Development and validation of reverse phase HPLC method for determination of Simvastatin and Ezetimibe in tablet dosage form

Sushil P Narkhede¹*, Gali Vidyasagar², Anil G Jadhav³, Sachin B Narkhede³ and Atul R Bendale³

¹Suresh Gyan Vihar University, Jaipur (RJ), India
²Veeraytan Institute of Pharmacy, Bhuj (GJ), India
³Smt. B.N.B Swaminarayan Pharmacy College, Salvav, Vapi (GJ), India

ABSTRACT
In present study reverse phase HPLC method was developed for simultaneous estimation of simvastatin (SM) and ezetimibe (EZ) in tablet formulation. The separation was achieved by Eurosphere-100 C₁₈ column and Methanol: Acetonitrile: Water (50:30:20 v/v, pH 6.8 with phosphate buffer) as a mobile phase, at a flow rate of 1.0 mL/min. The UV detection was carried out at 240nm. The retention time of SM and EZ was found to be 5.077 min and 6.633 min respectively. The assay method was found to be linear in range of 5-25 µg/mL for SM and 5-30 µg/mL for EZ.

Keywords: Simvastatin, Ezetimibe, RP-HPLC, Hypercholesterolemia.

INTRODUCTION
The pharmaceutical dosage forms containing of drugs are very much useful in therapies. Drug combinations are commonly used clinically and analyst is required to develop suitable method of their analysis.

Simvastatin and ezetimibe is antihyperlipoproteinemic. The marketed survey revealed that simvastatin (SM) and ezetimibe (EZ) in combination are recently introduced in the market. It is available in market as film coated tablet formulation. It is indicated for the treatment of hypercholesterolemia. [1-2]

In literature separate analytical methods are reported for estimation of simvastatin and ezetimibe in biological fluids these include LC-MS-MS, LC-MS, GC-MS, and no method have been reported for estimation of these drugs in formulations. So there is an immense need to develop
RP-HPLC method for its estimation in formulation. Accordingly a simple, rapid, precise and accurate method was developed for quality control of drugs formulation. [3-18]

Simvastatin [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-naphthalenyl ester.] is Hypolipidemic agent. It acts by competitively inhibiting 3-hydroxy-3-methyl glutaryl co-enzyme-A (HMGCoA) reductase, an enzyme involved in cholesterol synthesis. These agents specifically competitively inhibit 3-hydroxy-3-methyl glutaryl co-enzyme A (HMG- Co A) reductase, an enzyme that catalyzes the conversion of HMG- CoA to mevalonate, which is an early rate limiting step in cholesterol biosynthesis, HMG-CoA reductase inhibitor increase HDL (high density lipoprotein) cholesterol and decreases LDL (low density lipoprotein) cholesterol, VLDL cholesterol and plasma triglycerides. This agent highly bound to plasma proteins (95%). Excreted through urine, feces and bile. [19-23]

Ezetimibe [(3R, 4S)-1-(4-Flurophenyl)-3-[(3S)-3-(4-Fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)-2-azetidinone] Antihyperlipoproteinemic. It is selective cholesterol absorption inhibitor, since it inhibits cholesterol absorption across the small intestinal brush order membrane. It reduces blood cholesterol by inhibiting the absorption of cholesterol by small intestine. It also reduces total-C, LDL-C, Apo-B and triglycerides (TG) and increases HDL-C in patients with hypercholesterolemia. After oral administration, ezetimibe is absorbed and extensively conjugated to a pharmacologically active phenolic glucuronide (ezetimibe-glucuronide). It is primarily metabolized in the small intestine and liver via glucuronide conjugation with subsequently biliary and renal excretion. [24-26]

**MATERIALS AND METHODS**

**Chemicals and reagents** [27-29]
The drug Simvastatin was a gift sample from Cipla Ltd. Kurkumbh (MS, India). Ezetimibe gifted by MSN Laboratories (Hyderabad, India). HPLC grade acetonitrile, methanol and water were obtained from Qualigens fine chemicals, Mumbäi. All the other chemicals used of analytical grade.

**Instrumentation**
Chemito isocratic HPLC system with LC 6600 solvent delivery system (pump), UV-Visible detector, and C2000 1.7 (2) data station was used for analysis. Analysis was carried out at 240nm with Eurosphere-100 C18 reverse phase column (250×4.6 mm, 5 µm) at ambient temperature. The mobile phase consisting of Methanol: Acetonitrile: Water (50:30:20 v/v, pH 6.8 with phosphate buffer) was set at flow rate of 1 mL/ min.

**Preparation of solutions**
**Standard stock solution**
- **Simvastatin (SM) Standard Stock Solution:** (1000 µg/mL)
Standard SM (25 mg) was accurately weighed and transferred to a volumetric flask (25 mL) and dissolved in methanol. The flask was shaken and volume was made up to mark with methanol to get a solution of SM (1000 µg/mL).
Ezetimibe (EZ) Standard Stock Solution: (1000 µg/mL)

Standard EZ (25 mg) was accurately weighed and transferred to a volumetric flask (25 mL) and dissolved in methanol. The flask was shaken and volume was made up to mark with methanol to get a solution of EZ (1000 µg/mL).

Calibration standards

Calibration standards of simvastatin were prepared at concentration range of 5, 10, 15, 20, 25 µg/mL and ezetimibe at concentration range of 5, 10, 15, 20, 25, 30 µg/mL from stock solution by appropriate dilution with methanol. An aliquot of 20 µL of these solutions was injected for HPLC analysis to obtain linearity curve.

Sample preparation

Twenty tablets were weighed accurately and finely powdered. The powder equivalent to SM (10 mg) and EZ (10 mg) was accurately weighed and transferred to volumetric flask (100 mL) containing of methanol (50 mL). Then the content was shaken for 30 min. and volume was made up to mark by addition of methanol. The above solution was filtered through Whatman Filter Paper No.1. This solution was again filtered through 0.45 µ Millipore Membrane Filter. 1 mL of this solution was diluted to 10 mL by addition of mobile phase to get the SM (10 µg/mL) and EZ (10 µg/mL) solution. Tablet solution (20 µL) was injected and chromatograms are recorded for 10 min.

Method validation: [30-35]

Accuracy

Accuracy is the measure of how close the experimental value to the true value. Accuracy studies were performed by standard addition method at the 80, 100 and 120% levels as stated in ICH Guideline. The results are shown in Table 1.

Table 1: Determination of Accuracy

<table>
<thead>
<tr>
<th>Amount of sample</th>
<th>Amount of drug added</th>
<th>%Recovery</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>SM µg/mL</td>
<td>EZ µg/mL</td>
<td>SM µg/mL</td>
<td>EZ µg/mL</td>
</tr>
<tr>
<td>10.0</td>
<td>10.0</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>10.0</td>
<td>10.0</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>10.0</td>
<td>10.0</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

Precision

The precision was determined by repeatability and intermediate precision studies.

Table 2: Repeatability Data for SM and EZ

<table>
<thead>
<tr>
<th>Conc. (µg/mL)</th>
<th>Area of * SM</th>
<th>Area of* EZ</th>
<th>tR of* SM</th>
<th>tR of* EZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:10</td>
<td>704.831</td>
<td>1326.479</td>
<td>5.070</td>
<td>6.629</td>
</tr>
<tr>
<td>S.D.</td>
<td>0.256</td>
<td>0.844</td>
<td>0.012</td>
<td>0.006</td>
</tr>
<tr>
<td>% CV</td>
<td>0.036</td>
<td>0.063</td>
<td>0.23</td>
<td>0.093</td>
</tr>
</tbody>
</table>

*mean of five determinations
• **Repeatability**
Standard mixture solution containing SM (10 µg/mL) and EZ (10 µg/mL) was injected and chromatograms were recorded each time. Area and retention time (tR) were measured and % CV was calculated. Results are shown in Table 2.

• **Intra and Inter Day Precision:**
Variation of results within the same day (intraday), variation of results between days (inter day) were analyzed.

Intraday precision was determined by analyzing SM and EZ for three times in the same day. Inter day precision was determined by analyzing both the drugs daily for three days. The results are shown in Table 3 and 4.

### Table 3: Determination of Precision for SM

<table>
<thead>
<tr>
<th>Conc. (µg/mL)</th>
<th>Intra-day (n=5)</th>
<th>% CV</th>
<th>Inter-Day (n=5)</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>352.018±0.198</td>
<td>0.056</td>
<td>355.275±0.211</td>
<td>0.059</td>
</tr>
<tr>
<td>10.0</td>
<td>710.806±0.276</td>
<td>0.038</td>
<td>706.905±0.296</td>
<td>0.041</td>
</tr>
<tr>
<td>15.0</td>
<td>1028.120±0.311</td>
<td>0.030</td>
<td>1034.469±0.346</td>
<td>0.033</td>
</tr>
</tbody>
</table>

### Table 4: Determination of Precision for EZ

<table>
<thead>
<tr>
<th>Conc. (µg/mL)</th>
<th>Intra-day (n=5)</th>
<th>% CV</th>
<th>Inter-Day (n=5)</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>657.253±0.587</td>
<td>0.089</td>
<td>649.574±0.589</td>
<td>0.090</td>
</tr>
<tr>
<td>10.0</td>
<td>1319.811±0.813</td>
<td>0.061</td>
<td>1320.669±0.829</td>
<td>0.062</td>
</tr>
<tr>
<td>15.0</td>
<td>1957.292±0.962</td>
<td>0.049</td>
<td>1963.842±0.919</td>
<td>0.046</td>
</tr>
</tbody>
</table>

• **Linearity and Range:**
The linearity curve was constructed by calibration solutions and evaluated by its correlation coefficients. Results are shown in Table 5.

### Table 5: Statistical Data of SM and EZ by RP-HPLC Method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SM</th>
<th>EZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear Range (µg/mL)</td>
<td>5-25 µg/mL</td>
<td>5-30 µg/mL</td>
</tr>
<tr>
<td>Slope</td>
<td>69.161</td>
<td>131.26</td>
</tr>
<tr>
<td>Intercept</td>
<td>5.709</td>
<td>0.892</td>
</tr>
<tr>
<td>% CV</td>
<td>0.9996</td>
<td>0.9998</td>
</tr>
</tbody>
</table>

### Table 6: Results of Ruggedness Testing

*Mean of five estimations*
• **Ruggedness:**
It is the reproducibility results when the method is performed under actual use conditions. Ruggedness of method was determined by different analysts. The results are shown in Table 6.

• **System Suitability Parameters:**
The system suitability parameters were assessed prior to analysis. These parameters include plate number (N), tailing factor (As), capacity factor (K’), resolution (Rs) as shown in Table 7.

<table>
<thead>
<tr>
<th>System Suitability Parameters</th>
<th>Proposed Method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SM</td>
</tr>
<tr>
<td>Retention Time (t_R)</td>
<td>5.077 min</td>
</tr>
<tr>
<td>Peak area</td>
<td>710.806</td>
</tr>
<tr>
<td>Capacity Factor (K’)</td>
<td>0.60</td>
</tr>
<tr>
<td>Theoretical Plate Number (N)</td>
<td>8448</td>
</tr>
<tr>
<td>Tailing Factor (As)</td>
<td>1.24</td>
</tr>
<tr>
<td>Resolution Factor (Rs)</td>
<td>-</td>
</tr>
</tbody>
</table>

**RESULTS AND DISCUSSION**

**Method development and optimization**
Simvastatin and ezetimibe were almost insoluble in aqueous solutions, whereas it was freely soluble in organic solvents like methanol and acetonitrile. During the development phase, the use of methanol, acetonitrile and water as the mobile phase resulted in asymmetric peak with greater tailing factor. The addition of phosphate buffer to the mobile phase resulted in reduction in peak tailing. At pH 6.8 the tailing factor was within the acceptable limit resulting in good peak symmetry and resolution. Increasing the flow rate to 1.5 mL/min resulted in poor resolution between the drugs. A flow rate of 0.5 mL/min results in increasing drug retention time. Hence, the mobile phase was optimized at the flow rate of 1 mL/min with the retention time of 5.066 min for Simvastatin and 6.626 min for Ezetimibe. The peak shape and symmetry were found to be good when mobile phase composition of 50:30:20 (v/v, methanol: acetonitrile: water) was used with better resolution of the drugs.

**Application of method to dosage form**
Tablet containing simvastatin 10mg and ezetimibe 10mg (SIMVOTIN-EZ, Ranbaxy) was evaluated for amount of simvastatin and ezetimibe present in the formulation. The amount of simvastatin and ezetimibe in SIMVOTIN-EZ was 99.86% and 100.29% respectively. None of the tablet ingredients interfere with the analyte peak as seen in the fig 1.

**Validation of method**
• **Accuracy:**
Three replicate injections, each of three different test concentrations in the range of 80%, 100% and 120% of labeled claim of tablet under study had yielded the results within 99 to 100% of true concentration of each drug. These results indicate the accuracy of the method.
Fig. 1: Typical Chromatogram of the Tablet Solution. Simvastatin elutes at 5.077 min. and Ezetimibe at 6.633 min

- **Precision:**
  Precision studies were carried out using parameters like different days, repeatability. Results showed that the % CV was in the range of 0.1%-0.4% i.e. less than 2% for different day’s shows reproducibility of the method.

- **Linearity and Range:**
The Method showed linear response in the range 5-25 µg/mL for SM and 5-30 µg/mL for EZ.

- **Ruggedness:**
  Ruggedness studies were carried out using different analyst parameter. Results showed that the % CV was in the range of 0.08-0.2, i.e. less than 2 for different analyst studies. This study signifies the ruggedness of the method under varying conditions of its performance.

- **System suitability:**
  System suitability tests are used to verify that the resolution and reproducibility of the chromatographic method are adequate for the analysis to be done. The %CV of retention time and peak area for both drug are within 2% indicates suitability of the system. The efficiency of the column as expressed by number of theoretical plates 8448 and 9518 for SM and EZ resp (N > 2000). The tailing factor 1.24 and 1.11 for SM and EZ resp. (As ≤2), signifies system suitability.

**CONCLUSION**

A rapid, specific RP-HPLC method has been developed for determination of simvastatin and ezetimibe using UV detector the method was validated for accuracy, precision, linearity and
ruggedness. The method uses simple mobile phase composition, easy to prepare with little or no variation. The rapid run time of 10 min. and the relative flow rate (1 mL/min) allows the analysis of large number of samples with less mobile phase that proves to be cost effective. Hence this RP-HPLC method can be used for the routine drug analysis.

REFERENCES