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Original Article

Chemometrics Assisted Spectrophotometric Method Development and Validation for Simultaneous Estimation of Abacavir, Lamivudine and Dolutegravir in Dosage Form

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ABSTRACT

Aim: Multivariate methods, particularly Classical Least Square, Inverse Least Square (ILS), Partial Least Square and Principal Component Regression are invented for the quantitation of the Abacavir sulphate (ABA), Lamivudine (LAM) and Dolutegravir sodium (DOL) in their combined tablet formulation. **Materials and Methods:** Chemometrics is the integration of statistical and mathematical approaches to analytical data in order to extract as much information as possible. The calibration and validation sets were built using fractional factorial design. 32 ternary mixtures of calibration sets and 16 mixtures of validation set were prepared. The absorbance data matrix was created by measuring absorbance in the range of 230 to 308 nm (Δλ = 3 nm) at 27 distinct wavelengths. The models were developed with the aid of MATLAB 2018a, Minitab 16.1.1 and MS Excel 2010. **Results:** The recovery values were close to 100% with low standard deviation (SD) justified the high accuracy of the proposed methods. For chemometrics approaches, the RMSECV, RMSEC, and RMSEP values produced show good accuracy and precision. The values of model diagnostic tools were lowest for ILS method. **Conclusion:** The ILS was shown to be the most appropriate method among produced chemometric models. The chemometric method is more accurate and precise than conventional methods as the total absorbance of the ternary mixture was measured and can be utilised in simultaneous estimation. With great recoveries and precision, the proposed approach was successfully used to the assay of formulation.

Keywords: Abacavir sulphate, Lamivudine, Dolutegravir sodium, Chemometrics, Spectophotometric, Validation.

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INTRODUCTION

AIDS is considered as the final chapter in the life of HIV infected patients. Pre-exposure prophylaxis (PrEP) with oral antiretroviral medicines has recently been shown to minimise the risk of HIV infection.[1] To prevent the repeated dose and to achieve better efficacy generally fixed-dose combinations are used.[2] The research which is presented here is done on combination of integrase inhibitor (Dolutegravir) and reverse transcriptase inhibitor (Abacavir and Lamivudine). From the view of analytical method development for these multi drugs, it is difficult to develop but it is easier for patient comfort as it reduces the dose frequency.

The drug abacavir sulphate (ABA) is used to treat HIV and AIDS infections.[3] Chemically, it is {(1S, 4R)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl] cyclopent-2-en-1-yl} methanol (Figure 1). Lamivudine (LAM) is a drug used in treatment of Hepatitis B and HIV-1.[4] Chemically, it is known as 4-amino-1-[(2R, 5S)-2-(hydroxymethyl)-1, 3- oxathiolan-5-yl]-1, 2-dihydropyrimidin-2-one (Figure 1). These both drugs act by inhibiting the reverse transcriptase enzyme thereby block the viral growth. Dolutegravir Sodium (DOL) is a intergrase inhibitor[5] and chemically is (4R,12aS)-N-(2,4-difluorobenzyl)-7-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2H-pyrido 1', 2': 4, 5] pyrazino [2,1-b][1,3]oxazine-9-carboxamide. The structure of Dolutegravir Sodium is shown in Figure 1. INBEC is a multidrug combination therapy comprised of 600 mg Abacavir sulphate, 300 mg Lamivudine, 50 mg Dolutegravir and is approved by FDA for treatment of HIV-1 infection in adults as a complete regimen.

Literature Survey reveals there are several analytical methods like HPLC and HPTLC[6-15] reported for quantitation of Abacavir

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Sulphate, Lamivudine and Dolutegravir sodium in combination. A UV-spectroscopic method is reported for simultaneous quantitation of Abacavir Sulphate, Lamivudine and Dolutegravir Sodium in dosage form.[16] But, till date no chemometric method has apparently been reported for quantitation of Abacavir Sulphate, Lamivudine and Dolutegravir Sodium in combined dosage form. As a result, a multivariate approach for resolving complex spectra was developed and validated.

Chemometrics is a sort of multivariate analysis that considers multiple variables at once. When this tool is implemented for UV-spectrophotometric method, then many wavelengths as variable and absorbance at each wavelength is taken into consideration. This tool has an advantage over traditional methods like sensitive, minimise noise, does not require prior separation, can be easily applied, save time and energy.[17-19] In chemometrics, there are two types of dataset namely, calibration and validation set. The results of calibration set were utilised to calculate the amount of components in unknown sample. The additional advantage of chemometric method was calibration can be performed by taking the consideration of amount of analyte of interest without considering the amount of all other components present in the mixture. The chemometric method was found suitable for the resolution of overlapping spectra for quantitative determination. The detailed description of chemometric techniques is given in literature.[20-24] In the present study, four different chemometric models are successfully developed for the quantitation of cited drugs.

MATERIALS AND METHODS

Instrumentation and Softwares

The absorbance of all the solutions was measured using a Shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer equipped with a pair of 10 mm matched quartz cells. UV Probe software was employed for recording the spectra of different calibration set and validation set. Chemometric calculations were performed using MATLAB-R2018a, Minitab 16.1.1 and Microsoft Excel 2010. MVC1toolbox (with MATLAB) was employed to estimate Figures of merit for multivariate calibration models.

Materials

Abacavir Sulphate, Lamivudine and Dolutegravir Sodium bulk powder was procured from Cipla Pharmaceuticals, Ltd., Mumbai. The Commercial Tablets (INBEC) comprising of 600 mg ABA, 300 mg LAM and 50 mg DOL was purchased from the local market. Methanol (A.R.) (SD fine grade chemicals Ltd.), Distilled water and other chemicals utilised were of analytical grade. Whatman filter paper no. 41 was used.

Preparation of Stock Solution and Working Standard Solution

Each drug was accurately weighed (50 mg) and taken into a separate 50 mL volumetric flask, where it was dissolved in methanol and the volume was built up to the mark with methanol (1000 µg/mL). Aliquot 10 mL of each drug's standard stock solution and transferred to a separate 100 mL volumetric flask and diluted with distilled water to obtain a working standard solution with a concentration of 100 µg/mL and further dilutions were made to produce resultant concentration. The overlain spectrum of 10 µg/mL of ABA (λ_{max} = 283.4 nm), LAM (λ_{max} = 268.4 nm) and DOL (λ_{max} = 259 nm) in range of 210 – 400 nm is shown in Figure 2.

Construction of Calibration set and Validation Set

A set of calibration and validation samples was created using fractional factorial design. Total 32 ternary mixture solutions and 16 mixture solutions were constructed by mixing known amount of drugs under study in different proportions (Table 1). For each of the medications investigated, the ranges had previously been proven to follow Beer's law. The mixtures were made by carefully transferring aliquots of working solutions into a series of 10 mL volumetric flasks, which were then filled with distilled water to complete the volume. The overlain spectra of calibration set and validation set is represented in Figure 3 (a) and Figure 3 (b) respectively.

All the mixtures (calibration and validation) were scanned at 200 to 400 nm range. From the spectra of mixture of drugs, absorbance data from spectral region at selected wavelength points was selected as chemometric region.

Classical Least Square

In MATLAB2018a programme, the CLS model was developed by adding absorbance (A) and concentration matrix (C) data. Absorbance matrix A is made up of 27 wavelength points with zero order spectra between 230 and 308 nm at 3 nm intervals. The built model incorporated absorbance values of samples at 27 different wavelength points, and quantities of ABA, LAM, and DOL in the validation data set, as well as tablet formulations, were predicted.

Inverse Least Square

Moore–Penrose pseudo inverse, a MATLAB2018a special function, was used to create the approach. The sample absorbance values were included into the calibrations at 27 different wavelength points in the spectrum band from 230 nm to 308 nm. In the validation data set, as well as tablets, the amounts of ABA, LAM, and DOL were predicted.

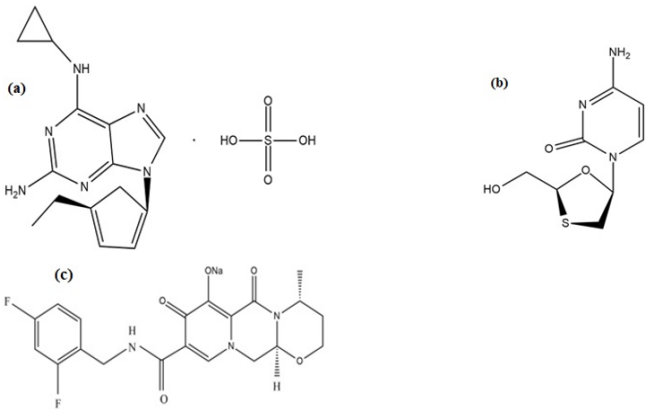

Figure 1: Structures of (a) ABA (b) LAM and (c) DOL.

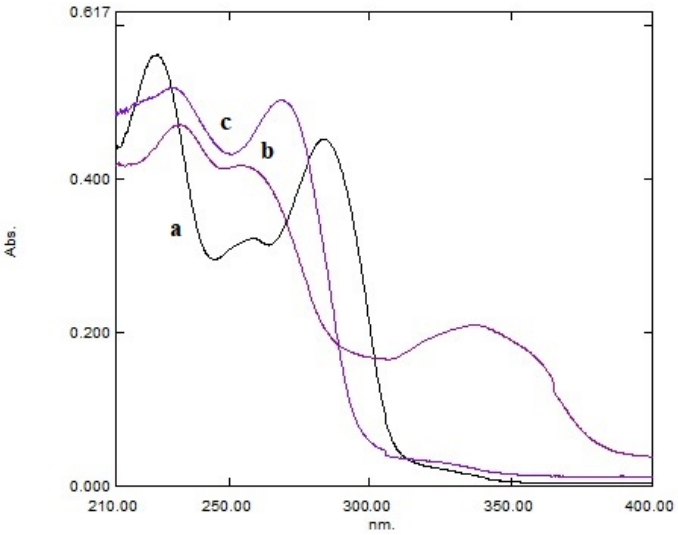

Figure 2: Overlay Spectra of 10.0 µg/mL solution of drugs a; ABA, b; DOL, c; LAM.

Partial Least Square and Principal Component Regression

Minitab 16.1.1 introduced the Absorbance (A) and Concentration (C) data matrix. For PCR and PLS calibrations, an adequate number of principal components or factors must be chosen. The number of components should account for as much of the experimental data as possible without overfitting. In the validation set and formulation, the amounts of ABA, LAM, and DOL were anticipated.

Validation of Developed Methods

Precision

Intra-day and Inter-day precision study was confirmed by triplicate analysis of ternary mixture containing different proportions of ABA, LAM and DOL (10/5/20 µg/mL, 20/10/5 µg/mL, 5/20/10 µg/mL) on similar day and on three consecutive days respectively. The outcomes were stated in % RSD.[25]

Accuracy

The method's accuracy was determined by applying the analytical approach to synthetic mixtures of drug product components (placebo) containing known amounts of the drug substance to be examined. Accuracy of the method was studied in triplicate at three different levels (80%, 100% and 120%). The established quantities of standard solutions containing ABA (16.0, 20.0, and 24.0 µg/mL), LAM (8.0, 10.0, 12.0 µg/mL) and DOL (1.2, 1.6, and 1.9 µg/mL) were spiked to placebo sample solutions to accomplish 80, 100 and 120% levels.

Assay of Formulation

Calculate the average mix content by weighing 10 tablets. In a 50 mL volumetric flask, the tablet powder equivalent to 100 mg of ABA (50 mg of LAM and 8.3 mg of DOL) was transferred. Methanol (30 mL) was added to it and sonicated for 20 min, after which the volume was adjusted with methanol to the desired level. The solution was filtered. Aliquot 1 mL from the above filtrate in 100 mL volumetric flask and dilute it upto the mark with Distilled water that will represent 20 µg/mL of ABA, 10.0 µg/mL of LAM and 1.6 µg/mL of DOL. At the designated wavelengths, the absorbance of the sample solution was measured, and the concentration of each component was determined.

Calibration Statistical Parameters

Statistical tools such as RMSEC, RMSECV, RMSEP, PRESS and R^2 were determined for validation of various mathematical models. Figure of merits (FOM)[26-28] such as sensitivity (SEN), analytical sensitivity (γ), limit of detection (LOD) and Limit of Quantitation (LOQ) are determined to compare various developed models and also for validation of chemometric methods.

RESULTS AND DISCUSSION

The conventional UV multicomponent spectroscopic methods hinder the simultaneous analysis of three compounds, Lamivudine, Abacavir Sulphate and Dolutegravir Sodium due to spectral interferences and overlapping. So, in this case, as an alternative to sophisticated methods like chromatography or LC-MS, a Chemometric method is used. The chemometric method is more accurate and precise than conventional methods as the total absorbance of the ternary mixture was measured. The routine analysis like in process quality control, dissolution testing, content uniformity, etc can be done with the developed methods as it will reduce the time because once the data is stored in the computer and the model is built, the concentration of sample can be deduced from the equations by placing the respective absorbance at respected wavelength.

The training set as well as validation set were prepared by using Fractional Factorial design. A considerable degree of spectral overlapping observed in the region from 220 to 350 nm. The absorbance data from the spectrum regions of 200 nm to 220

Table 1: Composition of calibration set and validation set for mixtures.

Calibration Set							
MIX	Concentrations (µg/mL)			MIX	Concentrations (µg/mL)		
	A	L	D		A	L	D
1	10	20	15	17	40	5	5
2	10	25	20	18	5	25	5
3	40	25	15	19	5	10	1
4	30	10	15	20	30	5	20
5	5	10	20	21	10	5	20
6	30	10	5	22	10	25	1
7	40	20	1	23	10	10	5
8	40	10	1	24	10	5	1
9	5	5	5	25	40	20	5
10	5	25	15	26	5	10	1
11	30	20	15	27	40	5	15
12	40	10	20	28	30	5	1
13	30	25	20	29	10	10	15
14	40	20	20	30	40	25	5
15	30	25	1	31	10	20	5
16	5	5	15	32	5	20	20
Validation Set							
MIX	Concentrations (µg/mL)			MIX	Concentrations (µg/mL)		
	A	L	D		A	L	D
1	5	15	15	9	40	25	20
2	10	10	10	10	40	5	1
3	20	20	15	11	40	10	5
4	20	15	10	12	40	5	20
5	10	10	1	13	5	25	1
6	5	25	20	14	10	5	5
7	5	10	5	15	10	10	1
8	5	5	1	16	5	5	20

nm with noise and 350 nm to 400 nm with zero reading were eliminated because they were not necessary for the chemometric approach. A wavelength range of 230 nm to 308 nm was chosen to produce minimal RMSEC, RMSECV values.

The equations obtained for the respective models were utilised to anticipate the concentrations of analyte in validation set as well as in formulation. The proposed approach is validated as per ICH Q2(R1) and Figures of merit were estimated using MVC1 software.

Classical Least Square

The value of calibration coefficient can be calculated by using equation:

$$K = pinv(c)*A$$

Where, pinv(c) is the pseudo inverse of concentration matrix and

A is matrix of absorbance of mixture.

$$K_{cal} = pinv(K)$$

Where, pinv(K) is pseudo inverse of K matrix

Concentration of unknown:

$$C = K_{cal} *A$$

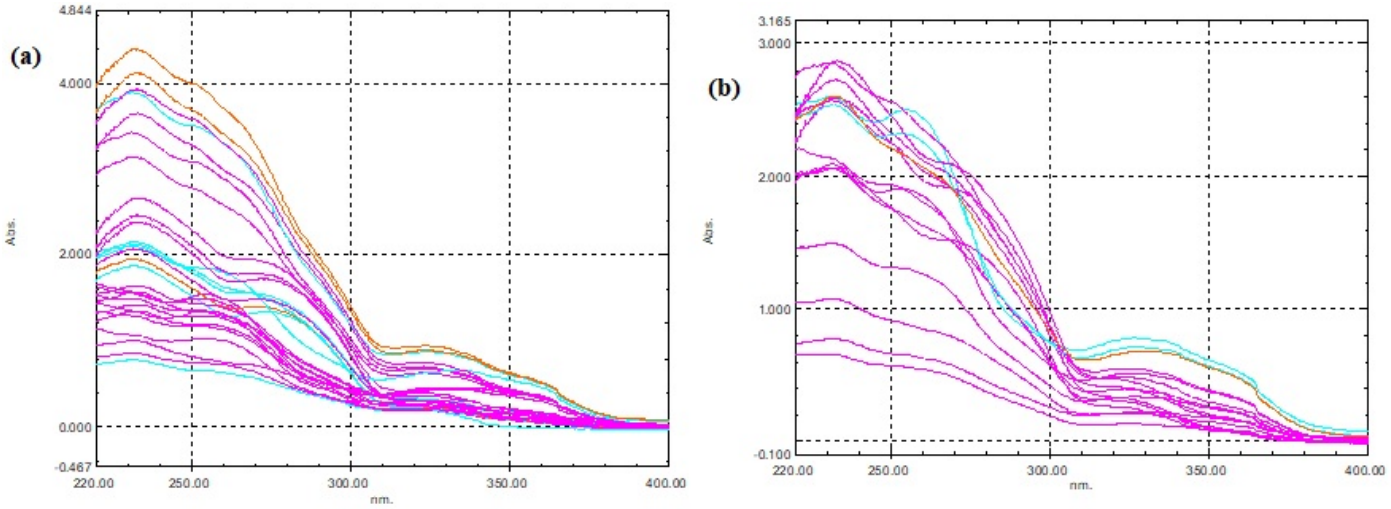

Figure 3: Overlay Spectra of (a) Calibration set; (b) Validation set in the range of 230–380 nm.

$$\underset{\text{Conc matrix}}{\begin{bmatrix} C_{comp1} \\ C_{comp2} \\ C_{comp3} \end{bmatrix}} = \underset{\text{Kcal Matrix}^{\#}}{\begin{bmatrix} Comp1 & Comp2 & Comp3 \\ 1.6452 & 0.014 & -0.0954 \\ 0.5064 & -0.4143 & 1.2792 \\ -1.1111 & -0.2967 & 2.5416 \\ -2.564 & 0.1682 & 3.3258 \\ -3.2105 & 0.4642 & 3.5667 \\ -3.0175 & 0.1425 & 3.6595 \\ -2.45 & -0.7288 & 3.9594 \\ -1.9813 & -1.6214 & 4.3843 \\ -1.8204 & -1.8868 & 4.5031 \\ -1.7968 & -1.4469 & 4.0853 \\ -2.0546 & 0.0947 & 2.885 \\ -2.5564 & 2.128 & 1.4296 \\ -2.5102 & 4.3272 & -0.6749 \\ -1.5644 & 5.7864 & -2.894 \\ 0.101 & 7.0403 & -5.5748 \\ 1.9638 & 7.332 & -7.5472 \\ 3.7968 & 6.7119 & -8.6613 \\ 5.2639 & 5.1992 & -8.6305 \\ 6.3957 & 2.41 & -7.1223 \\ 7.1063 & -1.2744 & -4.4111 \\ 7.5576 & -5.3796 & -1.0818 \\ 7.428 & -8.5231 & 1.8751 \\ 6.5874 & -10.1692 & 4.0816 \\ 4.957 & -10.4143 & 5.6899 \\ 2.6882 & -9.9812 & 7.2374 \\ 0.6056 & -9.4934 & 8.584 \\ -0.694 & -9.4463 & 9.6597 \end{bmatrix}} \times \underset{\text{Abs matrix } (Mixture)}{\begin{bmatrix} A230 \\ A233 \\ A236 \\ A239 \\ A242 \\ A245 \\ A248 \\ A251 \\ A254 \\ A257 \\ A260 \\ A263 \\ A266 \\ A269 \\ A272 \\ A275 \\ A278 \\ A281 \\ A284 \\ A287 \\ A290 \\ A293 \\ A296 \\ A299 \\ A302 \\ A305 \\ A308 \end{bmatrix}}$$

$$\underset{\text{Conc matrix}}{\begin{bmatrix} C_{comp1} \\ C_{comp2} \\ C_{comp3} \end{bmatrix}} = \underset{P \text{ Matrix}}{\begin{bmatrix} Comp1 & Comp2 & Comp3 \\ 43.1218 & 3.8269 & 32.9657 \\ 0.1232 & -8.7433 & 45.1049 \\ -50.2816 & -11.1682 & 24.5201 \\ -6.5641 & 10.5536 & -13.3866 \\ 33.1628 & 21.7784 & -74.3249 \\ -18.982 & 25.8078 & -37.6984 \\ 45.665 & 25.0556 & -73.5978 \\ -15.7063 & 35.3858 & 27.2542 \\ -9.2258 & -10.1218 & 14.6867 \\ 33.4178 & -15.8901 & 20.5751 \\ 35.5331 & -38.786 & 0.6279 \\ -73.6883 & -14.2252 & 67.739 \\ -34.0481 & -9.4875 & -6.8407 \\ -20.3182 & -3.8245 & 23.595 \\ 69.9633 & -38.5956 & -57.2415 \\ -1.0114 & 9.2975 & 20.1238 \\ 57.4862 & 33.9441 & -35.1949 \\ -56.743 & 34.216 & -4.6465 \\ -69.2619 & 20.1813 & 22.5748 \\ -11.2367 & -10.8813 & -10.1561 \\ -3.3328 & -14.2106 & 0.5109 \\ 67.8089 & -21.4164 & -5.9658 \\ 19.0923 & -32.3338 & -6.4114 \\ 14.5305 & 6.5231 & -38.6276 \\ -1.634 & -9.7298 & -23.0299 \\ 48.5551 & 1.1088 & -2.31 \\ -123.4156 & -8.2945 & 112.0764 \end{bmatrix}} \times \underset{\text{Abs matrix } (Mixture)}{\begin{bmatrix} A230 \\ A233 \\ A236 \\ A239 \\ A242 \\ A245 \\ A248 \\ A251 \\ A254 \\ A257 \\ A260 \\ A263 \\ A266 \\ A269 \\ A272 \\ A275 \\ A278 \\ A281 \\ A284 \\ A287 \\ A290 \\ A293 \\ A296 \\ A299 \\ A302 \\ A305 \\ A308 \end{bmatrix}}$$

Predicting the Unknown Concentration

Spectra of solutions comprising unknown amount of drugs were recorded in the optimised range of wavelength and absorbance matrix A were generated. Using the calibration coefficient matrix K, the concentration was computed.

* For representation of matrix conveniently, Kcal values are shown in transposed form,

Where, A is the absorbance values at 27 points corresponding to the 230–308 nm spectral range at interval of 3 nm and $Comp_1$, $Comp_2$ and $Comp_3$ are the concentrations of Abacavir Sulphate, Lamivudine, and Dolutegravir sodium, respectively.

Inverse Least Square

The value of calibration coefficient can be calculated by using following equation:

$$P = pinv(A) * C,$$

Where, **P** is the matrix of the unknown calibration coefficients relating the concentrations to the spectral intensities.

Predicting the unknown concentration

Spectra of solutions comprising unknown amount of drugs mixture were recorded in the optimised range of wavelength and absorbance matrix A were generated. Using the calibration

coefficient matrix P, the concentration was computed using equation,

$$C = P * A$$

Where, A is the absorbance values at 27 points corresponding to the 230–308 nm spectral range at interval of 3 nm and $Comp_1$, $Comp_2$ and $Comp_3$ are the concentrations of Abacavir sulphate Lamivudine, and Dolutegravir sodium, respectively.

Partial Least Square and Principal Component Regression

The number of factors should be selected for the experimental data in such a way that could not result in over fitting. Number of PCs in PLS selected using model selection plot (Figure 4a, 4b and 4c); scatterplot of the cross validated R^2 and fitted R^2 values as a function of the number of components. Four numbers of components selected on the basis of retaining components with identical R^2 values of validated R^2 and fitted R^2. For PCR, Three PCs selected on the basis of retaining components with eigenvalues greater than 1 and it was confirmed using scree plot (Figure 4d).

The equations for the PLS method was obtained as:

C_{ABA} = - 0.835 + 0.253 × **A1** + 4.643 × **A2** + 5.653 × **A3** + 3.123 × **A4** + 0.967× **A5** - 0.142 × **A6** + 2.2 × **A7** + 1.709 × **A8** + 1.07× **A9** - 0.377× **A10** - 1.36× **A11** - 5.73 × **A12** - 4.925 × **A13** - 4.094× **A14** + 1.268 × **A15** + 2.868 × **A16** + 3.301 × **A17** - 3.957 × **A18** - 8.197 × **A19** - 10.977 × **A20** - 7.033 × **A21** + 0.191 × **A22** + 16.237 × **A23** + 45.227 × **A24** + 56.377 × **A25** + 2.809× **A26** -110.641 × **A27**

C_{LAM} = 3.5172 - 0.0961 × **A1** + 1.7923× **A2** + 4.1755 × **A3** + 5.1243 × **A4** + 6.8132 × **A5** + 6.9257 × **A6** + 6.0438 × **A7** + 5.844 × **A8** + 3.3075 × **A9** + 2.386 × **A10** + 1.5499 × **A11** - 1.695 × **A12** - 3.2005 × **A13** - 4.3558× **A14** - 3.454 × **A15** - 2.1178 × **A16** - 0.4708 × **A17** + 1.1633 × **A18** + 1.3754 × **A19** + 0.0309 × **A20** - 2.1704 × **A21** - 2.2424 × **A22** - 2.9642 × **A23** - 6.6107 × **A24** -9.3628 × **A25** - 34.8879× **A26** - 37.5808 × **A27**

C_{DOL} = - 5.648 + 29.434 × **A1** + 15.196 × **A2** + 6.672 × **A3** + 2.724 × **A4** - 8.978 × **A5** - 14.941 × **A6** - 20.228 × **A7** - 19.446 × **A8** - 14.856 × **A9** - 14.344 × **A10** - 8.635 × **A11** + 7.295 × **A12** + 10.865 × **A13** + 12.393 × **A14** +4.03 × **A15** + 2.566 × **A16** - 4.451 × **A17** - 3.779 × **A18**+ 1.799 × **A19** + 5.974 × **A20** + 11.343 × **A21** - 1.667 × **A22** - 22.055 × **A23** - 27.289 × **A24** - 52.829 × **A25** + 47.614× **A26** + 138.268 × **A27.**

The equations for the PCR method were obtained as

C_{ABA} = - 5.1497 + 0.896 × **A1** + 0.177 × **A2** - 0.831 × **A3** - 1.862 × **A4** -2.407 × **A5** - 2.368 × **A6** -2.009 × **A7** - 1.704 × **A8** - 1.582 × **A9** - 1.499 × **A10** - 1.591 × **A11** - 1.817 × **A12** - 1.646 × **A13** - 0.846 × **A14** + 0.459 × **A15** + 1.862 × **A16** + 3.267 × **A17** + 4.496 × **A18** + 5.509× **A19** + 6.312 × **A20** + 6.971 × **A21** + 7.280× **A22** + 7.067× **A23** + 6.080× **A24** + 3.705× **A25** - 0.354 × **A26** - 4.653× **A27**

C_{LAM} = 1.656 + 0.774 × **A1** + 0.699× **A2** + 0.820× **A3** + 1.080× **A4** + 1.228× **A5** + 1.080× **A6** + 0.656 × **A7** + 0.262× **A8** + 0.163× **A9** + 0.370× **A10** + 1.051× **A11** + 1.938× **A12** + 2.915× **A13** + 3.483× **A14** + 3.923× **A15** + 3.935× **A16** + 3.539× **A17** + 2.799 × **A18** + 1.426× **A19** - 0.587 × **A20** - 3.153× **A21** - 5.655× **A22** - 7.710× **A23** - 9.598 × **A24** - 12.235 × **A25** - 15.649× **A26** - 18.764 × **A27**

C_{DOL} = 0.494 -0.400× **A1** + 0.343× **A2** + 1.186× **A3** + 1.929× **A4** + 2.310× **A5** + 2.401× **A6** + 2.436× **A7** + 2.495× **A8** + 2.466× **A9** + 2.208× **A10** + 1.703× **A11** + 1.141× **A12** + 0.129× **A13** - 1.117 × **A14** - 2.728 × **A15** - 4.064× **A16** - 5.054× **A17** - 5.579× **A18** - 5.356 × **A19** - 4.377 × **A20** - 2.784× **A21** - 0.909× **A22** + 1.070× **A23** + 3.647× **A24** + 8.193× **A25** + 14.972× **A26** + 21.660× **A27**

Here A is the absorbance values at 27 points corresponding to the 230–308 nm spectral range at interval of 3 nm and $C_{ABA,}$ C_{LAM} and C_{DOL} are the concentrations of Abacavir Sulphate, Lamivudine and Dolutegravir Sodium, respectively.

The graphical representation of actual concentration of analyte in validation set and predicted concentration are shown in Figure 5, Figure 6, Figure 7 and Figure 8. The graph of residual versus actual concentration are also summarised.

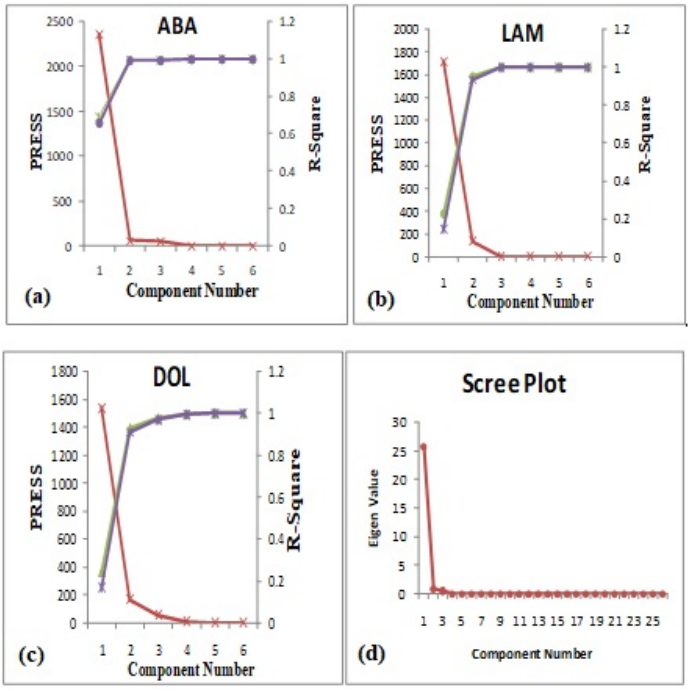

Figure 4: Plots for selection of number of component in PLS method for ternary mixture ABA (a), LAM (b) and DOL (c) and Scree Plot (d) for selection of component in PCR.

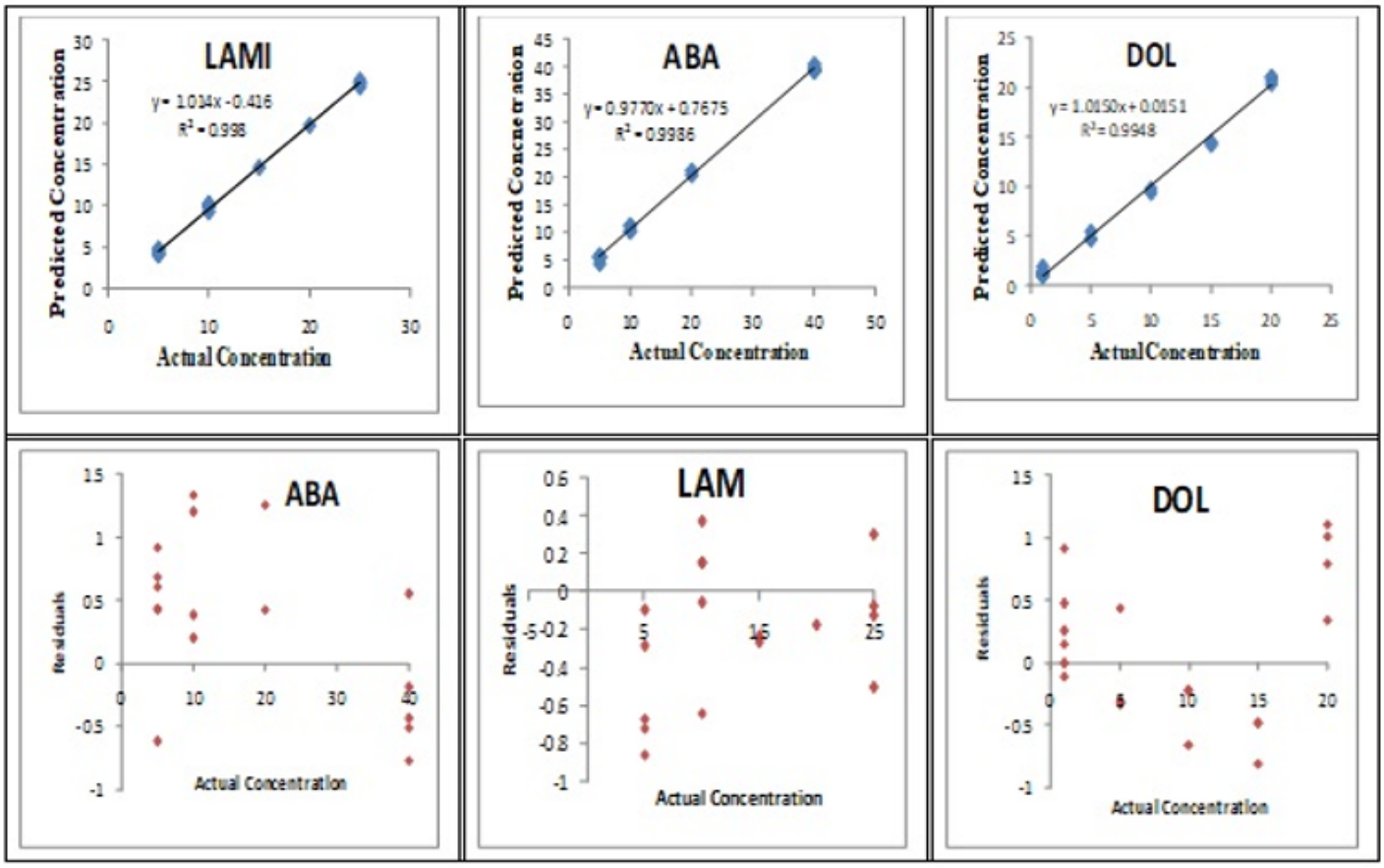

Figure 5: Predicted concentrations of ABA, LAM and DOL by applying CLS.

Validation of Proposed Method

Precision

Method reproducibility was illustrated by repeatability and intermediate precision measurements of % RSD in chemometric methods for each title ingredient. The obtained results within and between days trials (Table 2 and Table 3) are in acceptable range indicating good precision of the proposed methods.

Accuracy

The parameter of the approach was estimated by calculating recoveries of spiked known concentration of ABA, LAM and DOL. The mean percent recoveries for ABA, LAM and DOL reported in Table 4. The recovery values were close to 100% with low SD justified the high accuracy of the proposed methods (Acceptable range is 98% - 102%).

Assay of Tablets

The proposed chemometric method was applied for the quantitation of ABA, LAM and DOL in tablet formulations. The results were satisfactory and in line with the label claim. Table 5 shows that the method is suitable for simultaneously estimating ABA, LAM, and DOL without influence from common excipients.

Calibration Statistical Parameters

Leave One Out (LOO) approach was employed for cross validation technique using calibration set of 32 mixtures. Each calibration sample's anticipated quantities were compared to the known values of substances.

RMSECV and RMSEP were calculated to validate the model. These values must be as low as possible for a particular model. The RMSECV, RMSEC and RMSEP values obtained for chemometrics methods are shown in (Table 6, Table 7 and Table 8) indicating good accuracy and precision. Analytical Figure of Merits (FOM) are critical for quantifying the quality of an approach or comparing methods. Several FOM have been observed in multivariate calibration, including sensitivity (SEN), analytical sensitivity, Limit of Detection (LOD), and Limit of Quantitation (LOQ). Table 6 to Table 8 represents the FOM results.

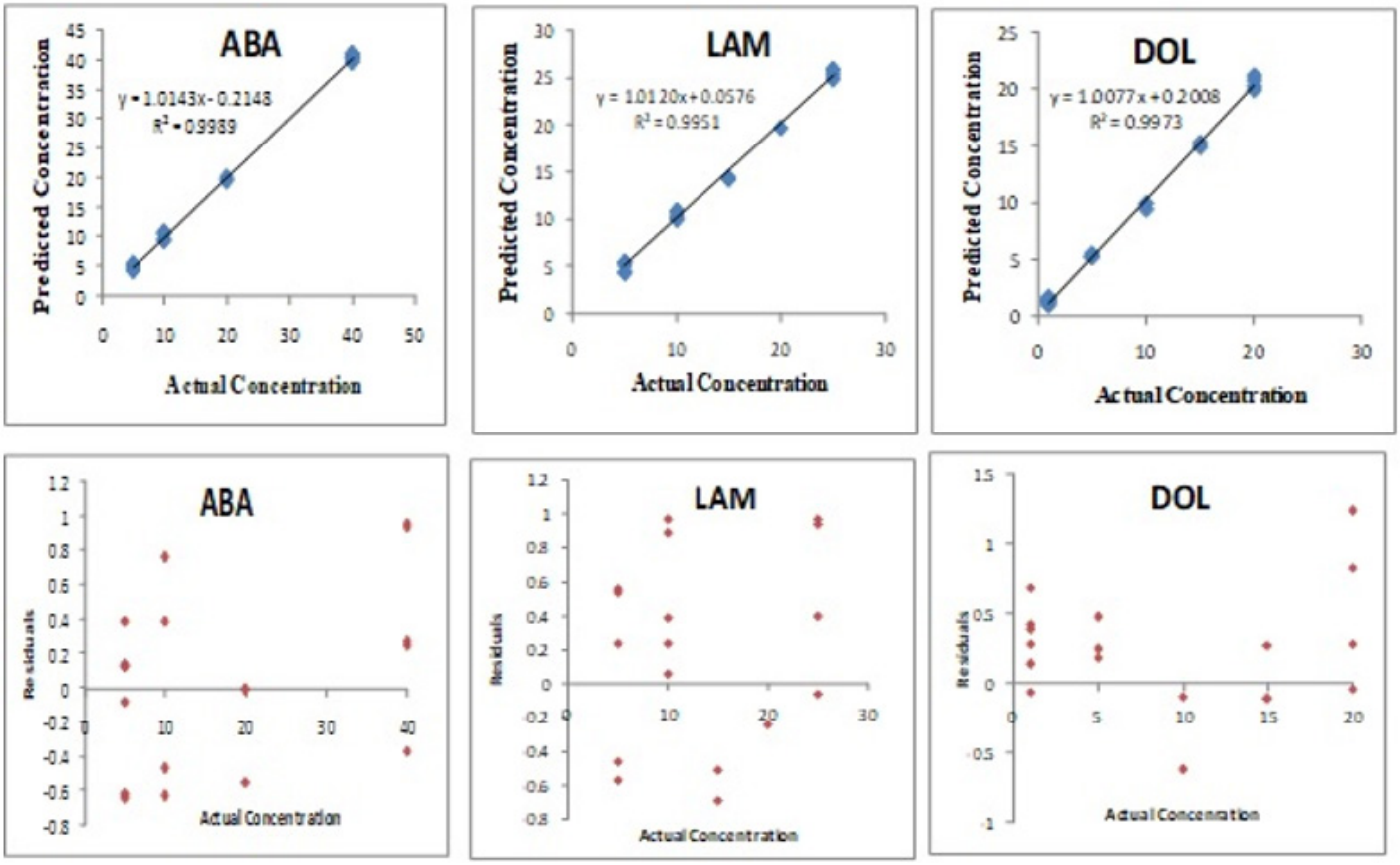

Figure 6: Predicted concentrations of ABA, LAM and DOL by applying ILS.

Table 2: Precision data for ABA, LAM and DOL by Chemometric methods.

Chemometric model	Ternary Mixture (µg/mL) (ABA/LAM/DOL)	% Recovery, Mean ± SD (*n* = 3)		
		Intra - Day		
		ABA	LAM	DOL
CLS	1 (10/5/20)	99.59 ± 0.819	99.54 ± 1.102	99.82 ± 0.581
	2 (30/20/15)	99.74 ± 0.467	99.45 ± 0.902	100.12 ± 0.901
	3 (5/25/5)	99.47 ± 1.419	100.11 ± 0.642	100.19 ± 1.51
ILS	1 (10/5/20)	99.67 ± 0.851	99.74 ± 1.332	100.14 ± 0.751
	2 (30/20/15)	99.87 ± 0.318	99.84 ± 0.579	100.34 ± 0.743
	3 (5/25/5)	99.87 ± 1.419	100.18 ± 0.594	99.59 ± 1.201
PLS	1 (10/5/20)	100.17 ± 1.251	99.54 ± 1.223	100.37 ± 0.501
	2 (30/20/15)	99.94 ± 0.434	100.02 ± 0.936	99.87 ± 0.677
	3 (5/25/5)	99.74 ± 1.405	100.06 ± 0.613	99.47 ± 1.027
PCR	1 (10/5/20)	99.85 ± 1.267	100.4 ± 1.401	100.32 ± 0.514
	2 (30/20/15)	99.79 ± 0.399	99.79 ± 0.527	100.38 ± 0.567
	3 (5/25/5)	99.87 ± 1.302	99.83 ± 0.372	99.79 ± 1.312

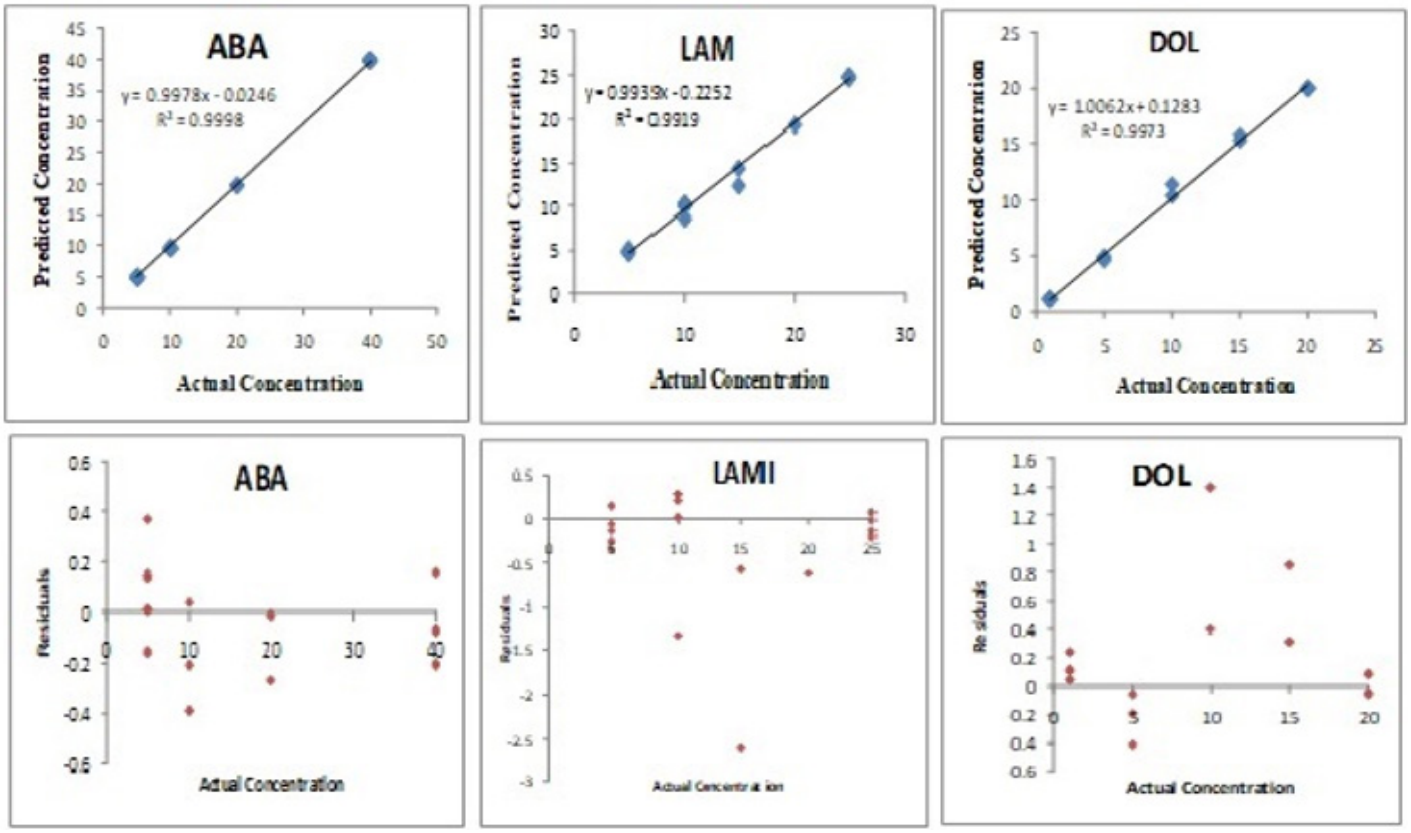

Figure 7: Predicted concentrations of ABA, LAM and DOL by applying PLS.

Table 3: Precision data for ABA, LAM and DOL by Chemometric methods.

Chemometric model	Ternary Mixture (µg/mL) (ABA/LAM/DOL)	% Recovery, Mean ± SD (*n* = 3)		
		Inter-Day		
		ABA	LAM	DOL
CLS	1 (10/5/20)	99.95 ± 1.064	100.25 ± 1.257	99.85 ± 0.584
	2 (30/20/15)	99.91 ± 0.249	100.02 ± 0.519	100.03 ± 0.571
	3 (5/25/5)	99.94 ± 1.101	99.96 ± 0.449	99.8 ± 1.012
ILS	1 (10/5/20)	100.12 ± 1.193	100.25 ± 1.279	99.67 ± 0.559
	2 (30/20/15)	99.88 ± 0.349	99.84 ± 0.485	99.42 ± 0.545
	3 (5/25/5)	100.05 ± 1.261	99.89 ± 0.324	100.05 ± 1.352
PLS	1 (10/5/20)	99.65 ± 1.175	100.05 ± 1.372	100.05 ± 0.439
	2 (30/20/15)	99.92 ± 0.275	99.84 ± 0.392	99.98 ± 0.634
	3 (5/25/5)	99.87 ± 1.154	99.95 ± 0.409	100.27 ± 1.029
PCR	1 (10/5/20)	99.87 ± 1.009	100.34 ± 1.101	100.05 ± 0.456
	2 (30/20/15)	100.03 ± 0.249	100.02 ± 0.369	99.85 ± 0.489
	3 (5/25/5)	99.96 ± 1.326	100.06 ± 0.294	99.94 ± 1.039

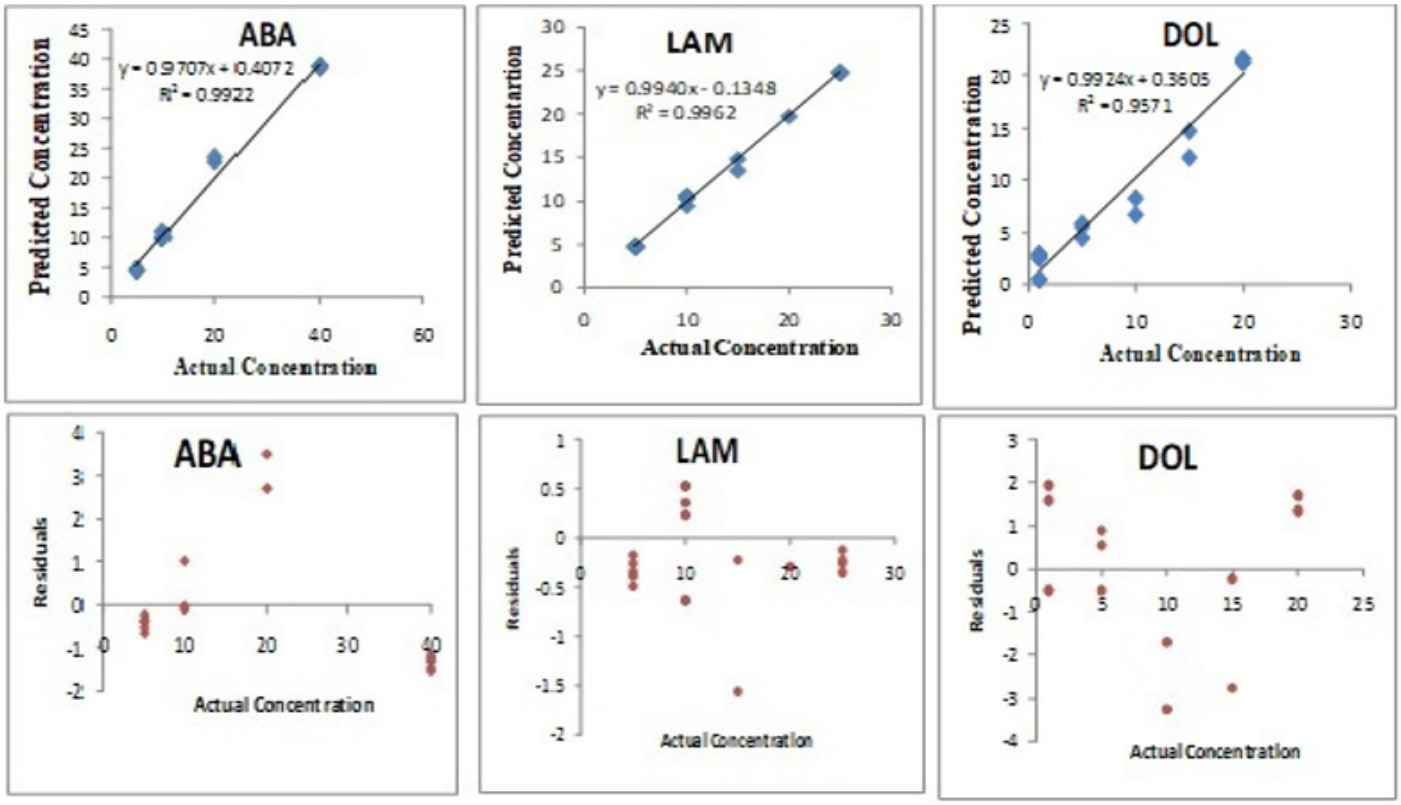

Figure 8: Predicted concentrations of ABA, LAM and DOL by applying PCR.

Table 4: Accuracy data of ABA, LAM and DOL by Chemometric methods.

Drug	Level (%)	Std. spiked (µg/mL)	% Recovery Mean ± SD (*n* = 3)			
			CLS	ILS	PLS	PCR
ABA	80	16	100.44 ± 0.512	100.15 ± 0.764	100.44 ± 0.659	100.38 ± 0.472
	100	20	99.85 ± 0.551	100.11 ± 0.777	99.85 ± 0.551	100.12 ± 0.729
	120	24	100.14 ± 0.608	100.03 ± 0.528	100.14 ± 0.313	100.19 ± 0.388
LAM	80	8	100.75 ± 0.944	100.21 ± 0.939	100.42 ± 1.202	100.67 ± 0.814
	100	10	99.59 ± 0.855	99.67 ± 0.863	99.67 ± 0.961	100.39 ± 1.082
	120	12	99.81 ± 0.835	100.39 ± 0.709	100.62 ± 0.669	100.23 ± 0.509
DOL	80	1.2	100.98 ± 1.254	99.49 ± 1.146	100.03 ± 1.182	99.87 ± 1.088
	100	1.6	99.79 ± 1.349	100.15 ± 1.119	99.28 ± 1.102	100.65 ± 1.065
	120	1.9	100.79 ± 1.564	99.81 ± 1.194	100.16 ± 1.126	100.22 ± 0.914

Table 5: Content of ABA, LAM and DOL by Chemometrics method.

Tablet: INBEC				
Drug		**ABA**	**LAM**	**DOL**
Label Claim (mg/tablet)		**600**	**300**	**50**
% Label claim, Mean ± SD, % CV (*n* =3)	CLS	100.35 ± 0.958, 0.954	99.57 ± 1.053, 1.057	100.93 ± 0.769, 0.761
	ILS	98.72 ± 1.152, 1.166	100.15 ± 0.729, 0.727	99.72 ± 1.189, 1.192
	PLS	101.29 ± 1.268, 1.251	99.83 ± 0.425, 0.425	100.43 ± 1.142, 1.137
	PCR	100.53 ± 0.729, 0.725	99.78 ± 1.154, 1.156	99.92 ± 0.812, 0.812

Table 6: Statistical parameters and figure of merits for ABA.

Component	ABA			
Parameters/ Model	**CLS**	**ILS**	**PLS**	**PCR**
RMSEC	0.2902	2.739×10^{-4}	0.2438	0.6852
RMSECV	0.2608	0.5378	0.2871	0.4648
RMSEP	1.5918	0.5555	1.3371	3.7578
R^2 Calibration	0.9995	0.9996	0.9996	0.9989
R^2 Prediction	0.9986	0.9989	0.9998	0.9922
Intercept	0.767	-0.214	-0.024	0.407
Slope	0.977	1.014	0.997	0.970
PRESS	2.1768	4.6287	1.2716	6.9148
Sensitivity (SEN)	0.0085	0.0088	0.010	0.0130
Selectivity (SEL)	0.0484	0.0495	0.0315	0.0739
LOD, µg/mL	0.2279	0.2469	0.5604	0.5452
LOQ, µg/mL	0.6907	0.7481	1.6984	1.6521
Analytical sensitivity (γ), mL/µg	13.163	12.150	5.364	5.5118

Table 7: Statistical parameters and figure of merits for LAM.

Component	LAM			
Parameters/ Model	**CLS**	**ILS**	**PLS**	**PCR**
RMSEC	0.4919	1.23×10^{-4}	0.4040	0.5154
RMSECV	0.1147	0.5614	0.3758	0.3739
RMSEP	3.7167	0.5798	3.0531	3.8942
R^2 Calibration	0.9997	0.9996	0.9977	0.9977
R^2 Prediction	0.9982	0.9951	0.9919	0.9962
Intercept	-0.416	0.057	-0.225	-0.134
Slope	1.014	1.012	0.993	0.994
PRESS	0.4214	5.0439	2.2196	4.4739
Sensitivity (SEN)	0.0171	0.0168	0.0243	0.0300
Selectivity (SEL)	0.0866	0.0859	0.0491	0.1517
LOD, µg/mL	0.1134	0.1868	0.3288	0.2362
LOQ, µg/mL	0.3438	0.5661	0.9963	0.7157
Analytical sensitivity (γ), mL/µg	26.804	16.059	9.1238	12.701

Table 8: Statistical parameters and figure of merits for DOL.

Component	DOL			
Parameters/ Model	CLS	ILS	PLS	PCR
RMSEC	0.1674	1.77×10^{-4}	0.9450	1.2409
RMSECV	0.1057	0.4932	0.6745	0.8601
RMSEP	1.8847	0.5094	10.639	13.97
R^2 Calibration	0.9998	0.9996	0.9921	0.9871
R^2 Prediction	0.9948	0.9973	0.9973	0.9571
Intercept	0.015	0.200	0.128	0.360
Slope	1.015	1.007	1.006	0.992
PRESS	0.3577	3.8925	1.7214	13.678
Sensitivity (SEN)	0.0078	0.0071	0.0147	0.0158
Selectivity (SEL)	0.0408	0.0395	0.0243	0.0824
LOD, µg/mL	0.2477	0.2139	0.4110	0.4482
LOQ, µg/mL	0.7507	0.6481	1.245	1.3583
Analytical sensitivity (γ), mL/µg	12.108	14.025	7.2983	6.6925

CONCLUSION

The aim of work is to develop and validate the chemometrics assisted spectrophotometric method development and validation for simultaneous estimation of ABA, LAM and DOL in combined dosage form. The proposed method, which does not require the use of mobile phase or any other separation apparatus, is a good alternative to chromatographic separations in quantitation of quality control samples. Chemometrics techniques are usually very efficient techniques for the simultaneous analysis of multiple components in which the overlap of the active compound's spectra creates an interference that prevents the concentrations of each compound from being determined using traditional linear regression equations. The RMSEC, R^2 (calibration), RMSEP, and R^2 (validation) values for the ILS approach were determined to be the lowest, according to our findings. As a result, ILS may be the most appropriate among the developed chemometric techniques. Another feature of the suggested method is that no derivatization or ratio spectra modes were used in the analysis, which are both costly and time-consuming. Furthermore, chemometric calibration methods are simple since they may analyze a large number of samples in a short amount of time as reliably and precisely as chromatographic procedures. The observed results showed that the proposed spectrophotometric method can be used as a feasible alternative approach for simultaneous assessment of ABA, LAM and DOL in pharmaceutical industry for quality control analysis.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ABA: Abacavir Sulphate; **LAM:** Lamivudine; **DOL:** Dolutegravir Sodium; **CLS:** Claasical Least Square; **ILS:** Inverse Least Square; **PLS:** Partial Least Square; **PCR:** Principal Component Regression; **LOD:** Limit of Detection; **LOQ:** Limit of Quantitation; **PRESS:** Predicted Residual Sum of Squares; **RMSEC:** Root mean standard error of calibration; **RMSECV:** Root mean standard error of cross validation; **RMSEP:** Root mean standard error of prediction; **FOM:** Figure of Merit; **LOO:** Leave one out; **SD:** Standard Deviation; **RSD:** Relative Standard Deviation; **PC:** Principal Component; **R^2:** Coefficient of determination; **HIV:** Human Immunodeficiency Virus; **AR:** Analytical grade reagent; **SEN:** Sensitivity; **SEL:** Selectivity.

SUMMARY

The multicomponent investigation of antiviral medications like ABA, LAM and DOL in formulation was carried out using various chemometric models (CLS, ILS, PLS, and PCR) with no separation step. The training and validation sets were built using a fractional factorial approach. There were 32 calibration sets and 16 validation sets prepared. The equations were built at 27 wavelengths corresponding to the 230–308 nm spectral range at 3 nm intervals and utilised for ABA, LAM, and DOL determination in tablet formulation. MATLAB and MS Excel were utilised for the chemometric calculations. The applicability and efficacy of the proposed models for regular analysis and quality control with satisfactory precision (lowest RMSECV) were demonstrated by good percentage recoveries and proper statistical data collected

with the tablets. When compared to HPLC, HPTLC, and other advanced methods, the proposed chemometric approaches are less expensive, mostly non-polluting, and equally reliable.

REFERENCES

1. Howland RD, Mycek MJ, Harvey RA, Champe PC. Lippincott's illustrated reviews: pharmacology. 1st ed. New Delhi: Wolters Kluwer Health; 2007.
2. Rang HP, Flower RJ, Henderson G, Ritter JM, Flower RJ. Rang and Dale's pharmacology. 6th ed. New York: Churchill Livingstone; 2007.
3. Srihari G, Reddy NR, Chakravarthi IE. Spectrophotometric methods for the determination of abacavir sulphate in pharmaceutical preparations. Glob J Pharmacol. 2011;5(3):172-5.
4. Deepali G, Elvis M. UV spectrophotometric method for assay of the anti-retroviral agent lamivudine in active pharmaceutical ingredient and in its tablet formulation. J Young Pharm. 2010;2(4):417-9. doi: 10.4103/0975-1483.71628, PMID 21264106.
5. Bhavar GB, Aher KB, Thorat RS, Kakad SJ, Pekamwar SS. Development and validation of UV-spectrophotometric Method for estimation of Dolutegravir sodium in tablet dosage form. Malays J Anal Sci. 2015;19(6):1156-63.
6. Monica M, Sankar DG. Simultaneous RP-HPLC determination of abacavir, lamivudine and Dolutegravir in bulk API dosage forms. Int J Pharm Anal Res. 2015;4(3):168-81.
7. Tol T, Kadam N, Raotole N, Desai A, Samanta G. A simultaneous determination of related substances by high performance liquid chromatography in a drug product using quality by design approach. J Chromatogr A. 2016;1432:26-38. doi: 10.1016/j.chroma.2015.12.080, PMID 26792450.
8. Dave K, Desai S. Factorial design for development of a high-performance thin-layer chromatography method for the simultaneous estimation of abacavir sulfate, lamivudine hydrochloride, and dolutegravir sodium. JPC - Journal of Planar Chromatography - Modern TLC. 2018;31(6):489-95. doi: 10.1556/1006.2018.31.6.9.
9. Dubey S, Duggirala M. Simultaneous estimation of lamivudine, abacavir and Dolutegravir by UPLC method. Int J Appl Pharm. 2018;10(1):46-52. doi: 10.22159/ijap.2018v10i1.21156.
10. Pal N, Rao AS, Ravikumar P. Simultaneous HPLC method development and validation for estimation of lamivudine, abacavir and dolutegravir in combined dosage form with their stability studies. Asian J Chem. 2016;28(2):273-6. doi: 10.14233/ajchem.2016.19116.
11. Prava R, Seru G, Pujala KV. RP-HPLC method development and validation for the simultaneous determination of lamivudine, abacavir and Dolutegravir in pharmaceutical dosage forms. World J PharmSci. 2017;5(5):168-81.
12. Nagubandi N, Pappula N. Stability indicating UPLC method for simultaneous estimation of abacavir, lamivudine and Dolutegravir from its tablet dosage form. World J PharmSci. 2015;3(10):2135-40.
13. Khaleel N, Rahaman SA. A validated stability indicating RP-HPLC Method for simultaneous determination of abacavir, lamivudine and Dolutegravir in bulk and pharmaceutical dosage form. World J Pharm Res. 2015;4(7):1453-76.
14. Saidulu P, Mastanamma S. Stability Indicating Gradient RP-HPLC Method for the simultaneous estimation of lamivudine, abacavir and Dolutegravir in bulk and their combined dosage form. Int J Pharm Sci Rev Res. 2016;37:249-57.
15. Purnima BV, Santha M, Ramu G. Validated reversed phase HPLC method for assay and degradation studies of lamivudine, abacavir sulphate and dolutegravir in combined dosage form. Int J Educ Appl Res. 2016;6(2):86-95.
16. M. Rathod S, U. Patel P. Novel Spectrophotometric Method Development and Validation for Simultaneous Estimation of Abacavir Sulphate, Lamivudine And Dolutegravir Sodium in Dosage Form. Indian Drugs. 2022;59(7):44-51. doi: 10.53879/id.59.07.12415.
17. Kramer R. Chemometric techniques for quantitative analysis. New York: Marcel Dekker Inc; 1998.
18. Beebe KR, Pell RJ, Seasholtz MB. Chemometrics: a practical guide. 1st ed. New York: John Wiley and Sons; 1998.
19. Miller JN, Miller JC. Statistics and chemometrics for analytical chemistry. 6th ed. London: Pearson Education Limited; 2005.
20. Goicoechea HC, Olivieri AC. Simultaneous determination of rifampicin, isoniazid and pyrazinamide in tablet preparations by multivariate spectrophotometric calibration. J Pharm Biomed Anal. 1999;20(4):681-6. doi: 10.1016/S0731-7085(99)00072-2, PMID 10704137.
21. Patel NS, Nandurbarkar VP, Patel AJ, Patel SG. Simultaneous spectrophotometric determination of celecoxib and diacerein in bulk and capsule by absorption correction method and chemometric methods. Spectrochim Acta A Mol Biomol Spectrosc. 2014;125:46-52. doi: 10.1016/j.saa.2014.01.036, PMID 24530708.
22. Nejem RM, Issa MM, Shanab AA, Shaat NT. Double divisor mean centering of ratio spectra as a developed spectrophotometric method for the analysis of five-component mixture in drug analysis. J Saudi Chem Soc. 2017;21:S283-92. doi: 10.1016/j.jscs.2014.03.001.
23. Rathod SM, Patel PU. Chemometrics Assisted Spectroscopic Method Development and Validation for Simultaneous Determination of Sofosbuvir and Daclatasvir dihydrochloride in Tablet Formulation. Indian Drugs. 2020;57(3):37-46. doi: 10.53879/id.57.03.12273.
24. Patel NC, Patel AP, Patel JK. Development and Validation of Chemometric-assisted UV-spectrophotometric Method for Epigallocatechin Gallate and Curcumin in Tablet Formulation. Indian Drugs. 2020;57(4):45-51. doi: 10.53879/id.57.04.12286.
25. International Conference on Harmonization. Technical Requirements for Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedures: validation of analytical procedures: text and Methodology, ICH. Vol. Q2(R1); 1996;2005.
26. Lorber A, Faber K, Kowalski BR. Net analyte signal calculation in multivariate calibration. Anal Chem. 1997;69(8):1620-6. doi: 10.1021/ac960862b.
27. Olivieri AC, Goicoechea HC, Iñón FA. MVC1: an integrated MATLAB toolbox for first-order multivariate calibration. Chemometr Intell Lab Syst. 2004;73(2):189-97. doi: 10.1016/j.chemolab.2004.03.004.
28. Olivieri AC, Faber NM, Ferré J, Boqué R, Kalivas JH, Mark H. Uncertainty estimation and Figures of merit for multivariate calibration (IUPAC Technical Report) [IUPAC technical report]. Pure Appl Chem. 2006;78(3):633-61. doi: 10.1351/pac200678030633.

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