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Liquid chromatography and tandem mass spectrometric method for the quantification of Tepotinib in plasma samples

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ABSTRACT

A precise and accurate liquid chromatographic tandem mass spectrometric technique was developed to measure the levels of Tepotinib in plasma. Axitinib and Tepotinib were separated from the plasma sample solution using a protein precipitation method. A chromatographic isolation was carried out using a ZorbaxC18 stationary phase (2.1 × 100 mm, 3 µm) and mobile phase proportion of 0.1 % HCOOH and acetonitrile (15:85). The analytes were quantified using positive ionization methodology with electrospray ionization. Mass transitions for Tepotinib were m/z 493.23 → 296.17 and for Axitinib (IS) they were m/z 387.12 → 220.08. No interference from any components of blank plasma or additional substances was identified. Correlation between Tepotinib concentrations and the respective area of the peaks ratio to Axitinib showed a linear pattern across a range of 1.5–1200 ng/mL. The precision of Tepotinib was excellent, with an intra-day precision of ≤4.92% (n = 10) and an inter-day precision of ≤04.76% (n = 20, over three days). The measured average extraction recoveries of Tepotinib were 98.32%. Tepotinib underwent various stability tests at low and high-quality control levels. The results showed that Tepotinib remained stable and was within 95.41%–102.43%. The developed technique can be valuable for regular quantification of Tepotinib plasma samples in clinical organizations, various industries, and forensic laboratories.

INTRODUCTION

Tepotinib is chemically designated as 3-{1-[(3- {5-[(1-methyl piperidin-4-yl) methoxy] pyrimidin 2-yl} phenyl) methyl] -6-oxo-1, 6-dihydropyridazin3-yl} benzonitrile with formula and weight of $C_{29}H_{28}N_6O_2$ and 492.583 g·mol^{-}1 (Fig. 1A). Tepotinib is approved for use in patients detected with metastatic non small cell lungs cancers (NSCLC) and have definite genetic alterations known as mesenchymal epithelial transitions (MET) factors exon14 skipping [1–3]. Tepotinib is a medication planned to inhibit and target the activities of MET tyrosine kinases, commonly found in certain types of solid tumors that have an overexpression of MET. The development of this treatment began in 2009 through a collaboration between the University of Texas M.D. Anderson Cancer Center and EMD Serono [4]. Since then, researchers have studied its potential in treating various types of cancer, including neuroblastoma, NSCLC, gastric cancers, and hepatocellular carcinomas. As a result, targeting MET has become a desirable approach in their treatment.

Tepotinib was granted its initial approval for use in Japan for the management of NSCLC with MET alterations in 2020 March [5]. This oral medication is the first of its kind to specifically target MET tyrosine kinase and can be taken once a day. This feature is beneficial as it helps reduce the number of pills that patients need to take, which can be a burden when undergoing chemotherapy. Europe approved the use of Tepotinib in February 2022 [4–6]. In the present study, Axitinib was utilized as an internal standard (IS), with similar chromatographic behavior as that of analyte, for the estimation of Tepotinib in biological samples [7]. It is chemically designated as *N*-Methyl-2-[[3-[(*E*)-2-pyridin-2-ylethenyl]-1*H*-indazol-6-yl] sulfanyl] benzamide with formula and weight of $C_{22}H_{18}N_4OS$ and 386.47 g·mol^{-}1 (Fig. 1B) [8].

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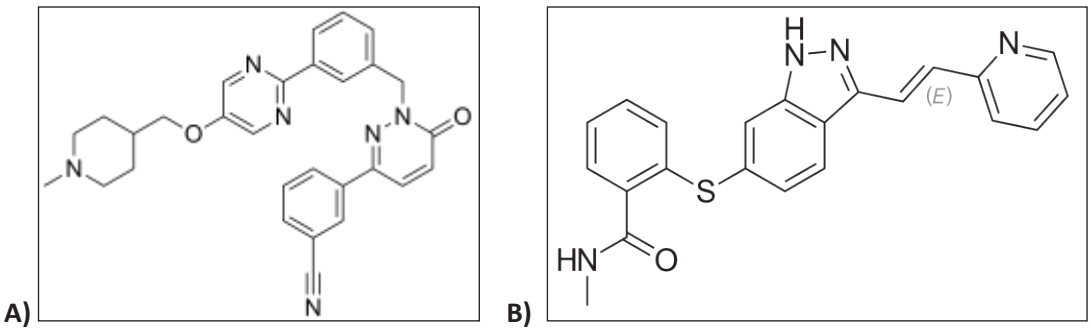

Figure 1. A) Tepotinib and B) Axitinib chemical structures.

There is only one analytical approach that has been reported for quantifying Tepotinib using LC-MSMS, according to the available literature. There are alternative techniques mentioned in the literature, such as HPLC [9,10], UV [11], and LC-MS/MS [12]. The reported method is applied to study in human liver microsomes and there were no studies revealed about plasma samples. In the present scenario, there is a requirement for bioanalytical methods for bioequivalence and bioavailability studies. Therefore, there is a need to develop a bioanalytical method for the quantification of Tepotinib levels in plasma samples.

MATERIAL AND METHODS

Reagent chemicals

Tepotinib and Axitinib were provided by Dr. Reddy's, Hyderabad, India. We purchased chromatographic grade methyl alcohol, acetonitrile, and HCOOH (formic acid) of analytical grade attained from JT Bakers, Hyderabad, India. The mobile and cleaning solvents were prepared with water obtained from the Milli-Q®RO system. Human K2EDTA plasma was attained from Vivekananda blood bank, HYD, India.

Liquid chromatography tandem mass spectrometry(LC-MS/MS)

An LCMS/MS system was used for chromatography, with a Water 2695 Alliance separation module for sample introduction and solvent delivery. The system also included a Micromass Quattro micro API bench-top triple quadruple mass spectrometer connected to an ESI Z-spray source as a detector. At room temperature, an analysis was conducted using a reversed-phase ZorbaxC18 stationary phase (2.1 × 100mm, 3 µm) and mobile phase composition of 0.1%HCOOH and acetonitrile(15:85). Movable phase flow was monitored at 0.4 ml/minute. The Version 4.0Mass Lynx software was utilized on an MS Windows XP Professional operating system to manage the device, retrieve the data, calculate the signal-to-noise (S/N) ratio, and process the peaks through integration and smoothing.

LC and mass system conditions

Chromatography was established with ZorbaxC_{18} stationary phase (2.1 × 100 mm, 3 µm) and a mobile phase of 0.1% HCOOH and can (15:85% V/V), sent at a flow rate of 0.4 ml/minute. The ESI (electrospray ionization) interfaces were operated in positive ionization mode. Mass spectrometric parameters were as follows: 450°C: temperature of desolvation, 150°C: temperature of source, 4kV: capillary voltage. N_2 was utilized as both cone gas and desolvation gas at 40 l/hour and 750 l/hour flow rates, respectively. Argon was utilized as the collisional gas at 0.17 ml/minute flow rate in the collisional cell. Collisional energy and voltage of the cone for Tepotinib and Axitinib were optimized as 18V/20V and 20V/28V, respectively. The quantitation of drug and IS was executed using positive ionization with ESI at the mass transitions of (m/z): for Tepotinib were m/z 493.23 → 296.17 and for Axitinib was 387.12 → 220.08.

Processing of standard and quality controls

Tepotinib and Axitinib stock solutions (1,000 µg/ml) were processed in a mobile solvent individually. The substances were further solubilized using plasma to produce a 10 µg/ml concentration. A set of 8 calibration standards was processed using human plasma (after the sample preparation), covering within the concentrations range of 1.5–1,200 ng/ml. Additionally, 4 quality control (QC) sample solutions were also processed, with concentrations of 1.5, 4.5, 600, and 900 ng/ml [13] LLOQ, LQCs, MQCs, and HQCs, respectively. The linearity controls and QCs were thoroughly mixed for 1 minute, and then precise 1.0 ml portions were carefully relocated into borosilicate Teflon-lined (13 × 100 mm) glass tubing. These samples were then stored at −20˚C till they were desired.

Sample extraction

Ensure that the 1 ml sample of QC, linearity curve, and plasma blank samples are brought to room temperature. The final concentrations of each and every tubing were 150 ng/ml of IS. To achieve this, 250 µl of the IS solution was added to each tube and vortex for 20 seconds. They received a dosage of 5 ml of acetonitrile for treatment. The resultant solutions were then centrifuged at an RPM of 15,000 for a duration of 25 minutes [14]. The organic phase was isolated and dried using a N_2 gas stream in a heated block at 45°C. The remaining solution was mixed with 100 µl of a mobile solvent system, transferred to an auto-sampler container, and 10 µl was then infused into an LC-MSMS instrument.

Stability study

By storing the processed QC solutions in an autosampler maintained at 5**°C** ± 3°C temperature for the duration of 2 days, 20 hour, and 27 minutes, their stability in the auto sampler could be evaluated. After undergoing rigorous 2-month storage at −20**°C, long-term stability at the** low-quality control (LQC) (4.5 ng/ml) and high-quality control (HQC) (900 ng/ml) levels. The stock solution stability for the analytes was tested at the LQC (4.5 ng/ml) and HQC (900 ng/ml) levels by keeping it at a temperature of 2–8**°C for 10 days. Three freeze**/thaw cycle stability solutions were executed at room temperature and −20°C. The analytes underwent short-term stability testing by being stored at room temperature for 8 hours. The stability of the QC spiked samples was tested by keeping them at room temperature for a duration of 17 hours and 28 minutes. The stability of the dry extract in spiked QC solutions was assessed over a time of 2 days by preserving them at −28**°C** ± 5**°C** [15].

Validation

Developed method subjected for the validation as per the recommended guidelines of USFDA. Validation criteria included factors such as specificity, accuracy, linearity, precision, stability, and recovery [13–20].

RESULTS AND DISCUSSION

Optimization LC and mass parameters

Parent and product ionic components of Tepotinib and Axitinib were analyzed by injecting a standard 1.0 **µg/ml** solution in acetonitrile into the mass instrument. The analytes were measured using positive ionization with ESI in the MRM approach. The resulting transitions were: Tepotinib, 493.23/296.17.09 and Axitinib, 387.12/220.08. The following mass limitations were employed: The desolvation temperature is 450°C, the source temperature is 150°C, and the capillary voltage is 4kV. A cone and desolvation gas consisting of N_2 was used at flow rates of 40 l h^{-1} and 750 l h^{-1}, correspondingly. An argon gas mixture was subjected to collisional processing at a flow rate of 0.17 ml/minute in a collision cell. The collisional energy and voltages for Tepotinib and Axitinib cones were tuned at 18V/20V and 20V/28V, respectively.

Specificity

To assess the specificity six batches of plasma blank and IS were examined. The chromatogram seen in Figure 2A exemplifies a standard chromatogram of plasma blank, utilized in the generation of QC and standard samples. Figure 2B displays a plasma sample spiked with drug and Axitinib [16–19].

Calibration curve

The test's linear plot was established by estimating a set of standard blends including Tepotinib and IS in human plasma. The blends were tested at eight different concentration levels, ranging from 1.5 to 1,200 ng/ml. Regression assessment was conducted on peak height ratios associated with the IS and the concentrations [18–21]. The equations for Tepotinib were determined to be y = 0.0012x + 0.0035, with an R^2 value of 0.9997 (n = 6). The suitability of the linearity plots for application was established by performing back calculations to ascertain the concentrations of Tepotinib in plasma samples, using the linearity graph provided in Table 1. All calculated

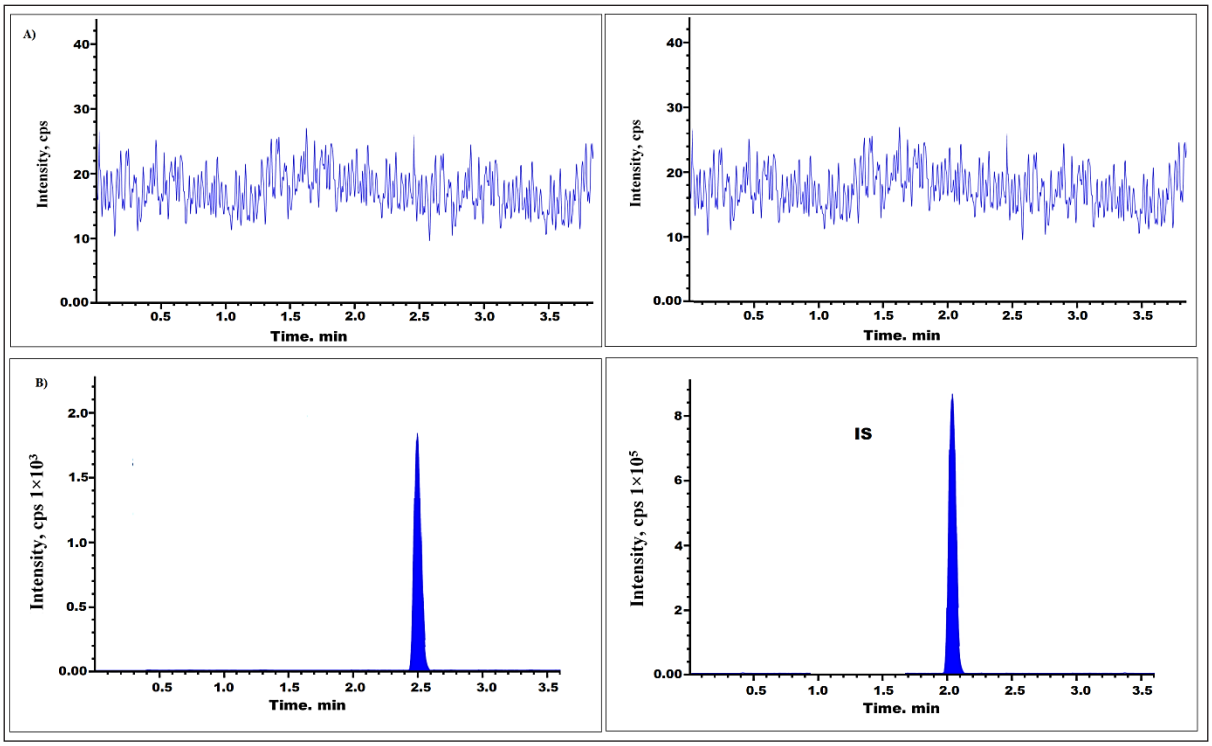

Figure 2. Chromatogram of Tepotinib (A) plasma blank and (B) LLOQ levels.

Table 1. Tepotinib calibration standards data.

LS-ID	Concentration (ng/ml)	Average concentration (ng/ml)	Average response	IS response	Analyte/IS response
LS -1	1.5	1.56	1,791	975,581	0.001,836
LS -2	6	5.956	6,845	975,697	0.007,015
LS -3	60	60.86	69,984	975,841	0.071,717
LS -4	150	149.85	172,327	975,637	0.17,663
LS -5	350	376.13	432,547	975,584	0.443,372
LS -6	630	634.67	729,874	974,952	0.748,626
LS -7	900	901.06	1,036,214	974,873	1.062,922
LS -8	1200	1205.68	1,386,527	976,034	1.420,572

LS: Calibration standard.

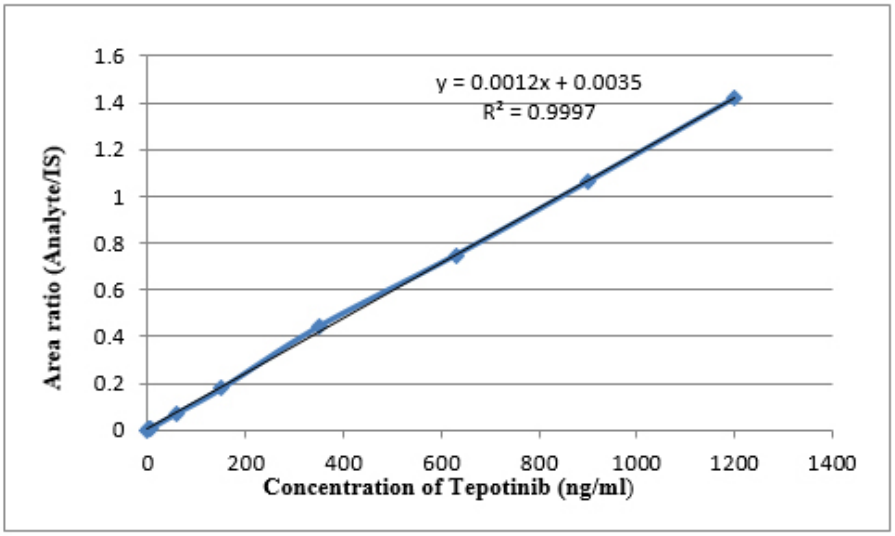

Figure 3. Linearity of Tepotinib.

concentrations were far lower than the maximum allowable limit of ICH and FDA validation guidelines [17,18]. Figure 3 depicts the average linearity graph of Tepotinib. LLOQQC for Tepotinib was estimated to be 1.5 ng/ml, with a S/N ratio greater than 10. Because, if the noise is more, the response of the drug and IS will be merged in the noise and cannot be identified. This level of sensitivity is enough for precise measurement of Tepotinib in plasma samples.

Precision and accuracy

Precision and accuracy were estimated for 4 QC levels (1.5, 4.5, 600, and 900 ng/ml). Intra ($n = 10$) and inter ($n = 20$, 3 days) day precisions were ≤4.92% and ≤ 04.76% for Tepotinib (Fig. 4 and Table 2). Intra and Interday bias was −0.33%–2.56% and −1.97%–2.67%, respectively [20–22].

Recovery

The extraction recoveries of Tepotinib at 3 different concentration levels (4.5, 600, and 900 ng/ml) and the Axitinib at 150 ng/ml were estimated by paralleling the peak areas of spiked samples before and after the extracting procedure. This assessment was conducted using six sets of spiked solutions [19]. The extraction recoveries of Tepotinib were found to be 98.32% on average (Table 3 and Fig. 4).

Matrix effect

The effect of matrix quantification was conducted by equating the peak intensities of Tepotinib in the presence and absence of the elements of the matrix such as lipids, proteins, electrolytes, and metabolites. The six sample solutions were infused to test at both low and higher QC standards. %CV findings for LQC and HQC levels were 3.37 and 3.32, respectively (Table 4).

Stability

In this study, both the stability of Tepotinib and Axitinib in processed and unprocessed plasma solutions was investigated at both low and high-quality controls. Table 5 provides a summary of the conclusions from the studies into the stability.

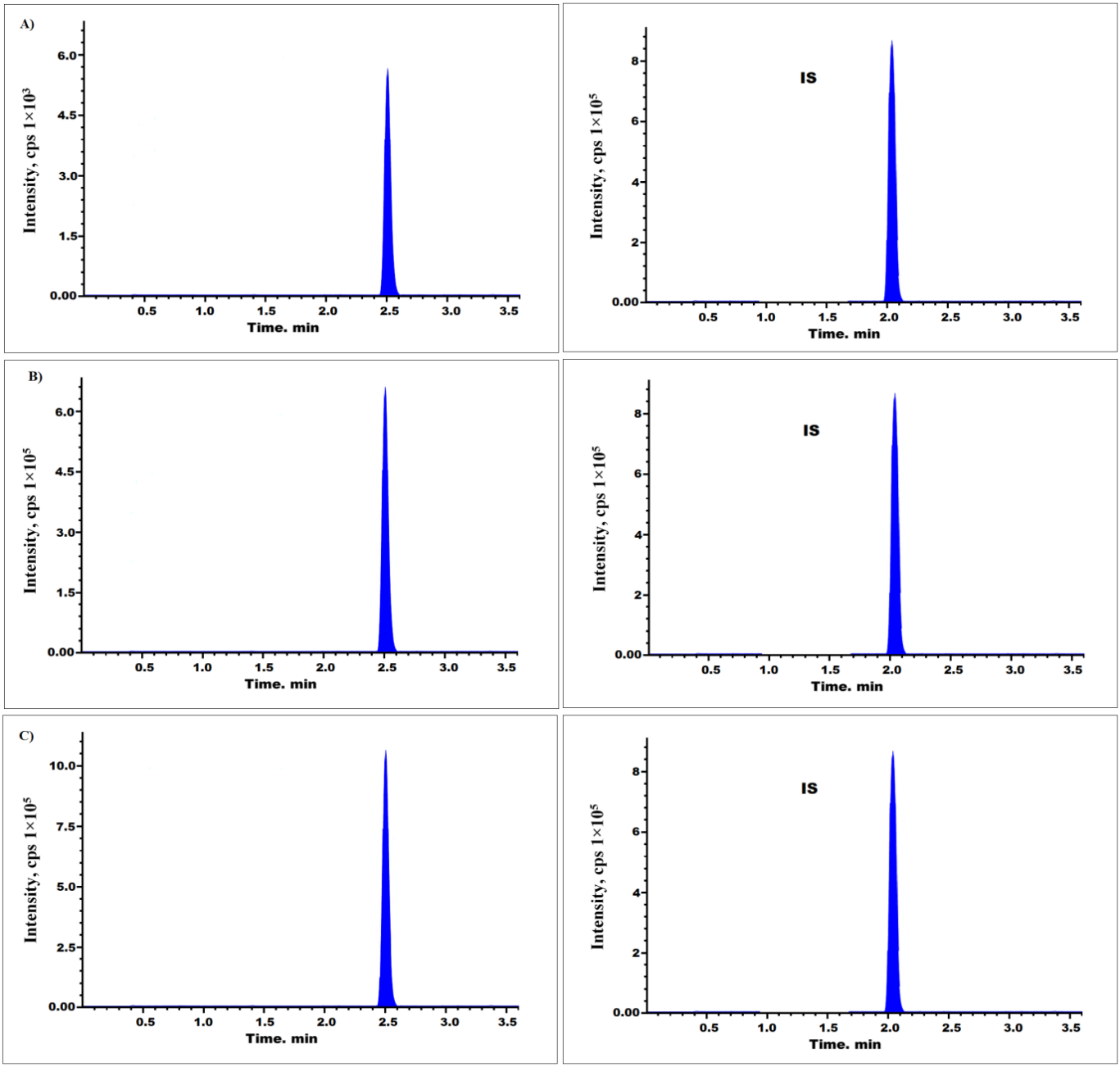

Figure 4. Chromatogram of Tepotinib at A) low-QC level, B) median-QC level, and C) high-QC levels.

Table 2. Tepotinib accuracy and precision for inter and intra batches.

Concentration level	Nominal concentration (ng/ml)	Intra-batch			Inter-batch		
		Mean (SD)	%CV	%Bias	Mean (SD)	% CV	% Bias
LLOQ	1.5	1.49 (0.06)	4.04	−0.93	1.47 (0.05)	3.40	−1.98
LQC	4.5	4.62 (0.17)	3.68	2.56	4.62 (0.19)	4.11	2.67
MQC	600	598.05 (18.78)	3.14	−0.33	588.14 (20.94)	3.56	−1.98
HQC	900	923.02 (45.41)	4.92	2.54	882.21 (41.95)	4.76	−1.97

Table 3. Recoveries of analytes after the extraction.

Concentration levels	C	D	% Recoveries	% Average recovery	% RSD
LQC	5,175	5,033	97.27	98.32	3.01
MQC	690,000	657,846	95.34		
HQC	1,035,000	1,059,426	102.36		
IS	975,236	959,729	98.41		

C, average recoveries of unextracted solutions; D, mean recovery of extracted solutions.

Table 4. Tepotinib findings for matrix factor.

	LQC			HQC		
S.No	Area of peak without a matrix	Area of peak with a matrix	Matrix factors	Area of peak in absence of matrix	Area of peak with a matrix	Matrix factor
1	5,191	4,964.1,533	95.63	1,035,954	1,069,312	103.22
2	5,156	5,048.2,396	97.91	1,035,742	997,834	96.34
3	5,137	5,282.8,908	102.84	1,035,861	992,251	95.79
4	5,194	5,024.6,756	96.74	1,036,638	1,016,009	98.01
5	5,167	5,057.4,596	97.88	1,035,159	1,064,558	102.84
6	5,185	5,378.4,005	103.73	1,034,954	1,006,803	97.28
Mean			99.12			98.91
± SD			3.34			3.28
% CV			3.37			3.32

Table 5. Stability data of Tepotinib.

Stability study	Concentration level	Comparison samples area mean	Stability samples area mean	% Mean stability
Auto sampler stability	HQC	1,024,461	1,049,353	102.43
	LQC	5,171	5,022	97.11
Long term stability	HQC	1,024,461	983,055	95.96
	LQC	5,171	4,934	95.41
Freeze thaw stability	HQC	1,024,461	1,005,717	98.17
	LQC	5,171	5,017	97.02
Bench top stability	HQC	1,024,461	1,005,671	98.16
	LQC	5,171	4,991	96.51
Short term stabilities	HQC	1,024,461	997,382	97.36
	LQC	5,171	5,017	97.01
Dry extract stability	HQC	1,024,461	992,898	96.92
	LQC	5,171	5,046	97.58
Stocks solution stabilities	HQC	1,024,461	1,012,933	98.87
	LQC	5,171	5,030	97.27

CONCLUSION

A precise, targeted, and accurate LC-MS/MS technique was established to measure the levels of Tepotinib in plasma. Axitinib and Tepotinib were separated from the plasma sample solution using a protein precipitation method. A chromatographic isolation was carried out using a ZorbaxC18 stationary phase (2.1 × 100 mm, 3 µm) and mobile phase composition of 0.1% formic acid and acetonitrile (15:85). The analytes were quantified using positive ionization mode with ESI. Mass transitions for Tepotinib were 493.23/296.17.09, and for Axitinib (IS) they were 387.12/220.08. No interference from any components of blank plasma or other substances was identified. The relationship between Tepotinib concentrations and their corresponding area of the peak ratios to Axitinib showed a linear pattern across a range of 1.5–1,200 ng/ml. The precision of Tepotinib was excellent, with an intra-day precision of ≤4.92% (n = 10) and an inter-day precision of ≤04.76% (n = 20, over 3 days). The measured average extraction recoveries of Tepotinib were 98.32%.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

FUNDING

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CONFLICTS OF INTEREST

The author reports no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

The study protocol was approved by the Institutional Ethics Committee (Approval No.: HECA-280/A, Date: 23rd Dec 2024).

DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

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USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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