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DEVELOPMENT AND VALIDATION OF SIMPLE, RAPID AND SENSITIVE UV, HPLC AND HPTLC METHODS FOR THE ESTIMATION OF PITAVASTATIN IN TABLET DOSAGE FORM

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ABSTRACT

UV, HPLC and HPTLC methods were developed and validated for the quantitative determination of Pitavastatin, is an inhibitor of HMG-CoA reductase, the enzyme that catalyses the first step of cholesterol synthesis. Chromatography was carried out by isocratic technique on reversed phase Lichrospher®100, C18 column (150 x 4.6 mm, 5µm) with mobile phase consisting of Methanol: Phosphate buffer (pH adjusted to 5.0) (85:15 v/v) at flow rate 1mL/min. By using stationary phase precoated Silica Gel 60 F254 TLC Plate using mobile phase Toluene: Methanol, 8:2 v/v TLC was carried out. The UV spectrophotometric determination was performed at 244nm using solvent methanol. According to ICH Q2-(R1) guidelines the proposed methods were validated. The linearity range for Pitavastatin was 5-50µg/mL for HPLC, 50-400ηg/band for HPTLC and 10-60µg/mL for UV method. These methods were accurate and precise with recoveries in the range of 99.50-101.45 and relative standard deviation < 2%. The developed methods were successfully applied for determination of Pitavastatin in tablets.

KEYWORDS

UV, HPLC, HPTLC, Pitavastatin and Anti-hypertensive agent.

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INTRODUCTON

Pitavastatin [Figure No.1] is (E)-7-[2-cyclopropyl-4-(4-fluorophenyl) qui-nolin-3-yl]-3, 5-dihydroxy-hept-6-enoic acid. Calcium salt of Pitavastatin is a novel member of the medication class of statins. Like the other statins, it is an inhibitor of HMG-CoA reductase, the enzyme that catalyses the first step of cholesterol synthesis. It has been available in Japan since 2003, and is being marketed under license in South Korea and in India. It is likely that

pitavastatin will be approved for use in hypercholesterolemia[1,2].

Literature search revealed that methods like Review article on Analytical techniques for estimation of pitavastatin[3], validated stability indicating UPLC method[4], Simple LC–MS/MS methods for simultaneous determination of pitavastatin and its lactone metabolite in human plasma and urine[5], Determination of pitavastatin in human plasma via HPLC-ESI-MS/MS[6], Simultaneous determination of pitavastatin Ca and ezetimibe by liquid chromatography[7], HPLC determination of pitavastatin calcium in pharmaceutical dosage forms[8,9], Novel spectrophotometric method for the assay of pitavastatin calcium in pharmaceutical formulations[10], Quantification of pitavastatin calcium in Pharmaceutical Dosage Forms by HPTLC[11-13]. In this paper we are reporting a better assay including UV and HPTLC along with HPLC method for routine quality control of Pitavastatin formulations.

EXPERIMENTAL

Chemicals and Materials

A request sample of Pitavastatin was received from Mylan Laboratories Ltd., Hyderabad. Methanol AR, acetonitrile HPLC and water HPLC, toluene (AR) grade was purchased from Merck India limited. Film coated tablets of Pitavastatin (PIVASTA 4mg, zydus cadila) were procured from local market. The chromatographic system comprised of a Shimadzu HPLC system equipped with Shimadzu LC-20AD prominence with LC solution software, SPDM-20A prominence PDA detector, Shimadzu DGU-20A$_5$ prominence degasser. Pre-filtered sample were injected in to Lichrospher®100, C18 column (150 x 4.6 mm, 5µm) using Rheodyne syringe loading sample injector (Rheodyne injector 7725i with 20 µl loop). The mobile phase consisted of Methanol: Phosphate buffer (pH adjusted to 5.0) (85:15 v/v). HPTLC instrument consists of CAMAG Linomat-IV TLC applicator, CAMAG TLC-Scanner-III (version 4.0.1) supported with CAMAG Wincats® software (version 4.0.1), CAMAG twin trough glass chamber (10 cm × 10 cm) for TLC development and Hamilton Syringe (100µL). The mobile phase consisted of toluene- methanol (8:2 v/v). A spectrophotometric system consists of a double beam Shimadzu UV/VIS spectrophotometer, UV-1700 having two matched 10 mm quartz cells with 1cm light path equipped with Shimadzu UV probe software was employed in spectrophotometric analysis.

Preparation of standard solution

To prepare standard solution, accurately weighed 100 mg of Pitavastatin was transferred in to 100 mL volumetric flask, dissolved and diluted up to the mark with methanol to obtain stock solution containing 1.0mg/mL of the drug.

Preparation of sample solution

The sample solution was prepared by following method; twenty tablets containing Pitavastatin were accurately weighed and finely powdered. Accurately weighed amount of powder equivalent to 100mg of Pitavastatin was transferred to 100mL volumetric flask and 50mL of methanol was added. The mixture was sonicated for 30 min, diluted to mark with methanol and filtered through whattman filter paper no.41 from the stock solution 1.0mL was accurately transferred to 10mL volumetric flask and diluted with methanol to obtain an intermediate solution of 100µg/mL of Pitavastatin. Aliquot of 1.0mL of this solution was accurately diluted to 10mL with diluents to obtain the final sample solution containing 10µg/mL of Pitavastatin. This was filtered through 0.45µm nylon filter, degassed by sonication and volume of 20µl was injected in HPLC system. As that same in high performance thin layer chromatography from the stock solution 1.0 mL was accurately transferred to 10mL volumetric flask and diluted with methanol to obtain an intermediate solution of 100µg/mL of Pitavastatin was applied on HPTLC plate. In UV spectrophotometric sample preparation was same as that of HPTLC from the stock solution 1.0 mL was accurately transferred to 10mL volumetric flask and diluted with methanol to obtain an intermediate solution of 100µg/mL of Pitavastatin. Aliquot of 1.0mL of this solution was accurately diluted to 10mL with diluents to obtain the final sample solution containing 10µg/mL of Pitavastatin.

Development of Spectrophotometric and Chromatographic methods

The UV absorption spectrum of Pitavastatin in methanol depicted λmax at 244 nm and a shoulder peak at 207 nm (Figure No.2A). Wavelength showing the maximum absorbance 244 nm was selected for measurement of absorbance. The HPLC conditions for Pitavastatin was optimal with isocratic mode Methanol: Phosphate buff (pH 5) 85:15 v/v at flow rate 1mL/min in a total run time of ten minutes and was used to check the system suitability study. Figure No.2B exhibits the corresponding chromatogram of standard Pitavastatin in optimized HPLC conditions showing retardation time for Pitavastatin at 4.58 min. HPTLC was performed on aluminium-backed Silica Gel 60F254 HPTLC plate from Merck, India. Sample solution was applied as 6 mm band and 4 mm spacing at a constant application rate of 5 sec µl-1 by means of Linomat IV sample applicator fitted with a 100-µl syringe. The Linear ascending development was performed in a CAMAG twin trough glass chamber with mobile phase consisting of toluene: methanol (8:2 v/v). The optimum time for saturation of the chamber with mobile phase vapour was 15 min at ambient temperature (25 ± 2ºC) and relative humidity 60 ± 5%. The plates were allowed to develop at distance of 7.0 cm. Subsequent to development; the plates were dried in current of dry air by using compact hot air dryer. Plates were evaluated by densitometry at 245 nm by means of a CAMAG TLC scanner III with Wincat software version 4.0.1. Figure No.2C displays the corresponding chromatogram of standard Pitavastatin in optimized HPTLC condition.

Validation of the methods

The proposed methods were validated according to International Conference on Harmonization ICH Q2 (R1) guidelines. The method was validated using various parameters such as precision, accuracy, specificity and linearity[14-17]. The system suitability and validation parameters for determination of Pitavastatin by HPLC method are depicted in Table No.1. Linear correlation was found between peak areas versus concentration of Pitavastatin in the range of 5-50µg/mL the result of injection repeatability (RSD<1%), intraday and interday precision (RSD<1%) and method reproducibility (RSD<2%) proved the method to be precise. The % recovery was found to be in the range of 99.50-101.45% (Table No.2). The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.96 and 2.92µg/mL of Pitavastatin respectively. The specificity of the method was assessed by comparing chromatograms of Pitavastatin obtained from standard solution of drug and that obtained from formulation sample solutions (Figure No.2). The retardation time of the drug from standard and sample solution was identical. The comparison of chromatograms confirmed that excipients did not interfere in the separation of Pitavastatin. Peak purity check of Pitavastatin peaks obtained from standard solution was found to be 0.9972, conforming to the specificity of the method. This was further supported by good correlation (r=0.997) between the PDA spectrum of Pitavastatin from standard solution and that from sample solution of formulation. The change (%5) in proportion of organic solvent, the change ± 2 in wavelength and flow rate did not show significant variation in the area for Pitavastatin (%RSD<2). The stability of working standard solution of Pitavastatin was evaluated to verify that any spontaneous degradation occurs when the sample were prepared. The stability profile for sample solution of Pitavastatin was studied at different conditions such as normal, acidic, basic, oxide, heat and sunlight. The results were expressed as percentage of drug remaining. The data obtained showed that sample solution was stable during all the conditions with degradation less than 2% except in acidic condition. For acidic condition 0.01 N HCl was used because it readily degraded, in higher concentration of HCl.

The validation parameters for determination of Pitavastatin by HPTLC method are depicted in Table No.1. Linear correlation was found to be in the range of 50-400ng/spot respectively. The result of repeatability (RSD<1%), intraday and interday precision (RSD<2%) and method reproducibility (RSD<2%) proved the method to be precise. The % recovery was found to be in the range of 99.27-

101.87% (Table No.2). The specificity of the method was assessed by comparing chromatograms of Pitavastatin obtained from standard solution of drug and that obtained from formulation sample solutions (Figure No.2C). The Rf value of the drug from standard solution and from sample solution were identical. The comparison of chromatograms confirmed that excipients did not interfere in the separation of Pitavastatin. This was further supported by good correlation (r=0.997). The change (%5) in proportion of mobile phase and change ±2 in wavelength did not show significant variation in the area for Pitavastatin (%RSD<2). The stability of working standard solution of Pitavastatin was evaluated to verify that any spontaneous degradation occurs when the sample were prepared. The stability profile for sample solution of Pitavastatin was studied at different conditions such as normal, acidic, basic, oxide, heat and sunlight. The results were expressed as percentage of drug remaining. The data obtained showed that sample solution were stable during all the conditions with degradation less than 2%. Validation parameters for determination of Pitavastatin by UV method are depicted in Table No.1. The Beer's range was found to be in the range of 10-60µg/mL of Pitavastatin. The result of regression analysis and validation for determination of Pitavastatin by UV method are shown in Table No.1. The variability in the precision study was found within limits proving the method to be precise. The % recovery in accuracy study was found to be 98.20-102.2% (Table No.2). The limit of detection (LOD) and (LOQ) was found to be 0.98 and 2.96, respectively. The working solution of Pitavastatin in methanol was found to be stable for at least five days.

Tablet powder was sonicated with methanol for 30 min to ensure complete dissolution of drug. The developed UV, HPLC and HPTLC methods were applied to estimate content of Pitavastatin in marketed tablet formulation by our method. The amount of Pitavastatin was calculated using the respective regression equation obtained from calibration graph of UV, HPLC and HPTLC methods. Result of analysis of pharmaceutical formulation containing Pitavastatin is shown in Table No.3.

RESULTS AND DISCUSSION

The earlier reported HPLC method lacks accuracy with respect to peak sharpness and a single method is reported. We have successfully utilized an isocratic elution technique at room temperature with UV detection for the determination of Pitavastatin in marketed formulation. This method is void of tedious sample preparation procedure and is highly economic. The real advantage of the proposed HPLC method is low retardation time of 4.58 min for Pitavastatin, with sharp result. The real advantages of the proposed HPTLC method are high sample throughput with small amount of solvent requirement and simple sample preparation techniques.

Table No.1: Validation parameters for determination of Pitavastatin by proposed UV, HPLC and HPTLC methods

S.No	Validation of developed methods	
1	**Parameters**	**UV Spectrophotometric method**
2	Linearity range	10-60 µg/mL
3	Regression equation	y = 0.059x
4	Correlation coefficient	0.997
5	Intra-day precision (%RSD, n=3)	1.20
6	Inter-day precision (%RSD, n=3)	1.50
7	Reproducibility	1.90
	Parameters	**HPLC method**
8	Retardation time ± SD (min)	4.58±0.008
9	Capacity factor	1.32
10	Tailing factor	1.45
11	Theoretical plate	3916
12	Injection repeatability (%RSD, n=5)	0.32
13	Linearity range (µg/mL)	5-50 µg/mL
14	Regression equation (y= mx + c)	Y= 71278X – 21082
15	Correlation coefficient	0.997
16	Intra-day precision (%RSD, n=3)	0.98
17	Inter-day precision (%RSD, n=3)	0.67
18	Reproducibility (%RSD, with different analyst)	1.23
19	**Parameters**	**HPTLC method**
20	Regression equation	y=535.887+16.64*x
21	Correlation coefficient	0.997
22	Linearity	50-400ηg/band
23	Precision(intraday)%RSD	1.45
24	Precision(interday)%RSD	1.57
25	LOD	1.05
26	LOQ	3.21
27	Specificity	Specific
28	Robustness	Method is robust
29	%Assay	99.12±1.58%
30	R_f	0.50±0.02

SD=standard deviation, RSD=relative standard deviation, y=concentration of Pitavastatin in µg/mL, x=peak area of Pitavastatin.

Table No.2: Recovery studies for UV, HPLC and HPTLC method

S.No	Recovery Studies			
	Method	Amount taken (µg/mL)	Amount added (mg)	%Recovery (mean ± SD)
1	UV	10	3.2	100.50±1.40
			7.5	99.95 ±1.20
			11	100.05±1.15
2	HPLC	10	3.2	101.45±1.25
			7.5	99.89±1.50
			11	99.50±1.65
3	HPTLC	10	3.2	99.27±1.10
			7.5	99.85±1.20
			11	100.45±0.90

Table No.3: % Estimation of Pitavastatin formulation

S.No	Dosage form	Labeled claim (mg)	Methods	% Content of drug ± SD
1	Tablet	4	UV	101.23 ± 1.65
			HPLC	100.10 ± 1.15
			HPTLC	100.03 ± 1.05

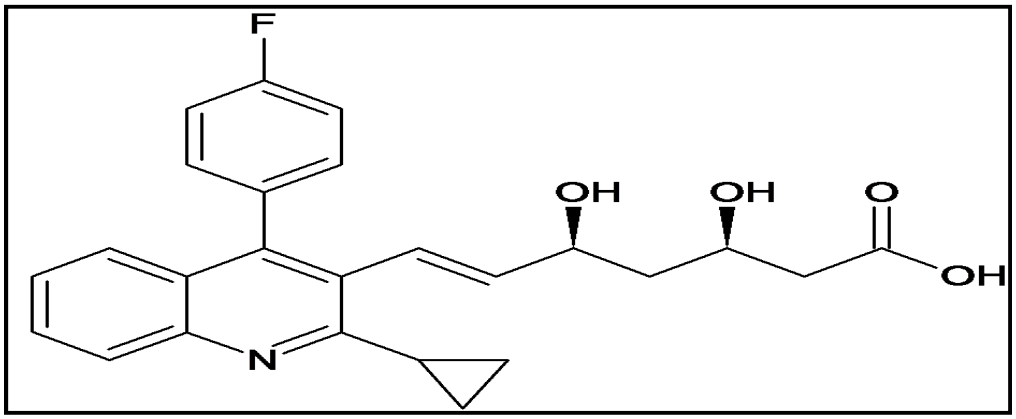
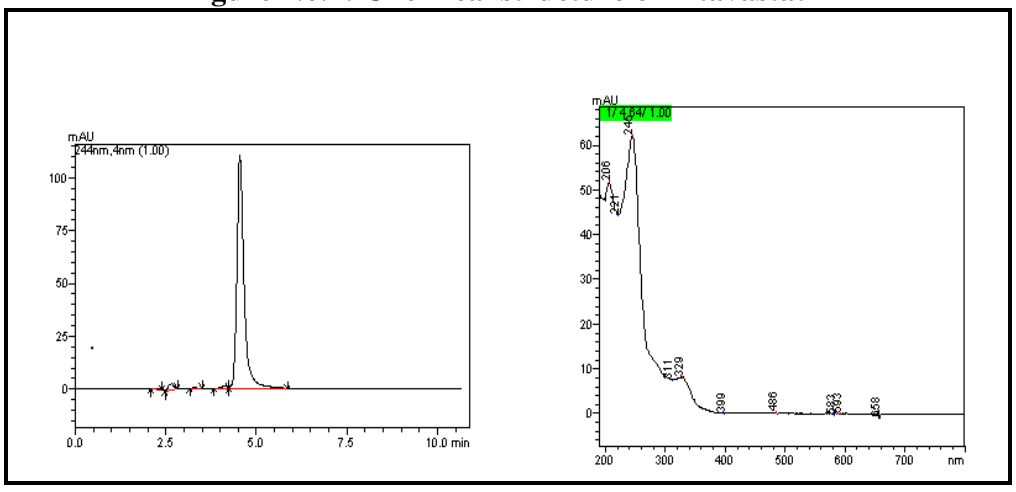
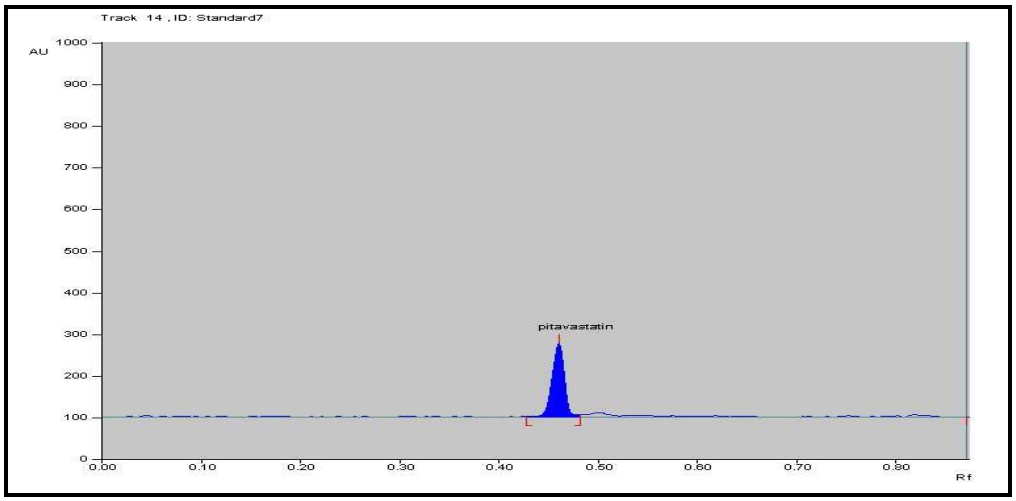


Figure No.1: Chemical structure of Pitavastatin



(a) (b)



(c)

Figure No.2: (a) UV spectra of Pitavastatin, (b) Chromatogram of Pitavastatin (c) Densitogram of Pitavastatin

CONCLUSION

To conclude with, reported UV, HPLC and HPTLC method for the determination of Pitavastatin in pharmaceutical formulation were found to be highly economical, simple, sensitive, accurate, precise and rapid. Moreover, the HPTLC method presented was found to be rather simple and time saving alternative to HPLC method of analysis for Pitavastatin for routine quality control of pharmaceutical formulation.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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