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Development and Validation of Stability Indicating RP-HPLC Method for Estimation of Fluvastatin Sodium in Bulk and Capsule Dosage Form

Ashok H. Akabari
C. K. Pithawalla Institute of Pharmaceutical Science and Research, INDIA
Bhanubhai N. Suhagia
Dharamsinh Desai University, INDIA
Mahesh G. Saralai
C. K. Pithawalla Institute of Pharmaceutical Science and Research, INDIA
Vishnu A. Sutariya
Shree Dhanvantary Pharmaceutical Analysis and Research Centre (SDPARC), INDIA

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ABSTRACT
A sensitive, specific and stability-indicating reversed phase high performance liquid chromatography-diode array detection method was developed for the quantitative determination of fluvastatin sodium in the presence of its degradation products. The chromatographic separation was performed on a Phenomenex Luna C18 column (150 X 4.0 mm, 5µm) in isocratic mode using acetonitrile and 0.02M potassium phosphate buffer (50 + 50, v/v, pH 5.0 adjusted with potassium hydroxide) as the mobile phase at a flow rate of 1.0 ml/min. The quantification was performed with a photodiode array detector at 235nm based on peak area. The method showed good linearity over the concentration range of 5-40 µg/mL with a detection limit of 1.1µg/mL and quantification limit of 3.3µg/mL. The proposed LC method was used to investigate the kinetics of acidic and oxidative degradation of fluvastatin sodium. The acidic and oxidative degradation had shown an apparent first-order kinetics and rate constants were found to be 0.0191µg/mL/min and 0.0048µg/mL/min, respectively.
Keywords: fluvastatin, kinetic study, stability indicating method, anti hyperlipidemic agent, development and validation

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 substance inhibit, by competition, the 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-Co A), thus preventing this substance from catalyzing the conversion to mevolanate and consequently inhibiting the first stage in cholesterol biosynthesis in humans [1].

Correspondence: Ashok H. Akabari, *C.K.Pithawalla Institute of Pharmaceutical Science and Research, India.*
ashokakabari@yahoo.com

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. FVS 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 FVS 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 FVS in bulk and pharmaceutical dosage form has been reported [22].

To date, all analytical methods described in the literature for the determination of FVS in biological fluids such as plasma involve voltammetric techniques, CE, HPLC, LC-MS and GC-MS methods and require a tedious procedure for sample pre-treatment. The kinetic spectrophotometric method was based on the formation of colored product between FVS and 4-chloro-7-nitrobenzofurazan (NBD-Cl) in acetone medium at 55 ± 2^{o}C. The reaction was followed spectrophotometrically by measuring the increase in absorbance at 462 nm as a function of time [8]. Mielcarek et al. has been studied a photodegradation of fluvastatin in methanol or water, in accordance with the ICH recommendations of the first version by HPTLC [21]. Gomes et al. developed the stability indicating HPLC method for determination of FVS in pharmaceutical [17]. Gomes et al. studied limited stress condition like acidic, neutral, alkaline and chemical oxidative degradation was discussed [17]. Gomes et al. was not studied for the specificity, peak purity or correlation between spectra of standard and test FVS in the presence of the degradation product that are likely to be present as an impurity in their formulations. Literature survey reveals that there is no stability-indicating RP-HPLC method has been reported to study the effect of temperature and photostability of FVS and for the systematic degradation of FVS in acidic and oxidative conditions. Moreover, kinetic studies and accelerated stability experiments are important to solve problems encountered in quality control and to predict the expiry dates of pharmaceutical products. Since literature cites that water or methanol plays a role in photodegradation of fluvastatin, therefore the original stock solution of fluvastatin was prepared in water and immediately diluted by mobile phase (acetonitrile and phosphate buffer mixture) [21].

Nowadays, photo stability studies are an integral part of the drug development process and are widely recognized as one of the most important procedures in the registration of pharmaceutical products [23]. Knowledge of the photochemical and photophysical properties of the compound is necessary for appropriate handling, packaging and labelling the drug substance and drug product [24]. Radiation has two main effects on the drugs. The first is the influence of light on the stability of the drug substances and drug formulations. The second aspect of drug–light interactions is that of the biological effects caused by the reaction of drugs,

photoproducts or metabolites of drugs with light and biomolecules, resulted in drug induced photosensitivity [25-26].

Most of the degradation reactions of pharmaceuticals occur at finite rates and are chemical in nature. These reactions are affected by conditions such as solvent, concentration of reactants, temperature, pH of the medium, radiation energy, and the presence of catalysts. The order of the reaction is described based on the reaction rate and the concentration of the reactant. The degradation of most pharmaceuticals can be classified as zero order, first order, or pseudo first order. Thus, kinetic studies of the decomposition of drugs using stability testing techniques are essential for the quality control (QC) of such products. Therefore, kinetic determinations of acid degradation and oxidative degradation were carried out to predict the expiry date of the drug [27-29].

The purposes of study were, 1) To develop and validate more sensitive, short analysis time, specific and fully stability indicating RP-HPLC-DAD method for determination of FVS in the presence of its degradation products according to ICH guidelines. 2) To obtain more detail knowledge on the degradation kinetics of FVS in acidic and oxidative conditions. Furthermore, the developed method was used to investigate the kinetics of the FVS in acid and oxidative degradation at different temperatures. The kinetic parameters, such as apparent order degradation rate constant (k), half-life ($t_{1/2}$), and energy of activation for FVS were calculated. Determine the shelf life, t_{90} for the FVS in acidic and oxidative conditions, assuming that the product is satisfactory until at the time at which it has decomposed to 90% of its original concentration (i.e., 10% of drug remain) at different temperatures. The proposed method has several advantages over published methods, including high sensitivity and wider linearity range of the proposed method, whereas the calibration range of the proposed method is 5- 40 µg/ml while the calibration range of the published HPLC method [17] was 12- 28 µg/ml. Also, the proposed method can be used for quantitative determination of degradation product FVS during the stability study with kinetic investigation. The proposed method is studied for the specificity and the effect of light, temperature was discussed.

EXPERIMENTAL

Materials and Reagents

Fluvastatin sodium was supplied by Intas pharmaceutical ltd. Ahmedabad, India. HPLC grade acetonitrile and water, hydrogen peroxide, ortho phosphoric acid and potassium dihydrogen phosphate was procured from Merck, Mumbai, India.

Apparatus and Chromatographic Conditions

The liquid chromatography instrument (Shimadzu technologies, Kyoto, Japan) was equipped with pump, photo diode array (PDA) detector, degasser, auto sampler and class-VP chromatography software. Separation and quantitation were made on a Phenomenex Luna C_{18}

column (5µm particle size, 150 x 4.6 mm id). The detection was performed at 235 nm, where the maximum absorption was observed.

The mobile phase was prepared by mixing of acetonitrile and 0.02M phosphate buffer with pH adjusted to 5.0 with potassium hydroxide, in ratio of 50:50, v/v, filtered through a 0.45µm nylon membrane filter (Rankem, New Delhi, India) and degassed before use. The flow rate was 1.0 ml/min and the injection volume was 20 µL.

Preparation of stock solution

Stock standard solution of FVS (1 mg/mL) was prepared in water. An aliquot of this solution was diluted in the mobile phase to obtain a standard working solution of 100 µg/mL FVS.

Method Validation

Validation of the developed LC method was carried out as per the International Conference on Harmonization (ICH) guidelines Q2 (R1) [29-33].

Specificity

The specificity of the HPLC method was evaluated to ensure that there was no interference from the excipients contained in the pharmaceutical product or from products resulting from forced degradation products. The specificity of the method was established by comparing the chromatogram of FVS standard and FVS sample solution of the capsule dosage form. Solutions containing 1 mg/mL of the FVS were prepared in water for the stress degradation studies. The samples for degradation studies were prepared by transferring 5 ml solution of FVS (1 mg/ml) to four different 50 mL volumetric flask. Sodium hydroxide solution (5 ml, 0.1M), hydrochloric acid solution (5 ml, 0.1M HCl and 1M HCl) and hydrogen peroxide solution (5 ml, 3%H_2O_2) were added in separate flasks for alkaline hydrolysis, acidic hydrolysis and oxidative degradation, respectively. The solutions were heated at 70^{o}C for 120 min in thermostatically controlled water-bath. Aliquots were withdrawn after 120 min cooled to room temperature, and neutralized (for acid and base induced degradation), appropriately diluted with mobile phase to obtain a final concentration of 10 µg/ml of FVC and analyzed by HPLC method.

Pure solid drug (in a 1 mm thick layer in a petri plate) was exposed to dry heat at 80°C in an oven for 24 h to study the thermal stability of the drug. To study photostability, FVS was exposed to direct sunlight for 24 h. Samples after 24 h were transferred to stoppered volumetric flask and diluted with mobile phase and analyzed by HPLC method. The stress degradation was performed in the dark to exclude the potential degradation effect of light. The peak purity determination was performed with a PDA detector, that the analyzed chromatography peak did not contain more than one substance. Peak purity of FVS was assessed by comparing acquired spectra at the peak start (S), peak apex (M) and peak end (E).

Linearity and Range

The standard working solution (100 µg/ml) was diluted with the mobile phase to prepare sample solutions in the concentration range of 5.0–40.0 µg/ml FVS. The linearity and range of the method were determined by plotting a calibration curve over the concentration range of 5.0–40.0 µg/ml. The calibration curve was plotting by peak areas of FVS against concentrations of FVS and the regression equation was calculated.

Precision

The repeatability was evaluated at the concentration of 20 µg/ml FVS solution six times on the same day and under the same experimental conditions. The intraday precision was evaluated at three different concentrations 10, 20 and 40 µg/ml of FVS by estimating the corresponding responses three times on the same day and under the same experimental condition. The interday precision was evaluated at three different concentrations of 10, 20 and 40 µg/ml of FVS by estimating the corresponding responses three times on the three consecutive days and under the same experimental condition. The results were reported in terms of percentage RSD.

Accuracy (% Recovery)

The accuracy of the method was studied at three concentrations of FVS, i.e., recovery studies (80, 100 and 120%). Known amounts of standard solutions of FVS (8, 10, and 12 µg/ml) were added to a pre-quantified sample solution of FVS (10µg/ml). All solutions were prepared in triplicate and analyzed. The percentage recovery of spiked drug was calculated from the regression equation.

LOD and LOQ

Limit of detection *(L*OD) and Limit of quantitation (LOQ) of FVS for the developed method were calculated using equation, LOD = 3.3 ´ s/S and LOQ = 10 ´ s/S, where s is the average standard deviation of the response and S is the mean slope of the calibration curve.

Robustness

The robustness of the method was evaluated by changing the composition of the mobile phase, flow rate, pH of the mobile phase and column oven temperature over small ranges.

System suitability test

The system suitability tests were performed to prove that the resolution and repeatability of the HPLC system were adequate for the analysis intended. The system suitability test was carried out using six replicate injection of a standard solution containing 10µg/mL of FVS. The parameters measured were FVS retention time, chromatographic peak symmetry (tailing factor) and theoretical plates.

Analysis of capsule dosage form

To prepare a sample solution, powder content of 20 capsules (Lescol- 20 mg FVS) were weighed. The amount of powder equivalent to 100 mg of FVS was transferred in to 100ml of volumetric flask, 60ml water is added. The resulting solution was sonicated for 15 min. It was diluted to the mark with water and filter through 0.45µm nylon membrane filter. An appropriate aliquot was diluted to the mark with mobile phase to obtain final solution containing 10µg/mL of FVS and analyzed by HPLC.

Kinetic Investigation

Kinetic investigation of FVS in acidic degradation

The kinetics of the acid degradation of FVS was evaluated in 0.1 M HCl. Solutions containing 1 mg/mL of the FVS were prepared in water. An appropriate aliquot of these solutions were transferred into separate stoppered volumetric flasks, and diluted with 0.1 M HCl to give a final concentration of 100µg/ml FVS. The flask were placed in a thermostatic oven at different temperatures (50, 60, 70 80°C) for different time intervals of 30 min, 60 min and 120 min. After the specified time, 1 ml aliquots taken were transferred to a 10 mL volumetric flask and immediately cooled to room temperature and neutralized with 0.1 M NaOH was checked by using pH strip and these solutions were diluted with mobile phase. These solutions were injected in the HPLC system and the concentration of the remaining FVS was calculated at each temperature and time interval.

Kinetic investigation of FVS in oxidative degradation

The kinetics of the oxidative degradation of FVS was evaluated in 3% H_2O_2. Solutions containing 1 mg/mL of the FVS were prepared in water. An appropriate aliquot of these solutions were transferred into separate stoppered volumetric flasks, and diluted with 3% H_2O_2 to give a final concentration of 100µg/ml FVS. The flask was placed in a thermostatic oven at different temperatures (50, 60, 70 80°C) for different time intervals of 30 min, 60 min and 120 min. After the specified time, 1 ml aliquots transferred to a 10 ml volumetric flask and immediately cooled to room temperature and this solution was diluted with mobile phase. These solutions were injected in the HPLC system and the concentration of the remaining FVS was calculated at 70°C temperature and each time interval.

The kinetic determinations were performed in the dark to exclude the possible degradation effect of light. The first-order kinetics plots is ln of concentration versus time and the kinetic parameters, such as the apparent order degradation rate constant (k), half-life (t1/2) and energy of activation were determined..

RESULTS AND DISCUSSION

Method development and optimization

The main criterion is to develop successful HPLC method for the determination of FVS and its degradation product. The developed method should be able to determine FVS and its degradation product in a single run. This method should be accurate, reproducible, robust, stability indicating, free of interference from degradation products and straight forward enough for routine use in quality control laboratory.

Regulatory agencies recommend the use of stability-indicating methods for the analysis of stability of samples. Thus, stress studies are required in order to generate the stressed samples, method development, and method validation [29-33]. In order to separate FVS and degradation products produced under stress conditions, different mobile phases were used and adjusted to obtain a rapid and simple assay method with a less retention time, reasonable run time, and a sharp peak. Methanol, water and acetonitrile were evaluated in a different ratio to obtain a sharp peak of FVS, well resolved from its degradation products. When only acetonitrile and water were evaluated in a different ratio, retention time of fluvastatin reduced but satisfactory resolution was not achieved as well as the peak was broad with tailing. These problems were solved when water was replaced with phosphate buffer solution. Since the buffer forms an ion pair with free silanol groups of the column, it reduces the tailing. Buffer pH played a major role in separating all the degradation products of FVS. Further, as the pKa of fluvastatin is 4.56, the probability of the drug remaining in the unionized form in acidic pH is higher, which in turn has an effect on peak shape and retention time (Rt). The final mobile phase pH was 5 which are adjusted with potassium hydroxide solution. Improved peak shape, decrease in tailing, and reproducible response was observed at pH 5. The resolutions among all degradation products were improved. Therefore, a retention time between 4 and 5 min was chosen for the drug since it allowed both a rapid analysis of the drug, which is important for routine analysis, and a complete drug separation from its degradation products.

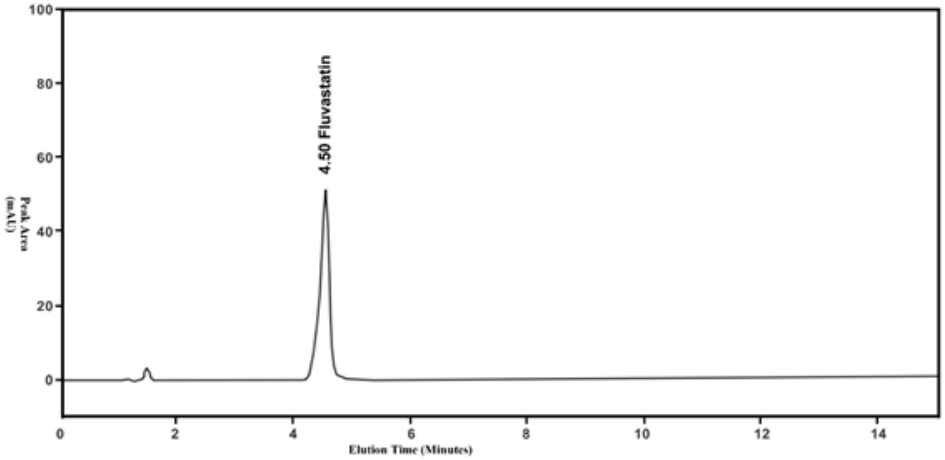

Figure 1. Liquid chromatogram of standard fluvastatin sodium at 235nm

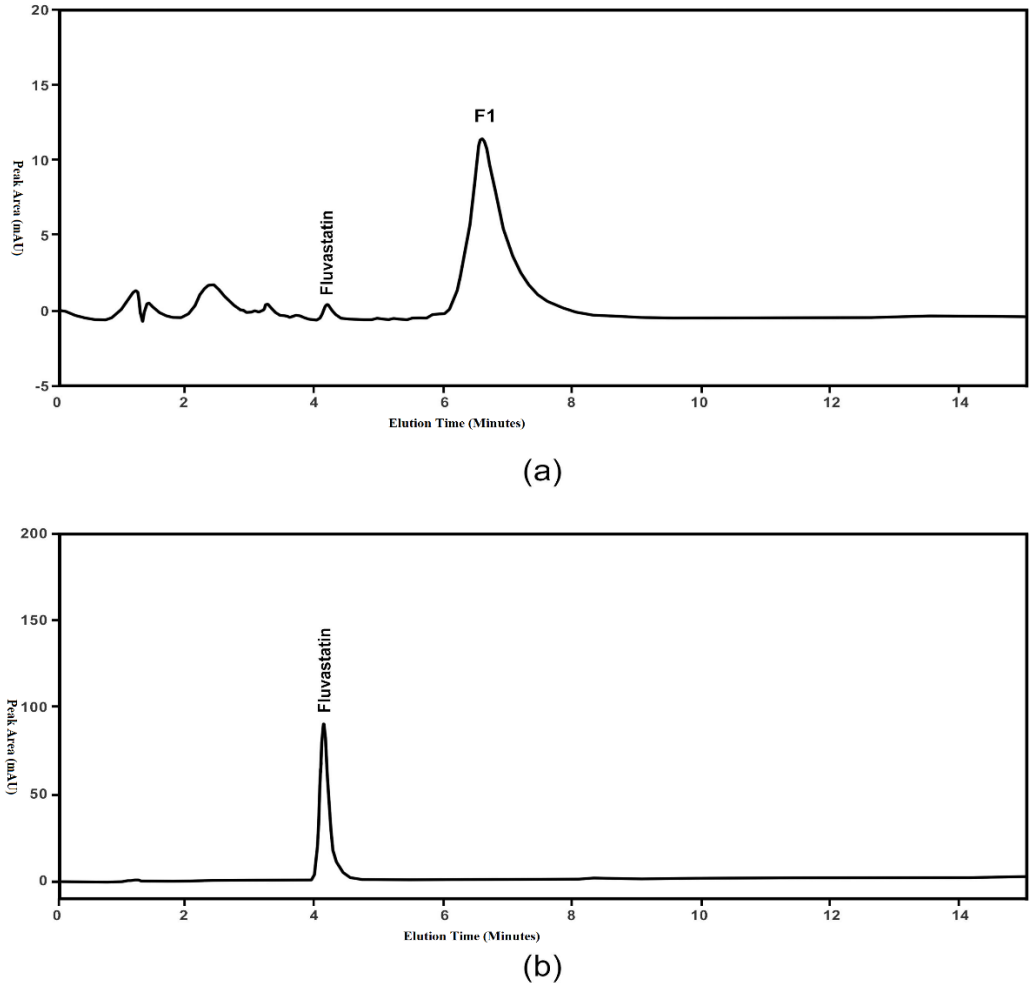

Figure 2. Chromatograms obtained of FVS at 70 °C. (a) Acid Hydrolysis, 1M HCl 30 mins, Major degradation product F1, (b) Alkaline Hydrolysis, 0.1M NaOH 120 mins

A satisfactory separation with good peak symmetry and steady baseline was achieved by using 0.02M phosphate buffer (pH 5.0 adjusted with potassium hydroxide) and acetonitrile (50 + 50, v/v) as mobile phase and flow rate was 1.0 ml/min. The retention time of FVS was 4.50 ± 0.05 min (**Figure 1**). This mobile phase gave good resolution for the separation of FVS and its degradation products and was selected for the proposed stability-indicating method. The optimized conditions of the HPLC method were validated for the analysis of FVS in capsule formulations and application for QC. **Figure 1** shows a typical chromatogram obtained by the proposed RP-HPLC method, demonstrating the resolution of the symmetrical peak corresponding to FVS. The retention time observed (4.5 min) allows a fast determination of the drug, which is suitable for QC laboratories.

Method Validation

Specificity

Forced degradation or stress testing is undertaken to demonstrate specificity when developing stability-indicating methods, particularly when little information is available about potential degradation products. The ICH guideline entitled "Stability Testing of New Drug Substances and Products" requires stress testing to be carried out to elucidate the inherent stability characteristics of the active substances [33].

The chromatographic peak purity index for fluvastatin in presence of degradation products resulting from stress conditions was higher than 0.9999. These results indicated that the proposed method is specific and stability-indicating, and can be applied for stability studies and QC analysis of FVS in pharmaceutical products, with advantages when compared to the previously published methods.

The acid hydrolysis was carried out in 0.1 M HCl and 1M HCl at 70°C for 120 min and 30 min respectively. FVS is unstable and degrades rapidly (approximately 45%/h) when exposed to acidic conditions in 0.1MHCl. The decomposition rate of FVS in 1 M HCl was fast compared to 0.1MHCl indicate that the concentration of HCl increase decomposition rate of FVS also increases. Drug recovery at levels of 10.1% of the acid hydrolysis sample at 70°C in 0.1 M HCl (**Figure 3(a)**) and negligible amount recovered in 1M HCl (**Figure 2(a)**).

The alkaline degradation was carried out in 0.1 M NaOH at 70°C for 2 hr and the percentage of drug decomposition was found to be 61.2%. During alkaline degradation condition decrease in the original drug peak area was observed and no additional peaks were observed in the chromatogram (**Figure 2(b)**).

The oxidative degradation was carried out in 3% H_2O_2 at 70°C for 2 hr and the percentage of drug decomposition was found to be 43%. During oxidative degradation condition decrease in the original drug peak area was observed and no additional peaks were observed in the chromatogram (Figure 4(c)). The FVS has undergone thermal and photolytic degradation very slightly i.e. 2.65 and 6.47% respectively, no additional peaks were observed in the chromatogram, suggested significant stability under these conditions compare to acid, alkaline hydrolysis and oxidative conditions. The drug FVS and the degradation product formed under various stress condition showed good resolution and not interfere in the determination FVS. A regular decrease in the concentration of FVS with increasing time intervals was observed in acidic, alkaline hydrolysis and oxidative condition. The degradation study indicated that FVS was found to be susceptible to acid, alkaline hydrolysis and oxidative reaction. The number of the degradation product with their retention time, the content of fluvastatin sodium remained, and percentage recovery is listed in **Table 1.**

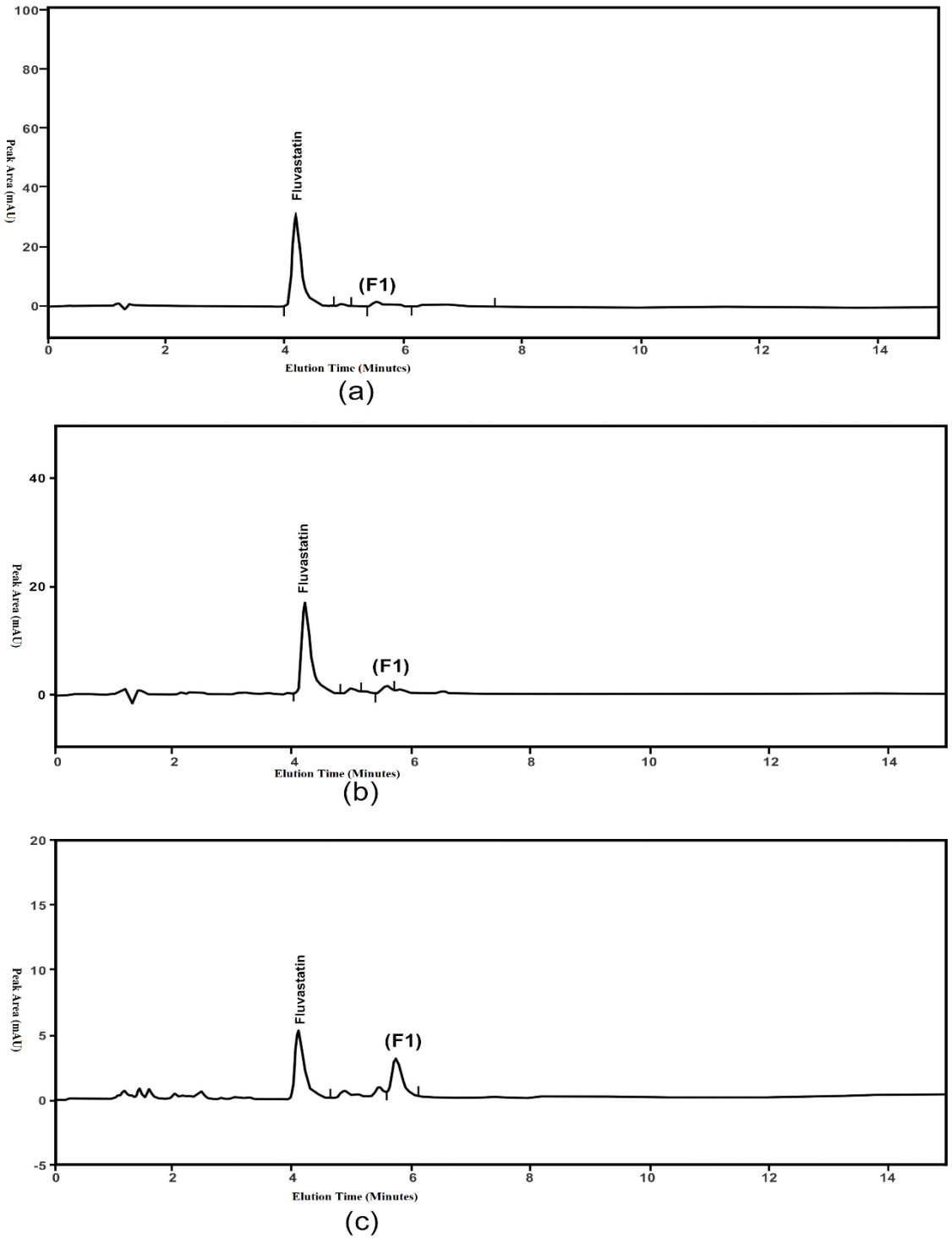

Figure 3. Chromatograms obtained of FVS at 70 °C. (a) Acid Hydrolysis, 0.1M HCl 30 mins, Major degradation product F1, (b) Acid Hydrolysis, 0.1M HCl 60 mins, Major degradation product F1, (c) Acid Hydrolysis, 0.1M HCl 120 mins, Major degradation product F1

Table 1. Summary of Forced Degradation Studies

Condition	Time	% Drug remain unchanged	Retention time of degradation product
0.1M HCl (70°C)	2 hr	10.1%	6.54min
0.1M NaOH (70°C)	2 hr	38.80%	
3% H2O2 (70°C)	2 hr	57.0 %	
Dry Heat (80°C)	24 hr	97.92 %	
Direct sun light (photolysis)	24 hr	94.32 %	

Table 2. Accuracy study of proposed method

Amount of Drug Taken (mg)	Amount of drug added (mg)	% Recovery ± SD[a]	% RSD
10	0	100.47 ± 0.43	0.4291
10	8	99.02 ± 0.57	0.5807
10	10	100.14 ± 0.30	0.3001
10	12	101.27 ± 0.601	0.5938

a=Average of three determination at each

Linearity

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. FVS showed linearity over the concentration range of 5-40 µg/mL. Correlation coefficient was found to be 0.999.

Precision

The results of the repeatability, intraday and inter day precision are shown in **Table 4**, respectively. The RSD values for intraday and interday studies were found to be 0.165–0.642% and 0.515–0.754%, respectively. The RSD value for repeatability was found to be 0.44. The RSD values were lower than 2% for repeatability, intraday and inter day precision indicated that the proposed method precise and repeatable.

Accuracy

According to ICH guidelines, the accuracy study was performed by a standard addition method. The mean recovery of FVS was found to be in the range of 99.02 % - 101.27 %, indicating good accuracy of the proposed methods (**Table 2**).

Table 3. Robustness data of proposed method

Parameters	Normal Condition	Change in condition	Change in % RSD
Flow Rate	1.0 ml/min	0.9 ml/min	0.25
		1.1 ml/min	0.11
pH	5	4.8	0.17
		5.2	0.83
Mobile phase ratio	Acetonitrile + 0.02M phosphate buffer (50+50)	40+60	0.79
		60+40	0.13
Column temperature	25 ° C	23 ° C	0.89
		27 ° C	0.24

Robustness

The robustness of an analytical procedure is a measurement of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the proposed method was determined by changing the composition of the mobile phase, flow rate, pH of the mobile phase and column oven temperature over small ranges. The RSD values were less than 2, indicate that proposed method was robust. Robustness study results are shown in **Table 3**.

LOD and LOQ

According to ICH recommendations, the standard deviation of the response and mean slope of the calibration curve were used for determination of LOD and LOQ. The LOD and LOQ of FVS were 1.1µg/ml and 3.3µg/ml, respectively which indicate the sensitivity of proposed methodd.

System suitability

In order to prove the adequate resolution and reproducibility of the developed methodology, system suitability parameters, peak area, retention time, asymmetry factor and a theoretical plate number were measured. The results are shown in **Table 4**.

Table 4. Summary of Validation parameter and System suitability test parameter

Parameter	Fluvastatin sodium
Linearity	5-40 µg/ml
Corelation coefficient	0.999
LOD[a]	1.1 µg/ml
LOQ[b]	3.3µg/ml
Accuracy	99.02 – 101.27 %
Repeatability (RSD[c] %) (n=6)	0.44
Precision (RSD[c] %)	
Interday (n=3)	0.515-0.754
Intraday (n=3)	0.165-0.642
Retention Time	4.50 ± 0.05 min
Theoretical Plate number	5270
Asymmetry Factor	0.87

[a] LOD =Limit of Detection
[b] LOQ = Limit of Quantitation
[c] RSD = Relative Standard Deviation

Analysis of Marketed Formulation

The proposed stability indicating LC method was successfully applied to the determination of FVS in capsule dosage forms. The assay result was found to be 101.75% ± 0.26, which is good agreement with the label claim.

Kinetic investigation

Kinetic investigation of FVS in acidic degradation

The kinetics of degradation of FVS was investigated in 0.1MHCl. The increased concentration of acid increased the formation of the main degradation product, and the area of the FVS peak decreased drastically (**Figure 2(a), (b), (c), 2-(a)**). A regular decrease in the concentration of intact FVS with increasing time intervals was observed. During the acidic degradation major degradation product was observed at 6.54mins without interfering the elution of drug peak (FVS=4.5mins) (**Figure 3(a), (b), (c), 2-(a)**). At the selected temperature (50, 60, 70 80°C) the acid degradation of FVS processes first-order kinetics (**Figure 5(a)**). The apparent first order degradation rate constant and the half life at each temperature are shown in **Table 5**. The influence of temperature on the reaction rate constant (kobs) in 0.1MHCl was given by the Arrhenius equation.

$$\ln k_{obs} = \ln A - \frac{E_a}{RT} \quad (1)$$

where A is the frequency factor, E the energy of activation, R the universal gas constant, and T is the absolute temperature.

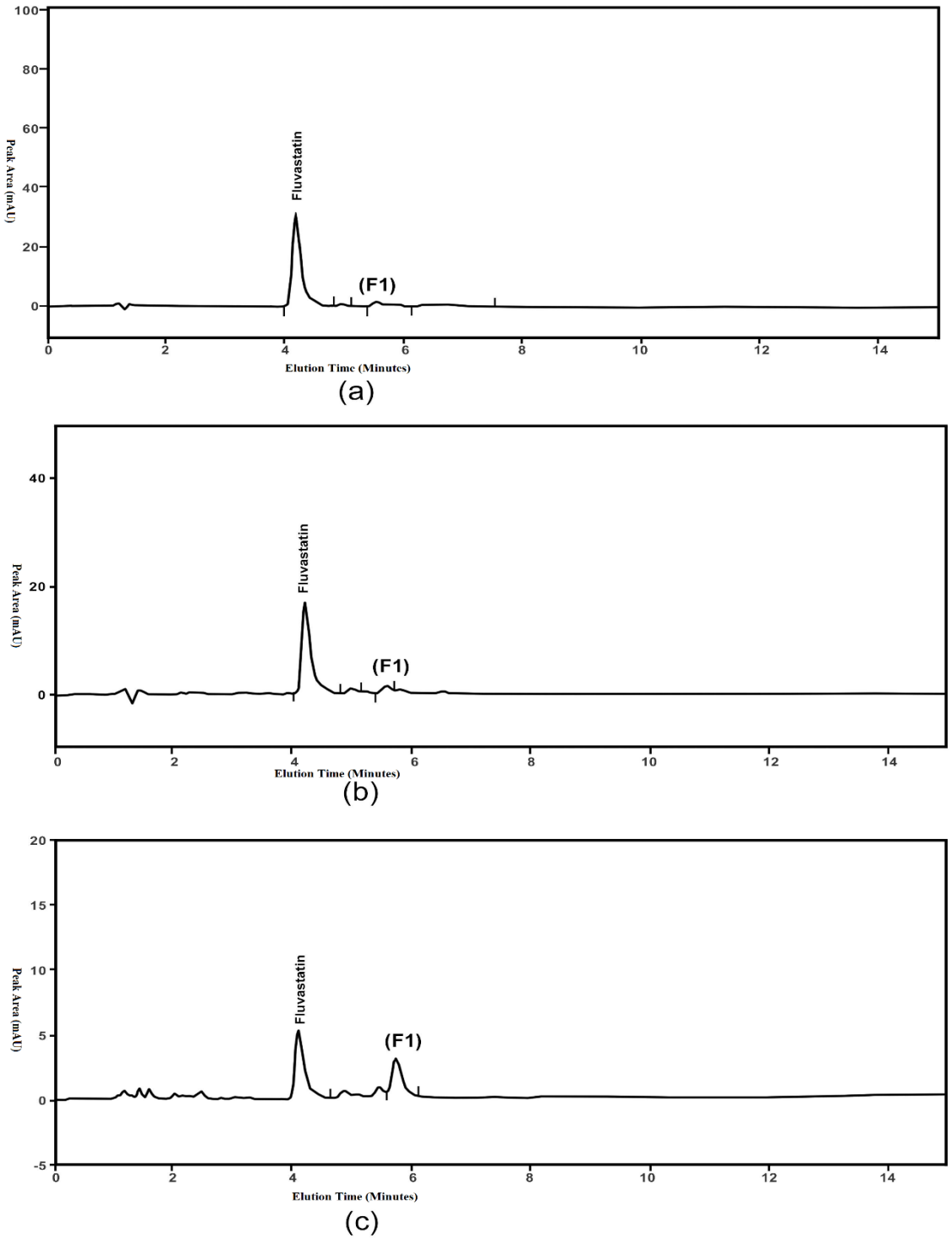

Figure 4. Chromatogram obtained of FVS at 70 °C. (a) Oxidative degradation, 3% Hydrogen peroxide, 30mins, (b) Oxidative degradation, 3% Hydrogen peroxide, 60mins, (c) Oxidative degradation, 3% Hydrogen peroxide, 120mins

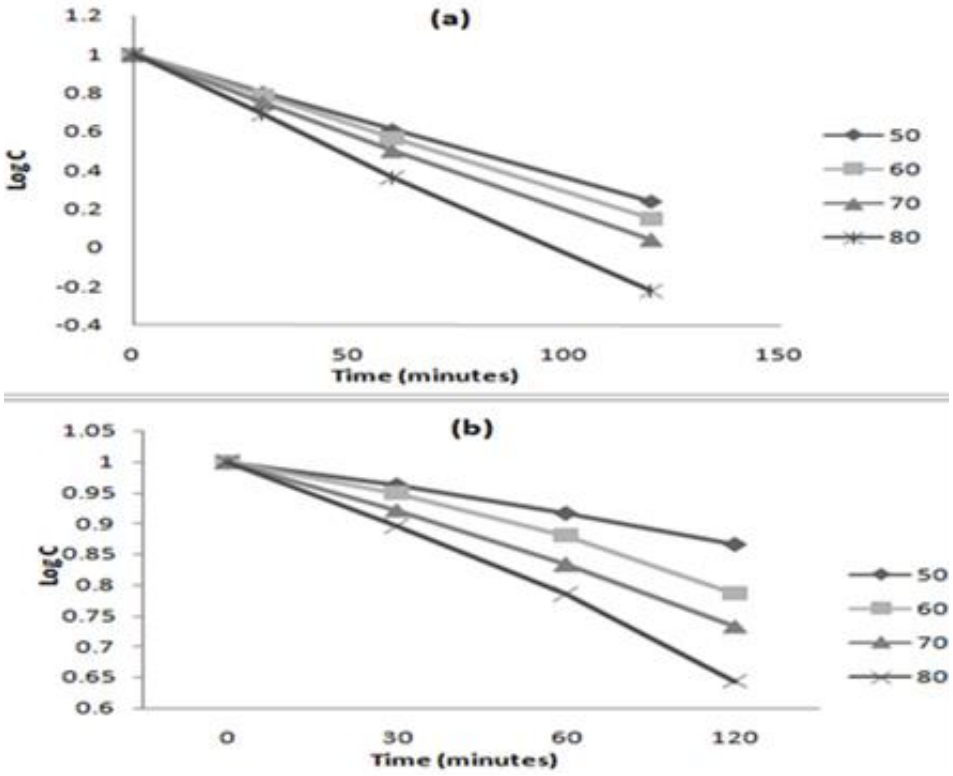

Figure 5. First order plot of the FVS in (a) 0.1M hydrochloric acid and (b) 3% hydroxide peroxide at different temperature by LC method.

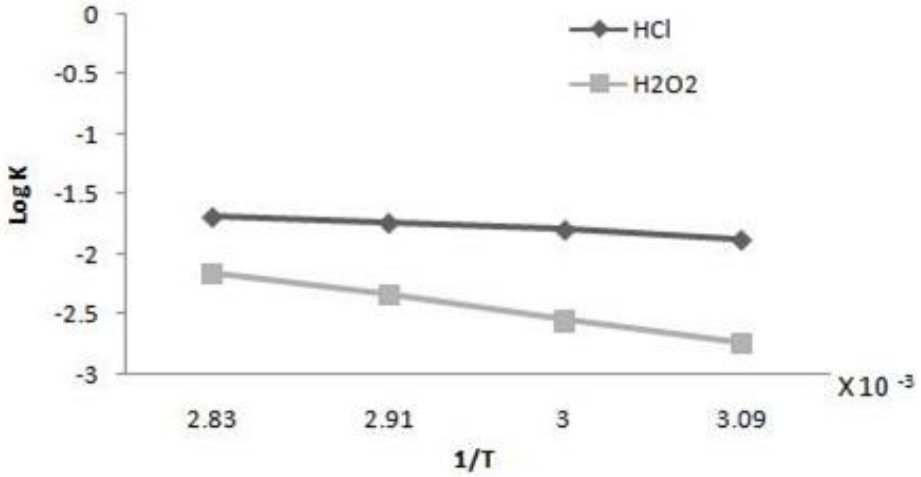

Figure 6. Arrhenius plots for the degradation of fluvastatin in (a) 0.1M hydrochloric acid and (b) 3% hydroxide peroxide by LC method. K is apparent first order degradation rate constant and T is temperature in Kelvin scale.

Table 5. *Degradation Rate Constant (kobs) and half-life (t1/2) of fluvastatin sodium*

Temperature (°C)	Acidic degradation (0.1M HCl)			Oxidative degradation (3% H_2O_2)		
	K_{obs} (min^{-1})	$t_{1/2}$ (min)	t_{90} (min)	K_{obs} (min^{-1})	$t_{1/2}$ (min)	t_{90} (min)
50	0.0133	52.10	7.81	0.001842	376.13	56.44
60	0.0161	43.04	6.45	0.00276	250.75	37.63
70	0.0184	37.66	5.65	0.00460	150.45	22.57
80	0.0206	33.59	5.04	0.00690	100.30	15.05

Plotting log K values versus 1/T, the Arrhenius plots (**Figure 6**) were obtained, which were found to be linear in the temperature range 60–90°C for the acidic degradation (r= 0.99). The activation energy was calculated for FVS which was found to be 3.27cal/mol.

Kinetic investigation of FVS in oxidative degradation

The kinetic determinations of FVS in oxidative conditions with 3% hydrogen peroxide investigated. A regular decrease in the concentration of intact FVS with increasing time intervals was observed, and no additional peaks were observed in the chromatogram (**Figure 4 (a), (b), (c)**). At the selected temperature (50, 60, 70 80°C) the oxidative degradation of FVS processes first-order kinetics (**Figure 5(b)**). The apparent first order degradation rate constant and the half life at each temperature are shown in **Table 5**. The influence of temperature on the reaction rate constant (kobs) in 3% hydrogen peroxide was given by the Arrhenius equation (1). Plotting log K values versus 1/T, the Arrhenius plots (**Figure 6**) were obtained, which were found to be linear in the temperature range 60–90°C for the oxidative degradation (r=0.99). The activation energy was calculated for FVS which was found to be 10.22cal/mol.

CONCLUSION

The developed RP-HPLC-DAD method is sensitive, precise, specific, accurate, robust, stability indicating and validates according to ICH guidelines. In this method the susceptibility of the FVS towards the environmental conditions, including light, temperature, acid hydrolysis, alkaline hydrolysis and chemical oxidation were studied. Based on the peak purity results obtained from the analysis of forced degradation samples using the described method, it can be concluded that there is no other co-eluting peak with the main peaks, and the method is specific for the determination of FVS in the presence of degradation products. The forced degradation study revealed that degradation products were formed under acidic conditions. The acidic and oxidative degradation of fluvastatin followed a first order reaction. It may be extended to the determination of the degradation kinetics of fluvastatin sodium in biological fluids. Because the method separates the drug from its degradation products, it can be used as stability indicating. The proposed method was successfully applied to study the kinetic degradations of FVS in acidic and oxidative conditions.

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