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Development and validation of HPTLC method for niacin and simvastatin in binary combination

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ABSTRACT

A simple, sensitive and validated HPTLC method has been developed to determine Niacin and simvastatin simultaneously in synthetic mixture form. Chromatographic separation was achieved on a RP18 plate using a mixture of Methanol: Water: Acetic acid (60: 40:0.1) at a wavelength of 237 nm. Linearity of the method was found to be in the concentration range of 5000.0-25000.0 µg/ml for niacin and 100.0-500.0 µg/ml for simvastatin with correlation coefficient greater than 0.999. The method can be used for simultaneous determination of Niacin and Simvastatin.

Keywords: HPTLC; Methanol; Niacin; Simvastatin

1. 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 [2-8]. These include determination of niacin by liquid chromatography–mass spectrometry [9], HPLC [9-12], 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-dimethy-lbutanoate. It is used for the treatment of hypercholes-terolemia [13]. An HMG-CoA reduces ase inhibitor, acts by decreasing cholesterol synthesis and by increasing low density lipoprotein (LDL) catabolism via increased LDL receptor activity [14]. Different analytical methods have been reported for the determination of simvastatin, which include HPLC [15-17], HPLC-MS/MS [18], spectrophotometer [19].

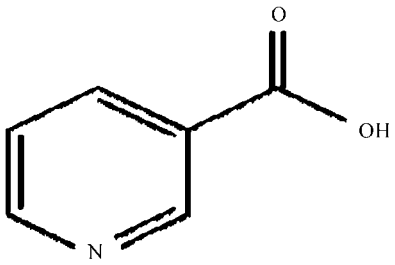

Figure 1. Niacin.

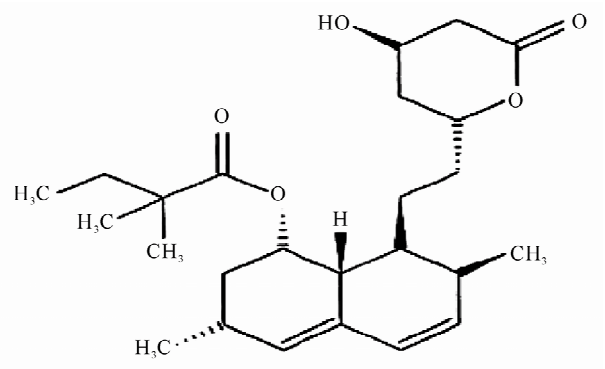

Figure 2. Simvastatin.

It was found that Simvastatin plus niacin provides marked clinical and angiographic ally measurable benefits in patients with coronary disease and low HDL levels [20]. 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 [21] The FDA has approved maximum dose of niacin 1000mg and 20 mg 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 pharmaceutical preparation.

We describe in this paper a simple, sensitive and validated HPTLC method for the simultaneous determination of Niacin and Simvastatin. The developed method can be applied successfully for quality control and for other analytical purposes.

2. MATERIAL AND METHODS

2.1. Chemicals and Reagents

Niacin and simvastatin reference substances respectively were taken from Precise Pharma (Turbhe, mumbai). Methanol (HPLC grade), Triethyl amine and acetic acid (analytical reagent grade) were purchased from Merck (Mumbai). All excepients used were of pharmaceutical grade.

2.2. Chromatographic Conditions

The chromatographic estimation were performed using RP18 pre-coated on aluminum sheet (10 × 10 cm, prewashed with methanol and dried in oven at 50℃ for 5 min) mobile phase, Methanol: Water: Acetic acid (60: 40:0.1), chamber and plate saturation time of 45 min, migration distance allowed was 75 mm, wavelength scanning was done at 237 nm, keeping the slit dimension at 5 × 0.45 mm.

2.3. Preparation of System Suitability Solution

A Stock solution of niacin and simvastatin was prepared at about 100000 µg/ml and 2000 µg/ml respectively in diluent. Then dilute 1 ml of stock solution to 10 ml with diluent to give 10000 µg/ml and 200 µg/ml niacin and simvastatin respectively. Spotted 2.5 µl of system suitability solution in five replicates. And found RSD of peak area and Rf is below 2%.

2.4. Determination of Simvastatin and Niacin in Their Combined Dosage Forms

The content of twenty tablets where taken and weighed .powder equivalent to Niacin 1000 mg and 20 mg simvastatin in 10.0 ml volumetric flask add 6 ml 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. Then diluted 1.0 ml of filtrate to 10.0 ml with diluent.

2.5. Linearity

Linearity of the proposed method was checked by analyzing solutions in the range of 5000-25000 µg/ml for niacin (5000, 7500, 10000, 12500, 15000, 20000, 25000 µg/ml) and 100-500 µg/ml for simvastatin (100,150,200, 250,300 ,400,500 µg/ ml). Each level was made in triplicate and spot 2.5 µl of each solution.

2.6. 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. Four levels of solutions were made which correspond to 0, 50, 100 and 150% of the nominal analytical concentration. Each level was made in triplicate and spot 2.5 µl of each solution.

2.7. Specificity

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

2.8. Precision

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

3. RESULTS

In the present work conditions were optimized for the development and validation of a simple and accurate HPTLC method for the simultaneous determination of niacin and simvastatin in synthetic mixture form.

Method development was started with water and methanol in the ratio of 50:50 (v/v). At this composition although both components were resolved but peak shape were not good. With the mobile phase composition of Methanol: Water: Acetic acid (60:40:0.1) good resolution and better peak shape were obtained.

Under the described experimental conditions, sharp peaks that belong to niacin and simvastatin were obtained at retention factor of 0.23 and 0.57 minutes respectively as shown in **Figure 3**.

The developed chromatographic method was validated using ICH guidelines [22]. Validation parameters performed include linearity, specificity, accuracy and precission. The calibration curve was linear over the concentration range of 5000-25000 µg/ml for niacin and 100-500 µg/ml for simvastatin. The correlation coefficient in both cases were found to be greater than 0.999 which manifests a linear relationship between concentration and the peak area. The linear regression equation for niacin was found to be Y = 3.2342 X + 149.26 with correlation coeffi cient equal to 0.9999.

The linear regression equation for simvastatin was found to be Y = 11.499 X + 4.8303 with value of correlation coefficient equal to 0.9999.

The recovery and the relative standard deviation for each of the analytes are given in **Table 1**, **Table 2**, **Table 3** and **Table 4** mean recovery for Niacin is found 100.48% & for simvastatin is found 99.80%.

The results of three successive day and precision are presented in **Table 5**.

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

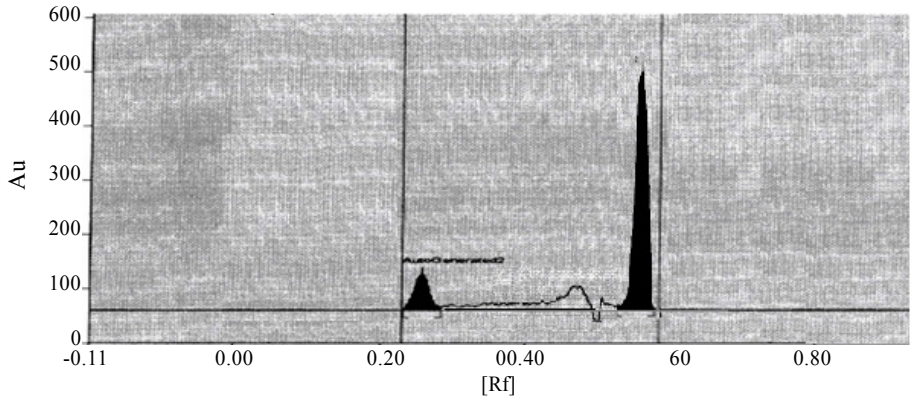

Figure 3. Chromatograms of Niacin and Simvastatin in standard solution.

Table 1. Accuracy of Niacin.

Level	Wt. of sample (mg)	Wt. of std added in (mg)	Amount of Niacin found in (mg)			Mean (mg)	S.D	%R.S.D
			1	2	3			
0	1352.66	0	1000.35	1000.19	1000.17	1000.24	0.10	0.01
50	1349.25	501.48	1501.48	1502.32	1504.80	1502.87	1.73	0.11
100	1351.41	1001.12	2000.35	2000.14	2000.18	2000.22	0.11	0.01
150	1351.23	1501.21	2512.36	2521.46	2521.48	2518.43	5.26	0.21

Percent Recovery $= \frac{N(\sum XY)-(\sum X)(\sum Y)}{N(\sum X^2)-(\sum X)^2} \times 100$,Where, N = Number of observations; X = Amount of the standard added (mg); Y = Amount of the standard found (mg).

Table 2. Accuracy of Niacin.

Level	X	Y	X^2	X Y
0	3 × 0	3 × 1000.24	3 × $(0.00)^2$	3 × 0 × 1000.24
1	3 × 501.48	3 ×1502.87	3 × $(501.48)^2$	3 × 500.10 × 1502.87
2	3 × 1001.12	3 × 2000.22	3 × $(1001.12)^2$	3 × 1000.02 × 2000.22
3	3 × 1501.21	3 × 2518.43	3 × $(1501.21)^2$	3 × 1500.06 × 2518.43
∑	9011.43	21065.28	9770627.04	19588924.19

$(\sum X)^2$ 81205870.64

No. of Observations N = 12

Therefore % Recovery $= \frac{12(19588924.19)-(9011.43)\cdot(21065.28)}{12(37552304.73)-(10519061.37)} \times 100$

$= \frac{45238794.13}{45022865.76} \times 100$

$=100.48\%$

Table 3. Accuracy of Simvastatin.

Level	Wt. of sample (mg)	Wt. of std added in (mg)	Amount of Simvastatin found in (mg)			Mean (mg)	S.D	%R.S.D
			1	2	3			
0	1352.66	0	20.03	20.10	20.21	20.11	0.09	0.45
50	1349.25	10.21	30.25	30.16	30.29	30.23	0.07	0.22
100	1351.41	20.23	40.23	40.16	40.52	40.30	0.19	0.47
150	1351.23	30.15	50.12	50.23	50.16	50.17	0.06	0.11

Table 4. Accuracy of Simvastatin.

Level	X	Y	X^2	X Y
0	3 × 0	3 × 20.11	3 × $(0.00)^2$	3 × 0 × 20.11
1	3 × 10.21	3 × 30.23	3 × $(10.21)^2$	3 × 10.11 × 30.23
2	3 × 20.23	3 × 40.30	3 × $(20.23)^2$	3 × 20.74 × 40.30
3	3 × 30.15	3 × 50.17	3 × $(30.15)^2$	3 × 30.16 × 50.17
∑	181.77	422.43	4367.56	7909.63

$(\sum X)^2$ 33040.33

No. of Observations N = 12

$$\text{Therefore \% Recovery} = \frac{12(7909.63) - (181.77)\cdot(422.43)}{12(33040.33) - (4267.56)} \times 100$$

$$= \frac{18134.09}{18170.37} \times 100$$

$$= 99.80\%$$

Table 5. Precision of the proposed HPTLC method.

Analyte	Mean Peak Area			Mean	S.D.	% R.S.D
	DAY 1	DAY 2	DAY 3			
	16250.39	16389.00	16309.86	16316.42	69.54	0.43
Niacin	32423.63	32438.20	32453.41	32438.41	14.89	0.05
	48207.62	48485.72	48529.63	48407.66	174.62	0.36
	1154.14	1150.11	1147.11	1150.45	3.52	0.31
Simvastatin	2319.85	2318.79	2349.88	2329.51	17.65	0.76
	3463.75	3474.25	3452.86	3463.62	10.69	0.31

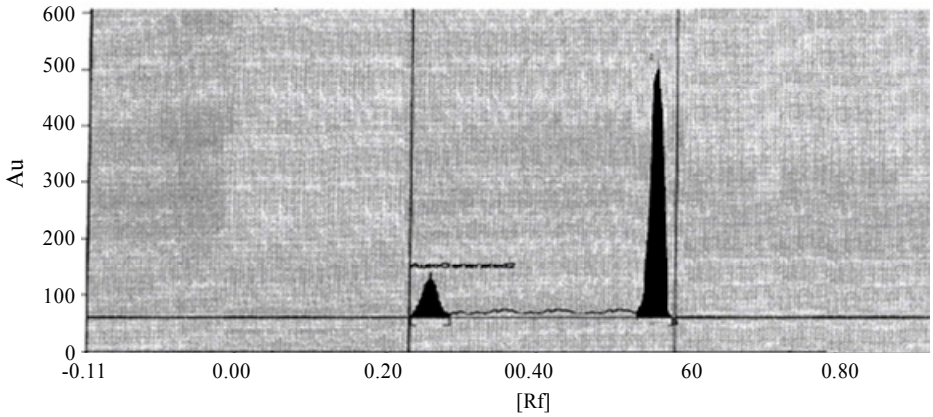

Figure 4. Chromatograms of Niacin and simvastatin in sample solution.

4. CONCLUSIONS

A simple and accurate reverse phase HPTLC method has been developed for the simultaneous determination of niacin and simvastatin. The method was validated by testing its linearity, accuracy, precision and specificity.

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