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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF SIMULTENIOUS ESTIMATION OF TENOFOVIR AND EMTRICITABINE IN BULK AND PHARMACEUTICAL DOSAGEFORMS BY USING RP-HPLC

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ABSTRACT

A simple, rapid reverse phase high-performance liquid chromatographic method was developed and validated for the simultaneous estimation of Tenofovir and Emtricitabine in bulk and pharmaceutical dosage forms. Chromatography was carried out by using Hypersil, 250 X 4.6 mm, 5µ internal diameter with a mixture of Buffer: acetonitrile in the ratio of 60:40 (v/v) as mobile phase. Determination of the different analytical parameter such as linearity, accuracy, precision and specificity, limit of detection (LOD), limit of quantification (LOQ) was done. The calibration curve was found to be linear for each analyte in the desired concentration range. The % recovery was found to be 99.85 and 99.99 for emtricitabine and tenofovir respectively. The proposed method is highly sensitive, precise, and accurate, which was evident from the LOD value of 0.4 and 1.3 for emtricitabine and tenofovir respectively and hence the present method can be applied successfully for the quantification of active pharmaceutical ingredient (API) content in the combined formulation of emtricitabine and tenofovir.

KEYWORDS

Anti viral (HIV) and Nucleoside reverse transcriptase inhibitor (NRTI) for the treatment of HIV.

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INTRODUCTION

Emtricitabine (FTC) (Figure No.1), with trade name Emtriva (formerly Coviracil), is a nucleoside reverse transcriptase inhibitor (NRTI) for the treatment of HIV infection in adults and children. Emtricitabine is also marketed in a fixed-dose combination with tenofovir (Viread) under the brand name Truvada. Emtricitabine is an analogue of cytidine. The drug

works by inhibiting reverse transcriptase, the enzyme that copies HIV RNA into new viral DNA. By interfering with this process, which is central to the replication of HIV, emtricitabine can help to lower the amount of HIV, or "viral load", in an patient's body and can indirectly increase the number of immune system cells (called T cells or CD4+ T-cells). Both of these changes are associated with healthier immune systems and decreased likelihood of serious illness. Emtricitabine is indicated in combination with other antiretroviral agents for the treatment of HIV infection in adults. Emtricitabine is commercially available and is approved by the FDA for treatment of HIV infection. Emtricitabine exhibits clinical activity against the hepatitis B virus (HBV). Among individuals with chronic HBV infection, emtricitabine treatment results in significant histologic, virologic, and biochemical improvement. The safety profile of emtricitabine during treatment is similar to that of a placebo[1-5]. Emtricitabine, however, cures neither HIV nor HBV infection. In a study involving individuals with HBV infection, symptoms of infection returned in 23% of emtricitabine-treated individuals who were taken off therapy. In studies involving individuals with chronic HIV infection, viral replication also resumes when study subjects are taken off therapy[6-8]. Emtricitabine is not approved by the FDA for treatment of HBV infection. As with drugs used to treat HIV infection, drugs used to treat HBV infection may have to be used in combination to prevent the evolution of drug resistant strains. Lamivudine is also active against HBV virus and commercially available. Like emtricitabine, lamivudine, when used on its own, does not completely suppress viral replication. This allows drug resistant strains to emerge[9-12]. The effectiveness of emtricitabine in combination with other anti-HBV drugs has not been established. Clinical trials are still ongoing. In clinical practice, toxicity with emtricitabine is unusual[13-15]. The most common treatment-related adverse events are diarrhea, headache, nausea, and rash. These symptoms are generally mild to moderate in severity, but they caused 1% of clinical trial patients to give up treatment. Skin discoloration, which is typically reported as hyper pigmentation and usually affects either the palms of the hands or the soles of the feet, is reported in less than 2% of individuals and is almost exclusive to patients of African origin. Among the more severe side effects patients may experience are hepatotoxicities or a lactic acidosis.

Tenofovir disoproxil fumarate (TDF or PMPA1) (Figure No.2), 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. Tenofovir was discovered through a collaborative research effort between Antonín Holý at the Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic (IOCB) in Prague, and Erik DeClercq, Rega Institute for Medical Research, Catholic University of Leuven, Belgium. Tenofovir is indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection in adults. This indication is based on analyses of plasma HIV-1 RNA levels and CD4 cell counts in controlled studies of tenofovir in treatment-naive and treatment-experienced adults. There are no study results demonstrating the effect of tenofovir on the clinical progression of HIV. It also has activity against wild-type and lamivudine-resistant HBV. The most common side effects associated with tenofovir include nausea, vomiting, diarrhea, and asthenia. Less frequent side effects include hepatotoxicity, abdominal pain, and flatulence[5]. Tenofovir has also been implicated in causing renal toxicity, particularly at elevated concentrations[16-23].

MATERIALS AND METHOD[24-30]

Chemical and Reagents

Emtricitabine and tenofovir as pure standard reference drug were purchased from local market were used for this present study water (TKA), acetonitrile methonal and orthophosphoric acid, tri ethyl amine (all HPLC grade) were purchased from merck specialities private limited, Mumbai, India.

Instruments

To develop a high pressure liquid chromatography method for quantitative estimation of emtricitabine and tenofovir (Figure No.3) an isocratic waters 2695 separation model HPLC instrument with Hypersil, 250 x 4.6 mm, 5µ. was used. The instrument with 7 Software Empower V 1.2.2.1 PDA detector 2990and variable wavelength programmable Lc 7000 Uv-detector. Analytical Balance Afcos, ER-180A, Sartorius- M500P, Meter, AG 104, Microbalance Sartorius-M500P, pH Meter Thermo scientific, pH Meter Thermo scientific.

Mobile phase

Buffer and Acetonitrile taken in the ratio 60:40.

Buffer Preparation

Accurately weighed 1.36gm of potassium dihyrogen phosphate in 1000ml of Volumetric fiask add about 900ml of milli-Q water, sonicate to dissolve and make up to 1000ml with milli-Q water add 1ml of Triethylamine and then is adujuted to pH 3.7 with dil.OPA solution.

Standard Preparation

Accurately Weighed and transferred 200mg of Emtricitabine and 300mg of Tenofovir working Standards into a 100 ml clean dry volumetric flask, add 70ml of diluent , sonicated for 5 minutes and make up to the final volume with diluents.

Sample Preparation

5 tablets were weighed and crushed into powder, in order to calculate the average weight of each tablet. From that powder weight equivalent to 200mg of Emtricitabine and 300mg of Tenofovir were transferred into a 500 mL volumetric flask, 450mL of diluents is added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Label Claim

200mg of Emtricitabine + 300mg of Tenofovir.

RESULTS AND DISCUSSION

Method and Validation

Linearity

The linearity of the response for emtricitabine and tenofovir assay method was determined by preparing and injecting standard solutions of emtricitabine and tenofovir. The linear regression data for the calibration curves (Figure No.4 and Figure No.5) indicates that the response is linear over the concentration range studied with correlation coefficient (r^2) value, slope and intercept as shown in Table No.1 and 2.

Precision

The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculate from six replicate injections of freshly prepared Emtricitabine and Tenofovir combined test solution in the same equipment at a concentration value of 50ppm on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak areas of the drugs were determined and precision as % RSD was reported (Table No.3 and 4). Shown graphs on intraday Figure No.6 and inter day Figure No.7.

Recovery

The recovery of the standard solutions was done by adding them to pre-analysed sample at different levels i.e. 50%, 100%, and 150% separately to study the accurate of the above method. The corresponding results were recorded (Table No.5).

Specificity

Specificity was performed to exclude the possibility of interference with excipients in the region of elution of Tenofovir and Emtricitabine. The specificity and selectivity of the method was tested under normal conditions and the results of the tests proved that the components other than the drug did not produce a detectable signal at the retention place of Tenofovir and Emtricitabine.

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ were determined from standard deviation of y-intercept of regression line and slope method as per ICH guidelines (Table No.6).

Rubustness

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. In this study, the chromatographic parameters monitored were retention time, area, capacity factor, tailing factor and theoretical plates. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above.

Table No.1: Linearity of the response for Emtricitabine and Tenofovir

S.No	Pipetted from stock (Ml)	Volume of flask (mL)	Tenofovir				%Linearity Level
			Con(ppm)	Peak area	Con(ppm)	Peak area	
1	0.25	10	50	642191.3	75	425107	25
2	0.50	10	100	1295718	150	873548	50
4	0.75	10	150	1922463	225	1266288	75
5	1.00	10	200	2501859	300	1684996	100
6	1.25	10	250	3119127	375	2081388	125
7	1.50	10	300	3690467	450	2457058	150

Table No.2: Validation Parameters

S.No	Parameters	Tenofovir	Emtricitabine
1	Slope	8210	8201
2	Intercept	23857	36595
3	Calibration range	75-450	50-300
4	Correlation coefficient(r^2)	0.999	0.999

Table No.3: Intraday Precision

S.No	Concentration	Emtricitabine Peak area	Tenofovir Peak area
1	50ppm	1678326	2526833
2	50ppm	1678382	2520891
3	50ppm	1674008	2526154
4	50ppm	1673468	2535331
5	50ppm	1673981	2525751
6	50ppm	1679489	2527371
% RSD		0.22	0.18

TableNo.4: Inter day Precision

S.No	Concentration	Emtricitabine Peak area	Tenofovir Peak area
1	50ppm	1583532	2418879
2	50ppm	1582948	2412484
3	50ppm	1583039	2414951
4	50ppm	1587154	2418775
5	50ppm	1574125	2413279
6	50ppm	1565952	2395141
% RSD		0.5	0.4

Table No.5: The recovery of the standard solutions

S.No	Conc. in ppm Tenofovir	Tenofovir amount recovery	% of recovery Tenofovir	Conc in ppm Emtri	Emtricitabine recovery	% of amount recovery emtricitabine
1	100	99.75	99.747	150	150.04	100.03
2	200	200.4	100.222	300	302.09	100.69
3	300	301.9	100.645	450	447.40	99.20
			Avg 100.2%			Avg Recovery 99.97%

Table No.6: System suitability and Validation parameters

S.No	Parameters	Emtricitabine	Tenofovir
1	Theoretical plates	11953	9261
2	Retention Time	3.3	4.5
3	Tailing factor	1.17	1.68
4	LOD	0.58	0.58
5	LOQ	1.77	1.77
6	% RSD	0.06	0.07

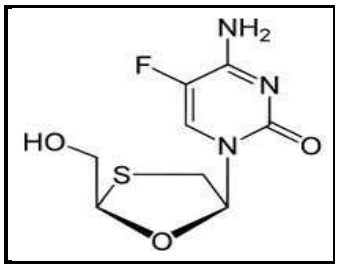


Figure No.1: Structure of Emtricitabine

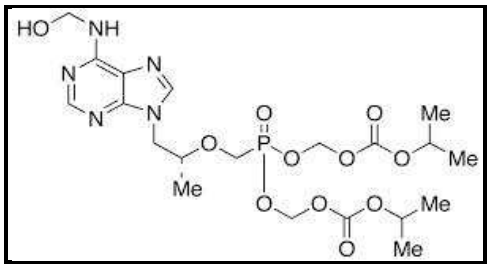
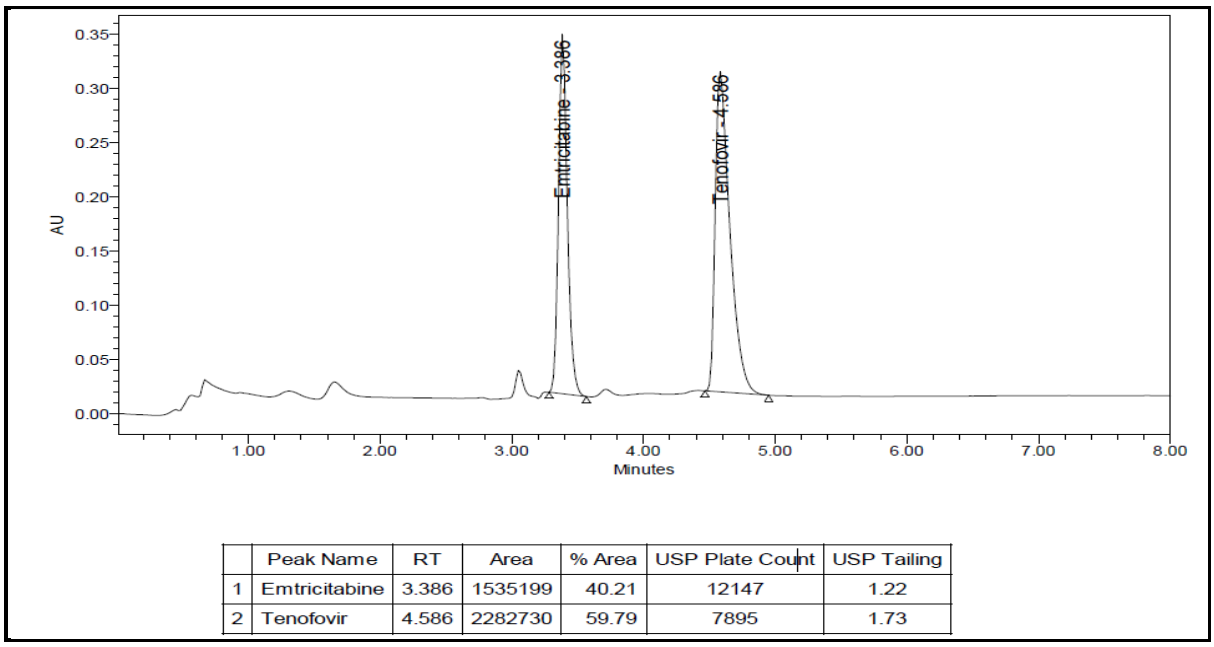


Figure No.2: Structure of Tenofovir



	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing
1	Emtricitabine	3.386	1535199	40.21	12147	1.22
2	Tenofovir	4.586	2282730	59.79	7895	1.73

Figure No.3: Method development

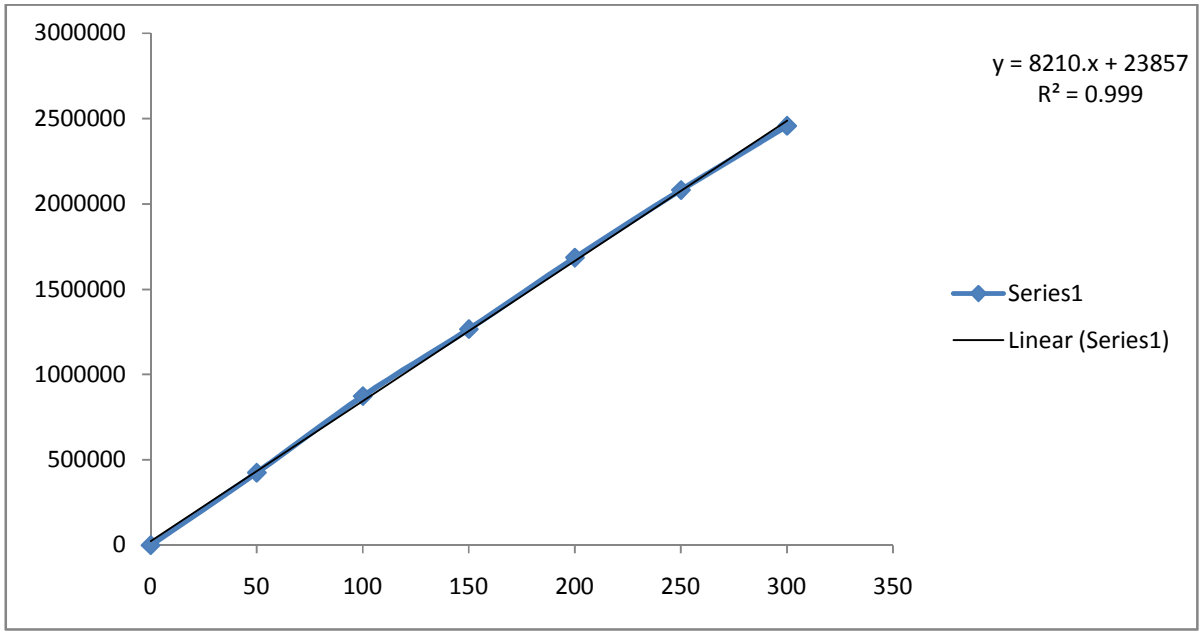


Figure No.4: Calibration curve for Emtrivitabine

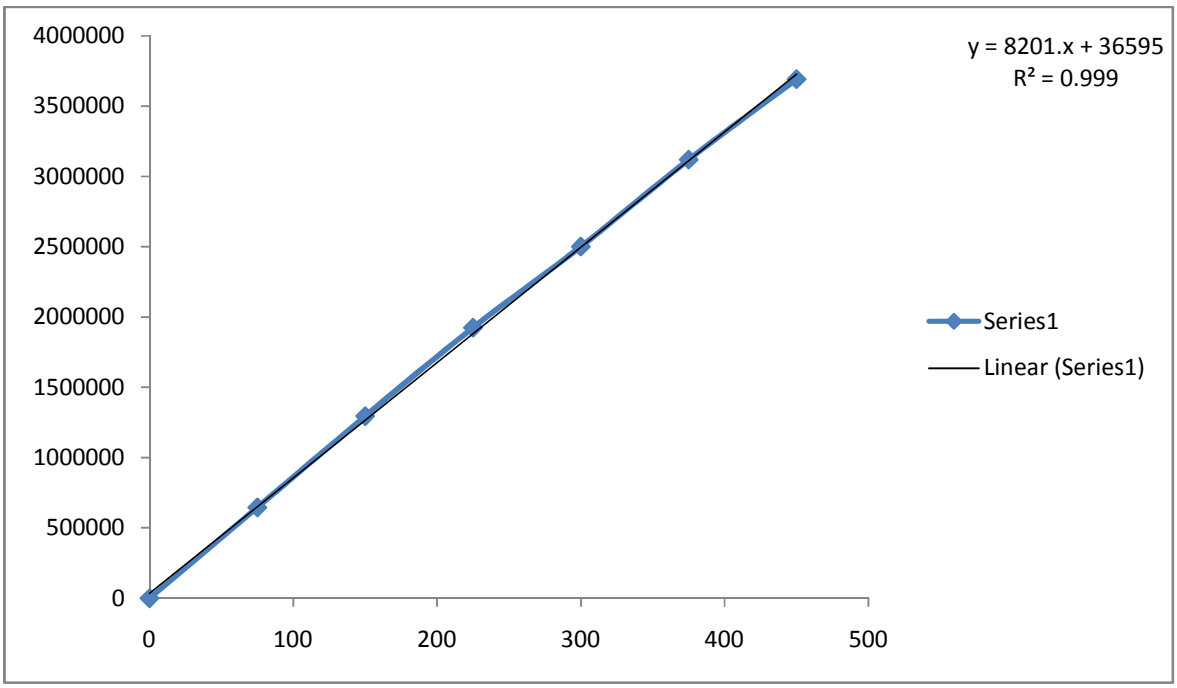


Figure No.5: Calibration curve for Tenofovir

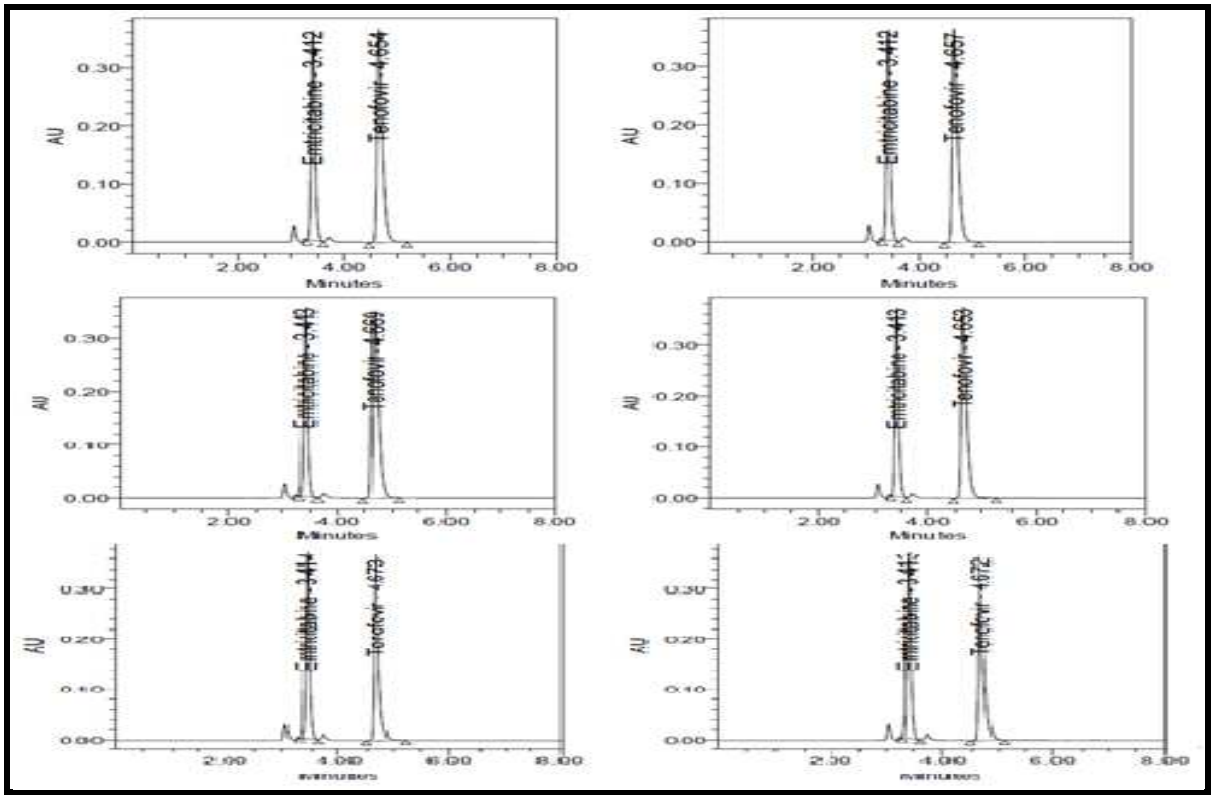


Figure No.6: Intraday precision

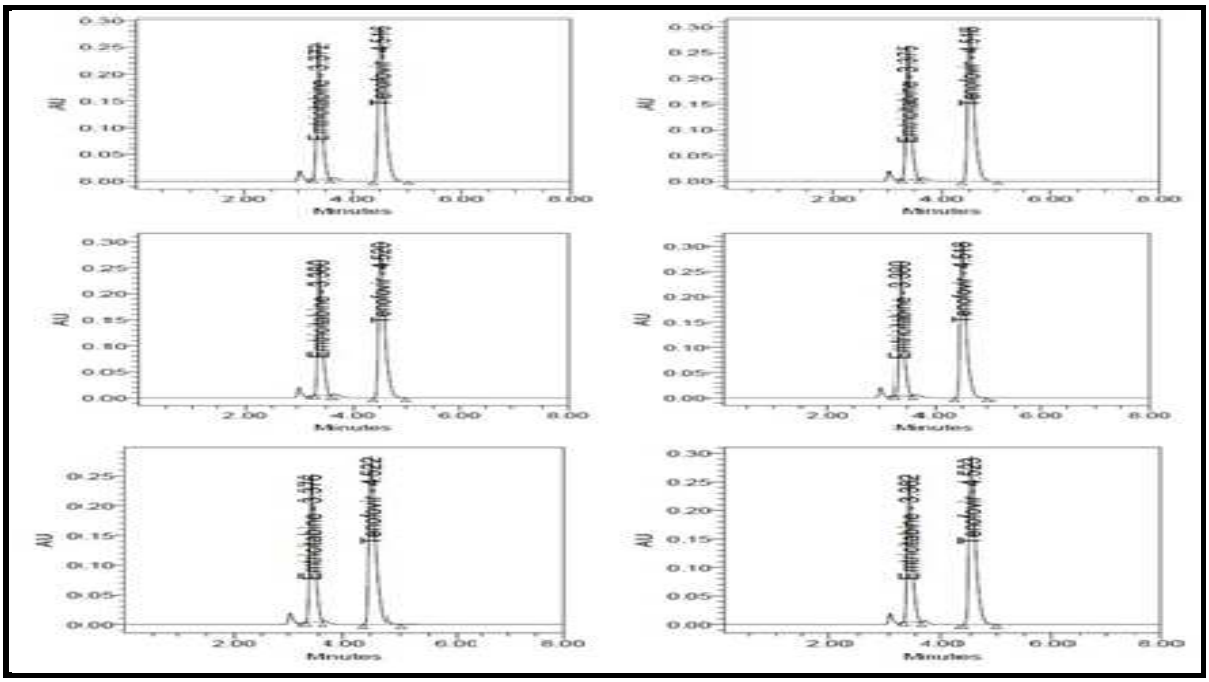


Figure No.7: Intraday precision

CONCLUSION

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. In this study, the chromatographic parameters monitored were retention time, area, capacity factor, tailing factor and theoretical plates. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above.

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