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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETERIC METHOD FOR SIMULTANEOUS ESTIMATION OF EMITRICITABINE AND TENOFOVIR DISPROXIL FUMARATE IN BULK AND TABLET FORMULATION BY SIMULTANEOUS EQUATION METHOD.

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ABSTRACT

The simple, accurate, rapid and precise method was developed for the simultaneous determination of tenofovir disoproxil fumarate (TEF) and emtricitabine (EMT) in combined tablets. The method involves the Simultameous equation method.Tenofovir (TEF) and Emtricitabine (EMT) at their respective λmax 260 nm and 280nm shows linearity in a concentration range of 5-30 µg/mL and 5-30 µg/mL respectively. Method employs formation and solving of simultaneous equation using 260nm and 280nm as two analytical wavelengths for both drugs using Distilled water. Reliability and analytical performance of the proposed methods, including linearity, range, precision, accuracy, detection and quantitation limits, were validated according to ICH guideline. The methods were successfully applied for the determination of EMT and TEF in laboratory-prepared mixtures and in their combined tablets.

INTRODUCTION:

Emtricitabine (4-amino-5-fluoro-1-[(2*S*,5*R*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one), an analogue of cytidine, and Tenofovir disoproxil fumarate (({[(2*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl]oxy}methyl)phosphonic acid), are nucleoside reverse transcriptase inhibitor (NRTI) that are used for the treatment of HIV infection.

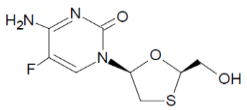
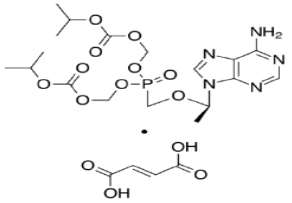


Fig 1: Chemical Structure of Emitricitabine

The chemical name of tenofovir disoproxil fumarate is 9- [(R) - 2[[bis[[(isopropoxycarbonyl)oxy]methoxy]phosphinyl]methoxy]propyl] adenine fumarate. Tenofovir disoproxil fumarate (tenofovir DF) is a bioavailable prodrug of tenofovir, a potent nucleotide analogue reverse-transcriptase inhibitor with activity against human immunodeficiency virus (HIV) and hepatitis B virus. **Fig 2: Chemical Structure of Tenofovir disoproxil fumarate**



A literature survey revealed that liquid chromatography techniques have been reported for the simultaneous determination of emtricitabine, and tenofovir disoproxil fumarate in pure drug, pharmaceutical dosage forms and biological samples. Hence, the authors have attempted to develop a simple, rapid, precise and accurate method for the simultaneous estimation of these drugs in tablet dosage forms. Confirmation of the applicability of the developed method was validated according to the International Conference on Harmonization (ICH) for the simultaneous determination of emtricitabine, and tenofovir disoproxil fumarate in bulk and in tablet dosage forms.

MATERIAL AND METHODS:

Chemicals and Reagents:

Pharmaceutical grade of Emitricitabine and tenofovir disproxil fumarate were kindly supplied as gift sample by Lupin. Distilled water was used as solvent. The marketed formulations were purchased from local market were obtained from retail pharmacist.

Instruments:

UV-Visible Spectrophotometer-ELICO SL 164 with cuvette cells of one cm light path were used for the measurement of absorbance. Electronic Balance 200 D was used for weighing the samples. Class 'A' volumetric glassware were used. UltraSonicator- wensar was used for sonication purpose.

Procedure:

UV Spectrometric methods for determination of Emitricitabine and tenofovir Disproxil fumarate:

Selection of Solvent:

The solvent was selected by determining the solubility of Emitricitabine and tenofovir Disproxil fumarate in various solvents namely Distilled water, Hydrochloric Acid, Sodium Hydroxide Solution, Methanol. Finally, Distilled water was chosen as the solvent for Emitricitabine and tenofovir Disproxil fumarate depending on absorption at its analytical wavelength.

Preparation of standard stock solution:

An accurately weighed quantity of about 20mg Emitricitabine and tenofovir Disproxil fumarate was taken in a 100 mL volumetric flask and was dissolved in Distilled wtaer with sonication. The volume was made upto mark with Distilled water to get the concentration of 200ppm.

Preparation of Working Standard Solution of Emitricitabine and tenofovir Disproxil fumarate:

The aliquot portion of standard stock solution of Emitricitabine and tenofovir Disproxil fumarate was diluted appropriately with Distilled water obtaining concentration 10 µg/mL. Solution was taken in 1 cm cell and scanned in the range 200 nm to 400 nm and spectrum was recorder as showed in Fig.

Application of the proposed procedures for the simultaneous determination of EMT and TEN in laboratory prepared mixtures Mixtures of the two drugs were prepared by diluting 1.0 mL of 200ppm stock solution of EMT and TEN with distilled water upto 10 mL. The concentrations of both EMT and TEN were determined by measuring the absorbance of the prepared mixtures at 280 nm and 260 nm. From these absorbance values, the concentrations of EMT and TEN were determined using Simultaneous equation method.

Application of the proposed procedure for the determination of dosage form Twenty tablets were weighed accurately and average weight was calculated. The tablets were triturated to a fine powder. An accurately weighed quantity of tablet powder equivalent to 200 mg of EMT was weighed and transferred into 100 mL volumetric flask and added a minimum quantity of distilled water to dissolve the substance. The solution was sonicated for 15 minutes and and made up to the volume with the same. The solution was filtered through Whatmann filter paper No. 41. From the clear solution, further dilutions were made by diluting 1.0 mL to 10 mL with distilled water to obtain 20 µg/ mL solution of EMT and which also contains 30 µg/ mL of TEN theoretically. The samples containing two absorbing species EMT and TEN (X & Y) each of which absorbs at the λ max of the

other. So the absorbance of each drugs were measured at both wavelengths λ1 & λ2 respectively. Both the drugs were determined by simultaneous method (Vierodt's method). The absorptivity of EMT (X) at λ1 (281) and λ2 (260) is ax1 and ax2, respectively. The absorptivity of TEN (Y) at λ1 (281) and λ2 (260) is ay1 and ay2, respectively. The absorptivity of each solution was calculated by using the following formula:

Absorptivity=Absorbance/concentration (gm/100 ml. The absorbance of the sample (formulation) at λ_1 (281) and λ_2 (260) is A_1 and A_2 respectively. The total absorbance of the mixture is equal to the sum of individual absorbance of X and Y.

$A_1 = ax_1bCx + ay_1bCy$

$A_2 = ax_2bCx + ay_2bCy$

$Cx= A_2ay_1-A_1ay_2/ ax_2 ay_1- ax_1 ay_2$

$Cy=A_1ax_2-A_2ax_1/ ax_2 ay_1- ax_1 ay_2$

Cx – concentration of EMT

Cy – concentration of TEN

Method Validation:

Specificity

Specificity was studied by measuring the absorbance of EMT, TDF individually at 280 nm and 260 nm against the blank and comparing the absorbance of drugs solutions to the blank. No interference was observed.

Linearity and range:

Five aliquots of each drug solutions were taken from standard stock solution and transferred to 10ml volumetric flask to get a final concentration of 5, 10, 15, 20, 25 and 30 µg/ml of Emitrictabine and 5, 10, 15, 20, 25 and 30 µg/ml µg/ml of Tenofovir Disoproxil Fumarate and the volume was made upto the mark with the distilled water and each flask content was measured to determine the absorbance at the selected wavelength. For simultaneous equation method the absorbance of all standard solutions were measured at 260nm and 280nm, the calibration curves of absorbance vs. concentration was plotted and correlation coefficient and regression line equations for both EMT and TDF were determined. Straight line equations were obtained from these calibration curves. The linear regression equation of Emitricitabine y=0.0351x-0.0671 (R^2 = 0.9972) and Tenofovir Disoproxil Fumarate was y = 0.0252x +0.0140 R^2 = 0.9999. The result were reported in Table No 1& 2.

Precision

In intraday study concentration of two drugs were calculated on the same day after 6 hour. In inter day study the concentration of drug contents were calculated on two different days. In both intra and inter-day precision study for the methods % RSD were calculated. The result were reported in Table No 3,4,6.

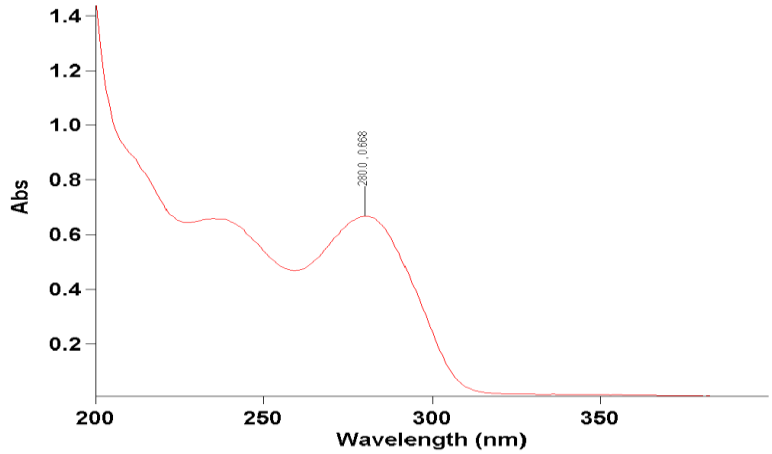
Limit of Detection and Limit of Quantification:

ICH guideline describes several approaches to determine the detection and quantification limits. These include visual evaluation, signal-to-noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and were calculated according to the 3.3 × (SD/Slope) and 10 × (SD/Slope) criteria, respectively; where SD is the standard deviation of y-intercept of regression line and S is the slope of the calibration curve.

Accuracy: The accuracy of the proposed method was confirmed by Standard addition method. To the pre analyzed formulation a known amount of raw material was added and it can be analyzed by proposed methods. Recovery studies were carried out by at three different levels (50%, 100%, and 150% level). The amount of each drug recovered was calculated. The procedure was repeated for three times for each concentration. The results for recovery analysis are shown in table No 6.

RESULTS AND DISCUSSION:

A reliable Simultaneous Equation method was developed for simultaneous estimation of Emitricitabine and Tenofovir disoproxil Fumarate in bulk and Tablet by UV Spectrophotometry. Beers law was obeyed in concentration range of 5-30 µg/ml for Emitricitabine and 5-30 µg/ml for Tenofovir disoproxil Fumarate at 280 nm and 260 nm.



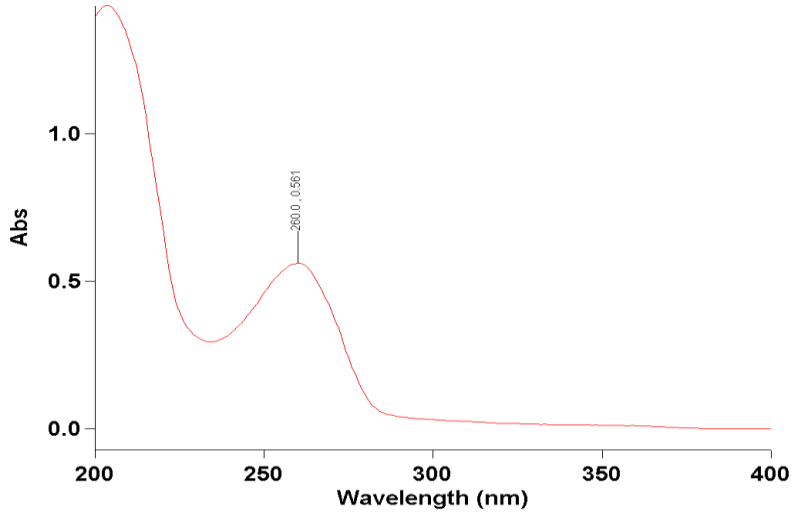


Fig 1& 2: UV Spectrum of Emitricitabine and UV spectrum of Tenofovir Disoproxil Fumarate

Table No.1 --Calibration data forEmitricitabine

S.no	Concentration in ppm	Absorbance
1	5	0.228
2	10	0.446
3	15	0.575
4	20	0.781
5	25	0.938
6	30	1.120

Table No. 2 -Calibration data for Tenofovir Disoproxil Fumarate

S.no	Concentration in ppm	Absorbance
1	5	0.1368
2	10	0.2670
3	15	0.3933
4	20	0.5200
5	25	0.6396
6	30	0.7682

Table No 3: Intraday Precision

S.no.	Absorbance		Concentration of EMI	Concentration of THF	% assay of EMI	% assay of THF
	280nm	260nm				
1	1.2798	0.9265	20.04	30.29	100.81	100.95
2	1.2742	0.9236	19.98	30.12	99.9	100.4
3	1.2721	0.9245	20.01	30	100.07	99.99
4	1.2658	0.9209	19.94	29.82	99.71	99.39
5	1.2788	0.9289	19.87	30.42	100.53	100.56
6	1.2769	0.9256	20.02	30.18	100.12	100.61
				Mean	100.08	100.32
				SD	0.28	0.55
				% R. S.D	0.28	0.56

Table No 4: Intraday Precision after 6 hr

Sr.No.	Absorbance		Concentration of EMI	Concentration of THF	% assay of EMI	% assay of THF
	280nm	260nm				
1	1.2796	0.9266	20.01	30.28	100.2	100.92
2	1.2740	0.9233	19.97	30.12	99.86	100.4
3	1.2719	0.9248	20.02	29.98	100.12	99.93
4	1.2655	0.9216	19.96	29.78	99.82	99.28
5	1.2785	0.9285	20.1	30.17	100.49	100.56
6	1.2765	0.9254	20.02	30.17	100.1	100.57
				Mean	100.09	100.27
				SD	0.24	0.58
				% R. S.D	0.22	0.58

Table No 5: Intraday Precision 2nd day

S.no.	Absorbance		Concentration of EMI	Concentration of THF	% assay of EMI	% assay of THF
	280nm	260nm				
1	1.28	0.9265	20.06	30.09	100.28	100.31
2	1.2749	0.9235	19.97	30.16	99.87	100.52
3	1.2765	0.9263	20.05	30.14	100.23	100.48
4	1.2628	0.9215	19.97	29.66	99.86	98.88
5	1.2750	0.9242	19.99	30.14	99.97	100.46
6	1.2789	0.9263	20.03	30.25	100.17	100.84
				mean	100.06	100.24
				SD	0.18	0.69
				% RSD	0.18	0.69

Table No 6: Accuracy Data for emitricitabine and tenofovir Disoproxil fumarate in tablet sample

Sr.No.	Level	Absorbance		Concentration of EMI	Concentration of THF	% assay of EMI	% assay of THF
		280nm	260nm				
1	50	1.9105	1.3925	30.17	44.93	100.57	99.84
2	50	1.9201	1.3920	30.11	45.38	100.38	100.85
3	50	1.9195	1.3909	30.09	45.39	100.29	100.86
4	100	2.5421	1.8595	40.33	59.58	100.82	99.3
5	100	2.5462	1.8605	40.34	59.74	100.85	99.57
6	100	2.5470	1.8613	40.36	59.75	100.89	99.59
7	150	3.1802	2.3105	50.02	75.01	100.03	100.02
8	150	3.1981	2.3235	50.3	75.44	100.59	100.58
9	150	3.1897	2.3231	50.32	75.07	100.65	100.09
					Mean	100.56	100.08
					SD	0.29	0.54
					% RSD	0.28	0.54

Table No 7: Validation Data for emitricitabine and tenofovir Disoproxil fumarate

Parameters	Emitricitabine	Tenofovir disoproxil Fumarate
Concentration range (µg/ml)	5-30 µg/ml	5-30 µg/ml
Regression equation	f(x)=0.0351x+0.0671	f(x)=0.0252x+0.0140
Slope	0.035	0.025
Intercept	0.067	0.014
Correlation Coefficient	0.9972	0.9999
Accuracy (% recovery, n=3)	100.03%-100.89%	99.3%-100.86 %
Precision (%RSD, n=6)	0.28	0.55
Intraday after 6hr (%RSD, n=6)	0.22	0.58
Interday (%RSD, n=6)	0.18	0.69
LOD (µg/ml)	0.95 µg/ml	1.43 µg/ml
LOQ (µg/ml)	2.89 µg/ml	4.33 µg/ml

The correlation coefficient Emitricitabine and Tenofovir Disoproxil fumarate was found to be R2= 0.9972 and 0.9999. The mean % recoveries were found to be in the range of % 100.03-100.89% and 99.3-100.86 % Emitricitabine and Tenofovir Disoproxil Fumarate respectively. Precision (% RSD) of Emitricitabine and Tenofovir Disoproxil Fumarate was found to be within the limit. The LOD and LOQ were 0.95 µg/ml and 2.89 µg/ml Emitricitabine and 1.43 µg/ml and 4.33 µg/ml of Tenofovir Disoproxil Fumarate, respectively. The proposed method was precise, accurate and reproducible and acceptable recovery of the analytes, which can be applied for the analysis of Emitricitabine and Tenofovir disoproxil Fumarate in bulk and Tablet. The result were reported in Table No 7.

CONCLUSION

A Simple, Specific, accurate, precise simultaneous equation method by UV has been developed which can be used accurately for quantitative estimation of emitricitabine and tenofovir disoproxil fumarate for routine analysis of drugs in combined dosage form. Method was validated as per ICH Q2 (R2) as it can be used by analytical department.

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REFERENCES:

1. International Conference On Harmonisation Guideline On Validation Of Analytical Procedures: Text And Methodology; Q2 (R1), 2005
2. A.H.Becket And J.B.Stenlak, Practical Pharmaceutical Chemistry, 4th Edition Part- 2,284-285.
3. S.Budhavari, The Merck Index (monograph#3521), 14, 598, (2006).
4. S.Budhavari, The Merck Index (monograph#3565), 14, 606, (2006).
5. Ashutoshkar, Pharmaceutical Drug Analysis 2 Nd Edition. New Age Publisher 2005 P.No:16-17
6. Deepthi Komaroju, G. Nagarjuna Reddy, K. Dhanalakshmi "Method Development And Validation For Simultaneous Estimation Of Emtricitabine And Tenofovir Disoproxil Fumarate In Pure And

Tablet Dosage Form By Using RP-HPLC" International Journal Of Pharma Research & Review, Oct 2013; 2(10):1-11.

7. Swapnil A Ghorpade, Monali S. Sali, Atul H. Kategaonkar, Dhaval M. Patel, Vishnu P Choudhari, Bhanudas S Kuchekar "Simultaneous Determination Of Emtricitabine And Tenofovir By Area Under Curve And Dual Wavelength Spectrophotometric Method" J. Chil. Chem. Soc., 55, Nº 1 (2010).
8. Bala Rami Reddy.Yenumula, Mutta Reddy.Singampalli, "Simultaneous Estimation Of Emtricitabine And Tenofovir Disoproxil Fumarate In Tablet Dosage Form By Reverse Phase High-Performance Liquid Chromatography"Soj Chromatograph Sci 1(1): 6.
9. Maithilee Joshi, A. P. Nikalje, M. Shahed, M. Dehghan "Hptlc Method For The Simultaneous Estimation Of Emtricitabine And Tenofovir In Tablet Dosage Form" Indian J. Pharm. Sci., 2009, 71 (1): 95-97.