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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF TEZACAFTOR, IVACAFTOR AND ELEXACAFTOR IN BULK AND IT' S COMBINED DOSAGE FORM

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ABSTRACT

A simple, accurate, precise quantitative method was developed for the simultaneous estimation of the Tezacaftor, Ivacaftor and Elexacaftor in solid dosage form. Chromatogram was run using Ascentis C18, 150×4.6mm, 5µ column with mobile phase containing 0.1% orthophoshoric acid and acetonitrile in the ratio of 60:40 *v/v* which was pumped through the column at a flow rate of 1ml/min. Temperature was maintained at 30°C. Optimized wavelength was 260.0 nm. Retention times of Tezacaftor, Ivacaftor and Elexacaftor were found to be 2.336 min, 2.901 min and 3.517 min, respectively. %RSD of system precision and method precision was found to be less than 2% for three studied drugs. Mean % recovery was obtained as 99.61%, 99.92% and 99.57% for Tezacaftor, Ivacaftor and Elexacaftor respectively. LOD, LOQ values obtained from regression equations of Tezacaftor, Ivacaftor and Elexacaftor were 0.28 µg/ml, 2.36 µg/ml, 0.59 µg/ml and 0.84 µg/ml, 7.15µg/ml, 1.79µg/ml respectively.

INTRODUCTION

Ivacaftor (also known as Kalydeco or VX-770) 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 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 lungs, pancreas, liver, digestive system, and reproductive tract [1]. Tezacaftor is a small molecule that can be used as a corrector of the CFTR gene function. It was developed by Vertex

Pharmaceuticals and FDA approved Tezacaftor in combination with Ivacaftor; a CFTR potentiator that allows the proteins at the cell surface to open longer and improve nutrient transport [2]. Elexacaftor (previously known as VX-445) is a small molecule, next-generation corrector of the CFTR protein. It received FDA approval in October 2019 in combination with tezacaftor and ivacaftor as the combination product TrikaftaTM. Elexacaftor is considered a next-generation CFTR corrector as it possesses both a different structure and mechanism as compared to first generation correctors like tezacaftor [3]. While dual corrector/potentiator combination therapy has proven useful in the treatment of a subset of CF patients, and their use is typically limited to patients who are

homozygous for the F508del-CFTR gene [3]. The chemical structures of Ivacaftor, Tezacaftor and Elexacaftor were shown in fig 1.

From the literature, it was observed that few methods have been reported for the estimation of Ivacaftor, Tezacaftor and Elexacaftor individually and in combined with other dosage forms by using UV, HPLC and bioanalytical methods [4, 5, 6]. There is no method reported for the simultaneous estimation of Ivacaftor, Tezacaftor and Elexacaftor in combined dosage form. So, present research work aimed to develop an accurate, precise, sensitive, selective, reproducible and rapid analytical method using HPLC technique for cost effective estimation of Tezacaftor, Ivacaftor and Elexacaftor in combination.

MATERIALS AND METHODS

Chemicals and reagents:

Drug Samples were obtained from SS Pharma Labs. HPLC grade water, acetonitrile and AR grade triethyl amine, potassium dihydrogen phosphate and orthophosphoric acid was used in the study. All the reagents were purchased from Rankem.

Instruments:

Waters HPLC 2695 series with quaternary pumps, photodiode array detector and auto sampler integrated with empower software. UV double beam spectrophotometer of Lab India make. BVK enterprises made pH meter were used in this study.

Preparation of Standard Stock Solutions:

Accurately weighed 5mg of Tezacaftor, 7.5 mg of Ivacaftor and 10mg of Elexacaftor were transferred to three 50ml volumetric flasks separately. 10ml of diluent was added to the flasks and sonicated for 20mins. Flasks were made up to the mark with diluent and labelled as standard stock solution 1, 2 and 3. (100µg/ml of Tezacaftor, 150µg/ml of Ivacaftor and 200 µg/ml of Elexacaftor)

Preparation of standard working solutions (100% solution):

1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent (10µg/ml of Tezacaftor, 15µg/ml of Ivacaftor and 20µg/ml of Elexacaftor)

Preparation of Sample stock solutions:

5 Trikafta tablets (label clam per one tablet: 50 mg of Tezacaftor, 75 mg of Ivacaftor and 100 mg of Elexacaftor) were weighed individually and average weight of each tablet was calculated. Weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 25ml of diluent added and sonicated for 50 min, further the volume was made up to the mark with diluent and filtered. (500µg/ml of Tezacaftor, 750µg/ml of Ivacaftor and 1000 µg/ml of Elexacaftor)

Preparation of sample working solutions (100% solution):

From the filtered solution, 0.2ml was pipetted out into a 10 ml volumetric flask and made up to 10ml with diluent. (10µg/ml of Tezacaftor, 15µg/ml of Ivacaftor and 20µg/ml of Elexacaftor).

Optimized chromatographic conditions:

Column used : Ascentis C18, 150 × 4.6 mm, 5 µ.
Mobile phase : 0.1% orthophosphoric acid: acetonitrile (60:40 *v/v*)
Flow rate : 1.0ml/min
Diluent : Water: acetonitrile (50:50 *v/v*).
Wavelength : 260.0 nm
Temperature : 30°C
Injection volume: 10µl

Observation: Tezacaftor, Ivacaftor and Elexacaftor were eluted at 2.336 min, 2.901 min and 3.517 min respectively with good resolution (fig. 2). Plate count and tailing factor was very satisfactory, so this method conditions were optimized and applied the same conditions during validation.

Degradation studies:

To perform the forced degradation studies the standard stock solutions of Tezacaftor, Ivacaftor and Elexacaftor were subjected to various stress conditions such as 1 ml of 20% hydrogen peroxide (for oxidative degradation), 1ml of 2N hydrochloric acid(for acidic degradation),1 ml of 2N sodium hydroxide (for basic degradation). The prepared solutions were refluxed at 60°C for 30 min. The standard solutions were also exposed to the UV light (for light-based degradation) and thermal (dry heat degradation) conditions. For degradation study, the resultant solutions were diluted to obtain 10µg/ml, 15µg/ml and 20µg/ml of Tezacaftor, Ivacaftor and Elexacaftor, respectively. 10µl samples were injected into the system and the chromatograms were recorded to assess the stability of sample.

Method validation

Method validation was carried on according to ICH guidelines Q_2R_1. The

validation parameters include system suitability, specificity, linearity, accuracy, precision, LOD& LOQ and robustness [12, 13].

RESULTS AND DISCUSSION

System suitability parameters:

The system suitability parameters were determined by preparing standard solutions of Tezacaftor (10 µg/ml), Ivacaftor (15 µg/ml) and Elexacaftor (20 µg/ml) and the solutions were injected six times. The parameters like peak tailing, resolution and USP plate count were determined. USP Plate count was more than 2000 and tailing factor was less than 2 for 3 drugs in combination. All the system suitable parameters were passed and were within the limits. The results were shown in table 1.

Specificity:

Checking of the interference in the optimized method. Retention times of Tezacaftor, Ivacaftor and Elexacaftor were 2.3 min, 2.9 min and 3.5 min respectively. We did not find any interfering peaks in blank and placebo sample chromatograms at retention times of these drugs in this method. So, this method was said to be specific. The chromatograms for specificity were given in fig 3, 4 and 5

Linearity:

Six linear concentrations of Tezacaftor (2.5-15µg/ml), Ivacaftor (3.75-22.50µg/ml) and Elexacaftor (5-30µg/ml) were injected in triplicate manner. Correlation coefficients obtained were greater than 0.999 for all the three drugs. The results were given in table 2 and fig 6, 7 and 8.

Precision:

Repeatability:

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations (Tezacaftor 10 µg/ml, Ivacaftor 15 µg/ml and Elexacaftor 20 µg/ml) were prepared, each injection from each working sample solution was given and obtained areas were mentioned in table 3. Average area, standard deviation and % RSD were calculated for the three drugs and obtained as 0.8%, 0.5% and 0.4%, respectively for Tezacaftor, Ivacaftor and Elexacaftor. As the limit of precision was less than "2%" the system precision was passed for this method. The data was shown in table 3.

Intermediate Precision:

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations (Tezacaftor 10 µg/ml, Ivacaftor 15 µg/ml and Elexacaftor 20 µg/ml) were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the table 4. Average area, standard deviation and % RSD were calculated for the three drugs and obtained as 0.9%, 1.5% and 1.4% respectively for Tezacaftor, Ivacaftor and Elexacaftor. As the limit of precision was less than "2%" the intermediate precision was passed for this method. The results are depicted in table 4.

Accuracy:

Three levels of accuracy sample were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %recovery was obtained as 99.61%, 99.92% and 99.58% for Tezacaftor, Ivacaftor and Elexacaftor, respectively. The results were given in tables 5, 6 & 7. As good recover values were obtained, the accuracy was passed for this method.

LOD & LOQ:

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (s). The LOD was calculated according to the formula:

$$LOD = 3.3 \times SD/s$$

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (s) according to the formula:

$$LOD = 10 \times SD/s$$

The results were shown in table 8. The chromatograms of LOD and LOQ are shown in fig 9 and 10, respectively.

Robustness:

Robustness conditions like flow minus (0.9ml/min), flow plus (1.1ml/min), mobile phase minus (65:35*v/v*), mobile phase plus (55:45 *v/v*), temperature minus (25°C) and temperature plus(35°C) was maintained and samples (Tezacaftor 10 µg/ml, Ivacaftor 15 µg/ml and Elexacaftor 20 µg/ml) were injected in duplicate manner. %RSD was calculated and found to be within the limit. The data was given in table 9.

Assay: The label claim of Trikafta tablets was Ivacaftor 75mg Tezacaftor50 mg Elexacaftor 100mg per unit formulation. Assay was performed with the above formulation. Average percent assay for Tezacaftor, Ivacaftor and Elexacaftor obtained were99.45%, 99.06% and 99.71% respectively.

Degradation Studies:

Degradation studies were performed with the stock standard solution and the degraded samples were analysed using proposed method. Assay percent of Tezacaftor, Ivacaftor and Elexacaftor in the injected samples was calculated and all the samples passed the limits of degradation. The results were shown in table 10.The chromatograms obtained in degradation studies are shown in fig 11,12,13,14 and 15.

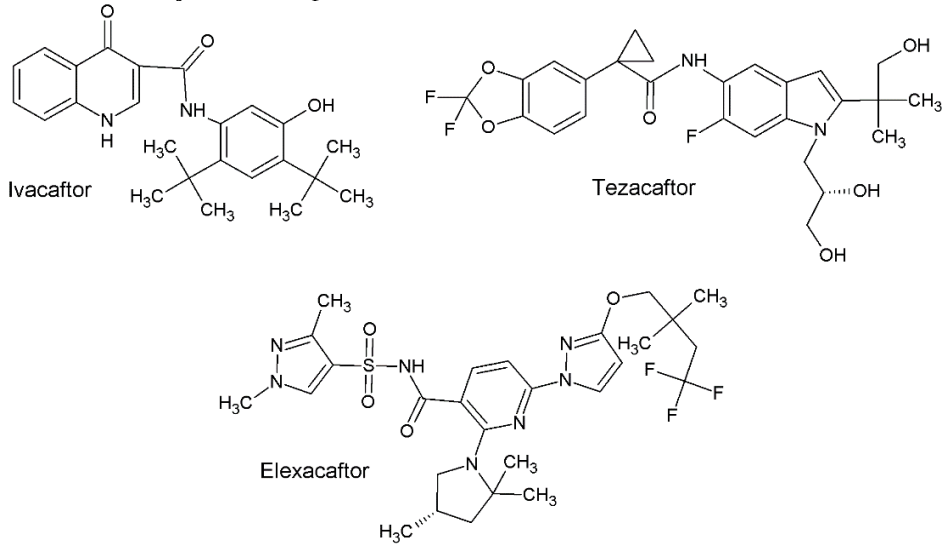

Figure No 1: Chemical structures of Ivacaftor, Tezacaftor and Elexacaftor

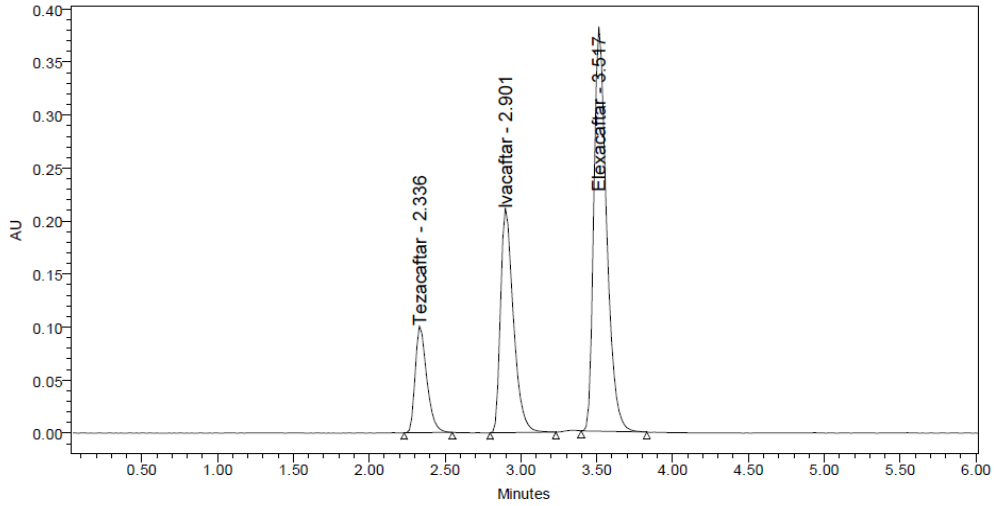

Figure No 2: Optimized Chromatogram for Ivacaftor, Tezacaftor and Elexacaftor

Table No 1: System suitability parameters for Tezacaftor, Ivacaftor, and Elexacaftor

S no	Tezacaftor			Ivacaftor			Elexacaftor		
Inj	RT (min)	TP	Tailing factor	RT (min)	TP	Tailing factor	RT (min)	TP	Tailing factor
1	2.32	4058	1.34	2.86	5395	1.43	3.43	7925	1.35
2	2.32	4268	1.35	2.86	5410	1.39	3.44	7916	1.39
3	2.32	4320	1.35	2.87	5207	1.43	3.46	8005	1.35
4	2.33	4211	1.35	2.88	5427	1.43	3.46	7928	1.40
5	2.33	3967	1.33	2.88	5409	1.40	3.46	8091	1.39
6	2.33	4191	1.34	2.88	5478	1.42	3.46	8024	1.39

S no. – Sample number; RT-retention time; TP-theoretical plates

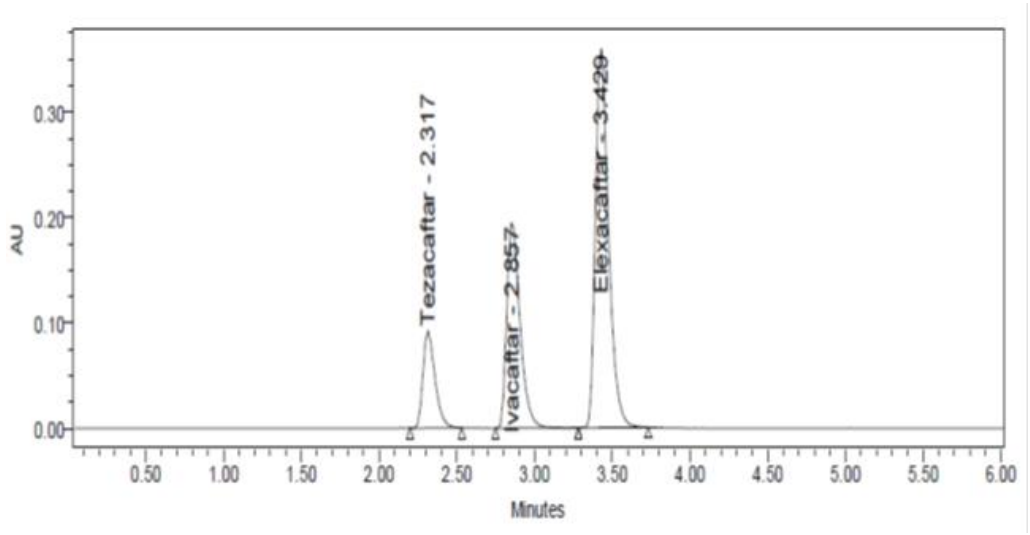

Figure No 3: Standard solution chromatogram

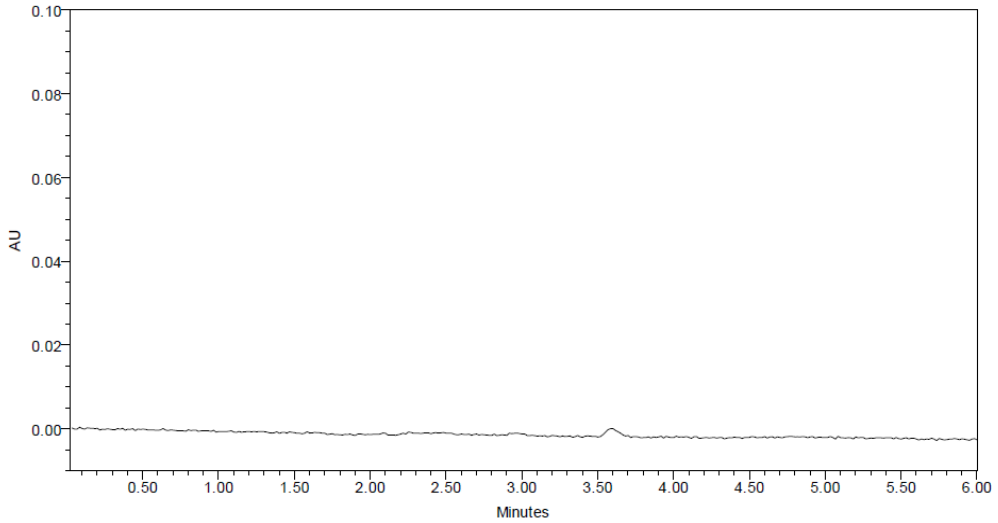

Figure No 4: Diluent blank chromatogram

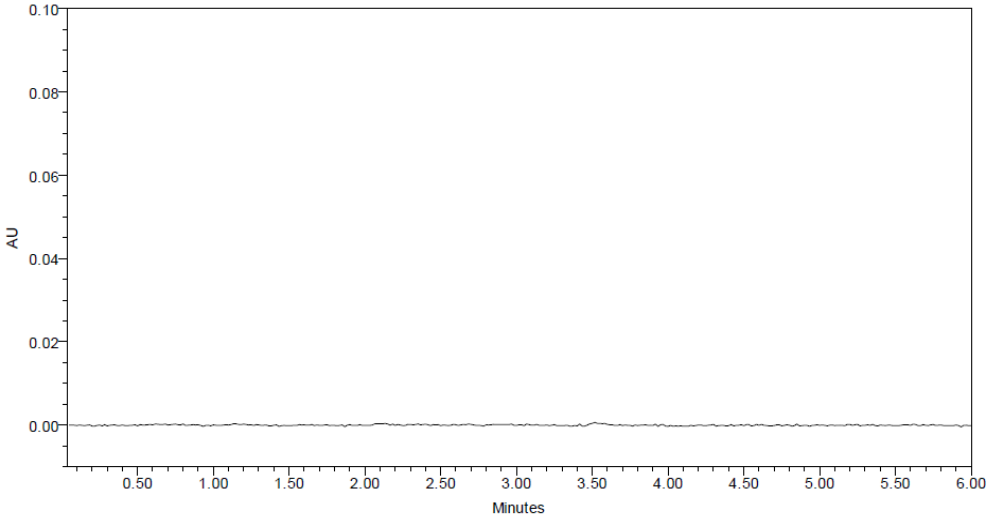

Figure No 5: Placebo blank chromatogram

Table No 2: Linearity table for Tezacaftor, Ivacaftor and Elexacaftor

Tezacaftor		Ivacaftor		Elexacaftor	
Conc (µg/ml)	Peak area	Conc (µg/ml)	Peak area	Conc (µg/ml)	Peak area
2.5	207562	3.75	564437	5	1060859
5	403155	7.5	1123830	10	2042504
7.5	602356	11.25	1684813	15	3031715
10	807326	15	2263196	20	4032131
12.5	1010415	18.75	2802718	25	5054211
15	1203289	22.5	3327885	30	6051007

Conc. – Concentration of analyte

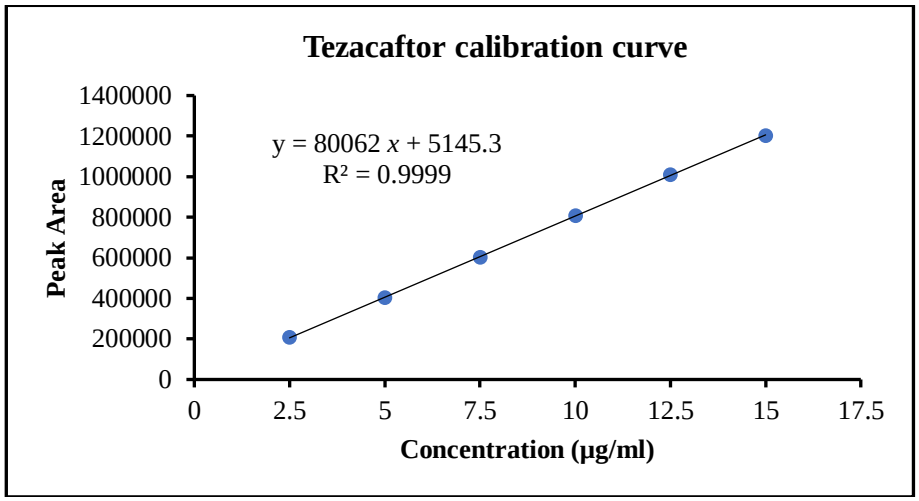

Figure No 6: Calibration curve of Tezacaftor

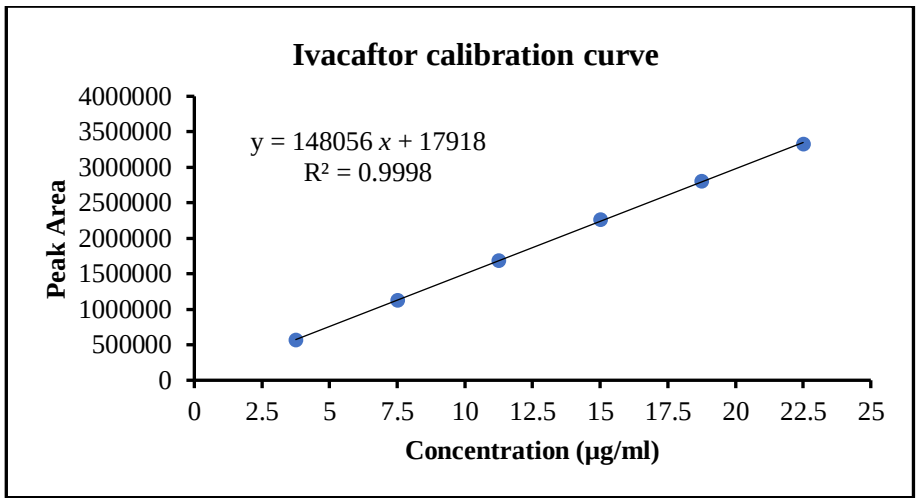

Figure No 7: Calibration curve of Ivacaftor

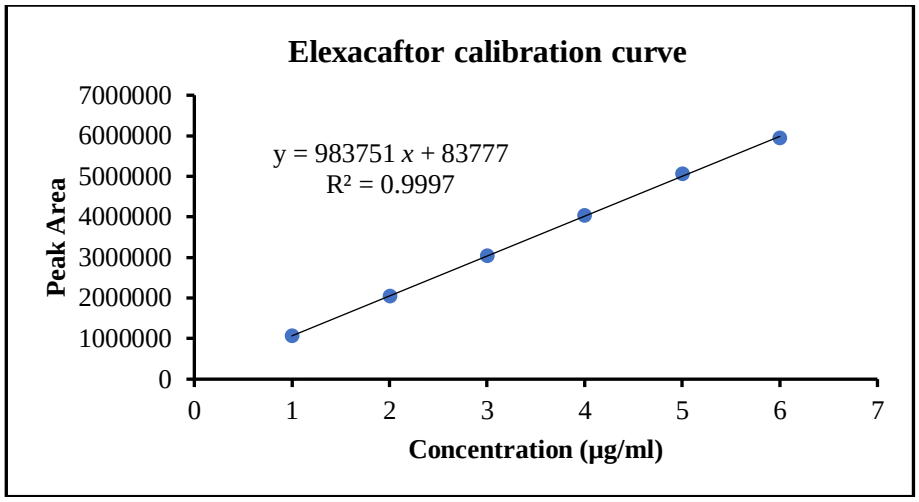

Figure No 8: Calibration curve of Elexacaftor

Table No 3:Repeatability results of Tezacaftor, Ivacaftor and Elexacaftor

S. No	Peak area of Tezacaftor	Peak area of Ivacaftor	Peak area of Elexacaftor
1.	806357	2246978	4024866
2.	808057	2237341	4039984
3.	804174	2248021	4052661
4.	790645	2264471	4069485
5.	804851	2243697	4045938
6.	808891	2230231	4022650
Mean	803829	2245123	4042597
S.D	6706.3	10580.5	17632.4
%RSD	0.8	0.5	0.4

S.D- standard deviation; %RSD – percent relative standard deviation

Table No 4: Intermediate precision results of Tezacaftor, Ivacaftor andElexacaftor

S. No	Peak area of Tezacaftor	Peak area of Ivacaftor	Peak area of Elexacaftor
1.	791089	2117293	3989025
2.	796806	2193861	4069189
3.	793257	2170154	3903221
4.	785895	2193900	3973502
5.	807806	2140581	3992209
6.	791197	2194972	3931175
Mean	794342	2168460	3976387
S.D	7488.0	32843.4	57320.1
%RSD	0.9	1.5	1.4

S.D- standard deviation; %RSD – percent relative standard deviation

Table No 5: Accuracy Tezacaftor

% Level	Amount Spiked (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean %Recovery
50%	5	4.98	99.66	99.61
	5	4.93	98.60	
	5	4.93	98.66	
100%	10	9.85	98.52	
	10	9.97	99.65	
	10	9.95	99.47	
150%	15	15.09	100.59	
	15	15.16	101.09	
	15	15.03	100.20	

Table No 6: Accuracy of Ivacaftor

% Level	Amount Spiked (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean % Recovery
50%	7.5	7.39	98.53	99.92
	7.5	7.48	99.73	
	7.5	7.36	98.13	
100%	15	15.14	100.93	
	15	15.07	100.47	
	15	15.16	101.07	
150%	22.5	22.40	99.56	
	22.5	22.57	100.31	
	22.5	22.63	100.58	

Table No 7: Accuracy of Elexacaftor

% Level	Amount Spiked (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean % Recovery
50%	10	9.93	99.30	99.57
	10	10.07	100.70	
	10	10.06	100.60	
100%	20	19.62	98.10	
	20	20.05	100.25	
	20	20.01	100.05	
150%	30	29.56	98.53	
	30	29.62	98.73	
	30	29.97	99.90	

Table No 8:Sensitivity data of Tezacaftor, Ivacaftor andElexacaftor

Drug	LOD(µg/ml)	LOQ(µg/ml)
Tezacaftor	0.28 µg/ml	0.84 µg/ml
Ivacaftor	2.36 µg/ml	7.15 µg/ml
Elexacaftor	0.59 µg/ml	1.79 µg/ml

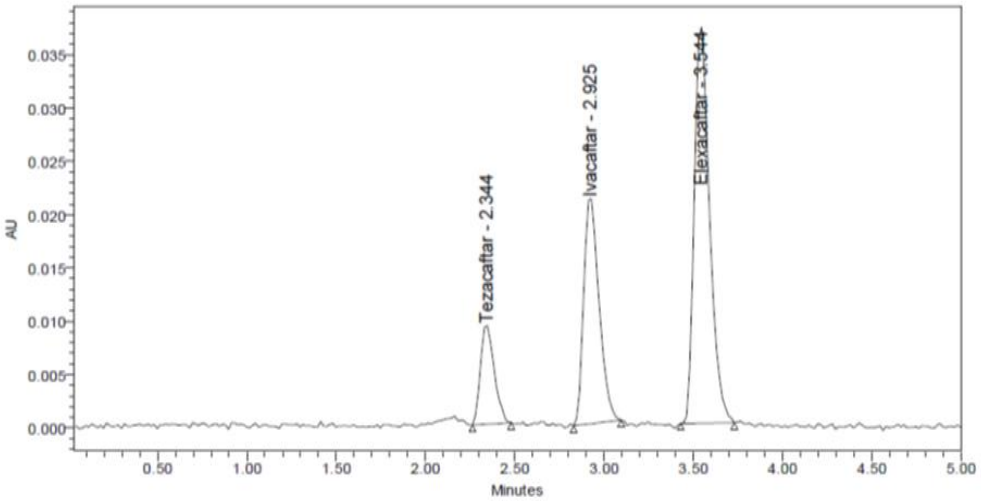

Figure No 9: LOD chromatogram of Tezacaftor, Ivacaftor and Elexacaftor

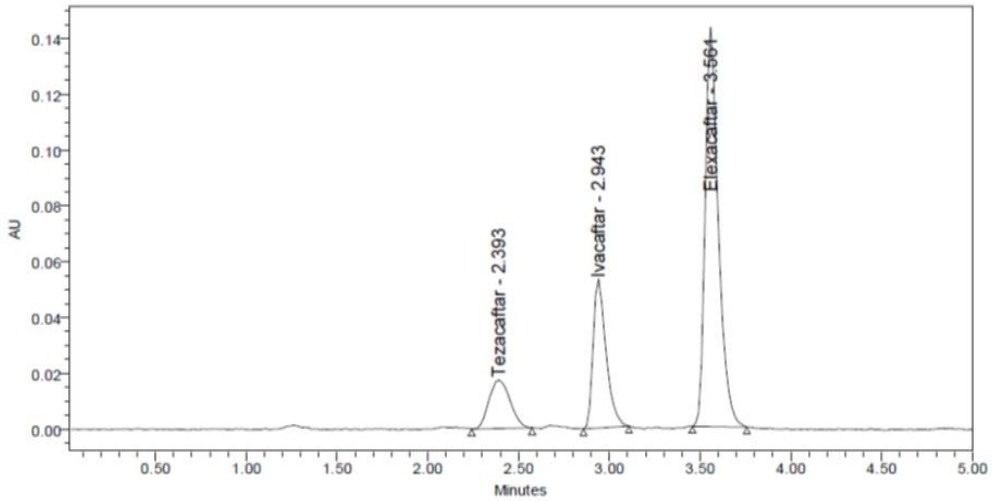

Figure No 10: LOQ chromatogram of Tezacaftor, Ivacaftor and Elexacaftor

Table No 9: Robustness data for Tezacaftor, Ivacaftor andElexacaftor.

S.no	Condition	%RSD of Tezacaftor	%RSD of Ivacaftor	%RSD of Elexacaftor
1	Flow rate (-) 0.9ml/min	1.08	1.16	1.41
2	Flow rate (+) 1.1ml/min	0.60	0.88	0.89
3	Mobile phase (-) 65:35*v/v*	1.21	1.18	0.80
4	Mobile phase (+) 55:45*v/v*	1.33	1.48	0.99
5	Temperature (-) 25°C	0.77	1.07	0.71
6	Temperature (+) 35°C	0.66	0.44	0.64

Table No 10: Degradation Data of Tezacaftor

S.No	Degradation Condition	% Drug degraded		
		Tezacaftor	Ivacaftor	Elexacaftor
1	Acidic	3.9	5.3	8.1
2	Base	3.5	4.0	5.5
3	Oxidation	1.3	8.4	1.8
4	Dry heat	0.9	2.9	1.2
5	Light based	0.6	2.5	0.7

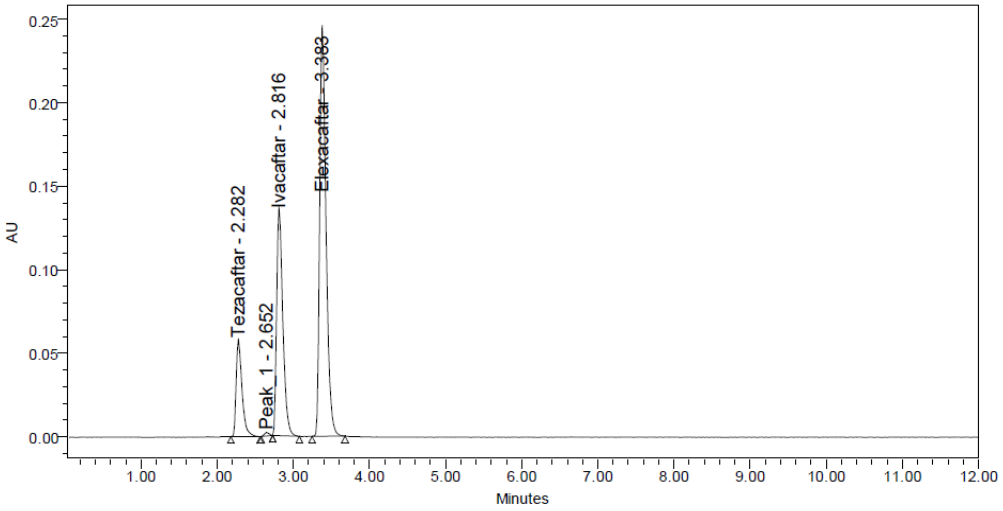

Figure No 11: Acid degradation chromatogram of Tezacaftor, Ivacaftor and Elexacaftor

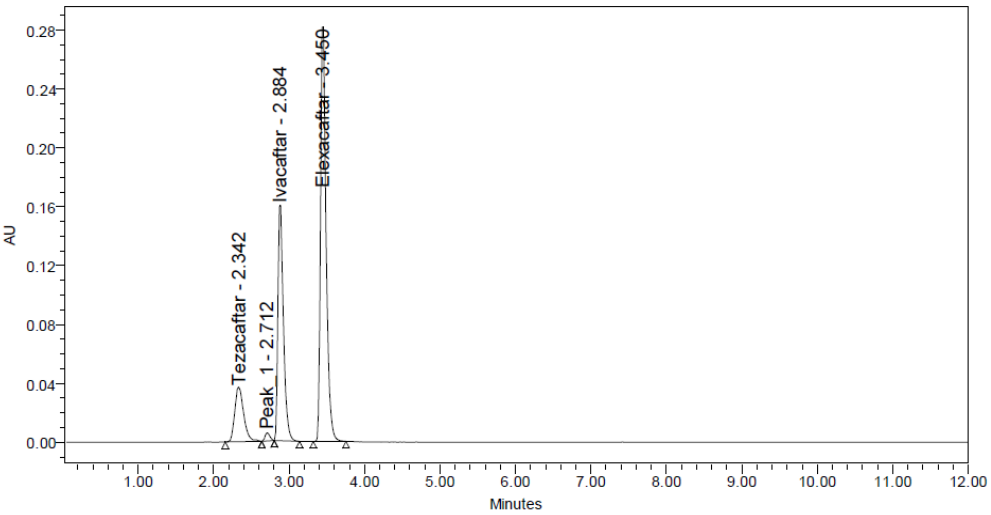

Figure No 12: Base degradation chromatogram of Tezacaftor, Ivacaftor and Elexacaftor

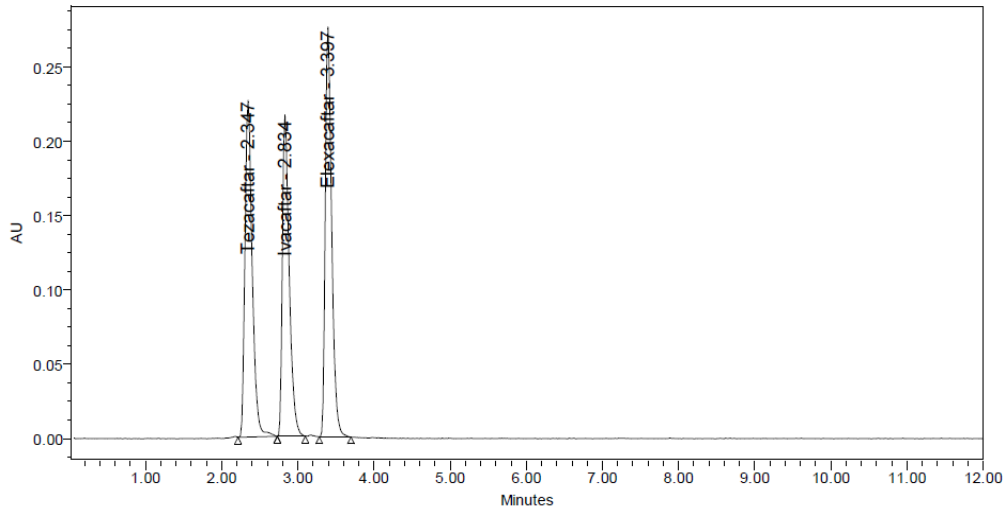

Figure No 13: Peroxide degradation chromatogram of Tezacaftor, Ivacaftor and Elexacaftor

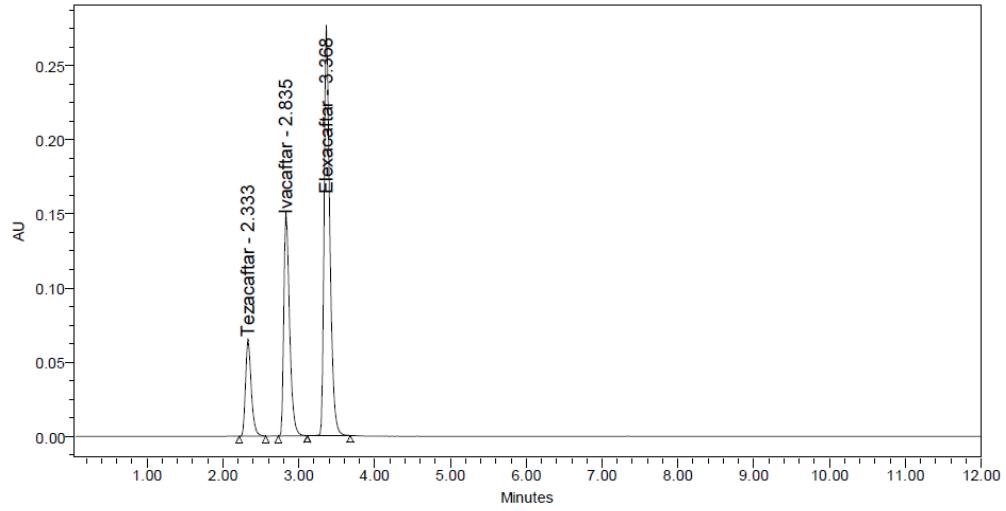

Figure No 14: Thermal degradation chromatogram of Tezacaftor, Ivacaftor and Elexacaftor

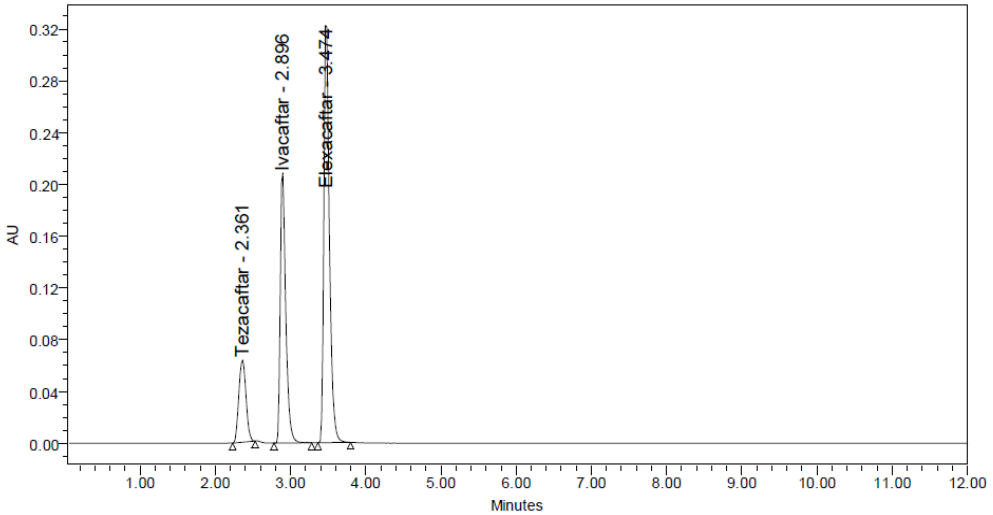

Figure No 15: UV degradation chromatogram of Tezacaftor, Ivacaftor and Elexacaftor

CONCLUSION

A simple, accurate, precise method was developed for the stability indicating RP-HPLC method for the simultaneous estimation of the Tezacaftor, Ivacaftor and Elexacaftor in Tablet dosage form. The developed method was satisfying the all the validation parameters and results were found to be within the limit. Present method when compared to the past reported works decrease the retention times. So the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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