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Research Article

ANALYTICAL QUALITY BY DESIGN BASED DEVELOPMENT AND VALIDATION OF A UPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF DEUTIVACAFTOR, TEZACAFTOR AND VANZACAFTOR

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ABSTRACT:
For the simultaneous measurement of deutivacaftor, tezacaftor, and vanzacaftor in bulk and pharmaceutical dose forms, a UPLC technique based on analytical quality by design (aqbd) was created and verified using a mobile phase made up of buffer and organic solvent in a 60:40 (v/v) ratio at a flow rate of 0.3 ml/min, the chromatographic separation was accomplished on by HSS 100Å 100 x 2.1mm, 1.8µm column. 270 nm was the ideal wavelength for detection. retention times were shown as deutivacaftor at 0.942 minutes, vanzacaftor at 1.109 minutes, and tezacaftor at 1.304 minutes. SST %RSD values of 0.7%, 0.3%, and 0.5% for deutivacaftor, tezacaftor, and vanzacaftor, while method precision yielded %RSD values of 0.4, 0.1, and 0.4, indicating excellent reproducibility. the percentage recoveries were found to be 99.28% for deutivacaftor, 99.72% for tezacaftor, and 100.10% for vanzacaftor, confirming the method's accuracy. the regression equations were determined as y = 10286x + 2613.8 for deutivacaftor, y = 10059x + 1491.2 for tezacaftor, and y = 15330x + 162.2 for vanzacaftor, demonstrating strong linearity.

K**ey Words:** Deutivacaftor. Tezacaftor, Vanzacaftor, RP-UPLC, QbD, Method Validation, Quality Control

INTRODUCTION:

CENTRAL COMPOSITE DESIGN

The experimental data was analyzed using surface plots and ANOVA, which made it easier to see how flow rate and methanol concentration interacted while determining the statistical significance of each element in connection to the chromatographic responses. The factorial design technique assured a procedure that is resilient and trustworthy in conformity with QbD principles by making it easier to find the appropriate chromatographic parameters and providing insights into the interaction between the two variables. A 3^2 full factorial design was utilized to optimize the RP-UPLC method for the simultaneous detection of Vanzacaftor, Tezacaftor and Deutivacaftor , emphasizing on two independent variables: the flow rate and the methanol content in the mobile phase. The flow rate was evaluated at 0.9, 1.0, and 1.10 mL/min, and the methanol concentration was altered to three different levels: 35%, 40%, and 45% (v/v with Ammonium Acetate). This setup resulted in twenty experimental runs, which allowed for a thorough evaluation of how variations in methanol concentration and flow rate affected significant chromatographic responses, including tailing factor, theoretical plate count, and retention duration.

Cystic fibrosis is an inherited disease caused by mutations in a gene called the cystic fibrosis transmembrane conductance regulator (CFTR). Both life expectancy and quality of life may be significantly impacted. The median anticipated survival is 45.[1] years, while the median age of individuals who have passed away is currently 28 years.[2] for the Treatment of Cystic Fibrosis Alyftrek a combination of two CFTR correctors Vanzacaftor, Ttezacaftor and CFTR potentiator Deutivacaftor is used for Individuals 6 years of age and older with at least one responsive mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene,

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such as F508del.[3] Vanzacaftor is a small molecule cystic fibrosis transmembrane conductance regulator (CFTR) corrector. It is used alongside other CFTR correctors and CFTR potentiators to increase the quantity and function of CFTR at the cell surface in patients with cystic fibrosis. [4,5] it is also known as (14S)-8-[3-(2-{dispiro[2.0.2^{4}.1^{3}]heptan-7-yl}ethoxy)-1H-pyrazol-1-yl]-12,12-dimethyl-2lambda6-thia-3,9,11,18,23-pentaazatetracyclo[17.3.1.1^{11,14}.0^{5,10}]tetracosa-1(22),5,7,9,19(23),20-hexaene-2,2,4-trione.[6]
Vanzacaftor exerts its therapeutic effects by facilitating the expression CFTR on the cell surface[5] Vanzacaftor is a CFTR corrector that aims to repair mutant CFTR cellular misprocessing. as co-administered with tezacaftor, vanzacaftor binds to a distinct location on the CFTR protein, which has an additional effect on the cellular processing and trafficking of mutant CFTR as compared to either agent alone.[6] Tezacaftor is a CFTR corrector known as 1-(2,2-difluoro-2H-1,3-benzodioxol-5-yl)-N-{1-[(2R)-2,3-dihydroxypropyl]-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl}cyclopropane-1-carboxamide.[7] Clinical studies have shown a significant decrease in sweat chloride and an increase in the forced expiratory volume (FEV), a measure of lung function, following Tevacaftor/Ivacaftor therapy.[8] Tezacaftor is a CFTR corrector that aims to repair F508del cellular misprocessing.[9] This is done by modulating the position of the CFTR protein on the cell surface to the correct position, allowing for adequate ion channel formation and increased in water and salt movement through the cell membrane.[10] Deutivacaftor is a CFTR potentiator written as N-{2-tert-butyl-5-hydroxy-4-[2-(2H3)methyl(1,1,1,3,3,3-2H6)propan-2-yl]phenyl}-4-oxo-1,4-dihydroquinoline-3-carboxamide [11] It is used alongside CFTR correctors to increase the quantity and function of CFTR.[4,5] Deutivacaftor exerts its therapeutic effects by increasing open probability of cell surface CFTR proteins. Through these combined mechanisms, Alyftrek improves CFTR function, helping to restore salt and water flow across mucosal surfaces reducing thick, sticky mucus in lungs and digestive tract that cause most CF symptoms.[12]

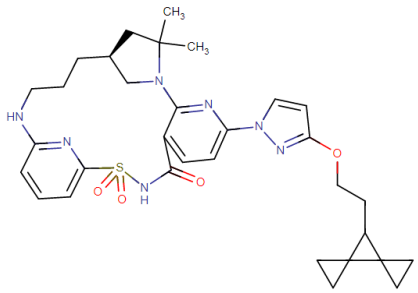


Figure 1: Structure of Vanzacaftor

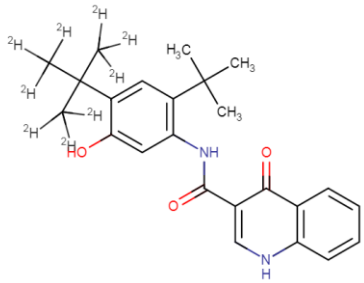


Figure 2: Structure of Deutivacaftor

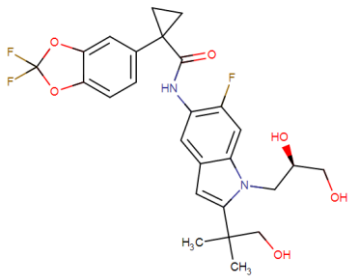


Figure 3: Structure OF Tezacaftor

A QbD is defined as “A systemic approach to the method development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management5.” The QbD approach emphasizes product and process understanding with quality risk management and controls, resulting in higher assurance of product quality, regulatory flexibility, and continual improvement. The QbD method was based on the understanding and implementation of guidelines ICH Q8 Pharmaceutical Development, ICH Q9 Quality Risk Management, and ICH Q10 Pharmaceutical Quality System[13,14,15]. Analytical science is considered to be an integral part of pharmaceutical product development and hence go simultaneously during the entire product life cycle. Analytical QbD defined as a science and risk-based paradigm for analytical method development, endeavouring for understanding the predefined objectives to control the critical method variables affecting the critical method attributes to achieve enhanced method performance, high robustness, ruggedness, and flexibility for continual improvement[16,17]. Extensive literature research has unearthed a multitude of recorded analytical procedures[18-20], including the discovery of more economically efficient ways. Nevertheless, there is currently no documented approach for UPLC approach. Hence, a reliable and cost-effective approach is suggested for assessing the QbD and stability of Vanzacaftor,

Tezacaftor and Deutivacaftor, and their medicinal dose form using RP-UPLC must be validated and developed as per ICH guidelines.

Materials and Methods:

Tezacaftor, Vanzacaftor, Tezacaftor and Deutivacaftor (API), and Alyftrek Combination (vanzacaftor, Tezacaftor, deutivacaftor) tablet, Acetonitrile, Methanol, Ortho Phosphoric Acid, Distilled water. All of the solvents and chemicals were of UPLC quality and obtained from Rankem Chemicals Pvt Ltd.

Instrumentation:

The Method Development and Validation was performed by Acquity UPLC Model equipped with TUV Detector and Empower 2 Software. For QbD Design Expert 13 Software, Analytical weighing Balance, Ultrasonicator, pH Meter, Hot air oven.

Chromatographic Condition:

An Isocratic Elution carried out by using **Acetonitrile and Phosphate Buffer 40:60 v/v** as the Mobile Phase, Diluent used was Combination of Acetonitrile and Water in 1:1 ratio. **HSS C18** (2.1 x 50mm, 1.8µm) column was used to determine the Method at a flow rate of 0.3ml/min, by maintaining the column Temperature at 30^0C. In addition, with an injection volume of 1µL and the wavelength detected at 270nm.

API Formulation

Preparation of Standard stock solutions and Working Solution: Accurately weighed 4mg of Vanzacaftor, 50mg of Deutivacaftor and 20mg of Tezacaftor working Standards into a 50 ml clean dry volumetric flasks separately. 10ml of Diluent was added to flasks and sonicated for 20mins. Flasks were make up with the diluents and labeled as Standard stock solution 1, 2 and 3. (80µg/ml of Vanzacaftor, 1000µg/ml of Deutivacaftor, 400µg/ml of Tezacaftor.) from this sol 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (8µg/ml of Vanzacaftor, 100µg/ml of Deutivacaftor, 40µg/ml of Tezacaftor.)

Sample Formulation

Preparation of Sample stock solutions and Working Solution: 10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to tablet was transferred into a 100ml volumetric flask and until the Tablets are completely dispersed. Equilibrate to room temperature, 25ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by UPLC filters (200µg/ml of Vanzacaftor, 2500µg/ml of Deutivacaftor, 1000µg/ml of Tezacaftor.) From this 0.4ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent to make (8µg/ml of Vanzacaftor, 100µg/ml of Deutivacaftor, 40µg/ml of Tezacaftor).

Method Validation

The established technique is validated in accordance with ICH criteria for the purpose of validating analytical methods. The validation metrics were: system appropriateness, accuracy, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, robustness, specificity, and degradation studies.

System suitability parameters:

The chromatographic analysis was done in accordance with the designed and optimized parameters after the working standard solution was introduced into the UPLC system six times. By computing the % RSD of retention times, theoretical plates, and peak areas from six duplicate injections, the system appropriateness parameters were established.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Precision:

Repeatability (intraday) and intermediate precision (inter-day) were used to assess the developed analytical method's precision. The usage of an analytical process in a laboratory over a brief period of time that was evaluated by assaying the samples on the same day is known as repeatability. Intermediate precision was assessed via comparison of the assays on several days. SD and %RSD were determined.

Linearity:

Standard calibration curves were created with six distinct concentrations including the LOD and LOQ by making repeated volume to volume dilution of stock solution I over the range of 10-60 µg/ml for Tezacaftor, 2-12 µg/ml for Vanzacaftor and 25-150 µg/ml for Deutivacaftor. Linear calibration curves were produced between peak area and medication concentration. The linearity was tested using linear regression, which was calculated by the least square regression method

- 25 µg/mL: Take 0.25 mL of stock solution and dilute to 10 mL
- 50 µg/mL: Take 0.5 mL of stock solution and dilute to 10 mL
- 75 µg/mL: Take 0.75 mL of stock solution and dilute to 10 mL
- 100 µg/mL: Take 1.0 mL of stock solution and dilute to 10 mL
- 125 µg/mL: Take 1.25 mL of stock solution and dilute to 10 mL
- 150 µg/mL: Take 1.5 mL of stock solution and dilute to 10 mL

Accuracy:

Accuracy was performed in triplicate for various concentrations of Vanzacaftor, Tezacaftor and Deutivacaftor equivalent to 50%, 100% and 150% of the standard amount were injected into the UPLC system per the test procedure. Dilution were as follows.

- 50 µg/mL: Take 0.1 mL of stock solution and dilute to 10 mL
- 100 µg/mL: Take 0.2 mL of stock solution and dilute to 10 mL
- 150 µg/mL: Take 0.3 mL of stock solution and dilute to 10 mL

Sensitivity:

Limit of detection and Limit of Quantification

Limits of detection (LOD) and limit of quantitation (LOQ) were established using the signal-to-noise ratio. The detection limit was stated to as the lowest concentration level resulting in a peak area of three times the baseline noise. The lowest concentration level that produced a peak area with a signal-to-noise ratio greater than ten was referred to as the quantitation limit.

Based on the response's standard deviation and calibration curve's slope, the LOD and LOQ can be estimated. The formulae given below can be used to calculate LOD and LOQ:

$$LOD = 3.3\sigma/S$$

$$LOQ = 10\sigma/S$$

where S is calibration curve of the slope and σ is the response of the standard deviation.

Sensitivity Stock solution: Take 0.25 mL of stock solution and dilute to 10 mL

- **LOD:** From above take 0.3 ml solution and dilute to 10 mL
- **LOQ:** From above take 0.9 ml solution and dilute to 10 mL

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guidelines.

Assay:

For the brand (Alyftrek) with label claims of Vanzacaftor (10mg) tezacaftor (50mg) deutivacaftor (125mg), the assay and percentage purity were computed. The value that was determined was compared against that of standard value without interference from the excipients used in the tablet dosage form

Degradation Studies

These investigations are carried out under various stress situations to describe the stability of the pure pharmacological material and are useful in establishing the best storage settings. These research cover base, peroxide, acid, neutral hydrolysis, photo, and heat degradation.

Oxidation:

After adding 1ml of a stock solution to 10ml of a 20% volume fraction of H2O2 and allowing it to sit in an oven at 60°C for 30 minutes, a chromatogram was produced by injecting a 100µg/ml, 40µg/ml and 8µg/ml solution at 10 µl into UPLC

Acid Degradation Studies:

1 ml of hydrochloric acid was added to 10 ml of vf with 1 ml stock and refluxed for 30 minutes at 60 ºC. A 100µg/ml, 40µg/ml and 8µg/ml solution was injected at 10 µl into the system, resulting in the formation of a chromatogram.

Alkali Degradation Studies:

A mixture of 1 ml of stock and 1 ml of NaOH in 10 ml of vf was refluxed for 30 minutes at 60°C. A 100µg/ml, 40µg/ml and 8µg/ml solution was injected at 10 µl into UPLC, resulting in the production of a chromatogram.

Dry Heat Degradation Studies:

The stock solution was allowed to undergo thermal deterioration in an oven set at 105oC for 6 hours. Subsequently, a chromatogram was prepared by injecting a 100µg/ml, 40µg/ml and 8µg/ml solution at 10 µl into UPLC.

Photo Stability studies:

The stock underwent degradation by exposure to UV radiation in the laboratory for a duration of 7 days. Upon injecting a 100µg/ml, 40µg/ml and 8µg/ml solution at a volume of 10 µl into UPLC, a chromatogram was developed.

Neutral Degradation Studies:

After refluxing the stock for 6 hours at 60 degrees Celsius, a chromatogram was prepared by injecting a 100µg/ml, 40µg/ml and 8µg/ml solution at 10 µl into UPLC.

RESULT AND DISCUSSION

Parameter Selection

Various preliminary UPLC trials were carried out for selection of Column and organic modifier. The choice of C18 column based on the preliminary investigation was done using **HSS C18 (50×2.1 mm, 1.8µm),** Selection of a suitable organic modifier is also important to get better selectivity with adequate separation of all analytes. Commonly used organic solvents for the reversed phase HPLC include Acetonitrile and Methanol, from that trials acetonitrile showed to be an ideal and suitable organic modifier compared to Methanol, because Vanzacaftor, tezacaftor, and deutivacaftor. was solubilized in acetonitrile compare to methanol. Therefore, Acetonitrile was selected and finalized as the organic modifier for further optimization study.

Optimization of method

The method was optimized via Central composite design. The earliest trials are needed to optimize the final approach. It was necessary to tune the organic concentration, flow rate, and column temperature. In order to maximize these characteristics, which were adjusted over three levels (high, mid, and low), central composite design was employed. different ranges of parameters ranging from **31.59-48.41% Aqueous Phase**, **temperature 24.95 ^{0}C – 35.05 ^{0}C** and **0.24-0.35ml/min flow rate** respectively were taken and counter and 3D surface plot showing the effect of each parameter on Retention Time, Theoretical plates and Resolution (CQA) were generated. A desirability function used to the optimal settings to estimate retention period, asymmetry, theoretical plates

Table 1 Optimized Condition

Parameter	*Condition*
Mobile phase	*Acetonitrile: 0.1% OPA(40:60 v/v)*
Flow rate	*1 ml/min*
Column	*HSS C18 (2.1 x 50mm, 1.8µm)*
Detector wave length	*270nm*
Column temperature	*30°C*
Injection volume	*2 µL*
Diluent	*Water and Acetonitrile in the ratio 50:50*

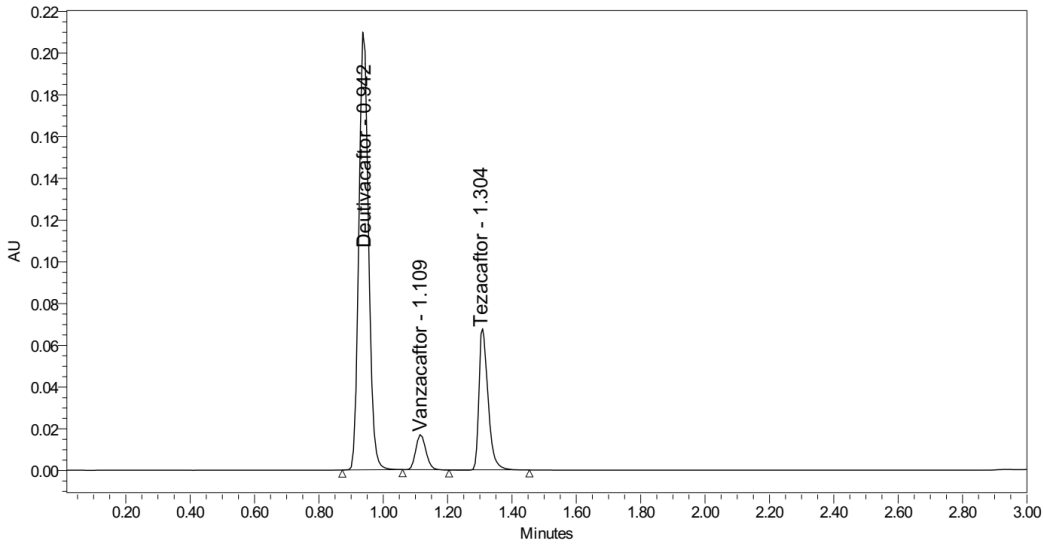


Figure 4: Optimized Chromatogram

Design summary of CCD

Design Summary					
File version: DX 13.0.0 ***Study Type: Response surface*** ***Design Type: central composite design*** ***Design Model: Quadratic***				***ATP: Robustness*** ***CQA: Retention time, Theoretical plates and Tailing factor*** ***Runs: 20***	
CMPs	***Unit***	*Type*	*Subtype*	***Min.***	***Max.***
Aqueous Phase	***%***	*Numeric*	*Continuous*	***31.59 %***	***48.41 %***
Flow rate	***ml/min***	*Numeric*	*Continuous*	***0.24 ml/min***	***0.35 ml/min***
Temp	***^{0}C***	*Numeric*	*Continuous*	***24.95 ^{0}C***	***35.05 ^{0}C***

Factors

Factor	*Name*	*Level*	*Low Level*	*High Level*	*Std. Dev.*	*Coding*
A	*FR*	*0.30*	*0.270*	*0.330*	*0.0000*	*Actual*
B	*MP*	*40.00*	*35.00*	*45.00*	*0.0000*	*Actual*
C	*Temp*	*30.00*	*27.00*	*33.00*	*0.0000*	*Actual*

The Responses of Trial

		Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4	Response 5	Response 6	Response 7	Response 8
Std	**Run**	**A:FR**	**B:MP (Organic phase)**	**C:Temp**	**RT1**	**RT2**	**RT3**	**RS1**	**RS2**	**NTP1**	**NTP2**	**NTP3**
		ml/min	%	0 C	min	min	num	min	num	min	num	num
1	7	0.27	35	27	1.411	1.764	2.118	3.5	3.6	9589	11158	12531
2	4	0.33	35	27	0.908	1.158	1.402	3.2	3.4	8467	10036	11282
3	20	0.27	45	27	1.29	1.52	1.769	2.8	3.3	9255	10824	12207
4	2	0.33	45	27	0.812	0.965	1.124	2.5	3	8205	9774	10997
5	8	0.27	35	33	1.13	1.43	1.72	3.3	3.4	8938	10507	11936
6	10	0.33	35	33	0.723	0.938	1.14	3	3.1	7984	9553	10722
7	12	0.27	45	33	1.037	1.231	1.43	2.7	3.1	8563	10132	11667
8	15	0.33	45	33	0.638	0.765	0.89	2.4	2.7	7707	9276	10445
9	9	0.24955	40	30	1.382	1.667	1.98	3.1	3.6	9224	10793	12530
10	14	0.35045	40	30	0.634	0.792	0.954	2.6	3.1	7675	9244	10439
11	6	0.3	31.591	30	1.065	1.404	1.685	3.6	3.4	8960	10529	11709
12	17	0.3	48.409	30	0.913	1.057	1.2	2.5	2.8	8504	10073	11175
13	1	0.3	40	24.9546	1.19	1.442	1.72	3	3.4	9013	10582	11925
14	5	0.3	40	35.0454	0.755	0.933	1.125	2.7	3	7855	9424	11002
15	19	0.3	40	30	0.951	1.158	1.381	2.8	3.3	8270	9839	11523
16	11	0.3	40	30	0.953	1.165	1.395	2.8	3.3	8378	9947	11491
17	13	0.3	40	30	0.954	1.166	1.397	2.9	3.3	8377	9946	11487
18	18	0.3	40	30	0.964	1.175	1.405	2.8	3.3	8463	10032	11496
19	3	0.3	40	30	0.966	1.178	1.406	2.8	3.3	8349	9918	11469
20	16	0.3	40	30	0.967	1.18	1.407	2.8	3.2	8308	9877	11500

Final Responses

Response	Name	Units	Observations	Minimum	Maximum	Mean	Std. Dev.	Ratio
R1	RT1	min	20.00	0.634	1.411	0.9821	0.2194	2.23
R2	RT2	min	20.00	0.765	1.764	1.20	0.2686	2.31
R3	RT3	min	20.00	0.89	2.118	1.43	0.3222	2.38
R4	RS1	num	20.00	2.4	3.6	2.89	0.3210	1.50
R5	RS2	num	20.00	2.7	3.6	3.23	0.2342	1.33
R6	NTP1	num	20.00	7675	9589	8504.20	521.85	1.25
R7	NTP2	num	20.00	9244	11158	10073.20	521.85	1.21
R8	NTP3	num	20.00	10439	12531	11476.65	587.37	1.20

Factor Coding: Actual

All Responses

Design Points

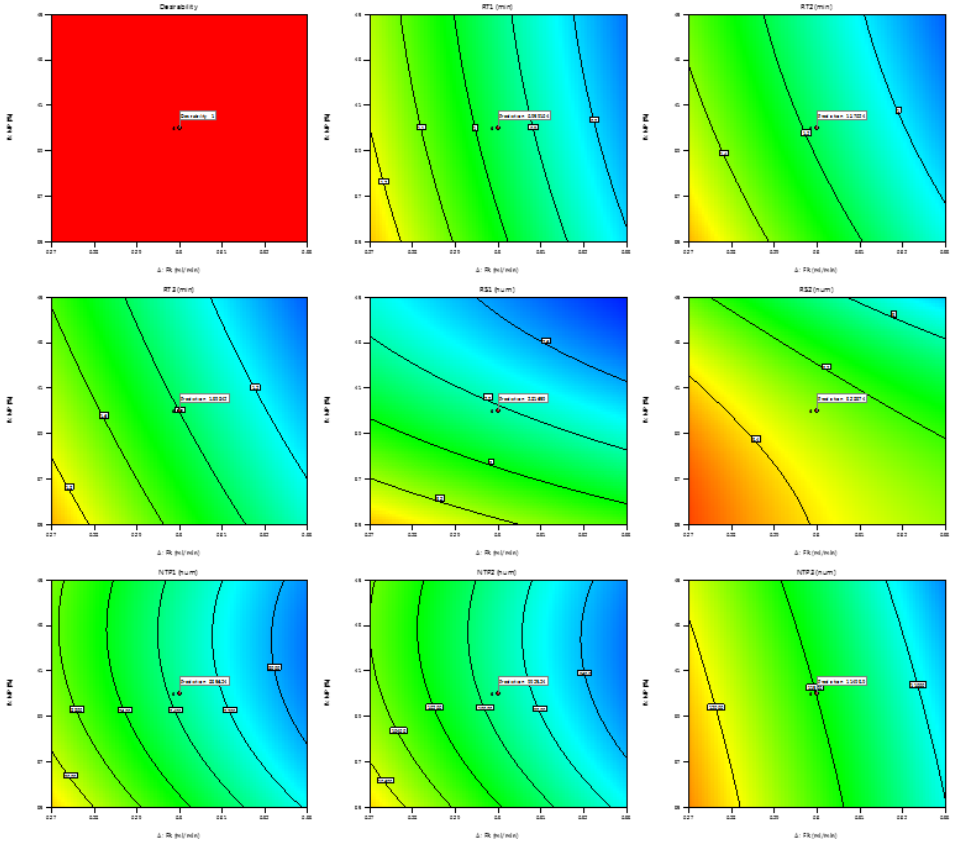
0 1

X1 = A

X2 = B

Actual Factor

C = 30



Factor Coding: Actual

All Responses

Design Points

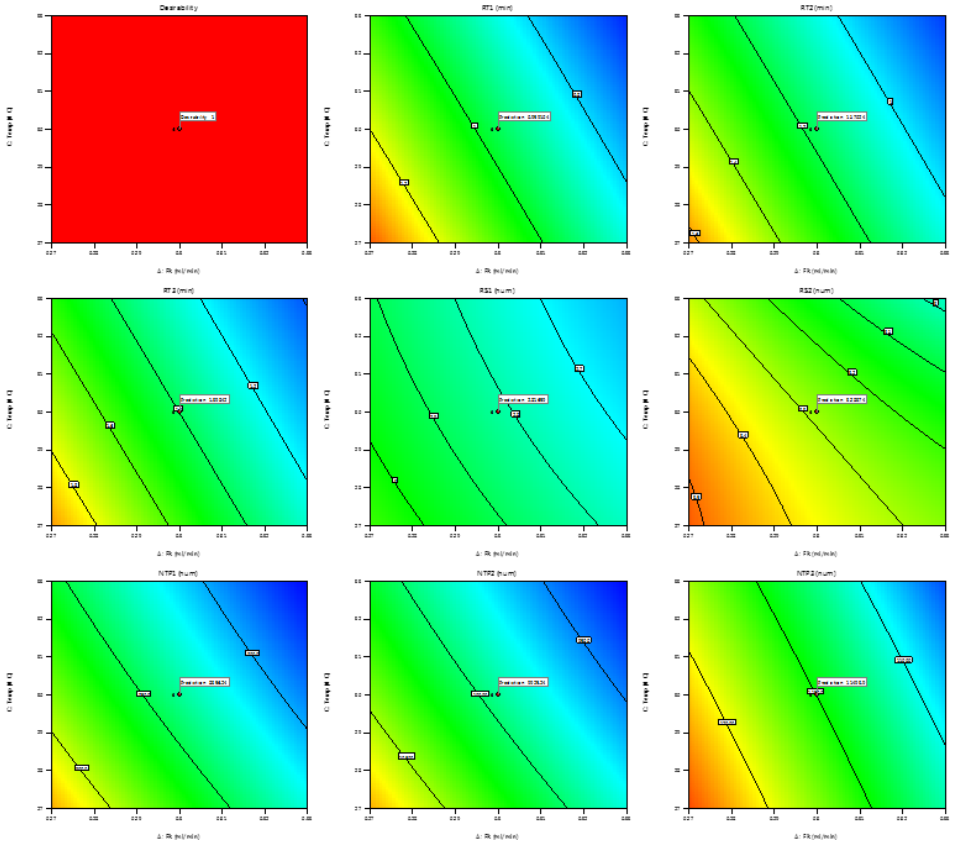
0 1

X1 = A

X2 = C

Actual Factor

B = 40



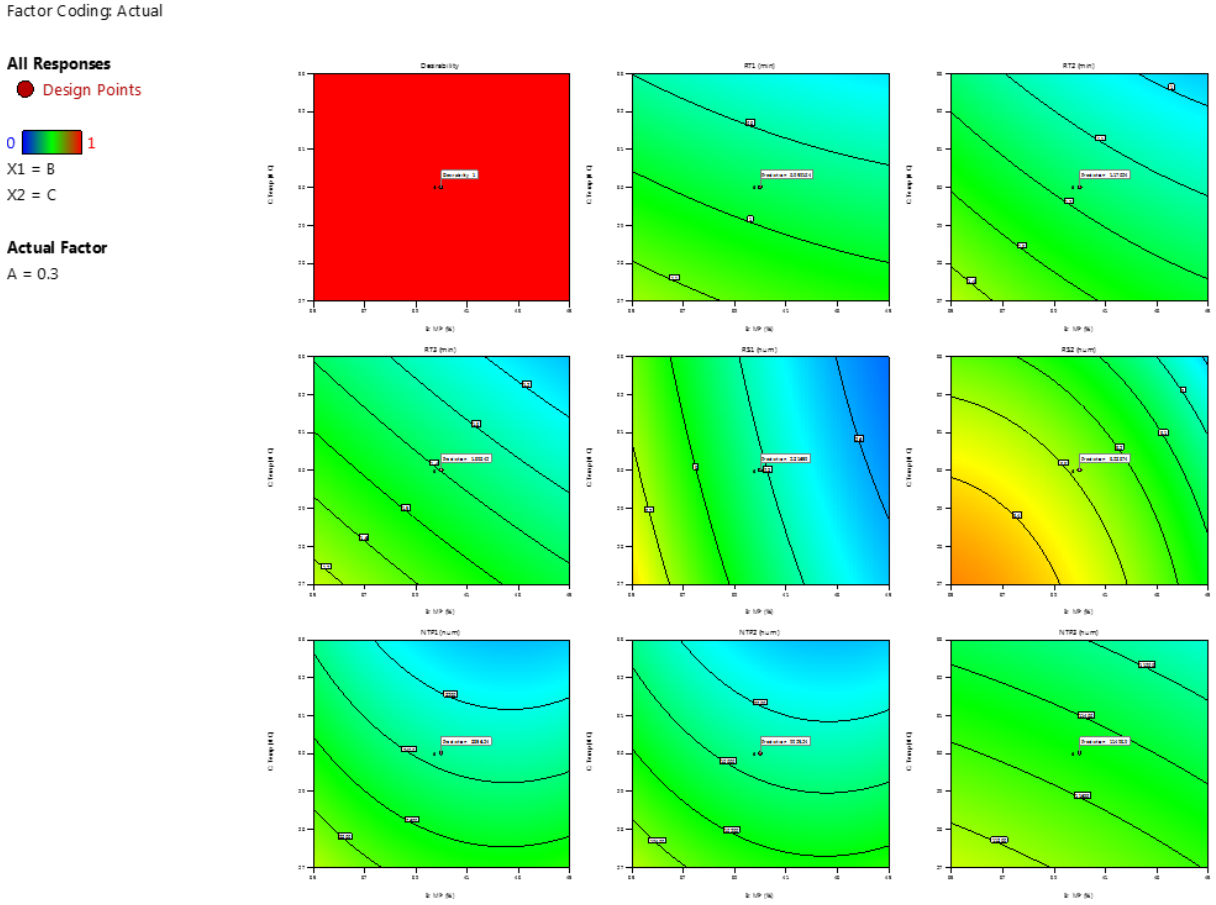


Figure 5: Multi Contour plots of all Response

Validation

System Suitability:

Table 2: System Suitability

	Deutivacaftor			
Injection	***RT***	***area***	***Plate Count***	***Tailing***
Injection-1	*0.945*	*1023793*	*8342*	*1.42*
Injection-2	*0.945*	*1033449*	*8365*	*1.41*
Injection-3	*0.946*	*1039072*	*8361*	*1.42*
Injection-4	*0.948*	*1044422*	*8359*	*1.43*
Injection-5	*0.951*	*1040849*	*8355*	*1.43*
Injection-6	*0.953*	*1033423*	*8358*	*1.43*
Mean		*1035835*		
Std ev		*7292.1*		
RSD		*0.7*		

Vanzacaftor					
Injection	***RT***	***area***	***Plate Count***	***Tailing***	***Resolution***
Injection-1	*1.118*	*126336*	*9975*	*1.22*	*2.7*
Injection-2	*1.120*	*125748*	*9983*	*1.23*	*2.8*
Injection-3	*1.120*	*124864*	*9980*	*1.22*	*2.7*
Injection-4	*1.123*	*125853*	*9978*	*1.23*	*2.8*
Injection-5	*1.128*	*126022*	*9985*	*1.22*	*2.8*
Injection-6	*1.130*	*125023*	*9973*	*1.24*	*2.8*
Mean		*125641*			
Std ev		*578.0*			
RSD		*0.5*			

Vanzacaftor					
Injection	**RT**	**area**	**Plate Count**	**Tailing**	**Resolution**
Injection-1	1.340	407512	11463	1.35	3.2
Injection-2	1.343	406615	11488	1.33	3.2
Injection-3	1.345	409070	11479	1.35	3.2
Injection-4	1.346	407774	11496	1.32	3.2
Injection-5	1.351	410193	11497	1.34	3.2
Injection-6	1.354	407970	11477	1.33	3.2
Mean		408189			
Std ev		1260.8			
RSD		0.3			

Six injections of the standard solution were performed, and the chromatograms that corresponded to each injection were acquired. Observations showed that the percent RSD was less than 2%, the USP tailing was less than 2, and the theoretical plate count surpassed 2,000. Every condition for system appropriateness was satisfied and falls within permissible bounds.

Linearity:

Six concentrations ranging were prepared and linearity was estimated in a duplicate manner. For the calibration curve over the concentration range, the data have shown a good correlation.

Table 3: Linearity Data

Deutivacaftor		*Tezacaftor*		*Vanzacaftor*	
Concentration (ppm)	****Peak area***	***Concentration (ppm)***	****Peak area***	***Concentration (ppm)***	****Peak area***
0	*0*	***0***	*0*	***0***	*0*
25	*259849*	*10*	*101469*	*2*	*30644*
50	*515051*	*20*	*203572*	*4*	*60901*
75	*777117*	*30*	*302712*	*6*	*90884*
100	*1035351*	*40*	*408325*	*8*	*125623*
125	*1293737*	*50*	*505387*	*10*	*155786*
150	*1537400*	*60*	*601268*	*12*	*181158*
y:	*10286x + 2613.8*	***y:***	*10059x + 1419.2*	***y:***	*15330x + 162.2*
R^2	*0.999*	***R^2***	*0.999*	***R^2***	*0.999*
Slope	*10286*	***Slope***	*10059*	***Slope***	*15341*
Intercept	*2613.8*	***Intercept***	*1419.2*	***Intercept***	*151.48*
LOD	*0.16 µg/ml*	***LOD***	*0.06 µg/ml*	***LOD***	*0.03 µg/ml*
LOQ	*0.48 µg/ml*	***LOQ***	*0.17 µg/ml*	***LOQ***	*0.08 µg/ml*

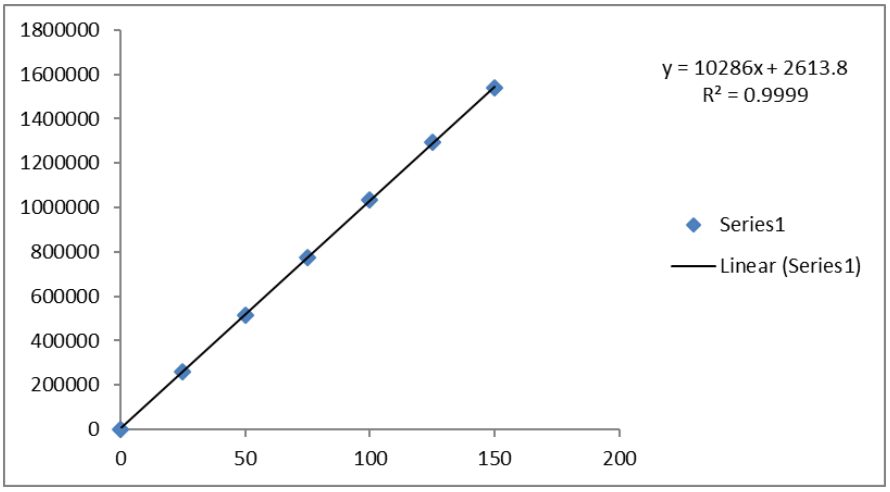


Figure 6: Calibration Curve of Deutivacaftor

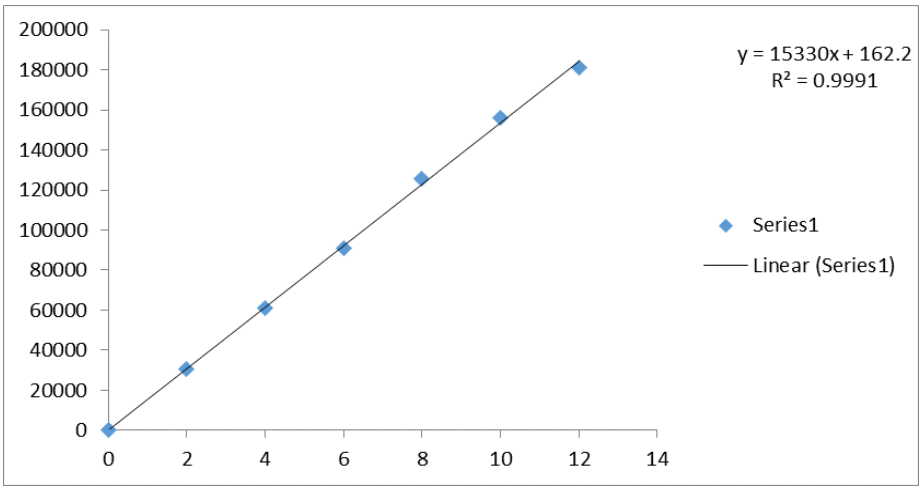


Figure 7: Calibration Curve of Vanzacaftor

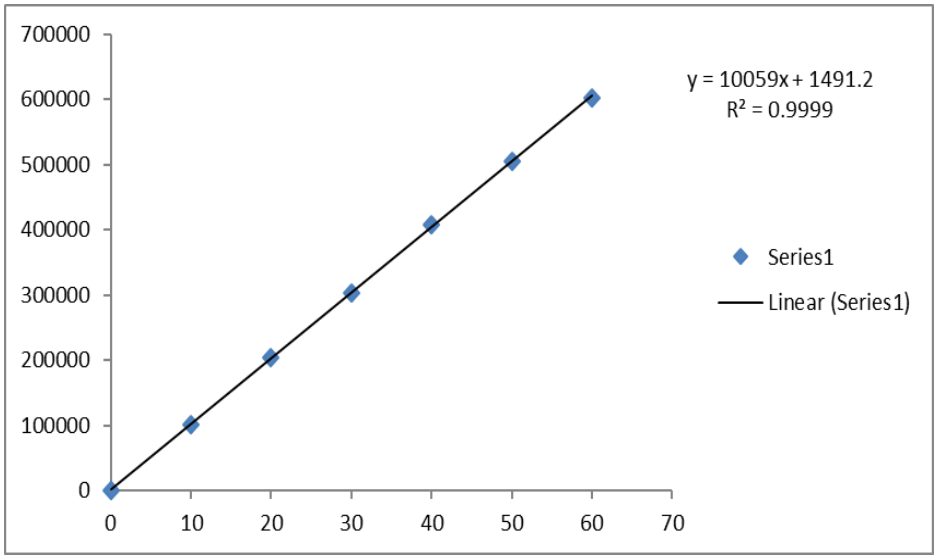


Figure 8: Calibration Curve of Tezacaftor

Accuracy:
Three doses were given at each level, and the mean % recovery was calculated. The recovery rate was observed to be between 99% and 100.%, which is within the acceptable ranges

Table 4: Accuracy Data

	Deutivacaftor			Tezacaftor			Vanzacaftor		
% Level	Amount Spiked	Amount recovered	% Recovery	Amount Spiked	Amount recovered	% Recovery	Amount Spiked	Amount recovered	% Recovery
50%	50	49.62	99.25	20	19.86	99.31	4	3.98	99.58
		49.65	99.29		19.85	99.25		3.98	99.73
		49.59	99.18		19.86	99.30		3.98	99.61
100%	100	99.17	99.17	40	39.64	99.10	8	8.02	100.31
		99.20	99.20		39.64	99.11		8.04	100.56
		99.30	99.30		39.63	99.09		7.96	99.55
150%	150	149.5	99.64	60	60.38	100.64	12	12.12	101.01
		148.9	99.24		60.51	100.85		12.07	100.61
		148.8	99.22		60.48	100.80		11.99	99.89
% recovery		99.28			99.72%			100.10	

Precision:

Table 5: Precision Data

	Deutivacaftor		***Tezacaftor***	
S. No	***Day 1***	***Day 2***	***Day 1***	***Day 2***
Injection-1	*1028806*	*987120*	*408105*	*407915*
Injection-2	*1041091*	*997672*	*408201*	*403213*
Injection-3	*1037250*	*987193*	*407891*	*404070*
Injection-4	*1034008*	*998063*	*406732*	*410172*
Injection-5	*1038680*	*999802*	*407804*	*406942*
Injection-6	*1040020*	*996095*	*407970*	*403914*
Mean	*1036643*	*994324*	*407784*	*406038*
S.D	*4562.2*	*5676.0*	*534.8*	*2749.4*
%RSD	*0.4*	*0.6*	*0.1*	*0.7*

	Vanzacaftor	
S. No	***Day 1***	***Day 2***
Injection-1	*125720*	*123845*
Injection-2	*125135*	*123776*
Injection-3	*125036*	*125512*
Injection-4	*126130*	*123623*
Injection-5	*125515*	*124531*
Injection-6	*124813*	*124716*
Mean	*125392*	*124334*
S.D	*489.0*	*725.0*
%RSD	*0.4*	*0.6*

Robustness:

Table 6: Robustness data for Deutivacaftor, Tezacaftor and Vanzacaftor

Parameter	Optimized condition	Used condition	Deutivacaftor	Tezacaftor	Vanzacaftor
			Obtained %RSD		
Flow rate (±0.1ml/min)	**0.3ml/min**	0.2ml/min	0.2	0.4	1.0
		0.4 ml/min	0.9	0.9	0.7
MP (5%v/v)	**60:40**	65:35	0.6	0.3	0.4
		55:45	0.9	0.7	0.2
Column temp. (±3^{0}c)	**30^{0}c**	27 ^{0}C	0.7	0.9	0.4
		33 ^{0}C	0.9	0.2	0.4

Assay

Table 7: % Assay Purity Data

Formulation	*Label claim(mg)*	*% Assay**
Alyftrek	***Deutivacaftor 125 mg***	***99.98 %w/w***
	Vanzacaftor 10mg	***99.60 %w/w***
	Tezacaftor 50mg	***99.80 %w/w***

Degradation studies:

Table 8: Force Degradation Studies of Deutivacaftor, Vanzacaftor and Tezacaftor

		Deutivacaftor	*Vanzacaftor*	*Tezacaftor*	
S.No	***Stress Conditions***	***% Degradation***	***% Degradation***	***% Degradation***	***Peak Purity***
1	*Acid*	*3.92*	*6.26*	*6.34*	*Passes*
2	*Base*	*4.89*	*6.17*	*6.60*	*Passes*
3	*Oxidation*	*4.88*	*4.80*	*6.20*	*Passes*
4	*Thermal*	*2.33*	*2.55*	*3.04*	*Passes*
5	*Photolytic*	*1.49*	*1.75*	*1.87*	*Passes*
6	*Hydrolytic*	*0.54*	*0.98*	*0.91*	*Passes*

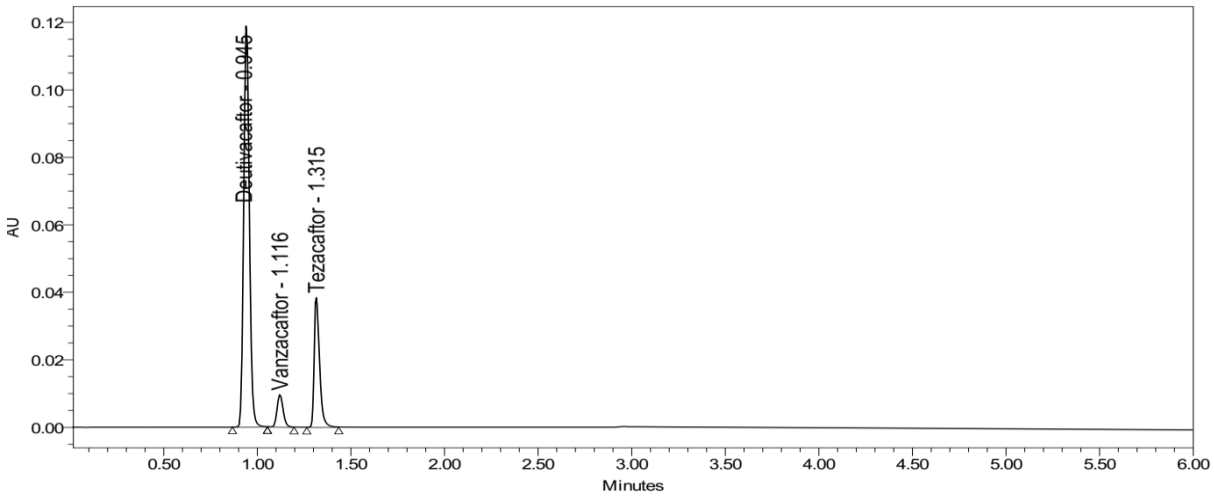


Figure 9: Acid Chromatogram

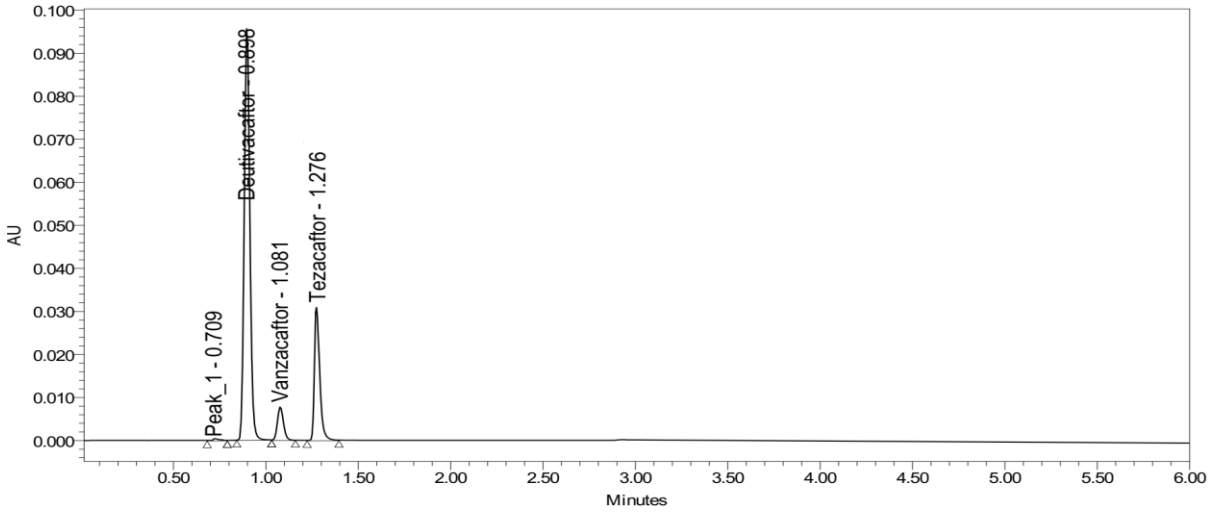


Figure 10: Base Chromatogram

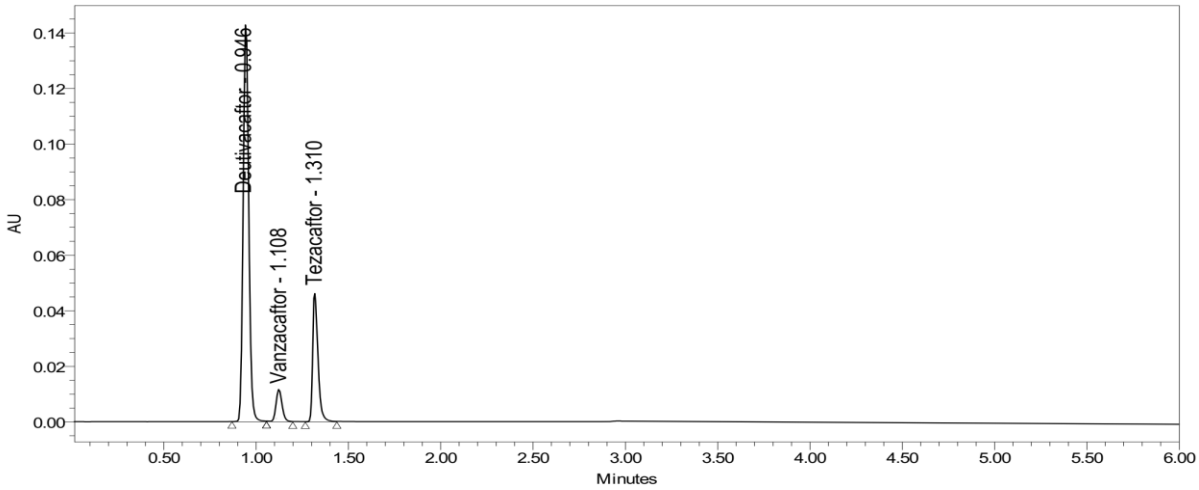


Figure 11: Oxidative Chromatogram

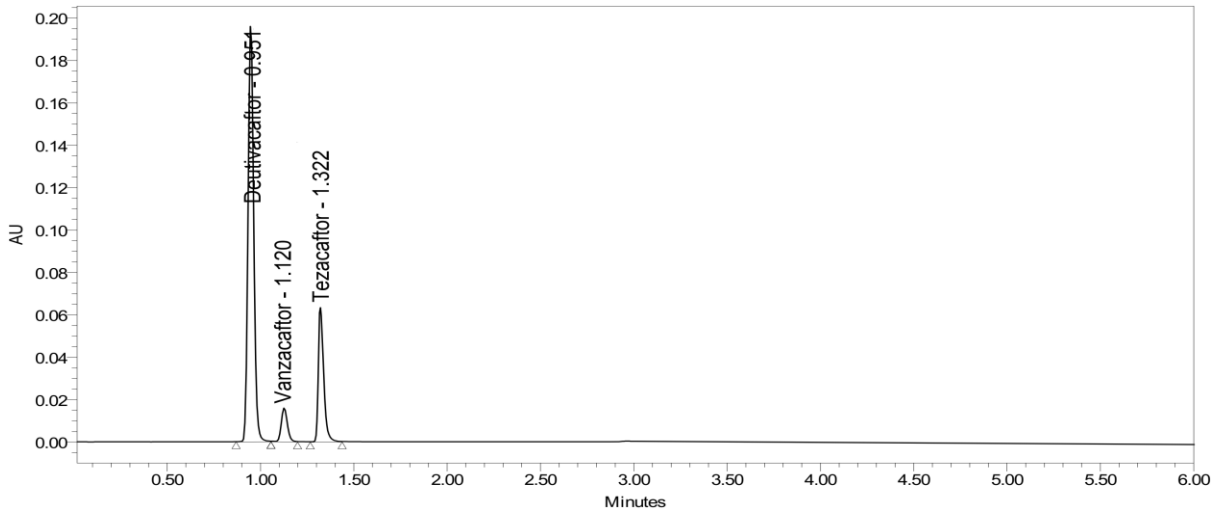


Figure 12: Thermal Chromatogram

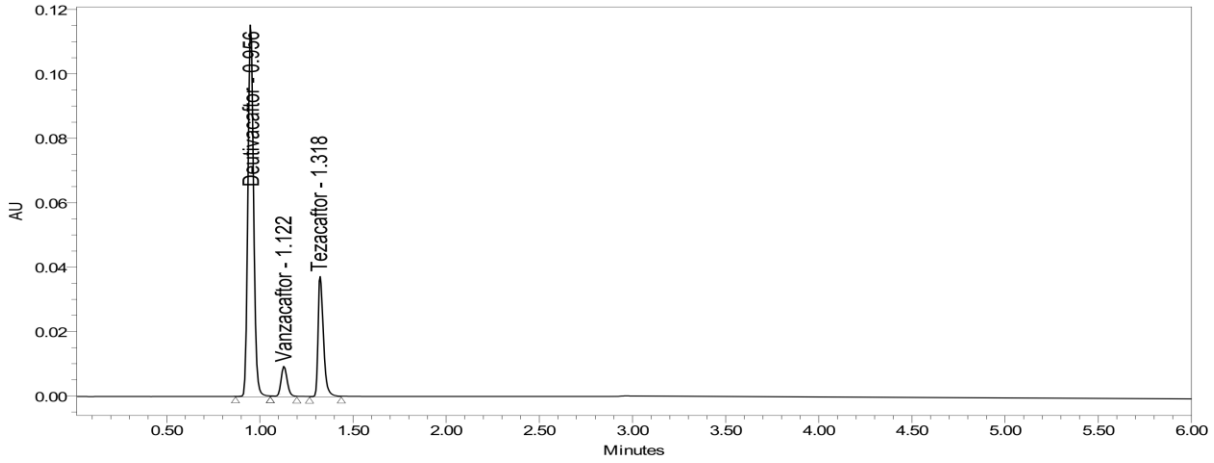


Figure 13: Photolytic Chromatogram

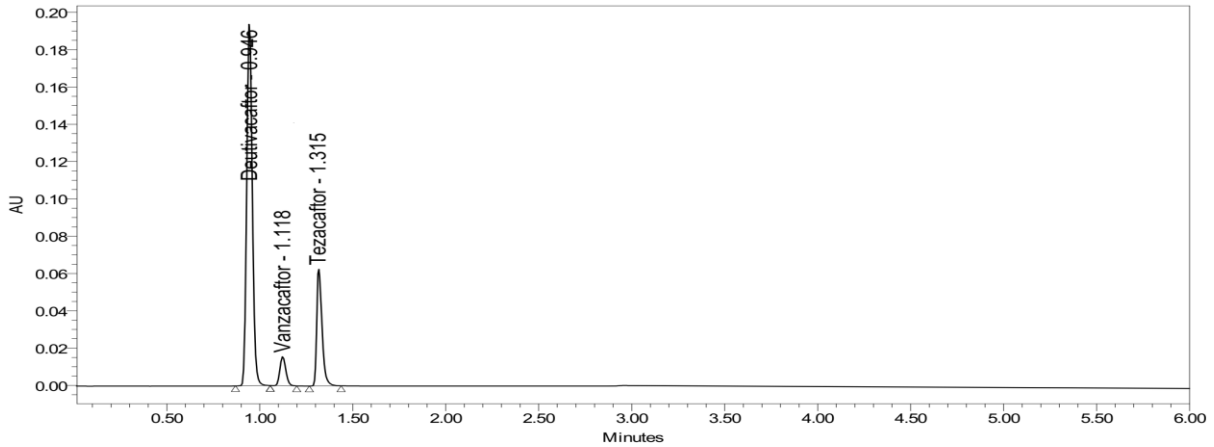


Figure 14: Water Chromatogram

CONCLUSION:

The present study was aimed at developing a sensitive, precise and accurate stability indicating UPLC method for the analysis of Vanzacaftor, Tezacaftor and Deutivacaftor in bulk drug and pharmaceutical dosage forms by using QbD approach using Design Expert® software. The Central composite design experimental design describes the interrelationships of mobile phase and pH at three different level and responses to be observed were retention time, theoretical plates, and peak asymmetry with the help of the Design Expert 13.0.5.0 version. The QbD approach to analytical method development was used for better understanding of method variables with different levels. it is a faster way of developing method, which helps choosing much better method condition during the development process through design space. The validation results confirmed the usefulness of the method. The method employed a HSS column, and chromatographic separation was achieved with a retention time of Deutivacaftor, Vanzacaftor and Tezacaftor as 0.942, 1.109 and 1.304 minutes respectively, highlighting the efficiency of the developed HPLC method. Excellent linearity was observed in the concentration range of 25–150 µg/mL for Deutivacaftor, 10–60 µg/mL for Tezacaftor and 2–12 µg/mL Vanzacaftor, with the regression equation $y = 10286x + 2613.8$, $y = 10059x + 1491.2$ and $y = 15341x + 151.48$, indicating a strong correlation and method sensitivity respectively.

In conclusion, The proposed UPLC method was also applied for the forced degradation studies on Vanzacaftor, Tezacaftor and Deutivacaftor under a variety of conditions like acid and base hydrolysis, oxidation, and heat and photo stability. The drugs were found to be stable except in acidic, Basic, Peroxide stress conditions. The drug peaks in these degradations were found to be homogenous No major degradants were found in Neutral stress, photo stability and Thermal degradation studies. As the developed method could effectively separate Vanzacaftor, Tezacaftor and Deutivacaftor from the degradants, it can be employed as a stability indicating assay. System suitability testing was performed prior to the study of each validation parameter and the verified parameters like tailing factor (< 2.0), resolution (> 2.0), column efficiency (> 2000) and repeatability (% RSD <

2) ensured that the equipment, electronics, and analytical operations for the samples analysed could be constituted as an integral system that can be evaluated as a whole.

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REFERENCES:

1. National Heart, Lung and Blood Institute: Cystic Fibrosis
2. Abdulghani Sankari; Sandeep Sharma: Cystic Fibrosis, StatPearls.
3. https://www.alyftrek.com
4. Rowe SM, Verkman AS: Cystic fibrosis transmembrane regulator correctors and potentiators. Cold Spring Harb Perspect Med. 2013 Jul 1;3(7). pii: 3/7/a009761
5. FDA Approved Drug Products: ALYFTREK (vanzacaftor, tezacaftor, and deutivacaftor tablets), for oral use
6. Drugbank: Vanzacaftor
7. Drugbank: Tezacaftor
8. Donaldson SH, Pilewski JM, Griese M, Cooke J, Viswanathan L, Tullis E, Davies JC, Lekstrom-Himes JA, Wang LT: Tezacaftor/Ivacaftor in Subjects with Cystic Fibrosis and F508del/F508del-CFTR or F508del/G551D-CFTR. Am J Respir Crit Care Med. 2018 Jan 15;197(2):214-224.
9. Rowe SM, Verkman AS: Cystic fibrosis transmembrane regulator correctors and potentiators. Cold Spring Harb Perspect Med. 2013 Jul 1;3(7).
10. Ratjen F, Bell SC, Rowe SM, Goss CH, Quittner AL, Bush A: Cystic fibrosis. Nat Rev Dis Primers. 2015 May 14;1:15010.
11. Drugbank: Deutivacaftor
12. Faria Munir: Treating Cystic Fibrosis With Alyftrek,
13. The International Conference on Harmonisation ICH Technical Requirements for Registration of Pharmaceuticals for Human Use on Pharmaceutical Development Q8(R2) (2009) https://database.ich.org/sites/default/files/Q8%28R2%29%20Guideline.pdf
14. The International Conference on Harmonisation ICH Technical Requirements for Registration of Pharmaceuticals for Human Use on Quality Risk Management Q9 (2005) https://database.ich.org/sites/default/files/Q9%20Guideline.pdf
15. The International Conference on Harmonisation ICH Technical Requirements for Registration of Pharmaceuticals for Human Use on Pharmaceutical Quality System Q10 (2008) https://database.ich.org/sites/default/files/Q10%20Guideline.pdf
16. Borman P, Nethercote P, Chatfield M, Thompson D, Truman K (2007) The application of quality by design to analytical methods. Pharm Tech 31:142–152
17. Schweitzer M, Pohl M, Hanna BM, Nethercote P, Borman P, Hansen G, Smith K, Larew J (2010) Implications and opportunities of applying QbD principles to analytical measurements. Pharm Tech 34:52–59.
18. K Dhanalakshmi, M .Guruva Reddy, Stability Indicating Method Development and Validation for Simultaneous Estimation of Tezacaftor and Ivacaftor and Elexacaftor in Bulk and Pharmaceutical Dosage Form by HPLC, Ijppr.Human, 2020; Vol. 19 (4): 651-67
19. Bachanaboina Shivaradha, Satla Shobha Rani, RP- HPLC method development and validation for the simultaneous determination of elexacaftor, ivacator and tezacaftor in pharmaceutical dosage forms, World J Pharm Sci 2022; 10(10): 12-21;
20. Madhuri Donakonda et al., A rapid RP- HPLC method for the simultaneous estimation of Ivacaftor and Tezacaftor and in silico study of their metabolitic products, Future Journal of Pharmaceutical Sciences, 7, 118 (2021).