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# DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF LAMIVUDINE AND TENOFOVIR DISOPROXIL FUMARATE IN COMBINED DOSAGE FORM USING QUALITY BY DESIGN APPROACH

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**Keywords:**

Lamivudine, Tenofovir Disoproxil Fumarate, UV- spectrophotometric method, Simultaneous estimation, QbD

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**ABSTRACT:** A new, simple, rapid, accurate, and economical method has been developed for the simultaneous estimation of Lamivudine and Tenofovir Disoproxil Fumarate in formulation by using a quality by design approach. Design expert software was used for QbD analysis. 03 level factorial quadratic design models were used to analyze the response. The model generated was found to be significant. The optimized conditions of factors were concentration 10ug/ml and wavelength ($\lambda_{max}$) 272 nm for LAM and 259 nm for TDF. The distilled water was used as a solvent for analysis. The linearity was observed in the concentration range of 02-100 µg/ml for Lamivudine and Tenofovir Disoproxil Fumarate, both drugs. The simultaneous equation method was used for estimation, and the method was validated as per ICH guidelines. The recovery of Lamivudine and Tenofovir Disoproxil Fumarate was found in the range of 98.90-100.77% and 101.63-102.43%. The stability testing was done as per ICH guidelines. The developed method may be used by industries for analyzing their products.

**INTRODUCTION:** Acquired immunodeficiency syndrome (AIDS) was first recognized in 1981 and the human immunodeficiency virus (HIV) that causes AIDS was identified in 1983. By the end of 2015, there were 36.7 million people living with HIV worldwide, and as of June 2016, only 18.2 million HIV-infected people had routine access to antiretroviral therapy. Lamivudine (LAM) is chemically - 4 - Amino - 1 - [(2*R*, 5*S*) – 2 - (hydroxymethyl)-1, 3-oxathiolan-5-yl] pyrimidin-2-one is a reverse transcriptase inhibitor reported to be active against HIV-1, HIV-2, and hepatitis B virus.

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Lamivudine has been used for treatment of chronic hepatitis B at a lower dose than for treatment of HIV. Tenofovir disoproxil fumarate (TDF) is (2R)-1- (6-Aminopurin-9-yl)propan-2-yl] oxymethyl -(propan -2-yloxycarbonyl - oxymethoxy) phospho-ryl] oxymethyl propan-2-yl carbonate;(E)-but-2-enedioic acid belongs to the class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors (nRTIs), which blocks reverse transcriptase, an enzyme crucial to viral production in HIV-infected people. Tenofovir is the first nucleotide analogue approved for HIV-1 treatment [1-3].

The quality by design approach has been used by several professional platforms to optimize the newly developed process and product. The application of the QbD approach enhances product quality, analytical, and manufacturing productivity. Therefore, this approach can extend to newly develop analytical methods [4-7].

A thorough literature survey revealed that there are no analytical methods reported using QbD approach for quantitative estimation of Lamivudine and Tenofovir Disoproxil Fumarate in combination or alone in the solid dosage form. In the present work a successful attempt made to develop suitable analytical UV method and validated as per ICH guideline by using quality by design approach [8-11].

**Structure of Drugs [12-13]:**

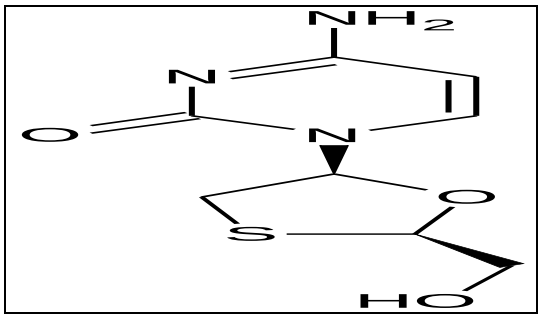

**FIG. 1: STRUCTURE OF LAMIVUDINE**

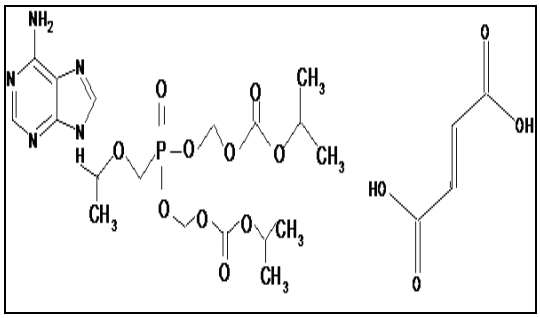

**FIG. 2: STRUCTURE OF TENOFOVIR DISOPROXIL FUMARATE**

## MATERIALS AND METHODS:

**Chemicals and Reagents:** The standard drug samples of Lamivudine and Tenofovir Disoproxil Fumarate were provided as gift samples from Macleods pharmaceuticals Mumbai. Distilled water AR grade is used as a solvent for UV Spectrophotometric dilutions; all solutions were filtered through Whatman filter paper no. 41 before use in UV spectrophotometer. The tablet formulation Tenvir-L was purchased from the local market, its detail is given below.

**TABLE 1: MARKETED FORMULATION**

| Brand Name | Drugs | Label Claim | Manufactured by |
|---|---|---|---|
| Tenvir-L | Lamivudine (IP) | 300 mg | Cipla Pharmaceuticals |
| | Tenofovir Disoproxil Fumarate (IP) | 300 mg | |

**Instruments:** Shimadzu UV 1800 with UV probe 2.33 software and 10 mm matched quartered cell and precision balance model Citizen Cy220 were used.

**Selection of Solvent:** Distilled water was selected as the suitable solvent for simultaneous estimation of Lamivudine and Tenofovir Disoproxil Fumarate after trials of several solvents.

**Preparation of Standard Stock Solutions:**

**Standard Stock Solution (A):** An accurately weighed quantity of Lamivudine equivalent to LAM (10.0 mg) was dissolved in distilled water in a volumetric flask (10.0 ml). The volume was made up to mark with distilled water. Appropriate dilutions were made from the resulting solution with distilled water so as to get a concentration of 100 µg/ml.

**Standard Stock Solution (B):** An accurately weighed quantity of Tenofovir Disoproxil Fumarate equivalent to TDF (10.0 mg) was dissolved in distilled water in a volumetric flask (10.0 ml).The volume was made up to mark with distilled water. Appropriate dilutions were made from the resulting solution with distilled water so as to get a concentration of 100 µg/ml.

**Mixed Standard Stock Solution (C):** An aliquots portion of stock solution A and stock solution B in the ratio of 1:1 were mixed in a volumetric flask (10.0 ml), and volume was adjusted up to mark with distilled water.

**Quality by Design Approach for Optimization of Method by UV:** The quality by design approach was used for this analytical method development process by UV. Firstly the critical quality attributes were identified for this method. 32 factorial designs were applied for the optimization of the processes. The numerical representation 32 is read as the model will have 3 levels of study, including minimum, maximum, and average value. The number 2 present at the superscript represents that there are two factors that directly affect the final response. The response in the present study was considered as absorbance of the solution. Design Expert ® software was used for the QbD studies.

The critical quality attributes (CQA) identified for this method were wavelength and concentration of drug solution. The critical analytical attribute (CAA) for this method was absorbance of the solution of drug to be analyzed.

**Preparation of Calibration Curve:** The standard stock solution of LAM and TDF were diluted with distilled water to get series of a standard solution having concentrations 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/ml. Similarly, laboratory mixtures were diluted with distilled water to get the concentration in the range of 2:2, 5:5, 10:10, 20:20, 30:30, 40:40, and 50:50 µg/ml. Calibration curves were plotted as concentration versus absorbance is shown in **Fig. 11, 12 & 13,** respectively.

**Preparation of Sample Solution:** Twenty tablets were weighed accurately and finely powdered. The powder equivalent to 10 mg of LAM and 10 mg of TDF was dissolved in distilled water, shake properly and make up the volume. The solution was sonicated for 5 min, filtered through Whatman filter paper an aliquot portion of the filter was diluted with distilled water to get a conc. of 10 µg/ml and 10 µg/ml of LAM and TDF respectively from the resulting solution

**Simultaneous Equation Method [14-15]:** From the spectra, two wavelengths were selected as 272 nm for LAM and 259 nm for TDF. The absorptive values of LAM and TDF were determined at selected wavelengths. The concentration of two drugs in the mixture can be calculated using the following equations,

$$Cx = A2ay1 – A1ay2 / ax2ay1 – ax1ay2 \ldots\ldots \text{ [Eq.1]}$$

$$Cy = A1ax2 – A2ax1 / ax2ay1 – ax1ay2 \ldots\ldots \text{ [Eq.2]}$$

Where, CX = Concentration of LAM, CY = Concentration of TDF, A1 = Absorbance of mixture at 259 nm, A 2 = Absorbance of mixture at 272 nm, ax1 & ax2 = Absorptivity of LAM at 259nm and 272 nm, ay1 & ay2 = Absorptivity of TDF at 259 nm and 272 nm.

**Method Validation [16]:**

**Linearity & Range:** The linearity of the proposed methods was evaluated by linear regression analysis, which was calculated by the least square method. Calibration standards were prepared by dilution of working standard solution 100 µg/ml of LAM and 100 µg/ml TDF into different 10ml volumetric flasks and volume made with distilled water to y get concentrations of 2, 5 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100µg/ml of LAM and TDF both. The absorbance of the drugs was measured.

**Accuracy:** The accuracy of the methods was determined at three different concentration levels *i.e.* 80%, 100% and 120% in triplicate for each drug as per ICH guidelines.

**Precision:** Precision was studied to find out intra and inter-day variations in the test method of LAM and TDF. Intraday precision was determined by analyzing three concentrations in three replicate measurements within the linearity range of drugs three different times in the same day. Inter-day precision was conducted during routine operation of the system over a period of 3 consecutive days. Also, precision was carried out by using different analysts.

**Limit of Detection (LOD) and Limit of Quantification (LOQ):** LOD is the lowest amount of analyte in a sample that can be detected but not necessarily quantify under the stated experimental conditions. LOQ is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under stated experimental conditions**.** The LOD and LOQ for LAM and TDF were determined according to the ICH guideline.

$$LOD = 3.3\sigma / S$$

$$LOQ = 10\sigma / S$$

Where, σ = Standard deviation of the y-intercept of calibration curves. S = Slope of the calibration curve.

**Stress Degradation Study [17-18]:** The stress degradation study of LAM and TDF was carried out as per ICH Q1A (R2) and photostability as per ICH Q1B guidelines. All the stressed samples were analyzed by the proposed method, and % labeled claim was calculated.

**Acidic Hydrolysis Study:** Acidic hydrolysis studies were performed using a sample with 0.1 N HCl. An equivalent weight of LAM and TDF (100mg each) was first dissolved in a small portion of distilled water and then mixed with 10 ml of 0.1

N HCl in a volumetric flask (25 ml). This solution was then kept for 24 h. The samples were further diluted with distilled water to get a concentration of 10 µg/ml of LAM and 10 µg/ml of TDF. The absorbance of the solution was taken, and % labeled claim was calculated.

**Alkaline Hydrolysis Study:** Alkaline hydrolysis studies were performed using a sample with 0.1 N NaOH. An equivalent weight of LAM and TDF (100 mg each) was first dissolved in a small portion of distilled water and then mixed with 10 ml of 0.1 N NaOH in a volumetric flask (25 ml). This solution was then kept for 24 h.

The samples were further diluted with distilled water to get a concentration of 10 µg/ml of LAM and 10 µg/ml of TDF. The absorbance of the solution was taken, and % labeled claim was calculated.

**Oxidative Degradation Study:** It was performed in 3 % H2O2 for 24 h. Equivalent weight of LAM and TDF (100 mg each) was first dissolved in a small portion of distilled water and then mixed with 10 ml of 3 % $H_2O_2$ in a volumetric flask (25 ml).

This solution was then kept for 24 h. The samples were further diluted with distilled water to get a concentration of 10 µg/ml of LAM and 10 µg/ml of TDF. The absorbance of the solution was taken and % labeled claim was calculated.

**Neutral Hydrolysis Study:** Neutral hydrolysis studies were performed using a sample with water. The equivalent weight of LAM and TDF (100 mg each) was first dissolved in a small portion of distilled water, and then 10 ml of distilled water is added in a volumetric flask (25 ml).

This solution was then kept for 24 h. The samples were further diluted with distilled water to get a concentration of 10 µg/ml of LAM and 10 µg/ml of TDF. The absorbance of the solution was taken, and % labeled claim was calculated.

**Photolytic Degradation Study:** For photostability study, the standard drugs were exposed to UV light in the photostability chamber for 24 h at 254 nm. Appropriate dilutions were made using distilled water. The absorbance of the solution was taken and % labeled claim was calculated.

**Thermal Degradation Study:** Thermal degradation studies were carried out by exposing the pure drug to the temperature of 60 ºC for 24 h and the samples were analysed after 24 h. The absorbance was taken, and % labeled claim was calculated.

## RESULTS AND DISCUSSION:

### Study of Various Parameters:

**Location of $\lambda_{max}$:** An aliquots portion of standard stock solution A and B were appropriately diluted with distilled water to get a concentration 10 µg/ml. The solutions were scanned in the range of 200 nm to 400 nm against solvent blank.

The wavelength 272 nm was selected for LAM and 259 nm for TDF. The UV absorbance overlain spectrum of the drug is depicted in **Fig. 3.**

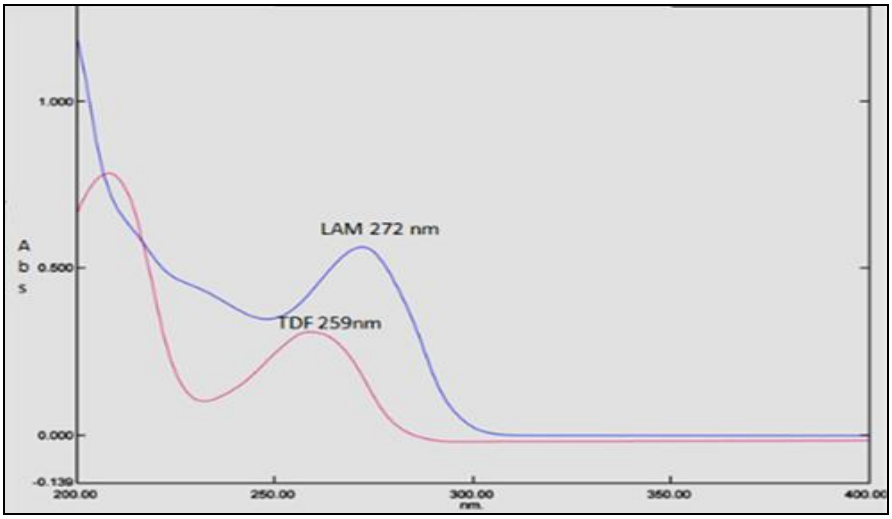


**FIG. 3: OVERLAIN SPECTRUM OF LAM AND TDF**

**Data of QbD Approach:** The 3D plot of final response was found to be, the contour plot and overlay plot of response were found as follows,

**TABLE 2: EXPERIMENTAL DATA FOR STUDY OF QBD BY UV FOR LAM**

| Std | Run | Factor 1 A: Concentration ug/ml | Factor 2 B: Wavelength nm ( λmax.) | Response 1 Absorbance Abs LAM |
|---|---|---|---|---|
| 7 | 1 | 8 | 273 | 0.47 |
| 13 | 2 | 10 | 272 | 0.49 |
| 3 | 3 | 12 | 271 | 0.52 |
| 12 | 4 | 10 | 272 | 0.49 |
| 2 | 5 | 10 | 271 | 0.46 |
| 6 | 6 | 12 | 272 | 0.53 |
| 5 | 7 | 10 | 272 | 0.49 |
| 1 | 8 | 8 | 271 | 0.45 |
| 11 | 9 | 10 | 272 | 0.49 |
| 9 | 10 | 12 | 273 | 0.56 |
| 10 | 11 | 10 | 272 | 0.49 |
| 4 | 12 | 8 | 272 | 0.46 |
| 8 | 13 | 10 | 273 | 0.50 |

03 level factorial quadratic design models were used to analyze the response. The model generated was found to be significant. The optimized conditions of factors were concentration 10 ug/ml and wavelength (λmax.) 272nm for LAM.

**TABLE 3: THE DESIGN SUMMARY FOR LAMIVUDINE**

| Factor | Name | Units | Type | Low Actual | High Actual | Low Coded | High Coded |
|---|---|---|---|---|---|---|---|
| A | Concentration | ug/ml | Numeric | 8.0 | 12.0 | -1.000 | 1.000 |
| B | Wavelength (λmax) | Nm | Numeric | 271 | 273 | -1.000 | 1.000 |
| **Response** | **Name** | **Units** | **Obs** | **Analysis** | **Minimum** | **Maximum** | **Mean** |
| Y1 | Absorbance LAM | Abs | 13 | Polynomial | 0.450 | 0.560 | 0.492 |

The final equation of actual factors derived was found to be, LAM Absorbance = 201. 224830. 72204* Concentration+1.49236*Wavelength LAM+2.50000E-003 * Concentration * Wavelength LAM+3.06034E-003 * Concentration 2-2.75862E-003 * Wavelength LAM2.

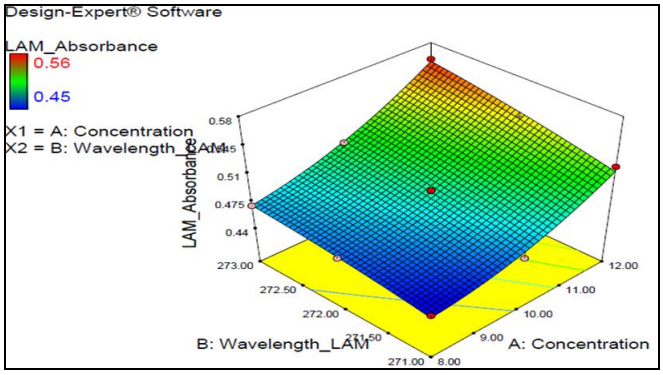


**FIG. 4: 3D PLOT OF RESPONSE OF LAMIVUDINE**

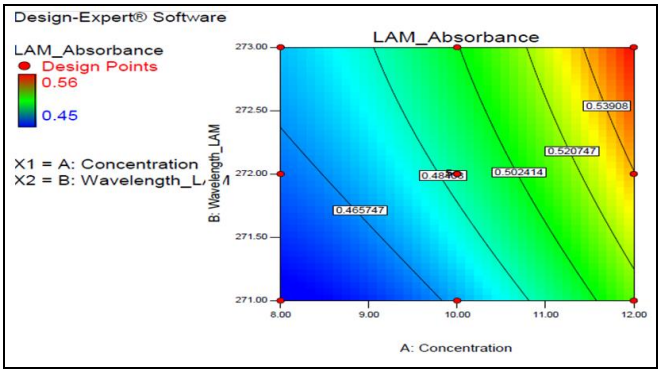


**FIG. 5: CONTOUR PLOT OF RESPONSE OF LAMIVUDINE**

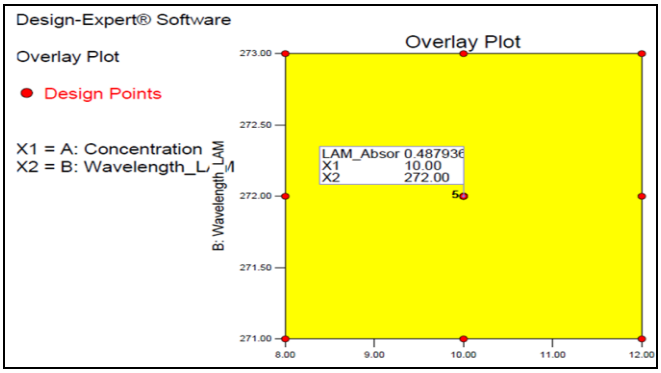


**FIG. 6: OVERLAY PLOT OF RESPONSE OF LAMIVUDINE**

**TABLE 4: EXPERIMENTAL DATA FOR STUDY OF QBD BY UV FOR TDF**

| Std | Run | Factor 1 A: Concentration ug/ml | Factor 2 B: Wavelength nm (λmax.) | Response 1 TDF Absorbance Abs |
|---|---|---|---|---|
| 3 | 1 | 12 | 258 | 0.192 |
| 11 | 2 | 10 | 259 | 0.189 |
| 1 | 3 | 8 | 258 | 0.185 |
| 12 | 4 | 10 | 259 | 0.189 |
| 5 | 5 | 10 | 259 | 0.189 |
| 6 | 6 | 12 | 259 | 0.193 |
| 9 | 7 | 12 | 260 | 0.196 |
| 7 | 8 | 8 | 260 | 0.185 |
| 4 | 9 | 8 | 259 | 0.185 |
| 8 | 10 | 10 | 260 | 0.194 |
| 2 | 11 | 10 | 258 | 0.186 |
| 10 | 12 | 10 | 259 | 0.189 |
| 13 | 13 | 10 | 259 | 0.189 |

03 level factorial quadratic design model was used to analyse the response. The model generated was found to be significant. The optimized conditions of factors were concentration 10 ug/ml and wavelength ($\lambda_{max}$.) 259nm for TDF.

**TABLE 5: THE DESIGN SUMMARY FOR TENOFOVIR DISOPROXIL FUMARATE**

| Factor | Name | Units | Type | Low Actual | High Actual | Low Coded | High Coded |
|---|---|---|---|---|---|---|---|
| A | Concentration | ug/ml | Numeric | 8.0 | 12.0 | -1.000 | 1.000 |
| B | Wavelength ($\lambda_{max}$) | Nm | Numeric | 258 | 260 | -1.000 | 1.000 |
| **Response** | **Name** | **Units** | **Obs** | **Analysis** | **Minimum** | **Maximum** | **Mean** |
| Y1 | Absorbance TDF | Abs | 13 | Polynomial | 0.185 | 0.196 | 0.189 |

The final equation of actual factors derived was found to be, TDF Absorbance =-0.35036+2.16667E-003 * Concentration+2.00000E-003 * Wavelength TDF

The 3D plot of final response was found to be, the contour plot and overlay plot of response were found as follows,

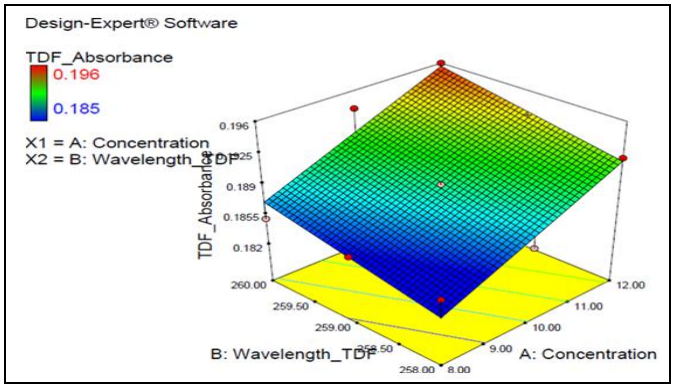


**FIG. 7: 3D PLOT OF RESPONSE OF TENOFOVIR DISOPROXIL FUMARATE**

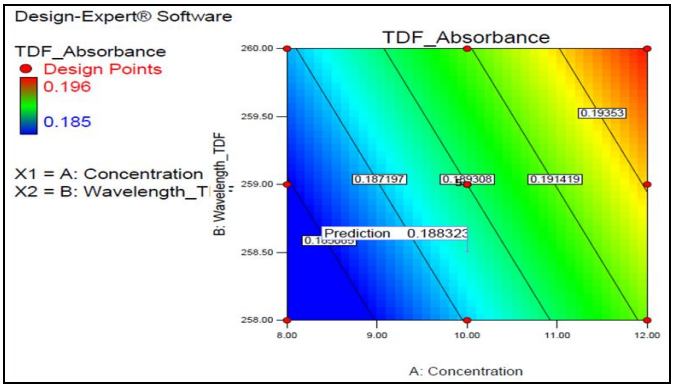


**FIG. 8: CONTOUR PLOT OF RESPONSE OF TENOFOVIR DISOPROXIL FUMARATE**

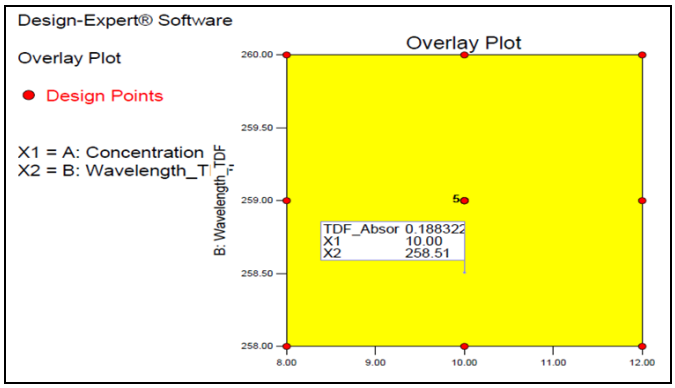


**FIG. 9: OVERLAY PLOT OF RESPONSE OF TENOFOVIR DISOPROXIL FUMARATE**

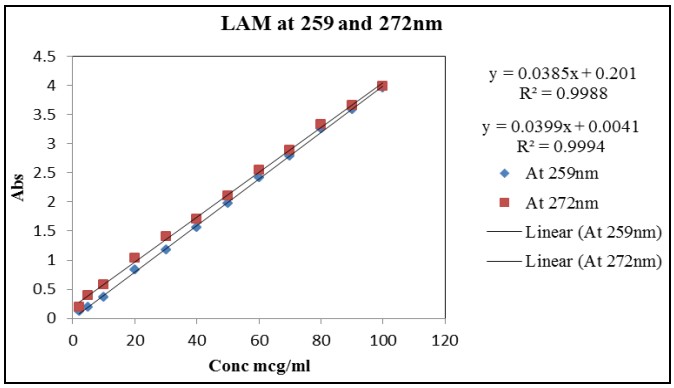


**FIG. 10: CALIBRATION CURVE OF LAM**

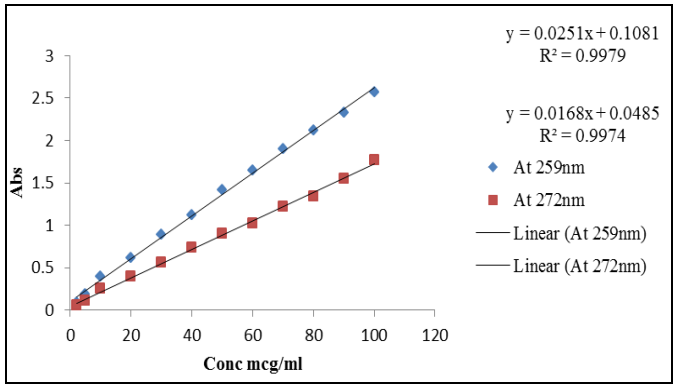


FIG. 11: CALIBRATION CURVE OF TDF

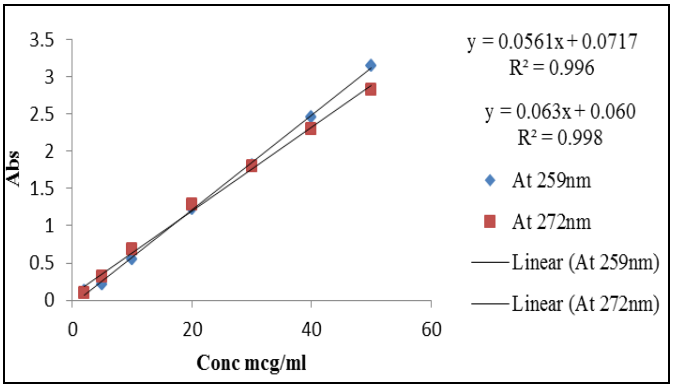


FIG. 12: CALIBRATION CURVE OF LABORATORY MIXTURE OF LAM AND TDF

**Determination of Absorptivity at Analytical Wavelength:** Absorbance of the individual drug was divided by the concentration in g/100 ml to get the absorptive coefficients of this drug which were determined at a selected wavelength.

(1%, 1cm) = Absorbance \ Concentration(g / 100 ml)

**TABLE 6: ABSORPTIVITY COEFFICIENT OF DRUG**

| Drugs | Absorptive of Drugs | |
|---|---|---|
| | 259 nm | 272 nm |
| LAM | 361 | 490 |
| TDF | 189 | 92 |

**Analysis of Laboratory Mixture by Proposed Method:** The laboratory mixture of LAM (10 µg/ml) and TDF (10 µg/ml) was prepared from stock solution in distilled water and their absorbance value at two selected wavelength was recorded. The procedure was repeated five times for analysis of homogenous standard mixture.

The concentration of each drug was then calculated by using [Eq.03] and [Eq.04] given below.

By using Simultaneous Equation Method

Cx = 0.003181 A2 – 0.0015488 A1 ……[Eq.3]

Cy = 0.008249 A1 – 0.006077 A2 ……[Eq.4]

Where, CX = Concentration of LAM, CY = Concentration of TDF. A1 = Absorbance of mixture at 259 nm. A 2 = Absorbance of mixture at 272 nm. The result is given in **Table 7.**

**TABLE 7: DATA OF ANALYSIS OF LAM AND TDF IN LABORATORY MIXTURE**

| S. no. | Amount of Drug Taken for Assay (µg/ml) | | Amount of Drug Estimated (µg/ml) | | % Drug Estimated | |
|---|---|---|---|---|---|---|
| | LAM | TDF | LAM | TDF | LAM | TDF |
| 1 | 10 | 10 | 10.22 | 10.20 | 102.20 | 102.00 |
| 2 | 10 | 10 | 10.19 | 10.47 | 101.90 | 104.70 |
| 3 | 10 | 10 | 09.93 | 10.49 | 99.30 | 104.90 |
| 4 | 10 | 10 | 10.15 | 09.97 | 101.50 | 99.70 |
| 5 | 10 | 10 | 10.27 | 10.26 | 102.70 | 102.60 |

**Statistics:**

| Drugs | Mean | ± SD | R.S.D |
|---|---|---|---|
| LAM | 101.52 | 1.383 | 1.176 |
| TDF | 102.77 | 1.426 | 1.381 |

**Analysis of Marketed Formulation by Proposed Method:** The results of analysis of the marketed formulation are given in **Table 8.**

**TABLE 8: DATA OF ANALYSIS OF MARKETED FORMULATION**

| S. no. | Amount of Tablet Powder Taken for Assay (g) | Amount of Drug Estimated (g/tablet) | | % Labeled Claim | |
|---|---|---|---|---|---|
| | | LAM | TDF | LAM | TDF |
| 1 | 0.0375 | 0.3063 | 0.3078 | 102.12 | 102.82 |
| 2 | 0.0376 | 0.3093 | 0.2997 | 103.10 | 99.94 |
| 3 | 0.0373 | 0.3096 | 0.3003 | 103.26 | 100.11 |
| 4 | 0.0372 | 0.3033 | 0.3090 | 101.10 | 103.10 |
| 5 | 0.375 | 0.2973 | 0.3123 | 99.10 | 104.10 |

Average weight of tablet = 1.1371g ( Each tablet contains 300mg of LAM and 300mg of TDF).

**Statistics:**

| Drugs | Mean | ± SD | R.S.D |
|---|---|---|---|
| LAM | 101.736 | 1.709 | 1.76 |
| TDF | 102.214 | 1.639 | 1.60 |

**Validation Parameters:** The proposed method was validated as per ICH guidelines for linearity and range, precision, specificity, accuracy, ruggedness and robustness, LOD and LOQ,

**Linearity and Range:** The series of solution of LAM and TDF were analyzed in the range of 2:2, 5:5, 10:10, 20:20 and 30:30, 40:40, 50:50 µg/ml. Results are shown in **Table 9.**

**TABLE 9: LINEARITY STUDY**

| Concentration of Drug (µg/ml) LAM:TDF | Absorbance of Mixture 259 nm | 272 nm |
|---|---|---|
| 2:2 | 0.132 | 0.1 |
| 5:5 | 0.209 | 0.315 |
| 10:10 | 0.557 | 0.69 |
| 20:20 | 1.23 | 1.288 |
| 30:30 | 1.812 | 1.795 |
| 40:40 | 2.46 | 2.291 |
| 50:50 | 3.15 | 2.829 |

**Precision:** Precision of an analytical method is expressed as S. D and C.V of series of measurements. It was ascertained by replicate estimation of all the drugs by proposed methods in **Table 7.**

**Specificity:** The studies were carried out by attempting deliberate degradation of the tablet sample with exposure to various stress condition results are drawn in **Table10.**

**TABLE 10: RESULTS OF SPECIFICITY STUDY**

| Drug | Room temperature | Acid ( 0.1N HCl) | Alkali (0.1N NaOH) | Oxide ( 3% $H_2O_2$) | Heat 24 H (60ºC) |
|---|---|---|---|---|---|
| LAM | 101.20 | 95.47 | 74.00 | 88.10 | 85.31 |
| TDF | 102.55 | 85.81 | 97.34 | 93.11 | 91.53 |

**Ruggedness:** The study for ruggedness was carried out at two different conditions.

1**.** Different elapsed times (intraday and interday)

2. Different analysts, the results are shown in **Table 11.**

**TABLE 11: RESULT OF RUGGEDNESS STUDY**

| Drugs | Parameters | Intermediate precision | | |
|---|---|---|---|---|
| | | Intraday | Interday | Different analyst |
| LAM | Mean | 101.5 | 101.74 | 101.36 |
| | ± S.D | 0.964 | 0.8671 | 0.907 |
| | % RSD | 0.949 | 0.852 | 0.90 |
| TDF | Mean | 103.86 | 103.26 | 103 |
| | ± S.D | 1.619 | 1.42 | 1.646 |
| | % RSD | 1.558 | 1.37 | 1.59 |

**Accuracy:** Accuracy of the proposed method was ascertained on the basis of recovery studies performed by the standard addition method. The results are shown in **Table 12.**

**TABLE 12: DATA OF RECOVERY STUDY**

| S. no. | Amount of Pure Drug Added (µg/ml) | | Amount of Drug Estimated (µg/ml) | | % Drug Estimated | |
|---|---|---|---|---|---|---|
| | LAM | TDF | LAM | TDF | LAM | TDF |
| **80% Recovery** | | | | | | |
| 1 | 08 | 08 | 07.96 | 08.16 | 99.50 | 102 |
| 2 | 08 | 08 | 07.90 | 08.02 | 98.87 | 100.25 |
| 3 | 08 | 08 | 08.09 | 08.21 | 101.22 | 102.67 |
| **100% Recovery** | | | | | | |
| 1 | 10 | 10 | 09.81 | 10.11 | 98.12 | 101.15 |
| 2 | 10 | 10 | 10.17 | 10.13 | 101.7 | 101.33 |
| 3 | 10 | 10 | 10.15 | 10.32 | 101.5 | 103.20 |
| **120% Recovery** | | | | | | |
| 1 | 12 | 12 | 11.78 | 12.13 | 98.22 | 101.10 |
| 2 | 12 | 12 | 11.94 | 12.27 | 99.56 | 102.33 |
| 3 | 12 | 12 | 11.87 | 12.46 | 98.95 | 103.87 |

**Statistics:**

| Drug | Mean | ± S.D | % RSD |
|---|---|---|---|
| **80% Recovery** | | | |
| LAM | 99.86 | 1.216 | 1.21 |
| TDF | 101.63 | 1.250 | 1.22 |
| **100% Recovery** | | | |
| LAM | 100.77 | 1.642 | 1.61 |
| TDF | 101.89 | 1.135 | 1.11 |
| **120% Recovery** | | | |
| LAM | 98.90 | 0.672 | 0.679 |
| TDF | 102.43 | 1.388 | 1.35 |

## LOD and LOQ:

**TABLE 13: LOD AND LOQ OF LAM AND TDF (UV)**

| S. no. | Drug | LOD (µg/ml) | LOQ (µg/ml) |
|---|---|---|---|
| 1 | LAM | 0.220 | 0.618 |
| 2 | TDF | 0.250 | 0.799 |

**CONCLUSION:** The QbD approach was applied for the optimization of UV method and was found significant. The proposed UV spectrophotometric method was developed for the determination of LAM and TDF in the pharmaceutical formulation were simple, accurate, sensitive, and reproducible. Statistical analysis proves that the methods were repeatable and selective for the analysis of LAM and TDF in bulk drugs as well as in pharmaceutical formulation. The method was completely validated as per ICH Q2 (R1) guidelines showing satisfactory data for all the parameters tested.

The QbD approach can be used for the optimization of various parameters like degradation in UV. The proposed method is simple and suitable for the determination of LAM and TDF in pure and pharmaceutical preparations alone or in combination and could be applied for routine analysis in quality control laboratories. In the future the developed UV method can be used for bio-analysis of LAM and TDF in the single or combined dosage form.

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