Simultaneous Determination of Ezetimibe and Simvastatin in Pharmaceutical Dosage form by Validated RP-HPLC and UV- Spectrophotometric Methods

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ABSTRACT
A simple, rapid and precise method is developed for the quantitative simultaneous determination of Ezetimibe and Simvastatin in combined pharmaceutical-dosage forms. Two methods are described for the simultaneous determination of Ezetimibe and Simvastatin. The first method was based on UV-Spectrophotometric determination of two drugs, using simultaneous equation method. It involves absorbance measurement at 228.8 nm ($\lambda_{\text{max}}$ of Ezetimibe) and 238.4 nm ($\lambda_{\text{max}}$ of Simvastatin) Methanol: Water (90:10); For UV Spectrophotometric method, linearity was obtained in concentration range of 2 – 18 µg/ml, for both the drugs; with regression 0.999 and 0.999, intercepts 0.004 and 0.005 and slope 0.040 and 0.062 for Ezetimibe and Simvastatin respectively. Recovery was in the range of 99 –107%; the value of standard deviation and % R.S.D. were found to be < 2 %; shows the high precision of the method.. The second method was based on HPLC separation of the two drugs in reverse phase mode using these C18, 4.5µ (250 X 4.6 mm). The accuracy and reliability of the method was assessed by evaluation of linearity (1-18 µg/spot for both Ezetimibe and Simvastatin), precision (intra-day and inter-day % RSD >2 for Ezetimibe and Simvastatin), accuracy (98-102% for Ezetimibe and Simvastatin) and specificity, in accordance with ICH guidelines. Both these methods have been successively applied to pharmaceutical formulation and were validated according to ICH guidelines.

Key words: RP-HPLC, UV-spectrophotometer, fixed dose combinations, Ezetimibe, Simvastatin.

INTRODUCTION
Ezetimibe (Fig 1), (1-(4-flurophenyl)-3(R)-(3(S)-(4-flurophenyl) –3-hydroxypropyl) –4(S) (4 –ydroxyphenyl) azetidin- 2-one), which belongs to a group of selective and very effective 2-azetidione cholesterol absorption inhibitors acts at the level of cholesterol entry into enterocytes [9]. It prevents transport of cholesterol through the intestinal wall by selectively blocking the absorption of cholesterol from dietary and biliary sources. This reduces the overall delivery of cholesterol to the liver, thereby promoting the synthesis of LDL receptors and a subsequent reduction in serum LDL-C [4-5]. Clinical studies have shown that coadministration of ezetimibe with statins could provide an additional reduction in LDL cholesterol as well as total cholesterol [8].

Simvastatin (Fig 2) is a methylated analog of lovastatin, is the lactone form of (1S, 3R, 7S, 8S, 8aR)-8-[2-{2(R, 4R)-4-hydroxy-6-oxo oxotetraHydro-2-y1ethyl}-3, 7-dimethyl-1, 2, 3, 7,8,8a-hexahydroNaphthalen-1-y1]2,2 dimethylbutanoate. This drug, which acts by inhibiting 3-hydroxy-3 methyl lutarylcoA reductase is used in the treatment of hypercholesterolemia.

A few methods based on HPLC [7-9], UV [10], LC-MS [11, 12] and GC-MS [13] were reported earlier for the determination of Simvastatin individually and in combination with other drugs. A few analytical procedures were also proposed for the determination of ezetimibe in dosage forms [14] in human serum, urine and feces [15]. Although the combinational use of Simvastatin and ezetimibe is continuously increasing, simultaneous analysis of these two components in their pharmaceutical preparation is not official in Indian Pharmacopoeia, British Pharmacopoeia, United states and European Pharmacopoeia. There is an urgent need to develop and validated analytical methods for the simultaneous analysis of Simvastatin and Ezetimibe in pharmaceutical dosage forms. We describe herein a simple, sensitive and validated stability indicating HPLC method utilizing isocratic mobile phase with short retention time for the simultaneous determination of these two components in pharmaceutical formulations like tablets. The developed method can be successfully applied to quality control and other analytical purposes.

Fig 1- Structure of Ezetimibe.

Fig 2- Structure of Simvastatin.
EXPERIMENTAL

Materials and Reagents
Working standards of pharmaceutical grade Simvastatin and Ezetimibe obtained as generous gifts from Hetero Drugs Erragadda (Hyderabad, India), respectively. Fixed-dose combination tablets (Simvas–EZ), containing 10 mg Simvastatin and 10 mg Ezetimibe were procured from Hetero Drugs. Chemicals and reagents of analytical-grade were purchased from Merck grade and were purchased from Qualigens fine Chemicals, Mumbai, India.

Preparation of Mobile Phase and Stock Solution

UV- Spectrophotometer
UV-Vis Double beam spectrophotometer 2200 (Systronics) with spectral bandwidth of 2 nm and 10nm matched quartz cells was used. Standard stock 10 mg of each in 10 mL of (MeOH; H2O-9:1). From these stock solutions, working standard solutions having concentration 10 µg / mL each were prepared by appropriate dilutions. They were scanned in the wavelength range of 200– 400 nm and the overlap spectrum was obtained (Fig 3). Two wavelengths 228.8 nm (λmax of Ezetimibe) and 238.4 nm (λmax of Simvastatin) were selected for the formation of simultaneous equation. The calibration curves were found to be linear in the concentration range of 2- 18 µg/mL each, for each drug. The absorbivity coefficients of each drug at both wavelengths were determined. The concentration of two drugs in the mixture were calculated using equations,

\[ C_{\text{Eze}} = A_2 a y_1 - A_1 a y_2 / a x_1 a y_1 - a x_2 a y_2 \]  

\[ C_{\text{Sim}} = A_1 a x_2 - A_2 a x_2 / a x_1 a y_2 - a x_2 a y_2 \]  

Where A1 and A2 are absorbance of mixture at 228.8 nm and 238.4 nm respectively; ay1 and ay2, absorptivities of Ezetimibe at 228.8 nm and 238.4 nm, respectively; ax1 and ax2, absorptivities of Simvastatin at 228.8 nm and 238.4 nm, respectively. C_{\text{Eze}} and C_{\text{Sim}} are concentrations of Ezetimibe and Simvastatin in mixture.

Analysis of Pharmaceutical Dosage Forms

To determine the content of Ezetimibe and Simvastatin simultaneously in tablets (label claim: 10 mg Simvastatin and 10 mg Ezetimibe, film coated); twenty tablets were weighed; their average weight determined and were finely powdered. The correct amount of powder was dissolved in mobile phase by stirring for 30 min. The excipients were separated by filtration. Appropriate aliquots were subjected to above methods and the amount of Simvastatin and Ezetimibe were determined and was reported in Table 1.

Table 1: Analysis Data of Tablet Formulations.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>UV-Spectrophotometer</th>
<th>RP-HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Simvastatin</td>
<td>Ezetimibe</td>
</tr>
<tr>
<td>1</td>
<td>Label Claim (mg)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>% Drug Content</td>
<td>10.03</td>
<td>10.5</td>
</tr>
<tr>
<td>3</td>
<td>% S.D.</td>
<td>0.014</td>
<td>0.212</td>
</tr>
<tr>
<td>4</td>
<td>% R.S.D.</td>
<td>0.282</td>
<td>0.140</td>
</tr>
</tbody>
</table>

*Value for drug content (mg) is the mean of 3 estimations; S.D is Standard Deviation and R.S.D is Relative Standard Deviation.

Recovery Studies

To check the accuracy of the developed methods and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method, at 80, 100 and 120 % level. From the total amount of drug found, the percentage recovery was calculated. The results are reported in Table 2.

Table 2: Recovery Studies.

<table>
<thead>
<tr>
<th>UV-Spectrophotometer</th>
<th>RP-HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excess Drug</td>
<td>*Recovery</td>
</tr>
<tr>
<td>Ezetimibe</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>9.04±0.014</td>
</tr>
<tr>
<td>100</td>
<td>9.93±0.021</td>
</tr>
<tr>
<td>120</td>
<td>10.88±0.014</td>
</tr>
<tr>
<td>Simvastatin</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>8.83±0.049</td>
</tr>
<tr>
<td>100</td>
<td>10.04±0.028</td>
</tr>
<tr>
<td>120</td>
<td>11.14±0.028</td>
</tr>
</tbody>
</table>

*Recovery is the mean of Three Estimations.

RESULTS AND DISCUSSION

Both, UV Spectrophotometric, RP-HPLC methods were found to be simple, accurate, economic and rapid for routine simultaneous estimation of Ezetimibe and Simvastatin, in tablet dosage forms. For UV Spectrophotometric method, linearity was obtained in concentration range of 2 – 18 µg / mL, for both the drugs; with regression 0.9998 and 0.9999, intercept – 0.004 and −0.005 and slope 0.04 and 0.062 for Ezetimibe and Simvastatin, respectively. Recovery was in the range of 100 – 105 %; the value of standard deviation and % R.S.D. were found to be < 2 %; shows the high precision of the method. The all parameters are shown in Table 3.
In HPLC method, HPLC conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to separate drugs. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity factor), run time etc. The system with Methanol: Water (90:10 v/v) with 1 ml/min flow rate is quite robust. A typical UV spectrum and HPLC chromatogram for Ezetimibe, Simvastatin is shown in Fig 4 and 5. The optimum wavelength for detection was 232 nm at which better detector response for drugs was obtained. The average retention times for Ezetimibe and Simvastatin was found to be 3.573 and 5.687 min, respectively. According to USP XXIV (621), system suitability tests are an integral part of chromatographic method. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The parameters obtained are shown in Table 4. The calibration was linear in concentration range of 1 – 18 µg/mL, with regression 0.999 and 0.999, intercept -14296 and – 555.9 and slope 12200 and 14446 for Ezetimibe and Simvastatin, respectively. The low values of % R.S.D. indicate the method is precise and accurate. The mean recoveries were found in the range of 98 – 102 %.

The proposed methods are accurate, simple, rapid and selective for the simultaneous estimation of Ezetimibe and Simvastatin in tablet dosage forms. Hence, it can be conveniently adopted for the routine quality control analysis in the combination formulations. As the drug combination is available in market, hence, work is toward development of an analysis.

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