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APPLICATION OF RP-HPLC FOR SIMULTANEOUS NIACIN AND LOVASTATIN SR IN BULK AND DOSAGE FORMS

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Abstract

A simple, specific, sensitive, precise and reproducible Reverse Phase High Performance Liquid Chromatography method has been developed for simultaneous estimation of Lovastatin and Niacin. Niacin and Lovastatin is Anti-hyperlipidemic Sustained Release drug. The determination was carried out by using symmetry C-18 column with ACN:0.05M Phosphate Buffer (PH4) (85:15) as the mobile phase and with the detection wavelength of 239,245 nm respectively. The flow rate is 0.6 ml/ min. The Retention time of Lovastatin, Niacin was 5.0 min and 3.2 min respectively. Linearity for the Niacin and Lovastatin were found in the range of 5-70 µgm and 5 - 50 µgm respectively. The limit of quantification for both drugs was found to be 30, 10 ng respectively. The recoveries of Niacin and Lovastatin were found to be in the range of 99.25-101.15% and 99.57-100.15%, respectively. The proposed method was validated suitably and can be used for routine analysis.

Key words: RP-HPLC, Lovastatin, Niacin, Sustained Release.

Introduction

Lovastatin (LOV) and Niacin (NIA) combination tablets are newly marketed and both are used as hyperlipidemic agents. LOV is chemically (1S,3R,7S,8S,8aR)-8-{2[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl(2S)-2-methylbutanoate¹. Lovastatin is an inhibitor of 3-hydroxy-3methylglutaryl-coenzyme A reductase (HMG-CoA reductase), an enzyme that catalyzes the conversion of HMG-CoA to mevalonate. NIA Niacin (also called Nicotinic Acid) and Niacinamide (also

called Nicotinamide), two compounds of water-soluble vitamin B complex, are active in the metabolism of body. Chemically they are 3-Pyridinecarboxylic acid and 3-Pyridinecarboxamide respectively. Commercially, niacin is obtained from beta -picoline or from quinoline, Niacin binds to and stimulates a G-protein-coupled receptor, GPR109A, which causes the inhibition of fat breakdown in adipose tissue. Nicotinamide does not bind this receptor which explains why it does not affect blood lipid levels. Lipids that are liberated

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from adipose tissue are normally used to build very-low-density lipoproteins (VLDL) in the liver, which are precursors of low-density lipoprotein (LDL) or "bad" cholesterol. Because niacin blocks the breakdown of fats, it causes a decrease in free fatty acids in the blood and, as a consequence, decreases the secretion of VLDL and cholesterol by the liver². It is needed to develop a method without any drawbacks and only very few methods have been reported for estimation of LOV and for NIA by spectrophotometric method UV-spectrophotometric method³ for simultaneous estimation individually and by HPLC method⁴⁻⁵.

Materials and methods

Pharmaceutical grade LOV and NIA were supplied by Orchid chemicals, Chennai, India, with the purity of 99.09% and 99.78% respectively on dried basis. Acetonitrile HPLC grade of Rankem, ortho phosphoric acid HPLC grade Rankem, Hydrochloric acid HPLC grade Rankem, Water HPLC grade of Qualigens were used for the analytical purpose. Waters HPLC System, with 515 pump system, Symmetry C₁₈ column (250x4.5 mm, 0.5 μm) and Dual λ Absorbance Detector 2487, worked in room temperature.

Chromatographic conditions

The separation the drugs were performed by using symmetry C₁₈ (250 × 4.6 mm, 5μm particle size) column. Mobile Phase consisted of a mixture of Methanol: water (85: 15 v/v) with a Flow rate of 0.6 ml/minute. Volume of injection is 20 μl and the detection wavelength was 245 nm. Mobile Phase was prepared by mixing 850 ml of Acetonitrile, HPLC Grade with 150 ml of Phosphate buffer in a 1000 ml

standard flask to get the proportion of 85:15 v/v. The mobile phase was filtered through 0.45 micron membrane filter and degassed by Ultrasonication for 15 min. The standard stock solutions of 1000 μg/ml of LOV and NIA were prepared by dissolving 100 mg of LOV and NIA in mobile phase, in a 100ml volumetric flask and made up to the volume. Further dilutions were made to 100 μg/ml and the solutions were stored under refrigeration. From the above solutions the dilutions of working standards were made from 5-70 μg/ml and 5-50 μg/ml for LOV and NIA respectively.

Calibration curve

The calibration curves were constructed for the determination of the linearity and the curves were plotted with the concentration range verses area must obey Beer's law. The linearity was evaluated by analysis of the serially diluted sample in the range of 5-70 μg/ml and 5-50 μg/ml for LOV and NIA respectively. An aliquot was injected using mixture of Acetonitrile and water in the ratio of 85:15 v/v. The retention times were 5.0 min and 3.2 min for LOV and NIA respectively with a good resolution of 9.46.

Analysis of formulations

Analysis of the tablets was conducted in two brands. Twenty tablets of both the brands were weighed and powdered separately. A quantity equivalent to 10 mg of LOV and 10 mg of NIA were transferred to 100 ml volumetric flask and dissolved on about 50 ml of Mobile phase. The solution was ultrasonicated for 10 min and filtered through Whatmann filter paper No.41 and the final filtration was done in 0.45 micron membrane volume made up to mark with same solvent system. Above solution was taken to

prepare a dilution of 50 µg/ml and the amount of drug was determined. Similar method was performed in other brand and three replicate injections were done for each formulation.

Result and discussion

In method development phase, initially both the drugs showing asymmetry factor more than 2 in Methanol: water, with a run time of more than 10 min. Then the mobile phase was shifted to Acetonitrile: Phosphate buffer, showed a good result. At the reported Mobile phase proportion of 85:15, LOV and NIA showed a retention time of 5.0 min and 3.2 min respectively at the flow rate of 0.6 ml/min. The wavelength for the determination was selected at 239,245 nm for both the drugs. The tailing factor, resolution and peak shape were found to be good in the finally reported condition for both the drugs. The peaks are shown in Fig 1.

As per ICH guidelines ⁶, system suitability tests were carried out by five replicate injections, with a constant concentration 50 µg/ml. The % Relative standard deviation of peak area and the retention time was within the limit of ±2%. This indicates that the method was system suitable. The reports are tabulated below in Table 1. The linearity of LOV and NIA were determined by calibration curves and the linearity based on the area observed in the range of 5-70 µg/ml and 5-50 µg/ml respectively. The regression co-efficient value (r^2) for LOV and NIA is 0.9998 and 0.9992 respectively. Precision was measured for both inter and intra-day, and checked with repeatability and the %RSD for the repeatability was found to be 0.177% and 0.256% for LOV and NIA respectively. The

RSD was found to within the limit and tabulated in Table 2. Since both the drugs were stable for 24 hr only even under refrigeration condition; only intra-day precision studies were conducted. The limit of quantification was determined by injecting minimum concentration of the drugs. The limit of quantification was found to be 30, 20 ng/ml for LOV and NIA respectively.

Analysis of the tablets was performed in two brands containing 500/20 mg of both the drugs as label claim. An average quantity of LOV and NIA were 20.931 ± 0.01 and 500.943 ± 0.004 respectively in Brand 1 and 20.126 ± 0.006 and 500.037 ± 0.002 respectively in Brand 2 and has conformation with the label claim. The results are tabulated in Table 3.

The accuracy was studied by the recovery studies. The recovery studies are usually made by spiking the known amount of pure drug with the formulation. It is usually done by adding 80 %, 100 % and 120 % of the pure drug with the formulation taken for analysis. The average % recovery for LOV and NIA was found to be 99.25 % and 101.15 % respectively for Brand 1 and 99.57 % and 100.15 % respectively for Brand 2. The results are tabulated below in Table 4.

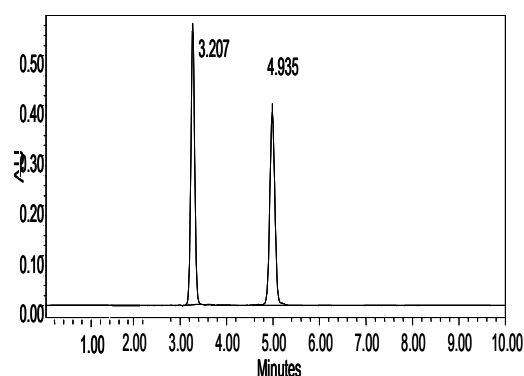


Fig 01: A Typical Chromatogram for Lovastatin and Niacin (Concentration of 50 mcg)

Table 01: System Suitability Parameters

| PARAMETERS | LOV | NIA |
|--|---------|---------|
| Calibration Range (mcg/ml) | 05-50 | 05-50 |
| Correlation Coefficient(r ²) | 0.9998 | 0.9992 |
| Retention time(Min) | 5.0±0.2 | 3.2±0.2 |
| Regression equation(y=mx+c) | | |
| Slope (m) | 627266 | 635879 |
| Intercept(c) | -639679 | -625887 |
| Asymmetry | 1.03 | 1.06 |
| Theoretical Plates | 9453 | 6669 |
| Resolution factor | 9.43 | -- |
| Tailing Factor | 1.01 | 1.03 |
| Selectivity | 1.78 | -- |
| Repeatability %RSD (n=5) | 0.177% | 0.256% |
| Limit of quantification (ng/ml) | 30 | 20 |

System suitability parameters are the data's performed to check the system testing and the data's are based on the ICH Guidelines.

Table 02: Intra-day precision study

| LOV | | NIA | |
|------------|-------|------------|-------|
| Conc µg/ml | % RSD | Conc µg/ml | %RSD |
| 10 | 0.228 | 10 | 0.952 |
| 20 | 0.273 | 20 | 0.558 |
| 30 | 1.637 | 30 | 0.692 |
| 40 | 0.515 | 40 | 0.517 |
| 50 | 1.594 | 50 | 0.198 |

Intraday precision studies are done to confirm the repeatability and the stability in a day. RSD stands for Relative standard deviation taken for three readings.

Table 3: Analysis of Marketed Formulation

| Formulation | LOV | | | NIA | | |
|-------------|--------------------|----------------------------|---------------|--------------------|----------------------------|---------------|
| | Label claim mg/tab | Amount found* mg/tab ± RSD | % assay ± RSD | Label claim mg/tab | Amount found* mg/tab ± RSD | % assay ± RSD |
| Brand I | 20 | 19.92±0.0105 | 99.25±0.103 | 500 | 500.96±0.0045 | 99.57±0.044 |
| Brand II | 20 | 20.21±0.0066 | 101.15±0.066 | 500 | 500.05±0.0021 | 100.15±0.021 |

Formulation analysis was done in two different brands. * stands for the average reading taken in three readings.

Table 04: Recovery studies of niacin and lovastatin combined dosage form

| Formulation | LOV | | | NIA | | |
|-------------|---------|--------------------|------------------|---------|--------------------|------------------|
| | % added | % recovered* ± RSD | % recovery ± RSD | % added | % recovered* ± RSD | % recovery ± RSD |
| Brand I | 80 | 80.29±0.308 | 100.36±0.380 | 80 | 79.89±0.015 | 99.86±0.015 |
| | 100 | 99.95±0.010 | 99.95±0.010 | 100 | 99.98±0.010 | 99.98±0.010 |
| | 120 | 119.87±0.085 | 99.57±0.221 | 120 | 120.07±0.085 | 100.15±0.065 |
| Brand II | 80 | 79.29±0.230 | 99.11±0.130 | 80 | 79.98±0.315 | 99.96±0.361 |
| | 100 | 100.05±0.030 | 100.05±0.030 | 100 | 101.28±0.250 | 101.28±0.250 |
| | 120 | 119.11±0.071 | 99.25±0.081 | 120 | 121.97±0.356 | 101.15±0.296 |

Recovery experiment data for Niacin and Lovastatin showing the amount of drug recovered from sample solution at each level (n=3), percentage recovery and the average percentage recovery.

Conclusions

The isocratic RP-HPLC method developed for quantitative determination of Niacin and Lovastatin simple, specific, sensitive, precise and reproducible. The method was completely validated and satisfactory results were obtained for all the method validation data tested.

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