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METHOD DEVELOPMENT AND VALIDATION USING HPLC-ESI-MS/MS FOR DETERMINING STABILITY OF TALAZOPARIB IN BIOLOGICAL MATRICES

K. Poorna Chandra Rao [*1] and K. R. S. Sambasiva Rao [2]

Jawaharlal Nehru Technological University [1], JNTUK, Kakinada - 533003, Andhra Pradesh, India.
Mizoram Central University [2], Aizawl - 796004, Mizoram, India.

Keywords:

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Correspondence to Author:
K. Poorna Chandra Rao

Research Scholar,
Jawaharlal Nehru Technological University, JNTUK, Kakinada - 533003, Andhra Pradesh, India.

E-mail: poorna7575@gmail.com

ABSTRACT: A highly sensitive, simple, accurate and precise HPLC-ESI-MS/MS method was developed and validated for the quantitative determination of talazoparib in biological matrices. The present study describes the determination of Talazoparib using validated protein precipitation method from human plasma employing Talazoparib 13C D4 as internal standard by electron spray ionization technique. Combination of 0.1% v/v formic acid and methanol (25:75) % v/v was used as mobile phase. Ascentis Express C18 (50 mm × 4.6 mm, 2.7 µm) column was employed for the separation and the run time was 3 minutes. Flow rate was set at 0.6 ml/min and column oven temperature at 40°C for the chromatographic separation method. 10µl injection volume was selected for mass spectrometer to obtain good ionisation. Parent (Q1) and Product (Q3) ion mass transitions for Talazoparib and Talazoparib-13C-D4 were found at m/z 381.5→180.5 and 386.4→180.5 respectively. The linear regression data for the calibration plot showed a good relationship with high correlation coefficient. Correlation coefficient (r^2) value from the standard curve (5-10000 pg/ml) found to be more than 0.9982.

INTRODUCTION: Breast cancer develops from breast tissues and typically starts in the cells that line the ducts. The human genes BRCA1 and BRCA2 create tumour suppressor proteins that aid in DNA repair and so contribute to the integrity of the cells genetic material [1]. Most breast cancer victims are over 50 years old. However, those with a BRCA gene mutation frequently get breast cancer at an earlier age [2-3]. Talazoparib (TZ) (see **Fig. 1A**) is an inhibitor of poly ADP-ribose polymerase (PARP).

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Inhibition of PARP can lead to cancer cell death in cells lacking in other DNA repair pathways, such as those observed with BRCA gene abnormalities, inhibition of PARP can lead to cancer cell death [4-8].

For breast cancer that has spread locally or to other areas of the body containing a germline BRCA mutation, TZ is a novel therapy option. More research is being done to demonstrate how effectively TZ works and that it is safe to use in order to treat patients whose first chemotherapy treatment has failed and whose cancer has spread significantly. A variety of methods were reported on the pharmacokinetics of TZ in human plasma [9-12] and only one method was reported for quantification of TZ by using HPLC [13]. From the literature review it was concluded that there is no method reported for estimation of TZ using deuterated internal standard in biological samples.

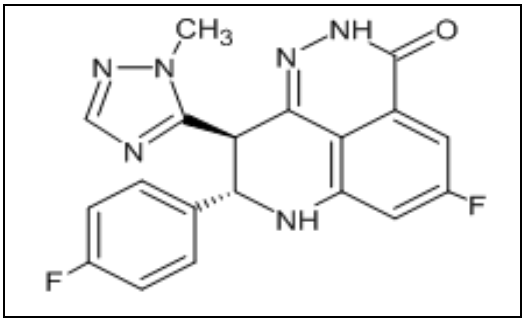

FIG. 1A: TZ

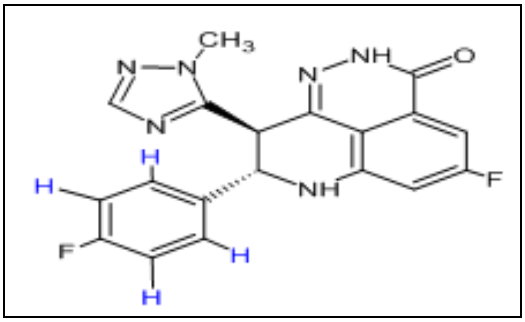

FIG. 1B: TZ 13C D4

The main goal of the present study is to develop and validate the novel simple, sensitive, selective, rapid, rugged and reproducible analytical method for quantitative determination of Talazoparib in human plasma by HPLC-ESI-MS/MS with a small amount of sample volume. In majority of the described methods used for determination of TZ internal standards employed were not deuterated or stable. Hence it is aimed to use a deuterated/stable isotope of the analyte to control the extraction of the analyte, injection volume variability and ionization variability. Deuterated Talazoparib 13C D4 (see **Fig. 1B**) is used as an internal standard in the current work, which has an advantage over the other approaches already described.

MATERIALS & METHODS:

Chemical and Reagents: TZ was obtained from MedKoo Biosciences, USA and TZ 13C D4 obtained from Clearsynth, Mumbai, India.

Chromatographic Parameters: Chromatographic separation with symmetrical peak shape and minimum baseline noise was achieved on Ascentis Express C18 column (50mm x 4.6mm, 2.7µm) with 0.1% v/v formic acid in methanol (25:75) % v/v mobile phase, 0.6 ml/min flow rate, 3 minutes runtime and column oven temperature 40°C. 10µl injection volume was selected for mass spectrometer to obtain good ionisation.

Detection: 10 ng/mL concentration of TZ and TZ 13C D4 solutions have been prepared with methanol and introduced into positive ion mode mass spectrometer at flow rate 5 µL/min. Multiple reaction-monitoring positive ion mode of analysis have been performed with mass transitions of m/z 381.5→180.5 and 386.4→180.5 for TZ and TZ 13C D4 respectively. (See **Fig. 2 & 3**).

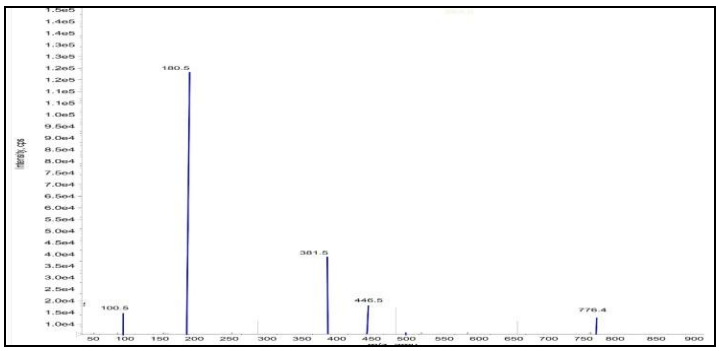

FIG. 2: MASS TRANSITIONS OF TZ PARENT (Q1) AND PRODUCT (Q3) IONS

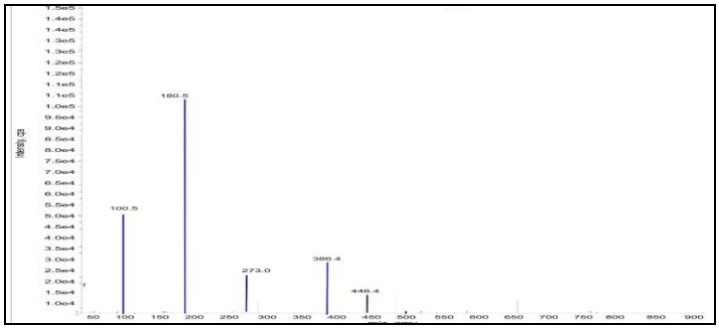

FIG. 3: MASS TRANSITIONS OF TZ 13C D4 PARENT(Q1) AND PRODUCT(Q3) IONS

Preparation of Standard Stock and Quality Control Sample: Standard stock solutions of TZ and TZ-13C-D4 (1000 µg/ml have been prepared with methanol. TZ 13C D4 spiking solution of 500 ng/ml prepared with 75% methanol using TZ 13C D4 stock solution.

All the stock solutions of TZ, TZ 13C D4 and intermediate spiking solutions have been stored at 2-8°C refrigerated conditions till the analysis is completed.

Sample Extraction: Protein precipitation methods have been used to extract TZ as well as TZ 13C D4. 50 µl of TZ 13C D4 500 ng/ml and 100 µl of plasma sample were transferred into each marked polypropylene tube and 0.25 ml of acetonitrile was added before being vortexed for five minutes and further centrifuged at 4000 rpm for 10 minutes at 20°C. Organic phase was then transferred to auto sampler vials containing 100 µl of 0.1% v/v formic acid and introduced into instrument.

Validation:

Linearity: Calibration standards in the range 5–10000 pg/ml were prepared and injected in five replicates on 5 different days.

Selectivity and Specificity: Blank plasma samples of ten batches have been analyzed to select six batches which were interference free, for assessment of selectivity and specificity. The endogenous/potential interfering peak areas for blank samples must be not greater than 20% of the lower limit of quantification (LLOQ) peak area of TZ and not greater than 5% of LLOQ peak area of TZ 13C D4.

Limit of Quantification: 6 LLOQ standards prepared using screened plasma lot were spiked with internal standard 500ng/ml and signal to noise ratio was calculated from analyst software.

Precision: One complete set of calibration standards and another set containing four different quality control (QC) standards (lower limit quality control - 5 pg/ml, Low quality control - 15 pg/ml, Mid quality control- 3000 pg/ml and high quality control - 7000 pg/ml) have been prepared with screened plasma. Each QC standard was injected in 6 replicates on the same day and 5 different days to determine intraday and interday precisions.

Matrix Effect: Blank plasma samples six in number were extracted in three replicates and spiked with un-extracted concentration of mid QC (3000.00 pg/ml) and compared with un-extracted standards of the same concentration.

Recovery: Protein precipitation method was used to recover the samples.The extraction recovery was determined by comparing average of 6 replicate injections of extracted QC standards with un-extracted QC standards at three different concentrations i.e low (15.00 pg/ml), mid (300.00 pg/ml) and high (7000.00 pg/ml).

Stability Studies:

Bench top Stability: *S*ix replicates of samples spiked at low and high concentrations have been maintained at ambient temperature for 24 hr. samples have been analyzed and compared with that of freshly prepared samples of low and high concentrations.

Freeze and thaw Stability: Six replicates of samples of low and high concentrations maintained at frozen temperature -30°C were subjected to three freeze-thaw cycles of 24hr, 36hr and 48 hr (-30°C - room temperature) and compared with that of fresh samples of low and high concentrations.

Autosampler Stability: Six replicates of samples of low and high concentrations were stored for 65 hours at 2-8°C and compared with that of freshly prepared samples of low and high concentrations.

Long-term Stability: Six replicates of samples of low and high concentrations stored at -30°C for a period of 45 days were compared compared with that of freshly prepared samples of low and high concentrations.

RESULTS AND DISCUSSION:

Method Development: HPLC-MS/MS was selected for simple method development and determination of TZ in human plasma. Separation of the TZ was tried with different brands of C_{18} columns in RP-HPLC mode. Isocratic mobile phase with 10mM ammonium formate and acetonitrile was used for Initial separation. Various combinations of above mobile phase were tried which ended with little response. Better responses were obtained with mobile phase 0.1%v/v acetic acid in water: acetonitrile (20:80 % v/v) and 0.1%

v/v acetic acid in water: methanol (20:80 % v/v) but peak shape was found to be poor.

Series of trials were performed with 0.1%v/v formic acid in water with varying quantities of either methanol or acetonitrile. Mobile phase consisting of 0.1%v/v formic acid in water and methanol (25:75) %v/v, produced better signal with peak symmetry. Baseline noise was low for Ascentis Express C_{18} (50 mm × 4.6 mm, 2.7 µm) analytical column at the flow rate 0.6 ml/min. Run time was set at 3 min. Column oven temperature 40°C and auto sampler temperature 4°C were opted to get better results. Injection volume of sample was fixed at 10 µl for better ionization and chromatography. Afatinib dimaleate, Imatinib mesylate and Lenvatinib mesylate were tried as internal standard. But TZ-13C-D4 was selected as internal standard finally due to its better compatability.

RT Values of TZ and TZ-13C-D4 were found to be at 1.42 ± 0.2 min and 1.44 ± 0.2 min respectively. Different extraction procedures were tried, among those Protein precipitation was selected due to ease in extraction, more recovery and low ion suppression effect on drug and internal standard.

Maximum response was obtained in Electro spray ionization (ESI) when compared to atmospheric pressure chemical ionization (APCI) mode. The instrument was optimised to produce high sensitivity and signal stability during infusion of the analyte in the continuous flow of mobile phase to electro spray ion source at a flow rate of 20µl/min. TZ produced more response in positive ion mode rather than negative ion mode.

Source dependent parameters were optimized like nebulizer gas flow 30 psi, CAD gas and curtain gas flow 25 psi, ion spray voltage 5500 V and temperature 500°C to get high intense productions.

Major peaks at m/z 381.5 and 386.4 in the primary ESI spectra correspond to MH^+ ions of TZ and TZ-13C-D4 respectively. Product ions of TZ and TZ-13C-D4 were scanned in quadrupole-3 after colliding with nitrogen in quadrupole-2 which had a m/z of 180.5. The parent and product ions mass spectra of TZ and TZ-13C-D4 -13C-D4 were shown in **Fig. 2** & **3.**

Method Validation:

Specificity and Selectivity, Limit of Quantification: When compared to LLOQ and blank spiked with internal standard, no significant response was observed at retention times of TZ and TZ-13C-D4 in blank plasma. The lowest concentration of the calibration curve i.e, 5.0 pg/ml is the limit of quantification for this method. Representative chromatograms were shown in **Fig. 4.**

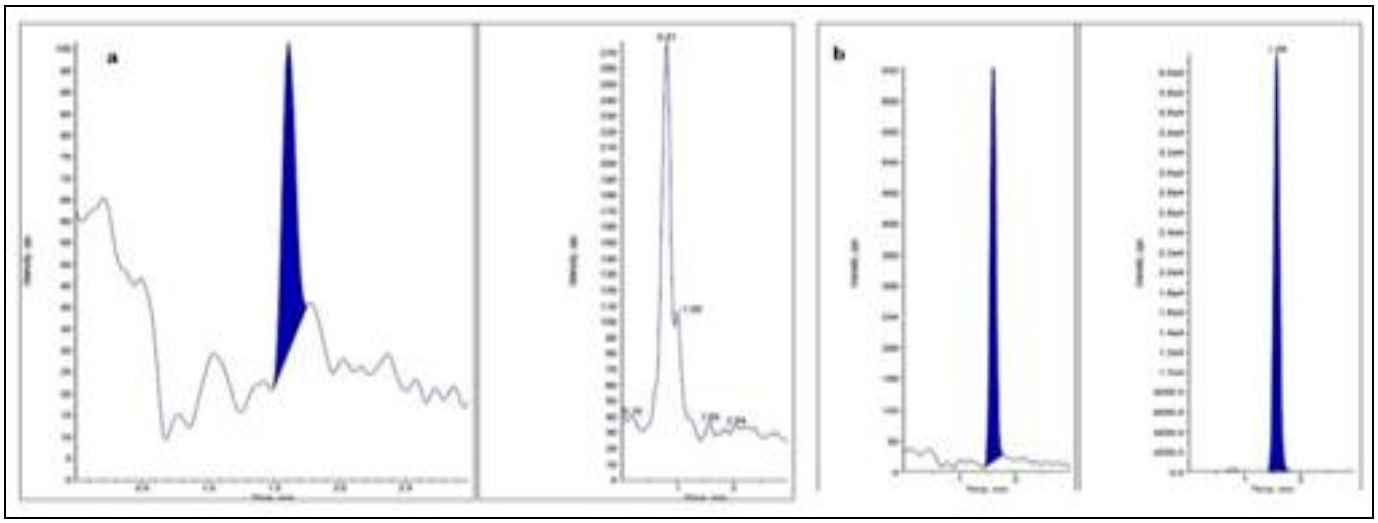

FIG. 4: CHROMATOGRAMS OF TZ IN PLASMA SAMPLES A) BLANK PLASMA CHROMATOGRAM FOR INTERFERENCE FREE TZ & TZ-13C-D4 B) CHROMATOGRAM OF LLOQ SAMPLE (TZ & TZ-13C-D4).

Linearity: Calibration curve was found to be linear for TZ from 5-10000 pg/ml. Correlation coefficient found to be greater than 0.9980 for TZ. % CV was found to be below 15 and mean % accuracy was found to be 99.40 - 102.67. (see **Table 1**).

TABLE 1: TZ CALIBRATION CURVE DATA

Plasma concentration spiked in pg/ml	Concentration measured in pg/ml		Percentage CV (n = 5)	Percentage Accuracy
	Mean	Standard deviation		
5	4.99	0.01	1.4	99.9
10	10.24	0.02	3.6	101.7
50	49.89	0.15	2.7	101.3
100	100.24	0.22	2.5	100.1
500	501.6	0.27	3.8	100.1
1000	1004.22	0.21	2.6	101.7
2000	1999.18	1.02	3.1	99.4
4000	4001.35	1.10	3.4	101.7
6000	6003.76	1.11	1.7	102.6
8000	8001.12	1.96	3.8	101.5
10000	10000.07	1.23	2.5	100.5

Precision & Accuracy: Intra as well as inter-batch % accuracy for TZ have been ranging at 94.16-96.01 and 91.66 to 99.33. % coefficient of variation is 2.16 to 5.65 and 1.65 - 8.00 .

TABLE 2: PRECISION AND ACCURACY (ANALYSIS OF SPIKED SAMPLES AT THREE DIFFERENT ONCENTRATIONS) OF TZ

Plasma concentration spiked in pg/ml	(Intra-day)			(Inter-Day)		
	Concentration measured in pg/ml (mean± standard deviation)	Percentage CV (n=6)	Percentage Accuracy	Concentration measured in pg/ml (mean± standard deviation)	Percentage CV (n=6)	Percentage Accuracy
15.00	14.8±0.07	5.6	98.9	14.9±0.08	3.2	99.93
3000.00	3002.34±1.23	2.1	102.4	2999.78±2.56	1.6	99.45
7000.00	6999.47±2.45	3.7	99.8	7004.33±3.61	2.4	103.45

Recovery: Mean % recoveries obtained for samples at Low QC, Mid QC and High QC levels of TZ were found to be 99.86, 95.31 and 93.55. TZ mean % recovery and % coefficient of variation (CV) for overall quality control levels were found to be 96.22 and 3.39 respectively. The mean % recovery and % CV for TZ 13C D4 were found to be 91.68 and 7.09.

Matrix Effect: Substantial matrix impact was not observed from different sources of human plasma examined for TZ, TZ-13C-D4. The % CV was found to be 3.71.

Stability Studies: Mean % Accuracy values after quantification of the TZ in plasma after subjecting to 3 freeze–thaw cycles was 95.21 & 95.11 for high QC and low QC samples, Autosampler (processed) was 86.67& 93.78, room temperature (Bench top) was 93.02 & 85.91 and for long-term stability was 86.55 & 95.33. All these results were found to be within the specified limits. Mean % Accuracy should be 85-115 % and % CV should ≤ 15%.No significant degradation of the TZ was observed, which confirmed the stability of TZ in human plasma.

TABLE 3: TZ STABILITY STUDIES DATA

Spiked Plasma concentration (pg/ml)	Rt stability		processed sample stability		long term stability		freeze & thaw stability	
	24 hr		65 hr		45 days		Cycle (48 hr)	
	Concentration measured in pg/ml (mean± standard deviation)	Percentage CV (n=6)	Concentration measured in pg/ml (mean± standard deviation)	Percentage CV (n=6)	Concentration measured in pg/ml (mean± standard deviation)	Percentage CV (n=6)	Concentration measured in pg/ml (mean± standard deviation)	Percentage CV (n=6)
15.00	14.9±0.12	7.8	15.5±2.16	5.3	15.2±1.54	8.8	14.8±0.12	5.8
7000.00	7005.3±0.14	8.9	7001.3±1.23	9.5	6999.563±0.12	9.4	7001.4±2.55	2.7

CONCLUSION: Method proposed in this article has been developed and validated in human plasma at a concentration range between from 5-10000 pg/ml. % CV for batch precisions (intra and inter) were found to be below 6. % Accuracy results obtained were ranged from 98.9–102.4. The overall %recovery for TZ and TZ-13C-D4 was greater than 90%.The method developed was quick and simple

to apply for analysis of samples. This method is accurate, precise, selective and also applicable for analysing stability samples. This simple method uses rapid protein precipitation extraction technique with less run time of 3.0 min is recommended for high-throughput analysis of biological samples containing TZ.

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CONFLICT OF INTEREST: Nil

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