



Development and Validation of RP- HPLC Method for Simultaneous Determination of *Niacin* (Extended Release) and *Lovastatin* in Oral Solid Dosage Form

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ABSTRACT

A simple, precise and rapid HPLC method has been developed and validated for the estimation of Lovastatin and niacin simultaneously in combined dosage form. Chromatographic separation of the two drugs was performed on a Purospher BDSC8 column (150mm×4.6mmid, 5µm particlesize). The mobile phase used was a mixture of 0.1% v/v triethylamine (pH 5.0), containing 20 mM of ammonium acetate buffer: methanol (30:70% v/v). Detection was performed at 237nm and sharp peaks were obtained for *niacin* and *Lovastatin* at retention times of 3.2±0.01 min and 6.4±0.01 min respectively. The calibration curve was linear in the concentration range 100-700µg/ml for niacin 3-18µg/ml for *Lovastatin*; the correlation coefficients were 0.9991 and 0.9992, respectively. The optimized method showed good performance in terms of specificity, linearity, detection and quantitation limits, precision and accuracy in accordance with the International Conference on Harmonization (ICH) Q2 (R1) guidelines. This assay was demonstrated to be applicable for routine quantitation of Lovastatin and niacin in tablet dosage form.

Key words: Lovastatin, Niacin, Validation, Ion Pair RP-HPLC.

INTRODUCTION

Hyperlipidemia (HLP) is a group of lipid metabolism disorders with various pathogenesis. Its characteristic feature is an increase in cholesterol level, especially the low density lipoprotein (LDL) level or triglyceride level in blood. An increase in total and LDL cholesterol level is related to the increased risk of ischaemic heart disease as well as

cerebral, coronary and peripheral circulation disorders¹⁻².

Lovastatin (LS) is a HMG-CoA reductase inhibitor¹ used in the treatment of hypercholesterolaemia. It is chemically, [1S-[1α(R*), 3α, 7β, 8β(2S*, 4S*), 8αβ]]-1, 2, 3, 7, 8, 8a-hexahydro-3, 7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-1-

naphthalenyl 2-methylbutanoate³. The combination of astatin with Niacin(NI) can be an attractive option because both have excellent records of improving cardiovascular outcomes and can effectively correct all abnormalities of atherogenic dyslipidemia in patients with diabetes⁴.

Literature survey revealed spectrophotometric⁵, capillary electrophoresis⁶⁻⁷ and chromatographic methods⁸⁻¹¹ reported formulation of LS and NI individually or in combination with other drugs. One derivative spectrophotometric method¹² method reported for this combination. However, there is no analytical method reported for the simultaneous determination of these drugs in a pharmaceutical formulation. Present work describes simple, rapid, accurate and precise High Performance Thin Layer Chromatographic method for simultaneous determination of ATS and MET in capsules. The proposed method was validated as per ICH guidelines¹³.

Reagents and chemicals

HPLC grade methanol and water were procured from E. Merck, Mumbai, India. Triethylamine, Ammonium acetate AR grade were procured from Qualigens Fine Chemicals, Mumbai. A standard bulk drug sample niacin was provided by Madras pharmaceuticals, Chennai and of Lovastatin was kindly supplied by Cadila Health care pvt Ltd., Ahmedabad, India. Advicor® containing 500 mg of niacin and 20 mg of Lovastatin was obtained as a commercial source.

Instrumentation

Analysis was performed on Alliance HPLC System (Water's Corporation, USA) consisting of 2695 separation module and 2998 Photo Diode Array (PDA) detector. Chromatographic data was acquired using waters empower software.

HPLC Condition

HPLC was performed on an ODS C₈ column (250×4.6 mm i.d.; 5 µm particle size). The mobile phase consisted of 0.1% v/v triethylamine (pH 3.0), containing 1 mM of Sodium 1-hexanesulfonate: methanol (30:70% v/v). The mobile phase was filtered through a nylon 0.45 µm membrane filter and was degassed before use. The flow rate was 1.0 ml/min. The determination was

carried out at 237 nm, and the injection volume was 20 µL. The total run time was 10 min. The data were analyzed by waters empower software.

METHODS

Preparation of standard solutions and calibration curve

Standard stock solutions of NI and LS were prepared separately by dissolving 100mg of each drug in 100ml volumetric flask with 10ml of mobile phase and the solutions were sonicated for about 15 min. Then the volume was made up to the mark with mobile phase to get 1mg/ml standard stock solution. Several aliquots of these standard stock solutions were taken in different 10ml volumetric flask and diluted up to the mark with mobile phase such that the final linearity concentrations of niacin and lovastatin were 100-700 µg/ml and 3-18 µg/ml, respectively.

Assay standard solution preparation

A working standard solution containing 500 µg/ml of NI and 10 µg/ml of LS were prepared from the above standard stock solution.

Analysis of tablet formulation

Twenty tablets of the formulation were weighed and the average weight of one tablet was calculated. All 20 tablets were crushed and grounded to a fine powder. Powder equivalent to 1000 mg of niacin and 20 mg of lovastatin was transferred into a 100 ml of volumetric flask and diluted up to the mark with the mobile phase and mixed well, then the solution was filtered through a 0.45 µm filter to obtain a clear filtrate. This solution was suitably diluted and used for analysis. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, a fixed volume of 20 µl of the sample solution was loaded by an automatic sampler. The solution was injected, and chromatograms were recorded. The injections were repeated six times, and the peak area were recorded.

Method validation

The chromatographic conditions were validated by evaluating linearity, recovery, method and system precision, accuracy, system suitability, solution stability, limit of detection (LOD), Limit of

Quantification (LOQ), robustness, ruggedness studies in accordance with ICH guideline Q2(R1).

The system suitability was assessed by five replicate analysis of the drug at a concentration as per standard preparation. System suitability of the method was evaluated by analyzing the repeatability, peak symmetry (symmetry factor), theoretical plates of the column, resolution between the peaks, capacity factor, and relative retention. Specificity was tested against standard compounds and against potential interferences in the presence of placebo.

The linearity of the calibration curve was checked over the range of niacin and lovastatin were 100-700 µg/ml and 3-18 µg/ml. LOD and LOQ were calculated using σ (standard deviation of the response) and b (slope of the calibration curve) using the following formulae: $LOD = (3.3 \times \sigma) / b$ and $LOQ = (10 \times \sigma) / b$.

The accuracy of the method was determined by recovery experiments. It was confirmed by spiking known amount of working standard with placebo at three concentration levels

(50%, 100%, and 150%) and analyzed and the analysis being done in replicate. The results obtained were compared with the expected results.

The precision of the method was demonstrated by repeatability, intra-day and inter-day variation studies at three different concentration level of analytes covering the concentration range. In the intra-day studies, three repeated measurements of standard and sample solutions were made in a day and three repeated measurements of standard and sample solutions were made on three consecutive days for inter-day variation studies. Percentages Relative Standard Deviation (RSD) were calculated for intra-day and inter day variation.

The ruggedness of the method was determined by carrying out the experiment on different instrument like Waters HPLC and Shimadzu HPLC by different operators using different columns of similar type like Phenomenex C₈, Hypersil C₈. Robustness studies were performed on method precision by making slight variations in flow rate, amount of the mobile phase and pH changes.

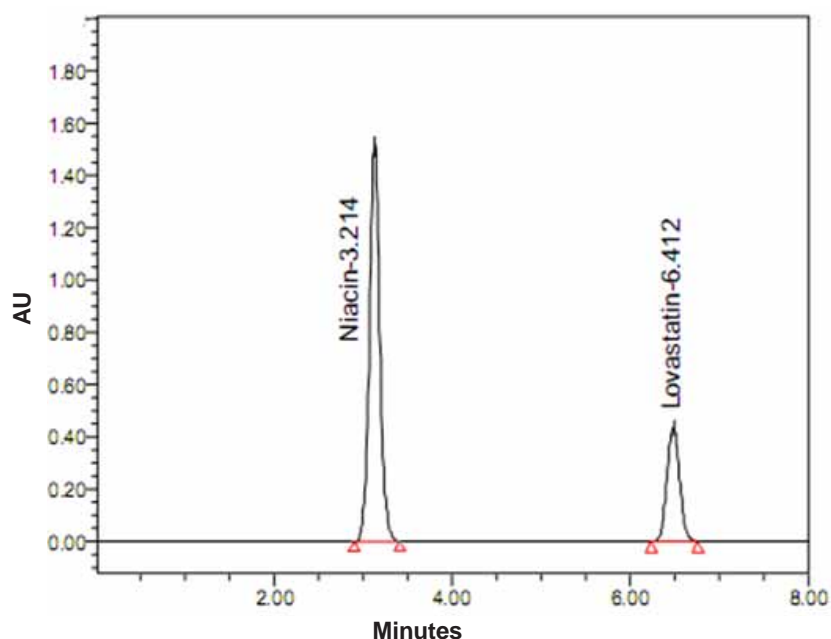


Fig. 1: Standard chromatogram of Sample

RESULTS AND DISCUSSION

Optimization of solvent system and chromatographic condition

The primary target in developing this method is to achieve simultaneous determination of NI and LS in the tablet formulation under common conditions that will be applicable for routine quality control of the product in laboratories. Various development trials were taken for the development of a chromatographic system for the estimation of LS and NI in their fixed dosage form. On the basis of their structural formula, the reversed - phase liquid chromatography was selected. Trials were performed using C18 stationary phase (4.6 mm, 5 μ m) with varying column lengths from 250 to 150 mm and various brands (Thermo Hypersil C18, YMC-PackPro C18, Phenomenex C18 gemini, Waters XTerra RP18). Chromatographic separation using Purospher C 8, 250 \times 4.6 mm, 5- μ m column gave higher retention time of 4.6 min for NI and 8.6 min for LS. The shorter column length (150mm \times 4.6 mm, 5 μ m) of the same brand reduced the retention time to 3.2 min for NI and 6.4 min for LS with better resolution. The trial using Ammonium acetate buffer pH 5 with methanol in the ratio of 30:70 (v/v) gave desirable chromatographic separation with better

peak shape, tailing factor, plate count, and resolution. As the UV spectrum showed good response at 237 nm for both components, this wavelength was found suitable for simultaneous determination of both components. The standard chromatogram of sample was shown in Fig 1.

System suitability studies

System suitability testing is used to verify that the proposed method was able to produce good resolution between the peak of interest with high reproducibility. The system suitability was determined by making six replicate injections from freshly prepared standard solutions of assay and dissolution method and analyzing each solute for their retention time, theoretical plates, resolution, capacity factor and tailing factor and the results are shown in Table.1

Linearity

Linearity of the method was studied by spotting seven concentrations of the drug prepared in the mobile phase in the range of 100-700 μ g/ml and NI and 3-18 μ g/ml, for LS. The correlation coefficient (r) values were >0.999 ($n = 7$). Typically, the regression equations for the calibration curve were found to be $Y = 1550 + 19.46 * X$ for NI and $Y = 654.5 + 21.01 * X$ for LS and results were shown in Table.2.

Table.1: System suitability studies

| Parameters | Niacin | Lovastatin |
|--------------------|--------|------------|
| Theoretical plates | 6135 | 5148 |
| USP tailing | 0.92 | 1.24 |
| Resolution | 5.36 | |
| Capacity factor | 4.15 | 1.16 |
| Retention time | 3.21 | 6.42 |

Tablet studies

The proposed method was successfully applied to the analysis of marketed products (Advicor®) containing NI and LS. Three replicate determinations were performed on accurately weighed amounts of the tablets Table.2

Table.2: Regression analysis of calibration curves, sensitivity and formulation analysis

| S.No | Parameters | Niacin | Lovastatin |
|------|-----------------------------------|--------------------|-----------------|
| 1 | Linearity range | 100-700 μ g/mL | 3-18 μ g/mL |
| 2 | Slope | 199.1 | 369.1 |
| 3 | Intercept | 1373 | 26.8 |
| 4 | Correlation coefficient(r^2) | 0.999 | 0.999 |
| 5 | Limit of Detection | 5 μ g/mL | 0.42 μ g/mL |
| 6 | Limit of Quantification | 20 μ g/mL | 1 μ g/mL |
| 7 | Formulation analyzed (assay,%RSD) | 100.49,0.45 | 99.97,0.28 |

Table. 3: Intra-day and inter-day precision (n=3)

| Drug name | Concentration µg/mL | Measured concentration µg/mL, RSD% | |
|------------|------------------------|------------------------------------|-------------|
| | | Intra-day | Inter-day |
| Niacin | 400 µg/mL | 399.68,0.24 | 400.11,0.65 |
| | 700 µg/mL | 701.23,0.14 | 699.23,0.38 |
| | 900 µg/mL | 899.04,0.89 | 900.12,0.58 |
| Lovastatin | 6 µg/mL | 6.04,0.41 | 5.98,0.21 |
| | 18 µg/mL | 18.01,0.24 | 17.99,0.29 |
| | 24 µg/mL | 23.97,0.47 | 24.02,0.59 |

Table. 4: Robustness data for tablet analysis

| Condition | Change | Niacin | | Lovastatin | |
|----------------------|----------|----------|-------|------------|-------|
| | | %Content | %RSD* | %Content | %RSD* |
| pH | Normal | 100.64 | 0.46 | 99.91 | 0.48 |
| | -0.2unit | 100.55 | 0.68 | 99.87 | 0.41 |
| | +0.2unit | 101.44 | 0.87 | 99.86 | 0.99 |
| Flow Rate | Normal | 99.91 | 0.35 | 0.11 | 0.33 |
| | -10% | 100.85 | 0.24 | 0.67 | 0.41 |
| | 10% | 101.21 | 0.96 | 0.28 | 0.40 |
| Mobile phase ratio | Normal | 100.11 | 0.57 | 0.99 | 0.88 |
| | -2% | 99.97 | 0.68 | 0.98 | 0.41 |
| | 2% | 101.23 | 0.47 | 0.94 | 0.56 |
| Detection wavelength | Normal | 100.21 | 0.68 | 0.85 | 0.39 |
| | -0.2unit | 99.56 | 0.74 | 0.84 | 0.41 |
| | +0.2unit | 100.35 | 0.64 | 0.94 | 0.42 |

*Average of six determinations

Precision and accuracy

The method was found to be precise, as indicated by % RSD not more than 2. Intra-day and inter-day studies [Table 3] support the precision of the method. The proposed method when used for estimation of NI and LS from the pharmaceutical dosage form after spiking with the working standard afforded recovery of 99-101% Table.4

Sensitivity of the method

The limit of detection was found to be 5 µg/mL and 20µg/mL, while the limit of quantitation was found to be 0.42 µg/mL and 1 µg/mL for NI and LS, respectively. The low value of LOD and LOQ indicates that the method is sensitive.

Ruggedness

The ruggedness of an analytical method is the degree of reproducibility of the test results

obtained by the samples under a variety of conditions, such as different laboratories, different analysts, different instruments, different lots of reagents, and different days. The %RSD of below 2% indicated that the method was accurate with high precision.

Robustness

Robustness is a measure of the performance of a method when small deliberate changes are made to the conditions of the method. The results of the robustness study are summarized in Table 5.

Specificity

There is no interference was detected at the retention time of niacin and lovastatin in sample solution confirms the specificity of the method.

Table. 5: Recovery studies for spiked concentration of Niacin and Lovastatin

| Name of the Drug | Level of % Recovery | Amount of standard | | % Recovery |
|------------------|---------------------|--------------------------|------------------------------|------------|
| | | Added $\mu\text{g/mL}^*$ | Recovered $\mu\text{g/mL}^*$ | |
| Niacin | 50 | 250 | 749.96 | 99.98 |
| | 100 | 500 | 1001.19 | 100.1 |
| | 150 | 750 | 1248.12 | 99.84 |
| Lovastatin | 50 | 5 | 14.99 | 99.91 |
| | 100 | 10 | 20.34 | 101.7 |
| | 150 | 15 | 25.3 | 101.2 |

*Average of six determinations

CONCLUSION

The proposed method gave good resolution between NI and LT with in short analysis time (<8.0min). Ruggedness of the method was confirmed as no significant changes were observed on analysis using different instrument. High percentage recovery of drug shows the method is

free from inference of excipients present in the formulation. The proposed method was found suitable for simultaneous analysis of NI and LT can be used for routine quality control of their bulk drug mixture and their combined dosage form. Its advantages are low costs reagents, speed and simplicity of sample treatment, satisfactory precision and accuracy.

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