ABSTRACT
A simple, rapid and economic UV method for the determination of simvastain in bulk and dosage form has been developed. The method proved linear in the range 1-20 µg ml\(^{-1}\) and exhibited good correlation coefficient (\(r^2= 0.997\)). The method was also validated statistically for linearity, precision, repeatability, and reproducibility. The obtained result proved that method can be satisfactorily employed for the routine analysis of simvastatin.

KEY WORDS Validation, Spectrophotometry, Precision.

INTRODUCTION
Simvastatin Statins are a group of 3-hydroxyl–3- methylglutaroyl--coenzyme A (HMG-CoA) reductase inhibitors used in heterozygotic hypercholesterolaemia and hyperlipidemia \(^{[1, 2]}\). Simvastatin (Figure 1) is a prodrug \(^{[2, 3]}\) which is biotransformed in liver into an active form of simvastatin (β-hydroxyacid) by ring opening reaction of the lacton. Chemically, simvastatin is (1S,3R,7S,8S, 8aR)-8-[2-[(2R,4R)-4-hydroxy-2H-pyran-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a hexahydronaphthalen-1-yl 2,2 dimethyl—butanoate. The inhibition of the HMGCoA causes a decrease in LDL, low density lipoprotein (20–40 %), triglycerides (10–20 %), while it increases HDL, high-density lipoprotein (5–15%) and LDL receptor expression \(^{[3, 4]}\). So, it is most commonly prescribed for the prevention of atherosclerosis and heart disease.

Validation is an integral part of any good analytical practice. It is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. The results obtained are used to judge the quality, reliability and consistency of analytical result
A survey of literature has revealed few UV-spectrophotometry \[6, 7\], HPLC \[8, 9\], and voltammetry\[10, 11\] methods for estimation of simvastatin in bulk drug and formulations. The aim of present work was to validate a simple, rapid, precise, accurate and reproducible UV method of Simvastatin estimation in raw materials as well as marketed dosage formulations.

MATERIAL AND METHOD

MATERIAL

Simvastatin was obtained as gift sample from Cipla Pharmaceutical Pvt Ltd (Mumbai, India). All chemicals used were of analytical grade.

INSTRUMENT

UV/VIS spectrophotometer (Shimadzu 1700, Kyoto, Japan) having double beam detector configuration was used.

PREPARATION OF CALIBRATION PLOT

Standard stock solution (100 µg ml\(^{-1}\))

Primary stock solution of simvastatin 100 µg.ml\(^{-1}\) was prepared in methanol by dissolving 10 mg of drug in 100 ml media. The standard solutions in the range of 1-20 µg.ml\(^{-1}\) were prepared by dilutions of stock with methanol.

Determination of \(\lambda_{\text{max}}\)

The standard solution of Finasteride was scanned in the wavelength range of 200 nm - 400 nm using UV spectrophotometer for obtaining the absorption spectra.

STABILITY

The different concentrations of drug solution were prepared from stock solution and preserved for 24 h at room temperature and analyzed on the following day to test for short-term stability.

VALIDATION

The developed UV spectrophotometric method was validated for linearity range, accuracy, precision, selectivity, limit of detection, limit of quantification and robustness parameters as per ICH guidelines\[5\].
Accuracy
A 10 µg ml\(^{-1}\) of drug solution was prepared and analyzed. The solution was further divided into 9 sub parts (10 ml each). Each sub part was spiked with 0.2, 1.0, or 2.0 ml of 100 µg.ml\(^{-1}\) drug solution. In each case, the percent recovery was reported by the assay of known added amount of drug in the sample. The percentage recovery of the added pure drug was calculated using following equation:

\[
\text{% Recovery} = \left(\frac{C_v - C_u}{C_a}\right) \times 100
\]

Where, 
\(C_v\) = total drug concentration measured after standard addition
\(C_u\) = drug concentration in 10 ml sample
\(C_a\) = drug concentration added

Linearity
Linearity was evaluated by measuring area responses at the concentration range of 1-20 µg mL\(^{-1}\). Serial dilutions were prepared and each sample was injected in triplicate. Linear regression analysis was performed after drawing the calibration curve.

Precision
i. **Repeatability** It was determined by taking observations for five consecutive days as inter-day variation. Three different drug concentrations in triplicates were analyzed and standard deviation was determined.

ii. **Intermediate precision** It was determined by calculating intra-day variations. The intra-day variations were calculated by analyzing three concentrations in triplicates, three times a day. Standard deviation and percent standard deviation values were then determined from the data.

Selectivity
The selectivity of the method for the estimation of the drug in the presence of the various excipients was investigated. A placebo (in-house mixture of excipients) comprising 25% w/w each of sodium chloride, fructose, lactose, potassium chloride was prepared. A 1:1 blend of drug and placebo was prepared. The drug was extracted from blend using methanol and absorbance was measured at \(\lambda_{\text{max}}\).

Limit of Detection
Analysis was performed in triplicate on five different drug concentrations and the LOD was measured using standard deviation of response and its slope. The LOD may be expressed as:
where $\sigma = \text{the standard deviation of the response}$
$S = \text{the slope of the calibration curve}$

**Limit of Quantification**

Based on the standard deviation of the response, the LOQ was calculated using following equation:

$$\text{LOQ} = \frac{10 \sigma}{S}$$

where
$\sigma = \text{the standard deviation of the response}$
$S = \text{the slope of the calibration curve}$

**RESULTS**

**DETERMINATION OF $\Lambda_{\text{MAX}}$**

The scan of simvastatin showed absorption maxima at 237.5 nm (Figure 2) which was in the close proximity to maxima reported in USP (2004)\textsuperscript{12} as 238 nm.

**STABILITY**

Stability study’s results were within the acceptance range (Table 1) and indicated the samples stability over 24 h (short-term).

**VALIDATION**

**Accuracy**

The validity and reliability of the proposed method was evaluated by recovery studies from standard addition method. The mean percentage recoveries (% RSD) for 0.2 ml, 1 ml and 2 ml in standard addition studies were found to be 97.94 (0.73), 98.13 (0.34) and 98.10 (0.36) respectively. The high mean percent recovery and very low standard deviation indicated high accuracy of develop method (Table 2).

**Linearity**

The observed absorbance at different drug concentrations showed in Table 3. The calibration graph was constructed by plotting graph between absorbance and concentration (Figure 3).
Calibration curve was found to be linear within the concentration range of 1 to 20 µg mL\(^{-1}\). The \(r^2\) value of 0.997 (P < 0.0001) satisfied the prerequisites of linearity.

**Precision**

Precision is the degree of agreement among individual test results, when the procedure is applied repeatedly to multiple sampling of homogeneous samples (US FDA 2000). The study was conducted by analyzing samples at different time intervals as explained below:

**i. Repeatability**

System repeatability gives the precision of a method under the same operating conditions over a short period of time. Repeatability is also termed as inter-day precision. Table 4 comprising of system repeatability data shows that the percent RSD values ranged from 0.271% to 0.794%. These values were well within the limits of 2% \(^{[5]}\).

**ii. Intermediate precision**

Intermediate precision expresses variations within days, analytes, equipments etc. It is also called as intra-day precision. Table 5 shows the mean peak area, SD and percent RSD data for observation made during intermediate precision. The % RSD value varies from 0.189 % to 0.548 % and was well within the limits of 2% \(^{[5]}\).

**Selectivity**

The UV-spectrum of simvastatin was not changed in the presence of various excipients. Paired student t-test (table 6) was performed to determine the level of significance between pure drug sample and placebo sample using Graph pad prism 5 software (demo version). There was statistically no difference between mean absorbance of solution prepared from pure drug sample and placebo sample. Therefore, proposed method is selective for the drug.

**Limit of Detection**

The limit of detection (LOD) is the lowest concentration of analyte in a sample that can be detected but not necessarily to be determined quantitatively under specified experimental conditions. The limit of detection, as calculated statistically for simvastatin was found to be 115 ngmL\(^{-1}\). The low value of LOD suggests that very low concentration of drug can be detected.

**Limit of Quantification**

The limit of quantification (LOQ) is the lowest concentration of analyte in a sample that can
be quantitatively determined within an acceptable level of accuracy and precision under the stated operational conditions of the method. The limit of quantification was found to be 536 ngmL\(^{-1}\). Hence, a very low concentration of drug can be quantified satisfactorily.

Figure 1: Simvastatin Structure

![Simvastatin Structure](image1)

Figure 2: Calibration

![Calibration Plot](image2)

Figure 3: UV Absorption Spectra Of Simvastatin

![UV Absorption Spectra](image3)
Table 1: Stability Studies

<table>
<thead>
<tr>
<th>Concentration declared (µg ml(^{-1}))</th>
<th>Concentration observed* (µg ml(^{-1}))</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.989±0.003</td>
<td>0.30334</td>
</tr>
<tr>
<td>10</td>
<td>9.941±0.015</td>
<td>0.15089</td>
</tr>
<tr>
<td>15</td>
<td>14.523±0.014</td>
<td>0.096399</td>
</tr>
</tbody>
</table>

* Each value is average of three independent determinations

Table 2: Determination of Accuracy

<table>
<thead>
<tr>
<th>Absorbance of 10 µg ml(^{-1}) drug solution</th>
<th>Spiked Samples* (ml)</th>
<th>%RSD</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.555</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>0.021 ± 0.021</td>
<td>0.24 ± 0.01</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>0.642 ± 0.033</td>
<td>0.36 ± 0.18</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>0.640 ± 0.014</td>
<td>0.55 ± 0.31</td>
<td>0.36</td>
</tr>
</tbody>
</table>

* Each value is average of three independent determinations

Table 3: Absorbance of Concentrations Obeying Beer’s Law

<table>
<thead>
<tr>
<th>Concentration (µg ml(^{-1}))</th>
<th>(Mean absorbance ± SD)*</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.072 ± 0.002</td>
<td>1.269</td>
</tr>
<tr>
<td>5</td>
<td>0.289 ± 0.003</td>
<td>0.788</td>
</tr>
<tr>
<td>10</td>
<td>0.511 ± 0.005</td>
<td>0.790</td>
</tr>
<tr>
<td>15</td>
<td>0.817 ± 0.004</td>
<td>0.509</td>
</tr>
<tr>
<td>20</td>
<td>1.051 ± 0.008</td>
<td>0.779</td>
</tr>
</tbody>
</table>

* Each value is average of three independent determinations

Table 4: Intra-Day Precision

<table>
<thead>
<tr>
<th>Concentration (µg ml(^{-1}))</th>
<th>Predicted Concentration (µgml(^{-1}))</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>2.351±2.601</td>
<td>1.031</td>
</tr>
<tr>
<td>6.5</td>
<td>6.443±6.796</td>
<td>1.063</td>
</tr>
<tr>
<td>14.5</td>
<td>14.335±14.870</td>
<td>1.098</td>
</tr>
</tbody>
</table>

* Each value is average of three independent determinations
Table 5: Inter-Day Precision

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Levels</th>
<th>% RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>1.</td>
<td>2.5</td>
<td>1.03</td>
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<tr>
<td></td>
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<td>2.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.95</td>
</tr>
<tr>
<td>2.</td>
<td>6.5</td>
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<td>2.93</td>
</tr>
<tr>
<td>3.</td>
<td>14.5</td>
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</tr>
<tr>
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<td>1.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.08</td>
</tr>
</tbody>
</table>

* Each value is average of three independent determinations

Table 6: Paired T-Test

<table>
<thead>
<tr>
<th>Concentration (µg.ml⁻¹)</th>
<th>Degree of Freedom</th>
<th>t_Calc</th>
<th>t_Crit*</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>5</td>
<td>0.1851</td>
<td>2.015</td>
<td>No</td>
</tr>
<tr>
<td>15</td>
<td>5</td>
<td>0.05834</td>
<td>2.015</td>
<td>No</td>
</tr>
</tbody>
</table>

*a: Theoretical value of t (5) at α = 0.05 level of significance.

CONCLUSION
The developed UV Spectrophotometric method was found to be simple, economic, easy, accurate, precise, reproducible and highly sensitive and can be used for routine estimation of simvastatin.

CONFLICT OF INTEREST Author declares no conflict of interest.

REFERENCES