RP-HPLC method for simultaneous estimation of Atorvastatin Calcium and Fenofibrate in tablet dosage forms.

Rupali Hirave,* Ravindra Bendagude 1, Manish Kondawar.

Aapasaheb Birnale College of Pharmacy, South Shivajinagar, Sangli. 416416(MS), SVERR’S College of Pharmacy, Pandharpur.

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ABSTRACT

A reverse phase high performance liquid chromatographic method was developed for simultaneous estimation of atorvastatin calcium and fenofibrate in tablet formulation. The separation was achieved by HiQ sil C-8 (4.6×250mm) column with a mobile phase consisting of methanol: water pH 3.2 (90:10v/v), at a flow rate of 1ml/min. Detection was carried out at 260 nm. Retention time of atorvastatin calcium and fenofibrate was found to be 3.32 and 4.51 min, respectively. The method has been validated for linearity, accuracy and precision. Developed method was found to be accurate, precise, selective and rapid for simultaneous estimation of atorvastatin calcium and fenofibrate in tablets.

Key words: RP-HPLC, simultaneous determination, atorvastatin calcium, fenofibrate, development and validation.

INTRODUCTION

Atorvastatin calcium (8 R, d R)-2-(4-fluorophenyl)-ß,d-dihydroxy-5-(1-methylethyl)-3-phenyl-4-(phenyl amino) carbonyl)-1 H-pyrole-1-hepatic acid as the calcium salt belongs to the group of statins. 1 All the statins, including atorvastatin reduce the production of cholesterol in the liver by the competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase the rate limiting enzyme in the biosynthesis of cholesterol. 1 Fenofibrate is 2-[4-(4-chlorobenzoyl) phenoxy]-2-methyl-propanoic acid, 1-methylethyl ester. 2 It is indicated for the treatment of hypercholesterolemia and mixed dyslipidemia. 3 Tablet formulation containing 10 mg of atorvastatin calcium 160 mg of fenofibrate is available. (Atorlip-F) The literature survey revealed some HPLC methods 5-13 & Spectrophotometric methods 14-16 methods for determination of atorvastatin calcium and Fenofibrate individually and in combination with other drugs. The present work describes the development of simple, precise, and accurate isocratic reverse phase HPLC method for simultaneous estimation of atorvastatin calcium and fenofibrate in tablets.

Experimental

Chemicals: The drug sample, atorvastatin calcium and fenofibrate were obtained as gift samples from the Emcure Pharmaceuticals Ltd, Pune. Acetonitrile and Methanol (HPLC Grade) were purchased from Merk Chemical division Ltd. Mumbai. Triple distilled water was used for analysis.

Equipments: A gradient HPLC (JASCO PU-2080) was used equipped with Intelligent HPLC pumps UV-2075, and Intelligent UV / VIS Detector.

Chromatographic conditions: Chromatographic conditions were obtained by using a stainless steel column HiQ Sil C-8 (4.6 × 250 mm). A mixture of methanol and water in the ratio of (90:10 v/v) was used as a mobile phase and the pH was adjusted to 3.2 with o-phosphoric acid. It was filtered through 0.45µ membrane filter and degassed. The flow rate of mobile phase was maintained at 1 ml/min. Detection was carried out at 260 nm at 25º.

Preparation of standard stock solutions: The standard stock solution was prepared to contain 10 µg/ml of atorvastatin calcium and fenofibrate in a mixture of methanol and water (90:10v/v) as diluents. From the standard stock solution, a mixed standard solution was prepared using mobile phase to get the final concentration of 4 µg/ml atorvastatin calcium and 64 µg/ml fenofibrate. Chromatographic chromatography was recorded and results are shown in fig no.1.

Analysis of formulation: Twenty tablets of Atorlip-F each tablet containing 10 mg of atorvastatin calcium and 160 mg of fenofibrate were weighed and finely powdered. Powder equivalent to 10 mg of atorvastatin calcium and 160 mg of fenofibrate were weighed and transferred to a 100 ml dried volumetric flask. Sufficient amount of mobile phase was added to dissolve the content and shaken for 20 min. The volume was made up to 100 ml with mobile phase, filtered & degassed through membrane filter. From these solution appropriate dilutions of atorvastatin calcium and fenofibrate were made to get the final concentrations of 4 µg/ml atorvastatin calcium and 64 µg/ml fenofibrate. Results are shown in table no.1 the chromatogram recorded is shown in fig.2.

Table no.1 Analysis of test formulation

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Amount present in(mg)</th>
<th>Amount found in(mg)</th>
<th>% Label claim</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Atorvastatin Fenofibrate</td>
<td>Atorvastatin Calcium</td>
<td>Atorvastatin Fenofibrate</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>160.07</td>
<td>100.74</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>159.2</td>
<td>99.26</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>159.16</td>
<td>99.34</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>160.8</td>
<td>99.62</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>160.9</td>
<td>99.56</td>
</tr>
</tbody>
</table>

*Corresponding author.

Rupali Hirave*
Appasaheb Birnale College of Pharmacy,
Sangli, Maharashtra, India-416416
Tel.: +919623694452
E-mail: rupalahirave_29@rediffmail.com
The retention time of atorvastatin calcium and fenofibrate was found to be 3.32 and 4.51 min respectively. The proposed method was validated as per ICH guidelines. Each of the sample was injected 5 times and the same retention time was observed in all the cases. Linearity: Linearity of atorvastatin calcium and fenofibrate was determined by plotting peak area versus concentration. The response was observed in all the cases. Linearity: Linearity of atorvastatin calcium and fenofibrate was determined by plotting peak area versus concentration. The response was observed in all the cases.

CONCLUSION:
The results of the study indicate that the proposed HPLC method was simple, precise, highly accurate, specific and less time consuming.

ACKNOWLEDGEMENT
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REFERENCES

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Table 2: Recovery study

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Atorvastatin</td>
<td>3.2</td>
<td>7.12 ± 0.34</td>
<td>99.12</td>
<td>0.35</td>
</tr>
<tr>
<td>calcium</td>
<td>100</td>
<td>7.86 ± 0.31</td>
<td>98.91</td>
<td>0.31</td>
</tr>
<tr>
<td>Fenofibrate</td>
<td>51.2</td>
<td>114.29 ± 0.21</td>
<td>99.23</td>
<td>0.21</td>
</tr>
<tr>
<td>Calcium</td>
<td>120</td>
<td>8.83 ± 0.18</td>
<td>100.81</td>
<td>0.18</td>
</tr>
<tr>
<td>Fenofibrate</td>
<td>64.4</td>
<td>127.14 ± 0.21</td>
<td>99.14</td>
<td>0.21</td>
</tr>
<tr>
<td>Fenofibrate</td>
<td>76.8</td>
<td>139.46 ± 0.40</td>
<td>99.05</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Fig no.2. Chromatogram of sample solution.

RESULT & DISCUSSION:
The retention time of atorvastatin calcium and fenofibrate was found to be 3.32 and 4.51 min respectively. The proposed method was validated as per ICH guidelines. Each of the sample was injected 5 times and the same retention time was observed in all the cases. Linearity: Linearity of atorvastatin calcium and fenofibrate was determined by plotting peak area versus concentration. The response was found to be linear in the range of 10-50 µg/ml for atorvastatin calcium and 16-80 µg/ml for fenofibrate. Correlation coefficient "r" values (n=5) for both atorvastatin calcium and fenofibrate were 0.999. Accuracy of the method was calculated by recovery studies (n=3) at three levels. Standard drug solutions containing drugs in the range of 1-5 µg/ml for atorvastatin calcium and 16-80 µg/ml for fenofibrate were added to previous analysed test solution. Amount of drug recovered at each level (n=3) was determined. Percent recovery at each level were found to be 99.61 % & 99.14 % for atorvastatin calcium & fenofibrate respectively. Results are shown in Table 2. High percentage recovery at each level (n=3) was determined. Percent recovery at each level were found to be 99.61 % & 99.14 % for atorvastatin calcium & fenofibrate respectively. Results are shown in Table 2. High percentage recovery at each level (n=3) was determined. Percent recovery at each level were found to be 99.61 % & 99.14 % for atorvastatin calcium & fenofibrate respectively. Results are shown in Table 2. High percentage recovery at each level (n=3) was determined. Percent recovery at each level were found to be 99.61 % & 99.14 % for atorvastatin calcium & fenofibrate respectively. Results are shown in Table 2. High percentage recovery at each level (n=3).