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Stability Indicating RP-UPLC-PDA Method Development, Validation of Multi drug Combination of Emtricitabine,Tenofovir Alafenamide and Rilpivirine In Bulk drug & its tablet formulation

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ABSTRACT

Stability representing Ultra Performance LC method was developed for Assay of multi drug Combination of Rilpivirine, Emtricitabine and Tenofovir alafenamide in bulk active pharmaceutical ingredients & its tablet formulation, Validation was performed for all parameters .Retention times of Reference Standard Emtricitabine, Tenofovir Alafenamide and Rilpivirine was found to be 0.965, 1.528, 2.186 respectively, with the flow rate of 0.3 mille liters per minute by Injecting Volume of 2 micro liters by maintaining Run time of 3 minutes. Developed method was subjected to forced degradation studies under specified conditions, which meets the required criteria. Degradation product at 1.975 Rt was collected under various stress condition, cleavage of imidazole ring of tenafovir alafenamide confirmed with Proton NMR, ESI –MASS in + MODE.

Keywords: RP-UPLC-PDA, Emtricitabine, Tenofovir Alafenamide, Rilpivirine,

INTRODUCTION

Emtricitabine, Rilpivirine, and Tenofovir Alafenamide combination is used in the treatment of HIV-1 as initial therapy as a part of anti retroviral therapy[1-3].This combination was approved in March 2016 by US-FDA. Literature survey indicate no UPLC –PDA (stability indicating) method for this new fixed dose Alafenamide derivative combination [4-6].

As the Tenofovir disproxil fumarate causing the release of formic acid with frequent administration in HIV-I patients, which ultimately cause GI disturbances, henceforth alternative

analogue that is Tenafovir Alafenamide was proved alternative drug to that of previous analogue, The current work describes the stability indicating accurate, Precise Ultra Performance LC method for quantifying Emtricitabine, Rilpivirine, Tenofovir Alafenamide in bulk drug &its formulation by using PDA detector.

The ICH recommend stability testing for combined drug formulation for enhancing safety and to avoid the interference of degradation products during the routine analysis [7,8]. The Objective of current work was to develop the method for quantifying and to study the degradation process under various stress condition for assay of long term stability testing samples and in routine analysis.

MATERIALS &METHODS

Details of Chemicals

Emtricitabine, Rilpivirine, and Tenofovir Alafenamide Standards (Aurabindo Pharma Pvt Ltd), KH_2PO_4 (FINE chemical LTD), Water and Methanol for UPLC (LICHROSOLV (MERCK), Acetonitrile (Batch: IA51F65129, Merck) **.**

Table1: Percentage Degradation

Degradation aid	Emtricitabine	Tenofovir Alafenamide	Rilpivirine
Standard	0.8	1.2	1.3
Acid	3.15	3.01	8.08
Base	5.77	4.27	3.44
Peroxide	4.15	4.97	8.37
Thermal	8.07	6.47	8.74
Photo	5.31	4.37	6.08

INSTRUMENTS USED

1. UPLC: WATERS, Aquity Empower, 2695 separation module, PDA detector
2. UV/vis spectrophotometer, LABINDIA UV 3000
3. Weighing Machine (afcoset ER-200A))

Preparation of Diluents

Buffer

0.1M tri ethyl amine buffer was prepared , P^H was adjusted to 3 with Ortho phosphoric acid and filtered through membrane filter of pore size 0.45 microns.

Mobile phase

To 35% of 0.1M tri ethyl amine buffer add 65% Acetonitrile HPLC Grade, degassed and filtered through membrane filter under vacuum condition.

Standard Solution Preparation

300 ppm of Emtricitabine, 450 ppm of Tenofovir Alafenamide and 37.5 ppm of Rilpivirine was prepared by using the mobile phase and injected into liquid chromatography (Standard Stock Solution).

Sample Solution Preparation

Accurately weigh 10 tablets, crush and take equivalent to 200 mg of Emtricitabine, 300 mg of Tenofovir Alafenamide and 25 mg of Rilpivirine powder sample in a 100 mL volumetric flask, transfer 65 mL of mobile phase and subject to sonication for 30 minutes make up the volume with diluent. Then it is filtered through 0.45 micron filter (Sample Stock solution) From the above take aliquots to prepare 300 ppm of Emtricitabine, 450 ppm of Tenofovir Alafenamide and 37.5 ppm of Rilpivirine.

Table 2: Accuracy results for Tenofovir Alafenamide

%Concentration (at specification Level)	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%(n=6)	150	151.87	101.25	100.48
100%(n=6)	300	301.88	100.63	
150%(n=6)	450	448.04	99.57	

Procedure

Inject 2 µL of the standard, sample (each six replicates) into the UPLC system and calculate the percentage RSD and calculate the %Assay by using the formulae.

Method Validation

Precision

Procedure

The standard solution was injected for six times and measure the area for all six replicate samples in UPLC.

INTERMEDIATE PRECISION/RUGGEDNESS

Procedure

The standard solutions prepared in the precision were injected on the other day, for six times and measure the area for all six samples in UPLC.

Acceptance Criteria

The % RSD for the area of six standard injections results should not be more than 2%.

Limit of Detection of Tenofovir Alafenamide

450 µg/ml, 0.18 µg/ml solutions were Prepared by taking suitable aliquots from Stock by using mobile Phase as diluents.

Calculation of S/N Ratio

Average Baseline Noise obtained from Blank : 66µV

Signal Obtained from LOD solution : 198µV

S/N = 198/66 = 3.00

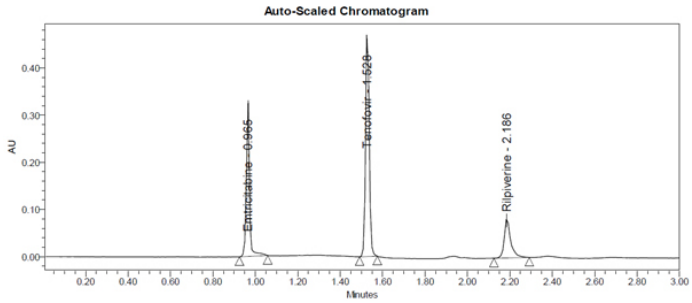

Fig.1: Typical chromatogram of Standard

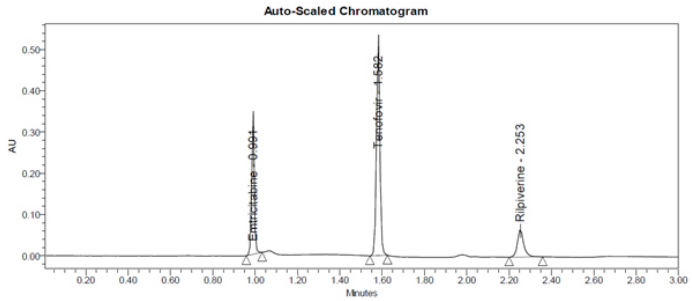

Fig. 2: Typical chromatogram of Sample

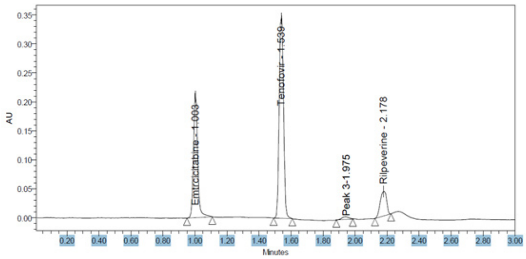

Fig. 3: Typical chromatogram of base degradation

Limit of Quantification of Tenofovir Alafenamide

45 µg/ml, 0.61 µg/ml solutions were Prepared by taking suitable aliquots from Stock by using mobile Phase as diluents,degassed,filtered through membrane filter .

Calculation of S/N Ratio

Average Baseline Noise obtained from Blank : 66 µV

Signal Obtained from LOQ solution :659µV

S/N = 659/66 = 9.98

Acceptance Criteria

S/N Ratio value shall be 10 for LOQ solution..

LINEARITY

From the stock solution, suitable aliquots were taken to prepare the following samples of various drug concentrations by using mobile phase as diluent.

Sample– I

100 ppm of Emtricitabine, 150 ppm of Tenofovir Alafenamide and 12.5 ppm of Rilpivirine.

Sample – II

200 ppm of Emtricitabine, 300 ppm of Tenofovir Alafenamide and 25ppm of Rilpivirine.

Sample – III

300 ppm of Emtricitabine, 450 ppm of Tenofovir Alafenamide and 37.5 ppm of Rilpivirine.

Sample – IV

400 ppm of Emtricitabine, 600 ppm of Tenofovir Alafenamide and 50ppm of Rilpivirine.

Sample – V

500 ppm of Emtricitabine, 750 ppm of Tenofovir Alafenamide and 62.5 ppm of Rilpivirine.

Procedure

2 micro liters of each level sample was injected into liquid chromatography, peak areas were measured, calculate correlation co efficient value by plotting the graph.

DEGRADATION STUDIES

Hydrolytic degradation under acidic condition

Transfer 1.5 ml of standard stock to volumetric flask of 10ml and add 3 ml of 0.1N Hydrochloric acid, flask was subjected to temperature of 60ºC for a period of 6 hours and the sample is neutralized with 0.1 N Sodium hydroxide and make up the volume to 10ml with mobile phase diluents, filter ,inject into the system under optimized chromatographic condition .

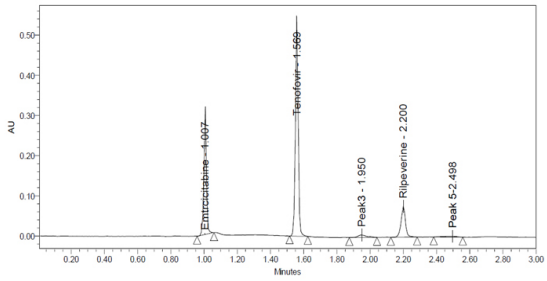

Fig. 4: Typical chromatogram of acid degradation

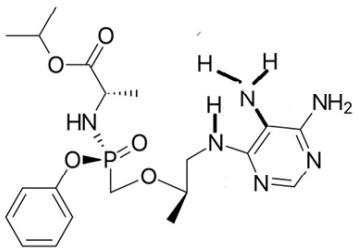

Fig. 5: Structure of degradation Product at 1.975 Rt

Hydrolytic degradation under alkaline condition

Transfer 1.5 ml of standard stock to the volumetric flask of 10ml and add 3 ml of 0.1 N Sodium hydroxide , flask was subjected to temperature of 60ºC for a period of 6 h and the sample is neutralized with 0.1N hydrochloric acid and make up the volume to 10ml with mobile phase diluents, filter ,inject into the system under optimized chromatographic condition.

Thermally induced degradation

Emtricitabine, Tenofovir Alafenamide and Rilpivirine sample was taken in Petri dish and kept in Hot air oven at 110^0 C for 24 hours. Then the sample was taken and diluted with diluents and injected into UPLC and analyzed.

Oxidative degradation

Transfer 1.5 ml of standard stock to the volumetric flask of 10ml and add 1 ml of 3% w/v of hydrogen peroxide, make up the volume to the mark and keep the flask at room temperature for a period of 15 minutes, filter, inject into the system under optimized chromatographic condition.

RESULTS & DISCUSSIONS

Optimized Chromatographic Conditions

Temperature	:	Ambient
Column	:	Thermosil Octa Decyl Column (4.6 x 50mm, 1.7 mm)
Buffer	:	0.1M Tri Ethyl Amine of P^H 3.0
Mobile phase	:	35% buffer 65% Acetonitrile
Rate of flow into UPLC:		0.3 mille liters per minute
Wavelength	:	260 mμ
Injection volume	:	2 μl
Sample Run time	:	3.0min

The %RSD for the area of six replicate samples was found to be within the specified limits for Precision, Intermediate Precision.

The Method Validation was done for all parameters and the results were within the Acceptance criteria. Acid, base, peroxide ,photolytic degradation product at the retention time of 1.975 was collected by using preparative UPLC , cleavage of imidazole ring of tenafovir alafenamide confirmed with Proton NMR, ESI –MASS in + MODE and developed method Proved to be effective even in the presence of degradation products.

CONCLUSION

Forced degradation studies were performed for the developed method as per US FDA, ICH guidelines, hence proved that developed method is stability indicating, can be very effective in bulk drug synthetic sites & even in formulation premises for quantifying the amount of drug in the tablet formulation .

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