World Inventia Publishers

Journal of Pharma Research
Available online through
www.jprinfo.com

JPR INFO
Journal of Pharma Research
USA CODEN: JPROK3

Vol. 05 Issue 12, 2016 ISSN: 2319-5622

Original Article

# ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF TENOFOVIR IN TABLET DOSAGE FORM BY RP-HPLC TECHNIQUE

**Y. Naveen Kumar[1] *, B. Divya[2], Dr. J Sreekanth[3]**

*** [1]Scientific Officer, Drugs Control Administration, Vengalrao Nagar, Hyderabad 500038, Government of Telangana, India.**
**[2]Assistant Professor, Brilliant Institute of Pharmacy, Abdullapur (V), Abdullapurmet (M), Rangareddy (D), Hyderabad, 501505, India.**
**[3]Managing Director, Progenerics Pharma Private limited. Plot no.73a, Export Promotion Industrial Park Pashamylarm, Patancheru, Telangana – 502307, India**

**Received on: 22-11-2016 Revised and Accepted on: 31-12-2016**

***ABSTRACT***

*A simple, Accurate, precise method was developed for the estimation of the Tenofovir in Tablet dosage form. Chromatogram was run through C18Inertsil 5µ, 250mm×4.6mm column using phosphate buffer:acetonitrile:methanol(40:20:40) as mobile phase was pumped through column at a flow rate of 1.0 ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 257.0 nm. Retention time of Tenofovir was found to be 4.5min. %RSD of the Tenofovir was found to be 0.21. The method is linear over a concentration range ofTenofovir50 to 300µg/ml. The method was validated for system suitability, accuracy, precision, linearity and ruggedness. The system suitability parameters were within limit, hence it was concluded that the method was suitable to perform the assay. It was also used for determining lower concentration of drug in its solid dosage forms. Therefore it was concluded that the proposed method can be used for analysis of Tenofovir Disoproxil Fumerate in Pharmaceutical dosage forms.*

***Key words**: Tenofovir, Stability Indicating, Validation RP- HPLC*

## INTRODUCTION

Tenofovir is chemically ({[(2R)-1-(6 –amino-9H – purin-9-yl)propan -2-yl] oxy} methyl) phosphonicacid is a nucleotide analog indicated in the treatment of HIV infections. tenofovir is activated by a bi-phosphorylation it acts as an antiviral acyclic nucleoside phosphonate. It is a potent inhibitor of the viral reverse transcriptase with an inhibitory constant. The literature review reveals that few RP- HPLC methods for the estimation of Lamivudine and Tenofovirare available alone and in combination with other drugs. Few methods are also reported for estimation of both drugs from formulation [1-5]. Weintend to develop a Stability indicating RP-HPLC method by simultaneous determination with simple, rapid, greater sensitivity and faster elution.

***Corresponding author:**
**Y. Naveen Kumar**
Scientific Officer, Drugs Control Administration, Vengalrao Nagar, Hyderabad 500038, Government of Telangana, India.
**Email:** naveen.yed@gmail.com
**DOI:** https://doi.org/10.5281/zenodo.14201891

## 2. MATERIALS AND METHODS

Tenofovirpure drug (API) received as gift sample from Aurobindo pharma Ltd. Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, perchloric acid Ortho-phosphoric acid. All the above chemicals and solvents are purchased from Merck.

### 2.1. Preparation solutions:

#### 2.1.1. Preparation of Standard solutions:

Transfer an accurately weighed quantity of about 40mg of Tenofovir Disoproxil Fumerate working standard in to 100ml volumetric flask add 75ml of Mobile phase and sonicate to dissolve the content, and make up to the volume with mobile phase and further dilute 10ml in to 100 ml with diluents, mix.

#### 2.1.2. Samples Preparation

10 Tablets of contents were weighed and triturated in glass mortar. The quantity of powder equivalent to 100 mg of active ingredient present in Tenofovir was transferred into a 100 ml clean dry volumetric flask, 7 ml of diluent was added to it and

was shaken by mechanical stirrer and sonicated for about 30 minutes by shaking at intervals of five minutes each and was diluted up to the mark with diluent to give a concentration of 1000 µg/ml and allowed to stand until the residue settles before taking an aliquot for further dilution (stock solution). 3 ml of upper clear solution was transferred to a 10 ml volumetric flask and diluted with diluent up to the mark to give the respective concentrations as per with standard solution. The solution was filtered through 0.45 m filter before injecting into HPLC system.

**2.1.3. Cc standards:**

Calibration curve standards were prepared by pipetting suitable aliquots from stock solution into separate 10 ml volumetric flasks and the volume was made up to the mark with diluent to obtain the CC standards in the range of 50 - 300 µg/ml concentrations of Tenofovir.

**2.2. Diluent: Mobile phase is used as diluent.**

**2.3. Chromatographic conditions:**

The new HPLC method for estimation of Tenofovir was developed and validated using C18Develosil ODS HG-5RP 150mm×4.6mm column.65 volumes of HPLC grade 40 volumes of 0.01MPhosphate buffer adjusted to pH 5.020 Volumes of Acetonitrile and 40 volumes of Methanol and(40:20:40% v/v) as mobile phase. Separation was achieved through isocratic elution mode at 0.8 mL/min flow rate and the effluent was monitored at 257nm.

**2.4. System suitability:**

• The system suitability parameters were determined by preparing standard solution of Tenofovir. The solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The RSD for the peak area of Tenofovir Disoproxil Fumerate for 5 replicate injections should not be more than 2%. Tailing Factor of Tenofovir Disoproxil Fumerate should be not more than 2.

**2.5. Method validation**

The method validation was performed in accordance with ICH guidelines

**2.5.1. Linearity**

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

**2.5.2. Accuracy**

Accuracy was determined by the recovery studies of the analyte. It is determined by standard addition method where the test solution of known quantity is spiked with standard solutions at three levels i.e., 50%, 100% & 150% in triplicate. Mean percentage recoveries at all the levels were calculated.

**2.5.3. Precision**

The precision of an analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision of an analytical procedure is usually expressed the variance, standard deviation of coefficient of variation of a series of measurement. Precision of the method is determined in terms of System precision and Method precision

**2.5.4. Robustness**

Small deliberate changes in method like Flow rate and mobile phase ratio, are made. The actual flow rate is 1.0 ml/min. Change the flow rate ± 0.2 ml/min and observed USP tailing and USP plate count. Actual mobile phase ratio was buffer: ACN is 60:40 .It was changed to 65:35 and 55:45 and observed USP tailing and USP plate count. Standard solutions were injected in sextet. System suitability parameters are evaluated by making the deliberate changes.

**2.5.5. Specificity:**

The specificity was studied by establishing the interference of placebo with the drug. A sample of placebo was injected into the HPLC system as per the test procedure. Chromatogram of placebo should not show any peak at the retention time of analyte peak.

**3. RESULTS AND DISCUSSION**

**3.1 Assay of formulation:**

Assay of the formulation is performed as per the givem procedure. This was done in triplicate. The amount of drug present in the formulation was calculated from standard graph. The % assay of Tenofovir obtained was found to be 98.48 %.

**3.2 System suitability**

System suitability parameters were determined according to ICH guidelines. Plate count was more than 2000, tailing factor was less than 2 and resolution was more than 2. All the system suitable parameters were passed and were within the limits.

**3.3 Validation**

**3.3.1. Linearity**

The linearity was determined at six concentrations in the range of Tenofovir 50 - 300 µg/ ml. The Peak areas against concentration were plotted and the calibration curve was constructed. The Correlation coefficient (r2) was greater than 0.99 within the concentration range for both the drugs.

**3.3.2. Accuracy**

 

Accuracy of the method wsa established at three levels of concentrations by standard addition method. Triplicate injections were given at each level of accuracy and percentage recoveries were calculated. The mean % Recovery was obtained was 100.22 % for Tenofovir.

### 3.3.3. Precision:

The precision of the method was studied by considering system precision and method precision. System precisionwas studied by taking six replicate injections from same homogenous standard solution and peak areas were determined. Average area, standard deviation and % RSD were calculated for two drugs. Method precision was studied by taking six replicate injections from test solution and peak areas were determined. Average area, standard deviation and % RSD were calculated for two drugs. The % RSD of Tenofovir for System precision was found to be 0.01 and 0.02.

### 3.3.4. Robustness:

Robustness of the method was studied by making deliberate changes in flow rate, column oven temperature and mobile phase ratio. After making each change in the conditions, chromatograms were recorded by injecting the standard solutions in six replicates. System suitability parameters were checked at each level. System suitability parameters were not much affected and all the parameters were passed. % RSD was within the limit.

### 3.3.5. Specificity

The Chromatograms of Standard and Sample are identical with nearly same Retention time. No interference due to Placebo and Sample at the retention time of analyte which shows that the method was specific.

## 4. CONCLUSION

In the present study a new RP-HPLC method was developed for the estimation of Tenofovir Disoproxil Fumerate in Pharmaceutical dosage forms and Bulk drugs. The analysis is resolved by using a on C18Inertsil 5µ, 250mm×4.6mm column using phosphate buffer: acetonitrile: methanol (40:20:40) as mobile phase the flow was quite satisfactory. The flow rate was 0.8ml/min and the analyte was monitored at 257nm at which better detector response for drugs were obtained. The retention time for Tenofovir Disoproxil Fumerate was found to be 4.5min. The method was validated for system suitability, accuracy, precision, linearity and ruggedness. The system suitability parameters were within limit, hence it was concluded that the method was suitable to perform the assay. It was also used for determining lower concentration of drug in its solid dosage forms. Therefore it was concluded that the proposed method can be used for analysis of Tenofovir Disoproxil Fumerate in Pharmaceutical dosage forms.

## REFERENCES:

1. Ananda kumar Karunakaran (2012). Analytical method development and validation for simultaneous estimation of Lamivudine and Tenofovir. Eurasian J Anal Chemistry.volume; 56-66.

2. Anjaneyulu. N(2013). Validation for simultaneous estimation of Lamivudine and Tenofovir. Asian Journal of Biomedical and Pharmaceutical Sciences. Vol3 ,Issue 23, 7-11.

3. P. Vivek Sagar , T. Samidha. M. Vamshi Krishna and S. Shobha Rani. A Validated RP-HPLC method for simultaneous estimation of Aspirin and Prasugrel in tablet dosage form. International journal of Pharmaceutical Sciences and Research, 2014;5 (11):1000-06.

4. Sindhura D (2013).Analytical Method Development and Validation for Simultaneous Estimation of Lamivudine, Zidovudine and Efavirenz by RP-HPLC. International Journal of Research in pharmacy and Biotechnology. pp; 583-588.

5. Naga sandhya and Manikanta (2013). Development and Validation of RP-HPLC method for simultaneous estimation of Lamivudine and Efavirenz . pp; 147-155.

6. P. VivekSagar, S. Sushma, P. Shivani, and S. Shobha Rani. Stability indicating RP-HPLC Method for estimation of Agomelatine in tablet dosage form. International journal of Pharmacy and Biological Sciences, 2015; 5(4): 74-81

**Table 1: Assay Data**

| S.no | Peak area of Tenofovir |
|---|---|
| 1 | **4222121** |
| 2 | **4220120** |
| 3 | **4219232** |
| Avg | **4220491** |
| **Regression equation** | **y = 5404.x + 1432.1** |
| **% Assay** | **98. 32%** |

**Table 2: System suitability parameters forTenofovir**

| SAMPLE | Rt | Peak Area | USP plate count | USP Tailing |
|---|---|---|---|---|
| TENOFOVIR | **4.31** | **1193159** | **1302** | **1.0** |

**Table 3: Linearity data of Tenofovir**

| **Tenofovir** | |
|---|---|
| Conc (µg/mL) | **Peak area** |
| 50 | **1083182** |
| 100 | **2140868** |
| 150 | **3178742** |
| 200 | **4234699** |
| 250 | **5283960** |
| 300 | **6383864** |

 

**Table 4: Accuracy data of Tenofovir**

| S No | Accuracy--80% | Accuracy--100% | Accuracy--120% |
|---|---|---|---|
| | Area | Area | Area |
| Injection-1 | 3393262 | 4222121 | 5072521 |
| Injection-2 | 3353232 | 4220120 | 5035654 |
| Injection-3 | 3366565 | 4219232 | 5091236 |
| Avg | 3371020 | 4220491 | 5066470 |
| amt Recovered | 79.35 | 99.32 | 119.27 |
| %Recovery | 99.19 | 99.32 | 99.39 |

**Table 5: Method Precision data of Tenofovir**

| S No | Name | Tenofovir | |
|---|---|---|---|
| | | RT | Area |
| 1 | M-Precision-1 | 4.323 | 4201252 |
| 2 | M-Precision-2 | 4.319 | 4199998 |
| 3 | M-Precision-3 | 4.315 | 4222215 |
| 4 | M-Precision-4 | 4.316 | 4201213 |
| 5 | M-Precision-5 | 4.315 | 4215222 |
| 6 | M-Precision-6 | 4.316 | 4212121 |
| Average | | 4.317 | 4208670 |
| Standard Deviation | | 0.003 | 9210.24 |
| %RSD | | 0.07 | 0.22 |

**Table 6: System Precision data of Lamivudine and Tenofovir**

| S No | Name | Tenofovir | |
|---|---|---|---|
| | | RT | Area |
| 1 | S-Precision-1 | 4.313 | 4209541 |
| 2 | S-Precision-2 | 4.312 | 4212874 |
| 3 | S-Precision-3 | 4.312 | 4232293 |
| 4 | S-Precision-4 | 4.312 | 4228294 |
| 5 | S-Precision-5 | 4.311 | 4250605 |
| 6 | S-Precision-6 | 4.311 | 4248839 |
| Average | | 4.312 | 423408 |
| Standard Deviation | | 0.001 | 17311.94 |
| % RSD | | 0.02 | 0.41 |

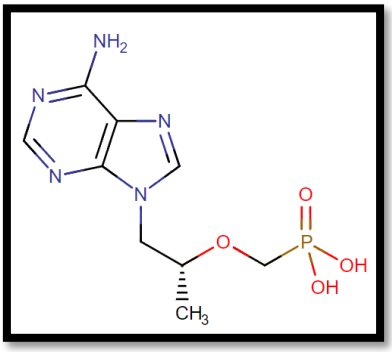


**Fig. 1: Structure of Tenofovir**

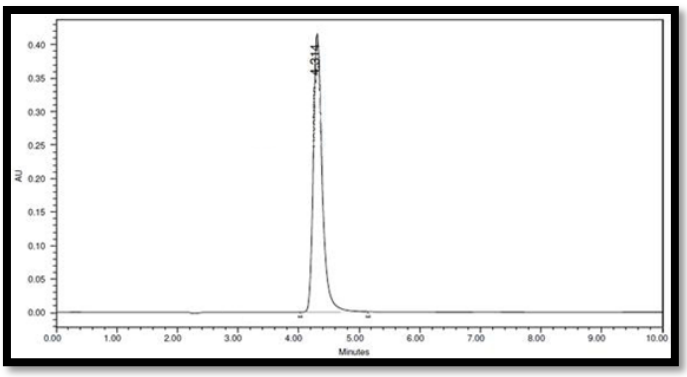

**Fig 2: Representative Chromatogram of working standard solution**

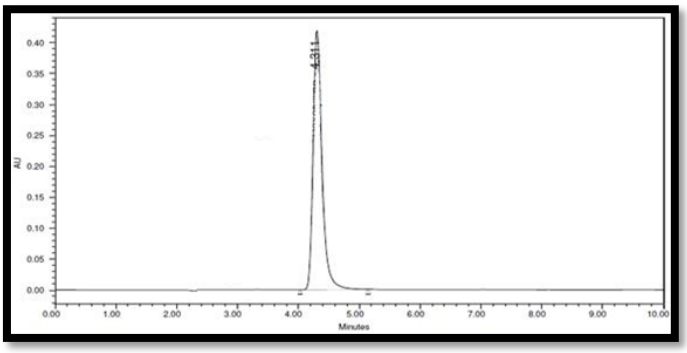

**Fig 3: Representative Chromatogram of working sample solution**

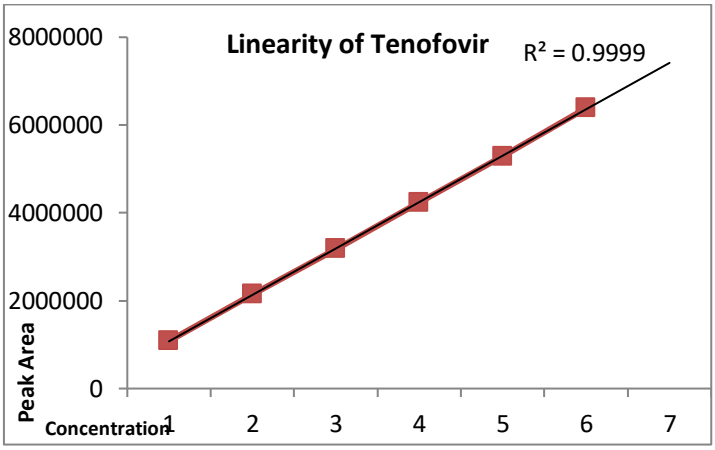


**Fig 4: Standard graph of Tenofovir**

**How to cite this article:**
**Y. Naveen Kumar*, ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF TENOFOVIR IN TABLET DOSAGE FORM BY RP-HPLC TECHNIQUE Pharma Res, 2016; 05(12): 293-297.**
**DOI: https://doi.org/10.5281/zenodo.14201891**

**Conflict of interest**: The authors have declared that no conflict of interest exists.
**Source of support:** Nil

 