Simultaneous Determination of Tenofovir disoproxil fumarate and Lamivudine by UV Spectrophotometric Method

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Abstract
A simple and rapid UV spectrophotometric method has been developed for simultaneous estimation of Tenofovir disoproxil fumarate (TDF) and Lamivudine (LAM). The absorption maxima of both drugs were found at 260nm and 280nm and obeyed Beer’s law in the range of 5-45µg/ml \((y = 0.021x + 0.002; r^2 = 0.999)\) and 2-16µg/ml \((y = 0.061x + 0.004; r^2 = 0.998)\) respectively for TDF and LAM in acetonitrile : 0.1N HCl (20:80) solvent system. Accuracy and reproducibility of the proposed method was statistically validated by recovery studies. This method is found to be precise and accurate and can easily be employed in the laboratory for the routine estimation of drugs.

Key words: Tenofovir disoproxil fumarate, Lamivudine, Simultaneous equation, UV spectrophotometry

INTRODUCTION

Tenofovir Disoproxil Fumarate (TDF) chemically, it is 9-[(R)-2-[[bis[(Isopropoxycarbonyl) oxy]methoxy] phosphinyl]methoxy]propyl]adenine fumarate (1:1), is an antiretroviral agent belonging to the class of nucleotide reverse transcriptase inhibitors\(^1,2,3,4\). It has a molecular formula of \(C_{19}H_{30}N_5O_{10}P \cdot C_4H_4O_4\) and a molecular weight of 635.52 (Figure - 1). TDF is the first nucleotide analog approved for HIV-1 treatment and remains in cells for longer periods of time than many other antiretroviral drugs\(^5,6\). TDF is a prodrug of tenofovir and converted to an acyclic nucleoside phosphate in vivo by competing with the natural DNA substrates to inhibit reverse transcriptase and subsequently decreasing or preventing HIV replication in infected cells with a view to block HIV replication\(^7,8\).

Lamivudine (LAM), chemically it is (2R-cis)-4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H) pyrimidinone, is a synthetic nucleoside analogue with potent activity against human immune deficiency (HIV) and hepatitis B viruses (HBV) through inhibition of reverse transcriptase activity\(^9,10\). It has a molecular formula of \(C_8H_{11}N_3O_3S\) and a molecular weight of 229.3 (Figure - 1). TDF and LAM are extensively used for antiretroviral therapy for HIV infection to delay disease progression by prolonged suppression of HIV replication. The combination may be given to patients, those are not responding to mono-therapy of HIV\(^11,12\).

Literature survey has revealed a number of analytical methods for determination of both TDF and LAM. TDF was estimated individually by UV\(^8,13,14\), RP-HPLC\(^15,16\) and HPTLC\(^17\). TDF with other drug combinations were determined by UV\(^18,19,20\), RP-HPLC\(^21,22\) and HPTLC\(^23\). LAM was estimated individually by UV\(^12,24,25,26\) and its combination with other drugs by UV\(^27,28\) and RP-HPLC\(^29,30\) techniques. Simultaneous estimation of TDF and LAM with distilled water\(^31\) and methanol\(^32\) was reported. Hence it has much attracted us to carry out the development of simultaneous determination of Tenofovir Disoproxil Fumarate (TDF) and Lamivudine (LAM) by UV spectrophotometric method with acetonitrile : 0.1N HCl (20:80) solvent system. The present study illustrates a simple,
accurate and reproducible procedure and so far, this UV spectrophotometric method has not been reported and it can be utilized for routine quality assurance.

MATERIAL AND METHODS

Instrument
UV-Visible Spectrophotometer T60 (model), Analytical technologies Limited, connected to the digital system loaded with UVWin software ver.5.1.1 have an wavelength accuracy of ±5.0nm with quartz cells of 1cm path length.

Reagents and Materials
Working standards of pharmaceutical grade Tenofovir Disoproxil Fumarate (IP) and Lamivudine (IP) were procured locally and other chemicals used were of AR grade and purchased from SD fine chemicals, Mumbai.

Selection of solvent system
TDF and LAM were dissolved separately in acetonitrile solvent and final volume was made up with 0.1N HCl (pH 1.2). The absorbances of TDF and LAM at respective wavelengths were determined.

Preparation of TDF and LAM Standard Stock Solutions
Standard stock solutions of TDF and LAM (10 mg of each) were prepared separately in 20 ml acetonitrile and made up to 100 ml with 0.1N HCl to get the final concentration of 100µg/ml.

Selection of wavelength (λ<sub>max</sub>)
Standard solutions were scanned in the range of 200-400nm, against (20:80) acetonitrile: 0.1N HCl solvent system as reference. TDF (Figure-2) and LAM (Figure-3) were showed absorbance maxima (λ<sub>max</sub>) at 260nm and 280nm respectively.

Calibration standards
From the standard stock solution of TDF and LAM, different concentrations were prepared respectively in the range of 5-45µg/ml and 2-16µg/ml and measured absorbance at 260nm (Figure-4) and 280nm (Figure-5). The calibration curves were plotted (Figure-6 and Figure-7) and data presented in Table -1 and Table-2.

Simultaneous equation method
The simultaneous Spectrophotometric determination of TDF and LAM in a acetonitrile : 0.1N HCl (20:80) solvent system is provided without reaction between these two drugs. The amount of TDF and LAM were calculated using the simultaneous equation given below

\[ A_1 = a_1b_x + a_yb_y \]
\[ A_2 = a_2b_x + a_yb_y \]

\[ C_{TDF} = \frac{A_yb_y - A_1b_y}{a_xb_y - a_1b_y} \]
\[ C_{LAM} = \frac{A_xb_x - A_2b_x}{a_xb_y - a_1b_y} \]

Where
\[ A_1 = \text{Absorbance of Mixture at 260nm} \]
\[ A_2 = \text{Absorbance of Mixture at 280nm} \]
\[ a_1 = \text{Absorptivity of TDF at 260nm} \]
\[ a_2 = \text{Absorptivity of TDF at 280nm} \]
\[ a_y = \text{Absorptivity of LAM at 260nm} \]
\[ a_y = \text{Absorptivity of LAM at 280nm} \]
\[ C_{TDF} = \text{Concentration of Tenofovir disoproxil fumarate} \]
\[ C_{LAM} = \text{Concentration of Lamivudine} \]
The wavelengths were selected to coincide with the absorption maxima of two drugs: the absorption spectra of two drugs should not overlap appreciably; so that TDF absorbs strongly at $\lambda_1$ (260nm) and weakly at $\lambda_2$ (280nm), and LAM absorbs strongly at $\lambda_2$ (280nm) and weakly at $\lambda_1$ (260nm). The absorbances of pure TDF and LAM in the concentration of each 10µg/ml and a combination of TDF and LAM in the concentration of each 5µg/ml were measured at two wavelengths 260nm and 280nm (Figure-8). Absorptivity values were calculated from the absorbance values of both drugs at two wavelengths are shown in Table -3. The drug content in the combination was quantified by using above simultaneous equations and that results were represented in Table -4.

Validation parameters

**Linearity** - Linear correlation was obtained between absorbance and concentration of TDF and LAM in the range of 5 - 45µg/ml and 2 - 16µg/ml respectively. Data of regression analysis was summarized in Table -2.

**Accuracy** - The recovery experiments were carried out by the standard addition method. The recoveries obtained were 99.6±0.3% and 100.2±0.2% for TDF and LAM respectively.

The high percentage recovery and low %RSD values indicate that method is accurate.

**Method Precision** – The RSD values for TDF and LAM were found to be 0.3003 and 0.2081% respectively. The RSD values were found to be below 2% which indicate that the proposed method is repeatable (Table-4).

**Intermediate Precision** – The RSD were found to be below 2% which indicate that the proposed method is reproducible.

**LOD and LOQ** – LOD for TDF and LAM were found to be 0.094µg/ml and 0.141µg/ml respectively. LOQ for TDF and LAM were found to be 0.296µg/ml and 0.429µg/ml respectively. These data show that microgram quantity of both drugs can be accurately determined.

**RESULTS AND DISCUSSION**

Literature review indicated that various methods have been reported for the analysis of TDF and LAM by UV, RP-HPLC and HPTLC etc. But no analytical methods were reported for the estimation of these drugs using this solvent system [acetonitrile: 0.1N HCl (20:80)] in UV Spectroscopic.
Table 1: Calibration data of Tenofovir Disoproxil fumarate (TDF) and Lamivudine (LAM) (each value is result of nine separate determinations)

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Absorbance at 260nm(±SD*)</th>
<th>%RSD**</th>
<th>Concentration (µg/ml)</th>
<th>Absorbance at 260nm(±SD*)</th>
<th>%RSD**</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.114±0.0007</td>
<td>0.62</td>
<td>2</td>
<td>0.125±0.0007</td>
<td>0.56</td>
</tr>
<tr>
<td>15</td>
<td>0.335±0.001</td>
<td>0.29</td>
<td>6</td>
<td>0.377±0.009</td>
<td>2.57</td>
</tr>
<tr>
<td>25</td>
<td>0.547±0.0007</td>
<td>0.14</td>
<td>10</td>
<td>0.644±0.005</td>
<td>0.81</td>
</tr>
<tr>
<td>35</td>
<td>0.766±0.001</td>
<td>0.13</td>
<td>14</td>
<td>0.849±0.001</td>
<td>0.11</td>
</tr>
<tr>
<td>45</td>
<td>0.991±0.0006</td>
<td>0.06</td>
<td>16</td>
<td>0.993±0.002</td>
<td>0.29</td>
</tr>
</tbody>
</table>

*Standard deviation
**Relative standard deviation
Table 2: Statistical data of regression equations and validation parameters for Tenofovir disoproxil fumarate (TDF) and Lamivudine (LAM) (each value is result of nine separate determinations)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>TDF</th>
<th>LAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>260</td>
<td>280</td>
</tr>
<tr>
<td>Beer’s law limit (µg/ml)</td>
<td>5-45</td>
<td>2-16</td>
</tr>
<tr>
<td>Molar absorptivity (l mol⁻¹ cm⁻¹)</td>
<td>1.57 × 10⁴</td>
<td>4.36 × 10⁴</td>
</tr>
</tbody>
</table>

Regression equation*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Intercept (c)</th>
<th>Slope (m)</th>
<th>Regression coefficient (r²)</th>
<th>LOD (µg/ml)</th>
<th>LOQ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.002</td>
<td>0.021</td>
<td>0.999</td>
<td>0.094</td>
<td>0.141</td>
</tr>
</tbody>
</table>

* y = mx+c; where y = absorbance at respective λmax, x = concentration of the analyte

LOD – limit of detection, LOQ – limit of quantification.

Table 3: The absorptivity values of Tenofovir disoproxil fumarate (TDF) and Lamivudine (LAM)

<table>
<thead>
<tr>
<th>Absorptivity values</th>
<th>Wavelength (nm)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ax1</td>
<td>260</td>
<td>-</td>
</tr>
<tr>
<td>ax2</td>
<td>-</td>
<td>0.0063</td>
</tr>
<tr>
<td>ay1</td>
<td>280</td>
<td>-</td>
</tr>
<tr>
<td>ay2</td>
<td>-</td>
<td>0.064</td>
</tr>
<tr>
<td>A1</td>
<td></td>
<td>0.249</td>
</tr>
<tr>
<td>A2</td>
<td></td>
<td>0.359</td>
</tr>
</tbody>
</table>

Table 4: The results of analysis and recovery studies

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount added (µg/ml)</th>
<th>Amount found (µg/ml)</th>
<th>% Recovery</th>
<th>SD</th>
<th>%RSD</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDF</td>
<td>5</td>
<td>4.98</td>
<td>99.6</td>
<td>0.3</td>
<td>0.3003</td>
<td>0.03001</td>
</tr>
<tr>
<td>LAM</td>
<td>5</td>
<td>5.01</td>
<td>100.2</td>
<td>0.2</td>
<td>0.2081</td>
<td>0.02082</td>
</tr>
</tbody>
</table>

SD – Standard deviation, RSD – Relative standard deviation
SE – Standard error

by simultaneous equation method. The absorption maxima of TDF and LAM were found at 260nm and 280nm respectively. In these wave lengths absorbances of TDF and LAM and mixture was noted. The results showed an excellent correlation between absorbances and concentration of the drugs. Validation parameters like accuracy, precision and linearity found low %RSD values which indicates that the method is sensitive. The percentage recovery of TDF and LAM were found to be 99.6±0.3 and 100.2±0.2 respectively. The main advantage of the proposed method is suitability for routine determination of TDF and LAM without their prior separation.

CONCLUSION

Simple UV spectrophotometric methods were developed for the simultaneous determination of TDF and LAM in bulk. To the best of our knowledge, the present study is the first report for the purpose. The present method succeeded in adopting a simple sample preparation and achieved satisfactory percentage recovery and therefore it can be concluded that use of this method can save analysis time and money. The proposed method is accurate and precise for the determination of TDF and LAM in combined form. Hence, it can be employed for routine analysis in Quality Control Laboratories.
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