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RP-HPLC SIMULTANEOUS DETERMINATION OF EMTRICITABINE, RILPIVIRINE, TENOFOVIR IN BULK AND ITS DOSAGE FORM

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ABSTRACT

A simple and selective LC method is described for the determination of Emtricitabine, Rilpivirine and Tenofovir dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of mixed Phosphate buffer pH: 4: Acetonitrile (40:60v/v/v), with detection of 262nm. Linearity was observed in the range 32.5-97.5 µg /ml for Emtricitabine (r^2 =0.9976) 40-120µg/ml for Rilpivirine (r^2 =0.996)& 2-6µg /ml for Tenofovir (r^2 =0.993) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

KEY WORDS: Liquid chromatography (LC), RSD Relative standard deviation,r^2 correlation coefficient.

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INTRODUCTION

Pharmaceutical analysis simply means analysis of pharmaceuticals. Webster' dictionary defines a pharmaceutical is a medical drug. A more appropriate term for a pharmaceutical is active pharmaceutical Ingredient (API) or active ingredient to distinguish it from a formulated product or drug product is prepared by formulating a drug substance with inert ingredient (excipient) to prepare a drug product that is suitable for administration to patients. Research and development (R&D) play a very comprehensive role in new drug development and follow up activities to ensure that a new drug product meets the established standards is stable and continue to approved by regulatory authorities, assuring that all batches of drug product are made to the specific standards utilization of approved ingredients and production method becomes the responsibility of pharmaceutical analysts in the quality control (QC) or quality assurance department The methods are generally developed in

an analytical R&D department and transferred to QC or other departments as needed. At times they are transferred to other divisions. By now it should be quite apparent that pharmaceutical analysts play a major role in assuring the identity, safety, efficacy, and quality of drug product, safety and efficacy studies required that drug substance and drug product meet two critical requirements. Established identity and purity and established bio availability/dissolution.

Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI) for the treatment of HIV infection in adults. Emtricitabine is an analogue of cytidine. The drug works by inhibiting reverse transcriptase, the enzyme that copies HIV RNA into new viral DNA. Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1). Emtricitabine helps to block HIV reverse transcriptase, a chemical in your body (enzyme) that is needed for HIV to multiply. Emtricitabine is always used with other anti-HIV medicines to treat people with HIV infection. Emtricitabine may lower the amount of HIV in the blood (viral load). Emtricitabine may also help to increase the number of T cells called CD4 cells. Lowering the amount of HIV in the blood lowers the chance of death or infections that happen when your immune system is weak (opportunistic infections). People taking emtricitabine may still get opportunistic infections or other conditions that happen with HIV infection.

Rilpivirine is non-nucleoside reverse transcriptase inhibitor (NNRTI) which is used for the treatment of HIV-1 infections in treatment-naive patients. It is a diarylpyrimidine, a class of molecules that resemble pyrimidine nucleotides found in DNA. Because of its flexible chemical structure, resistance of rilpivirine is less likely to develop than other NNRTI's. FDA approved on May 20, 2011. Rilpivirine is the most potent NNRTI and has a EC50 of 0.73 nM in vitro against HIV-1 because its chemical structure allowed for better binding to reverse transcriptase.

Tenofovir disoproxil fumarate (a prodrug of tenofovir), marketed by Gilead Sciences under the trade name Viread®, belongs to a class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors (nRTIs), which block reverse transcriptase, an enzyme crucial to viral production in HIV-infected people. [Wikipedia] In vivo tenofovir disoproxil fumarate is converted to tenofovir, an acyclic nucleoside phosphonate (nucleotide) analog of adenosine 5'-monophosphate.

Tenofovir belongs to a class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors (NtRTIs), which block reverse transcriptase, an enzyme crucial to viral production in HIV-infected people. Tenofovir is currently in late-stage clinical trials for the treatment of hepatitis B. Tenofovir disoproxil fumarate is an acyclic nucleoside phosphonate diester analog of adenosine monophosphate. Tenofovir requires initial diester hydrolysis for conversion to tenofovir and subsequent phosphorylations by cellular enzymes to form tenofovir diphosphate. Tenofovir diphosphate is a weak inhibitor of mammalian DNA polymerases α, β, and mitochondrial DNA polymerase γ (1-4).

Analytical method development provides the support to track the quality of the product from batch to batch. Method development involves considerable trial and error procedures. The most difficult problem usually is where to start, what type of column is worth trying with what kind of mobile phase. Single dosage forms with combination of drugs are widely used today due to their advantages and their simultaneous estimation of individual component is a challenging task.

Literature review reveals that very few methods are reported to estimate Emtricitabine, Rilpivirine and Tenofovir . Hence aim of the study is to develop new RP HPLC method for the simultaneous estimation of Emtricitabine, Rilpivirine and Tenofovir in pharmaceutical dosage form.

MATERIALS AND METHOD (5)

Determination of Working Wavelength (λmax)

In simultaneous estimation of three drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate three drugs accurately.

Preparation of standard stock solution of Emtricitabine

13 mg of Emtricitabine was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 160 µg /ml of solution by diluting 1.6 ml to 10ml with methanol.

Preparation of standard stock solution of Rilpivirine

16.2 mg of Rilpivirine was weighed in to 100ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 0.1ml to 10ml with methanol.

Preparation of standard stock solution of Tenofovir

20 mg of Tenofovir was weighed in to 100ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 5µg/ml of solution by diluting 0.5 ml to 10ml with methanol.

Preparation of mixed standard solution

Weigh accurately 37.5mg of Emtricitabine and 75mg of Rilpivirine and 5mg of Tenofovir in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase From above stock solution 160µg/ml of Emtricitabine and 100µg/ml of Rilpivirine and 50µg/ml of Tenofovir is prepared by diluting 5.3ml to 10ml with mobile phase. This solution is used for recording chromatogram.

VALIDATION (6)

Specificity by Direct comparison method

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

Preparation of samples for Assay

Preparation of mixed standard solution

Weigh accurately 13mg of Emtricitabine and 1.62mg of Rilpivirine and 20mg of Tenofovir in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase From above stock solution 13µg/ml of Emtricitabine and 1.62µg/ml of Rilpivirine and 20µg/ml of Tenofovir is prepared by diluting 5.3ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Preparation of sample solution

5tablets (each tablet contains 200mg of Emtricitabine and 25mg of Rilpivirine and 300mg of Tenofovir) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. weight equivalent to 34.62mg of Emtricitabine , Rilpivirine and Tenofovir and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 13µg/ml of Emtricitabine and 1.62µg/ml of Rilpivirine and 20µg/ml of Tenofovir was made by adding 5.3ml of stock solution to 10 ml of mobile phase.

Linearity and range

Preparation of mixed standard solution

Weigh accurately 13mg of Emtricitabine and 1.62mg of Rilpivirine and 20mg of Tenofovir in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. This stock solution contains 13µg/ml of Emtricitabine and 1.62µg/ml of Rilpivirine and 20µg/ml of Tenofovir. This solution is used for recording chromatogram.

RESULTS AND DISCUSSION

Optimized chromatographic conditions

Efficiency and resolution and shape of the peaks were good. Hence the method was optimized. Optimized chromatograph was recorded and shown in Fig-1.

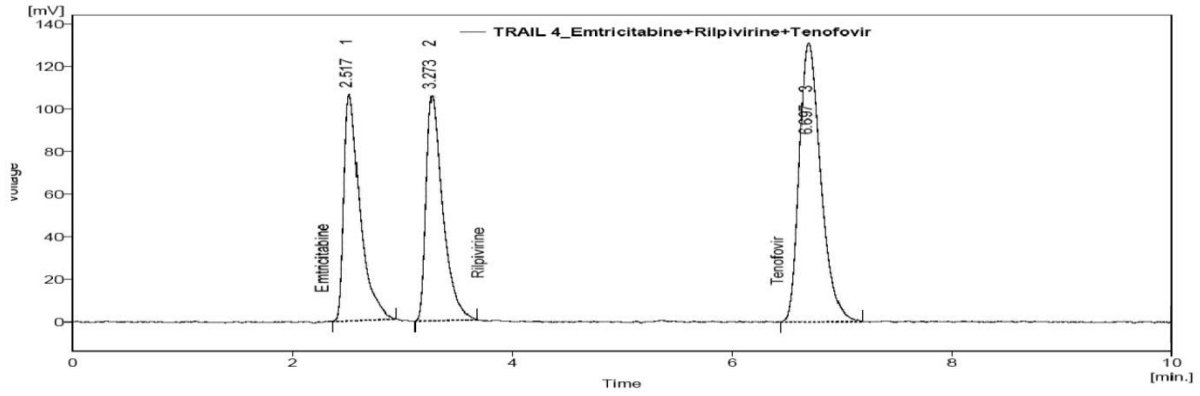

Figure-1 Chromatogram of Emtricitabine,Rilpivirine and Tenofovir

The amount of Emtricitabine, Rilpivirine and Tenofovir present in the taken dosage form was found to be100.04 %,99.77 % and 102.41% respectively (Table-1).

Table-1 Assay Results

Emtricitabine			**Rilpivirine**		**Tenofovir**	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	1077.431	1077.348	1094.978	1073.394	1775.284	1739.803
Injection-2	1082.305	1087.415	1094.085	1095.614	1760.260	1735.753
Injection-3	1079.334	1068.454	1085.067	1069.418	1748.738	1741.096
Injection-4	1088.743	1084.874	1090.770	1105.547	1732.934	1762.022
Injection-5	1075.047	1079.334	1081.967	1092.076	1741.886	1746.089
Average Area	1080.572	1079.485	1089.373	1087.21	1751.820	1744.953
Assay(%purity)	**100.04**		**99.74**		**102.41**	

The relationship between the concentration of Emtricitabine, Rilpivirine and Tenofovir and area of Emtricitabine, Rilpivirine and Tenofovir should be linear in the specified range and the correlation should not be less than 0.99 (Table-2-4 and Fig-2-4).

Table-2 Linearity of Emtricitabine

S.No.	**mcg**	**Area**
1	24	631.586
2	32	907.713
3	40	1091.004
4	48	1339.312
5	56	1549.123

Table-3 Linearity of Rilpivirine

S.No.	mcg	Area
1	3	659.236
2	4	919.393
3	5	1086.050
4	6	1348.518
5	7	1552.332

Table-4 Linearity of Tenofovir

S.No.	mcg	Area
1	30	1229.584
2	40	1482.509
3	50	1750.266
4	60	2124.626
5	70	2413.579

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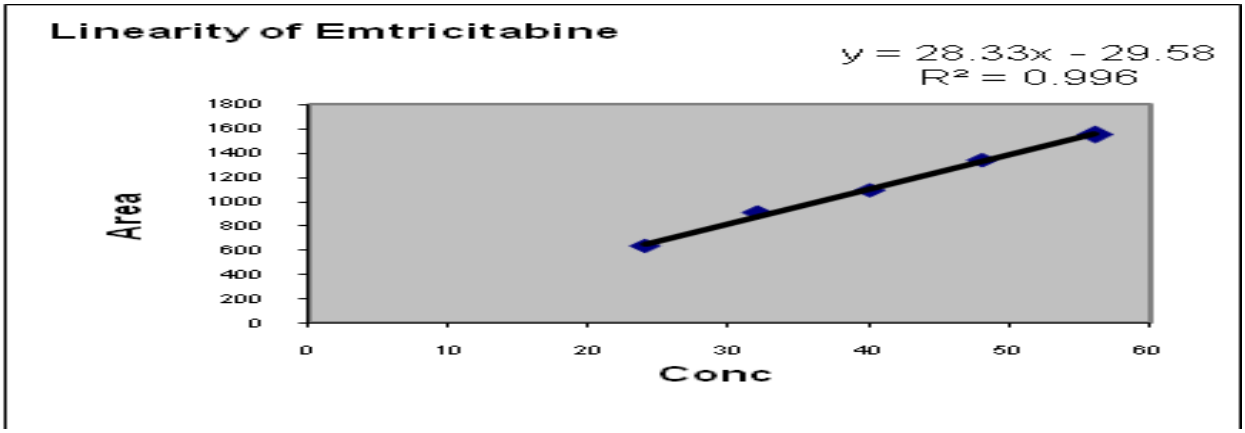

Fig-2 Linearity graph of Emtricitabine

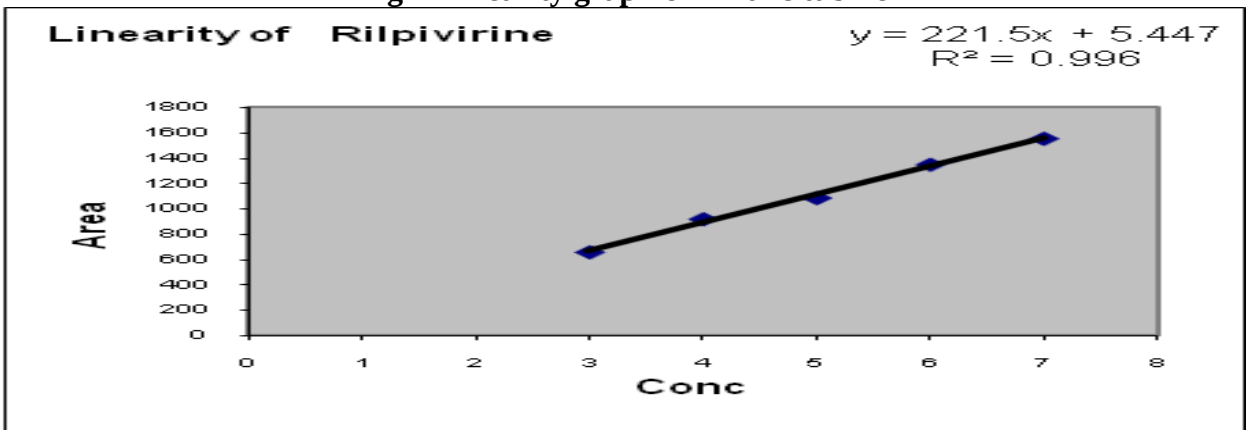

Fig-3 Linearity graph of Rilpivirine

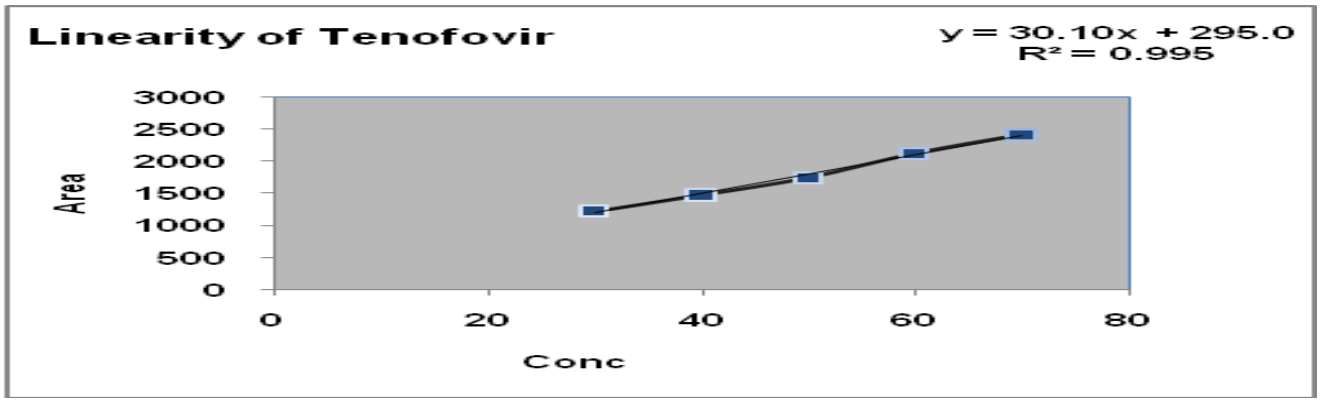

Fig-4 Linearity graph of Tenofovir

The percentage mean recovery of Emtricitabine, Rilpivirine and Tenofovir is 100%, 101%, and 99% respectively.

Test results for Emtricitabine, Rilpivirine and Tenofovir are showing that the %RSD of Assay results are within limits (Table-5 and 6).

Table-5 Results for Method precision of Emtricitabine, Rilpivirine

Emtricitabine		
S.No.	Rt	Area
1	2.520	1087.803
2	2.517	1089.666
3	2.523	1067.836
4	2.520	1097.279
5	2.517	1059.014
6	2.517	1071.854
avg	2.5190	1078.909
stdev	0.0024	14.836
%RSD	**0.10**	**1.38**

Rilpivirine		
S.No.	Rt	Area
1	3.277	1094.372
2	3.270	1101.764
3	3.277	1073.595
4	3.273	1108.428
5	3.270	1079.236
6	3.273	1075.584
avg	3.273	1088.830
stdev	0.003	14.708
%RSD	**0.10**	**1.35**

Table-6 Results for Method precision of Tenofovir

Tenofovir		
S.No.	Rt	Area
1	6.707	1753.454
2	6.690	1774.741
3	6.707	1729.087
4	6.707	1774.492
5	6.693	1729.167
6	6.693	1727.320
Avg	6.700	1748.044
Stdev	0.008	22.745
%RSD	**0.12**	**1.30**

CONCLUSION

A simple and selective LC method is described for the determination of Emtricitabine, Rilpivirine and Tenofovir dosage forms. From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of Emtricitabine, Rilpivirine and Tenofovirwas found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

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