Development and validation of uv spectroscopy method for simvastatin in pH 6.8 phosphate buffer
* S.Prasanthi¹, Dr.A.Rajendra Prasad¹, Y.Ganesh Kumar², Dr.K.Shantha Kumari³

¹Department of pharmaceutics, Nirmala College of Pharmacy, Mangalagiri, Andhra Pradesh, India.
²Department of Pharmaceutics, Research scholar at JNTU, Kukatpally, Hyderabad-500085, Telangana, India.
³Department of pharmaceutical analysis, Nirmala college of Pharmacy, Mangalagiri, Andhra Pradesh, India.

* Corresponding author: Srikakulapu.Prasanthi
E-mail id: srikakulapusanthi@gmail.com.

ABSTRACT
The aim of present work is to develop and validate simple, sensitive and specific Spectrophotometric method for the determination of simvastatin, a hypolipidemic drug in pure form and in pharmaceutical formulations. UV-Spectrophotometric method, which is based on measurement of absorption of U.V. The maximum wavelength in solvent system employed for determination of simvastatin was estimated at 233 nm in pH 6.8 phosphate buffer. The linearity range was found to be 0.01-0.08 µg/mL (R²=0.999). The developed method was validated with respect to linearity, accuracy (recovery), precision and specificity. The optimum conditions for analysis of the drug were established. The drug obeyed the Beer’s law and showed good correlation. Beer’s law was obeyed in concentration range 0.01-0.08µg/mL. The method was found to be simple, accurate, precise, economical and robust. This method has been statistically validated and is found to be precise and accurate.

Keywords: Dimethylsulphoxide, Simvastatin, UV-Spectroscopy, pH 6.8 phosphate buffer, Validation.

INTRODUCTION
Simvastatin
Simvastatin⁴ (SIM) butanoic acid, 2, 2-dimethyl-1, 2, 3, 7, 8, 8a-hexahydro-3,7-dimethyl-8- [2(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)-ethyl]-1-napthalenyl ester, shown in figure-1² is a lipid-lowering agent that is derived synthetically from fermentation products of Aspergillus-terreus³. After oral ingestion SIM, which is an inactive lactone, is hydrolyzed to corresponding β-hydroxy acid form⁴ leading to the inhibition of 3-hydroxy 3-methylglutaryl – coenzyme A. (HMG-CoA) reductase, responsible for catalyzing the conversion of HMG CoA to mevalonate, which is an
early and rate limiting step in cholesterol biosynthesis\textsuperscript{[5]}. Simvastatin is rapidly absorbed from the gastrointestinal tract after oral administration but undergoes extensive first-pass metabolism in the liver. It is inactive lactone prodrug and hydrolyzed in the gastrointestinal tract to the actives of hydroxy derivative.

\textbf{Structure of simvastatin}

Several methods were reported for the determination of SIM individually in plasma samples by HPLC\textsuperscript{[6]} with simvastatin acid\textsuperscript{[7][8][9]} and with metoprolol\textsuperscript{[10]} by LC-MS/MS. The available methods are used either for the determination of SIM individually or for determination of these analytes along with its metabolites or with other drugs. The aim of our work was developed a simple, sensitive, rapid, economical and validated analytical method for the quantification of SIM in pH 6.8 phosphate buffer. Present work focuses on the development of rapid and simple analytical method for quantification of SIM respectively. Further this method was applied for Invitro dissolution studies. The developed method may also be expected to provide a support for carrying out of the dissolution studies.

\textbf{MATERIALS AND METHODS}

\textbf{INSTRUMENTS USED}

The work was performed by using UV-Visible single beam spectrophotometer of Aquamateplus i.e., from Thermo scientific corporation. The absorption spectra analysis for the reference and test solutions were carried out in a quartz cell of the measured length of 1 cm over the specific $\lambda_{\text{max}}$ at 200-400nm.

pH meter of ELICO® Li 120 was used for measuring of pH of the prepared pH 6.8 phosphate buffer.

\textbf{CHEMICALS USED}

Simvastatin was received from the Mylan pharmaceuticals, Hyderabad as the gift sample. The drug received was having the accurate purity of 99.99% w/w and was used for the analysis without the purification of the drug simvastatin. Dimethylsulphoxide (DMSO), potassium dihydrogen phosphate and sodium hydroxide were purchased from Loba chemie Pvt.Ltd. Mumbai. The chemicals and the reagents used for the analysis of the work is of the analytical grade. Simvotin® tablets of 10 mg manufactured by Ranbaxy are chosen for the estimation of drug content by this developed method.

\textbf{PREPARATION OF PRIMARY STANDARD STOCK SOLUTION}

Accurately weighed 10 mg of simvastatin and transferred into 100 mL volumetric flask and the volume was made up to the mark with DMSO to get 100 μg/mL solution.

\textbf{PREPARATION OF SECONDARY STANDARD STOCK SOLUTION}

1mL of primary standard stock solution was diluted up to 10 mL in a volumetric flask using DMSO as a solvent to get 10 μg/mL solution.

\textbf{DETERMINATION OF \lambda MAX}

1 mL of secondary standard stock solution was diluted up to 10 mL in a volumetric flask using pH 6.8 phosphate buffer as a solvent to get the 1 μg/mL solution. Spectrum of this solution was run from 200-400nm range in UV spectrophotometer. $\lambda$ max of simvastatin was found at 233nm.

\textbf{PROCEDURE FOR CONSTRUCTION OF CALIBRATION CURVE}

Aliquots of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 mL were withdrawn from above prepared 1μg/mL of drug
solution and diluted to 10 mL in 10 mL volumetric flask with pH 6.8 phosphate buffer so as to get 0.01, 0.02, 
0.03, 0.04, 0.05, 0.06, 0.07, 0.08 μg/ mL. Absorbance of 

STUDY OF LINEARITY 

An aliquot of concentration of 0.01-0.08 μg/mL were 
prepared and absorbance were measured as per the 
developed method to confirm the linearity\textsuperscript{10}\textsuperscript{11}. The 
calibration curve was constructed for the obtained 
absorbance values of simvastatin and its concentrations 
at λ max of 233nm and linearity was evaluated by linear 
regression equation. The slope intercept and correlation 
coefficient values are recorded.

PRECISION 

The precision\textsuperscript{11}\textsuperscript{12} of the assay was determined by 
repeatability and reported as %RSD. For this 0.03μg/mL 
concentration was taken and measured six times in a day 
and same was measured in the next day. The %RSD was 
calculated.

ACCURACY 

The accuracy of method was evaluated through standard 
addition method \textsuperscript{11}\textsuperscript{12}. In this known amount of 
standard simvastatin was added in pre-analysed sample. 
This was done for 0.03 μg/mL concentration taking it as 
a 100% for three times. It was done for 80%, 100% & 
120% and the recovery studies were performed and 
finally the %RSD was calculated.

ROBUSTNESS 

It is a study of small but deliberate variations in method 
parameters\textsuperscript{11}\textsuperscript{12} such as absorption maxima, pH and 
ratio of mobile phase solvents. In this present work the 
absorption maxima was decreased and increased by 2nm 
and carried the process for 0.03 μg/mL solution for 6 
times. The %RSD was calculated.

RUGGEDNESS 

It is the study\textsuperscript{11}\textsuperscript{12} of degree of reproducibility of test 
results obtained by the variety of conditions like 
different analysts, reagents, laboratories, days, 
each solution was measured by UV spectrometer at 233 
nm using pH 6.8 phosphate buffer as blank.

equipment etc. The present work was performed by the 
change of analyst. Then nearly same results were 
obtained which are similar to that of first analyst.

ASSAY OF SIMVASTATIN IN MARKETED 
PRODUCT 

Simvotin 10 tablets manufactured by Ranbaxy 
laboratories limited was selected and analyzed for 
simvastatin using the newly developed and validated 
method. 0.03μg/mL of simvastatin was taken as a 
reference standard solution. 10 tablets were accurately 
weighed and powdered equivalent to average weight of 
tablet was accurately weighed and transferred to 100mL 
DMSO. 1 mL of this solution was diluted up to 10 mL 
in a volumetric flask using pH 6.8 phosphate buffer as a 
solvent and blank. From this 0.3 mL was withdrawn and 
diluted to 10 mL in 10 mL volumetric flask with pH 6.8 
phosphate buffer. Absorbance of this solution was 
measured by UVspectrometer at 233 nm.

RESULTS AND DISCUSSION 

A simple, selective, accurate, precise spectroscopic 
method for estimation of simvastatin in bulk and 
pharmaceutical dosage form has been developed and 
validated. The linearity range was in the concentration 
range of 0.01-0.08μg/mL (r2=0.999), it indicated that 
the concentrations of simvastatin had good linearity. 
The assay of simvastatin was found to be 98.56%. it 
indicated that by the precision of method was 
confirmed by the repeatable analysis of solution. The % 
RSD was found to be 0.631 and 0.771% respectively for 
the intra and interday precision. It indicated that method had 
good precision. The procedure for accuracy was 
repeated for 3 times by taking 0.03μg/mL as 100%. The 
recovery was calculated for 80%, 100% and 120%. The 
% RSD was found to be 1.157%. Because the % RSD 
were below 2 the method developed is highly accurate 
and precise.
Figure 1: Calibration curve of simvastatin in pH 6.8 phosphate buffer

![Calibration curve of simvastatin in pH 6.8 phosphate buffer](image)

\[
y = 9.975x + 0.021 \\
\text{r}^2 = 0.999
\]

TABLE 1: PRECISION OF DEVELOPED METHOD

<table>
<thead>
<tr>
<th>S.No</th>
<th>Intraday precision</th>
<th>% RSD</th>
<th>Inter day precision</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.382</td>
<td>0.631</td>
<td>0.377</td>
<td>0.771</td>
</tr>
<tr>
<td>2</td>
<td>0.383</td>
<td>0.378</td>
<td>0.380</td>
<td>0.375</td>
</tr>
<tr>
<td>3</td>
<td>0.380</td>
<td>0.375</td>
<td>0.386</td>
<td>0.382</td>
</tr>
<tr>
<td>4</td>
<td>0.385</td>
<td>0.382</td>
<td>0.385</td>
<td>0.382</td>
</tr>
<tr>
<td>5</td>
<td>0.386</td>
<td>0.381</td>
<td>0.386</td>
<td>0.382</td>
</tr>
</tbody>
</table>

TABLE 2: ACCURACY DATA OF DEVELOPED METHOD

<table>
<thead>
<tr>
<th>Conc. (µg/mL)</th>
<th>Spike level</th>
<th>Mean absorbance</th>
<th>Amount found (mg)</th>
<th>% Recovery</th>
<th>Mean % RSD</th>
<th>Total Mean % RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.03</td>
<td>80%</td>
<td>0.582</td>
<td>0.0534</td>
<td>98.88</td>
<td>0.334</td>
<td></td>
</tr>
<tr>
<td>0.03</td>
<td>100%</td>
<td>0.645</td>
<td>0.0594</td>
<td>98.66</td>
<td>0.806</td>
<td>0.436</td>
</tr>
<tr>
<td>0.03</td>
<td>120%</td>
<td>0.711</td>
<td>0.0655</td>
<td>98.78</td>
<td>0.168</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 3: ROBOUSTNESS OF DEVELOPED METHOD

<table>
<thead>
<tr>
<th>S.NO</th>
<th>231nm %RSD</th>
<th>235nm %RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.316</td>
<td>0.315</td>
</tr>
<tr>
<td>2</td>
<td>0.317</td>
<td>0.316</td>
</tr>
<tr>
<td>3</td>
<td>0.315</td>
<td>0.317</td>
</tr>
<tr>
<td>4</td>
<td>0.318</td>
<td>0.317</td>
</tr>
<tr>
<td>5</td>
<td>0.314</td>
<td>0.318</td>
</tr>
</tbody>
</table>

CONCLUSION

The proposed method is simple, accurate, precise, specific and selective for estimation of simvastatin in bulk and pharmaceutical dosage forms. The method is economical, rapid and do not require any sophisticated instruments in contrast to chromatographic method. Hence it can be effectively applied for routine analysis of simvastatin in bulk and marketed formulation.

www.ijpar.com
~ 19~
ACKNOWLEDGEMENTS
The authors like to acknowledge the staff and management of the Nirmala College of Pharmacy, Atmakuru (Gunturu), Andhra Pradesh, India for providing necessary facilities to carry out the research work.

REFERENCES