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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF NIACIN AND SIMVASTATIN IN TABLET DOSAGE FORM

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Abstract:

A simple RP-HPLC method has been developed for the simultaneous estimation of Niacin and Simvastatin in tablet dosage form. Chromatographic separation was achieved on Hypersil BDS C18 (250 x 4.6 mm, 5µm column using Acetonitrile and Ammonium dihydrogen phosphate buffer in the ratio of 80:20 v/v, pH 5, with flow rate of 1 ml/min. The detection wavelength was set at 254 nm. The retention time of niacin was 2.0852 and simvastatin was found to be 5.7052. The method was applied to tablet dosage forms, without any interference from excipients. The calibration curve was linear over the range of 20-120 ug/ml. The performance of the method was validated according to ICH guidelines and it was found suitable for the analysis of Niacin and Simvastatin in tablet dosage forms.

Keywords: *Niacin, Simvastatin, RP-HPLC, Method Development, Validation, ICH guidelines.*

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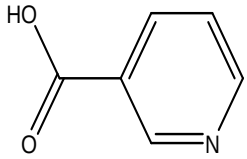
INTRODUCTION:

The US Food and Drug Administration (FDA) have approved a fixed-dose combination of extended release (ER) Niacin and Simvastatin for use in patients with complex lipid abnormalities where treatment with Niacin or Simvastatin alone is not sufficient. The drug, known as Simcor **(**GENERIC NAME(S): Niacin-Simvastatin**)** (Abbott, Abbott Park, IL), is approved to lower total- and low-density lipoprotein (LDL)-cholesterol levels and triglycerides and to raise high-density lipoprotein (HDL)-cholesterol levels. The approval is based on safety and efficacy data from 641 patients with mixed dyslipidemia and type 2 dyslipidemia, a study in which patients treated with Simcor 1000/20 mg achieved significantly better improvements in cholesterol end points than simvastatin 20 mg [1].

Niacin is chemically pyridine-3-carboxylic acid (Figure 1), official in IP, has been used to treat dyslipidemia, a high risk factor in Cardiac Heart Disorders (CHD) [2]. It has shown to reduce coronary death and nonfatal myocardial infarction and, all-causing mortality [3]. Niacin reduces total cholesterol, LDL-C (Low density lipoprotein cholesterol) and triglycerides and increases HDL-C (High density lipoprotein cholesterol) [4].



pyridine-3-carboxylicacid

Fig. 1: Structure of Niacin

Simvastatin is (1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxotetrahydro-2H-pyran-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate. (Figure 2) The molecular formula of simvastatin is C25H38O5 and its molecular weight is 418.57. Statins lower cholesterol by inhibiting the synthesis of mevalonic acid, which is a key precursor in cholesterol synthesis [5]. Simvastatin, a lipid lowering agent that is derived synthetically from a fermentation product of *Aspergillus terreus* has been found to lessen both normal and elevated LDL-C concentrations [6].

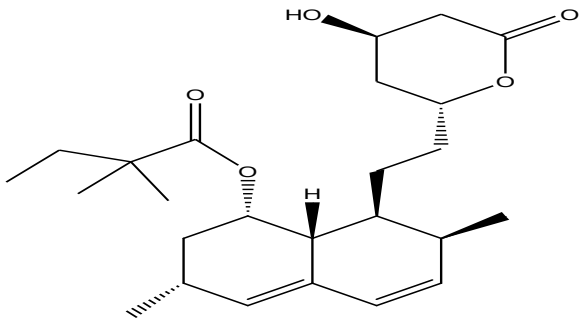


Fig. 2: Structure of Simvastatin.

Literature survey reveals that, only few spectrophotometric methods and few analytical methods have been reported for the simultaneous quantitative estimation of Simvastatin and Niacin in bulk drug and pharmaceutical formulation [7-10]. Hence an attempt has been made to develop new HPLC methods for its estimation in bulk and pharmaceutical formulation with good precision, accuracy, linearity and reproducibility. Conformation of the applicability of the developed method was validated according to ICH guidelines [11].

MATERIALS AND METHODS:

Experimental Procedure

Chemicals, solvents and drugs:

Ammonium dihydrogen phosphate, triethylamine of GR grade and Acetonitrile, water HPLC grade was purchased from Merck chemicals limited. Niacin and Simvastatin was obtained from obtained as gift samples from NATCO, Hyderabad. . SIMCOR tablets were procured from local market.

Equipment and chromatographic conditions:

The chromatographic system consisted of Shimadzu HPLC LC-2010 CHT using LC solutions as data handling systems. Hypersil BDS C18 (250 x 4.6 mm, 5µm) was used for this method. All chromatographic runs were carried out in isocratic mode with flow rate of 1.0 ml/min. Acetonitrile and Ammonium dihydrogen phosphate in the ratio of 80:20 with pH adjusted to 5 is used as buffer solution. The detector wavelength was set at 254nm. The injection volume was 20µL.

Table 1: Optimized chromatographic conditions

Stationary phase	Hypersil BDS C18 (250 x 4.6 mm), 5µm
Operating temperature	Room temperature
Mobile phase	ACN: Ammonium dihydrogen phosphate, pH-5, (80:20) v/v.
Injection volume	20 microlitre.
Flow rate	1.0 mL/min
Selected wave length	254 nm.
Diluent	ACN: Ammonium dihydrogen phosphate, pH-5, (80:20) v/v.
Run time	10 min.

Standard solutions:
Preparation of Niacin and Simvastatin stock solutions
Accurately weighed quantity of 50 mg Niacin and 2 mg Simvastatin was transferred to a 100 ml volumetric flask, dissolved in 50 ml of mobile phase, sonicated for 15 min and the volume was made up to 100 ml with mobile phase.

Preparation of working standard solutions:
Working standards solutions of Niacin and Simvastatin were prepared by diluting the 1ml, 2 ml, 3ml, 4 ml, 5 ml, and 6 ml of stock solution in six different 25 ml volumetric flasks with the mobile phase to the give the following concentrations.
Niacin: 20 µg /ml, 40 µg /ml, 60 µg /ml, 80 µg /ml, 100 µg /ml and 120 µg /ml
Simvastatin: 0.8 µg /ml, 1.6 µg /ml, 2.4 µg /ml, 3.2 µg /ml, 4 µg /ml and 4.8 µg /ml

Sample preparation:
Accurately weigh 10 tablets, average weight is taken and powdered. Amount equivalent to 50 mg Niacin and 2 mg Simvastatin was accurately weighed and taken in a 100 ml volumetric flask and 50 ml of mobile phase was added. The mixture was subjected to sonication for 20 min with intermediate shaking for complete extraction of drugs. Filtered and cooled to room temperature and solution was made up to mark with mobile phase. From the above solution 5 ml is taken and further diluted in 25 mL volumetric flasks with mobile phase. To acquire a concentration of 100 µg of Niacin and 4 µg of Simvastatin.

Procedure for assay:
It employs a separate injection of a fixed volume of sample and standard Solution into liquid chromatogram and the peak areas are integrated and concentration is calculated.

$$\% \text{ percentage content} = \frac{\text{Sample area} \times \text{Sample dilution} \times \text{Avg weight} \times \text{standard weight} \times \text{purity of working standard} \times 100}{\text{Standard area} \times \text{standard dilution} \times \text{label claim} \times \text{sample weight}}$$

RESULTS AND DISCUSSION:

During initial method optimization studies C18 columns with different column lengths were tried. Finally chromatographic conditions (Table 1) were finalized after evaluating column efficiency parameters like theoretical plates and tailing. Wavelength was selected by scanning standard solution of drug with diluent as blank, over a wavelength region of 200nm to 400nm. Using mobile phase, base line separation was achieved. The retention time was found to be 2.0852 for Niacin and 5.7052 for Simvastatin.

System suitability studies: System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (*R*T), number of theoretical plates (*N*), tailing factor (*T*), and peak asymmetry (*A*S), resolution (RS) were evaluated for five replicate injections of the drug. The system suitability test was performed using five replicate injections of standards before analysis of samples.

Table 2: System suitability parameters

Parameters	Niacin	Simvastatin	Acceptance Criteria
Peak asymmetric factor	1.278	1.5964	NMT 2.0
No. of theoretical plates	2692	7945	NLT 2000
% RSD of Peak areas	0.97	1.97	NMT 2.0
Retention time	2.0852	5.7052	
Resolution	15.779		NLT 2.0

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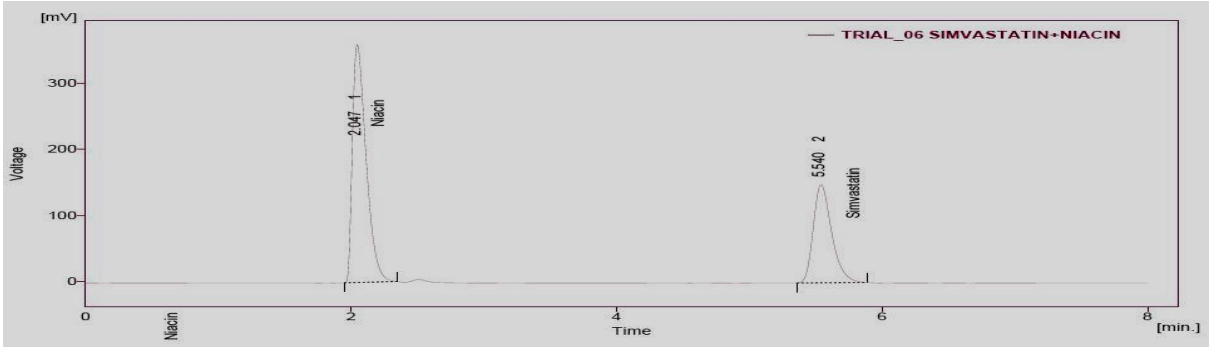

Fig 3: Representative chromatogram obtained from the analysis of Niacin and Simvastatin in optimized chromatographic conditions

Linearity:

The calibration curve was plotted for the drug and it was found to be linear over the concentration range of 20% to 120%. The regression plot was computed by least square regression method and is shown in Figure 4. The correlation coefficient is 0.99 and %RSD studied for each concentration studied was less than 2. The linearity data is shown in table 3.

Table 3: Linearity data

Concentration of Niacin (µg/mL)	Peak area (mV.s)	Concentration of Simvastatin (µg/mL)	Peak area (mV.s)
20	391.222	0.8	134.936
40	794.883	1.6	281.806
60	1278.733	2.4	427.687
80	1690.916	3.2	563.173
100	2111.890	4.0	682.895
120	2510.840	4.8	811.054

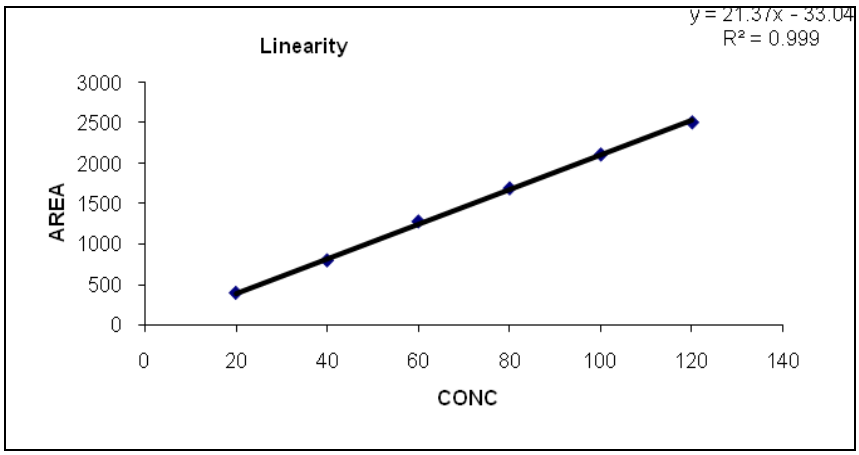

Fig 4: Linearity plot for Niacin

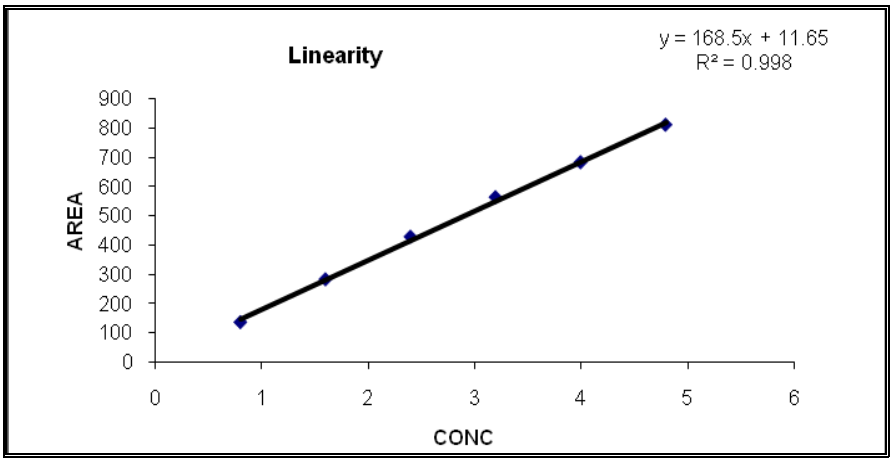

Fig 5: Linearity plot for Simvastatin

Accuracy and Precision:

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery and standard deviation of the percentage recovery were calculated and represented in table 4. The high percentage of recovery indicates that the proposed method is highly accurate. The precision of the method was demonstrated by system precision and method precision variation studies. Five replicate injections of the sample solutions were made and the percentage RSD was calculated and represented in table 5. From the data obtained the developed RP-HPLC method was found to be precise.

Table 4: Accuracy data of the proposed method

Analyte	Concentration level	Mean recovery	% Mean recovery
Niacin	80%	89.5645 µg	99.51
	100%	108.9256 µg	99.02
	120%	129.3773 µg	99.52
Simvastatin	80%	3.569183 µg	99.14
	100%	4.370849 µg	99.33
	120%	5.160354 µg	99.23

Table 5: Precision data for proposed method

Analyte		**System precision**	**Method precision**
Niacin	**Mean peak area**	2018.073	2125.503
	SD	19.59803	18.19356
	%RSD	0.97	0.855365
Simvastatin	**Mean peak area**	663.566	699.575
	SD	13.0877	12.90105
	%RSD	1.97	1.844127

Robustness and Ruggedness:

For demonstrating the robustness and ruggedness of the developed method, experimental conditions were purposely altered and evaluated. The robustness of the method was studied by carrying out experiments by changing conditions like wavelength and flow rate and ruggedness by changing analysts. The results are summarized in table 6 and 7. The developed method is found to be robust and rugged.

Table 6: Robustness

Parameter	**Variation**	**Rt of Niacin (min)**	**Rt of Simvastatin (min)**
Flow rate ml/min	0.9 ml/min	2.250	6.220
	1.1 ml/min	1.860	5.140
Detection wavelength (nm)	252 nm	2.037	5.607
	256 nm	2.037	5.610

Table 7: Ruggedness from analyst to analyst

Analysts	**Rt of Niacin (min)**	**Rt of Simvastatin (min)**	**Area of Niacin (mV.s)**	**Area of Simvastatin (mV.s)**
Analyst 1	2.053	5.627	2323.225	764.595
Analyst 2	2.050	5.607	2280.562	749.582
Mean	2.0515	5.617	2301.894	757.0885
S.D	0.00212	0.01414	30.1673	10.6157
% R.S.D	0.10340	0.25177	1.31054	1.40218

Limit of Detection and Quantification:

The LOD and LOQ values are given in the table 8.

Table 8: LOD and LOQ Data

Sample	**LOD**	**LOQ**
Niacin	3.598 µg	10.904 µg
Simvastatin	42.486 µg	128.746 µg

ASSAY

The commercial tablet formulation **SIMCOR** tablets (Niacin-500 mg and Simvastatin- 20 mg) were analysed by the proposed method. The purity of working standard of Niacin is 99.86% and of Simvastatin is 99.85%. The assay procedure was performed and the assay percentage was calculated as per the given formula. The value was found to be in good agreement with the labelled amounts, which confirms the suitability of the method for the analysis of Niacin and Simvastatin in pharmaceutical dosage forms.

$$\% \text{ percentage content} = \frac{\text{Sample area} \times \text{Sample dilution} \times \text{Avg weight} \times \text{standard weight} \times \text{purity of working standard} \times 100}{\text{Standard area} \times \text{standard dilution} \times \text{label claim} \times \text{sample weight}}$$

The assay chromatograms and results are given in the table 9.

Table 9: Summary of Assay results

Drug	Peak areas of standard	Mean Standard area (mV.s)	Peak areas of sample	Mean Sample area (mV.s)	% Assay	Amount present	Labeled amount
Niacin	2132.082	2117.967	2103.007	2116.881	99.642	498.21 mg	500 mg
	2103.853		2130.756				
Simvastatin	701.129	702.854	697.274	701.444	99.087	19.817 mg	20 mg
	704.579		705.614				

CONCLUSION:

The proposed RP-HPLC method is sensitive, precise and accurate and can be used for routine quality control analysis for the determination of Niacin and Simvastatin in its tablet dosage form.

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