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RESEARCH ARTICLE

K_3edta Plasma Samples: Unique Bio-Analytical *Lc-Ms/Ms* Method Development & Validation Of *Niraparib*

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Background: authors want to define the procedure for LC-ESI-MS/MS, a technique utilized in bio-analytical research to establish a simple technique for determining the level of Niraparib in K3EDTA plasma from human beings, is a combination of liquid chromatography, electro spray ionization and mass spectro-photometry. Niraparib in plasma samples has been measured using a sensitive and efficient technique.
Materials and methods: Chromatographic elution was done by using 10mM Ammonium acetate pH-5.0 (A) : acetonitrile (B) in the ratio of 10:90v/v as mobile phase having flow rate of 1.0 milli-liter per minute, Gemini 5µ C18150 x 4.6 mm column. 70% division of flow was used for chromatographic separation of Niraparib with an ISTD as Niraparib-D5.
Results: System Suitability: Area ratio < 2.06%, ISTD RT, and analyte RT% CV ≤ 2.06%. System performance: internal standard residual ≤ 1.07, analyte carryover < 6045, signal to noise ratio ≥ 270.0. Over LOQ & LOQQC 0.0005 µg/ml, LQC 0.00134 µg/mL, MQC 0.02 µg/mL and HQC 0.04 µg/mL, this approach is verified, ULOQ 0.05 µg/mL, and linear concentration range of 0.0005 µg/mL to 0.05 µg/mL with a correlation coefficient (r^2) of ≥ 0.9997. Stability investigations indicated that the developed conduct was suitable for use with K_3EDTA plasma samples when it was validated.
Summary: The *LC-MS/MS* technique that was created to quantify the amount of Niraparib in the biological matrix worked well for routine blood sample analysis from patients for pharmacokinetics research and medication monitoring.

Keywords: Niraparib, Niraparib-D5, LC-ESI-MS/MS, LQC, MQC, HQC, ULOQ, Validation, Stability.

INTRODUCTION:

The Niraparib (Figure 1) Chemical name: ((S)-2-(4-(piperidin-3-yl) phenyl)-2H-indazole-7-Carboxamide . Chemical formula: $C_{19}H_{20}N_4O$ and Molecular weight: 320.396 g/mol [1]. Niraparib (ZEJULA*) preferably blocks PARP 1&2 Enzymes [2-3]. The primary cause of death from gynaecological cancer is ovarian cancer. Germ-line mutations in BRCA 1&2 have been found to be associated with an increased incidence of both familial ovarian cancer & breast cancer. These genes are engaged in the processes that repair DNA damage. Enzymes in the PARP family are part of the base excision repair (BER) system. Synthetic lethality is associated with the use of PARP medicines in individuals with ovarian cancer harboring a BRCA mutation [4-6]. It is an inhibitor of PARP-1&2, two polymerase enzymes involved in DNA repair. Studies conducted in-vitro have shown that Niraparib induced cyto-toxicity may be caused by decreased PARP activity as an enzyme and increased PARP-DNA complex formation, which results in DNA damage, necrosis and cell death [7-9]. Niraparib enhanced cyto-toxicity and decreased tumor growth in both mouse Xeno-graft models of human cancer cell lines with BRCA-1-1/2 defects and human patient-derived xenograft tumour models with homologous stem cells. [10-12]. According to review of literature, only a few *LC-MS/MS* methods for estimating Niraparib in rat & human plasma in integrated forms and individual have been published [13–14].We found that the published techniques had numerous issues with stability and reproducibility for long-term analysis. The goal of the approach is to improve an analyte's sensitivity in comparison to previously published methods, either as a single analyte or in combination with other analytes in various biological matrices. It also aims to have a short chromatographic run time of just over five minutes per sample, making the method applicable to high-throughput bioanalysis.

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Nevertheless, analysts may find it challenging to completely understand the potential medications due to the complexities of clinical drug combinations. A patient's medication history is frequently not fully documented, and the hospital medical records of 61% of patients contain one or more unregistered prescriptions. Combinations of drugs are also frequent. It would be difficult to find signal suppression by co-elution if the combination medication is co-eluted with the analytes in the biological analysis and is not included in the drug history. [15-20]

The current study aims to develop and evaluate a bio-analytical method for *LC-ESI-MS/MS*-based Niraparib estimation in K3EDTA human plasma samples. According to US-FDA criteria, compare with the corresponding internal standard as deuterated Niraparib (Niraparib-D5) (Figure: 1). Additionally a straightforward extraction procedure that is extremely sensitive and linear with minimal plasma consumption needs to be devised.

MATERIALS AND METHODS

Chemicals and reagents

Niraparib standard drug (STD), Niraparib-D5 internal standard (ISTD), Acetonitrile, Methanol, Ethyl-acetate (HPLC grade). Ammonium-formate, Ammonia Solution and Ammonium-acetate (AR grade). Glacial-acetic acid (LR grade), Water (Milli-Q/HPLC grade). Human plasma having K_3EDTA as an anti-coagulant. Other chemicals consumed for this study were AR grade and solvents were HPLC grade.

Instrumentation

The HPLC system used was Shimadzu LC-20 Series. An API 3000 triple quadruple instrument (MDS Sciex) was used for ESI (turbo Ion Spray) mass spectrometric detection employing multiple reaction mode (MRM). It made use of a Turbo Ion Spray interface operating in positive ionization mode. The Watson LIMS Version 7.3 package (SCIEX) and Analyst version 1.4.2 were used for data processing. Niraparib and Niraparib-D5 optimized chromatogra phic conditions and mass parameters have been provided in (Table 1a, b, c).

Preparation of Solution:

Preparation of standard Drug (STD) Stock Solution (1mg/mL): To get the final concentration of Niraparib corresponding to 1 mg/mL, precisely weigh the Niraparib standard, which is equivalent to 2 mg of Niraparib. Then, add the required volume of methanol. Adjust the final Niraparib concentration based on its potency and the actual weight, then store it in the refrigerator at 50±30C. After the preparation date, use this solution within seven days.

Preparation of internal Standard (ISTD) Stock Solution (50µg/mL): To get the final concentration of Niraparib-D5 equivalent to 50µg/mL, accurately weigh the Niraparib-D5 standard, which is equivalent to 2mg of Niraparib-D5. Then, add the required volume of methanol. Adjust the final Niraparib-D5 concentration based on its potency and the actual weight, and then store it in the refrigerator at 5±30. After the preparation date, use this solution within seven days.

Sample Preparation:

a) Extracted Sample Preparation: Take out the necessary quantity of spiked or plasma samples from the deep freezer, defrost them either alone or in a room-temperature water bath, and then vortex the tubes to combine them.. Fill the tube with the sample (0.3 mL) before labeling it. To all the samples except STD Blank, add 50µL of ISTD dilution of 30 ng/mL and vortex to combine. To each sample, add 300 micro-liters of 100mM ammonium formate (pH: 7.0), then vortex to combine. Cap the tubes, add 2.5 mL of ethyl acetate, and extract for 15 minutes at 50 rpm on an extractor. For five minutes, centrifuge the samples at 4000 rpm and 10±2°C. Empty approximately 2.0 mL of supernatant into tubes that have already been labeled, and then evaporate the samples under nitrogen at a temperature of around 40₱5°C until they are completely dry. Mix the dried samples by vortexing them with 100 micro-liters of Mobile Phase (10mM Ammonium acetate with pH 5.0 and Aceto-nitrile in the ratio of 10:90 v/v). Fill vials labeled as pre-sampler samples, place them in the auto sampler, and inject the samples into the LC-ESI-MS/MS.

b) Aqueous Sample Preparation: Fill pre-labeled tubes with 440 micro-liters of mobile phase, 500 micro-liters of ISTD dilution of 0.03 µg/mL concentration), 60µL of the appropriate spiking solution, and vortex to combine. Fill pre-labeled Auto sampler vials with the necessary amount of samples, then inject into an LC-ESI-MS/MS instrument.

VALIDATION

The US-FDA guidelines were followed in the full validation of the bio-analytical method for estimating Niraparib in K_3EDTA human plasma and its pharmacokinetic study. This included the plot of calibration curve with linearity concentration values, sensitivity, selectivity, precision & accuracy, recovery, and analyte Stability (short & long - term). Analyte stability in K_3EDTA blood plasma are as auto-sampler, bench-top. Freeze thaw equilibrium. The instruments were calibrated using SOP or the previously specified settings. [21-23]

Selectivity and specificity: To evaluate selectivity, blank plasma samples set of six different batches were compared with an extra lipidimic group and hemolyzed group to check for the interference during the analyte retention time. A blank sample's Niraparib peak area shouldn't be more than 5% of the LOQ of the Niraparib mean peak area. It is recommended that the peak-area for Niraparib-D5 in a blank sample should not exceed 5% of the average peak-area of the Limit of quantification (LOQ) of Niraparib-D5.

Precision and accuracy: Replica analysis of Quality-control samples (n=6) at LQC, HQC and MQC were used to determine the precision & accuracy levels. The percentage coefficient of variation should be less than 15%. Furthermore, accuracy ought to be within 15%, except for Lower limit of quantification (LLOQ) when accuracy ought to be within 20%.

Matrix effect: The matrix effect arising from plasma was used to assess the improvement of ion suppression in a signal by comparing the absolute response of the QC samples during pretreatment (liquid-liquid extraction with diethyl ether) with that of the reconstituted samples. For MQC-level experiments, six distinct batches of plasma were used in duplicate. The precision (%CV) was maintained at less than 15%.

Recovery: Six duplicates of each Quality-control concentration levels for Niraparib and one concentration for Niraparib-D5 were analysed in order to evaluate the extraction efficiencies of Niraparib and Niraparib-D5. Comparing the peak-area of the extracted & non-extracted standards (spiked into mobile phase), the percent recovery was calculated.

Limit of Detection and Quantification (LOD & LOQ): The lowest concentration in a sample can be identified without measurement, above background S/N (signal to noise) ratio is referred to as the "limit of detection" (LOD). It was calculated by comparing test results from samples with known analyte concentrations with blank samples using a signal-to-noise ratio (S/N) of 3:1. The limit of quantification (LOQ) is the lowest analyte concentration that can be found with a reasonable level of accuracy and precision. By examining a set of mobile phase and plasma standards with a known concentration of Niraparib, the LOQ was discovered.

Ruggedness: The degree of repeatability of test findings achieved using various standard test settings, including but not limited to different labs, analysts, instruments, lots of reagents, elapsed periods, temperatures, days, etc. 0.98 should be the regression (r2 value).With the exception of the LLOQ standard, all CC standards should have an accuracy percentage between 85.00 and 115.00%.The LLOQ standard's accuracy percentage should fall between 80.00 and 120.00%. The acceptance criteria should be met by at least 75% of the calibration curve standards, including at least one LLOQ and one ULOQ.

Concomitant medication: The combination medications are especially prone to co-elution in a short analysis period when numerous compounds are detected simultaneously, potentially doubling the validation effort. It is indisputable that a tactic for the detected concurrent medicine that may cause interference is the application of standards for validation. The LQC and HQC samples' accuracy should be within ±15.000 percent. Not less than 50% of QC samples should be at the same level as at least 67% of the samples (4 out of 6) should meet the previously stated acceptance requirements.

STABILITY: (Bench top, Auto sampler, Freeze-thaw, Short term, Long term, Wet & Dry extract, re-injection, Ruggedness)

Stock solution stability: Standard and internal standard area responses for stability preparations were compared to determine stability in stock solution. Using the sample's area response made from a solutions prepared at that time.

Stability studies in plasma: Six repetitions were used for each concentration level to test the stability of plasma samples at the LQC, MQC, and HQC levels. According to US-FDA criteria, the standard preparation was considered as stable, if the percentage change should be less than 15%.

Bench top stability: The bench-top stability for low and high quality control sample concentrations. The mean accuracy for the back-calculated concentrations of the samples kept on the bench at room temperature (24±4^{0}C) for around 48hrs was compared to the nominal concentration. Within the 15% acceptability level, the precision for low-quality control samples was 1.4%, while for excellent-quality control samples, it was 1.2%. The percentage accuracy for low & high-quality control samples as 95.3% & 93.1% respectively within the permissible scale of 85% – 115%.

Auto -sampler stability: By comparing the extracted plasma samples that were injected immediately (time 0 h) with the samples that were re-injected after storing in the auto-sampler for 38 Hours at 5±30C, the mean accuracy concentrations for the back calculated samples (extended P&A) stored in the auto-sampler minus 20^{o}C was evaluated for 28 hours. As a result, the auto sampler stability of

low- and high-quality control sample concentrations could be established.

Freeze thaw stability: Freeze thaw stability was performed by collates newly spiked quality control samples with stability samples that had been frozen for five (V) Cycles at -20±5^{0}C & -78±8^{0}C and thawed 3 times. Six aliquots of each concentration level—LQC, MQC, and HQC—were used to assess the freeze-thaw stability. Within the 15% acceptance level, the precision values for the control samples with low and high quality were 3.4% and 6.5%, respectively. Within the allowable range of 85–115%, the percentage accuracy for low- and high-quality control samples was 112.5% and 88.8%, respectively. **Stability for Short term stock solution**: The evaluation of Niraparib's stability for short term stock solution involved comparing the average area response from six replicate injections of aqueous ULOQ and LLOQ samples of analyte stock that were stored at room temperature (24±40C) for ninety-two minutes and ninety-eight minutes, respectively, to that of aqueous ULOQ and LLOQ samples of analyte stock that were stored between two and eight degrees Celsius. The ULOQ and LLOQ accuracy percentages were 102.1 and 100.7, respectively, with an acceptability range of 90-110%.

Stability for Long term stock solution: By comparing the average area response from six replicate injections of aqueous ULOQ and LLOQ samples created from analyte stock stored in a refrigerator at 2-8^{0}C for 71 days with that of aqueous samples freshly prepared ULOQ and LLOQ, the stability for long-term stock solution of Niraparib was evaluated. LLOQ and ULOQ's percentage accuracy was 98.1% and 101.8%, respectively, falling within the acceptable range of 90 to 110% (Table No. 4). To evaluate the long-term stock solution stability of the ISTD stock, the mean area response from six replicate injections of the stock dilution, kept in a refrigerator at 2-8^{0}C for seven days and four hours, was compared to a freshly prepared hat of dilution made from fresh ISTD stock.

Dry extract stability: For both low- and high-quality control concentrations, the dry extract stability was assessed by comparing the precision of the mean of the back-calculated concentrations of the samples stored in the refrigerator (2–80C) after evaporating for 21 hours and 42 minutes to the nominal concentration. The acceptable criteria of 15% was not met by the precision values of 1.3% and 1.1%, respectively, for the low-quality control samples. The percentage accuracy for the high- and low-quality control samples was 99.6% and 90.2%, respectively, falling within the acceptability range of 85 to 115%.

RESULTS&DISCUSSION

Method development

In this work, we intend to design and validated a simple, fast, sensitive assay method for the quantitative determination of Niraparib from plasma samples. In clinical pharmacokinetic evaluations, *LC-MS/MS* has been recognised as one of the most successful analytical methods due to its selectivity, sensitivity, and reproducibility. To perform *LC-MS/MS* optimisation for Niraparib & Niraparib-D5 (Figure: 1) solutions were injected into the turbo ion spray ionization of the mass spectrometer. Key parameters including source dependent like curtain gas, temperature, ion spray voltage, nebulizer & collision gas, and other compound dependent like de-clustering, focussing, entrance potential, collision energy, collision cell exit potential and dwell time, modifying both these parameters to obtain a acceptable spray shape and ionisation to generate the relevant output for the elution of Niraparib and Niraparib-D5 molecules. Standard Blank plasma chromatogram of interference free (Figure: 3) and Standard Zero plasma chromatogram of interference free (Figure: 4) are obtained. Chromatographic conditions optimized through numerous trials, the mobile phase composition and column selection were specifically tuned to produce the optimized resolution and boost the standard and internal standard signals. Several techniques for extraction, such as solid-phase extraction, precipitation techniques, liquid-liquid extraction was refined to extract Niraparib and Niraparib-D5 from the plasma sample by using 10mM ammonium formate (pH 7) a satisfactory separation and elution were accomplished. The optimized chromatographic conditions are mobile phase pH 5.0 ammonium acetate : acetonitrile (10:90v/v), 1.0 mL/min flow rate, 70% flow splitting, and 5µL injection volume were utilized. Analytes were extracted from human plasma using liquid-liquid extraction with a 0.3 mL sample processing volume and K_3EDTA used as an anticoagulant. With a total runtime of 3.5 minutes for each, the retention times were optimized to be 2.20 and 2.19 minutes respectively for STD and ISTD. Obtained an optimized chromatograms for LLOQ for Standard (Figure: 5) and for sample (Figure: 6) were noted.

Validation parameters for the developed method

Linearity

The peak area ratio (Niraparib / Niraparib-D5) against (Niraparib) concentration was used to plot the calibration cure. It was discovered that the calibration was linear in the 0.50–50.0 ng/mL concentration range. (Table. 2) & (Figure: 2) with weighting of "1/(x*x)" "linear" regression: y=0.19x+-0.00075 (r=0.9995)

Selectivity
The method's selectivity was evaluated using a comparison of blank plasma chromatograms. For both Niraparib and Niraparib-D5, no notable endogenous peaks were seen at the corresponding retention times. The findings show that the procedure demonstrated good selectivity and specificity. (Figure: 3& 4)

Precision and Accuracy
The precision and accuracy of this approach were controlled by computing the inter-run (n=6) and intra-run (n=25) fluctuations at three spiked concentrations (0.50, 1.340, 20.0, and 40.0 ng/mL) of QC samples were recorded (Table 3). The precision and accuracy within a run ranged from 0.69 to 1.86 and 96.27 to 108.5%, respectively, while the precision and accuracy across runs varied from 2.73 to 3.87. These results demonstrate that the repeatability and reliability of this method are adequate within the analytical range.

Matrix effect
At the MQC level, the ion suppression/ enhancement in the signal was found to be %CV 1.30. These findings suggest that both ion enhancement and ion suppression are unaffected.

Recovery
The recovery percentage for both ISTD and each QC level should be within 15.00%.
Three distinct concentrations of LQC (1.34ng/mL), HQC (20.0ng/mL), and MQC (40.0ng/mL) Found to be 73.21±2.4, 78.84±1.7 and 79.16±1.6 respectively the, were found to be the extraction recoveries of Niraparib. The results showed that the overall average was 96.0±2.8 and 98.76 ± 4.47. Analyte and ISTD recoveries that is reliable, accurate, and consistent.

Ruggedness:
For the toughness batch containing Low quality control (LQC), Middle quality control (MQC) and High quality control (HQC) samples, the intra-batch precision should be within 15.00%, and for the Lower limit of quantification quality control it should be within 20.00%. For LQC, MQC, and HQC samples, the within batch percentage mean accuracy should fall between 85.00 and 115.00% and for LLOQQC it should fall between 80.00 and 120.00%. The study was conducted with three parameter variations such as change of another column, different analyst and different model equipment, the results were found to be within the given limits as per guidelines (Table 4).

Concomitant Drug Experiment
For unpredictable drug combinations such as Acetamin, Cetirizine, Diclofenac, Domperido, Ibuprofen, Omeprazole, and Ranitidine, a more reasonable and reliable correction technique is needed; this study further validated its efficacy in addressing ion interference caused by co-eluting medications. Experiment six out of six human plasma passed for Concomitant Medication (Table 5).

Stability studies: (Freeze-Thaw, Auto-sampler, Room-temperature, long-term)
The stability of Niraparib & Niraparib-D5 stock solutions prepared in methanol and kept in a refrigerator between minus 20 & 75 degrees Celsius was tested using stock solution stability. Niraparib & Niraparib-D5 percentage changes of -0.02% and -0.03% respectively, show that the stock solutions were steady for at least 26 days. At both the LQC and HQC levels, room temperature and auto-sampler stability were examined. The findings showed that at room temperature, Niraparib remained stable in plasma for at least 72 hours. 78 hours in an automated sampler. It was established that the stability of plasma samples spiked with Niraparib at low quality control and high quality control levels were unaffected by five cycles of repeated freezing and thawing.
Long-term stability of stock solutions Drug and ISTD mean stability percentages at room temperature should range from 90.00 to 110.00%, and CV should not exceed ±15.00% (Table 6). The stock solutions that were made recently and those that were made earlier than 38 hours at 5±30C in Mobile Phase were prepared for analysis in order to ensure repeatability of auto sampler re-injection (Table 7). The stability of Freeze Thaw (Table 8), Bench Top (Table 9), Dry Extract (DE), and Wet Extract (DE) indicates that the percentage CV and mean accuracy (%RE) of HQC and LQC samples should be within the limit as per guidelines.

Table 1a: Niraparib and Niraparib-D5 mass parameters optimized.

Quantification using molecular ion to production transitions									
Molecule Name	Ionization molecule (m/z)				Ionization product (m/z)				
Niraparib	868.12[M+H]$^+$				313.20 $^+$				
Niraparib-D5	876.90[M+H]$^+$				316.20$^+$				
	Dependents parameters on source (psi - units**)**				**Dependent parameters on compound (**potential in Volts)				
	Curtain-gas	Collision-gas	Nebulizer-gas	Temp (°C)	Entrance	De-clustering	Collision	Coll. Cell exit	Focusing
Niraparib	12	10	6	300	10	80	30	15	80
Niraparib-D5	12	10	6	300	10	80	30	15	80

Common mass parameters for NIRAPARIB, NIRAPARIB-D5	
Voltage for Ion-spray	3000 volts
Maintained temperature	400°C
Type of scan	Multiple Reaction Mode
Exist-time	200milliseconds
Ionization type	Ion-spray Turbo

Table 1b: Chromatographic conditions

Column	Gemini 5µC18150x4.6mm, 3.5nm 80 Analytical column
Column over temperature	40±3^{0}C
Mobile phase	Composition of A) 10mm Ammonium Acetate pH: 5.0 **:** B) Acetonitrile. In the ratio of 10(A) : 90(B) v/v
Flow-rate	1.0 mL/minute, with flow-splitting as 70%
Injection-volume	5µL
Auto sampler temperature	50±3^{0}C
Detector	Mass detector with MRM and TIS
Run time	3.5 minutes
STD elution time	2.20 minute
ISTD elution time	2.19 minute

Table 1c: Detection Mass Parameters

Mode of synchronization	*LC-MS/MS*
Ion Supply	Ion spray turbo
Type of Scan	Multiple-reaction-mode
Solubility	STD & ISTD polar-soluble
MRM Transition	STD 313.20/256.20 (m/z) [m/z (amu) 321.5 → 195.4 is the choice] ISTD 316.20/256.20 (m/z) [m/z (amu) 325.4 → 195.4 is the choice]

Table 2: Calibration curve details Niraparib**.** (All concentration in ng/mL)

S. No.	**STD-8 0.500**	**STD-7 1.00**	**STD-6 2.13**	**STD-5 3.80**	**STD-4 5.00**	**STD-3 10.00**	**STD-2 25.00**	**STD-1 50.00**
1	0.49	0.997	2.15	3.87	4.86	10.1	24.8	50.7
2	0.515	0.983	2.13	3.81	4.86	9.96	25.7	49.7
3	0.483	1.05	2.17	3.74	4.98	10.1	25.4	50.6
4	0.496	1.03	2.2	3.71	4.8	9.93	24.4	49.3
5	0.525	1	2.11	3.89	5.07	10.2	23.7	50.3
6	0.469	1.03	2.11	3.79	4.82	10.3	25.6	48.7
Mean	0.499	1.01	2.14	3.75	4.96	9.98	25.1	50.4
SD	0.0179	0.0347	0.0471	0.141	0.167	0.197	0.7	0.963
%CV	3.59	3.44	2.2	3.76	3.37	1.97	2.79	1.91

Table 3: Accuracy and Precision Data
(analysis by using three distinct amounts of spiked plasma samples**).**

Spiked plasma concentration (ng/mL)	**Intra-run (n=6)**			**Inter-run (n=25)**		
	Measured concentration (ng/mL) (Mean±S.D)	**Precision %CV**	**Percentage Accuracy**	**Measured concentration (ng/mL) (Mean±S.D)**	**Precision %CV**	**Percentage Accuracy**
0.50	0.492±0.0094	1.86	98.40	0.488±0.0179	3.61	97.60
1.340	1.29±0.00894	0.69	96.27	1.36±0.0447	3.29	101.49
20.0	21.7±0.342	1.58	108.5	21.9±0.597	2.73	109.50
40.0	40.2±0.472	1.17	100.0	43.1±1.67	3.87	107.75

Table 4: Ruggedness data

	Different column				**Different analyst**				**Different equipment**			
S.No	LLOQ 0.500 ng/mL	LQC 1.34 ng/mL	MQC 20.0 ng/mL	HQC 40.0 ng/mL	LLOQ 0.500 ng/mL	LQC 1.34 ng/mL	MQC 20.0 ng/mL	HQC 40.0 ng/mL	LLOQ 0.500 ng/mL	LQC 1.34 ng/mL	MQC 20.0 ng/mL	HQC 40.0 ng/mL
1	0.469	1.34	~23.5	41.1	0.511	1.35	20.7	40.8	0.491	1.38	21.7	41.3
2	0.455	1.34	23	40.8	0.507	1.35	21.2	39.4	0.486	1.34	21.3	41.8
3	0.464	1.34	21.6	40.4	0.501	1.29	20.1	39.7	0.498	1.4	21.4	41.6
4	0.459	1.31	21	39.6	0.496	1.33	20.2	39.7	0.496	1.38	21.3	42.2
5	0.474	1.36	21.4	40	0.517	1.31	20	39.5	0.506	1.41	21.4	40.3
Mean	0.464	1.34	22.1	40.4	0.506	1.33	20.4	39.8	0.495	1.38	21.4	41.4
SD	0.0076	0.0179	1.09	0.602	0.00823	0.0261	0.503	0.563	0.00754	0.0268	0.164	0.716
%CV	1.64	1.34	4.93	1.49	1.63	1.96	2.47	1.41	1.52	1.94	0.77	1.73

Table 5: Concomitant Drug Experiment data

DRUGS	Acetamin	Cetirizine	Diclofenac	Domperido	Ibuprofen	Omeprazole	Ranitidine	STD
LQC (1.34ng/mL)	1.36± 0.0208	1.35± 0.0289	1.36± 0.0361	1.36± 0.0153	1.36± 0.0173	1.34± 0.0231	1.33± 0.0153	1.36± 0.0252
%CV	1.53	2.14	2.65	1.13	1.27	1.72	1.15	1.85
% Accuracy	101.49	100.75	101.49	101.49	101.49	100	99.25	101.49
HQC (40.0 ng/mL)	44± 0252	44.3± 1.41	43.3± 0.115	44± 0.0.88	43.5± 0.666	44.1± 0.153	43.8± 0.819	43.5± 0.216
%CV	0.57	3.18	0.27	1.93	1.53	0.35	1.87	0.5
% Accuracy	110	110.75	108.25	110	108.75	110.25	109.5	108.75

Table 6: Long term Matrix stability

S.No	LQC 1.34ng/mL			MQC 20.0ng/mL	HQC 40.0ng/mL		
	Ambient	(-20±5°C)	(-78±8°C)	Ambient	Ambient	(-20±5°C)	(-78±8°C)
1	1.42	1.24	1.23	20.6	42.1	38.2	39.0
2	1.44	1.23	1.23	20.5	40.9	39.5	38.1
3	1.42	1.31	1.23	20.6	42.7	39.2	36.6
4	1.44	1.25	1.24	20.5	40.9	38.6	37.7
5	1.43	1.24	1.2	20.6	40.8	39.9	37.7
Mean	1.43	1.25	1.23	20.6	41.5	39.1	37.8
SD	0.0141	0.0321	0.0152	0.0707	0.849	0.683	0.864
%CV	0.99	2.57	1.24	0.34	2.05	1.75	2.29
%Accuracy	106.72	93.28	91.79	103	103.75	97.75	94.5

Table 7: Auto sampler re-injection reproducibility (38 hours at 5±3^{0}C in mobile phase)

S.No.	LLOQ QC 0.500ng/mL	% Bias	LQC 1.34ng/mL	% Bias	MQC 20.0ng/mL	% Bias	HQC 40.0ng/mL
1	0.481	-3.8	1.28	-4.48	21.4	7	40.4
2	0.49	-2	1.3	-2.99	22	10	40.5
3	0.489	-2.2	1.29	-3.73	22	10	40.4
4	0.506	1.2	1.3	-2.99	21.9	9.5	40.5
5	0.494	-1.2	1.3	-2.99	21.3	6.5	39.4
Mean	0.492		1.29		21.7		40.2
SD	0.00914		0.00894		0.342		0.472
%CV	1.86		0.69		1.58		1.17
%Accuracy	98.4		96.27		108.5		100.5

Table 8: Freeze thaw Stability of Niraparib in human plasma samples**.**

S. No.	FT-I LQC		FT-V LQC		LQC	MQC	FT-I HQC		FT-V HQC		HQC
	(-20±5°C) 1.34ng/mL	(-78±8°C) 1.34 ng/mL	(-20±5°C) 1.34 ng/mL	(-78±8°C) 1.34 ng/mL	1.34 ng/mL	20.0 ng/mL	(-20±5°C) 40.0ng/mL	(-78±8°C) 40.0ng/mL	(-20±5°C) 40.0 ng/mL	(-78±8°C) 40.0ng/mL	40.0 ng/mL
1	1.45	1.45	1.45	1.37	1.27	21.7	42.3	41.9	42.1	42	45.4
2	1.42	1.4	1.54	1.49	1.36	21.9	42.3	44.2	42.2	42.5	42.1
3	1.43	1.44	1.51	1.39	1.33	21.4	42.3	42.9	42	42.4	44.6
4	1.42	1.49	1.44	1.42	1.31	21.7	41.3	41.8	42.2	43.2	43.2
5	1.41	1.45	1.43	1.43	1.32	21.9	42.3	43.1	43.8	40.9	43.1
Mean	1.43	1.45	1.47	1.42	1.32	21.7	42.1	42.8	42.5	42.2	43.8
S.D.	0.0152	0.0321	0.0483	0.0458	0.0377	0.206	0.447	0.983	0.754	0.846	1.47
% CV	1.06	2.21	3.29	3.23	2.86	0.95	1.06	2.3	1.77	2	3.36
%Accuracy	106.72	108.21	109.7	105.97	98.51	108.5	105.25	107	106.25	105.5	109.5

Table 9: Bench top Stability and Dry & Wet extract stability of Niraparib in human plasma

S.No.	BT LQC	DE LQC	WE LQC	LQC (1.34ng/mL)	MQC 20.0ng/mL	BT HQC	DE HQC	WE HQC	HQC (40.0ng/mL)
1	1.3	1.45	1.27	1.21	21.4	40.4	42.6	41.4	41.5
2	1.32	1.47	1.24	1.21	20.9	40.7	43.3	39.9	40.8
3	1.3	1.32	1.27	1.22	21.1	39	42.2	39.9	41.2
4	1.31	1.33	1.23	1.21	21.2	39.6	43	39.9	40.9
5	1.26	1.3	1.27	1.20	21.2	40.4	42.5	40	41.1
Mean	1.3	1.42	1.26	1.21	21.2	40	42.7	40.2	41.2
S.D.	0.0228	0.103	0.0195	0	0.354	0.701	0.432	0.661	0.495
% CV	1.75	7.25	1.55	0	1.67	1.75	1.01	1.64	1.2
% Accuracy	97.01	105.97	94.03	90.3	106	100	106.75	100.5	103

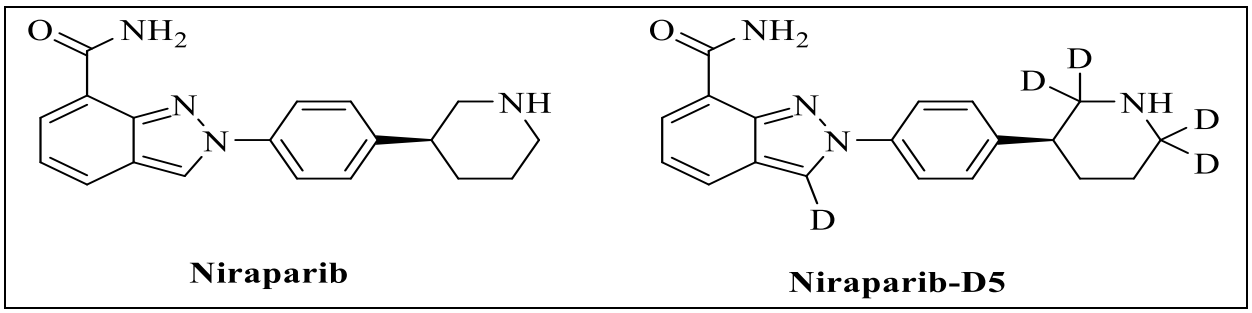

Figure 1: STD (Niraparib) & ISTD (Niraparib-D5)

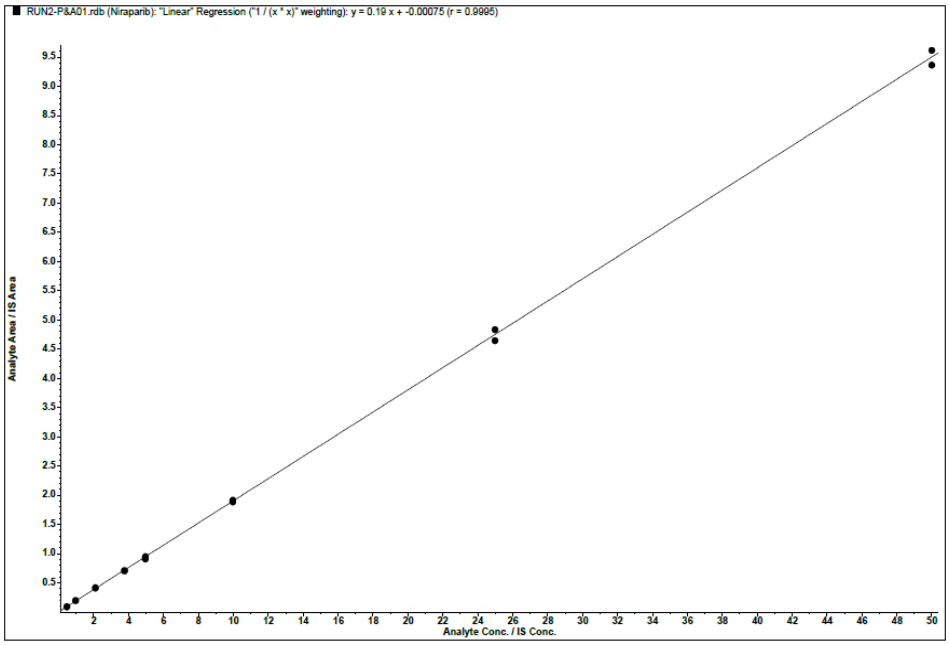

Figure 2: Calibration curve for Niraparib standard.

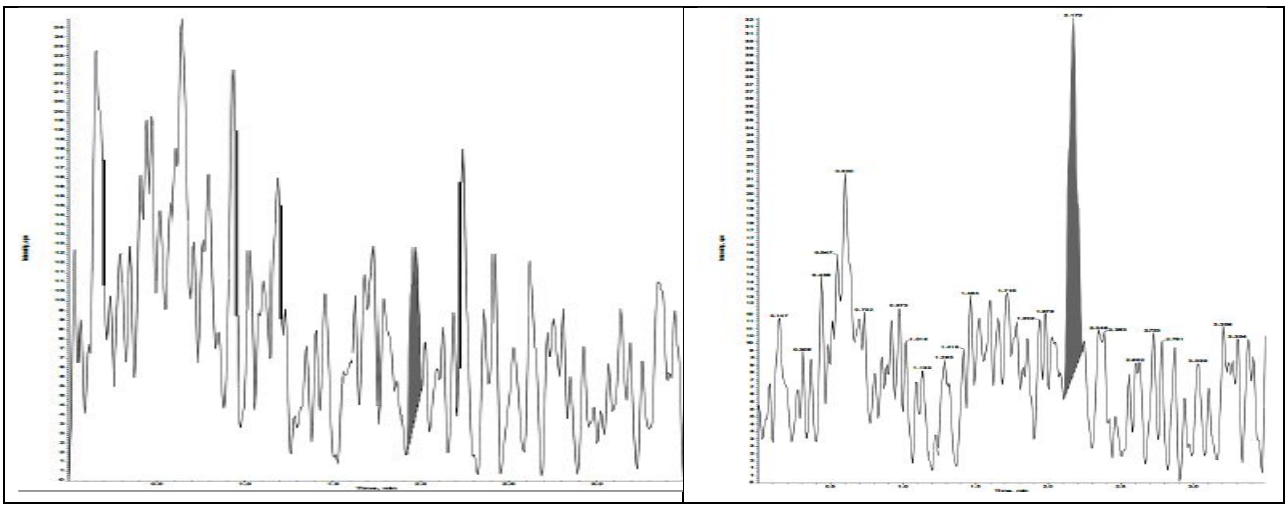

Figure 3: Standard blank plasma chromatogram of interference free, Niraparib&Niraparib-D5.

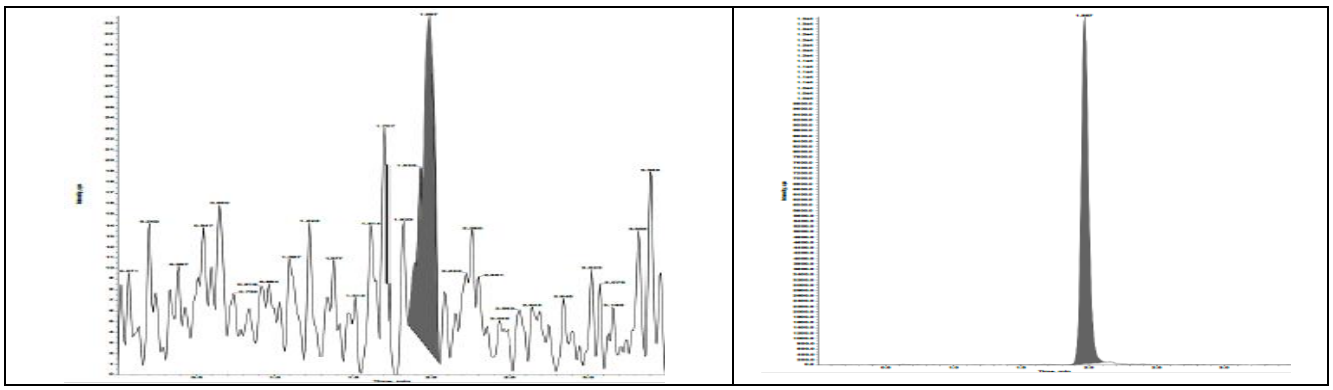

Figure 4: Standard Zero plasma chromatogram of interference free Niraparib&Niraparib–D5.

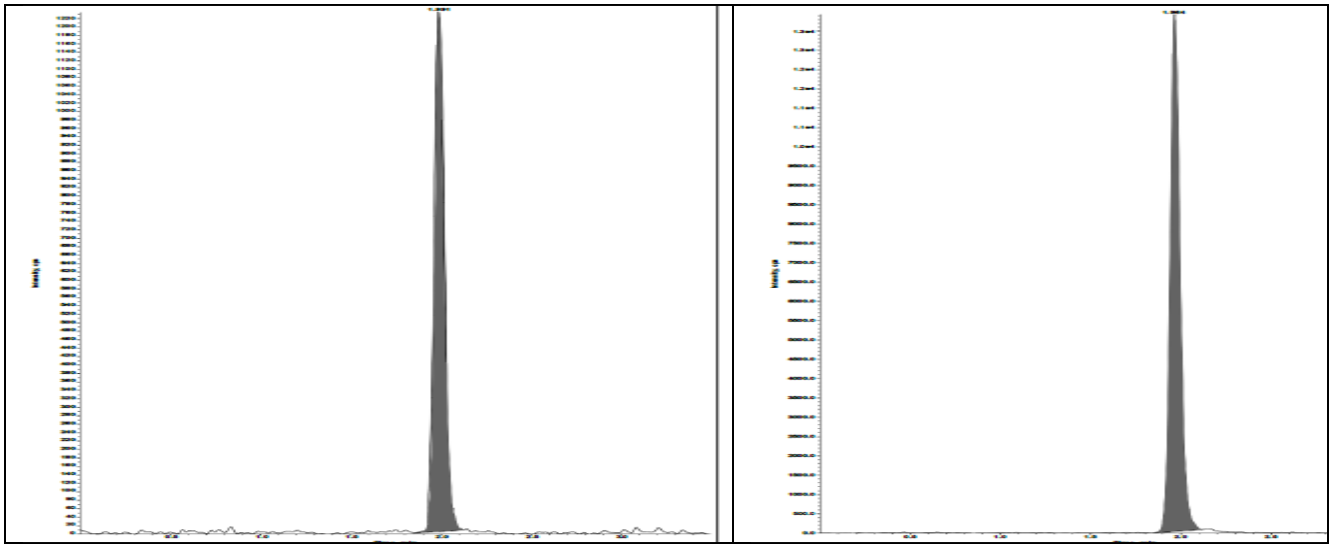

Figure 5: Chromatogram of LLOQ Standard (Niraparib & Niraparib–D5).

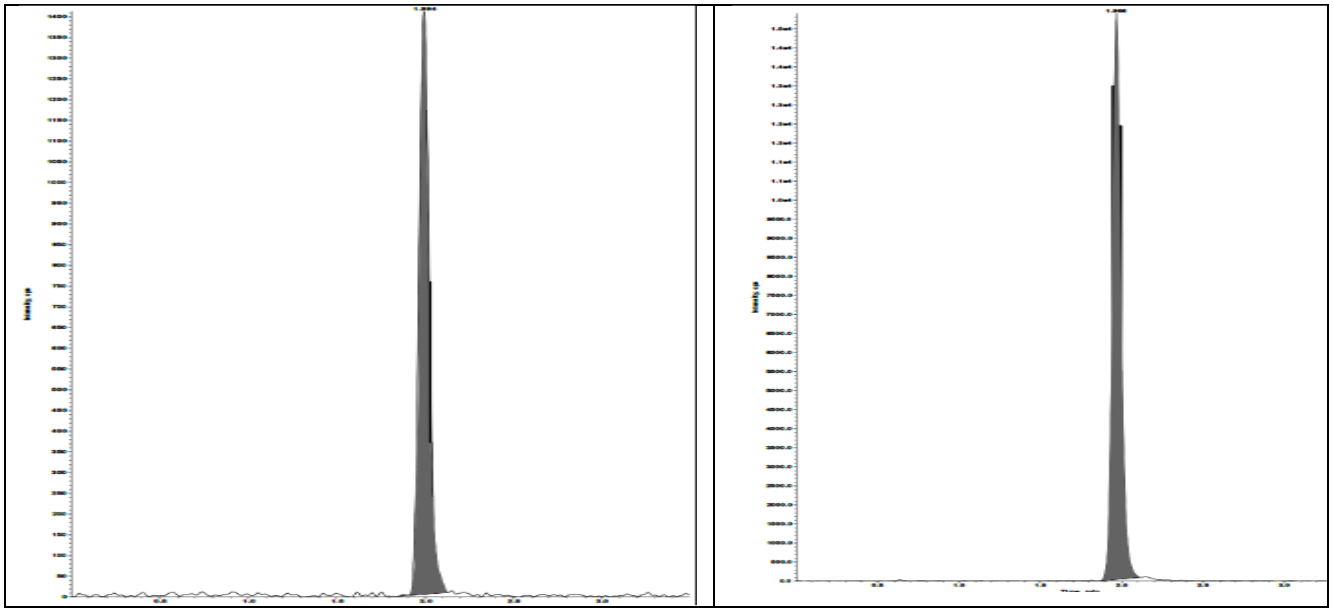

Figure 6: Chromatogram of LLOQ Sample (Niraparib and Niraparib–D5).

CONCLUSION

The proposed study endeavour is quite selective and has various advantages over other previously stated methodologies because of the intrinsic selectivity of Tandem mass spectrometry. The quantification of Niraparib was compared to the internal standard tagged with the relevant isotope. The analyte and ISTD were extracted using liquid-liquid extraction. For analysis, improvements could be made to the injection volume, plasma utilisation volume, flow

rate, column, linearity range, and mobile phase. The assay sensitivity is adequate to precisely track the Niraparib pharmacokinetics. As a result in terms of sensitivity, selectivity, repeatability and linearity, this approach is far superior to previously described approaches.

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