Validation of Fluvastatin Sodium by HPLC Method

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Abstract:-The HPLC method was developed for Fluvastatin Sodium for the pure drug. The determination was performed on LC, column part no: 00G-4252-EQ, dimension: 250x4.6 mm, mobile phase containing Methanol & Acetonitrile:1-Octane sulphonic acid at pH 2.5 (60:40), with a flow rate 1ml/min and retention time was 3-4 min using UV detector with wave length 230 nm was used. The linearity of Fluvastatin sodium was found to be in the concentration range of 2-12 µg/ml with regression coefficient of 0.999 was observed. The percentage recovery data was found to be within 98-102% indicating that method is accurate. The LOD and LOQ were found to be 5.4 and 1.7 µg/ml respectively. The percentage recovery data was found to be within 98-102%. HPLC method for Fluvastatin sodium was validated as per ICH guidelines.

Key words: Fluvastatin sodium, Validation, HPLC, ICH guidelines.

INTRODUCTION

The statins are the most efficient and best tolerated drugs, used for the treatment of hyperlipidemia. These drugs are competitive inhibitors of HMG CoA reductase [1]. It is a weak acid with pKa 5.5. There is no official pharmacopoeia method has been found for validation of Fluvastatin sodium. The main objective is to develop and validate the new quantitative high performance liquid chromatography method using UV detector according to ICH guidelines. Various parameters were performed for method validation [2] like system suitability, linearity, accuracy, precision, limit of detection, limit of quantification, and robustness.

MATERIALS AND METHODS

Materials: Fluvastatin sodium drug was collected gift sample from Biocon India Ltd. Bengaluru and HPLC grade Methanol, other solvents and chemicals were analytical grades.

Chromatographic condition: Type of instruments: LC, column part no: 00G-4252-EQ, Model no: Phenomenex, mobile phase containing Methanol + Acetonitrile:1-Octane sulphonic acid at pH 2.5 (60:40), with a flow rate 1ml/min, Wave length: 230 nm, Flow rate: 1ml/minute, Injection volume: 20 µl, Diluent: HPLC grade methanol, Column dimension: 250x4.6 mm, 5 µ, Column temperature: Ambient Gradient: High pressure, Retention time: 3-4 min.

Validation Methods

System suitability: A portion of 1 ml of 100 µg/ml of standard stock solution of Fluvastatin sodium was taken in a 10 ml volumetric flask. Volume was adjusted up to 10 ml with HPLC grade methanol to get 10 µg/ml. The resulting solution was sonicated for 5 min, 20 µl of this mixed standard stock solution was injected into HPLC system. Retention time, peak area and peak resolution in chromatogram for the drug were observed.

Specificity: Pipette out 1ml of 100 µg/ml drug from microcapsules into volumetric flask and diluted up to 10 ml with HPLC grade methanol to get 40 µg/ml and 20 µg/ml concentration of Fluvastatin sodium. Blank was injected and chromatogram was recorded.

Linearity: About 100 mg of Fluvastatin sodium was accurately weighed and transferred into 100 ml volumetric flask then made it up to the mark using methanol. From this solution 1ml was pipette out into 10 ml volumetric flask & the volume was made up to the mark. The final concentration obtained was 100 µg/ml. This was used as stock for calibration curve. Final concentrations of stock solution obtained for Fluvastatin sodium were 2-12 µg/ml.

Accuracy: The accuracy of an analytical method was closeness to the test result obtained by that method to the true value. The accuracy was performed by addition of known amounts of standard drug to the test and compared with added concentration.

Solution1: A portion of 1ml sample solution and 2 ml of 1000 µg/ml standard stock solutions of Fluvastatin sodium were mixed and adjusted the volume with HPLC grade methanol.
Solution2: A portion of 1ml sample solution and 4 ml of 1000 µg/ml standard stock solutions of Fluvastatin sodium were mixed and adjusted the volume with HPLC grade methanol.
Solution3: A portion of 1ml sample solution and 6 ml of 1000 µg/ml standard stock solutions of Fluvastatin sodium were mixed and adjusted the volume with HPLC grade methanol.

Precision:

The precision of an analytical procedure expresses the closeness of agreement between the value which was accepted either as a conventional value or an accepted reference value. The precision of the analytical method is determined by assaying sufficient number of samples and relative standard deviation was calculated. The precision of the instrument was determined by assaying the same consecutively, for number of times and relative standard deviation was calculated.

Intraday precision: Stock solution of 100 µg/ml containing Fluvastatin sodium were diluted to get 10 µg/ml by using HPLC grade methanol and 20 µl was injected to HPLC column, chromatogram was recorded. Repeated the procedure on same day, chromatogram was recorded and assessed. The results were presented in terms of % relative standard deviation.

Interday precision: Stock solution of 100 µg/ml containing Fluvastatin sodium were diluted to get 10 µg/ml by using HPLC grade methanol and 20 µl was injected to HPLC column, chromatogram was recorded. Repeat the procedure on different day, chromatogram was recorded and assessed. The results were presented in terms of % relative standard deviation.

Limit of Detection and Limit of Quantification:

LOD and the LOQ of the drug were calculated using the following equation as per ICH guidelines. LOD= 3.3 × σ/S, LOQ= 10 × σ/S Where, σ = Standard deviation of the response, S= Slope of calibration curve.

Robustness:

The robustness of an analytical method is a measure of its capacity to remain unaffected by small variations in method parameters like change in flow rate, change in mobile phase ratio and change in wave length. The robustness was performed for the solution containing for the concentration of drug is 10 µg/ml [2, 3]**.**

1. **Change in flow rate:** The optimized flow rate 1ml/ min, and 2 ml/min were selected for study. 20 µl of sample solution was injected into HPLC system.

2. **Change in mobile Phase:**

   The optimized mobile phase ratio was 60:40; the mobile phase ratio is changed to 58:42. About 20 µl was injected to HPLC column.

3. **Change in wave length:**

   The wave length was changed from 230 nm to 227 nm and 232 nm, chromatogram was recorded.

RESULTS:

i. **System suitability**

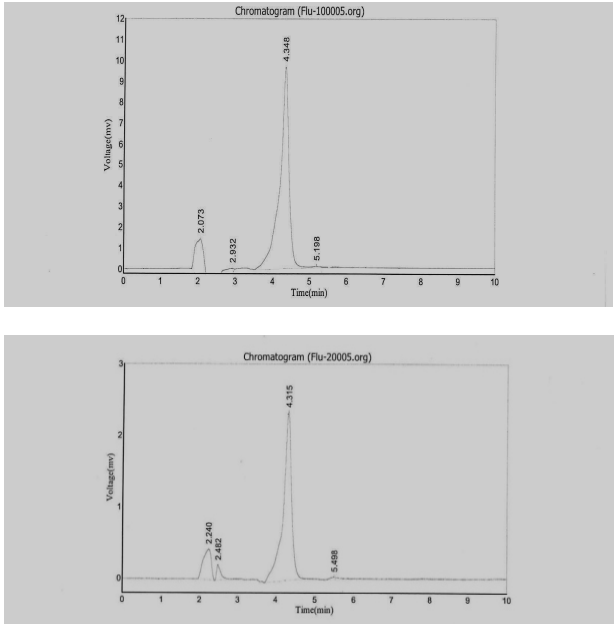


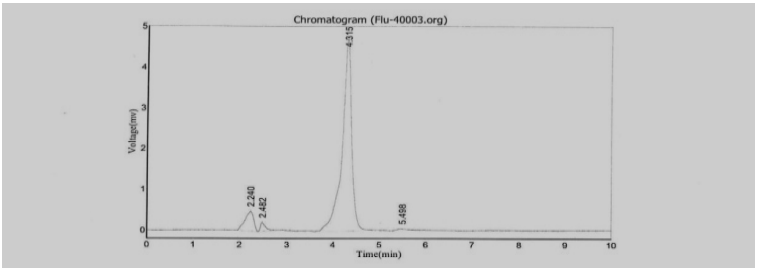
Fig 1: Chromatogram (Flu-10 µg/ml)

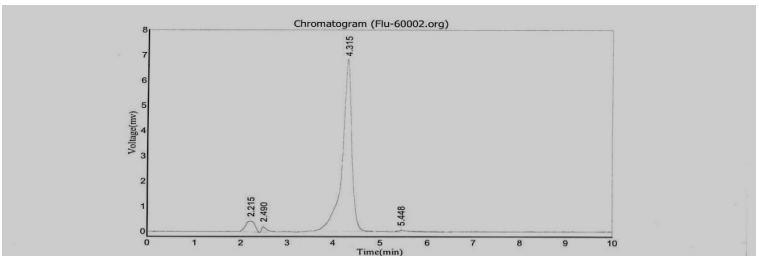
Table1 : System suitability parameters from chromatogram (Flu-10 µg/ml)

Peak No	Ret time (min)	Height (µm)	Area (mV)	Conc (%)
1	2.073	2039.872	40141.801	17.7147
2	2.932	198.966	17799.400	7.8549
3	4.348	9660.032	167502.172	73.9194
4	5.198	58.106	1157.733	0.5109
Total		11956.976	226601.106	100.0000

Peak	Half peak	Theoretical	Resolution	Tail factor	Asymmetry
No	width (cm)	levels			
1	0.298	267.569	0.000	1.327	1.664
2	0.283	593.100	1.476	1.741	2.507
3	0.202	2575.652	2.921	0.719	0.478
4	0.333	1347.350	1.589	1.593	1.971

ii) Linearity





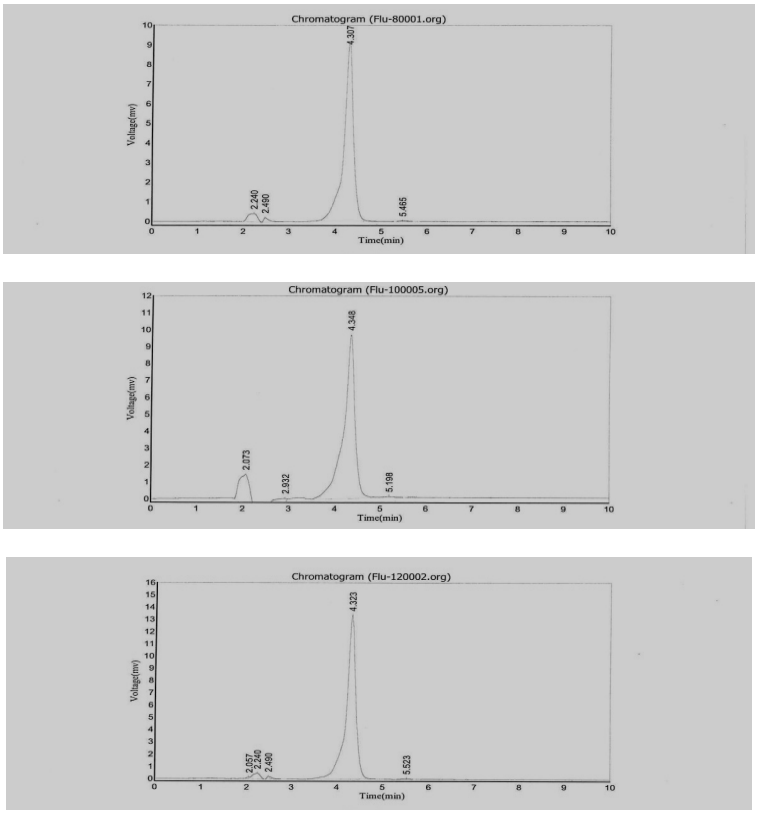


Fig 2: Chromatograms linearity range from 2 to12 µg/ml

Table 2: Linearity data of Fluvastatin Sodium

Sl no	Drug Concentration(µg/ml)	Peak Area*±SD (mV)
1	2	34254.949±0.0028
2	4	66189.299±0.0040
3	6	97164.935±0.0029
4	8	129429.632±0.0045
5	10	161460.015±0.0056
6	12	190450.781±0.0067

* Each value is an average of three determinations

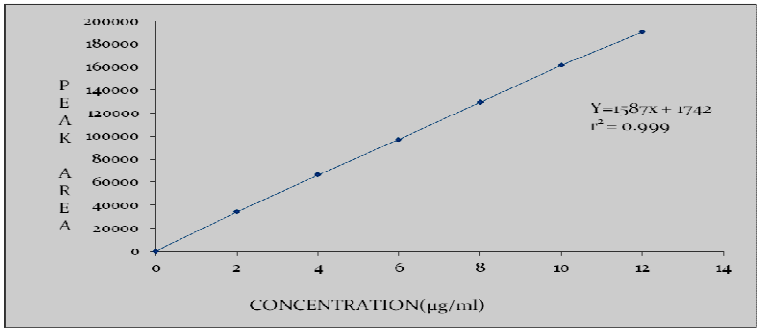


Fig 3: Linearity graph of Fluvastatin sodium

ii. Accuracy

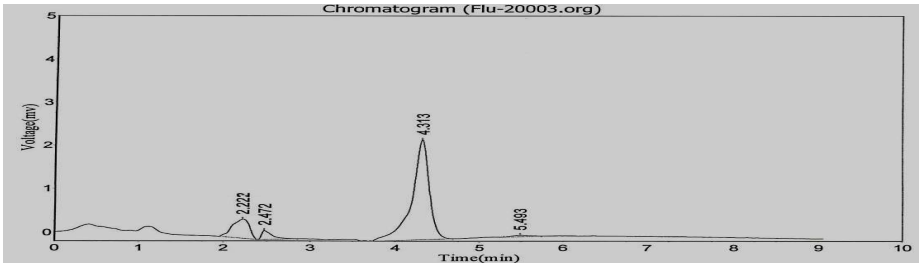


Fig 4: Chromatogram at 80% level

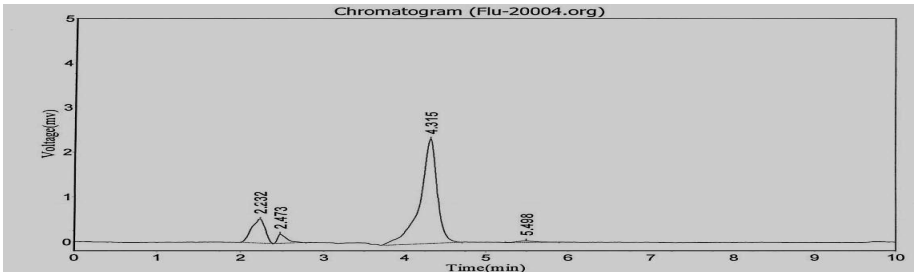


Fig 5: Chromatogram at 100% level

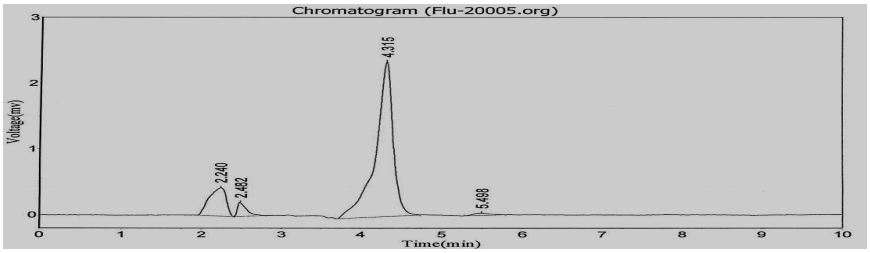


Fig 6Chromatograt120% level

Table 3: Recovery data of Fluvastatin sodium

	Level of addition (%)	Peak area of standard Flu (2 µg/ml)	Peak area theoretical (mV)			% Recovery
			1	2	3	
1	80	35479.801	32951.098	34333.949	35479.801	98.72
2	100					99.62
3	120					99.24

iii. Robustness:

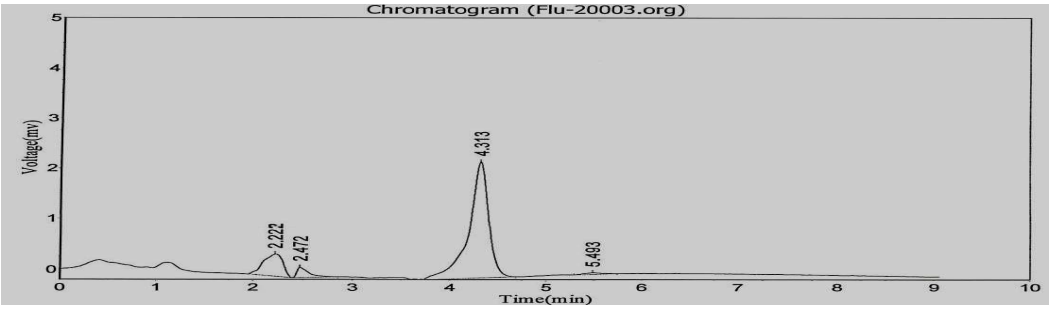


Fig 7: Chromatogram with respect to increase in flow rate

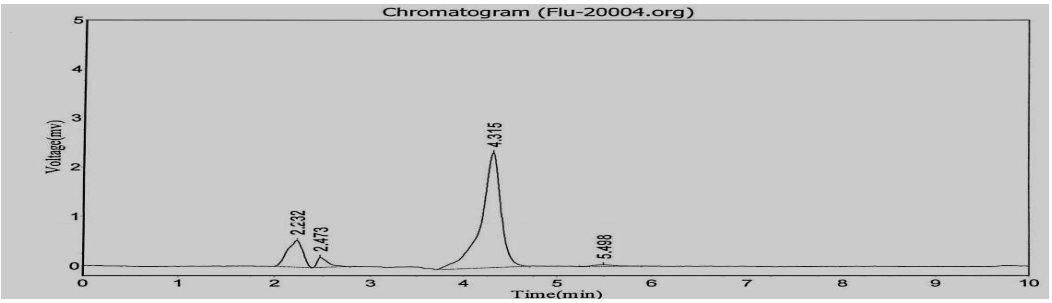


Fig 8: Chromatogram with respect to decrease in flow rate

Table 4: Robustness data with respect to change in flow rate

Flow rate	1.2 ml/min	1.0 ml/min
Rt of Fluvastatin sodium	4.313	4.315
Changes in Rt of Fluvastatin sodium	-0.002	-

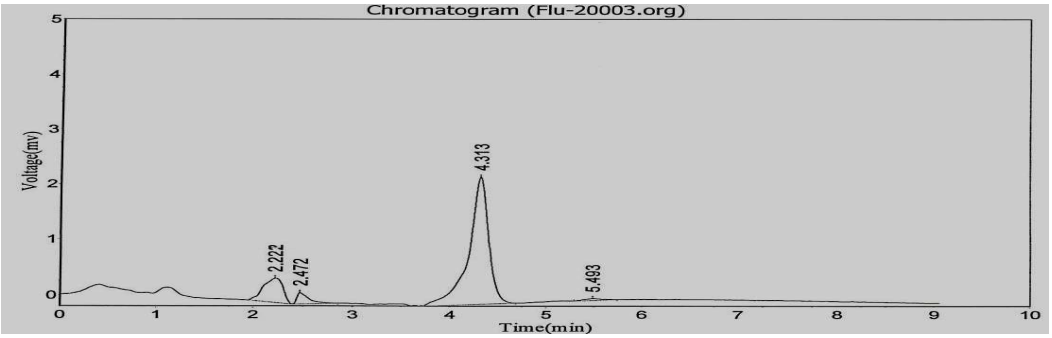


Fig 9: Chromatogram with respect to increase in wave length

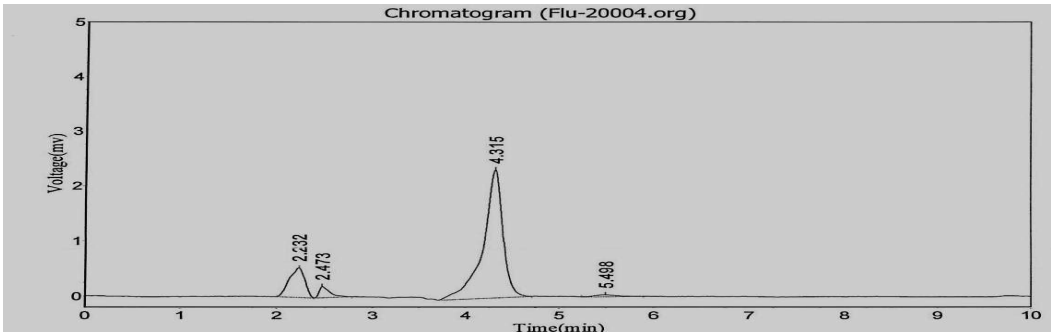


Fig 10: Chromatogram with respect to decrease in wave length

Change in λ_{max} nm	227 nm	232 nm
Rt of Fluvastatin sodium	4.313	4.315
Changes in Rt of Fluvastatin sodium	-002	-

Table 5: Robustness Data with respect change in wave length

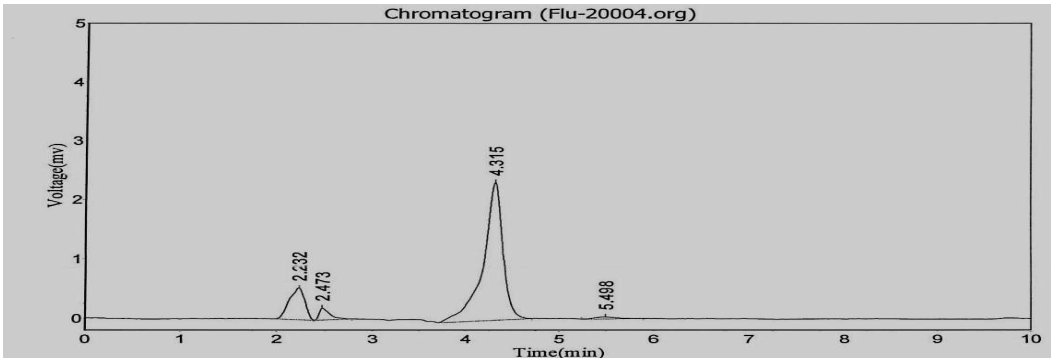


Fig 11: Chromatogram with respect to increase in mobile phase ratio

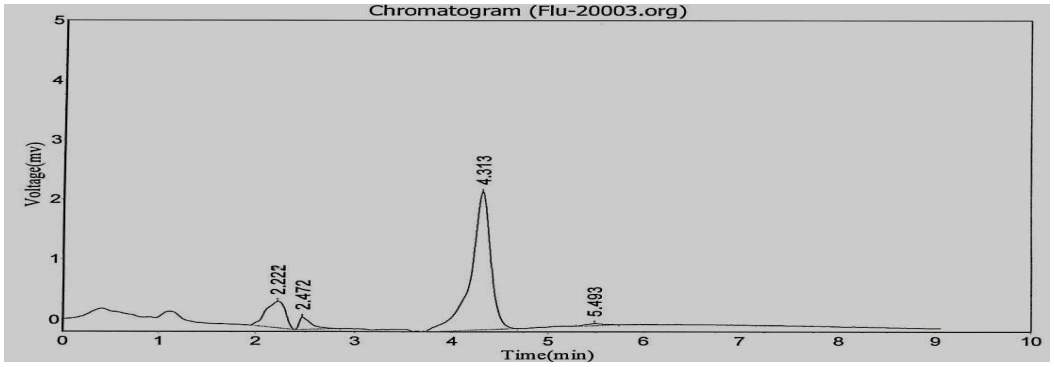


Fig 12: Chromatogram with respect to decrease in mobile phase ratio

Change in mobile ratio	60:40 v/v	58:42 v/v
Rt of Fluvastatin sodium	4.315	4.313
Change in Rt of Fluvastatin sodium	-	-0.002

Table 6: Robustness data with respect to change in mobile ratio

iv. Precision:

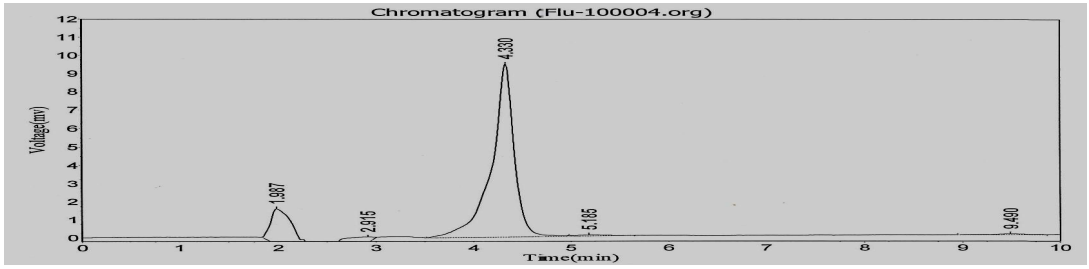


Fig13: Trial 1-Intraday precision of Fluvastatin sodium

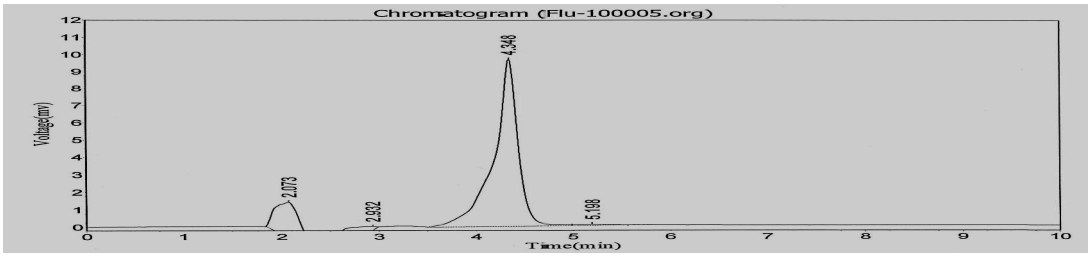


Fig 14: Trial 2- Intraday precision of Fluvastatin sodium

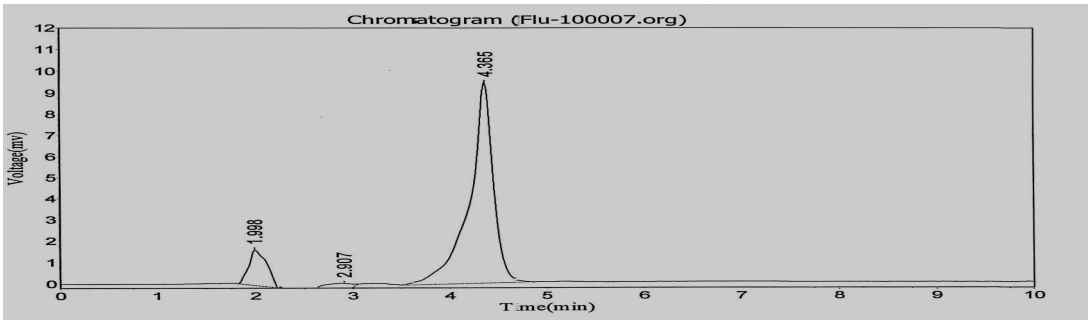


Fig 15: Trial 1-Interday precision of Fluvastatin sodium

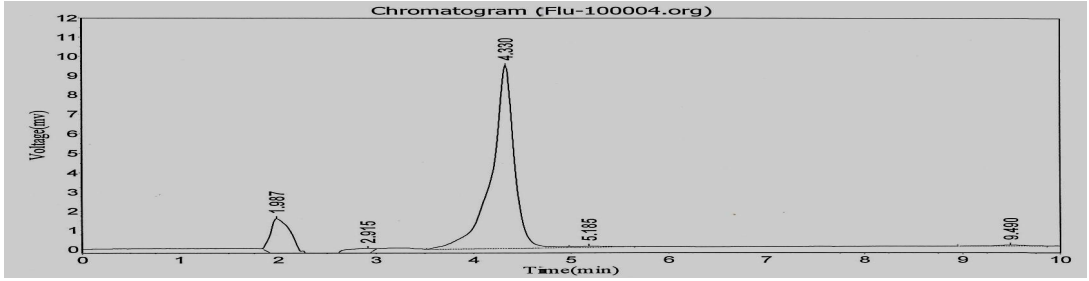


Fig 16: Trial 2-Interday precision of Fluvastatin sodium

Table 7: Intraday and Interday precision data:

Parameters	Avg peak area of Fluvastatin sodium	SD	%RSD
Concentration	10 µg/ml		
Intraday*	166074	2018.7	1.24
Interday*	162518	407.29	1.56

*Mean average of 3 determinations

v. Assay:

Table 8: Drug Eluted From Microcapsules

Concentration	Peak area of std	Peak area of Sample	% Assay
10	9660.032	9460.010	97.9

LOD and LOQ:

Parameters	Conc (µg/ml)
LOD	5.498
LOQ	1.755

Table 9: LOQ and LOD data

1. Linearity	Slope Intercept Correlation coefficient	1587 1742 0.999
2. Accuracy (% Recovery)	High Middle Low	99.24 99.62 98.72
3. Precision (%RSD)	Intra day Inter day	1.24 1.56
4. % Assay	-	97.9
5. LOQ (µg/ml) LOD (µg/ml)	-	5.498 1.755

Table 10: Cross validation data

DISCUSSION:

The system suitability was assessed by analysis of repeatability, Retention time, Height, Area, Concentration in percentage, theoretical plates of the column, half peak width, and symmetry factor in the each peak. Fluvastatin Sodium was spiked at a concentration of 10 µg/ml in pure drug. It was checked by UV detector. Typical system suitability was given in the Table1. System suitability parameters from chromatogram was interpretated. The interpretated data shows highest peak at retention time was 4.348 minutes with a peak area of 167502.172 mV as showed in the Figure1.

Linearity is the primary specification for a detector used for the quantitative analysis. Concentration range over which the detector response is linearly related to the concentration of drug passing through it. The linearity of Fluvastatin sodium was found to be in the concentration range of 2-12 µg/ml as showed in the chromatogram Figure2 with regression coefficient of 0.999 was observed as showed in the Figure 3. The linearity data was as showed in the Table 2.

The % recovery data was found to be within 98-102 % indicating that method is accurate as showed in the Figure 4, 5 & 6. The recovery data was as showed in the Table 3. Very small variation was found in the retention time with respect to change in mobile ratio, flow rate and wave length detection indicating that method is robust. The robustness data with respect to chromatogram is as shown in the Table 4 & Figure 9 to 12. Very small variation was found in the retention time with respect to change in mobile ratio, flow rate and wave length detection indicating that method is robust as showed in the Figure 7 to 10 and Table 5 & 6. Precision is the indication of reproducible results. The % RSD for Fluvastatin sodium drugs was found to be less than 2, indicating that the method and system was précised as showed in the Figure 12 to 15. The intraday and interday variations are shown in the Table 7. The drug was eluted from the microcapsules dosage form. The % assay of Fluvastatin sodium was found to be 97.9 % as showed in the Table 8. LOD and LOQ was calculated from equation as per ICH guidelines. The LOD and LOQ were found to be 5.4 µg/ml and 1.7 µg/ml respectively as shown in the Table 9. The cross validation data was established as showed in the Table 10.

CONCLUSION:

HPLC method for Fluvastatin sodium was validated as per ICH guidelines. The validation results of accuracy, precision, linearity, LOD, LOQ and robustness were within acceptable limits. The qualitative and quantitative method for Fluvastatin sodium was successfully developed.

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