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Research Article

DEVELOPMENT AND VALIDATION OF A REVERSE-PHASE LIQUID CHROMATOGRAPHIC METHOD FOR DETERMINATION OF RELATED SUBSTANCES OF PITAVASTATIN FOR 2 AND 4 MG TABLETS

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ABSTRACT

Objective: The main objective of current study was to develop and validate RP-HPLC, simple, precise, accurate and specific chromatographic method for the determination of related impurities of pitavastatin in pharmaceutical formulations.

Methods: A high performance liquid chromatography instrument and Phenomenex, Kinetex C18, 75 X 4.6 mm, 2.6 µ 100A were used for determination of pitavastatin and its related impurities (Desfluoro impurity, anti isomer, Z-isomer, methyl Ester impurity, lactone impurity and tertiary butyl ester impurity). Buffer was prepared by using 0.82 g of sodium acetate in 1000 mL of water and adjusts its pH to 3.8 with acetic acid. Filter this solution through 0.22 µm nylon filter and sonicate to degas. The mobile phase-A was prepared by mixing of buffer and acetonitrile in the ratio of 0 90:10(v/v).The mobile phase-B was prepared by mixing acetonitrile and water in the ratio of 90:10(v/v).The flow rate of 1.0 mL/min was set with gradient program, the temperature of column compartment maintained at 25°C and Ultra violet detection done at 250nm wavelength. The pitavastatin and its related impurities peaks eluted at 9.13, 7.41, 9.71, 10.78, 14.86, 15.54 and 21.82 minutes and then run time was set as about 30 minutes.

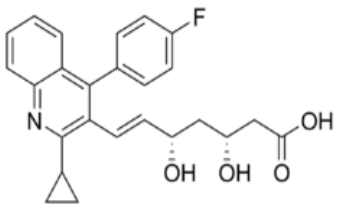
Results: The correlation coefficient (≥ 0.998) shows the linearity of response against concentration over the range of LOQ to 200%. The observed result shows that the method was rapid, precise, accurate and simple. The method was validated as per ICH guidelines.

Conclusion: The developed and validated High performance liquid chromatographic method was suitable for determination of pitavastatin and its related impurities in pharmaceutical formulations which is more useful with respect to regular Laboratory analysis.

Keywords: Pitavastatin, Related substances, Validation, RP-HPLC.

INTRODUCTION

Pitavastatin Calcium is a member of the medication class of statins. The IUPAC name (3*R*, 5S, 6E)-7-[2-cyclopropyl-4-(4-fluorophenyl) quinolin-3-yl]-3, 5-dihydroxyhept-6-enoic acid with molecular formula $C_{25}H_{24}FNO_4$ (421.461).Pitavastatin completely inhibits HMG CoA reductase, the rate-determining enzyme in hepatic cholesterol synthesis. Consequently, LDL-C receptors in the liver are increased, thereby increasing the removal of LDL-C from the blood. The primary route of metabolism of pitavastatin was hepatic glucuronidation with minimal metabolism by cytochrome P450 2C9 (CYP 2C9) and CYP 2C8 [1, 2].The plasma concentration of pitavastatin and lactone, atorvastatin and lactone and 2-hydroxy atorvastatin acid were quantified by the liquid chromatography-mass spectrometry mehtods.LC-MS method with electro spray ionization was developed for the simultaneous determination of pitavastatin and its lactone in human plasma and urine [3, 4]. HPTLC method was developed for determination of pitavastatin calcium in table dosage form by using mobile phase toluene, methanol and glacial acetic acid and wavelength of detector 238nm [5, 6]. HPLC and UV spectroscopy methods were developed for determination of pitavastatin in tablet dosage form with different mobile phase combinations [7, 8, 9, and 10].All above reported methods were used for only determination of pitavastatin calcium. But there was no method for determination of related substances of pitavastatin calcium in tablet dosage form. The novelty of present research work was this is the first method reported for related substances of pitavastatin in tablet dosage form. The present work describes a simple, gradient RP-HPLC method for the determination of Pitavastatin and its related substances in tablet dosage form as per ICH guidelines [11, 12 and 13].



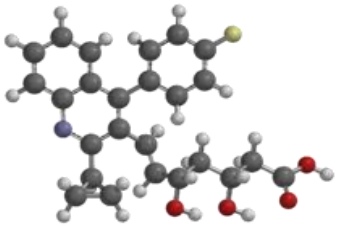


Fig. 1: Structure of Pitavastatin Calcium

MATERIALS AND METHODS

Chemicals

Qualified standards for drug substance and impurities were obtained from Bio-Leo analytical laboratory and were used without any further purification. The chemicals like Sodium acetate and Acetonitrile (ACN) were purchased from Merck, Mumbai. Millipore water generated from Millipore Water System. The analytical column used was Phenomenex, Kinetex C18, 75 X 4.6 mm, 2.6 µ 100A.

Instruments

A Waters Alliance HPLC system equipped with a quaternary UFLC LC-20AD pump, a DGU-20A$_5$ degasser, a SPD-M20A diode array detector, a SIL-20AC auto sampler, a CTO-20AC column oven and CBM-20A communications bus module was used for method development and validation studies. Second HPLC system Agilent 1200 series with high pressure liquid chromatographic instrument provided with Auto sampler and VWD UV detector, thermostatted column compartment connected with EZ Chrome software.

Standard Stock preparation

Weighed and transferred 10 mg Pitavastatin calcium into 100 mL amber colored volumetric flask, add 60 mL diluent, sonicated to dissolve and dilute to volume with diluent. Further dilute 1 mL to 100 mL with diluent, Acetonitrile: Water (50:50)

Placebo preparation

Transferred equal amount of the placebo powder present in 10 tablets into 50 mL volumetric flask add 30 mL of diluent, sonicated to dissolve for 20 min and diluted to volume with diluent. Further filtered the solution through 0.22 µm Nylon filter, discard first 5 mL of the filtrate.

Preparation of sample for 2mg

Transferred 10 tablets into 100 mL amber colored volumetric flask add 60 mL of diluent, sonicated to dissolve for 20 min and dilute to volume with diluent. Further filter the solution through 0.22 µm Nylon filter, discard first 5 mL of the filtrate.

Preparation of sample for 4mg

Transferred 10 tablets into 200 mL amber colored volumetric flask add 120 mL of diluent, sonicated to dissolve for 20 min and diluted to volume with diluent. Further filter the solution through 0.22 µm Nylon filter, discard first 5 mL of the filtrate.

Chromatographic conditions

The chromatographic column used was Phenomenex, Kinetex C18 column with dimensions of 75 mm X 4.6 mm with 2.6 µm particle size. The gradient program was employed with the mobile phase-A was buffer and Acetonitrile in the ratio of 90:10(v/v).The mobile phase-B was prepared by mixing acetonitrile and water in the ratio of 90:10(v/v).The column temperature was maintained at 25.0^{o}C and detection was monitored at a wavelength of 250 nm. Injection volume was 10 µl and the mobile phase flow was set at 1.0 mL/min. The acetonitrile and water in the ratio (50:50 v/v) was used as diluents for preparation of solutions. The gradient program was given in Table 1.

Table 1: Gradient Program

Time in (min)	%Mobile phase-A	%Mobile phase-B	Flow rate (mL/min)
0	72	28	1.0
5	72	28	1.0
19.5	40	60	1.0
20.5	40	60	1.0
21.5	72	28	1.0
30	72	28	1.0

Method Validation

The developed method for determination of Pitavastatin Calcium and its related substances was validated for system suitability along with method selectivity, specificity, linearity, range, precision (Repeatability and Intermediate precision),accuracy, limits of detection and limit of quantification according to the ICH guidelines.

System suitability

The system suitability was conducted using diluted standard preparation and evaluated by injecting six replicate injections.

Specificity

Specificity is the ability of analytical method to assess un equivocally the analyte in the presence of component that may be expected to be present, such as impurities, degradation products and matrix components. Performed the specificity parameter of the method by injecting Diluent, standard preparation, and sample preparation spiked with impurities into the chromatographic system and evaluated by making three replicate injections.

Linearity

Performed the linearity with pitavastatin standard and impurities in the range of LOQ to 300% of specification limit. Recorded the area response for each level and calculated slope, intercept & correlation coefficient. Also performed precision at higher level by injecting six times into the chromatographic system.

Precision and Accuracy

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation of series of measurements. The system precision was conducted using all the impurities spiked to Pitavastatin and evaluated by making six replicate injections. The Accuracy of the method by recoveries of all the impurities was determined by analyzing Pitavastatin sample solutions spiked with each impurity at three different concentration levels ranging from LOQ to 300%.

LOD and LOQ

The LOD and LOQ were determined for Pitavastatin and each of the impurities based on the signal to noise ratio method as per ICH guidelines.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Method development includes selection of appropriate chromatographic conditions/factors like detection wave length, selection and optimization of stationary and mobile phases. The longer wavelength of 250 nm was selected since it produces less noise, which minimizes problems that may exhibit around the active ingredient when attempting to quantify Pitavastatin and its related substances. Moreover, all related impurities were also detected satisfactorily at the same wavelength and hence it was selected as detection wavelength. Preliminary development trials were performed with various C_{18} columns of different types and dimensions from different manufacturers were tested for the peak shape and the number of theoretical plates for specification concentrations. Finally by switching to Phenomenex, Kinetex C18, 75 X 4.6 mm, 2.6 µ 100A there was a substantial increase in the theoretical plates (30794) with a significant improvement in the peak shape. It also produced adequate resolution between Pitavastatin and its related substances.

System suitability

The RSD from six replicate injections of diluted standard preparation was 0.36 %. Theoretical plates for Pitavastatin peak 30794.The system suitability of pitavastatin given in the table-2.The system suitability chromatogram shown in the figure-2.

Table 2: System Suitability results of Pitavastatin

Parameters	Observed Results	Acceptance criteria
Resolution	2.87	NLT 1.5
Theoretical plates	30794	NLT 3000
Asymmetry	0.96	NLT 2.0
% RSD of standard preparation	0.36	NLT 2.0

Selectivity

Performed the specificity parameter of the method by injecting diluent, standard preparation, sample preparation, placebo preparation, impurities and sample spiked with impurities into the chromatographic system. This was further demonstrated by determining the peak purity of analyte and known impurity peaks. The results of the peak purity values of analyte and known impurities were given in the below table-3.The selectivity chromatograms of pitavastatin shown in the figure-3, 4.

Table 3: Selectivity results of Pitavastatin calcium

Compound Name	Peak Purity
Pitavastatin	1.000
Desfluoro impurity	1.000
Anti isomer	1.000
Z-isomer impurity	1.000
Methyl ester impurity	1.000
Lactone impurity	1.000
Tertiary butyl ester impurity	1.000

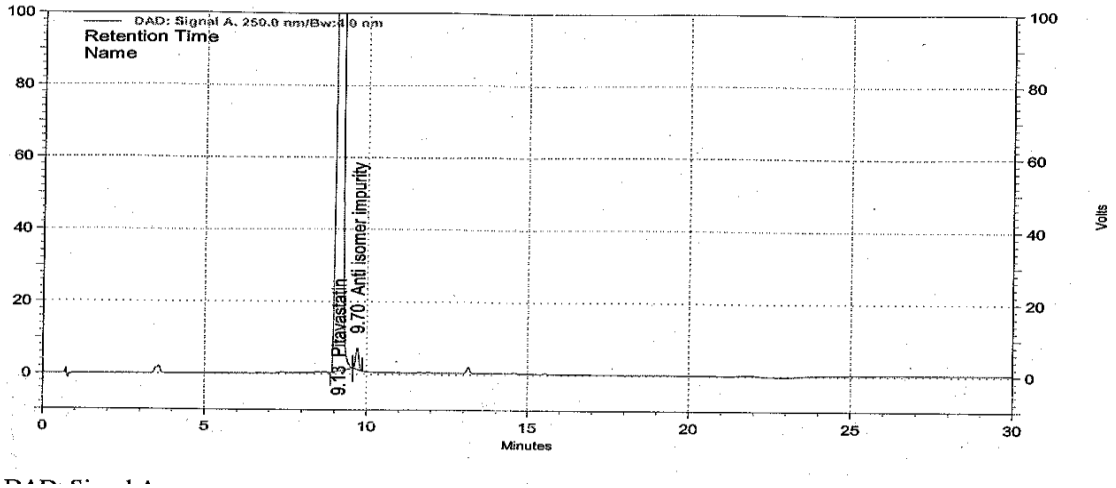
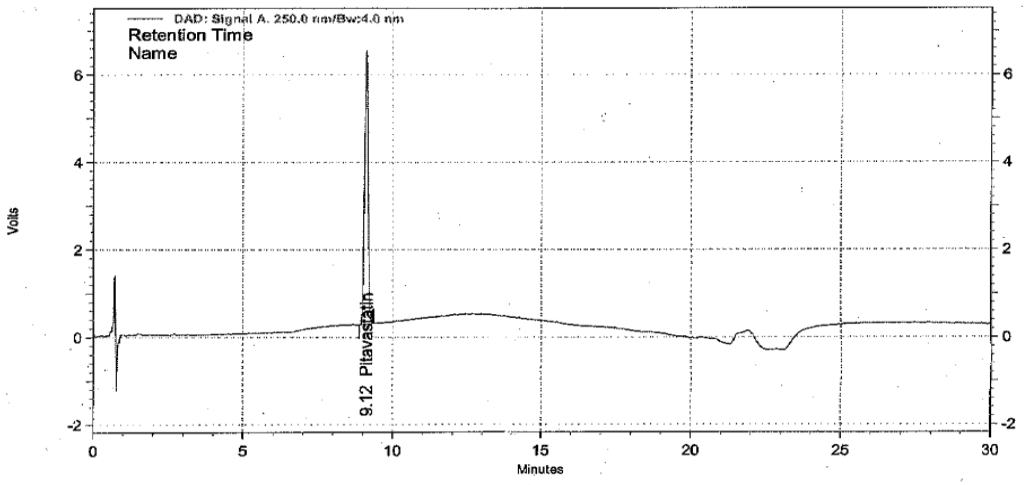


Fig: 2 System suitability chromatogram of Pitavastatin



DAD: Signal A, 250.0 nm/Bw:4.0 nm Results

Name	Retention Time	Area	Theoretical plates (USP)	Asymmetry
Pitavastatin	9.12	6584220	30605	0.99

Fig. 3: Pitavastatin standard chromatogram

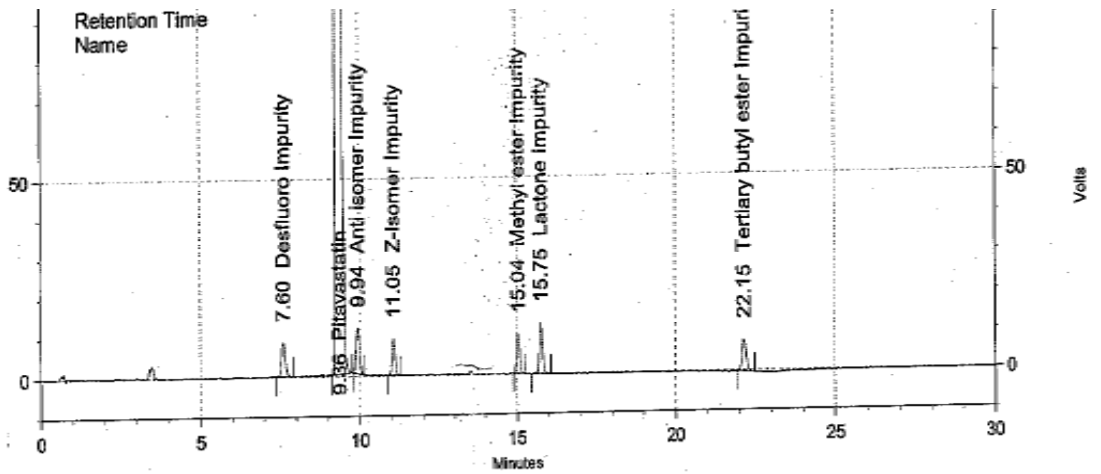


Fig. 4: Pitavastatin sample with its related impurities chromatogram

Linearity

To demonstrate the linearity with Pitavastatin Calcium standard, impurities in the range of LOQ to 200% of specification limit. Correlation coefficient of Pitavastatin and its related compounds was 1.000. Plotted a graph of Pitavastatin standard and impurities concentration (ppm) on X-axis and Area responses on Y-axis. Linearity data of pitavastatin and its relative impurities given in the below table 4.

Accuracy

Accuracy study found that the mean % of recovery was more than 90% and less than 110% at each level LOQ to 200% of concentration levels, hence method is accurate. The accuracy results are given table-5, 6, 7, 8 and 9.

Precision

The % RSD of the Retention time for peaks obtained from 6 injections of standard preparation was less than 10.0%. The RSD of % impurities from six spiked sample preparations should not be more than 10% by different instruments, analysts, columns and days. The RSD of % impurities from twelve spiked sample preparations should not be more than 15.0% by different instruments, analysts, columns and days. The precision data for different strengths given in the table-10, 11, 12.

LOD and LOQ

The distinct visible peak observed at LOD level concentration. The LOD and LOQ were established as per signal to noise ratio method data given table-13, 14.

Robustness

The method robustness was studied by injecting the system suitability solution at change in the percentage of organic modifier, flow rate, and column temperature. The results were obtained as shown in the below table-15

Table 4: Linearity results of Pitavastatin and its Impurities

Name	Correlation coefficient	Slope	Y-Intercept	Residual sum square
Pitavastatin	0.9998	6519577	-86701	2.8084×10^{11}
Desfluoro Imp	0.9997	5955400	69392	2.5691×10^{11}
Anti isomer	0.9987	6396207	205557	1.3685×10^{12}
Z-isomer Impurity	0.9998	4948762	12688	1.2063×10^{11}
Methyl ester Impurity	0.9995	474794	10929	9.5204×10^{10}
Lactone Impurity	0.9999	7271965	41028	1.3058×10^{11}
Tertiary butyl ester Impurity	0.9995	5933905	-23418	3.6593×10^{11}

Table5: Recovery of Desfluoro Impurity

S. No.	Level in %	% Mean Recovery	% RSD
1.	LOQ	99.19	1.99
2.	50%	106.67	0.92
3.	100%	99.58	0.86
4.	150%	98.66	0.45
5.	200%	97.76	0.41

Table: 6 Recovery of Anti isomer Impurity

S. No.	Level in %	% Mean Recovery	% RSD
1.	LOQ	98.32	1.59
2.	50%	101.28	0.59
3.	100%	99.35	0.32
4.	150%	97.49	0.13
5.	200%	98.44	0.33

Table: 7 Recovery of Z-isomer Impurity

S. No.	Level in %	% Mean Recovery	% RSD
1.	LOQ	95.53	1.67
2.	50%	104.97	0.90
3.	100%	98.91	0.73
4.	150%	97.90	0.15
5.	200%	98.90	0.43

Table: 8 Recovery of Methyl ester Impurity

S. No.	Level in %	% Mean Recovery	% RSD
1.	LOQ	103.27	0.76
2.	50%	101.26	2.04
3.	100%	94.84	1.38
4.	150%	100.53	0.29
5.	200%	100.05	0.29

Table: 9 Recovery of Tertiary butyl ester Impurity

S. No.	Level in %	% Mean Recovery	% RSD
1.	LOQ	101.47	0.73
2.	50%	104.92	1.91
3.	100%	100.40	1.32
4.	150%	101.17	0.11
5.	200%	100.59	0.52

Table 10: Precision results of 1 mg Pitavastatin

S. No.	Compound Name	%RSD
1.	Desfluoro Impurity	1.62
2	Anti isomer	1.38
3.	Z-isomer Impurity	1.25
4.	Methyl ester	1.07
5.	Lactone Impurity	1.85
6.	Tertiary butyl ester Impurity	1.09

Table 11: Precision results of 2 mg Pitavastatin

S. No.	Compound Name	%RSD
1.	Desfluoro Impurity	1.37
2	Anti isomer	0.77
3.	Z-isomer Impurity	1.11
4.	Methyl ester	1.05
5.	Lactone Impurity	1.34
6.	Tertiary butyl ester Impurity	1.35

Table 12: Precision results of 4 mg Pitavastatin

S. No.	Compound Name	%RSD
1.	Desfluoro Impurity	1.54
2.	Anti isomer	1.48
3.	Z-isomer Impurity	1.00
4.	Methyl ester	0.84
5.	Lactone Impurity	1.06
6.	Tertiary butyl ester Impurity	0.66

Table 13: LOQ establishment data

S. No.	Compound Name	S/N ratio	% level of comp. w.r.t to sample conc.
1.	Desfluoro Impurity	10.42	0.0230
2.	Anti isomer	10.08	0.0243
3.	Z-isomer Impurity	10.05	0.0385
4.	Methyl ester	10.00	0.0225
5.	Lactone Impurity	10.14	0.0308
6.	Tertiary butyl ester Impurity	9.75	0.0398

Table 14: LOD establishment data

S. No.	Compound Name	S/N ratio	% level of comp. w.r.t to sample conc.
1.	Desfluoro Impurity	2.37	0.0069
2.	Anti isomer	2.57	0.0072
3.	Z-isomer Impurity	2.79	0.0115
4.	Methyl ester	2.55	0.0067
5.	Lactone Impurity	2.68	0.0072
6.	Tertiary butyl ester Impurity	2.35	0.0119

Table 15: Robustness Results

Condition	Resolution	Theoritical plates	Asymmetry	% RSD for STD preparation
Limits	**NLT 1.5**	**NLT 3000**	**NMT 2.0**	**NMT 5.0**
Normal Condition	3.29	42857	1.00	0.26
Flow rate 1.1ml/min	3.29	37174	0.99	0.26
Flow rate 0.9/min	3.25	49037	1.05	0.26
Column Temperature 30°C	3.03	38859	1.12	0.38
Column Temperature 20°C	3.33	40600	1.02	0.19
Buffer pH changed to 4.0	1.96	9411	0.91	0.95
Buffer pH changed to 3.6	1.97	6594	0.93	2.70

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CONCLUSIONS

A simple gradient RP-HPLC method has been developed and validated for the determination of related substances of pitavastatin tablets. The developed method has been found to selective, sensitive, precise and robust. The method can be directly adopted in quality control laboratories for routine analysis with respect to determination of related substances of Pitavastatin calcium in tablet formulations.

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