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Analytical Method Development and Validation of Tezacaftor and Ivacaftor by RP-HPLC Method in Bulk and Marketed Formulation

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Abstract
A RP-HPLC technique based assay procedure is developed, validated and applied for quantification of ivacaftor and tezacaftor simultaneously in tablet dosage forms. Procedure is based on separation and analysis of ivacaftor and tezacaftor in Kromosil C18 column with 0.1M KH_2PO_4: methanol (65:35 v/v) mixture as mobile phase. The elution time values for ivacaftor and tezacaftor were 3.128 min and 4.044 min, respectively. Linear ranges for ivacaftor and tezacaftor were 75-225 µg/ml and 50-150 µg/ml, respectively with regression coefficients of >0.9990. The sensitivity values were 0.056 µg/ml (LOD) and 1.819 µg/ml (LOQ) for ivacaftor and 0.405 (LOD) µg/ml and 1.351 µg/ml (LOQ). Validation parameters are tested as per guidelines of ICH and all values are well acceptable. The method was applied to tablet dosage forms with excellent percent assay values.

Keywords
Ivacaftor, Stability indicating RP-HPLC Method, Tezacaftor, Validation.

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INTRODUCTION:
Tezacaftor is chemically 1-(2, 2-Difluoro-1, 3-benzodioxol-5-yl)-*N*-[1-[(2*R*)-2, 3-dihydroxypropyl]-6-fluoro-2-(2-hydroxy-1, 1-dimethylethyl)-1*H*-indol-5-yl]-cyclopropane carboxamide and it is helps to move the CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) protein to the correct position on the cell surface and designed to treat people with F508del mutation. Ivacaftor is chemically *N*-(2, 4-Di-*tert*-butyl-5-hydroxyphenyl)-4-oxo-1, 4-dihydroquinoline-3-carboxamide and it is a Potentiator for Cystic Fibrosis Transmembrane Conductance Regulator, Chloride channel agonists.

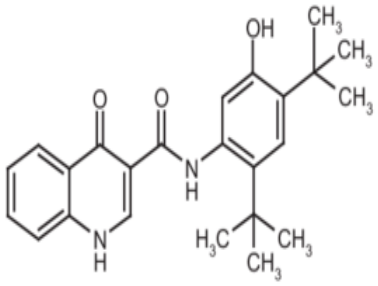

Fig 1: Ivacaftor chemical structure

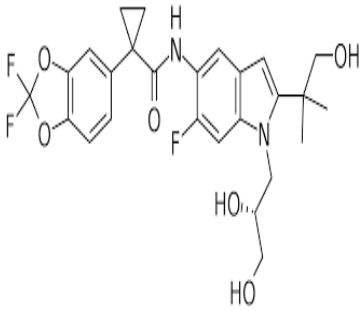

Fig 2: Tezacaftor chemical structure

MATERIALS AND METHODS:

Chemicals and reagents:

Ivacaftor and Tezacaftor were received as a reference sample from Lara Drugs Private Limited, Telangana, India. Tezacaftor and ivacaftor combination is available as tablets with brand name Symdeko (strength of each tablet is 100 mg tezacaftor and 150 mg ivacaftor). Methanol of HPLC grade (Merck specialties Ltd, India) and potassium dihydrogen phosphate (KH2PO4) of Analytical grade (SD Fine-Chem Limited, India) were taken.

Chromatographic study:

The HPLC system consisted of Waters Alliance 2695 model separation module, uv detector (Photodiode array), Empower (version 2) software was used for mathematical computations and data acquisition. Ambient temperature was used for performing the analysis.

Preparation of mobile phase:

0.1 M KH_2PO_4 and methanol are mixed in ratio 65:35 volumes/ volume. Prior to utilize passed via membrane filter with pore size 0.45 0.45µm and sonicated for degassing. The same mixture is used to prepare stock and working standard solutions.

Preparation of standard stock solutions:

100 mg and 150 mg of tezacaftor and ivacaftor, respectively are weighed precisely and transferred to 100 ml flask. Then 30 ml of mobile phase was added and sonicated 10 min. Mobile phase was further added to finish the volume to 100 ml (final concentration: 1000 µg/ml of tezacaftor and 1500 µg/ml of ivacaftor). This is tezacaftor and ivacaftor stock solution.

Preparation of standard working solution:

Tezacaftor and ivacaftor working solution is made by diluting 1.0 ml of stock tezacaftor and ivacaftor solution to 10 ml by mobile phase (final concentration: 100 µg/ml of tezacaftor and 150 µg/ml of ivacaftor).

Calibration curves of tezacaftor and ivacaftor:

Tezacaftor and ivacaftor calibration solutions are made by diluting 0.5, 0.75, 1.0, 1.25 and 1.5 ml of above prepared stock solution to 10 ml with mobile phase to prepare calibration solutions with concentrations:

75 µg/ml, 112.5 µg/ml, 150 µg/ml, 187.5 µg/ml and 225 µg/ml – ivacaftor

50 µg/ml, 75 µg/ml, 100 µg/ml, 125 µg/ml and 150 µg/ml – tezacaftor

Assay of tezacaftor and ivacaftor:

The sample solution with concentration1000 µg/ml of tezacaftor and 1500µg/ml of ivacaftor was analyzed thrice as described above. The quantity of tezacaftor and ivacaftor tablet dosage form was calculated using calibration curve or regression equation of tezacaftor and ivacaftor.

RESULTS AND DISCUSSION

Method development:

Through literature search, chemical and physical properties of tezacaftor and ivacaftor were obtained. The assay method was developed to choose preliminary RP-HPLC conditions, like - mobile phase composition and ratio, stationary phase, and detection wavelength.

Table 1: Parameters of Method Validation

Mobile Phase composition and ratio	:	0.1 M potassium dihydrogen orthophosphate and methanol mixed in ratio 65:35 vol/vol
Flow rate run	:	0.8 ml/min
Column tested	:	Kromosil, C18, 25 cm × 4.6 mm, 5µm
Temperature set	:	25˚C
Volume of sample for injection	:	10 µl
Run time for single analysis	:	6 min

Wavelength for detection	:	217
P^H	:	4.1

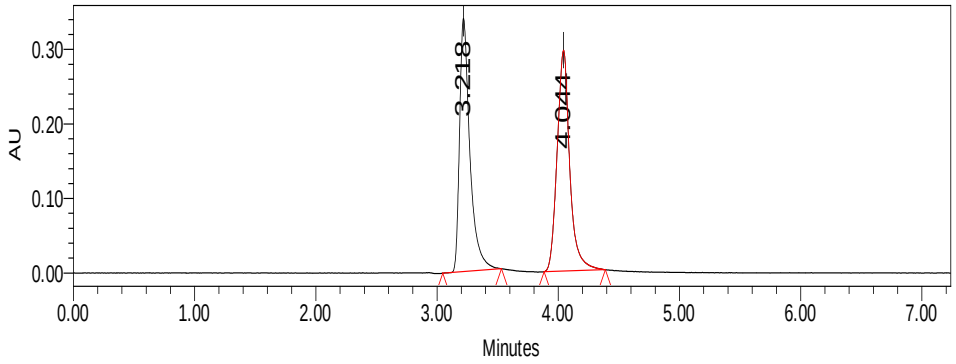

Fig 3: Chromatogram of Tezacaftor and Ivacaftor

Method validation:

Validation was performed in harmony to ICH guideline. System suitability, selectivity, linearity, sensitivity, accuracy, precision, specificity and robustness were determined.

System suitability:

Ten µl of tezacaftor and ivacaftor working standard solution (100 µg/ml of tezacaftor and 150 µg/ml of ivacaftor) was injected five times. The parameter for system suitability such as peak area, retention time, theoretical plates number, tailing factor and separation of tezacaftor and ivacaftor peaks (resolution) were studied. As revealed in Table 1, all values in the studied parameters area within satisfactory limits.

Table 2: Tezacaftor and ivacaftor system suitability data

Sample no.	Tezacaftor (100µg/ml)					Ivacaftor(150µg/ml)				
	RT	PA	PC	PT	RS	RT	PA	PC	PT	RS
1	4.062	2158051	8289	1.20	4.96	3.219	1988732	7498	1.64	-
2	4.065	2125575	8211	1.18	4.94	3.218	1975574	7403	1.64	-
3	4.073	2129831	8769	1.15	5.12	3.221	1984270	7844	1.64	-
4	4.072	2137583	8300	1.18	4.99	3.220	1975171	7457	1.63	-
5	4.071	2154870	8693	1.18	5.12	3.217	1973723	7690	1.63	-
Mean		2141182.0					1979494.1			
% RSD		0.7					0.3			

RT - retention time, PC - plate count, PA - peak area, PT - peak tailing & Rs - resolution

Selectivity:

Selectivity was proved by injection of working standard solution (concentration - 100 µg/ml of tezacaftor and 150 µg/ml of ivacaftor) tablet sample solution (concentration - 100 µg/ml of tezacaftor and 150 µg/ml of ivacaftor), placebo blank and mobile phase blank. Then chromatograms of the above said solution were checked for retention times of tezacaftor and ivacaftor. No peaks were seen at the retention times of tezacaftor and ivacaftor in chromatograms of blank mobile phase and placebo. This proves the noninterference of components in mobile phase and placebo. Hence the method is considered selective.

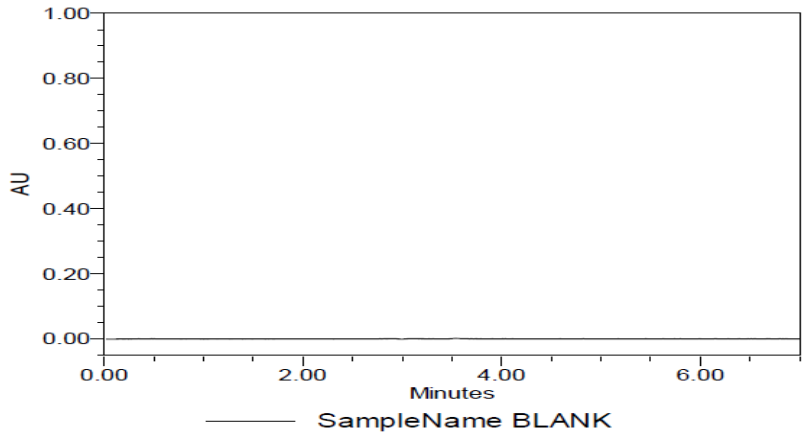

Fig 4: Blank mobile phase chromatogram

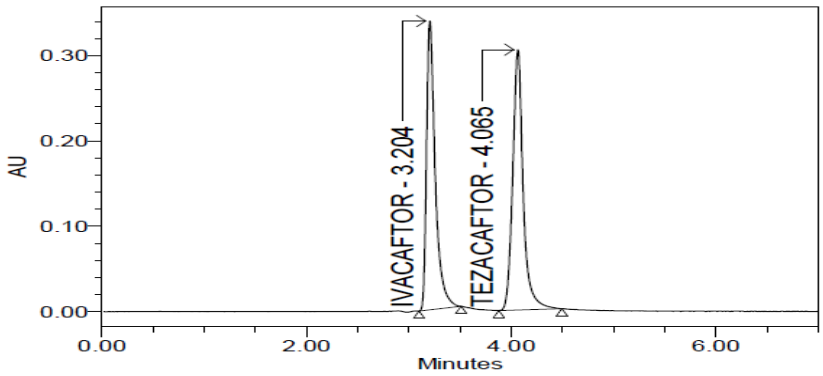

Fig 5: Tezacaftor and ivacaftor working standard chromatogram

Linearity:

Five calibration solutions, 50 to 150 µg/ml of tezacaftor and 75 to 225 µg/ml of ivacaftor were analyzed by the proposed procedure. Analytical responses of tezacaftor and ivacaftor were documented and calibration curves are made by plotting area response against tezacaftor and ivacaftor concentration. The linearity of tezacaftor and ivacaftor were evaluated by determining y-intercept, slope and regression coefficient (R^2) by least square regression. Calibration curves of tezacaftor and ivacaftor were linear in range 50 - 150 µg/ml and 75 - 225 µg/ml, respectively. The regression equations (y = mc + x) were:

y = 19811 c – 4647, R^2 = 0.9999 - Ivacaftor

y = 21459 c – 1969, R^2 = 0.9998 - Tezacaftor

y = peak area response of ivacaftor or tezacaftor, m = slope of calibration curve, c = concentration of ivacaftor or tezacaftor, x = interceopt on x-axis

The regression coefficients for ivacaftor and tezacaftor were larger than 0.999 which revealed a degree of high correlation and good quality method linearity.

Table 3: Tezacaftor and ivacaftor linearity information

Concentration (%)	Tezacaftor		Ivacaftor	
	µg/ml	Area	µg/ml	Area
50	50	1070865	75	989571
75	75	1607640	112.5	1480065
100	100	2146934	150	1970183
125	125	2674267	187.5	2473142
150	150	3219919	225	2969442

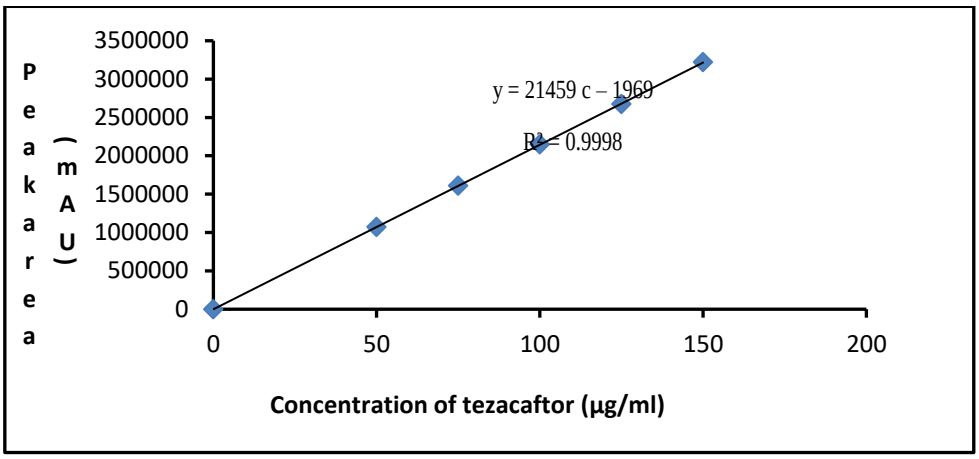

Fig 6: Tezacaftor linearity curve

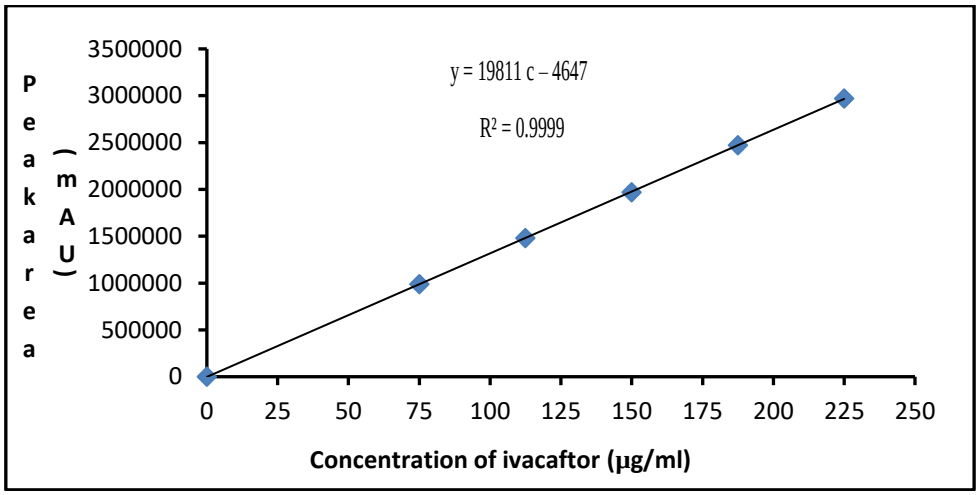

Fig 7: Ivacaftor linearity curve

Limit of detection and limit of quantification:

These parameters are estimated on basis of signal to noise ratio 3.1 and 10.1 for limit of detection (LOD) and limit of quantification (LOQ), respectively. The values were 0.056 µg/ml (LOD) and 1.819 µg/ml (LOQ) for ivacaftor and 0.405 (LOD) µg/ml and 1.351 µg/ml (LOQ). This supports that the proposed method has offered adequate sensitivity.

Precision:

Method precision was determined with standard solution of concentration 100 µg/ml of tezacaftor and 150 µg/ml of ivacaftor. Method precision was checked through six replicate analysis of standard solution. Precision was expressed by percent relative standard deviation (%RSD) for peak areas and percent assay of tezacaftor and ivacaftor. As per ICH guideline for validation, low value for %RSD (<2%) revealed high method precision.

Table 4: Tezacaftor and ivacaftor method precision information

S.No	Ivacaftor peak area	Tezacaftor peak area	Ivacaftor assay %	Tezacaftor assay %
I	1978729	2144568	99.76	99.86
II	1973364	2141127	99.49	99.7
III	1970166	2140589	99.33	99.67
IV	1973849	2144133	99.52	99.84
V	1975905	2142419	99.62	99.76
VI	1975162	2140806	99.58	99.68
Average	1974529	2142274	99.55	99.75
RSD %	0.145	0.081	0.144	0.083

S.No. – sample number

Accuracy and recovery (standard addition method): The accuracy and recovery of method was demonstrated through by spiking tablet sample solution with known quantities of tezacaftor and ivacaftor at concentration of three levels (50%, 100% and 150%). Method accuracy and recovery was checked through three replicate analysis of above prepared sample solution. The results are expressed by percent recovery of tezacaftor and ivacaftor. As per ICH guideline for validation, the percent recovery of tezacaftor and ivacaftor were between 98 to 102% suggesting high method accuracy of the method

Table 5: Accuracy and recovery of Ivacaftor and Tezacaftor

Spiked conc%	Concentration of Ivacaftor (µg/ml)					Concentration of Tezacaftor (µg/ml)				
	Area	Added	Determined	Recovery(%)	Mean (%)	Area	added	Determined	Recovery	Mean (%)
	989816	75.000	74.86	99.81		1074297	50.000	50.02	100.05	
	989000	75.000	74.79	99.72	99.77	1073461	50.000	49.98	99.97	100.10
50%	989644	75.000	74.84	99.79		1077001	50.000	50.15	100.30	
	1974276	150.000	149.31	99.54		2148639	100.000	100.05	100.05	
	1970493	150.000	149.02	99.35	99.55	2143090	100.000	99.79	99.79	99.92
100%	1978572	150.000	149.63	99.75		2145822	100.000	99.92	99.92	
	2965865	225.000	224.29	99.69		3215965	150.000	149.75	99.83	
	2966848	225.000	224.37	99.72	99.68	3216569	150.000	149.77	99.85	99.84
150%	2963969	225.000	224.15	99.62		3216751	150.000	149.78	99.85	

Conc: concenteration

Robustness:
To investigate method robustness, a little change was done in the flow rate, mobile phase pH, column temperature, detection wavelength and composition of mobile phase.
Ratio of methanol - changed by ±5%; pH of buffer – changed by ± 0.2 units; Flow rate - changed by ± 0.1 ml/min; Column temperature - changed by ± 2 °C; Wavelength – changed by ± 2 nm
In all changed conditions, system suitability parameters were determined for ivacaftor and tezacaftor. System suitability parameters shown in table revealed that my method was robust when little changes were made.

Table 6: Ivacaftor and Tezacaftor robustness information

Parameter	Ivacaftor				Tezacaftor			
	Changed value	TF	TP	RS	Changed value	TF	TP	RS
Temperature (°C)	23	1.65	7643	-	23	1.20	8432	5.09
	27	1.65	7403	-	27	1.20	8180	5.06
Flowrate (ml/min)	0.7	1.64	6991	-	0.7	1.21	7984	4.96
	0.9	1.59	7213	-	0.9	1.19	8082	4.90
Mobile phase pH (units)	4.3	1.63	7619	-	4.3	1.17	8641	5.08
	3.9	1.64	7587	-	3.9	1.18	8579	5.11
Percent of methanol in mobile phase (%)	30	1.64	6991	-	30	1.21	7984	4.96
	40	1.65	7643	-	40	1.20	8432	5.09
Wavelength (nm)	215	1.64	7546	-	215	1.12	8525	4.99
	219	1.65	7394	-	219	1.19	8223	4.93

TF: Tailing factor; TP: Theoretical plate; RS: Resolution

Tezacaftor and ivacaftor simultaneous assay in tablets:
To study method applicability, test sample solutions of ivacaftor and tezacaftor were prepared from Symdeko tablets (labeled content: tezacaftor 100 mg and ivacaftor 150 mg) at concentration 100 µg/ml of tezacaftor and 150 µg/ml of ivacaftor. 10 µl of solutions was injected to get their chromatograms and peak area responses. Good separation, percent assay and relative standard deviation values of tezacaftor and ivacaftor has pointed out high selectivity, accuracy and precision of the method to assay tezacaftor and ivacaftor simultaneously in tablet dosage forms.

Table 7: Assay of Tezacaftor and Ivacaftor in tablet

Tezacaftor				Ivacaftor			
content in tablet (mg)	determined (µg/ml)	Assay (%)	Statistical value	content in tablet (mg)	determined (µg/ml)	Assay (%)	Statistical value
100	100.10	100.10	Mean: 99.95%	150	149.66	99.77	Mean: 99.67 %
100	99.92	99.92	SD: 0.133%	150	149.33	99.55	SD: 0.111%
100	99.84	99.84	RSD: 0.133%	150	149.52	99.68	RSD: 0.111%

CONCLUSION:

In this investigation, a simple and sensitive RP-HPLC method was developed for quantification of tezacaftor and ivacaftor simultaneously. Experimental conditions of chromatography including mobile phase components, pH of mobile phase and flow rate run was validated in conformity of ICH Q_2 (R_1) guideline. The method was appropriate for quantification of tezacaftor and ivacaftor simultaneously in tablet samples with good linearity, accuracy and precision.

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