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A Simple, Reliable, Rapid and Stability Indicating Ultra Performance Liquid Chromatographic Method for the Quantitation of Emtricitabine

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ABSTRACT

A novel, simple, rapid and stability-indicating reversed-phase ultra performance liquid chromatographic method was developed and subsequently validated for quantitation of Emtricitabine (ECB) from drug substance matrix. The separation was achieved in less than 2.0 minutes on Waters ACQUITY UPLC BEH C_{18} (50 x 2.1) mm, 1.7µm column in isocratic mode with flow rate 0.25 mL/min. Mobile phase used was 0.015 M potassium dihydrogen phosphate buffer pH 2.2 and acetonitrile in ratio 75:25 v/v. Detection was carried out at the maximum wavelength of 284 nm using a photodiode array detector. The retention time of emtricitabine was 1.2 minutes. A forced degradation study was performed. Specificity of the method was established on drug substance by hydrolytic and oxidative stress conditions. Validation of analytical method was carried out as per the current ICH guidelines for linearity, recovery, precision, limit of detection, limit of quantification and robustness parameters.

Keywords: Emtricitabine (ECB), Ultra Performance Liquid Chromatography, Antiretroviral, Stability indicating, ICH

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INTRODUCTION

Emtricitabine is chemically 4-amino-5-fluoro-1-[2-(hydroxy methyl)-1,3-oxathiolan-5-yl]-pyrimidin-2-one with molecular mass 247.25 g/mol[1]. It is used as a nucleoside reverse transcriptase inhibitor (NRTI) for the treatment of HIV infection in adults [1]. Literature survey revealed that several analytical methods have been published for the determination of ECB by TLC/HPTLC [1-3],RP-HPLC[4-8], LC MS[9], UV-Visible Spectrophotometer[10] and by UPLC[11]. Those published methods were having minimum 4 minutes run time by HPLC and 3 minutes by LCMS techniques. However, the exhaustive literature survey revealed that none of the most recognized pharmacopoeias or any journals published the method with less than 3 minutes run time in isocratic mode of elution. So we developed ultra performance liquid chromatographic isocratic procedure with less than 2.0 minutes run time which will serve as rapid, reliable, accurate and sensitive and stability indicating for estimation of ECB. Thus, here is an attempt to develop a new, simple, less time consuming and less expensive specific method of analysis for the given drug by using UPLC method and to perform its forced degradation studies as per the ICH guidelines. The chemical structure of ECB is presented in (Figure 1).

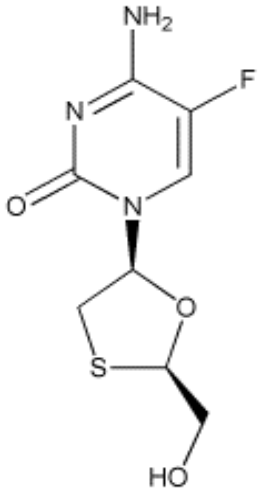

Figure. 1 Chemical structure of ECB

MATERIALS AND METHODS

Chemicals and Reagents

Potassium dihydrogen phosphate (GR grade), Orthophosphoric acid (GR grade) and Acetonitrile (HPLC grade) were purchased from Merck Fine Chemicals (Mumbai, India). The 0.22 µm nylon filters were purchased from Advanced Micro Devices Pvt Ltd., India. High purity Emtricitabine (ECB) was purchased from LGC Promochem India Ltd. Double distilled water was used throughout the experiment. Other chemicals used were of AR or GR grade.

Instrumentation

Waters acquity UPLC system comprised of degasser, quaternary pump, auto injector, column compartment with heater & chiller facility, photodiode array detector and a system control was used as a chromatographic system throughout the study. The data collection and data processing was done by using Waters Empower chromatography data software.

Chromatograpic conditions

The Waters ACQUITY UPLC ultra performance liquid chromatographic system was used comprised of degasser, quaternary pump, auto injector, column compartment with heater & chiller facility, photodiode array detector and system control. Data collection and data processing were accomplished by using Waters Empower chromatography data software. The separation of ECB was achieved on Waters ACQUITY BEH C_{18} (50 x 2.1) mm, 1.7µm column in isocratic mode. Mobile phase consisted of 0.015M potassium dihydrogen phosphate buffer pH 2.2 and acetonitrile in ratio of 72:25 v/v. The mobile phase pumped through column with flow rate of 0.25 mL/min in isocratic mode. Injection volume was 1.0 µL throughout the study. Based on the response of ECB peak the optimum wavelength 284 nm was selected.

Diluents Preparation

Prepared 0.015 M potassium dihydrogen phosphate buffer pH 2.2 and acetonitrile in ratio 90:10 v/v .

Blank Preparation

Diluent was used as a blank solution throughout the study.

Standard solution preparation

Dissolved 50 mg of ECB standard into 50 mL diluent and mixed. 5 mL of this solution was further diluted to 50 mL with diluent and mixed to achieve the concentration of ECB 100 µg/mL.

Sample solution preparation

Dissolved 50 mg of ECB sample into 50 mL diluent and mixed. 5 mL of this solution was further diluted to 50 mL with diluent and mixed to achieve the concentration of ECB 100 µg/mL.

METHOD VALIDATION

The optimized RP- HPLC method was validated according to ICH guidelines [13], with respect to specificity, linearity, robustness, limit of detection and limit of quantification, precision, recovery and solution stability parameters. System suitability parameters were also assessed during the experiment.

System suitability test

The system suitability test was evaluated according to United States Pharmacopoeia.

Forced degradation studies

Forced degradation studies were performed. The drug was subjected to stress conditions of acid hydrolysis, alkali hydrolysis, oxidation, thermal degradation and neutral degradation Stress testing of the drug substance can help to identify the likely degradation products, which can in turn help to establish the degradation pathways and intrinsic stability of the molecule. Specificity is the ability of the method to measure the analyte response in presence of its potential impurities. For hydrolysis degradation the sample solutions containing ECB 100µg/mL were treated with 5 mL 0.1N hydrochloric acid and 5 mL 0.1N sodium hydroxide solutions for 1 hr on water bath at 80°C. All the solutions prepared were quenched to their original pH before injection. For oxidation degradation the sample solution was treated with 5 mL of 5% v/v Hydrogen Peroxide and exposed to 80°C on water bath for 1 hr. All stressed sample solutions were studied for the purity angle and purity threshold values. In all the above stressed solution chromatograms the purity angle found less than the purity threshold which proved the homogeneity of the analyte peaks. The data interpretation confirmed that there were no co-eluting peaks with the analytes which proved the ability of the method to assess unequivocally the analyte of interest in the presence of potential interference. Major degradation observed in oxidative stressed condition at 5 mL 5 % v/v hydrogen peroxide.

Precision

The precision of the analytical method was established by method precision and intermediate precision. Method precision was evaluated by assaying six sample solutions of ECB of same batch where as intermediate precision was carried out on next day by using different instrument and different column by using the same batch used under method precision study. The RSD of six replicate method precision results and intermediate precision results was studied and found less than 1 %.

Accuracy

Accuracy of the method was evaluated by determining percent recovery of the ECB spiked in triplicate at three concentration levels 50, 100 and 150% respectively of test concentration in diluent. Accuracy of the method was evaluated at three different levels in triplicate on spiked samples.

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ for ECB was determined by signal to noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations. The LOD and LOQ for ECB were established from the RSD of six replicate injections.

Linearity

The linearity of ECB was established in the range of 50 to 150% of test concentration (50.38 µg/ml to151.13 µg/ml) using serial dilutions of standard solutions. The linearity plot of ECB was studied and the following regression equation was established.

$y = 9069 x - 11981$ ($R^2 = 0.9992$).

Robustness

The robustness is the capacity of method to remain unaffected by small but deliberate changes in chromatographic conditions. Robustness was established by testing the influence of small changes in column temperature ($\pm 5^0$C), change in flow rate (±10%) and changes in organic composition of mobile phase (±2 %). The robustness data obtained was evaluated for the system suitability criteria as per the United States pharmacopoeia. The data obtained confirmed that the method is robust for the above parameters.

Solution Stability

To demonstrate the stability of standard and sample solution, the solutions were analyzed over a period of 12 hrs stored at room temperature and the peak area counts observed were studied for all time points. The results confirmed that the peak areas of the ECB remained within acceptable range and no significant degradation was observed during this period. Thus the study concluded that the solutions can be used for the analysis after the preparation of 12 hrs.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The mechanism of retention in the reverse phase packing is due to the partitioning of the molecule into the lipophilic stationary phase, which primarily depends upon the lipophylicity of the compound. The other factor that influences the degree of retention is the polarity of the mobile phase. The reversed-phase chromatography allows efficient separation of substances with different polarities by altering the composition/polarity of the mobile phase[12]. Retention, selectivity and peak symmetry of compounds are strongly influenced by the sorbents/stationary phases. Strongly distorted peaks of the compounds are often observed when unsuitable RP sorbents are used, due to the interaction of the compounds with free silanol groups on the sorbent matrix. ECB is weak acid compound with pKa value 2.65. The main objective of this investigation was to develop a rapid and reliable method on reverse phase stationary phase which will be helpful to analyze the samples in very short time and subject the drug to various stress studies. We initiated the development by using the Waters ACQUITY BEH C_{18} column on

UPLC by using the potassium dihydrogen phosphate as a buffer in mobile phase and methanol as organic modifier. Due to low percentage of organic modifier used, the retention of ECB observed higher on the stationary phase with peak symmetry more than 1.5. The higher retention observed due to the low polarity of the mobile phase. Hence we optimized the acetonitrile composition in mobile phase to achieve the retention time 1.2 minutes with symmetry near to 1.0. The optimum performance of chromatography was achieved in 0.015M potassium dihydrogen phosphate buffer pH 2.2 and acetonitrile in ratio 75:25 v/v as a mobile phase with 0.25 mL/min flow are in isocratic mode. The 284 nm wavelength of analysis was selected based on the spectral analysis of ECB peak by using the PDA. Typical UPLC chromatogram of Blank is shown in Figure 2. Standard chromatogram is presented in Figure 3. Sample chromatogram is presented in Figure 4. The % degradation, purity angle and purity threshold values are of all stressed conditions are tabulated in Table 1. Percentage RSD of method precision and intermediate precision average % recovery results are tabulated in Table 2.The results of system suitability test are also tabulated in Table 2. The data of LOD and LOQ for ECB is tabulated in Table 2.

Table 1 Forced degradation data of ECB

Parameters	% Assay	% Deg[a]	PA[b]	PT[c]
Control sample	100.32	NA	0.038	1.031
Acid Deg. Sample(5ml 0.1NHCl/1hr)	100.67	0.00	0.038	1.029
Base Deg. sample(5ml 0.1NHCl/1hr)	100.07	0.25	0.036	1.030
Peroxide Deg. sample(5ml 5% H_2O_2/1hr)	43.81	56.51	0.066	1.066

[a] Percentage Degradation. [b] Purity angle. [c] Purity Threshold

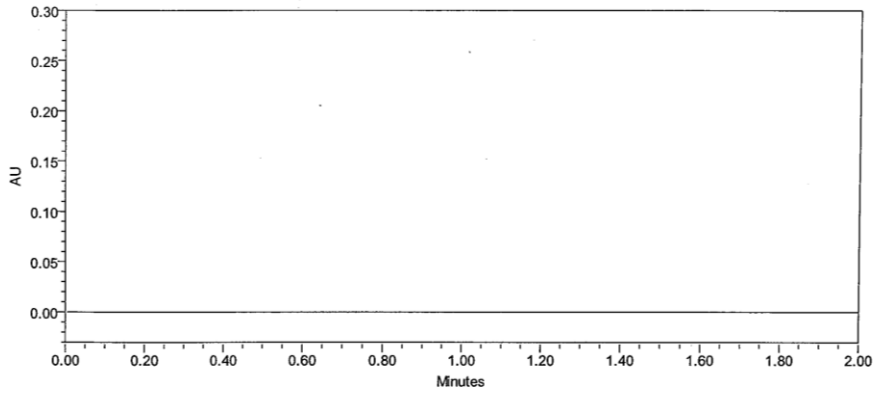

Figure. 2 Typical UPLC chromatogram of Blank

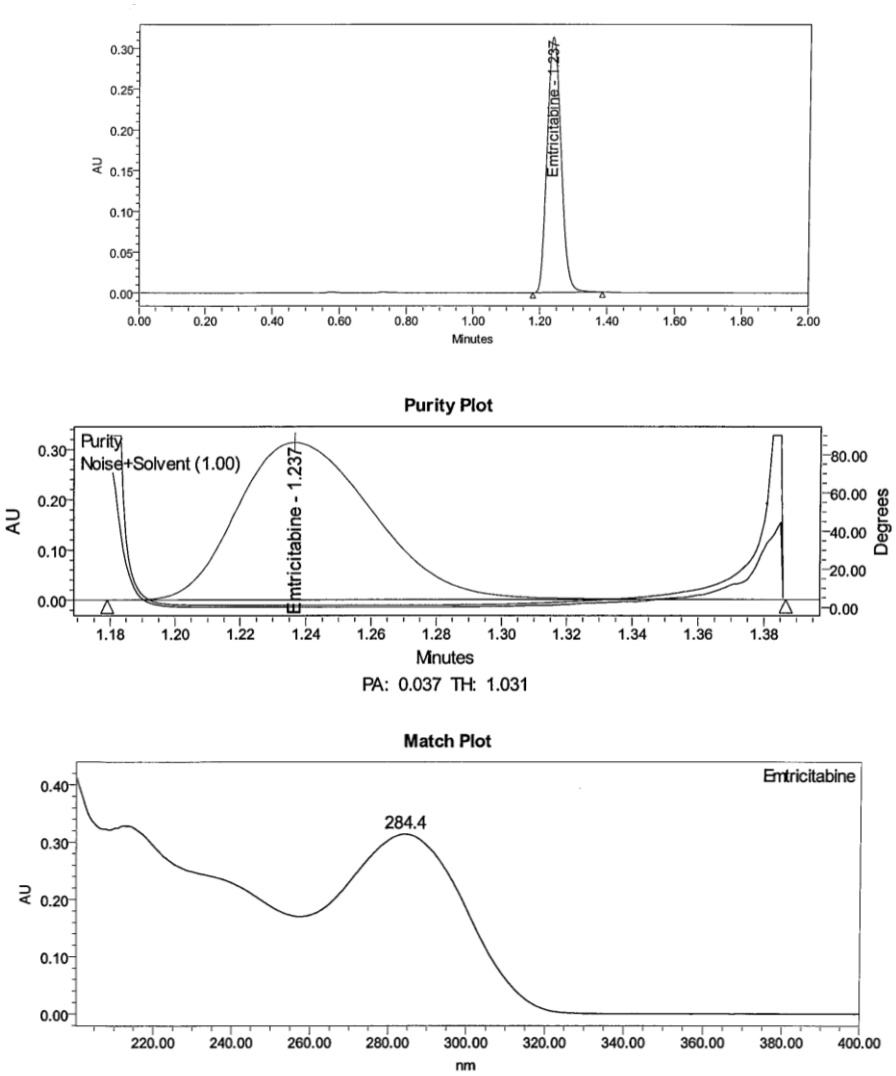

Figure. 3 Typical UPLC chromatogram, Purity profile and UV spectra of ECB Standard

Table 2 Method validation results of ECB

Parameters	ECB
System precision (% R.S.D.)	0.30
Tailing factor(NMT 2)	1.28
Theoretical plates (NLT 2000)	4221
Method Precision [a]	100.32
Method Precision (% R.S.D.)	0.35
Intermediate Precision	100.58
Intermediate Precision (% R.S.D.)	0.68
Accuracy (% Recovery at 50%)[b]	100.94
Accuracy (% Recovery at 100%)[b]	99.50
Accuracy (% Recovery at 150%)[b]	100.43
LOD (µg/mL)[a]	0.5038
LOQ (µg/mL)[a]	1.5113

[a] Average of six determinations, [b]Average of three determinations

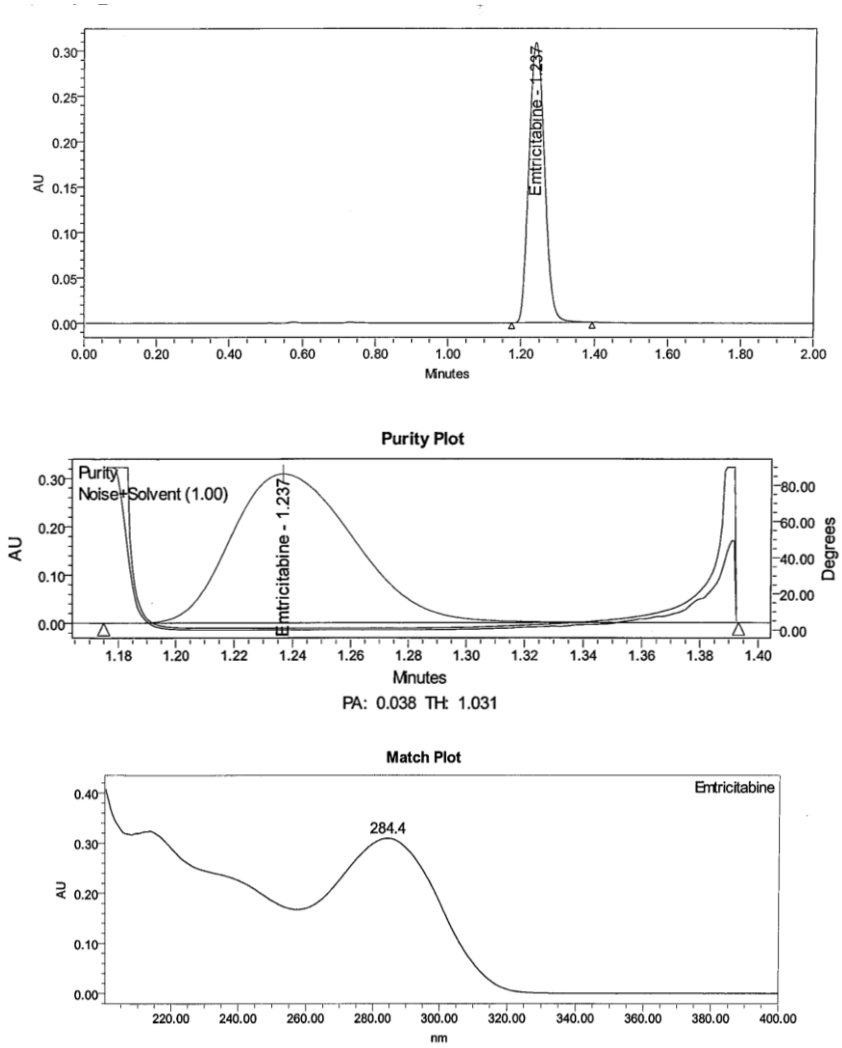

Figure. 4 Typical UPLC chromatogram, Purity profile and UV spectra of ECB Sample

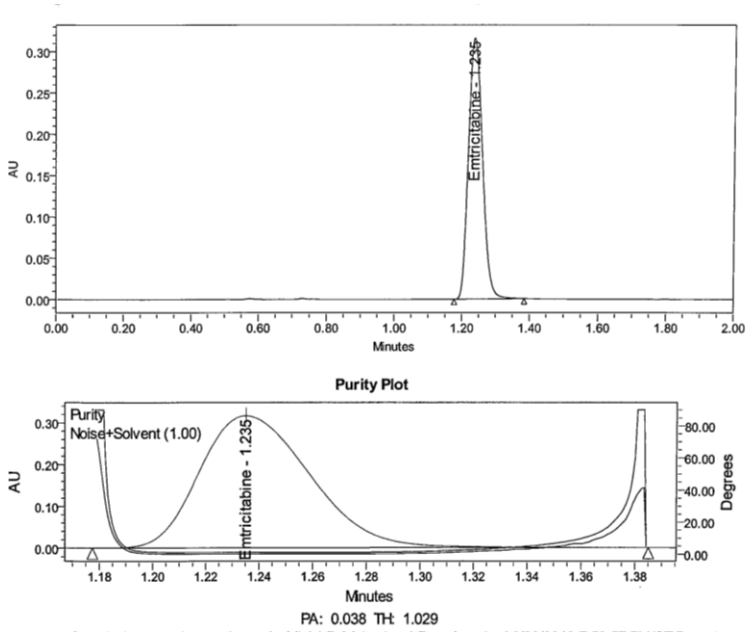

Figure. 5 Typical UPLC chromatogram and Purity profile of ECB acid Stressed Sample

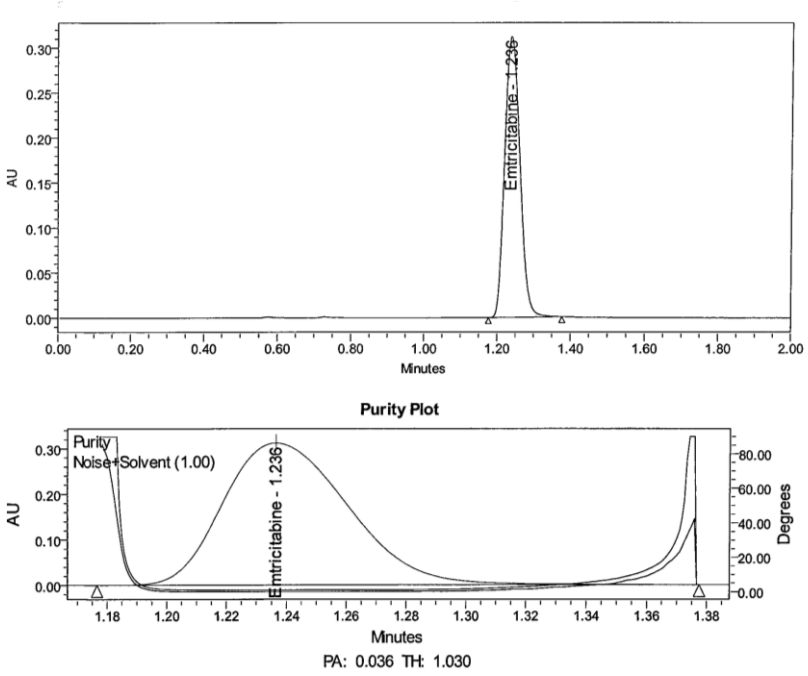

Figure. 6 Typical UPLC chromatogram and Purity profile of ECB base Stressed Sample

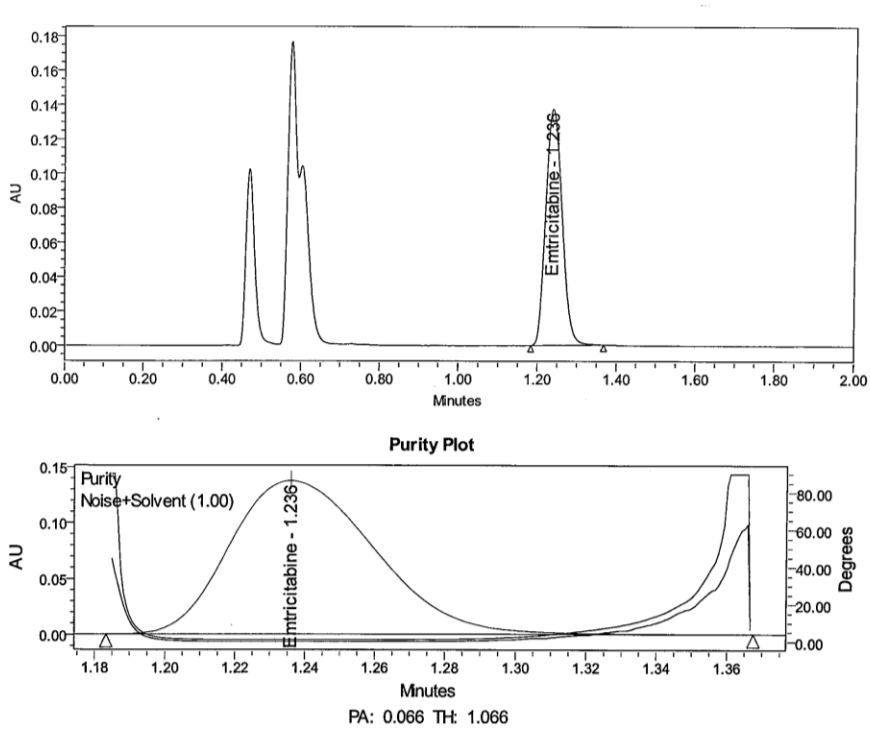

Figure. 7 Typical UPLC chromatogram and purity profile of ECB Oxidation Stressed sample

The stressed sample chromatograms of ECB along with peak purity profile and spectra are presented in Figure 5, Figure 6 and Figure 7. The representative chromatograms of ECB LOD and LOQ are presented in Figure 8 and Figure 9.

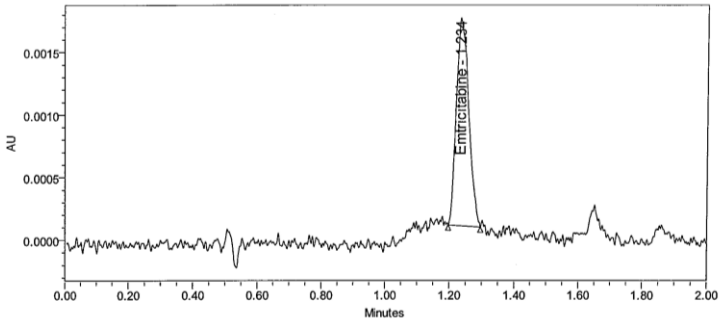

Figure. 8 Typical LOD chromatogram of ECB

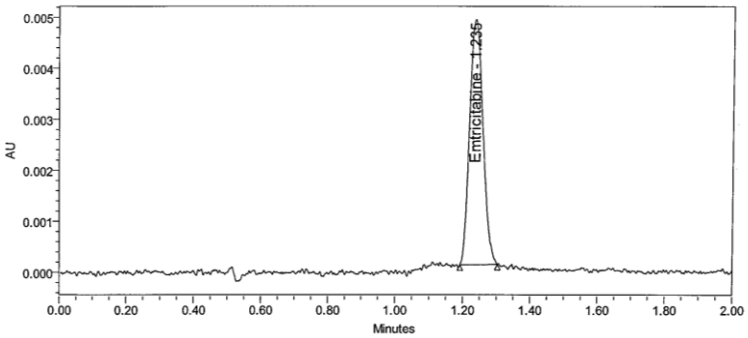

Figure. 9 Typical LOQ chromatogram of ECB

CONCLUSION

A new rapid RP-UPLC method with less than 2 minutes run time was successfully developed for the quantitation of emtricitabine from drug substance matrix. The validation of proposed method was carried out as per current ICH guidelines. The method validation results confirmed that the method is selective, precise, accurate, linear, robust and stability indicating. The proposed method provides optimum selectivity between the analyte peak and the degradants formed under degradation study. As the run time is very short we can analyze more number of samples in a very short time. In the proposed method emtricitabine elutes at 1.2 minutes and the analysis can be completed in less than 2 minutes. Hence this method can be used for the release and stability testing in quality control laboratories. Moreover, this method can also be used for the other dosage forms after establishment of the specificity studies. Under validation study sample solution found stable for 12 hrs at room temperature which can be helpful to cater the multiple unattended sample analysis or any breakdown of the instrument during analysis.

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