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ORIGINAL RESEARCH

New cost effective RP-HPLC method development and evaluation for quantitative estimation of Pitavastatin in pharmaceutical formulation

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Abstract

A simple, sensitive, reliable and rapid reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for the determination of pitavastatin in bulk and pharmaceutical dosage form. The chromatographic system consisted of waters (784), 515 binary Pump, data Ace with UV-Visible detector. Separation was achieved on the thermo C18 (250 x 4.60), 5 µ particle size column in isocratic mode at room temperature. The sample was introduced through an injector valve with a 20 µl, sample loop. 20mM KH_2PO_4 (pH-3.0 with OPA): Acetonitrile 20:80 (%, v/v), was used as mobile phase with flow rate of 1 ml/min. UV detection was performed at 250 nm. A calibration graph was plotted which showed a linearity range between5-25 µg/ml with the correlation coefficient of 0.997. The LOD was 0.095µg/ ml, while the LOQ was 0.271 µg/ml. Validation studies revealed the method is specific, rapid, reliable and reproducible. To study the validity of the method, recovery studies and repeatability studies were carried out using the same optimum conditions. The system suitability studies were also calculated which includes column efficiency, resolution, capacity factor and peak asymmetrical factor. Therefore the proposed method is reliable, rapid, precise and selective so may be used for the quantitative analysis of pitavastatin.

Keywords: HPLC, Pitavastatin, Pharmaceutical dosage form, Method Validation

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Introduction

Pitavastatin (PVT; (E, 3R, 5S)-7-[2-cyclopropyl-4-(4-fluorophenyl) quinolin-3-yl]-3, 5-dihydroxyhept-6-enoic acid; Fig. 1) is a novel, fully synthetic statin which has a more potent cholesterol-reducing action than other drugs in its class. PVT is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMGCoA) reductase, used as the calcium salt in the treatment of hyperlipidemia [1, 2]. This enzyme catalyses the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis [3]. The short-term and long-term lipid-modifying effects of pitavastatin have already been investigated in subjects with primary hypercholesterolemia, heterozygous familial hypercholesterolemia, hypertriglyceridemia and type-2 diabetes mellitus accompanied by hyperlipidemia. Within the range of daily doses, from 1 to 4 mg, the efficacy of pitavastatin as a lipid lowering drug seems to be similar, or potentially superior, to that of atorvastatin. According to results from pharmacokinetic studies, pitavastatin has a favorable and promising safety profile. It was only slightly metabolized by the cytochrome P450 (CYP) system. It could be concluded that pitavastatin could be a new and potentially better therapeutic choice for lipid modifying therapy than currently available statins [4, 5]. PVT is an unofficial drug substance, available as the bulk material and the tablet dosage form [6]. Literature survey revealed several analytical methods such as spectrophotometry [7,8] simple high performance thin layer chromatography [9,10], simple and column-switching high performance liquid chromatography (HPLC) with UV and PDA detection [11-14], HPLC with fluorescence detection [15], stability indicating HPLC [16, 17] , ultra fast performance chromatography [18], LC-MS/MS [19, 20] and LCESI- MS [21] have been reported for the determination of PVT in pharmaceutical dosage forms and or in biological samples. In the present work, we are therefore focused on to achieve the optimum chromatographic conditions for the determination of PVT in a formulation. The developed method could be applied to quality control of the tablet dosage form. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines, [22] which are mandatory also.

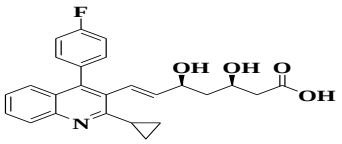


Figure 1 Chemical structure of Pitavastatin

Materials and methods

Instrument

Liquid chromatographic system from Waters model no 784 comprising of manual injector, water 515 binary pump for constant flow and constant pressure delivery and UV-Visible detector connected to software Data Ace for controlling the instrumentation as well as processing the generated data.

Reagents and chemicals

Pitavastatin was obtained as pure sample from Zydus Cadila (Ahmedabad, India), as gift samples along with their analytical reports. HPLC grade methanol and acetonitrile was obtained from Merck (India) limited. All other chemical used were of analytical grade. Triple distilled water was used for whole experiment was generated in house. Tablet Pitava 2, 2 mg Zydus Cadila Ahmedabad, India was purchased from local market.

Chromatographic conditions

The isocratic mobile phase consisted of 20mM KH_2PO_4 (pH-3.0 with OPA): Acetonitrile (20:80 v/v), flowing through the column at a constant flow rate of 1.0 ml/ min. The mobile phase was filtered through nylon 0.22 µm membrane filters and was degassed before use (30 min). A Thermo (C-18) Column (5 µm, 250mm x 4.60mm) was used as the stationary phase. By considering the chromatographic parameter, sensitivity and selectivity of method for drugs, 250 nm was selected as the detection wavelength for UV-Visible detector.

Standard stock solution

Accurately weighed 10 mg of PVT was transferred into 10 ml volumetric flask, dissolved in 5ml of methanol and volume was made up to 10ml with methanol to get concentration of solution 1000 µg/ml (Stock-A), 5ml of stock-A was taken and diluted up to 50ml to get concentration of 100µg/ml (Stock-B).

Working standard solution

Working standard solutions were prepared by taking dilutions ranging from 5-25 µg/ml for PVT.

Sample preparation

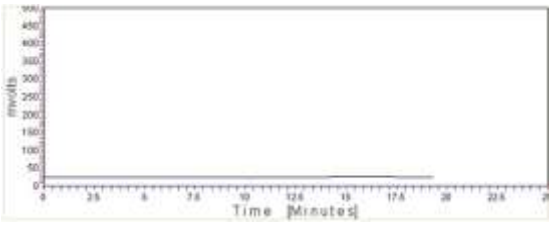
Commercial formulations pitavastatin of Pitava 2 was selected for analysis. Twenty tablets of Pitava 2 were weighed and powdered. Weight equivalent to 2mg PVT was dissolved in 10 ml diluents and then sonicated for 15min. and filtered through Whatmann paper no. 41. Then different concentration of solution were prepared by serial dilution technique, as per standard and each dilution was analyzed.

Results and discussion

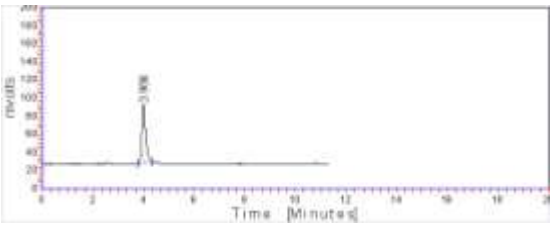
Chromatography

The mobile phase was chosen after several trials with methanol, isopropyl alcohol, acetonitrile, water and buffer solutions in

various proportions and at different pH values. A mobile phase consisting of 20mM KH_2PO_4 (pH-3.0 with OPA): Acetonitrile (20:80 v/v) was selected to achieve maximum separation and sensitivity. Flow rates between 0.5 and 1.5 min were studied. A flow rate of 1 ml/min gave an optimal signal-to-noise ratio with a reasonable separation time. Using a reversed-phase C18 column, the retention times for PVT was observed to be 3.986 ± 0.3 min. Total time of analysis was less than 6 min. The maximum absorption of PVT was detected at 250 nm, and this wavelength was chosen for the analysis Fig. 2



(A)



(B)

Figure 2 Chromatograms of (A) Blank mobile phase (B) PVT (15µg/ml) as reference substances

System suitability

System suitability parameters such as number of theoretical plates, HETP and peak tailing are determined. The results obtained are shown in Table 1. The number of theoretical plates for PVT was 3553.33.

Table 1 Results of system suitability parameters

Parameters	Pitavastatin
AUC*	845.789
No. of Theoretical Plates	3553.33± 7.46
Tailing Factor*	0.955
Retention time*	3.986
Calibration range (µg/ml)	5-25

*Each value is the mean ± SD of six determinations

Linearity

The calibration curve was linear over the concentration range of 5-25 µg/ml for PVT. The linearity was represented by a linear regression equation as follows:

Y (PVT) = 83.34 conc. + 25.96(r2 = 0.997)

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve table 2.

Table 2 LOD and LOQ

Name	LOD (µg/ml)	LOQ (µg/ml)
Pitavastatin	0.095	0.271

Accuracy

Method accuracy was performed by adding known amounts of PVT to the preanalysed tablet solution and then comparing the added concentration with the found concentration. Three levels of solutions were made which correspond to 80%, 100%, and 120% of the nominal analytical concentration (10µg/ml for PVT). Each level was made in triplicate table 3. The mean percentage recoveries obtained for PVT was 99.78%, respectively, and RSD was less than 1.

Table 3 Results of recovery study

Statistical data	Pitavastatin		
	80%	**100%**	**120%**
% Mean*	99.62	99.71	99.78
SD*	0.084	0.091	0.170
%R.S.D*.	0.084	0.092	0.170

*Mean of nine determinations (three replicates at three concentration level)

Precision

Repeatability

Five dilutions in three replicates were analyzed in the same day for repeatability and results were found within acceptable limits (RSD < 2) as shown in table 4.

Intermediate precision

Five dilutions in three replicates were analyzed on two different days and by two analysts for day-to-day and analyst-to-analyst variations and results were found within acceptable limits (RSD < 2) as shown in table 4.

Table 4 Statistical data for precision

Statistical parameter	Pitavastatin		
	Mean*	**S.D***	**R.S.D***
Repeatability	99.74	0.028	0.028
Intermediate Precision (I) (A day to day)	99.27	0.041	0.041
(II) Analyst to Analyst	99.62	0.99	0.99
Robustness	99.45	0.065	0.065

*Mean of 15 determinations (three replicates at five concentration level)

Robustness

As per ICH norms, small, but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, 20mM KH_2PO_4 (pH-3.0 with OPA): Acetonitrile (20:80 % v/v), to (15: 85% V/V) and method is found robust as RSD is again found < 2.0 table 4.

Specificity and selectivity

Commonly used excipients were spiked in to a pre weighed quantity of drugs. The chromatogram was taken by appropriate dilution and the quantities of drug were determined. The specificity of the HPLC method is illustrated in Fig. 3. Where complete separation of PVT in presence of tablet excipients.

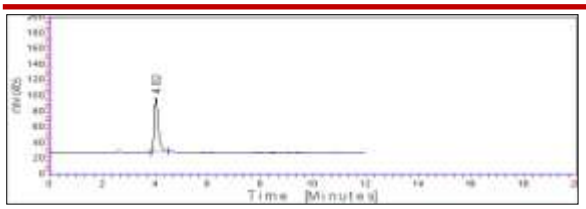


Figure 3 Chromatograms of PVT (15µg/ml) in a tablet formulation

Analysis of Tablets

The concentration of PVT in the tablet formulation was found to be 99.98%. The low values of % RSD indicate that the method is precise and accurate in table 5.

Table 5 Results of tablet analysis

S.NO.	Parameter	Sample
		Pitavastatin
1	% Found	99.98
2	S.D.	0.125
3	% R.S.D.	0.125
4	SEσ*	0.243

* Mean of nine determinations

Conclusion

The developed HPLC methods for Pitavastatin in tablet formulation using mobile phase 20mM KH_2PO_4 (pH-3.0 with OPA): Acetonitrile (20:80 % v/v), at the flow rate of 1 ml/min. A calibration graph was plotted which showed a linearity range between5-25 µg/ml with the correlation coefficient of 0.997. The proposed method is simple, sensitive and accurate with good precision and is suitable for routine analysis of Pitavastatin in formulations.

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