



Development and Validation of rp-HPLC Method of Cabozantinib in Active Pharmaceutical Ingredient and Pharmaceutical Dosage form

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Authors' contributions

This work was carried out in collaboration among all authors. Author AAC designed the study, performed the statistical analysis, wrote the protocol, managed the literature searches, manages the analysis of the study and wrote the first draft of the manuscript. Author AVS and AGJ revised the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

A specific, accurate rp-HPLC (reversed-phase high performance liquid chromatographic) method was developed for the quantification of Cabozantinib. The effective separation was achieved through reversed-phased C18 column 4.6 x 250 mm, 5µm using a mobile phase Methanol: phosphate buffer (ph. 3.00) with orthophosphoric acid (OPA) (55:45 % v/v). The flow rate of the mobile phase was found to be 0.8 mL/min. The detection was carried at a wavelength of 244 nm. The retention time of Cabozantinib was found to be 3.702 min. The correlation coefficient was found to be 0.9999. The developed method was accurately validated in the terms of accuracy, linearity range, precision, system suitability, robustness, limit of detection and limit of quantification. The details presented in this test will be useful for industrial application for determining Cabozantinib in active pharmaceutical ingredient and pharmaceutical dosage form.

Keywords: Cabozantinib; rp-HPLC; method development; method validation.

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1. INTRODUCTION

Cabozantinib is a drug used to treat medullary thyroid malignancy, renal cell carcinoma, and hepatocellular carcinoma. It is a bio available molecule inhibitor of the tyrosine kinases c-Met and VEGFR2, and furthermore represses AXL and RET [1,2]. Cabozantinib suppresses metastasis, angiogenesis, and oncogenesis by inhibiting receptor tyrosine kinases. Cabozantinib inhibits specific receptor tyrosine kinases such as VEGFR-1, -2 and -3, KIT, TRKB, FLT-3, AXL, RET, MET, and TIE-2. Cabometyx and Cometriq are the brand names of Cabozantinib [3,4].

The chemical name of Cabozantinib (Fig. 1) is 1-N- [4- (6, 7-dimethoxyquinolin-4-yl) oxyphenyl]-1-N'- (4- fluorophenyl) cyclopropane- 1, 1-dicarboxamide. The literature survey uncovers that there are not many techniques for determination of Cabozantinib by rp-HPLC method [5]. So, the current technique was intended to develop and validate accurate, precise and conservative RP-HPLC technique. The proposed method was validated according by ICH guidelines [6,7,8,9].

2. MATERIALS AND METHODS

Materials: Cabozantinib active pharmaceutical ingredient (API) and pharmaceutical dosage form was safely and kindly gifted by Reliable's Shree Industrial Training Centre And Research Laboratory, Jalgaon, Maharashtra. HPLC grade water, methanol is of Merck Specialities Pvt. Ltd. Shiv Sager Estate 'A' Worli, Mumbai and orthophosphoric acid used was of Avantor Performance material India Ltd. Thane, Maharashtra Instruments: Instruments like HPLC of company name Agilent (1100) Gradient System with auto injector, UV-Spectrophotometer of company name Analytical Technologies Limited, Column(C18) of company name AgilentC18 (250mmX 4.6mm,5µm),

Balance of company name WENSAR™ High Resolution Balance and pH meter of company name VSI pH meter are used for developing this technique.

Instrumentation and chromatographic conditions: All separations were carried out on HPLC waters with a UV detector (DAD) G1314 S.NO. DE71365875. The effective chromatographic separation was carried through reversed-phased C18 column 4.6 x 250 mm, 5µm. Quaternary Gradient (G130A) S.NO.DE9180834) was used with ambient column (4.6 x 250 mm) temperature (26 D, C). The ingredients of the mobile phase used for this gradient elution are Methanol: phosphate buffer (ph. 3.00) with orthophosphoric acid (OPA) (55:45 % v/v) with flow rate as 0.8ml/min. The injection volume was 20 µl. Detection was carried out at 244nm with a UV detector (DAD).

2.1 Preparation of Solution

Preparation of mobile phase: Methanol and phosphate buffer with OPA: taken in the ratio 55:45. 0.01N Phosphate Buffer: Accurately weighed 1.36 gm of Potassium dihydrogen Orthophosphate in a 1000ml of volumetric flask add about 900 ml of milli-Q water added and degas to sonic ate and finally made up the volume with water then pH adjusted to 3.32 with dil. Orthophosphoric acid solution. 10 Mm Na2Hpo42 h20 (1.7799 gm in 1000 ml water) ph adjust 3 with OPA Preparation of diluent: Mobile Phase (Methanol and phosphate buffer with OPA: taken in the ratio 55:45) is used as diluent.

Preparation of standard stock solution: Accurately weighed 10 mg Cabozantinib working standard was transferred into 10 mL volumetric flasks. Drug was dissolved in 10 mL diluent and diluted up to mark with diluent, to achieve final concentration of 1000 µg/mL. Label it as stock solution 1.

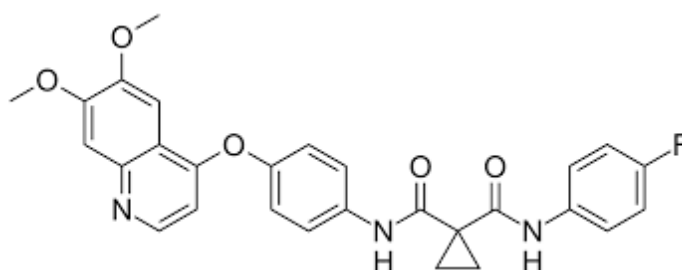


Fig. 1. Cabozantinib structure

Preparation of working standard solution: From the standard stock solution, pipetted out 0.1 mL into 10 mL volumetric flasks and diluted up to the mark with diluent to prepare 10 µg/mL of Cabozantinib. Label it as standard solution 1. From the standard stock solution, pipetted out 0.2 mL into 10 mL volumetric flasks and diluted up to the mark with diluent to prepare 20 µg/mL of Cabozantinib. Label it as standard solution 2. From the standard stock solution, pipetted out 0.3 mL into 10 mL volumetric flasks and diluted up to the mark with diluent to prepare 30 µg/mL of Cabozantinib. Label it as standard solution 3. From the standard stock solution, pipetted out 0.4 mL into 10 mL volumetric flasks and diluted up to the mark with diluent to prepare 40 µg/mL of Cabozantinib. Label it as standard solution 4. From the standard stock solution, pipetted out 0.5 mL into 10 mL volumetric flasks and diluted up to the mark with diluent to prepare 50 µg/mL of Cabozantinib. Label it as standard solution 5.

Preparation of sample solution: 20 tablets (COMETRIQ) were triturated and weigh their average weight. The total weight of 20 powered tablets was 4.184 gm and the average powder weight is 0.2092 gm/tablet. The tablet powder equivalent to 10 mg (i.e., $10 \times 209.2 \div 80 = 26.15$ mg) is 26.15 mg. Take 26.15 mg of tablet powder into a 1000 mL volumetric flask and dissolved in 10 mL diluent to produce 1000 µg/mL of Cabozantinib. Sonicate for 20 min and then the solution was filtered through 0.45 µm membrane filter and the residues were washed thoroughly with diluent. The filtrate and washings were combined in a 1000 mL volumetric flask and diluted to the mark with diluent to get a final concentration of 1000 µg/mL of Cabozantinib. The concentration of sample solution was found from regression equation of CABO. Label it as stock solution 2.

2.2 Method Validation

The developed rp-HPLC method was accurately validated as per ICH guidelines in terms of accuracy, linearity and range, precision, system suitability, robustness, limit of detection and limit of quantification.

2.3 Linearity and Range

In the chromatographic technique, linearity produces test result which is directly proportionate to the concentration of an analyte within the given range. The range refers to the interval between the upper limit and the lower limit of the analytes present in the samples. The

linearity of Cabozantinib has the range of 10-60 µg/mL. The chromatograms and the area for each concentration were recorded. The correlation coefficient was found to be 0.9999.

2.4 Precision

2.4.1 Method precision (% repeatability)

The repeatability studies were carried out by determining the response of concentration of Cabozantinib (20 µg/mL). The result was evaluated in terms of relative standard deviation (%RSD).

2.4.2 Intermediate precision (reproducibility)

The intra-day and inter-day precision (intermediate precision) were carried out by determining the responses three times on the same day and the different days of three different concentration of Cabozantinib (20, 30, 40 µg/mL). The results were evaluated in terms of relative standard deviation (%RSD).

2.4.3 Accuracy (recovery study)

Recovery studies (% recovery) were determined to validate the accuracy of developed technique. To pre-analyzed tablet solution, a definite concentration standard drug (80%, 100%, and 120%) was added and then its recovery was determined. Statistical validation of recovery studies was carried out.

2.4.4 Robustness

The robustness of a method is the ability of a method to resist change without adapting its initial stable configuration. The robustness of a method was carried out by deliberately changing the experimental conditions like flow rate and wavelength.

2.5 System Suitability

The determination of system suitability was significant part of the method development to evaluate the suitable adequate performance of the chromatography system. The determination of system suitability was carried out before validation runs. Retention time, Theoretical Plates, Tailing Factor were evaluated.

2.5.1 Limit of detection (LOD)

The LOD is the lowest limit that can be detected. The LOD can be determined by the Standard Deviation (SD) of the response and the slope (S).

$$\text{LOD} = 3.3(\text{SD})/(\text{S})$$

Whereas,
 SD = Standard Deviation of Y- intercept
 S = slope
 Limit of Detection = $3.3 \times 5.39 \div 85.27 = 0.2085$ ($\mu\text{g/ml}$)

2.5.2 Limit of quantitation

The LOQ is the lowest concentration that can be quantitatively measured. The LOQ can be determined by the Standard Deviation (SD) of the response and the slope (S).

$$\text{LOQ} = 10(\text{SD})/(\text{S})$$

Whereas,
 SD = Standard Deviation of Y- intercept
 S = slope
 Limit of Quantitation = $10 \times 5.39 \div 85.27 = 0.6321$ ($\mu\text{g/ml}$)

2.6 Analysis of Tablet Formulation

Pipette out 0.3ml from the above stock solution 2 into a 10 ml volumetric flask and dilute up to the mark with the mobile phase. The accurate chromatogram of test Cabozantinib is shown in (Fig. 2). The amount of Cabozantinib per tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated two times with tablet formulation. Tablet Assay for % Label claim and for % RSD is calculated.

3. RESULT AND DISCUSSION

3.1 Linearity and Range

The linearity was evaluated for concentration 10-60 $\mu\text{g/ml}$. The correlation coefficient was found to be 0.9999. The linearity results were performed in the Table 1 and co-relation graph is shown in Figs. 2,3.

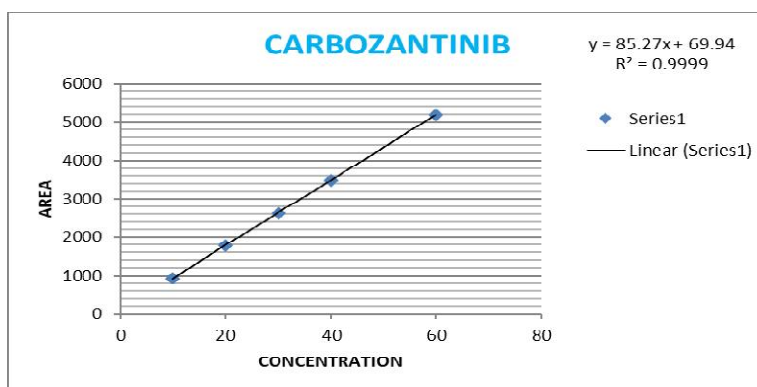


Fig. 2. Linearity graph

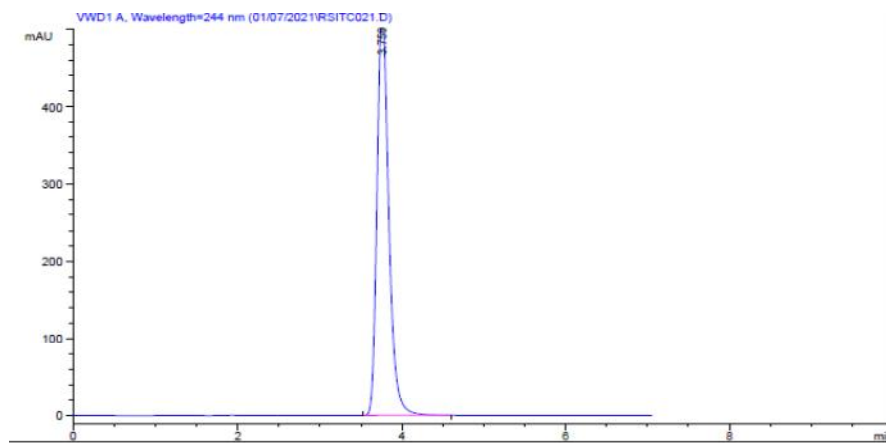


Fig. 3. Chromatogram of linearity

Table 1. Linearity and range

Ser no.	Concentration ($\mu\text{g/ml}$)	Peak area
1	10	928.78
2	20	1773.58
3	30	2623.22
4	40	3477.22
5	60	5191.23

3.2 Precision

Method Precision (% Repeatability): The RSD value for repeatability was found to be 0.08% (Table 2). The relative standard deviation (RSD) (less than 2%) indicates that the determined method is repeatable. Chromatograph of precision is shown in Fig. 4.

Intermediate Precision (Reproducibility): The RSD of inter-day was found to be 0.05-0.17% and the RSD of intra-day was found to be 0.06-0.44%. The results indicates that the determined method is precise. (Table 3,4)

3.3 Accuracy

The % mean recovery of accuracy was found to be 99.97-100.06 %. (Table 5). The results of accuracy indicates that the determined method is accurate. (Figs. 5,6,7)

3.4 Robustness

The mobile phase composition was changed in ($\pm 1 \text{ ml/min}$) proportion, the flow rate was varied by ($\pm 1 \text{ ml/min}$), and wavelength change ($\pm 2 \text{ ml/min}$) of optimized chromatographic condition. The results of robustness studies are shown in (Table 6). Robustness studies were also found satisfactory and hence the analytical method would be concluded.

3.5 System Suitability

The retention time was found to be 3.702min, the theoretical plates was found to be 3663 and the tailing factor was found to be 0.72.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): The LOD and LOQ were determined theoretically. The LOD was found to be 0.2085 ($\mu\text{g/ml}$) and the LOQ was found to be = 0.6321 ($\mu\text{g/ml}$).

Table 2. Method precision (% repeatability)

Ser no.	Concentration ($\mu\text{g/ml}$)	Peak area	Amt. found (mg)	% Amt. found	SD	% RSD
1	20	1772.52	19.96	99.83	1.44	0.08

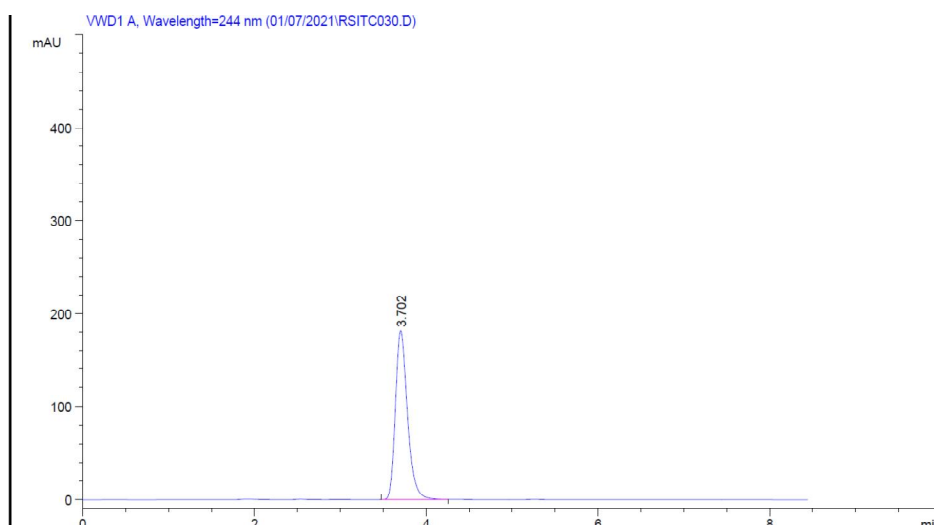
**Fig. 4. Chromatogram of precision (% repeatability)**

Table 3. Intermediate precision (reproducibility) result of intra-day precision

Ser no.	Concentration (µg/ml)	Peak area	Amt. found (mg)	% Amt. found	SD	% RSD
1	20	1778.58	20.05	100.27	0.96	0.16
2	30	2611.15	29.80	99.33	11.40	0.44
3	40	3461.64	39.77	99.43	2.10	0.06

Table 4. Intermediate precision (reproducibility) result of inter-day precision

Ser no.	Concentration (µg/ml)	Peak area	Amt. found (mg)	%Amt. found	SD	% RSD
1	20	1774.80	19.99	99.95	0.96	0.05
2	30	2619.16	29.89	99.63	4.49	0.17
3	40	3465.77	39.82	99.55	4.54	0.13

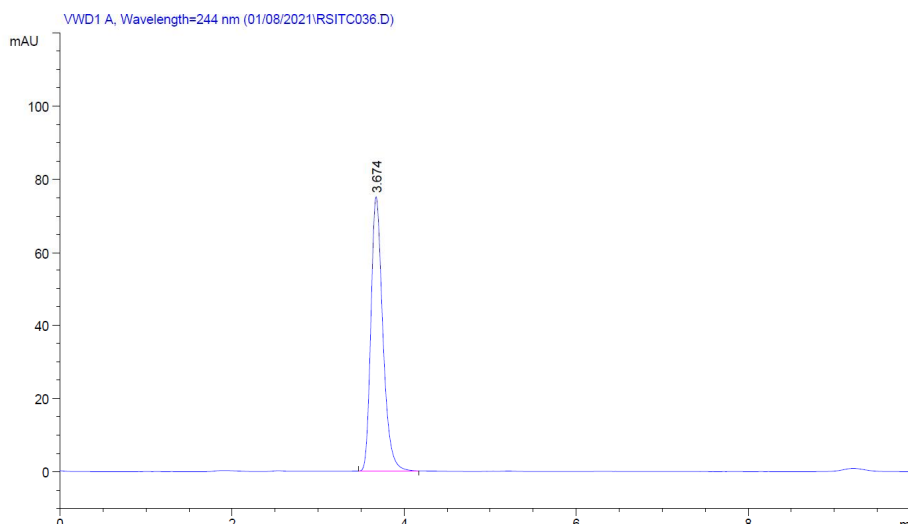


Fig. 5. Chromatogram of accuracy (80%)

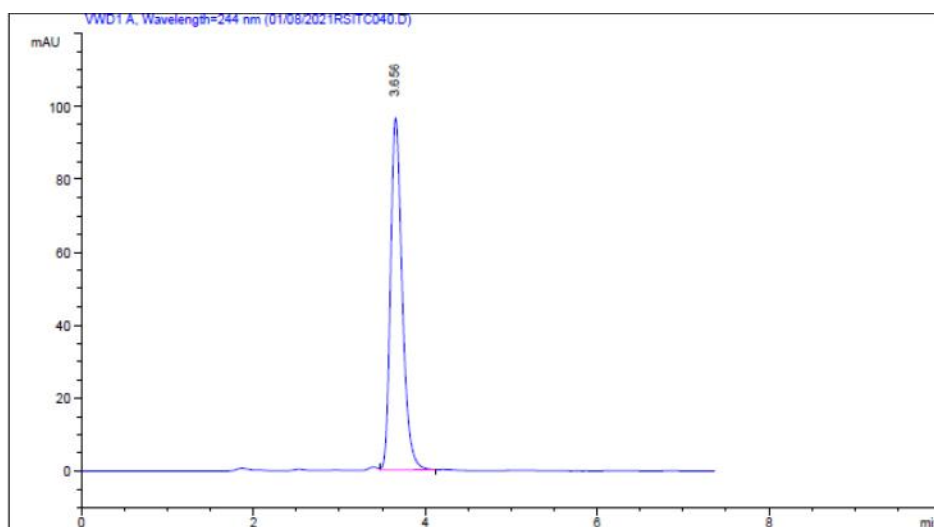


Fig. 6. Chromatogram of accuracy (100%)

Table 5. Accuracy

Ser no.	Level %	Area	Amt. added	Amt. found	Amt. received	Recovery	% Mean Recovery	SD	% RSD
1	80	1610.35	8	18.06	8.06	100.75	100.06	0.98	0.98
2	80	1600.46	8	17.94	7.94	99.36			
3	100	1773.194	10	19.98	9.98	99.83	99.73	0.15	0.15
4	100	1772.103	10	19.96	9.96	99.62			
5	120	1939.790	12	21.92	11.92	99.33	99.97	0.27	0.27
6	120	1942.96	12	21.96	11.96	99.71			

Table 6. Robustness

Parameter	Concentration ($\mu\text{g/ml}$)	Mean Area	SD	% RSD
Chromatogram of flow rate change 0.7ml	10	853.69	2.02	0.24
Chromatogram of flow rate change 0.7ml	10			
Chromatogram of flow change 0.9ml	10	695.25	1.58	0.23
Chromatogram of flow rate change 0.9ml	10			
Chromatogram of mobile phase change (56+49)	10	918.9	2.02	1.75
Chromatogram of mobile phase change (56+49)	10			
Chromatogram of mobile phase change (54+46)	10	920.35	0.22	0.19
Chromatogram of mobile phase change (54+46)	10			
Chromatogram of wavelength change 243	10	840.1	113.23	13.48
Chromatogram of wavelength change 243	10			
Chromatogram of wavelength change 243	10	796.6	2.02	0.25
Chromatogram of wavelength change 243	10			

Analysis of marketed tablet formulation (Assay): Current studies indicates the suitability of reversed-phase column procedure for the analysis of Cabozantinib in Pharmaceutical dosage form. The percentage of Cabozantinib was found to be satisfactory, which is

comparable with the corresponding claim amount results given in Table 7. The labeled amount of Cabozantinib 20 mg/tablet recovered 98.87-99.07%. It indicates that the method is simple, accurate, and precise. (Fig 8)

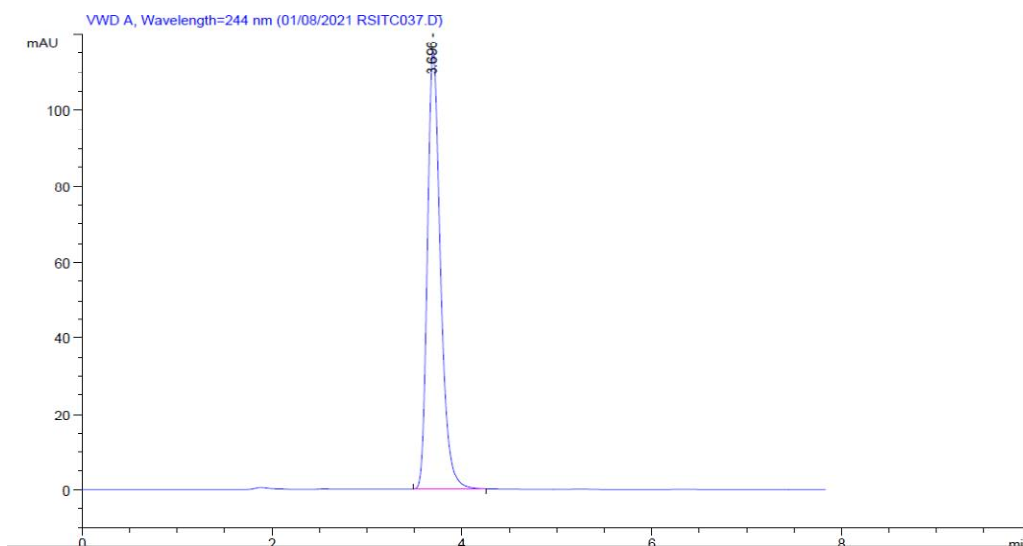


Fig. 7. Chromatogram of accuracy (120%)

Table. 7 Analysis of marketed formulation

Concentration (µg/ml)	Area	Amt. found	% Label Claim
30	2599.478	29.66	98.87
30	2604.840	29.72	99.07
Mean	2602.16	24.89	98.97
SD	3.79	0.04	0.14
% RSD	0.15	0.17	0.14

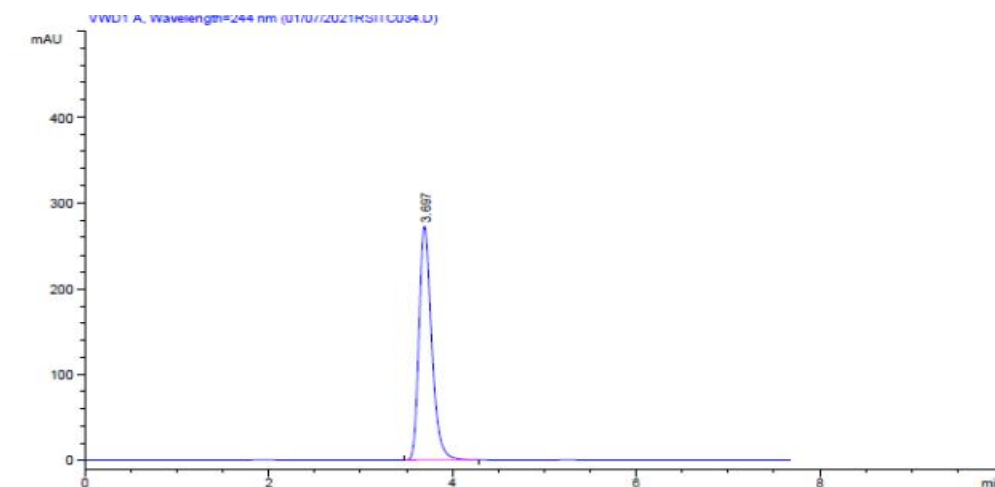


Fig. 8. Chromatogram of analysis of marketed tablet formulation

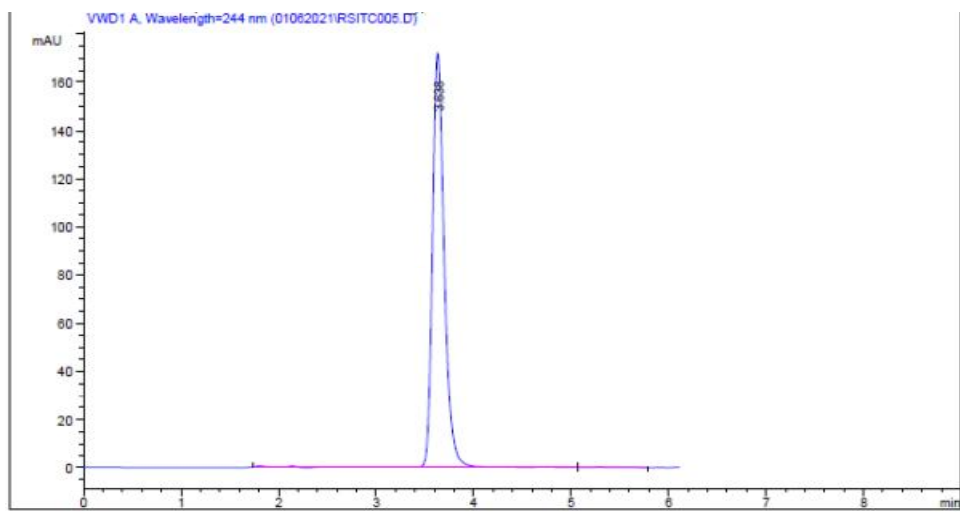


Fig. 9. Chromatogram of final of cabozantinib

4. 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 results of rp-HPLC method development and its validation indicates that the method carried out is accurate and precise. The system developed could be used effectively for the purpose of analysis and quality control of the pharmaceutical dosage type.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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