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Development and validation of a stability-indicating RP-HPLC method for the estimation of Fluvastatin sodium in bulk and tablet dosage form

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Abstract

A simple and gradient RP- HPLC method has been validated and developed for Fluvastatin Sodium in bulk and tablet dosage form. The proposed method was validated to obtain official requirements including stability, accuracy, precision, linearity and selectivity. The method was developed on Hypersil ODS C18 column (150 x 4.6 mm, 5micron) using the mobile phase consists of methanol: 20mM Phosphate buffer (pH 3.2 adjusted with Phosphoric acid): acetonitrile (55: 30: 15 v/v) was delivered at a flow rate of. The flow rate was set as 1.1 ml/minute and the maximum absorption were observed at 234 nm. The Fluvastatin Sodium drug showed a precise and good linearity at the concentration ranges of 3-15 µg/ml. The RP-HPLC, assay showed the highest purity ranging 99.88 % to 100.09 % for Fluvastatin Sodium tablet formulation and 100.02 % was the mean percentage purity. The Fluvastatin Sodium retention time was found to be 5.5 minutes. The method accuracy was showed by statistical analysis. The developed RP-HPLC method can be adopted for the routine analysis of Fluvastatin Sodium in bulk and pharmaceutical dosage forms in quality control laboratories. The developed method was validated according to the ICH guidelines.

Keywords: Fluvastatin Sodium; Methanol; Buffer; Acetonitrile; HPLC

1. Introduction

Fluvastatin Sodium (FVS) chemically is 7-[3-(4-fluorophenyl)-1-(1-methylethyl)-1*H*-indol-2-yl]-3, 5 dihydroxy-6-heptenoic acid monosodium salt. These substances inhibit, by competition, the 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-Co A), thus preventing this substance from catalyzing the conversion to mevalonate and consequently inhibiting the first stage in cholesterol biosynthesis in humans [1].

Literature revealed that some analytical methods, such as differential plus voltammetry [2-3], square-wave adsorptive-stripping voltammetry [4], cyclic voltammetry [5] and other voltammetric techniques [6] have been reported for the determination of FVS in bulk and pharmaceutical dosage form. First derivative spectrophotometry [7] and kinetic spectrophotometric [8] determination of Fluvastatin in pharmaceutical preparations have been reported. Fluvastatin have been determined by capillary electrophoresis [9] (CE), high performance liquid chromatography (HPLC) in biological fluids such as human and rat plasma [10-17]. Liquid chromatography- mass spectrometry [18-19] and gas chromatography- mass spectrometry [20] have been reported for the determination of Fluvastatin in biological fluids. Photo degradation studies of Fluvastatin by HPTLC and spectrophotometry method have been reported [21]. Simple and sensitive HPTLC method for the determination of Fluvastatin in bulk and pharmaceutical dosage form has been reported [22].

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2. Material and methods

2.1. Chemicals

The Fluvastatin Sodium reference standard (RS) was purchased from Sigma, Germany. The marketed Fluvastatin Sodium 80mg tablet brand name Lescol® XL 80 tablet, manufactured by Novartis purchased from USA.The HPLC grade acetonitrile, water and methanol were purchased from Sigma, Germany. Stock solutions of Fluvastatin Sodium were prepared from mobile phase. Fresh working solutions were prepared daily. All solutions were filtered (0.45µm) and degassed by sonicator.

2.2. RP-HPLC instrumentation

Shimadzu LC-20 AT HPLC system, using SPD-10 detector (SPD- M20A, Japan). The Chromatographic separation was carried out on a Hypersil ODS C18 column (150 x 4.6 mm, 5micron). The column temperature was maintained at a 27°C, and the flow rate was 1ml/min. The sample injection volume is 20µl and the wavelength was set as 234nm, the HPLC run time was set for 15 minutes.

2.3. Preparation of Mobile phase

The mobile phase consists of HPLC grade methanol: 20mM Phosphate buffer (pH 3.2 adjusted with Phosphoric acid): HPLC grade acetonitrile (55: 30: 15 v/v.), filtered through a 0.45µm membrane filter and sonicated for 15 minutes.

2.4. Preparation of Fluvastatin Sodium stock solution

2.4.1. Standard Fluvastatin Sodium solution

Fluvastatin Sodium (15 mg) weighed accurately and transferred to 100 ml volumetric flask and mixed with 100 ml of mobile phase solution, the resulting solution were kept in the sonicator for 5 minutes. The concentration of 3-15 µg/ml was achieved by diluting the standard stock solution with mobile phase. Fluvastatin Sodium powder was freely soluble in methanol.

2.4.2. Preparation of Fluvastatin Sodium tablet solution

1 gm of marketed sample of Fluvastatin Sodium tablet was analyzed by this method. Ten tablets were accurately weighed, and their average weight was determined. The tablets were then crushed to fine powder and powder equivalent to 10 mg was transferred into 25ml volumetric flask and dissolved with 25 ml of mobile phase and filtered through Whatman 1 filter paper. Further dilutions were made based on the required concentrations.

2.5. Solution stability

The prepared drug solution stability was analysed during the time of analysis and repeated the same analysis method on same day with different time intervals. The same analysis was repeated after 24 hrs by keeping the drug solution under laboratory temperature (37 ± 1°C) and in refrigeration (5 ± 1°C).

2.6. Method validation

The proposed method was preceded to achieve a new, sensitive, and easy method for estimation of Fluvastatin Sodium by RP-HPLC. The experimental analysis was validated according to the ICH (Q2 B) guidelines, recommendations, and USP-30.

2.7. System suitability

The resolution, retention time, tailing factor and column theoretical plates parameters were performed by six replicates of standards and three replicates of sample preparation.

3. Results and discussion

3.1. Method optimization

Chromatogram with good shape peaks and good retention time shows good resolution for Fluvastatin Sodium. The typical RP-HPLC conditions are presented in Table 1. The good separation of Fluvastatin Sodium shows the success of

the method. The HPLC chromatogram of Fluvastatin Sodium standard and Fluvastatin Sodium tablet is presented in figure 1 and 2.

Table 1 RP-HPLC conditions for estimation of Fluvastatin Sodium

Parameters	Description
Column	ODS C18 column (150 x 4.6 mm, 5micron)
Mode of operation	Isocratic
Column temperature	27 ± 1°C
Mobile phase	Methanol: Buffer: Acetonitrile (50:30:15 v/v).
Detection	Photodiode array detection at 234 nm
Injection volume	20 µl
Flow rate	1.1 ml/ min
Run Time	15 Min

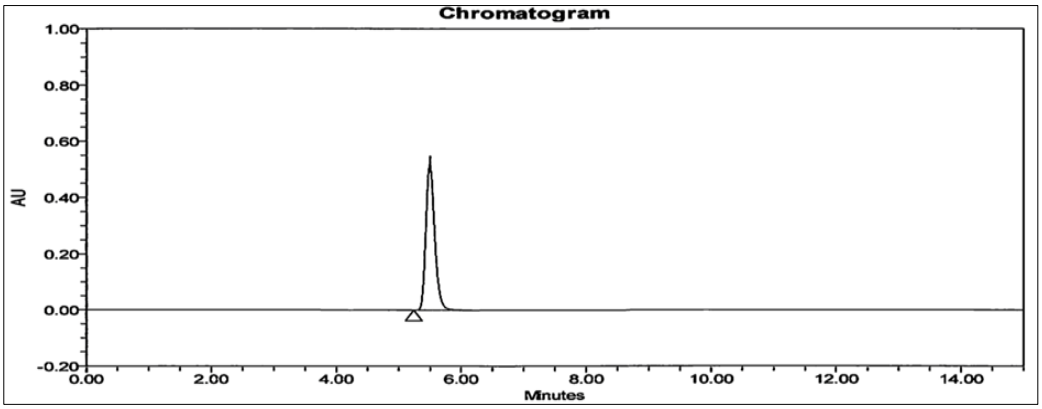

Figure 1 A Chromatogram of Fluvastatin Sodium Standard

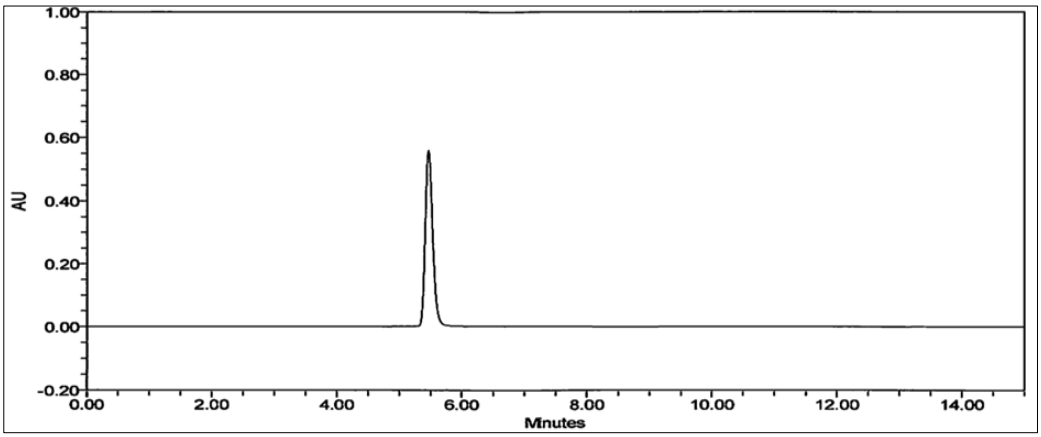

Figure 2 A Chromatogram of Fluvastatin Sodium tablet formulation

3.2. Linearity

The proposed method linearity was examined for five concentrations. The concentration ranges from 3-15µg/ml. The Fluvastatin Sodium standard linearity was determined by the plotting graph concentration vs absorbance. By

absorbance as a functional of analyte concentration linearity was evaluated for Fluvastatin Sodium. The linearity graph presented in figure 3, and data presented in Table 2. The system suitability is demonstrated by the linearity analysis.

Table 2 RP- HPLC linearity for Fluvastatin Sodium

Concentration (µg/ml)	Peak area
3	520437
6	1041321
9	1562440
12	2084353
15	2605454

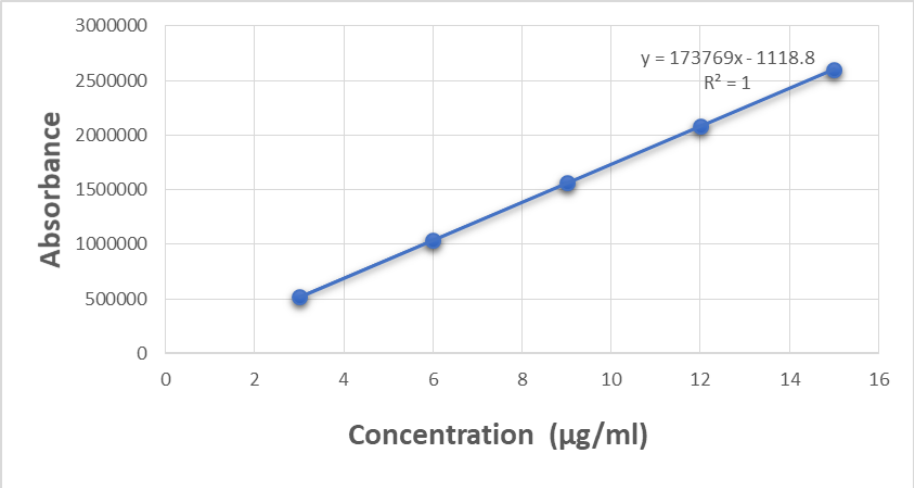

Figure 3 Calibration graph of Fluvastatin Sodium 3-15 µg/ml precision

3.3. Accuracy

The recovery experiment shows the accuracy of the method. The good recovery shows the method was accurate. The analysis for recovery was performed by known amount of Fluvastatin Sodium working standard added to pre-analyzed solution of formulation in the test concentration range of (80%, 100% and 120 %). For each recovery level three samples were prepared and repeated for 3 consecutive days. The statistical results for recovery study are well within the range (S.D. < 2.0). The Fluvastatin Sodium bulk recovery results are presented in Table 3.

Table 3 Recovery studies of Fluvastatin Sodium in pure form

Recovery Level (%)	Amount added (µg/ml)		Amount Found (µg/ml)	% Recovery	Mean recovery
	Standard	Test			
80	12	5	17.98	99.85	99.83
100	15	5	19.96	99.81	
120	18	5	22.97	99.83	

Parameters	Concentration (µg/ml)	Recovery concentration	% Recovery	Mean recovery
Assay	3.0	3.001	100.03	99.99
	6.0	5.998	99.97	
	9.0	8.989	99.88	
	12.0	12.011	100.09	
	15.0	14.997	99.98	

3.4. Precision

The proposed method precision (repeatability) experiment results of are shown in Table 4. In the proposed method intraday and interday precision was examined by analyzing the responses of the sample on the same day for 4 repetitions and 3 alternate days for 20µg/ml concentration range of Fluvastatin Sodium. The obtained results are represented in % RSD. The % CV of the proposed method was precise as the values < 1.0 % for the repeatability study. The precision data are presented in Table 5.

Table 4 Method precision data of Fluvastatin Sodium by RP-HPLC (n = 4)

Fluvastatin Sodium 3µg/ml (n=4)	Retention time	Area
1	5.51	520654
2	5.50	520876
3	5.56	520432
4	5.52	520453
Mean	5.55	520603
S.D [a]	0.0264	0.152
% CV[b]	0.74	2.33

Table 5 Intermediate precision data of Fluvastatin Sodium by RP-HPLC nc = 4 observations

Fluvastatin Sodium µg/ml	Inter-day measured mean area ± S.D.[a]	%CV[b] (n[c]=4)	Intra-day measured mean area ± S.D.[a]	%CV[b] (n[c]=4)
09	520545± 2.09	0.0462	520745± 2.61	0.0145
12	2085323±1.12	0.0547	2075311±2.10	0.0107
15	2605124±1.52	0.0632	2602104±1.26	0.1019

3.5. Specificity

The standard reference and the drug formulation show specificity of the method. The RP-HPLC chromatogram of Fluvastatin Sodium both bulk and the tablet formulation are presented in figure 1, 2. The bulk and tablet formulation retention time were found to be 5.5 minutes. For the tablet formulation there was no excipient interference was detected, which shows the specificity of the method. The proposed method shows the ability to determine the analyte in presence of excipients.

3.6. Limit of detection and quantitation

The limit of detection and quantification for Fluvastatin odium is presented in table 6. Limit of detection (LOD) and limit of quantification (LOQ): LOD and LOQ were examined by minimum detectable peak area by injecting known concentration of drug solution. As per the International Conference on Harmonization guidelines the results are multiplied thrice to get LOD and 10 times to get LOQ. LOD and LOQ were found at concentrations of 3.211µg/mL and 1.135 µg/mL respectively. The limit of detection and quantification for Fluvastatin Sodium is presented in table 6.

Table 6 Limit of detection and quantification

Parameters	Results (µg/ml)
Limit of detection (LOD)	3.211
Limit of quantification (LOQ)	1.135

3.7. System suitability

For the system suitability parameters five repeats of standards and two repeats of sample preparation are injected, the data is presented in table 7. The Assay data of Fluvastatin Sodium is presented in table 8

Table 7 Results of system suitability parameters

Sr. No	Parameters	Fluvastatin Sodium
1.	Theoretical plates	14100
2.	Tailing factor	0.779
3.	Resolution factor	1.38
4.	Retention time	5.51± 0.1
5.	Calibration range or Linear dynamic range	3-15µg/ml

Table 8 Quantitative estimation (Assay) data of Fluvastatin Sodium

Sr. No	Formulation	Labeled Amount (mg/tab)	Amount found (mg/tab)	% Recovery	Mean recovery
1	Lescol XL	80	80.012	100.06	100.02
2			80.007	100.00	
3			80.002	100.00	

3.8. Statistical parameters

The obtained assay results are subjected to the coefficient of variation; statistical analysis, regression equation and standard deviation are presented in table 9

Table 9 Results of statistical parameters

Sr. No	Parameters	Fluvastatin Sodium
1.	Standard deviation (SD)	1.27
2.	Relative standard deviation (RSD)	0.0576
3.	% RSD	0.216
4.	Standard error (SE)	0.01321
5.	Correlation Coefficient (r)	1.0000
6.	Slope (a)	3317
7.	Intercept (b)	1118.8

4. Conclusion

The proposed and validated RP-HPLC method was performed according to the guidelines of International Conference on Harmonization (ICH), 1the developed RP-HPLC method shows the accuracy, sensitive and stability indicating. The developed method is rapid, reproducible. The developed method can be used for the routine analysis for Fluvastatin Sodium tablet formulations.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors has declared that no competing interests exist.

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