Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

VALIDATED RP-HPLC METHOD FOR FLUVASTATIN SODIUM IN BULK AND ITS DOSAGE FORM

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Abstract

A Simple, selective and rapid reverse-phase high Performance Liquid Chromatography (RP-HPLC) method for the analysis of fluvastatin sodium in bulk and in tablet dosage form has been developed and validated. The method was carried out on a Hypersil® ODS C_{18} (150 x 4.6 mm, 5micron) column. The mobile phase consists of methanol: 20mM Phosphate buffer (pH 3.0 adjusted with Phosphoric acid): acetonitrile (5: 3: 2 v/v.) was delivered at a flow rate of 1.2 ml/min at ambient temperature and the retention time was about 7.65 minutes with symmetrical peaks. Studies was performed on an HPLC System equipped with uv/vis detector at 235nm.The method is specific to fluvastatin sodium and able to elute the peak from formulation excipients. The calibration curve was linear over the concentration range of 1 - 6 µg/ml (R^2=0.9998). The proposed method was accurate (the accuracy results were 98.31- 99.70 for fluvastatin recoveries. precise (the intraday and interday precision of CVs were 0.2073- 0.8976 % for fluvastatin sodium recoveries) and linear within the desired range. the lower limits of detection for FVS was found to be 0.0194µg/ml and the quantitation limit was about 0.0588 µg/ml and therefore could be employed as more convenient and efficient for the analysis of fluvastatin sodium in bulk and its formulation.

Keywords: *Fluvastatin sodium; method validation; C_{18} column; RP- HPLC determination;*

Introduction

Fluvastatin sodium [1] (FVS), chemically is [*R**, *S**-(*E*)]-(±)-7-[3-(4-fluorophenyl)-1-(1-methylethyl)-1*H*-indol-2-yl]-3, 5dihydroxy-6-heptenoic acid monosodium salt (fig.1).It is lipid lowering agent and for reduction of elevated total cholesterol. Fluvastatin sodium [2] is a water soluble cholesterol lowering agent which acts through the inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. Literature survey revealed estimation of Fluvastatin sodium by several techniques such as chiral Evaluation in human plasma [3] and serum [4], capsules by Differential pulse voltammetry [5] in human plasma by LC-MS [6] and HPLC [7] methods have been reported for the quantitation of fluvastatin sodium in the formulation.

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This technique requires sophisticated instruments and reagents. The present work describes a simple, precise and

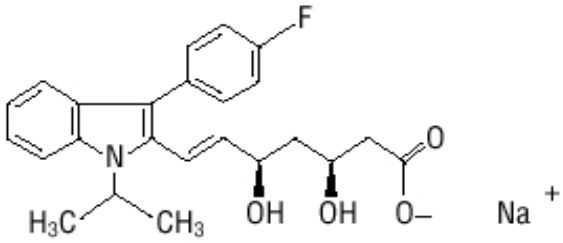


Fig.1 Structure of fluvastatin sodium

accurate reversed phase HPLC method for the estimation of FVS in bulk and in tablet dosage form.

Experimental conditions

Instrumentation

A Shimadzu HPLC System equipped with LC- 10 ATVP pump and variable wavelength programmable SPD -10AV VP UV/VIS detector was utilized. Chromatographic system was integrated

via shimadzu model CBM 102 communication bus module to P-IV Computer loaded with winchrom software for data acquisition and mathematical calculations. Rheodyne manual injector fitted with 20µl fixed loop, a Hypersil ODS column C_{18} (150 x 4.6 mm, 5micron) column and shimadzu online degasser In addition electronic balance, microlitre syringe and microphore filteration assembly was used in this assembly.

Materials and Reagents

Fluvastatin sodium was a kind gift sample from Orchid Chemicals and Pharmaceuticals ltd, Chennai and formulation was known as LESCOL® 80 mg were obtained from Granada medicals, Qatar. Methanol, Water (Qualigens India Ltd) used were HPLC grade. Ortho-phosphoric acid and Potassium dihydrogen phosphate (LOBA Chemicals Ltd., Mumbai) were of analytical grade. HPLC grade water was used to prepare the mobile phase for HPLC. 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.

Preparation of Solutions

A stock solution of fluvastatin sodium was 10µg/ml were prepared in mobile phase and serially diluted to required concentrations. Twenty tablets of FVS were weighed and finely powdered and an amount equivalent to 10mg was mixed with 50ml of mobile phase to give a concentration of 200 µg/ml. This mixture was allowed to stand for 1 hour with intermittent sonication to ensure complete solubility of the drug. This stock solution was filtered and clear filtrate was diluted to the concentrations of 30µg/ml and finally is made up to the 3µg/ml.

Chromatographic Conditions

The mobile phase was methanol/Phosphate pH3 buffer/acetonitrile (5:3:2 v/v). The pH of this mobile phase was adjusted to 3.0 with Phosphoric acid (85%). Prior to delivering into the system. It was filtered through 0.45 µm filter and degassed using a Sonicator. The samples were introduced by injector with a 20µL sample loop. The analysis was carried out under isocratic conditions using a flow rate 1.2 ml/min at ambient temperature. Chromatograms were recorded at 235 nm using a SPD-10AV VP Shimadzu uv visible detector.

Validation procedures

The method was validated for the parameters like system suitability, specificity, range and linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, ruggedness and robustness. The system suitability was assessed by six replicate analyses of the drug at a concentration of 10µg/ml. System suitability of the method was evaluated by analyzing the repeatability, peaks symmetry (symmetry factor), theoretical plates of the column, between the peaks of fluvastatin sodium, mass distribution ratio (capacity factor) and retention time. The specificity of the chromatographic method was determined to ensure separation of standard and fluvastatin sodium. Specificity was also determined in the presence of excipients used in formulation, FVS was spiked (at a concentration of 10µg/ml) in drug product and chromatogram was observed and compared with that of raw material. The purity of the peak was checked using a UV-Visible detector. To evaluate the

linearity, the LOD and LOQ of the method in reference drug, serial dilutions were made from the standard stock solution in the working range of 1.0-6.0 µg/ml and volume was made up with diluent which was a mixture of 5:3:2 methanol: Phosphate buffer (adj to pH3): acetonitrile on a C_{18} column with UV detection at 235nm. To determine accuracy of the method and recovery of fluvastatin sodium in dosage formulation, working standard of FVS was prepared in the range of 1.0- 6.0µg/ml. Samples for recovery studies were also prepared by spiking known amount of drug in the raw materials at three concentration levels (25, 50, and 75 %) and analyzed. The precision of the method was investigated with respect to repeatability. To determine intermediate precision, standard solutions of FVS at six concentration levels were analyzed three times within the same day (intra-day variation) and on three different days (inter-day variation). Generally acceptable repeatability of the results within one day and day-to-day was observed. Robustness studies were performed on method precision sample concentration 3µg/ml by making slight variations in flow rate, amount of methanol and pH changes one at a time.

Assay in formulations

In case of marketed formulations, twenty accurately weighed tablets of each brand were crushed to a fine powder and an amount equivalent to 10 mg of FVS was added into different 50 ml volumetric flasks and volume was made up with methanol/Phosphate pH3 buffer/acetonitrile (5:3:2 v/v) pH of this mobile phase was adjusted to 3.0 with Phosphoric acid (85%) to give a concentration of approximately 4 µg/ml. This mixture was allowed to stand for 1 hour with intermittent sonication to ensure complete solubility of the drug (stock solution). The samples were filtered through a 0.45µm membrane filter and serial dilutions (1, 2, 3, 4, 5, 6µg/ml) were made in 25ml volumetric flask and were injected for HPLC analysis.

Results and Discussion

The use of HPLC methods for the quantitation of drug has become a routine consideration in the quality control of drugs and drug products. The goal of this study was to develop a rapid, easy accurate, precise reliable and least time consuming HPLC method for the analysis of FVS from raw materials, bulk drug samples, tablets or other formulations using the most commonly employed C_{18} column with UV detector. Development and optimization of isocratic HPLC conditions Initial method development was conducted on a Hypersil, ODS, C_{18} (150×4.6 mm, 5 micron) column for separation at ambient temperature. This column provides efficient and reproducible separation of the components while minimizing solvent usage. Consequently, it was selected for the method development. Among the suitable isocratic conditions to fluvastatin sodium on C_{18} column, the mobile phase methanol/Phosphate pH3 buffer/acetonitrile (5:3:2 v/v) adjusted pH 3 with phosphoric acid to 3.1 was found to provide a reproducible, baseline and peak. Optimal retention times (7.65 minutes) were achieved. When the pH of mobile phase was adjusted to pH 3 with 85% phosphoric acid. The chromatographic conditions were optimized with respect to specificity and time of analysis. The specificity of the method was established through the study of FVS peak from the standard peak. Peaks were identified using retention

times compared with those of standards. The method has been successful in determining FVS in concentration, as low as 1µg, with retention time of only 7.65 minutes.

Method validation

The newly developed method has been validated and holds well for the determination of drug in raw materials and its dosage forms. For validation of analytical methods [9] have recommended the accomplishment of accuracy test, precision, specificity, linearity, ruggedness and robustness of the method.

System suitability testing

Typical system suitability results are summarized in table.1. All the values for the system suitability [10] parameters were within limits. System suitability test are an integral part of chromatographic methods and reproducibility of the system are adequate for the analysis to be performed.

Range and linearity

The range of an analytical method is the interval between the upper and lower analytical concentration of a sample where the method has shown to demonstrate acceptable accuracy, precision, and linearity [11].The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in samples within a given range. The linearity of the method was observed in the expected concentration range demonstrating its suitability for analysis. The calibration curve was constructed with six concentrations including the LOQ ranging from 1 to 6 µg/ml (fig. 3). The regression statistics are shown in table 2. The goodness-of-fit is measured by R^2 value which was found to be > 0.999 and value of intercept was less than 2% of the response of 100% concentration in all the cases indicating functional linear relationship between the concentration of analyte and area under the peak.

Accuracy

Accuracy [12] of an analytical method is the closeness in agreement between the accepted true value or a reference value and the actual result obtained. Accuracy studies are usually evaluated by determining the recovery of a spiked sample of the analyte into the matrix of the sample to be analyzed. The results of accuracy studies are shown in table 3. Recoveries of fluvastatin sodium were 98.31-99.70% and coefficients of variation ranged from 0.168% to 1.903% which is evident that the method is accurate within the desired range.

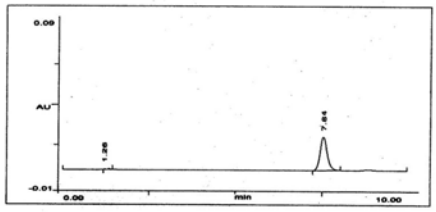
Precision

Precision is a measure of the ability of the method to generate reproducible results. The precision of a method is evaluated using three separate determinations for repeatability, intermediate precision, and reproducibility. Repeatability refers to the use of the analytical procedure within a laboratory over a short period of time using the same analyst with the same equipment and is expressed as the % RSD. The results of intra-and inter-day variations are shown in table 4. The accuracy of the method ranged from 0.2073% to 0.8987%. The results obtained from intermediate precision (interday) also indicated a good method precision. All the data were within the acceptance criteria

Detection and quantitation limit

The detection limit or LOD [13] is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated. LOD was expressed as a concentration that gives a signal to noise

Fig.2. Typical chromatogram of sample solution



(Chromatogram showing the sample solution of FVS at the approximate concentration of 10µg/ml of FVS)

Fig.3: Calibration curve showing linearity over the concentration range of 1-6 µg/ml

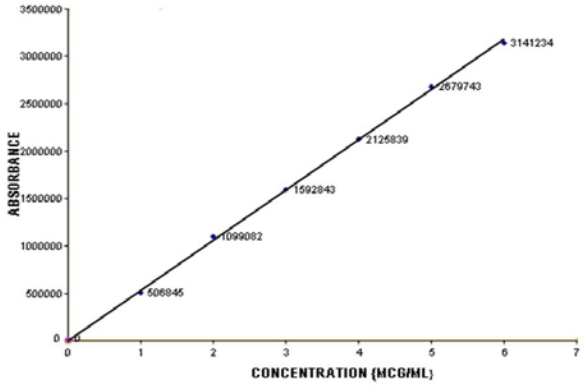


Table 1: System suitability parameters

Parameters	Minimum	Maximum	Average	%RSD
Retention time (min)	7.45	7.68	7.57	2.40
Peak area of FVS	1592843	1614072	1597296	0.87
Capacity factor(k')	4.40	4.56	4.46	1.24
Tailing factor (T)	1.18	1.21	1.19	1.01
Asymmetric factor	1.27	1.31	1.29	0.98
Theoretical plates (N)	7108	8130	7619	4.69

Table 3: Accuracy / Recovery data for fluvastatin sodium in pure form

Parameters	Concentration (µg/ml)	Recovered concentration	% Recovery	% RSD
Assay	1.0	0.997	99.70	0.595
	2.0	1.989	99.45	0.266
	3.0	2.986	99.53	0.5825
	4.0	3.976	99.40	0.3033
	5.0	4.920	98.42	0.7867
	6.0	5.898	98.31	1.903
Spiking level				
25%	3.75	3.71	98.93	0.168
		3.64	99.06	0.764
		3.73	99.46	0.564
50%	4.50	4.40	99.77	0.467
		4.35	99.66	0.243
		4.42	99.22	0.486
75%	5.25	5.17	99.40	0.383
		5.19	99.20	0.743
		5.08	99.21	0.785

Table.2.Regression statistics of the proposed method of Fluvastatin sodium

Concentration range (µg/ml)	Goodness of fit (R^2)	Slope	Intercept	Standard error
1.0-6.0	0.99979	535522	-5627	22622

Table 4: Intra-and inter-day, variation /intermediate precision of the method

Concentration (µg/ml)	Day 1 (%RSD)	Day 2 (%RSD)	Day 3 (%RSD)
1	0.8381	0.8590	0.8987
2	0.2073	0.2172	0.2235
3	0.4428	0.6184	0.5441
4	0.6485	0.6188	0.4748
5	0.5192	0.5208	0.3497
6	0.4349	0.4307	0.3764

Table 5: Robustness data for fluvastatin sodium

Parameter	% RSD	% Recovery
Flow rate **1.2ml/min**	0.235	99.82
Methanol: phosphate buffer: water	0.664	98.34
pH 3.0	0.39	99.59

Table 6: Assay of FVS in marketed formulations

S. No	Formulation	Labeled Amount (mg/tab)	Amount found* (mg/tab)	% Recovery	S.D	%R.S.D	S.E
1	Lescol	80	80.484	100.60			
2			79.605	99.50	±0.665	±0.666	0.018
3			79.525	99.40			
		Mean	79.871	99.83			

ratio of 2:1 or 3:1. Quantitation limit or LOQ [14], on the other hand is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. LOQ is measured in terms of signal to noise ratio of 10:1. LOD and LOQ were calculated by the equation given in ICH guidelines[8]. This may be expressed as LOD = 3.3 σ /S and LOQ = 10 σ / S, where σ is the standard deviation of the response, S is the slope of the calibration curve which may be estimated from the calibration curve of the analyte. The limit of detection and limit of quantification of the proposed method at 236 nm were found

Specificity

Specificity [13, 14] is the ability of a method to discriminate between the analyte of interest and other components that are present in the sample. The specificity of the method was evaluated to ensure separation of FVS and was demonstrated by assaying samples of fluvastatin sodium. The method demonstrated good separation between the peaks and was found to be free of interference. For demonstrating the specificity of the method for drug formulation, the drug was spiked, wherein the excipients used in different formulation products did not interfere with the drug peak and thus the method was specific for FVS.

Ruggedness

The ruggedness [13] of an analytical method is the degree of reproducibility of the test results obtained by the same samples under a variety of conditions, such as different laboratories, different analysts, different instruments, different lots of reagents and different days. The assay result indicated that the method was capable with high precision (table.4). Results of % RSD (0.2073-0.8987) of three different days prove the ruggedness of developed method.

Robustness

Robustness is a measure of the performance of a method when small, deliberate changes are made to the conditions of method [14]. The results of the robustness study are summarized in table.5

Applicability of method for FVS analysis

This developed HPLC method was sensitive and specific for the quantitative determination of fluvastatin sodium from dosage formulations. The method was applied to the determination of FVS content in marketed formulations. The assay results are shown in table.6 demonstrating the suitability of method.

Conclusion

A rapid, precise, accurate, low cost and least time consuming RP-HPLC method for the qualitative and quantitative analysis, determination and quantification of fluvastatin sodium in raw material as well as dosage formulation has been successfully developed. The proposed RP-HPLC method enables the determination of fluvastatin sodium because of good separation of chromatographic peaks. The accuracy and precision of the method were confirmed by the statistical parameters. Reliability, rapidness, simplicity, sensitivity, economical nature and good recovery of this RP-HPLC method.

Acknowledgements

The authors are grateful to M/s orchid Pharmaceuticals Chennai, Novartis Pharma ltd, Switzerland for providing the raw material, formulations of Fluvastatin

sodium. Authors also thankful to the management of Arulthiru Amma, Thirumathi Amma and Dr.T.Ramesh M.D., ACMEC trust, Melmaruvathur for providing the necessary facilities to carry out this research.

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