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IMPLEMENTING STABILITY-INDICATING ASSAY UHPLC METHOD FOR SIMULTANEOUS QUANTIFICATION OF EMTRICITABINE AND TENOFOVIR DISOPROXIL FUMARATE IN PHARMACEUTICAL MATRICES

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ABSTRACT

An economical, rapid, and precise stability-indicating UHPLC method for simultaneous quantification of HIV therapeutic agents (emtricitabine and tenofovir disoproxil fumarate) in pure standard and pharmaceutical matrices. The UHPLC separation analysis was achieved on UPLC BEH C_{18} (150 mm × 2.1 mm) with 1.7 µm particle size column at ambient temperature using a solvent system in a proportion of (70:30 % v/v) potassium dihydrogen orthophosphate buffer: methanol; pH 2.8 ± 0.03 was adjusted with 0.1 % OPA. The PDA detector has functioned as a mode for analysis for identification and confirmation of peak purity. In order to evaluate the assay procedure's stability-indicating efficacy, emtricitabine and tenofovir disoproxil fumarate were experienced to various stress procedures of forced degradation analysis like acidic, alkaline, and neutral hydrolysis, oxidative, photodegradation, and thermal degradation (dry and wet heat). The designed approach was successful in distinguishing the selected therapeutics from the peaks of their respective degradation products. Furthermore, the designed approach demonstrated calibration plots for emtricitabine and tenofovir disoproxil fumarate in over wide concentration ranges of 3 – 18 µg/mL and 10 – 60 µg/mL, respectively, with determination coefficients (r^2) of 0.9983 and 0.9999. The method validation assays had relative standard deviations of less than 2 % for accuracy, precision (intra- and inter-day assay

variance), repeatability, and robustness. Therefore, simultaneous quantification of both HIV therapeutic agents in tablet matrices was accomplished.

Keywords*:* Emtricitabine; Forced degradation study; Stability-indicating assay; UHPLC method; Tenofovir disoproxil fumarate

1. INTRODUCTION

Emtricitabine (EMT) and Tenofovir Disoproxil Fumarate (TDF) are belonging to the nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs) class of therapeutic agents. EMT, chemically is 4-amino-5-fluoro-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl] pyrimidin-2-one and chemically, Tenofovir Disoproxil Fumarate (TDF) is [[(2*R*)-1-(6-aminopurin-9-yl)propan-2-yl]oxymethyl-(propan-2-yloxycarbonyloxymethoxy)phosphoryl] oxymethylpropan-2-yl carbonate;but-2-enedioic acid depicted in **Figure 1**. They play a significant role in preventing HIV from spreading throughout the body. Since these therapeutic agents will not help treat HIV, they may reduce the risk of getting AIDS and HIV-related problems, including serious infections or cancer. Together with safer sex and other lifestyle modifications, these therapeutic agents may lower the risk of contracting HIV or transferring it to others [1–3]. The fixed-dose combination (FDC) of EMT 200 mg and TDF 300 mg given orally once daily, is commonly used as part of first-line HIV-1 treatment regimens was approved in the United States for pre-exposure prophylaxis in high-risk persons who are not infected, in combination with safer sex practices, to minimize the risk of sexually acquired HIV-1 [1].

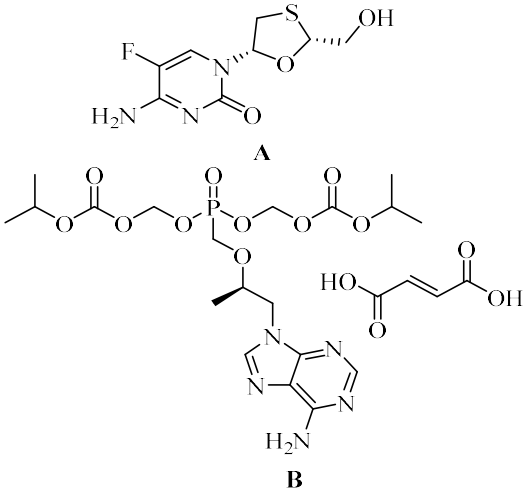


Figure 1: Chemical structures of Emtricitabine (A) and Tenofovir Disoproxil Fumarate (B)

The development of pharmaceutical matrices using a combination of drugs to obtain a better clinical impact is gaining popularity to address a non - tested therapeutic gap. Antiviral therapeutic agents can help minimize Human Immunodeficiency Virus (HIV)-related morbidity, increase survival, and minimize HIV transmission. Today's HIV therapy currently consists of three-drug, while HIV pre-exposure prophylaxis currently consists of two-drug regimens; both require once-daily dosing and long-term reduction of viral infection [4, 5].

For quantification of cited HIV therapeutic agents, many literature findings have been documented for analysis and quantification of EMT and TDF in the various pharmaceutical and biological matrices, including; LC-MS/MS[6–13], HPLC[14–20], HPTLC[21–24], spectrophotometry [25–28]. The use of higher consumption of organic solvents and the need for close monitoring of flow rate, injection volume, flow gradient, and use of tedious sample preparation procedures are all significant drawbacks associated with these appraised reports coherent method performance during the development process. Establishing an efficient, robust, and rugged stability-indicating UHPLC method is beneficial for analysis of the drug molecules is essential in understanding the intrinsic chemical stability of therapeutic agents. Moreover, the design of the stability-indicating analytical method for the aforementioned pharmacological products can be challenging for an analytical chemist, especially when each medication has distinctive physicochemical properties such as pH, pKa, and solubility.

To the best of our knowledge, no stability-indicating UHPLC method has been reported for the simultaneous determination of EMT and TDF in FDC matrices so far with the application of the UHPLC system. Thus, the main objective of this study was to develop a rapid, sensitive, and stability-indicating UHPLC method to estimate and assess the EMT and TDF in pharmaceutical matrices.

2. MATERIAL AND METHOD

2.1 standard and reagents

Pharmaceutical grade of Emtricitabine (purity label claim as 99.99 %) and Tenofovir Disoproxil Fumarate (purity label claim as 100.01 %) used in the design study was supplied as gift samples from Mylan Pharmaceuticals Pvt. Ltd., India, and Abbott Pharmaceuticals Pvt. Ltd., India. Water (HPLC grade), Methanol (HPLC grade), potassium dihydrogen orthophosphate (HPLC grade), and orthophosphoric acid (OPA) were purchased from Merck, Mumbai, India

2.2 Preparation of standard and working solution

EMT (20 mg) and TDF (30 mg) were precisely weighed into a neat, dry 100 mL of a calibrated flask and solubilized in 20 mL of methanol with stirred manually for 10 min; the final volume of the calibrated flask was made up to 100 mL using same. The working solution of EMT and TDF was 5 µg/mL and 15 µg/mL (concentration), respectively.

2.3 Sample preparation

Into a two-separate neat, dry 100 mL of a calibrated flask, the portion of one tablet powder of Truvada^{TM} and Ricovir EM tablets (equivalent to label claim EMT- 200 mg and TDF- 300 mg) were weighed and solubilized in 20 mL of methanol by sonication for 15 min and finally made-up to 100 mL using methanol. The working solution of EMT and TDF were 6 µg/mL and 20 µg/mL (concentrations), respectively.

2.4 Instrumentation and chromatographic analysis of UHPLC method

Design method development and validation were achieved using an Agilent UPLC system (Agilent Technologies, USA) installed with a solvent delivery system, ACQ-PDA detector, autosampler, and a thermostatically controlled column compartment. UHPLC chromatographic quantifications of EMT, TDF, and their degradant products were tested on UPLC BEH C_{18} (150 mm × 2.1 mm) with a 1.7 µm particle size column as a stationary phase. The optimized gradient method comprised (70:30 % v/v) potassium dihydrogen orthophosphate buffer: methanol (pH 2.8 ± 0.03 was adjusted with 0.1 % OPA) at a 0.5 mL/min rate of flow. The solvent system was filtered through a 0.2 µm membrane (Ultipor N_{66} Nylon 6, 6) and degassed by sonication for 15 min aforementioned to use. The operational thermostatically controlled column compartment was set at about 30 ℃. The 10 µL of fixed volume (working solution) was injected with an autosampler. The PDA detector was set at 190 – 380 nm range of wavelength, and EMT and TDF were simultaneously monitored at 273 nm. Empower® 3 software (Agilent Technologies, USA) was used to analyze the recorded data.

2.5 UHPLC method validation

With the use of the following number of parameters, the established specific approach for determining EMT and TDF in pharmaceutical matrices has been confirmed concerning the International Conference on Harmonization Q2(R1) [29].

2.5.1 System suitability: The working standard solution comprising EMT (5 µg/mL) and TDF (15 µg/mL) samples was

introduced and estimated as six determinations. The tailing factor, theoretical plate number (USP plate count), resolution, and percent relative standard deviation (% RSD) values for EMT and TDF peaks were determined.

2.5.2 Calibration curves: Using the optimized chromatographic conditions, unique calibration curves for EMT and TDF were constructed. Six concentration levels were used to establish calibration curves for EMT (3 – 18 µg/mL) and TDF (10 – 60 µg/mL), and each concentration was examined five times. The least-square method was used to perform regression analysis on the data.

2.5.3 Accuracy: The accuracy of the design UHPLC method for EMT and TDF was addressed in the context of % recovery and accomplished at three distinct levels, i.e., 80%, 100%, and 120%. The % recovery was exercised by adding a fixed EMT and TDF standard to the pre-analyzed tablet solution (EMT- 6 µg/mL and TDF- 20 µg/mL). The resulting solution was ultimately addressed using the established method. The % recovery of the planned method was determined through the formula; Recovery (%) = A-B/C×100; where, A-total concentration of EMT and TDF; B- initial concentration of EMT and TDF and C- concentration added of EMT and TDF.

2.5.4 Precision: For intra- and inter-day variability and repeatability, the precision analysis of the design UHPLC method for EMT and TDF was explored. The results were calculated as a % RSD. The three different concentrations of EMT (9, 12, and 15 µg/mL) and 30, 40, and 50 µg/mL of TDF (30, 40, and 50 µg/mL) were addressed using assay at different time frames on the same day of analysis for intra-day precision and as a result of continuous analysis for three consecutive days for inter-day regarding the ICH guidelines. In addition, six determinations of 9 µg/mL EMT and 30 µg/mL TDF concentrations were used to examine repeatability variability.

2.5.5 Sensitivity: The devised approach was used to determine the limit of detection (LOD) and limit of quantification (LOQ) by introducing the lower concentration levels of the EMT (3 – 7 µg/mL) and TDF (10 – 30 µg/mL) standard solutions. The LOD and LOQ for EMT and TDF were determined using the equations LOD = 3.3 × N/B and LOQ = 10 × N/B, respectively, utilizing the standard deviation (N) of EMT and TDF results (n=3) and calibration curve slope (B).

2.5.6 Robustness: Attempts were made to make considerable modifications in flow

rate, wavelength detection, and the amount of methanol in a solvent system as part of the robustness analysis. For the peak areas of EMT and TDF, the influence of each of the independent variables was identified. The independent variables for this study were flow rate (0.4 – 0.6 mL/min), wavelength detection (271 – 275 nm), and methanol percentage (20 – 40 % *v/v*), and were addressed with EMT and TDF concentrations of 15 µg/mL and 50 µg/mL, respectively.

2.5.7 Selectivity and specificity: The designed UHPLC method's selectivity and specificity were confirmed by testing the test sample and using known contaminants at their specified levels. The data on their peak-purity spectra were recorded.

3. FORCED DEGRADATION ANALYSIS

Additionally, standard EMT and TDF compounds were subjected to forced degradation analysis to demonstrate the stability-indicating capability and selectivity of the established method as per the stability testing of new drug substances and products by ICH[30, 31]. The stressed conditions explored for the degradation analysis are acid hydrolysis, alkaline hydrolysis, photolytic, oxidation, photolytic, and thermal following the ICH recommendation. Acid hydrolytic stress, the samples were refluxed at 80°C for 6 h for EMT and 80°C for 12 h for TDF on a thermostatic water bath using 1 M methanolic HCl for EMT and 0.5 M methanolic HCl for TDF, respectively. For the quantification of EMT, TDF, and its degradants, additional dilutions were performed to the analyte concentration. For alkaline stress, samples were exposed to 2 M methanolic NaOH; for EMT was preserved in the dark at room temperature for 3 days, and for TDF was refluxed at 80°C for 6 h. The oxidation stress study was accomplished with 3% and 6 % hydrogen peroxide (H_2O_2) solution for 2 days at room temperature. The photolytic stress experiment was executed by subjecting to the illumination of ≥360Wh/m^2 at 30°C with UV radiation, i.e., for short UV-254 nm and long UV-360 nm for 14 consecutive days. Whereas, dry heat thermal stress experiments were performed at 80 ℃ for 10 h for EMT and 60°C for 10 h TDF and wet heat thermal stress experiment was performed at 80 ℃ for 5 h for EMT and 60 ℃ for 10 h for TDF. The cited HIV therapeutic agents and degradants were measured from all stress samples. Each sample was neutralized and diluted with the solvent system to a final concentration of 10 µg/mL before being assessed under optimum chromatographic conditions at the end of the exposure. Secondary peaks and a decline in

the EMT and TDF peaks area were regarded as signs of degeneration. To prevent errors, appropriate blanks and controls were utilized each time.

4. RESULTS AND DISCUSSION

Although EMT and TDF have different physiochemical properties, choosing the appropriate solvent to dissolve these two medicines in FDC matrices is a complex job in designing the stability-indicating UHPLC method. Before method development, we used methanol as a solvent to produce optimum solubility of EMT and TDF contained in FDC matrices. The concentrations of EMT and TDF in the working solution were 5 µg/mL and 15 µg/mL, respectively.

4.1 Optimization of chromatographic conditions

The achievement of baseline resolution of pharmaceuticals and degradation products, if any, with good peak symmetry in a short run time analysis, especially when particle sizes in column are smaller than 2 µm, is the most significant consideration in a stability-indicating approach. Various experiments were carried out to optimize the solvent systems in order to achieve this. Initially, acetonitrile and potassium dihydrogen orthophosphate buffer were tested as solvent systems to assess EMT and TDF resolution as standard samples. In this case, both analytes displayed a splitting with poor resolution. Further, methanol was used as a polar organic solvent (organic modifier) with potassium dihydrogen orthophosphate buffer. Increasing methanol proportion in the solvent system caused an early elution of EMT and TDF. Though the higher proportion of the methanol in the solvent system improved the resolution, it had an adverse impact on the tailing of peak shapes of both analytes. Therefore, the pH of the buffer in the solvent system was adjusted to 2.8 ± 0.03 with 0.1 % OPA. Finally, the excellent UHPLC separation method was achieved using a gradient method composed of solvent system (A) methanol and (B) potassium dihydrogen orthophosphate buffer, and a UPLC BEH C_{18} (150 mm × 2.1 mm) with 1.7 µm particle size column. The selection of a specific absorption range for EMT and TDF samples was crucial for ensuring method specificity. This was achieved by monitoring the spectra of the respective analytes during peak elution. In the designed UHPLC method, maximum absorption of EMT and TDF was observed at 273 nm, respectively. UHPLC chromatographic separation of the peaks corresponding to EMT and TDF are presented in **Figure 2**.

4.2 Validation of UHPLC method

Thus, according to analytical method validation recommendations of ICH, the suggested UHPLC method was verified in terms of system suitability evaluation, calibration analysis, accuracy, precision, Sensitivity, method robustness, selectivity/specificity, and EMT and TDF matrices stability [32, 33]. Before addressing the stability-indicating characteristics of UHPLC protocol, system suitability criteria such as EMT and TDF peak tailing factors, UPLC column efficiency, resolution, and percent RSD of the peak areas of working standard solutions of EMT and TDF from six successive injections were assessed, and the results are presented in **Table 1**. Calibration plots of the UHPLC method were addressed by investigating standard solutions of the EMT and TDF at six different calibration concentrations ranging from 3 – 18 µg/mL and 10 – 60 µg/mL, and the determination coefficients (r^2) estimates for EMT and TDF were 0.9983 and 0.9999, respectively. The standard addition technique was employed to determine the % recovery of the UHPLC method. The EMT and TDF standards were added to the pre-assessed tablet solution at 80, 100, and 120 % in triplicate to investigate the accuracy experiment. The outcomes documented in **Table 2** revealed that the proposed method is entirely accurate for EMT and TDF with excellent % recovery.

The precision assay variability was assessed with the three distinct concentrations of EMT and TDF calibration plots on different time intervals on the same day (intra-day) and three successive days (inter-day). The measurement of repeatability assay variability was assessed using six determinations of EMT and TDF. The outcomes of precision assay variability were expressed in terms of % RSD for all outcomes. The results are presented in **Table 3**. The % RSD values for intra-day, inter-day and repeatability variability were below 2%, suggesting high precision.

With the view to address the Sensitivity of the UHPLC method, LOD and LOQ were estimated with lower levels of calibration plots of EMT and TDF. According to the proposed method the LOD and LOQ values were recorded to be 0.15 µg/mL and 0.46 µg/mL for EMT and 0.44 µg/mL and 1.34 µg/mL for TDF, correspondingly. As a result, the ultimate Sensitivity of the UHPLC method to the solvent system was identified.

A robustness experiment was carried out by evaluating the EMT and TDF standards at the same concentration level utilized under consistency when modifying the control variables indicated in **Table 4**. The % RSD

of the amount determined after evaluating the same samples with slight deliberate changes in the method parameters was calculated and found to be less than 2%, demonstrating that the approach is robust. Furthermore,

the simultaneous measurement of EMT and TDF in their FDC matrices indicated that the proposed UHPLC approach was unaffected by commonly used diluents and additives. The proposed UHPLC approach successfully analyzed FDC matrices, including EMT and TDF in Truvada^{TM} and Ricovir EM tablets. Further, it was also investigated by stress studies, and no involvement was noticed from degradants. For 48 hours, the EMT and TDF working solution was required to leave at room temperature. The areas of the peaks of 5 µg/mL and 15 µg/mL had a % RSD of 1.45 % and 1.83 %, respectively, showing that the prepared solution was stable for up to 48 hours.

4.3 Force degradation studies

A forced degradation study was conducted on EMT and TDF standard (EMT 10 µg/mL and TDF 10 µg/mL) under acidic, alkaline, oxidative, photolytic, thermal (dry and heat) stress conditions following the ICH reference. The developed UHPLC method was specific and sensitive enough to separate the cited HIV therapeutic agents from their degradation products.

When the EMT standard was subjected to 1 M HCl reflux for 80 °C for a period of 6 h, EMT degraded, and degradation was recorded to be 9.06 % (**Figure 3a**). Alkaline induced stress studies at 2 M NaOH for 3 days, EMT was degraded about 12.22 % (**Figure 3b**). The 5.38 % degradation was observed on exposing EMT to ≥360Wh/m^2 at 30°C with UV radiation for 14 consecutive days (**Figure 3c**), while considerable degradation, i.e., 13.67 %, was pointed out in wet heat 80 ℃ for 5 h (**Figure 3d**). At the same time, EMT was stable in neutral, H_2O_2 induced oxidative conditions and dry heat stress conditions. Similarly, the TDF standard was subjected to 0.5 M HCl for 80°C for 12 h, TDF was degraded, and 18.20 % (**Figure 4a**) degradation was noticed. In alkaline and H_2O_2 induced oxidative conditions, TDF was degraded, and degradation was about 11.87 % (**Figure 4b**) and 5.68 % (**Figure 4c**). Furthermore, TDF was degraded by about 12.79 % (**Figure 4d**), by illumination of 360Wh/m^2 for 14 days at 30°C, and 12.45 % (**Figure 4e**) TDF was noticed after exposing to 60 ℃ for 10 h. **Table 5** summarizes the degradation behavior of EMT and TDF under various stress environments. In each of these conditions, the capacity of the planned UHPLC method to evaluate intact EMT and

TDF with no impurities (degradation products) indicates the stability-indicating potential of the planned study and, as a result, addresses the method's specificity.

4.4 Analysis of pharmaceutical matrices

Using the proposed UHPLC method, quantification of EMT and TDF in their Truvada™ and Ricovir EM tablets was carried out using 6 µg/mL for EMT and 20 µg/mL for TDF. The assay was evaluated in triplicate. Satisfactory results were obtained for each tablet matrix in good agreement with the label claim. The result of the analysis of pharmaceutical matrices is shown in **Table 1**.

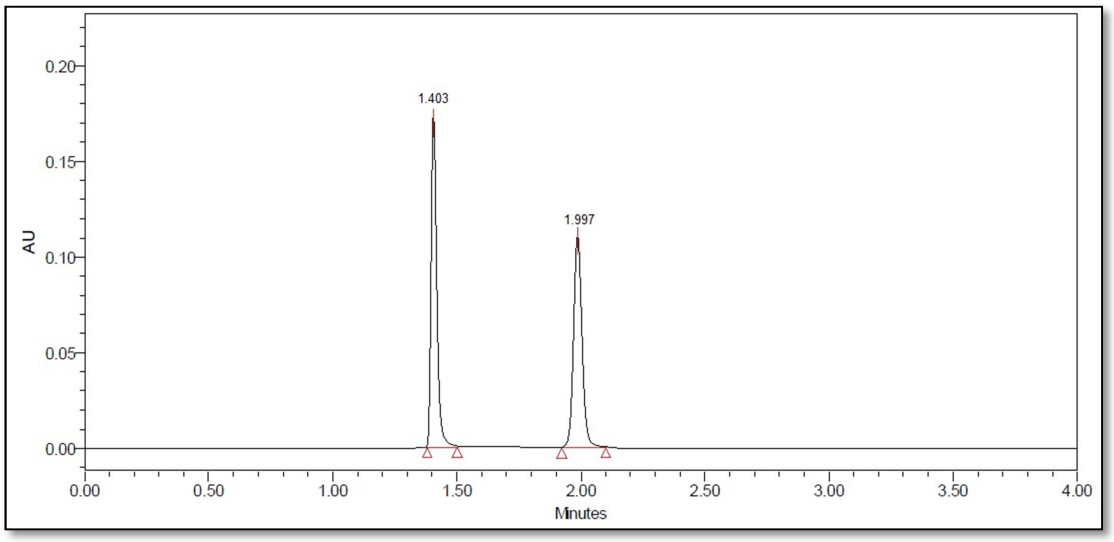


Figure 2: Optimized chromatogram of EMT and TDF

Table 1: System suitability analysis and an assay of EMT and TDF in pharmaceutical matrices

	System suitability parameters				Assay analysis	
	Tailing factor	Theoretical plates	Resolution	Retention time [min ±SD; n=6]	Truvada™ tablets [% Amount found ± SD]	Ricovir EM tablets [% Amount found ± SD]
					[Label claim EMT- 200 mg TDF- 300mg]	
EMT	1.23	2356.58	2.05	1.403 ± 0.012	99.48 ± 0.39	99.33 ± 0.65
TDF	1.48	2681.81		1.997 ± 0.018	99.88 ± 0.52	99.44 ± 0.33

*** n= number of determinations, SD= standard deviation**

Table 2: Assessment of the accuracy for the quantification of EMT and TDF using the proposed UHPLC method by ICH guideline

Addition of standard [µg/mL]	Average % recovery ±SD[a]	Grand average ± SD[b]	% RSD[c]
	EMT		
4.8	100.41 ± 0.46	100.11 ± 0.30	0.30
6.0	99.89 ± 0.12		
7.2	100.05 ± 0.33		
	TDF		
16	100.05 ± 0.66	99.88 ± 0.40	0.40
20	99.94 ± 0.35		
24	99.66 ± 0.19		

[a] Average %recovery of the three different solutions at each level concentration for each analyte.
[b]Average of % recovery of the three different levels concentration.
c Average of % standard deviation of all recoveries for three different concentration levels.

Table 3: Determination of the precision assay for the quantification of EMT and TDF

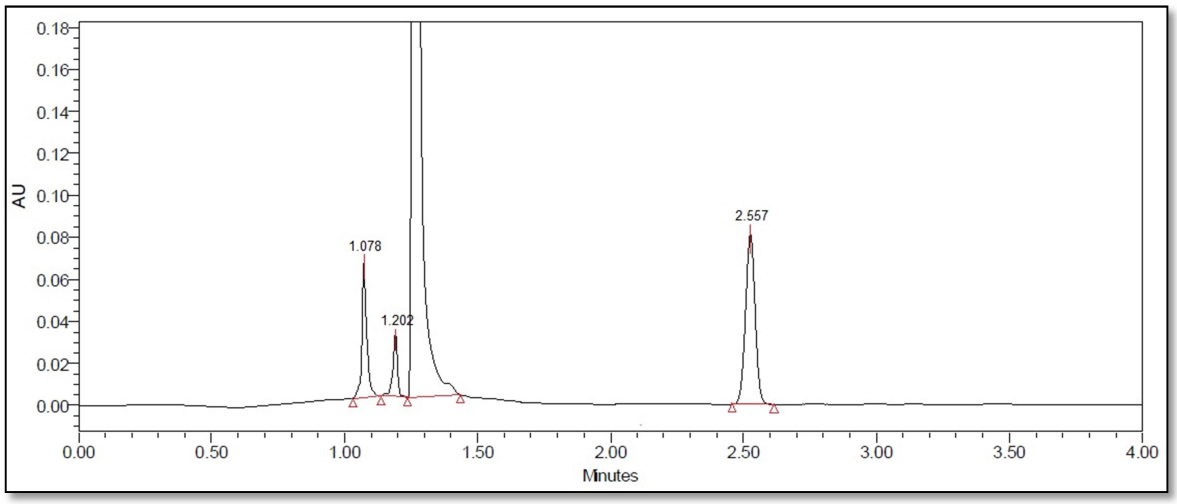
Standard concentration level [µg/mL]		Intra-day				Inter-day			
		% Amount found [n=3]		% RSD		% Amount found [n=3]		% RSD	
EMT	TDF	EMT	TDF	EMT	TDF	EMT	TDF	EMT	TDF
9	30	99.42	99.52	0.82	0.64	99.30	99.35	1.00	0.53
12	40	99.46	99.72	1.10	0.33	99.55	98.96	1.12	0.57
15	50	99.23	100.25	0.90	0.54	98.73	100.05	0.46	0.53
Repeatability assay variability									
EMT	TDF	% Amount found [n=6]		% RSD		% Amount found [n=6]		% RSD	
9	30	99.68 ± 0.90		0.91		99.78 ± 0.62		0.62	

*** n= number of determinations, %RSD= percent relative standard deviation**

Table 4: Evaluation of robustness experiment for determination of EMT and TDF

Parameters		% Amount found ±SD [n=6]		Average % Amount found ±SD		% RSD	
		EMT	TDF	EMT	TDF	EMT	TDF
Flow rate [mL/min]	0.4	100.55 ± 0.27	100.04 ± 0.13	100.87 ± 0.58	100.30 ± 0.11	0.58	0.11
	0.6	101.20 ± 0.90	100.57 ± 0.10				
Detection of wavelength [nm]	271	99.42 ± 0.20	99.14 ± 0.19	99.70 ± 0.26	99.14 ± 0.28	0.26	0.28
	275	99.99 ± 0.32	99.15 ± 0.37				
Proportion of methanol [% *v/v*]	20	100.80 ± 1.16	99.71 ± 0.30	100.68 ± 0.99	99.99 ± 0.27	0.99	0.26
	40	100.56 ± 0.83	100.27 ± 0.24				

*** n= number of determinations, SD= standard deviation, and %RSD= percent relative standard deviation**



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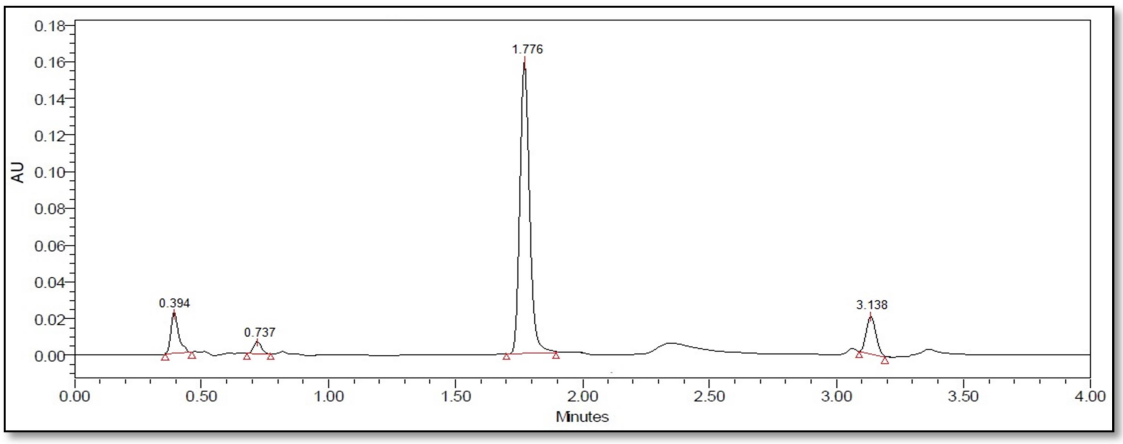

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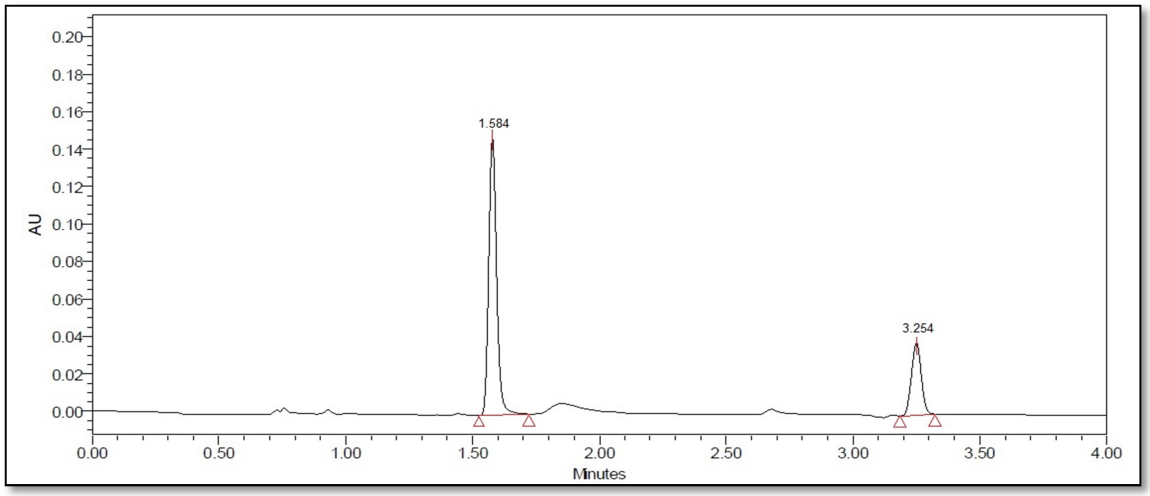

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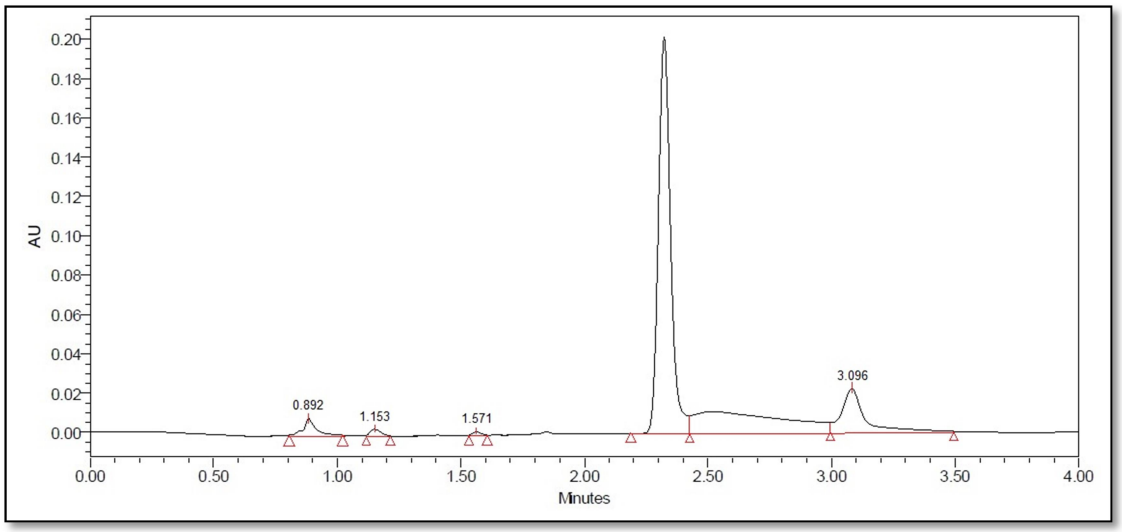
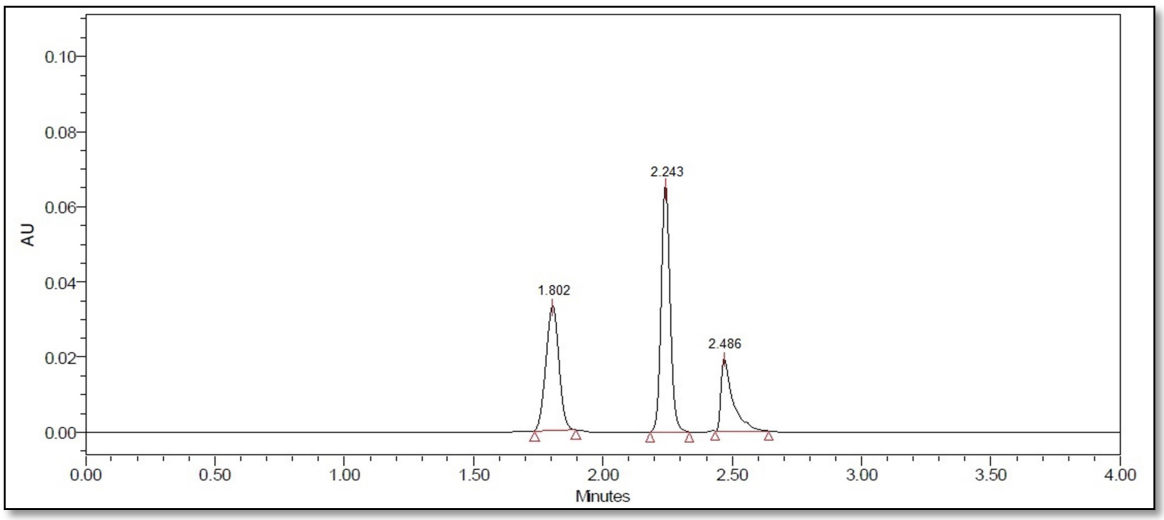


[d]

Figure 3: UHPLC chromatograms of a working standard solution of EMT 10 µg/mL after acid hydrolysis [a], alkaline hydrolysis [b], photolysis [c], and wet heat [d]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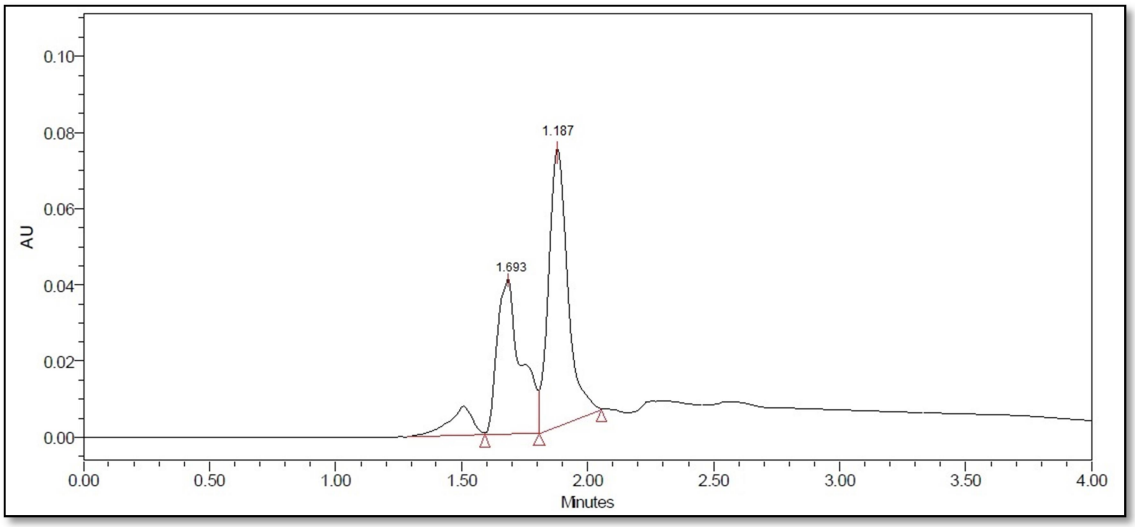
[a]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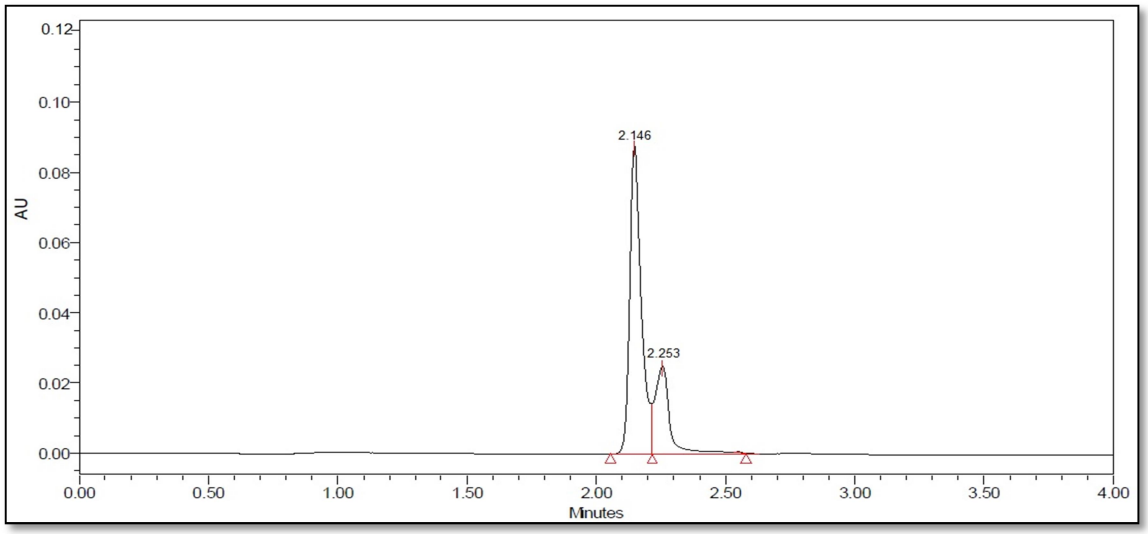
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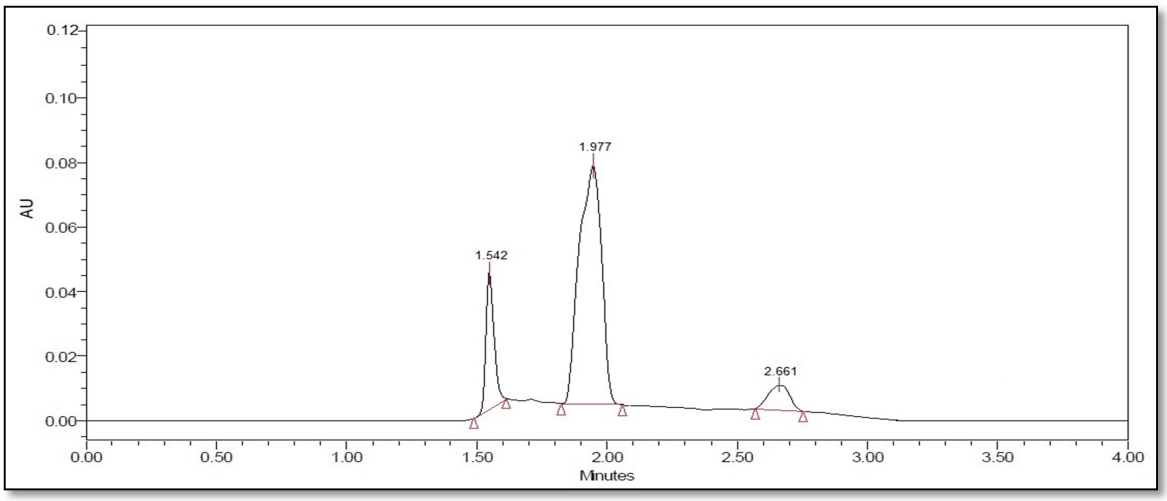

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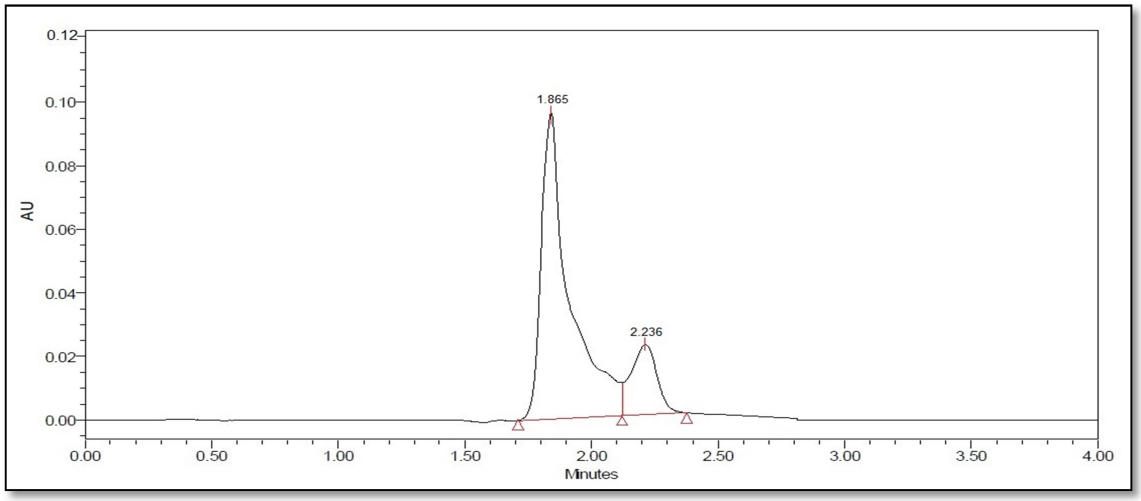

[e]

Figure 4: A characteristic UHPLC chromatograms of a working standard solution of TDF 10 µg/mL after acid hydrolysis [a], alkaline hydrolysis [b], oxidative [c], photolysis [d], and wet heat [e]

Table 5: Summary of data on EMT and TDF degradation under different stress conditions

Degradation conditions	Number of impurities	Rt of impurities [min]	% Degradation
For EMT			
Acidic hydrolysis-1 M HCl reflux for 80 °C for 6 h	03	1.078, 1.202, 2.557	9.06
Alkaline hydrolysis- 2 M NaOH for 3 days	03	0.394, 0.737, 3.138	12.22
Photolysis- ≥360Wh/m^2 at 30°C for 14 consecutive days	01	3.254	5.38
Wet heat- Digital controlled thermostatic hot air oven at 80 °C for 5 h	04	0.892, 1.153, 1.571, 3.096	13.67
For TDF			
Acidic hydrolysis- 0.5 M HCl for 80°C for 12 h	02	1.802, 2.486	18.20
Alkaline hydrolysis- 2 M NaOH at 80°C for 6 h	01	1.693	11.87
Oxidation- 6 % H_2O_2 *v/v* for 2 days	01	2.253	5.68
Photolysis- ≥360Wh/m^2 at 30°C for 14 consecutive days	02	1.542, 2.661	12.79
Wet heat- Digital controlled thermostatic hot air oven at 80 °C for 5 h	01	2.236	12.45

5. CONCLUSION

Finally, for the simultaneous quantification of EMT and TDF in FDC tablets, we devised a reverse-phase stability-indicating UHPLC method. Since this method was specific, sensitive, accurate, linear, exact, and repeatable, it could be used to analyze EMT and TDF in various pharmaceutical matrices. The advantages of the proposed method over the previously reported ones are the use of advanced column packaging UPLC columns with particle sizes of less than 2 µm allowed for excellent and efficient separation of the analyte concentration from the degradation products.which ultimately savings on operating costs and revealed excellent performance and analysis of results. EMT and TDF were successfully separated from their degradation products using optimized chromatographic conditions. The analytical approach satisfied all of the validation guidelines' acceptance criteria and may be used to acquire stability data by simultaneously estimating EMT and TDF in pharmaceutical matrices.

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Competing interests

The authors declare that they have no competing interests.

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