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

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ABSTRACT

This work is concerned with application of simple, accurate, precise and highly selective reverse phase high performance liquid chromatographic (RP-HPLC) method for simultaneous estimation of Niacin (NA) and Simvastatin (SIM) in combined dosage form. Chromatographic separation was achieved by isocratic mode using a reverse phase C18 column (phenomenx, 150 x 4.6 mm i.d.). The mobile phase composed methanol:water in ratio 85:15 water consisting of Triethylamine (TEA) (0.05%v/v) v/v at flow rate of 1 ml/min pH was adjusted to 4 with orthophosporic acid. Detection was carried out using a UV-vis detector at 250 nm. The mean retention time of NA and SIM was found to be 1.8 min and 8.5 min. respectively. The method was found to be linear in the range of 80-120 µg/ml with mean recovery of 99.39% for NA and 99.18% for SIM. The correlation coefficients for both NA and SIM were close to 1. The developed method was validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values. Thus the proposed method was successfully applied for simultaneous determination of NA and SIM in routine analysis of formulation.

Key Words: *Niacin, Simvastatin, Reverse phase high performance liquid chromatographic (RP-HPLC), isocratic mode.*

INTRODUCTION

Niacin (NIA) (Figure 1) chemically designated as Pyridine-3-carboxylic acid used in treating hyperlipedimic condition and has found to effective for increasing serum HDL levels [1]. It has also been demonstrated that this drug lowers the occurrence of coronary heart disease in subjects [1]. Literature indicates number of analytical methods for determination of NIA in pharmaceutical formulations or in biofluids either alone or in combination with other drugs [2-8]. The methods include determination of niacin by LC-MS [9], HPLC [9-11], flow injection and spectrofluorimetric analysis.

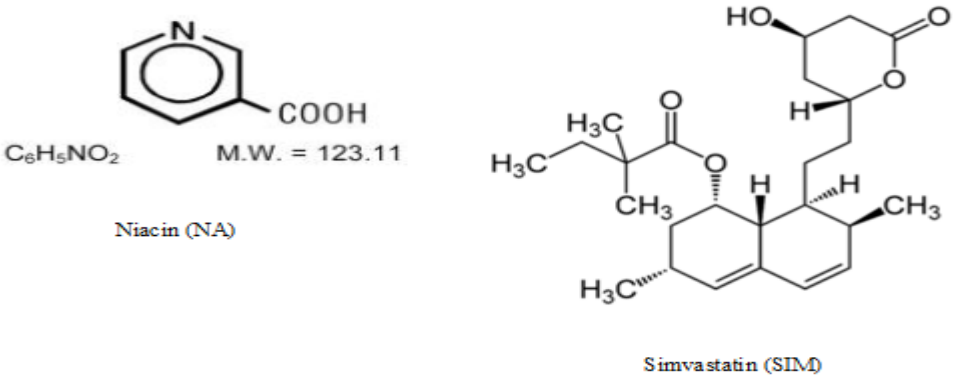

Fig.1. Chemical strucrure of Niacin and Simvastatin

Simvastatin (SIA)(Fig.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-dimethyl butanoate. SIA an HMG-CoA reductase inhibitor acts by decreasing cholesterol synthesis and by increasing low density lipoprotein (LDL) catabolism via increased LDL receptor activity [12]. It is used in treatment of hypercholesterolemia [13]. Literature supports number of analytical methods for the determination of simvastatin alone or in combination with other drugs by HPLC [14], LC-MS/MS [15], micelle electro kinetic chromatographic method [16], voltametric technique [17] and spectrophotometry [18].

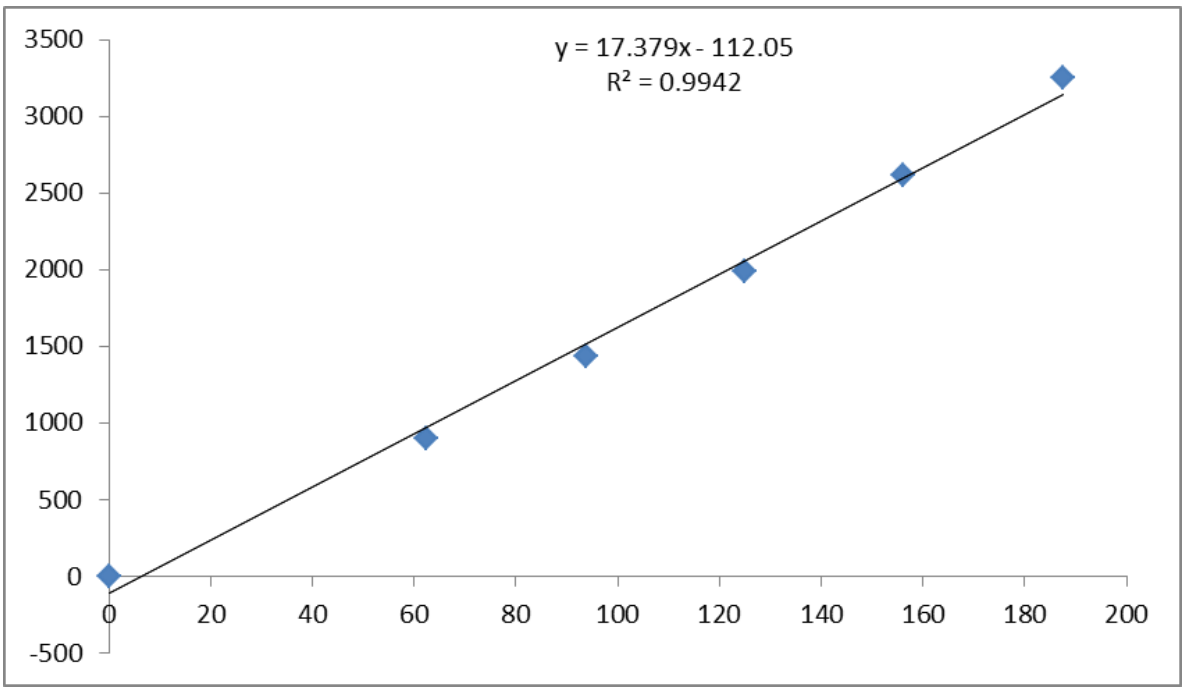

Fig. No.2: Plot of linearity and range study for NA

Simvastatin in combination with Niacin has provided benefits in patients with coronary disease and low HDL levels [19]. A fixed-dose combination comprising niacin 1000mg and 20 mg of simvastatin per tab has been approved by U.S. Food and Drug Administration (FDA) for treating patients with complex lipid abnormalities where

treatment with niacin or simvastatin alone is not sufficient [20].

Literatures suggested a HPLC method for selected drug combination [21] using HPLC. The reported method made use of 10Mm phosphate buffer and acetonitrile. Use of buffer is always regarded to diminish column life and is damaging to stationary phase also the method made use of acetonitrile which is a costlier organic phase as compared to methanol. Present work reports development of simple and rapid HPLC method involved with UV detection which makes use of water as a component of mobile phase offering advantage and not making use of buffer as component of mobile phase also methanol made the process of analysis cheap moreover the method proved to be rapid with total run time of 10 min.

EXPERIMENTAL

Chemicals & Reagents:

NA and SIM were obtained as gift samples from Umedica Pvt Ltd. Vapi and Piramal Pvt. Ltd. Indore, India. All chemicals and reagents were of analytical grade. HPLC grade methanol, water and TEA from Loba chem. A commercial sample of tablets of containing NA and SIM in ratio of 125 mg: 5 mg respectively was procured from local market.

Instrumentation and chromatographic conditions:-

The HPLC system consisted of a Thermo separation products quaternary gradient system equipped with HPLC pump spectra system P4000 with online degasser and a variable UV-visible detector spectra system UV 1000. Stationary phase used was a reverse-phase C18 column (phenomenax, 150mm x4.6mm i.d. particle size 5µ), including a manual Rheodyne injector with 20 µl fixed loop. The data was acquired by a chromatography module connected to a personal computer and processing was performed running DATA-ACE software. The chromatographic conditions were optimized by varying the concentration and pH of water and the percentage of organic solvent. The mobile phase consisted of methanol:water 85:15 (v/v) in a isocratic mode with water containing 0.05% Triethylamine (TEA), pH was adjusted to 4 with orthophosporic acid, flow rate was 1.ml/min. The mean retention time for NA and SIM was 1.8 and 8.5 min respectively.

Preparation of standard solution:-

Accurately weighed quantity of NIA 50 mg was transferred to 100 ml volumetric flask, shaken vigorously for five minutes and volume was made up to mark with methanol. And accurately weighed quantity of SIM 20 mg was transferred to 100 ml volumetric flask, shaken vigorously for five minutes and volume was made up to mark with methanol. The standard solution of NA & SIM were mixed and diluted with methanol properly to obtain laboratory mixtures containing a concentration 20 µg /ml of SIM and 500 µg /ml of NA. The nominal concentrations in range of 80, 90, 100, 110,120 µg/ml, were prepared for calibration. Tablets of NA and SIM combination are available in 1:25 ratio.

Sample Preparation:-

Twenty tablets were weighed and average weight was determined. Tablets were finely powdered and weighed tablet powder equivalent to 125 mg of NA and 5 mg SIM was transferred in a 100 ml volumetric flask and Methanol was added. It was shaken vigorously for 5 to 10 minutes and sonicated. Later the volume was made up to mark with methanol. The solution was filtered through 0.45 µm membrane filter. Further dilution was done with methanol to get concentration of 20 µg /ml of SIM and 500 µg /ml of NA.

RESULT AND DISCUSSION

Method development and optimization of chromatographic conditions:

Chromatographic separation was achieved on C18 stationary (phenomenax, 150mm x4.6mm i.d. particle size 5µ) phase by varying concentration of organic phase and water simultaneously pH was varied. Mobile phase was optimized for method advantage by making use of water instead of buffer hence in the process of optimization success was achieved by making use of methanol: water 85:15 (v/v) in a isocratic mode with water modified with 0.05% Triethylamine (TEA), pH was adjusted to 4

with orthophosporic acid, flow rate was 1.ml/min. both NA and SIM were well separated from each other with mean retention time for NA and SIM as 1.8 and 8.5 min respectively.

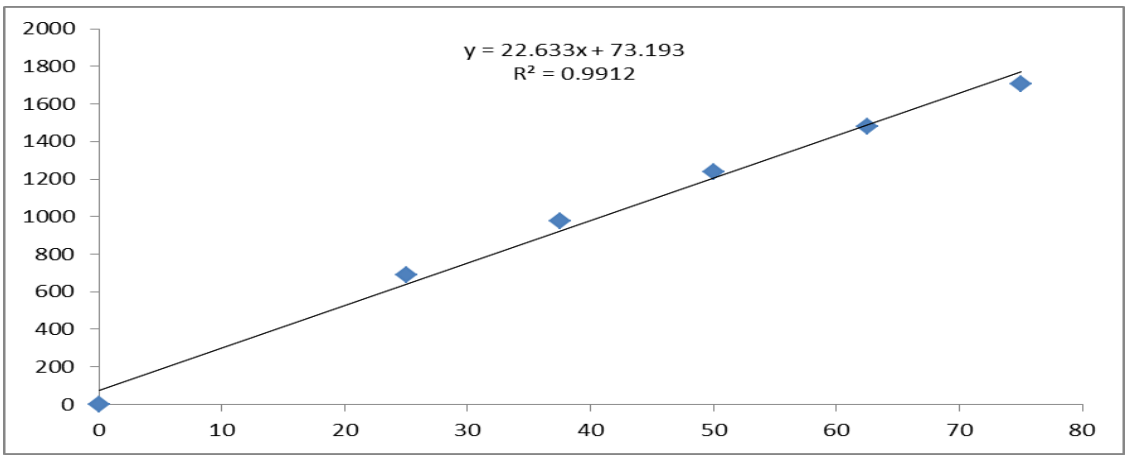

Fig. No.3: Plot of linearity and range study for SIM

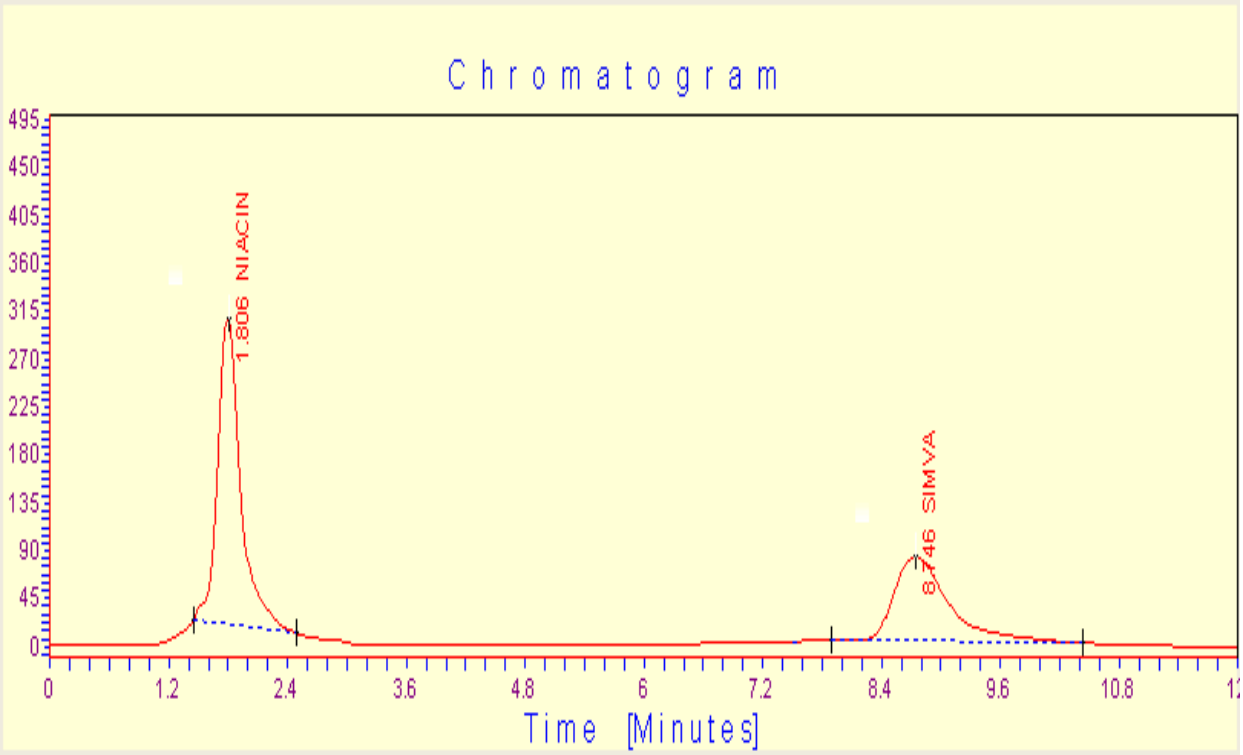

Figure 4: HPLC chromatogram for Niacin and Simvastatin in developed chromatographic conditions

Method validation:

Linearity:

The method was linear in the range of 80 µg/ml to 120µg/ml for both NA and SIM standards. Linear regression data was given in Table 1.

Table 1: Linear regression data for calibration curves

Parameter	**Niacin**	**Simvastatin**
Linearity range (µg/ml)	80 - 120	80 – 120
Correlation coefficient	0.994	0.991
Intercept	112.05	73.793

Table 2: Summary of laboratory mixture and marketed formulation Analysis by RP-HPLC Method

Sr. No.	**Sample**	**Statistical data**	**% Estimation**		**%Recovery ReRecovery**	
			NA	**SIM**	**NA**	**SIM**
1.	**Standard Laboratory mixture**	Mean	99.93	101.56		-
		S.D.	1.45	0.79	-	-
		%RSD	1.45	0.78	-	-
2.	**Simvotin**	Mean	100.4	99.46	99.39	99.18
		S.D.	0.19	0.53	0.45	0.67
		%RSD	0.19	0.53	0.45	0.67

Table 3: Summary of results of Ruggedness by RP-HPLC method

Parameter	**Statistical data**	**% Estimation by RP-HPLC method**	
		NA	**SIM**
Interday	**Mean**	99.97	99.56
	S.D	0.21	0.42
	%RSD	0.21	0.42
Intraday	Mean	99.61	98.81
	S.D	0.87	0.55
	%RSD	0.87	0.55
Different analyst	Mean	100.10	99.40
	S.D	0.24	0.37
	%RSD	0.23	0.37

Precision:

For the precision study, repeatability study was carried out for short time interval under the same chromatographic condition. The sample was injected in three replicate. The peak area for all the three replicate was recorded. The mean and % relative standard deviation (%RSD) was calculated. The intraday %RSD for NA and SIM were found to be 0.87 and 0.55 respectively. The interday %RSD for NA and SIM were found to be 0.21 and 0.42 respectively. From the data obtained the developed RP-HPLC method was found to be precise.

Accuracy:

The accuracy of the method was determined by recovery experiments. Known concentration of working standard was added to the fixed concentration of the pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of working standard. For both the drugs, recovery was performed in the same way. The recovery studies were performed in triplicate. This standard addition method was performed at 80%, 100%, 120% level and the percentage recovery was calculated. Percent recovery was within the range of 99.31 for NA and 99.18 for SIM which indicates that the method was accurate.

Specificity Studies:

Specific studies were done by assessing any interferences from placebo. In the study subsequent chromatographic runs of

diluents was done and any interference at the retention of analytes was observed. Study concluded that there were no interfering peaks for the diluents in developed chromatographic conditions.

CONCLUSION

The proposed RP-HPLC method allows for accurate, precise and reliable measurement of NA and SIM simultaneously in combined dosage form. The developed RP-HPLC method was found to be simple, rapid, selective, accurate and precise for the concurrent estimation of drugs in respective two component tablet dosage form of NA and SIM. The RSD for all parameters was found to be less than two, which indicates the validity of method and assay results obtained by this method are in fair agreement. The developed method can be used for routine quantitative simultaneous estimation of NA and SIM in multicomponent pharmaceutical tablet dosage form.

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