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Method development and validation of RP-HPLC for simultaneous analysis of two component tablet formulation containing Tezacaftor and Ivacaftor in bulk and marketed formulations

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Abstract
Analytical Method Development and Validation for Tezacaftor and Ivacaftor in bulk and Combine Dosage Form by RP-HPLC, New method was established for simultaneous estimation of Tezacaftor and Ivacaftor by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Tezacaftor and Ivacaftor by using Symmetry C18 5µm (4.6 x 150mm), flow rate was 1.0 ml/min, mobile phase ratio was Phosphate buffer (0.02M) pH-3.8: Methanol: Acetonitrile (60:20:20%v/v), detection wavelength was 260nm. The retention times of Tezacaftor and Ivacaftor were found to be 2.324mins and 4.314mins respectively. The % purity of Tezacaftor and Ivacaftor was found to be 99.865% and 99.658% respectively. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Tezacaftor and Ivacaftor was found in concentration range of 0µg-36µg and 0µg-39µg and correlation coefficient (r2) was found to be 0.9995 and 0.9998, % recovery was found to be 100.280, %RSD for repeatability was 0.174 and 0.709, % RSD for intermediate precision was 0.093 and 0.937 respectively. The precision study was precise, robust, and repeatable. LOD value was 1.377 and 1.079, and LOQ value was 4.174 and 3.272 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Tezacaftor and Ivacaftor in API and Pharmaceutical dosage form.

Keywords: Tezacaftor and Ivacaftor, Method Development, Validation, Accuracy

1. Introduction

Tezacaftor1 is a drug of the cystic fibrosis transmembrane conductance regulator (CFTR) potentiator class. It was developed by Vertex Pharmaceuticals and FDA approved in combination with [Ivacaftor] to manage cystic fibrosis. This drug was approved by the FDA on February 12, 2018. Cystic Fibrosis is an autosomal recessive disorder caused by one of several different mutations in the gene for the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein, an ion channel involved in the transport of chloride and sodium ions across cell membranes. CFTR is active in epithelial cells of organs such as of the lungs, pancreas, liver, digestive system, and reproductive tract. Alterations in the CFTR gene result in altered production, misfolding, or function of the protein and consequently abnormal fluid and ion transport across cell membranes. As a result, CF patients produce thick, sticky mucus that clogs the ducts of organs where it is produced making patients more susceptible to complications such as infections, lung damage, pancreatic insufficiency, and malnutrition. The IUPAC Name of Tezacaftor2 is 1-(2, 2-difluoro-1, 3-benzodioxol-5-yl)-N-[1-[(2R)-2, 3-dihydroxy propyl]-6-fluoro-2-(1-hydroxy-2-methyl propan-2-yl) indol-5-yl] cyclopropane-1-carboxamide. The Chemical Structure of Tezacaftor3 is

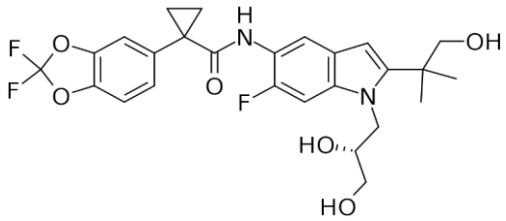

Fig 1: Chemical Structure of Tezacaftor

Ivacaftor is an aromatic amide obtained by formal condensation of the carboxy group of 4-oxo-1, 4-dihydroquinoline-3-carboxylic acid with the amino group of 5-amino-2, 4-di-tert-butylphenol. Used for the treatment of cystic fibrosis. It has a role as a CFTR potentiator and an orphan drug. It is a quinolone, a member of phenols, an aromatic amide and a monocarboxylic acid amide. Ivacaftor is a Cystic Fibrosis Transmembrane Conductance Regulator Potentiator. The mechanism of action of Ivacaftor[4] is as a Chloride Channel Activation Potentiator, and Cytochrome P450 2C9 Inhibitor, and P-Glycoprotein Inhibitor, and Cytochrome P450 3A Inhibitor. When used as monotherapy as the product Kalydeco, Ivacaftor is indicated for the treatment of cystic fibrosis (CF) in patients aged four months and older who have one mutation in the CFTR gene that is responsive to Ivacaftor[5] potentiation based on clinical and/or in vitro assay data. The IUPAC Name of Ivacaftor [6] is N-(2, 4-ditert-butyl-5-hydroxy phenyl)-4-oxo-1H-quinoline-3-carboxamide. The Chemical Structure of Ivacaftor is following

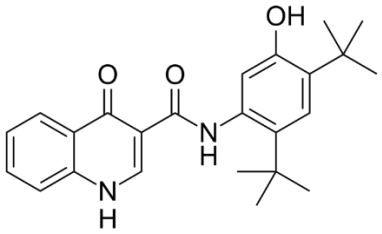

Fig 2: Chemical Structure of Ivacaftor

As per the literature review [36-40], some methods was reported for the estimation of Tezacaftor and Ivacaftor and its degradation products in bulk form and finished dosage forms using RP-HPLC. The present work describes the simultaneous estimation of Tezacaftor and Ivacaftor and its degradation products in tablet dosage forms using RP-HPLC and also developed method gives a sensitive, specific, and stability indicating [7] method for the determination of Tezacaftor and Ivacaftor in a short run time by RP-HPLC.

Materials and Methods

Table 1: List of Equipments

S.No.	Instrument	Model No.	Software	Manufacturer's Name
1	HPLC Alliance	Waters 2695	Empower	Waters
2	UV Double Beam Spectrophotometer	UV 3000	UV Win 5	Lab India
3	Digital Weighing Balance	BSA224SCW	-	Sartorius
4	pH meter	AD102U	-	Lab India
5	Ultra Sonicator	SE60US	-	-
6	Suction Pump	VE115N	-	-

Table 2: List of Chemicals

S.No.	Chemical	Manufacturer	Grade
1	Water	Merck	HPLC Grade
2	Methanol	Merck	HPLC Grade
3	Acetonitrile	Merck	HPLC Grade
4	Potassium dihydrogen orthophosphate	Merck	A.R

Selection of Wavelength: The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm.

Method Development

Preparations and Procedures

Preparation of Phosphate buffer: (pH: 3.8): Weighed 0.136086 grams of KH_2PO_4 was taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water, adjusted the pH to 3.8 with ortho phosphoric acid.

Preparation of Mobile Phase: A mixture of pH 3.8 Phosphate buffer 600 mL (60%), 200 mL of MEOH (20%) and 200 mL of Acetonitrile are taken and degassed in ultrasonic water bath for 15 minutes. Then this solution is filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: Mobile phase is used as Diluent[8].

Preparation of the individual Tezacaftor standard preparation:

10mg of Tezacaftor working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of Diluent is added. Then it is sonicated to dissolve it completely and made volume up to the mark with the diluent. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of the individual Ivacaftor standard preparation: 10mg of Ivacaftor working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of Diluent is added. Then it is sonicated to dissolve it completely and made volume up to the mark with the diluent. (Stock solution). Further 10.0ml from the above stock solution is pipette into a 100 ml

volumetric flask and was diluted up to the mark with diluent. **Preparation of Sample Solution: (Tablet)** Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to 10 mg of Tezacaftor and Ivacaftor (marketed formulation) sample into a 10mL clean dry volumetric flask and about 7mL of Diluents is added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution) Further 3 ml of above stock solution was pipetted into a10ml volumetric flask and diluted up to the mark with diluent.

Procedure: 20µL of the standard, sample are injected into the chromatographic system and the areas for Tezacaftor and Ivacaftor peaks are measured and the %Assay[9] is calculated by using the formula.

Results and Discussion

Wavelength Detection

The overlay spectrum of Tezacaftor and Ivacaftor was obtained and the isobestic point of Tezacaftor and Ivacaftor showed absorbance's maxima at 260 nm.

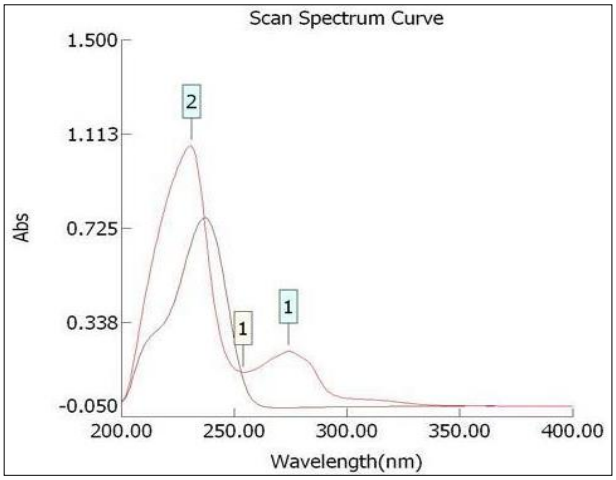

Fig 3: Overlay Spectrum for Tezacaftor and Ivacaftor

Optimized Chromatographic Method:

Optimized Chromatographic Conditions:

Column : Symmetry C18 5µm (4.6 x 150mm)
Mobile phase ratio: Phosphate buffer (0.02M) pH-3.8: Methanol: Acetonitrile (60:20:20%v/v)
Detection wavelength : 260nm
Flow rate : 1ml/min
Injection volume : 20µl
Column temperature : Ambient
Auto sampler temperature : Ambient

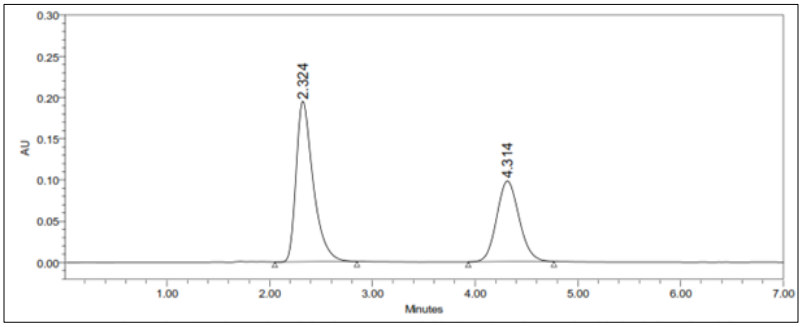

Fig 4: Chromatogram of Optimized Method

Method Validation

System Suitability: System Suitability [10, 11] was the checking of a system to ensure system performance before or during the analysis of unknowns. Parameters such as tailing factor, resolution, plate count and reproducibility are determined and compared against the specification suitable for the method.

Table 3: Data of System Suitability Test for Tezacaftor

S.No.	Injection No.	RT	Area	USP Plate Count	USP Tailing	Resolution
1	Injection 1	2.327	946257	5245	1.3	8.6
2	Injection 2	2.328	946325	5326	1.2	8.7
3	Injection 3	2.319	946859	5124	1.3	8.9
4	Injection 4	2.320	945875	5296	1.3	8.6
5	Injection 5	2.323	946396	5248	1.2	8.9
6	Injection 6	2.328	946548	5295	1.3	8.7
Mean			**946376.7**			
S.D			**325.8936**			
%RSD			**0.034436**			

Table 4: Data of System Suitability Test for Ivacaftor

S.No.	Injection No.	RT	Area	USP Plate Count	USP Tailing
1	Injection 1	4.331	112543	3854	1.4
2	Injection 2	4.341	111652	3965	1.5
3	Injection 3	4.299	112854	3874	1.3
4	Injection 4	4.313	111485	3698	1.5
5	Injection 5	4.325	113526	3785	1.4
6	Injection 6	4.341	112985	3965	1.6
Mean			**112507.5**		
S.D			**795.4945**		
%RSD			**0.707059**		

Linearity: Linearity is the ability of the method to elicit test results that are directly proportional to analyte concentration within a given range. Linearity[12] is generally reported as the variance of the slope[13] of the regression line.

Table 5: Chromatographic Data for Linearity Study of Tezacaftor

Concentration µg/ml	Average Peak Area
0	0
12	523864
18	764875
24	999874
30	1235658
36	1488542

Table 6: Chromatographic Data for Linearity Study of Ivacaftor

Concentration µg/ml	Average Peak Area
0	0
13	65698
19.5	98254
26	128587
32.5	160648
39	191874

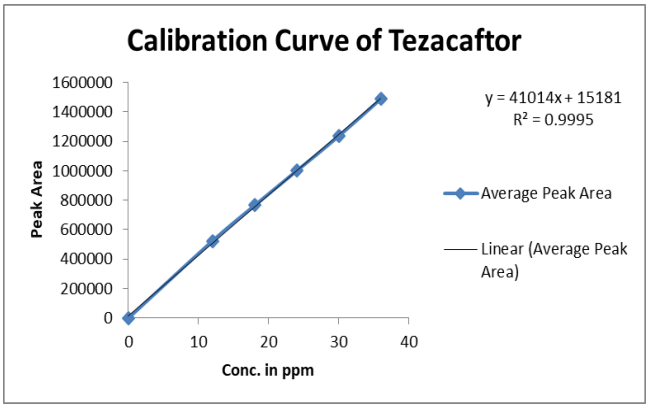

Fig 5: Calibration Curve of Tezacaftor

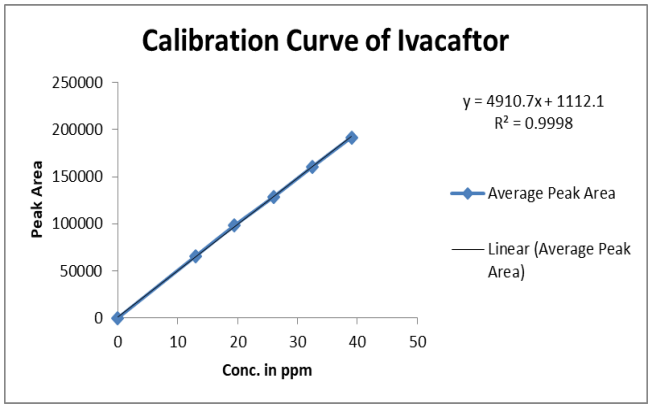

Fig 6: Calibration Curve of Ivacaftor

Linearity Plot

The plot of Concentration (x) versus the Average Peak Area (y) data of Tezacaftor is a straight line.

Y = mx + c

Slope (m) = 41014

Intercept (c) = 15181

Correlation Coefficient (r) = 0.99

Validation Criteria: The response linearity is verified if the Correlation Coefficient [14] is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 15181. These values meet the validation criteria.

Linearity Plot

The plot of Concentration (x) versus the Average Peak Area (y) data of Ivacaftor is a straight line.

Y = mx + c

Slope (m) = 4910.7

Intercept (c) = 1112.1

Correlation Coefficient (r) = 0.99

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 1112.1. These values meet the validation criteria.

Accuracy:

The accuracy [15] of the present method was carried out by injecting the solution at three different concentration levels of 50%, 100% and 150% to their specification limit, in triplicate determinations. Percent recovery [16] and the mean percentage recovery were calculated for known Concentration.

Table 7: Accuracy results of Tezacaftor

%Concentration (at specification Level)	Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean Recovery
50%	508367	12	12.024	100.200%	100.150%
100%	999100.3	24	23.989	99.954%	
150%	1496200.3	36	36.110	100.305%	

Table 8: Accuracy results of Ivacaftor

%Concentration (at specification Level)	Area	Amount Added(mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	65093.67	13	13.029	100.223%	100.280%
100%	129339.3	26	26.111	100.426%	
150%	178242.7	39	36.070	100.194%	

Acceptance Criteria

The percentage recovery was found to be within the limit (98-102%).

Observation: The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Precision:

The precision [17, 18] of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Repeatability

Obtained five (5) replicates of 100% accuracy solution as per experimental conditions [19]. Recorded the peak areas and calculated % RSD [20].

Table 9: Results of repeatability for Tezacaftor

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Resolution
1	Tezacaftor	2.321	946253	155465	5326	1.36	8.25
2	Tezacaftor	2.317	947845	154578	5246	1.37	8.26
3	Tezacaftor	2.323	945867	155845	5478	1.35	8.34
4	Tezacaftor	2.322	948572	155698	5425	1.38	8.37
5	Tezacaftor	2.324	949857	154857	5326	1.36	8.39
Mean			**947678.8**				
Std. Dev			**1649.66**				
%RSD			**0.174074**				

Table 10: Results of Repeatability for Ivacaftor

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Ivacaftor	4.304	111563	13254	3869	1.42
2	Ivacaftor	4.300	111254	13425	3852	1.43
3	Ivacaftor	4.308	111672	13254	3896	1.45
4	Ivacaftor	4.310	112654	13265	3962	1.42
5	Ivacaftor	4.314	113123	13154	3874	1.48
Mean			**112053.2**			
Std. Dev			**795.2614**			
%RSD			**0.709718**			

Intermediate Precision/Ruggedness

Table 11: Results of Intermediate precision for Tezacaftor

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USPPlate Count	USPTailing	Resolution
1	Tezacaftor	2.328	956325	156325	5246	1.35	8.24
2	Tezacaftor	2.326	958741	157854	5367	1.38	8.26
3	Tezacaftor	2.327	957542	156986	5265	1.34	8.47
4	Tezacaftor	2.326	956895	158547	5384	1.39	8.29
5	Tezacaftor	2.331	957486	156985	5297	1.35	8.34
Mean			**957397.8**				
Std. Dev.			**899.5091**				
% RSD			**0.093954**				

Table 12: Results of Intermediate precision for Ivacaftor

S.No.	Peak Name	Rt	Area (µV*sec)	Height (µV)	USPPlate count	USPTailing
1	Ivacaftor	4.335	121231	13458	3896	1.52
2	Ivacaftor	4.336	121457	13674	3785	1.54
3	Ivacaftor	4.334	123142	13485	3969	1.58
4	Ivacaftor	4.337	121325	13958	3859	1.57
5	Ivacaftor	4.340	123654	13875	3789	1.59
Mean			**122161.8**			
Std. Dev.			**1145.733**			
% RSD			**0.937882**			

Method Robustness: Influence of small changes in chromatographic conditions [21] such as change in flow rate [22] (± 0.1ml/min), Temperature (±2^{0}C), Wavelength of detection [23] (±5nm) & acetonitrile content in mobile phase [24] (±2%) studied to determine the robustness [25] of the method are also in favour of (Table-13, % RSD < 2%) the developed RP-HPLC method for the analysis of Tezacaftor (API).

Table 13: Result of Method Robustness Test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.96
Flow (0.9 ml/min)	0.84
Temperature (27^{0}C)	0.81
Temperature (23^{0}C)	0.94
Wavelength of Detection (265 nm)	0.56
Wavelength of detection (255 nm)	0.17

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^{0}C), Wavelength of detection (±5nm) & acetonitrile content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (Table-14, % RSD < 2%) the developed RP-HPLC method for the analysis [26] of Ivacaftor (API).

Table 14: Result of method robustness test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.58
Flow (0.9 ml/min)	0.64
Temperature (27^{0}C)	0.72
Temperature (23^{0}C)	0.91
Wavelength of Detection (265 nm)	0.86
Wavelength of detection (255 nm)	0.78

Limit of detection (LOD) & Limit of quantification (LOQ):

The detection limit [27] (LOD) and quantitation limit (LOQ) may be expressed as:

L.O.D. = 3.3 (SD/S).

L.O.Q. = 10 (SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

Result & Discussion: The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified [28] (LOQ) were found to be 1.377µg/ml & 4.174µg/ml respectively for Tezacaftor.

The LOD was found to be 1.079µg/ml and LOQ was found to be 3.272µg/ml for Ivacaftor which represents that sensitivity of the method is high.

Estimation of Tezacaftor & Ivacaftor in TABLET Dosage Form

Twenty tablets were taken and the I.P. method was followed to determine the average weight [29]. Finally the weighed tablets are powdered and triturated well by using mortar and pestle. A quantity of powder which is equivalent [30] to the 100mg of drugs were transferred to a clean and dry 100ml of volumetric flask and add 70 ml of mobile phase and the resulted solution was sonicated for 15 minutes by using ultra sonicator, Then the final volume was make up to the mark with the mobile phase. The final solution was filtered [31] through a selected membrane filter (0.45 µm) and in order to sonicate to degas the mobile phase (Solvent system). From this above stock solution (1 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system [32] (Mobile phase).

The prepared solutions were injected in five replicates into the HPLC system and the observations were recorded.

A duplicate injection (Blank Solution) of the standard solution also injected into the HPLC system and the chromatograms and peak areas were recorded and calculated. The obtained data are shown in Table-15.

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Average weight} = \text{mg/tab}$$

Where:

AT = Test Preparation Peak Area

AS = Standard preparation Peak Area

WS = Working standard weight taken in mg

WT = Sample weight taken in mg

DS = Standard solution dilution[33]

DT = Sample solution dilution

P = Working standard percentage purity

The assay was performed as explained in the previous chapter (Above). The results which are obtained are following:

Table 15: Assay of Tezacaftor & Ivacaftor Tablets

Brand Name of Tablets	Labelled Amount of Drug (mg) Tezacaftor & Ivacaftor	Mean (±SD) amount (mg) found by the proposed method (n=6)	Mean (± SD) Assay (n = 6)
Symkevi Tablet 10s (Fredun Pharmaceuticals Limited)	100 mg/150 mg	99.387 (±0.09) /149.685 (±0.08)	99.865 (±0.245) /99.658 (±0.354)

Result and Discussion: The assay of Symkevi Tablets containing Tezacaftor and Ivacaftor was found to be 99.865% and Ivacaftor was found to be 99.658%.

Forced Degradation Studies

Following protocol was strictly adhered to for forced degradation [34, 35] of Tezacaftor and Ivacaftor Active Pharmaceutical Ingredient (API). The API (Tezacaftor and Ivacaftor) was subjected to keep in some stress conditions in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage and/or after administration to body. It is one type of accelerated stability studies of the drugs that is used to help us to determining the total fate of the drug that is likely to happen after long time storage, within a very short time as compare to the real time or long term stability testing. The different types of forced degradation pathways/studies are studied here are acid hydrolysis, basic hydrolysis, thermal degradation and oxidative degradation.

Results of Degradation Studies: The results of the forced degradation studies indicated the specificity of the developed method that has been developed. Tezacaftor and Ivacaftor were stable only in acidic and thermal stress conditions. The results of stability studies are given in the following Tables-16 & 17.

Table 16: Results of Forced Degradation Studies of Tezacaftor API

Stress Condition	Time (hours)	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1N HCl)	24Hrs.	87.316	12.684	100.00
Basic Hydrolysis (0.IN NaOH)	24Hrs.	78.155	21.845	100.00
Thermal Degradation (60 ^{0}C)	24Hrs.	86.215	13.785	100.00
UV (254nm)	24Hrs.	76.346	23.654	100.00
3% Hydrogen Peroxide	24Hrs.	75.104	24.896	100.00

Table 17: Results of Forced Degradation Studies of Ivacaftor API

Stress Condition	Time (hours)	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1N HCl)	24Hrs.	85.155	14.845	100.00
Basic Hydrolysis (0.IN NaOH)	24Hrs.	77.514	22.486	100.00
Thermal Degradation (60 ^{0}C)	24Hrs.	84.522	15.478	100.00
UV (254nm)	24Hrs.	74.251	25.749	100.00
3% Hydrogen Peroxide	24Hrs.	73.015	26.985	100.00

Summary and Conclusion

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for simultaneous analysis of Tezacaftor and Ivacaftor different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here Symmetry C18 5µm (4.6 x 150mm) column was preferred because using this column peak shape, resolution and absorbance were good. Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Tezacaftor and Ivacaftor it is evident that most of the HPLC work can be accomplished in the wavelength range of 240-300 nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 20 µl were found to be the best analysis. The result shows the developed method is yet another suitable method for assay & stability which can help in the simultaneous analysis of Tezacaftor and Ivacaftor in different formulations. A sensitive & selective RP-HPLC method has been developed & validated for the simultaneous analysis of Tezacaftor and Ivacaftor API. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. The result shows the developed method is yet another suitable method for assay, purity & stability which can help in the simultaneous analysis of Tezacaftor and Ivacaftor in different formulations.

References

1. https://go.drugbank.com/drugs/DB11712
2. https://pubchem.ncbi.nlm.nih.gov/compound/Tezacaftor
3. https://en.wikipedia.org/wiki/Tezacaftor
4. https://go.drugbank.com/drugs/DB08820
5. https://pubchem.ncbi.nlm.nih.gov/compound/Ivacaftor
6. https://en.wikipedia.org/wiki/Ivacaftor
7. "Principles of Instrumental Analysis", 5th edition, Harcourt Publishes Int Company, Skoog, Holler and Nieman, Chapter 28, p.726-766.
8. "HPLC Columns" Theory, Technology and Practice. Uwe D. Neue, Wiley-VC.
9. Handbook of HPLC, Vol.78, by Elena Katz *et al*. Marcel Dekker Inc.
10. "Instrumental Methods of Chemical Analysis", 5th

Edition, Galen W. Ewing, McGraw Hill Book Company 1988.
11. "HPLC in Pharmaceutical Industry", Fong and Long, Marcel Dekker Series
12. "Instrumental Method of Chemical Analysis" by Chatwal Anand, Himalaya Publishing House, p.no.615-623.
13. Dr. Kealey and P.J Haines, Analytical Chemistry, 1stedition, Bios Publisher, (2002), P1-7.
14. "Practical Pharmaceutical Chemistry", 4th edition, Part 2, by Beckett and Stenlake, CBS Publishers and Distributors, P.No.157-174.
15. Govt. of India, Ministry of Health and Family Welfare. Vol. 2. Delhi: Publication by Controller of Publication; 2007. Indian Pharmacopoeia; pp. 484–554.
16. British Pharmacopoeia. (International Ed.) 1993; Vol. 1:429, 483. Published on the Recommendation of the Medicines Commissions Pursuant to Medicines Act 1968, 1993.
17. United States Pharmacopoeia 29 NF 24, Published on the Recommendation of the Medicines Commissions Pursuant to Medicines, page no. 587.
18. Skoog, West, Holler, Crouch. Fundamentals of analytical chemistry", eighth edition, (Indian edition), Cengage learning India Pvt ltd, New Delhi, 2009, Page no. 271-280.
19. AV Kasture, KR Mahadik, SG Wadodkar, HN More. A textbook of pharmaceutical analysis, Instrumental methods", Nirali Prakashan, vol.2, 9th edition, page no. 5-7, 28-30.
20. Settle FA. In: Handbook of Instrumental Techniques for Analytical Chemistry. 1st Ed, Singapore, Pearson Education Inc, 2004.
21. Willard HH, Dean AJ. Instrumental Methods of Analysis. CBS Publishers and distributors, 7th Ed, 1986, 513-515.
22. Connors AK. In: A Text Book of Pharmaceutical Analysis. A Wiley Interscience Publication, 3rd ed, 2005, 373-400.
23. Ahuja S. In: High Pressure Liquid Chromatography of Comprehensive Analytical Chemistry. Elsevier Publications, 2006.
24. Principles and Methods. In: Amesham Biosciences of Reversed Phase Chromatography. 6-8.
25. Synder LR, Kirkland JJ and Glajch JL. In: Practical HPLC Method Development, 2nd Ed, John Wiley and Sons Inc. Canada, 1997.
26. Mohammad T *et al*., HPLC Method Development and Validation for Pharmaceutical Analysis- A Review. International Pharmaceutica Sciencia. 2012; 2(3):14.
27. Snyder LR, Kirkland JJ, Glajch JL. In: Practical HPLC Method Development. 2nd ed, 2001.
28. Vibha G *et al*., Development and validation of HPLC method - a review. International Research Journal of Pharmaceutical and Applied Sciences. 2012, 2(4):22-23.
29. Bliesner DM. In: Validating Chromatographic Methods. John Wiley & sons Inc, 2006, 88-92.
30. Validation of Analytical Procedures: Methodology. ICH-Guidelines Q2B, Geneva. 1996, 11. (CPMP/ICH/281/95).
31. Development and validation of HPLC method - A Review, Vibha Gupta *et al*, International Research Journal of Pharmaceutical and Applied Sciences. 2012; 2(4):17-25.
32. A Review: HPLC Method Development and Validation, Santosh Kumar Bhardwaj *et al*. International Journal of Analytical and Bioanalytical Chemistry, accepted 20 November 2015.
33. Method Development: A Guide to Basics Quantitative & Qualitative HPLC, LC, GC chromacademy.
34. Lalit V Sonawane* Bioanalytical Method Validation and Its Pharmaceutical Application- A Review Pharmaceutica Analytical Acta 2014, 5:3Center for Drug Evaluation and Research (CDER) Reviewer Guidance.
35. ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology.
36. Narendra Singh, Parveen Bansal, Mukesh Maithani, Yashpal Chauhan, Development and Validation of a Novel Stability-Indicating RP-HPLC Method for Simultaneous Determination of Tezacaftor and Ivacaftor in Fixed Dose Combination, Journal of Chromatographic Science, Volume 58, Issue 4, April 2020, Pages 346–354, https://doi.org/10.1093/chromsci/bmz120.
37. Dharmamoorthy G. 1, G. Sarath Kumar*1, Poornima B. 1, P. Jayachandra Reddy1 and K. Chandan Kumar1, Stability Indicating RP-HPLC Method Development and Validation for the Simultaneous Estimation of Ivacaftor and Tezacaftor in Bulk and Pharmaceutical Dosage Form, European Journal of Pharmaceutical and Medical Research, ejpmr, 2020,7(1), 342-346.
38. Ramanjaneyulu K. V, Venkata Ramana K, M. Prasada Rao. Stability indicating LC Method Development and Validation for the Simultaneous analysis of Cystic Fibrosis Drugs - Ivacaftor and Tezacaftor in Pharmaceutical Formulations. Research J. Pharm. and Tech 2020; 13(5):2076-2080. Doi: 10.5958/0974-360X.2020.00373.X.
39. G. Indira Priyadarshini*, V. Mounika and G. Anjani, B. Sowmya, Stability Indicating RP-HPLC Method Development and Validation for the Simultaneous Estimation of Tezacaftor and Ivacaftor in Bulk and Pharmaceutical Dosage Form, World Journal of Pharmacy and Pharmaceutical Sciences, Volume 9, Issue 1:1420-1431.
40. Gadeela Srimounika1, Shyamala*1, J V C. Sharma2, A. Swarupa3, A New Stability- Indicating Method for Simultaneous Estimation of Ivacaftor and Tezacaftor by RP-HPLC in Bulk and Its Dosage Form, International Journal of Research and Analytical Reviews (IJRAR), 2018 IJRAR December 2018, Volume 5, Issue 4, Pages: 774-785.