INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND NOVEL SCIENCES

IJPRNS

NEW RP HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF EMTRICITABINE, RILPIVIRINE AND TENOFOVIRIN PHARMACEUTICAL DOSAGE FORM

Adapa Sowmya*, Yarrarapu Anjanadevi

Department of Pharmaceutical Analysis, Sri Siddhartha Pharmacy College, Ammavarithota, Nuziveedu, Andhra Pradesh, India.

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.

Author for correspondence
Adapa Sowmya,
Department of Pharmaceutical Analysis,
Sri Siddartha Pharmacy College, Ammavarithota,
Nuziveedu, Andhra Pradesh, India.
 Email id: adapa.sowmya@gmail.com

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. Chromatography is a family of analytical chemistry techniques for the

separation of mixtures. It involves passing the sample, a mixture that contains the analyte, in the "mobile phase", often in a stream of solvent, through the "stationary phase." The stationary phase retards the passage of the components of the sample. When components pass through the system at different rates they become separated in time, like runners in a marathon. Ideally, each component has a characteristic time of passage through the system. This is called its "retention time. A physical separation method in which the components of a mixture are separated by differences in their distribution between two phases, one of which is stationary (stationary phase) while the other (mobile phase) moves through it in a definite direction. The substances must interact with the stationary phase to be retained and separated by it. A chromatograph takes a chemical mixture carried by liquid or gas and separates it into its component parts as a result of differential distributions of the solutes as they flow around or over a stationary liquid or solid phase. Various techniques for the separation of complex mixtures rely on the differential affinities of substances for a gas or liquid mobile medium and for a stationary adsorbing medium through which they pass; such as paper, gelatin, or magnesium silicate gel. Analytical chromatography is used to determine the identity and concentration of molecules in a mixture. Preparative chromatography is used to purify larger quantities of a molecular species. 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. 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. Quality investigation plays a very important role in quality specification establishment of chemical drugs. The number of drugs introduced into the market every year .very often there is a time lag from the date of introduction of a drug into the market to the date of its

inclusion in pharmacopoeias. Hence, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs. 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 (1-5).

MATERIALS AND METHODS
Determination of Working Wavelength (λmax) (6-8)
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:
13mg of Emtricitabinewas 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:
16.2mg of Rilpivirinewas 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:
20mg of Tenofovirwas 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.

Assay
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 Emtricitabineand 1.62µg/ml ofRilpivirineand 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 ofEmtricitabine ,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 ofEmtricitabine and 1.62µg/ml of Rilpivirineand 20µg/ml of Tenofovirwas made by adding 5.3ml of stock solution to 10 ml of mobile phase.

Linearity and range

Weigh accurately 13mg of Emtricitabineand 1.62mg of Rilpivirine and 20mg of Tenofovirin 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 Emtricitabineand 1.62µg/ml ofRilpivirine and 20µg/ml of Tenofovir. This solution is used for recording chromatogram

RESULTS AND DISCUSSION

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra are shown in the fig-1.
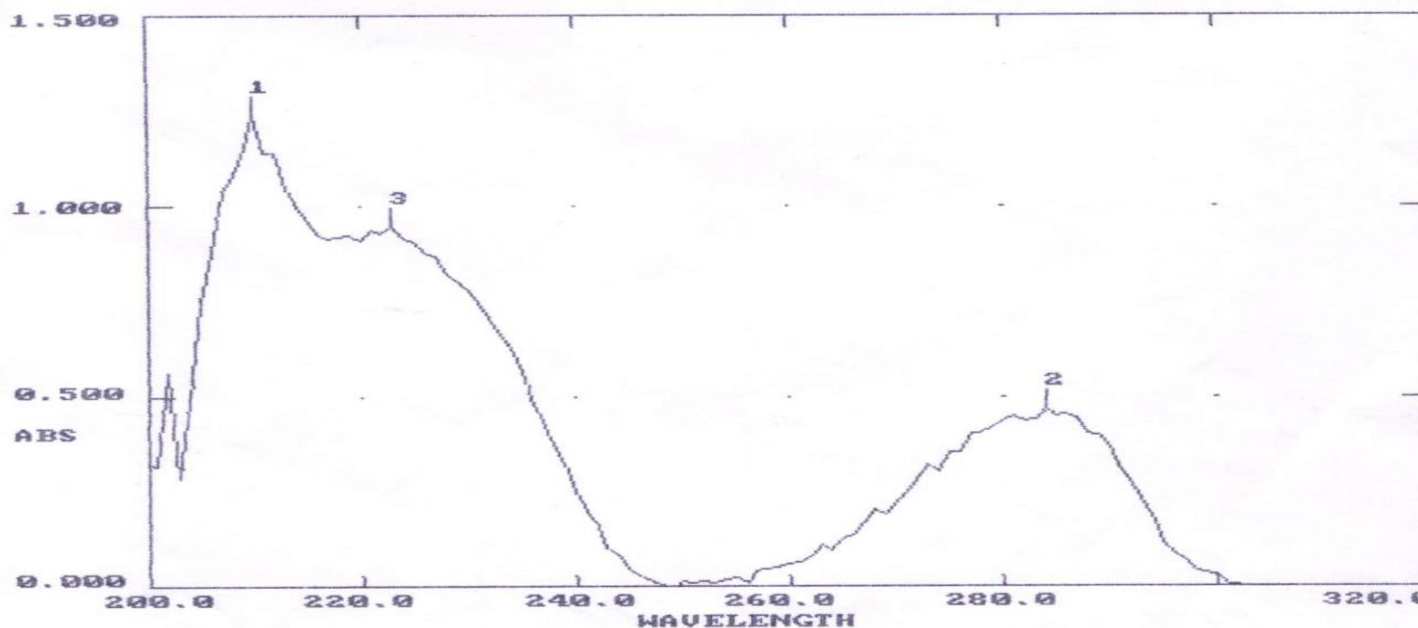

Fig-1 The Isosbestic point was found to be 275nm for Emtricitabine, Rilpivirine and Tenofovirin combination
The amount of Emtricitabine, Rilpivirine and Tenofovirpresent in the taken dosage form was found to be100.04 %, 99.77% and 102.41% respectively (Table-1)

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 correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of Emtricitabine, Rilpivirine and Tenofoviris 0.997, 0.988 and 0.981. The relationship between the concentration of Emtricitabine, Rilpivirine and Tenofovirand area of Emtricitabine, Rilpivirine and Tenofoviris linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits. The % recovery of Emtricitabine, Rilpivirine and Tenofovirshould lie between 98% and 102% (Fig-2-4). Test results for Emtricitabine, Rilpivirine and Tenofovirare showing that the %RSD of Assay results are within limits. From the observation the %RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged (Table-2).

Table-2 Results for Ruggedness

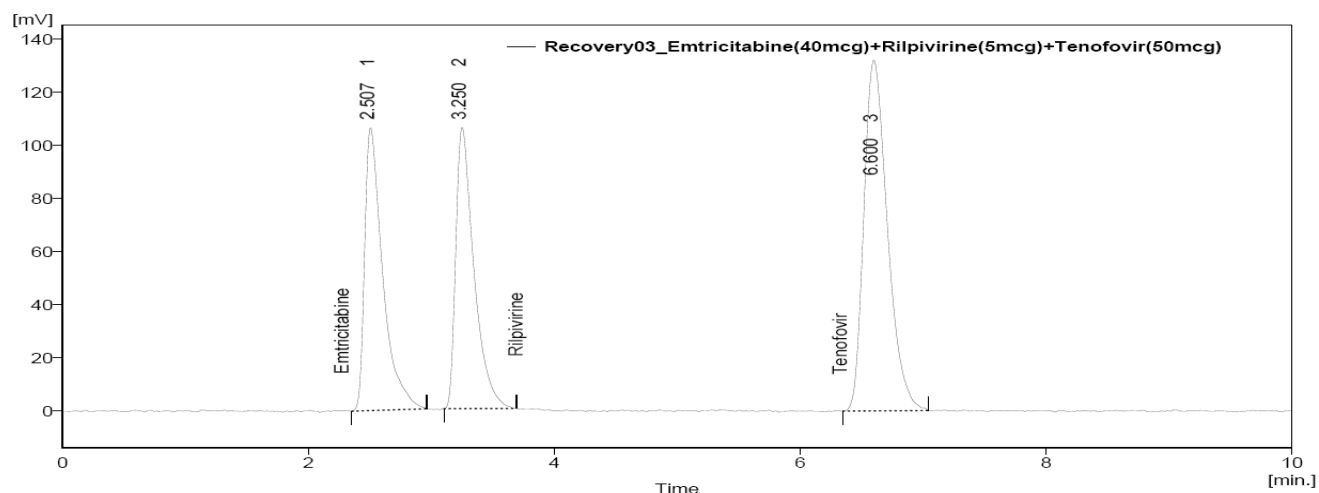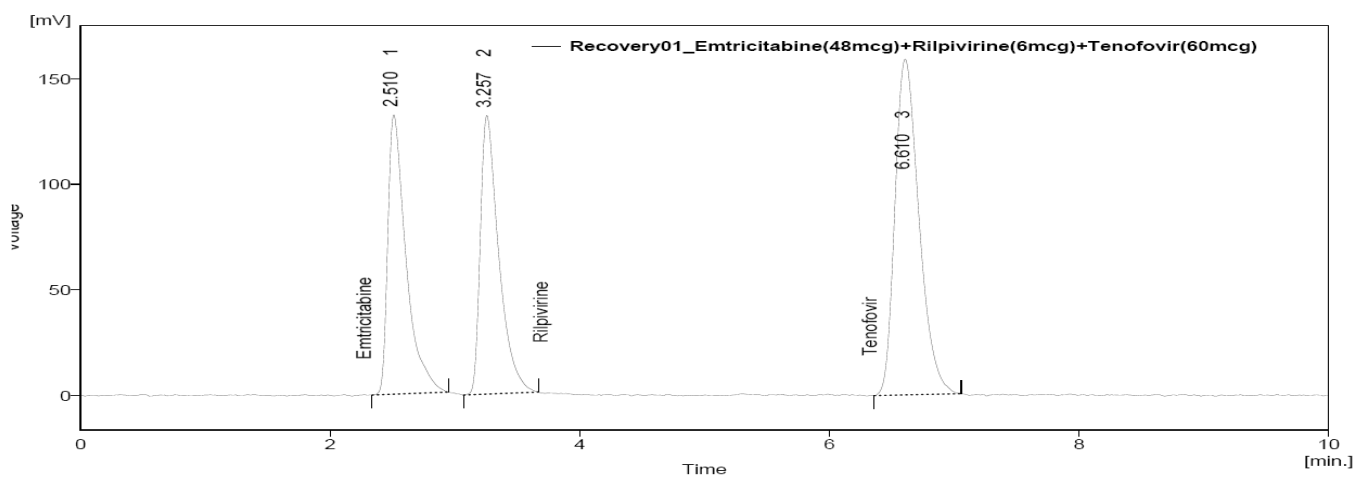| Emtricitabine	%Assay	Rilpivirine	%Assay	Tenofovir	%Assay
Analyst 01	100.86	**Analyst 01**	100.479884	**Analyst 01**	100.723731
Analyst 02	99.97565	**Analyst 02**	100.51467	**Analyst 02**	99.1048846

Fig-2 Chromatogram of 50% recovery

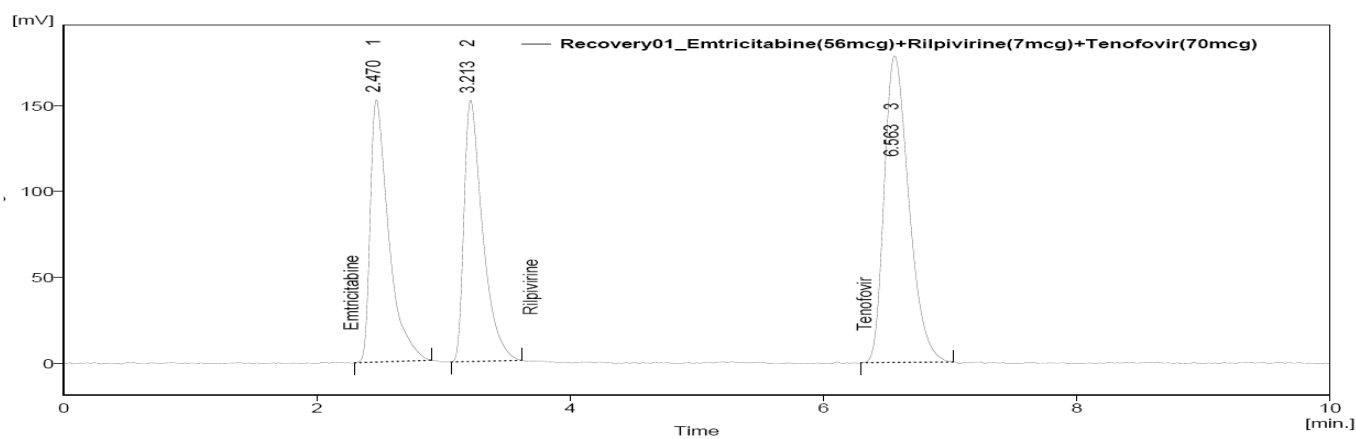**Fig-3 Chromatogram of 100% recovery**

Fig-4 Chromatogram of 150% recovery

CONCLUSION

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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