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A SIMPLE RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF TENOFOVIR DISOPROXIL FUMARATE AND EMTRICITABINE IN TABLET DOSAGE FORM

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ABSTRACT

A rapid, specific, sensitive and simple high performance liquid chromatography was developed for simultaneous estimation of Tenofovir Disoproxil Fumarate and Emtricitabine in tablet formulation. The separation was achieved by Thermoscientific C_{18} column (4.6×250 mm, particle size 5µm) with a mobile phase consisting of Acetonitrile: water (70:30v/v, PH 3.5 adjusted with orthophosphoric acid), at a flow rate of 1.5ml/min. Detection was carried out at 270nm.Retention time of Emtricitabine and Tenofovir Disoproxil Fumarate were found to be 2.82 and 4.38 min, respectively. The linear dynamic range was 200-400µg/ml and 100-300µg/ml Tenofovir Disoproxil Fumarate and Emtricitabine respectively. The method is validated for Accuracy, Precision, Ruggedness and Robustness. The proposed method is successfully applied for the simultaneous determination of both drugs in commercial tablet preparation. The results of the analysis have been validated statistically and by recovery studies.

Key words: Tenofovir Disoproxil Fumarate, Emtricitabine, High performance liquid chromatography, Simultaneous estimation.

INTRODUCTION

Tenofovir disoproxil fumarate (TDF) 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 chemically it is 9-[(R)-2-[[(isopropoxcarbonyl)-oxy]methoxy]phosphiny]methoxy]propyl]adeninefumarate[1,2,3]. Emtricitabine (ETB) is an analogue of cytidine, chemically it is 5-fluoro-1-(2R,5S)-[2(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine[1,2,3], works by inhibiting reverse transcriptase, the enzyme that copies HIV RNA into new viral DNA. By interfering with this process, which is central to the replication of HIV, emtricitabine can help to lower the amount of HIV, or "viral load", in a patient's body and can indirectly increase the number of immune system cells (called T cells or CD4+ T-cells). J. A. H. Droste et al., Determined Emtricitabine in Human Plasma using HPLC by Fluorometric Detection[7]. A Shirkhedar etal., estimated Tenofovir Disoproxil Fumarate in tablets by UV-Spectrophotometric method[9]. Hence, it is necessary to develop a rapid, accurate and validated RP-HPLC method for the determination of TDF and ETB from combined dosage form. The method proved to be simple model since it does not contain a buffer system. This paper describes the development and validation of reliable, simple, robust, time and money saving reversed phase HPLC method, using UV detection, for the simultaneous estimation of TDF and ETB in tablet dosage forms. The developed method validated according to ICH guidelines [13] The present manuscript describes a novel LC method which is simple, rapid, precise and accurate isocratic reverse phase HPLC for simultaneous determination of Tenofovir Disoproxil Fumarate and Emtricitabine.

MATERIALS AND METHODS

Equipment

Chromatographic separation was performed on Waters HPLC system consist of model 2695 having PDA detector and Rheodyne injector with 20µl loop volume. Waters Empower software was applied for data collecting and processing.

Reagents and chemicals

Acetonitrile and water of HPLC grade were procured from Rankem lab ltd. TDF and ETB standards were received as gift samples from Hetero Drugs Limited Hyderabad, India, respectively. Ortho phosphoric acid A.R grade was purchased from E.Merck chemicals Mumbai, India. Tablet Tenof-EM having combination of TDF (300mg) and ETB (200mg) was used.

HPLC Conditions

A Thermo scientific C_{18} (25cm×4.6mm, 5µ) column was used as the stationary phase. A mixture of Acetonitrile and water in the ratio of (70:30v/v) was used as a mobile phase and P^H 3.5 adjusted with orthophosphoric acid. It was filtered through 0.45µ membrane filter and degassed. The mobile phase was pumped at 1 ml/min. The eluents were monitored at 270nm.The injection volumes of sample and standard were 20µl.

Standard solutions

A stock solution containing 1000µg/ml of TDF and ETB were prepared separately by dissolving in acetonitrile. A working standard solution containing 200-400µg/ml and 100-300µg/ml of TDF and ETB were prepared from the above stock solution. All the stock solutions were covered with aluminum foil to prevent photolytic degradation until the time of analysis.

ASSAY OF TABLET FORMULATION

Twenty tablets were weighed, each containing 300mg of TDF and 200 mg of ETB were weighed and finely powdered. A quantity of powder equivalent to 300mg of TDF and 200mg of ETB was weighed and transferred to a 50ml standard flask. The drug was initially dissolved in Acetonitrile and sonicated for 10 minutes. The volume was made up to 50ml with mobile phase. The solution was filtered using 0.2µm membrane filter. The aliquot was then suitably diluted to get final concentrations of 300µg/ml of TDF and 200 µg/ml of ETB. Then 20µl of these solutions was injected in to the column, recorded and chromatogram was shown in Fig.1. Concentrations of TDF and ETB in the tablet formulation were calculated by comparing area of the sample with that of standard. The percentage assay of individual drug was calculated and presented in Table1.

VALIDATION OF THE METHOD

Linearity and range

The developed method has been validated as per ICH guidelines. Every 20µl of the working standard solution of TDF in the concentration range of 200-400µg/ml **(Fig 2)** and for ETB in the concentration range of 100- 300µg/ml **(Fig 3)** were injected into the chromatographic system. The chromatograms were developed and

the peak area was determined for each concentration of the drug solution. Calibration curves of TDF and ETB were obtained by plotting the peak area ratio versus the applied concentrations of TDF and ETB. The linearity curves of TDF and ETB were shown in Figure 3 and 4 and Linearity data's were shown in Table.2.

LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as $3.3\partial/S$ and $10\partial/S$, respectively as per ICH guidelines, where ∂ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOQ is the smallest concentration of the analyte which gives response that can be accurately quantified (signal to noise ratio of 10). (Table. 2)

System suitability

The resolution and peak symmetry were calculated for the standard solutions. (Table. 3).The values obtained demonstrated the suitability of the system for the analysis of this drug combination and the system suitability parameters fall within ±3% standard deviation range during performance of the method. Here tailing factor for peaks of TDF and ETB was less than 2 and resolution was satisfactory. The peaks obtained for TDF and ETB were sharp and have clear base line separation.

Accuracy

Recovery studies were carried out by applying the standard addition method. A known amount of standard TDF and ETB corresponding to 80%, 100%, and 120% of the label claim was added to pre analyzed sample of tablet dosage form separately. The recovery studies were carried out three times, at each level of recovery. The data's of accuracy were shown in (Table. 4)

System precision and Method Precision

The Precision of the method was demonstrated by system precision and method precision studies. In the system precision studies, six replicate injections of the working standard solution prepared as per the proposed method and chromatograms were recorded. Standard deviation and relative standard deviation for the area was calculated and presented in (Table .5). In the method precision studies, six replicate injections of the analyte solution prepared as per the proposed method and chromatograms were recorded. Standard deviation and relative standard deviation for the area was calculated and presented in (Table.6).

Solution Stability

Solution stability was evaluated at room temperature for 48hrs. The percentage difference of relative standard deviation was not more than 2% from initial assay value result, thus indicated that both sample and standard solutions were stable for 24hrs, which was sufficient to complete the whole analytical process.

Ruggedness and Robustness

The ruggedness of the method was determined by carrying out the experiment on different instrument like Waters HPLC and Shimadzu HPLC by different operators using different columns of similar type like Phenomenex C_{18},Hypersil C_{18}.Robustness of the method was determined by making slight changes in the experimental conditions such as the composition of the mobile phase, pH of the mobile phase, and flow rate of the

Mobile phase and the chromatographic characteristics were evaluated.

RESULTS AND DISCUSSION

The proposed method was found to be simple and sensitive with linearity in the concentration range of 200-400µg/ml TDF and 100-300µg/ml for EBT. System suitability parameter indicates good resolution of both the peaks >2. In addition high column efficiency was indicated from the large number of theoretical plates (>1000). The degree of asymmetry was also evaluated using the tailing factor which did not exceed the critical value (1.5) indicating acceptable degree of peak asymmetry. The method was found to be accurate and precise as indicated by results of recovery studies and precision studies %RSD not more than 2%. There were no marked changes in the chromatograms which confirmed the ruggedness of the method. The standard deviation of % assay for sample was calculated for each parameter in robustness studies and relative standard deviation was found less than 2%. The low RSD value confirms the robustness of the method.

CONCLUSION

The developed RP-HPLC method for simultaneous determination of TDF and ETB can be used for routine analysis of both these components in combined dosage form.

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Table 1: Report for Assay

S.No	Drug	Amount present	Amount found* (mg/tab)	% label claim*
1	TDF	300	300.21	100.07%
2	ETB	200	200.17	100.08%

Table 2: Statistical data of calibration curve of TDF and ETB

S.No	Parameters	Tenofovir Disoproxil Fumarate	Emtricitabine
1	Linearity range µg/ml	200-400 µg/ml	100- 300 µg/ml
2	Correlation Coefficient	0.999	0.999
3	LOD µg/ml	2.5 µg	5.2 µg
4	LOQ µg/ml	3.2 µg	6.2 µg

Table 3: System Suitability Report

S.No	Parameters	Tenofovir Disoproxil Fumarate	Emtricitabine
1	Theoretical plate	11246	9264
2	Asymmetry of the peak	0.84	0.42
3	Retention time	4.38	2.82
4	Resolution	3.46	

Table 4: Recovery studies of TDF and ETB

Drug	%Concentration	Amount added(µg/ml)	Total amount found (µg/ml)	Recovery (%)	Mean Recovery (%)
TDF	80%	240	239.24	99.68	99.96
	100%	300	300.08	100.02	
	120%	360	360.67	100.18	
ETB	80%	160	160.12	100.07	100.07
	100%	200	200.26	100.13	
	120%	240	240.06	100.02	

Table 5: System Precision Report

Parameters	Area of Tenofovir Disoproxil Fumarate	Area of Emtricitabine
Trail 1	2561876	1475126
Trail 2	2562346	1474924
Trail 3	2563204	1475247
Trail 4	2561943	1474612
Trail 5	2563921	1474578
Trail 6	2561023	1474934
Average	2562386	1474904
Standard deviation	1033.544	268.243
%RSD	0.040335	0.018187

Table 6: Method Precision Report

Parameters	Area of Tenofovir Disoproxil Fumarate	Area of Emtricitabine
Trail 1	2562432	1474532
Trail 2	2568475	1474876
Trail 3	2565782	1475287
Trail 4	2569673	1474563
Trail 5	2563567	1474885
Trail 6	2567258	1475134
Average	2566198	1474880
S.D	2816.449	300.5986
%RSD	0.109752	0.020381

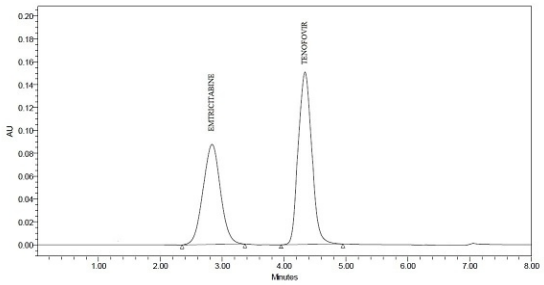

Fig 1. Chromatogram of the sample

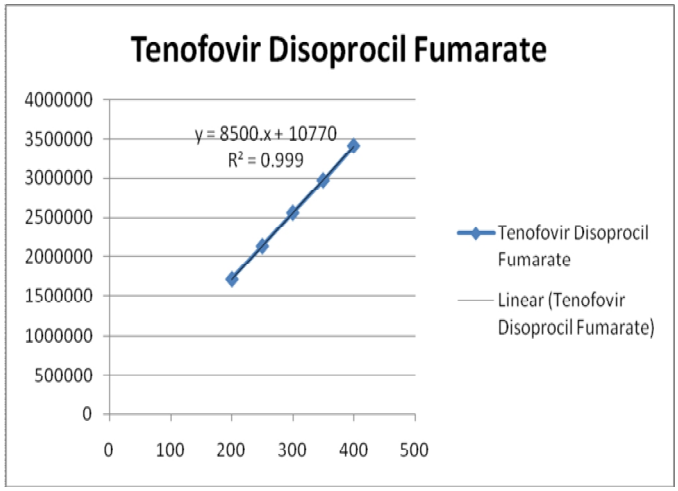

Fig 2: Linearity of detector response in HPLC method for Tenofovir Disoproxil Fumarate Concentration level Verses Analyte response

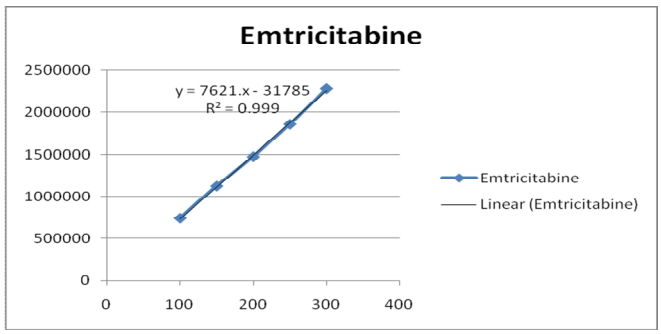

Fig 3: Linearity of detector response in HPLC method for Emtricitabine Concentration level Verses Analyte response

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