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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF PRAVASTATIN IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A rapid and precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Pravastatin, in its pure form as well as in tablet dosage form. Chromatography was carried out on Apollo C18 (4.6×150mm, 5µ) column using a mixture of Methanol: water (65:35 v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 235nm. The retention time of the Pravastatin was 2.6 ±0.02min. The method produce linear responses in the concentration range of 20-100µg/ml of Pravastatin. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

KEYWORDS

Pravastatin, RP-HPLC and Validation.

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INTRODUCTON

Pravastatin is a HMG-CoA Reductase Inhibitor[1]. The mechanism of action of Pravastatin is as a hydroxy methyl glutaryl-CoA Reductase Inhibitor. Pravastatin is commonly used cholesterol lowering agent (statin) that is associated with mild, asymptomatic[2] and self-limited serum amino transferase elevations during therapy, and rarely with clinically apparent acute liver injury. Pravastatin is a cholesterol-lowering agent that belongs to a class of medications known as statins. It was derived from microbial transformation[3,4] of mevastatin, the first statin discovered. It is a ring-opened dihydroxy acid with a 6'-hydroxyl group

that does not require *in vivo* activation. Pravastatin[5,6,7] is one of the lower potency statins; however, its increased hydrophilicity is thought to confer advantages such as minimal penetration through lipophilic membranes[8,9] of peripheral cells, increased selectivity for hepatic tissues, and a reduction in side effects compared with lovastatin and simvastatin. Pravastatin is structurally[10] similar to the HMG, a substituent of the endogenous substrate of HMG-CoA reductase. Unlike its parent compound, mevastatin, and statins such as lovastatin and simvastatin, Pravastatin does not need to be activated *in vivo*[11,12]. Its hydrolyzed lactone ring mimics the tetrahedral intermediate produced by the reductase allowing the agent to bind with a much greater affinity[13] than its natural substrate. The bicyclic portion of Pravastatin binds to the coenzyme[17,18] A portion of the active site. Pravastatin sodium produces its lipid-lowering effect in two ways. First, as a consequence of its reversible inhibition[14] of HMG-CoA reductase activity, it effects modest reductions in intracellular pools of cholesterol. This results in an increase in the number[15,16] of LDL-receptors[20] on cell surfaces and enhanced receptor-mediated catabolism[19] and clearance of circulating LDL. Second, Pravastatin inhibits LDL production by inhibiting hepatic synthesis of VLDL, the LDL precursor.

The IUPAC Name[25-29] of Pravastatin is (3R, 5R)-7-[(1S, 2S, 6S, 8S, 8aR)-6-hydroxy-2-methyl-8-{[(2S)-2-methylbutanoyl] oxy}-1, 2, 6, 7, 8, 8a-hexahydronaphthalen-1-yl]-3, 5-dihydroxyheptanoic acid and the chemical formula is $C_{23}H_{36}O_7$. The Chemical Structure of Pravastatin is as follows.

MATERIAL AND METHODS

HPLC METHOD DEVELOPMENT TRAILS

Preparation of standard solution

Accurately weigh and transfer 10 mg of Pravastatin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.0ml of the above Pravastatin stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was Water, Methanol: Water and Acetonitrile: Water, with varying proportions. Finally, the mobile phase was optimized to Methanol and Water in proportion 65:35 v/v respectively.

Optimization of Column

The method was performed with various C18 columns like ODS column, X Bridge, and Symmetry C18 column. Apollo C18 (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used	:	Waters HPLC with auto sampler and PDA detector 996 model.
Temperature	:	35ºC
Column	:	Apollo C18 (4.6 x 150mm, 5µm)
Mobile phase	:	Methanol: Water (65:35% v/v)
Flow rate	:	1ml/min
Wavelength	:	235nm
Injection volume	:	10µl
Run time	:	5minutes

VALIDATION

Preparation of mobile phase

Accurately measured 650ml (65%) of HPLC Methanol and 350ml of HPLC Water (35%) were mixed and degassed in a digital ultra sonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

VALIDATION PARAMETERS

System Suitability

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

Further pipette 0.6ml of the above Pravastatin stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

SPECIFICITY STUDY OF DRUG

Preparation of Standard Solution

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

Further pipette 0.6ml of the above Pravastatin stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Pravastatin 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 0.6ml of the above Pravastatin stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

PREPARATION OF DRUG SOLUTIONS FOR LINEARITY

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

Preparation of Level - I (20µg/ml of Pravastatin)

Pipette out 0.2ml of stock solution in to a 10ml volumetric flask and make up the volume up to mark by using diluent.

Preparation of Level - II (40µg/ml of Pravastatin)

Pipette out 0.4ml of stock solution in to a 10ml volumetric flask and make up the volume up to mark by using diluent.

Preparation of Level - III (60µg/ml of Pravastatin)

Pipette out 0.6ml of stock solution in to a 10ml volumetric flask and make up the volume up to mark by using diluent.

Preparation of Level - IV (80µg/ml of Pravastatin)

Pipette out 0.8ml of stock solution in to a 10ml volumetric flask and make up the volume up to mark by using diluent.

Preparation of Level - V (100µg/ml of Pravastatin)

Pipette out 1.0ml of stock solution in to a 10ml volumetric flask and make up the volume up to mark by using diluent.

Procedure

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

PRECISION

Repeatability

Preparation of Pravastatin Product Solution for Precision

Accurately weigh and transfer 10 mg of Pravastatin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and

sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.6ml of the above Pravastatin stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

INTERMEDIATE PRECISION

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure

Analyst 1

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Analyst 2

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy

For preparation of 50% Standard stock solution

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

Further pipette 0.3ml of the above Pravastatin stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 100% Standard stock solution

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

Further pipette 0.6ml of the above Pravastatin stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 150% Standard stock solution

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

Further pipette 0.9ml of the above Pravastatin stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Pravastatin and calculate the individual recovery and mean recovery values.

ROBUSTNESS

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For preparation of Standard solution

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

Further pipette 0.6ml of the above Pravastatin stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Effect of Variation of flow conditions

The sample was analyzed at 0.9ml/min and 1.1ml/min instead of 1.0ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded

Effect of Variation of mobile phase organic composition

The sample was analyzed by variation of mobile phase i.e. Methanol: Water was taken in the ratio

and 60:40, 70:30 instead of 65:35 remaining conditions are same.10µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

HPLC Method Development

METHOD VALIDATION

The proposed method was subjected to validation for various parameters like linearity and range, precision, accuracy, and robustness in accordance with International Conference on Harmonization Guidelines.

Linearity

Appropriate aliquots of Pravastatin working standard solutions were taken in different 10mL volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 20, 40, 60, 80, and 100μg/mL of Pravastatin. The solutions were injected using a 10 μL automatic injecting system and chromatograms were recorded. Calibration curves are constructed by plotting average peak area versus concentrations and regression equation was computed for the Pravastatin.

Precision

The repeatability studies were carried out by estimating response of Pravastatin (60μg/mL) five times and results were reported in terms of relative standard deviation. The intraday and inter day precision studies (intermediate precision) were carried out by estimating the corresponding responses 6 times on the same day and on 2 different days for the different concentrations of Pravastatin 60μg/mL, and the results were reported in terms of relative standard deviation.

Intermediate precision or ruggedness

The ruggedness of the method was verified by analyzing six samples of the same batch used for method precision as per proposed method by different analyst.

The repeatability of sample applications and measurement of peak area were expressed in term of %RSD since their %RSD is <2.0 %, and hence, the developed method was found to be precise. Data obtained from intermediate are summarized in Table No.6 and 7.

Accuracy

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out at three levels of 50%, 100% and 150% and the percentage recovery was calculated and presented in Table No.8. Recovery was within the range of 98%-102% which indicates accuracy of the method.

LOD and LOQ

The LOD and

LOQ were calculated using the following equation as per ICH guidelines:

$$\mathrm{LOD} = 3.3 \times \frac{\sigma}{S}, \quad \mathrm{LOQ} = 10 \times \frac{\sigma}{S},$$

Where σ is the standard deviation of y-intercepts of regression

Lines and S is the slope of the calibration curve.

The results of LOD and LOQ are summarized in Table No.9.

Robustness

Robustness of the method was studied by deliberately changing the experimental conditions like flow rate and percentage of mobile phase ratio. The study was carried out by changing 5% of the mobile phase ratio and 0.1 mL/min of flow rate.

System Suitability

A system suitability test was an integral part of the method development to verify that the system is adequate for the analysis of Pravastatin to be performed. System suitability test of the chromatography system was performed before each validation run. Five replicate injections of a system suitability standard and one injection of a check standard were made. Area, retention time (RT), tailing factor, asymmetry factor, and theoretical plates for the five suitability injections were determined.

INSTRUMENTS USED

Table No.1: Instruments Used

S.No	Instruments And Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA Detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

CHEMICALS USED

Table No.2: Chemicals Used

S.No	Chemical	Brand names
1	Pravastatin (Pure)	Sura labs
2	Water and Methanol for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for HPLC	Merck

Table No.3: Peak Results for Optimized Chromatogram

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Pravastatin	2.693	631521	92254	1.12	9544

Table No.4: Calibration Data of Pravastatin

S.No	Concentration Level (%)	Concentration µg/ml	Average Peak Area
1	33	20	191834
2	66	40	400444
3	100	60	631299
4	133	80	864659
5	166	100	1063774

Table No.5: Results of Repeatability for Pravastatin

S.No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Pravastatin	2.611	631744	92281	9644	1.2
2	Pravastatin	2.600	631937	92284	9765	1.2
3	Pravastatin	2.605	631877	92204	9284	1.2
4	Pravastatin	2.612	631983	92274	9783	1.2
5	Pravastatin	2.621	631573	92285	9837	1.2
Mean			631822.8			
Std.dev			166.0217			
%RSD			0.026277			

Table No.6: Results of Intermediate Precision Analyst 1 for Pravastatin

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Pravastatin	2.641	631847	92274	9184	1.2
2	Pravastatin	2.642	631922	92857	9004	1.2
3	Pravastatin	2.655	631884	92018	9771	1.2
4	Pravastatin	2.642	631183	92271	9448	1.2
5	Pravastatin	2.625	631840	92276	9019	1.2
6	Pravastatin	2.633	631443	92206	9764	1.2
Mean			631686.5			
Std. Dev.			302.1898			
% RSD			0.047839			

Table No.7: Results of Intermediate Precision Analyst 2 for Pravastatin

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Pravastatin	2.611	631831	92281	9847	1.2
2	Pravastatin	2.643	630696	92277	9164	1.2
3	Pravastatin	2.625	633829	92201	9755	1.2
4	Pravastatin	2.623	638575	92274	9174	1.2
5	Pravastatin	2.613	630228	92265	9575	1.2
6	Pravastatin	2.618	631181	92210	9333	1.2
Mean			632723.3			
Std. Dev.			3129.739			
% RSD			0.494646			

Table No.8: The Accuracy Results for Pravastatin

S.No	%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
1	50%	315573.7	30	29.8	99.7%	99.7%
2	100%	631448.7	60	59.9	99.7%	
3	150%	947420.3	90	89.8	99.8%	

Table No.9: LOD and LOQ Values of Pravastatin

S.No	LOD	LOQ
1	3.2	9.8

Table No.10: Results for Robustness of Pravastatin

S.No	Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
1	Actual Flow rate of 1.0 mL/min	631521	2.693	9544	1.12
2	Less Flow rate of 0.9 mL/min	631633	3.008	8474	1.2
3	More Flow rate of 1.1 mL/min	631047	2.303	8575	1.4
4	Less organic phase	631141	2.943	7285	1.17
5	More organic phase	634271	2.917	7264	1.2

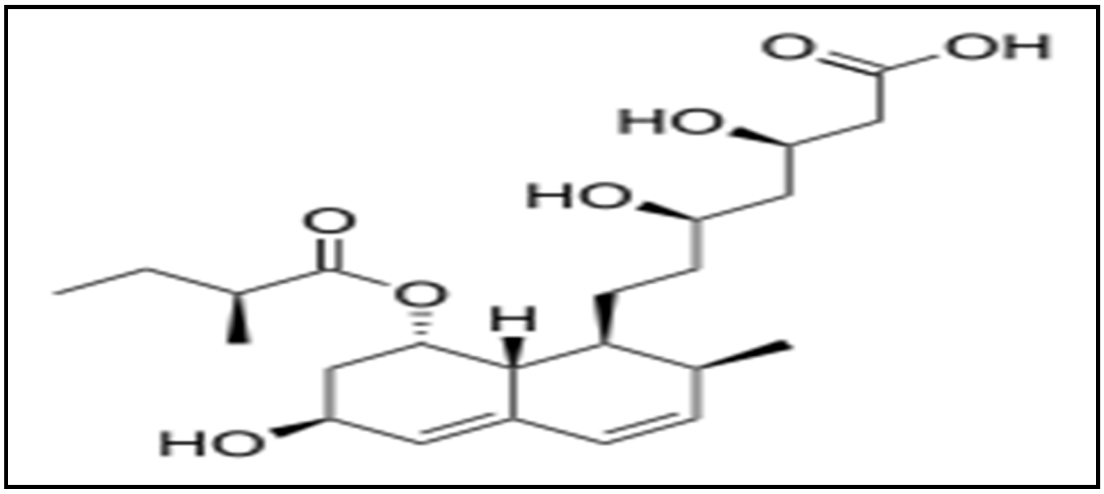

Figure No.1: Chemical Structure of Pravastatin

Optimized Chromatographic Condition

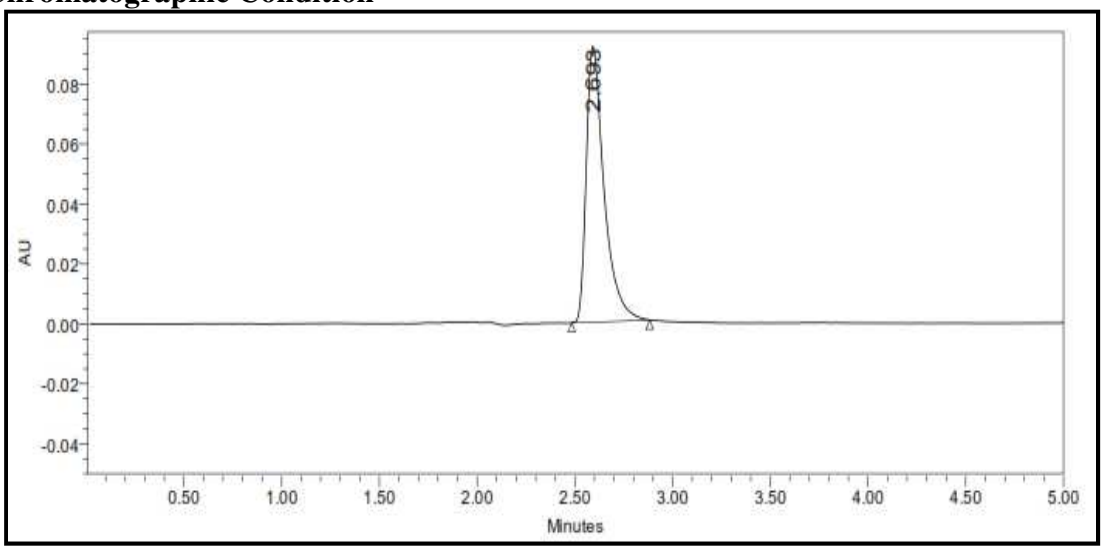

Figure No.2: Optimized Chromatographic Condition

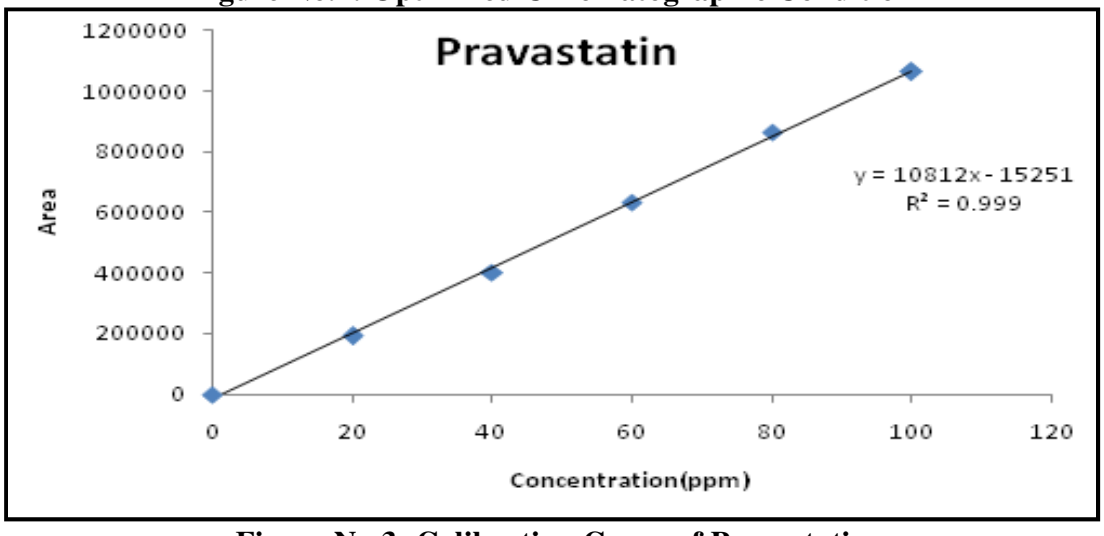

Figure No.3: Calibration Curve of Pravastatin

CONCLUSION

A new method was established for estimation of Pravastatin by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Pravastatin by using Apollo C18 (4.6×150mm) 5µ column, flow rate was 1ml/min, and mobile phase ratio was Methanol: water (65:35% v/v), detection wave length was 235nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2. The retention times were found to be 2.693mins. The % purity of Pravastatin was found to be 99.9%. The system suitability parameters for Pravastatin such as theoretical plates and tailing factor were found to be 1.12, 9544 and the resolution was found to be within the limits. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Pravastatin was found in concentration range of 20µg-100µg and correlation coefficient (r2) was found to be 0.999, % mean recovery was found to be 99.7%, %RSD for repeatability was found to be 0.026, % RSD for intermediate precision was found to be 0.047 and 0.494 respectively. The precision study was precise, robust, and repeatable. LOD value was 3.2, and LOQ value was 9.8 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Pravastatin in bulk and Pharmaceutical tablet dosage form.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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