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Research Article

Development and Validation of Bioanalytical Method for Estimation of Niraparib in Rat Plasma Using High Performance LC-MS/MS And Its Application to Pharmacokinetic Study

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ABSTRACT

Niraparib, a PARP Inhibitor which is a novel ovarian cancer drug. For applicability to pharmacokinetic study, a LC-MS/MS based method for monitoring plasma levels of niraparib was developed. The analyte and Olaparib(IS) were chromatographed on YMC Pack ODS column C_{18} (50×4.6 mm i.d., 3µ) using MeOH:Ammonium Acetate (2 mM) as binary gradient mobile phase at flow rate 1mL/min with splitter (1:1) over 5 min RT. Detection of analytes were performed on LC-MS/MS system in SRM mode. The method was validated over concentration range of 4.38 -1121.35 ng/mL and lower LOQ was 4.38 ng/mL for the analyte. Recoveries from spiked controls were >83% for the analyte and IS at all QC levels. Within batch and between batch accuracy for Niraparib was found within 94.2 -105.8% and 98.9-102.0%. Within batch and between batch precision for niraparib was found <11.2% CV at all concentration levels. This method was successfully applied to monitor PK profile of niraparib on oral and IV administration to rats.

Introduction

Inflammation In March 2017, the US FDA approved Niraparib for maintenance therapy of recurrent gynecologic cancers (Epithelial ovarian, Primary peritoneal and Fallopian tube carcinomas) which are sensitive to previous platinum-based chemotherapy irrespective of BRCA mutation and HRD status [20,21]. It is the third drug in this class to receive FDA approval, following Olaparib and Rucaparib and is the first global approval for maintenance therapy for cancers. Niraparib preferably blocks PARP 1 and PARP 2 enzymes[22-24]

The objective of method is to develop higher sensitivity of the analyte compared to other reported methods either as a single analyte or with combination in different biological matrices, and also to have short chromatographic run time of 5min per sample which renders the method useful in high-throughput bioanalysis [1-5]

Niraparib has also several important pharmacokinetic features including negligible interaction with food; once daily dosing regimen; less likely to interact with other co administered drugs since it is primarily metabolized by hydrolytic and conjugative pathways, and lower dosage requirement than previously approved PARP inhibitors (olaparib and rucaparib). Chemically, Niraparib is ((S)-2-(4-(piperidin-3-yl) phenyl)-2H-indazole-7-carboxamide ZEJULA), the epical formula is $C_{19}H_{20}N_4O$, and molecular weight of 320.396 g/mol.(Fig.1)[19-29]

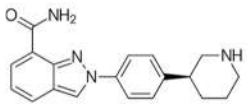

Fig 1. Chemical structure of Niraparib

Materials and Methods

Chemicals: Niraparib (Standard) and Olaparib (Internal Standard) are supplied by Adooq Biosciences, Methanol supplied by RCI Lab scan, India, ACN by Merck Specialties, India, Ammonium Acetate by Sigma

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Table1: Calibration Standard Preparation for Niraparib

Details	WSConc. (ng/mL)	WS Vol taken (µL)	Diluent volume (µL)	Vol made (µL)	Int Conc. (ng/mL)	Int Vol taken (µL)	Final Vol (µL)	Final Conc. (ng/mL)
CC Std-1	160192.31	70	930	1000	11213.462	5	50	1121.35
CC Std-2	11213.46	500	500	1000	5606.731	5	50	560.67
CC Std-3	5606.73	500	500	1000	2803.365	5	50	280.34
CC Std-4	2803.37	500	500	1000	1401.683	5	50	140.17
CC Std-5	1401.68	500	500	1000	700.841	5	50	70.08
CC Std-6	700.84	500	500	1000	350.421	5	50	35.04
CC Std-7	350.42	500	500	1000	175.21	5	50	17.52
CC Std-8	175.21	500	500	1000	87.605	5	50	8.76
CC Std-9	87.61	500	500	1000	43.803	5	50	4.38

Aldrich, India, Water (Milli Q), Matrix by Wistar rat plasma (with K2EDTA as anticoagulant)Blank plasma obtained of Incozen Therapeutics Pvt. Ltd. After IAEC (Institutional Animal Ethics Committee) approval. Experimented in the year 2019.

*Stock solution, calibration standards and quality control samples:*Standard stock solutions of Niraparib and olaparib were prepared in methanol with a final concentration of 1.0 mg/mL. The IS stock solution was diluted to achieve a final concentration of 250 ng/mL in methanol. These solutions were stored at 2-8°C until use. Intermediate calibration standards were prepared in Methanol : Water (80:20) with serial dilutions to be used for preparation of calibration curve standards in rat plasma over 4.38-1121.35 ng/mL range for niraparib**(Table1).**
Intermediate QC standards are prepared separately of calibration standards with serial dilutions to be used for QC standards at four different concentration levels 4.38,12.52, 400.48, 800.96 ng/ml for Niraparib as LLOQ, Low, Middle and High QC respectively(**Table2**).

*SampleProcessing***:**Sample preparation involved protein precipitation extraction method using750µL IS containing methanol.The processing volume of plasma was fixed as 50µL. Samples vortex mixed (Genius 3, IKA Vortex India) prior to the addition of the extraction solvent. After mixing thoroughly with vortex mixer for 3 min and centrifugation (Eppendorf centrifuge 5810 R, Germany) at 14,000 rpm for 10 min, 5µL of the supernatant was injected into the LC–MS/MS system.[6-7]
Instrumentation Operating Conditions:

Liquid chromatography: Estimation of Niraparib in rat plasma is carried out by high performance liquid chromatography(Shimadzu LC Prominence), Analysis is performed using the column of YMC Pack ODS AM 50*4.6*3µ, the mobile phase composed of (A) 2mM Ammonium (B) Acetonitrile, with flow rate of 1.0 mL/min, Splitter of 1:3 (Source: Waste), the method is Gradient**(Table3)**, with the injection volume of 5 µL, Column oven temperature was set to 40°C ± 2°C and the Sample cooler temperature was 15°C ± 2°C. The divert valve is set in place to direct the flow to mass spectrophotometer[5,13-14]

Mass spectrometry: Mass spectrometric detection was performed on Thermo Scientific Quantum Ultra system equipped with a heated ESI probe as ionization source and triple quadrupole analyzer with positive ionization mode. In mass spectrometer, nitrogen gas was used as nebulizing gas, sheath gas and ion sweep gas were constantly supplied from a gas generator (Peak Scientific, USA). Argon gas was used as collision activated dissociation (CAD) gas was constantly supplied from argon gas cylinder. MS and MS/MS condition for pure standards of niraparib and IS were optimized by continuous infusion at 10µl/min using syringe pump (Model '11', Harvard apparatus, Inc., Holliston, MA, USA). MS/MS analysis of all analytes was performed using argon as Collision gas. The most stable product ion of each component was selected to build selective reaction-monitoring (SRM) method. The transitions monitored 321.3 > 304.09 m/z and 435.25> 280.98 for niraparib and IS respectively. Nebulizing gas, sheath gas, ion spray voltage and vaporizer temperature were set to 40, 450, 4500Volt and 150°C respectively. Collision energy was selected 22 and 32 eV for niraparib and olaparib (IS) respectively. Collision gas pressure was kept 1.5 mTorr. Scan type was SRM, the mass spectrometer was operated in unit resolution for both Q1 and Q3 in the SRM mode, with a dwell time of 200 mSec per SRM channel. All data were acquired with auto tuning using Quantum tune software (Thermo Scientific Quantum Ultra Mass spectrometer). SRM pattern and Instrument parameters finalized from these trials are in (**Table 4a & 4b**).[8,9,10,11]

Table3:Gradient table

Time (min)	Buffer(mL)	Acetonitrile(mL)
0.01	80	20
1.2	20	80
3.0	20	80
3.2	80	20
5.0	80	20

*ValidationProcedures***:**
A complete validation of the bioanalytical method for estimation of niraparib in rat plasma and it's pharmacokinetic study was performed according to the US FDA guidelines and included calibration curve, sensitivity, selectivity, linearity, precision and accuracy, recovery, stability of analyte (short term stability, long term stability), stability of analyte in plasma (Bench top stability, Auto sampler stability, Freeze thaw stability, long term stability). Validation was performed on the instruments with the settings as mentioned above.[5,16-18]

Table.2: Quality control Standard Preparation for Niraparib

Details	WS Conc. (ng/mL)	WS Vol taken (µL)	Dilu -ent vol (µL)	Vol made (µL)	Int Conc. (ng/mL)	Int Vol taken (µL)	Final Vol (µL)	Final Conc .(ng/mL)
HQC	160192.31	50	950	1000	8009.62	5	50	800.96
MQC	8009.62	500	500	1000	4004.81	5	50	400.48
LQC	250.3	500	500	1000	125.15	5	50	12.52
LOQQC	125.15	500	500	1000	62.575	5	50	4.38

Table 4a: Compound dependent parameters SRM modes

Analyte	Parent ion(m/z)	Product ion(m/z)
Niraparib	321.23	304.09
IS	435.25	280.98
Tube Lens (V)	109	119
Skimmer offset (V)	10	10
Collision Energy (eV)	22	32

Table 4b: Source Dependent Parameters for Analyte and IS

Parameters	Niraparib	IS
Ionization Mode	Positive	Positive
Spray Voltage(V)	4500	4500
Sheath gas Pressure(psi)	50	50
Auxiliary gas Pressure(psi)	40	40
Temperature (°C)	300°C	300°C
Vaporizer temperature (°C)	150°C	150°C
Dwell time(mSec)	200	200

Results and Discussion

Selectivity: The specificity of the LC-MS/MS method was established by screening the standard blanks of different lots of Wistar rat plasma contain Six different lots of plasma were screened for the specificity experiment. Six out of six batches were of intended anticoagulant (Na2-EDTA) plasma (In house ID: RPL-1,RPL-2,RPL-3, RPL-4,RPL-5 &RPL-6) were found to be free of significant interferences at the retention time of both analytes and IS (i.e. area of the peak at the retention time of both analytes in standard blank samples was ≤ 20.00 % of the area of the drug in the extracted LLOQ sample; area of the peak at the retention time of ISTD in standard blank samples was ≤ 5.00 % of the area of the ISTD in the extracted LLOQ sample). Plasma lots (In house ID: RPL-1, RPL-2, RPL-3, RPL-4, RPL-5 &RPL-6) were pooled and used as a blank matrix to prepare calibration curve standards and quality control samples. Representative chromatograms of Standard Blank and LLOQ standard using pooled plasma are shown in **(Fig 2,3,4 and 5)** respectively. The results are summarized in the **(Table5a & 5b)** for interference at Niraparib and IS respectively.

Table5a: Selectivity Data for Niraparib

Plasma ID	Blank area	Niraparib LOQQC Area	% Interference
RPL-1	1000	12921	7.74
RPL-2	800	10559	7.58
RPL-3	598	9670	6.18
RPL-4	675	9955	6.78
RPL-5	897	14027	6.39
RPL-6	1200	13590	8.83

Table5b: Selectivity Data for IS

Plasma ID	Blank area	IS area	%Interference
RPL-1	1200	2249068	0.05
RPL-2	1456	2287534	0.06
RPL-3	1248	2209505	0.06
RPL-4	1258	2246694	0.06
RPL-5	1567	2322270	0.07
RPL-6	1589	2328793	0.07

Sensitivity: The Sensitivity of the method was evaluated by analyzing 6 LLOQ (Lower Limit of Quantification) at 4.38ng/mL for Niraparib the precision and accuracy for Niraparib at LLOQ level were found to be 9.30 %CV and 95.02 to 92.21 % nominal respectively. Acceptance criteria at least 67% of the sample should be within 80-120% of nominal and precision should be <20% CV. The results are summarized in the **(Table6)**.

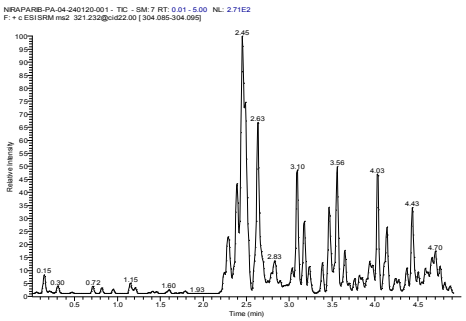


Fig.2 Blank Plasma of Niraparib

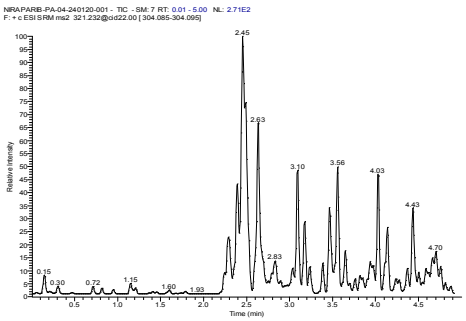


Fig.3 Blank Plasma of IS-Olaparib

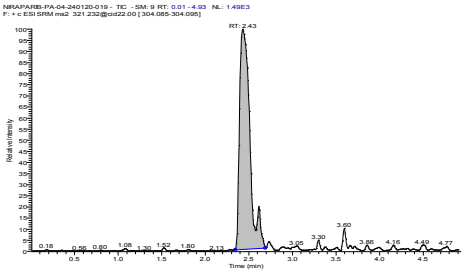


Fig.4 LOQ QC of Niraparib

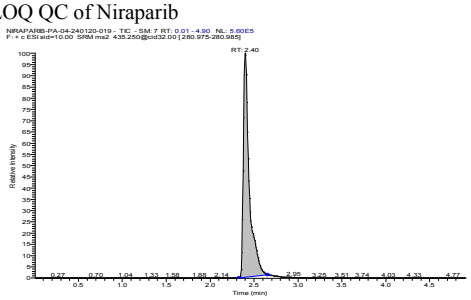
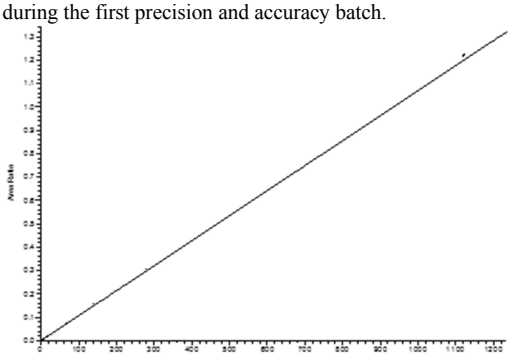


Fig.5 LOQ QC of IS

Table 6: Sensitivity Data

Niraparib Nominal Conc.(ng/ml)	Niraparib Calculated Conc.(ng/mL)	%Nominal Niraparib
4.38	4.85	103.29
4.38	4.63	95.02
4.38	4.09	93.47
4.38	4.04	92.21
4.38	5.01	114.41
4.38	4.85	110.62
Mean	101.50	
STD.Dev	9.44	
%CV	9.30	

*Linearity:*The Linearity of the method was determined by using a 1/x2 weighted least square regression analysis of standard plots associated with a ten-point standard curve. All the four calibration curves analyze during the course of validation were found to be linear for the standards concentration ranging from 4.38-1121.35 ng/mL range for Niraparib. A representative calibration curve is shown in **(Fig 6)**, which is obtained during the first precision and accuracy batch.



(Fig.6 Niraparib Calibration curve)

The average correlation coefficient (r2) was observed to be ≥ 0.99 during validation. Data of calculated calibration standard conc. are shown in (**Table 7a**). Results are summarized in **(Table 7b).**

Table 7a: **Calibration curve (Linearity) data for Niraparib**

Nominal Conc. (ng/ml)	Calculated Conc. Avg. (ng/mL)	S.D.	%CV
4.38	4.41	0.21	4.7
8.76	8.50	0.63	7.4
17.52	16.82	0.93	5.5
35.04	35.52	1.34	3.8
70.08	70.99	1.15	1.6
140.17	142.69	3.87	2.7
280.34	280.21	6.20	2.2
560.67	571.47	14.47	2.5
1121.35	1104.14	35.49	3.2

Precision: The precision of the LC-MS/MS method was evaluated by the % CV at different concentration levels corresponding to LLOQ QC, LQC, MQC and HQC during the course of validation. Representative chromatograms for all QC levels are shown in(**Fig.7 to Fig.11).**

Table 7b: Summary parameters of four different linearities for Niraparib

Linearity	Intercept	Slope	Correlation coefficient (r2value)
1	-0.0018	0.0012	1.000
2	-0.0001	0.0012	0.998
3	-0.0005	0.0011	0.997
Mean	-0.0008	0.0012	0.998

Within Batch Precision: The % CV of back calculated concentrations for all quality control samples of LLOQ QC, LQC, MQC and HQC concentration levels with six replicates for Niraparib. The % CV of back calculated concentrations for all concentration's levels of Niraparib was within 9.3%. Acceptances criteria are that at least 67% of QC samples must be within 15% except LLOQ QC where limit is within20%. The results are shown in (**Table8a)** summarized in the (**Table.8b& 8c).**

Accuracy: The precision of the LC-MS/MS method was evaluated by the %Nominal at different concentration levels corresponding to LLOQ QC, LQC, MQC and HQC during the course of validation. Representative chromatograms for all QC levels are shown in (**Fig.7 to Fig.11).**

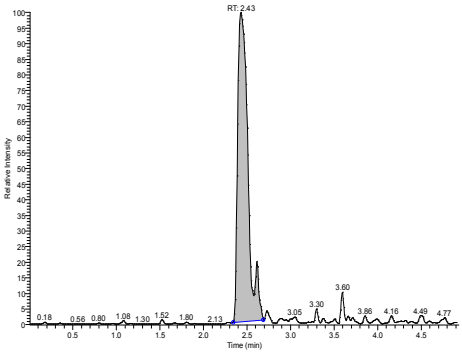


Fig.7 At LLQC level Niraparib

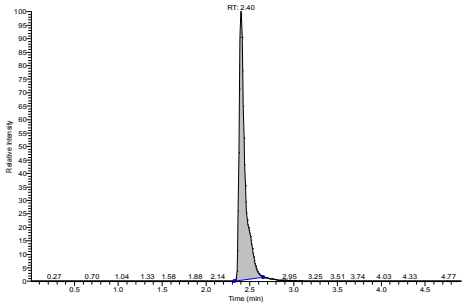


Fig.8 IS(Olaparib)

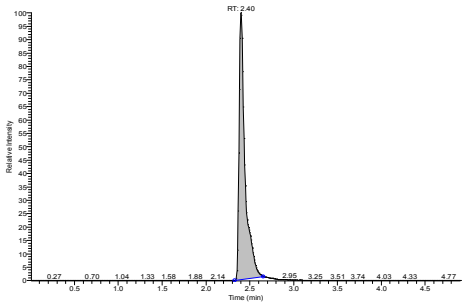


Fig.9 At LQC Level Niraparib

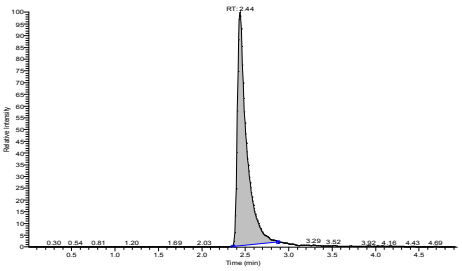


Fig.10 At MQC Level Niraparib

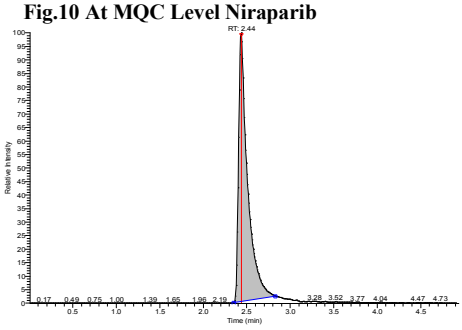


Fig.11 At HQC Level Niraparib

(Representative Chromatograms P&A batches at all QC levels)

Between Batch Precision: The % CV of back calculated concentrations for all quality control samples at LLOQ QC, LQC, MQC and HQC concentration levels from three different bathes of six replicate at each QC levels were found ≤11.2 for Niraparib. Acceptances criteria are that at least 67% of QC samples must be within 15% except LLOQ QC where limit is within 20%. The results of three P&A batches are shown in (**Table 8a**) summarized in the (**Table.8b&8c).**

Within Batch Accuracy: The % Nominal of back calculated concentrations for all quality control samples of LLOQ QC, LQC, MQC and HQC concentration levels with six replicates for Niraparib. The % Nominal of back calculated concentrations for all concentration's levels of Niraparib were within 94.2-105.8% respectively. Acceptances criteria are that at least 67% of QC samples must be within 15% except LLOQ QC where limit is within 20%. The results are shown in (**Table 8a)** and summarized in the **(Table 8b).**

Table8a: Precision and Accuracy Data

	LOQ QC	LQC	MQC	HQC
Nominal conc.(ng/ml)	4.38	12.52	400.48	800.96
P&A 1	5.19	11.40	380.16	765.94
	4.52	12.48	381.74	771.98
	4.16	11.83	369.66	799.91
	4.66	11.99	378.07	749.24
Average	**4.63**	**11.93**	**377.41**	**771.76**
S.D.	**0.4**	**0.45**	**5.38**	**21.08**
%CV	**9.2**	**3.8**	**1.4**	**2.73**
% Average Nominal	**105.8**	**95.3**	**94.2**	**96.4**
P&A 2	5.01	11.32	403.67	819.38
	4.85	11.79	398.50	812.03
	3.62	12.76	403.08	818.03
	4.01	11.78	406.45	830.94
Average	**4.37**	**11.91**	**402.93**	**820.09**
S.D.	**0.67**	**0.61**	**3.30**	**7.90**
%CV	**15.24**	**5.11**	**0.82**	**0.96**
% Average Nominal	**99.8**	**95.1**	**100.6**	**102.4**
P&A 3	4.85	11.91	418.50	808.23
	4.63	12.56	403.61	839.84
	4.09	12.96	397.34	843.53
	4.04	13.03	412.03	841.48
Average	**4.40**	**12.62**	**407.87**	**833.27**
S.D.	**0.40**	**0.51**	**9.30**	**16.76**
%CV	**9.06**	**4.07**	**2.28**	**2.01**
% Average Nominal	**100.5**	**100.8**	**101.8**	**104.0**

Table 8b: Summary of Accuracy

Drug	Conc. (ng/ml)	Within run Accuracy	Between run Accuracy
Niraparib	4.38	105.8	102.0
	12.52	95.3	97.1
	400.48	94.2	98.9
	800.96	96.4	100.9

Table 8c: Summary of precision

Drug	Conc. (ng/ml)	Within run precision	Between run precision
Niraparib	4.38	9.2	11.2
	12.52	3.8	4.3
	400.48	1.4	1.5
	800.96	2.73	1.9

Between Batch Accuracy: The % Nominal of back calculated concentrations for all quality control samples of LLOQ QC, LQC, MQC and HQC concentration levels with six replicates of three different batches for both Niraparib. The % Nominal of back calculated concentrations for all concentration's levels of Niraparib was within 98.9-102.0% respectively. Acceptances criteria are that at least 67% of QC samples must be within 15% except LLOQ QC where limit is within 20%. The results are shown in **(Table 8a)** and summarized in the **(Table 8b&c).**

Recovery: The % mean recoveries were determined by measuring the responses of the extracted plasma quality control samples against unextracted quality control samples at LQC, MQC and HQC levels. The % mean recovery for Niraparib at LQC, MQC and HQC levels were found to be 100.51, 101.14, 98.49% respectively. Overall recovery and % CV at all QC levels was 1.39% respectively, which is within the acceptance limit of 15.0% except for LLOQ QC where limit is 20%. **(Table 9a & 9b).**

Table 9a: Recovery data of Niraparib

Sample Details	Aqu std QC Area	Ext QC Area	% Rec	% Avg Rec	SD	% CV
LQC-1	33636	30950	92.0	100.5	6.05	6.0
LQC-2	33104	33260	100.5			
LQC-3	33338	35205	105.6			
LQC-4	30932	32153	103.9			
MQC-1	1106374	1132784	102.4	101.1	2.50	2.5
MQC-2	1127228	1105091	98.0			
MQC-3	1094824	1098601	100.3			
MQC-4	1102512	1144212	103.8			
HQC-1	2288550	2271410	99.3	98.5	1.86	1.9
HQC-2	2318922	2219590	95.7			
HQC-3	2301113	2282865	99.2			
HQC-4	2266078	2260790	99.8			
Global Recovery						
AvgeRec	100.04					
SD	1.39					
% CV	1.39	**Limit** : Global % CV should be <15%				

Table 9b: Recovery data of IS (Olaparib)

Sample Details	Aqu std QC Area	ExtQC Area	% Rec	% Avg Rec	SD	% CV
LQC-1	2288491	2244681	98.1	96.6	1.80	1.9
LQC-2	2361916	2314236	98.0			
LQC-3	2313210	2261877	97.8			
LQC-4	2307258	2239636	97.1			
MQC-1	2340985	2283813	97.6			
MQC-2	2330861	2256900	96.8			
MQC-3	2309911	2218118	96.0			
MQC-4	2301465	2291044	99.5			
HQC-1	2385295	2255767	94.6			
HQC-2	2370652	2224275	93.8			
HQC-3	2376557	2270880	95.6			
HQC-4	2355717	2213997	94.0			

The % mean recovery for IS(Olaparib) at LQC, MQC and HQC levels were found to be 96.57% respectively. Overall recovery and % CV at all QC levels was 1.9% respectively, which is within the acceptance limit of 15.0% except for LLOQ QC where limit is 20%.

Stability of Analytes (in stock solutions)

Short term stock solution stability of analytes and IS: Short term stock solution stability for the Niraparib at concentration 1000000 ng/mL was determined by using aqueous standard equivalent to concentration of 1000 ng/mL Niraparib, after storage of stock solution over a period of 6 hours minutes at room temperature. Stability was assessed by comparing against the freshly prepared stock concentration of Niraparib at conc. 1000000

ng/ml and prepared aqueous standard equivalent to concentration of 1000 ng/mL. The % mean stability was found to be 97.6% for Niraparib which is within the acceptance limit of 90.00 – 110.00 %. The results are summarized in the **(Table10).**

Short term stock solution stability for the ISTD at concentration (1000µg/mL) was determined by using ISTD dilution concentration 250 ng/mL after storage of ISTD stock solution over a period of 6 at room temperature. Stability was assessed by comparing against the freshly weighed stock concentration (1000 µg/mL) was determined by using ISTD dilution concentration of 250 ng/mL. The % mean stability was found to be 99.4%, which is within the acceptance limit of 90.00 – 110.00 %. The results are summarized in the **(Table10).**

Table10: Short term stock solution stability data

Replicate No.	Fresh stock area	Old Stock area	%Stability	Mean % Stability
Niraparib				
1	1063521	1004523	94.5	97.6
2	1031642	996532	96.6	
3	1012634	1015679	100.3	
4	1032897	998516	96.7	
5	1023654	997934	97.5	
6	1017268	1019486	100.2	
Olaparib (IS)				
1	3501000	3527591	100.8	99.4
2	3496582	3684392	105.4	
3	3597135	3496237	97.2	
4	3674521	3561239	96.9	
5	3527693	3487246	98.9	
6	3694217	3600761	97.5	

Long Term Stock Solution Stability for Analyte and Internal Standard: Long term stock solution stability for Niraparib at concentration 1000000 ng/mL was determined by using aqueous standard equivalent to aqueous HQC concentration of Niraparib, after storage of stock solution over a period of 20 days at 2-8°C.

Stability was assessed by comparing against the freshly prepared stock concentration of Niraparib at conc. 1000000 ng/ml and prepared aqueous standard equivalent to aqueous HQC concentration of Niraparib. The % mean stability was found to be 92.5% for Niraparib which is within the acceptance limit of 90.00 – 110.00 %. The results are summarized in the **(Table11).**

Long term stock solution stability for the ISTD at concentration (1000µg/mL) was determined by using ISTD dilution concentration 250 ng/mL after storage of ISTD stock solution over a period of 20 days at 2-8°C. Stability was assessed by comparing against the freshly weighed stock concentration (1000 µg/mL) was determined by using ISTD dilution concentration of 250 ng/mL. The % mean stability was found to be 96.2%, which is within the acceptance limit of 90.00 – 110.00 %. The results are summarized in the **(Table 11).**

Stability of analyte in plasma:

Bench Top Stability: Bench top stability of the spiked quality control samples was determined for a period of 8 hr. stored at room temperature. Stability was assessed by comparing them against the freshly spiked calibration standards. The % mean stability for LQC & HQC was found to be 93.9 & 95.8 % for Niraparib, which is within the acceptance limit of 85.00 – 115.00 %. The results are summarized in the **(Table 12).**

Autosampler Stability: Autosampler stability of the processed quality control samples was determined for a period of 42 hours by storing them in autosampler maintained at temperature 15°C. Stability was assessed by comparing against the freshly spiked calibration standards. The % mean stability for LQC & HQC was found to be 99.58 & 100.60 % for Niraparib, which is within the acceptance limit of 85.00 – 115.00 %. The results are summarized in the **(Table 13).**

Table 11: Long term stock solution stability data

Replicate No.	Fresh stock area	Old Stock area	%Stability	Mean% stability
NIRAPARIB				
1	1063521	956327	89.9	92.5
2	1038297	953267	91.8	
3	1031642	914756	88.7	
4	1017268	967823	95.1	
5	1013642	965723	95.3	
6	1023698	965214	94.3	
OLAPARIB(IS)				
1	3501000	3485672	99.6	96.2
2	3961724	3298635	83.3	
3	3597351	3546874	98.6	
4	3762541	3564784	94.7	
5	3964852	3928563	99.1	
6	3527693	3586421	101.7	

Table12: Bench Top Stability

QC Sample	Specified Conc.(ng/ml)	Calculated Conc(ng/ml)	% Stability	% Avg Stability
LC-1	12.52	12.03	96.09	93.9
LC-2	12.52	11.76	93.90	
LC-3	12.52	11.186	89.35	
LC-4	12.52	12.036	96.13	
HC-1	800.96	758.902	94.75	95.8
HC-2	800.96	776.997	97.01	
HC-3	800.96	752.013	93.89	
HC-4	800.96	781.106	97.52	
Auto Sampler Stability data				
LC-1	12.52	11.40	91.01	99.58
LC-2	12.52	12.48	99.70	
LC-3	12.52	12.96	103.5	
LC-4	12.52	13.03	104.1	
HC-1	800.96	765.94	95.63	100.60
HC-2	800.96	771.98	96.38	
HC-3	800.96	843.53	105.3	
HC-4	800.96	841.48	105.1	

Freeze Thaw Stability: Freeze thaw stability of the spiked quality control samples was determined after three freeze thaw cycles stored at -80 °C. Stability was assessed by comparing them against the freshly spiked calibration standards. The %mean stability for LQC & HQC was found to be 100.92 & 102.36 % for Niraparib, which is within the acceptance limit of 85.00 – 115.00 %. The results are summarized in the **(Table 14).**

Table 14: Freeze Thaw Stability data

Sample	Specified Conc. (ng/ml)	Calc Conc. (ng/ml)	% Stability	Avg Stability	SD	%CV
LC-1	12.52	11.79	94.17	100.92	3.98	3.9
LC-2	12.52	12.76	101.9			
LC-3	12.52	12.96	103.5			
LC-4	12.52	13.03	104.1			
HC-1	800.96	808.23	100.9	102.36	1.52	1.5
HC-2	800.96	839.84	104.9			
HC-3	800.96	819.38	102.3			
HC-4	800.96	812.03	101.4			

Long Term Stability: Long term stability of the spiked quality control samples was determined after stored at -80 °C for 22 days. Stability was assessed by comparing them against the freshly spiked calibration standards. The %mean stability for LQC & HQC was found to be 95.14 &

102.39 % for Niraparib, which is within the acceptance limit of 85.00 – 115.00 %. The results are summarized in the **(Table15) .**

Table15: Long term Stability data

Sample	Specified Conc. (ng/mL)	Calculated Conc.(ng/mL)	% Stability	Avg Stability
LC-1	12.52	11.32	90.38	95.14
LC-2	12.52	11.79	94.17	
LC-3	12.52	12.76	101.92	
LC-4	12.52	11.78	94.07	
HC-1	800.96	819.38	102.30	102.39
HC-2	800.96	812.03	101.38	
HC-3	800.96	818.03	102.13	
HC-4	800.96	830.94	103.74	

Table16a: Individual and mean plasma concentrations of Niraparib by single dose (10mg/kg) oral administration(Conc.(ng/mL)

Time(h)	Mean	SD	CV%
0	0	0	
0.25	330.99	48.742	14.7
0.5	549.963	10.721	1.9
1	747.517	145.846	19.5
2	697.11	127.247	18.3
4	704.92	274.975	39
6	623.433	242.835	39
8	448.993	176.231	39.3
24	65.79	13.322	20.2

Application to pharmacokinetic study:

The method was successfully applied to generate the plasma concentration niraparib) in oral and IV pharmacokinetic samples. PK study was done on 3 male Wistar rats after IAEC (Institutional animal ethics committee) approval. Oral formulation was prepared in suspension form by triturating accurately weighed amount of niraparib powdered compound in Polysorbate 80 (10% v/v) and 90% methyl cellulose solution (0.5%, w/v water) Suspending agent. IV formulation was prepared with drug dissolved in cosolvents in the following pattern of 10%NMP + 20%PEG200 + 20%PG + 50% (Water: PEG200) was administered by IV route.

Table16b: Individual and mean pharmacokinetic parameters of Niraparib by single dose oral administration

Parameters	Mean	SD	CV%
Cmax (ng/mL)	776.81	180.362	23.2
AUC last (h*ng/mL)	9119.253	3000.751	32.9
AUC Inf-obs(h*ng/mL)	9660.105	3060.857	31.7
Tmax (h)	2.333	1.528	65.5
HL Lambda_ z (h)	5.77	0.533	9.2
Lam-bda_z (1/h)	0.121	0.011	9.3

Oral dose (10 mg/kg) of Niraparib was administered using an oral gavage at 10 mL/kg volume in rats and IV dose(1mg/kg) administered by injecting iv formulation to lateral caudal vein of rat using 1ml syringe, after overnight fasting (12 hrs) and these animals were continued for fasting till 4th hr post dose. The blood samples (0.17 ml) were collected from retro orbital sinus at Predose, 15, 30 min and 1, 2, 4, 6, 8 and 24 hrs post dose of niraparib. After blood collection samples were kept on ice bath till further processing. These samples were separated for plasma by centrifuging at 4°C for 10 min at 4000 rpm and then stored at –80°C till further analysis. These samples were analyzed for estimation of the levels of niraparib. Results for conc. level vs. time profile are tabulated in **(Table16).** Results of pharmacokinetic parameters are summarized in **(Table17)**. **(Fig.12&13)** shows conc. level vs. time profile for Niraparib[20]

Table17a: Individual and mean plasma concentrations of Niraparib by single dose (1 mg/kg) IV administration

Parameters	Mean	SD	CV%
C0(ng/mL)	1132.04	47.003	4.2
Cmax (ng/mL)	822.917	33.335	4.1
AUC last (h*ng/mL)	737.711	64.212	8.7
AUC Inf-obs(h*ng/mL)	801.857	64.406	8
Tmax (h)	0.017	0	0
HL Lambda_ z (h)	7.519	0.749	10
Lam-bda_z (1/h)	0.093	0.009	9.4
Vz_obs(Lkg)	13.594	1.786	13.1
Cl_Obs(mL/min/kg)	20.874	1.658	7.9

Table17b: Individual and mean pharmacokinetic parameters of Niraparib by single dose (1 mg/kg) IV administration (Conc.(ng/mL))

Time(h)	Mean	SD	CV%
0.02	822.917	33.335	4.1
0.08	252.667	16.981	6.7
0.25	163.737	13.175	8
0.5	124.367	9.051	7.3
1	90.53	7.895	8.7
2	63.793	7.301	11.4
4	43.423	4.977	11.5
6	31.757	2.039	6.4
8	24.873	2.992	12
24	5.863	0.871	14.9

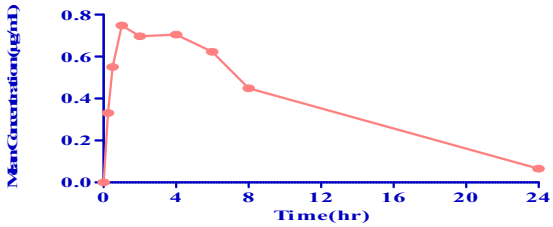


Fig.12: Time Vs Mean Plasma concentration of Niraparib after oral administration of 1 mg/kg

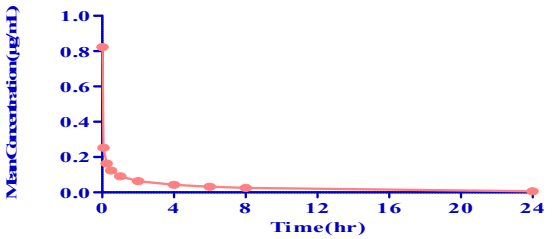


Fig.13: Time Vs Mean Plasma concentration of Niraparib after oral administration of 1 mg/kg

Summary and Future aspects

This method was used to estimate niraparib levels in rat plasma with and the linearity range of 4.38-1121.35 ng/mL. The method is valid as per the USFDA guideline for bioanalytical method validation. Sample processing step involves very simple protein precipitation technique with high recovery for both analyte and IS. Within run and between runs precision is within 9.3 and 11.02% respectively for Niraparib. Within run accuracy and between run accuracy is within 94.2-105.8% and 98.9-102.0% respectively for Niraparib.

Absorption was rapid and with maximum plasma concentrations of 0.78 at 1hr and 0.82 µg/mL at 0.02min after oral and IV administration of niraparib. Absorbed niraparib were eliminated with a half-life of 5.74hr and 7.47hr for oral and IV respectively. Area under plasma curve (AUC0-24) were 9.12 and 0.74 µg.h/mL for oral and IV of niraparib. Plasma

concentrations were observed up to 24.0 hr for niraparib after oral and IV administration.

Conclusion

The developed LC-MS/MS method for the quantitation of niraparib in rat plasma was fully validated as per US-FDA guidelines. The proposed method has a much higher sensitivity for the analyte compared to other reported methods either as a single analyte or with combination in different biological matrices. Efficiency of protein precipitation extraction and chromatographic run time of 5min per sample renders the method useful in high-throughput bioanalysis. Absence of matrix interference is effectively shown by the precision values for the calculated slopes of calibration curves in different plasma sources. The validated method showed acceptable data for all the validation parameters, with adequate sensitivity and selectivity for their simultaneous quantification in a clinical setting.

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Conflict of Interest

The author(s) confirm that this article content has no conflict of interest.

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