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# Spectroscopic and HPTLC Method for Quantification of Simvastatin in Tablet Dosage Forms

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Two simple methods, ultra violet spectroscopy and high performance thin layer chromatography for the determination of simvastatin in tablet dosage form are described. Detection wavelength for spectrophotometric and high performance thin layer chromatography methods was found to be 239 nm. For the spectrophotometric method, the linearity was found to be in the range of 3-15 µg/mL with correlation co-efficient of 0.9995 and for the high performance thin layer chromatography; The linear regression analysis data for the calibration plots showed good linear relationship with correlation co-efficient $r^2 = 0.9974$ in the concentration range 200-500 ng per spot The average recovery was found to be 99.61 ± 0.29 % and 99.89 ± 0.31 % for spectrophotometric and high performance thin layer chromatography, respectively. The drug was satisfactorily resolved with $R_f$ value 0.602 ± 0.03 in HPTLC method.

**Key Words: Simvastatin, HPTLC method, Spectrometric method.**

## INTRODUCTION

Chemically, simvastatin is butanoic acid, 2,2-dimethyl-1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-{2-[tetrahydro-4-hydroxy-6-oxo-2*H*-pyran-2-yl]-ethyl}-1-naphthyl-ethyl ester. It is a HMG CoA reductase inhibitor, which blocks the synthesis of cholesterol in liver by competitively inhibiting HMG CoA reductase activity. It is clinically useful in the treatment of primary hypercholesterolemia and mixed dyslipidemia[1]. Simvastatin is official in British Pharmacopoeia and also in United States Pharmacopoeia. A literature survey reveals a few analytical methods include UV spectrophotometry[2-5] and HPLC[6,7] for determination of simvastatin. HPTLC is currently becoming a routine analytical technique for analysis of drugs[8-10]. It has proved a very useful technique because of its low operating cost, high sample-throughput and need for minimum sample clean-up. The major advantage of HPTLC is in reducing analysis time and cost per analysis. Only few HPTLC methods are available for simultaneous estimation of simvastatin in combination form[11-13]. The present work reports two simple, precise and accurate spectrophotometric and HPTLC methods for the estimation of simvastatin alone from tablet dosage form.

## EXPERIMENTAL

All chemicals and reagents used were of analytical grade and purchased from S.D. Fine Chemicals Ltd., Mumbai and Qualigens Fine Chemicals Ltd., Mumbai. A Shimadzu Double Beam UV-Spectrophotometer1600A was used for absorbance measurements and Camag HPTLC system with TLC scanner 3, Wincats Software and Linomat 5 as application device was employed for peak area measurement.

**Preparation of standard stock solution:** For UV method, the standard stock solutions of 100 µg/mL of simvastatin was prepared using acetonitrile:water in the ratio of 1:1 suitable dilutions were made and the solutions was scanned over 200-400 nm against blank. The $\lambda_{max}$ for simvastatin was found to be 239 nm. For second method, The standard stock solutions of 1000 µg/mL of simvastatin was prepared by weighing 100 mg of simvastatin and dissolved in methanol in 100 mL volumetric flask and made up to the volume. Further dilutions were made to 100 µg /mL of simvastatin with methanol.

**Chromatographic conditions:** For HPTLC method, the mobile phase used in the estimation for simvastatin comprising of chloroform:methanol:toluene (6:2:2 v/v/v). The TLC Plates were pre-washed by methanol and activated at 110 ºC for 0.5 h prior to application of sample. The chamber and plate saturation time was 0.5 h. The spotting of 5 µL of standard solution of simvastatin was done and the plate was allowed to run for a time of 0.5 h to a distance of 80 mm. The wavelength of scanning was done at 239 nm.

**Calibration curve:** For the spectrophotometric method, aliquots of standard simvastatin solutions ranging from 3-15 µg/mL from stock solution of 100 µg/mL were prepared using acetonitrile: water in the ratio of 1:1 and absorbance were noted at 239 nm. Calibration curve was drawn by plotting absorbance of simvastatin *versus* concentration of respective drug solutions (Fig. 1).

For HPTLC method, the stock solution was further diluted with methanol to obtain a series of concentrations ranging from 0.02-0.05 µg/mL of simvastatin. Five microlitres from these solutions were applied on precoated TLC plate. The plate was analyzed photometrically and chromatograms recorded. Calibration curve was drawn by plotting area obtained of simvastatin *versus* concentration of respective drug solutions (Fig. 2).

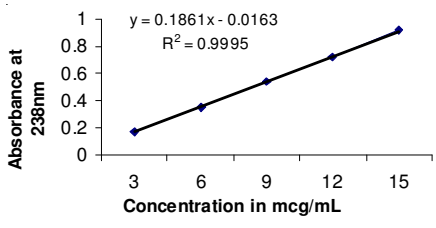


Fig. 1. Calibration curve of simvastatin (UV method)

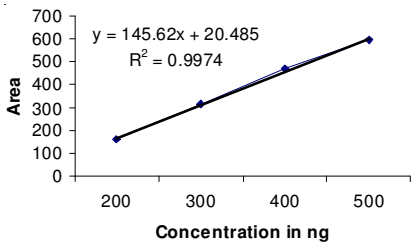


Fig. 2. Calibration curve of Simvastatin (HPTLC method)

**Validation:** The developed method was validated as per ICH guidelines[14] for linearity, specificity, accuracy and precision. The calibration curves constructed with the concentration range must obey Beer's law. The linearity was evaluated by regression analysis. The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. It was determined for both inter and intra-day variations. Precision was measured for both inter and intra-day and checked with repeatability.

**Analysis of formulation:** Twenty tablets of simvastatin were weighed and average weight was calculated. Quantity equivalent to 10 mg was weighed accurately and transferred to 100 mL volumetric flask. The active ingredient was extracted with acetonitrile: water (1:1) and volume was made up with acetonitrile:water (1:1) and filtered. The filtered solution was further diluted to get requisite concentrations and analyzed as described under the procedure for pure sample. The concentration of simvastatin in tablet formulation was calculated from calibration graph. Results are given in Table-1.

TABLE-1
VALIDATION PARAMETERS

| Parameters | Results | |
|---|---|---|
| | UV | HPTLC |
| $R_f$ value | – | 0.602±0.03 |
| Linearity range | 3-15 µg/mL | 200-500 ng/mL |
| Correlation co-efficient ($r^2$) | 0.9995 | 0.9980 |
| Repeatability (% RSD) n = 5 | 0.937 | 0.1058 |
| Recovery studies (± SD) n = 3 | | |
| 80 % level | 99.29±1.12 | 98.87±0.40 |
| 100 % level | 100.50±0.98 | 99.98±0.33 |
| 120 % level | 99.05±0.53 | 100.82±0.19 |

RSD = Relative standard deviation.

For HPTLC method, twenty tablets of simvastatin were weighed and average weight was calculated. Quantity of powder equivalent to 10 mg was weighed accurately, dissolved and volume was made up to 100 mL with methanol. This solution was filtered and further diluted to get requisite concentrations and analyzed as described under procedure for pure sample. The concentration of simvastatin in tablet formulation was calculated from calibration graph. The results are given in Table-1. The sample solutions were plated along with the standard to check the specificity. One plate spotted with 5.0 µL of sample and allowed to develop in appropriate mobile phase and detect the spots as described earlier. From the peak area recorded the amount of the drug in the formulation was determined and reported in Table-2.

TABLE-2
ANALYSIS OF SIMVASTATIN IN TABLET DOSAGE FORM

| Drug | Label amount (mg/tablet) | Amount found (mg/tablet ± RSD*) | | % Assay | |
|---|---|---|---|---|---|
| | | UV | HPTLC | UV | HPTLC |
| A | 10 | 10.11±0.03 | 9.927±0.105 | 101.10 | 99.27 |
| B | 10 | 9.973±0.09 | 9.983±0.275 | 99.73 | 99.83 |

Where A and B are two brands of tablet formulation.
*RSD = Relative standard deviation (n = 5).

## RESULTS AND DISCUSSION

The developed methods were validated for parameters like accuracy, precision and stability. The regression equation and validation parameters are given in Table-1. Accuracy was established by performing recovery studies. These were carried out at 80, 100 and 120 % levels. Recovery values close to 100 % indicates accuracy of method. For HPTLC method, limit of detection (LOD) and limit of quantitation (LOQ) were found to be 100 and 200 ng/mL, respectively. Precision was studied under intra-day precision, inter-day precision and repeatability.

For all these parameters, % RSD values were found to be less than two which indicates that the developed methods have good precision. Stability studies were also carried out. Drug solution was found to be stable for 3 h at room temperature and the developed TLC plate was found to be stable for *ca.* 4 h. The developed UV spectrophotometric and HPTLC method are precise and accurate. From the two methods developed for estimation of simvastatin, the HPTLC method was found to be more precise. However, both techniques can be applied for routine analysis of simvastatin from tablet dosage forms.

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