Determination of Simvastatin, Pravastatin Sodium and Rosuvastatin Calcium in Tablet Dosage Forms by HPTLC

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Simple and reproducible HPTLC method was developed for the separation and quantitation of simvastatin, pravastatin sodium and rosuvastatin calcium, cholesterol lowering agents in pharmaceutical dosage forms. The stationary phase used was precoated silica gel $60F_{254}$. The mobile phase used was a mixture of chloroform:methanol:toluene (6:2:2, v/v/v). The method has been completely validated and proved to be rugged. Calibration curves were linear over the studied ranges with correlation coefficients grater than 0.999. All the drugs were extracted from the respective tablets using methanol. The percentage recoveries ranged from 100 to 101 for simvastatin, 98 to 101 for pravastatin sodium and 98 to 102 for rosuvastatin calcium. The LOD for simvastatin, pravastatin sodium and rosuvastatin calcium were found to be 15, 9 and 8 ng/spot, respectively and LOQ were 200 ng/spot for simvastatin and 100 ng/spot for pravastatin sodium and rosuvastatin calcium. The method can be useful in the quality control of bulk manufacturing and tablet dosage forms.

Simvastatin[1] (SMV), 2,2-dimethyl butyric acid, 8-ester with (4R,6R)-6-[2-[(1S,2S,6R,8S,8aR)-1,2,6,7,8a-hexahydro-4hydroxy-2,6-dimethyl-1-naphthyl]ethyl]tetrahydro-4hydroxy-2H-pyran-2-one; pravastatin sodium[2] (PRV), sodium (+)-(3R,5R)-3,5-dihydroxy-7-[(1S,2S,6S,8S,8aR)-6-hydroxy-2-methyl-8-[(S)-2-methylbutyryloxy]-1,2,6,8,8a-hexahydro-1-naphthayl] heptanoate and rosuvastatin calcium (RSV), (3R,5S,6E)-7-[4-(fluorophenyl)-6(1-methyl ethyl)-2-[methyl(methyl sulfonyl)amino]-5-pyrimidinyl]-3,5-dihydroxy-6-heptenoic acid calcium salt, are selective and competitive inhibitors of HMG-CoA reductase. Literature survey revealed that HPLC[3-11] methods, electrophoresis[12] and UV spectroscopic[13] methods have been reported for the analysis of these drugs and metabolites in biological fluids. So far no HPTLC method was reported for the quantitative determination of these drugs in pharmaceutical dosage forms. The present paper describes development of simple, accurate, precise and reproducible method for the determination of drugs in tablet dosage form.

Working standards of simvastatin (SMV), pravastatin sodium (PRV) and rosuvastatin calcium (RSV) were procured as gift samples from Torrent Research Centre, Ahmedabad, India. Tablets containing 10 mg each of SMV (Simvastol®$_{10}$, Themis Medicare Ltd., Vapi), PRV sodium (pravator™, Slolus-Ranbaxy Labs Ltd., Dewas) and RSV calcium (rozavel 10, Sun Pharma. Industries, Jammu) were purchased form a local pharmacy. All chemicals used were of analytical grade. 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 were used during study.

To prepare standard and sample solutions, about 10 mg of SMV, PRV and RSV working standards were accurately weighed and dissolved separately in 100 ml of methanol to get concentration of 100 µg/ml and used as standard stock solution. The contents of 20 tablets each of SMV, PRV and RSV were ground to a fine powder. Weight equivalent to 10 mg each of SMV, PRV and RSV were transferred to three separate conical flasks and dissolved in methanol. The solutions were sonicated for 15 min. The extracts were filtered through Whatmann filter paper No. 41 and residue was washed with methanol. The extracts and washing were pooled and transferred to three separate 100 ml volumetric flask and volume was made with methanol to get about concentration of 100 µg/ml of each.

The chromatographic estimations were performed using stationary phase, precoated silica gel $60F_{254}$ aluminum sheets (10×10 cm, prewashed with methanol and dried in an oven at 50° for 5 min); mobile phase, chloroform: methanol: toluene (6:2:2, v/v/v); chamber and plate saturation time of 30 min; migration distance allowed was

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TABLE 1: RECOVERY DATA FOR THE PROPOSED HPTLC METHOD

Label claim mg/tablet	Amount added (%)	Total amount added (mg)	Amount recovered* (mg)±SD	% Recovery±SD	% RSD
Simvastatin 10	20	2.0	2.02±0.019	101.0±0.943	0.933
	40	4.0	4.001±0.05	100.02±1.34	1.34
	60	6.0	6.014±0.07	100.24±1.2	1.39
Pravastatin sodium 10	50	5.0	4.96±0.05	99.31±0.95	0.96
	100	10.0	10.17±0.07	101.68±0.75	0.73
	150	15.0	14.98±0.19	99.87±1.3	1.3
Rosuvastatin calcium 10	16.66	1.67	1.65±0.03	98.87±1.53	1.55
	33.33	3.33	3.312±0.05	99.47±1.38	1.39
	50.0	5.00	4.95±0.07	99.09±1.31	1.322

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

74 mm; wavelength scanning was done at 239 nm, 238 nm and 310 nm, respectively for SMV, PRV and RSV, keeping the slit dimension at 5×0.45 mm.

To prepare calibration curves, from standard solutions aliquots of 2, 5, 6, 8, 10 µl for SMV, 1, 2, 5, 8, 10 µl for PRV and 1, 2, 3, 4, 5, 6 µl for RSV were applied respectively on the TLC plates. The TLC plates were dried, developed and analyzed photometrically as described earlier. The developed method was validated in terms of linearity, accuracy, specificity, limit of detection, limit of quantification, intra-day and inter-day precision and repeatability of measurement as well as repeatability of sample application. For analysis of the formulations, 5 µl (SMV), 4 µl (PRV) and 3 µl (RSV) of the filtered solutions of the formulations were spotted on to the separate TLC plates followed by development scanning. The analysis was repeated in triplicate. The content of the drugs were calculated from the peak areas recorded.

To develop a precise, accurate and suitable HPTLC method for the quantitative determination of simvastatin, pravastatin and rosuvastatin, different solvent systems were employed and the proposed chromatographic condition was found appropriate for the quantitative determination. The mobile phase consisted of chloroform: methanol: toluene (6:2:2, v/v/v) and Rf value of SMV, PRV and RSV were found to be 0.59, 0.45 and 0.53, respectively. Detection was carried out at 239, 238 and 310 nm for SMV, PRV and RSV, respectively.

The proposed method has been validated for assay of SMV, PRV and RSV in bulk and tablet dosage forms using following parameters[14,15]. The target analyte concentration of all the three drugs was fixed as 100 µg/ml. Linear calibration plots were obtained over the calibration ranges tested, i.e., 200 to 1000 ng/spot, 100 to 1000 ng/spot and 100 to 600 ng/spot for SMV, PRV and RSV, respectively. The corresponding linear regression equations, with correlation coefficient ≥0.999, were y= 3.4091x+541.84; y= 4.9873x+131.07 and y= 4.7199x+47.80 for SMV, PRV and RSV, respectively. Linearity was checked for three consecutive days for the same concentration range from the same stock solutions.

Accuracy of the method was checked by recovery study using standard addition method, known amount of standard SMV, PRV and RSV were added into pre analyzed samples separately and subjected them to the proposed HPTLC method. Results of recovery studies are shown in Table 1. These studies were carried out at three levels i.e., multiple level recovery studies.

The intra- and inter-day precision were carried out at three different concentration levels, i.e., 400, 600, 800 ng/spot; 400, 500, 600 ng/spot and 200, 300, 400 ng/spot for the determinations of SMV, PRV and RSV, respectively. The low values of percentage relative standard deviation (% RSD) for intra-and inter-day variation as shown in Table 2 reveal that the proposed method is precise.

The limit of detection (LOD) represents the concentration of analyte that would yield a signal-to-noise ratio of 3[7]. The limit of quantification (LOQ) represents the concentration of the analyte that would yield a signal-to-noise ratio of 10[7]. The LOD and LOQ were found to be 15 and 200 ng/spot, respectively for SMV; 9 and 100 ng/spot, respectively for PRV and 8 and 100 ng/spot, respectively drug RSV.

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 SMV, PRV and RSV in bulk and tablet dosage forms were comparable with the value of labeled claim. The obtained results are given in Table 3.

TABLE 2: PRECISION DATA FOR THE PROPOSED HPTLC METHOD

Drug	Concentration (ng/spot)	Intra-day precision % RSD	Inter-day precision % RSD
Simvastatin	400	0.94	1.41
	600	1.04	1.25
	800	0.503	1.06
Pravastatin	400	0.872	1.64
sodium	500	1.06	1.3
	600	0.79	1.42
Rosuvastatin	200	0.53	1.29
calcium	300	0.66	0.95
	400	0.91	1.58

RSD=Relative standard deviation

TABLE 3: ANALYSIS OF MARKETED FORMULATIONS OF DRUG SMV, PRV, AND RSV BY PROPOSED HPTLC METHOD

Label claim (mg/tablet)	Amount found* mg	% of drug found*	% RSD
Simvastatin 10	9.85	98.48	1.139
Pravastatin 10	9.903	99.03	0.6003
Rosuvastatin calcium 10	9.92	99.24	1.52

*Each value is mean of three determinations

The proposed HPTLC method is a simple, rapid and accurate for the determination of simvastatin, pravastatin sodium and rosuvastatin calcium in bulk and tablet dosage forms. The statistical analysis proved that method is selective and precise and can be used for the routine quality control analysis.

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