Int. J. Drug Res. Tech. 2016, Vol. 6 (3), 228-238 ISSN 2277 - 1506

International Journal of Drug Research and Technology

Available online at http://www.ijdrt.com

Original Research Paper

DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR THE ESTIMATION OF ANTIRETROVIRAL DRUGS AND THEIR PHARMACEUTICAL FORMULATIONS

Naresh Chandra Joshi, Pradeep Kumar and **Rakesh Kumar Jat***

Institute of Pharmacy, Shri Jagdishprasad Jhabarmal Tibrewala University, Vidya Nagri, Jhunjhunu-333001, Rajasthan, India

ABSTRACT

New, basic, delicate, exact & powerful superior dainty layer chromatographic (HPTLC) strategy was created for synchronous estimation of TENO & EMTRI in pharmaceutical measurement shapes. After improvement of plate, Camas TLC scanner III (examining speed 20 mm sec-1) was utilized for densitometry checking with WinCATs programming (opening miniaturized scale, 6 x 0.45 mm). Investigation of plate in absorbance mode at 274 nm was done for both medications all through trial. Framework was found to give smaller spots for TENO & EMTRI with RF (Retardation variable) estimation of 0.41±0.01and 0.56±0.01 separately. Data for adjustment plots demonstrated great direct association with r2 = 0.9985 & 0.9979 in fixation scope of 100-600ng/ml for both medications. Present technique was accepted by Conference on Harmonization (ICH) rules. Measurable investigation of information demonstrated that strategy is reproducible & exceptionally specific for estimation of TENO & EMTRI in quality control labs.

Keywords: Tenofovir**,** Emtricitabine, HPTLC, Validation, WinCATs software.

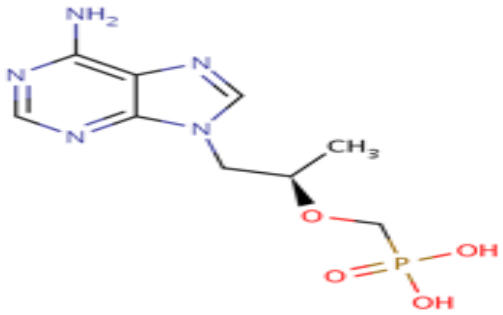
INTRODUCTION

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.



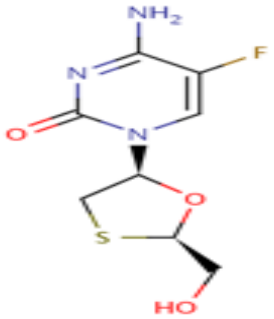
Chemical Structure of Tenofovir

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 sullying. 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

Perfect standard of Tenofovir & Emtricitabine (Assigned flawlessness 99.98%) were obtained as gift test from Ranbaxy labs Pvt. Ltd, Gurgaon, India. favoring test was used as standard without further purging. Silica gel 60 F 254 TLC plates (20x10cm) were used as stationary stage. All chemicals & reagents used were of investigative survey & got from Qualigens & S.D. fine chemicals, Mumbai, India. Business pharmaceutical arranging Truvada which was proclaimed to contain 300mg of Tenofovir & 200mg of Emtricitabine was used as major aspect of examination all through trial. Delivered structure & faultlessness of representation got was confirmed by TLC, IR, Melting point considers. The instrument used as component of present study was Camag Linomat V-adjusted test contraption, Hamilton syringe (100µl), Camag TLC scanner 3, Cagmag Twin through gathering of reasonable size, Analytical distributing evening (Shimadzu AX 200), Sonicator (model SONIC2200MH) were used all through trial. Cagmag WinCATs creating PC tasks was used for getting, assessment & most extreme of chromatographic data.

Coordinating of Standard Solution

Stock course of action of solution was prepared by dissolving 60 mg of Pure Tenofovir & 40 mg of impeccable Emtricitabine in 100 ml volumetric carafes containing tasteful measure of refined water (HPLC assessment) to separate pharmaceutical, sonicated for around 15 min & after that made up to volume with refined water. Deliberate working standard methodologies of Tenofovir & Emtricitabine was prepared by trading 01 ml of this stock strategy in 100 ml volumetric jug & made up to volume with supportive stage. Six courses of action of game plan approach were prepared at focalizing of 100-600 ng/ml by trading 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml, 5.0 ml, 6.0 ml of systematic working standard diagrams in 100 ml volumetric cup & brief time span later made up to volume with adaptable stage up to 100 ml.

Blueprint of Sample Solution (From Formulation)

Twenty tablets were weighed unequivocally & powdered. Measure of powder equivalent to 500 mg (300 mg Tenofovir & 200 mg Emtricitabine) (substance of one tablet) was rotted in 50 ml of refined water (HPLC grade). Procedure was mixed for 10 min using drawing in stirrer, sonicated for around 15 min & while later isolated into 100 ml volumetric glass through 0.45 µm film channel. Store was washed 3 times with 10 ml of refined water, & after that volume was done to 100 ml with same dissolvable. Controlled working standard outlines of Tenofovir & Emtricitabine was prepared by trading 0.2 ml of this stock course of action in 100 ml volumetric compartment & made up to volume with adaptable stage. Six systems of drug course of action were prepared at union of 100-600 ng/ml by trading 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml, 5.0 ml, 6.0 ml of dependably working standard outlines in 100 ml volumetric holder & after that made up to volume with accommodating stage up to 100 ml.

Prewashing of TLC Plates

HPTLC was performed on 20 cm × 10 cm precoated silica gel 60 F 254 TLC plates. Adsorbent has far reaching surface district; it may ingest air & different corruptions from air, particularly exceptional sullying influences, after pack has been opened. Non-exceptional dirtying influences adsorbed by layer can affect sporadic case in taking gander at densitometry. To avoid possible obstacle from such defilements in quantitative examination, plates were prewashed with methanol, dried, & started for 30 min. at 110 C, with plates being put between two sheets of glass to decline bowing of aluminum in midst of warming.

Framework

Distinctive groupings of Tenofovir & Emtricitabine in level of 100-600 ng/ml were connected on plates as 8 mm packs, 8 mm restricted & 1 cm from edge of plate, by technique for Camag Linomat V adjusted test execute fitted with 100 µl Hamilton syringe. Methanol clear was associated with parallel track. flexible stage, toluene: ethyl acidic damaging insincerity: methanol: acidic acid (6.3:4:4:0.7, v/v) was filled twin trough glass chamber & glass chamber left to equilibrate for 10 min at 25 ± 20 C. After that plate was set in Camag twin trough glass chamber. After development, plate was removed from chamber, dried in current of hot air, & researched at 274 nm, using deuterium light, by system for Camag TLC scanner III densitometer. Densitogram were grabbed by HPTLC at various focus leisure activities. This framework was taken after for each & every quantitative investigation. WinCATs making PC tasks was used for data getting & get readied of plate. Top tallness & top zone were entwined for entire track. Arrangement curve was delivered by plotting got top area on ordinate against taking gander at spotlight on abscissa.

RESULTS AND DISCUSSION

Linearity

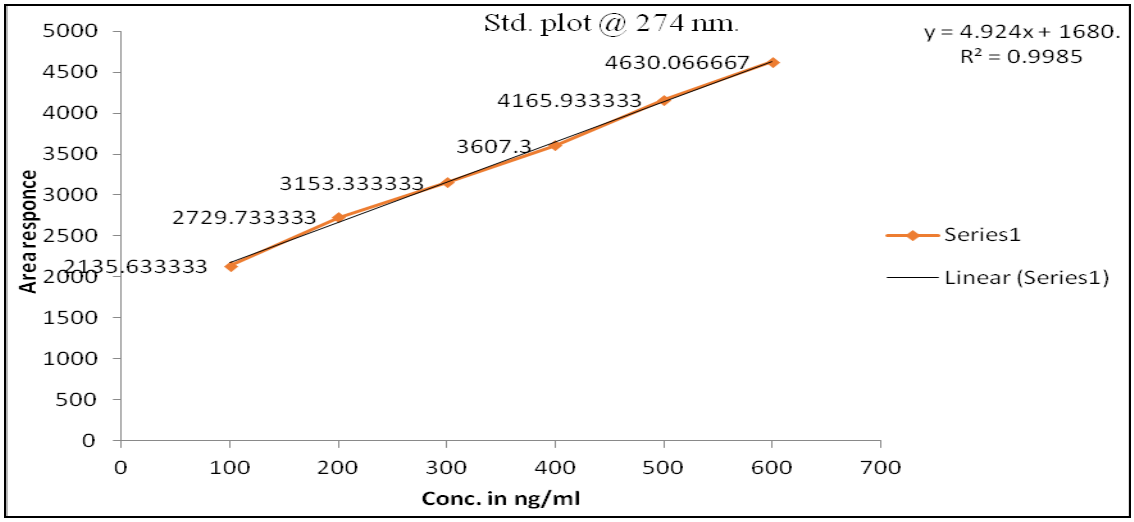


Figure 1: Standard plot of Tenofovir

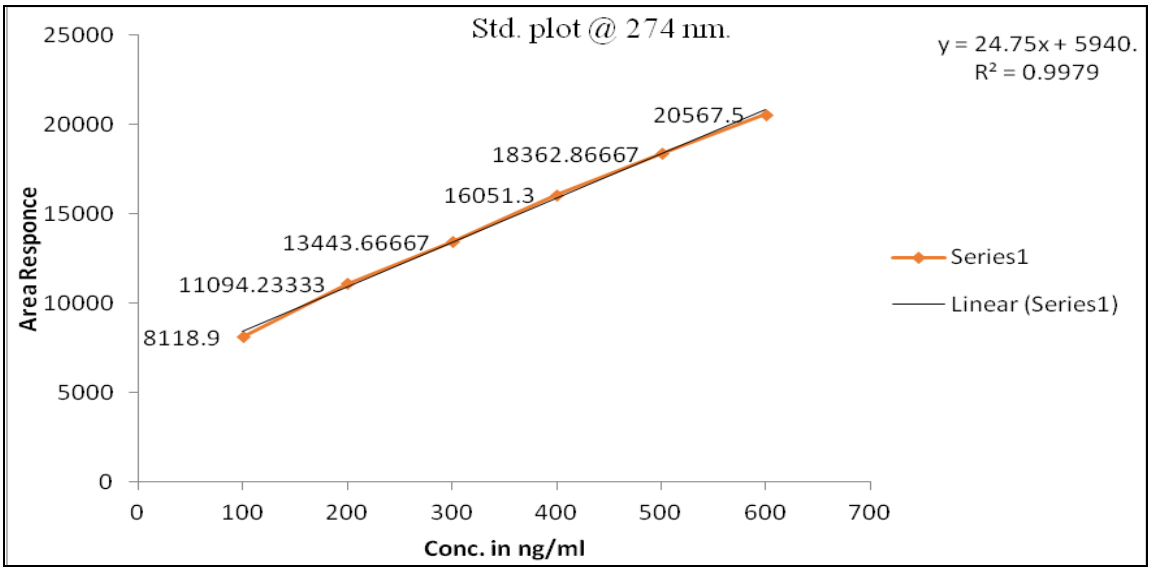
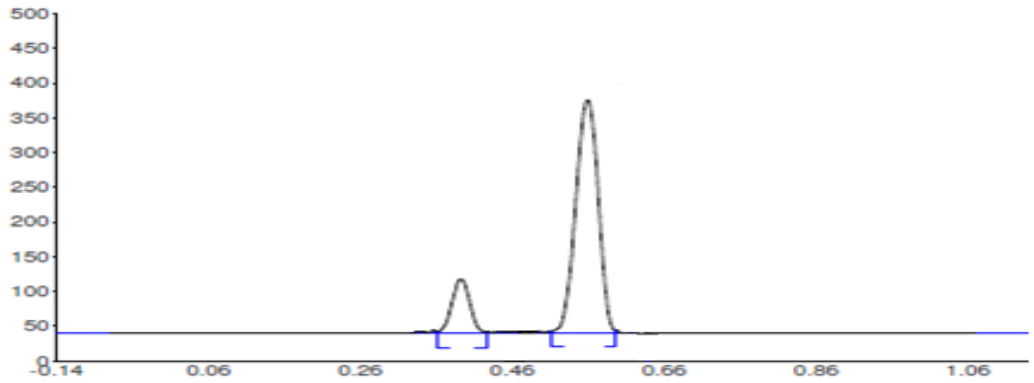


Figure 2: Standard plot of Emtricitabine



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.38	3.3	0.41	123.9	100.00	0.44	0.5	2147.8	100.00

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
2	0.51	3.2	0.56	336.1	100.00	0.60	4.2	8126.1	100.00

Figure 3: Chromatogram showing RF value

Table 1: Peak Area* of Tenofovir

Replicate	Dilution I	Dilution II	Dilution III	Dilution IV	Dilution V	Dilution VI
1	2147.8	2726.5	3132.4	3594.5	4189.9	4617.2
2	2129.9	2731.1	3163.5	3605.9	4154.3	4618.7
3	2129.2	2731.6	3164.1	3621.5	4153.6	4654.3
Average	2135.633	2729.733	3153.333	3607.3	4165.933	4630.067
SD	10.54245	2.811287	18.13128	13.55434	20.75869	21.00008
RSD%	0.493645	0.102988	0.574988	0.375747	0.498296	0.453559

*Average of three readings

Table 2: Peak Area* of Emtricitabine

Replicate	Dilution I	Dilution II	Dilution III	Dilution IV	Dilution V	Dilution VI
1	8126.1	11087.8	13429.6	16047.5	18360.1	20562.5
2	8113.4	11098.6	13448.9	16052.3	18362.9	20569.9
3	8117.2	11096.3	13452.5	16054.1	18365.6	20570.1
Average	8118.9	11094.23	13443.67	16051.3	18362.87	20567.5
SD	6.518435	5.688878	12.31435	3.411744	2.750152	4.331282
RSD%	0.080287	0.051278	0.0916	0.021255	0.014977	0.023587

*Average of three readings

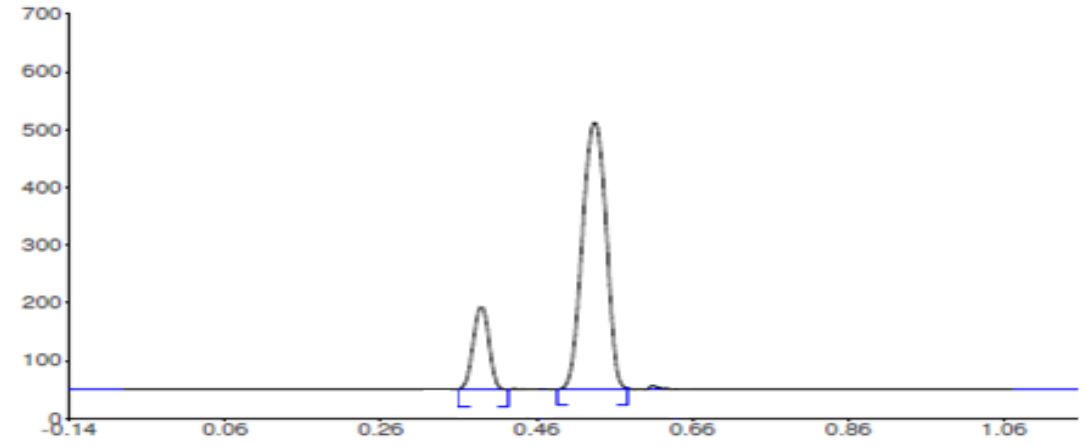
Acceptance Criteria

For linearity Coefficient of relationship worth (r2) should be more important than 0.998 (Regression regard in direct plot).

RESULT

Relationship coefficient (r2) for Tenofovir & Emtricitabine was seen to be 0.9985 & 0.9979 exclusively exhibiting linearity & system is straight between groupings of 100-600ng/ml for both drugs with Rf regard 0.41±0.01and 0.56±0.01 independently.

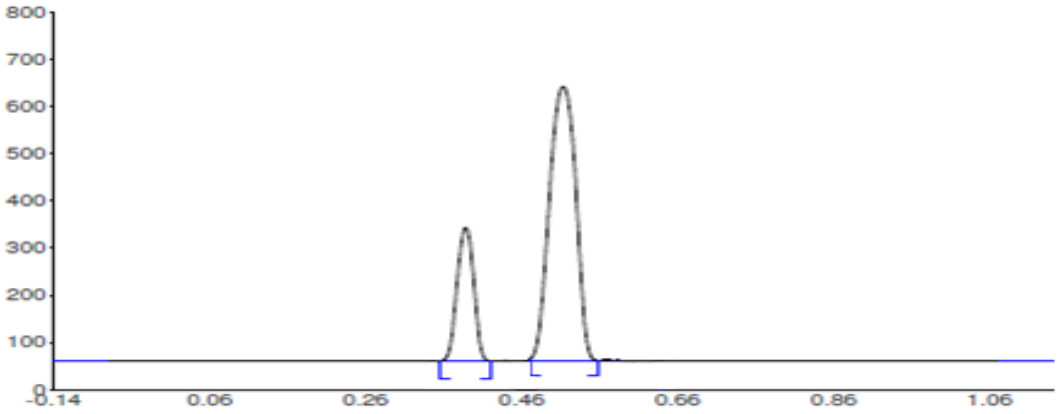
Accuracy



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.38	3.3	0.41	123.9	100.00	0.44	0.5	2686.84	100.00

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
2	0.51	3.2	0.56	336.1	100.00	0.60	4.2	10945.74	100.00

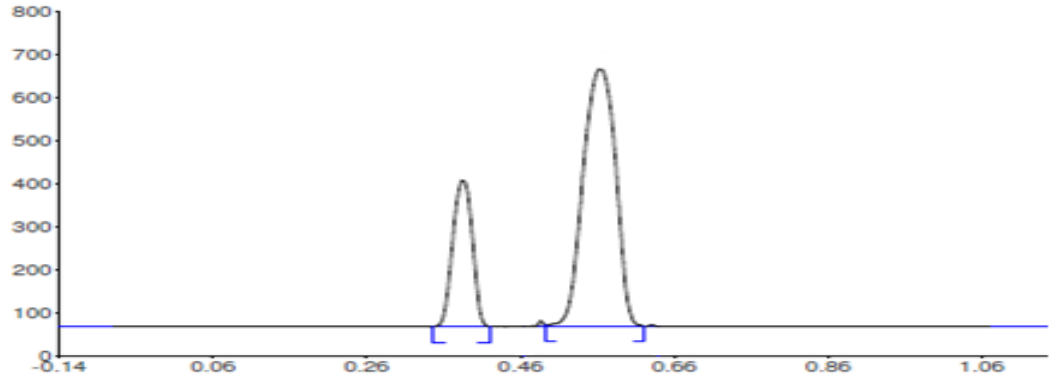
Figure 4: Dilution 1 (200 ng/ml)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.38	3.3	0.41	123.9	100.00	0.44	0.5	3585.56	100.00

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
2	0.51	3.2	0.56	336.1	100.00	0.60	4.2	15859.36	100.00

Figure 5: Dilution 2 (400 ng/ml)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.38	3.3	0.41	123.9	100.00	0.44	0.5	4573.19	100.00

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
2	0.51	3.2	0.56	336.1	100.00	0.60	4.2	20461.21	100.00

Figure 6: Dilution 3 (600 ng/ml)

Table 3: Results of recovery studies of drug

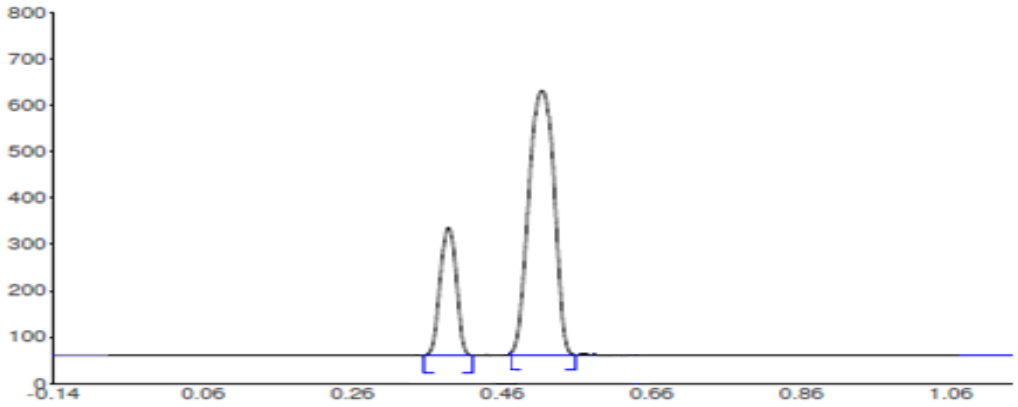
Conc. taken (ng/ml) (A)	Std addition (ng/ml) (B)	Total drug conc. (ng/ml) (A+B)	Peak Area*		% Recovery	
			Teno.	Emtri.	Teno.	Emtri.
100	100	200	2686.84	10945.74	98.42878	98.66156
300	100	400	3585.56	15859.36	99.39733	98.80421
500	100	600	4573.19	20461.21	98.77157	99.48321

*Average of three readings

RESULT

Percentage recovery by proposed method was ranging from 98.42 to 99.48 % indicating no interference of tablet excipients with drug under analysis.

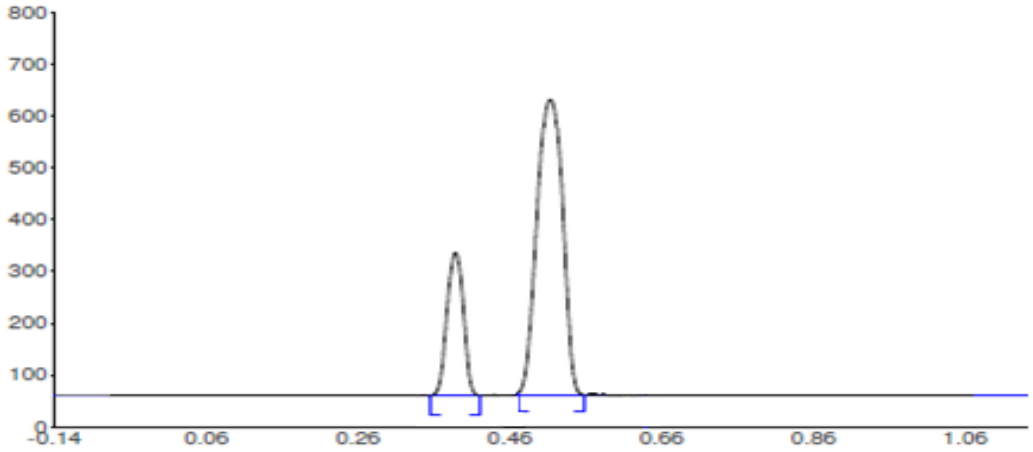
Precision



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.38	3.3	0.41	123.9	100.00	0.44	0.5	3606.9	100.00

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
2	0.51	3.2	0.56	336.1	100.00	0.60	4.2	16954.36	100.00

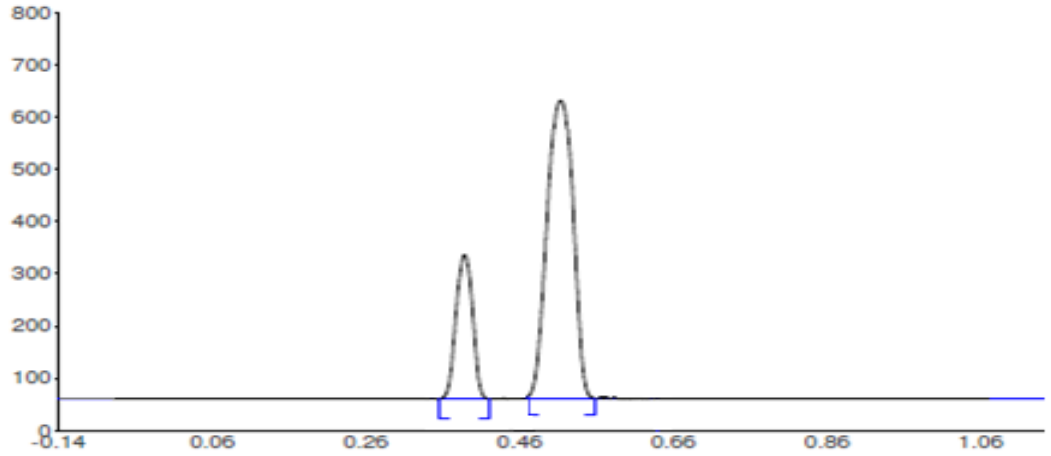
Figure 7: Replicate 1 (400 ng/ml)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.38	3.3	0.41	123.9	100.00	0.44	0.5	3597.1	100.00

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
2	0.51	3.2	0.56	336.1	100.00	0.60	4.2	16962.23	100.00

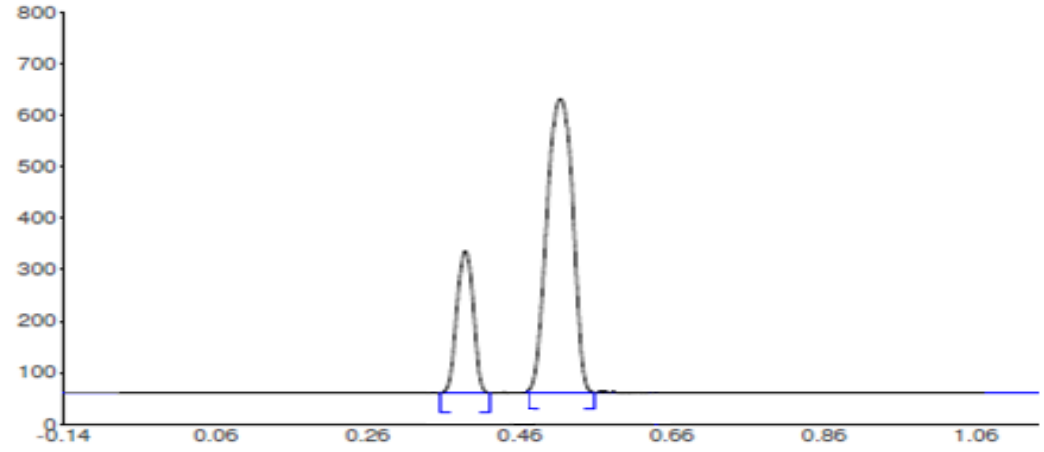
Figure 8: Replicate 2 (400 ng/ml)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.38	3.3	0.41	123.9	100.00	0.44	0.5	3605.8	100.00

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
2	0.51	3.2	0.56	336.1	100.00	0.60	4.2	16945.27	100.00

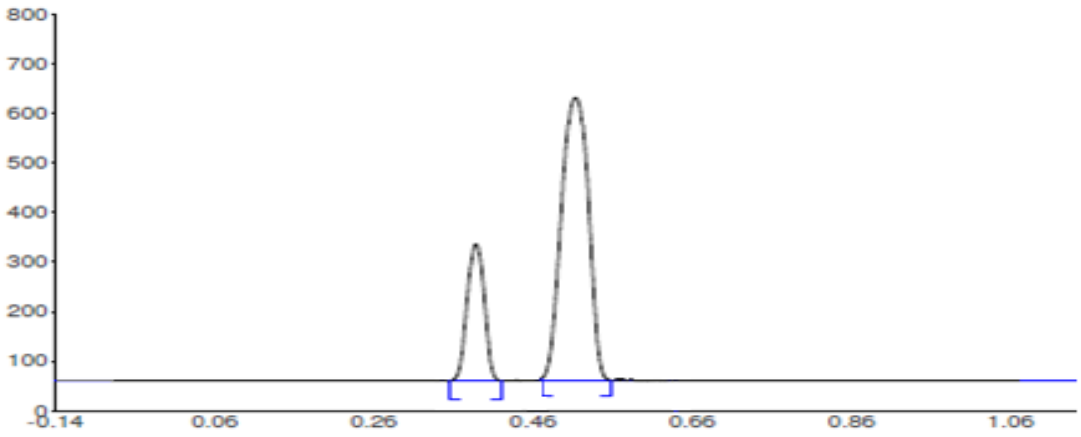
Fig 9: Replicate 3 (400ng/ml)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.38	3.3	0.41	123.9	100.00	0.44	0.5	3615.5	100.00

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
2	0.51	3.2	0.56	336.1	100.00	0.60	4.2	16998.96	100.00

Fig 10: Replicate 4 (400ng/ml)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.38	3.3	0.41	123.9	100.00	0.44	0.5	3586.3	100.00

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
2	0.51	3.2	0.56	336.1	100.00	0.60	4.2	16943.23	100.00

Figure 11: Replicate 5 (400 ng/ml)

Table 4: Precision

S. No.	Area Response	
	Teno.	Emtri.
1.	3606.9	16954.36
2.	3597.1	16962.23
3.	3605.8	16945.27
4.	3615.5	16998.96
5.	3586.3	16943.23
Average	3602.32	16960.81
S.D	11.07574	22.6362
R.S.D	0.307461	0.133462

RESULT

From above analytical data is observed that RSD value for assay is 0.307% & 0.133% for Tenofovir & Emtricitabine respectively which indicates that method is precise & reproducible (According to ICH guidelines).

Specificity

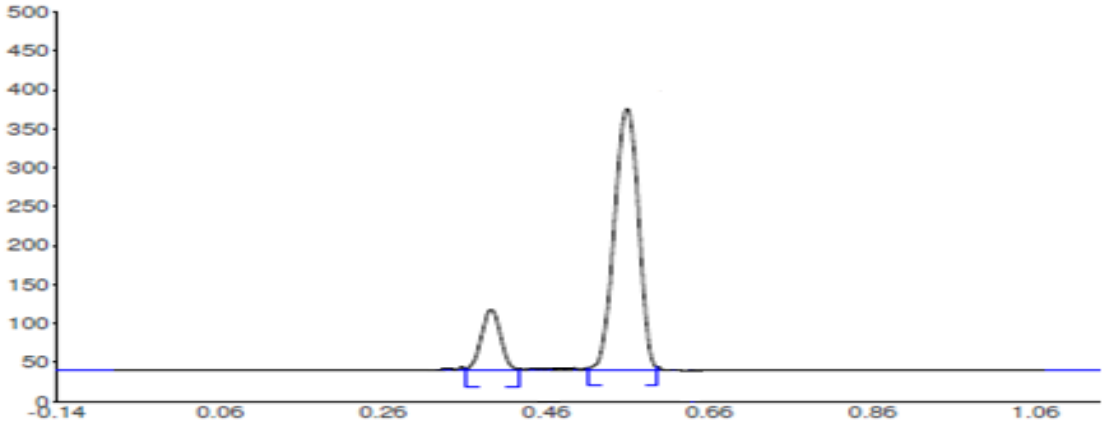


Figure 12: Chromatogram showing specificity

RESULT

Excipients used as piece of different specifying things don't interfere with prescription top which shows that procedure is specific.

Limits of Detection & Quantification

Limit of revelation (LOD) is most lessened measure of analyte in example that can be recognized, however not inflexibly quantized under communicated test conditions. It may be conveyed as center that gives sign to-tumult extent of 2:1 or 3:1. Lower uttermost spans of acknowledgment are 3.6941ng/ml & 0.1422 ng/ml for Tenofovir & Emtricitabine independently in reference material & definition. Limit of Quantification (LOQ) is most lessened total analyte in example that can be determined with sufficient precision & exactness under communicated test conditions. Sign to-hullabaloo extent of 10:1 can be taken as LOQ of method. LOQ qualities were seen to be 11.1945ng/ml & 0.4309ng/ml for Tenofovir & Emtricitabine independently for rough material & definitions.

Range

Scope was learned from linearity graph. Specific degree can be gotten from linearity chart. Degree which is immediate, correct & correct amidst lower & higher obsession is mix's extent of technique. Degree for was seen to be 100-1000 ng/ml.

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.

ACKNOWLEDGMENT

I thank to Satiate Research & Anatech Pvt. Ltd. for providing required facilities to carry out this research work.

REFERENCES

1. M. Minnis & NS, Padian (2004), “Effectiveness of female controlled barrier methods in preventing sexually transmitted infections & HIV: current evidence & future Res. Directions”, ***Sex Transm Infect.***, 81, 193-200.
2. Narendra (2013), “Determination of Emtricitabine by Derivative Spectrophotometric Method in Pharma. Dosage Forms”, ***Chem Sci Trans.***, 2, 3, 978-982.
3. Amand, M (2013), “Uptake of tenofovir & emtricitabineinto non-monocytic female genital tract cellswith & without hormonal contraceptives”, ***J. of Experimental Pharmacology***, 5, 55-64.
4. Anand, K and Vetrichelvan, Thangarasu (2010), “Development & Validation of First-Derivative Spectrophotometric Method for Simultaneous Estimation of Lamivudine & Tenofovir disoproxil fumerate in Pure & in Tablet Formulation”, ***Der Pharmaci. Lettre***, 2, 5, 221-228.
5. Ann, P (2013), “Simple, Reliable, Rapid & Stability Indicating UltrPerformance Liquid Chromatographic Method for Quantitation of Emtricitabine”, ***Am. J. PharmTech Res.***, 3, 1, 621-632.
6. Anthony, E and Gregory, Braden (2005), “Tenofovir-Associated Acute & Chronic Kidney Disease: Case of Multiple Drug Interactions”, ***CID***, 42,283-290.
7. Ashok, G (2013), “Method Development & Validation for Simultaneous Estimation of Lamivudine & Tenofovir Disoproxil Fumarate in Pharma. Dosage Forms by RP-HPLC”, ***IJARPB***, 2, 1, 22-32.
8. Prathap (2013), “Method Development & Validation of Efavirenz in Bulk & Pharma. Dosage Form by UV Spectrophotometer”, ***Int. J. of Innovative Pharma. Res.***, 4, 1, 269-273.
9. Bartr, S (2011), “Process for obtaining Emtricitabine”, ***European Patent Application***, EP2377862A1, 1-13.

10. Bojj, S (2012), "Simultaneous Determination of Tenofovir disoproxil fumarate & Lamivudine by UV Spectrophotometric Method", ***Int. J. of Pharm. & Pharma. Sci. Res.***, 2, 1, 9-15.
11. CH, Venkat (2012), "Development & Validation of Stability Indicating HPLC Method for Combination Tablet Dosage Form of Efavirenz, Lamivudine & Tenofovir in Tablet", ***Int. J. Pharm. Phytopharmacol. Res.***, 2, 1, 40-45.
12. Ch and rashekar, K (2013), "New Analytical Method Development For active Pharma. ingredients using Visible Spectrophotometry", ***Int. J. of General Medicine & Pharm.***, 2, 2, 55-64.
13. Claire, L and Pat, A Tookey (2007), "Antiretroviral therapy & premature delivery in diagnosed HIV-infected women in United Kingdom & Irel", ***AIDS***, 21, 8, 1019–1026.
14. Dahl (2014), "Method & composition for Pharma. Product", ***U.S. Patent Application Publication***, US2014/0370102A1, 1-10.
15. Evanthi, Dova & Rit, Ramos (2010), "***Wet Granulation of Tenofovir, Emtricitabine & Efavirenz***".
16. G, Bhargavi & Ravindrreddy, Y (2011), "Method development & validation of Lamivudine, Tenofovir & Efavirenz in combined dosage form by RP-HPLC", ***J. of Pharm. Res.***, 5, 12, 711-714.
17. Geet, B (2012), "Spectrophotometric determination of tenofovir using 1, 2-napthaquinone-4-sulfonic acid sodium in Pharma. dosage form", ***Analytic Acta***, 3, 7, 83.
18. Girij, P (2011), "Novel Polymorph of Emtricitabine & Process for Preparing of same", ***United States Patent Application Publication,*** US2011/0288298A1, 1-7.
19. Heb, K (2013), "New simple spectrophotometric method for determination of antiviral mixture of emtricitabine & tenofovir disoproxil fumarate", ***Arabian J. of Chemistry***, 1-7.
20. Hemlat, M & Mahesh, B Fadake (2010), "Application of U.V Spectrophotometric method for estimation of Emtricitabine in bulk & capsule", ***IJPRD***, 2, 8, 77-79.
21. Hymavathi, K (2012), "Development & validation of RP HPLC method for simultaneous estimation of Tenofovir Disproxil Fumerate & Lamiudine in combined dosage form", ***Int. J .A. PS. BMS***, 1, 3, 277-284.
22. Indukuri (2014), "Process for preparation of Tenofovir", ***U.S. Patent Application Publication***, US2014/0303368A1, 1-10.
23. Johnson (2011), "Spectrophotometric Determination of Tenofovir Disoproxil Fumarate after Complexation with Ammonium Molybdate & Picric Acid", ***Int. J. of Drug Development & Res.***, 3, 1, 199-204.
24. Johnson, O (2011), "Quantitative Ana. of Tenofovir by Titrimetric,Extractive ion-pair Spectophotometric & Charge- Transfer Complexation Methods", ***Tropical J. of Pharma. Res.***, 10, 1, 89-96.

Correspondence Author:

Rakesh Kumar Jat*

Institute of Pharmacy, Shri Jagdishprasad Jhabarmal Tibrewala University, Vidya Nagri, Jhunjhunu-333001, Rajasthan, India

Cite This Article: Naresh, Chandra Joshi; Pradeep, Kumar and Rakesh, Kumar Jat (2016), "Development and validation of HPTLC method for the estimation of antiretroviral drugs and their pharmaceutical formulations", ***International Journal of Drug Research and Technology***, Vol. 6 (3), 228-238.