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METHOD DEVELOPMENT AND VALIDATION OF IVACAFTOR IN BULK & PHARMACEUTICAL DOSAGE FORM BY UV-VISIBLE SPECTROPHOTOMETRY

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Abstract:

Objective: *The objective of the present work is to develop a simple, efficient, and reproducible spectrophotometric method for the quantitative estimation of drug - Ivacaftor in active pharmaceutical ingredient (API) form and in pharmaceutical dosage form****Methods:*** *The developed ultraviolet spectrophotometric method for the quantitative estimation of Cystic fibrosis drug Ivacaftor - based on measurement of absorption at a wavelength maximum (λ_{max}) of 255 nm using Acetonitrile as solvent.****Results:*** *The method was validated in terms of , precision, linearity, accuracy, and robustness ,LOD,LOQas per the ICH guidelines. The method was found to be linear in the range of 50-150% for Ivacaftor . The percentage recovery values were in the range of 99.9-100.9% for Ivacaftor at different concentration levels. Relative standard deviation for precision and intermediate precision results were found to be <2%. The correlation coefficient value observed for Ivacaftor drug substances was not <0.99, 0.99, respectively. Results obtained from the validation experiments prove that the developed method is quantified for the estimation of Ivacaftor drug substances.****Conclusion:*** *The developed method can be successfully applied for Ivacaftor routine analysis, quality control analysis, and also suitable for stability analysis of in API & its pharmaceutical dosage form as per the regulatory requirements.*

Keywords: Ivacaftor, Method development, Validation, Ultraviolet-visible spectrophotometry.

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INTRODUCTION:

The chemical name of ivacaftor [1] is N- (2,4ditert-butyl-5-hydroxyphenyl)-4-oxo-1H- quinoline-3-carboxamide. This drug is used for the treatment of cystic fibrosis. ivacaftor is a white to yellow crystalline non-hygroscopic powder. It is freely soluble in water, methanol, ethanol (95%), acetonitrile and practically insoluble in dichloromethane, tetrahydrofuran, acetone, and ethyl acetate.

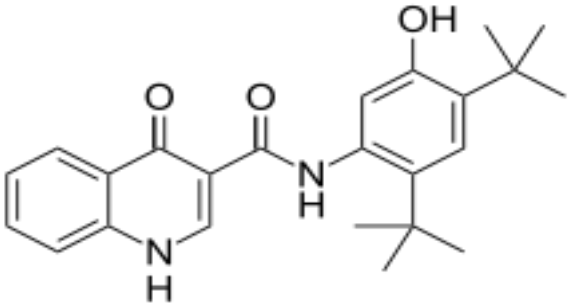


FIG 1.0: Structure of Ivacaftor

- ✓ **Category:** Treating cystic fibrosis
- ✓ **Mechanism of action:**

Cystic fibrosis [2] is caused by any one of several defects in the CFTR protein, which regulates fluid flow within cells and affects the components of sweat, digestive fluids, and mucus. One such defect is the G551D mutation, in which the amino acid glycine (G) in position 551 is replaced with aspartic acid (D). G551D is characterized by a dysfunctional CFTR protein on the cell surface. In the case of G551D, the protein is trafficked to the correct area, the epithelial cell surface, but once there the protein cannot transport chloride through the channel. Ivacaftor, a CFTR potentiator, improves the transport of chloride through the ion channel by binding to the channels directly to induce a non-conventional mode of gating which in turn increases the probability that the channel is open.

Table: 2.1 Characteristic profile of drug

S.No	Parameters	Characteristics
1	Drug name	Ivacaftor
2	Molecular formula	$C_{24}H_{28}N_2O_3$
3	Molecular Weight:	392.499g/mol
4	CAS number	873054-44-5
5	Colour	white crystalline colour
6	Odor	Odorless
7	Taste	Bitter
8	Appearance	non-hygroscopic powder
9	Melting range	292°C-295°C
10	Solubility	water, methanol, ethanol (95%), acetonitrile and practically insoluble in dichloromethane, tetrahydrofuran, acetone, and ethyl acetate.
11	Therapeutic category	cystic fibrosis

LITERATURE REVIEW

1. Janardhana Reddy et al[3], developed a easiest, precise, accurate means of analysis of the drug is by UV Spectrophotometry. In this regard, Ivacaftor drug is analyzed by method development and validation for UV spectrophotometric study. The linearity studies obeyed Beer and Lambert's law where the linear regression coefficient value was found to be 0.9973 at λmax 202nm. The linearity range considered for the present study being 1-5 µg/ml. The test validation parameters and the recovery value satisfy the acceptance criteria by the method developed. Therefore the present method developed can be used for routine analysis of Bulk product and formulated dosage forms of Ivacaftor.

EXPERIMENTAL PROCEDURE

- ❖ **Materials and methods**

Ivacaftor tablets (label claim 150mg, brand name Kalydeco) were obtained from local pharmacy. HPLC grade Acetonitrile was obtained from Rankem (Mumbai, India).

API was obtained as gift sample from alembic pharmaceuticls pvt Ltd.

- ❖ **Instrumentation**

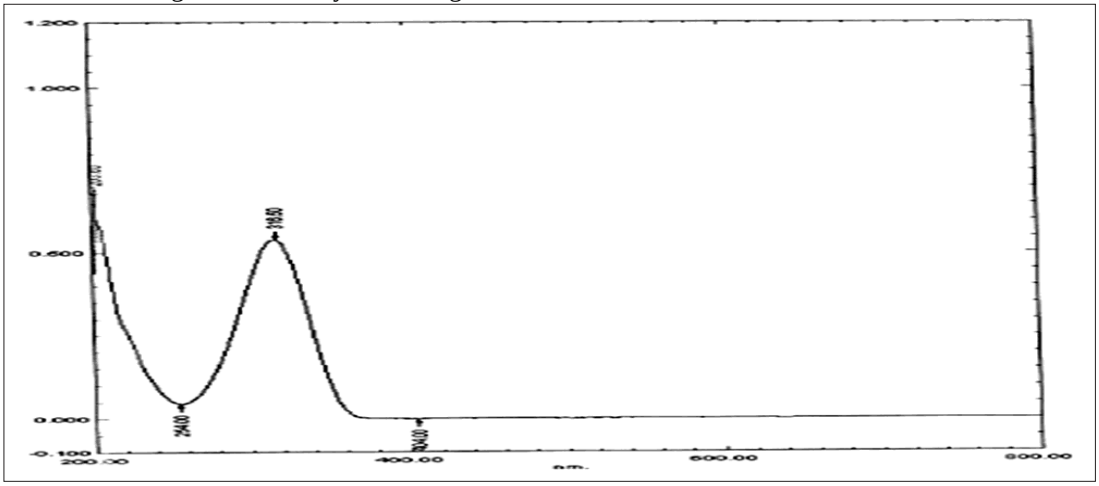
A double beam UV-vis spectrophotometer (Lasa) having two matched quartz cells with 1 cm light path length and loaded with UV probe software was used for recording of spectra and measuring absorbance for method development and validation study.

- ❖ **Method development**
- ✓ ***Selection of diluent***

Acetonitrile was used as diluent for the preparation of standards for Ivacaftor Formulation based on the solubility characteristics of the drug substances.

✓ ***Selection of suitable wavelength detection***

Spectra for Ivacaftor was measured from 200 to 400 nm for wavelength maxima by recording UV-vis spectrum of standard solution. The corresponding spectrum of Ivacaftor is shown in Fig. 3.8. Maximum absorbance (λmax) was shown at 255 nm for standard solution of Ivacaftor . Based on the spectra maxima, 255 nm were selected for identification and quantification of drug Ivacaftor.



❖ **Preparation of Standard stock solution**

A standard stock solution of Ivacaftor was prepared by dissolving 10 mg of Ivacaftor in a 10ml clean dry volumetric flask, 7mL of Acetonitrile was added and sonicated for about 10min and then made up to 10mL with Acetonitrile to get a 1 µg/mL standard stock solution.

Calibration curve were prepared by dilution of above stock solution in the range of 25µg/mL- 150 µg/mL.

Table:1.1 Preparation of Standard stock solution

S.No	Pipetted from stock (mL)	Volume of flask (mL)	Concentration in ppm
1	0.25	10	25
2	0.5	10	50
3	0.75	10	75
4	1.0	10	100
5	1.25	10	125
6	1.50	10	150

❖ **Preparation of Sample solutions**

Ivacaftor (label claim 150mg)

Five tablets containing 150 mg of Ivacaftor were weighed and then Powdered. An amount of powder equivalent to 1 mg of Ivacaftor and was transferred in a 100 mL volumetric flask, with 70mL of ACN and sonicated for 25min, to ensure complete solubility of the drug, and volume made up with the diluent (Acetonitrile) and filtered through 0.45µm membrane filter. From this 1ml was pipetted out and transferred to 10 mL volumetric flask and made up to 10mL with Acetonitrile to get the concentration of 1µg/mL of Ivacaftor.

RESULTS AND DISCUSSION:

❖ **METHOD VALIDATION**

The method was validated for the Parameters like linearity, precision, limit of detection (LOD), limit of quantification (LOQ), Accuracy, , robustness based on ICH/CPMP guidelines(14-16).

❖ **PRECISION**

Precision was measured in terms of repeatability of application and measurement. Repeatability of standard application was carried out using six replicates of same standard concentration. Repeatability of sample measurement was carried out in six different sample preparations from same homogenous blend of marked sample. The results of Precision studies are expressed in Table 1.2

Table 1.2 : Method precision of Ivacaftor

Sample preparation	**% Assay** Ivacaftor
1	100.72
2	100.81
3	100.41
4	100.19
5	101.20
6	103.20
Mean	101.08
± SD	0.995987
% RSD	0.89

The % RSD for repeatability of sample preparation is 0.89%. This shows that precision of the method is satisfactory as % relative standard deviation is not more than ± 2.0%

❖ **Accuracy**

Accuracy of the method was determined by analysis of standard at three different levels. Values were found to be within the limit given in (Table 2.3). The mean recovery was in the range of 99.43-100.54% which shows there is no interference from excipients.

% Recovery = b-a/c×100

Where,

a = The amount of drug found before the addition of standard drug

b = The amount of drug found after the addition of standard drug

c = The amount of standard drug added

Table1.3: Recovery studies of Ivacaftor

concentration	**Sample preparation**	**% Recovery of** Ivacaftor	**AVG**	**±SD**	**%RSD**
	1	99.54			
50%	2	99.15	99.43	0.245	0.246
	3	99.61			
	1	99.17			
100%	2	100.84	100.06	0.836	0.835
	3	100.16			
	1	100.98			
150%	2	99.88	100.54	0.58	0.57
	3	100.76			

❖ **Linearity**

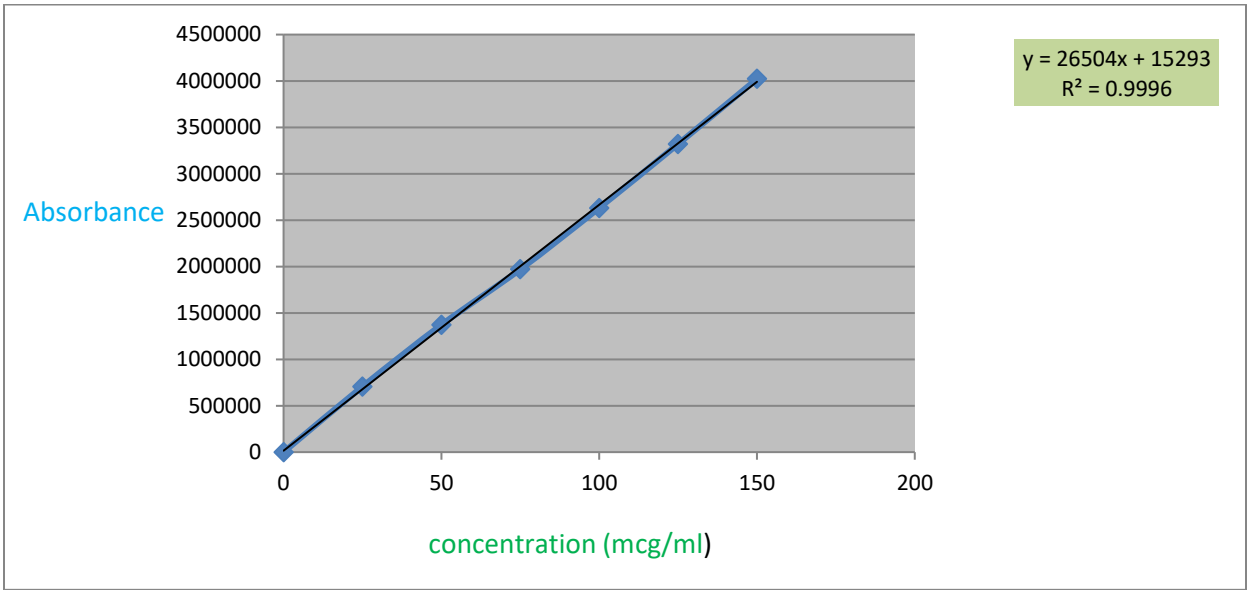
✓ Aliquots of Ivacaftor[4] standard stock solution were taken in different 10ml volumetric flasks and diluted up to the mark with the diluents such that the final concentrations of daclasatavir are in the range of 25-150 µg/mL.. Evaluation was performed with PDA detector at 248nm and a Calibration graph was obtained by plotting area versus concentration of Ivacaftor.

✓ The plot of area of Ivacaftor each sample against respective concentration of was found linear in the range of 25-150 µg/mL with correlation coefficient of 0.999.. The respective linear regression equation being Y= 26504x+152930. The regression characteristics were calculated for this method and given in Table 2.4.

Table 1.5: Linear regression data for calibration curves

Drug	Ivacaftor
Linearity Range	25 -150 µg/mL
Slope(m)	265040
Y Intercept(b)	152930
Correlation coefficient	0.9998

Fig 1.1: Calibration curve of Ivacaftor



❖ **Limit of Detection and Limit of Quantification**

The sensitivity of measurement of Ivacaftor by use of proposed method was estimated in terms of the limit of quantification (LOQ) and the lowest concentration detected under the chromatographic condition as the limit of detection (LOD). The LOQ and LOD were calculated by the use of equations LOD=3×N/B and LOQ=10×N/B where N is the standard deviation of the absorbance of the drug and B is the slope of corresponding calibration plot. (LOD) limit of detection and (LOQ) limit of quantification were found to be 1.9 µg/mL and 5.7 µg/mL respectively.

❖ Robustness

The robustness of the proposed method was performed by preparing the standard a change in wavelength for absorbance readings. The wavelength selected was ±2 nm to the λ_{max}, i.e 253 and 257 nm for Ivacaftor drug, for standard solutions. In the robustness condition (wavelength variation of ±2 nm to the λ_{max}), the assay values of Ivacaftor were not <99%. % Assay results for robustness parameters were shown table.

Table 1.6: robustness parameters

Determination	**% Assay of** Ivacaftor **at 253nm**	**% Assay of** Ivacaftor **at 257nm**
Determination-1	99.5	99.5
Determination-2	99.3	99.3
Determination-3	99.4	99.4
Average	99.4	99.4
SD	0.11	0.11
%RSD	0.11	0.12

SUMMARY AND CONCLUSION:

A simple, rapid, accurate, precise, robust method was developed for estimation of Ivacaftor in bulk and its formulation by UV-Visible spectrophotometry.
A good linear relationship (r=0.999) was observed between concentration range of 25-150 µg/ml. The assay of tablets was recovered which indicates high accuracy of the method.

The method was found to be robust by changing the wavelength. (±2 nm to the λ_{max}, i.e257 and 253 nm) In the robustness condition(wavelength variation of ±2 nm to the λ_{max}), the assay values of were not <99%.

The method was found to be accurate and precise with % RSD of 0.55 &0.89%. respectively.This shows that precision of the method is satisfactory as % relative standard deviation is not more than ± 2.0%.

Ivacaftor (LOD) limit of detection and (LOQ) limit of quantification were found to be 1.9 µg/mL and 5.7 µg/mL respectively.

Thus, the developed method can be easily used for the routine quality control of bulk and tablet dosage form of within a short analysis is time.

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