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HPLC Determination of Pitavastatin Calcium in Pharmaceutical Dosage Forms

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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 calcium using paracetamol as internal standard. The chromatographic system consisted of Shimadzu LC-10ATVP Pump, SPD-M10 AVP with PDA detector. Separation was achieved on the phenomenex C_{18} (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. 0.5% Acetic acid: Acetonitrile 35:65 (%, v/v), was used as mobile phase with flow rate of 1 ml/min. UV detection was performed at 245 nm. A calibration graph was plotted which showed a linearity range between 1-5 µg/ml with the correlation coefficient of 0.9986. The LOD was 5 ng/ml, while the LOQ was 20 ng/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 calcium.

Keywords: HPLC; Pitavastatin calcium; Pharmaceutical dosage form; Method Validation

Introduction

Pitavastatin is a new synthetic 3-hydroxy-3-methyl glutaryl coenzyme A 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 (Figure 1). Metabolism of pitavastatin by the cytochrome P450 (CYP) system is minimal, principally through CYP 2C9, with little involvement of the CYP 3A4 isoenzyme, potentially reducing the risk of drug-drug interactions between pitavastatin and other drugs known to inhibit CYP enzymes [1,2]. [3] investigated the effect of repeated intake of grapefruit juice (GFJ) on the pharmacokinetics of pitavastatin and atorvastatin in Japanese subjects. The Plasma concentrations of pitavastatin acid and lactone, atorvastatin acid and lactone, and 2-hydroxy atorvastatin acid were quantified by the liquid chromatography-mass spectrometry methods. The mobile phase consist of acetonitrile and either 0.1% ammonium hydroxide in water (for pitavastatin compounds) or 10 mm ammonium acetate with 0.4% ammonia hydroxide in water (for atorvastatin) were used [3]. LC-MS/MS method with electrospray ionization (ESI) were developed for the simultaneous determination of pitavastatin and its lactone in human plasma and urine. The calibration curves of pitavastatin and its lactone were obtained in the concentration range of 1-200 ng/ml, with a lower limit of quantitation of 1 ng/ml using methanol-0.2% acetic acid in water (70: 30, v/v) as the mobile phase [4]. [5] studied the pharmacokinetics of pitavastatin in patients with liver cirrhosis [5]. [6] studied the inhibitory effect of NK-104, a potent inhibitor of HMG-CoA reductase, on taxol metabolism were examined using radio-TLC. This method is described for in vitro measurement of taxol metabolites as an alternative to the commonly used HPLC assay [6]. A simple and sensitive column-switching HPLC method were developed for the simultaneous determination of NK-104 (HMG-CoA reductase inhibitor) and its lactone in human and dog plasma. The calibration curves of NK-104 and NK-104 lactone were linear over the ranges 0.5 to 100 ng/ml for human plasma samples and 0.5 to 500 ng/ml for dog plasma, respectively [7]. [8] reported an analytical method for the determination of the drug from human plasma making use of the fluorescent property of the drug. Plasma is extracted from the drug using ethyl acetate and then analyzed by reversed-phase high performance liquid chromatography (HPLC) with fluorescence detection using C_{18} HPLC column with gradient flow of mobile phase (0.01 mol/L monobasic potassium phosphate (pH 3.20) acetonitrile, 63:37, v/v) [8]. HPTLC determination of pitavastatin calcium in tablet dosage form were studied with the mobile phase of ethyl acetate-methanol-ammonia-1 drop formic acid (7:2:0.8) [9]. Another HPTLC method were developed by [10] in that method they used toluene-
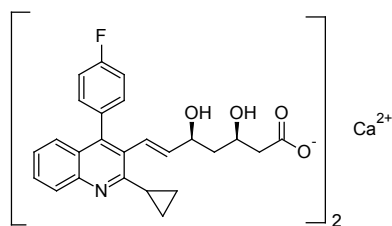

Figure 1: Chemical structure of pitavastatin calcium.

methanol-glacial acetic acid 7.6:2.36:0.04 (v/v) as mobile phase and quantified at 238 nm [10]. HPLC and UV Spectroscopy method has been reported for Pitavastatin calcium in tablet formulation by [11]. In that study [11] developed a method with different mobile phase containing acetonitrile-water-triethylamine (80 + 19.8 + 0.2, v/v/v), adjusted to pH 3.5 +/- 0.05 with orthophosphoric acid at a flow rate of 1.5 ml/min were used for the separation of Pitavastatin calcium. The disadvantage of the method is that its run time is about 5.70 min. Our study is to report an isocratic RP-LC method for the estimation of Pitavastatin Calcium using paracetamol as internal standard and the mobile phase consist of 0.5% Acetic acid: Acetonitrile 35:65 (%, v/v), with the flow rate of 1 ml/min. The retention time of Paracetamol and Pitavastatin Calcium were 2.5 and 4.2 min respectively. And it shows good peak purity and correlation. The developed method is simple, rapid, accurate and sensitive for estimating Pitavastatin calcium in tablet formulations than the previously reported method.

Experimental

Chemicals and reagents

Pitavastatin calcium was from Micro Laboratories. HPLC-grade Acetonitrile and methanol was purchased from Merck, India. High-purity water was prepared using Millipore purification system. Other chemicals and reagents were of AR grade Pitava 1 and Flovas 1mg were obtained commercially.

Instrumentation and chromatographic conditions

HPLC analysis was performed on a Shimadzu LC-10ATVP pump, SPD-M10 AVP with PDA detector. Separation was achieved on the phenomenex C_{18} (250 x 4.60); 5μ particle size column at room temperature with the mobile phase constituted by 0.5% acetic acid: acetonitrile (35:65) at the flow rate of 1 ml/min. The mobile phase was filtered through 0.45μm nylon filter (Millipore, USA) and degassed by sonication prior to use. The detection was performed at 245 nm. The Standard and sample was introduced through an injector valve with a 20 μl, sample loop. Data acquisition was performed using CLASS-VP software by the measurement of peak areas.

Preparation of standard solution

Standard stock solution of pitavastatin calcium and paracetamol (1000 μg/ml) was prepared in mobile phase containing 0.5% Acetic acid: Acetonitrile 35:65 (%, v/v). Working standard solutions for the calibration curve were prepared by dilution of the above stock solution in a range of 1-5 μg/ml with mobile phase. To each dilution a fixed concentration of 1 μg/ml of internal standard paracetamol was added.

Preparation of sample solution

Twenty tablets of Pitava and Flovas containing 1 mg of Pitavastatin calcium were weighed to get the average weight and then powdered. An amount of powder equivalent to 1 mg of Pitavastatin calcium was transferred to a 100 ml volumetric flask with 50 ml mobile phase 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.

Method development and choice of internal standard

A variety of mobile phases and internal standards were investigated in the development of an HPLC method suitable for analysis of Pitavastatin Calcium in the tablet formulations. Finally, the condition adopted was 0.5% acetic acid: acetonitrile 35:65 (%, v/v), at the flow rate of 1 ml/min. Paracetamol was selected as the suitable internal standard for this method.

Method validation

The method was validated for the parameters like specificity, range and linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, and precision based on ICH/CPMP guidelines [12-15].

Results and Discussion

For RP-HPLC of Pitavastatin Calcium, various mobile phases were tried like methanol–water, 75:25 (% v/v), acetonitrile - water, 75:25, methanol - water, 50:50, methanol - water, 95:5, methanol - phosphate buffer (pH 3.5–6.5), 80:20, and acetonitrile - phosphate buffer (pH 3.5–6.5), 80:20. The suitability of the mobile phase was decided on the basis of the sensitivity of the assay, suitability for stability studies, time required for the analysis, ease of preparation, and use of readily available cost-effective solvents. The same solvent mixture was used for extraction of the drug from the formulation containing excipients. Among the various mobile phases used, the mobile phase containing 0.5 % acetic acid and acetonitrile in the ratio of 35:65 (v/v) was found to be best for analysis, at the flow rate of 1 ml/min. An injection volume of 20 μl and UV detection at 245 nm was used. For choosing an appropriate internal standard for the analysis of Pitavastatin Calcium, four drugs like Aspirin, Diclofenac Sodium, Paracetamol and Nimuslide were used. Among these, Paracetamol was chosen because it met all the typical requirements of an internal standard. It was stable during the analysis, readily available, was well resolved, its peak shape was good (tailing factor 1.24), and its elution time was short. The developed method was also found to be specific, accurate, precise and robust with acceptable values of LOD and LOQ.
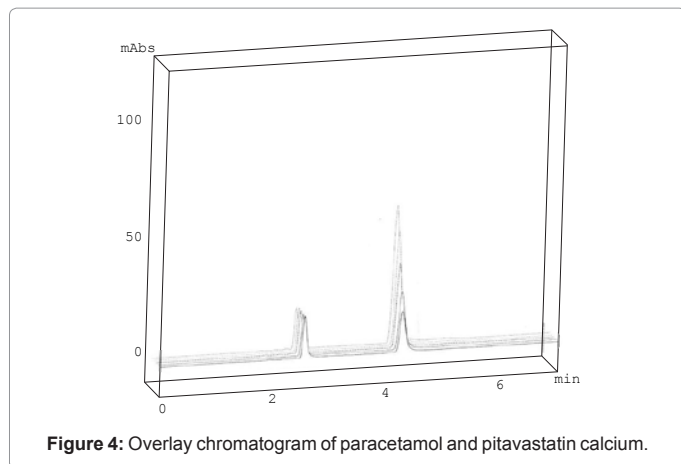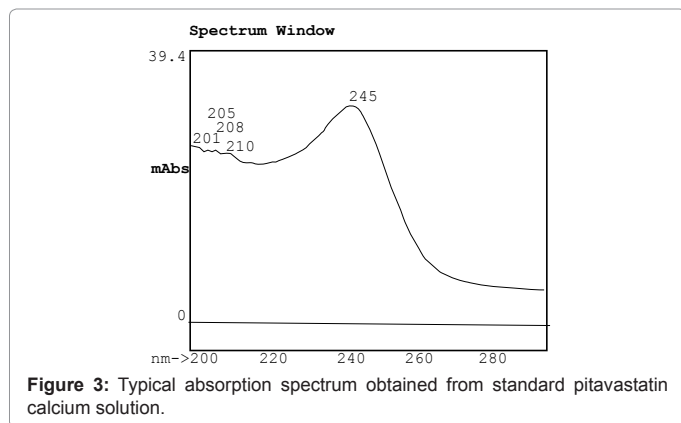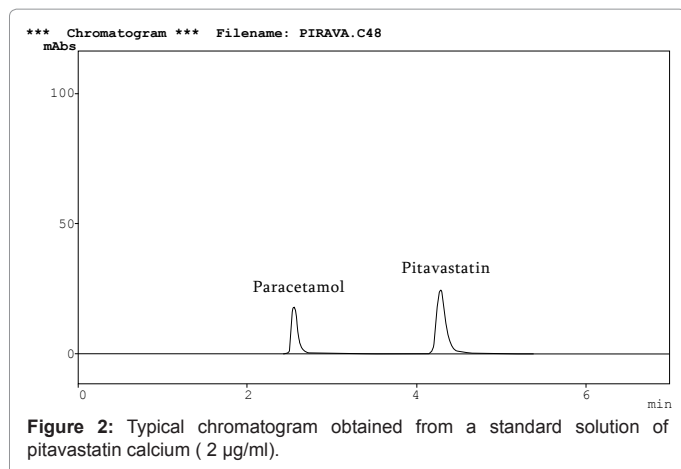
Linearity

Linearity of the proposed method was verified by analyzing five solutions in the range of 1-5 μg/ml for pitavastatin calcium (1, 2, 3, 4 and 5 μg/ml). Each concentration was made in triplicate. Peak area ratios of standards compounds were plotted against theoretical concentration of standards. The linearity was expressed as a correlation coefficient by linear regression analysis. A calibration graph was plotted which showed a linearity range between 1-5μg/ml with the correlation coefficient of r^2 = 0.9986.

Concentration [μg/mL]	Intraday precision (RSD [%] n=3)	Interday precision (RSD [%] n=3)
1	0.93	1.32
2	0.72	1.28
3	0.81	1.16

Table1: Result from determination of precision of analysis of pitavastatin calcium.

Sample	Label claim [mg per tablet]	Initial amount [μg/ml]	Amount added [%]	Recovery [%]	RSD [%]
Brand I [PITAVA]	1	2	0	99.27	0.14
		2	80	99.86	0.23
		2	100	99.91	0.08
		2	120	99.79	0.05
Brand II [FLOVAS]	1	2	0	99.48	0.17
		2	80	99.70	0.21
		2	100	99.87	0.27
		2	120	99.43	0.12

Table 2: Result from recovery studies.

Figure 2: Typical chromatogram obtained from a standard solution of pitavastatin calcium (2 µg/ml).

Figure 3: Typical absorption spectrum obtained from standard pitavastatin calcium solution.

Figure 4: Overlay chromatogram of paracetamol and pitavastatin calcium.

Limit of detection and limit of quantification

The sensitivity of measurement of pitavastatin calcium by use of proposed method was estimated in terms of the limit of quantification (LOQ) and the lowest concentration detected under the chromatographic condition as the limit of detection (LOD). The LOQ and LOD were calculated by the use of equations LOD=3 × N/B and LOQ =10 × N/B where N is the standard deviation of the peak area of the drug (n=3), taken as a measure of noise and B is the slope of the corresponding calibration plot. Solutions of Pitavastatin Calcium were prepared in the range of 2 to 500ng/ml and injected in triplicate.

Average peak area of three analyses was plotted against concentration. The limit of quantification (LOQ) was 20 ng and limit of detection (LOD) was 5 ng.

Precision

Precision was measured by analysis of sample solution containing Pitavastatin Calcium at concentrations covering the entire calibration range. The precision of the method in terms of intra day variation (% CV) was determined by analysis of pitavastatin calcium standard solution 1 -5 µg/ml (n=6). Inter-day precision (% CV) was assessed by analysis of the same solution on three different days. The results of precision studies are expressed in Table 1. The results showed that the proposed method is highly reproducible. Intra-day and Inter-day precision ranged from 0.72 to 0.93 % and 1.16 to 1.32% respectively. Intra-day and inter-day precision was also within acceptability criteria of %RSD ≤2.0 and ≤3.0, respectively, indicating method precision was good [16].

Accuracy

The accuracy of the method was determined by analysis of standard at three different levels, ie. Multiple level recovery studies. A stock solution of a tablet formulation was prepared containing 2 µg/ml Pitavastatin Calcium. Amount of standard drug solution equivalent to 80%, 100% and 120% of the concentration in the table solution were added and recovery [%] was determined. Values were found to be within the limit given in (Table 2). Intra-day and inter-day accuracy were within acceptability criteria for bias, ±5% [16].

Specificity

The mobile phase designed for the method resolved the drug very efficiently, as is shown in (Figure 2). The retention time of Paracetamol and Pitavastatin Calcium were 2.5 and 4.2 min respectively and typical absorption spectrum and overlay chromatogram of pitavastatin calcium is shown in (Figure 3 and 4). The wavelength 245 nm was selected for the detection because it resulted in better detection sensitivity for the drug.

Repeatability

The repeatability of sample application was assessed by injecting 2 µg/ml of Pitavastatin Calcium six times. The RSD [%] for peak-area value of pitavastatin calcium were found to be 0.93 and 0.96 respectively.

Conclusion

The developed HPLC methods for Pitavastatin Calcium in tablet formulation using mobile phase 0.5% acetic acid: acetonitrile 35:65 (%, v/v), at the flow rate of 1 ml/min. A calibration graph was plotted which showed a linearity range between 1-5 µg/ml with the correlation coefficient of 0.9986. The proposed method is simple, sensitive and accurate with good precision and is suitable for routine analysis of Pitavastatin Calcium in formulations.

Acknowledgments

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