APPLICATION OF UV –SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF LAMIVUDINE IN TABLETS

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ABSTRACT

Two new, simple and cost effective UV-spectrophotometric and first order derivative methods were developed for estimation of Lamivudine in bulk and tablets. Lamivudine was estimated at 270 nm in methanol-water (3:7). In first order derivative, it showed amplitude at 300 nm. In both the methods linearity was found to be in the range of 5 - 40 µg/ml; for UV-spectrophotometric method \( Y = 0.02586 X + 0.0083; r^2 = 0.9999 \) and for first order derivative spectrophotometric method \( Y = 0.00132 X + 0.00035; r^2 = 0.9995 \), respectively. These methods were tested and validated for various parameters according to USP guidelines.

The quantitation limits were found to be 1.546 and 1.986 µg/ml, for both the methods. The proposed methods were successfully applied for the determination of Lamivudine in pharmaceutical formulations.

The results demonstrated that the procedure is accurate, precise and reproducible (relative standard deviation <2%), while being simple, cheap and less time consuming and can be suitably applied for the estimation of Lamivudine in different dosage forms.

KEYWORDS: Lamivudine, UV-Spectrophotometric Method, First Order Derivative Spectrophotometry

INTRODUCTION

Lamivudine is antiretroviral drug and acts by blocking reverse transcriptase [1]. Chemically Lamivudine is 4-amino-1-[(2R,5S)-2- (hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one [2].

The dose of Lamivudine is 300 mg per day. Several combinations of Lamivudine with other antiretroviral drugs are available in the market for treatment of HIV infected patients [3].

Literature survey revealed, few analytical methods which include simultaneous determination of lamivudine and stavudine in human serum using HPLC with tandem mass spectrometry [4], development and validation of normal phase HPTLC method for analysis of lamivudine, stavudine and nevirapine in fixed dosed combination tablet [5], simultaneous determination of HIV nucleoside analogues of reverse transcriptase inhibitors lamivudine, didanosine, stavudine, zidovudine and abacavir in human plasma using reverse phase high performance liquid chromatography [6]. Simultaneous determination of lamivudine, stavudine and nevirapine in antiretroviral fixed dose combination by high performance liquid chromatography [7].

Validation of high-performance liquid chromatography methods for determination of zidovudine, stavudine, lamivudine and indinavir in human plasma [8]. Lamivudine is not official in IP, BP and USP.

The present work deals with estimation of Lamivudine in tablets by UV-Spectrophotometry and first order derivative spectrophotometry [9].
MATERIALS AND METHODS

Instruments

- UV-visible spectrophotometer (2450 Shimadzu with UV probe 2.21 software), 10 mm quartz cell and spectral bandwidth 1nm
- Micropipette, Variable volume 20 - 200 µL Biosystem classic

Reagents

- Methanol
- Double Reverse Osmosis (R.O.) water

Preparation of Standard Stock Solution

Standard stock solution containing 100 µg/ml of Lamivudine was prepared in methanol – water (3:7). From the stock, different aliquots were taken and diluted to 10 ml mark with water to obtain series of concentrations. The solutions were scanned on spectrophotometer in the UV range 200 - 400 nm. Lamivudine showed absorbance maxima at 270 nm (fig.1).

![UV-Spectrum of Lamivudine in Methanol: Water (3:7)](image)

The same solutions were subjected to first order derivative, using UV probe software of instrument, where Δλ = 2 (fig.2). The amplitudes of the corresponding troughs were measured at 300 nm. In both the methods, drug follows linearity in the concentration range of 5 - 40 µg/ml (Y = 0.02586 X + 0.0083, \( r^2 = 0.9999 \) and Y = 0.00132 X + 0.00035, \( r^2 = 0.9995 \)), respectively.
Application of UV–Spectrophotometric Methods for Estimation of Lamivudine in Tablets

**Preparation of Sample Solution**

For analysis of commercial formulation, twenty tablets were weighed, average weight determined and crushed into fine powder. An accurately weighed quantity of powder equivalent to 150 mg of Lamivudine was transferred into 100 ml volumetric flask containing 30 ml, methanol – water (3:7), shaken manually for 10 min., volume was made up to mark with same solvent and filtered through Whatmann filter paper no. 41.

An appropriate aliquot was transferred to 10 ml volumetric flask, volume was adjusted to the mark and absorbance was recorded at 270 nm. The same solution was subjected for first order derivative using UV-probe software and amplitude of the trough was recorded at 300 nm. The concentrations of the drug was calculated from linear regression equation; results are shown in Table II.

**RESULTS AND DISCUSSIONS**

The zero order UV spectrum of Lamivudine in methanol – water (3:7) has showed maximum absorbance at 270 nm. The first derivative spectrum of Lamivudine has sharper and well-defined peak. The structural features are sharpened to give improved resolution of overlapping peaks. In first order derivative spectrum, the amplitude of the trough was recorded at 300 nm. The amount of drug determined was in the good agreement with the label claim as shown in Table I.

**Table 1: Results of Assay**

<table>
<thead>
<tr>
<th>Label Claim</th>
<th>*Amount Found (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV-Spectrophotometric ±SD</td>
<td>First Order Derivative Spectrophotometry ±SD</td>
</tr>
<tr>
<td>Lamivudine (300mg/tablet)</td>
<td>100.75 ± 0.528</td>
</tr>
</tbody>
</table>

*mean of six determinations
The methods were validated for accuracy, precision, ruggedness and robustness. The accuracy of the methods was assessed by recovery studies at three different levels i.e. at 80%, 100% and 120%. The precision of the methods were studied as intra-day, inter-day and repeatability. The % RSD values less than 2 indicate the methods are accurate and precise. Ruggedness of the proposed methods was studied with the help of two analysts.

Robustness of the methods was studied in two different laboratories using UV- visible spectrophotometer. The results did not show any statistical difference between operators and environmental conditions, suggesting that methods developed were rugged and robust. The results from validation studies are shown in Table 2.

Table 2: Summary of Validation Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>UV-Spectrophotometric</th>
<th>First Order Derivative Spectrophotometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity and range (µg/ml)</td>
<td>5.00 - 40.00</td>
<td>5.00 - 40.00</td>
</tr>
<tr>
<td>LOD</td>
<td>0.51</td>
<td>0.655</td>
</tr>
<tr>
<td>LOQ</td>
<td>1.546</td>
<td>1.986</td>
</tr>
<tr>
<td>Accuracy (% Recovery) (n = 9)</td>
<td>99.62%</td>
<td>100.15%</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.297</td>
<td>0.118</td>
</tr>
<tr>
<td>Precision (%RSD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intra-day (n = 3)</td>
<td>0.139 - 0.466</td>
<td>0.297 - 0.581</td>
</tr>
<tr>
<td>Inter-day (n = 3)</td>
<td>0.247- 0.475</td>
<td>0.101- 0.466</td>
</tr>
<tr>
<td>Repeatability (%RSD) (n = 6)</td>
<td>0.701</td>
<td>0.349</td>
</tr>
<tr>
<td>Ruggedness (%RSD) (n = 3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Analyst I (% label claim)</td>
<td>0.247</td>
<td>0.139</td>
</tr>
<tr>
<td>Analyst II (% label claim)</td>
<td>0.475</td>
<td>0.466</td>
</tr>
<tr>
<td>Robustness (% RSD) (n = 3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laboratory - I</td>
<td>0.442</td>
<td>0.459</td>
</tr>
<tr>
<td>Laboratory - II</td>
<td>0.364</td>
<td>0.466</td>
</tr>
</tbody>
</table>

CONCLUSIONS

Both these methods are simple, rapid and accurate and precise and can be used for routine analysis of Lamivudine from tablet formulations

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REFERENCES


