DEVELOPMENT AND VALIDATION OF UPLC METHOD FOR ESTIMATION OF % RELEASE OF SOME ANTI-HIV DRUGS IN FIXED DOSE COMBINATION

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ABSTRACT: A simple, accurate and precise UPLC method was developed and validated for dissolution testing of Lamivudine, Tenofovir disoproxil fumarate and Efavirenz in tablet dosage form. The separation was achieved under optimized chromatographic condition on an Acquity UPLC BEH (50 mm × 2.1 mm, 1.7 µm) column with mobile phase consist of 20 mM potassium dihydrogen phosphate buffer pH 2.5: acetonitrile with gradient elution at a flow rate of 0.37 mL/min using 35 °C column oven temperature with UV detection at 260 nm. The method was validated as per ICH and USP guideline and the values were found to be within the limits. So, the proposed method was found to be simple, linear, accurate, precise, robust and specific.

Keywords:

Lamivudine, Tenofovir disoproxil fumarate, Efavirenz, UPLC, Dissolution

INTRODUCTION: Treatment with HIV medicines is called antiretroviral therapy (ART). Antiretroviral drugs are used for the treatment of HIV infection. Lamivudine {4-amino-1-[(2R, 5S)-2-(hydroxyl methyl)-1, 3-oxathiolan-5- yl]- 1, 2-dihydro pyrimidin-2-one} is an NRTI used in the treatment of HIV infection and chronic hepatitis B virus (HBV) **Fig. 1A** [7-8]. Tenofovir disoproxil fumarate {9-[(R)-2- [[bis [[isopropoxycarbonyl] oxy] methoxy] phosphonyl] methoxy] popyl] adenine fumarate} is a nucleotide analog reverse transcriptase inhibitor (NRTI) and is used for treating HIV infection in adults, in combination with other antiretroviral agents **Fig. 1B** [8].

Efavirenz [(4S)-6-chloro-4-(cyclopropylethynyl)-1, 4-dihydro-4-(trifluoromethyl)-2H-3, 1-benzoxazin-2-one] is a non-nucleotide reverse transcriptase inhibitor (NNRTI) used in the combination treatment of HIV infection (AIDS) **Fig. 1C** [9]. The combination of Lamivudine (300 mg), Tenofovir disoproxil fumarate (30 mg) and Efavirenz (600 mg) were tentatively approved by US Food and Drug Administration (USFDA) on 9 March 2009 for the treatment of HIV infection in adults.

Drug absorption from a solid dosage form after oral administration depends on the release of the drug substance from the drug product, the dissolution or solubilization of the drug under physiological conditions, and the permeability across the gastrointestinal tract. Because of the critical nature of the first two of these steps, *in-vitro* dissolution may be relevant to the prediction of *in-vivo* performance. Based on this general consideration, *in-vitro* dissolution tests for immediate release solid oral dosage forms, such as tablets and capsules, are used to (1) assess the lot-to-lot quality of a drug product; (2) guide development of new formulations and (3) ensure continuing product quality and performance after certain changes, such as changes in the formulation, the manufacturing process, the site of manufacture, and the scale-up of the manufacturing process. There are multiple reported articles available for simultaneous estimation of all three drugs but not a single article reported for dissolution testing of Lamivudine, Tenofovir disoproxil fumarate and Efavirenz in their fixed dose combination.

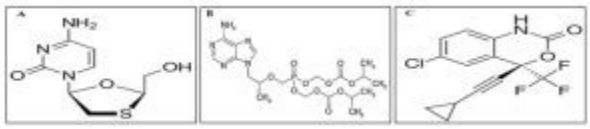

FIG. 1: STRUCTURES OF ANTI-HIV DRUGS. A) (-) LAMIVUDINE; B) (-) TENOFOVIR DISOPROXIL FUMARATE; C) (-) EFAVIRENZ

The objectives of this study were: (i) to perform BCS solubility study and to select suitable dissolution media; (ii) to set dissolution parameters for testing of formulation and set specifications for IR release formulation as per USFDA requirement; (iii) to develop a robust and reliable analytical method for the quantification of Lamivudine, Tenofovir disoproxil fumarate and Efavirenz in tablet dosage form by HPLC; (iv) to validate the obtained method and transfer it from HPLC to UPLC.

MATERIALS AND METHODS:

Chemicals / Reagents: Potassium dihydrogen phosphate, O-phosphoric acid, triethylamine, sodium acetate trihydrate, glacial acetic acid, hydrochloric acid (35%), sodium lauryl sulfate and HPLC grade acetonitrile were purchased from Merck (India). Gift samples of analytical standards were provided by Hetero Labs Ltd., (India). TenoLam-E tablet formulation purchased from the market.

Selection of Dissolution Medium: BCS solubility was performed to select suitable dissolution medium. The solubility of Lamivudine, Tenofovir disoproxil fumarate and Efavirenz was performed in different pH buffer as well as with surfactant. The concentration of surfactant was also evaluated.

Procedure: For BCS solubility highest dose of API needs to be dissolved in 250 ml of respective buffer media, but to avoid the use of a large amount of API, the volume of buffer and API weights were scaled down to maintain same concentration as in 250 ml of buffer.

TABLE 1: ACTUAL AMOUNT AND SCALED DOWN AMOUNT OF API AND BUFFER VOLUME

Table 1 shows the actual amount and scaled down the amount of API and volume of the buffer medium. Samples were kept in thermomixer at 900 RPM and 37 °C temperature for 24 h. After 24 h samples were centrifuged and suitable dilution carried out with respective buffer. Samples were analyzed using UV spectrophotometer. Analytical standards were prepared in methanol due to the high solubility of all API in methanol. All API were dissolved in water, 0.1N HCl, pH-4.5 acetate buffer, pH-6.8 phosphate buffer, 1% SLS in water, 1.5% SLS in water, and 2% SLS in water. Maximum solubility of all three APIs was in 2% SLS in water to achieve sink condition. **Table 2** shows the results of the solubility study. Based on these results, 2% SLS in water selected as a dissolution medium.

TABLE 2: RESULTS OF SOLUBILITY STUDY

Selection of Dissolution Parameters: Dissolution parameters selected as per the guidance for Industry-Dissolution Testing of immediate release solid oral dosage forms. All dissolution parameter mentioned in **Table 3**.

TABLE 3: DISSOLUTION PARAMETERS

Method Development for Dissolution Testing of Anti-HIV Drugs: Waters acquity UPLC was used with PDA detector and autoinjector module to perform analysis of samples. 20mM phosphate buffer pH-2.5 ± 0.05 with 0.1% TEA was selected as a mobile phase-A and acetonitrile 100% as a mobile phase-B. Due to the high difference in polarity of all three active gradient elution was performed for separation. Samples were injected in C18 column (UPLCBEH 50 × 2.1 mm; 1.7 µm) which was eluted at 0.5 mL/min. Injection volume kept 1µL. UPLC column temperature was set to 35 °C, and autosampler temperature kept ambient.

Selected gradient was as follows: 0-0.30 min, isocratic 5% B 0.33-0.62 min, linear gradient 5-80% B; 0.62-1.45 min, isocratic 80% B; 1.45-1.70 min, linear gradient 80-5%; 1.70-2.65 min.

Sample Preparation: Individual tablets were weighed and transferred to each six individual dissolution bowl having a 1000 ml of 2% SLS in water which was pre-equilibrated at 37°C ± 0.5°C. RPM was set to 50, and dissolution was run. After 45 min (Q time point)' sample aliquots collected and filtered through 10 µm PVDF filters after discarding 5mL of the filtrate. Pipetted out 5.0 ml of the filtered solution into a 10 ml volumetric flask and diluted up to the mark with dissolution media and mixed well

Preparation of Analytical Standards: Accurately weighed and transferred about 15 mg Tenofovir disoproxil fumarate, 15.0 mg of Lamivudin, and 30 mg Efavirenz into 25 ml of clean, dry volumetric flask. Added 5 ml of methanol and sonicated to dissolve. Diluted up to the mark with dissolution medium. Pipetted out 5.0 ml of this solution into a 20 ml volumetric flask and diluted up to the mark with dissolution media and mixed well.

Method Optimization: Our finalized method for the chromatographic separation of anti-HIV is described further on in Section 2.8. The parameters that were optimized are described below.

Alternative Chromatographic Conditions: Dissolution samples were analyzed by UPLC (Waters) equipped with a quaternary pump, autosampler, column oven, and photodiode array detector. Mobile phases and HPLC conditions tested were:

2. UPLC BEH column and a mobile phase consisting of 20mM phosphate buffer pH-2.5 ± 0.05 with 0.1% TEA (A), acetonitrile (B). Samples were eluted with a gradient of (B),0-0.33 min, isocratic 3% B 0.33-0.70 min, linear gradient 3-80% B; 0.70-1.60 min, isocratic 80% B; 1.60-1.90 min, linear gradient 80-3%; 1.90-3.00 min, isocratic 3% at a flow rate of 0.4 mL/min. Detection was achieved using wavelengths 260 nm.

3. UPLC BEH column and mobile phase consisting of 20mM phosphate buffer pH-2.5 ± 0.05 with 0.1% TEA (A), acetonitrile (B). Samples were eluted with a gradient of (B), 0-0.33 min, isocratic 3% B 0.33-0.90 min, linear gradient 3-80% B; 0.90-1.75 min, isocratic 80% B; 1.75-2.03 min, linear gradient 80-3%; 2.03-3.45 min, isocratic 3% at a flow rate of 0.37 mL/min. Detection was achieved using wavelengths 260 nm.

Optimized Method for Analysis of Dissolution Samples: The final in-house method developed for dissolution testing of anti-HIV drugs in tablet formulation. Individual tablets were weighed and transferred to each six individual dissolution bowl having a 1000 ml of 2% SLS in water which was pre-equilibrated at 37°C ± 0.5°C. RPM was set to 50 and dissolution was run.

After 45 min (Q time point)' sample aliquots collected and filtered through 10 µm PVDF filters after discarding 5 ml of filtrate. Pipetted out 5.0 ml of the filtered solution into a 10 ml volumetric flask and diluted up to the mark with dissolution media and mixed well. For chromatographic separation of the anti-HIV drugs, dissolution samples were analyzed by waters acquity UPLC equipped with a quaternary pump, autosampler, column oven, and photodiode array detector.

The selected column was a UPLC BEH (50 × 2.1 mm; 1.7 µm) (Waters). Mobile phase consisted of 20mM phosphate buffer pH-2.5 ± 0.05 with 0.1% TEA (A), acetonitrile (B). Samples were eluted with an increasing gradient of (B), 0-0.33 min, isocratic 3% B 0.33-0.90 min, linear gradient 3-80% B; 0.90-1.7 min, isocratic 80% B; 1.75-2.03 min, linear gradient 80-3%; 2.03-3.45 min, isocratic 3% at a flow rate of 0.37 mL/min. The total run time was 3.45 min. The injection volume was 1 µL. The column temperature was held at 35°C. The PDA detection wavelength was 260 nm.

Method Validation: The performance characteristics considered for validation of the optimized method were: specificity, linearity and working range, accuracy, precision, and robustness.

Specificity: Specificity was performed by checking interference from dissolution medium and placebo (excipients of formulation) at the retention time of all three active in standard preparation **Table 4**.

Linearity and Working Range: Linearity was assessed visually and using a lack-of-fit test. The working range was defined as the interval between the upper and the lower levels of the analytes within the calibration curve **Table 5**.

Accuracy: Accuracy of the analytical method was evaluated by recovery study. A known amount of API and placebo spiked in 1000 ml of dissolution medium at different level (20%, 100%, and 120%) **Table 7**.

System Precision: The five replicate injections of standard preparation were injected to determine the reproducibility of the instrument.

Method Precision: Six sample sets were injected to determine the repeatability of the analytical method **Table 6**.

Robustness: Robustness of an analytical method was evaluated by changes in column oven temperature, detection wavelength, and buffer pH. System suitability monitored during robustness study.

RESULTS AND DISCUSSION:

Method Optimization:

Alternative Chromatographic Conditions: Optimization of the chromatographic separation of anti-HIV drugs was based on the polarity of drugs and appropriate mobile phase techniques. Analyte peak identification was based upon retention time match with the reference standards.

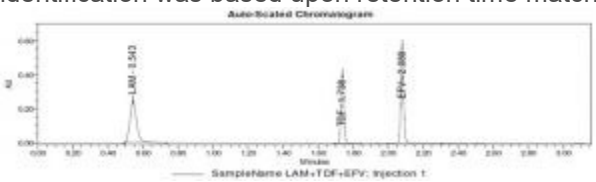

FIG. 2: CHROMATOGRAPHIC SEPARATION OF ANTI-HIV DRUGS IN STANDARD PREPARATION

The HPLC method was adopted from Parth Patel *et al.,* which is described in section 2.7.1 and converted into UPLC method using acquity UPLC column calculator for separation of all three drugs but due to early elution of Lamivudine, this method was not suitable for quantification purposes. In the second trial mobile phase gradient and flow rate were modified for separation of all three drugs. Good symmetrical peaks observed and good resolution achieved using this method. **Fig. 2** shows the separation of all three drugs using this method.

CONCLUSION: UPLC method was developed and validated for estimation of Lamivudine, Tenofovir disoproxil fumarate and Efavirenz in tablet dosage form. All system suitability parameters were passed in acceptable range. Linearity of the developed method was near to 1.0 within the specified range. % RSD was found to be less than 2 for repeatability. % Recoveries for all three drugs were found to be within 98-102% across all levels. These results indicate that the developed method is accurate, precise, specific, robust and less time consuming. It can be used in the routine quality control of marketed dosage form.

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CONFLICTS OF INTEREST: Nil

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