Simultaneous Estimation of Emtricitabine and Tenofovir Disoproxil Fumarate in Tablet Dosage Form by Reverse Phase High-performance Liquid Chromatography


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Abstract

A simple, rapid reversed-phase high performance liquid chromatographic method had been developed and validated for estimation of emtricitabine and tenofovir disoproxil fumarate in tablet dosage form. The estimation was carried out on Phenomenex-Luna C18 (25 cm x 4.60 mm, particle size 5 µm) column with a mixture of 10mM phosphate buffer (pH 6.8): Acetonitrile; 40: 60 (v/v) as mobile phase. UV detection was performed at 260 nm. The method was validated for linearity, accuracy, precision, specificity and sensitivity as per ICH norms. The developed and validated method was successfully used for the quantitative analysis of commercially available dosage form. The retention time was 2.81 and 7.42 min for emtricitabine and tenofovir disoproxil fumarate respectively and total run time was 10 min at a flow rate of 1.0 mL/ min. The calibration curve was linear over the concentration range of 40.00 - 240.00 µg/mL for emtricitabine and 60.00 - 360.00 µg/mL for tenofovir disoproxil fumarate. The LOD and LOQ values were found to be 1.54 and 4.54 µg/mL for emtricitabine and 4.60 and 13.65 µg/mL for tenofovir disoproxil fumarate respectively. The high percentage of recovery and low percentage coefficient of variance confirm the suitability of the method for the simultaneous estimation of emtricitabine and tenofovir disoproxil fumarate in tablet dosage form.

Keywords: Emtricitabine; Tenofovir disoproxil fumarate; RP-HPLC; Validation

Introduction

Emtricitabine (ETB) is a nucleoside reverse transcriptase inhibitor (NRTIs). Chemically it is 5-fluoro-1-[(2R, 5S)\-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine (Figure 1). FTC is the (-) enantiomer of thio analog of cytidine which differs from other cytidine analogs, in that it has fluorine in 5th position. FTC is an antiviral agent used for the prevention of perinatal HIV-1 reverse transcriptase [1]. It is also active against Hepatitis B virus [2,3].

Tenofovir disoproxil Fumarate (TDF) is fumaric acid salt of the bisisopropoxycarbonyl- oxiemethyl ester derivative of tenofovir. Chemically it is 9-[(R)-2-[(isopropoxycarbonyl) oxy]-methoxy] phosphinyloxyemethoxypropyl]adenine fumarate (Figure 1). It is used in combination with other antiretroviral for the treatment of HIV infection [2,3]

Literature survey reveals that few RP-HPLC [4-6] methods are reported for estimation of ETB, TDF and efavirenz in pharmaceutical formulation. TDF is estimated individually by UV [7], derivative-HPLC [8], HPTLC [9] in pharmaceutical formulation. ETB, TDF were estimated in biological matrices by HPLC [11,12] and LC/ MS/ MS [13-25], methods. The reported methods [4-6] for estimation of ETB, TDF in pharmaceutical formulations have some drawbacks in terms of sensitivity, ruggedness and robustness. The purpose of this study was to develop simple, rapid, precise and accurate RP-HPLC method for the simultaneous estimation of both the drugs in combined tablet dosage form.

Experimental

Apparatus

RP-HPLC was performed with an Agilent chromatographic system equipped with 1200 series isocratic pump UV-visible and a Rheodyne universal loop injector of injection capacity 50 µL. The monitoring software was Ezichrome Elite. The equipment was controlled by a PC workstation. Compounds were separated
on a 25 cm x 4.6 mm id, 5-μm particle, Phenomenex-Luna C\textsubscript{18} column under reversed-phase partition chromatographic conditions. The flow rate was 1.0 mL/ min and injection volume was 20 μL, analyte were monitored at 260 nm and run time was 10 min.

Chemicals and reagents

Working Standards of pharmaceutical grade ETB and TDF were obtained as gift samples from Micro labs, Bangalore. The tablet dosage form, manufactured by Hetero Drugs Limited, Hyderabad, India (Label claim: ETB 200 mg and TDF 300 mg), was procured from the local pharmacy. All the chemicals and reagents used were of HPLC grade and purchased from Merck, Mumbai, India.

Mobile phase

The mobile phase selected was acetonitrile: 10 mM phosphate buffer (pH 6.8) (60:40, v/v), and filtered through 0.45 μm pore size membrane filter. Before analysis mobile phase was degassed.

Preparation of standard stock solution

Standard stock solution of Emtricitabine and Tenofovir pure drug prepared by accurately weighing about 100 mg drugs and transferring in to 100 mL volumetric flask and dissolved in acetonitrile.

Construction of calibration curve

A series of standard concentrations were prepared from 50 % to 150 % of the target concentration of ETB and TDF. Linearity was assessed by performing single measurement at several analyte concentration varying quantities of stock standard solution diluted with the mobile phase to get final concentrations of 40,80,120,160,200,240 μg/mL of ETB and 60,120,180,240,300,360 μg/mL of TDF.

Sample preparation

A total of 20 tablets were accurately weighed and powdered in a mortar. An amount equivalent to 66.5 mg (tablet Containing 20 mg of ETB and 30 mg of TDF) was transferred to 50 mL volumetric flask and added 10ml of mobile phase and sonicated for 10 min and make up to 50 mL with mobile phase. The solution was filtered 0.45 μm pore size membrane filter. The filtered sample solution 5mL was diluted to 10 mL with mobile phase to get the solution containing ETB and TDF in 200 & 300 μg/mL proportions respectively. The test solution was injected in to HPLC and % assay was calculated. The results are depicted in Table 1.

Result and Discussion

HPLC method development and optimization

Column chemistry, solvent type, solvent strength, detection wavelength and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimized so that the components were not interfered from the solvent and excipients.

After series of trials with various C\textsubscript{n} and C\textsubscript{18} columns, the final choice of stationary phase giving satisfactory resolution and run time was the reversed phase Phenomenex-Luna C\textsubscript{18} column. Mobile phase and flow rate selection was based on peak parameters (height, area, tailing, theoretical plates, capacity factor and resolution) and run time. The best result was obtained by use of acetonitrile: 10 mM phosphate buffer (pH 6.8) (60:40, v/v), with 1.0 mL/min. From the overlay UV spectra, suitable wavelength considered for monitoring the drugs was 260 nm (Figure 2).

Table 1: Assay of Tablet Formulation

<table>
<thead>
<tr>
<th>Tablet</th>
<th>Label claimed (mg)</th>
<th>Conc. found (mg)</th>
<th>% Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDF</td>
<td>ETB 20.0, TDF 30.0</td>
<td>ETB 20.05, TDF 29.98</td>
<td>ETB 100.21, TDF 98.97</td>
</tr>
</tbody>
</table>

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Figure 2: Chromatogram of Placebo.

Figure 3: Chromatogram of standard ETB and TDF.

Figure 4: Chromatogram of ETB and TDF in test formulation.

Table 2: Linearity and range of ETB and TDF.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Concentration µg/ ml</th>
<th>Area of Emtricitabine</th>
<th>Concentration µg/ ml</th>
<th>Area of Tenofovir</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>22988.567</td>
<td>60</td>
<td>16998.424</td>
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<tr>
<td>2</td>
<td>80</td>
<td>47605.237</td>
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<td>33602.702</td>
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<td>3</td>
<td>120</td>
<td>68028.039</td>
<td>180</td>
<td>50459.14</td>
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<tr>
<td>4</td>
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<td>5</td>
<td>200</td>
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<td>300</td>
<td>84014.837</td>
</tr>
<tr>
<td>6</td>
<td>240</td>
<td>130654.429</td>
<td>360</td>
<td>101710.108</td>
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</table>

Concentration range µg/ ml 40-240 60-360

Slope (m) 543.85 281.04

Correlation co-efficient ($r^2$) 0.993 0.999

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Figure 5: Linearity of Emtricitabine.

Figure 6: Linearity of Tenofovir.

Table 3: Recovery Studies.

<table>
<thead>
<tr>
<th>Level of % recovery</th>
<th>Target Conc. (µg/ml)</th>
<th>Amount of drug spiked (µg/ml)</th>
<th>Drug recovered (µg/ml)</th>
<th>%Recovery</th>
<th>Mean</th>
<th>SD</th>
<th>%RSD</th>
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<tr>
<td>80</td>
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<td>80</td>
<td>181.13</td>
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<td>99.95</td>
<td>1.42</td>
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<td>179.85</td>
<td>99.98</td>
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<td>100</td>
<td>100</td>
<td>100</td>
<td>200.10</td>
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<td>1.23</td>
<td>1.12</td>
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<td>220.41</td>
<td>100.42</td>
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</tbody>
</table>

Table 4: Recovery Studies.

| Sample. No | Intra day | | Inter day |
|------------|-----------| |-----------|
|            | ETB % Label Claim | | TDF % Label Claim |
|            | TDF % Label Claim | | TDF % Label Claim |
| 1          | 100.31     | | 99.67  |
| 2          | 99.41      | | 99.12  |
| 3          | 99.34      | | 100.42 |
| 4          | 99.22      | | 99.98  |
| 5          | 100.33     | | 100.1  |
| 6          | 100.14     | | 100.22 |
| Mean       | 99.75      | | 99.26  |
| S.D        | 0.2262     | | 0.3742 |
| % R.S.D    | 0.2264     | | 0.3742 |
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Precision: The concentrations of both the drugs were measured three times on the same day at intervals of 1 h and on three different days for intra and interday study respectively. The results are depicted in Table 4.

Limit of detection and Limit of Quantification: LOD is found to be 1.5456 µg/ mL for Emtricitabine and 0.0712 µg/ mL for Tenofovir and LOQ are found to be 4.5924 µg/ mL for Emtricitabine and 13.931µg/ mL for Tenofovir.

Conclusion

A new, reversed-phase HPLC method has been developed for simultaneous analysis of ETB and TDF in a tablet formulation. It was shown that, the method was linear, accurate, reproducible, repeatable, precise, selective and specific proving the reliability of the method. The run time is relatively short (10 min), which enables rapid determination of many samples in routine and quality control analysis of tablet formulations. Hence, the proposed method was successfully applied to analyze preparation containing ETB and TDF.

Acknowledgement

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References

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