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***ORIGINAL RESEARCH ARTICLE***

# Development and Validation of A HPTLC Method for the Estimation of Simvastatin and Ezetimibe.

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## ABSTRACT

A simple, precise, accurate and rapid high-performance thin-layer chromatographic method has been developed and validated for the estimation of Simvastatin and ezetimibe simultaneously in combined dosage forms. The stationary phase used was precoated silica gel 60F 254. The mobile phase used was a mixture of chloroform: benzene: methanol: acetic acid (6.0:3.0:1.0:0.1 v/v/v/v). The detection of spots was carried out at 250 nm. The method was validated in terms of linearity, accuracy, precision and specificity. The calibration curve was found to be linear between 0.8 and 4.0 µg/spot for Simvastatin and 0.1 and 1.0 µg/spot for ezetimibe. The limit of detection and the limit of quantification for Simvastatin were found to be 170 ng/spot and 570 ng/spot respectively, and for ezetimibe, 20 ng/spot and 70 ng/spot respectively. The proposed method can be successfully used to determine the drug content of marketed formulation.

**KEY WORDS:** Simvastatin, Ezetimibe, HPTLC, ICH

## INTRODUCTION

Simvastatin (SIM) butanoic acid, 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 a lipid-lowering agent that is derived synthetically from fermentation products of *Aspergillus terreus*[1]. After oral ingestion SIM, this is an inactive lactone, is hydrolyzed to corresponding ortho-hydroxy acid leading to theinhibition of 3-hydroxy 3-methyl glutaryl – coenzyme A. (HMG-CoA) reductase, responsible for catalyzing the conversion of HMG CoA to mevalonate [2], which is an early and rate limiting step in cholesterol biosynthesis.Ezetimibe (EZ), 1- (4- Fluorophenly) – 3 (R) - [3-(4-fluorophenyl) - 3 (S)hydroxy propyl]-4 (S)–(4-hydroxyphenyl) – 2 azetidinones, is a therapeutically beneficial drug that works by inhibiting the protein transporters on small intestinal brush border, which brings about this active transport of cholesterol. In addition,it also inhibits phytosterol absorption [3]. Clinical studies have shown that co-administration of ezetimibe with statins could provide an additional reduction in LDL cholesterol as well as total cholesterol [4]. In addition, it also inhibits phytosterol absorption. [5] EZ has no inhibitory effect on absorption of lipid soluble vitamins triglycerides or bile acids, as do statins. This distinct mechanism of action results in a synergistic cholesterol lowering effect, when used together with statins that inhibits cholesterol synthesis by liver [6]. A few methods based on HPLC [7-11] UV [12,13] LC-MS [14,15] and GC-MS [16] was 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 [17] in human serum[18-20], urine and feces[21]. This paper now describes an HPTLC method for the determination of simvastatin and ezetimibe in tablets. The method is rapid, accurate and precise.

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## MATERIALS AND METHODS:

Simvastatin and Ezetimibe working standards were procured as gift samples from Torrent Research Centre, Ahmedabad. Silica gel 60F 254 TLC plates (E. Merck, Mumbai) were used as a stationary phase. Tablets containing 10 mg each of Simvastatin and ezetimibe were purchased from the local market (Simvas EZ Simlo 10, Simcard EZ.). A Camag HPTLC system comprising of Camag Linnomate V automatic sample applicator, Hamilton syringe, Camag TLC Scanner 3, Camag WinCATS software, Camag twin-trough chamber and ultrasonicator was used during the study.

## PREPARATIONS OF STANDARD SOLUTION:

Working standards of Simvastatin and ezetimibe (10 mg each) were weighed accurately and diluted with methanol to obtain a final concentration of 1 mg/ml for Simvastatin and 100 µg/ml for ezetimibe. The contents of 20 tablets were ground to a fine powder. Weight equivalent to 25 mg each of Simvastatin and ezetimibe was transferred to a conical flask and dissolved in methanol. The solution was sonicated for 15 min. The extract was filtered through Whatman filter paper No. 41, and the residue was washed with methanol. The extract and washing were pooled and transferred to a 25 ml volumetric flask, and volume was made with methanol. Required dilutions were made to obtain 1000 µg/ml of Simvastatin and 100 µg/ml of ezetimibe in two different 10 ml volumetric flasks.

## CHROMATOGRAPHIC CONDITIONS:

The chromatographic estimations were performed using stationary phase, precoated silica gel 60F 254 aluminium sheets (20 × 10 cm, prewashed with methanol and dried in an oven at 50° for 5 min); mobile phase, chloroform: benzene: methanol: acetic acid (6:3:1:0.1 v/vv/v); chamber and plate saturation time of 30 min. Migration distance allowed was 72 mm; wavelength scanning was done at 250 nm **[Figure-1]**

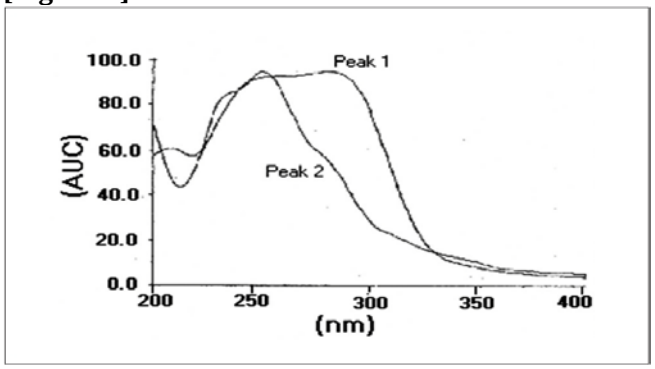


**Figure-1.Selection of Wavelength:**

## VALIDATION PROCEDURE:

Aliquots of 0.8, 0.9, 1, 2, 3 and 4 µl of standard solution of Simvastatin and 1, 3, 6, 8 and 10 µl of standard solution of ezetimibe were applied on the TLC plate. The TLC plate was dried, developed and analyzed photometrically as described earlier. The calibration curves were prepared by plotting peak area versus concentration (µg/spot) corresponding to each spot. The method was validated [22-24] by establishing linearity, accuracy, inter-day and intra-day precision, specificity, repeatability of measurement of peak, as well as repeatability of sample application. The limit of detection and limit of quantification were also determined. The related impurities were determined by spotting higher concentration of the drugs so as to detect and quantify them.

## SAMPLE PREPARATION:

For the analysis of the marketed formulations, 2 µl (for simvastatin) and 5 µl (for ezetimibe) of filtered solutions of the marketed formulations were spotted onto the same plate, followed by development scanning. The analysis was repeated six times. The spots were resolved into two peaks in the chromatogram of drug samples extracted from the marketed formulations. The content of the drug was calculated from the peak areas recorded. A solvent system that would give dense and compact spots with appropriate and significantly different Rf values was desired for quantification of Simvastatin and ezetimibe in pharmaceutical formulations. The mobile phase consisting of chloroform: benzene: methanol: acetic acid (6:3:1:0.1 v/v/v/v) gave Rf values of 0.3 (±0.04) and 0.53 (±0.04) for simvastatin and ezetimibe respectively [Figure - 2]. Linearity range for Simvastatin and ezetimibe was found to be in the range of 0.8-4.0 µg/spot and 0.1-1.0 µg/spot, with a correlation coefficient of 0.9992 and 0.9995, respectively. The LOD and LOQ for Simvastatin were found to be 170 ng/spot and 570 ng/spot for ezetimibe, 20 ng/spot and 70 ng/spot respectively.

## PRECISION:

The intra-day and inter-day precision (RSD) values were determined for standard Simvastatin (0.8-4.0 µg/spot) and ezetimibe (0.1-1.0 µg/spot) six times on the same day and over a period of 1 w. The intra-day and inter-day coefficients of variation are given in [**Table - 1**].

**Table – 1 .A summary of validation parameters of Simvastatin and Ezetimibe.**

| Parameters | Results | |
|---|---|---|
| | Simvastatin | Ezetimibe |
| Linearity | 0.8-4.0 | 0.1-1.0 |
| Correlation coefficient | 0.9992 | 0.9995 |
| Precision(%CV) | 1.05-1.15 | 1.12-1.32 |
| Intra day (n=6) | 1.39-1.50 | 1.45-1.89 |
| Inter day (n=6) | 1.09 | 1.17 |
| Repeatability of sample application (n=6) | 0.14 | 0.07 |
| Repeatability of Peak area (n=6) | 570 | 70 |
| Limit of Detection (ng/spot) | Specific | |
| Limit of Quantification(ng/spot) | Specific | |
| Specificity | | |

## RESULTS AND DISCUSSION:

Repeatability of sample application was assessed by spotting 2 µl of Simvastatin and 5 µl of ezetimibe solution six times on a TLC plate, followed by development of plate and recording the peak area for 6 spots. The % RSD for peak area values of Simvastatin and ezetimibe was found to be 1.09 and 1.17 respectively [Fihure-2]. Repeatability of measurement of peak area was determined by spotting 2 µl of Simvastatin and 5 µl of ezetimibe solution on a TLC plate and developing the plate. The separated spot was scanned five times without changing the position of the plate and % RSD for measurement of peak area of simvastatin and ezetimibe was found to be 0.143 and 0.072 respectively. To confirm the specificity of the proposed method, the solution of the formulation was spotted on the TLC plate, developed and scanned. It was observed that the excipients present in the formulation did not interfere with the peaks of Simvastatin and ezetimibe.

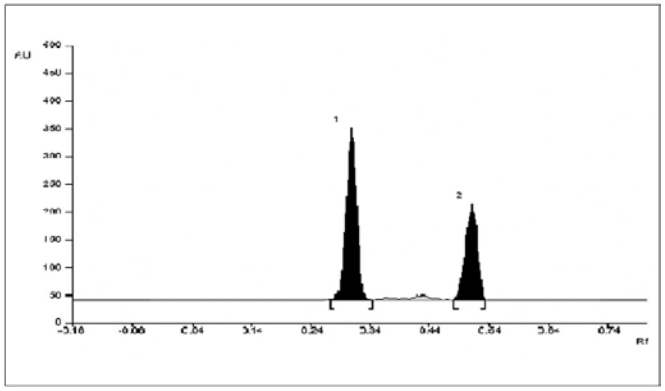

**Figure-2. Representative chromatogram peak of Simvastatin and Ezetimibe:**

## RECOVERY STUDY:

Recovery studies of drugs were carried out for accuracy parameters. These studies were carried out at three levels, i.e., multiple level recovery studies. Sample stock solution from tablet formulation of 1 mg/ml and 100 µg/ml of simvastatin and ezetimibe respectively was prepared. To the above prepared solution, 50%, 100%, 150% of the standard simvastatin solution and 20%, 40% and 60% of the standard ezetimibe solution were added. Dilutions were made and recovery studies were performed. Percentage recovery was found to be within limits, as listed in [**Table - 2**]. For the detection of the related impurities, Simvastatin and ezetimibe (0.1 g each) were dissolved separately in 10 ml of methanol, and these solutions were termed as sample solutions (10 mg/ml). One millilitre of each sample solution was diluted to 10 ml with methanol, and these solutions were termed as standard solutions (1000 µg/ml). Aliquots of both the standard solutions (2 µl) and sample solutions (20 µl) were spotted on the plate and chromatography performed as described earlier. The spot other than the principal spot and the spot of the starting point from the sample solution were not intense than the spot from the standard solution. The sample solution of Simvastatin showed three unknown additional spots at Rf of 0.06, 0.41 and 0.47. The sample solution of ezetimibe showed three unknown additional spots at Rf of 0.37, 0.70 and 0.76. However, the areas of these spots were found to be less than 0.04% as compared to the areas of standard solution spots.

**Table - 2. Recovery study of simvastatin and ezetimibe.**

| Label Claim mg/tablet | Amount added | Total amount added (mg) | Amount recovered* (mg) ± SD | % Recovery ± SD | % RSD |
|---|---|---|---|---|---|
| Simvastatin 10 | 50 | 15 | 15.36 ± 0.20 | 102.4 ± 1.36 | 1.36 |
| | 100 | 20 | 19.60 ± 0.33 | 98.00 ± 1.63 | 1.63 |
| | 150 | 25 | 25.68 ± 0.29 | 102.7 ± 1.16 | 1.16 |
| Ezetimibe | 20 | 12 | 11.98 ± 0.12 | 99.87 ± 1.02 | 1.02 |
| | 40 | 14 | 14.37 ± 0.22 | 102.65± 1.59 | 1.59 |
| | 60 | 16 | 16.20 ± 0.16 | 101.23± 0.72 | 0.72 |

Recovery study of simvastatin and ezetimibe.* indicates that each value is a mean ± Standard deviation of three determinations.

**ASSAY:**

The assay value for the marketed formulation was found to be within the limits, as listed in [Table - 3]. The low RSD value indicated the suitability of the method for routine analysis of simvastatin and ezetimibe in pharmaceutical dosage forms.

**Table – 3. Assay:**

| Label Claim (mg/tablet) | Amount Found * | % of Drug found* | % RSD |
|---|---|---|---|
| Simvastatin | 10.04 | 100.40 | 1.96 |
| Ezetimibe | 9.84 | 98.40 | 0.767 |

*Each value is mean of six determinations

**CONCLUSION:**

The developed HPTLC technique is simple, precise, specific and accurate, and the statistical analysis proved that method is reproducible and selective for the analysis of simvastatin and ezetimibe in bulk drug and tablet formulations.

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