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Simultaneous Determination of Niacin and Lovastatin in Tablet Dosage Form by Reverse Phase HPLC

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A simple, specific and sensitive reverse phase high performance liquid chromatographic method was developed and validated for simultaneous determination of niacin and lovastatin in bulk drugs and formulation. The separation was effected on a kromasil C_{18} column (250 mm × 4.6 mm; 5µ) using a mobile phase consisting of acetonitrile and 0.02 M potassium dihydrogen phosphate and 0.01 M dipotassium hydrogen orthophosphate (pH 5) in the ratio of 80:20 (v/v) at a flow rate of 1 mL/min. UV detector was programmed at 254 nm. The retention times for niacin and lovastatin were 2.1 and 5.4 min respectively. All the validation parameters were in acceptable range. The developed method was effectively applied to quantitate amount of niacin and lovastatin and from tablets. Calibration curves were linear over the ranges of 0.4-2.4 µg/mL for lovastatin and 10-60 µg/mL for niacin. The proposed method was validated as per the ICH and USP guidelines. The method is accurate and precise and found to be suitable for the quantitative analysis of both the drugs individually and in combination in tablet dosage forms.

Key Words: Niacin, Lovastatin, HMG-CoA reductase and HPLC method.

INTRODUCTION

Lovastatin (LVS) is [(1S,3R,7S,8S,8aR)-8-[2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl]-3,7-dimethyl -1,2,3,7,8,8a-hexahydronaphthalen-1-yl](2S)-2-methyl butanoate Fig. 1(a). Lovastatin is known as reversible inhibitor of the microsomal enzyme HMG-CoA reductase, which catalyzes an early rate-limiting step, the conversion of HMG-CoA to mevalonate in cholesterol biosynthesis[1]. Lovastatin acts as an inhibitor of the action because it is structurally similar to HMG-CoA and the inhibition is competitive and highly specific. Inhibition of HMG-CoA reductase by lovastatin decreases intracellular cholesterol biosynthesis, which then leads to transcriptionally raised production of microsomal HMG-CoA reductase and cell surface low density lipoprotein receptors. Subsequently, additional cholesterol is provided to the cell by de novo synthesis and by receptormediated uptake of low density lipoprotein-cholesterol from the blood[2]. Haasis and Berger[3] have reported that lovastatin (20 mg per day) reduces low density lipoprotein-cholesterol to 19.3 %. Hypercholes-terolaemia plays a crucial role in the development of atherosclerotic disease in general and coronary heart disease (CHD) in particular. Several angiographic studies of hypercholestero-laemic patients with clinical symptoms of coronary heart disease have showed that

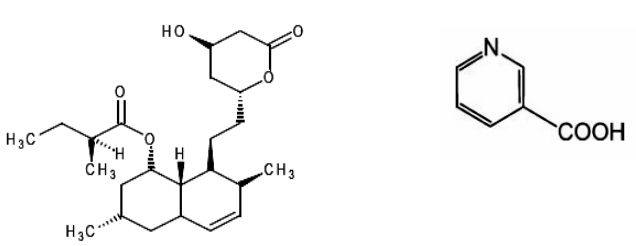

Fig. 1. Chemical structures of (a) Lovastatin, (b) Niacin.

progression of atherosclerosis or cardiovascular problems can be efficiently retarded by treatment with lovastatin[4,5]. Lovastatin increases bone formation when injected subcutaneously over the calvaria of mice and increases cancellous bone volume when orally administered to rats. Thus, in appropriate dosage, lovastatin may have therapeutic applications for the treatment of osteoporosis[6,7]. A number of analytical techniques had been applied to investigate the absorption, distribution, metabolism and excretion of lovastatin. However, most of the methods involve in-series ultraviolet and electrochemical detection and gas chromatography/mass spectrometry and are fairly complicated and costly[8,9]. Although some HPLC methods have been developed, most of them involve the use of C_{10} solid-phase extraction cartridges for sample

clean-up, which is time-consuming and can lead to low recovery[10]. We have developed a simple and sensitive HPLC method for the determination of niacin and lovastatin in tablet dosage form.

Niacin (NIA) is 3-pyridinecarboxylic acid Fig. 1(b). Niacin (3-pyridine-carboxylic acid or nicotinic acid) is a water soluble vitamin, belonging to the vitamin B family. It represents an important therapeutic option in large doses (1-6) for the treatment of dyslipidemia[11] because it favorably alters all major lipid subfractions. Niacin takes a major role in reduction of the levels of total cholesterol, low density lipoprotein (LDL) and triglycerides, while it increases the high density lipoprotein (HDL)[12]. Alone or in combination with simvastatin or lovastatin, it promotes regression of coronary artery diseases, decreases coronary events, stroke and total mortality[13]. *In vivo*, niacin undergoes metabolism *via* two major pathways [14-16]. One is through a simple conjugation with glycine to form nicotinuric acid (NUR) and the other contributes to the formation of niacinamide (NAM), which is a component of nicotinamide adenine dinucleotide (NAD), an essential coenzyme for many cellular oxidation-reduction reactions. Niacin status is also of increasing interests due to its possible association with HIV-related conditions[17] and cancer[18]. Capillary electrophoresis[16,19,20] and liquid chromatographic methods with ultraviolet detection[21,22] or fluorescence detection[23] have been attempted to quantitate niacin in biological matrices. The sensitivity of ultraviolet detection is not sufficient, while fluorescence detection has a relatively high sensitivity but the tedious and labor intensive derivatization and extraction procedure is a bottleneck, especially in the face of high throughput demands. The present work is aimed at development of a sensitive, specific and validated reverse phase high performance liquid chromatographic method for simultaneous determination of niacin and lovastatin from the dosage form.

EXPERIMENTAL

All the solvents and reagents used were of analytical grade. Acetonitrile of HPLC grade (Merck), ammonium acetate (Aldrich) and water purified by Milli-Q system (Millipore) were used for the preparation of the mobile phase. All solutions and solvents were filtered through a membrane filter 0.45 μm pore size.

Apparatus and chromatographic conditions: A Shimadzu HPLC system with a kromasil C_{18} column (250 mm × 4.6 mm; 5μ), a sample injector (rheodyne 7125) with a 20 μL loop and a UV detector (UV-2075) connected to a model 720 data module integrator was employed for the analysis.

A mobile phase consisting of a mixture of acetonitrile and 0.02 M potassium dihydrogen phosphate and 0.01 M dipotassium hydrogen orthophosphate (pH 5) in the ratio of 80:20 (v/v) was prepared, filtered through a 0.45 μm membrane filter and degassed prior to use.

Drug samples: Working standard samples of niacin and lovastatin used in this study were gifted by Aurobindo Pharma Ltd., Hyderabad and Dr. Reddy's Laboratories Ltd., Hyderabad respectively. A commercial sample of tablets of niacin and lovastatin is from abbott, containing 500 mg niacin and 20 mg of lovastatin was used in this study.

Preparation of drug solutions: Primary stock solution of niacin (1000 μg/mL) and lovastatin (40 μg/mL) was prepared by dissolving them in the mobile phase. These solutions were further diluted to obtain the working standard solutions in the concentration range of 10-60 μg/mL for niacin and 0.4-2.4 μg/mL for lovastatin. The working standard solutions of both the drugs were prepared individually as well as in combination.

RESULTS AND DISCUSSION

In this work the aim was to develop a simple, isocratic, accurate and sensitive HPLC method for the simultaneous determination of niacin and lovastatin in their fixed dose combination. Initially various mobile phases and stationery phases were tested to obtain the best separation and resolution between niacin and lovastatin. A mobile phase consisting of a mixture of acetonitrile and 0.02 potassium dihydrogen phosphate and 0.01M dipotassium hydrogen orthophosphate (pH 5) in the ratio of 80:20 (v/v) and kromasil C_{18} column were found to be the most appropriate for the separation of both the components at a the flow rate of 1.0 mL/min. Using the mentioned chromatographic conditions, well resolved sharp peaks can be obtained at retention time of 2.17 and 5.46 min for niacin and lovastatin respectively. The chromatograms of standard and tablet solutions of niacin and lovastatin are shown in Figs. 2 and 3, respectively.

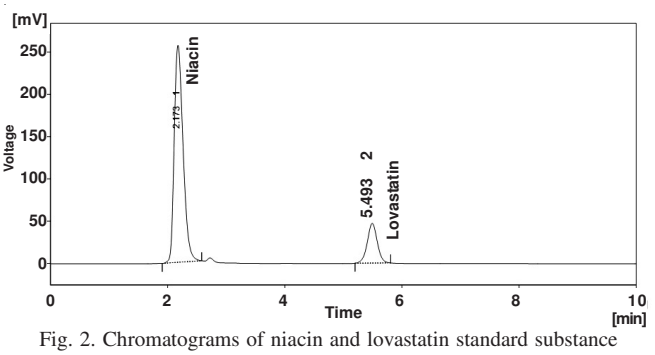

Fig. 2. Chromatograms of niacin and lovastatin standard substance

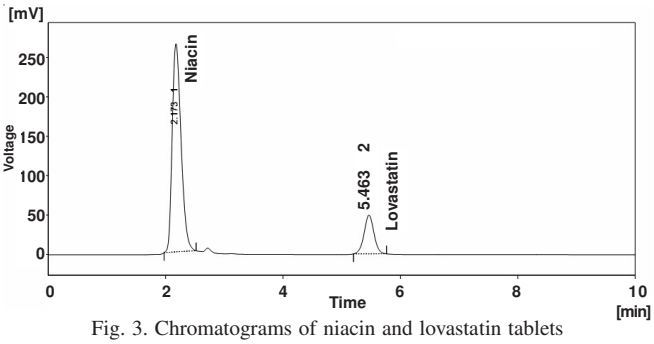

Fig. 3. Chromatograms of niacin and lovastatin tablets

Method development was started with different mobile phases with changing concentrations of acetonitrile, (less polar mobile phase 40 % acetonitrile) and buffers however, broadening peaks could be obtained. The chromatographic conditions were optimized for the simultaneous determination of niacin and lovastatin within a short analysis time (<10 min) and an acceptable peak resolution (Rs > 2). To accomplish these objectives, the chromatographic column was first chosen based on peak shapes and resolution. In preliminary

experiments, the sample retention time increased with a decrease in concentration of acetonitrile and, resulted in peak overlap between them, in order to avoid long run-times and overlaps. The polarity of the mobile phase was then increased by the increase in concentration of acetonitrile and addition of buffer. A ratio of 80:20, (v/v) for acetonitrile and 0.02 potassium dihydrogen phosphate and 0.01 M dipotassium hydrogen orthophosphate resulted in good separation and sharp peaks. The optimum mobile phase composition was found to be acetonitrile and 0.02 potassium dihydrogen phosphate and 0.01 M dipotassium hydrogen orthophosphate (pH 5.0) in the ratio of 80:20 (v/v).

Method validation: The developed chromatographic method for the simultaneous determination of niacin and lovastatin was validated using ICH guidelines. Validation parameters performed include linearity, limit of detection/ quantitation, specificity, accuracy, precision and robustness of solutions.

System suitability: The system suitability was assessed using three replicate analyses of drugs at concentration of 60 µg/mL for niacin and 2.4 µg/mL for lovastatin. The kromasil C_{18} column was stabilized for 0.5 h in the optimized conditions before replicate injections were made. The system suitability parameters like resolution, number of theoretical plates, assymetric factor were presented in Table-1.

TABLE-1
SYSTEM SUITABILITY DATA FOR MIXED STANDARD SOLUTION

Parameter	Niacin	Lovastatin
Therotical plate	2905	5141
Assymetric factor	1.60	1.02
Resolution	–	11.13

Linearity: Linearity was assessed by performing single measurement at several analyte concentration varying quantities of stock standard solution diluted with the mobile phase to give a concentration in the range of 10-60 µg/mL for niacin and 0.4-2.4 µg/mL for lovastatin. Good linearity was observed over the above range for both niacin and lovastatin. The calibration curve was made using concentration of the analytes *versus* peak area. The coefficient of determination from the linear regression analysis was calculated and found to be greater than 0.9990 in case of both the analytes. This indicates that there exists a good linear relationship between concentration of drugs and the peak area. The linear regression equation for niacin was Y = 43.831x + 63.484 with value of coefficient of determination equal to 0.9990 whereas the linear regression equation for lovastatin was Y = 205.4x + 13.57 with 0.9990 as the value of coefficient of determination.

Accuracy: The accuracy of the method was evaluated by the addition of known amounts niacin and lovastatin to the sample solution. The results obtained were compared with the theoretical concentration containing various amounts of niacin and lovastatin (40, 50 and 60 µg/mL for niacin and 1.6, 2.0 and 2.4 µg/mL for lovastatin) and then analyzed by the proposed method. The mean percentage recovery and the % RSD were calculated from recovery experiments. The recovery range and the relative standard deviation for each of the analytes were found to be 99.21-100.15 and 0.14-0.45 %, respectively.

Precision: The precision of the proposed method was determined by the analysis of three different concentrations in terms of % RSD. The within-day precision was based upon the results of five replicate analysis of three different concentrations of analytes on a single day. The between-day precision was determined from the same samples analyzed for five consecutive days. The results of within-day and between-day precision are given in Table-2.

Limit of detection and limit of quantitation: Two types of solutions, *i.e*., blank and spiked with known concentrations of each analyte, were prepared and analyzed. The limit of detection and quantification were then established by evaluating the signal to noise ratio of 3:1 and 10:1 respectively. The limit of detection was found to be 0.68 µg/mL and 0.04 µg/mL for niacin and lovastatin respectively. The limit of quantification was found to be 2.07 µg/mL and 0.13 µg/mL for niacin and lovastatin.

Robustness: Robustness of the method was performed by intentionally but slightly modifying the chromatographic conditions such as flow rate and temperatures. The results showed that the slight change in the chromatographic conditions had no pronounced effects on the chromatographic parameters. The results of the robustness study are given in Table-3.

Conclusion

A rapid, simple and specific reverse phase HPLC method has been developed for simultaneous determination of niacin and lovastatin from tablet dosage form. The method was validated for accuracy, precision, linearity. So it can be used

TABLE-2
INTRA AND INTER DAY PRECISION OF THE PROPOSED HPLC METHOD

Parameters	Niacin			Lovastatin		
	40	50	60	40	50	60
Day 1						
Means + SD	39.95+0.12	49.86+0.19	59.58+0.16	39.71+0.05	49.89+0.11	59.83+0.27
% RSD	0.31	0.39	0.27	0.14	0.23	0.45
Day 2						
Means + SD	40.03+0.09	49.67+0.14	59.75+0.21	39.63+0.07	49.72+0.09	59.32+0.12
% RSD	0.35	0.47	0.33	0.25	0.41	0.29
Day 3						
Means + SD	40.20+0.17	50.07+0.07	59.90+0.23	39.73+0.11	49.9+0.22	59.43+0.09
% RSD	0.22	0.30	0.41	0.32	0.47	0.56

for the simultaneous determination of niacin and lovastatin in pharmaceutical formulations. The total run time for the both components is less than 6 min. From these values it is concluded that the new HPLC method is suitable for the simultaneous determination of niacin and lovastatin in their pharmaceutical formulations.

TABLE-3
ROBUSTNESS STUDY OF NIACIN AND LOVASTAIN

Robustness study of niacin			
Conditions	RT (min)	Theoretical plates	Resolution
Flow rate (0.8 mL)	2.87	1040	–
Flow rate (1.1 mL)	2.02	1013	–
Temperature at 25 ºC	2.16	1013	–
Temperature at 27 ºC	2.00	1013	–
Temperature at 30 ºC	1.98	1013	–
Robustness study of lovastain			
Flow rate (0.8 mL)	7.25	5208	11.54
Flow rate (1.1 mL)	5.15	5104	11.42
Temperature at 25 ºC	5.46	5104	11.42
Temperature at 27 ºC	5.31	5104	11.54
Temperature at 30 ºC	4.99	5104	11.54

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