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# Study of Validation Procedure for Ezetimibe and Simvastatin in its Pure and Pharmaceutical Dosage Form using HPLC and its Behavior in FT-IR

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**A B S T R A C T**
A new method was established for simultaneous estimation of Ezetimibe and Simvastatin by RP-HPLC method. The chromatographic conditions were success fully developed for the separation of Ezetimibe and Simvastatin by using Thermosil C18 column (4.0×125mm) 5µ , flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: Sodium acetate buffer pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 252nm. The retention times were found to be 2.566mins and 3.417mins. The % purity of Ezetimibe and Simvastatin was found to be 101.27% and 99.97% respectively. The system suitability parameters for Ezetimibe and Simvastatin such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Ezetimibe and Simvastatin was found in concentration range of 5µ g-25µ g and 50µ g-250µ g and correlation coefficient ($r^2$ ) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.86 and 0.82, % RSD for intermediate precision was 0.44 and 0.19 respectively. The precision study was precise, robust, and repeatable. LOD value was 3.17 and 5.68, and LOQ value was 0.0172 and 0.2125 respectively. Hence the suggested RP-HPLC

**Keywords:** Thermosil C18 column, Ezetimibe and Simvastatin, RP-HPLC

**A R T I C L E I N F O**

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**Contents**



## 1. Introduction

Ezetimibe is a drug that lowers plasma cholesterol levels. It acts by decreasing cholesterol absorption in the small intestine. It may be used alone (marketed as Zetia or Ezetrol), when other cholesterol-lowering medications are not tolerated, or together with statins (e.g., ezetimibe/simvastatin, marketed as Vytorin or Inegy) when statins alone do not control cholesterol. Simvastatin, marketed under the trade name Zocor

among others, is a lipid-lowering medication.t is used along with exercise, diet, and weight loss to decrease elevated lipid (fat) levels. It is also used to decrease the risk of heart problems in those at high risk. It is taken by mouth. Serious side effects may include muscle breakdown, liver problems, and increased blood sugar levels. Common side effects include constipation, headaches, and nausea. A lower dose may be needed in people with kidney problems. There is evidence of harm to unborn babies when taken during pregnancy and it should not be used by those who are breastfeeding. It is in the statin class of medications and works by decreasing the manufacture of cholesterol by the liver.

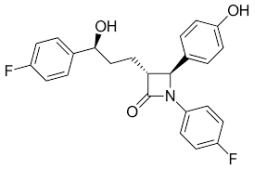


Fig.1. Ezetimibe

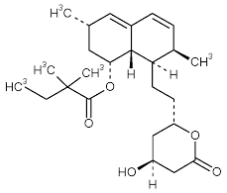


Fig.2. Simvastatin

## 2. Materials and Methods

**Materials:** Methanol, KH2PO4, Ortho phosphoric acid, Acetonitrile, K2HPO4, Water

**Methodology**

**HPLC Method Development**

**Method development for the simultaneous estimation of Simvastatin and ezetimibe by using RP-HPLC.**

1. Selection of mobile phase
2. Selection of detection wavelength
3. Selection of column
4. Selection of solvent delivery system
5. Selection of flow rate
6. Selection of column temperature
7. Selection of diluent
8. Selection of test concentration and injection volume

**1. Selection of mobile phase**

- Sodium acetate buffer : Methanol (30 : 70% v/v)
- Buffer pH should be between 2 to 8.
- Below 2: siloxane linkages are cleaved.
- pH selected: 3 ±0.05
- pH controls the elution properties by controlling the ionization characteristics.
- To decrease the retention, improve separation. Good Response, Area, Tailing factor, Resolution.

**2. Selection of wavelength:**

10mg of Simvastatin and ezetimibe was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Simvastatin and ezetimibe. The isobestic point was taken as detection wavelength.

Trial -5 (optimised method): Chromatographic conditions

Column : Thermosil C18 (4.0×125 mm) 5.0µ m
Mobile phase ratio : Methanol: Sodium acetate buffer (70: 30 % v/v) Detection wavelength : 252 nm
Flow rate: 0.7 ml/min
Injection volume : 10µ l Column
Temperature : Ambient
Auto sampler temperature : Ambient
Run time : 8min
Retention time : 2.449 & 3.191 mins

**Method Validation**

**1. Specificity**

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

**2. Linearity**

1 mg of ezetimibe and 10 mg of Simvastatin working standard were accurately weighed and were transferred into a 10ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

**3. Range**

Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 5µ g/ml-25µ g/ml and 50µ g/ml-250µ g/ml of Simvastatin and ezetimibe respectively.

**4. Accuracy**

1mg of ezetimibe and 10mg of Simvastatin working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent

**5. Precision**

1mg of ezetimibe and 10 mg of Simvastatin working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Intermediate Precision/ Ruggedness. To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on different days by using different make column of same dimensions.

**Limit of detection (LOD)**

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

**Limit of quantification**

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

**Robustness**

As part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method.

- The flow rate was varied at 0.4ml/min to 0.6 ml/min. Standard solution 15ppm of ezetimibe and 150 ppm of Simvastatin was prepared and analyzed using the varied flow rates along with method flow rate.
- The organic composition in the mobile phase was varied from 65% to75 %standard solution 15 µ g/ml of ezetimibe and 150 µ g/ml of Simvastatin were prepared and analyzed using the varied mobile phase composition along with the actual mobile phase composition in the method.

**System suitability**

1mg of ezetimibe and 10 mg of Simvastatin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of Simvastatin and ezetimibe from the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

## 3. Results and Discussion

**Table.1** Linearity Results for Simvastatin

| S.No | Linearity Level | Concentration | Area |
|---|---|---|---|
| 1 | I | 5 ppm | 471543 |
| 2 | II | 10 ppm | 656277 |
| 3 | III | 15 ppm | 794999 |
| 4 | IV | 20 ppm | 946124 |
| 5 | V | 25 ppm | 1002139 |
| Correlation Coefficient | | | 0.9990 |

**Table.2** Linearity Results for ezetimibe

| S.No | Linearity Level | Concentration | Area |
|---|---|---|---|
| 1 | I | 50ppm | 56472 |
| 2 | II | 100 ppm | 73841 |
| 3 | III | 150ppm | 92655 |
| 4 | IV | 200ppm | 111541 |
| 5 | V | 250ppm | 130567 |
| Correlation Coefficient | | | 0.9990 |

**Table.3.** Showing accuracy results for Simvastatin

| %Concentration (at specification level) | Average area | Amount added (mg) | Amount found (mg) | % Recovery | Mean recovery |
|---|---|---|---|---|---|
| 50% | 2630409 | 5 | 4.96 | 99.91% | 99.56% |
| 100% | 5277055 | 10 | 9.98 | 99.18% | |
| 150% | 7514836 | 15 | 15.02 | 99.60% | |

**Table.4.** Showing accuracy results for ezetimibe

| %Concentration (at specification level) | Average area | Amount added (mg) | Amount found (mg) | % Recovery | Mean recovery |
|---|---|---|---|---|---|
| 50% | 1366666 | 0.5 | 0.99 | 99.53% | 99.56% |
| 100% | 2777487 | 1.0 | 1.05 | 99.38% | |
| 150% | 4151234 | 1.5 | 1.495 | 99.52% | |

**Table.No.5.** Showing% RSD results for Simvastatin

**Peak Name: simvastin**

| | Peak Name | RT | Area | Height (µV) 1 |
|---|---|---|---|---|
| | **simvastin** | 3.616 | 2742453 | 238643.4 |
| 2 | **simvastin** | 3.634 | 2762750 | 271543.5 |
| 3 | **simvastin** | 3.460 | 2797670 | 281711.6 |
| 4 | **simvastin** | 3.446 | 2793578 | 274499.8 |
| 5 | **simvastin** | 3.437 | 2778483 | 276713.0 |
| Mean | | | 2774987 | |
| Std. Dev. | | | 22806.9 | |
| % RSD | | | 0.82 | |

**Table.No.6.** Showing %RSD results for ezetimibe

**Peak Name: ezetimibe**

| | Peak Name | RT | Area | Height (µV) 1 |
|---|---|---|---|---|
| | **ezetimibe** | 2.755 | 5223559 | 541538.3 |
| 2 | **ezetimibe** | 2.687 | 5208511 | 485548.5 |
| 3 | **ezetimibe** | 2.632 | 5323569 | 574440.4 |
| 4 | **ezetimibe** | 2.612 | 5259147 | 557413.5 |
| 5 | **ezetimibe** | 2.616 | 5273463 | 565020.1 |
| Mean | | | 5257650 | |
| Std. Dev. | | | 45206.4 | |
| % RSD | | | 0.86 | |

**Table.7.** Showing results for intermediate precision of Simvastatin

**Peak Name: simvastin**

| | Peak Name | RT | Area | Height (µV) 1 |
|---|---|---|---|---|
| | **simvastin** | 3.617 | 2624315 | 231325.6 |
| 2 | **simvastin** | 3.635 | 2623598 | 231315.4 |
| 3 | **simvastin** | 3.461 | 2623541 | 231250.1 |
| 4 | **simvastin** | 3.447 | 2624987 | 231342.6 |
| 5 | **simvastin** | 3.438 | 2635698 | 231765.2 |
| Mean | | | 2626428 | |
| Std. Dev. | | | 5215.78 | |
| % RSD | | | 0.19 | |

**Table.8.** Showing results for intermediate precision of ezetimibe

**Peak Name: ezetimibe**

| | Peak Name | RT | Area | Height (µV) |
|---|---|---|---|---|
| 1 | **ezetimibe** | 2.756 | 5698542 | 539568.1 |
| 2 | **ezetimibe** | 2.688 | 5682534 | 536985.4 |
| 3 | **ezetimibe** | 2.633 | 5695846 | 539584.1 |
| 4 | **ezetimibe** | 2.613 | 5689452 | 534569.8 |
| 5 | **ezetimibe** | 2.617 | 5636591 | 534985.5 |
| Mean | | | 5600593 | |
| Std. Dev. | | | 203577.3 | |
| % RSD | | | 0.44 | |

**Table.9** Showing results for Limit of Detection

| **Drug name** | **Standard deviation(σ)** | **Slope(s)** | **LOD(µg)** |
|---|---|---|---|
| Simvastatin | 373625.50 | 581075863 | 3.1 |
| ezetimibe | 5772.40 | 476579210 | 0.0172 |

**Table.10.** Showing results for Limit of Quantitation

| **Drug name** | **Standard deviation(σ)** | **Slope(s)** | **LOQ(µg)** |
|---|---|---|---|
| Simvastatin | 372727.80 | 574265980 | 5.8 |
| ezetimibe | 576 | 478828490 | 0.21 |

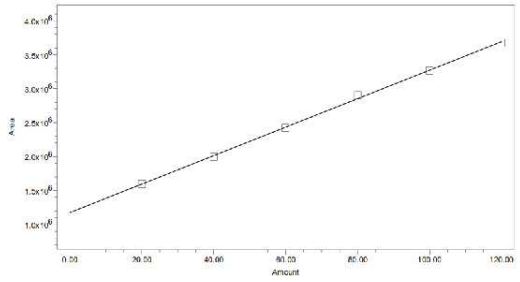

**Fig.3.** Linearity Results for Simvastatin

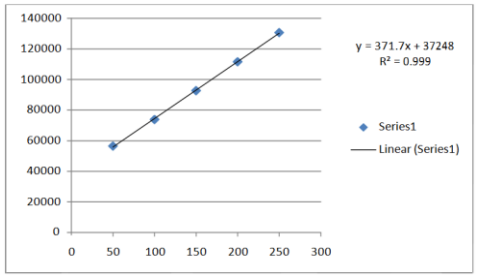


**Fig.4.** Showing calibration graph for ezetimibe

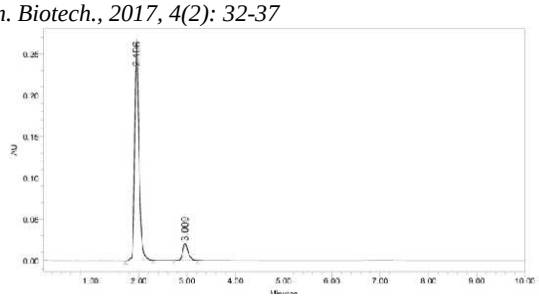

**Fig.5.** Chromatogram showing more organic phase ratio

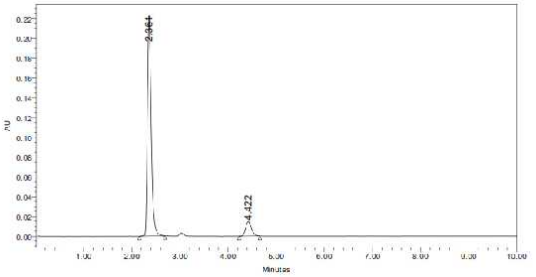

**Fig.6.** Chromatogram showing less organic phase ratio

## 4. Conclusion

The chromatographic conditions were success fully developed for the separation of Ezetazimide and Simvastatin by using Thermosil C18 column (4.0×125mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: Sodium acetate buffer pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 252nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2690, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.566mins and 3.417mins. The % purity of Ezetazimide and Simvastatin was found to be 101.27% and 99.97% respectively. The system suitability parameters for Ezetazimide and Simvastatin such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH,Q2(R1)). The linearity study n Ezetazimide and Simvastatin was found in concentration range of 5µ g-25µ g and 50µ g-250µ g and correlation coefficient (r2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.86 and 0.82, % RSD for intermediate precision was 0.44 and 0.19 respectively. The precision study was precise, robust, and repeatable. LOD value was 3.17 and 5.68, and LOQ value was 0.0172 and 0.2125 respectively.

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