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# Validated Simultaneous Estimation of Simvastatin and Ezetimibe by RP-HPLC in Pure and Pharmaceutical Dosage Form

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A simple, precise RP-HPLC method was developed for the estimation of ezetimibe and simvastatin in pure and pharmaceutical dosage forms. The quantification was carried out using a C-18 column 250 × 4.6 mm i.d., 5 µm particle size in isocratic mode, with mobile phase comprising of buffer and acetonitrile in the ratio of 45:55 (v/v) pH 7. The flow rate was 1 mL/min and the detection was carried out UV detector at 210 nm. The retention times were 12.06 and 18.97 min for ezetimibe and simvastatin, respectively. The method produced linear response in the concentration range of 25-125 µg/mL for ezetimibe and simvastatin. The percentage recovery was found to be 99.8 and 100 % for ezetimibe and simvastatin, respectively. Atorvastatin used as an internal standard in the present study. The method validated by evaluation of required parameters.

**Key Words: RP-HPLC, Ezetimibe, Simvastatin.**

## INTRODUCTION

Simvastatin[1,2] is a hypolipidemic drug for oral administration. Chemically it is 2,2-dimethyl butanoic acid (1S,3R,7S,8S,8aR)-1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-[(2R,4R)-tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl]ethyl]-1naphthalenyl ester. It is empirical formula is $C_{25}H_{38}O_5$ and molecular weight is 418.56. Simvastatin is HMG-CoA reductase inhibitor synthetic analog of lovastatin[3,4].

Ezetimibe[1] is a novel and selective cholesterol absorption inhibitor drug for oral administration, chemically it is (3R,4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)-2-azetidinone. Its empirical formula is $C_{24}H_{21}F_2NO_3$ and molecular weight is 409.42. Ezetimibe is an azetidione-based cholesterol absorption inhibitor that blocks the intestinal absorption of cholesterol, resulting in lowered plasma total cholesterol and LDL-C levels[3,4].

The combination of simvastatin 10 mg and ezetimibe 10 mg provides 52 % reduction in LDL-C. The combination of simvastatin plus ezetimibe promises an additional margin of safety.

The literature survey[5-9] indicates that simvastatin and ezetimibe has been determined individually by using UV-spectrophotometry, high performance liquid chromatography and LC-MS in pharmaceutical and biological fluids preparations. There is no method has been reported for estimation of simvastatin and ezetimibe simultaneously. In the present investigation an attempt was made to develop a simple and economical validated RP-HPLC with greater precision, accuracy and sensitivity for the simultaneous estimation of simvastatin and ezetimibe in pure and tablet dosage forms.

## EXPERIMENTAL

Pure standards of ezetimibe and simvastatin were obtained as gift samples from AEON Pharmaceuticals Ltd., Chennai. The purities of these standards were 99.5 and 98.6 %, respectively. Acetonitrile, methanol, potassium dihydrogen phosphate and water used were of HPLC grade (qualigens). Simlol-EZ (IPCA Laboratories Ltd.) was employed in the study. An isocratic HPLC (Shimadzu Tokyo) with a single pump LC-10 ATVp equipped with universal injector (Rheodyne) with injection volume 20 µL, ultra violet visible detector (UV-Vis) SPD-10AV$_A$-Shimadzu series and Shimadzu class Vp software. A thermo hypersil key stone C-18 ODS column 250 × 4.6 mm i.d. with 5 µm particles. Detection was carried out by UV detection at 210 nm.

**Chromatographic conditions:** Freshly prepared 45:55 (v/v) buffer and acetonitrile were filtered through 0.45 µm membrane filter and sonicated before used. The flow rate of mobile phase was maintained at 1 mL/min. The column temperature was maintained at ambient temperature. The detection was carried out by a 210 nm. The injection volume was 20 µL and run time was 20 min.

**Preparation of mobile phase:** Phosphate buffer and acetonitrile in the ratio of 45:55 (v/v) was used as a mobile phase for present study. Phosphate buffer was prepared by taking accurately weighed quantity of 1.3609 g of potassium hydrogen phosphate is dissolved in HPLC grade water and make up to 1000 mL. The pH of the solution was adjusted to 7 by adding 0.1 N potassium hydroxide.

**Preparation of internal standard solution:** Atorvastatin was used as internal standard in the present study. About 100 mg of atorvastatin was accurately weighed, transferred to 100 mL volumetric flask. It was dissolved in mobile phase and volume made up to 100 mL so as to give 1000 µg/mL stock solution. Take 1.5 mL of stock solution and make up to 10 mL with mobile phase to give concentration about 150 µg/mL.

**Preparation of stock solution of simvastatin and ezetimibe:** About 50 mg of pure samples of simvastatin and ezetimibe were accurately weighed and transferred to a 50 mL volumetric flask. Then they are dissolved in mobile phase and the solution was made up to volume with the same. Each mL of stock solution contained 1000 µg/mL. 10 mL of this stock solution was diluted to 50 mL with mobile phase to give 200 µg/mL solution (working standard).

**Procedure for assay:** From working standard solution, 1.26-6.25 mL of solutions was transferred to 10 mL volumetric flasks. To these solutions 1.5 mL of internal standard solution was added and the volume was made up to 10 mL with mobile phase to give 25, 50, 75, 100 and 125 µg/mL of standard samples and 150 µg/mL of internal standard. The standard solutions prepared as above were filtered through 0.4 µm membrane filter and filtrate was injected 5 times into the column at a flow rate 1 mL/min. The ratio of the drug peak area to that of internal standard for each of the drug concentrations was calculated. The regression of the drug concentration over the ratio of drug peak area to that of internal standard was obtained.

**Estimation of simvastatin and ezetimibe in tablet dosage form:** 10 Tablets were weighed, pulverized and an accurately weighed sample of powder tablets equivalent to 10 mg of simvastatin and ezetimibe was taken in 100 mL volumetric flask. Add 50 mL of methanol and extracted by sonication to ensure complete solubility of the drug. The mixture was then made up to 100 mL with mobile phase, thoroughly mixed and filtered through a 0.45 µm membrane filter. An aliquot of this filtrate was transferred to a 10 mL volumetric flask along with appropriate volume of internal standard solution and made up to volume with mobile phase to give required concentration of 50 µg/mL of simvastatin and ezetimibe and 150 µg/mL of atorvastatin. Then the solution was injected five times in to the column. All the determinations were conducted five times from the peak areas. The drug content in the tablets was quantified using the regression equation obtained from the pure samples.

## RESULTS AND DISCUSSION

The present study carried out to develop a simple, rapid, accurate and precise RP-HPLC method for the analysis of simvastatin and ezetimibe in pharmaceutical dosage forms using most commonly employed RP C-18 column with UV detection. The retention times for ezetimibe and simvastatin were 10.06 and 18.97 min, respectively. The total run time of the proposed method was set as 20 min.

The ratio of the peak area of simvastatin and ezetimibe to peak area of internal standard for different concentrations set up as above were calculated and the average values of five such determinations given in Table-1.

By using peak area ratios LOD, LOQ of simvastatin and ezetimibe were calculated.

TABLE-1
CONCENTRATION *vs.* MEAN PEAK AREA RATIO OF SIMVASTATIN AND ESETIMIBE

| Drug | Drug conc. (µg/mL) | Mean peak area ratio (n = 5)* | Coefficient of variance (%) |
|---|---|---|---|
| Simvastatin | 25 | 0.207 | 0.54 |
| | 50 | 0.423 | 0.12 |
| | 75 | 0.645 | 0.14 |
| | 100 | 0.847 | 0.24 |
| | 125 | 1.053 | 0.35 |
| Ezetimibe | 25 | 0.235 | 0.36 |
| | 50 | 0.474 | 0.54 |
| | 75 | 0.686 | 0.24 |
| | 100 | 0.913 | 0.12 |
| | 125 | 1.178 | 0.13 |

*Mean of 5 values.

A good linear relationship (r = 0.9999) was observed between the simvastatin and respective peak area ratio. The calibration equation was found to be Y = 17.375X - 0.0007 (where Y is the ratio of peak area of drug to that of internal standard, X = concentration of simvastatin).

A good linear relationship (r = 0.9992) was observed between the ezetimibe and respective peak area ratio. The calibration equation was found to be Y = 19.128X - 0.0003 (where Y is the ratio of peak area of drug to that of internal standard, X = concentration of ezetimibe).

The intra-day and inter-day variations of the method were determined using five replicate injections of three different concentration which were prepared and analyzed on the same day and three different days over a period of 2 weeks, a low coefficient of variation was observed and the results are given in Table-2.

TABLE-2
PRECISION OF METHOD

| Drug | Conc. (µg/mL) | Observed concentration (n = 5)* | | | |
|---|---|---|---|---|---|
| | | Intra-day | CV (%) | Inter-day | CV (%) |
| Simvastatin | 25 | 25.106 | 0.18 | 25.006 | 0.11 |
| | 50 | 50.138 | 0.22 | 50.142 | 0.13 |
| | 75 | 75.182 | 0.14 | 75.112 | 0.18 |
| Ezetimibe | 25 | 25.013 | 0.16 | 25.011 | 0.21 |
| | 50 | 50.124 | 0.28 | 50.021 | 0.13 |
| | 75 | 75.218 | 0.17 | 75.130 | 0.12 |

*Mean of 5 values.

To ensure the reliability and accuracy of the method recovery studies were carried out by mixing a known quantity of drug with pre-analyzed sample and contents were reanalyzed by the proposed method. The values were given in Table-3. About 100.13 % of simvastatin and ezetimibe could be recovered from the pre-analyzed samples indicating the high accuracy of the proposed HPLC method.

TABLE-3
RECOVERY STUDIES

| Drug | Amount added (mg) | Amount recovered (mg) | Mean amount found (n = 5)* | Mean recovery (%) |
|---|---|---|---|---|
| Simvastatin | 40 | 40.13 | 40.15 | 100.32 |
| | 50 | 50.35 | 50.24 | 100.70 |
| | 60 | 60.12 | 60.23 | 100.20 |
| Ezetimibe | 40 | 40.19 | 40.17 | 100.47 |
| | 50 | 50.82 | 50.75 | 99.64 |
| | 60 | 60.87 | 60.82 | 99.78 |

*Mean of 5 values.

The drug content in the tablets was quantified using the proposed analytical method. The mean amount of simvastatin and ezetimibe estimated and SD were calculated and the results are given in Table-4. The systems suitability parameters are given in Table-5.

TABLE-4
ESTIMATION OF AMOUNT PRESENT IN TABLET DOSAGE FORM

| Brand name | Label claim (mg) | Amount estimated (mg) | Mean (±SD) mean (mg) found by the proposed method (n = 5)* | Mean (±SD) % labelled amount (n = 5)* |
|---|---|---|---|---|
| Simvastatin | 10 | 9.76 | 9.77 ± 0.051 | 100.6 ± 0.504 |
| Ezetimibe | 10 | 10.06 | 10.18 ± 0.089 | 98.1 ± 0.874 |

*Mean of 5 values.

TABLE-5
SYSTEM SUITABILITY PARAMETERS

| Parameters | Simvastatin | Ezetimibe |
|---|---|---|
| Resolution factor | 11.57 | 11.57 |
| Theoretical plates | 11750.5 | 9308.3 |
| Linearity range (μg/mL) | 25-125 | 25-125 |
| Limit of detection (LOD) (μg/mL) | 0.122 | 0.143 |
| Limit of quantitation (LOD) (μg/mL) | 0.407 | 0.478 |
| Relative standard deviation (RSD) | 0.874 | 0.504 |

It can be concluded that the proposed HPLC method is simple, sensitive, rapid and reproducible for the analysis of simvastatin and ezetimibe in pharmaceutical dosage forms.

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