An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTIFICATION OF SELECTED ANTI RETROVIRAL DRUG

Muchu Balaji * , K.Thejomoorthy, Sreenivasa prasanna

Malineni Lakshmaiah College of Pharmacy, Kanumalla , Singarayakonda, Prakasam -523101, Andhra Pradesh, India

ARTICLE INFO

Key Words

RP-HPLC

Tenofovir disproxil

Validation

ABSTRACT

The Present work is aimed to develop, simple, fast, rapid, accurate, efficient and re producible by RP-HPLC method for the analysis of Tenofovir Disoproxil Fumarate. The RP-HPLC Isocratic method was developed by utilizing Phenomenex luna C_{18} column (250×4.6mm, 5µ) and using mobile phase was Methanol: Water (0.1%OPA) in the ratio of 55:45%v/v. The retention time of Tenofovir disproxil fumarate was found to be 4.7 minutes. The linearity concentration was obtained from 10-60µg/ml and correlation coefficient was found to be 0.9995. Considering all the results of validation parameters simplicity of the method and the cost effectiveness of the overall procedure, it is possible to conclude that the developed method can be suitable for the regular quality control determination of Tenofovir disproxil fumarate in bulk as well as pharmaceutical dosage form.

Key words: Tenofovir disproxil fumarate, Isocratic, RP-HPLC, Mobile phase, Validation

INTRODUCTION

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. This enables the management of HIV viral load through decreased viral replication. It is indicated in combination with other antiretroviral agents for the management of HIV-1 infection in adults and pediatric patients 2 years of age and older [1,2]. It is also used for the clinical management of chronic hepatitis B in adults and pediatric patients 12 years of age and older. From the literature survey reveals that few analytical methods have been reported for the analysis of the Tenofovir Disoproxil Fumarate by Spectroscopy and RP-HPLC [59]. The reported methods shown quantification of Tenofovir Disoproxil Fumarate was expensive. Hence we felt that need of new analytical method development for the estimation of Tenofovir Disoproxil Fumarate in less time of analysis for the determination of this drug in API and pharmaceutical dosage form. The developed method will be validated according to ICH guidelines [10].

MATERIALS AND METHODS

Materials:

API of Tenofovir disproxil fumarate gift sample was procured from Reddy's labs, Hyderabad. HPLC grade methanol and water were purchased from Merck labs, Mumbai, India. AR grade ortho phosphoric acid was obtained from SD fine chemicals ltd, Mumbai.

Instruments and specifications:

Ultra-fast liquid chromatographic system was made up of shimadzu, Japan, it proving software was LC solutions -20AD along with PDA detector. Vibra shinko denshi co.ltd manufactured analytical balance and ultra sonicator was

manufactured by Spincotech Pvt.,Ltd also used for the determination of Tenofovir Disoproxil Fumarate.

Preparation of mobile phase:

Preparation of mobile phase by using Methanol and water in the ratio of 55:45 v/v. Mobile phase P^H was adjusted with 0.1% OPA (P^H 4). The mobile phase was filtered through 0.45 µ Nyoln 66 (N66) 47 mm membrane filter paper. After filtration it was ultra sonicated for 20 minute on ultra sonicator.

Preparation of stock solution of Tenofovir Disoproxil Fumarate:

API of Tenofovir Disoproxil Fumarate (10mg) accurately weighed and transferred to 10 ml volumetric flask, dissolved in sufficient quantity of methanol and then diluted to the mark with mobile phase. The solution contains 1000ug/ml of Tenofovir Disoproxil Fumarate.The solution was filtered through 0.45 um Nylon 66 (N66) 47 mm membrane filter paper and firs few drops of filtrate were discarded.

Preparation of sample solution:

Take 25 mg equivalent tablet powder of Tenofovir and dilute with 25 ml of mobile phase up to the mark. Pipette out 0.4 ml of this solution in the 10 ml volumetric flask and make the volume with mobile phase, Sonicate it for 10 minutes. Filter the solution through Whatmann filter paper no. 41. This solution was used as sample solution.20 µL of the blank, standard and sample was injected in to the chromatographic system and areas for the Tenofovir Disoproxil Fumarate the peaks were used for calculating the % assay by using the formulae.

Method development:

The chromatographic method development for the Tenofovir Disoproxil Fumarate was optimized by several trails for various parameters as different column, flow rate and mobile phase; finally the following chromatographic method was selected for the separation and quantification of Tenofovir Disoproxil Fumarate in API and pharmaceutical dosage form by RP-HPLC method.

Optimized Chromatographic conditions:

Column	**:**Phenomenex Luna C18 column 250x4.6mm; 5µm
Mobile phase	**:**Methanol:Water (0.1%OPA) (55:45%V/V)
Detection wavelength	**:**295 nm
Flow rate	**:**1.0 ml/min
Injection volume	**:**20µl
Column temperature	**:**Ambient
Run time	**:**12 min
Retention time	**:**4.7 min
Elution	**:**Isocratic

System Suitability: System suitability is a integral part of many analytical procedures and the various parameters like theoretical plates, efficiency and tailing factor were found to be with in the limit. Then selected system was precise. Tailing factor for the peaks due to Tenofovir Disoproxil Fumarate in standard solution should not be more than 1.5. Theoritical plates for the Tenofovir Disoproxil Fumarate peaks in standard solution should not be less than 1.5.

Method Validation

Specificity: In the case of assay, demonstration of assay specificity is required to show that the procedure is unaffected by the impurities or exipients. Specificity of an analytical method indicates that the analytical method is its able to measure accurately and specifically the analyte of interest without any interference from blank.

Linearity: API of Tenofovir Disoproxil Fumarate (10mg) accurately weighed and transferred to 10 ml volumetric flask, dissolved in sufficient quantity of methanol and then diluted to the mark with mobile phase. Further respected dilutions were prepared from stock solution, and then obtained linearity concentration in the range of 10-60µg/ml. Correlation coefficient should be not less than 0.999.

Accuracy: The accuracy of the test method is demonstrated by % of recovery.

Table 1: Specificity Data

S.No	Peak Name	Observation	
1	Blank	Nil	
2	Placebo	Nil	
3	Standard	R_t: 4.7 min	λ max: 295 nm

Table 2: Results of System Suitability

Parameter	Result	Acceptance Limit
Number of theoretical plates (N)*		More than 2000
Tailing factor (T)*		Less than 2
* Number of injections: 6 replicates		

Table.3: linearity results

S. No	Concentration (µg/ml)	Peak Area
1	10	1045452
2	20	1615705
3	30	2306987
4	40	2891876
5	50	3538425
6	60	4202516

Table 4: Results for intraday and inter day precision

S.No.	Intraday precision Area	Inter day precision Area
1	2810632	2879654
2	2831462	2908789
3	2904681	2831354
4	2865401	2790216
5	2891876	2860675
6	2854980	2913458
Mean	2859839	2864024
Std Dev	32450.74	43239.81
%RSD	1.13	1.50

Table 5: Accuracy results

Spiked Concentration (µg/ml)	Peak area	Amount added (µg/ml)	Amount Found (µg/ml)	Recovery	% Mean Recovery
20	1480105	20.01	20.38	101.84	100.91
	1468972		20.22	101.08	
	1450566		19.97	99.81	
40	2904681	40.02	40.00	99.93	99.34
	2865401		39.45	98.58	
	2891876		39.82	99.49	
60	4316545	60.03	59.44	99.00	99.00
	4325498		59.56	99.21	
	4306545		59.30	98.77	

Table 6(a): Change of Flow rate (± 0.1mL)

S.No	Robust condition	0.9ml/min	1ml/min	1.1ml/min
1	Flow Rate	2906452	2860675	2905465
2		2865620	2913458	2860654
3		2840645	2887067	2850498
4	Mean	2870905.66	2887067	2872206
5	Std dev	27124.33	21548.57	23880.59
6	% RSD	0.94	0.74	0.83

Table 6(b): Change in Temperature (± 5ºC)

S.No	Robust condition	30 ºC	35 ºC	40 ºC
1	Temperature	2810632	2904681	2810632
2		2831462	2865401	2831462
3		2904681	2891876	2880654
4	Mean	2848925	2887319	2840916
5	Std dev	40332.12	16356.49	29357.61
6	% RSD	1.41	0.56	1.03

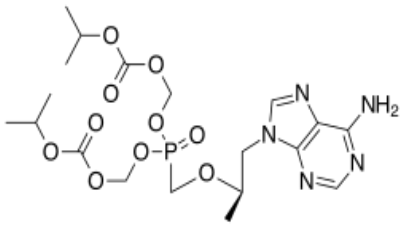

Fig.1: Chemical structure of Tenofovir Disoproxil Fumarate

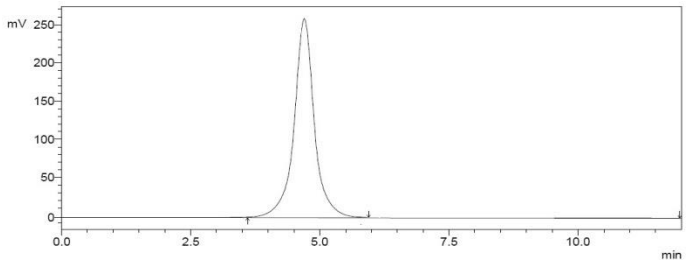

Fig.2: Optimized chromatogram of Tenofovir Disoproxil Fumarate

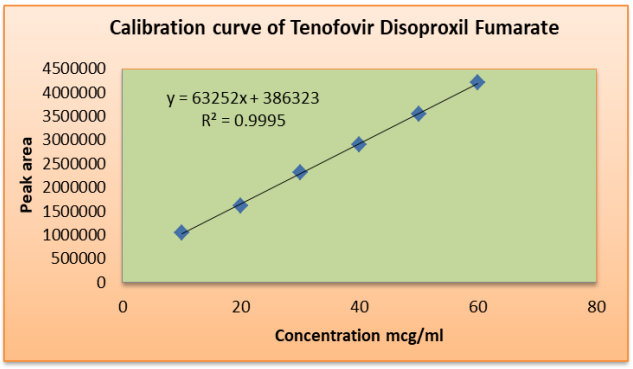

Fig.3: Calibration curve of Tenofovir Disoproxil Fumarate

Accuracy was performed in three different three concentration levels and injected three times. The standard solutions of accuracy 50%, 100% and 150% were injected in to chromatographic system. Calculate the amount found and amount added for Tenofovir Disoproxil Fumarate and calculate the individual % recovery and mean % recovery values. % Recovery at each spike level shall be not less than 98.0 and not more than 102.0.

Precision: The standard solution was injected for five times and measured the area for all five injections in HPLC. The % RSD for the area of five replicate injections was found to be within the specified limits.

Robustness: As Part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method.

RESULTS AND DISCUSSION

Specificity: There is no interference of mobile phase, and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form. The data was shown in table 1.

System suitability: System suitability test was an integral part of method development and has been used to ensure adequate performance of the chromatographic system. The results are given in table 2.

Linearity: Each solution was injected in to the chromatographic system and peak area was measured. Plot a graph of peak area versus concentration (on x –axis concentration and on y axis peak area) and the correlation was calculated. The results were shown in table 3.

Accuracy: Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The data was shown in table 5.

Robustness: The flow rate was varied at ± 10%. Standard solution 40 µg/ml of Tenofovir Disoproxil Fumarate was prepared and analysed using the varied flow rates along with method flow rate. The Temperature was varied (± 5^{o}C) Standard solution 40 µg/ml of Tenofovir Disoproxil Fumarate was prepared and analysed using the varied flow rates along with method flow rate. Results were shown in table 6a & 6b.

DISCUSSION

A new method was established for estimation of Tenofovir Disoproxil Fumarate by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Tenofovir Disoproxil Fumarate bu using Phenomenex Luna C_{18} column 250x4.6mm 5µm, flow rate was 1.0 ml/min, mobile phase ratio was (55:45%v/v) Methanol: Water (0.1%OPA) ,detection wavelength was 295 nm. The retention time was found to be 4.7 mins. The % purity of Tenofovir Disoproxil Fumarate was found to be 100.443% respectively. The system suitability parameters for Tenofovir Disoproxil Fumarate such as theoretical plates and tailing factor were found to be more than 2000, the resolution was found to be less than 2. The analytical method was validated according to ICH guidelines (ICH, Q_2 (R_1). The linearity study for Tenofovir Disoproxil Fumarate was found in concentration range of 10µg-60µg/ml and correlation coefficient (r^2) was found to be 0.999, % recovery was found to be 99.75%, % RSD for repeatability was 1.134, % RSD for intermediate precision was 1.509 respectively.

CONCLUSION

The present research work concluded that developed RP-HPLC method was found to be simple, sensitive, precise and reproducible. Hence the suggested RP-HPLC method can be used for routine analysis of Tenofovir Disoproxil Fumarate in API and pharmaceutical dosage form.

REFERENCES

1. https://www.drugbank.ca/drugs/DB00300
2. Breaux J and Jones K, understanding and implementing efficient analytical methods development and validation. J pharma tech 2003; 5: 110- 114.
3. Anindita Behera, Aurobinda Parida, Amit Kumar Meher, Dannana Gowri

Sankar, Swapan Kumar Moita, Sudam Chandra Si, Development and Validation of Spectrophotometric Method for Determination of Emtricitabine and Tenofovir Disoproxil Fumarate in Bulk and Tablet dosage form. Int J PharmTech Res 2011; 3: 1874-1882.

4. Rajesh Sharma, Pooja Gupta, A Validated RP- HPLC Method for Simultaneous Estimation of Emtricitabine and Tenofovir Disoproxil Fumarate in a Tablet Dosage Form. Eur j Anal Chem 2009; 4(3):276-284.
5. Shirkhedkar Atul A, Bhirud Charushila H, Surana Sanjay J, Application of UVSpectrophotometric methods for Estimation of Tenofovir Disoproxil Fumarate in tablets. Pak. J. Pharm. Sci 2009; 22: 27-29.
6. Manish Yadav, Puran Singhal,Sailendra Goswami, Umesh C Pande, Mallika Sanyal, Pranav S, Shrivastav. Selective Determination of Antiretroviral agents Tenofovir, Emtricitabine, and Lamivudine in human plasma by a LC-MS-MS method for a Bioequivalence study in healthy Indian Subjects. Jof chrom sci 2010; 48: 704-13.
7. Narendra Devanaboyina, Satyanarayana T, Ganga Rao B, HPLC Method Development and Validation for Simultaneous Estimation of Tenofovir and Emtricitabine in combined pharmaceutical dosage form. IntJof Res Pharmaand Biomed Sci 2012: 3(1): 361-367.
8. P.Chandra, A.S.Rathore, L.Sathiyanarayanan, K.R.Mahadik, Application of High- Performance Thin-Layer Chromatographic method for the Simultaneous Determination of Lamivudine and Tenofovir Disoproxil Fumarate in pharmaceutical dosage form. J. Chil. Chem. Soc 2011; 56: 702-705.
9. R.Sharma, K.Mehta, Simultaneous Spectrophotometric Estimation of Tenofovir Disoproxil Fumarate and Lamivudine in three component tablet formulation containing Efavirenz. IndJof PharmaSci 2010; 72(4): 527-530.
10. ICH Q 2A, Validation of analytical methods, definitions and terminology, ICH Harmonized tripartite guideline, (1999).
11. Code Q2B, "Validation of analytical procedures; Methodology. ICH Harmonized tripartite guidelines. Geneva, Switzerland, 1996, PP 1-8.