VALIDATED GRADIENT STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS QUANTIFICATION OF 11 RELATED SUBSTANCES IN THE COMBINED DOSAGE FORMS OF LAMIVUDINE AND TENOFOVIR DISOPEOXIL FUMARATE

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ABSTRACT

Objective: Development of a stability-indicating reverse phase liquid chromatographic (RP-HPLC) method for the simultaneous quantification of 11 impurities in the combined dosage forms of lamivudine and tenofovir disopropil fumarate drug substances.

Methods: Efficient chromatographic separation of all analytes was achieved on a Waters X-terra RP18 column (150 x 4.6 mm, 3.5 µm) using mobile phase A (ammonium acetate buffer, pH adjusted to 5.0±0.05 with dilute orthophosphoric acid) and mobile phase B (mixture of methanol and ammonium acetate buffer in the ratio of 20:80) with the flow rate of 1.0 ml/min in gradient elution mode at 260 nm.

Results: The method was validated in terms of the limit of detection, limit of quantification, linearity, accuracy, precision and robustness according to the international conference on harmonisation (ICH Q2R1). Regression analysis showed that the correlation coefficient (r²) is greater than 0.997 for individual active drug substances as well as their related substances. The method has proven very accurate (94.6 % to 102.8 % with % RSD not more than 4.9), highly precise (% RSD of the Intra-day and the inter-day study was not more than 8.9) and robust enough to deliver accurate results, when the chromatographic conditions were altered intentionally. Forced degradation studies were conducted in acidic, basic, thermal, photolytic, humid and peroxide stress conditions, where all the degradation peaks were monitored. Highest degradation of lamivudine was observed under oxidative stress condition and tenofovir was more susceptible to degradation under acid and alkaline conditions.

Conclusion: The present method is able to separate all the related compounds with each other and with the main drug substances with the resolution more than 2.0. The test solution was found to be stable in diluent up to 24 h. The mass balance of forced degradation of formulations, close to 99 %, made this method as a stability indicating method.

Keywords: Related Substances, Lamivudine and Tenofovir disopropil fumarate, HPLC method development, Validation

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INTRODUCTION

Tenofovir disoproxil fumarate (9-[[R]-2-[[bis [[isopropoxycarbonyl] oxy] methoxyl] phosphonyl] methoxyl] 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. Lamivudine (4-amino-1-[[(2R, 5S)-2-(hydroxyl methyl)-1,3-oxathiolan-5-yl]-1,2-dihydro pyrimdin-2-one} is another NRTI used in the treatment of HIV infection and chronic hepatitis B virus (HBV) [1-2]. The combination of these two drugs is available in the market with brand names of Forstavir 30s tablet, Tavin 30s tablet, Ricovir 30s tablet, Tenolam 30s tablet, Envir 30s tablet with 300 mg strength of each rugs substance. Total eleven specified impurities are present in both lamivudine and tenofovir out of which, four impurities are related to lamivudine and seven impurities (including one dimer) are related to tenofovir. As per the ICH guidelines, all these related substances must be controlled below 0.2 % of the drug concentration except monoester and isopropyl ethers and limits should not exceed 0.2 % at various storage conditions.

As per the USP monograph, the specification limits for tenofovir monoester and tenofovir isopropyl impurities are 1.0 % and 0.3 % respectively. Under various environmental conditions, a change in the purity of these drug substances is common and it results in change in the amount of impurities. In order to maintain the quality of these drug substances, degradation study of combined dosage forms is of prime importance for the determination of the various degraded impurities.

Fig. 1: Chemical structures of lamivudine, tenofovir and its impurities
The literature review revealed that most efforts were put in developing analytical methods for estimating the lamivudine and tenofovir drug substances, either individually or in combination with other drugs in different dosage forms or biosamples which includes spectrophotometric method [3-6], high performance liquid chromatography [7-18], ultra-performance liquid chromatography [19, 20] and mass spectrometry [21-23]. Rao et al. [18], reported a stability indicating method for the simultaneous estimation of lamivudine, tenofovir and dolutegravir in bulk and their dosage forms. But, no any analytical method was reported for the simultaneous estimation of related substances in a combined dosage form of lamivudine and tenofovir. Therefore, the present study is aimed to develop a stability indicating high performance liquid chromatographic method for the determination of 11 impurities including two drug substances in the dosage forms. The structures of both the drug substances and related substances are presented in Fig. 1 and chemical names are given in Table 1. The method was validated as per the guidelines are given by food and drug administration (FDA) and international conference on harmonization (ICH) with respect to the limit of detection, limit of quantification, linearity, precision, accuracy, specificity and stability studies.

### Table 1: Chemical names of lamivudine, tenofovir disoproxil fumarate and their impurities

| S. No. | Name of the compounds | IUPAC name |
|-------|-----------------------|------------|
| 1     | Lamivudine            | (R)-5-(4-amino-2-oxopyrimidin-1(2H)-yl)-1,3-oxathiolane-2-carboxylic acid |
| 2     | Impurity-A            | (2RS,5S(R)-5-(4-amino-2-oxopyrimidin-1(2H)-yl)-1,3-oxathiolane-2-carboxylic acid) |
| 3     | Impurity-F            | (1R,2S)-2-(2-amino-5-(4-methoxy-2-oxo-1,3-oxathiolan-2-yl)phosphonic acid) |
| 4     | Impurity-H            | (R)-9-[2-phosphonooxymethyl)propyl]adminine |
| 5     | Tenofovir             | ((2R)-1-[(6-amino-9H-purin-9-yl)-2-propanyloxy]methyl)phosphonate |
| 6     | Tenofovir disoproxil fumarate | Bis[[isopropoxycarboxyl]oxy)methyl](((2R)-1-[(6-amino-9H-purin-9-yl)-2-propanyloxy]methyl)phosphonate |
| 7     | Adenine impurity      | 6-Amino purine |
| 8     | Mono ester impurity   | 1-methyl-ethoxymethyl]-phosphonic acid monoisopropoxycarboxyloxymethyl ester |
| 9     | Ethyl impurity        | (R)-9-[2-phosphonooxymethyl]propyl]adminine |
| 10    | Isopropyl impurity    | (R)-9-[2-mono isopropoxycarbonyloxy methyl phosphine methyl]propyladine |
| 11    | n-propyl impurity     | (R)-9-[2-mono isopropoxycarbonyloxy methyl phosphine methyl]propyladine |
| 12    | Tenofovir impurity    | (R)-1-(6-Amino-9H-purine-9-y1) propane-2-yloxymethyl |
| 13    | Dimer impurity        | (2-[6-[[9-(bis-isopropoxycarboxyl oxy methoxy phosphonyl-methoxy)]propyl]-9Hpurin-6-ykyno]methyl)amino]-9H-purin-9-y1)-1-methyl-ethoxymethyl]-phosphonic acid diisopropoxycarboxyloxymethyl ester |

### MATERIALS AND METHODS

#### Chemicals and reagents

Reference standards of drug substances and related compounds are supplied by GSN Pharmaceuticals private limited, Hyderabad as gift samples. Lamivudine and tenofovir tablet dosage forms were purchased from local market. HPLC grade acetonitrile was procured from Qualigens, India. Ammonium acetate and orthophosphoric acid were purchased from Merck, India. All other chemicals and solvents of analytical grade were supplied by Merck, India. Water used in the HPLC analysis was purified by the water purifier (Milli-Q Millipore). The mobile phase and all the solutions were filtered through a 0.45 µm filter. Mobile phase B prepared by mixing of methanol and ammonium acetate buffer in the ratio of 20:80 (v/v). Diluent is prepared mixing of methanol and ammonium acetate buffer in the ratio of 80:20 (v/v).

#### Instrumentation

The HPLC system was composed of 2695 Waters alliance system fixed column temperature at 35 °C, gradient time program as set as a time in minutes/% of mobile phase B composition 0/0, 45/90, 54/80, 60/0. Before delivering the mobile phase into the system, it was degassed and filtered through 0.45 µm nylon filter using the vacuum. The injection volume was 10 µl and the detection was performed at 260 nm using a photodiode array (PDA) detector.
Method validation

The aim of method validation is to confirm that the present method is suitable for its intended use purpose. The described method has been extensively validated in terms of specificity, limit of detection (LOD) and quantification (LOQ), linearity, accuracy, precision, robustness and solution stability. The precision was expressed in terms of intraday and interday variation in the expected drug concentrations. The accuracy was expressed in terms of percent recovery by adding a known amount of impurities to the sample preparation.

System suitability

The system suitability was evaluated by a series of six injections of the standard solution with the concentration of 1 µg/ml. The system suitability criteria like % RSD, USP tailing factor and USP theoretical plates summarized in Table 2. The corresponding standard chromatogram is shown in Fig. 2. As per the results tabulated in Table 2 system suitability parameters are fixed as % RSD of all the injections should be less than 5 and tailing factor should not more than 2.0 and the limit for theoretical plates should more than 5000 for both the peaks.

Table 2: System suitability results

| S. No | Retention time (min) | Peak area | Tailing factor | Theoretical plates | Retention time (min) | Peak area | Tailing factor | Theoretical plates |
|-------|---------------------|-----------|----------------|-------------------|---------------------|-----------|----------------|-------------------|
| 1     | 7.85                | 27222     | 1.18           | 12579             | 30.11               | 37119     | 1.18           | 13213             |
| 2     | 7.84                | 27121     | 1.17           | 12614             | 30.10               | 37108     | 1.18           | 13196             |
| 3     | 7.87                | 27037     | 1.18           | 12678             | 30.09               | 37155     | 1.18           | 13233             |
| 4     | 7.82                | 27069     | 1.18           | 12689             | 30.14               | 37160     | 1.17           | 13254             |
| 5     | 7.80                | 27175     | 1.18           | 12632             | 30.18               | 37110     | 1.18           | 13252             |
| 6     | 7.81                | 27268     | 1.17           | 12589             | 30.12               | 37083     | 1.18           | 13264             |
| Mean  |                     | 27149     |                |                   |                     | 37120     |                |                   |
| SD    |                     | 89.323    |                |                   |                     | 30.773    |                |                   |
| %RSD  |                     | 0.33      |                |                   |                     | 0.08      |                |                   |

Fig. 2: Typical chromatogram of standard solution

Table 3: Specificity results of spiked sample

| S. No | Name                        | RT  | RRT | Purity angle | Purity threshold | Peak purify |
|-------|-----------------------------|-----|-----|--------------|-----------------|-------------|
| 1     | Fumaric acid                | 1.62| 0.21| 12.501       | 14.254          | Pass        |
| 2     | Impurity-E                  | 2.12| 0.27| 10.124       | 15.587          | Pass        |
| 3     | Impurity-F                  | 2.49| 0.32| 0.584        | 2.157           | Pass        |
| 4     | Impurity-A                  | 2.78| 0.36| 5.687        | 6.789           | Pass        |
| 5     | Impurity-H                  | 3.63| 0.46| 1.242        | 3.610           | Pass        |
| 6     | Adenine                     | 5.96| 0.76| 3.547        | 4.587           | Pass        |
| 7     | Tenofovir impurity          | 6.35| 0.81| 9.125        | 13.524          | Pass        |
| 8     | Lamivudine                  | 7.81| 1.00| 14.254       | 16.245          | Pass        |
| 9     | Mono ester impurity         | 19.36| 2.48| 0.54        | 1.021           | Pass        |
| 10    | Di ethyl impurity           | 25.39| 3.25| 3.014        | 3.541           | Pass        |
| 11    | Isopropyl impurity          | 27.37| 3.50| 6.647        | 6.847           | Pass        |
| 12    | Tenofovir disoproxil        | 30.11| 3.85| 1.201        | 1.542           | Pass        |
| 13    | n-Propyl impurity           | 30.66| 3.93| 3.125        | 4.852           | Pass        |
| 14    | Tenofovir dimer impurity    | 44.17| 5.65| 2.325        | 2.854           | Pass        |

RT= Retention time, RRT= Relative retention time

Specificity

The specificity of the present method was checked by injecting blank, placebo preparations and samples by spiking with appropriate levels of impurities and demonstrated the separation of these impurities individually and/or from other components in the sample matrix of tenofovir, lamivudine. The chromatograms of the blank and spiked sample for the specificity study are included in fig.
3 and fig. 4. The results of the specificity in terms of retention time (RT) and relative retention time (RRT), purity angle, purity threshold and peak purity of all analytes are included table 3. The chromatograms of blank and placebo in fig. 3 and fig. 4 showed that no any peak was found at the retention times of impurities. The spiked chromatogram presented in fig. 5 showed that all the peaks are well separated with each other with resolution more than 2.0 confirmed the specificity of the present method is good. The results presented in table 3 indicate that there is no interference between the peaks of impurities with main drug substances and the specificity of the method is confirmed by their peak purities i.e. purity angle is less than purity threshold. Hence, the chromatographic system used for the estimation of related substances in tenofovir and lamivudine is very selective and specific. Rao et al. [18], presented a stability indicating a method for three drug combination formulations without impurity profile study. But, the present method is able to quantify all the individual impurities that arise during storage of both active pharmaceutical ingredient and tablet dosage forms.

Forced degradation studies

Intentional degradation was attempted at various stress conditions like the thermal sample (at 60 °C for 7 d), photolytic sample (1.2 million lux h), humidity (at 90 % related humidity for 7 d), acid hydrolysis (using 0.5 N hydrochloric acid, 1h at room temperature), base hydrolysis (using 0.5 N sodium hydroxide, 1h at room temperature) and oxidative degradation (using 10 % hydrogen peroxide, 1h at room temperature) to evaluate the ability of the proposed method to separate degradation products from each other and active ingredients as well and to provide an indication of stability indicating property of the method. To check and ensure the homogeneity (peak purity) of all peaks in the stressed sample solutions, photodiode array detector was employed. The results of forced degradation study presented in table 4 indicate that tenofovir is susceptible for degradation in acid, base and high humidity stress conditions, whereas lamivudine is susceptible to peroxide and high humidity stress conditions.

Limit of detection and quantification

Limit of detection (LOD) and limit of quantification (LOQ) for related substances are determined by injecting a series of solutions of known concentration till the signal-to-noise ratio became as 3:1 and 10:1, respectively, and the corresponding values are summarized in table 5. The found LOQ values are sufficient to quantify these impurities below the 0.2 % of the drug concentration as per the limits defined by pharma regulating agencies. The LOD and LOQ values are determined as 0.048 µg/ml and 0.160 µg/ml for lamivudine and 0.035 µg/ml and 0.115 µg/ml for tenofovir respectively, which are lower values when compared to earlier methods [18].
The results in table 6 indicate that the detector response was found. The accuracy of the method is established in terms of conventional true value or an accepted reference value and the value found. The accuracy of an analytical method is an expression of the methodology. The relative retention factor (RRF) is critical for quantification of impurities. Establishment of the RRF is required to avoid the stability issues with impurity standards, to reduce the cost on the preparation of impurity standards, to reduce maintenance of impurity standards due to the lack of donation of impurity standards and difficulty in the synthesis and isolation of impurity standards. RRF is used to correct the difference in detector response of impurities with analyte peak. RRF is established by the linearity slope method with a linear range of solutions and corresponding RRF values of this method is summarized in table 6.

The accuracy of an analytical method is an expression of the agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy of the method is established in terms of recovery. Sample solutions for accuracy study were prepared in triplicate by spiking all impurities at the specification level (Not more than 0.2 % of each impurity) to the test sample at LOQ, 50 %, 100 % and 150 % of the specification level and injected into the HPLC system. Individual % recovery, mean % recovery and % RSD at each level are presented in table 7. The recovery of samples was found to be within the range of 90 % to 110 % and agreement with the ICH guidelines.
To evaluate the method precision for related substances, six replicates test preparations (n=6) of lamivudine and tenofovir tablets were prepared and spiked all individual known impurities at the specification level (0.2 % of drug substance) and analyzed as per method. The % of individual known impurities and % of total impurities were calculated and reported in table 8. The % RSD values were found to be below 8.9 for all impurities and found to be more precise as per the ICH guidelines. The intermediate precision of the method was evaluated by adopting the same method by using a different HPLC system, the different column of the same make at different days and the results are tabulated in table 9. The % RSD values were found to be below 7.9 for all impurities and found to be method is more ruggedness.

| S. No | Name                  | Impurity (µg/ml)            | % mean ±SD | % RSD |
|-------|-----------------------|----------------------------|------------|-------|
|       |                       | Spl-1 | Spl-2 | Spl-3 | Spl-4 | Spl-5 | Spl-6 |
| 1     | Impurity-E            | 1.05  | 1.00  | 0.90  | 1.10  | 0.95  | 1.05  | 1.01±0.07   | 7.3  |
| 2     | Impurity-F            | 0.95  | 1.10  | 0.95  | 0.90  | 1.00  | 1.10  | 1.00±0.08   | 8.4  |
| 3     | Impurity-A            | 1.05  | 0.95  | 1.00  | 1.05  | 0.95  | 1.00  | 1.00±0.04   | 4.5  |
| 4     | Impurity-H            | 1.10  | 1.00  | 1.05  | 0.95  | 1.05  | 1.00  | 1.03±0.05   | 5.1  |
| 5     | Adenine               | 1.05  | 1.00  | 1.10  | 1.10  | 1.00  | 1.05  | 1.05±0.04   | 4.3  |
| 6     | Tenofovir impurity    | 1.00  | 0.95  | 1.00  | 0.95  | 0.90  | 0.95  | 0.96±0.04   | 3.9  |
| 7     | Monoester impurity    | 5.10  | 5.50  | 5.25  | 5.30  | 4.95  | 5.40  | 5.25±0.20   | 3.8  |
| 8     | Di ethyl impurity     | 1.05  | 1.00  | 0.90  | 1.00  | 0.95  | 1.05  | 0.99±0.06   | 5.9  |
| 9     | Isopropyl impurity    | 1.60  | 1.50  | 1.55  | 1.45  | 1.40  | 1.65  | 1.53±0.09   | 6.1  |
| 10    | n-Propyl impurity     | 1.10  | 1.05  | 1.00  | 1.05  | 1.00  | 0.95  | 1.03±0.05   | 5.1  |
| 11    | Tenofovir dimer impurity | 1.05  | 0.95  | 1.00  | 0.95  | 0.90  | 1.15  | 1.00±0.09   | 8.9  |
| 12    | Total impurities      | 16.10 | 16.00 | 15.70 | 15.80 | 15.05 | 16.35 | 15.83±0.45  | 2.8  |

S = Sample

Robustness

The robustness of the method was studied by injecting the blank and standards with deliberate changes in flow rate of the mobile phase (±0.1 ml/min), column temperature (±5 °C) and pH of the mobile phase buffer (±0.2). The results of the robustness are presented in table 10. Results tabulated in table 10 showed that method is not affected by intentional changes in the method parameters which was proved by tailing factors of standards are less than 2 and % RSD of six replicate standards is less than 2.
Stability of analytical solutions

To evaluate the stability, standard solution and the spiked sample solution were prepared and kept on the bench top (25 °C) and refrigerator (2-8 °C) conditions for 24 h. Similarity factor was calculated for a standard solution with a freshly prepared standard solution and the % of the difference of individual impurities and total impurities were calculated with the initial results. It was concluded that the standard solution is stable up to 24 h in bench top and refrigerator conditions. Sample solutions were unstable at bench top and refrigerator. Hence, samples were prepared freshly and injected immediately.

CONCLUSION

A specific, linear, precise and more accurate stability indicating high performance liquid chromatographic method has been developed for the quantification of 11 related substances including the tenofovir dimer impurity. The method has been validated for specificity, linearity, accuracy, precision, robustness and stability. This method is able to quantify all the impurities in the presence of tenofovir dimer impurity. The method has been validated for degradation study of tenofovir disoproxil fumarate in bulk and combined pharmaceutical dosage form by RP-HPLC. Asian J Biomed Pharm Sci 2013;6:226-31

Table 10: Robustness results

| S. No. | Robustness parameter | Lamivudine | Tenofovir |
|--------|----------------------|------------|-----------|
|        | % RSD | Tailing factor | Theoretical plates | % RSD | Tailing factor | Theoretical plates |
| 1      | Low flow (0.9 ml/min) | 1.2 | 1.0 | 12451 | 0.8 | 1.1 | 13512 |
| 2      | High flow (1.1 ml/min) | 1.0 | 1.1 | 10147 | 1.1 | 1.0 | 13602 |
| 3      | Low buffer pH (4.8) | 0.8 | 1.0 | 11458 | 0.2 | 1.1 | 12915 |
| 4      | High buffer pH (5.2) | 0.4 | 1.2 | 10987 | 0.1 | 1.0 | 13025 |
| 5      | Low column temp. (30 °C) | 1.1 | 1.0 | 12142 | 1.5 | 1.0 | 12854 |
| 6      | High column temp. (40 °C) | 0.5 | 1.1 | 11411 | 1.2 | 1.1 | 12965 |

CONFLICTS OF INTERESTS

Declared none

REFERENCES

1. The Merck Index. In: Budavari S. editor. 13th ed. Whitehouse Station, NJ: Merck and Co Inc; 2001.
2. Martindale: The complete drug reference. In: Sweetman SC. editors. 33rd ed. London: The Pharmaceutical Press; 2002.
3. Sharma R, Mehta K. Simultaneous spectrophotometric estimation of tenofovir disoproxil fumarate and lamivudine in three component tablet formulation containing efavirenz. Indian J Pharm Sci 2010;72:527-30.
4. Anandakumar K, Kamaraj K, Vetrichelvan T. Development and validation of emtricitabine and tenofovir disoproxil fumarate in pure and in fixed-dose combination by UV spectrophotometry. Digest J Nanomater Biostructures 2011;6:1085-90.
5. Sutar SV, Patil SS, Pishvikar SA, More BN. Spectrophotometric method for degradation study of tenofovir disoproxil fumarate. Int J Pharm Sci Res 2012;3:4363-6.
6. Anindita B, Aurobinda P, Kumar MA, Chandra T. Development and validation of spectrophotometric method for determination of emtricitabine and tenofovir disoproxil fumarate in bulk and tablet dosage form. Int J Pharm Tech Res 2011;3:125-35.
7. Nyamweru B, Kaale E, Mugoyola V, Chambuso M. Development and validation of an HPTLC-densitometric method for simultaneous analysis of lamivudine, tenofovir disoproxil fumarate, and efavirenz (LTE) in tablets. J Planar Chromatogr Mod TLC 2013;6:226-21.
8. Prasad LAR, Rao JVLS, Srinivasu P, Hemalatha J. New stability indicating HPLC method for simultaneous estimation of lamivudine, tenofovir and nevirapine in extended release tablets. Int J Pharm 2013;3:136-44.
9. Goud VM, Rao AS, Kumar SR. Method development and validation for simultaneous determination of lamivudine and tenofovir in tablet dosage form by RP-HPLC. Int J Pharm Sci 2013;5:215-8.
10. Sonawane PH, Panzade PS, Kale MA. Simultaneous estimation of lamivudine and Tenofovir disoproxil fumarate in bulk and combined pharmaceutical dosage form by HPLC method. Asian J Biomed Pharm Sci 2013;3:27-30.
11. Anandakumar K, Kamaraj K, Vetrichelvan T. A validated RP-HPLC method for simultaneous estimation of lamivudine and tenofovir disoproxil fumarate in pure and in tablet dosage form. Asian J Pharm Clin Res 2012;5:108-10.
12. Sharma T, Mishra N, Swapan K, Sudam CSI, Sankar DG. Validated RP-HPLC method for estimation of tenofovir disoproxil fumarate in bulk and pharmaceutical formulation. Asian J Pharm Clin Res 2012;5:108-10.
13. Dhara SB, Bhavini NP, Chaghanbhai NP. RP-HPLC method for simultaneous estimation of tenofovir disoproxil fumarate, lamivudine, and efavirenz in combined tablet dosage form. Pharm Methods 2012;3:73-8.
14. Shweta H, Dhaneshwar SR. Development and validation of a stability-indicating LC method for the determination of tenofovir disoproxil fumarate in a pharmaceutical formulation. Songkla University Br J Pharm Sci Technol 2012;34:615-22.
15. Srinath A, Sneha B, Akhila A, Ahmed R, Kulkarni RG. Method development and validation for simultaneous estimation of lamivudine, tenofovir and efavirenz in combined tablet dosage form by RP-HPLC and UV-spectroscopic method. Int J Pharm Sci Res 2014;5:5491-7.
16. Anandakumar K, Abirami G, Murugan S, Ashok B. RP-HPLC method for simultaneous estimation of lamivudine, tenofovir disoproxil fumarate and efavirenz in tablet formulation. J Anal Chem 2013;68:215-21.
17. Dunge A, Varalakshmi C, Daisy WV, Sanjana D, Jos H, Ervin A. Development of a validated liquid chromatographic method for the determination of related Substances and assay of tenofovir disoproxil fumarate. J Sep Sci 2010;33:1708-16.
18. Rao NM, Sankar DG. Development and validation of stability-indicating HPLC method for simultaneous determination of lamivudine, tenofovir, and deluggravir in bulk and their tablet dosage form. J Pharm Bio Sci 2015;6:807-21.
19. Madessh SK, Ismail Y, Gunasekaran V. Development and validation for simultaneous estimation of lamivudine, tenofovir disoproxil fumarate and efavirenz by UPLC. Int J Pharm 2012;2:656-60.
20. Purnima BV, Reddy TVB, Rao YS, Ramu G. Stability indicating RP-UPLC method for the assay of emtricitabine and tenofovir disoproxil fumarate in bulk and dosage forms. Am J Anal Chem 2015;6:807-21.
21. Kurmi M, Singh DK, Tiwari S, Sharma P, Singh S. Stability behaviour of antiretroviral drugs and their combinations. characterization of interaction products of emtricitabine and tenofovir disoproxil fumarate by mass spectrometry. J Pharm Biomed Anal 2016;128:438-46.
22. Krishna MM, Laxminarayana B, Rao PN, Kumar IJ, Rao JVLNS. A novel LC-MS/MS method for simultaneous quantification of tenofovir and lamivudine in human plasma and its application to a pharmacokinetic study. Biomed Chromatogr 2012; 26:1202-9.

23. Krishna MM, Rao PN, Kumar IJ, Laxminarayana B, Rao JVLNS. Simultaneous quantitation of lamivudine, zidovudine and nevirapine in human plasma by liquid chromatography-tandem mass spectrometry and application to a pharmacokinetic study. Acta Pharm Sin B 2012;2:472–80.

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