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A Stability-Indicating RP-LC method for the Determination of Related Substances in Simvastatin

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Abstract

A gradient reverse-phase liquid chromatographic (RP-LC) method was developed for the determination of related substances in Simvastatin drug substance. Simvastatin is a potent competitive inhibitor of HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis, can be used in treatment of hypercholesterolemia. Good chromatographic separation was achieved for all the listed known impurities along with some new process impurities from the peak of interest. The current USP HPLC method for the determination of related substances of simvastatin is not able to well separate some critical impurities that may form during the synthetic process or carried over from the starting materials. The chromatographic separation achieved on a ZORBAX column. The LC method employs solutions A and B as mobile phase. The solution A contains a mixture of phosphate buffer with pH 3 and Acetonitrile (50:50, v/v) and solution B contains pure Acetonitrile and the detection wavelength used 238 nm. The resolution between simvastatin and its closely eluting potential impurity, namely methyl ester of simvastatin was found to be greater than 2. Forced degradation studies were performed for Simvastatin bulk drug using acidic condition, basic condition, oxidation, heat and light. All the degradants products are well resolved in the developed method. The method was validated with respect to linearity, accuracy, precision, robustness and forced degradation studies prove the stability-indicating power of the developed method.

Key words: Related substances and degradants, Simvastatin, Stability-Indicating LC method

Introduction

Simvastatin a hypolipidemic drug belonging to the class of pharmaceuticals called statins, is chemically designated as [(1S, 3R, 7R, 8S, 8aR)-8-[2-[(2R, 4R)-4-hydroxy-6-oxo-oxan-2-y1l] ethyl]-3,7-dimethyl-1, 2,3,7,8,8a-hexahydronaphthalen-1-y1] 2,2-dimethylbutanoate. It is used for the treatment of hypercholesterolemia [1] . Following conversion of this lactone prodrug to its hydroxyl acid form, the compound is a potent competitive inhibitor of HMG-CoA reductase, the rate limiting enzyme in cholesterol biosynthesis [2] . Various analytical methods have been reported for the determination of simvastatin, which includes HPLC [3-6], HPLC-MS/MS [7], derivative spectrophotometry [8] and Volta metric techniques[9].

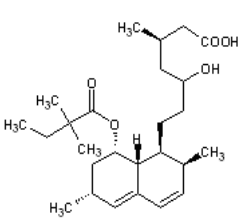
The current USP monograph method for the determination of related substances of simvastatin by HPLC [10] is not able to well resolve some potential impurities that may form during the synthetic process or carried over from the starting materials. It is felt necessary to develop a suitable stability-indicating LC method with an objective to resolve all USP listed impurities and also the other potential impurities which the current USP monograph method deficient to separate. The present work also deals with the forced degradation.

Materials and Methods

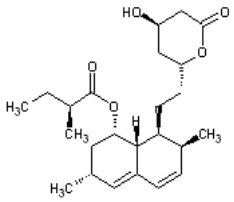
Experimental

2.1 Chemicals

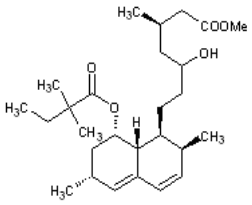
Samples of simvastatin, related impurities of diol lactone, hydroxy acid, lovastatin, methyl ester of simvastatin, acetate ester, methoxy impurity of simvastatin and anhydro simvastatin, except methylene derivative of simvastatin and dimer of simvastatin (Fig.1) were received from business unit of Dr. Reddy's Laboratories Limited, Hyderabad, India. HPLC grade acetonitrile was purchased from Merck, Germany. Analytical reagent grade potassium dihydrogen orthophosphate and orthophosphoric acid was purchased from RANKEM, India. High pure water was prepared by using Millipore Milli Q plus purification system.



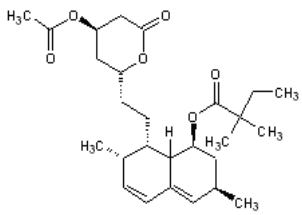
Hydroxy acid impurity



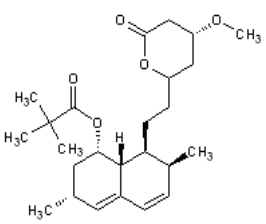
Lovastatin impurity



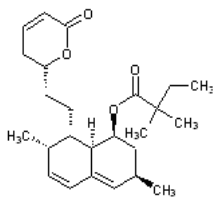
Methyl ester of Simvastatin



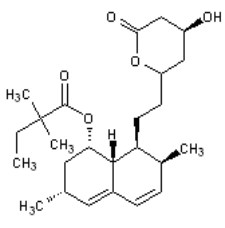
Acetate ester of Simvastatin



Methoxy impurity of Simvastatin



Anhydro simvastatin impurity



Simvastatin

2.2 Equipment

The LC system used for method development and forced degradation studies were Waters 2695 series LC system with a diode array detector. The out put signal was monitored and processed using Millenium32 chromatography manager software on Pentium computer from Digital Equipment Co. Shimadzu Prominence LC system with a diode array detector also employed in method development. The out put signal was monitored and processed using LC solution software on Pentium computer from Digital Equipment Co. The LC system, used for method validation was Agilent 1100 series LC system with a diode array detector. The out put signal was monitored and processed using Chemstation software, Agilent Technologies USA, on Pentium computer from Digital Equipment Co.

2.4 Preparation of solutions

A stock solution of simvastatin (500 µg mL^{-1}) was prepared by dissolving appropriate amount in the diluent. Stock solution was further diluted with diluent to obtain a standard solution of 0.25 µg mL^{-1} for related substance determination. A stock solution of each impurity diol lactone, hydroxy acid, lovastatin, methyl ester, acetate ester, methoxy impurity of simvastatin and anhydro simvastatin was prepared separately in diluent at 500 µg mL^{-1} concentration.

3. Investigations, Results and discussion

3.1 Method development and optimization

Number of attempts was made to develop a suitable stability-indicating, precise, linear and accurate RP-LC method for Simvastatin. *(*3R, 5R)-methyl 7-((1S,2S,6R,8S,8aR)-8-(2,2-dimethylbutanoyloxy)-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl)-3,5-dihydroxy-heptanoate (methyl ester) and (1S,3R,7S,8S,8aR)-8-(2-((4R)-4-methoxy-6-oxotetrahydro-2H-pyran-2-yl)ethyl)-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate (methoxy impurity of simvastatin) new impurities were observed about 0.1% area level in simvastatin bulk samples. The above two impurities namely methyl ester and methoxy impurity of simvastatin were not able to well resolve under USP recommended chromatographic conditions. The major objective during the development of the chromatographic method was to achieve the separation of closely eluting impurities set of simvastatin and methyl ester of simvastatin, similar way acetate ester of simvastatin, methoxy impurity of simvastatin and anhydro simvastatin impurities were co-eluted by using different stationary phases like C18, C8 and polar amide functional group of RP and different mobile phases phosphate buffer with different pH (3-6), Tri fluoro acetic acid (TFA), Triethyl amine (TEA) additives and using organic modifiers like acetonitrile and methanol. pH of the buffer has played a significant role in achieving the separation of the related impurities, tailing of the impurities, simvastatin and solution stability of simvastatin.

The chromatographic separation was achieved on a ZORBAX SB C18, 150 mm x 4.6mm, 3.5 µm column by using solutions A and B as mobile phase. The solution A contains a mixture of 20 mM phosphate buffer pH 3.0: acetonitrile (50:50, v/v) and solution B contains pure acetonitrile. The column temperature (25°C) has improved the peak shape of simvastatin. In the optimized conditions, simvastatin and methyl ester of simvastatin were separated with a resolution of greater than 2 and the typical retention times for diol lactone, hydroxy acid, lovastatin, methyl ester, acetate ester, methoxy impurity of simvastatin, anhydro simvastatin and simvastatin were about 3.9, 10.4, 13.1, 16.9, 23.3, 24.1, 24.6 and 16.0 min respectively (Fig.2). The system suitability results were given in Table-2 and the developed LC method was found to be specific for simvastatin and its all related potential impurities. The various trials performed during the method development with conclusions were captured in Table-1.

Table-1. Results of various trials

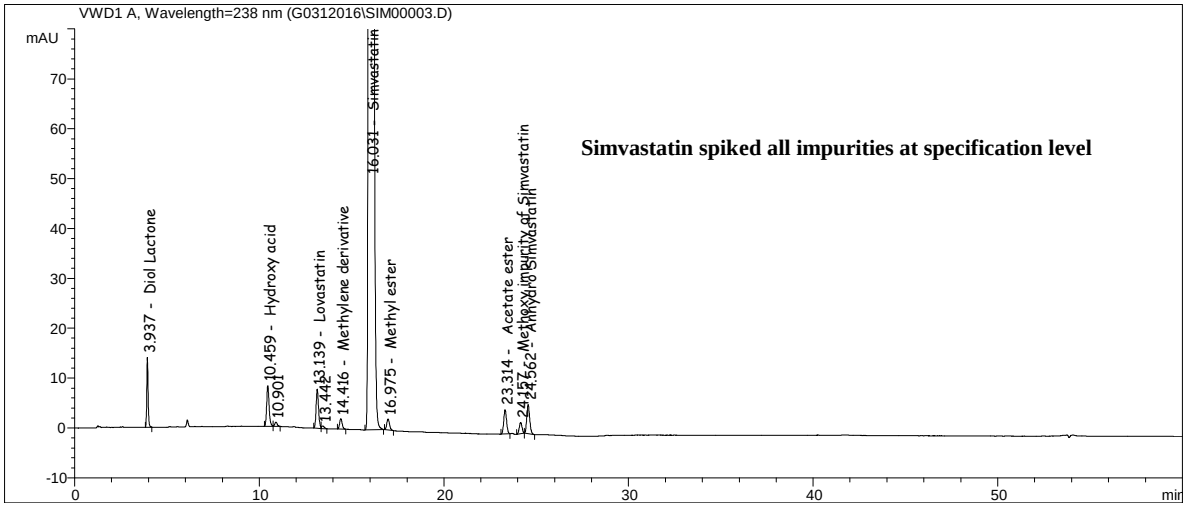
Trial No.	HPLC conditions	Retention factor (*k*)	Remarks
1	Column: Develosil ODS MG-3 33 x 4.6 mm, 3 µm Mobile phase -A: 0.1%H3PO4: Acetonitrile (50:50, v/v) Mobile phase –B: Acetonitrile Flow rate: 3.0 mL min^{-1} Gradient program: Time/%Mobile phase-B 0/0, 4.5/0, 4.6/5,8/75,11.5/75,11.6/0,15/0 Column temperature: 25 °C	Simvastatin: 3.1 Methyl ester: 3.1 Acetate ester 6.0 Methoxy impurity 6.2	Simvastatin & methyl ester, acetate ester & methoxy impurity co--eluted.
2	Column: Develosil ODS MG-3 33 x 4.6 mm, 3 µm Mobile phase -A: 0.1%H3PO4: Acetonitrile (50:50, v/v) Mobile phase –B: Acetonitrile Flow rate: 3.0 mL min^{-1} Gradient program: Time/%Mobile phase-B 0/0, 4.5/0, 4.6/5,8/75,11.5/75,11.6/0,15/0 Column temperature: 50 °C	Simvastatin: 2.8 Methyl ester: 2.8 Acetate ester 5.2 Methoxy impurity 5.3	Simvastatin & methyl ester, acetate ester & methoxy impurity co--eluted.
3	Column: Symmetry C8 150 x 4.6 mm, 5 µm Mobile phase -A: : 0.05% TFA in aqueous (v/v) Mobile phase –B: 0.05% TFA in acetonitrile Flow rate: 1.0 mL min^{-1} Gradient program: Time/%Mobile phase-B 0/20, 25/80, 34/80, 35/20 &40/20 Column temperature: 25 °C	Simvastatin: 22.3 Methyl ester: 23.4 Acetate ester : 29.1 Methoxy impurity 29.8	R_S between the simvastatin & methyl ester: 1.8 acetate ester & methoxy impurity :1.1
4	Column: Symmetry shield RP18 150 x 4.6 mm, 5 µm Mobile phase -A: : 0.05% TFA in aqueous (v/v) Mobile phase –B: 0.05% TFA in acetonitrile Flow rate: 1.0 mL min^{-1} Gradient program: Time/%Mobile phase-B 0/20, 25/80, 34/80, 35/20 & 40/20 Column temperature: 25 °C	Simvastatin: 18.2 Methyl ester:19.5 Acetate ester : 24.5 Methoxy impurity 25.1	R_S between the simvastatin & methyl ester: 1.6 acetate ester & methoxy impurity :1.1
5	Column: Symmetry shield RP18 150 x 4.6 mm, 5 µm Mobile phase -A: : 0.05% TFA in aqueous (v/v) Mobile phase –B: 0.05% TFA in acetonitrile Flow rate: 1.0 mL min^{-1} Gradient program: Time/%Mobile phase-B 0/20, 25/80, 34/80, 35/20 & 40/20 Column temperature: 40 °C	Simvastatin: 17.5 Methyl ester: 18.8 Acetate ester :23.8 Methoxy impurity 24.6	R_S between the simvastatin & methyl ester: 1.7 acetate ester & methoxy impurity :1.43
6	Column: Zorbax SB C18 150 x 4.6 mm, 3.5 µm Mobile phase -A: : 0.05% TFA in aqueous (v/v) Mobile phase –B: 0.05% TFA in acetonitrile Flow rate: 1.0 mL min^{-1} Gradient program: Time/%Mobile phase-B 0/20, 25/80, 34/80, 35/20 & 40/20 Column temperature: 25 °C	Simvastatin: 17.9 Methyl ester: 19.2 Acetate ester :24.1 Methoxy impurity 25.2	R_S between the simvastatin & methyl ester: 2.5 acetate ester & methoxy impurity :1.5

Table-2. System suitability test results in optimized conditions

Compound (n=3)	USP Resolution (Rs)	USP Tailing factor (T)	No. of theoretical plates (*N*) Tangent method
Diol lactone	-	1.2	14669
Hydroxy acid	35.9	1.2	31912
Lovastatin	11.7	1.1	49753
Simvastatin	11.8	1.2	60351
Methyl ester	3.4	1.1	59130
Acetate ester	23.4	1.1	113492
Methoxy impurity	3.0	1.1	114466
Anhydro Simvastatin	1.5	1.1	121385

n = Number of determinations

Fig.2: Typical impurities spiked chromatograms



Time (min)

3.2 Optimized Chromatographic conditions

ZORBAX SB C18 column with 150 mm length, 4.6 mm ID and 3.5 µm particle size used as chromatographic column. The gradient LC method employs solutions A and B as mobile phase. The solution A contains a mixture of phosphate buffer with pH 3.0 and Acetonitrile (50:50, v/v) and solution B contains pure Acetonitrile. The HPLC gradient flow program was set as time /flow: 0/1.0, 25/1.0, 26/2.0, 59/2.0, 60/1.0 and 65/1.0. The gradient program of mobile phase composition was set as time/ % solution B: 0/0, 25/60, 26/60, 59/60, 60/0 and 65/0. The column was maintained at 25°C and the detection was monitored at a wavelength of 238 nm. The injection volume was 10 µL. A mixture of 10 mM of potassium dihydrogen orthophosphate buffer pH 4.0 adjusted with dilute phosphoric acid: acetonitrile. (40:60, v/v) was used as a diluent.

3.3 Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities [11]. The specificity of the developed LC method for Simvastatin was carried out in the presence of its related potential impurity namely diol lactone, hydroxy acid, lovastatin, methyl ester, acetate ester, methoxy impurity of simvastatin and anhydro simvastatin.

Forced degradation studies were performed for Simvastatin bulk drug to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress conditions of light as per ICH Q1B Step 4 version 1996, heat (60 °C), acid (0.01 N HCl), base (0.005 N NaOH) and oxidation (3% H_2O_2) to evaluate the ability of the proposed method to separate Simvastatin from its degradation products. For heat and light studies, time period for stress was 3 days where as for acid, base and oxidation, it was 48 h. To check and ensure the homogeneity of Simvastatin peak in the stressed sample solutions, diode array detector was employed.

3.4. Method validation

3.4.1 Precision

The precision of the related substance method was checked by injecting six individual preparations of simvastatin spiked with 0.40% of hydroxy acid, acetate ester, anhydro simvastatin, spiked with 1.0% of lovastatin and along with spiked 0.15% level of diol lactone, methyl ester and methoxy impurity of simvastatin with respect to simvastatin analyte concentration (500 μg mL^{-1}). Intermediate precision of the above method was confirmed by different analyst, using a different make instrument in the same laboratory.

The % RSD of %w/w of each related impurity in related substance method precision study was with in 3.0%. The % RSD of %w/w of the above-related impurities results obtained in intermediate precision study was also below 5.0 %, representing good precision of the developed method.

3.4.2 Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ were estimated at a signal-to-noise ratio of 3:1 and 10:1 respectively for each related impurity by injecting a series of dilute solutions with known concentration [12]. Precision study was also carried at the LOQ level by injecting six individual preparations of each related impurity and calculating the % RSD for the area.

The limit of detection (LOD) of diol lactone, hydroxyl acid, lovastatin, methyl ester, acetate ester of simvastatin, methoxy impurity of simvastatin, anhydro simvastatin and simvastatin were 0.002, 0.003, 0.003, 0.003, 0.005, 0.005, 0.003 and 0.003 % for 10 µL injection volume. The limit of quantification (LOQ) of diol lactone, hydroxyl acid, lovastatin, methyl ester, acetate ester of simvastatin, methoxy impurity of simvastatin, anhydro simvastatin and simvastatin were 0.005, 0.010, 0.010, 0.010, 0.015, 0.015, 0.010 and 0.010 % for 10 µL injection volume. The method precision for each impurity at LOQ level was below 6.5 %.

3.4.3 Linearity

Linearity test solutions for related substance method were prepared by diluting the each related impurity stock solution at five concentration levels from LOQ to 200% of the specification level. The calibration curve was drawn by plotting the peak area of each related impurity versus its corresponding concentration.

Linear calibration plot for each related impurity corresponding linear regression equations were $y = 174.3918 x + 0.5453$, $y = 139.1419 x + 1.3196$, $y = 157.2044 x + 4.8988$, $y = 150.6812 x – 0.0204$, $y = 131.9943 x + 2.2060$, $y = 126.8666 x + 0.7582$, $y = 157.5548 x + 2.5539$ with correlation coefficients greater than 0.997. Linearity was checked for related substance method over the same concentration range for three consecutive days. The % RSD values of the slope and intercept of the calibration curves were 2 and 9 respectively. The

results show that an excellent correlation existed between the peak area and concentration of each related impurities of Simvastatin.

3.4.4 Accuracy

Standard addition and recovery experiments were conducted to determine accuracy of the related substance method for the quantification of each related impurities in bulk drug samples. The study was carried out in triplicate at 50, 100 and 150 percent of each related impurity at specification level of the simvastatin analyte concentration (500 µg mL^{-1}). The percentage recovery of the above related impurities of simvastatin in bulk drugs samples were ranged from 88.7 to 105.6. HPLC chromatogram of spiked with all related impurities at specification level (of the analyte concentration 500 µg mL^{-1}) in simvastatin bulk drug sample were shown in Fig. 2

3.4.5 Robustness

To determine the robustness of the developed method experimental conditions were deliberately altered and the resolution between simvastatin and methyl ester of simvastatin was evaluated. To study the effect of flow rate on the resolution, mobile phase gradient was changed by 0.1 units from initial flow rate while the other mobile phase components were held constant as stated in section 3.3. The effect of percent organic strength on resolution was studied by varying acetonitrile percentage from - 3 to + 3% while the other mobile phase components was held constant as stated in section 3.3. The effect of column temperature on resolution was studied at 20 °C and 30 °C instead of 25 °C while the other mobile phase components were held constant.

In all the varied chromatographic conditions (flow rate, percentage organic strength, buffer pH, detector wavelength and column temperature) the resolution between simvastatin and methyl ester was greater than 2.0, illustrating the robustness of the method.

3.4.6 Solution stability and mobile phase stability

The solution stability experiments data confirms that simvastatin sample solutions used for during related substances determination were stable for at least 48 h in refrigerator conditions, but at room temperature (25 ± 2 °C) condition stable up to 12 h. The mobile phase stability experiments data confirms that simvastatin sample solutions used for during related substance determination were stable up to 48 h at room temperature (25 ± 2 °C).

3.4.7 Results of Forced degradation experiments

No degradation was observed for simvastatin during stress conditions like light, heat, and oxidation except in acid and base hydrolysis. Simvastatin was degraded into hydroxy acid during acid and base hydrolysis and it was confirmed by co-injection with pure hydroxy acid. Peak purity test results confirm simvastatin is homogeneous in all the stress conditions tested and more over the un affected resolution in the presence of all related impurities and degradation products confirms the stability indicating power of the method. The summary of forced degradation studies were presented in Table 3.

Table 3. Summary of forced degradation results

Stress condition	Time	% Purity of Active substance	% total degradation impurities	Remarks
Acid hydrolysis (0.01N HCl)	3 h	93.3	6.74	Simvastatin was degraded into hydroxyl acid
Base hydrolysis (0.005N NaOH)	1 h	87.0	13.0	Simvastatin was degraded into hydroxyl acid
Oxidation (3% H_2O_2)	48 h	98.1	1.86	Degradation products formation rate is very slow
Thermal (60 °C)	5 days	99.5	--	No degradation products formed
Light	11 days	99.5	--	No degradation products formed

Conclusion

The new RP-LC method developed for quantitative determination of related substances in simvastatin is precise, accurate and stability-indicating. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method is stability-indicating and can be used for assessing the stability of bulk samples of simvastatin. The developed method is superior to the current USP monograph method and able to well separate critical impurities such as methyl ester of simvastatin and methoxy impurity of simvastatin.

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