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Analytical method development and validation of assay test of pravastatin sodium tablets

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ABSTRACT

A simple, accurate, precise and stability indicating Ultra performance liquid chromatographic method for determination of pravastatin sodium in tablet dosage form. The separation was carried on Acquity UPLC ® HSS C_{18}, 2.1 × 100mm, 1.8µm ID column, with mobile phase comprising of mixture of pH 5.5 buffer: methanol in the ratio of 30 : 70 v/v, as the mobile phase at a flow rate 0.2 ml/min and the detection was carried out using UV-visible detector at 238nm. The method was validated by evaluation of different parameters such as accuracy, precision, linearity, ruggedness, robustness, filter equivalency, solution stability. The retention time were found to be 1.5 min. Calibration curves were linear with correlation coefficient (r^2) 0.999. The Percent assay of Pravastatin sodium tablet was found to be 98.4%. The developed methods were validated as per the ICH guidelines.

Keywords: Pravastatin sodium (PVS), UPLC, Method Validation.

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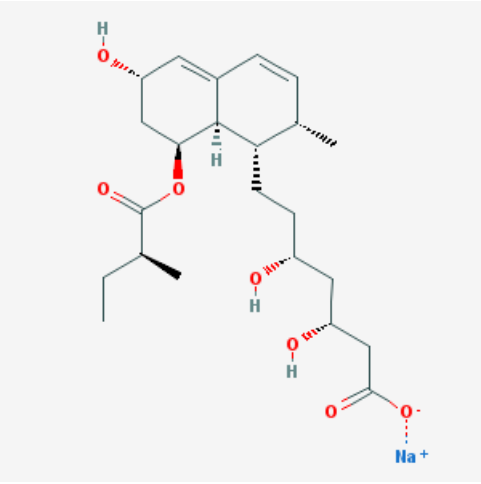
INTRODUCTION

Chromatography is a non-destructive procedure for resolving a multi-component mixture of trace, minor, or major constituents into its individual fractions. UPLC refers to Ultra Performance Liquid Chromatography.[1-5] It improves in three areas: chromatographic resolution, speed and sensitivity analysis. It uses fine particles and saves time and reduces solvent consumption. UPLC comes from HPLC. HPLC has been the evolution of the packing materials used to effect the separation. An underlying principle of HPLC dictates that as column packing particle size decreases, efficiency and thus resolution also increases. As particle size decreases to less than 2.5µm, there is a significant gain in efficiency and it's doesn't diminish at increased linear velocities or flow rates according to the common Van Demeter equation. By using smaller particles, speed and peak capacity (number of peaks resolved per unit time) can be extended to new limits which is known as Ultra Performance.[6]

Drug Profile:

Name: Pravastatin Sodium



Structure of Pravastatin sodium

Chemical Name : sodium (3*R*,5*R*)-3,5-dihydroxy-7-((1*R*,2*S*,6*S*,8*R*,8a*R*)-6hydroxy 2-methyl-8- {[(2*S*)-2-methylbutanoyl]oxy}-1,2,6,7,8,8a-hexahydronaphthalen-1-yl)-heptanoic acid

Molecular Formula: $C_{23}H_{35}O_7Na$

Molecular Weight : 446.5

Category: Anti-hyperlipidemic

Description: Pravastatin is white to yellowish white power or crystalline Powder, hygroscopic.

BCS Class: High solubility, low permeability.

Solubility: Freely soluble in water and in methanol, soluble in ethanol.

Melting point: 172-173 °C

pH: 7.2 - 9.0

Mode of Action: Pravastatin sodium is one of a new class of lipid- lowering compounds. The HMG-CoA reductase inhibitors, which reduce cholesterol biosynthesis. These agents are competitive inhibitors of 3-hydroxy-3-methylglutaryl-co-enzyme A (HMG-CoA) reductase, the enzyme catalyzing the early rate-limiting step in cholesterol biosynthesis, conversion of HMG-CoA to mevalonate.[7-11]

MATERIALS AND METHODS

Instruments:

1) Acquity Waters,Acquity PDA ,Empower 3 Software Build 3471
2) Balance RADWAG Wagl Elektroniczne, model-XA 82|220|2X
3) PH-meter used of LABINDIA
4) Sonicator chiller
5) Calibrated glasswares were used for the whole experimental work

Reagents and Chemicals:

All chemicals and solvents used for the experimental are from MERCK, RANKEM etc. enlisted as follows;

1) HPLC/AR Grade Methanol
2) HPLC Grade Water (Milli-Q)
3) AR Grade Triethylamine
4) Pravastatin Sodium, Glenmark Phramaceuticals Ltd.Taloja (Mumbai)

Spectral study of Pravastatin Sodium:

A solution of Pravastatin Sodium was prepared in methanol having concentration of 10 ppm. The above solution was taken in a 1 cm cell in order to obtain the Ultra violet (UV) spectrum of drug in the range 200-400 nm using UV spectrophotometer was recorded. The absorbance maxima or lambda max (λ_{max}) of the drug was found to be 238 nm.

Preparation of Diluent: Methanol: water (HPLC grade) 50:50v/v was used as diluent

Preparation of Mobile Phase A:

Added 5.0 ml glacial acetic acid in 1.5 liter of water. Adjusted the pH to 5.5 ± 0.05 with Triethylamine. Filtered through 0.45µ nylon membrane filter.

Preparation of Mobile Phase B: Methanol.

Preparation of Standard Solution:

Accurately weighed and transfered about 25 mg of Pravastatin Sodium working standard into a 50 ml volumetric flask. Added 30 mL of diluents and sonicated to dissolve. Made up the volume upto the marked and mixed. Diluted 5 ml of the above solution to 50 ml with diluents (50ppm)

Preparation of Sample Solution:

Taken 5 intact tablets to 100 mL volumetric flask and 60 mL of diluent added then sonicated it for complete dispersion with intermittent swirling. Further sonicated for 15 minutes and diluted to volume with diluent. Allowed it to stand for 10 minutes, diluted 5 mL of supernatant solution to 50 mL with diluent. Further Filtered through 0.45 µ nylon membrane filter. As shown in table no.2

Optimized chromatographic method for Assay:

Table1: Data for Assay of Pravastatin Sodium tablet

Parameter	Condition
UPLC System	Wasters UPLC
Stationary Phase	Acquity UPLC ® HSS C_{18} (2.1 × 100mm, 1.8µ)
Mobile Phase	Buffer(pH 5.5) : Methanol(30:70)
Flow Rate	0.2ml/min
Detection	238nm
Pump Mode	Gradient
Injection Volume	2 µl
Run Time	3 min
Column Temperature	40^0C
Retention Time	1 to 2 min

Evaluation of System suitability:

Inject the standard solution five times. The relative standard deviation of five replicate injections should not be more than 2.0%. The Tailing factor for the Pravastatin Sodium peak should not be more than 2.0 and the number of theoretical plates should not be less than 2500.

Method validation

Method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Method validation provides the method development extremely specific, Linear, Precise, accurate & sensitive. [12-22]

Linearity

Linearity is the ability of the method to elicit test results that are directly proportional to analyte concentration within a given range. Linearity is generally reported as the variance of the slope of the regression line. It is demonstrated directly on the drug substance by dilution of a standard stock solution of the drug product components, using the proposed procedure. For the establishment of linearity, minimum of five concentrations is recommended by ICH guideline. The value of correlation co-efficient should fall around 0.99. The regression equation and correlation coefficient was calculated and found to be within the required limits as shown in Tables no.4 and figure no.3 respectively.

Accuracy

The test for accuracy is intended to demonstrate the closeness of agreement between the value found and the value that is accepted either as a conventional true value or as an accepted reference value. Thus the accuracy of a method is the closeness of the measured value to the true value for the sample. The accuracy can also be demonstrated by recovery of the impurity spiked to a drug substance or into a placebo with drug substance. The percentage recovery with the certain acceptance criteria at each defined level is

reported. Accuracy should be assessed using a minimum of nine determinations at a minimum of three concentration levels covering the specified range. The recovery results were shown in table no.5.

Precision:

The precision of analytical procedure expresses the closeness of agreement between series of measurements obtained from multiple sampling of the same homogeneous sample.The precision of an analytical procedure is usually expressed as the variance,standard deviation.The precision results were shown in table no.6 .

Ruggedness

Ruggedness is the degree of reproducibility of the results obtained under a Varity of conditions, expressed as %RSD. These conditions include different laboratories, analyst, instruments, reagents, days etc.The ruggedness results was shown in table no.7.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters. The robustness of a method is evaluated by varying method parameters such as percent organic, pH, wavelength, temperature, etc., and determining the effect on the results of the method. The results of robustness study were shown in Tables no 9, 10, 11 respectively.

RESULTS AND DISCUSSION

Assay data: The percent Assay of pravastatin sodium tablet was found to be 98.4%

Table 2: Data for Assay of Pravastatin Sodium tablet

Wt.of Std (mg)	Area of Standard	Area of Sample	Purity of Standard	% Assay
25	1359265 1361568 1356933 1365771 1361791	1368275 1365143	98%	98.4%
Mean	1361066	1366709		
SD	3287.854			
% RSD	0.24			

Specificity: The retention time of the Pravastatin Sodium peak in the chromatogram of the Sample preparation corresponds to that of the Pravastatin Sodium peak in the chromatogram of the Standard preparation. The Pravastatin Sodium peak is pure in Standard solution and Sample solution. Blank and Placebo should not show any peak at the retention time of Pravastatin sodium.

Table 3: Specificity table for Pravastatin Sodium tablet

SR. No.	Name	Purity Angle	Purity Threshold
1	Standard solution	0.418	1.640
2	Sample solution 40 mg	0.381	1.616

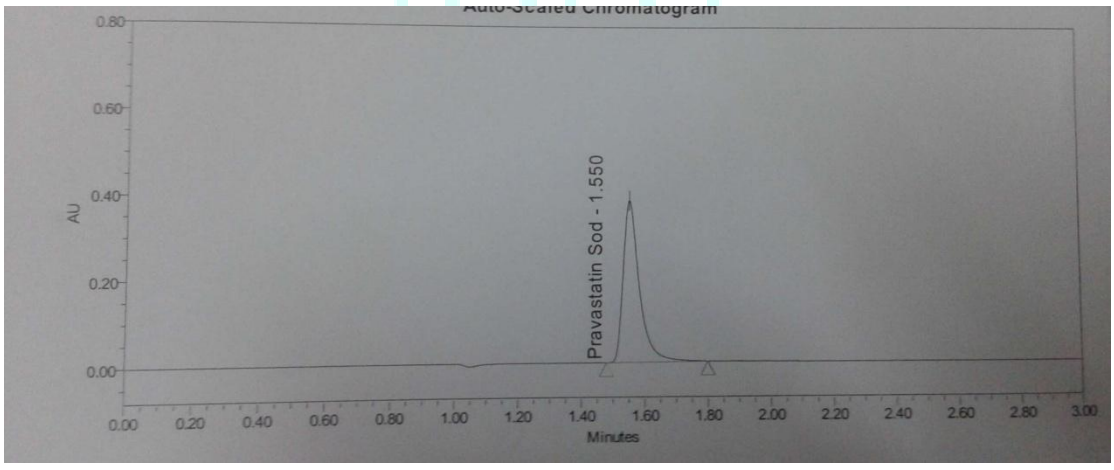


Figure 1: Chromatogram of Specificity Standard Solution.

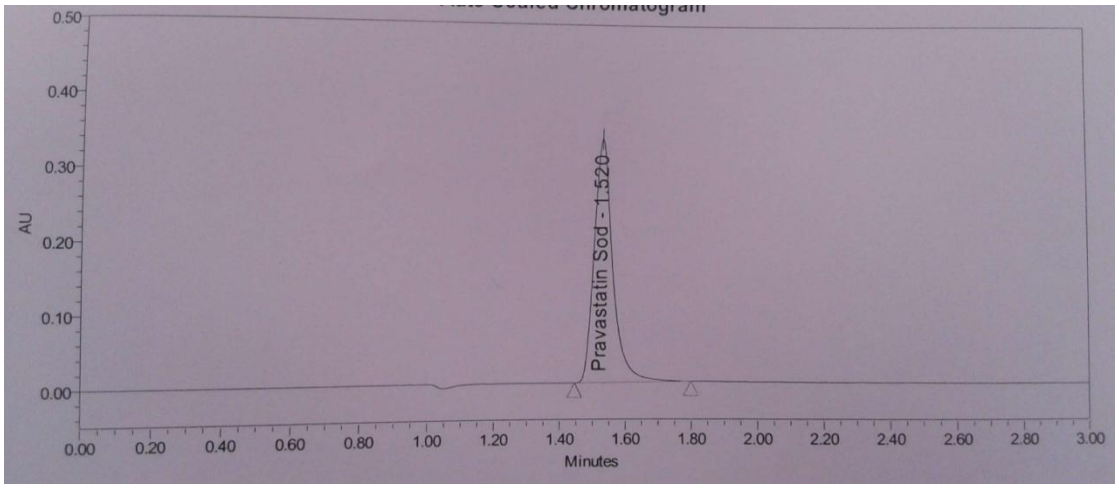


Figure 2: Chromatogram of Specificity Sample Solution

Linearity: A series of solutions of Pravastatin Sodium Standard was prepared over a range of 30% to 70% of the standard concentration of Pravastatin Sodium in Pravastatin Sodium tablets. Since the standard concentration is 50 µg/ml. Linear calibration graph was obtained between absorbance versus concentration of pravastatin sodium drug. Correlation coefficient was 0.99993. Therefore, the UPLC method for the determination of Pravastatin Sodium in Pravastatin Sodium tablets was linear.

Table 4: Linearity for Pravastatin Sodium tablets

% Concentration	Concentration (µg per ml)	Response (Area)	Statistical analysis	
30%	30.432	831281	**Slope**	27168.2
40%	40.576	1107490		
50%	50.720	1389802	**Intercept**	-6284.1
60%	60.864	1659407		
70%	71.008	1933293	**Correlation Coefficient**	0.99997

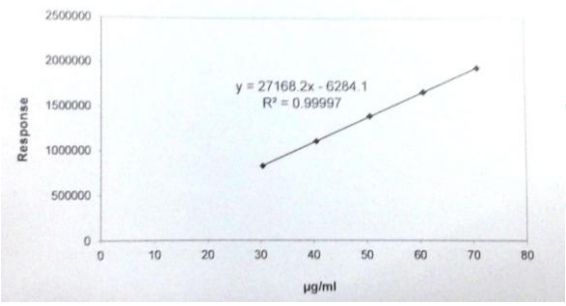


Figure 3: Linearity Graph of Pravastatin Sodium.

Accuracy (Recovery)

Placebo of Pravastatin Sodium tablets 40 mg was spiked with Pravastatin Sodium Drug Substance at three different levels: 80%, 100% and 120% of the label claim in triplicate (in total nine determinations) and then proceeded with Sample solution as described under Methodology. Each of the Sample solution was injected in duplicate and the average area count was taken for calculation. Mean recovery is 100.5% & RSD is 1.02 %. Therefore, the UPLC method for the determination of Pravastatin Sodium in Pravastatin Sodium tablets was accurate.

Table 5: Accuracy for Pravastatin Sodium tablets

Sample No.	Amount added (mg)	Amount recovered (mg)	% Recovery
Acc. 80% -1	31.85	31.90	100.2
Acc. 80% -2	31.56	31.86	101.0
Acc. 80% -3	31.48	31.83	101.1
Acc. 100% -1	39.45	40.06	101.5
Acc. 100% -2	39.32	39.87	101.4
Acc. 100% -3	40.62	40.19	98.9
Acc. 120% -1	48.59	48.33	99.5
Acc. 120% -2	48.66	48.28	99.2
Acc. 120% -3	48.27	48.88	101.3
Mean			100.5
SD			1.024
% RSD			1.02

Precision

System Precision: Five replicate injections of the Standard preparation were injected into the UPLC using the method as described under Methodology**.**

Method Precision: Six sample solutions of Pravastatin Sodium tablets 40 mg were prepared and injected into the UPLC using the method as described under Methodology.

Table 6: System Precision for Pravastatin & Method Precision of Pravastatin Sodium tablet

System Precision for Pravastatin	
Injection	**Area**
1	1321824
2	1321593
3	1324619
4	1316424
5	1321550
Mean	1321202
SD	2965.048
%RSD	0.224
Method Precision of Pravastatin Sodium tablet	
Sample	**% Label claim**
1	99.4
2	99.2
3	99.2
4	98.9
5	99
6	99
Mean	99
SD	0.183
%RSD	0.18

Robustness

Three Sample preparations of the Pravastatin Sodium tablets 40 mg were prepared as described under Methodology. The Six sample preparations of the Pravastatin Sodium Tablets 40 mg were made by a different analyst, using different column on a different day and injected in duplicate into a different UPLC using the method as described under Methodology, along with Standard preparation.

Table 7: Ruggedness for Pravastatin Sodium tablet

Sample	**Analyst -1 % Label claim**	**Analyst -2 % Label claim**
1	100.3	99.9
2	99.9	99.8
3	100.3	97.3
4	100.4	99.6
5	100.3	99.8
6	99.9	99.8
Mean	100	99
SD	0.223	1.017
%RSD	0.22	1.02
Overall Mean	99.5	
Overall SD	0.62	

Stability of Analytical solution

The sample and standard preparations were stored at room temperature and tested against freshly prepared standard preparations for 24 hours.

Table 8: Stability of Analytical solution at Room Temperature

Sr. No.	**Name**	**% Content 0 hours**	**% Content 24 hours**	**% Correlation**
1	Standard Solution	--	99.0	99.0
2	Sample solution	98.9	100.6	101.7

samples along with standard were injected in duplicate under different chromatographic condition as shown below.

Change in mobile phase composition (± 2% absolute):

Table 9: Table for mobile phase composition (± 2% absolute)

Control		**+2% absolute**	**-2% absolute**
99.9	99.6	99.9	100.5
99.8	99.8	98.5	98.8
97.3	99.8	99.1	99.4
Mean		99.3	99.4
SD		0.883	0.918
%RSD		0.89	0.92

Change in Flow rate (± 0.1%):

Table 10: Table for Change in Flow rate (± 0.2 mL/min).

Control		**+0.2ml/min**	**-0.2ml/min**
99.9	99.6	98.8	100.0
99.8	99.8	97.5	98.8
97.3	99.8	98.0	99.3
Mean		98.9	99.4
SD		1.075	0.859
%RSD		1.09	0.86

Change in wavelength (± 5nm)

Table 11: Table for Change in wavelength (λmax 238)

Control		+ 5 (243nm)	-5 (233nm)
99.9	99.6	100.2	100.2
99.8	99.8	99.5	99.5
97.3	99.8	100.1	100.1
Mean		99.6	99.6
SD		0.873	0.873
%RSD		0.88	0.88

CONCLUSION

The developed UPLC method offers several advantages such as rapidity, usage of simple mobile phase and sample preparation steps. Further, improved sensitivity makes it specific and reliable for its intended use. Hence, this method can be applied for the analysis of pharmaceutical dosage forms. From the present study it can be concluded that the proposed method was Simple, Linear, Precise, and Accurate for Assay of Pravastatin sodium in its tablet dosage form. Hence this method can be introduced into routine use for the assay & dissolution study of Pravastatin in Pravastatin sodium tablets. Results of validation parameters demonstrated that the analytical procedure is suitable for its intended purpose and meets the criteria defined in ICH Q2A/B.

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