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NEW RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF NIACIN AND LOVASTATIN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple and selective LC method is described for the determination of niacin and lovastatin in tablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 55 volumes of water and 45 volumes of Methanol with detection of 240nm. Linearity was observed in the range 20-100 µg/ml for niacin (r^2 =0.998) and 10-30 µg /ml for lovastatin (r^2 =0.993) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Kev Words: niacin. lovastatin. selective LC method

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INTRODUCTION

Pharmaceutical analysis simply means analysis of pharmaceuticals. Webster' dictionary defines a pharmaceutical is a medical drug. A more appropriate term for a pharmaceutical is active pharmaceutical ingredient (API) or active ingredient to distinguish it from a formulated product or drug product is prepared By formulating a drug substance with inert ingredient (excipient) to prepare a drug product that is suitable for administration to patients. Research and development (R&D) play a very comprehensive role in new drug development and follow up activities to ensure that a new drug product meets the established standards is stable and continue to approved by regulatory authorities, assuring that all batches of drug product are made to the specific standards utilization of approved ingredients and production method becomes the responsibility of pharmaceutical analysts in the quality control (QC) or quality assurance department. The methods are generally developed in an analytical R&D department and transferred to QC

or other departments as needed. At times they are transferred to other divisions.

Reverse phase chromatography uses hydrophobic bonded packing, usually with an octadecyl or octyl functional group and a polar mobile phase, often a partially or fully aqueous mobile phase. Polar substances prefer the mobile phase and elute first. As the hydrophobic character of the solutes increases, retention increases. Generally, the lower the polarity of the mobile phase, the higher is its eluent strength. The elution order of the classes of compounds in table is reversed (thus the name reverse-phase chromatography) (1-2).

Niacin-A water-soluble vitamin of the B complex occurring in various animal and plant tissues. It is required by the body for the formation of coenzymes NAD and NADP. It has pellagra-curative, vasodilating, and antilipemic properties. Niacin binds to Nicotinate D-ribonucleotide phyrophsopate phosphoribosyltransferase, Nicotinic acid phosphoribosyltransferase, Nicotinate N-methyltransferase and the Niacin receptor. Niacin is the precursor to nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), which are vital cofactors for dozens of enzymes. The mechanism by which niacin exerts its lipid lowering effects is not entirely understood, but may involve several actions, including a decrease in esterification of hepatic triglycerides. Niacin treatment also decreases the serum levels of apolipoprotein B-100 (apo B), the major protein component of the VLDL (very low-density lipoprotein) and LDL fractions.

Niacin and niacinamide are indicated for prevention and treatment of vitamin B3 deficiency states. Vitamin B3 (Niacin) also acts to reduce LDL cholesterol, triglycerides, and HDL cholesterol. The magnitude of individual lipid and lipoprotein responses may be influenced by the severity and type of underlying lipid abnormality. The increase in total HDL is associated with a shift in the distribution of HDL subfractions (as defined by ultra-centrifugation) with an increase in the HDL2:HDL3 ratio and an increase in apolipoprotein A-I content. Vitamin B3 (Niacin) treatment also decreases the serum levels of apolipoprotein B-100 (apo B), the major protein component of the VLDL (very low-density lipoprotein) and LDL fractions, and of lipoprotein-a, a variant form of LDL independently associated with coronary risk.

Lovastatin is a cholesterol-lowering agent that belongs to the class of medications called statins. It was the second agent of this class discovered. It was discovered by Alfred Alberts and his team at Merck in 1978 after screening only 18 compounds over 2 weeks. The agent, also known as mevinolin, was isolated from the fungi *Aspergillus terreus*. Research on this compound was suddenly shut down in 1980 and the drug was not approved until 1987. Interesting, Akira Endo at Sankyo Co. (Japan) patented lovastatin isolated from *Monascus ruber* four months before Merck. Lovastatin was found to be 2 times more potent than its predecessor, mevastatin, the first discovered statin. Like mevastatin, lovastatin is structurally similar to hydroxymethylglutarate (HMG), a substituent of HMG-Coenzyme A (HMG-CoA), a substrate of the cholesterol biosynthesis pathway via the mevalonic acid pathway. Lovastatin is a competitive inhibitor of HMG-CoA reductase with a binding affinity 20,000 times greater than HMG-CoA. Lovastatin differs structurally from mevastatin by a single methyl group at the 6' position. Lovastatin is a prodrug that is activated by *in vivo* hydrolysis of the lactone ring. It, along with mevastatin, has served as one of the lead compounds for the development of the synthetic compounds used today. Lovastatin is structurally similar to the HMG, a substituent of the endogenous substrate of HMG-CoA reductase. Lovastatin is a prodrug that is activated *in vivo* via hydrolysis of the lactone ring to form the β-hydroxyacid. The hydrolyzed lactone ring mimics the tetrahedral intermediate produced by the reductase allowing the agent to bind to HMG-CoA reductase with 20,000 times greater affinity than its natural substrate. The bicyclic portion of lovastatin binds to the coenzyme A portion of the active site. The primary cause of cardiovascular disease is atherosclerotic plaque formation. Sustained elevations of cholesterol in the blood increase the risk of cardiovascular disease. Lovastatin lowers hepatic cholesterol synthesis by competitively inhibiting HMG-CoA reductase, the enzyme that catalyzes the rate-limiting step in the cholesterol biosynthesis pathway via the mevalonic acid pathway.

Decreased hepatic cholesterol levels causes increased uptake of low density lipoprotein (LDL) cholesterol and reduces cholesterol levels in the circulation. At therapeutic doses, lovastatin decreases serum LDL cholesterol by 29-32%, increases high density lipoprotein (HDL) cholesterol by 4.6-7.3%, and decrease triglyceride levels by 2-12%. HDL cholesterol is thought to confer protective effects against CV disease, whereas high LDL and triglyceride levels are associated with higher risk of disease (3-5).

Aim is to develop new RP HPLC method for the simultaneous estimation of niacin and lovastatin pharmaceutical dosage form.

MATERIALS AND METHODS

Determination Of Working Wavelength (λmax) (6-8)

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

Preparation of standard stock solution of niacin

10 mg of NIACIN was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard stock solution of lovastatin

10 mg of LOVASTATIN was weighed in to 100ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Assay

Preparation of samples for Assay

Preparation of mixed standard solution

weigh accurately 10 mg of niacin and 10 mg of lovastatin in 25ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 40µg/ml of NIACIN and 40µg/ml of lovastatin is prepared by diluting 1.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets (each tablet contains lovastatin– 20 mg NIACIN -1000 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of lovastatin and niacin (µg/ml) were prepared by dissolving weight equivalent to 10 mg of Lovastatinand 10 mg of niacin and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 10ml with mobile phase. Further dilutions are prepared in 5 replicates of 20µg/ml of lovastatin and 40µg/ml of niacin was made by adding 1.5 ml of stock solution to 10 ml of mobile phase.

Validation

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated. Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100%, 150%.

RESULTS AND DISCUSSION

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra are shown in the fig-1 and the absorption curve shows characteristic absorption maxima. The isobestic point was found to be 240 nm for the combination.

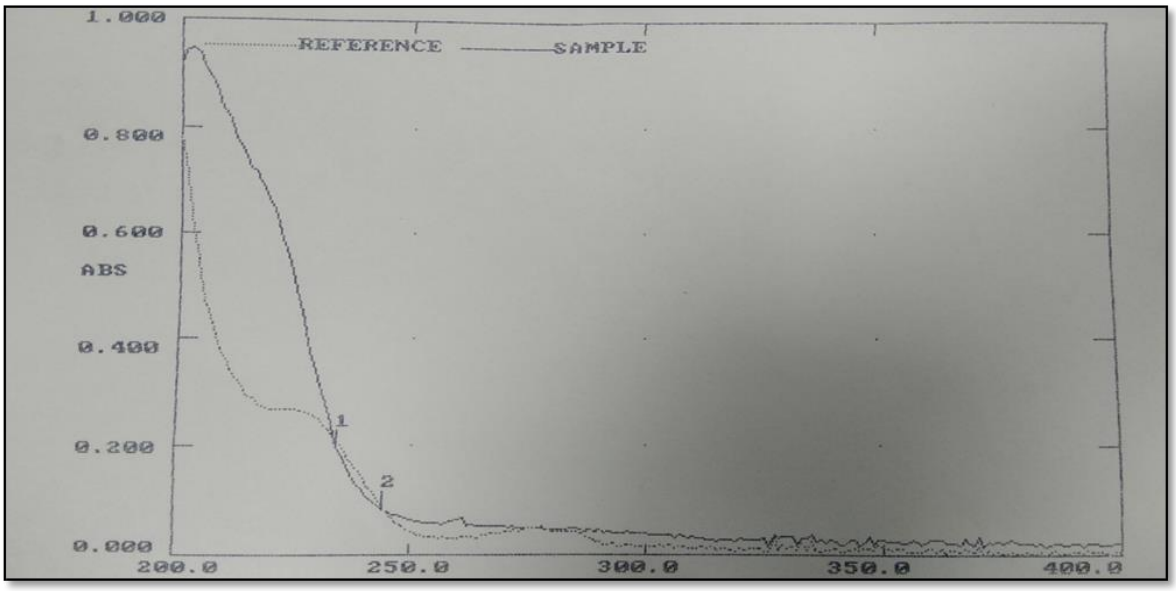
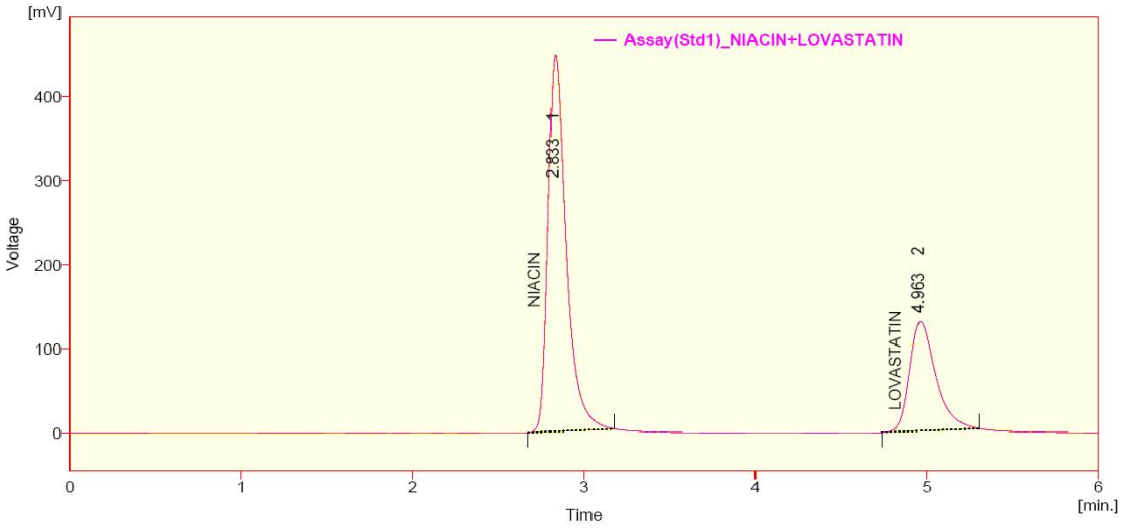


Fig-1 UV-VIS spectrum of niacin and lovastatin and the isosbestic point was 240nm

The amount of niacin and lovastatin present in the taken dosage form was found to be 98.40% and 99.87 % respectively (Fig-2 and table-1).



Result Table (Uncal - Assay(Std1)_NIACIN+LOVASTATIN

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.833	3275.556	447.475	70.43
2	4.963	1374.953	130.029	29.57
	Total	4650.509	577.503	100.00

Column Performance Table (From 50% - Assay(Std1)_NIACIN+LOVASTATIN

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.833	0.110	1.792	3676	36755	-
2	4.963	0.160	1.722	5331	53311	9.284

Fig-2 Chromatogram of Assay preparation

Table-1 Assay Results

	Niacin		Lovastatin	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	1374.953	1357.053	3275.556	3265.567
Injection-2	1377.148	1358.43	3264.35	3263.581
Injection-3	1367.963	1337.954	3251.196	3272.933
Injection-4	1373.965	1363.191	3264.87	3264.071
Injection-5	1389.462	1356.846	3277.547	3246.908
Average Area	1376.698	1354.695	3266.704	3262.612
Standard deviation	9.702838		9.551679	
%RSD	0.716238		0.292762	
Assay(%purity)	**98.40**		**99.87**	

The % RSD for the retention times and peak area of niacin and lovastatin were found to be less than 2%. The plate count and tailing factor results were found to be satisfactory and are found to be within the limit (Table-2 and 3).

Table-2 Results for system suitability of niacin

Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	4.963	1374.953	5331	1.722
2	4.947	1377.148	5766	1.714
3	4.957	1367.963	5789	1.743
4	4.943	1373.965	5758	1.743
5	4.953	1389.462	5781	1.771
Mean	4.9526	1376.698	-	-
SD	0.007925	7.904694	-	-
%RSD	0.16001	0.574178	-	-

Table-3 Results for system suitability of lovastatin

Injection	Retention time (min)	Peak area	Theoretical plates	Tailing factor
1	2.833	3275.556	3676	1.792
2	2.833	3264.350	3909	1.750
3	2.827	3251.196	3658	1.870
4	2.817	3264.870	3863	1.792
5	2.833	3277.547	3909	1.826
Mean	2.8286	3266.704	-	-
SD	0.006986	10.55144	-	-
%RSD	0.246967	0.323	-	-

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of niacin and lovastatin is 0.998 and 0.993. The relationship between the concentration of niacin and lovastatin and area of niacin and lovastatin is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits. The % recovery of niacin and lovastatin should lie between 98% and 102%.

Test results for lovastatin and niacin are showing that the %RSD of Assay results are within limits. From the observation the between two analysts Assay values not greater than 2.0%, hence the method was rugged (Table-4).

Table-4 Results for Ruggedness

Niacin	%Assay	Lovastatin	%Assay
Analyst 01	100%	**Analyst 01**	99.37%
Anaylst 02	100.82%	**Anaylst 02**	99.58%

CONCLUSION

The above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation niacin and lovastatin was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

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