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Validated Spectroscopic Methods for the Determination of Fluoxetine HCl and Lamivudine in Bulk and Marketed Formulations

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ABSTRACT
A simple, convenient analytical methods were developed for the estimation of Fluoxetine HCl (FLU) and Lamivudine (LAM) in bulk and pharmaceutical dosage forms. The method for Fluoxetine HCl is based on the reaction between FLU and crystal violet in presence of Chloramine T and Sulphuric Acid. The blue coloured complex obeyed the Beer-Lamberts law in the concentration range of 0-2.5µg/ml at λ-max 603nm. The correlation coefficient was found to be 0.9991. This method was validated for linearity, sensitivity, accuracy, precision, LOD, LOQ and robustness. In the case of Lamivudine (LAM) the colour reaction is based on reaction involving the formation of greenish blue complex between Lamivudine and malachite green in the presence of Chloramine T (CT) and sulphuric acid. It obeys the Beer-Lamberts law in the concentration range of 0.3-2.7 µg/ml at λ-max of 623nm. The correlation coefficient was found to be 0.9997. The method was validated for linearity, sensitivity, accuracy, precision, LOD, LOQ and robustness.

Keywords: Fluoxetine HCl, Lamivudine, Crystal violet, Malachite green, Chloramine T, LOD, LOQ.

1. INTRODUCTION:
Fluoxetine HCl [1, 2] is chemically N-methyl-3-phenyl-3-(4-(trifluoromethyl)phenoxy)propan-1-amine hydrochloride [3, 4]. It is an anti-depressant with selective serotonin – reuptake inhibitor action.

Lamivudine [5, 6] is chemically 4-amino-1-((2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl)pyrimidin-2(1H)-one [7, 8]. It is a potent nucleoside analogue with reverse transcriptase inhibitor action (n RTI). It is used for the treatment of chronic hepatitis B. It is widely used and well tolerated. It is also used to inhibit both type 1 and 2 of HIV reverse transcriptase.

2. MATERIALS AND METHODS:
Instrument: UV-Visible Spectrophotometer, JASCO V-360, Shimadzu 1700, with one pair of matched cells.
Reagents and chemicals: Fluoxetine HCl and Lamivudine in bulk form and marketed sample, Chloramine T (0.01M), Sulphuric Acid (2M), Crystal violet (0.02%), Malachite...
green (0.02%).

**Standard stock solution of FLU:** 100mg of pure FLU was accurately weighed and taken in a 100ml calibrated volumetric flask, dissolved in distilled alcohol and the final volume was made up to 100ml.

**Working standard solution of FLU:** 10ml of standard stock solution was taken in 100ml calibrated volumetric flask, dissolved in distilled alcohol; final volume was made up to 100ml. The final concentration was 100µg/ml.

**Standard stock solution of LAM:** 100 mg of pure LAM was weighed accurately and taken in a 100 ml calibrated volumetric flask, dissolved in distilled alcohol and final volume was made up to 100 ml.

**Working standard solution of LAM:** 10 ml of standard stock solution was taken in a 100 ml calibrated volumetric flask. It was dissolved in distilled alcohol and final volume was made up to 100 ml. The final concentration was 100µg/ml.

**Preparation of 0.01 M Chloramine T solution:** Weighed accurately 0.280 gm of CT and transferred to 100 ml volumetric flask and made up the volume with distilled water.

**Preparation of 2M sulphuric acid:** 10.8 ml of con. Sulphuric acid was transferred to 100 ml volumetric flask and made up the volume with distilled water.

**Preparation of malachite green solution:** Weighed accurately 20mg of malachite green and added in 100 ml volumetric flask. It was diluted to 100 ml with distilled water.

**Determination of absorption maximum (λ-max) of FLU:** 1ml of working standard solution (100µg/ml) was taken in 10ml volumetric flask to that 0.7ml of 0.01M CT solution, 0.4ml of 2M Sulphuric Acid was added, the reactants were allowed to react for 20 minutes. 0.3ml of 0.02% crystal violet solution was added after 5 minutes, the final volume was made up with distilled alcohol. The solution was used to determine the absorption maximum (λ-max). The absorption maximum was found to be 603nm.

**Determination of absorption maximum (λ-max) of LAM:** 1ml of working standard solution (100µg/ml) was taken in 10ml volumetric flask to that 0.7ml of 0.01M CT solution, 0.4ml of 2M Sulphuric Acid was added, the reactants were allowed to react for 20 minutes. 0.3ml of 0.02% malachite green was added and kept aside the reaction mixture for 15 minutes. Later, it was made up to the volume with distilled alcohol. The solution was used to determine the absorption maximum (λ-max) the absorption maximum was found to be 623nm.
Optimum concentrations of all the reagents used in this study were determined to get the stable blue colour complex for the determination of concentration of range of FLU and LAM as per the Beer-Lamberts law [17, 18].

2.1 Stability of Colour Complex FLU:

Colour was developed using the reagents and procedure as indicated above. The absorbance readings were recorded for every 10 minute interval up to 90 minutes. It was observed that colour complex was stable up to 90 minutes in the concentration of 10 µg/ml and 20 µg/ml.

Table 1: Stability study of colour complex for FLU

<table>
<thead>
<tr>
<th>SR.NO</th>
<th>TIME IN MINUTES</th>
<th>ABSORBANCE (10µg/ml)</th>
<th>ABSORBANCE (20µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>0.2474</td>
<td>0.4652</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>0.2409</td>
<td>0.4609</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>0.2414</td>
<td>0.4593</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>0.2368</td>
<td>0.4629</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>0.2371</td>
<td>0.4689</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
<td>0.2369</td>
<td>0.4589</td>
</tr>
<tr>
<td>7</td>
<td>70</td>
<td>0.2383</td>
<td>0.4623</td>
</tr>
<tr>
<td>8</td>
<td>80</td>
<td>0.2415</td>
<td>0.4655</td>
</tr>
<tr>
<td>9</td>
<td>90</td>
<td>0.2409</td>
<td>0.4668</td>
</tr>
</tbody>
</table>
2.2 Stability of colour complex LAM:

Colour was developed using the reagents and procedure as indicated above, absorbance reading was recorded for every 10 minute intervals up to 90 minutes. It was observed that coloured complex was stable for 90 minutes in the concentration of 6µg/ml and 12µg/ml.

**Table 2:** Stability studies of colour complex for LAM

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>TIME IN Min</th>
<th>ABSORBANCE 6 µg/ml</th>
<th>ABSORBANCE 12 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>0.1056</td>
<td>0.2213</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>0.1291</td>
<td>0.2594</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>0.1272</td>
<td>0.2589</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>0.1289</td>
<td>0.2584</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>0.1275</td>
<td>0.2574</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
<td>0.1259</td>
<td>0.2566</td>
</tr>
<tr>
<td>7</td>
<td>70</td>
<td>0.1265</td>
<td>0.2575</td>
</tr>
<tr>
<td>8</td>
<td>80</td>
<td>0.1257</td>
<td>0.2543</td>
</tr>
<tr>
<td>9</td>
<td>90</td>
<td>0.1249</td>
<td>0.2568</td>
</tr>
</tbody>
</table>
2.3 Determination of linearity and calibration graph for FLU:

Standard graph was obtained in the concentration range of 0.5-2.5µg/ml of FLU at 603nm. 5 volumetric flasks of 10ml capacity was taken, to each flask 0.5ml of 0.1M CT, 0.7ml 2M H₂SO₄ followed by 0.5,1.0,1.5,2.0 and 2.5ml working standard solution of FLU. The flasks were kept aside for some time. Then to each flask 0.2ml of 0.02% crystal violet solution was added and made up the volume with distilled alcohol. Absorbance values were recorded against reagent blank at 603nm.

![Stability study for LAM (6µg/ml)](image1)

![Stability study for LAM (12 µg/ml)](image2)

![Standard calibration curve of FLU at 603nm](image3)
2.4 Determination of linearity and calibration graph for LAM [15]:

Standard calibration graph [16] was obtained in the concentration range of 0.3-2.7 µg/ml of LAM at 623nm. Nine volumetric flasks of 10ml capacity was taken, to each flask 0.5 ml of 0.01M CT, 0.7 ml of 2 M H₂SO₄ was added, kept aside for 20 minutes. 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.1, 2.4 and 2.7 ml of working standards of LAM was added to each flask and kept aside for 10 minutes. The 0.2 ml of 0.02% of malachite green solution was added, kept aside for 10 minutes, made up the volume with distilled alcohol. Absorbance was taken against reagent blank at 623 nm.

![Calibration Curve LAM](image)

Fig 10: Standard calibration curve of LAM at 623nm

3. VALIDATION : [9,10,11]

These include study of specificity, selectivity, LOD and LOQ. The parameters are summarised in table 3 and 4.

Table 3: Summary of Validation Parameters of FLU

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fluoxetine HCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength λ (nm)</td>
<td>603</td>
</tr>
<tr>
<td>Beer-Lamberts Limit (µg/ml)</td>
<td>0.5-2.5</td>
</tr>
<tr>
<td>Limit of Detection (µg/ml)</td>
<td>0.117</td>
</tr>
<tr>
<td>Limit of Quantitation (µg/ml)</td>
<td>0.354</td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.0213</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.9991</td>
</tr>
<tr>
<td>Relative Standard Deviation</td>
<td>0.372</td>
</tr>
</tbody>
</table>

Table 4: Summary of Validation parameters of LAM

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Lamivudine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength λ (nm)</td>
<td>623</td>
</tr>
<tr>
<td>Beer –Lamberts limit (µg/ml)</td>
<td>0.3-2.7</td>
</tr>
<tr>
<td>Limit of detection (µg/ml)</td>
<td>0.128</td>
</tr>
<tr>
<td>Limit of quantitation (µg/ml)</td>
<td>0.390</td>
</tr>
</tbody>
</table>
4. RESULT AND DISCUSSION:

In the method described, only analytical and validation parameters [13] were studied. No other procedure was investigated. Accuracy, recovery, and precision studies which include intraday, interday, and values were also determined. Limit of detection (LOD) and Limit of quantitation (LOQ) were determined as per the ICH guidelines [11, 12]. The same studies were carried out on marketed formulation, and found appreciably reliable results were observed.

Proposed Coloured reaction of Fluoxetine-HCl is as follows:

\[
\begin{align*}
\text{Fluoxetine HCl} & \quad \text{HCl} \quad + \quad \text{Chloramine T} \\
\text{Colourless compound} & \quad \text{HCl} \quad + \quad \text{p-toluene sulphonamide}
\end{align*}
\]

\[
\begin{align*}
\text{Crystal violet (Coloured)} & \quad \text{Unreacted Chloramine T} \\
\text{Crystal violet (Leuco form)} & \quad \text{H} \quad \text{(Coloured)}
\end{align*}
\]
Summary of Reaction: Reaction of FLU is based on oxidation reaction [14]; FLU reacts with Chloramine T, a strong oxidizing agent in presence of sulphuric acid. It produced colourless complex of FLU. After completion of reaction known amount of crystal violet is added, and excess of Chloramine T reacts with crystal violet, oxidize it and produce Leucoform of dye. Remaining unreacted molecules of crystal violet give dark blue colour. So the intensity of the colour depends on amount of drug present.

Proposed Coloured reaction of Lamivudine is as follows:

\[
\text{Lamivudine} + \text{Chloramine T} + \text{NaCl} \rightarrow \text{Leucoform of dye.} \\
\text{Unreacted Chloramine T} + \text{Malachite green (Leuco form)} \\
\]

5. CONCLUSION:
These colorimetric methods were found to be convenient, easily reproducible and cost effective. The results obtained and validation parameters studied were noteworthy. All the reagents used were of analytical grade, glasswares were calibrated. Hence these methods could be considered as simple, specific and sensitive for the determination of Fluoxetine HCl and Lamivudine in pure and pharmaceutical formulations.
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Conflicts of interest: The authors declare no conflict of interest.

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