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NOVEL ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY-PDA METHOD DEVELOPMENT AND VALIDATION FOR RUCAPARIB IN BULK AND ITS APPLICATION TO MARKETED DOSAGE FORM

Nabajyoti Parida [1]*, H K Sundeep Kumar [2] and Mrityunjay Banerjee [3]

[1] Research Scholar, Biju Patnaik University of Technology,
Rourkela, Odisha, India. (*Corresponding Author)
[2,3] Institute of Pharmacy and Technology, Salipur, Cuttack, Odisha, India.

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Abstract

To develop a novel ultra-performance liquid chromatographic method for estimation of Rucaparibin a bulk and tablet dosage form and validate.The chromatographic separation was achieved using DIKMA Endoversil (2.1 x 50mm, 1.7µm) column. A mixture of 80% methanol and 20% water was utilize as a mobile phase with the isocratic elution mode and eluent was monitored at 283 nm using PDA detector. The method was continued and validated in accordance with International conference on harmonization guidelines. Validation study revealed the specificity and reliability of the method. In this method rucaparib was eluted with retention time of 0.523 minutes. Calibration curve plots were found linear over the concentration ranges 10-50 µg/mL for rucaparib. Limit of detection was 0.02µg/ml and limit of quantification was found 0.07µg/mL. The present method was also found stable in force degradation study.The empirical evidences of all the study results revealed the suitability of the estimation of rucaparib in bulk and tablet dosage form without any interference from the excipients.

Keywords: Rucaparib, Method Development, UPLC, Method Validation, ICH Guidelines.

INTRODUCTION

Small molecule poly(ADP-ribose) polymerase (PARP) inhibitor rucaparib (Rubraca®) has significant efficacy against PARP-1, -2, and -3. It is licenced for the treatment of adult BRCA-mutated ovarian cancer patients in the USA and the EU who have had two or more lines of chemotherapy. For use as maintenance therapy in adult patients with recurrent or relapsed ovarian cancer who are in a full or partial response to platinum-based chemotherapy, rucaparib is also authorised in the USA and the EU[1,2,3]. Rucaparib is a member of the class of azepinoindoles that is 1,3,4,5-tetrahydro-6H-azepino[5,4,3-cd]indol-6-**(Figure 1)** one carrying additional 4-[(methylamino)methyl]phenyl and fluoro substituents at positions 2 and 8 respectively[4].

After reviewing the literature in extensive manner, it was observed that only one HPLC method, one LCMS method [5] was available for the estimation of Rucaparib. The method developed by Suchitra et al[6], utilized phosphate buffer and methanol, with the volume ratio of 65:35 at pH 4.8. Though phosphate buffer is common solvent for mobile phase still it should be avoid because of the salt deposition chance, if cleaning was not proper[7,8]. The obtained retention time is 5.484 min, which can be minimize utilizing the advanced technique like ultra performance column. The possibility to increase the sensitivity was also there, by reducing the level of limit of quantitation.

After considering the above drawbacks from the existing method, efforts was taken to developed astability indicating estimation method for rucaparib using ultra performance liquid chromatography. The authors successfully resolved the drawbacks in this present method, by significantly reducing the retention time and increased the linearity range with a high resolutionoptimized chromatogram of rucaparib. In the

present method is also consider sensitive as the detection and quantitation limit were very less in comparison to other reported methods. Thereforethe present research work describes simple, stability indicating, accurate, specific, robust, rugged and rapid UPLC (Ultra performance liquid chromatography) method and its subsequent validation study as per International Conference on Harmonization (ICH) Guidelines Q2 (R1)[9, 10], for the estimation of rucaparib in active pharmaceutical ingredient and in its dosage forms.

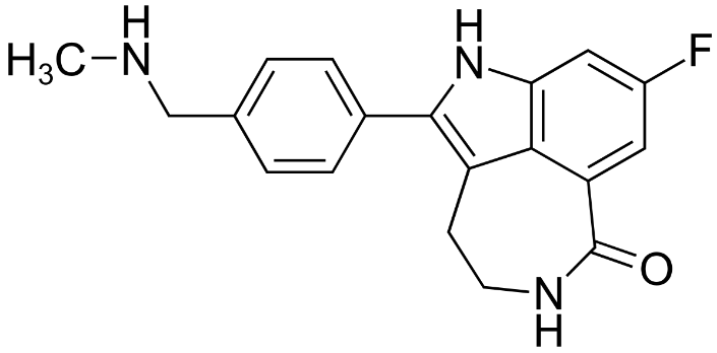

Figure 1: Chemical Structure of Rucaparib

MATERIALS AND METHODS

Chemicals and Reagents

Pharmaceutical grade working standards Rucaparib (99.97%) was procured from Manus Aktteva Biopharma LLP, Ahmedabad, India.The tablets (200mg) were obtained from Local Market of Bhubaneswar, Odisha, manufactured by BDR Pharmaceutical, Vadodara, India. All chemicals and reagents were required for the method development and validation and stability Studies were purchased from Fisher Scientific, Merck and Finer chemical Ltd.

Instrumentation Conditions

The analysis was performed using Ultra performance liquid chromatography (UPLC) Acquity Waters, PDA detector. Software: Empower 2 equipped with Auto Sampler and PDA detector. The column used is DIKMA Endoversil (2.1 x 50mm, 1.7μm) UPLC Column with the flow rate 0.3ml/min (isocratic) Detection was carried out at 283nm. The analytical balance 0.01mg Sensitivity (CAS analytical balance, CAUW220D), pH meter (Elico), Ultra Sonicator (PG scientific works). Labjal UV Lab India Laboratory Water Purification System was utilised for the HPLC water procurement.

Preparation of Mobile Phase

Accurately measured 800 ml (80%) of methanol and 200 ml of acetonitrile HPLC grade (20%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45µ filter under vacuum filtration.

Standard Solution Preparation

Accurately weigh and transfer 50mg of rucaparib working standard into a 50ml clean dry volumetric flask add about 10ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (stock solution). Further pipette 0.2 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent to obtain 20 µg /mL of rucaparib.

Assay of Marketed Dosage Form

Accurately weigh 5 rucaparib tablets and triturated in a mortor and pestle and transfer equivalent to 50mg Rucaparib (marketed formulation=64mg of tablet Powder) sample into a 25ml clean dry volumetric flask add about 10 ml of diluent and sonicate it up to 30 mins to dissolve completely and make volume up to the mark with the same solvent. The solution was shaken in a separating funnel for 10 minutes for proper extraction of drug form the excipients. Then it is filtered through 0.44-micron injection filter which is considered as stock solution. Further pipette 0.2 ml of solution from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Inject 10 µL of the standard and sample solution in triplicate into the chromatographic system and measure the areas for rucaparib peaks and calculate the assay percentage.

Method Validation

Specificity

The study was carried out using placebo interference test of the sample solution using 500 mg of placebo equivalent to one tablet dissolved in 100ml of mobile phase and the placebo solution was treated like a standard solution. The solution was injected to the chromatographic system to evaluate the possible interfering peaks.

System Suitability

The system suitability study wascarriedout to justify whether analytical system is working properly. It was carried out by injecting the six replicates of standard solution of rucaparib. The %RSD of the various optimised parameters like theoretical plates, peak area, retention time and asymmetric factor were calculated.

Accuracy

To justify the accuracy of the developed method recovery study was conducted at various levels (80%, 100%, and 120%) of pure rucaparib. The various amounts of standard rucaparibwere added to a fixed concentration to rucaparib tablet sample solution to achieve the various levels. This study were carried out three times and the percentage recovery as well as percentage mean recovery were calculated.

Intraday& Inter Day Precision

The precision of the method was studied by determining the 100% concentration rucaparib solution. It was evaluated by analysing the six sample solutions in triplicate (n=6) of 10µg/mL of rucaparib solution. The intra- and inter-day precision was determined by analysing for six times on same day (intra-day study) and repeated on the second and third day (inter-day study). The chromatograms were recorded.The peak area and retention time of rucaparib was determined and relative standard deviation (RSD) was calculated.

Detection and Quantitation Limit

The limit of detection was defined as the concentration for which a signal-to-noise ratio of 3 was obtained and for Quantitation limit, a signal-to-noise ratio of 10 was considered. Standard solutions of rucaparibwere prepared by sequential dilution and injected in to the chromatographic system in decreasing order of concentration in the range of 0.02-10µg/ml of rucaparib.

Linearity

To carry out the linearity, working standard solution of rucaparib were prepared as describe earlier, aliquot from this solution was diluted with mobile phase in five different concentrations to 5-50 µg/ml of rucaparib. Calibration curve plotted for both the drug under study considering concentration versus peak area, obtained data was subjected to regression analysis.

Robustness

Robustness of the developed UPLC method for rucaparib was studied by deliberately changing the chromatographic conditions. Six sample solutions were prepared and analysed in triplicate under the established condition by varying the analytical conditions like flow rate, mobile phase ratio, and detection wavelength at three different levels. One factor was changed at one time to estimate the effect. All the optimised parameter was found within the limit. For the calculation of percentage RSD, the tailing factor was considered.

Force Degradation Study of Rucaparib

Force degradation study [11] was carried out by following different ICH prescribed stress condition such as acidic, basic, oxidative, thermal and photolytic stress conditions.All the types of degradation studies have been performed in triplicate and mean peak area has been considered for the calculation.

Acid Degradation

The acid degradation study for rucaparib was performed in environmental test chamber (Acamus Technologies, India) at 60^0C and 75% relative humidity using 1M HCL. 0.3 ml of rucaparib stock solution (1 mg/mL) was taken in 10 ml of volumetric flask, 0.3 ml of 1 M HCL was added to the flask, kept in environmental test chamber for 16 hours. After the stress period solution was neutralized using 1M NaOH and make up the volume with mobile phase.

Base Degradation

Base degradation study of rucaparib was performed at 60^0C and 58% relative humidity using same environmental chamber. 0.3 ml of stock solution was taken in 10 ml volumetric flask mixed with 1 M 0.3 ml of 1M NaOH for 16 hr. After the suitable stress period the solution was neutralized with I M HCL and make up the volume with mobile phase.

Oxidative Degradation

It was performed in an environmental chamber at 40^0C, 75% relative humidity using 6% H_2O_2. For this purpose, 0.3 ml of stock solution was taken in 10 ml volumetric flask and 0.3 ml of 6% H_2O_2 was added in to flask and kept at 60^0C for 16 hr, finally make up the volume up to mark with mobile phase.

Thermal Degradation

The thermal degradation study has been carried out in environmental chamber at 40^0C, 75% relative humidity in oven at 105^0C, 0.3 ml of stock solution of rucaparib was taken in 10 ml volumetric flask and kept in chamber for 144 hr. and for dry heat thermolysis 1 mg of dry drug in solid form was placed in oven at 110^0C for 2 days.

Photolytic Degradation

Photolytic degradation study was carried out in sunlight (60000- 70000 lux) during day time and in U.V light at 254 nm for the period of 48 hr. 0.3 ml of stock solution of rucaparib was taken in 10 ml volumetric flask and make up the volume up to the mark with mobile phase was used for the study.

RESULT AND DISCUSSION

Method Optimization

Initially the various mobile phase has been tried with different ratios of buffer & oganic solvents eg. Methanol, acetonitrile. Finally, the mobile phase was optimized with methanol and water with the ratio of 80:20. Various columns like C18 column, BEH column, Phenominex, and Endoversil column has been tested for optimised separation. Finally, Endoversil (2.1cm x 50mm, 1.7µm) was found to be ideal as it shows good peak shape and parameters in acceptable range at the flow rate of 0.3 ml/min at ambient temperature. The photo diode array (PDA) detection was considering 283 nm. Under these optimised condition rucaparib was separated at 0.523 minute, which is consider too fast separation. The optimised chromatogram was shown in **Figure 2**.

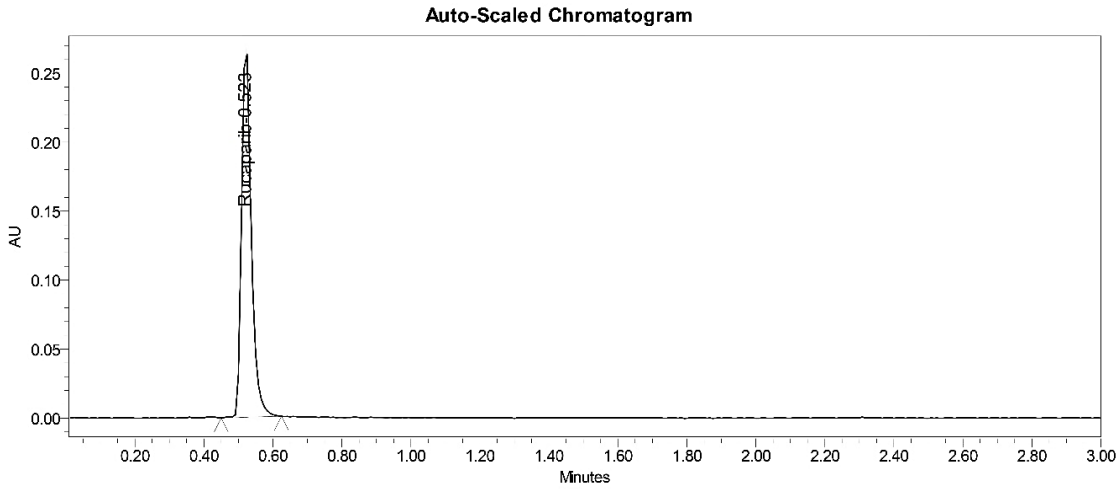

Figure 2: Optimized Chromatogram of Rucaparib

Method Validation

For the subsequent validation study and force degradation study the optimized chromatographic conditions was utilized and validated as per ICH guidelines. Fixed dose tablet dosage from “BDPARIB”, from BDR Pharmaceutical Ltd, containing 200 mg of rucaparib was selected for the application of the developed method. The obtained amount the drug was found to be significantly close to the label claimed amount. The content of the tablet was found 197.68 mg, with a percentage purity of 98.84%. The result was presented in **Table 1**. And the chromatogram of assay was shown in **Figure. 3.**

Table 1: Assay of Marketed Formulation

Brand Name of Rucaparib	Labeled amount of Drug (mg)	Mean (± SD) amount (mg) found	Assay % (± SD)
Rucaparib Tablets (BDPARIB) (200mg) BDR Pharmaceuticals	200mg	196.17	98.43%

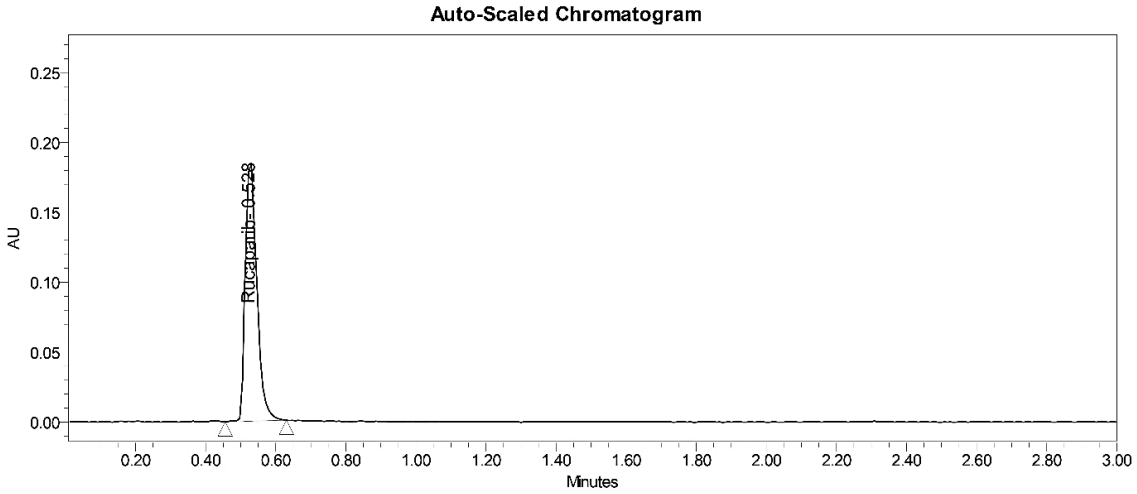

Figure 3: Assay of Rucaparib of Marketed Dosage Form

The accuracy of test method was performed at three different concentration levels (80%, 100% and 120%). The prepared solutions for these levels were injected in a triplicate manner and average % recovery was calculated, which were found 98.83% for 80% level, 98.30% for 100% level and 101.40% for 120% level of the labeled amount with an overall average of 99.51%. The results were summarized in **Table 2**. The %RSD of peak area, theoretical plates, tailing factors and retention time were 0.32%, 0.43%, 0.54% and 0.61% respectively, which confirms the suitability of the instrument to carry out the validation study. Precision study was conducted under the division of repeatability and intermediate precision. In repeatability the amount found was calculated and % RSD was found to be 0.73. In Intermediate precision the % amount found was calculated and % RSD was found to be 0.64, shown in **Table 2**.

Table 2: Summary of Validation Parameters of Rucaparib

Parameters	Rucaparib
Linearity range (µg/ml)	5 to 50
Co-relation co-efficient	0.991
LOD µg/ml	0.02
LOQ µg/ml	0.07
Repeatability (% RSD)	0.73
Intermediate precision (% RSD)	0.64
Mean % recovery	99.51
Assay (Rucaparib Tablets, each tablet contains 200mg) (% Assay)	98.43

The result of linearity values of the developed method was expressed by plotting a graph between concentration of the test solution on X-axis and response of the corresponding solutions on Y-axis and correlation co-efficient was found 0.999, as shown in Table 1. The Limit of detection was found 0.021µg/ml and limit of quantitation was 0.072 µg/ml, for the robustness study (**Table 3**) the % RSD of tailing factor was considered. In the robustness study the change in flow rates from ±0.03 mL/min

(%RSD=0.353), the mobile phase ratio of ± 2 (%RSD=0.512), and also with change of column temperature ± 2^0C (%RSD=0.332).

Table 3: Robustness Study

Parameters	Tailing Factor	% RSD
Flow Rate (-0.03 ml/min)	0.811	0.353
Flow Rate (+ 0.03 ml/min)	0.855	0.113
Column temperature (-5^0c)	0.559	0.332
Column temperature (+5^0c)	0.543	0.353
M.P ratio (- 2)	0.663	0.512
M.P ratio (+2)	0.661	0.41

The decomposition was seen on exposure of rucaparib drug solution to acidic (12.14 %), alkaline (8.37 %), oxidation (5.34 %). The details of the result indicated that the drug found significantly resistant towards the above degradations as shown in **Table 4**. And chromatograms of degradations studies were shown in **Figure 4.**

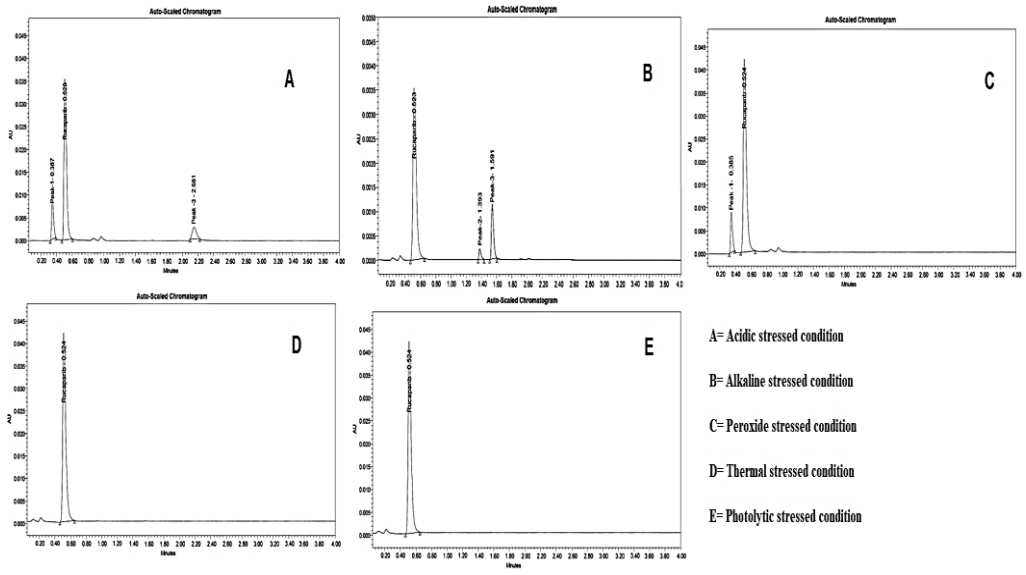

Figure 4: Degradation Chromatogram of Rucaparib

Table 4: Degradation Results for Rucaparib Solution

Sample Name	Rucaparib				
	Mean Area	% Degraded	Purity Angle	Purity Threshold	Peak purity
Acid	459367	12.14	0.577	1.637	Passes
Base	475075	8.37	0.863	2.544	Passes
Peroxide	519367	5.34	0.321	0.948	Passes
Thermal	530258	0	0.341	2.131	Passes
Photo	536473	0	0.737	3.768	Passes

DISCUSSION

The optimized chromatographic conditions were developed after several trials in the current research paper. Rucaparib was eluted with a good peak form, very short retention time using a mixture of methanol and water with a volume ratio of 80:20 and a flow rate of 0.3 mL / min by the use of the developed optimized condition.

The amount found was measured in the intraday and interday accuracy analysis and the calculated percent RSD was found acceptable and within the limits. The findings of the precision study showed that the methodology developed was found to be specific. The retention time of 0.523 minutes obtained for rucaparib. In accordance with the guidelines, accuracy and accuracy were determined and the percent recovery was found as a result of accuracy within the acceptable limit, i.e. within 95-105%as shown in the result, confirming the accuracy of the method developed. The percentage assay of 98.43 % in the marketed dosage form, which indicates the suitability of the method developed for the study of Rucaparib in the marketed dosage form. In the linearity study of the method the correlation coefficient was found 0.991 for rucaparib which indicates its specified linearity. The least squares method was used to establish the regression line and the curve was found linear. The limit of detection and quantitation values (0.021 and 0.072 µg/ml) proved sensitivity of the developed method. In the specificity result it was observed that no excipients peaks were found at the retention time of the rucaparib and justifies the specificity of the method. System suitability study was conducted to verify the proper working of the equipment used for analytical measurements. Several parameters like tailing factor and theoretical plates were be taken into consideration. In system suitability study. In the robustness study, the tailing factor was considered for the measurement tool and the % RSD of the tailing factor was found less than 2.0 which validates the robustness of the established approach, because no there was no significant changes were observed on the deliberate changes in the optimized parameters. The result of degradation studies of Rucaparib indicated that acidic, alkaline stressed condition some degradation was observed.In peroxide stressed condition leads to little degradation in compare to other stressed condition, whereas in photolytic and thermolytic condition no degradation was established. In every stressed condition, the chromatograms of Rucaparib were found very specifically eluted in presence of other degradation products. Based on peak purity results, obtained from the analysis of forced degradation samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of indicated that the developed method is specificfor the estimation of Rucaparib in presence of degradation products.

CONCLUSION

Based on the empirical evidences of this developed method, authors can be strongly claim about the novelty of the present developed method over the available methods. This is the first stability indicating UPLC method which is ‘rapid’ as it significantly reduced the total analysis time within 0.523 minutes, which is the lowest analysis time required. The present method is “stability indicating” as this has shown less degradation pattern in stressed conditions and good separation of famciclovir among the other degraded peaks. The results of all validation parameters were within the acceptance criteria of ICH Q2B guidelines. Hence the present developed method can be employed as a novel, reliable, validated useful method can be apply for routine analytical and quality control assay of rucaparib in the tablet dosage form.

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Conflict of Interest:

There is no conflict of interest

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