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Method Development and Validation for Simultaneous Estimation of Emtricitabine, Tenofovir Disoproxil Fumarate And Isoniazid In Bulk And Pharmaceutical Dosage Form By RP – HPLC

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Abstract

A new developing method was established for simultaneous estimation of Emtricitabine, Tenofovir Disoproxil Fumarate and Isoniazid by RP-HPLC method. The chromatographic condition were successfully developed for the elution of Emtricitabine (EMT), Tenofovir Disoproxil Fumarate (TDF) and Isoniazid (INH) by using Sunfire C18 column (4.6×150mm) 5µm, flow rate was 0.6ml/min, mobile phase ration was (60: 25: 15v/v) Acetonitrile: 0.02M Potassium dihydrogen Ortho- phosphate Buffer: HPLC grade Water. The detection of wavelength was 260nm. The developed and validated method was successfully used for the quantitative analysis of commercially available dosage form. The instrument used was Shimadzu LC-20AR, Labsolution software. The retention time was found to be Emtricitabine 2.334mins, Tenofovir Disoproxil Fumarate 3.835mins and Isoniazid 5.209mins. The % purity of Emtricitabine, Tenofovir Disoproxil Fumarate and Isoniazid were found to be 99.55%, 98.99% and 99.87% respectively. The linearity study of Emtricitabine, Tenofovir Disoproxil Fumarate and Isoniazid was found in the concentration range of 10-50µg/ml, 15-75µg/ml and 15-75µg/ml and correlation coefficient (r^2) was found to be 0.999, 0.999 and 0.999, Accuracy was found to be 100.3%, 100.2% and 100.3% for Emtricitabine, Tenofovir Disoproxil Fumarate and Isoniazid and all other validation parameters were found to be the limits as per ICH Guidelines..

Keywords: Emtricitabine, Tenofovir Disoproxil Fumarate, Isoniazid, RP-HPLC.

INTRODUCTION

Emtricitabine (EMT) is a cytidine analogue and it is a nucleoside reverse transcriptase inhibitor. Chemically it is 5-fluoro-1-(2R, 5S)-[2-(hydroxymethyl)-1, 3-oxathiolan-5yl] cytosine. It inhibits the reverse transcriptase and the enzyme that is needed for the HIV to multiply and that copies HIV RNA into new viral DNA. It is used for the treatment of HIV and active against Hepatitis B virus. **(Figure I)**

Tenofovir Disoproxil Fumarate (TDF) is an acyclic nucleoside phosphonatediester analogue of adenosine monophosphate. Chemically it is (9-[(R)-2-[[bis [(isopropoxycarbonyl) propyl) oxy] methoxy] phosphinyl] methoxy] propyl] adenine fumarate. It is nucleotide reverse transcriptase inhibitors, which block reverse transcriptase, an enzyme necessary to viral production in HIV infected persons. It is used to the treatment of Hepatitis B in adults and childrens and combined with other drugs to treat the immunodeficiency virus or HIV. **(Figure II)**

Isoniazid (INH) is a hydrazide of isonicotinic acid. Chemically it is pyridine-4-carbohydrazide. It is the first-line anti-tubercular drug. It prevents the synthesis of mycolic acid in the mycobacterial cell wall and works in part by disrupting the formation of the bacteria cell wall which results in cell death. It is an antibiotic used to treatment of tuberculosis. A review of literature reveals that no method was developed for simultaneous estimation of Emtricitabine, Tenofovir Disoproxil Fumarate and Isoniazid. It reveals that various analytical methods has been reported for determination of Emtricitabine, Tenofovir Disoproxil Fumarate and Isoniazid individually or combination with other drugs. But till date no method was found for simultaneous determination of these three drugs in bulk and Pharmaceutical formulation. Hence, the aim of this work was directed to the simple, sensitive, selective and validated spectrophotometric method for the simultaneous estimation of Emtricitabine, Tenofovir Disoproxil Fumarate and Isoniazid in bulk and Pharmaceutical dosage form. . **(Figure III)**

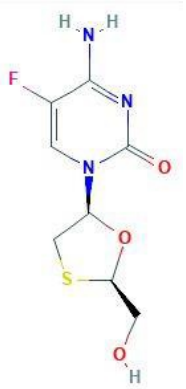

Fig. I Emtricitabine

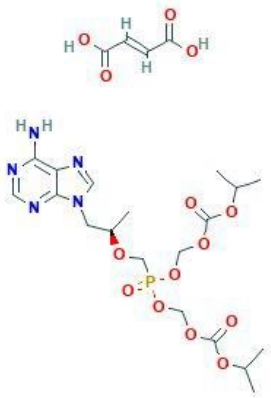

Fig. II Tenofovir Disoproxil Fumarate

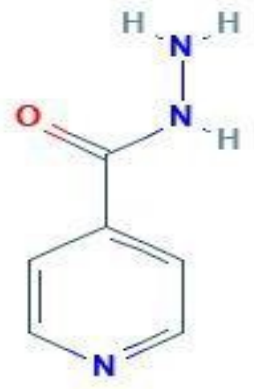

Fig. III Isoniazid

MATERIALS AND METHODS

Chemicals and Reagents:

The reference standard of Emtricitabine and Tenofovir Disoproxil Fumarate were procured from Macleods Pharmaceutical Limited., Baddi and Isoniazid were procured from Amsal chem. Pvt. Limited., Mumbai. The commercial product were procured from the market. HPLC grade Acetonitrile were obtained from sigma Aldrich, India, HPLC grade water were obtained from Thermo Fisher Pvt. Ltd and Potassium dihydrogen Orthophosphate from Himedia Labs Pvt Ltd.

Intrumentation:

The Shimadzu LC-20AR HPLC is a dual reciprocrating plunger parallel-flow solvent delivery module system using a Sunfire C18 column (4.6×150mm) 5µm and Shimadzu SPD-M20A Photodiode Array UV-Vis Detector and the Labsolution was used. The mobile phase was sonicated using Ultra Sonic Sonicator. The detection wavelength was fixed utilizing UV- 1650PC, Shimadzu.

Preparation of Mobile Phase:

A mixture of Acetonitrile: 0.02M Potassium dihydrogen Orthophosphate: HPLC grade water in the ratio of 60: 25: 15 and was sonicated for 15 minutes.

Preparation of 0.02M Potassium dihydrgen Orthophosphate Buffer:

3.12gm off potassium dihydrogen Orthophosphate in 1000ml HPLC grade water and the pH 5.3.

Preparation of Standard solution:

Weigh accurately the working standard 10mg of Emtricitabine and 15mg of both Tenofovir Disoproxil Fumarate and Isoniazid was taken in dry clean 10ml volumetric flask and make up with using the mobile phase and sonicated. From the above stock solution pipette out 1ml of solution in the 10ml volumetric flask and make up with HPLC grade water and then again pipette out 1ml of solution in the 10ml volumetric flask and make up with HPLC grade water to obtain the concentration of 10-50µg/ml of Emtricitabine and 15-75µg/ml of both Tenofovir Disoproxil Fumarate and Isoniazid respectively. The calibration curve was derived by plotting the ratio of peak area of drug versus concentration.

Preparation of Sample solution:

The commercial product 10 tablets of Emtricitabine, Tenofovir Disoproxil Fumarate and Isoniazid was individually taken, weighed and the amount of the tablets and weigh to be taken to be determined. Finally 10mg of Emtricitabine and 15mg of both Tenofovir Disoproxil Fumarate and Isoniazid was extracted using the mobile phase. The concentration of 10-50 µg/ml of Emtricitabine and 15-75µg/ml of both Tenofovir Disoproxil Fumarate and Isoniazid was prepared and injected. **(Table a)**

Table (a) Analysis of Formulation

S.No	Drug	Amount (mg)		% Label Claim	% RSD
		Labelled	Found		
1	Emtricitabine	200	184.8	92.4	0.8
2	ofovir disoproxil Fumarate	300	273	91	0.7
3	Isoniazid	300	283.9	94.62	0.6

RESULTS AND DISCUSSION

Selection of Detection Wavelength:

The reference standard 10mg of Emtricitabine and 15mg of both Tenofovir Disoproxil Fumarate and Isoniazid was taken and dissolved in HPLC grade water was scanned in the range of 200-400nm and the isobestic point of these three drugs was found to be 260nm. **(Figure IV)**

Optimization of Chromatographic Conditions:

For the several trials of the drugs were performed using the mobile phase mixtures of organic phases, aqueous phases and acids with the standard solutions. Thus the final mobile phase for this thesis was found to be Acetonitrile: 0.02M Potassium dihydrogen Orthophosphate: HPLC grade water in the ratio of 60: 25: 15. This specific ration gives a sharp peak with good resolution. The Sunfire C18 (4.6×150mm) 5µm column of stationary was used. Flow rates of 0.2ml/min, 0.4ml/min,0.6ml/min and 0.8ml/min were tried and finally 0.6ml/min was fixed for the study. **(Figure V, VI, VII, VIII)**

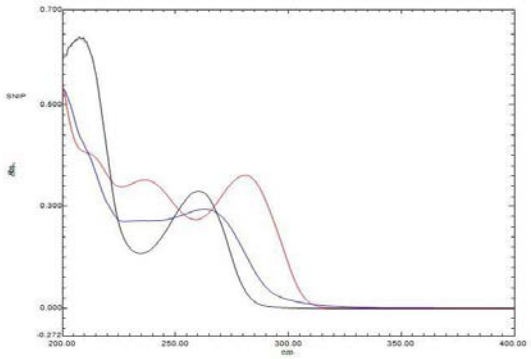

Figure IV Overlay Spectrum of Emtricitabine, Tenofovir Disoproxil Fumarate and Isoniazid

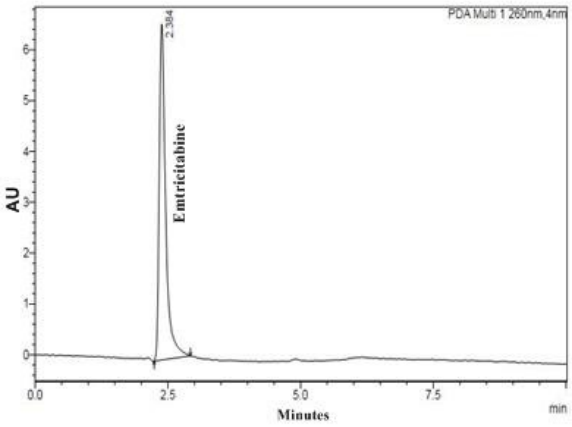

Figure V Standard Chromatogram of Emtricitabine (EMT)

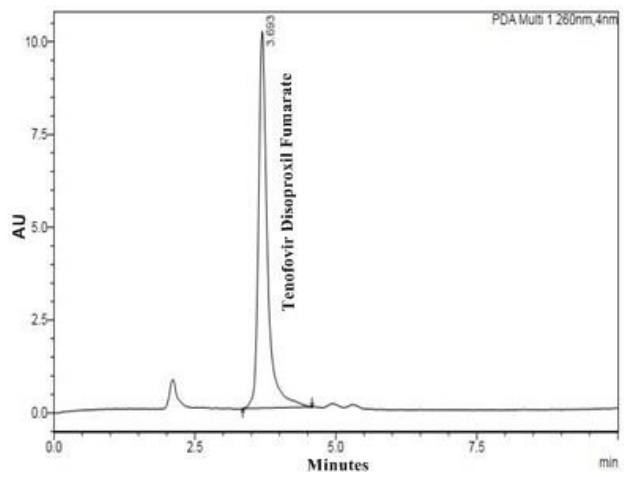

Figure VI Standard Chromatogram of Tenofovir Disoproxil Fumarate(TDF)

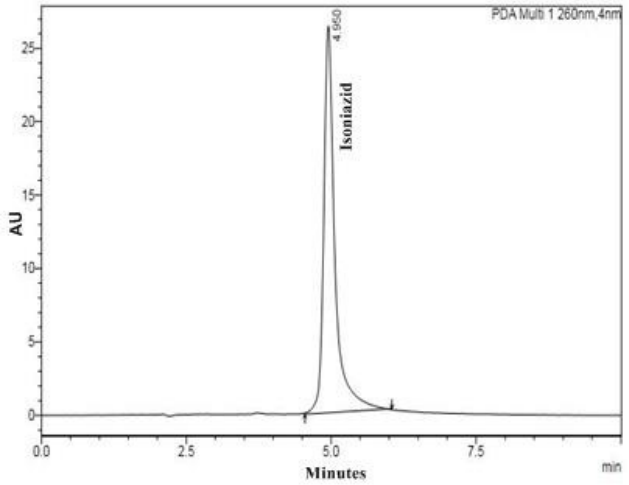

Figure VII Standard Chromatogram of Isoniazid(INH)

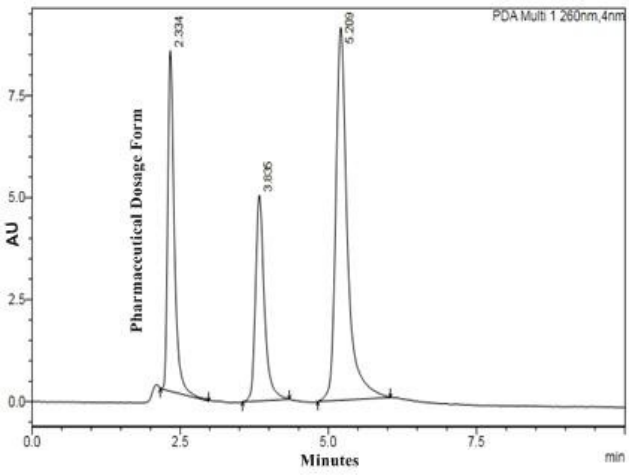

Figure VIII Chromatogram of Pharmaceutical Dosage Form (Emtricitabine, Tenofovir Disoproxil Fumarate and Isoniazid)

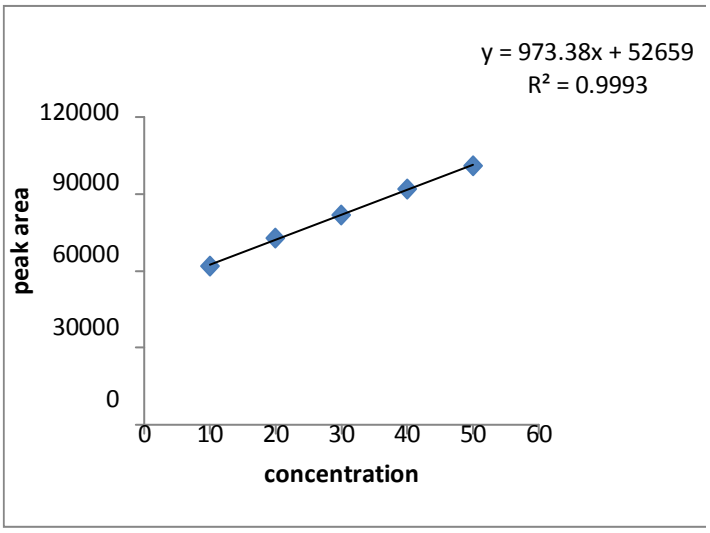

Figure IX Calibration Curve of Emtricitabine (EMT)

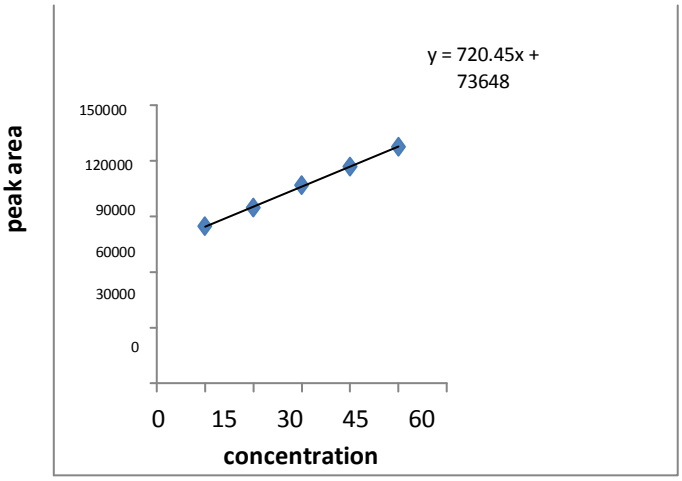

Figure X Calibration Curve of Tenofovir Disoproxil Fumarate(TDF)

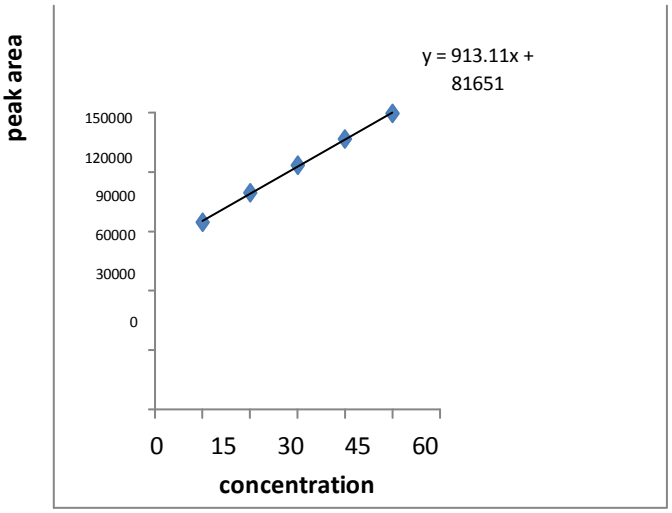

Figure XI Calibration Curve of Isoniazid(INH)

Table (b) 1 Intraday Precision

S.No	Concentration (µg/ml)			Peak area			%RSD		
	EMT	TDF	INH	EMT	TDF	INH	EMT	TDF	INH
1	20	30	30	72678	94675	109562	0.2	0.3	0.2
				72492	94219	109259			
				72798	94751	109757			
2	30	45	45	81728	106789	123491	0.2	0.2	0.1
				81509	106243	123270			
				81376	106575	123511			
3	40	60	60	91904	116749	136528	0.2	0.2	0.1
				91722	116453	136279			
				92094	116997	136798			

Table (b) 2 Interday Precision

S.No	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 (b) 3 Repeatability

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 (c) 1 Calculated Concentration and Percentage Recovery of Emtricitabine

Level	LQC		MQC		HQC	
Analyte Concentration	10µg/ml		30µg/ml		50µg/ml	
S.No	Calculated Concentration (µg/ml)	Accuracy	Calculated Concentration (µg/ml)	Accuracy	Calculated Concentration (µg/ml)	Accuracy
1	10.15	101.5	29.85	99.5	49.95	99.9
2	10.05	100.5	28.95	96.5	50.25	100.5
3	10.4	104	30.25	100.8	49.86	99.7
4	9.89	98.9	29.98	99.9	50.55	101.1
5	10.08	100.8	30.06	100.2	49.99	99.99
6	9.97	99.7	30.52	101.7	50.38	100.7
Mean	100.3					
%RSD	0.8					

Table (c) 2 Calculated Concentration and Percentage Recovery of Tenofovir Disoproxil Fumarate

Level	LQC		MQC		HQC	
Analyte Concentration	10µg/ml		30µg/ml		50µg/ml	
S.No	Calculated Concentration (µg/ml)	Accuracy	Calculated Concentration (µg/ml)	Accuracy	Calculated Concentration (µg/ml)	Accuracy
1	15.02	100.1	45.09	100.2	75.12	100.1
2	15.12	100.8	45.67	101.5	74.45	99.3
3	14.95	99.7	44.93	99.84	75.18	100.2

4	15.23	101.5	45.47	101	75.57	100.7
5	15.07	100.4	44.86	99.68	74.97	99.9
6	14.78	98.5	45.01	100.2	74.56	99.4
Mean	100.2					
%RSD	0.7					

Table (c) 3 Calculated Concentration and Percentage Recovery of Isoniazid

Level	**LQC**		**MQC**		**HQC**	
Analyte Concentration	**10µg/ml**		**30µg/ml**		**50µg/ml**	
S.No	**Calculated Concentration (µg/ml)**	**Accuracy**	**Calculated Concentration (µg/ml)**	**Accuracy**	**Calculated Concentration (µg/ml)**	**Accuracy**
1	14.95	100.2	45.08	100.2	75.12	100.2
2	15.14	100.9	45.49	101.09	75.75	101
3	15.37	102.4	45.01	100.02	74.91	99.8
4	14.89	99.3	45.63	101.4	74.87	99.82
5	15.09	100.6	45.16	100.3	75.19	100.2
6	15.16	101.5	44.81	99.58	75.46	100.6
Mean	100.3					
%RSD	1.3					

Fixed Chromatographic Conditions

Stationary phase : Sunfire C18 (250 x 4.6 mm) 5µm
Mobile phase : Acetonitrile: 0.02M Potassium dihydrogen Orthophosphate Buffer: HPLC grade water
Mobile phase ratio : 60: 25: 15
Detection Wavelength : 260nm
Flow Rate : 0.6ml/min
Injection Volume : 20µl
Temperature : 25^{O} C

Method Validation

The developed method validation parameters were employed by ICH Guidelines. In this study all the validation parameters results within the limits were confirmed.

Linearity:

The calibration curve of Emtricitabine, Tenofovir Disoproxil Fumarate and Isoniazid were plotted by Concentration v/s Peak area and the regression equation was calculated. The linearity of Emtricitabine concentration range was 10-50µg/ml and both Tenofovir Disoproxil Fumarate and Isoniazid concentration range was 15-75µg/ml. These concentrations were prepared by diluting with HPLC grade water and the calibration curve was plotted. The correlation coefficient of Emtricitabine, Tenofovir Disoproxil Fumarate and Isoniazid were found to be 0.999, 0.999 and 0.999. **(Figure IX, X, XI)**

Precision:

It should be measured using a minimum of three determinations per concentration. The concentration solution were prepared using 20,30,40µg/ml of Emtricitabine and 30,45,60µg/ml of both Tenofovir Disoproxil Fumarate and Isoniazid. In Intraday Precision were found by carrying out the analysis at three different concentrations in a same day. In Interday Precision were found by carrying out the analysis at three concentrations in a week. **(Table b1, b2, b3)**

Accuracy:

The recovery studies were performed to validate the accuracy of the newly developed method with different concentrations (lowest, middle and highest) of pre analyzed sample solution of tablets. **(Table c1, c2, c3)**

Limit of Detection and Limit of Quantification:

The limit of Detection and limit of Quantification of the developed method was establishing by injecting the lowest concentration of the Standard solution using RP-HPLC method.

LOD=3.3×SD/Slope and LOQ=10×SD/Slope. (Table d) Specificity:

Specificity is the ability of an analytical method to differentiate and quantify the analyte in presence of other components in the sample. Selectivity is evaluated by injecting the blank and comparing with the response of extracted LLOQ samples processed with Mobile Phase. Endogenous interferences were not detected at the retention time of sample and standard. These observations show that the developed assay method is specific.

Table (d) Limit of Detection and Limit of Quantification

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

Robustness:

Even on the slightly varied of detection wavelength, temperature, flow rate and pH for the developed method there was no changes noted in the retention time of the drugs so it can be claimed to be the robust.

System Suitability:

System Suitability was studied by injecting six consecutive replicates of the standard solution and the HPLC parameters like Tailing Factor, Theoretical Plates, Capacity Factor and Resolution were calculated. The results were found to be in the tolerable limits. **(Table e)**

Table (e) System Suitability

PARAMETERS	DRUGS		
	EMT	TDF	INH
Tailing Factor	1.42	1.38	1.51
Theoretical Plate(N)	2079	3388	4093
Resolution	1.5		

CONCLUSION

The developed method was found to be suitable for simultaneous estimation of Emtricitabine, Tenofovir Disoproxil Fumarate and Isoniazid in bulk and Pharmaceutical dosage form by using RP-HPLC. In this newly developed method was found to simple, precise, reliable, robust, economical and less time consuming. The retention time was found to be 2.334min, 3.835min and 5.209min for Emtricitabine, Tenofovir Disoproxil Fumarate and Isoniazid. By reverse phase HPLC an isocratic method was developed with an elution time of less than 10 minutes. The correlation coefficient for these three drugs was found to be 0.999. All the validation parameters like Accuracy, Precision, LOD, LOQ and System Suitability were found to be within the limits according to ICH Guidelines. Thus we firmly determine that the proposed method development employed for other Pharmaceutical dosage forms and also for Bio-analytical methods.

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REFERENCES

[1] Hadija H Semvua, Charles M Mtabho, Quirine Fillekes, Jossy van den Boogaard, Riziki M Kisonga, Liberate Mleoh, Arnold Ndaro, Elton R Kisanga, Andre van der Ven, Rob E Aarnoutse, Gibson S Kibiki, Martin J Boeree, David M Burger. Antiviral Therapy 2013; 18:105-113.

[2] Text book of Practical HPLC method development, 2nd edition Lloyd R. Snyder, Joseph J.

[3] Dr. Santosh Kumar Bhadrwaj, A Review: HPLC Method Development and Validation, 2015.

[4] Indian Pharmacopoeia vol (2), 1936-1938 &2321-2322.

[5] Indian Pharmacopoeia vol (3), 3327-3332.

[6] ICH, Q2 (R1) Validation of analytical procedure(1994).

[7] K. Anathakumar, K. Kannan, T. Vetrichelvan. **"**Development and Validation of Emtricitabine and Tenofovir Disoproxil Fumarate in pure and in Fixed Dose Combination by UV Spectrophotometry". Digest Journal of Nanomaterials and Biostructures Vol. 6. No.3, 2011, p.1085-1090.

[8] Syeda Kulsum, G Vidya Sagar. "Determination of Tenofovir and Emtricitabine by Simultaneous Equation Spectrophotometric Method". IOSR Journal of Pharmacy and Biological Sciences, p-ISSN: 2319-7676. Volume 11, Issue 3 Ver. III (May- Jun.2016), PP 111-115.

[9] Mariana Tilinca, Gabriel Hancu, EleonoraMircia, Diana Iriminescu, Aura Rusu, Robert AlexandruVlad, EnikoBarabas. **"**Simultaneous Determination of Isoniazid and Rifampicin by using Spectrophotometry". Farmacia 2017, Vol 65, 2.

[10] Rajesh Sharma and Pooja Gupta. **"**A Validated RP-HPLC Method for Simultaneous Estimation of Emtricitabine and Tenofovir Disoproxil Fumarate in a Tablet Dosage Form". Eurasian Journal of Analytical Chemistry 2009; 4(3):276- 284.

[11] Ajay D. Mali and Uttam B. **"**RP-HPLC Method for Simultaneous Estimation of impurities from Emtricitabine and Tenofovir Disoproxil Fumarate Tablet". IJPSR, 2016; Vol. 7(4): 1662-1669.

[12] MD AbdulSattar and Suneetha Achanta. **"**Analytical Method Development and Validation for the Determination of Emtricitabine and Tenofovir Disoproxil Fumurate using RP-HPLC Method in Bulk and Tablet Dosage Form". J. Pharma. Sci. & Res. Vol. 10(5), 2018, 1207-1212.

[13] B. Venkateswara Rao, S. Vidyadhara, B. Nagaraju and SK Jhonbi. **"**A Novel Stability Indicating RP-HPLC Method Development and Validation for the Determination of Tenofovir Disoproxil Fumarate and Emtricitabine in Bulk and Pharmaceutical formulations". IJPSR, 2017; Vol. 8(5): 2168-2176.

[14] BommakantiValliPurnima, TummalaVijayaBaskara Reddy, YadlapalliSrinivas Rao, GolkondaRamu, DittakaviRamachandran. **"**Stability Indicating RP- UPLC Method for Assay of Emtricitabine and Tenofovir Disoproxil Fumarate in Bulk and Dosage Forms". American Journal of Analytical Chemistry, 2015, 06(10): 807-821.

[15] SaiDatriArige and Lakshmana Rao A. **"**RP-HPLC Method Development and Validation for Simultaneous Estimation of Isoniazid and Pyrazinamide". IJAPS 2017, 1-11.

[16] Yateen Shah, S. Khanna, K.C. Jindal & V.S. Dighe. **"**Determination of Rifampicin and Isoniazid in Pharmaceutical Formulations by HPLC". Journal Drug Development and Industrial Pharmacy, Vol. 18, 1992- issue 14.

[17] GampaVijaya Kumar, Dr. D. Jayaprakash. **"**Analytical Method Development and Validation by RP-HPLC for Simultaneous Estimation of Isoniazid and Ethambutol in Combined Tablet dosage Form". JPBMAL 2015, 3(2): 251- 258.