World Journal of Pharmaceutical Science and Research
www.wjpsronline.com
Research Article
ISSN: 2583-6579
SJIF Impact Factor: 5.111
Year - 2024
Volume: 3; Issue: 6
Page: 329-341

RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF ABACAVIR, DOLUTEGRAVIR AND LAMIVUDINE IN COMBINED PHARMACEUTICAL DOSAGE BY ANALYTICAL QBD

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Article Received: 04 November 2024 | | ***Article Revised: 23 November 2024*** | | ***Article Accepted: 15 December 2024***

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DOI: https://doi.org/10.5281/zenodo.14576822

How to cite this Article: Divyesh Vanparia, Ronak Dedania and Zarna Dedania (2024). RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF ABACAVIR, DOLUTEGRAVIR AND LAMIVUDINE IN COMBINED PHARMACEUTICAL DOSAGE BY ANALYTICAL QBD. World Journal of Pharmaceutical Science and Research, 3(6), 329-341. https://doi.org/10.5281/zenodo.14576822

ABSTRACT

A high-performance liquid chromatographic approach was developed and validated in order to gauge the effectiveness of three antiviral drugs in a combined pharmaceutical dosage form: Abacavir (ABA), Dolutegravir (DOLU), and Lamivudine (LAMI). For the intent of simultaneous drug quantification, the Analytical Quality by Design (AQbD) RP-HPLC method evolved using the Box-Behnken Design and the Design Expert® software (Version 11.0). The optimal conditions for the mobile phase's composition Methanol: Acetonitrile: Buffer of phosphate pH 3.5, 50:20:30 % v/v/v Using a Shimadzu C18 column on an HPLC system incorporating ultraviolet (UV) illumination at 230 nm was employed. The number of theoretical plates and the asymmetry factor reside within the bound. The suggested means was validated by obeying to the ICH standard. There are no findings of excipient involvement in the drug mixture assay. The computed percentage RSD was discovered in the acceptance criteria, demonstrating the accuracy, precision, and reproducibility of the procedure.

KEYWORDS: Abacavir, Dolutegravir, Lamivudine, Analytical QbD, RP-HPLC.

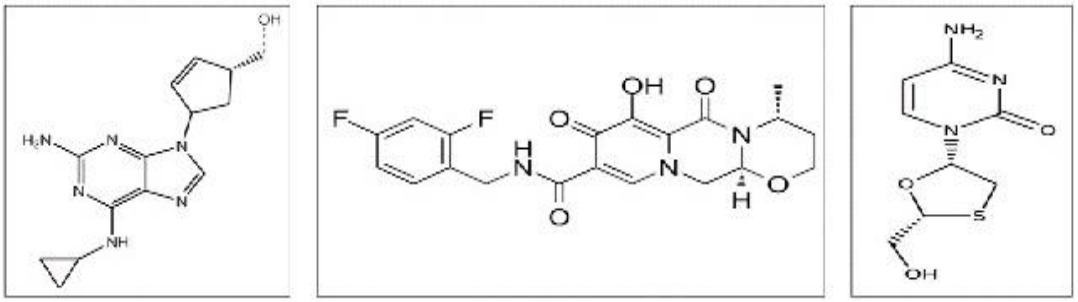
INTRODUCTION

Abacavir (ABA), [(1*S*,4*R*)-4-[2-amino-6-(cyclopropylamino)purin-9-yl]cyclopent-2-en-1-yl]methanol (**Figure 1**), an authorized by the Food and medication is used conjointly with additional antiretroviral medications for the relief of infection. Abacavir is usually used in combination with other drugs, similar to other kinds of nucleoside reverse-transcriptase inhibitors (NRTIs). It is not advised to take abacavir alone.[1] For recipients younger than three months, abacavir is an approved medical care option that can be supervised orally as a tablet or solution.[2] Abacavir is often laid

out in combination with other drugs, notably abacavir/zidovudine/lamivudine, abacavir/dolutegravir/lamivudine, and abacavir/lamivudine.[3]

Dolutegravir (DOLU), (3S,7R)-N-[(2,4-difluorophenyl)methyl]-11-hydroxy-7-methyl-9,12-dioxo-4-oxa-1,8-diazatricyclo[8.4.0.0^{3,8}]tetradeca-10,13-diene-13-carboxamide**,** conversely to non-dolutegravir comprising ART, commencing care in naive patients with dolutegravir-containing ART has a higher chance for accomplishing the suppression of viral infections Those with a high baseline viral load show the greatest the norm gain.[4] Abacavir's molecular structure is shown in **Figure 1**.

Lamivudine (LAMI), (4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2- dihydropyrimidin-2-one)[1] **(Figure 1)** nucleoside analogues. HIV-1 is treated with Lamivudine. Those who have persistent hemopatitis B, in which the virus has evolved and resulted in liver damage, additionally receive therapy with Lamivudine at smaller dosages. This composite is a member of the 3'–thia pyrimidine nucleosides group of organic substances.[5]



(a) **(b)** **(c)**

Figure 1 (a): Structure of Abacavir (b) Structure of Dolutegravir (c) Structure of Lamivudine.

Following a thorough assessment of the literature, it was found that a variety of previously recorded ways to analysis have been used to evaluate ABA, DOLU and LAMI.[6-14] There isn't currently a DOE concept in place to construct the HPLC method of ABA, DOLU and LAMI and achieve the required separation.

As a result, an effort was made to create an RP-HPLC method that is straightforward, sensitive, accurate, and robust for the quantification of ABA, DOLU and LAMI using AQbD approach. The study's goals include using box-behnken design to help select influencing variables in advance, thoroughly analyzing how variables interact statistically, optimizing methods using the desirability method, and validating it in accordance with ICH guideline.

Experimental

Standard API, Chemicals and Materials

The API ABA, DOLU, and LAMI were supplied by the reputable Ahmedabad, Gujarat-based pharmaceutical company Emcure Ltd. Acetonitrile, methanol, and water have been allocated by Finar Ltd. in Gujarat; sodium hydrogen phosphate, O-phosphoric acid, and potassium dihydrogen orthophosphate were supplied by Chemdyes Lab in Gujarat.

Instrumentation

The Inertsil ODS 3V, C18 (250 mm x 4.6 mm x 5 µm) Column featuring UV-visible multiple wavelength detector was the component of the Shimadzu HPLC that was utilized. Software through Lab solution was used to deal with and keep an eye on the concluded stream.

Preparation of standard stock solutions

To make stock solutions of ABA, DOLU, and LAMI standards (1200 µg/mL, 100 µg/mL, and 600 µg/mL), a meticulously measured quantity of drug standard was dissected in medium (methanol).

Preparation of working solutions

Using dilution solvent the standard base solution was further diminished and poured to a volumetric flask up till the quantity present achieved the necessary level, preparing a working concentration of 120 µg/mL of ABA, 10 µg/mL of DOLU, and 60 µg/mL of LAMI.

Preparation of mobile phase

To produce the mobile phase Methanol: Acetonitrile: Buffer of phosphate pH 3.5, 50:20:30 % v/v/v.

Method optimization applying DOE

The essential analytical parameters that demonstrate an impact on the efficiency of the method were determined to be the mobile phase ratio (Methanol), pH of the mobile phase and flow rate based on the risk analysis. The drug resolution between peaks 1 and 2, which most likely co-elute and frequently result in methodology collapse, were the chosen technique answers. The Design Expert 11.0 application will be used to optimize the most suitable chromatographic circumstances. Below **Table 1** lists the levels of the chosen technique replies.[15-23]

Table 1: Dependent and Independent Variables Selection.

Factors	Coded values given factor	Levels		
		-1	0	+1
Methanol (%)	A	45	50	55
Buffer pH	B	3.0	3.5	4.0
Flow Rate	C	0.8	1	1.2

The central composite design was chosen, and 14 chromatogram runs were carried out in accordance with the DoE design. **Table 2** lists the method responds for each run.

Table 2: Box-Behnken Design Arrangement and Response.

Trial runs	Organic phase (Methanol) (%)	pH	Flow rate (mL/min)	RS 1-2	RS 2-3
1	45	3.0	1.0	7.787	3.777
2	55	3.0	1.0	4.543	1.588
3	50	3.0	0.8	11.42	3.146
4	50	3.0	1.2	3.576	1.576
5	45	3.5	0.8	10.99	4.993
6	55	3.5	0.8	6.812	1.802
7	45	3.5	1.2	5.324	5.083
8	55	3.5	1.2	5.168	1.168
9	50	3.5	1.0	9.787	2.887
10	50	3.5	1.0	9.987	3.095
11	45	4.0	1.0	6.132	6.123
12	55	4.0	1.0	4.707	1.777
13	50	4.0	0.8	11.97	4.073
14	50	4.0	1.2	3.412	1.716

The linear exponential equations are the result of the analysis of variance, or ANOVA. The total statistics of the algorithm's recommendations showed that the equation using quadratics would perform best in the current study.

Validation

The course of action was granted authorization. The technique validation was conducted pursuant to with the points of view made by the International Conference Harmonization (ICH).[24]

Specificity

The percentage of additive disruption was assessed using a standard, diluent, and placebo in order to confirm specificity.

Linearity

Six calibrations have been made in the concentration range of 30–180 µg/mL for ABA, 2.5–15 µg/mL for DOLU, and 15–90 µg/mL for LAMI in order to evaluate the linearity (**Table 4**). Employing a straight-line equation to plot Peak Area v/s Concentration on the graph, the calibration curve was obtained.

Precision

To gauge the repeatability, an identical concentration working solution was looked at six times **(Table 5)**, Three distinct times of the day were used to assess the intraday precision of the drug mixture, and three separate days were used to analyze the interday precision of the drug mixture. RSD percentage was computed for accuracy. **(Table 6)**

Accuracy

This has been ascertained by applying the traditional additive approach to compute the amount of drug mixture recovery. A placebo was injected into the API to assess accuracy at 50%, 100%, and 150%. **(Table 7)**

Limit of detection and quantification

The drug mixture's LOD and LOQ have been established using an equation in accordance with the ICH guideline.[24] **(Table 8)**

Robustness

The degree to which an analytical procedure can tolerate small, deliberate modifications to the three method parameters—flow rate, mobile phase composition, and detection wavelength—is a measure of its resilience. **(Table 9)**

Assay

Weigh and powder twenty tablets, transfer equivalent weight of a powder containing 300 mg of ABA (50 mg of DOLU and 150 mg of LAMI) in 100 mL volumetric flask. Add 50 mL of methanol sonicated for five minutes, make up the volume upto mark with methanol, filtered, and the filtrate used to confirm the steps that were previously explained. To a 10 mL volumetric flask, 0.2 mL of the previously mentioned solution was further diluted, and the volume was modified with diluent. A final concentration of 60 µg/mL of ABA, 5 µg/mL of DOLU, and 30 µg/mL of LAMI had been added to the HPLC system in accordance with ideal chromatographic conditions. The analytical information displayed in **Table 10**.

RESULTS AND DISCUSSION

Selection of Detection wavelength

To determine the wavelength, an ultraviolet spectrometer was employed. 230 nm was selected as the HPLC measurement wavelength as all three drugs shown the significance absorbance. **(**Error! Reference source not found.**)**

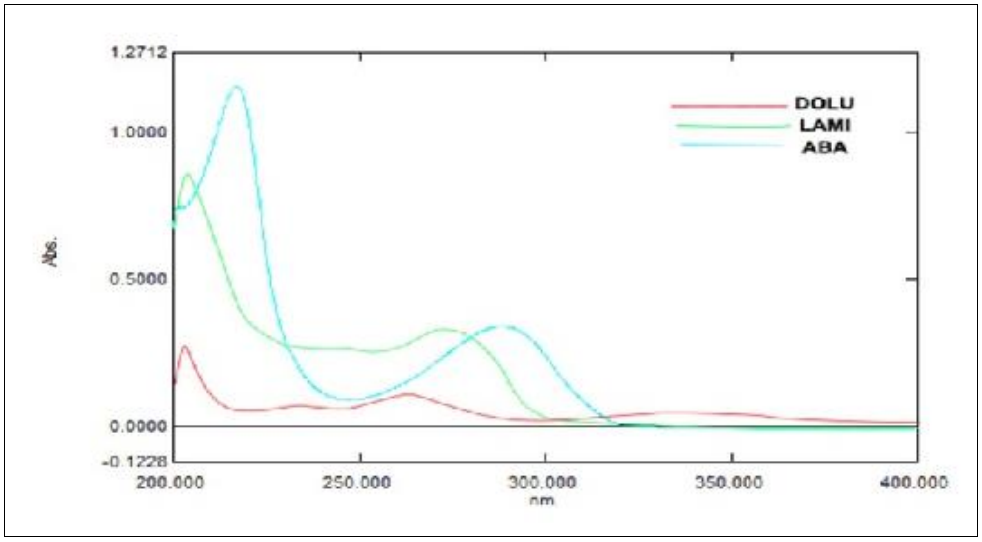


Figure 2: Overlain UV Spectrum in Methanol (ABA+DOLU+LAMI).

Determination of Mobile phase

Different solvent amounts and pH values were employed in the various mobile phases. For appropriate peak travel, separation, and resolution, the mixture of Methanol: Acetonitrile: Buffer of phosphate pH 3.5, (50:20:30 % v/v/v) offered the ideal polarity. In these circumstances, the elution peak was clear, distinct, and appeared despite tailing.

Assessment of outcomes and choice of optimal technique parameters

It will be crucial to optimize the ideal chromatographic conditions with the Design Expert® (Version 11.0, Stat-ease Inc., and M M).

Statistical analysis of model

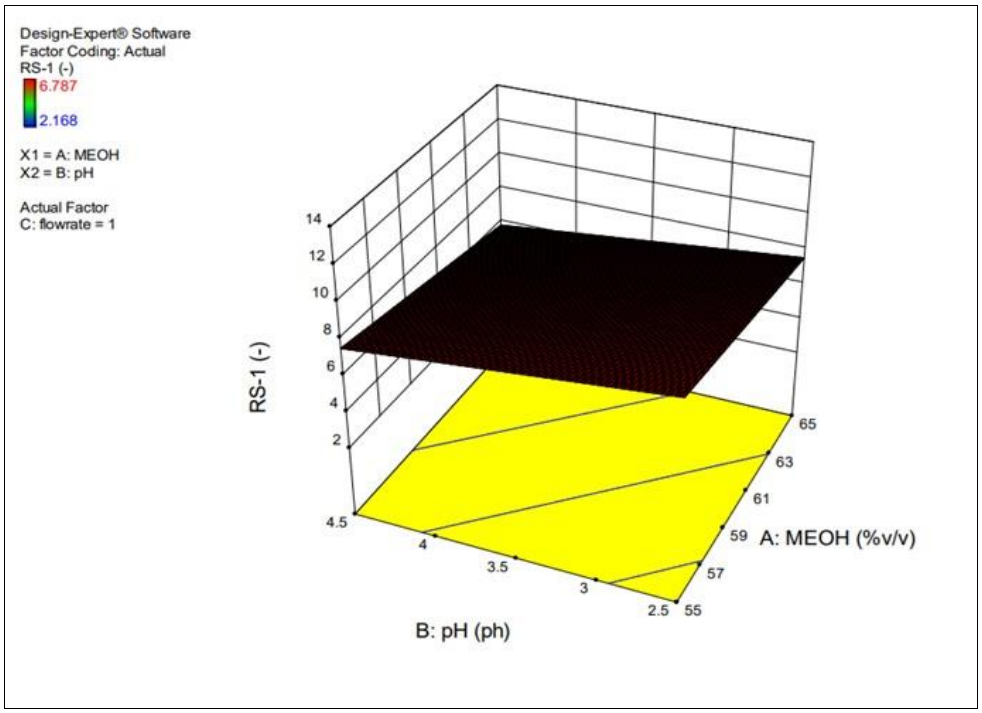
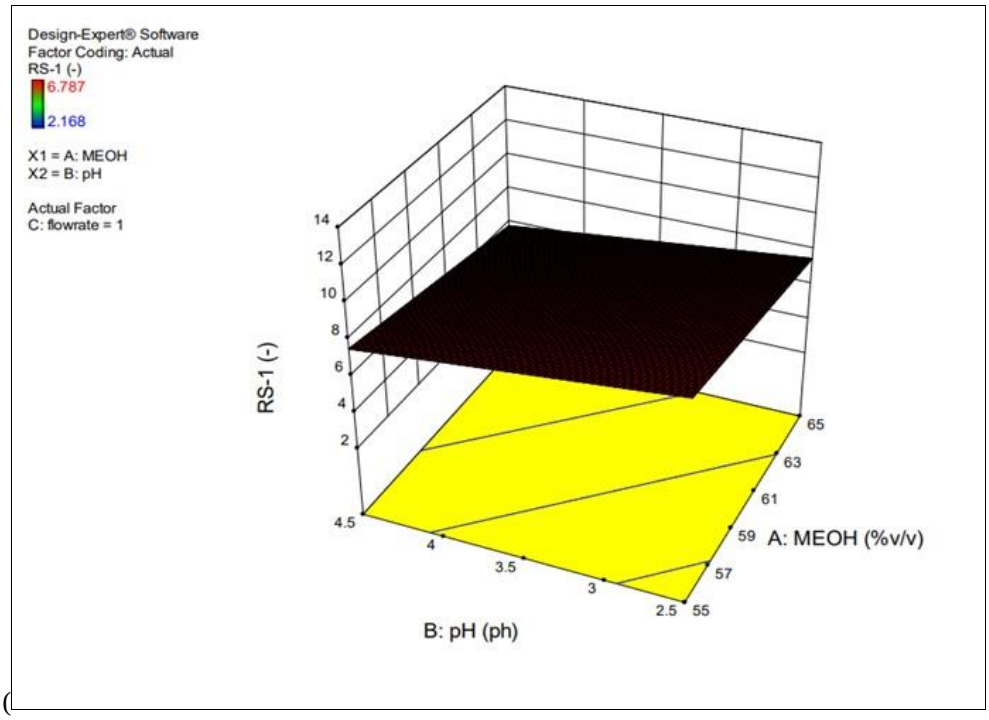


Figure 3: RESPONSE 1: ANOVA for Response Surface Linear model.

The model is significant, as indicated by the model's F-value of 3.89. A huge F-value like this could only happen by 4.0% probability because of noise. Model terms are considered significant when P-values are less than 0.0500. The 49.6 Lack of Fit F-value suggests that the Lack of Fit is not statistically significant in comparison to the pure error. A significant Lack of Fit F-value has a 10.9% probability of being caused by noise. A negligible mismatch is Non-significant. (**Figure *3***)



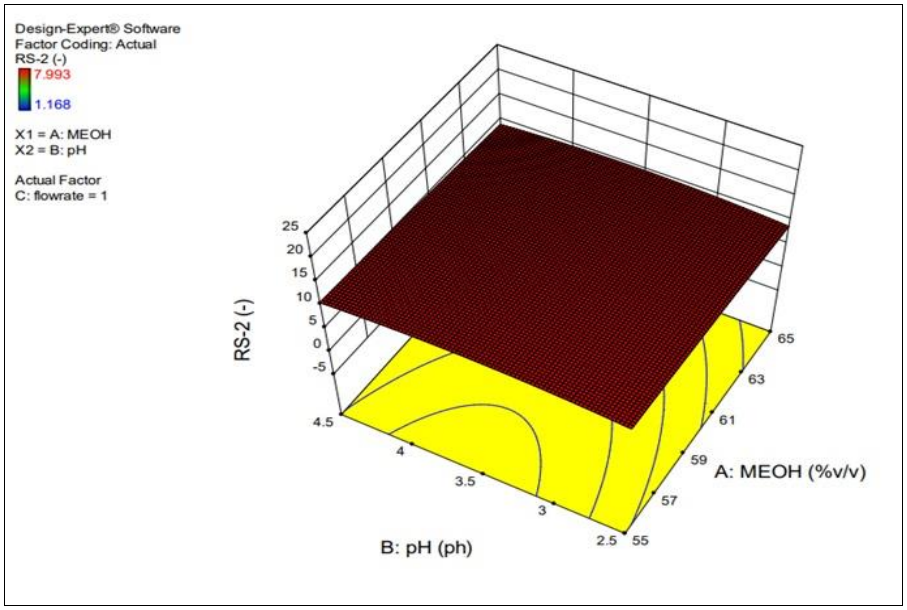


Figure 4: RESPONSE 2: ANOVA for Partial sum of squares – Type III.

The model is significant, according to the model's F-value of 63.46. This kind of big F-value has a 0.06% probability of being caused by noise. Model terms are considered significant when P-values are less than 0.0500. The 5.61 F-value for lack of fit indicates that there is no significant difference between the lack of fit and the pure error. A significant Lack of Fit F-value has a 29.8% probability of being caused by noise. A negligible mismatch is non-significant. (**Figure 4**)

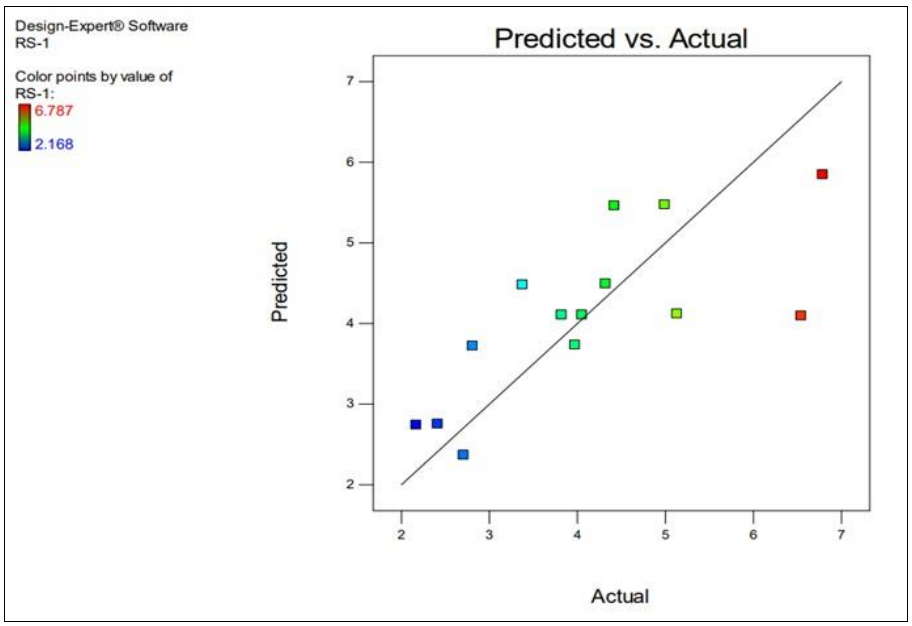


Figure 5: Predicted vs Actual Response 1.

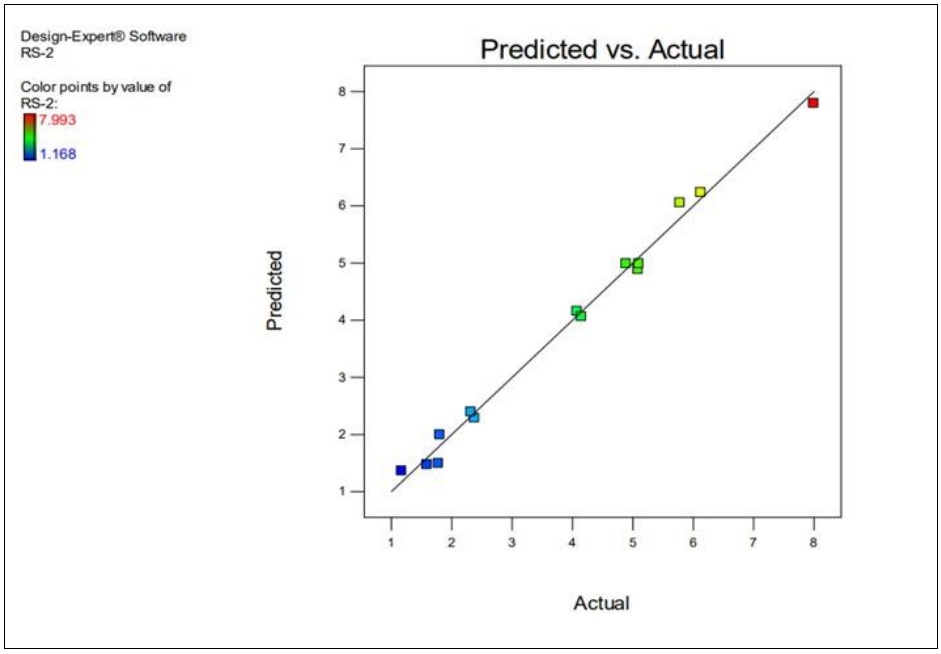


Figure 6: Predicted Vs Actual Response 2.

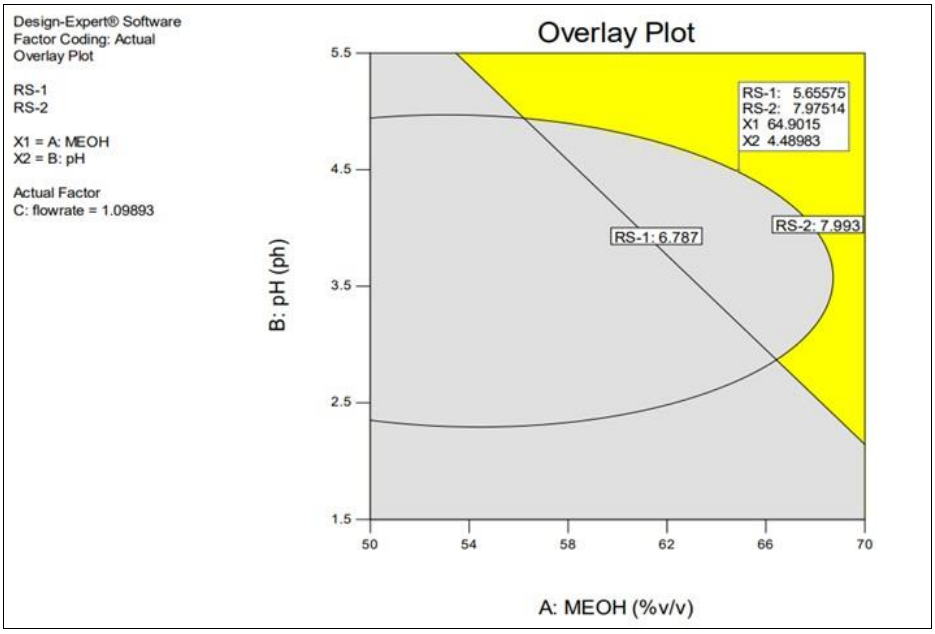


Figure 7: Overlain Plot Response 1 and Response 2.

Table 3: Optimization of RP-HPLC chromatographic condition.

Parameters	**Optimized attributes**		
Column	C18 (250 mm×4.6 mm×5 µm)		
Mobile Phase composition	Methanol: Acetonitrile: Buffer of phosphate pH 3.5 (50:20:30 % v/v/v)		
Flow Rate	1.0 mL/min		
Detection Wavelength	230 nm		
Injection Volume	10 µl		
pH	3.5		
Retention time (min)	ABA	DOLU	LAMI
	9.25	4.12	8.05

Validation

Specificity

By comparing the chromatograms of the blank, standard, and sample preparation solutions, it was demonstrated that there was no additive influence with the ABA, DOLU, and LAMI peaks.

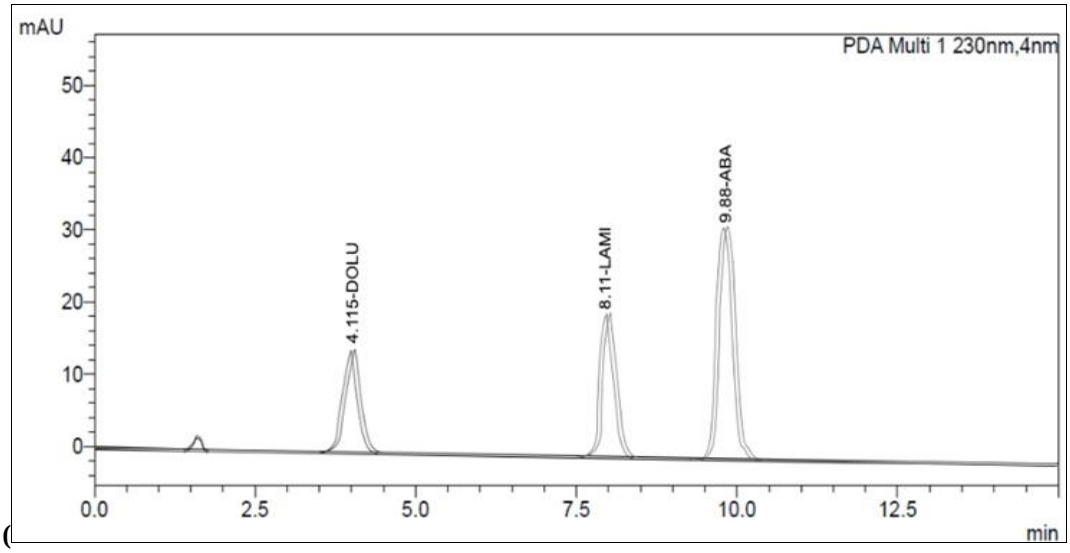


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Figure *8*)

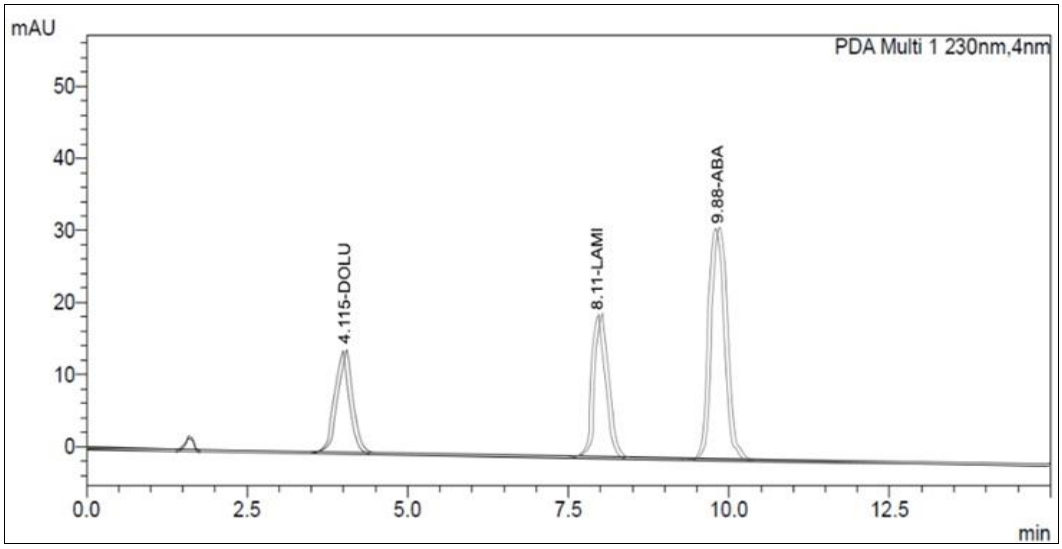

Figure 8: Overlain HPLC Chromatogram of standard and formulation.

Linearity

Table 4: Linearity data.

ABA			DOLU			LAMI		
Conc. (µg/mL)	**Peak Area ± SD**	**RSD (%)**	**Conc. (µg/mL)**	**Peak Area ± SD**	**RSD (%)**	**Conc. (µg/mL)**	**Peak Area ± SD**	**RSD (%)**
30	94499.29 ± 1683.52	1.78	**2.5**	53114.67 ± 891.02	1.68	**15**	57535.70 ± 540.58	0.94
60	193253.39 ± 2519.19	1.30	**5.0**	88728.80 ± 748.19	0.84	**30**	115841.91 ± 1298.95	1.12
90	308105.78 ± 2075.50	0.67	**7.5**	118246.82 ± 1313.84	1.11	**45**	162262 ± 1636.78	1.01
120	383599.12 ± 6981.12	1.82	**10.0**	162939.83 ± 1377.69	0.85	**60**	238953 ± 2319.11	0.97
150	479521.58 ± 2939.83	0.61	**12.5**	184618.26 ± 2082.78	1.13	**75**	295542 ± 1706.05	0.58
180	593016.36 ± 4019.81	0.68	**15.0**	223416.73 ± 2034.36	0.91	**90**	345935 ± 3945.39	1.14

Precision

Table 5: Repeatability data.

ABA		DOLU		LAMI	
Conc. (µg/mL)	**Area**	**Conc. (µg/mL)**	**Area**	**Conc. (µg/mL)**	**Area**
90	309543.28	**7.5**	118362.36	**45**	162643.25
	309543.28		117161.66		160403.27
	307432.23		117142.46		163623.36
	306140.33		117302.13		162423.29
	305432.28		117152.21		160843.41
	309543.28		119361.11		164642.42
SD	1870.66	**SD**	1873.66	**SD**	1611.76
% RSD	0.61	**% RSD**	1.57	**% RSD**	0.99

Table 6: Intraday and Inter day data.

Conc. (µg/mL)	**Intraday precision**		**Interday precision**	
	Peak Area (Mean ± SD)^n	**% RSD**	**Peak Area (Mean ± SD)^n**	**% RSD**

ABA				
30	94482.60 ± 652.56	0.69	94548.98 ± 1549.92	1.66
90	308172.93 ± 1588.25	0.51	308038.63 ± 2197.79	0.72
180	593208.29 ± 2291.32	0.39	592824.43 ± 5919.17	1.00
DOLU				
2.5	53173.36 ± 582.02	1.09	53147.97 ± 998.50	1.88
7.5	118221.85 ± 1016.84	0.86	118271.79 ± 1810.97	1.53
15	223062.23 ± 993.01	0.44	223119.90 ± 1913.96	0.86
LAMI				
15	57485.80 ± 612.26	1.07	57585.60 ± 536.94	0.93
45	162889.27 ± 1624.11	1.00	162302.27 ± 2401.76	1.48
90	345425.77 ± 1774.42	0.51	345660.40 ± 2942.23	0.85

Accuracy

Table 7: Accuracy data.

Level (%)	Target Conc. (µg/mL)	Spiked Conc. (µg/mL)	Total Conc. (µg/mL)	Conc. Found (µg/mL)	% Recovery
0	60	0	60	58.82	98.03
50	60	30	90	91.75	101.95
100	60	60	120	122.21	101.84
150	60	90	150	152.88	101.92
0	5.0	0	5.0	5.08	101.69
50	5.0	2.5	7.5	7.37	98.22
100	5.0	5.0	10.0	10.19	101.92
150	5.0	7.5	12.5	12.27	98.14
0	30	0	30	30.37	101.23
50	30	15	45	44.32	98.50
100	30	30	60	61.12	101.86
150	30	45	75	76.27	101.70

LOD and LOQ

Table 8: LOD and LOQ data.

	ABA	DOLU	LAMI
LOD (µg/mL)	1.82	0.78	1.87
LOQ (µg/mL)	5.55	2.38	5.67

Robustness

Table 9: Robustness data.

Effect of change in volume of Mobile Phase (methanol) - ± 2 % v						
	48 mL		**50 mL**		**52 mL**	
	Peak Area ± SD	**% RSD**	**Peak Area ± SD**	**% RSD**	**Peak Area ± SD**	**% RSD**
ABA (60 µg/mL)	193541.71 ± 3264.56	1.7	192875.05 ± 1600.90	0.83	193575.05 ± 2223.22	1.15
DOLU (5.0 µg/mL)	88788.51 ± 1107.71	0.94	887221.85 ± 1016.84	0.86	887555.18 ± 2127.61	1.82
LAMI (30 µg/mL)	115055.39 ± 1769.78	1.51	115388.71 ± 1253.87	1.08	116055.38 ± 1792.26	1.51
Effect of change in Flow Rate - ± 0.2 mL/min						
	0.8 ml/min		**1.0 ml/min**		**1.2 ml/min**	
	Peak Area ± SD	**% RSD**	**Peak Area ± SD**	**% RSD**	**Peak Area ± SD**	**% RSD**
ABA (60 µg/mL)	192575.05 ± 3321.44	1.73	192875.05 ± 1600.90	0.83	193875.05 ± 3056.58	1.6
DOLU (5.0 µg/mL)	88745.18 ± 1138.20	0.96	88821.85 ± 1016.84	0.86	88701.84 ± 1342.31	1.14
LAMI (30 µg/mL)	116355.38 ± 2203.69	1.89	116388.71 ± 1253.87	1.08	117455.38 ± 1845.07	1.57
Effect of change in Detection wavelength - ± 2λ						
	228 nm		**230 nm**		**232 nm**	
	Peak Area ± SD	**%**	**Peak Area ± SD**	**%**	**Peak Area ± SD**	**%**

		RSD		RSD		RSD
ABA (60 µg/mL)	192975.05 ± 3079.62	1.25	192875.05 ± 1600.90	0.83	193566.05 ± 2130.58	1.11
DOLU (5.0 µg/mL)	88672.05 ± 1420.85	1.22	88721.85 ± 1016.84	0.86	88655.38 ± 1500.74	1.29
LAMI (30 µg/mL)	117981.51 ± 1409.15	1.19	116388.71 ± 1253.87	1.08	119221.84 ± 891.55	0.75

Assay of synthetic mixture

Table 10: Analysis of Synthetic mixture.

Drugs	Conc. (µg/mL)	% Assay
ABA	60	101.64 ± 0.77
DOLU	5	101.23 ± 1.07
LAMI	30	98.60 ± 1.65

CONCLUSION

An approach to establish the desired quantity of AMA + DOLU + LAMI in API and its form as a tablet was established using RP-HPLC. The process was successfully validated while complying with the ICH Q2R1 recommendation. Along with risk evaluation, the QbD strategy enables us to comprehend the impacting elements and their impact on method responses. Box-Behnken central composite design was used to examine the interactions between all the variables. On individual method replies, the significant impacts of each factor and the interaction between the factors were examined. By utilizing the desirability function, the ideal chromatographic condition setting was found in the analytical design space. The specifications were determined to be within the recommendations' limitations. Thus, it can be said that the newly designed method can be often applied to the examination of Abacavir, Dolutegravir and Lamivudine.

Declarations

Acknowledgement

We acknowledge our sincere thanks to Bhagwan Mahavir Centre for Advance Research, Surat for providing facilities to carry out the research work.

Funding

Not applicable.

Conflict of interest

The authors declared that they have no competing interests.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Data availability

Data will be made available on request.

Aauthors contribution statement

Divyesh Vanparia: Methodology, Project administration, Investigation, Formal analysis, Validation.

Ronak R. Dedania, Zarna R. Dedania: Conceptualization, Resources, Investigation.

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