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HPLC method development for estimation of pitavastatin in liquid dosage form

Shubham Verma [1,2,*], Nitin Kumar [3], Umesh Kumar [4], Gaurav Jain [5]

[1]School of Pharmacy, Monad University, Pilkhuwa, Hapur, Uttar Pradesh, India
[2]Department of Pharmacy, Uttrakhand Technical University, Dehradun, Uttrakhand, India
[3]School of Medical & Allied Sciences, K R Mangalam University, Gurugram, India
[4]Om Bio Sciences & Pharmacy College, Roorkee, Uttrakhand, India
[5]Faculty of Pharmacy, Jamia Hamdard, New Delhi, India

*corresponding author e-mail address: ***shubh.verma678@gmail.com***

ABSTRACT

For the determination of pitavastatin calcium nanoemulsion liquid dosage form a simple, sensitive, reliable and rapid reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated. 5 µ particle size column in isocratic mode at 25ºC temperature. The sample was injected through an injector valve with a 10 µl, sample loop. Phosphate buffer (pH 6.4): Methanol (50:50 v/v) was used as a mobile phase with a flow rate of 1 ml per min at 286 nm wavelength. A calibration graph was plotted range between 25-200 µg/ml with the correlation coefficient of 0.999 which showed linearity. Validation studies revealed the method is specific, rapid, reliable, and reproducible. The validity of the method, degradation studies were carried out using the same optimum conditions. Therefore the proposed method is reliable, rapid, precise and selective and may be used for the quantitative analysis of nanoemulsion liquid dosage form of pitavastatin calcium.

Keywords: *HPLC; Nanoemulsion; Pitavastatin; Sonicator.*

1. INTRODUCTION

Pitavastatin is a new synthetic 3-hydroxy-3-methyl glutaryl coenzyme reductase inhibitor, it is chemically (3R, 5S)-7-(2-cyclopropyl-4-(4-fluorophenyl) quinolin-3-yl) - 3,5-dihydroxy 6(E) -heptenoic acid calcium salt [1]. It is an inhibitor of HMG CoA reductase enzyme that accelerates the first step of cholesterol synthesis [figure 1].

Pitavastatin is odorless and found as white to pale yellow powder. Pitavastatin is moisture sensitive and slightly unstable in light. It was discovered by Nissan chemical industries limited, Japan and developed further by kowa pharmaceuticals Tokyo, Japan [2-3]. Pitavastatin is less likely to interact with drugs that are metabolized via CYP3A4, which might be important for elderly patients who need to take multiple medicines. We are now reporting a simple sensitive and selective RP-HPLC method for the validation of pitavastatin and its related impurities [4].

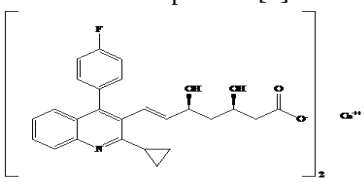

Figure 1. Structure of Pitavastatin calcium.

2. MATERIALS AND METHODS

2.1. Drug, chemical & solvent: Pitavastatin gift sample obtained from Zydus health care, Ahmedabad, Gujarat**,** HPLC grade methanol was purchased from Rankem Fine Chemicals Ltd., Mumbai. HPLC grade water was purchased from CDH laboratories, New Delhi.

2.2. Equipment & chromatographic conditions: Agilent 1120 compact LC fitted with a diode array detector (model 2996) and running on EZ chrom software was employed in the study. An Agilent HC C-18(2) column (150 x 4.6 mm; 5µ) was used for analyzing the drug.

All the chromatographic runs were carried out by using a mobile phase consisting of a mixture of phosphate buffer (pH 6.4) and methanol (50:50 v/v) in isocratic mode at a flow rate of 1.0 ml/min. The injection volume of the samples was 10 µL. The detector wavelength was set at 286 nm. The chromatographic run time was set as 10.0 min. Under these optimized conditions, the retention time obtained for pitavastatin was 4.321 min.

2.3. Preparation of the phosphate buffer [5]: The phosphate buffer was prepared by dissolving 1.79 disodium hydrogen phosphate, 1.36 gm of potassium dihyrogen phosphate, 7.02g sodium chloride in a beaker containing 1000 mL of water and the contents were sonicated. Ortho phosphoric acid was used to adjust the pH of the solution to 6.4. It was then filtered through a 0.45µ membrane filter.

2.4. Preparation of the mobile phase: The optimized mobile phase consisted of a mixture of the above above-mentioned phosphate buffer (pH 6.4) and methanol in the ratio of 50:50 v/v.

2.5. Preparation of the diluents: Mobile phase was used as the diluents.

2.6. Preparation of standard sample solution [6]: 1 mg of pitavastatin calcium was weighed & transferred to a 100 ml volumetric flask with 50 ml mobile phase i.e. phosphate buffer (pH 6.4) and methanol (50:50 v/v) and shaken for 10 min, followed by making up to volume with mobile phase. This solution was filtered using 0.2 µ membrane filter. From this, 1 ml were pipetted out and transferred to 10 ml volumetric flask, 1 ml of internal standard solution (µg/ml) was added to each and made up to the volume to get the concentration of 1 µg/ml of pitavastatin calcium.

2.7. Calibration plot: Solutions of pitavastatin at different concentration levels including the working standard concentration were prepared with the diluent. 20 µl of each concentration was injected three times into the HPLC system (n=3). The response was read at 286nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas at the different concentration levels were calculated and the linearity plot of mean peak areas over their concentrations was constructed.

2.8. Estimation of the drug from the liquid dosage forms: 10 mg of pitavastatin was transferred into a 10 ml volumetric flask. 8 ml of diluent was added and sonicated for 30 min. The volume was made up with the diluent and the contents were mixed well. This mixture was filtered through a 0.45µ membrane filter (discarding the first few ml of the filtrate). 1 ml of the filtrate was transferred into a 10 ml volumetric flask and made up to volume with diluent. This solution was then chromatographed six times. From the chromatograms obtained, the average drug content in the formulation was calculated [7].

3. RESULTS

During the method optimization studies, various combinations and proportions of the solvents and buffers were examined on an Agilent HC C-18(2) column for efficient separation of pitavastatin. Using a mobile phase consisting of a mixture of phosphate buffer (pH 6.4) and methanol in the ratio of 50:50 v/v, a good resolution and baseline separation of the drug peak was obtained. All the chromatographic conditions were optimized by evaluating the column efficiency parameters like theoretical plates and tailing factor [Table 1].

Table 1. Optimization of HPLC chromatographic conditions.

Stationary Phase	Agilent HC
Mobile Phase	Phosphate buffer(6.4 pH) : Methanol = 50:50 v/v
Column Temperature	25º C
Injection Volume	10 µl
Flow Rate	1 ml per minute
Wavelength	286 nm

Under these optimized conditions, the retention time obtained for pitavastatin was 4.321 min [Figure 2] in a run time of 10.0 min. The method was then validated as per the ICH guideline. The proposed method was also found to be applicable for the analysis of pitavastatin in nano-emulsion formulations.

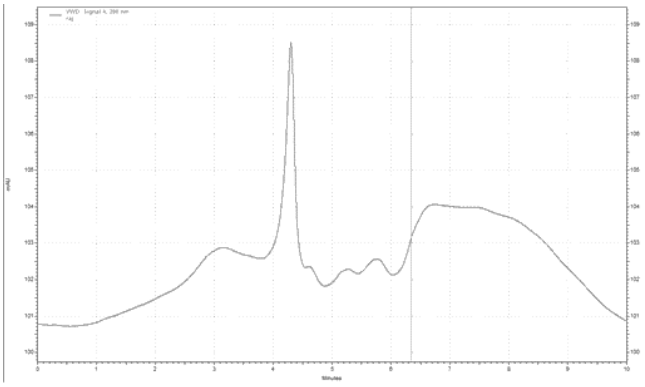

Figure 2. Chromatogram of Standard solution of Pitavastatin.

3.1. Specificity.

A good analytical method should be capable to determine the analytes accurately in the presence of probable interferences from its solvent and from the excipients of its formulation. Figure 2 shows good chromatographic baseline separation of pitavastatin from its working standard solution. Figure 3 demonstrates that no interfering peaks were observed at the retention time of pitavastatin arising due to the excipients.

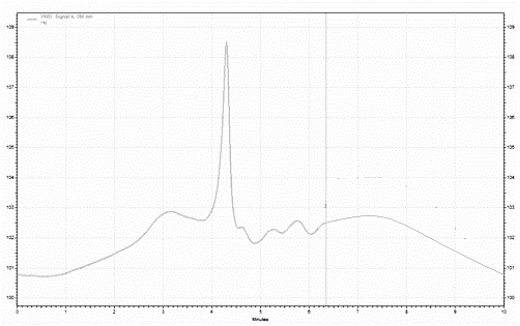

Figure 3. Chromatogram of nanoemulsion liquid dosage form of Pitavastatin

3.2. Linearity.

The calibration curve (n=3) constructed for the drug was linear over the concentration range of 25–200 µg/mL. The regression of the plot was computed by the least square regression method and is shown in Figure 4. The correlation coefficient is greater than 0.99 and the % RSD at each concentration studied was less than 2.

Table 2. Linearity result for pitavastatin.

Linearity level	Amount of pitavastatin (ppm)	Average areas of pitavastatin
LOQ	0.025	141011
25.0%	0.68	3812919
50.0%	1.38	7101718
100.0%	2.45	15327680
150.0%	3.71	23556121
200.0%	4.78	31921679

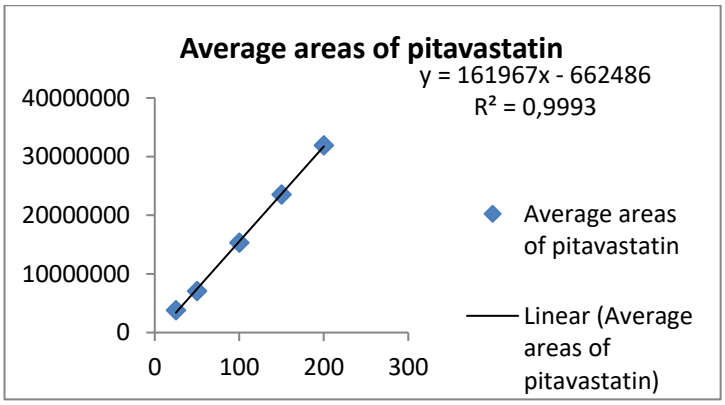

Figure 4. Linearity plot of Pitavastatin.

3.3. Accuracy and precision.

Recovery experiments were used to determine the accuracy of the method. The experiment was carried out and the percent recovery with its standard deviation was calculated [Table 3]. The accuracy of the proposed method is directly proportional to the

percentage of recovery. Six replicate injections of sample solutions were made and the percent RSD was calculated [Table 2].

Table 3. Accuracy data of the proposed method

S. No.	Amount of the analyte taken (µg/ml)	Mean recovery (µg/ml) ± SD	% Mean recovery (µg/ml) ± SD
1	25	25.07 ± 0.17	100.26 ± 0.83
2	50	50.13 ± 0.08	100.26 ± 0.12
3	100	100.5 ± 0.29	100.5 ± 0.2
4	150	150.48 ± 0.32	100.32 ± 0.18
5	200	200.2 ± 0.13	100.1 ± 0.05

3.4. System suitability parameters.

System suitability parameters were studied with six replicate injections of the standard solution and the results are presented in Table 4.

Table 4. System suitability parameters of the proposed method.

S. No.	Parameter	Value
1	Retention time	4.321
2	Tailing factor	1.6
3	Theoretical factor	8106
4	HETP	0.0152

3.5. Degradation studies.

Acid-degradation: 1 ml of stock solution of pitavastatin was transferred into a 10 ml volumetric flask. 1 ml of 2M hydrochloric acid was added to it and refluxed for 30 mins at 60°C. The resultant solution was diluted with diluent to obtain 100 µg/ml solution of pitavastatin. 10 µl of this solution was injected into the system and the chromatogram was recorded [Figure 5].

Base-degradation: 1 ml of stock solution of pitavastatin was transferred into a 10 ml volumetric flask. To it, 1mL of 2 M sodium hydroxide was added and refluxed for 30min at 60°C. The resultant solution was diluted with diluent to obtain 100 µg/ml solution of pitavastatin. 10 µl of this solution was injected into the system and the chromatogram was recorded [Figure 6].

Neutral-degradation: Under neutral conditions, stress testing was studied by refluxing the drug in water for 6 hours at a temperature of 60°C. The resultant solution was diluted to get 100 µg/ml solution of pitavastatin and 10 µl was injected into the system and the chromatograms were recorded [Figure 7].

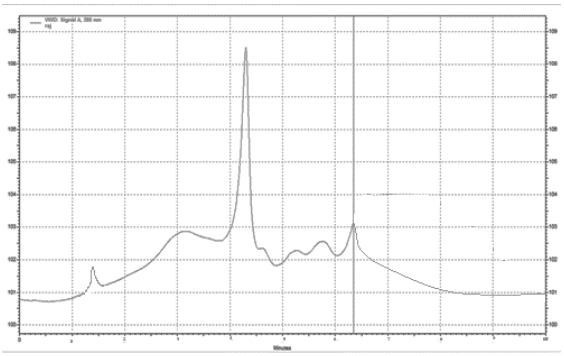

Figure 5. Chromatogram of acid degradation of Pitavastatin.

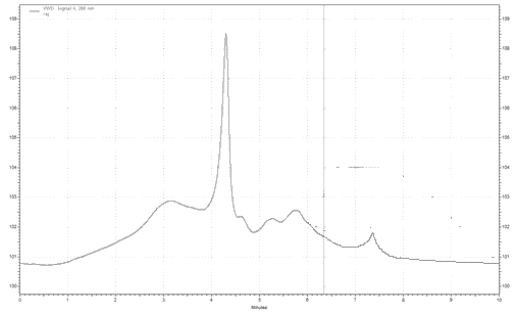

Figure 6: Chromatogram of base degradation of Pitavastatin

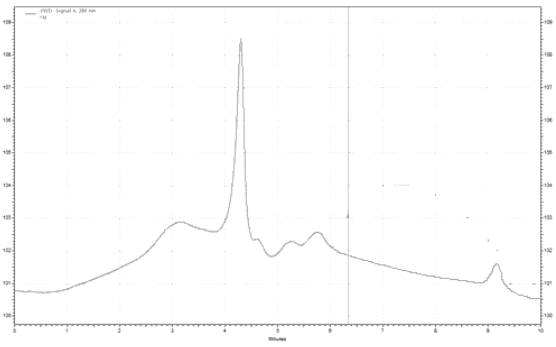

Figure 7. Chromatogram of neutral degradation of Pitavastatin.

3.6. Method suitability.

The nanoemulsion liquid dosage form was analyzed by the proposed method. The suitability of the method for the analysis of pitavastatin in nanoemulsion liquid dosage form was confirmed by recovery obtained (100.1%) to be in good agreement with the labelled amount of the drug.

4. CONCLUSIONS

The developed HPLC method for Pitavastatin Calcium in nanoemulsion liquid dosage form using mobile phase phosphate buffer & methanol (50:50 % v/v) at 1 ml/min flow rate. A calibration graph was plotted which showed linearity with the correlation coefficient of 0.999. The proposed RP-HPLC method is sensitive, precise, accurate and stability-indicating and can be used for the routine determination of pitavastatin in its nanoemulsion liquid dosage form.

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6. ACKNOWLEDGEMENTS

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