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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF TENOFOVIR ALAFENAMIDE FUMARATE AND EMTRICITABINE IN DRUG PRODUCT

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ABSTRACT
The aim of the method was to develop and validate a rapid, sensitive and accurate method for simultaneous estimation of Emtricitabine and Tenofovir alafenamide fumarate in drug product by liquid chromatography. The chromatographic separation was achieved on Phenyl column (Eclipse XDB-Phenyl 250*4.6, 5um) at ambient temperature. The separation achieved employing a mobile phase consists of 0.1%v/v Trifluoroacetic acid in water: Acetonitrile: Methanol (30:10:60). The flow rate was 1.2ml/ minute and ultraviolet detector at 260nm. The average retention time for Emtricitabine and Tenofovir alafenamide fumarate found to be 2.370 min and 2.897 min. The proposed method was validated for selectivity, precision, linearity, and accuracy. All validation parameters were within the acceptable range. The assay methods were found to be linear from 50.0 – 150.0µg/mL for Emtricitabine and 6.3 –18.8µg/mL of Tenofovir alafenamide fumarate.

KEYWORDS: Emtricitabine, Tenofovir alafenamide fumarate, Isocratic, HPLC, Phenyl, Trifluoroacetic acid, Acetonitrile, Methanol, and validation.

1. INTRODUCTION

Emtricitabine

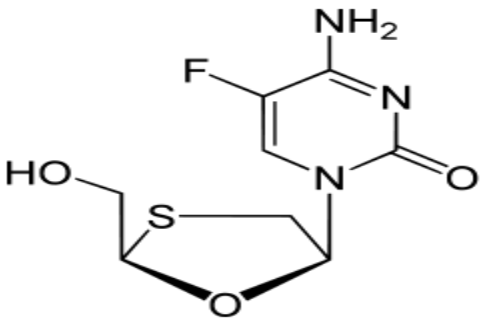


Fig. 1: Chemical structure: Emtricitabine.

Emtricitabine (**2'-deoxy-5-fluoro-3'thiacytidine**, **FTC**), with trade name **Emtriva** (formerly **Coviracil**), is a nucleoside reverse transcriptase inhibitor (NRTI) for the prevention and treatment of HIV infection in adults and children.

Emtricitabine is chemically designated as 4-amino-5-fluoro-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one. Its molecular formula is $C_8H_{10}FN_3O_3S$, and its molecular weight is 247.248 g/mol.

Tenofovir alafenamide fumarate

Tenofovir alafenamide (INN/USAN; trade name **Vemlidy**) is a nucleotide reverse transcriptase inhibitor and a prodrug of tenofovir. It was developed by Gilead Sciences for use in the treatment of HIV infection and chronic hepatitis B, and is applied in the form of tenofovir alafenamide fumarate (TAF). Closely related to the commonly used reverse-transcriptase inhibitor tenofovir disoproxil fumarate (TDF), TAF has greater antiviral activity and better distribution into lymphoid tissues than that agent.

Tenofovir alafenamide fumarate is chemically designated as Isopropyl (E)-but-2-enedioic acid;propan-2-yl (2S)-2-[[[(2R)-1-(6-aminopurin-9-yl)propan-2-yl]oxymethyl-phenoxyphosphoryl]amino]propanoate. Its molecular formula is C46H62N12O14P2, and its molecular weight is 1069.02 g/molg/mol.

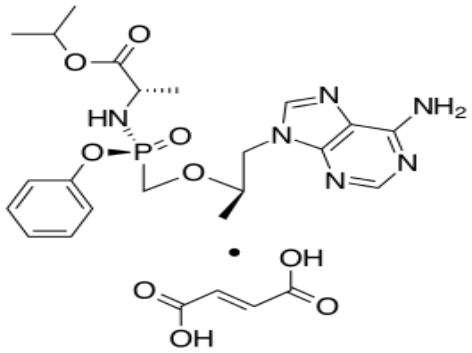


Fig. 2: Chemical structure: Tenofovir alafenamide fumarate.

2. MATERIALS AND METHODS

2.1 Equipments: The chromatographic technique performed on a waters 2695 with 2487 detector and Empower2 software, reversed phase Phenyl column (Eclipse XDB-Phenyl 250*4.6, 5µm) as stationary phase, Ultrasonic cleaner, Scaletech analytical balance and Vacuum micro filtration unit with a 0.45µ membrane filter.

2.2 Materials: Pharmaceutically pure sample of Emtricitabine/Tenofovir alafenamide fumarate were obtained as gift samples from Fortune pharma training institute, Sri Sai Nagar colony, KPHB, Hyderabad, India.

HPLC-grade Methanol and Acetonitrile were obtained from qualigens reagents Pvt Ltd. Trifluoroacetic acid (AR grade) was from sd fine chem.

2.3 Chromatographic conditions The sample separation was achieved on an (Eclipse XDB-Phenyl 250*4.6, 5µm) Phenyl column, aided by mobile phase mixture of 0.1%v/v Trifluoroacetic acid in water: Acetonitrile: Methanol (30:10:60). The flow rate was 1.2 ml/ minute and ultraviolet detector at 260nm that was filtered and degassed prior to use, Injection volume is 10 µl and ambient temperatures.

Preparation of mobile phase

Buffer Preparation: Taken accurately 1ml of Trifluoroacetic acid in 1000mL of water.

Mobile phase: Then added 30 volumes of buffer, 10 volumes of Acetonitrile and 60 volumes of Methanol mixed well and sonicated for 5 min.

Diluents: Water: Methanol: 50:50 v\v

2.4 Preparation of solutions

2.4.1 Standard solution: 40 mg of pure Emtricitabine and 5 mg of Tenofovir alafenamide fumarate were weighed and transferred to 10 ml of volumetric flask and dissolved in the diluent. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution. From the above solution 0.25ml of the solution is pipetted out into a 10 ml volumetric flask and volume was made up to mark with water to give a solution containing 100.0µg/ml of Emtricitabine and 12.5µg/ml Tenofovir alafenamide fumarate.

2.4.2 Preparation of sample solution: Accurately weighed twenty tablets were ground to obtain fine powder equivalent to 40mg of Emtricitabine and 5mg of Tenofovir alafenamide fumarate sample and transferred to 10 ml of volumetric flask and dissolved in the diluent. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution. From the above solution, 0.25 ml of solution is pipetted out into a 10 ml volumetric flask and volume was made up to mark with diluents to give a solution containing 100.0µg/ml of Emtricitabine and 12.5µg/ml Tenofovir alafenamide fumarate.

2.5 Method validation

2.5.1. System suitability

The typical values for evaluating system suitability of a chromatographic procedure are RSD <2%, tailing factor <1.5 and theoretical plates >3000. The retention time, peak area, theoretical plates and tailing factor were evaluated for system.

2.5.2. Linearity

Linearity was studied by analyzing five standard solutions covering the range of 50.0 -150.0µg/ml for Emtricitabine and 6.3 -18.8µg/ml Tenofovir alafenamide fumarate. From the primary stock solution 0.125ml,0.187ml,0.25ml,0.312ml,0.375 ml of aliquots are pipetted into 10 ml volumetric flasks and made up to the mark with the water to give a concentrations of 50.0 µg /mL, 75.0µg/mL, 100.0µg/mL,125.0µg/mL and 150.0µg/mL of Emtricitabine and 6.3g/mL, 9.4µg/mL, 12.5µg/mL, 15.6µg/mL and 18.8 µg/mL of Tenofovir alafenamide fumarate.

A calibration curve with concentration verses peak areas was plotted by injecting the above-prepared solutions and the obtained data were subjected to regression analysis using the least squares method.

2.5.3. Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve.

$$LOD = 3.3\ \delta/S$$

$$LOQ = 10\ \delta/S$$

Where,

δ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

2.5.4. Method precision

The precision of the method was checked by repeated preparation(n=6) of 100.0µg/ml of Emtricitabine and 12.5µg/ml Tenofovir alafenamide fumarate without changing the parameter of the proposed chromatographic

method. And measured the peak areas and retention times.

2.5.5. Accuracy

The accuracy of the method was determined by calculating the recoveries of Emtricitabine and Tenofovir alafenamide fumarate by analyzing solutions containing approximately 50%, 100% and 150% of the working strength of Emtricitabine and Tenofovir alafenamide fumarate.

2.5.6. Robustness

Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied ±2nm and flow rate was varied ±0.2 ml/min.

3. RESULTS AND DISCUSSIONS

Determination of Working Wavelength (λ max): 10 mg of the Emtricitabine and Tenofovir alafenamide fumarate standard drug is taken in a 10 ml volumetric flask and dissolved in diluent and volume made up to the mark, from this solution 0.1ml is pipette into 10 ml volumetric flask and made up to the mark with the Water to give a concentration of 10 µg/ml. The above-prepared solution is scanned in UV between 200-400 nm using Water as blank. The λmax was found to be 260nm.

After several initial trails with mixtures of methanol, water, Acetonitrile and buffer in various combinations and proportions, a trail with a mobile phase mixture of 0.1%v/v Trifluoroacetic acid in water: Acetonitrile: Methanol (30:10:60). At flow rate was 1.2mL/ minute brought sharp peaks. The chromatogram was shown in Fig 3.

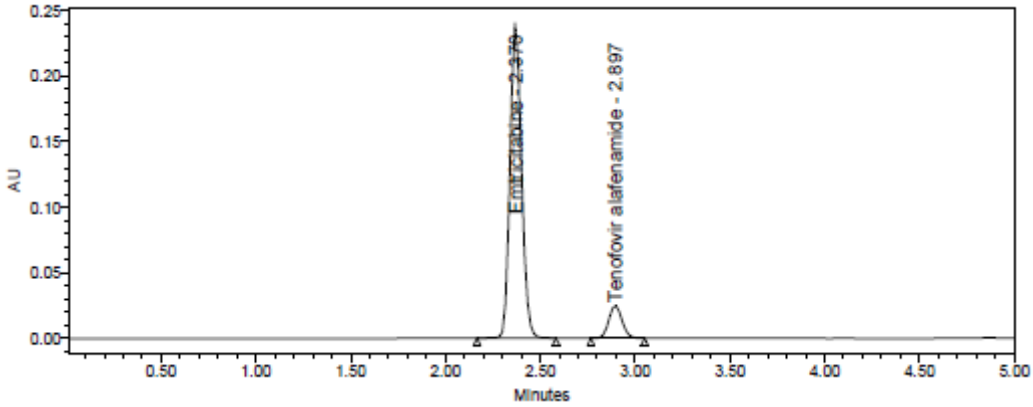


Fig. 3: Chromatogram of Emtricitabine and Tenofovir alafenamide fumarate.

System suitability

The system suitability of the method was checked by repeated preparations for Tenofovir alafenamide fumarate and Emtricitabine. The typical values for evaluating system suitability of a chromatographic procedure are RSD <2%, tailing factor <1.5 and theoretical plates >3000. The retention time, peak area, theoretical plates and tailing factor were evaluated for the system, System suitability data of Tenofovir alafenamide fumarate and Emtricitabine are shown in Table 1.

Table 1: System suitability data of Emtricitabine and Tenofovir alafenamide fumarate.

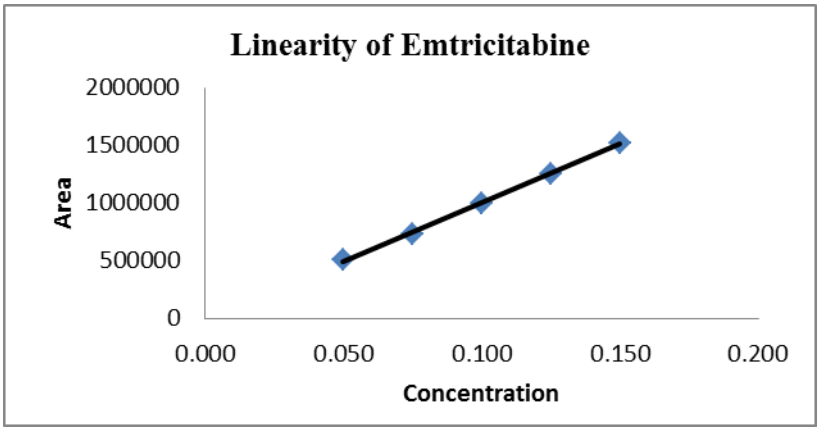
Parameter	Emtricitabine	Tenofovir alafenamide fumarate	Acceptance criteria
Retention time	2.369	2.897	+-10
Theoretical plates	7227	7980	>3000
Tailing factor	1.11	1.06	<1.50
% RSD	0.14	0.20	<2.00

Linearity

Linearity was studied by analyzing five standard solutions covering the range of 50.0 -150.0µg/ml for Emtricitabine and 6.3 -18.8µg/ml Tenofovir alafenamide fumarate. From the primary stock solution 0.125ml,0.187ml,0.25ml,0.312ml,0.375 ml of aliquots are pipetted into 10 ml volumetric flasks and made up to the mark with the water to give a concentrations of 50.0 µg /mL, 75.0µg/mL, 100.0µg/mL, 125.0µg/mL and 150.0µg/mL of Emtricitabine and 6.3g/mL, 9.4µg/mL, 12.5µg/mL , 15.6µg/mL and 18.8 µg/mL of Tenofovir alafenamide fumarate in Table 2 and Table 3.

A linear relationship between peak areas versus concentrations was observed for Emtricitabine and Tenofovir alafenamide fumarate in the range of 50% to 150% of nominal concentration. The correlation coefficient was 0.9996 and 0.9993 for Emtricitabine and Tenofovir alafenamide fumarate.

A



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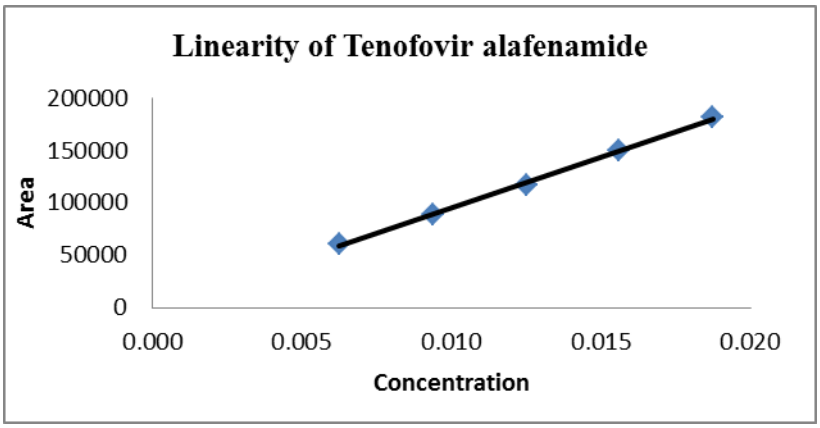

Fig. 4 Calibration curve: (A) Emtricitabine: (B) Tenofovir alafenamide fumarate.

Table 2: Linearity data of Emtricitabine.

Level	Concentration (mg/mL)	Peak area
50%	0.15	504197
75%	0.075	728128
100%	0.100	995636
125%	0.125	1253650
150%	0.150	1514911
Correlation		0.9996

Table 3: Linearity data of Tenofovir alafenamide fumarate.

Level	Concentration (mg/mL)	Peak area
50%	0.006	60649
75%	0.009	88129
100%	0.013	116620
125%	0.016	149852
150%	0.019	181028
Correlation		0.9993

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (1) and (2), respectively.

LOD = 3.3 σ /S ……. (1)

LOQ =10 σ /S ……. (2)

Where,

σ = the standard deviation of the response (STEYX)

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Table 4: LOD and LOQ values Calculated from the calibration curve.

	Emtricitabine mg	Tenofovir alafenamide fumarate mg
LOD	0.004	0.001
LOQ	0.013	0.002

Method precision (repeatability)

The precision of the method was checked by repeated preparation(n=6) of 50.0µg/ml of Emtricitabine and 12.5µg/ml Tenofovir alafenamide fumarate without changing the parameter of the proposed chromatographic method. And measure the peak areas and retention times. The precision of the method (% RSD) was found to be <1% showing good repeatability. The values of percentage RSD for Emtricitabine and Tenofovir alafenamide fumarate are shown in Table 5 and Table 6.

Table 5: Summary of peak areas for method precision of Emtricitabine.

	Retention time	Peak area	% Assay
1	2.370	1002849	99.8
2	2.369	1000291	99.5
3	2.37	997682	98.9
4	2.369	999434	99.0
5	2.369	1002599	98.6
6	2.369	996938	99.0
Mean	2.369	999966	99.1
%RSD	**0.02**	**0.25**	**0.45**

Table 6: Summary of peak areas for method precision of Tenofovir alafenamide fumarate.

Sample No	Retention time	Peak area	% Assay
1	2.902	118483	99.7
2	2.901	119223	98.7
3	2.902	118552	99.4
4	2.900	117598	99.0
5	2.901	118428	99.1
6	2.900	118197	99.7
Mean	2.901	118414	99.3
%RSD	**0.03**	**0.45**	**0.40**

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of Emtricitabine and Tenofovir alafenamide fumarate by analyzing solutions containing approximately 50%, 100% and 150% of the working strength of Emtricitabine and Tenofovir alafenamide fumarate. The percentage recovery results obtained are listed in Table 7 & 8.

Table 7: Recovery data of Emtricitabine.

LEVEL	S.NO	%Recovery of Emtricitabine	Average
50	1	99.4	99.4%
	2	99.7	
	3	98.9	
100	1	998	99.4%
	2	99.5	
	3	98.9	
150	1	99.1	99.8%
	2	98.5	
	3	98.7	

Table 8: Recovery data of Tenofovir alafenamide fumarate.

LEVEL	S.NO	%Recovery of Tenofovir alafenamide fumarate	Average
50	1	99.5	99.8%
	2	99.8	
	3	100.3	
100	1	99.7	99.3%
	2	98.7	
	3	99.4	
150	1	99.5	99.7%
	2	99.8	
	3	99.9	

Robustness: Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied ±2nm and flow rate was varied ±0.2 ml/min. The results were shown in (Table 9&10) The results of Robustness of the present method had shown that changes are not significant was found to be the method is Robust.

Table 9: Results of Emtricitabine.

parameter	Rt of Emtricitabine	Theoretical plates	Asymmetry
Decreased flow rate (1.1ml/min)	2.584	7674	1.14
Increased flow rate (1.3ml/min)	2.190	6186	1.13
Wave Length 258nm	2.370	7210	1.11
262nm	2.369	7277	1.10

Table 10: Results of Tenofovir alafenamide fumarate.

parameter	Rt of Tenofovir alafenamide fumarate	Theoretical plates	Asymmetry
Decreased flow rate (1.1ml/min)	3.170	8438	1.09
Increased flow rate (1.3ml/min)	2.685	7321	1.07
Wave Length 258nm	2.897	8048	1.06
262nm	2.898	7936	1.06

Ruggedness: The ruggedness of the method was studied by analyzing the sample and standard preparations by two analysts. The results were shown in Table 11&12. The %RSD assay values between two analysts were calculated, this indicates the method was rugged.

Table 11: Ruggedness data for Emtricitabine.

		%Assay	%RSD
Analyst-1	**EMTRICITABINE**	99.8	0.21%
Analyst-2		99.5	

Table 12: Ruggedness data for Tenofovir alafenamide fumarate.

		%Assay	%RSD
Analyst-1	**TENOFOVIR ALAFENAMIDE FUMARATE**	99.7	0.71%
Analyst-2		98.7	

CONCLUSION

From the above experimental results it was concluded that, newly developed method for the simultaneous estimation of EMTRICITABINE and TENOFOVIR ALAFENAMIDE FUMARATE was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost-effective and it can be effectively applied for routine analysis in research institutions, quality control department in pharmaceutical industries, approved testing laboratories.

REFERENCES

1. ICH, Q2A validation of analytical procedure: Methodology International Conference on Harmonization, Geneva, October 1994.
2. ICH, Q2B Validation of analytical procedure: Methodology International Conference on Harmonization, Geneva, March 1996.
3. http://www.ich.org/
4. https://www.drugbank.ca/drugs/DB00879
5. https://www.drugbank.ca/drugs/DB09299
6. https://en.wikipedia.org/wiki/Emtricitabine
7. https://en.wikipedia.org/wiki/Tenofovir_alafenamide
8. Development and Validation of Stability-Indicating HPLC-DAD Method for Simultaneous Determination of Emtricitabine, and Tenofovir in their Tablet Dosage Forms Mallikarjuna rao Nagasarapu1 * and Gowri Sankar Dannana2.
9. A Validated Stability-Indicating RP-HPLC Method for the Simultaneous Determination of Tenofovir, Emtricitabine, and a Efavirenz and Statistical Approach to Determine the Effect of Variables Prashant S. Devrukhakar, 1,2 Roshan Borkar,2 Nalini Shastri,1 and K. V. Surendranath2.
10. A Validated Stability-Indicating RP-HPLC Method for the Simultaneous Determination of Tenofovir, Emtricitabine, and a Efavirenz and Statistical Approach to Determine the Effect of Variables.
11. Simultaneous Estimation of Tenofovir and Emtricitabine in Human Plasma Using HPLC after Protein Precipitation Extraction.