WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 14, 213-219. Review Article ISSN 2277– 7105

APPLICATION OF UV SPECTROPHOTOMETERIN METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANIOUS ESTIMATION OF TEZACAFOR AND IVACAFTOR IN PHARMACEUTICAL DOSAGE FORM

Sonawane M. D.[1]*, Gade S. T.[2] and B. M. Narwate[2]

[1]*,[2]Assistant Professor of Pharmaceutical Chemistry, MES College of Pharmacy, Sonai, Ahmednagar, (MS), India, 414707.

Article Received on
30 May 2018,

Revised on 20 June 2018,
Accepted on 10 July 2018

DOI: 10.20959/wjpr201814-12464

***Corresponding Author**
Sonawane M. D.
Assistant Professor of Pharmaceutical Chemistry, MES College of Pharmacy, Sonai, Ahmednagar, (MS), India, 414707.

ABSTRACT

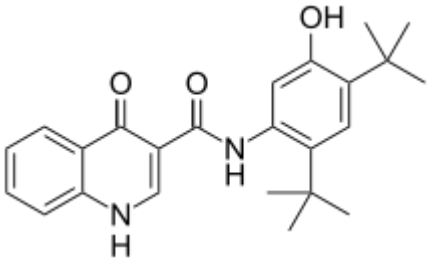
Two precise, simple, accurate, reproducible, rapid and economical UV spectrophotometric methods have been developed for simultaneous estimation of tezacaftor and ivacaftor in tablet dosage form by using ethanol as solvent. Method I is based on formation and solving of simultaneous equation method, known as vierodt's method. tezacaftor and ivacaftor show absorbance maxima at 239 nm and 351 nm respectively, so absorbance was measured at the same wavelength for the estimation of tezacaftor and ivacaftor in tablet combination. Method II is based on principle of Q-analysis, known as absorbance ratio method. Absorbance was measured at 265 nm (isobestic point) and 274 nm (λmax of ivacaftor). tezacaftor and ivacaftor obeys beer's law in the concentration range of 5 to 30 µg/ml. Percentage estimation of tezacaftor and ivacaftor in tablet dosage form by method I is 99 and 99.85 and by method II is 99 and 99.89 respectively with standard deviation ≤2. Methods are validated according to ICH guidelines and can be applied for routine analysis of drugs in tablet dosage form.

KEYWORDS: Tezacaftor and ivacaftor.

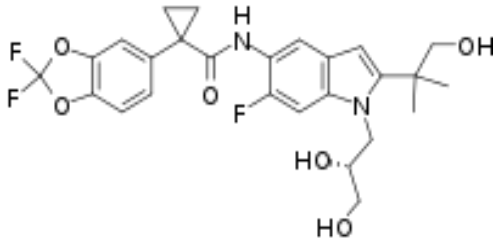
INTRODUCTION

Ivacaftor (trade name Kalydeco) is a drug used to treat cystic fibrosis in people with certain mutations in the cystic fibrosis transmembrane conductance regulator (CFTR).[1] Ivacaftor was developed by Vertex Pharmaceuticals in conjunction with the Cystic Fibrosis Foundation

and is the first drug that treats the underlying cause rather than the symptoms of the disease.[2] Cystic fibrosis 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. 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.[3] Ivacaftor is approximately 99% bound to plasma proteins.[4]



Tezacaftor, also known as VX-661, is a drug approved by the FDA to treat some cases of cystic fibrosis. Tezacaftor helps move the CFTR protein to the correct position on the cell surface, and is designed to treat people with the F508del mutation.[5]



The review of literature revealed that several methods are available for the determination of tezacaftor and ivacaftor individually. Reported methods for estimation of tezacaftor were Spectrophotometric[6], HPLC[7,8], HPTLC[9], and LC-MS[10] and for ivacaftor were Spectrophotometric[11], HPLC[11] and HPTLC.[12] But there is no any analytical method yet reported for simultaneous estimation of these drugs in combination.

UV Spectrophotometric method

MATERIALS AND METHODS

UV-Visible double beam spectrophotometer, (Jasco model 2201) with spectral bandwidth of 1 nm, wavelength accuracy of ± 0.3 nm and a pair of 1mm matched quartz cell was used. The commercially available tezacaftor and ivacaftor was procured from local market.

Preparation of standard stock solution and calibration curve

The standard stock solution of tezacaftor and ivacaftor were prepared by dissolving 10 mg of tezacaftor and ivacaftor in ethanol in a separate 100 ml volumetric flask and final volume was adjusted with the same solvent in 100 ml of volumetric flask to get a solution containing 100 µg/ml of tezacaftor and 100 µg/ml of ivacaftor respectively.

Working standard solutions of 10µg/ml for tezacaftor and ivacaftor were scanned in the entire UV range of 200-400 nm to determine their λmax. The λmax of tezacaftor and ivacaftor is found to be 239 nm and 351 nm respectively and isobestic point is at 265 nm from overlain spectra as shown in **Fig.3**. Six working standard solutions with concentration 5, 10, 15, 20, 25, 30µg/ml for tezacaftor and ivacaftor were prepared in ethanol from stock solution. The absorbance of resulting solutions were measured at their respective λmax and isobestic point and plotted a calibration curve to get the linearity and regression equation as shown in **Fig. 4 and 5**.

Method I (Simultaneous equation method)

Simultaneous equation method of analysis is based on the absorption of both drugs at their wavelength maximum. Two wavelengths selected for the development of the simultaneous equation are 239 nm and 351 nm. The absorptivity values were determined for tezacaftor are 0.14621(ax_1), 0.08121 (ax_2) and for ivacaftor are 0.07101 (ay_1), 0.1871 (ay_2) at 239nm and 351 nm respectively. These values are means of six estimations. The absorbances and absorptivity at these wavelengths were substituted in equation 1 and 2 to obtain the concentration of these drugs.

$$C_{\text{tezacaftor}} = \frac{(A_2 \times ay_1) - (A_1 \times ay_2)}{ax_2 \times ay_1 - ax_1 \times ay_2} \quad \text{.....Eqn.1}$$

$$C_{\text{ivacaftor}} = \frac{(A_1 \times ax_2) - (A_2 \times ax_1)}{ax_2 \times ay_1 - ax_1 \times ay_2} \quad \text{.....Eqn.2}$$

Where C_{FLX} and C_{QTF} are concentration of tezacaftor and ivacaftor respectively in µg/ml. A_1 and A_2 were the absorbance of the sample at 239 nm and 351 nm respectively.

Method II (Absorbance ratio method)

Absorbance ratio method of analysis is based on the absorbance at two selected wavelengths, one of which is an isobestic point and the other being the absorption maximum of one of the two drugs. From overlain spectra (**Fig.3**) 265 nm (isobestic point) and 351 nm (λmax of

ivacaftor) are selected for the formation of Q absorbance equation (Eqn.3 and 4). The absorptivity values determined for tezacaftor are 0.08121 (ax_1), 0.04958 (ax_2) and for ivacaftor are 0.17710 (ay_1), 0.17794 (ay_2) at 351 nm and 265 nm respectively. These values are means of six estimations. The absorbance and absorptivities at these wavelengths were substituted in equation 3 and 4 to obtain the concentration of these drugs.

$$C_{\text{tezacaftor}} = \frac{Q_M - Q_Y}{Q_Y - Q_X} \times \frac{A_1}{ax_1} \quad \text{..... Eqn.3}$$

$$C_{\text{ivacaftor}} = \frac{Q_M - Q_X}{Q_X - Q_Y} \times \frac{A_1}{ay_1} \quad \text{..... Eqn.4}$$

Q_M, Q_X and Q_Y were obtained from Eqn.no.5, 6, 7 respectively.

$$Q_M = \frac{A_2}{A_1} \quad \text{..... Eqn.5}$$

$$Q_X = \frac{ax_2}{ax_1} \quad \text{..... Eqn.6}$$

$$Q_Y = \frac{ay_2}{ay_1} \quad \text{..... Eqn.7}$$

Where C_{FLX} and C_{QTF} are concentration of tezacaftor and ivacaftor respectively in µg/ml. A_1 and A_2 were the absorbance of the sample at 265 nm and 351 nm respectively.

Validation of developed methods

Linearity

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed method. For method I and II, the Beer-Lambert's concentration range was found to be 5-30 µg/ml for both tezacaftor and ivacaftor. The linearity data of both methods are presented in **Table.1.**

Accuracy

To check the accuracy of the proposed methods, recovery studies were carried out at 80, 100 and 120% of the test concentration as per ICH guidelines. The recovery study was performed three times at each level. The results of the recovery studies are shown in **Table.2.**

Repeatability

To check the degree of repeatability of these methods, suitable statistical evaluation was carried out. Repeatability was performed for six times with synthetic mixture. The standard

deviation and coefficient of variation were calculated. The results of statistical evaluation are given in **Table.2.**

Intermediate Precision (Interday and Intraday precision)

The Interday and intraday precision was determined by assay of the sample solution on the same day and on different days at different time intervals respectively. The results of the same are presented in **Table.3**.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of tezacaftor and ivacaftor. by proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S respectively. Where S is the slope of the calibration curve and σ is the standard deviation of response. The results of the same are quoted in **Table.3**.

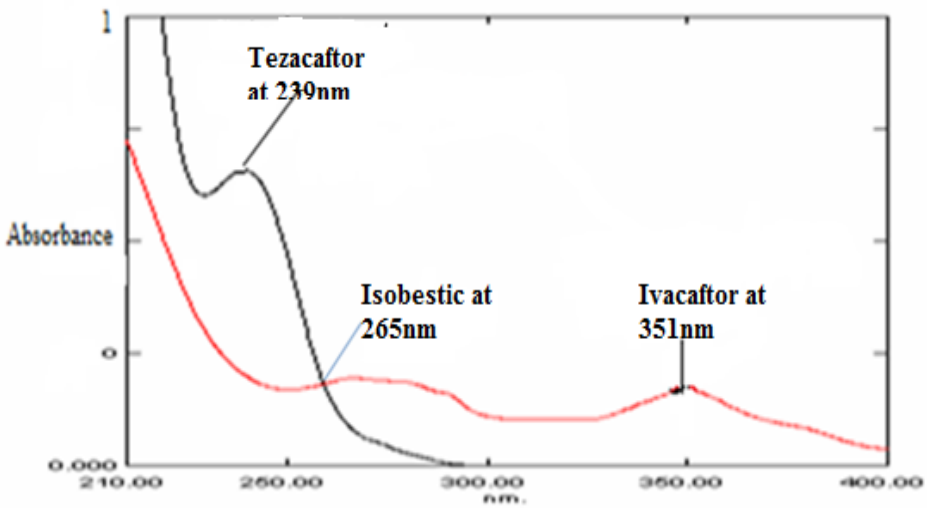


Fig. 3: Overlain spectra of tezacaftor and ivacaftor.

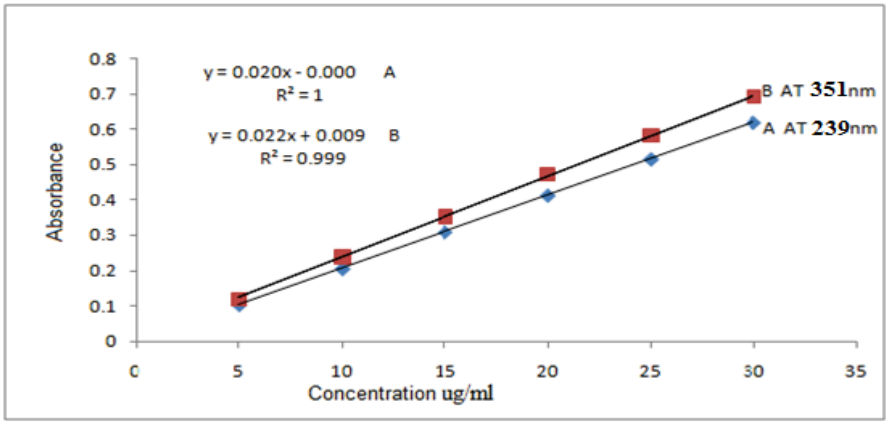


Fig. 4: Calibration curve and regression equation of Tezacaftor in ethanol.

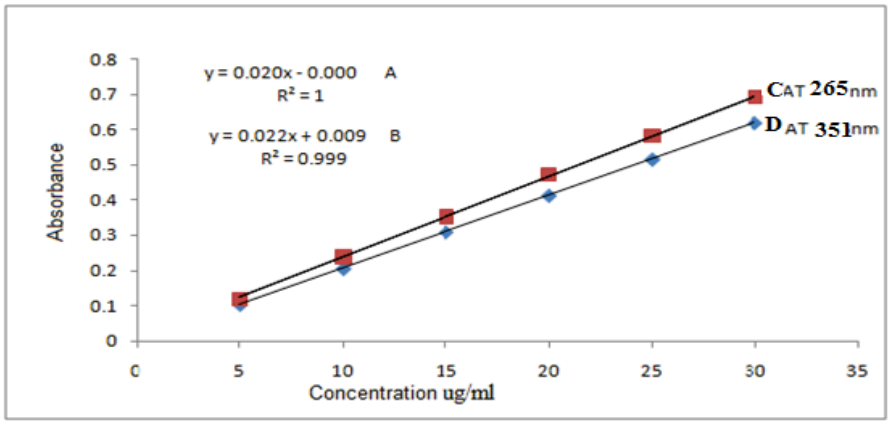


Fig. 5: Calibration curve and regression equation of Ivacaftor in ethanol.

Table 1: Optical Characteristics for tezacaftor and ivacaftor.

Parameters	Tezacaftor		Ivacaftor	
Working wavelength	283nm	285nm	274nm	285nm
Beer's law limit(µg/ml)	5-30	5-30	5-30	5-30
Absorptivity*	0.1462	0.0495	0.1771	0.1779
Correlation coefficient*	0.999	0.997	0.998	0.998
Intercept*	0.008	0.026	0.036	0.002
Slope*	0.026	0.047	0.052	0.065

**Average of six estimations.*

Table 2: Recovery studies.

Method	Level of recovery (%)	% Recovery ±S.D.#	
		Tezacaftor	Ivacaftor
I	80	98.5±0.45	99.45±0.82
	100	99.42±0.60	100.5±0.45
	120	99.60±0.97	101.2±0.54
II	80	98.56±0.54	100.54±0.23
	100	99.45±0.65	101.2±0.12
	120	100.56±0.78	99.60±0.45

#mean of three determinations, SD: Standard Deviation

ACKNOWLEDGMENTS

We would like to acknowledge MES College of pharmacy, sonai Principal Dr.V.K.Deshmukh and vice principal Dr.V.B.Ghawate of our institute for providing necessary facility to carry out this work and guide for his diligent guidance and counsel on the planning and execution of this study.

REFERENCE

1. Riordan J, Rommens J, Kerem B, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science., 1989; 245(4922): 1066–1073.
2. Huaux F, Noel S, Dhooghe B, et al. Dysregulated proinflammatory and fibrogenic phenotype of fibroblasts in cystic fibrosis. PLoS One., 2013; 8(5): e643413.
3. Marcorelles P, Friocourt G, 3.Uguen A, Ledé F, Férec C, Laquerrière A. Cystic fibrosis transmembrane conductance regulator protein (CFTR) expression in the developing human brain: comparative immunohistochemical study between patients with normal and mutated CFTR. J Histochem Cytochem., 2014; 62(11): 791–801.
4. Gao Z, Sun HY, Lau CP, Chin-Wan Fung P, Li GR. Evidence for cystic fibrosis transmembrane conductance regulator chloride current in swine ventricular myocytes. J Mol Cell Cardiol., 2007; 42(1): 98–105.
5. Bonfield TL, Hodges CA, Cotton CU, Drumm ML. Absence of the cystic fibrosis transmembrane regulator (Cftr) from myeloid-derived cells slows resolution of inflammation and infection. J Leukoc Biol., 2012; 92(5): 1111–1122.
6. Di A, Brown ME, Deriy LV, et al. CFTR regulates phagosome acidification in macrophages and alters bactericidal activity. Nat Cell Biol., 2006; 8(9): 933–944.
7. Painter RG, Valentine VG, Lanson NA, et al. CFTR expression in human neutrophils and the phagolysosomal chlorination defect in cystic fibrosis. Biochemistry, 2006; 45(34): 10260–10269.
8. Zengerling S, Tsui LC, Grzeschik KH, Olek K, Riordan JR, Buchwald M. Mapping of DNA markers linked to the cystic fibrosis locus on the long arm of chromosome 7. Am J Hum Genet., 1987; 40(3): 228–236.
9. Tsui L, Buchwald M, Barker D, et al. Cystic fibrosis locus defined by a genetically linked polymorphic DNA marker. Science., 1985; 230(4729): 1054–1057.
10. Gadsby DC, Vergani P, Csanady L. The ABC protein turned chloride channel whose failure causes cystic fibrosis. Nature., 2006; 440(7083): 477–483.
11. Sebastian A, Rishishwar L, Wang J, et al. Origin and evolution of the cystic fibrosis transmembrane regulator protein R domain. Gene., 2013; 523(2): 137–146.
12. Cheng SH, Rich DP, Marshall J, Gregory RJ, Welsh MJ, Smith AE. Phosphorylation of the R domain by cAMP-dependent protein kinase regulates the CFTR chloride channel. Cell., 1991; 66(5): 1027–1036.