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Original Research Paper

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF ANTIRETROVIRAL DRUGS AND THEIR PHARMACEUTICAL FORMULATIONS

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ABSTRACT

Quick, exact, precise, particular & basic RP-HPLC technique was created & accepted for synchronous estimation of TENO & EMTRI in its business plans by utilizing plasma. Agilent 1100 arrangement HPLC Auto sampler High execution fluid chromatograph with U.V-Visible indicator was utilized all through examination, with versatile stage piece of Methanol: Phosphate cushion [68:32 % v/v]. Stream rate was kept up 1.0 ml min⁻¹ with UV location at 259 nm. Maintenance time of TENO & EMTRI were 5.54±0.02 & 9.48±0.02 minutes individually. Linearity was seen over focus scope of 4-40µg/ml for both medications. Lower breaking points of identification were observed to be 0.1571µg/ml & 0.1622µg/ml & cutoff points of measurement qualities were observed to be 0.4760µg/ml & 0.4917µg/ml for TENO & EMTRI individually for crude material & definitions. Precision of proposed strategy was dictated by recuperation examines & observed to be 98.12 to 101.31 % for TENO & EMTRI individually. Business plans & research center arranged blends were effectively broke down utilizing created strategy. Proposed technique was approved for different ICH parameters like linearity, farthest point of recognition, cutoff points of evaluation, exactness, accuracy, specificity, & extent & framework reasonableness.

Keywords: Tenofovir, Emtricitabine, Plasma, R P-HPLC, Method validation.

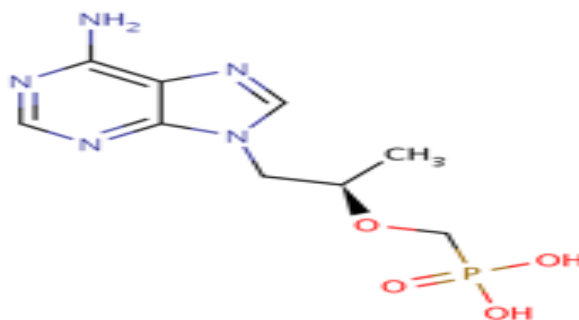
INTRODUCTION

Tenofovir

Tenofovir has spot with class of antiretroviral pharmaceuticals known as nucleotide direct invert transcriptase inhibitors (NtRTIs), which square turn transcriptase, protein earnest to viral creation in HIV-dirtied people. Tenofovir is starting now in late-mastermind clinical trials for treatment of hepatitis B. Tenofovir disoproxil fumarate is non-cyclic nucleoside phosphonate diester clear of adenosine monophosphate. Tenofovir requires starting diester hydrolysis for change to tenofovir & coming about phosphorylations by cell main thrusts to format tenofovir diphosphate. Tenofovir diphosphate is slight inhibitor of mammalian DNpolymerases α , β , & mitochondrial DNpolymerase γ . In any case, not at all like standard deoxy nucleotides substrates, NRTIs & NTRTIs (nucleoside/tide reverse transcriptase inhibitors) don't have 3'- hydroxyl pack on deoxyribose moiety. Thusly, taking after breaker of NRTI or NtRTI, running with drawing closer deoxy nucleotide can't shape running with 5'- 3' phosphodiester bond expected that would reinforce DNchain. As necessities be, time when NRTI or NtRTI is mixed, viral DNmix is halted, strategy known as chain end. All NRTIs & NtRTIs are named effective substrate inhibitors.

Chemical Name

[(2R)-1-(6-aminopurin-9-yl) propan-2-yl] oxymethylphosphonic acid.



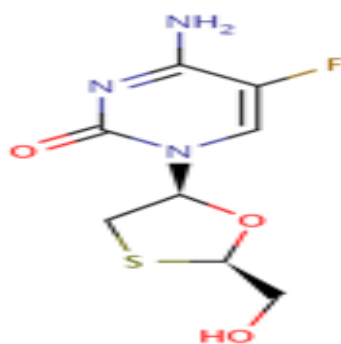
Chemical structure of Tenofovir

Emtricitabine

Emtricitabine is nucleoside reverse transcriptase inhibitor (NRTI) with progress against Human Immunodeficiency Virus Type 1 (HIV-1). Emtricitabine pieces HIV reverse transcriptase, substance in your body (blend) that is required for HIV to increase. Emtricitabine is constantly utilized with other handicapping to HIV meds to treat individuals with HIV sullyng. Emtricitabine may divide down measure of HIV in blood (viral weight). Emtricitabine may in like way escalate measure of T cells called CD4 cells. Hacking down measure of HIV in blood severs down probability of death or weights that happen when your protected structure is slight (entrepreneurial defilements). Individuals taking emtricitabine may at present get wily corruptions or particular conditions that happen with HIV illness. By controlling HIV-1 reverse transcriptase, emtricitabine can cut down measure of HIV, or "viral weight", in patient's body & can indirect assembling measure of safe structure cells (called T cells or CD4+ T-cells). Both of these headways are connected with more important safe structures & decreased probability of guaranteed contamination.

Chemical Name

4-amino-5-fluoro-1-[(2R, 5S)-2-(hydroxyethyl)-1, 3-oxathiolan-5-yl] pyrimidin-2-one.



Chemical structure of Emtricitabine

MATERIALS AND METHODS

Collection of Solvents

Clear human plasm was given by Ranbaxy Research Labs (India). Taking after HPLC grade chemicals were used as component of study: methanol (Qualigens Fine Chemicals, Mumbai), Perchloric harming, Disodium hydrogen phosphate, Potassium dihydrogen phosphate (S.D. fine chemicals, Mumbai, India). HPLC grade water was gotten from Millipore water cleansing unit. Each & every other dissolvable are grabbed from Merk association

Collection of Reference Standards

Unadulterated standard of Tenofovir & Emtricitabine (Assigned faultlessness 99.98%) were gotten as gift test from Ranbaxy labs Pvt. Ltd, Gurgaon, India. favoring tests were used as standard without further purifying. created structure & flawlessness of case got was avowed by TLC, IR, Melting point considers.

Collection of Samples

Business pharmaceutical masterminding Truvada which was guaranteed to contain 300mg of Tenofovir & 200mg of Emtricitabine was utilized as part of examination all through examination.

Collection of Equipments

Diverse sorts of supplies viz Analytical apportioning evening, HPLC framework, Column, Sonicator, Water purging structure, Vacuum channel pump, Glass vacuum versatile stage structure, Water shower, Sample filtration get together were utilized all through trial. All are collected from Satiare Research & Anatech Pvt. Ltd., Barwala, & Panchkul Haryana. Chem Station composing PC projects was utilized for getting, assessment & cutoff of chromatographic information.

Collection of Materials

Distinctive sorts of dish sets, for occasion, volumetric compartment, pipettes, receptacles, measuring barrel, channel & so on were amassed from Satiare Research & Anatech Pvt. Ltd., Barwala, Panchkul Haryana.

Chromatographic Conditions

Agilent 1100 game-plan HPLC Autosampler High execution fluid chromatograph with U.V.-Visible pointer was utilized all through examination running with HPLC condition was kept up all through test.

- *Mobile Phase*

Methanol: Phosphate cushion [68:32 % v/v]: I) Solvent A: - Methanol II) Solvent B: - Phosphate Buffer (pH 6.8± 0.04): Dissolve 28.80 g of disodium hydrogen phosphate & 11.45 g of potassium dihydrogen phosphate in adequate water to make 1000 ml.

- *Wavelength*

Tenofovir & Emtricitabine have ingestion maxima 259 nm. Properly, wavelength was picked as 259 nm. For estimation of this blend of meds.

- *Detector*

UV Detector.

- *Flow Rate*

1.0 ml/min.

- *Tablet Volume*

20µl.

- *Data Procurement*

Using suitable programming i.e. Chemstation programming.

- *Record Chromatogram*

Record chromatogram with running with motivations behind interest: Retention time, Peak range, & % Area.

- For figuring fixation, Mean, Standard Deviation, Relative Standard Deviation, Relative upkeep time, Theoretical plate, Resolution, Asymmetry, Tailing portion & for plotting Graph EXCELL Software was utilized.

Preparation of Mobile Phase, Standard Solution & Sample Solution

Organizing of Mobile Phase

HPLC grade solvents of Methanol & Phosphate Buffer were utilized for organizing of flexible stage in degree of 68:32 % (v/v). Substance of versatile stage were segregated before use through 0.45µm layer channel, sonicated & pumped from dissolvable supply to section at stream rate of 1 ml/min.

Masterminding of Standard Solution

Stock strategy of medication was readied by dissolving 60 mg of Pure Tenofovir & 40 mg of flawless Emtricitabine in 100 ml volumetric glasses containing tasteful measure of refined water (HPLC evaluation) to partitioned arrangement, sonicated for around 15 min & while later made up to volume with refined water. Reliably working standard blueprints of Tenofovir & Emtricitabine was readied by exchanging 10 ml of this stock game-plan in 100 ml volumetric container & made up to volume with advantageous stage. Six arrangements of medication strategy were readied at union of 4-24 µg/ml by exchanging 0.4 ml, 0.8 ml, 1.2 ml, 1.6 ml, 2.0 ml, 2.4 ml of dependably working standard strategies in 10 ml volumetric flask & while later made up to volume with clear human plasma up to 10 ml.

Protein precipitation was refined by including 200 µl of perchloric harming to 500 µl of plasma test, & cases were again vortex mixed. These were subjected to refrigerated focus point for 15 min at 10000 rpm. After centrifugation supernatant was traded to HPLC auto sampler vials & 50 µl of course of action was mixed into HPLC zone for examination. Each of these game plan outlines, (50µl) six debilitating were inserted six times in three repeats into part, beat district & support times were recorded.

Philosophy for Test Course of Action Preparation (From Formulation)

Twenty tablets were weighed convincingly & powdered. Measure of powder practically identical to 500 mg (300 mg Tenofovir & 200 mg Emtricitabine) (substance of one tablet) was segregated in 50 ml of refined water (HPLC grade). Technique was blended for 10 min using connecting with stirrer, sonicated for around 15 min & after that separated into 100 ml volumetric compartment through 0.45 µm layer channel. Store was washed 3 times with 10 ml of refined water, & after that volume was done to 100 ml with same dissolvable. Directed exchanging to work standard methodology was prepared 02 ml of this stock outline in 100 ml volumetric glass & made up to volume with flexible stage. Six plans of pharmaceutical system were prepared at meeting of 4-24 µg/ml by trading 0.4 ml, 0.8 ml, 1.2 ml, 1.6 ml, 2.0 ml, 2.4 ml of controlled working standard game-plans in 10 ml volumetric holder & brief traverse later made up to volume with clear human plasm up to 10 ml.

Protein precipitation was refined by including 200 µl of perchloric destructive to 500 µl of plasm test, & illustrations were again vortex mixed. These were subjected to refrigerated rotator for 15 min at 10000 rpm. After centrifugation supernatant was traded to HPLC auto sampler vials & 50 µl of game plan was mixed into HPLC area for examination. All determinations were coordinated in triplicate, top area & upkeep times were recorded.

Table 1: HPLC instrumentation & chromatographic conditions for Tenofovir & Emtricitabine

S. No.	Parameters	Description
1.	Instrument	HPLC instrument (Agilent 1100 series)
2.	Column	Promosil C-18, (250 mm, 4.6 mm, 5µm)
3.	Mobile Phase	Different mobile phase used for Trial 1 to 6
4.	Flow Rate	1.0 mL/minute
5.	Detection wavelength	259 nm
6.	Tablet Volume	20 µL
7.	Run Time	20 minutes

Impeccable state of advantageous stages were asked about in progress of HPLC method appropriate for examination of in mass medication. These included Methanol: Acetonitrile: Phosphate Buffer (50:20:30)

(% v/v), Methanol: Acetonitrile: Phosphate Buffer (60:20:20), Methanol: Phosphate cushion (50:50), Methanol: Phosphate Buffer (70:40), Methanol: Phosphate Buffer pH 6.8± 0.04 (90:10), & Methanol: Phosphate Buffer pH 6.8± 0.04 (68:32).same dissolvable blend was utilized for extraction of pharmaceutical from separating containing excipients.

Standard Strategy of Tenofovir & Emtricitabine

Unequivocally weigh & exchange around 20 mg of Tenofovir & Emtricitabine working standard into 100 mL volumetric holder, & around 70 mL of diluents, sonicate to crumble, weaken to volume with diluents & blend. Channel game-plan through 0.45µ.

RESULTS AND DISCUSSION

Linearity

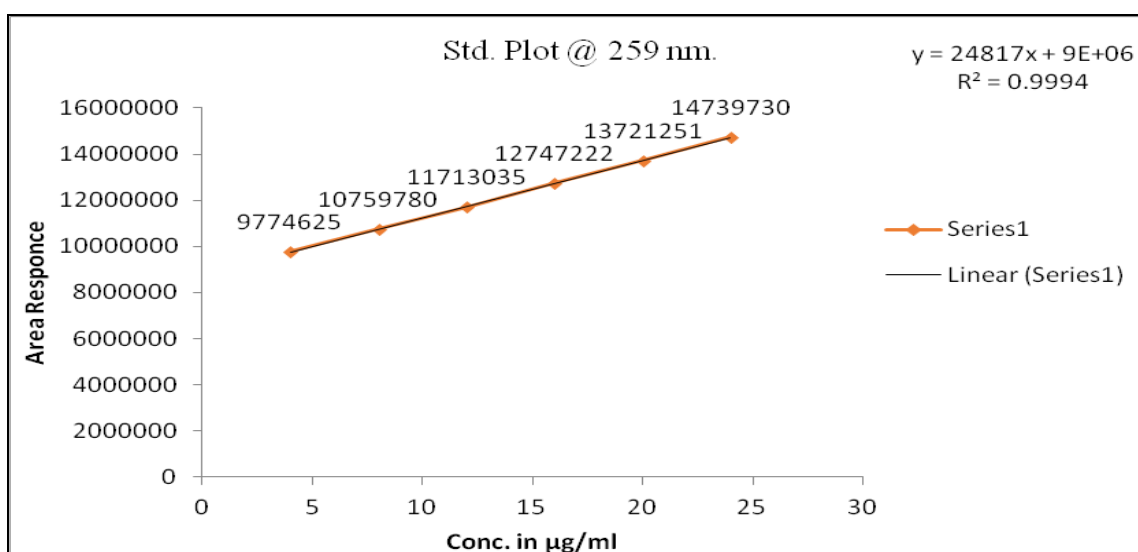


Figure 1: Standard plot of Tenofovir

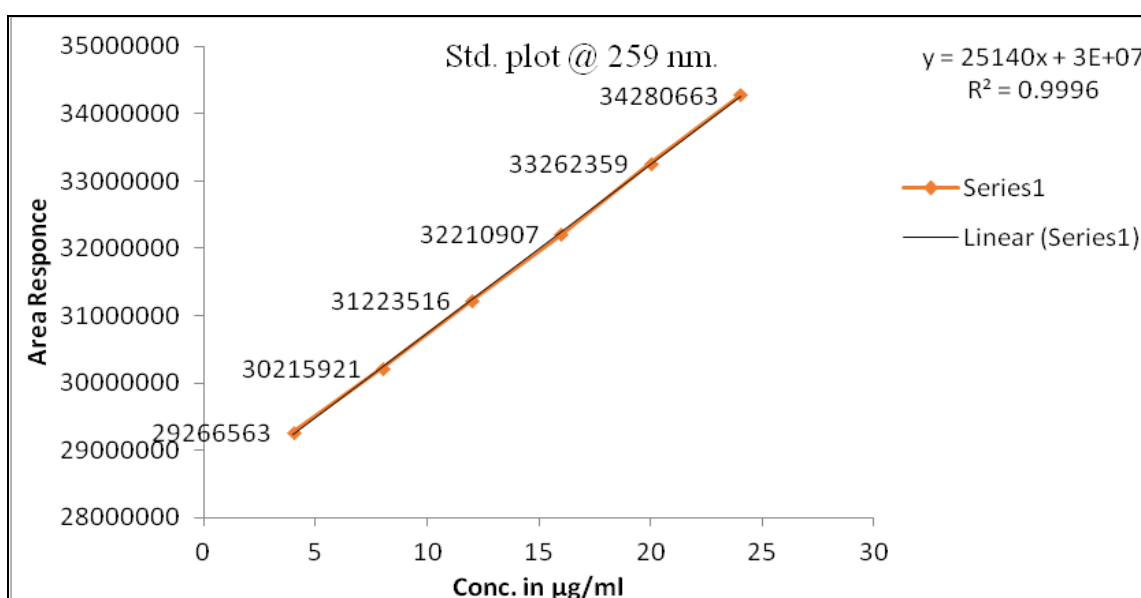


Figure 2: Standard plot of Emtricitabine

Table 2: Peak Area* of Tenofovir

Replicate	Dilution I	Dilution II	Dilution III	Dilution IV	Dilution V	Dilution VI
1	9778436	10756075	11725812	12778954	13724297	14725418
2	9772653	10742186	11712279	12741253	13726596	14701275
3	9772785	10781079	11701014	12721459	13712859	14792498
Average	9774625	10759780	11713035	12747222	13721251	14739730
SD	3301.371	19709.430	12416.274	29208.569	7357.744	47265.644
RSD%	0.03	0.18	0.11	0.23	0.05	0.32

*Average of three readings

Table 3: Peak Area* of Emtricitabine

Replicate	Dilution I	Dilution II	Dilution III	Dilution IV	Dilution V	Dilution VI
1	29272453	30212645	31225148	32216363	33292422	34266557
2	29263664	30232561	31220152	32201083	33224324	34278864
3	29263573	30202558	31225249	32215275	33270331	34296568
Average	29266563	30215921	31223516	32210907	33262359	34280663
SD	5100.804	15267.474	2914.036	8525.208	34741.891	15086.164
RSD%	0.02	0.05	0.01	0.03	0.10	0.04

*Average of three readings

Acceptance Criteria

For linearity Coefficient of correlation value (r^2) should be greater than 0.998 (Regression value in linear plot).

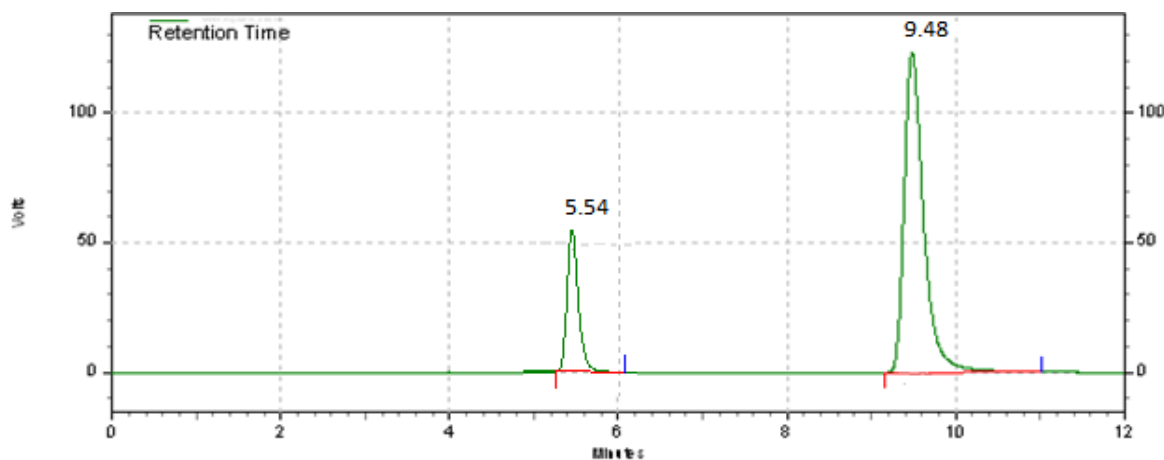


Figure 3: Chromatogram showing Retention time

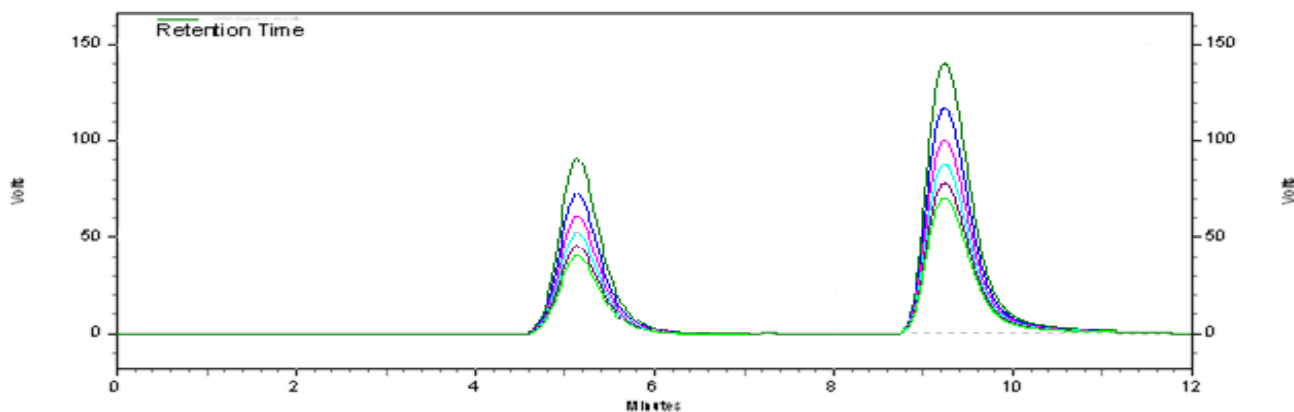


Figure 4: Overlay spectra for Linearity

RESULTS

Correlation coefficient (r^2) for Tenofovir & Emtricitabine was found to be 0.9994 & 0.9996 respectively indicating linearity & method is linear between concentrations of 4-40 μ g/ml for both drugs with retention time 5.54 \pm 0.02 & 9.48 \pm 0.02 respectively.

Accuracy

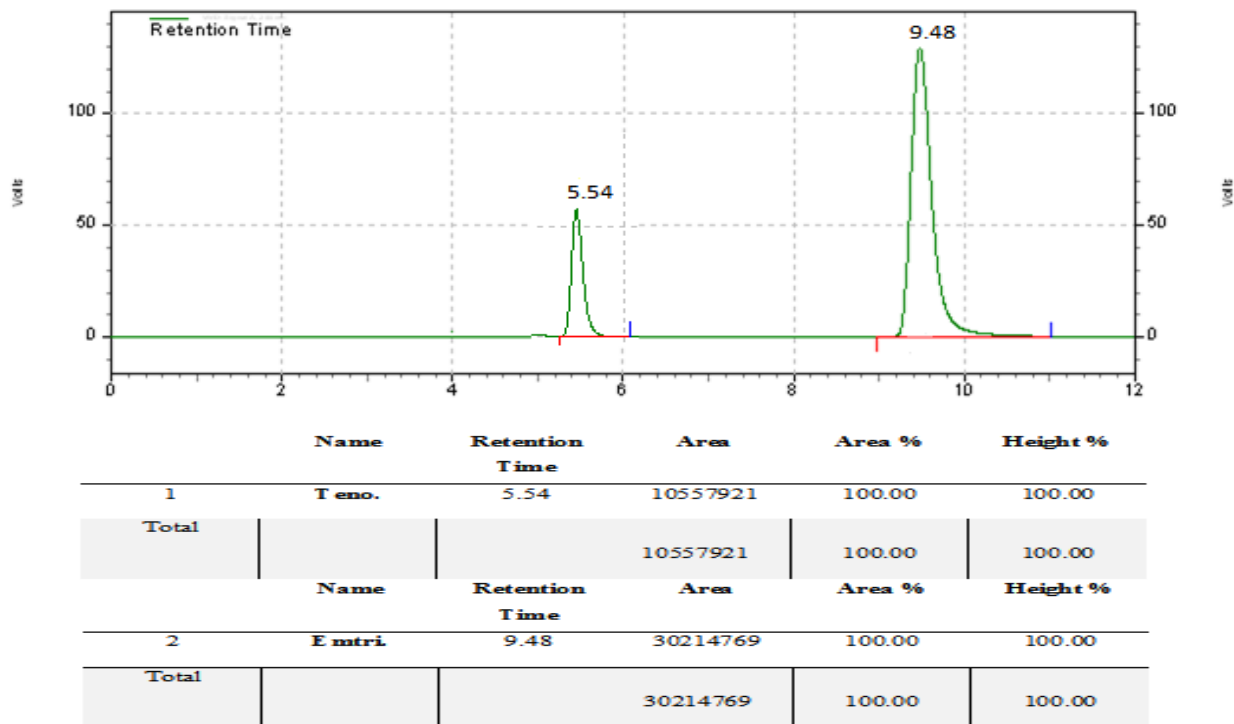


Figure 5: Dilution 1 (8 μ g/ml)

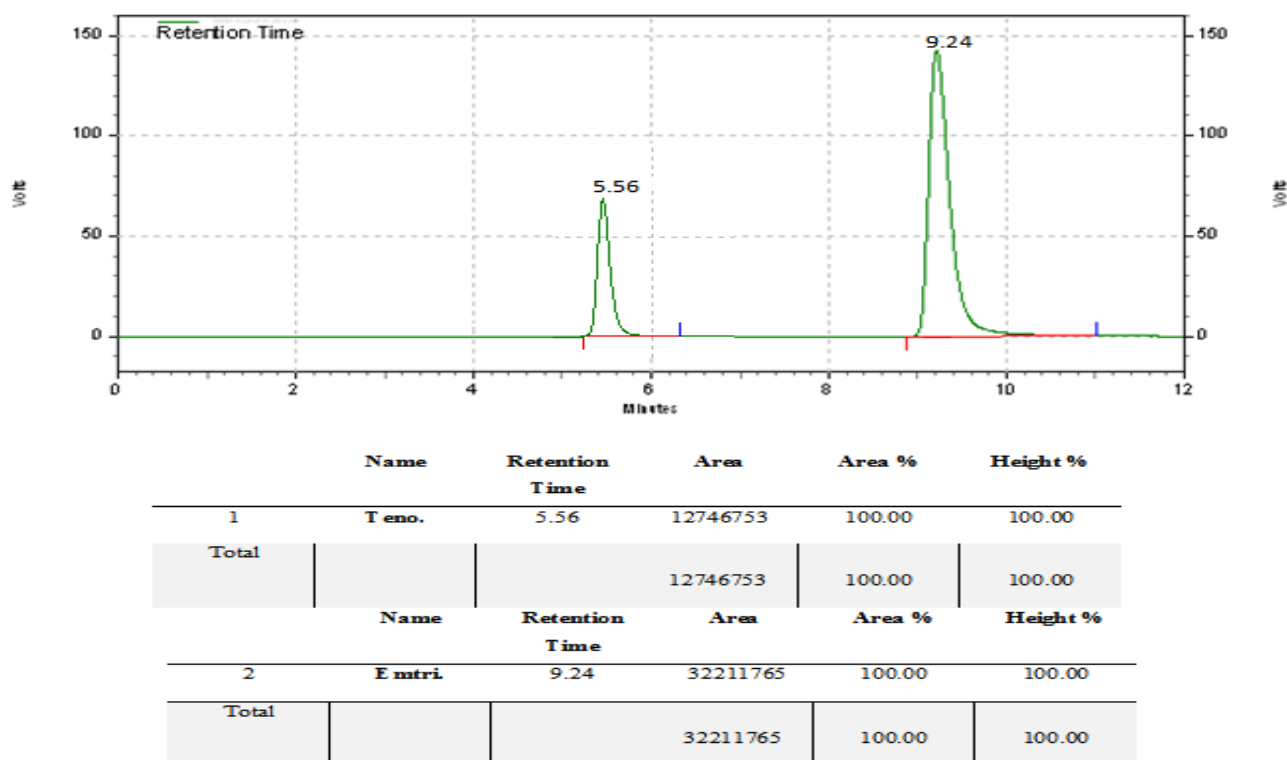


Figure 6: Dilution 2 (16 μ g/ml)

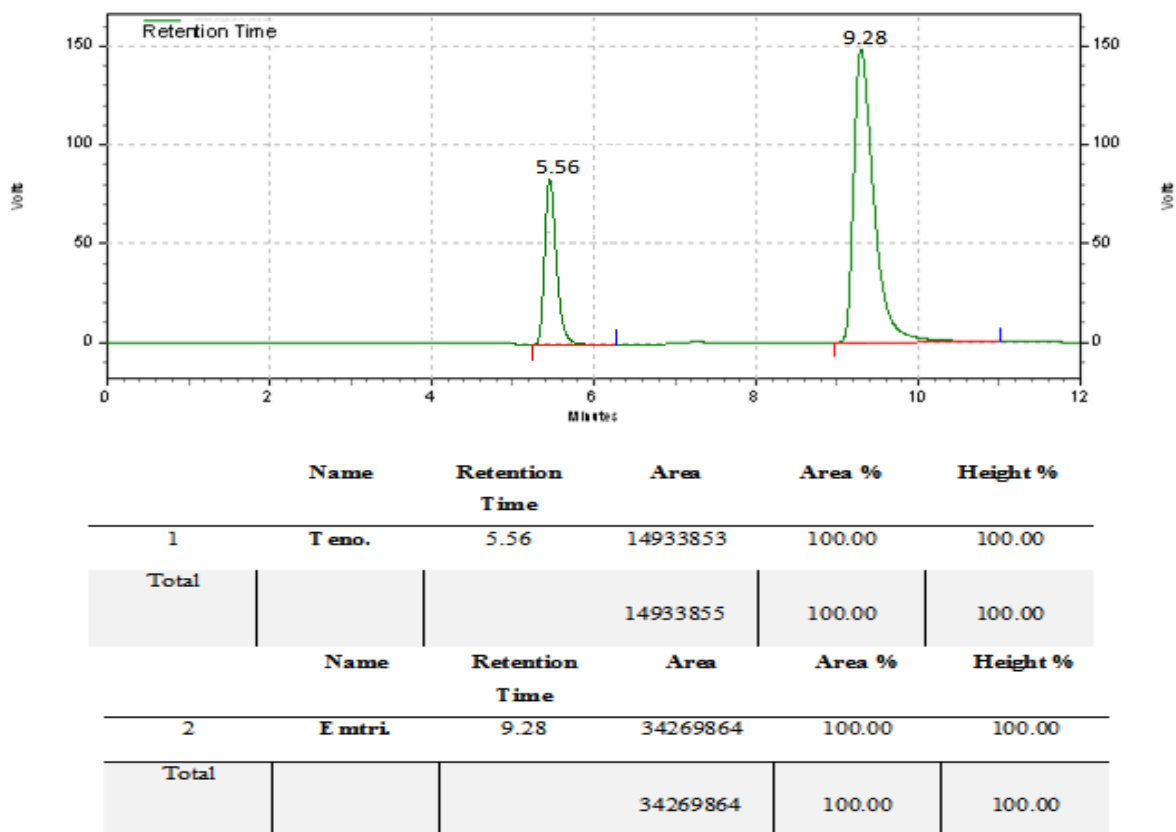


Figure 7: Dilution 3 (24 µg/ml)

Table 4: Results of recovery studies of drug

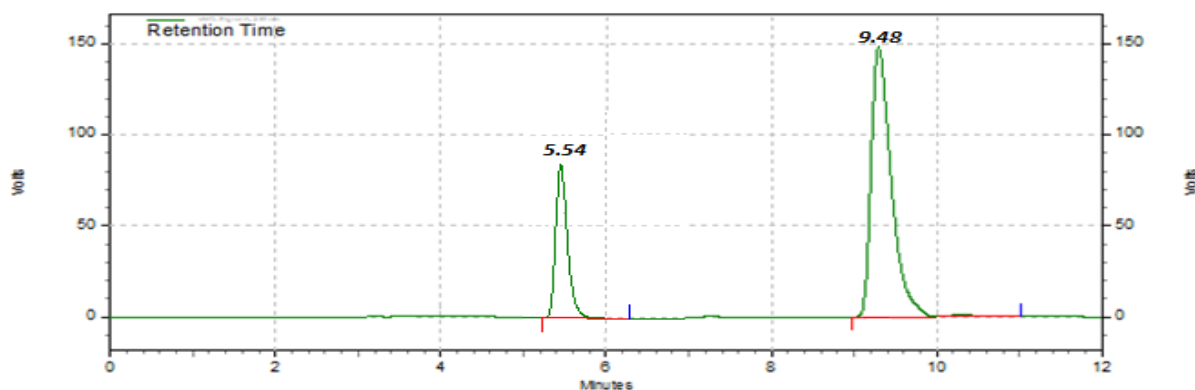
Conc. taken in µg/ml (A)	Std addition in µg/ml (B)	Total drug conc. in µg/ml (A+B)	Peak Area*		% Recovery	
			Teno.	Emtri.	Teno.	Emtri.
04	04	08	10557921	30214769	98.12395	99.99619
12	04	16	1274673	32211765	99.99632	100.0027
20	04	24	14933855	34269864	101.317	99.9685

*Average of three readings

RESULTS

Percentage recovery by proposed method was ranging from 98.12 to 101.31 % indicating no interference of tablet excipients with drug under analysis.

Precision



	Name	Retention Time	Area	Area %	Height %
1	T eno.	5.54	13724247	100.00	100.00
Total			13724247	100.00	100.00

	Name	Retention Time	Area	Area %	Height %
2	E mtri.	9.48	33292463	100.00	100.00
Total			33292463	100.00	100.00

Figure 8: Replicate 1 (20µg/ml)

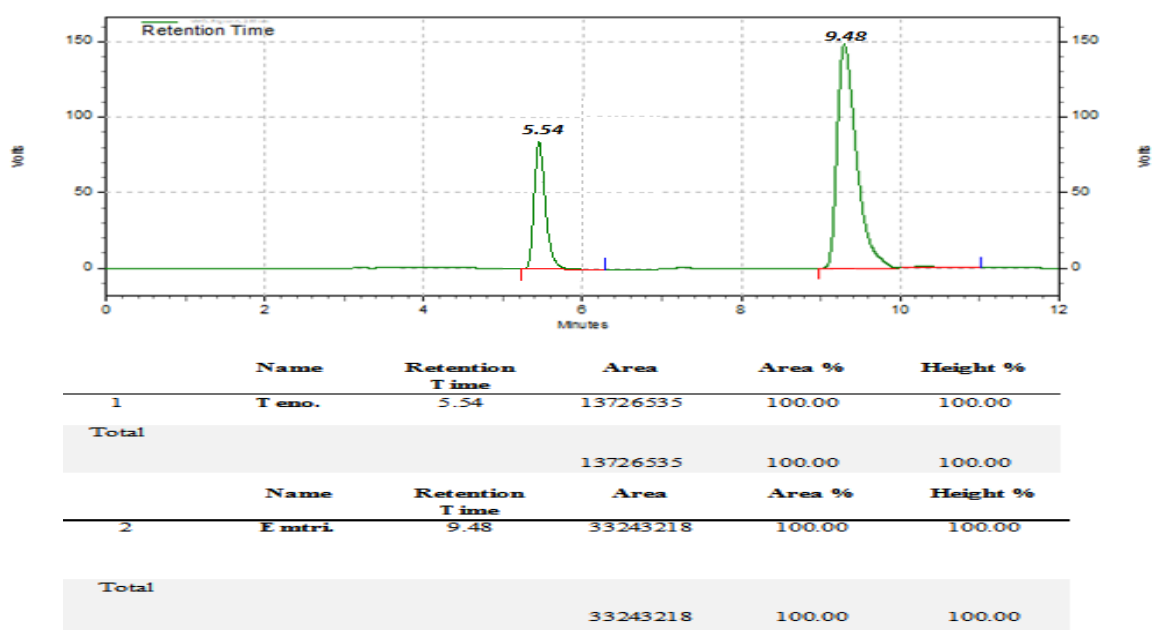


Figure 9: Replicate 2 (20µg/ml)

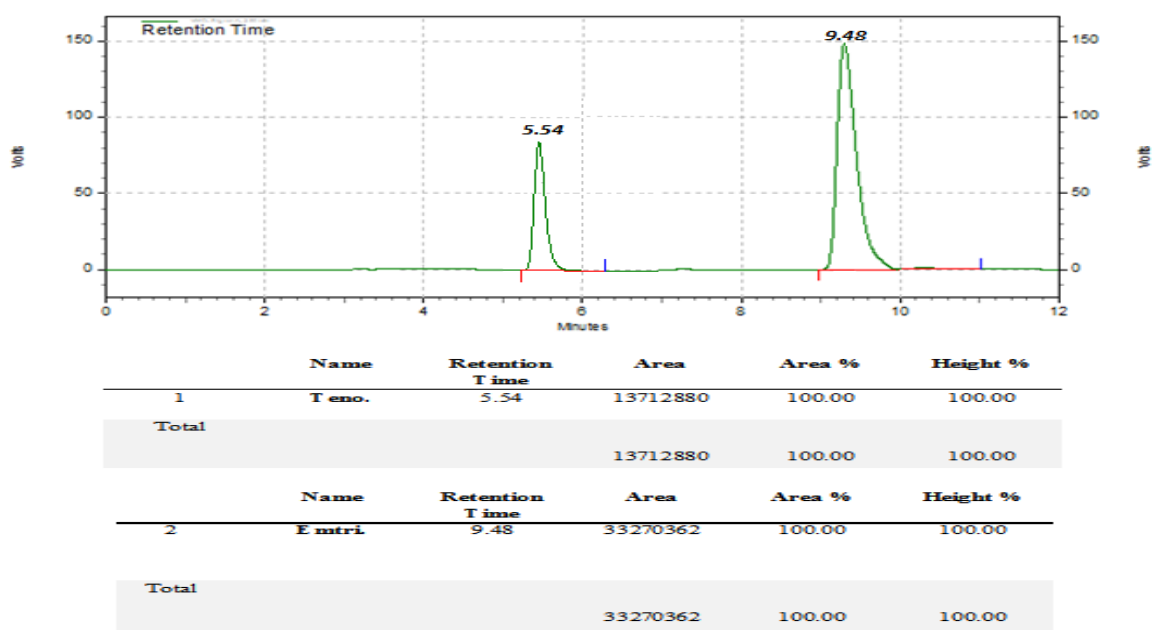


Figure 10: Replicate 3 (20 µg/ml)

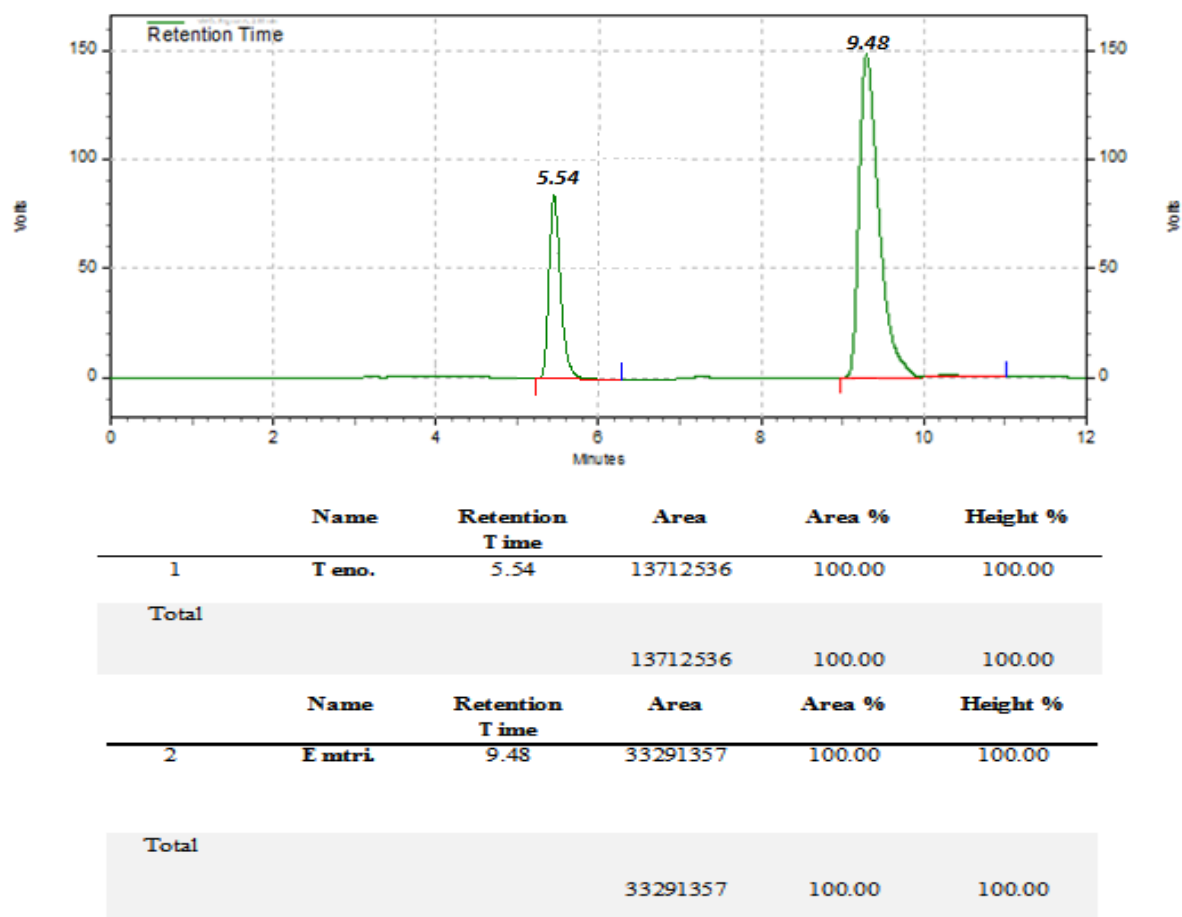


Figure 11: Replicate 4 (20 µg/ml)

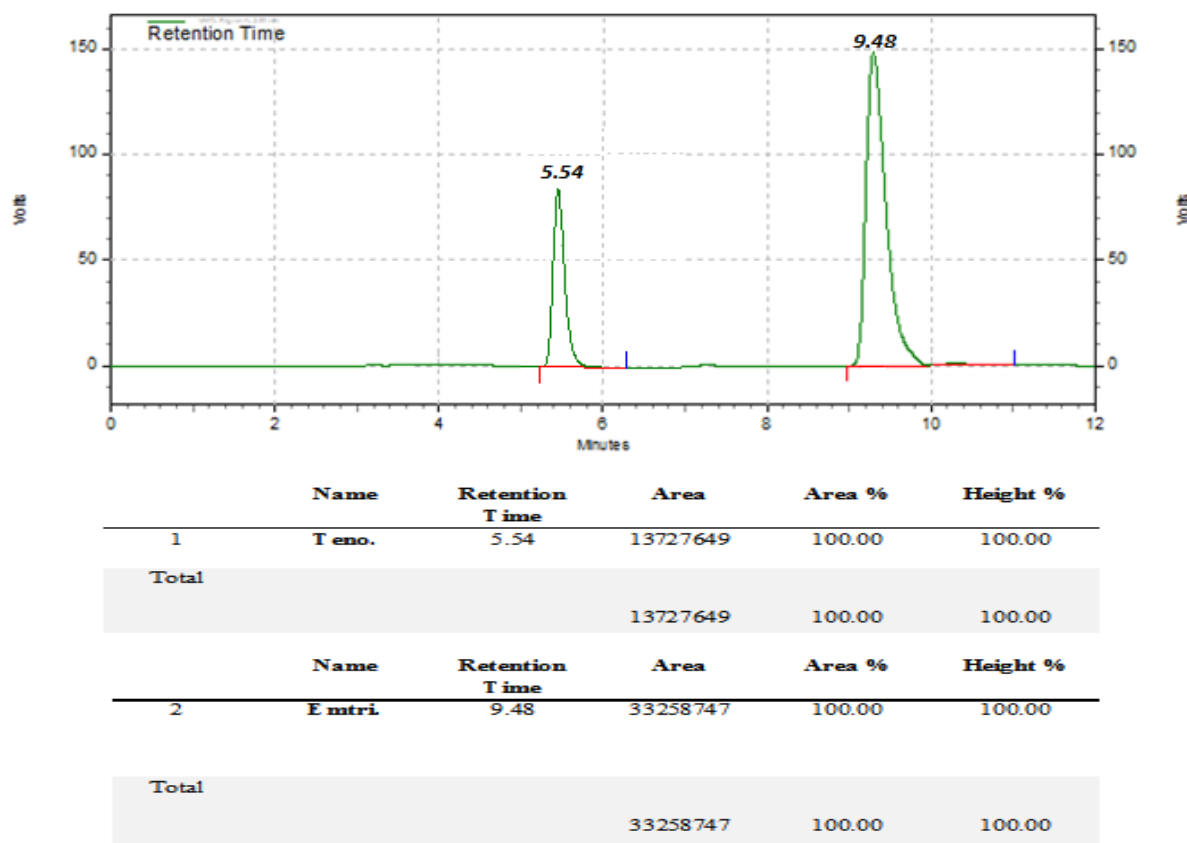


Figure 12: Replicate 5 (20µg/ml)

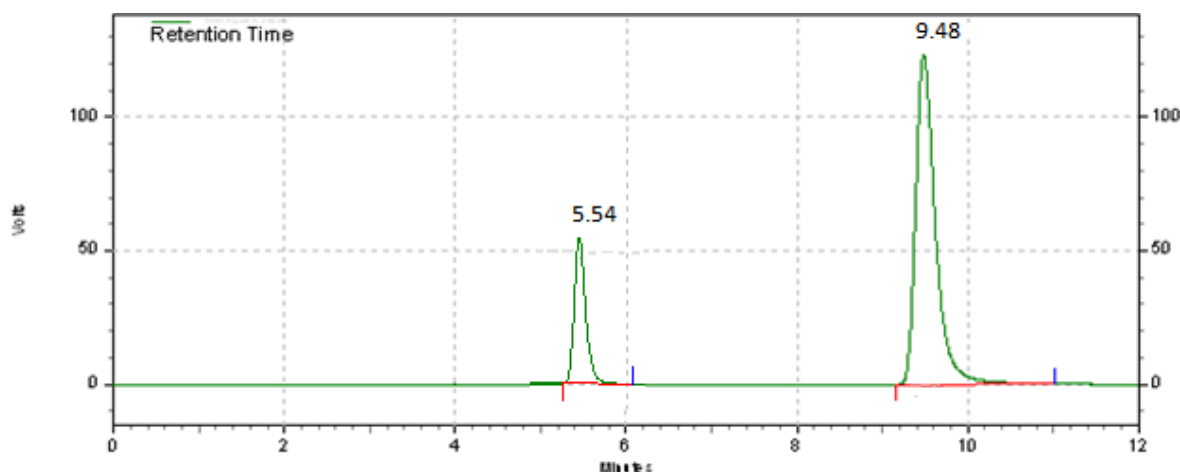
Table 5: Precision

S. No.	Are Response	
	Teno.	Emtri.
1.	13724247	33292463
2.	13726535	33243218
3.	13712880	33270362
4.	13712536	33291357
5.	13727649	33258747
Average	13720769	33271229
S.D	7461.5054	21196.623
R.S.D	0.0543811	0.0637086

RESULTS

From above analytical data it is observed that RSD value for assay is 0.054% & 0.063% for Tenofovir & Emtricitabine respectively which indicates that method is precise & reproducible (According to ICH guidelines).

Specificity

**Figure 13:** Chromatogram for Specificity

RESULTS

Excipients used in different formulation products do not interfere with drug peak which shows that method is very specific.

Range

Range was calculated from linearity graph. Specific range can be obtained from linearity graph. Range which is linear, accurate & precise between lower & higher concentration is beer's range of method. Linear range was found to be 4-40 μ g/ml.

Limits of Detection & Limits of Quantification

Limit of acknowledgment (LOD) is most negligible measure of analyte in illustration that can be perceived, however not as is normally done quantized under communicated trial conditions. It may be imparted as center that gives sign to-racket extent of 2:1 or 3:1. Lower uttermost ranges of acknowledgment are 0.1571 μ g/ml & 0.1622 μ g/ml for Tenofovir & Emtricitabine independently in reference material & specifying. Most remote purpose of Quantification (LOQ) is most negligible entirety analyte in sample that can be determined with commendable precision & accuracy under communicated test conditions. Sign to-

disturbance extent of 10:1 can be taken as LOQ of methodology. LOQ qualities were seen to be 0.4760µg/ml & 0.4917 µg/ml for Tenofovir & Emtricitabine separately for rough material & definitions.

System Suitability

Stock solution of pharmaceutical preparation (Approx. 20 µg/ml) was prepared & injected in system in five replicates, after obtaining chromatogram system suitability parameters were calculated (Figure 13).

Table 6: system suitability parameters

Parameters	Data obtained	
	Teno.	Emtri.
Theoretical plates per column	4982	4754
Symmetry factor/Tailing factor	1.08	1.03

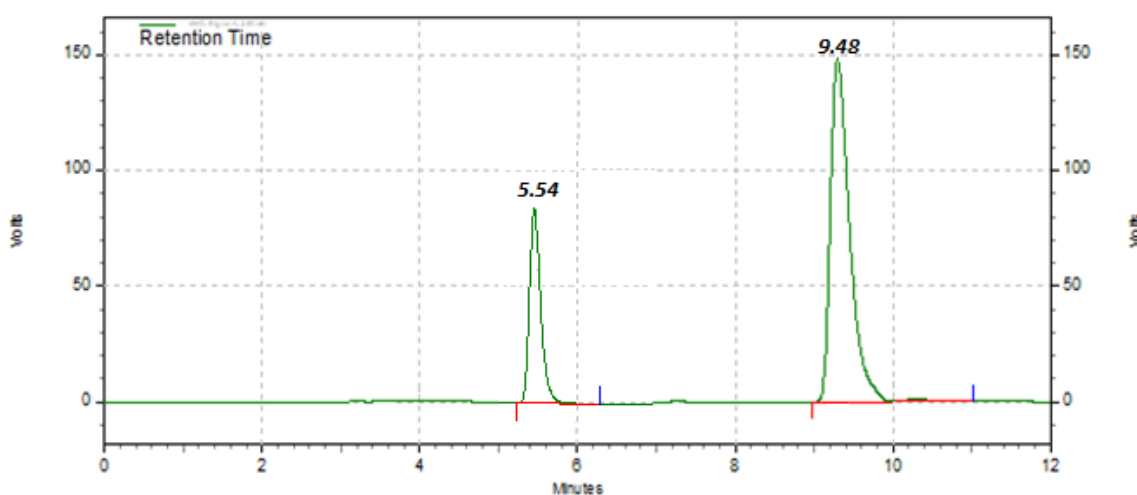


Figure 14: Chromatogram for system suitability

RESULTS

Results obtained for system suitability parameters such as tailing factor was found within limit i.e. less than 2.0

CONCLUSION

Present strategy was acknowledged by Conference on Harmonization (ICH) rules. Quantifiable examination of data showed that procedure is reproducible & uncommonly particular for estimation of TENO & EMTRI in quality control labs.

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