A reliable UPLC method for the analysis of bulk and pharmaceutical dosage forms forms of Emtricitabine, Tenofovir Alafenamide and Doravirine

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Abstract:

A stability-indicating RP-UPLC method was developed to simultaneously assess the concentrations of Emtricitabine, TAF and Doravirine in pharmaceutical dosage forms.

Materials and Methods: *HSS C18 100 x 2.1 mm, 1.8 m column was used for separation. The mobile phase that was utilized was a 60:40 mixture of ACN and buffer (0.01N KH_2PO_4). The wavelength used for detection was 265 nm, with 0.4mL min rate of flow.*

Results: *Retention time of Emtricitabine, TAF, and Doravirine were obtained as 0.692,1.285 and 2.108. LOD and LOQ were found to be 0.58,0.10,0,28µg/ml and 1.76,0.30,0.85µg/ml respectively. R^2 obtained for drugs were 0.9999,0.9996,0.9993 respectively.*

Conclusion: *The linearity, accuracy, and precision of this approach were verified. It was discovered that the approach indicated stability as well.*

Key words: *Emtricitabine, TAF, Doravirine, RP-UPLC, ACN, KH_2PO_4, Accuracy, Precision, Linearity.*

1. Introduction:

Emtricitabine is prescribed in conjunction with other drugs for the management of HIV-1 infections in both adults and children. Different emtricitabine products are approved for patients with specific features; consult the individual medicine product for patient eligibility about therapy. It can be utilized for pre-exposure prophylaxis of HIV-1.[1]

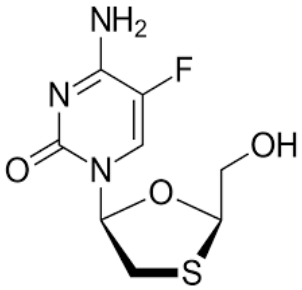

Figure. 1 Structure of Emtricitabine

TAF is a phosphonic acid that is classified as a methyl phosphonic acid. One component of combination therapy for HIV infection is the fumaric acid salt of the bis ester prodrug. It inhibits the HIV-1 reverse transcriptase and functions as an antiviral; it is a pharmacological metabolite. It is a nucleoside analogue and a member of the phosphonic acid family. [2]

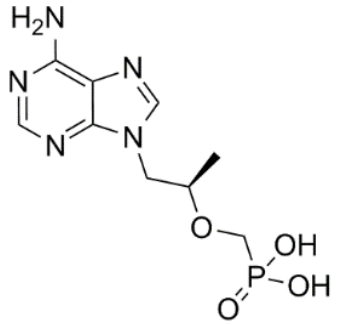

Figure. 2 Structure of TAF

To treat HIV-1 infections, Doravirine, a NNRT inhibitor, given in conjunction with other antiretrovirals in elderly patients without a history of antiretroviral therapy. Individuals who are immunity deprived, it is also recommended to switch out their current antiretroviral regimen with one that is stable with no past of failed treatment, and are not Doravirine resistant.[3]

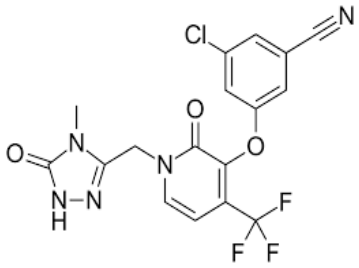

Figure. 3 Structure of Doravirine

2. Literature:

The formulation of this combination is under trials in various pharmaceutical companies and is not available in Indian market yet. But as per the literature available, this combination can be given due to its antiretroviral properties. Many combinations were used to treat HIV, but darunavir/cobicistat/emtricitabine/TAF was used for long time as an effective medication, but due to side effects like nausea the combination was revised as Doravirine + Emtricitabine/TAF. According to a review article published in 2023 this combination was found to be safe, secure, potent when compared to other combinations used to treat HIV in highly treatment experienced multi drug resistant patients. A case report was published supporting the efficacy and safety of this combination in HIV patients with hemodialysis/peritoneal dialysis [(4-6)]. There are few articles which shows the use of this combination and other different combinations of antiviral drugs in treatment of HIV [7-16]. There are various analytical methods like HPLC, UPLC and LC/MS-MS reported in different combinations of antiviral drugs along with few conducted in human plasma. There was no reported method yet and hence there is a high need to develop an analytical method for this combination as this combination was proved to be better than many other anti-retroviral regimens in various research findings [17-26].

3. Experimental Parameters:

3.1 Apparatus:

Table 1. Apparatus Used

Instrument	**Manufacturing Company**
Electronics Balance	Denver
Digital pH meter 7007	Digisun Electronics Hyderabad
Ultrasonicator	Labman
UPLC instrument	WATERS
UV-VIS spectrophotometer	PG Instruments T60
Vacuum pump	Crompton
Hot Air Oven	Servewell Instrument PVT LTD, Bangalore.
Electronics Balance	Denver

3.2 Chemicals and Reagents:

Table 2. Chemicals used

Material	**Source**
CH_3CN CH_3OH H_2O	Merck chemical division, Mumbai
KH_2PO_4 H_3PO_4 $NaH_2PO_4.2H_2O$	Rankem, Avantor performance material India Limited

3.3 Optimized Method:

Precisely weighed 1.36 grams of KH_2PO_4 transfer to a 1000 ml volumetric flask together with roughly 900 ml of H_2O. The mixture was then allowed to degas and sonicate, and the volume was eventually made up with water and pH was corrected to 4.8 with 0.1% OPA. Mobile phase used was ACN: KH_2PO_4 in the ratio of 60:40v/v. Figure 4 and Table 3 shows the optimized chromatogram and optimized method respectively.

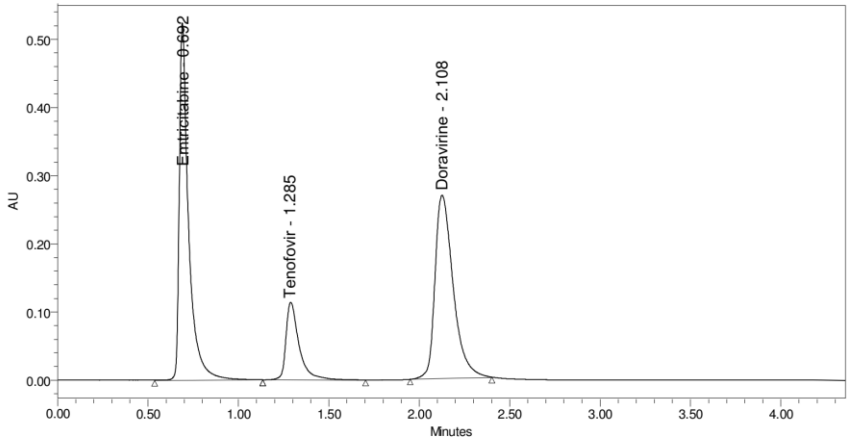

Figure 4. Optimised Chromatogram of Drug

Table 3. Optimized Chromatographic Conditions

Characteristics	Result
Mobile Phase	ACN: KH_2PO_4 (60:40 v/v)
Flow Rate	1mL/min
Column	HSS C_{18} 100 x 4.6mm, 5µ
Detector Wavelength	265nm
Column Temperature	30^0C
Injection Volume	1.0µL
Run Time	10 minutes

Table 4. System Suitability results

Compound	USP Tailing Factor	USP Resolution	USP Plate Count
Emtricitabine	1.3	-	3227.6
TAF	1.7	5.6	2570.0
Doravirine	1.4	6.1	2916.9

4. Standard Preparation:

After careful weighing, 50 milligrams of Emtricitabine, and 6.25 milligrams of TAF ,25 milligrams of Doravirine, were put to 50 millilitres of clean, dry volumetric flasks and combined.

Ten minutes were then spent sonicating the mixture, and diluents were added to bring the volume up to the final level. (1000 PPM Emtricitabine, 125 PPM TAF, and 500 PPM Doravirine).

4.1 Standard Working Preparation: A 10-milliliter volumetric flask was filled with one milliliter of filtered sample stock solution, which was then diluted. (100 PPM Emtricitabine, 12.5 PPM TAF, and 50 PPM Doravirine).

4.2 Sample Preparation: Accurately weighed equivalent weight of one tablet synthetic mixture, powder it and transfer into a 100mL volumetric flask. Following a 25-minute sonication period and the addition of 50 ml of diluent, the volume was again adjusted with diluent and passed through HPLC filters containing 2000 ppm Emtricitabine, 250 ppm TAF, and 1000 ppm Doravirine.
The sample working preparation involved transferring 0.5ml of the filtered sample solution to a 10ml volumetric flask and diluting it with diluent. (150 ppm Emtricitabine, 1500 ppm TAF, and 150 ppm Doravirine).

4.3 Assay:
Emtricitabine 500 mg, TAF 20 mg, and Doravirine 15 mg per unit formulation are stated on the label. The assay utilized the formulation stated before. TAF, Doravirine, and emtricitabine assay average percentage. 99.94%, 99.22%, and 99.17% were attained, respectively.

5. Degradation Studies:

Oxidation was performed by adding 1mL of 20% hydrogen peroxide (H_2O_2) separately to one milliliter of the stock solution that contained Emtricitabine, TAF and Doravirine. 30 minutes were spent storing the solutions at 60^0C. A dilution of 10 µl of the final solution yielded solutions with concentrations of 100 ppm, 12.5 ppm, and 50 ppm, which were then used to assess the stability of the sample for the UPLC analysis.

5.1 Acid Degradation Studies:

Add One ml of 2N HCl to one ml of the stock solution containing Emtricitabine, TAF, and Doravirine. The solution was then refluxed for thirty minutes at 60^0C. 10 µL of obtained solution was transferred into the system, and chromatograms were recorded to evaluate the sample's steadiness. The resulting solution was then diluted to obtain solutions at 100 ppm, 12.5 ppm, and 50 ppm.

5.2 Alkali Degradation Studies:

Add 1 ml of 2 N NaOH to 1 ml of solution containing Emtricitabine, TAF, Doravirine. The mixture was refluxed for 30 minutes at 60^0 C. After diluting the resulting solution to create solutions at 100 ppm, 12.5 ppm, and 50 ppm, 10 µl was transferred into the system, and chromatograms were observed to evaluate the sample's stability.

5.3 Dry Heat Degradation Studies:

For six hours, the standard solution was heated to 105^0C in an oven to examine dry heat deterioration. To know the stability of the sample for the HPLC analysis, 10µl of the resulting mixture was transferred into the system and diluted to 100 ppm, 12.5 ppm, and 50 ppm.

5.4 Photo Stability studies:

The drug (1000 ppm, 125 ppm, and 500 ppm) solution was exposed to UV light and keeping the beaker in a UV chamber for seven days. For the UPLC analysis, the final solution was diluted to produce solutions that were 100 ppm, 12.5 ppm, and 50 ppm. After injecting 10µl into the system, the steadiness of the sample was assessed.

5.5 Neutral Degradation Studies:

Drug stress testing under neutral settings was investigated by refluxing the medication in water at 60ºC for six hours. In order to evaluate the stability of the sample, 10 µl of the resulting solution was injected into the UPLC system after it had been diluted to 100 ppm, 12.5 ppm, and 50 ppm. Table 5 shows the degradation results of the developed method.

Table 5. Degradation Results

S. No	Degradation Condition	% Assay			% Degraded		
		E	T	D	E	T	D
1	Acid	94.11	94.10	93.92	5.89	5.90	6.08
2	Alkali	95.27	94.99	95.27	4.73	5.01	4.73
3	Oxidation	95.92	95.50	96.17	4.08	4.10	3.83
4	Thermal	97.14	97.23	97.29	2.86	2.77	2.71
5	UV	98.24	98.15	98.38	1.76	1.85	1.62
6	Water	99.14	98.97	99.22	0.86	1.03	0.78

6. Validation:

In accordance with ICH guidelines, method validation was carried out to know the amount of Emtricitabine, TAF and Doravirine in the formulations.

6.1 Precision:

An analytical method's precision provides information about random mistakes. It indicates the degree of agreement between a number of measurements made under specified conditions from repeated samplings of the same homogenous sample. Three distinct drugs were determined to have average area, S.D, %RSD of 0.3%, 0.5%, and 0.9%, respectively. The system precision was less than "2". Precision data for the method are shown in Table 6.

Table 6. Precision Results

S. No	Variable	% RSD of E	%RSD of T	%RSD of D
1	System Precision	0.5	0.5	0.6
2	Intermediate Precision	0.3	0.5	0.9
3	Repeatability	0.8	0.3	1.2

6.2 Accuracy:

Accuracy expresses how closely the derived result and the reference value match. The standard addition method was used to construct three tiers of the Accuracy sample. Three injections were given for each accuracy level, and the mean percentage recovery for Emtricitabine, TAF and Doravirine, was determined to be 100.11%, 99.90%, and 99.71%, respectively. accordingly. Table 7 shows Accuracy data of the method.

Table 7. Accuracy data

S. No	Drug	Recovery Levels	% Recovery (n=3)	% RSD
1	E	50	100.72	0.25
		100	100.24	0.34
		150	99.59	0.16
2	T	50	99.96	0.39
		100	100.38	0.53
		150	99.46	0.59
3	D	50	100.74	0.31
		100	100.96	0.16
		150	100.37	0.26

6.3 (LOQ) and (LOD):

The LOD and LOQ were determined by putting a series of diluted solutions with known concentrations. Table 8 shows the LOD and LOQ values of the drugs.

Table 8. LOD and LOQ values

Molecule	LOD(µg/ml)	LOQ(µg/ml)
Emtricitabine	0.58 µg/ml	1.76 µg/ml
TAF	0.10 µg/ml	0.30 µg/ml
Doravirine	0.28 µg/ml	0.85 µg/ml

6.4 Linearity:

In a triplicate injection protocol, six linear dosages of Emtricitabine (25-150 µg/ml), TAF (3.125-18.75 µg/ml), and Doravirine (12.5-75µg/ml) were injected. The linearity equations for Emtricitabine (y = 30525x + 10702), TAF (y = 72058x + 9745.8), and Doravirine (y = 77511x + 8806.9) were obtained. Correlation coefficient was found to be 0.999. Linearity values are shown in Table 9.

Table 9. Linearity values of drug

Parameters	E	T	D
Range (mg/mL)	25-150	3.125-18.75	12.5-75
R^2	0.9999	0.9996	0.9993
Slope	30525	72058	77511
Y- intercept	10702	9745.8	8806.9

6.5 Robustness:

A method is said to be robust if it can withstand even minor changes in the environment. Sample was kept in triplicate with robustness setting such as temperature (±10^{0}C), mobile Phase (±10), flow Rate (±0.2ml/min). It passed all the parameter with minimal impact. %RSD was within the limit. Table10 shows the robustness values of the drugs.

Table 10. Robustness results

S.no	Condition	%RSD of E	%RSD of T	%RSD of D
1	Flow rate (-) 0.9ml/min	0.2	0.2	0.9
2	Flow rate (+) 1.1ml/min	0.5	0.4	0.8
3	Mobile phase (-) 55:45	0.5	0.6	1.0
4	Mobile phase (+) 45:55	0.3	0.3	0.2
5	Temperature (-) 25°C	0.6	0.9	0.6
6	Temperature (+) 35°C	0.2	0.5	0.2

7. Conclusion:

A linear, precise, reliable, robust, specific and quick isocratic RP-UPLC method was developed for the quantitative analysis of Emtricitabine, TAF and Doravirine in pharmaceutical dosage forms. All of the parameters examined yielded satisfactory results, and the newly developed UPLC method for the assay determination was able to provide faster retention durations while keeping good resolution. It is a stability-indicating approach that is appropriate for quick analysis of Emtricitabine, TAF and Doravirine in API and pharmaceutical dosage forms according to the results of stress testing that were conducted as per the ICH guidelines.

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