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ESTIMATION OF TENOFOVIR BY UV VISIBLE SPECTROSCOPY

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ABSTRACT
In view of the need for a suitable Spectroscopic method for routine analysis of Tenofovir(antiretroviral medicine) in formulations, attempts were made to develop simple, precise and accurate analytical method for estimation of Tenofovir in formulation. As Validation is a necessary and important step in both framing and documenting the capabilities of the developed method it was done in accordance with USP and ICH guideline for the assay of active ingredient. The method was validated for parameters like linearity, precision, accuracy, specificity, 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.The utility of the developed method to determine the content of drug in commercial formulation was also demonstrated.
KEY WORDS: *Tinofovir,Validation,UV Visible spectroscopy.*

DRUG PROFILE
TenofovirDisoproxil Fumarate
Drug category : Antiretroviral
Structure

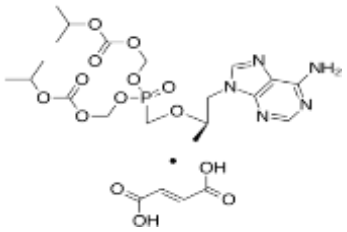

Fig :1 Structure of Tenofovir

IUPACName : ({[(2R)-1-(6-amino-9H-purin-9 yl)propan2yl]oxy}methyl) phosphonic acid.

Molecular Formula: $C_{19}H_{30}N_5O_{10}P \bullet C_4H_4O_4$

Molecular Weight : 635.52gm/mole.

PHYSICOCHEMICAL PROPERTIES: It is white to off-white crystalline powder
Freely soluble in distilled water at 25°C**.** Having a M.P of 276-280°C Recommended Dose in Adults and Pediatric Patients 12 Years of Age and Older (35 kg or more) For the treatment of HIV-1 or chronic hepatitis B: The dose is one 300 mg VIREAD tablet once daily taken orally, without regard to food. For patients unable to swallow VIREAD tablets, the oral powder formulation (7.5 scoops) may be used. In the treatment of chronic hepatitis B, the optimal duration of treatment is unknown. Safety and efficacy in pediatric patients with chronic hepatitis B weighing less than 35 kg have not been established.

Mechanism of action: Tenofovir diphosphate inhibits HIV-1 reverse transcriptase and the Hepatitis B polymerase by direct binding competition with the natural deoxyribonucleotide substrate (deoxyadenosine 5'-triphosphate) and, after integration into DNA, causes viral DNA chain termination [24], [Label].

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Materials and Methods

S.No	Instruments And Glasswares	Model
1	UV-Visible spectroscopy	Labindia
2	Weighing machine	Sartorius
3	Volumetric flasks	Borosil
4	Pipettes and Burettes	Borosil
5	Beakers	Borosil

Table 1 :List of Instruments and glassware's used

CHEMICALS USED

S.No	Chemical	Brand names
1	Tenofovir	Sura labs
2	Methanol LR Grade	Merck
3	Acetonitrile for Spectroscopy	Merck

Table 2 :List of Chemicals

METHOD DEVELOPMENT

SOLUBILITY TEST

The solubility test for drug Tenofovir was performed by using various solvents. Solvents were methanol, chloroform, methanol (100% v/v) was chosen as solvent for developing this method.

VALIDATION

Preparation of mobile phase:

Accurately measured 500 ml (50%) of Acetonitrile and 500 ml of Double distilled water (50%) were mixed in a 1000ml of volumetric flask.

Diluent Preparation:

The Mobile phase was used as the diluent.

VALIDATION PARAMETERS

SPECIFICITY STUDY OF DRUG

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Tenofovir working standard into a 10ml of clean dry volumetric flasks add about 7ml of diluent .Then sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15 ml of the above Tenofovir stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents. Measure the absorbance of standard solution five times and calculate the %RSD for the absorbance was found to be within the specified limits.

Preparation of Sample Solution

Take average weight of one Tablet and crush in a mortor by using pestle and weight 10 mg equivalent weight of Tenofovir sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.15 ml of Tenofovir above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

Measure the absorbance of sample solutions and calculate the assay by using formula:

$$Assay\,\% = \frac{sample\ area}{Standard\ area} \times \frac{dilution\ sample}{dilution\ of\ standard} \times \frac{P}{100} \times \frac{Avg.wt}{Lc} \times 100$$

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PREPARATION OF DRUG SOLUTIONS FOR LINEARITY

Accurately weigh and transfer 10 mg of Tenofovir working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluent then sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (5 ppm of Tenofovir)

Take 0.05ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – II 10 ppm of Tenofovir)

Take 0.1ml of stock solution in to 10ml of volumetric flask and make up the volume upto mark with diluent.

Preparation of Level – III (15 ppm of Tenofovir)

Take 0.15ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – IV (20 ppm of Tenofovir)

Take 0.2ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – V (25 ppm of Tenofovir)

Take 0.25ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Procedure: Check the each level into the spectroscopic system and measure the absorbance.

Plot a graph of absorbance versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

PRECISION AND REPEATABILITY

Preparation of Tenofovir Product Solution for Precision

Accurately weigh and transfer 10 mg of Tenofovir working standard into a 10ml of clean dry volumetric flasks add about 7ml of diluent then sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of the above Tenofovir stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents. Measure the absorbance of standard solution five times and calculate the %RSD for the absorbance was found to be within the specified limits.

INTERMEDIATE PRECISION

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure

DAY 1

Measure the absorbance of standard solution six times and calculate the %RSD for the absorbance was found to be within the specified limits.

DAY 2

Measure the absorbance of standard solution six times and calculate the %RSD for the absorbance was found to be within the specified limits.

Accuracy

For preparation of 50% Standard stock solution

Accurately weigh and transfer 10 mg of Tenofovir working standard into a 10ml of clean dry volumetric flasks add about 7mL of diluent. Then sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.075ml of the above Tenofovir stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 100% Standard stock solution

Accurately weigh and transfer 10 mg of Tenofovir working standard into a 10ml of clean dry volumetric flasks add about 7mL of diluent .Then sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of the above Tenofovir stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 150% Standard stock solution

Accurately weigh and transfer 10 mg of Tenofovir working standard into a 10ml of clean dry volumetric flasks add about 7mL of diluent .Then sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.225ml of the above Tenofovir stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

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Procedure
Check the three replicate absorbance of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the absorbance. Calculate the Amount found and Amount added for Tenofovir and calculate the individual recovery and mean recovery values.

ROBUSTNESS
The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

For preparation of Standard solution
Accurately weigh and transfer 10 mg of Tenofovir working standard into a 10ml of clean dry volumetric flasks add about 7mL of diluent.Then sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.15ml of the above Tenofovir stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation in wavelength
The sample was analyzed at 249nm and 251nm instead of 250nm and measures the absorbance at different wavelength.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT SOLUBILITY STUDIES
The solubility test for drug Tenofovir was performed by using various solvents. Solvents were methanol, chloroform, methanol (100% v/v). Methanol (100% v/v) was shows maximum absorbance and solubility to compare with other solvents. So it was chosen as solvent for developing this method.

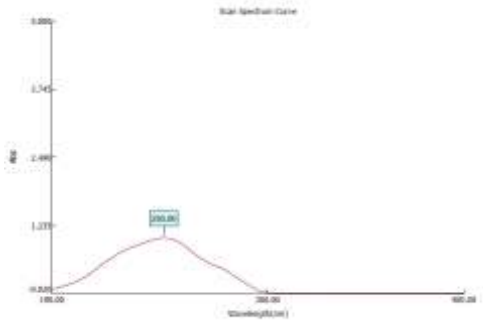

Fig. 2: Selection of Wavelength

Observation: Above spectrum shows maximum absorbance at 250nm wavelength by using methanol (100% v/v) as diluents

VALIDATION
Blank

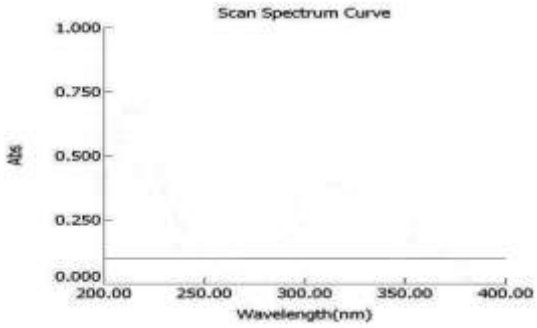

Fig.10 : UV Scan Spectrum for Blank

SPECIFICITY
The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitate Tenofovir in drug product.

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Assay (Standard):

S.no	Drug Name	Absorbance
1	Tenofovir	0.474
2	Tenofovir	0.475
3	Tenofovir	0.475
4	Tenofovir	0.476
5	Tenofovir	0.475
Mean		0.475
Std. Dev.		0.000707
% RSD		0.148865

Table 3: Assay results for standard

Acceptance Criteria

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

Assay (Sample)

S.no	Drug Name	Absorbance
1	Tenofovir	0.474
2	Tenofovir	0.475
3	Tenofovir	0.475
Mean		0.475

Table 4 : Assay Results for sample

%Assay = 0.475/0.475×10/15×15/0.0281×99.6/100×0.8452/300×100= 99.8%
The % purity of Tenofovir in pharmaceutical dosage form was found to be 99.8%

LINEARITY

Concentration Level (%)	Concentration µg/ml	Absorbance
33	5	0.168
66	10	0.343
100	15	0.48
133	20	0.642
166	25	0.807

Table 5 : CHROMATOGRAPHIC DATA FOR LINEARITY STUDY

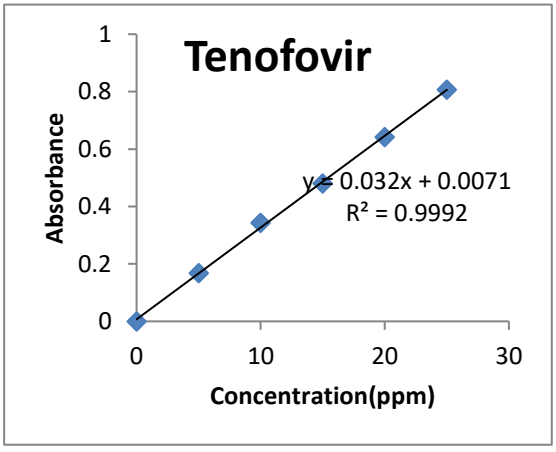

Fig 11: Linearity graph of Tenofovir

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LINEARITY PLOT

The plot of Concentration (x) versus the Average Peak Area (y) data of Tenofovir is a straight line.

Y = mx + c
Slope (m) = 0.032
Intercept (c) = 0.007
Correlation Coefficient (r) = 0.999

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is C 0.007 these values meet the validation criteria.

PRECISION

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

REPEATABILITY

Obtained five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the absorbance and calculated % RSD.

S.no	Drug Name	Absorbance
1	Tenofovir	0.474
2	Tenofovir	0.475
3	Tenofovir	0.475
4	Tenofovir	0.474
5	Tenofovir	0.474
Mean		0.4744
Std. Dev.		0.000548
% RSD		0.115456

Table 6 : Repeatability results for Tenofovir

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

	Drug Name	Absorbance
1	Tenofovir	0.493
2	Tenofovir	0.493
3	Tenofovir	0.494
4	Tenofovir	0.494
5	Tenofovir	0.493
6	Tenofovir	0.493
Mean		0.493333
Std. Dev.		0.000548
% RSD		0.111025

Table 7: Intermediate precision results by analyst 1

Acceptance criteria:

- %RSD of six different sample solutions should not more than 2

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S.no	Drug Name	Absorbance
1	Tenofovir	0.454
2	Tenofovir	0.453
3	Tenofovir	0.454
4	Tenofovir	0.454
5	Tenofovir	0.454
6	Tenofovir	0.454
Mean		0.453833
Std. Dev.		0.000447
% RSD		0.098541

Table8 : Intermediate precision results by analyst 2

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2

ACCURACY:

Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated.

Accuracy50%

S.no	Drug Name	Absorbance
1	Tenofovir	0.247
2	Tenofovir	0.247
3	Tenofovir	0.247
Mean		0.247

Table9 : Accuracy results for Tenofovir 50%

Accuracy100%

S.no	Drug Name	Absorbance
1	Tenofovir	0.476
2	Tenofovir	0.489
3	Tenofovir	0.485
Mean		0.483333

Table10 : Accuracy results for Tenofovir 100%

Accuracy150%

S.no	Drug Name	Absorbance
1	Tenofovir	0.725
2	Tenofovir	0.726
3	Tenofovir	0.726
Mean		0.725667

Table11 : Accuracy results for Tenofovir 150%

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%Concentration (at specification Level)	Absorbance	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	0.247	7.5	7.5	100.2	100.6%
100%	0.483	15	15.1	100.1	
150%	0.725	22.5	22.9	101.6	

Table12 : The accuracy results for Tenofovir

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.

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.

$$LOD = 3.3 \times \sigma / s$$

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

Result

LOD =3.3×0.009541/0.031
= 1.0µg/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.

$$LOQ=10\times\sigma/S$$

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

Result:

LOQ =10×0.009541/0.031
= 3.0µg/ml

Robustness

The robustness was performed for the wavelength variations from 249nm to 251nm and mobile phase ratio variation from more organic phase to less organic phase ratio for Tenofovir. The method is robust only in less wavelength condition and the method is robust even by change in the Mobile phase ±5%.

S.No	Drug Name	Wavelength variation±1	Absorbance
1	Tenofovir	249(less)	0.469
2	Tenofovir	250(actual)	0.478
3	Tenofovir	251(more)	0.470

Table13 : Variation in wavelength

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate Spectroscopic method was developed for the quantitative estimation of Tenofovir in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Tenofovir was freely soluble in Methanol was chosen as

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the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise.The results expressed in Tables for Spectroscopic method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Tenofovir in bulk drug and in Pharmaceutical dosage forms.

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