Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 12 [12] November 2023 : 291-301

Online ISSN 2277-1808
Journal's URL: http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Simultaneous Estimation of Emtricitabine, Tenofovir Alafenamide, and Efavirenz via a State-of-the-Art RP-UPLC Method in Drug Formulation

Kanchan Sunil Nautiyal[1], Naresh N[2], Nagaraju Pappula[2*], Balaji Kodali[3] and P. Jitendra Kumar[4]
[1]Gitam University, Goregaon West, Mumbai, Maharashtra - 400104, India
[2]Hindu College of Pharmacy, Amaravathi Road, Guntur – 522002, A.P., India
[3]Chaitanya Techno School opposite, Nujvid road, Bapulapadu Mandal, Krishna District – 521105, A.P., India
[4]Vignan Pharmacy College, Vadlamudi, Guntur, A.P., India
*Corresponding Author E-mail: pappulanagaraju@gmail.com

ABSTRACT
Emtricitabine, tenofovir alafenamide, and efavirenz in their tablet format were all simultaneously quantified using an accurate and dependable RP-UPLC method that was developed and validated. Waters UPLC with an HSS C18 column (100 x 3 mm, 1.7 µm) was used for the separation and estimation. The analytes were successfully eluted at a flow rate of 0.3 mL/min with detection at a wavelength of 265 nm thanks to the mobile phase, which was composed of acetonitrile and phosphate buffer in a 50:50% v/v ratio. The concentration ranges for emtricitabine, tenofovir alafenamide, and efavirenz were 50-300 µg/mL, 2.5-15 µg/mL, and 150-900 µg/mL, respectively, where the linear detector response was seen. The results indicated that the limits of detection and quantification for efavirenz, tenofovir alafenamide, and emtricitabine were 0.91 and 2.6 µg/mL, 0.03 and 0.10, and 0.6 and 1.82 µg/mL, respectively, within acceptable bounds. Following ICH guidelines, the method's validation revealed that all validation parameters fell within the allowed ranges.
Keywords: *RP-UPLC, Emtricitabine, Tenofovir Alafenamide, Efavirenz*

Received 06.10.2022 Revised 24.10.2023 Accepted 25.11.2023

INTRODUCTION

Emtricitabine 1 has the chemical formula 4-amino-5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl].Pyrimidine-2-one belongs to the class of nucleoside reverse transcriptase inhibitors, which includes anti-HIV-1 drugs. This powder is white to off-white in color, dissolves in water at a rate of around 112 mg/mL at 25°C, and has the chemical formula C8H10FN3O3S with a molecular weight of 247.244 g/mol [1]. Figure 1 shows that emtricitabine has a pKa of 2.65 and a log P of -0.43. Tenofovir alafenamide fumarate2 (TAF) is a novel ester prodrug that inhibits nucleotide reverse transcriptase and is an antiviral medication. Its chemical name is propan-2-yl (2S).C21H29N6O5P is the chemical formula for -2-[[[(2R)-1-(6-aminopurin-9-yl)propan-2-yl]oxymethyl-phenoxyphosphoryl]amino]propanoate. It is a solid powder that dissolves in water at a rate of 4.86 mg/mL [2]. The log P for this medication is 1.6 and its pKa is 3.96 (figure 2).Efavirenz3 is (4S), chemically speaking.-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-1H-3,1-benzoxazin-2-one is one of the medications of the HIV-1Non-Nucleoside Analog Reverse Transcriptase Inhibitor family that are used to treat HIV. Its chemical formula is C14H9ClF3NO2, and its weight is 315.68 g/mol. It is a white to slightly pink crystalline powder that dissolves in water at a rate of around 0.093 mg/L at 25 °C. The log P for this medication is 4.6, and its pKa is 10.2, 12.52 (figure-3).

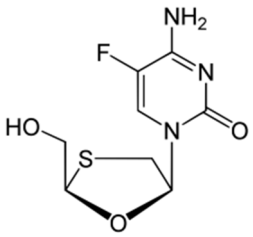

Figure 1: The emtricitabine structure

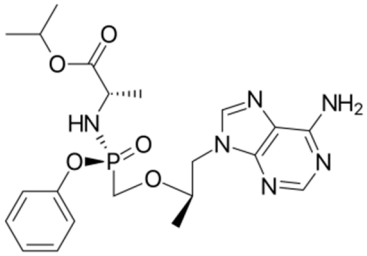

Figure 2: Structure of tenofovir alafenamide

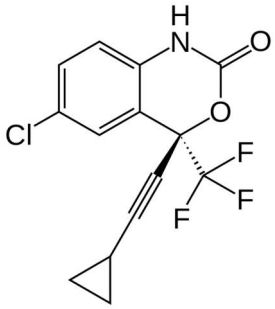

Figure 3: Structure of efavirenz

Emtricitabine, tenofovir alafenamide, and efavirenz alone as well as in combination with other medications have all been determined using a variety of analytical techniques, including spectroscopy [4] and chromatography [5-37]. As per ICH guidelines [38], the author has attempted to develop and validate a simple, fast, precise, and accurate UPLC method for the simultaneous determination of emtricitabine, tenofovir alafenamide, and efavirenz in combined tablet dosage form, since there is currently no available UPLC method for this purpose.

MATERIAL AND METHODS

EXPERIMENTAL:

Chemicals and reagents:

M/s. Mylan Labs Pvt. Ltd., Hyderabad, India provided the reference samples of emtricitabine, tenofovir alafenamide, and efavirenz. M/s. Rankem Chemicals Ltd., in Mumbai, India, provided analytical quality dihydrogen orthophosphate, orthophosphoric acid, and UPLC grade methanol and acetonitrile. During the course of the study, Milli-Q water sourced from the Milli-Q water purification system (Millipore, Merck KGaA, Darmstadt, Germany) and filtered through a 0.22 µ filter was employed.

Instrumentation:

The underwater UPLC employed the Empower 2695 separation module, auto-sampler, and PDA detector. Every spectral measurement was performed using a LAB INDIA Ultraviolet (UV)-visible spectrophotometer. Af coset ER-200A was utilized for the weight measurement, and Adwa -AD 1020 pH meter was used to correct the pH.

Chromatographic conditions:

The study utilized an HSS C18 column (100 x 3 mm, 1.7 µm). Gradient separation was achieved by combining 500 milliliters of phosphate buffer with 500 milliliters of acetonitrile, followed by degassing in an ultrasonic water bath for five minutes. The resulting mixture was filtered using a 0.45 µm filter under vacuum filtration. The mobile phase flow rate was set at 0.3 milliliters per minute. Detection was

monitored at 265 nm, and the column temperature was maintained at a constant 25°C.

Phosphate buffer preparation: To prepare 0.01 M potassium dihydrogen orthophosphate solution, 1.36 grams of potassium dihydrogen orthophosphate were accurately weighed and transferred to a 1000 milliliter volumetric flask. Around 900 milliliters of milli-Q water were added, and the mixture underwent sonication to eliminate any residual gas. Subsequently, one milliliter of triethylamine was introduced, and the pH was set to 4.8 by the addition of a diluted orthophosphoric acid solution.

Preparedness of the mobile phase:

ACN and phosphate buffer are taken at a 50:50 ratio.

Preparing the Diluent: A 50:50 mixture of water and acetonitrile is used.

Making stock solutions:

Accurate amounts of 50 mg emtricitabine, 2.5 mg tenofovir alafenamide, and 150 mg efavirenz were precisely weighed and placed into a 25 mL clean and dry volumetric flask. Subsequently, 10 mL of diluent was added, followed by sonication for ten minutes, and the remaining volume was filled with diluent. For the preparation of solutions at concentrations of 200 µg/mL, 10 µg/mL, and 600 µg/mL, 1 mL of the aforementioned stock solution was pipetted into a 10 mL volumetric flask, and diluent was added to complete the volume.

Setting up a sample solution:

The commercial sample was measured into twenty tablets and then finely powdered. A 100 milliliter volumetric flask holding 70 milliliters of the diluent was filled with a precisely weighed part of the powdered sample, which was equal to the weight of one tablet (200 mg of emtricitabine, 10 mg of tenofovir alafenamide, and 600 mg of efavirenz). To ensure that the medications were completely dissolved, the flask's contents were sonicated for approximately ten minutes. The volume was then adjusted using additional diluent. Subsequently, a 0.45 µ membrane filter was used to filter this combination. For additional analysis, this filtrate was utilized.

Choosing a wavelength by scanning the region between 200 and 400 nm, the UV spectrum was captured. A wavelength of 265 nm was chosen from the UV spectrum since all three medications exhibit good absorption.

RESULTS AND DISCUSSION

Method development:

I order for the three components to be eluted concurrently, the first trails aimed to select appropriate and optimal chromatographic settings. Using different flow rates for each parameter—such as the ideal mobile phase and its ratios, the ideal PH, different columns, and the concentration of the standard solutions—a comprehensive analysis was carried out. Phosphate buffer and acetonitrile mixed 50:50 v/v at a flow rate of 0.3 mL/min yielded the best separation outcomes with respectable peak geometries (Figure 4).

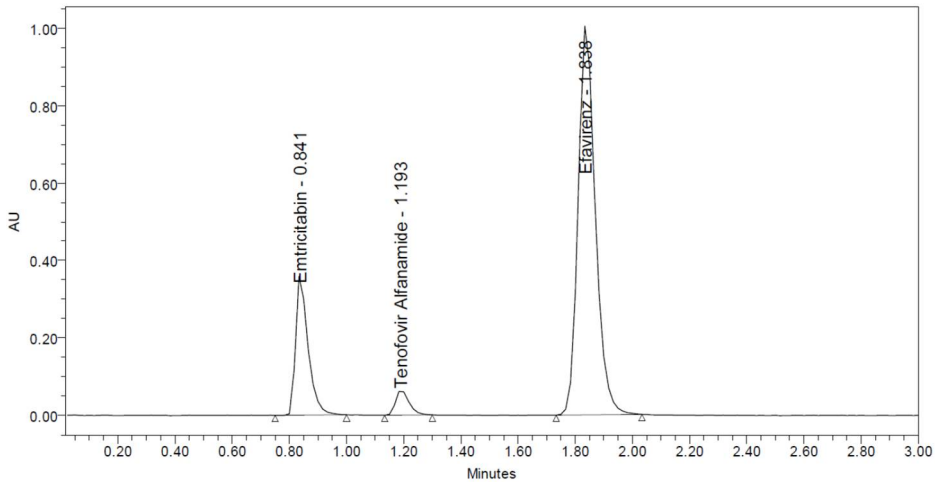

Figure 4: Emtricitabine, tenofovir alafenamide, and efavirenz are separated from the mixed standard solution in this chromatogram.

Method validation:
Emtricitabine, tenofovir alafenamide, and efavirenz were all simultaneously estimated using the developed method, which was then subjected to a thorough evaluation that included system suitability, specificity, linearity, accuracy, precision, robustness, and the determination of the limit of detection (LOD) and limit of quantitation (LOQ).
Accuracy:
Three levels of recovery experiments using the usual addition approach were carried out in order to evaluate the method's accuracy. Based on the results, which are shown in Table 1, the mean percentage recoveries for efavirenz, tenofovir alafenamide, and emtricitabine are 99.10%, 99.80%, and 99.18%, respectively.

Table 1: Emtricitabine, Tenofovir Alafenamide, and Efavirenz Recovery Experiments' Outcomes

Pre-analysed amount (µg/mL)		Spiked amount (µg/mL)				% recovered		
Emreicitabine	Tenofovir Alafenamide	Efavirenz	Emreicitabine	Tenofovir Alafenamide	Efavirenz	Emreicitabine	Tenofovir Alafenamide	Efavirenz
200	10	600	100	5	300	99.43	99.07	99.16
200	10	600	100	5	300	99.25	99.26	98.39
200	10	600	100	5	300	99.06	99.60	100.18
200	10	600	200	10	600	98.13	100.38	98.59
200	10	600	200	10	600	99.53	100.33	99.04
200	10	600	200	10	600	99.30	99.34	99.60
200	10	600	300	15	900	99.01	100.69	98.44
200	10	600	300	15	900	99.16	99.78	9936
200	10	600	300	15	900	99.72	99.75	99.13
				MEAN		99.18	99.80	99.10
				SD		0.45	0.56	0.58
				%RSD		**0.46**	**0.56**	**0.58**

Linearity: The linearity investigation demonstrated that emtricitabine exhibited linearity in the concentration range of 50-300 µg/mL, tenofovir alafenamide in the range of 2.5-15 µg/mL, and efavirenz in the range of 150-900 µg/mL. The correlation coefficients (r2) for all three drugs were within the range of 0.998 to 0.999. Detailed results can be found in Table 2 and Figures 5-7.

Table 2: A recapitulation of the outcomes related to the linearity parameters for emtricitabine, tenofovir alafenamide, and efavirenz is provided.

S.No.	Emtricitabine		Tenofovir Alafenamide		Efavirenz	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	50	258775	2.5	46125	150	1049027
2	100	522892	5	95572	300	2114913
3	150	758485	7.5	141395	450	3251994
4	200	1021049	10	194584	600	4242896
5	250	1249545	12.5	238647	750	5391363
6	300	1515417	15	277870	900	6322139

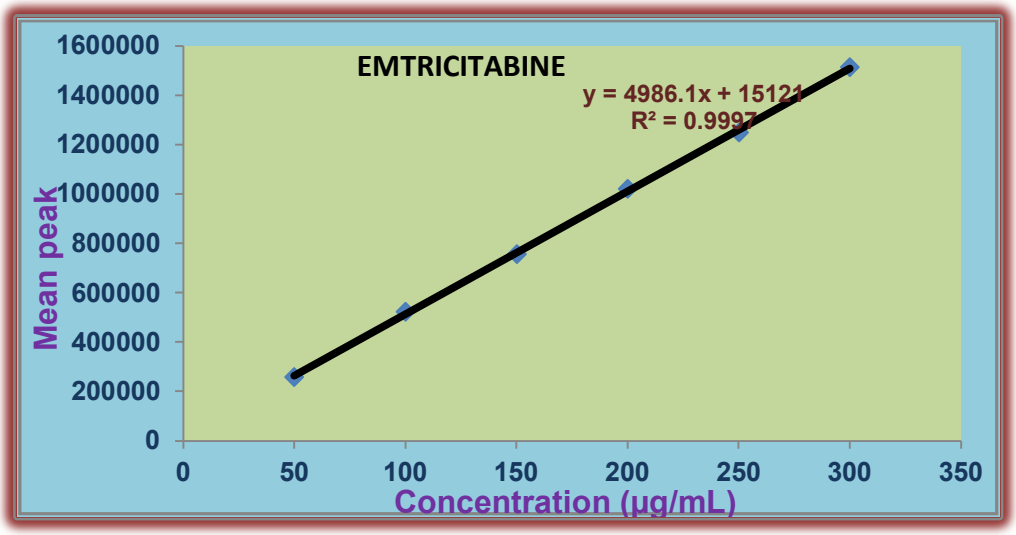

Figure 5: Linearity graph for emtricitabine

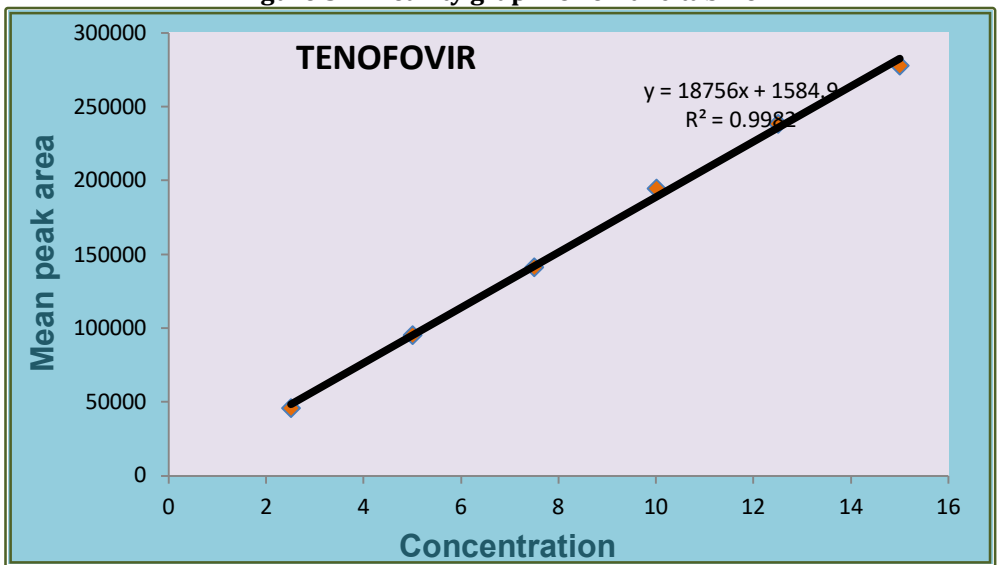

Figure 6: Linearity graph for tenofovir alafenamide

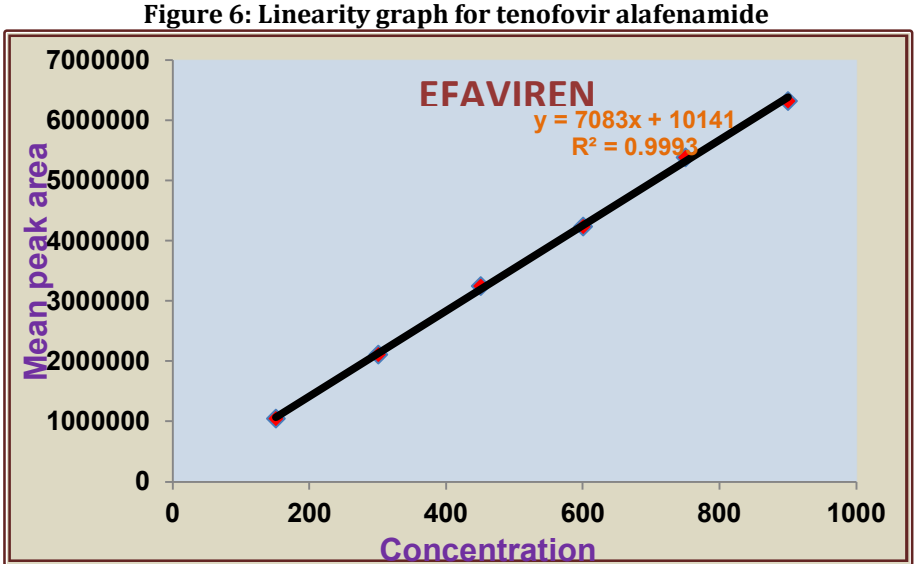

Figure 7: Linearity graph for efavirenz

Precision:
The repeatability of an analytical process under standard operating conditions is known as precision. To assess the system precision—a measure of procedure variability that can be predicted for a certain analyst performing the analysis—six replicate analyses of the identical working solution were carried out. Relative standard deviation percentages for efavirenz, tenofovir alafenamide, and emtricitabine were 0.3, 0.6, and 0.2, respectively. Table 3 presents the findings.

Table 3: Results of repeatability of emtricitabine, tenofovir alafenamide and efavirenz

S. No.	Emtricitabine			Tenofovir Alafenamide			Efavirenz		
	Area	USP Plate Count	USP tailing	Area	USP Plate Count	USP tailing	Area	USP Plate Count	USP tailing
1	1013584	5771	1.41	198688	2810	1.23	4213969	4580	1.18
2	1018931	5727	1.28	198947	2891	1.24	4245455	4417	1.19
3	1015804	5579	1.30	199241	2816	1.25	4208198	4573	1.19
4	1014595	5414	1.40	197814	2683	1.26	4218495	4554	1.17
5	1017275	5831	1.42	197159	2829	1.25	4211589	4628	1.19
6	1017094	5824	1.42	196381	2873	1.23	4216703	4472	1.19
Mean	1016214			198038			4219068		
Std. Dev.	1948.8			1119.3			13433.4		
% RSD	**0.2**			**0.6**			**0.3**		

Intermediate precision:
To confirm the variation in precision, six duplicate injections of the same dilution were examined on two distinct days. Emtricitabine, tenofovir alafenamide, and efavirenz were found to have % RSDs of 0.3,0.4, and0.7, respectively, which are within the acceptable range of ≤2. As such, the procedure can be repeated. This shows how accurate the process is. The outcomes are displayed in Table 4a–c.
Acceptance standards: There shouldn't be a percentage RSD for the area of six standard injection results greater than 2%.

Table 4a: Equilibrium precision results for emtricitabine

S. No.	Average area (n=6)	USP Plate Count	USP Tailing
Day 1	1008061	5597	1.36
Day 2	1008059	5659	1.34
Overall average	1008060	5636	1.36
SD	3424.8		
% RSD	**0.3**		

Table 4b: Intermediate precision results for tenofovir alafenamide

S. No.	Average area (n=6)	USP Plate Count	USP Tailing
Day 1	195169	2729	1.27
Day 2	195165	2983	1.27
Overall average	195167	2998	1.27
SD	775.3		
% RSD	**0.4**		

Table 4c: Findings from efavirenz's intermediate precision

S. No.	Average area (n=6)	USP Plate Count	USP Tailing
Day 1	376548	4079	1.19
Day 2	222582	3927	1.14
Overall average	299565		
SD	2125.8		
% RSD	0.7		

Limit of Quantitation (LOQ) and Limit of quantification (LOQ):
The limit of detection (LOD) refers to a process's capacity to reliably distinguish an analyte's concentration from the background levels. The limit of quantification (LOQ) is the lowest concentration on the standard curve that can be measured with accuracy, precision, and reliability. By applying the formulas 3.3×SD/S and 10×SD/S, respectively, to determine the Limit of Detection (LOD) and Limit of Quantification (LOQ), the ICH requirements were followed. SD stands for standard deviation of the response (Y intercept), and S for slope of the calibration curve. In Table 5, the results of these computations are shown.

Table 5: Limit of Detection and Limit of Quantitation (LOQ) data for emtricitabine, tenofovir alafenamide and efavirenz

Drug name	LOD(µg/mL)	LOQ(µg/mL)
Emtricitabine	0.6	1.82
Tenofovir Alafenamide	0.03	0.10
Efavirenz	0.91	2.6

Robustness:
A method's robustness is its ability to withstand small, intentional changes in its parameters. In order to evaluate robustness, intentional modifications were made to the experimental parameters, such as the mobile phase's composition and flow rate. The assay's % relative standard deviation (R.S.D.) was computed for every variation after a mixed standard solution was injected under each changed circumstance. As a measure of the method's dependability for regular quality analysis, Table 6 displays the results' reproducibility.

Table 6: Robustness parameter summary for emtricitabine, tenofovir alafenamide, and efavirenz

Condition	Emtricitabine		Tenofovir Alafenamide		Efavirenz	
	Mean area	% assay	Mean area	% assay	Mean area	% assay
Optimized	1078319	99.7	209914	99.1	4266264	98.9
Flow rate at 0.2 mL/min	1075986	99.1	213148	100.9	4266014	98.6
Flow rate at 0.4 mL/min	1072518	98.6	212401	100.5	4249107	98.3
Mobile phase:						
Buffer-acetonitrile (55:45)	1082742	100.9	209789	98.6	4286926	100.2
Buffer-acetonitrile (45:55)	1081287	100.2	214442	101.3	4308627	100.9
Column Temperature:	1094325	101.1	212144	100.1	4270193	99.3
• at 25°C	1103863	101.2	215357	101.7	4274522	99.7
• at 35°C						

Degradation studies:
The effectiveness of the suggested method in distinguishing emtricitabine, tenofovir alafenamide, and efavirenz from their degradation products was assessed through exposure to various stress conditions. These circumstances included thermal degradation (heated to 110°C for 24 hours), oxidative hydrolysis (using 20% H2O2), base hydrolysis (using 1 N NaOH), and acid hydrolysis (using 1 N HCl). Table 7 displays the findings from these assessments.

Table 7: Tenofovir alafenamide, efavirenz, and emtricitabine degradation data

	Emtricitabine	% degraded	Tenofovir Alafenamide	% degraded	Efavirenz	% degraded
Standard	1018525		198797		4222619	
Acid	993684	2.73	191687	3.77	3984829	5.73
Base	989884	3.10	189856	4.69	4030378	4.65
Peroxide	983443	3.73	190140	4.55	4074947	3.59
Thermal	994225	2.68	192461	3.38	4126900	2.36

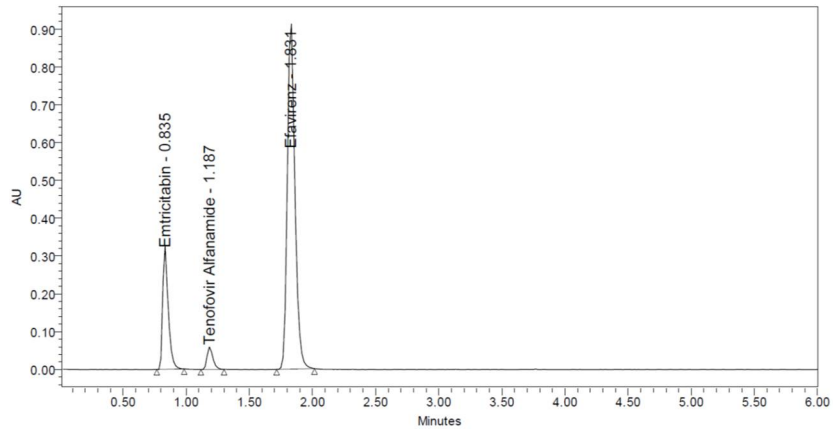

Figure 8: Acid degradation chromatogram

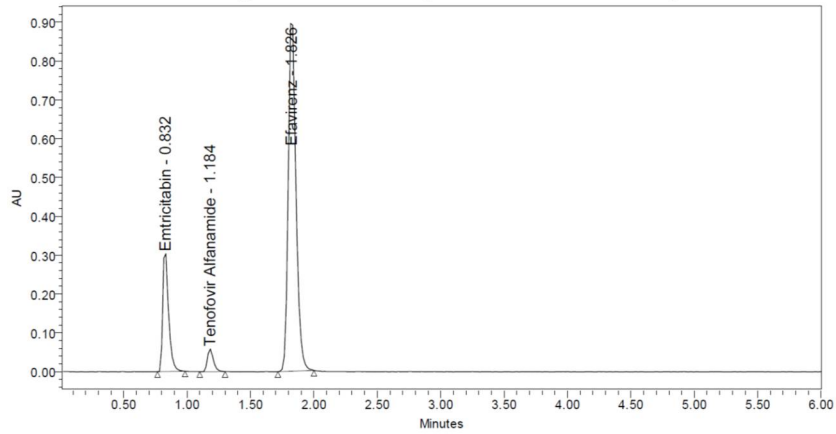

Figure 9: Base degradation chromatogram

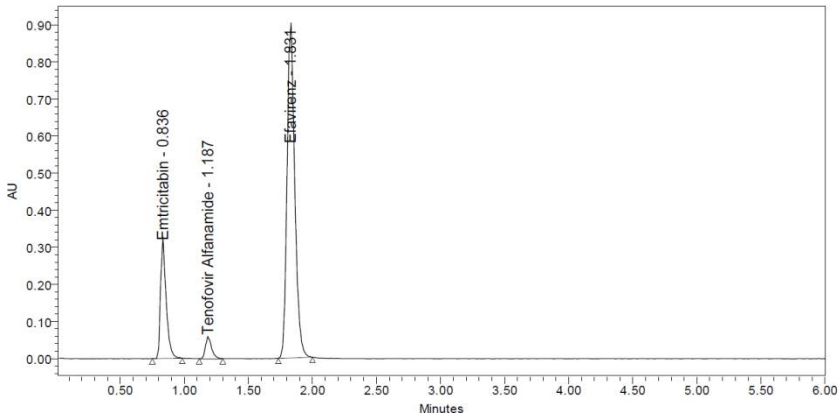

Figure 10: Peroxide degradation chromatogram

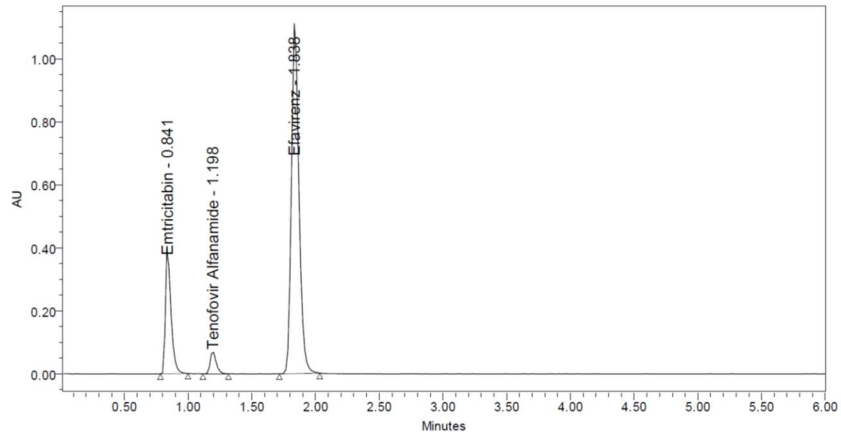

Figure 11: Thermal degradation chromatogram

CONCLUSION

Concurrent estimation of emtricitabine, tenofovir alafenamide, and efavirenz in their combination dose form using the current RP-UPLC method was established and validated in accordance with ICH guidelines. Emtricitabine, tenofovir alafenamide, and efavirenz were shown to be linear with correlation coefficients (r2) more than 0.998 in the concentration ranges of 50-300 µg/ml, 2.5-15 µg/ml, and 150-900 µg/ml, respectively. The developed approach was proven to be accurate as the percentage recoveries of efavirenz, tenofovir alafenamide, and emtricitabine were obtained within the acceptable range of 98-102%, with an RSD of less than 2. Simple, linear, sensitive, quick, accurate, robust, and focused is the method that was developed. The experiments on forced deterioration employed 1N HCl, 1N NaOH, 20% H2O2, thermal, photolytic, and water degradation. The combination dose form of emtricitabine, tenofovir alafenamide, and efavirenz can therefore be routinely analyzed for quality control using the proposed UPLC method.

Funding

No Funding

Conflict of Interest

Author are declared that no Conflict of Interest

Ethical Considerations

Not Required

Author Contribution

All authors are Contributed equally

REFERENCES

1. Villani P, Regazzi MB, Castelli F, Viale P, Torti C, Seminari E,Maserati R,(1999). Pharmacokinetics of efavirenz (EFV) alone and in combination therapy with nelfinavir (NFV) in HIV-1 infected patients, Brazilian Journal of Clinical Pharmacology, 48(5): 712–715.
2. Julien Fonsart, Sentob Saragosti, Milad Taouk Gilles, Peytavin, Lane Bushman, Isabelle Charreau, Allan Hance, Lauriane Goldwirt, Stephane Morel, Fabrizio Mammano, Bendicte Loze, Catherine Capitant, Francois Clavel, Nadia Mahjoub, Laurence Meyer, Peter L Anderson, Constance Delaugerre, Jean-Michel Molina, (2017). Single-dose pharmacokinetics and pharmacodynamics of oral tenofovir and emtricitabine in blood, saliva and rectal tissue: a sub-study of the ANRS IPERGAY trial, *Journal of Antimicrobial Chemotherapy*, 72 (2): 478–485.
3. Joseph M Custodio, Marshall Fordyce, William Garner, Mona Vimal, Kah Hiing J Ling, Brian P Kearney, Srinivasan Ramanathan, (2016). Pharmacokinetics and Safety of Tenofovir Alafenamide in HIV-uninfected Subjects with Severe Renal Impairment, *Antimicrobial Agents and Chemotheraphy*; 60 (9): 5135-5140.
4. Venkatesan S, Kannappan N, (2014). Simultaneous spectrophotometric method for determination of emtricitabine and tenofovir disoproxil fumarate in three-component tablet formulation containing rilpivirine hydrochloride, International Scholarly Research Notices, 1-8.
5. Kiran Kumar V, Appala Raju N, (2009). Estimation of emtricitabine in tablet dosage form by RP-HPLC, *Asian Journal of Chemistry*, 21(8): 5979-5983.
6. Mastanamma Sk, Asha Jyothi J, Saidulu P**,** Varalakshmi M**, (2018).** Development and validation of RP-HPLC method for the simultaneous estimation of lamivudine, tenofovir alafenamide and dolutegravir bulk and their combined dosage form, *Pharm Methods,* 9(2): 49-55.
7. Purnachandra Rao P, Mogili Reddy D, Ramachandran D, (2014). Stability indicating HPLC method for simultaneous estimation of emtricitabine, tenofovir disoproxyl fumarate, cobicistat and elvitegravir in pharmaceutical dosage form, *World Journal of Pharmaceutical Sciences*, 2(12): 1822-1829.
8. Sk Mastanamma Sk, Venkata Reddy D, Saidulu P, Varalakhimi M, (2017). Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of emtricitabine, tenofovir alafenamide bulk and their combined dosage form, *Journal of Chemical and Pharmaceutical Research*, 9(9):70-80.
9. Benzil D, Ravichandran C, Ramachandraiah C, Devanna N, (2017). Development and validation of RP-HPLC method for the simultaneous estimation of emtricitabine and tenofovir alafenamide in bulk and tablet dosage form, *European Journal of Biomedical and Pharmaceutical sciences*, 4(10): 663-668.
10. Prabhakar Reddy A, Chandra Teja U, Ashraf Sultana SK, M. Vijayalakshmi M, Buchi N. Nalluri, (2014). Development and validation of RP-HPLC-PDA method for the simultaneous estimation of emtricitabine, tenofovir disoproxil fumarate and rilpivirine hydrochloride in bulk, pharmaceutical dosage forms and in dissolution samples, *Indo American Journal of Pharmaceutical Research*,; 4 (11): 5226-5234.
11. Mallikarjuna rao N, Gowri Sankar D, (2016). Development and Validation of Stability-Indicating HPLC-DAD method for simultaneous determination of emtricitabine, elvetegravir, cobicistat and tenofovir in their tablet dosage forms, *Indian Journal of Pharmaceutical Education and Research*, ; 50(1): 205-211.

12. Vanitha C, Pradeep kumar P, Rajani sekhar V, Swarnalatha G, Sekar V, (2014). Simulataneous estimation of emtricitabine and tenofovir disoproxil fumarate in a tablet dosage form by RP-HPLC method, *International Journal of Pharmaceutical Development & Technology*, 4(4): 279-283.
13. Shah AU, Kotadiya VV, Ajmera AA, (2016). Analytical method development and validation for simultaneous estimation of emtricitabine and tenofovir disoproxil fumarate in tablet dosage form, *Research journal of Pharmacy and Technology*, 9(4): 463-468.
14. Venkatesan S, Kannappan N, Sai Sandeep M, (2014). Stability-indicating HPLC method for the simultaneous determination of HIV tablet containing emtricitabine, tenofovir disoproxil fumarate and rilpivirine hydrochloride in pharmaceutical dosage forms, *International Scholarly Research Notices*.
15. Akram MD, Umamahesh M, (2017). A new validated RP-HPLC method for the determination of emtricitabine and tenofovir AF in its bulk and pharmaceutical dosage forms, Journal of Chemical and Pharmaceutical Sciences, 10(4): 54-59.
16. Rajesh Sharma, Pooja Gupta, (2009). A validated RP-HPLC method for simulataneous estimation of emtricitabine and tenofovir disoproxil fumarate in a tablet dosage form, *Eurasian Journal Analytical Chemistry*, 4(3): 276-284.
17. Uttam Prasad P, Sunil Kumar Reddy A,(2015). A novel validated RP-HPLC method for the simultaneous estimation of emtricitabine, tenofovir disoproxil fumarate and rilpivirine in bulk and pharmaceutical tablet dosage forms, *Der Pharmacia Lettre*, 7 (1):303-314.
18. Anandakumar, Karunakaran, Kannan Kamarajan, Vetrichelvan T, (2010). A validated RP - HPLC method for simultaneous estimation of emtricitabine and tenofovir disoproxil fumarate in pure and in tablet dosage form, *Der Pharmacia Sinica*, 1 (2): 52-60.
19. Chinnalalaiah R, Ravi Kumar P, Srinivasa Rao A, (2016). A Validated Stability Indicating RP-HPLC method for the determination of emtricitabine, tenofovir disoproxil fumarate, elvitegravir and cobicistat in pharmaceutical dosage form, *Journal of Chromatographic Science*, 54(5): 759–764.
20. Bala Rami Reddy Y, Mutta Reddy S, Bala Sekhara Reddy CH,(2015). Simultaneous estimation of emtricitabine and tenofovir disoproxil fumarate in tablet dosage form by reverse phase high-performance liquid chromatography, *International Journal of Analytical Techniques,* 1(1): 1-6.
21. Godasu SK, Sreenivas SA, (2018). Determination of Simultaneous Estimation HPLC method for elvitegravir, tenofovir disoproxil fumarate, emtricitabine and cobicistat it's pure and tablet form, *Der Pharma Chemica,* 10(3): 158-165.
22. Pranitha D, Vanitha C, Prince Francis, Alagar Raja M, Vishnu vardan P, Surendar M, David Banji, (2012). Simultaneous estimation of emtricitabine, tenofovir disoproxil fumarate, and rilpivirine in bulk form by RP-HPLC method, *Journal of Pharmacy Research*, 5(8): 4600-4602.
23. Abdul Sattar MD, Suneetha A, (2018). Analytical method development and validation for the determination of emtricitabine and tenofovir disoproxil fumarate using reverse phase HPLC method in bulk and tablet dosage form, *Journal of Pharmaceutical Sciences and research*, 10(5): 1207-1212.
24. Lavanya B, Hariprasad P, Venkata praveen A, Prasanna Lakshmi D, Ramireddy M, (2012). Simultaneous estimation of emtricitabine and tenofovir disproxil fumerate by HPLC method, *Der Pharmacia Lettre*, 4(6):1855-1862.
25. Ashok G, Sumanta Mondal, (2018). Development and validation of stability indicating method for the simultaneous quantification of emtricitabine, tenofovir disoproxil fumarate and rilpivirine hydrochloride in pharmaceutical dosage forms by RP-HPLC, *Saudi Journal of Medical and Pharmaceutical Sciences*, 4(2): 175-183.
26. Palavan C, Ramaprasad LA, Srinivasu P, Seshagiri Rao JVLN, (2013). A new RP-HPLC method for the simultaneous estimation of emtricitabine, efavirenz and tenofovir in tablet dosage forms, *American Journal of PharmTech Research.*; 3(4): 547-554.
27. Varma D, Lakshmana Rao A, (2019). Stability-indicating RP-HPLC method for the simultaneous estimation of efavirenz, tenofovir and emtricitabine in pharmaceutical formulations, *Research Communication*, 1-17.
28. Prashant SD, Roshan B, Nalini S, Surendranath KV, (2013). A validated stability-indicating RP-HPLC method for the simultaneous determination of tenofovir, emtricitabine and efavirenz and statistical approach to determine the effect of variables, *ISRN Chromatography*, 1-8.
29. Srinivasa Rao A, Naveen Kumar G, Srilekha K, Aruna Kumari N, (2016). Stability indicating method for the simultaneous estimation of tenofovir, emtricitabine and efavirenz in pure and pharmaceutical dosage form by RP-HPLC, *International Journal of Advanced Research In Science And Engineering*, 5(5):188-200.
30. Saminathan J, Vetrichelvan T, (2016). Development and validation of HPTLC method for simultaneous estimation of emtricitabine, rilpivirine and tenofovir disoproxil fumarate in combined dosage form, *Bangladesh Pharmaceutical Journal,* 19(1): 114-121.
31. Bhushan PB, Moreshwar PM, Sanjay DS, (2017). Development and validation of RP-HPLC method for the simultaneous estimation of tenofovir alafenamide and emtricitabine in bulk and tablet dosage form, International Journal of ChemTech Research, 10(5): 731-739.
32. Rajesh Sharma, Pooja Gupta, (2009). A validated RP - HPLC method for simulataneous estimation of emtricitabine and tenofovir disoproxil fumarate in a tablet dosage form, Eurasian Journal of Analytical chemistry, 4(3): 276-284.
33. Imam Pasha S, Murali Balaram V, Ibrahim Mohammed, (2017). Stability indicating RP-UPLC-PDA method development, validation of multi drug combination of emtricitabine, tenofovir alafenamide and rilpivirine in bulk drug & its tablet formulation, oriental journal of chemistry, 33(2): 925-929.

34. Kavitha KY, Geetha G, Hariprasad R, venkatnarayana R, Kaviarasu M, (2013). Development and validation of RP-UPLC analytical method for simultaneous estimation of Emtricitabine, Rilpivirine, Tenofovir disoproxil fumarate and its pharmaceutical dosage forms, international research journal of pharmacy, 4(1):150-155.
35. Valli Purnima B, Vijaya Bhaskara Reddy T, Srinivas Rao Y, Ramu G, Ramachandran D,(2015). Stability indicating RP-UPLC method for assay of emtricitabine and tenofovir disoproxil fumarate in bulk and dosage forms, American Journal of Analytical Chemistry, 6: 807-821.
36. Pravish Tiwari, Ravi Yadav, Avinash K, Vaidya V, Sathe VPA, Deepali G, (2010). Development and validation of UPLC method for emtricitabine, tenofovir and efavirenz in pharmaceutical preparation, *Analytical chemistry an indian journal,* 9(2):247-251.
37. Babu C, L Narasimha Rao K, Devanna, Suresh Reddy KVN, (2017). RP-UPLC method for the simultaneous quantification of related substances in emtricitabine, tenofovir disoproxil fumarate and efavirenz pharmaceutical dosage forms,*Journal of Chemical and Pharmaceutical Research*, 9(5):45-52.
38. ICH Harmonised Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology, Q2 (R1), USA, 2005:1-13.

CITATION OF THIS ARTICLE

Kanchan S N, Naresh N, Nagaraju P, Balaji K and P. Jitendra Kumar. Simultaneous Estimation of Emtricitabine, Tenofovir Alafenamide, and Efavirenz via a State-of-the-Art RP-UPLC Method in Drug Formulation. Bull. Env.Pharmacol. Life Sci., Vol 12 [12] November 2023: 291-301