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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS QUANTIFICATION OF TEZACAFTOR AND IVACAFTOR IN COMBINED TABLET DOSAGE FORM

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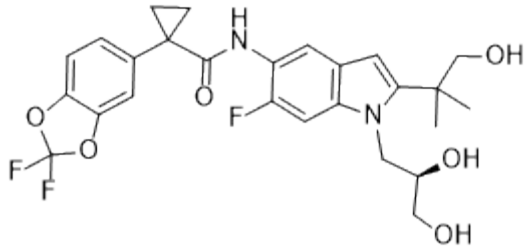
ABSTRACT

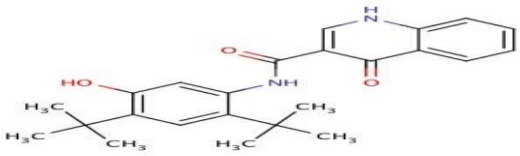
A new, simple, precise, accurate, and reproducible RP-Hplc method for simultaneous estimation of its Tezacaftor and Ivacaftor of tablet dosage form. Separation of Tezacaftor and Ivacaftor was successfully achieved by using Inertsil ODS column, flow rate were 1ml/min, mobile phase composition containing (30:10:60v/v)ACN, Methanol and 0.1% OPA(pH 3.0) . The retention time of 3.101min and 4.205min for Tezacaftor and Ivacaftor respectively. The linearity study of Tezacaftor and Ivacaftor were found in the concentration range 62.5µg/ml-312.55µg/ml and 100µg/ml-500µg/ml and correlation coefficient(R2) be 0.999 and 0.999, %recovery were found to be 100.13 and 100.53, %RSD for repeatability 0.8 and 0.8, %RSD for intermediate precision were 0.7 and 0.6 respectively. The LOD and LOQ for Tezacaftor were found to be 0.12 and 0.37. The LOD and LOQ for Ivacaftor were found to be 0.27 and 0.81. This method was extensively validated according to ICH guidelines for accuracy, precision, linearity, robustness, specificity and system suitability.

INTRODUCTION

Tezacaftor is a small molecule that can be used as a corrector of the cystic fibrosis transmembrane conductance regulator (CFTR) gene function. It was developed by Vertex Pharmaceutical and FDA approved in combination with Ivacaftor, a CFTR potentiator that allows the proteins at the cell surface to open longer and improve nutrient transport. Ivacaftor is a drug used for the management of cystic Fibrosis (CF) in patients aged 2 years and older. Cystic Fibrosis is an autosomal recessive disorder caused by one of several different mutatations 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 membrane. CFTR is active in epithelial cells of organs such as lungs, pancreas, liver, digestive system, and reproductive tract. Alterations in the CFTR gene result in the altered production, misfolding ,or function of the protein and consequently abnormal fluid and ion transport across cell membranes. CF patients produce a 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



Structure of Tezacaftor



STRUCTURE OF IVACAFTOR

HPLC METHOD DEVELOPMENT

Instrumentation

The chromatographic system consisted of a Waters H-Class UPLC (Model 2695) chromatograph equipped with CHS C18 50X 2.1mm 1.8m column, LC-20AD pumps and an SPD-20A photo diode array(PDA) detector.Samples were injected into the system through a Rheodyne 7725 injector valve via a 1µL loop. The output signal was monitored and integrated by Empower-2 software. Solubility of the compound was enhanced by sonication on an ultrasonicator (PCI Analytics PCI81). All the weighings in the experiments were done with a Sartorius balance (model CPA225D). PVDF membrane filters used for filtration were purchased from Merck Millipore.

Drugs and Chemicals

The reference standard samples of Tezacaftor and Ivacaftor were obtained from Suven life sciences Ltd. Acetonitrile and Orthophosphoric acid used were of HPLC grade, while Sodium hydroxide, hydrogen peroxide was of GR grade (Merck Ltd. Mumbai, India). Milli-Q water was used throughout the analysis.

Preparation of pH 2.2 buffer(0.1% Ortho phosphoric acid)

Transferred 1mL of ortho phosphoric acid solution in a 1000ml of volumetric flask, added 100ml of milli-Q water and final volume make up to 1000mL with milli-Q water.

Preparation of the mobile phase

pH 2.2 buffer and acetonitrile was mixed in the ratio of of 60:40 % v/v and sonicated to degas.

Preparation of Diluent

Water and Acetonitrile was mixed in the ratio of 50:50 % v/v and used as diluent for preparing drug solutions.

Preparation of the mixed working standard Solution of Tezacaftor and Ivacaftor

Weighed and transferred 10mg of Tezacaftor and 15mg of Ivacaftor working standards into a 25ml clean dry volumetric flask, added 10ml volume of diluent, sonicated for 5 minutes and made up to the final volume with diluents (400 µg/mL of Tezacaftor and 600 µg/mL of Ivacaftor). A Quantity of 5ml from the above two stock solutions was taken into a 50ml volumetric flask and made up to 50ml (40 µg/mLof Tezacaftor and 60 µg/mL of Ivacaftor).

METHOD VALIDATION

ACCURACY

To determine the accuracy of the proposed method, different amounts of bulk sample of Tezacaftor and Ivacaftor within linearity limits were taken and analyzed by the proposed method.Accuracy for Tezacaftor and Ivacaftor was conducted by spiking the drug to the placebo powder at three different levels of the test concentration (i.e. 50%, 100%, and 150%) and each level three times. The mean % Recovery and % RSD values were calculated. The % Recovery value was found to be in between 98.0 % to 102.0 %.

PRECISION

To ascertain the effectiveness of method system suitability tests were carried out on freshly prepared standard stock solution containing 40 µg/mL of Tezacaftor and 60 µg/mL of Ivacaftor.1µL of solution was injected into the optimized chromatographic system.For system suitability 6 replicates of working standard samples were injected and the peak response of sample were calculated.

The inter-day precisions were determined by analyzing a mixed solution containing 40 µg/mL of Tezacaftor and 60 µg/mL of Ivacaftor. The intermediate precision was determined on two consecutive days different instrument.

LINEARITY

To establish linearity, a stock solution containing 500 µg/mL Tezacaftor and 750 µg/mL Ivacaftor were prepared using diluent and further diluted to yield solutions in the concentration range of 10-60 µg/mL and 15-90µg/mL ofIvacaftor. The solutions were prepared and analyzed in triplicate. The experiment was repeated thrice by preparing different solution and analyzed by injecting 1 µL in UPLC.

SYSTEM SUITABILITY

System suitability was assessed by analyzing the mixed standard drug solution (40 µg/mL of Tezacaftor and 60 µg/mL of Ivacaftor) and calculating the chromatographic parameters such as resolution, theoretical plates and tailing factor.

SPECIFICITY

Specificity is the extent to which the procedure applies to analyte of interest and is checked by examining the formulation samples for any interfering peaks. The specificity of the methodwas evaluated with regard to interference due to presence of excipients. The excipients usedin formulation did not interfere with the drug peaks and thus th method is specific.The UPLC chromatograms recorded for the drug matrix (mixture of the drug and the excipients) showed almost no interfering peaks within retention times ranges.

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION: LOD and LOQ values were calculated from the average standard deviation and slope from the calibration curve as per ICH guideline.

ROBUSTNESS: Robustness study was done by applying small deliberate changes in the chromatograohic conditions and studying the system suitability parameters of both the drugs.The conditions selected for testing were the flow rate, column oven temperature and composition of the mobile phase. The study was conducted on a mixed standard solution containing 40 µg/mL of Tezacaftor and 60 µg/mL of Ivacaftor.

FORCED DEGRADATION STUDIES

Preparation of standard stock solution of Tezacaftor and Ivacaftor

10mg of Tezacaftor and 15mg of Ivacaftor were weighed accurately and transferred working standards into a 25 mL clean dry volumetric flask, added 10ml volume of diluent,sonicated for 5 minutes and made up to the final volume with dilutents (400 µg/mL of Tezacaftor and 600 µg/mL of Ivacaftor).

Acid induced degradation study

In acid hydrolytic degradation, to 5 ml of standard stock solution of Tezacaftor and Ivacaftor 5 ml of 0.1 N Hydrolytic acid was added and refluxed for 60min at 60^0C and then neutralized with 5 mL of 0.1N sodium hydroxide solution. The resultant solution was diluted to obtain 40 µg/mL of Tezacaftor and60 µg/mL of Ivacaftor, 1µL solutions were injected into the UPLC system and the chromatograms were recorded to assess the stability of sample.

Base induced degradation study

For base hydrolytic degradation, to 5 mL of standard stock solution of Tezacaftor and Ivacaftor 5 mL of 0.1N sodium hydroxide solution was added and refluxed for 60 min at 60^0C and then neutralized with 5 mL of 0.1N hydrochloric acid. The resultant solution was diluted to obtain 40µg/mL of Tezacaftor and 60µg/mL of Ivacaftor,1µL solutions were injected into the UPLC system and the chromatograms were recorded to assess the stability of sample.

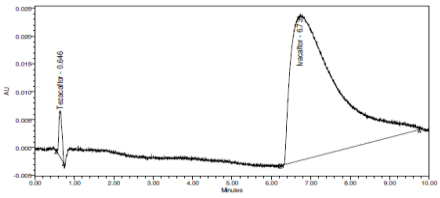
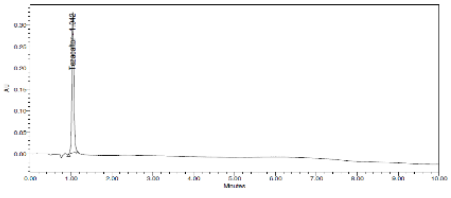
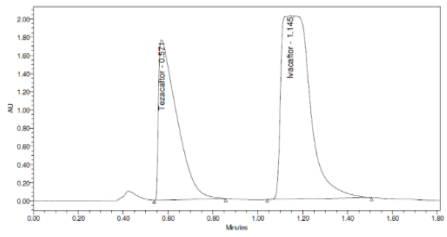
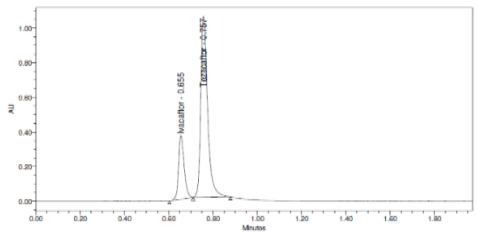
Peroxide induced degradation study: In peroxide degradation, 5 mL of 10% hydrogen peroxide (H_2O_2) was added to 5 mL of standard stock solution of Tezacaftor and Ivacaftor. The solutions were kept on Bench top for 30 min. For UPLC study, the resultant solution was diluted to obtain 40 µg/mL of Tezacaftor and 60 µg/mL of Ivacaftor, 1µL solutions were injected into the UPLC system and the chromatograms were recorded to assess the stability of sample.

Thermal induced degradation study: Inthermal degradation, 5 mL of standard stock solution of Tezacaftor and Ivacaftor was placed inoven at 105°C for 6 h. For UPLC study, the resultant solution was diluted to obtain 40 µg/mL ofTezacaftor and 60 µg/mL of Ivacaftor, 1 µL solutions were injected into the UPLC system and thechromatograms were recorded to assess the stability of sample.

Neutral degradation study: In Neutral degradation, To 5 mL of standard stock

solution of Tezacaftor and Ivacaftor. 5 mL of waterwas added and refluxed for 1 h at 60^0C. The resultant solution was diluted to obtain 40 µg/mL ofTezacaftor and 60 µg/mL of Ivacaftor, 1µL solutions were injected into the UPLC system and thechromatograms were recorded to assess the stability of sample.

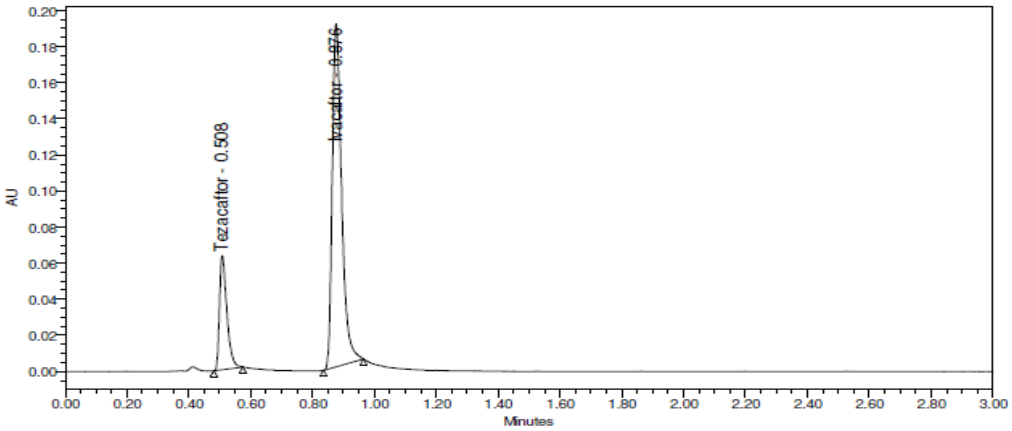
Photolytic degradation study: The photochemical stability of the drug was also studied by exposing the 5 mL of standard stock solution of Tezacaftor and Ivacaftor to UV Light by keeping the beaker in UV Chamberfor 1hours or 200-Watthours/m^2 in photo stability chamber. For UPLC study, the resultant solution was diluted to obtain Tezacaftor and 60 µg/mL of Ivacaftor,1 µL solutions were injectedinto the UPLC system and the chromatograms were recorded to assess the stability of sample.

	
Trial-1 X-Bridge C18 100 X 2.1mm 1.8µ column, Acetonitrile: 0.1% OPA (50:50%v/v)	Trail-2 Hibar C18 100 X 2.1mm 1.7µ column, Acetonitrile: 0.1% OPA (50:50%v/v)
	
Trial-3 HSS C18 50 X 2.1mm 1.8µ column, Acetonitrile: 0.1% OPA (50:50%v/v)	Trial-4 CHS C18 50 X 2.1mm 1.8µ column, Acetonitrile: 0.1% OPA (50:50%v/v)

OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS AND METHOD DEVELOPMENT

S.NO	PARAMETER	VALUE
1	Stationary phase	CHS C18 50 X 2.1mm 1.8 µ
2	Mobile phase	0.1% OPA (pH2.2): Acetonitrile (60:40% v/v)
3	Flow rate	0.3 mL/min
4	Column temperature	30ºC
5	Volume of injection	1 µL
6	Detection wavelength(λ_{max})	292 nm
7	Run time (min)	3 min

OPTIMIZED CHROMATOGRAPHIC CONDITIONS OF THE PROPOSED METHOD

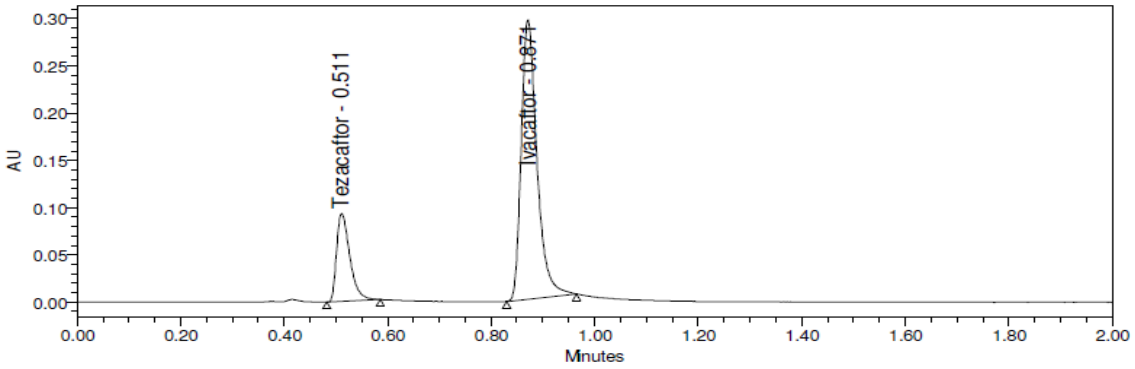


TYPICAL CHROMATOGRAM OF THE MIXED STANDARD SOLUTION OF TEZACAFTOR AND IVACAFTOR

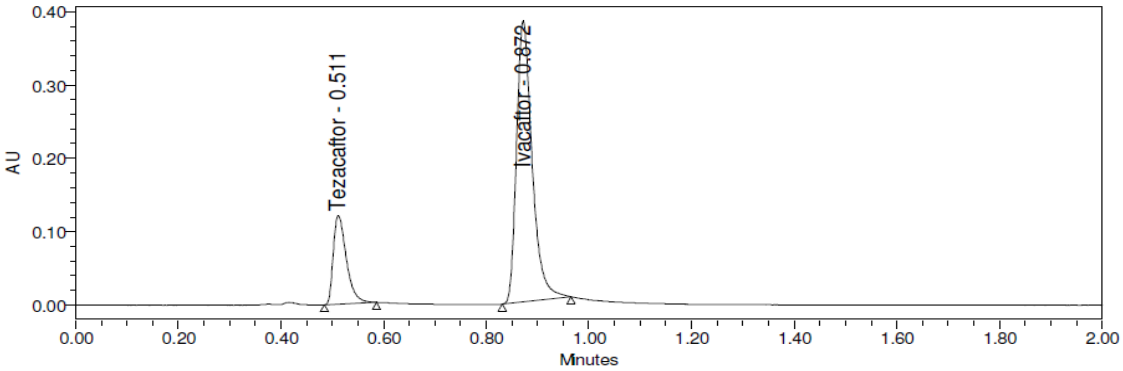
Concentration Level	Peak area difference	Amount added (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Assay
		ACCURACY OF IVACAFTOR			
50%	52781			99.42	100.39
	53564	20	20.178	100.89	
	53543	20	20.170	100.85	
100%	105733	40	39.680	99.20	99.33
	105946	40	39.760	99.40	
	105937	40	39.756	99.39	
150%	160713	60	60.233	100.39	100.77
	162063	60	60.738	101.23	
	161207	60	60.418	100.70	

ACCURACY OF TEZACAFTOR

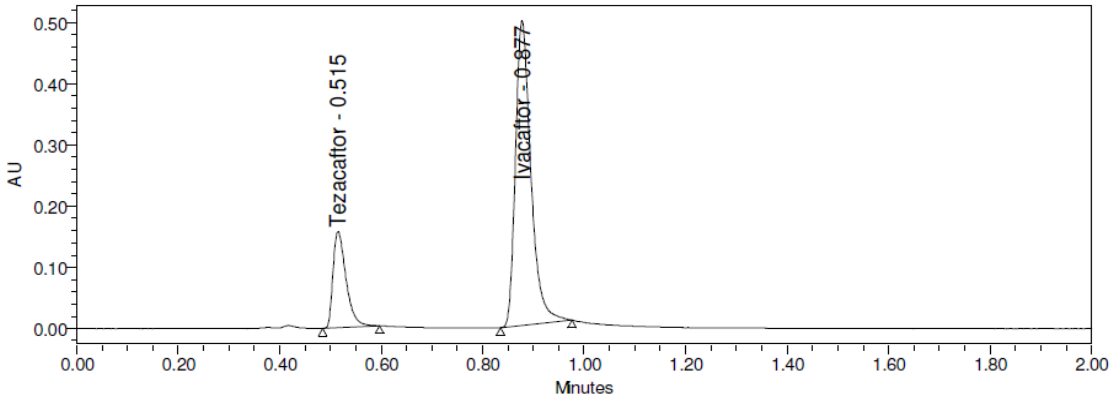
Concentration Level	Peak area difference	Amount added (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Assay
50%	198730	30	30.16604	100.55	100.31
	198578	30	30.14336	100.48	
	197425	30	29.97132	99.90	
100%	399639	60	60.14351	100.24	100.12
	399011	60	60.04981	100.08	
	398873	60	60.02922	100.05	
150%	597213	90	89.62337	99.58	99.72
	598533	90	89.82032	99.80	
	598483	90	89.81286	99.79	



TYPICAL CHROMATOGRAM OF ACCURACY AT 50% LEVEL



TYPICAL CHROMATOGRAM OF ACCURACY AT 100% LEVEL



TYPICAL CHROMATOGRAM OF ACCURACY AT 150% LEVEL

INTRA-DAY PRECISION DATA

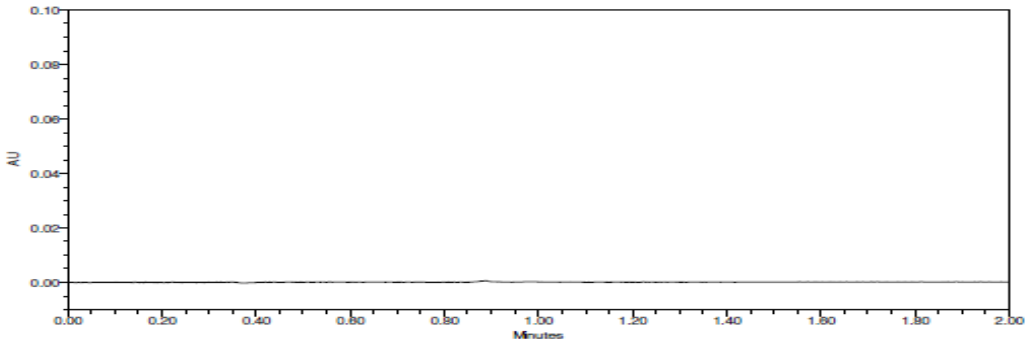
S.No	Injection	Tezacaftor	Ivacaftor
	Injection-1	107235	406882
	Injection-2	107310	407321
	Injection-3	107313	408665
	Injection-4	107722	408018
	Injection-5	107208	407805
	Injection-6	107871	409533
Mean		**1251326**	**107443**
SD		**9447.2**	**280.8**
% RSD		**0.8**	**0.3**

INTERMEDIATE PRECISION DATA

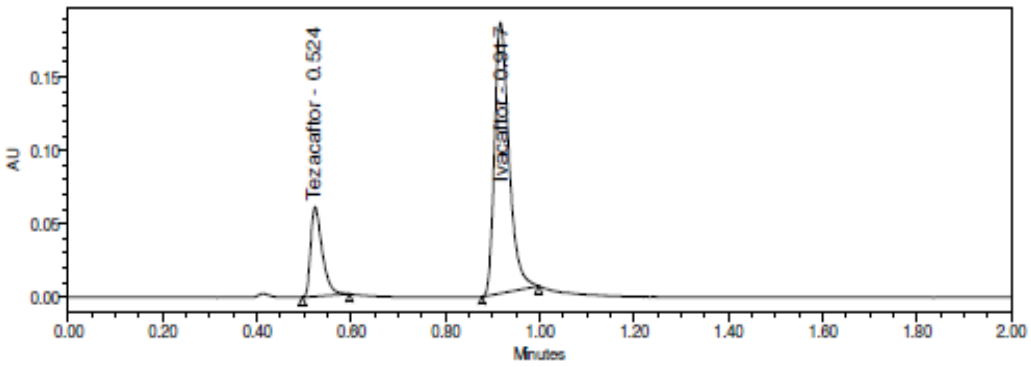
S.No	Injection	Tezacaftor		Ivacaftor	
		Day-1	Day-2	Day-1	Day-2
	Injection-1	107235	105395	406882	399046
	Injection-2	107310	105735	407321	399785
	Injection-3	107313	105680	408665	399188
	Injection-4	107722	105550	408018	395168
	Injection-5	107208	105951	407805	397998
	Injection-6	107871	105941	409533	399875
	Mean	106576		403274	
	SD	936.99		5156.73	
	% RSD	0.88		1.28	

METHOD PRECISION RESULTS OF TEZACAFTOR AND IVACAFTOR

S.No	% Assay	
	Tezacaftor	Ivacaftor
1	99.63	99.82
2	99.70	99.93
3	99.71	100.26
4	100.09	100.10
5	99.61	100.05
6	100.23	100.47
Mean	**99.83**	**100.10**
SD	**0.261**	**0.23**
%RSD	**0.3**	**0.2**



PRECISION TYPICAL CHROMATOGRAM OF PLACEBO



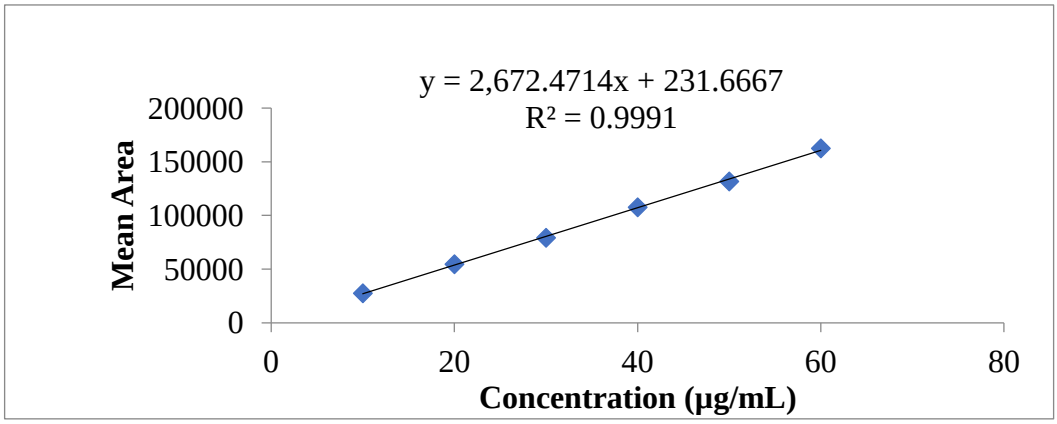
PRECISION TYPICAL CHROMATOGRAM OF TEZACAFTOR AND IVACAFTOR IN SYMDEKO

LINEARITY OF TEZACAFTOR

Concentration of Tezacaftor (µg/mL)	Peak Area	Mean Area	RSD
10	26906	27387	1.59
	27757		
	27499		
20	53812	54475	1.75
	55567		
	54045		
30	79897	79102	0.99
	78338		
	79070		
40	108280	107535	0.95
	106375		
	107951		
50	129818	131629	1.21
	132287		
	132781		
60	162729	162481	0.55
	163227		
	161488		

LINEARITY PLOT OF TEZACAFTOR

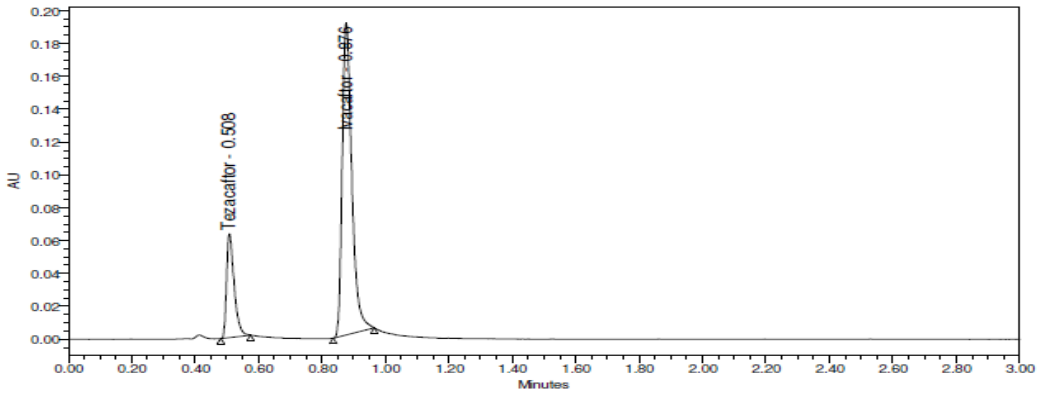
Concentration of Ivacaftor (µg/mL)	Peak Area	Mean Area	RSD
15	100419	27387	5.25
	102047		
	103283		
30	203950	54475	4.07
	208304		
	205402		
45	301829	79102	4.52
	297911		
	294687		
60	409720	107535	4.02
	407860		
	401481		
75	492220	131629	2.10
	497754		
	495149		
90	606954	162481	1.35
	610091		
	611183		



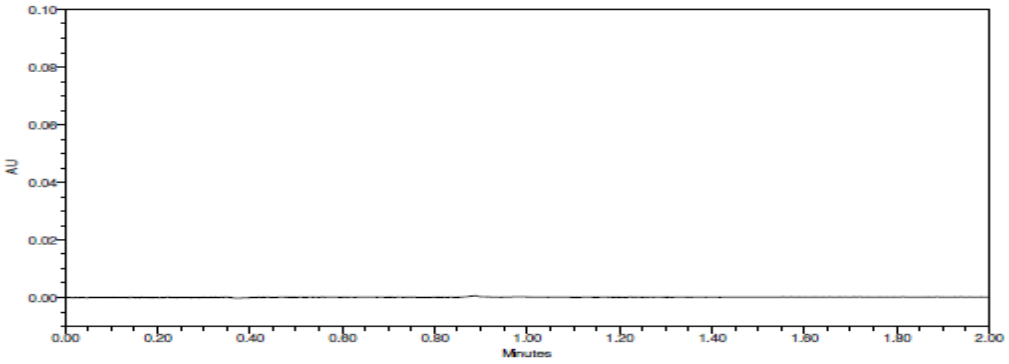
LINEARITY OF IVACAFTOR

SYSTEM SUITABILITY

Parameter	Tezacaftor	Ivacaftor
Retention time (min)	0.508	0.876
Peak area	104750	398640
Resolution	-	7.4
Theoretical Plates	2222	4262
Tailing Factor	1.6	1.4



SPECIFICITY TYPICAL CHROMATOGRAM OF TEZACAFTOR AND IVACAFTOR



SPECIFICITY TYPICAL CHROMATOGRAM OF PLACEBO

Chromatographic conditions	Ivacaftor				
	% assay	Theoretical Plates	Asymmetry	Resolution	Retention time
Water: Methanol (60:40% v/v)	99.40	4025	1.37	6.3	0.758
Water: Methanol (40:60% v/v)	99.12	4168	1.29	8.3	1.048
0.2 mL/min	99.15	4203	1.31	7.1	1.063
0.4 mL/min	100.10	4037	1.41	7.1	0.751
28°C	100.7	4201	1.35	6.7	0.805
32°C	99.93	4163	1.31	7.3	0.967

LOD AND LOQVALUES OF THE METHOD

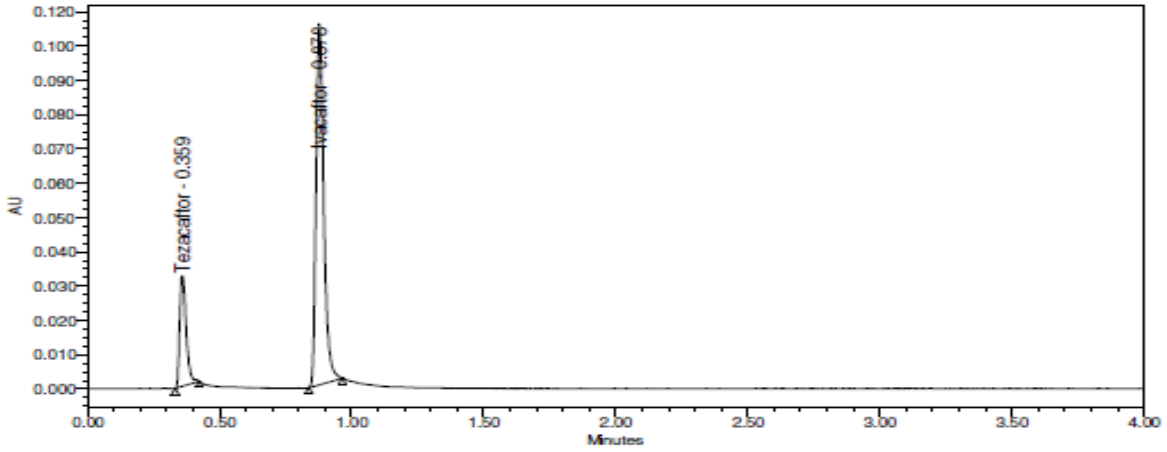
S. No	Parameter	Tezacaftor	Ivacaftor
1	LOD	0.12	0.27
2	LOQ	0.37	0.81

ROBUSTNESS DATA OF TEZACAFTOR

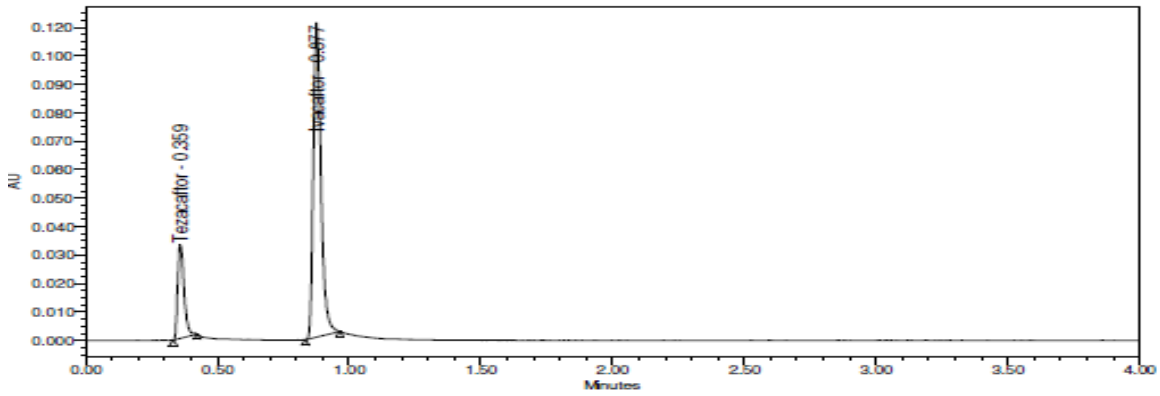
Chromatographic conditions	Tezacaftor			
	assay	Theoretical Plates	Asymmetry	Retention time
Water: Methanol (60:40% v/v)	100.05	2158	1.51	0.473
Water: Methanol (40:60% v/v)	99.96	2112	1.47	0.587
0.2 mL/min	98.79	2076	1.46	0.623
0.4 mL/min	99.77	2217	1.55	0.446
28°C	100.02	2067	1.44	0.492
32°C	99.18	2193	1.43	0.554

ROBUSTNESS DATA OF IVACAFTOR

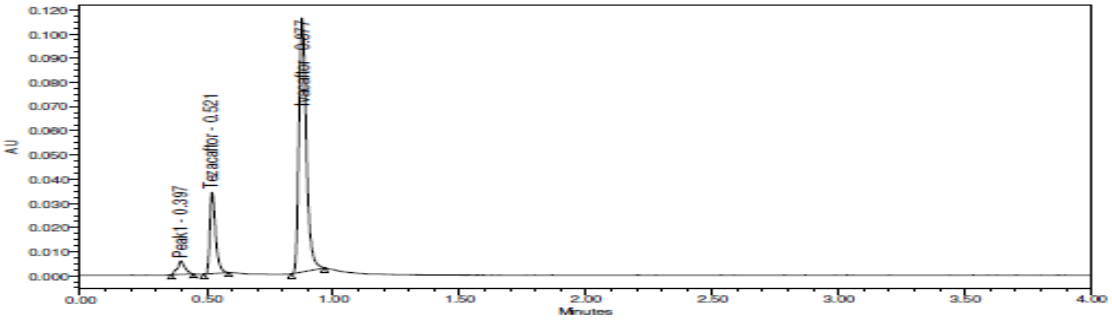
FORCED DEGRADATION STUDIES:



TYPICAL CHROMATOGRAM OF ACID STRESSED SAMPLE



TYPICAL CHROMATOGRAM OF BASE STRESSED SAMPLE



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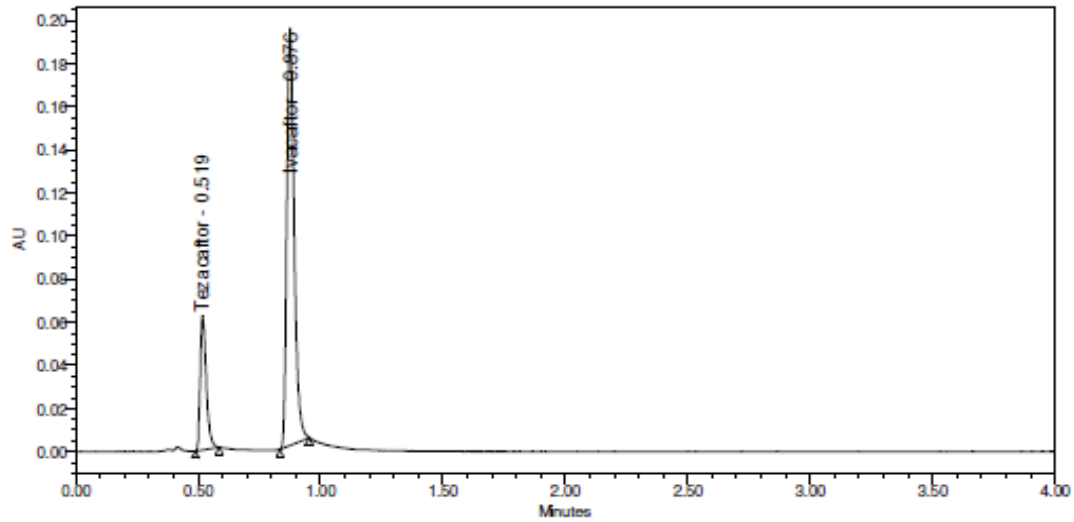
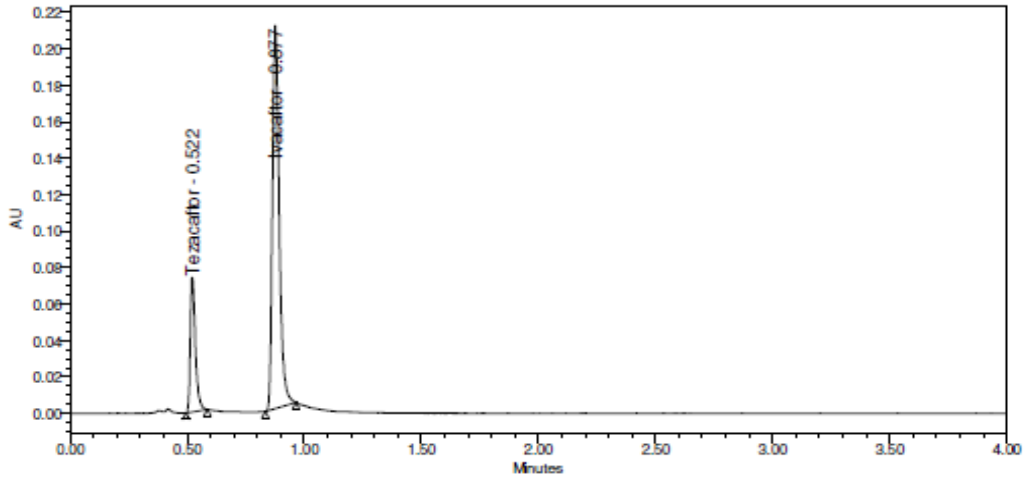
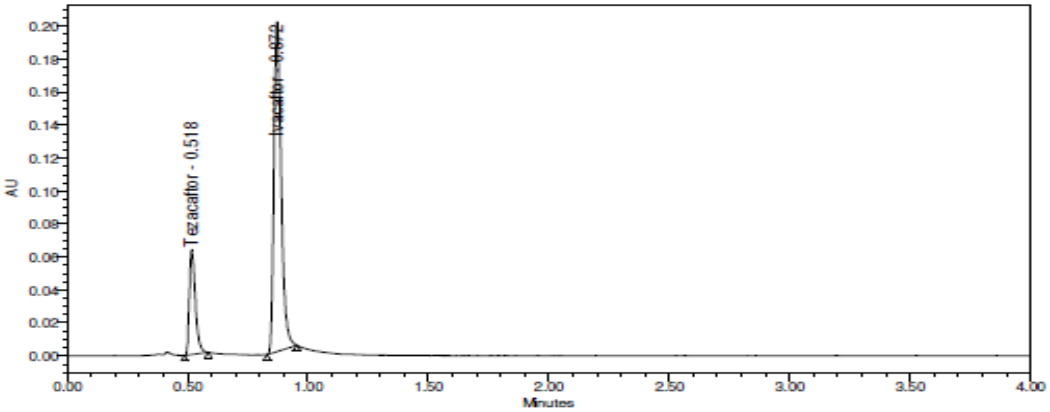


RAM OF PEROXIDE STRESSED SAMPLE

TYPICAL CHROMATOGRAM OF THERMAL STRESSED SAMPLE



TYPICAL CHROMATOGRAM OF WATER STRESSED SAMPLE



TYPICAL CHROMATOGRAM OF PHOTOLYTIC DEGRADATION SAMPLE

FORCED DEGRADATION DATA OF TEZACAFTOR AND IVACAFTOR

Stress sample	**Stress conditions**	**Tezacaftor**		**Ivacaftor**	
		%Assay	%Degradation	% Assay	% Degradation
Control sample		99.83	-	100.10	-
Acid sample	0.1N HCl refluxed for 60 min at 60°C	95.69	4.14	96.80	3.3
Base sample	0.1N NaOH refluxed for 60 min at 60°C	96.50	3.33	97.78	2.32
Peroxide sample	10% peroxide for 30 min Bench top	92.64	7.19	93.29	6.81
Thermal sample	oven at 105°C for 6 h	97.78	2.05	9.21	90.89
UV sample	in UV Chamber for 1h or 200-Watt hours/m^2	98.88	0.95	98.51	1.59
Water sample	Water refluxed for 1h at 60^0C	99.65	0.18	99.00	1.10

PEAK PURITY DATA OF TEZACAFTOR AND IVACAFTOR

Nature of degradation	Stress conditions	Tezacaftor		Ivacaftor	
		Purity of angle	Purity of Threshold	Purity of angle	Purity of Threshold
Acid sample	0.1N HCl refluxed for 60 min at 60°C	0.156	0.161	0.109	0.642
Base sample	0.1N NaOH refluxed for 60 min at 60°C	0.741	0.927	0.081	0.162
Peroxide sample	10% peroxide for 30min Bench top	0.184	0.321	0.037	0.703
Thermal sample	oven at 105°C for 6 h	0.109	0.270	0.584	1.013
UV sample	in UV Chamber for 1h or 200-Watt hours/m^2	0.105	0.831	0.121	0.321
Water sample	Water refluxed for 1h at 60^0C	0.050	0.311	0.147	0.170

SUMMARY AND CONCLUSION

The present analytical method was developed by studying different parameters. The column used for the study was CHS C18 50 X 2.1mm 1.8µ column because it gave good separation and peak shapes. Ideal λ max for both the drugs was found to be at 292 nm as the peak purity was good. Injection volume was selected to be 1µL which gave a good peak area. The flow rate was fixed at 0.3 mL/min for giving satisfactory retention times. A mixture of 0.1% OPA (pH2.2): Acetonitrile (60:40% v/v) was found to be ideal for the proposed study as it resulted in °good resolution of the drugs. Run time was selected to be 3 min because the analysis gave peaks around 0.508 and 0.76 ±0.02 min of Tezacaftor and Ivacaftor respectively. The percent recovery was found to be in between 98.0 to 102.0%. The analytical method was found to be linear over the range 10-60 µg/mL of Tezacaftor and 15-90 µg/mL of Ivacaftor of the target concentration. The analytical method passed both the robustness and ruggedness tests. In both the cases, relative standard deviation was below 2.0.

The developed method is simple, sensitive, precise and accurate for simultaneous quantitative estimation of Tezacaftor and Ivacaftor in tablet dosage forms and has certain advantages over the methods reported in literature. This method is simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps. The solvent system used in this method is economical due to the use of OPA and Acetonitrile. The % RSD values were within 2.0 and the method was found to be precise. The results of the validation parameters of the method lie within the prescribed limits. The method did not show any interference from excipients. These results confirm the suitability of the proposed RP-UPLC method for accurate and precise analysis of Tezacaftor and Ivacaftor in combined tablet dosage forms.

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