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Original Research Article

Development and Validation of RP-HPLC method of Emtricitabine and Tenofovir in Bulk and Pharmaceutical Dosage form

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ABSTRACT

A new method was established for simultaneous estimation of Emtricitabine and Tenofovir Disoproxil Fumarate by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Emtricitabine and Tenofovir Disoproxil Fumarate by using Inspire C18 column (250×4.6mm) 5.0µm, flow rate was 1.0ml/min, mobile phase ratio was (0.1% OPA) Ortho-phosphoric acid Buffer (adjust the pH 3 (60:40 Methanol: Water) .The detection of wavelength was 272nm.The developed and validated method was successfully used for the quantitative analysis of commercially available dosage forms. The instrument used was HPLC Agilent (1100) Gradient System with auto injector, DED Detector.Equipped with reverse Phase(Agilent) C18 Column (4.6mm x 250mm; 5 µm), 20 µl injection loop and UV730D Absorbance detector and running Chemstation-10.1 software.The retention times of Emtricitabine, it has been observed that, using mobile phase of Methanol + water(0.1% OPA) PH 3(60 :40%)v/v) 271nm,0.7 ml, pH 3.0 gave adequate retention time at 3.477min and 4.989min. With good peak shape (Theoretical plates of 8066 of Emtricitabine & 8594 of Tenofovir. Accuracy of RP-HPLC method is ascertained by recovery studies performed at different levels of concentrations (80%, 100% and 120%). The % recovery was found to be within 99-101%.. Repeatability studies on RP-HPLC for Emtricitabine and Tenofovir was found to be ,The % RSD was less than 2%, which shows high percentage amount found in between 98% to 102% indicates the analytical method that concluded . All the solution was analyzed thrice in order to record any intra-day & inter-day variation in the result that concluded. Robustness parameters were also found satisfactory; hence the analytical method would be concluded. The LOD and LOQ of Emtricitabine was found to be 0.48 (µg/mL) and 1.459(µg/mL), analytical method that concluded. The LOD and LOQ of Tenofovir was found to be 0.99 (µg/mL) and 3.022(µg/mL), analytical method that concluded.) The amounts of Emtricitabine and Tenofovir per tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with tablet formulation. Tablet Assay for % Lable claim for %RSD Calculated. Analysis of marketed formulation were also % Lable Claim was found to be 98-101% Satisfactory are concluded.

Introduction

Analytical chemistry deals with quantitative analysis of composition of substances and complex materials in various matrices by measuring a physical or chemical property of a distinctive constituent of the components of interest. Analytical methods are classified according to the property of the analyst measured[1]. The pharmaceutical analysis is one of the most important fields in analytical chemistry. Modern analytical chemistry is dominated by instrumental analysis. There are so many different types of instruments used today that, it seems like a confusing array of acronyms rather than a unified field of study[2]. The analytical methods should be accurate as required and not as accurate as possible[3].

Several instrumental methods are used in pharmaceutical analysis, amongst these some important methods are separation techniques, spectrometric techniques and other analytical techniques[4]. Pharmaceutical analysis is the integral part of the pharmaceutical sciences. In pharmaceutical analysis section, the research analyst is responsible for three important functions viz: Development of analytical method for raw materials, active ingredients and chemical intermediates of the product.

Development of analytical methods for selective analysis of drug, excipients, degradation products and impurities along with identification of degradation product, degradation pathway and extent of degradation when stored at ambient and accelerated conditions.

Development of analytical method for micro and semi micro quantities of drugs and its metabolites in biological system[5].

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice. It is the process of defining an analytical requirement, and confirms that the method under consideration has performance capabilities consistent with what the application requires. Use of equipment that is within specification, working correctly

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and adequately calibrated is fundamental to the method validation process[6]. To device an accurate estimation procedure for each ingredient of such multicomponent dosage form containing several therapeutically active drugs is not an easy task, as they are present in widely divergent proportions[6]. To device an accurate estimation procedure for each ingredient of such multicomponent dosage form containing several therapeutically active drugs is not an easy task, as they are present in widely divergent proportions[7]. Chemical/classical methods are Titrimetric (acid-base titration, oxidation-reduction titration, non-aqueous titration, complex formation), gravimetric and volumetric methods, etc[8].

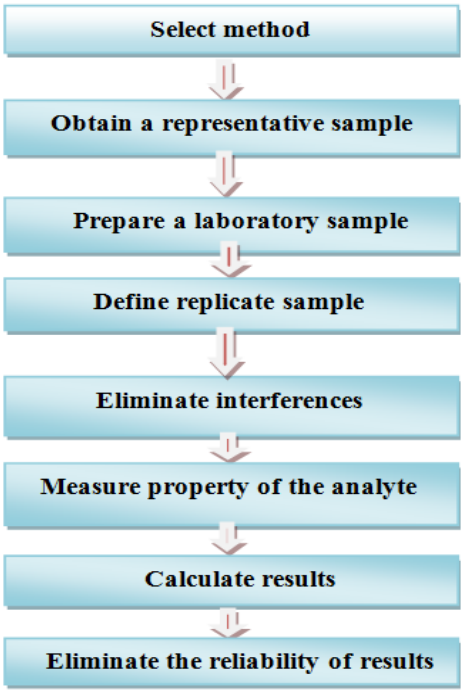

Figure No. 1: Steps involved in quantitative analysis[9]

Liquid chromatography though more troublesome than gas chromatography, has the main advantage of operating at low temperature and can be used with advantage for separation of substance as proteins, nucleosides which are thermolabile. In conventional liquid chromatography, a dilute solution of sample is passed through a column packed with solid particles. Thus, liquid is passed through vertical columns under gravitational flow. This is passed with slow speed and especially if the packing granules were small enough to give efficient separation, then the delivery under gravity decreases even upto a few drops per minute. The obvious way to increase the flow rate and get efficient separation is to force the liquid by a positive displacement pump or by gas pressure. This versatility can be achieved by making certain modification in column[10].

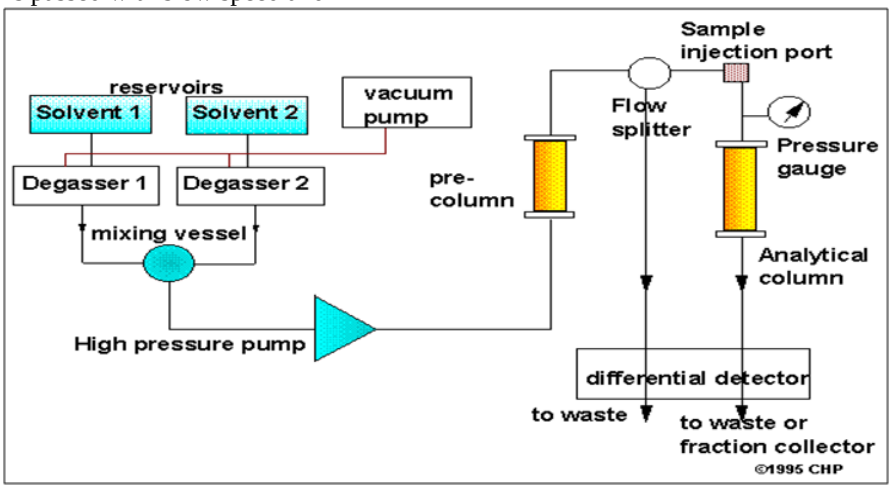

Figure No. 2: Schematic diagram of HPLC system[11]

Method development in HPLC

Method development and optimization in liquid chromatography is still an attractive field of research for theoreticians. Complex mixtures or samples required systematic method development involving accurate modelling of the retention behaviour of the analyte. Among all, the liquid chromatographic methods, the reversed phase systems based

on modified silica offers the highest probability of successful results. However, a large number of (system) variables (parameters) affect the selectivity and the resolution[12].

"Best column, best mobile phase, best detection wavelength, efforts in separation can make a world of difference while developing HPLC method for routine analysis. Determining the ideal combination of these factors assures faster delivery of desired results- a validated method of separation".

Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI) for the treatment of HIV infection in adults. Emtricitabine is an analogue of cytidine. The drug works by inhibiting reverse transcriptase, the enzyme that copies HIVRNA into new viral DNA. IUPAC name: 4-amino-5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one.Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1). Emtricitabine helps to block HIV reverse transcriptase, a chemical in your body (enzyme) that is needed for HIV to multiply. Emtricitabine is always used with other anti-HIV medicines to treat people with HIV infection. Emtricitabine works by inhibiting reverse transcriptase1, the enzyme that copies HIV RNA into new viral DNA. Emtricitabine is a synthetic nucleoside analogue of cytidine. It is phosphorylated by cellular enzymes to form emtricitabine 5'-triphosphate 2, which is responsible for the inhibition of HIV-1 reverse transcript[13-16].

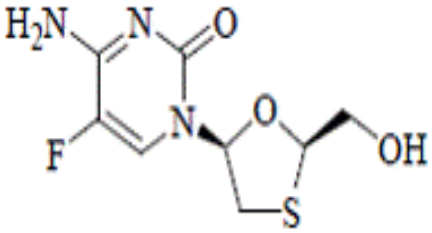

Figure No. 3: Structure of Emtricitabine

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), which block reverse transcriptase, an enzyme crucial to viral production in HIV-infected people. *In-vivo* tenofovirdisoproxilfumarate is converted to tenofovir, an acyclic nucleoside phosphonate (nucleotide) analog of adenosine 5'-monophosphate.IUPAC: is ({[(2R)-1-(6-amino-9H-purin-9-yl)propan-yl]oxy}methyl) phosphonic acid. Tenofovir belongs to a class of antiretroviral drugs known as nucleotide4 analogue reverse transcriptase inhibitors (NtRTIs), which block reverse transcriptase, an enzyme crucial to viral production in HIV-infected people. Tenofovir is currently in late-stage clinical trials for the treatment of hepatitis B. Tenofovir disoproxil fumarate is an acyclic nucleoside phosphonatediester analog of adenosine monophosphate. Tenofovir inhibits the activity of HIV reverse transcriptase by competing with the natural substrate deoxyadenosine 5'-triphosphate and, after incorporation into DNA, by DNA chain termination. Specifically, the drugs are analogues of the naturally occurring deoxynucleotides needed to synthesize the viral DNA and they compete with the natural deoxynucleotides5 for incorporation into the growing viral DNA chain. Hence in the present communication we would like to report a simple, economic, feasible, rapid, sensitive and validated6-12 specific RP-HPLC method for the simultaneous estimation of Emtricitabine and Tenofovir disoproxil fumarate in Bulk and formulation[17-20].

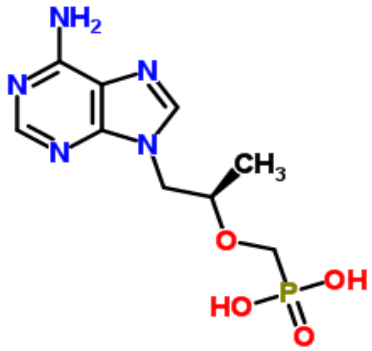

Figure 4: Structure of Tenofovir

Materials and methods

Table No. 1: List of instruments

S. No.	Name of Instrument	Company Name
1	HPLC Instrument	Agilent (1100) Gradient System with auto injector
2	UV-Spectrophotometer	Analytical Technologies Limited
3	Column(C18)	AgilentC18 (250mmX 4.6mm,5µm)
4	pH meter	VSI pH meter(VSI 1-B)
5	Balance	WENSAR™ High Resolution Balance

Table No. 2: Chemicals and reagents

Ingredients	Grade	Suppliers
Emtricitabine	API	R.S.I.T.C Jalgaon.
Tenofovir	API	R.S.I.T.C Jalgaon.
Orthophopsphoric acid(OPA)	HPLC	Avantor Performance material India Ltd. Thane, Maharashtra
Methanol	HPLC	Merck Specialities Pvt. Ltd. Shiv Sager Estate 'A' Worli, Mumbai
Water	HPLC	Merck Specialities Pvt. Ltd.Shiv Sager Estate 'A' Worli, Mumbai

Selection of Wavelength

UV spectrum of 10 µg/ml Emtricitabine and Tenofovir DF in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 272. At this wavelength both the drugs show good absorbance.

Preparation of buffer and mobile phase

Methanol + Water (0.1% OPA) pH3

(60:40v/v)

Preparation of mobile phase

Mix a mixture of above buffer 300 ml (30%) and 700 ml Methonol HPLC (70%) and degas in ultrasonic water bath for 5 minutes. Filter through 045 µ filter under vacuum filtration.

Diluent Preparation

Use the Mobile phase as Diluents.

Optimized chromatographic conditions

Instrument used: High performance liquid chromatography equipped with Auto Sampler and DAD or UV detector

Temperature: Ambient

Column: Inspire C 18 (4.6 x 150mm, 5.0µm)

Buffer: Ortho phosphoric acid pH 2.5

Mobile phase: 40% buffer: 60% Methanol

Flow rate: 1.0 ml per min

Wavelength: 272 nm

Injection volume: 20 µl

Run time: 7min

Standard Solution Preparation

Accurately weigh and transfer 10mg of Emtricitabine & 15mg of Tenofovir DF working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

Further pipette 1ml of Emtricitabine & Tenofovir DF of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Further pipette 3ml of Emtricitabine & Tenofovir DF of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Sample Solution Preparation

Accurately weigh and transfer equivalent to 10mg of Emtricitabine & 15mg Tenofovir DF equivalent weight of the sample into a 10ml clean dry volumetric flask add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

Further pipette 1ml of Emtricitabine & Tenofovir DF of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Further pipette 3ml of Emtricitabine & Tenofovir DF of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for the Emtricitabine & Tenofovir DF peaks and calculate the % Assay by using the formulae.

Results and discussion

Linearity

From Emtricitabine standard stock solution, different working standard solution (5-25µg/ml) were prepared in mobile phase Likewise from Tenofovir standard stock solution different working standard solution (6.25-31.25µg/ml) were prepared in mobile phase 20 µl of sample solution was injected into the chromatographic system using fixed volume loop injector. Chromatograms were recorded. The areas for each concentration were recorded (Table No. 3, 4).

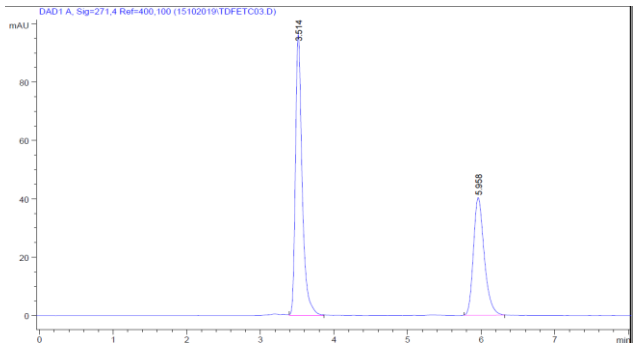

Figure No. 5: Chromatogram of linearity

Table No. 3: Linearity of Emtricitabine

Concentration µg/ml	Area Emtricitabine
10	413.08
20	823.29
30	1194.13
40	1626.10
50	2064.20

Table No. 4: Regression equation data for Emtricitabine

Regression Equation Data Y=mx+c	
Slope(m)	41.051
Intercept(c)	7.355
Correlation Coefficient	0.999

Table No. 5: Linearity of Tenofovir

Concentration µg/ml	Area Tenofovir
15	610.58
30	1206.17
45	1766.74
60	2386.68
75	3032.52

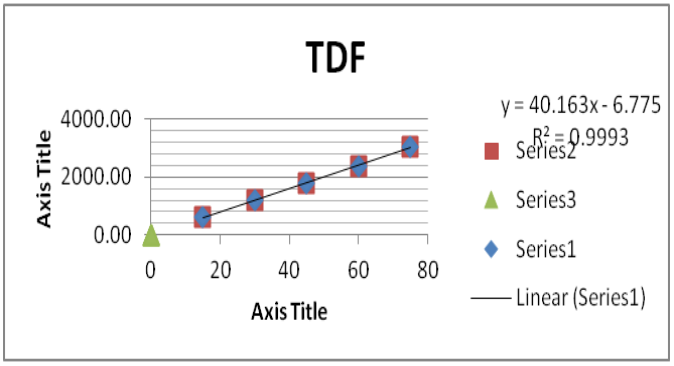

Figure No. 6: Calibration graph of Tenofovir for HPLC method

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To pre analyzed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed (Table No. 6). Statistical validation of recovery studies shown in (Table No. 6)

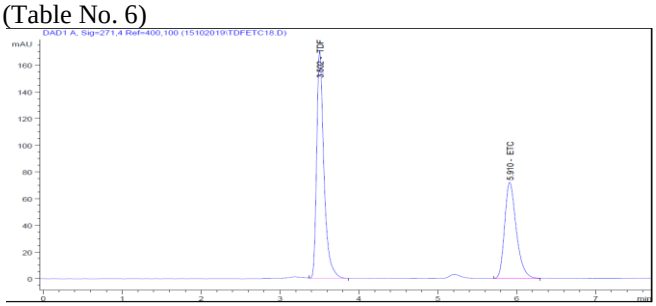

Figure No. 7: Chromatogram of Accuracy 80%

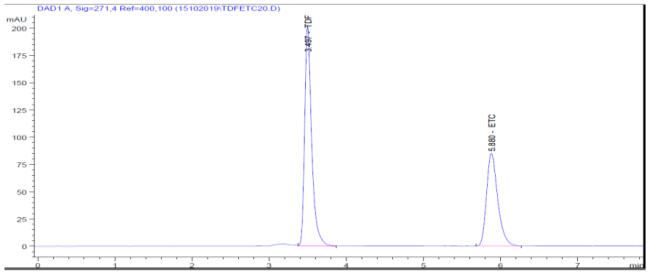

Figure No. 8: Chromatogram of Accuracy 100

Recovery

Table No. 6: Result of Recovery data for Emtricitabine and Tenofovir

Method	Drug	Level (%)	Amount. taken (µg/ml)	Amount Added (µg/ml)	Area Mean* ± S.D	Amount. recovered Mean *±S.D	% Recovery Mean *± S.D
RP-HPLC Method	EMT	80%	10	8	18.17± 0.08	1.02± 0.01	99.91 ±0.06
		100%	10	10	20.14± 0.01	20.58 ±0.01	100.1±0.04
		120%	10	12	22.27±0.01	1.03 ±0.00	101.58±0.15
	TENO	80%	15	12	26.92± 0.10	11.99± 0.10	99.94 ±0.80
		100%	15	15	30.10± 0.01	20.58 ±0.01	100.66±0.99
		120%	15	18	33.01±0.04	18.01 ±0.04	100.05±0.23

Accuracy of RP-HPLC method is ascertained by recovery studies performed at different levels of concentrations (80%, 100% and 120%). The % recovery was found to be within 99-101% (Table No. 6, 7).

System suitability parameters: (Repeatability)

To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of Emtricitabine and Tenofovir system suitability parameters were studied. The result shown in below (Table No. 7)

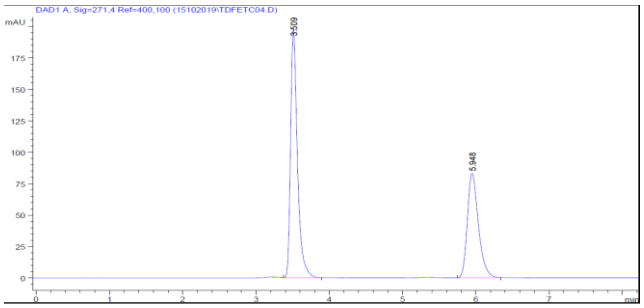

Figure No. 9: Chromatogram of System suitability No. 2

Table No. 7: Repeatability studies on RP-HPLC for Emtricitabine and Tenofovir

Method	Concentration of Emtricitabine and Tenofovir (mg/ml)	Peak area	Amount found (mg)	% Amount found
RP-HPLC Method for EMT	20	827.85	20.30	101.62
	20	824.34	20.32	101.30
		Mean	20.31	
		SD	2.48	
		%RSD	0.30	
RP-HPLC Method for TENO	30	1201.92	29.87	99.57
	30	1202.35	30.10	100.35
		Mean	29.98	
		SD	0.30	
		%RSD	0.03	

Repeatability studies on RP-HPLC for Emtricitabine and Tenofovir was found to be, the % RSD was less than 2%, which shows high percentage amount found in between 98% to 102% indicates the analytical method that concluded (Table No. 7, 8).

Precision

The method was established by analyzing various replicates standards of Emtricitabine and Tenofovir. All the solution was analyzed thrice in order to record any intra-day & inter-day variation in the result that concluded. The result obtained for intraday is shown in (Table No. 8) respectively.

Chromatogram of Precision

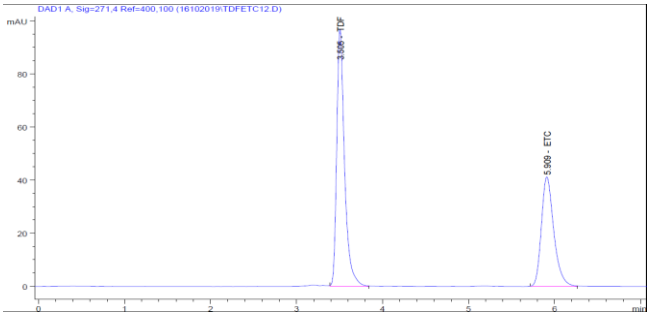

Figure No. 10: Chromatogram of Precision

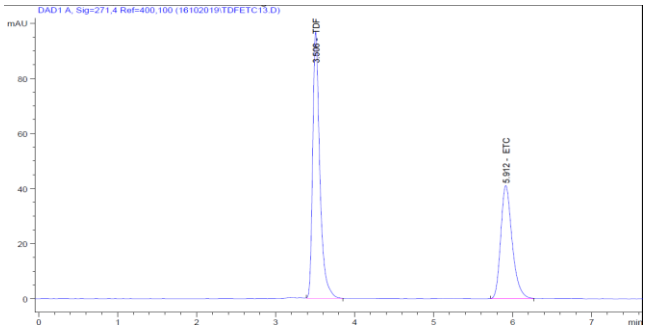

Figure No. 11: Chromatogram Intra-day precision

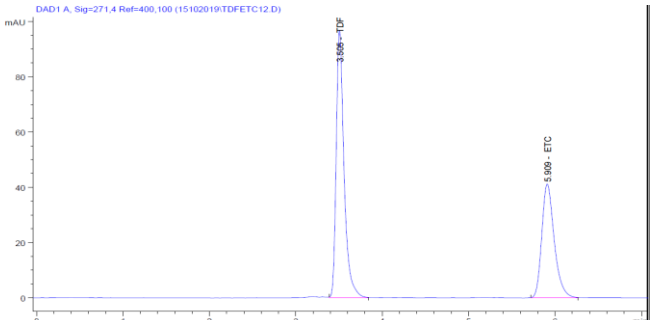

Figure No. 12: Chromatogram Inter-day precision

Table No. 8: Result of Intraday and Inter day Precision studies on RP-HPLC for Emtricitabine and Tenofovir

METHOD	Drug	Concn (µg/ml)	Intraday Precision		Interday Precision	
			Mean± SD	% Amount Found	Mean± SD	% Amount Found
Rp-HPLC METHOD	EMT	10	412.79 ±0.40	100.10	409.04 ±0.03	101.14
		30	1196.13±0.99	99.33	1193.19± 0.10	97.47
		50	2042.45±0.99	100.81	2040.25±0.28	99.97
	TENO	15	609.09±10.45	102.27	605.18± 0.96	101.53
		45	1761.80±0.80	99.36	1760.39± 0.80	97.76
		75	2999.00±1.50	99.77	2997.39±0.62	99.83

*Mean of each 3 reading for RP-HPLC

Intraday and Inter day Precision studies on RP-HPLC for Emtricitabine and Tenofovir which shows the high precision % amount in between 98% to 100% indicates to analytical method that concluded.

Robustness

The Robustness of a method is its ability to remain unaffected by small deliberate changes in parameters. To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate, wavelength on retention time and tailing factor of drug peak was studied.

The mobile phase composition was changed in(±1 ml/min-1) proportion and the flow rate was varied by (±1ml/min-1), and wavelength change (±1 ml/min-1) of optimized chromatographic condition. The results of robustness studies are shown in (Table No.9, 10). Robustness parameters were also found satisfactory; hence the analytical method would be concluded.

Table No. 9: Result of Robustness Study of Emtricitabine

Parameters	Conc. (µg/ml)	Amount of detected (mean ± SD)	% RSD
Chromatogram of flow change 0.6 ml	40	1899.05±0.08	0.0
Chromatogram of flow change 0.8 ml	40	1897.88 ±0.49	0.03
Chromatogram of comp change 59 ml MEOH +41ml water	40	1625.58 ±0.24	0.01
Chromatogram of comp change 61 ml MEOH + 39ml water	40	2516.88± 0.05	0.00
Chromatogram of comp change wavelength change 270 nm	40	1724.7± 0.21	0.01
Chromatogram of comp change wavelength change 272 nm	40	1724.66± 0.22	0.01

Robustness Study of Emtricitabine

The changes were did flow rate (±1 ml/ min-1) PH of mobile phase composition (±1 ml/ min-1), and Wavelength (±1 ml/ min^{-1}). Percent RSD for peak area was calculated which should be less than 2%.the result shown in analytical method that concluded. (Table No. 9)

Table No. 10: Result of Robustness Study of Tenofovir

Parameters	Conc.(µg/ml)	Amount of detected (mean ±SD)	% RSD
Chromatogram of flow change 0.6ml	60	2798.29 ±4.39	0.16
Chromatogram of flow change 0.8 ml	60	2093.17±4.83	0.23
Chromatogram of comp change 59ml MEOH + 41ml WATER	60	2394.6±6.56	0.27
Chromatogram of comp change 61ml MEOH + 39ml WATER	60	2399.54± 18.1	0.76
Chromatogram of comp change wavelength change 270nm	60	2346.7 ±16.9	0.72
Chromatogram of comp change wavelength change 272nm	60	2470.83± 20.4	0.83

Robustness Study of Tenofovir

The changes were did flow rate (±1 ml/ min^{-1}), PH of mobile phase composition (±1 ml/ min^{-1}), and Wavelength (±1 ml/ min^{-1}). Percent RSD for peak area was calculated which should be less than 2%.the result shown in analytical method that concluded (Table No. 10).

Limit Detection

The LOD is the lowest limit that can be detected. Based on the S.D. deviation of the response and the slope the limit of detection (LOD) may be expressed as:

LOD = 3.3 (SD)/S

Where, SD = Standard deviation of Y intercept

S = Slope

Limit of detection = 3.3 X 5.99/41.051= 0.48 (µg/mL)

Limit of Quantitation = 10 X 5.99/41.051= 1.459 (µg/mL)

The LOD and LOQ of Emtricitabine was found to be 0.48 (µg/mL) and 1.459 (µg/mL), analytical method that concluded.

Limit Quantification

The LOQ is the lowest concentration that can be quantitatively measured. Based on the S.D. deviation of the response and the slope,

The quantitation limit (LOQ) may be expressed as:

LOQ = 10 (SD)/ S

Where, SD = Standard deviation Y intercept

S = Slope

Limit of detection = 3.3X12.14/40.163 =0.99 (µg/mL)

Limit of Quantitation = 10 X12.14/40.163=3.022 (µg/mL)

The LOD and LOQ of Tenofovir was found to be 0.99 (µg/mL) and 3.022 (µg/mL), analytical method that concluded.

Analysis of tablet formulation

Weigh of 20 tablets Emtricitabine and Tenofovir combination tablets weigh 8.15gms and calculated the average weight of powder 0.4075 gm, accurately weigh and transfer the sample equivalent to 20.37 mg Emtricitabine and Tenofovir into 10 ml volumetric flask. Add about 10ml MEOH of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45 µm filter. Further pipette 0.4ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents. The simple chromatogram of test Emtricitabine and Tenofovir Shown in (Figure No. 4). The amounts of Emtricitabine and Tenofovir per tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with tablet formulation. Tablet Assay for % Label claim for % RSD Calculated, Result was shown in (Table No. 11.)

Brand Name: TRAVIN-EM (Emcure pvt. ltd)

Total weight of 20 tab wt. = 8.15 Gms -
Average Weight = 0.407 Gms./Tab
Eq.wt for 15 mg = 15X 407.5 / 300 = 20.37 mg
Take 20.37 mgs in 10 ml Methanol sonicate 10 min
i.e. 1000 µgm/ml EMTri and 1500 µgm/m ----- STOCK -I
Take 20.37 mgs in 10 ml Methanol= 1000 µgm/ml EMT and 1500 µgm/ml TENO.

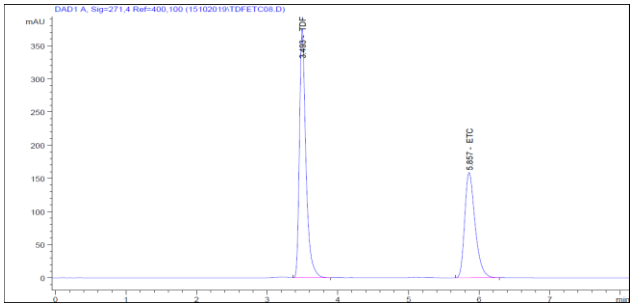

Figure No. 13: Chromatogram for Marketed Formulation

Table No. 11: Analysis of marketed formulation

Assay	Drug	Label Claimed	Amount Found	% Label Claim	SD	%RSD
Rp-HPLC Method	EMT	40	39.37	98.43	1.82	0.11
	TENO	60	59.34	101.94	3.79	0.16
	EMT	40	20.82	98.23	0.04	0.01
	TENO	60	59.21	98.68	0.09	0.07

Analysis of marketed formulation were also % Lable Claim was found to be 98-101% Satisfactory are concluded (Table No.11).

Summary and conclusions

A combination of Emtricitabine and Tenofovir is clinically used in combination in the treatment of anti-HIV agent, The present work deals with Development and validation of rp-HPLC method of Emtricitabine and Tenofovir in bulk and pharmaceutical dosage form

Summary of Stability indicating for RP-HPLC method AND UV Method

Attempts were made to develop RP-HPLC method for simultaneous estimation of Emtricitabine and Tenofovir from tablet. For the RP HPLC method, agilent GradientSystem DAD Detector and C18 column with 250mm x4.6 mm i.d and 5µm particle size Methanol: Water (60:40v/v) pH 3 was used as the mobile phase for the method. The detection wavelength was 271 nm and flow rate was 0.7 ml/min. In the developed method, the retention time of Emtricitabine and Tenofovir were found to be 3.515min and 5.963min. The developed method was validated according to the ICH guidelines. The linearity, precision, range, robustness was within the limits as specified by the ICH guidelines. Hence the method was found to be simple, accurate, precise, economic and reproducible.
So, it is worthwhile that, the proposed methods can be successfully utilized for the routine quality control analysis Emtricitabine and Tenofovir in bulk drug as well as in formulations.

Conclusion

Simple, rapid, accurate and precise RP-HPLC as well as spectrophotometric methods have been developed and validated for the routine analysis of Emtricitabine and Tenofovir in API and tablet dosage forms. Both methods are suitable for the simultaneous determination of Emtricitabine and Tenofovir in multi-component formulations without interference of each other. The developed methods are recommended for routine and quality control analysis of the investigated drugs in two component pharmaceutical preparations. The amount found from the proposed methods was in good agreement with the label claim of the formulation.

References

[1]. Rashmin. An introduction to analytical method development for pharmaceutical formulations. Pharma info.net, 2008: 6(4):1.

[2]. Agrawal, U. and Govindasamy, J. Development and Validation of RP-HPLC method for Simultaneous Estimation of Metformin and Pioglitazone. Indian Journal of Pharmaceutical and Biological Research. 2017;5(1):48-50.

[3]. Sethi. PD. In; HPTLC Quantitative analysis of pharmaceutical formulations, 1st ed. CBS publishers and distributors, New Delhi, 2001; preface IIV, p.3.

[4]. Douglas AS., Holler FJ., Crouch SR. Principle of Instrumental Analysis, 6th ed, Thomson Publication, 2007: p.1.

[5]. Sharma BK., Instrumental Methods of Chemical Analysis, 23rd ed, Goel Publishing House, Meerut, 2002: p.7-8.

[6]. kalra K. Method Development and Validation of Analytical Procedures, Quality Control of Herbal Medicines and Related Areas, Prof. Yukihiro Shoyama (Ed.), ISBN: 978-953-307-682-9, InTech:2011:p.1-5.

[7]. Jeffery GH. Besset J. Mendham J. Denney RC. Vogel's Text Book of Quantitative Chemical Analysis. 5th ed. Longman Scientific and Technicals. 1999.p.3.

[8]. Akhilesh Sharma, Anurag Mishra and Sanjay Sharma. Stability indicating simultaneous validation of hydrochlorothiazide, dihydralazine, and propranolol with thestudy of forced degradation behavior through RP-HPLC in thepharmaceutical dosage form. Asian Pacific Journal of Health Sciences. 2017;4(3): 292-305.

[9]. Skoog DA, West DM, Holler FJ. Analytical chemistry – An Introduction, 6th ed. Saunder college Publishing. p.3.

[10]. Kasture AV, Wadodkar SG, Mahadik KR, More HM. A Textbook of Pharmaceutical Analysis. 10th ed. Vol. II. Pune: Nirali Prakashan; 2004.p.48.

[11]. Singh, R., Pathak, A. and Chawla, P. Method Development and Validation for Simultaneous Estimation of Ketorolac and Sparfloxacin by RP-HPLC. Indian Journal of Pharmaceutical and Biological Research. 2013;1(4): 95-101.

[12]. Sethi PD. High Performance Liquid Chromatography Quantitative Analysis of Pharmaceutical Formulation. Ist ed. New Delhi: CBC Publication and Distributors; 2001.p.35.

[13]. https://en.wikipedia.org/wiki/Emtricitabine

[14]. Oxenius A; Price DA; Günthard HF; et al. (October 2002). "Stimulation of HIV-specific cellular immunity by structured treatment interruption fails to enhance viral control in chronic HIV infection". Proc. Natl. Acad. Sci. U.S.A. 99 (21): 13747–52.

[15].

[16]. O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Cambridge, UK: Royal Society of Chemistry, 2013., p. 657.

[17]. https://www.drugbank.ca/drugs/DB00879

[18]. http://en.wikipedia.org/wiki/Tenofovir.

[19]. www.chemspider.com/Chemical-Structure.408154.html

[20]. https://www.drugs.com › Drugs A to Z › Tenofovir

[21]. Shttps://pubchem.ncbi.nlm.nih.gov/compound/Tenofovir

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