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UV Spectrophotometric Method Validation for Ivacaftor in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

Cystic fibrosis (CF) is an inherited disorder that causes severe damage to the lungs, digestive system and other organs in the body. A protein that controls the flow of salt into and out of cells are altered in cystic fibrosis due to a flaw (mutation) in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. As a result, sweat becomes more salted and the respiratory, digestive, and reproductive systems produce thick, sticky mucus. A novel medicinal drug called ivacaftor modifies the function of the CFTR channel. Ivacaftor is indicated for treatment of CF in patients homozygous for the Phe-508del CFTR gene mutations. Using ethanol as a solvent, two exact, straightforward, accurate, repeatable, quick, and affordable UV spectrophotometric techniques have been established for the simultaneous quantification of ivacaftor in tablet dose form. The methods are designed to be employed to perform regular drug analysis in tablet dosage form and have been validated in accordance with ICH criteria. A straightforward, exact, and accurate method was created for the simultaneous estimate of the Ivacaftor dose form in tablet form. This research mainly focuses on the method development and validation of Ivacaftor by UV spectroscopy to improve the novelty of the drug.

Key words: *Cystic fibrosis, ivacaftor, cystic fibrosis transmembrane conductance regulator, UV spectroscopy, validation.*

INTRODUCTION

Cystic fibrosis (CF) is a multisystemic autosomal recessive disease caused by a defect in the expression of CFTR (Cystic fibrosis transmembrane conductance regulator) protein, i.e. chloride channel present in the apical membrane of respiratory, digestive, reproductive and sweat glands epithelium.

Cystic fibrosis (CF) affects more than 30,000 people in the United States and 80,000 people worldwide, according to the American Cystic Fibrosis Foundation patient registry. As per the 2018 Registry Report by the Cystic Fibrosis Foundation, the average estimated survival time for CF patients in the United States is roughly 47.4 years.

Ivacaftor is an aromatic amide that synthesizes via the condensation of the 5-amino-2,4-di-tert-butyl phenol amino group with the carboxy group of 4-oxo-1,4-dihydroquinoline-3-carboxylic acid. utilized as a cystic fibrosis therapy. It functions as both an orphan medication and a CFTR potentiator. It is an aromatic amide, a monocarboxylic acid amide, a quinolone, and a member of the phenol family [1-3].

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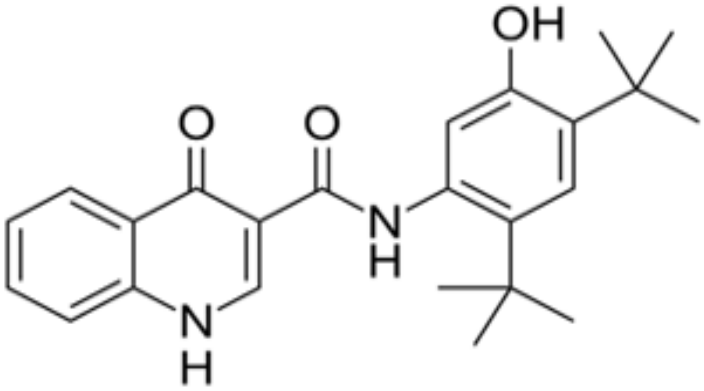

Figure 1: Chemical structure of ivacaftor

In this present investigation, an attempt has been made to develop an accurate, precise and economical UV method for the estimation of ivacaftor in bulk and in pharmaceutical dosage form.

MATERIALS AND METHODS

Selection of solvent

A number of trails were done to find out the ideal solvent for dissolving the drug. The solvents such as double distilled water, methanol and acetonitrile and ethanol were tried based on the solubility of the drug. Ivacaftor was found to be freely soluble in ethanol.

Selection of wavelength (λmax)

Appropriate volume 1 ml of standard stock solution of Ivacaftor was transferred into a10 ml volumetric flask, diluted to a mark with ethanol to give concentration of 10 µg/ml. The resulting solution was scanned in the UV range (200-400 nm).

Preparation of stock solution [4,5]

Stock solution of Ivacaftor was prepared by dissolving 10mg of Ivacaftor in 10ml of standard volumetric flask and solution was sonicated 5min and then made up to the mark with solvent to get a concentration of 1mg/ml. Subsequent dilutions of this solution made with solvent. The standard solution prepared as were injected into the quartz cuvette.

Preparation of sample solution

From the stock solution take 1ml of solution in a volumetric flask and dilute with 10ml of ethanol up to the mark to makeup the concentration 10µg/ml.

Assay for pharmaceutical formulation

The solution was filtered through Whatman filter paper No. 41. 0.5 ml of this solution was transferred to 10 ml volumetric flask and final volume was made with ethanol. It gives 0.5 µg/ml. It was scanned on a spectrophotometer in the UV range 200-400 nm. The spectrum was recorded at 310 nm against blank solution of ethanol. Determine the amount of % Ivacaftor in tablet according to the following formula:

$$\%\ \textbf{Assay} = \frac{\textbf{WS X AT X Sample D. F. X Avg. wt.}}{\textbf{AS X Standard D. F. X WT X LC}}\ \textbf{X PS}$$

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Where,

WS = Weight of standard,
WT = Weight of sample,
AT = Absorbance of apremilast in the test solution,
AS = Absorbance of apremilast in the standard solution,
Standard D.F. = Standard dilution factor,
Sample D.F. = Sample dilution factor,
PS = Purity of working standard [%],
LC = Label claim.

Method development [6-9]

For developing the method, a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. The following studies were conducted for this purpose.

Wavelength detection

The spectrum of 10ppm solution of the in ivacaftor-ethanol was recorded separately on UV spectrophotometer. The spectra of ivacaftor were showed maximum absorbance at 310 nm.

Validation of the proposed method

The proposed method was validated as per ICH guidelines. The parameters studied for validation was linearity, precision, accuracy, LOD and LOQ.

Linearity

In order to assess the linearity of the method, five doses of the reference substance (5mg,10mg, 15mg, 20mg, 25mg) were used at UV spectroscopy for standard curves. The calculation of regression line was employed by the method of least squares. The responses were read at 310nm and the corresponding values were recorded.

Precision

The precision is a measure of ability of the method to generate reproducible results. The method was performed as intraday precision. To study the inter day precision, six replicate standard solutions (15ppm) of Ivacaftor were injected. The percent relative standard deviation (%RSD). For this, 15µg/ml of the sample was measured five times in the day and the same procedure was repeated in next three days. The precision results are showed good reproducibility with percent relative standard deviation (%RSD) was below %. This indicated the method was highly precise.

Accuracy

Recovery studies were performed to judge the accuracy of the method. These studies are carried out by a known quantity of pure drug adding to pre-analyzed formulation and the proposed method was followed by the amount of drug found, the percentage recovery was calculated. Accuracy was carried out three levels 80%, 100%, 120% for the correction of 15µg/ml. The solution was analyzed in triplicate at each level. The percentage recover and % RSD was calculated and results were presented in satisfactory recovery were obtained by the proposed method and this indicates that was accurate.

Robustness

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The sample solution is taken in the cuvette and run under UV spectroscopy at different wavelengths with respect to the standard wavelength of 310nm.The difference of wavelength is 310 ± 5 nm.

Ruggedness

The sample solution is taken in the cuvette and run under UV spectroscopy at different wavelength of 310 nm. But the sample is diluted with the different ratio of solvent. The solvent is prepared by the mixture of ethanol and water in the ratio of 9:1.

RESULTS AND DISCUSION

Validated analytical methods are aimed for the estimation of Ivacaftor in API and its formulation. Simple, precise, rapid, accurate methods were developed for the estimation of Ivacaftor in formulation by using UV spectroscopy method. In case UV spectroscopy method solubility is the important parameter. Solubility parameter was studied and ethanol was selected as the solvent, since it gave a maximum absorbance and a good spectral pattern when compared with other solvents. The marketed formulation was extracted and diluted to get the concentration in the linearity range. The solution was scanned and measured at 310 nm. Percentage recovery, linearity, stability studies were also carried out. The above method gave a satisfactory recovery value and found to be stable, linear, hence it can be used for routine analysis of the drug formulation. Solutions of Ivacaftor and its marketed product were prepared by using ethanol and UV spectrum of each was recorded by scanning between 200-400 nm.

Absorbance maxima (λ max)

The absorbance maximum of Ivacaftor was found to be 310 nm.

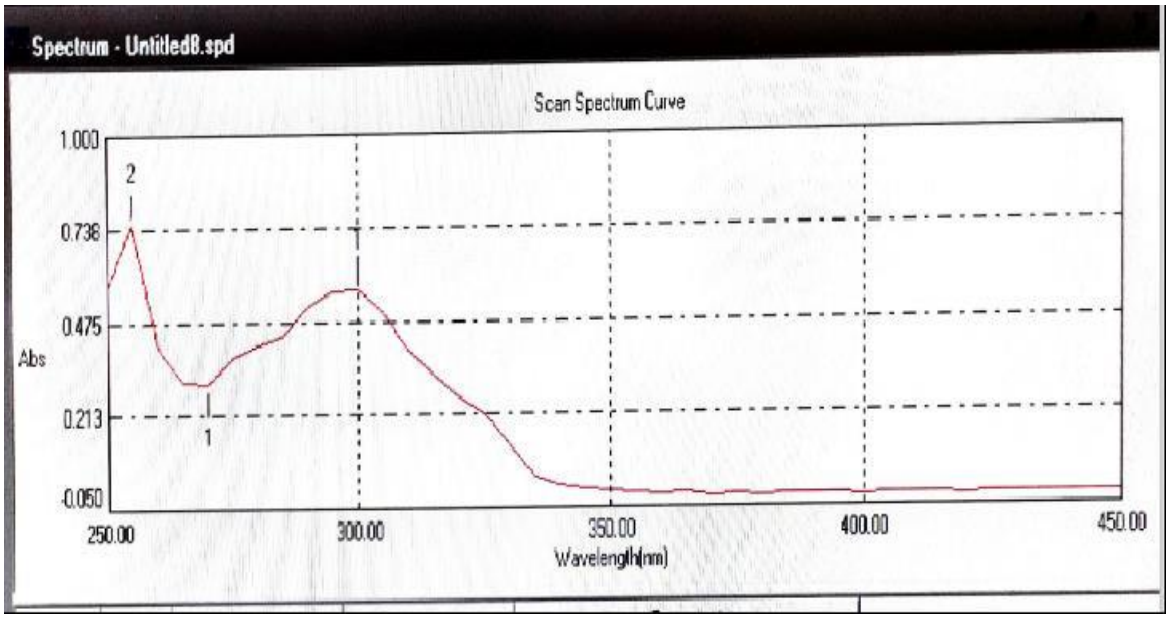

Figure 2: Maximum absorbance of Ivacaftor

Table 1: Assay

Drug	Labelled amount	Amount found	Assay
Ivacaftor	150 mg	150.99	100.66%

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Method Validation

The proposed method was validated as per ICH Q2 guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experimental work. The analysis of Ivacaftor was achieved by using UV spectroscopy.

Linearity

The linearity was checked in different concentrations range from 5-25 µg/mL.

Table 2: Linearity

Concentration (ppm)	Absorbance
0	0.00
5	0.159
10	0.372
15	0.541
20	0.709
25	0.900

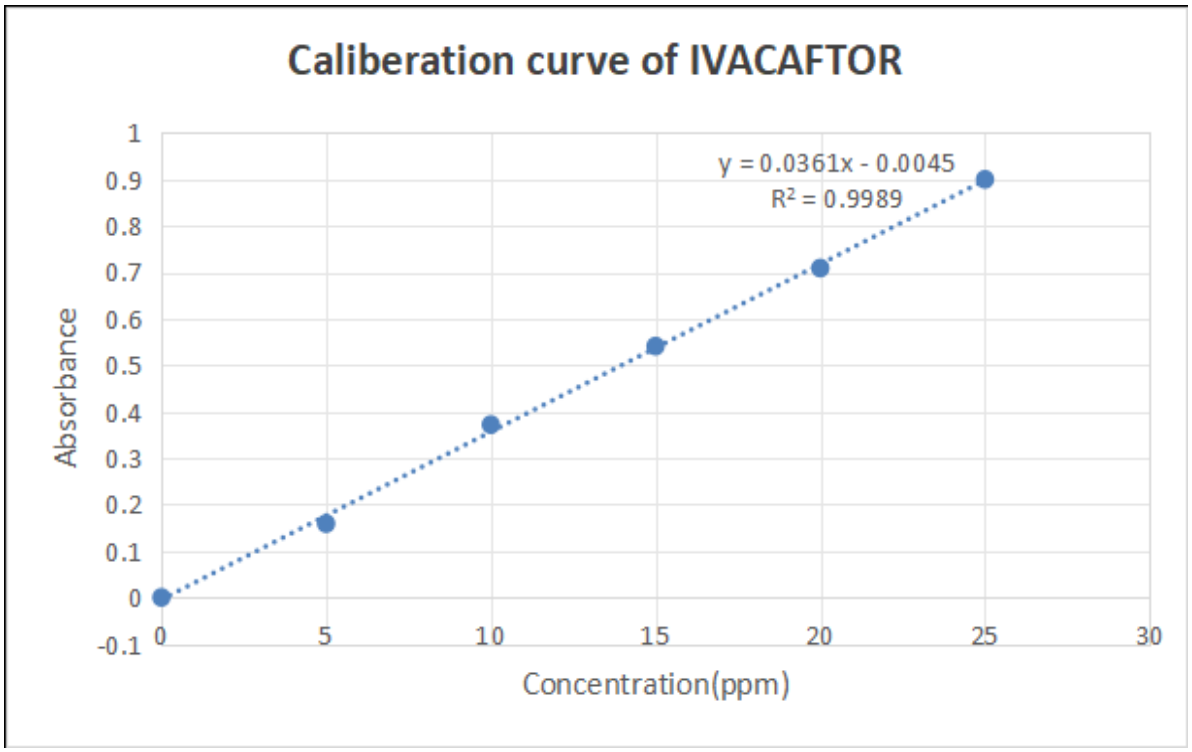

Figure 3: Linearity

Precision

The Precision studies were carried in terms of intraday and interday. The Percentage Relative Standard Deviation (%RSD) values were found to be less than 2, which indicate that the method is precise.

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Table 3: Intra-day Precision

Parameter	Conc. (µg/ml)	Intra-day precision (Mean ± SD)	%RSD
Morning	6	0.158667±0.000516	0.325461
Afternoon	6	0.158333±0.000816	0.515682
Evening	6	0.158167±0.000753	0.475936

Table 4: Inter-day Precision

Parameter	Conc. (µg/ml)	Inter-day precision (Mean ± SD)	%RSD
Morning	6	0.158833±0.000408	0.257029
Afternoon	6	0.158167 ±0.000753	0.475936
Evening	6	0.157833±0.000753	0.476941

Accuracy:

Table 5: Accuracy

15ppm taken as 100% and 1.5 ml is taken as 100%						
Parameter	**Sample (Tablet)**	**Standard (6ppm)**	**Mean**	**SD**	**%RSD**	**% Recovery**
50%	1.5	0.75	0.151	0.001	0.662252	101.1111
	1.5	0.75				
	1.5	0.75				
100%	1.5	1.5	0.198	0.001	0.505051	99.62963
	1.5	1.5				
	1.5	1.5				
150%	1.5	2.25	0.245	0.001	0.408163	99.1358
	1.5	2.25				
	1.5	2.25				

The recovery studies were carried out to ensure the reproducibility and reliability of the method by adding known amount of standard drugs and analysis was carried out as per formulation procedure. The recovery values were within the limits indicating that the method is accurate.

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Limit of Detection (LOD) and Limit of Quantification (LOQ):
LOD and LOQ were carried out according to ICH guidelines and were found. In order to test the appropriateness of the developed method to the pharmaceutical formulation, an assay of Ivacaftor tablets was performed at working concentration.

Table 6: LOD & LOQ

Drug	LOD	LOQ
Ivacaftor	0.374 µg/ml	1.135 µg/ml

The proposed UV spectroscopy method for the quantification of Ivacaftor in API and tablet formulation is simple, accurate, and rapid and can be employed for the routine analysis. Once the absorbance of the sample is determined, it requires only simple calculation. This method can be applied for the substances which obey Beer's law. The low standard deviation and good percentage recovery indicated the reproducibility and accuracy of the method.

Robustness:

Table 7: Robustness

Conc. in µg/ml	Parameter	Wavelength	
		305 nm	315 nm
15	Mean	0.541	0.541333
15	SD	0.001	0.000577
15	%RSD	0.184843	0.106653

Ruggedness:

Table 8: Ruggedness

Parameter	Conc. in µg/ml	Mean	SD	%RSD
Analyst – 1	15	0.294333	0.000577	0.196155
Analyst – 2	15	0.293333	0.000577	0.196824

CONCLUSION

From present research work, it is concluded that the method validation is economical and reproducible. The method was developed and validated as per ICH Q2 (R1) guidelines. The proposed methods can be employed for routine analysis of Ivacaftor in bulk and from pharmaceutical dosage form (Tablets). The results obtained on the validation parameters met ICH Q2 and USP requirements. It is inferred that the methods were found to be simple, accurate, precise and linear. The methods were found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision. The precision was

measured in terms of repeatability, which was determined by sufficient number of aliquots of a homogeneous sample. The results showed that the recovery of marketed product by the proposed method was satisfactory. Application of this method for the analysis of ivacaftor tablet dosage form showed that. there was no interference of excipients in the determination. The method is advantageous over most of the reported methods in-terms of sensitivity, simplicity, cost-effectiveness and experimental conditions. The method does not involve any tedious procedural steps; do not require any extra reagents or longer analysis time and a very simple instrument are required. The method can be used to determine the purity of the drug available from various sources. Because of cost-effective and minimal maintenance, the present UV spectroscopy method can be preferred at small scale industries and successfully applied and suggested for the quantitative analysis of Ivacaftor in pharmaceutical formulations for QC, where economy and time are essential and to assure therapeutic efficacy. Many pharmaceutical industries manufacture their formulation of all mentioned drugs either in combination or in single dosage form. Most of the pharmaceutical industries use time consuming method and different solvents for different dosage form of drugs. But with the proposed method developed, time and cost required for changing different solvent could be saved, because only one solvent can be used for six drugs and their combinations. This makes the method suitable for routine analysis in quality control laboratories.

Ethical Approval: Not required.

Competing Interest Statement: All authors declare that there is no conflict of interests regarding publication of this paper.

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