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DEVELOPMENT AND VALIDATION OF DIFFERENT SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF TENOFOVIR DISOPROXIL FUMARATE FROM BULK DRUG AND TABLETS

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ABSTRACT: Simple, sensitive and accurate UV- spectrophotometric methods have been developed for the determination of an anti-HIV drug, Tenofovir Disoproxil fumarate (TDF), in raw material and in tablets. The drug shows maximum absorbance at 259 nm in selected four different media namely gastric fluid simulated (HCl) pH 1.5, vaginal fluid simulated (VFS) pH 4.2, phosphate buffer (PB) pH 6.8 and double distilled water. Beer's law is obeyed in the concentration range of 5-45 µg/mL of drug. The limits of detection and limits of quantification are found to be 1.37 and 4.17 µg/ml in gastric fluid simulated, 1.27 and 3.85 µg/ml in vaginal fluid simulated, 1.22 and 3.71 µg/ml in phosphate buffer and 1.30 and 3.95 in double distilled water respectively. The methods have been successfully applied for the determination of TDF in tablets and bulk drugs. Results are validated statistically as per ICH guidelines. It is found that the excipients present in the commercial formulation do not interfere with the methods and hence the UV-method permits a rapid and economical quantification of drug in bulk and in tablet dosage form.

INTRODUCTION: Tenofovir disoproxil fumarate (TDF) belongs to the class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors (nRTIs), which blocks reverse transcriptase, an enzyme crucial to viral production in HIV-infected people [1-3]. Chemically TDF is 9[(R)-2-[[bis [[(isopropoxycarbonyl) oxy] methyl] phosphinyl] methoxy]proply] adenine fumarate [4] (**fig. 1**). TDF is the first nucleotide analogue approved for HIV-1 treatment.

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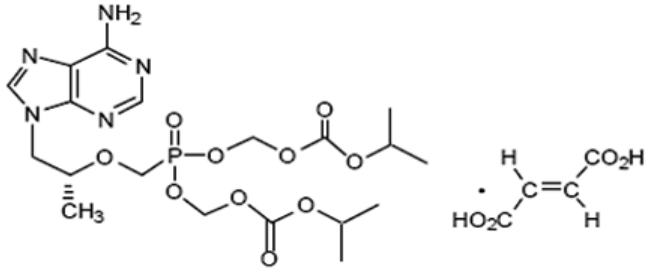

FIGURE 1: CHEMICAL STRUCTURE OF TENOFOVIR DISOPROXIL FUMARATE

TDF is used in combination with other antiretroviral for the treatment of HIV infections [5-7]. Literature survey has revealed that number of methods have been published for the estimation of TDF. Tenofovir in plasma RP-HPLC [8, 9], derivative-HPLC [10] and LC-MS/MS [11-13] methods were reported for analysis.

Most of these methods are tedious and time-consuming involving complex sample preparation.

The aim of the present work is to develop simple, accurate and precise analytical methods for the quantitative estimation of TDF from different simulated body fluid from bulk and in solid dosage form. The literature survey does not reveal any UV-spectrophotometric method together for the determination of the drug in bulk and in pharmaceuticals, in different simulated, physiologic body fluids like gastric, vaginal, phosphate buffer and in double distilled water.

This paper reports a study on the development of new validated UV-spectrophotometric methods for the estimation of TDF in bulk and solid dosage form in different simulated buffer media i.e. gastric fluid simulated (HCl) pH 1.5, vaginal fluid simulated (VFS) pH 4.2, phosphate buffer (PB) pH 6.8 and double distilled water. The methods were validated according to the International Conference on harmonization (ICH) guidelines [14, 15].

MATERIALS AND METHODS:

Apparatus: A double beam UV-spectrophotometer (pharmaSpec-1700, shimadzu, Japan) connected to computer that was loaded with spectral bandwidth of 1 nm and wave length accuracy of ± 0.3 nm with a pair of 1 cm matched quartz cell. All weights were taken on electronic balance (Vibra, DJ-150S-S, Shinko Denshi, Japan).

Materials: Pure sample of tenofovir disoproxil fumarate was gifted by Bioequivalence Study Centre, Department of Pharmaceutical Technology, Jadavpur University, Kolkata. Tablets of brand name Tenohep (Batch no.-GL2201) containing 300 mg of tenofovir disoproxil fumarate, were procured form a local pharmacy. Double distilled water was used as the solvent for the experiment.

Hydrochloric acid (HCl), lactic acid, acetic acid, sodium chloride, potassium hydroxide, calcium hydroxide, urea, glucose, glycerol, disodium hydrogen phosphate, and potassium dihydrogen phosphate were purchased from Qualigens (Fisher), Mumbai, India. Bovine serum albumin (BSA) was purchased from Himedia, Mumbai, India. All the above chemicals were of analytical grade.

Preparation of standard solutions and calibration curves: Standard solutions of tenofovir disoproxil fumarate were prepared by dissolving 10 mg of the standard drug separately in different medium such as HCl (pH 1.5), VFS (pH 4.2), PB (pH 6.8) and double distilled water diluted up to 100 mL by respective media to obtain a stock solution of final concentration 100 µg/mL.

Aliquots (0.5-5.0 mL) of stock solution of tenofovir disoproxil fumarate were transferred into a series of 10 mL volumetric flasks and volume was made up to the mark with different buffers to produce the concentration range 5-45 µg/mL. The absorbance of each solution was measured at 259 nm against the respective medium as blank. The calibration curves were prepared by plotting graph between absorbance and concentration (**fig. 2**).

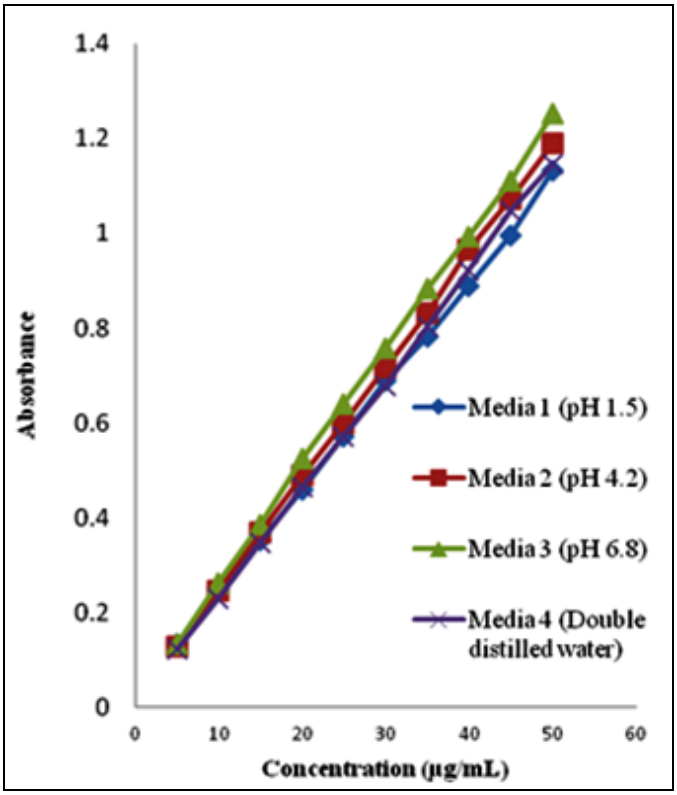

FIGURE 2: CALIBRATION CURVES OF STANDARD DRUG IN DIFFERENT SIMULATED BODY FLUID MEDIA

Estimation of tenofovir disoproxil fumarate in bulk and in tablets: For the analysis of drug in bulk, accurately weighed 10 mg drug was dissolved in 100 mL of four different media in a volumetric flask. After suitable dilution, the absorbances of final contents were recorded against the respective blanks at 259 nm. For the analysis of tablets, 20 tablets of tenofovir disoproxil fumarate (300 mg)

were ground to fine powder and mixed thoroughly. A quantity of powder equivalent to 10 mg of the drug was transferred into a 100 mL volumetric flask and dissolved separately with the HCL, VFS, PB and double distilled water by sonication at 30 min. the solutions were filtered through 0.22 µm membrane filters. The membrane was washed with the same media. The washing was added to the filtrate and the final volume was made up to 100 mL. After suitable dilutions, the absorbances of final solutions, corresponding to the 30 µg/mL, were recorded at 259 nm against the respective media as blank.

Methods validation: The methods were validated according to International Conference on Harmonization (ICH) guidelines for validation of analytical procedures [14, 15].

Linearity: The absorbance of the standard solutions, in different media at 5-45 µg/mL range was measured at 259 nm. Calibration curves were constructed by plotting average absorbance versus concentrations. Linearity was determined by regression equation for all media solutions (**Table 1**).

Recovery studies: Pure drug at five levels was added to a fixed amount of drug in tablet powder and the total amount was determined to calculate the percentage recovery of the drug (**Table 2**).

Specificity: The specificity of the methods was evaluated by interaction study obtained from scan reports (UV) of the standard drug solution, sample solution (of tablet) and placebo tablet matrix solution (these matrices solutions were prepared in a manner similar to the sample solution using placebo tablet matrix instead of tenofovir disoproxil fumarate tablets). This UV method was found to be specific for TDF, as none of the excipients interfered with the calculation of TDF.

Accuracy (by standard addition method): Accuracy can be analysed by percentage recovery of added standard drug solutions to fixed concentration of sample solutions. For stock solutions, an accurately weighted amount of standard and sample tablet powder equivalent to 10 mg of drug was transferred separately in to 100 mL volumetric flasks to get 100 µg/mL in HCl, VFS, PB and double distilled water medium respectively. In a separate dilution five different 10 mL volumetric flasks each having 10 µg/mL of sample stock solution were added with 0 µg/mL, 5 µg/mL, 10 µg/mL, 15 µg/mL and 20 µg/mL of standard stock solution to get final concentration of 10, 15, 20, 25 and 30 µg/mL after diluting with HCl (pH 1.5) medium. The same were repeated with diluting media VFS, PB and double distilled water. All solutions were prepared in triplicate and assayed for percentage recoveries of added standards TDF. The accuracy was reported as percentage recovery by the assay of known added amount of analyte in the sample.

Precision: Repeatability was calculated by analysing three independent TDF standard solutions (10, 20 and 30 µg/mL), in triplicate, in different media. The intermediate precision was evaluated on three independent TDF standard solutions (10 µg/mL) on same day and also on three consecutive days. The precision was expressed as the standard deviation, percentage relative standard deviation (coefficient of variation) and confidence interval of each mean (Table 1).

Limit of Detection and limit of Quantification: Limit of detection (LOD) and limit of quantification (LOQ) are based on the slope of the calibration curves and standard deviation of y-intercept of regression lines (Table 1).

RESULTS AND DISCUSSION: The methods were validated according to the guidelines of international conference on harmonization (ICH). The proposed UV- spectrophotometric methods were found to be specific and selective for analysis of TDF in bulk and in tablet, where no interference was observed at 259 nm by the excipients of tablet, when compared with standard and sample TDF solution. The absorbance spectra of TDF in different pH solutions of HCL, VFS, PB and double distilled water are shown in **fig. 3**. The average λ_{max} was found to be 259 nm. A standard calibration curve of the drug was constructed by plotting absorbance versus concentration. Linear absorbance concentration gives regression equations $y = 0.0218x + 0.0257$, $y = 0.0236x + 0.0109$, $y = 0.0245x + 0.0189$ and $y = 0.023x + 0.0013$ with correlation coefficients (r^2) indicate a good linearity between absorbance and concentration in the range of 5-45 µg/mL (Table 1).

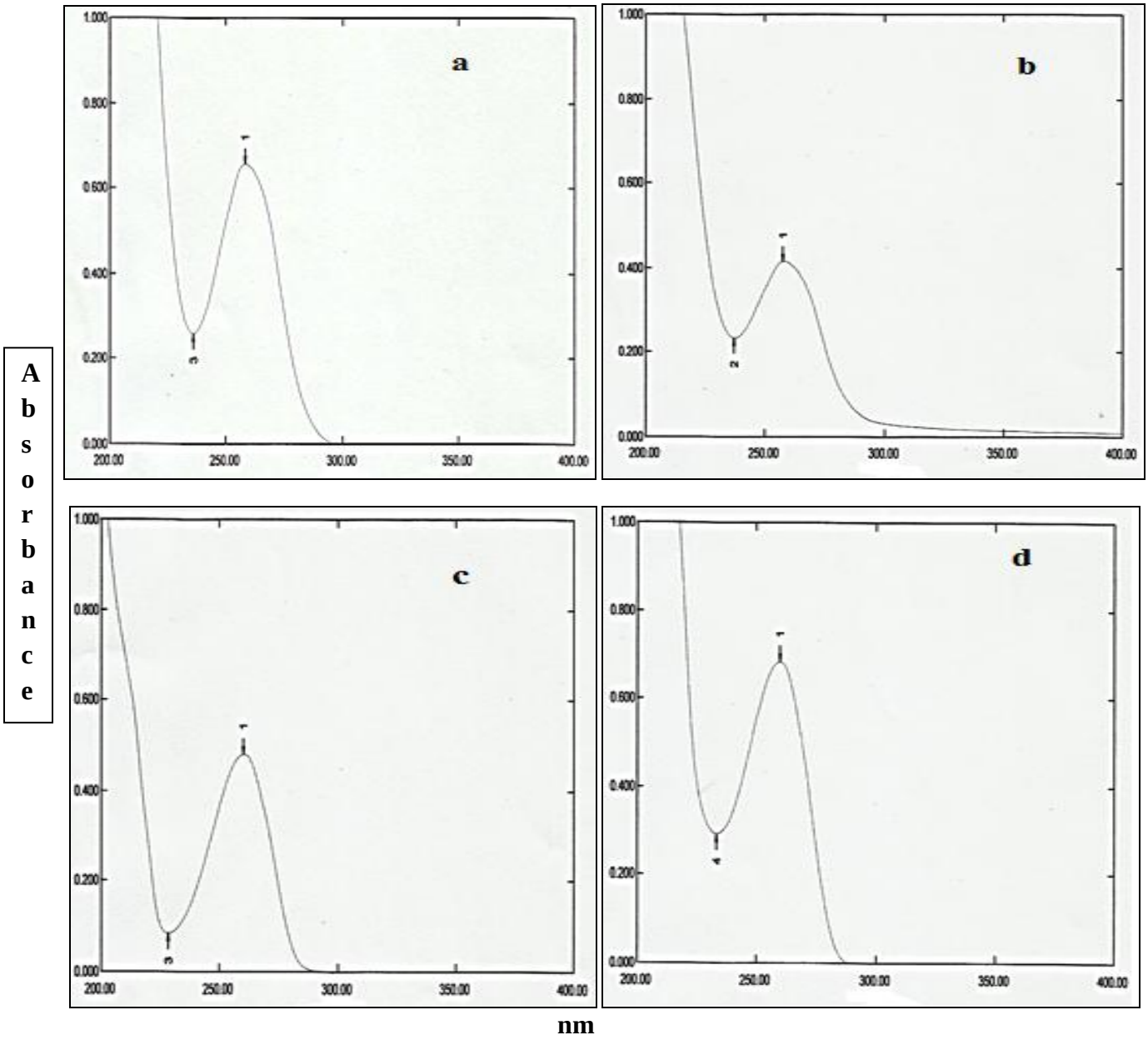

FIGURE 3: UV-SCANNING CURVES OF TDF IN FOUR DIFFERENT FLUIDS (a) HCl pH 1.5 (b) VFS pH 4.2 (c) PB pH 6.8 (d) DOUBLE DISTILLED WATER.

The accuracy of the proposed method by standard addition method was determined for tablet and the mean recovery (n=5) is found to be 99.93±0.011, 99.41±0.010, 102.01±0.009 and 98.19±0.012 in different types of media, like gastric fluid simulated, vaginal fluid simulated, phosphate buffer and double distilled water respectively (Table 2) which was in the near agreement with the labelled amount and thus indicates the accuracy of the method. The standard and sample solutions were found to be stable for 48 h (**Table 3**). Results of assay of tablet solid dosage form of TDF by proposed UV- method are reported in **table 4**.

The assay results obtained by the different proposed methods are found to be 98.96±0.015, 97.86±0.011, 101.68±0.007 and 98.03±0.018 respectively which are acceptable agreement with the pharmacopoeia limits. The assay result of proposed UV-methods when compared using student t-test do not reveal significant difference between the experimental values obtained for the standard drug and sample drug analysis by the two methods (Table 4).The repeatability (% RSD or percentage relative standard deviation) and intermediate precision (% RSD) were observed for analysis of three independent in replicate samples.

The repeatability values are found to be 0.358, 0.284, 0.341 and 0.462 in HCl buffer, VFS, PB and double distilled water respectively and intermediate precision (% RSD) for selected samples in three consecutive different days are found to be 0.801, 0.432, 0.572 and 0.710 in HCl, VFS , PB and double distilled water respectively. The low value of both percentage relative standard deviation and intermediate precision confirm the high degree of precision and accuracy of the proposed method. The LOD and LOQ are found to be 1.37 and 4.17 µg/ml in hydrochloric acid buffer, 1.27 and 3.85 µg/ml in vaginal fluid simulated, 1.22 and 3.71 µg/ml in phosphate buffer and 1.30 and 3.95 in double distilled water respectively.

TABLE 1: REGRESSION ANALYSIS AND SYSTEM SUITABILITY PARAMETERS FOR THE QUANTIFICATION OF TENOFOVIR DISOPROXIL FUMARATE FROM UV-SPECTROPHOTOMETER IN DIFFERENT MEDIA

Parameter	Media 1	Media 2	Media 3	Media 4
Linearity range µg/ml	5-45	5-45	5-45	5-45
Regression equation	y = 0.0218x + 0.0257	y = 0.0236x + 0.0109	y = 0.0245x + 0.0189	y = 0.023x + 0.0013
Correlation coefficient (r^2)	0.9994	0.9998	0.9995	0.9996
Molar absorptivity(ε) (lit./mole/cm)	1.4489×10^4	1.5227×10^4	1.6269×10^4	1.4489×10^4
Sandell's sensitivity (µg/ml/cm-^{2}/0.001)	0.0438	0.0417	0.0390	0.0438
95% confidence interval for slope	< 0.0001	< 0.0001	< 0.0001	< 0.0001
95% confidence interval for intercept	0.0028	0.0146	0.0169	0.8032
Standard error of slope	0.0002	0.0001	0.0002	0.0002
Standard error of intercept	0.0061	0.0035	0.0063	0.0049
Repeatability[a] (%RSD)	0.358	0.284	0.341	0.462
Intermediate[b] precision (%RSD)	0.801	0.432	0.572	0.710
LOD, µg/ml	1.37	1.27	1.22	1.30
LOQ, µg/ml	4.17	3.85	3.71	3.95

Media 1- Gastric fluid simulated (GFS), pH= 1.5, Media 2- Vaginal fluid simulated (VFS), pH=4.2, Media 3- Phosphate buffer (PB), pH=6.8, Media 4- Double distilled water, [a]Relative standard deviation (RSD) of 6 independent determination in a day. [b]Relative standard deviation (RSD) of 9 independent determinations (Three independent samples per day for 3 days).

TABLE 2: ACCURACY TEST RESULTS IN DIFFERENT SIMULATED MEDIA FOR TENOFOVIR DISOPROXIL FUMARATE IN BULK AND IN SOLID DOSAGE FORM BY UV-SPECTROPHOTOMETER

Media type	Sample conc. (mcg/ml)	Conc. of added standard (mcg/ml)	Percentage recovery ± SD	Mean percentage recovery ± SD
Media 1 (pH 1.5)	10	0	100.59 ± 0.013	99.93 ± 0.011
	10	5	99.17 ± 0.007	
	10	10	98.69 ± 0.016	
	10	15	99.50 ± 0.012	
	10	20	101.72 ± 0.009	
Media 2 (pH 4.2)	10	0	99.19 ± 0.011	99.41± 0.010
	10	5	100.30 ± 0.017	
	10	10	100.23 ± 0.002	
	10	15	98.48 ± 0.005	
	10	20	98.88 ± 0.018	
Media 3 (pH 6.8)	10	0	102.89 ± 0.010	102.01 ± 0.009
	10	5	102.60 ± 0.002	
	10	10	101.74 ± 0.003	
	10	15	101.89 ± 0.012	
	10	20	100.96 ± 0.018	
Media 4 (Double distilled water)	10	0	96.82 ± 0.019	98.19 ± 0.012
	10	5	99.33 ± 0.017	
	10	10	97.97 ± 0.008	
	10	15	97.33 ± 0.005	
	10	20	99.52 ± 0.011	

SD- Standard deviation (n=3)

TABLE 3: STABILITY OF THE STANDARD AND SAMPLE SOLUTIONS OF TENOFOVIR DISOPROXIL FUMARATE IN DIFFERENT MEDIA BY UV METHODS

Media	Time Interval n	UV method			
		Standard solution		Sample solution	
		Recovery (n=3), %	Difference, %	Recovery (n=3), %	Difference, %
Media 1 (pH 1.5)	0	99.87	0.00	101.48	0.00
	24	99.10	0.77	100.73	0.75
	48	99.07	0.03	100.40	0.33
Media 2 (pH 4.2)	0	99.12	0.00	96.49	0.00
	24	99.10	0.02	97.04	0.55
	48	99.30	0.20	97.28	0.24
Media 3 (pH 6.8)	0	102.05	0.00	100.93	0.00
	24	102.53	0.48	100.96	0.03
	48	102.67	0.14	100.80	0.16
Media 4 (Double distilled water)	0	98.49	0.00	101.38	0.00
	24	98.50	0.01	100.84	0.54
	48	99.64	1.14	100.27	0.57

TABLE 4: ASSAY RESULT OF MARKETED TENOFOVIR DISOPROXIL FUMARATE (TENOHEP) DOSAGE FORMS BY UV-METHOD

Releasing media	Percentage Drug in standard solution ± SD	Percentage Drug in dosage form solution ± SD
Media 1 (pH 1.5)	99.62 ± 0.009	98.96 ± 0.015
Media 2 (pH 4.2)	99.30 ± 0.002	97.86 ± 0.011
Media 3 (pH 6.8)	101.97 ± 0.012	101.68 ± 0.007
Media 4 (Double distilled water)	98.67 ± 0.015	98.03 ± 0.018

SD- Standard deviation

CONCLUSION: The methods are found to be very simple, rapid, precise, accurate and sensitive. The validated UV- methods can be used for the drug analysis in routine for bulk and solid dosage forms, at four different physiological conditions.

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