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Original Article

A STABILITY-INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF SIMVASTATIN AND NIACIN IN A COMBINED DOSAGE FORM

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ABSTRACT

Objective: To develop a simple, selective and rapid stability-indicating reverse phase high performance liquid chromatography (RP-HPLC) method and validate as per ICH guidelines for simultaneous determination of simvastatin and niacin in a combined dosage form.

Methods: The chromatographic separation of the two cholesterol lowering drugs were achieved using Inertsil CN (5 µm, 250 mm x 4.6 mm i. d. column), maintained at 30 °C throughout the analysis. The drugs were separated in isocratic elution mode with a mobile phase of 0.1% acetic acid buffer-methanol (50:50, v/v) at a flow rate of 1.0 mL/min and a detection wavelength of 237 nm using a UV-PDA detector.

Results: The linearity and range for niacin and simvastatin were 0.05 to 0.150 mg/mL (R^2> 0.9999) and 0.004 to 0.012 mg/mL (R^2> 0.9992), respectively. Mean recoveries observed for niacin and simvastatin were 99.36% and 99.93%, respectively. The precision of the method obtained was 99.66% for niacin and 99.34% for simvastatin with a relative standard deviation less than 2%. The lower degree of % RSD that was obtained for intermediate precision has proved that the method is robust and rugged.

Conclusion: A simple and rapid stability-indicating RP-HPLC method was developed and validated for simultaneous determination of niacin and simvastatin in a combined dosage form and hence, it can be used in the quality control analysis of an active pharmaceutical ingredient and pharmaceutical dosage form.

Keywords: Simvastatin, Niacin, RP-HPLC, Stability, Validation.

INTRODUCTION

Statins, the 3-hydroxy-3-methyl-gultaryl-coenzyme A (HMG-CoA) reductase inhibitors are the most effective cholesterol lowering drugs which catalyse the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in the biosynthesis of cholesterol [1]. There are currently six statins approved for treatment in the United States, which are simvastatin, pravastatin, lovastatin, fluvastatin, rosuvastatin and atorvastatin [2]. Simvastatin (Fig. 1), synthetically-derived from a fermented product of *Aspergillus terreus,* is the most prescribed drug in clinical practice and is chemically known as {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, 8-ahexahydrona-phtha-len-1-yl]2, 2-dimethy-butanoate [3].

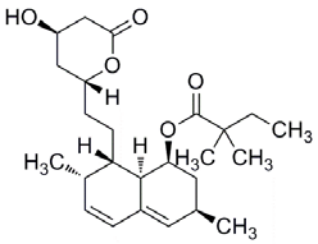

Fig. 1: Chemical structure of simvastatin

The successful discovery of the niacin-protein-coupled receptor HM74 (GPR109b) and HM74A (GPR109A) has shown a great potential for the development of new drugs to treat multiple lipid abnormalities [3], thus extending the scope of lipid-related therapy. Niacin (Fig. 2), or also known as nicotinic acid, is a water-soluble vitamin belonging to the vitamin B complex [4] and has been reported to be one of the most effective drugs in elevating high density lipoprotein cholesterol (HDL-C) levels, reducing triglyceride and low density lipoprotein cholesterol (LDL-C) levels, and improving lipoprotein particle size [5-7].

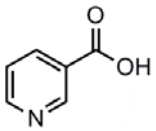

Fig. 2: Chemical structure of niacin

A new drug developed by Abbott Laboratories called Simcor is a combined dosage form of niacin extended-release (ER) coated with simvastatin, which has been approved in the United States recently for the treatment of hypercholesterolemia and dyslipidaemia [8]. Several clinical studies on the safety and efficacy of this combined drug have been conducted in patients with dyslipidaemia, and the results demonstrated remarkable improvement of HDL-C, triglyceride and lipoprotein levels in patients [9-13]. Furthermore, it has been proven that the combined drug of niacin extended release (ER) and simvastatin has synergistic effect, compared to single dose treatments or with other combinations such as ezetimibe, which in turns delay or relapse the progress of atherosclerosis development [7] and [8]. According to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH, Q3) guidelines [14], quality control analysis with a validated analytical method is necessary for monitoring active pharmaceutical ingredient (API), as well as known and unknown impurities in stability studies of drug substance and drug products [14].

Several HPLC methods have been reported for individual analysis of simvastatin and niacin in API, dosage forms or biological fluids [15-20] and it is also officially described in pharmacopoeias [4, 21]. However, no HPLC method is provided in pharmacopoeias for simultaneous determination of simvastatin and niacin [4, 21, 22].

Since separate analysis of drugs is time-consuming, incurs high costs and delays both production and batch release, simultaneous determination will likely reduce these effects in the laboratory. Hence, the main objective of the present work is to establish a stability-indicating HPLC method for simultaneous determination of simvastatin and niacin in combined dosage form. The validated method would be applicable in both formulation development and routine quality control analysis.

MATERIALS AND METHODS

Chemicals and reagents

The working standard of niacin was purchased from Sigma Aldrich (St. Louis, MO, USA). Raw material of niacin was obtained from Merck (Darmstadt, Germany). Excipients, raw material and working standard of simvastatin were obtained as gift samples from Innovax Sdn. Bhd, R&D Centre of CCM Pharmaceuticals, Malaysia. Simcor, a combination product of simvastatin and ER of niacin (Label claim: Niacin ER 500 mg and Simvastatin 40 mg), was procured from Abbott Laboratories (Illinois, USA). Methanol (HPLC grade), acetonitrile (HPLC grade), hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from Fisher Scientific (Leics, UK), glacial acetic acid and hydrogen peroxide (H_2O_2) were purchased from Merck (Darmstadt, Germany) and uracil was purchased from Acros (Leicestershire, UK).

Instruments

The HPLC analysis was performed using Waters Alliance 2695 Separation Module equipped with Alliance autosampler, 2996 photodiode array detector PDA, column heater, and Empower 2 software. The Inertsil CN (5 µm, 250 mm x 4.6 mm, i. d. column) was used as the column in this study. All mobile phase solutions were filtered by vacuum filter assembly using nylon membrane filters (47 mm, 0.22 µm) and degassed ultrasonically by sonicator (Branson 5510). XS 204 analytical balance (Mettler Toledo) were used to measure the mass of the standards and samples. As for the forced degradation studies, stability chamber (MMM medcentre), oven (Shelab), refrigerator (Samsung SR-569) and UV lamp (INC, USA) were used.

Method

Chromatographic conditions

The method development was initiated with Inertsil C8 (5 µm, 150 mm x 4.6 mm & 250 mm x 4.6 mm, i. d. column), Inertsil ODS C18 (5 µm, 150 mm x 4.6 mm & 250 mm x 4.6 mm, i. d. column), Inertsil Silica (5 µm, 250 mm x 4.6 mm, i. d. column), Phenomenex Phenyl (5 µm 150 mm x 4.6 mm, i. d. column) and Inertsil CN (5 µm, 250 mm x 4.6 mm, i. d. column). The optimum chromatographic separations were achieved by using an Inertsil CN, (5 µm, 250 mm x 4.6 mm i. d. column) (GL Sciences Inc, Tokyo, Japan), maintained at 30 °C using a column heater. The optimal composition of mobile phase was determined to be 0.1% acetic acid buffer-methanol (50:50, v/v), eluted at a flow rate of 1.0 mL/min. The injection volume used was 10 µL and the wavelength detector was set at 237 nm. For development analysis, the PDA was scanned ranging from 210 to 400 nm and the data acquisition was performed using Empower 2 chromatographic workstation software. The mobile phase was used as a diluent for standard and sample preparations.

Buffer preparation

Acetic acid buffer was prepared by mixing 1 mL of concentrated glacial acetic acid in 1 L of water and 500 mL of this buffer was then added into 500 mL of methanol in order to obtain the optimal mobile phase composition.

Mobile phase preparation

Mobile phase was prepared by mixing 0.1% acetic acid buffer and methanol in the ratio 50:50, v/v and later it was sonicated for 30 minutes for removal of air bubbles.

Preparation of standard solution

Twenty milligrams of simvastatin reference standard was accurately weighed and transferred into a 100 mL volumetric flask while 5 mg of niacin reference standard was weighed and transferred into a 50 mL volumetric flask followed by addition of 2 mL of simvastatin standard stock solution into the same flask. The final concentrations were 0.008 mg/mL and 0.1 mg/mL of simvastatin and niacin, respectively. The standard was filtered using 0.45 µm polyvinylidene difluoride (PVDF) syringe filter. An aliquot was collected in a vial for HPLC analysis and the standard was analysed based on the condition mentioned in the chromatographic conditions section.

Preparation of sample solution

Twenty tablets of Simcor were weighed to determine the average tablet weight and it was further crushed into powder using a mortar and pestle. An amount of samples equivalent to 4 mg of simvastatin and 50 mg of niacin was weighed and transferred into a 100 mL volumetric flask and made up to volume with diluent. Further dilutions were made to obtain final concentrations of 0.008 mg/mL and 0.1 mg/mL of simvastatin and niacin, respectively. The samples were filtered using 0.45 µm PVDF syringe filter. An aliquot was collected in a vial for HPLC analysis. The samples were analysed based on the conditions mentioned in the chromatographic conditions section.

Forced degradation studies

To evaluate the stability indicating properties of the developed HPLC method, forced degradation studies were carried out in accordance with the ICH guidelines. The drug substance, finished products and placebos of simvastatin and niacin were exposed to acidic, basic, neutral, oxidative, thermal and photolytic stress conditions.

All solutions used in forced degradation studies were prepared by weighing drug substances, drug product and placebos respectively followed by dissolving in small volume of methanol before further diluting with diluent (control), distilled water, 1.0 N HCl, 1.0 N NaOH and 0.3% H_2O_2.

The drug substances, drug product and placebo that were subjected to stress under the acidic, basic and oxidation degradation conditions were placed in controlled temperature oven at 50 °C for 24 hours while those drug substances, drug product and placebo treated with water was placed in oven at 90 °C for 24 hours. On the other hand, photolytic stress condition was carried out on UV exposure for 24 hours. Control sample was kept in refrigerator for comparison.

After 24 hours of stress studies under various conditions and temperatures, the stock degradant solutions were further diluted to obtain final concentrations of 0.008 mg/mL and 0.1 mg/mL of simvastatin and niacin, respectively. All the exposed drug substances, drug product and placebo under the degradation conditions were then analysed after 24 hours. The degradation studies were continued till 20% - 80% of degradation occured in both simvastatin and niacin in order to assess the potential impurities that developed during the stability study of formulation drugs.

Method validation

The developed RP-HPLC method was validated as per ICH guidelines [23] with respect to parameters such as specificity, linearity and range, accuracy, precision and robustness.

Specificity

According to ICH and United States Pharmacopeia (USP) guidelines, specificity is performed to demonstrate the ability to assess potential impurities, degradants, excipients and matrix that may develop during the stability studies of the drug product and the ability of the developed method to separate simvastatin and niacin from its potential impurities. The specificity of the method was established through the forced degradation studies using API and tablets of simvastatin and niacin.

Linearity and range

Linearity and range of method was assessed by performing regression analysis of the linear plot of peak response over concentration of simvastatin and niacin in the range of 50% to 150%

of the targeted working concentrations (0.008 mg/mL of simvastatin and 0.1 mg/mL of niacin). The linear correlation coefficients, y-intercepts and slopes of the calibration curve were then used for data evaluation [23].

Accuracy

Accuracy of an analytical procedure expresses the closeness of agreement between the theoretical value and actual value. The accuracy of sample preparation was assessed by performing in triplicate recovery experiments with externally spiked known amount of drug substances to placebos. It was prepared at three levels of analyte concentrations ranging from 50% to 150% of the targeted concentration of 0.008 mg/mL of simvastatin and 0.1 mg/mL of niacin. Accuracy experiments were carried out as described by Guzik *et al* (2012) [15].

Precision

The precision of stability-indicating RP-HPLC method expresses the closeness of agreement within a series of analysis obtained from multiple samples from the same pool of homogenous sample under the developed method. The precision was determined on two different levels: Repeatability and intermediate precision [23].

Repeatability

Repeatability expresses the precision under the same condition and was measured using standards and drug product of simvastatin and niacin prepared at targeted working concentrations (0.008 mg/mL of simvastatin and 0.1 mg/mL of niacin). The standard and drug products were analysed in six replicates on the same day and mean % assay and % relative standard deviation (RSD) were then calculated.

Intermediate precision

Intermediate precision expresses the potential variations within laboratories. The study was conducted based on the same procedure as repeatability by using standard and drug products of simvastatin and niacin prepared at targeted working concentration. The standard and drug products were analysed in six replicates using different instruments, different batch of columns by different analysts on different days. The mean % assay and % RSD were then calculated.

Robustness

Robustness expresses the capacity of the analytical method to remain unaffected by small but significant changes in chromatographic conditions and provides an assurance of its reliability during quality control analysis. The assay of sample preparations (as per procedure in repeatability) was studied by changing the mobile phase composition (±2%) and % of acetic acid (±2%) in the mobile phase. A series of system suitability parameters was established to evaluate the robustness [23].

Stability studies

Stability studies on standard and drug products of simvastatin and niacin were carried out by keeping the solutions at room temperature for a predefined time. All the test solutions used in the precision test were then injected after being kept for 24 hours at room temperature inside the HPLC auto sampler. The analysis was conducted with the same mobile phase and chromatographic system as used in the precision test. The test solutions were analysed by the optimised HPLC method relative to freshly prepared standards.

RESULTS AND DISCUSSION

Method development

The physicochemical properties including solubility and polarity of simvastatin and niacin differ significantly. Thus, it was very challenging to develop a HPLC method that could analyse both drugs simultaneously. The absorption maxima of simvastatin was found to be 230, 238 and 246 nm, whereas niacin showed absorption maxima at 215 and 262 nm [24].

As part of method development efforts, various silica-based reverse-phase columns of different dimensions were checked for system suitability [25]. A cyano-propyl (CN) bonded stationary phase resulted in a good separation without compromising the system suitability parameters. Several experiments were carried out in order to optimise the chromatographic conditions.

The optimum LC separation was achieved by Inertsil CN (5 µm, 250 mm x 4.6 mm, i. d. column), maintained at 30 °C, with isocratic elution of mobile phase consisting of 0.1% acetic acid and methanol (50:50 v/v), and at flow rate of 1.0 mL/min. As the peak area and precision of six injections for both substances at 237 nm was found to be within 2%, this wavelength was selected for further analysis. The developed method was simple and rapid with simvastatin and niacin having retention times of 10.78 and 6.87, respectively (Fig.3 and Fig. 4). The tailing factor for simvastatin and niacin was found to be less than 1.5 which meets the general requirements of system suitability [4] and [25].

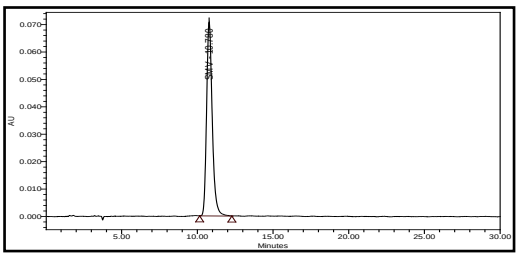

Fig. 3: Typical chromatogram for simvastatin

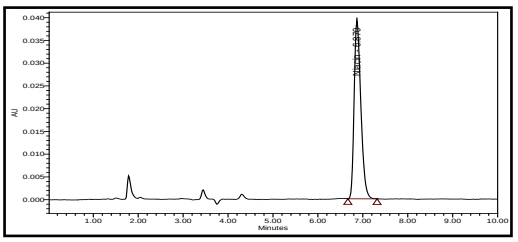

Fig. 4: Typical chromatogram for niacin

Forced degradation studies

In order to ascertain whether the analytical method and assay were stability-indicating, forced degradation studies were performed on placebo, simvastatin, niacin and their combination drug product under acidic, basic, neutral, oxidative, thermal and photolytic conditions. Peak purity and resolution of drug substances and their impurities were monitored for all the samples that were subjected to forced degradation.

Decomposition level of 20% to 80% of the drug substances was achieved within 24 hours of forced degradation under acidic, basic and oxidative stress conditions. Placebos that were subjected to stress did not exhibit any degradation even after exposure for prolonged duration.

Simvastatin completely degraded within 24 hours in 1 N HCl at 50 °C while it degraded 90% in finished product form. As simvastatin is a prodrug and expected to hydrolyse to its active beta-hydroxyacid form, simvastatin acid, the drug decomposed completely under acidic conditions [26] and [27]. Although the drug was totally degraded, no impurity peak was observed due to lower strength of simvastatin. Similar results were observed when simvastatin was subjected to oxidative stress at 50 °C and neutral stress condition at 90 °C. However, simvastatin was found to be stable in basic and photolytic conditions.

Niacin samples were found to degrade about 20% under acidic, basic and oxidative stress conditions. No impurity peaks observed except under oxidative stress condition wherein the relative retention time

(RRT) and resolution were found to be 0.93 and 1.80, respectively. However, when the finished product was subject to stress conditions, impurity peaks were observed under basic and neutral conditions only, with RRT of 0.90 and 1.05, respectively. The impurity profile is very similar to that of simvastatin.Thus it can be concluded as potential degradation of simvastatin. Resolution between the impurities, simvastatin and niacin was more than 1.0. Based on the results obtained from forced degradation studies of simvastatin, niacin and their combined dosage form as shown in Table 1 and Table 2, simvastatin was found to be more sensitive compared to niacin.

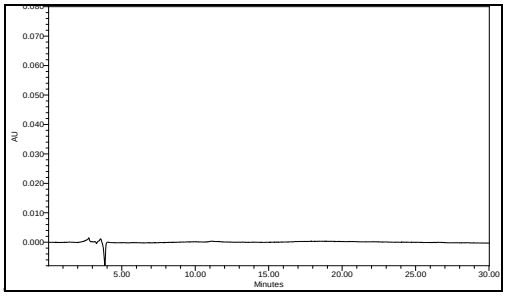

Fig. 5: Chromatogram of acid hydrolysis of simvastatin after 24 hours

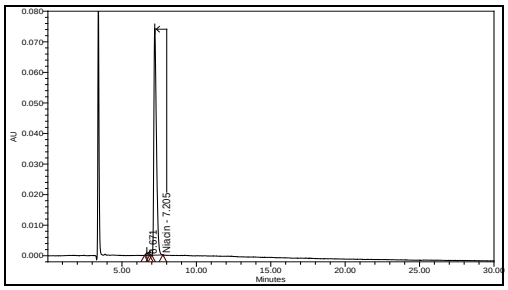

Fig. 6: Chromatogram of oxidative degradation of niacin after 24 hours

Method validation

Specificity

Chromatograms of both substances in all stress conditions were well resolved from its potential impurities. The obtained results demonstrated selectivity of the developed method for the simultaneous determination of simvastatin and niacin in combined dosage form [23].

The chromatograms of simvastatin in acidic stress condition and niacin in oxidative degradation condition are shown in Fig. 5 and Fig. 6 respectively.

Linearity and range

Linearity was established over the range of 50% to 150% of standard working concentrations for niacin and simvastatin. The five point calibration curve obtained by plotting the peak area (y axis) against concentration (x axis) of simvastatin and niacin was found to be linear within the concentration range from 0.004 mg/mL to 0.012 mg/mL and 0.050 mg/mL to 0.150 mg/mL respectively (Fig. 7 and Fig. 8).

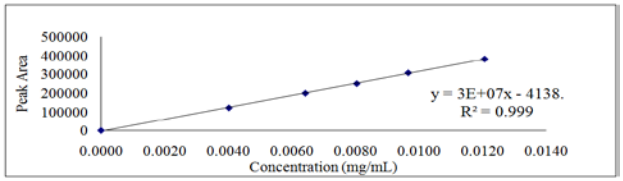

Fig. 7: Linearity curve for simvastatin

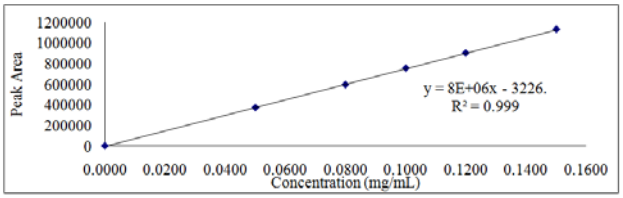

Fig. 8: Linearity curve for niacin

Regression analysis was used to determine the correlation coefficient, slope and intercept. Correlation coefficient (R^2) for simvastatin and niacin obtained from the linear curve were 0.9992 and 0.9999, respectively (Table 5 and Table 6).

Accuracy

The accuracy of the stability-indicating assay method was evaluated in triplicate at three concentrations ranging from 50% to 150% of the targeted working concentration of drug substances. The accuracy data for simvastatin and niacin are shown in Table 7. The recovery results indicate that there is no significant difference between sthe concentration of amount externally spiked-in and amount found from the HPLC analysis for both substances. The recovery of simvastatin was within the range 99.00% to 100.81%, with the % mean recovery of 99.93. For niacin, the recovery was within the range 98.38% to 100.09%, with the % mean recovery of 99.36. In both cases, the % RSD of total recovery was within 2.

Table 1: Forced degradation studies data for simvastatin and niacin in drug substances

Degradation condition	Temperature (°C)	% degradation of simvastatin	% degradation of niacin
Acidic (1N HCl)	50	100.0	20.0
Basic (1N NaOH)	50	6.8	26.0
Oxidation (0.3% H_2O_2)	50	82.8	20.3
Neutral	90	71.8	2.3
Photolytic	-	4.4	9.2

Table 2: Forced degradation studies data for simvastatin and niacin in drug product

Degradation condition	Temperature (°C)	% degradation of simvastatin	% degradation of niacin
Acidic (1N HCl)	50	89.5	0.0
Basic (1N NaOH)	50	0.0	0.0
Oxidation (0.3% H_2O_2)	50	80.1	0.0
Neutral	90	76.3	0.0
Photolytic	-	4.8	0.0

Chromatographic peak purity was analysed for drugs and potential impurities. The peak purity angle of all the peaks was found to be smaller than peak purity threshold in all the chromatograms, indicating that all the peaks were pure (Table 3 and Table 4).

Table 3: Peak purity from the forced degradation studies in drug substances after 24 hours

Degradation condition	Simvastatin		Niacin		Impurities	
	Purity angle	Purity threshold	Purity angle	Purity threshold	Purity angle	Purity threshold
Acidic	-	-	0.10	0.45	-	-
Basic	1.81	3.43	0.10	0.44	-	-
Oxidation	6.48	30.05	0.06	0.31	2.14	5.28
Water	5.68	21.74	0.07	0.32	8.14	64.95
Photolytic	1.31	3.60	0.07	0.34	-	-

Table 4: Peak purity from the forced degradation studies in drug product after 24 hours

Degradation condition	Simvastatin		Niacin		Impurities	
	Purity angle	Purity threshold	Purity angle	Purity threshold	Purity angle	Purity threshold
Acidic	20.62	67.44	0.14	0.52	-	-
Basic	0.90	3.19	0.08	0.45	6.57	23.98
Oxidation	5.88	33.83	0.10	0.47	37.50	90.00
Water	2.43	8.50	0.07	0.42	3.18	12.02
Photolytic	1.22	4.38	0.09	0.42	-	-

Table 5: Linearity for simvastatin

Value No.	Level (%)	Expected concentration (mg/mL)	Peak area (mAU)
1	50	0.0040	120192
2	80	0.0064	197764
3	100	0.0080	247830
4	120	0.0096	306583
5	150	0.0121	381631

Table 6: Linearity for niacin

Value No.	Level (%)	Expected concentration (mg/mL)	Peak area (mAU)
1	50	0.0501	370557
2	80	0.0801	594060
3	100	0.1001	756260
4	120	0.1201	898878
5	150	0.1502	1129371

Table 7: Accuracy for simvastatin and niacin

Analyte	Accuracy Level (%)	% Mean Recovery	% RSD
Simvastatin	50	100.00	2.0
	100	99.00	1.1
	150	100.81	0.6
Niacin	50	100.09	2.0
	100	99.61	1.4
	150	98.38	0.2

The precision of standards for accuracy experiment is within 2.0%. The resolution between both peaks was greater than 8.0. The system suitability for both drug substances at all concentrations remained within the acceptance criteria.

Precision

Repeatability

Repeatability was studied on intra-day variation using standard and samples of simvastatin and niacin at the working concentration of 0.008 mg/mL and 0.10 mg/mL, respectively. Repeatability of the method was assessed on standard preparation and six replicate samples preparation (Table 8). System suitability parameters and precision of standard and samples were analysed in replicate.

Based on the data shown in the Table 8, the precision of retention times of standard and sample for each drug was within 1.0%, which shows that each of the respective peaks does not shift over time. Precision of six injections of simvastatin and niacin standards are 1.4% and 0.5% respectively, which meets the USP guidelines. The mean assay of simvastatin and niacin was found to be 99.34% and 99.66% respectively, with precision less than 1.0% and resolution of 9.8, for both substances. The intra-day variation data demonstrated that the method was precise for the determination of simvastatin and niacin in combined dosage form.

Intermediate precision

Intermediate precision was studied on an inter-day variation using standard and samples of simvastatin and niacin at the working concentration of 0.008 mg/mL and 0.10 mg/mL, respectively. Based on the intermediate precision data shown (Table 9), slight difference was observed in the retention time of simvastatin using the two instruments and different batch of CN columns. However, no significant difference was observed in the retention time of niacin. The second analyst found the retention time of simvastatin to be 10.3 minutes. In both cases, precision in standard and samples were within 2%. The mean assays of simvastatin and niacin determined by the two analysts were found to be 99.34% and 100.98%, 99.66% and 99.84%, respectively. The system suitability parameters comply with the acceptance criteria in both cases. There is no significant difference in inter-day variation and hence the method was precise for the determination of simvastatin and niacin in combined dosage form.

Robustness

Robustness data of simvastatin and niacin in variable conditions are summarised in Table 10. The precision of standard and samples in

all chromatographic conditions was found to be within 2%. The mean assay of simvastatin and niacin was within the range of 97% to 100% and the difference of the mean assay of both substances was found to be less than 2.

Table 8: Repeatability for simvastatin and niacin standard and sample preparations

Repeatability										
Analyte	**Mean RT of standard (n=6) (min)**	**%RSD**	**Mean Peak Area of standard (mAU) (n=6)**	**%RSD**	**Mean RT of sample (n=6) (min)**	**%RSD**	**Mean Peak Area of sample (mAU) (n=6)**	**%RSD**	**Assay (%)**	**%RSD**
S	12.200	0.2	260643	1.4	12.154	0.1	254288	0.5	99.34	0.5
N	6.732	0.1	829795	0.5	6.709	0.1	785948	0.2	99.66	0.2

S-Simvastatin; N-Niacin

Table 9: Intermediate precision for simvastatin and niacin by two different analysts

Intermediate precision										
	Analyte	**Mean RT of standard (n=6) (min)**	**%RSD**	**Mean Peak Area of standard (mAU) (n=6)**	**%RSD**	**Mean RT of sample (n=6) (min)**	**%RSD**	**Mean Peak Area of sample (mAU) (n=6)**	**%RSD**	**Assay (%)**	**%RSD**
Analyst 1	S	12.200	0.2	260643	1.37	12.154	0.1	254288	0.5	99.34	0.5
Analyst 2	S	10.291	0.2	249940	1.3	10.210	0.4	250529	0.9	100.98	0.9
Analyst 1	N	6.732	0.1	829795	0.5	6.709	0.1	785948	0.2	99.66	0.2
Analyst 2	N	6.594	0.5	767163	1.3	6.591	0.1	735986	0.4	99.84	0.4

S-Simvastatin; N-Niacin

The method was found to be robust with no significant effect in all the deliberately varied chromatographic conditions, indicating that the method is reliable and can be used to analyse simvastatin and niacin in a combined dosage form.

Stability studies

The stability of standard and sample solutions was determined at room temperature by reanalysing the solutions that were kept in HPLC auto sampler over a period of 24 hours (Table 11). The precision of six replicate injections of standard and samples at beginning of the experiment and after 24 hours were found to be within 2%. The difference of standard peak area for simvastatin was found to be more than 2%. However, there is no significant difference in standard peak area of niacin after reanalysis over a period of 24 hours. These results suggest that the analysis of these drugs should be performed with freshly prepared solutions during the quality control analysis.

Table 10: Robustness for simvastatin and niacin

Condition	**Precision of total 6 standard injections (% RSD)**		**Precision of total 6 sample preparations (% RSD)**		**Mean assay of 6 samples (%)**		**Difference (%)**	
	S	**N**	**S**	**N**	**S**	**N**	**S**	**N**
Standard condition	1.5	0.5	1.2	0.6	98.10	98.62	-	-
Organic content (+2%)	1.4	0.3	0.7	0.8	99.48	99.96	1.38	1.34
Organic content (-2%)	1.1	0.3	0.7	0.4	97.69	97.71	0.41	0.91
Acid content (+2%)	1.1	0.2	1.7	0.5	99.56	99.18	1.46	0.56
Acid content (-2%)	1.0	0.2	1.8	0.7	98.16	100.18	0.06	1.56

S-Simvastatin; N-Niacin

There is no significant difference in retention time of simvastatin and niacin in all conditions except when the organic solvent was increased by 2%. Retention time of simvastatin reduced to 10.32 minutes, with resolution more than 2.0. Thus, it shows that retention of simvastatin is highly dependent on mobile phase composition as reported by Trone et al (2005) [28]. In all conditions, peak tailing, k', resolution and tangent meet the general requirements.

Table 11: Stability of standard and sample solutions

Peak	**Solution**	**Parameter**	**Initial**	**24 hours**	**Difference (%)**
S	Standard	Peak area	254375	248200	2.43
	Sample	Assay	98.10	97.02	1.14
N	Standard	Peak area	804745	798927	0.72
	Sample	Assay	98.62	100.25	1.63

S-Simvastatin; N-Niacin

CONCLUSION

A simple and rapid stability-indicating RP-HPLC method was developed and validated for simultaneous determination of niacin and simvastatin in a combined dosage form. The results have proved that the method was specific, linear, accurate, precise and robust to analyse the drugs in the presence of degradation products and excipients.

ABBREVIATIONS

API, Active Pharmaceutical Ingredient; ER, Extended release; HCl, Hydrochloric acid; HDL-C, High density lipoprotein cholesterol; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-coenzyme A; H_2O_2, Hydrogen Peroxide; ICH, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; LDL-C, Low density lipoprotein cholesterol; NaOH, Sodium hydroxide; PDA, Photodiode array detector; PVDF, Polyvinylidene difluoride; RRT, Relative retention time; RSD, Relative standard deviation; R^2, Correlation coefficient; USP, United States pharmacopeia.

CONFLICT OF INTEREST

Declared none

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