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Research Article

Spectrophotometric Method Development and Validation for Simultaneous Estimation of Tenofoir disoproxil fumarate and Emtricitabine in Bulk Drug and Tablet Dosage form

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Abstract
Two simple, accurate, economical and reproducible spectrophotometric methods for simultaneous estimation of two-component drug mixture of Tenofovir disoproxil fumarate and Emtricitabine in bulk and combined tablet dosage form had been developed. The first method employs formation and solving of simultaneous equations using 259 nm and 286 nm as two analytical wavelengths. The second method is absorption ratio method, which uses 286 nm and 247.6 nm as two analytical wavelengths. Both methods were statistically validated according to International Conference on harmonization and recovery studies confirmed the accuracy of the proposed method.

Keywords: Simultaneous equation, Absorption ratio, Tenofovir disoproxil fumarate and Emtricitabine.

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. Chemically TDF is 9[(R)-2-[[bis [[(isopropoxycarbonyl) oxy] methoxy] phosphinyl] methoxy] propyl] adenine fumarate. [1] Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI) and chemically, it is 4-amino-5-fluoro-1-[2-(hydroxymethyl)-1, 3-oxathiolan-5-yl]-pyrimidin-2-one.[2] The drugs are prescribed individually, as well as multi component dosage forms available in the market. A number of methods have been published for the estimation of the above said analytes. Tenofovir in plasma RP-HPLC [3] derivative-HPLC [4] and LC-MS/MS [5-7] methods were reported for analysis. Method for estimation of Emtricitabine in human plasma by HPLC with fluorometric detection [8] was published. Stability-indicating LC was also reported in the literature. [9] Most of these methods are tedious and time-consuming involving complex sample preparation. Even though various methods are reported in the literature, for estimation of Tenofovir disoproxil fumarate and Emtricitabine individual or in combination with other drugs, no method had been reported so far for simultaneous estimation of these two drugs using simultaneous equations in bulk and tablet dosage form. The present study was aimed at the simultaneous estimation of Tenofovir disoproxil fumarate and Emtricitabine by simultaneous equations method. This method was validated according to the International Conference on harmonization (ICH) guidelines. [10]

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EXPERIMENTAL

Materials : Gift sample of Tenofovir disoproxil fumarate and Emtricitabine were procured from Emcure Pvt. Ltd., Pune (MH.) India. The commercial fixed dose combination product (Tenvir-EM containing 300 mg Tenofovir sisoproxil fumarate and 200 mg Emtricitabine) was procured from the local Pharmacy. HCl AR grade was procured from Qualigens fine chemical Mumbai, India.

Equipment: Shimadzu UV-1700; UV-Vis spectrophotometer with 1 cm matched quartz cells was used for the measurement of the absorbance. Shimadzu - AX -200 electronic balance was used for weighing the samples.

Preparation of stock solutions: Standard stock solution (1000µg/ml) of Tenofovir disoproxil fumarate and Emtricitabine were prepared separately in 0.01N HCl by transferring accurately weighed 100mg portion of Tenofovir disoproxil fumarate and Emtricitabine in separate 100 ml volumetric flasks and volume was made up with 0.01N Hcl. The Working standard solutions were prepared and further diluted.

Development of the method

Simultaneous Equation (Method 1)

Selection of analytical wavelengths was done by diluting the stock solution of Tenofovir disoproxil fumarate and Emtricitabine at a concentration of 10 µg /ml. They were scanned in the wavelength range of 200 to 400nm in overlain spectra mode (Fig.1), wavelengths 259.0 and 286.0 nm were selected for the formation of simultaneous

Table 1 : Summary of Optical Characteristics

Parameters	Tenofovir disoproxil fumarete		Emtricitabine	
	Method-I	Method-II	Method-I	Method-II
λ_{max} (nm)	259	247.6	286	286
Beer's Law Limit (µg/ml)	5-45	5-45	3-27	3-27
Molar absorptivity (lit/mole/cm)	1.586×10^4	1.556×10^4	9.5680×10^3	9.5680×10^3
Sandell's sensitivity (µg / ml / cm^{-2}/ 0.001)	4.0071×10^{-2}	4.0091×10^{-2}	2.5841×10^{-2}	2.5841×10^{-2}
Correlation coefficient (r^2)	0.9997	0.9995	0.9998	0.9998
Slope	9.498×10^{-2}	2.41×10^{-2} 0.0241	4.07×10^{-2}	4.07×10^{-2}

Table 2: **Recovery studies**

Name of the Drug	Amount added (in mg)	Amount recovered (in mg) n=3		Percentage recovery		Average percentage recovery	
		Method -I	Method –II	Method –I	Method -II	Method -I	Method -II
Tenofovir disoproxil fumarete	10	310.22	309.92	100.072	99.97		
	20	319.98	320.04	99.9958	100.01	99.90	99.90
	30	328.87	329.09	99.6576	99.72		
Emtricitabine	10	209.67	210.1	99.846	100.04		
	20	220.29	219.97	100.134	99.98	99.86	100.16
	30	229.10	231.12	99.6116	100.48		

'n' is number of replication of experiments

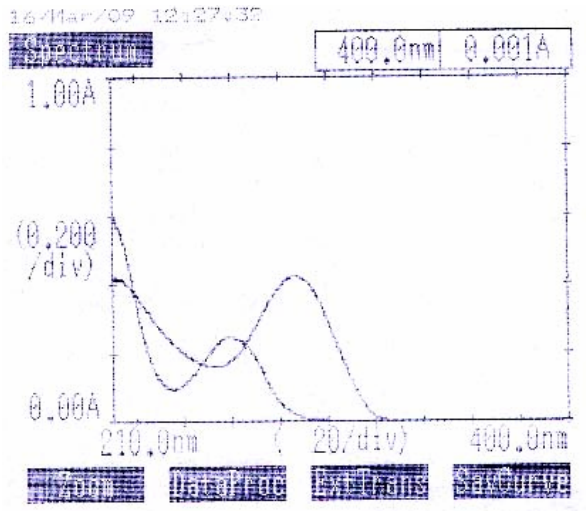

Figure: 1 : Overlain spectra of Tenofovir disoproxil fumarate and Emtricitabine in 0.01N Hcl

equation for construction of calibration curves. Two series of different concentration in range of 5-45 µg/ml for Tenofovir disoproxil fumarate and 3-27 µg/ml for Emtricitabine were prepared from the working standard solutions. The calibration curves were plotted at 259.0 and 286.0 nm. LOD is the limit of detection while LOQ is limit of quantification, RSD is relative standard deviation and r^2 is correlation coefficient.

The absorptivities of both the drugs at both the wavelength were determined. These calculated values were the mean of five independent determinations. The absorbance and absorptivity value at the particular wavelength were calculated and substituted in the following equation to obtain the concentrations:

$A_1 = 240.04Cx + 167.74Cy$……(1) and

$A_2 = 22.82\ Cx + 409.29Cy$…….(2)

Where A_1 and A_2 are absorbance of sample solution at 259 nm and 286 nm respectively; Cx and Cy are concentration of Tenofovir disoproxil fumarate and Emtricitabine respectively (mole /lit) in sample solution. The validity of formed equation was checked by preparing five mixed standards measuring their absorbance at respective wavelength and comparing them with the absorbance calculated using above formed equations.

Absorption ratio method (method 2)

From the overlain spectra of Tenofovir disoproxil fumarate and Emtricitabine, 286.0 nm was taken as λ_{max} for Emricitabine and 247.6 nm as isobestic point for estimation of Tenofovir disoproxil fumarate. Series of different concentraions in range of 5-45 µg/ml for Tenofovir disoproxil fumarate and 3-27 µg/ml for Emtricitabine were prepared from the working standard solutions. The calibration curves were plotted at 286.0 and 247.6 nm. The absorptivities (A1%, 1 cm) of both the drugs at both the wavelength were determined. These calculated values were the mean of five independent determinations. The concentration of sample was measured by this equation,

$$\mathbf{C_1} = \frac{Qo - Q_2}{Q1 - Q2} \cdot \frac{A}{ax_1} \quad \ldots 3$$

$$\mathbf{C2} = \frac{Qo - Q_1}{Q2 - Q1} \cdot \frac{A}{ax_1} \quad \ldots 4$$

Estimation from formulation:

Twenty tablets were weighed and finely powdered. A quantity of powder equivalent to 150 mg of TDF and 100mg of ETB was transferred to 100 ml volumetric flask containing 40 ml of 0.01N HCl, sonicated for 10 min and volume was made up to the mark with the same solvent and filtered through Whatmann filter paper (no.41). Aliquot portion 1ml was transferred to 100 ml volumetric flask and volume was adjusted to mark. The absorbance (A_1 and A_2) were recorded. The concentrations of two drugs in sample were determined using above methods.

Recovery studies: To study the accuracy, reproducibility and precision for all the three developed methods, recovery studies were carried out by the addition of standard drug solution to pre-analyzed tablet sample with

proper dilutions at three different concentration levels within the range of linearity for both the drugs. Results of recovery studies were found to be satisfactory and are reported in (Table 2).

Table 3: Summary of analytical method validation.

Validation parameters	Tenofovir disoproxil fumarete		Emtricitabine	
	Method-I	Method-II	Method-I	Method-II
Recovery (%)	99.90	99.90	9	100.16
Intraday precision (%RSD)	0.2654	0.3152	0.4730	0.4793
Interday precision (%RSD)	0.2314	0.2383	0.2179	0.2198
Linearity (r^2)	0.9998	0.9997	0.9986	0.9988
Robustness (%RSD)	0.2132	0.3584	0.5473	0.5469
LOD(µg/ml)	0.2152	0.1843	0.7142	0.7131
LOQ (µg/ml)	1.2142	1.3371	2.2471	2.2432
Specificity	Specific	Specific	Specific	Specific

RESULTS AND DISCUSSION

The proposed methods developed for simultaneous analysis of TDF and ETB in combined tablet dosage form were found to be simple, accurate, rapid, economical and sensitive to be applied in routine analysis of tablets. In the described methods there were no additional extraction or separation procedures to extract the drug from the formulation excipient matrix. The elimination of these procedures thereby decreasing the error in quantitation. First developed method involving formation and solving of simultaneous equation is based on absorptivity coefficient of two drugs at λ_{max}. Once the equation is framed, the absorbance of sample solution at selected wavelengths was to measure followed by simple calculation. Framed equation was validated using laboratory prepared mixed standards of two drugs which gave satisfactory results.

In second developed method (absorption ratio method) for estimation of TDF and ETB, two wavelengths were selected from overlain spectra. Out of which one is isobestic point and another is λ_{max} of one of the drugs. The spectra of TDF and ETB when overlaid, indicated that the isobestic point was at 247.6 nm at which estimation of TDF was done and estimation of ETB was done at its λ_{max} 286.0 nm.

By observing the validation parameters (Table 3), both the methods was found to be specific, accurate, precise, repeatable and reproducible. However, absorption ratio method has an advantage of simpler calculations over the simultaneous equations method; both methods can be employed for routine analysis of tablet for assay.

CONCLUSION

Both of the above methods were simple, specific, and easy to perform and require short time to analyze the samples. Low limit of quantification and limit of detection makes these methods suitable for use in quality control. The methods were found to be accurate, precise, linear, robust and rugged.

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