Factorial Design for Development of a High-Performance Thin-Layer Chromatography Method for the Simultaneous Estimation of Abacavir Sulfate, Lamivudine Hydrochloride, and Dolutegravir Sodium

Krupali Dave and **Sonal Desai***

Key Words:

Abacavir sulfate
Dolutegravir sodium
High-performance thin-layer chromatography
Lamivudine hydrochloride

Summary

A high-performance thin-layer chromatography (HPTLC) method has been developed for the simultaneous estimation of abacavir sulfate (ABC), lamivudine hydrochloride (LAM), and dolutegravir sodium (DTG) in an in-house physical mixture and it was validated as per the International Conference on Harmonization (ICH) guidelines. A 2^3 full factorial design was utilized to aid in method development and optimization. Effective chromatographic separation was achieved using pre-coated silica gel aluminum HPTLC plate 60 F_{254} as the stationary phase and ethyl acetate–ethanol–acetone–ammonia (4.478:0.740:0.50:0.15, *v/v*) as the mobile phase. The optimized chamber saturation time was kept at 30 min. Densitometric scanning was performed in the absorbance–reflectance mode at a detection wavelength of 267 nm. Quality by design approach was applied to evaluate the effect of all three factors, *i.e.,* volume of ethyl acetate, volume of ethanol, and volume of acetone, on the chromatographic response retardation factor (R_F) of each drug. The R_F values of ABC, LAM, and DTG were found to be 0.65, 0.34, and 0.26, respectively. The calibration curves were found to be linear by taking independently weighed standard solutions with concentrations of 4.8, 7.2, 9.6, 12.0, and 14.4 µg per band for ABC, 2.4, 3.6, 4.8, 6.0, and 7.2 µg per band for LAM, and 0.4, 0.6, 0.8, 1.0, and 1.2 µg per band for DTG. The limit of detection and limit of quantification values were found to be 0.9972 µg per band and 3.0218 µg per band for ABC, 0.2544 µg per band and 0.7711 µg per band for LAM, and 0.1004 µg per band and 0.3043 µg per band for DTG, respectively. The % recoveries of the drugs by the developed method were found in the range of 98.0955–100.9813% for ABC, 98.2616–99.9900% for LAM, and 98.4666–101.3000% for DTG, respectively. The proposed method was found to be novel, simple, accurate, reproducible, and robust. The developed HPTLC method was successfully applied for the quantitative determination of ABC, LAM, and DTG in an in-house physical mixture.

1 Introduction

Abacavir sulfate (ABC), chemically known as [(1S,4R)-4-[2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl]cyclopent-2-en-1-yl]methanol **(Figure 1),** and lamivudine hydrochloride (LAM), chemically known as (2*R*-cis)-4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1*H*)pyrimidinone (Figure 1) are nucleoside reverse transcriptase inhibitors (NRTIs), a carboxylic nucleoside category of anti-retroviral drugs used in the treatment of human immunodeficiency virus type 1 (HIV-1) infection [1, 2]. Dolutegravir sodium (DTG), chemically known as (RS)(4R,12aS)-*N*-(2,4-difluorobenzyl)-7-hydroxy-4-methyl-6,8-dioxo3,4,6,8,12,12a-hexahydro-2*H*-pyrido[11,21,4,5]pyrazino[2,1-b][1,3]oxazine-9-carboxamide (Figure 1), is an integrase strand transfer inhibitor (INSTI), a carboxylic nucleoside category of anti-retroviral drugs used in the treatment of HIV-1 infection [3]. A combination of all these three drugs is available with a brand name of Triumeq® as single-tablet regime for HIV medication.

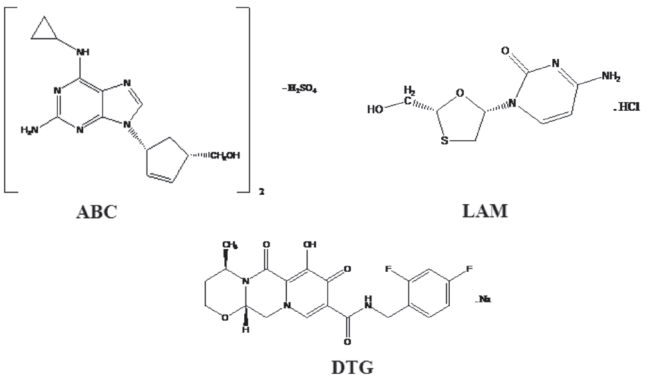

Figure 1

Chemical structures of abacavir sulfate (ABC), lamivudine hydrochloride (LAM), and dolutegravir sodium (DTG).

Several analytical methods, such as ultraviolet (UV), high-performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC), ultra-performance liquid

K. Dave and S. Desai, Department of Quality Assurance, S.S.R. College of Pharmacy, S.S.R. Campus, Sayli Road, Silvassa 396230, India.
*E-mail: sdesai6381@gmail.com

chromatography (UPLC), and spectrofluorimetric methods have been reported for the determination of ABC, LAM, and DTG alone. A few UV, HPLC, stability-indicating HPLC, and HPTLC methods have been described for the estimation of all three drugs individually and in combination [4–23]. A few HPLC, stability-indicating HPLC methods and ultra-high-performance liquid chromatography (UHPLC) methods have been reported for the simultaneous estimation of ABC, LAM, and DTG [24–28]. However, to the best of our knowledge, no HPTLC method for the simultaneous estimation of ABC, LAM, and DTG in combined dosage form has been reported.

HPTLC is the most advanced form of instrumental TLC that is controlled by integrated software which ensures the highest possible degree of usefulness, reliability, and reproducibility of generated data. It is preferred over HPLC because of a wide range of choice of mobile phase, low solvent consumption, low analysis cost, less sample clean-up steps, and simultaneous analysis of many samples [29–30].

Quality by design (QbD)-based analytical method development helps to recognize and reduce sources of variability that may lead to poor method performance. Quality is built into the development of the method itself, resulting in improved separations. A design space is a multi-dimensional combination of input variables (*e.g.,* material attributes), their interactions, and process parameters that have been demonstrated to provide assurance of quality. Working within the design space is not considered as a change. Movement out of the design space is considered to be a change and would normally initiate a regulatory post approval change process. Design of experiments (DOE) is a structured and organized system to determine the relationship among factors that influence outputs of a process or method. Factorial designs (full or fractional) and the response surface methodology (RSM) are characteristic tools for this kind of application. Among the various experimental designs, factorial design as a response surface was preferred for prediction of nonlinear response and also due to its flexibility, in terms of experimental runs and information related to the factors interaction effects.

This research paper describes the development of a HPTLC method for the simultaneous estimation of ABC, LAM, and DTG using factorial design.

2 Experimental

2.1 Materials

Reference standards of ABC, LAM, and DTG were procured with purity 98% *w*/*w* from an authentic source. All solvents and chemicals used were purchased from Merck Specialities Pvt. Ltd. (Mumbai, India). The physical mixture used in this study was prepared in-house.

2.2 Instrumentation and Software

HPTLC studies were performed on pre-coated silica gel 60 F_{254} aluminum plates (10 cm × 10 cm, 100-mm thickness; Merck, Darmstadt, Germany) as the stationary phase. Samples were applied with the help of a microsyringe (Linomat syringe 659.0014 – Hamilton, Bonaduz, Switzerland; CAMAG, Muttenz, Switzerland); sample application was performed using Linomat V applicator (CAMAG), and the plates were developed using a twin-trough chamber (20 cm × 10 cm; CAMAG). The plates were visualized using a UV chamber (CAMAG) and were scanned using a TLC Scanner IV (CAMAG).

All the data obtained in HPTLC studies were analysed by winCATS version 1.4.6 software (CAMAG). Factorial design was performed by Design-Expert trial version 10.0.0 (Stat-Ease Inc., Minneapolis, MN, USA). Statistical calculations for method validation were performed by use of Microsoft Excel 2013 software (Microsoft Corporation, Redmond, WA, USA).

2.3 Preparation of Standard Solutions

Accurately weighed quantity of 100 mg each of ABC and LAM and 10 mg of DTG were transferred into 10-mL separate volumetric flasks, respectively. To each flask, small amount of methanol was added, sonication was carried out for 15 min, and dilution was performed up to mark with methanol. This resulted in solution having a concentration of 10,000 µg mL^{-1} each of ABC and LAM and 1000 µg mL^{-1} of DTG. From the above stock solution, accurately measured 1 mL of each solution of ABC, LAM, and DTG was withdrawn and transferred into 10-mL separate volumetric flasks, and the volume was made up to the mark to get a concentration of 1000 µg mL^{-1} for both ABC and LAM and 100 µg mL^{-1} for DTG.

2.4 Preparation of Test Solution

An in-house physical mixture taking into consideration all the drug-excipient parameters was prepared in a ratio similar to the commercially available marketed formulation. A portion of powder equivalent to 600 mg of ABC, 300 mg of LAM, and 50 mg of DTG, *i.e.,* an amount of 1254 mg of the in-house powder mixture was accurately weighed and taken into a 100-mL volumetric flask; an amount of about 50 mL of methanol was added into the flask and sonicated for 15 min. The solution was further diluted up to the mark with methanol, mixed well and filtered to obtain solutions containing 6000 µg mL^{-1} for ABC, 3000 µg mL^{-1} for LAM and 500 µg mL^{-1} for DTG.

2.5 Initial Method Optimization

Appropriate volumes of standard sample solutions were applied in the form of band having 6-mm length on pre-coated silica gel aluminum plate 60 F_{254}, (10 cm × 10 cm) of 100-mm thickness using a CAMAG Linomat V sample applicator. The chamber saturation time was 30 min. The distance travelled by the mobile phase was fixed at 8 cm. Ascending development technique was used for plate development. The TLC plates that were then dried and scanned in the absorbance reflectance mode at a wavelength of 267 nm. The source of radiation was a deuterium lamp. The slit dimension was 6.0 mm × 0.30 mm. The scanning speed was 20 mm s^{-1}, and the data resolution was 100 mm per step. The concentration of each drug was determined from peak areas using linear regression analysis.

Initial mobile phase optimization was carried out based on one factor at a time (OFAT) approach. Different solvents in different combinations were tried. The use of ethyl acetate in combination with ethanol, acetone, and ammonia produced the desired peak shapes, peak intensity, and separation. This mobile phase was further optimized using factorial design.

2.6 Software-Aided Method Optimization

A 2^3 factorial design was applied for all three drugs to optimize the composition of mobile phase. 2^3 factorial design indicates that there were two levels and three factors involved in it. The two levels were low (−1) and high (+1), whereas the three factors were the volume of ethyl acetate (*A*), the volume of ethanol (*B*), and the volume of acetone (*C*). The chromatographic responses evaluated in the trial for all three drugs were R_F of ABC, R_F of LAM, and R_F of DTG. This design was specifically selected since it required fewer runs (8) as compared to the others. It was suitable for exploring response surface and creating different models with Design Expert® software (Version 10.0.0, trial version). The values of *A*, *B*, and *C* were 3 mL, 0.5 mL, and 0.5 mL for low level and 5 mL, 1 mL, and 1 mL for high level, respectively. The chromatographic conditions along with a range of dependent variables are specified in **Table 1.**

Table 1

Observed responses for 8 experimental runs for ABC, LAM, and DTG.

		Factors			Response		
	Level	Volume of ethyl acetate	Volume of ethanol	Volume of acetone	R_F of ABC	R_F of LAM	R_F of DTG
R1	+1,+1,+1	5	1.0	1.0	0.77	0.52	0.46
R2	−1,−1,−1	3	0.5	0.5	0.93	0.61	0.28
R3	+1,−1,−1	5	0.5	0.5	0.55	0.22	0.11
R4	−1,+1,−1	3	1.0	0.5	0.80	0.56	0.51
R5	−1,+1,+1	3	1.0	1.0	0.86	0.63	0.59
R6	+1,+1,−1	5	1.0	0.5	0.71	0.40	0.32
R7	+1,−1,+1	5	0.5	1.0	0.59	0.24	0.11
R8	−1,−1,+1	3	0.5	1.0	0.72	0.46	0.41

A total of 8 trials with different mobile phase ratios were performed, and the R_F value for all drugs was measured. The response values (R_F value of all drugs) were added to the Design Expert software, and the data were analyzed.

2.7 Model Validation

Analysis of variance (ANOVA) was applied for validating the model to the response to examine the significance of model. Lack-of-fit test, which indicates insignificant lack-of-fit value corresponding to a higher *p*-value as compared to model *F*-value, was used to examine the model. The design calculated the standard deviation, R^2, predicted R^2, adjusted R^2, and *%CV.* "Adeq Precision" measured the signal-to-noise ratio. A ratio greater than 4.0 was desirable for "Adeq Precision". For visualization of effects of independent variable and their interactions on the responses, 3D response surface plots and perturbation plots were obtained. Equations were generated for response showing its relationship with independent variables.

The expected criteria for each response was chosen, and based on that, various solutions with desirability were obtained. The solutions for the optimized mobile phase were tried in laboratory. The experimental values of each response obtained were compared with the expected values, and the optimum mobile phase composition was finalized.

2.8 Chromatographic Conditions

The following chromatographic conditions were used for the HPTLC analysis:

- Stationary phase: pre-coated silica gel G F_{254} plates
- Mobile phase: ethyl acetate–ethanol–acetone–ammonia (4.478:0.740:0.50:0.15, *v/v*)
- Chamber saturation time: 30 min
- Development distance: 8 cm
- Sample volume: 5 µL
- Band width: 6 mm
- Detection mode: absorbance–reflactance
- Detection wavelength: 267 nm

2.9 Method Validation

The method was validated as per the International Conference on Harmonization (ICH) guidelines Q2 (R1) [31] for various parameters that include specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, and robustness. The specificity of the method was ascertained by analyzing peak purity of all three drugs in standard solution and test solution. After chromatographic development, the R_F values and spectra of all the three drugs were compared to those of standards. The peak purity of each of the three drugs was assessed by comparing the spectra at 3 points, *i.e.,* leading edge of the peak, peak maxima, and tailing edge of the peak. The presence of any interference was checked. Linearity was evaluated by performing 5 measurements of independently weighed standard solutions with concentrations of 4.8, 7.2, 9.6, 12.0, and 14.4 µg per band for ABC, 2.4, 3.6, 4.8, 6.0, and 7.2 µg per band for LAM, and 0.4, 0.6, 0.8, 1.0, and 1.2 µg per band for DTG. The homoscedasticity of the variances along the regression line of each drug was verified using the Bartlett's test. Although the homoscedasticity requirement was fulfilled for regression lines, the standard deviation of the slope and the intercept were calculated using ordinary least squares. Calibration plots were prepared for each drug, and the regression coefficient was calculated. To assess the sensitivity of the proposed method, LOD and LOQ were calculated based on a standard deviation method. Accuracy of the proposed method was checked by performing recovery at 3 concentration levels (50%, 100%, and 150%). Recovery studies were carried out by spiking 3 different amounts of ABC standard (2.4 µg per band, 4.8 µg per band, and 7.2 µg per band) to test (4.8 µg per band), LAM standard (1.2 µg per band, 2.4 µg per band, and 3.6 µg per band) to test (2.4 µg per band), and DTG standard (0.2 µg per band, 0.4 µg per band, and 0.6 µg per band) to test (0.4 µg per band) by a standard addition method. Triplicate measurements were performed at each level and % recovery was calculated.

Method precision was evaluated by performing intra-day and inter-day precision studies at 3 concentration levels. Intra-day precision was carried out by triplicate analysis of independently prepared test solutions with concentrations of 4.8 µg

per band, 9.6 µg per band, and 14.4 µg per band for ABC, 2.4 µg per band, 3.6 µg per band, and 7.2 µg per band for LAM, and 0.4 µg per band, 0.8 µg per band, and 1.2 µg per band for DTG, respectively. The peak area was measured at each level and *%RSD* was calculated. Intra-day precision studies were carried out on the same day at different time intervals, whereas inter-day studies were carried out for 3 consecutive days. The robustness of the method was evaluated by varying method parameters, such as saturation time (25 min and 35 min), the distance travelled (7 cm and 9 cm), and detection wavelength (266 nm and 268 nm). Each parameter was varied at a time. It was assessed by using triplicate analysis of independently prepared standard solutions with concentrations of 4.8 µg per band of ABC, 2.4 µg per band of LAM, and 0.4 µg per band of DTG) and calculating the values of mean area and *%RSD*.

2.10 Analysis of In-House Physical Mixture

From test solution, accurately measured 1.6 µL of the filtered solution (containing 9.6 µg per band of ABC, 4.8 µg per band of LAM, and 0.8 µg per band of DTG) was applied on the HPTLC plate. The plate was developed and scanned. The analysis was repeated in triplicate. The content of each drug in the physical powder mixture was calculated.

3 Results and Discussion

Solubility of ABC, LAM, and DTG was checked using a variety of solvents like distilled water, methanol, acetonitrile, and chloroform of analytical reagent (AR) grade. It was found that ABC and LAM were completely soluble in methanol and distilled water, whereas DTG was slightly soluble in distilled water and completely soluble in methanol after sonication. Due to the more volatile nature of methanol as compared to water, methanol was selected as the solvent for preparation of standard and test solution. To determine the appropriate wavelength, UV spectra of all three drugs using CAMAG TLC Scanner IV were recorded, and it was found that all three drugs produced high intensity at a wavelength of 267 nm **(Figure 2).** Hence, 267 nm was selected as detection wavelength for analysis. Initial method development was carried out based on trial and error. Mobile phase optimization was carried out in different solvent systems and different ratios of various solvents were tried such as *n*-hexane, chloroform, toluene, ethyl acetate, acetonitrile, acetone, ethanol, and methanol. Ethyl acetate–ethanol–acetone–ammonia in a ratio of 4:0.7:0.5:0.15 (*v*/*v*) produced better separation as compared to other mobile phases. This mobile phase was further optimized by design expert software. In the software-aided optimization step, the effects of the three factors, *i.e.,* volume of ethyl acetate, volume of ethanol, and volume of acetone on chromatographic responses were evaluated. The 3D surface plot and perturbation plot for all the three drugs were generated by the software.

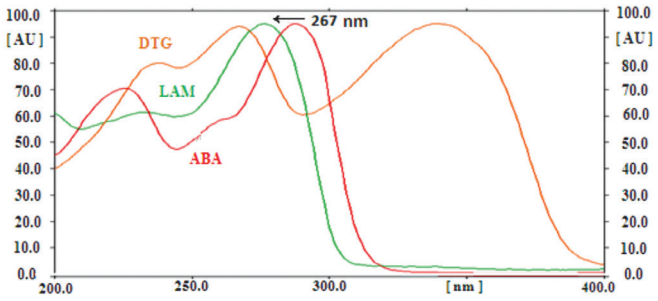

Figure 2

Overlaid spectra of ABC, LAM, and DTG standards.

The 3D plot **(Figure 3A)** indicates that factor *A* (volume of ethyl acetate) has a negative effect and factors *B* (volume of ethanol) and *C* (volume of acetone) have no effect on the R_F of ABC. As the proportion of ethyl acetate in the mobile phase was increased, the R_F decreased. The 3D plot **(Figure 3B)** indicates a negative effect of factor *A* (volume of ethyl acetate) and a positive effect of factor *B* (volume of ethanol) on R_F of LAM. As the proportion of ethyl acetate in the mobile phase was decreased and the volume of ethanol was increased, the R_F of LAM also increased. The 3D plot **(Figure 3C)** shows that all variables affected the R_F of DTG. This plot indicates a negative effect of factor *A* (volume of ethyl acetate) and positive effects of factor *B* (volume of ethanol) and factor *C* (volume of acetone) on response R_F of DTG. As the proportion of ethyl acetate in the mobile phase was decreased and the volumes of ethanol and acetone were increased, the R_F of DTG increased. As per 3D plots, a decrease in the ethyl acetate content of the mobile phase resulted in an increase in the R_F of all three drugs. Perturbation plots reveal the effect of factors and deviation in response from its nominal value with all other factors held constant at a reference point, and the steepest slope or curvature indicates sensitiveness to specific factors. Perturbation plots indicate that

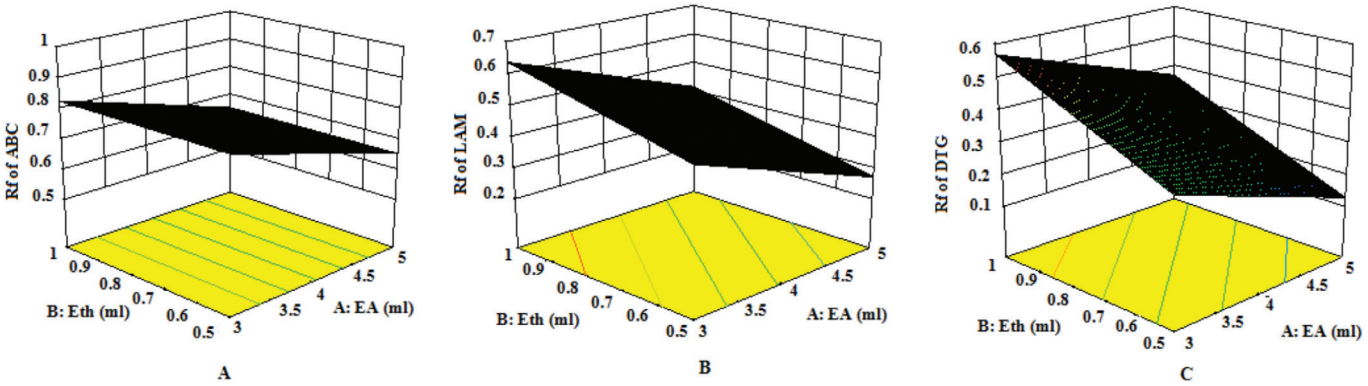

Figure 3

3D surface plot of (A) ABC, (B) LAM, and (C) DTG for response R_F.

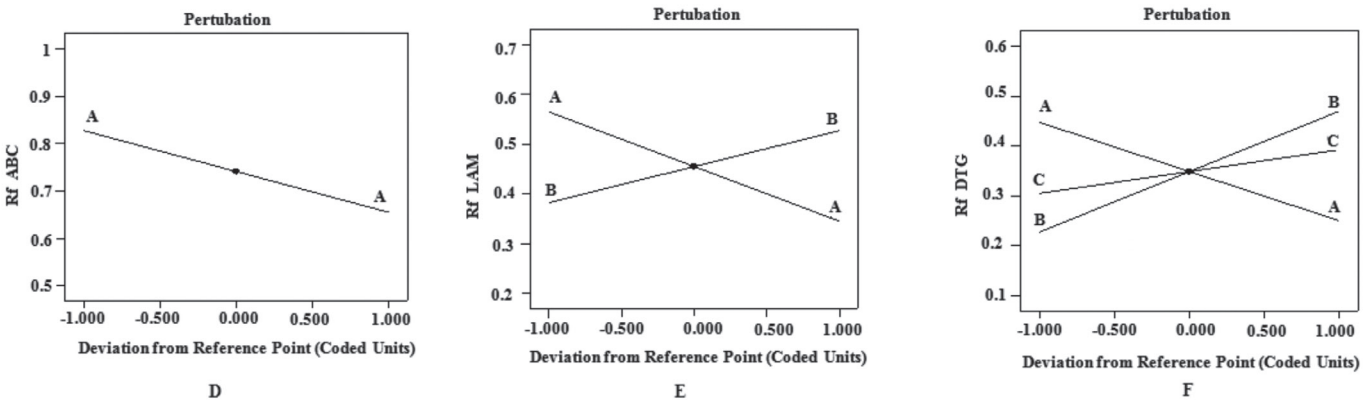

Figure 4

Perturbation plot showing effect of factors on R_F values of ABC (D), LAM (E), and DTG (F).

a small variation in factor *A* (volume of ethyl acretate) had a significant effect on the R_F of all three drugs and factor *B* (volume of ethanol) had a significant effect on the R_F of LAM and DTG, whereas factor *C* (volume of acetone) had an effect only on the response R_F of DTG as shown in **Figures 4D–4F.**

Analysis of variance (ANOVA) was used to validate the model **(Table 2).** The polynomial equation in terms of coded factors for each response was obtained to predict the response for given levels of each factor. Factor coefficients of equation were used to evaluate the relative impact of the factors. The negative value of the coefficient for factor *A* confirmed that the volume of ethyl acetate had a negative impact on the R_F values of all three drugs. Meanwhile, positive values of coefficients for factors B and C confirmed that the volume of ethanol and the volume of acetone had a positive effect. All model terms were significant as the *p*-value was less than 0.05. The value of adequate precision greater than 4.0 indicated an adequate signal. The low % coefficient of variance (CV) indicated good relationship between the experimental data and those of the fitted models.

As per the desirability factors provided by the software, different combinations of ethyl acetate, ethanol, and acetone at suggested proportions were tried, and the responses for all three drugs were evaluated. In order to validate the model, trials were conducted with the suggested optimized mobile phase. From the trials suggested by the software, the best mobile phase was found to be ethyl acetate–ethanol–acetone–ammonia (4.478:0.740:0.50:0.15, *v*/*v*). With this mobile phase, well defined bands of ABC at $R_F = 0.65$, LAM at $R_F = 0.34$, and DTG at $R_F = 0.26$ were obtained when the chamber saturated time for the mobile phase was kept at 30 min at room temperature **(Figure 5).** The peak purity of all three drugs in the in-house mixture was evaluated by comparing with the overlaid spectra of standard at the leading edge of the peak, peak maxima, and tailing edge of the peak **(Figures 6G–6I),** showing good correlation, *i.e., r* (S,M) and *r* (M,E) were 0.9994 and 0.9972 for ABC, 0.9998 and 0.9935 for LAM, and 0.9986 and 0.9893 for DTG, respectively. As there was no interference in the spectra of all the three drugs and as the peak purity was more than 0.99, it indicated that the proposed method was specific. The developed and optimized method was validated. In linearity studies, the linear relationship between area and concentration was observed by taking independently prepared standard solutions with concentrations of 4.8, 7.2, 9.6, 12.0, and 14.4 µg per band for ABC, 2.4, 3.6, 4.8, 6.0, and 7.2 µg per band for LAM, and 0.4, 0.6, 0.8, 1.0, and 1.2 µg per band for DTG **(Table 3).**

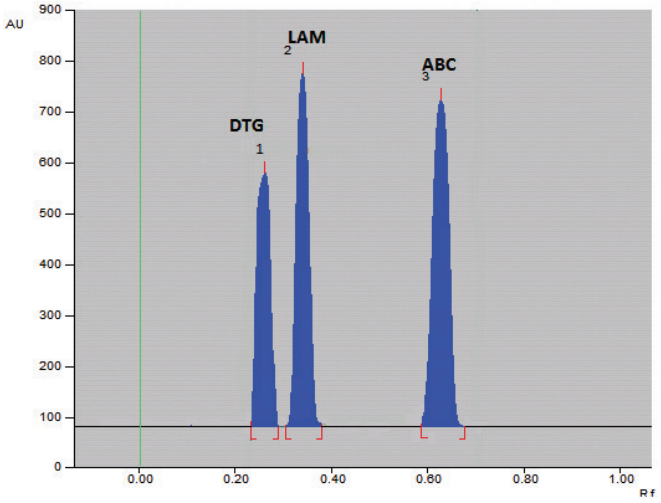

Figure 5

Densitogram using ethyl acetate–ethanol–acetone–ammonia (4.478:0.740:0.50:0.15, *v*/*v*).

Table 2

Predicted response models and statistical parameters by ANOVA.

Response (R_F value)	Polynomial equation for retardation factor	Model *p*-value	% Coefficient of variance	Adequate precision
ABC	$+0.74 - 0.86A$	0.0441	12.96	4.591
LAM	$0.46 - 0.11A + 0.073B$	0.0188	18.53	7.069
DTG	$+0.35 - 0.999A + 0.12B + 0.044C$	0.0031	13.56	15.771

Bartlett's test was used to evaluate the homoscedasticity of variance. Low value of $c\chi^2$ than tabulated value confirmed that all three drugs showed homogenous variance. The limits of detection (LOD) and quantification (LOQ) values were found to be 0.9972 µg per band and 3.0218 µg per band for ABC, 0.2544 µg

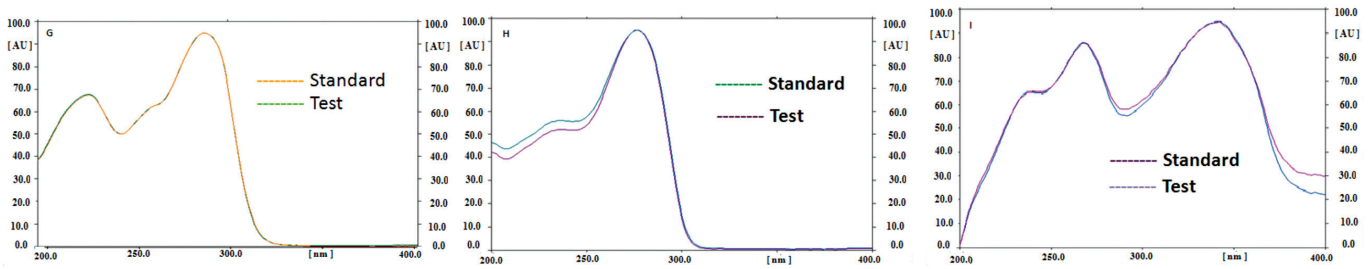

Figure 6

Overlaid spectra of samples with standard showing peak purity: ABC (G), LAM (H), and DTG (I).

Table 3

Linear regression parameters for ABC, LAM, and DTG by the HPTLC method.

Parameters	ABC	LAM	DTG
Calibration range[a] [µg per band]	4.8–14.4	2.4–7.2	0.4–1.2
Regression equation	$y = 1.1368x + 15048$	$y = 1.0748x + 12767$	$y = 5.6929x + 4108.2$
Correlation coefficient	0.9967	0.9966	0.9940
Standard deviation of slope	0.0259	0.0129	0.2216
Confidence limit of slope[b]	1098 to 1162	1064 to 1089	5461 to 5945
Standard deviation of intercept	341.47	83.01	173.56
Confidence limit of intercept[b]	14765.23–15595.60	12682.68–12884.86	3914.42–4358.16
Bartlett's test[c] (χ^2)	0.0139	0.0046	0.0437

[a]Five replicates, SD = standard deviation, *%RSD* = relative standard deviation
[b]Confidence interval at 95% confidence level and 4 degrees of freedom ($t = 2.57$)
[c]$c\chi^2$ critical value = 9.488 at $\alpha = 0.05$

per band and 0.7711 µg per band for LAM, and 0.1004 µg per band and 0.3043 µg per band for DTG, respectively. The proposed method was found to be accurate, as % recovery was found in the range of 98.0955–100.9813% for ABC, 98.2616–99.9900% for LAM, and 98.4666–101.3000% for DTG. The method was found to be precise as the values of *%RSD* of peak area for both intra-day and inter-day precision studies for all three drugs at all levels were less than 2.0. The method was robust as small deliberate changes in method parameters like chamber saturation time, distance travelled by mobile phase, and detection wavelength did not significantly affect the method performance as values of *%RSD* of peak area were less than 2.0 for all changed conditions.

The prepared in-house physical mixture was analyzed using the developed method. The in-house physical mixture of all three drugs showed three peaks at R_F of 0.65, 0.34, and 0.26 for ABC, LAM, and DTG, respectively, that was found to be at the same R_F for all three respective standards. The recovery of ABC, LAM, and DTG was found to be 100.0069%, 100.1146%, and 99.55%, respectively, indicating good agreement between the amounts measured and the label claims **(Table 4).**

The developed method was found to be novel, specific, simple, accurate, reproducible, and robust for the simultaneous estimation of ABC, LAM, and DTG in the in-house physical mixture. The utilization of factorial design in method optimization allowed the study of the effects of various solvents on R_F of three drugs. It was found that the volume of ethyl acetate had possibly significant effects on the R_F of all three drugs. The proposed method was successfully applied for the simultaneous analysis of all three drugs and can be used for the routine quality control of dosage containing these drugs.

Table 4

% Recovery of in-house physical mixture at 267 nm.

Drugs	Amount spotted [µg per band]	% Assay[a]	Mean ± SD	*%RSD*
ABC	9.6	99.8906 100.1313 99.9989	100.0069 ± 0.1205	0.1205
LAM	4.8	100.1910 99.9205 100.2104	100.1146 ± 0.1608	0.1606
DTG	0.8	99.9202 99.5500 99.1701	99.5500 ± 0.3750	0.3766

[a]Average of 3 determinations
SD = standard deviation; *%RSD* = relative standard deviation

4 Conclusion

Factorial-design-aided HPTLC method has been developed for the simultaneous estimation of ABC, LAM, and DTG.

A simple, rapid, linear, accurate, and precise HPTLC method was developed utilizing QbD approach for the simultaneous determination of ABC, LAM, and DTG. A 2^3 factorial design was applied to optimize chromatographic parameters such as volume of ethyl acetate, volume of ethanol, and volume of acetone with respect to responses like R_F of ABC, R_F of LAM, and R_F of DTG. The main aim of implementing analytical QbD in method optimization was to identify the failures and the critical quality attributes as well as to establish a design space because moving within a design space would not require post approval changes, thereby reducing the cost involved. The developed method could detect all the drugs at the microgram level.

The method represents a good procedure for determination of ABC, LAM, and DTG in bulk pharmaceuticals and dosage forms. The method was applied for the simultaneous estimation of ABC, LAM, and DTG in an in-house physical mixture.

Conflicts of Interest

All authors have no conflict of interest.

Acknowledgments

The authors are grateful to Anand Pharmacy College, Anand, India, for providing necessary facilities to conduct HPTLC studies. The authors are also thankful to Mr. Vinod Ramani, Assistant Professor, Sumandeep Vidyapeeth University, Vadodara, India, for providing assistance in statistical analysis.

References

[1] *H.P. Rang, M.M. Dale, J.M. Ritter, P.K. Moore,* Pharmacology, 5th edn., Elsevier Science, Churchill Livingstone, London, 2003.

[2] *K.D. Tripathi,* Essentials of Medical Pharmacology, 6th edn., Jaypee Brothers Medical Publishers (P) Ltd., New Delhi, 2008.

[3] *L. Bruton, K. Parker, D. Blumenthal, I. Buxton,* Goodman and Gilman's Manual of Pharmacology and Therapeutics, 13th edn., McGraw-Hill, New York, NY, 2013.

[4] *R. Appala, V. Rao, V. Prakash, K. Mukkanti,* E-J. Chem. **5** (2008) 511–514.

[5] *N. Kumar, R. Appala, K. Mukkanti,* Int. J. Chem. Sci. **6** (2008) 2066–2072.

[6] *R. Venkatamahesh, D. Dhachinamoorthi,* Int. J. Pharm. Tech. Res. **3** (2011) 356–359.

[7] *M. Lenkalapally, K. Vangala, S. Takkadapalliwar, S. Bandapalli, P. Pasham,* Vedic Res. Int. Phytomed. J. **1** (2013) 64–68.

[8] *A. Kumari, K. Prakash, K.E.V. Nagoji, M.E.B. Rao,* Asian J. Chem. **19** (2007) 3412–3416.

[9] *B. Venkata, B.S. Rao, S.S. Dubey,* Int. J. Pharm. Sci. Res. **3** (2012) 2590–2598.

[10] *P. Vukkum, G.R. Deshpande, J.M. Babu, R. Murlikrishna, P. Jagu,* Sci. Pharm. **80** (2012) 903–921.

[11] *S. Lavanya, S.K. Mansura Begum, K. Nagamalleswara Rao, K. Gayathri,* Int. J. Pharm. Sci. Res. **5** (2014) 790–799.

[12] *P.K. Saini, C.L. Jain, R.M. Singh, S.C. Mathur, G.N. Singh, M. Naslam,* J. Pharm. Res. **15** (2016) 2213–2220.

[13] *A. Singh, L. Nath, N. Pani,* J. Pharm. Anal. **1** (2011) 251–257.

[14] *A. Manikanta, B. Naga Sandhya, M. Nasare, V.V.L.N Prasad, P. Diwan,* J. Adv. Pharm. Edu. Res. **2** (2012) 210–216.

[15] *P. Hariprasad, P. Patel, D. Vijaysree, K. Vamshi,* Int. J. Pharm. Sci. Res. **3** (2012) 416–421.

[16] *P. Venkatesh, M. Daggumati,* J. Pharm. Anal. **2** (2012) 152–155.

[17] *K. Shaikh, S. Subramaniam, M. Sundaram, A. Sivasubramaniam,* Int. J. Pharm. Tech. Res. **5** (2013) 1321–1331.

[18] *B. Naga, A. Manikanta, M. Nasare, V. Kumar, S. Jetta, P. Diwan,* Pharm. Lett. **5** (2013) 148–155.

[19] *G. Abirami, K. Anandakuma, S. Murugan, B. Ashok,* J. Anal. Chem. **68** (2013) 815–821.

[20] *B.G. Balasaheb, A.K. Balasaheb, R.S. Throat, K. Jijabapu, P.S. Sudhakar,* Malaysian J. Anal. Sci. **19** (2015) 1156–1163.

[21] *N.M. Rao, D.G. Sankar,* Future J. Pharm. Sci. **1** (2015) 73–77.

[22] *K. Tarari,* Pharm. Chem. **9** (2017) 117–127.

[23] *G. Bhavar, S. Pekamwar, K. Aher, R. Throat, S. Chadhari,* Sci. Pharm. **84** (2016) 305–320.

[24] *G. Shankar, M. Monika*, Int. J. Pharm. Anal. Res. **4** (2015) 391–398.

[25] *N. Khaleel, A. Rahaman,* World J. Pharm. Res. **4** (2015) 1453–1476.

[26] *N. Nagubandi, N. Pappula,* World J. Pharm. Sci. **3** (2015) 2135–2140.

[27] *N. Pal, A.S. Rao, P. Ravikumar,* Asian J. Chem. **28** (2016) 273–276.

[28] *P. Saidulu, S.K. Mastanamma,* Int. J. Pharm. Rev. Res. **37** (2016) 249–257.

[29] *M. Attimarad, K.K. Mueen Ahmed, B.E. Aldhubaib, S. Harsha,* Pharm. Methods **2** (2011) 71–75.

[30] *A. Hala,* HPTLC Instrumentation: An Overview. Pharmatutor. Pharmacy infopedia (www.pharmatutor.org) (accessed on 20/09/2016).

[31] ICH harmonized tripartite guideline, Validation of analytical procedures: text and methodology, Q2 (R1), Geneva, 2005, 1–13.

Ms received: April 24, 2018
Accepted: September 24, 2018