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METHOD DEVELOPMENT AND VALIDATION OF TENOFOVIR DISOPROXIL FUMARATE IN PURE FORM AND SOLID DOSAGE FORM BY USING UV SPECTROPHOTOMETRY

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ABSTRACT

A new simple and cost-effective UV-spectrophotometric method had been developed for estimation of Tenofovir disoproxil fumarate (TDF) in bulk and tablet dosage form. The wavelength (λ max) was found to be 259 nm by using methanol as solvent. The linearity of this drug at selected wavelengths lies between 5-40µg/ml. Beer's law was obeyed in this concentration range with a correlation coefficient of 0.999. The analysis results were validated as per ICH guidelines. The method has good reproducibility with %RSD less than 2%. Thus proposed method can successfully applied for Tenofovir disoproxil fumarate (TDF) in routine analysis work.

INTRODUCTION

Tenofovir disoproxil fumarate: TDF chemically is (2E) -but- 2-enedioicacid;bis ({[(propan-2-yloxy)carbonyl]oxy} methyl) {[(2R)-1-(6-amino-9H-purin-9-yl)propan-2-yl]oxy}methanephosphonate (fig1) . Tenofovir disoproxil is a nucleotide analog reverse-transcriptaseinhibitor (NtRTI) Tenofovir disoproxil is used for HIV-1 infection and chronic hepatitis B treatment [1-2]Tenofovir has a melting point of 279 °C (534 °F). Tenofovir disoproxil fumarate is a white to off-white Crystalline powder Tenofovir disoproxil fumarate is an acyclic nucleoside phosphonate diester analog of adenosine monophosphate. Tenofovir disoproxil fumarate requires initial diester hydrolysis for conversion to tenofovir and subsequent phosphorylation by cellular enzymes to form tenofovir diphosphate. Tenofovir diphosphate inhibits the activity of HIV-1 reverse transcriptase by competing with the natural substrate deoxyadenosine 5'-triphosphate and, after incorporation into DNA, by DNA chain termination. Tenofovir disoproxil fumarate is a solid dosage form available in tablet form in the market.

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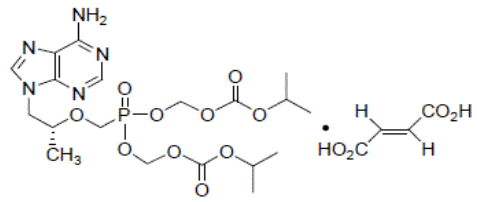

Fig1: Structure of Tenofovir disoproxil fumarate

Literature survey reveals that few methods based on UV [3], HPLC [4-9]. This present work describes the development and validation of UV Spectrophotometry which quantifies the tenofovir disproxil fumarate. The main objective of this method is to develop a simple, accurate, precise, and rapid spectrophotometric method for estimation of Tenofovir disoproxil fumarate in bulk and pharmaceutical dosage form. And validate the method according to ICH guidelines [10].

MATERIALS AND METHODS

Materials: The pure form of Tenofovir disoproxil fumarate was obtained from spectrum labs as a gift sample. Methanol obtained from Thermo Fischer Scientific Pvt Ltd was used for dilutions. The commercial form of Tenofovir disoproxil fumarate tablets with brand name Tenohip® containing 300mg was purchased from the local pharmacy. Class A grade glassware was used.

Instruments: Absorption spectral measurements were performed in LABINDIA (T60) double beam UV/Visible Spectrophotometer by using 1cm quartz matched cuvettes. The weighing was carried out in ELITE analytical balance. For data analysis, Microsoft Excel 2007 was used.

Preparation of standard stock solution: A stock solution of 1000 µg/mL was prepared by dissolving 50 mg of TDF in methanol which has taken in a clean, dry 50 mL volumetric flask. The solution was made up to mark with the same solvent.

Preparation of working standard solution: From this stock solution, 100 µg/mL working standard was prepared. It was done by transferring 10mL of standard stock into a 100 mL volumetric flask and was made up to mark with methanol. The sample solution of 20 µg/mL was prepared by taking 2ml from 100µg/ml solution and made up the solution to the mark in a 10mL volumetric flask.

Selection of λ max: The prepared stock solution was scanned in UV Spectrophotometer between the range 400nm and 200nm using methanol as blank. The maximum absorbance was found at 259nm.

Assay: Commercial tablets containing 300 mg of TDF per tablet was assayed by weighing and powdering 5 numbers of tablets accurately. Powder equivalent to 100 mg was calculated and weighed the required amount of drug powder and transferred into dry 100 mL volumetric flask which contains 50 mL methanol and kept for sonication for 10 minutes. The solution was then filtered through Whattman filter paper and made up to the mark with methanol. From this, 10mL of the solution was taken into another dry 100 mL volumetric flask and made up to the mark by using a diluent to obtain the concentration of 100 µg/mL solution. The sample solution of 20 µg/mL was prepared by taking 2ml from 100 µg/ml solution and made up the solution to the mark in a 10mL volumetric flask.The objective of method validation is to demonstrate that the method is suitable for its intended purpose. The method was validated for linearity, precision, accuracy, robustness, ruggedness, LOD & LOQ.

Linearity: Linearity can be determined by taking a series of dilutions of standard solutions that are used as working standards. So, the working range was observed as 5-40 µg/mL at 259 nm. Regression data was given in Table 2 and Figure 2.

Precision: The Repeatability of the method was checked by scanning 20 µg/ml solution for 6 times represented in [Table 3]. Intraday precision was determined by checking the absorbance of (20 µg/ml) on the same day (morning, afternoon, evening) and the results were represented in [Table 4]. Inter-day precision was determined by checking the absorbance of (20 µg/ml) on three different days and the obtained results were represented in [Table 4].

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Table 1: Assay of tenofovir disoproxil fumarate tablets

Formulation (Mfr)	Brand	Wavelength (nm)	Amount of drug taken from tablet let (mg)	%Purity
tenofovir disoproxil fumarate tablets (Zydus Heptiza)	Tenohep 300mg	259	100	98.58

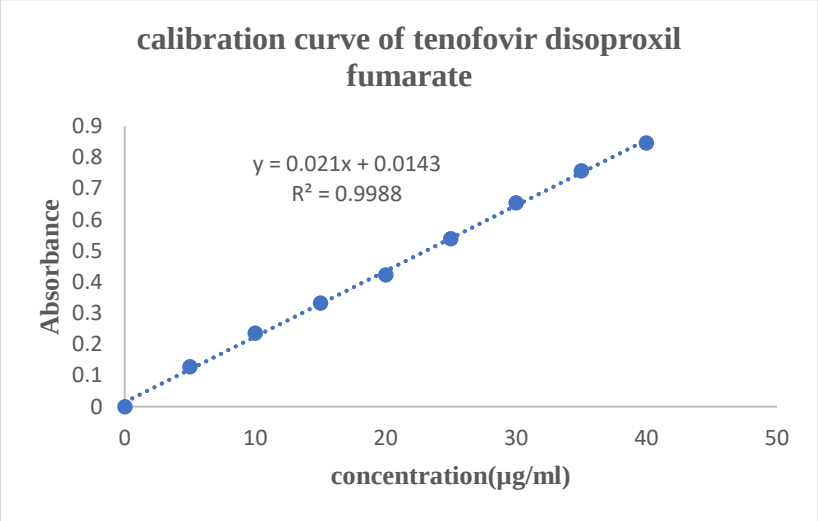

Fig2: Calibration curve of Tenofovir disoproxil fumarate

Table 2: Results of Linearity

S.No.	Parameters	Method wavelength (nm)
1	Absorption maxima (nm)	259
2	Beer's law limit (µg/mL)	5- 40
3	Correlation coefficient	0.999
4	Regression equation (y = mx+c)	y = 0.020+0.023
5	Slope (m)	0.020
6	Intercept	0.023

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Table 3 repeatability data

Concentration (ug/ml)	Absorbance	statistical analysis
20	0.4122	Mean=0.412
20	0.4117	%RSD=0.42%
20	0.4119	
20	0.4126	
20	0.4120	
20	0.416	

Table 4 Results of Intraday and Interdayprecision

Concentration (µg/mL)	Intraday (Morning, Afternoon, Evening)		Interday (Day 1, 2, 3, 4, 5)	
	%RSD	Avg %RSD	%RSD	Avg %RSD
20	0.42		0.42	
20	0.14	0.29	0.13	
20	0.32		0.15	0.18
			0.10	
			0.10	

Table 5: Results of accuracy

Level of addition (%) (µg/mL)	Tablet amount (µg/mL)	Amount added	Drug found	%Recovery	Avg recovery (µg/mL)
80	20	16	15.84	99.03	
100	20	20	19.67	98.37	98.76
120	20	24	23.7	98.9	

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Table 6: Results of Robustness (± 1nm of actual wavelength)

Concentration (µg/mL)	λ1 (258nm)		λ2 (259nm)		λ3 (260nm)	
	Absorbance	Statistical analysis	Absorbance	Statistical analysis	Absorbance	Statistical analysis
20	0.3825	Mean=0.383	0.4122	Mean=0.412	0.4128	Mean=0.412
20	0.3832	%RSD= 0.18	0.4117	%RSD= 0.42	0.4123	%RSD=0.12
20	0.3839		0.4119		0.4120	
20	0.3841		0.4126		0.4131	
20	0.3837		0.4120		0.4134	
20	0.3845		0.4163		0.4126	

Table7: Results of Ruggedness

Concentration µg/mL)	Analyst 1		Analyst 2	
	Absorbance	Statistical analysis	Absorbance	Statistical analysis
20	0.4122	Mean=0.412	0.4006	Mean= 0.400
20	0.4117	%RSD=0.42	0.4008	%RSD=0.11
20	0.4119		0.4011	
20	0.4126		0.4003	
20	0.4120		0.3998	
20	0.4163		0.4005	

Table 8: Results of Sensitivity

Limit of detection	Limit of quantification
0.01µg/ml	0.03µg/ml

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Table 9: Validation parameters of Tenofovir disoproxil fumarate

Parameters	Results
Absorption maxima (nm)	259
Linearity range (µg/mL)	5-40 µg/mL
Regression Equation	y= 0.020x - 0.023
Correlation coefficient (R^2)	0.999
Molar extinction coefficient	8.2204×10^{-9}
LOD (µg/mL)	0.01
LOQ (µg/mL)	0.03
Accuracy (% Recovery)	98.76
Sandell's sensitivity	
(µg/cm^2/0.001Absorbance units)	6.889×10^{-8}
Precision	
Inter-day (%RSD)	0.18
Intraday (%RSD)	0.29

Accuracy: An Accuracy study was conducted by spiking at three concentration levels (80%, 100%, and 120%). At each level, triplicate samples were scanned and the percentage recovery was determined and presented in the [Table5]

Robustness: Robustness is the capacity of a sample to remain unchanged by small after changing the conditions. The robustness of the method was determined by altering the experimental conditions deliberately and the assay was performed in the same conditions. The wavelength effect was observed at two different wavelengths like 258 and 260 which is ±1nm to actual wavelength (259 nm). The assay of TDF at all the altered conditions was within 98-102%. The results were given in [Table 6].

Ruggedness: Ruggedness is a measurement of the reproducibility of the sample at different conditions like laboratories, analysts, instruments, reagents, etc. By using two different analysts, the sample was analyzed and absorbances were recorded. The results were given in Table 7.

Sensitivity: Sensitivity was obtained by performing Limit of Detection (LOD) and Limit of Quantification (LOQ) calculations as per the equation given in ICH guidelines.

Limit of Detection: It is the lowest amount of the drug in the sample that can be detected, but not necessarily quantified.

$$\mathbf{LOD} = \frac{3.3X\sigma}{S}$$

Where, S=standard deviation

Limit of Quantification: It is an amount of analyte that can be quantified with a specified limit of accuracy and precision.

$$\mathbf{LOQ} = \frac{10X\sigma}{S}$$

Where, S= standard deviation

RESULTS AND DISCUSSION

Spectrum scan was performed to the standard solution over a range of 200 – 400nm to decide the detection wavelength. The maximum absorbance (λ_{max}) of TDF was found to be 259nm. Excipients in the tablet solution did not

interfere with the absorbance of the standard solution of TDF at 259 nm. Hence, quantitative analysis and validation were performed at this wavelength. The Beer-Lambert's law was obeyed by this drug in the linearity range of 5-40 µg/mL. Regression equation was found to be y = 0.020x - 0.023 with slope and intercept as 0.020 and 0.023 respectively. The regression coefficient (R^2) was found to be 0.999 and % recovery was found to be 99.3 – 100.6 % at 259 nm. % recovery of the sample represents that there were no interferences of excipients present in the tablet formulation. Relative standard deviations from the measurements were found to be always less than 2%. By observing %RSD values of intraday and inter-day precision which is less than 2%, the developed method was found to be precise. The method was also found to be accurate where recoveries were ranging from 98 – 102%. LOD & LOQ were found to be 0.01 µg/mL and 0.03 µg/mL respectively. It indicates that the method is sensitive. Robustness and ruggedness were performed by analyzing the sample at different wavelengths and by different analysts respectively. As the % RSD of robustness and ruggedness were found to be less than 2%, the method was found to be robust and rugged. Molar Extinction Coefficient and Sandell's Sensitivity were calculated. Validated parameters were given in Table 9.

CONCLUSION

The method development in UV spectrophotometer for the determination of tenofovir disoproxil fumarate has the advantage of being simple, rapid, inexpensive and applicable to various concentration ranges with high precision and accuracy. This method was validated as per the ICH guidelines. The validation results were found to be satisfactory. Hence, this method can be successfully applied to analyze the dosage forms.

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