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R E S E A R C H A R T I C L E

Analytical Method Development and Validation of tenofovir distroxil fumarate (TDF) in bulk and formulations by using folin-ciocalteau reagent through UV- Visible Spectroscopy

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A B S T R A C T

A simple, economical, rapid, accurate, precise UV- Visible spectrophotometric method has been developed and validated according to ICH guidelines for the Tenofovir Distroxil Fumarate (TDF) as active pharmaceutical ingredient (API) by UV – Visible Spectrophotometric method. The absorption maximum of TDF was found to be at 763nm wavelength using in ethanol water as a solvent. Linearity range was found to be 5-25ug/ml, with the correlation coefficient being more than 0.999. The relative standard deviation was found to be < 2 %. The percentage recovery was within the range of 98% -105%, indicating that there is no significant interference from the other ingredients present in the formulation. The molar absorptivity ans sandell's sensitivity were found to be 0.278X104L mol-1cm-1 and 17.6 µg/cm2 and the of drug: FC reagent was found to be 1:2 in distilled water. The method can be applied for the routine analysis of TDF as API in pharmaceutical preparation.

Keywords: Tenofovir Disproxil Fumarate, Ethanol, Routine analysis, UV–Visible Spectrophotometry.

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Tenofovir Disoproxil Fumarate (TDF)[1-2] is a medication used to treat chronic hepatitis B and to prevent HIV/AIDS. It is recommended for use with other antiretroviral. It may be used for prevention of HIV/AIDS among those at high risk before exposure, an after a needle stick injury or other potential exposure. It is available as by mouth as a tablet or powder.

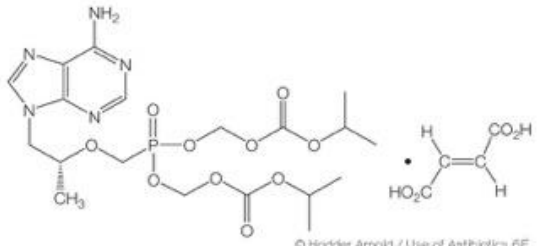


Figure 1: Tenofovir Disoproxil Fumarate (TDF)

FC Reagent

Folin-ciocalteau reagent was a heteropoly acid which is phosphomolybdotungstic acid. It produces blue color with phenolic group, so used for colorimetric estimation of drugs containing phenols and amines. Hexavalent phosphomolybdotungistic acid complexes with the following structures formed in the solution.

$$3H_2O_5.P_2O_513WO_35MoO_3.10H_2O$$
$$3H_2O.P_2O_5.14WO_3.4MoO_3.1OH_2O$$

When FC reagent reacts with drug in presence of reducing agents like $SnCl_2$, ascorbic acid hydrazine, probably drug effects reduction of one or more oxygen atoms from tungstate or molybdate. In the FC reagent, there by producing one or more possible reduced species which have characteristic intense blue color. Literature review revealed that methods based on HPLC, HPTLC and very few colorimetric methods for the determination of TDF were reported hence a new analytical method was developed for the estimation of TDF by Complexation with FC reagent by using UV-Visible spectrophotometer and the method was validated according to ICH guidelines.

2. Materials and Methods

2.1 Chemicals: A sample gift of Tenofovir Disoproxil Fumarate as a gift sample from Hetero Pharma Limited was obtained from Hetero Pharma limited. Ethanol, Sodium Chloride, Hydrochloric acid, Distilled water all are analytical Grade.

FC Reagent used is purchased from LOBA CHEME Ltd.

Instrument: Lab India UV-Visible Spectrophotometer 3000+

2.2 Preparation of solutions

1. Selection of solvent:

The solubility of Tenofovir Disoproxil Fumarate was determined in a variety of solvents as per Indian Pharmacopoeia Standards. Solubility test was carried out in different acids and bases and non polar solvents from the solubility studies, ethanol& water was selected as suitable solvent for proposed method.

2.2. Preparation of Solutions

Preparation of primary Stock Solution: Standard stock solution was prepared by dissolving, accurately measured 100mg of Tenofovir Disoproxil Fumarate in little amount of ethanol and make up to 100ml with distilled water (1000µg/ml).

Preparation of secondary stock solution: Secondary stock solution was prepared by taking 10 ml of drug solution from primary standard and make up to 100 ml with distilled water (100µg/ml).

Preparation of FC Reagent: FC solution (Folin-ciocalteu). This reagent is diluted in water. This solution has to be prepared in 1:2 ratio i.e., 5 ml of FC reagent is taken and added 10 ml of water. For any concentration, FC reagent has to be prepared in 1: 2 ratio.

Determination of Absorbance Maxima: 1ml of secondary stock solution was taken in10 ml standard volumetric flask dilute to 10 ml with water to get conc. of 10µg/ml. The absorbance of resulting solution was measured against respective blank solution (water) in the UV –Visible region of 200 – 800nm, which shows maximum absorbance at 763nm.

Determination of Linearity Concentration range:

For preparation of different concentrations, aliquots of stock solution of suitable concentrations of Tenofovir Disoproxil Fumarate were transferred into a series of 10 ml standard flasks and volumes were made up to mark with distilled water. Five different concentrations were prepared in the range of 5 - 30µg/ml and the absorbance were measured at 763nm against solvent (water) blank. The obtained absorbance values are plotted against the concentrations of Tenofovir Disoproxil Fumarate to get the calibration graph.

Precision:

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples.

Intraday and Inter day precision:

A variation of results within the same day (intraday), variation of results between days (inter day) was analyzed. Intraday precision was determined by analyzing Tenofovir Disoproxil Fumarate for six times in the same day at 763nm. Inter day precision was determined by analyzing the drug once for six days at 763nm.

Recovery Studies:

In order to study the accuracy, powder of Tenofovir Disoproxil Fumarate was taken, and used to carry out the analysis. Recovery studies were carried out by addition of standard drug solution to the sample at 3 different concentration levels (50%, 100% and 150%) and the percentage recovery was determined by using the formula.

$$Amount\ of\ drug\ recovered = \frac{Recovery\ percentage}{Amount\ of\ drug} \times 100$$

Limit of Detection (LOD) and Limit of Quantification (LOQ): Preparation of calibration curve from the serial dilutions of standard was repeated for six times. The limit of detection and the limit of quantification was calculated

by using the average value of slope and standard deviation of intercept.

Determination of Composition of Tdf-Fc Complex

a) Job's continuous variation method[17-18]

The metal ion solution and the complex agent are mixed in different volume ratios if keeping the total volume of the mixture constant. Both the species should be of equimolar concentrations. The mixture is made up to known volume. The absorbance of each of the solution is measured against the reagent blank and graph is drawn between the mole fraction of the metal ion or the reagent and absorbance. This results in an invert or 'S 'or 'U' shaped curve and mole fraction ratio of the metal ion to the drug corresponding to the maximum absorbance gives the composition the complex. While job's method[26] gave satisfactory results for several complexes, instance of in conclusive results were reported in literature. Cooper et al reported that the method is still useful if a modified procedure is adopted. In this method equimolar solutions of metal ion and drug were mixed in different simple mole ratios and the absorption spectra of solutions were recorded .In the modified procedure the optical measurements were made at various wavelengths covering the entire range instead at the wavelength corresponding to the maximum .If the same conclusions were obtained at all the wavelengths it can be assumed single complex is formed and job's method can be successfully applied for the elucidation of the composition of the complex

b) Mole ratio method

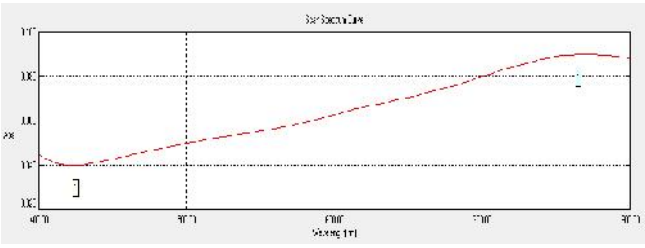
This method was introduced by Yoe and jone[27] in 1944 for the study of composition of a complex .In this method a series of solution are prepared in which the concentration of one of the reactants (very often the metal) is held constant at a high value while that of the other is varied .A plot of absorbance versus mole fraction of the reactant is prepared. The graph drawn between the absorbance and the composition consists of two linear segments intersecting at a point. The linear segment corresponding to higher concentration range of the varied component while be generally parallel to the concentration axis. The mole ratio corresponding to the point of section intersection of the linear segment gives composition of the complex.

3. Results and Discussions

1. Determination of λ – max: The λ max of Tenofovir Disoproxil Fumarate (TDF) in FC reagent in alkaline medium was determined and it was found to be 763 nm.

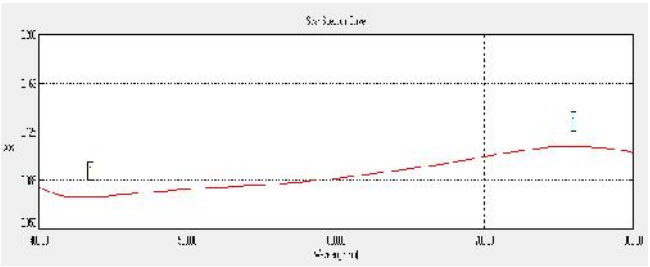
2. Method development: By varying TDF concentration with constant amounts of FC reagent and sodium carbonate solution we optimize the method through some trails following

Trail-1



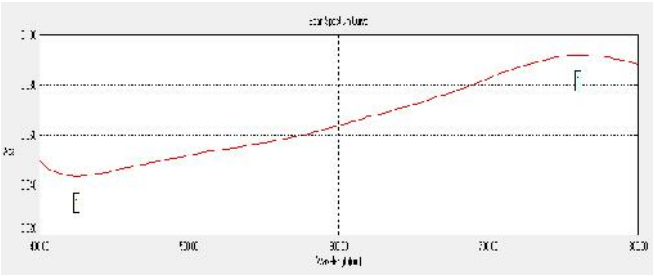
Observed peak of [TDF-FC] at wavelength - 763nm at 1ml

Trail-2



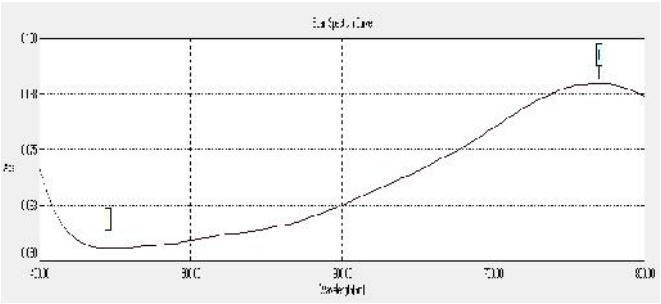
Observed peak at wavelength -763 nm at 2 ml.

Trail-3



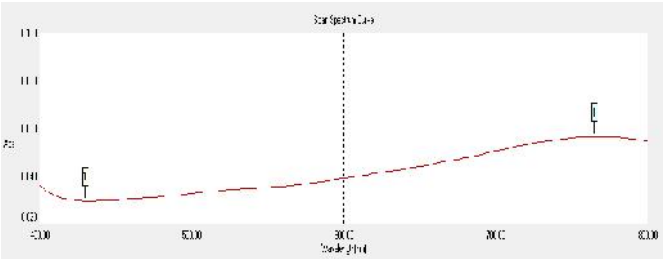
Observed peak at wavelength - 763 nm at 3ml.

Trail-4



Observed peak at wave length- 763nm at 4ml.

Trail-5



Observed peak at wavelength -763nm at 5ml.

Inference: From the spectrum obtained in the five trails revealed that the method was optimized by taking 1.5ml of FC reagent and 3.5 ml of 10% sodium carbonate and the absorption maxima was found to be 763nm

3. Study of Beer Lambert's Law:

Different concentration of Tenofovir Disproxil Fumarate standard was prepared ranging from 5 to 30µg/ml linearity curve was concentrated and the regression coefficient was found to greater than to be 0.99.

Table 1: Study of linearity range of Tenofovir Disproxil Fumarate by proposed method

S.No	Concentration (µg/ml)	Absorbance at 763 nm
1	5	0.028
2	10	0.048
3	15	0.070
4	20	0.088
5	25	0.108
6	30	0.124

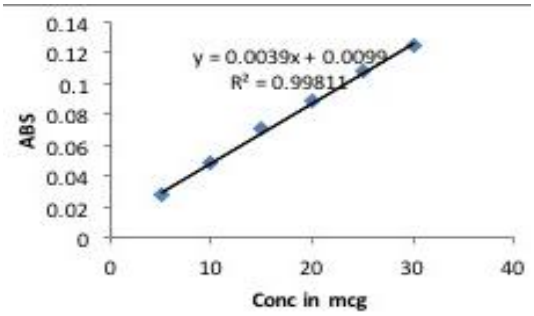


Figure 2: Linearity graph of TDF by Proposed method

Table 2: Results of Linearity and Range Studies

Parameters	At 763 nm
Linear dynamic range (µg/ml)	5 to 30µg/ml
Slope	0.003
Correlation &coefficient	0.998

Determination of molar absorptivity coefficient and Sandell's Sensitivity: The molar absorptivity and sandell's sensitivity were found to be 0.278X104L mol-1cm-1 and 17.6 µg/cm2.

Composition of Drug –FC reagent complex: by job's method and molar ratio method and 1:2 complex of Drug – FC reagent complex formed.

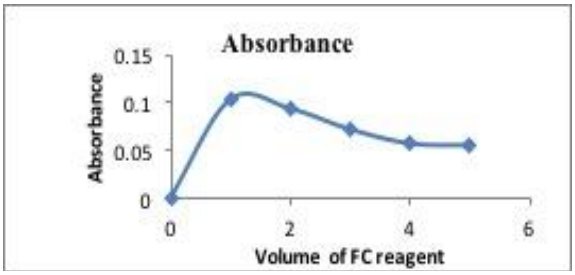


Figure 3: Job's continuous Curve for [TDF-FC]

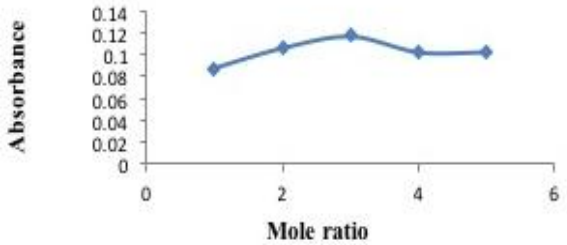


Figure 4: Mole Ratio graph

6. Method validation: This method is validated according to ICH guide lines

Precision:

Table 3: Intraday precision of Tenofovir Disoproxil Fumarate by proposed method

S.NO	Concentration in µg/ml	Absorbance at 763nm
1	15	0.0625
2	15	0.0610
3	15	0.0615
4	15	0.0620
5	15	0.0625
6	15	0.0640

SD -0.000946

Table 4: Interday precision of Tenofovir Disoproxil Fumarate by proposed method

S.NO	Concentration in µg/ml	Absorbance at 763nm
1	15	0.0595
2	15	0.0605
3	15	0.057
4	15	0.06
5	15	0.0595
6	15	0.0605

SD-0.0595

Recovery studies:

Table 5: Recovery studies of TDF by proposed method: (50%)

S. No	Concentration (µg/ml)	Absorbance	% Recovery
1	15	0.122	96.8
2	15	0.124	98.4
3	15	0.123	97.6

Table 6: Recovery studies of TDF by proposed method: (100%)

S. No	Concentration (µg/ml)	Absorbance	%Recovery
1	7.5	0.105	111.06
2	7.5	0.099	104.70
3	7.5	0.103	108.93

Table 7: Recovery studies of TDF by proposed method: (150%)

S. No	Concentration (µg/ml)	Absorbance	% Recovery
1	22.5	0.168	106.6
2	22.5	0.165	104.7
3	22.5	0.163	103.4

Limit of detection: The limit of Detection was found to be 1.0406µg

Limit of quantification: The limit of quantification was found to be 3.1533 µ

Application of present method for the estimation of the marketed formulation of Tenofovir Disoproxil Fumarate

Assay:

Table 8: Method for the assay of TDF by proposed method: Brand name: Tenvir

S.NO	Equivalent in µg/ml	Absorbance	%Recovery
1	15	0.061	96.8
2	15	0.0605	96.03
3	15	0.062	98.41
4	15	0.063	100
5	15	0.063	100
6	15	0.062	98.41

Mean=98.275 %, SD =1.483425 ;RSD=1.509466

4. Conclusions

The developed method is simple, accurate, precise, economical and ecofriendly .The colour development is based on reduction of one or more oxygen atoms of tungstic acid and tungstate showing an absorption maxima at 763nm having intense blue colour in alkaline medium (10% sodium carbonate) and donot involve any complicated reactions and donnot show any interference with the excipients and the TDF-FC complex showed stable colour for one week at 25 ^{o} . The present method can be applied for quality control and routine analysis of TDF in pure and its dosage form.

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