Analytical method development and validation of anticancer agent (Cabozantinib) by using UV method

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ABSTRACT:
Cancer is a broad phrase. It describes how disease arises from uncontrolled cell growth and division brought on by biological changes. While some cancer types stimulate quick cell proliferation, others induce cells to grow and divide more slowly than others. A cell is given the okay to die so that the body can replace it with a more youthful, effective cell. The components that instruct healthy cells to stop multiplying and die are lacking in cancerous cells. For Differentiated thyroid carcinoma (DTC), Cabozantinib (CBZ) is a recently created tyrosine kinase inhibitor (TKI). Hepatic cellular carcinoma (HCC), Medullary thyroid carcinoma (MTC), and Renal Cell Carcinoma (RCC). Develop and validate a simple, rapid, accurate, economic and precise UV/ VI'S method for Cabozantinib form S in bulk formulation. Cabozantinib showed maximum absorbance at 244 nm.

KEYWORDS: Cabozantinib, UV-spectrophotometry, Carcinoma, Analytical methods, Hepatic cellular carcinoma.

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INTRODUCTION : -

Any disease that can affect any region of the body is referred to as cancer. Neoplasms and malignant tumours are other words that are used. One characteristic of cancer is the quick development of aberrant cells that expand outside of their normal borders, infiltrate other body components, and eventually move to other organs. This process is known as metastasis. The main reason why cancer patients die is because of widespread metastases. One of the leading causes of death worldwide is cancer, which is the body's unchecked cell growth and development. The damaged tissue or organ of the human body is used to classify the roughly 100 different forms of cancer that exist. As a multifactorial disease, cancer alters the genome in numerous ways as a result of interactions with the environment of the patient. Currently, cancer can be healed using non-traditional or complementary therapeutic techniques, such as hormone treatment, immunotherapy, nano therapy, etc., in addition to standard tonic procedures, such as surgery, radiation therapy, and chemotherapy. However, many current cancer treatment methods have negative side effects and primarily cause discomfort to healthy cells, tissues, and organs.
MTC is a cancer that develops in the thyroid, where parafollicular C cells become malignant and proliferate out of control. It can spread to other organs and lymph nodes, and up to 25%

of diagnoses have inherited forms. Patients with MEN2A may also develop parathyroid tumours or pheochromocytomas.

A condition known as renal cell cancer, also known as kidney cancer or renal cell adenocarcinoma, is characterised by the presence of malignant (cancer) cells in the lining of the kidney's tubules (very tiny tubes). Above the waist, on either side of the backbone, are two kidneys. The blood is filtered and cleaned by tiny tubules in the kidneys. They remove trash and produce urine. A lengthy tube called a ureter transports the urine from each kidney and into the bladder. Urine is stored in the bladder until it exits the body through the urethra.

The most typical form of primary liver cancer is hepatocellular carcinoma (HCC). People with chronic liver illnesses, such as cirrhosis brought on by hepatitis B or hepatitis C infection, are most likely to develop hepatocellular carcinoma. Hepatocellular carcinoma (HCC) is a primary liver cancer that most commonly affects people who have cirrhosis and underlying chronic liver disease.

Cabozantinib (S)-malate (CBZ), as it is chemically known, "N-(4-fluorophenyl)-N-(4-fluorophenyl) cyclopropane-1,1-dicarboxamide, (25)-hydroxybutanedioate". It belongs to the antineoplastic drug class. CBZ is described as "a white to off-white crystalline powder that is naturally non-hygroscopic." Due to the fact that the freebase is water insoluble, CBZ contains the (m-salt). It is well known that CBZ's molecular structure is non-chiral. One of the two crystalline forms—"N-1" and "N-2," which have a similar structure—exists in amorphous form. CBZ is highly protein bound in vitro in human plasma, binding 299.7% of the total amount of protein. Preliminary protein binding (with albumin) was determined from the continuing hepatic impairment (2.7–4.3 g/dl). Multiple RTKs (receptor tyrosine kinases) involved in tumor growth, angiogenesis, and the spread of metastatic disease are known to be inhibited by CBZ.

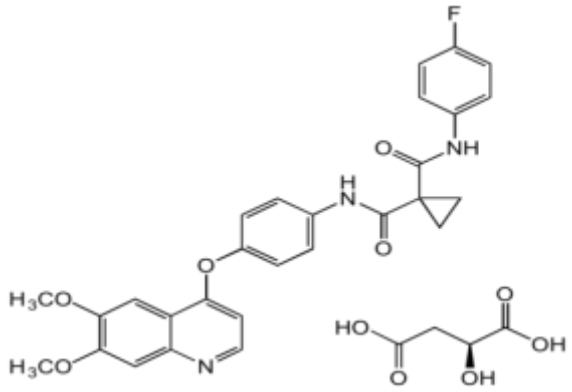

Fig.1. Strucutre of CBZ.

The molecular weight of CBZ is 635.59.

The molecular formula of CBZ $C_{32}H_{30}FN_3O_{10}$.

MATERIALS AND METHODS: -

Chemicals and Reagents: -

All of the chemicals and solvents were of analytical quality, and methanol (Rankem). Cabozantinib was acquired from MSN Laboratories Pvt. Ltd, Sangareddy District, Telangana India. The only other compounds were analytical-grade

Preparation of Standard Stock Solutions: -

Separately weighed 10 mg of Cabozantinib were put into 10 mL volumetric flasks. By using a sonicator. To get the component's final concentration of 1000 g mL^{-1}, the volume was then brought up to the required level using the same solvent.

Preparation of Working Standard Solutions: -

To obtain the concentration range of 0.5,1,2,2.5,3,4,4.5, mg mL^{-1} for Cabozantinib, suitable aliquots of 1000 mg mL-1 solution were diluted up to the mark with Methanol. At 244 nm, the absorbance was measured.

Selection of wavelength: -

The UV spectrum was used to determine the analytical wavelength for cabozantinib (100ppm). The Cabozantinib solution was scanned in the range of 200-400 nm, with 244 nm being the maximum measured against methanol.

Method validation: -

According to the ICH criteria for the validation of analytical procedures, the technique was created and validated in accordance with the analytical procedure to ascertain for the analyte, linearity, accuracy, precision, Intraday, Intraday, LOD, LOQ.

Linearity: -

By examining the various concentrations of the standard solution of Cabozantinib, the linearity was assessed. The range of Beer-concentration Lambert's was discovered.

Approximately 0.5-4.5g mL-1 for Cabozantinib. Plotting the calibration curves for Cabozantinib, which are presented in Table, allowed researchers to ascertain the linearity of the connection between absorbances and concentration.

Table.1. Linearity

Conc(ppm)	Absorbance
0.5	0.096
1	0.195
2	0.399
2.5	0.504
3	0.602
4	0.800
4.5	0.921

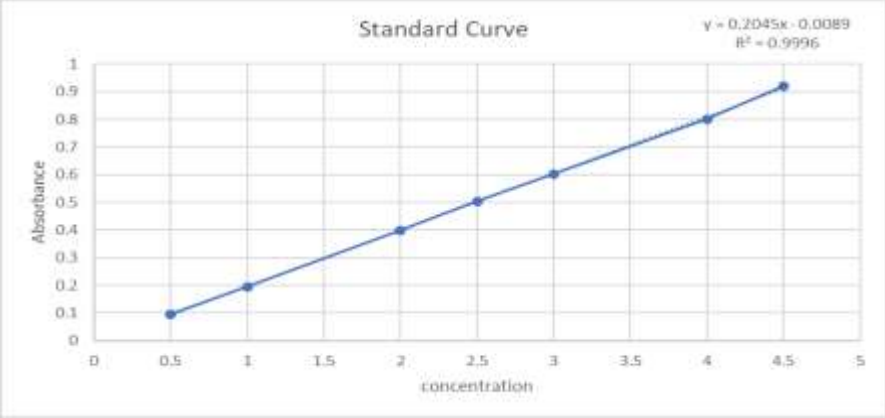

Fig.2. Standard Curve of CBZ

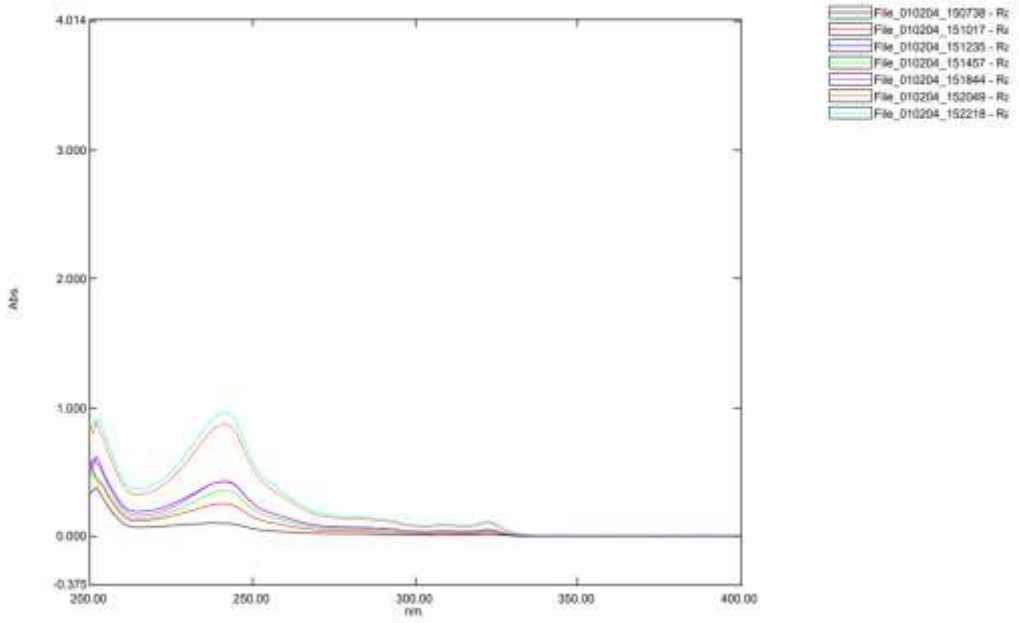

Fig.3. linearity graph of CBZ

Accuracy (% Recovery): -

The traditional addition method was used to conduct the accuracy investigation. Cabozantinib 2.5g mL-1 sample solution was pre-quantified and spiked with an additional expients of the usual Cabozantinib. At 244 nm, absorbances were recorded, and the drug concentration was calculated. Calculations were made at each concentration to determine the samples' percentage recovery, percent RSD, and percentage**.**

Recovery Studies: -

Table.2a. Accuracy

S.NO	Conc (µg/ml)	Absorbance Std sol^{n}	Conc. Found (µg/ml)	Absorbance spiked sample	Conc. Found (µg/ml)
1	2	0.399	1.99	0.398	1.99
		0.398	1.99	0.397	1.98
		0.399	1.99	0.397	1.98
2	2.5	0.504	2.50	0.501	2.49
		0.504	2.50	0.502	2.5
		0.505	2.51	0.503	2.50
3	3	0.602	2.99	0.589	2.92
		0.603	2.99	0.588	2.92
		0.602	2.99	0.589	2.92

Table.2b. Recovery Studies

S.no	Conc.	Mean Conc. (std)	Mean Conc. (spiked sample)	Recovery
1	2	0.398	0.397	100%

2	2.5	0.504	0.502	100%
3	3	0.602	0.588	102%

Intraday Precision: -
A solution (2.5 ppm) was chosen for the intraday fluctuation investigation and was examined three times over the course of two days (i.e., morning, afternoon, evening). Calculations were made for mean, standard deviation, and RSD.

Table.3. Intraday

Sample no.	Conc. (µg/ml)	Absorbance	wavelength	RSD	SD
1	2.5	0.312	245	0.48699	0.001528
2	2.5	0.314	245		
3	2.5	0.315	245		

Interday: -
On a different day, the interday precision for a solution (2.5 ppm) was calculated and examined for the three measurements RSD was determined.

Table.4. Interday

Time of sample	Sample (µg/ml)	Absorbance	Wavelength	RSD	SD
1hrs	2.5	0.305	245	0.81796	0.002517
2hrs	2.5	0.308	245		
3hrs	2.5	0.310	245		

Blank Readings: -
0.001, 0.001, 0.002, 0.001, 0.002, 0.000
Mean=0.001167
SD =0.000753

DETECTION LIMIT: -
The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

$$LOD=\frac{3.3\ X\ SD}{S}=\frac{3.3X0.000753}{0.204}=0.012180$$

QUANTITATION LIMIT: -
The lowest amount of analyte in a sample that can be quantitatively measured with enough precision and accuracy is the quantitation limit of a specific analytical process. One of the parameters in quantitative assays for low quantities of substances in sample matrices, and is particularly useful for identifying contaminants and/or degradation products.

$$LOQ=\frac{10\ X\ SD}{S}=\frac{10X0.000753}{0.204}=0.0313$$

Conclusion: -
In order to regularly analyse Cabozantinib in pure form on a qualitative and quantitative level, an UV technique was created and validated. For showing and identifying any potential the approach is stability-indicating, making it qualified and dependable.

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