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Research Article

STABILITY-INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF CABOZANTINIB IN PHARMACEUTICAL DOSAGE FORMS BY ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Objective: The proposed study aimed to develop a stability-indicating ultra-performance liquid chromatography (UPLC) method for the estimation of cabozantinib in pharmaceutical dosage form and validate the method in accordance with the International Conference on Harmonization guidelines.

Methods: The optimized conditions for the developed UPLC method are Acquity UPLC Hibar C18 (100 mm × 2.1 mm, 1.7 µ) column maintained at 30°C with mobile phase consisting of 0.1% orthophosphoric acid and acetonitrile in the ratio of 55:45% v/v on isocratic mode at flow rate of 0.3 mL/min. The sample was detected at 244 nm.

Results: The retention time for cabozantinib was deemed 1.3 min. The developed method was validated for accuracy, precision, specificity, ruggedness, robustness, and solution stability. The method obeyed Beer's law in the concentration range of 20 µg/mL and 120 µg/mL with correlation coefficient of 0.9997. Forced degradation studies were conducted by exposing the drug solution to numerous stress conditions such as acidic, basic, peroxide, neutral, photolytic, and thermal conditions. The net degradation was considered within the limits, indicating that drug is stable in stressed conditions.

Conclusion: The developed method for the estimation of cabozantinib can be utilized for the routine analysis of pharmaceutical dosage form.

Keywords: Cabozantinib, Stability indicating, Method development, Validation, Ultra-performance liquid chromatography.

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INTRODUCTION

Cabozantinib (Fig. 1) [1-5] chemically defined as N-(4-(6,7-dimethoxyquinolin-4-yloxy)phenyl)-N'-(4-fluorophenyl)cyclopropane-1,1-dicarboxamide, (2S)-hydroxybutanedioate. It is white to off-white solid, practically insoluble in aqueous media. It has pKa values of 5.9 and 13.46. It belongs to anticancer category which acts by inhibiting tyrosine kinase activity of receptors that participate in pathologic processes such as oncogenesis, metastasis, tumor angiogenesis, and maintenance of tumor microenvironment. It is used in the treatment of medullary thyroid cancer and a second-line treatment for renal cell carcinoma.

Ultra-performance liquid chromatography (UPLC) [6] is specially designed to withstand higher system pressure during chromatographic analysis so that it enables a significant decrease in separation time and solvent consumption. UPLC columns packed with 1.7 µm sized particles provide not only increased efficiency but also the ability to work at an increased linear velocity without loss of efficiency, providing both resolution and speed. Using advantages of UPLC, a number of applications in different fields including pharmacy, clinical analysis, and pesticide analysis have been recorded.

The literature survey reveals that there are only few methods developed for the estimation of cabozantinib using LC coupled with tandem mass spectrometry method (LC–MS/MS) [7,8], spectrofluorimetric method [9] and UPLC coupled with tandem mass spectrometry method (UPLC–MS/MS)[10].

As there was no method developed using UPLC, the present study aimed to develop and validate a UPLC stability-indicating method for the estimation of cabozantinib in pharmaceutical dosage form.

METHODS

Reagents and chemicals

Cabozantinib working standard was procured from Spectrum Labs, Hyderabad, as a gift sample. The Cometriq capsules were purchased from a local pharmacy. All the chemicals used were of AR grade purchased from Merck, Mumbai. All the solvents used were of high-performance LC grade purchased from Sigma-Aldrich, Mumbai.

Chromatographic conditions and instruments

The ACQUITY UPLC [11] system equipped with binary solvent manager, sample manager, ultraviolet (UV) detector, and Hibar C18 (100 mm × 2.1 mm, 1.7 µ) column was used for the determination of cabozantinib. The optimized conditions included 0.1% orthophosphoric acid and acetonitrile (55:45%v/v) as mobile phase run on an isocratic mode at a flow rate of 0.3 mL/min. The column was held at 30°C and detection was done at 244 nm. Further, equipment included pH meter, ultrasonic bath sonicator, and weighing balance.

Preparation of mobile phase

Mixture of 0.1% orthophosphoric acid and acetonitrile in the ratio of 55:45% v/v was used as mobile phase.

Preparation of standard and sample solutions

About 80 mg of cabozantinib working standard was dissolved in 100 mL of diluent. Then, 1 mL of the above stock solution was diluted to 10 mL using diluent to get a concentration of 80 µg/mL.

20 capsules (Cometriq) were weighed accurately and average weight was calculated. An amount equivalent to 80 mg of the drug was

dissolved in 100 mL of diluent, filtered the solution, and diluted 1 mL of the above solution to 10 mL with diluent.

Method validation
The developed method was validated in accordance with the International Conference on Harmonization (ICH) guidelines [12,13].

Specificity
The specificity of the method was determined by comparing the drug solution to the placebo solution and observed for the interference of placebo peak with drug peak.

Accuracy
Accuracy of the method was determined in accordance with percentage recovery. The drug solution along with sample was prepared in three concentration levels, i.e. 50%, 100%, and 150%. Then, the percentage recovery was calculated.

Precision
Precision of the method was established by injecting the standard solution 6 times into the UPLC system and percentage relative standard deviation (RSD) was calculated.

Linearity
Linearity of the method was determined by preparing a series of dilutions ranging from 20 µg/mL to 120 µg/mL and injecting them into UPLC system.

Ruggedness
Ruggedness was determined by injecting the standard solution into UPLC 6 times for different days. The percentage RSD was calculated.

Robustness
Robustness of the method was determined by varying the optimized analytical conditions such as mobile phase composition by ±5%, flow rate by ±0.1 mL/min, and column oven temperature by ±5°C.

Solution stability
Solution stability determined on the basis of analyzing the standard drug solution after storage for 24 h under laboratory conditions.

Forced degradation studies
Forced degradation studies [14,15] were carried out for drug by exposing the drug solution to the various stress conditions such as acidic (2 N hydrochloric acid for 30 min at 60°C), basic (2 N sodium hydroxide for 30 min at 60°C), peroxide (20% hydrogen peroxide [H_2O_2] for 30 min at 60°C), neutral (refluxing the drug in water for 6 h at 60°C), photolytic (105°C for 6 h), and thermal (exposing the drug solution to UV light by keeping the beaker in UV chamber for 7 day or 200 Wt h/m^2 in photostability chamber) conditions.

RESULTS

From the UV spectrum, detection wavelength for Cabozantinib was found to be 244nm.

DISCUSSION

For the development of a method for the estimation of cabozantinib in pharmaceutical dosage form, initially, many mobile phases and many columns were tried to elute the drug peak with less tailing factor and more plate count. Acquity UPLC Hibar C18 (100 mm × 2.1 mm, 1.7 µ) column and 0.1% OPA:acetonitrile (55:45% v/v) as mobile phase were selected based on peak parameters. The detection wavelength was found to be 244 nm as shown in Fig. 2 of the UV spectrum.

Prepared standard solution, sample solution, and the blank solution were injected into the UPLC system, and system suitability parameters were noted as summarized in Table 1 along with chromatograms as showed in Fig. 3a-c, respectively.

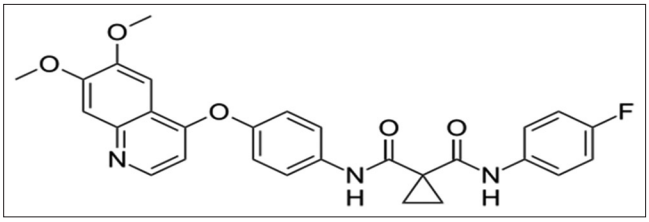

Fig. 1: Chemical structure of cabozantinib

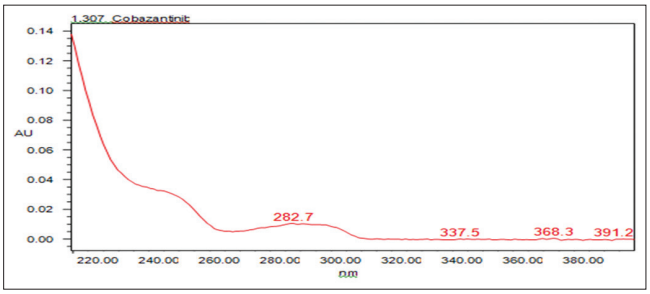

Fig. 2: Ultraviolet spectrum of cabozantinib

Table 1: System suitability and validation parameter results

Parameter	Result	Result
Precision (%RSD, n)	0.3	
Accuracy (% recovery, n)	99.57–99.91%	
Specificity	Specific, no interference	
Linearity range (µg/mL)	20–120	
Correlation coefficient, r	0.9997	
Limit of detection (µg/mL)	0.15	
Limit of quantitation (µg/mL)	0.47	
Ruggedness (%RSD, n)	Day 1	Day 2
	0.5	0.3
Robustness (%RSD, n)	Less flow rate	More flow rate
	0.4	0.5
	Less mobile phase (organic)	More mobile phase (organic)
	1.0	0.4
	Less column temperature	More column temperature
	0.7	0.3
Solution stability (%RSD, n)		
USP plate count		
USP tailing factor		
Day 1 (0 h)	Day 2 (after 24 h)	
0.5	0.3	
	2943	
	1.28	

n: Number of samples, i.e., 6 samples, %RSD: % Relative standard deviation

The developed method was identified to obey Beer's law in the concentration range of 20 µg/mL–120 µg/mL with correlation coefficient of 0.9997. A linearity graph was plotted between concentration and peak area as showed in Fig. 4 and results as presented in Table 1.

The method was found to be accurate as the percentage recovery was 99.57–99.91% and was within the limits. The percentage RSD was determined to be 0.3, which indicates that the method was precise. The method was shown to be specific, as there is no interference of retention time of placebo peak with that of drug peak. The placebo chromatogram was displayed in Fig. 5.

Forced degradation studies results indicate that the drug was reported to be stable in various stress conditions as net degradation was found to

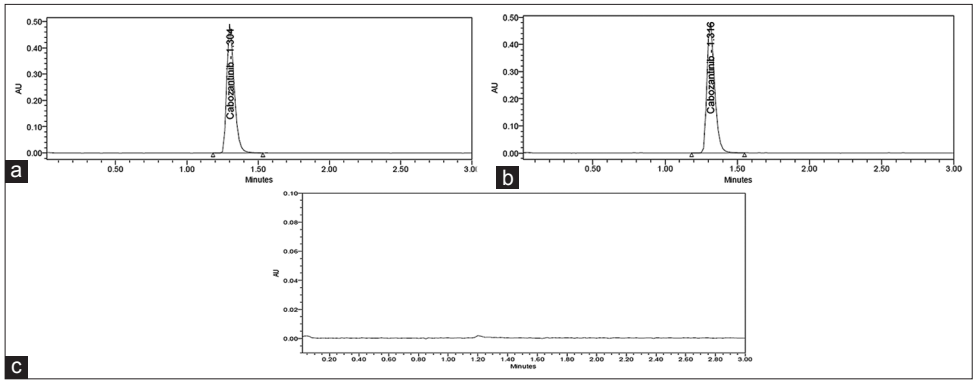

Fig. 3: (a) Ultra-performance liquid chromatography (UPLC) chromatogram of a standard solution, (b) UPLC chromatogram of sample solution, (c) UPLC chromatogram of blank solution

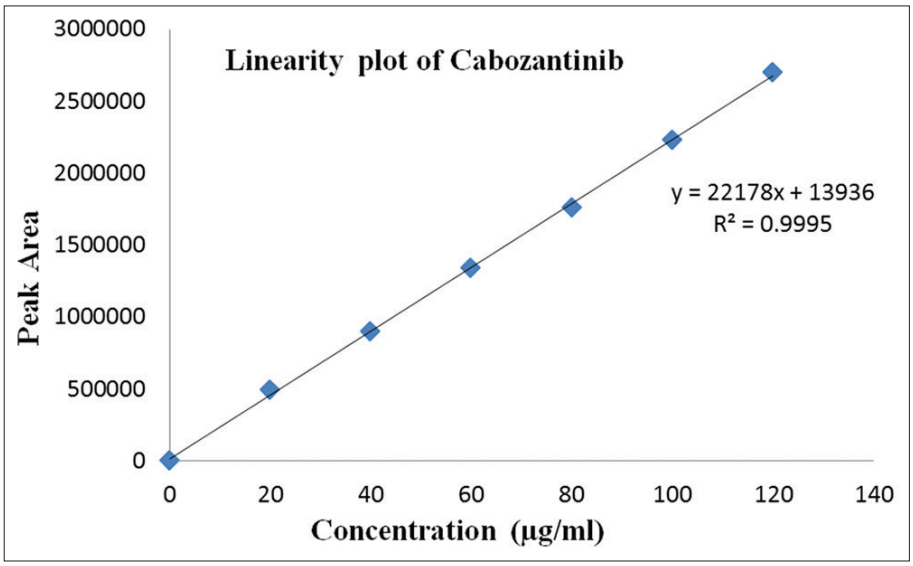

Fig. 4: Linearity plot of cabozantinib

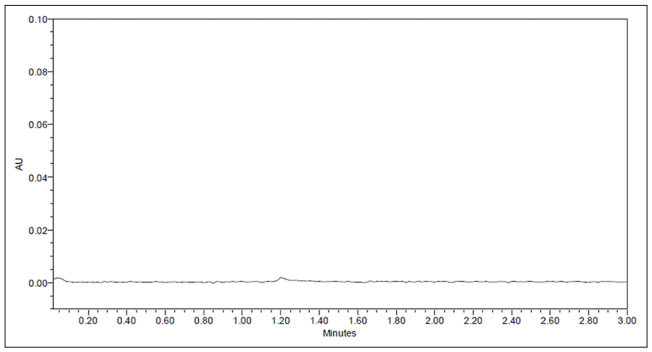

Fig. 5: Ultra-performance liquid chromatography chromatogram of placebo solution

Table 2: Forced degradation studies results

Stress condition	% assay	% area of degradation peak	% degradation
2 N HCl for 30 min at 60°C	97.69	0.08	2.31
2 N NaOH for 30 min at 60°C	96.87	0.02	3.13
20% H_2O_2 for 30 min at 60°C	98.26	-	1.74
Water for 6 h at 60°C	99.45	-	0.55
UV light 200 Wts/h or 7 day	99.05	-	0.95
105°C for 6 h	98.70	-	1.30

be within the limits. The chromatograms were illustrated in Fig. 6 and results were outlined in Table 2.

CONCLUSION

A specific, accurate, and precise stability-indicating method was developed for the estimation of cabozantinib in pharmaceutical dosage form using UPLC. The method was validated using numerous validation parameters, and the method was found to be linear, precise, accurate, specific, and robust. From the degradation studies conducted, it infers that cabozantinib was more stable at more concentrations of acid, base, peroxide, thermal, UV, and water stress study conditions. The run time was 3 min which enables rapid quantitation of many samples in routine and quality control analysis of capsule formulation.

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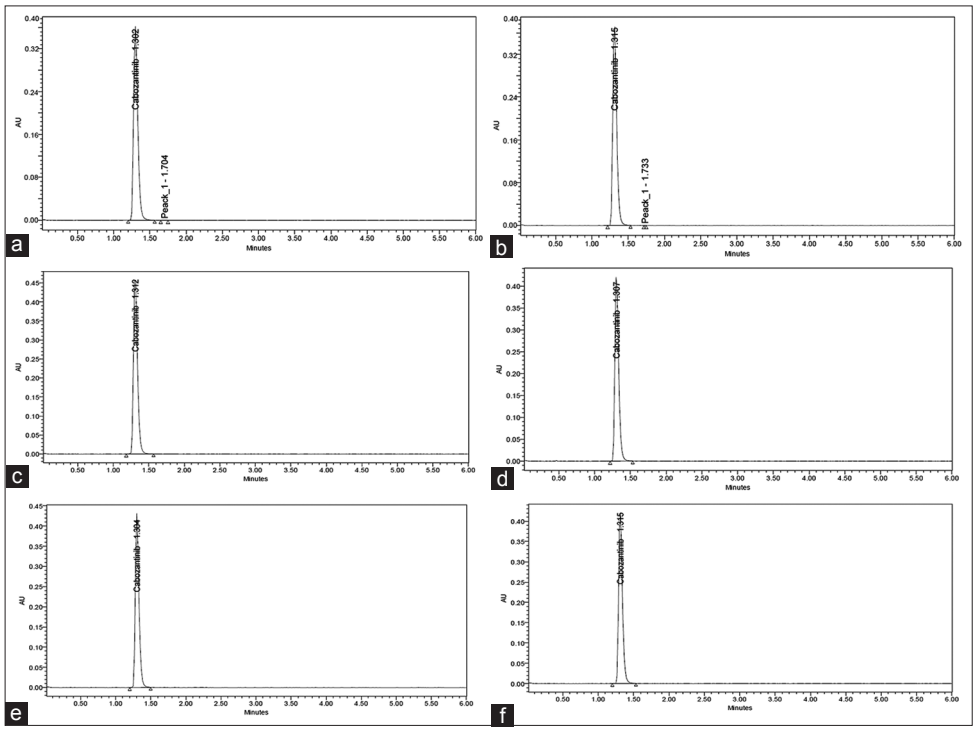

Fig. 6: (a) Acid degradation ultra-performance liquid chromatography (UPLC) chromatogram, (b) base degradation UPLC chromatogram, (c) peroxide degradation UPLC chromatogram, (d) water stress study UPLC chromatogram, (e) photostability degradation UPLC chromatogram, (f) dry heat study UPLC chromatogram

AUTHOR'S CONTRIBUTIONS

All authors contribute equally to this manuscript.

CONFLICTS OF INTEREST

The authors claim that they have no conflicts of interest. It has not meant to publish elsewhere. Moreover, it has not meant simultaneously presented for publication elsewhere. All authors have decided to the submission to the journal.

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