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High Performance Thin Layer Chromatographic Estimation of Simvastatin in Bulk and Tablet Dosage Form

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A simple, fast, specific, accurate and precise HPTLC method has been developed and validated for the estimation of simvastatin in pure and in tablet dosage forms. Aluminium backed silica gel $60F_{254}$ were used as stationary phase and a mixture of toluene:ethyl acetate:formic acid in the ratio 5:1.5:0.5 v/v/v were used as mobile phase. Densitometric analysis was carried out in absorbance mode at 242 nm. The R_f value of simvastatin was found to be 0.26 ± 0.01. The method was validated in terms of linearity, accuracy, precision and recovery. The linearity curve was found to be linear over 200-1000 ng per spot. The limit of detection and limit of quantification were found to be 50 and 160 ng per spot, respectively. The proposed method can be useful for estimation of simvastatin in the quality control of bulk and tablet dosage forms.

Key Words: Simvastatin, HPTLC, Validation.

INTRODUCTION

Simvastatin[1] is chemically, (1S,3R,7S,8S,8aR)-1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-[(2R,4R)-tetrahydro-4-hydroxy-6-oxo-2*H*-pyran-2-yl]ethyl]-1-naphthalenyl ester; 2,2-dimethyl butyric acid 8-ester with (4R,6R)-6-[2-[(1S,2S,6R,8S,8aR)-1,2,6,7,8,8a-hexahydro-8-hydroxy-2,6-dimethyl-1-naphthyl]ethyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one. Simvastatin is a synthetic lipid-lowering agent, analogue of lovastatin and is used in the treatment of hypercholesterolaemia[2]. It is a selective and competitive inhibitor of HMG-CoA reductase, that catalyzes the conversion of HMG-CoA to mevalonate, the rate-limiting step in cholesterol biosynthesis. Literature survey reveals various spectrophotometric[3,4], HPLC[5], HPTLC[6,7] and LC-MS[8] methods have been reported for the estimation of simvastatin in bulk and table dosage forms. In present investigation an attempt has been made to develop simple, accurate, precise and specific HPTLC method for the estimation of simvastatin in bulk and tablet dosage forms.

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EXPERIMENTAL

Camag HPTLC system comprising of Linomat-IV automatic sample applicator, TLC Scanner-III with CATS4 software were used in the study. Hamilton syringe (100 µL) and Telsonic ultrasonix sonicator were used for the present study.

Drugs and chemicals: Simvastatin working standard was obtained as a gift sample from Krebs Biochemicals & Industries Limited, Hyderabad. All the chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, Mumbai, India.

Chromatographic conditions: Aluminium-backed silica gel $60F_{254}$ TLC plates coated with 0.2 mm layers of silica gel $60F_{254}$ (E.Merck, Germany) were used as a stationary phase. Mobile phase used in the study was a mixture of toluene:ethyl acetate:formic acid in the ratio of 5:1.5:0.5 v/v/v. Development chamber used was a Camag twin trough glass chamber (20 cm × 10 cm) saturated with filter paper for 10 min. Seperation was carried out by ascending development. The size of the plate used was 20 cm × 20 cm. Sample application position on plate was at 10 mm distance from the base and solvent front was developed to 80 mm. Band of length 8 mm was applied under a stream of nitrogen gas. Dueterium lamp was used and UV detection was carried out at 242 nm. The scanning speed used was 20 mm/s.

Preparation of standard solution: A standard stock solution of simvastatin (5 mg) was weighed accurately and transferred to 50 mL volumetric flask and dissolved in 50 mL of methanol. The resulting solution was a concentration of 0.1 mg/mL. 2 mL of above solution is made upto 10 mL with methanol to give standard stock solution of concentration of 0.2 mcg/mL or 0.2 ng/mL.

For analysis of the tablet dosage form, 20 tablets of simvastatin were weighed, finely powered and their average weight was calculated. An accurately weighed quantity of tablet powder equivalent to 10 mg simvastatin was transferred to 10 mL volumetric flask and dissolved in 10 mL methanol. The solution was sonicated for 15 min. Then it was filtered through Whatmann filter paper and the filterate was diluted with methanol to get a final concentration of 200 ng/spot of simvastatin.

Calibration curve: Aliquots of 20, 40, 60, 80 and 100 µL of standard stock solution of simvastatin were applied on the TLC plate with the help of a Camag Linomat IV sample applicator to give concentrations of 200, 400, 600, 800 and 1000 ng/spot. TLC plate was dried, developed and the bands were evaluated at 242 nm. The standard calibration curve was plotted using regression analysis.

Validation of the method: The developed method was validated in terms of linearity, accuracy, specificity, limit of detection, limit of quantification, intra-day and inter-day precission and repeatability of measurement as well as repeatability of sample application.

Analysis of the marketed formulations: 20 µL of sample solution of the marketed formulation was spotted on to the plate followed by development scanning. The analysis was repeated in triplicate. The content of the drug was calculated from the peak area recorded.

RESULTS AND DISCUSSION

To develop a precise, accurate and simple HPTLC method for the quantitative determination of simvastatin, different solvent systems were employed and the proposed chromatographic condition was found appropriate for the quantitative determination. The mobile phase consisting of toluene:ethyl acetate:formic acid (5:1.5:0.5 v/v/v) gave R_f value of 0.26 ± 0.01. Detection was carried out at 242 nm.

Good linear relationship was observed over a concentration range of 200-1000 ng/spot. Linearity was checked for three consecutive days for the same concentration range from the same stock solutions. Calibration curve was constructed by plotting concentration against peak area. The proposed method has been validated for estimation of simvastatin in bulk and tablet dosage forms using following parameters. The target analyte concentration of simvastatin was fixed at 200 µg/mL. The corresponding regression equation with correlation coefficient = 0.999, where y = 6.1224x - 304.22.

Accuracy of the method was checked by recovery study using standard addition method, known amounts of standard simvastatin were added into pre-analyzed samples and subjected to the proposed HPTLC method. The results of recovery studies and assay values are shown in Table-1. The results revealed no interference of excipients.

TABLE-1
RECOVERY STUDIES AND ASSAY OF SIMVASTATIN

Dosage form	Label claim (mg/tablet)	Amount added (mg)	Amount recovered* (mg ± SD)	% Recovery* ± SD	% Assay*
Brand-1	10	100	99.01 ± 0.006	99.81 ± 1.25	101.05 ± 1.29
Brand-2	10	100	100.24 ± 0.021	101.75 ± 0.47	101.45 ± 1.64

*Each value is a mean ± standard deviation of three determinations.

The intra-day precision was determined at concentration range of 200-600 ng/spot of simvastatin for three times on the same day while inter-day precision was determined daily over a period of 1 week. The low values of percentage relative standard deviation (% RSD) for intra- and inter-day variation data are given in Table-2, reveal that the proposed method is precise.

TABLE-2
PRECISION DATA OF SIMVASTATIN

Concentration (ng/spot)	Intra-day precision % RSD	Inter-day precision % RSD
200	100.41 ± 0.75	101.38 ± 1.61
400	101.10 ± 1.54	101.65 ± 0.49
600	101.60 ± 0.35	101.92 ± 1.74

RSD = Relative standard deviation.

Repeatability of sample application was assessed by spotting drug solution as spots on a TLC plate by five times followed by development of plate by recording the peak area for spots. The % RSD for peak area values of simvastatin was found

to be 0.56. Repeatability of measurement of peak area was determined by spotting drug solution on to a TLC plate and developing the plate. The separated spot was scanned for 5 times without changing the position of the plate and % RSD for measurement of peak area of simvastatin was found to be 0.42. 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.

The limit of detection (LOD) and limit of quantification (LOQ) of simvastatin were found to be 51 and 168 ng/spot, respectively. For ruggedness, study was carried out for two different parameters *i.e.*, days and analyst. The results of estimation by proposed method are very much similar under variety of conditions. The assay results of simvastatin in bulk and tablet dosage forms were comparable with the value of labelled claim. The various statistical parameters and the method validation parameters were shown in Table-3.

TABLE-3
METHOD VALIDATION PARAMETERS

Parameters	Value
Linearity range (ng/spot)	200-1000
Correlation coefficient (r)	0.999
Regression equation (y = mx + c)	
Slope (m)	6.1224
Intercept (c)	-304.22
Limit of detection (LOD)	51 ng/spot
Limit of quantification (LOQ)	168 ng/spot
R_f value	0.26 ± 0.01
Repeatability of application (n = 5)	0.56
Repeatability of measurement (n = 5)	0.42
Specificity	Specific
Robustness	Robust

The proposed HPTLC method for the determination of simvastatin is simple, rapid, precise, specific, accurate, sensitive and economical. The statistical analysis proved that method is selective and reproducible for the analysis of simvastatin in bulk and tablet dosage forms and can be used for the routine quality control analysis.

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