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"METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE ESTIMATION OF PITAVASTATIN IN API FORM AND MARKETED TABLET DOSAGE FORM BY RP-HPLC"

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ABSTRACT

A new, simple, rapid, precise, accurate and reproducible RP-HPLC method for estimation of Pitavastatin in bulk form and marketed formulation. Separation of Pitavastatin was successfully achieved on a Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.e. column in an isocratic mode of separation utilizing Methanol: Phosphate buffer (0.02M, pH3.6) in the ratio of 45:55% v/v at a flow rate of 1.0 mL/min and the detection was carried out at 255nm. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The response was found to be linear in the drug concentration range of 12-28mcg/mL for Pitavastatin. The correlation coefficient was found to be 0.9995 for Pitavastatin. The LOD and LOQ for Pitavastatin were found to be 5.004µg/mL and 15.164µg/mL respectively. The proposed method was found to be good percentage recovery for Pitavastatin, which indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard solution with the sample solution. Therefore, the proposed method specifically determines the analyte in the sample without interference from excipients of pharmaceutical dosage forms.

Keywords: *Pitavastatin, RP-HPLC, Accuracy, Precision, Robustness, ICH Guidelines*

INTRODUCTION

HPLC METHOD DEVELOPMENT:

A decent technique development16 procedure ought to require just the same number of trials keeps running as are important to accomplish the coveted last outcome. At long last strategy, advancement ought to be as straightforward as could be expected under the circumstances, and it ought to permit the utilization of modern instruments, for example, PC demonstrating. Amid introductory strategy improvement, an arrangement of starting conditions (locator, segment, mobile phase) is chosen to acquire the main "exploring" chromatograms of the example. By and large, these depend on turned-around stage partitions on a C18 segment with UV l.

DETERMINATION OF CHROMATOGRAPHIC MODE

Turned around stage chromatography (RPC), the most widely recognized mode for little natural particles.

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Note that ionizable mixes (acids and bases) are regularly isolated by RPC with cushioned versatile stages (to keep the analytes in a non-ionized state) or with particle matching reagents. In switch stage mode, the versatile stage is similarly more polar than the stationary stage. For the partition of polar or modestly polar intensifies, the most favored mode is the turnaround stage. The idea of the analyte is the essential factor in the choice of the method of partition. A second factor is the idea of the grid.

TEST SAMPLE PREPARATION:

Tests come in different structures

Arrangements prepared for infusion

Arrangements that require weakening, buffering, expansion of an inside standard or other volumetric control

Solids must be broken down or separated

Tests that expect pretreatment to expel impedances and additionally shield the section or hardware from harm.

DECISION OF THE COLUMN:

Choice of the section is the first and the most critical advance in technique improvement. The fitting decision of the partition segment demonstrates three distinct methodologies.

A portion of the essential parameter considered while choosing chromatographic sections

Length and diameter of the column

~ Packing material

~ Shape of the particles

~ Size of the particles

~ % of Carbon loading

~ Pore volume

~ Surface area

~ End capping

DETERMINATION OF SOLVENT DELIVERY SYSTEM:

Chromatographic division with isocratic elution i.e., all constituents of the versatile stage are combined and pumped as a solitary dissolvable, is constantly ideal be that as it may, inclination elution is a great instrument in accomplishing partition between nearly eluting mixes or mixes having generally contrasting in polarities.

The imperative eventual fate of inclination elution is that the extremity and ionic quality of the mobile phase can be changed amid the run. The versatile stages are brought into the segment in two distinctive ways, low-weight and high-weight slope frameworks. Low weight inclination can be received when not over 80% of the natural stage is to be pumped or the other way around. While enhancing inclination elution particularly low thick solvents like Acetonitrile and phosphate support, it is prescribed to blend around 10% watery bit ideally a similar cushion utilized in mobile phase to abstain from pumping issues.

CHOICE OF MOBILE PHASE:

In fluid chromatography, the solute retention is administered by the solute dissemination factor, which mirrors the diverse communications of the solute-stationary stage, solute-versatile stage, and mobile phase stationary stage. For a given stationary stage, the nature and the creation of which must be prudently chosen to get fitting and required solute retention. The versatile stage must be adjusted as far as elution quality (solute retention) and dissolvable selectivity (solute partition). Dissolvable extremity is the watchword in chromatographic detachments since a polar versatile stage will offer ascent to low solute retention in the typical stage and high solute retention in switch stage LC. The selectivity will be especially changed if the cushion pH is near the pka of the analytes. The accompanying are the parameters, which will be thought about while choosing and advancing the versatile stage.

• Buffer and its quality

• pH of the support or pH of the mobile phase

• Mobile stage piece

BUFFERS IF ANY AND ITS STRENGTH:

Support and its quality assume an essential job in choosing the pinnacle symmetries and partitions. Probably the most usually utilized supports are

Phosphate supports are arranged utilizing salts like KH2PO4, K2HPO4, NaH2PO4, Na2HPO4 and so forth.

Phosphoric corrosive cushions are arranged to utilize H3PO4.

Acetate cushions Ammonium acetic acid derivation, Sodium acetic acid derivation and so forth.

Acetic corrosive cushions were arranged to utilize CH2COOH.

pH OF THE BUFFER:

pH assumes an imperative job as it controls the elution properties by controlling the ionization qualities. In RP-HPLC the retention of analytes is identified with their hydrophobicity. The more hydrophobic the analyte, the more it is held. In this way, corrosive shows diminish in retention with expanding pH while base shows an increment in retention.

SELECTION OF BUFFER:

The Ideal buffering limit happens at a pH equivalent to the pKa of the support. All of the pH-related change in retention happens for pH esteems inside ±1.5 units of pka esteem. Outside this range, the compound is either ionized or unionized, and its retention doesn't change much with pH.

Accompanying tablet:

The connection between RPC retention and mobile phase pH is more confounded for exacerbates that contain different acidic or potentially fundamental gatherings. On the off chance that these gatherings are for the most part same (acidic, essential) retention as a component of pH is comparative. Support quality of 10-50 mM is by and large sufficient, yet 25mM are trade off and appropriate. The supports indicating UV absorbance beneath 220 nm were best. An investigation was directed utilizing cradles having distinctive pH to get the required divisions. In the wake of evaluating the outcomes, the pH was chosen which is rough for at any rate ±0.2 units of the chose pH. Rundown of usually utilized cushions given in the accompanying tablet.

CHOICE OF FLOW RATE:

For the most part flow rate will not be in excess of 2.0 ml/min. The flow rate will be chosen in light of the accompanying information.

Retention time
Column back pressure
Resolution between the peaks
Peak symmetries

The flow rate which gives the slightest retention times, great pinnacle symmetries, minimum back weights and better partition will be chosen.

ANALYTICAL METHOD VALIDATION:

Technique validation can be characterized according to ICH as," Establishing recorded confirmation, which gives a high level of affirmation that a particular action will reliably create a coveted outcome or item meeting its foreordained details and quality attributes".

SPECIFICITY/SELECTIVITY:

The terms selectivity and specificity are frequently utilized conversely. As indicated by ICH, the term particular for the most part alludes to a strategy that delivers a reaction for a solitary analyte just while the term specific alludes to a technique which gives reactions to various substance elements that might be recognized from one another. In the event that the reaction is recognized from every other reaction, the technique is said to be specific. Since there are not very many techniques that react to just a single analyte, the term selectivity is typically more suitable.

LINEARITY:

Linearity of an investigative technique is its capacity (inside an offered extent) to get test results which are specifically relative to the focus (sum) of the analyte in the example. A straight relationship ought to be assessed over the scope of the explanatory system. It might be exhibited straightforwardly on the medication substance (by weakening a standard stock arrangement) as well as the partitioned weighing of engineered blends of the medication item segments, utilizing the proposed strategy.

RANGE:

The range is the interim between the upper and lower centralization of the analyte in the example for which it has a reasonable level of exactness, precision and linearity.

ACCURACY:

Exactness is the proportion of the closeness of the trial esteem is to the genuine esteem. Precision ought to be built up over the predefined scope of the diagnostic system.

Precision ought to be evaluated on tests (tranquilize substance/medicate item) spiked with known measures of polluting influences. In situations where it is difficult to get tests of specific polluting influences as well as debasement items, it is viewed as worthy to look at results gotten by a free strategy. The reaction factor of the medication substance can be utilized. It ought to be clear how the individual or aggregate polluting influences are to be resolved e.g., weight/weight or zone percent, in all cases as for the major analyte. Exactness ought to be surveyed utilizing at least 9 judgments over at least 3 fixation levels covering the predefined run (e.g., 3 fixations/3 reproduces every one of the aggregate scientific techniques).

PRECISION:

Accuracy is the proportion of how shut the information esteems are to one another for a progression of estimations under the same explanatory conditions acquired from various inspecting of the same homogeneous example. Accuracy might be considered at three levels: repeatability, moderate exactness and reproducibility.

Repeatability
Intermediate precision
Reproducibility

LIMIT OF DETECTION:

The breaking point of identification is the least convergence of analyte in an example which can be recognized, yet not really quantitated, as a correct incentive under the expressed trial conditions.

A. In view of Visual Evaluation

In view of Signal-to-Noise

In view of the Standard Deviation of the Response and the Slope As far as possible (DL) might be communicated as Where, σ = the standard deviation of the reaction

S = the slant of the adjustment bend

$$DL = \frac{3.3\ \sigma}{S}$$

LIMIT OF QUANTIFICATION:

The breaking point of measurement is the most reduced centralization of the analyte in an example which can be quantitatively decided with satisfactory exactness and precision under the expressed trial conditions.

A few methodologies for deciding as far as possible will be conceivable, contingent upon whether the technique is Non-instrumental or instrumental. Methodologies other than those recorded beneath might be adequate.

In light of Visual Evaluation

In light of the Signal-to-Noise Approach

In light-weight of the quality Deviation of the Response and therefore the Slope

As way as doable (QL) may well be communicated as:

Where, σ = the quality deviation of the reaction

S = the slope of the standardization curve

The slope S is also calculable from the standardization curve of the analyte.

RUGGEDNESS:

Ruggedness 10 isn't tended to in the ICH archives (4,5) Its definition has been supplanted by reproducibility, which has indistinguishable importance from roughness, characterized by the USP as the level of reproducibility of results acquired under an assortment of conditions, for example, unique research facilities, examiners, instruments, ecological conditions,

administrators and materials. Roughness is a proportion of reproducibility of test results under typical, expected operational conditions from research centre to lab and from examiner to expert. Toughness is controlled by the examination of aliquots from homogeneous parts in various labs.

ROBUSTNESS:

ICH characterizes strength as a proportion of the technique's ability to remain unaffected by very little, but assumes varieties in strategy parameters and offers a symptom of its unwavering quality amid typical utilization. Sincerity will be incompletely bonded with nice framework appropriateness determinations. The assessment of strength needs to be thought about amid the development stage and depends upon the type of system below investigation.

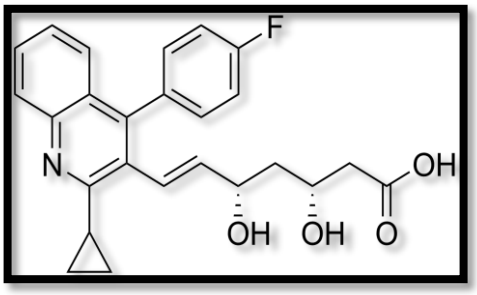
2. DRUG PROFILE

Drug Name: Pitavastatin

Synonyms: Pitavastatia, Pitavastatin, Pitavastatina, Pitavastatine and Pitavastatinum

Description: Pitavastatin 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.

Drug Category: Lipid-Lowering Drug



Chemical Structure

IUPAC Name: (3R, 5S, 6E)-7-[2-cyclo propyl-4-(4-fluoro phenyl) quinolin-3-yl]-3, 5-dihydroxy hept-6-enoic acid

Molecular Formula: C25H24FNO4

Molecular Weight: 421.4608g/mol

Physical Appearance: White to pale-yellow, odourless powder.

Solubility: Pitavastatin was found to be is freely soluble in pyridine, chloroform, dilute hydrochloric acid, and tetrahydrofuran, soluble in ethylene glycol, sparingly soluble in octanol, slightly soluble in methanol, very slightly soluble in water or ethanol, and practically insoluble in Acetonitrile or diethyl ether.

Pharmacodynamics: 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.

Pharmacokinetics:

Absorption: Pitavastatin peak plasma concentrations are achieved about 1 hour after oral administration. Both Cmax and AUC0-inf increased in an approximately dose-proportional manner for single Pitavastatin doses from 1 mg to 24 mg once daily. The absolute bioavailability of Pitavastatin oral solution is 51%. The Cmax and AUC of Pitavastatin did not differ following evening or morning drug administration. In healthy volunteers receiving 4 mg Pitavastatin, the percent change from baseline for LDL-C following evening dosing was slightly greater than that following morning dosing. Pitavastatin was absorbed in the small intestine but very little in the colon.

Metabolism: The principal route of Pitavastatin metabolism is glucuronidation via liver uridine 5'-diphosphate glucuronosyl transferase (UGT) with subsequent formation of Pitavastatin lactone. There is only minimal metabolism by the cytochrome P450 system. Pitavastatin is marginally metabolized by CYP2C9 and to a lesser extent by CYP2C8. The major metabolite in human plasma is the lactone, which is formed via an ester-type Pitavastatin glucuronide conjugate by UGTs (UGT1A3 and UGT2B7).

Mechanism of action: Pitavastatin is a statin medication and a competitive inhibitor of the enzyme HMG-CoA (3hydroxy-3-methylglutaryl coenzyme A) reductase, which catalyzes the conversion of HMG-CoA to mevalonate, an early ratelimiting step in cholesterol biosynthesis.3Pitavastatin 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

INTERACTIONS:

DRUG INTERACTIONS:

Abiraterone: The metabolism of Pitavastatin can be decreased when combined with Abiraterone.

Acebutolol: The serum concentration of Pitavastatin can be increased when it is combined with Acebutolol.

Acenocoumarol: The metabolism of Acenocoumarol can be decreased when combined with Pitavastatin.

Acetaminophen: The serum concentration of Pitavastatin can be increased when it is combined with Acetaminophen. Acetohexamide: The metabolism of Pitavastatin can be decreased when combined with Acetohexamide.

CONTRAINDICATIONS:

The use of LIVALO is contraindicated in the following conditions:

Patients with a known hypersensitivity to any component of this product. Patients with active liver disease which may include unexplained persistent elevations of hepatic transaminase levels.

SIDE EFFECTS: muscle pain, back pain, joint pain, pain in your arms and legs, diarrhoea, constipation, skin rash and headache.

MEDICAL USES:

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.

MARKETED FORMULATION:

S.No	Drug Name	Label Claim	Brand Name	Flower
1	Pitavastatin	1, 2 & 4mg	Livalo Tablets	Lilly

3. LITERATURE REVIEW

Nanjappan Satheesh Kumar, et al. (2011):

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 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. 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 5ng/ ml, while the LOQ was 20ng/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.

K. Sujatha, et al. (2014):

An accurate and stability indicating high performance liquid chromatographic method was developed for quantification of Pitavastatin in its tablet dosage forms. Ideal separation of the drug was achieved on an Agilent Eclipse XDB C18 column (150 x 4.6 mm; 5µ) by eluting with a mobile phase consisting of a mixture of phosphate buffer (pH 3.4) and acetonitrile (65:35 v/v) at a flow rate of 0.9 mL/min. The drug in the eluates was monitored at 244 nm. Under optimized conditions, the retention time obtained for the drug was 3.905 min. The calibration plot was linear in the concentration range of 25 150 µg/mL of the drug. The validation of the method was done by following the ICH guidelines. The proposed method could be applied for determination of Pitavastatin in its tablet dosage forms without any interference from normal excipients.

The method thus, is suitable for routine quality control analysis of Pitavastatin.

Vinod Kumar D. Ramani, et al. (2019):

The optimization of HPLC method involves several variables whose influence has been widely studied. However, in most of the cases, only process variables are taken into account. In this work, the influence of mixture composition on peak quality parameters of Pitavastatin calcium in bulk and tablet dosage form has been studied using a mixture simplex design. A simplex centroid design with axial points in a pseudo-component representation was generated from the pure mixture components. Twelve ternary mixture mobile phases corresponding to augmented design points were tested to separate the drug in sample. The statistical analysis was performed to generate the polynomial equation for each response. The desirability approach was used to determine the optimal mobile phase composition. Furthermore, the method was validated as per the ICH guidelines using specificity, linearity, accuracy, precision, sensitivity, system suitability, and robustness. The results of experimental design were statistically tested for full and in portion to get best fitted model which accurately describe changes in the proportion of these solvents in the mobile phase close to the region of optimal peak quality. The method demonstrated optimum chromatographic separation with isocratic elution of the mobile phase containing a mixture of acetonitrile-water (pH 3.0)-tetrahydrofuran (43:55:02, v/v/v) with a flow rate at 1.0 ml/minute. Design of experiment optimization strategy is a powerful tool to acquire the maximum quality data while performing minimum number of experiments. The mobile phase composition was successfully optimized using simplex centroid mixture design with desirability approach. Additionally, developed method can be applied for routine quantitative analysis of Pitavastatin calcium in bulk and tablet dosage form as it was found to be simple, sensitive, and robust.

4. AIM, OBJECTIVE AND PLAN OF WORK

Aim:

A survey of literature reveals that good analytical methods are not available for the drug Pitavastatin. Despite the fact that not very many strategies for estimation of above medications are accessible, a significant number of them experience the ill effects of one drawback or the other, for example, low affectability, absence of selectivity and straightforwardness and so on.

The current physicochemical techniques are insufficient to meet the necessities; consequently, it is proposed to enhance the current strategies and to grow new strategies for the assay & stability studies of Pitavastatin in pharmaceutical dosage forms adapting different available analytical techniques like UV spectrophotometry and HPLC.

Objective:

As per the writing review it was discovered that couple of diagnostic techniques, for example, (HPLC, UV-Visible investigation and LC-MS) were accounted for the estimation of Pitavastatin. The goal of the proposed technique is to create basic and precise strategies for the assurance of Pitavastatin by RP-HPLC strategy in pharmaceutical measurement frames and its soundness characteristic examinations.

Security testing shapes a vital piece of the procedure of medication item improvement. The motivation behind security testing is to give confirm on how the nature of a medication substance shifts with time affected by an assortment of ecological factors, for example, temperature, dampness and light, and empowers proposal of capacity conditions, retest periods, and time span of usability to be built up.

The measure of Pitavastatin API (Active Pharmaceutical Ingredient) in solidness test should be resolved utilizing steadiness demonstrating techniques.

PLAN OF WORK

Collection of literature for the selected drug.

Extensive literature survey for selection of appropriate solvents to dissolve respective selected drug and preparation of the stock solution.

- Study of the drug profile
- Procurement of samples, standards and other chemicals.
- Selection of chromatographic conditions
- Selection of mobile phase
- Method trials on HPLC by using different solvents and columns.
- Development of RP-HPLC method which is different from the finished articles.
- Optimization of the developed method by varying mobile phase
- Conditions, and temperature.

Validation of the developed method for the following parameters:

- Accuracy
- Precision
- Specificity
- Limit of detection
- limit of quantitation
- Linearity
- Robustness
- System Suitability

5. METHOD DEVELOPMENT

Wavelength Detection (Or) Selection of Wavelength:

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The UV spectrum of Pitavastatin was obtained and the Pitavastatin showed absorbance's maxima at 255nm. The UV spectra of drug are follows:

Observation:

While scanning the Pitavastatin solution we observed the maxima at 255nm. The UV spectrum has been recorded on T60-LAB INDIA make UV – Vis spectrophotometer model UV-2450

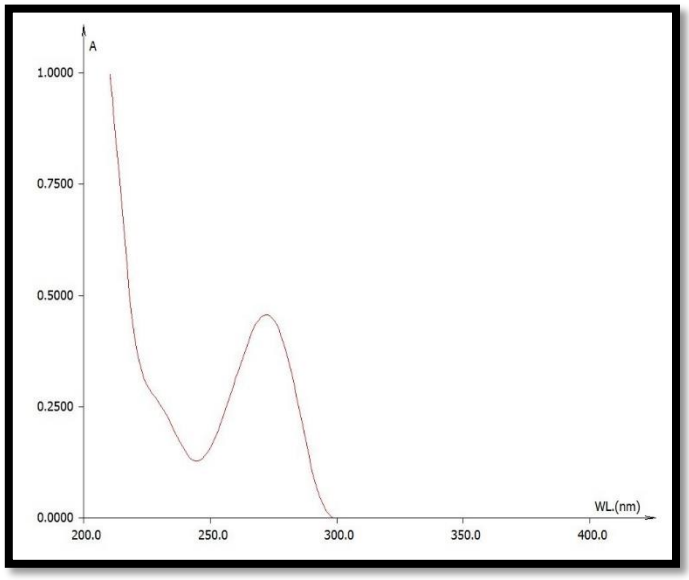


Fig 1-: UV Spectrum of Pitavastatin

Table 1: Trials for the Method Development and Results

S.No.	Column Used	Mobile Phase	Flow Rate	Wavelength	Observation	Result
1	SymmetryC18, 5µm, 25cmx4.6mm i.d.	ACN : Water = 70 : 30	0.8 ml/min	255nm	Early elution of peak	Method rejected
2	Waters C18, 5µm, 25cmx4.6mm i.d.	Methanol : ACN = 40 :60	1.0 ml/min	255nm	Tailing Peaks	Method rejected
3	Waters C18, 5µm, 25cmx4.6mm i.d.	ACN: Phosphate buffer (0.02M) = 70:30	1.0 ml/min	255nm	Low-resolution peak	Method rejected
4	DevelosilODS HG-5 RP C18, 5µm,15cmx4.6mm i.d.	Methanol : Phosphate buffer (0.01M) = 50:50 (pH-3.8)	1.0 ml/ min	255nm	Many Peaks	Method rejected
5	Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d.	Methanol : Phosphate buffer (0.02M) = 65:35 (pH-2.6)	1.0 ml/min	255nm	Many Peaks	Method rejected
6	Develosil ODS HG-5 RP C18,5µm, 15cmx4.6mm i.d.	Methanol : Phosphate buffer (0.02M) = 45:55 (pH-3.6)	1.0 ml/min	255nm	Good Peaks	Method Accepted

Chromatographic Condition:

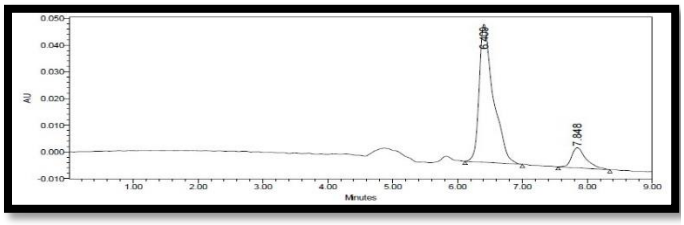


Fig 2-: Chromatogram for Trial-1

Table 2-: Results of Trial-1

S. No.	Drug Name	RT	Peak	Theoretical Plates	Tailing Factor
1	Pitavastatin	6.409	395478	3586	1.08

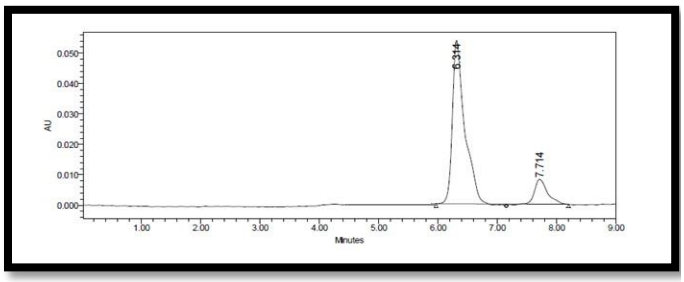


Fig 3-: Chromatogram for Trial-2

Table 3-: Results of Trial-2

S. No.	Drug Name	RT	Peak	Theoretical Plates	Tailing Factor
1	Pitavastatin	6.314	412547	3878	1.07

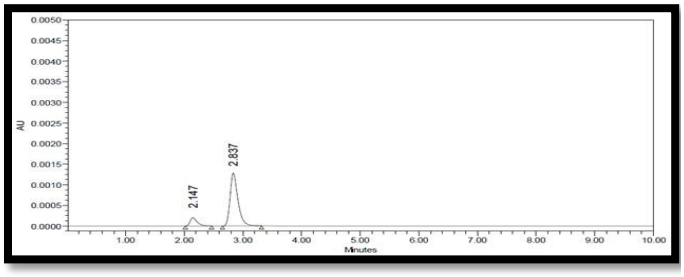


Fig 4-: Chromatogram for Trial-3

Table 4-: Results of Trial-3

S. No.	Drug Name	RT	Peak	Theoretical Plates	Tailing Factor
1	Pitavastatin	2.147	238675	2176	1.26

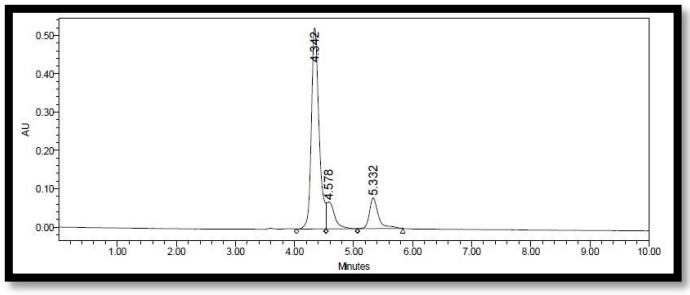


Fig 5-: Chromatogram for Trial-4

Table 5-: Results of Trial-4

S. No.	Drug Name	RT	Peak	Theoretical Plates	Tailing Factor
1	Pitavastatin	4.342	431561	3579	1.14

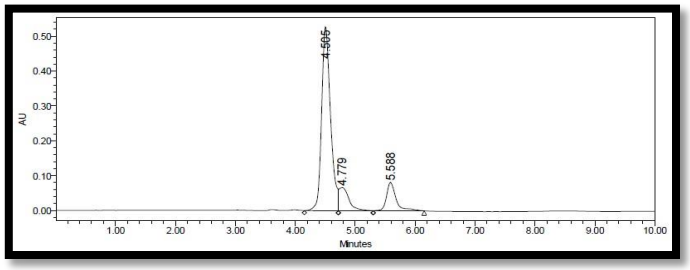


Fig 6-: Chromatogram for Trial-5

Table 6-: Results of Trial-5

S. No.	Drug Name	RT	Peak	Theoretical Plates	Tailing Factor
1	Pitavastatin	4.342	431561	3579	1.14

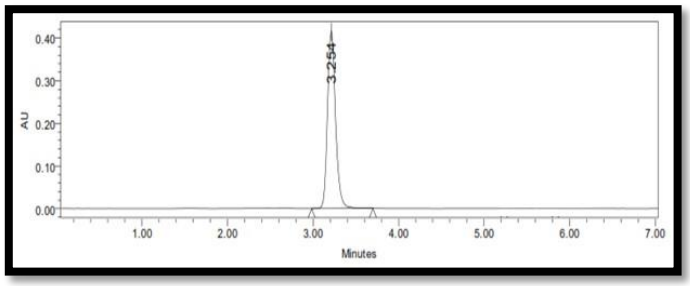


Fig 7-: Chromatogram for Trial-6

Table 7-: Results of Trial-6

S. No.	Drug Name	RT	Peak	Theoretical Plates	Tailing Factor
1	Pitavastatin	3.254	283261	7258	1.25

Optimized Chromatographic Method:

Mobile phase	**Methanol : Phosphate buffer (0.02M, pH-3.6) =**
Column	45:55
Column Temperature	Develosil ODS HG-5 RP C18, 5□m, 15cmx4.6mm i.d.
Detection Wavelength	Ambient
Flow rate	255 nm
Run time	1.0 ml/ min.
Temperature of Auto sampler	07 min.
Diluent	Ambient
Injection Volume	Mobile Phase
Type of Elution	20µl

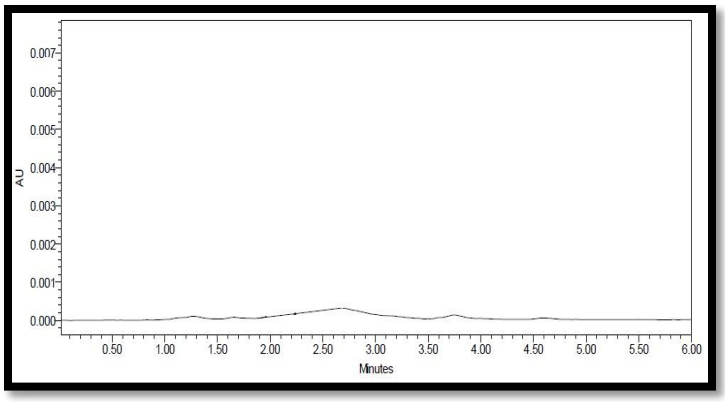


Fig 8: Chromatogram of Blank Solution

Table 8: Peak Result of Optimized Chromatogram

S. No.	Drug Name	RT	Peak	Theoretical Plates	Tailing Factor
1	Pitavastatin	3.254	283261	7258	1.25

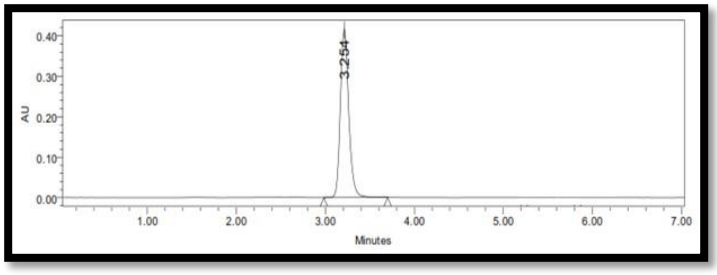


Fig 9: Chromatogram of Pitavastatin in Optimized Chromatographic Condition:

6. METHOD VALIDATION

System Suitability:

System suitability testing 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 analyzed constitute an integral system that can be evaluated as such.

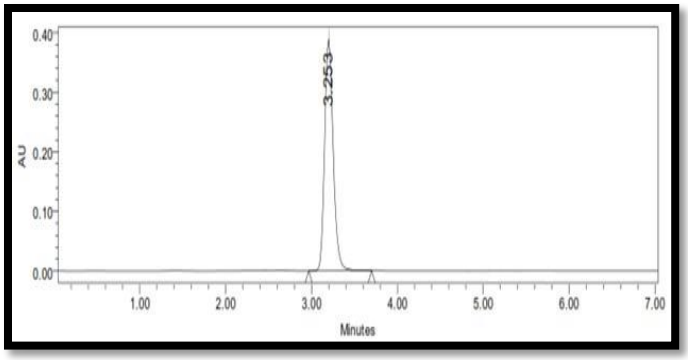


Fig 10-: Chromatogram for System Suitability Injection-1

Table 9-: Results for System Suitability Injection-1

S. No.	Drug Name	RT	Peak	Theoretical Plates	Tailing Factor
1	Pitavastatin	3.254	283261	7258	1.25

Table 10-: System suitability results for Pitavastatin (Flow rate)

S.No.	Parameter	Limit	Result
1	Asymmetry	T≤ 2	Pitavastatin = 0.12
2	Theoretical plate	N>2000	Pitavastatin = 7368
3	Tailing Factor	(Tf) < 2	Pitavastatin = 1.26

Linearity: 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 0-28µg/ml for Pitavastatin. The prepared solutions were filtered through Whatman filter paper (No.41). From these solutions, 20µl injections of each concentration were injected into the HPLC system and chromatographed under the optimized conditions. Calibration curve was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis).

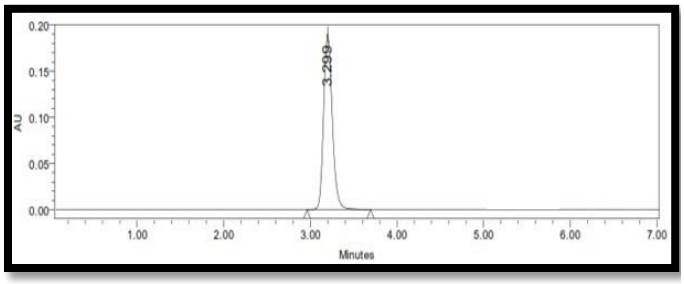


Fig 11-: Chromatogram for linearity-1

Table 11-: Results of Linearity-1

S. No.	Drug Name	RT	Peak	Theoretical Plates	Tailing Factor
1	Pitavastatin	3.299	192164	7657	1.26

Observation: Linearity range was found to be 0-28µg/ml for Pitavastatin. The correlation coefficient was found to be 0.9995, the slope was found to be 55283 and intercept was found to be 12871 for Pitavastatin.

Observation: Linearity range was found to be 0-28µg/ml for Pitavastatin. The correlation coefficient was found to be 0.9995, the slope was found to be 55283 and intercept was found to be 12871 for Pitavastatin.

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
12	690316
16	910621
20	1121057
24	1328903
28	1554666

Accuracy:

Accuracy 80%

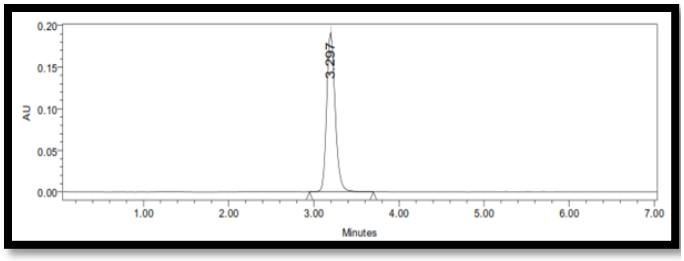


Fig 12: Chromatogram showing accuracy 80%injection-1

Table: Results of Accuracy -80%injection -1

S. No.	Drug Name	RT	Peak Area	Theoretical Plates	Tailing Factor
1	Pitavastatin	3.297	458679	7236	1.25

Accuracy 100%:

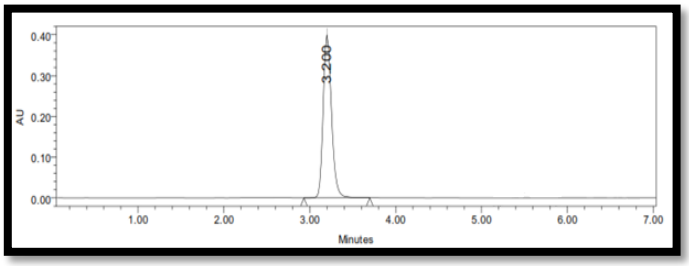


Fig 13: Chromatogram showing accuracy 100% injection-1

Table: Results of Accuracy -100% injection -1

S. No.	Drug Name	RT	Peak Area	Theoretical Plates	Tailing Factor
1	Pitavastatin	3.200	559767	7469	1.28

Accuracy 120%

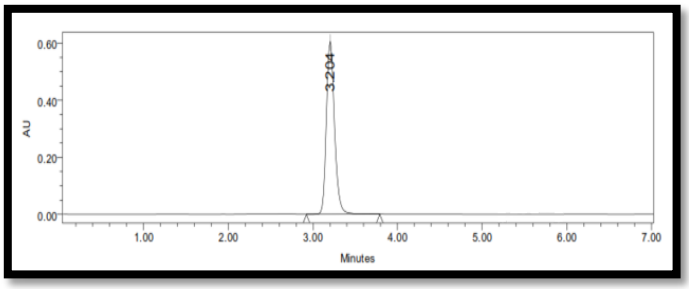


Fig 14: Chromatogram showing accuracy 120% injection-1

Observation: The mean recoveries were found to be 100.411, 100.664 and 100.463% for Pitavastatin. The limit for mean % recovery is 98-102% and as both the values are within the limit, hence it can be said that the proposed method was accurate.

Precision:

1) Repeatability

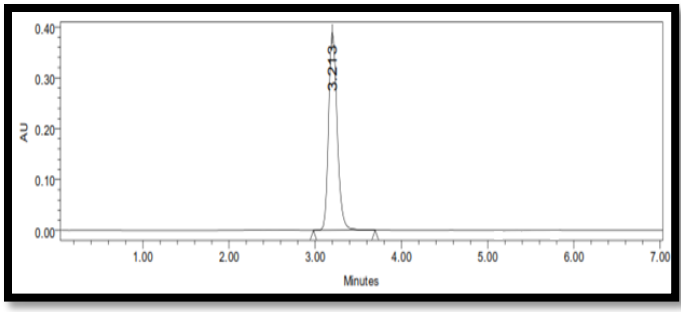


Fig 15: Chromatogram for repeatability-1

Table: Results of Repeatability-1

S. No.	Drug Name	RT	Peak Area	Theoretical Plates	Tailing Factor
1	Pitavastatin	3.213	285479	7598	1.28

Table-: Repeatability Results of Pitavastatin

HPLC Injection Replicates	AUC for Pitavastatin
Replicate – 1	285479
Replicate – 2	284571
Replicate – 3	286954
Replicate – 4	283261
Replicate – 5	285964
Replicate – 6	284259
Average	285081.3
Standard Deviation	1318.666
% RSD	0.462558

Observation: The repeatability study which was conducted on the solution having the concentration of about 20µg/ml for Pitavastatin (n=6) showed a RSD of 0.462558% for Pitavastatin. It was concluded that the analytical technique showed good repeatability.

2) Intermediate Precision / Ruggedness:

Conc. of Pitavastatin (API) (µg/ml)	Observed Conc. of Pitavastatin (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=3)	% RSD	Mean (n=3)	% RSD
8	8.21	0.76	8.23	0.46
10	10.37	0.33	10.36	0.57
12	12.56	0.23	12.56	0.75

Observation: Intraday and inter-day studies show that the mean RSD (%) was found to be within acceptance limit (≤2%), so it was concluded that there was no significant difference for the assay, which was tested within day and between days. Hence, method at selected wavelength was found to be precise.

Robustness: Robustness is defined as the capacity of that method to be unaffected by even small deliberate changes that occur in the method parameters. The evaluation of robustness of a method is done by varying the chromatographic parameters such as pH, temperature, flow rate, mobile phase proportions change, ionic strength etc., and determining any possible effect on the results obtained by that method.

Change in parameter	% RSD
Flow (0.8 ml/min)	0.554
Flow (1.2 ml/min)	0.867
More Organic	0.886
Less Organic	0.817
Wavelength of Detection (257 nm)	0.813
Wavelength of detection (253 nm)	0.794

Observation: Influence of small changes in chromatographic conditions such as a change in flow rate (±0.1ml/min), Temperature (±2^{0}C), Wavelength of detection (±2nm) & organic phase (±5%) studied to determine the robustness of the method are also in favour of (Table-38, % RSD < 2%) the developed RP-HPLC method for the analysis of Pitavastatin (API).

LOD: The limit of detection (LOD) is the lowest concentration of analyte in a sample which can be detected, but not quantitated. LOD is a limit test that specifies whether an analyte is above or below a certain value. Signal-to-noise ratio of three-to-one is used to determine LOD.

Observation: The LOD was found to be 5.004µg/ml for Pitavastatin.

LOQ: The Limit of Quantitation (LOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. Signal-to-noise ratio of ten-to-one is used to determine LOQ.

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

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

Observation: The LOQ was found to be 15.164µg/ml for Pitavastatin.

7. RESULTS AND DISCUSSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Pitavastatin, different chromatographic conditions were applied & the results observed are presented in previous chapters.

Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution.

In case of RP-HPLC various columns are available, but here Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.e. column was preferred because using this column peak shape, resolution and absorbance were good.

Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, acetonitrile, water, 0.1N NaOH, 0.1NHCl).

The drug was found to be freely soluble in DMSO, soluble in Methanol and Acetonitrile, Very Slightly Soluble in Octanol, Ethanol and Water. Using these solvents with appropriate composition newer methods can be developed and validated.

Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Pitavastatin it is evident that most of the HPLC work can be accomplished in the wavelength range of 255 nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 20µl were found to be the best analysis.

The result shows the developed method is yet another suitable method for assay which can help in the analysis of Pitavastatin in different formulations.

8. CONCLUSION

• A sensitive & selective RP-HPLC method has been developed & validated for the analysis of Pitavastatin API.

• Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

• The result shows the developed method is yet another suitable method for assay, purity which can help in the analysis of Pitavastatin in different formulations.

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