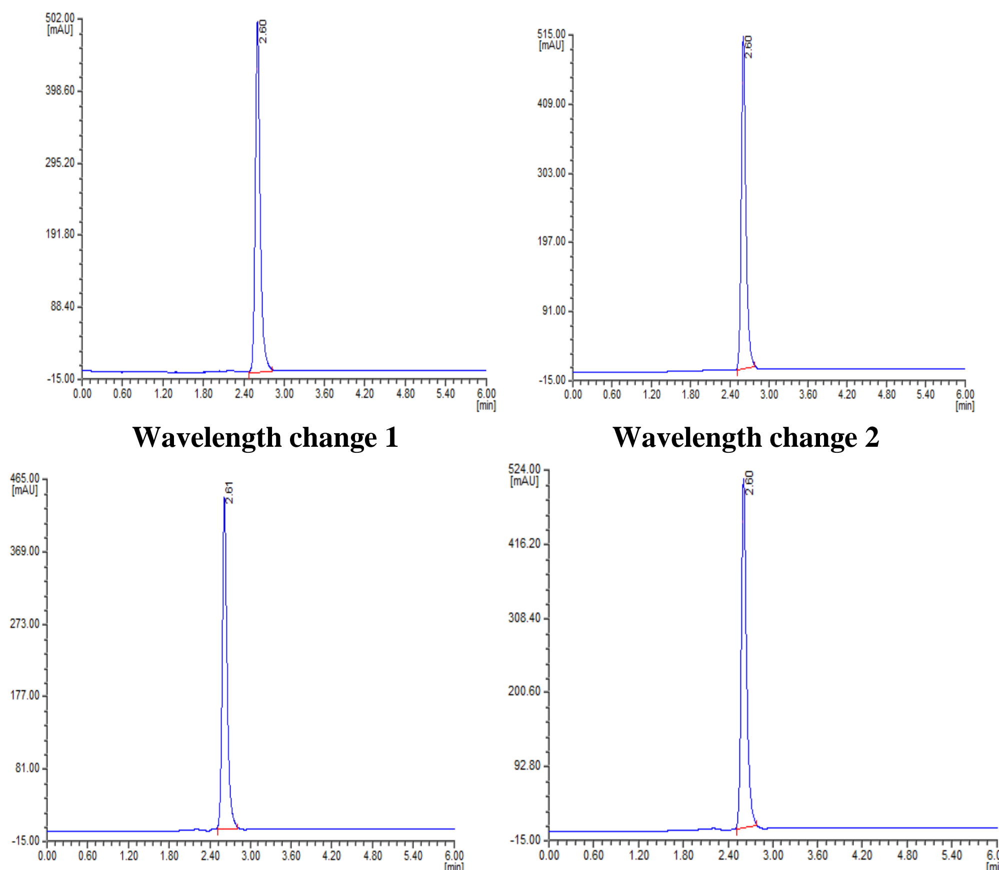


Figure 11: Robustness chromatograms

Method Sensitivity:

The sensitivity of the method was confirmed by determining the detection limit (LOD) and quantification limit (LOQ). LOD and LOQ were found to be 0.03 µg/mL and 0.10 µg/mL for Talazoparib. Results confirmed that the method was sensitive and can be useful for the detection and analysis of drugs at very lowest concentrations.

Forced Degradation studies:

When stress conditions were applied to Talazoparib, the HPLC results showed that there was no interference between the tested drug and the degradation products. Peak purity results were also within the acceptable limit for all the degradation conditions studies confirms that the Talazoparib peak is homogeneous in all stress conditions tested thus establishing the specificity and confirming the stability indicating power of the assay method.

S No	Condition studied	No of degradation compounds separated	% assay	% degradation
1	Acid	3	92.35	6.25
2	Base	3	93.08	8.51
3	Peroxide	3	96.52	4.22
4	Thermal	2	97.92	4.19
5	UV light	4	94.32	8.76

Table 04: Forced degradation study results

Formulation analysis:

The developed method was applied for the estimation of Talazoparib in its formulation solution prepared from Talzenna®brand tablets of Talazoparib. The % assay in formulation analysis was found to be 98.95 forTalazoparib in the developed method. More than 98% assay was observed in the developed method. Hence the method was found to be suitable for the routine analysis ofTalazoparib in bulk drug as well as formulations. Results of the formulation analysis were given in table 5and formulation chromatograms were given in figure 12.

S.No	Drug	Brand	Label claim	Concentration prepared	Concentration found	% assay
1	Talazoparib	Talzenna®	1.0 mg	60 µg/mL	59.37µg/mL	98.95

Table 05: Formulation results

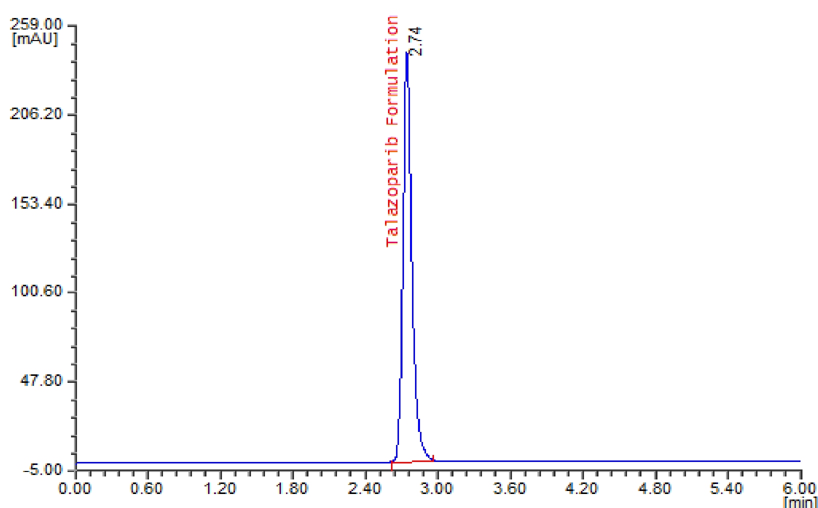


Figure 12: Formulation chromatogram

CONCLUSION

An efficient and simple HPLC method has been developed and validated for the determination of Talazoparib in bulk and was applied on marketed Talazoparib products. The mobile phase used for the chromatographic runs consisted of Methanol: acetonitrile: 0.1 % sodium perchlorate in the ratio of 25:75:05 (v/v). The separation was achieved on an Spherisorb ODS2 C18 column (250mm × 4.5 mm; 5µm) column using isocratic mode. Drug peak was well separated and were detected by a UV detector at 269 nm. The method was linear at the concentration range of 15–90 µg/ml for Talazoparib. The method has been validated according to ICH guidelines with respect to system suitability, specificity, precision, accuracy and robustness. LOD and LOQ were found to be 0.03 µg/mL and 0.10 µg/mL for Talazoparib. Results confirmed that the method was sensitive and can be useful for the detection and analysis of drugs at very lowest concentrations. The method can effectively separate the forced degradation products formed during the stress study confirms the stability indicating nature of the method. Hence it the method reported in the present study was used for the separation and quantification of Talazoparib in bulk drug, formulations as well as stability testing.

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