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# STABILITY-INDICATING RP-HPLC METHOD FOR CONCURRENT ESTIMATION OF LAMIVUDINE, TENOFOVIR DISOPROXIL FUMARATE, AND EFAVIRENZ IN BULK AND PHARMACEUTICAL DOSAGE FORM

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## ABSTRACT

The main aspiration of this research work is to assess and validate the Lamivudine, tenofovir disoproxil fumarate and efavirenz in bulk and marketed formulation. The exploration was carried out by using Hypersil BDS column, (150×4.6mm, particle size 5µ) and PDA detector at 258nm. The mobile phase containing Methanol:0.1%TFA: acetonitrile(30:40:30v/v) in isocratic mode pumped into a column at a flow rate 0.8mL/min. The method was validated according to ICH Q2(R1) guidelines. The linearity was perceived in the range of 10-50µg/mL for lamivudine, tenofovir disoproxil fumarate and 20-100µg/mL for efavirenz. The method was accurate with % recovery of 100.41%, 99.69% and 99.75% for Lamivudine, Tenofovir

disoproxil fumarate and Efavirenz respectively. The percentage relative standard deviation was NMT 2, indicating that it was precise and robust. Under stress instances, execution of forced degradation studies such as acidic (0.1N HCL), Basic (0.1N NaOH), oxidative (3%$H_2O_2$), Photolytic (95%RH) and thermal ($80^0$C). The extent of degradation was accomplished within the acceptable limits i.e., 5-20%. The acquired method was precise, accurate and can be utilized for the estimation of lamivudine, tenofovir disoproxil fumarate and efavirenz in bulk and tablet dosage form.

**Keywords: Lamivudine, Tenofovir disoproxil fumarate, Efavirenz, HPLC, Forced degradation studies**

## INTRODUCTION

Lamivudine (LAMI) is a nucleoside analogue and reverse transcriptase inhibitor that is used to treat HIV and hepatitis B virus infections. Before it can be used, it must be phosphorylated to the triphosphate form. It is phosphorylated to an active 5′-triphosphate metabolite within the cell. After integration of the nucleoside analogue into viral DNA, lamivudine triphosphate suppresses HIV-1 reverse transcriptase activity by terminating DNA chains **[1]**. Chemical name for lamivudine is 4-amino -1- [(2R, 5S)-2-(hydroxymethyl)-1, 3-oxathiolan-5-yl] -1, 2-dihydropyrimidin-2-one **(Figure 1)**.

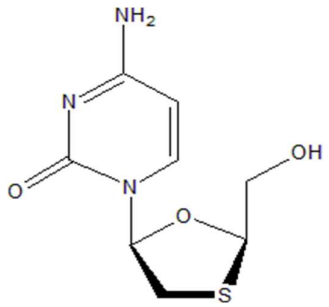


**Figure 1: Structure of Lamivudine**

Tenofovir disoproxil fumarate (TDF) is a drug that is used to treat HIV-1 infection and chronic hepatitis B. Tenofovir is used in combination with other antiretroviral drugs to treat HIV-1 infection in people aged 2 and above. It is a prodrug that can overcome pharmacokinetic limitations caused by the parent drug's restricted permeability across the intestinal mucosa **[2]**. Its chemical name is [[(2*R*)-1-(6-aminopurin-9-yl)propan-2-yl]oxymethyl-(propan-2-yloxycarbonyloxymethoxy)phosphoryl]oxy methyl propan-2-yl carbonate;but-2-enedioic acid **(Figure 2)**.

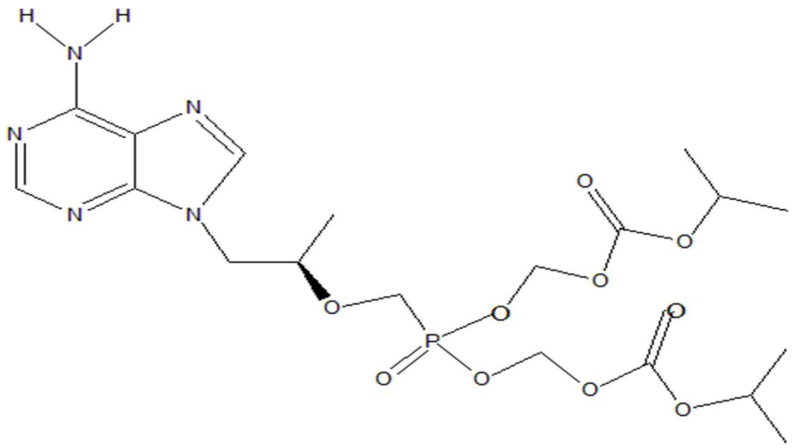

**Figure 2: Structure of Tenofovir disoproxil fumarate**

Efavirenz (EFV) is an anti-HIV medicine that acts by reducing the immune system's damage and preventing AIDS-related disorders. It belongs to the nonnucleoside reverse transcriptase inhibitors family of medicines that convert single-stranded viral RNA to double-stranded DNA. Efavirenz is metabolized extensively, mostly by the cytochrome P-450 isoenzyme CYP2B6, which is known to have a lot of inter-individual variability **[2]**. Its chemical name is (4S)-6- chloro-4(2-cyclopropylethynyl)-4-(tri-fluoromethyl)-2,4-dihydro1H-3,1-benzoxazin-2-one **(Figure 3)**.

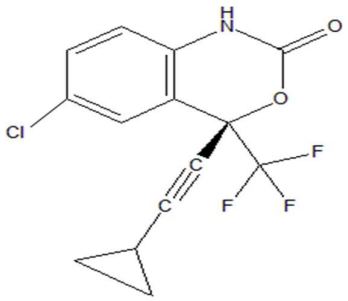

**Figure 3: structure of Efavirenz**

**MATERIALS AND METHODS:**

**MATERIALS:**

National Scientific Products provided analytical quality reagents and solvents such as methanol, acetonitrile, trifluoracetic acid, and HPLC water. From the local market, a (vonaday®) containing lamivudine, tenofovir 300mg and efavirenz 600 mg was purchased.

**INSTRUMENTATION:**

The data was analyzed using the LAB SOLUTIONS software on a Shimadzu HPLC 2030 C 3D Plus apparatus equipped with a PDA (Photo Diode Array) detector.

**PREPARATION OF STANDARD STOCK SOLUTIONS:**

About 10 mg of each lamivudine, tenofovir disoproxil fumarate and efavirenz was properly weighed and deposited into a 10 mL clean dry volumetric flask, to which 3/4

of the volume of methanol was added, and the flask was made up to the mark with methanol to attain 1000µg/ml.

**PREPARATION OF WORKING STANDARD SOLUTIONS:**

From the stock solution 1 mL of Lamivudine, Tenofovir disoproxil fumarate and efavirenz was pipette out and transferred into 10mL clean dry volumetric flask and each volumetric flask made upto the mark with methanol to obtain 100µg/ml.

**PREPARATION OF SAMPLE SOLUTION:**

With a mortar and pestle, 20 tablets were weighed and finely crushed. Lamivudine, tenofovir disoproxil fumarate and efavirenz tablet powder were carefully weighed about 53mg was placed in a 10ml volumetric flask and the volume was built up to the mark with diluent, and then filtered with 0.45 Millipore Nylon filter. 0.3 ml of solution was pipette out and deposited into a 10 ml volumetric flask, and the volume was filled up with methanol.

**CHROMATOGRAPHIC CONDITIONS:**

The Hypersil BDS column (150×4.6mm, particle size 5µ) was used to separate the samples, with the mobile phase containing methanol:0.1 percent TFA: acetonitrile in a ratio of 30:40:30 v/v/v with a flow rate of 0.8mL/min and a detection wavelength of 258 nm with an injection volume of 10µL and a run duration is 15 minutes.

**OPTIMIZED CHROMATOGRAPHIC CONDITIONS:**

**Column** **:** Hypersil BDS (150 x 4.6, 5µm)

**Mobile phase** **:** Methanol: Buffer (0.1%TFA): Acetonitrile (30:40:30v/v)

**Flow rate** **:** 0.8ml/min

**Run time** **:** 15 mins

**Wave length** **:** 258nm

**Injection volume** **:**10µl

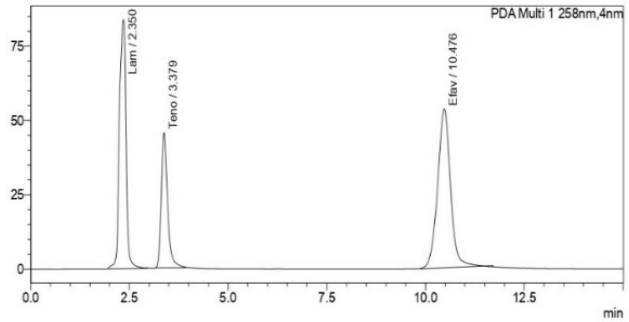


**Figure 4: Optimized chromatogram of standard**

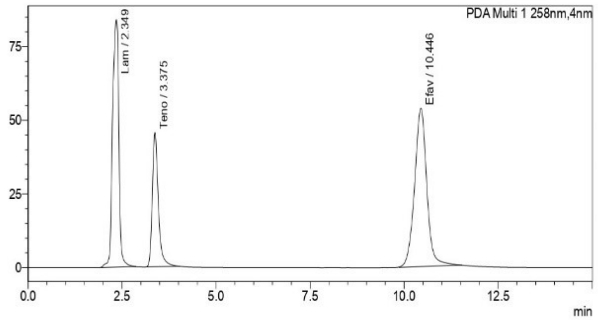


**Figure 5: Optimized chromatogram of sample**

## RESULTS AND DISCUSSION

### SYSTEM SUITABILITY:

The chromatographic conditions were used to optimize the HPLC system. 10 µl standard solutions were used. six injections into the chromatographic system. To determine whether the system is suitable for the parameters of the suggested approach, such as the number of theoretical plates, the retention duration the resolution, tailing factor, and percent RSD were all calculated and compared to a standard system specification and the data was represented in **Table 1**.

### SPECIFICITY:

Lamivudine, tenofovir disoproxil fumarate and efavirenz chromatograms are compared to assess specificity, and any interference with the analyte is noticed. The chromatograms were obtained after the standard, sample, and diluent solutions were prepared according to the test procedure and injected into the chromatographic system. There was no interaction of the blank with the analyte of the standard and sample.

### LINEARITY:

Triplicate injections of solutions containing standard Lamivudine, Tenofovir disoproxil fumarate and Efavirenz were used to determine linearity. For Lamivudine, Tenofovir disoproxil fumarate, the linearity range was 10 to 50 µg/ml, while for Efavirenz, it was 20 to 100 µg/ml data was represented in **Table 2**.

### PRECISION:

The technique's precision was established by applying a standard solution to determine system and method precision. Six injections of Lamivudine and tenofovir disoproxil fumarate 30µg/ml and Efavirenz 60µg/ml standard solutions was mentioned in **Table 3**.

### ACCURACY:

The accuracy was accomplished by producing known quantities of samples at 50, 100, and 150 percent. At each level, they were injected three times**.** Data was represented in **Table 4**.

### LIMIT OD DETECTION (LOD) and LIMIT OF QUANTIFICATION(LOQ):

According to the methodology, the calibration curves were used to compute the limit of detection (LOD) and limit of quantitation (LOQ) values.

### ROBUSTNESS:

By altering chromatographic parameters such as the flow rates of 0.6ml/min and 1.0ml/min as well as change in wavelength to 253nm and 263nm of the standard solution data was mentioned in **Table 5**.

### % ASSAY:

The percentage purity of Lamivudine, Tenofovir disoproxil fumarate and efavirenz was found to be 99.45%, 99.20% and 99.17% respectively. Data was represented in **Table 6**.

**FORCED DEGRADATION STUDIES:**

Lamivudine, Tenofovir disoproxil fumarate and Efavirenz pharmaceutical formulation (VONDAY®) was exposed to acidic (0.1N HCl), Basic (0.1N NaOH), and peroxide (3% $H_2O_2$) conditions at room temperature for 24 hours, as well as thermal degradation at $80^0$C hot air oven and under the sunlight for 24 hours. The solutions were allowed to cool to room temperature before being diluted with solvent to get a sample solution containing 300 µg/ml of Lamivudine, 300 µg/ml of Tenofovir disoproxil fumarate and 600 µg/ml of Efavirenz. With a PDA detector, the percent assay of the drug recovered from the stressed sample was evaluated and compared to chromatograms of untreated pharmaceuticals in tablet solution, as well as data from degradation tests. Data was represented in **Table 7**.

**Table 1: Data of System suitability**

| Injection No | Lamivudine | | Tenofovir disoproxil fumarate | | Efavirenz | |
|---|---|---|---|---|---|---|
| | $R_t$(min) | Peak area | $R_t$(min) | Peak area | $R_t$(min) | Peak area |
| 01 | 2.349 | 978175 | 3.375 | 504263 | 10.446 | 1213623 |
| 02 | 2.350 | 981444 | 3.374 | 500356 | 10.476 | 1211768 |
| 03 | 2.346 | 981608 | 3.378 | 514086 | 10.464 | 1240268 |
| 04 | 2.348 | 975257 | 3.375 | 509881 | 10.461 | 1237267 |
| 05 | 2.345 | 978830 | 3.379 | 508489 | 10.467 | 1248412 |
| 06 | 2.351 | 971426 | 3.381 | 506663 | 10.478 | 1209018 |
| Mean | | 977790 | 507290 | | 1226726 | |
| Standard Deviation | | 3900.64738 | 4732.287 | | 17168.49 | |
| %RSD | | 0.40 | 0.93 | | 1.40 | |

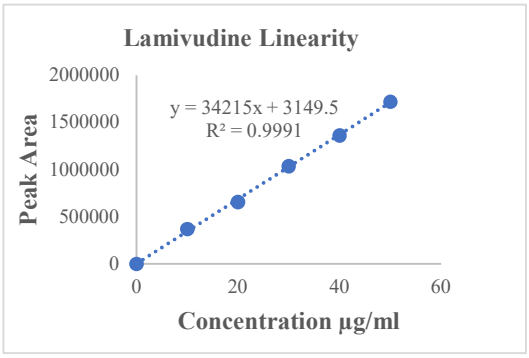


**Figure: 06 Linearity plot of Lamivudine**

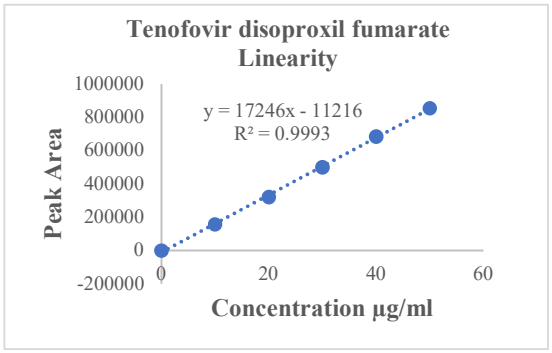


**Figure: 07 Linearity plot of tenofovir disoproxil fumarate**

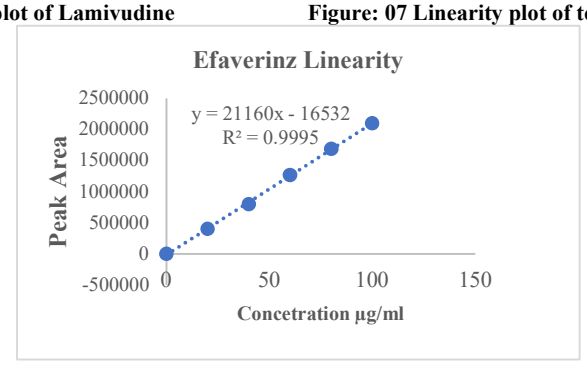


**Figure: 08 Linearity plot of Efavirenz**

**Table 2: Linearity data for Lamivudine, Tenofovir disoproxil fumarate and efavirenz**

| Lamivudine | | Tenofovir disoproxil fumarate | | Efavirenz | |
|---|---|---|---|---|---|
| Conc(µg/ml) | Peak Area | Conc(µg/ml) | Peak Area | Conc(µg/ml) | Peak Area |
| 10 | 370254 | 10 | 157393 | 20 | 401534 |
| 20 | 656340 | 20 | 321654 | 40 | 797466 |
| 30 | 1037923 | 30 | 500439 | 60 | 1265405 |
| 40 | 1364422 | 40 | 685650 | 80 | 1686649 |
| 50 | 1722254 | 50 | 854543 | 100 | 2097736 |
| $R^2$=0.9991 | | $R^2$=0.9993 | | $R^2$=0.9995 | |

**Table 3: Data of system precision and method precision**

| S. No | System Precision | | | Method Precision | | |
|---|---|---|---|---|---|---|
| | Lam | TDF | Efavirenz | Lam | TDF | Efavirenz |
| | Peak area | Peak area | Peak area | Peak area | Peak area | Peak area |
| 01 | 977125 | 505243 | 1242323 | 976168 | 505253 | 1223724 |
| 02 | 981234 | 502356 | 1212248 | 982344 | 502342 | 1221489 |
| 03 | 982607 | 512075 | 1230278 | 971804 | 518046 | 1250268 |
| 04 | 974228 | 507951 | 1248257 | 978249 | 506841 | 1237267 |
| 05 | 979720 | 508276 | 1237212 | 976530 | 508139 | 1248412 |
| 06 | 972524 | 507562 | 1198014 | 971528 | 504562 | 1199018 |
| AVG | 977906.3 | 507243.8 | 1228055 | 976103.8 | 507530.5 | 1230030 |
| SDV | 3989.214 | 3252.725 | 19250 | 4078.087 | 5519.8 | 19361.34 |
| %RSD | 0.41 | 0.64 | 1.57 | 0.42 | 1.09 | 0.57 |

**Table 4: Accuracy data for Lamivudine, Tenofovir disoproxil fumarate, Efavirenz**

| % Level | Lamivudine | | | | Tenofovir disoproxil fumarate | | | |
|---|---|---|---|---|---|---|---|---|
| | Standard peak area | Sample Peak area | % Recovery | Mean % recovery | Standard peak area | Sample Peak area | % Recovery | Mean % recovery |
| 50% | 977790 | 491232 | 100.29 | 100.41% | 507290 | 256589 | 100.57 | 99.69% |
| | 977790 | 492423 | 100.19 | | 507290 | 258954 | 101.15 | |
| | 977790 | 493025 | 100.81 | | 507290 | 254859 | 100.04 | |
| 100% | 977790 | 985367 | 100.43 | | 507290 | 507148 | 99.23 | |
| | 977790 | 986367 | 100.39 | | 507290 | 508452 | 99.34 | |
| | 977790 | 985654 | 100.26 | | 507290 | 507589 | 99.12 | |
| 150% | 977790 | 1469754 | 100.07 | | 507290 | 758956 | 99.20 | |
| | 977790 | 1479891 | 100.85 | | 507290 | 759189 | 99.32 | |
| | 977790 | 1475651 | 100.42 | | 507290 | 759589 | 99.24 | |

| % Level | Efavirenz | | | |
|---|---|---|---|---|
| | Standard peak area | Sample Peak area | % Recovery | Mean % recovery |
| 50% | 1226726 | 615689 | 99.49 | 99.75% |
| | 1226726 | 617854 | 99.50 | |
| | 1226726 | 614889 | 99.51 | |
| 100% | 1226726 | 1228457 | 99.10 | |
| | 1226726 | 1229465 | 99.04 | |
| | 1226726 | 1229889 | 99.01 | |
| 150% | 1226726 | 1864581 | 100.48 | |
| | 1226726 | 1856941 | 100.16 | |
| | 1226726 | 1884521 | 101.51 | |

**Table 5: Data of robustness**

| S. No | Parameters | Lamivudine | | | Tenofovir disoproxil fumarate | | | Efavirenz | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Rt (min) | Peak area | %RSD | Rt (min) | Peak area | %RSD | Rt (min) | Peak area | %RSD |
| 01 | Flow rate 0.6ml/min | 3.067 | 1794471 | 0.83 | 4.583 | 7365284 | 0.87 | 12.356 | 2219837 | 1.00 |
| | | 3.075 | 1815682 | | 4.586 | 7456423 | | 12.361 | 2251432 | |
| 02 | Flow rate 1ml/min | 1.867 | 1054344 | 0.48 | 2.854 | 429288 | 0.53 | 8.661 | 1341359 | 0.60 |
| | | 1.871 | 1061465 | | 2.859 | 432504 | | 8.629 | 1352741 | |
| 03 | Change in wavelength 253nm | 2.342 | 709267 | 1.15 | 3.378 | 412604 | 0.98 | 10.428 | 2245843 | 1.03 |
| | | 2.330 | 697870 | | 3.376 | 406904 | | 10.449 | 2278720 | |
| 04 | Change in wavelength 263nm | 2.373 | 1491538 | 0.44 | 3.477 | 468727 | 0.50 | 10.527 | 664215 | 0.86 |
| | | 2.378 | 1500849 | | 3.514 | 472036 | | 10.599 | 672348 | |

**Table 6: Data of percentage Assay:**

| Tablet sample | Label claim (mg) | % Assay |
|---|---|---|
| Lamivudine | 300 mg | 99.45% |
| Tenofovir disoproxil fumarate | 300 mg | 99.20% |
| Efavirenz | 600 mg | 99.17% |

**Table 7: Data of Forced degradation studies**

| S. No | Degradation study | Degradation conditions | Peak area | | | %Assay | | |
|---|---|---|---|---|---|---|---|---|
| | | | Lam | TDF | Efavirenz | Lam | TDF | Efavirenz |
| 01 | Tablet assay | None | 975367 | 506788 | 1228852 | 99.45% | 99.20% | 99.17% |
| 02 | Acid degradation | 0.1N HCL | 918967 | 475773 | 1148852 | 93.70% | 93.13% | 92.72% |
| 03 | Base degradation | 0.1N NaOH | 938967 | 485773 | 1188852 | 95.74% | 95.09% | 95.94% |
| 04 | Oxidative degradation | 3% H2O2 | 916093 | 465773 | 1158852 | 94.43% | 93.13% | 95.94% |
| 05 | Thermal degradation | 80°C | 917093 | 462873 | 1157852 | 93.51% | 90.61% | 93.44% |
| 06 | Photolytic degradation | 90%RH | 926093 | 475773 | 1168852 | 94.43% | 93.13% | 94.33% |

## DISCUSSION:

In optimized chromatogram, Lamivudine, Tenofovir disoproxil fumarate and efavirenz are eluted at 2.360, 3.379, 10.476 respectively. The linearity was perceived in the range of 10-50µg/ml for lamivudine, tenofovir disoproxil fumarate and 20-100µg/ml for efavirenz. The method was accurate with % recovery of 100.41%, 99.69% and 99.75% for Lamivudine, Tenofovir disoproxil fumarate and Efavirenz respectively. Precision results were found to be within the acceptable limits. The method was validated according to ICH guidelines. Even with the changes in robustness parameters, separation was not affected and persists within the acceptable limits. Under stress instances, execution of forced degradation studies such as acidic (0.1N HCL), Basic (0.1N NaOH), oxidative (3%$H_2O_2$) and thermal ($80^0$C). The extent of degradation was accomplished within the acceptable limits i.e., 5-20%.

**CONCLUSION:**

A simple, rapid and specific method was developed and validated for the estimation of Lamivudine, tenofovir disoproxil fumarate and efavirenz in bulk and marketed formulation. The exploration was carried out by using Hypersil BDS column, (150×4.6mm, particle size 5µ) and PDA detector at 258nm. The mobile phase containing Methanol: 0.1%TFA: acetonitrile (30:40:30v/v) in isocratic mode pumped into a column at a flow rate 0.8ml/min. The method was validated according to ICH Q2(R1) guidelines. According to a review of the literature, no stability-indicating RP-HPLC procedures for simultaneous estimation have been devised for Lamivudine, tenofovir disoproxil fumarate with efavirenz. The current work exemplifies the creation and testing of a Stability straight forward, precise, and accurate simultaneous estimation method.

**ACKNOWLEDGEMENT:**

I am very thankful to the administration of Chalapathi Institute of Pharmaceutical Sciences for providing the required resources and guidance.

**CONFLICT OF INTEREST:**

In this inquiry, the authors have no competing interests.

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