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A Quality by Design Approach to Estimate Cabotegravir and Rilpivrine Simultaneously in Pharmaceutical Dosage Form and API Using RP-UPLC

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Abstract: A straightforward, accurate, and precise approach was created to estimate the amounts of Rilpivirine and Cabotegravir in pharmaceutical dosage form and bulk. Using the central composite model in Quality by Design, Design Expert software 11.0.0 version, chromatographic conditions were optimised. HSS100 x 2.1 mm, 2 m column was used to run the chromatogram. The mobile phase, which included 0.01N phosphate buffer, acetonitrile was injected through the column at a ratio of 55 : 45 (%v/v) was forced through the column at a 0.31 ml/min flow rate. A constant 30°C temperature was maintained. The chosen optimised wavelength was ACQUITYT UV 260.0 nm. The retention times for Rilpivirine and Cabotegravir were determined to be 1.109 and 0.899 minutes, respectively. The percentage RSD for both substances was found to be 1.5 and 0.8. The recovery rates were 100.23 for Rilpivirine and 99.77% for Cabotegravir. Regression equations for both Cabotegravir and Rilpivirine yielded LOD and LOQ values of 0.32, 0.98, 0.67, and 2.04, respectively. The equation for Cabotegravir is y = 3108.8x + 661.44, whereas that for Rilpivirine is = 5694.3x + 1309.5. The method that was created was easy to use and cost-effective, making it suitable for routine quality control testing in industries. Both the retention times and the run time were reduced.

Key Words: Cabotegravir, Rilpivirine, QbD Approach, Method development, RP-UPLC

1. Introduction:

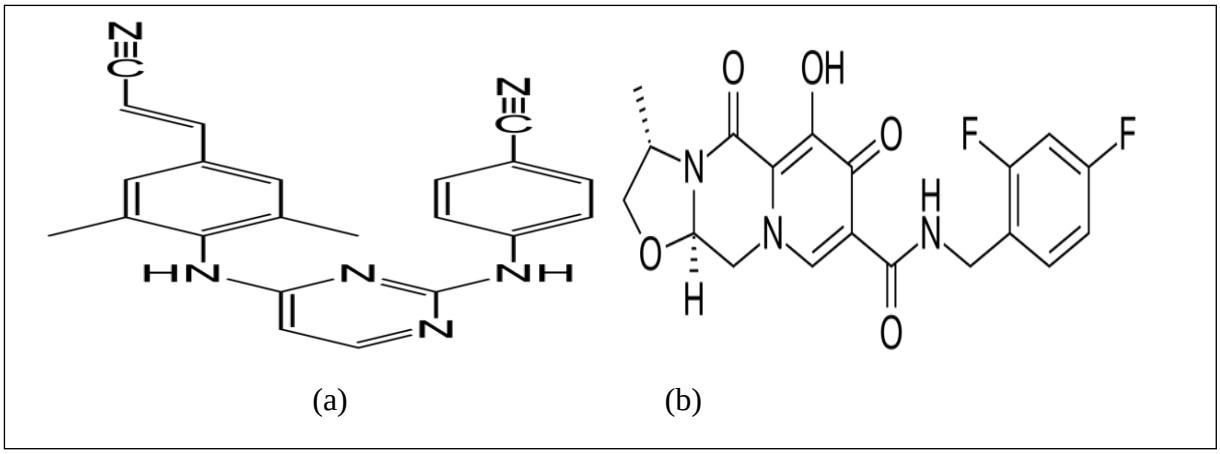
Integrase inhibitors are a relatively new family of HIV medications that work by stopping the virus from integrating its DNA into the genome of the host. Integrase specifically attaches to viral DNA and connects it with host DNA. Integrase may create covalent connections with DNA thanks to the divalent cations in its catalytic centre. Cellular repair processes then take place, sealing the viral DNA into the chromosome. Inhibitors of integrase stop covalent connections from forming with host DNA. HIV cannot enter the host DNA as a result (1). Reverse transcriptase inhibitors that do not contain nucleosides (NNRTIs) are regarded as non-competitive inhibitors that modify the structure of reverse transcriptase (RT) and significantly reduce catalysis. Secondly. A type of antiviral medication called capegravir prevents the human immunodeficiency virus (HIV) from integrating into and is used to treat acquired immunodeficiency syndrome (AIDS) and HIV infection in conjunction with rilpivirine, a non-nucleoside HIV reverse transcription inhibitor (3).

Chemically, cabotegravir is referred to as N-((2,4-Difluorophenyl) methyl).Three-methyl-5,6-hydroxySeven-dioxo-2,3,5,7,11,11a-hexahydro (1,3) pyrido(1,2-d) pyrazine-8-carboxamide (C19H17F2N3O5), 405.358 g·mol−1 (4). Chemically, rilpivirine is known as 4-{[4-(~4-[(E)-2-cyanovinyl]Aminobenzonitrile (C22H18N6), 366.428 g·mol−1, -2,6-dimethylphenylamino)pyrimidin-2-yl (5).

According to current trends, the International Conference on Harmonisation (ICH) recommends using design-based experiments to apply quality in the development of analytical methods and pharmaceutical products. The process of optimising the UPLC method is highly complex since different independent variable parameters, including buffer strength, mobile phase pH, flow rate, detection wavelength, and others, affect separation and other performance criteria. Any significant interactions between these independent factors could result in the inability to the values of the other variables involved in the method optimisation may be related to the impact of one variable on the result in the univariate procedure. Since there is a reduction in the number of tests, chemometric technique has emerged as a novel and superior concept for optimising the RP-HPLC method compared to the previous strategy based on erratic trial and error methodologies. The methodology of experimental design illustrates the correlation between the chromatographic parameters and the sensitivity of the independent variables, which is a crucial factor in determining the method's quality (6).

A literature review notes that a number of techniques, including LC-MS [22, 24], UPLC [17, 24], HPLC [7-16, 18-21, 23], and bioanalytical, have been developed for the simultaneous detection of Rilpivirine and Cabotegravir in pharmaceutical formulations and biological fluids.

To the best of our knowledge, there has never been a way for developing and validating a Quality by Design approach for these medications. Therefore, the authors' primary goal is to create an RPUPLC technique using the Quality by Design Approach that has statistically optimised chromatographic parameters and the simplest mobile phase possible, and to validate it in accordance with ICH criteria [25].



(a) (b)

MATERIALS AND METHOD

Table: 1 Chemicals and reagents

S.No.	Chemicals and Reagents	Make
1.	Pure Cabotegravir and Rilpivirine	Spectrum pharma lab (Hyderabad)
2.	Hydrochloric acid AR grade (HCL)	Rankem, India
3.	sodium hydroxide AR grade (NAOH)	Rankem, India
4.	Hydrogen Peroxide (H_2O_2)	Qauligens
5.	Acetic acid AR grade	Fisher scientific, India and S.D. Fine chem Ltd
6.	Potassium dihydrogen orthophosphate	S.D. Fine chem Ltd and Merck India Pvt Ltd.
7.	orthophosphoric acid	S.D. Fine chem Ltd and Merck India Pvt Ltd.
8.	UPLC grade Acetonitrile (ACN)	Fischer scientific
9.	UPLC grade methanol (MeOH)	Fischer scientific
10.	UPLC grade water	Merck milli-Q

Table: 2 Instrumentation

S.No.	Apparatus and Equipment	Make
1.	Acquity UPLC SYSTEM	Equipped with Binary pumps, ACQUITY UPLC Tunable UV (TUV) and Auto sampler integrated with Empower 2 Software
2.	UV-VIS spectrophotometer	PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for

1.36 grammes of potassium dihydrogen orthophosphate were put to a 1000 millilitre volumetric flask along with roughly 900 millilitres of milli-Q water. The mixture was then allowed to degas and sonicate, and the volume was eventually made up with water. A solution

of dilute orthophosphoric acid was then used to adjust the pH to 4.0. As a diluent, a 50:50 v/v solution of water and acetonitrile was employed.

In order to prepare the stock solutions, 40 mg of CAB and 60 mg of RIL were added to a 100 ml clean, dry volumetric flask along with 50 ml of diluent. The flask was then sonicated for 20 minutes, and diluents (400 µg of CAB and 600 µg of RIL) were used to make up the final volume. With diluent, the stocks are further diluted to concentrations of 40µg/ml CAB and 60µg/ml RIL.

Conditions for chromatography:

Acquity HSS C18 100x 2.1 x 2m column was used for the chromatographic separation. The mobile phase consisted of 0.01N potassium dihydrogen phosphate buffer (pH 4.0) and acetonitrile in a 55:45 v/v ratio. For both CAB and RIL, the UV detection wavelength was set at 260 nm, and the mobile phase flow rate was set at 0.3 mL minute−1. The column temperature was set to 30°C and the injection volume was set at 1 µL. The method's total chromatographic run time was five minutes.

Software and computations:

Empower 2 software was used to collect chromatographic responses. The experimental design and run selection were done using the Design Expert 11.0.0 trial edition (Stat-Ease Inc., Minneapolis, MN, USA). The consequences of parameters and their statistical interpretation used to construct analytical methods was examined and computed.

Method validation:

Linearity:

By serial volume to volume dilution of stock solution I over the range of 10-60 µg/ml for CAB and 15-80 µg/ml for RIL, standard linearity curves were created with seven distinct concentrations, including the LOQ. The Y-axis peak area and the X-axis drug concentration were calibrated using linear curves. This was developed for the assay evaluation of marketed formulations. The linearity was investigated using linear regression, which was computed by the least square regression approach.

Precision:

The repeatability of the procedure was used to evaluate its precision. The samples of CAB and RIL, weighing 40 µg/ml and 60 µg/ml, respectively, were analysed for the precision studies. Three assay values (n = 3) were computed to determine the percentage RSD. The study for intraday precision took place on the same day at different times, while the study for interday precision was carried out on three separate days, namely day 1, day 2, and day 3.

Accuracy:

The two drugs were spiked at three predefined concentration levels (50, 100, and 150%) in order to assess the accuracy of the established procedure and determine their respective percentage recoveries. Triplet doses of 20, 40, and 60 µg/ml for CAB and 30, 60, and 90 µg/ml for RIL were used to check the study. In each case, the percentage of drugs recovered were calculated.

Robustness:

Samples were injected in duplicate under robustness conditions comprising Flow minus (0.2 ml/min), Flow plus (0.4 ml/min), Mobile Phase minus (50:50), Mobile Phase plus (60:40), Temperature minus (25°C), and Temperature plus (35°C). All of the system suitability parameters passed with little to no impact.

Limits of detection and quantitation:

According to ICH guidelines Q2 (R1), the slope of the charted calibration curve (n=3) and the standard deviation of the response were used to determine the limit of quantification (LOQ) and limit of detection (LOD). LOD and LOQ are primarily related to the method's sensitivity.

Degradation studies:

Forced deterioration experiments (FDS) were carried out, and once the degradation process was finished, all of the FDS samples were diluted using diluent (Table 5). In order to eliminate any influence from the method, blank and placebo solutions were made similarly, especially in terms of deterioration. Sample solutions for forced degradation were introduced into the UPLC device, and the chromatograms were recorded. In order to confirm any interference from blank and placebo during the retention period of cabotegravir and rilpivirine, peak purity was ascertained for each drug.

Assay:

CABENUVA (400 mg/600 mg) CAB 400 mg and RIL 600 mg per unit formulation are stated on the label. A 500 mL volumetric flask was filled with 2 ml of Vial (equivalent to 400 mg/600 mg of CAB/RIL), 100 mL of diluent, sonicated for 25 minutes, then centrifuged for 25 minutes at 3000 rpm. Subsequently, the material was collected in a second volumetric flask, its volume was adjusted with diluent, and it was filtered using 0.25µm nylon filters. (1200µg of RIL and 800µg of CAB per millilitre). A 10ml volumetric flask was filled to capacity with 0.5ml of the sample stock solution and diluted with diluent (40µg/ml of CAB and 60µg/ml of RIL).

The average percentage of assay obtained for RIL and CAB was 99.67% and 100.74%, respectively.

Results and Discussion:

Optimization of experimental conditions:

The primary goal of the RP-UPLC method's development is to simultaneously estimate CAB and RIL in bulk and dosage form that are separated from one another with good resolution (RS >2), a sufficient number of theoretical plates (NTP >2000), a good peak shape (TF≤2), and a retention time (RT<5min) for both drugs. These goals can be attained by adjusting crucial UPLC parameters. To investigate their impact on the responses, a variety of mobile phase compositions (% buffer), column temperature, and flow rates were evaluated during the initial testing. A face-centered central composite design (CCD) was used in the optimisation phase to identify a number of crucial UPLC parameters whose permutation

affects the separation of both medications. This allowed for the determination of the best combination and response pattern. As indicated in Table 1, three independent variables were used: flow rate, percentage mobile phase, and column temperature, each at three levels. A design of experiment based on response surface methodology (RSM) and CCD was used to determine the optimal combination of column temperature, flow rate, and buffer (KH2PO4) on the chromatographic responses. We looked into the combined effects of independent variables, each at triplet levels, on the chromatographic responses.

Twenty experimental runs using CCD-aided RSM were conducted in order to examine the effects of the aforementioned variables at three different levels on the chromatographic responses, retention times (RTCAB and RTRIl), theoretical plates, tailing factor, and resolution (RSCAB – RSRIL) (Table 2). Figure 2a-2f shows a few of the experiment's produced chromatograms.

Table : 3 Experimental variables and coded levels considered in the Central composite design.

Variables	Levels		
	(-1) Low	(0) Medium	(1) High
Independent			
Flow rate(ml/min)	0.2700	0.3182	0.3300
Mobile Phase	55.00	55.77	65.00
Temperature(^{0}c)	27.00	30.62	33.00
Dependent			
RT $_{(Cab)}$	Retention Time of Cabotegravir		
RT $_{(Ril)}$	Retention Time of Rilpivirine		
RS $_{(Cab- Ril)}$	Resolution Factor between Cab and Ril		
NTP	Number of theoretical plates		
TF	Tailing Factor		

Table :4 Experimental runs given by CCD for the 3 variables at triplet levels and their observed values.

		Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4	Response 5
Std	Run	A:FR	B:MP	C:TEMP	RT(Cab)	RT(Ril)	RS(Cab-Ril)	NTP	TF
		ml/min	%	0 C	min	min	num	num	num
1	13	0.27	55	27	1.096	1.297	2.5	2997.5	1.31
2	17	0.33	55	27	0.821	1.12	1.9	3106.1	1.29
3	12	0.27	65	27	1.42	2.113	4.8	3024.8	1.41
4	18	0.33	65	27	1.053	1.739	5	2983.1	1.34
5	14	0.27	55	33	0.986	1.193	2.1	3132.4	1.31
6	3	0.33	55	33	0.868	1.04	1.9	2667	1.33
7	20	0.27	65	33	1.017	1.701	3.2	3009.8	1.36

8	11	0.33	65	33	0.973	1.587	3.3	2078.6	1.34
9	8	0.249546	60	30	1.176	1.523	3.3	3139	1.33
10	9	0.350454	60	30	0.883	1.155	3.1	2552.2	1.31
11	5	0.3	51.591	30	0.949	1.268	1.9	2864.3	1.3
12	2	0.3	68.409	30	1.212	2.24	4.6	2438.5	1.4
13	7	0.3	60	24.9546	1.135	1.404	2.9	3427.3	1.36
14	1	0.3	60	35.0454	0.866	1.125	1.8	3011.3	1.35
15	6	0.3	60	30	1.004	1.291	2.5	3184	1.3
16	16	0.3	60	30	1.006	1.298	2.5	3186	1.3
17	19	0.3	60	30	1.006	1.298	2.5	3185	1.3
18	4	0.3	60	30	1.016	1.299	2.5	3180	1.3
19	10	0.3	60	30	1.017	1.313	2.5	3176	1.3
20	15	0.3	60	30	1.02	1.319	2.5	3185	1.3

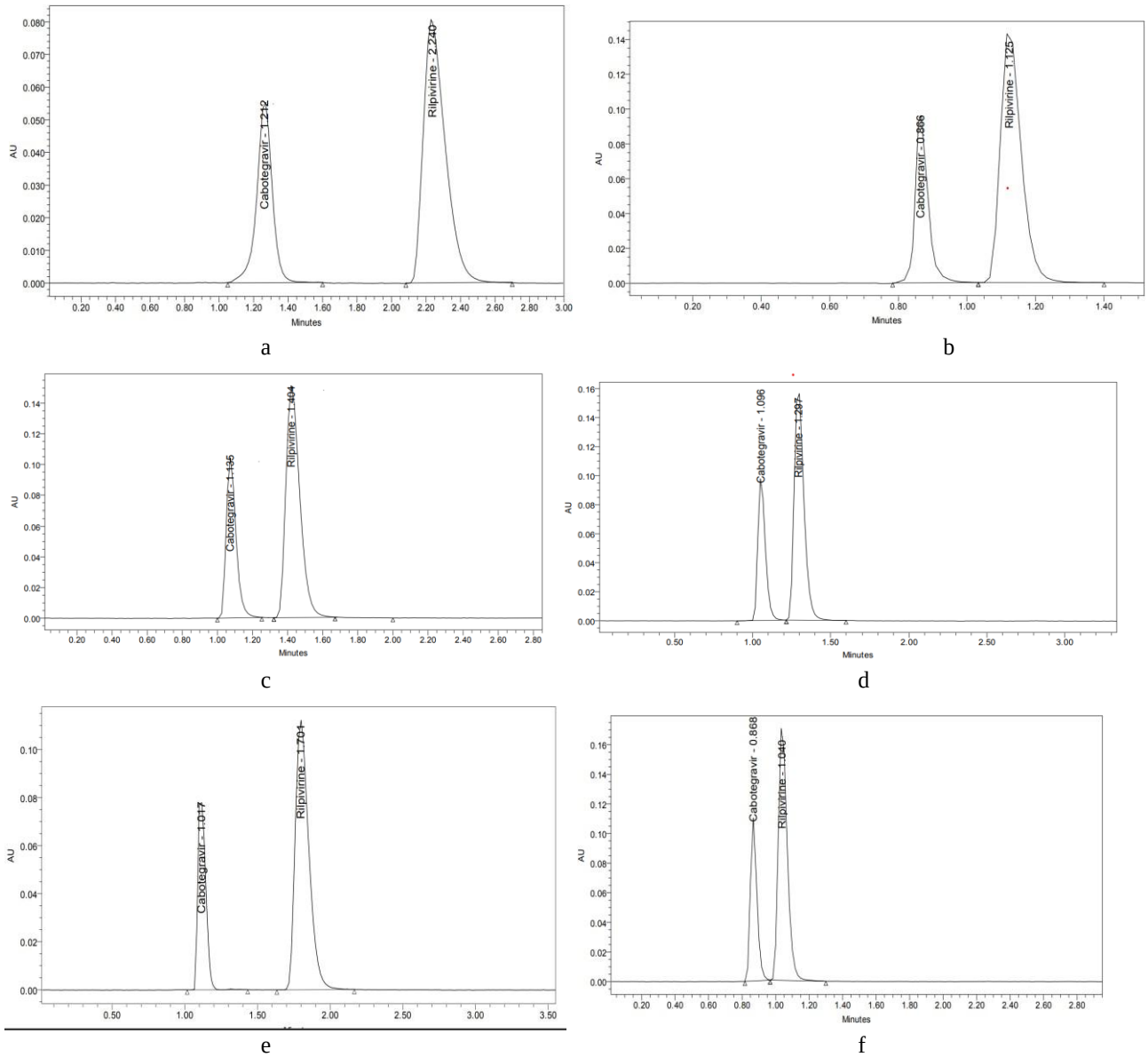


Figure 2: CCD generated some trial runs and their respective chromatograms.

Polynomial models were generated by RSM computations using Design Expert 11.0.0 software. The fit summary, which indicates the degree of associability between the variables and the answers, was the first factor considered while assessing the chosen model. Table 3 presented the statistical parameters obtained from the analysis of variance (ANOVA) results for this technique. The model's values were obtained without any modifications. All of the highly significant model terms have probability (Prob > F) (P value) < 0.05. The significant and predictability of the model are indicated by the high values of the adjusted R2 for the model, which show a strong relationship between the experimental and anticipated values of the responses.

Table:5 Analysis of variance for the screened chromatographic responses.

	RT (Cab)		**RT(Ril)**		**RS(Cab-Ril)**		**NTP**		**TF**	
Source	**F-value**	**p-value**	**F-value**	**p-value**	**F-value**	**p-value**	**F-value**	**p-value**	**F-value**	**p-value**
Model	74.29	< 0.0001	210.10	< 0.0001	73.57	< 0.0001	114.64	< 0.0001	69.24	< 0.0001
A-FR	235.52	< 0.0001	148.44	< 0.0001	1.99	0.1888	213.77	< 0.0001	31.33	0.0002
B-MP	180.21	< 0.0001	1223.19	< 0.0001	440.17	< 0.0001	92.37	< 0.0001	293.14	< 0.0001
C-Temp	139.61	< 0.0001	106.52	< 0.0001	87.60	< 0.0001	147.35	< 0.0001	1.47	0.2526
AB	0.0775	0.7864	3.06	0.1106	5.87	0.0358	25.81	0.0005	28.34	0.0003
AC	55.09	< 0.0001	9.90	0.0104	0.4369	0.5235	145.64	< 0.0001	28.34	0.0003
BC	42.18	< 0.0001	17.72	0.0018	40.83	< 0.0001	25.74	0.0005	28.34	0.0003
A^2	0.5433	0.4780	3.31	0.0988	42.17	< 0.0001	116.81	< 0.0001	11.74	0.0065
B^2	13.92	0.0039	371.46	< 0.0001	47.77	< 0.0001	285.17	< 0.0001	103.29	< 0.0001
C^2	0.9317	0.3572	1.73	0.2184	0.3797	0.5515	0.7934	0.3940	127.37	< 0.0001

Adjusted R^2	0.9720	0.9900	0.9717	0.9818	0.9700
Adeq. Precision **	35.9365	52.8915	27.4587	43.7660	28.0355

F - Fisher ratio; P – Probability

For examining the interactions between the variables and their effects on the responses, the perturbation plots (Figures 3a–3f) and the three-dimensional (3D) response surface plots (Figures 4a–4f) are highly helpful. Conciliating the various responses (Table 4) leads to the final composition independent variables for the UPLC method optimisation, which allowed for greater peak resolution, a decent tailing factor, and a minimum analysis time. Figure 5 displayed the bar graph for the optimization's desirability. On the other hand, Figure 6 depicted the desirability ramp for this approach and made the desirability criteria and changing limitations very evident. The desirability was analysed graphically as follows:

Predicted Error(P E) =[(Observed- Predicted)/(Predicted)] X 100 [Eq-1]

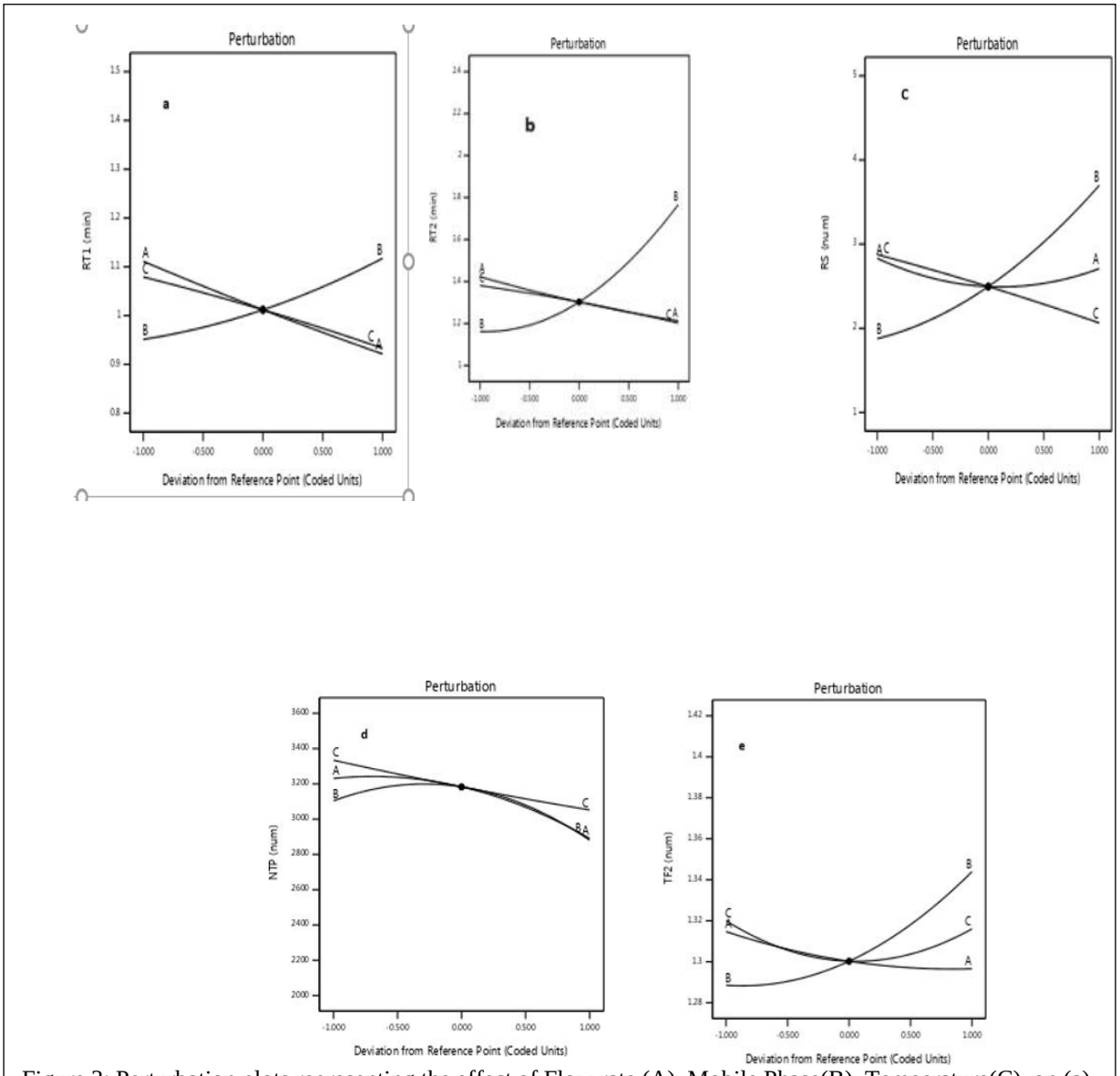


Figure 3: Perturbation plots representing the effect of Flow rate (A), Mobile Phase(B), Temperature(C), on (a) RT (Cab),
(b) RT(Ril), (c) RS (Cab-Ril), (d) NTP, (e) TF

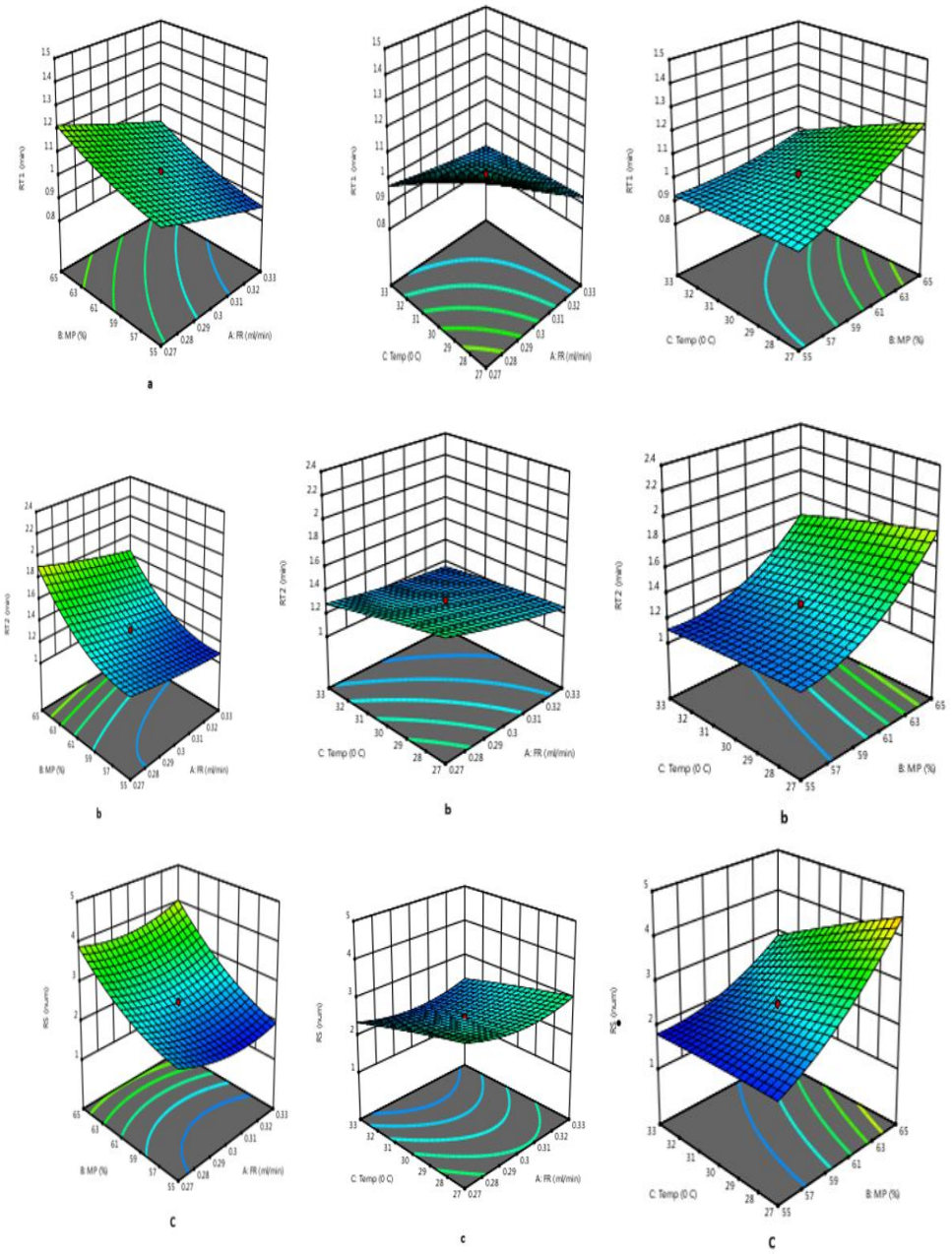


Figure 4: 3D Response surface plots representing the effect of Flow rate (A), Mobile Phase(B), Temperature(C), on (a) RT (Cab), (b) RT(Ril), (c) RS (Cab-Ril), (d) NTP, (e) TF

ame	Goal	Lower Limit	Upper Limit
A:FR	is in range	0.27	0.33
B:MP	is in range	55	65
C:Temp	is in range	27	33
RT(Cab)	is in range	0.821	1.42
RT(Ril)	is in range	1.04	2.24
RS(Cab-Ril)	is in range	1.8	5
NTP	is in range	2078.6	3427.3
TF	is in range	1.29	1.41

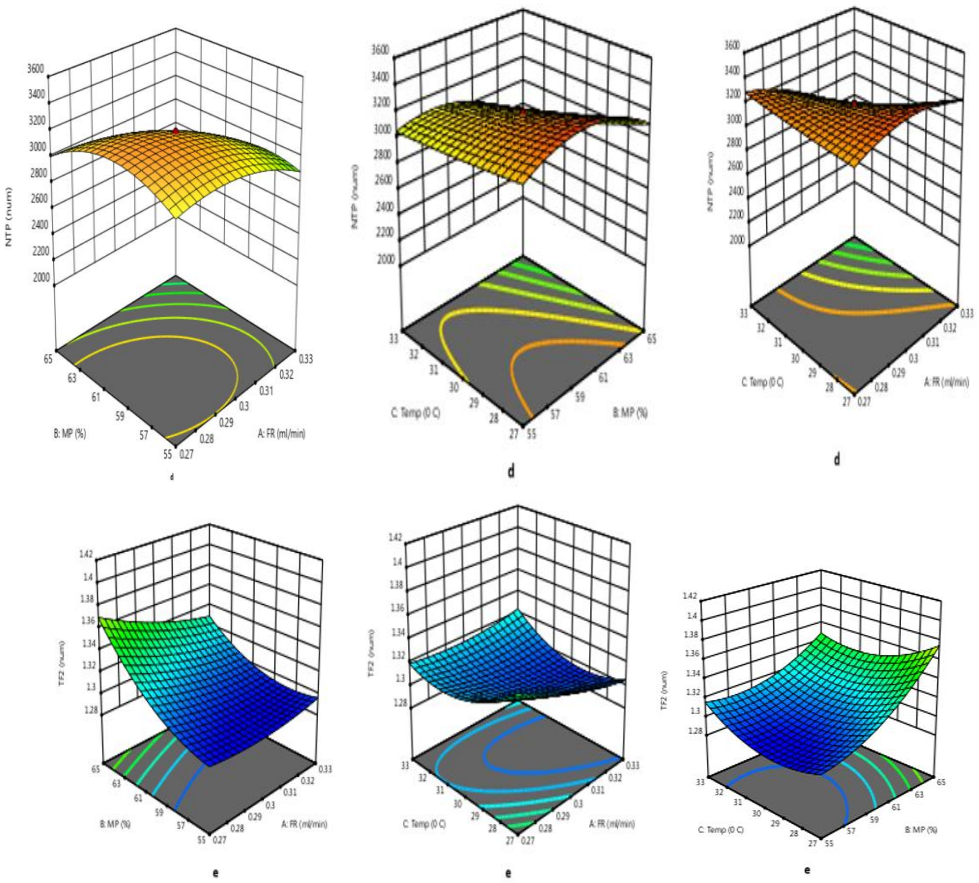


Figure 4: 3D Response surface plots representing the effect of Flow rate (A), Mobile Phase(B), Temperature(C), on (a) RT (Cab), (b) RT(Ril), (c) RS (Cab-Ril), (d) NTP, (e) TF

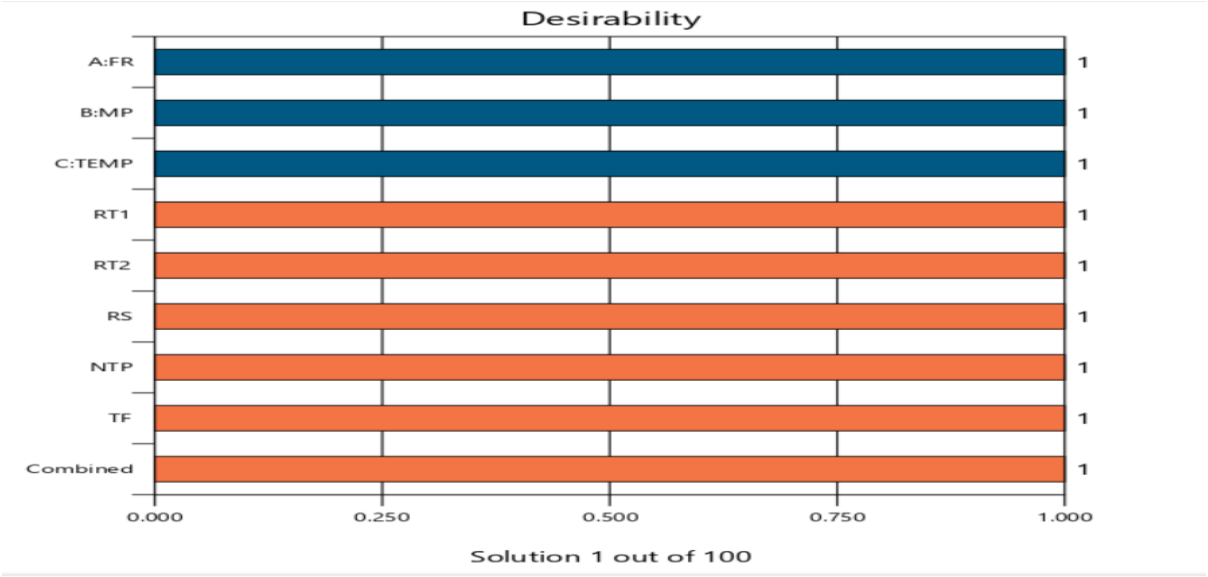


Figure 5: The desirability bar graph for the responses

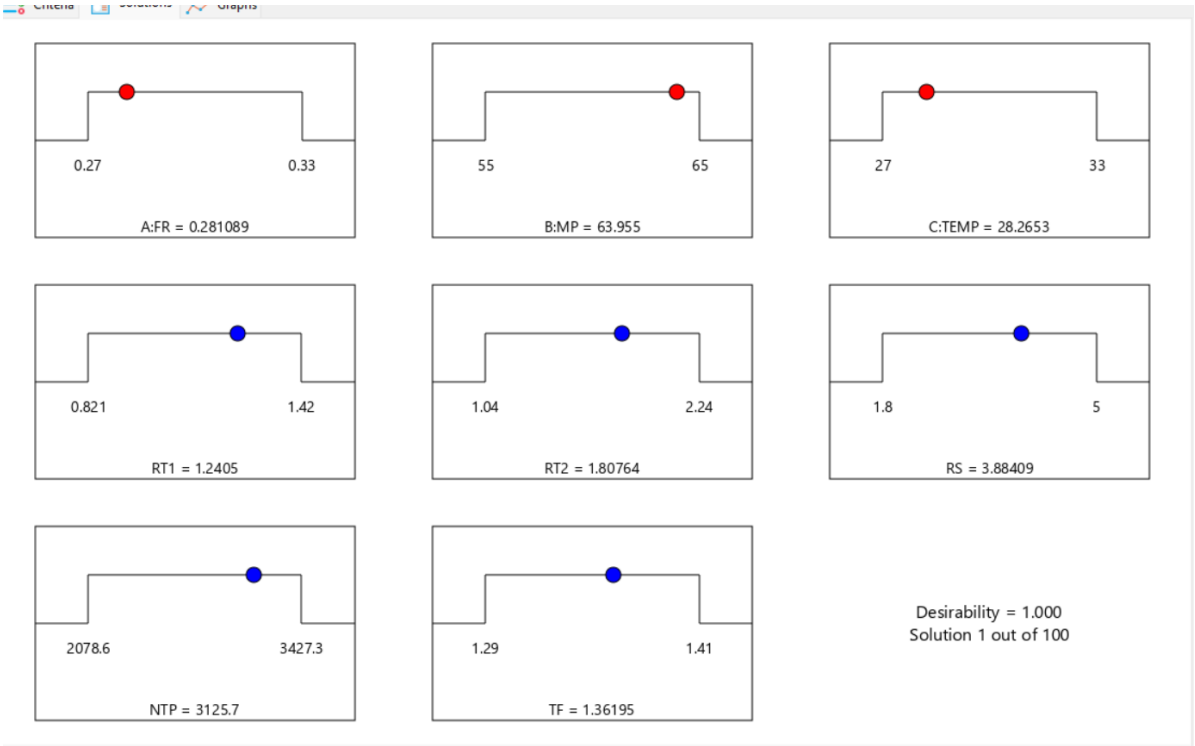


Figure 6: The desirability ramp representing the optimization of the independent variables for the better responses

Using the design expert software with higher desirability, a total of 100 runs were provided. These were tested, and the percentage prediction error (P.E.) was computed using Equation 7 and displayed in Table 5. The ideal approach is determined by looking at the run with the mean percentage prediction error being the lowest when compared to the other runs. The desired results are obtained by using a mixture of 0.01N potassium dihydrogen phosphate buffer and acetonitrile (55:45, v/v) as the mobile phase (Isocratic mode), with a flow rate of 0.3182 ml/min and a temperature of 30.62. RT (Cab)=0.904, RT (Ril)=1.1088,RS(Cab-Ril)=1.911, NTP= 1982.61, TF= 1.294 were recorded under these conditions, and Figure 7 depicted the chromatograph.

Table:6 Table for optimization.

Flow Rate	**Mobile Phase**	**Temperature**	**Solution 1 of 100 Response**	**Predicted Mean**	**Observed**	**Std Dev**	**SE Pred**	**Data Mean**
0.318245	55.7714	30.6167	RT1	0.904028	0.899	0.0228651	0.0251415	0.899
			RT2	1.1088	1.109	0.0319132	0.0350904	1.109
			RS	1.91103	1.9	0.160459	0.176434	1.9
			NTP1	1982.61	3146	30.9486	34.0298	3146
			TF2	1.29427	1.2	0.00597704	0.0065721	1.2

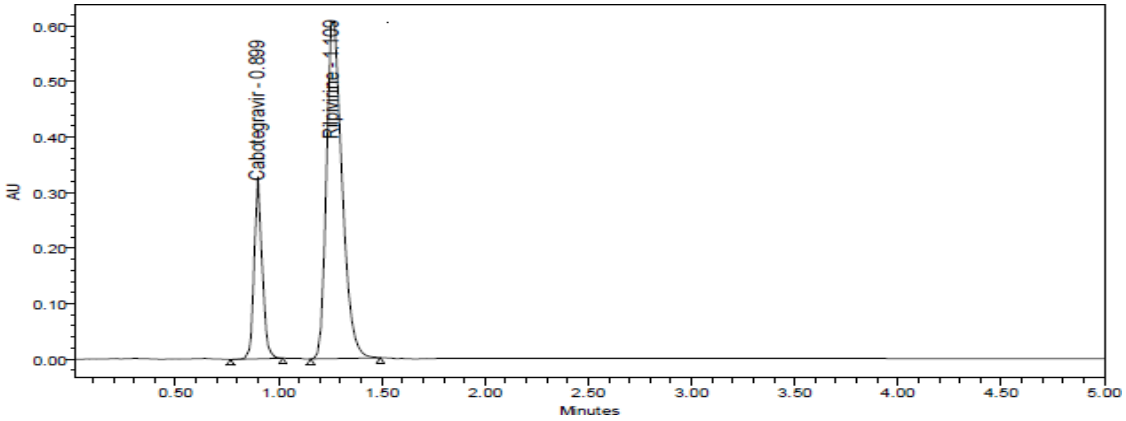


Figure 7: Chromatogram of Cabotegravir and Rilpivirine

Degradation studies:

Carried out the forced degradation studies (FDS), when the degradation process was finished, all of the FDS samples were diluted using diluent (Table 5). To eliminate any influence from the method, blank and placebo solutions were made in a manner that was comparable to each other, especially in terms of degradation. Sample solutions for forced degradation were introduced into the UPLC equipment, and the chromatograms were recorded. In order to confirm any interference from blank and placebo during the retention period of Cabotegravir and Rilpivirine, peak purity was ascertained for each drug.

Table: 7 Forced degradation studies

Degradation condition	**Optimized condition**	**% Degradation**	
		Cabotegravir	**Rilpivirine**
Acid	2N Hydrochloricacidand refluxed for 30mins	6.75	6.42
Base	2 N sodium hydroxideand refluxed for30mins	5.64	5.91
Peroxide	20% hydrogen peroxide (H2O2)solutionsfor 30min	3.68	3.27
Thermal	Sample in ovenat105^{0}c for6h	2.49	1.58
UV	Sample in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber	1.63	1.89
Water	Refluxingthedruginwaterfor6h r s atatemperature of 60º	0.30	0.63

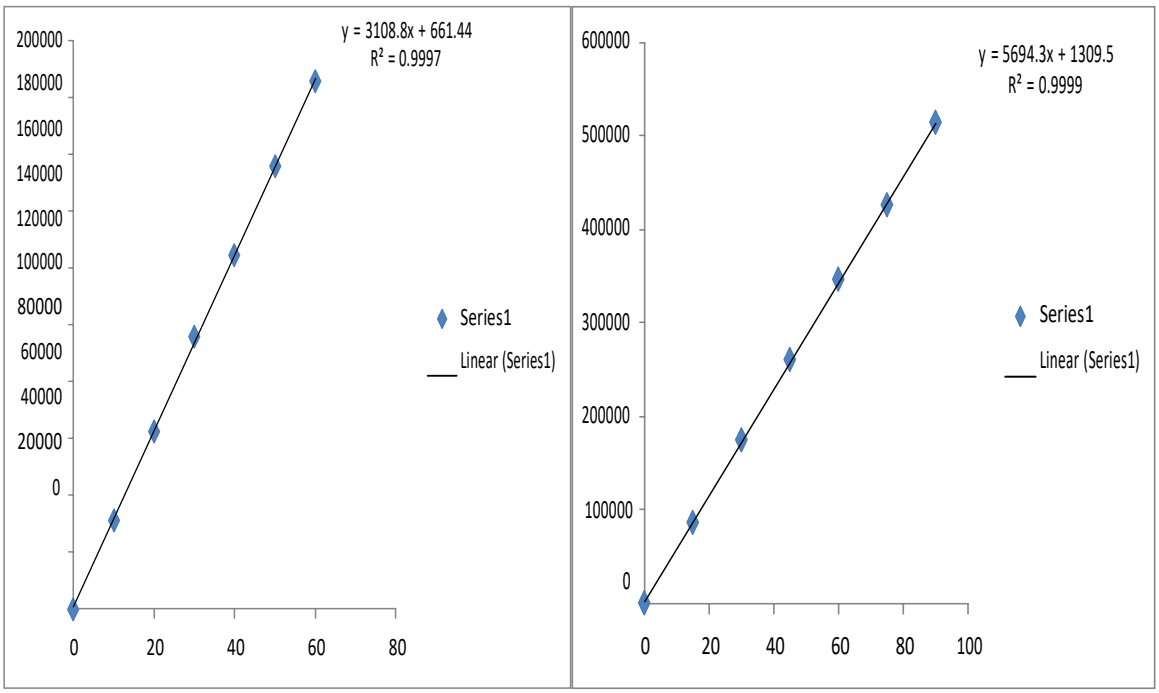
Method validation: In accordance with the ICH criteria from 2005, the method was validated for system suitability, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), specificity, and robustness.

Linearity: In the concentration range of 10–60 µg mL−1 for CAB and 15–80 µg mL−1 for RIL, linearity is followed (Table 7). Figures 8a–8b display the linearity graphs of the various samples for RIL and CAB, respectively. For CAB and RIL, the corresponding linear regression equations were found to be y = y = 3108.8x + 661.44 (r2 = 0.9997) and y = y = 5694.3x + 1309.5 (r2 = 0.9999). Table 7 displayed each drug's limit of quantitation (LOQ) and limit of detection (LOD).

Table:8 linearity data of Cabotegravir and Rilpivirine

Parameters	**Cabotegravir**	**Rilpivirine**
Concentration Range (µg/ml)	10-60 µg/ml	15-80 µg/ml
Regression equation (y= mx + c)	y = 3108.8x + 661.44	y = 5694.3x + 1309.5
Slope (m)	3109	5694
Intercept (c)	661	1310
Correlation Coefficient (r2)	0.9997	0.9999
LOD(µg/ml)	0.32	0.67
LOQ(µg/ml)	0.98	2.04

Figure 8: Linearity graphs of (a)Cabotegravir and (b)Rilpivirine



(a) **(b)**

Precision: Repeatability and intermediate precision demonstrated the analytical method's precision. Table 8 displays the percentage RSD results for repeatability for CAB and RIL, which were 0.7 and 0.7, respectively. For CAB and RIL, the % RSD between two analyst values was 1 and 0.7, respectively (Table:9). The procedure was reproducible because the six assay results' % RSD did not exceed 2.0. Since the two analysts' assay findings had a percentage RSD of less than 2.0, moderate precision was considered acceptable.

Table: 9 Repeatability (intraday) data of CAB and RIL

Concentration	**Cabotegravir**	**Rilpivirine**
Area* (NMT-2%)	126170	344662
± SD	858.8	2502.5
%RSD	0.7	0.7

Table:10 Interday precision data for estimation of CAB and RIL

S.No.	**Cabotegravir**	**Rilpivirine**
Concentration(µg/ml)	40	60
Area Mean ± S.D. (n=3)	128399 ± 1315.2	343390±2443.7
%RSD	1	0.7

Accuracy:

The suggested UPLC method was used to analyse the drugs CAB and RIL, which were spiked to the standard drug at 50%, 100%, and 150% percentage levels relative to the sample concentration. The recovery of RIL and CAB was found to be between 100.2% and 100.5% and 99.6% to 99.9%, respectively. Furthermore, every individual result met the 98.0–102.0%

threshold for both CAB and RIL. Tables 10 and 11 provided a summary of the accuracy results.

Table:11 Recovery data of CAB

Sr.No.	Conc Level%	Sample Amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	%Mean Recovery ± S.D
1	50%	40	20	20.1	100.5	99.6 ± 0.819
2		40	20	19.8	99.0	
3		40	20	20.1	100.4	
4	100%	40	40	40.0	100.0	99.90 ±0.2742
5		40	40	40.0	100.1	
6		40	40	39.8	99.6	
7	150%	40	60	59.6	99.3	99.46 ±0.4371
8		40	60	60.0	99.9	
9		40	60	59.5	99.1	

Table:12 Recovery data of RiIL

Sr.No.	Conc Level%	Sample Amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	%Mean Recovery ± S.D
1	50%	60	30	30.18	100.59	100.5 ± 0.38
2		60	30	30.27	100.89	
3		60	30	30.04	100.13	
4	100%	60	60	60.39	100.66	100.01 ± 0.64
5		60	60	59.63	99.38	
6		60	60	59.99	99.99	
7	150%	60	90	89.77	99.74	100.14±0.36
8		60	90	90.39	100.44	
9		60	90	90.21	100.24	

Robustness: One of the validation parameters is robustness, which is a measure of the method's ability to withstand little, intentional changes in chromatographic circumstances. It was investigated by examining the effects of minor variations in temperature (±5%), flow rate (±5%), and organic content (±5%) in the mobile phase.

Table:13 Robustness data for CAB and RIL

S.no	Condition	%RSD of Cabotegravir	%RSD of Rilpivirine
1	**Flow rate (-) 0.28ml/min**	1.2	0.8
2	**Flow rate (+) 0.34ml/min**	1.3	1.2
3	**Mobile phase (+) 60:40A**	1.7	1.3
4	**Mobile phase (-) 50:50A**	1.1	0.9
5	**Temperature (-) 25°C**	0.9	1.2
6	**Temperature (+) 35°C**	0.9	1.4

*Mean of triplet replicates

Analysis of commercial formulations :

CABENUVA (400 mg/600 mg) Cabotegravir 400 mg and Rilpivirine 600 mg per unit formulation are stated on the label. The above formulation was assayed using the suggested methodology. The average percentage of assays obtained for Ril and Cab was 99.67% and 100.13%, respectively. Ril has a retention time of 1.109 and cab of 0.899, respectively. Figure 7 displays the final chromatograms that were produced for commercial formulations.

Conclusions: For the simultaneous, quick quantification of Cabotegravir and Rilpivirine in bulk and pharmaceutical medication products, the UPLC method was created using a design-of-experiments methodology. The method that was developed completed validation in accordance with the International Council for Harmonization's ICH Q2 (R1) validation of analytical processes. The process was discovered to be simple, adaptive, accurate, precise, and selective. The new approach demonstrated stability, as seen by the lack of interference from degradation products or placebo during the Cabotegravir and Rilpivirine retention period. This method lowers the cost of analysis due to the reduced solvent use, faster analysis, and higher work throughput because of its two-minute run time. The created technique can therefore be applied to regular assay examination of samples for stability and quality control of pharmaceutical dosage forms in bulk in finished form.

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References:

1. https://www.sciencedirect.com/topics/medicine-and-dentistry/integrase-inhibitor
2. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6629025/
3. https://www.ncbi.nlm.nih.gov/books/NBK592772/
4. https://en.wikipedia.org/wiki/Cabotegravir
5. https://en.wikipedia.org/wiki/Rilpivirine
6. Mukthinuthalapati Mathrusri Annapurna*, et.al.,2017, Quality by Design Approach (QbD) for the Simultaneous Determination of Anti-Hypertensive Drugs (Candesartan, Irbesartan and Hydrochlorothiazide) by RP-HPLC, *Journal of Analytical & Pharmaceutical Research*, Volume 4 Issue 5 –, pg:1-17.
7. Inken K. Ramoller, Marco T.A. Abbate, Lalitkumar K. Vora.et.al.,(2022), HPLC-MS method for simultaneous quantification of the antiretroviral agents rilpivirine and

cabotegravir in rat plasma and tissues, *Journal of Pharmaceutical and Biomedical Analysis*, Vol 213.

8. C.Parthiban, et.al., (2022), RP-HPLC Method Development and Validation for the Simultaneous Estimation of Cabotegravir and Rilpivirine in Pharmaceutical Dosage Form, *International Journal of Pharmacy and Pharmaceutical Sciences*, Vol. 26, Issue 1, pg: 65-79.
9. K. Srinivas Reddy, et.al., (2020), Validated Stability Indicating Rp-Hplc Method For The Simultaneous Estimation Of Rilpivirine And Dolutegravir In Bulk Form, *International Journal of Pharmaceutical Sciences and Research*, Vol. 11, Issue 10 pg: 4991-4997.
10. Challamalla Pavani, et.al., (2022), The Development Of A Novel StabilityIndicating Rp-Hplc Method For The Simultaneous Evaluation Of Rilpivirine And Cabotegravir In Pure Api Form And Tablet Dosage In Accordance With Ich Guidelines,*Eur.Chem. Bull*Vol 11, Issue 5, pg:64 – 70.
11. Pindi Shravani, et.al., (2022), Analytical Method Development And Validation For The Simultaneous Estimation Of Cabotegravir & Rilpivirine By Rp Hplc Method, *Indo American Journal of Pharmaceutical Sciences*, Vol 09 Issue 10, pg:495-505.
12. S. Venkatesan, N. Kannappan, and Sai Sandeep Mannemala, (2014) Stability-Indicating HPLC Method for the Simultaneous Determination of HIV Tablet Containing Emtricitabine, Tenofovir Disoproxil Fumarate, and Rilpivirine Hydrochloride in Pharmaceutical Dosage Forms, *International Scholarly Research Notices,* Vol 2014.
13. Anugu Prasanna, et.al., (2022), A Validated Stability Indicating RP-HPLC Method Development for Simultaneous estimation of Cabotegravir and Rilpivirine in Pharmaceutical Dosage form,*World Journal of Pharmaceutical Sciences* ; Vol 10, Issue 10, pg: 22-29.
14. Yogi Pandya, (2022), RP-HPLC stability method development & validation for anti-HIV drugs cabotegravir & rilpivirine in I.M. injection and in human plasma, *International Journal of Health Sciences*, Vol 6 Issue S1,pg: 9104–9117.
15. B M S Kumar, et.al., (2019), Development and Validation of Rilpivirine in Pharmaceutical Formulation by RP-HPLC, *American Journal of PharmTech Research*, Vol 9, Issue 03, pg:343-353.
16. Veeraswami B, et.al., (2019), Development And Validation Of Rp-Hplc Method For The Estimation Of Dolutegravir And Rilpivirine In Bulk And Pharmaceutical Dosage Form And Its Application To Rat Plasma, *Asian Journal of Pharmaceutical and Clinical Research*, Vol 12, Issue 2, pg: 267-271.
17. M. M. Eswarudu, et.al., (2023), A Review On Analytical Profile Of Cabotegravir And Rilpivirine In Pharmaceutical Formulations And Biological Matrices, *International Journal of Creative Research Thoughts*, Volume 11, Issue 10, pg-257-265.
18. Padmabhushana Chary Vemuluri, et.al., (2023), Stability Indicating Reverse Phase-High Performance Liquid Chromatography Method for Simultaneous Estimation of Cabotegravir and Rilpivirine, *Indian Journal of Pharmaceutical Education and Research*, Vol 57, Issue 3(Suppl), pg:766-771
19. A Suneetha, et.al., (2022), Development and Validation of Stability Indicating RP-HPLC Method for the Simultaneous Determination of Cabotegravir and Rilpivirine in Bulk and Injection Dosage Form, *Journal of Pharmaceutical Research*, Vol 21, Issue

4 pg:144–150.

20. Anuradha Vejendla, et.al., (2021), Method development and validation for Cabotegravir and Rilpivirine by using HPLC and its degradants are characterized by LCMS and FTIR, *Future Journal of Pharmaceutical Sciences*, 7:226, pg 2-18.
21. Dr. Ch. V. Suresh, et.al., (2023), Development Of Stability Indicating Rp-Hplc Method And Validation For The Estimation Of Cabotegravir And Rilpivirine In Pure Form And Marketed Pharmaceutical Dosage Form, *YMER*, VOLUME 22 : ISSUE 02 (Feb), pg:703-725.
22. L.A.H. Bevers, et.al., (2023), Development, validation and clinical implementation of a UPLC-MS/MS bioanalytical method for simultaneous quantification of cabotegravir and rilpivirine E-isomer in human plasma, *Journal of Pharmaceutical and BiomedicalAnalysis.*
23. Bhaskar O. Aher, et.al., (2023), Method Development and Validation of Cabotegravir by Using HPLC and Characterization of its Degradants by LC-MS, *Eur. Chem. Bull.* ,Volc12(Special Issue 4), pg:10597-10611 .
24. Lidija Kovac, et.al., (2022), Development of an Analytical Method for Determination of Related Substances and Degradation Products of Cabotegravir Using Analytical Quality by Design Principles, *ASC Omega Publications*, Vol 7, pg:8896-8905.
25. ICH (2005) Q2 (R1): validation of analytical procedures: text and methodology. International Conference on Harmonization, USA.