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A NOVEL RP- HPLC METHOD FOR THE QUANTIFICATION OF CABOZANTINIB IN ACTIVE PHARMACEUTICAL INGREDIENTS AND PHARMACEUTICAL DOSAGE FORMS

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Keywords:

Cabozantinib, RP-HPLC, Validation, Method Development

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ABSTRACT: A simple, specific, accurate reversed-phase high performance liquid chromatographic method was developed for the quantification of Cabozantinib. Although extensive studies on Cabozantinib have been developed for determining Cabozantinib in human place and urine by LC-MS, studies on the pharmaceutically active ingredient and formulation are scarce. The effective separation was achieved through BDS C18 150 × 4.6 mm, 5µ using a mobile phase KH_2PO_4 Buffer and ACN (55:45% v/v). The flow rate of the mobile phase was 1.0 mL/min, and the detection was carried at a wavelength of 210 nm. The retention time of Cabozantinib was 2.932 nm. The correlation coefficient is 0.9998. The developed method was validated in terms of system suitability, specificity, linearity range, precision, accuracy, limits of detection and quantification. Degradation studies were performed on Cabozantinib to indicate the stability property and specificity of the proposed method. The information presented in this study will be useful for industrial application for determining Cabozantinib in active pharmaceutical ingredient and pharmaceutical dosage form.

INTRODUCTION: Cabozantinib is a medication used to treat medullary thyroid cancer and a second line treatment for renal cell carcinoma among others. It is a small molecule inhibitor of the tyrosine kinases c-Met and VEGFR2 and also inhibits AXL and RET. Cabozantinib is used in two forms. A capsule form is used since 2012 to treat medullary thyroid cancer [1, 2] and a tablet form is used since 2016 as a second line treatment for renal cell carcinoma [3, 4]. Pharmacologically it inhibits the following receptor tyrosine kinases: MET (hepatocyte growth factor receptor protein) and VEGFR, RET, GAS6 receptor (AXL), KIT), and Fms-likely tyrosine kinase-3 (FLT3).

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As analytical methods must be validated before use in the pharmaceutical process, the proposed method was validated by ICH (International Conference on Harmonization) guidelines [5, 6, 7]. Cabozantinib **Fig. 1** is chemically 1- N- [4- (6, 7-dimethoxyquinolin-4-yl)oxyphenyl]-1- N'- (4- fluorophenyl) cyclopropane- 1, 1-dicarboxamide

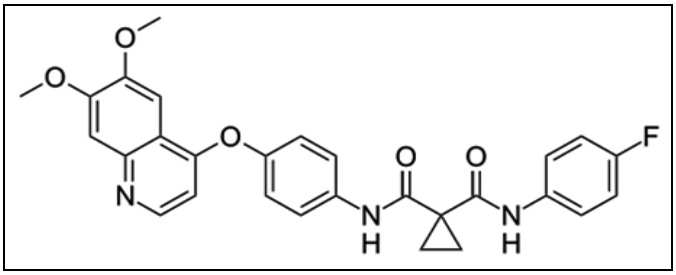


FIG. 1: STRUCTURE OF CABOZANTINIB

From the literature survey, it was known that there were no methods developed and validated for the determination of Cabozantinib with RP-HPLC. So this study on RP-HPLC was developed to keep this research method simple, sensitive and accurate.

This method was developed and validated with parameters like accuracy, linearity, LOD, LOQ, precision, specificity, system suitability and forced degradation studies [8].

MATERIAL AND METHODS:

Materials: The active pharmaceutical ingredient and pharmaceutical dosage form were kindly gifted by Spectrum Laboratories, Hyderabad. HPLC grade water, acetonitrile is of Merck, Mumbai, and potassium dihydrogen othro phosphate used was of SD fine chem limited, Mumbai. Instruments like Waters HPLC (Empower software), ultrasonicator (Make: Labman) and pH meter (Make: Adwa) are used for developing this method.

Instrumentation and Chromatographic Conditions: All separations were carried out on HPLC waters with a PDA detector. The data was analyzed by using Empower software. Chromatographic separation was performed on column BDS C18 150 × 4.6 mm, 5 µ. Gradient binary pump was used with ambient column temperature. The components of the mobile phase used for this gradient elution are KH_2PO_4 Buffer and ACN (55:45) with flow rate as 1.0ml/min. The injection volume was 20 µl. Detection was performed at 210 nm with a PDA detector.

Selection and Preparation of Mobile Phase: Buffer and ACN: taken in the ratio 55:45. 0.01N KH_2PO_4 Buffer: Accurately weighed 1.36 gm of Potassium dihydrogen Orthophosphate in a 1000 ml of Volumetric flask add about 900 ml of milli-Q water added and degas to sonicate and finally made up the volume with water then pH adjusted to 3.32 with dil. Orthophosphoric acid solution.

Preparation of Standard Solution: Accurately Weighed and transferred 5.0 mg of Cabozantinib, working Standards into a 25 ml clean dry volumetric flasks, added 10 ml of diluents, sonicated for 10 minutes and made up to the final volume with diluents (200 µg/ml Cabozantinib).

Preparation of Sample Solution: Accurately weighed equivalent weight of the powder sample transfer into a 50 ml volumetric flask, 25ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (400 µg/ml Cabozantinib) 0.5ml of filtered sample stock solution was transferred to 10 ml volumetric flask and made up with diluent (20 µg/ml Cabozantinib).

Method validation:

Specificity: Commonly used excipients were spiked into a pre-weighed quantity of drugs. The chromatogram was taken by appropriate dilutions, and the quantities of drugs were determined.

System Suitability: System suitability test was an integral part of method development and is used to ensure adequate performance of the chromatographic system.

Linearity and Range: In the chromatographic method, linearity generates test effects which are directly proportionate to the concentration of an analyte within the given range. The range is defined as an interval between the upper and lower extent of analytes in the sample. Cabozantinib had linearity in the range of 5 - 30 µg/ml. The correlation coefficient was found to be 0.9998.

Precision: The intraday and interday precision study of Cabozantinib was carried out by estimating the corresponding responses five times on the same day and different days. The results were reported in terms of relative standard deviation.

The Repeatability studies were carried out by estimating the response of five different concentrations of Cabozantinib and results are reported in terms of relative standard deviation (% RSD).

Accuracy: The accuracy of the developed method was determined by calculating recovery of Cabozantinib by spike method, a known amount of Cabozantinib was added to a pre quantified sample solution, and the amount of Cabozantinib was estimated by measuring the peak areas and by fitting these values to the straight-line equation of calibration curve.

Detection and Quantification Limits: The level of quantification (LOQ) and detection (LOD) were conducted by a signal to noise ratio method.

Degradation Studies:

Oxidation: To 1 ml of stock solution of Cabozantinib, 1 ml of 20% hydrogen peroxide

(H_2O_2) was added separately. The solutions were kept for 30 min at 60 ºC. For HPLC study, the resultant solution was diluted to obtain (20ppm) solution and 10 µl were injected into the system, and the chromatograms were recorded to assess the stability of the sample.

Acid Degradation Studies: To 1 ml of stock solution Cabozantinib 1 ml of 2N Hydrochloric acid was added and refluxed for 30 min at 60 ºC. The resultant solution was diluted to obtain (20ppm) solution, and 10 µl solutions were injected into the system, and the chromatograms were recorded to assess the stability of the sample.

Alkali Degradation Studies: To 1 ml of stock solution Cabozantinib 1 ml of 2 N sodium hydroxide was added and refluxed for 30 min at 60 ºC. The resultant solution was diluted to obtain (20ppm) solution and 10 µl were injected into the system, and the chromatograms were recorded to assess the stability of the sample.

Dry Heat Degradation Studies: The standard drug solution was placed in an oven at 105 ºC for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to (20 ppm) solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability Studies: The photochemical stability of the drug was also studied by exposing the (400 ppm) solution to UV Light by keeping the beaker in UV Chamber for 7 days or 200 Watt-hours/min photostability chamber.

For HPLC study, the resultant solution was diluted to obtain (20 ppm) solutions and 10 µl were injected into the system, and the chromatograms were recorded to assess the stability of the sample.

Neutral Degradation Studies: Stress testing under neutral conditions was studied by refluxing the drug in water for 6 h at a temperature of 60 ºC. For HPLC study, the resultant solution was diluted to (20ppm) solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSION:

Specificity: This RP-HPLC method was specific. The retention time for Cabozantinib was 2.932 min. Specificity results were represented in **Table 1**, **Fig. 2a** for blank and **Fig. 2b** for the drug.

TABLE 1: SPECIFICITY DATA FOR RP-HPLC

S. no.	Peak Name	Observation	
1	Blank	Nil	
2	Placebo	Nil	
3	Standard	R_t = 2.932 min	λ_{max} = 210 nm

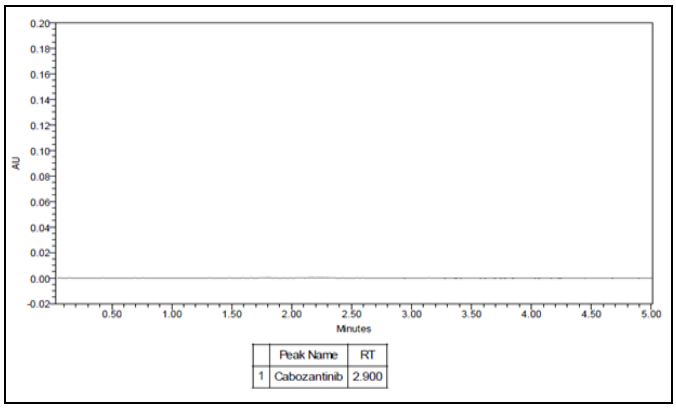


FIG. 2A: SPECIFICITY CHROMATOGRAM FOR BLANK

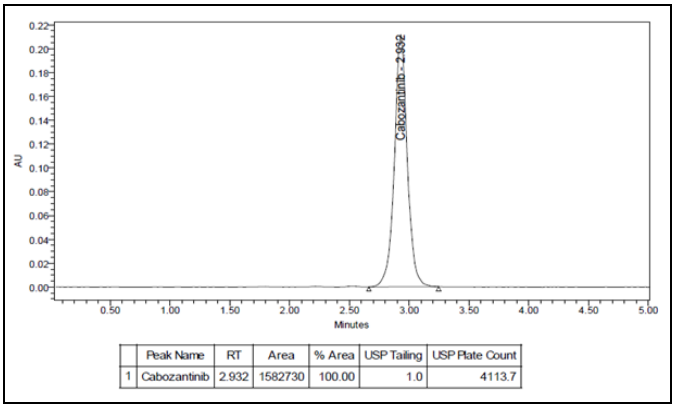


FIG. 2B: SPECIFICITY CHROMATOGRAM FOR DRUG

System Suitability: Tailing factor was 1.0 (T), and some theoretical plates were found to be 4113 (N). System Suitability results were represented in **Table 2**.

TABLE 2: RESULTS OF SYSTEM SUITABILITY

Parameter	Result	Acceptance Limit
Retention time (Rt)	2.932 min	--
Resolution factor	NA	--
Number of theoretical plates (N)	4113	More than 2000
Tailing factor (T)	1.0	Less than 2

Linearity and Range: Linearity was determined for concentration 5 to 30 µg/ml. The correlation coefficient was 0.9998. Linearity results were represented in **Table 3** and co-relation graph in **Fig. 3**.

Precision: In method validation, intraday and interday precision determined. The % RSD for interday precision is 0.2 and intraday is 0.2. Precision results were represented in **Table 4**.

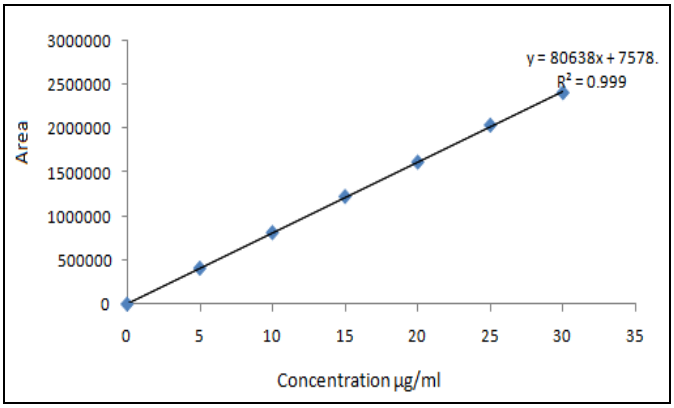


FIG. 3: CORRELATION GRAPH

TABLE 3: LINEARITY AND RANGE

S. no.	Concentration (µg/ml)	Peak Area
1	5	410289
2	10	815536
3	15	1226729
4	20	1618912
5	25	2039133
6	30	2409400

TABLE 4: RESULTS FOR INTRADAY AND INTERDAY PRECISION

S. no.	Intraday precision Area	Interday precision Area
1	1618816	1605676
2	1612553	1608530
3	1617141	1603472
4	1614090	1604331
5	1612355	1605164
6	1610192	1612555
Mean	1614191	1606621
Std Dev	3229.5	3377.4
% RSD	0.2	0.2

Accuracy: The % Mean recovery for accuracy was in the range of 98.98 to 100.49. Accuracy results were represented in **Table 5**.

TABLE 5: RESULTS FOR ACCURACY

Spiked Concentration (µg/ml)	Peak area (n=3)	Amount added (µg/ml)	Amount Found (µg/ml)	Recovery	% Mean Recovery
50	2418734	10	9.85	98.51	98.98
	2423571		9.91	99.11	
	2425220		9.93	99.32	
100	3256107	20	20.21	101.07	100.56
	3246153		20.09	100.46	
	3241004		20.03	100.14	
150	4062142	30	30.19	100.63	100.49
	4058979		30.15	100.50	
	4055178		30.10	100.34	

Detection and Quantification Limits: The sensitivity for detection was demonstrated by determining the LOD. The LOD was found to be 0.03 µg/ml, and the quantification limit was the lowest concentration of a substance that can be quantified with precision and accuracy. The LOQ was determined to be 0.10 µg/ml for Cabozantinib. The level of quantification (LOQ) and detection (LOD) were represented in **Table 6** and **Fig. 4**, **Fig. 5**.

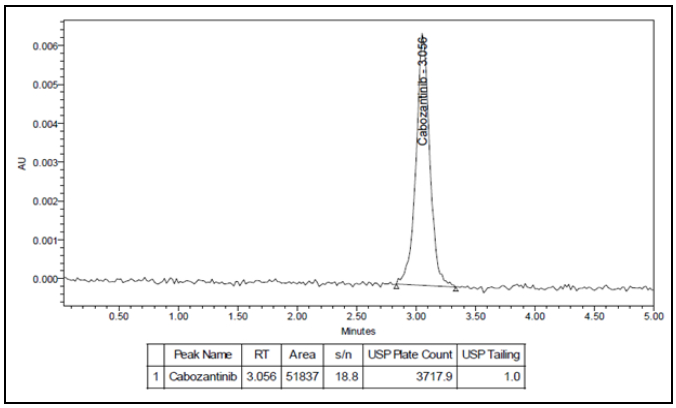


FIG. 4: LOD CHROMATOGRAPH

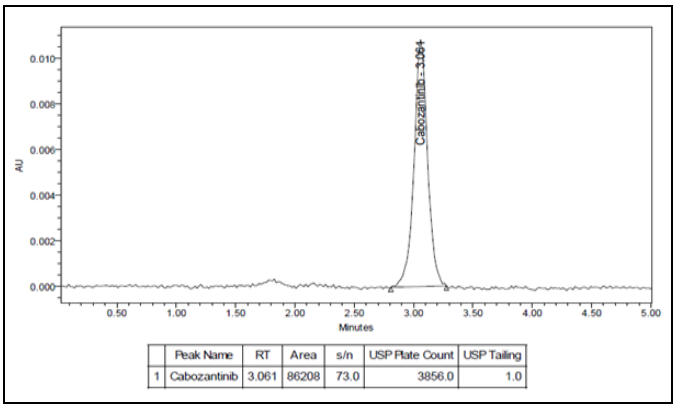


FIG. 5: LOQ CHROMATOGRAPH

TABLE 6: LOD & LOQ

Parameter	Results	
	LOD	LOQ
RT	3.056	3.061
Area	51837	86208
s/n value	18.8	73.0
Plate count	3717.9	3856.0

Degradation Studies: Degradation studies related to acid, base, peroxide, thermal, UV and water were represented in **Table 7**. **Fig. 6** represents acid degradation chromatograph and **Fig. 7** represents acid degradation purity plot. **Fig. 8** represents base degradation chromatograph and **Fig. 9** represents the base degradation purity plot. **Fig. 10** represents peroxide degradation chromatograph and **Fig. 11** represents the peroxide degradation purity plot. **Fig. 12** represents thermal degradation chromatograph and **Fig. 13** represents the thermal degradation purity plot. **Fig. 13** represents UV degradation chromatograph and **Fig. 14** represents the UV degradation purity plot. **Fig. 15** represents water degradation chromatograph and **Fig. 16** represents water degradation purity plot.

Degradation studies were performed on Cabozantinib to indicate the stability property and specificity of the proposed method. Degradation study included light, heat, acid hydrolysis (2N HCl), base hydrolysis (2N NaOH) and oxidation (20% H_2O_2). The samples were exposed to 105 ºC for 6 h for dry heat degradation, the refluxed drug in water for 6 h at a temperature of 60 ºC for neutral degradation studies.

TABLE 7: FORCED DEGRADATION STUDY

S. no.	Study	RT	Area	USP Plate count	USP Tailing
1	Acid degradation	2.935	1510221	4312	1.0
2	Base degradation	2.986	1534158	4099	1.0
3	Peroxide degradation	2.984	1566756	3926	1.0
4	Thermal degradation	2.991	1572366	3928	1.0
5	UV degradation	3.020	1594712	4198	1.0
6	Water degradation	3.029	1608728	4343	1.0

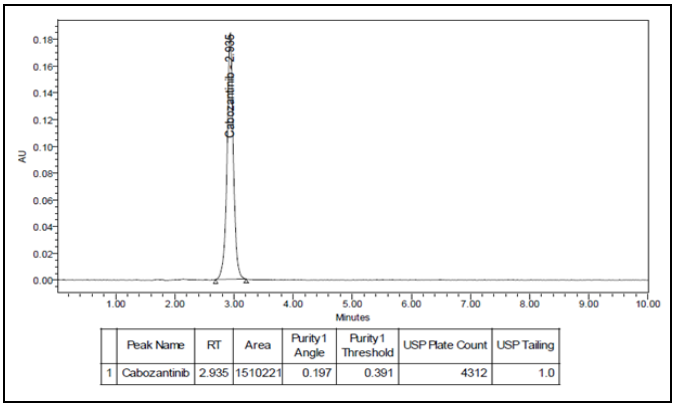


FIG. 6: ACID DEGRADATION CHROMATOGRAPH

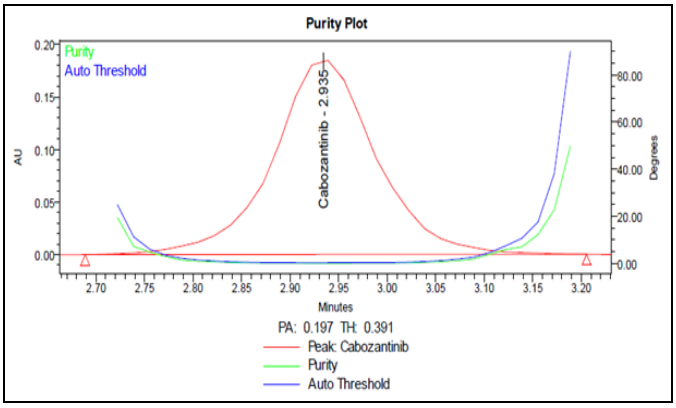


FIG. 7: ACID DEGRADATION PURITY PLOT

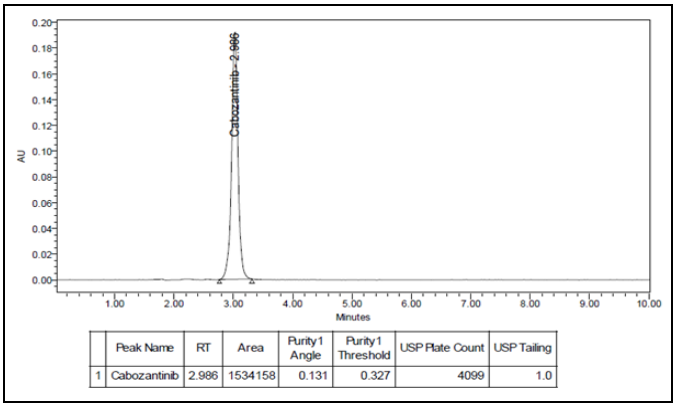


FIG. 8: BASE DEGRADATION CHROMATOGRAPH

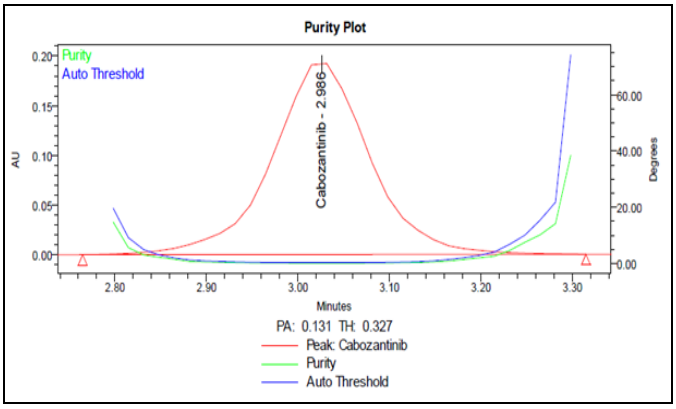
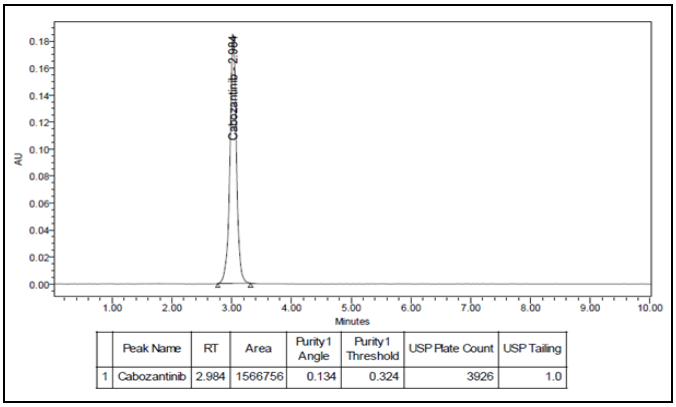


FIG. 9: BASE DEGRADATION PURITY PLOT



	Peak Name	RT	Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	Cabozantinib	2.984	1566756	0.134	0.324	3926	1.0

FIG. 10: PEROXIDE DEGRADATION CHROMATOGRAPH

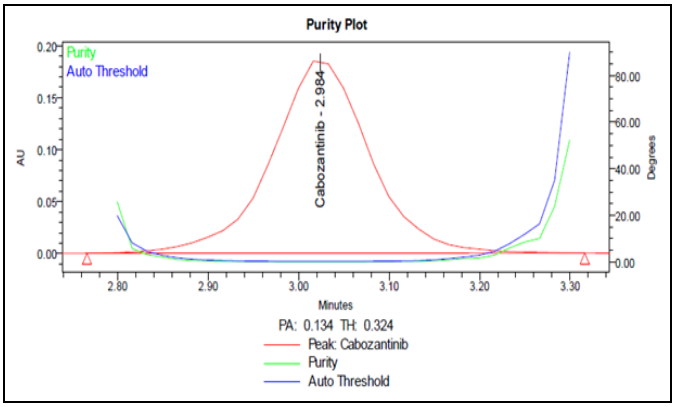
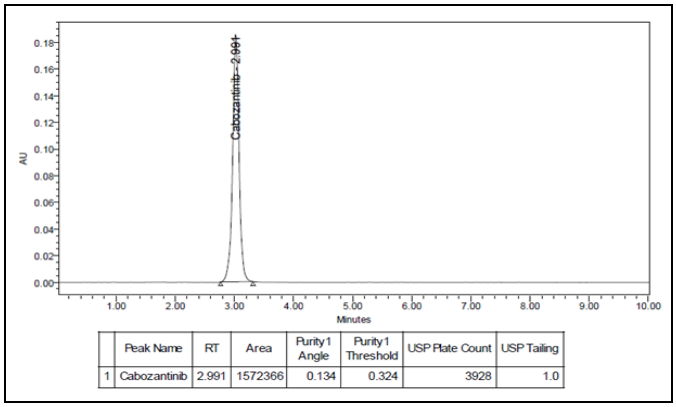


FIG. 11: PEROXIDE DEGRADATION PURITY PLOT



	Peak Name	RT	Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	Cabozantinib	2.991	1572366	0.134	0.324	3928	1.0

FIG. 12: THERMAL DEGRADATION CHROMATOGRAPH

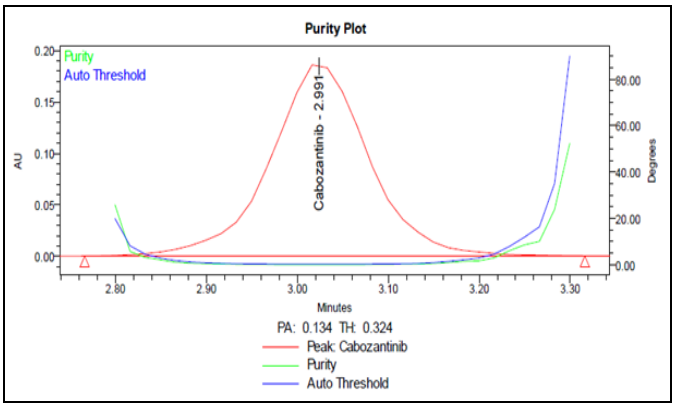
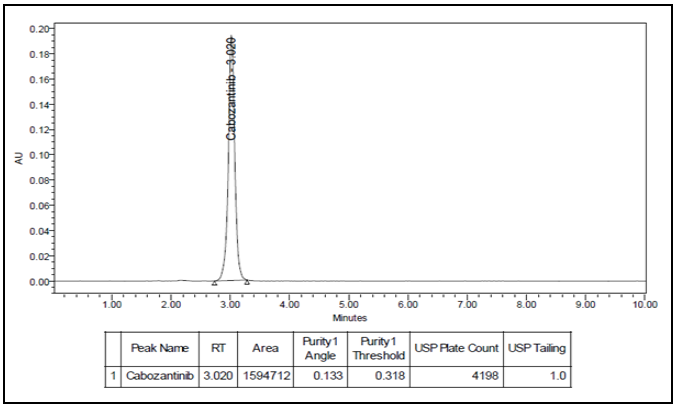


FIG. 13: THERMAL DEGRADATION PURITY PLOT



	Peak Name	RT	Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	Cabozantinib	3.020	1594712	0.133	0.318	4198	1.0

FIG. 14: UV DEGRADATION CHROMATOGRAPH

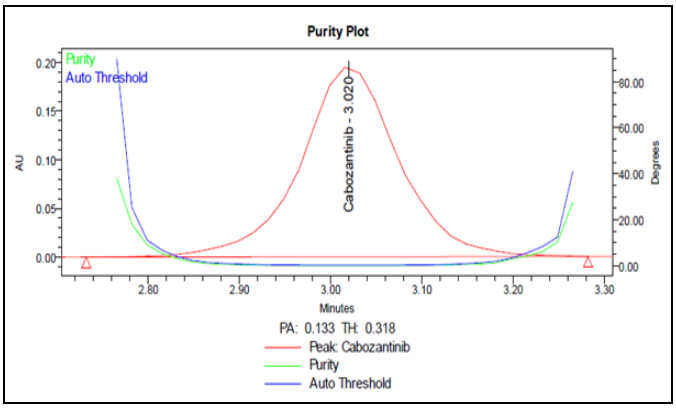
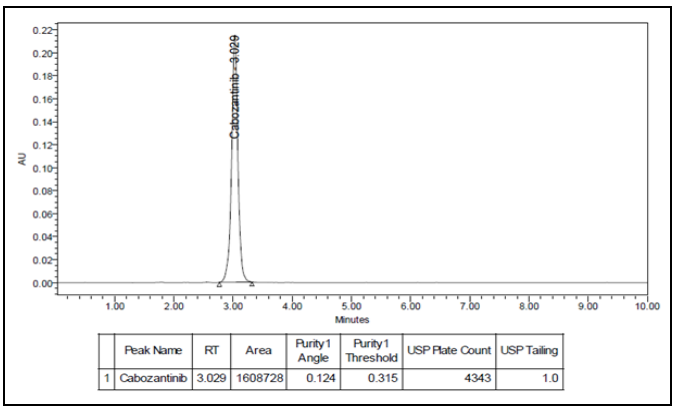


FIG. 15: UV DEGRADATION PURITY PLOT



	Peak Name	RT	Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	Cabozantinib	3.029	1608728	0.124	0.315	4343	1.0

FIG. 16: WATER DEGRADATION CHROMATOGRAPH

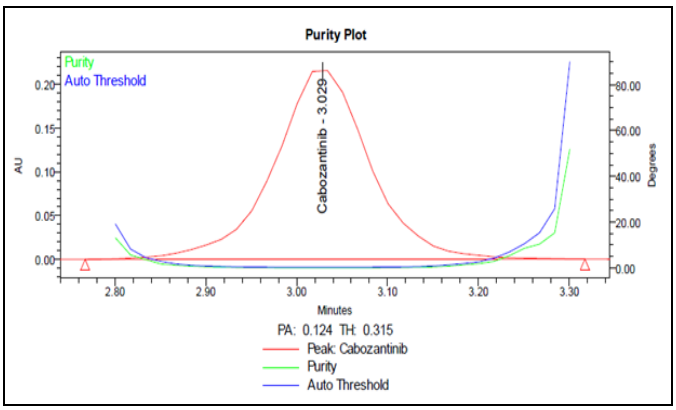


FIG. 17: WATER DEGRADATION PURITY PLOT

CONCLUSION: A simple, linear, accurate and precise HPLC method was developed and validated for the analysis of Cabozantinib in active pharmaceutical ingredient and pharmaceutical dosage form. The developed method and its validation results indicate that the method is precise and accurate. The developed method could be successfully applied for routine analysis and quality control of pharmaceutical dosage forms.

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CONFLICT OF INTEREST: The authors declare no conflicts of interest.

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