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Gradient Stability Indicating RP-HPLC Method for Impurity Profiling of Simvastatin in Tablet Dosage Forms

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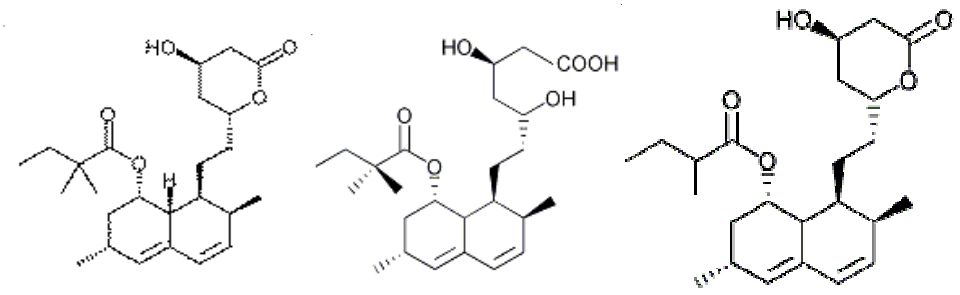
Gradient, reversed phase high performance liquid chromatographic (RP-HPLC) method was developed for quantitative estimation and validation of simvastatin impurities which are generated during formulation and storage of simvastatin in tablet dosage forms. The chromatographic separation was achieved on column intersil ODS (150 mm × 4.6 mm, 5 µm) by following gradient flow using mobile phase A and B containing 0.1 % phosphoric acid, acetonitrile in the ratio of 47:53 and 90:10, respectively. Flow rate was 1.0 mL/min. The photo diode array detector was operated at 238 nm. Forced degradation studies were performed on tablets powder which contain simvastatin using acid hydrolysis, base, peroxide, water and UV, thermal, sunlight, humidity degradations. The method was validated for specificity, linearity, precision, accuracy and limit of quantification. The degree of linearity of the calibration curves, the recoveries of simvastatin impurities, the limit of detection and quantification for the HPLC method were determined. The method was found to be simple, specific, precise, accurate and reproducible. The method was applicable for the quality control of commercial simvastatin tablets to quantify the drug and its related substances and to check the formulation content uniformity.

Key Words: Simvastatin, Impurity profiling, Reversed phase HPLC.

INTRODUCTION

Simvastatin[1] is chemically [(1S,3R,7R,8S,8aR)-8-[2-[(2R,4R)-4-hydroxy-6-oxo-oxan-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl]-2,2-dimethylbutanoate) is a hypolipidemic drug belonging to the class of pharmaceuticals called 'statins'. Simvastatin is a synthetic derivate of a fermentation product of *Aspergillus terreus*. It is used to control hypercholesterolemia (elevated cholesterol levels) and to prevent cardiovascular disease. The active ingredient simvastatin has five impurities (Fig. 1) in this process. These impurities may be present in small quantities and reduce the quality of simvastatin. Therefore separation and quantification of simvastatin and its impurities are quiet important not only for quality assurance but also for monitoring reactions involved in process development. Literature survey revealed that different UV[2,3], HPLC[4-6] and GC-MS[7,8] methods for determination of impurities of simvastatin were reported. The proposed method is simple, fast, accurate and precise for estimation of simvastatin impurities in tablets.



Simvastatin Hydroxy acid Lovastatin

Acetate ester Dehydro simvastatin

Fig. 1. Structures of simavastatin and its impurities

EXPERIMENTAL

The waters LC system with a photo diode array detector was used for method development and forced degradation studies. The output signal was monitored and processed using EMPOWER software.

The waters LC system, used for method validation was water alliance HPLC consisted of 2695 separation module, variable wavelength programmable UV detector waters 2996, EMPOWER software and intersil ODS 3V column 150 mm × 4.6 mm, 5 µm particle size was used.

Chromatographic conditions: The mobile phase A was made by first preparing a 0.1 % v/v orthophosphoric acid (1 mL of orthophosphoric acid into a 1000 mL of milli-Q-water). 470 volumes of this 0.1 % v/v orthophosphoric acid in water was mixed with 530 volumes of acetonitrile to yield mobile phase A.

The mobile phase B was made by first preparing a 0.1 % v/v orthophosphoric acid (1 mL of orthophosphoric acid into a 1000 mL of acetonitrile). 900 volumes of this 0.1 % v/v orthophosphoric acid in acetonitrile was mixed with 100 volumes of Milli Q water to yield mobile phase B.

Separations were performed on a intersil ODS 3V column 150 mm × 4.6 mm, 5 µm particle size. The mobile phase flow rate was 1.0 mL/min (gradient) with 27 ºC column temperature, 20 µL injection volume and UV detection at 238 nm. Run time of 75 min.

Gradient program:

Time	Mobile phase A%	Mobile phase B%
0.0	100	0
15.0	100	0
35.0	16	84
40.0	16	84
42.0	0	100
62.0	0	100
64.0	100	0
75.0	100	0

Procedure

Diluent preparation: Mix acetonitrile and phosphate buffer (dissolve 1.36 g of potassium dihydrogen phosphate in 1000 mL milli Q water and adjust pH of solution to 4.0 with phosphoric acid) in the ratio of 60:40. Adjust pH of the solution to 4.0 with orthophosphoric acid.

Standard solution: Weigh accurately 80 mg of simvastatin working standard in a 100 mL volumetric flask. Dissolve and dilute to volume with diluent. Transfer 5 mL of above solution to 50 mL volumetric flask and dilute to volume with diluent. Transfer 5 mL of above solution into a 100 mL volumetric flask, add 10 mL 0.1 % phosphoric acid and dilute to volume with diluent.

Sample preparation: The test solution was prepared by taking 10 tablets (400 mg) in 500 mL volumetric flask. Add 50 mL of 0.1 % phosphoric acid and sonicate. Dilute to volume with diluent and mix well to get 0.8 mg/mL of simvastatin.

Method validation: The precision of test method was evaluated by analyzing 6 samples prepared by spiking test preparation with simvastatin impurities blend solution to get 0.5 % of acetate ester, 1.0 % lovastatin, 1.5 % of simvastatin dimer, dehydro simvastatin and 2.5 % of hydroxy acid. The relative standard deviation was calculated for the response of each impurities.

Limit of detection for simvastatin impurities were established by identifying the concentration, which gives signal to noise ratio of about 3. Limit of quantification for simvastatin impurities were established by identifying the concentration, which gives signal to noise ratio of about 10. Precision and accuracy studies were also carried out at the limit of quantification (LOQ) level by injecting six individual preparations of impurities and calculated the % RSD of the area.

Linearity test solution for related substance method was prepared from the impurities stock % of the specification. Plotting the peak areas solution at 10 concentration levels from LOQ to 150 of impurities *versus* its corresponding concentration. Calculate the slope, Y-intercept and correlation coefficient for each impurity.

An accurate study of simvastatin impurities from spiked samples of simvastatin test preparation was conducted. Sample were prepared in triplicate by spiking test preparation with 25, 50, 75, 100 and 150 % to the target concentration of simvastatin

impurities blend solution. (0.5 % of acetate ester, 1.0 % lovastatin, 1.5 % of simvastatin dimer, dehydro simvastatin and 2.5 % of hydroxy acid). % Recoveries of individual impurities were calculated by external standard method.

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. According to ICH guidelines forced degradation studies were performed on simvastatin tablets powder to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress conditions of UV light, heat, acid, base, oxidation, water, sunlight and humidity to evaluate the ability of the proposed method to separate simvastatin from its degradation products.

RESULTS AND DISCUSSION

The present study was carried out to develop a simple, fast, accurate and precise HPLC method for analysis of simvastatin impurities in tablets. In this process hydroxy acid, lovastatin, acetate ester, dehydro simvastatin, dimer are potential impurities for simvastatin. The chromatographic separation was achieved by following gradient flow using mobile phase A and mobile phase B containing 0.1 % orthophosphoric acid and acetonitrile in the ratio of 47:53 and 90:10. A typical chromatogram was shown in Fig. 2. The retention time for simvastatin and its impurities hydroxy acid, lovastatin, acetate ester, dehydro simvastatin, dimer are about 25.93 and 16.35, 21.55, 34.16, 35.24, 59.66 min, respectively. The relative retention time for simvastatin and its impurities hydroxy acid, lovastatin, acetate ester, dehydro simvastatin, dimer were about 1.00 and 0.63, 0.83, 1.32, 1.36, 2.30, respectively. The results are given Table-1.

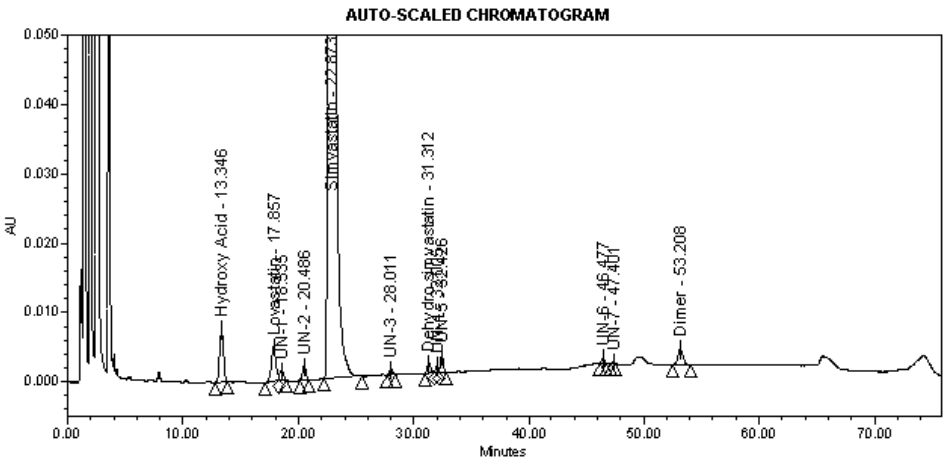


Fig. 2. Representative chromatogram for simvastatin and its impurities

Linearity results shows that good correlation existed between the peak area and concentration of impurities. The results are summarized in Table-2.

TABLE-1
RETENTION TIME, RELATIVE RETENTION TIME AND RELATIVE RESPONSE FACTOR'S OF SIMVASTATIN AND ITS IMPURITIES

Name of the impurity	Retention time	Relative retention time	Relative response factor
Hydroxy acid	16.35	0.63	0.90
Lovastatin	21.55	0.83	1.02
Simvastatin	25.93	1.00	–
Acetate ester	34.16	1.32	0.87
Dehydro simvastatin	35.24	1.36	0.83
Dimer	59.66	2.30	0.84

TABLE-2
LINEARITY DATA OF SIMVASTATIN IMPURITIES

Name of the impurity	Concentration range (µg/mL)	Coefficient of correlation (r)	Slope (b)	Intercept (a)
Hydroxy acid	0.199-34.849	0.99983	58791.20904	1187.9535
Lovastatin	0.160-14.040	0.99982	65040.32015	-3864.4606
Acetate ester	0.081-7.069	0.99980	57096.95345	-152.6960
Dehydro simvastatin	0.115-20.185	0.99987	54935.09170	-2635.6283
Dimer	0.717-14.947	0.99974	19406.18333	-3550.6907

The % RSD of response of hydroxy acid, lovastatin, acetate ester, dehydro simvastatin, dimer during precision and intermediate precision was found to be less than 15.0 %. The results are summarized in Table-3.

TABLE-3
PRECISION OF THE PROPOSED HPLC METHOD

Sample No.	Simvastatin impurities				
	Hydroxy acid	Lovastatin	Acetate ester	Dehydro simvastatin	Dimer
01	3.156	1.563	0.617	1.765	1.621
02	3.179	1.560	0.615	1.763	1.620
03	3.363	1.659	0.658	1.880	1.732
04	3.182	1.571	0.625	1.773	1.636
05	3.171	1.571	0.625	1.772	1.635
06	3.227	1.596	0.635	1.798	1.662
Average	3.213	1.587	0.629	1.792	1.651
% RSD	2.400	2.400	2.500	2.500	2.600

The recovery studies were performed from 25 to 150 % of target concentration (0.5 % of acetate ester,1.0 % lovastatin, 1.5 % of simvastatin dimer, dehydro simvastatin and 2.5 % of hydroxy acid). The % mean recoveries simvastatin in simvastatin tablets are satisfactory. The results are summarized in Table-4.

TABLE-4
ACCURACY OF HPLC METHOD FOR DETERMINATION OF SIMVASTATIN IMPURITIES IN TABLETS

Sample No.	Spike level (%)	Average 'µg/mL' added	Average 'µg/mL' found	Mean % recovery
Hydroxy acid	50	10.1661	10.8160	106.4
	100	20.3322	26.3680	110.0
	150	30.4983	31.4213	103.0
Lovastatin	50	4.0320	4.1760	103.6
	100	8.0640	8.5786	106.4
	150	12.0960	12.5120	103.4
Acetate ester	50	2.1351	2.2213	104.3
	100	4.2702	4.2740	100.1
	150	6.4053	5.8320	91.1
Dehydro simvastatin	50	5.7554	6.2180	108.1
	100	11.5108	12.4020	107.7
	150	17.2662	18.5013	107.1
Dimer	50	5.8890	6.5120	105.0
	100	11.7780	11.6300	98.8
	150	17.6670	17.7440	100.4

The limit of detection and limit of quantification for all impurities were established by signal to noise method and precision and accuracy as verified at LOQ level. The results are summarized in Table-5.

TABLE-5
LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION OF SIMVASTATIN IMPURITIES

Name of the impurity	Test name		Signal to noise ratio		% Impurity at LOQ level
	Limit of detection (µg/mL)	Limit of quantification (µg/mL)	LOD	LOQ	
Hydroxy acid	0.056	0.176	3.10	9.70	0.022
Lovastatin	0.056	0.168	2.81	10.20	0.021
Acetate ester	0.024	0.088	2.73	10.20	0.011
Dehydro simvastatin	0.032	0.120	2.70	9.70	0.015
Dimer	0.248	0.776	3.22	9.93	0.097

From forced degradation studies peak purity has been verified and purity angle is found to be less than purity threshold. The results are summarized in Table-6.

Conclusion

The RP-LC method developed for related substance determination of simvastatin in simvastatin tablets are precise, accurate, specific and selective. The method was validated shown satisfactory data for all the method validation parameters tested. The developed method is stability indicating and can be used for assessing the simvastatin impurities in simvastatin tablets.

TABLE-6
FORCED DEGRAGATION STUDIES OF SIMVASTATIN

Stress condition	Simvastatin		
	% Degradation	Purity angle	Purity threshold
Acid degradation	5.13	0.177	0.518
Base degradation	2.07	0.100	0.363
Peroxide degradation	2.43	0.137	0.434
Water degradation	1.47	0.123	0.412
UV degradation	0.52	0.137	0.422
Thermal degradation	3.54	0.101	0.353
Sunlight degradation	1.15	0.173	0.518
Humidity degradation	0.89	0.172	0.510

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