DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE DETERMINATION OF PITAVASTATIN IN BULK FORM AND MARKETED PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC METHOD

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***ABSTRACT:** The current investigation was pointed at developing and progressively validating novel, simple, responsive and stable RP-HPLC method for the Quantitative Determination of Pitavastatin in active pharmaceutical ingredient and Marketed Pharmaceutical Dosage form.A simple, selective, validated and well-defined stability that shows isocratic RP-HPLC methodology for the quantitative determination of Pitavastatin. The chromatographic strategy utilized Symmetry C18, 250 mm x 4.6 mm i.d.5µm particle size, using isocratic elution with a mobile phase consists of Methanol and Phosphate Buffer (0.02M) (pH-3.8) was taken in the ratio of 70: 30% v/v. A flow rate of 1.0 ml/min and a detector wavelength of 245nm utilizing the UV detector were given in the instrumental settings. Validation of the proposed method was carried out according to an international conference on harmonization (ICH) guidelines.LOD and LOQ for the active ingredients were established with respect to test concentration. The calibration charts plotted were linear with a regression coefficient of R2>0.999, means the linearity was within the limit. Recovery, specificity, linearity, accuracy, robustness, ruggedness were determined as a part of method validation and the results were found to be within the acceptable range.The proposed method to be fast, simple, feasible and affordable in assay condition. During stability tests, it can be used for routine analysis of the selected drugs.*

***Key Words:** Pitavastatin, RP-HPLC, Method Development, Validation, Accuracy, Precision.*

I. INTRODUCTION

Pitavastatin[1] is a relatively newly developed cholesterol lowering agent (statin) that is associated with mild, asymptomatic and self-limited serum aminotransferase elevations during therapy, but has had limited use and has yet to be linked with clinically apparent acute liver injury. Pitavastatin is an oral Antilipemic agent which inhibits HMG-CoA reductase. It is used to lower total cholesterol, low density lipoprotein-cholesterol (LDL-C), apolipoprotein B (apoB), non-high density lipoprotein-cholesterol (non-HDL-C), and trigleride (TG) plasma concentrations while increasing HDL-C concentrations. High LDL-C, low HDL-C and high TG concentrations in the plasma are associated with increased risk of atherosclerosis and cardiovascular disease. The total cholesterol to HDL-C ratio is a strong predictor of coronary artery disease and high ratios are associated with higher risk of disease. Increased levels of HDL-C are associated with lower cardiovascular risk. By decreasing LDL-C and TG and increasing HDL-C, Rosuvastatin reduces the risk of cardiovascular morbidity and mortality. Pitavastatin[2] is a statin medication and a competitive inhibitor of the enzyme HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase, which catalyzes the conversion of HMG-CoA to mevalonate, an early rate-limiting step in cholesterol biosynthesis. Pitavastatin acts primarily in the liver, where decreased hepatic cholesterol concentrations stimulate the up regulation of hepatic low density lipoprotein (LDL) receptors which increase hepatic uptake of LDL, thereby reducing circulating LDL-C levels. Pitavastatin[3] is indicated for the treatment of

adult patients with primary hyperlipidemia or mixed dyslipidemia to reduce elevated total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), apolipoprotein B (Apo B), triglycerides (TG), and to increase high-density lipoprotein cholesterol (HDL-C). It is also indicated for the treatment of Pediatric patients aged 8 years and older with heterozygous familial hypercholesterolemia (HeFH) to reduce elevated TC, LDL-C, and Apo B. Pitavastatin is used along with a proper diet to help lower "bad" cholesterol and fats (such as LDL, triglycerides) and raise "good" cholesterol (HDL) in the blood. It belongs to a group of drugs known as "statins." It works by reducing the amount of cholesterol made by the liver. The IUPAC name of Pitavastatin is (3R, 5S, 6E)-7-[2-cyclo propyl-4-(4-fluoro phenyl) quinolin-3-yl]-3, 5-dihydroxy hept-6-enoic acid. The Chemical Structure of Pitavastatin is shown in fig-1.

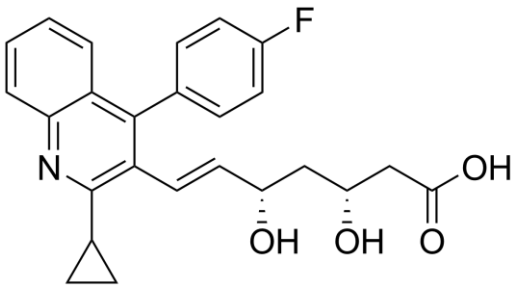

Fig.1. Chemical Structure of Pitavastatin

II. EXPERIMENTAL METHODS

Materials and Instruments:

The following are the list of instruments/Equipments, chemicals/reagents and standards to perform the HPLC Analysis[4-7] of the drug Pitavastatin.

Equipments:

Table-1: List of Equipments

S.No.	Instruments/Equipments/Apparatus
1.	HPLC WATERS with Empower2 Software with Isocratic with UV-Visible Detector.
2.	T60-LABINDIA UV – Vis spectrophotometer
3.	High Precision Electronic Balance
4.	Ultra Sonicator (Wensar wuc-2L)
5.	Thermal Oven
6.	Symmetry C_{18} Column, 250 mm x 4.6 mm and 5µm particle size
7.	P^H Analyser (ELICO)
8.	Vaccum Filtration Kit (Labindia)

Chemicals and Reagents:

Table-2: List of Chemicals used

S.No.	Name	Grade	Manufacturer/Supplier
1.	HPLC grade water	HPLC	Sd fine-Chem ltd; Mumbai

2.	Methanol	HPLC	Loba Chem; Mumbai.
3.	Ethanol	A.R.	Sd fine-Chem ltd; Mumbai
4.	Acetonitrile	HPLC	Loba Chem; Mumbai.
5.	DMSO	A.R.	Sd fine-Chem ltd; Mumbai
6.	DMF	A.R.	Sd fine-Chem ltd; Mumbai

HPLC Instrumentation & Conditions: The HPLC system[8] employed was **HPLC WATERS** with Empower2 Software with Isocratic with UV-Visible Detector.

Standard preparation for UV-Spectrophotometer Analysis:

The Standard Stock Solutions – 10 mg of Pitavastatin standard was transferred into 10 ml volumetric flask, dissolved & make up to volume with Methanol. Further dilutions were done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with methanol to get 10ppm concentration.

It scanned in the UV spectrum[9] in the range of 200 to 400nm. This has been performed to know the maxima of Pitavastatin, so that the same wave number can be utilized in HPLC UV detector for estimating the Pitavastatin.

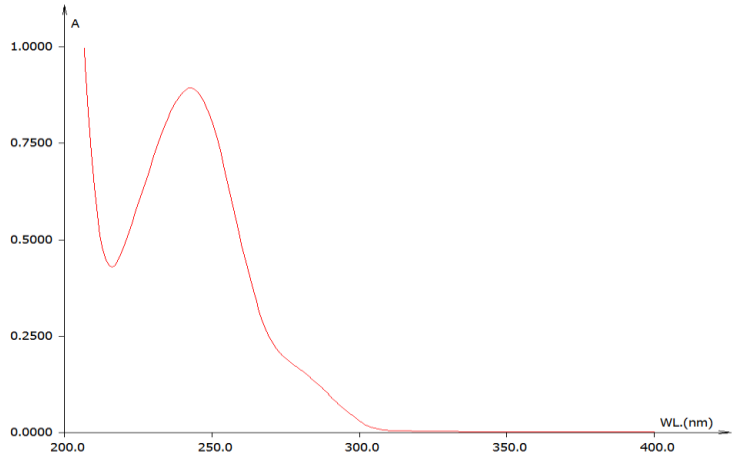

Fig.2. UV-Spectrum for Pitavastatin (245nm)

Observation: While scanning the Pitavastatin solution we observed the maxima at 245nm.

Different Trials For Chromatographic Conditions:

Table-3: Different Chromatographic Conditions

Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Develosil C_{18}, 250 mm x 4.6 mm and 5µm Column	Acetonitrile : Water = 65 : 35	0.8 ml/min	245nm	Base line noise is high	Method rejected
Develosil C_{18}, 250 mm x 4.6 mm and 5µm Column	Acetonitrile : Water = 55 : 45	0.8ml/min	245nm	Tailing is more	Method rejected
Zorbax C_{18}, 250 mm x 4.6 mm and 5µm Column	Methanol : Acetonitrile = 30 : 70	0.9 ml/min	245nm	Extra peaks	Method rejected

Phenomenex C_{18}, 250 mm x 4.6 mm and 5µm Column	Methanol : Acetonitrile = 60 : 40	1.0 ml/min	245nm	Good sharp peak	Method accepted
Symmetry C_{18}, 250 mm x 4.6 mm and 5µm Column	Methanol : Acetonitrile = 50 : 50	1.0 ml/min	245nm	Improper peak separation	Method rejected
Symmetry C_{18}, 250 mm x 4.6 mm and 5µm Column	Methanol : Phosphate Buffer (0.01M) (pH-2.8) = 40 : 60	1.0 ml/min	245nm	Tailing peaks	Method rejected
Symmetry C_{18}, 250 mm x 4.6 mm and 5µm Column	Methanol : Phosphate Buffer (0.02M) (pH-3.2) = 60 : 40	1.0 ml/min	245nm	Tailing peaks	Method rejected
Symmetry C_{18}, 250 mm x 4.6 mm and 5µm Column	Methanol : Phosphate Buffer (0.02M) (pH-3.8) = 70 : 30	1.0 ml/min	245nm	Proper Peak	Method Accepted

Preparation of 0.02M Phosphate Buffer (pH-3.8): Prepare 800 mL of distilled water in a suitable container. Add 2.72172g of Potassium dihydrogen Phosphate to the solution to the solution. Adjust solution to final desired pH 3.8 using diluted solution of orthophosphoric acid and add distilled water until volume is 1 Litre.

Preparation of Mobile Phase: Mix a mixture of 0.02M Phosphate Buffer (pH-3.8) 700 ml (70%) and 300 ml Methanol HPLC (30%) and degas in ultrasonic water bath for 15 minutes. Filter through 4.5 µ filter under vacuum filtration.

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Pitavastatin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents[10] and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette out 0.1ml of Pitavastatin from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution:

Weight 10 mg equivalent weight of Pitavastatin sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.1ml of Pitavastatin above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

III. RESULTS AND DISCUSSION

Development of Analytical Method:

Optimization of Method:

Optimized Chromatographic Conditions:

Column : Symmetry C18, 250 mm x 4.6 mm i.d.5µm particle size
Mobile Phase : Methanol: Phosphate Buffer (0.02M) (pH-3.8) (70: 30% v/v)
Flow Rate : 1.0ml/minute
Wave length : 245 nm
Injection volume : 10 µl
Run time : 7 minutes
Column temperature : Ambient

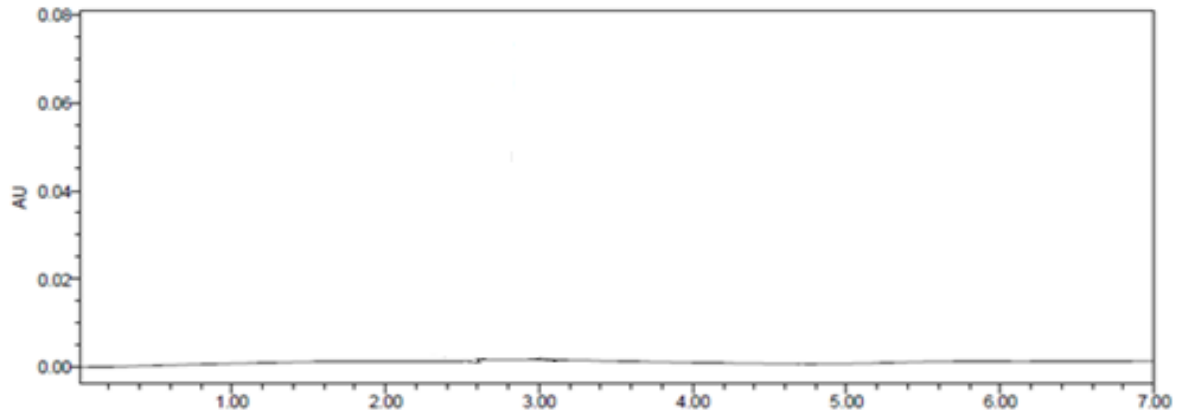

Fig.3. Chromatogram for Blank Solution

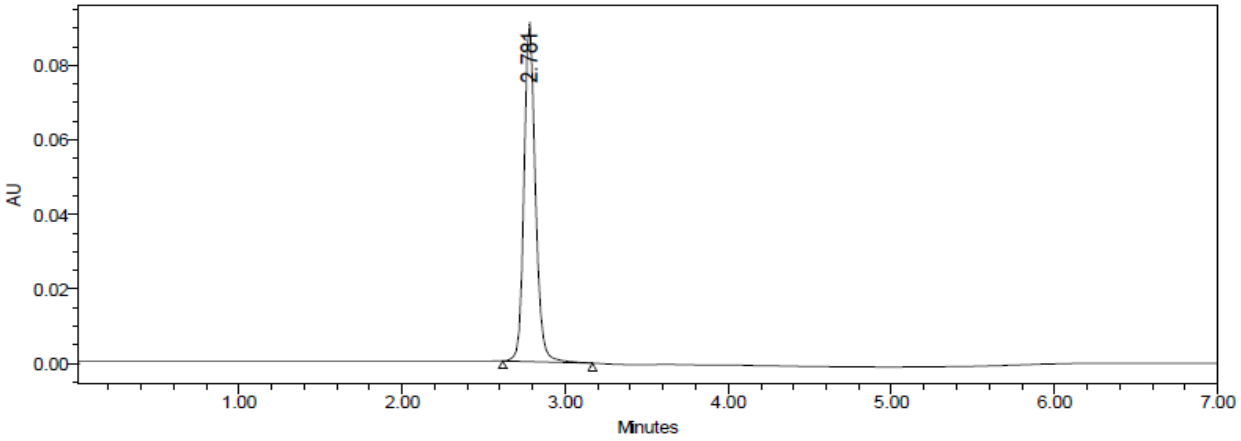

Fig.4. Optimized Chromatogram for Pitavastatin

Result: The selected and optimized mobile phase was Methanol: Phosphate Buffer (70: 30% v/v) and conditions optimized were flow rate (1.0 ml/minute), wavelength (245nm), Run time was 07 mins. Here the peak has shown better theoretical plate count and symmetry. The proposed chromatographic conditions[11] were found appropriate for the quantitative determination of the Pitavastatin drug.

Validation of Analytical Method:

The developed method was validated as per ICH guidelines[27,32] in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ) and system suitability.

System Suitability Test

System suitability testing[11-14] is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analysed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established. The data are shown in Table-4.

Table-4: Acceptance Criteria and Result

S.No.	Parameter	Limit	Result
1	Tailing factor	$T \leq 2$	1.36
2	Theoretical plate	$N > 2000$	5821.5

Accuracy:

Recovery Study:

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Pitavastatin were taken and 3 replications of each has been injected to HPLC system[15]. From that percentage recovery values were calculated from the linearity equation y = 74143x + 7294.9. The results were shown in table-5.

Table-5: Accuracy Readings

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Mean % Recovery	
	Amount Injected	Amount Recovered				
S_1 : 80 %	8	8.013	601425	100.162	Mean = 100.195%	% Mean Recovery = 100.364%
S_2 : 80 %	8	8.012	601396	100.150		
S_3 : 80 %	8	8.022	602123	100.275		
S_4 : 100 %	10	10.038	751584	100.380	Mean = 100.356	
S_5 : 100 %	10	10.039	751642	100.390		
S_6 : 100 %	10	10.030	750969	100.300		
S_7 : 120 %	12	12.057	901253	100.475	Mean = 100.541	
S_8 : 120 %	12	12.073	902431	100.608		
S_9 : 120 %	12	12.065	901864	100.541		

Precision:

Repeatability

The precision[16] of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug Pitavastatin (API). The percent relative standard deviation[17] was calculated for Pitavastatin.

Table-6: Results of Repeatability readings

HPLC Injection Replicates of Pitavastatin	Retention Time	Peak Area	Theoretical Plates	Tailing Factor
Replicate – 1	2.777	716984	5986	1.36
Replicate – 2	2.795	715698	5897	1.37
Replicate – 3	2.789	716859	5869	1.39
Replicate – 4	2.797	718548	5967	1.37
Replicate – 5	2.797	714895	5984	1.35
Replicate – 6	2.799	715986	5879	1.38
Average		**716495**	**5930.333**	**1.37**
Standard Deviation		**1268.126**		

% RSD		**0.17699**		

Intermediate Precision:

The Intermediate Precision[18-20] consists of two methods:-

Intra Day: In Intra Day process, the 80%, 100% and 120% concentration are injected at different intervals of time in same day.

Inter Day: In Inter Day process, the 80%, 100% and 120% concentration are injected at same intervals of time in different days.

Table-7: Peak results for Intra-Day Precision

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Pitavastatin	2.784	716587	48685	1.38	5954	1
2	Pitavastatin	2.768	717845	48698	1.39	5935	2
3	Pitavastatin	2.786	716857	46989	1.36	5798	3
4	**Average**		**717096.3**	**48124**	**1.376**	**5895.66**	
5	**S.D**		**662.2698**				
6	**% RSD**		**0.092354**				

Table-8: Peak results for Inter-Day Precision

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Pitavastatin	2.780	716987	49867	1.34	5968	1
2	Pitavastatin	2.794	718695	48574	1.33	5998	2
3	Pitavastatin	2.775	718542	48569	1.39	5859	3
4	**Average**		**718074.7**	**49003.33**	**1.353333**	**5941.667**	
5	**S.D**		**945.0483**				
6	**% RSD**		**0.131609**				

Linearity & Range:

To evaluate the linearity, serial dilution of analyte were prepared from the stock solution was diluted with mobile phase to get a series of concentration ranging from 6-14µg/ml. The prepared solutions were sonicated. From these solutions, 10µl injections of each concentration were injected into the HPLC system and chromatographed under the optimized conditions[21]. Calibration curve[22] was constructed by plotting the mean peak area (Y-axis) against the

concentration (X-axis).

Table-9: Linearity Concentrations of Pitavastatin

S.No.	Concentration (in ppm)	Peak Area
1	0	0
2	6	457896
3	8	607574
4	10	752268
5	12	896587
6	14	1036579

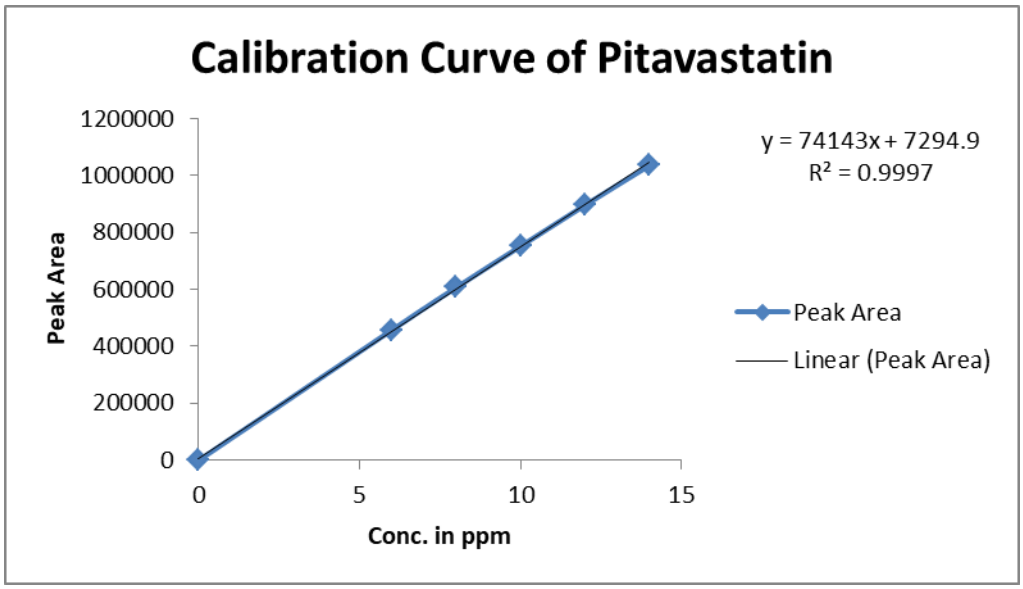

Fig.5. Calibration Curve of Pitavastatin

Specificity: Specificity[23] of the pharmaceutical analysis is the ability to measure accurately and specifically the concentration of API, without interference from other active ingredients, diluents, mobile phase. Solutions of mobile phase, sample solution, standard solution were injected into liquid chromatography. Retention times of samples and standard were compared.

Method Robustness: Influence of small changes in chromatographic conditions such as change in flow rate 1ml (± 0.1ml/min), Wavelength of detection 245nm (±2nm) & organic phase content in mobile phase 60 (±5%) studied to determine the robustness[24-26] of the method are also in favour of (Table-10, % RSD <2%) the developed RP-HPLC method for the analysis of Pitavastatin (API).

Table-10: Results of Method Robustness Test

Change in Parameter	Theoretical Plates	Tailing Factors
Flow (1.1 ml/min)	5954	1.35
Flow (0.8 ml/min)	6188	1.39
More Organic (70+5)	5748	1.41
Less Organic (70-5)	6185	1.48

Wavelength of Detection (250 nm)	6184	1.69
Wavelength of detection (240nm)	6247	1.47
Temperature (30 ^{0}C)	6324	1.34
Temperature (20 ^{0}C)	6985	1.32

LOD & LOQ: The detection limit (LOD) and quantization limit (LOQ) may be expressed as:

L.O.D. = 3.3(SD/S).

L.O.Q. = 10(SD/S)

Where, SD = Standard deviation[28] of the response

S = Slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

The Minimum concentration level at which the analyte can be reliable detected[29] (LOD) & quantified (LOQ) were found to be 0.507 & 1.539 µg/ml respectively.

Estimation of Pitavastatin in Pharmaceutical Dosage Form

Twenty tablets were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 10 mg of drug were transferred to 10 ml volumetric flask, and 8 ml of mobile phase was added and solution was sonicated for 15 minutes, there after volume was made up to 10 ml with same solvent. Then 1ml of the above solution was diluted to 10 ml with HPLC grade methanol. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. From this stock solution (1.0 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system.

The solution prepared was injected in five replicates into the HPLC system and the observations were recorded.

A duplicate injection of the standard solution was also injected into the HPLC system and the peak areas were recorded. The data are shown in Table-11.

ASSAY

$$\% \text{ Assay} = AT/AS \times WS/DS \times DT/WT \times P/100 \times AW/LC \times 100$$

Where:

AT = Peak Area of Pitavastatin obtained with test preparation

AS = Peak Area of Pitavastatin obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

Results obtained are tabulated below:

Table-11: Assay of Pitavastatin

Brand name of Tablets/Capsules	Labelled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=5)	Assay + % RSD
Livalo Tablets	4mg	3.652 (± 0.568)	99.698 % (± 0.487)

Result & Discussion: The % Purity[30] of Livalo Tablet containing Pitavastatin was found to be 99.698% (±0.487).

Stability Studies

Results of Degradation Studies: The results of the stress studies indicated the specificity of the method that has been developed. Pitavastatin was stable in Acidic, Photolytic & Oxidative conditions. The results of forced degradation studies[31] are given in the following table-12.

Table-12: Results of Forced Degradation Studies of Pitavastatin

Stress Condition	Time	Assay of Active Substance	Assay of Degraded Products	Mass Balance (%)
Acid Hydrolysis (0.1N HCl)	24Hrs.	87.635	12.365	100

Basic Hydrolysis (0.1N NaOH)	24Hrs.	94.154	5.846	100
Thermal Degradation (60^0C)	24Hrs.	90.311	9.689	100
UV (254nm)	24Hrs.	91.205	8.795	100
3% Hydrogen peroxide	24Hrs.	89.346	10.654	100

IV. SUMMARY

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 245nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was Symmetry C18, 250 mm x 4.6 mm i.d.5µm particle size because it was giving good peak. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Methanol: Phosphate Buffer (0.02M) (pH-3.8) (70: 30% v/v) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Run time was selected to be 7min because analyze gave peak around 2.768min and also to reduce the total run time. The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. The analytical method was found linearity over the range of 6-14ppm of the Pitavastatin target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

V. CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Pitavastatin in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps. Methanol and Phosphate Buffer (0.02M) (pH-3.8) in the ratio of 70: 30% v/v was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method and validation was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Pitavastatin in bulk drug and in Pharmaceutical dosage forms.

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