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DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF NIACIN AND LOVASTATIN IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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Abstract:

A simple specific, sensitive, precise reverse phase high performance liquid chromatography method has been developed for simultaneous estimation of lovastatin and niacin. The determination was carried out by using Symmetry C_{18} (4.6 x 250mm, 5µm) column with the mobile phase containing acetonitrile: phosphate buffer (pH 4) in the ratio of 65:35 v/v. The optimized flow rate was 0.7ml/min and the UV detection was carried out at 240 nm. The retention time of lovastatin and niacin were found to be 3.093 min and 6.196 min respectively. The method was found to be linear in the concentration range 2.0-10µg/ml for lovastatin and niacin respectively. The method was validated as per ICH guidelines. The proposed method was successfully applied for the estimation of lovastatin and niacin in pharmaceutical dosage forms.

Key words: *Lovastatin, Niacin, HPLC, ICH guidelines.*

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INTRODUCTION:

Pharmaceutical analysis derives its principles from various branches of sciences like physics, microbiology, nuclear science, and electronics etc. Qualitative analysis reveals the chemical identity of the sample. Quantitative analysis establishes the relative amount of one or more of these species or analytes in numerical terms. Qualitative analysis is required before a quantitative analysis can be undertaken.

Lovastatin is an inhibitor of 3-hydroxy-3-methyl glutaryl coenzyme A Reductase (HMG-CoA Reductase), an enzyme that catalyses the conversion of HMG-CoA to mevalonate. Mevalonate is a required building block for cholesterol biosynthesis and lovastatin interferes with its production by acting as a reversible competitive inhibitor for HMG-CoA, which binds to the HMG-CoA Reductase. 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-hydroxy acid. The hydrolysed 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.

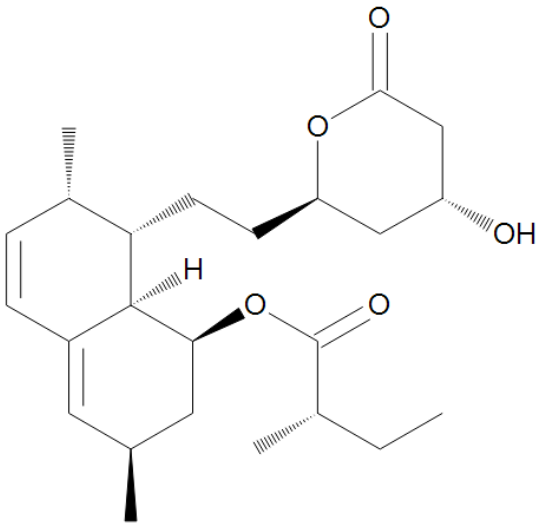

Figure 1: Structure of lovastatin

Niacin, known as Vitamin B_3 or nicotinic acid, is chemically pyridine-3-carboxylic acid official in IP (Indian Pharmacopoeia, 2007); which is a colourless, water soluble solid. It has the ability to reduce low density lipoprotein cholesterol (LDL), very low-density lipoprotein cholesterol (VLDLC), and triglycerides (TG), and also effectively increase high density lipoprotein cholesterol (HDL). Niacin binds to Nicotinate D-ribonucleotide pyrophosphate phosphoribosyl transferase, Nicotinic acid phosphoribosyl transferase, Nicotinate N-methyltransferase and the Niacin receptor.

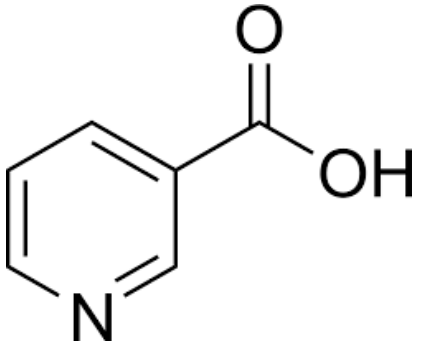

Figure 2: Structure of niacin

In the present study a successful attempt has been made to develop a rapid, precise, accurate and comparatively economical RP-HPLC method for quantitative estimation of Isotretinoin in. The developed method validated and recovery studies were conducted and studied by using various statistical parameters according to ICH guidelines.

MATERIALS AND METHODS:

Instruments:

The HPLC system (WATERS model 2487) consisting of dual λ absorbance detector containing 515 HPLC pump, Rheodyne injector (7725i) with 20µl fixed loop. The output signal was monitored and integrated using Empower 2 software.

Chemicals:

Acetonitrile and water HPLC grade, orthophosphoric acid, potassium dihydrogen phosphate of AR grade was obtained from Merck, Mumbai, India.

Selection of detection wavelength:

10 mg of Niacin and Lovastatin was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Niacin and Lovastatin. The isobestic point was taken as detection wavelength.

Preparation of mobile phase:

The mobile phase was prepared by mixing 0.01M potassium dihydrogen phosphate adjust to 4 PH with ortho phosphoric acid and acetonitrile in the ratio of (35:65% v/v).

Diluent preparation:

Mobile phase is used as Diluent.

Preparation of standard stock solution:

Initially 50mg of Lovastatin was weighed accurately and transferred to 100ml volumetric flask, about few ml of methanol was added and sonicated to dissolve. The final volume was made up to mark with methanol and 1ml of this solution transferred to 100ml volumetric flask, volume was made up to the mark with 100ml of mobile phase to obtain 50µg/ml of Lovastatin solution. Finally, 4ml of this solution transferred to10ml volumetric flask, volume was made up to the mark with mobile phase to obtain final concentration of Lovastatin solution as 20µg/ml. Different aliquots 1,2,4,6,8,10 ml of standard stock solutions was transferred into 10ml volumetric flasks and volume was adjusted to mark to obtain the concentration in the ranging from 5µg/ml to 50µg/ml.

Methods development:

The developed method was fully validated for the parameters as per ICH guidelines. **System Suitability** System suitability is done by replicate analysis of 6 injections at the concentration of 16µg /mL of Lovastatin and 200µg /mL of Niacin were injected six times and the chromatograms were recorded for the same.

Linearity:

Linearity is determined by a series of three to six injections of five or more standards. Peak areas (or heights) of the calibration standards are usually plotted in the Y-axis against the nominal standard concentration, and the linearity of the plotted curve is evaluated through the value of the co-relation coefficient (r^2). The methods were linear in the range of 8-24 ppm lovastatin 100-300 ppm for niacin and inject each level into the chromatographic system and measure the peak area.

Preparation of stock solution: Accurately 20mg of Lovastatin and 250mg of Niacin working standard were weighed and transferred into a 100ml of clean dry volumetric flask and about 70mL of Diluents was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent (Stock Solution).

Accuracy:

Accuracy of the method was determined by Recovery studies. To the formulation (preanalyzed 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.

Limit of detection and limit of quantification:

The limit of detection and quantification were calculated using signal to noise ratio. The LOD for lovastatin and niacin were tested at specific level i.e. 0.115µg/ml and 0.121µg/ml. The LOQ for lovastatin and niacin were tested at specific level i.e. 0.384µg/ml and 0.036µg/ml.

RESULTS AND DISCUSSION:

Wavelength detection:

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Niacin and Lovastatin was obtained and the isobestic point of Niacin and Lovastatin showed absorbance's maxima at 242 nm.

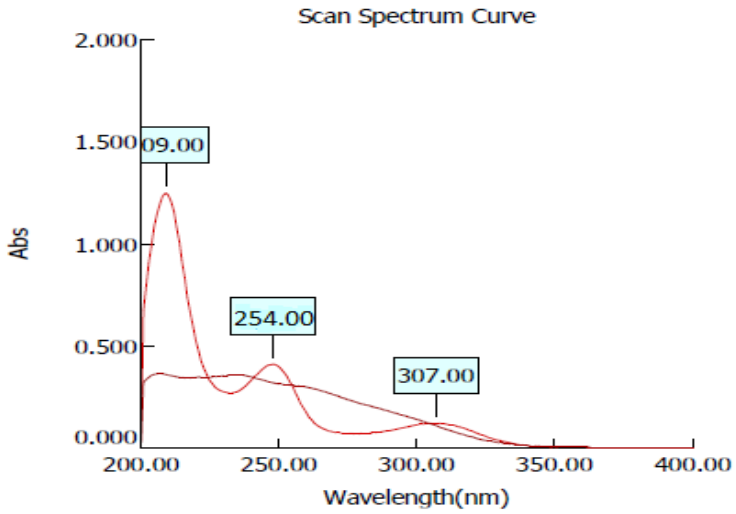

Figure 3: Overlay spectrum of Niacin and Lovastatin

System suitability:

System suitability tests were carried out on freshly prepared standard solutions. Linearity The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of Lovastatin and Niacin is 0.999and 0.999 respectively. It shows that the good correlation exists between the drug and response.

Specificity:

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study was performed by injecting blank.

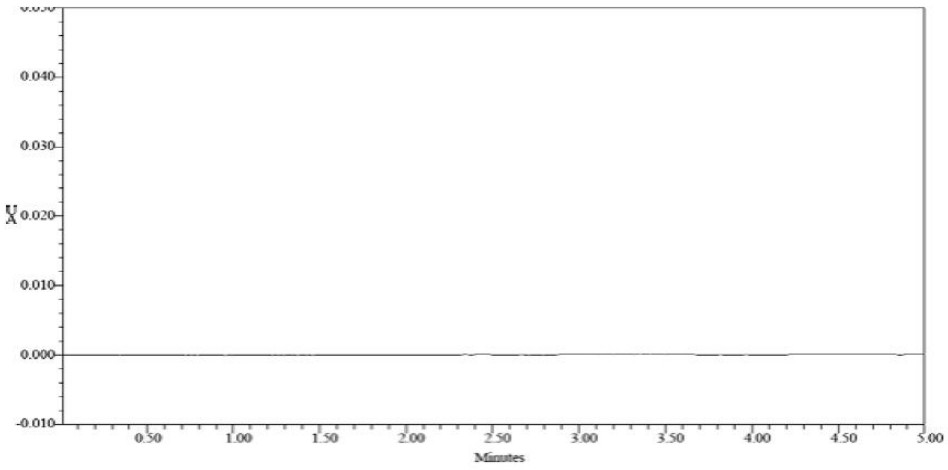

Figure 4: Chromatogram of blank Injection

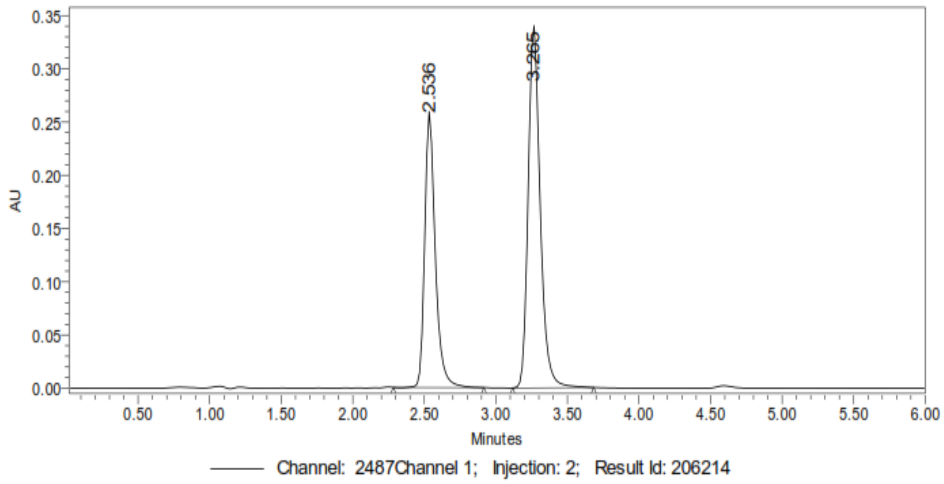

Figure 5: Chromatogram of sample Injection

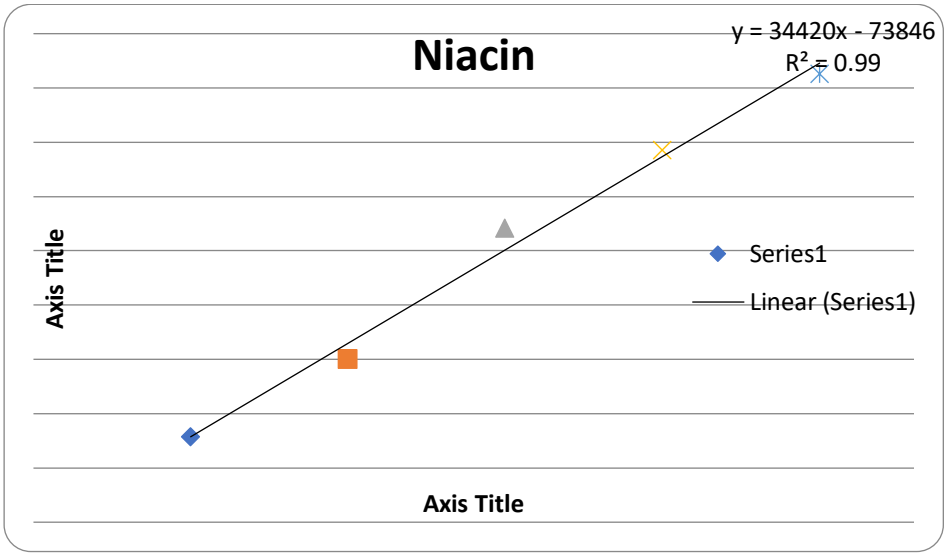

Figure 6: Calibration curve of Lovastatin

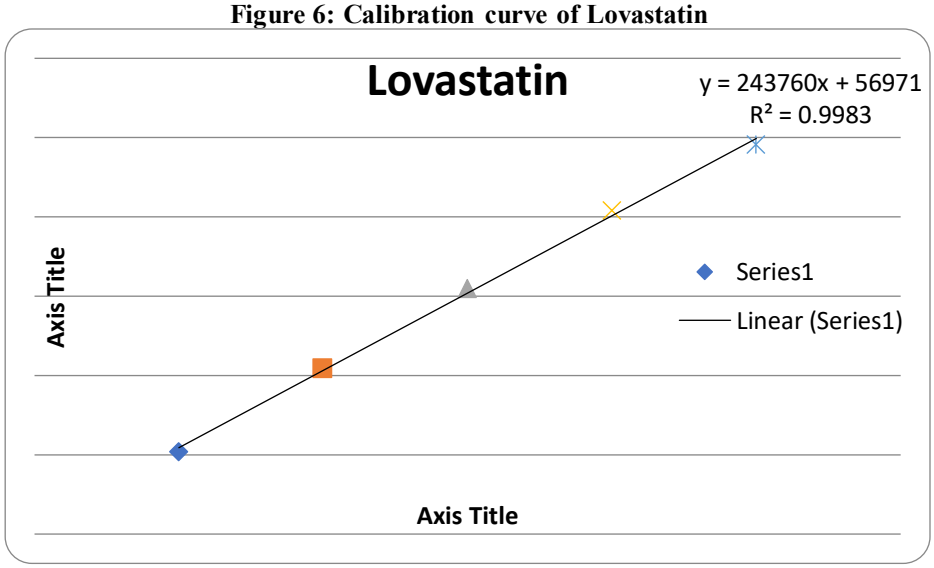

Figure 7: Calibration curve of Niacin

CONCLUSION:

A new method was established for simultaneous estimation of Lovastatin and Niacin by RP-HPLC method. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2. The retention times were found to be 3.503 mins and 2.577 mins. The % purity of Lovastatin and Niacin was found to be 100.3% and 101.1% respectively. The system suitability parameters for Lovastatin and Niacin such as theoretical plates and tailing factor were found to be 1.3, 5824.4 and 1.2, 2936.0 the resolution was found to be 9.4. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Lovastatin and Niacin was found in concentration range of 20µg-100µg and 20µg-100µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % mean recovery was found to be 102.5% and 101.0%, %RSD for repeatability was 0.6 and 0.5, % RSD for intermediate precision was 0.7 and 0.6 respectively. The precision study was precise, robust, and repeatable. LOD value was 3.1 and 3.02, and LOQ value was 10.1 and 10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Lovastatin and Niacin in API and Pharmaceutical dosage form.

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