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# Simultaneous stability indicating rp-hplc method development and validation of abacavir, dolutegride and lamivudine in bulk and pharmaceutical formulation

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**Abstract**

A fresh analytical approach was created to estimation of Abacavir, Dolutegravir and Lamivudine in bulk and its pharmaceutical formulation. The sensitive, précised and accurate method were developed by using waters HPLC system equipped with quaternary gradient pump. The column was used Agilent TC-$C_{18}$ (2) 5µm [4.6mm x 250mm] and mobile phase was Methanol: Water (70:30). The mobile phase flow rate was 1 mL/min, and the PDA detector detected it at 257 nm. The procedure was carried out at room temperature. The retention time of the ABV, DTV and LVD were found to be 2.6, 2.9 and 6.6 min. The % RSD values in precision was >2%. It was discovered that the method's accuracy ranged from 99.95% -100.01% for ABV, 99.95%-100.13% for DTV and 99.95%-100.04% for LVD. The limits of detection and quantification values were obtained 0.57µg/mL and 1.89µg/mL respectively. The range of linearity concentration was found to be 150-450µg/mL for ABV, 12.5-37.5µg/mL for DTV and 75-225µg/mL for LVD, it shown wider linearity concentration range. The technique demonstrated good robustness. The parameters of flow rate (±0.2 mL) and wavelength (±2 nm) were changed and obtained good % assay values. The technique demonstrated its capacity to withstand various stress conditions, including UV, peroxide, acidity, and alkalinity. The acidity and alkalinity stress studies were performed by 0.1N HCl and 0.1N NaOH. The peroxide stress study was carried out by 3% hydrogen peroxide at room temperature. UV-Light carried out the UV degradation investigation. The technique was applied to the routine HPLC analysis of bulk Abacavir, Dolutegravir, and Lamivudine as well as its pharmaceutical dosage form.

## INTRODUCTION

The combinational therapy used for the treatment of the HIV efficiently. Abacavir inhibiting the HIV-I reverse transcriptase activity[1-3]. The molecular formula is $C_{14}H_{18}N_6O$ and soluble in water, buffer, methanol. Dolutegravir used for the treatment of HIV infection, approved by FDA and inhibitor of integrase strand transfer. It is a two-step process that is carried out by the viral integrase enzyme that preferentially prevents the strand transfer stage of the viral genome's integration into the DNA of the host cell[4-9]. The molecular formula is $C_{20}H_{19}F_2N_3O_5$ and soluble in water and methanol. Lamivudine is more potent and act against Human Immuno Virus (HIV) and Hepatitis B Virus (HBV), it inhibits the reverse transcriptase enzyme. Its molecular formula is $C_8H_{11}N_3O_3S$, and it is soluble in water, only a little bit in methanol, and almost none at all in acetone[10-18]. According to a review of the literature, spectrophotometric, HPLC-MS/MS, and chromatographic methods have been developed for the individual and concurrent quantification of LVD, DTV, and ABV in bulk and mixed dose forms. Therefore, utilizing isocratic elution mode, the author attempted to build a stability demonstrating the RP-HPLC method's specificity, sensitivity, accuracy, and precision. According to ICH Q2 R1 requirements, the developed approach was validated[19]. Few simultaneous analytical methods were reported on these combined dosage forms by using HPLC, among all those methods the current method shown good linearity, precision, accuracy and stability after applied some stress conditions.

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## MATERIALS & METHODOLOGY

### Chemicals and Reagents

This experiment employed analytical and HPLC grade chemicals and reagents. After double distillation, the water was filtered using a membrane filter. To make a mobile phase, methanol of HPLC quality (Merck, India) was utilized. Standard pharmaceutical grade API's were donated by Hetero Laboratories, located in Hyderabad. The tablet formulation, which was bought at the Nellore local market, comprises 600 mg of ABV, 300 mg of LVD, and 50 mg of DTV.

### Instrumentation and chromatographic conditions:

The developed and validated method was performed on Waters HPLC system (Waters, Milford, MA, USA) equipped with quaternary gradient pump, photodiode array detector and column was used Agilent TC-$C_{18}$ (2) 5µm [4.6mm x 250mm] for the separation. The separation and estimation was carried out by using Methanol: Water in the ratio of 70:30 v/v. The mobile phase was filtered using a membrane filter, and the flow rate of the mobile phase was kept constant at 1 mL/min. The effluent was examined at 257 nm using PDA detection. The HPLC column was filled with a 10µL solution.

### Preparation of Standard solution:

Weigh accurately 10 mg of ABV, DTV and LVD and transferred in to a individual 10 mL volumetric flasks, add small extent of mobile phase. The solution was sonicated for 5 min and volume was made 10 mL with mobile phase and the concentration of ABV, DTV and LVD was obtained 1000µg/mL. The optimized concentration was created by diluting the solutions further to obtain final concentrations of 300 µg/mL ABV, 25 µg/mL DTV, and 150 µg/mL LVD, respectively. The solution was utilized as a typical working solution.

### Preparation of Sample Solution

The 20 pills were weighed and finely ground in a mortar. The average weight of the tablets was found. The powder equivalent 60 mg of ABV was weighed and placed in a 10 mL volumetric flask. The 10mL of the mobile phase were added to the volumetric flask, and the tablet powder was thoroughly broken down over the course of five minutes with an ultrasonicator. In order to get the final concentrations of 300µg/mL of ABV, 25µg/mL of DTV, and 150µg/mL of LVD, the aliquot portion of the filtrate was further diluted. A membrane filter was used to filter the mixture. The HPLC system was injected with 10µL of the solution.

### Selection of Mobile phase

Different mobile phases were used for the separation of combined dosage form. The good separation was achieved with the composition of Methanol: Water in the ratio of 70:30 v/v. This ratio resulted in strong theoretical plates, a small tailing factor, and a strong peak area.

## Results and Discussion

### Method Development

Different buffers and organic solvents were used to develop the chromatographic technique. The method was optimized by using a mobile phase that had Methanol: Water in a 70:30 v/v ratio and that shown strong separation, high theoretical plates, symmetrical peaks, and a short retention period for mixed pharmaceuticals.

### Method Validation

Different validation parameters were examined for the developed approach in accordance with ICH regulations. The approval level of the ICH recommendations was indicated by all validation parameters[19].

### System suitability study

After system suitability analysis, good tailing factor <2 and theoretical plates >5000 were obtained, along with good peak regions. Figures No.1 displayed the chromatograms.

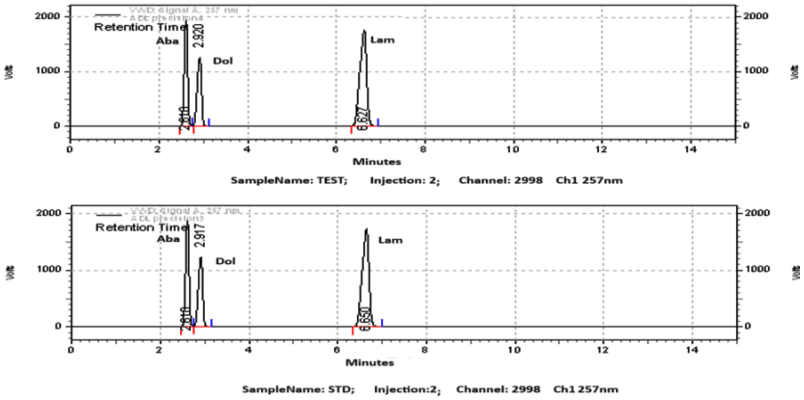


**Figure: I** Sample and Standard chromatograms of ABV, DTV & LVD

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**Specificity:**
The method's specificity is indicated by the force degradation studies. In the chromatograms, no further peaks were seen. The method is shown good ability to determine the active moieties in the presence of other products.

**Linearity:**
The standard solution had linear concentrations of 150–450 µg/mL for ABV, 12.5–37.5 µg/mL for DTV, and 75–225 µg/mL for LDV (Figure 5). The linearity curve between concentration and peak area was plotted. The calibration curve's linearity over the concentration range was established, and its $R^2$ value was 0.999 results were shown table 1.

**Table: 1 Results of Linearity Studies**

| S.No | **Abacavir** | | **Dolutegravir** | | **Lamivudine** | |
|---|---|---|---|---|---|---|
| | Concentration **(µg/mL)** | Peak Area | Concentration **(µg/mL)** | Peak Area | Concentration **(µg/mL)** | Peak Area |
| 1 | 150 | 34678841 | 12.5 | 26235298 | 75 | 61484695 |
| 2 | 225 | 69737065 | 18.75 | 52287603 | 112.5 | 118044097 |
| 3 | 300 | 100262412 | 25 | 78718068 | 150 | 178788394 |
| 4 | 375 | 132065042 | 31.25 | 104960696 | 187.5 | 242570568 |
| 5 | 450 | 165785755 | 37.5 | 131230072 | 225 | 306340955 |
| Regression Coefficient Value($R^2$) | | 0.999 | 1 | | 0.999 | |

**Precision**
System and method precision were combined to determine the method's level of precision. Through assay, the method's precision was evaluated. The HPLC chromatographic system was filled with sample solutions at optimized concentrations in order to examine the precision of the system and method. Good precision was reported in the approach outcomes. The results showed that the %RSD values ranged from 0.11 to 0.41.

**Accuracy**
The technique's accuracy was evaluated by recovery process (Table 2). The accuracy of the method was performed with different concentrations of solutions 50%, 100%, 150%. The % mean recovery values were found to be 99.95-100.01% for ABV, 99.95-100.13% for DTV and 99.90-100.04% LVD. The method was shown good ability towards accuracy and chromatograms shown in figure no 2.

**Table No: 2 Accuracy results**

| **S.No** | **Recovery Level** | **Amount Added (µg/mL)** | | **Peak Area** | **Amount Found (µg/mL)** | **% Recovery** |
|---|---|---|---|---|---|---|
| | | **Standard** | **Sample** | | | |
| **Abacavir** | | | | | | |
| 1 | 50%* | 10 | 150 | 53473286 | 159.92 | 99.95 |
| 2 | 100%** | 10 | 300 | 103604492 | 309.95 | 99.98 |
| 3 | 150%* | 10 | 450 | 153735698 | 460.05 | 100.01 |
| **Dolutegravir** | | | | | | |
| 1 | 50%* | 10 | 12.5 | 70846271 | 22.53 | 100.13 |
| 2 | 100%** | 10 | 25 | 110205285 | 34.91 | 99.74 |
| 3 | 150%* | 10 | 37.5 | 119177198 | 47.48 | 99.95 |
| **Lamivudine** | | | | | | |
| 1 | 50%* | 10 | 75 | 101219432 | 84.92 | 99.90 |
| 2 | 100%** | 10 | 150 | 190707620 | 160.07 | 100.04 |
| 3 | 150%* | 10 | 225 | 280101823 | 234.89 | 99.95 |

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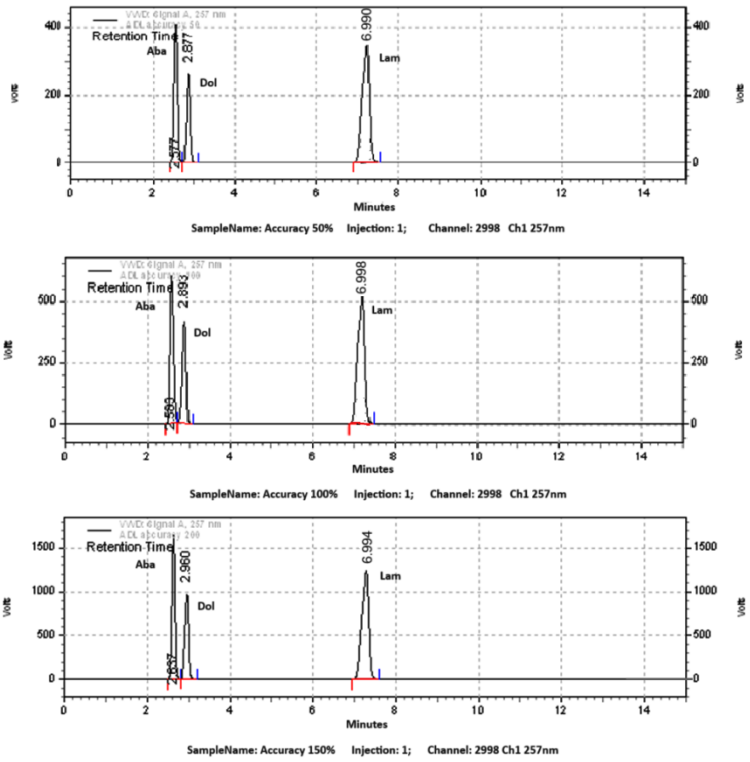


**Figure:** 50%, 100%, 150% Chromatograms of Accuracy

**Limit of detection and quantification**

The limit of detection and quantifications were studied by slope and standard deviation. The detection limit and quantifications were found to be 0.56-0.89 µg/mL respectively. The method was shows ability to detect the Apalutamide at lower concentration hence method proved as high sensitivity to detect the Apalutamide. The chromatograms were shown in figure no 6&7.

**Robustness**

By purposefully altering the flow rate and wavelength of the process, the resilience of the approach was tested. The changed parameters were not affected the method and obtained good %RSD values (Table 3). The %RSD values were in between 0.05-1.49% for wavelength variation and 0.04-0.94% for rate of flow variation. The method was proved as robust for said conditions.

**Table: 3 Robustness results**

| S.No | Wavelength Variation (±2 nm) | Abacavir | Dolutegravir | Lamivudine |
|---|---|---|---|---|
| | | Peak Area | Peak Area | Peak Area |
| Mean | 255 Low | 100202364 | 77536565 | 177508556 |
| SD | 255 Low | 55582 | 1206182 | 903348 |
| %RSD | 255 Low | 0.05 | 1.53 | 0.51 |
| Mean | 259 High | 100302364 | 77519898 | 177925223 |
| SD | 259 High | 51716 | 1156409 | 1281272 |
| %RSD | 259 High | 0.05 | 1.49 | 0.72 |
| **Flow rate Variation (±0.2 mL/min)** | | | | |
| Mean | 0.8 Low | 100292364 | 76886565 | 177508556 |
| SD | 0.8 Low | 84337 | 564574 | 751102 |
| %RSD | 0.8 Low | 0.08 | 0.73 | 0.42 |
| Mean | 1.2 High | 100324031 | 178175223 | 77253232 |
| SD | 1.2 High | 47078 | 412974 | 730184 |
| %RSD | 1.2 High | 0.04 | 0.23 | 0.94 |

**Assay of Apalutamide**

Weighed 20 tablets and finely powdered in to a mortar. The average tablets weight was determined. The powder equivalent to 60 mg of ABV was weighed and transferred in to a 10 mL volumetric flask. The mobile phase was added 10 mL to the volumetric flask to disintegrate tablet powder completely by using ultra sonicator for 5 min. The aliquot portion of the filtrate was further diluted to get final concentration 300µg/mL of ABV, 25µg/mL of DTV and 150 µg/mL

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of LVD. The solution was filtered through membrane filter. The 10µL of the solution was injected into HPLC system.

**Assay in % =** *Area in sample/Area in STD× Weight of STD/ Dilution of STD× Dilution of sample/Weight of sample× Average weight/Labelled claim× Purity of ST*

**Force degradation studies**

The combination dose form was subjected to the stress conditions. There was no interference between the degradants and blank in the method. Different force indicating studies were performed with acid, base, peroxide and UV light. All stress conditions resulted in a %degradation of up to 7.68%. The ABV, DTV, and LVD assay findings from all stress conditions averaged 92.32-94.57%, 93.56-95.12%, and 94.33-95.12%, respectively. The force degradation study findings were shown in Table No. 8.

**Preparation of samples stock solutions**

The forced degradation studies were carried out by weighed accurately 60 mg of ABV and transferred in to 10 mL volumetric flask. The sample was dissolved to sonicate for 5min, diluent was used to bring the volume up to mark.

**Acid degradation studies (3H, at 50℃ with 0.1N HCl)**

Take 0.5 mL of the sample solution from the test stock solution, transfer it to a 10 mL volumetric flask, add 1 mL of 0.1N HCl, and heat it on a water bath for 3 Hrs at 50°C. Take the volumetric flask out of the water bath, allow the solution to cool to ambient temperature, and then add 1 mL of 0.1N NaOH to neutralize the 0.1 N HCl. Use the diluent to dilute the solution up to the mark. To assess the acid stability, 5µL of sample solution was injected into the HPLC chromatographic system, and the chromatogram was recorded.

**Alkaline degradation studies (4H, at 60℃ with 0.1N NaOH)**

A 0.5 mL sample solution was taken from the test stock solution and put into a 10 mL volumetric flask. 1 mL of 0.1N NaOH was then added, and the mixture was heated for 4 Hrs at 60°C on a water bath. The volumetric flask should then be taken out of the water bath, the solution cooled to room temperature, and 1 mL of 0.1N HCl added to neutralize the 0.1 N NaOH. Add diluent to the solution to dilute it to the mark. A 5µL sample solution was injected into the UPLC chromatographic machine, and the chromatogram was recorded in order to evaluate the alkaline stability.

**Peroxide stability (3% v/v $H_2O_2$)**

Collect 0.5 mL of the test stock solution and transfer it to a 10mL volumetric flask, then add 1mL of 3% v/v $H_2O_2$ and leave it at room temperature for 6 Hrs. Dilute the solution to the mark with diluent. To determine the peroxide stability, 5µL of sample solution was injected into an HPLC chromatographic system and the chromatogram was recorded.

**Photolytic degradation (At ≤ 200 nm for 5 days)**

The photolytic degradation study was performed by, accurately weighed equivalent quantity 60 mg of ABV and transferred in to petridish. During five days, the sample was subjected to UV light in a photolytic chamber at < 200 nm. The sample was collected from photolytic chamber in petridish and transferred in to 10 mL volumetric flask. The sample was dissolved and made the volume up to mark with diluent. After passing the sample solution through 0.22µ whatman filter paper, put 0.5 mL of the stock solution into a 10mL volumetric flask, top it off with diluent, and measure the final concentration, which should be 45µg/mL. To assess the stability against light degradation, 5µL of sample solution was injected into the HPLC chromatographic system, and the chromatogram was recorded.

**Table: 4 Degradation Studies**

| Stress Condition | | % Assay of Active Moiety | | | | | |
|---|---|---|---|---|---|---|---|
| | | ABA | % Degradation | DTV | % Degradation | LVD | % Degradation |
| Acid (0.1N HCl refluxed for 3H at 50℃) | | 92.32 | 7.68 | 94.22 | 5.78 | 95.12 | 4.88 |
| Base (0.1N NaOH refluxed for 4H at 60℃) | | 94.23 | 5.77 | 93.56 | 6.44 | 94.46 | 5.54 |
| $H_2O_2$ (3% $H_2O_2$ stored at room temperature for 6 H) | | 94.57 | 5.43 | 95.12 | 4.88 | 94.43 | 5.57 |
| UV Light (256 nm for 5 days) | | 94.33 | 5.67 | 93.87 | 6.13 | 94.33 | 5.57 |

**CONCLUSSION**

The selective, précised and accurate technique was created for the estimate of ABV, DTV and LVD in bulk and its pharmaceutical dosage form. The retention time of drugs were found to be 2.56, 2.86 and 6.79 min ABV, DTV and LVD respectively with high plate count, low tailing factor. The mobile phase was kept at 257 nm with a 70:30 v/v ratio of methanol to water. It was discovered that the linearity range was 150 - 450µg/mL for ABV, 12.5-37.5 µg/mL for DTV and

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75-225 µg/mL for LVD. The range of linearity is wider and regression co-efficient values were found to be 0.999 and its shown good linearity. The limit of detection and quantification limits were found to be 0.57µg/mL and 1.89µg/mL respectively. The drugs were found and measured at low concentration level it indicates the method was highly sensitive. The % RSD values of precision were found to be >2%. The accuracy of the method was found to be between 99.95-100.01% for ABV, 99.95-100.13% for DTV 99.95-100.01% for LVD and results proved as an accurate. The method was shown ability towards forced degradation studies. Different stress conditions were of acid, base, peroxide, photolytic degradation studies were performed on combined dosage form. Few methods were reported on these drugs in combined dosage form and other combinations by using HLPC. The proposed method was more selective, précised, accurate and stability indicated, compared with other methods; it was shown few parameters were good. The ABV, DTV, and LVD in bulk and medicinal dose form were routinely analysed using this approach.

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**Conflict of Interest**
This paper has no conflict of interest, according to the authors.

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