CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

https://doi.org/10.5281/zenodo.7813782

Available online at: http://www.iajps.com *Research Article*

ANALYTICAL METHOD VALIDATION REPORT FOR ASSAY OF TENOFOVIR DISOPROXIL FUMARATE, EMTRICITABINE AND ISONIAZID BY RP-HPLC

H.padmalatha

Gyanajyothi College of Pharmacy, uppal, Hyderabad,Telangana

Abstract:

A New method was established for simultaneous estimation of Emtricitabine, Tenofovir disoproxil fumarate and Isoniazid by RP-HPLC method. proposed analytical methods are simple, novel, economical, rapid, sensitive, reproducible and accurate for the Simultaneous estimation of Tenofovir Disoproxil Fumarate, Emtricitabine and Isoniazid in Bulk and Phamaceutical dosage form by using RP-HPLC. This Method gives reliable assay results with short analysis time using mobile phase of Acetonitrile: 0.02M Potassium Dihydrogen Orthophosphate: water (pH 5.3) in the ratio of 60: 25: 15 respectively. Retention time was found to be 2.3 min, 3.7min and 4.9 min for Tenofovir Disoproxil Fumarate, Emtricitabine and Isoniazid respectively. System suitability parameters were in the desired Limit. This method has been developed and optimized as per ICH Q2 (R1) guidelines.

Keywords: *Emtricitabine, Tenofovir disoproxil fumarate and Isoniazid, RP-HPLC, Simultaneous estimation.*

Corresponding author:

H.padmalatha,

Gyanajyothi college of pharmacy
uppal.
E-mail: Padmalatha.malthar@gmail.com

QR code

Please cite this article in press H.padmalatha, ***Analytical Method Validation Report For Assay Of Tenofovir Disoproxil Fumarate, Emtricitabine And Isoniazid By RP-HPLC.,*** *Indo Am. J. P. Sci, 2023; 10 (03).*

INTRODUCTION:

Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI) indicated for the treatment of HIV infection in adults or combined with tenofovir alafenamide for the prevention of HIV-1 infection in high-risk adolescents and adults.[1] Emtricitabine is a cytidine analogue.[2] The drug works by inhibiting HIV reverse transcriptase, preventing transcription of HIV RNA to DNA. IUPAC Name: 4-amino-5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one. Chemical Formula is C8H10FN3O3S. Molecular Weight is 247.247 g·mol−1. Emtricitabine is a white to off-white powder with a solubility of approximately 112 mg/mL in water at 25 °C. The log P for emtricitabine is -0.43 and the pKa is 2.65.

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).[3] This drug is prescribed in combination with other drugs for the management of HIV infection as well as for Hepatitis B therapy. Tenofovir belongs to a class of antiretroviral drugs known as nucleotide analog reverse transcriptase inhibitors (NtRTIs), which block reverse transcriptase, an enzyme necessary for viral production in HIV-infected individuals.[4] This enables the management of HIV viral load through decreased viral replication. IUPAC Name (2E)-but-2-enedioic acid; bis({[(propan-2 yloxy) carbonyl]oxy}methyl){[(2R)-1-(6-amino-9H-purin-9-yl)propan-2yl]oxy}methanephosphonate. Chemical Formula is C23H34N5O14P. Molecular Weight is 635.51 g·mol−1

Isoniazid is an antibiotic used to treat mycobacterial infections; most commonly use in combination with other antimycobacterial agents for the treatment of active or latent tuberculosis.[5] Isoniazid is a prodrug and must be activated by bacterial catalase. Specficially, activation is associated with reduction of the mycobacterial ferric KatG catalase-peroxidase by hydrazine and reaction with oxygen to form an oxyferrous enzyme complex.[6] Once activated, isoniazid inhibits the synthesis of mycoloic acids, an essential component of the bacterial cell wall.[7] At therapeutic levels isoniazid is bacteriocidal against actively growing intracellular and extracellular Mycobacterium tuberculosis organisms. Specifically, isoniazid inhibits InhA, the enoyl reductase from Mycobacterium tuberculosis, by forming a covalent adduct with the NAD cofactor.[8] It is the INH-NAD adduct that acts as a slow, tight-binding competitive inhibitor of InhA. IUPAC Name pyridine-4-carbohydrazide. Molecular Formula C6H7N3O. Molecular Weight 137.13.

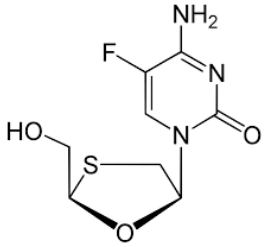

Fig no: 1 Structure of Emtricitabine

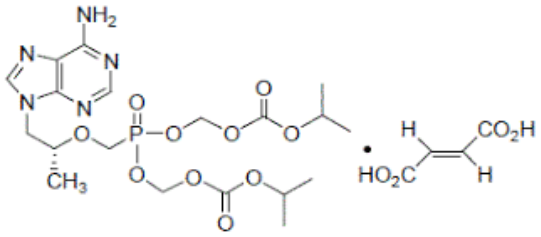

Fig no: 2 Structure of Tenofovir D.F

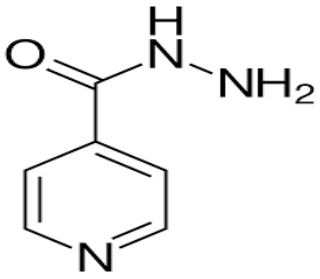

Fig no: 3 Structure of Isoniazid

The literature survey revealed that There are very few methods reported in the literature for analysis of Emtricitabine, Tenofovir disoproxil fumarate and Isoniazid alone or in combination with other drugs in the pure form and pharmaceuticals formulations. There was some HPLC[9-22] methods which have been reported for analysis of these drugs alone or combination with other drugs. Hence, an attempt was made to develop RP-HPLC method for simultaneous estimation of Emtricitabine, Tenofovir disoproxil fumarate and Isoniazid in pharmaceutical dosage form. It can be adopted in regular quality control test in industries and laboratories.

MATERIALS AND METHODS:

Chemicals and Reagents: The Reference standard of Tenofovir Disoproxil Fumarate, Emtricitabine were procured from Macleods Pharmaceuticals Ltd., Baddi and Isoniazid were procured from Amsal Chem Pvt. Ltd., Mumbai. The commercial product of Tavin EM (Emcure Pharmaceuticals Ltd.,) and Isonex of (Pfizer Ltd.,) was bought market separately. HPLC grade Acetonitrile was purchased from Sigma Aldrich Chemicals Pvt Ltd., Mumbai. Ortho Phosphoric acid were purchased from Thermo fischer Pvt. Ltd. Ultrapure water was obtained from Mille Q- water purification system from Millipore (Miliford) USA.

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, PDA detector and Empower 2 software. Analysis was carried out at 260 nm with an SunfireTM C18 (250 x 4.6 mm),5µm dimensions at ambient temperature. The optimized mobile phase consists of Acetonitrile: 0.02M Potassium Dihydrogen Orthophosphate Buffer: Water. Flow rate was maintained at 0.6 ml/min and run time for 10 min.

Preparation of solutions:

Preparation of Mobile Phase :

A Mixture of Acetonitri: 0.02M Potassium Dihydrogen Orthophosphate buffer: Water (60:25:15) was prepared. The pH of the mobile phase was checked. The Resultant mobile phase was degassed in an ultra sonicator for 15 min.

0.02 M Potassium Dihydrogen Orthophospahte buffer were prepared by dissolving 3.12g of Potassium Dihydrogen Orthophosphate in 1000ml of water.

Preparation of Standard Stock solution:

Transfer an accurately weighed quantity of about 10 mg of Tenofovir Disoproxil Fumarate, 10mg of Emtricitabine and 10mg of Isoniazid separately in 10ml Standard flask and added 10ml with Distilled Water.

From the Stock solution, working standard solution of drugs was prepared by appropriate dilution with mobile phase. Stock calibration curve were prepared from 15- 75µg/ml for Tenofovir Disoproxil Fumarate, 10-50µg/ml for Emtricitabine and 15-75µg/ml for Isoniazid.

Preparation of Sample solution:

Weigh and powder 10 tablets. Transfer an accurately weighed quantity of finely powdered tablets equivalent to 10 mg of Emtricitabine and 15 mg of Tenofovir Disoproxil Fumarate and Isoniazid in 10 ml volumetric flask, add about 10ml of mobile phase is added and sonicate for 15min, then filter it with Whatmann filter paper (No.41) and dilute to volume with mobile phase and mix.

Procedure:

Inject 10 µL of the standard, sample into the chromatographic system and measure the areas for Emtricitabine, Tenofovir disoproxil fumarate and Isoniazid peaks and calculate the %Assay by using the formulae.

METHOD:

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 0.6 ml/min for 10 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into SunfireTM C18 (250 x 4.6 mm),5µm, the mobile phase of composition Acetonitrile: 0.02M Potassium Dihydrogen Orthophosphate Buffer: Water was allowed to flow through the column at a flow rate of 0.6 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Emtricitabine, Tenofovir disoproxil fumarate and Isoniazid in their pharmaceutical dosage form. The result obtained for Emtricitabine,

Tenofovir disoproxil fumarate and Isoniazid was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method:

Linearity and Range: A Calibration curve is the relationship between the instrument response and known concentration of analyte. It was observed that the optimized methods were linear with in a specific concentration range for individual drugs. The Calibration curve was constructed by plotting the Peak area Vs Concentration of calibration standards. The Standards were found to be linear in the concentration range of 10-50µg/ml for Emtricitabine ,15-75µg/ml for Tenofovir Disoproxil Fumarate and 15-75 µg/ml for Isoniazid.The results are shown in table 3.

Accuracy studies: Accuracy of the optimized method was determined by absolute and relative recovery. It was found out by replicate analysis of samples containing known amount of the analyte. A minimum of three concentrations in the range of expected study sample was recommended. Based on the calibration curve, accuracy of Emtricitabine, Tenofovir Disoproxil Fumarate and Isoniazid was found by using three concentrations (10, 30, 50 µg/ml in Emtricitabine) and (15, 30, 45 µg/ml in Tenofovir Disoproxil Fumarate and Isoniazid) as LQC (Low quality Control), MQC (Middle quality control), HQC (High quality Control). Each concentration range was injected six times.The results are shown in table 4.

Precision Studies: precision was calculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 5.

Ruggedness: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day. The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 6.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 7.

RESULTS AND DISCUSSION:

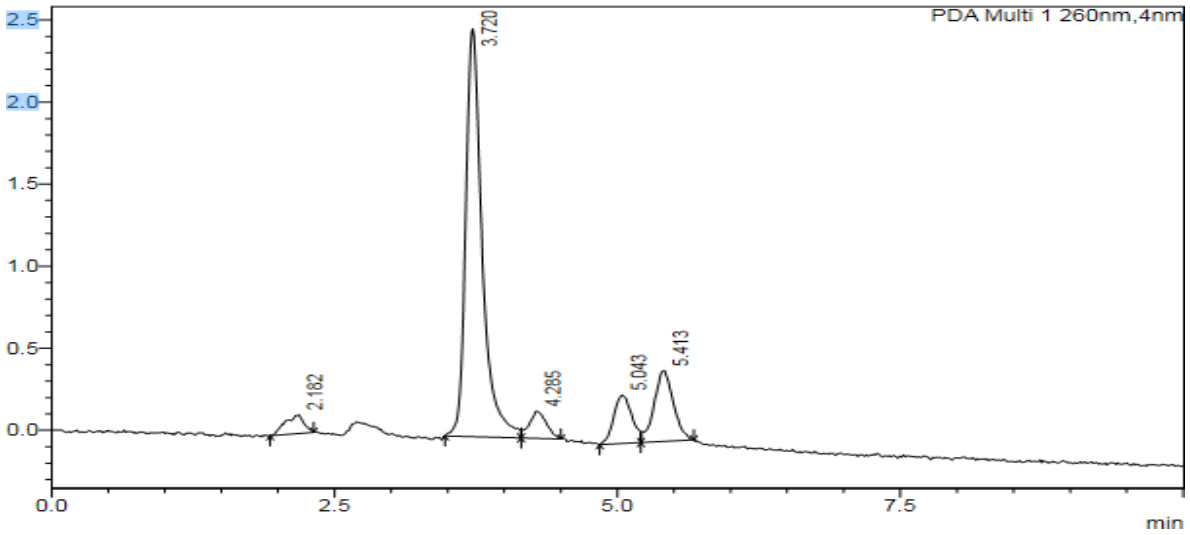

Figure 4: Standard chromatogram

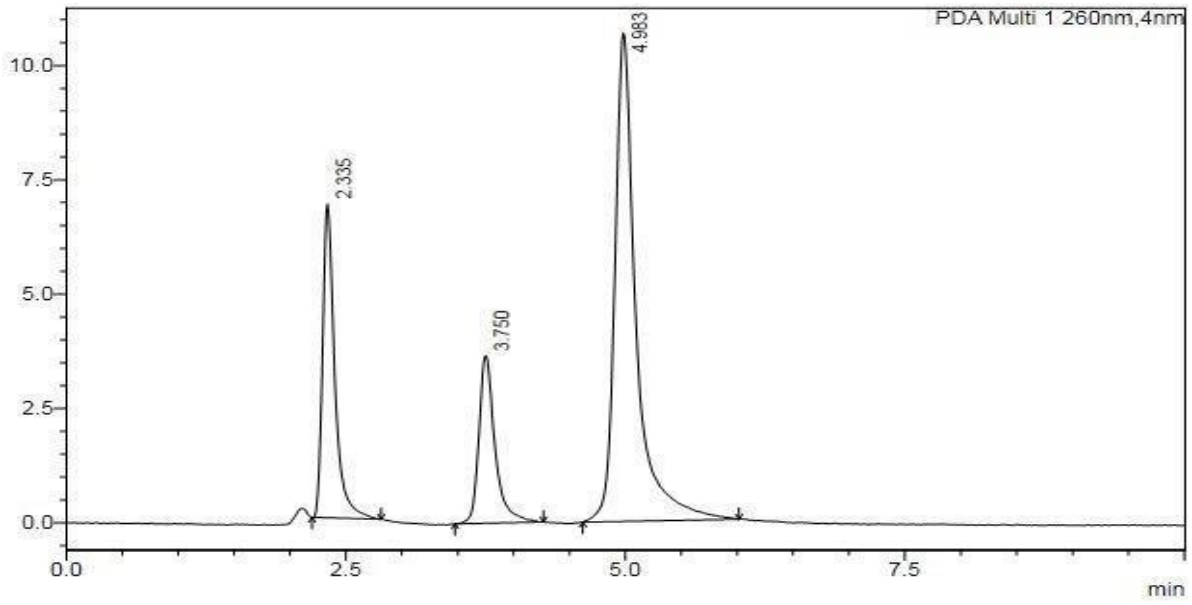

Figure 5: Sample chromatogram

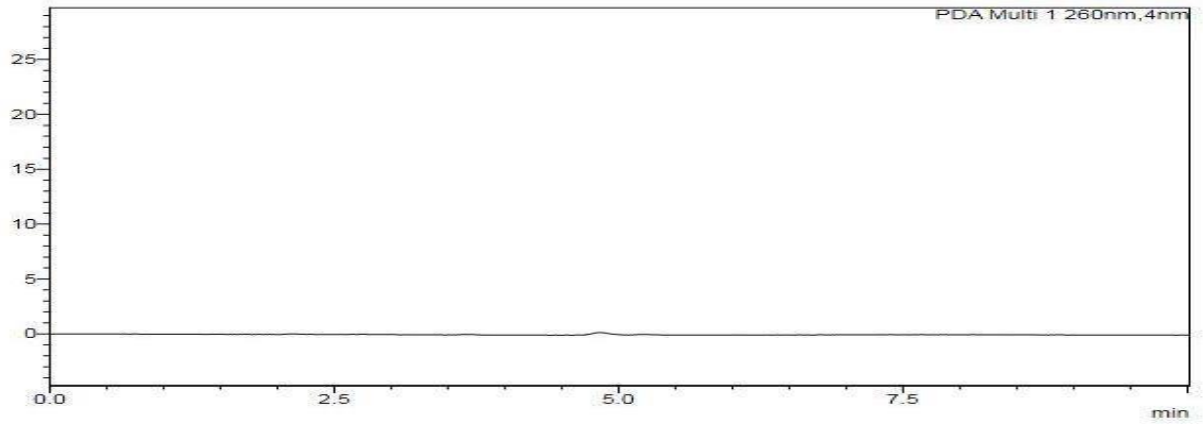

Figure 6: Blank chromatogram

Table 1: System suitability parameters for Emtricitabine, Tenofovir disoproxil fumarate and Isoniazid

PARAMETERS	DRUGS		
	EMT	TDF	INH
Tailing Factor	1.42	1.38	1.51
Resolution	1.1	1.6	1.3
Theoretical Plate(N)	2079	3388	4093

Table 2: Assay results for Emtricitabine, Tenofovir disoproxil fumarate and Isoniazid

	Label Claim (mg)	% Assay
Metformin	10	99.80
Empagliflozin	10	102.33
Linagliptin	10	99.80

Table 3: Linearity results for Emtricitabine, Tenofovir disoproxil fumarate and Isoniazid

S. No	**Emtricitabine**		**Tenofovir disoproxil fumarate**		**Isoniazid**	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	10	61968	15	84567	15	94562
2	20	72678	30	94675	30	109562
3	30	81728	45	106789	45	123491
4	40	91904	60	116749	60	136528
5	50	101024	75	127564	75	149562

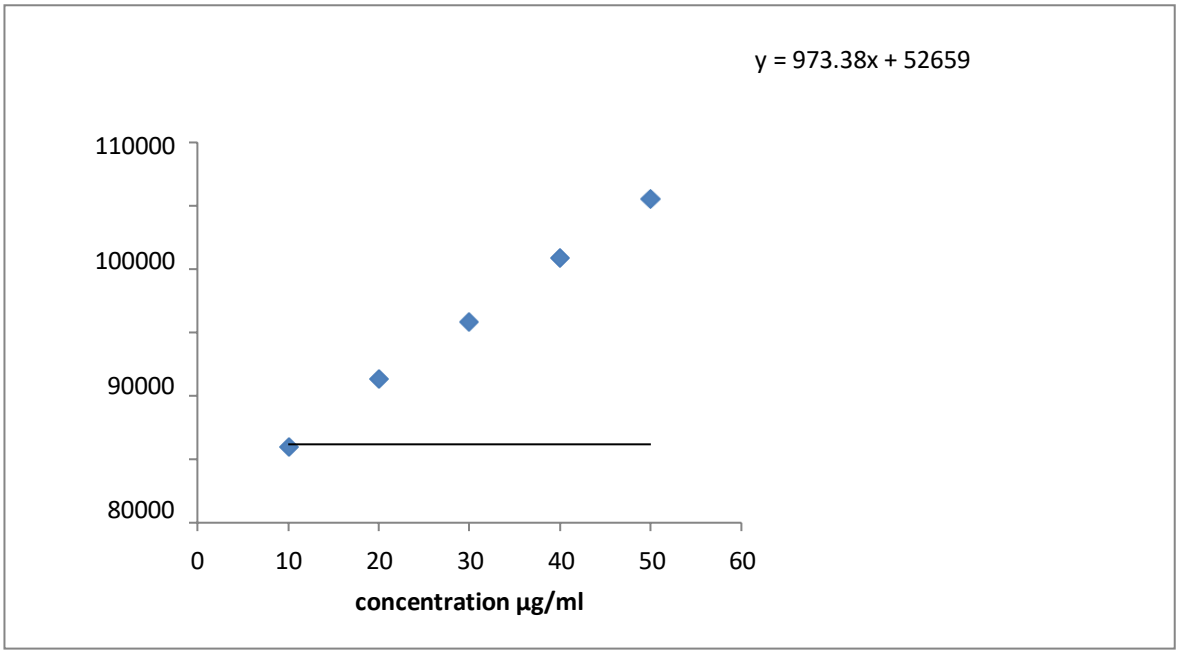

Figure 7: Linearity graph for Emtricitabine

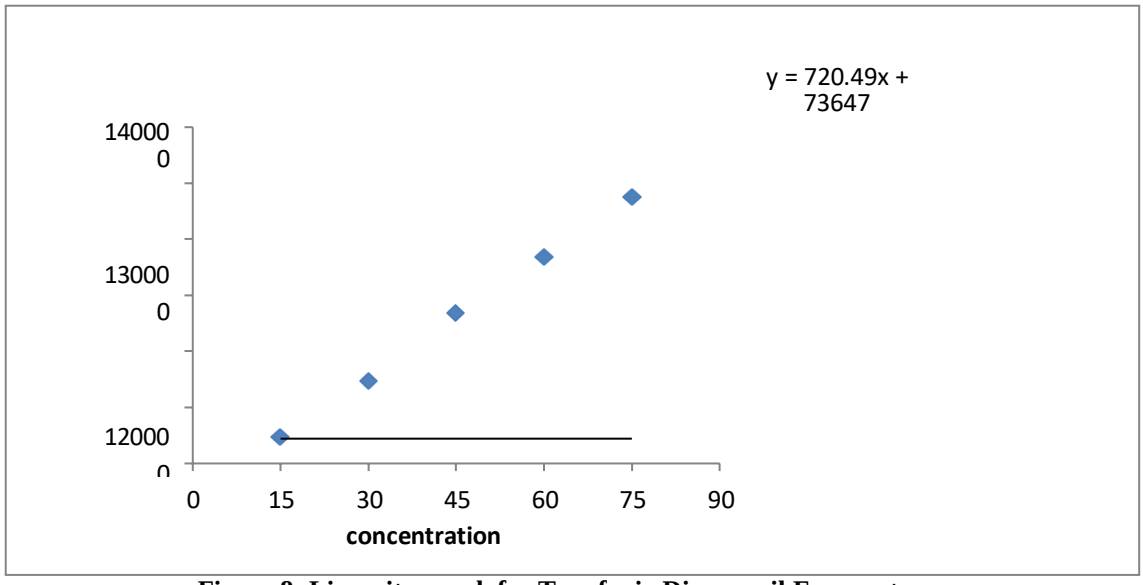

Figure 8: Linearity graph for Tenofovir Disoproxil Fumarate

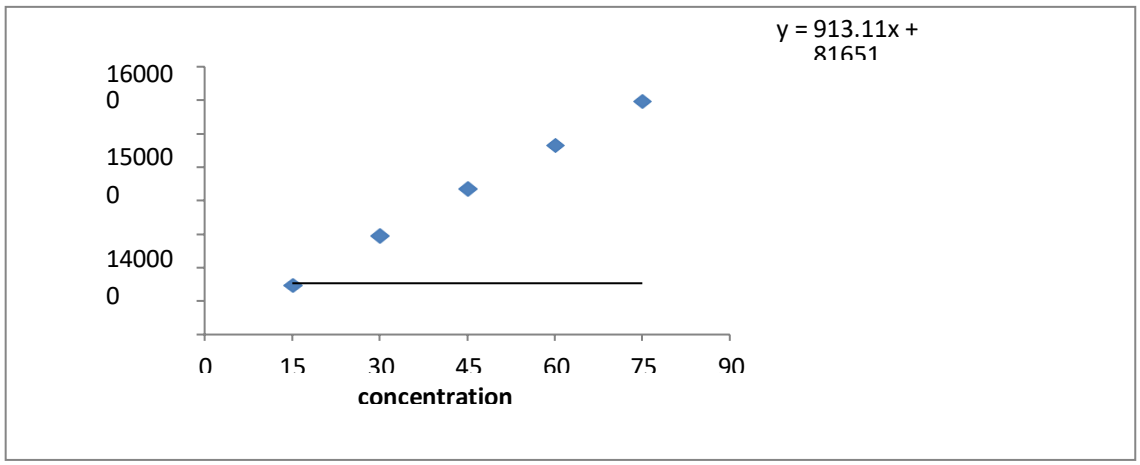

Figure 9: Linearity graph for Isoniazid

Table 4: Accuracy results for Emtricitabine, Tenofovir disoproxil fumarate and Isoniazid

S.no	Level	% Recovery			% RSD		
		EMT	TDF	INH	EMT	TDF	INH
1	80%	100.9	100.2	100.4	1.3	0.7	0.8
2	100%	99.8	100.4	100.4			
3	120%	100.3	99.98	100.3			

Table 5: Precision results for Emtricitabine, Tenofovir disoproxil fumarate and Isoniazid

Concentration(µg/ml)			Peak area			%RSD		
EMT	TDF	INH	EMT	TDF	INH	EMT	TDF	INH
30	45	45	81728	112464	123420	1.35	1.4	1.02
			82456	113678	124567			
			81610	112245	124900			
			84956	113638	122356			
			82559	112564	126432			
			83231	116876	124764			

Table 6: Ruggedness results for Emtricitabine, Tenofovir disoproxil fumarate and Isoniazid

Leve l	Concentration (µg/ml)			Peak area			%RSD		
	EMT	TDF	INH	EMT	TDF	INH	EMT	TDF	INH
1	20	30	30	73351	98799	108976	0.8	0.6	0.6
				74156	99874	107856			
				74567	98794	109189			
2	30	45	45	83781	112464	125759	0.6	0.5	0.9
				84795	112544	125299			
				84463	113664	123420			
3	40	60	60	92795	118759	137555	0.7	0.9	0.7
				93821	116634	136789			
				92509	117985	135476			

Table 7: Results for LOD and LOQ

Drugs	Parameters	
	LOD	LOQ
EMT	1.08	3.2
TDF	1.69	5.1
INH	1.10	3.3

CONCLUSION:

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Emtricitabine, Tenofovir disoproxil fumarate and Isoniazid in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Emtricitabine, Tenofovir disoproxil fumarate and Isoniazid in pure and its pharmaceutical dosage forms.

REFERENCES:

1. Storch CH, Theile D, Lindenmaier H, Haefeli WE, Weiss J: Comparison of the inhibitory activity of anti-HIV drugs on P-glycoprotein. Biochem Pharmacol. 2007 May 15;73(10):1573-81.
2. Soriano V, Labarga P, Fernandez-Montero JV, Mendoza C, Benitez-Gutierrez L, Pena JM, Barreiro P: Drug interactions in HIV-infected patients treated for hepatitis C. Expert Opin Drug Metab Toxicol. 2017 Aug;13(8):807-816.
3. FDA Approved Drug Products: Emtriva (Emtricitabine) Oral Capsules
4. FDA Press Announcements: FDA approves second drug to prevent HIV infection as part of

ongoing efforts to end the HIV epidemic.
5. Pym AS, Domenech P, Honore N, Song J, Deretic V, Cole ST: Regulation of catalase-peroxidase (KatG) expression, isoniazid sensitivity and virulence by furA of Mycobacterium tuberculosis. Mol Microbiol. 2001 May;40(4):879-89. [Article]
6. Heym B, Alzari PM, Honore N, Cole ST: Missense mutations in the catalase-peroxidase gene, katG, are associated with isoniazid resistance in Mycobacterium tuberculosis. Mol Microbiol. 1995 Jan;15(2):235-45. [Article]
7. Wilson TM, de Lisle GW, Collins DM: Effect of inhA and katG on isoniazid resistance and virulence of Mycobacterium bovis. Mol Microbiol. 1995 Mar;15(6):1009-15. [Article]
8. Vilcheze C, Jacobs WR Jr: The mechanism of isoniazid killing: clarity through the scope of genetics. Annu Rev Microbiol. 2007;61:35-50. [Article]
9. MD Abdul Sattar* and Suneetha Achanta. Analytical Method Development and Validation for the Determination of Emtricitabine and Tenofovir Disoproxil Fumarate Using Reverse Phase HPLC Method in Bulk and Tablet Dosage Form. /J. Pharm. Sci. & Res. Vol. 10(5), 2018, 1207-1212
10. Shah A.U., Kotadiya V. V., Ajmera A. A. Department of Quality Assurance, K.B. Raval College Pharmacy, Gandhinagar-382423, India. Research J. Pharm. and Tech. 2016; 9(4): 463-468.
11. Ramaswamy, Anton Smith Arul Gnana Dhas, Development and validation of analytical method for quantitation of Emtricitabine, Tenofovir, Efavirenz based on HPLC, Arabian Journal of Chemistry,2018,11(2),275-281
12. Tej Kumar Kokkirala & Duvvuri Suryakala (2019) RP-HPLC method development and validation for the estimation of Emtricitabine, Bictegravir and Tenofovir alafenamide in bulk and pharmaceutical dosage form, Journal of Taibah University for Science, 13:1, 1137-1146.
13. Rao BV, Vidyadhara S, Nagaraju B and Jhonbi SK: A novel stability indicating RP-HPLC method development and validation for the determination of tenofovir disoproxil fumarate and emtricitabine in bulk and pharmaceutical formulations. Int J Pharm Sci Res 2017; 8(5): 2168-76
14. Reddy BRY, Reddy MT, Reddy BSC (2015) Simultaneous Estimation of Emtricitabine and Tenofovir Disoproxil Fumarate in Tablet Dosage Form by Reverse Phase High-performance Liquid Chromatography. SOJ Chromatograph Sci 1(1): 6.
15. Sharma R, Gupta P. A Validated RP - HPLC Method for Simulataneous Estimation of Emtricitabine and Tenofovir Disoproxil Fumarate in a Tablet Dosage Form. Eurasian Journal of Analytical Chemistry. 2009;4(3):276-284.
16. Prasanthi B, Vijaya ratnar. S, Phani Ch. Development and validation of RP-HPLC method for simultaneous estimation of rifampicin, isoniazid and pyrazinamide in human plasma. J of Anal Chem.2015; 70(8):1015-1022.
17. Khuhawar M.Y, F. M. A. Rind, and A. D. Rajper. High-performance liquid chromatographic determination of isoniazid, pyrazinamide, and indomethacin in pharmaceutical preparations. Acta chromatographica. 2005; 15:205-207.
18. S.K. Dhal and R. Sharma. Development and Validation of RP-HPLC Method for Simultaneous Determination of Pyridoxine Hydrochloride, Isoniazid, Pyrazinamide and Rifampicin in pharmaceutical Formulation. Chem. Anal. (Warsaw).2009; 54:1487.
19. Rohit Bhandari and Indu Pal Kaur, A Sensitive HPLC Method for Determination of Isoniazid in Rat Plasma, Brain, Liver and Kidney Chromatography Separation Techniques.J Chromat Separation Techniq. 2012; 3:3-7.
20. Suchada Jongrungruangchok, Thanapat Songsak. Validation of HPLC methods for determination of isoniazid, rifampicin, pyrazinamide, and ethambutol in fixed-dose combination antituberculosis. Bulletin of Health, Science and Technology.2015; 13(1): 17-28.
21. O. E Bouazzi, N. Badrane, F. Zalagh, R. Soulaymani Bencheikh, Benguedour R. and L. Ait Moussa. Optimization and validation of rapid and simple method for determination of Isoniazid and Pyrazinamide in plasma by HPLC-UV. Journal of Chem and PharmaRes. 2016; 8(3):165- 169.
22. L.Ait Moussa, C. Khassouani, R.Soulaymani, M.Jana, G.Cassanas and R.Alric, et al. Therapeutic isoniazid monitoring using a simple high performance liquid chromatographic method with ultraviolet detection. J Chromatogr B 2002b; 766: 181–187.