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AN INNOVATIVE STABILITY INDICATING HPLC METHOD WITH IMPURITY PROFILING OF NIRAPARIB-AN ANTICANCER DRUG IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A novel and sensitive HPLC method has been developed, and subsequently validated for the analysis of Niraparib and its impurity 1, Impurity 1 Acyl Glucuronide, and impurity 2 in formulations. The separation of Niraparib and its impurities was achieved on Inertsil 3V ODS (250mm × 4.6mm, 5µm) as stationary phase, methanol, 20mM ammonium formate and 0.05% of triethylamine in 80:15:5 (V/V) at pH 5.5 (adjusted with formic acid) at 1.0 mL/min isocratic flow and 246 nm wavelength. In the optimized conditions, the retention time was observed at 8.10, 11.01, 13.50, and 5.18 min respectively for Niraparib, impurity 1, Impurity 1 Acyl Glucuronide, and impurity 2. The method was confirmed to be sensitive with a detection limit of 0.015, 0.045, and 0.024 µg/mL for impurity 1, Impurity 1 Acyl Glucuronide, and impurity 2 respectively. Good linear correlation was observed in the concentration level of 15–120 µg/mL for Niraparib and 0.15 – 1.20 µg/mL for the impurities studied. Stress degradation studies were carried for evaluating the effectiveness of the method for the separation of stress degradation compounds along with known impurities. based on the results, it can be confirmed that the method was stable that can resolve the degradation compounds and the analytes in the study. The % degradation of Niraparib was confirmed to be less than 10% in all the stress degradation conditions studied. Hence the method can effectively be utilized for the routine analysis of Niraparib and its impurity 1, Impurity 1 Acyl Glucuronide, and impurity 2 in the formulation as well as stability studies.

Keywords: Niraparib, Impurity Analysis, Impurity 1 Acyl Glucuronide, Forced Degradation Study, Method Validation.

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INTRODUCTION

Niraparib is a small molecule poly ADP ribose polymerase 1 and 2 inhibitors prescribed for treating ovarian cancer in adult patients.[1] It is also used to treat primary peritoneal cancer and fallopian tube cancer for patients who are showing partial or complete response to platinum-based chemotherapy.[2] Niraparib works by irreversible multiple breaks in the double strand with Breast cancer susceptibility protein (BRCA) type 1 and 2 mutations leading to the death of the cells. The DNA in the uninfected cells doesn't replicate as often as in cancer cells and the patients recover from cancer.[3] Fatigue, Nausea, Constipation, Vomiting, and decreased platelet and neutrophil count are the possible side effects while using Niraparib. These side effects are often predictable in terms of their onset, duration, and severity and there is no relation between the presence of side effects and the effectiveness of Niraparib.[4]

In the extensive literature review, it was confirmed that no analytical HPLC method was reported for assaying the impurities of Niraparib in dosage forms. In the literature, one UPLC method reported for the simultaneous assay of Niraparib in combination with regorafenib, dabrafenib, cobimetinib, cabozantinib, olaparib, vemurafenib and regorafenib M2 which is its metabolite in human plasma.[5] Bio-analytical LCMS MS methods published for quantifying Niraparib in single[6,7], in combination with five PARP-inhibitors[8] and its metabolite M1.[9]

Hence the present work intended to establish an analytical HPLC method for the estimation of Niraparib and its impurities 1, Impurity 1 acetylgluconide, and impurity 2 in dosage forms. Niraparib and its impurities' molecular structure was given in Fig.-1.

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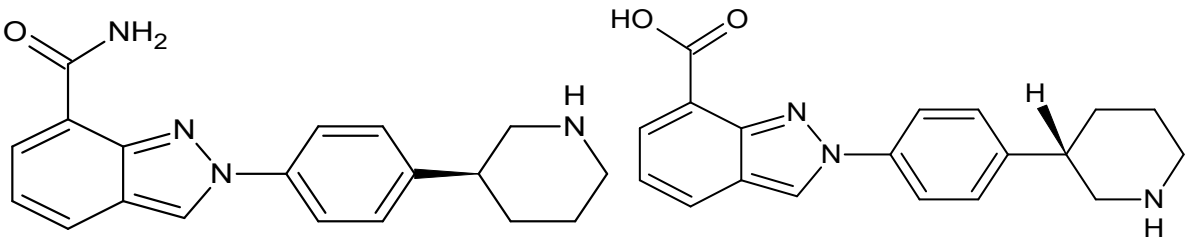
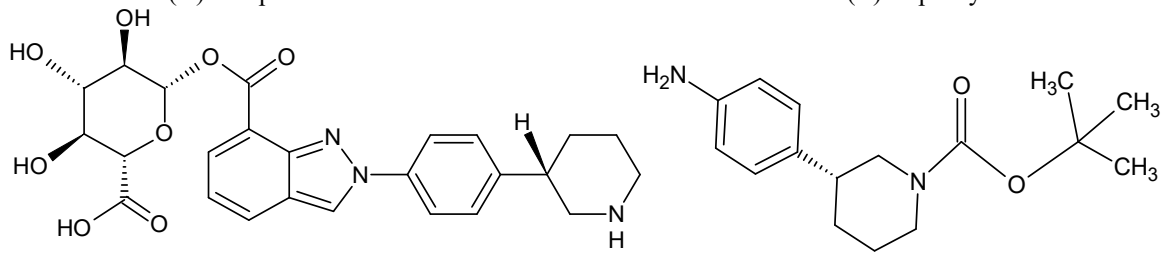


(A) Niraparib (B) Impurity 1



(C) Impurity 1 Acyl Glucuronide (D) Impurity 2

Fig.-1: Chemical Stricture of Niraparib and its Impurities

EXPERIMENTAL

Chemicals and Solvents

The pure standard drug Niraparib (98.62% purity), Impurity 1, Impurity 1 Acyl Glucuronide and Impurity 2 in the study were obtained from GlaxoSmithKline (GSK) Pharmaceutical Ltd, Hyderabad, Telangana. Ultra-Pure (Milli-Q®) Water, methanol, and acetonitrile (HPLC grade) were obtained from Merck chemicals.

Instrumentation

The analysis was performed on Agilent (USA) 1100 HPLC that consists of Quaternary (G1311 A) pump, 0.1 – 1500 µL injectable G 1329A auto sampler with thermostatic and programmable G 1314 A UV detector and the eluents were recorded through Agilent chem software.

Niraparib and Impurity Solution

The standard solution of Niraparib, Impurity 1, Impurity 1 Acyl Glucuronide and Impurity 2 were separately prepared by mixing 50 mg of analyte in 50 mL methanol. Then selected concentration of Niraparib and its impurities prepared separately, and equal volumes of known concentrations were mixed while performing the experiment.

Formulation Solution

The formulation brand Zejula® tablets having 100 mg of Niraparib was used for preparing formulation solution. The tablets were made in to fine powder using mechanical grinding and from the fine powder an amount equivalent to 50 mg of pure Niraparib was weighed and dissolved in 50 mL solvent. Then the content was sonicated to dissolve Niraparib in solvent and filtered through 0.45 µ pore size filters. Then it was diluted to required concentration prior to the analysis.

Method Development

Prior to the development of the method, the UV iso-absorption wavelength of Niraparib, Impurity 1, Impurity 1 Acyl Glucuronide, and Impurity 2 was determined using a UV spectrophotometer. The mobile phase composition, flow, pH and stationary phase was optimised by change in different conditions and analysing the standard solution containing Niraparib, Impurity 1, Impurity 1 Acyl Glucuronide and Impurity 2. The area response, system suitability conditions were observed for selecting the suitable conditions for validation.[10-13]

Method Validation

The placebo, blank and standard solution containing Niraparib, Impurity 1, Impurity 1 Acyl Glucuronide and Impurity 2 was analysed in the developed method for determining the system suitability and specificity of the method.

The standard solution of Niraparib spiked with 1 % impurity was used for evaluating the linearity range of the analysis. The linear graph was plotted by considering the peak area of each analyte against the concentration prepared. In the calibration range, the spiked recovery was performed at three spiked levels i.e. 50, 100, and 150 %. The % recovery for Niraparib and its impurities was calculated and a % recovery of 98-102 was found to be acceptable. The known standard solution of Niraparib containing 1 % solution of each impurity was analyzed one day (n =6), three different days (n =6), and three different analysts (n = 6) for intraday, interday precision and ruggedness respectively. The % RSD (relative standard deviation) of the peak area response of Niraparib, Impurity 1, Impurity 1 Acyl Glucuronide, and Impurity 2 was calculated for evolution, and % RSD of less than 2 was considered as the precise and rugged nature of the method. The method robustness was ascertained by determining the change in the separation and analysis of Niraparib and its impurities when small changes were made in the optimized conditions. The % change in the peak area response and the system suitability conditions of the resultant chromatograms was evaluated. The standard drug was exposed to acid (25 mg in 25 mL of 0.1 N HCl), base (25 mg in 25 mL of 0.1 N NaOH), peroxide (25 mg in 25 mL of 3% peroxide), thermal (25 mg kept in an air oven at 80 ^{0}C) and UV light (at 254nm) degradation studies. The number of degradation compounds identified and the % degradation in each degradation study were determined for evaluating the method effectiveness for separating the degradation compounds of Niraparib. The sample solution prepared with Zejula® tablets of Niraparib was analyzed in the current optimized conditions and the % assay of Niraparib and its impurities was evaluated.[10-13]

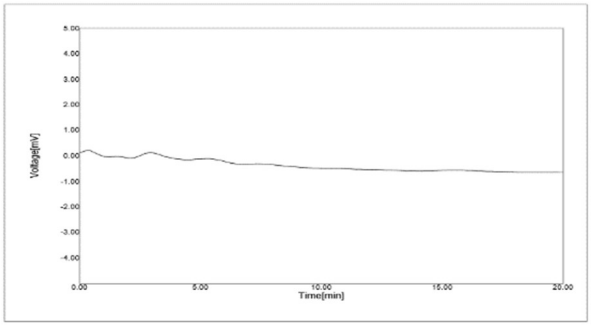
RESULTS AND DISCUSSIONS

The present study was designed to develop a simple and sensitive analytical HPLC method for quantifying the impurities of Niraparib in formulations. The method was confirmed by performing systematic method development trials and the results achieved in significant trials were summarized in Table-1.

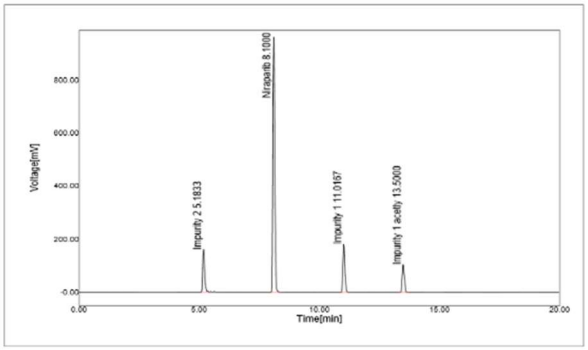
Table-1: Trails Performed and Results Achieved During the Optimization of Method

S. No.	Mobile Phase composition	Result	Conclusion
1	Inertsil 250 mm ODS 3V (5 µ id) column, sodium acetate buffer pH 6.8 and acetonitrile in 45:55 (V/V) at 1.0 mL/min and 246 nm wavelength	Separation was not achieved. Compounds were not identified	Method Rejected
2	Inertsil 250 mm ODS 3V (5 µ id) column, sodium acetate buffer pH 5.8 and acetonitrile in 70:30 (V/V) at 1.0 mL/min and 246 nm wavelength	Peak overlap was observed. No clear identification of compounds	Method Rejected
3	ProntoSIL 250 mm ODS C18 (5 µ id) column, acetate buffer pH 5.6, and methanol in 75:25 (V/V) at 1.0 mL/min and 246 nm wavelength	Individual peaks were identified but the separation was acceptable and peak response was found to be very poor.	Method Rejected
4	Inertsil 250 mm ODS 3V (5 µ id) column, Phosphate buffer pH 5.7, and methanol in 35:65 (V/V) at 1.0 mL/min and 246 nm wavelength	The separation between the standard, impurity 1, and Impurity 1 Acyl Glucuronide was found to be very less.	Method Rejected
5	Inertsil 250 mm ODS 3V (5 µ id) column, Phosphate buffer pH 5.1, and methanol in 80:20 (V/V) at 1.0 mL/min and 246 nm wavelength	The peak observed for Impurity 1 Acyl Glucuronide was found to be split and the elution was found to be not acceptable. The peak area response of Impurity 1 Acyl Glucuronide was also found to be very less.	Method Rejected
6	Inertsil 250 mm ODS 3V (5 µ id) column, methanol, 20 mM ammonium formate in 75:25 (V/V) at 1.0 mL/min and 246 nm wavelength	Individual peaks were observed for Niraparib and its impurities but the separation is not satisfactory and peaks doest satisfy the system's suitable conditions.	Method Rejected
7	Inertsil 250 mm ODS 3V (5 µ id) column, methanol, 20 mM ammonium formate, and 0.05 % of triethylamine in the ratio of 80:15:5 (V/V) at pH 5.5 (adjusted with formic acid) at 1.0 mL/min and 246 nm wavelength	Symmetric peaks with acceptable peak shape and acceptable system suitability were observed.	Method Accepted

The separation of Niraparib, impurity 1, Impurity 1 Acyl Glucuronide, and impurity 2 was attained on Inertsil (250 mm × 4.6 mm, 5 µm) ODS 3V column as stationary phase, methanol, ammonium formate (20 mM) and triethylamine (0.05 %) in 80:15:5 (V/V) at pH 5.5 as mobile phase at 1.0 mL/min flow is 245 nm wavelength. In the finalized method condition, the blank (without analytes) and standard (with analytes) were given in Fig.-2a and 2b respectively.



(A) Blank



(b) Standard

Fig.-2: Chromatograms Obtained for Blank and Standard Solutions in the Developed Method

The limit of detection (LOD) was observed as 0.015, 0.045, and 0.024 µg/mL for Impurity 1, Impurity 1 Acyl Glucuronide, and Impurity 2 respectively. Based on the LOQ, the limit of quantification (LOQ) was calculated and obtained as 0.05, 0.15, and 0.08 µg/mL for Impurity 1, Impurity 1 Acyl Glucuronide, and Impurity 2 respectively. The high LOQ level concentration of impurities i.e 0.15 µg/mL was considered as the initial concentration of construction of the calibration curve. The standard Niraparib solution was taken such that the standard contains 1 % of each impurity. Based on this, the linear calibration curve with a high correlation coefficient was obtained in the concentration range of 15 – 120 µg/mL for Niraparib and 0.15 – 1.20 for all the studied impurities. The regression equation was found to be $y = 10723x + 18928$ ($R^2 = 0.999$), $y = 89385x - 183.2$ ($R^2 = 0.999$), $y = 68818x - 122.9$ and $y = 92512x + 2933$ ($R^2 = 0.999$) for Niraparib, impurity 1, Impurity 1 Acyl Glucuronide, and impurity 2 respectively. Linearity results were given in Table-2.

Table-2: Results Achieved

S. No.	Niraparib		Impurity 1		Impurity 1 Acyl Glucuronide		Impurity 2	
	Con*	Peak Area	Con*	Peak Area	Con*	Peak Area	Con*	Peak Area
1	15	186353	0.15	14282	0.15	10285	0.15	16353
2	30	349251	0.30	26357	0.30	19936	0.30	31164
3	45	495686	0.45	39683	0.45	30595	0.45	45991
4	60	662166	0.60	53485	0.60	41588	0.60	57843
5	75	810025	0.75	66194	0.75	51994	0.75	71517
6	90	965684	0.90	79504	0.90	62340	0.90	85453
7	105	1151951	1.05	93051	1.05	72061	1.05	99547
8	120	1320515	1.20	108654	1.20	81833	1.20	115163

The chromatographic results obtained for analysing the Niraparib standard solution spiked with 1 % of studied impurities were summarized for determining the system suitability of the method. The reproducibility in retention time, relative retention time, theoretical plates, tail (asymmetric) factor, and resolution factor for all the analytes was considered for evaluating the system's suitability. The results were tabulated in Table-3 and based on the results, it was confirmed that the method gives all the system suitability results under the acceptable levels proving that the method obeys system suitability.

Table-3: System Suitability Results

Compound	Concentration in µg/mL	Retention Time (min)#	RRT#	Theo plate	Tail Factor	Resolution
Niraparib	45	8.111±0.019	---	9519	0.92	11.52
	60	8.122±0.039	---	9638	0.92	11.51
	75	8.106±0.025	---	9472	0.91	11.56
Impurity 1	0.45	11.038±0.026	1.361±0.006	14251	0.96	12.68
	0.60	11.033±0.017	1.358±0.007	14332	0.98	12.71
	0.75	11.022±0.025	1.360±0.005	14746	0.97	12.65
Impurity 1 Acyl Glucuronide	0.45	13.564±0.126	1.672±0.018	19365	1.09	8.91
	0.60	13.494±0.010	1.661±0.009	19556	1.08	8.96
	0.75	13.494±0.010	1.665±0.004	19542	1.08	8.94
Impurity 2	0.45	5.200±0.017	0.641±0.003	6258	1.02	---
	0.60	5.233±0.180	0.644±0.019	6419	1.03	---
	0.75	5.144±0.054	0.635±0.006	6326	1.01	---

n=3

The Niraparib at 60 µg/mL concentration solution spiked with 1% of each impurity was analyzed in the developed method to confirm method repeatability (precision) and reproducibility (ruggedness). The % RSD in the peak area response was 0.34, 0.57, 0.81, and 0.48 in intraday precision, 0.44, 0.68, 0.93, and 0.42 in interday precision and 0.87, 0.46, 0.44 and 0.78 in ruggedness respectively for Niraparib, impurity 1, Impurity 1 Acyl and 2. This proves that the method was rugged and precise. The precision level concentration was analyzed by small variations in the established method conditions. The positive and negative change in the solvent ratio of mobile phase, mobile phase pH, and detector wavelength was made in the method conditions and the standard solution was analyzed in each altered condition. The peak area of the individual analyte was compared with the same level standard calibration results and % change in each altered condition was calculated. The % RSD of the peak area response was under permissible levels of less than 2 for Niraparib and its impurities. The system suitability conditions were also evaluated for standard, and its impurities were studied in all the changed conditions results found that there was no considerable change in the results observed (Table-4) confirms that the method is robust.

Table-4: Robustness Results

S. No.	Analyte	Change	Peak Area	% Change	Plate Count	Tail factor	Resolution
1	Niraparib	MP 1	657888	0.65	9641	0.92	11.55
2		MP 2	656637	0.83	9655	0.91	11.47
3		pH 1	657242	0.74	9643	0.90	11.43
4		pH 2	668095	0.90	9644	0.93	11.44
5		WL 1	666499	0.65	9685	0.95	11.45
6		WL 2	667986	0.88	9632	0.91	11.41
7	Impurity 1	MP 1	53161	0.61	14251	0.96	12.75
8		MP 2	53563	0.15	14009	0.97	12.74
9		pH 1	53221	0.49	14323	0.95	12.71
10		pH 2	53935	0.84	14632	0.96	12.77
11		WL 1	53520	0.07	14415	0.95	12.75
12		WL 2	53941	0.85	14752	0.96	8.99
13	Impurity 1 Acyl Glucuronide	MP 1	41212	0.90	19658	1.09	8.91
14		MP 2	41358	0.55	19505	1.08	8.93
15		pH 1	41553	0.08	19332	1.10	8.95
16		pH 2	41439	0.36	19426	1.08	8.92

17		WL 1	41431	0.38	19176	1.09	8.91
18		WL 2	41277	0.75	19410	1.08	8.93
19	Impurity 2	MP 1	57312	0.92	6153	1.03	---
20		MP 2	57526	0.55	6108	1.03	---
21		pH 1	57502	0.59	6113	1.05	---
22		pH 2	57627	0.37	6154	1.04	---
23		WL 1	57583	0.45	6135	1.03	---
24		WL 2	57403	0.76	6239	1.05	---

MP (mobile phase) 1: methanol, ammonium formate (20 mM) and triethylamine (0.05 %) in 75:20:5 (V/V); MP 2: methanol, ammonium formate (20 mM) and triethylamine (0.05 %) in 85:10:5 (V/V); WL (wavelength) 1: 241 nm; WL 2: 251 nm; pH 1: 5.4; pH 2: 5.6

Method accuracy/recovery test was conducted by using spiked recovery and was performed at 50 %, 100 %, and 150 % spiked levels by considering 30 µg/mL of Niraparib and 0.3 µg/mL of impurities as the target. Based on the results as shown in Table-5, it was observed that the results were under the permissible level for standard and both impurities in all spiked levels confirm that the method was accurate.

Table-5: Results Obtained in Recovery

S. No.	Analyte	Recovery Level	Concentration in µg/mL			Amount found* Mean ± SD	% recovered* Mean ± SD	% RSD of Recovery
			Target	Spiked	Final			
1	Niraparib	50 %	30	15	45	44.888±0.020	99.75±0.045	0.05
2		100 %	30	30	60	59.696±0.084	99.49±0.140	0.14
3		150 %	30	45	75	74.603±0.046	99.47±0.061	0.06
4	Impurity 1	50 %	0.3	0.15	0.45	0.447±0.002	99.41±0.339	0.34
5		100 %	0.3	0.3	0.60	0.593±0.002	98.83±0.333	0.34
6		150 %	0.3	0.45	0.75	0.745±0.004	99.29±0.468	0.47
7	Impurity 1 Acyl Glucuronide	50 %	0.3	0.15	0.45	0.446±0.004	99.11±0.801	0.81
8		100 %	0.3	0.3	0.60	0.595±0.003	99.11±0.481	0.49
9		150 %	0.3	0.45	0.75	0.743±0.002	99.07±0.267	0.27
10	Impurity 2	50 %	0.3	0.15	0.45	0.445±0.003	98.96±0.559	0.57
11		100 %	0.3	0.3	0.60	0.593±0.002	98.83±0.333	0.34
12		150 %	0.3	0.45	0.75	0.740±0.003	98.71±0.429	0.43

* n=3

The results of the stress degradation study confirm that peroxide degradation shows high % degradation followed by UV light degradation. In base degradation, the % degradation of Niraparib was 9.84 % and two degradation compounds were separated and retained at a retention time of 6.4 and 9.4 min [Fig.-3A]. In the UV light degradation study, the % degradation of Niraparib was found to be 9.05%. The chromatogram of UV light degradation shows three additional degradation compounds at a retention time of 4.4, 7.5, and 15.1 min [Fig.-3B]. In the acid degradation study, the % degradation was 6.25 % with two degradation compounds identified in the chromatogram [Fig.-3C] at a retention time of 3.3 and 15.9 min. In the peroxide [Fig.-3D] and thermal [Fig.-3E] degradation study, the % degradation was 5.84 and 5.92 % respectively. In both degradation conditions, the degradation compounds were effectively separated, retained, and identified. In all the stress degradation conditions, the studied impurities viz., impurity 1, Impurity 1 Acyl Glucuronide, and impurity 2 were identified and retained the same as the unstressed standard. Based on the results, it was proved that the method can efficiently and adequately suitable for the separation and detection of known impurities and degradants.

The developed method is applied for the estimation of Niraparib, impurity 1, Impurity 1 Acyl Glucuronide, and impurity 2 in the formulation. The formulation assay was calculated to be 98.69 %, 0.24, 0.09, and 0.21 % for Niraparib, impurity 1, Impurity 1 Acyl Glucuronide, and impurity 2 respectively. There are no excipients were detected in the formulation chromatogram [Fig.-4] and the retention time of Niraparib and its impurities studied was found to be very similar to the standard proving that the method is applicable for the routine analysis of Niraparib and its impurities.

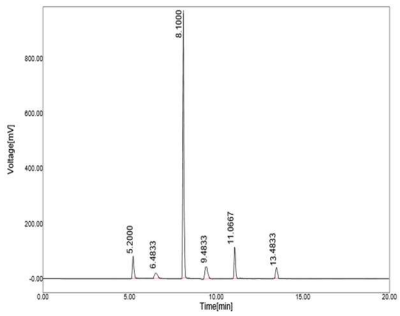


Fig.-3A: Base Degradation

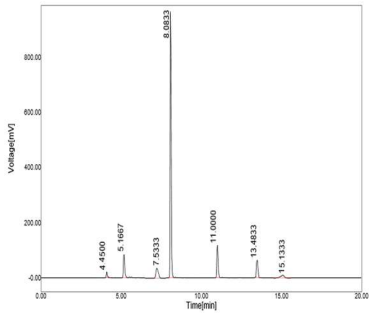


Fig.-3B: UV Light Degradation

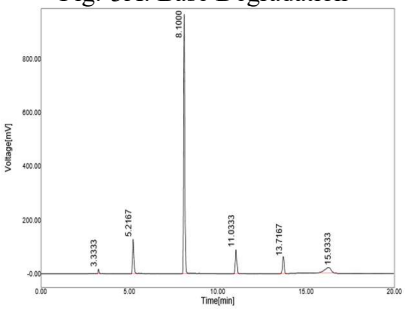


Fig.-3C: Acid Degradation

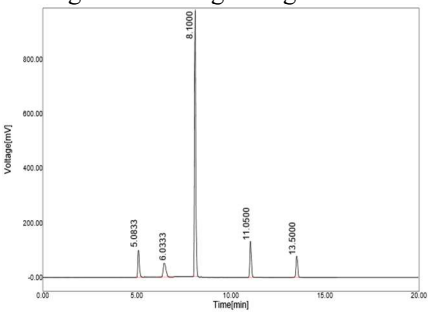


Fig.-3D: Peroxide Degradation

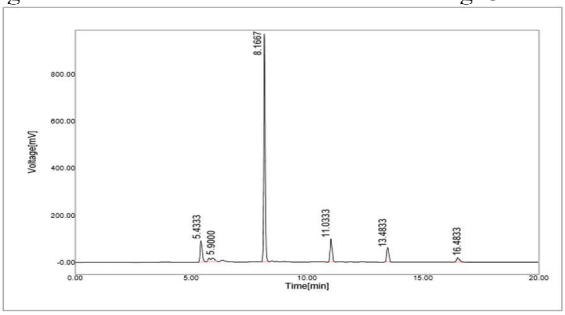


Fig.-3E: Thermal Degradation

Fig.-3: Forced Degradation Chromatograms of Niraparib

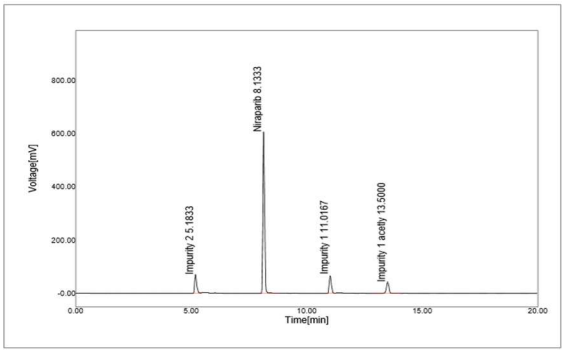


Fig.-4: Formulation Chromatogram of Niraparib

CONCLUSION

The analytical RP-HPLC method satisfies all the method validation parameters such as specificity, system suitability, accuracy, linearity of detector response, precision, ruggedness (change in two different analysts), and robustness (variation in composition, flow rate, and pH of mobile phase). Meanwhile, the method satisfactorily separates the unknown degradation compounds formed during the forced degradation study along with studied known impurities. It confirms that the method is more stable and suitable for the analysis of Niraparib and its impurities. Based on the findings it can be concluded that the

method can effectively be utilized for the assay of Niraparib and its impurities in bulk compounds as well as in dosage forms.

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