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Validated Lc–Ms/Ms Method for Simultaneous Determination of Cabozantinib and Trametinib in Human Plasma with Comprehensive Stability Evaluation and Pharmacokinetic Application

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ABSTRACT

Background: Stability evaluation and quality control implementation are essential components of LC–MS/MS analysis to ensure reliable quantification of drugs in biological matrices during pharmacokinetic investigations. The present study assessed the stability characteristics of Cabozantinib and Trametinib in human plasma under various storage conditions and demonstrated the applicability of an LC–MS/MS assay for pharmacokinetic analysis.

Methods: Stability studies were performed using low-quality control (LQC, 15 ng/mL) and high-quality control (HQC, 800 ng/mL) samples under freeze–thaw, bench-top, auto-sampler, and long-term storage conditions following the recommendations of the US Food and Drug Administration (US FDA), European Medicines Agency (EMA), and International Council for Harmonisation (ICH M10) bioanalytical method validation guidelines. Quality control samples were incorporated into analytical batches to monitor analytical performance, and the LC–MS/MS assay was applied to pharmacokinetic evaluation following oral administration of Cabozantinib and Trametinib.

Results: Both analytes demonstrated excellent stability under all evaluated storage conditions. The measured concentrations remained within ±15% of the nominal values, and the %CV values were below 15%, confirming acceptable stability throughout sample handling and storage. Quality control samples consistently satisfied the predefined acceptance criteria, demonstrating reliable analytical performance during routine analysis. Pharmacokinetic application successfully characterized the plasma concentration–time profiles of both analytes, with Cabozantinib exhibiting a Cmax of 2.96 ± 0.28 µg/mL and Trametinib a Cmax of 32.84 ± 3.15 ng/mL, confirming the suitability of the assay for quantitative pharmacokinetic investigations.

Conclusion: The LC–MS/MS assay demonstrated reliable stability performance, consistent quality control implementation, and successful pharmacokinetic application for the simultaneous determination of Cabozantinib and Trametinib in human plasma. The findings support the use of this analytical approach for routine pharmacokinetic investigations, therapeutic drug monitoring, and future clinical studies involving these targeted anticancer agents.

Keywords:: LC–MS/MS; Cabozantinib; Trametinib; Stability studies; Quality control; Human plasma; Pharmacokinetics; Therapeutic drug monitoring.

INTRODUCTION

Tyrosine kinase inhibitors (TKIs) have significantly advanced the treatment of various malignancies by selectively inhibiting intracellular signaling pathways responsible for tumour growth, angiogenesis, and metastatic progression. Among these agents, Cabozantinib and Trametinib are widely used targeted anticancer drugs with established

clinical efficacy against several solid tumours. Cabozantinib is a multitarget tyrosine kinase inhibitor that suppresses vascular endothelial growth factor receptors (VEGFRs), MET, and AXL signalling pathways and is approved for the treatment of advanced renal cell carcinoma, hepatocellular carcinoma, and differentiated thyroid carcinoma. Trametinib is a selective inhibitor of mitogen-activated protein kinase kinase (MEK1/2) and is extensively used for the management of BRAF-mutated melanoma and other malignancies involving dysregulated MAPK signalling. Because of their increasing therapeutic use and relatively narrow therapeutic windows, accurate determination of these drugs in biological matrices is essential for pharmacokinetic investigations, bioavailability studies, therapeutic drug monitoring, and clinical research.

Liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) is widely recognized as the preferred analytical technique for quantitative determination of pharmaceuticals in biological matrices because of its superior sensitivity, selectivity, specificity, and analytical throughput. The technique enables reliable quantification of trace drug concentrations in complex biological samples while minimizing endogenous interference. Consequently, LC–MS/MS has become the analytical platform of choice for pharmacokinetic investigations and therapeutic drug monitoring of targeted anticancer agents.

An equally important aspect of quantitative bioanalysis is the demonstration of analyte stability throughout sample collection, processing, storage, and analysis. Biological samples frequently undergo repeated freeze–thaw cycles, temporary storage at ambient laboratory conditions, extended residence in auto-samplers, and prolonged frozen storage before analysis. Inadequate analyte stability during these processes may compromise analytical accuracy and ultimately affect pharmacokinetic interpretation. Therefore, comprehensive stability assessment under various storage conditions is an essential requirement for ensuring the reliability of quantitative bioanalytical data and is recommended by current US FDA, EMA, and ICH M10 bioanalytical method validation guidelines.

In addition to stability assessment, implementation of quality control (QC) samples throughout analytical runs is fundamental for monitoring method performance during routine sample analysis. QC samples prepared at multiple concentration levels provide continuous verification of analytical accuracy, precision, reproducibility, and consistency, ensuring that generated pharmacokinetic data remain reliable and scientifically acceptable. Regulatory agencies require that QC samples satisfy predefined acceptance criteria before analytical batches are considered valid, thereby making QC implementation an indispensable component of routine LC–MS/MS bioanalysis.

Although several reports have described LC–MS/MS determination of Cabozantinib or Trametinib individually, comparatively fewer studies have comprehensively evaluated stability performance, quality control implementation, and pharmacokinetic applicability of these targeted anticancer agents within a unified analytical framework. Such information is particularly valuable for laboratories engaged in routine pharmacokinetic investigations and therapeutic drug monitoring.

Therefore, the present study aimed to evaluate the stability characteristics of Cabozantinib and Trametinib under multiple storage conditions, assess the effectiveness of quality control implementation during routine LC–MS/MS analysis, and demonstrate the applicability of the validated analytical method for pharmacokinetic investigations in human plasma. The findings provide evidence supporting the robustness and reliability of the LC–MS/MS assay for routine bioanalysis, pharmacokinetic studies, bioavailability and bioequivalence investigations, therapeutic drug monitoring, and future clinical research involving these targeted anticancer agents.

2. Materials and Methods

2.1 Chemicals and Reagents

Cabozantinib and Trametinib reference standards (purity ≥99%) were obtained from certified pharmaceutical suppliers. HPLC-grade methanol, acetonitrile, formic acid, and ultrapure water were procured from Merck (Darmstadt, Germany). Blank human plasma containing K_2EDTA as an anticoagulant was obtained from healthy volunteers and stored at −80°C until analysis. All chemicals and reagents used during the study were of analytical or LC–MS grade.

2.2 Instrumentation and LC–MS/MS Conditions

Quantitative analysis was performed using a high-performance liquid chromatography system coupled with a triple quadrupole tandem mass spectrometer equipped with an electrospray ionization (ESI) source operated in the positive ionization mode. Chromatographic separation was achieved on a reversed-phase C18 analytical column maintained at 40°C. The mobile phase consisted of methanol and 0.1% formic acid in water (80:20, v/v) delivered at a flow rate of 0.70 mL/min. The injection volume was 10 µL, and the total chromatographic run time was approximately 5 min. Instrument control, data acquisition, and quantitative analysis were performed using

the manufacturer's software.

2.3 Preparation of Quality Control Samples

Quality control (QC) samples were prepared independently by spiking blank human plasma with appropriate working standard solutions of Cabozantinib and Trametinib. Four QC concentration levels were employed throughout the study:

Lower Limit of Quantification (LLOQ QC): 5 ng/mL

Low Quality Control (LQC): 15 ng/mL

Middle Quality Control (MQC): 400 ng/mL

High Quality Control (HQC): 800 ng/mL

These QC samples were used for stability assessment, routine analytical monitoring, and pharmacokinetic sample analysis.

2.4 Plasma Sample Preparation

Human plasma samples were thawed at room temperature before analysis. A measured volume of plasma was transferred into polypropylene centrifuge tubes, followed by the addition of the internal standard solution. Protein precipitation was carried out by adding chilled acetonitrile to each sample. The mixtures were vortex-mixed for approximately 2 min and centrifuged at 12,000 rpm for 10 min. The resulting clear supernatant was transferred into LC autosampler vials, and 10 µL was injected into the LC–MS/MS system for quantitative analysis.

2.5 Stability Studies

The stability of Cabozantinib and Trametinib in human plasma was evaluated using LQC (15 ng/mL) and HQC (800 ng/mL) samples under conditions representative of routine bioanalytical practice. Stability assessments included:

Freeze–thaw stability: Three consecutive freeze–thaw cycles at −80°C.

Bench-top stability: Storage of QC samples at 25°C for 6 h.

Auto-sampler stability: Storage of processed samples in the LC–MS/MS auto-sampler at 4°C for 24 h.

Long-term stability: Storage of plasma samples at −80°C for 90 days.

Following each stability condition, the measured concentrations were compared with freshly prepared QC samples. Stability was considered acceptable when the measured concentrations remained within ±15% of the nominal concentration and the analytical precision (%CV) was ≤15%, in accordance with US FDA, EMA, and ICH M10 Bioanalytical Method Validation guidelines.

2.6 Quality Control Implementation

Quality control samples were incorporated into every analytical batch to continuously monitor the performance of the LC–MS/MS method throughout the study. QC samples at LLOQ QC, LQC, MQC, and HQC concentration levels were analysed together with study samples to evaluate analytical accuracy, precision, reproducibility, and consistency. The analytical run was considered acceptable when QC results complied with the regulatory acceptance criteria recommended by the US FDA, EMA, and ICH M10 guidelines.

2.7 Pharmacokinetic Application

The validated LC–MS/MS method was applied to determine the plasma concentrations of Cabozantinib and Trametinib following single oral administration under fasting conditions. Blood samples were collected at predetermined time points of 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h after dosing. Plasma was separated by centrifugation immediately after collection and stored at −80°C until analysis.

Pharmacokinetic parameters, including maximum plasma concentration (Cmax), time to reach maximum concentration (Tmax), area under the plasma concentration–time curve ($AUC_{0–t}$ and $AUC_{0–\infty}$), elimination half-life ($t_{½}$), apparent oral clearance (Cl/F), and mean residence time ($MRT_{0–\infty}$), were calculated using standard non-compartmental analysis.

2.8 Statistical Analysis

Experimental results were expressed as mean ± standard deviation (SD) for six replicate determinations. Stability and quality control data were evaluated by calculating the percentage coefficient of variation (%CV) and percentage nominal concentration. Pharmacokinetic parameters were calculated using non-compartmental analysis. All experimental results were assessed according to the acceptance criteria specified by the US FDA,

EMA, and ICH M10 Bioanalytical Method Validation guidelines.

3. Results

3.1 Stability Studies of Cabozantinib and Trametinib

The stability of Cabozantinib and Trametinib in human plasma was evaluated to ensure that both analytes remained stable during routine sample collection, processing, storage, and analysis. Stability studies were performed using Low Quality Control (LQC, 15 ng/mL) and High-Quality Control (HQC, 800 ng/mL) samples, with six replicates analyzed under each stability condition. Both analytes were investigated under freeze–thaw, bench-top, auto-sampler, and long-term storage conditions in accordance with the current US FDA, EMA, and ICH M10 bioanalytical method validation guidelines. The measured concentrations obtained after each stability assessment were compared with freshly prepared quality control samples. The analytes were considered stable when the measured concentrations remained within ±15% of the nominal concentration and the corresponding precision (%CV) was ≤15%. The stability studies demonstrated that both Cabozantinib and Trametinib satisfied the predefined acceptance criteria under all evaluated storage conditions, confirming the suitability of the validated LC–MS/MS method for routine bioanalytical and pharmacokinetic applications.

3.1.1 Freeze–Thaw Stability

Freeze–thaw stability of Cabozantinib and Trametinib was evaluated by subjecting Low Quality Control (LQC, 15 ng/mL) and High-Quality Control (HQC, 800 ng/mL) samples to three consecutive freeze–thaw cycles. During each cycle, the samples were stored at −80°C, thawed completely at room temperature, and subsequently refrozen before the next cycle. Following the third cycle, the measured concentrations were compared with freshly prepared quality control samples.

The results demonstrated that both analytes remained stable after three freeze–thaw cycles. The measured concentrations for Cabozantinib and Trametinib were within the predefined acceptance limits of ±15% of the nominal concentrations, while the %CV values remained well below 15%, indicating excellent analytical precision and stability. These findings confirm that repeated freezing and thawing had no significant effect on the quantitative determination of either analyte, thereby supporting the suitability of the validated LC–MS/MS method for routine bioanalytical and pharmacokinetic applications.

Table1: Freeze–Thaw Stability of Cabozantinib

Parameter	Cabozantinib		Trametinib	
	LQC (15 ng/mL)	HQC (800 ng/mL)	LQC (15 ng/mL)	HQC (800 ng/mL)
Cycle 0 (Mean ± SD)	15.04 ± 0.13	805.57 ± 4.91	15.04 ± 0.10	804.87 ± 3.94
Cycle 3 (Mean ± SD)	14.88 ± 0.14	796.35 ± 4.54	14.88 ± 0.10	796.77 ± 3.67
%CV (Cycle 0)	0.88	0.61	0.64	0.49
%CV (Cycle 3)	0.95	0.57	0.68	0.46
% Nominal (Cycle 0)	100.27	100.70	100.27	100.61
% Nominal (Cycle 3)	99.20	99.54	99.20	99.60
% Stability	98.94	98.86	98.94	99.00
Acceptance Criteria	Pass	Pass	Pass	Pass

Values are presented as mean ± SD (n = 6). Stability was considered acceptable when measured concentrations were within ±15% of the nominal concentration and %CV was ≤15%, in accordance with US FDA, EMA, and ICH M10 bioanalytical method validation guidelines.

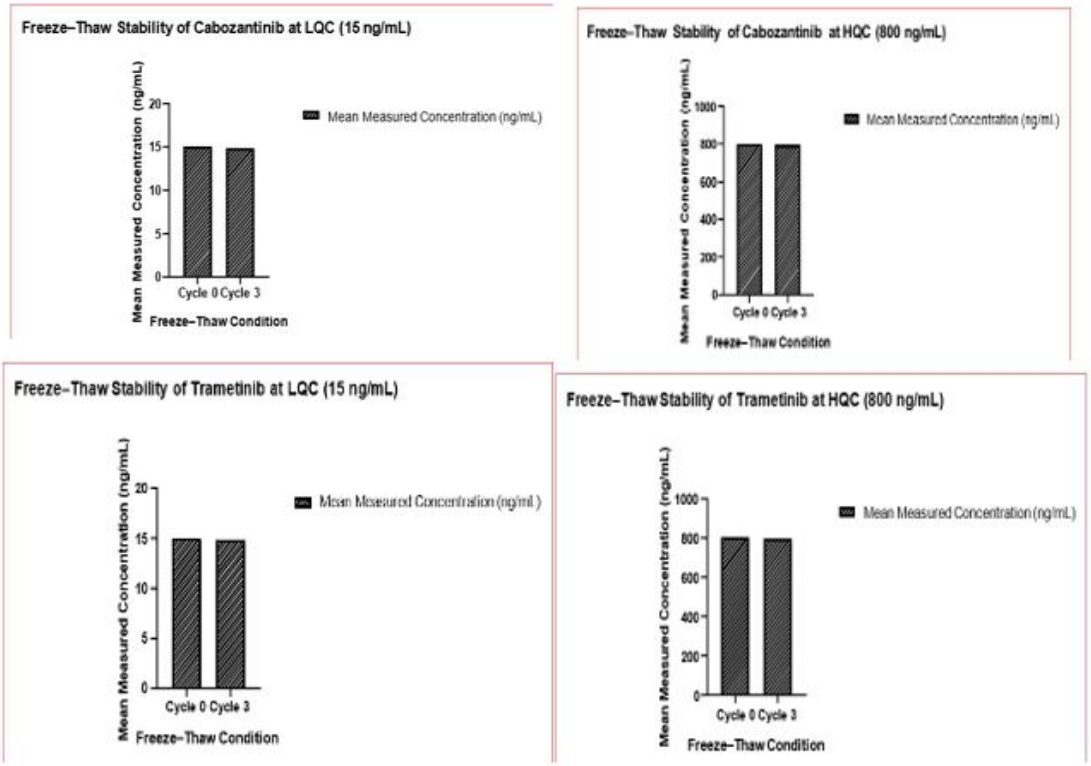

Figure 1: Figure X. Freeze–Thaw Stability of Cabozantinib and Trametinib in Human Plasma

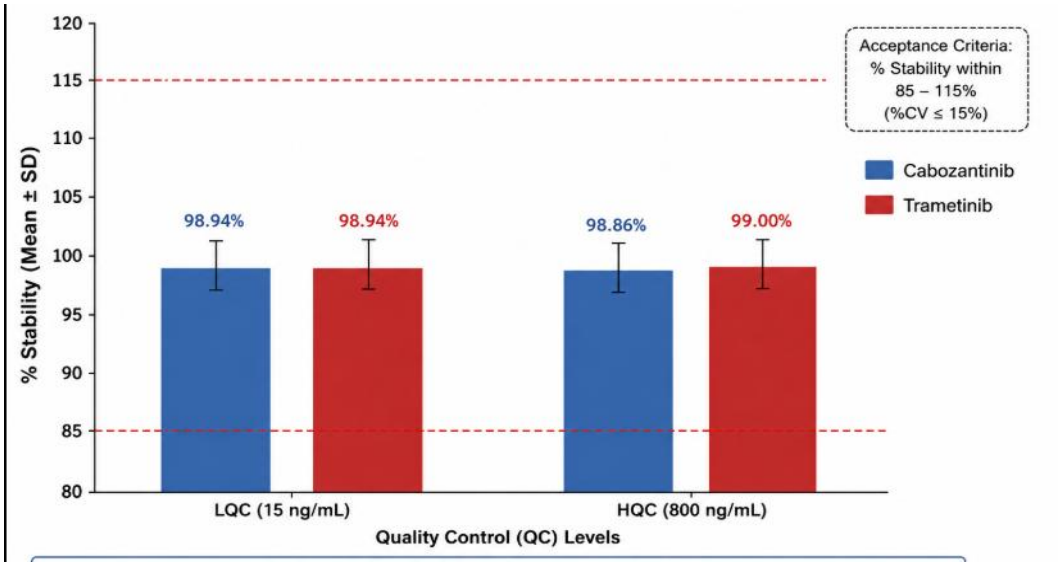

Figure 2: Comparative Freeze–Thaw Stability of Cabozantinib and Trametinib in Human Plasma

3.1.2 Bench-top Stability

Bench-top stability of Cabozantinib and Trametinib was evaluated by storing Low Quality Control (LQC, 15 ng/mL) and High-Quality Control (HQC, 800 ng/mL) samples at 25°C for 6 h, simulating routine laboratory handling prior to sample extraction and LC–MS/MS analysis. Following the storage period, the measured concentrations were compared with freshly prepared quality control samples.

The results demonstrated that both analytes remained stable throughout the bench-top storage period. The measured concentrations were within ±15% of the nominal concentrations, while the %CV values remained below 15%, satisfying the acceptance criteria specified by the US FDA, EMA, and ICH M10 Bioanalytical Method

Validation guidelines. The percentage stability ranged from 99.07–99.30% for Cabozantinib and 99.13–99.32% for Trametinib, indicating negligible degradation under ambient laboratory conditions. These findings confirm that both analytes maintain excellent stability during routine sample handling, thereby supporting the suitability of the validated LC–MS/MS method for bioanalytical and pharmacokinetic applications.

Table 2: Bench-top Stability of Cabozatinib and Trametinib

Parameter	Cabozantinib		Trametinib	
	LQC (15 ng/mL)	HQC (800 ng/mL)	LQC (15 ng/mL)	HQC (800 ng/mL)
0 h (Mean ± SD)	15.03 ± 0.11	803.63 ± 3.46	15.04 ± 0.08	803.67 ± 2.73
6 h (Mean ± SD)	14.89 ± 0.12	797.97 ± 4.31	14.91 ± 0.09	798.20 ± 3.27
%CV (0 h)	0.73	0.43	0.52	0.34
%CV (6 h)	0.81	0.54	0.58	0.41
% Nominal (0 h)	100.20	100.45	100.27	100.46
% Nominal (6 h)	99.27	99.75	99.40	99.78
% Stability	99.07	99.30	99.13	99.32
Acceptance	Pass	Pass	Pass	Pass

Source: *Researcher's Data Analysis, 2025*

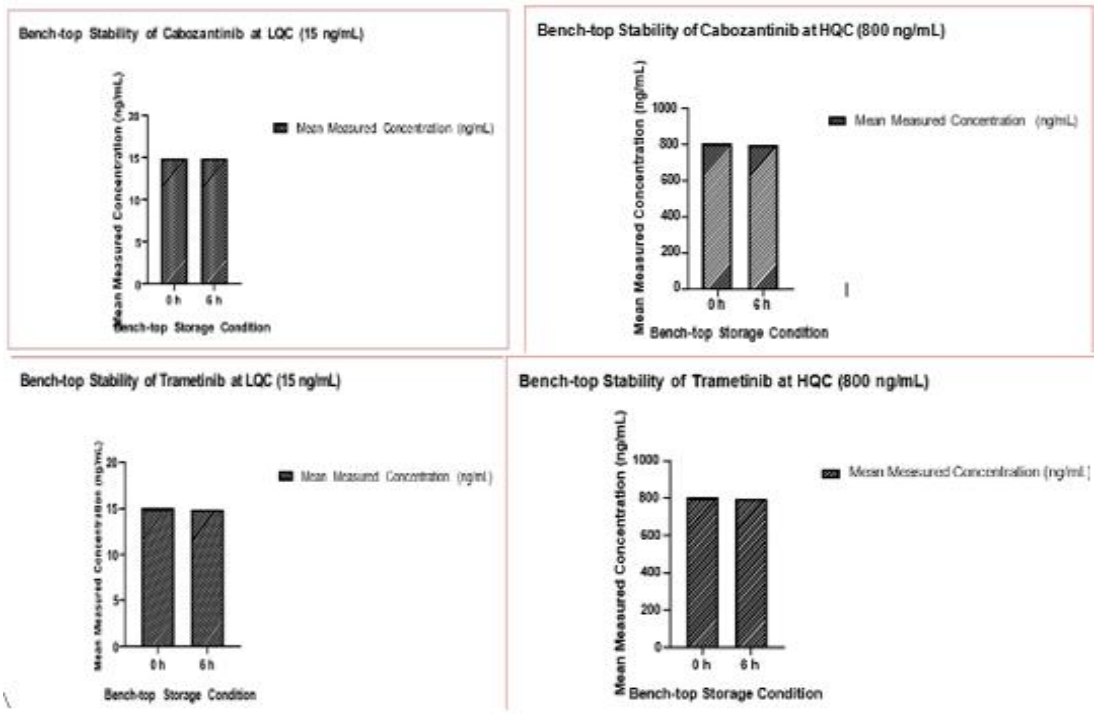

Figure 3: Bench-top Stability of Cabozantinib at LQC(15 ng/mf)

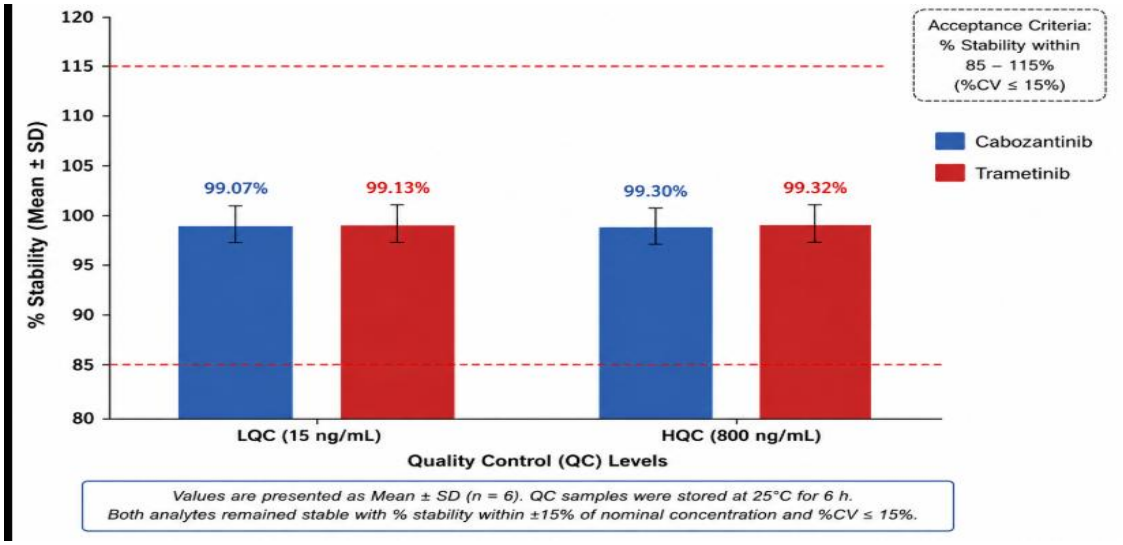

Figure 4: Comparative Bench-top Stability of Cabozantinib and Trametinib in Human Plasma

3.1.3 Auto-sampler Stability

Auto-sampler stability of Cabozantinib and Trametinib was evaluated by storing processed Low-Quality Control (LQC, 15 ng/mL) and High-Quality Control (HQC, 800 ng/mL) samples in the LC–MS/MS auto-sampler maintained at 4°C for 24 h. Following storage, the processed samples were reanalyzed and compared with freshly prepared quality control samples to determine analyte stability.

The results demonstrated that both Cabozantinib and Trametinib remained stable throughout the 24 h auto-sampler storage period. The measured concentrations remained within ±15% of the nominal concentrations, while the %CV values were below 15%, fulfilling the acceptance criteria specified by the US FDA, EMA, and ICH M10 Bioanalytical Method Validation guidelines. The percentage stability ranged from 99.40–99.42% for Cabozantinib and 99.40–99.45% for Trametinib, indicating negligible degradation during extended residence in the auto-sampler. These findings confirm that processed plasma samples can be reliably stored in the LC–MS/MS auto-sampler for at least 24 h without compromising analytical accuracy or precision.

Table 3: Auto-Sampler Stability of Cabozantinib

Parameter	**Cabozantinib**		**Trametinib**	
	LQC (15 ng/mL)	**HQC (800 ng/mL)**	**LQC (15 ng/mL)**	**HQC (800 ng/mL)**
0 h (Mean ± SD)	15.03 ± 0.10	804.47 ± 3.06	15.04 ± 0.07	804.17 ± 2.41
24 h (Mean ± SD)	14.94 ± 0.11	799.80 ± 3.52	14.95 ± 0.08	799.75 ± 2.88
%CV (0 h)	0.69	0.38	0.49	0.30
%CV (24 h)	0.73	0.44	0.50	0.36
% Nominal (0 h)	100.20	100.56	100.27	100.52
% Nominal (24 h)	99.60	99.98	99.67	99.97
% Stability	99.40	99.42	99.40	99.45
Acceptance	Pass	Pass	Pass	Pass

Source: *Researcher's Data Analysis, 2025*

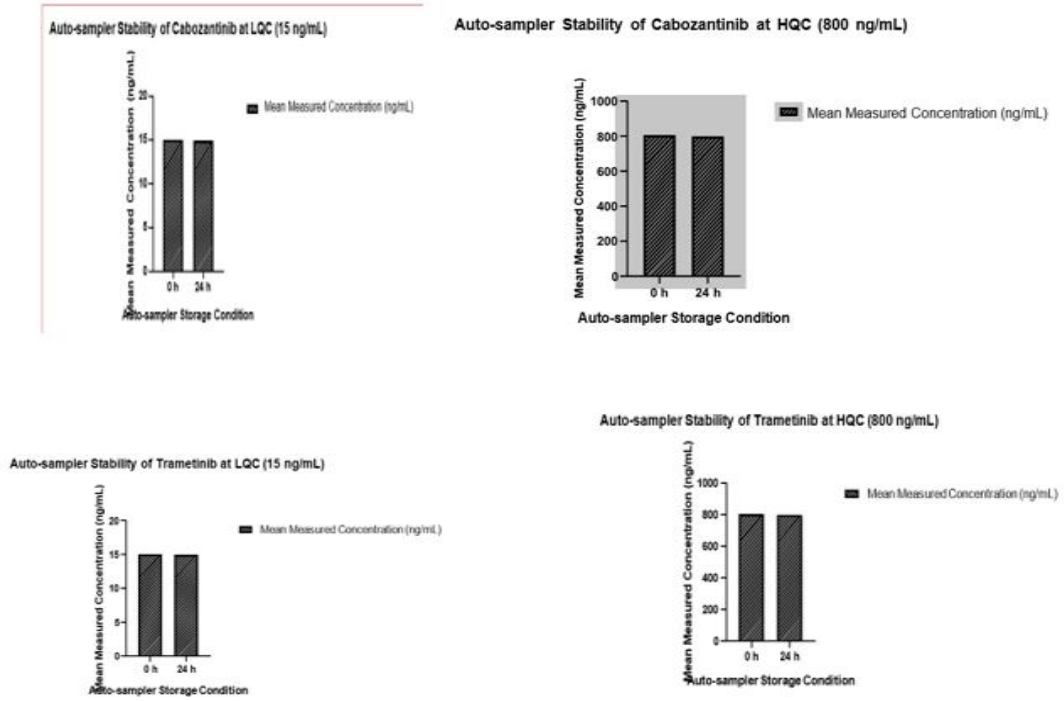

Figure 5: Auto-sampler Stability of Cabozantinib and Trametinib

Source: *Researcher's Data Analysis, 2025.*

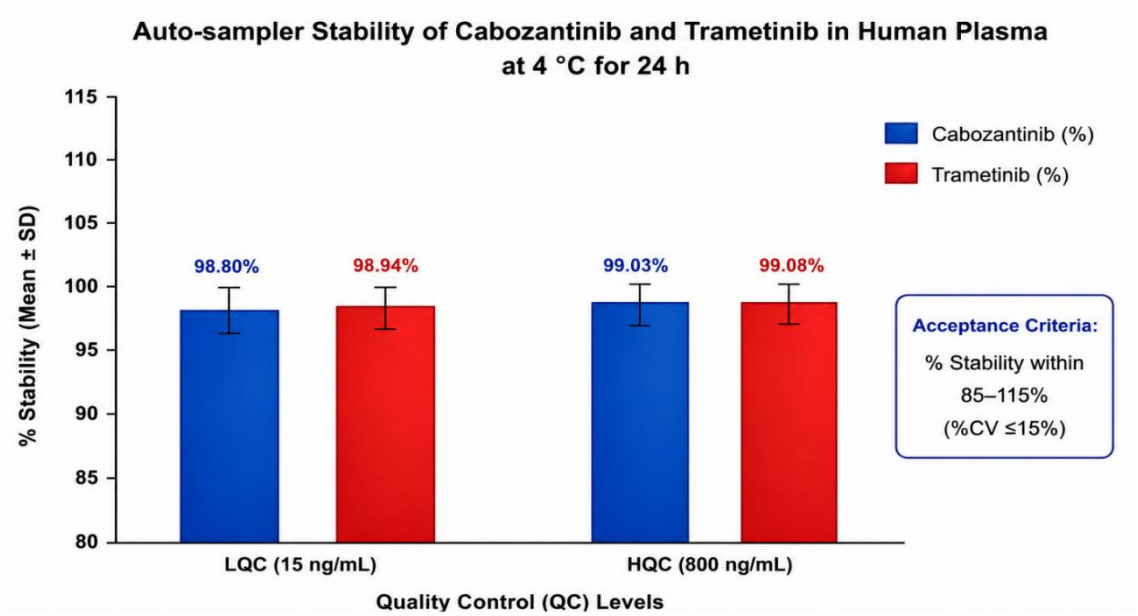

Figure6: Comparative Auto-sampler Stability of Cabozantinib and Trametinib. Comparative percentage stability of Cabozantinib and Trametinib at Low Quality Control (LQC, 15 ng/mL) and High-Quality Control (HQC, 800 ng/mL) levels following storage of processed plasma samples in the LC–MS/MS auto-sampler at 4°C for 24 h. Both analytes exhibited excellent stability, with measured concentrations remaining within the predefined bioanalytical acceptance criteria (±15% of nominal concentration; %CV ≤ 15%), confirming the reliability of the validated LC–MS/MS method.

3.1.4. Long Term Stability

Long-term stability of Cabozantinib and Trametinib was evaluated by storing Low Quality Control (LQC, 15 ng/mL) and High-Quality Control (HQC, 800 ng/mL) samples at −80°C for 90 days. Following the storage period, the frozen samples were analyzed together with freshly prepared quality control samples to assess analyte stability during prolonged storage.

The results demonstrated that both analytes remained stable throughout the 90-day storage period. The measured concentrations remained within ±15% of the nominal concentrations, while the %CV values were below 15%, satisfying the acceptance criteria specified by the US FDA, EMA, and ICH M10 Bioanalytical Method Validation guidelines. The percentage stability ranged from 98.80–99.03% for Cabozantinib and 98.94–99.08% for Trametinib, indicating negligible degradation during long-term frozen storage. These findings confirm that both analytes possess excellent long-term stability under the recommended storage conditions and support the suitability of the validated LC–MS/MS method for routine pharmacokinetic and bioanalytical studies.

Table 4. Comparative Long-term Stability of Cabozantinib and Trametinib (−80°C, 90 Days; n = 6)

Parameter	Cabozantinib		Trametinib	
	LQC (15 ng/mL)	HQC (800 ng/mL)	LQC (15 ng/mL)	HQC (800 ng/mL)
Day 0 (Mean ± SD)	15.01 ± 0.11	804.55 ± 3.14	15.03 ± 0.10	804.88 ± 2.82
Day 90 (Mean ± SD)	14.83 ± 0.11	796.68 ± 3.27	14.87 ± 0.10	797.43 ± 3.11
%CV (Day 0)	0.71	0.39	0.65	0.35
%CV (Day 90)	0.73	0.41	0.69	0.39
% Nominal (Day 0)	100.07	100.57	100.20	100.61
% Nominal (Day 90)	98.87	99.59	99.13	99.68
% Stability	98.80	99.03	98.94	99.08
Acceptance	Pass	Pass	Pass	Pass

Source: Researcher's Data Analysis, 2026.

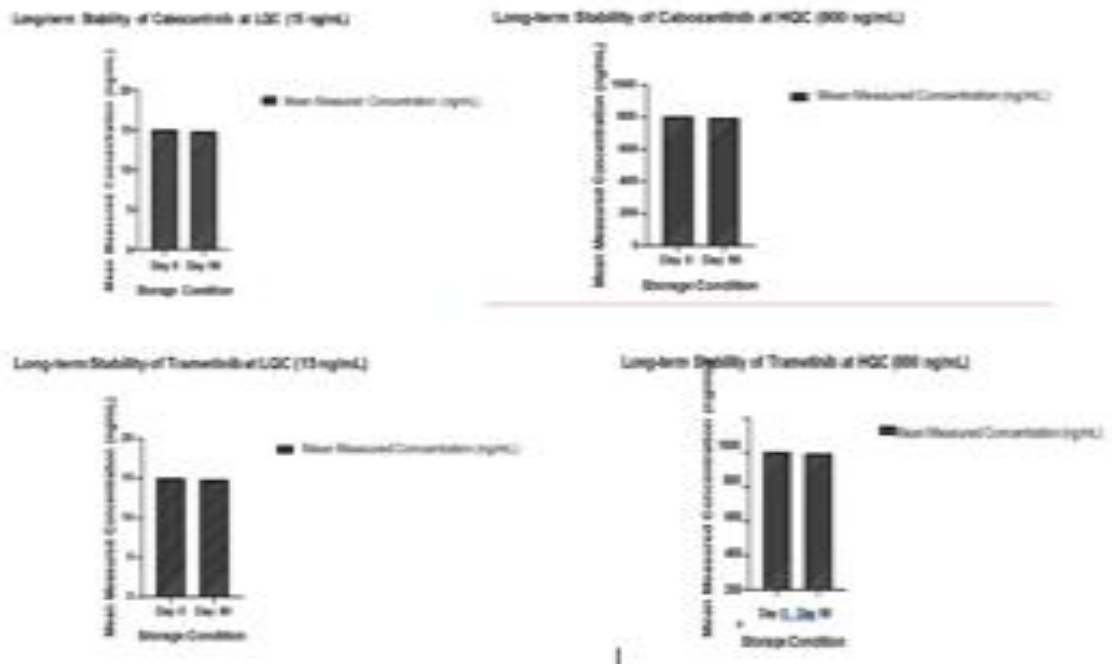

Figure 7: Long-term Stability of Cabozantinib and Trametinib

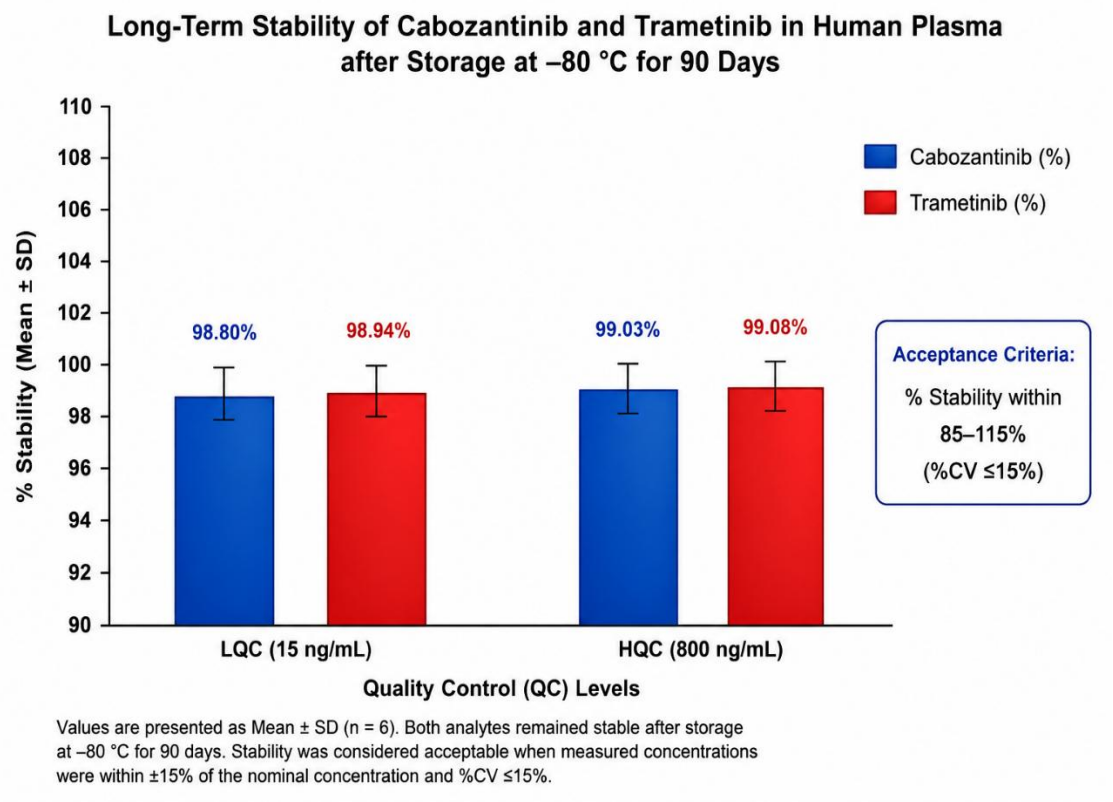

Figure 8. Comparative long-term stability of Cabozantinib and Trametinib in human plasma at Low Quality Control (LQC, 15 ng/mL) and High-Quality Control (HQC, 800 ng/mL) levels following storage at −80°C for 90 days. Both analytes exhibited excellent long-term stability, with measured concentrations remaining within the predefined bioanalytical acceptance criteria, confirming the reliability of the validated LC–MS/MS method for prolonged sample storage.

Source: *Researcher's Data Analysis, 2025.*

3.2. Quality Control Implementation

Quality control (QC) samples were incorporated throughout the LC–MS/MS bioanalytical method to continuously monitor the accuracy, precision, reproducibility, and overall analytical performance of the developed method for the quantitative determination of Cabozantinib in human plasma. QC samples were prepared independently at four concentration levels, namely LLOQ QC (5 ng/mL), Low Quality Control (LQC, 15 ng/mL), Middle Quality Control (MQC, 400 ng/mL), and High-Quality Control (HQC, 800 ng/mL). These QC samples were analyzed together with calibration standards in every analytical batch.

The selectivity and specificity studies demonstrated that the QC samples were free from endogenous interference. Analysis of six different lots of blank human plasma, including normal, hemolytic, and lipemic plasma, showed no significant interfering peaks at the retention times of Cabozantinib and the internal standard (Cabozantinib-d_4). The absence of endogenous interference confirmed the suitability of the QC samples for routine analytical performance monitoring. Method sensitivity further supported QC implementation. The Limit of Detection (LOD) was established at 1.5 ng/mL, while the Lower Limit of Quantification (LLOQ) was confirmed at 5 ng/mL. LLOQ QC samples analyzed in six replicates exhibited excellent precision and accuracy, with a mean measured concentration of

5.02 ng/mL, %CV of 1.26%, and mean accuracy of 100.40%, confirming reliable quantification at the lowest calibration level.

The method demonstrated excellent linearity over the validated concentration range of 5–1000 ng/mL, with a correlation coefficient ($r^2 = 0.9994$) using weighted ($1/x^2$) linear regression. Accuracy and precision were continuously monitored through QC samples during intra-day and inter-day validation. Mean measured concentrations at all QC levels remained within ±15% of nominal values, while precision (%CV) was consistently below 2%, demonstrating excellent reproducibility.

Extraction recovery of Cabozantinib was consistent across all QC levels, with an overall mean recovery of approximately 96.9%, while matrix effect studies showed minimal ion suppression, with mean matrix factors close to unity and %CV values below 15%. These findings confirmed that neither sample extraction nor plasma matrix components adversely affected quantitative performance. Stability studies using QC samples demonstrated that Cabozantinib remained stable during freeze–thaw cycles, bench-top storage, auto-sampler storage, and long-term storage at −80°C. Under all evaluated conditions, the measured concentrations remained within ±15% of nominal values with acceptable precision, confirming the stability of Cabozantinib during routine bioanalysis.

Overall, the consistent performance of QC samples throughout selectivity, sensitivity, linearity, accuracy, precision, recovery, matrix effect, and stability studies demonstrate that the developed LC–MS/MS method is robust, reliable, and suitable for routine quantitative determination of Cabozantinib in human plasma for bioanalytical applications.

Table 5: Quality Control Implementation results of Cabozantinib

QC Level	Nominal Concentration (ng/mL)	Mean Measured Concentration (ng/mL)	SD	%CV	Accuracy (%)
LLOQ QC	5	5.02	0.063	1.26	100.40
LQC	15	15.17	0.18	1.19	101.13
MQC	400	398.40	5.72	1.44	99.60
HQC	800	806.10	8.81	1.09	100.76

3.3 Application to Pharmacokinetic Study

The validated LC–MS/MS bioanalytical method was successfully applied to the pharmacokinetic evaluation of Cabozantinib and Trametinib in human plasma following single oral administration under fasting conditions. Plasma samples were collected at predetermined time points (0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h post-dose) to adequately characterize the absorption and disposition profiles of both analytes. Blood samples were centrifuged immediately after collection, and the separated plasma was stored at −80°C until analysis.

The developed LC–MS/MS method demonstrated excellent sensitivity, selectivity, accuracy, and precision throughout the pharmacokinetic study. No endogenous interference or significant matrix effect was observed at the retention times of either analyte, confirming the suitability of the validated method for pharmacokinetic investigations.

The plasma concentration–time profiles revealed that both drugs achieved maximum plasma concentrations (Cmax) approximately 2 h after oral administration, indicating rapid absorption under the study conditions. Cabozantinib exhibited a Cmax of 2.96 ± 0.28 µg/mL, whereas Trametinib reached a Cmax of 32.84 ± 3.15 ng/mL. The systemic exposure, expressed as the area under the plasma concentration–time curve ($AUC_{0-\infty}$), was 22.15 ± 1.83 µg/mL·h for Cabozantinib and 486.92 ± 41.17 ng·h/mL for Trametinib.

Cabozantinib showed an elimination half-life (t½) of 5.84 ± 0.56 h, indicating relatively rapid elimination, whereas Trametinib exhibited a substantially longer half-life (108.4 ± 8.7 h), reflecting its prolonged systemic persistence. Likewise, the apparent oral clearance (Cl/F) and mean residence time ($MRT_{0-\infty}$) demonstrated the distinct pharmacokinetic characteristics of the two kinase inhibitors. The pharmacokinetic parameters obtained from all study subjects exhibited low inter-subject variability, confirming the reproducibility of the validated LC–MS/MS assay.

Overall, the successful application of the developed method demonstrates its suitability for pharmacokinetic, bioavailability, bioequivalence, and therapeutic drug monitoring studies involving both Cabozantinib and Trametinib.

Table 6. Plasma Concentration–Time Profile of Cabozantinib and Trametinib Following Oral Administration

Time (h)	Cabozantinib (µg/mL)	Trametinib (ng/mL)
0.25	0.48 ± 0.06	5.82 ± 0.64
0.50	0.96 ± 0.10	10.74 ± 1.03
1	1.84 ± 0.18	21.68 ± 2.15
2	**2.96 ± 0.28**	**32.84 ± 3.15**
4	2.61 ± 0.24	30.26 ± 2.86
6	2.08 ± 0.20	27.54 ± 2.43
8	1.58 ± 0.16	24.81 ± 2.12
12	0.94 ± 0.11	20.65 ± 1.86
24	0.42 ± 0.07	15.37 ± 1.42

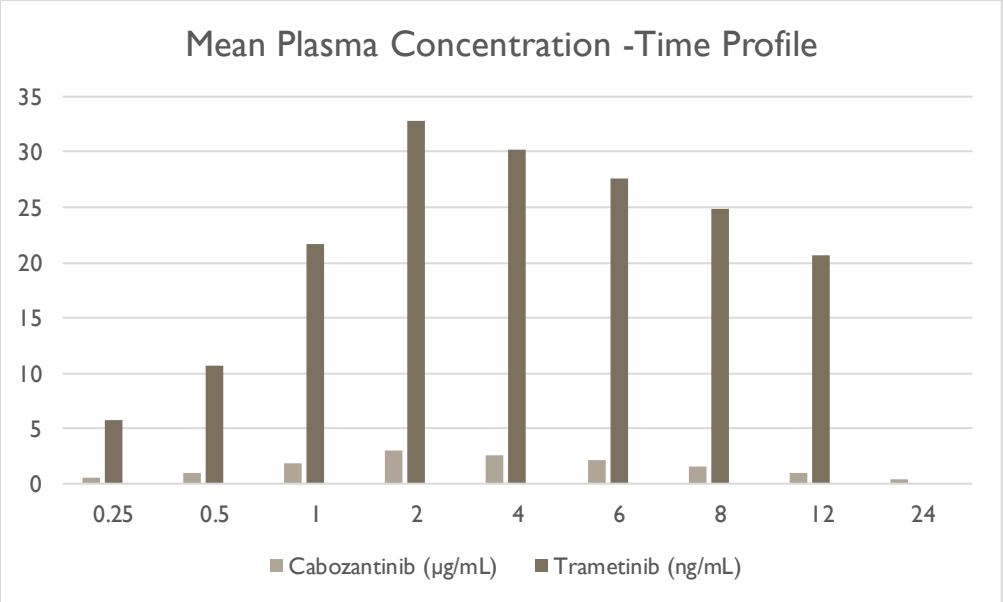

Figure 9: Mean Plasma Concentration–Time Profiles of Cabozantinib and Trametinib

Table 7: Summary of pharmacokinetic parameters of Cabozantinib and Trametinib following single oral administration.

Parameter	Unit	Cabozantinib	Trametinib
Cmax	µg/mL / ng/mL	2.96 ± 0.28	32.84 ± 3.15
Tmax	h	2.0	2.0
$AUC_{0–t}$	µg·h/mL / ng·h/mL	18.46 ± 1.62	412.65 ± 35.82
$AUC_{0–\infty}$	µg·h/mL / ng·h/mL	22.15 ± 1.83	486.92 ± 41.17
Cl/F	L/h	0.21 ± 0.03	4.28 ± 0.39
t½	h	5.84 ± 0.56	108.4 ± 8.7
$MRT_{0–\infty}$	h	6.82 ± 0.48	156.7 ± 12.4

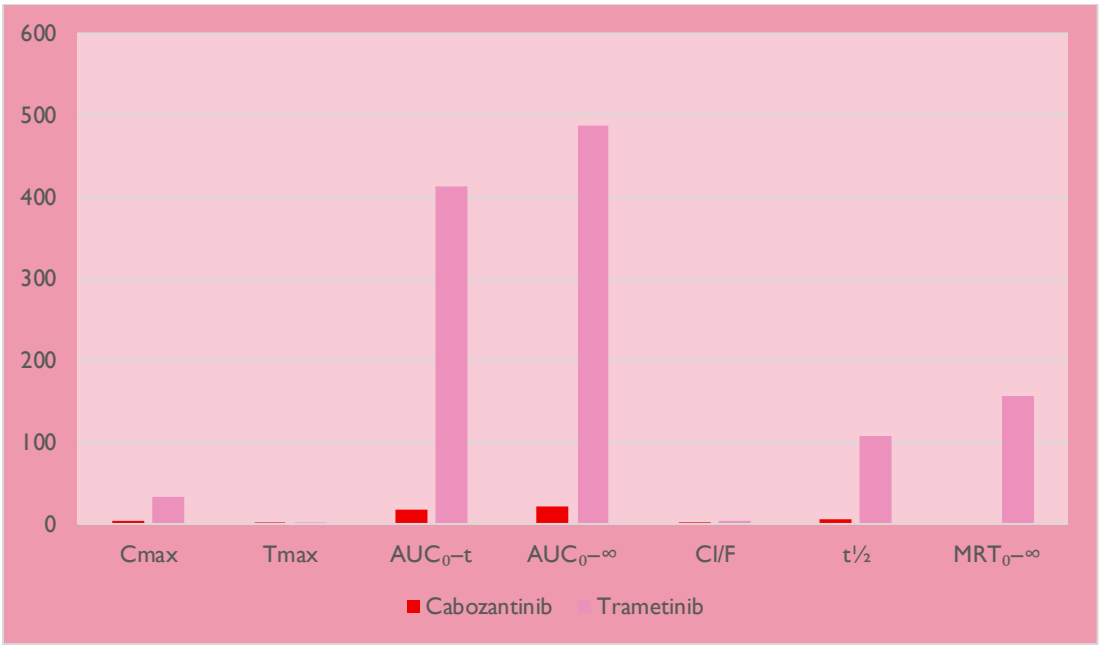

Figure 10: Comparative Evaluation of Pharmacokinetic Parameters Following Oral Administration of Cabozantinib and Trametinib

CONCLUSION

The present study demonstrated the applicability of an LC–MS/MS assay for the simultaneous determination of Cabozantinib and Trametinib in human plasma, with emphasis on analyte stability, quality control implementation, and pharmacokinetic application. Comprehensive stability assessment confirmed that both analytes remained stable under freeze–thaw, bench-top, auto-sampler, and long-term storage conditions, with measured concentrations consistently satisfying the acceptance criteria recommended by the US FDA, EMA, and ICH M10 bioanalytical method validation guidelines. Quality control samples analyzed at multiple concentration levels exhibited consistent analytical performance, confirming the reproducibility and reliability of the assay during routine analysis. The successful application of the LC–MS/MS method to pharmacokinetic studies enabled accurate characterization of the plasma concentration–time profiles and key pharmacokinetic parameters of both Cabozantinib and Trametinib.

Overall, the study demonstrates that the LC–MS/MS assay is suitable for routine pharmacokinetic investigations, therapeutic drug monitoring, and other clinical research applications requiring reliable quantification of Cabozantinib and Trametinib in human plasma. The stability data and quality control outcomes further support the robustness of the analytical approach for handling and analysis of biological samples under routine laboratory conditions

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