



DEVELOPMENT & VALIDATION OF STABILITY INDICATING HPLC METHOD FOR THE DETERMINATION OF CHROMATOGRAPHIC PURITY OF FLUVASTATIN SODIUM

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Abstract: A precise and simple HPLC technique was developed and validated for the measurement of Fluvastatin sodium. The statin family of drugs, which include fluvastatin sodium, inhibits the reductase enzyme that breaks down 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA). The drug is used to treat ischemic coronary artery disease and hypertension. Additionally, it is used to treat dyslipidemia and lower "bad cholesterol" levels. The method was used on a C18 column (250 4.6mm 1.d; 5) in isocratic mode with acetonitrile and water as the mobile phases in a 75:25 v/v ratio. The flow rate is 0.6 millilitres per minute, and the wavelength is 242 nm. With a correlation coefficient of 0.9998, the linearity between 10 and 60 microgram per ml was identified. Because the suggested method is straightforward, rapid, accurate, precise, and repeatable, it can be utilised for routine quality control analysis of fluvastatin both pure and tablet dosage forms. The medicine has a retention time of 3.643.

Index Terms : Fluvastatin sodium, HPLC, Hypertension ischemic coronary artery disease, Mobile phase, linearity.

1. INTRODUCTION

Chemically, Fluvastatin sodium (FVS) is [R*, S*-(E)]-7- [3-(4-fluorophenyl)-1-(1-methylethyl)-1H-indol-2-yl]-3, 5 dihydroxy-6-heptenoic acid monosodium salt [1]. It is a lipid-lowering drug. Fluvastatin sodium is a water-soluble medication that lowers cholesterol by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase [2,3]. A review of the literature revealed that Fluvastatin sodium has been quantified in formulations using a variety of techniques, including chiral evaluation in human plasma and serum [4], differential pulse voltammetry in capsules, and HPLC [5]. Due to their capacity to reduce LDL and triglycerides and their capacity to inhibit the enzymes involved in the synthesis of endogenous cholesterol and isoprenoid formation, statins are used as the first-line treatment for primary hyperlipidaemia [6]. Statins may cause gastrointestinal side effects when taken orally, such as indigestion, constipation, diarrhoea, stomach cramps, nausea, and vomiting [7]. enhance the risk of hepatotoxicity (with symptoms of hepatomegaly, jaundice, elevated liver aminotransferase, bilirubin, and prothrombin levels) [8] and diminish systemic absorption [9]. To address these problems and the negative effects of statins taken orally, the transdermal delivery technique was investigated. The development and validation of a novel HPLC method for statins. For detecting incredibly small quantities, this technique is sensitive, suitable, and accurate. For statins, a unique HPLC approach was created and validated. This method is sensitive, appropriate, and accurate for detecting extremely low quantities.

2. DRUG PROFILE

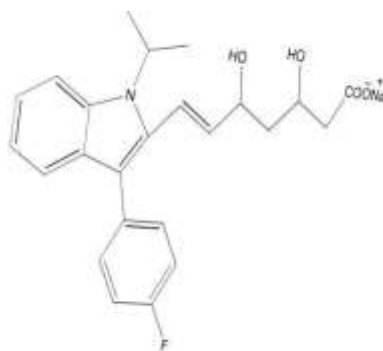


Fig. (1). structure of Fluvastatin sodium(7-[3-(4-fluorophenyl)-1-(1-methylethyl)-1H-indol-2-yl]-3,5-dihydroxy-6-heptenoic acid, sodium Salt).

Molecular weight: 433.45,

Solubility: Freely soluble in methanol, ethanol & water,

Appearance: yellowish, hygroscopic powder, **pKa:-5.5**

Melting point: 195-198°C.

3. MATERIALS & METHOD

Table 3.1 List of chemicals.

Sr. no	Name	Specification	Manufacturer / supplier
1	Orthophosphoric acid	AR Grade	Merck specialities Pvt. Ltd, Mumbai .
2	Methanol	HPLC Grade	Merck specialities Pvt. Ltd, Mumbai .
3	Acetonitrile	HPLC Grade	Merck specialities Pvt. Ltd, Mumbai .
4	Hydrochloric acid	GR Grade	Merck specialities Pvt. Ltd, Mumbai.
5	Hydrogen peroxide	GR Grade	Merck specialities Pvt. Ltd, Mumbai.
6	Potassium dihydrogen phosphate	AR Grade	Merck specialities Pvt. Ltd, Mumbai .
7	Sodium hydroxide pellet	AR Grade	Merck specialities Pvt. Ltd, Mumbai .
8	Milli Q Water	HPLC Grade	Merck specialities Pvt. Ltd, Mumbai .
9	Triethylamine	HPLC Grade	Merck specialities Pvt. Ltd, Mumbai .

Table 3.2 List of Standards.

Sr. No.	Name	Manufacturer
1	Fluvastatin sodium working standards	Ind- swift labs Mohali
2	Fluvastatin sodium working sample	Ind- swift labs Mohali
3	Fluvastatin sodium impurities working standard	Ind- swift labs Mohali

Table 3.3 List of Instruments.

Sr. no.	Name	Model	Manufacturer

1	pH Meter	pH : 5 -10	Eutech instruments
2	HPLC System	SC- 133	Dionex
3	columns	Ultimate -3000	Waters, GL Science, Thermoelectron corporation
4	Analytical macro balance	Kromasil, Hypersil, Interstill , Zorbax, Symmetry	Sartorius
5	Analytical micro balance	CP225D	
6	Water bath	MC215	Julabo
7	UV Spectrophotometer	-	Shimadu

3.1 Characterization of Drugs

The samples of FLS working standard were collected from Ind-shift labs (R&D)Mohali & characterized by observing the following parameter.

3.2. Description: Reddish pale-yellow powder

3.3 Melting Point

A capillary tube fused from one side filled with the drug was installed into the melting point apparatus. The temperature noted at which solid drugs convert into liquid by visual observation. experimental & reported values of the melting point of Fluvastatin were compared.

3.4 Solubility

The solubility of FLS was determined in different solvents. The drug was usually weighed (usually 1g) & dissolved in a known amount of solvent like water, methanol, acetonitrile, benzene, dimethyl sulphoxide, hexene etc.

3.5 pH:

Prepare 1% w/v solution of FLS & pH was measured immediately after the preparation of solution by pH meter.

3.6 Moisture Content

Take about 25 ml of methanol in the flask containing the electrode, then conditioning was done with the Karl Fischer reagent. Then weighted accurately about 500 mg of the sample was added & water content was calculated by using the formula.

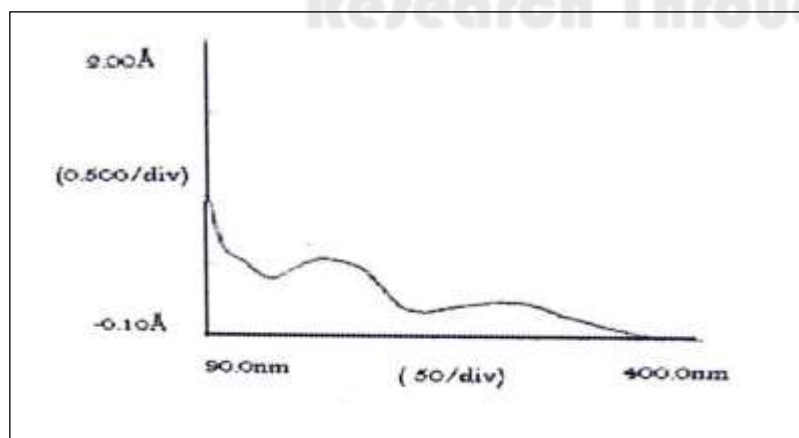
$$\text{Moisture content} = \frac{\text{volume of karl fischer reagent used} \times \text{factor}}{\text{weight of the sample (in mg)}} \times 100$$

Observation

volume of Karl Fischer reagents used = 2.19 ml weight of the sample taken = 500.13 mg Factor = 6.33

3.7 Calculation of λ_{max}

Weigh accurately 25 mg of the drug in a 50 ml volumetric flask & add 30 ml of water to dissolve then make up the volume up to the mark with water. Then take about 1ml of this solution in 50 ml of a volumetric flask & make up the volume to the mark with water. Then the solution was scanned in UV at 400 nm -190 nm.



Observation

UV spectra of FLS

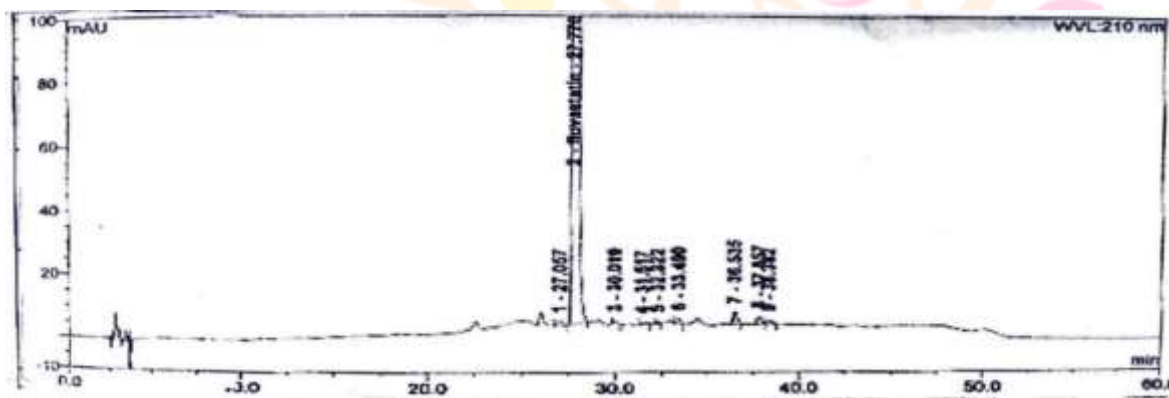
The λ max was found to be at 305.0 nm & 235.5 nm with the absorbance of 0.266 & 0.537 respectively.

4. METHODOLOGY

After taking trials the final method is developed & selected for validation. The final method is as follows:

Reagent Acetonitrile (HPLC grade), Methanol (HPLC grade), Milli Q water (HPLC grade), Potassium dihydrogen orthophosphate

PARAMETERS	CHROMATOGRAPHIC CONDITION
column	Inertsil-ODS (250×4.6 mm); 5 μ m
Flow rate	1 ml / min
wavelength	305 nm
Injection volume	20 μ l
Run time	60 min
Column temp	35 \pm 2 $^{\circ}$ C
Method	Gradient



Chromatogram of Final Method

4.1 Preparation of Solution

4.1.1 Preparation of Mobile Phase A

Weigh accurately 1.36 gm of potassium dihydrogen orthophosphate. Take it into the 1000ml volumetric flask & dissolve it in a small amount of water. Then make up the volume to mark with the water. Adjust the pH to 4.5 with OPA.

4.1.2 Preparation of mobile phase B

The ACN of HPLC grade as mobile phase B

4.1.3 Preparation of Diluents

Mix methanol & water in a ratio of 50:50.

4.1.4 Impurity of stock Solution

Weigh & transfer accurately about 5 mg of each impurity 3-K-5-H, 3-H-5-K, SCA, HD, N-ETA into 10 ml volumetric flask & dissolve in a small amount of methanol & then make up the volume to mark with the diluent.

4.1.5 System suitability solution

Weigh & transfer accurately 50 mg of Fluvastatin sodium reference/working standard in a 100 ml volumetric flask & dissolve in a small amount of methanol & add about 1 ml of impurity stock solution & then make up the volume to mark with the diluents.

(Note: the system suitability solution is stable for up to 1 month if stored in a refrigerator)

4.1.6 Preparation of Standard solution

Weigh & transfer accurately about 25 mg of Fluvastatin sodium, reference / working standard in a 50 ml volumetric flask & dissolve in a small amount of methanol & then make up the volume to the mark with the diluent.

4.1.7 Preparation of Test solution

Weigh & transfer accurately about 25 mg of the test sample in a 50 ml volumetric flask & dissolve in a small amount of methanol & then make up the volume up to the mark with the diluents.

Table 4.1 RT & RF of impurities w.r.t Fluvastatin sodium.

Name of the components	Relative retention time(RRT)	Relative response factor (RRF)	Limit(%)
3-H-5-K	0.35	0.0032	0.10
3-K-5-H	0.70	0.002	0.10
SCA	0.80	0.003	0.10
HD	0.83	0.064	0.10
N-ETA	0.90	1.43	0.10
FLS	1.00	1	1
Fluvastatin anti isomer	1.03	0.02	0.80

5. VALIDATION OF DEVELOPED METHOD

The developed chromatographic method for chromatographic purity of Fluvastatin sodium was found to be adequate so it was subjected to validation.

5.1 Specificity

To assess the peak purity, inject a blank solution followed by a specificity solution. The concentration of the specificity solution of all the impurities was 500 μ l/ ml. All the impurities were injected individually at the method which is developed & their retention time & resolution for each impurity were recorded.

5.2 Limits of Detection & Quantification

The limit of detection & quantification of the impurities were determined by injecting a blank solution & the sample was injected in decreasing concentration. limits of detection of impurities were established by calculating the signal-to-noise(S/N) ratio.it is quoted as the concentration yielding a signal-to-noise ratio of approximately 3:1 in the case of detection &10:1 in the case of quantitation & is confirmed by analysing some samples near this value.

5.3 Linearity

The linearity of an analytical method is to ability to elicit test results that are directly proportional to the concentration of analyte in the sample within the given range. The different conc. ranging from 80-120% of drug substances (40, 45,50,50,55,60 μ g/ml) were injected in triplicate. Then mean value of the three concentrations was calculated & a graph between concentration and area was plotted.

Preparation of impurity stock

Weigh & transfer accurately about 25 mg of each impurity in each 50 ml volumetric flask separately & dissolve in a small amount of methanol & then make up the volume to mark with the diluents.

Table 5.2 Linearity.

Conc. (µg/ml)	Dilution	Level	3-H-5-K		3-5-H-K		SCA		HD		N-ETA		FLS		
			Area	Mean	Area	Mean	Area	Mean	Area	Mean	Area	Mean	Area	Mean	
40	0.1 ml of impurity stock in 10 ml	80%	1/1	0.330	0.321	0.062	0.064	15.497	15.496	1.271	1.272	22.226	22.224	209.061	209.062
			½	0.319		0.063		15.487		1.269		22.221		209.055	
			1/3	0.314		0.067		15.504		1.276		22.225		209.070	
45	0.9 ml of impurity stock in 10 ml	90%	2/1	0.361	0.357	0.068	0.073	17.451	17.454	1.431	1.432	24.968	24.973	235.185	235.195
			2/2	0.354		0.076		17.456		1.428		24.978		235.195	
			2/3	0.356		0.075		17.452		1.437		24.973		235.204	
50	1.0 ml of impurity stock in 10 ml	100%	3/1	0.407	0.398	0.082	0.081	19.598	19.591	1.593	1.589	27.663	27.632	261.523	261.521
			3/2	0.392		0.077		19.589		1.586		27.628		261.517	
			3/3	0.395		0.084		19.586		1.588		27.635		261.523	
55	1.0 ml of impurity stock in 10 ml	110%	4/1	0.469	0.431	0.098	0.089	21.296	21.291	1.754	1.751	30.546	30.547	288.109	288.112
			4/2	0.463		0.077		21.284		1.748		30.541		288.113	
			4/3	0.475		0.084		27.293		1.571		30.554		288.114	
60	1.0 ml of impurity stock in 10 ml	120%	5/1	0.469	0.469	0.098	0.097	23.363	23.369	1.907	1.901	33.354	33.346	312.945	312.943
			5/2	0.463		0.096		23.368		1.897		33.345		312.949	
			5/3	0.475		0.097		23.370		1.899		33.339		312.935	

5.4 Robustness study

The robustness study was done by changing the parameters like column, pH, flow rate, and column temperature. The sample was prepared according to the method which was developed. By changing these parameters, the change in the retention time & asymmetry of the peak was observed.

5.5 Recovery studies Impurities stock solution

Weigh & transfer accurately about 25 mg of each impurity in each 50 ml volumetric flask separately & dissolve in a small amount of methanol & then make up the volume to the mark with the diluents.

Table 5.3 Recovery of 3-Hydroxy-5-keto Fluvastatin.

Level	Dilution	Injection	Area of standard	Mean	Area of impurity in the sample	Area of the spiked sample	% Recovery
80%	0.8 ml in 10 ml	1/1	0.334	0.331	0.064	0.383	103.63
		1/2	0.328			0.379	102.42
		1/3	0.331			0.381	103.02
100%	1.0ml in 10 ml	2/1	0.401	0.402		0.452	102.49
		2/2	0.406			0.457	103.73
		2/3	0.398			0.450	101.99
120%	1.2 ml in 10 ml	3/1	0.475	0.473	0.522	101.90	
		3/2	0.474		0.512	101.85	
		3/3	0.469		0.514	99.79	
Mean							102.31
S.D							1.18
% R.S.D							1.15

Table 5.4 Recovery of 3-keto -5-Hydroxy Fluvastatin.

Level	Dilution	Injection	Area of standard	Mean	Area of impurity in the sample	Area of spike in the sample	% Recovery
80%	0.8ml in 10 ml	1/1	0.065	0.063	0.008	0.070	98.41
		1/2	0.062			0.060	95.24
		1/3	0.061			0.066	92.06
100%	1.0 ml in 10 ml	2/1	0.083	0.079		0.085	97.47
		2/2	0.071			0.083	94.94
		2/3	0.084			0.081	92.41
120%	1.2 ml in 10 ml	3/1	0.104	0.098	0.104	97.96	
		3/2	0.097		0.099	92.86	
		3/3	0.093		0.109	103.06	
Mean							96.05
S.D							3.56
%R.S.D							3.71

6. FORCED DEGRADATION STUDY Blank solution Diluents as a blank solution

Control sample Weigh & transfer accurately about 25 mg of the test sample in a 50 ml volumetric flask & dissolve in a small amount of methanol & then make up the volume to mark with the diluent.

Forced degradation by UV degrade about 500 mg sample of Fluvastatin into UV for 72 h & take 50 mg of the sample into 100 ml volumetric flask & dissolve into small amount of methanol & than make up the volume up to the mark with the diluents.

Thermal degradation Degradation about 500 mg sample of Fluvastatin into the oven at 80 °c for 72 hours & take 50 mg of the sample into a 100 ml volumetric flask & dissolve it in a small amount of methanol & then make up the volume to mark with the diluents.

Humid degradation Degrade about 500 mg of sample of Fluvastatin into Petri dish & the dish is placed into the sodium sulphate solution which also placed in a oven at 60 °c for 72 h& take 50 mg of the sample into 100 ml volumetric flask & dissolve in small amount of methanol & make the volume with the help of diluents.

Forced degradation by visible light Degrade about 500 mg sample of Fluvastatin into visible light for 72 h& take 50mg of the sample into 100 ml volumetric flask & dissolve in small amount of methanol & than make up the volume up to the mark with the help of diluents.

Acid degradation Take about 50 mg of sample & 0.5N HCL & keep for 30 min, than dissolve in small amount of methanol & then make up the volume up to the mark with the help of diluents.

Base degradation Take about 50 mg of the sample & 0.5N NaOH & keep for 30 min, than dissolve in small amount of methanol & then make up the volume up to the mark with the diluents.

Peroxide degradation Take about 50 mg of the sample & add 6% hydrogen peroxide & keep for 30 min, then dissolve in small amount of methanol & than make up the volume up to the mark with the diluents.

Table 6.1 Degradation study of Fluvastatin sodium.

Degradation type	% Purity	% Degradation	RRT of the major degradation peaks obtained from degradation parameter.	Peak purity at 10% of the peak height
Control sample	99.21	0.79	No peak was observed	995
Thermal (80 °c, 72 h)	99.13	0.87	0.92	987
Humid (60 °c, 72h)	98.90	1.10	0.61,0.63,0.83,0.85,0.86,0.93,0.96.	969
UV light (72 h)	63.52	36.48	0.63, 1.07	981
Visible (72 h)	98.65	1.35	0.83,0.85,0.86,0.92,1.06	975
Acid treatment (5.0 N HCL, 30 min)	59.28	40.70	1.02	989
Base treatment (5.0 N NaOH, 30 min)	97.74	2.26	No peak was observed	996

Peroxide treatment (6.0% H ₂ O ₂ ,30 min)	98.92	1.08	0.84, 0.86	979
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7. RESULTS

7.1 Results of characterization

The melting point & pH of the Fluvastatin was found to be 193°C -196°C & 8.32 respectively. It was found that the Fluvastatin sodium was freely soluble in water, ethanol, methanol & the moisture content of Fluvastatin was found to be 2.77%. From the mass spectra & FT-IR, it is confirmed that the given compound is Fluvastatin sodium & an intense yellow colour in the flame shows the confirmation of sodium. The λ max of Fluvastatin sodium was found to be 305.0 nm.

7.2 Results of Method Development

Table 7.1 RRT & RRF of Impurities.

Name of the component	Relative retention time (RRT)	Relative response factor (RRF)	Limit (%)
3-H-5-K	0.35	0.032	0.10
3-K-5-H	0.70	0.002	0.10
SCA	0.80	0.003	0.10
HD	0.83	0.064	0.10
N-ETA	0.90	1.43	0.10
FLS	1.00	1	1
Fluvastatin antiser	1.03	0.02	0.80

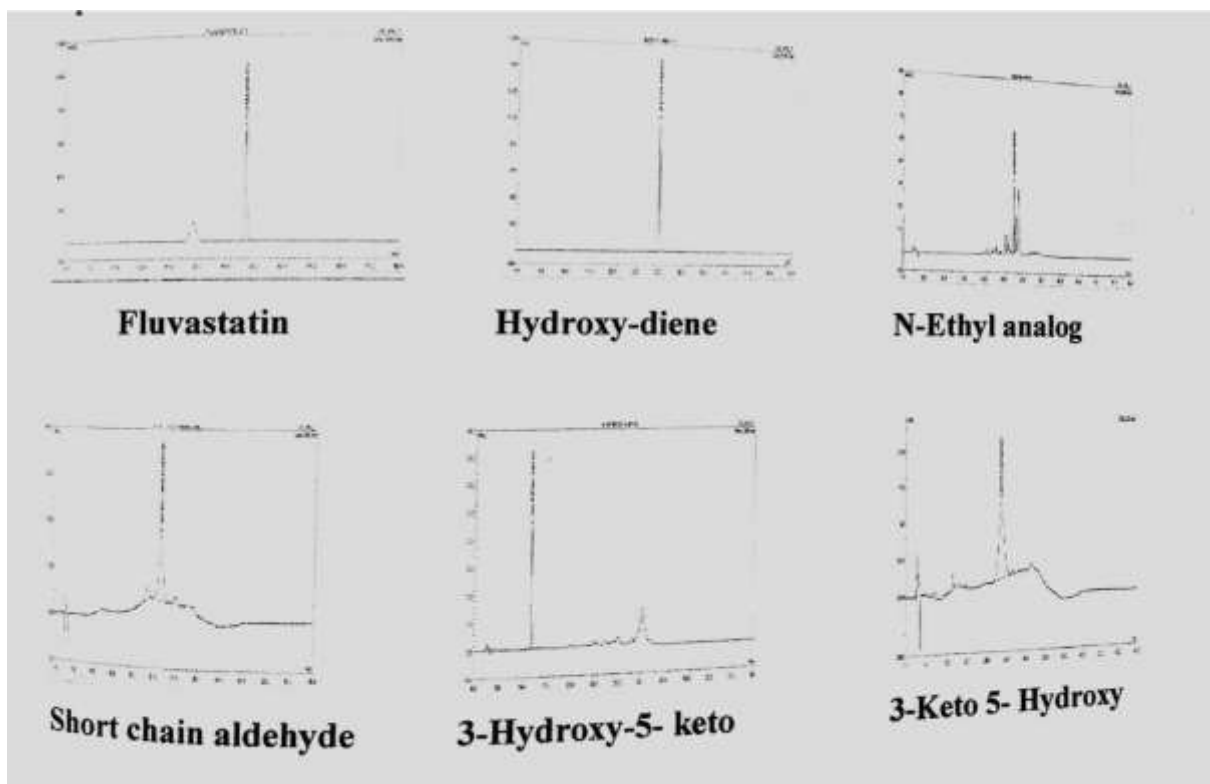


Fig. (2). Specificity Chromatograms of specificity

Table 6.2 Specificity.

Name of the compound	RT	RRT	Peak purity	Acceptance criteria
3-H-5-K	11.63	0.35	992	All the peaks should be resolved
3-K-5-H	23.75	0.70	987	
SCA	26.75	0.80	983	
HD	27.61	0.83	994	
N-ETA	30.09	0.90	986	
Fluvastatin sodium	33.38	1.00	998	

Table 6.3 LOD & LOQ.

Sr No.	Impurity	LOD ($\mu\text{g/ml}$)	LOQ ($\mu\text{g/ml}$)
1	3-H-5-K	0.56	2.5
2	3-K-5-H	0.67	3.1
3	SCA	0.71	2.7
4	HD	0.63	2.5
5	N-ETA	0.23	0.91
6	Fluvastatin sodium	0.5	2.5

Linearity Graph

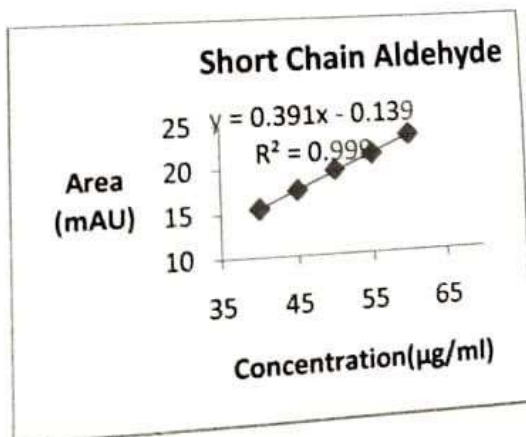
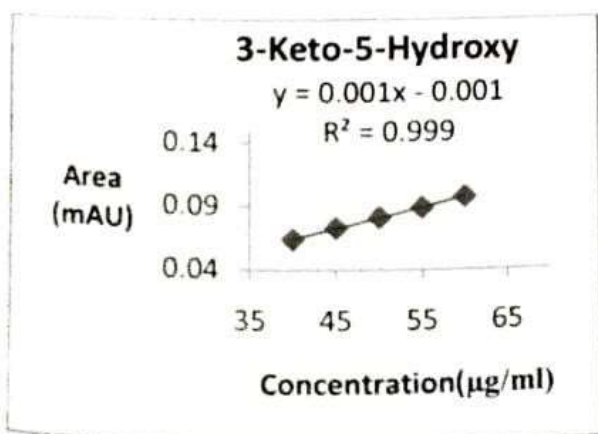
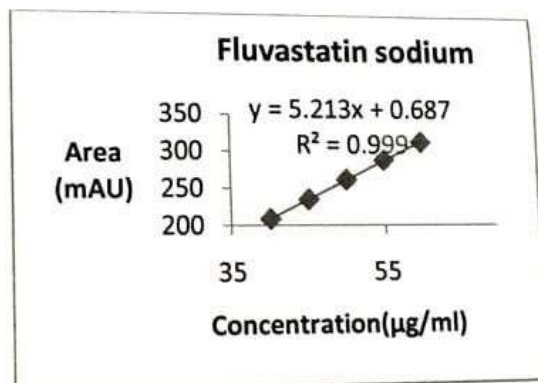
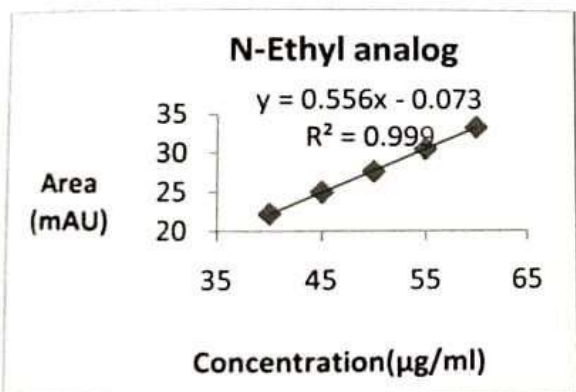
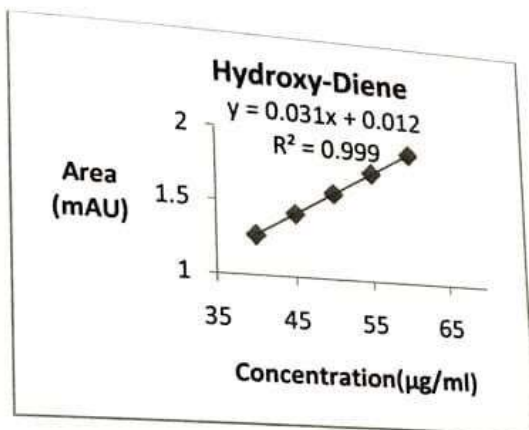
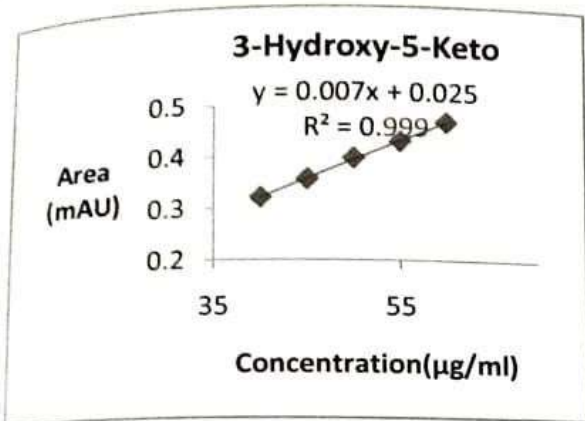


Table 6.4 linearity results

Impurity	slope	intercept	r ²	Acceptance criteria
3-H-5-K	0.0074	0.0252	0.9992	The r ² should not be less than 0.999
3-K-5-H	0.0016	0.0012	0.9994	
SCA	0.3916	0.1392	0.9991	
HD	0.0315	0.0120	0.9999	

Table 6.5 Recovery results

Sr no.	Name of the component	% Recovery (mean)	SD	RSD	Acceptance criteria
1	3-H-5-K	102.31	1.18	1.15	% recovery should be with in range of 90% -110%
2	3-H-5-K	96.05	3.56	3.71	
3	SCA	100.01	0.08	0.08	
4	HD	99.02	0.54	0.54	
5	N-ETA	99.95	0.04	0.04	

CONCLUSION

The methods which are available in the literature are not adequate & reliable. In the literature, there is no stability indicating the method is available for the analysis of chromatographic purity. So a simple, precise, accurate, routine reversed-phase HPLC method was developed & validated for simultaneous analysis of five impurities of Fluvastatin sodium, viz., 3-hydroxy-5-keto, 3-keto-5-hydroxy, short chain aldehyde, hydroxyl-diene, N-ethyl analogue & validation of the developed method was performed according with the parameters as per ICH guidelines including specificity, linearity, system precision, robustness & recovery. After the collection of the sample, characterization of the sample was done to confirm the given sample was Fluvastatin sodium. For the conformation melting point, pH, and solubility were done. Spectral study (UV, IR, Mass) was also done. From the characterization results we can conclude the given sample is Fluvastatin sodium. After taking various trials the final method is selected. Chromatographic separation of the drug was performed at ambient temperature on a C₁₈ column stationary phase with gradient elution with a mobile phase consisting of a mixture of phosphate buffer 0.01M, pH 4.5 & acetonitrile, at a flow rate min 1 ml/ min. The detection was at 305nm. The retention time of 3-Hydroxy-5-keto Fluvastatin, 3-keto-5-hydroxy Fluvastatin, Fluvastatin short-chain aldehyde, Fluvastatin hydroxyl diene, Fluvastatin N-Ethyl analogue, Fluvastatin sodium was found to be 11.63, 23.75, 26.76, 27.61, 30.09 & 33.58 respectively. The suitability of this HPLC method for quantitative analysis of the drug was proved by validation in accordance with International Conference for Harmonization (ICH) guidelines. In the specificity chromatograms, all the peaks are well separated & linearity, system precision, robustness study, and recovery studies were done. Since the validation is within the acceptance criteria for all validation parameters, therefore, the method is considered as validation & suitable for the analysis of chromatographic purity of Fluvastatin sodium. The degradation study was done to support the suitability of the analytical method as stability indicating & to optimize the storage condition of the drug. Through the degradation study intrinsic stability of the Fluvastatin sodium was evaluated. In this, the sample was degraded under different conditions like UV, Visible, Humid, thermal, acid, Base, and peroxide & then the sample was evaluated by the method developed. After the degradation study, the peak was not arising at the RRT of the 3-Hydroxy-5-keto Fluvastatin, 3-keto-5-hydroxy Fluvastatin, Fluvastatin short-chain aldehyde,

Fluvastatin hydroxyl diene, Fluvastatin N-Ethyl analogue, Fluvastatin sodium so the method is stability indicating. Hence we conclude that the developed methods are simple reliable & adequate. The developed analytical methods are simple, reliable & adequate. The developed analytical methods find importance in various fields like research institutions, quality control departments in industries, approved testing laboratories, bio-pharmaceutical & bio-equivalence studies & clinical pharmacokinetic studies.

LIST OF ABBREVIATIONS CONSENT FOR PUBLICATION

Not applicable.

FUNDING

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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