HPLC METHOD DEVELOPMENT OF ATORVASTATIN BY RP-HPLC IN ITS BULK DOSAGE FORMS

S. Navaneetha Krishnan*, P. Suresh Kumar, V. Naveen Kumar, Sunil, Y. Surendernath
Browns College of Pharmacy, Khammam, Andhra Pradesh, India-507305

Received on: 11/08/11 Revised on: 22/09/11 Accepted on: 08/10/11

*Corresponding author
Email: nasveen@gmail.com

ABSTRACT
A fast, simple, sensitive, precise, accurate and reproducible Reverse phase high performance chromatographic method was developed and validated for the analysis of atorvastatin in bulk dosage forms. The separation was conducted by using C-18 RP-HPLC column, which was maintained at ambient temperature. The mobile phase consisting of potassium di-hydrogen ortho phosphate, methanol and acetonitrile in the ratio 30:20:50 was delivered at a rate of 1 ml/min. The analysis was detected by using UV detector at the wave length of 240nm. The method is validated for its Precision, Limit of quantitation (LOQ), Limit of detection (LOD), linearity and robustness. The method was found to be linear over the concentration range 10-100 µg/ml ($r^2=0.999$). The retention time for Atorvastatin was found to be 8.32±0.25 min. Limit of quantification of the method is 0.189 µg/ml and limit of detection is 0.086 µg/ml.

KEY WORDS: Atorvastatin, C-18 column, RP-HPLC, Acetonitrile

INTRODUCTION
Atorvastatin is chemically known as (3R,5R)-7-[2-(4-fluorophenyl)-3-phenyl]-4-(phenyl carbamoyl)-5-(propan-2-yl)-1H-pyrrol-1-yl]-3,5-dihydroxy heptanoic acid. Atorvastatin selectively and competitively inhibits the hepatic enzyme hydroxy methyl glutaryl Co A reductase (HMG CO A). As HMG CO A reductase is responsible for converting HMG Co A to mevalonate. This results in a decrease in mevalonate, a precursor of cholesterol and a subsequent decrease in hepatic cholesterol levels and increase in uptake of LDL and cholesterol. The Various methods are used for the determination of drugs in pharmaceutical formulations. These methods include titrimetry, fluorimetry, UV spectrophotometry, infra red spectroscopy, differential scanning calorimetry (DSC), chromatographic methods etc. Among these, chromatographic methods are frequently used for the qualitative & quantitative analysis of drug substances, drug products and raw materials. This paper describes a fast, sensitive, rapid and accurate method for developed and validated the analysis of atorvastatin in bulk dosage forms by using Reverse phase-High performance liquid chromatography (RP-HPLC). The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines.

MATERIALS AND METHODS

Instrumentation
Quaternary isocratic HPLC (Younglign HPLC YL9000 series) with YL 9110 Pump and with “autochrom 3000” software and UV-Vis detector YL9120, electronic balance (Shimadzu) was used for weighing the samples, were injected on to HPLC system using Hamilton micro syringe.

Reagents and chemicals
Atorvastatin was obtained from FDC limited Goa and acetonitrile and water employed for the preparation of mobile phases were of HPLC grade (Qualigens fine chemicals, Mumbai). All the other chemicals and solvents viz. Phosphate buffer, methanol, Acetonitrile are of ambient grade.

Chromatographic conditions
The mobile phase for the proposed method, potassium di hydrogen ortho phosphate, methanol and acetonitrile (30:20:50) was filtered through a 0.45-µm membrane filter, degassed with a helium sparge for 20 min and pumped from the respective solvent reservoir to the column inertsil C 18 column (250 × 4.6mm) at a flow rate, 1.0 ml/min. The run time was set at 10 min the column temperature was maintained at room temperature. Prior to injecting the drug solution in to the column, the column was equilibrated for atleast 1 hour with the mobile phase flowing through the system. The eluent was monitored at 240 nm. The data was stored and analyzed with the soft ware “autochrom-3000” (youngling).

Selection of mobile phase
The solution of Atorvastatin was injected into the HPLC system and run in different solvent systems. Different mobile phases containing methanol, water, acetonitrile and phosphate buffer in different proportions were tried and finally Phosphate buffer, methanol and acetonitrile (30:20:50) was selected as an appropriate mobile phase which gave good resolution and acceptable peak parameters for Atorvastatin.

Preparation of mobile phase
Mobile phase comprised of Potassium di-hydrogen ortho phosphate. (Adjusted to pH 3.5±0.05 with Ortho phosphoric acid), methanol and acetonitrile (30:20:50), diluent (pH 7) used was water. Mobile phase was filtered through a 0.45-µm membrane filter, degassed with a helium sparge for 20 min and pumped from the respective solvent reservoir to the column (flow rate, 1.0 ml/min), which yield a column back pressure of 653-720 psi. Run time was set as 10 min; column was equilibrated for 60 min with mobile phase flowing through the system. Eluents were monitored at 240 nm and data were acquired, stored and analyzed with the software “Autochrom-3000” (Young Lin).

EVALUATION OF ANALYTICAL METHODS

Linearity
Aliquots ranging from 10-100µg/ml were prepared by suitable dilution of standard stock solution using mobile phase. Though linear response was obtained at lower concentrations for Atorvastatin, the higher concentration range was used to improve signal to noise. Linearity was determined by analyzing five working standard solutions over the concentration range of 10-100µg/ml for Atorvastatin.

Precision
Five sets of aliquots with same concentration (50 µg/ml) were prepared and these solutions were analyzed to record any intra and inter day variations in the results.

Limit of Detection (LOD)
The limit of detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value.
LOD is calculated from the formula,

\[ \text{LOD} = \frac{3.3\sigma}{S} \]

LOD = 0.086µg/ml

Where,

\( \sigma \) = standard deviation of the response
S = slope of the calibration curve

**Limit of quantification (LOQ)**
The limit of quantitation is the lowest amount of analyte in the sample that can be quantitatively determined with precision and accuracy.

LOQ is calculated from the formula,

\[ \text{LOQ} = 10\sigma \]

Where,

\( \sigma \) = standard deviation of the response
S = slope of the calibration curve

**RESULTS AND DISCUSSION**
The calibration graph was found to be linear in the range of 10-100 µg/mL (Table 1 & 2) (Fig.1-6). When the Atorvastatin solution was analyzed by the proposed RP-HPLC method, in intra and inter day variation studies, Inter day and intraday precision was determined by analyzing the drug sample at three different concentration levels. The results are presented in the form of %RSD which is shown (Tables 3A & 3B) that the proposed HPLC method was highly precise. Limit of quantification of the method is 0.189µg/ml and limit of detection is 0.086 µg/ml (Table 4). The drug content in the Capsules was quantified using the proposed analytical method. The proposed reversed phase HPLC method was found to be simple, precise, highly accurate, specific and less time consuming.

**CONCLUSION**
The drug content in the Capsules was quantified using the proposed analytical method. The proposed reversed phase HPLC method was found to be simple, precise, highly accurate, specific and less time consuming.

**ACKNOWLEDGEMENT**
Browns college of pharmacy management for granting permission for the publication of this work.

**REFERENCES**

**TABLE NO. 1 RESOLUTION OF DRUG MATERIAL**

<table>
<thead>
<tr>
<th>Drug</th>
<th>RT (min)</th>
<th>Peak Area</th>
<th>Height</th>
<th>Plates</th>
<th>HETP</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATROVASTATIN</td>
<td>8.32</td>
<td>3798546</td>
<td>43314</td>
<td>5689</td>
<td>0.0456</td>
</tr>
</tbody>
</table>

**TABLE NO. 2 LINEARITY OF ATORVASTATIN**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration (µg/ml)</th>
<th>Retention Time (min)</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>8.322</td>
<td>868456</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>8.323</td>
<td>2364251</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>8.325</td>
<td>3798546</td>
</tr>
<tr>
<td>4</td>
<td>75</td>
<td>8.326</td>
<td>5556486</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>8.328</td>
<td>7213945</td>
</tr>
</tbody>
</table>

**TABLE NO. 3A INTRA-DAY PRECISION FOR ATORVASTATIN**

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Peak Area</th>
<th>Mean (n=5)</th>
<th>S.D</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td></td>
<td>3785469.5</td>
<td>52007.66</td>
<td>0.12</td>
</tr>
<tr>
<td>50</td>
<td>3873448</td>
<td>3883621</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>3785469</td>
<td>3923748</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>3798546</td>
<td>3924568</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE NO. 3B INTER DAY PRECISION OF ATORVASTATIN**

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Peak Area</th>
<th>Mean (n=5)</th>
<th>S.D</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>3785469</td>
<td>3785469.5</td>
<td>56735.38</td>
<td>0.14</td>
</tr>
<tr>
<td>50</td>
<td>3873448</td>
<td>3883621</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>3785469</td>
<td>3923748</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>3798546</td>
<td>3924568</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE NO. 4 LIMIT OF DETECTION AND QUANTIFICATION (LOQ&LOD)**

<table>
<thead>
<tr>
<th>Limit of Detection</th>
<th>Limit of Quantification</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD = 0.086µg/ml</td>
<td>0.189µg/ml</td>
</tr>
</tbody>
</table>

**Figure 1**: A typical chromatogram for atorvastatin (10µg/ml) standard solution

**Figure 2**: A typical chromatogram for atorvastatin (25µg/ml) standard solution

**Figure 3**: A typical chromatogram for atorvastatin (50µg/ml) standard solution
Figure 4: A typical chromatogram for atorvastatin (75 µg/ml) standard solution

Figure 5: A typical chromatogram for atorvastatin (100 µg/ml) standard solution

Figure 6: Calibration curve of atorvastatin

\[ y = 760630.183x + 8303.194 \]
\[ R^2 = 0.999 \]

Source of support: Nil, Conflict of interest: None Declared