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# A novel rp- hplc method development & validation for simultaneous estimation of emtricitabine and tenofovir alafenamide in active pharmaceutical ingredients and marketed combined tablet dosage forms

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## ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Emtricitabine and Tenofovir Alafenamide, in its pure form as well as in tablet dosage form. Chromatography was carried out on X bridge C18 (4.6×150mm) 5µ column using a mixture of Methanol: Phosphate Buffer pH3 (60:40v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 252nm. The retention time of the Emtricitabine and Tenofovir Alafenamide was 2.6, 3.8±0.02min respectively. The method produces linear responses in the concentration range of 5-25µg/ml of Emtricitabine and 20-100µg/ml of Tenofovir Alafenamide respectively. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

**Keywords:** Emtricitabine, Tenofovir Alafenamide, RP-HPLC, Accuracy, Precision, ICH Guidelines.

## INTRODUCTION TO HPLC

HPLC is also called as high pressure liquid chromatography since high pressure is used to increase the flow rate and efficient separation by forcing the mobile phase through at much higher rate. The pressure is applied using a pumping system. The development of HPLC from classical column chromatography can be attributed to the development of smaller particle sizes. Smaller particle size is important since they offer more surface area over the conventional large particle sizes. The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages.

1. Improved resolution of separated substances
2. column packing with very small (3,5 and 10 µm) particles
3. Faster separation times (minutes)
4. Sensitivity
5. Reproducibility
6. continuous flow detectors capable of handling small flow rates
7. Easy sample recovery, handling and maintenance.

### Types of HPLC Techniques
### Based on Modes of Chromatography

These distinctions are based on relative polarities of stationary and mobile phases

## Reverse phase chromatography

In this the stationary phase is non-polar and mobile phase is polar. In this technique the polar compounds are eluted first and non polar compounds are retained in the column and eluted slowly. Therefore it is widely used technique.

## Normal phase chromatography

In this the stationary phase is polar and mobile phase is non-polar. In this technique least polar compounds travel faster and are eluted first where as the polar compounds are retained in the column for longer time and eluted. (Fig 1)

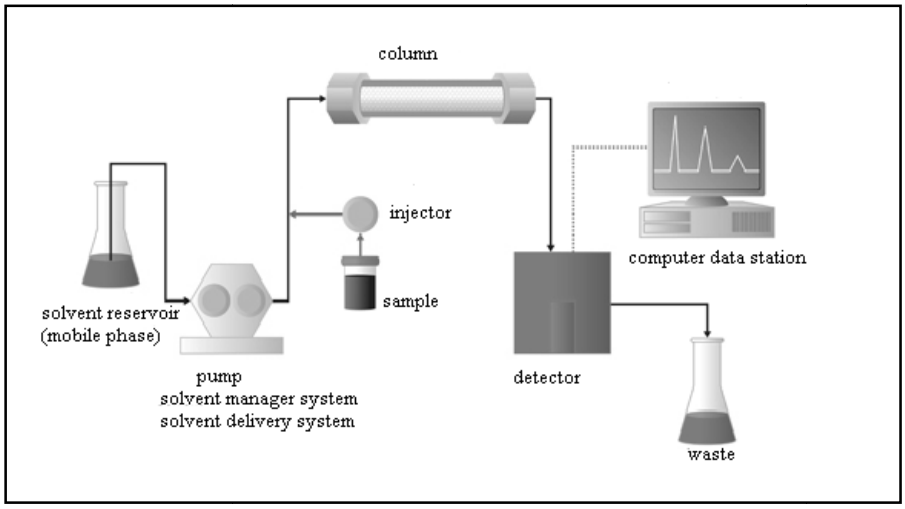


**Fig.1: Components of HPLC instrument block diagram.**

## Analytical method validation

Method validation as per ICH can be defined as "Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics.

## Objective of validation

There are two important reasons for validating assays in the pharmaceutical industry. The first, and by for most important is that assay validation is an integral part of the quality control system. The second is that current good manufacturing practice regulation requires assay validation. In industry it would be difficult to confirm that the product being manufactured is uniform and that meet the standards set to assure fitness for use. The varying nature of the differences between the analytical development laboratory and quality control laboratory is a good reason for validation program.

Method validation study includes Specificity / Selectivity, Linearity, Accuracy, Precision, Limit of detection, Limit of Quantitation, Robustness, System suitability and Stability criteria (Table 1)

**Table 1: Acceptance criteria of validation for HPLC.**

| S.No | Characteristics | Acceptance criteria |
|---|---|---|
| 1 | Accuracy | 98-102% |
| 2 | Precision | RSD<2 |
| 3 | Specificity | No interference |
| 4 | Detection limit | S/N >3:1 |
| 5 | Quantitation limit | S/N > 10:1 |
| 6 | Linearity | $R^2$> 0.99 |

## Aim and objectives

- ✓ To develop new simple, sensitive, accurate and economical analytical method for the simultaneous estimation of Emtricitabine (EMT) and Tenofovir Alafenamide (TEN).
- ✓ To validate the proposed method in accordance with USP and ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the Emtricitabine (EMT) and Tenofovir Alafenamide (TEN) in dosage form.
- ✓ The utility of the developed method to determine the content of drug in commercial formulation was also demonstrated. Validation of the method was done in accordance with USP and ICH guideline for the assay of active ingredient. The method was validated for parameters like system suitability, linearity, precision, accuracy, specificity, ruggedness and robustness, limit of detection and limit of quantification. This method provides means to quantify the component. This

proposed method was suitable for the analysis of Pharmaceutical dosage forms.

## MATERIALS AND METHODS

**Table 2: Instruments used**

| S.No | Instrument | Model |
|---|---|---|
| 1 | HPLC | WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA Detector. |
| 2 | UV/VIS spectrophotometer | LABINDIA UV |
| 3 | pH meter | Lab India |
| 4 | Weighing machine | Sartorius |
| 5 | Pipettes and Burettes | Borosil |
| 6 | Beakers | Borosil |
| 7 | Digital ultra sonicator | Labman |

**Table 3: chemicals used**

| S.No | Chemical | Brand names |
|---|---|---|
| 1 | Emtricitabine | Sura labs |
| 2 | Tenofovir Alafenamide | Sura labs |
| 3 | Water and Methanol for HPLC | LICHROSOLV (MERCK) |
| 4 | Anhydrous di hydrogen phosphate | Finar chemicals |
| 5 | Phosphate Buffer | Finar chemicals |
| 6 | Citric Acid | Finar chemicals |

## HPLC METHOD DEVELOPMENT

### Mobile Phase Optimization

Initially the mobile phase tried was Water: Methanol and ACN: Methanol with varying proportions. Finally, the mobile phase was optimized to phosphate buffer (pH 3), Methanol in proportion 60:40 v/v respectively.

### Optimization of Column

The method was performed with various columns like C18 column ODS column, Zodiac column, and Xterra C18 column. Xbridge C18 (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

### Optimized chromatographic conditions

Instrument used : Waters HPLC with auto sampler and PDA detector 996 model.
Column : X bridge C18 (4.6×150mm) 5 µ
Buffer : Phosphate buffer (pH-3)-Dissolve 0.9g of anhydrous di hydrogen phosphate and 1.298 g of Citric acid mono hydrate in sufficient water to produce 1000ml. Adjust the pH 3 by using ortho phosphoric acid.
PH : 3
Mobile phase : Methanol: Phosphate Buffer pH3 (60:40v/v)
Flow rate : 1.0 ml per min
Wavelength : 252 nm
Injection volume : 10 µl
Run time : 10 min.

## RESULTS AND DISCUSSION

### (Optimized Condition)

Mobile phase : Methanol: Phosphate Buffer pH3 (60:40v/v)
Column: X Bridge (4.6 × 150 mm, 5 µ)
Flow rate : 1.0 ml/min

Wavelength : 252 nm
Column temp : Ambient
Injection Volume : 10 µl
Run time : 8 min

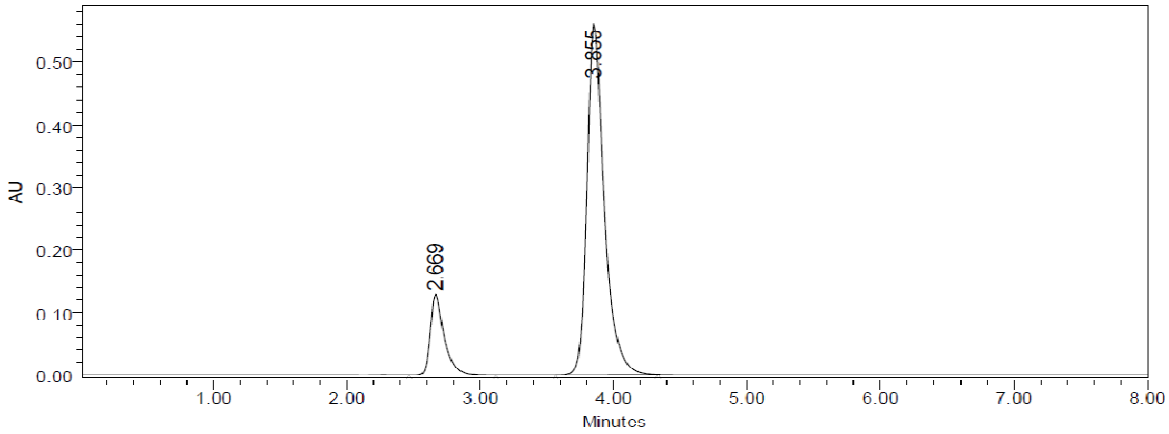


**Figure-2: Chromatogram for Trail 7**

**Table-4: Peak Results for Trail 7**

| S. No. | Peak name | $R_t$ | Area | Height | USP Resolution | USP Tailing | USP plate count |
|---|---|---|---|---|---|---|---|
| 1 | Emtricitabine | 2.669 | 917816 | 128672 | | 1.5 | 3551.0 |
| 2 | Tenofovir Alafenamide | 3.855 | 5040174 | 562209 | 1.7 | 1.4 | 4675.7 |

## Observation

This trial shows improper separation sample peaks, baseline and show very less plate count in the chromatogram. So it's required more trials to obtain good peaks.

From the above chromatogram it was observed that the Tenofovir Alafenamide and Emtricitabine peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial. (Table 5)
Retention time of Emtricitabine– 2.669min
Retention time of Tenofovir Alafenamide –3.855min

**Table 5: Results of system suitability parameters for Emtricitabine and Tenofovir Alafenamide**

| S.No. | Name | Retention time(min) | Area (µV sec) | Height (µV) | USP resolution | USP tailing | USP plate count |
|---|---|---|---|---|---|---|---|
| 1 | Emtricitabine | 2.669 | 918737 | 128687 | | 1.5 | 3549.3 |
| 2 | Tenofovir Alafenamide | 3.855 | 5040174 | 562209 | 1.7 | 1.4 | 4675.7 |

## Acceptance Criteria

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

## Validation parameters

**Table 6: Showing assay standard results**

| S.No. | Name | Rt | Area | Height | USP Resolution | USP Tailing | USP plate count | Injection |
|---|---|---|---|---|---|---|---|---|
| 1 | Emtricitabine | 2.669 | 918296 | 128680 | | 1.5 | 3550 | 1 |
| 2 | Tenofovir Alafenamide | 3.855 | 5041296 | 562209 | 1.7 | 1.4 | 4675 | 1 |
| 3 | Emtricitabine | 2.669 | 918482 | 128625 | | 1.5 | 3548 | 2 |
| 4 | Tenofovir Alafenamide | 3.855 | 5040174 | 562162 | 1.7 | 1.4 | 4592 | 2 |
| 5 | Emtricitabine | 2.654 | 918215 | 128721 | | 1.5 | 3595 | 3 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 6 | Tenofovir Alafenamide | 3.849 | 5040154 | 562481 | 1.7 | 1.4 | 4618 | 3 |

**Table- 7: Showing assay sample results**

| S.No. | Name | Rt | Area | Height | USP Resolution | USP Tailing | USP plate count | Injection |
|---|---|---|---|---|---|---|---|---|
| 1 | Emtricitabine | 2.669 | 918296 | 128680 | | 1.6 | 3550.1 | 1 |
| 2 | Tenofovir Alafenamide | 3.855 | 50401746 | 562209 | 1.7 | 1.4 | 4675 | 1 |
| 3 | Emtricitabine | 2.651 | 919583 | 128700 | | 1.5 | 3547.8 | 2 |
| 4 | Tenofovir Alafenamide | 3.849 | 15041294 | 562209 | 1.7 | 1.4 | 4675 | 2 |
| 5 | Emtricitabine | 2.621 | 918296 | 128680 | | 1.5 | 3550.1 | 3 |
| 6 | Tenofovir Alafenamide | 3.840 | 5040215 | 562209 | 1.7 | 1.4 | 4675 | 3 |

**Table-8: Showing Assay Results**

| S.No | Name of compound | %purity |
|---|---|---|
| 1 | Emtricitabine | 98 % |
| 2 | Tenofovir Alafenamide | 99% |

The retention time of Tenofovir Alafenamide and Emtricitabine was found to be 2.669min and 3.855mins respectively. The % purity of Emtricitabine and Tenofovir Alafenamide in pharmaceutical dosage form was found to be 98% and 99% respectively. (Table 9 & 10)

**Table-9: Results of method precession for Emtricitabine**

| S.No. | Name | Rt | Area | Height | USP plate count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Emtricitabine | 2.669 | 918296 | 128680 | 3550 | 1.5 |
| 2 | Emtricitabine | 2.659 | 918356 | 128712 | 3546 | 1.5 |
| 3 | Emtricitabine | 2.671 | 918247 | 128614 | 3574 | 1.5 |
| 4 | Emtricitabine | 2.669 | 918636 | 128647 | 3564 | 1.5 |
| 5 | Emtricitabine | 2.669 | 919578 | 128652 | 3712 | 1.5 |
| Mean | | | 918622.6 | | | |
| Std. Dev | | | 554.9295 | | | |
| % RSD | | | 0.060409 | | | |

**Table 10: Results of method precision for Tenofovir Alafenamide**

| S.No. | Name | Rt | Area | Height | USP plate count | USP Tailing | USP Resolution |
|---|---|---|---|---|---|---|---|
| 1 | Tenofovir Alafenamide | 3.855 | 5040174 | 562209 | 4675 | 1.4 | 1.7 |
| 2 | Tenofovir Alafenamide | 3.842 | 5046151 | 562219 | 4765 | 1.4 | 1.7 |
| 3 | Tenofovir Alafenamide | 3.850 | 5053141 | 561436 | 4512 | 1.4 | 1.7 |
| 4 | Tenofovir Alafenamide | 3.845 | 5076521 | 562148 | 4155 | 1.4 | 1.7 |
| 5 | Tenofovir Alafenamide | 3.855 | 5063147 | 571542 | 4951 | 1.4 | 1.7 |
| Mean | | | 5055827 | | | | |
| Std. Dev | | | 14384.71 | | | | |
| % RSD | | | 0.284518 | | | | |

## Acceptance criteria

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

## Intermediate precision/Ruggedness

**Table-11: Results of Intermediate precision for Emtricitabine**

| S.No. | Name | Rt | Area | Height | USP plate count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Emtricitabine | 2.669 | 918296 | 128675 | 3684 | 1.5 |
| 2 | Emtricitabine | 2.529 | 908296 | 128457 | 3564 | 1.5 |
| 3 | Emtricitabine | 2.669 | 907194 | 128475 | 3579 | 1.5 |
| 4 | Emtricitabine | 2.569 | 909291 | 128621 | 3569 | 1.5 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 5 | Emtricitabine | 2.569 | 908296 | 128632 | 3546 | 1.5 |
| 6 | Emtricitabine | 2.669 | 908458 | 128419 | 3550 | 1.5 |
| Mean | | | 909971.8 | | | |
| Std. Dev | | | 4132.316 | | | |
| % RSD | | | 0.454115 | | | |

**Table-12: Results of Intermediate precision for Tenofovir Alafenamide**

| S.No. | Name | Rt | Area | Height | USP plate count | USP Tailing | USP Resolution |
|---|---|---|---|---|---|---|---|
| 1 | Tenofovir Alafenamide | 3.845 | 4940174 | 562182 | 4678 | 1.4 | 1.7 |
| 2 | Tenofovir Alafenamide | 3.795 | 4951174 | 562493 | 4675 | 1.4 | 1.7 |
| 3 | Tenofovir Alafenamide | 3.855 | 4942175 | 562198 | 4624 | 1.4 | 1.7 |
| 4 | Tenofovir Alafenamide | 3.840 | 4840174 | 563541 | 4684 | 1.4 | 1.7 |
| 5 | Tenofovir Alafenamide | 3.855 | 4950176 | 562184 | 4675 | 1.4 | 1.7 |
| 6 | Tenofovir Alafenamide | 3.855 | 4942312 | 562487 | 4621 | 1.4 | 1.7 |
| Mean | | | 4927698 | | | | |
| Std. Dev | | | 43117.6 | | | | |
| % RSD | | | 0.875005 | | | | |

## Acceptance criteria

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

## Accuracy

**Table-13: Results of Accuracy standard values**

| S.No. | Name | Rt | Area | Height | USP Resolution | USP Tailing | USP plate count | Injection |
|---|---|---|---|---|---|---|---|---|
| 1 | Emtricitabine | 2.669 | 918296 | 128680 | | 1.5 | 3550 | 1 |
| 2 | Tenofovir Alafenamide | 3.855 | 5040174 | 562209 | 1.7 | 1.4 | 4675 | 1 |
| 3 | Emtricitabine | 2.658 | 918594 | 128541 | | 1.5 | 3514 | 2 |
| 4 | Tenofovir Alafenamide | 3.849 | 5040214 | 562152 | 1.7 | 1.4 | 4621 | 2 |
| 5 | Emtricitabine | 2.661 | 918364 | 128632 | | 1.5 | 3599 | 3 |
| 6 | Tenofovir Alafenamide | 3.851 | 5046512 | 568421 | 1.7 | 1.4 | 4625 | 3 |

**Table-14: Results of Accuracy sample 50% values**

| S.No. | Name | Rt | Area | Height | USP Resolution | USP Tailing | USP plate count | Injection |
|---|---|---|---|---|---|---|---|---|
| 1 | Emtricitabine | 2.668 | 576127 | 80301 | | 1.5 | 3594 | 1 |
| 2 | Tenofovir Alafenamide | 3.865 | 3113550 | 346575 | 1.7 | 1.4 | 4785 | 1 |
| 3 | Emtricitabine | 2.659 | 577153 | 80321 | | 1.5 | 3561 | 2 |
| 4 | Tenofovir Alafenamide | 3.862 | 3120597 | 346693 | 1.7 | 1.4 | 4162 | 2 |
| 5 | Emtricitabine | 2.696 | 577763 | 80333 | | 1.5 | 3951 | 3 |
| 6 | Tenofovir Alafenamide | 3.858 | 3125881 | 346769 | 1.7 | 1.4 | 4824 | 3 |

**Table-15: Results of Accuracy sample 100% values**

| S.No. | Name | Rt | Area | Height | USP Resolution | USP Tailing | USP plate count | Injection |
|---|---|---|---|---|---|---|---|---|
| 1 | Emtricitabine | 2.658 | 917816 | 128672 | | 1.5 | 3551.0 | 1 |
| 2 | Tenofovir Alafenamide | 3.854 | 5149522 | 562209 | 1.7 | 1.4 | 4518.1 | 1 |
| 3 | Emtricitabine | 2.641 | 918737 | 128687 | | 1.5 | 3549.3 | 2 |
| 4 | Tenofovir Alafenamide | 3.849 | 5751221 | 562147 | 1.7 | 1.4 | 4695.1 | 2 |
| 5 | Emtricitabine | 2.670 | 917816 | 128672 | | 1.5 | 3551.0 | 3 |
| 6 | Tenofovir Alafenamide | 3.851 | 5012452 | 569142 | 1.7 | 1.4 | 4672.3 | 3 |

**Table-16: Results of Accuracy sample 150% values**

| S.No. | Name | Rt | Area | Height | USP Resolution | USP Tailing | USP plate count | Injection |
|---|---|---|---|---|---|---|---|---|
| 1 | Emtricitabine | 2.671 | 1288229 | 777609 | 1.7 | 1.5 | 3521 | 1 |
| 2 | Tenofovir Alafenamide | 3.896 | 1288154 | 181646 | 1.7 | 1.4 | 4484 | 1 |
| 3 | Emtricitabine | 2.662 | 1275416 | 777502 | 1.7 | 1.5 | 3562 | 2 |
| 4 | Tenofovir Alafenamide | 3.831 | 1289142 | 182651 | 1.7 | 1.4 | 4456 | 2 |
| 5 | Emtricitabine | 2.677 | 1289352 | 777518 | 1.7 | 1.5 | 3526 | 3 |
| 6 | Tenofovir Alafenamide | 3.815 | 1289327 | 181459 | 1.7 | 1.4 | 4562 | 3 |

**Table-17: Accuracy (recovery) data for Emtricitabine**

| %Concentration (at specification Level) | Area | Amount Added (ppm) | Amount Found (ppm) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 577153 | 7.5 | 7.47 | 98% | 98.8% |
| 100% | 918737 | 15 | 14.92 | 99.2% | |
| 150% | 1288229 | 22.5 | 22.49 | 99.3% | |

## Acceptance Criteria

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate. (Table-18)

**Table-18: Accuracy (recovery) data for Tenofovir Alafenamide**

| %Concentration (at specification Level) | Area | Amount Added (ppm) | Amount Found (ppm) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 3120597 | 30 | 29.8 | 98% | 99.1% |
| 100% | 5040174 | 60 | 59.9 | 99.9% | |
| 150% | 7087906 | 90 | 89.8 | 99.6% | |

## Acceptance Criteria

- The % Recovery for each level should be between 98.0 to 102.0%. (Figure 3)

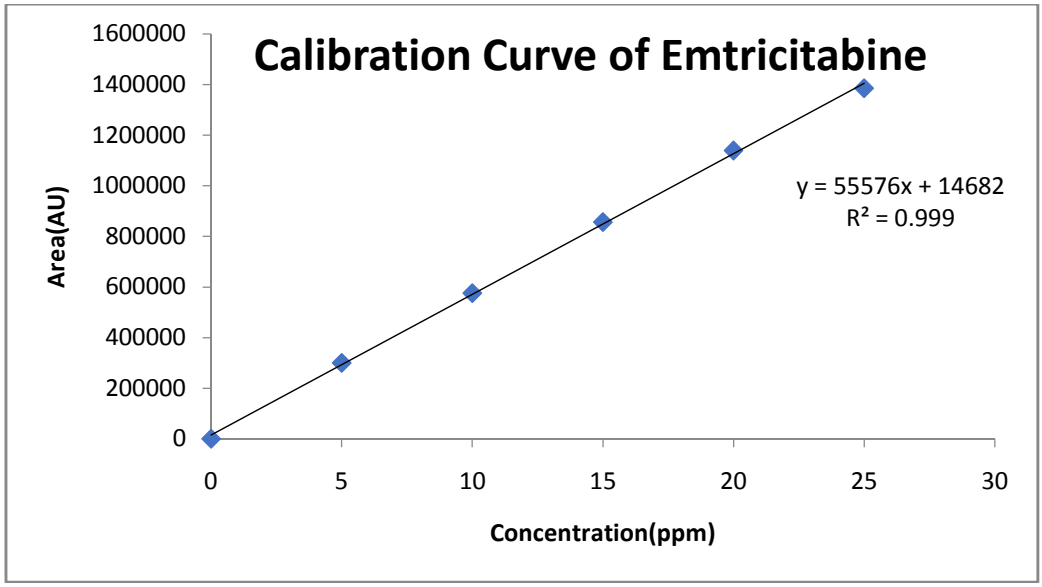


**Figure 3**

## Linearity Results: (for Emtricitabine)

**Table 19**

| S.No | Linearity Level | Concentration(ppm) | Area |
|---|---|---|---|
| 1 | I | 5 | 300010 |
| 2 | II | 10 | 575361 |
| 3 | III | 15 | 856266 |
| 4 | IV | 20 | 1139178 |
| 5 | V | 25 | 1385477 |
| | Correlation Coefficient | | 0.999 |

**Acceptance Criteria:** Correlation coefficient should be not less than 0.999 (Figure 4)

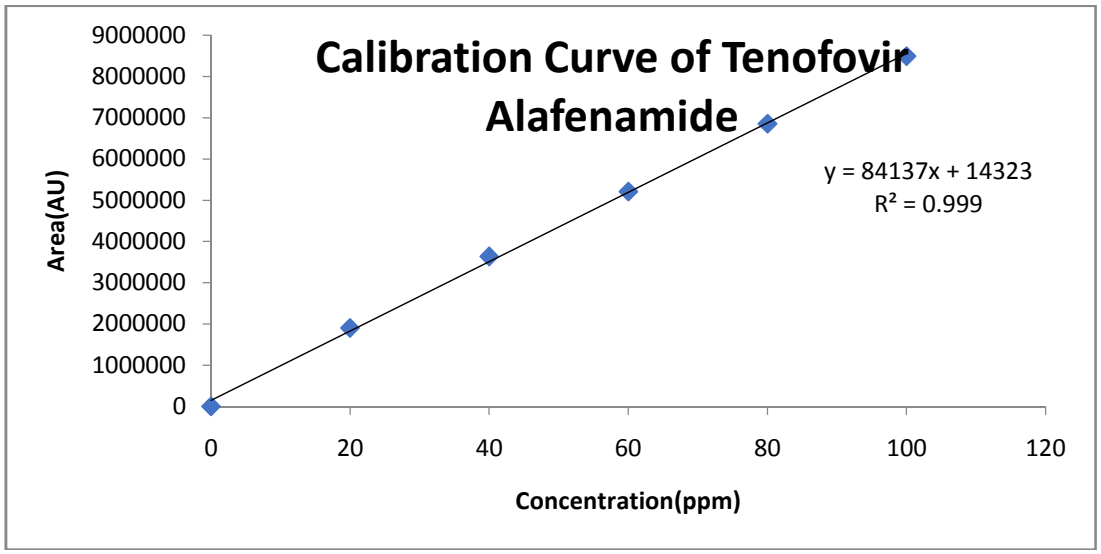


**Figure 4 calibration graphs for Tenofovir Alafenamide**

## Linearity Results: (for Tenofovir Alafenamide)

**Table 20**

| S.No | Linearity Level | Concentration(ppm) | Area |
|---|---|---|---|
| 1 | I | 20 | 1903922 |
| 2 | II | 40 | 3637044 |
| 3 | III | 60 | 5210174 |
| 4 | IV | 80 | 6856370 |
| 5 | V | 100 | 8493149 |
| | Correlation Coefficient | | 0.999 |

## Acceptance Criteria

- Correlation coefficient should be not less than 0.99.

**Table-21: Analytical performance parameters of Emtricitabine and Tenofovir Alafenamide**

| Parameters | Emtricitabine | Tenofovir Alafenamide |
|---|---|---|
| Slope (m) | 27788 | 84137 |
| Intercept (c) | 14682 | 14323 |
| Correlation coefficient ($R^2$) | 0.999 | 0.999 |

## Acceptance criteria

Correlation coefficient ($R^2$) should not be less than 0.999

## Limit of detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

**LOD= 3.3 × σ / s**

Where, σ = Standard deviation of the response, S = Slope of the calibration curve

## Emtricitabine

**Result:** =1.4µg/ml

## Tenofovir Alafenamide

**Result:** =1.5µg/ml

## Quantitation limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

**LOQ=10×σ/S**

Where, σ = Standard deviation of the response, S = Slope of the calibration curve

## Emtricitabine

**Result:** =4.2µg/ml

## Tenofovir Alafenamide

**Result:** =4.7µg/ml

## System suitability results for Emtricitabine

**Table 22 * Results for actual flow (1.0 ml/min) have been considered from Assay standard.**

| | | System Suitability Results | |
|---|---|---|---|
| **S.No** | **Flow Rate (ml/min)** | **USP Plate Count** | **USP Tailing** |
| 1 | 0.9 | 3462 | 1.5 |
| 2 | 1.0 | 3578 | 1.5 |
| 3 | 1.1 | 3421 | 1.5 |

## System suitability results for Tenofovir Alafenamide

**Table 23 * Results for actual flow (1.0ml/min) have been considered from Assay standard.**

| | | System Suitability Results | |
|---|---|---|---|
| **S.No** | **Flow Rate (ml/min)** | **USP Plate Count** | **USP Tailing** |
| 1 | 0.9 | 4675 | 1.4 |
| 2 | 1.0 | 4675.6 | 1.4 |
| 3 | 1.1 | 4085 | 1.4 |

## System suitability results for Emtricitabine

**Table 24**

| **S.No** | **Change in Organic Composition in the Mobile Phase** | **System Suitability Results** | |
|---|---|---|---|
| | | **USP Plate Count** | **USP Tailing** |
| 1 | 10% less | 4819.3 | 1.5 |
| 2 | *Actual | 3550.3 | 1.5 |
| 3 | 10% more | 4721.8 | 1.5 |

### System suitability results for Tenofovir Alafenamide

**Table 25 * Results for actual mobile phase have been considered from Assay standard.**

| S.No | Change in Organic Composition in the Mobile Phase | System Suitability Results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 10% less | 5834.2 | 1.4 |
| 2 | *Actual | 4675.6 | 1.4 |
| 3 | 10% more | 5235.6 | 1.4 |

## SUMMARY AND CONCLUSION

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Emtricitabine and Tenofovir Alafenamide was done by RP-HPLC. The Phosphate buffer was $p^H$ 3 and the mobile phase was optimized with consists of Methanol: Phosphate buffer (pH-3) mixed in the ratio of 60:40 % v/v. An Xbridge column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The solutions were chromatographed at a constant flow rate of 1.0 ml/min. The linearity range of Emtricitabine and Tenofovir Alafenamide were found to be from 5-25µg/ml, 20-100µg/ml respectively. Linear regression coefficient was not more than 0.999, 0.999.

The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-99% of Emtricitabine and Tenofovir Alafenamide.LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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