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A Facile RP-HPLC Method for the Determination of Capmatinib in Human Plasma: Development and Validation

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KEYWORDS
Capmatinib (CPTB), Erlotinib (ETB), Bio-analytical, Internal Standard.

ABSTRACT:
A simple, precise, accurate, sensitive, repeatable, and RP-HPLC technique was established and validated for assessing Capmatinib in human plasma. A Hypersil C18 column (250mm 4.6mm; 5 m) was employed for the chromatographic separation. The orthophosphoric acid buffer, methanol, and acetonitrile ratio of the mobile phase has been optimized at a flow rate of 1.0 ml/min. A model UV detector with a variable wavelength was used to measure the detection wave length at 252 nm. Capmatinib (CPTB) and Erlotinib (ETB), which was employed as an internal standard, were perfectly eluted from plasma samples using the liquid-liquid extraction procedure with methanol as the solvent. The CPTB and ETB (IS) retention times using the developed method are determined to be 3.1 and 5.9 min, respectively. The assay shows a linear ($r2 > 0.99$) calibration range over the concentration range of 5-30µg/mL of plasma analyte concentration. This method also demonstrated an intra and inter-day precision within the range of 0.41%-0.86% and 0.44%-0.94%, respectively. The obtained LOQ value was calculated as 5.0µg/mL with precision and accuracy. The mean recovery of CPTB was 98.95%–100.12%.Utilizing human plasma, a simple, rapid, accurate, linear, precise, and robust bio-analytical approach was developed and validated. The method was firmly validated in accordance with ICH recommendations. The results obtained demonstrate that the proposed method can be successfully used for routine Capmatinib analysis in human plasma.

Journal of Chemical Health Risks
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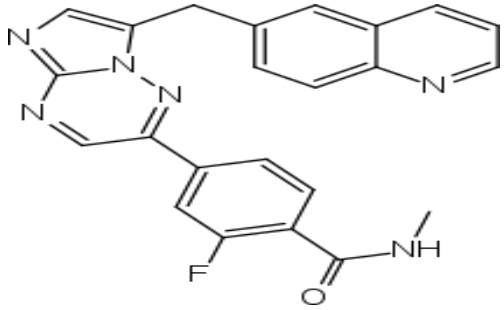
1. INTRODUCTION

A kinase inhibitor called Capmatinib is used to treat adults, who had metastatic non-small cell lung cancer (NSCLC) whose tumours have been found to have an exon 14 skipping mutation in the MET (mesenchymal epithelial transition) gene, which codes regarding the membrane receptor HGFR (hepatocyte growth factor receptor).

Fig.-1: Chemical structure of Capmatinib



It is a MET factor inhibitor that is orally bioavailable and has anti-cancer activity. It gained its initial approval in the USA on May 6th, 2020. Fig. 1 depicts the CPTB's organisational structure. In tablet dose forms, the medication can be consumed orally. It is an oral MET inhibitor with potent anticancer activity and excellent MET kinase selectivity, is available [1-6]. CPTB has confirmed preclinical activity in multiple trials for advanced multiforme, glioblastoma, hepatocellular carcinoma, and non-small-cell lung cancer. It can be used alone or in combination with other drugs (NCT01911507, NCT01737827, and NCT02386826).

For instance, a patient with stage Ib very inadequately differentiated squamous carcinoma of the lung (LSCC) responded to CPTB therapy over a 13-month period, resulting in a 61% decrease in tumour size. Additionally, Capmatinib promotes hepatocyte growth factor apoptosis and renovates sensitivity to Erlotinib in NSCLC mice that are resistant to the drug [7–15]. Peripheral edoema, tiredness, vomiting, dyspnea, and decreased appetite are the most frequent side effects.

Despite being established in an analytical investigation using LC-MS/MS to measure CPTB in plasma [16], Xiaoguang Fan et al. did not publish details about sample preparation, quantitative ion pairs, mobile phase, or method validation in the literature review. By utilizing UPLC-MS/MS techniques, ChunlingZhou et., al. explained only in rat plasma [17]. Due to recent disclosures by Shubhada Dalvi et., al. and Suresh Dhone et., al. regarding formulation using HPLC methods, there is currently no bio-analytical RP-HPLC method for Capmatinib drug in human plasma. [18–19]. The primary goal is to create an RP-HPLC technique for the assessment of Capmatinib in human plasma based on the literature.

2. EXPERIMENTAL

2.1. Chemicals and Reagents:

Pharmaceutical grade CPTB and ETB drug samples were obtained as a gift sample. Pooled drug- free expired frozen human plasma (K2-EDTA as anticoagulant) was procured from collections at local diagnostic laboratories. Merck Chemicals,

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(4), 221-234 | ISSN: 2251-6719

Mumbai, India, supplied all other chemicals and buffers. Milli-Q System HPLC-grade water was used.

2.2. Instrumentation:

The HPLC system used for method development of Capmatinib was equipped with a pump (model G1311), an automatic injection (ALS) with a capacity of 0.1 to 1500 l (model G1329A), COLCOM G1316A column temperature control with thermostat, and a G1314A model variable wavelength UV detector with standard flow cell. Chromatographic separation was performed on this system. The chromatographic separation was achieved with the Xtimate C18 column (250mm×4.6mm; 5µm id) column (Paisley, UK) at ambient temperature. The instrument was controlled using Software (Chemstation-Version Rev B.04.03 (16)), was utilized for data processing, analysis, and reporting.

2.3. Preparation of stock solutions of CPTB and ETB (IS):

The standard stock solution used during the LC method development stage was prepared by dissolving CPTB (1.0mg/mL). It was made by exactly weighing 10 mg of CPTB in a 10mL volumetric flask after diluting it with methanol. By accurately weighing 10mg of the ETB (IS) taken in a 10mL volumetric flask and volume made up with diluent, main stock solutions of the ETB (IS) were prepared separately.

A 10mL volumetric flask was filled with 1.0mL of the stock solution and 1.0mL of diluent to establish a 10g/mL solution. Both stock solutions are stored at 2-8°C. By combining and further diluting the stock solution with methanol, a series of working standard solutions of each analyte were prepared at the proper concentrations. To create an appropriate standard solution of the IS (5g/mL), the stock solution of the IS was diluted with methanol. In order to prepare the calibration curve and quality control (QC) samples in human plasma, these diluted working standard solutions were employed.

2.4. Calibration standards and quality control samples preparation:

Prior to spiking, blank human plasma performed screening to confirm that it was devoid of endogenous interference at the retention times of the CPTB and ETB internal standards. To create the working solutions needed for the calibration curve plotting, working standard solution was spiked into blank human plasma to produce seven different concentrations of CPTB that ranged from 5 to 35 g/mL (i.e., 5, 10, 15, 20, 25, and 35 g/mL). To create the low (5 g/mL, LQC), medium (20 g/mL, MQC), and high (35 g/mL, HQC) concentrations of CPTB, suitable volumes of QC- working solutions (from a separate batch to that for the calibration standard) were added to blank human plasma. All samples contained an internal standard concentration of 20 g/mL.

2.5. Liquid-liquid extraction procedure:

Simple liquid extraction (LLE) technique with performed by taking the 250µL plasma sample, 100µL of CPTB and 100µL ETB internals standard working standard concentrations were placed into a centrifuge tube and 2mL of methanol was added and allowed for cyclomixer for 30Sec. This was followed by the addition of 3mL of methanol and vortexed for 10 minutes and finally centrifuged for 5 minutes at 4000 rpm. After centrifugation, the organic supernatant layer was collected and filtered through 0.45µm syringe driven membrane filter unit. Finally, a 20µL of each sample of CPTB was transferred into

Journal of Chemical Health Risks
www.jchr.org
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auto sampler vials as well as injected into RP-HPLC.

2.6.Chromatographicconditions:

RP-HPLC separation was achieved on a Waters Alliance-e2695 using a Hypersil C18 column (250mm×4.6mm; 5µm id). To achieve good resolution and symmetric peak shapes for the CPTB, the chromatographic conditions, including the mobile phase composition, flow rate, selection of the appropriate column, injection volume, column oven temperature, auto sampler temperature, and short time, were optimized through several trials. Different mobile phase compositions were tried to achieve optimum detection of analytes. The optimum results were obtained at a mobile phase composition of 0.5% orthophosphoric acid (pH 4.8) (25%), methanol (35%), and acetonitrile (40%). Prior to use, the mobile phase system was filtered through a 0.45µm filter membrane, and later degassed. At a flow rate of 1.0mL/min, the mobile phase was pumped through the column. The column temperature was ambient, and the injection volume was 20µL.The runtime was 10.0min. The analyte was analyzed at a single wavelength of 252nm.

2.7. Method Validation:

The method was verified in accordance with the standards for Q2 (R1) of the ICH (International Conference on Harmonization) bio-analytical method validation. Additionally, it was found that the specificity, matrix effect, sensitivity, accuracy, precision (repeatability and reproducibility), recovery, ruggedness, and stability of analytes in plasma were evaluated as validation parameters. By examining drug-free human plasma (without the IS or the analytes) for the exclusion of any endogenous co-eluting interferences at the peak area of each analyte and the IS, the specificity of the approach was evaluated. Six sequential injections of a standard solution to the MQC concentration of the CPTB calibration curve and a 20 g/ml internal standard were used to validate the system's applicability. The ratio of peak area was calculated.

2.8. Specificity and Linearity:

The proposed method's specificity was determined by screening the standard blanks from various plasma batches. The linearity of the proposed method was assessed by utilized a seven-point standard curve. By adding the proper volumes of working solution to the blank plasma, a seven- point calibration solution is created that contains final concentrations (5, 10, 15, 20, 25, and 30 g/mL) of CPTB as well as 20 g/ml of internal standard. A regression model was used to obtain correlation coefficients (r^2) across the entire range of tested concentrations.

2.9. Sensitivity and Recovery:

Analyzing six LOQ samples (5 g/ml) allowed researchers to determine the method's sensitivity. The coefficient of variation is used to describe precision (CV). The recovery is determined by comparing the reaction of a blank plasma sample that had been processed and then spiked with the analyte to the mean peak area of a biological sample that had been spiked and processed. Replicating the analysis at least three times at different concentration levels allows for the evaluation of recovery (LQC, MQC, and HQC).

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(4), 221-234 | ISSN: 2251-6719

2.10. Ruggedness, Precision and Robustness:

Ruggedness was assessed using various columns and various analysts. For the LQC, MQC, and HQC samples, precision needs to be shown both within a single run and between different runs. The proposed method's robustness was confirmed by purposefully making small modifications to the experimental conditions. It was altered to see how it might affect the methodology. To ascertain the impact of change on the method, robustness should be shown for the LQC, MQC, and HQC samples for each case, both within a single run and between different runs.

2.11. Studies on the CPTB's stability:

Long-term, short-term, and freeze-thaw stability studies are used to evaluate stability investigations. The CPTB in QC samples was also tested for long-term stability after 30 days of storage at 10 to 30 °C. The stability of the bench top was investigated for a full day(24-hours). Together with the stability samples, the freshly spiked calibration curve standards were processed and extracted. According to the clinical guidelines, freeze-thaw cycles The samples were kept at a cycle of 10 to 30 degrees Celsius for 24 hours. Then, all samples were thawed and examined with freshly prepared calibration samples. By comparing the stability stock solution's results to the analyte's freshly generated stock solution, stability was determined. If the determined concentration differed from the nominal concentration by more than 15%, the compound was considered unstable.

3. RESULTS AND DISCUSSION

3.1. Method Development:

Based on the molecular structure and solubility data, CPTB was a non-polar molecule. Since it meets the requirements for analytical methods' sensitivity and selectivity, RP-HPLC is a especially effective technique for pharmacokinetic research studies. The instrumental RP-HPLC conditions were optimized to maximize the analyte response. Additionally, the response to optimization was examined using UV spectra of individual compounds in the 200–400 nm wavelength range. The selection of 252nm as the wavelength allowed for sufficient sensitivity in the detection of CPTB, which was considered to be satisfactory. A 35:40:25 (v/v/v) ratio of methanol, acetonitrile, and buffer containing 0.5% orthophosphoric acid was eventually chosen as the mobile phase after numerous different attempts. It was discovered that the formic acid was required to lower the pH, protonate the CPTB, and produce an acceptable peak shape.

This peak shape was maintained while being consistent by optimizing the formic acid content. The high amount of organic solvent produced good peak shapes, allowed for a run time of 10.0 minutes,

and eluted the CPTB and ETB internal standards at retention times of 3.1 minutes and 5.0 minutes, respectively.

3.2. Liquid-liquid extraction (LLE):

In this work, liquid-liquid extraction (LLE) was used to prepare samples. The samples can be cleaned with the aid of LLE. To reduce ion suppression and the matrix impact in RP-HPLC analysis, clean samples are crucial. A number of organic solvents and their mixes in various ratios and combinations were assessed. Finally, it was revealed that methanol generated a clear chromatogram for a sample of blank plasma and gave the maximum recovery of the internal standards for CPTB and ETB

Journal of Chemical Health Risks
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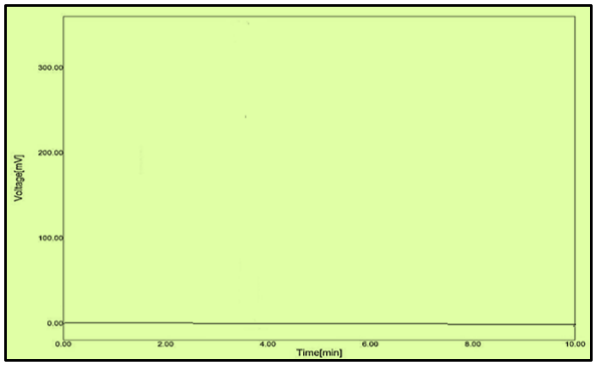
JCHR (2024) 14(4), 221-234 | ISSN: 2251-6719

from plasma.

No substantial direct interferences were found at the relevant retention times, and clear chromatograms were produced. In order to test the method's selectivity, blank human plasma extracts (n = 6) were analyzed. The result of one sample of blank plasma is shown in Fig. 2, and the absence of interference is comparable to that of other samples that were also examined. Endogenous substances were not found to significantly affect the blank plasma traces during the analyte's retention time in drug-free human plasma.

Fig. -2: The blank chromatogram of Capmatinib



The deviation of retention time (Rt) and area of 2% and 5% CV, respectively, were accepted as satisfactory results of the system-suitable study, and the UV detector analysis of the plasma samples revealed no interference of endogenous peaks with either the CPTB or the ETB (IS). With the method conditions indicated in Table 1 and Fig. 3, selectivity or specificity should be assessed to determine the interference at the RT of the CPTB and ETB (IS).

Table -1:Capmatinib's final optimized chromatographic conditions

S. No.	Condition	Results
1	Mobile phase	Methanol: Acetonitrile: 0.5% Orthophosphoric acid buffer in the ratio of 35:40:25 (v/v/v)
2	Pump mode	Isocratic
3	pH	5.1
4	Diluents	Mobile phase
5	Column	Hypersil C18 column (250mm×4.6mm; 5µmid)
6	Column Temp	Ambient
7	Wavelength	252nm
8	Injection Volume	20µl
9	Flow rate	1.0ml/min.
10	Run time	10minutes

Journal of Chemical Health Risks
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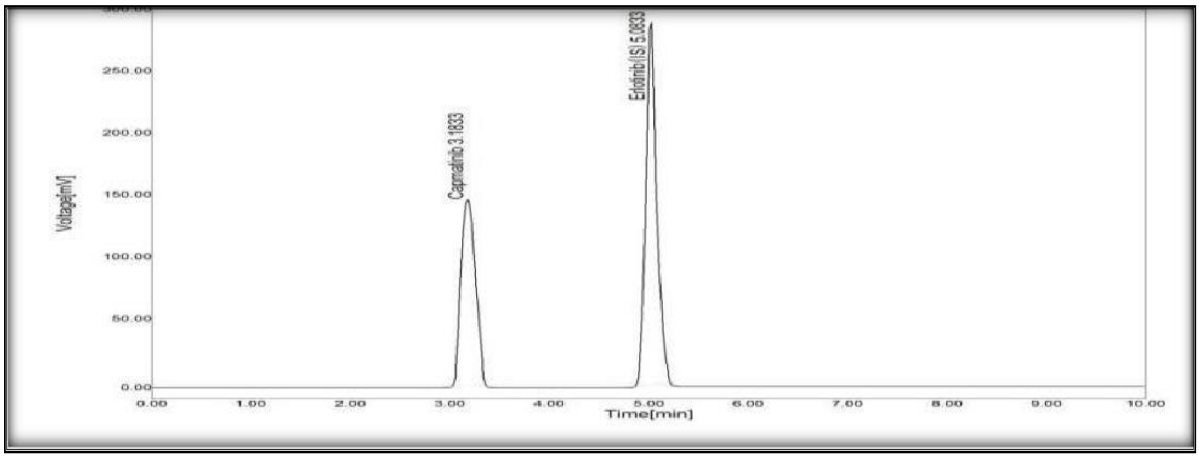

Fig. -3: Standard chromatogram of Capmatinib

The proposed method for CPTB was validated to be employed at concentrations between 5 and 30 g/mL. It was shown that the analyte and calibration curve of y = 0.1272x-0.2802, where y is the CPTB's peak-area ratio and ETB (IS) and the CPTB concentration is x in plasma, exhibit good linearity with a value of r^2 above 0.999. (Table 2).The calibration graph was drawn using the area response ratio on the y-axis and the concentration of spiked plasma on the x-axis (Fig. 4).

Table -2: Linearity results of CPTB in plasma sample

S.No.	Sample Id	Concentration in ng/ml	Peak Area observed for		Ratio of Standard/IS
			Capmatinib –St.	Erlotinib- IS	
1	LIN- 1	5	22361.2	55263.1	0.405
2	LIN- 2	10	55122.5	54362.7	1.014
3	LIN- 3	15	87943.7	55698.5	1.579
4	LIN- 4	20	117264.1	53218.2	2.203
5	LIN- 5	25	159854.3	55621.4	2.874
6	LIN- 6	30	195627.1	54918.3	3.562
7	LIN- 7	35	235164.7	55849.6	4.211

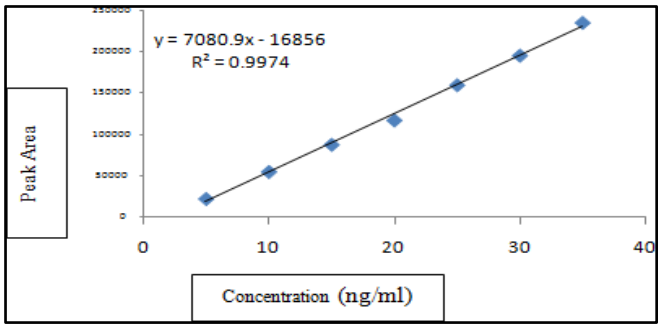

Fig. -4: Calibration curve CPTB in plasma sample

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(4), 221-234 | ISSN: 2251-6719

Acceptance criteria for validation parameters should be between and within batch CV for low, middle, and HQC levels should be ≤15% and for the LOQ QC level should be ≤20%. To determine the LOQ, the signal and noise ratio (S/N) values were calculated for 6 injections of CPTB, and a value of 11.93 was found at the LOQ concentration. By examining a minimum of three validation batches, comprising intra- and inter-day runs, the precision of the method was estimated. The intra- day and inter-day precision ranged from 0.38 to 0.86% and 0.44 to 0.94%, respectively. The recoveries for CPTB at 50 %, 100%, and 150 % concentrations with three replicate injections each showed 98.95 – 99. 84 %, 99.63 – 100.12 % and 99.48 – 100.03%. The overall recovery of CPTB was found to be 98.95–100.12%. Similarly, recoveries of CPTB were high, precise, and reproducible. Table 3 provided a summary of the results of the recovery. By adjusting the wavelength and making slight pH and chemical composition changes to the mobile phase, the robustness of the proposed method was tested.

According to the results, small changes in the chromatographic conditions had little impact on the chromatographic parameters. The proposed method was therefore very accurate and suitable for the intended usage, as shown by the recovery studies. As a result, the assay has demonstrated its robustness in high-throughput bio-analysis.

Table-3: Results of recovery

S. No.		Concentration in ng/ml				% Recovery
	Level	Target	Spiked	Total	Recovered	
1		10	5	15	14.98	99.84
2	50% 1	10	5	15	14.93	99.55
3		10	5	15	14.84	98.95
4		10	10	20	19.93	99.63
5	100% 1	10	10	20	20.02	100.12
6		10	10	20	19.99	99.97
7		10	15	25	25.01	100.03
8	150%1	10	15	25	24.92	99.67
9		10	15	25	24.87	99.48

The ruggedness of the method was carried out by changing by column, mobile phase combination, and by a different analyst. The % RSD of the ruggedness study was found to be 0.83% (HQC),
0.26% (MQC), and 0.34% (LQC) of the developed method, and its results were not more than 2.0% as recommended by the ICH guideline. The conditions that are very probable to arise during ordinary sample handling as well as analysis should be reflected in the stability testing. By measuring the CPTB in plasma that had undergone stress tests, the method's stability was assessed. By the way of comparing the stability of long-term stock solutions versus freshly prepared stock solutions over a 30-day period at a temperature of -28°C, long-term stock solution stability studies were evaluated.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(4), 221-234 | ISSN: 2251-6719

The HQC, MQC, and LQC of CPTB were reported to have 100%, 99.2%, and 99.9% mean stability (at 28°C) correspondingly. Tables 4 to Table 6 provide a summary of the results.

Table -4: Results of long term stability at HQC

At HQC							
			Peak Area Obtained		**Ratio of Peak area of standard/IS**	**Amount of Drug estimated**	
S.No.	**Test**	**Sample ID**	**Standard**	**IS**			**% Assay**
1		LTS-H1	233961.2	55236.2	4.236	35.20	100.59
2		LTS-H2	231567.1	55167.5	4.198	34.89	99.69
3	Long term Stability at HQC	LTS-H3	233187.8	55418.3	4.208	34.98	99.93
4		LTS-H4	235071.5	55684.2	4.222	35.09	100.26
5		LTS-H5	231871.4	55148.1	4.205	34.95	99.85
6		LTS-H6	234695.2	55986.4	4.192	34.84	99.56
	Average		233392.4	55440.1	4.2	35.0	100.0
	SD		1451.0	334.6	0.0	0.1	0.4
	% RSD		0.62	0.60	0.38	0.38	0.38

Table -5: Results of long term stability at MQC

At MQC							
			Peak Area Obtained		**Ratio of Peak area of standard/IS**	**Amount of Drug estimated**	
S. No.	**Test**	**Sample ID**	**Standard**	**IS**			**% Assay**
1		LTS-M1	116352.7	53123.4	2.190	19.88	99.42
2		LTS-M2	115967.2	53241.7	2.178	19.77	98.85
3	Long term Stability at MQC	LTS-M3	116284.3	53621.5	2.169	19.68	98.42
4		LTS-M4	117926.1	53847.1	2.190	19.88	99.39
5		LTS-M5	117105.8	53284.2	2.198	19.95	99.74
6		LTS-M6	116352.4	53068.1	2.193	19.90	99.50
	Average		116664.8	53364.3	2.2	19.8	99.2
	SD		722.7	305.4	0.0	0.1	0.5
	% RSD		0.62	0.57	0.49	0.49	0.49

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(4), 221-234 | ISSN: 2251-6719

Table -6: Results of long term stability at LQC

At LQC							
S. No.	Test	Sample ID	Peak Area Obtained		Ratio of Peak area of standard/IS	Amount of Drug estimated	% Assay
			Standard	IS			
1		LTS—L@1	22359.1	55251.4	0.405	5.00	100.01
2	Long term Stability at LQC	LTS—L@2	22158.7	55306.4	0.401	4.95	99.02
3		LTS—L@3	22634.1	55418.3	0.408	5.05	100.94
4		LTS—L@4	22471.5	55361.2	0.406	5.02	100.32
5		LTS—L@5	22284.3	55484.7	0.402	4.96	99.26
6		LTS—L@6	22351.4	55205.5	0.405	5.00	100.06
	Average		22376.5	55337.9	0.4	5.0	99.9
	SD		162.6	104.5	0.0	0.0	0.7
	%RSD		0.73	0.19	0.70	0.70	0.70

After being exposed to CPTB for 12 hours on a bench top, its bench top stability in human plasma was assessed in 6 replicates at 3 different concentrations (LQC, MQC, and HQC). It was discovered that the CPTB's mean stability HQC, MQC, and LQC results were 100.1%, 98.9%, and 99.9%, respectively. The results are displayed in Tables 7 to Table 9. It was able to assess the freezer stability of CPTB in plasma by assessing samples in six replicates that were held at 20°C for 24 hours for the stability study. Results of freeze-thaw stability tests at 28°C showed that the mean stability of LQC, MQC, and HQC CPTB was 100.1%, 99.5%, and 99.4%, respectively. The results were presented in Table 10 to Table 12. Throughout the whole period of sample storage, preparation, and analysis, there was no significant degradation (losses were within 4.0%). These outcomes imply that the proposed RP-HPLC method is accurate, precise, and reproducible for assessment of CPTB in plasma.

Table -7: Results of short term stability at HQC

At HQC:							
S.No.	Test	Sample ID	Peak Area Obtained		Ratio of Peak area of standard/IS	Amount of Drug estimated	% Assay
			Standard	IS			
1		STS-H1	234851.4	55267.8	4.249	35.32	100.91
2		STS-H2	230548.1	55364.1	4.164	34.61	98.90
	Short term Stability at HQC						
3		STS-H3	235117.5	55847.7	4.210	34.99	99.98
4		STS-H4	232684.7	55298.5	4.208	34.98	99.93
5		STS-H5	236187.3	55824.7	4.231	35.17	100.48
6		STS-H6	234987.2	55476.2	4.236	35.21	100.60
	Average		234062.7	55513.2	4.2	35.0	100.1
	SD		2066.6	260.3	0.0	0.2	0.7
	%RSD		0.88	0.47	0.71	0.71	0.71

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(4), 221-234 | ISSN: 2251-6719

Table -8: Results of short term stability at MQC

At MQC							
			Peak Area Obtained		**Ratio of Peak area of standard/IS**	**Amount of Drug estimated**	
S. No.	**Test**	**Sample ID**	**Standard**	**IS**			**% Assay**
1		STS-M1	115216.7	53264.1	2.163	19.64	98.19
2		STS-M2	114875.9	53104.5	2.163	19.63	98.17
3	Short term Stability at MQC	STS-M3	117874.6	53140.3	2.218	20.13	100.67
4		STS-M4	115098.4	53247.9	2.162	19.62	98.10
5		STS-M5	116364.5	53316.7	2.183	19.81	99.05
6		STS-M6	116174.8	53078.6	2.189	19.87	99.33
	Average		115934.2	53192.0	2.2	19.8	98.9
	SD		1126.2	97.0	0.0	0.2	1.0
	%RSD		0.97	0.18	1.01	1.01	1.01

Table -9: Results of short term stability at LQC

At LQC							
Peak Area Obtained							
S. No.	**Test**	**Sample ID**	**Standard**	**IS**	**Ratio of Peak area of standard/IS**	**Amount of Drug estimated**	**% Assay**
1		STS-L1	22136.4	55416.5	0.399	4.94	98.72
2		STS-L2	22415.7	55248.1	0.406	5.01	100.27
3	Short term Stability at LQC	STS-L3	22514.8	55123.7	0.408	5.05	100.94
4		STS-L4	22418.5	55287.3	0.405	5.01	100.21
5		STS-L5	22395.7	55641.5	0.402	4.97	99.47
6		STS-L6	22318.3	55164.2	0.405	5.00	99.99
	Average		22366.6	55313.6	0.4	5.0	99.9
	SD		129.0	190.5	0.0	0.0	0.8
	%CV		0.58	0.34	0.76	0.76	0.76

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(4), 221-234 | ISSN: 2251-6719

Table -10: Results of freeze thaw stability at HQC

At HQC - Peak Area Obtained							
S. No.	**Test**	**Sample ID**	**Standard**	**IS**	**Ratio of Peak area of standard/IS**	**Amount of Drug estimated**	**% Assay**
1		FTS-H1	234014.2	55063.2	4.250	35.32	100.92
2	Freez Thaw Stability at HQC	FTS-H2	233965.3	55248.7	4.235	35.20	100.57
3		FTS-H3	232568.1	55263.3	4.208	34.98	99.95
4		FTS-H4	231084.5	55847.5	4.138	34.39	98.27
5		FTS-H5	231896.7	55230.7	4.199	34.90	99.72
6		FTS-H6	234471.9	55174.1	4.250	35.32	100.93
	Average		233000.1	55304.6	4.2	35.0	100.1
	SD		1356.5	275.8	0.0	0.4	1.0
	%RSD		0.58	0.50	1.01	1.01	1.01

Table -11: Results of freeze thaw stability at MQC

At MQC - **Peak Area Obtained**							
S. No.	**Test**	**Sample ID**	**Standard**	**IS**	**Ratio of Peak area of standard/IS**	**Amount of Drug estimated**	**% Assay**
1		FTS-M1	118157.4	53264.7	2.218	20.14	100.69
2	Freez Thaw Stability at MQC	FTS-M2	115684.2	53497.1	2.162	19.63	98.14
3		FTS-M3	114986.5	53216.4	2.161	19.61	98.06
4		FTS-M4	117485.3	53264.5	2.206	20.02	100.10
5		FTS-M5	118264.1	53748.1	2.200	19.97	99.86
6		FTS-M6	117742.3	53341.4	2.207	20.04	100.18
	Average		117053.3	53388.7	2.2	19.9	99.5
	SD		1377.9	201.8	0.0	0.2	1.1
	%RSD		1.18	0.38	1.12	1.13	1.13

Table -12: Results of freeze thaw stability at LQC

At LQC - **Peak Area Obtained**							
S. No.	**Test**	**Sample ID**	**Standard**	**IS**	**Ratio of Peak area of standard/IS**	**Amount of Drug estimated**	**% Assay**
1		FTS-L1	22451.3	55654.1	0.403	4.98	99.70
2	Freez Thaw Stability at LQC	FTS-L2	22373.7	55134.7	0.406	5.01	100.29
3		FTS-L3	22234.2	55246.5	0.402	4.97	99.46
4		FTS-L4	22034.7	55346.3	0.398	4.92	98.39
5		FTS-L5	22261.5	55197.4	0.403	4.98	99.67
6		FTS-L6	22179.3	55455.7	0.400	4.94	98.84
	Average		22255.8	55339.1	0.4	5.0	99.4
	SD		146.5	191.5	0.0	0.0	0.7
	%RSD		0.66	0.35	0.68	0.68	0.68

Journal of Chemical Health Risks
www.jchr.org
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4. CONCLUSIONS

When used in pharmaceutical analysis, the current HPLC approach satisfies the acceptance requirements typically established for bio-analytical assays. The validation data also shows that the method can accurately and precisely measure the drug in biological samples at low concentrations, such as 5.0µg/mL, and that it can be used to quantify CPTB. However, the confirmation of the HPLC approach should be helpful for developing pharmacokinetic studies and monitoring inhuman plasma concentrations. The approach proved simple, accurate, linear, robust, and selective enough to allow the analysis of CPTB in human plasma samples within the explored range. The signal suppression impact might be compensated for and accuracy issues could be reduced by using the internal standard ETB chose as structural analogue of CPTB.In terms of overall analytical performance, the proposed methodology gives a clear advantage over those previously developed. Consequently, the new approach has applications in pharmacokinetic investigations and therapeutic drug monitoring in human plasma.

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