Original Research Article

Quantitative Determination of the PARP Inhibitor Olaparib (AZD 2281) in Rat Plasma using LC-MS/MS: Application to Pharmacokinetic Studies

ABSTRACT

Purpose: Olaparib, a highly selective PARP inhibitor in advanced treatment of ovarian cancer. The method describes a simple, rapid, sensitive, specific LC-ESI-MS/MS assay for the simultaneous detection and accurate measurement of olaparib in rat plasma using telmisartan as an internal standard as per the regulatory guidelines.

Method: Chromatographic separation was carried out on a Liquid Chromatography with tandem mass spectrometry (LC-MS/MS) unit with a Kinetex EVO C18 column (50 × 4.6 mm, 5 µ) using a gradient mobile phase of acetonitrile and 5mM ammonium acetate in water. No endogenous interfering compounds were discovered at the retention time of olaparib (1.66 min) and telmisartan (IS, 1.77 min).

Results: The MS/MS detection was performed in positive mode and MRM transitions were m/z 435.22→366.96 and 515.21→276.16 for olaparib and IS, respectively. This method was assessed to be stable, selective and no matrix effect in three concentrations (4, 500, 800 ng/mL). The intra and inter-day precisions were less than 7.55 % and accuracy ranged from 98.00 % to 106.38 %. The extraction recovery was within acceptable limits. Additionally, the method had good linearity in the range of 1-1000 ng/mL.

Conclusion: The validated method was successfully applied to the pharmacokinetic study of rats through oral and intravenous administration routes.

Keywords: Olaparib, LC-MS/MS, Rat plasma, Pharmacokinetics, Ovarian cancer

Introduction

"Ovarian cancer remains the leading cause of gynecologic cancer mortality". [1]. "The chemical structures of Olaparib and telmisartan (IS) are shown in Fig. 1. In ovarian cancer, poly [adenosine diphosphate (ADP)-ribose] polymerase (PARP) has emerged as a significant target, particularly for women with BRCA gene pathway mutations. Olaparib has shown significantly improved PFS in patients with platinum-sensitive recurrent ovarian cancer, regardless of BRCA mutation" [2, 3]. "Few methods have been described for quantification of olaparib in biological fluids including, HPLC with a diode-array detector" [4]; "Liquid chromatography with tandem mass spectrometry (HPLC-MS/MS)" [5-7]. The previous methods used HPLC coupled with mass spectrometry employed with liquid-liquid extraction for sample preparation [6. 7]. The fluid extraction frequently needs high-virtue organic solvents, is troublesome to handle, and requires an evaporation step. It very well may be a costly and tedious cycle. The plasma samples were prepared by single-step protein precipitation with acetonitrile. In this study, we attempt to develop and validate a sensitive method of the estimation of olaparib in rat plasma. Further studies were also conducted after oral and intravenous administration in rats.

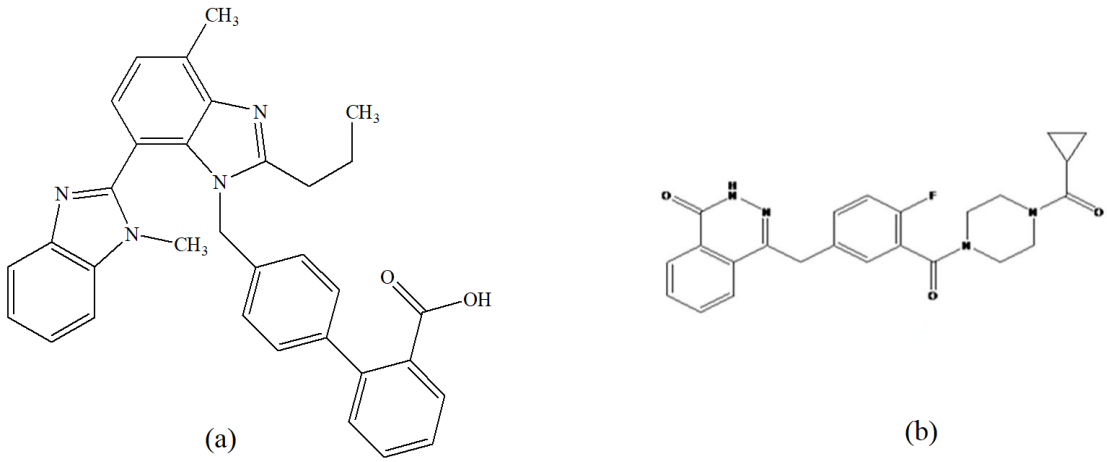

Fig. 1 Chemical structure of telmisartan (IS, a) and olaparib (b).

EXPERIMENTAL METHODS

Chemicals and reagents

Pharmaceutical grade olaparib was purchased from Medkoo Biosciences (Morrisville, USA) 99.0%) and telmisartan, (98.0 %), used as internal standard (IS), was procured from Sigma-

Aldrich (Bangalore, India). Methanol and acetonitrile (HPLC grade) were acquired from Merck Ltd (Mumbai, India). Formic acid and ammonium acetate were acquired from Sigma-Aldrich. Deionized water was prepared through the Milli-Q plus ultra-pure water system (Millipore Corporation, Bedford, MA, USA). Blank rat plasma was collected in our laboratory from male Sprague Dawley rats purchased from Tanconic Laboratories (Vivo Biotech, Hyderabad, India).

Instrument and chromatographic conditions

The LC-MS/MS instrument consisted of an accela pump and auto-sampler and a triple quadrupole mass spectrometer with heated electrospray ionization (Thermo Fisher Scientific, San Jose, CA, USA). The equipment was controlled by Thermo Fisher Xcalibur software (version 2.07). Liquid chromatographic separation was achieved using Kinetex EVO C18 column (50 × 4.6mm, 5µ) (Phenomenex). An injection volume of 10 µL was used for each analysis. Gradient elution consisted of acetonitrile and ammonium formate solution as mobile phase. The flow rate of the mobile phase was set at 1.0 ml/min. The column and autosampler were maintained at 25 °C ± 2 °C and 15 °C ± 2 °C.

Mass spectrometry conditions

Ionization and detection of the analyte and IS were carried out on a triple quadrupole mass spectrometer, equipped with electrospray ionization (ESI) in the positive ion mode using multiple-reaction monitoring (MRM) of transitions of the protonated molecular ions. The MRM transitions were m/z 435.22→366.96 and m/z 515.21→276.16 for olaparib and IS, respectively. The unit resolution was applied to both Q1 and Q3. Dwell time was set at 0.15 s for both analytes. Nitrogen was utilized as the nebulizer, auxiliary, collision, and curtain gas. The source parameters of the mass spectrometer were improved and kept up are electrospray capillary voltage of 5500 V, a source of the temperature of 50 ºC, and a desolvation temperature of 550ºC. The collision activated dissociation (CAD) gas 8, curtain gas (CUR) 30, nebulizer (gas 1), and heater (gas 2) 50. Other optimized compound parameters for monitoring olaparib were set as follows: declustering potential (DP) 80, entrance potential (EP) 10, collision energy (CE) 26, and collision cell exit potential (CXP) 12 V.

Preparation of standard solutions, calibration, and quality control samples

A stock solution of olaparib was prepared in methanol at a concentration of 1.00 mg/mL for subsequent working solution preparation. The working solution of the analyte over the desired

concentration was prepared by further dilution of stock solution with methanol-water (50:50, v/v). A Calibration curve (CC) and quality control (QC) standards were prepared by diluting the individual solutions. All solutions were stored at 4 ºC and placed for 10 min at room temperature before use. The calibration samples were prepared by spiking 2 µL of individual analyte stock solution to 48 µL of blank rat plasma to obtain concentrations of 1, 2, 10, 50, 200, 500, 800, 900, 1000 ng/mL for individual analytes. QC standards were prepared separately by spiking the analyte solution to blank rat plasma to achieve the final concentrations of 4 (low QC), 500 (medium QC), and 800 (high QC) ng/mL. From internal standard stock solution (1 mg/mL) was prepared with acetonitrile and diluted in acetonitrile: water (50:50, v/v) to give a final concentration of 200 ng/mL and stored at 2-8ºC in the refrigerator. All plasma samples were stored in a -20 ºC until use.

Sample preparation

To induce the precipitation of plasma proteins, rat plasma samples were thawed at room temperature for approximately 20 min and then vortexed for 10 s. 50 µL of plasma sample and 10 µL of the IS working solution (200 ng/mL) were transferred into 1.5 mL tubes, and then a 200 µL of precipitant consisting of 70:30 acetonitrile and methanol (v/v) was added. The resulting mixture was mixed vigorously for 10 min and then centrifuged at 13000 rpm for 15 min, at 20 ºC. Finally, a sample of the supernatant (50 µL) separated and placed in an auto-sampler vial and an aliquot (15 µL) of this solution was injected directly into the LC-MS/MS system. Serial standard and quality control samples (QC samples) were prepared following the method described above.

Method validation

The developed method was validated with respect to selectivity, linearity, the lower limit of detection, matrix effect, precision and accuracy, recovery, and stability according to the acceptance criteria of the Food and Drug Administration Guidance for bioanalytical method validation [8-11]. The stability test of room temperature stability as bench-top study, post-preparative stability, freeze-thaw stability was evaluated. Bench-top stability was performed by placing samples at room temperature for 6 h. post-preparation stability of processed samples was investigated by placing it in an autosampler at 8 °C for 24 h. Freeze (-20°C) and thaw (room temperature) was also evaluated after three cycles for 3 days.

Method application for pharmacokinetic study [12]

Six male Sprague-Dawley rats (240 ± 20 g) were supplied by the Taconic laboratories (Vivo Biotech, Hyderabad, India), and then were randomly divided into two groups (n =3). Animals were maintained in a controlled environment animal facility (22 ± 2 ºC and 55 ± 5 % relative humidity on a 12 h light/12 h dark cycle) for 5 days before the experiment. Before the pharmacokinetic studies, the rats have fasted overnight with free access to water ad libitum. Blood samples (0.3 mL) were collected into heparinized microcentrifuge tubes from the fossa orbitalis vein at 0.25, 0.5, 1, 2, 4, 8, and 24 h after a single oral administration of olaparib (5 mg/kg in an oral suspending vehicle). Similarly, after intravenous administration (1 mg/kg in saline), blood samples were collected at 0.08, 0.25, 0.5, 1, 2, 4, 8 and 24 h. The samples were centrifuged at 10000 rpm for 5 min immediately after collection to afford the plasma. All the samples were stored at −20 ºC until analysis. The pharmacokinetic parameters were calculated with Phoenix Win Nonlin version 6.3.0.395 using a non-compartment model.

Results

In this study, liquid chromatography with tandem mass spectrometer was selected to quantify olaparib in rat plasma. To optimize the LC conditions, different mobile phases and ionizing agents including methanol, acetonitrile, formic acid, and ammonium acetate were used. Initially, olaparib was eluted in a solvent consisting of acetonitrile and water (30:70, v/v). However, poor linearity was observed in the concentration range of 1-1000 ng/mL when analyzing the working solutions, which may be attributed to strong peptides adsorption. Then the elution strength of the solvent was changed to acetonitrile and 5 mM ammonium acetate in water [13]. The results showed that the linearity of the working solutions in the range of 1-1000 ng/mL was good. Therefore, peptides adsorption is directly related to the elution strength of the solvent, and the presence of ammonium ions is beneficial for the stabilization of the peptides and proteins. Different types of columns including C8 and C18 reverse phase columns, i.e., X-bridge column, Zorbax Eclipse plus C8 column (Agilent, 100 × 4.6 mm, 5 µ) showed a poor peak shape and tailing. Based on the shape of the peak and signal response in MS, acetonitrile (A) and 5 mM ammonium acetate in water (B), in contrast, Kinetex EVO C18 column (50 × 4.6mm, 5µ) demonstrated good resolution, symmetrical peaks, and less co-elution between target compounds due to its effectiveness in polarity and aromatic selectivity. Gradient elution was established based on the shape of the olaparib peak to increase the throughput of the method. In addition,

both positive and negative scan modes were tested. The results showed that the positive scan mode was more sensitive. Using ESI, both olaparib and IS were successfully ionized and produced predominantly protonated precursor molecule (MH^+) at m/z 435.22 and 515.21. In the MS/MS compound optimization mode, each precursor ion was fragmented to yield the various product ions (figure 1). Based on the intensity of the product ions, those at m/z 366.96 for olaparib and m/z 276.16 for IS were selected as the main product ions for the purpose of this study. Consequently, the precursor-product ion pairs of m/z 435.22→366.96 for olaparib and m/z 515.21→ 276.16 for IS were selected for multiple reaction monitoring analysis. For precipitating proteins, methanol and acetonitrile were evaluated according to their protein precipitating efficacy. When acetonitrile was used as precipitant, olaparib with plasma proteins resulted in lower recovery. In addition, the acetonitrile and methanol (70:30, v/v) provided sensitivity and higher recovery.

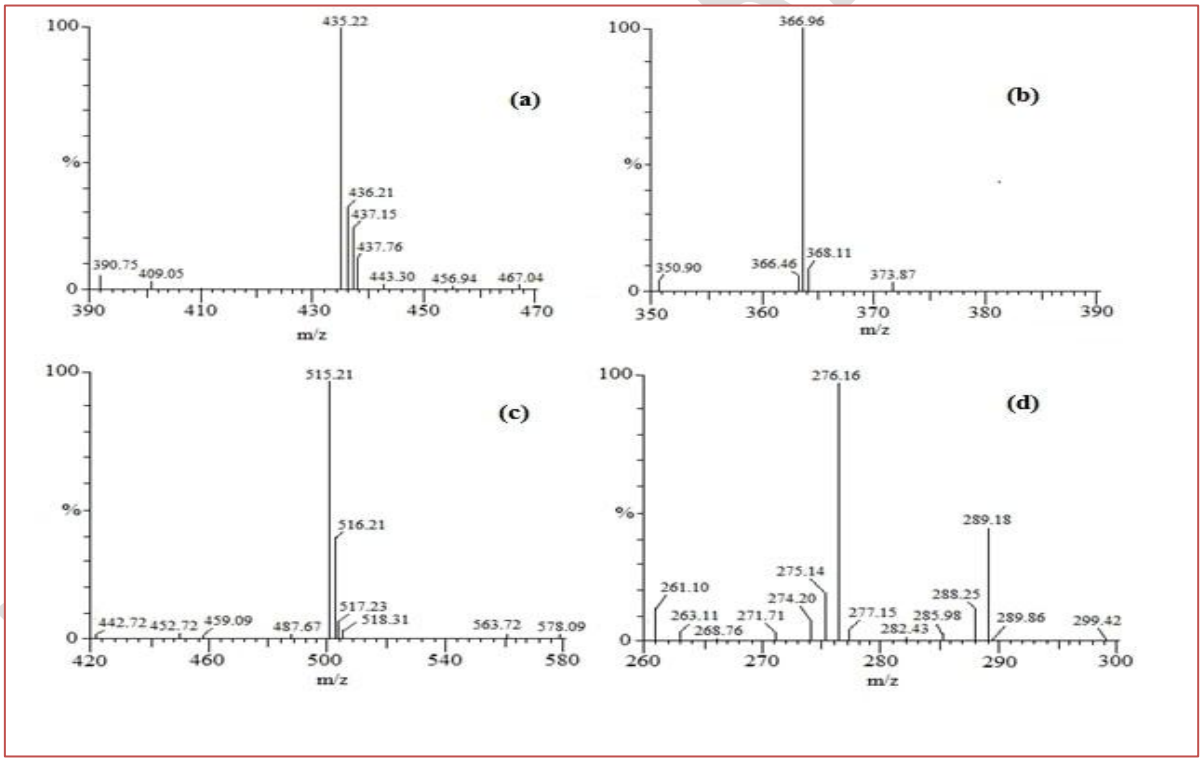

Fig. 2 Parent and daughter mass spectra for olaparib (a and b) and for internal standard (c and d)

Selectivity was assessed by comparing chromatograms of blank plasma from six Sprague-Dawley rats, plasma samples spiked with olaparib and IS, and a plasma sample after

oral administration of olaparib. Fig. 2 shows representative HPLC-MS/MS chromatograms of olaparib and of the internal standard.

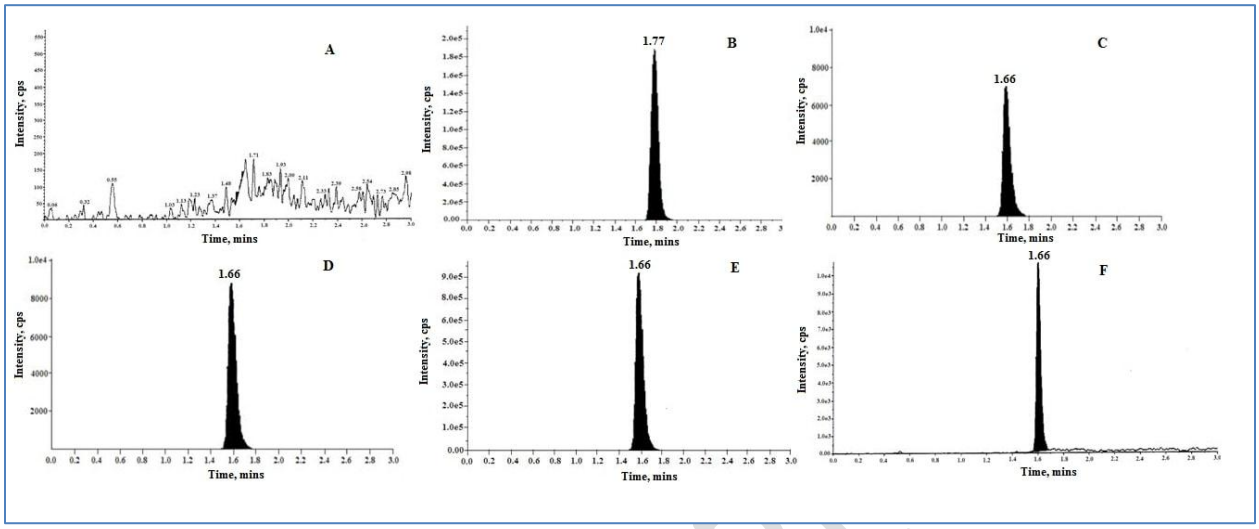

Fig. 3 Representative MRM chromatograms of olaparib and IS in rat plasma. A) Blank plasma; B) Blank plasma spiked with an internal standard, 200 ng/mL; C) Plasma spiked with olaparib at 1.00 ng/mL (LLOQ); D) Low quality control sample E) Upper limit of quantitation F) A rat plasma obtained at 25 mins after IV administration, 1 mg/kg.

Standard curves were established by plotting the ratios of chromatogram peak areas of olaparib to those of the IS. The curves showed a correlation coefficient of 0.9967 and exhibited good linearity over the concentration range of 1-1000 ng/mL. The lowest concentration with the CV < 20 % was taken as LLOQ and found to be 1.00 ng/mL.

Table 1 summarizes the precision and accuracy data for the intra and inter-day assays of the QC samples. The LLOQ was 1 ng/mL (nominal concentration ± 20 % and RSD 7.55 %, n=3). The recovery of olaparib was 96.80 %. The matrix effect of olaparib at three concentration levels of 4, 500 and 800 ng/mL were 101.44 %, 100.0 %, 101.38 % (n = 6), respectively. The variability (% CV) of matrix effect at each concentration level were found to be less than 15 [14].

Table 1: Intra- and Inter batch precision and accuracy for determination of olaparib in rat plasma

Concentration (ng/mL)	**Intra-batch (n = 6)**			**Inter-batch (n = 18)**		
	Measured[a] (ng/mL)	**CV (%)**	**Accuracy (%)**	**Measured[a] (ng/mL)**	**CV (%)**	**Accuracy (%)**
LLOQ (1.00)	0.98 ± 0.074	7.55	98.00	1.05 ± 0.041	3.90	105.00
LQC (4.00)	4.23 ± 0.13	3.07	105.75	4.1 ± 0.2	4.87	102.50
MQC (500)	531.93 ± 9.63	1.81	106.38	508.0 ± 5.6	1.10	101.60
HQC (800)	827.00 ± 11.93	1.44	103.37	826.3 ± 9.1	1.10	103.28

The stability of olaparib was tested at three levels under different conditions. The results (Table 2) showed that the percentage nominal value and relative standard deviation were all within ± 15%.

Table 2: Stability data of the analyte in rat plasma

Stability study	Quality control	Olaparib Measured[a] (ng/mL)	CV (%)	Accuracy (%)
Bench-top[a]	LQC	4.13 ± 0.36	8.71	103.25
	MQC	508.00 ± 29.1	5.72	101.60
	HQC	795.01 ± 64.5	8.11	99.37
Post-preparative [b]	LQC	3.97 ± 0.39	9.79	99.25
	MQC	497.61 ± 50.3	10.12	99.52
	HQC	802.54 ± 39.1	4.87	100.31
Freeze-thaw [c]	LQC	3.86 ± 0.37	9.58	96.5
	MQC	485.19 ± 44.7	9.21	97.03
	HQC	787.36 ± 58.3	7.40	98.42

[a] 6 h, room temperature, *n = 6.*
[b] 24h, 8 º C, *n = 6.*
[c] Three cycles, − 20 ºC/room temperature, *n = 6.*

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Application to pharmacokinetic study

The method was used to determine the pharmacokinetics of olaparib in male Sprague-Dawley rats after IV (1 mg/kg) and oral administration of a 5 mg/kg dose. The mean plasma concentration versus time profile of olaparib is shown in figure 3. The non-compartmental analysis parameters are given in Table 3. Olaparib pharmacokinetic studies in rats provided an overview of the absorption and disposition behavior. It showed moderate oral bioavailability of 28 % [15]. It showed a high volume of distribution indicating that olaparib rapidly distributed to highly perfused tissues.

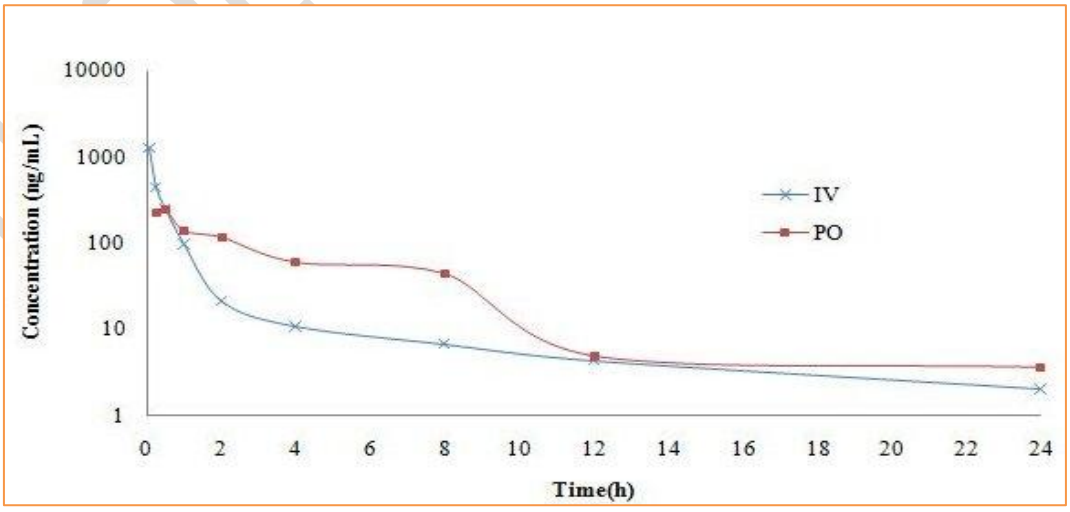

Fig. 4 Mean plasma concentration vs. Time profile of olaparib in rats following intravenous and per oral administration of 1 mg/kg and 5 mg/kg, respectively.

Table 3 Mean pharmaceutical parameters of Olaparib after oral and intravenous administration (Mean ± SD),

Parameters	**Intravenous (1 mg/kg)**	**Oral (5 mg/kg)**
C_{max} (ng/mL)	1288.66 ± 12.013	270.33 ± 38.17
T_{max} (h)	-	0.416 ± 0.144
$T_{1/2}$ (h)	9.433 ± 0.472	-
$AUC_{0\text{-}t}$ (ng/mL.h)	554.00 ± 52.201	807.66 ± 45.654
$AUC_{0\text{-}\infty}$ (ng/mL.h)	581.666 ± 53.454	828.66 ± 39.878
CL (mL/min/kg)	29.000 ± 2.645	-
Ke (1/h)	0.36 ± 1.03	0.308 ± 0.89
$MRT_{0\text{-}t}$ (h)	3.330 ± 0.577	5.033 ± 0.763
Bioavailability (F) %	-	28.33 ± 3.785

Discussion

Compared with the previous methods [6, 7] the protein precipitation extraction used for the treatment of plasma samples in this work was simple and cost-effective. Additionally, to minimize contamination of ion source, the eluent from 0 to 3 min was delivered directly to waste through a divert valve. The plasma calibration curve was constructed using nine calibration standards over the concentration range of 1-1000 ng/mL. The calibration curves were fitted using a weighted ($1/x^2$) least-squares linear regression method by measuring the peak area ratio of the analytes to the IS. The lowest acceptable point of olaparib on the calibration curves was regarded as the lower limit of quantitation (LLOQ). Good linearity was obtained in this concentration range with a correlation coefficient (r) greater than 0.9967. The LLOQ was confirmed to be 1 ng/mL, at which the accuracy was in the range of 92.1-111.4 % and the precision were below 8.0 %. Carry over was investigated by injecting blank samples after higher concentration of calibration curve sample in three analytical runs. No peak was found at the retention time of olaparib and IS in the blank sample, indicating that there is no quantifiable carry over was obtained and the rinsing solution consisting of acetonitrile:isopropanol:water (45:10:45, v/v) cleans the injector appropriately. The values of accuracy and precision of intra and inter-day were found to be within the acceptance criteria of FDA ± 15 %; except ± 20% at LLOQ [16]. These results proposed that the extraction method could provide high extraction efficiency, and there was no significant matrix effect on the method response to analytes.

Conclusion

In conclusion, an accurate, precise, sensitive and rapid HPLC-MS/MS method was developed and validated to quantify olaparib in rat plasma. This method showed high throughput (1.66 min each sample) and good sensitivity with an LLOQ of 1.0 ng/mL. The method was successfully applied to quantify olaparib in pharmacokinetics studies in Sprague-Dawley rats. The absolute bioavailability was calculated and found to be about 28 %. The developed method has advantages of simple preparation procedure and high extraction recovery.

Abbreviations

PARP: Poly (ADP-ribose) polymerase; PFS: Progression-free survival; BRCA: Breast Cancer Gene; LLE: Liquid-liquid extraction; IS: Internal standard; MRM: Multiple reaction monitoring; CAD: collision activated dissociation; ESI: Electrospray ionization; QC: Quality control standards; MRT: Mean residence time; AUC: Area under curve; Ke: Elimination constant; C_{max}: Maximum concentration; T_{max}: Time taken to reach Cmax; $T_{1/2}$: Half-life.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors

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