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Validated liquid chromatographic method for niacin and simvastatin in pharmaceutical preparation

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ABSTRACT

A simple, sensitive and validated HPLC method has been developed to determine niacin and simvastatin simultaneously in pharmaceutical preparation. Chromatographic separation was achieved on a C-8 column using a mixture of phosphate buffer pH 3.0 and methanol in the ratio of 20:80 (v/v) at a wavelength of 237 nm. Linearity of the method was found to be in the concentration range of 75-525µg/ml for niacin and 1.5 -10.5µg/ml for simvastatin with correlation coefficient greater than 0.999. The total eluting time for the two components is less than ten minutes. The method can be used for simultaneous determination of niacin and simvastatin.

KEYWORDS

HPLC;
Methanol;
Niacin;
Simvastatin.

INTRODUCTION

Niacin (Figure 1) chemically designated as Pyridine 3 carboxylic acid reduce triglyceride, levels, is also effective for increasing serum HDL levels[1]. It has also been demonstrated that this drug lowers the incidence of coronary heart disease in humans[1]. A number of analytical methods have been developed for its determination in pharmaceutical formulations or in biofluids either alone or in combination with other drugs. These include determination of niacin by liquid chromatography– mass spectrometry[4], HPLC[2-5], flow injection and spectrofluorimetric analysis.

Simvastatin (Figure 2), 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-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8ahexahydronaphthalen-1-yl] 2,2-dimethylbutanoate. It is used for the treatment of hypercholesterolemia an HMG-CoA reduce ase inhibitor, acts by decreasing cholesterol synthesis and by increasing low density lipoprotein (LDL) catabolism via increased LDL receptor activity. Different analytical methods have been reported for the determination of simvastatin, which include HPLC[7-9], HPLC-MS/MS[6], spectrophotometer[10].

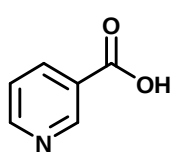

Figure 1 : Niacin

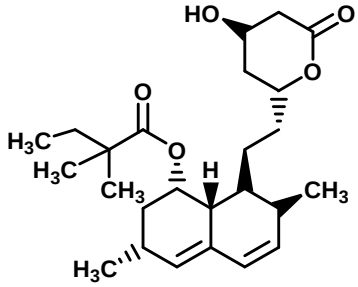

Figure 2 : Simvastatin

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It was found that simvastatin plus niacin provides marked clinical and angiographic ally measurable benefits in patients with coronary disease and low HDL levels[11].The US Food and Drug Administration (FDA) has approved a fixed-dose combination of niacin and simvastatin for use in patients with complex lipid abnormalities where treatment with niacin or simvastatin alone is not sufficient[12] .The FDA has approved maximum dose of niacin 1000mg and 20mg of simvastatin per tab. The combine dosage form of Niacin and simvastatin are available in market.

According to the information collected from literature there is no reported method for simultaneous determination of niacin and simvastatin. In the present work we have focused on deciding the optimum chromatographic conditions for the simultaneous determination of niacin and simvastatin in a tablet.

We describe in this paper a simple, sensitive and validated HPLC method with total run time less than ten minutes for the simultaneous determination of niacin and simvastatin the developed method can be applied successfully for quality control and for other analytical purposes.

MATERIAL AND METHODS

Chemicals and reagents

Niacin and simvastatin reference substances with claimed purity of 99.70% and 99.65% respectively were taken from Precise Pharma (Turbhe, Mumbai) methanol (HPLC grade), triethyl amine and orthophosphoric acid (analytical reagent grade) were purchased from Merck (Mumbai). All excepients used were of pharmaceutical grade. Mobile phase was filtered using 0.45μm cellulose acetate filters made by Millipore (USA) whereas; Whatmann filter papers No.41 (purchased from the local market) were used in the preparation of sample solution.

Apparatus and chromatographic conditions

HPLC apparatus consisting of Jasco system equipped with a pump model PU980, an UV detector model uv -998 (set at 237nm), and thermostat column compartment and injector port model As-2057 a interface module with Browin HPLC software 1.21 was used for development and evaluation of this method. A Water spherisorb C 8 column (150mm, 4.6mm, i.d., 5μm particle sizes) was selected. The mobile phase was composed of a mixture of 0.1% TEA in water pH 3.0 ± 0.05 with orthophosphoric acid and methanol in the ratio of 20: 80 (v/v). An external standard method was used and the flow rate was 1 ml/min.and column temp is 45°C the HPLC system was operated at room temperature 25° + 20°C. And diluent is mixture of 1% TEA in water and methanol in the ratio of 20: 80 (v/v).

Preparation of standard solution

A stock solution of niacin and simvastatin was prepared at about 10,000μg /ml and 200μg/ml respectively in diluent. The working standard solution 300μg/ml for niacin and 6μg/ml for simvastatin were prepared by diluting the stock solution with mobile phase.

Determination of simvastatin and niacin in there combined dosage forms

Twenty tablets were crushed and weighed powder equivalent to niacin 1000mg and 20mg simvastatin in 100 ml volumetric flask add 50ml of diluent and flask was sonicated for 5 min .The flask was sonicated and the volume was diluted to the mark with diluent .The above solution was filtered using what man filter paper No .1. Appropriate volume of aliquot was diluted with mobile phase to obtain a solution containing 300μg/ml for niacin and 6μg/ml for simvastatin.

Linearity

Linearity of the proposed method was checked by analyzing seven solutions in the range of 75-525μg/ml for niacin (75, 150, 225, 240, 300, 360, 450, 525μg /ml) and 1.50-10.5μg/ml for simvastatin (1.5, 3, 4.5, 6, 7.5, 9, 10.5μg /ml). Each level was made in triplicate.

Accuracy

Method accuracy was performed by adding known amounts of niacin and simvastatin to the pre analyzed sample and then comparing the added concentration with the found concentration. Three levels of solutions were made which correspond to 50, 100 and 150% of the nominal analytical concentration. Each level was made in triplicate.

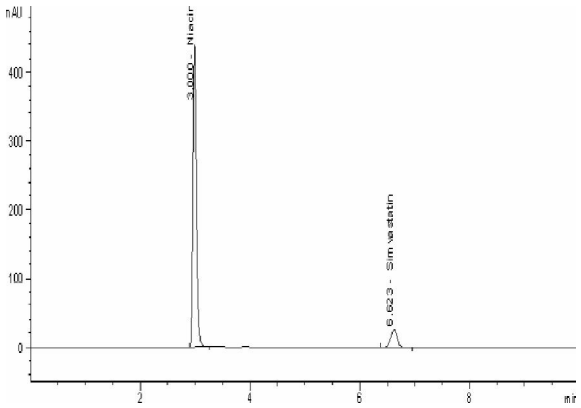

Figure 3 : Chromatograms of niacin and simvastatin reference substance

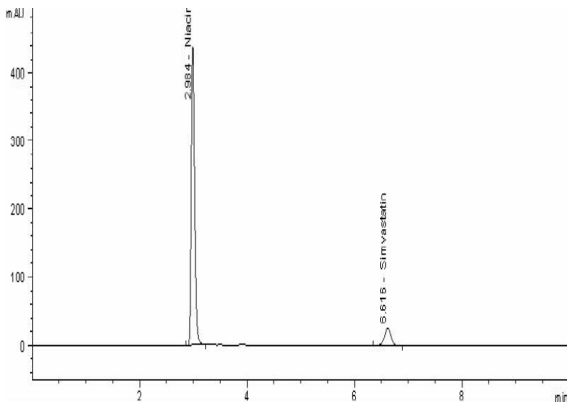

Figure 4 : Chromatograms of niacin and simvastatin in sample solution

Specificity

Commonly used exceipients (starch, microcrystalline cellulose and magnesium stearate, lactose, opadry, BHA, PEG) were spiked in to a pre weighed quantity of drugs .The chromatogram was taken by appropriate dilution and the quantities of drug were determined.

Robustness

Robustness of the method was performed by intentionally modifying the chromatographic conditions such as composition and flow rate of the mobile phase and pH of the buffer solution. The chromatographic parameters of each analyte such as retention time, tailing factor, resolution and number of theoretical plates were measured at each changed conditions.

Precision

For evaluating the within-day precision, results of five replicate analyses of three different concentrations of samples were calculated on a single day. The between-day precision was calculated from the same samples analyzed on different days.

LOD and LOQ

For calculating the LOD and LOQ values, solutions with known decreased concentrations of analytes were injected into the HPLC system. The limit of detection (LOD) and quantification (LOQ) were then measured by calculating the minimum level at which the analytes can be readily detected (signal to noise ratio of 3:1) and quantified (signal to noise ratio of 10:1) with accuracy, respectively.

RESULTS

In the present work conditions were optimized for the development and validation of a simple and accurate HPLC method for the simultaneous determination of niacin and simvastatin in pharmaceutical preparation .Method development was started with water and methanol in the ratio of 50:50 (v/v). At this composition although both components were eluted but retention time of simvastatin was about 25 minutes. & niacin about 7min and peak shape was also not good. The methanol contents of the mobile phase were then increased to decrease resolution and retention time and pH of water adjusted to 3 with the help of Orthophosphoric acid and Triethyl amine .At the composition of 20:80 (0.1%TEA pH 3.0 with OPA and Methanol) both components were eluted with a good resolution and good peak shape. The most appropriate mobile phase composition was thus found to be 0.1%TEA in water pH 3.0 by OPA and Methanol in the ratio of 20:80 (v/v). Under the Described experimental conditions, sharp peaks that belong to niacin and simvastatin were obtained at retention times of 3.00and 6.62 minutes respectively as shown in figure 3.

The developed chromatographic method was validated using ICH guidelines[20]. Validation parameters performed include linearity, limit of detection and quantitation, selectivity, robustness, accuracy and repeatability the calibration curve was linear over the concentration range of 75-525 μg/ml for niacin and 1.5-10.5 μg/ml for simvastatin. The correlation coefficient in both cases was found to be greater than 0.999 which manifests a linear relationship between concentration

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TABLE 1 : Accuracy of the proposed HPLC method

Compound	Level (%)	n	Added Conc.µg/ml	Found Conc. µg/ml	% Recovery	RSD (%)
Niacin	50	3	150	149.13	99.42	0.68
	100	3	300	302.13	100.71	0.33
	150	3	450	451.12	100.25	0.35
Simvastatin	50	3	3.0	3.02	100.66	1.63
	100	3	6.0	5.96	99.33	0.21
	150	3	9.0	9.03	100.33	0.67

TABLE 2 : Precision of the proposed HPLC method

Compound	Conc. µg/ml	n	Within a day precision		Between a day precision	
			Mean	Rsd (%)	Mean	RSD (%)
Niacin	150	3	147.31	0.14	147.18	0.16
	300	3	305.24	0.244	304.07	0.12
	450	3	451.00	0.111	450.14	0.03
Simvastatin	3	3	2.91	0.73	2.91	0.47
	6	3	6.09	0.872	5.96	0.23
	9	3	8.76	0.382	8.70	0.24

TABLE 3a : Robustness study of niacin

Condition	t R (min)	Theoretical plate	Tailing
Methanol : Buffer (86:14)	2.961	8029	1.196
Methanol : Buffer (80:20)	2.998	11528	1.099
Methanol : Buffer (74:26)	3.139	11138	1.269
Flow Rate (1.1ml/min)	2.745	6312	1.674
Flow Rate (0.9ml/min)	3.372	6742	1.720
Buffer p H (3.2)	3.141	7744	1.285
Buffer p H (2.8)	3.108	11131	1.255

TABLE 3b : Robustness study of simvastatin

Condition	t R (min)	Theoretical plate	Tailing	Resolution
Methanol : Buffer (86:14)	4746	15827	1.069	12.62
Methanol : Buffer (80:20)	6.619	16398	0.994	22.71
Methanol : Buffer (74:26)	11.769	14365	0.888	33.67
Flow Rate (1.1ml/min)	6.189	13224	1.069	19.45
Flow Rate (0.9ml/min)	7.614	14931	1.089	20.48
Buffer p H (3.2)	6.646	14114	1.285	19.10
Buffer p H (2.8)	6.531	15706	0.998	20.95

and the peak area. The linear regression equation for niacin was found to be Y= 6.324 X + 6.900 with correlation coefficient equal to 0.999. The linear regression equation for simvastatin was found to be Y= 34.064 X + 0.453 with value of correlation coefficient equal to 0.99997. In this study, the LOD was found to be 1.25µg/ml and 0.1416µg/ml for niacin and simvastatin respectively. The LOQ was found to be 3.75µg/ml and 0.425µg/ml for niacin and simvastatin respectively. The recovery and the relative standard deviation for each of the analytes are given in TABLE 1.

The results of within-day and between-day precision are presented in TABLE 2.

Chromatogram of niacin and simvastatin in sample in given in figure 4 showing selectivity of the proposed method.

Robustness of the method was performed by intentionally modifying the chromatographic conditions. The results showed that the variance of the conditions had no appreciable effects to that of actual. The results of the robustness study are given in TABLE 3a & 4b.

CONCLUSION

A simple and accurate reverse phase HPLC method has been developed for the simultaneous determination of niacin and simvastatin. The method was validated by testing its linearity, accuracy, precision, limits of detection and quantization, selectivity and robustness. The run time of less than ten Minutes allows its application for the routine determination of niacin and simvastatin.

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