RESEARCH ARTICLE

Novel RP-HPLC Method Development and Validation for Estimation of Pravastatin in Pure and Pharmaceutical Formulation

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Abstract

Introduction: A simple, rapid, precise, accurate, sensitive and stability indicating RP-HPLC method for the determination of Pravastatin in pure and tablet dosage form.

Materials & Methods: HPLC Method was developed using Zorbax ODS (250×4.6 mm ×5 µ) with the mobile phase of 0.1% formic acid pH adjusted to 3 and methanol in the ratio 50:50 v/v. Pravastatin peak was monitored at 238 nm, and the retention time was 4.44 minutes.

Results and Discussion: ICH guidelines were followed to validate the proposed method regarding specificity, precision, linearity, accuracy, system suitability, and robustness. The method was found to be linear in the range of 10–50 µg/mL, and also the regression equation was found to be y=124936 x+19884 R^2=0.997. For intra- and inter-day precision, the %RSD for Pravastatin was 1.05 and 0.917%. Percentage mean recovery was found to be 98.36%. LOD and LOQ values were 0.231 and 0.701 µg/mL, respectively. Pravastatin stability was inspected under various forced degradation conditions, and it was found to be easily degraded in acidic and basic conditions.

Conclusion: The developed method was found to be having a suitable application for routine quality control analysis of Pravastain in pharmaceutical formulations.

Keywords: Degradation, Pravastatin, RP-HPLC. Validation.

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Introduction

Pravastatin is chemically known as (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.[1] Pravastatin (Figure 1) is a specific inhibitor of the hepatic HMG-CoA reductase in humans. The inhibition of this enzyme produces a reduction in cholesterol biosynthesis as HMG-CoA reductase activity is an early-limiting step in cholesterol biosynthesis.[2] Pravastatin is also used to lower the risk of stroke, heart attack, and other heart complications.[3]

Literature survey reveals that very few HPLC[4-8] methods were reported to estimate Pravastatin in pharmaceutical dosage forms. In the present work an attempt has been made to develop a novel, rapid and economic RP-HPLC method for estimation of Pravastatin in pure and tablet dosage form.

Materials and Methods

Instrument

Agilent 1260 infinity binary pump HPLC equipped with PDA detector and EZ Chrome open lab software was used for chromatographic studies. The column used was Zorbax ODS with dimensions 250 mm×4.6 mm ×5 µ.

Chemicals

Pravastatin pure drug was purchased from Yarrow Chemicals, Mumbai. HPLC grade methanol, formic acid, and all other chemicals were purchased from Merck Limited, Mumbai. Triple distilled water was used throughout the study. Pravastatin tablets were procured from local pharmacy.

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Conflict of interest: None

Preparation of Standard Stock Solution

A standard stock solution was prepared by dissolving 10 mg of Pravastatin in 10 mL mobile phase, then sonicated for about 10 minutes to get the primary standard stock solution containing 1000 µg/mL of Pravastatin. Working standard solution was prepared by further dilution with mobile phase.

Preparation of Sample Solution

5 tablets of Pravastatin were weighed and crushed to a fine powder, and a quantity of tablet powder equivalent to 10 mg of PRT was transferred to 10 mL volumetric flask and dissolved in the mobile phase, and the volume was adjusted up to the mark with mobile phase. The mixture was allowed to stand for 30 min with intermittent sonication to ensure complete dissolution. The resulting solution was filtered through a 0.22 µm membrane filter. The filtrate was diluted further with the mobile phase to get the working sample solution.

Mobile Phase

A 50:50 v/v of 0.1% formic acid pH was adjusted to 3 and methanol was mixed to get the mobile phase. The mobile phase was then filtered through 0.22 µm nylon membrane vacuum filtration and degassed by sonication.

Detection of Wavelength

The spectrum of diluted solutions contains 10 µg/mL of Pravastatin in mobile phase were recorded separately on UV spectrophotometer and the solutions were scanned between 200-400 nm by using mobile phase as blank. The peaks of maximum absorbance wavelengths were observed. The maximum wavelength was found to be 238 nm for Pravastatin.

Results

Method Development

Several concurrent trails developed the proposed method to establish the preferred chromatographic conditions, which would be helpful to conduct a complete validation study. The mobile phase for consisting of 0.1% Formic acid (pH 3): methanol (50:50 v/v) at 1-mL/min flow rate and detection wavelength 238 nm was optimized, which gave sharp peak, minimum tailing factor with short run time for Pravastatin. The retention time for Pravastatin was found to be 4.44 minutes (Figure 2).

Method Validation

System Suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated. The results were presented in Table 1.

Specificity

The specificity was studied to examine the presence of interfering components, while in the comparison of Chromatograms, there was no interference from blank and standard Chromatogram.

Linearity

Linearity was performed by preparing a standard solution of PRT at different concentration levels, i.e., 10–50 µg/mL. The absorbance was measured at 238 nm. Each measurement was carried out in triplicate. Linearity was proven by regression analysis by the least square method. The correlation coefficient and linearity results were presented in Table 2, and the linearity curve was represented in Figure 3.

Precision

Precision was studied to find out intra-day and inter-day variation in the test methods of PRT for 6 times on the same day and different day. The intra-day and inter-day precision obtained was %RSD (<2.0) indicates that the proposed method is quite precise and reproducible, and results are shown in Table 3.

Accuracy

The accuracy of the method was determined by the standard addition method. A known standard drug was added to the fixed amount of pre-analyzed drug sample solution. The standard addition method was performed at three concentration levels in triplicate at 50%, 100%, and 150%. Percent recovery (Table 4) was calculated by comparing the peak area before and after adding the standard drug.

Robustness

To demonstrate the method's robustness, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and wavelength. The results for robustness are represented in Table 5.

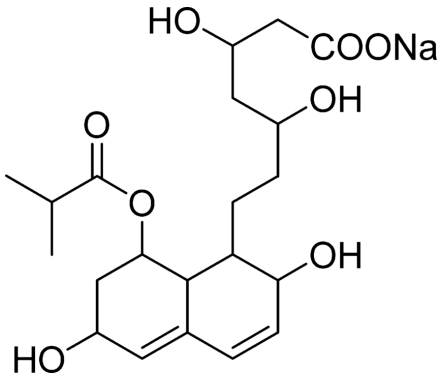

Figure 1: Structure of Pravastatin

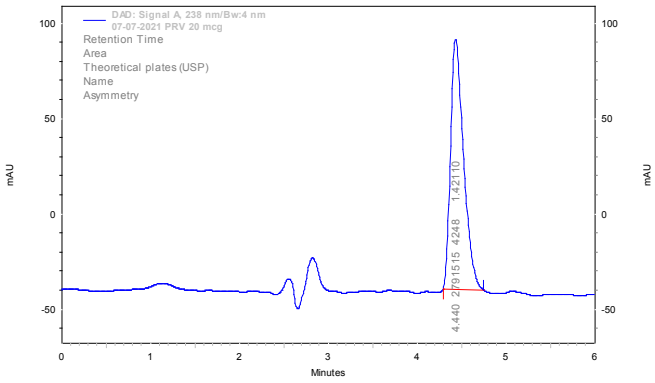

Figure 2: Optimized Chromatogram of Pravastatin

Table.1: Results for system suitability of PRT

Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	4.441	2791515	4280	1.52
2	4.44	2683204	4235	1.5
3	4.427	2786415	4257	1.52
4	4.44	2795612	4264	1.51
5	4.44	2767821	4238	1.52
6	4.442	2714561	4216	1.54
7	4.443	2745923	4251	1.52
8	4.44	2789632	4236	1.51
9	4.442	2754926	4285	1.5
10	4.441	2762451	4269	1.48
Mean	4.4396	2759206	-	-
%RSD	0.1025	1.328	-	-

Table 2: Results for Linearity of PRT

S. No.	Conc.(µg/mL)	Peak area	Statistical Analysis	
1	0	0	Slope	124936
2	10	1425912	Intercept	198484
3	20	2791515	Regression equation	y = 124936x +1984744
4	30	4027939		
5	40	5107667	Correlation coefficient	R^2 = 0.997
6	50	6395401		

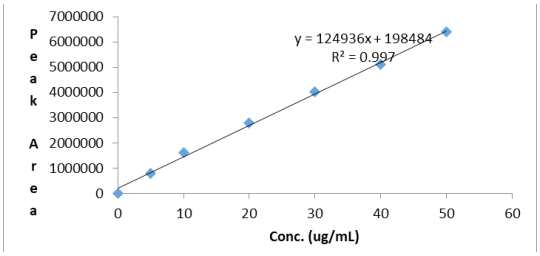

Figure 3: Calibration curve of Pravastatin

Table 3: Intra-day and Inter-day Precision results

	Intra-day		Inter-day	
S. No.	Time (Hours)	Peak area	Days	Peak area
1	0	2791515	1	2791515
2	3	2785621	2	2736425
3	6	2736924	3	2745923
4	9	2796514	4	2792516
5	12	2732615	5	2745861
6	15	2746921	6	2745692
Mean		2765018	Mean	2759655
SD		29275.59	SD	25331.2
%RSD		1.05	%RSD	0.917

Table 4: Results for Accuracy of PRT

Recovery/ Spike level at about [%]	Amount of PRT added (ppm)	Peak Area	Conc. Found (µg/mL)	% Recovery	% Mean recovery
50	10	4192561	9.82	98.25	
50	10	4211562	9.95	99.58	99.01
50	10	4206123	9.92	99.2	
100	20	5593021	20.071	100.35	
100	20	5582362	19.99	99.97	99.97
100	20	5572431	19.92	99.6	
150	30	6791026	29.78	99.29	
150	30	6746342	29.54	98.18	98.71
150	30	6765923	29.6	98.67	

Table 5: Results for robustness of PRT

S. No.	Parameter	Optimised	Used	Rt (min)	Peak area	%RSD
1	Flow rate	0.8 mL/min	0.8 mL/min	4.52	2789632	0.52
			1.0 mL/min	4.44	2696834	0.64
			1.2 mL/min	4.38	2745862	0.21
2	Wavelength	230 nm	236 nm	4.46	2689732	0.51
			238 nm	4.44	2696834	0.35
			240 nm	4.42	2715356	0.48
3	Mobile phase	MeOH:Buffer (70:30)	68:32	4.5	2658127	0.39
			70:30	4.44	2696834	0.51
			72:28	4.41	2786324	0.72

Ruggedness

The analysis of samples confirmed different analysts did the ruggedness of the method. Samples of 100 µg/mL concentration were analyzed by different analysts. It was observed that there were no marked changes in absorbance, which demonstrated that the developed method was rugged in nature.

Table 6: LOD and LOQ of PRT

Parameter	*Measured value(µg/mL)*
Limit of detection	0.231
Limit of quantification	0.701

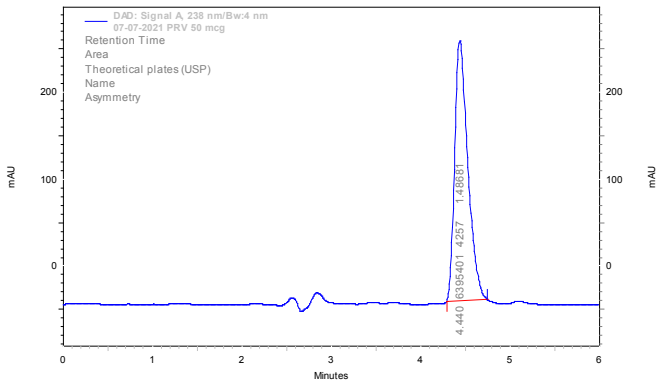

Figure 4: Sample Chromatogram of PRT

Table 7: Assay results of PRT formulation

Formulation		*Label claim*	*Amount found*	*%Assay*
PRAVASTATIN	PRAVACHOL	20 mg	19.91	99.55

Limit of Detection and Limit of Quantification

Limit of detection (LOD) is defined as the lowest concentration of analyte that gives a detectable response. Limit of quantification (LOQ) is defined as the lowest concentration that can be quantified reliably with a specified level of accuracy and precision. The results are furnished in Table 6.

Assay of Tablet Dosage Form

The assay results of Pravastatin in tablet dosage form were comparable with the value claimed on the label. The obtained results were presented in Table 7 indicated the suitability of the method for routine analysis. The sample chromatogram is shown in Figure 4.

Degradation Studies

Acid Degradation Studies

To 1-mL of stock solution of Pravastatin, 1-mL of 2N Hydrochloric acid was added and refluxed for 30 minutes at 60°C. The resultant solution was diluted to obtain 10 µg/mL solution, 10 µL of that solution was injected into the system, and the chromatograms were recorded to assess the stability of the sample (Table 8).

Alkali Degradation Studies

To 1-mL of stock solution of Pravastatin, 1 mL of 2N sodium hydroxide was added and refluxed for 30 minutes at 60°C. The resultant solution was diluted to obtain 10 µg/mL solution, 10 µL of that solution was injected into the system and the chromatograms were recorded to assess the stability of the sample.

Table 8: Degradation data of Pravastatin

	Pravastatin		
Type of degradation	*Area*	*%Recovered*	*% Degraded*
Acid	212564	89.18	10.82
Base	2548632	91.41	8.59
Peroxide	365214	92.39	7.61
Neutral	387324	99.23	0.77

Table 9: Summary of validation parameters of RP-HPLC

Validation Parameters	*Results*
Detection wavelength (λmax)	238 nm
Regression Equation	Y=126453x + 130070
Correlation coefficient(R^2)	0.997
Flow rate	1.0 mL/min
Retention time(min.)	4.41
Accuracy (% recovery)	98.71-99.07%
Limit of Detection (µg/mL)	0.231
Limit of Quantification (µg/mL)	0.709
Intra-day Precision (% RSD)	1.05
Inter-day Precision (% RSD)	00.97
Assay (%)	99.95

Oxidative Degradation Studies

To -mL of stock solution of Pravastatin, 1-mL of 20% hydrogen peroxide (H^2O^2) was added separately. The solutions were kept for 30 min at 60°C. The resultant solution was diluted to obtain 10 µg/mL solution, and 10 µL solution was injected into the system and the chromatograms were recorded to assess the stability of the sample.

Neutral Degradation Studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 6 hours at a temperature of 60°C. For HPLC study, the resultant solution was diluted to 10 µg/mL solution and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Summary

The method was developed using C^{18} (250 x 4.6 mm, 5 µ) as stationary phase with mobile phase containing mixture of 0.1% formic acid (pH 3) and methanol (50:50). The eluted compound was monitored at 238 nm. The developed method was validated for specificity, linearity, precision, accuracy, limit of detection, limit of quantification, and robustness as per approved ICH guidelines.[9] The results were summarized in Table 9.

Conclusion

The present study demonstrated a validated stability-indicating RP-HPLC method to estimate Pravastatin available

as a tablet dosage form. The method was completely validated and showed satisfactory results. The method was free from the interference of the other active ingredients and additives used in the formulation. The RP-HPLC method for estimating Pravastatin has various advantages: low solvent consumption, less retention time, excellent peak symmetry, highly sensitive, precise, accurate, and robust. Hence it can be concluded that this method may be employed for the routine quality control analysis of Pravastatin in active pharmaceutical ingredient (API) and pharmaceutical preparations.

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