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Original Article

Spiked Human Plasma Samples: Stability Indicating Method Development and Validation of Pexidartinib by LCMS

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ABSTRACT

Aim: Pexidartinib (PDTB) was utilized in the development of a novel bioanalytical LCMS method in human plasma, with Talazoparib (TZPB) serving as the IS (Internal standard). **Materials and Methods:** The chromatographic separation was accomplished using an Xbridge C_{18} column (50 mM×4.6 mM, 5 µM) with an Acetonitrile-based simple isocratic mobile phase composition: Throughout the study, 0.6 mL/min of methanaol:0.1% orthophosphoric acid (35:35:30) was flow-regulated. **Results and Discussion:** In the positive ion mode, mass spectra of Pexidartinib (PDTB) and Talazoparib (TZPB) were discovered at m/z 418→153 and m/z 381→108, respectively. The study took 5 min to complete and had a strong linearity in the 0.5-500 ng/mL range with a correlation coefficient (r) of 0.999. The original ranges for the % RSD of the method and the intermediate precision of the PDTB were 0.29-1.13 and 0.13-1.01, respectively. LOD and LOQ were 0.5 µg mL^{-1} and 0.05 µg mL^{-1}, respectively, and more than 90.0% of the samples were recovered. **Conclusion:** The bioanalytical method's validation was conducted in compliance with the ICH recommendations. The proposed LC-MS method has been effectively applied for routine analysis and bioanalysis, thanks to the produced promising results that are simple, precise, reliable, sensitive, and robust.

Keywords: Pexidartinib, Talazoparib, LC-MS, Bioanalytical method.

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INTRODUCTION

Pexidartinib (PDTB) (Figure 1) is a multi-kinase inhibitor that is used as an antineoplastic agent in the treatment of tenosynovial giant cell tumors. It is approved in 2019 by FDA as a first-in-class medication for those in their adult years who have rare disease symptomatic (TGCT) Tenosynovial Giant Cell Tumors.[1-6] The synovium and tendon sheaths thicken and develop excessively in TGCT, a rare type of non-malignant tumour that damages the joint tissue around it. The mechanism of action of PDTB is to inhibit the immunological responses triggered by tenosynovial giant cell tumours. Tenosynovial giant cell tumour growth is suppressed because it prevents the activation and signalling of receptor tyrosine kinases and tumor-permissive cytokines, which are essential for tumour cell survival and proliferation.[7-9] PDTB is available in tablet dosage for oral administration. Side effects with the usage of PDTB include loss of hair color, increased alanine aminotransferase (enzymes that are primarily in the liver and kidney), lactate dehydrogenase (proteins that help produce energy in the body), increased aspartate aminotransferase (enzymes that are mostly in the liver but also in muscles), and increased cholesterol.[10,11]

Review of literature

There are very few methods described for the analysis of PDTB. Two UPLC/MS/MS methods[12,13] and 2 HPLC stability-indicating method[14,15] have been reported. The aim of the present work is to develop the bioanalytical method for the quantitative analysis of PDTB in human plasma. A known concentration of Talazoparib (TZPB) drug was added to the sample solution as Internal Standards (IS) to improve the precision of quantitative analysis.

MATERIALS AND METHODS

Chemicals and Materials

Reputable organizations provided the pharmaceutical standards for the medications Pexidartinib and its internal standard, talazoparib (IS). Before being used, control buffered (K2-EDTA) human plasma was kept at -20°C after being acquired from diagnostic labs in Guntur. Merck Chemicals in Mumbai provided the following solvents: acetonitrile (≥99.9%) of HPLC grade,

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methanol (≥99.9%) of HPLC grade, water (≥99.9%) of HPLC grade, and orthophosphoric acid (98-100% extra-pure) of analytical grade.

Instrumentation

Analyses were performed on the Water Alliance 2695 HPLC (Waters Mass Lynx software (Version 1.6.3) Liquid Chromatographic (LC) and Waters ZQ (LAA 1369) Mass Spectrophotometer (MS) system. The LC-MS is attached with an online degasser attached to a quaternary gradient pump, automatic sampler system controller (SCL-10Avp) was used to inject 20 µL aliquots of the processed samples and a temperature-controlled column chamber that is linked to UV 487 waters Detection by mass spectrometry was carried out using a triple quadrupole API 4000 (AB Sciex, Canada). PDTB and IS were separated from the plasma matrix on an Xbridge C18 column (50 mM×4.6 mM, 5 µM) column analytical column. To enhance the solubility, an ultrasonicator (Model-D3) made by Make-GT professional ultrasonic cleaner, China, was utilised, along with an analytical balance (Model-CP225D, Germany) for weighing standards and samples.

Standard stock and calibration standards preparation stocked items

Primary stock solutions of PDTB standard and TZPB internal standard solution were prepared. 100 mg of the reference standard were dissolved in 100 mL of methanol to create the PDTB stock solution, which had a final concentration of 1000 µg/mL. The primary stock solutions were stored at 4°C, which were found to be stable and successively diluted with methanol to prepare working solutions to prepare Calibration Curves (CC). Appropriate CC solutions of seven concentrations of PDTB stock solution was made in methanol to produce the final concentrations of 0.5, 5, 25, 50, 150, 300 and 500 ng/mL for CC freshly prepared from a primary prepared stock solution. The method of liquid-liquid extraction was employed to extract both medicines. Three concentrations of the calibration solution were designated as LQC (Low-Quality Control), MQC (Middle-Quality Control), and HQC (High-Quality Control) standard solutions, correspondingly: 0.5 ng/mL, 50 ng/mL, and 500 ng/mL respectively. All the CC and QC solutions were stored at-20°C till the method of analysis.

RESULTS

Method Development and Optimization

Developing a unique, straightforward, and sensitive method for determining PDTB in plasma was the primary goal of this work. Through systematic adjustments to the chromatographic parameters and trial-and-error methodology, the LC-MS process was optimized for the measurement of PDTB. To improve separation and get symmetric peak morphologies for all analytes, including the internal standard, in a short run time, various mobile phase ratios, such as methanol and acetonitrile, were explored. Regarding the analyte's peak form, retention duration, sensitivity, carry-over, and baseline noise. Development trails were shows in Table 1.

In the end, it was determined that the X-bridge C18 column (50 mM×4.6 mM, 5 µM) was ideal since it had a nice peak shape and retention time. As a result, it was determined that acetonitrile, methanol, and 0.1% orthophosphoric acid (35:35:30) with a pH of 4.8 would make an appropriate mobile phase. At ambient temperature for the autosampler and column oven, respectively, the optimal flow rate was 0.6 mL/min. Figure 5 shows that the analyte's retention time is 1.87±0.3 min and the IS is 3.84±0.3 min.

For optimization of mass spectrometric parameters, the mixture of PDTB and TZPB (internal standards) solutions were injected directly into the electrospray ionization source of the MS (Figure 6). The mass spectrometer was operated in positive mode using in the mass range of 40-1000 amu and analyzed in the triple quadruple analyzer. Mass spectrometer was operated in positive

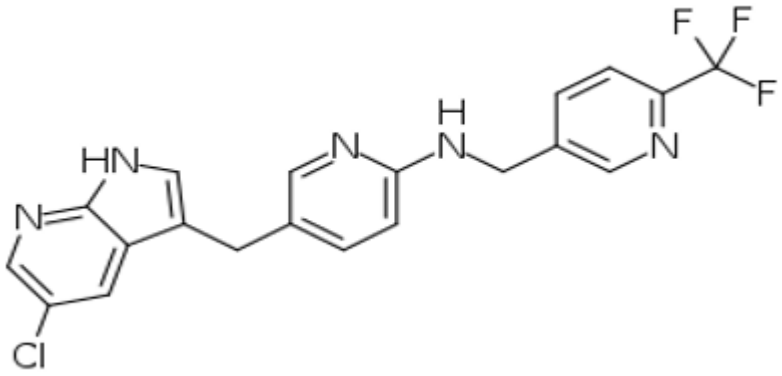

Figure 1: Chemical structure of PDTB.

Table 1: **Development trails.**

Mobile Phase	Column	flow rate (ml. min^{-1})	Run Time (min)	Mass Range (amu)	Chromatography Condition
Methanol: Acetonitrile (30:70 v/v).	Zorbax C8 (100×4.6 mM, 5 µM).	0.6	5	40-1000	These two trails no separation peaks were not observed (Figure 2).
Methanol: Acetonitrile (50:50 v/v).	Zorbax C8 (100×4.6 mM, 5 µM).	0.6	5	40-1000	
Methanol: Acetonitrile (50:50 v/v).	Phenomenex Luna C18 (250×4.6 mM, 5 µM).	0.6	5	40-1000	These two trails separation peaks were observed but base line drift and low tailing factor were observed (Figure 3).
Methanol: Acetonitrile (75:30 v/v).	Phenomenex Luna C18 (250×4.6 mM, 5 µM).	0.6	5	40-1000	
Methanol: Acetonitrile: Water; 45:30:25 v/v/v).	Phenomenex Luna C18 (250×4.6 mm, 5 µM).	0.6	5	40-1000	In these trail peaks are separated but parameters are not in ICH guidelines, hence go to next trail (Figure 4).

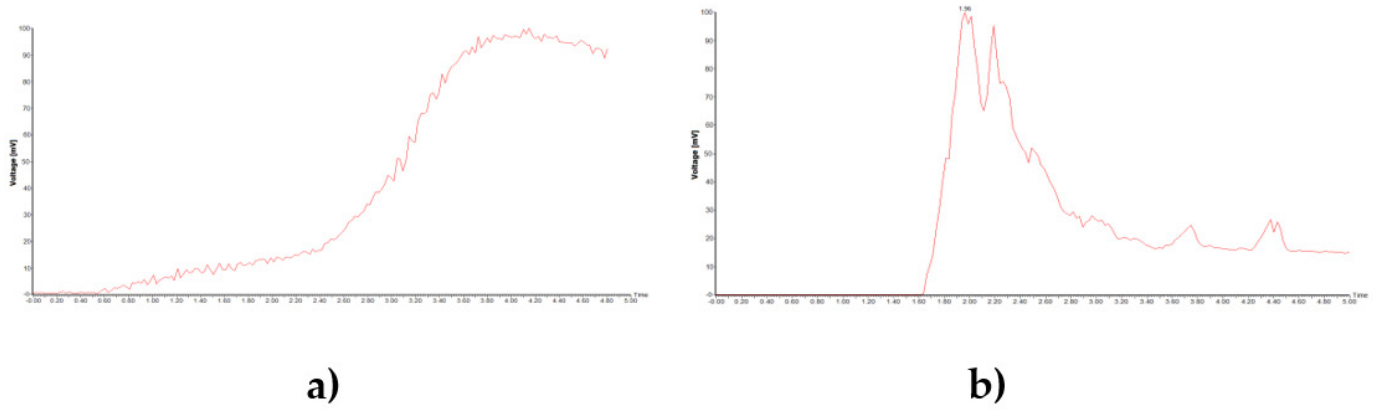

Figure 2: Chromatogram Obtained from (Methanol: Acetonitrile (a) 70:30 v/v and (b) 50:50) Mobile Phase with Zorbax C8 (100×4.6 mM, 5 µM) column.

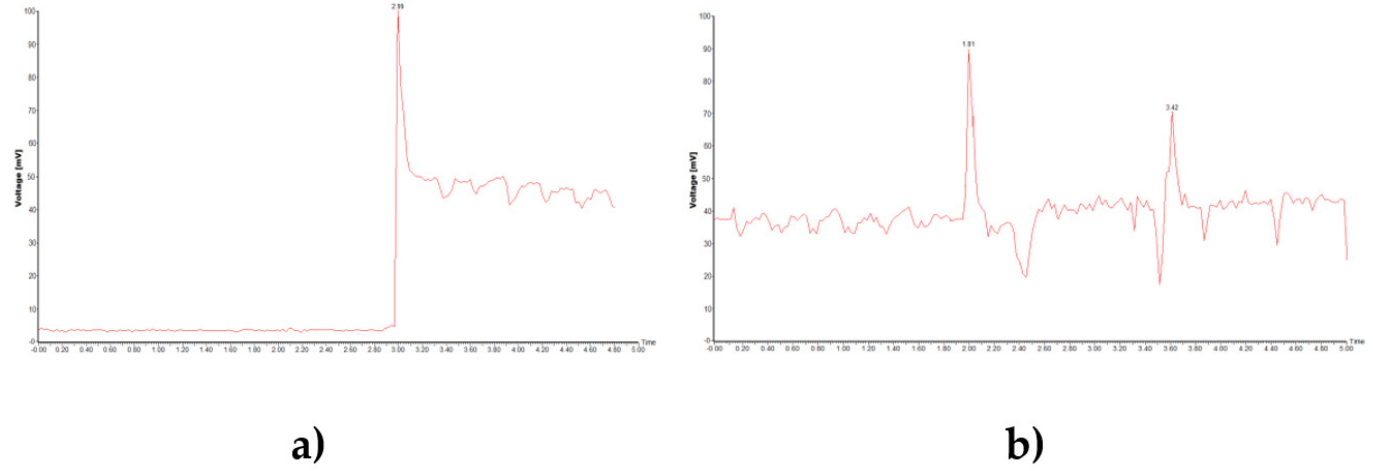

Figure 3: Analysed chromatogram acquired from (Methanol: Acetonitrile (a) 50:50 v/v (b) 75:35) Mobile Phase with Phenomenex Luna C18 (250×4.6 mM, 5µM) column.

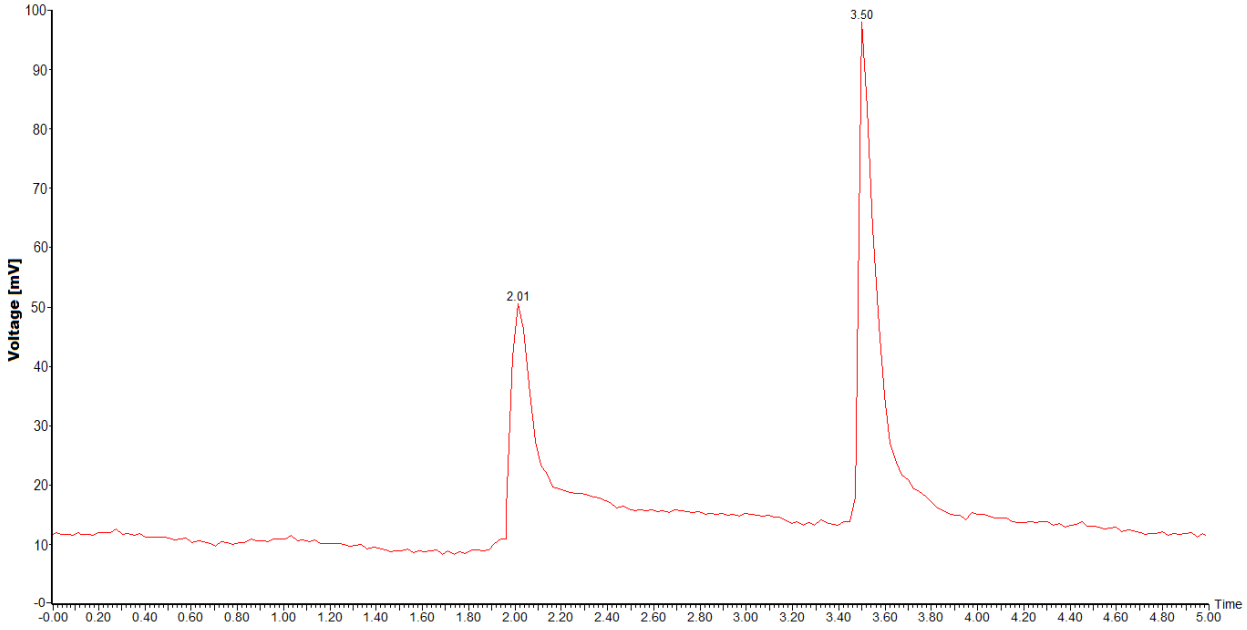

Figure 4: Analysed chromatogram acquired from (Methanol: Acetonitrile: water (45:30:25; v/v/v:) Mobile Phase with Phenomenex Luna C18 (250×4.6 mM, 5 µM).

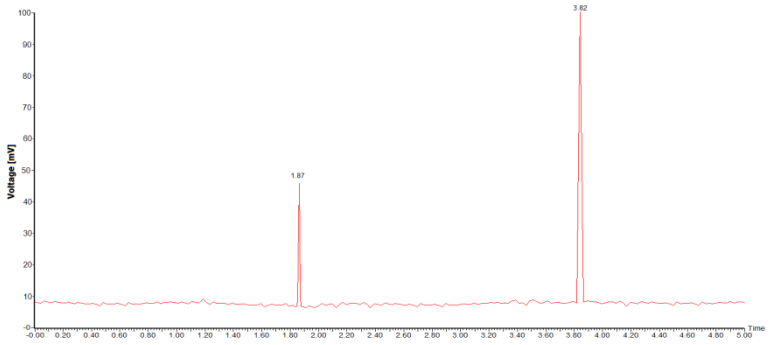

Figure 5: Chromatogram Obtained from (Acetonitrile: Methnaol:0.1% orthophosphoric acid (35:35:30; v/v/v) Mobile Phase with Xbridge C18 column (50 mM×4.6 mM, 5 µM) column.

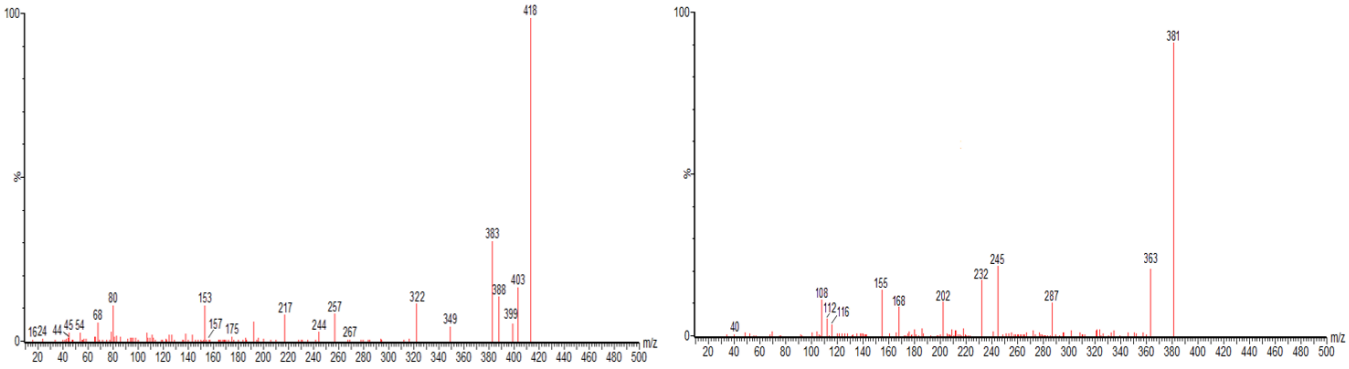

Figure 6: Mass fragmentation of (a) Pexidartinib (b) Talazoparib internal standard.

mode using the parameters of the ion source: In a mass spectral analysis with a fixed MS tuning temperature of 35°C, nitrogen gas (320 psi) was employed as the carrier gas at a flow rate of 0.6 mL/min. The capillary voltage was 3.5 KV, the nebulizer pressure was 310 kPa, the cone voltage was 50V, and the extractor voltage was 3.0 V. The mass resolution (2.5 amu) for the PDTB standard is employed, along with the m/z transitions m/z 418→153 for PDTB eV and m/z 381→108 Evat collision energy of 85 eV for TZPB IS. Optimized conditions are given in Table 2.

DISCUSSION

Method Validation

Liquid chromatography and mass spectroscopy were used to validate the above-optimized bio-analytical method of Pexidartinib in spiking human plasma samples in accordance with the ICH guidelines.[16-19]

Table 2: Optimized chromatographic conditions of the method.

Sl. No.	Parameter	Optimized Condition
1	Column	Xbridge C18 column (50 mM×4.6 mM, 5 µM) column.
2	Mobile Phase	Acetonitrile: Methanol: 0.1% orthophosphoric acid (35:35:30).
3	Mobile phase pH	5.2
4	Mobile phase flow rate (mL/min).	0.6
5	Elution	Isocratic
6	Wavelength (nm)	242 nm
7	Sample volume (µL)	10
8	Run time (min)	5
9	Diluent	Mobile Phase

Table 3: System Suitability Parameters.

System Suitability Parameter	Ruggedness	
	Pexidartinib	Talazoparib
USP resolution	----	9.18
USP tailing factor	1.09	0.96
USP plate count	4429	6128
Retention time (min)	1.90	3.89
Mean Peak area±SD	963256.7±3572.22	125124.6±1225.55
% RSD of area	0.37	0.97

*From 6 standard injections at 500 µg mL^{-1} of Pexidartinib.

System Suitability, Specificity and System Precision

Confirmed that the system performed as expected based on the system suitability characteristics that were found, and the related tabulated parameters (Table 3). At PDTB retention times, no appreciable influence from plasma was detected (Figure 7). The PDTB was retained for roughly 1.83 min. Table 4 tabulates the system suitability results that were obtained together with the validation parameters. Figure 8 displays typical chromatograms demonstrating system precision and appropriateness. Six replicate injections of standard preparations were used to determine system precision, and the percentage RSD was assessed. The method applied to plasma samples for the pharmacokinetic investigation showed good specificity and selectivity, according to the results.

PDTB's stability in human plasma

All the QC (HQC (500 µg mL^{-1}), MQC (50 µg mL^{-1}), and LQC (0.50 µg mL^{-1})) standards were unprotected to different stability conditions (short-term stability was observed at normal temperature for 8 hr, Long-term stability was observed at -85°C for 65 days,[13] and freeze-thaw stability were assessed -80°C) and evaluated to analyze the stability of PDTB in human plasma.[13] The accuracy values of (long-term, Short-term and Freeze-thaw) stability in between 92.414-101.76 at all QC levels (Table 5). No significant degradation of the PDTB was detected even afterward an 8 hr storage period in the auto sampler tray. These results have established the stability of PDTB in human plasma for at least 65 days at -85°C.

Linearity

The linearity of the proposed method was determined by the study of seven standardization curves (Figure 9) containing 0.5-500 ng/mL for PDTB with TZPB. The calibration curve fit and QC imprecision the regression equation (y=1915.1x-2355) for PDTB and TZPB in the developed method was found with Residual sum of squares (r^2) of 0.999 indicating the overall performance of regression models that shows good linearity (Table 6).

Table 4: Validation Parameters.

Sl. No.	Parameter	Results observed
		Pexidartinib
1	Concentration of APIs (µg mL^{-1}).	500
2	Linearity (µg mL^{-1}).	0.5-500
3	Method precision (% RSD).	0.29-1.13
4	Intermediate precision (% RSD).	0.13-1.01
5	% Recovery	85.64-99.16
6	LOQ (µg mL^{-1})	0.5
7	LOD (µg mL^{-1})	0.05

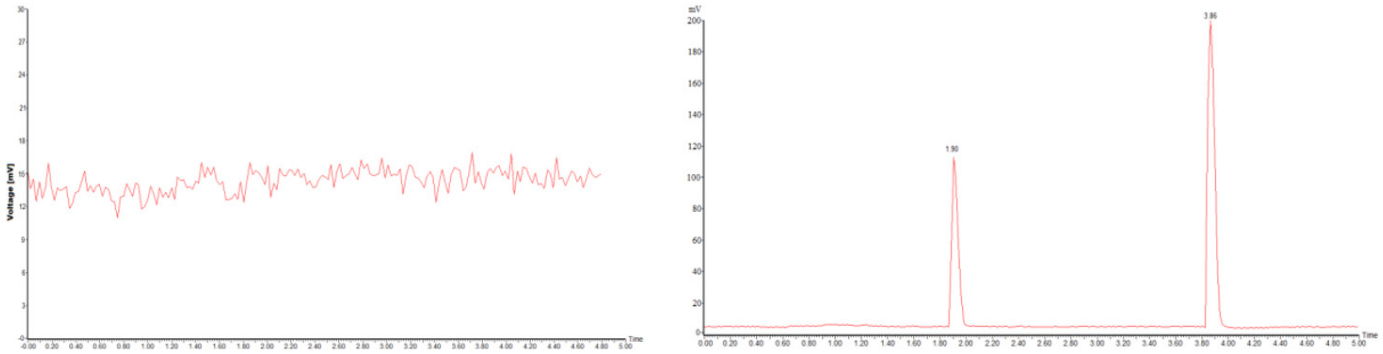

Figure 7: Typical Chromatogram of a) Placebo and Blank b) System Suitability.

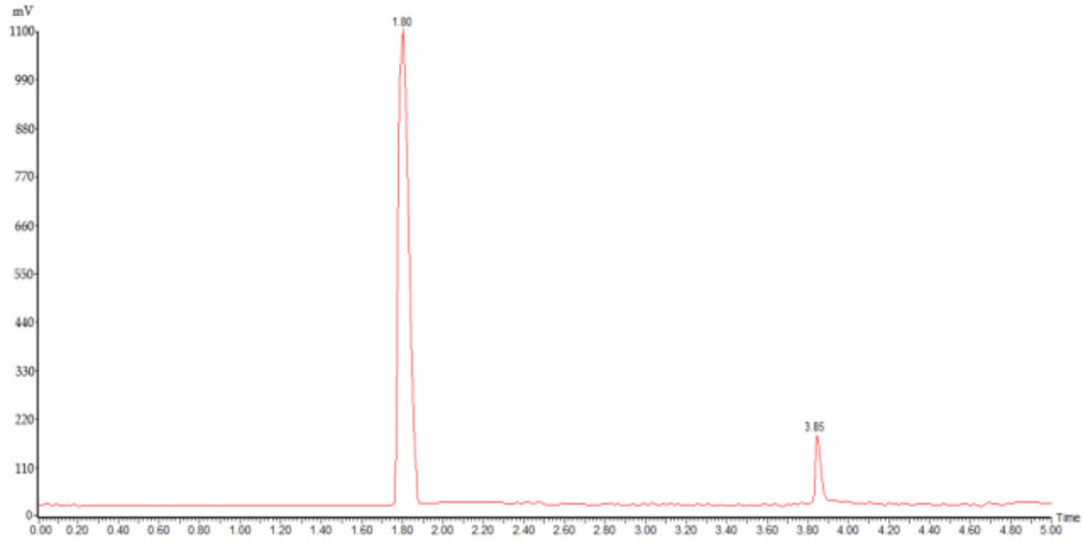

Figure 8: Standard Chromatogram of Typical Precision in the System.

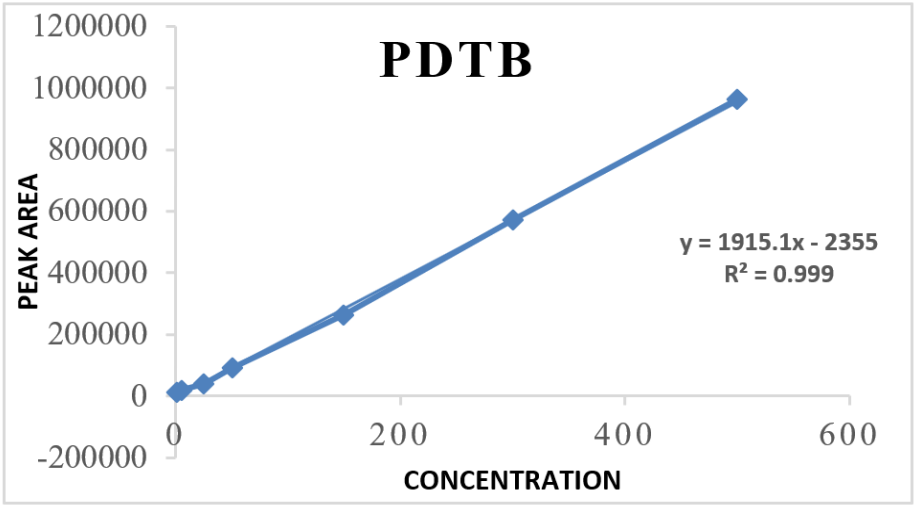

Figure 9: Calibration Graphs of Pexidartinib.

Table 5: **Stability of PDTB in human plasma.**

Spiked plasma concentration (ng/mL)		Concentration measured (*n*=6) (ng/mL) (mean±S.D.)	%RSD (*n*=6)	Accuracy (%)
Long term stability	HQC	508.50±4.25	0.836	101.70
	MQC	46.21±0.17	0.372	92.41
	LQC	6.27±0.154	2.451	96.43
Short term stability	HQC	508.80±4.92	0.966	101.76
	MQC	48.30±0.209	0.432	96.60
	LQC	6.53±0.013	0.198	100.39
Freeze Thaw stability	HQC	503.24±1.79	0.356	100.65
	MQC	48.10±0.227	0.473	96.21
	LQC	6.24±0.067	1.076	95.95

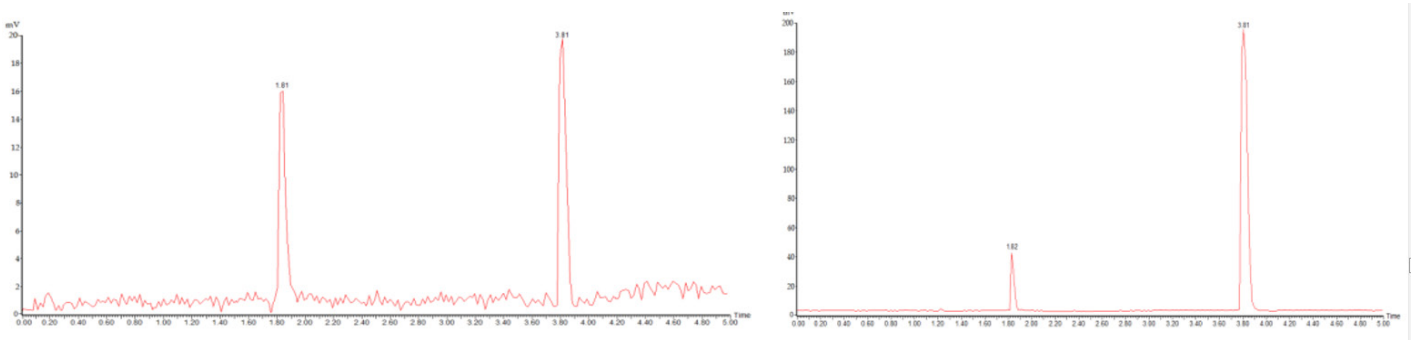

Figure 10: LOD and LOQ Chromatograms of Pexidartinib.

Table 6: **Summary of Regression Parameters.**

Sl. No.	Parameter	Acquired Values (PDTB)
1	Residual sum of squares	0.999
2	Slope	1915.1
3	Y-Intercept	2355

Precision of Method (M.P.) and Precision of Intermediate (I.P.)

Precision of Method (M.P.) were assessed and analyzed the six samples same day, whereas Intermediate Precision were assessed through repeated analysis over 3 days and precision was determined by calculating percentage % RSD values calculated at each concentration level of all QC sample. The examination of the spiked plasma samples has revealed that Method Precision of the assay was varied between 101.36 to 96.60 at QC levels and the intermediate precision of the assay was varied between 101.13 to, 96.51 at all QC level for PDTB respectively (Table 7).

Robustness

Through the modification of the conditions of the experiment, the robustness (Table 8) of the approaches was investigated. The retention time shift of PDTB did not show significance for variance. Modifying the experimental parameters (pH, composition, flow rate, etc.,) did not result in any discernible changes to the chromatographic parameters.

Recovery

Newly prepared QC set and quality controls have been introduced alongside a total of six duplicates of unconstrained low, medium, and high QC samples to measure the method matrix effect, overall mean, and recovery. At three distinct doses, the overall mean, SD, and %CV were computed along with the method matrix effect. The percentage recovery values for HQC, MQC, and LQC are 102.25, 103.10, and 100.24, respectively, indicating a significant recovery for the approach (Table 9). There were no discernible variations between the concentrations in these data.

LOD and LOQ

The smallest amount of linearity is referred to LOQ value (Figure 10). It was discovered that the technique was sensitive enough to find out PDTB's pharmacokinetic analysis in plasma. However, these values could be impacted by the application of non-HPLC quality solvents, experimental adjustments (like pumping systems and detectors), separation conditions (such column, reagents, and instrumentation and data systems), and other factors that could alter signal to noise ratios.[20-26] LOD and LOQ were 0.5 µg mL^{-1} and 0.05 µg mL^{-1}, respectively.

Table 7: Comparison of method precision (MP) and intermediate precision (IP).

Sl. No.	% Assay					
	HQC		MQC		LQC	
	M.P	I.P	M.P	I.P	M.P	I.P
1	101.3625	101.0187	96.60968	96.84507	100.8752	100.8833
2	101.2842	101.1327	97.4488	97.75281	100.7901	100.8013
3	101.1653	100.9433	96.79056	97.02637	100.5804	100.7025
4	101.0747	100.752	97.13091	96.73333	100.5716	100.6125
5	100.3308	100.0106	97.44421	97.04527	100.1868	100.8833
6	101.1657	100.8226	96.8268	96.5135	100.5218	100.7105
Mean±SD	101.0 ±0.34	100.8 ±0.37	97.04 ±0.32	96.99 ±0.39	100.6 ±0.22	100.8 ±0.01
%RSD	0.337	0.363	0.334	0.399	0.218	0.099

Table 8: Results of robustness/ruggedness experiment.

Altered parameter	Actual cond.	Altered cond.	RT (Min)	Tailing factor	Theor plates	peak area (mean±SD)	% RSD
HQC							
Control	------	35:35:30	1.90	1.09	4429	963259.7	----
Mobile phase	35:35:30 (V/V/V)	40:30:30	1.82	1.07	4591	964215.7	0.68
		30:40:30	1.84	1.01	4574	969343.9	0.31
Flow ($mL.min^{-1}$)	0.5	0.4	1.87	1.08	4578	963407.5	0.38
		0.6	1.83	1.04	4525	967296.2	0.68
pH	4.8	4.9	1.84	1.07	4414	966484.2	0.56
		4.7	1.90	1.05	4436	965318.4	0.61
MQC							
Control	------	35:35:30	1.83	1.09	4536	90203.4	0.35
Mobile phase	35:35:30 (V/V/V)	40:30:30	1.90	1.07	4511	90468.8	0.45
		30:40:30	1.86	1.07	4497	90555.1	0.26
Flow ($mL.min^{-1}$)	0.5	0.4	1.84	1.08	4591	90707.5	0.28
		0.6	1.88	1.07	4426	90620.9	0.26
pH	4.8	4.9	1.90	1.07	4427	90107.6	0.32
		4.7	1.88	1.05	4597	90308.8	0.26
LQC							
Control	------	35:35:30	1.83	1.01	4581	10202.1	----
Mobile phase	35:35:30 (V/V/V)	40:30:30	1.87	1.02	4464	11089.0	0.65
		30:40:30	1.87	1.02	4503	11130.1	0.69
Flow ($mL.min^{-1}$)	0.5	0.4	1.83	1.03	4574	10820.6	0.59
		0.6	1.85	1.04	4477	11089.1	0.48
pH	4.8	4.9	1.80	1.08	4575	11056.1	0.40
		4.7	1.88	1.05	4474	11121.6	0.52

Table 9: Recovery.

Elevated concentration of plasma (ng/mL)	Calculated value (*n*=6) (ng/mL) (mean±S.D.)	%RSD (*n*=6)	Accuracy (%)
HQC	511.2415±0.883122	0.172741	102.25
MQC	51.55095±0.130317	0.252793	103.10
LQC	7.016735±0.039941	0.569227	100.24

CONCLUSION

The present study describes a new, highly selective, sensitive, and rugged bioanalytical LC-MS method for the estimation of PDTB in human plasma with TZPB as IS. The method uses simple Liquid-Liquid Extraction (LLE) method for the extraction of drugs from plasma. The method using Reverse Phase-Symmetry C18 X-bridge C18 column (50 mM×4.6 mM, 5 µM) column with a simple isocratic mobile phase composition of Acetonitrile: Methnaol:0.1% orthophosphoric acid (35:35:30). The drug was eluted within 5 min suggests high throughput of the proposed method. This proposed method has good recovery, accuracy, and precision and is suitable for sample analysis to support bioequivalence/bioavailability and/or pharmacokinetic studies involving formulations of PDTB over the concentration range from 0.5 to 500 µg/mL.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

PDTB: Pexidartinib; **TZPB:** Talazoparib; **LCMS:** Liquid Chromatography-Mass Spectroscopy; **HPLC:** High Pressure Liquid Chromatography; **IS:** Internal stranded; **LLE:** Liquid-liquid extraction; **LOD:** Limit of Detection; **LOQ:** Limit of Quantitation; **TGCT:** Tenosynovial giant cell tumors.

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